THE HERPETOFAUNA OF THE VIRUNGA MOUNTAINS, WITH AN EMPHASIS ON TWO SPECIES OF ALBERTINE RIFT ENDEMIC TREEFROGS IN THE GENUS *LEPTOPELIS*

by

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ABSTRACT

THE HERPETOFAUNA OF THE VIRUNGA MOUNTAINS, WITH AN EMPHASIS ON TWO SPECIES OF ALBERTINE RIFT ENDEMIC TREEFROGS IN THE GENUS *LEPTOPELIS*

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I present a short description of the biodiversity crisis, the Albertine Rift as a biodiversity hotspot, and a literature review of the taxonomy of the genus *Leptopelis.* I then present a list of the reptiles and anurans from the *Parc National des Volcans* (PNV)(01°43'S, 29°52'W), an area in the west and north provinces of the Republic of Rwanda in the Albertine Rift region of Africa. Fieldwork was conducted for two to six days per week from June through August of 2007 and 2008. I also conducted literature searches of all historical expeditions within the park for species records. Seventeen species of reptiles and anurans are recorded from the PNV. Nine of the species were anurans, distributed in five families: Arthroleptidae (3), Bufonidae (1), Hyperoliidae (3),

Phrynobatrachidae (1), and Pipidae (1). Eight species of reptiles were recorded from five families: Chamaeleonidae (1), Lacertidae (2), Scincidae (2), Colubridae (2), and Viperidae (1). Eight of the seventeen species found in the PNV are endemic to the Albertine Rift.

The previously unreported tadpole of *Leptopelis karissimbensis,* an endangered treefrog from Rwanda, is described. Tadpoles were collected, photographed, measured, and examined for standard metrics of tadpole morphology. Larvae of *L. karissimbensis* resemble other tadpoles in the genus *Leptopelis* in being muscular, elongate, and eel-like. The lateral tooth row formula for *L. karissimbensis* is 4/3. In late stage larvae, tadpoles of *L. karissimbensis* exhibit a prominent white spot below the eye. Larvae of this species were often abundant in suitable habitat at approximately 2800 meters in elevation. Larval habitat for *L. karissimbensis* includes seasonally flooded marshes, forest pools, and permanent ponds in the Virunga Mountains, the only known range of this species.

I examined the taxonomy of two frequently confused Albertine Rift endemic treefrogs, *Leptopelis karissimbensis* and *L. kivuensis*. Included is a review of the literature regarding geographic distribution of the two species and historical characters used to diagnose the species from each other. We present new evidence discounting the use of some characters previously considered diagnostic for the two species. Three previously unrecognized characters, one molecular, one morphological, and one behavioral are provided for diagnostic purposes and we extend the known range of *L. karissimbensis,* an IUCN endangered species, to the west and south into Democratic Republic of the Congo. Based on the findings presented in this paper, we recommend that *L. karissimbensis* be downlisted in conservation status by the IUCN, as it does not meet the criteria to be listed as endangered.

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CHAPTER 1

LITERATURE REVIEW

The Biodiversity Crisis

There can no longer be any doubt that the Earth is in the midst of a biodiversity crisis. Individual species are disappearing at a rate estimated to be over 1000 times the background rate of extinction (Sisk et al., 1994; Singh, 2002). Some authors would argue that even though the idea of species conservation is nothing new, we need to realize the fact that the Earth's entire assemblage of species is necessary for a fully functioning planet as we know it (Savage, 1995). Biodiversity has a philosophical and intrinsic value, but it also has great practical value to humans. Besides the obvious benefits of providing food and shelter, biodiversity provides medicine and probably most importantly, a multitude of ecosystem services such as gas exchange and waste filtration (Savage, 1995; Ando et al., 1998). From a systematics perspective, the preservation of biodiversity is crucial to understand evolutionary history, as missing taxa in phylogenetic analyses can significantly alter the results of these studies, leading to incorrect conclusions about the pattern and process of evolution within a group or for a trait (Wheeler, 1995; Myers and Knoll, 2001; Wheeler et al., 2004).

How we preserve biodiversity by preventing extinction of individual species is a hotly debated topic. Because of human needs, some areas of the world have to be used for food production and physical space to live. With the world's current population nearing seven billion, prioritizing areas for conservation is one of the core issues in the discipline of conservation biology (Ando et al., 1998; Margules et al., 1998). It is logical to attempt to preserve areas and habitats that contain the largest number of species. To achieve this goal, adequate knowledge of where individual species exist is required (Alberch, 1993; Wheeler, 1995). Lack of adequate taxonomic surveys and specimen identifications by trained taxonomists is hampering our knowledge of biodiversity. Some taxonomic groups (various non-vertebrates, plants, some microbes) may have only a small handful of researchers working on a set of particular taxa (Savage, 1995). We cannot attempt to compare potential conservation areas without accurate information about the distribution of biodiversity within those areas if our comparisons are to be fair and unbiased (Cole and Landres, 1996; Ando et al., 1998, Polasky et al., 2000). Clearly, one cannot attempt to conserve a species or even an entire ecosystem unless you know it exists.

It has been estimated that for non-marine species about 50% of all the described species on the planet exist in less than 3% of the land area of the globe (Mittermeier et al., 1998; Myers et al., 2000). This means that regardless of how well we preserve existing habitat elsewhere in the world, if that critical 3% of land area is not preserved, we risk losing the majority of all species that exist. These “biodiversity hotspots,” represent mostly areas of tropical forest in equatorial or subtropical latitudes. There are two criteria a region must meet to qualify as a hotspot according to Myers et al. (2000): the region must contain at least 1,500 species of vascular plants and must have lost at least 70% of its primary vegetation. Criticism of these criteria has been widespread, leading some authors to implement other criteria, such as including other taxa in species richness estimates and considering ecosystem processes (Mittermeier, 2004). Now that the issue has had years of debate and peer review, new, more agreed upon lists of hotspots have emerged (Brooks et al., 2006).

One hotspot that has only recently been recognized as such is the Albertine Rift. This region of Africa currently is one of the most protected in terms of national parks and forest reserves, but suffers from incredible anthropogenic population pressure (Cordeiro et al., 2007). Some of the countries that encompass the Albertine Rift have some of the highest population densities in the developing world. An example is Rwanda, which has a population density of about 400 people per square kilometer, the highest figure for any nation in Africa and the highest of any underdeveloped nation (Cordeiro et al., 2007). Like all biodiversity hotspots, the Albertine Rift contains a massive number of species, many of which are in danger of extinction. One particular group in danger of extinction is the amphibians. Amphibians worldwide are declining at an alarming pace (Duellman and Trueb, 1994; Alford and Richards, 1999; Stuart et al., 2004) and have not been adequately studied in the region, for reasons that will be explained later. The combination of small distributional ranges, anthropogenic pressures, and potential sensitivity to climate change threaten many of these species. Especially since the region has only recently been labeled as a hotspot, there is a need for increased knowledge of all components of the biodiversity of the Albertine Rift, including the reptiles and amphibians.

The Albertine Rift

The Albertine Rift is the western branch of the Great Rift Valley of Africa. From a biological perspective, the Great Rift of Africa exists from where the Red Sea meets the Gulf of Aden at the junction of the African and Arabian tectonic plates south to central Mozambique (Pavitt, 2001; Saundry, 2009). The rift itself forms the boundary between the two subdivisions of the African tectonic plate (Nubian and Somalian). At the southern end of the rift near lake Malawi, it bifurcates into two branches. One branch continues south, while the other extends northwards and westwards. The eastern branch of the Great Rift Valley is known as the Gregory Rift and named for the 19th century British explorer John Walter Gregory. The western branch of the rift is known as the Albertine Rift and is named after Prince Albert of England. Both braches of the Great Rift Valley are being formed as the Nubian Plate remains stationary, while the Somalian Plate moves northwards and eastwards. This action is pulling the continent of Africa apart and has created two enormously long valley complexes, complete with large, deep lakes and high levels of volcanism (Saundry, 2009).

The Albertine Rift itself extends from Lake Malawi in the south to Lake Albert in the North. All of Africa's "Great Lakes" were formed by the creation of the Albertine Rift and most of these lakes lie within the border of the rift itself, despite the fact that they drain to many separate river systems. While Lakes Albert, Edward, and Victoria empty into the Nile drainage, Lakes Kivu and Tanganyika flow into the Congo drainage, and Lake Malawi is part of the Zambezi drainage (Pavitt, 2001). There are two major mountain ranges on the Albertine Rift, the Ruwenzoris and the Virungas. The Ruwenzori

Mountains are higher, with the highest peak being Mount Stanley at approximately 5,100 m in elevation. These mountains were formed by a pressure uplift and are not volcanoes in themselves (Schlüter, 1997). The second set of mountains, the Virunga range, is truly volcanic in origin. There are eight volcanoes in the chain, one of which is active. The highest mountain in the range is the inactive volcano Mt. Karissimbi at approximately 4500 m in elevation. Each of the individual peaks of the Virungas was created by one or repeated volcanic eruptions.

The Albertine Rift represents a distinct biotic region, despite influences from the two bordering biotic provinces (Pomeroy, 1993). The Congo region lies to the west and is mostly low elevation (below 1500 m). Historically, this region was mostly forested and part of the second largest rainforest in the world. To the east lies the East African biotic province. This region is considerably drier than either the Congo or Albertine Rift regions. Like the Congo region, it is much lower in elevation than the Albertine Rift. Instead of forest, much of the region is covered in different types of savannah vegetation ranging from grass-dominated communities to *Acacia* thornscrub or even thorn forest (Greenway, 1973). The differences in elevation between the Albertine Rift and the surrounding areas make the mountains and highlands of the rift “islands in the sky” and like other isolated ecosystems, this makes the rift an area of high endemism regarding species richness (Plumptre et al., 2003, 2007).

The Albertine Rift is generally narrow along most of its length (< 100 km wide) (Saundry, 2009). This narrowness has allowed many species, even those with high site fidelity or philopatric tendencies, to disperse from either the East African or Congo biotic provinces into the Albertine Rift. Because of the proximity of these two regions of largely different habitats, it creates a narrow zone within the rift where species from all three biotic provinces occur. This interesting biogeographic circumstance, combined with the biogeographic nature of isolated montane habitats, leads to the high species level richness in the Albertine Rift.

Knowledge of species richness in the Albertine Rift is incomplete because adequate surveys for many taxonomic groups (fungi, insects) have not been completed (Plumptre et al., 2003). Some results are summarized from Plumptre et al. (2003, 2007). There are about 1,050 species of birds reported from the Albertine Rift. This represents over 50% of the species known to exist on the African continent at some time of the year, including seasonal migrants. Forty-one of these birds are endemic to the Albertine Rift. There are about one hundred seventy five species of reptiles known from the rift, about 15% of the total reptile species richness of Africa. Sixteen reptile species are Albertine Rift endemics. About 140 amphibians are found in the region, compromising a total of about 20% of the total number of species in Africa. Thirty-four amphibians are endemic to the rift. Over 400 mammal species are reported from the Albertine Rift. This number represents about 40% of all the species on the continent. Thirty-four of these are endemic to the region. Plant and fish richness is not well sampled across the continent of Africa, so continental comparisons are difficult, but about 6,000 plant species and about 400 fish species have been identified from the Albertine Rift. Some of the total numbers of species reported are low for their respective taxonomic groups (amphibians, fish) and this is likely the result of undescribed, cryptic species level diversity.

Regarding herpetological diversity, the Albertine Rift usually has at least one endemic representative species from most reptile and amphibian genera found across the continent of Africa. Examples of snakes include the viperid, *Atheris nitschei,* which bears the common name Great Lakes Bush Viper in reference to another name for the Albertine Rift region. *Philothamnus ruandae,* the Rwanda Forest Green Snake is another rift endemic. Among the lizards, the Scincid genus *Leptosiaphos* has only seventeen described species, but four of these are putative Albertine Rift endemics: *Leptosiaphos graueri, L. hackarsi, L. luberoensis,* and *L. meleagris.* Several more species in the genus are Congo Basin species, but occur in the Albertine Rift at the western edge. Three notable species of chameleons are endemic to the Albertine Rift: *Chamaeleo johnstoni, Kinyongia adolfifriderici,* and *Rhampholeon boulengeri.* Examples from the amphibians are numerous. Only one species of caecilian, *Boulengerula fischeri,* is reported from the Albertine Rift and is known only from one specimen collected in southern Rwanda. The most species-rich genus of frogs in Africa, *Hyperolius,* has at least three Albertine Rift endemics: *Hyperolius alticola, H. castaneus,* and *H. frontalis.* The genus *Leptopelis* has two species endemic to the region: *Leptopelis karissimbensis* and *L. kivuensis. Xenopus* has two endemic species: *Xenopus ruwenzoriensis* and *X. wittei.* Two other notable genera with nearly continent-wide distributions have species endemic to the Albertine Rift: *Phrynobatrachus versicolor* and *Amietia ruwenzorica*. All of the reptile or amphibian species endemic to the Albertine Rift share one common trait; they are tolerant of cool or even cold (near 0 C) temperatures. Sub-Saharan Africa itself is a plateau rising out of the ocean several hundred meters and when this baseline elevation is added to the elevations found in the highlands of the Albertine Rift, the result is often a cooler environment, with snow at the highest altitudes in the Virungas and glaciers in the Ruwenzoris. This dissertation will largely focus on the herpetofauna of the Virunga Mountains with a specific focus on the taxonomy, natural history, and conservation biology of the two Albertine Rift endemic treefrog species of the genus *Leptopelis.*

Taxonomic History of the genus *Leptopelis*

The genus *Leptopelis,* Günther, 1859, contains 51 recognized species distributed across Sub-Saharan Africa (Frost, 2008). Originally, this genus was paired with *Nesionixalus*, Perret, 1976, and given the higher order taxonomic name of Leptopelini by Laurent (1972) to recognize a tribe of frogs within the Hyperoliidae. *Nesionixalus* was subsequently placed into synonomy with *Hyperolius*, Rapp, 1842 by Drewes (1984) leaving the Leptopelini to contain only *Leptopelis*. These frogs were then placed in the subfamily Leptopelinae by Dubois in 1981 and then elevated to the family level by Scott in 2005. More recently, the group was downgraded to subfamily status and placed in the genus Arthroleptidae by Frost et al. (2006) on the basis of DNA sequence data.

