The LINNEAN SOCIETY of London ZOOLOGICAL Journal of the Linnean Society

Zoological Journal of the Linnean Society, 2010, **160**, 397–419. With 10 figures

Molecular and morphometric variation in two sibling species of the genus *Praomys* (Rodentia: Muridae): implications for biogeography

VIOLAINE NICOLAS[1]*, BERTIN AKPATOU[2,3], WIM WENDELEN[4], JULIAN KERBIS PETERHANS[5], AYODEJI OLAYEMI[6], JAN DECHER[7], ALAIN-DIDIER MISSOUP[1], CHRISTIANE DENYS[1], PATRICK BARRIERE[8], CORINNE CRUAUD[9] and MARC COLYN[8]

[1]*Muséum National d'Histoire Naturelle, Département de Systématique et Evolution, UMR 7205, Laboratoire Mammifères et Oiseaux, 57 rue Cuvier, CP 51, 75005 Paris, France*
[2]*Université de Cocody, UFR Bio-Sciences, Laboratoire de Zoologie et Biologie Animale, 22 B.P. 582 Abidjan 22, République de Côte d'Ivoire*
[3]*Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, 01 B.P. 1303 Abidjan 01, Km 17, route de Dabou, Abidjan, République de Côte d'Ivoire*
[4]*Royal Museum for Central Africa, Zoology Department, Vertebrate Section, Leuvensesteenweg 13, Tervuren, Belgique*
[5]*Roosevelt University and the Field Museum of Natural History, Chicago, IL 60605, USA*
[6]*Obafemi Awolowo University, Ile-Ife, Osun, Nigeria*
[7]*Department of Biology, University of Vermont, Burlington, VT 05405, USA*
[8]*UMR CNRS 6553 Ecobio, Université de Rennes 1, Station Biologique, 35380 Paimpont, France*
[9]*Genoscope, Centre National de Sequençage, 2 Rue Gaston Crémieux, CP5706, 91057 Evry Cedex, France*

Received 17 September 2008; accepted for publication 11 May 2009

The rodent genus *Praomys* is widely distributed in the African tropics. The species are cryptic, rendering the species taxonomy unclear. There are differences of opinion concerning the specific status of *Praomys misonnei* and *Praomys tullbergi*, and their geographical distribution. We sequenced the cytochrome *b* and/or the *16S* gene of 221 specimens from 12 countries in order to evaluate the genetic variability within these two species, and to precisely determine their geographical distribution. Morphological and morphometrical analyses on the sequenced specimens were also performed to find criteria useful for the identification of museum specimens. Our results confirm that *P. misonnei* and *P. tullbergi* are two valid species that can be separated by molecular data. However, no single discrete morphological character or simple metric measurement can be used to discriminate them. The percentage of misclassified individuals in multivariate discriminant analysis is relatively high (10%). The two species have allopatric distributions: *P. tullbergi* occurs in West Africa, from eastern Guinea to western Ghana, and *P. misonnei* is widely distributed from eastern Ghana to western Kenya. Within *P. misonnei* we identified three or four major geographical clades: a West Central African clade, an East African clade, a Nigerian clade, and a possible West African clade. Within *P. misonnei*, high geographical morphometrical variability was also identified. The role of both rivers and Pleistocene forest refugia in promoting speciation within the genus *Praomys* is discussed.

doi: 10.1111/j.1096-3642.2009.00602.x

ADDITIONAL KEYWORDS: *16S* – Africa – cytochrome *b* – morphometry – phylogeny – refuge theory – sibling species – speciation – taxonomy – Volta River.

*Corresponding author. E-mail: vnicolas@mnhn.fr

INTRODUCTION

'Sibling species' are similar in appearance, but are nonetheless reproductively isolated from one another (Ridley, 2003). As sibling species are difficult to distinguish morphologically, they are often unrecognized. However, during the past decades, numerous sibling species have been recognized as a result of the development of cytogenetic and molecular techniques (e.g. Westheide & Hass-Cordes, 2001; Volobouev *et al.*, 2002; Bickford *et al.*, 2007; Craul *et al.*, 2007). Species diversity is not equally distributed on earth: for mammals it is higher in the tropics than in the northern and southern temperate zones (Platnick, 1991). However, tropical biodiversity is still largely unknown, even for mammals. Over the past decade, many species have been described or resurrected, and most are sibling species (e.g. Bonvicino, Lima & Almeida, 2003; Verheyen *et al.*, 2003; Nicolas *et al.*, 2006; Huhndorf, Kerbis Peterhans & Loew, 2007; Lavrenchenko *et al.*, 2007; Reeder, Helgen & Wilson, 2007; Nicolas *et al.*, 2008). The detection and description of sibling species is fundamental to the estimation of the world's biodiversity. There is a critical need for rigorously delineating species, not only for producing accurate species inventories, but also because most questions in evolutionary biology (e.g. speciation), ecology (e.g. ecosystem development), conservation biology (e.g. conservation priorities) or biogeography (e.g. diversification processes) depend on species inventories and our knowledge of species' distribution (Dayrat, 2005).

Before we attempt to recognize species, we need a clear concept of what species are. deQueiroz (1998) suggested that despite the long history of dispute over species concepts, most species concepts agree fundamentally that species are lineages. What previous authors have generally disagreed about are the best criteria for recognizing these lineages (deQueiroz, 1998). A number of studies have demonstrated the utility of mitochondrial DNA (mtDNA) sequence variation in illuminating potential species boundaries between morphologically similar taxa (Avise, 2000). A major problem with this approach is that processes unrelated to speciation (e.g. deep coalescence) may be responsible for patterns such as reciprocal monophyly of mtDNA haplotype lineages within a species, as well as for patterns of nonmonophyly in reproductively isolated taxa (Avise *et al.*, 1983; Maddison, 1997). Although we recognize the potential for nonmonophyletic gene trees to be embedded within reproductively cohesive species as a result of incomplete lineage sorting (Pamilo & Nei, 1988) or recurrent gene flow (Slatkin & Maddison, 1989), mtDNA haplotypes are expected to coalesce much more rapidly relative to nuclear markers, because of their smaller effective population size (Moore, 1995). They should therefore serve as relatively early indicators of reproductive isolation. In this paper, we used mtDNA sequence variation to solve systematic difficulties within the genus *Praomys*.

