Baseline for Amphibian monitoring in some Albertine Rift sites

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Comprehensive Monitoring for Climate Change Adaptation and Management in the Albertine Rift Protected Area Network

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Introduction

Tropical forests are among the most biologically rich ecosystems on Earth, though they are all being rapidly jeopardized by habitat conversion. They are also vulnerable to other large-scale environmental changes (Laurance and Peres, 2006), and particularly global warming (Williams et al., 2003; Tewksbury et al., 2008; Colwell et al., 2008). However, some degree of uncertainty exists about the nature and magnitude of anthropogenic impacts on tropical forest ecosystems (see: Houghton et al., 2001; Laurance, 2004; Vera et al., 2006 for projecting models of global temperatue and CO2 levels increasing; Hemp and Beck, 2001; Cochrane, 2003 for increasing vulnerability to fire due to changes in rainfall levels; Phillips et al., 1998; Laurance et al., 2004; Lewis et al., 2009; Clark et al., 2003; Feeley et al., 2007 for the debate on the effect of increasing CO2 levels on tropical ecosystem functioning). Similarly, the effects of rising temperatures on tropical biota are still under debate. Many tropical organisms are supposed to be thermally specialized, being adapted to response to limited temperature variability (Janzen, 1967; Huey, 1976). Those species that experience low temperature variation in nature are thought to have reduced thermal tolerances (van Berkum, 1988; Addo-Bediako et al., 2000; Ghalambor et al., 2006; Deutsch et al., 2008; Jiguet et al., 2006). Such thermal specialization should have produced the key consequence of elevational specialization in tropical species with many thermally specialized organisms having narrow elevational ranges alike (Janzen, 1967; Ghalambor et al., 2006; Colwell et al., 2008; McCain, 2009; for a review see Laurence et al., 2011). Elevational specialization partly explains the striking richness and endemicity of montane tropical species (Fjeldså and Lovett, 1997; Rahbek, 1997; Ricketts et al., 2005). Tropical high-elevation specialists are suggested to be among the most threatened species on Earth due to global warming (Williams et al., 2003; Hilbert et al., 2004; Ricketts et al., 2005; Thomas et al., 2004; Williams et al., 2007; Raxworthy et al., 2008; Sekercioglu et al., 2008). Actually, currently available data are still scarce, but suggest that montane species are increasingly shifting towards higher elevations (Pounds et al., 1999; Raxworthy et al., 2008; Chen et al., 2009). Nonetheless, many low altitudes species, particularly reptiles and amphibians, could also be vulnerable to global warming in ways similar to their counterparts at higher elevations, since they may have narrow thermal optima, low capacity to adapt to higher temperatures and high rates of evaporative water loss (Huey and Webster, 1976; Hertz, 1979; van Berkum, 1988; Deutsch et al., 2008; Weathers, 1997). Among the highest priorities of the challenging threats that global

warming is posing to tropical ecosystems, and particularly to montane areas, is the monitoring of on going elevational shifts in montane species (Raxworthy et al., 2008; Sekercioglu et al., 2008; Chen et al., 2009). Up to now, upslope displacement leading to the extinction of species pushed towards and off the tops of mountains has been showed in the field only one time (Pounds et al., 1999). However, upslope displacement has been recently documented by various studies (Grabherr et al., 1994; Parmesan, 1996; Pauli et al., 1996; Kullman, 2001; Erasmus et al., 2002; Epps et al., 2003; Konvicka et al., 2003; Pauli et al., 2007), but the information regarding tropical ecosystems are still scarce (IPCC, 2007, Raxworthy et al. 2008). In particular tropical montane regions, which usually show high levels of endemicity and are topically inhabited by species adapted to live in narrow elevational zones close to summits (Ricketts et al., 2005), have been poorly investigated (Rull & Vegas-Vilarrúbia, 2006). Few studies addressed the vulnerability of the herpetofauna of tropical mountains; despite results seem to confirm that amphibians and reptiles are highly susceptible to global warming, due to their physiological and ecological constraints. Still, few evidences of upslope displacement have been reported from tropical regions (Bustamante et al., 2005; Stuart et al., 2004). Moreover, there is evidence that the impact of the well known chytrid fungus disease can be boosted by warming thus working in synergy with global warming to drive herpetological assemblages to decline (Pounds et al., 2006; Lips et al., 2005, 2006; Whitfield et al., 2007).

<u>Aims</u>

The Albertine Rift region has been targeted to investigate warming trends and their effects on its highly susceptible herpetological assemblages. The Albertine Rift region in East Africa is one of the world's hotspots for biodiversity (Plumptre 2002; Plumptre et al. 2003; 2007; Cordeiro et al. 2007). It is also one of the most threatened, due to dense intensive smallholder agriculture, high levels of land and resource pressures, and high rates of habitat loss and conversion, making it a high priority area for conservation (Brooks et al. 2001). Regional warming trends in the Albertine Rift region have not been widely explored and the potential vulnerability of herpetological montane assemblages to global warming has never been addressed. Namely, the aim of our study will be to determine whether there is supporting evidence for upslope shifts of reptiles and amphibians in the Albertine Rift region and to assess their potential extinction vulnerability. Here we provide details on species assemblages at different elevation occurring at selected sites, in order to provide the baseline data for subsequent monitoring activities on selected assemblages.

Methods

Field survey

At each site, spatial occurrence and altitudinal distribution of herpetological assemblages were recorded during surveys conducted between 2008 and 2011. Details on survey periods are provided below for each area. Sample sites within each area have been selected as representative of significant habitats at various elevations. Sites have been sampled through opportunistic searching, visual encounter surveys and acoustic surveys. Where needed, anuran vocalizations were recorded by means of a Olympus linear PCM digital LS-10 Recorder. Surveys were conducted during both the day and night (with the help of headlamps) to sample the highest number of species. Specimens have also been sampled opportunistically during forest walks in touristic trails, and along roads, where present. Surveys were conducted along linear transect with a searching buffer of 10-20 m around, or within plots (details on each surveyed site are provider hereafter) depending on terrain conditions. Transects and plots elevation was recorded by means of GPS units (Garmin GPS60X). Survey dates, geographical coordinates, HD (Herpetologist Days, each day including both daytime and night-time surveying) are reported for each site. In addition, we report here also data collected in previous surveys that we conducted in the same areas (Nyungwe Forest NP). Data on amphibian assemblages on Bwindi, Uganda, are included despite being recorded in a less standardized manner. They represent an important update on species occurrence and their altitudinal distribution and constitute an important source of information on a site where investigations on amphibians have been subject of interest in earlier years.



