HABITAT FRAGMENTATION, PATTERNS OF DIVERSITY AND PHYLOGEOGRAPHY OF SMALL MAMMAL SPECIES IN THE ALBERTINE RIFT

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Declaration

The undersigned, Prince K. Kaleme, hereby declares that this dissertation is my own original work and that I have not previously, in its entirety or in part, submitted it for a degree at any academic institution for obtaining any qualification. The experimental work was conducted in the Department of Botany and Zoology, Stellenbosch University, the Royal Museum for Central Africa, Tervuren, Belgium and the Field Museum of Natural History, Chicago, USA.

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PRINCE K.K. KALEME

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For Martine, David, Jonathan and Gradi

Mum and Dad

with love

Abstract

The Albertine Rift is characterized by a heterogeneous landscape which may, at least in part, drive the exceptional biodiversity found across all taxonomic levels. Notwithstanding the biodiversity and beauty of the region, large areas are poorly understood because of political instability with the inaccessibility of most of the region as a contributing factor. The majority of studies in the Albertine Rift have focussed on charismatic mega fauna, with other taxa receiving less attention. One of the taxonomically and numerically more abundant small mammal genera is the genus *Praomys,* an African endemic with a wide distribution range spanning most of west, central and east Africa. Four species are typically recognized from the Albertine Rift namely *P. degraaffi*, *P. jacksoni*, *P. misonnei* and *P. verschureni*. In this study I used a combination of DNA sequence data (mitochondrial control region, mitochondrial cytochrome b and 7th intron of the nuclear ß-fibrinogen gene) as well as morphometric data (traditional and geometric) to investigate the systematics of the *Praomys* taxa occurring in the Albertine Rift. To allow meaningful DNA assessments and in an attempt to identify potential drivers of diversifications, other *Praomys* species were also included from public sequence data bases for comparisons. The main focus was on *P. jacksoni* (the numerically most abundant taxon; also, up to 2005, all *Praomys* in the Albertine Rift were mostly collected as "*jacksoni*") and *P. degraaffi* (an Albertine Rift endemic). A surprising finding was the presence of *P. mutoni*; this represents a range extension for this species into the Albertine Rift. Distinct evolutionary lineages were found in both *P. jacksoni* (confirmed by sequence data as well as morphometrics) as well as *P. degraaffi* (based only on sequence data; insufficient samples precluded a full morphometric investigation). These lineages (in both *P. jacksoni* as well as *P. degraaffi*) appear to be separated along a north – south gradient; however, further investigations should confirm this.

To further investigate the genetic patterns at local scales across the Albertine Rift, as well as introgression between species as revealed by sequence data, a species-specific microsatellite library was developed for *P. jacksoni*. Twelve polymorphic markers were identified of which nine also amplified in *P. degraaffi*. Introgression was confirmed between the two focal species with almost 20% of the individuals analysed being *jacksoni-degraaffi* hybrids. This is perhaps not so surprising given that there is considerable overlap in their ranges (between ~ 1500 m a.s.l. to 2450 m a.s.l.) as well as the relative ages of the species (the divergence time between these two species were estimated at 3.8 Mya). The presence of distinct lineages within each of these species was confirmed by microsatellite analyses (these lineages diverged approcimately at same time at *ca.* 3.4 Mya). As suggested by sequence and morphometric data, these lineages had a largely north – south distribution but with considerable overlap in the central Albertine Rift in the vicinity of Lake Kivu. The phylogeographic patterns obtained for both focal species were not consistent with the physical barriers such as the rivers, lakes or mountains, nor were they exclusively associated with Pleistocene phenomena such as the change of the course of the rivers or uplift; rather, the lineages predate the Pleistocene and fall firmly in the Pliocene (>3 Mya). Biogeographically, the north - south location of lineages with a centrally - located

contact zone could be a result of parapatric speciation due to habitat fragmentation or past climate change, followed by secondary contact.

Barcoding using genetic information provides a useful tool to identify unknown taxa, cryptic diversity or where different life stages are difficult to identify. From an invasion biology perspective, it allows for the rapid identification of problem taxa against a known data base. By adopting such a barcoding approach (*senso lato*), the presence of three invasive rodents was confirmed in the Democratic Republic of the Congo (DRC); these are *Rattus rattus* (black rat), *R. norvegicus* (Norway rat) and *Mus musculus domesticus* (house mouse). A comparison with global data available for these species revealed two possible introduction pathways namely via the shipping port at Kinshasa/Matadi (with strong links to Europe) and via the slave trade routes in the east (strong links to the Arab world and the east). Of these three taxa, only *R. rattus* is currently documented from the DRC although the others have received mention in the gray literature. These findings draw attention to the lack of any official policy regarding biosecurity in the DRC, and argue for the development of strict control measures to prevent further introductions.

Opsomming

Die Albertine Rift word gekenmerk deur 'n heterogene landskap wat kan, ten minste gedeeltelik, die uitsonderlike biodiversiteit wat oor al die taksonomiese vlakke gevind word teweeg bring. Nieteenstaande die biodiversiteit en die skoonheid van die streek, is groot gebiede onbekend as gevolg van politieke onstabiliteit met die ontoeganklikheid van meeste van die streek as 'n bydraende faktor. Die meerderheid van studies in die Albertine Rift het gefokus op die charismatiese mega fauna, met ander taxa wat minder aandag ontvang. Een van die taksonomies en numeries meer volop klein soogdier genera is die genus *Praomys*, 'n Afrika endemiese groep met 'n wye verspreiding wat strek oor die grootste deel van van wes-, sentraal en oos-Afrika. Vier spesies word tipies erken van die Albertine Rift naamlik *P. degraaffi*, *P. jacksoni*, *P. misonnei* en *P. verschureni*. In hierdie studie het ek 'n kombinasie van DNA volgorde data (mitochondriale beheer streek, mitochondriale sitochroom b en 7de intron van die kern ß-fibrinogeen geen) sowel as morfometriese data (tradisioneel en meetkundig) gebruik om die sistematiek van die *Praomys* taxa te ondersoek. Om betekenisvolle DNA aanslae toe te laat en in 'n poging om potensiële aandrywers van diversiteit te identifiseer, is ander *Praomys* spesies van openbare volgorde data basisse vir vergelykings ingesluit. Die hooffokus is op *P. jacksoni* (die numeries volopste takson, ook, tot en met 2005 is alle *Praomys* in die Albertine Rift meestal as "*jacksoni*" versamel) en *P. degraaffi* ('n Albertine Rift endemiese spesie). 'n Verrassende bevinding was die teenwoordigheid van *P. mutoni*, dit verteenwoordig' n verspreidingsuitbreiding vir hierdie spesie in die Albertine Rift. Bepaalde evolusionêre ontwikkelingslyne was in beide *P. jacksoni* (bevestig deur die volgorde data sowel as morfometrie) sowel as *P. degraaffi* (wat slegs gebaseer is op die volgorde data, onvoldoende monsters verhinder 'n volledige morfometriese ondersoek). Hierdie lyne (in beide *P. jacksoni* sowel as *P. degraaffi*) word geskei langs 'n noord - suid gradiënt, maar verdere ondersoeke moet dit bevestig.

Om die genetiese patrone op plaaslike skaal oor die Albertina Rift verder te ondersoek, sowel as introgressie tussen spesies soos geopenbaar deur die volgorde data, is 'n spesie-spesifieke mikrosatelliet biblioteek ontwikkel vir *P. jacksoni*. Twaalf polimorfiese merkers is geïdentifiseer waarvan nege ook amplifiseer in *P. degraaffi*. Introgressie is bevestig tussen die twee brandpunt spesies met byna 20% van die individue wat ontleed is as jacksoni-degraaffi basters. Dit is miskien nie so verbasend gegee dat daar aansienlike oorvleueling is in hul gebiede (tussen ~ 1500 m bo seespieel tot 2450 m bo seespieel), sowel as die relatiewe ouderdomme van die spesies (die divergensie tussen hierdie twee spesies is geskat op 3,8 Mya). Die teenwoordigheid van verskillende lyne in elk van hierdie spesies is bevestig deur mikrosatelliet ontleding (hierdie lyne het gedivergeer ongeveer 3,4 Mya). Soos voorgestel deur die DNA volgorde en morfometriese data, het hierdie lyne 'n grootliks noorde – suid verspreiding, maar met 'n aansienlike oorvleueling in die sentrale Albertine Rift in die omgewing van die Kivumeer. Die filogeografiese patrone wat vir beide die brandpunt spesies gevind is nie in ooreenstemming met die fisiese struikelblokke soos die riviere,

mere of berge nie, en hou ook nie uitsluitlik verband met die Pleistoseen verskynsels soos die verandering van die loop van die riviere nie; die afstammelinge is eerder veel ouer as die Pleistoseen en val binne die Plioseen (> 3 Mya). Biogeografies, die noorde – suid plasing van die lyne met 'n sentraal geleë kontak sone kan die gevolg wees van parapatriese spesiasie te danke aan habitatfragmentasie as gevolg van verandering in die klimaat, gevolg deur 'n sekondêre kontak.

Strepieskodering met behulp van genetiese inligting verskaf 'n nuttige instrument om onbekend taxa, kriptiese diversiteit of waar verskillende lewensfases moeilik is om te identifiseer, te identifiseer. Vanuit 'n indringerbiologie perspektief, maak hierdie benadering dit moontlik om vinnige identifikasies van die probleem taksa teen' n bekende data basis te bekom. Deur gebruik te maak van so 'n strepieskoderingsbenadering (*senso lato*), is die teenwoordigheid van drie indringende knaagdiere bevestig in die Demokratiese Republiek van die Kongo (DRK), naamlik *Rattus rattus* (swart rot), *R. norvegicus* (Noorweë rot) en *Mus musculus domesticus* (huis muis). 'n Vergelyking met die globale data wat beskikbaar is vir hierdie spesies het aan die lig gebring dat twee moontlike betree-roetes bestaan, naamlik via die skeepshawe by Kinshasa / Matadi (met sterk skakels na Europa), en via die slawehandel roetes in die ooste (sterk skakels na die Arabiese wêreld en die ooste) . Van hierdie drie taxa, is tans slegs *R. rattus* van die Demokratiese Republiek van die Kongo gedokumenteer, hoewel die ander melding ontvang in die grys literatuur. Hierdie bevindinge vestig die aandag op die gebrek aan enige amptelike beleid ten opsigte van biosekuriteit in die Demokratiese Republiek van die Kongo, en argumenteer vir die ontwikkeling van streng beheermaatreëls om verdere indringerspesies te voorkom.

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List of abbreviations

AMOVA: Analysis of molecular variance

ANOVA: Analysis of variance

a.s.l.: Above sea level

BP: Before present

CS: Centroid size

CVA: Canonical variate analysis

DFA: Discriminant function analysis

DRC: Democratic Republic of Congo

GPA: Generalized Procrustes Analysis

H_E: Expected heterozygosity

H_O: Observed heterozygosity

HWE: Hardy–Weinberg equilibrium

IBD: Isolation by distance

LGM: Last glacial maximum

MANOVA: Multivariate analysis of variance

mtDNA: Mitochondrial DNA

My: Million years

Mya: Million years ago.

Nm: Number of migrants

NPA: Number of private alleles

OUT: Operational taxonomic unit

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PLS: Partial least squares

PCR: Polymerase chain reaction

SGS: Spatial genetic structure

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Chapter 1

General introduction and background information

1.1 Aims and objectives of the study

The Albertine Rift is biologically diverse, yet poorly explored. Notwithstanding a paucity of information, all indications are that it is an area of global biodiversity significance. In addition, several biogeographical regions have been identified across the spatially complex montane circle of East Africa; these include the Albertine Rift, Kenyan Highlands, Crater Highlands, Northern Eastern Arc Mountains (Mts.), Central and Southern Eastern Arc Mts., Malawi Rift and Ufipa Plateau (Udvardy 1975; Bowie *et al*. 2006; Moodley & Bruford 2007). Climatic cycling has been the most widely cited explanation for the biogeographical patterns observed and may have significantly contributed to the fragmentation of forest biomes (see e.g. deMennocal 2004, Fjeldså & Bowie 2008, Voelker *et al*. 2010), while the uplift of East African volcanic highlands (e.g. Virunga in the Albertine Rift, Crater Highland in northwestern Tanzania) could have caused the vegetation changes observed in some localities (Bonnefile *et al*. 1990; Partridge *et al*. 1995b; deMenocal 2004). The formation of the Virunga Volcanoes also altered the hydrology of the region (Beadle 1981; Hamilton 1982).Today the Albertine Rift is comprised of isolated montane and lowland forests separated by areas of dry savanna, rivers and lakes. The overall goal of this thesis is to further our knowledge on the Albertine Rift's biodiversity for use in conservation planning, and to form a basis for drafting documentation to deal with threats such as alien invasive species, which have never been systematically assessed for the region. To achieve these objectives, small mammal (rodent) taxa are investigated.

This study focuses (albeit not exclusively) on the rodent taxa of the genus *Praomys* from the Albertine Rift; specifically the two most abundant (of the four known) species, *P. degraaffi* and *P. jacksoni*. Whether genetic differentiation between the two species exists has never been determined. In addition, two alien invasive taxa, *Mus musculus* and *Rattus* sp. were also sampled across the Democratic Republic of Congo (DRC).

The aims of the study are to determine the effects of habitat fragmentation on species richness, the spatial pattern of genetic diversity (if any) and hence the taxonomic status of the species in the Albertine Rift montane forests, and to assess the spread of alien invasive species (mice and rats).

The specific objectives were to:

1. assess the phylogeny and taxonomic status of the *Praomys* species in the Albertine Rift by combining data from morphometrics (traditional and geometric) and molecular (mitochondrial and nuclear) DNA sequences;

2. reconstruct the phylogeography of *P. degraaffi* and *P. jacksoni*;
3. document the presence and the spread of two alien invasive taxa, *Mus musculus* and *Rattus* sp. in the DRC using mitochondrial DNA data and historical records.

1.2 The Albertine Rift

1.2.1 Description and location

The East African Rift System forms part of a significant geologic structure that extends from Jordan in the Middle East to Mozambique in southern Africa and is 6,400 km long and averages 48–64 km in width. It has been forming for *c*. 30 million years since Africa and the Arabian Peninsula separated (Baker *et al*. 1972); a process which is still ongoing. The system's main branch, the Eastern Rift Valley, encompasses the Jordan River, the Dead Sea and the Gulf of Aqaba to the north, and continues south along the Red Sea through Kenya and Tanzania, to the Indian Ocean near Beira at the lower Zambezi River in Mozambique (Johnson 1984; Rosendahl 1987; Partridge *et al.* 1995b). The second branch, the Western Rift Valley (which includes the Albertine Rift), extends north from the northern end of Lake Malawi in an arc that includes the lakes Rukwa, Tanganyika, Kivu, Edward, George and Albert (Rosendahl 1987). The Albertine Rift specifically (Figure 1.1) extends from *c*. 30 km north of Lake Albert to the southern tip of Lake Tanganyika and encompasses a diverse array of habitats and altitudinal zones including ice fields on the Ruwenzori massif (> 5,000 m a.s.l.), active volcanoes in the Virunga National Park, ericaceous shrubs (above 3,000 m), bamboo forests (mostly above 2,400 m), montane forests (above 1,500 m), lowland broad-leaved forests (above 600 m) (see Jolly *et al*. 1997; Plumptre *et al*. 2007a) as well as Africa's deepest lake, Lake Tanganyika.

1.2.2 Geology and climate

The origin of the mountains of the Albertine Rift is subject to debate. Two mechanisms can be invoked to explain the rift's formation (see Rosendahl 1987): active and passive rifting. The first (Gregory 1896; Dainelli 1943; Le Bas 1971; Cerling & Powers 1977) supports the idea that rifts are a tensile response to doming, arching, and/or uplift on a regional scale. The mechanism is variable and its formation and extension takes into account the role and timing of volcanism, causes and scale of doming and their episodicity. The second mechanism, namely passive rifting, supports the hypothesis that subsidence (stretching or thinning of the lithosphere) is the first expression of rifting, and any doming is a consequence of thermal events (Baker *et al*. 1972; Chapin 1979; Faller & Soper 1979). Rosendahl (1987) argued that the Albertine Rift Mountains were formed from a combination of uplifted Pre–Cambrian basement rocks and volcanic activity. The uplift and volcanism are also associated with the origin of Africa's Great Rift valleys

and lakes that were created by the clockwise rotation of the continent, producing cracks extending down the eastern side of Africa (Livingstone 1967, 1975; Rosendahl 1987).

The evolution of the Western Rift differs from that of the Eastern Rift in that the doming in the central section was of smaller amplitude during the early Miocene; rifting began during the mid Miocene and led to the first lacustrine sedimentation *c*. 12 Mya, while the major subsequent faulting occurred at about 5.0, 2.8 and 2.4 Mya (Pickford *et al*. 1993). Pickford (1990) and Hartnady & Partridge (1995) suggested that the major uplift due to rifting occurred in the mountains of the Western Rift only around the late Pliocene (*c*. 3 Mya). The uplift of the rift's shoulders averaged ± 1,500 m but reached 4,300 m in the Ruwenzori Mountains, the only East African mountain range of non–volcanic origin (Partridge *et al.* 1995b). From 2 Mya to 35,000 years BP, the uplifting of the areas west of Lake Victoria caused the reversal of rivers creating some of the present day lakes in the Western Rift (Bootsma & Heckey 1993). Investigations in the basin of the proto-Lake Kivu (Degens *et al.* 1973) found that the thickness of the sediments (west of Idjwi) was consistent with an age of *c*.1 My or more. During the Pleistocene, the volcanic activity of the Virunga dramatically altered the central Albertine Rift. During the mid–Pleistocene, proto-Lake Kivu flowed to the north into a large lake encompassing the area of present day lakes Edward and George (Beadle 1981). Volcanic eruptions in the mid- to late Pleistocene dammed the rivers and formed Lake Kivu (15,000 – 10,000 years BP) when its northern drainage was blocked by lava flows. Lake Kivu now flows out to the south via the Ruzizi River toward Lake Tanganyika. The filling-in of the valleys created Idjwi Island within Lake Kivu.

The relationship between tectonically controlled uplift and climate change was demonstrated for other areas such as the Tibetan plateau (Raymo & Ruddiman 1992; Molnar *et al*. 1993). Volcanic activity and rifting in combination with climatic change during the mid to late Pleistocene created complex patterns of habitat fragmentation with expansion/contraction cycles of montane vegetation (Morrison 1968; Morrison & Hamilton 1974; Hamilton 1982). This relationship can similarly be extrapolated to the African Rift Valley and specifically the Albertine Rift.

It is well established that sub-Saharan Africa became much more arid during glacial periods in the Northern Hemisphere (Hamilton 1982; deMenocal 1995, 2004). The shift from rainforest to savanna habitat appears to have occurred at least three times, at approximately 2.8, 1.7, and 1.0 Mya (deMenocal 1995). The expansion of savannas could have resulted in the montane rainforest remnants becoming fragmented from each other and from the lowland rainforests that once connected them. Due to the wealth of niches and habitats in combination with climatic change and the effect on habitat, tropical and montane forests are of importance for investigating speciation and evolutionary

processes in the fauna and flora (Dieterlen 1990; Prigogine 1985; Vande Weghe 1988a, b; Stattersfield *et al*. 1998; Olson & Dinerstein 2002; Burgess *et al*. 2004; Brooks *et al*. 2004) and may, at least in part, be drivers for the exceptionally high biodiversity that characterizes the region.

1.2.3 Biodiversity features

The Albertine Rift contains several global conservation priority sites and more vertebrate species than anywhere else on the African continent (Burgess *et al*. 2004; Plumptre *et al*. 2007a). Based on taxonomic inventories (Prigogine 1979, 1984, 1985; Kerbis Peterhans *et al*. 1998), the fauna and flora of the Albertine Rift Mountains are remarkably rich; exceptionally high numbers of endemic species characterize all taxonomic groups at all altitudes; these high levels of endemism extend into the lower altitude forests on the western margin of the Albertine Rift which forms a border with the Congo basin lowland forests (Olson & Dinerstein 2002; WWF *et al*. 2007). According to Denys *et al*. (1986) and Partridge *et al.* (1995b), the local structures and the rifts impeded migration and may account for the unusually high endemism that is evident, particularly in micro-mammalian faunas, up to the end of Pliocene.

The botanical and invertebrate diversities are poorly known because many families have not been studied. The best studied invertebrate group is butterflies, of which 117 species are endemic (Plumptre *et al*. 2007a). Among vertebrates, birds are characterized by exceptional levels of endemism with 41 (Albertine Rift and the eastern Congo lowlands combined) of the 1,061 species being endemic, there are 14 endemic amphibian species and 16 endemic reptile species (Plumptre *et al*. 2007a). The aquatic biodiversity is also unique (Verheyen *et al*. 2003); 366 fish species are endemic to the Albertine Rift (Plumptre *et al*. 2007). Four hundred and two (402) mammal species have been reported in the Albertine Rift with 34 globally threatened (Critically Endangered, Endangered or Vulnerable) and 35 endemic species, most of which are rodents (13) and shrews (18) (Kityo *et al*. 2003; Plumptre *et al*. 2007a).

Present diversity measures are likely to be under-estimates of species diversity because of sampling biases. I.e. sites in Uganda and the Kahuzi Biega National Park (KBNP) in the DRC are among the few sites that are thoroughly surveyed. Notwithstanding, charismatic mammals include the gorillas (the mountain gorilla *Gorilla beringei beringei* and the eastern lowland gorilla *G.b. graueri*), the eastern chimpanzee (*Pan troglodytes schweinfurthi*), the savanna and forest elephants (*Loxodonta africana* and *L. cyclotis*), and the okapi (*Okapia johnstoni*). Endemic small mammal species include, amongst others, the Kivu shrew (*Crocidura kivuana*), the western rift bush-furred rat (*Lophuromys medicaudatus*), Rahm's bush-furred rat (*L. rahmi*), the Ruwenzori otter shrew (*Micropotamogale ruwenzori*), Shaller's

mouse shrew (*Myosorex schalleri*), Degraaff's praomys (*Praomys degraaffi*), Verschuren's praomys (*P. verschureni*), the Ruwenzori horseshoe bat (*Rhinolophus ruwenzori*) and Hill's horseshoe bat (*R. hilli*). IUCN red listed small mammal species include Carruther's mountain squirrel (*Funisciurus carruthersi*), Dent's vlei rat (*Otomys denti*) and the Mount Kahuzi African climbing mouse (*Dendromus kahuziensis*).

The richness in biodiversity is likely a consequence of several factors including geological instability as a result of uplift (Livingstone 1967, 1975), volcanism (which commenced *c*. 11 to 9 Mya and which is still ongoing; Kampunzu *et al*. 1998) and natural climatic oscillations most notably during the Plio – Pleistocene (Beadle 1981). In a region of predominantly dry climate, the mountains are conspicuous areas of high rainfall (deMenocal 1995, 2004). Prior to the early Pliocene, the region of the Western Rift was covered with tropical forest but the rifting produced cold and wet Afromontane conditions favorable for the spread of Afromontane-adapted taxa (White 1983). Bonnefile *et al.* (1990) noticed that the pollen spectra from Hadar in the Ethiopian Rift indicated that the vegetation between 3.3 and 2.8 Mya has no analogue in the semi-desert steppe flora that characterizes the area today; it is therefore similar to the modern vegetation occurring at altitudes from 1,600 to 2,200 m, receiving two to three times the present rainfall. Likewise, herbaceous *Cliffortia* spp. (Family Rosaceae), which occurred in marginal vegetation around the Kuwasenkoko swamps in Rwanda at *c*. 2,340 m (Hamilton 1982) are now associated with scrubby vegetation above 2,500 to 2,700 m in East Africa (Taylor 1988; Jolly *et al*. 1997).

The uplift of East Africa may have contributed to the vegetation changes observed at some localities (Bonnefile *et al*. 1990). However, climatic cycling during the Pleistocene is currently the most widely postulated explanation in the literature for the biogeographical patterns observed in Africa today and may have significantly contributed to the fragmentation of forest biomes (Cerling 1992; Cerling *et al*. 1997; deMennocal 2004; see also Fjeldså & Bowie 2008). These changes would have had the most significant effect on small mammal taxa, particularly taxa adapted to specific habitats. It is therefore not surprising that rodent taxa are species rich in the region, some of which are forest dwellers such as *Praomys*, *Hylomyscus*, *Lophuromys* and *Thamnomys* (Dieterlen 1990), whereas other taxa as *Lemniscomys* and *Arvicanthis* are adapted to xeric environments (Kingdon 1997). The composition and extent of forests have varied during the Holocene; these changes were probably driven by climatic and endogenic factors (e.g. succession at different rates of dispersal) during the early to mid-Holocene (Jolly *et al*. 1997).

High species diversity characterizes the Albertine Rift (Fjeldså & Lovett 1997), which could be explained by the refuge model (Haffer 1974, 1997). However, patterns of several taxa of animals and plants reveal not only vicariance between different montane areas, but also a large number of interconnections between highlands, possibly as a consequence of dispersal. Furthermore, there is ecoclimatic instability in certain parts where a high incidence of anomalous events (such as discontinuities in forest cover or microclimate) leads to high turnover rates of species (Fjeldså & Lovett 1997). For instance, bird species that occur at higher altitude occupy a greater number of mountain blocks; more widespread species occur over a wide range of altitudes and most species with restricted range occur in small bands at lower edge of mountain forest or below, in the transitional forest (Bober *et al.* 2001).

The region is unfortunately not without anthropogenic pressures; much of the interlacustrine highlands have been transformed to cultivated and grazed land (Jolly *et al*. 1997; Plumptre *et al*. 2003) as evident in Rwanda. The farming activities of rural people and hunting (including poaching) in and outside protected areas are the largest threat to conservation in the region, associated with a high human population density that averages 300 inhabitants / km^2 (Plumptre *et al*. 2007a). Highland forests have been largely cleared but blocks of montane forest are still intact in all the countries (WWF *et al*. 2007). A wide variety of invasive species occur in the region but the extant occurrence and threat to biodiversity have to be assessed. No national or regional strategy exists on invasive species and only a handful of opportunistic, unpublished studies on alien species have been carried out to date in universities and colleges. Clearly, the socio-economic problems of the region must be resolved in order for conservation efforts to have a chance to succeed (Kerbis Peterhans & Hutterer 2009).

1.3 Natural history of the genus *Praomys*

1.3.1 Rodent taxonomy and account of the Praomys complex

The Order Rodentia represents the most diverse order of mammals, with ± 2,277 species recognized, nearly half of all mammalian species. Among rodents from the family Muridae, the subfamily Murinae (Old World rats and mice) exemplifies one episode of this diversification with up to 125 genera and *c.* 560 species (Musser & Carleton 2005). The subfamily originated 12 – 14 Mya (Jacobs & Pilbeam 1980; Jacobs *et al*. 1989) and diversified in Eurasia, Southeast Asia, Australia and Africa. This successful radiation can be attributed to their ability to adapt to a variety of habitats and their radiation reflects two aspects of their ecology namely diet (omnivorous, herbivorous, insectivorous or seedeaters) and life history traits (cursorial, arboreal, burrowing, amphibious, richochetal) (Dieterlen 1986, 1990).

Several factors account for the lack of, or little consensus in rodents' systematics as highlighted by Luckett & Hartenberger (1985a), Michaux *et al.* (2001) Steppan *et al.* (2005) and Musser & Carleton (2005):

- an extensive radiation during the Eocene – Oligocene;
- an incomplete fossil record;
- the existence of a large number of species and families that makes it difficult to study and evaluate any character complex in most of the taxa;
- emphasis having traditionally been placed on the discovery of ancestor–descendent relationships while less attention has been devoted to assess the sister group relationships among taxa.

African rodents occupy diverse habitats including forests (e.g. genus *Praomys*; Lecompte *et al.* 2001), xeric environments (e.g. genus *Gerbillus;* Shenbrot & Krasnow 2001) or mesic habitats (e.g. genera *Dasymys* and *Colomys*; Musser & Carleton 2005). However, the majority of taxa tolerate diverse habitats such as the genera *Arvicanthis* (Ducroz *et al.* 1998) and *Aethomys* (Chimimba 2000b).

The *Praomys* complex is one of the most abundant and successful groups of Old World rodents with 50 species belonging to the genera *Colomys* (1 species), *Heimyscus* (1 species), *Hylomyscus* (13 species), *Mastomys* (8 species), *Myomys* (4 species), *Praomys* (17 species), *Stenocephalamys* (4 species) and *Zelotomys* (2 species) (Lecompte *et al*. 2005; Musser & Carleton 2005; Van der Straeten 2007). It represents a group of African murine rodents which is taxonomically diverse and often abundant at the population level (Lecompte *et al*. 2002a). According to Rosevear (1969), Lecompte *et al.* (2002a, b, 2005), Nicolas *et al*. (2005) and Carleton *et al*. (2006), the systematics of the group has long been unstable due to the low level of morphological differentiation among species.

Accounts by Rosevear (1969) and Carleton *et al*. (2006) state that the genus *Praomys* has been known since 1860 when Gray described one of Richard Burton's collections from Cameroun as *Mus maurus* (now *morio* Troussart). The next species named in 1892, collected by Burton, was assigned to *Mus* by Thomas as *M. burtoni*, which was later changed to *M. tullbergi* Thomas. The same generic assignation was applied to the species described by de Winton in 1897 as *Mus jacksoni*. They became Troussart's *Epimys* and remained so until Thomas (1915) assigned them to the new subgenus *Praomys*, diagnosed first by the mammary formula 1–2 = 6, then in 1925 he added a range of other characters both external and cranial. Thomas (1926) created the genus *Hylomyscus* to separate certain small African murines that had been associated with *Praomys*; specifically, species of *Hylomyscus* exhibited more arboreal adaptations. In a subsequent classification, Ellerman (1941) placed *Hylomyscus, Mastomys, Myomys* and *Praomys*

as subgenera of a broadly defined *Rattus*. Misonne (1969a) argued for an *in situ* African origin for *Praomys* and kin and their distant phylogenetic relationship to *Rattus sensu stricto*, although he maintained them in the generic groupings within his *Rattus* division.

More recently, Heim de Balsac & Aellen (1965), Brosset *et al*. (1965), Rosevear (1969) and Musser & Carleton (1993) considered them as full genera while Misonne (1969) considered *Hylomyscus*, *Mastomys* and *Myomyscus* as subgenera of *Praomys* in his *Rattus* division. Taxonomically informative morphological studies by authorities (Rosevear 1969; Robbins *et al*. 1980; Musser & Carleton 1993) and molecular studies (Watts & Baverstock 1995; Lecompte *et al*. 2002a, b; Jansa & Weksler 2004) supported the exclusion of *Hylomyscus*, *Mastomys*, *Myomys* and *Praomys* from *Rattus*. Misonne (1969) pointed out that *Hylomyscus* is the most derived taxon to the genus *Praomys*. Likewise, *Malacomys* shows morphological affinities with *Praomys* (Dieterlen & Van der Straeten 1984; Van der Straeten & Dudu 1990). Indeed, individuals from the genus *Praomys* have been identified as belonging to *Malacomys* in museum collections. However, there is moderate support for uniting the genus *Malacomys* near the base of the rapid radiation of the core murine genera (Steppan *et al*. 2005). Nevertheless, the relationships between taxa within the *Praomys* group and species biogeographic limits are still not well understood (Lecompte *et al*. 2002 a, b; Chevret *et al*. 2003; Michaux *et al*. 2007). This is mainly due to the low level of morphological differentiation among the species and between genera (Lecompte *et al*. 2002b) as well as poor sampling within the range of occurrence of the species.

1.3.2 Genus Praomys

The genus *Praomys* is diverse; individuals are abundant and distributed in a wide range of habitats. *Praomys* species (soft-furred mice) are rats characterized by soft fur without long bristles, the tail is thin and finely haired, much longer than the head and body, ears dark; upper parts smoky brown and under parts grey, naked and very long; the zygomatic plate projected forward (Thomas 1915, 1926). The *Praomys* species are morphologically similar, making their separation using traditional taxonomic techniques difficult (Nicolas *et al*. 2005). As such, their diversity may be underestimated due to the probable existence of sibling species as suggested by Meester (1988), Taylor (2000) and Musser & Carleton (2005) or incomplete sampling in some parts of their range. Species identification problems are especially pronounced when dealing with juveniles. The genus currently comprises 17 species (Table 1) of which four (*P. degraaffi*, *P. jacksoni*, *P. misonnei* and *P. verschureni*) occur in the Albertine Rift.

The focus of the present study is on two species, *P. jacksoni* and *P. degraaffi*. *Praomys jacksoni* is abundant and widespread in Central and East Africa (Dieterlen 1983; Van der Straeten & Dudu 1990) ranging from Nigeria to Kenya, as far south as Angola and Zambia. It was described from Entebbe, Uganda (Thomas 1915) in the Albertine Rift. To date, *P. jacksoni* remains a systematic concern because the type specimen is a juvenile and most research suggested that *P. jacksoni* consists of different subspecies or species composites (Van der Straeten & Dudu 1990; Nicolas *et al*. 2005; Lecompte *et al*. 2002b). All the Albertine Rift *Praomys* species were described from *P. jacksoni*. *Praomys degraaffi* was described in the Albertine Rift where it ranges in mountains above 1,400 m (Van der Straeten & Kerbis Peterhans 1999). It is endemic to the region, but populations are stable despite habitat loss. It was described from three sites namely, Bwindi Impenetrable National Park and Mgahinga Gorilla National Park in Uganda and Kibira National Park in Burundi. A subsequent study (Kaleme *et al.* 2007) has since confirmed its presence in Kahuzi-Biega National Park in the DRC. However, its northern and southern limits of occurrence are to still be established.

1.3.3 Life history and ecological traits

The biogeographical analyses suggest that *Praomys* occurs in tropical rain forest ranging from closed (primary forest) to semi-closed (secondary forest, fringes, gallery forest and fallow), woodlands, moist savanna–forest mosaics and montane habitats which harbor abundant and available food resources throughout the year (Rosevear 1969; Dieterlen 1990). The genus ranges from the Gambia River in Gambia and Senegal to Kenya and Tanzania and south to Angola and Malawi (Figure 1.2; Kingdon 1997). The genus appears to be a habitat generalist and shows obvious trends of adaptation to arboreal life (Dieterlen1990). Studies of the ecology of African forest rodents (Dieterlen 1985a,b, 1986, 1990) demonstrated that they are omnivorous, feeding on invertebrates, fruits, seeds and leaves to a varying degree while the main food is made up of plant materials, especially fruits and seeds. Population dynamics are linked to the periodicity of flowering and fruiting; reproduction occurs all year round with seasonal periodicity in relationship with rainfall, peaking when abundant fallen fruit is available. The mean litter size is 2 to 3 young (Dieterlen 1985b, 1986, 1990).

1.3.4 Fossil record

The oldest (known) rodent fossil belongs to the genus *Tribosphenomys* from Paleocene – Eocene deposits in Mongolia, China (Meng *et al.* 1994). From the late Eocene to middle Oligocene the rodent families rapidly diversified, producing over half the extant families (Vaughan 1986). It is possible that in the Eocene the two major groups leading to the suborders Sciurognathi and Hystricognathi split (Carleton 1984). Although there is evidence of a fossil record

for *Praomys* (Musser & Carleton 2005), it has not been easy to trace the history within the *Praomys* complex. The fossil records are mostly represented by *P. skouri* from late Pliocene sediments in Morocco, when the environment may have been similar to the present conditions in east and southern Africa (Geraads 1995, 1998; Musser & Carleton 2005) and by *P. derelbeidae* from the mid-Pleistocene at the time when the environment was open and dry (Geraads 1994; Musser & Carleton 2005). According to Misonne (1969), it is likely that the Murine radiation formed during the lower Miocene, and at the latest in the Oligocene. Strata in Chad suggest that the *Praomys* radiation has at least been present since the early Pliocene, *c*. 5 Mya (Lecompte 2003).

1.4 Relevance of combined morphological and molecular data

Morphology is an obvious source of evidence for genealogical relationships. However, morphological characters are prone to convergent or parallel evolution, which may cause distinct taxa to look alike because they have adapted to similar environmental demands (Monteiro *et al.* 2003; Straney & Patton 1980). As such, morphological characters alone may be inadequate for defining species boundaries because two species may be so similar that their specific status would remain undetected (Donnellan & Alpin 1989). Likewise, morphologically distinct forms may just represent polymorphisms (ecotypes) within a single interbreeding population (Moritz *et al*. 1989). Similarly, molecular data alone are prone to shortcomings such as the often weak resolution provided by a single gene, which should be supplemented by other genes and morphological data (Lecompte *et al*. 2005). Patton *et al*. (2007) found that the species boundaries are often fuzzy, as a result of the retention of ancestral polymorphism (see also Ward *et al*. 2002), lack of complete lineage sorting or reticulation due to hybridization subsequent to initial divergence of the respective lineages (Bulgin *et al*. 2003). As such, a combined approach which incorporates information from different spheres provides an overall (and optimal) assessment of genealogical relationships.

1.4.1 Morphometrics

During the early twentieth century, biologists began the transition from a descriptive field to a quantitative science, and the analysis of morphology saw a similar quantitative revolution (Bookstein 1997a).

1.4.1.1 Linear measurements

The linear measurements, also known as traditional morphometrics, were used where counts, ratios, and angles were included. Covariation in the morphological measurements was quantified and patterns of variation within and

among samples could be assessed (Adams *et al.* 2004). Modern morphometrics involves the application of multivariate statistical analyses to sets of quantitative variables such as length, width, and height (Adams *et al.* 2004). This field applies univariate and multivariate statistics on the linear measurements of specimens corresponding to the distance between two identifiable points on the surface of objects, in this case animal skulls (Rohlf & Marcus 1993; Marcus & Corti 1996). Approaches for character selection include ANOVA (analysis of variance) and correlation between characters (Pimentel & Smith 1986; Chimimba 1997). The former is applied to multi-group studies where character redundancy is ignored (Pimentel & Smith 1986), whereas the second summarizes correlations among characters by principal component analysis (PCA; Thomas 1968) and cluster analysis (Taylor *et al*. 1993). The development of statistical methods such as ANOVA (Fisher 1935) and PCA further advanced quantitative rigor (Rohlf & Marcus 1993; Zelditch *et al*. 2004), which requires accuracy (the closeness of a measurement or estimate to its true value) and precision (the closeness of repeated character or measurements to each other) in data recording. Accuracy depends on the level of precision relative to the total variability among individuals in a group (Bailey & Byrnes 1990).