Much of the following is adapted from Idris (2004), who compiled an excellent literature review of the genus *Leptopelis* and the Old World treefrogs. Phylogenetic, phylogeographic, and systematic studies of the reptiles and amphibians of Africa are more rare than those of North and South America. The relationships of climate and geologic events to speciation and biogeography are not well understood. Most studies of African herpetology have focused on alpha taxonomy, although systematic revisions based on phylogeny are becoming more common with increasing use of molecular data.

The currently recognized families of Old World treefrogs are distributed across Africa, Asia, Madagascar, and several smaller islands in Indonesia and the Indian Ocean. They were originally placed in the family Polypedatidae by Ahl in 1931, which included twelve genera and 527 species. Characters uniting the family were distal intercalary cartilages, a firmisternal pectoral girdle, and slightly to not dilated sacral diapophyses. Since Ahl’s study was published in 1931, huge advances have been made in the knowledge of phylogenetic relationships of higher level taxa of Old World treefrogs. Three main disagreements have arisen regarding the systematics of the group. The first conflict involves simply which families belong in the common grouping known as the Old World treefrogs. Families that have historically been placed in the group include the Hyperoliidae, Astylosternidae, Rhacophoridae, Arthroleptidae and Scaphiophrynidae. The second conflict involves the phylogenetic relationships among the families mentioned above. The third conflict involved the elucidation of the relationships within the afore mentioned families. A specific example related to this dissertation involves the placement of *Leptopelis* into the family Arthroleptidae by Frost et. al. in 2006. Liem (1970) attributed at least some of these problems to character choice and phylogenetic reconstruction methods based on morphological data. Even with the increased use of molecular data in anuran systematics, researchers still issues of character choice and character homology.

In 1951, Raymond Laurent recognized two African frog families with a firmisternal pectoral girdle: the Hyperoliidae and the Ranidae. The synapomorphies for the Hyperoliidae were the unfused astragalus and calcaneum and a cartilaginous metasternum. It should be noted that only two frog families worldwide exhibit the presence of a fused astragalus and calcaneum (Centrolenidae and Pelodytidae), so the absence of this character in the Hyperoliids is not of tremendous phylogenetic significance. This group, as recognized by Laurent, included all the treefrogs of Africa except *Heterixalus* from Madagascar and the Seychelles and the Rhacophorid *Chiromantis.* It also included several non-arboreal lineages, such as frogs of the currently recognized families Arthroleptidae, Astylosternidae, and Scaphioprhynidae. Frogs such as *Chiromantis* and the mantellines of Madagascar were placed in the family Ranidae. Scaphiophrynidae has since been though to be closely related to either Ranids or Microhylids.

Based on thirty-six external, osteological, cartilaginous, and mycological characters, Liem (1970) recognized three families of Old World frogs: the Rhacophoridae, Ranidae, and Hyperoliidae. He stated that the Rhacophorids and Hyperoliids were from different ancestral stocks of Ranids. As classified by Liem, Rhacophorids included many Asian treefrog genera, four from Madagascar, and the African *Chiromantis.* The Arthroleptids and Astylosternids were removed from Hyperoliidae based on their terrestrial lifestyle and morphology. Laurent (1972a, 1979) then refuted many of these taxonomic changes and elevated *Hemisus* to the family level. Laurent did agree with Liem (1970) in that the Astylosternidae represented the ancestral stock of both the Arthroleptids and Astylosternids.

Using morphological characters alone to assess the phylogenetic relationships of frog families in Africa has proven problematic. Morphological and some molecular characters have suggested doubtful relationships and are often highly impacted by the effects of homoplasy (Channing, 1989; Maxson, 1992; Richards and Moore, 1996; Wilkinson and Drewes, 2000; Vences et al., 2003; Frost et al., 2006). Laurent was the first person to challenge the alleged Hyperoliid-Rhacophorid relationship in 1981. Drewes (1984) examined most of the known genera of Hyperoliids and supported the sister group relationship between Rhacophorids and Hyperoliids. Duellman and Trueb later lumped the mantellas and their allies with the Hyperoliids on the basis that both share an unfused second tarsal. However, both Liem (1970) and Ford (1989) showed this trait to be absent in three genera of mantelline frogs: *Mantella, Mantidactylus,* and *Aglyptidactylus.* Liem (1970) found that mantellines and Hyperoliids did share a fused second carpal. Channing (1989) reanalyzed Liem's (1970) and Drewes' (1984) data and supported the sister group relationship between Hyperoliids and Rhacophorids. He found thirteen synapomorphies for Hyperoliids with six that he considered to be the most informative: the presence of a medial dentomentalis muscle, the absence of a nuptial pad, claw shaped terminal phalanges, a vertical pupil, a cartilaginous sternum, and an absence of the posterolateral process of the hyoid. Channing did not include the Astylosternids and Arthroleptids, but did consider "ranids" his outgroups, even though the two families mentioned above have the last three characters. Drewes (1984) considered the most important synapomorphies to be the lack of fusions in the secondary carpals and tarsals, both traits considered to be paedomorphic by him. Laurent suggested that the two groups (Rhacophorids and Hyperoliids) were united by the presence of a vertical pupil, a cartilaginous metasternum, a free second tarsal and a free third carpal. Ford and

Cannatella (1993) questioned the sister taxa of Channing (1989) and Liem (1970) on the basis that Channing inflated the number of actual synapomorphies and Liem did not include appropriate outgroup taxa. Bloomers-Schlosser (1993) considered Rhacophorids to be a group within the Ranidae and proposed Arthroleptids as the sister taxon to Hyperoliids. Emerson et al. (2000) suggested that the subfamily Leptopelinae was closely related to the Scaphiophryninae, which was a subfamily within the Microhylidae. In 2003, Vences et al. stated that Leptopelinae was the sister group to the Astylosternidae but found a weakly supported sister relationship with *Hemisus* and the Arthroleptids. In 2005, Scott suggested that the Leptopelinae was the sister taxon to the Arthroleptids (including the Astylosternines) based on molecular and morphological evidence. Frost et al. (2006) placed the Astylosternines within the Arthroleptids, but also removed the Leptopelinae from Hyperoliidae and placed the Leptopelinae into Arthroleptidae. Odierna et al. (2001) then confirmed that the Leptopelinae was phylogenetically far from Hyperoliidae on the basis of karyological data. Van der Meijden et al. (2007) provided molecular evidence that Arthroleptidae is paraphyletic with regard to Leptopelinae. These most recent studies provide evidence that despite its morphology, the genus *Leptopelis* of the subfamily *Leptopelinae* is closely allied with the currently recognized family Arthroleptidae and not the Hyperoliidae.

Phylogenetic and taxonomic issues within the genus *Leptopelis* have been difficult to address because many new species have been discovered in recent years, tissue samples for DNA sequence analysis are not available for many species, and species in the genus often lack readily diagnosable characters (Laurent, 1981; Poynton, 1985).

Excellent reviews of known frogs in the genus *Leptopelis* are provided by Schiotz (1975, 1999) and several authors who have worked on the herpetofauna of specific countries or regions (Laurent, 1953 for the Democratic Republic of the Congo; Perret, 1966 for Cameroon; Stewart, 1967 for Malawi; Largen, 1977 for Ethiopia; Lanza, 1981 for Somalia; Poynton, 1985 and Poynton and Broadley, 1987 for South Africa; Lambiris, 1989 for Zimbabwe; Amiet, 1991 for Cameroon; Rödel, 2000 for west African savannah; Channing, 2001 for central and southern Africa; and Channing and Howell, 2006 for East Africa).

CHAPTER 2

HERPETOFAUANA OF THE VIRUNGA MOUNTAINS IN THE PARC NATIONAL DES VOLCANS: AN ANNOTATED CHECKLIST OF SPECIES

Introduction

The *Parc National des Volcans* (PNV) in the Republic of Rwanda is situated in the northwest corner of the country along the border with the Democratic Republic of Congo (DRC) and the Republic of Uganda. The park encompasses an area of approximately 120 km^2 at an elevation of approximately 2,600 – 4,500 m (Sleeman et al., 2000). Within the boundaries of the PNV are portions of the Virunga Mountain range, a volcanic massif that is part of the Albertine Rift, which itself makes up the Central African highlands region (Saundry, 2009). The PNV contains the sixth highest mountain in Africa (Mt. Karissimbi) and is one of two remaining areas with a population of mountain gorillas (*Gorilla beringei beringei)* (Plumptre et al., 2007). The Albertine Rift has more endemic vertebrate species richness than any other region in Africa and contains approximately 20% of the known amphibian species, 40% of the known bird species, and 20% of the known mammal species on the continent of Africa (Plumptre et al., 2007). One species of endangered treefrog, *Leptopelis karissimbensis*, is known to exist within the borders of the PNV (IUCN, 2009).

The PNV is part of a wider protected area that makes up most of the central Albertine Rift. This area was once a single national park called Albert National Park, although only a very small percentage of this original park now lies within the borders of Rwanda (< 5 %). In addition, the government of Rwanda annexed much of the lower elevations in the park during the 1970's and 80's in order to provide more land for human settlement, which almost completely eliminated all the true montane forest within the park. While de Witte (1941) wrote an excellent opus on the reptiles and amphibians of Albert National Park and Laurent (1972) published another book length review of the amphibians of the greater Virunga region, no comprehensive list exists for the herpetofauna of the PNV in its current form as a national park of Rwanda. Accurate data concerning species distribution is a necessary component of biodiversity conservation. In this paper, I report on surveys we carried out in the PNV and provide a historical literature review in order to make a list of all the reptiles and amphibians currently found in the PNV, as well as any species that may occur within the borders of the park or have been extirpated. Many of the other protected areas formerly in Albert National Park such as Virunga National Park in DRC and Queen Elizabeth National Park in Uganda have lower elevation habitats (<2000 m), which often have a high number of amphibian and reptile species (Channing and Howell, 2006). In contrast, the lowest elevations in the PNV are around 2,600 m.

Materials and Methods

Study Site

The study site is the *Parc National des Volcans* and its neighboring areas, situated in the North Province of Rwanda in the northwestern corner of the country (Figures 1 and 2). The PNV has an area of approximately 15,000 ha and elevations above 2,600 m.

Since the park is topographically diverse, there are several distinct vegetation zones: a bamboo zone dominated by *Arundinaria alpina*, which occurs between the park boundary and approximately 3,200 m; an upper forest zone dominated by large trees in the genera *Hypericum* and *Hagenia* between 3,200 m and 3,800 m; montane meadow communities dominated by *Senecio, Lobelia,* and various grasses occur interspersed throughout the forest at elevations between 3,000 m and 3,800 m; above 3,800 m, grasses, mosses, and lichens dominate the alpine zone, which exists all the way to the top of the highest mountains in the chain. The Virungas are one of only two areas in central Africa to have true Afromontane vegetative communities (Owiunji et al., 2005).

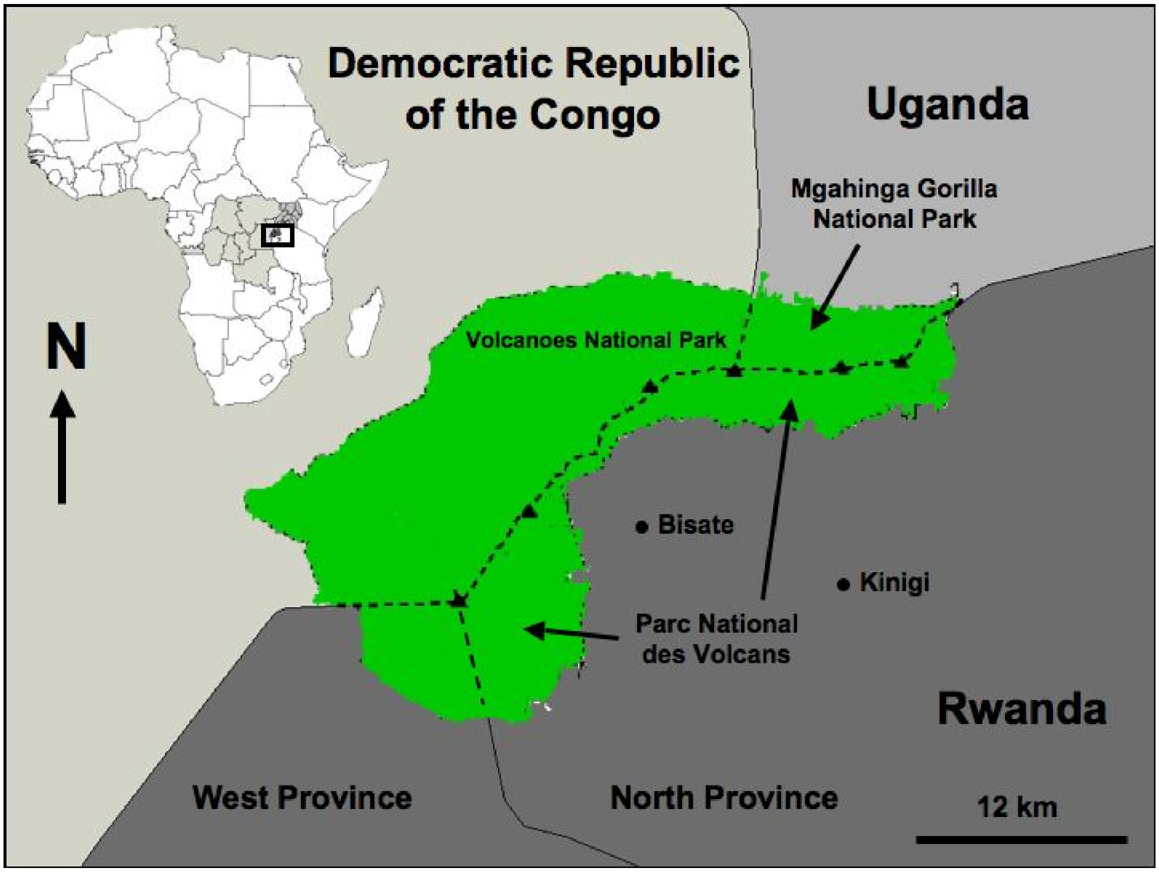

Figure 1 Map of the three parks that encompass the Virunga Mountains on the borders of Rwanda, Uganda, and the Democratic Republic of the Congo.