Molecular analyses

DNA analyses of the specimens collected in Nyungwe Forest and Kahuzi-Biega NPs have been carried out in order to assess and confirm the taxonomic status of the species and to evaluate genetic diversity across the investigated landscapes. For the total genomic DNA extraction, the "DNeasy © Blood and Tissue Handbook" using the kit from QIAGEN (Hombrechtikon, Switzerland) was applied. Tissue samples of 25mg were cut into small pieces and were put into 180µl of ATL buffer (QIAGEN). Then 20µl of Proteinase K (QIAGEN) was added, vortexted and incubated on a Thermomixer® (Eppendorf) at 55°C for \geq 3 hours, until the tissue is completely digested. After vortexing and adding 200µl of AL buffer (QIAGEN), the tissues were incubated at 70°C for ten minutes. Then 200µl of ethanol (96%) was added and the tissues sample was mixed by vortexting for 15 seconds. The digested samples were pipetted into the corresponding Spin Columns and centrifuged at 8'000 revolutions per minutes (rpm) for one minute. Then the through-flow was discarded and 500µl of AW1 buffer (QIAGEN) was added to the samples. After centrifuge at 8'000rpm for one minute, the through-flow was discarded and 500µl of AW2 buffer (QIAGEN) was put into the column. Subsequently the samples were put into the centrifuge for another 3 minutes at 14'000rpm. The columns were then placed into Eppendorf tubes

and 100µl of AE buffer (QIAGEN) was added directly into the center of the column. After incubating at room temperature for one minute and centrifuge at 8'000rpm for another minute, the DNA elution was completed. To ensure the total elution of the DNA, the last step was repeated in a second Eppendorf tube to gain a "back-up" sample.

Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) was used to amplify approximately 600 base pairs (bp) of the 16S rRNA genes using the following 16S primers:

- 1) 16s 16SAL modified (Palumbi et al. 1991)
 5'-CGC CTG TTT ACC AAAAAC AT-3'
- 2) 16s L2510 f (16SAL from Palumbi et al. 1991)5'-CGC CTG TTT ATC AAAAAC AT-3'
- 3) 16s H3062 r (16SBH modified Palumbi et al. 1991)5'-CCG GTT TGAACT CAGATC A-3'

For this study, PCR beads from illustraTM puReTag Ready-To-Go (GE healthcare, UK) were used. For each bead, 15µl of doubled-distilled H2O, 5µl of the extracted template, 2,5µl of forward and 2,5µl of reverse primer was added. A Labcycler (SensoQuest, Switzerland) was used for amplification of the DNA followed the standard PCR conditions. The following PRC thermal cycle was used for 16S PCR: 5 minutes at 95°C, followed by 35 cycles of 95°C for 60s, 51°C for 30s and 72°C for 90s, and a final extension phase at 72°C for 7 minutes. Then the PCR product was cooled down and kept at 4°C. All amplified PCR products were verified using electrophoresis on a 1.0% agarose gel. PCR products were purified using the QIAGEN DNeasy DNA Purification System according to manufacturer's recommendations. DNA sequences of both strands were sequenced by Microsynth AG (Balgrist, Switzerland). Raw DNA sequences where aligned in CodonCode Aligner version 3.7.1.2 (Centerville, USA) and corrected by hand. A consensus sequences was made of both strands and exported. Sequences were aligned using MUSCLE (Edgar 2004) with default settings (maximum number of Iterations: 8) in the bioinformatics tool suit Geneious Pro 5.5.4 (Auckland, New Zealand). Alignment ambiguities were identified using GBLOCKS version 0.91b (Talavera & Castresana 2007) with default parameter settings for block selection. For each gene partition the best-fit models of nucleotide substitution were identified using the Akaike information criterion (Akaike 1974) as

implemented in jModeltest version 0.1.1 (Posada 2008). The datasets were analyzed using Bayesian inference (BI) using the "Maia Cluster" at the University of Basel. Bayesian analysis was conducted with MrBayes 3.2.1 (Huelsenbeck & Ronquist 2001; Ronquist et al. 2012) by running four simultaneous Markov chains for 10 million generations, which were sampled every 1000 generations and discarding the first one million generations as burn-in to prevent sampling before reaching stationary. Support for internal branches was evaluated using posterior probabilities calculated in MrBayes and p-distances were calculated using Geneious.

Training of local personnel

From 29th to 30th November 2010, a workshop have been organized, in order to provide basic knowledge on Albertine Rift herpetofauna, survey training skills and herpetological data collection techniques to WCS staff and to the various NPs personnel involved in surveys. Chytrid fungus sampling as well as biosecurity procedures (namely aimed at preventing chytrid disease spreading) and basic knowledge on climate change and its effects on tropical environments were provided. All the trainers and participants were also involved in the following survey in NFNP.

Instructors and scientists involved were as follows:

- Michele Menegon (MdS, Trento)
- Fabio Pupin (MdS, Trento)
- Tracie Seimon (WCS, NY)
- Anton Seimon (WCS, NY)
- James Watson (WCS, NY)
- Liana Jospeh (WCS, NY)

Workshop participants were respectively from:

- WCS (DRC: Guillain Mugilegile Mitamba, Emmanuel Muhindo Wasukundi; Rwanda: Martin Sindikubwabo, Augustin Ntamunoza)
- Karisoke Research Centre (Deo Tuyisingize, Valens Musemakweli)

- Makerere University & Institute for Tropical Forest Conservation (Robert Sekisambu)
- Rwanda Development Board (Evariste Musonera, Daniel Nsabimana).



Study area

Albertine Rift

The Albertine Rift is recognised as the richest region for vertebrates in Africa (Brooks et al., 2001; Plumptre et al., 2003). It is defined as the region extending from 30 km north of Lake Albert to the southern tip of Lake Tanganyika, including the valley, flanks of the escarpment and associated protected areas, and the range of species endemic to it (Plumptre et al., 2003). Various sources recognize it as an area of high conservation concern: it is an Endemic Bird Area according to Bird-Life International (Stattersfield et al., 1998), a 'Global-200' priority ecoregion according to WWF (Olson and Dinerstein, 1998; Burgess et al., 2004), and is recognized as a part of the Eastern Afromontane Hotspot by

Conservation International (CI) (Brooks et al., 2004), though these publications have some difference in geographical coverage, mirroring the different criteria they adopted to define the region (see Plumptre et al., 2007, for a comprehensive definition of the region).

Selected areas overview

Nyungwe Forest National Park (Rwanda)

NFNP is considered among the most diverse and important montane forests of East Africa, mainly due to the presence of 13 species of primates and 285 birds, 25 of the bird species being local endemics. Also flora and vegetation are particularly diverse, with 280 Albertine Rift endemics and 47 local endemic species. Interestingly, NFNP and particularly Cyamudongo Forest, represent the eastern distribution limit for many plants of the Guineo-Congolian region. Floristic discontinuities can be observed both on latitudinal and altitudinal clines, according with the complex topographic, geological and climatic diversity of the forest. Between 2008 and 2011 we have been surveying 6 sites in NFNP. Besides data provided in the following sites, additional records have been collected in the surroundings of Gisakura HQ and Uwinka Park Centre by opportunistic surveys.

Kahuzi-Biega National Park (DRC)

The Kahuzi-Biega National Park (KBNP) has been gazetted in 1970 with the main goal to protect some of the last Grauer's gorillas (*Gorilla beringei graueri*). Named after the higher peaks within the area, which are in fact extinct volcanoes, Kahuzi (3.300 m a.s.l.) and Biega (2.790 m a.s.l.), it comprises about 6.000 square kilometres of forest, and it ranges from the lowland sector at 600 m a.s.l. to the top of Kahuzi peak. The mountain sector lies entirely north to Bukavu town, along the lake Kivu, thus in one of the most densely populated area of DRC. KBNP is a World Heritage Site since 1990 and, unfortunately, is listed as a World Heritage Site in Danger since 1997, due to the unceasing conflicts, political instability and economic interests that shaped this side of DRC, which still today threaten the future of the forest. However, the Institut Congolais pour la Conservation de la Nature (ICCN), and some international institutions as Wildlife Conservation Society (WCS)

are actively protecting and monitoring the park. Between 11/11/2011 and 20/11/2011 we have been surveying three sites in the mountain sector of KBNP. Due to poor security, we have not been able to survey any site in the lower sector of the park.