1.4.1.2 Geometric morphometrics

Geometric morphometrics involves the comparison of the geometry (shape) of objects (the skull or the mandibles) where landmark coordinates (X, Y and/or Z) are used in the description and analysis of shape variation (Bookstein 1991; Rohlf & Marcus 1993; Corti *et al*. 2000). It provides a robust methodology to analyze evolutionary relationships between taxa, because it incorporates both size and shape components (Bookstein *et al*. 1985). The landmarks carry information specific to the geometric location of various points of the image that is positioned in the digitising plane, referred to as figure space. Because linear measurements are usually highly correlated with size (Bookstein *et al*. 1985), methods for size correction are incorporated so that size-free shape variables could be extracted, and patterns of shape variation elucidated (Sundberg 1989; Jungers *et al*. 1995; Adams *et al*. 2004).

The vertebrate skull is made up of bony segments held together by sutures; it is the rigidity of the structures as a whole that facilitates landmark determination for replicable measurements. Scale, position and orientation effects (non shape variation) are removed from the data through the superimposition method which consists of overlaying specimens according to some optimization criteria; that is why allometry (changes of shape with an increase or decrease in size) is investigated (Jolicoeur 1963). The generalized procrustes analysis (GPA, also called generalized least squares, GLS) that was used superimposes landmark configurations using the least–squares estimates for translation and rotation parameters (Bookstein *et al*. 1985; Zelditch *et al*. 2004). Differences in shape

can be described by differences in deformation grids depicted in the object and the parameters can be used as shape variables to assess variations between populations (Adams *et al*. 2004).

Principal component analyses and relative warp analyses are explorative techniques that enable identification of discrete groupings of individuals, for example according to age and sex (Chimimba & Dippenaar 1995; Chimimba 1997; Gaubert *et al*. 2005) as well as to geographic variations (Burnett 1983a; Corti *et al*. 1996; López-González & Presley 2001; Gaubert *et al.* 2005). In the case of PCA, the group assignment is not known *a priori*. The canonical variate analysis (CVA) is an inferential analysis, relying on the *a priori* grouping of individuals. It employs a Wilks' lambda and Pillai trace as indices of the multivariate analysis of variance (MANOVA) to test for significance of morphological difference between OTU means. Canonical variate analysis is derived from eigenvectors computed through the comparison of the average within – group variance – covariance matrix to the between – group variance – covariance matrix (Rohlf 1996; Slice *et al*. 1996).

1.4.2 DNA sequence data

Genetic variation can be measured using chloroplast DNA in plants, mitochondrial DNA (mtDNA) in animals and nuclear DNA in both groups. Evolutionary processes (e.g. gene flow) can be distinguished from historical events, such as vicariance and dispersal, by an analysis of the relative ages and historical relationships of alleles in a geographic context (Hare 2001). These processes leave their imprints in the distribution of intra- and inter-population variation (Tajima 1983; Slatkin & Maddison 1989; Hewitt 2000) that can be traced back in time using coalescent and/or phylogenetic analyses. The coalescent theory is a retrospective model of population genetics that attempts to trace all alleles of a gene shared by all members of a population to a single ancestral copy, known as the most recent common ancestor (MRCA) or coancestor (Arenas & Posada 2007). Coalescent Bayesian analyses run models of genetic drift backwards in time to investigate the genealogy of antecedents (Arenas & Posada 2007). In the simplest case, it assumes no recombination, no natural selection, no gene flow or population structure. The probability that two lineages coalesce in the immediately preceding generation is the probability that they share a parent.

Phylogeny is a hierarchical stream of gene transmission or the historical relationships among lineages or organisms (Hillis *et al*. 1996) which provides a branching diagram showing ancestral relationships among populations, species or other taxonomic groupings (Ridley 2004). An understanding of the microevolutionary forces affecting species

throughout their history depends on quantification of how gene flow interacts with genetic drift, mutation and natural selection in forming spatial or temporal population structure (Bohanak 1999). Specifically, Bayesian coalescent methods challenge the traditional practice of phylogeography in that they separate the effects of historical vicariance, isolation and ancestral polymorphism (incomplete lineage sorting) from migration (processes of gene flow) through the simultaneous estimation of population parameters such as migration rate, time of population divergence and time to most common recent ancestor (Nielsen & Wakely 2001).

Phylogenetic analysis can answer a variety of questions about the taxonomic relationships of species: e.g. the evolution of gene families, the evaluation of evolutionary rates in different lineages, the dating of past historical events, the study of the coevolution of host – parasite relationships or by clarifying the sources of epidemic diseases (Zhang & Nei 1996; Pamilo & O'Neill 1997; Klicka & Zink 1997).

Phylogeography denotes the overlay of haplotypes and phylogeny on geographical locations (Avise 2004). Population differentiation at the geographical scale may be caused by factors including mating system, social structure, dispersal and habitat fragmentation that may result in limited gene flow, genetic recombination, natural selection, and random drift (Kvist 2000). It allows historical scenarios which caused the present-day spatial arrangements of organisms to be assessed including the processes that formed these patterns such as vicariance, dispersal, population expansions, bottlenecks and/or migration (Hare 2001; Knowles & Maddison 2002). The neutral genetic markers (microsatellites, animal mtDNA [but see Brown *et al*. 1979, 1982; Shoemaker *et al*. 2000, 2002; Keller *et al*. 2004; Dyer & Jaenike 2004] and chloroplast DNA) can be used to study population structure stemming from habitat fragmentation or genetic differentiation.

1.4.2.1 Mitochondrial DNA

Mitochondrial DNA is a small circular molecule which is maternally inherited (Nei 1987). It evolves faster than nuclear DNA (Brown *et al*. 1982; Avise *et.al.* 1988). With few exceptions, genetic rearrangements are relatively stable within major taxonomic groups but vary between groups (Avise *et al*. 1987; Palumbi 1996). Mitochondrial sequence data are frequently used to reconstruct recent evolutionary events although some of the slower evolving genes may be useful in resolving deeper nodes (Palumbi 1996). Different regions of the mtDNA such as cytochrome b, the cytochrome oxidase subunits (I, II) or the control region evolve at different rates (Saccone *et al.* 1991) allowing appropriate regions to be chosen for specific studies (Kvist 2000). Although sequence evolution of animal mtDNA is

typically rapid, certain regions are highly conserved (Dowling *et al*. 1996). The extraordinary rate of substitution in mammalian mtDNA is thought to result from a rapid rate of evolution (i.e. high rate of mutation accumulation) during mtDNA replication (Hartl & Clark 1989). The popularity of mtDNA for studies at the population level is due to a combination of its maternal inheritance, its clonal inheritance, its relatively rapid rate of base substitution and the ease with which it is isolated and analyzed (Dowling *et al.* 1996).

A review by Zink & Barrowclough (2008) reveals the existence of relatively few cases in which nuclear markers contradict mitochondrial markers in a pattern not consistent with coalescent theory. Furthermore, it suggests that geographically structured mtDNA trees may be suggestive of long-term population isolation and therefore offers new perspectives for species delimitation.

Two mtDNA fragments namely the control region and the cytochrome b genes were used in this study.

- *Control region* (*sometimes called the displacement loop "D-loop*") is the region of the mitochondrial genome that controls replication and transcription. Some taxa other than mammals have the control region organized differently, often without an obvious D-loop (Palumbi 1996). It is extensively used to understand the population biology of mammals through DNA sequencing because it contains many polymorphic sites (Martin & Palumbi 1993a; Palumbi 1996).
- *Cytochrome b* is a protein coding gene in the electron transport chain. It has a wide variety of conserved and variable (at the 3' end of the sense strand) domains that are associated with the function of this gene in the mitochondrial membrane (Martin & Palumbi 1993a; Palumbi 1996). It is widely used in vertebrate studies and is often a marker of choice for studying phylogeny (also the phylogeography) of African rodents (Lecompte *et al*. 2002a, b, 2005; Nicolas *et al*. 2005, 2006, 2008; Dobigny *et al.* 2008).

1.4.2.2 Nuclear markers

- *The 7th intron of the Beta-fibrinogen gene* is often used in studies at the population (intraspecific) level because it is a relatively fast evolving intron (Palambi 1996). PCR amplification of this segment is relatively straight forward as the primer binding sites are situated in the flanking coding regions and therefore conserved across a diverse array of taxa. The 7th intron of the Beta-fibrinogen is characterized by a low transition–transversion ratio and lower homoplasy in introns than mtDNA (Prychitko & Moore 2000, 2003). It evolves more slowly than mtDNA, and has been used to assess the phylogenetic signal of bird species (Prychitko & Moore 1997, 2000) and to define the

geographical hybrid zone between two divergent groups of lizards (Godinho *et al*. 2006). In mammals, it has been used to supplement morphometric and the cytochrome b sequence data to help resolve rodent phylogenies in the Nearctic (Patton *et al*. 2007).

- *Microsatellites* are loci where short sequences of DNA are repeated at tandem. The lengths of the sequences are typically di-, tri, or tetra-nucleotides (Selkoe & Toonen 2006). They are useful because the number of times the sequence (e.g. CA) is repeated at the same location in the genomic DNA often varies between individuals, within populations, and/or between species (Li *et al*. 2002). The high microsatellite mutation rates, coupled with the likelihood of physical and selective constraints on the allelic size imply that mutations to some allelic states occur independently in different populations (Goldstein & Schlötterer 1999; Mank & Avise 2003). Microsatellites have become the marker of choice in many studies because of their high level of variability, ease and reliability of scoring, co-dominant inheritance and short lengths (Luikart & England 1999). Microsatellite markers have been extensively and successfully used on rodents in the neotropics (Patton *et al*. 2007), as well as in tropical Africa (see for example Burland *et al. 2002*; Galan *et al*. 2004; Brouat *et al*. 2007; Loiseau *et al*. 2007; Meyer *et al*. 2009) where they have been demonstrated to be effective at resolving recently evolved clades and the shallower nodes. Their major use in phylogeography is for detecting hybridization.

1.5 Motivation for this study

Gaining an understanding of the nature of metapopulations in these montane ecosystems is fundamental to achieving sustainable management goals for the protection of critical habitats and the vulnerable fauna and flora of the Albertine Rift. Because much of the biological diversity may be of a cryptic nature (detectable only by methods such as molecular analyses) there is a risk that genetically distinct forms will be lost due to the lack of taxonomic recognition (Avise *et al*. 1989, 2004; Kahindo *et al*. 2007). Despite featuring exceptional levels of endemism and the threat of habitat loss, the Albertine Rift has typically been overlooked in identifying regions of high biodiversity (e.g. “biodiversity hotspots”; see e.g. Myers *et al*. 2000) because it has not been sufficiently documented. Recently some progress has been made towards documenting biodiversity for several taxonomic groups (see e.g. Kerbis Peterhans *et al*. 1998; Bober *et al*. 2001; Kasangaki *et al*. 2003; Plumptre *et al*. 2003, 2007a; Kaleme *et al*. 2007; Thorn & Kerbis Peterhans 2009). These efforts have drawn attention not only to biodiversity features, but have also highlighted conservation concerns and areas of possible cryptic diversity (Thorn & Kerbis Peterhans 2009; Kerbis Peterhans & Hutterer 2009). Notwithstanding this progress, qualitative and quantitative data on species distributions and abundance are lacking, and vast areas still remain to be sampled.

Genetic diversity is an under-appreciated aspect of biodiversity, yet can provide valuable insights about the health and distinctness of populations that could go undetected with traditional monitoring methods (e.g. Kahindo *et al*. 2007). For a loosely connected and naturally fragmented region such as the Albertine Rift, these studies can provide valuable information on evolutionary history, natural movement of individuals and be of great use in setting conservation priorities.

Studies investigating the genetic diversity and respective genetic patterns for species in the Albertine Rift are scarce, and focused almost exclusively on birds. They aimed to broadly assess areas with higher diversity for setting conservation priorities (Fjeldså 1991, 1994, Danielsen 1997), while others dealt with the genetic diversity and systematic status of populations in tropical African mountains (Fjeldsa *et al*. 1997; Bowie *et al*. 2004a, b, 2006; Kahindo 2005; Fjeldså & Bowie 2008). However, a rich body of works exists on fish communities (see Verheyen *et al.* 2003; Koblmüller *et al.* 2006; Anseeuw *et al.* 2008, 2011; Nevado *et al.* 2009, 2011). For plants, Kadu *et al*. (2010) assessed the phylogeography of *Prunus africana* in Africa, and this study included Albertine Rift populations. Investigation of phylogenetic structure in mammals include studies by Jensen-Seaman & Kidd (2001) and Matsubara *et al*. (2005) on gorillas, Taylor *et al*. (2004, 2009) on *Otomys*, Huhndorf *et al*. (2007) on three endemic rodents (*Hybomys lunaris, Hylomyscus denniae* and *Lophuromys woosnami*) and Huhndorf (2007) on the genus *Lophuromys*.

It is noteworthy that a zone with more predictable ecoclimatic conditions crosses the Albertine Rift from the Ituri lowlands north of the Ruwenzori Mountains into the lowlands of western Uganda, south to *c*. 4 - 5° S along the southern edge of Itombwe Forest (Fjeldså *et al*. 1997). The Albertine Rift is one of the most speciose, endemic–rich and highly threatened montane ecosystems in Africa and was identified as one of the top five geographic regions of conservation priority in sub–Saharan Africa (Brooks *et al*. 2001). The disjunct distribution of the forests has shown how volcanic activity and increased aridity during periods of aridification at higher latitudes have shaped the diversity of tropical forest-dependent organisms. Different studies have suggested complex patterns of genetic differentiation in the study taxa, including the discovery of a number of previously unknown highly genetically distinct populations (e.g. birds: Roy *et al*. 1998, 2001; Bowie *et al*. 2004a, b, 2006; rodents: Huhndorf *et al.* 2007). The alteration of the vegetational composition due to volcanism may have divided continuously distributed populations or created barriers to dispersal for forest species significantly affecting diversification in this biodiversity hotspot.

Morphology is an obvious source of evidence of genealogical relationships because related organisms can be similar in structure. However, morphological characters are also prone to convergent or parallel evolution, which may cause distinct taxa to look alike because they are adapted to similar environmental demands. Elsewhere phylogenetic/phylogeographic studies based on mtDNA and/ or nuclear DNA have been used in a wide variety of organisms, providing valuable information that can be compared and contrasted with other data sets on morphology and behavior. In this study, a combination of techniques (morphometrics, two mtDNA and two nuclear DNA genes) was adopted in order to increase the resolution of the analyses. Indeed, whereas widespread species comprise geographically differentiated populations, they may be more likely to give rise to new, rare species by isolation or local adaptation.

According to Sites & Marshall (2004), species are routinely used as the fundamental units of analysis in biogeography, ecology, macro-evolution and conservation biology. Literature about species concepts is abundant but the issue of empirically testing species boundaries has received little attention relative to the debate over what a species is. Different operational criteria may fail to delimit species boundaries properly or, more likely, give conflicting results (Patton *et al*. 2007). That is why the final decision on taxonomic boundaries recognized for any taxonomic group relies on the judgement of the investigators rather than on any definable criteria (Site & Marshall 2003). Herein, a species is considered as an entity in nature that has a unique evolutionary trajectory and that is diagnosable by defendable means including morphological, molecular or chromosomal characters (Patton *et al*. 2007). Reproductive isolation required by many is difficult to test, especially when it concerns individuals in nature. Some works on fish have shown that valid species can indeed exchange genes (see e.g. Nevado *et al*. 2011). Practically, we use the concordance of tree topologies including the two mtDNA fragments (the control region and cytochrome b) and the nuclear 7th intron of the β fibrinogen geen in association with the analyses of morphological traits (traditional and geometric morphometrics) to define the lineages within the Albertine Rift *Praomys*. Microsatellites were used to assess introgression/hybridization between populations/species.

1.6 Organization of the dissertation

Most of the information contained in this dissertation is prepared in the form of scientific publications. This has, to some extent, impacted on the format and organization of the work. The layout of the thesis is as follows:

Chapter 1: General introduction and background information.

Chapter 2: Prince K Kaleme, Emanuela Solano, Rauri C.K. Bowie, John M. Bates, Julian C. Kerbis Peterhans & Bettine Jansen van Vuuren (*in preparation*) Phylogeny and taxonomic assessment of *Praomys* in the Albertine Rift, east – central Africa: evidence for the role of paleoclimate and geology in the intraspecific differentiation.

Chapter 3: Prince K. Kaleme, Celine Born, Rauri C.K. Bowie, John M. Bates & Bettine Jansen van Vuuren (*in preparation*) Unraveling some of the complexity in the *Praomys jacksoni* species complex across the Albertine Rift.

Chapter 4: Kaleme, P.K., Bates, J.M., Belesi, H.K., Bowie, R.C.K., Gambalemoke, M., Kerbis-Peterhans, J., Michaux, J., Mwanga, J.M., Ndara, B.R.,Taylor, P.J., Jansen van Vuuren, B. (2011) Origin and putative colonization routes for invasive rodent taxa in the Democratic Republic of Congo. *African Zoology* 46(1): 133 – 145.

Chapter 5: General conclusion

References

Appendix

Table 1. Taxonomic classification of *Praomys* species with the habitat association and distribution. The species with indication (*) occur in the Albertine Rift.

Species	*Original descriptors*	*Habitat association*	*Distribution*
Praomys coetzeei	Van der Straeten 2007	Primary wood, secondary wood, gallery wood and tropical wood stripe along rivers	Northwest (and possibly northeast) Angola,
Praomys daltoni	Thomas 1902	Dry savanna, rocky and urban areas	Senegal to west Sudan, south to Central African Republic
Praomys degraaffi (*)	Van der Straeten & Kerbis Peterhans 1999	Subtropical and tropical moist mountain forest	Burundi, eastern DRC, Rwanda and Uganda (Albertine Rift)
Praomys delectorum	Thomas 1920	Subtropical or tropical moist montane forest	Northeast Zambia, Malawi to southwest Kenya
Praomys derooi	Van der Straeten & Verheyen 1978	Dry savannas and urban areas	Ghana to west Nigeria
Praomys hartwigi	Eisentraut 1968	subtropical or tropical moist montanes	West Cameroon
Praomys jacksoni (*)	de Winton 1897	Subtropical or tropical moist lowland forest, tropical moist montanes, arable lands and heavily degraded forest	Nigeria to south Sudan – Kenya to northeast Angola and Zambia
Praimys lukolelae	Hatt 1937	Subtropical or tropical moist lowland forest	North and North east DRC
Praomys minor	Hatt 1934	Subtropical and tropical moist lowland forest	Lukolela, centre DRC
Praomys misonnei (*)	Van der Straeten & Dieterlen 1987	Subtropical or tropical moist lowland forest, subtropical or tropical moist montanes, and arable land.	East (Irangi) and north east DRC
Praomys morio	Trouessart 1881	Subtropical or tropical moist montanes	Bioko, Mount Cameroon in Cameroon
Praomys mutoni	Van der Straeten & Dudu 1990	Subtropical or tropical swamps and rivers	Batiabongena in the north of DRC and also found at the foot of

			Mount Kahuzi at Bushema – Lutinguru near Irangi (*this study*)
Praomys obscurus	Hutterer & Dieterlen 1992	Fern-grassland, swamp and gallery forest in subtropical or tropical moist montanes	Mts Oku in Cameroon and Gotel Mts. in Nigeria
Praomys petteri	Van der Straeten, Lecompte & Denys 2003	Subtropical or tropical moist lowland forests.	South Cameroon, Central African Republic and Republic of Congo
Praomys rostratus	Miller 1900	Subtropical or tropical moist lowland forests and subtropical or tropical moist montanes	Liberia to Ivory Coast, limit unresolved
Praomys tullbergi	Thomas 1894	Subtropical or tropical moist lowland forests and subtropical or tropical moist montanes	Gambia to northwest Kenya and northwest Angola
Praomys verschureni (*)	Verheyen & Van der Straeten 1977	Subtropical or tropical moist lowland forest	North and north-east DRC
Praomys kikwit	Kennis *et al*. (in press)		South west DRC.

Source: Thomas (1915, 1926), Rosevear (1969), Dieterlen & van der Straeten (1984), van der Straeten & Dieterlen (1987), van der Straeten & Dudu (1990), Musser & Carleton (1993, 2005), Kerbis-Peterhans *et al*. (1998), van der Straeten & Kerbis-Peterhans (1999), Carleton *et al*. (2006), Van der Straeten (2007), Nicolas *et al*. (2008) and Kennis *et al.* (2011).

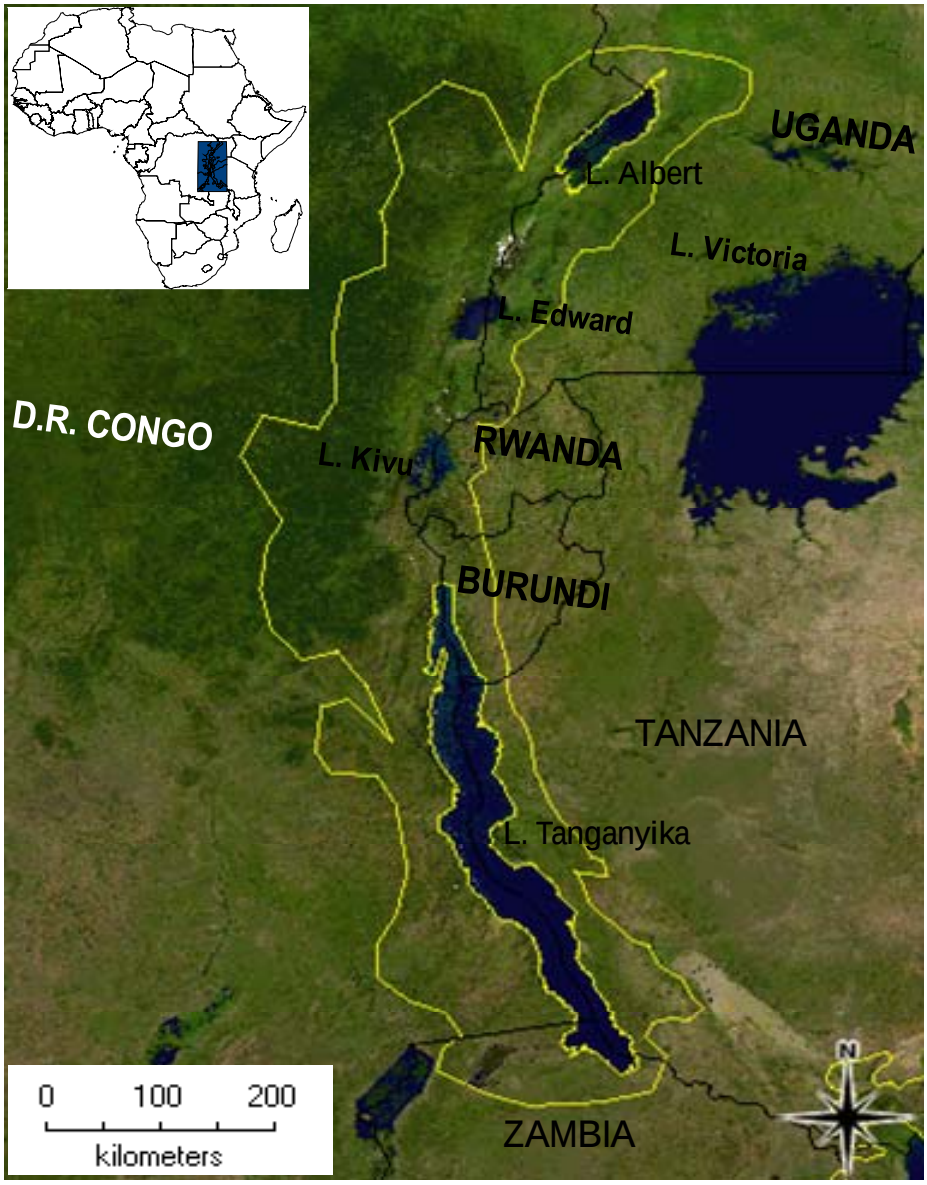

Figure 1.1. Map of the Albertine Rift. Dark green areas indicate the tropical rainforest. Yellow line indicates the limit of the Albertine Rift. Black lines are the country boundaries (adapted from Plumptre *et al*. 2003)

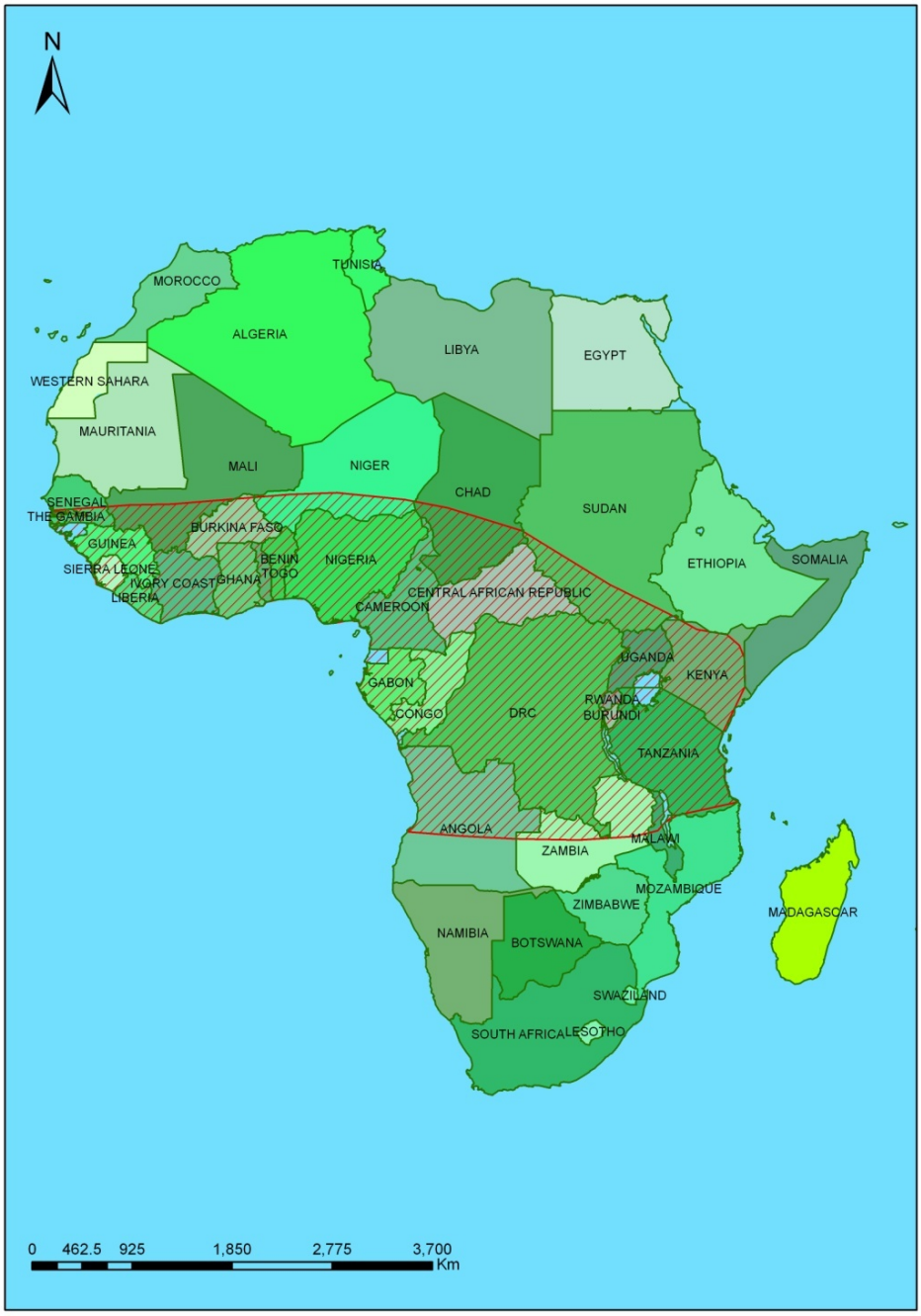

Figure 1.2. Map of Africa depicting the distribution of the genus *Praomys* (striped area enclosed by the red lines).

Chapter 2

Phylogeny and taxonomic assessment of *Praomys* in the albertine rift, east – central Africa: evidence for the role of paleoclimate and geology in the intraspecific differentiation

2.1. Introduction

The Albertine Rift forms the western boundary of the large East African Rift System, which moving clockwise also includes the Kenyan Highlands, Eastern Arc Mountains in Tanzania and south-eastern Kenya, the mountains of the Malawi Rift, and the uplifted escarpments of the Ufipa Plateau (Rosendahl 1987). The Albertine Rift, which extends from *c*. 30 km north of Lake Albert to the southern tip of Lake Tanganyika (Plumptre *et al.* 2007a), encompasses a diverse array of habitats and altitudinal zones including ice fields on the Ruwenzori Massif (> 5,000 m a.s.l.), active volcanoes in the Virunga National Park, ericaceous shrublands (above 3,000 m), bamboo forests (mostly above 2,400 m), montane forests (above 1,500 m) and the lowland broad-leaved woodlands (above 600m; see Jolly *et al*. 1997; Plumptre *et al*. 2007a) down to Africa's deepest lake, Lake Tanganyika (Figure 2.1).

The Albertine Rift is characterized by instability as a result of uplift (Livingstone 1967, 1975; Rosendahl 1987), volcanism, which commenced *c*. 11 to 9 Mya and which is still ongoing (Kampunzu *et al*. 1998), and natural climatic oscillations (most notably during the Plio / Pleistocene); the latter would further have added to the heterogeneity of the region by causing altitudinal shifts in the distribution of species (Beadle 1981; Livingstone 1967, 1975; Bálint *et al.* 2011). These factors, when taken in concert, may account at least in part for the rich fauna and flora (Plumptre *et al*. 2007a) including exceptional species endemism (Prigogine 1985; Vande Weghe 1988a, b; Stattersfield *et al*. 1998; Olson & Dinerstein 2002; Plumptre *et al.* 2007a, b; Burgess *et al*. 2004; Brooks *et al*. 2004). The region is unfortunately not without anthropogenic pressures. For example, much of the interlacustrine highlands (in Rwanda) have been transformed to cultivated and grazed land (Jolly *et al*. 1997). Further, there has been an overall marked decrease in the number of large mammals in the savannas of the Greater Virunga Landscape (and all the associated protected areas) as a direct result of civil war that affected different countries at different times, which unfortunately led to rampant poaching of mammals, birds and reptiles, as well as trade in gorilla infants and ivory (Plumptre *et al*. 2007b).

Species inventories, systematic investigations, or studies that document the spatial distribution of organisms provide a valuable means with which to investigate species richness patterns and to test various hypotheses regarding processes that drive species occurrence. A surprisingly limited number of studies have been carried out in the Albertine Rift, however, perhaps because of the political instability that has characterized much of the region. Fieldwork in the region has focussed almost exclusively on charismatic groups such as birds (Prigogine 1960, 1979, 1984, 1985; Vande Weghe 1988a, b) and larger mammals (Schaller 1963; Weber & Vedder 1983; Yamagiwa *et al*. 1993, 1996, 2003; Hall *et al*. 1998; Omari *et al*., 1999) with small mammals receiving only cursory attention (but see Rahm 1966, 1967; Rahm & Christiaensen 1966; Dieterlen 1967, 1976a, b, 1985a, b; Kasangaki *et al*. 2003; Kaleme *et al*. 2007) while very little is known about invertebrates or plants (Plumptre *et al*. 2007a). Very few studies have

included aspects of genetic diversity (but see Fjeldså & Lovett 1997; Fjeldså *et al*. 1997; Saltonstall *et al*. 1998; Jensen-Seaman & Kidd 2001; Bowie *et al*. 2004a, b, 2006; Matsubara *et al*. 2005; Kahindo 2005; Fjeldså & Bowie 2008) with small mammals being largely overlooked despite their obvious species richness (but see Taylor *et al*. 2004b, 2009; Huhndorf *et al*. 2007). However, aquatic ecosystems are well studied (see e.g. Verheyen *et al.* 2003; Koblmüller *et al.* 2006; Anseeuw *et al.* 2008, 2011; Nevado *et al.* 2009, 2011)

One of the most abundant African small mammal groups is the *Praomys* complex that conservatively comprises four genera (*Hylomyscus*, *Mastomys*, *Myomys* and *Praomys*). Until recently, the taxonomy of the *Praomys* complex was based on morphology alone (see Thomas 1915, 1926; Verheyen & Brackes 1966; Misonne 1969a; Rosevear 1969; Van der Straeten & Verheyen 1981; Van der Straeten & Dieterlen 1987, 1992; Van der Straeten & Dudu 1990; Weltz 1998; Van der Straeten & Kerbis Peterhans 1999; Van der Straeten *et al*. 2003; Van der Straeten 2007). As considerable morphological similarities exist among taxa (Misonne 1969a; Rosevear 1969; Musser & Carleton 2005), the taxonomic status of several species has remained uncertain. Following more robust methods of analyses and the inclusion of genetic data (Watts & Baverstock 1995; Lecompte *et al*. 2002a, b, 2005; Jansa & Weksler 2004; Nicolas *et al*. 2005) it is now generally accepted that *Praomys*, *Mastomys*, *Myomys* and *Hylomyscus* represent distinct genera. Although the majority of these genera are considered monophyletic, *Praomys sensu stricto* appears paraphyletic with two groups recognized namely the *P. tullbergi*- and the *P. jacksoni*-groups. These two groups appear geographically and ecologically separated with the *P. tullbergi*-group occurring in West and Central Africa mainly in primary forest, and the *P. jacksoni*-group having a more Central / East African distribution in secondary forests and along forest fringes (Lecompte *et al*. 2002a).

The majority of studies to date have focussed on the taxonomic uncertainties in the group, and not until more recently has attention been paid to phylogeographic patterns within species. Although some of these species have restricted ranges such as *P. degraaffi* (Van der Straeten & Kerbis Peterhans 1999; Kaleme *et al.* 2007) others such as *P. jacksoni* and *P. tullbergi* appear widespread, and for these taxa, several distinct genetic clades have invariably been found (Lecompte *et al*. 2002a, b, 2005; Nicolas *et al*. 2005). The formation of these clades is most often attributed to Plio-Pleistocene climatic fluctuations confining habitat specialist taxa to forest refugia (Endler 1982; Prigogine 1988; Daimond & Hamilton 1980; Roy 1997; Moritz *et al.* 2000), with major rivers having acted as putative barriers to gene flow (Caperella 1991; Gascon *et al.* 1996; Hackett 1996; Moritz *et al.* 2000; Quérouil *et al.* 2003) and less frequently to ecological gradients or local adaptation resulting from isolation-by-distance (Endler 1977; Patton & Smith 1992; Patton & da Silva 1998; Fjeldsa 1995). No study to date has included material from across the Albertine Rift (i.e. broad geographic coverage). Consequently, whether populations among the isolated montane blocks within this biodiversity hotspot are interconnected remains largely unknown.

In this paper we provide, to our knowledge, the first phylogenetic comparison that includes all members of the *P. jacksoni*-group occurring in the (entire) Albertine Rift. We further focus on the two numerically more abundant taxa namely *P. jacksoni* and *P. degraaffi*, and document the distribution of genetic variation across their range in the Albertine Rift. The null hypothesis was that there is an association between the genetic structure and the geological features in the Rift and / or the Plio-Pleistocene refugia, and this may have driven, either singly or in concert, the formation of regional clades in these forest specialist mice. To test this hypothesis, we infer the evolutionary relationships among taxa using both molecular (mitochondrial and nuclear genes) and morphological differences (employing traditional and geometric morphometric methods) and date the origin of putative clades using a Bayesian approach. *Praomys degraaffi* and *P. jacksoni* are both distributed throughout the Rift, but are separated altitudinally with *P. degraaffi* found at higher elevations, typically 1,500m and above, in a variety of habitats from swamps and primary forest to less disturbed secondary forest (Kaleme *et al*. 2007). *Praomys jacksoni* is common (Rosevear 1969; Van der Straeten & Dudu 1990), has no habitat restriction and ranges from lowland to montane forests extending to *c*. 2,450m.

2.2 Material and methods

2.2.1. Sampling and DNA processing

A total of 179 *Praomys* specimens were collected from twelve mountain and four lowland areas (Table 2.1; Figure 2.2) distributed throughout the Albertine Rift. These specimens were collected during surveys conducted in 1990–91 and again in 1996–97 in Uganda and Burundi, and from 2001–08 in the DRC (Table 2.1). Voucher specimens are deposited at both the Field Museum of Natural History in Chicago (FMNH) and at the Centre de Recherche en Sciences Naturelles (CRSN) at Lwiro, DRC (see Appendix 2.1).

Total genomic DNA was extracted from fresh tissue samples using the DNeasy Blood and Tissue Kit (QIAGEN). Two mitochondrial (cytochrome b and the control region) and one nuclear (the 7th intron of the ß-fibrinogen gene, ß*fib7*) segments were targeted using published primer sequences (control region: Rosel *et al*. 1994; cytochrome b: Pääbo & Wilson 1988; ß*fib7*: Wickliffe *et al*. 2003, adapted from Prychitko & Moore 1997). Amplicons obtained from standard PCR methodologies (initial denaturation at 96°C for 5 min followed by 30 cycles of 96°C for 1 min, primer-specific annealing for 1 min, elongation for 1 min at 72°C and a final extension step at 72°C for 5 mins) were directly cycle-sequenced using BigDye chemistry (Applied Biosystems) and analyzed on an ABI 3137 automated sequencer (Applied Biosystems). Electropherograms were checked using Geneious 5.1 Software (Biomatters Ltd, 2010) and

aligned manually using MacClade 4.06 (Maddison & Maddison 2000). Sequences were submitted to GenBank under accession numbers JN636327 to JN636751.

