

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

P.K. Kaleme^{1,2*}, J.M. Bates³, H.K. Belesi⁴, R.C.K. Bowie⁵, M. Gambalemoke⁶, J. Kerbis-Peterhans^{3,7}, J. Michaux^{8,9}, J.M. Mwangi², B.R. Ndara², P.J. Taylor¹⁰ & B. Jansen van Vuuren¹

¹Evolutionary Genomics Group, Department of Botany and Zoology, Stellenbosch University, South Africa

²Centre de Recherche en Sciences Naturelles, Lwiro, Democratic Republic of Congo

³Department of Zoology, The Field Museum of Natural History, Chicago, U.S.A.

⁴Laboratory of Forest Resource Management, Department of Environment, University of Kinshasa, Democratic Republic of Congo

⁵Museum of Vertebrate Zoology and Department of Integrative Biology, University of California Berkeley, U.S.A.

⁶Faculté des Sciences, Université de Kisangani, Democratic Republic of Congo

⁷College of Professional Studies, Roosevelt University, Chicago, U.S.A.

⁸Institut de Botanique, University of Liège, Belgium

⁹Centres de Biologie et de Gestion des Populations, Campus International de Baillarguet, Montferrier le Lez, France

¹⁰Department of Ecology and Resource Management, School of Environmental Sciences, University of Venda, South Africa

Received 13 December 2010. Accepted 22 February 2011

The threat posed by biological invasions is well established. An important consideration in preventing the spread of invasives and also subsequent introductions lies in understanding introduction pathways. The Democratic Republic of the Congo (DRC) houses a large percentage of the world's biodiversity, yet no national strategy exists to deal with the growing number of invasive alien species. Amongst these are the house mouse and ship and Norwegian rats. By comparing our result to published data, we show that species were possibly introduced into the DRC via two routes. The first is via the western seaport at Kinshasa where specimens of *M. m. domesticus* and *R. rattus* on the western and northwestern side of the DRC show ties with European haplotypes. The second is via the east where specimens of *R. rattus* appear linked to Arab and southeast Asian haplotypes. Future work should consider more comprehensive sampling throughout the DRC to more accurately investigate the occurrence of invasive species throughout the country as well as extend sampling to other African countries.

Key words: control region, invasion, mitochondrial DNA, Democratic Republic of the Congo, *Mus musculus*, *Rattus rattus*, *Rattus norvegicus*, introduction pathway.

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 on 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). Fewer studies attempt to reconstruct biological invasion pathways (but see 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

*Author for correspondence. E-mail: pkaleme@gmail.com

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 species new to science being 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; Borchert *et al.* 2007; McGinley 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 species have been carried out to date.

With the exception of biological control, a large percentage of 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 tracks 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 biogeographical barriers which previously prevented their spread (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 aliens are *Mus musculus* and *Rattus rattus* and the successful spread of these taxa are 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, online at: 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). Little is

known about the current occurrence of *Mus* in the DRC with the exception of an earlier report by Misonne (1963) which includes it from Bukavu and Mount Ruwenzori in the eastern DRC. *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). No information is available for *R. norvegicus* in the DRC.

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 mouse and rat populations present in the DRC, (2) provide information toward documenting the extent of occurrence of these species throughout the DRC, and (3) reconstruct the colonization history of species using mitochondrial DNA data and historical records.

