Comparative phylogeography of three endemic rodents from the Albertine Rift, east central Africa

MICHAEL H. HUHNDORF,*† JULIAN C. KERBIS PETERHANS†‡ and SABINE S. LOEW*†

Department of Biological Sciences, Behaviour, Ecology, Evolution and Systematics Section, Illinois State University, Normal, Illinois 61790-4120, USA, †Department of Zoology, The Field Museum, Chicago, Illinois, 60605-2496, USA, ‡University College, Roosevelt University, Chicago, Illinois, 60605, USA

Abstract

The major aim of this study was to compare the phylogeographic patterns of codistributed rodents from the fragmented montane rainforests of the Albertine Rift region of east central Africa. We sampled individuals of three endemic rodent species, *Hylomyscus denniae*, *Hybomys lunaris* and *Lophuromys woosnami* from four localities in the Albertine Rift. We analysed mitochondrial DNA sequence variation from fragments of the cytochrome *b* and control region genes and found significant phylogeographic structuring for the three taxa examined. The recovered phylogenies suggest that climatic fluctuations and volcanic activity of the Virunga Volcanoes chain have caused the fragmentation of rainforest habitat during the past 2 million years. This fragmentation has played a major role in the diversification of the montane endemic rodents of the region. Estimation of the divergence times within each species suggests a separation of the major clades occurring during the mid to late Pleistocene.

Keywords: Africa, Albertine Rift, control region, cytochrome *b*, phylogeography, rodent

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Introduction

Different biogeographical processes than those of the Northern Hemisphere have shaped the evolutionary history of taxa of east central Africa. The advance and retreat of glaciers during the Quaternary were a major force in shaping the biodiversity on the continents of the Northern Hemisphere. These glacial periods corresponded to cool and dry periods in tropical west and east Africa (deMenocal 1995), which presumably led to the periodic fragmentation of the lowland rainforest of west and central Africa. The 'refuge theory' (Haffer 1969) postulates that periods of climatic fluctuation caused rainforests to become fragmented and isolated and then reconnected thus leading to the high species diversification seen in the lowland tropics. Climatic change during the Plio–Pleistocene has also been suggested as the major force driving diversification in east African montane rainforests (Fjeldsa & Lovett 1997; Roy 1997; Roy *et al.* 2001). Additionally, geologic activity, i.e. volcanic activity, during this time period may have played an equal role in shaping the diversification of the Albertine Rift.

Correspondence: Michael H. Huhndorf, Fax: 309-438-3722; E-mail: mhhuhnd@ilstu.edu

The Albertine Rift is the western branch of the Great Rift Valley in central and east Africa. It lies in the eastern Democratic Republic of Congo (DR Congo), western Uganda, Rwanda, Burundi and Tanzania. The Albertine Rift has been formed by uplifting of pre-Cambrian rock during the Miocene and by volcanic activity that began during the Plio–Pleistocene and that continues to this day. The main feature of the Albertine Rift is a chain of mountains stretching from Lake Albert in the north to the southern end of Lake Tanganyika (Fig. 1). The ecoregion is dominated by montane rainforest [1500–3500 metres above sea level (m a.s.l.) altitude] but is bordered by a lowland forest/savanna mosaic to the east and west down to altitudes of 500–800 m a.s.l.

Volcanic activity and rifting in combination with climatic change during the mid- to late-Pleistocene created complex patterns of habitat fragmentation and expansion/contraction cycles of montane vegetation in the Albertine Rift (Morrison 1968; Morrison & Hamilton 1974; Hamilton 1982). These patterns are consistent with proposed hypotheses (Fjeldsa & Lovett 1997; Roy *et al.* 2001) of diversification in montane rainforests. At several historical stages volcanic activity reduced or eliminated the vegetation around the eight present volcanoes of the Virungas chain (Fig. 1). This may have separated the Albertine Rift into northern and southern biogeographical regions. Additionally, the volcanic activity
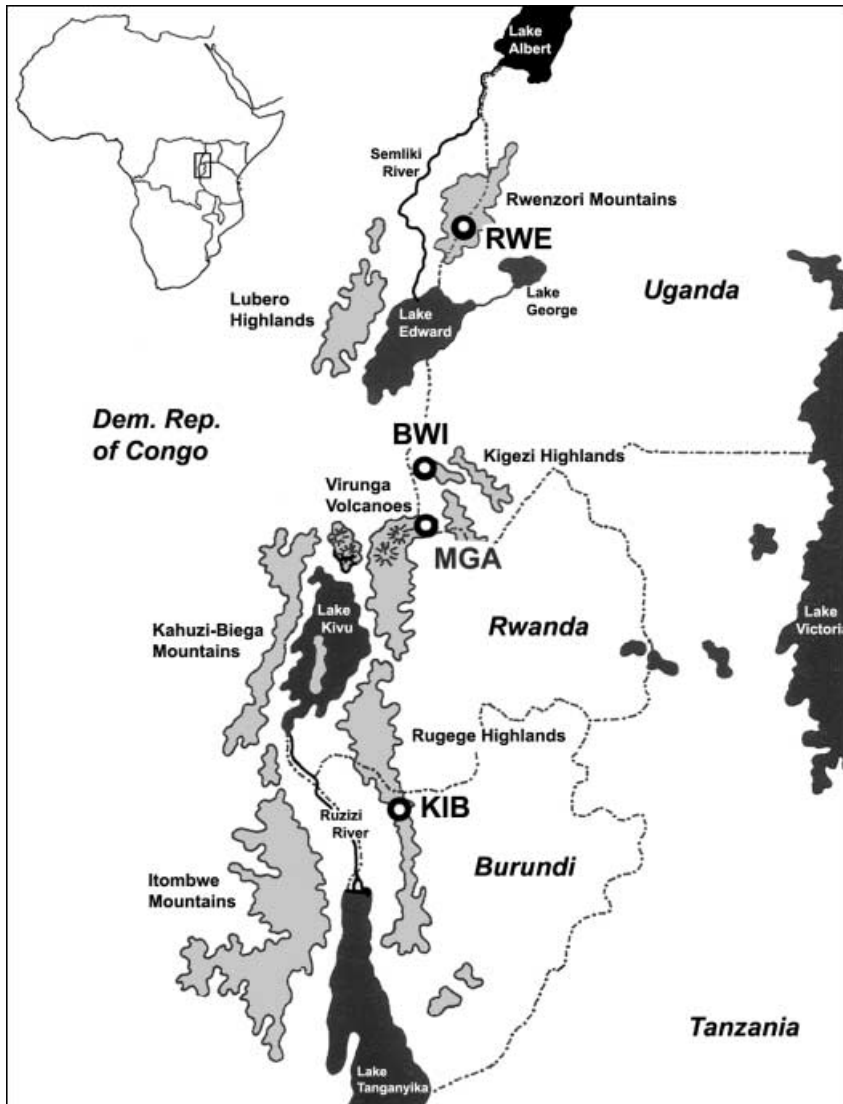
Fig. 1 Montane regions (grey) of the Albertine Rift. Modified from Dieterlen (1976). Black circles with white dots indicate the four sampling locales included in this study (Appendix). RWE, Rwenzori Mountains National Park; BWI, Bwindi Impenetrable Forest National Park; MGA, Mgahinga Gorilla National Park; KIB, Kibira National Park. All three taxa are distributed across the montane forests of the Albertine Rift.