Bwindi Impenetrable National Park (Uganda)

Bwindi Impenetrable National Park is located in South Western Uganda at the edge of the western arm of the Albertine Rift. The park covers about 331 Km2 characterized by steep hills and dense forest at an elevation from 1200 to 2600 m a.s.l. with varying vegetation cover associated with the high faunal biodiversity and endemicity. An earlier herpetological survey of amphibians in Bwindi revealed 24 species of which 10 were endemic to the Albertine Rift (Drewes and Vindum 1992). A survey was conducted between December 2010 and March 2012 to find out the diversity and distribution of amphibians in Bwindi INP. Sampling was done in 9 sectors of the park namely Ruhija, Mubwindi swamp, Kitahurira, Buhoma, Rushaga, Nkuringo, Ngoto and Ndego valley.

Nyungwe Forest National Park monitoring sites



Cyamudongo

Cyamundongo is a forest patch of around 5 square km, about 10 km from Nyungwe Forest, ranging from 1600 to 2000 m a.s.l. Due to the small size of the forest and terrain conditions, two survey plots were established. Since there isn't a proper wet area within the forest, and the streams crossing the area seemed too steep to be suitable for surveys, Plot 1 was established within a wet area with openings in a small valley adjacent to the road and Plot 2 in the western border of the forest. Opportunistic walks were made along the many paths of the forest and around campsite.



Cyamudongo	S	E	HD	
Camp site	-2.545290962	28.98507756	20	
Plot 1	-2.544752089	28.98456811	8	
Plot 2	-2.545522889	28.98193644	7	

Campsite (and surroundings):

Campsite was established near the road arriving to the forest, at the beginning of a chimptracking trail. Surroundings (due to the small size of the forest most of it can be surveyed within few hours walking) have been surveyed along trails. It is particularly important to actively search under the leaf litter after raining, while randomly searching along trails, in order to contact any *Boulengerula fischeri* emerging from the underground.

Plot1:

Adjacent to the road and the campsite, an opening on the forest border in a small valley with a wet bottom and ephemeral ponds have been surveyed for amphibians.

Plot2:

It has been identified as good searching plot by the calls heard from the main road. It's represented by a wide wet valley at the forest edge, surrounded by cultivation.

Genus	note	species	Camp site and surroundings	Plot1	Plot2
Amietophrynus	sp.	1	X		
Arthroleptis	sp.	1	X		
Arthroleptis	cf.	schubotzi	X		
Boulengerula		fischeri	X		
Hyperolius		castaneus		х	x
Hyperolius	cf.	kivuensis			x
Leptopelis	cf.	karissibensis		х	
Xenopus	cf.	wittei			x
Adolfus		africanus	X		
Atheris		nitschei	X		
Cnemaspis	cf.	quattuorseriata	X		
Lepidothyris		hinkeli	x		
Leptosiaphos	cf	graueri 1	x		
Lygodactylus	cf.	gutturalis	X		
Polemon		collaris	x		
Rhampholeon		boulengeri	x		
Telothornis		kirtlandi	x		1
Trachylepis	cf.	maculilabris	x		

Kamiranzovu

Kamiranzovu Swamp lies at around 1950 m a.s.l. and includes a huge wet opening surrounded by forest. Two plots were established. Plot 1 focuses on an opening and low density forest mixed area, that has been surveyed by random walk along the stream within the plot, the ponds and along the vegetation within the area. Plot 2 contacts both the forest border facing the swamp and the open area of the swamp itself. Also, random walks were conducted along the path linking the camp site to the swamp, and around the camp site.



Kamiranzovu	S	E	HD
Camp site	-2.477220604	29.15814073	6
Plot 1 (Dood Troop)	1) -2. 476580897	1) 29.15815271	6
Plot 1 (Dead Trees)	2) -2.476803772	2) 29.15852303	0
Plat 2 (Orabid Trail)	1) -2.481001765	1) 29.15491386	C
Plot 2 (Orchid Trail)	2) -2.480707057	1) 29.15306381	6

Campsite (and surroundings):

the campsite has been built up near the border between the open swamp and the surrounding forest. Some ponds can be found around the campsite within few minutes walking, though their water level can vary among seasons. Smaller ephemeral water bodies are also present in the surroundings

Plot1 (Dead Trees):

it has been established around a stream near the campsite. It is easily recognizable by the dead trees in the clear openings within the area. Terrain conditions prevented us to establish a proper transect along the stream. The surroundings of the stream, as well as the stream itself and the forest edges has be surveyed.

Plot 2 (Orchid Trail):

this plot covers the border of the forest and the adjacent open swamp area, including part of the path going to the Orchid Trail, part of the Orchid Trail itself, and covers a roundish area having a diameter of about 300 m, from coordinates 1 to 2 (see the table). In particular, ephemeral water ponds within the open area should be thoroughly investigated for amphibians.

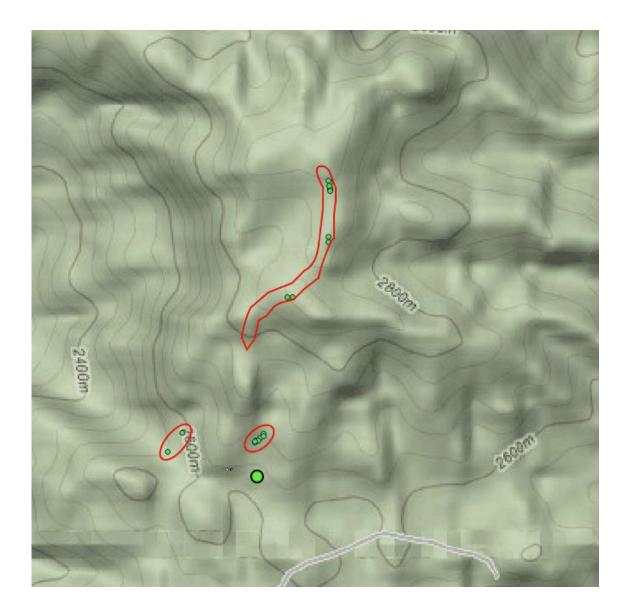
Opportunistic walks:

the trail from the campsite to Plot 2 can be opportunistically surveyed with particular attention to any basking reptile in open spots during the day, and for sleeping chameleons during the night. It should be underlined that opportunistic surveys in the open area of the swamp have not been successful and no amphibian has been detected there, while they all have been contacted on the border of the swamp and in the nearby forest.

Genus	note	species	Camp site and surroundings	Plot1	Plot2
Afrixalus		laevis	x	x	X
Amietia	sp.	1	x	x	X
Arthroleptis	cf.	schubotzi	x	x	X
Hyperolius		castaneus	X	x	x
Hyperolius		chrysogaster		X	
Leptopelis	cf.	karissimbensis		x	
Atheris		nitschei	x		x
Congolacerta		vauereselli			x
Rhampholeon		boulengeri	x		x
Xenopus		wittei		x	x

Bigugu

With its 2950 m a.s.l., it is the highest peak in NFNP. Here we established two plots, Plot 1 and Plot 2, each focused on a streams along the south side of the mountain. Plots were covering both the stream and the surrounding vegetation. A transect was also established along the trail to the mountain summit, with a buffer of about 5/10 m on each side. Random walks around the campsite and on the top of the mountain were also conducted.