MATERIALS & METHODS

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 northeastern

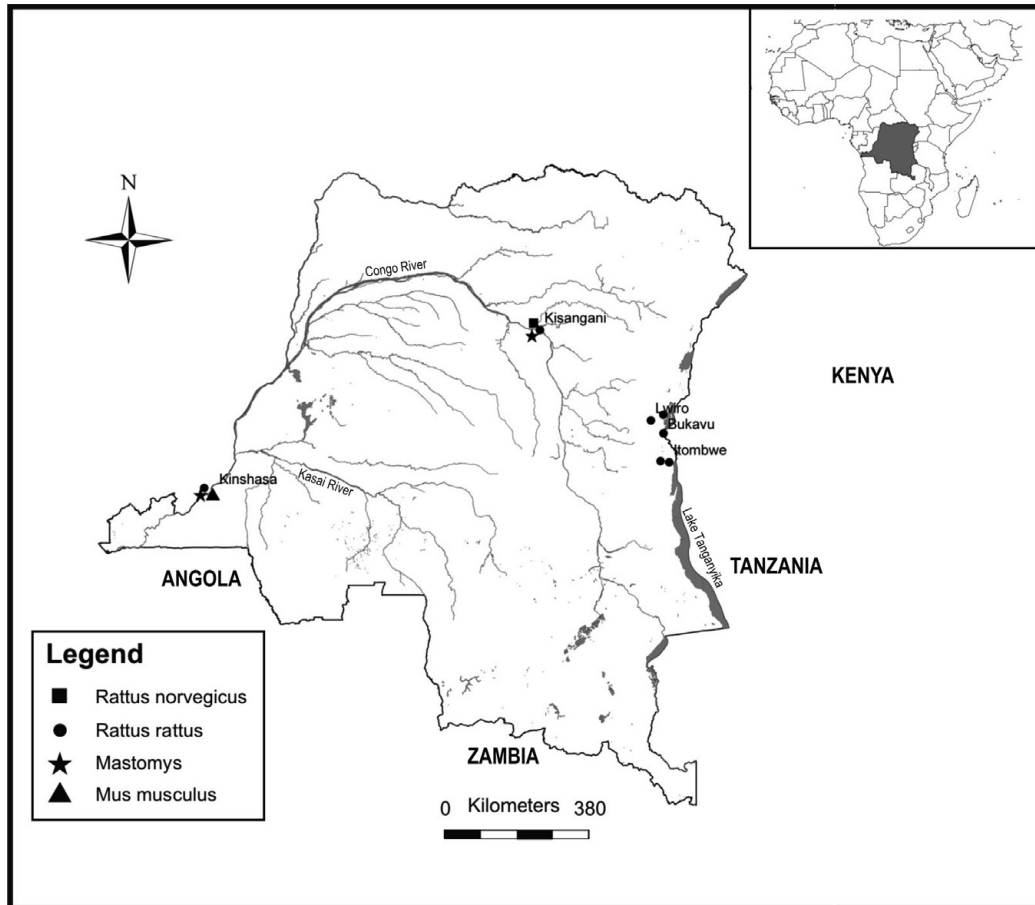


Fig. 1. Map of the Democratic Republic of Congo showing the geographic location of the main cities and town mentioned in the text and the collection localities for species.

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 Fig. 1). Twelve samples were collected from Kinshasa and four samples 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. 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 was collected from four localities across the DRC (Fig. 1 and Table 1). These samples were collected as 'rats'; 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.

Laboratory methods

DNA was extracted from tissue samples using a commercial DNA extraction kit (DNeasy Tissue

