Revised occurrence of rodents from the tribe Praomyini (Muridae) in Zambia based on mitochondrial DNA analyses: implications for biogeography and conservation

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Abstract. The taxonomy and distribution of rodents in Zambia was comprehensively summarized in 1978 by W.F.H. Ansell in his excellent book Mammals of Zambia. Despite the fact that during the last three decades many new taxonomic revisions of African rodents were published and extensive new material collected, not much work has been done on Zambian rodents since the book publication. Here we summarize the current knowledge of one of the most speciose group of African rodents, the tribe Praomyini, in Zambia. We review available historical records and revise our recently collected material by sequencing the mitochondrial DNA gene of cytochrome *b*. The presence of eight species of Praomyini in Zambia is documented and the pattern of their geographical distribution is described and discussed. Two species, *Praomys minor* and *Mastomys coucha*, are reported for the first time from Zambia and *Praomys* cf. *jacksoni* probably represents a new undescribed species. On the other hand, the actual occurrence of *Colomys goslingi*, known in Zambia only from one historical record, is questionable. The results document the usefulness of the DNA barcoding approach for description of species diversity of taxonomically complicated groups with many cryptic species.

Introduction

Rodents are a very important group of mammals forming more than 42 % of mammalian species diversity (Musser & Carleton 2005). They have a practical importance for humans, being suitable models for research e.g. in biomedicine (e.g. laboratory mouse or rat) or evolutionary ecology (e.g. some voles as models in population demography). They are also significant pests in agriculture and vectors of many infectious diseases (e.g. Gratz 2006, Singleton et al. 2010). Besides intensive research of rodents across the globe over the last century, there are still many geographical areas, where even basic data are missing and the rodent diversity is poorly described. Filling the gap in this knowledge has many important consequences, e.g. for proposing new areas for biodiversity conservation or for epidemiological studies.

Among the least known areas in this sense, some parts of tropical Africa belong to the most intriguing for many reasons. During last five millions years many groups of African rodents underwent a rapid radiations linked with the speciation processes, resulting in extreme cryptic diversity. The use of DNA-based methods led to the discovery of many monophyletic genetic lineages within phenotypically

uniform groups that were subsequently described as separate biological species (e.g. Hoffmann et al. 2009). This approach is very useful, because it allows quick identification of the centres of genetic diversity even in the lack of classical taxonomical knowledge (e.g. Kan Kouassi et al. 2008, Dobigny et al. 2011). The Zambezian phytochorion, and Zambia particularly, is an important geographical area, where the diversity of mammalian fauna has been studied only marginally by molecular-genetic approaches. Rodents of Zambia were intensively studied up to the 1970s of the last century and the knowledge of their taxonomy and distribution are summarized in an excellent book, The Mammals of Zambia by W.F.H. Ansell (Ansell 1978). Since that time, however, the rodents of Zambia were studied only occasionally as part of geographically localized ecological studies (e.g. Chidumayo 1977, 1979, 1980a, b, 1984, Kawalika 2004) and a few individuals from Zambia were also used as a part of recent systematic reviews of some taxonomic groups (e.g. Burda et al. 1999, Castiglia et al. 2002, Verheyen et al. 2003, Castiglia et al. 2003, Corti et al. 2004, 2005, Mullin et al. 2004, 2005, Carleton & Stanley 2005, Taylor et al. 2009, Verheyen et al. 2011). The lack of systematic research of Zambian rodents in the last 40 years has led to the absence of up-to-date patterns of distribution of rodent species, especially in the taxonomically complicated groups, where the genetic approaches recently led to the description of many new cryptic species (e.g. Van Daele et al. 2007). Absence of recent systematic research in this area is quite surprising, because the Zambezian Region is the largest major phytochorion in Africa after the Sahara (White 1983). Zambia itself is a large country and despite the fact that the most landscape is covered with relatively uniform miombo woodland, there is still a wide spectrum of other habitats suggesting higher diversity of small mammals than expected so far. In lower altitudes, dominant miombo is substituted with mopane woodland, and there are also other types of savannah woodlands and shrubs across the country. Several types of forests such as evergreen forests especially in northern Zambia, gallery forest along rivers and patches of Afromontane evergreen forest in the eastern highlands together with seasonal floodplains, dambos and wetlands, grasslands and thickets contribute to a high diversity of habitats suitable for small mammals.

The tribe Praomyini (sensu Lecompte et al. 2008) belongs to the family Muridae (subfamily Murinae) and it is one of the most intensively studied groups of African rodents. This endemic African taxon is interesting and important for several reasons. It comprises one of the most important agricultural pests (e.g. in the genus *Mastomys*), and their population ecology, demography and direct impact in agriculture have been intensively studied (e.g. Granjon et al. 2005, Sluydts et al. 2009). They are also hosts of many pathogens and some of them are very important for human health (e.g. Lassa virus in western Africa; Lecompte et al. 2006). A recent description of the highly pathogenic virus Lujo (Briese et al. 2009) led to intensive searches of new pathogens, which resulted in discoveries of new arenaviruses (Coulibaly-N'Golo et al. 2011, Ishii et al. 2011), or polyomaviruses (Orba et al. 2011) hosted by Praomyini rodents. Despite this importance for human being, the basic knowledge of many taxa is still very poor. Several genera of the tribe were recently revised using a combination of DNA-based methods and morphology and candidates for new species were identified (e.g. Dobigny et al. 2008, Nicolas et al. 2008a, 2010, Bryja et al. 2010, Kennis et al. 2011). More detailed information about the distribution and ecology of these speciose genera is strongly required, for example, for the improved understanding of the epidemics and dynamics of emerging infectious diseases (Fichet-Calvet et al. 2007, 2008, Coulibaly-N'Golo et al. 2011).

In this study, we review the occurrence of species of the Praomyini tribe in Zambia. Based on the recent definition of the tribe (Lecompte et al. 2008) and faunistic data of Ansell (1978), the Praomyini is in Zambia represented by six species in the genera *Mastomys*, *Praomys*, *Hylomyscus*, *Zelotomys* and *Colomys*. However, the first three genera underwent important taxonomic revisions during last decade, which can obscure previous faunistic data. Within our recent projects, two field expeditions to Zambia were organized in 2009-2010 and numerous material of rodents from diverse habitats at many localities across the whole country were collected. Here we use the sequences of mitochondrial DNA and phylogenetic methods to assign individuals into species of recently revised genera. Together with previously published data, we present the current status of knowledge on distribution of these interesting rodents in Zambia and we highlight the prospective directions of future research of this group.

Material and Methods

Geography of Zambia

Zambia is a land-locked country covering 752620 km^2 in south-central Africa, between 10 and 18 degrees south of the Equator. It is bordered to the east by

Malawi and Tanzania, north by the Democratic Republic of the Congo (DRC), west by Angola and to the south by Namibia, Botswana, Zimbabwe and Mozambique (Jachmann 2000) (Fig. 1). The country is largely occupied by the undulating Central African Plateau, varying from about 900 to 1400 m above sea level. At some places, particularly at the northern-eastern rim it rises to over 2000 m, reaching 2320 m on the Mafingas, whereas it drops down to 329 m in the Zambezi Valley. At the east, the Plateau is delimited by the Great Escarpment, the southern extension of the Great Rift Valley. The southern border of Zambia is made by the River Zambezi, on which the famous Victoria Falls (Mosi-o-Tunya) is located.