Bigugu	S	E	HD

Camp site	-2.457320374	29.24936484	
Plot 1	1) -2.455565287	1) 29.2492775	8
	2) -2.455083579	2) 29.24972191	0
Plot 2	1) -2.456037775	1) 29.24474037	1
	2) -2.455052985	2) 29.24549625	1
Transect 1	1) -2.448060215	1) 29.25098958	8
	2) -2.442183075	2) 29.25321498	0

Campsite:

The campsite was established on a small flat area on the right side of the trail going to the peak summit. The surroundings of the campsite have been opportunistically surveyed though no encounter has been recorded.

Plot 1:

the plot extends from coordinates 1 to 2 on a stream near the campsite, which has been surveyed by walking inside the stream bed and thoroughly inspecting the small ponds herein present, as well as the vegetation along the water. The stream crosses an open area mostly covered by ferns, which proved to be a suitable habitat for *T. rudis*.

Plot 2:

same as Plot 1, a small stream crossing an open area covered by ferns, on the left side of the trail going to the peak summit. The plot has been surveyed similarly to Plot 1.

Transect 1:

the transect has been established from coordinates 1 to 2 along the trail going to the peak summit, with a buffer of 5/10 m depending on terrain conditions. Surroundings are mostly represented by *Erica johnstoni* stands, bushes and rocks. Though highly elusive, rocks along the trail should be accurately surveyed for *Leptosiaphos* cf. *graueri*. Night surveys have resulted entirely in *T. rudis* encounters. Also, the peak summit has been intensively surveyed by opportunistic walks along *E. johnstoni* bushes and trees.

Genus	note	species	Plot1	Plot2	Transect
Hyperolius	cf.	discodacylus	x	Х	
Phrynobatrachus	sp.	1			Х
Leptosiaphos	cf.	graueri			Х
Trioceros		rudis	x		Х

Karamba



Karamba	S	E	HD
Camp site and Plot 1	-2.47866	29.22654	8

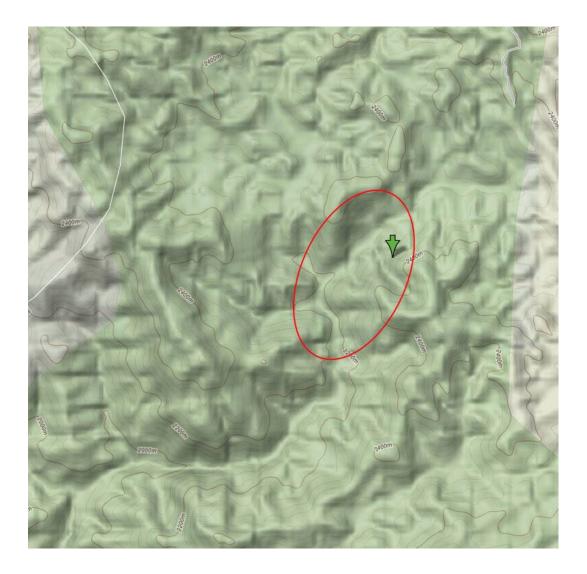
Campsite:

the camp site has been established near the main road crossing the park, in a small open area and surroundings have been opportunistically surveyed. Plot 1 has been established

in the nearby, where a wet area and openings are present. Also, diurnal search of basking or dead reptiles along the main road have been conducted.

Genus	note species		Plot1
Amietia	sp.	2	X
Arthroleptis	cf.	schubotzi	Х
Hyperolius		jackie	x
Hyperolius		castaneus	X
Hyperolius	cf.	chrysogaster	x
Leptopelis	cf.	kivuensis	x
Leptopelis	sp.	Karamba	X
Xenopus		wittei	X
Lycophidion		ornatum	x
Philotamnus		carinatus	X
Rhamnophis		ethiopissa	Х

Nshili



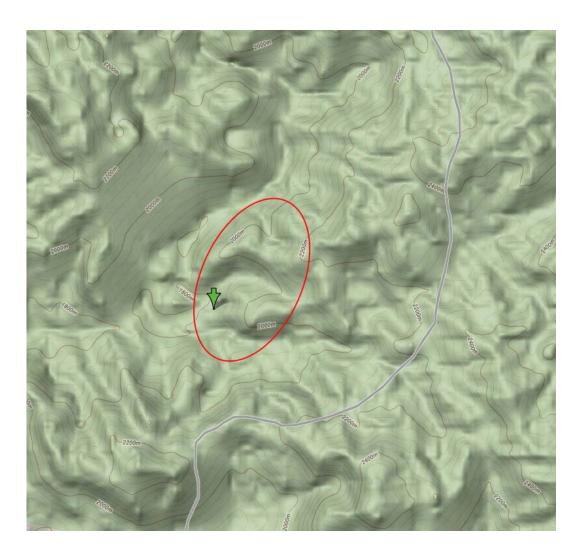
Nshili	S	E	HD
Camp site and Plot 1	-2.739167	29.390361	6

The campsite has been established near a stream in a valley at the border of the forest. Surroundings within the forest as well the stream and the open area nearby have been surveyed by random walks both during the day and night.

Surveys have been conducted also in the nearby patch of bamboo forest though the absence of any water element seemed to affect herpetofauna presence.

Genus	note	species	Camp site
Hyperolius	cf.	chrysogaster	Х
Hyperolius		frontalis	Х
Leptopelis	sp.	red	Х
Leptopelis	cf.	kivuensis	х
Rhampholeon		boulengeri	х

Busoro

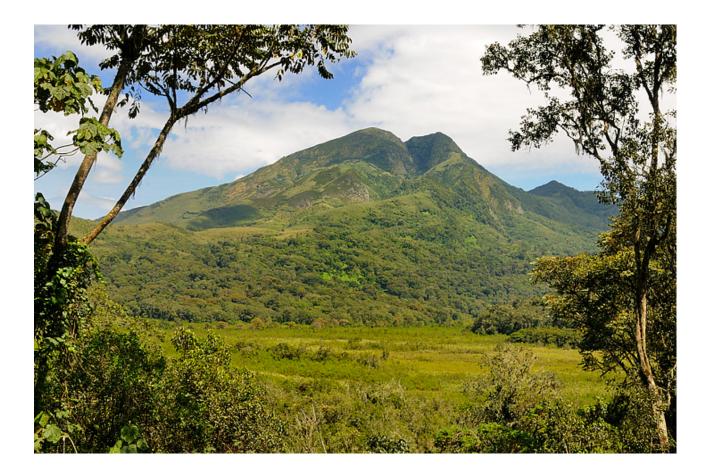


The collecting site at Busoro is a particularly interesting due to its relatively lower elevation. Day and night search took place along small stream and ponds in the forest surrounding the campsite. The valley bottoms are often strongly altered by past mining activities; the excavations have left series of water filled ponds of different size, where the frog *Xenopus wittei* is common.