2.2.2. Phylogenetic analyses

Because individuals were collected as "*Praomys*" in the field with only limited attempts to identify specimens to species level (given the morphological similarity between species), phylogenetic trees were constructed as a first step to assign species status to specimens, and to assess their mitochondrial phylogeny (a barcoding approach). For this, the two mitochondrial DNA fragments were considered separately from the nuclear fragment to identify possible cases of introgression. Identifications were confirmed by comparison of voucher specimens to paratypes or museum series at the FMNH (with Julian Kerbis) or the Royal Museum for Central Afrrica (RMCA) at Tervuren, Belgium (with Wim Wendelen). A combined analysis was also performed to indicate possible hybridization (which is an unfrequent event) by including maternally and bi-parentally inherited genes. For reference purposes, representative sequences of *Praomys* species that co-occur in the region or neighbouring regions were obtained from the website http://projects.biodiversity.be/africanrodentia/ (unless otherwise stated). These are *P. misonnei* (n=3 [AF518362, AF518363 and AF518364]), *P. delectorum* (n=1 [TZ20426] from Nicolas Violaine, MNHN, Paris), *P. verschureni* (n=1 [AF518373]), *P. tullbergi* (n=2 [EU349730 and EU349779] from GenBank), *P. petterri* (n=1 [AF518368]), *P. daltoni* (n=1 [AF518348]), *P. derooi* (n=1 [AF518350]), *P. hartwigi* (n=1 [AF518366]) and *P. mutoni* (n=1 [R27374]). Parsimony trees were constructed in PAUP4.0b10 (Swofford 2000) using a heuristic search with random additions of taxa with 1000 replicates. Nodal support was assessed with 500 bootstrap pseudo-replicates. Bayesian trees were constructed in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2003). Five independent Markov chains were run for $5x10^6$ generations with sampling at intervals of 100 generations. To ensure that the analyses were not trapped on local optima, the runs were repeated and checked for convergence and adequate effective sampling of parameter and tree space (ESS values > 200). The first 10% of all trees were discarded as burn-in (burn-in % was verified using the "sump" parameter). Divergence times among the main groups were estimated using Beast v1.6.1 (Drummond & Rambaut 2007) on the cytochrome b data using the uncorrected log-normal clock. The model of substitution selected by jModeltest (Posada 2008) was HKY + G for cytochrome b, and TIM2 + I + G for the combined data. Calibration points included the *Mus* – *Rattus* split (12 Mya; Jacobs & Downs 1994) and the oldest (5 Mya) known *Praomys* fossil (see Geraads 1994, 1995, 1998, 2001; Lecompte 2003; Musser & Carleton 2005). In addition to *Mus* and *Rattus*, *Malacomys longipes* was included as outgroup. The program Tracer (Rambaut & Drommond 2007) was used to check that the stationary state was reached by graphically monitoring the fluctuating value of the likelihood scores. Genealogical patterns to visualize statistical parsimony networks for each species were also constructed in TCS 1.21 (Clement *et al*. 2004).

2.2.3. Traditional morphometrics

'Adult' intact skulls of 386 *P. degraaffi* and *P. jacksoni* from the Albertine Rift and adjacent regions were measured during visits to the museum collections at the Royal Museum for Central Africa (RMCA) in Tervuren (Belgium), the Muséum de l'Histoire Naturelle (MNHN) in Paris (France), the Field Museum of Natural History (FMNH) in Chicago (USA) and the Museum of Vertebrate Zoology (MVZ), University of California at Berkeley (USA).

As suggested by Chimimba & Dippenaar (1995) and Chimimba (2000a, b), character selection is required in order to reject measurements that may be prone to high error and hence confound results. Such error prone characters usually encompass: (1) characters that had an unclear recording point which would make the placement of the calliper tips difficult and subject to error; or if (2) there was high variability among individuals, and (3) if there was an association with frequently damaged parts of the skull. In this study, the measurements were selected in accordance to the commonality of use in previous studies on rodent taxa and their ability to provide comprehensive characterization of rodents' cranial morphology (Van der Straeten & Dieterlen 1987, 1992; Van der Straeten & Dudu 1990; Van der Straeten & Kerbis Peterhans 1999; Carleton & Stanley 2005; Carleton *et al*. 2006), as well as the amount of information the character carries regarding cranial configuration.

Twenty three (23) cranial variables were measured with a WURTH Sylvac system digital calliper to 0.01mm accuracy *sensu* Taylor & Kumirai (2001) and Taylor *et al*. (2009). The measurements (with respective abbreviations, see Table 2.2) were adapted from Verheyen & Brake (1966), Rosevear (1969), Carleton & Van der Straeten (1997), Van der Straeten & Kerbis Peterhans (1999), Carleton *et al*. (2006) and Patton *et al*. (2007). All the measurements were taken by the same person (Prince Kaleme) to avoid interpersonal errors as recommended by Verheyen & Brake (1966), Van der Straeten & Dieterlen (1992) and also Palmeirim (1998). Data recording was limited to individuals judged adults based on the possession of a fully erupted third molar (Rosevear 1969; Van der Straeten & Kerbis Peterhans 1999; Carleton *et al*. 2006). We used three age classes (AC): AC 1: young adult, AC 2: moderate adult and AC 3: heavy (old) adult, following the pattern of coronal tooth wear (Carleton & Martinez 1991, Carleton *et al*. 2006). To test for the precision of the measurements, a sample of 10 skulls was measured on three consecutive days and the Student t-test was performed in STATISTICA (StatSoft. Inc. 2009) to evaluate the standard error on each measurement. The error (see Table 2.3) was negligible for all the measurements and is hence very unlikely to have affected our results. The species *misonnei* (n=1) and *mutoni* (n=2) were excluded from the morphometric analyses because of the small sample sizes.

The analyses were performed on log-transformed data to correct for size differences in case of unequal contribution to variance among linear variables for multivariate analyses (Marcus 1990; Carleton & Byrne 2006; Taylor *et al*.

2009). To test for significant differences between the species or the sexes, the Students t–test was performed in STATISTICA 9. To test for sexual dimorphism, the Leven's test for equality of variances and the Students t–test were performed in SPSS. One-way ANOVA performed in Past 1.97 (Hammer 2001) was used to test for significant differences in character means of populations, age classes and clades. When there was no *a priori* pattern of interrelationship between individuals, principal component analysis (PCA) was used. In traditional morphometric analysis, the first PC accounts for the greatest variation among samples and is often referred to as a size vector (Blackith & Reyment 1971; Zelditch *et al*. 2004), while the second PC describing the shape is referred to as a shape component (Pimentel 1979). If the eigenvalue of PC 3 would be large, it might combine with PC 2 scores to more clearly define a posteriori groups by "shape" features of the skull. To test for a pattern between individuals when the groups were assigned *a priori*, the canonical variate analysis (CVA) was used. The significance of the differences was estimated through MANOVA's statistical indices, Wilk's Lambda and the Pillai trace; the significance of pairwise comparison among groups was estimated by the Hotelling test (Zelditch *et al.* 2004).

2.2.4. Geometric morphometrics

Three hundred and seventy (370) *Praomys* (*degraaffi* and *jacksoni*) from the Albertine Rift and adjacent regions were photographed during visits to the museum collections at the Royal Museum for Central Africa (RMCA) in Tervuren (Belgium), the Muséum de l'Histoire Naturelle (MNHN) in Paris (France), the Field Museum of Natural History (FMNH) in Chicago (USA), and the Museum of Vertebrate Zoology (MVZ), University of California at Berkeley (USA). Two photographs (dorsal and ventral views) of each specimen were taken using a FUJI (Finepix) S6000fd digital camera mounted on a frame at a fixed distance of 25 cm from the skull; skulls were placed on graph paper. Because the aim was to determine the species status of the individuals, analyses were restricted to 105 skulls for which cytochrome b sequence data were available (Appendix 2.2) as identifications could be verified using DNA clustering (assuming that the DNA sequences can be used as barcodes to identify a species) and confirmed by comparing the specimen with the museum collections. TpsDig version 2.1 (Rolf 2006a) was used to capture the landmarks in two dimensions. Nineteen landmarks were placed in the dorsal view and 22 in the ventral view. The position of landmarks was chosen according to the biological significance of the cranial suture/bone to provide comprehensive characterization of rodents' cranial morphology (Pavlinov 2001; Taylor *et al*. 2004b, 2009). The geometric morphometric analyses were performed using the software MorphoJ version 1.10 (Klingenberg 2011), unless otherwise stated. The specimens were divided into three groups following the clustering of our genetic data namely *P. degraaffi, P. jacksoni* Clade 1 and *P. jacksoni* Clade 2 (see Results). Clade 2 of *P. degraaffi* had only five available skulls, hence the species was analysed as a single group.

As a measure of the size of the skull, centroid size (CS) was used (Bookstein 1991a, Zelditch *et al*. 2004). Specimens were aligned using the Generalized Procrustes Analysis (GPA; Rolf & Slice 1990). To test for sexual dimorphism and size difference among species, One-Way ANOVA of the CS was performed in Past 1.97 (Hammer 2001). To test for allometry, the regression of size on shape was performed using the regression analysis. To test for differences among species and clades, the discriminant function analysis (DFA) was performed in Past. For shape analyses, the CVA was used to test for differences among species, clades or age classes to find the shape features that best distinguish among multiple groups of specimens. These differences were estimated using Wilk's lambda and Pillai trace, as well as via a Hotelling pairwise comparison test performed in Past 1.97. Since no difference was found between the dorsal and the ventral views, we only present the results for the dorsal view.

The shape of each specimen can be described as the deviation from the consensus configuration and is visualized as the deformation of the consensus coordinate grid by using a thin plate spline function (Barčiová 2009). To evaluate the possible occurrence of adaptive components of shape related to environmental variation (Rohlf & Corti 2000; Corti *et al*. 2001), the presence of co-variation between shape and climatic variables (altitude, latitude, longitude, mean annual rainfall and mean annual temperature), Partial Least Squares ([PLS], Rolf & Corti 2000) were calculated using the software tpsPLS (Rohlf 2006b). Climate data were obtained from the "Albertine Rift climate change" website (http://programs.wcs.org/albertineclimate).

2.3. Results

2.3.1. DNA sequence variation and phylogenetic analyses

A total of 1957 base pairs (346 bp of the control region, 987 bp of the cytochrome b gene and 624 bp of the ßfib7 intron) were obtained of which 562 were variable and 228 parsimony informative. The control region contributed less to the number of variable characters (n=112) while the nuclear ß*fib7* contributed more (n=36). Largely congruent topologies were retrieved irrespective of the method of analysis (MP and BI) or whether genes were considered singly or combined. The Bayesian topology from combined (concatenated) analyses is shown in Figure 2.3. Our sequences are attributed to four currently recognized species (see Figure 2.3); these include our two focal species *P. jacksoni* (n=112) and *P. degraaffi* (n=64) as well as *P. misonnei* (n=1) and *P. mutoni* (n=2). The recovery of *P. mutoni* was unexpected as this is the first record for the species in the Albertine Rift. Two clades were uncovered within both *P. jacksoni* and *P. degraaffi*. Sequence divergence (cytochrome b) separating the clades was between 2.1 and 5.67 %. The times of divergence among the main lineages are shown in Figure 2.4. The split between *P. jacksoni* and *P. degraaffi* is estimated at 3.8 Mya (4.29 Mya – 3.31 Mya). The time of separation of the clades within each of these species is very similar, and placed at *c*. 3.45 Mya.

2.3.2. Phylogeography

Considering only the two focal species, 141 haplotypes were identified for the combined data (cytochrome b: 113; control region: 99; ß*fib7*: 39). The largest proportion of these was recovered in *P. jacksoni* (91 haplotypes for 112 specimens) with fewer found in *P. degraaffi* (50 haplotypes for 64 specimens). Very few haplotypes were shared among individuals (*P. jacksoni*: 6 shared haplotypes; *P. degraaffi*: 5) or localities (*P. jacksoni*: 2 shared haplotypes; *P. degraaffi*: 0) with the majority of haplotypes being unique.

The presence of distinct genetic clades within the focal species recovered through phylogenetic analyses and the F-statistics also showed that there is some structure (P. jacksoni: $\Phi_{ST} = 0.40$, $p < 0.001$; P. degraaffi: $\Phi_{ST} = 0.37$, $p < 0.001$). The clustering of P. jacksoni clades has some geographical pattern with a more northern lineage comprising sites 1 – 13, and a more southern lineage (sites 10 – 16) with admixture at sites 10 – 14 (in the vicinity of Lake Kivu) (see Figure 2.5). Although not as pronounced, there was also some degree of geographic patterning for P. degraaffi with a transition zone around the central Albertine Rift (Figure 2.5).

2.3.3. Traditional morphometrics

The Leven's test for equality of variances and the Students t–test for the interaction of "species – sex" were not statistically significant for either of the two focal species ($p > 0.05$), our present data did not allow us to detect any degree of sexual dimorphism. As such, individuals of both sexes were pooled in subsequent analyses. The Students t–test performed on the 386 specimens showed significant differences between the two species for six measurements (Table 2.3): the breath of the nasal (BNAS), the length of the braincase (LBRCA), the breath of the braincase (BBRCA), the diastema length (DIAL), the palatal foramina length (PAFL) and the length of the auditory bulla (BULL). Two measurements, the length (LNAS) and the breath of the nasal (BNAS), were statistically significant in separating the two *P. jacksoni* clades. The PCA graph distinguishes the two species (Figure 2.6A), but the *P. jacksoni* clades overlapped and could not be distinguished (PC1 explains much of the variance with 48.19% [Eigenvalue: 11.07], PC2: 12.42 %, PC3: 5.6%). The CVA of the cranial variables revealed differentiation between the groups as displayed by the indices of significance in MANOVA (Wilk's $\lambda = 0.06024$, $F = 5.07$ and Pillai's = 1.573, $F = 3.673$); the putative species were recovered as well as the two clades of *P. jacksoni* (Figure 2.6B).

2.3.4. Geometric morphometrics

No significant sexual dimorphism for size (F=0.45, p=0.504) or shape (F=1.002, p=0.5) was found for either species (Wilk's λ_{dorsal}=0.27, p=4.96, Wilk's $\lambda_{ventral}$=0.19, p=0.52). The Regression analysis (ANOVA) to test for allometry showed no correlation of the change in size on the variation in shape between the two species ($p < 0.0001$), within species ($p < 0.0001$), or between clades ($p < 0.0001$) for both the dorsal and ventral sides. Consequently, the subsequent analyses were performed pooling together the sexes. The CVA (Figure 2.7A) showed significant differences between *P. degraaffi* and *P. jacksoni* ($p < 0.0001$) for both the dorsal and ventral view. Shape differences were also detected between *P. jacksoni* clades as shown by the deformation grids (Figure 2.7B). The discriminant function analysis to test for differences between species and clades (procrustes distance=0.013, Mahalanobis distance=2.27, p <0.0001) support the CVA indexes (Hotelling test for species=4.96, *P. degraaffi* vs. *P. jacksoni* Cl1=0.050, *P. degraaffi* vs. *P. jacksoni* Cl2=2.15E-05, *P. jacksoni* Cl1 vs. Cl2=0.49).

A combination of factors, namely altitude, latitude, longitude, mean rainfall and mean temperature were used to test for the association of climate variables on shape. These analyses recovered four groups with slight differences in the grouping between dorsal and the ventral views (Figure 2.8). The first group on the dorsal view was composed of individuals from site 1 and site 4. These sites are located at the same latitude (*c.* 1.50° N) and are lowland sites (maximum altitude: *c.* 1,300 m a.s.l.) with similar (mean) rainfall patterns (> 1,700 mm/yr). The second group is composed of individuals from site 3. Site 2 individuals were in the first group in the dorsal view and with site 3 in the ventral view. The third group was formed of individuals from site 15, the site with the lowest mean temperatures (*c.* 20º C) and the highest rainfall (1,150 mm/yr) amongst the mountain block sampled. The fourth group consisted of the remaining sites, and represents mountain forests with rainfall ranging between 970 and 1600 mm and temperature patterns (between 20º and 23.5º C).

2.4. Discussion

Several notable findings emerged from our study. First, the results presented here reveal a consistent pattern between the molecular and morphological data, which is also in line with the biogeography and history of the region. Specifically, *Praomys* species in the Albertine Rift show relatively deep divergences (early Pliocene) while distinct lineages are also present in our focal species (divergences occurred during the mid-Pliocene). There is some indication that these distinct lineages within the focal species are geographically structured, however, with notable overlap in their distributions. Secondly, DNA data revealed the presence of *P. mutoni* which was an unexpected finding as it represents a new species for the region, but failed to recover *P. verschureni*, perhaps due to inadequate

sampling in lowland sites which are the prime habitat for this species. These findings are discussed in more detail below.

2.4.1. Phylogenetic analyses and haplotype variation

DNA data, in combination with other lines of evidence, provide a powerful way to confirm (the presence/ absence of) species diversity and the geographic distribution thereof (Kahindo *et al.* 2007). This is especially true in regions of high yet poorly known biodiversity (such as the Albertine Rift) as well as areas where species distributions change as a result of climate change (again, such as the Albertine Rift). All probabilities point to the fact that cryptic and undescribed diversity far exceeds known diversity (see e.g. Mora *et al*. 2011). Although large areas within the Albertine Rift are poorly sampled as a result of political instability, lack of funding or the difficulty of gaining access to remote areas, many research initiatives and institutions (such as the CRSN Research Station at Lwiro, Wildlife Conservation Society, WWF and the Field Museum of Natural History in Chicago [list not exclusive]) have dedicated significant resources to sample and catalogue the rich diversity in the Albertine Rift. As such, a wealth of material is available in the form of collections for further investigations using modern techniques. The need for these follow-up studies is accentuated by the fact that some species, especially smaller and less well-known ones, are often collected under generic terms rather than as specific taxa.

A case in point concerns *Praomys* where, until recently, all soft-fur mice were collected as *P. jacksoni*. Careful examination of these collections has led to the description of *P. verschureni* (Dieterlen & Van der Straeten 1984), *P. misonnei* (Van der Straeten & Dieterlen 1987) and *P. degraaffi* (Van der Straeten & Kerbis Peterhans 1999). The specimens sequenced here confirm the presence of three of the four *Praomys* species in the Albertine Rift; *P. verschureni* was not found as its preferred habitat was not included. Surprisingly, two of the "*Praomys*" specimens sequenced were *P. mutoni*. This species is not typically reported from the Albertine Rift, and its retrieval from site 9 (Bushema forest, northwest of Mount Kahuzi) may therefore represent a range expansion for this taxon. However, as only 2 specimens belonging to *P. mutoni* were recorded, it is unsure whether these animals truly represent a range expansion or perhaps, because of poor sampling in the area, it could have only been recorded now.

Patton *et al*. (2007) and Mora *et al.* (2011) found that cryptic lineages may be expected in species that occur over large geographic distances and / or very heterogeneous habitat. Perhaps not surprisingly therefore, two divergent evolutionary lineages, separated by 4.65 % sequence divergence (for the cytochrome b) were uncovered in *P. jacksoni* (these lineages were also retrieved by the morphometric analyses). These lineages have an apparent north – south distribution with lineage 1 being found in sites 1 to 14 while lineage two is found predominantly in sites 6 to 16 (although it is found in site 1, it was not recorded in sites 7 and 8). There is, however, an overlap in their

distributions in the central Albertine Rift in the vicinity of Lake Kivu. Phylogenetic analyses similarly unveiled two lineages within *P. degraaffi*. These lineages were not found with the morphometric analyses because of the small sample sizes. Although these lineages within *P. degraaffi* appear to also have a north – south distribution, there is more overlap between them in the central Albertine Rift.

In a review by Johns & Avise (1998) the range of sequence divergence values between rodent sister taxa for the cytochrome b gene fragments varied between 1.3 and 13% (see also Bradley & Baker 2001). The mean sequence divergence between the *P. jacksoni* lineages was 4.65% (3.90 – 5.67%), which is consistent with the reports by other workers based on cranio–dental (Verheyen & Bracke 1966; Rosevear 1969; Van der Straeten & Dieterlen 1987; Van der Straeten & Dudu 1990) and mtDNA sequence variation (Lecompte *et al*. 2002a, b; Nicolas *et al*. 2005). Following from this, it is possible that *P. jacksoni* is composed of several cryptic forms which need formal species descriptions. Taken together, the patterns exhibited by the DNA sequence and morphometric data suggest deep evolutionary divergences in *P. jacksoni* from the Albertine Rift and are in agreement with Lecompte *et al*. (2002b) and Nicolas *et al*. (2005) in suggesting that different populations within this species might have experienced a rapid succession of cladogenetic events. The possible existence of distinct clades within *P. degraaffi*, as hinted at by the DNA data, needs further investigation (additional specimens need to be included).

2.4.2. Evolutionary time frame of lineages

Using the *Mus – Rattus* divergence of 12 Mya and the oldest *Praomys* fossil of 5 Mya (Lecompte 2003; Musser & Carleton 2005), the divergence estimates between our two focal taxa were *c.* 3.8 (± 0.490) Mya. The divergence times for the lineages within *P. jacksoni* and *P. degraaffi* are very similar at 3.4 Mya. Our results are in agreement with the estimates of Lecompte (2003), Lecompte *et al*. (2008) and Nicolas *et al*. (2005) based on the cytochrome b gene, and Moritz *et al*. (2000) who found that most speciation events in tropical rainforest vertebrate taxa predate the Pleistocene. Classical studies such as Hamilton (1982) and Crowe & Crowe (1982) found that the Pleistocene was a critical period in the speciation of African vertebrates (particularly birds). However, phylogenetic studies on African birds (Roy *et al*. 2001; Bowie *et al*. 2004a, b, 2005, 2006; Fjeldså & Bowie 2008; Voelker *et al*. 2010) and small mammals (Avise 2000; Lecompte *et al*. 2002b, 2005; Querouil *et al*. 2001, 2003; Nicolas *et al*. 2005, 2006) have revealed that most speciation events predate the Pleistocene, and rather dated in the Miocene and Pliocene.

Although the first rifting dates back to *c.* 70 Mya, most of the landscape uplifting and volcanic eruptions took place between 10 and 2 Mya (Partridge *et al.* 1995b), which correlates with the time of divergence for the Albertine Rift *Praomys*. Rifting was also associated with global climate change and the onset of considerable drying of northern Africa, causing the repeated fragmentation of the montane forest habitats over the past 5-6 Mya when the continent

became steadily more arid (Partridge *et al.* 1995a, b). The major uplift occurred in the flanking mountains of the Western and Eastern Rift as recently as the late Pliocene (Pickford 1990; Partridge *et al.* 1995b), but the process (in the Albertine Rift) may have continued until the mid-Pleistocene (McClanahan & Young 1996). Using geological evidence, Pickford (1990), Partridge *et al*. (1995b) and deMenocal (2004) concluded that prior to the Pliocene the region of the Western Rift was covered with tropical rain forest; then rifting produced cold and wet Afromontane conditions, favourable for the spread of mountain adapted taxa (such as *Praomys* or *Hylomyscus*). Under the molecular clock hypothesis, the divergence date between lineages of the *Praomys* complex has been estimated between 4 and 3 Myr (Chevret *et al*. 1994; Lecompte *et al*. 2002a) which is in agreement with the findings presented here.

2.4.3. Phylogeography and distribution of haplotypes

The genetic diversity within sites is high as depicted by the large number of unique haplotypes. The distribution of *P. jacksoni* clades reflects either a long-term extrinsic geographic barrier (with limited gene flow) around Lake Kivu where the two groups show a break, or the extinction of intermediate haplotypes. According to Avise (2000), these types of patterns are formed when formerly contiguous populations have become isolated for long periods, usually due to extrinsic barrier to gene flow. However, a study on Andean tree frog species by Guarnizo *et al*. (2009) found that deep phylogenetic breaks could be formed within a continuously disturbed species even in the absence of barriers to gene flow, as observed for the two *Praomys* species studied here.

The high sequence divergence and Φ-statistics within populations, in association with the fact that *P. jacksoni* lineages were retrieved with a combination of markers, suggests a parapatric divergence with secondary contact. The clustering of sites such as 1 and 10, 1 and 13 or 2 and 4 could be explained by past climate, vegetation changes, landscape history or geological features. According to Vrba *et al*. (1995), the period between 4.3 and 4 Myr was humid and the hottest of the Pliocene. This led to the development in East Africa of forested biotopes, reaching their maximal extension between 3.4 and 3.3 Myr (Williamson 1985; Vrba *et al*. 1995), characterized by a mosaic environment and expansion of tree-savannah and forest (Lecompte *et al.* 2002a).

- *The northern clades*

The northern lineages comprised individuals from sites 1, 3 and 4 to the north, site 2 within Lake Victoria, and site 14 to the south and encompassed populations amongst the paratypes of the original species described from Entebbe in Uganda. Sequences from Kakamega (GenBank) in Kenya (for *P. jacksoni* and sites 5 to 8 and 14 (for *P. degraaffi*). The geology and historical climatic factors in the region may have shaped the species distribution. Site 3 is isolated

from the southern sites by Lake Edward, Lake George, the Kazinga Channel (that connects lakes Edward and George) and to the western Congo basin (site 4) by Semliki River. According to Beadle (1981), prior to mid-Pleistocene, the area of present-day Lakes Edward and George was occupied by Semliki Lake, which might have acted as biogeographical barriers. Our data do not provide support for this argument because Ruwenzori (site 3) shares haplotypes with site 6 to the southeast, site 2 within Lake Victoria and site 1, west of Lake Albert.

Bootsma & Hecky (1993) suggest that during the Pleistocene, the climate fluctuated three times when Lake Victoria became shallow. This could have caused gene flow to occur between site 2 (in Lake Victoria) and the mainland. During the LGM, areas that are dry savannah today were much wetter. The retreat of forest habitat resulting from the expansion of savannas caused the fragmentation of the forest biomes, separating populations. Geologically, the Virunga Volcanoes and Kigezi Highlands are the main features that stand in the central Albertine Rift. The volcanoes have been active since the Plio–Pleistocene, but some (Muhavura, Mgahinga and Sabinyo) are extinct (Kampunzu *et al.* 1998). The prediction that the Virunga volcanoes would act as a barrier to dispersal between adjacent forests and populations from Kigezi Highlands was not upheld.

- *The southern clades*

Lake Kivu isolates site 13 (Idjwi Island) from the mainland; Lake Tanganyika and Ruzizi River stand between site 14 and the sites 15 (Itombwe massif) and 16 (Mount Kabobo). Although a dry savannah and the Kilembwe River separate these forest blocks (sites 15 and 16), the two populations clustered together in the different trees or parsimony networks (Figure 2.5). The null hypothesis that site 13 would share haplotypes with site 10, 11 and 12 to the west and site 14 to the east was upheld, but only 2 mtDNA haplotypes out of 7 were shared with the western sites vs. 5 for the eastern site. Studies on the sediments by Beadle (1981) and McClanahan & Young (1996) suggest that the proto Lake Kivu occupied the areas of the present day Lake Kivu approximately 1 Mya. We speculate that the proto Lake Kivu might have been located in the west of Idjwi where a large water body exists today, while Idjwi Island could have formed a continuous landmass with Nyungwe–Kibira (site 14) in the east. The formation of Lake Kivu and Ruzizi River (its outlet) separating these areas with Itombwe and Kabobo is recent, 15,000 – 10,000 years BP.

2.4.4. Taxonomic considerations and existence of cryptic lineages

Following an account by Rosevear (1969), the genus *Praomys* has been known since 1860 when Gray described one of Burton's collections from Cameroun Mountains as *Mus maurus* (now *morio* Troussart). The next species named, collected by Burton in 1892 was assigned to *Mus jacksoni*. They became the species of Troussart's *Epimys*

where they remained until Thomas (1915) assigned them to his new subgenus *Praomys*, diagnosed first by the mammary formula 1–2 = 6, then in 1925 he added a range of other characters both external and cranial.

The phylogeographic patterns, the sequence divergence and the divergence times between clades raise concerns on whether what is a single species may represent more than one taxon. Below is an account for the Albertine Rift *Praomys* species considered:

Praomys degraaffi Van der Straeten & Kerbis Peterhans 1999 was described from the Albertine Rift (Bwindi, Kibira and Nyungwe). Subsequent sampling to the west confirmed its occurrence in Kahuzi–Biega National Park in the DRC (Kaleme *et al.* 2007) and other sites where the range is limited to mountain forests from site 5 (northern limit) through site 10 and sites 15 to site 16 (southern limit) in altitudes ranging from c. 1500 to > 3000m. The species is endemic to the Albertine Rift and near threatened because of habitat destruction (Musser & Carleton 2005; Plumptre *et al*. 2007b). This species requires further investigations with more samples as our data found two lineages.

Praomys jacksoni (de Winton, 1897) named as such by Thomas (1915), type specimen from Entebbe (Uganda). The distribution spans from Kenya and Tanzania in east Africa to Nigeria in West Africa, south to Angola and Zambia (Musser & Carleton 2005, Van der Straeten 2007). The IUCN status is "data deficient" because of the taxonomic confusion between *P. jacksoni*, *montis* and *peromyscus* (Musser & Carleton 2005). Further taxonomic investigations are required to clarify the systematic position of the composite groups.

Praomys misonnei Van der Straeten & Dieterlen 1987 was described from Irangi (eastern DRC) in the Albertine Rift. One specimen in our data from Bushema, 20 km west of Irangi, belonged to this species. The comparison of the skull with the known ones in the museum collection confirmed our finding. The IUCN status is "lower risk". It is sympatric with *P. jacksoni*, *P. mutoni* and *P. verschureni* in the lowland Congo basin.

Praomys mutoni Van der Straeten & Dudu 1990 described from Masako, 15 km north of Kisangani, northeast of the DRC. Specimens were collected at Bushema, 20 km east of Irangi and identified as such by mtDNa sequence data. Comparison of skulls with specimens in the museum confirmed the findings. We hereby document the extension of its home range to the south. The IUCN status is "lower risk".

Praomys verschureni (Verheyen & Van der Straeten, 1977) described from Mamiki in the Oriental Province, northeastern DRC as *Malacomys verschureni* from *P. jacksoni*. It was subsequently recorded at Irangi (Dieterlen & Van der Straeten 1984) and Ituri forest (Katuala *et al*. 2005). Molecular studies by Lecompte *et al*. (2002 a,b, 2005)

reclassified it as a member of the genus *Praomys.* Nevertheless, it was not recorded in this study. It is endemic to the Albertine Rift and near threatened.

Two lineages of *P. jacksoni* and *P. degraaffi* co-occur in the Albertine Rift with range overlap at some locations. The northern lineages encompass the type locality of the type species (Entebbe, Uganda for *P. jacksoni* and Bwindi-Mgahinga [Uganda]-Kibira [Burundi] for *P. degraaffi*), to site 14 in the south. Huhndorf *et al.* (2007) obtained a similar pattern within *Hybomys lunaris*, one from Kibira Forest, separated from the northern populations in the Virunga and Ruwenzori at an average sequence divergence of 6.8%.

The diversification of *Praomys* species may correspond to a period of pronounced shifts in African climate, which resulted in major changes in the distribution and composition of the vegetation (Morley 2000). According to Partridge *et al*. (1995b), the Late Miocene (10 – 5 Mya) was characterized by a period of expansion of savannas while the Pliocene (5 – 3.5 Mya) was characterized by moist climates that favoured the expansion and diversification of rain forests, and retraction of savannas. The late Pliocene and early Pleistocene (3.5 – 1.6 Mya) corresponded to pronounced climatic changes with several drying and cooling phases, resulting in an extension of savannas and open environments in tropical Africa and concomitant contraction of humid forests. These climatic and environmental changes during the Miocene, Pliocene and Pleistocene could have promoted speciation within the major genera such as *Praomys.* The parapatric speciation due to change in the habitat (habitat fragmentation or climate change) followed by secondary contact could well be deduced from the observation of the Albertine Rift *Praomys* and explain the sympatric distribution observed within the species today.

The emergence of the forest species could be explained according to the refuge theory proposed by Haffer (1969, 1982) stating that some forest areas subsisted permanently during phases of vegetation contraction. These areas would have acted as refuges for forest species. Such refuge areas have been proposed (in Africa) on the basis of various data sets (Grubb 1982; Colyn *et al*. 1991; Maley 1996), among which the Eastern Congo basin refuge (possibly the Albertine Rift). The distribution of *P. misonnei*, *P. mutoni* and *P. degraaffi* corresponds to this Eastern Congo refuge.

2.4.6. Concluding remarks

Kerbis Peterhans & Hutterer (2009) acknowledged that many mammalian taxa probably remain to be discovered, a situation that can only be changed with comprehensive and wide-range surveys. Further studies would require a combination of molecular and morphometrics data on species from Central and East Africa. The Albertine Rift is important for conservation (Plumptre *et al.* 2007a, b) and we acknowledge the importance of continued surveys even in areas that may be considered fairly well known and that the current base of knowledge in the distribution of species still has immense gaps for a number of species which could/might range further than currently documented.

Table 2.1. List of sites and number of specimens (per species) recorded in the Albertine Rift. For the sites where altitude is given as a range, samples were collected in many locations in the given range. Sites are listed from north to south.

Site	Site No as on map	Longitude	Latitude	Altitudes	*Praomys degraaffi*	*Praomys jacksoni*	Other	Observation
Budongo FR	1	31.53083	1.71056	965 m	-	4		Lowland site
Sese Island	2	32.31900	-0.31300	1133 m	-	3		Lowland site
Ruwenzori NP	3	29.98330	0.36660	1646 – 2652 m		10		Mountain site
Okapi FR	4	28.64970	1.57500	814 m	-	7		Lowland site
Mt. Tshiabirimu	5	29.44039	-0.10013	1950 – 3050 m	11	1		Mountain site
Bwindi I. NP	6	29.66139	-1.07778	2503 m	3	12		Mountain site
Mgahinga Gorilla NP	7	29.64194	1.38806	2980 m	3	-		Mountain site
Echuya FR	8	29.83306	-1.30028	2383 m	3	-		Mountain site
Bushema Lutunguru	9	29.39941	-2.73080	1090 m	-	3	*P. misonnei & P. mutoni*	Lowland site
Kahuzi Biega NP	10	28.46810	-2.13170	1900 – 2149 m	14	9		Mountain site
Lwiro	11	28.47146	-2.13183	1700 m		8		Mountain site
Mugeri	12	28.86380	-2.21600	1493 m	-	13		Mountain site
Idjwi Island	13	29.12850	-2.28908	2255 m	-	10		Mountain site
Kibira NP	14	29.56660	-3.21660	1543 – 2104 m	10	5		Mountain site
Itombwe	15	28.57289	-3.43270	2090 – 2908 m	9	20		Mountain site
Kabobo	16	29.16706	-5.28758	1250 – 1950 m	2	14		Mountain site

Table 2.2. List of the linear measurements and the respective abbreviation as used in the traditional morphometrics analyses as adapted from Verheyen and Brake (1966), Rosevear (1969), Carleton & Van der Straeten (1997), Dieterlen (1982), Carleton *et al*. (2006), Van der Straeten & Kerbis Peterhans (1999) and Patton *et al*. (2007). SE = standard error for each measurement. The mean here is irrespective of species (*degraaffi* and *jacksoni* combined)

No	Abbreviation	Description of measurement	Mean	SE
1	PRCO	Prosthion – condylion (condylobasal length)	27.95	1.89
2	HEBA	Henselion – basion (basilar lenth)	26.05	3.06
3	HEPA	Henselion – palation (palatilar length)	25.32	2.18
4	COBASL	Condylo-basilar length	28.82	0.04
5	GRLS	Greatest length of the skull	30.63	0.25
6	DIAL	Length of diastema (diastema length)	9.19	0.09
7	PAFL	Palatal foramen length	6.63	0.16
8	INTONAS	Length of tip of nasal to the center of inter orbital	16.29	0.14
9	LBRCA	Length of braincase	15.30	0.25
10	LNAS	Length of the nasals	11.83	0.49
11	LOTE	Length of the lower cheek teeth	4.61	0.89
12	M1BR	Breath of M^1	1.39	0.03
13	BULL	length of auditory bulla	4.75	0.46
14	INTE	Interorbital breath (constriction)	4.96	0.27
15	BBRCA	Braincase breath	12.21	0.06
16	BNAS	Breath of the nasals	3.81	0.15
17	PALPMAX	Palatal length between end of palatine and tip of premaxilla	15.44	0.06
18	ROBR	Greatest rostrum breast	4.99	0.06
19	PALL	Palatal breadth between M^1s	14.98	0.35
20	ZYGO	Zygomatic (plate) breadth	14.39	0.33
21	ROHE	Rostrum height at anterior border of M^1	5.78	0.4
22	HBRCA	Height of braincase	9.33	0.22
23	LEMA	Length of the mandible	16.11	0.11

Table 2.3: Basic statistics depicting the results of t-test performed to assess the difference between species *P. degraaffi* and *P. jacksoni*. Significant values are indicated in bold). SE is the standard error for each measurement. For both species using the set of data collected as explained in the methods.