Zambia has a mild tropical climate with three distinct seasons: the cool dry season from May to August, the hot dry season from September to October and the warm wet season from November to April. Maximum daytime temperatures in the hot season average 27-38 °C, with the highest temperatures in the south-west and in the rift valley areas, and the lowest temperatures in the north-east on the high plateau; while minimum daily cold-season temperatures average between 2-15 °C, with the lowest temperatures in the south-west (Jachmann 2000). Mean annual rainfall is between 600 mm in the South and 1600 mm in the North (Copperbelt, Luapula and Northern Province) (Fig. 1b). Zambia is drained by two major river basins. The Zambezi River Basin in the South (about 3/4 of the country's total area) consists of the Zambezi and its major tributaries (the Kafue and the Luangwa). The Congo Basin in the North has two major rivers, Chambeshi and Luapula. From the point of view of phytochorial classification, the whole of Zambia belongs (together with Malawi, Zimbabwe, large parts of Angola, Tanzania and Mozambique) to the so-called Zambezian Region, represented by nine ecoregions in four biomes. The most widespread are Miombo, Mopane and Baikiaea woodland savannah, with grasslands (mainly flooded) and small patches of evergreen forest (see White 1983; Mayaux et al. 2004, and Fig. 1a for more detailed information on vegetation types).

Sampling
Two field expeditions to Zambia in 2009 and 2010 allowed visiting a total of 57 localities (Fig. 1). Small mammals were captured along transect lines during 70 trapping nights using wooden snap traps and medium-sized (8 × 9 × 23 cm) Sherman live traps that were set on the ground 5-15 m apart from each other depending on habitat type. Trapping effort per locality varied significantly, but typically cca 50-200 traps (combination of different types) were installed for 1-2 nights. Captured animals were examined and provisionally identified to genera based on external morphology, measurements and visible external features. At least one individual from each genus per locality was used for genetic analysis. In most localities, however, we sequenced more individuals (see Supplementary Table S1) and we especially tried to analyse morphologically different animals (e.g. with shortest and longest tail). Preferably spleen or kidney tissues were collected from sacrificed individuals and toe clips from subsequently released live animals. Tissue samples were preserved in the 96 % ethanol and stored at –20 °C until DNA extraction. Voucher specimens are deposited in the Vertebrate Collection of the University of South Bohemia (České Budějovice, Czech Republic), mammalian collection of the Livingstone museum (Livingstone, Zambia) and in the Copperbelt Museum (Ndola, Zambia).

DNA extraction, PCR and phylogenetic analyses
The DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's instructions. The complete cytochrome *b* gene was amplified by polymerase chain reaction (PCR) using the primers L14723 and H15915 (Lecompte et al. 2002). We also extracted DNA from tissues (toes) of selected samples from the Livingstone Museum using the same extraction kit. Extracted DNA was however very fragmented and we had to use a set of additional internal primers H15553, L15408, L15146, H15149 and L15513 (Lecompte et al. 2002) to obtain a sequence of cytochrome *b*. The PCR conditions were the same for all primer pairs – the cycling procedure started at 94 °C of initial denaturation for 3 min, followed by 35 cycles of 94 °C (30 sec), 50 °C (30 sec) and 72 °C (3 min), ending with final extension at 72 °C for 10 min. Sequencing of obtained amplicons was performed using BigDyeTM terminator chemistry (Applied Biosystems). All sequences are available in GenBank under accession numbers JX126523-JX126621 (Supplementary Table S1).

Sequence electrophoretograms were edited in SeqScape v. 2.5.0 (Applied Biosystems) and subsequently aligned with available GenBank sequences for particular genera (representing at least one sequence per intraspecific clade identified in previous studies; see Supplementary Table S1 and below for more details). These datasets were used for phylogenetic analysis using a maximum likelihood (ML) method with the aim to identify the species of
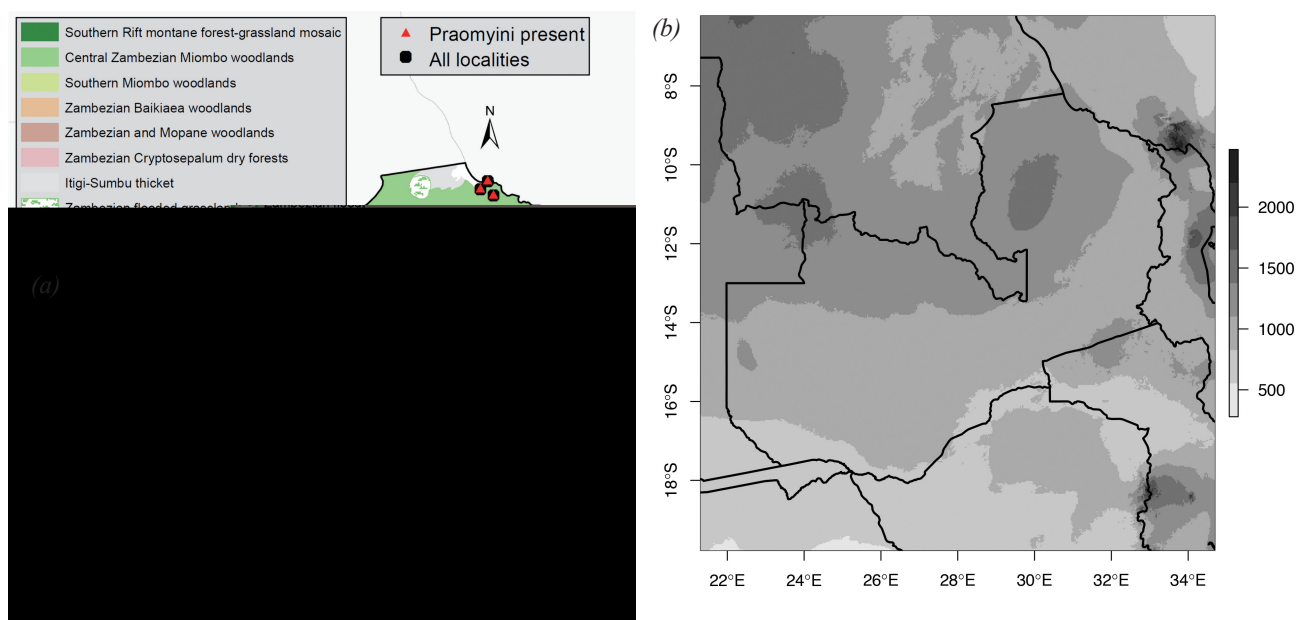
Fig. 1. *(a) Map of terrestrial ecoregions of Zambia (modified from Olson et al. 2001) with a distribution of trapping sites (black squares) and localities with the presence of species of the Praomyini tribe (red triangles) during our field expeditions. (b) Map of mean annual rainfall in Zambia.*