in the Virungas altered the hydrology of the central Albertine Rift (Beadle 1981) which may have created potential biogeographical barriers due to altered rivers and lakes that isolated certain montane areas from one another. This complex geological history and high biodiversity make the Albertine Rift ideally suited to test specific hypotheses on the relationship between biogeography and species diversification in east central Africa, nevertheless the comparative phylogeography of the montane dependent fauna of the Albertine Rift has received little attention and remains poorly understood.

Our study focuses on three rodent species endemic to the Albertine Rift montane forest biotic region, *Hylomyscus denniae*, *Hybomys lunaris* and *Lophuromys woosnami*. The complete limits of their distributions within the Albertine Rift is unknown as some areas have never been surveyed; however, it is presumed that they are found throughout the montane forest zone. These three taxa are codistributed in disjunct populations across the Albertine Rift but vary slightly in their habitat preferences. *Hylomyscus denniae*, climbing wood mouse, is restricted to mid to high-altitude montane forests and the afro-alpine zone (1900–4000 m a.s.l.), it is strictly arboreal in the montane forest but is more terrestrial in the higher afro-alpine zone.

Lophuromys woosnami, brush-furred mouse, is restricted to mid to high-altitude montane forests (1900–3000 m a.s.l.), is mainly terrestrial and found in wetter areas of the forests. *H. lunaris*, Rwenzori striped mouse, is restricted to low to mid-elevation montane forests (1500–2700 m a.s.l.), is terrestrial and prefers areas near water.

For all three taxa, we obtained DNA sequence data from two mitochondrial DNA (mtDNA) markers, the cytochrome-*b* gene and the noncoding control region to examine the evolutionary history of Albertine Rift fauna. Using the

principles of vicariance biogeography (Rosen 1978; Nelson & Platnick 1981), we can use phylogenies of mtDNA lineages to develop hypotheses about levels of endemism and area-relationships among the montane forests of the Albertine Rift. Congruent patterns among codistributed taxa are evidence that these taxa share a common history of response to vicariant events (Cracraft 1985). Using the general hypothesis that unique mtDNA lineages will evolve in allopatry, we specifically addressed the following three questions: (i) Are there distinct mtDNA lineages within *H. denniae*, *H. lunaris* and *L. woosnami*? (ii) How are mtDNA lineages geographically distributed within the Albertine Rift? (iii) What is the degree of phylogeographic concordance of mtDNA lineages among the three taxa? On the basis of the three species geographical distributions, the geological history and the current fragmentation seen among the montane rainforests we expect to see congruent phylogeographic patterns and make the following predictions. First, since all three taxa are dependent upon montane rainforest and are codistributed, historic biogeographical barriers will have affected them similarly and they will exhibit phylogenetic breaks in similar geographical locations. Second, we expect phylogenetic patterns to show a northern and southern region centred on the Virunga Volcanoes because the volcanic activity during the past 2 million years has altered the terrestrial and hydrologic landscape of the central Albertine Rift creating a potential barrier to gene flow. In order to understand the biogeographical history of *H. denniae*, *H. lunaris* and *L. woosnami* we have interpreted the phylogeographic patterns and the relative timing of major cladogenic events within the context of geological events of the Albertine Rift that occurred over the past 2 million years.

Materials and methods

Sampling design

Our analysis included a total of 21 individuals of *Hylomyscus denniae*, 20 individuals of *Lophuromys woosnami* sampled from the Rwenzori (RWE), Bwindi (BWI), Mgahinga (MGA) and Kibira (KIB) National Parks, and 13 individuals of *Hybomys lunaris* collected in the same localities except Mgahinga National Park (Fig. 1, Appendix 1). Two species, *Hylomyscus arcimontensis* and *Hylomyscus aeta* were chosen for outgroup comparisons with *H. denniae*, *Hybomys univittatus* was used for outgroup comparisons for *H. lunaris and Lophuromys medicaudatus* was used for outgroup comparison with *L. woosnami*. All specimens were collected during surveys conducted in 1990–91 and 1996–97 that included Rwenzori Mountains National Park (Kerbis Peterhans *et al.* 1998) among other montane forests of the Albertine Rift. All voucher specimens that tissue was obtained from are housed in the collections of The Field Museum (FM) (see

Appendix for details). Unique DNA sequences have been submitted to GenBank under Accession nos DQ902717–DQ902827.

DNA extraction and sequencing

Total genomic DNA was extracted from 0.25 g or less of liver, heart or muscle tissue using the commercially available DNeasy Tissue Kit (QIAGEN). For this study, we compared sequences from fragments of two mtDNA genes, cytochrome *b* and the control region (D-loop). Primers H14542 and L14115 (Smith & Patton 1991) were used to amplify a 426-bp segment of the mtDNA cytochrome *b* gene. Primer names correspond to the 3' base of the primer in the complete mtDNA sequence of *Mus* (Bibb *et al.* 1981). Primers CBT and MR1 (Morzunov *et al.* 1998), corresponding to bases 15 393 and 15 777 of the mtDNA *Mus* sequence, were used to amplify a 427-bp segment of the mtDNA D-loop. Fragments were amplified by polymerase chain reaction (PCR) using the following parameters for cytochrome *b*: denaturation at 93 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 1 min for a total of 30 cycles. The parameters used for the D-loop amplification were: denaturation at 93 °C for 30 s, annealing at 48 °C for 30 s and extension at 72 °C for 2 min for a total of 40 cycles. Amplified mtDNA was purified using the GENECLEAN II kit (BIO 101) and cycle sequenced using components of an ABI PRISM BigDye Terminator version 3.0 Cycle Sequencing Kit (Perkin-Elmer). Cycle sequencing parameters and purification of sequencing reactions by ethanol precipitation followed manufacturer's recommendations. Single-stranded sequence reactions were analysed with an ABI 310 Genetic Analyser (Perkin-Elmer). Sequence alignment among mtDNA sequences was performed by eye and using the computer program SEQUENCHER version 4.1.2 (Gene Codes).