Measurements	P degraaffi				P jacksoni					
	N	Mean ± Std.Dev.	Max	Min	N	Mean ± Std.Dev.	Max	Min	t-value	P
PRCO	130	27.67±1.63	30.09	16.61	256	28.07±1.71	31.58	18.83	-2.26	0.024
HEBA	130	25.74±1.47	28.37	16.64	256	26.23±1.51	29.64	21.1	-2.07	0.002
HEPA	130	25.09±1.23	27.31	20.6	256	25.50±1.55	30.56	20.7	-2.65	0.008
COBASL	130	27.05±1.37	32.90	24.57	256	28.80±1.39	32.20	23.40	-2.76	0.006
GRLS	130	30.42±1.27	33.4	25.6	256	30.76±1.43	34	25.8	-2.36	0.019
DIAL	130	8.69±1.13	11.2	7.3	256	9.25±0.98	11.7	7.08	-5.05	0.000
PAFL	130	6.53±0.45	7.7	5.44	256	6.68±0.49	8.1	4.2	-3.06	0.002
INTONAS	130	16.55±0.97	19	13.6	256	16.32±1.13	19.17	11.89	1.94	0.052
LBRCA	130	14.93±1.12	25.3	12.13	256	15.39±0.68	18.35	13.4	-5.01	0.000
LNAS	130	11.75±0.86	14.5	9.27	256	11.76±0.95	15.09	8.8	-0.10	0.918
LOTE	130	4.65±0.23	5.08	3.77	256	4.60±0.25	5.29	3.95	2.03	0.043
M1BR	130	1.47±0.56	4.49	0.82	256	1.38±0.15	1.82	1.1	2.33	0.021
BULL	130	4.76±0.29	5.63	3.99	256	4.76±0.39	5.94	3.7	-5.61	0.934
INTE	130	4.98±0.36	6.89	4.16	256	4.89±0.35	6.5	4.2	2.15	0.032
BBRCA	130	12.24±1.22	13.94	9.7	256	12.31±1.05	14.55	9.9	-5.28	0.571
BNAS	130	3.70±0.33	4.4	2.72	256	3.90±0.46	6.61	2.8	-4.41	0.000
PALPMAX	130	15.28±0.67	17	13.1	256	15.50±0.84	18	12.9	-2.63	0.009
ROBR	130	4.95±0.33	5.7	4	256	5.02±0.37	5.96	3.6	-1.59	0.112
PALL	130	14.91±0.66	16.4	12.9	256	15.04±0.84	17.12	12.4	-1.54	0.125
ZYGO	130	14.28±0.59	15.6	12.7	256	14.45±1.07	16.61	1.97	-1.63	0.104
ROHE	130	5.75±0.46	7.09	4.55	256	5.78±0.56	7.3	0.64	-0.62	0.538
HBRCA	130	9.27±0.38	10.42	8.1	256	9.38±0.62	14.4	7.7	-1.76	0.079
LEMA	130	15.87±0.75	17.35	13.3	256	16.10±1.01	18.8	13	-2.32	0.021

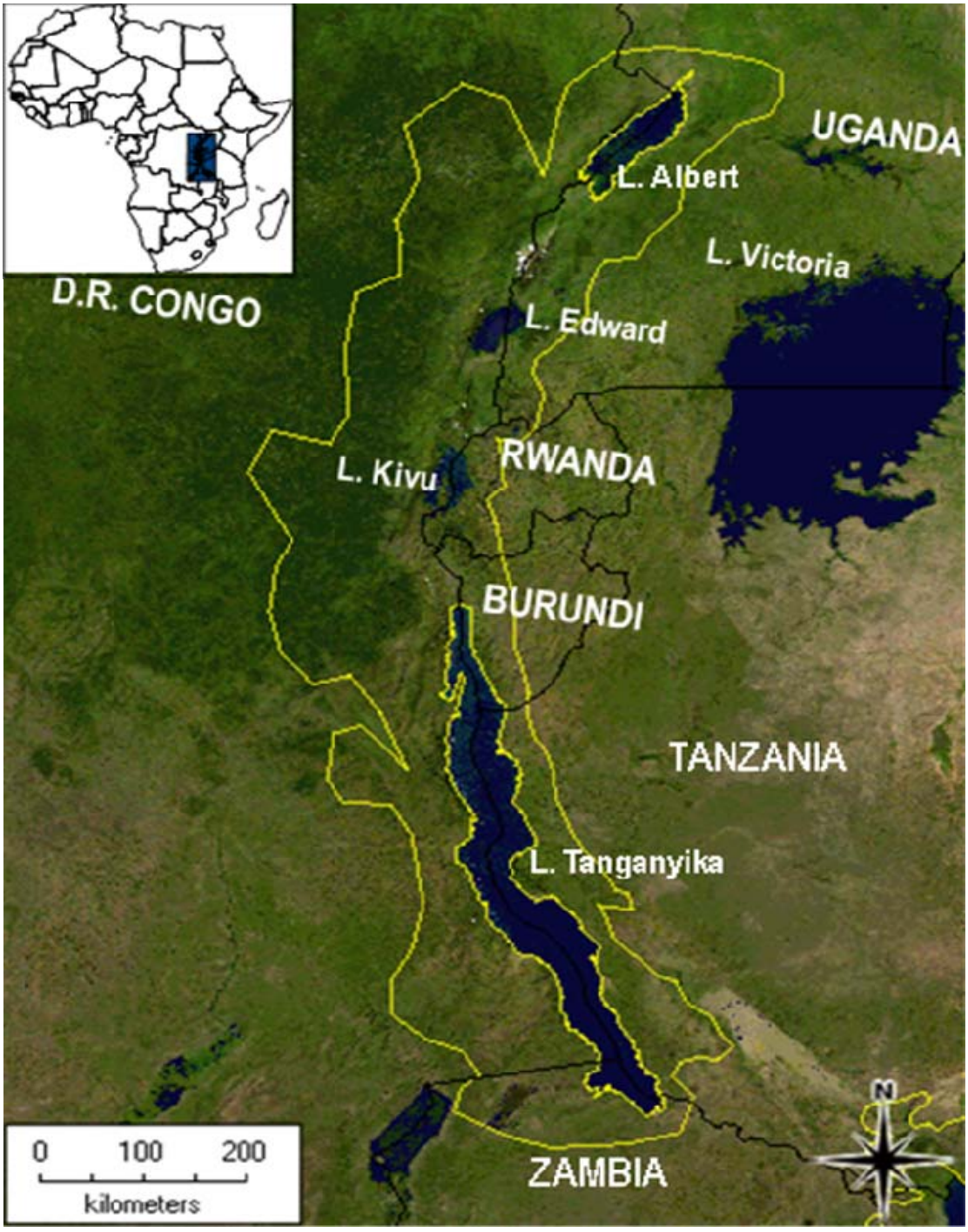

Figure 2.1. Map the Albertine Rift (yellow shape), adapted from *Roy et al*. 2001. Black line represents country limits and the blue shapes are the lakes (adapted from Plumtre *et al*. 2003).

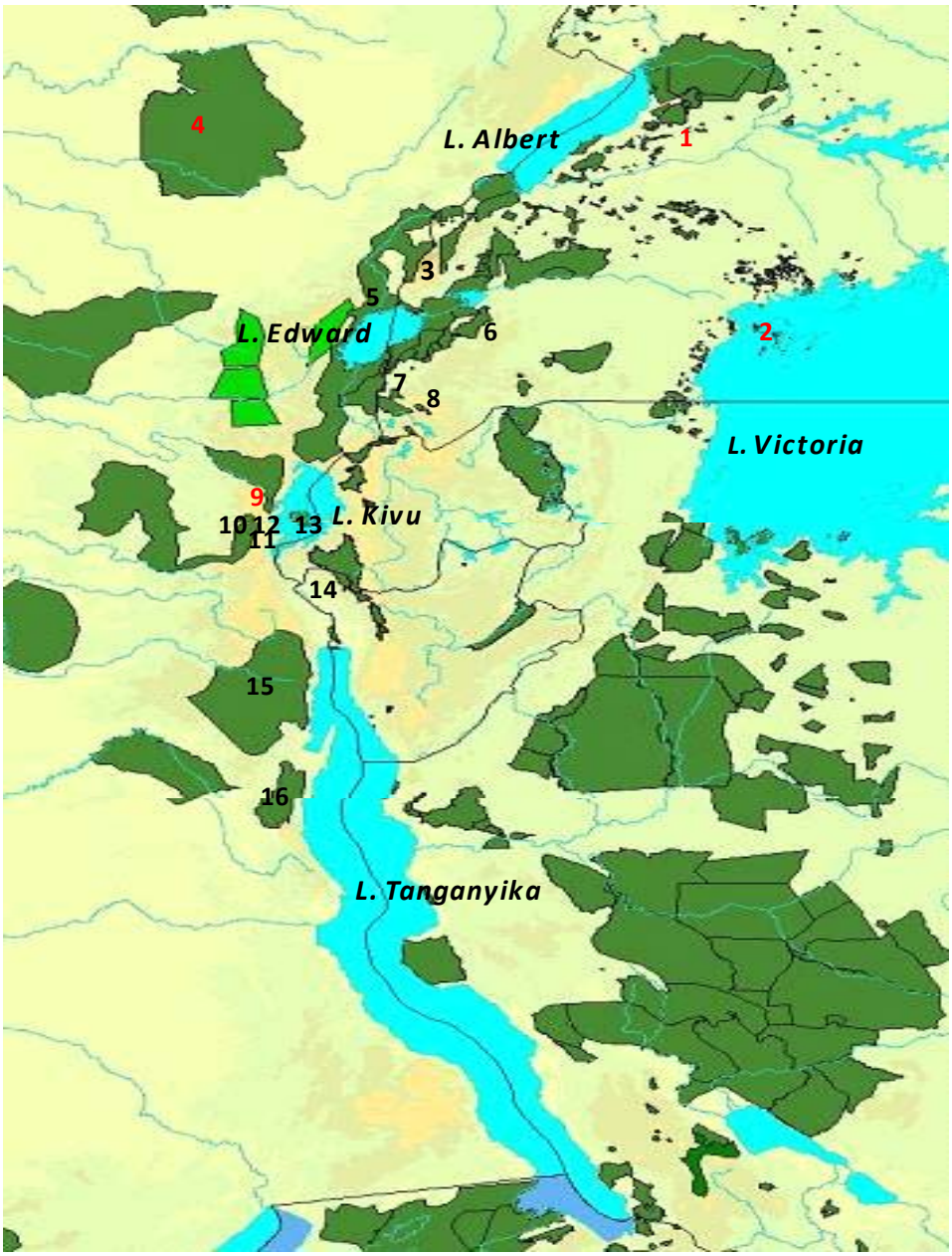

Figure 2.2. Map of the Albertine Rift showing the location of sampled sites. Numbers are from north to south. 1: Budongo FR, 2: Sesse Island, 3: Ruwenzori Mountains, 4: Okapi Faunal Reserve, 5: Mount Tshiabirimu, 6: Bwindi Impenetrable NP, 7: Echuya FR , 8: Mgahinga Gorilla NP, 9: Bushema – Lutunguru forest, 10: Kahuzi – Biega NP, 11: Lwiro Research station, 12: Mugeri Seminary, 13: Idjwi Island, 14: Kibira NP, 15: Itombwe massif and 16: Mount Kabobo. The numbers in red represent the lowland sites and the numbers in white, the mountain sites. Green areas represent protected or conservation areas (adapted from Plumptre *et al*. 2003).

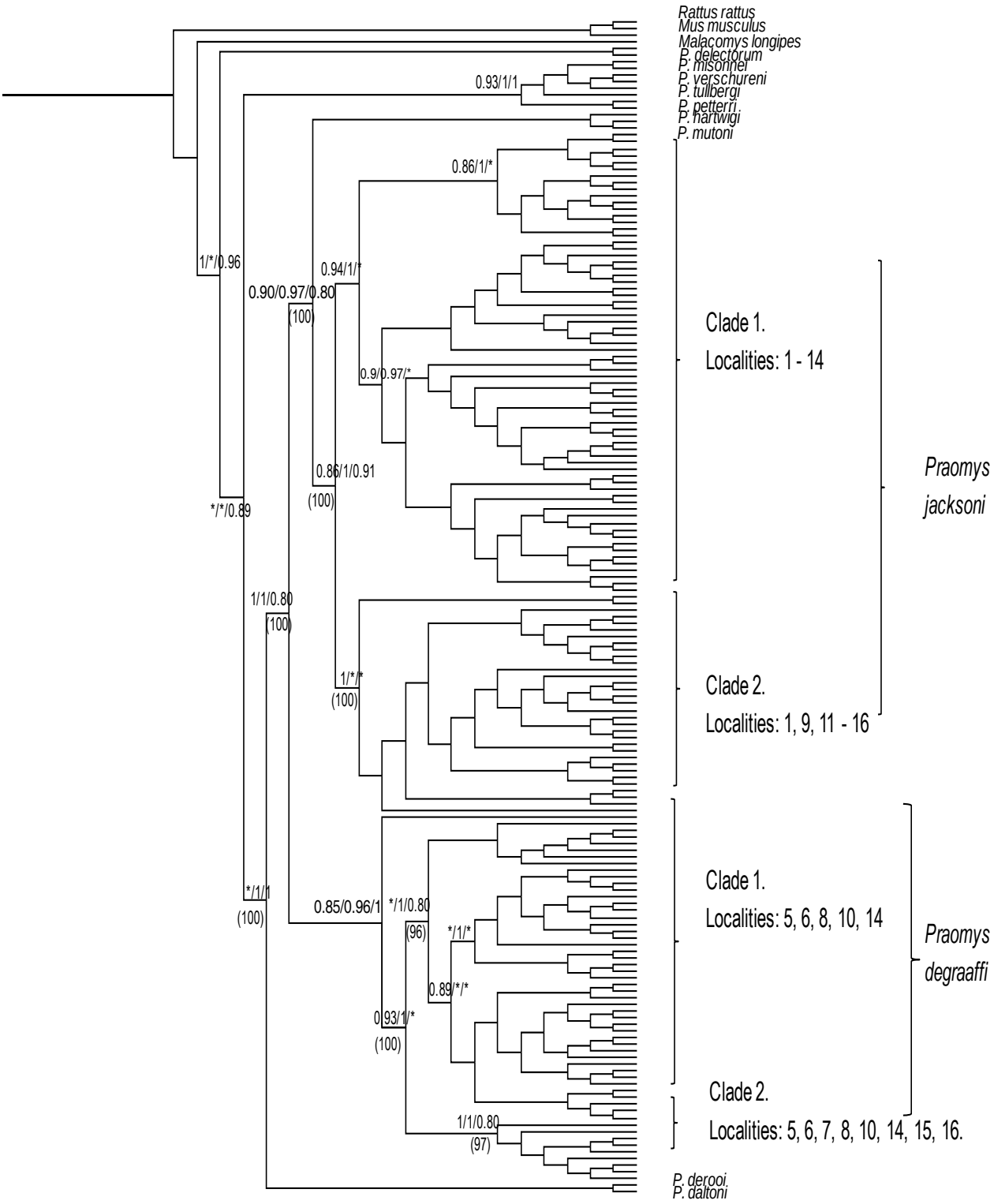

Figure 2.3. Bayesian tree for the Albertine Rift *Praomys*. Locality numbers refer Table 1. On the tree, numbers in brackets () indicate the bootstrap supports, while the three numbers x/x/x (decimals or 1) represent the posterior probabilities for cytochrome b, control region and the combined data respectively.

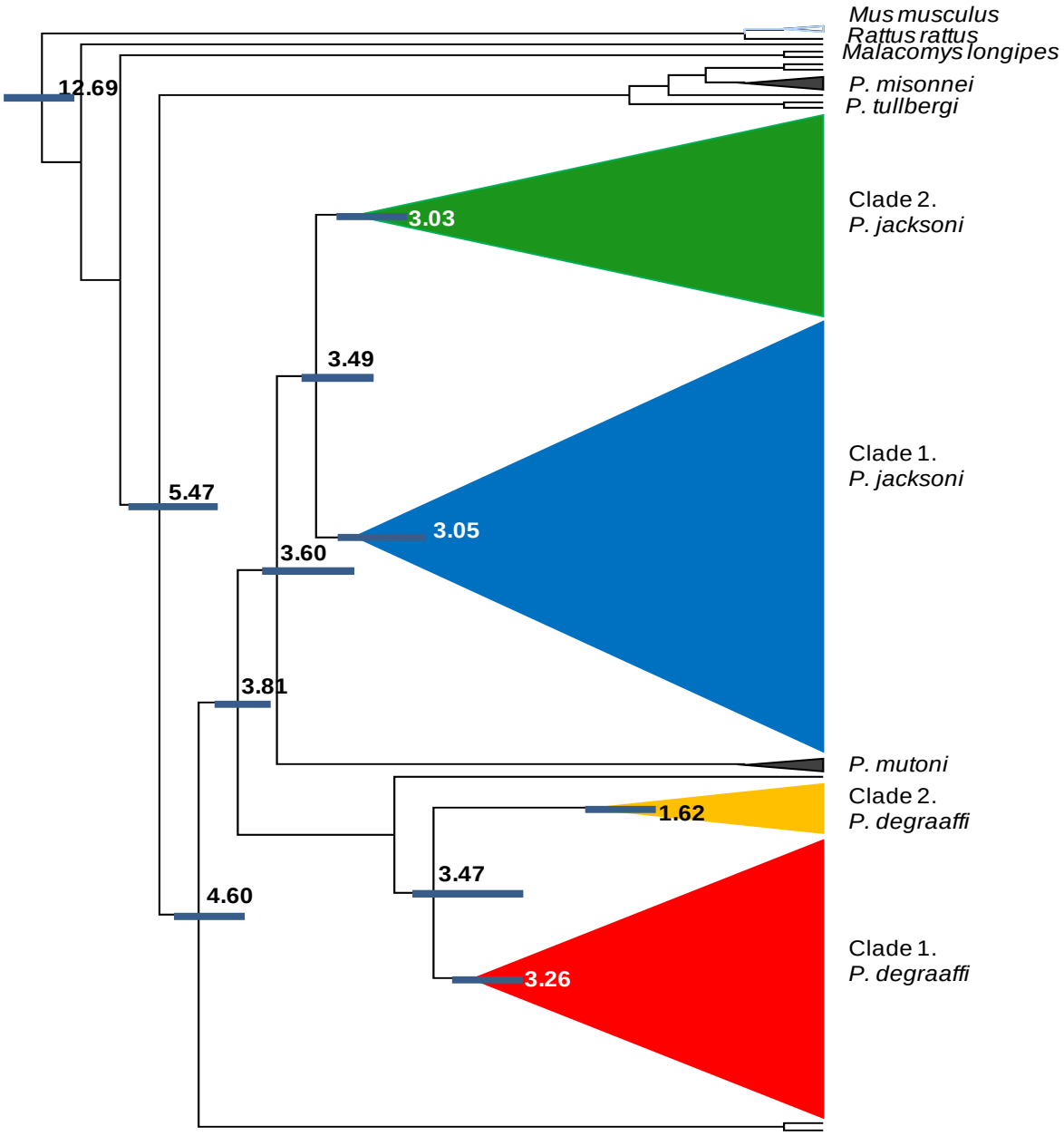

Figure 2.4. Bayesian tree of the Albertine Rift *Praomys* showing the divergence time of different species and clades for the focal species. Numbers are the divergence time in million years.

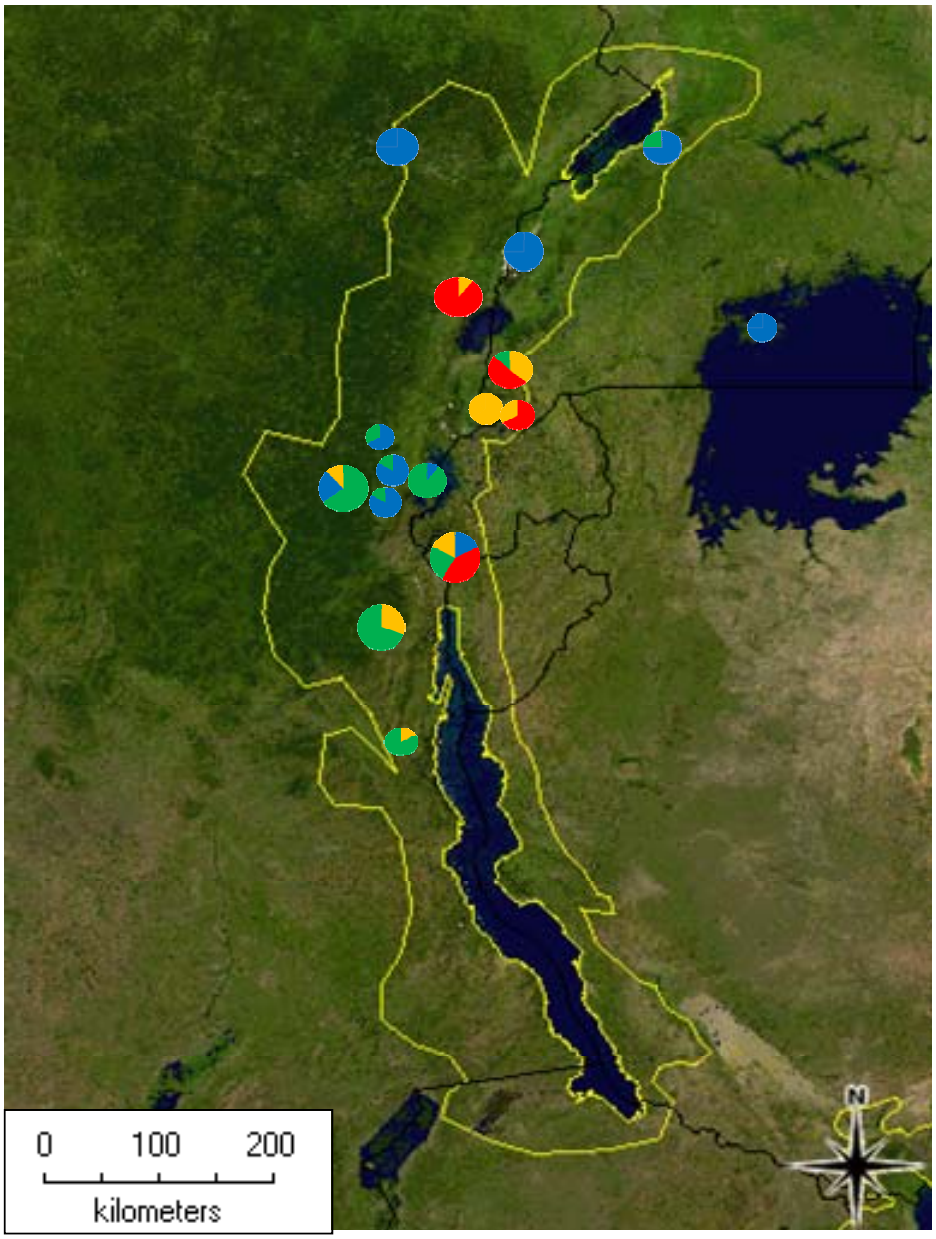

Figure 2.5. Schematic network of the clades for the two species *P. degraaffi* and *P. jacksoni* in the Albertine Rift. The colors, blue represents clade 1 – *P. jacksoni*, green: clade 2 – *P. jacksoni*, red: clade 1 – *P. degraaffi* and orange: clade 2 – *P. degraaffi*.

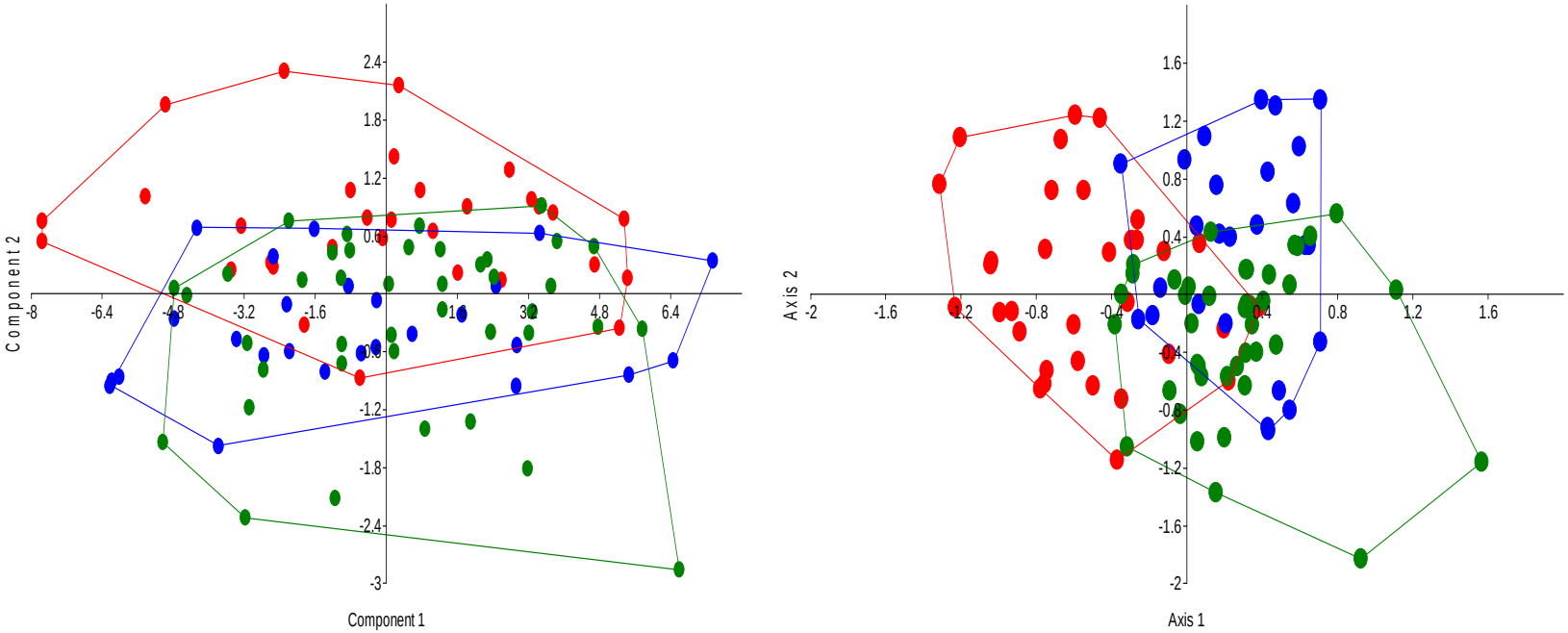

Figure 2.6. A: PCA and B: CVA of the *Praomys* grouping from the linear measurements. The red color represents *Praomys degraaffi*, blue: clade 1 - *P. jacksoni* and green, clade 2 - *Praomys jacksoni*.

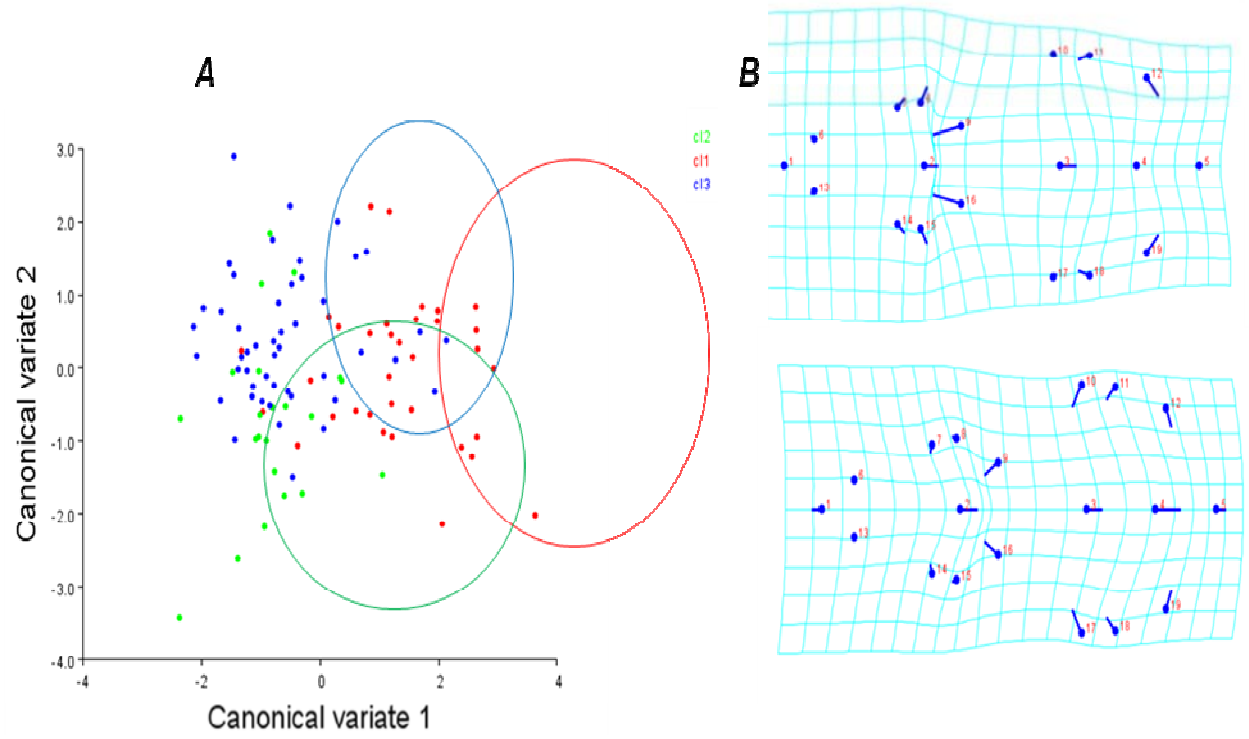

Figure 2.7. A: Scatter plots (CVA) of the geometic morphometric analyses for different groups of *Praomys* specimens from the Albertine Rift. The red color indicates *Praomys degraaffi*, blue: clade 1 - *P. jacksoni* and green, clade 2 - *Praomys jacksoni*. B: The deformation grids (dorsal view) of *P. jacksoni* PCs: on top: clade 1 and below: clade 2.

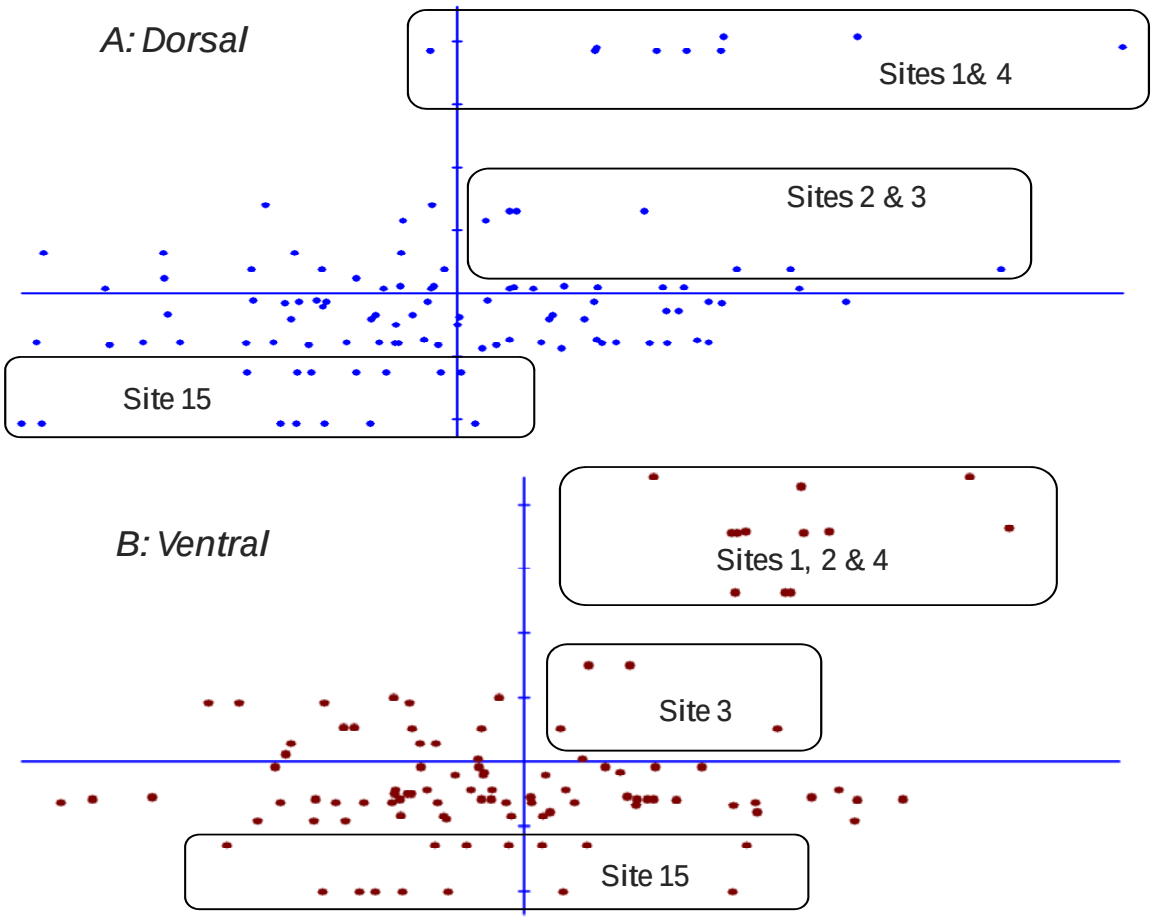

Figure 2.8. Graph of the Partial Least Square. Sites grouping using climate variables comprising rainfall, temperature and the covariates extracted from specimens' CSs in MorphoJ.

Chapter 3

Unraveling some of the complexity in the *Praomys jacksoni* species complex across the Albertine Rift

3.1. Introduction

The Albertine Rift, which forms part of the large East African Rift system, is defined as extending from ~30 km north of Lake Albert to the southern tip of Lake Tanganyika (see Figure 3.1; see also Figure 1 in Plumptre *et al*. 2007a). The landscape is notably heterogeneous and includes several lakes, valleys and mountains as well as a diversity of habitats across altitudinal gradients (Jolly *et al*. 1997; Plumptre *et al*. 2003, 2007a). The current layout is due to the fact that the region was characterized by instability as a result of uplift (Livingstone 1967, 1975; Rosendahl 1987), volcanism (Kampunzu *et al*. 1998) and natural climatic oscillations during the Plio - Pleistocene (Beadle 1981; deMenocal 2004). This may, at least in part, account for the rich fauna and flora (see e.g. Plumptre *et al*. 2003, 2007a) including exceptional levels of endemism (Prigogine 1985; Vande Weghe 1988a, b; Stattersfield *et al*. 1998; Olson & Dinerstein 2002; Burgess *et al*. 2004; Brooks *et al*. 2004; Plumptre *et al*. 2003, 2007a). Notwithstanding the exceptional diversity across all taxonomic levels, very little is known about the spatial distribution of genetic variation for the majority of taxa (exceptions include charismatic mega fauna such as gorillas - Jensen-Seaman & Kidd 2001; Matsubara *et al*. 2005) and as such, accurate inferences regarding the exact processes driving these patterns and also to some extend the high species diversity remain unclear.

Soft fur mice (members of the genus *Praomys*) are amongst the dominant rodent taxa in the region, with four species typically recognized in the Albertine Rift (*P. degraaffi, P. jacksoni, P. misonnei* and *P. verschureni*; see e.g. Dieterlen & Van der Straeten 1984; Van der Straeten & Dieterlen 1987; Van der Straeten & Dudu 1990; Van der Straeten & Kerbis Peterhans 1999). Of these, *P. degraaffi* and *P. verschureni* are Albertine endemics (Plumptre *et al*. 2007a). A recent study (see Chapter 2) provided genetic evidence for the presence of a fifth species (*P. mutoni*) from the Albertine Rift. These species are morphologically similar and are easily confused in the field. Indeed, earlier surveys typically included these taxa (*P. degraaffi, P. misonnei, P. verschureni* and possibly even *P. mutoni*) as *P. jacksoni*. As such it is not surprising that the description of these various taxa was all done from specimens previously believed to be *P. jacksoni*. These mice are typically found across a diverse array of habitats which include humid and shaded rain forests, secondary scrublands, woodlands, savanna-forest mosaics or mountain forests (Rosevear 1969; Kingdon 1997; Musser & Carleton 2005; Kaleme *et al*. 2007). Ecological studies found that *Praomys* is tolerant to disturbance (Rosevear 1969; Dieterlen 1990) and can reproduce throughout the year (Eisentraut 1971; Dieterlen 1990); traits which undoubtedly contribute to its abundance in the region.

Previous studies based on trapping (Van der Straeten & Kerbis Peterhans 1999; Kaleme *et al.* 2007) as well as DNA data (see Chapter 2) indicate that two of these species, *P. jacksoni* (type described from Entebbe, Uganda, typically considered a lowland species) and *P. degraaffi* (type described from 2,200 m a.s.l.) are parapatric over parts of their ranges with a zone of overlap between ~1,500 m to ~2,450 m. *Praomys misonnei, P. verschureni* and *P. mutoni* are

all strictly lowland species, and although they are sympatric with *P. jacksoni* across most of their ranges, they were not considered here as they were not collected at any of the intermediate to high altitude sites included in this study. To what extent introgression takes places between *P. jacksoni* and *P. degraaffi* is not known. Although the recent study by Kaleme *et al.* (Chapter 2) included both mitochondrial and nuclear genes for *P. jacksoni* and *P. degraaffi*, the resolution from the nuclear gene was not sufficient to address the question of introgression.

Praomys jacksoni occurs over a large geographic area from Nigeria in the west to Tanzania in the east, and it is not unfeasible to expect some degree of cryptic speciation or in the least, genetic differentiation over such a large area. Also, given the very heterogeneous landscape which characterizes the Albertine Rift, several taxonomic authorities (Dieterlen 1990; Prigogine 1985; Carleton *et al.* 2006) have highlighted the potential for cryptic speciation across this mountainous terrain in Central and East Africa. Indeed, a previous study by Kaleme *et al.* (see Chapter 2) indicated lineages with distinct evolutionary trajectories within both *P. degraaffi* and *P. jacksoni*.