Zambian individuals. The online program FindModel (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html) was used to evaluate the fit of 28 nested models of nucleotide substitution to the data and the best model for each alignment was selected on the basis of Akaike information criterion (AIC). ML analyses were performed using PHYML online web server (Guindon et al. 2005) with the NNI algorithm and BIONJ distance-based tree as the starting tree. Bootstrap analysis (1000 replicates) was used to estimate the robustness of internal nodes. The results were visualised in FigTree v. 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Analysis of published records and unpublished data from museum collections

Ansell (1978) revised the occurrence of all Zambian rodents up to 1978. We resurrected the GPS coordinates from the species distribution maps published in Ansell's book, by taking the centre of the particular quadrate as the position of the species occurrence. Furthermore, the data from the Livingstone museum (Livingstone, Zambia), African Rodentia (http://projects.biodiversity.be/africanrodentia, Access date: 20/12/2011) and GBIF (Vertebrate specimens, http://data.gbif.org/datasets/resource/541 and MVZ Mammal Catalog, http://data.gbif.org/datasets/resource/8121) databases collected after 1978 and additional data published after 1978 (Kawalika 2004) were reviewed and used for creating

distribution maps using the software R 2.14.0 (R Development Core Team 2011) and *mapdata* package.

Results and Discussion

In total, we sampled rodents at 57 localities in Zambia and species from the tribe Praomyini occurred at 47 of them (Fig. 1; see list of localities and sequenced individuals in Supplementary Table S1). Below we describe the results of genetic analysis and annotate the current list of all known Praomyini species in Zambia.

(a) Genus Mastomys

The genus *Mastomys* belongs to the most intensively studied rodents in Africa (Granjon et al. 1997, Lecompte et al. 2005). It harbours widely distributed species (like *M. natalensis* which is known from most of sub-Saharan Africa) as well as much more localized species (e.g. *M. awashensis* known only from a few localities in Ethiopia; Colangelo et al. 2010). Most species avoid continuous tropical forests and some of them are known to be synanthropic. Ansell (1978) reported one species from Zambia, however, sequencing of mtDNA confirmed the presence of a second species.

Mastomys natalensis (Smith, 1834)

Natal multimammate mouse was the most frequently captured animal during our fieldwork and it is distributed throughout the whole country (Fig. 2a,
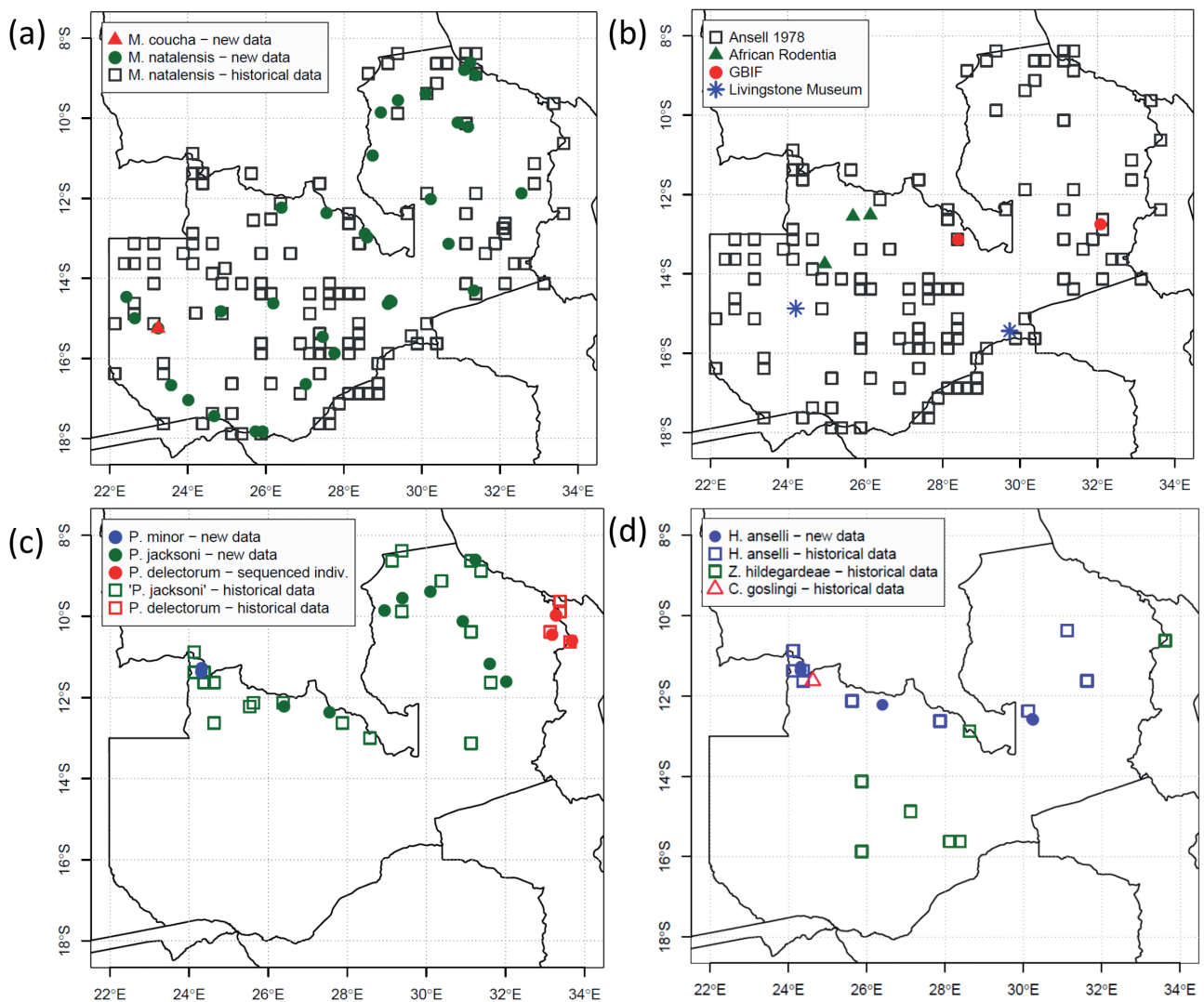
Fig. 2. *Distribution of eight species of Praomyini in Zambia. (a) genus* Mastomys; *(b) overview of historical records of* M. natalensis; *(c) genus* Praomys; *(d) genera* Hylomyscus, Zelotomys *and* Colomys. *In general, except part (b), filled symbols characterize the localities, where the material for our recent genetic analyses come from, while open symbols represent historical data ressurected from Ansell 1978 (+ the only more recent publication from Zambia, i.e. Kawalika 2004) and records from museum databases – Livingstone Museum (Livingstone, Zambia), African Rodentia (http://projects.biodiversity.be/africanrodentia, Access date: 20/12/2011) and GBIF data portal (Vertebrate specimens, http://data.gbif.org/datasets/resource/541 and MVZ Mammal Catalog, http://data.gbif.org/datasets/resource/8121).*

b). Phylogenetic analysis (Fig. 3a) clustered all Zambian individuals into a single cluster (but without bootstrap support) together with previously published sequences from Zambia and Botswana. Even usually strong barriers to gene flow, like Zambezi and Kafue Rivers, do not seem to have impact on the spatial structure of *M. natalensis* populations. However, we used only mtDNA sequences and more variable nuclear markers (like microsatellites) are necessary to identify the factors influencing genetic structure of this widely distributed rodent species.