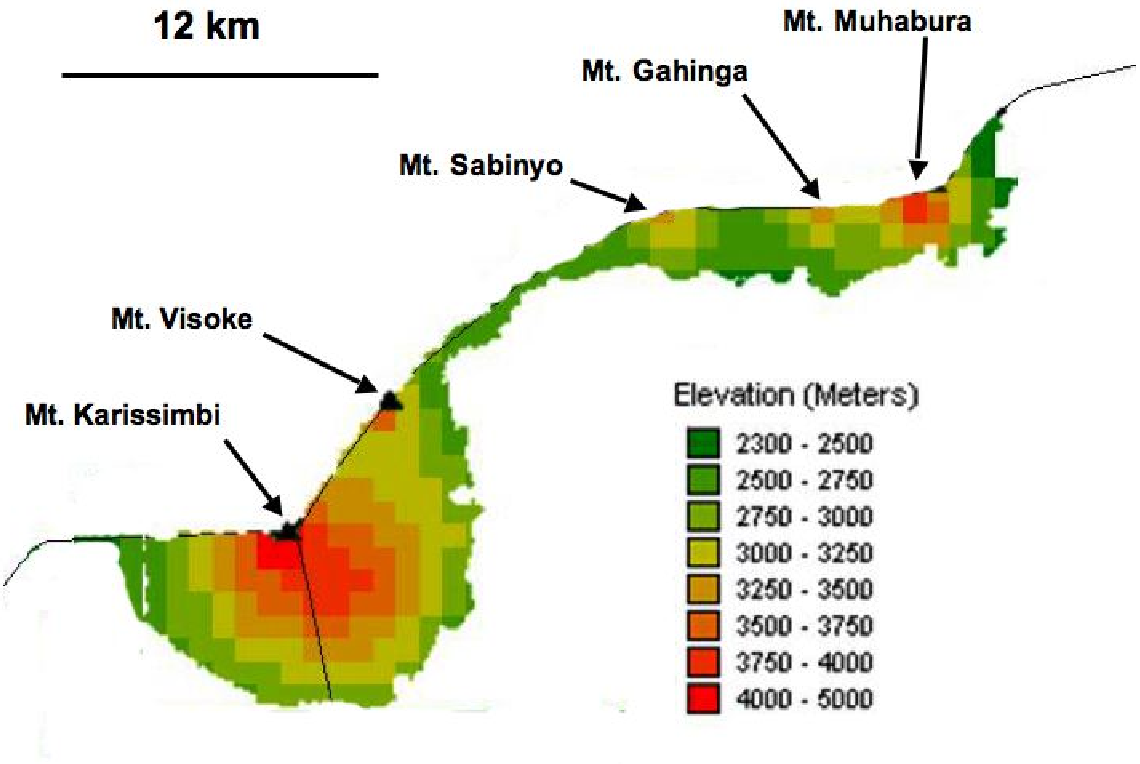

Figure 2 Map of the Parc National des Volcans, showing approximate elevation.

Data Collection

Fieldwork was conducted between two and six days per week depending on weather and logistical concerns from June through August 2007 and 2008. All searches were conducted during daylight hours in the PNV. Fieldwork at night was not possible due to security and wildlife concerns. Amphibians and reptiles were collected using the active search method (Franco et al., 2002). Some animals were captured with the aid of nets, snake hooks or snake tongs. Tadpoles were collected with nets. All voucher specimens were preserved according to techniques outlined by McDiarmid (1994) and deposited in the University of Texas at Arlington’s Amphibian and Reptile Diversity

Research Center collection (UTA A or UTA R series) or at the Karisoke Research Center in Ruhengeri, Rwanda. Collecting permits were provided by the Rwandan National Office of Tourism, Parks, and Recreation (ORTPN permit number: 62-06-1007). All research was conducted under the supervision of the University of Texas at Arlington's Institutional Animal Care and Use Committee (IACUC protocol number: A07.021). In addition to the fieldwork mentioned above, we have made an effort to canvass existing literature to find records of reptiles and amphibians recorded from within the current boundaries of the park. In the case of species that we did not personally observe, but are mentioned credibly in the literature, I have noted this disparity in the individual species accounts and Table 1.

Results

Table 1. Anuran and reptile species found in the Parc National des Volcans, Republic of Rwanda based on published literature records and collecting trips from June – August 2007 and 2008. 1 = Observation of adult specimen(s); 2 = observation of juvenile specimen(s); 3 = observation of tadpoles; 4 = recorded from published literature; 5 = Albertine Rift endemic.

FAMILY / SPECIES	1	2	3	4	5
Arthroleptidae Mivart, 1869					
Arthroleptis adolfifriederici Nieden, 1911	-	-	-	X	X
Leptopelis karissimbensis Ahl, 1929	X	X	X	X	X
Leptopelis kivuensis Ahl, 1929	X	X	X	X	X
Bufonidae Gray, 1825					
Amietophrynus kisoloensis Loveridge, 1932	-	-	-	X	-
Hyperoliidae Laurent, 1943					
Hyperolius castaneus Ahl, 1931	X	X	X	X	X
Hyperolius cinnamomeoventris Bocage, 1866	X	X	X	X	-
Hyperolius viridiflavus Duméril and Bibron, 1841	X	X	X	X	-
Phrynobatrachidae Laurent, 1941					
Phrynobatrachus graueri Nieden, 1911	-	-	-	X	-
Pipidae Gray, 1825					
Xenopus wittei Tinsley, Kobel, and Fischburg, 1979	-	-	-	X	X
Chamaeleonidae Rafinesque, 1815					
Chamaeleo rudis Boulenger, 1906	X	X	NA	X	-
Lacertidae Gray, 1825					
Adolfus jacksoni Boulenger, 1899	X	X	NA	X	-
Adolfus vauereselli Tornier, 1902	X	-	NA	X	-
Scincidae Gray, 1825					
Leptosiaphos graueri Sternfeld, 1912	X	-	NA	X	X
Mabuya striata Peters, 1844	X	X	NA	X	-
Colubridae Oppel, 1811					
Lamprophis olivaceus Duméril, 1856	-	-	NA	X	-
Philothamnus ruandae Broadley, 1998	X	-	NA	X	X
Viperidae Oppel, 1811					
Atheris nitschei Tornier, 1902	X	X	NA	X	X

Frogs and Toads: Order Anura

For all species accounts of anuran, taxonomy follows that of Frost (2009).

Arthroleptis adolfifriederici (Arthroleptidae) (Montane Squeaker)

De Witte (1941) recorded this species from the South versant of Mt. Visoke. This locality probably occurs within the current boundaries of the PNV in Rwanda. We did not encounter this species, but based on its montane distribution in other high elevation areas such as the Bwindi Impenetrable Forest (Drewes and Vindum, 1994), we feel that the species is likely to occur within the PNV.

Leptopelis karissimbensis (Arthroleptidae) (Karissimbi Treefrog)

Leptopelis karissimbensis was originally described by Ahl (1929) from the Virunga Mountains. The type locality is the slope of Mt. Karissimbi, likely within the borders of the PNV from Rwanda. This species is extremely morphologically similar to *L. kivuensis*, although diagnostic morphological features exist to diagnose the two species (Laurent, 1973; Schiøtz, 1999), which include a blue vocal sac in *L. karissimbensis* and a white vocal sac in *L. kivuensis*. Within the PNV, *L. karissimbensis* is common throughout the entire park near aquatic habitats in the bamboo zone, up to about 3,200 m in elevation. We observed that this species was particularly abundant in seasonally flooded depression marshes (Figure 3). In these habitats, adults (Figure 4), metamorphs, and putatively identified larvae were common. Without the use of molecular techniques (DNA sequence analysis), the tadpoles of this species and those of *L. kivuensis* are indistinguishable.

Figure 3 Flooded depression marsh at approximately 2800 m in elevation. *Hyperolius castaneus, H. cinnamomeoventris,* and *Leptopelis karissimbensis* were common at this site. *Atheris nitschei, Philothamnus ruandae,* and *Leptosiaphos graueri* were also found at this site.

Figure 4 Adult *Leptopelis karissimbensis*: green phase.

Leptopelis kivuensis (Arthroleptidae) (Kivu Treefrog)

Leptopelis kivuensis was described by Ahl (1929) from the immediate highlands surrounding the Virunga Mountains. Though the exact type locality is not known, the most accurate description by Ahl puts it near the town of Gisenyi on the Rwanda-DRC border, within 20 km of the nearest border of the PNV. Within the PNV, we collected this species in sympatry with *L. karissimbensis* at several localities, although *L. kivuensis* shows a distinct habitat preference for more wooded forest pools and may be more of a forest, rather than a meadow species as *L. karissimbensis* is thought to be (Schiøtz, 1975; Schiøtz, 1999). We found this species at slightly higher elevations than *L.*

karissimbensis, all the way into the *Hypericum-Hagenia* vegetation zone up to about 3,400 m. Adults, metamorphs, and putatively identified larvae were most common in and around shallow, water filled depressions in the forest.

Amietophrynus kisoloensis (Bufonidae) (Kisolo Toad)

We did not encounter any individuals of this species in the PNV. De Witte (1941) encountered this species in Uganda on the slopes of Mt. Sabinyo, very close to the border with Rwanda. Laurent (1972) lists one male specimen that was collected on the south slope of Mt. Karissimbi in Rwanda. This species is extremely common in disturbed habitats near the park boundary and probably would be easily located during breeding aggregations, but our inability to work at night at the beginning of the rainy season probably influenced our ability to locate any individuals of this species within the boundaries of the PNV.

Hyperolius castaneus (Hyperoliidae) (Ahl's Reed Frog)

This species is an Albertine Rift endemic and is found only at high elevations. It was described by Ahl (1931) from the volcanic area northeast of Lake Kivu. This can only refer to the Virungas, although the exact type locality is not known. Adults of this species were uncommonly collected, but newly metamorphosed and juvenile frogs were among the most commonly encountered anurans in the PNV (Figure 5). This species was found in all lentic habitats and tadpoles were extremely common and easily identifiable because of their brown coloration and dark lateral stripes. We encountered this species in the PNV from the lower park borders up to approximately 3,400 m.

Figure 5 Newly metamorphosed *Hyperolius castaneus.*

Hyperolius cinnamomeoventris (Hyperoliidae) (Cinnamon-Bellied Reed Frog)

This widely distributed species was another species of which we collected numerous individuals within the PNV. Adults were commonly collected in depression marshes and swamps. Juveniles and metamorphs were most common on sedge hummocks a few meters or more from standing water. This species is dimorphic as adults with the males being brown or green above with a light dorsolateral line and the females being green above and yellow on the venter with a dark line separating the two colors (Channing and Howell, 2006) (Figure 6). Males can easily be confused in the PNV with adults of *Hyperolius castaneus*, but the dorsolateral line on a male *H.*

castaneus is dark, while the line on a male *H. cinnamomeoventris* is pale. We collected this species at localities up to 3,200 m in the PNV.

Figure 6 Adult male (left) and female (right) *Hyperolius cinnamomeoventris.*

Hyperolius viridiflavus (Hyperoliidae) (Variable Reed Frog)

This extremely variable species is wide ranging across East and parts of Central Africa and is found within the borders of the PNV in wet meadows and swamps from the lower park boundaries up to 3,000 m. Only adults were located, sometimes by the calling of males during the day. This is a forest species (Drewes and Vindum, 1994) and it is likely that we could not locate individuals in thick forest because of their cryptic behavior and color pattern. While this species exhibits an amazing array of brilliant color patterns in other areas, individuals from the Virungas are dull grayish brown with yellow flecks on the dorsum and dark eyes (Figure 7).

Figure 7 Adult *Hyperolius viridiflavus*, photographed in situ.

Phrynobatrachus graueri (Phrynobatrachidae) (Grauer's Puddle Frog)

De Witte (1941) recorded this species from the slopes of Mt. Sabinyo in Uganda. We found this species to be common in leaf litter habitats below the boundaries of the PNV. We did not encounter it within the park borders. It is possible that this species occurs at low elevations within the PNV.