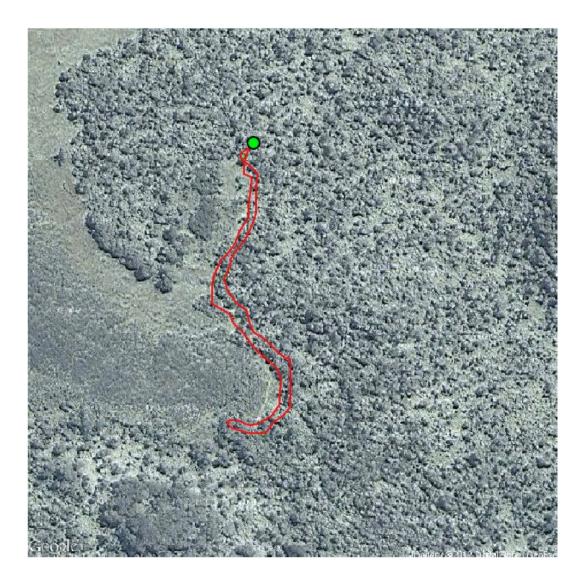
Busoro	S	E	HD
Camp site and Plot 1	-2.56753	29.23079	4

Genus	Genus note species		Plot1
Afrixalus		laevis	X
Amietophrynus		kisoloensis	X
Hyperolius		castaneus	X
Hyperolius		frontalis	X
Buhoma		depressiceps	X
Trioceros		johnstoni	x
Dasypeltis		atra	X
Lamprophis		olivaceus	Х
Philotamnus		carinatus	Х
Typhlops	sp.	1	Х

Kahuzi-Biega National Park monitoring sites



Bulugumiza

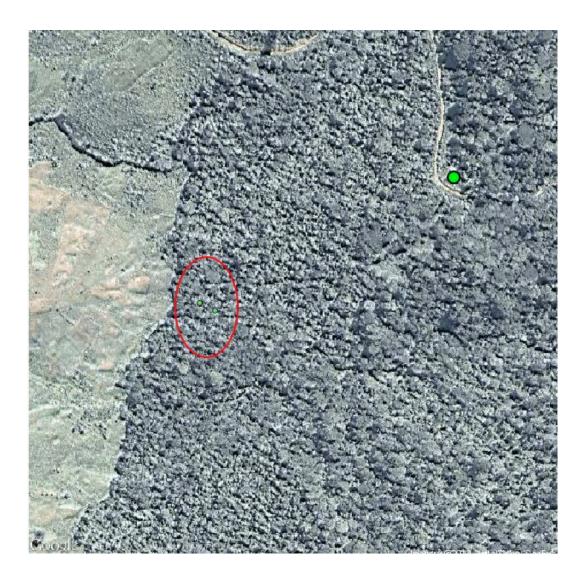


Bulugumiza	S	E	HD
Camp site	-2.325097024	28.729025	6
Plot1	1) -2.326861918	1) 28.72867447	6
	2) -2.328869384	2) 28.72917353	0

In Bulugumiza, the camp site has been established near an open swamp area, where we have established a survey plot (Plot 1) which included the open swamp borders, the surrounding vegetation and the water bodies within the swamp, represented by both standing water bodies and a slow-flowing stream.

Genus	note	species	Plot1
Afrixalus		laevis	X
Hyperolius		discodacylus	X
Hyperolius		castaneus	X
Leptopelis		karissimbensis karissimbensis	X
Leptopelis		kivuensis	X
Xenopus		wittei	X
Atheris		nitschei	x

Madidiri



Madidiri	S	E	HD
Madidiri HQ	-2.309264867	28.64935326	3
Plot 1 (centered)	-2.310998831	1) 28.64622764	6

In Madidiri the Park HQ have been used as camp and a plot (Plot 1) has been established along a stream in a steep valley. Water bodies along the stream as well as secondary small streams have been surveyed. Surrounding vegetation has been also included in the plot. The area is frequently visited by a forest elephant family, to which particular care is deserved. Nonetheless, along elephants paths, footprints should be thoroughly checked, since, when filled with water, they have proved to be a suitable micro-habitat for both tadpoles and adult amphibians. Species listed in Madidiri HQ have been collected by local people in the nearbies.

Genus	note	species	Madidiri HQ	Plot1
Amietophrynus		kisoloensis		X
Arthroleptis		adolfifriderici		X
Leptopelis		karissimbensis		X
Xenopus	sp.	KBNP		X
Xenopus		wittei		X
Atheris		nitschei	X	
Congolacerta		vaueresellii	X	X
Dasypeltis		atra	X	
Leptosiaphos	sp.	KBNP	X	
Rhampholeon		boulengeri		X
Trioceros		johnstoni	X	
Trioceros		rudis	X	x

Mugaba



Mugaba	S	E	HD
Park HQ	-2.273593582	28.66294434	3
Plot 1	1) -2.267188886	1) 28.66219198	6
Plot 1	2) -2.267768327	2) 28.66283177	0

In Mugaba we have been hosted in the Park HQ and a plot (Plot 1) has been established across an open swamp and the surrounding forest. The plot included the open vegetation of the swamp, as well as the water bodies in it and the forest vegetation at the border of the open has been surveyed as well. Encounters listed in Park HQ have been recorded during opportunistic walks along the road near the HQ.

Genus	note	species	Park HQ	Plot 1
Afrixalus		laevis		X
Amietia	sp.	Mugaba	X	
Hyperolius		castaneus		X
Leptopelis		karissimbensis		X
Leptopelis	sp.	KBNP		X
Leptopelis		kivuensis		X
Leptosiaphos	sp.	KBNP	X	
Kinyongia		adolfifriderici		X

Bwindi Impenetrable National Park monitoring sites

MANCA FOTO

Ruhija

Ruhija is located on the eastern edge of the park at an elevation of about 2350 m a.s.l. This is also where the Institute of Tropical Forest Conservation (ITFC) is stationed. Sampling was done in 3 narrow swamps, namely Bishayu, Rwizi and Hamukuubo, all located in the valleys between very steep cultivated hills. Additional sampling was done around the compound of ITFC station and along 2 already existing trails: one from Ruhija to Nyarucundura campsite and another from Ruhija ranger post to Kanyambogo campsite respectively.

Bishayu swamp is below the ITFC conservation center and stretches from the evidently less disturbed park boundary into community land where cultivation of yams and vegetables is evident. Rwizi swamp is located below an abandoned eco-tourism center about 2Km from ITFC along the park boundary. Hamukuubo swamp is an abandoned dam near the former Wolfram mine about 3Km from ITFC conservation center. The swamp is flooded throughout the year with tall grass vegetation measuring about 2 meters above ground. Most of the sampling here was done along the periphery to avoid drowning but also inside where it seemed safe to operate.

Ruhija	S	E	HD
Bishayu swamp (BIS)			
Rwizi swamp (RWI)			
Hamukuubo (HAM)			
ITFC compound (ITFC)			
Ruhija Nyarucundura trail (RNT)			
Ruhija Kanyambogo trail (RKT)			

A total area of 24000m² was sampled around Ruhija sector for 660 minutes by 3 peoples for 10 days between December 14th 2010 and March 3rd 2011. CONTROLLARE COME SONO DISTRIBUITI I 10 GIORNI SUI TRE SITI PER GLI HERPETOLOGICAL DAYS

Genus	note	species	BIS	RWI	HAM	ITFC	RNT	RKT
Amietia		angolensis	Х		Х			
Amietia	sp.	?			Х			
Leptopelis		kivuensis	Х	X	Х	Х		
Amietophrynus		kisoloensis			Х			
Ptychadena		mascareniensis		Х				
Xenopus	cf.	wittei	Х		Х			

Mubwindi Swamp

Mubwindi swamp is located at an elevation of about 2105 m a.s.l. at about 4km south of the ITFC station in Ruhija. The swamp is surrounded by steep densely forested edges and covers about 2 km2 and is the largest of all sites considered for the study. The wetland is characterized by tall grass (an average 1m from the ground) and it's accessible throughout the year although some parts get flooded during the rainy season. Sampling was done at 3 sites, namely Nyarucundura, Kajembejembe and Kanyambogo camping sites. Additional sampling was done during the day along the Nyarucundura - Kajembejembe trail and the Kanyambogo - Nyarucundura trail. A total area of 21,550m2 was sampled in a total of 390minutes by 24 people from January 15th 2011 to March 4th 2011.