Phylogenetic analysis

Data were analysed using the following partitions, each gene fragment separately and as a combined mtDNA data set. We used the computer program PAUP* 4.0b10 (Swofford 2002) to reconstruct phylogenetic relationships among individuals for both cyt-*b* and control region data using maximum parsimony (MP), maximum likelihood (ML) and neighbour joining (NJ) methods. In order to test for saturation in the protein coding cyt-*b* fragment, we performed partition homogeneity tests (ILD statistic of Farris *et al.* 1995a, b) for first and second codon positions vs. third positions. The partition homogeneity test was also used to assess congruence in the phylogenetic signal between the cyt *b* and control region fragments; all tests were performed, excluding uninformative characters, with 100 replicates, 10 random sequence additions per replicate, tree-bisection–

reconnection (TBR) branch swapping, and equal weights. The results suggest that transitions at the third codon position for cyt-*b* sequences are not saturated in any of the three species (*H. denniae*, P = 0.90; *H. lunaris*, P = 1.0; *L. woosnami*, P = 1.0). Based on these results, we chose equally weighted parsimony for all subsequent maximum-parsimony analyses. The null hypothesis that both, the cyt-*b* and control region data sets will reflect the same evolutionary history of these groups could not be rejected; therefore, the data were combined for all phylogenetic analyses. Maximum-parsimony trees were constructed using the heuristic search option, with random addition of taxa, and TBR branch swapping. We estimated support values for internal nodes of trees using the bootstrap resampling technique (Felsenstein 1985) with 1000 replicates and TBR branch swapping.

We employed hierarchical likelihood-ratio tests to select the most appropriate model of evolution for ML analyses. These tests were performed using the computer program MODELTEST version 3.06 (Posada & Crandall 1998). We determined that the best-fit model for *H. denniae* was the HKY model of substitution (Hasegawa *et al.* 1985) plus a gamma distribution (Γ) using the following estimated parameters, Γ = 0.1792 and Ti/Tv = 1.5542. For *H. lunaris,* we chose the HKY model of substitution plus a gamma distribution using the following parameters, Γ = 0.2945 and Ti/Tv = 1.4951. For *L. woosnami,* we employed the HKY model of substitution plus invariable sites (I) and gamma distribution using the following parameters, I = 0.6996, Γ = 0.3750 and Ti/Tv = 19.9176.

We applied the two-cluster test using LINTREE (Takezaki *et al.* 1995) to determine whether the lineages arising from the major nodes of our phylogenies are evolving in a clock-like manner. The two-cluster test creates a neighbour-joining tree (based on Tamura–Nei distance) and examines the hypothesis that two lineages created by an interior node of a tree are evolving according to a molecular clock.

Results

Phylogeographic relationships

Individual vs. combined gene analysis. The recovered topologies from the individual gene fragment data sets and the combined data sets were identical for all of the major nodes separating the populations. The alternative topologies of the equally parsimonious trees recovered from the cytochrome *b* and control region data were within the intrapopulation nodes. Combining the two mtDNA fragments for all three taxa is supported by the ILD tests (*Hylomyscus denniae*, P = 0.8; *Hylomyscus lunaris*, P = 1.0; *Lophuromys woosnami*, P = 0.48). We chose to present the topologies of the combined data set to represent the phylogenetic relationships of *H. denniae*, *H. lunaris* and *L. woosnami*.
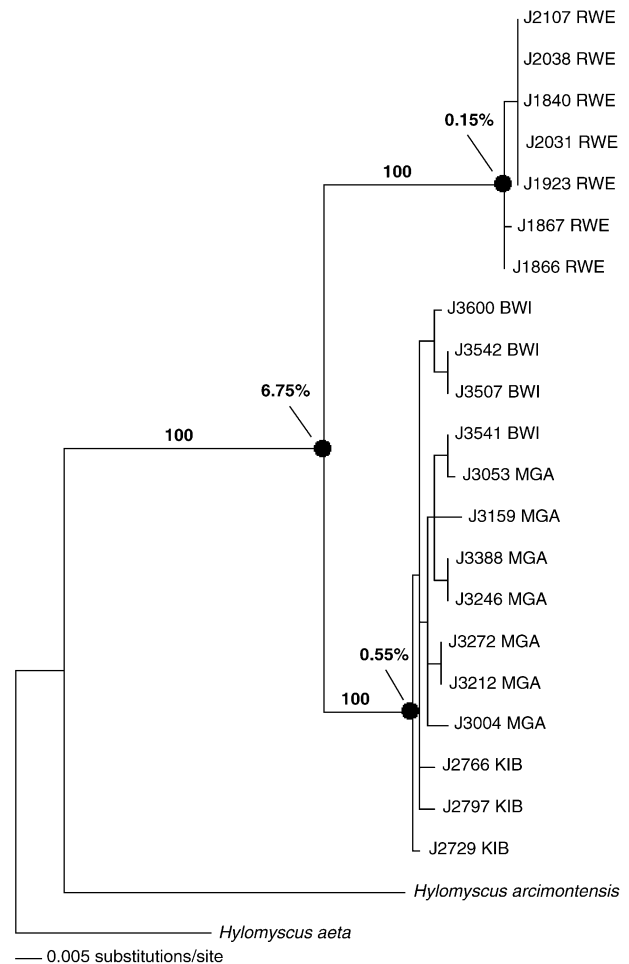
Fig. 2 Maximum-likelihood tree (ln L = −2012.40) for *Hylomyscus denniae* for the combined cytochrome *b* and control region data sets. Numbers above each branch indicate bootstrap values > 60% in 1000 replicates for the major clades. Average percentage sequence divergence among the two major clades is 6.75%. Average percentage sequence divergence within clades ranged from 0.15 to 0.55%.