Xenopus wittei (Pipidae) (de Witte's Clawed Frog)

We did not encounter this species in the PNV, but this was likely because of limited collecting effort directed towards this species. While we used small nets in many permanent bodies of water within the PNV (the preferred habitat of this frog), *X. wittei* are fast and agile underwater and may have evaded capture. It is also possible we never searched water deep enough to find either tadpoles or adults of *X. wittei*. This species is not mentioned as being found in the PNV by any previous publication (e.g. de Witte, 1941; Laurent, 1972), but *X. wittei* is common in many permanent bodies of water within 10 km of the park boundary and may exist in some lakes within the PNV.

Snakes and Lizards: Class Reptilia, Order Squamata

For all species accounts of reptiles, taxonomy follows that of Spawls et al. (2002).

Chamaeleo rudis (Chamaeleonidae) (Ruwenzori Side-striped Chameleon)

This species has two forms not currently recognized as separate species: the eastern form is found in northeastern Tanzania, while the western form is found along the Albertine Rift. This was the most commonly encountered reptile in the PNV and occurs all the way from the park boundary up to at least 4,000 m in elevation. It is the reptile species that occurs at the highest elevation within the park. At the upper limits of its occurrence, temperatures are extremely cold. We found this species active at air temperatures as low as 4-5 C. Mt. Karissimbi experiences what could be classified as “winter events” marked by snowfall and ice storms (Figure 8). Occasionally subfreezing temperatures extend far down the mountain’s slopes at night. Since most species of chameleons sleep exposed on vegetation and this species exhibits this behavior, it is likely that *C. rudis* in the Virunga Mountains has the ability to supercool to avoid freezing to death. We found these chameleons in every vegetation zone and habitat within the park, although they exhibit a preference for sunlight openings and clearings in the forest, where they can bask cryptically in arboreal habitats to raise their body temperature (Figure 9). We encountered over 100 specimens in our surveys of the PNV.

Figure 8 Mount Karissimbi after a "winter event."

Figure 9 Adult *Chamaeleo rudis*.

Adolfus jacksoni (Lacertidae) (Jackson's Forest Lizard)

The PNV is surrounded by an artificial volcanic rock wall intended to keep buffalo (*Syncerus caffer*) inside the park boundary. This wall is 1-2 m high across the entire boundary of the park within Rwanda. The habitat surrounding the park has been deforested and is almost exclusively agricultural fields. *Adolfus jacksoni* was extremely commonly sighted and captured along this wall and occurs within the PNV, but was never sighted anywhere past the wall in the park. This lizard is an animal of open canopy habitats that prefers to perch on elevated surfaces (Spawls et al., 2002). With the exception of the edges of forest clearings, few habitats of this nature occur within the PNV, but the buffalo wall provides ample habitat for *A. jacksoni.*

Adolfus vauereselli (Lacertidae) (Sparse-scaled Forest Lizard)

We encountered this species only on the buffalo wall mentioned in the account for *A. jacksoni*. These two species are sympatric in this region and were found basking within 3 m of each other on the wall. *Adolfus jacksoni* appeared to be more common than *A. vauereselli.* Spawls et al. (2002) mentions that this species is a true forest lizard, but we never encountered it in true forest or any other closed canopy habitat.

Leptosiaphos graueri (Scincidae) (Rwanda Five-toed Skink)

This species is an Albertine Rift endemic. Spawls et al. (2002) states that it a fossorial animal that often lives among the buttresses of trees in leaf litter. We found several specimens along rocky ledges covered in moss including one aggregation of three individuals in the open in some kind of social interaction. Other specimens were located while crossing trails. One specimen was found in a sedge swamp exposed on a hummock. Upon being sighted, the animal quickly dove into the sedge hummock and was located after considerable search within the center of the plant. Another specimen was found crossing a trail in the bamboo zone. We found this species up to 3,100 m in elevation.

Mabuya striata (Scincidae) (Striped Skink)

Within the PNV, we only encountered this species on the margin of the park on the buffalo wall, along with *Adolfus jacksoni* and *A. vauereselli.* Of the three lizard species mentioned that we found basking on the buffalo wall, *M. striata* was the most common. This is not surprising, as this is the most commonly seen reptile in disturbed and urban habitats in Rwanda. We feel that the buffalo wall represents excellent habitat for the three species of lizards mentioned because of its ecological resemblance to a forest clearing with the added benefit of escape cover in the form of volcanic rock crevices, which make predation on any of the species by birds difficult.

Lamprophis olivaceus (Colubridae) (Olive House Snake)

We found no individuals of this species in the PNV, but Spawls et al. (2002) mentions that several individuals were collected from “mid-altitude” forest on Mt. Karissimbi. Since this book does not include records from the DRC, we assume these animals were collected in Rwanda. It is likely that the forest mentioned is either remnant montane forest or *Hypericum-Hagenia* forest, which could also be classified as high elevation cloud forest. It is likely that this species occurs within the PNV.

Philothamnus ruandae (Colubridae) (Rwanda Forest Green Snake)

We encountered two individuals of this species during our surveys in the PNV and a primatologist observing Mountain Gorillas likely observed another specimen based on a credible description. Of the two specimens we observed, one specimen was an adult sitting on a horizontal bamboo stalk about 2 m above ground at the edge of a swamp. Another was found on the ground at the edge of the same swamp. This swamp is at approximately 2,900 m in elevation. This represents the highest elevation that this species has been found. Like many species of herpetofauna found in the PNV, this species is an Albertine Rift endemic (Figure 10).

Figure 10 Adult *Philothamnus ruandae.*

Atheris nitschei (Viperidae) (Great Lakes Bush Viper)

This species is another Albertine Rift endemic and is named for the large lakes along the length of the rift. We encountered two individuals of this species in the PNV and a primatologist encountered another, which could be easily identified from a photograph. All specimens were encountered in the bamboo zone at elevations between 2,600-2,800 m. One adult individual was encountered in thick, closed canopy bamboo forest draped across a horizontal stalk of bamboo about 2 m above the ground. Another adult was encountered along the margin of a swamp in the multiple small branches of new growth at the end of a horizontal bamboo stalk, about 1 m above the ground. The final specimen was a juvenile (Figure 11) that was encountered at the edge of a forest clearing on horizontal bamboo, about 1 m above the ground (Figure 12). The first individual, encountered by the primatologist, was not disturbed. The second two individuals, upon being disturbed, dropped backwards off their perches and attempted to burrow into vegetative cover on the ground in escape attempts. Before being preserved, the juvenile specimen was maintained for four days in a plastic shoebox and during this time, consumed an adult *Adolfus jacksoni* (Jackson's Forest Lizard) that was offered to it.

Figure 11 Juvenile *Atheris nitschei.*

Figure 12 Site where juvenile *Atheris nitschei* illustrated in Figure 11 was collected. *Chamaeleo rudis* was also common at this site and in these habitats.

During our surveys of the PNV, we collected twelve out of seventeen species of reptiles and amphibians historically recorded from the park. Of these seventeen species, eight are considered to be endemic to the Albertine Rift (Table 1). Nine of the species recorded from the PNV were anurans, distributed in five families (the number of species in each family is in parenthesis): Arthroleptidae (3), Bufonidae (1), Hyperoliidae (3), Phrynobatrachidae (1), and Pipidae (1). Eight species of reptiles were recorded from five families: Chamaeleonidae (1), Lacertidae (2), Scincidae (2), Colubridae (2), and Viperidae (1).

Discussion

According to the IUCN (2009), the eight reptile species that occur in the PNV are not listed for conservation status. Of the nine amphibian species that occur or likely occur within the boundaries of the PNV, six are listed in the “Least Concern” (LC) category because they have relatively large distributional ranges and do not appear to be in any danger of widespread population decline. *Leptopelis kivuensis* is listed “Near Threatened” (NT) because it occupies an area of occurrence not much greater than 20,000 km^2 and its montane habitat is declining. They cite this species as being close to qualifying for the more imperiled “Vulnerable” category. *Hyperolius castaneus* is listed as “Vulnerable” (V) because its area of occurrence is less than 20,000 km^2, and its habitat has become very fragmented. The IUCN states that the montane swamp habitat of *H. castaneus* is declining in both quality and extent. *Leptopelis karissimbensis* is listed as “Endangered” (EN), because its known range is less than 5,000 km^2, all known individuals are known from fewer than five locations, and its montane habitat is declining in both quality and extent.

The Virungas are among the highest mountains in Africa. The protected area that lies within the country of Rwanda is small in comparison with neighboring DRC and the lower park boundary is much higher. The lowest park boundary in Rwanda is approximately 2,600 m in elevation, while the lowest park boundary in DRC is approximately 1,900 m in elevation. This is important, because true montane forest communities do not usually exist in this region above about 2,500 m. There is virtually no true montane forest left within the park boundaries of the PNV, and consequently, reptile and amphibian species richness is much lower in the PNV than other forested Albertine Rift areas in close proximity. Two of these areas in Uganda, Bwindi Impenetrable National Park and Kibale National Park in Uganda, have had excellent surveys conducted for the presence of herpetofaunal diversity (Drewes and Vindum, 1994; Vonesh, 2001). Drewes and Vindum (1994) reported thirty-six reptile and twenty-nine amphibian species from Bwindi Impenetrable National Park (BINP), whose closest border to the PNV in Rwanda is only about 35 kilometers straight line distance to the North. Approximately 140 km North of the PNV, Vonesh (2001) reported fifty-three species of reptiles and thirty species of amphibians from Kibale Forest in Kibale National Park. Both of these parks are substantially lower in elevation than the PNV. The highest elevations in BINP are approximately 2,600 m in elevation and are approximately 1,600 m in elevation in Kibale National Park, but most of the area of both parks is considerably lower and consequently warmer. As mentioned earlier, the lowest elevations in the PNV are approximately 2,600 m in elevation.

The high elevations found in the PNV have a profound affect on herpetofaunal species richness. Ectotherm diversity at an ecosystem level is affected greatly by temperature (Vitt and Caldwell, 2009) and the Virunga Mountains are no exception. With only seventeen species of reptiles and amphibians reported from the park, herpetofaunal diversity at this site is considerably lower than at geographically proximate surveyed sites in the same region. Eight of the seventeen species mentioned are Albertine Rift endemics. While this appears to be a high proportion of endemic species for a taxonomic group at a specific site, it must be remembered that the Albertine Rift has large areas of protected habitat when compared with others areas of Africa (Plumptre et al., 2007) and that all the species found in the Virungas have been reported or are likely to occur in other protected sites in the Albertine Rift such as Bwindi Impenetrable National Park in southern Uganda and Nyungwe National Park in southern Rwanda. *Leptopelis karissimbensis* is the only amphibian or reptile species thought to be endemic to the Virungas (IUCN, 2009), although historical and recent research suggests that this species is more widely distributed throughout the Albertine Rift (de Witte, 1941; Hölting et al., 2009) than some literature (Schiøtz, 1975, 1999) and the IUCN report on the species would indicate. Based on these considerations, we feel that the herpetofauna of the PNV is adequately protected as it is likely that no reptile or amphibian species are endemic to the park itself and the PNV is one of the most highly controlled and protected areas in Africa, primarily because of the presence of mountain gorillas. The park exists largely for the protection of these mammals and their presence acts as a "conservation umbrella" for all other species found in the PNV, because as gorilla habitat remains protected, that habitat is thus protected for other species. Despite low herpetofaunal richness for an equatorial area, the Virunga Mountains ecosystem in Rwanda is amazing for its unique combination of geologic, historical, and biological wealth and stands as a model for conservation biology.

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CHAPTER 3

DESCRITPION OF THE LARVAL STAGE OF THE KARISSIMBI TREEFROG: *LEPTOPELIS KARISSIMBENSIS*

Introduction

Leptopelis Günther, 1859, contains 51 currently recognized species distributed across Sub-Saharan Africa (Frost, 2008). Most species of *Leptopelis* lay their eggs in a depression in moist soil. In some species, the eggs hatch during the beginning of the rainy season when the nest is inundated, while in other forms, the eggs hatch and the larvae wriggle to the water, where the tadpoles develop over several months (Schiøtz, 1975). One species, *Leptopelis karissimbensis* Ahl, 1929, is endemic to the Virunga region of the Albertine Rift in Rwanda, Uganda, and the Democratic Republic of the Congo (Laurent, 1972). It is considered "Endangered" by the IUCN because its total known range occupies an area less than 5000 km^2, all individuals are reported from less than five locations, and there is evidence that the natural habitat of the species is being degraded (IUCN, 2008). Once dead and in formalin and/or alcohol preservative, this species is considered to be indistinguishable from the closely related species, *L. kivuensis* Ahl, 1929, (Schiøtz, 1975, 1999). In life, adult males of *L. karissimbensis* can be distinguished from adult males of *L. kivuensis* by the presence of a prominent white spot below the eye and a blue vocal sac, which is white in the latter species (Laurent, 1973; Channing and Howell, 2006). These two species show some variation in advertisement call and appear to occupy different habitats. *Leptopelis karissimbensis* is thought to inhabit montane grassland, while *L. kivuensis* is reported to be a forest species (Schiøtz, 1975; Laurent, 1973). Some authors have considered the two species to be conspecific (Idris, 2004). The larval stage has not been adequately described for either species. The purpose of this paper is to describe the tadpole of *L. karissimbensis.*

Materials and Methods

Tadpole vouchers were collected during the long rainy season between 5 June and 11 July 2007. Specimens were collected in or at the border of the Parc National des Volcans (Volcanoes National Park) in the Republic of Rwanda. While several dozen *Leptopelis* tadpoles were collected, thirty tadpoles in Gosner developmental stages 29–42 (sensu Gosner, 1960) were fixed and stored in 10% formalin and examined for this paper. None could be reared until metamorphosis, although several individuals collected in advanced developmental stages (> stage 40) were compared with newly metamorphosed *L. karissimbensis*. Live, metamorphosed adult *L. karissimbensis* can be distinguished from *L. kivuensis* based on the presence of a large, prominent white spot below the eye, so we examined tadpoles in late developmental stages for this character. Several tadpoles and froglets exhibited the diagnostic white spot. All of the specimens have been deposited at the University of Texas at Arlington Amphibian and Reptile Diversity Research Center under the numbers UTA A 58616–58646. Adult *L. karissimbensis* were collected at several localities where larvae were present. Several specimens of adult *L. karissimbensis* were deposited under the numbers UTA A 58625–58630.