Table 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
<i>Mus musculus</i>	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 <i>et al.</i> 2009a
	Britain	FM211601			Searle <i>et al.</i> 2009a
	Britain	FM211602			Searle <i>et al.</i> 2009a
	Britain	FM211604			Searle <i>et al.</i> 2009a
	Bulgaria	EU194652			Rajabi-Maham <i>et al.</i> 2008
	Cameroon	AM182700			Ihle <i>et al.</i> 2006
	Cameroon	AM182703			Ihle <i>et al.</i> 2006
	Cameroon	AM182713			Ihle <i>et al.</i> 2006
	Cameroon	AM182714			Ihle <i>et al.</i> 2006
	Denmark	U47455			Prager <i>et al.</i> 1993
	Denmark	U47460			Prager <i>et al.</i> 1993
	England	U47430			Prager <i>et al.</i> 1993
	France	AM182717			Ihle <i>et al.</i> 2006
	France	AM182741			Ihle <i>et al.</i> 2006
	France	AM192742			Ihle <i>et al.</i> 2006
	Germany	AM182671			Ihle <i>et al.</i> 2006
	Germany	U47474			Prager <i>et al.</i> 1993
	Greece	AY551960			Tryfonopoulos <i>et al.</i> 2005
	Greece	AY551961			Tryfonopoulos <i>et al.</i> 2005
	Iran	EU194617			Rajabi-Maham <i>et al.</i> 2008
	Italy	U47471			Prager <i>et al.</i> 1993
Italy	U47477			Prager <i>et al.</i> 1993	
Italy	U47482			Prager <i>et al.</i> 1993	
Italy	EU194675			Rajabi-Maham <i>et al.</i> 2008	
Italy	EU194676			Rajabi-Maham <i>et al.</i> 2008	
Madeira	AJ313378			Gunduz <i>et al.</i> 2001	
Madeira	AJ313379			Gunduz <i>et al.</i> 2001	
Mauritania	AJ313380			Gunduz <i>et al.</i> 2001	
Morocco	AJ313381			Gunduz <i>et al.</i> 2001	
New Zealand	FM211635			Searle <i>et al.</i> 2009b	
New Zealand	FM211636			Searle <i>et al.</i> 2009b	
Sweden	AJ313361			Gunduz <i>et al.</i> 2001	
Turkey	AJ843837			Gunduz <i>et al.</i> 2001	
<i>Mastomys</i> 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

Continued on p. 137

Table 1 (continued)

Species	Locality	Accession Numbers/ Lab IDs	Latitude	Longitude	Reference
	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
<i>Mastomys natalensis</i>	South Africa	AF465342			Dawood <i>et al.</i> (unpubl.)
	South Africa	AY576886			Dawood <i>et al.</i> (unpubl.)
<i>Mastomys coucha</i>	South Africa	AF465344			Dawood <i>et al.</i> (unpubl.)
	South Africa	AY576888			Dawood <i>et al.</i> (unpubl.)
<i>Rattus rattus</i>	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 <i>et al.</i> 2005
	Ethiopia	GQ891583			Hingston <i>et al.</i> 2005
	French Polynesia	EF186354			Robins <i>et al.</i> 2007
	French Polynesia	EF186359			Robins <i>et al.</i> 2007
	India	GQ891569			Tollenaere <i>et al.</i> 2010
	India	GQ891570			Tollenaere <i>et al.</i> 2010
	India	GQ891571			Tollenaere <i>et al.</i> 2010
	India	GQ891572			Tollenaere <i>et al.</i> 2010
	India	GQ891573			Tollenaere <i>et al.</i> 2010
	Madagascar	DQ009781			Hingston <i>et al.</i> 2005
	Madagascar	GQ891602			Tollenaere <i>et al.</i> 2010
	Madagascar	GQ891603			Tollenaere <i>et al.</i> 2010

Continued on p. 138

Table 1 (continued)