Hylomyscus denniae. There were 10 cyt *b* and 12 control region haplotypes in the data sets. There are 14 haplotypes from the combined data set among the 21 individuals examined. No haplotypes were shared among the four localities. For the combined data sets of the cyt-*b* and control region fragments, the HKY pairwise sequence divergence between the two major clades ranged from 5.9 to 7.6%, within-clade diversity was low for the RWE clade (0.0–0.3%) and among the BWI/MGA/KIB clade (0.0–1.1%). The resulting topology from the combined data sets is shown in the maximum-likelihood tree (Fig. 2). Maximum-parsimony analysis resulted in 303 most parsimonious trees with identical topologies (with respect to the major clades) to the ML and NJ trees; the only differences were within clades. In *H. denniae*, there are two clearly
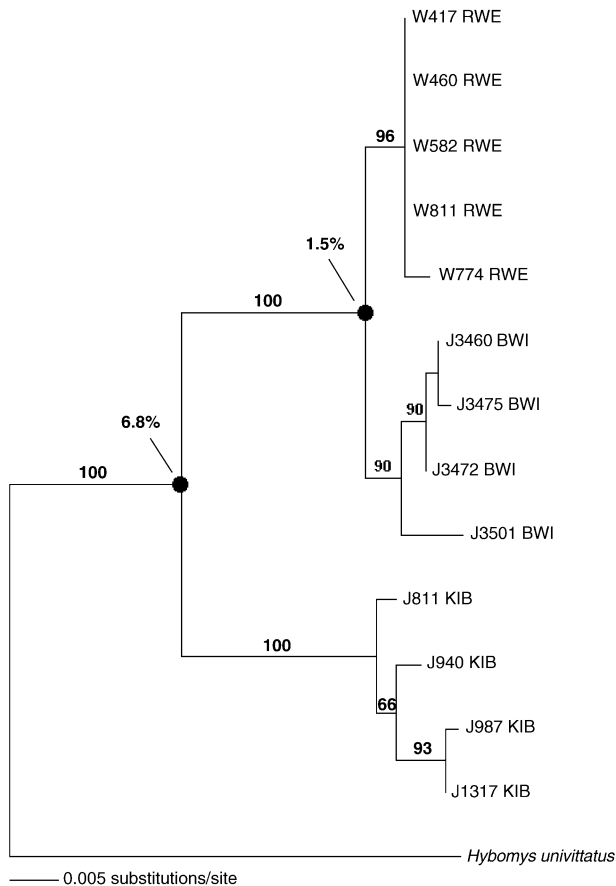
Fig. 3 Maximum-likelihood tree (ln L = −2088.18) for *Hybomys lunaris* for the combined cytochrome *b* and control region data sets. Numbers above each branch indicate bootstrap values > 60% in 1000 replicates for the major clades. Average percentage sequence divergence between the RWE/BWI clade and the KIB clade is 6.8%. Average percentage sequence divergence between RWE and BWI is 1.5%.

distinguishable clades, RWE (Rwenzori N.P.) and then all populations to the south, BWI (Bwindi Impenetrable Forest N.P.), MGA (Mgahinga Gorilla N.P.) and KIB (Kibira N.P.) (Fig. 2).

Hybomys lunaris. There are 5 cyt *b* and 8 control region haplotypes. Nine haplotypes from the combined data set were identified out of 13 individuals sequenced for *H. lunaris*. No haplotypes were shared among the three localities sampled. Uncorrected pairwise sequence divergence for the combined data sets between RWE and BWI, ranged from 1.0 to 1.7%. Within KIB, percentage sequence divergence ranged from 0.1 to 1.0%. Percentage sequence divergence between the RWE/BWI and KIB clades ranged from 5.9 to 7.7%. Maximum-parsimony analysis resulted in a single most parsimonious tree (149 steps; CI = 0.9597; RI = 0.9538); the MP tree had the same topology as the ML (Fig. 3) and NJ trees.
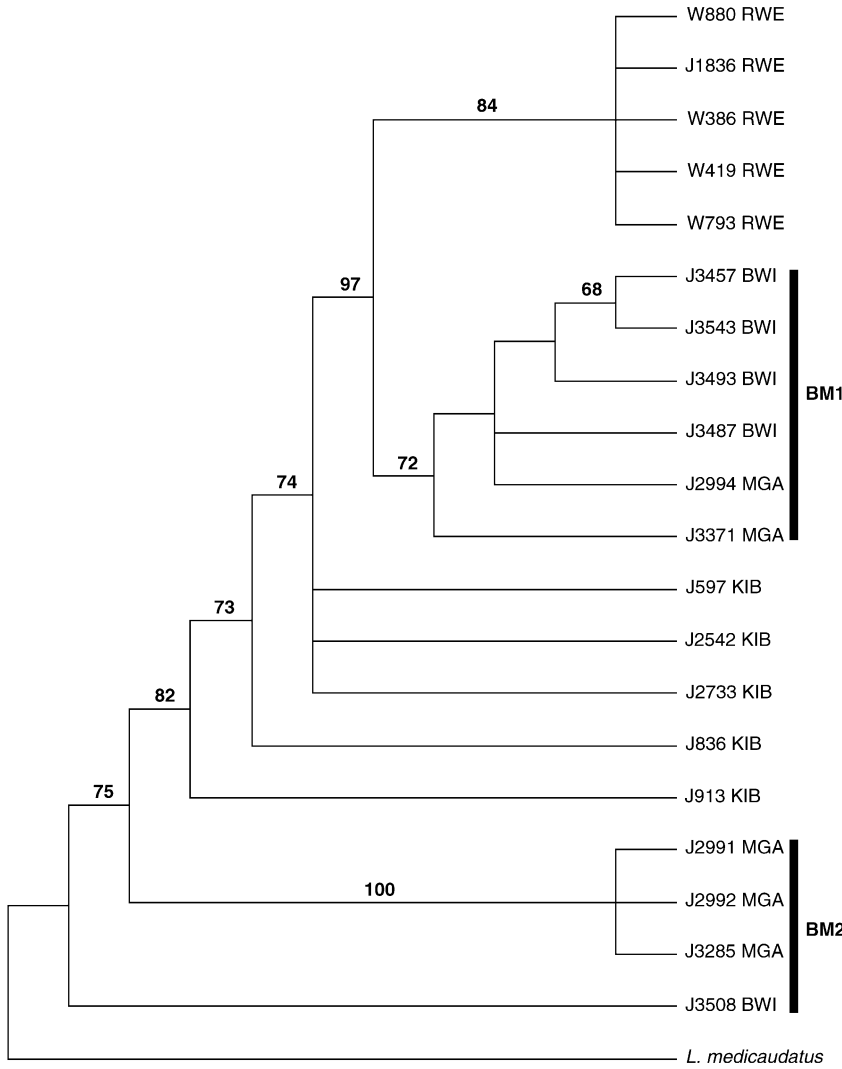
Lophuromys woosnami