To address the question of introgression as well as provide additional genetic information regarding possible cryptic speciation, we compare data from nine variable microsatellite markers for *P. jacksoni* and *P. degraaffi* from across their ranges in the Albertine Rift. We place our findings within the climatic and geological history of the region. At both the regional and local scale, habitat characteristics influence genetic structure through its effects on gene flow (Frankham *et al.* 2002) and effective population size (*Ne;* Wright 1931). In addition, characteristics such as mating system, sex ratio and/ or species dispersal capabilities affects the genetic structure through its impacts on mutation, genetic drift and selection on genetic structure (see e.g. Marko & Hart 2011). Although dispersal can be assessed through direct methods such as mark – recapture, radio tracking or direct observation, these methods may underestimate migration (see Schweizer *et al*. 2007). Despite some shortcomings, the use of genetic approaches provides an alternative to estimate dispersal parameters (Prugnolle & de Meeus 2002; Meyer *et al*. 2007).

A solid working knowledge of local biodiversity is essential to ensure successful conservation at local and broad regional scales; conservation policies based upon poorly defined categories which does not reflect the underlying genetic diversity is at best inefficient and at worst might reduce the long term evolutionary potential of species (see e.g. Alpers *et al*. 2004; Bunnefeld *et al*. 2011; Johnson 2011). This is especially true for (biodiversity rich) regions such as the Albertine Rift where funding priorities are often centered on human development projects or a few flagship species rather than global biodiversity conservation. However, it is important to bear in mind that recently diverged lineages often have incomplete reproductive barriers, allowing introgression of genetic material from one evolutionary lineage into the genomic background of others (Rieseberg *et al.* 1995; Castric *et al*. 2008). As such, the data presented here will enhance our understanding of the patterns and possible presence of cryptic lineages in two

of the soft fur mice; one being an Albertine Rift endemic and the other one of the most numerous (widespread) rodents in the region.

3.2. Materials and methods

3.2.1. Sampling

A total of 162 *Praomys* specimens were collected from 10 sites distributed throughout the Albertine Rift (see Table 3.1 and Figure 3.1). Collections were carried out during surveys conducted in 1990–91 and in 1996–97 in Uganda and Burundi, and in 2001–08 in the Demographic Republic of the Congo (DRC). Voucher specimens were deposited at the Field Museum of Natural History (Chicago) and at the "Centre de Recherche en Sciences Naturelles" (CRSN) at Lwiro (DRC) (see Appendix 3.1). Due to their close geographic proximity and in the absence of any obvious geographic separation between them, some sampling locations were pooled during all analyses (see Figure 3.1 and Table 3.1). *Praomys degraaffi* were collected at seven of these sites and *P. jacksoni* at nine sites (Table 3.1). Preliminary species status for all specimens included here was done *a priori* based on mitochondrial DNA data (see Chapter 2).

3.2.2. Analyses – Microsatellite library

An enriched microsatellite library for *P. jacksoni* was developed at the Field Museum of Natural History (Chicago) (see Feldheim *et al*. 2006 for a description of methodologies). Twelve of the clones sequenced contained repeat regions and sufficiently long flanking regions to design primers (Table 3.2) (GenBank accession numbers XX-XX). Primers were designed using PRIMER 3 web-based software (Rozen & Skalatsky 1998). In each instance, forward primers were 5'-dye-labelled with one of four fluorophores (6-FAM, NED, PET or VIC; see Table 3.2). The Multiplex PCR Kit (QIAGEN Inc.) was used following the recommended protocol using Q-Solution in a final reaction volume of 10 µl containing 6 µl of 2X QIAGEN Multiplex Master Mix, 1 µl of primer mix (mix of forward and reverse primers for one locus; final concentration of 2 µM), 1 µl of Q solution, 1 µl of H_2O and 3 µl of the template DNA. PCR conditions included an initial denaturation step at 95°C for 10 min followed by 30 cycles of 95°C for 60 sec, 60°C for 60 sec and 72°C for 60 sec. A 20 min elongation at 72°C completed the reactions. Amplifications were carried out in a GeneAmp 2700 Thermocycler (Applied Biosystems). PCR products (1 µl diluted - 1/20) were further diluted in 15 µl deionized formamide and genotyped on an ABI 3130 automatic sequencer, using 0.2 µl of GS500LIZ size standard (Applied Biosystems). Scoring was done using Genemapper 3.7 (Applied Biosystems). All loci were polymorphic for *P. jacksoni*, however, three of the markers failed to amplify in *P. degraaffi*. Hardy Weinberg equilibrium and linkage disequilibrium for both species were assessed in GenAlEx 6 (Peakall & Smouse 2006). To allow for comparable data

between *P. jacksoni* and *P. degraaffi*, all analyses were conducted on the nine markers that amplified in both species (see Table 3.2). To assess the population differentiation, pair wise population F_{ST} values were calculated in GenAlEx. To quantify the efficiency of each marker (locus), we assessed the typological value (TV), which is the contribution of the markers to the reference typology as described by Laloe *et al*. (2007) and Berthouly *et al*. (2008).

3.2.3. Analyses - Genetic diversity and introgression

Genetic diversity for the respective sampling sites was assessed through allelic richness (A), the effective number of alleles (Ne), the number of private alleles (NPA) as well as observed (Ho*)* and expected (H_E) heterozygosity (Genepop on the web version 4. 0.10; Raymond & Rousset 1995). Specimens were assigned to one of the two species (*jacksoni* or *degraaffi*) using a Bayesian clustering approach as implemented in STRUCTURE version 2.3.1 (Pritchard *et al*. 2000; Falush *et al.* 2003). To assess potential introgression between *P. jacksoni* and *P. degraaffi*, species assignments based on the nuclear (microsatellite) data was assessed against assignments based on the mitochondrial DNA data (for mitochondrial assignments, see Chapter 2).

3.2.4. Analyses – Geographic structure

To identify genetically homogenous groups of individuals within species, Bayesian model-based clustering was conducted on the microsatellite data as implemented in STRUCTURE version 2.3.1 (Pritchard *et al*. 2000; Falush *et al.* 2003) using the default values (Pritchard & Wen 2004). Five runs of 1,000,000 Markov chain Monte Carlo (MCMC) iterations were performed with the burn-in set to 10,000 (Evanno *et al*. 2005). We chose the admixture model and correlated allele frequencies between populations as this configuration is more appropriate when shallow population structure is expected (Falush *et al*. 2003). The degree of admixture (α) was inferred from the data. When α values are close to zero, the majority of individuals largely falls into a specific population (i.e. high membership to a specific population) while $\alpha > 1$ implies that the majority of individuals are admixed (Falush *et al*. 2003). The number of clusters (populations) *K* was assumed to fall between 1 and 10, with 10 iterations for each value of *K*. These analyses were conducted for all specimens included in the study as well as for *P. jacksoni* separately. Cluster analyses were not performed for *P. degraaffi* because only four of the sampling sites had a sample size larger than six individuals, and therefore precluded meaningful comparisons.

3.2.5. Characterization of spatial genetic structure (SGS)

Spatial genetic structure (SGS) was assessed using a procedure described by Vekemans & Hardy (2004), based on pair-wise kinship coefficients between individuals using the software SPAGEDI (Hardy & Vekemans 2002). Nason's

estimator of kinship coefficient (Loiselle *et al*. 1995) was chosen because it displays robust statistical properties (Vekemans & Hardy 2004). Kinship coefficient values (F_{ij}) were regressed on the spatial distance between individuals (d_{ij}) and its natural logarithm, ln(d_{ij}), providing the regression slopes b_d and b_{Ld}, respectively. Standard errors were assessed by jackknifing data over each locus. To assess if SGS better matched predictions of IBD in two dimensions (i.e. kinship decreasing linearly with the logarithm of the distance) or predictions of a contact between two spatially segregated differentiated gene pools (i.e. kinship decreasing linearly with the distance), the coefficients of determination were compared for each type of regression. To visualize SGS, kinship coefficient values (population relatedness) were averaged over a set of distance intervals (d), giving $F(d)$, and plotted against geographical distance. To test for SGS, spatial positions of individuals were permuted 9,999 times in order to obtain the frequency distribution of b_d and b_{Ld} under the null hypothesis that F_{ij} and *dij* or ln(d_{ij}) are uncorrelated (*cf*. Mantel test). To compare the extent of SGS among populations over the same spatial scale, we calculated $b10_{Ld}$, the regression slopes of F_{ij} on ln(d_{ij}) for d_{ij} = 100 km. We then calculated the statistic *Sp* (Vekemans & Hardy 2004; Hardy *et al*. 2006) as $Sp = -10Ld/(1 - FN)$, where *F*N is the mean F_{ij} between neighboring individuals, which was approximated by $F(d)$ for the first distance interval (d_{ij} < 300m).

The gene dispersal between males and females were assessed by comparing the SGS of females and males using the software SPAGEDI (Hardy & Vekemans 2002) as described above; the two species (*degraaffi* and *jacksoni*) were assessed separately. A student t-test was used to assess for significant difference between males and females.

3.3. Results

3.3.1. Microsatellite variability

The 12 markers developed for, and tested on *P. jacksoni*, were all polymorphic (see Table 3.2). However, three of these markers did not amplify for *P. degraaffi*, with the remaining nine being polymorphic (Table 3.2). As one of our aims was to provide a comparison between these two species, we include only the nine markers that amplified in both species in all subsequent analyses. All populations were in HWE, and no null alleles were detected for either *P. jacksoni* or *P. degraaffi*. Per locus observed heterozygosity (H_O) ranged from 0.21 to 0.92 for *P. degraaffi* and from 0.25 to 0.78 for *P. jacksoni*, while expected heterozygosity (H_E) ranged from 0.55 to 0.86 for *P. degraaffi* and from 0.50 to 0.88 for *P. jacksoni* (Table 3.2). The genetic variation at each locus across all populations (see Table 3.2 and Table 3.3) indicates that these loci are highly polymorphic across both species. All the markers contributed approximately equally to the overall typology as shown by the similarity values (Figure 3.2).

3.3.2. Species assignment and landscape genetic analysis

The result form individual assignment into species using STRUCTURE showed that, at least, two species occurred at each site (Figure 3.3). The modal value of ΔK was $K = 2$ (Figure 3.4), confirming the presence of the two species. Some individuals (17 %) depict a combination of characters from both species which may indicate introgression between *P. degraaffi* and *P. jacksoni* (see Figure 3.3). When considering only individuals belonging to *P. jacksoni*, two distinct lineages were found (see Figure 3.5). Again, considerable introgression (45 %) was evident between these (cryptic) lineages.

3.3.3. Population genetic analysis

Genetic diversity, as measured by the number of alleles and heterozygosity varied among the different sites included in this study (see Table 3.3). Specifically, the number of alleles detected per site varied between 3 and 12 for *P. degraaffi* and 6 to 14 for *P. jacksoni* (the effective number of alleles were lower). Private alleles were found in all of the sampled sites (Table 3.3). The observed heterozygosity (Ho) ranged from 0.40 (site 6) to 0.64 (site 9) for *P. jacksoni*; 0.50 (site 4) and 0.69 (site 6) for *P. degraaffi*.

3.3.4. Population differentiation

The lower pair wise population F_{ST} values were between site 6 and site 8 (0.038) for *P. degraaffi* and site 1 and site 6 as well as site 6 and site 9 (0.030) for *P. jacksoni*; while the higher values were between site 5 and site 9 (0.125) for *P. degraaffi*, sites 2 and 7 (0.083) for *P. jacksoni* (see Table 3.4), and showed some correlation with geographical distance.

3.3.5. Spatial genetic structure (SGS)

The values of the kinship coefficient over a set of distance classes were plotted against the geographical distance (Figure 3.6). Within each species, *bd* and *bLd* were both significant. For *P. degraaffi*, the kinship coefficient decreased with the log-linear relationship of the distance, as expected for SGS pattern resulting from IBD in a two-dimensional space. In contrast for *P. jacksoni*, the kinship coefficient decreased with the linear relationship of the geographical distance, as expected for a SGS pattern resulting from a contact zone between two differentiated gene pools (Figure 3.6), reflecting the presence of two genetic lineages. Patterns of SGS within each of these lineages are difficult to interpret because sample sizes become too small in the subgroups for any further analyses. Moreover, most individuals (45%) presented genetic admixtures so that it was not possible to completely remove the influences of historical events on SGS to assess the impact of the IBD.

The gene dispersal showed a biased toward females for the two species (Figure 3.7). The Student t-test performed on the slopes of regression revealed that the estimates are significant for both species ($p<0.005$; Table 3.5).

3.4. Discussion

3.4.1. Species assignment

Traditionally, phylogeographic studies of animals are based on mtDNA sequences (Zink & Barrowclough 2008), but the inferences could be affected by factors such as sex-biased dispersal (see Castella *et al*. 2001), absence of recombination that leads to separation of gene and species life histories (Lawson Handley & Perrin 2007), or introgression during past hybridization events (see Currat *et al*. 2008). As such, the inclusions of nuclear markers that are variable enough to detect variation are crucial to fully understand population patterns and processes. Our microsatellite data confirmed the presence of the two *Praomys* species, *P. degraaffi* and *P. jacksoni*, and revealed low levels of introgression between them (Figure 3.3). Within *P. jacksoni*, two lineages were obtained confirming the results of DNA sequence data (see Chapter 2). All individuals (109) but eleven (of which two were hybrids) had the same group membership (see Appendix 3). High levels of introgression were observed here (Figure 3.5). Although some analyses suggested the presence of two lineages within *P. degraaffi*, smaller sample sizes precluded firm conclusions about the validity of these lineages.

According to Arnold (2006), hybridization followed by introgression between recently diverged species with incomplete reproductive barriers is one of the main processes generating the genomic heterogeneity in species. Hence, in multi-allelic systems evolving under balancing selection, repeated exchanges of alleles promoted by adaptive introgression may be expected between closely related lineages as long as fertile hybrids can be formed (Castric *et al.* 2008).

3.4.2. Population genetic analysis

The allelic patterns (H_O, H_E, NPA and Nm) varied widely between populations (Table 3.3) that were at HWE at all loci. At population level, the results revealed significant intra-population genetic diversity (allelic richness and heterozygosity). Although the higher diversity (number of alleles) observed at site 6 might be the result of higher sample sizes, analyses of sequence data (see Chapter 2) suggest this central region as the contact zone between the two lineages (Figure 3.8). No deficiency in heterozygosity was detected in *Praomys* as observed for *Gerbillurus paeba* (Meyer *et al*. 2009). This diversity may be explained by the recurrent gene flow between populations

compared to other small mammals such as the Martino vole where fragmentation and geographic isolation of population led to lower genetic diversity (Buzan *et al*. 2010).

3.4.3. Population differentiation and phylogeography

Overall, the *Praomys* lineages retrieved resulted in clusters with no strong geographic association; specifically, the genetic discontinuities do not match any of the known geographic barriers such as lakes, rivers or mountains (Figure 3.8). This was a somewhat unexpected finding as fragmentation is typically reported for similar species with limited dispersal ability such as *Gerbillurus paeba* (Meyer *et al*. 2009) and *Dinaromys bogdanovi* (Buzan *et al.* 2010). The absence of significant geographic structure (measured by F-statistics, see Table 3.4) may not necessarily indicate high levels of gene flow (see Marko & Hart, 2011). However, we find no evidence to suggest that *Praomys* is significantly structured (at fine spatial scales) across its range. In fact, we find strong isolation by distance indicating that gene flow occurs over space and given enough time, may result in a more homogeneous genetic landscape for these rodent taxa.

3.4.4. Spatial genetic structure

Both species displayed a pattern of IBD. Smaller samples sizes in *P. degraaffi* precluded conclusive genetic analyses to determine whether the genetic variation is structured across the landscape. This species is confined to higher altitudinal areas and cannot freely move between these through the lower lying areas separating them. In contrast, altitude is not a limiting factor for *P. jacksoni.* At smaller spatial scales, it shows limited or no genetic differentiation between geographic localities and only separate into two genetic clusters at the scale of the Albertine Rift (i.e two differentiated gene pools recovered for the Albertine Rift with a contact zone in the vicinity of Lake Kivu). This structure is concordant with the demographic scenario retrieved from the DNA sequence data (see Chapter 2).

While some small mammal species have a male biased dispersal (e.g. *Microtis arvalis*: Hamilton *et al*. 2005; *Gerbillurus paeba*: Meyer *et al.* 2009), we found a female biased dispersal for *Praomys*. Although speculative, female may be the sex with the higher reproductive potential where competition is strongest, or they may simply try to avoid inbreeding between kin or may be males are territorial. However, to allow firm conclusions about dispersal, additional samples would need to be included.

3.4.5. Comparison of microsatellites, DNA sequence data and morphometrics with respect to landscape genetic analyses

Patterns of differentiation and variation recovered from microsatellite analyses match the phylogeographic structure obtained from (mitochondrial) DNA sequence data and morphometrics. Specifically, the presence of the two species as valid genetic entities, as well as the presence of cryptic lineages within these (Figure 3.9). These results agree with earlier reports based on the cranio-dental (Verheyen & Bracke 1966; Misonne 1969; Rosevear 1969; Van der Straeten & Dieterlen 1987; Van der Straeten & Dudu 1990; Musser & Carleton 2005; Carleton *et al*. 2006) and DNA sequence data (Lecompte *et al*. 2002a, b, 2005; Nicolas *et al*. 2005) stating that *P. jacksoni* was a species composite in need of taxonomic revision. Nevertheless, some aspects such as the recurrent hybridization and introgression, important variables in explaining the biogeographic history of the region, could only be detected with the microsatellites.

The assignment of individuals to species using different methods (field based identifications, mitochondrial DNA and microsatellite markers) failed to reach a consensus for some individuals. While the field identifications were confirmed by the clustering to a set of known individuals (from GenBank) for the mitochondrial DNA sequence data, assignment to species in the microsatellite was based on the use of highly variable codominant nuclear markers coupled with quantitative (i.e. Bayesian) methods. In addition, the parapatric distribution of the *P. jacksoni* lineages and the time to divergence suggest that what was recognized as a single species could well be a composite of cryptic species. Although reproductive isolation is not achieved, *P. jacksoni* lineages could have differentiated into ecotypes whose distribution would depend on factors to elucidate such as precipitation and vegetation were suggested for African primate sub-species (Dandelot 1965). Whether the cryptic diversity within *Praomys degraaffi* represent valid lineages needs further investigation (they probably do), however, the genetic lineages within *P. jacksoni* should at least be seen as distinct evolutionary lineages and may in fact represent valid species. Indeed, valid species can exchange genes (see e.g. Nevado *et al*. 2011).

Our data do not provide evidence of the populations being affected by the LGM or the Pleistocene changes (see also Pickford 1995; Livingstone 1967, 1975; Rosendahl 1987) that resulted in the formation of the lakes and the change of the course of the rivers (30,000 – 20,000 years BP; Beadle 1981; McClanahan & Young, 1996). In the absence of genetic patterns linked to physical barriers, the geological and climatic events prior to the Pleistocene most plausibly explain the divergence because the lineages predate the Pleistocene (>3 Mya; see Chapter 2). The impact of global climatic changes caused by Croll-Milankovitch cycles could have been accentuated by a general global cooling during the last 2.8 million years that led to large glacial peaks following the orbital precession cycles (see Kutzbach & Street-Perrott 1985; Bartlein & Prentice 1989; deMonocal 1995; Skinnes & Porter 1992), and might have caused

changes in the size and location of species geographical distributions (Dynesius & Jansson 2000; Bennett 2004). The discrepancy between the smaller sequence divergence estimates of *P. jacksoni* lineages and the geographic distances between sites separated by the barriers such as lakes, rivers or mountain ranges could mean that populations might have experienced successive phases of isolation before each range expansion; however, the short stable periods of isolation during Milankovitch oscillations or harsh climatic events were generally not long enough for gradual speciation to be completed before a new climatic shift reconnects the isolate with the main population (Coope 1995). Late Miocene (10-5 Mya) was a period of savannas expansion, while the early Pliocene (5-3.5 Mya) was characterized by moist climate associated with expansion and diversification of rain forests and the contraction of savannas (Partridge et al. 1995b) when forest blocs in the east were fragmented. The late Pliocene and early Pleistocene (3.5-1.6 Mya) corresponded to pronounced climatic changes with phases of drying and cooling, causing savannas expansion and open environment in tropical Africa associated with concomitant contraction of humid forests.

The diversity of lineages within the *Praomys* species across the Albertine Rift may indicate, as also stated by Robbrecht (1995) and Fjeldså & Lovett (1997) that the Albertine Rift acted as a mountain rain forest refuge. The remnant isolated populations surviving in refugia may have differentiated, giving rise to new species (Querouil *et al*. 2003) either within the refugia during isolation or at the margins of refugia during phases of populations expansion into new habitats (Graham *et al*. 2005). In primates (Telfer *et al*. 2003; Anthony *et al*. 2007), past fragmentation did not lead to speciation but strongly increased genetic differentiation between populations originating from recolonization from refugia. This can, at least in part, be true for *Praomys* where lineages might have differentiated in refugia from where, populations subsequently could have expanded.

1.5 Implications for conservation

This study is the first assessment where specimens are from the entire Albertine Rift (north to south, west to the sites adjacent to the Congo basin) as compared to the previous works (Carleton *et al*. 2006; Huhndorf *et al.* 2007), allowing inferences to apply for the region. Both the mitochondrial DNA and microsatellite variation revealed significant patterns of divergence between clades within the Albertine Rift allowing the designation of OTUs. Prudent conservation practices would maintain the integrity of this separation while investigations continue to unravel the status of each clade. Conservation priorities needs to incorporate scientific data on the existing taxa and the processes associated to the observed facts, highlighting the importance of taxa to ecosystem functioning and integrity. Long-term monitoring of species combining mark – recapture methods with molecular tools may provide reliable answers on the population trends.

Unfortunately, no study beyond simple trapping has been undertaken in the Albertine Rift where conservation priorities are based on flagship species such as gorillas, the other are overlooked while they are numerically important/abundant and can better show some patterns at local scale that can assist in explaining long-term fine scale trends. We suggest that while local genetic variability is of general importance, the short-term biological determinants of how this diversity arises and is monitored could be quite variable and follow different approaches from those usually envisaged.

Because the Albertine Rift has high endemism as a result of vicariance, climatic or geological events that occasioned the fragmentation of populations, we recommend the following three facets that require concerted attention to enhance comprehension of the biogeography of small mammal populations in Afromontane landscapes as suggested by Carleton *et al.* (2006): (1) convincingly vouch specific distributions and long-term site study to assess their ecological fidelity to Afromontane environments; (2) the need for improved taxonomic understanding of many genera and species using a combination of methods (e.g. molecular and morphological associated with site based monitoring); and (3) implicate historical biogeographic events in the genesis of kinship patterns.

Table 3.1. The geographic locations of the 10 sampling sites included in the present study. The specimens (species and number) collected at each site are indicated. Site numbers correspond to those presented in Figure 1 and the text. *Pd* = *Praomys degraaffi, Pj* = *Praomys jacksoni*. "–" indicates that the species was not recorded.

Site	Sampling locality	Longitude	Latitude	altitudes	*Pd*	*Pj*	Observation
Site 1. Okapi Faunal Reserve	Lenda	28.64970	1.57500	814 m	-	3	Lowland
	Epulu River right bank	28.57120	1.40300	758 m	-	4	Lowland
Site 2. Ruwenzori Mts. NP	Mubuku River bank	29.98330	0.36660	2652 m	-	10	Mountain
Site 3. Mts. Tshiabirimu	Musavaki valley	29.44039	-0.10013	1950 m	4	1	Mountain
	Kalibina River	29.44608	-0.12322	2335 m	3	-	Mountain
	Tukote	29.41980	-0.14089	2965 m	4	-	Mountain
Site 4. Bwindi Impenetrable NP	Buhoma	29.66139	-1.07778	2503 m	3	12	Mountain
Site 5. Echuya - Mgahinga	Echuya Forest Reserve	29.83306	-1.30028	2383 m	3	-	Mountain
	Mgahinga Gorilla NP	29.64194	1.38806	2980 m	3	-	Mountain
Site 6. Kahuzi Biega NP	Tshibati	28.46810	-2.13170	1900 m	4	2	Mountain
	Mbayo	28.47146	-2.13683	1870 m	5	3	Mountain
	Musisi swamp	28.80675	-2.28161	2149 m	5	2	Mountain
	Lwiro (Research Station)	28.47146	-2.13183	1700 m	-	8	Mountain
	Mugeri (Seminary)	28.86380	-2.21600	1493 m	-	13	Mountain
Site 7. Idjwi Island	Bigarhula	29.12850	-2.28908	2255 m	-	10	Mountain
Site 8. Kibira NP	Muramuya	29.56660	-3.21660	2104 m	6	4	Mountain
	Teza (Park headquarters)	29.58330	-3.23330	1543 m	4	1	Mountain
Site 9. Itombwe Forest	Lusasa	28.57289	-3.43270	2090 m	4	6	Mountain
	Nabindu	29.01400	-3.36874	2960 m	4	10	Mountain
	Miki	28.41070	-3.21480	2026 m	-	4	Mountain
Site 10. Mts. Kabobo	Mizimu	29.16706	-5.28758	1250 m	2	6	Mountain
	Talama	29.05353	-4.59068	2025 m	-	9	Mountain
TOTAL					54	108	

Table 3.2. Characteristics of the 12 microsatellite primer pairs developed for *Praomys jacksoni*. Repeat motifs, dye employed for genotyping, size range of the alleles in base pairs as well as the annealing temperature is presented. The number of alleles (A) as well as the mean observed (H_O) and expected (H_E) heterozygosities are given for *P. jacksoni* and *P. degraaffi*. N/A denotes those loci that failed to amplify in *P. degraaffi* and that excluded from subsequent analyses. *Pd = Praomys degraaffi, Pj = Praomys jacksoni.*

Locus	Primer sequence (5'- 3')	Repeat	DYE	Size (bp)	A		H_O/H_E		Tm
					Pd	*Pj*	*Pd*	*Pj*	
PK11(*)	F: GCAAAAGCCACAAGTGCTC R: AGGCACCTGTCCTCAAGTGT	$(TG)_{21}$	NED	92	N/A	31	N/A	0.52/0.75	60
PK12(*)	F: TGCAATAAAAGTGCCATCCA R: TAGAGGTCGCAGAGGCCTAA	$(TG)_{21}$	VIC	121	NA	16	N/A	0.78/0.88	60
PK13	F: GGCATATATTGAGAACACAGAAACA R: TTCAATTCCCAGCAATCACA	$(TG)_{19}$	PET	155	17	20	0.92/0.63	0.52/0.68	60
PK21	F: AGTGGCTGCTTAGGGTGATG R: GGGGTGAGTTTAAGGGCAAC	$(TGTC)_{11}$	NED	160	21	19	0.76/0.86	0.25/0.50	60
PK22	F: CATGGACATATGCTGCACAA R: AGGCTAGGACACAGGTTGGA	$(AC)_{21}$	VIC	188	15	18	0.82/0.84	0.67/0.80	60
PK23(*)	F: ATTATCCTCCGACCTCCACA R: GGAAAAAGTGTTTGGCTGAAA	$(AC)_{15}$	PET	214	N/A	26	N/A	0.71/0.83	60
PK24	F: TTCAGCTTTTAACAAACCAACAAA R: AATTTTGACACACAGCCCATT	$(AG)_{19}$	6–FAM	162	16	19	0.21/0.55	0.46/0.77	60
PK32	F: CACACTTGCACATTCACATAAA R: ATGTTGGGGTTGGTGTCATT	$(AG)_{21}$	VIC	247	18	28	0.55/0.73	0.51/0.78	60
PK33	F: GCAGACACCTTTCCCCTCTT R: GAAGGAGGAGGATAGAAGGACA	$(ATCC)_{11}$	PET	286	17	21	0.44/0.81	0.36/0.81	60
PK34	F: GCTGCAATGAAACATACATGC R: CCAGTTGGTCTCTGCTCTCC	$(TG)_{20}$	6–FAM	227	15	15	0.74/0.79	0.61/0.76	60
PK41	F: GCTTTGAAGCCAGATTTCCA R: GGATGGGTGGGCAGATAGAT	$(TATG)_{12}$	NED	297	21	26	0.48/0.67	0.54/0.80	60
PK52	F: GGACAGTGGCAAGAACCTGT R: CAGATGCCCTGGGACTAGAA	$(TG)_{20}$	VIC	356	16	18	0.68/0.84	0.45/0.78	60

Table 3.3. Genetic diversity for P. jacksoni (*Pj*) and P. degraaffi (*Pd*), as measured by the number of alleles (Na), effective number of alleles (Ne) as well as observed (H_O) and expected (H_E) heterozygosity values for each site (sample sizes (N) for each of the sites are given). Site numbers correspond to those given in Table 1. The fixation index (F) as well as the number of private alleles (NPA) are given. *Pd* : *P. degraaffi*; *Pj*: *P. jacksoni*.

Population	N		A		Ne		H_O		H_E		F		NPA	
	Pd	*Pj*	*Pd*	*Pj*	*Pd*	*Pj*	*Pd*	*Pj*	*Pd*	*P j*	*Pd*	*P j*	*Pd*	*P j*
Site 1	-	9	-	9	-	7.15	-	0.56	-	0.81	-	0.32	-	4
Site 2	-	9	-	7	-	5.08	-	0.57	-	0.77	-	0.24	-	5
Site 3	11	-	7	-	5.17	-	0.65	-	0.74	-	0.17	-	2	-
Site 4	4	14	5	12	4.41	8.06	0.50	0.54	0.76	0.86	0.34	0.37	6	3
Site 5	5	-	5	-	4.33	-	0.67	-	0.66	-	0.01	-	6	-
Site 6	15	30	12	14	8.56	8.74	0.69	0.64	0.87	0.84	0.21	0.24	19	9
Site 7	-	9	-	6	-	4.32	-	0.52	-	0.71	-	0.29	-	2
Site 7	11	6	9	7	5.65	4.94	0.58	0.47	0.80	0.76	0.29	0.40	14	1
Site 4	9	17	7	9	5.35	5.52	0.58	0.40	0.74	0.79	0.26	0.50	5	3
Site 5	2	14	3	11	2.70	6.78	0.67	0.53	0.61	0.82	0.10	0.36	4	8

Table 3.4. The measure of population differentiation (F_{ST}) for the pairwise combinations of *Praomys* in the Albertine Rift. Above the diagonal is *P. degraaffi*, below is *P. jacksoni*. – indicates that the species was not recorded.

Populations	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10
Site 1	-	-	-	-	-	-	-	-	-	-
Site 2	0.046	-	-	-	-	-	-	-	-	-
Site 3	-	-	-	0.075	0.116	0.058	-	0.076	0.100	0.115
Site 4	0.032	0.045	-	-	0.091	0.052	-	0.055	0.092	0.123
Site 5	-	-	-	-	-	0.080	-	0.058	0.125	0.124
Site 6	0.030	0.038	-	0.024	-	-	-	0.038	0.063	0.101
Site 7	0.067	0.083	-	0.062	-	0.056	-	-	-	-
Site 8	0.056	0.056	-	0.042	-	0.034	0.076	-	0.072	0.101
Site 9	0.048	0.068	-	0.041	-	0.030	0.064	0.045	-	0.107
Site 10	0.056	0.071	-	0.040	-	0.035	0.077	0.051	0.031	-

Table5. Indices of significance to test for dispersal distance between males and females of the species *P. degraaffi* and *P. jacksoni*.

Species	Sex	Jacknife - estimator	Slope	Value t-test	p value	Observation
P. jacksoni	M	0.0063	-0.000066	-0.0032	0.001748	significant
	F	0.1885	-0.000025			
P. degraaffi	M	0.9756	0.00001	6.2231	0.000012	significant
	F	0.0567	-0.00015			

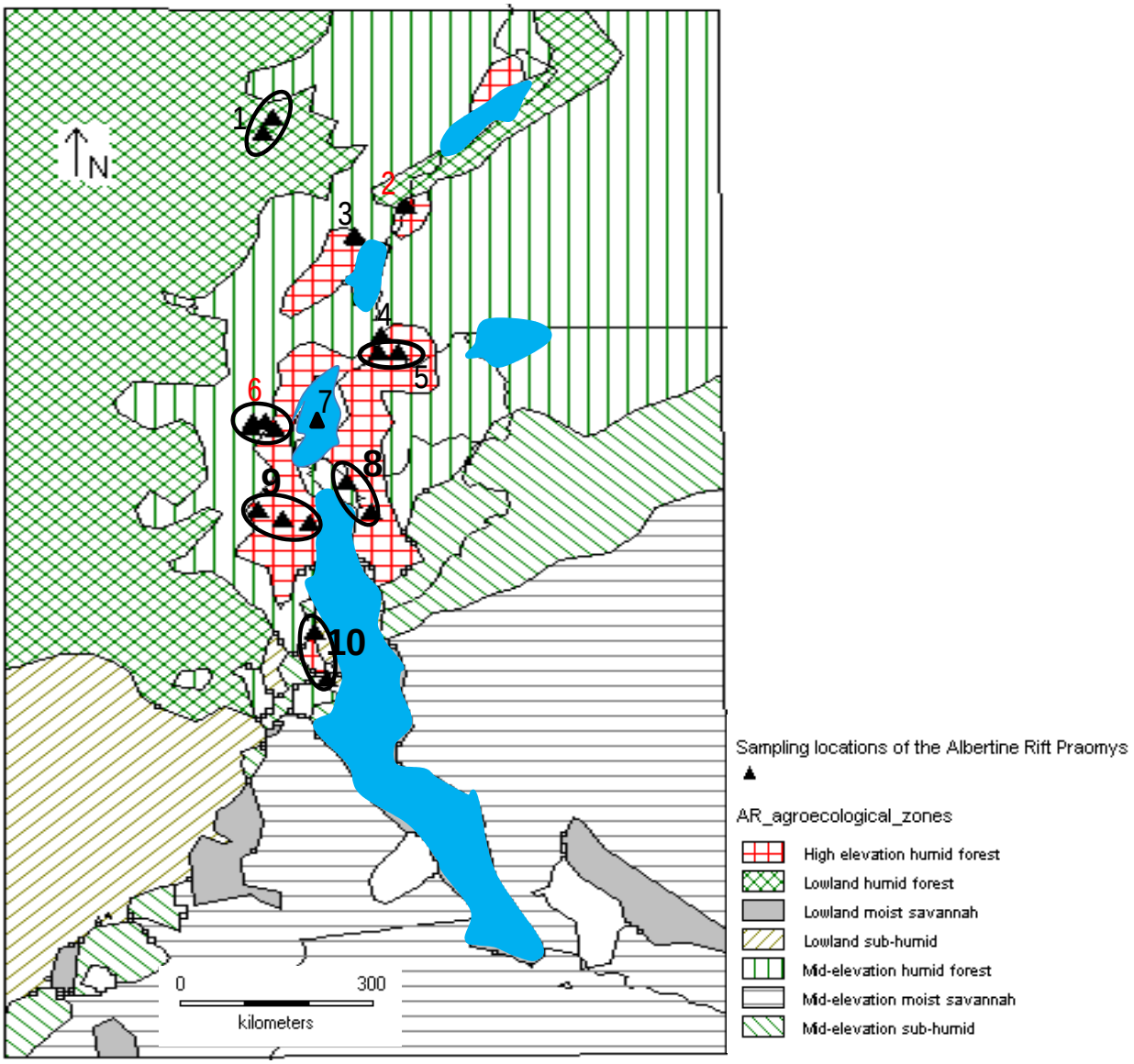

Figure 3.1. Geographic placement of the Albertine Rift (in white shading) indicating the geographic sampling sites included in the study. Sites are listed from north to south. site1: Okapi Faunal Reserve, site 2: Ruwenzori Mountains National Park, site 3: Mount Tshiabirimu, site 4: Bwindi Impenetrable National Park, site 5: Echuya Forest Reserve and Mgahinga Gorilla National Park, site 6: Kahuzi – Biega National Park, site 7: Idjwi Island, site 8: Kibira National Park, site 9: Itombwe forest, site 10: Mount Kabobo. In some instances, sampling sites in geographic close proximity and with no obvious geographic discontinuities separating them were considered a single locality; these sites are circled.

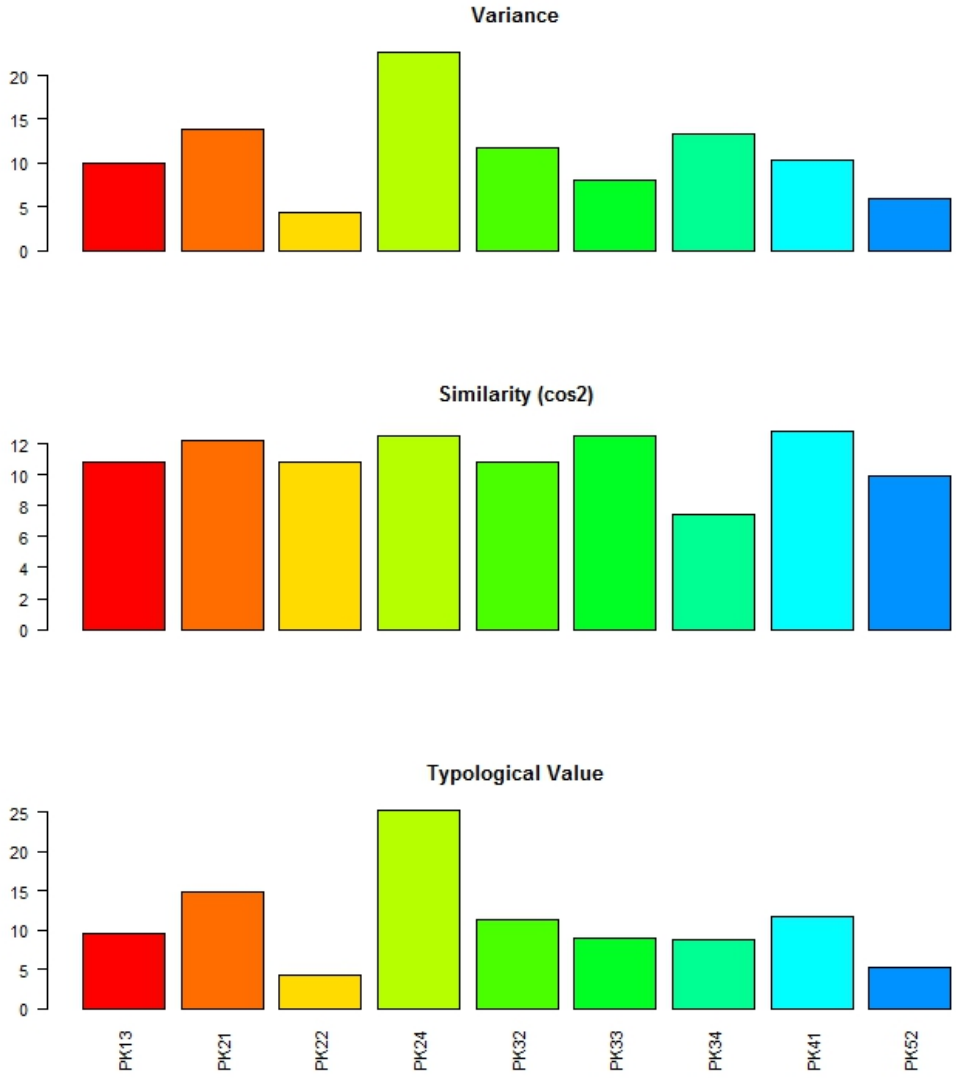

Figure 3.2. Diagrams of the typological value components in % for each locus used in the analyses. The similarity value shows the contribution of each marker.