Species	Locality	Accession Numbers/ Lab IDs	Latitude	Longitude	Reference
	Mozambique	GQ891588			Tollenaere <i>et al.</i> 2010
	Mozambique	GQ891589			Tollenaere <i>et al.</i> 2010
	New Zealand	EF186355			Robins <i>et al.</i> 2007
	Oman	GQ891574			Tollenaere <i>et al.</i> 2010
	Oman	GQ891575			Tollenaere <i>et al.</i> 2010
	Oman	GQ891577			Tollenaere <i>et al.</i> 2010
	Oman	GQ891578			Tollenaere <i>et al.</i> 2010
	Oman	GQ891579			Tollenaere <i>et al.</i> 2010
	Oman	GQ891580			Tollenaere <i>et al.</i> 2010
	Papua New Guinea	EF186357			Robins <i>et al.</i> 2007
	Réunion	GQ891607			Tollenaere <i>et al.</i> 2010
	Samoa	EF186360			Robins <i>et al.</i> 2007
	Senegal	FJ897498			Tollenaere <i>et al.</i> 2010
	Senegal	FJ897499			Tollenaere <i>et al.</i> 2010
	South Africa	GQ891608			Tollenaere <i>et al.</i> 2010
	Tanzania	GQ891584			Tollenaere <i>et al.</i> 2010
	Tanzania	GQ891586			Tollenaere <i>et al.</i> 2010
	Tanzania	GQ891587			Tollenaere <i>et al.</i> 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 <i>et al.</i> 1995
	United States of America	U13754			Usdin <i>et al.</i> 1995
	Yemen	GQ891581			Tollenaere <i>et al.</i> 2010
	Yemen	GQ891582			Tollenaere <i>et al.</i> 2010
<i>Rattus norvegicus</i>	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 <i>et al.</i> (unpublished)
	Britain	DQ897638			Modh-Zain <i>et al.</i> (unpublished)
	Denmark	AJ428514			Nilsson <i>et al.</i> 2003
	French Polynesia	EF186346			Robins <i>et al.</i> 2007
	Sweden	FJ91976			Tollenaere <i>et al.</i> 2010
	United Kingdom	DQ897633			Modh-Zain <i>et al.</i> (unpublished)
	United States of America	U13746			Usdin <i>et al.</i> 1995
	United States of America	U13747			Usdin <i>et al.</i> 1995

Kit, Qiagen). As the mitochondrial DNA control region is hypervariable, and given that data are available from public databases for these invasive species from localities across their native and introduced ranges, 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.

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).

RESULTS

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 (Fig. 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 them 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 to broaden our comparative sequence base for '*Mastomys*'. *Mastomys natalensis* has been reported from the DRC (Musser & Carleton 2005), and data for this species were available on GenBank for the control region (see also the work by Dobigny *et al.* 2008 for a discussion on *Mastomys* in north central and 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 (4 from Kisangani and 5 from Kinshasa) grouped within the genus *Mastomys* with high support (see Fig. 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 (barcoding) approach (see Fig. 2A). Two haplotypes separated by a single mutational change characterized the seven DRC specimens collected from Kinshasa (Fig. 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 (U.K.) and by two mutational steps from a haplotype from Belgium.

Rattus species

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. tanezumii*). *Rattus tanezumii* has been reported from southern Africa (Bastos *et al.* 2011) and it is possible that this species is spreading northwards. Following the phylogenetic analyses (Fig. 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 U.K., Denmark, the U.S.A., Sweden, and French Polynesia (Fig. 3B). For *R. rattus*, individuals from Kisangani ($n = 3$) and Kinshasa ($n = 6$) clustered with haplotypes from the U.S.A., Samoa, South Africa, Papua New Guinea, French Polynesia, New Zealand, Senegal and the U.K. One haplotype was

shared between the eastern DRC (Lwiro and Itombwe), one specimen from Kinshasa, Tanzania, Madagascar and Mozambique (Fig. 3C).

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 invasive species' distributions throughout the DRC and attempt to unravel putative source populations and colonization routes.

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 (Fig. 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 northeastern 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 the latter samples were collected at Kisangani, in and 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 dependent on agricultural (grain) food sources, as is the case for *Mus*.

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 one 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 U.S.A. *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-conti-

nent (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 Réunion close to those detected in the western DRC (Kinshasa and Kisangani) 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 Réunion 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 colonizations.

CONCLUSION

This paper contributes to a growing body of literature that documents the usefulness of DNA in establishing 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 the 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 northwestern 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 southeast 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.

ACKNOWLEDGEMENTS

Prince Kaleme is funded through the Belgium Technical Corporation (BTC). Graft Mugaragumbo is thanked for his help with logistics. Laboratory work was partially funded through the BTC and the Centre for Invasion Biology (BvV). The Ecorat project (www.nri.org/ecorat), and specifically Drs Loth Mulungu and Apia Massawe from Sekoine

University of Agriculture, Morogoro, Tanzania and Professor Jenny Lamb and Sarah Downs from University of KwaZulu-Natal are acknowledged for providing samples from Tanzania. The Mammalogy lab at Lwiro Research station kindly provided some material from the DRC. We thank Christiane Denys and an anonymous reviewer for helpful comments on our manuscript.