Measurements and stage of development follow Grosjean (2001) and are abbreviated as follows: GS, Gosner Stage; BH, maximum body height; BW, maximum body width; ED, maximum eye diameter; HT, maximum tail height; LF, maximum height of lower tail fin; NN, internarial distance; OWD, oral disc width; PP, distance between pupils; SS, distance from tip of snout to opening of spiracle; SU, distance from tip of snout to insertion of upper tail fin; SV, distance from tip of snout to opening of vent; TL, total length, UF, maximum height of upper tail fin; VMTH, distance vent-maximum height of tail; VT, distance vent-tip of tail. For exact definitions, follow measurements by Grosjean (2001). Labial tooth row formulae follows Dubois (1995). Photographs of preserved specimens were taken with a Nikon 8700 camera and drawings were done with the aid of a camera lucida by Roshanak Mehdibiegi.

Results

Description of Tadpole. – The following description is based on five larvae in stages 29–35 (UTA A–58618, 58619, 58630, 58633, 58687). Morphometric data of 30 tadpoles at stages 29–42 are provided in Table 2. In dorsal view, body elliptical, widest at spiracle, snout moderately truncate; eyes moderate in size (~0.90 times SV), slightly bulging, separated by distance equal to about 1.9 times internarial distance, directed more laterally than dorsolaterally, not visible in ventral view; nares circular, very small, not rimmed, directed and positioned dorsolaterally, visible in lateral view, closer to anterior margin of snout than to eye.

Table 2 Morphometric data (in millimeters) of tadpoles of *Leptopelis karissimbensis*. For abbreviations, see text and Grosjean (2001). The mean is followed by one SD, range, and N.

Stage	SS	SU	SV	VMHT	VT	TL	UF	LF	HT	BH	BW	PP	NN	ED	ODW
29	7.90	11.05	11.91	7.27	36.08	36.08	1.30	1.10	5.00	3.44	4.59	3.80	2.10	1.00	2.80
30	7.70	11.72	12.84	10.09	24.54	36.97	1.40	1.20	5.10	4.20	6.08	4.10	2.20	0.90	3.10
32	9.00	12.81	14.27	13.00	27.36	46.37	1.70	1.25	6.25	5.45	6.68	4.55	2.50	1.30	3.55
	+/− 1.70	+/− 1.97	+/− 4.17	+/− 5.06	+/− 8.05	+/− 1.93	+/− 0.57	+/− 0.35	+/− 1.48	+/− 1.07	+/− 1.10	+/− 1.20	+/− 0.28	+/− 0.42	+/− 0.78
	7.80−10.20	11.42−14.21	11.32−17.22	9.42−16.58	21.66−33.05	45.00−47.73	1.30−2.10	1.00−1.50	5.20−7.30	4.69−6.21	5.90−7.45	3.70−5.40	2.30−2.70	1.00−1.60	3.00−4.10
	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
34	8.40	12.15	13.53	15.23	28.76	42.57	1.40	1.10	5.80	4.35	5.92	4.60	2.50	2.00	2.90
36	9.10	13.17	15.20	14.34	31.79	46.77	1.70	1.50	6.30	5.04	7.16	5.00	2.50	1.40	3.60
37	9.78	13.95	15.87	13.99	32.50	48.26	1.78	1.42	5.78	5.51	7.17	5.11	2.56	1.43	3.44
	+/− 0.44	+/− 0.71	+/− 1.02	+/− 1.88	+/− 2.52	+/−2.45	+/− 0.19	+/− 0.19	+/− 0.52	+/− 0.29	+/− 0.53	+/− 0.17	+/− 0.37	+/− 0.13	+/− 0.26
	9.10−10.40	12.87−14.99	14.25−17.19	11.98−16.56	28.19−36.02	44.11−50.73	1.50−2.00	1.30−1.70	6.20−7.70	5.02−5.88	5.55−7.90	4.90−5.40	1.70−2.90	1.3−1.6	3.1−3.9
	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
38	9.77	13.47	16.55	13.30	32.23	48.00	1.97	1.53	7.37	3.53	7.13	4.93	2.60	1.47	3.50
	+/− 0.47	+/− 0.66	+/− 0.62	+/− 2.62	+/− 3.11	+/− 2.56	+/− 0.06	+/− 0.15	+/− 0.65	+/− 0.35	+/− 0.64	+/− 0.25	+/− 0.2	+/− 0.06	+/− 0.26
	9.40−10.30	12.78−14.09	15.87−17.09	10.63−15.86	29.29−35.48	46.26−50.94	1.90−2.00	1.40−1.70	6.70−8.00	5.16−5.85	6.67−7.86	4.70−5.20	2.40−2.80	1.4−1.5	3.3−3.8
	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
40	10.60	15.65	18.88	15.19	35.56	52.65	2.10	1.60	7.50	5.58	7.11	5.60	2.60	1.50	3.50
41	10.26	14.68	17.74	15.30	33.41	50.88	1.90	1.53	7.39	5.36	8.07	5.42	2.31	1.58	3.41
	+/− 0.66	+/− 0.65	+/− 1.11	+/−1.40	+/− 2.50	+/− 2.97	+/− 0.21	+/− 0.21	+/− 0.45	+/− 0.68	+/− 0.69	+/− 0.28	+/− 0.27	+/− 0.13	+/− 0.23
	9.40−11.50	13.73−15.07	15.47−18.82	13.86−17.46	29.61−37.02	46.29−54.39	1.50−2.20	1.40−2.00	6.70−7.90	4.59−6.47	6.89−8.83	5.00−5.90	2.20−2.70	1.40−1.70	3.10−.37
	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
42	9.70	14.62	17.42	12.24	32.62	51.40	1.40	1.00	6.70	5.10	7.32	5.60	2.10	1.50	3.00

In profile (Fig. 13), body depressed (BW/BH ~1.25), flattened dorsally and for posterior 2/3 of the venter; snout rounded but slightly truncate; spiracle sinistral, triangular, short tube attached to body wall, slightly closer to eye than to vent, positioned ventrolaterally and oriented posteriorly and slightly dorsally. Spiracular opening rounded, set approximately at horizontal plane of the hindlimbs and horizontal plane of ventral edge of caudal myotomes.

Tail musculature large and robust; about equal in size for the anterior 2/3 of tail, decreasing quickly thereafter, not reaching tip of tail; tail fins small in vertical dimension; dorsal tail fin not extending onto body, beginning on caudal musculature, reaching maximum height at length slightly past midpoint of tail, slightly convex, tail tip rounded; margin of tail fin convex, beginning distal to vent; vent tube medial, short, wide, linked to body, opening between hindlimbs; no apparent lateral line or glands.

Oral disc (Fig. 13) oriented anteriorly to slightly ventrally, nearly as wide as proximal end of body, bordered on lower edge by double row of short, rounded papillae and on lateral edges by single row of longer, rounded papillae; upper edge of the oral disc lacks papillae and consists of a slightly protuberant ridge. LTRF 4/3; all rows nearly equal in length and thin, innermost upper 2–3 rows incomplete; jaw sheaths strong, upper beak curved and much longer than lower beak, which is also curved.

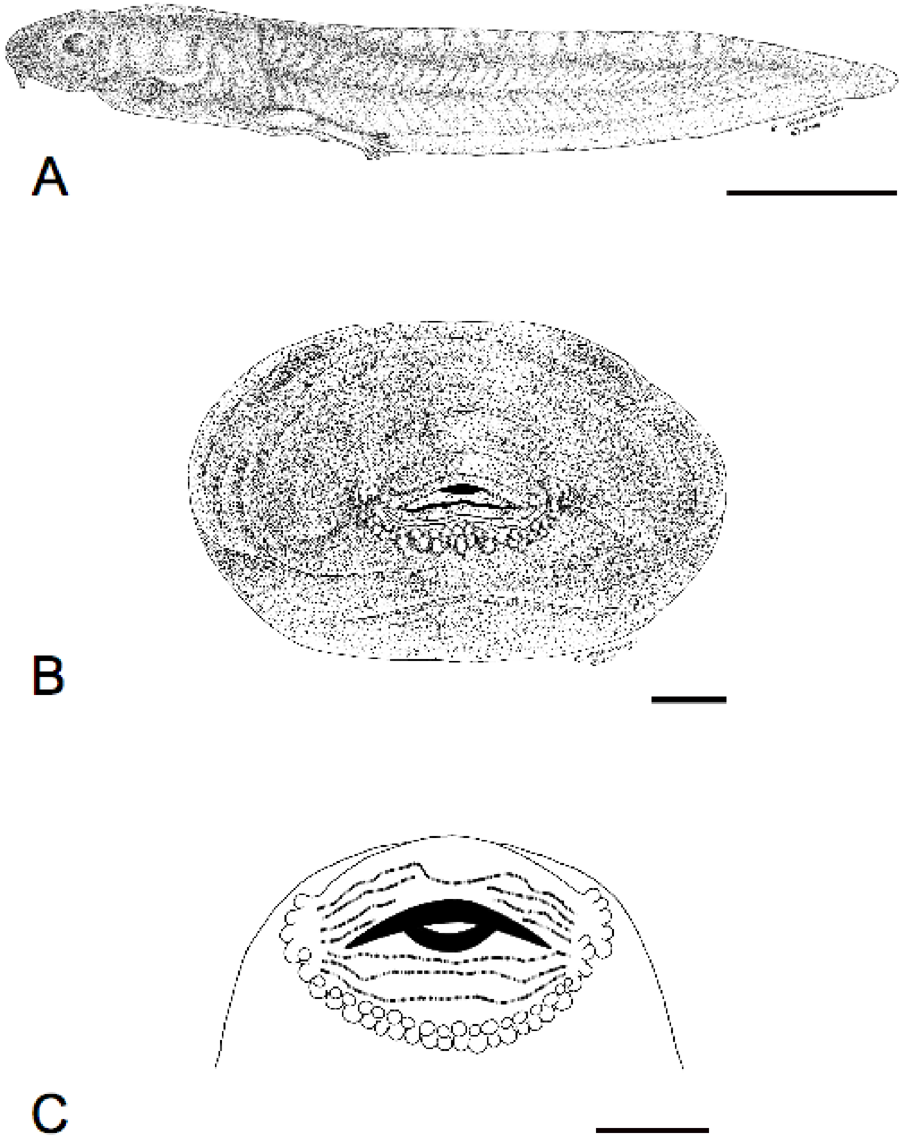

Figure 13 Illustrations of A) lateral aspect (scale bar represents 10 mm), B) frontal aspect (scale bar represents 1 mm), and C) larval mouth of *Leptopelis karissimbensis* (scale bar represents 1 mm)

In preservative, the back and flanks are dark green, almost black on the dorsum but lightening towards the venter, sometimes speckled evenly with tiny melanophores. A dark ring encircles the opening of the spiracle. The caudal musculature is dark green to brown and slightly mottled. The upper tail fin is lighter green than caudal musculature and moderately mottled. The lower tail fin is dark green near the border with caudal musculature, nearly transparent towards the distal edges and moderately mottled near the tail. The venter is dark green at the proximal end, lightening to nearly white or translucent at the vent and near the insertion of hind limbs. The coiled intestines are visible. The live coloration is very similar to color in preservative. Overall, the tadpole is dark dorsally, but countershaded.

Ecology. – Tadpoles were collected from cloud forest and upland meadows in bamboo (~ 2500 m) and *Hypericum-Hagenia* (~ 2900 m) zones. The habitat of *L. karissimbensis* is shown in Figure 14. Breeding occurred during the long rainy season, with tadpoles approaching metamorphosis soon after the end of the rains. Tadpoles occurred in temporary pools, semi-permanent ponds, and swamps at depths of 10--75 cm. Tadpoles often occupied heavily vegetated portions of the water column and during collection, emergent vegetation could be seen shaking from the escape activities of larval *Leptopelis* and *Hyperolius*. *Leptopelis* larvae were often outnumbered significantly in individual site collections by larvae of two species of *Hyperolius: H. castaneus* and *H. cinnamomeoventris*.

Figure 14 Flooded depression marsh. *Leptopelis karissimbensis, Hyperolius castaneus,* and *H. cinnamomeoventris* larvae were common at this site.

Discussion

Only about 4 of 51 currently recognized *Leptopelis* species have useful descriptions of larval forms (Drewes et. al., 1989; Channing and Howell, 2006). Larval descriptions are important to anuran biology and not having a description of one phase of a biphasic lifestyle leaves a significant gap in the life history of a species (Altig and Johnston, 1989). This becomes paramount when the species is considered endangered. Adult frogs are often active at night and require focused searches by researchers to find them. In contrast, larvae can be relatively easy to collect during the day and can be found at times when adults are inactive. Within the known range of *L. karissimbensis*, few researchers have been allowed to work at night because of security and wildlife concerns. One effective method of surveying for populations of this treefrog may be to search for tadpoles, as this will reflect current yearly reproduction in known breeding sites. Based on this description, it should be easier for future researchers to examine and identify *Leptopelis* tadpoles from the Virungas, which are likely to be *L. karissimbensis*.