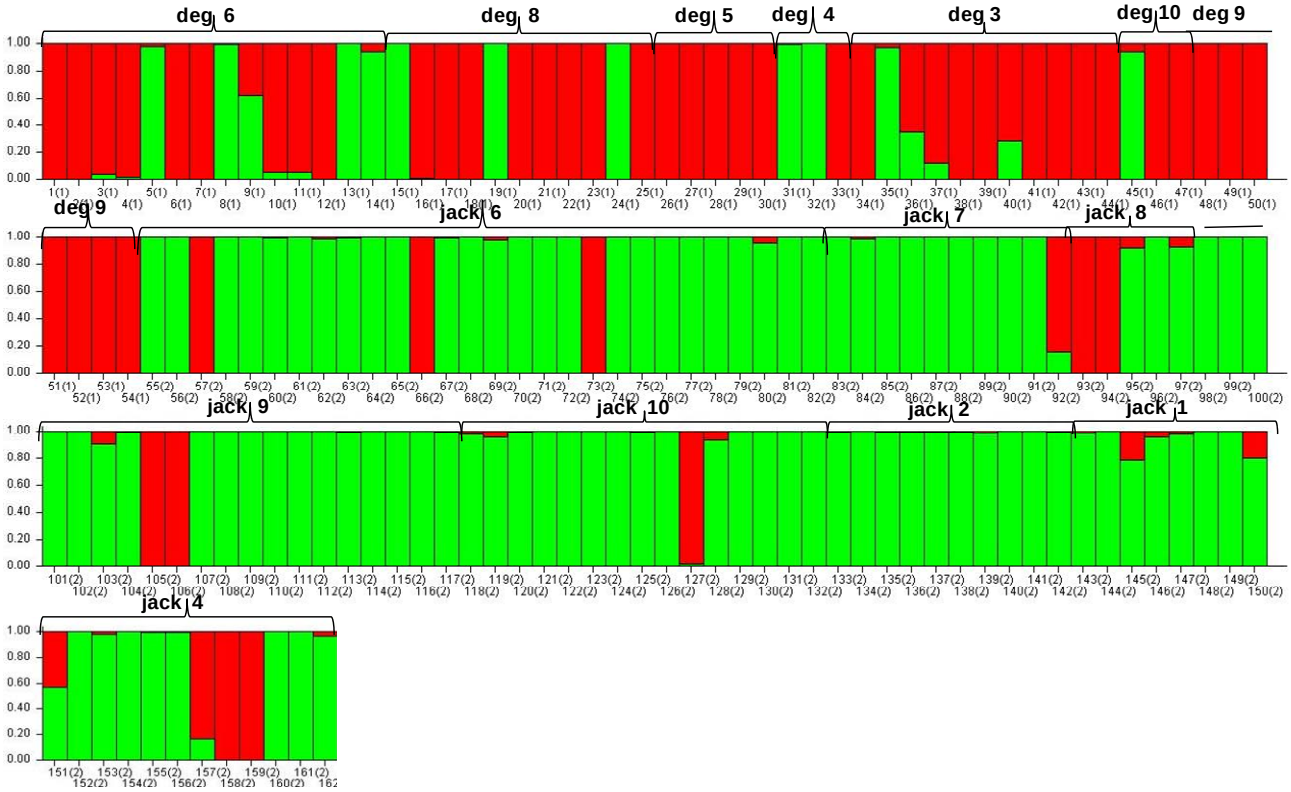

Figure 3.3. Clustering of individuals following the program Structure. Red is *Praomys degraaffi*, green is *P. jacksoni.* deg and jack represent the species *degraaffi* or *jacksoni* respectively. The associated number indicates the site the specimen was collected.

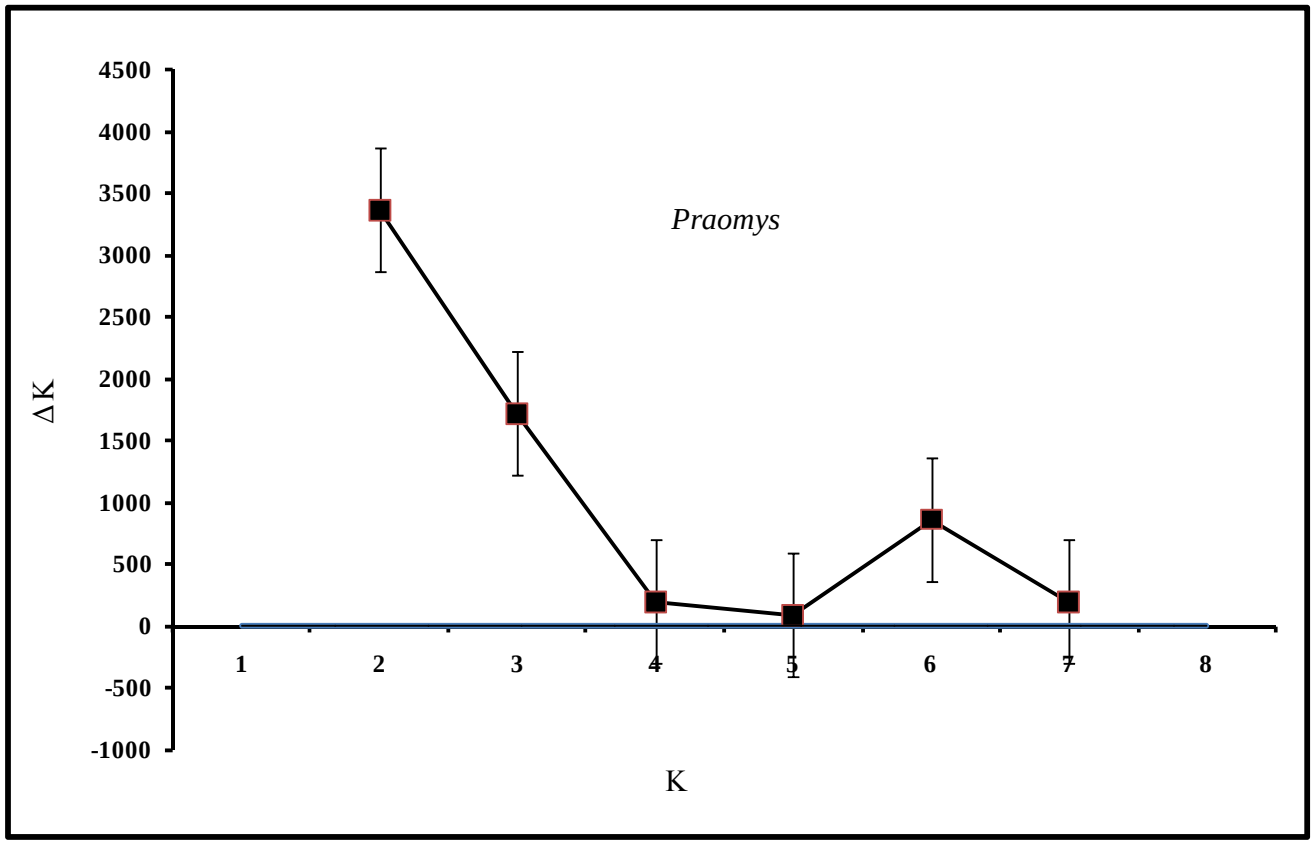

Figure 3.4. K value, the more probable number of groups (species in this case) for the sample considered. (D) Δ*K* calculated as Δ*K* = *m*|*L*"(*K*)|/ *s*[*L*(*K*)]. The modal value of this distribution is the true *K*(*) or the uppermost level of structure.

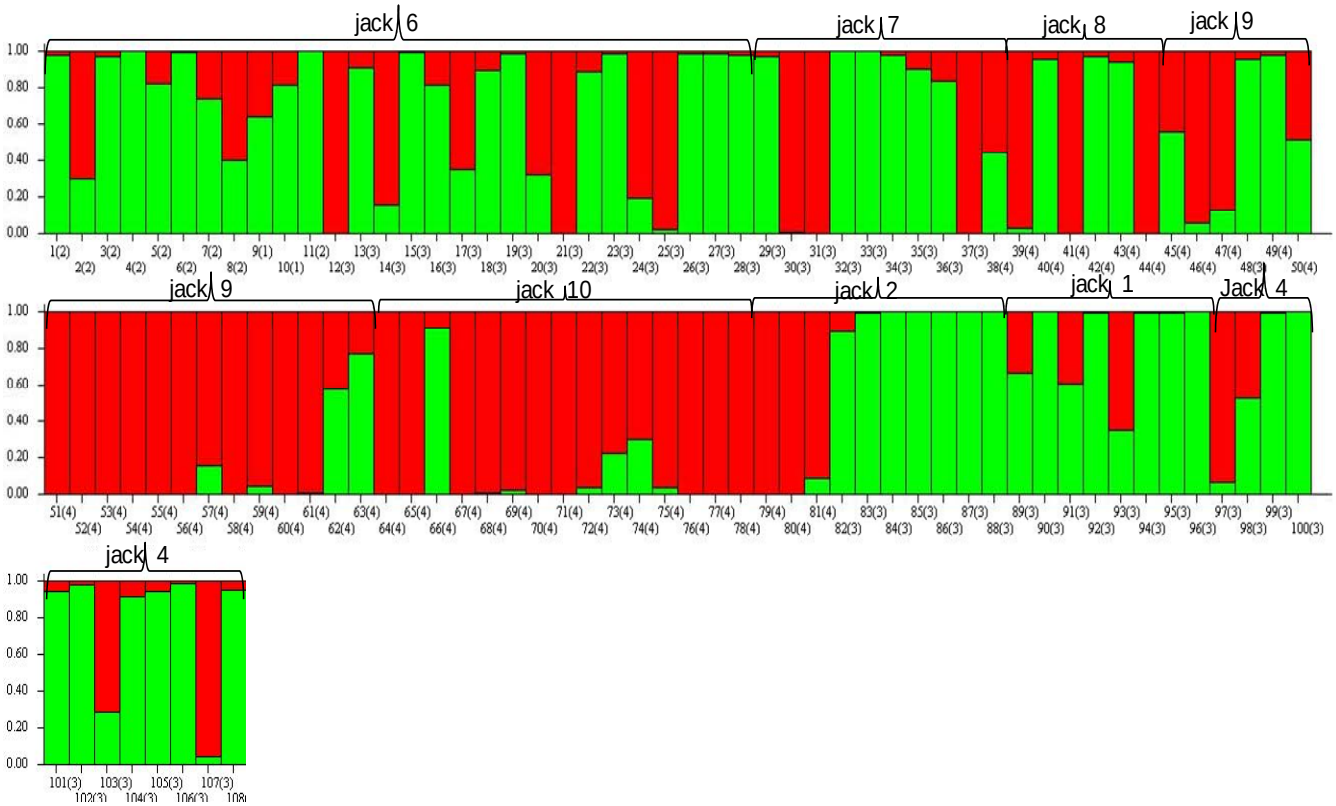

Figure 3.5. Clustering of individuals within *P. jacksoni* clades: green: clade 1 and red: clade 2.

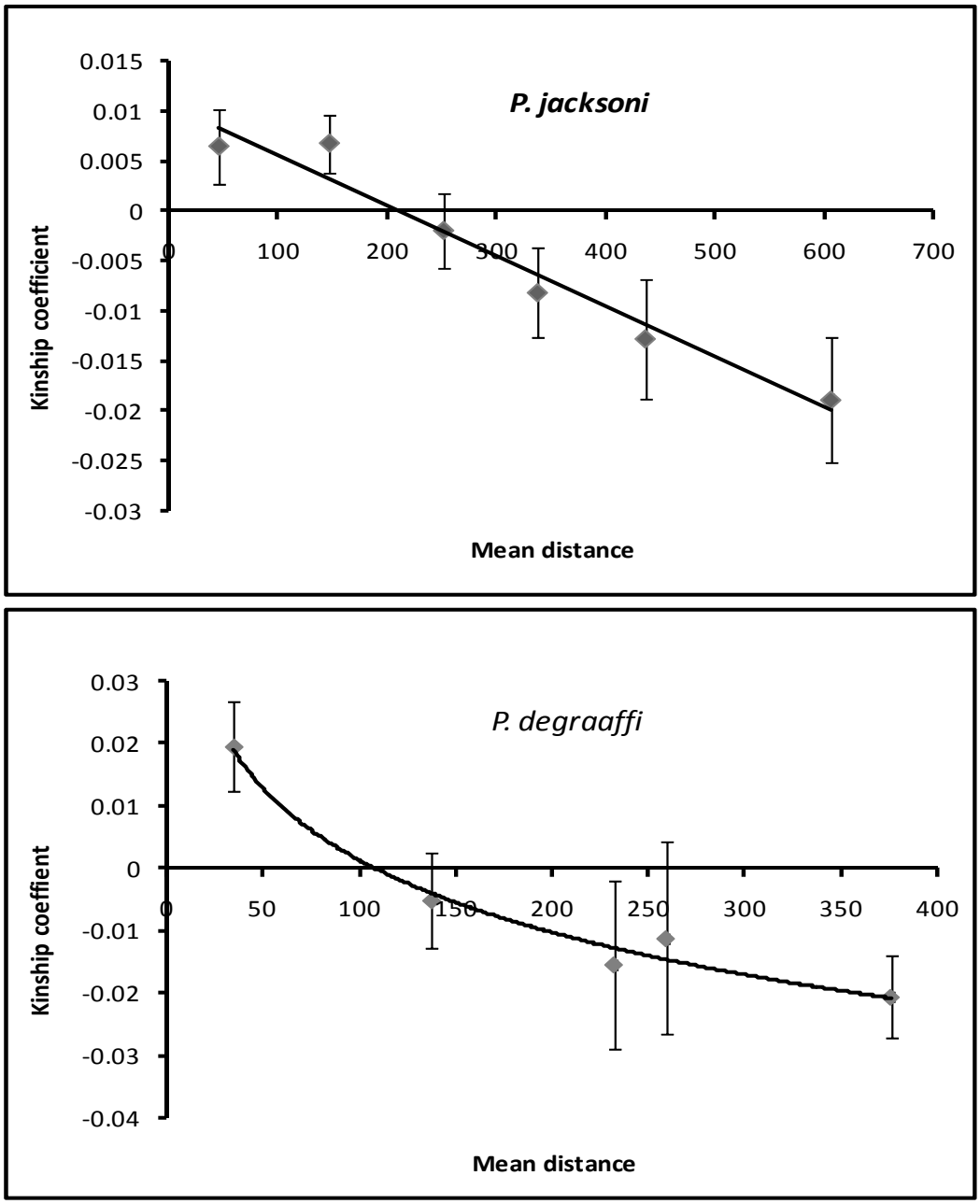

Figure 3.6. Kinship coefficient plotted against mean distance for both species used separately.

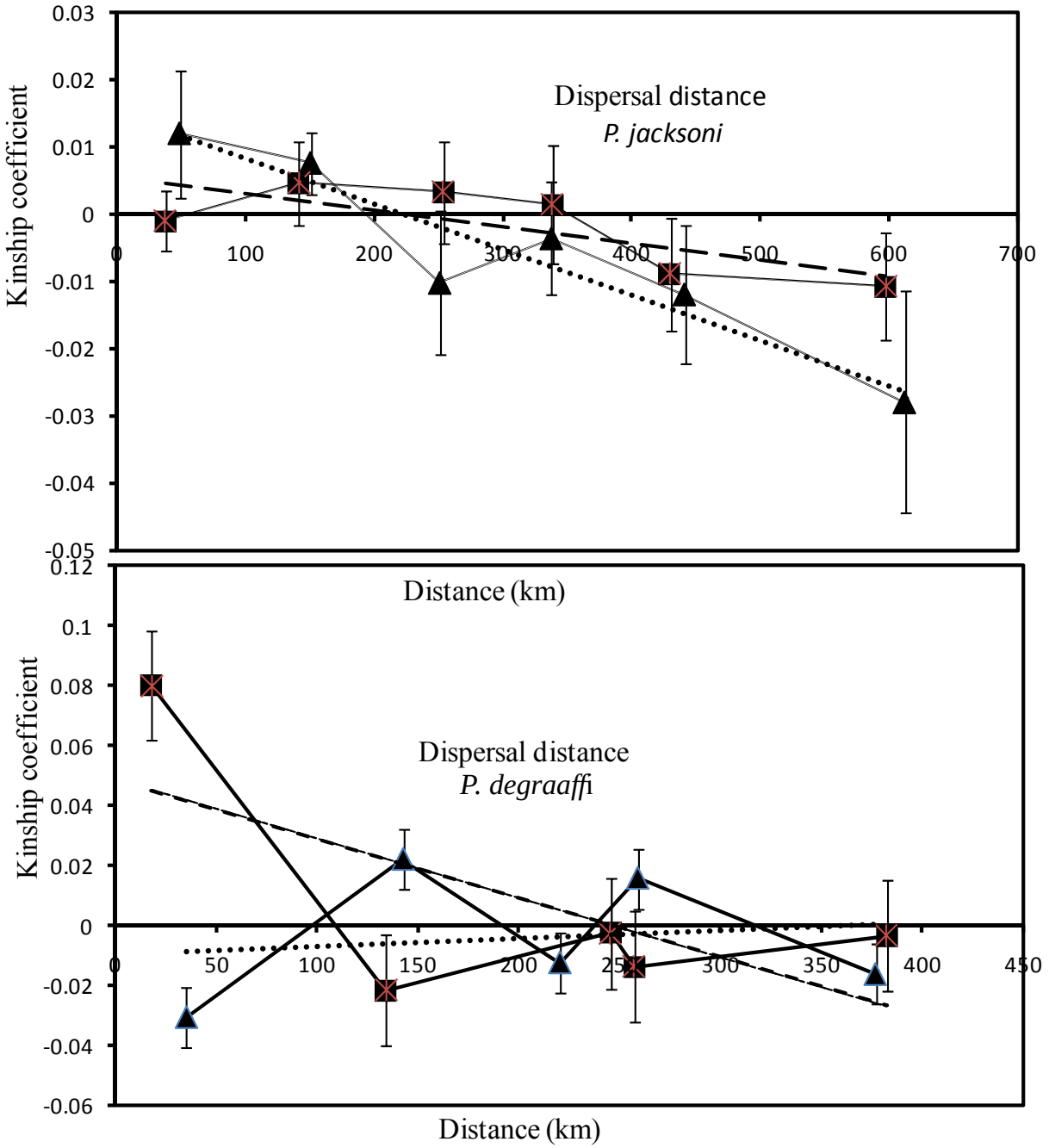

Figure 3.7. Regression of pairwise kinship coefficient on the geographic (dispersal) distance (km) for *P. degraaffi* and *P. jacksoni.* Triangle shapes represent males and squares, females.

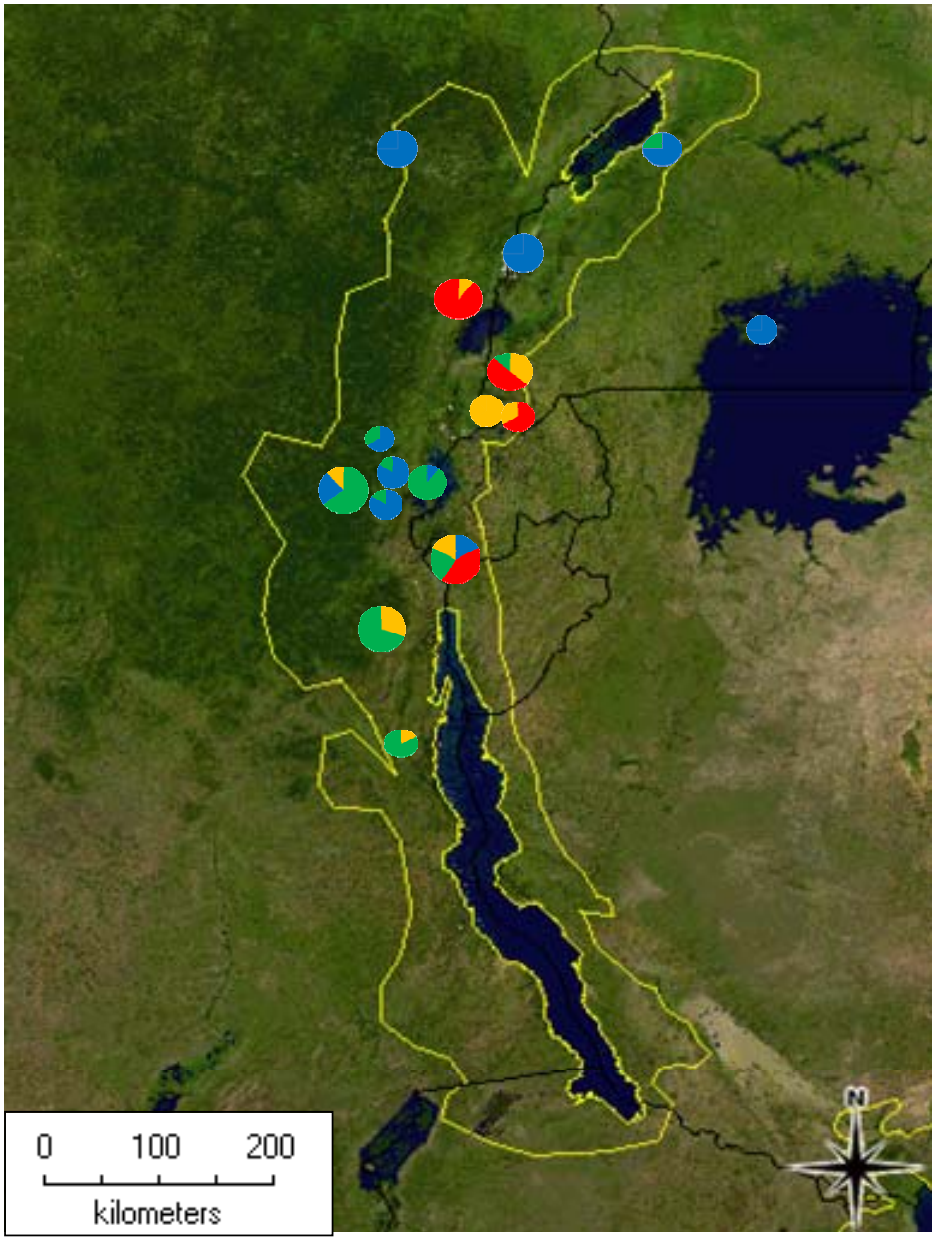

Figure 3.8. Population subdivision following the schematic network of the clades for the two focal *Praomys* species in the Albertine Rift. Blue color represents clade 1 – *P. jacksoni*, green: clade 2 – *P. jacksoni*, red: clade 1 – *P. degraaffi* and orange: clade 2 – *P. degraaffi* (Map adapted from Plumptre *et al.* 2003).

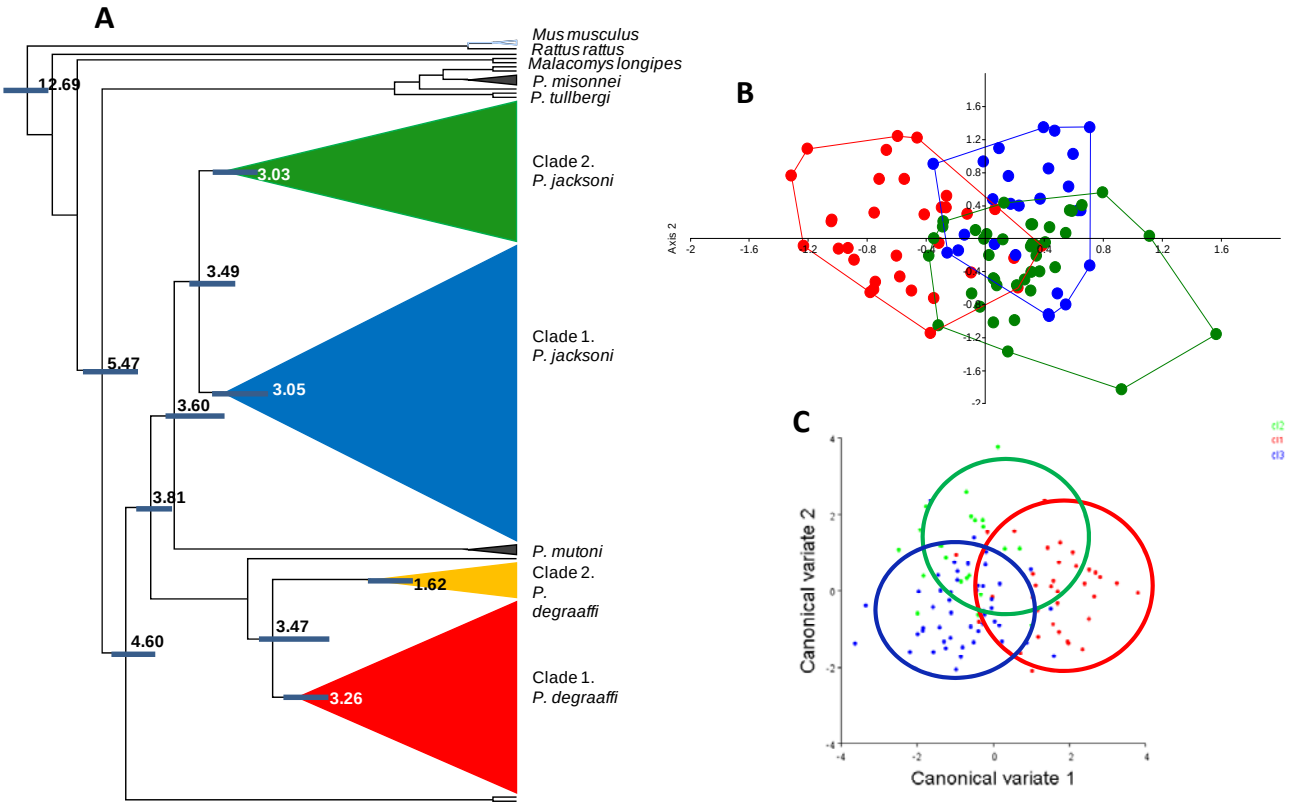

Figure 3.9. Bayesian tree (A) and CVA clusters for the traditional (B) and geometric (C) morphometrics for *Praomys* sp. from the Albertine Rift. Blue color represents clade 1 – *P. jacksoni*, green: clade 2 – *P. jacksoni*, red: clade 1 – *P. degraaffi* and orange: clade 2 – *P. degraaffi*. Numbers on the Bayesian tree are the divergence times.

Chapter 4

Origin and putative colonization routes for invasive rodent taxa in the Democratic Republic of Congo

4.1. Introduction

Biological invasions are a pervasive environmental and costly economic problem (McNely 2001; Hingston *et al*. 2005) and include threats to *in situ* conservation, the persistence of local endemics (Goodman 1995; Hingston *et al*. 2005) as well as agricultural yield and hence food security (Dieterlen 1966; Singleton *et al*. 1999; UNEP 2002; Olson 2006). The scientific literature has seen important debates surrounding theoretical concepts and terms used in biological invasions (see for example Valéry *et al*. 2009; Wilson *et al*. 2009a,b). In addition, numerous empirical studies have reported on invasive species and have contrasted various aspects among native and introduced ranges (Hingston *et al*. 2005; Searle 2008; van Wilgen *et al*. 2008; Hulme 2009). Surprisingly, fewer studies attempt to reconstruct biological invasion pathways (Dieterlen 1979; Hingston *et al*. 2005; Muirhead & Macisaac 2005; Jansen van Vuuren & Chown 2007; Searle *et al*. 2009a, b; Tollenaere *et al*. 2010). Routes of introduction increasingly form an integral part of risk assessments, and an understanding of the actual pathway of introduction can help to prevent further introductions (Sellens *et al*. 2007; van Wilgen *et al*. 2008; Hulme 2009). In this paper we provide the first preliminary evidence for reconstructing the introduction pathways for invasive rodent taxa (the house mouse, black/ship rat and brown/Norway rat) in the Democratic Republic of the Congo (DRC) using molecular data and information on historical human movements.

This Central African country is home to a significant portion of the world's biodiversity (WCMC 1992) with an increasing number of new species as being discovered and described (see Plumptre *et al*. 2007; Mukinzi *et al*. 2009 as well as unpublished reports). As is the case across the world, biodiversity within the DRC is threatened by various invasive species (see e.g. Pointier *et al.* 2005; McGinley 2007; Borchert *et al*. 2007; Khamis *et al.* 2008). Notwithstanding the obvious significant threats that invasive species pose, no national strategy exists on invasive species in the DRC and only a handful of opportunistic and unpublished studies on alien (exotic) species have been carried out to date.

With the exception of biological control, most introductions are accidental or opportunistic with species being passively transported through human or livestock movement (Searle *et al*. 2009a, b; Wilson *et al*. 2009a). As such, the colonization of several commensal species closely track the movement of humans and their livestock (Hingston *et al*. 2005; Rajabi-Maham *et al*. 2008; Searle *et al*. 2009a; Tollenaere *et al*. 2010). It is therefore not surprising that there was a drastic expansion in the distribution of several species closely associated with humans during the European Age of Exploration when colonial traders, explorers and surveyors transported organisms across major biogeographic barriers which previously prevented the spread of many species (Hingston *et al*. 2005; Robins *et al*. 2007). In addition, the African continent has a history of colonialism and slave trade and as such, many invasive alien species in Africa trace their origins back to countries actively involved in earlier colonial rule (Cloos 2003; Hingston *et al*. 2005).

Two of the most successful global invasive alien species are *Mus musculus* and *Rattus rattus* and the successful spread of both species is largely attributed to their close association with humans. Both species, together with *Rattus norvegicus*, are considered amongst the 100 worst global invaders (Lowe *et al*. 2000, Global Invasive Species Database www.issg.org). *Mus musculus* has its origin on the Indian subcontinent and with the spread of agriculture, expanded its range into the Middle East, Eurasia and is now found virtually worldwide (Nowak & Paradiso 1983; Musser & Carleton 2005; Pocock *et al*. 2005; Searle *et al*. 2009b). Reports from the eastern DRC include that of Misonne (1963) from Bukavu and Mount Ruwenzori. Four subspecies are generally recognized following Musser & Carleton (2005) (the eastern European house mouse *M. m. musculus*, the western European house mouse *M. m. domesticus,* the southeast Asian house mouse *M. m. castaneus* and *M. m. gentilulus*, found in Yemen) although the possibility of several other subspecies cannot be excluded (e.g. the Palestine house mouse *M. m. gazaensis*; Jaffa & Taher 2007). *Rattus rattus* whose origins trace to the Indian Peninsula (Nietammer 1975; Musser & Carleton 2005), may have spread to Egypt in the fourth century BC from where it spread globally along trade routes (see Hingston *et al*. 2005; Berdoy & Drickamer 2007; Tollenaere *et al*., 2010). Following a careful examination of skins and skulls, Dieterlen (1979) reported the presence of *R. rattus* in Tanzania, eastern Uganda and the eastern parts of the DRC, and traced the spread of the species along trading routes from Tanzania. More recent studies have suggested the presence of *Rattus* in the Ituri lowland and mountain forests in the eastern DRC (Katuala *et al*. 2005; Kaleme *et al*. 2007).

Although the presence of *Rattus* and *Mus* in the DRC is certain (Misonne 1963; Dieterlen 1985; Katuala *et al*. 2005; Kaleme *et al*. 2007), the exact taxonomic status for these alien species is uncertain as the earlier identifications were based on phenotype alone. Given morphological and phenotypic similarities between closely related species and subspecies, DNA sequence data provide a valuable means to confirm the presence of these pest species (down to subspecies level for *M. musculus*) in the DRC. By comparing the DNA profiles of these species from the DRC to those from other locations across Africa and Europe, and in concert with historical documentations of trade and other contact with Europeans, source populations can be identified and introduction routes inferred. This paper therefore aims to: (1) assess the taxonomic (species / subspecies) status of the populations present in the DRC, (2) provide information toward documenting the extent of occurrence of the species throughout the DRC, and (3) reconstruct the colonization history of the species using mitochondrial DNA data and historical records.

4.2. Material and methods

4.2.1. Samples

The known distribution of the house mouse in the DRC is largely confined to the western part of the country although older records suggest its presence in the east (Misonne 1963). More recently, anecdotal reports also raise the possibility of the

presence of the house mouse in the north-eastern part of the DRC, specifically around Kisangani. A total of 16 mouse samples were collected from the DRC in and around houses in two areas (see Figure 1). Twelve samples were from Kinshasa (five samples from the eastern suburbs [Kingasani and Masina] and seven samples from a suburb in the western part of Kinshasa [Kisenso]) and four samples were collected from Kisangani. Given the close historical ties and commercial links with Belgium, an additional four samples were included from Belgium (west of Brussels at Ceroux [n=2] and Louvain la Neuve [n=2]) as no data for the house mouse from Belgium are available on public databases. Sequences generated in the present study were aligned to data downloaded from GenBank which represent various countries and species from across the world. Table 1 provides a complete list of samples included in the present study.

The suspected distribution of rats (*Rattus* sp. *sensu lato*) in the DRC includes the entire country. It is believed that *R. rattus* has spread throughout the DRC, whereas *R. norvegicus* is restricted to the western and northern part of the DRC. To verify these anecdotal observations, a total of 35 rat samples were collected from four localities across the DRC (Figure 1 and Table 1). These samples were collected as 'rats' by co-authors on this project (Honoré Belesi, Benjamin Ndara, Sylvestre Gambalemoke, Prince Kaleme and Jacques Mwanga); no attempt was made to identify specimens in the field. For the Itombwe Forest, rats were collected away from homesteads (in the forest) as well as in and around houses. For Lwiro, Kinshasa and Kisangani samples were only collected in and around houses. Additional rat samples (n=5) were also collected from Tanzania (Ecorat project; see www.nri.org/projects/ecorat). Sequences generated in the present study were aligned to data downloaded from GenBank, representing various countries from across the world.

4.2.2. Laboratory methodology

DNA was extracted from tissue samples using a commercial DNA extraction kit (DNeasy Tissue Kit – Qiagen). As the mitochondrial DNA control region is hypervariable, and given that data are available from public databases, we targeted the 5' side of this DNA fragment. Standard polymerase chain reactions (PCR) were set-up following Jansen van Vuuren & Chown (2007). Amplicons were directly cycle sequenced using BigDye chemistry (version 3, Applied Biosystems). Electropherograms were checked using Geneious 4.8.5 (Biomatters Ltd 2010) and aligned manually using MacClade 4.06 (Maddison & Maddison 2000). Sequences were submitted to GenBank under accession numbers HM480108 to HM480167.

4.2.3. Data Analyses

Sequences generated were aligned to data downloaded from GenBank which represent various countries and species from across the world (see Table 1). For the mouse data, *Acomys chudeaui* and *R. norvegicus* were used as outgroups. For the rat data, *M. musculus* and *A. chudeaui* were used as outgroups. To investigate the species / subspecies status of

our samples, maximum likelihood trees (constructed in RaxML; Stamatakis *et al*. 2008) and Bayesian trees (constructed in MrBayes 3.1.2; Ronquist & Huelsenbeck 2003) were assembled. The models of evolution for the analyses were determined in jModeltest 0.1. (Posada 2008; Guindon & Gascuel 2003) using AIC criteria [*Mus*: HKY + $G_{0.264}$; *Rattus*: HKY + $G_{0.254}$]. For all analyses, individuals were treated as Operational Taxonomic Units. Posterior probabilities for the Bayesian trees was determined by running one cold and four heated chains for 1 000 000 generations with trees sampled every 100 generations. Parsimony networks depicting the relationships among haplotypes were constructed in TCS 1.21 (Clement *et al.* 2004).

4.3. Results

4.3.1. Mus musculus

Three hundred and twenty four base pairs of the mitochondrial control region were generated from 20 *Mus* specimens from the DRC (n=16) and Belgium (n=4). ML and Bayesian analyses resulted in congruent tree topologies (Figure 2), and revealed the presence of two very divergent lineages separated by > 16% uncorrected sequence divergence. A Blast Search in GenBank suggested one of these lineages to represent *Mastomys* sp. Although adult *Mastomys* and *Mus* are easily distinguished in the field, juveniles closely resemble one another and it is possible to confuse juveniles of both species in hand (but see Granjon & Duplantier 2009 for morphological differences between them). *Mastomys coucha* and *M. natalensis* control region sequences were added to our analyses to confirm the generic status of some of the "unknown" specimens in our study. *Mastomys coucha* is endemic to Southern Africa and although we have no reason to believe that it occurs in the DRC, we included it as data were available on GenBank for the control region and to broaden our comparative sequence base for "*Mastomys*". *Mastomys natalensis* has been reported from the DRC (Musser & Carleton 2005), and data for this species was available on GenBank for the control region (see also the work by Dobigny *et al*. 2008 for a discussion on *Mastomys* in western Africa). Other species of *Mastomys* such as *M. erythroleucus* and *M. pernanus* occur (or may occur) in the DRC (Musser & Carleton 2005) but representative specimens were not available, nor sequences in public data bases. Nine samples from the DRC grouped within the genus *Mastomys* with high support (see Figure 2A).