This genus of African murid rodents is widely distributed in the tropical zone, and provides one of the chief taxonomic problems in the study of African rodents. The genus was first downgraded by Ellerman (1941) to a subgenus of *Mastomys*, and was later re-elevated to genus level by Davis (1962). *Mastomys*, *Hylomyscus*, and *Myomyscus* were then considered to be subgenera of *Praomys* (Misonne, 1969; Honacki, Kinman & Koeppl, 1982; Happold, 1987), whereas Rosevear (1969), Robbins, Choate & Robbins (1980), Carleton & Musser (1984), and Musser & Carleton (2005) regarded *Mastomys*, *Hylomyscus*, and *Myomys* (referring to *Myomyscus*) as genera. The latter position is currently accepted as a general consensus, and the phylogenetic relationships between these genera were investigated by Lecompte, Granjon & Denys (2002a), Lecompte *et al.* (2002b), and Lecompte, Denys & Granjon (2005). Musser & Carleton (2005) recognized 16 species within the genus *Praomys*. Based on morphological characteristics, several species groups were defined within the genus *Praomys* (Van der Straeten & Dudu, 1990; Van der Straeten & Kerbis Peterhans 1999). The *Praomys tullbergi* complex includes the species *P. hartwigi*, *P. misonnei*, *P. morio*, *P. obscurus*, *P. petteri*, *P. rostratus*, and *P. tullbergi* (Hutterer, Dieterlen & Nikolaus, 1992; Lecompte *et al.*, 1999; Van der Straeten, Lecompte & Denys, 2003). Three of these are restricted to mountain forests (*P. morio*, *P. hartwigi*, and *P. obscurus* in the Cameroon region). Little is known about the taxonomy and distribution ranges of the species occurring in the lowland forests. *Praomys petteri* is restricted to West Central Africa, whereas *P. rostratus* is endemic to West Africa. The validity of these two species was recently confirmed by molecular data (Lecompte *et al.*, 2002b; Nicolas *et al.*, 2005), and morphometrical differences compared with other species of the *P. tullbergi* complex were highlighted (Van der Straeten *et al.*, 2003; Nicolas *et al.*, 2005; Akpatou *et al.*, 2007). In this paper, we will focus on the species *P. misonnei* and *P. tullbergi*. These two species are morphologically very similar, and, according to Musser & Carleton (2005), the possibility that *P. misonnei* simply represents populations of *P. tullbergi* at the eastern margins of its geographic range must be considered. Nevertheless, Lecompte, Denys & Granjon (2001), Lecompte *et al.* (2002a), and Lecompte (2003) found that several craniodental characters can be used to distinguish these two species. Moreover, Nicolas *et al.* (2005) and Lecompte *et al.* (2002b, 2005) showed that *P. misonnei* and *P.*

tullbergi are clearly differentiated, based on molecular studies (*16S* rDNA, cytochrome *b*, and *IRBP* gene sequencing), and represent two distinct species.

The distribution range of these two species is largely unknown. According to Musser & Carleton (2005), *P. misonnei* is restricted to northern and eastern parts of the Democratic Republic of Congo, whereas Lecompte *et al.* (2002b) consider that it is also present in Kenya and Uganda. According to Lecompte *et al.* (2001), *P. tullbergi* is widely distributed in West and West Central Africa. However, Nicolas *et al.* (2005) showed that populations from West Central Africa (Cameroon, Republic of Congo, Central African Republic, and Gabon) are molecularly (*16S* rDNA sequencing) closer to *P. misonnei*, and are clearly distinct from West African *P. tullbergi*. Moreover, based on craniometrical data, these populations more closely resemble *P. misonnei* than *P. tullbergi* (Nicolas *et al.*, 2005). This brief review of the literature clearly indicates discrepancies between studies concerning the geographical distribution of *P. misonnei* and *P. tullbergi*. These problems have arisen, in part, because no previous study tried to distinguished intra- from interspecific variation.

In this study we sequenced a large number of *Praomys* (cytochrome *b* and *16S* genes sequencing) in order to evaluate genetic variability within the species *P. misonnei* and *P. tullbergi*. Morphological and morphometrical analyses on sequenced specimens were also performed to find criteria useful in diagnosing historical voucher specimens. Based on a large data set, we were able to evaluate intra- and interspecific genetic and morphometric variation. Consequently, the role of rivers and Pleistocene forest refugia in promoting speciation within the genus *Praomys* are discussed.

MATERIAL AND METHODS

Molecular analyses

Based on craniodental characteristics, 221 specimens from 12 countries (52 localities) identified as *P. tullbergi* or *P. misonnei* were included in our molecular analyses (Table 1; Fig. 1; Appendix S1). See Lecompte *et al.* (2002a, 2005) for the list of morphological characters used to a priori define the species, and Akpatou *et al.* (2007) for a detailed analysis of the morphometrical differences between *P. rostratus* and *P. tullbergi*). A total of 269 specimens from 12 countries were included in our morphometrical analyses (Appendix S1). In order to get a better understanding of the phylogenetic relationships between *P. tullbergi* and *P. misonnei*, and with other species of the genus *Praomys*, 21 additional specimens of the genus *Praomys* were also included in our molecular analyses (Table 1). Based on morphological characteristics, several species groups were defined within the genus *Praomys* (Van der Straeten & Dudu, 1990; Van der Straeten & Kerbis Peterhans, 1999). All the species of the *P. tullbergi* complex were included in our study, except *P. morio* and *P. hartwigi*, for which no tissue sample are available. We also included three more distantly related species of the *Praomys jacksoni* complex: *P. mutoni*, *P. jacksoni*, and *P. degraaffi*.