Ruhija	S	E	HD
Nyarucundura (NYA)			
Kajembejembe KAJ)			
Kanyambogo (KAN)			
Nyarucundura - Kajembejembe trail (NKT)			
Kanyambogo - Nyarucundura trail (KNT)			

Genus	note	species	NYA	KAJ	KAN	NKT	KNT
Amietia		angolensis	Х	Х		Х	
Leptopelis		kivuensis	Х	Х	Х		
Leptopelis	sp.	2	Х				
Xenopus	cf.	wittei				Х	
Arthroleptis	sp.	1	Х				
Arthroleptis		adolfifriederici				Х	
Hyperolius		castaneus	Х	Х	Х		

Hyperolius	sp.	2	Х		Х	
Hyperolius	<mark>?</mark>	<mark>puslus</mark>	Х			
Hyperolius	Cf.	discodacylus	Х			
Hyperolius		cinnamomeoventris		Х		

Buhoma

Buhoma is located on the western side of the park, less than 1 km from the Uganda - DRC border at about 1450 m a.s.l. Sampling was done at 3 sites, namely along Munyaga river, around Habinyanja's swamps and along the trail between Munyaga river and Habinyanja's swamps.

Munyaga River is flowing along the park boundary in Buhoma –a busy tourism center for gorilla trekking. Sampling was done along an ecotourism footpath that goes between the fast flowing Munyaga river and a very slow moving stream from its start to the bridge. The fast flowing river has many rocks on the river bed yet the very slow moving stream had water logged soil. Sampling along River Munyaga was done for 90 minutes by 3 people on January 25th 2011. Habinyanja ponds are located about 2 hours walk from Munyaga river at an elevation of about 1830 m a.s.l. The swamp comprises 2 large ponds of about 50 m² in a relatively open area of high grasses. The surrounding area is dump with broad leaved trees. Some unidentified weeds seemed to slowly be growing to cover a significant area of the ponds. Sampling was done around the pond. Additional sampling was done in a plot laid 10 meters away from the swamp. This site has been sampled again during the rainy season of September 2010 to record any changes in amphibian composition. Several egg masses were also observed here.

Buhoma	S	E	HD
Munyaga river (MUN)			
Habinyanja swamp (HAB)			
Munyaga-Habinyanja trail (MHT)			

Genus	note	species	MUN	HAB	MHT
Amietia		angolensis	Х	Х	
Leptopelis		kivuensis	Х	Х	
Leptopelis		christyi	Х	Х	
Leptopelis		calcaratus	Х		

Hyperolius	sp.		Х	Х
Hyperolius	sp.	1		Х
Hyperolius	sp.	2		X
Amietophrynus		kisoloensis		X
Hyperolius		castaneus		X
Hyperolius		cinnamomeoventris		X
Hyperolius		lateralis		X
Hyperolius		adspersus		X
Hyperolius sp4	sp.	4		X
Hyperolius sp5	sp.	5		X
Hyperolius sp6	sp.	6		X
Xenopus cf wittei	cf.			X
Xenopus sp1	sp.	1		X
Afrixalus cf osorioi	cf.		Х	X

Rushaga

The camp was set at Rushaga ranger post located on the southern part of the park off Kisoro road at about 2000 m a.s.l. Sampling was done in 4 sites, namely Nshongi swamp, Rushaga falls, Mpororo stream, and Mpororo pond. Additional sampling was done along 3 trails connecting the sites. Although the swamp interior is generally accessible, some parts of the swamp have deep abandoned ponds covered with floating vegetation. The swamp is surrounded by a dense mature forest on a gentle slope and a slow moving stream along the park boundary. Rushaga falls is 45 minutes from the ranger post in a less frequently isolated bat cave surrounded by thick forest and rocks covered by algae. Mpororo stream and the adjacent pond (30 m2, 10 m from the stream) are located about 1 hour from the ranger post into the park, at 1940 m a.s.l and are surrounded by full grown forest. The pond is one of the remainings of the mining activity during the colonial eraWe learned that Mpororo pond was constructed for the purpose of gold mining that was going on in the area during the colonial era.

Rushaga	S	E	HD
Nshongi swamp (NSH)			
Rushaga falls (RUS)			
Mpororo stream and pond (MPO)			
Ranger post - Mpororo (RMT)			
Ranger post - falls trail (RFT)			

Genus	note	species	NSH	RUS	MPO	RMT	RFT
Xenopus	cf.	wittei	Х		Х		
Hyperolius		castaneus	Х		Х		
Hyperolius		cinnamomeoventris	Х		Х		
Leptopelis		kivuensis	Х		Х		
Amietophrynus		kisoloensis	Х		Х	Х	Х
Hyperolius	cf.	discodacylus			Х		
Xenopus	sp.				Х		
Ptychadena		chrysogaster				Х	

Nkuringo

Nkuringo is about 20 north east from Rushaga, at around 1660/1770 m a.s.l. The area is characterized by numerous extremely steep rocky hills. Tough the site did not have any swamp there are some streams, where the surveys have been focused. The camp site was built in the buffer zone near the ranger post below the UWA area office. Sampling was done along selected streams flowing through the buffer zone, at Nkuringo falls, along river Nkuringo. Additional sampling was done along the trail connecting Nkuringo to Buhoma.

Nkuringo	S	E	HD
Nkuringo river trail (NRT)			
Buffer zone (BUF)			
Nkuringo falls trail (NFT)			
Nkuringo falls (NKU)			

Genus	note	species	NRT	BUF	NFT	NKU
Amietia		angolensis	Х			Х
Amietia	sp.	(tadpoles)	Х	Х		
Hyperolius		frontalis	Х			
Ptychadena	sp.				Х	

Ngoto

Ngoto swamp is located in the northern sector of the park in Kanungu district at about 1280 m a.sl. The camp was set at on the playground of a church near the ranger post. Sampling was done at Ngoto community swamp (swamp 1), Ngoto papyrus swamp (swamp 2), along the trail from campsite to river Ishasha along Ngoto road side, along Ishasha river and along the trail on the Kiizi stream.