Mastomys coucha (Smith, 1834)
This species is largely endemic to South African subregion. It is found in South Africa, Lesotho, southern and western Zimbabwe, Mozambique, Botswana, central and northern Namibia to southern Angola (Coetzee et al. 2008). Phylogenetic analysis clearly identified mtDNA sequences of three Zambian individuals from the close proximity of the Newa village near Mongu as *M. coucha* (Fig. 3a), which constitutes the first record for Zambia and the northernmost record of this species (Supplementary

Table S1). The species was sympatric and even syntopic with *M. natalensis* in wet fallow fields near a stream.

(b) Genus Praomys

The species-rich genus *Praomys* consists of predominantly forest species widely distributed in tropical Africa. It was recently reviewed using both morphological and molecular data, one new species was described (Van der Straeten 2008) and several other candidates for the description of new species were identified (e.g. Akpatou et al. 2007, Bryja et al. 2010, Nicolas et al. 2005, 2008b, 2010, 2011). However, none of recent studies included material from the Zambezian phytochorion. Our molecular data provide clear evidence of the occurrence of three species in Zambia (Fig. 2c), one of them is reported for the first time from the country.

Praomys cf. jacksoni (de Winton, 1897)

"*Praomys jacksoni*" was reported from 18 quadrates by Ansell (1978), but the results of molecular analysis indicate the presence of two cryptic species in Zambia, *P.* cf. *jacksoni* and *P. minor* (Fig. 3b). *P.* cf. *jacksoni* is the most widespread species of *Praomys* in Zambia and the majority of previous records of "*P. jacksoni*" (sensu Ansell 1978) probably represent this species. It is distributed in the northern part of the country, i.e. in areas with more than 1100 mm annual rainfall (Fig. 1). All sequenced *P.* cf. *jacksoni* from Zambia form a monophyletic group that is a sister clade to *P.* cf. *jacksoni* Clade II (sensu Kennis et al. 2011) from the Kisangani region in DRC (Fig. 3b). The mean K2P distance between these two clades is 3.1 ± 0.63. The individuals from DRC are distributed on the right bank of the River Congo and they morphologically differ from nominate species (*P. jacksoni* s. str.) described from Uganda (see Fig. 4 in Kennis et al. 2011). The whole group (i.e. Clade II from DRC together with individuals from Zambia) could therefore represent a new species; however more material (especially from the eastern part of the Albertine rift), genetic data (nuclear genes) and morphological analyses (including e.g. geometric morphometric approach) are required for final decision about its specific status.

Praomys minor Hatt, 1934

All 18 sequenced individuals from two localities in the Mwinilunga District (Supplementary Table S1) in the north-western part of Zambia (where the River Zambezi springs) cluster to a separate group forming a monophyletic lineage together with Clade III from DRC (sensu Kennis et al. 2011) (Fig. 3b). The mean K2P distance between Zambian and DRC clades is 3.1 ± 0.59. The individuals from DRC occur at the left bank of the River Congo in the Kisangani region and are morphologically distinct from other clades of the *P. jacksoni* complex (see Fig. 4 in Kennis et al. 2011), especially they are significantly smaller at all skull measurements than other taxa from the complex (see Table 3 in Kennis et al. 2011). They are morphologically very similar to type series of *P. minor*, i.e. the species described on the basis of three specimens from Lukolela in DRC by Hatt (1934). The material published by Kennis et al. (2011) is thus very probably the first well-documented record of *P. minor* after its original description extending the geographical range of the species about 750 km to the east. Here, we extend its distribution range by more than 1300 km to the south. It is a new species for Zambia, where it reaches the southern border of its distribution. The species was very abundant at both localities in Zambia (gallery forest and moist evergreen forest called "mushitu") and occupied various habitats in Kisangani region in DRC (Kennis et al. 2011). This suggests that it was largely overlooked in previous studies of rodents in the Congo Basin, where it is probably largely distributed on the left bank of the River Congo. We have no evidence that *P. minor* at Mwinilunga District is sympatric with *P.* cf. *jacksoni*, suggesting that all (or most) of *P. jacksoni* sensu Ansell (1978) from this area are probably *P. minor*. However, more sequenced individuals from more localities are necessary to obtain a more precise record of the distribution of both species in north-western Zambia.

Praomys delectorum (Thomas, 1910)

This mountain species has been recorded from high plateaus and isolated mountains from northern Mozambique, through Malawi, north-eastern Zambia and Tanzania (Eastern Arc Mountains, Kilimanjaro, Mt. Meru) to south-eastern Kenya (Taita Hills) (Bryja et al., submitted). In Zambia, the species is abundant in the forest patches on Nyika Plateau and surrounding mountains (the Makutus and the Mafingas) (Ansell 1978; Fig. 2c). Recent detailed morphological study suggested that individuals from Zambia, northern Malawi and southern Tanzania should belong to a separate species *P. melanotus* Allen & Loveridge, 1933 (Carleton & Stanley 2012), however, in this study we still consider it as *P. delectorum*. Within the larger phylogeographic study of the species (Bryja et al., submitted), we sequenced historical DNA of five

(a)