There are 11 cyt *b* and 13 control region haplotypes. Fourteen unique haplotypes from the combined data set were recovered among the 20 individuals of *L. woosnami*. No haplotypes were shared among the four sampling localities. Individuals from BWI and MGA do not form a monophyletic clade, however, there is a clade composed of RWE and some individuals from BWI/MGA (henceforth referred to as BM1). Four individuals from BWI/MGA (henceforth referred to as BM2) differed greatly from other specimens from that locality, percentage sequence divergence between BM1 and BM2 ranged from 4.0 to 4.6%. The uncorrected pairwise sequence divergence between the RWE and BM1 clade and the KIB and BM2 clade ranged from 2.0 to 4.6%. Within clade divergence ranged from 1.6 to 2.5% between RWE and BM1 and ranged from 1.8 to 2.9% between KIB and BM2. The maximum-parsimony analysis resulted in five most parsimonious trees whose major clade topologies were identical to those of the maximum-likelihood tree (Fig. 4). Northern and Southern clades are not as clearly defined in the ML tree for *L. woosnami* as they are for *H. denniae* and *H. lunaris*. The clade composed of KIB and BM2 is not clearly defined in the ML tree and within-locality sequence divergence for KIB is as high as the between-locality divergence for RWE and BWI/MGA. In contrast, the NJ tree (Fig. 5) does show a clade made up of individuals from KIB and BM2; however, this clade does not have strong bootstrap support.

Estimates of divergence times

Results of the two-cluster test indicated that most of the nodes separating the major lineages in our three phylogenies are evolving in a clock-like manner. We used mean pairwise sequence divergence values from the cytochrome *b* data set to estimate divergence dates among the major lineages for each of our taxa. The percentage sequence estimates were based on our HKY model of evolution. We used a gamma-corrected rate of cytochrome *b* sequence divergence for rodents estimated to be 7.5–12% per million years (Arbogast *et al.* 2001) to calibrate our molecular clock. We used this rate and average values of estimated pairwise sequence divergence to approximate the divergence dates of the major clades in the ML phylogenies (Table 1).

We estimate that divergence of the major lineages for all three taxa occurred during the mid to late Pleistocene. The deepest split within *H. denniae*, separating the RWE clade from the BWI, MGA and KIB clade is dated at approximately 560 000–900 000 years ago. Two major splits have taken place within *H. lunaris* populations; the split between the RWE/BWI clade and the KIB clade is dated to approximately 560 000–910 000 years ago. The divergence between

Fig. 4 Maximum-likelihood tree (ln L = −1988.74) for *Lophuromys woosnami* for the combined cytochrome *b* and control region data sets. Numbers above each branch indicate bootstrap values > 60% in 1000 replicates. Clades BM1 and BM2 refer to the two distinct lineages from Bwindi Impenetrable Forest N.P. and Mgahinga Gorilla N.P.

Table 1 Estimates of divergence times for the major clades using HKY85 pairwise sequence divergence values for the cytochrome *b* data set, and based on a sequence divergence rate for cytochrome *b* of 7.5–12% per million years

Nodes	Sequence divergence	Divergence time estimates (years ago)
Hylomyscus denniae		
RWE and BWI/MGA/KIB	5.9–7.6	560 000–900 000
Hybomys lunaris		
RWE/BWI and KIB	5.9–7.7	560 000–910 000
RWE and BWI	1.0–1.9	125 000–200 000
Lophuromys woosnami		
RWE/BM1 and KIB/BM2	2.0–5.3	300 000–480 000
RWE and BM1	0.8–1.5	100 000–160 000
KIB and BM2	1.2–3.1	180 000–290 000
BM1 and BM2	2.0–3.2	220 000–350 000

RWE and BWI is dated to approximately 125 000–200 000 years ago. Divergence dates for *L. woosnami* indicate that the separation between the RWE/BM1 clade and the KIB/BM2 clade as indicated in the NJ tree is dated to approximately 300 000–480 000 years ago. The divergence between RWE and BM1 transpired approximately 100 000–160 000 years ago and the divergence between KIB and BM2 happened 180 000–290 000 years ago. The divergence between BM1 and BM2 arose approximately 220 000–350 000 years ago. Rates of molecular evolution are lineage specific and because age estimates are known to yield large confidence limits, the divergence dates estimated for our taxa are only mean approximations of the time since separation of major lineages and should be viewed as preliminary hypotheses with respect to the timing of historical events affecting the taxa examined.
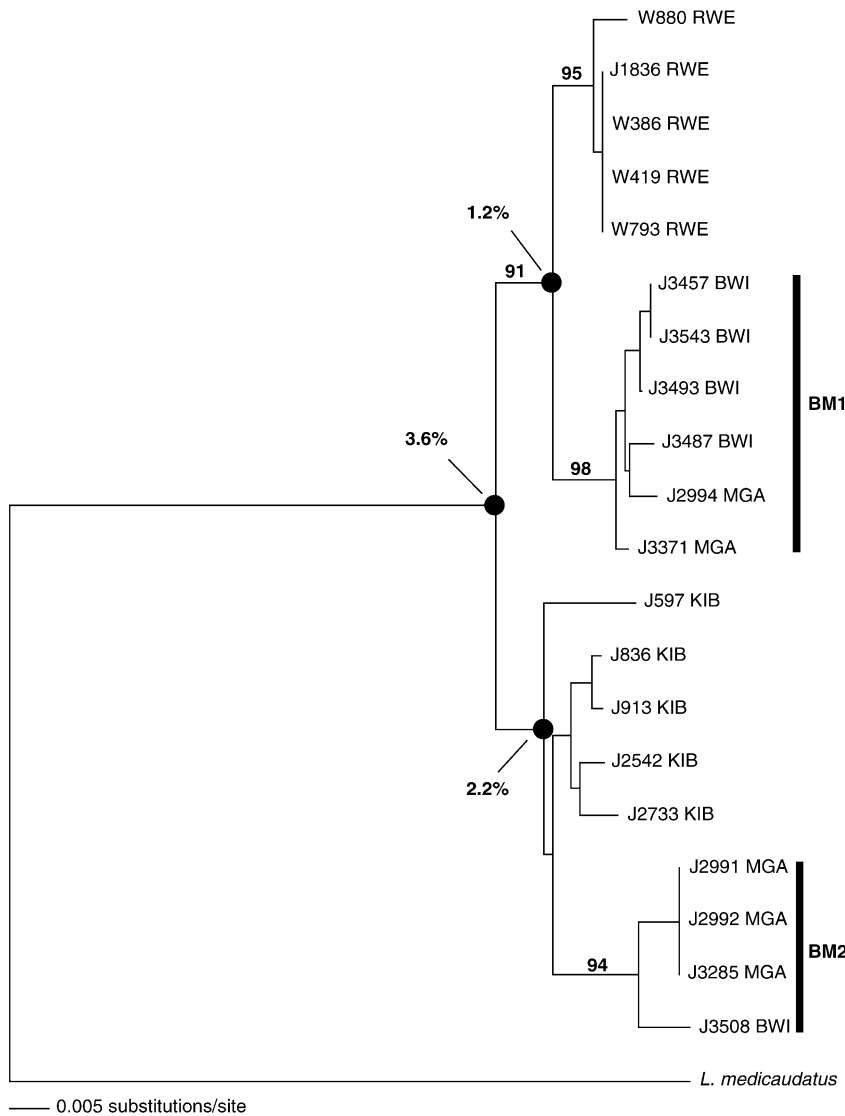
Fig. 5 Neighbour-joining tree for *Lophuromys woosnami* for the combined cytochrome *b* and control region data sets. Support in more than 60% of 1000 bootstrap replicates are shown for the major clades. Average pairwise sequence divergence between RWE/BM1 and KIB/BM2 is 3.6%. Average pairwise sequence divergence between RWE and BM1 is 1.2% and between KIB and BM2 is 2.2%. Clades BM1 and BM2 refer to the two distinct lineages from Bwindi Impenetrable Forest N.P. and Mgahinga Gorilla N.P.