Mitochondrial DNA sequencing and sequence alignment

DNA was extracted from ethanol-preserved muscle, liver, or heart by either the Chelex method (Walsh, Metzger & Higuchi, 1991) or the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Winnepenninckx, Backeljau & De Wachter, 1993). A large proportion of the cytochrome *b* was amplified using PCR primers L14723 (5′-CCAATGACATGAAAAATCATCG TT-3′), L14749 (5′-ACGAAACAGGCTCTAATAA-3′), 14896 (5′-TAGTTGTCGGGGTCTCCTA-3′), and 15915 (5′-TCTCCATTTCTGGTTTACAAGAC-3′). The *16S* gene was amplified using PCR primers Ar (5′-CGC CTGTTTAACAAAAACAT-3′) and Hm (5′-AGATCA CGTAGGACTTTAAT-3′). The PCR consisted of 35 cycles: 30 s at 94 °C, 40 s at 49–55 °C and 90 s at 72 °C. The double-stranded PCR products were purified using ethanol precipitation followed by automated sequencing on an ABI 3730 system (Applied Biosystems). Sequencing was conducted under BigDyeTM terminator cycling conditions.

Sequences were aligned using BIOEDIT 7.0.5.2 (Hall, 1999), and alignments were confirmed by eye. A 798-bp portion of the cytochrome *b* gene was retained for the analyses, as it was available for all specimens.

Phylogenetic analyses

Haplotypes were identified using TCS 1.13 (Clement, Posada & Crandall, 2000). Evolutionary relationships among all haplotypes were estimated by constructing phylogenetic trees using maximum-likelihood (ML) and Bayesian Markov-chain Monte Carlo phylogenetic analyses (MCMC). Two distantly related taxa were used as outgroups: *Mus musculus* (GenBank J01420) and *Rattus norvegicus* (GenBank 141848). The ML test was carried out with the PHYML program (Guindon & Gascuel, 2003), and MCMC was performed with MrBayes 3.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003).

The computer program Modeltest 3.04 (Posada & Crandall, 1998) was used to evaluate the fit of 56 nested models of nucleotide substitution to the data. Likelihood-ratio tests and the Akaike information criterion are commonly used to evaluate which model best fits the data. We used the Akaike information criterion, as it was recently shown to have several advantages over likelihood-ratio tests (Sullivan &

Table 1. List of specimens of *Praomys* included in the molecular analyses

Species	Country	Locality	Locality number of specimens		
			Number	Cyt *b*	*16S*
P. misonnei	Benin	Gotcha	18	6	5
		Igbéré	20	7	7
		Lougba	19	7	7
		Sakété	21	3	3
	Cameroon	Dja Faunal Reserve, Djomedjo	33	1	2
		Dja Faunal Reserve, Ekom	32	1	1
		Dja Faunal Reserve, Etou	31	1	1
		Ebogo	30	1	0
		Kribi	27	2	0
		Mt Bakossi, Kodmin	26	1	1
		Mt Kupe, Nyasoso	25	8	5
		Obala	29	2	0
		Sakbayémé	28	1	0
	Central African Republic	Ngotto Forest	34	3	7
	Democratic Republic of Congo	Ituri, Epulu right bank	48	4	2
		Maiko	47	2	1
		Masako	46	2	4
	Gabon	Carrière Four Place	38	0	1
		Franceville	36	3	3
		Kili	40	1	1
		Maybout	37	1	0
		Mont Mokekou	36	2	2
		Monts Doudou	41	3	6
		Mvoum	35	1	2
	Ghana	Agumasta Wildlife Sanctuary	16	2	2
		Apesokubi	17	3	3
		Shiare	15	2	2
	Kenya	Kakamega	52	1	0
	Nigeria	Asejire	22	3	1
		Ile-Ife	23	1	0
		Oshogbo	24	1	1
	Republic of Congo	Great Escarpement of Odzala, Baie de l'ombrette	44	1	1
		Odzala National Park, Ikessi	42	2	2
		Odzala National Park, Illego	45	0	1
		Odzala National Park, Tchombi	43	1	2
	Uganda	Budongo forest nature reserve, Businguro camp site	49	4	4
		Budongo forest, Nyabyeya forestry college	51	1	1
		Budongo forest, Royal Mile Area	50	6	4
P. tullbergi	Ghana	Ajenjua Bepo	13	3	3
		Katamanso	14	1	0
		Mamang River	12	27	8
	Guinea	Bamakama	3	1	1
		Ziama Forest, Balassou	1	1	0
		Ziama Forest, Malweta	2	6	1

Table 1. *Continued*

Species	Country	Locality	Locality number of specimens		
			Number	Cyt *b*	*16S*
	Ivory Coast	Agnibilekrou Kotdkossou	10	1	1
		Azagny National Park	6	4	2
		Bringakro Forest	8	4	0
		Dassioko Forest	5	16	1
		Divo	7	4	4
		Ehotilé National Park	9	15	2
		Niégré Forest	4	11	1
		Sanguiebo	11	1	1
P. petteri	Central African Republic	Ngotto Forest		1	1
	Gabon	Doudou Mounts		2	2
	Republic of Congo	Tchissangua		1	0
		Odzala National Park, Mbomo		1	1
P. rostratus	Guinea	Diécké		1	1
		Nimba Mount		1	1
		Ziama forest, Balassou		1	1
		Ziama forest, Malwéta		1	0
	Ivory Coast	Adiopodoumé		1	1
		Dassioko Forest		1	0
		Djidoubaye		1	1
		Gaourou		1	0
		Monagaga		1	0
		Niégré Forest		1	1
		Taï National Park		1	1
P. degraffi	Uganda			1	1
P. jacksoni	Gabon	Maybout		1	1
P. mutoni	Democratic Republic of Congo	Kisangani		1	1
P. obscurus	Nigeria			2	1

Locality numbers correspond to the localities of capture of *P. tullbergi* and *P. misonnei* (see Fig. 1). For specimens numbers and museum collections see Appendix S1.

Joyce, 2005). The Akaike information criterion revealed that the model that best fits the data (for both the cytochrome *b* and the *16S* genes) was the general time-reversible model, with a gamma-distributed rate variation across sites and a proportion of invariables sites. ML and MCMC analyses were conducted with the model selected by Modeltest.