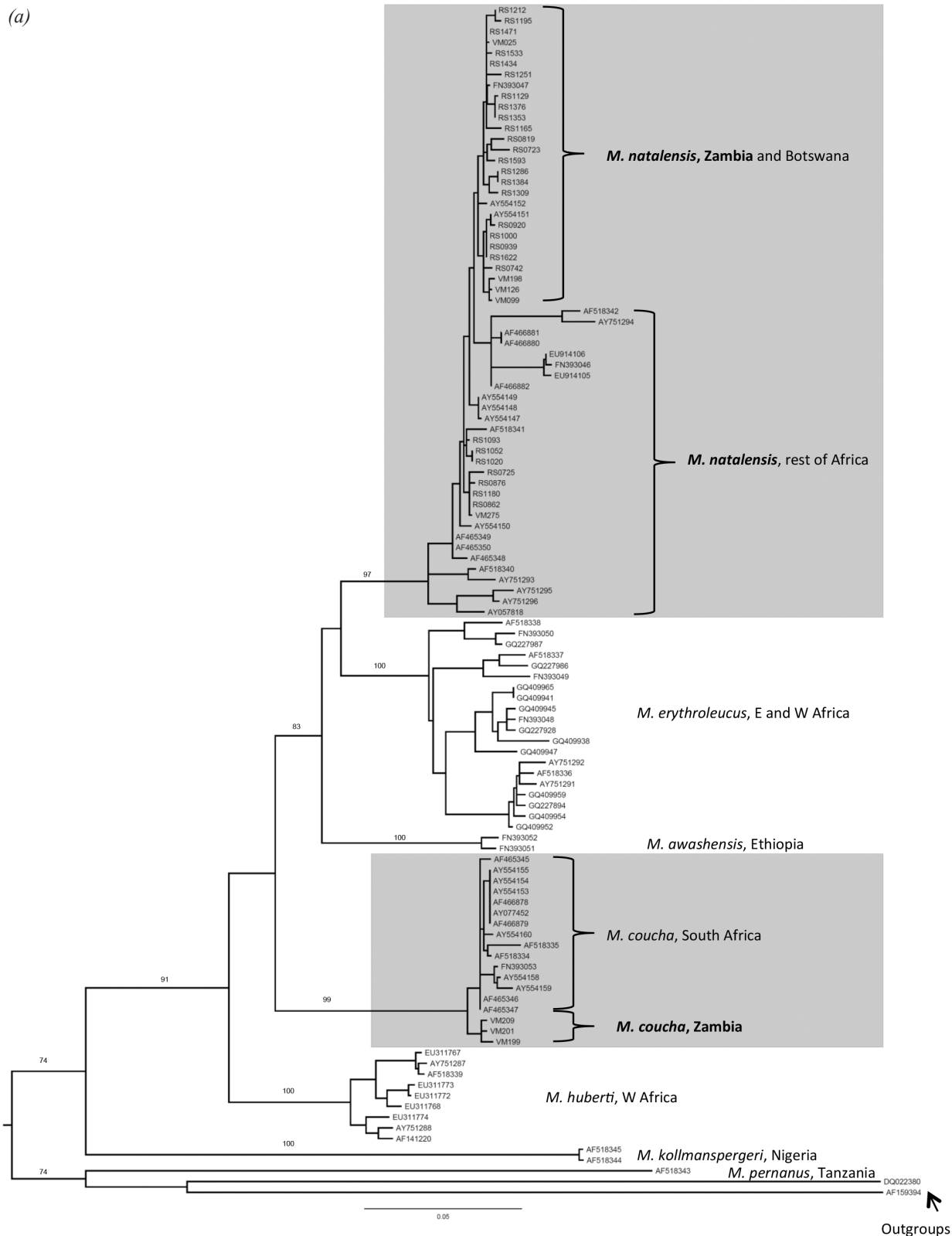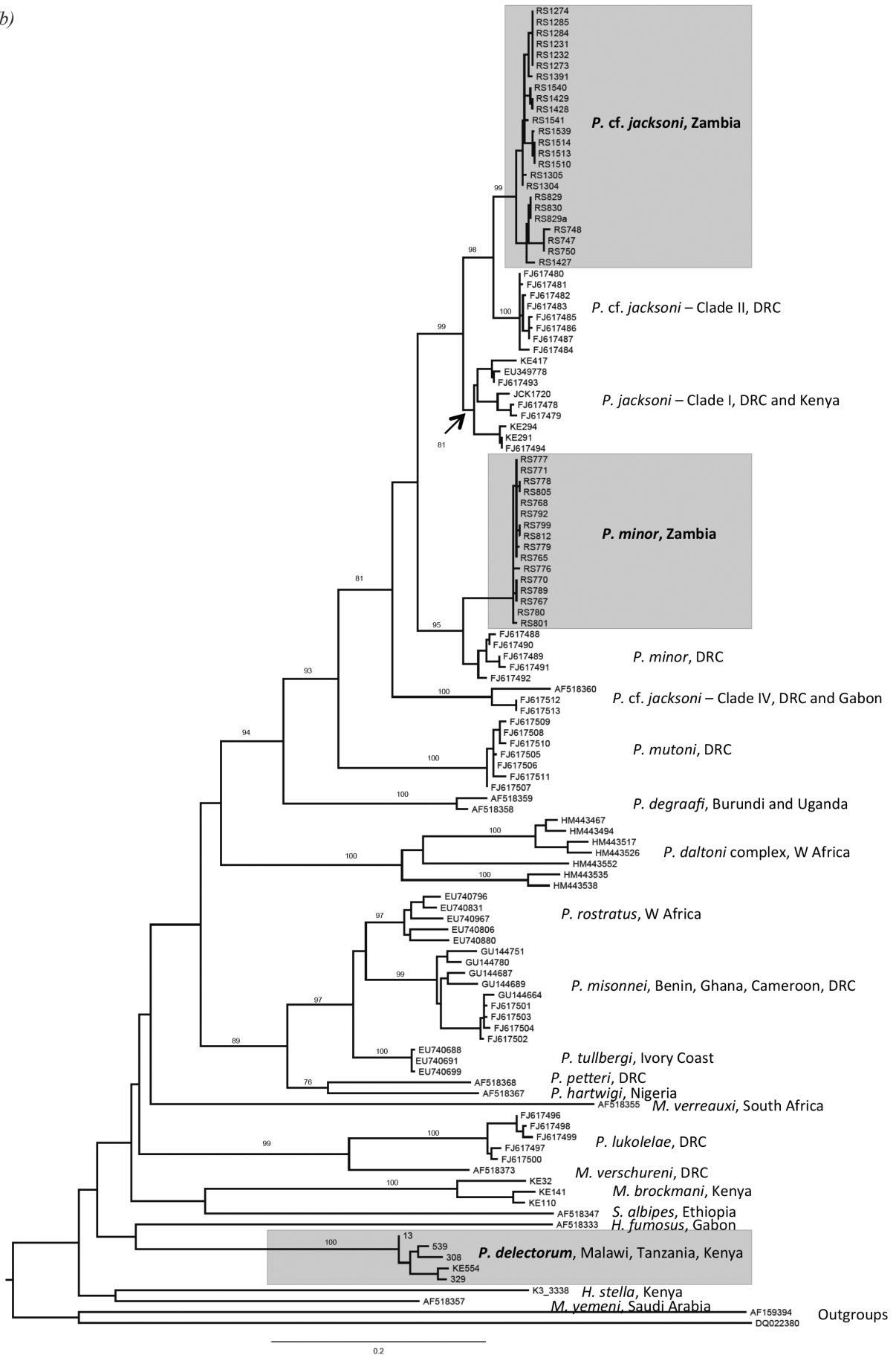**Fig. 3.** *Maximum likelihood phylogenetic trees of available sequences of cytochrome* b. *For more details of specimens used see Supplementary Table S1. Species occuring in Zambia are highlighted by grey background. The numbers above the branches represent the bootstrap values (1000 bootstraps in PhyML); only values higher than 70 are shown. (a) genus* Mastomys sensu lato*; (b) genus* Praomys *sensu lato; (c) genus* Hylomyscus*.*

(b)

RS1274
RS1285
RS1284
RS1231
RS1232
RS1273
RS1391
RS1540
RS1429
RS1428
RS1541
RS1539
RS1514
RS1513
RS1510
RS1305
RS1304
RS829
RS830
RS829a
RS748
RS747
RS750
RS1427