REFERENCES

- ALLEN, M.G. & LOVERIDGE, A. 1942. Scientific results of a forth expedition to forested areas in East and Central Africa. I. Mammals. *Bulletin of the Museum of Comparative Zoology at Harvard College*, Vol. LXXXIX, No. 4.
- APLIN, K.P., BROWN, P.R., JACOBS, J., KREBS, C.J. & SINGLETON, G.R. 2003. Field methods for rodent studies in Asia and the Indo-Pacific. *ACIAR Monograph* No. 100: 1–223.
- ATKINSON, A.E. 1985. The spread of commensal species of *Rattus* to oceanic islands and their effects on island avifauna. *ICBP Technical Publication* 3: pp. 35–81.
- BASTOS, A.D.S., NAIR, D., TAYLOR, P.J., BRETT-SCHNEIDER, H., KIRSTEN, F., MOSTERT, M.E., VON MALTITZ, E., LAMB, J.M., VAN HOOFT, P., BELMAIN, S.R., CONTRAFATTO, G., DOWNS, S. & CHIMIMBA, C.T. 2011. Genetic monitoring detects an overlooked cryptic species and reveals the diversity and distribution of three invasive *Rattus* congeners in South Africa. *BMC Genetics* 12: 26 doi: 10.1186/1471-2156-12-26.
- BERDOY, M. & DRICKAMER, L.C. 2007. Comparative social organization and life history of *Rattus* and *Mus*. In: *Rodents Societies: an Ecological and Evolutionary perspective*, (eds) J.O. Wolf & P.W. Sherman, pp. 380–392. University of Chicago Press, Chicago and London.
- BIOMATTERS LTD 2010. *Geneious* 5.3, 160 pp.
- BORCHERT, M., MULANGU, S., LEFEVRE, P., TSHOMBA, A., LIBANDE, M.L., KULIDRI, A., MUYEMBE-TAMFUM, J.J. & VAN DER STUYFT, P. 2007. Use of protective gear and the occurrence of occupational Marburg hemorrhagic fever in health workers from Watsa Health Zone, Democratic Republic of the Congo. *Journal of Infectious Diseases* 196 (Suppl. 2): S168–S175.
- CLEMENT, M., POSADA, D. & CRANDALL, K.A. 2004. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657–1660.
- CLOOS, P. 2003. *Histoire de la colonisation Belge*. Online at: <http://www.cobelco.info> (accessed February 2009)
- DE GRAAFF, G. 1981. *The Rodents of Southern Africa*. Butterworths, Durban.
- DIETERLEN, F. 1966. Importance économique au point de vue de l'agriculture de quelques rongeurs de la région du lac Kivu. *Chronique de l'IRSAC* 1(3): 16–20.
- DIETERLEN, F. 1979. Zur Ausbreitungsgeschichte der Hausratte (*Rattus rattus*) in Ostafrika. *Zeitschrift für angewandte Zoologie* 66: 173–184.
- DIETERLEN, F. 1985. Beziehungen zwischen Umweltfaktoren und Fortpflanzungsperiodik myomorpher