Ngoto	S	E	HD
Ngoto swamp 1 (NS1)			
Ngoto swamp 2 (NS2)			
Campsite – Ishasha river trail (CIT)			
Ngoto road (NGR)			
Ishasha river (ISH)			
Kiizi stream (KII)			

Genus	note	species	NS1	NS2	CIT	NGR	ISH	KII
Amietia		angolensis	Х					
Hyperolius	sp.	8	Х					
Hyperolius		lateralis	Х	X				
Hyperolius		cinnamomeoventris	Х					
Hyperolius		kivuensis	Х	X				
Leptopelis		kivuensis	Х	X		Х		
Ptychadena		mascareniensis	Х					
Ptychadena		chrysogaster	Х			Х		
Hyperolius		sp.1		Х				
Hyperolius		discodactylus		X				
Amietophrynus		kisoloensis			X			
Ptychadena		anchietae				Х		

Kitahurila

Kitahurila is located at the neck of the park that joins the southern to the norther sector, at around 1550 m a.s.l. It is a narrow forest crossed by river and a marum road to Buhoma. Sampling was done on a trail along Kitahurira stream from the road to to the junction between the stream and the river. *Afrixalus laevis* and *Hyperolius laevis* were found calling from leaves at about 8 meters from the road.

Kitahurila	S	E	HD
Kitahurila stream (KST)			
Kitahurila swamp (KSW)			
Opportunistic encounters (OPP)			
Hakikoome swamp (HAK)			

Genus	note	species	NS1	NS2	CIT	NGR
Afrixalus		laevis	Х			
Hyperolius		frontalis	Х			
Amietia		angolensis	Х			
Amietia	sp.					Х
Leptopelis		kivuensis		Х		
Hyperolius		cinnamomeoventris		Х	Х	Х
Hyperolius		castaneus			Х	
Hyperolius	sp.	1		Х	Х	
Hyperolius	sp.	7		Х		
Hyperolius	sp.	9		Х		Х
Phlyctimantis		verrucosus				Х
Ptychadena	cf.	chrysogaster		Х		
xenopus		wittei		X		

Maliburila

Maliburila is Sampling was done at a relatively small swamp (about 800 m²) located on Mbwa stream and along the Ruhija- Maliburila trail. Additional sampling was done along the trail from the road to Mbwa stream. Sampling was repeated the following day just in case any additional species would be encountered.

Maliburila	S	E	HD
Mbwa swamp (MBW)			
Ruhija- Maliburila trail (RMT)			

Genus	note	species	MBW	RMT
Ptychadena		mascareniensis	Х	
Amietia	sp.		Х	
Hyperolius	sp.	1	Х	
Leptopelis		kivuensis	Х	
Hyperolius		lateralis	Х	
Hyperolius		castaneus	Х	
Hyperolius		discodacylus	Х	

Ndego Valley

Ndego is located The site was chosen because of the presence of Kikugwa stream and evident agricultural activity up to the park boundary. Sampling was done along Kicugwa stream, a garden close to the park boundary, and at another small swamp located

Ndego Valley	S	E	HD
Kikugwa stream (KIK)			
Ndego uphill swamp (NSW)			
Ndego Valley Garden (NVG)			

Genus	note	species	KIK	NSW	NVG
Amietia		angolensis	Х		
Leptopelis		kivuensis	Х	Х	
Xenopus		laevis	Х	Х	
Hyperolius		lateralis	Х	Х	
Hyperolius	sp.	2	Х		
Hyperolius		castaneus		Х	
Amietophrynus		kisoloensis			Х
Ptychadena		mascareniensis		Х	
Ptychadena		chrysogaster		Х	

Altitudinal elevation

								Ele	eva	tior	ר (m	a.s	s.l.)			
Genus	note	species	Site	note	1800	1900	2000	2100	2200	2300	2400	2500	2600	2700	2800	2900
Africalis			NFNP		1	1										
Afrixalus		laevis	KBNP							1						
Amintin		4	NFNP			1										
Amietia	sp.	1	KBNP													
Amiatia	0.0	2	NFNP		1											
Amietia	sp.	2	KBNP													
Amiatia	0.0	2	NFNP													
Amietia	sp.	3	KBNP							1						
Amiotophymup	0.0	1	NFNP		1											
Amietophrynus	sp.	1	KBNP													
Amiotophymus		kisoloensis	NFNP		1											
Amietophrynus		KISOIOENSIS	KBNP			1										
Arthroloptic	of	schubotzi	NFNP		1	1	1	1	1	1						
Arthroleptis	cf.	SCHUDOTZI	KBNP													
Arthroloptic	of	a dalfifridariai	NFNP													
Arthroleptis	cf.	adolfifriderici	KBNP			1										
Arthroloptic	0.0	1	NFNP		1											
Arthroleptis	sp.	1	KBNP													
Poulongorulo		fischeri	NFNP		1											
Boulengerula		nschen	KBNP													
Lhung roliug	of	diagodogyływa	NFNP										1			
Hyperolius	cf.	discodacylus	KBNP							1						
Hyperolius			NFNP		1	1	1	1	1	1						
Hyperollus		castaneus	KBNP							1						
Hyperolius	cf.	chrysogaster	NFNP			1	1	1	1	1						
Typeronus	<i>C1.</i>	Chrysogaster	KBNP													
Hyperolius	cf.	cinnamomeoventris	NFNP			1										
nyperollus	CI.	Cirinamomeovenuis	KBNP													
Hyperolius		frontalis	NFNP		1	1	1									
riyperonus		nontans	KBNP													
Lhuparalius	of	kinganaia	NFNP		1											
Hyperolius	cf.	kivuensis	KBNP													
Hyporolius	00	1	NFNP		1	1	1	1	1	1						
Hyperolius	sp.	1	KBNP		1											
Lantonalia	of	korioaibionaia	NFNP		1											
Leptopelis	cf.	karissibiensis	KBNP			1	1	1	1	1						
Leptopelis	cf.	kivuensis	NFNP			1	1	1	1	1						

Baseline for herpetological monitoring in the Albertine Rift montane forests

			KBNP						1				
Leptopelis	sp.	1	NFNP			1							
Loptopono	00.		KBNP						1				
Phrynobatrachus	sp.	1	NFNP								1		
	901		KBNP										
Xenopus	cf.	wittei	NFNP	1	1	1	1	1	1				
	0.11		KBNP		1	1	1	1	1				
Xenopus	sp.	1	NFNP										
	- 1		KBNP		1								

Comments

boundary

Discussion

A total of 25 species of Amphibians and 24 species of Reptiles have been collected in the investigated areas so far. While almost all the collected reptile specimens have been identified at species level, with two exceptions (*Leptosiaphos* cf. *graueri* and *Trachylepis* cf. *maculilabris*), many of the amphibian identifications are at species level due to the poor taxonomic resolution of some of the taxa involved. Ongoing molecular analysis on the material collected will be critical in order to assess their taxonomic status.

The communities investigated in the two national parks share some of the typical highlands Albertine Rift species, like *Hyperolius castaneus* and *Leptopelis karissimbensis*, despite the overall similarities between amphibian communities, some of the species recorded seem to have particularly restricted distribution.

An unidentified *Leptopelis* species, similar in general morphology and call features to *L. kivuensis*, seems to occur at one site in KBNP only. Individuals of two *Leptopelis* populations, respectively from Nshili and Karamba, in Nyungwe Forest National Park, belong to quite distinctive lineages and seem not to be referable to any described taxon, they could thus be considered as putative new species.

The forest reed frogs *Hyperolius frontalis* and *Hyperolius chrysogaster* have been recorded for the first time in the NFNP and more in general in Rwanda. Both species seem to occur at low densities at two sites and three sites respectively. A further *Hyperolius* species from two sites in Nyungwe could represents a taxon new to science and it deserves further investigation. The leaf folding frog *Afrixalus laevis* was not recorder in Rwanda before, despite being common and widespread in suitable habitats.