Discussion

Comparative phylogeography

The strength of a comparative phylogeographic approach is that one can test whether codistributed taxa show congruent phylogeographic patterns that may be indicative of similar ecological requirements and/or shared geological and climatically altered histories. Such congruent phylogenetic patterns may predict similar partitioning of genetic variation for other codistributed taxa not yet examined. Phylogeographic patterns are generally formed when formerly contiguous populations have become isolated for long periods of time, usually due to some extrinsic barrier to gene flow (Avise 2000). Our study is the first comparative

phylogeographic study of rodents inhabiting the Albertine Rift region, and our goals were to determine the level of endemism of the isolated montane forests. Our phylogenetic analyses of *Hylomyscus denniae*, *Hybomys lunaris* and *Lophuromys woosnami* showed that some populations from separate mountain ranges are represented by monophyletic assemblages of haplotypes, and that the major mitochondrial DNA lineages probably diverged in the mid to late Pleistocene. The phylogeographic structuring recovered by our study suggests that historical biogeographical barriers in the Albertine Rift region have affected the three rodent taxa differently. While our initial prediction that the Virunga Volcanoes chain would be a complete barrier to dispersal for all three taxa was not upheld, it is clear that volcanic activity in the region did play a role in shaping the genetic

diversity of these three species. Additionally, historic climatic factors affecting the formation and alteration of lakes and rivers in the region and the general aridification of some lowland areas fragmenting the forests most likely created barriers that contributed to the high levels of diversity seen in our three species.

Our study has identified several interesting aspects of the phylogeographies of *H. denniae*, *H. lunaris* and *L. woosnami*. Here we discuss the main aspects of the phylogeographies for these taxa and compare them to each other. The discussion is ordered geographically beginning in the northern Albertine Rift and proceeding southwards (Fig. 1).

Rwenzori Mountains. Rising above 5000 m a.s.l., the Rwenzori Mountains make up the third highest mountain range in Africa. All three taxa show a monophyletic split between the Rwenzori populations and those to the south, which suggests that the Rwenzoris have been isolated for a significant period of time. The major barriers to gene flow to the south of the Rwenzori Mountains are Lake Edward, Lake George and the Kazinga Channel that connects the two lakes. Queen Elizabeth National Park lies in the area along the Kazinga channel, which is a mosaic of savannah and dry forest. During the mid-Pleistocene, Semliki Lake occupied the area of present-day Lakes Edward and George (Beadle 1981) suggesting that these bodies of water have acted as long-standing biogeographical barriers. The Semliki River which flows north out of Lake Edward to Lake Albert (Fig. 1) separates the Rwenzoris to the east from the lowland rainforests of the Congo basin to the west. However, to determine whether the Semliki River has acted as a barrier to gene flow we would need additional samples from the Lubero Mountains west of Lake Edward in the DR Congo.

Kigezi Highlands and the Virunga Volcanoes. Bwindi Impene-trable Forest National Park is one of the last remaining fragments of the montane forest ecosystem that was present in the Kigezi Highlands. These highlands lie to the north and northeast of the Virunga Volcanoes, there appears to have been no historic biogeographical barrier between these two highland regions and because of their close proximity (< 50 km), we assumed recurrent gene flow may have occurred between these areas. Geologically, the Virunga Volcanoes chain has helped shape the entire central Albertine Rift region, as the eight volcanoes have been active from the Plio–Pleistocene until the present day. Mgahinga Gorilla National Park is located in the eastern end of the Virungas chain, the three volcanoes within the park, Muhavura, Mgahinga and Sabinyo are extinct and among the older volcanoes in the chain. We initially predicted that the volcanoes created a barrier to dispersal between forests to the north and south of the Virungas and that all of the populations from the Virungas and the Kigezi Highlands would form a monophyletic clade for all three taxa. However, our analysis suggests that the Virungas have not acted as a permanent, consistent barrier to gene flow between southern populations (Kibira N.P.) and northern populations (Mgahinga and Bwindi N.P.). With respect to the Virunga Volcanoes, our three taxa do not show complete congruence in phylogeographic patterns rather the data suggest some historic gene flow between BWI/MGA and KIB for at least two of our taxa. For example, the pattern for *H. denniae* indicates that the Virungas were not a dispersal barrier with respect to the Bwindi/Mgahinga and Kibira populations. Specifically, the sequence divergence level between KIB and BWI/MGA is low, ranging from 0.5 to 1.0%. The recovered phylogeographic pattern suggests that there was continuous montane forest from the Rugege Highlands north to the Kigezi Highlands. Individuals of *L. woosnami* from BWI and MGA contained two distinct mtDNA lineages; one of these lineages (BM1) forms a clade with RWE haplotypes while the other lineage (BM2) is basal to the KIB haplotypes. This pattern may suggest that BWI and MGA were previously isolated and then may have come into secondary contact. However, we identified a large phylogenetic break for *H. lunaris* that is consistent with a significant geographical barrier to gene flow between the Kigezi and Rugege Highlands. *H. lunaris* has never been collected from the Virunga Volcanoes, which may be due to the lack of forest habitat or the region has not been completely sampled. Volcanic activity may have altered the historic habitat of Mgahinga National Park in such a way that there were no suitable dispersal corridors between Bwindi and Kibira; the latter question would need to be investigated further with samples from additional localities in the region.