Acknowledgments

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CHAPTER 4

TAXONOMY, NATURAL HISTORY, AND CONSERVATION STATUS OF TWO DISTINCT ALBERTINE RIFT TREEFROGS: *LEPTOPELIS KARISSIMBENSIS* AND *LEPTOPELIS KIVUENSIS* (ANURA: ARTHROLEPTIDAE)

Introduction

The Albertine Rift, another name for the western arm of the Great Rift Valley of Africa, is considered a biodiversity hotspot by both Conservation International (Myers et al., 2000; Mittermeier et al., 2004) and the Wildlife Conservation Society (Plumptre et al., 2003). The Rift has a multitude of habitats, ranging from lowland forest approximately 600 m to afromontane vegetation with glaciers at 5,100 m (Schutyser, 2007). Other habitats include cloud forest, both moist and dry savannah, swamps, and large lacustrine habitats (Plumptre et al., 2003). Biologically, the region is best known for the presence of many species of large mammals, including gorillas (*Gorilla beringei*; Eckhart and Lanjouw, 2008), okapis (*Okapia johnstoni*; Lindsey et al., 1999*)*, and forest elephants (*Loxodonta cyclotis*; Inogwabini et al., 2000), but the Rift also contains more species of vertebrates (1456 species) and more endemic species of vertebrates (152 species) than any other region in continental Africa (Plumptre et al., 2003; Burgess et al., 2004). Because the region is considered a biodiversity hotspot and has immense conservation challenges from anthropogenic causes (e.g., Hill et al., 2002; Mubalama and Bashige, 2006), increased attention is being focused on the region, including poorly known groups such as amphibians (e.g., Behangana et al., 2009).

Systematic studies of the amphibian genus *Leptopelis* have been hampered by the lack of recent collecting in Central Africa and the morphological similarity of several species in the genus (Laurent, 1973; Schiøtz, 1999; Köhler, 2009). Two putative species endemic to the Albertine Rift (*L. karissimbensis* and *L. kivuensis*) are especially problematic because both are of conservation concern (Stuart et al., 2008; IUCN, 2009), their natural history and geographic distribution are poorly known (Schiøtz, 1999), and both were described by Ernst Ahl, a notoriously careless herpetologist with approximately 84% of his taxonomic contributions relegated to synonymy (Adler, 2007).

This is underscored by the synonymy of *L. graueri* and *L. rugegensis* with either *L. karissimbensis* (Loveridge, 1936; de Witte, 1941) or *L. kivuensis* (Laurent, 1972a). *Leptopelis karissimbensis* is a small-sized (28–36 mm; Channing and Howell, 2006), endangered treefrog, and recent publications have noted the species from montane meadows in forest, savannahs and heathlands of extreme southwestern Uganda, western Rwanda, and adjacent eastern Democratic Republic of the Congo (DRC) between 2,000–2,800 m, and occasionally as low as 1,500 m (Schiøtz, 1999; Stuart et al., 2008; Hölting et al., 2009). The near-threatened species *L. kivuensis* is also a small-sized treefrog (26–36 mm; Channing and Howell, 2006), with recently published sources noting it from montane forests of southwestern Uganda, western Rwanda, and adjacent DRC above 1,500 m (Schiøtz, 1999; Stuart et al., 2008).

While most recent authors agree that the two forms are distinct species (e.g., Channing and Howell, 2006; Behangana et al., 2009; Hölting et al., 2009), Schiøtz (1975,

1999) stated that preserved specimens are impossible to separate, and at least two authors have considered the two putative species to be conspecific (de Witte, 1941; Idris, 2004). Several authors noted differences in color pattern, morphology, advertisement call and habitat of the two species. In the original descriptions, Ahl (1929) observed *Leptopelis karissimbensis* has a blue throat and tarsal spur, which contrasts with the white throat and lack of a tarsal spur in *L. kivuensis*. Schiøtz (1975) provided sonograms of both species from localities in Uganda, and stated that *L. karissimbensis* has "an atonal, rather uncharacteristic clack, sometimes a buzzing followed by a clack" and inhabits high-altitude savannah and heathland, whereas *L. kivuensis* inhabits high-altitude forest and has "a single or double clack... [one individual] was heard giving 3–4 very quiet clacks in rapid succession." Schiøtz (1975) added that he could not identify a difference between the calls of the two species, but based on disparate throat coloration and habitat preference, he continued to recognize both species as valid (Schiøtz, 1999). Genetic differences (16S rRNA) between the two species were discussed by Hölting et al. (2009), who also noted that juvenile *L. karissimbensis* have a combination of red ocular irises and white elbows and knees, whereas juvenile *L. kivuensis* have a different combination of green irises and yellow elbows and knees—juveniles of both species have a green dorsal coloration. In this paper, we evaluate the taxonomic status of these two species with morphological, acoustic and molecular data, and update their geographic distribution and conservation status in light of our new data and historical records.

Materials and Methods

Specimens and tissues were collected from northwestern Rwanda from May to August 2007 and June to July 2008, and eastern DRC from July to August 2007 and May to August 2008–09. Museum abbreviations are listed in Leviton et al. (1985). Specimens were preserved in 10% buffered formalin in the field, and transferred to 70% ethanol at the conclusion of each expedition. Tissues harvested from the liver or hind limb muscle of frogs were preserved in 95% ethanol. Color photographs of live frogs and associated whole-preserved specimens were examined to identify color patterns and external morphological characters that could be used to diagnose *L. karissimbensis* from *L. kivuensis* in life and once preserved. Thirty available adult specimens were dissected to check for the presence of testes or ovaries to confirm sex. Morphometric measurements were taken with a digital caliper to the nearest 0.1 mm. Abbreviations for morphological traits are: SVL (snout–vent length), HW (greatest head width at rear commisure of jaws), ED (horizontal eye diameter), END (eye-nostril distance), IOD (interorbital distance at midpoint of the orbits), TD (horizontal tympanic annulus diameter), TL (tibia length from the cloaca to the outer surface of the flexed knee), and FOTL (foot length including tarsus). To be consistent with previous studies of African anurans, we follow the webbing formula of Glaw and Vences (1994).

Breeding choruses of *L. karissimbensis* and *L. kivuensis* were discovered in a vegetated roadside verge in Kahuzi-Biega National Park near the ranger outpost of Mugaba, DRC during a light rain on 5 June 2008. Advertisement calls were recorded from seven male *L. karissimbensis* and five male *L. kivuensis* from this locality from 5–6

June 2008 with a Zoom H4 Handy Recorder (B&H Photo, New York, NY). One additional recording of a male *L. karissimbensis* was made in a flooded meadow adjacent to a stream near the village of Kizuka in the Itombwe Plateau, DRC during a light rain on 26 May 2009. Temperature was measured immediately after each recording to the nearest 1.0°C with a Sunto Core Multifunction Watch (REI, Sumner, WA) in 2008 and a Fisher Scientific Traceable Digital Hygrometer/Thermometer (Fisher Scientific, Houston, TX) in 2009; temperatures were recorded approximately 1 m above ground (the watch was suspended by a strap to eliminate bias from body heat), where the majority of frogs were perched and calling. Each recorded male was weighed within 8 h of recording to the nearest 0.1 g with an Avinet 10 g precision spring scale (Avient Inc., Dryden, NY). The most clear and complete single advertisement call from each recording was analyzed using Raven Lite© (Charif et al., 2006) and Canary© (Charif et al., 1995) software. We examined oscillograms (waveforms), audiospectrograms (sonograms), and results of the Fast Fourier Transformation (FFT; frequency spectrum) for spectral and temporal characters following methodology and terminology of Diesmos et al. (2002) and Brown et al. (2002). The number of components and pulses, call duration, and dominant frequency of each component was compared between all analyzed calls of both species.

A 575 base pair (bp) fragment of the 16S rRNA gene was amplified from 31 specimens of both focal species (*L. kivuensis* and *L. karissimbensis*) and two outgroups (*L. millsoni* and *L. palmatus*) that were identified from a preliminary phylogenetic analysis of the entire genus (Greenbaum, unpubl. data). As part of the latter analysis, a 716 bp fragment of the conservative BDNF (brain-derived neurotrophic factor) gene was amplified from six samples of each focal species with primers from van der Meijden et al. (2007). All sequences were deposited in GenBank. Genomic DNA was isolated from liver or muscle tissue samples with the Qiagen DNeasy tissue kit (Valencia, CA, USA). We used 25 µl PCR reactions with standard 16S primers (Palumbi et al., 1991) with an initial denaturation step of 95°C for 2 min, followed by denaturation at 95°C for 35s, annealing at 50°C for 35s, and extension at 72°C for 95s with 4s added to the extension per cycle for 32 cycles. Amplicons were visualized on a 1.5% agarose gel, and target products were purified with AMPure magnetic bead solution (Agencourt Bioscience, Beverly, MA, USA) and sequenced with BigDye® Terminator Cycle Sequencing Kits (Applied Biosystems, Foster City, CA, USA). Sequencing reactions were purified with CleanSeq magnetic bead solution (Agencourt Bioscience) and sequenced with an ABI 3130xl automated sequencer at the DNA Core Facility at the University of Texas at El Paso. Forward and reverse sequence contigs for each sample were assembled and edited using SeqMan (DNAStar, Maison, WI) to ensure accuracy.

Mitochondrial data generated for this study were combined with available 16S sequences from GenBank, but at the time of publication, *Leptopelis* sequences noted by Hölting et al. (2009) were not available on GenBank and could not be included for comparison. An initial alignment was produced in MEGALIGN (DNA Star) with the Clustal W algorithm, and manual adjustments were made in MacClade 4.08 (D. R. Maddison and W. P. Maddison, MacClade: Analysis of Phylogeny and Character Evolution, Sinauer, Sunderland, MA, 2005). No ambiguously aligned regions were observed, and as a result, no data were excluded from phylogenetic analyses.

Phylogenetic relationships among the samples were assessed with maximum parsimony (MP) and maximum likelihood (ML) optimality criteria in the programs PAUP* 4.0b10 (D. L. Swofford, PAUP*4.0b10. Phylogenetic Analysis Using Parsimony [*and Other Methods], Sinauer, Sunderland, MA, 2002) and GARLI version 0.96 (Zwickl, 2006), respectively. For MP analyses, the heuristic search algorithm was used with 25 random-addition replicates, accelerated character transformation and tree bisection-reconnection branch swapping, zero-length branches collapsed to polytomies, and gaps treated as missing data. We used non-parametric bootstrapping (1,000 pseudoreplicates) to assess node support in resulting topologies. For ML analyses, we used default settings in GARLI (including the general time reversible model of nucleotide substitution) with 1,000 bootstrap replicates to assess node support. Phylogenetic trees were visualized with FigTree (http://tree.bio.ed.ac.uk/software/figtree/). To be comparable with previous studies (Hölting et al., 2009), we calculated uncorrected pairwise (p) genetic distances for 16S data with PAUP*4.0b10. We also ran the data in a Bayesian phylogenetic analysis using MrBayes (Huelsenbeck and Ronquist, 2001). The analysis was run for 10,000,000 generations with a burn-in period of 1,000,000 trees. The model assumed for sequence evolution was GTR + I + Gamma.

Results

Morphology and Coloration.—Mensural data for adult specimens of *Leptopelis karissimbensis* and *L. kivuensis* are shown in Table 3. There is no significant difference in size among males of each species; sample sizes for females were too low for statistical comparison between species, but females are larger on average than males within each species. Webbing formulas for each species are virtually identical. The webbing formulas for *L. karissimbensis* are 1(1.75), 2i(1.75), 2e(1–1.5), 3i(2.5), 3e(2), 4(1.5) (manus) and 1(0.75–1), 2i(1), 2e(0.5), 3i(2), 3e(0.5), 4i(2–2.25), 4e(2), 5(0.5) (pes); relative lengths of digits are III > IV > II > I (manus), and IV > V > III > II > I (pes). The webbing formulas for *L. kivuensis* are 1(1.75), 2i(1.75), 2e(1–1.5), 3i(2.5), 3e(2), 4(1.5) (manus) and 1(0.75–1), 2i(1–1.25), 2e(0.5), 3i(2), 3e(0.5–0.75), 4i(2.25–2.5), 4e(2), 5(0.5–1) (pes); relative lengths of digits are III > IV > II > I (manus), and IV > V > III > II > I (pes). Our observations of webbing formulas are consistent with Ugandan specimens examined by Schiøtz (1975).

Field notes and examination of color photographs of live specimens from each species confirmed previously published observations of throat color in calling males. Every male specimen of *L. karissimbensis* possessed a blue vocal sac, and most individuals had extensive blue pigmentation on the ventral side of fore- and hind limbs (Figure 15). We did not note any female specimens of *L. karissimbensis* that exhibited extensive blue coloration or any specimens of *L. kivuensis* that exhibited any blue coloration. Juveniles and subadults of both species lacked blue pigment on the venter.