- Nager eines afrikanischen Tieflandregenwaldes (Ost Zaïre). *Zeitschrift für Säugetierkunde* **50**: 152–166.
- DOBIGNY, G., LECOMPTE, E., TATARD, C., GAUTHIER, P., BÂ, K., DENYS, C., DUPLANTIER, J.M. & GRANJON, L. 2008. An update on the taxonomy and geographic distribution of the cryptic species *Mastomys kollmannspergeri* (Muridae, Murinae) using combined cytogenetic and molecular data. *Journal of Zoology, London* **276**: 368–374.
- GOODMAN, S.M. 1995. *Rattus* on Madagascar and the dilemma of protecting the endemic rodent fauna. *Conservation Biology* **9**: 450–453.
- GRANJON, L. & DUPLANTIER, J.M. (eds) 2009. *Les rongeurs de l'Afrique sahélo-soudanienne*. Institut de recherche pour le développement/Muséum national d'Histoire naturelle, Paris.
- GUINDON, S. & GASCUEL, O. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology* **52**: 696–704.
- GUNDUZ, I., AUFRAY, J.C., BRITTON-DAVIDIAN, J., CATALAN, J., GANEM, G., RAMALHINHO, M.G., MATHIAS, M.L. & SEARLE, J.B. 2001. Molecular studies on the colonization of the Madeiran archipelago by house mice. *Molecular Ecology* **10**(8): 2023–2029.
- HINGSTON, M., GOODMAN, S.M., GANZHORN, J.U. & SOMMER, S. 2005. Reconstruction of the colonization of southern Madagascar by introduced *Rattus rattus*. *Journal of Biogeography* **32**: 1549–1559.
- HOCHSCHILD, A. 1998. *King Leopold's Ghost: a Story of Greed, Terror, and Heroism in Colonial Africa*. Houghton Mifflin Company, Boston, New York.
- HULME, P.E. 2009. Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology* **46**: 10–18.
- JANSEN VAN VUUREN, B. & CHOWN, S.L. 2007. Genetic evidence confirms the origin of the house mouse on sub-Antarctic Marion Island. *Polar Biology* **30**: 327–332.
- KALEME, P.K., BATES, J., KERBIS-PETERHANS, J., MWANGA, M.J. & NDARA, B.R. 2007. Small mammal diversity and habitat requirements in the Kahuzi-Biega National Park and surrounding areas, eastern Democratic Republic of Congo. *Integrative Zoology* **2**: 239–246.
- KASANGAKI, A., KITYO, R. & KERBIS, J. 2003. Diversity of rodents and shrews along an elevational gradient in Bwindi Impenetrable National Park, southwestern Uganda. *African Journal of Ecology* **41**: 115–123.
- KATUALA, P.G.B., HART, J.A., HUTTERER, R., LEIRS, H. & DUDU A. 2005. Biodiversity and ecology of small mammals (rodents and shrews) of the 'Réserve de Faune à Okapis', Democratic Republic of the Congo. *Belgian Journal of Zoology* **135**: 191–196.
- KHAMIS, F., KARAM, N., GUGLIELMINO, C.R., EKESI, S., MASIGA, D., DE MEYER, M., KENYA, E.U. & MALACRIDA, A.R. 2008. Isolation and characterization of microsatellite markers in the newly discovered invasive fruit fly pest in Africa, *Bactrocera invadens* (Diptera: Tephritidae). *Molecular Ecology Resources* **8**: 1509–1511.
- KILONZO, B.S. & MSANGI, A.S. 1991. Plague. In *Health and Disease in Tanzania*, (eds) G.M.P. Mwaluko, W.L. Kilama, M.P. Mandara, M. Murru & C.N.L. Macpherson, pp. 98–116. HarperCollins Academic, New York.