***P.* cf. *jacksoni*, Zambia**

99

98

FJ617480
FJ617481
FJ617482
FJ617483
FJ617485
FJ617486
FJ617487
FJ617484

100

P. cf. *jacksoni* – Clade II, DRC

99

KE417
EU349778
FJ617493
JCK1720
FJ617478
FJ617479
KE294
KE291
FJ617494

P. jacksoni – Clade I, DRC and Kenya

81

RS777
RS771
RS778
RS805
RS768
RS792
RS799
RS812
RS779
RS765
RS776
RS770
RS789
RS767
RS780
RS801

***P. minor*, Zambia**

95

FJ617488
FJ617490
FJ617489
FJ617491
FJ617492

P. minor, DRC

81

100

AF518360
FJ617512
FJ617513

P. cf. *jacksoni* – Clade IV, DRC and Gabon

93

100

FJ617509
FJ617508
FJ617510
FJ617505
FJ617506
FJ617511
FJ617507

P. mutoni, DRC

94

100

AF518359
AF518358

P. degraafi, Burundi and Uganda

100

100

HM443467
HM443494
HM443517
HM443526
HM443552
HM443535
HM443538

P. daltoni complex, W Africa

100

97

EU740796
EU740831
EU740967
EU740806
EU740880

P. rostratus, W Africa

99

GU144751
GU144780
GU144687
GU144689
GU144664
FJ617501
FJ617503
FJ617504
FJ617502

P. misonnei, Benin, Ghana, Cameroon, DRC

97

100

EU740688
EU740691
EU740699

P. tullbergi, Ivory Coast

89

76

AF518368
AF518367

P. petteri, DRC
P. hartwigi, Nigeria

AF518355 *M. verreauxi*, South Africa

99

100

FJ617496
FJ617498
FJ617499
FJ617497
FJ617500

P. lukolelae, DRC

AF518373 *M. verschureni*, DRC

100

KE32
KE141
KE110

M. brockmani, Kenya

AF518347 *S. albipes*, Ethiopia
AF518333 *H. fumosus*, Gabon

100

13

539
308
KE554
329

***P. delectorum*, Malawi, Tanzania, Kenya**

K3_3338 *H. stella*, Kenya
AF518357 *M. yemeni*, Saudi Arabia

AF159394
DQ022380

Outgroups

0.2

275

individuals from Nyika Plateau, the Makutu Mountains and the Mafinga Mountains from Livingstone Museum (individuals collected between 1969 and 1971; Supplementary Table S1). We failed to obtain complete sequences of cytochrome *b* from museum material, but partial sequences significantly clustered with *P. delectorum* from northern Malawi (not shown). Our phylogenetic analysis (in accordance with other recent analyses, e.g. Kennis et al. 2011, Nicolas et al. 2012) questions the inclusion of this species into the genus *Praomys* (Fig. 3b), but better taxon sampling and sequences from more genes (including nuclear ones) are required for more definitive conclusion.

(c) Other genera
Hylomyscus anselli (Bishop, 1979)
The genus *Hylomyscus* is widely distributed in African tropical forests with the highest diversity in central Africa (Nicolas et al. 2006, Kennis et al., submitted). Central and Western African species were recently reviewed by using sequences of mtDNA and morphometry, two new species were described and other candidates for new species were identified (Nicolas et al. 2006, 2008a, 2010, Kennis et al., submitted). Geographically restricted populations of *Hylomyscus* occur also outside continuous tropical forest areas, e.g. in Zambia and afromontane forests in eastern Africa (e.g. Tanzania, Kenya, Albertine rift). These populations were recently revised by Carleton & Stanley (2005) and Carleton et al. (2006), however only morphological traits were evaluated. The only genetic data from eastern Africa were provided by Huhndorf et al. (2007), which used short fragments of cytochrome *b* gene for analysis of phylogeographical structure of *H.* cf. *denniae* in mountains of the Albertine rift.

In Zambia, Ansell (1978) reported only one species of the genus (under the name *Praomys* (*Hylomyscus*) *denniae*). Carleton & Stanley (2005) analysed morphological data of *Hylomyscus* from Tanzania and found that a population from Mbizi Mountains in south-western Tanzania is very different from other Tanzanian populations, but very similar to the type series of the subspecies *Praomys* (*Hylomyscus*) *denniae anselli* Bishop, 1979 described from Mwinilunga District in Zambia. Furthermore, both Tanzanian groups are different from *H. denniae* that was described from Uganda. As a consequence, Carleton & Stanley (2005) described a new species *H. arcimontensis* distributed in Mount Rungwe and the Eastern Arc Mountains of central and eastern Tanzania, and *H. anselli* from south-western Tanzania and northern Zambia was elevated to species rank. In