The ML analyses were performed on the PHYML online web server (Guindon *et al.*, 2005). In the PHYML procedure, we used the BIONJ distance-based tree as the starting tree. Bootstrap analysis (1000 replicates) was used for ML analyses to estimate the robustness of internal nodes.

The program's default priors for parameters of the MCMC analyses were used for all analyses. We used three heated chains and a single cold chain in all MCMC analyses, and initiated runs with random trees. We conducted two independent MCMC runs with two million generations per run. We sampled trees (and parameters) every 100 generations. Stationarity was assessed by examining the average standard deviation of split frequencies. As the two runs converge onto the stationary distribution, we expect the average standard deviation of split frequencies to approach zero, reflecting the fact that the two tree samples become increasingly similar (Ronquist, Huelsenbeck & Van der Mark, 2005). Moreover, the potential scale reduction factor (PSRF) should approach 1.0 as the runs converge. For each run, the first 25% of sampled trees were discarded as burn-in.

To test some alternatives of the optimal tree, we used the Shimodaira–Hasegawa (SH) test, as implemented in PAUP (Shimodaira & Hasegawa, 1999). We generated the alternative topologies by introducing

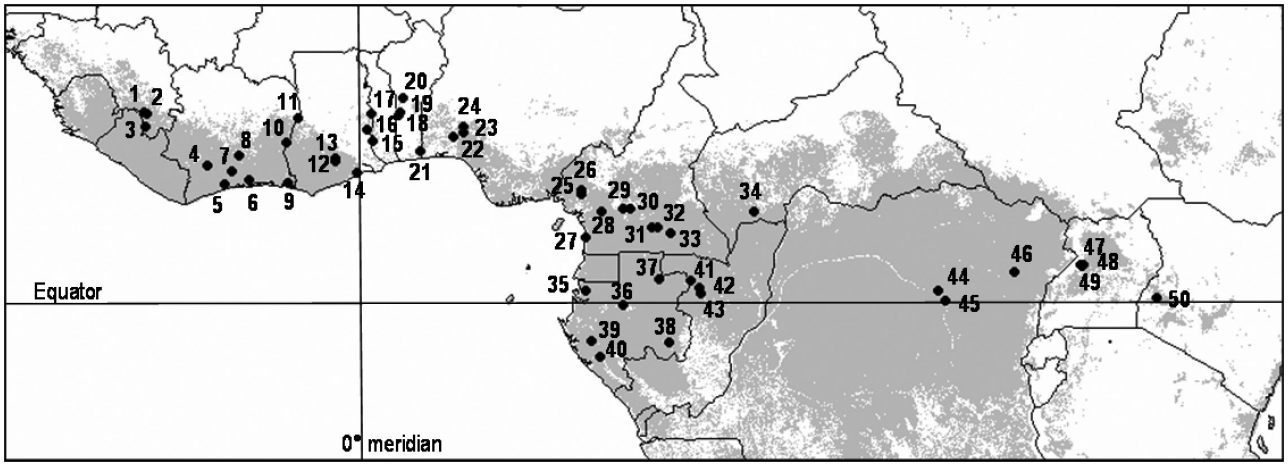

Figure 1. Map showing the 40 sites yielding the specimens of *Praomys tullbergi* and *Praomys misonnei* that were included in the molecular analyses. For the names of the localities, see Table 1. Forests are indicated in light grey (adapted from Mayaux *et al.*, 2004).

constraints during the search for the optimal tree using ML with the model for sequence evolution suggested by Modeltest.

Phylogenetic relationships between haplotypes were also inferred by constructing networks using the median-joining method available in NETWORK 4.500 (Bandlet, Forster & Röhl, 1999).

Times of divergence

Using clades identified in the phylogenetic analyses, a Bayesian MCMC approach was employed to calculate the time to the most recent common ancestor (TMRCA). The TMRCA estimates when genes last shared a common ancestor, and can therefore be used as a proxy for ancestral population age. Clades were dated using the program BEAST 1.4.6 (Drummond & Rambaut, 2007). Two independent runs of 10 million generations each, with burn-ins of one million, were performed. These two runs were then combined in TRACER 1.4 (Rambaut & Drummond, 2007), which also provides options for examining effective sample size (ESS) values and frequency plots in order to check that the mixing of the MCMC chain was adequate. MODELTEST was used to identify the model of sequence evolution used in BEAST 1.4.6, and a Bayesian skyline coalescent model of population size was specified. Divergence times and their credibility intervals were estimated using a relaxed clock model with branch rates drawn from an uncorrelated lognormal distribution. As *Praomys* has a poor fossil record, the calibration of a molecular clock for this genus is not possible. Four calibration points derived from palaeontological data were used for our estimation of the time of divergence based on cytochrome *b* sequences: the *Mus*/*Rattus* lineage split was estimated to have occurred 12 Mya (Jacobs & Downs, 1994), the appearance of *Otomys* was estimated to have occurred 6 Mya (Jansa, Barker & Heaney, 2006), the divergence between the 'big' *Apodemus mystacinus* and all of the 'small' Sylvaemus (*A. flavicollis*, *A. sylvaticus*, *A. alpicola*, *A. uralensis*) was estimated to have occurred 7 Mya (Michaux, Libois & Filippucci, 2005), and the divergence between *A. sylvaticus* and *A. flavicollis* was estimated to have occurred 4 Mya (Michaux *et al.*, 2005). Because these dates are only approximate, a normal distribution centred on the estimated date of divergence with a standard deviation of 1 million years for the *Mus*/*Rattus* split and a standard deviation of 0.5 million years for others estimates was used. We also based our estimates of the TMRCA on the *16S* sequences. However, as no sequences of *A. flavicollis* or *A. mystacinus* were available in the GenBank database, only two calibration points were used for this estimation: the *Mus*/*Rattus* lineage split and the appearance of *Otomys*.