Rugege Highlands. The montane forests of the Rugege High-lands currently are comprised of the Nyungwe Forest Reserve in southwestern Rwanda and the Kibira National Park in northeastern Burundi. These contiguous forests are one of the largest montane forest blocks remaining in Africa (Vedder *et al*. 1992). As discussed, *H. lunaris* showed a significant phylogeographic break between KIB and northern localities, ranging from 4.7 to 5.5% sequence divergence, while individuals from both *H. denniae* and *L. woosnami* indicated some degree of gene flow between KIB and BWI/MGA.

Temporal context of phylogenetic clades

Our data suggest that the divergences between the major phylogenetic clades for our species occurred during the mid to late Pleistocene. Forest fragmentation caused by volcanic activity and climatic fluctuations in the Albertine Rift appear to have been the major sources creating historical biogeographical barriers for the three rodent taxa examined

in our study. Volcanism in the Virunga rift region began during the late Miocene, approximately 9–11 million years ago based on the dating of tholeiitic rocks and sodium alkaline lava flows (Kampunzu *et al*. 1998). Rifting during this period began the uplift of the Rwenzoris. Volcanic activity in the Virungas during the Pleistocene dramatically altered the central Albertine Rift. During the mid-Pleistocene proto Lake Kivu outflowed north into a large lake encompassing the area of present-day Lakes Edward and George (Beadle 1981). Volcanic eruptions during the mid to late Pleistocene dammed rivers and created several smaller lakes in the region. This includes Lake Kivu, created 10 000–15 000 years ago when its northern drainage was blocked by lava flows, it now outflows to the south via the Ruzizi River to Lake Tanganyika. Additionally, the general aridification of the area around Lakes Edward and George (Queen Elizabeth N.P.) during the Quaternary has most likely constituted a significant barrier to the east for dispersal of forest dependent taxa.

Taxonomic considerations

While this study is not meant to be a systematic revision of these three taxa, the phylogeographic patterns, the levels of sequence divergence and the suggested ages of divergence among populations raises questions about whether the sampled populations of these three taxa may be represented by more than one species. The deepest phylogeographic divergence for *H. denniae* and for *H. lunaris* is the separation of populations into northern (Rwenzori) and southern (Kibira) subdivisions of the Albertine Rift. The maximum-likelihood HKY85 pairwise sequence divergence levels represented by this split is quite high for both taxa; levels ranged from 5.9 to 7.6% for *H. denniae* and 5.9 to 7.7% for *H. lunaris*. Initial divergences between clades for *H. denniae* and *H. lunaris* along the north–south subdivision probably occurred during the mid-Pleistocene.

Mus denniae (= *Hylomyscus denniae*) from the Rwenzori mountains was described by Thomas (1906) as a long-tailed, wooly pelaged mouse aligned with *Mus alleni*. It was noted to have long palatal foramina, square supra-orbital ridges without beading, and a zygomatic plate without much forward projection. Lonnberg & Gyldenstolpe (1925) described *Rattus* (*Praomys*) *denniae vulcanorum* as a smaller (e.g. tail, ear, hindfoot, snout, skull) subspecies of *H. denniae* from Mount Karisimbi and Mount Mikeno from the Virunga Volcanoes. This taxonomic arrangement was adopted by Allen (1939). In his morphometric review of east African *Hylomyscus*, Bishop (1979) recognized two taxa and further aligns specimens from Bwindi Impenetrable Forest N.P. with the Virunga specimens as *Praomys* (*Hylomyscus*) *denniae vulcanorum*. In fact, the morphological variation combined with the levels of mtDNA sequence divergence would seem to suggest that that these nomen be applied to

two species of *Hylomyscus* in the Albertine Rift, *H. denniae* restricted to the Rwenzori Mountains and *H. vulcanorum* in the fragmented montane forests to the south. Additional samples from other montane forest populations and the use of additional molecular markers will be necessary for a complete molecular systematic revision, but the data presented here coupled with a morphological revision of the Albertine Rift *Hylomyscus* (Carleton *et al*. 2006) further supports the separation of *H. d. denniae* and *H. d. vulcanorum*.

Hybomys lunaris was originally described from the Mubuku Valley of the Rwenzori Mountains of Uganda as a sub-species of *Mus* (*Hybomys*) *univittatus* Thomas (1906). *H. lunaris* is now recognized as a separate species based on karyological comparisons (Verheyen & Van der Straeten 1985) and morphometrical analysis (Van der Straeten *et al*. 1986). However, Van der Straeten *et al*.'s (1986) treatment did not consider the type specimen or additional topotypes from the Rwenzori Mountains. Our analysis based on partial mtDNA sequences would seem to suggest that more than one species of *Hybomys* is present in the Albertine Rift with *H. lunaris* being restricted to the montane forests north of the Virunga Volcanoes and an unnamed species inhabiting the forests south of the Virungas and in eastern DR Congo. A more complete molecular and morphometric analysis of populations from across the known range of *H. lunaris* is necessary before any taxonomic conclusions can be drawn.

As for both of our other taxa, *L. woosnami* was described by Thomas (1906) from the Rwenzoris. Thomas (1911) later described *Lophuromys prittiei* as a separate species from the Virunga Volcanoes region of Uganda; its status later reduced to subspecies (Allen 1939). The only substantive discussion on the validity of *L. prittei* was that of Gyldenstolpe (1928). A thorough review of Thomas' description led him to conclude that 'some of the characters given for distinguishing *L. prittiei* from *L. woosnami* are apparently not constant' (pp. 54, 56), although he upheld its status. Thorough systematic review and analysis of type specimens will be necessary before the status of *L. prittiei* can be determined. It is possible that the unique lineages from Kibira and Bwindi/Mgahinga (BM2) will confirm with specimens referable on morphological grounds to *L. prittiei*.

Conclusions

This study represents the first comparative mtDNA-based investigation of the phylogeographic structuring in endemic montane forest-dependent rodent species of the Albertine Rift region of east central Africa. We provide strong supporting evidence that volcanic activity combined with climatic fluctuations during the Plio–Pleistocene has shaped the biogeography of the Albertine Rift. While there is significant phylogeographic structure for the three endemic rodent taxa, the Virunga Volcanoes have not acted as a

consistent barrier to gene flow. The results of our study highlight several localities where more detailed research into phylogeographic structuring would provide a better understanding of the historical biogeographical processes of the Albertine Rift. For example, highly divergent lineages of *Lophuromys woosnami* from the Kigezi Highlands indicate a past connection to forests in the southern regions of the Albertine Rift. Our study has focused on the eastern side of the Albertine Rift were specimen and tissue collections are available for a limited number of localities. More individuals are needed from additional localities in the eastern Albertine Rift to fully understand the historical biogeography of the region. Also, the small mammal phylogeography of the western Albertine Rift is entirely understudied and can only be evaluated with sampling efforts in the montane forests of the eastern Democratic Republic of Congo. While the inferences of our study are based on small sample sizes, currently the only tissue specimens available from these sites, the results of our analysis are robust and would not likely change with an increase in the number of individuals from our sampled populations. Our study provides excellent baseline data for the sampling design of future phylogeographic studies and identifies areas needing increased conservation efforts across the Albertine Rift.