To confirm the taxonomic affinity of the house mouse in the DRC, data were aligned to *Mus* sequences downloaded from GenBank (see Table 1) representing three species (*M. caroli*, *M. musculus*, *M. spretus*) and five subspecies (*M. m. castaneus*, *M. m. domesticus*, *M. m. gentilulus*, *M. m. molossinus* and *M. m. musculus*). The house mouse in the DRC was confirmed as *M. m. domesticus* following our phylogenetic approach (see Figure 2A). Two haplotypes separated by a single mutational change characterized the seven DRC specimens collected from Kinshasa (Figure 2B). These haplotypes were shared with haplotypes reported from France, Cameroon, New Zealand and Morocco, and were separated by a

single mutational step to haplotypes from Germany and the United Kingdom (UK) and by two mutational steps from a haplotype from Belgium.

4.3.2. Rattus sp.

The data set for rat specimens from the DRC (n = 35) and Tanzania (n = 5) comprised 376 bp of the mitochondrial control region. Preliminary analyses revealed the presence of two divergent lineages separated by > 13% uncorrected sequence divergence. To confirm the species status of the rats in the DRC, specimens were aligned to sequences downloaded from GenBank (Table 1) representing five species (*R. exulans, R. norvegicus*, *R. rattus, R. tiomanicus* and *R. tanezumi*). *Rattus tanezumi* has been reported from Southern Africa (Bastos *et al*. 2011) and it is possible that this species is spreading northwards. Following the phylogenetic analyses (Figure 3A), three samples from Kisangani clustered with *R. norvegicus* whilst the remainder, collected from across the DRC and Tanzania, clustered within *R. rattus*.

For *R. norvegicus*, few sequences were available and our DRC samples clustered distantly (>10 mutational steps) with haplotypes from the UK, Denmark, the USA, Sweden, and French Polynesia (Figure 3B). For *R. rattus*, individuals from Kisangani (n=3) and Kinshasa (n=6) clustered with haplotypes from the USA, Samoa, South Africa, Papua New Guinea, French Polynesia, New Zealand, Senegal and the UK. One haplotype was shared between the eastern DRC (Lwiro and Itombwe), one specimen from Kinshasa, Tanzania, Madagascar and Mozambique (Figure 3C).

4.4. Discussion

Mitochondrial DNA sequence data unequivocally confirm the presence of *M. m. domesticus, R. norvegicus* and *R. rattus* in the DRC. In the absence of complete species representation for all *Mastomys* species, we cannot make any conclusive statements about the identity of the *Mastomys* samples collected, and merely draw attention to possible confusion with taxa when collecting juveniles in the field. Below we discuss our findings in the context of the species' distributions throughout the DRC and attempt to unravel putative source populations and colonization routes.

4.4.1. Species delimitation and occurrence in the DRC

The distribution of *M. musculus* seems restricted to the western DRC although more complete sampling throughout the DRC should be conducted to confirm this. Amongst the samples collected, *M. musculus* was found only in and around Kinshasa (Figure 1) with all samples collected as "mouse" in Kisangani being identified as *Mastomys*. Misonne (1963) documented *M. musculus* from Ruwenzori and Bukavu; although Ruwenzori lies far to the northeast, none of the samples from Lwiro (40 km north of Bukavu) were *M. musculus*, and only *Rattus* samples were caught in the eastern DRC.

Therefore, although more extensive sampling may reveal the presence of *M. musculus* in the eastern DRC, we found no evidence of this species here. In addition, several studies that compiled species lists for the north-eastern and eastern DRC do not include *M. musculus domesticus* (Allen & Loveridge 1942; Dieterlen 1966, 1985; Rahm & Christiaensen, 1966; Rahm 1967; Misonne 1969b; Katuala *et al*. 2005; Mukinzi *et al*. 2005; Kaleme *et al*. 2007). Despite being absent from our eastern and north-eastern DRC samples, Kilonzo & Msangi (1991) reported *M. musculus* from Tanzania, although Makundi (unpublished records) reports their distribution to be fragmented and confined to towns. Given the caveat of the need for additional sampling, an explanation for the absence of *M. musculus* from large areas in the DRC may be found in their inability to establish feral populations away from human settlements (Rosevear 1969), inter-specific competition with species such as *Mastomys* (de Graaff 1981), as well as the absence of permanent grain throughout most of Africa (de Graaff 1981).

The majority of rat samples collected throughout the DRC were assigned to R. *rattus* with only three samples assigned to *R. norvegicus*. All of these samples were collected at Kisangani, in an around homesteads. No *R. norvegicus* was collected from Kinshasa despite anecdotal information that this species occurs at Kinshasa (Lubini and Ifuta unpublished records). *Rattus rattus* was found throughout the DRC at all localities in and around human dwellings, less disturbed forests, in livestock yards and storage facilities (see also Aplin *et al*. 2003). This species has been shown to be highly adaptive with a wide tolerance for different habitat types (Goodman 1995; Kasangaki *et al*. 2003; Kaleme *et al*. 2007), has an opportunistic breeding strategy and rapid population growth when food is available (Berdoy & Drickamer 2007). This species is also omnivorous, an undeniable asset when colonizing new environments as they are typically faced with a wide variety of foods (Berdoy & Drickamer 2007) which makes them less dependant on agricultural (grain) food sources, as is the case for *Mus*.

4.4.2. Colonization history

Colonization of species into the DRC could have followed one of two main routes. The Atlantic Ocean seaports located on the western DRC allowed trade with European and other African countries. As *M. m. domesticus* was found only in Kinshasa, it is not surprising to find that the DRC individuals probably have a European ancestry. DRC individuals share haplotypes with France and New Zealand (the latter presumably reflecting colonization from the United Kingdom) with a haplotype from Germany being 1 step different. Haplotypes were also shared with Cameroon and Morocco (reflecting French involvement). Perhaps surprising, given the long-term association with Belgium, is that no haplotypes were shared between these two countries, although one of the haplotypes detected in Belgium was separated by two mutational steps (this may be a sampling artefact). Similarly, *R. rattus* caught at Kisangani and Kinshasa share haplotypes with the United Kingdom, Senegal, New Zealand, French Polynesia, South Africa, Samoa and the USA. *Rattus rattus* caught in the eastern DRC share haplotypes with Tanzania, Madagascar and Mozambique. Several studies have demonstrated the

close link between the Mascarene Islands and the Indian sub-continent (Tollenaere *et al*. 2010) as well as between African countries on the eastern seaboard such as Mozambique and India from where much of the trade presumably originated (Hingston *et al*. 2005). The clustering of a haplotype from Reunion close to those detected in the western DRC (Kisangani and Kinshasa) might reflect the involvement of France in both countries. Using historical evidence (see Moutou 1983; Atkinson 1985), Tollenaere and co-workers (2010) found that the settlement and colonization of Reunion by the black rat could have presumably been directly from Europe. Haplotype diversity was higher for the Tanzanian specimens (2 haplotypes found for 5 specimens) *cf*. the specimens collected in the DRC (2 haplotypes characterized 33 specimens). A possible explanation could be multiple colonisations into Tanzania from multiple entry points whereas the DRC may have seen fewer colonisations.

4.4.3. Conclusion

This paper contributes to a growing body of literature that documents the usefulness of DNA to establish the taxonomic affinity and provenance of morphologically difficult taxa. We provide the first molecular evidence for the presence of three invasive rodent species (*M. m. domesticus, R. norvegicus* and *R. rattus)* in the DRC. Using molecular data as well as historical records for the DRC we show that species were introduced into the DRC via two routes. The first is via the western seaport at Kinshasa where specimens of *Mus domesticus* and *Rattus rattus* on the western and north-western side of the DRC show close ties with European haplotypes. The second is via the east where specimens of *Rattus rattus* are closely tied to Arab and south-east Asian haplotypes (likely the slave trade). Future work should consider more comprehensive sampling throughout the DRC to investigate accurately the occurrence of invasive species as well as extend sampling to other African countries given the threat that invasive species pose to local biodiversity, agricultural yield and food security.

Table 4.1 Specimens included in the present study comprising sampling locality, coordinates (for specimens collected by us) or GenBank accession number and source (for those taken from the literature). Numbers in brackets associated with accession numbers for this study's specimens are the laboratory IDs.

Species	Locality	Accession Numbers/Lab IDs	Latitude	Longitude	Reference
Mus musculus	Kinshasa	HM480108 (PK33)	-4.40519	15.41132	Present study
	Kinshasa	HM480109 (PK34)	-4.40519	15.41132	Present study
	Kinshasa	HM480110 (PK35)	-4.40519	15.41132	Present study
	Kinshasa	HM480111 (PK36)	-4.40519	15.41132	Present study
	Kinshasa	HM480112 (PK37)	-4.40519	15.41132	Present study
	Kinshasa	HM480113 (PK586)	-4.40519	15.41132	Present study
	Kinshasa	HM480114 (PK590)	-4.40519	15.41132	Present study
	Ceroux	HM480117 (PK691)	50.66583	4.57052	Present study
	Ceroux	HM480118 (PK692)	50.66583	4.57052	Present study
	Louvain la Neuve	HM480116 (PK640)	50.67417	4.49933	Present study
	Louvain la Neuve	HM480115 (PK641)	50.67417	4.49933	Present study
	Britain	FM211599			Searle *et al*. 2009a
	Britain	FM211601			Searle *et al*. 2009a
	Britain	FM211602			Searle *et al*. 2009a
	Britain	FM211604			Searle *et al*. 2009a
	Bulgaria	EU194652			Rajabi-Maham *et al*. 2008
	Cameroon	AM182700			Ihle *et al*. 2006
	Cameroon	AM182703			Ihle *et al*. 2006
	Cameroon	AM182713			Ihle *et al*. 2006
	Cameroon	AM182714			Ihle *et al*. 2006
	Denmark	U47455			Prager *et al*. 1993
	Denmark	U47460			Prager *et al*. 1993
	England	U47430			Prager *et al*. 1993
	France	AM182717			Ihle *et al*. 2006
	France	AM182741			Ihle *et al*. 2006
	France	AM192742			Ihle *et al*. 2006

	Germany	AM182671			Ihle *et al*. 2006
	Germany	U47474			Prager *et al*. 1993
	Greece	AY551960			Tryfonopoulos *et al*. 2005
	Greece	AY551961			Tryfonopoulos *et al*. 2005
	Iran	EU194617			Rajabi-Maham *et al*. 2008
	Italy	U47471			Prager *et al*. 1993
	Italy	U47477			Prager *et al*. 1993
	Italy	U47482			Prager *et al*. 1993
	Italy	EU194675			Rajabi-Maham *et al*. 2008
	Italy	EU194676			Rajabi-Maham *et al*. 2008
	Madeira	AJ313378			Gunduz *et al*. 2001
	Madeira	AJ313379			Gunduz *et al*. 2001
	Mauritania	AJ313380			Gunduz *et al*. 2001
	Morocco	AJ313381			Gunduz *et al*. 2001
	New Zealand	FM211635			Searle *et al*. 2009b
	New Zealand	FM211636			Searle *et al*. 2009b
	Sweden	AJ313361			Gunduz *et al*. 2001
	Turkey	AJ843837			Gunduz *et al*. 2001
Mastomys sp.	Kisangani	HM480120 (PK567)	0.52603	25.20075	Present study
	Kisangani	HM480121 (PK568)	0.52603	25.20075	Present study
	Kisangani	HM480122 (PK572)	0.52603	25.20075	Present study
	Kisangani	HM480119 (PK576)	0.52603	25.20075	Present study
	Kinshasa	HM480123 (PK584)	-4.40519	15.41132	Present study
	Kinshasa	HM480124 (PK585)	-4.40519	15.41132	Present study
	Kinshasa	HM480125 (PK587)	-4.40519	15.41132	Present study
	Kinshasa	HM480126 (PK588)	-4.40519	15.41132	Present study
	Kinshasa	HM480127 (PK589)	-4.40519	15.41132	Present study
Mastomys natanensis	South Africa	AF465342			Dawood *et al*. (unpublished)
	South Africa	AY576886			Dawood *et al*. (unpublished)
Mastomys coucha	South Africa	AF465344			Dawood *et al*. (unpublished)

	South Africa	AY576888			Dawood *et al*. (unpublished)
Rattus rattus	Lwiro/DRC	HM480128 (PK038)	-2.13683	28.47146	Present study
	Lwiro/DRC	HM480129 (PK039)	-2.13683	28.47146	Present study
	Lwiro (Miti)/DRC	HM480130 (PK040)	-2.13563	28.46046	Present study
	Lwiro (Miti)/DRC	HM480131 (PK042)	-2.13563	28.46046	Present study
	Kinshasa King	HM480132 (PK044)	-4.40519	15.41132	Present study
	Itombwe 1 (Lusasa)/DRC	HM480133 (PK466)	-3.33569	28.75499	Present study
	Itombwe 1 (Lusasa)/DRC	HM480134 (PK467)	-3.33569	28.75499	Present study
	Itombwe 1 (Lusasa)/DRC	HM480135 (PK468)	-3.33569	28.75499	Present study
	Itombwe 1 (Lusasa)/DRC	HM480136 (PK469)	-3.33569	28.75499	Present study
	Itombwe 1 (Lusasa)/DRC	HM480137 (PK470)	-3.33569	28.75499	Present study
	Itombwe 2 (Nabindu)/DRC	HM480138 (PK471)	-3.36874	29.01400	Present study
	Itombwe 2 (Nabindu)/DRC	HM480139 (PK472)	-3.36874	29.01400	Present study
	Itombwe 2 (Nabindu)/DRC	HM480140 (PK473)	-3.36874	29.01400	Present study
	Lwiro/DRC	HM480141 (PK475)	-2.13683	28.47146	Present study
	Lwiro/DRC	HM480142 (PK476)	-2.13683	28.47146	Present study
	Lwiro/DRC	HM480143 (PK477)	-2.13683	28.47146	Present study
	Lwiro/DRC	HM480144 (PK478)	-2.13683	28.47146	Present study
	Lwiro/DRC	HM480145 (PK479)	-2.13683	28.47146	Present study
	Lwiro/DRC	HM480146 (PK480)	-2.13683	28.47146	Present study
	Lwiro/DRC	HM480147 (PK481)	-2.13683	28.47146	Present study
	Lwiro/DRC	HM480148 (PK482)	-2.13683	28.47146	Present study
	Lwiro/DRC	HM480149 (PK483)	-2.13683	28.47146	Present study
	Lwiro/DRC	HM480150 (PK484)	-2.13683	28.47146	Present study
	Kisangani/DRC	HM480151 (PK570)	0.52603	25.20075	Present study
	Kisangani/DRC	HM480155 (PK575)	0.52603	25.20075	Present study
	Kisangani/DRC	HM480156 (PK577)	0.52603	25.20075	Present study
	Kinshasa Kisenso/DRC	HM480157 (PK578)	-4.40519	15.41132	Present study
	Kinshasa Kisenso/DRC	HM480158 (PK579)	-4.40519	15.41132	Present study
	Kinshasa Kisenso/DRC	HM480159 (PK580)	-4.40519	15.41132	Present study

Kinshasa Kisenso/DRC	HM480160 (PK581)	-4.40519	15.41132	Present study
Kinshasa Kisenso/DRC	HM480161 (PK582)	-4.40519	15.41132	Present study
Kinshasa Kisenso/DRC	HM480162 (PK583)	-4.40519	15.41132	Present study
Britain	DQ009794			Hingston *et al*. 2005
Ethiopia	GQ891583			Hingston *et al*. 2005
French Polynesia	EF186354			Robins *et al*. 2007
French Polynesia	EF186359			Robins *et al*. 2007
India	GQ891569			Tollenaere *et al*. 2010
India	GQ891570			Tollenaere *et al*. 2010
India	GQ891571			Tollenaere *et al*. 2010
India	GQ891572			Tollenaere *et al*. 2010
India	GQ891573			Tollenaere *et al*. 2010
Madagascar	DQ009781			Hingston *et al*. 2005
Madagascar	GQ891602			Tollenaere *et al*. 2010
Madagascar	GQ891603			Tollenaere *et al*. 2010
Mozambique	GQ891588			Tollenaere *et al*. 2010
Mozambique	GQ891589			Tollenaere *et al*. 2010
New Zealand	EF186355			Robins *et al*. 2007
Oman	GQ891574			Tollenaere *et al*. 2010
Oman	GQ891575			Tollenaere *et al*. 2010
Oman	GQ891577			Tollenaere *et al*. 2010
Oman	GQ891578			Tollenaere *et al*. 2010
Oman	GQ891579			Tollenaere *et al*. 2010
Oman	GQ891580			Tollenaere *et al*. 2010
Papua New Guinea	EF186357			Robins *et al*. 2007
Reunion	GQ891607			Tollenaere *et al*. 2010
Samoa	EF186360			Robins *et al*. 2007
Senegal	FJ897498			Tollenaere *et al*. 2010
Senegal	FJ897499			Tollenaere *et al*. 2010
South Africa	GQ891608			Tollenaere *et al*. 2010

	Tanzania	GQ891584			Tollenaere *et al*. 2010
	Tanzania	GQ891586			Tollenaere *et al*. 2010
	Tanzania	GQ891587			Tollenaere *et al*. 2010
	Tanzania	HM480163 (PK642)			Present study
	Tanzania	HM480164 (PK643)			Present study
	Tanzania	HM480165 (PK644)			Present study
	Tanzania	HM480166 (PK645)			Present study
	Tanzania	HM480167 (PK693)			Present study
	United States of America	U13750			Usdin *et al*. 1995
	United States of America	U13754			Usdin *et al*. 1995
	Yemen	GQ891581			Tollenaere *et al*. 2010
	Yemen	GQ891582			Tollenaere *et al*. 2010
Rattus norvegicus	Kisangani/DRC	HM480152 (PK569)	0.52603	25.20075	Present study
	Kisangani/DRC	HM480153 (PK573)	0.52603	25.20075	Present study
	Kisangani/DRC	HM480154 (PK574)	0.52603	25.20075	Present study
	Britain	DQ897637			Modh-Zain *et al*. (unpublished)
	Britain	DQ897638			Modh-Zain *et al*. (unpublished)
	Denmark	AJ428514			Nilsson *et al*. 2003
	French Polynesia	EF186346			Robins *et al*. 2007
	Sweden	FJ91976			Tollenaere *et al*. 2010
	United Kingdom	DQ897633			Modh-Zain *et al*. (unpublished)
	United States of America	U13746			Usdin *et al*. 1995
	United States of America	U13747			Usdin *et al*. 1995

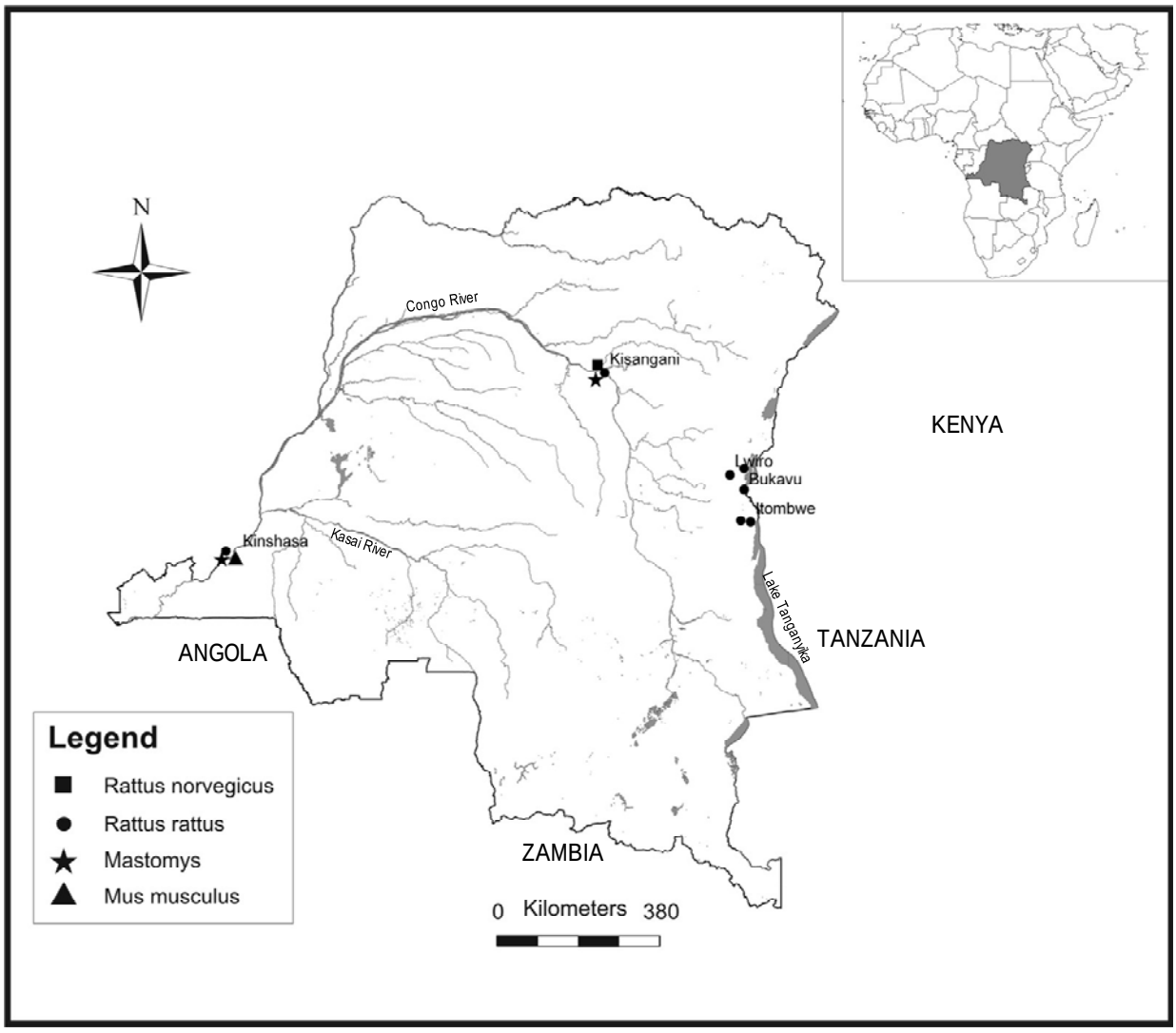

Figure 4.1. Map of the DRC showing the geographic location of the main cities and town mentioned in the text and the collection localities for species.

Figure 2 Maximum likelihood tree (A) indicating the taxonomic affinities of specimens collected as *Mus* in the DRC. Posterior probabilities and bootstrap support is indicated (BI / ML). The evolutionary relationships among haplotypes are shown by the parsimony haplotype networks (B). Haplotypes characterizing DRC specimens are shaded in grey.

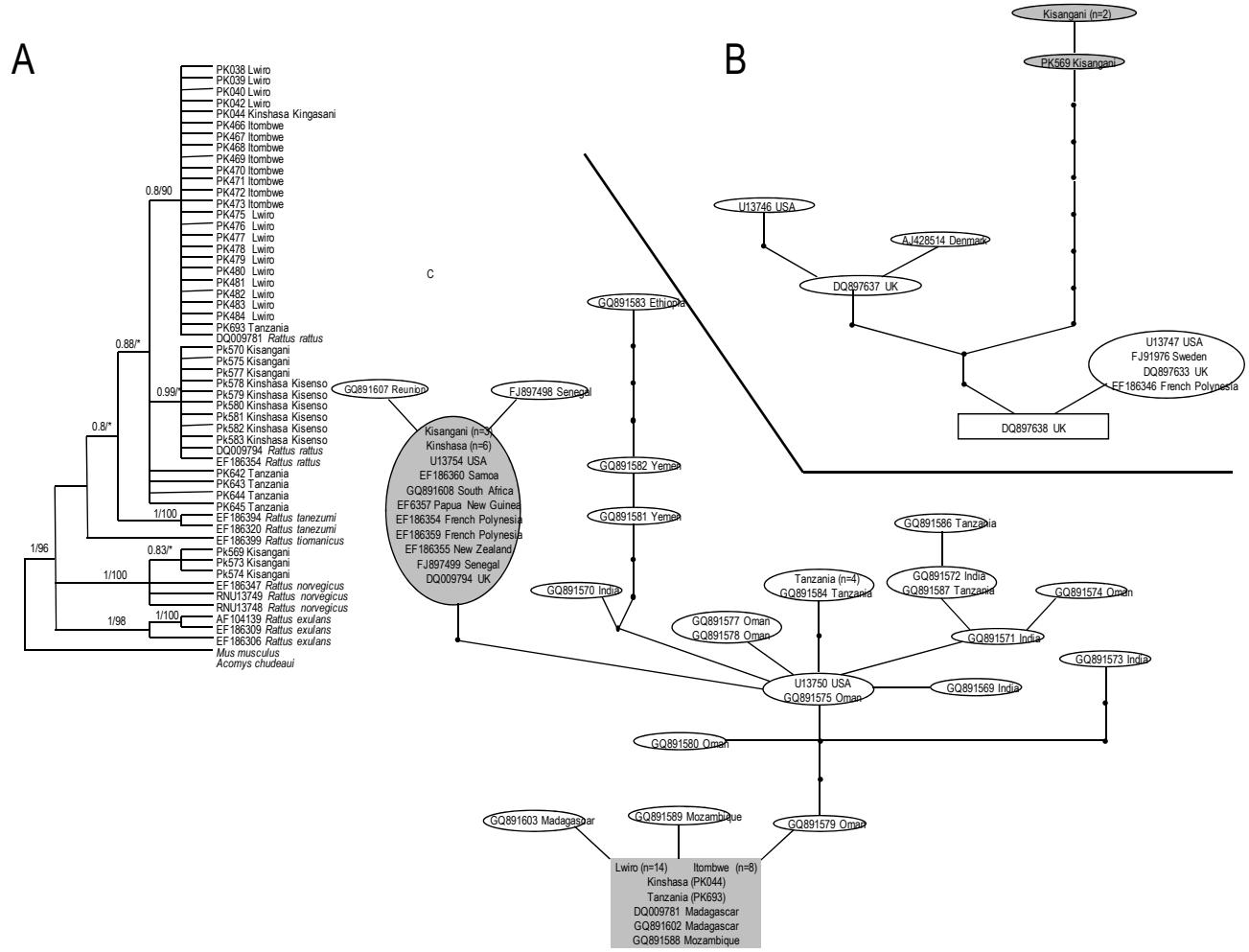

Figure 3 The maximum likelihood tree (A) indicating the taxonomic affinities of the rat samples. Posterior probabilities and bootstrap support is indicated (BI / ML). Parsimony haplotype networks showing the provenance of *R. norvegicus* samples (B) and *R. rattus* samples (C) collected from the DRC and Tanzania. Haplotypes characterizing DRC specimens are shaded in grey.

Chapter 5

General conclusion

Much effort has been put into understanding evolutionary processes in mammals as well as addressing the taxonomy of some African small mammal taxa (see e.g. Rosevear 1969; Dieterlen 1990; Kerbis Peterhans *et al*. 1998; Lecompte *et al*. 2002a, b, 2005, 2008; Nicolas *et al*. 2005, 2006, 2008; Carleton *et al*. 2006; Kerbis Peterhans & Hutterer 2009; Kerbis Peterhans *et al*. 2010). This effort includes molecular evolution and systematics as well as morphological, behavioural and ecological research. For many issues, a phylogenetic framework is of utmost importance for making interpretations and comparisons among and between species in order to draw conclusions based on reliable data.

The Albertine Rift is a biologically complex and rich area. The complexity of habitats is due to the rift structures and geology, and the past climatic cycling could have caused heterogeneity in the habitats that may have driven lineage diversification. It is known that areas with much habitat heterogeneity and climatic or geological instability appear to harbour more species (Moritz *et al*. 2000). It is therefore not surprising that the Albertine Rift is characterized by high species richness and exceptional endemism. The few taxa studied (such as birds and small mammals) have shown similar patterns with regard to taxonomy and phylogeography. The genus *Praomys* was an exciting model to investigate different hypotheses underlying the patterns of diversification in the Albertine Rift.

The results presented herein have contributed to understand the biogeography of the Albertine Rift as well as the mechanisms underlying species distribution and diversification from the Albertine Rift refugia. Our data suggest that the Albertine Rift *Praomys* species may have diverged during a rapid diversification event that occurred *c.*3.4 Mya; which adds evidence to the observation that most speciation events in tropical rainforests vertebrate taxa predate the Pleistocene (Moritz *et al*. 2000; Fjeldsá & Bowie 2008; Voelker *et al*. 2010). Within the *P. jacksoni*-group, the basal species *P. degraaffi* is found in mountain biotopes and the derived species *P. jacksoni* and *P. mutoni* in the lowlands. One potential evolutionary scenario to explain this distribution pattern is that during the humid period, the montane forests shifted to lower altitudes where lowland and mountain species intermixed while during the warmer periods, the mountain forests retracted to mountains tops, which may have isolated *P. degraaffi* in the mountains. *P. jacksoni*, a very adaptable (generalist) species, could have survived both in lowland and mountains whereas *P. mutoni* has adapted to areas along rivers in gallery forests (Van der Straeten & Dudu 1990). The lowland forest habitat probably expanded during the warm and humid period *c.* 3 - 4 Mya, during which *P. mutoni* may have differentiated from *P. jacksoni*.

The patterns of diversification can be explained by the vicariance events and the refuge hypothesis associated with the specific ecological requirement of species. Of the models of diversification proposed, the refugia model is based on the premise that climatic changes caused rainforests to contract to refugia separated by dry forests and savannas

and that this isolation promoted speciation through the accumulation of genetic divergences over time (Haffer 1969, 1997; Diamond & Hamilton 1980). The diversification of *Praomys* species corresponds to a period of pronounced shifts in African climate that resulted in major changes in the distribution and composition of the vegetation (Morley 2000).

At the local landscape level, the Virunga volcanoes, the only land mass that connects the Albertine Rift east – west on its length, could have played (or still play) an important role in the diversification of terrestrial organisms and could be acting as a corridor. At broader spatial scale, the Albertine Rift may have acted as a refuge within the larger central – east African landscape.

Very interesting questions can be formulated when considering genetic data in combination with the landscape. These include possible correlations between genetic variations and geographical location (i.e. are haplotypes distributed randomly or is there any pattern) or what processes are responsible for driving these patterns. The microsatellite data were used and compared with the DNA sequence data and the morphometrics. Different studies in the Albertine Rift have suggested complex patterns of genetic differentiation in many taxa, including the discovery of a number of previously unknown genetically distinct populations (e.g. birds: Roy *et al*. 2000; Bowie *et al*. 2004a,b, 2006; rodents: Huhndorf *et al.* 2007). The results for *Praomys* in the Albertine Rift are no exception to these findings as for other taxa.

Considerable introgression was found between the two focal species included in this study (*P. jacksoni* and *P. degraaffi*). The detected introgression between species and lineages may be due to the fact that, in the first stages of the split, reproductive barriers may be leaky; and the evolutionary mechanisms supposed to promote morphological or reproductive divergence among the isolated populations are rarely explicit (Moritz *et al*. 2000) and often difficult to investigate. What kind of an impact hybridization/introgression has on these species in their natural environment needs further investigation.

A key issue in conservation biology is the identification of operational taxonomic units (OTUs) within species that warrant attention because of their genetic and/or ecological distinctiveness. One approach to identify OTUs is to use genetic data to characterize phylogenetically distinct lineages (Moritz 1994), where additional ecological and behavioural data may be incorporated to strengthen these assignments. The identification of an OTUs may coincide with phylogeographic variation (indicative of the history of a taxon) and phenotypic variation (indicative of ecological distinctiveness), suggesting long-isolated lineages under sustained divergent selection pressures which have led to adaptive differences between populations (Crandall *et al*. 2000).

The Albertine Rift harbours high biodiversity features; but despite the threat they can have on local conservation, food security or introduction of diseases into new areas through alien species, no national or regional strategy exists to deal with the growing number of invasive alien species. Three species were recorded in the DRC: *Mus musculus*, *Rattus norvegicus* and *R. rattus*. If the DRC's species lists have mentioned *R. rattus*, it has not been the case for the other species. More alien/ invasive species may occur in the region and this paucity of knowledge needs to be addressed through additional studies.

The routes of introduction of the invasive species comprise trade (including slave trade), colonisation and explorations from Europe or other continents. Given the presence of alien/invasive species in the region and the constant movement of goods into and through countries, although *Mus musculus* has not been reported in the wild in the Albertine Rift, these species can spread across or beyond the boundaries.

Challenges and Recommendations

The main challenge is that the exact limit of the geographical distribution of many species remains unknown; as such it becomes increasingly difficult to understand the processes governing their distribution. These results highlight the need for further investigations involving a comprehensive sampling of the taxa throughout the range of occurrence to provide an accurate assessment of the specific diversity (including unmasking cryptic diversity), the taxonomic status and the geographical distribution of species to fill the gap between the population level and species level.

Reports are congruent in highlighting the importance of the region for biodiversity conservation, with new species recently described (Fahr *et al*. 2002; Carleton *et al*. 2006; Kerbis Peterhans & Hutterer 2009; Kityo *et al*. 2009; Kerbis Peterhans *et al*. 2010; Kerbis Peterhans *et al*. in preparation). The current base of knowledge in the distribution of species may still have immense gaps for many species which could range further than currently documented. The results show that many mammalian taxa probably remain to be discovered, a situation that can be changed with comprehensive and wide-ranging surveys that include DNA studies, karyotype data as well as morphometric information. There is a need to show (*i*) the importance of continued surveys of fauna even in areas that may be considered fairly well known and (*ii*) the value of museum specimens in acting as a database of information on species distribution and also as a basis for future verification of species identifications.

Conservation and government agencies need strategies that take into account factors such as:

For local biodiversity:

1. Improve regulation on biodiversity conservation and protected areas

2. Strengthen the regional collaboration on biodiversity conservation and control of country borders to dismantle illegal trade of biodiversity or wildlife products
3. Develop ecotourism to generate income for local communities through creative innovations
4. Limit anthropogenic activities within protected areas and, where possible, create regional conservation programmes such as trans-frontier parks or corridors to enable migration from site to site.

For invasive species:

1. Develop national and regional strategies including easily implemented policies to control the entry of alien organism (fauna and flora) to avoid the potential establishment and spread (i.e. species become truly invasive following Blackburn et al. 2011)
2. Assess the effect on alien species already in the country / region on local biodiversity, food security and human livelihood/health in order to envisage adequate control/eradication strategies
3. Prevent the introduction of diseases which can have devastating effects on local biodiversity (such as the case with the fungal infection that are currently decimating frogs from Central America).

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Appendix

Appendix 1. List of the specimens used to generate the DNA sequence data.