Morphological analyses

Multivariate morphometrical analyses

We recorded 21 cranial and dental measurements (Fig. 2): condylobasal length (PRCO); henselion–basion length (HEBA); henselion–palation length (HEPA); length of palatal foramen (PAFL); length of diastema (DIA1); distance between M^1 alveolus and cutting edge of upper incisor (DIA2); smallest interorbital breadth (INTE); zygomatic breadth on the zygomatic process of the squamosal (ZYGO); smallest palatal breadth between first upper molars (PALA); length of maxillary cheekteeth (UPTE); breadth of upper dental arch (UPDA); greatest breadth of M^1

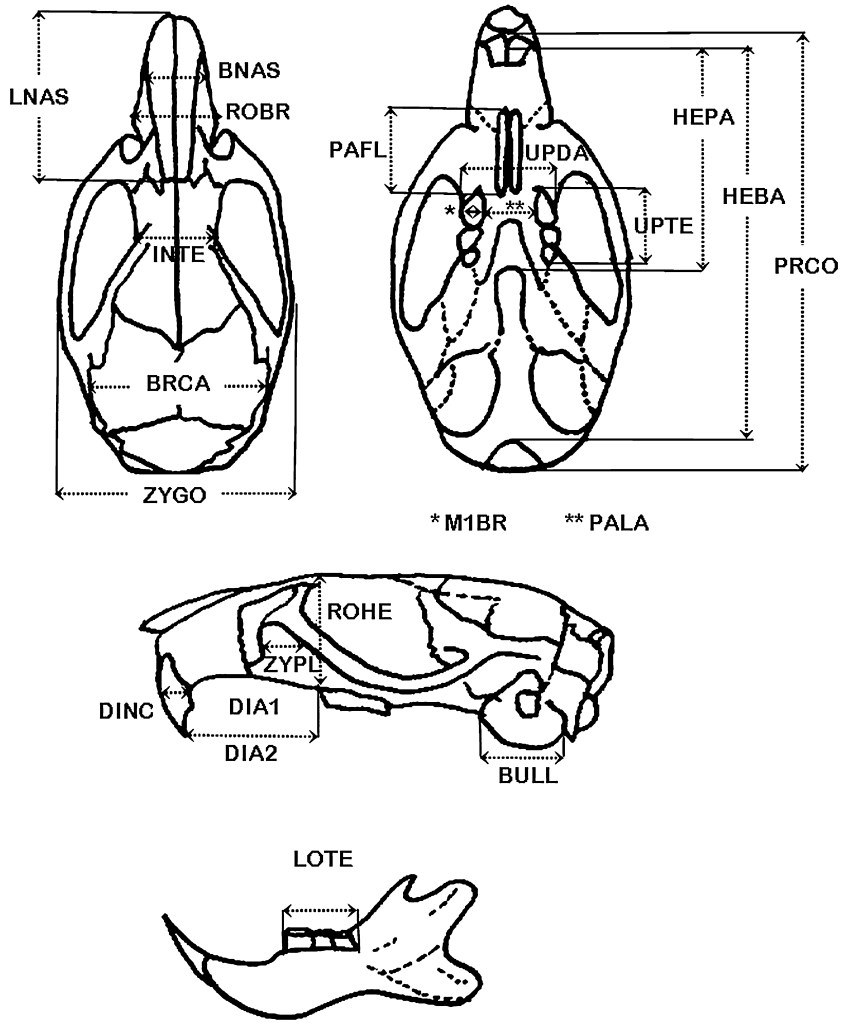

Figure 2. Schematic drawings of a skull (in dorsal, ventral, and lateral views) and mandibule of of *Praomys*, showing the 21 craniodental variables recorded in this study (see the Material and methods section for the definitions of these measurements).

(M1BR); smallest breadth of zygomatic plate (ZYPL); greatest breadth of nasals (BNAS); greatest length of nasals (LNAS); length of mandibular teeth (LOTE); length of auditory bulla (BULL); greatest breadth of braincase (BRCA); depth of I^1 (DINC); mediosagittal projection of rostrum height at anterior border of first upper molars (ROHE) and greatest rostrum breadth (ROBR). All measurements were recorded with digital calipers (Helios-digit, Helios, Germany), and were recorded to the nearest 0.05 mm.

Only adult specimens with similar tooth wear were used for morphometric analyses (i.e. tooth-wear class 2–4; Verheyen & Bracke 1966). These tooth-wear classes were grouped in our analyses because most cranial measurements were not significantly different between them (ANOVA). Moreover, the proportion of each tooth-wear class was similar between sexes and species.

Stepwise forward discriminant analysis was used to assess morphometric differences between operational taxonomic units (OTUs). Three analyses were performed.

In the first discriminant analysis we compared four OTUs: males and females of *P. tullbergi*, and males and females of *P. misonnei*. Only specimens previously identified by molecular analyses were included in this analysis.

Two additional discriminant analyses were performed on a larger data set (with both individuals identified by molecular analysis and individuals assigned to either *P. tullbergi* or *P. misonnei* based on their geographical origin), to test for possible morphometric variation within *P. misonnei*. Our molecular analyses showed that the two species have an allopatric geographical distribution; therefore, species identification based on the geographic origin of the

specimens is reliable. Moreover, all these specimens were previously compared with all other species of the *P. tullbergi* complex. We grouped *P. misonnei* specimens in several OTUs based on their geographical origin, to test if the geographical clades identified in the molecular analyses could also be distinguished morphometrically. Three OTUs were considered: 'West' (specimens from eastern Ghana, Togo, and Benin; corresponding to clade II of the molecular analysis), 'West Central' (Cameroon, Central African Republic, Republic of Congo, and Gabon; clade I), and 'Central + East' (Democratic Republic of Congo and Uganda; clade IV). We first compared eight OTUs: the six OTUs of *P. misonnei* (males and females from West, West Central, and Central + East Africa) and two OTUs of *P. tullbergi* (males and females). We then compared only the six OTUs of *P. misonnei* (males and females from West, West Central, and Central + East Africa).