- LOWE, S., BROWNE M., BOUDJELAS, S. & DE POORTER, M. 2000. *100 of the World's Worst Invasive Alien Species: a Selection from the Global Invasive Species database*. Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), Gland, Switzerland.
- MADDISON, D.R. & MADDISON, W.P. 2000. *MacClade 4: analysis of phylogeny and character evolution*. Sinauer Associates, Sunderland, Massachusetts.
- McGINLEY, M. 2007. Invasive alien species and development challenges in Africa. In: *Encyclopedia of Earth*, (ed.) C.J. Cleveland. Environmental Information Coalition, National Council for Science and the Environment, Washington, D.C. Online at: http://www.eoearth.org/article/Invasive_alien_species_and_development_challenges_in_Africa
- McNELLY, J. 2001. Invasive species: a costly catastrophe for native biodiversity. *Land Use and Water Resources Research* **1**: 1–10.
- MISONNE, X. 1963. Les rongeurs du Ruwenzori et des régions voisines. *Exploration du Parc National Albert*, Deuxième série, Fascicule **14**.
- MISONNE, X. 1969b. Quatre années de lutte antipesteuse au Congo. *Annales de la Société belge de médecine tropicale* **49**(1): 1–16
- MOUTOU, F. 1983. Introduction dans les îles, l'exemple de l'île de la Réunion. *Compte-Rendu de la Société de Biogéographie*, **59**: 201–211.
- MUIRHEAD, J.R. & MACISAAC, H.J. 2005. Development of inland lakes as hubs in an invasion network. *Journal of Applied Ecology* **42**: 80–90
- MUKINZI, I., KATUALA, P.G.B., KENNIS, J., GAMBALEMOKE, M., KADANGE, N., DUDU, A.M., COLYN, M. & HUTTERER, R. 2005. Preliminary data on the biodiversity of rodents and insectivores (Mammalia) in the periphery of Kisangani (D.R. Congo). *Belgian Journal of Zoology* **135**: 133–140.
- MUKINZI, I., HUTTERER, R. & BARRIÈRE, P. 2009. A new species of *Syloisorex* (Mammalia: Soricidae) from lowland forests north of Kisangani, Democratic Republic of Congo. *Mammalia* **73**: 130–134.
- MUSSER, G.G. & CARLETON, M.D. 2005. The Family Muridae. In *Mammal Species of the World: a Taxonomic and Geographic Reference*, (eds) D.E. Wilson & D.M. Reeder, pp. 501–775. Smithsonian Institution Press, Washington D.C.
- NIETAMMER, J. 1975. Zur taxonomie und ausbreitungsgeschichte der hausratte (*Rattus rattus*). *Zoologischer Anzeiger* **194**: 405–415.
- NOWAK, R.M. & PARADISO, J.L. 1983. *Walker's Mammals of the World*, 4th edn. Johns Hopkins University Press, Baltimore, Maryland.
- OLSON, L.J. 2006. The Economics of terrestrial invasive species: a review of the Literature. *Agricultural and Resource Economics Review* **35**: 178–194.
- PLUMPTRE, A.J., DAVENPORT, T.R.B., BEHANGANA, M., KITYO, R., EILU, G., SSEGAWA, P., EWANGO, C., MEIRTE, D., KAHINDO, C., HERREMANS, M., KERBIS PETERHANS, J., PILGRIM, J.D., WILSON, M., LANGUY, M. & MOYER, D. 2007. The bio-