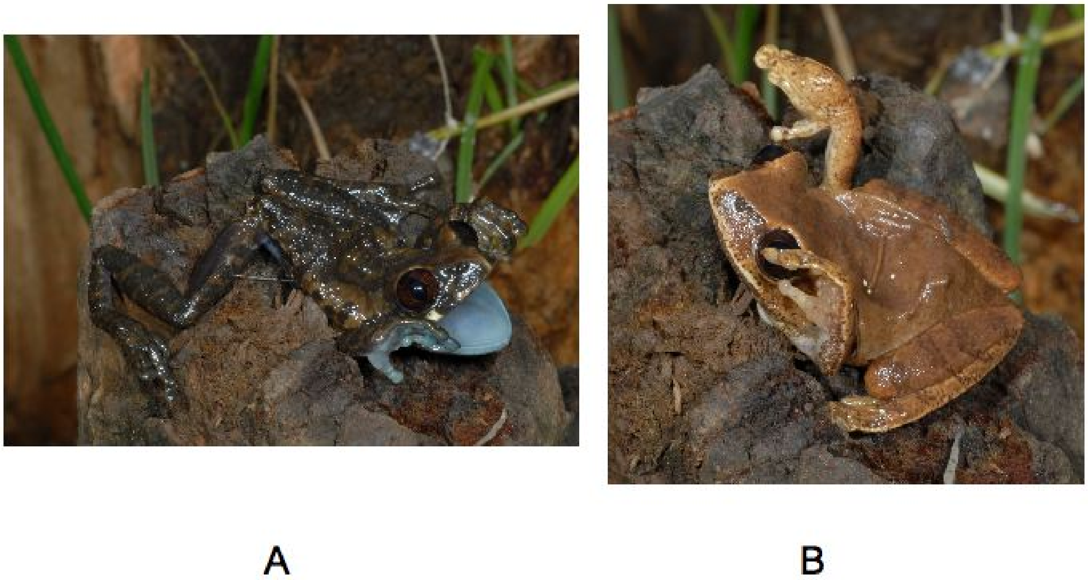

Figure 15 Male A) *Leptopelis karissimbensis* and B) *L. kivuensis* showing differences in coloration and defensive behavior.

Table 3 Means and standard deviations (in mm) for measured specimens of *Leptopelis kivuensis* and *Leptopelis karissimbensis* of both sexes. For abbreviations, see Materials and Methods.

Leptopelis kivuensis		SVL	HW	HL	ED	END	IOD	TD	TL	FOTL
Females	mean	33.42	14.07	10.42	3.87	3.12	8.08	2.12	14.58	25.65
n = 6	st. dev.	+/- 1.66	+/- 1.56	+/- 1.09	+/- 0.53	+/- 0.17	+/- 0.83	+/- 0.23	+/- 0.92	+/- 1.50
Males	mean	32.1	14.08	10.22	3.62	2.9	7.73	2.22	14.54	25.29
n = 11	st. dev.	+/- 2.63	+/- 0.78	+/- 0.99	+/- 0.42	+/- 0.38	+/- 0.49	+/- 0.26	+/- 1.05	+/- 1.63

Leptopelis karissimbensis		SVL	HW	HL	ED	END	IOD	TD	TL	FOTL
Females	mean	43.60	19.45	12.60	4.75	4.30	9.50	3.05	19.10	35.15
n = 2	st. dev.	+/- 4.95	+/- 4.03	+/- 1.98	+/- 0.49	+/- 0.99	+/- 0.99	+/- 0.21	+/- 6.22	+/- 5.73
Males	mean	33.26	14.05	10.70	3.55	2.95	7.59	2.16	15.96	28.05
n = 11	st. dev.	+/- 2.70	+/- 1.89	+/- 1.24	+/- 0.68	+/- 0.70	+/- 1.06	+/- 0.65	+/- 1.75	+/- 2.80

The results of our vocal analyses are shown in Table 4 and a representative sonogram and oscillogram for each species is shown in Figure 16. A complete advertisement call of *L. karissimbensis* consists of two components: a buzz and a clack. The call typically lasts almost a full second. More commonly, males of *L. karissimbensis* simply vocalize the clack portion of the call. Based on the analysis of calls from seven individual calling males, the mean call duration was 0.50 seconds. The mean dominant frequency of the first call component was 1.39 kHz and the mean dominant frequency of the second call component was 1.31 kHz. The advertisement call of *L. kivuensis* is a pulsed single clack repeated in rapid succession, although double clacks are occasionally heard. Calls of *L. kivuensis* were recorded for five calling males. The number of pulses in each call ranged from three to twelve pulses and the mean call duration was 1.64 seconds. In this species, the dominant frequency was extremely similar for all individual clacks within the call, and the mean dominant frequency was 1.48 kHz. As shown by Table 4 and Figure 16, the calls of the two species differ most notably in structure (two components in *L. karissimbensis,* one repeated component in *L. kivuensis)* call duration, and dominant frequency.

Table 4 Call characteristics measured from recordings of *Leptopelis karissimbensis* and *Leptopelis kivuensis* at the same site in the field (Kahuzi Biega National Park, Democratic Republic of the Congo).

Taxon	Museum No.	Date	Time	Temperature (° C)	Mass (g)	Number of Pulses	Call Duration (s)	Dominant Frequency: 1st Component (kHz)	Dominant Frequency: 2nd Component (kHz)
L. karissimbensis	EBG 1476	5 June 2008	20:02	13.0	4.4	1	0.34	1.12	—
L. karissimbensis	EBG 1479	5 June 2008	20:42	13.0	2.9	2	0.93	1.50	1.50
L. karissimbensis	EBG 1477	5 June 2008	21:28	13.0	7.1	1	0.20	1.12	—

Table 4 continued.

		5 June 2008	21:35	13.0	4.6	2	0.89	1.50	1.12
L. karissimbensis	EBG 1483	6 June 2008	19:46	14.0	4.0	1	0.34	1.49	—
L. karissimbensis	EBG 1484	6 June 2008	20:02	14.0	3.7	1	0.36	1.51	—
L. karissimbensis	EBG 1485	6 June 2008	20:20	13.0	4.3	1	0.46	1.50	—
L. karissimbensis	EBG 2124	26 May 2009	19:37	15.6	3.9	2	0.95	1.47	1.52
					Mean		Mean = 0.56	Mean = 1.40	Mean = 1.38

Table 4 continued.

					= 4.36				
L. kivuensis	EBG 1469	5 June 2008	19:15	15.0	2.8	12	2.84	1.50	—
L. kivuensis	EBG 1471	5 June 2008	19:23	15.0	2.7	7	1.58	1.50	—
L. kivuensis	EBG 1472	5 June 2008	21:19	13.0	3.0	6	1.54	1.49	—
L. kivuensis	EBG 1473	5 June 2008	21:50	13.0	4.0	3	0.62	1.46	—
L. kivuensis	EBG 1474	5 June 2008	21:57	13.0	3.3	7	1.60	1.47	—
					Mean	Mean = 7.0	Mean = 1.64	Mean = 1.48	

Table 4 continued.

= 3.16

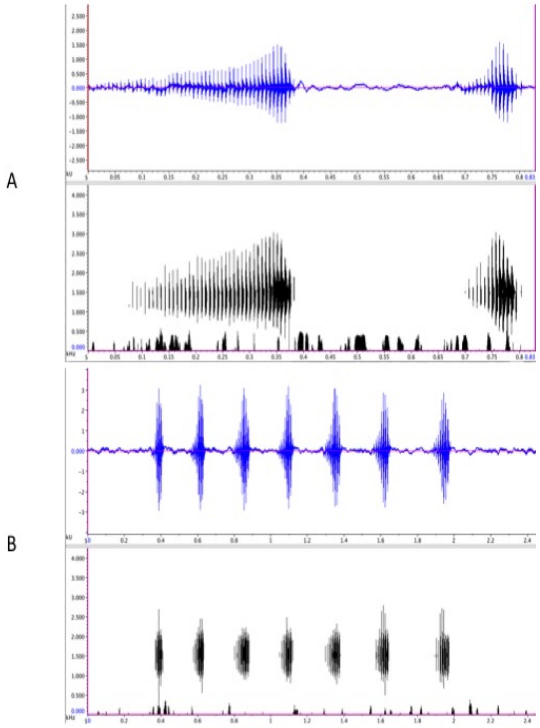

Figure 16 Sonogram and Oscillogram from calling male A) *Leptopelis karissimbensis* and B) *L. kivuensis.*

Phylogenetic Analyses.—The aligned 16S dataset contained 575 total characters, of which 64 were variable and 35 were parsimony-informative. The topology of trees obtained in MP, ML, and BI inferences were identical, and all relationships were highly supported (Fig. 17). Samples of *L. karissimbensis* and *L. kivuensis* occurred in well-supported clades, which were separated by moderate genetic differences (3.7–4.6% uncorrected p divergence). Populations of *L. kivuensis* showed evidence of genetic structure, with one well-supported clade from the Ruwenzori Mountains (DRC) and Bwindi Impenetrable National Park (Uganda), and another well-supported clade from Kahuzi Biega National Park (DRC); these clades were separated by minor genetic distances (1.4–1.5% uncorrected p divergence). The aligned BDNF dataset contained

716 characters. All six samples of *L. karissimbensis* had a three bp deletion at the 145th position that is present in all six samples of *L. kivuensis*; the deletion is at the 48th codon position for glycine in the latter species.

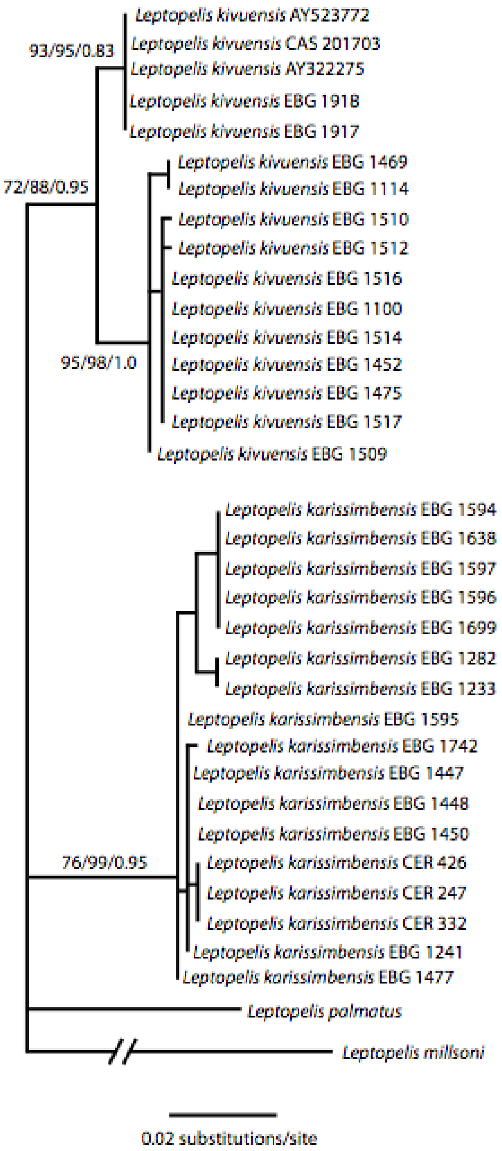

Figure 17 Phylogenetic hypothesis of relationships among individuals of *Leptopelis karissimbensis* and *L. kivuensis* based on 16s DNA sequences. Measures of support represent parsimony bootstrap values/maximum likelihood bootstrap values/Bayesian posterior probabilities

Discussion

At least two authors (Ahl, 1929, 1931; Hölting et al., 2009) have used morphological characters to diagnose *L. karissimbensis* from *L. kivuensis.* A review of these characters and the addition of a previously unrecognized diagnostic character follows. Ahl (1929) stated that mature males of *L. karissimbensis* possess a blue vocal sac, while males of *L. kivuensis* possess a white vocal sac. We found this to be a good character for diagnosis of live, mature male specimens, as every male specimen of *L. karissimbensis* that we had a color photo of (n = 9) possessed the blue vocal sac. We did not find any female specimens of *L. karissimbensis* that exhibited extensive blue coloration or any specimens of *L. kivuensis* that exhibited any blue coloration. Based on the relatively small number of specimens that could be examined for the presence of this character, we hypothesize that *L. karissimbensis* is sexually dimorphic, with mature males exhibiting blue coloration and all other age and sex classes exhibiting reduced blue or white coloration on the vocal sac. Furthermore, we noticed that all specimens exhibiting a blue vocal sac also exhibited copious amounts of blue coloration in most other areas of the venter (Figure 15).

Hölting et al. (2009) state that adult *L. karissimbensis* possess a slight heel spur or "fleshy angularity" (*sensu* Pickersgill, 2007) that *L. kivuensis* does not (Figure 18). This is a reasonably noticeable character on preserved specimens. We conducted a blind test on twenty-three specimens of adult *Leptopelis* of known specific identity from Kahuzi-Biega National Park in DRC. Specimens were mixed together and then sorted based on the presence or absence of the heel spur correctly classified nineteen of twenty-three specimens to species level. Positive identification of the two species may be more difficult because the heel spur is most prominent on the largest specimens of *L. karissimbensis*. All four specimens classified incorrectly were among the very smallest used in the blind test. Hölting et al. (2009) also state that juveniles of the two species can be diagnosed from each other based on a combination of characters. According to Hölting et al. (2009), juveniles of *L. kivuensis* possess yellow elbows and knees and have green ocular irises, while juvenile *L. karissimbensis* exhibit white elbows and knees and have red or reddish-brown ocular irises. We feel that the use of these character combinations is not informative as we found several specimens in the Virunga Mountains of the Parc National des Volcans exhibiting mixed characters. The juvenile specimen pictured in Figure 19 is one of several *L. karissimbensis* found with reddish ocular irises and yellow elbows and knees. Without molecular evidence, we cannot recommend a character to diagnose juveniles of the two species. During our examination of the specimens we collected, we did notice a difference in all adult specimens (n > 70) of the two species. Specimens of *L. karissimbensis* exhibit much darker ventral coloration of the front feet and usually the entire venter. The ventral surface of the foot in *L. kivuensis* is much lighter and typically white, but may have a small amount of pigment at the ends of the toes and edges of the foot (Figure 20). We propose that the presence of extensive pigmentation on the ventral surface of the foot is the best character for diagnosing adults of the two species in preservative. This morphological character has not previously been noted by any other publication on the two species.