The reed frog *Hyperolius discodacylus* seems to occur at elevation higher than 2300/2400 m als, suggesting a certain degree of physiological specialization to high altitude and it could represents a particularly suitable species for monitoring and detecting upslope displacement.

The herpetological community in Cyamudongo, the small forest fragment west of Nyungwe, formally included in the Nyungwe Forest National Park, based of the partial data so far collected, seems to be substantially different from the one recorder in the Nyungwe main forest block and it deserves further investigation. In fact, Cyamudongo is the only known Rwandese occurrence site of several reptile species like *Lepidothyris hinkeli* and *Polemon collaris*.

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Appendix 1: Species identification

Amphibians



Afrixalus laevis



Afrixalus laevis



Hyperolius alticola



Hyperolius alticola



Hyperolius castaneus



Hyperolius castaneus



Hyperolius castaneus



Hyperolius chrysogaster



Hyperolius frontalis



Hyperolius sp. blue



Hyperolius kivuensis



Hyperolius sp. web



Leptopelis karissimbensis



Leptopelis sp. Karamba



Leptopelis kivuensis



Leptopelis kivuensis



Leptopelis kivuensis



Leptopelis sp. red



Leptopelis sp. KBNP



Leptopelis sp. KBNP



Amietia sp. 1



Amietia sp. 2



Amietia sp. Mugaba



Amietophrynus kisoloensis



Arthroleptis adolfifriderici



Arthroleptis shubotzi



Boulengerula fischeri



Prhynobatrachus sp. Bigugu



Xenopus wittei



Xenopus sp. KBNP

Reptiles



Congolacerta vauereselli



Lepidothyris hinkeli



Leptosiaphos cf. graueri



Lygodactylus cf. gutturalis



Cnemaspis sp.1



Trachylepis cf. maculilabris



Kyniongia adolfifriderici



Trioceros ellioti



Trioceros johnstoni



Trioceros rudis



Rhampholeon boulengeri



Atheris nitschei



Buhoma depressiceps



Lamprophis olivaceus



Dasypeltis atra



Lycophidium ornatum



Phylotamnus carinatus



Phylotamnus ruandae



Polemon collaris



Rhamnophis aethiopissa



Telothornis kirtlandii

Appendix 2: species lists

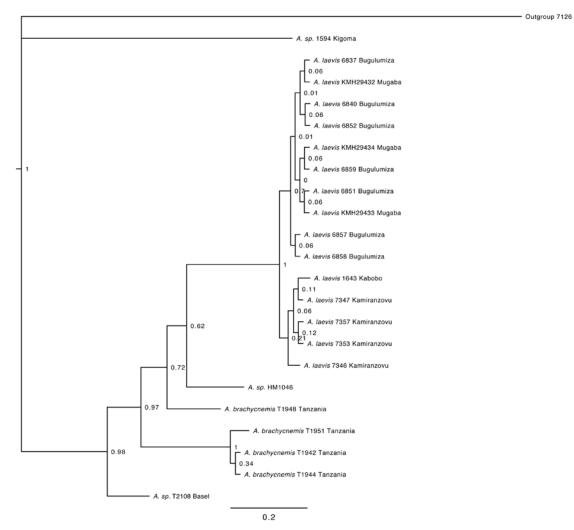
The list is based on the results of both morphological and molecular analysis. Molecular analysis has been completed for some of the groups and the id of some of the species include still need to be confirmed.

						NF	NP						
Genus	note	species	Comments	Cyamundongo	Nshili	Karamba	Kamiranzovu	Busoro	Bigugu	Bwindi	Bulugumiza	Madiriri	Mugaba
Afrixalus		laevis					х	х		х	х		х
Amietia	sp.	1					х						
Amietia	sp.	2				х							
Amietia	sp.	Mugaba											х
Amietophrynus	sp.	1		х									
Amietophrynus		kisoloensis						х		х		х	
Arthroleptis		adolfifriderici	at Uwinka	х						х		х	
Arthroleptis	cf.	schubotzi		х		х	х			х			
Boulengerula		fischeri		х									
Hyperolius		discodacylus							х	х	х		
Hyperolius		jackie				х							
Hyperolius		castaneus		х		х	х	х		х	х		х
Hyperolius	cf.	chrysogaster			х	х							
Hyperolius		frontalis			х			х		х			
Hyperolius	cf.	kivuensis		х						х			
Hyperolius	cf.	discodacylus			х					х			
Hyperolius		lateralis								х			
Leptopelis	sp.	Karamba				х							
Leptopelis	-	karissimbensis		х							х	х	х
Leptopelis	sp.	KBNP											х
Leptopelis	-	kivuensis			х	х	х			х	х		х
Leptopelis	sp.	red			х								
Leptopelis	-	calcaratus								x			
Leptopelis		chrysti								x			
Phlyctimantis		verrucosus								х			
Phrynobatrachus	sp.	1							х				
Ptychadena	-	chrysogaster								x			
Xenopus	sp.	KBNP										х	
Xenopus		wittei		х		х					x	х	
-													
Adolfus		africanus		x									
Atheris		nitschei		х			х			х	х	х	

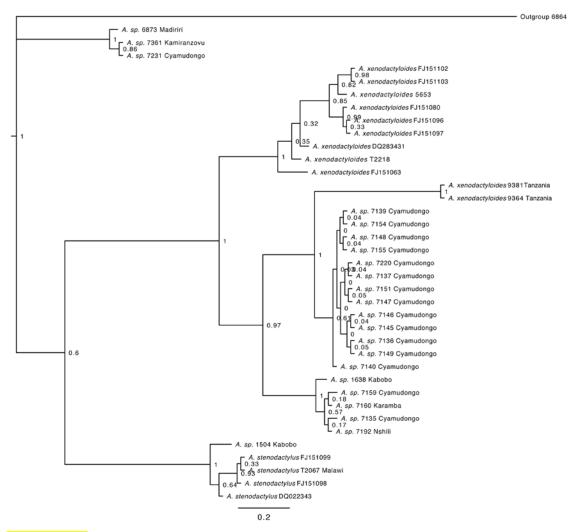
Buhoma		depressiceps						Х				
Trioceros		ellioti	at Gisakura									
Cnemaspis	sp.	1		х								
Congolacerta		vauereselli					х				х	
Dasypeltis		atra						х			х	
Dipsadoboa		unicolor						х		х		
Kinyongia		adolfifriderici	at Uwinka									х
Lamprophis		olivaceus						х				
Lepidothyris		hinkeli		х								
Leptosiaphos	cf	graueri		х					х		х	х
Lycophidion		ornatum				х						
Lygodactylus	cf.	gutturalis		х								
Philotamnus		carinatus				х		х				
Philothamnus		ruandae	at Gisakura									-
Polemon		collaris		х								
Rhamnophis		ethiopissa				х						
Rhampholeon		boulengeri		х	х		х			х	х	
Telothornis		kirtlandi		х								-
Trachylepis	cf.	maculilabris		х								-
Trioceros		johnstoni	at Uwinka					х		х	х	-
Trioceros		rudis					1		х		х	
Typhlops	sp.	1						х				

Hyperolius nasutus complex

Titolo molecular analyses



Afrixalus



Arthroleptis