- diversity of the Albertine Rift. *Biological Conservation* **134**: 178–194.
- POCOCKS, M.J.O., HAUFFE, H.C. & SEARLE, J.B. 2005. Dispersal in house mice. *Biological Journal of the Linnean Society* **84**: 565–583.
- POINTIER, J.P., DEJONG, R.J., TCHUEM TCHUENTE, L.A., KRISTENSEN, T.K. & LOKER, E.S. 2005. A neotropical snail host of *Schistosoma mansoni* introduced into Africa and consequences for the schistosomiasis transmission: *Biomphalaria tenagophila* in Kinshasa (Democratic Republic of Congo). *Acta Tropica* **93**: 191–199.
- POSADA D. 2008. ModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25**: 1253–1256.
- RAHM, U. & CHRISTIAENSEN, A. 1966. Les mammifères de la région occidentale du Lac Kivu. *Annales du Musée Royal pour l'Afrique Centrale. Terouren. Série 8, No. 118*: 1–83.
- RAHM, U. 1967. Les muridés des environs du lac Kivu et des régions voisines (Afrique Centrale) et leur écologie. *Revue Suisse de Zoologie* **74**(9): 437–519.
- RAJABI-MAHAM, H., ORTH, A. & BONHOMME, F. 2008. Phylogeography and postglacial expansion of *Mus musculus domesticus* inferred from mitochondrial DNA coalescent, from Iran to Europe. *Molecular Ecology* **17**: 627–641.
- ROBINS, J.H., HINGSTON, M., MATISOO-SMITH, E. & ROSS, H.A. 2007. Identifying *Rattus* species using mitochondrial DNA. *Molecular Ecology Notes* **7**: 717–729.
- RONQUIST, F. & HUELSENBECK, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
- ROSEVEAR, D.R. 1969. *The Rodents of West Africa*. Trustees of the British Museum (Natural History), London.
- SEARLE, J.B. 2008. The genetics of mammalian invasions: a review. *Wildlife Research* **35**: 185–192.
- SEARLE, J.B., CATHERINE S. JONES, C.S., GÜNDÜ, I., SCASCITELLI, M., JONES, E.P., HERMAN, J.S., RAMBAU, V.R., NOBLE, L.R., BERRY, R.J., GIMÉNEZ, M. D. & JÓHANNESDÓTTIR, F. 2009a. Of mice and (Viking) men: phylogeography of British and Irish house mice. *Proceeding of the Royal Society, Biological Sciences* **276**: 201–207.
- SEARLE, J.B., JAMIESON, P.M., GÜNDÜ, I., STEVENS, M.I, JONES, E.P., GEMMILL, C.E.C. & KING, C.M. 2009b. The diverse origins of new Zealand house mice. *Proceeding of the Royal Society, Biological Sciences* **276**: 209–217.
- SELLENS, L.J., MARKIEWICZ, A.J. & LANDIS, W.G. 2007. Risk evaluation of invasive species transport across the U.S.–Canada border in Washington State, Final Report. Report prepared for Border Policy Research Institute, Western Washington University, Bellingham, WA, U.S.A.
- SINGLETON, G.R., JUMANTA, S., TAN, T.Q. & HUNG, N.Q. 1999. Physical control of rats in developing countries. In *Ecologically-based Management of Rodent Pests*, (eds) G.R. Singleton, L.A. Hinds, H. Leirs & Z. Zhang. *ACIAR Monograph* **59**: 178–198.
- STAMATAKIS, A., HOOVER, P. & ROUGEMONT, J. 2008. A rapid bootstrap algorithm for the RAxML web-servers. *Systematic Biology* **57**: 758–771.
- TOLLENAÈRE, C., BROUAT, C., DUPLANTIER, J.M., RAHALISON, L., RAHELINIRINA, S., PASCAL, M., MONÉ, H., MOUAHID, G., LEIRS, H. & COSSON, J.F. 2010. Phylogeography of the introduced species *Rattus rattus* in the western Indian Ocean, with special emphasis on the colonization history of Madagascar. *Journal of Biogeography* **37**, 398–410.
- UNEP. 2002. Alien species that threaten ecosystems, habitats or species. In *Convention on Biological Diversity*. UNEP/ CBD/ COP/ INF/ 28, The Hague.
- UNESCO 2006. The central slave and ivory trade route. Online at: <http://whc.unesco.org/en/tentativelist/2095/>
- VALÉRY, L., FRITZ, H., LEFEUVRE, J.C. & SIMBERLOFF, D. 2009. Invasive species can be natives. *Trends in Ecology and Evolution* **24**: 585.
- VAN WILGEN, N.J., RICHARDSON, D.M. & BAARD, E.H.W. 2008. Alien reptiles and amphibians in South Africa: towards a pragmatic management. *South African Journal of Science* **104**: 13–20.
- WCMC 1992. *Global Biodiversity: Status of the Earth's Living Resources*. Chapman and Hall, London.
- WILSON, J.R.U., DORMONTT, E.E., PRENTIS, P.J., LOWE, A.J. & RICHARDSON, D.M. 2009a. Something in the way you move: dispersal pathways affect invasion success. *Trends in Ecology and Evolution* **24**: 136–144.
- WILSON, J.R.U., DORMONTT, E.E., PRENTIS, P.J., LOWE, A. J. & RICHARDSON, D.M. 2009b. Biogeographic concepts define invasion biology. *Trends in Ecology and Evolution* **24**: 58.

Responsible Editor: A. Monadjem