our data, *Hylomyscus* has very similar distribution as *P.* cf. *jacksoni*, i.e. in the northern part of the country with a mean annual rainfall higher than 1100 mm, but it seems to be less frequent (Fig. 2d). All sequenced individuals from Zambia cluster in a single group, which is very distinct from all available sequences from previous studies (Fig. 3c). Because no sequences of animals from *H. anselli* species group (i.e. *H. anselli* and *H. arcimontensis*) have been previously published, this suggests that all Zambian individuals belong to *H. anselli*.
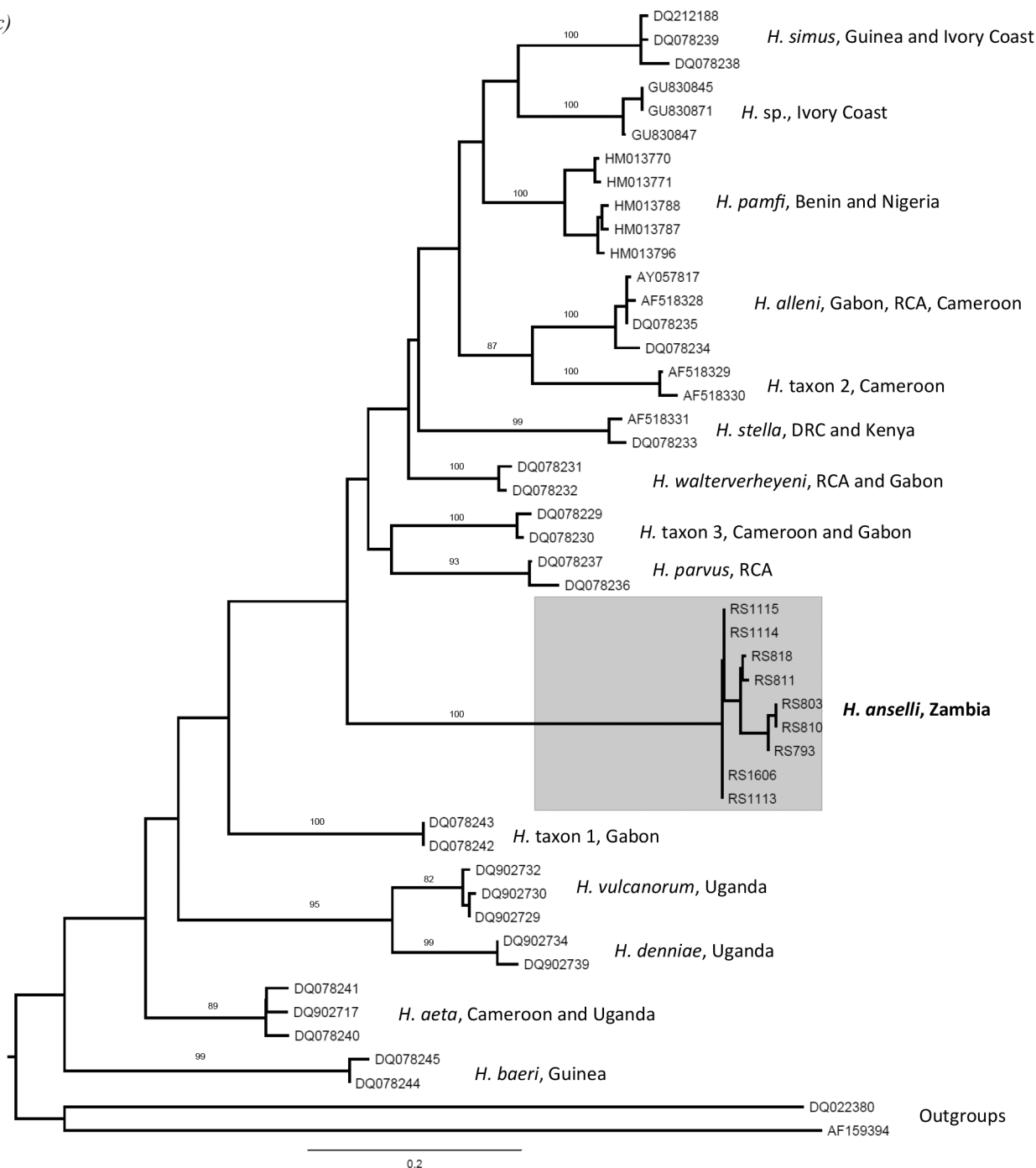
Zelotomys hildegardeae (Thomas, 1902)
This species is probably widely, although very sparsely, distributed in northern and especially southern savanna zones. It has been recorded from central Angola, Zambia, northern Malawi, western Uganda, Rwanda, Burundi, eastern DRC, Sudan and Central African Republic. This species is usually associated with moist grassy savanna and scrub, on the edge of swamps and forests and in tall grasslands; however, on the Nyika Plateau in Malawi it has been recorded from pine plantations and near houses (Coetzee & Van der Straeten 2008). In Zambia, this species was found in a relatively large area (Fig. 2d), but always in low numbers, which makes a more precise estimation of its distribution difficult (Ansell 1978). Scarcity of its occurrence is also indicated by its absence in a large collection by Mathias Kawalika, who carried intensive trapping and analysis of owl pellets in Ndola and surroundings (Kawalika 2004), despite Ndola being the type locality for *Z. h. shortridgei* (Ansell 1978). We did not capture any individuals of this species during our recent survey.

Colomys goslingi Thomas & Wroughton, 1907
Colomys goslingi possibly represents a complex of several similar species and further studies are needed to clarify the taxonomic status of populations currently allocated to this species (Kerbis Peterhans et al. 2008). It has been patchily recorded from large areas covered by more or less continuous tropical forest from Liberia in the west, to north-western Zambia in the south and western Ethiopia in the east. The range limits are not fully resolved, but it has not been recorded from the rainforests of Central African Republic or the forests of West Africa (except Gabon and Liberia). The species has been recorded up to 3200 m a.s.l., while the lowest elevation recorded is about 400 m, but generally it occurs above 800 m a.s.l. (Kerbis Peterhans et al. 2008). The only record in Zambia originates from a single lower mandible taken

(c)

DQ212188
DQ078239
DQ078238
100
H. simus, Guinea and Ivory Coast

GU830845
GU830871
GU830847
100
H. sp., Ivory Coast

HM013770
HM013771
HM013788
HM013787
HM013796
100
H. pamfi, Benin and Nigeria

AY057817
AF518328
DQ078235
DQ078234
100
H. alleni, Gabon, RCA, Cameroon

AF518329
AF518330
100
H. taxon 2, Cameroon

87

AF518331
DQ078233
99
H. stella, DRC and Kenya

DQ078231
DQ078232
100
H. walterverheyeni, RCA and Gabon

DQ078229
DQ078230
100
H. taxon 3, Cameroon and Gabon

DQ078237
DQ078236
93
H. parvus, RCA

RS1115
RS1114
RS818
RS811
RS803
RS810
RS793
RS1606
RS1113
100
H. anselli, Zambia

DQ078243
DQ078242
100
H. taxon 1, Gabon

DQ902732
DQ902730
DQ902729
82
95
H. vulcanorum, Uganda

DQ902734
DQ902739
99
H. denniae, Uganda

DQ078241
DQ902717
DQ078240
89
H. aeta, Cameroon and Uganda

DQ078245
DQ078244
99
H. baeri, Guinea

DQ022380
AF159394
Outgroups

0.2

from an owl pellet in 1964 at Mundwiji Plain (Ansell 1965) (Fig. 2d). Based on the facts that a follow-up expedition failed to collect it there (Ansell 1978), the absence of newer records and a rapid disappearance of suitable habitats, its current presence in Zambia is questionable.

Conclusions
Revised species list of the Praomyini tribe in Zambia
Based on historical records and our recent data, there are eight species that are currently placed in the tribe Praomyini in Zambia. By using analysis of mtDNA sequences, we report for the first time the presence of two new species in Zambia – *Praomys minor* and *Mastomys coucha*. Both species are so-called cryptic species of two more widely distributed species – *P.* cf. *jacksoni* and *M. natalensis*. Both species pairs can also be distinguished by detailed morphological analysis; however such analysis is still missing for Zambian populations. Here these two new species for Zambia reach the border of their distribution and probably occur only in a very limited number of localities.

The taxonomic status of three previously reported species was clarified based on genetic data. It was confirmed that *M. natalensis* is widely distributed all over the country, while two additional species are restricted to the northern part of the country with higher precipitation and remnants of forest habitats. First, we report the first sequences of *H. anselli*, which are very distinct from all other available *Hylomyscus*. Second, *P.* cf. *jacksoni* occurring in Zambia is genetically distinct from nominotypical *P. jacksoni* described from Uganda and probably represent a new species worth of further taxonomical studies (Kennis et al. 2011).

The remaining three species were not captured during our recent field work. *P. delectorum* was, however, confirmed genetically by analysis of museum samples. This species is in Zambia geographically limited to Nyika Plateau and neighbouring mountain ranges, and we captured it frequently in nearby localities in Malawi. *Z. hildegardae* was not found during our fieldwork, probably because of its relative rarity. It is reported to occur at several localities in Zambia but always in low densities or (alternatively) it is very trap-shy. The presence of the last Praomyini species, *C. goslingi*, in Zambia is questionable. The only record originates from owl pellets and is based on a single individual (Ansell 1965). The species has never been captured in Zambia and because it is a typical species of tropical forest, i.e. the habitat largely destroyed by humans in recent decades, we think that the probability that it is still a part of the mammal fauna of Zambia is very low.