Statistically significant differences between OTU centroids were evaluated by means of Mahalanobis distances and Wilks's lambda statistic (Klecka, 1980). All multivariate analyses were based on $\log_{10}$-transformed cranial morphometric data. All statistical analyses were performed using algorithms in STATISTICA 6.0 (StatSoft Inc., 2001).

Examination of visual morphological key characters

According to Lecompte *et al.* (2002a) and Lecompte (2003), *P. misonnei* and *P. tullbergi* can be distinguished by several morphological characters.

- The shape of the interorbital constriction: a gradual constriction, but more bold in the middle of the frontals (as a broken line) in *P. tullbergi*; a gradual constriction, amphora-shaped in *P. misonnei*.
- The size of the tympanic hook: bone quite short, squat with a thickening in *P. tullbergi*; bone long, thin, without thickening in *P. misonnei*.
- The posterior limit of the acoustic meatus: thin bone in *P. tullbergi*; thick bone with posterior pad in *P. misonnei*.
- The edge of the acoustic meatus: thick, origin of the anteroexternal extension wide in *P. tullbergi*; thin, origin of the anteroexternal extension thin in *P. misonnei*.
- The ratio of molar row length/maximal length of the skull: ratio > 0.15 in *P. tullbergi*, ratio < 0.15 in *P. misonnei*.
- The size and shape of the t3 cusp of the first upper molar: t3 not individualized, merged with t2 in *P. tullbergi*; t3 badly individualized, not very easily distinguished from t2 in *P. misonnei*.
- The t3 cusp on the third upper molar: t3 absent in *P. tullbergi*; t3 small or present as a crest in *P. misonnei*.
- The size and shape of the labial cingular conule 1 on the second lower molar: conule 1 quite large, elongated posteriorly and closely related with the corresponding cusp in *P. tullbergi*; conule 1 small, round and external in *P. misonnei*.

We have examined all of these characters on the specimens identified by molecular data to test if they could be used for species assignment.

RESULTS

Molecular phylogenetic analyses and divergence times based on molecular data

In total, 91 specimens of *P. misonnei* and 95 specimens of *P. tullbergi* were included in our cytochrome *b* molecular analyses. We identified 50 different haplotypes of *P. misonnei* and 41 different haplotypes of *P. tullbergi*. The trees obtained by ML and MCMC analyses have a similar topology: they only differ for the most terminal nodes. Consequently, only the tree obtained by ML is shown in Figure 3. All species of the *P. tullbergi* complex included in our study (*P. misonnei*, *P. obscurus*, *P. petteri*, *P. rostratus*, and *P. tullbergi*) form a highly supported monophyletic group. *Praomys misonnei* and *P. tullbergi* form two distinct highly supported genetic clades, and these clades are not sister clades. These results confirm that they are two distinct species. *Praomys misonnei* and *P. tullbergi* form a highly supported monophyletic group with the West African species *P. rostratus*. According to our phylogenetic tree the split between *P. misonnei* and *P. rostratus* could be more recent than the split between *P. tullbergi* and the two other species. However, the bootstrap support (71) and the posterior probability (0.86) for this node are not high. Furthermore, heuristic searches with a constrained phylogenetic position for the species *P. tullbergi* (either as the sister species of *P. misonnei* or the sister species of *P. rostratus*) produced trees with a log-likelihood that was not significantly different from the topology shown in Figure 3 (SH test, $P = 0.339$ for both tests). *Praomys tullbergi* is only found on the western side of the Volta River, whereas *P. misonnei* is found on the eastern side of the Volta River (Fig. 4).

For the *16S* gene, we identified 21 different haplotypes of *P. misonnei* (85 specimens) and six different haplotypes of *P. tullbergi* (26 specimens). In ML and MCMC analyses, *P. misonnei* and *P. tullbergi* form two distinct highly supported genetic clades, and these clades are not sister clades (Fig. 5). These results also confirm that they are two distinct species with an allopatric geographical distribution. Phylogenetic resolution within the genus *Praomys* is low for this gene.