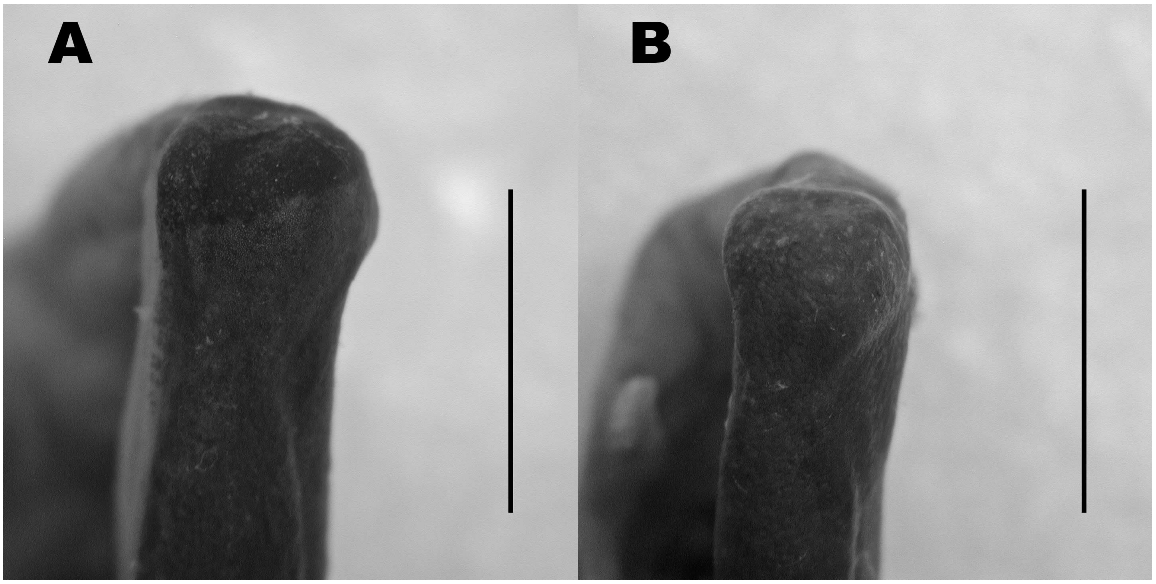

Figure 18 "Heel" region showing fleshy angularity on foot of *A) Leptopelis karissimbensis* and lack of this structure on foot of B) *L. kivuensis.* Scale bars represent 1 cm.

Figure 19 Juvenile *Leptopelis karissimbensis* from the Virunga Mountains, exhibiting the character state combination of reddish ocular irises and yellowish elbows and knees.

Figure 20 Ventral foot surfaces of A) *Leptopelis karissimbensis* and B*) L. kivuensis* showing diagnostic differences in coloration. Scale bars represent 1 cm.

During collection and photography of live specimens of the two species, differences between the two species in defensive behavior were noted. While our sample size for these observations was small (*L. karissimbensis* = 3, *L. kivuensis* = 4) and we acknowledge that some of the behaviors reported may represent individual variation, we feel these observations shed light on the natural history of the two species. When prodded, some individuals of both species will make their body concave while raising the hands (Figure 15). The frogs will often remain in this position for over a minute if harassment is ceased, but will continue as long as the animal continues to be bothered. In

L. karissimbensis, the mouth is opened, while in *L. kivuensis*, the mouth remains closed. In males of *L. karissimbensis*, this has the effect of making the blue vocal sac and ventral coloration very conspicuous and this may represent aposematic behavior that would discourage attack from a potential predator.

Figure 21 shows the current known distribution of *L. karissimbensis*. While previous authors have suggested the species is restricted in range to the Virunga Mountains (Ahl, 1929; Schiøtz, 1975, 1999), de Witte found the species in what is now known as Nyungwe Forest in Rwanda (de Witte, 1941). Based on the published literature (Ahl, 1929; de Witte, 1941; Schiøtz, 1975; Drewes and Vindum, 1994; Schiøtz, 1999; Hölting et al. 2009;) and this study, we have greatly increased our knowledge of the distribution of *L. karissimbensis.* The species is found in sympatry with *L. kivuensis* in the Virunga Mountains of Rwanda, the mountains and highlands of eastern DRC in Kahuzi-Biega National Park, and Nyungwe Forest in Rwanda. It is likely found in sympatry with *L. kivuensis* in southwestern Uganda in several localities (de Witte, 1941). Only *L. kivuensis* is found north of the Virungas in Bwindi Impenetrable National Park (Drewes and Vindum, 1994). It is likely that both species are found in sympatry in northern Burundi at Muramvya (Laurent, 1973). The discovery of *L. karissimbensis* in the forests around Mt. Kahuzi and Mt. Biega in DRC extends the known range of the species westward at least ninety km and the discovery of the species on the Itombwe Plateau extends the known range southward at least one hundred fifty km (Figure 19).

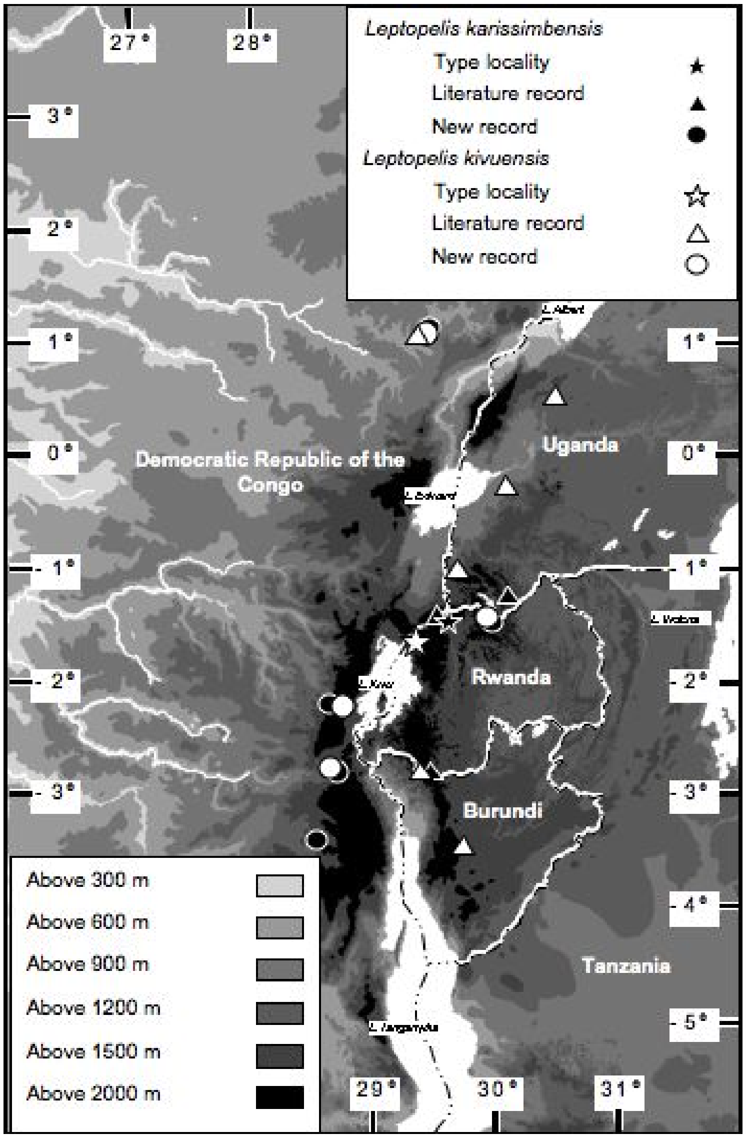

Figure 21 Map showing Albertine Rift region and records for *Leptopelis karissimbensis* and *L. kivuensis,* including all historical literature records and previously unpublished records discovered during this study.

Based on the results presented here, we can make several conclusions. *Leptopelis karissimbensis* and *L. kivuensis* are indeed distinct species. While the two species share many characteristics of gross external morphology, they exhibit multiple character states of different forms (morphological, molecular, and behavioral) that make at least adult animals of either species diagnosable from each other. They have measurable differences in the structure of their advertisement calls and each species appears to exhibit a distinct, stereotyped defensive behavior. The presence of apparent sexual dimorphism regarding vocal sac color in *L. karissimbensis* may be related to a visual mating signal, as has been reported with other anuran species (Rosenthal et al., 2004)

Some previous workers have confused the two species (de Witte, 1941; Laurent, 1973), making published locality records difficult to assign to either species. Based on credible literature records, we assume that *Leptopelis kivuensis* is known from Kibale Forest in Uganda (Vonesh, 2001), Bwindi Impenetrable Forest in Uganda (Drewes and Vindum, 1994), along the border where Rwanda, Uganda, and DRC abut (Ahl, 1929; de Witte, 1941); through western Rwanda (de Witte, 1941) and Burundi (Laurent, 1973; Hölting et al., 2009), and throughout eastern DRC (Schiøtz, 1999). Based on published records, we know that *L. karissimbensis* occurs in extreme southwest Uganda near the town of Kabale (Pickersgill, 2007), through the Virungas in eastern DRC (Ahl, 1929; Schiøtz, 1975, 1999; Roelke et al., 2009), and south through western Rwanda in Nyungwe Forest (Hölting et al., 2009). It is likely that at least some *Leptopelis* specimens examined by Laurent (1973) from northern Burundi are also *L. karissimbensis.*

While we have gained little new information on the distribution of *L. kivuensis*, we have determined that *L. karissimbensis* occupies a much larger geographic area than previously thought. Some authors stated that the distribution of *Leptopelis karissimbensis* is restricted to the Virunga Mountains (bordering DRC, Rwanda and Uganda), and that *L. kivuensis* occurs throughout most of the Albertine Rift (Laurent, 1972; Schiøtz, 1975, 1999; Channing and Howell, 2006). In 2009, Hölting et al. published a note claiming to extend the known range of *L. karissimbensis* over 130 km southwards from the Virunga Mountains to Nyungwe Forest in southern Rwanda. Nyungwe Forest has historically been known as Rugegewald (Schubotz, 1913; Kunkel and Kunkel, 1969; Laurent, 1973). G.F de Witte (1941), in his opus on the herpetofauna of Albert National Park, which is now mostly contained within the boundaries of Virunga National Park in DRC, lists several species of reptiles and amphibians found in Rwanda and eastern DRC. Among these listed species is *Hylambates rufus* (Nieden, 1912). In this volume, de Witte also provides a synonomy for *L. karissimbensis*, which includes *H. rufus*. Therefore, the note published by Hölting et al. (2009) does not represent a range extension for *L. karissimbensis*, as de Witte noted the presence of this species in Nyungwe Forest over sixty-five years ago. De Witte (1941) also lists this species as occurring at multiple localities in Uganda, such as the Ishasha River area around Lake Edward and from the vicinity of Lake Bunyonyi. Most other authors who have addressed the range of *L. karissimbensis* have also apparently missed this fact (Schiøtz, 1975, 1999; Channing and Howell, 2006). Drewes and Vindum (1994) recorded *L. kivuensis* from Bwindi Impenetrable National Park about 40 km north of the Virunga Mountains, but did not find *L. karissimbensis*. Based on literature records and some associated preserved specimens, we feel that Figure 19 represents the most accurate, currently known distribution of the two species. It is possible and likely that new localities for both species will be discovered in eastern DRC.

As stated in the introduction, the IUCN classifies *L. karissimbensis* as "endangered." This category represents the second highest conservation status of taxa assessed by the IUCN below "critically endangered." *Leptopelis kivuensis* is listed as "near threatened," which is the second lowest level of conservation status above "least concern" for assessed taxa. The species falls in this category because the IUCN states that the species occupies a geographic range smaller than 5000 km^2 and all individuals are found in less than five locations. Under the category of "conservation actions," the IUCN states, "Further survey work is needed to determine the current population status of this species and to investigate aspects of its breeding biology." One aspect of the breeding biology of *L. karissimbensis* has been published as a larval description by Roelke et al. (2009). Here we have attempted to clarify the current and historical distribution of the species. Based on historical records missed by other authors and new localities found in the course of fieldwork for this study, we have determined that the known range of *L. karissimbensis* is much larger than previously thought. The known range of *L. karissimbensis* is close in size to that of its close relative, *L. kivuensis*, and since *L. kivuensis* is listed as "near threatened," we propose that the IUCN downgrade the conservation status of *L. karissimbensis* from "endangered" to "near threatened." Both species can be abundant in suitable habitat and the Albertine Rift contains the greatest proportion of protected lands on the continent (Plumptre et al., 2003), so we feel that both species are adequately protected assuming continued protection of their habitats from timber harvest and other consumptive human activities. At least five national parks administered by three countries (DRC, Rwanda, and Uganda) protect the habitat of *L. karissimbensis* and *L. kivuensis* where they occur in sympatry. It is our hope that these natural reserves continue to be maintained and encourage research activities that will allow us further access to components of Africa's understudied biodiversity.

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BIOGRAPHICAL INFORMATION

Corey Roelke was born in Fort Lauderdale, Florida on December 7th, 1982. He moved to northern Indiana when he was almost two years old. He attended Penn High School in Mishawaka, Indiana and graduated in 2001. Corey attended Clemson University in Clemson, South Carolina and graduated with a Bachelors of Science Degree in Biological Sciences in 2005. He married Dr. Laura Hunt in September 2007. His research interests are wide and varied and include systematics, taxonomy, behavior, and natural history of vertebrates, but especially amphibians and reptiles. Corey's personal interests include hunting, fishing, herping, eating barbecue, exhibition poultry, curling, and travel. He is currently excited about the prospect of introducing his young son, Wyatt, to the rapidly dwindling wonders of the natural world. He greatly desires to spend more time on the continent of Africa and to visit Indonesia.