Acknowledgements

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Appendix

Localities and specimens included in this study. All voucher specimens are located in The Field Museum

Species	Country	General locality	Collector no.	FMNH no.
Hylomyscus denniae	Uganda	Rwenzori Mountains N.P.	JCK 1840	144457
Hylomyscus denniae	Uganda	Rwenzori Mountains N.P.	JCK 1866	144526
Hylomyscus denniae	Uganda	Rwenzori Mountains N.P.	JCK 1867	144527
Hylomyscus denniae	Uganda	Rwenzori Mountains N.P.	JCK 1923	144547
Hylomyscus denniae	Uganda	Rwenzori Mountains N.P.	JCK 2031	144588
Hylomyscus denniae	Uganda	Rwenzori Mountains N.P.	JCK 2038	144589
Hylomyscus denniae	Uganda	Rwenzori Mountains N.P.	JCK 2107	144608
Hylomyscus denniae	Burundi	Kibira N.P.	JCK 2729	149091
Hylomyscus denniae	Burundi	Kibira N.P.	JCK 2766	149094
Hylomyscus denniae	Burundi	Kibira N.P.	JCK 2797	149095
Hylomyscus denniae	Uganda	Mgahinga Gorilla N.P.	JCK 3004	157511
Hylomyscus denniae	Uganda	Mgahinga Gorilla N.P.	JCK 3053	157512
Hylomyscus denniae	Uganda	Mgahinga Gorilla N.P.	JCK 3159	157513
Hylomyscus denniae	Uganda	Mgahinga Gorilla N.P.	JCK 3212	157516
Hylomyscus denniae	Uganda	Mgahinga Gorilla N.P.	JCK 3246	157517
Hylomyscus denniae	Uganda	Mgahinga Gorilla N.P.	JCK 3272	157518
Hylomyscus denniae	Uganda	Mgahinga Gorilla N.P.	JCK 3388	157528
Hylomyscus denniae	Uganda	Bwindi Impenetrable Forest N.P.	JCK 3507	157903
Hylomyscus denniae	Uganda	Bwindi Impenetrable Forest N.P.	JCK 3541	157904
Hylomyscus denniae	Uganda	Bwindi Impenetrable Forest N.P.	JCK 3542	157905
Hylomyscus denniae	Uganda	Bwindi Impenetrable Forest N.P.	JCK 3600	157907
Hybomys lunaris	Burundi	Kibira N.P.	JCK 811	137708
Hybomys lunaris	Burundi	Kibira N.P.	JCK 940	137710
Hybomys lunaris	Burundi	Kibira N.P.	JCK 987	137711
Hybomys lunaris	Burundi	Kibira N.P.	JCK 1317	137712
Hybomys lunaris	Uganda	Bwindi Impenetrable Forest N.P.	JCK 3460	157900
Hybomys lunaris	Uganda	Bwindi Impenetrable Forest N.P.	JCK 3472	157871
Hybomys lunaris	Uganda	Bwindi Impenetrable Forest N.P.	JCK 3475	157873
Hybomys lunaris	Uganda	Bwindi Impenetrable Forest N.P.	JCK 3501	157880
Hybomys lunaris	Uganda	Rwenzori Mountains N.P.	WTS 417	144399
Hybomys lunaris	Uganda	Rwenzori Mountains N.P.	WTS 460	144401
Hybomys lunaris	Uganda	Rwenzori Mountains N.P.	WTS 582	144436
Hybomys lunaris	Uganda	Rwenzori Mountains N.P.	WTS 774	144444
Hybomys lunaris	Uganda	Rwenzori Mountains N.P.	WTS 811	144447
Lophuromys woosnami	Burundi	Kibira N.P.	JCK 597	137826
Lophuromys woosnami	Burundi	Kibira N.P.	JCK 836	137845
Lophuromys woosnami	Burundi	Kibira N.P.	JCK 913	137850
Lophuromys woosnami	Burundi	Kibira N.P.	JCK 2733	149164
Lophuromys woosnami	Burundi	Kibira N.P.	JCK 2542	149172
Lophuromys woosnami	Uganda	Mgahinga Gorilla N.P.	JCK 2991	157670
Lophuromys woosnami	Uganda	Mgahinga Gorilla N.P.	JCK 2994	157671
Lophuromys woosnami	Uganda	Mgahinga Gorilla N.P.	JCK 2994	157673
Lophuromys woosnami	Uganda	Mgahinga Gorilla N.P.	JCK 3285	157738
Lophuromys woosnami	Uganda	Mgahinga Gorilla N.P.	JCK 3371	157762
Lophuromys woosnami	Uganda	Bwindi Impenetrable-Forest N.P.	JCK 3457	157920
Lophuromys woosnami	Uganda	Bwindi Impenetrable-Forest N.P.	JCK 3487	157923
Lophuromys woosnami	Uganda	Bwindi Impenetrable-Forest N.P.	JCK 3493	157925
Lophuromys woosnami	Uganda	Bwindi Impenetrable-Forest N.P.	JCK 3508	157926
Lophuromys woosnami	Uganda	Bwindi Impenetrable-Forest N.P.	JCK 3543	157931
Lophuromys woosnami	Uganda	Rwenzori Mountains N.P.	JCK 1836	144840
Lophuromys woosnami	Uganda	Rwenzori Mountains N.P.	WTS 386	144827
Lophuromys woosnami	Uganda	Rwenzori Mountains N.P.	WTS 415	144831
Lophuromys woosnami	Uganda	Rwenzori Mountains N.P.	WTS 793	144879
Lophuromys woosnami	Uganda	Rwenzori Mountains N.P.	WTS 880	144889
Hylomyscus arcimontensis	Tanzania	East Usambara Mountains	WTS 1316	151251
Hylomyscus aeta	Uganda	Kibira N.P.	JCK 2794	149098
Hybomys univittatus	Gabon		SMG 10134	162162
Lophuromys medicaudatus	DR Congo	Mount Tschiaberimu	PK 125	Uncat.

N.P., National Park.