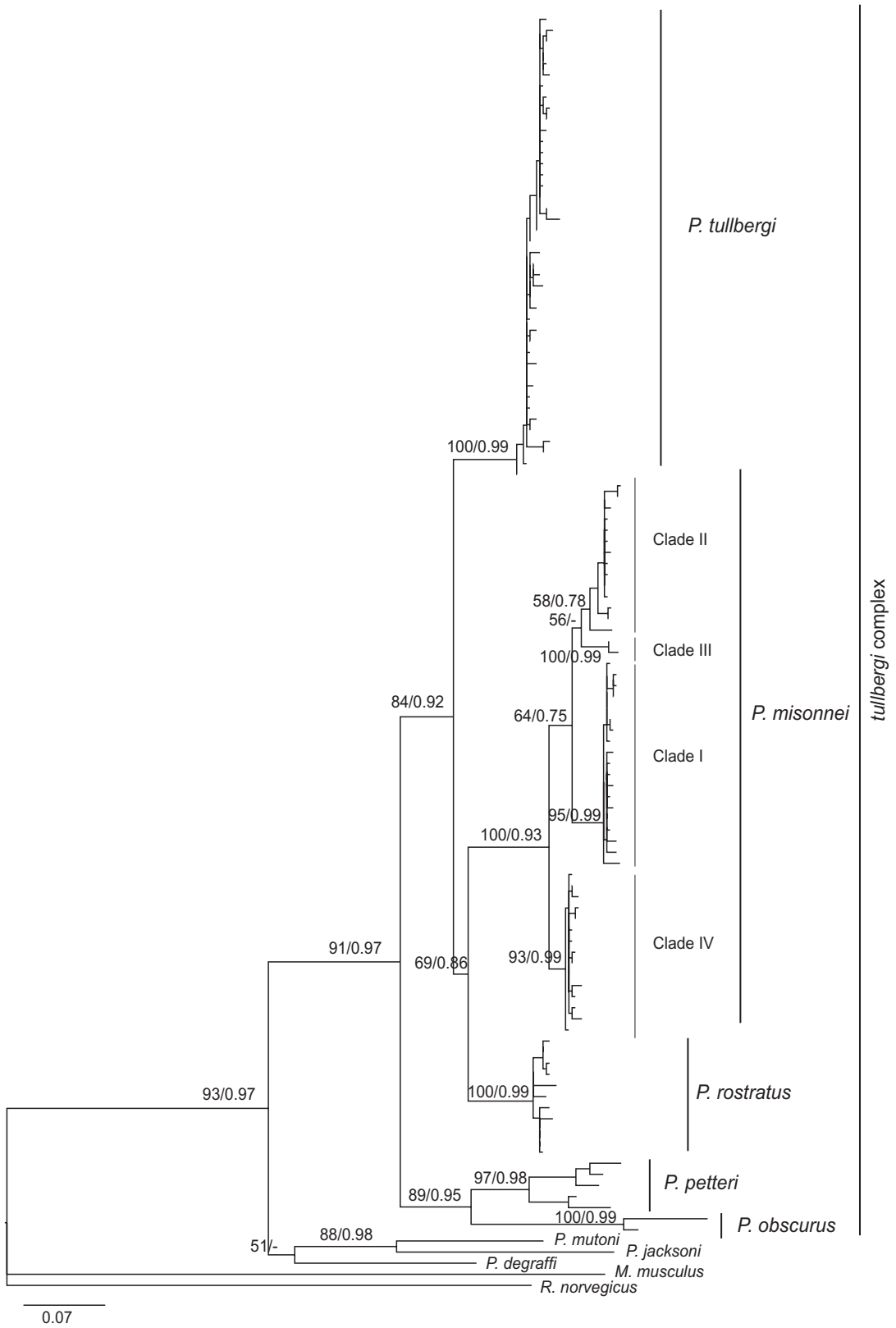

Figure 3. Phylogeny of cytochrome *b* haplotypes resulting from maximum-likelihood (ML) analysis (GTR + I + G model). Numbers at nodes represent ML bootstrap support (1000 replications), and Bayesian posterior probabilities.

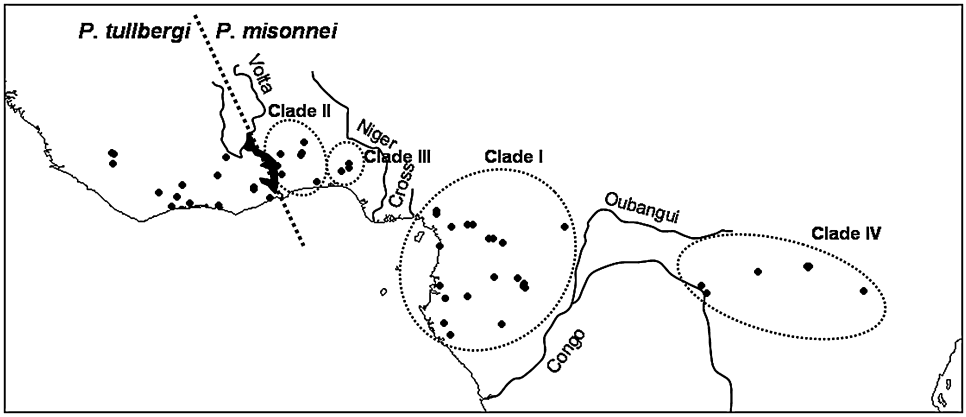

Figure 4. Schematic representation of the distribution of *Praomys tullbergi* and *Praomys misonnei*, and of the four *P. misonnei* populations (clades I–IV) resulting from phylogenetic analyses. Potential extrinsic barriers to gene flow (rivers) are indicated.

The net average number of nucleotide substitutions per site between *P. misonnei* and *P. tullbergi* is 0.061 for the cytochrome *b* gene and 0.019 for the *16S* gene. Within *P. misonnei*, the intraspecific divergence is 0.020 and 0.007 for the cytochrome *b* and *16S* genes, respectively. Within *P. tullbergi*, the intraspecific divergence is 0.006 and 0.001 for the cytochrome *b* and *16S* genes, respectively.

Within *P. tullbergi*, the genetic divergence is low for both genes, and no phylogeographical structure is apparent in both of the networks (Figs 6, 7) and the phylogenetic trees (Figs 3, 5).

Within *P. misonnei* the genetic divergence is higher, and several well-supported clades are observed for the cytochrome *b* gene (Figs 3, 6): one clade (clade I) is restricted to West Central Africa (Cameroon, Gabon, Republic of Congo, and Central African Republic), one clade (clade III) is restricted to Nigeria, and one clade (clade IV) is restricted to Central and East Africa (Democratic Republic of Congo, Uganda, and Kenya). One clade (clade II) is also observed in Benin and Ghana, but its support is low, and a quite high genetic variability is observed within this clade (net average number of nucleotide substitutions per site = 0.007). Clade II is phylogenetically closed to clade III (Fig. 3).

The resolution within *P. misonnei* is lower with the *16S* gene than with the cytochrome *b* gene. However, our results (Figs 5, 7) confirm the presence of two distinct clades in West Central Africa (clade I) and Central–East Africa (clade IV). The two specimens from Nigeria have the same haplotype (m21 in Fig. 7), and are grouped with specimens from Benin and Ghana. Once again, the genetic variability observed in Benin and Ghana is high, with one haplotype (m18) being very different (by at least five mutations) from all other haplotypes. Moreover, haplotype m02 (one specimen from Benin and one from Ghana) is not grouped with all of the other haplotypes from this region in the phylogenetic tree (Fig. 5).

The split between *P. tullbergi*, *P. misonnei*, and *P. rostratus* is estimated to have occurred *c*. 2.3 Mya (1.5–3.2 Mya). The TMRCA for the main clades recovered in our phylogenetic analyses are shown in Table 2. The estimated TMRCAs obtained with both DNA fragments are congruent, although the TMRCAs obtained with the *16S* gene are always slightly older than those obtained with the cytochrome *b* gene. The confidence intervals are always higher with the *16S* gene. Within *P. misonnei* all divergence events are estimated to have occurred later than 700 000 years ago.