Even if the research of small mammals in Zambia has been relatively intense in the past (especially in the middle of the last century), the list of Praomyini is not necessarily complete. Genetic data led to description of many new species in this group throughout Africa and it is possible that some of them could also occur in Zambia. (1) It concerns especially the species-rich genera *Praomys* and *Hylomyscus*, which are particularly species-rich in the tropical forests of the Congo Basin (e.g. Nicolas et al. 2005, Kennis et al. 2011) and the distribution of some of them may reach northern Zambia. (2) Endemic species of the Tanzanian mountains, *H. arcimontensis*, occurs in the Rungwe Mountains, which shares many species with Nyika Plateau (e.g. *P. delectorum*) and any *Hylomyscus* potentially found at this area will be very interesting. (3) The northernmost part of Zambia is also worthy of future faunistic studies, because it is relatively close to the Albertine rift, which is known for its very high rodent endemicism. For example, two endemic species of *Hylomyscus* (*H. denniae*, *H. vulcanorum*)

and one of *Praomys* (*P. degraafi*) are restricted only to mountain forests of the Albertine rift and they could have been neglected in previous studies of Zambian fauna, because they belong to complexes of cryptic species. (4) Another candidate for enriching Zambian fauna is *M. shortridgei*. Its presence in north-western Botswana (Ansell 1978, Coetzee & Griffin 2008) suggests its possible occurrence also in Zambia. Our effort to capture this cryptic species in Zambia was not successful; however, more intensive field research is necessary in the south-western part of the country.

Distribution of species from the Praomyini tribe in Zambia – biogeographical and conservation implications

All but one of the studied species of Praomyini are not widely distributed in the whole country. The only exception is *M. natalensis* and its occurrence in the whole country is probably at least partly related to its opportunistic habitat preferences and partial synanthropy. The remaining species, however, can be used as more general markers of important biogeographic areas in Zambia.

(a) Four Praomyini species are distributed in the northern part of the country, where mean annual rainfall is higher than cca 1100 mm (Fig. 2b, c). Two of them (*P.* cf. *jacksoni*, *H. anselli*) are relatively frequent in many forest habitats, but the remaining two (*P. minor*, *C. goslingi*) were recorded only in isolated forest habitats in the Mwinilunga District in north-western Zambia. This region would surely deserve to be given more attention and support by nature conservation efforts. Kawalika (2004) reviewed data from Ansell (1978) and found the highest diversity (31-33 species) of rodents in Zambia in this area. Based on Ansell (1978), there are also other rodents of humid tropical forest, whose distribution in Zambia is restricted to the Mwinilunga region (e.g. *Pelomys minor*, *Hybomys univittatus* and *Malacomys longipes*). Similar patterns also exist for other animals; for example one of Zambian endemic mammals, Ansell's musk shrew (*Crocidura ansellorum*), occurs only here (Hutterer & Dippenaar 1987). This relatively small area also hosts unique larger mammal fauna, which are found hardly anywhere else in Zambia. Among the most conspicuous, there are records of lowland forest mammals such as Angola pied-colobus (*Colobus angolensis*), red tailed monkey (*Cercopithecus ascanius*), African palm civet (*Nandinia binottata*), tree pangolin (*Manis tricuspis*), Thomas's bushbaby (*Galagoides thomasi*), giant otter shrew (*Potamogale velox*), and hammer bat (*Hypsignathus monstrosus*)

(Ansell 1978, Cotterill 2002, Leonard 2005, Van Daele in litt.). Very close faunal affinity with Guinean-Congolian biome is best documented in birds, because almost all Guinean-Congolian elements occurring in Zambia live just here (Leonard 2005). Unfortunately, this unique area, which should be among the most important conservation priorities in Zambia, is not included in any protected areas system. The only protection is guaranteed by owners of private commercial farms (Nchila Wildlife Reserve) or National Heritage Conservation Commission (Source of Zambezi National Monument). Adjacent areas without any protection are threatened especially by forest clearance (Cotterill 2002, Leonard 2005). According to Cotterill (2002), species richness and diversity here is higher than elsewhere in Zambia or southern Africa south of 10° S, which reinforces arguments for its conservation as biodiversity hotspot of regional and global importance.

(b) The Nyika Plateau and neighbouring mountains (Makutus and Mafingas) make up an afromontane region, which was identified as a rodent "biodiversity hotspot" (Kawalika 2004). This is consistent with the expectation based on the knowledge of high biodiversity in the Malawian part of the Nyika Plateau, just across the border (cf. Chitaukali et al. 2001 and literature cited therein), although sampling on the Zambian site was rather incidental and punctual both in space and time (Ansell 1978). This is the only truly montane region of Zambia belonging to the so-called "Southern Rift Mountains", i.e. the biogeographical area with many endemics (e.g. Taylor et al. 2009, Bayliss et al. 2010). There are several other rodent species, recorded in Zambia only from this area, e.g. undescribed species from *Otomys lacustris* complex (in Ansell 1978 as *O. denti*, in Taylor et al. 2009 as *O. sungae*), *Otomys uzungwensis* (in Ansell 1978 as *O. typus*), *Paraxerus lucifer*, *Beamys hindei*, *Rhabdomys dilectus*) (Ansell 1978 and subsequent actualizations in Rambau et al. 2003, Taylor et al. 2009, 2011). Most of the large mammals were heavily poached out in those mountain areas. Small mammals are threatened mainly by habitat destruction. For example, the area of a mature montane forest, i.e. the habitat preferred by *Praomys delectorum*, is estimated to be only about 200 ha in total making vertebrate species restricted to this specific habitat vulnerable on a national scale (Leonard 2005).

(c) The south-western part of the country (mainly to the west and southwest of the River Zambezi) is characterised by lower annual rainfall (less than 700 mm). It lies mainly on Kalahari sands and ideally belongs to the Southern African subregion (usually limited in the north by the River Zambezi). Besides Southern African large mammals, also several rodents occur in Zambia exclusively in this area, e.g. *M. coucha*, *Fukomys damarensis*, *Gerbilliscus* cf. *brantsii* and *Mus setzeri* have northern limits of their distribution here (Ansell 1978, this study and our unpublished data). Major habitats here are forest, grasslands and savannah woodland dominated with *Acacia* trees. The main conservation problem here is a rapid decrease and degradation of forest dominated by Zambezi teak (*Baikiaea plurijuga*) by illegal timber extraction and burning (Leonard 2005). The small mammal community is poorly known in this area and further sampling could reveal the presence of other species with confirmed distribution in neighbouring countries, such as *Mastomys shortridgei*, *Mus indutus* or *Gerbillurus paeba*.

Acknowledgements

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Supplementary online materials

Supplementary Table S1. *(a) List of sequenced individuals and their GenBank Accession numbers; (b) list of sequences retrieved from GenBank used in phylogenetic analyses (Excel file; URL: http://www.ivb.cz/folia/ download/bryja_et_al._supplementary_table_s1.xlsx).*