No	ID/ FieldNo	Species	Locality	Site No
1	137932	Praomys degaaffi	Kibira National Park	14
2	137953	Praomys degaaffi	Kibira National Park	14
3	137957	Praomys degaaffi	Kibira National Park	14
4	137977	Praomys degaaffi	Kibira National Park	14
5	137993	Praomys degaaffi	Kibira National Park	14
6	137996	Praomys degaaffi	Kibira National Park	14
7	138012	Praomys degaaffi	Kibira National Park	14
8	138016	Praomys degaaffi	Kibira National Park	14
9	138046	Praomys degaaffi	Kibira National Park	14
10	138047	Praomys degaaffi	Kibira National Park	14
11	138076	Praomys degaaffi	Kibira National Park	14
12	157790	Praomys degaaffi	Mgahinga Gorilla NP	7
13	157793	Praomys degaaffi	Mgahinga Gorilla NP	7
14	157795	Praomys degaaffi	Mgahinga Gorilla NP	7
15	157971	Praomys degaaffi	Bwindi Impenetrable NP	6
16	157973	Praomys degaaffi	Bwindi Impenetrable NP	6
17	157974	Praomys degaaffi	Bwindi Impenetrable NP	6
18	157982	Praomys degaaffi	Bwindi Impenetrable NP	6
19	160867	Praomys degaaffi	Bwindi Impenetrable NP	6
20	161127	Praomys degaaffi	Echuya FR	8
21	161128	Praomys degaaffi	Echuya FR	8
22	161130	Praomys degaaffi	Echuya FR	8
23	189041	Praomys degaaffi	Mt Tshiabirimu	5
24	189050	Praomys degaaffi	Mt Tshiabirimu	5
25	189051	Praomys degaaffi	Mt Tshiabirimu	5
26	189052	Praomys degaaffi	Mt Tshiabirimu	5
27	189053	Praomys degaaffi	Mt Tshiabirimu	5
28	189054	Praomys degaaffi	Mt Tshiabirimu	5
29	189055	Praomys degaaffi	Mt Tshiabirimu	5
30	189187	Praomys degaaffi	Kahuzi-Biega NP	10
31	189188	Praomys degaaffi	Kahuzi-Biega NP	10
32	189189	Praomys degaaffi	Kahuzi-Biega NP	10
33	189190	Praomys degaaffi	Kahuzi-Biega NP	10
34	189191	Praomys degaaffi	Kahuzi-Biega NP	10
35	189535	Praomys degaaffi	Kahuzi-Biega NP	10
36	189536	Praomys degaaffi	Kahuzi-Biega NP	10

37	189537	Praomys degaaffi	Kahuzi-Biega NP	10
38	189538	Praomys degaaffi	Kahuzi-Biega NP	10
39	189539	Praomys degaaffi	Kahuzi-Biega NP	10
40	189540	Praomys degaaffi	Kahuzi-Biega NP	10
41	189541	Praomys degaaffi	Kahuzi-Biega NP	10
42	189542	Praomys degaaffi	Kahuzi-Biega NP	10
43	195120	Praomys degaaffi	Kabobo	16
44	195121	Praomys jacksoni	Kabobo	16
45	195122	Praomys degaaffi	Kabobo	16
46	JCK5486	Praomys degaaffi	Lusasa, Itombwe	15
47	JCK5490	Praomys degaaffi	Lusasa, Itombwe	15
48	PK1001	Praomys degaaffi	Ruha, Itombwe	15
49	PK1004	Praomys degaaffi	Ruha, Itombwe	15
50	PK1006	Praomys degaaffi	Ruha, Itombwe	15
51	PK1007	Praomys degaaffi	Ruha, Itombwe	15
52	PK1012	Praomys degaaffi	Ruha, Itombwe	15
53	PK1039	Praomys degaaffi	Ruha, Itombwe	15
54	TCD2649	Praomys degaaffi	Lusasa, Itombwe	15
55	1699	Praomys jacksoni	Lwiro Research Station	11
56	1700	Praomys jacksoni	Lwiro Research Station	11
57	1726	Praomys jacksoni	Tshibati, Kahuzi	10
58	1742	Praomys jacksoni	Mugeri	12
59	1755	Praomys jacksoni	Tshibati, Kahuzi	10
60	1760	Praomys jacksoni	Mugeri	12
61	1783	Praomys jacksoni	Mugeri	12
62	1814	Praomys jacksoni	Mugeri	12
63	1815	Praomys jacksoni	Mugeri	12
64	1826	Praomys jacksoni	Mugeri	12
65	137954	Praomys jacksoni	Kibira	14
66	137956	Praomys jacksoni	Kibira	14
67	138013	Praomys jacksoni	Kibira	14
68	138026	Praomys jacksoni	Kibira	14
69	138085	Praomys jacksoni	Kibira	14
70	138095	Praomys jacksoni	Sese Isl, Bugala	2
71	138110	Praomys jacksoni	Sese Isl, Bugala	2
72	138111	Praomys jacksoni	Sese Isl, Bugala	2
73	145020	Praomys jacksoni	Mubuku River bank, Ruwenzori	3
74	145027	Praomys jacksoni	Mubuku River bank, Ruwenzori	3

75	145068	Praomys jacksoni	Mubuku River bank, Ruwenzori	3
76	145073	Praomys jacksoni	Mubuku River bank, Ruwenzori	3
77	145076	Praomys jacksoni	Mubuku River bank, Ruwenzori	3
78	145097	Praomys jacksoni	Mubuku River bank, Ruwenzori	3
79	145099	Praomys jacksoni	Mubuku River bank, Ruwenzori	3
80	145100	Praomys jacksoni	Mubuku River bank, Ruwenzori	3
81	145109	Praomys jacksoni	Mubuku River bank, Ruwenzori	3
82	145136	Praomys jacksoni	Mubuku River bank, Ruwenzori	3
83	149569	Praomys jacksoni	RFO	4
84	149579	Praomys jacksoni	RFO	4
85	149580	Praomys jacksoni	RFO	4
86	149582	Praomys jacksoni	RFO	4
87	149583	Praomys jacksoni	RFO	4
88	149585	Praomys jacksoni	RFO	4
89	149587	Praomys jacksoni	RFO	4
90	157976	Praomys jacksoni	Ruhizha, Bwindi	6
91	157980	Praomys jacksoni	Ruhizha, Bwindi	6
92	160873	Praomys jacksoni	Bwindi	6
93	160875	Praomys jacksoni	Bwindi	6
94	160876	Praomys jacksoni	Bwindi	6
95	160880	Praomys jacksoni	Bwindi	6
96	160881	Praomys jacksoni	Bwindi	6
97	160883	Praomys jacksoni	Buhoma, Bwindi	6
98	160885	Praomys jacksoni	Kinkizi, Bwindi	6
99	160894	Praomys jacksoni	Bufumbira, Bwindi	6
100	165304	Praomys jacksoni	Busingiro, Budongo	1
101	165313	Praomys jacksoni	Busingiro, Budongo	1
102	165314	Praomys jacksoni	Busingiro, Budongo	1
103	165315	Praomys jacksoni	Nyabyeya FC, Budongo	1
104	171673	Praomys jacksoni	Lwiro Research Station	11
105	171674	Praomys jacksoni	Lwiro Research Station	11
106	171675	Praomys jacksoni	Lwiro Research Station	11
107	171676	Praomys jacksoni	Lwiro Research Station	11
108	171677	Praomys jacksoni	Lwiro Research Station	11
109	171678	Praomys jacksoni	Lwiro Research Station	11
110	171688	Praomys jacksoni	Idjwi Island	13
111	171690	Praomys jacksoni	Idjwi Island	13
112	171691	Praomys jacksoni	Idjwi Island	13

113	171692	Praomys jacksoni	Idjwi Island	13
114	171693	Praomys jacksoni	Idjwi Island	13
115	171694	Praomys jacksoni	Idjwi Island	13
116	171695	Praomys jacksoni	Idjwi Island	13
117	171702	Praomys jacksoni	Idjwi Island	13
118	171703	Praomys jacksoni	Idjwi Island	13
119	171704	Praomys jacksoni	Idjwi Island	13
120	173430	Praomys jacksoni	Mugeri Seminary	12
121	173431	Praomys jacksoni	Mugeri Seminary	12
122	173432	Praomys jacksoni	Mugeri Seminary	12
123	173433	Praomys jacksoni	Mugeri Seminary	12
124	173434	Praomys jacksoni	Mugeri Seminary	12
125	173435	Praomys jacksoni	Mugeri Seminary	12
126	173436	Praomys jacksoni	Mugeri Seminary	12
127	188839	Praomys jacksoni	Burusi Tshiabiimu	5
128	189057	Praomys jacksoni	Burusi Tshiabiimu	5
129	189062	Praomys jacksoni	Burusi Tshiabiimu	5
130	189063	Praomys jacksoni	Burusi Tshiabiimu	5
131	189064	Praomys jacksoni	Burusi Tshiabiimu	5
132	189616	Praomys jacksoni	Kahuzi-Biega NP	10
133	189617	Praomys jacksoni	Kahuzi-Biega NP	10
134	189618	Praomys jacksoni	Kahuzi-Biega NP	10
135	189643	Praomys jacksoni	Kahuzi-Biega NP	10
136	189644	Praomys jacksoni	Kahuzi-Biega NP	10
137	189645	Praomys jacksoni	Kahuzi-Biega NP	10
138	189713	Praomys jacksoni	Kahuzi-Biega NP	10
139	189765	Praomys jacksoni	Kahuzi Biega NP	10
140	195131	Praomys jacksoni	Talama, Kabobo	16
141	195132	Praomys jacksoni	Talama, Kabobo	16
142	195153	Praomys jacksoni	Talama, Kabobo	16
143	195155	Praomys jacksoni	Talama, Kabobo	16
144	196154	Praomys jacksoni	Talama, Kabobo	16
145	ITWCS_02	Praomys jacksoni	Miki, Itombwe	15
146	ITWCS_03	Praomys jacksoni	Miki, Itombwe	15
147	ITWCS_13	Praomys jacksoni	Miki, Itombwe	15
148	ITWCS_16	Praomys jacksoni	Miki, Itombwe	15
149	ITWCS_19	Praomys jacksoni	Miki, Itombwe	15
150	ITWCS_30	Praomys jacksoni	Miki, Itombwe	15

151	ITWCS_34	Praomys jacksoni	Miki, Itombwe	15
152	ITWCS_35	Praomys jacksoni	Miki, Itombwe	15
153	MWWF 11	Praomys jacksoni	Bushema	9
154	MWWF 22	Praomys jacksoni	Bushema	9
155	MWWF 40	Praomys jacksoni	Bushema	9
156	ITWCS_224	Praomys jacksoni	Miki, Itombwe	15
157	PK_721	Praomys jacksoni	Mizimu, Kabobo	16
158	PK_723	Praomys jacksoni	Kabobo, Mizimu	16
159	PK_732	Praomys jacksoni	Mizimu, Kabobo	16
160	PK_745	Praomys jacksoni	Mizimu, Kabobo	16
161	PK_748	Praomys jacksoni	Mizimu, Kabobo	16
162	PK_756	Praomys jacksoni	Kabobo, Mizimu	16
163	PK1021	Praomys jacksoni	Ruha, Itombwe	15
164	PK1022	Praomys jacksoni	Ruha, Itombwe	15
165	PK907	Praomys jacksoni	Lusasa, Itombwe	15
166	PK912	Praomys jacksoni	Lusasa, Itombwe	15
167	PK915	Praomys jacksoni	Lusasa, Itombwe	15
168	PK916	Praomys jacksoni	Lusasa, Itombwe	15
169	PK917	Praomys jacksoni	Lusasa, Itombwe	15
170	PK922	Praomys jacksoni	Lusasa, Itombwe	15
171	PK936	Praomys jacksoni	Lusasa, Itombwe	15
172	PK942	Praomys jacksoni	Lusasa, Itombwe	15
173	MWWF 30	Praomys misonnei	Bushema	9
174	MWWF 2	Praomys mutoni	Bushema	9
175	MWWF 10	Praomys mutoni	Bushema	9
176	MWWF 16	Malacomys longipes	Bushema	9
177	MWWF 28	Malacomys longipes	Bushema	9

Appendix 2.2. List of specimens used for the geometric morphometric data.

ID	Species	Locality	Site	Sex	Age class	Clade
165313	P jacksonni	Budongo FR	1	F	2	2
165314	P jacksonni	Budongo FR	1	F	2	2
165315	P jacksonni	Budongo FR	1	F	2	1
138095	P jacksonni	Sese Island	2	F	2	1
138110	P jacksonni	Sese Island	2	M	2	1
138111	P jacksonni	Sese Island	2	M	2	1
160867	P degraaffi	Bwindi I NP	6	F	2	P deg
157980	P jacksonni	Bwindi I NP	6	M	2	1
160873	P jacksonni	Bwindi I NP	6	M	2	1
160875	P jacksonni	Bwindi I NP	6	F	2	1
160876	P jacksonni	Bwindi I NP	6	F	2	1
161127	P degraaffi	Echuya FR	8	M	2	P deg
161128	P degraaffi	Echuya FR	8	M	2	P deg
161130	P degraaffi	Echuya FR	8	F	3	P deg
171688	P jacksonni	Idjwi Island	13	F	2	2
171690	P jacksonni	Idjwi Island	13	M	3	2
171691	P jacksonni	Idjwi Island	13	M	3	2
171692	P jacksonni	Idjwi Island	13	F	3	2
171694	P jacksonni	Idjwi Island	13	M	2	2
171695	P jacksonni	Idjwi Island	13	F	2	2
171702	P jacksonni	Idjwi Island	13	M	3	2
171703	P jacksonni	Idjwi Island	13	F	3	2
171704	P jacksonni	Idjwi Island	13	M	2	1
204192	P degraaffi	Itombwe	15	M	2	P deg
204194	P degraaffi	Itombwe	15	M	2	P deg
204196	P degraaffi	Itombwe	15	M	3	P deg
204197	P degraaffi	Itombwe	15	M	3	P deg
204199	P degraaffi	Itombwe	15	M	3	P deg
204200	P degraaffi	Itombwe	15	F	2	P deg
204204	P degraaffi	Itombwe	15	M	3	P deg
204239	P degraaffi	Itombwe	15	M	3	P deg
204235	P jacksonni	Itombwe	15	F	2	2
204237	P jacksonni	Itombwe	15	M	2	2
204238	P jacksonni	Itombwe	15	F	2	2
204240	P jacksonni	Itombwe	15	F	2	2
204243	P jacksonni	Itombwe	15	M	2	2
204244	P jacksonni	Itombwe	15	F	3	2

195120	P degraaffi	Kabobo	16	M	2	2
195122	P degraaffi	Kabobo	16	F	2	
195131	P jacksonni	Kabobo	16	M	2	2
195134	P jacksonni	Kabobo	16	F	1	1
195135	P jacksonni	Kabobo	16	M	1	2
195147	P jacksonni	Kabobo	16	M	3	2
189189	P degraaffi	Kahuzi-Biega NP	10	M	2	P deg
189190	P degraaffi	Kahuzi-Biega NP	10	F	1	P deg
189535	P degraaffi	Kahuzi-Biega NP	10	F	2	P deg
189536	P degraaffi	Kahuzi-Biega NP	10	F	2	P deg
189537	P degraaffi	Kahuzi-Biega NP	10	F	2	P deg
189538	P degraaffi	Kahuzi-Biega NP	10	M	3	P deg
189539	P degraaffi	Kahuzi-Biega NP	10	M	2	P deg
189540	P degraaffi	Kahuzi-Biega NP	10	F	2	P deg
189541	P degraaffi	Kahuzi-Biega NP	10	F	2	P deg
189542	P degraaffi	Kahuzi-Biega NP	10	F	3	P deg
189616	P jacksonni	Kahuzi-Biega NP	10	F	2	1
189617	P jacksonni	Kahuzi-Biega NP	10	M	2	1
189618	P jacksonni	Kahuzi-Biega NP	10	F	2	1
189643	P jacksonni	Kahuzi-Biega NP	10	M	2	1
189644	P jacksonni	Kahuzi-Biega NP	10	M	2	1
189645	P jacksonni	Kahuzi-Biega NP	10	M	3	1
189713	P jacksonni	Kahuzi-Biega NP	10	M	3	1
137932	P degraaffi	Kibira NP	14	F	3	P deg
137953	P degraaffi	Kibira NP	14	M	1	P deg
137957	P degraaffi	Kibira NP	14	M	3	2
137977	P degraaffi	Kibira NP	14	M	2	P deg
137993	P degraaffi	Kibira NP	14	M	2	P deg
137996	P degraaffi	Kibira NP	14	F	1	P deg
138016	P degraaffi	Kibira NP	14	F	3	P deg
138076	P degraaffi	Kibira NP	14	M	3	P deg
137954	P jacksonni	Kibira NP	14	M	3	1
137956	P jacksonni	Kibira NP	14	M	3	1
138013	P jacksonni	Kibira NP	14	F	3	1
138085	P jacksonni	Kibira NP	14	M	3	2
171673	P jacksonni	Lwiro RS	11	F	3	1
171674	P jacksonni	Lwiro RS	11	M	3	1
171675	P jacksonni	Lwiro RS	11	M	2	1
171676	P jacksonni	Lwiro RS	11	M	3	1
171677	P jacksonni	Lwiro RS	11	F	2	1

171678	P jacksonni	Lwiro RS	11	M	3	1
173430	P jacksonni	Mugeri	12	F	2	1
173432	P jacksonni	Mugeri	12	F	3	1
173433	P jacksonni	Mugeri	12	F	3	1
173434	P jacksonni	Mugeri	12	F	3	1
173435	P jacksonni	Mugeri	12	M	2	1
173436	P jacksonni	Mugeri	12	F	2	1
149579	P jacksonni	Okapi FR	4	M	1	1
149580	P jacksonni	Okapi FR	4	F	2	1
149582	P jacksonni	Okapi FR	4	M	2	1
149583	P jacksonni	Okapi FR	4	M	2	1
149585	P jacksonni	Okapi FR	4	M	2	1
149587	P jacksonni	Okapi FR	4	M	2	1
145020	P jacksonni	Ruwenzori NP	3	M	2	1
145027	P jacksonni	Ruwenzori NP	3	M	3	1
145073	P jacksonni	Ruwenzori NP	3	F	3	1
145076	P jacksonni	Ruwenzori NP	3	M	2	1
145077	P jacksonni	Ruwenzori NP	3	M	2	1
145136	P jacksonni	Ruwenzori NP	3	M	2	1
189041	P degraaffi	Mt Tshiabirimu	5	M	2	P deg
189050	P degraaffi	Mt Tshiabirimu	5	M	2	P deg
189052	P degraaffi	Mt Tshiabirimu	5	F	2	P deg
189054	P degraaffi	Mt Tshiabirimu	5	F	2	P deg
189055	P degraaffi	Mt Tshiabirimu	5	M	1	P deg
189057	P jacksonni	Mt Tshiabirimu	5	M	2	P deg
189062	P jacksonni	Mt Tshiabirimu	5	M	3	1
189063	P jacksonni	Mt Tshiabirimu	5	F	3	P deg
189064	P jacksonni	Mt Tshiabirimu	5	M	2	P deg

Appendix 3.3. List of specimens used in the microsatellite analyses. Numbers of the sites are as presented in Table 3.1. Obs. = observation indicated whether it was a hybrid of not.

Individual ID	mtDNA "identification"	Microsat "identification"	Obs.	Locality	Site	Latitude (Y)	Longitude (X)
189187	*P. degraaffi* cl1	*P. degraaffi*	Pure	Karherere/Mbayo	Site 6	-2.26603	28.78205
189188	*P. degraaffi* cl1	*P. degraaffi*	Pure	Karherere/Mbayo	Site 6	-2.26603	28.78205
189189	*P. degraaffi* cl1	*P. degraaffi*	Hybrid	Karherere/Mbayo	Site 6	-2.26603	28.78205
189190	*P. degraaffi* cl1	*P. degraaffi*	Pure	Musisi swamp	Site 6	-2.28161	28.80675
189191	*P. degraaffi* cl1	*P. jacksoni*	Hybrid	Musisi swamp	Site 6	-2.28161	28.80675
189535	*P. degraaffi* cl1	*P. degraaffi*	Pure	Karherere/Mbayo	Site 6	-2.26603	28.78205
189536	*P. degraaffi* cl1	*P. degraaffi*	Hybrid	Karherere/Mbayo	Site 6	-2.26603	28.78205
189537	*P. degraaffi* cl1	*P. jacksoni*	Hybrid	Karherere/Mbayo	Site 6	-2.26603	28.78205
189538	*P. degraaffi* cl1	*P. jacksoni*	Hybrid	Karherere/Mbayo	Site 6	-2.26603	28.78205
189539	*P. degraaffi* cl1	*P. degraaffi*	Hybrid	Musisi swamp	Site 6	-2.28161	28.80675
189540	*P. degraaffi* cl1	*P. degraaffi*	Pure	Musisi swamp	Site 6	-2.28161	28.80675
189541	*P. degraaffi* cl1	*P. degraaffi*	Pure	Musisi swamp	Site 6	-2.28161	28.80675
189542	*P. degraaffi* cl1	*P. jacksoni*	Pure	Musisi swamp	Site 6	-2.28161	28.80675
137932	*P. degraaffi* cl2	*P. jacksoni*	Hybrid	Teza Park headquarters	Site 7	-3.2166	29.56660
137953	*P. degraaffi* cl2	*P. jacksoni*	Pure	Teza Park headquarters	Site 7	-3.2166	29.56660
137957	*P. degraaffi* cl2	*P. degraaffi*	Pure	Teza Park headquarters	Site 7	-3.2166	29.56660
137977	*P. degraaffi* cl1	*P. degraaffi*	Pure	Teza Park headquarters	Site 7	-3.2166	29.56660
137993	*P. degraaffi* cl2	*P. degraaffi*	Pure	Teza Park headquarters	Site 7	-3.2166	29.56660
137996	*P. degraaffi* cl1	*P. jacksoni*	Pure	Teza Park headquarters	Site 7	-3.2166	29.56660
138012	*P. degraaffi* cl1	*P. degraaffi*	Pure	Nyabudida	Site 7	-3.2166	29.56660
138016	*P. degraaffi* cl1	*P. degraaffi*	Pure	Nyabudida	Site 7	-3.2166	29.56660
138046	*P. degraaffi* cl1	*P. degraaffi*	Pure	Nyabudida	Site 7	-3.2166	29.56660
138047	*P. degraaffi* cl1	*P. degraaffi*	Pure	Nyabudida	Site 7	-3.2166	29.56660
138076	*P. degraaffi* cl1	*P. jacksoni*	Pure	Nyabudida	Site 7	-1.3003	29.83310
161127	*P. degraaffi* cl1	*P. degraaffi*	Pure	Rubanda /Echuya FR	Site 2	-1.3003	29.83310
161128	*P. degraaffi* cl1	*P. degraaffi*	Pure	Rubanda /Echuya FR	Site 2	-1.3003	29.83310
161130	*P. degraaffi* cl2	*P. degraaffi*	Pure	Rubanda /Echuya FR	Site 2	-1.38806	29.64194
157790	*P. degraaffi* cl1	*P. degraaffi*	Pure	Mgahinga	Site 2	-1.38806	29.64194
157793	*P. degraaffi* cl1	*P. degraaffi*	Pure	Mgahinga	Site 2	-1.38806	29.64194
157795	*P. degraaffi* cl1	*P. degraaffi*	Pure	Mgahinga	Site 2	-1.0778	29.66140
157971	*P. degraaffi* cl1	*P. jacksoni*	Pure	Ruhizha	Site 1	-1.0778	29.66140
157973	*P. degraaffi* cl1	*P. jacksoni*	Pure	Ruhizha	Site 1	-1.0778	29.66140
157982	*P. degraaffi* cl1	*P. degraaffi*	Pure	Ruhizha	Site 1	-0.10013	29.44039
188839	*P. degraaffi* cl1	*P. degraaffi*	Pure	Musavaki valley	Site 10	-0.10013	29.44039
189041	*P. degraaffi* cl1	*P. jacksoni*	Hybrid	Musavaki valley	Site 10	-0.12322	29.44608

189050	*P. degraaffi* cl2	*P. degraaffi*	Hybrid	Kalibina River bank	Site 10	-0.12322	29.44608
189051	*P. degraaffi* cl1	*P. degraaffi*	Hybrid	Kalibina River bank	Site 10	-0.12322	29.44608
189052	*P. degraaffi* cl1	*P. degraaffi*	Pure	Kalibina River bank	Site 10	-0.12322	29.44608
189053	*P. degraaffi* cl1	*P. degraaffi*	Pure	Ngwangwa forest	Site 10	-0.12322	29.44608
189054	*P. degraaffi* cl1	*P. degraaffi*	Hybrid	Ngwangwa forest	Site 10	-0.12322	29.44608
189055	*P. degraaffi* cl1	*P. degraaffi*	Pure	Ngwangwa forest	Site 10	-0.10013	29.44039
189057	*P. degraaffi* cl1	*P. degraaffi*	Pure	Musavaki valley	Site 10	-0.10013	29.44039
189063	*P. degraaffi* cl1	*P. degraaffi*	Pure	Musavaki valley	Site 10	-0.10013	29.44039
189064	*P. degraaffi* cl1	*P. degraaffi*	Pure	Kivulya slope	Site 10	-4.59068	29.05353
195120	*P. jacksoni* cl2	*P. jacksoni*	Pure	Mizimu	Site 5	-3.36874	29.01400
195121	*P. degraaffi* cl1	*P. degraaffi*	Pure	Talama	Site 5	-5.28758	29.16706
195122	*P. degraaffi* cl1	*P. degraaffi*	Pure	Talama	Site 5	-3.36874	29.01400
PK1001	*P. degraaffi* cl1	*P. degraaffi*	Pure	Nabindu	Site 4	-3.36874	29.01400
PK1006	*P. degraaffi* cl1	*P. degraaffi*	Pure	Nabindu	Site 4	-3.36874	29.01400
PK1007	*P. degraaffi* cl1	*P. degraaffi*	Pure	Nabindu	Site 4	-3.36874	29.01400
PK1012	*P. degraaffi* cl1	*P. degraaffi*	Pure	Nabindu	Site 4	-3.33569	28.75499
PK1039	*P. degraaffi* cl1	*P. degraaffi*	Pure	Nabindu	Site 4	-3.33569	28.75499
TCD2649	*P. degraaffi* cl1	*P. degraaffi*	Pure	Lusasa	Site 4	-3.33569	28.75499
JCK5486	*P. degraaffi* cl1	*P. degraaffi*	Pure	Lusasa	Site 4	-5.28758	29.16706
JCK5490	*P. degraaffi* cl1	*P. degraaffi*	Pure	Lusasa	Site 4	-3.21660	29.5666
1726	*P. jacksoni* cl1	*P. jacksoni*	Pure	Karherere/Mbayo	Site 6	-5.28758	29.16706
1755	*P. jacksoni* cl1	*P. jacksoni*	Pure	Tshibati	Site 6	-2.13170	28.46810
189617	*P. jacksoni* cl1	*P. jacksoni*	Hybrid	Bwamba/Tshibati	Site 6	-2.13170	28.46810
189618	*P. jacksoni* cl1	*P. degraaffi*	Pure	Bwamba/Tshibati	Site 6	-2.13170	28.46810
189644	*P. jacksoni* cl1	*P. jacksoni*	Pure	Lurhogole/Tshibati	Site 6	-2.13170	28.46810
189645	*P. jacksoni* cl1	*P. jacksoni*	Pure	Musisi	Site 6	-2.13170	28.46810
189713	*P. jacksoni* cl1	*P. jacksoni*	Hybrid	Musisi	Site 6	-2.13170	28.46810
189765	*P. jacksoni* cl1	*P. jacksoni*	Pure	Musisi	Site 6	-2.13170	28.46810
1699	*P. jacksoni* cl1	*P. jacksoni*	Hybrid	Lwiro	Site 6	-2.26603	28.78205
1700	*P. jacksoni* cl1	*P. jacksoni*	Hybrid	Lwiro	Site 6	-2.13180	28.47150
171673	*P. jacksoni* cl1	*P. jacksoni*	Hybrid	Lwiro	Site 6	-2.13180	28.47150
171674	*P. jacksoni* cl1	*P. jacksoni*	Hybrid	Lwiro	Site 6	-2.13180	28.47150
171675	*P. jacksoni* cl1	*P. degraaffi*	Pure	Lwiro	Site 6	-2.13180	28.47150
171676	*P. jacksoni* cl1	*P. jacksoni*	Pure	Lwiro	Site 6	-2.13180	28.47150
171677	*P. jacksoni* cl1	*P. jacksoni*	Pure	Lwiro	Site 6	-2.13180	28.47150
171678	*P. jacksoni* cl2	*P. jacksoni*	Hybrid	Lwiro	Site 6	-2.13180	28.47150
1742	*P. jacksoni* cl1	*P. jacksoni*	Pure	Mugeri Seminary	Site 6	-2.13180	28.47150
1760	*P. jacksoni* cl1	*P. jacksoni*	Pure	Mugeri Seminary	Site 6	-2.21600	28.86380
1783	*P. jacksoni* cl1	*P. jacksoni*	Pure	Mugeri Seminary	Site 6	-2.21600	28.86380
1814	*P. jacksoni* cl1	*P. degraaffi*	Pure	Mugeri Seminary	Site 6	-2.21600	28.86380

1815	*P. jacksoni* cl1	*P. jacksoni*	Pure	Mugeri Seminary	Site 6	-2.21600	28.86380
1826	*P. jacksoni* cl1	*P. jacksoni*	Pure	Mugeri Seminary	Site 6	-2.21600	28.86380
173430	*P. jacksoni* cl1	*P. jacksoni*	Pure	Mugeri Seminary	Site 6	-2.21600	28.86380
173431	*P. jacksoni* cl1	*P. jacksoni*	Pure	Mugeri Seminary	Site 6	-2.21600	28.86380
173432	*P. jacksoni* cl1	*P. jacksoni*	Pure	Mugeri Seminary	Site 6	-2.21600	28.86380
173433	*P. jacksoni* cl1	*P. jacksoni*	Pure	Mugeri Seminary	Site 6	-2.21600	28.86380
173434	*P. jacksoni* cl1	*P. jacksoni*	Hybrid	Mugeri Seminary	Site 6	-2.21600	28.86380
173435	*P. jacksoni* cl1	*P. jacksoni*	Pure	Mugeri Seminary	Site 6	-2.21600	28.86380
173436	*P. jacksoni* cl1	*P. jacksoni*	Pure	Mugeri Seminary	Site 6	-2.21600	28.86380
171688	*P. jacksoni* cl2	*P. jacksoni*	Pure	Washiha	Site 3	-2.21600	28.86380
171690	*P. jacksoni* cl2	*P. jacksoni*	Hybrid	Washiha	Site 3	-2.28910	29.12850
171691	*P. jacksoni* cl2	*P. jacksoni*	Pure	Washiha	Site 3	-2.28910	29.12850
171692	*P. jacksoni* cl2	*P. jacksoni*	Pure	Washiha	Site 3	-2.28910	29.12850
171693	*P. jacksoni* cl2	*P. jacksoni*	Pure	Washiha	Site 3	-2.28910	29.12850
171694	*P. jacksoni* cl2	*P. jacksoni*	Pure	Washiha	Site 3	-2.28910	29.12850
171695	*P. jacksoni* cl2	*P. jacksoni*	Pure	Washiha	Site 3	-2.28910	29.12850
171702	*P. jacksoni* cl2	*P. jacksoni*	Pure	Washiha	Site 3	-2.28910	29.12850
171703	*P. jacksoni* cl2	*P. jacksoni*	Pure	Washiha	Site 3	-2.28910	29.12850
171704	*P. jacksoni* cl1	*P. degraaffi*	Hybrid	Washiha	Site 3	-2.28910	29.12850
137954	*P. jacksoni* cl1	*P. degraaffi*	Pure	Nyabudida	Site 7	-2.28910	29.12850
137956	*P. jacksoni* cl1	*P. degraaffi*	Pure	Nyabudida	Site 7	-3.21660	29.56660
138013	*P. jacksoni* cl1	*P. jacksoni*	Hybrid	Nyabudida	Site 7	-3.21660	29.56660
138026	*P. jacksoni* cl1	*P. jacksoni*	Pure	Nyabudida	Site 7	-3.21660	29.56660
138085	*P. jacksoni* cl2	*P. jacksoni*	Hybrid	Nyabudida	Site 7	-3.21660	29.56660
ITWCS_02	*P. jacksoni* cl2	*P. jacksoni*	Pure	Miki	Site 4	-3.21660	29.56660
ITWCS_03	*P. jacksoni* cl2	*P. jacksoni*	Pure	Miki	Site 4	-3.43270	28.57289
ITWCS_13	*P. jacksoni* cl2	*P. jacksoni*	Pure	Miki	Site 4	-3.43270	28.57289
ITWCS_16	*P. jacksoni* cl2	*P. jacksoni*	Pure	Miki	Site 4	-3.43270	28.57289
ITWCS_19	*P. jacksoni* cl2	*P. jacksoni*	Pure	Miki	Site 4	-3.43270	28.57289
ITWCS_21	*P. jacksoni* cl2	*P. jacksoni*	Hybrid	Miki	Site 4	-3.43270	28.57289
ITWCS_30	*P. jacksoni* cl2	*P. jacksoni*	Pure	Miki	Site 4	-3.43270	28.57289
ITWCS_34	*P. jacksoni* cl2	*P. degraaffi*	Pure	Miki	Site 4	-3.43270	28.57289
ITWCS_35	*P. jacksoni* cl2	*P. degraaffi*	Pure	Miki	Site 4	-3.43270	28.57289
ITWCS_224	*P. jacksoni* cl2	*P. jacksoni*	Pure	Miki	Site 4	-3.43270	28.57289
PK907	*P. jacksoni* cl2	*P. jacksoni*	Pure	Lusasa	Site 4	-3.43270	28.57289
PK912	*P. jacksoni* cl2	*P. jacksoni*	Pure	Lusasa	Site 4	-3.33569	28.75499
PK915	*P. jacksoni* cl2	*P. jacksoni*	Pure	Lusasa	Site 4	-3.33569	28.75499
PK916	*P. jacksoni* cl2	*P. jacksoni*	Pure	Lusasa	Site 4	-3.33569	28.75499
PK917	*P. jacksoni* cl2	*P. jacksoni*	Pure	Lusasa	Site 4	-3.33569	28.75499
PK922	*P. jacksoni* cl2	*P. jacksoni*	Pure	Lusasa	Site 4	-3.33569	28.75499

PK936	*P. jacksoni* cl2	*P. jacksoni*	Pure	Nabindu	Site 4	-3.33569	28.75499
PK942	*P. jacksoni* cl2	*P. jacksoni*	Pure	Nabindu	Site 4	-3.33569	28.75499
PK1021	*P. jacksoni* cl2	*P. jacksoni*	Pure	Nabindu	Site 4	-3.33569	28.75499
PK1022	*P. jacksoni* cl2	*P. jacksoni*	Pure	Nabindu	Site 4	-3.36870	29.01400
PK_721	*P. jacksoni* cl2	*P. jacksoni*	Hybrid	Mizimu	Site 5	-3.36870	29.01400
PK_723	*P. jacksoni* cl2	*P. jacksoni*	Hybrid	Mizimu	Site 5	-5.28758	29.16706
PK_724	*P. jacksoni* cl2	*P. jacksoni*	Pure	Mizimu	Site 5	-5.28758	29.16706
PK_729	*P. jacksoni* cl2	*P. jacksoni*	Pure	Mizimu	Site 5	-5.28758	29.16706
PK_731	*P. jacksoni* cl2	*P. jacksoni*	Pure	Mizimu	Site 5	-5.28758	29.16706
PK_732	*P. jacksoni* cl2	*P. jacksoni*	Pure	Mizimu	Site 5	-5.28758	29.16706
PK_745	*P. jacksoni* cl2	*P. jacksoni*	Pure	Talama	Site 5	-5.28758	29.16706
PK_748	*P. jacksoni* cl2	*P. jacksoni*	Pure	Talama	Site 5	-5.28758	29.16706
PK_756	*P. jacksoni* cl2	*P. jacksoni*	Pure	Talama	Site 5	-5.28758	29.16706
195131	*P. jacksoni* cl2	*P. jacksoni*	Hybrid	Talama	Site 5	-4.59068	29.05353
195132	*P. jacksoni* cl2	*P. jacksoni*	Pure	Talama	Site 5	-4.59068	29.05353
195153	*P. jacksoni* cl2	*P. jacksoni*	Pure	Talama	Site 5	-4.59068	29.05353
196154	*P. jacksoni* cl2	*P. jacksoni*	Pure	Talama	Site 5	-4.59068	29.05353
195155	*P. jacksoni* cl2	*P. jacksoni*	Pure	Talama	Site 5	-4.59068	29.05353
145020	*P. jacksoni* cl1	*P. jacksoni*	Pure	Mubuku River bank	Site 9	0.36660	29.98330
145027	*P. jacksoni* cl1	*P. jacksoni*	Pure	Mubuku River bank	Site 9	0.36660	29.98330
145068	*P. jacksoni* cl1	*P. jacksoni*	Pure	Mubuku River bank	Site 9	0.36660	29.98330
145073	*P. jacksoni* cl1	*P. jacksoni*	Pure	Mubuku River bank	Site 9	0.36660	29.98330
145076	*P. jacksoni* cl1	*P. jacksoni*	Pure	Mubuku River bank	Site 9	0.36660	29.98330
145097	*P. jacksoni* cl1	*P. jacksoni*	Pure	Mubuku River bank	Site 9	0.36660	29.98330
145099	*P. jacksoni* cl1	*P. jacksoni*	Hybrid	Mubuku River bank	Site 9	0.36660	29.98330
145100	*P. jacksoni* cl1	*P. jacksoni*	Pure	Mubuku River bank	Site 9	0.36660	29.98330
145109	*P. jacksoni* cl1	*P. jacksoni*	Pure	Mubuku River bank	Site 9	0.36660	29.98330
145136	*P. jacksoni* cl1	*P. jacksoni*	Pure	Mubuku River bank	Site 9	0.36660	29.98330
149569	*P. jacksoni* cl1	*P. jacksoni*	Pure	Lenda	Site 8	1.40300	28.57120
149579	*P. jacksoni* cl1	*P. jacksoni*	Pure	Lenda	Site 8	1.40300	28.57120
149580	*P. jacksoni* cl1	*P. jacksoni*	Pure	Lenda	Site 8	1.40300	28.57120
149582	*P. jacksoni* cl1	*P. jacksoni*	Pure	Epulu River right bank	Site 8	1.40300	28.57120
149583	*P. jacksoni* cl1	*P. jacksoni*	Pure	Epulu River right bank	Site 8	1.57500	28.64970
149585	*P. jacksoni* cl1	*P. jacksoni*	Pure	Epulu River right bank	Site 8	1.57500	28.64970
149587	*P. jacksoni* cl1	*P. jacksoni*	Pure	Epulu River right bank	Site 8	1.57500	28.64970
157976	*P. jacksoni* cl1	*P. jacksoni*	Pure	Ruhizha	Site 1	-1.07780	29.66140
157980	*P. jacksoni* cl1	*P. jacksoni*	Pure	Ruhizha	Site 1	-1.07780	29.66140
160873	*P. jacksoni* cl1	*P. jacksoni*	Hybrid	Ruhizha	Site 1	-1.07780	29.66140
160875	*P. jacksoni* cl1	*P. jacksoni*	Pure	Ruhizha	Site 1	-1.07780	29.66140
160876	*P. jacksoni* cl1	*P. jacksoni*	Hybrid	Ruhizha	Site 1	-1.07780	29.66140

160880	*P. jacksoni* cl1	*P. jacksoni*	Pure	Ruhizha	Site 1	-1.07780	29.66140
160881	*P. jacksoni* cl1	*P. degraaffi*	Hybrid	Ruhizha	Site 1	-1.07780	29.66140
160883	*P. jacksoni* cl1	*P. degraaffi*	Pure	Ruhizha	Site 1	-1.07780	29.66140
160885	*P. jacksoni* cl1	*P. degraaffi*	Pure	Ruhizha	Site 1	-1.07780	29.66140
160894	*P. jacksoni* cl1	*P. jacksoni*	Pure	Ruhizha	Site 1	-1.07780	29.66140
157974	*P. jacksoni* cl1	*P. jacksoni*	Pure	Ruhizha	Site 1	-1.07780	29.66140
160867	*P. jacksoni* cl1	*P. jacksoni*	Hybrid	Ruhizha	Site 1	-1.07780	29.66140
189062	*P. jacksoni* cl1	*P. jacksoni*	Hybrid	Musavaki valley	Site 10	-0.10013	29.44039