Multivariate morphometrical analyses

In the first discriminant analysis, we compared four OTUs: males and females of *P. tullbergi* (41 and 37 specimens, respectively), and males and females of *P. misonnei* (40 and 27 specimens, respectively). Only specimens previously identified by molecular data were included in this analysis. After a stepwise forward discriminant analysis, 16 variables were retained (Table 3). We found that 74% of the total variation is expressed by the first canonical variate axis, whereas 19% of the total variance is explained by the second canonical variate axis (Fig. 8). For a given sex, the group centroids of the two species were statistically highly significantly different from each other ($P < 0.0001$). The group centroid of the OTU '*P. tullbergi* females' was also significantly different from the group centroid of the OTU '*P. tullbergi* males' ($P = 0.002$), indicating sexual dimorphism in this species. The group centroid of the OTU '*P. misonnei* females' was not significantly dif-

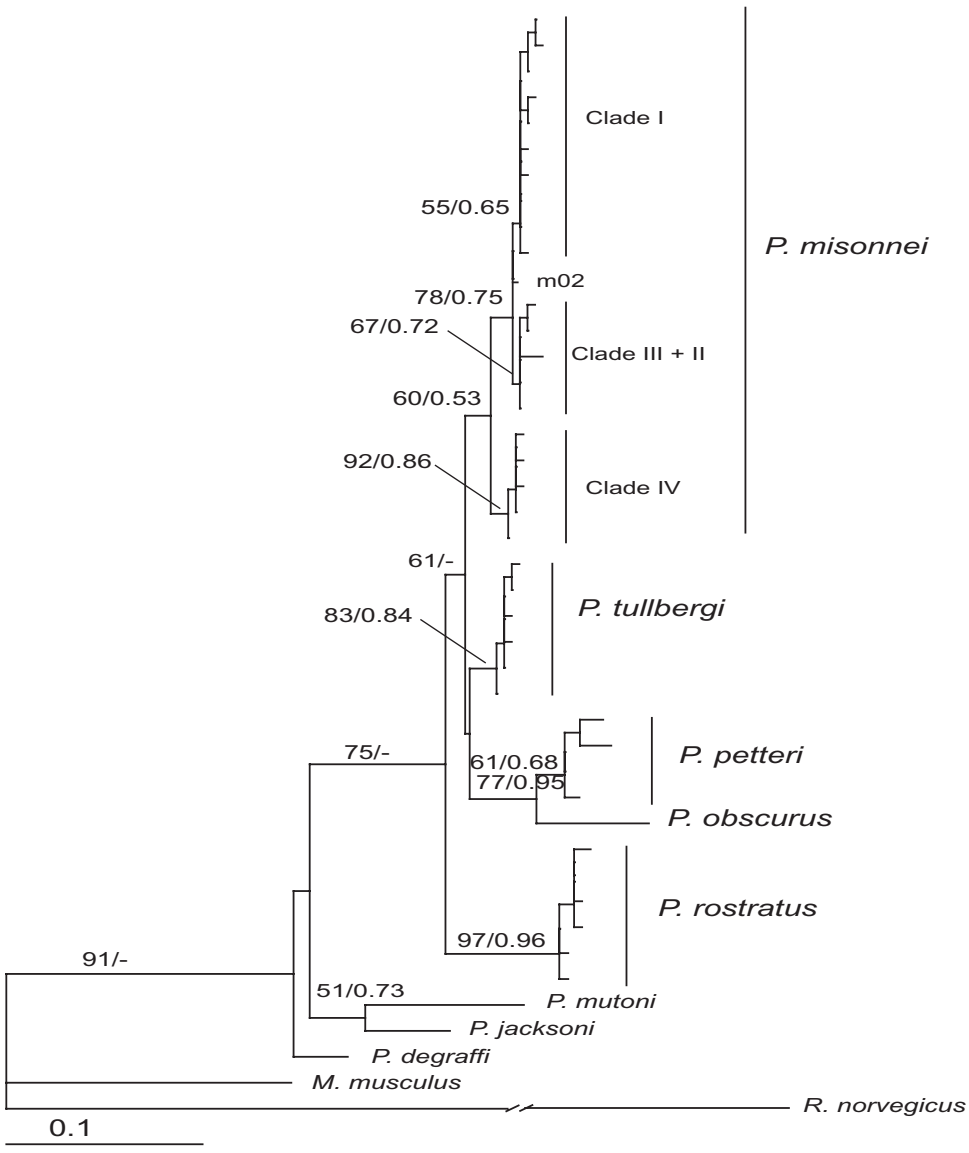

Figure 5. Phylogeny of *16S* haplotypes resulting from maximum-likelihood (ML) analysis (GTR + I + G model). Numbers at nodes represent ML bootstrap support (1000 replications), and Bayesian posterior probabilities.

ferent from the group centroid of the OTU '*P. misonnei* males' ($P = 0.092$), indicating no sexual dimorphism in this species.

The first canonical variate axis discriminates the two OTUs of *P. misonnei* from those of *P. tullbergi*. The factor structure matrix indicates that ROHE, ZYPL, PAFL, and DINC show the highest correlation with the first canonical axis (Table 3). Negative correlations with the first canonical axis are scarce, and are never strong. *Praomys misonnei* specimens have lower canonical variate scores on the first canonical variate axis than those of *P. tullbergi*, which means that they have lower values for ROHE, ZYPL, PAFL, and DINC (Fig. 8).

The second canonical variate axis tends to discriminate males of *P. tullbergi* from females of the same species. Nearly all variables are positively correlated with this second canonical axis (Table 3), indicating a size axis: most cranial and dental measurements are greater in males of *P. tullbergi* than in females (Fig. 8). This is particularly true for ZYPL, ZYGO, LNAS, HEBA, BRCA, ROHE, and DIA1.

To conclude, our discriminant analysis tends to discriminate the two species. However, this discrimination is not absolute: 10% of individuals are misclassified. Significant sexual dimorphism was recorded in *P. tullbergi*, but the percentage of misclassified individuals remains high (23%).

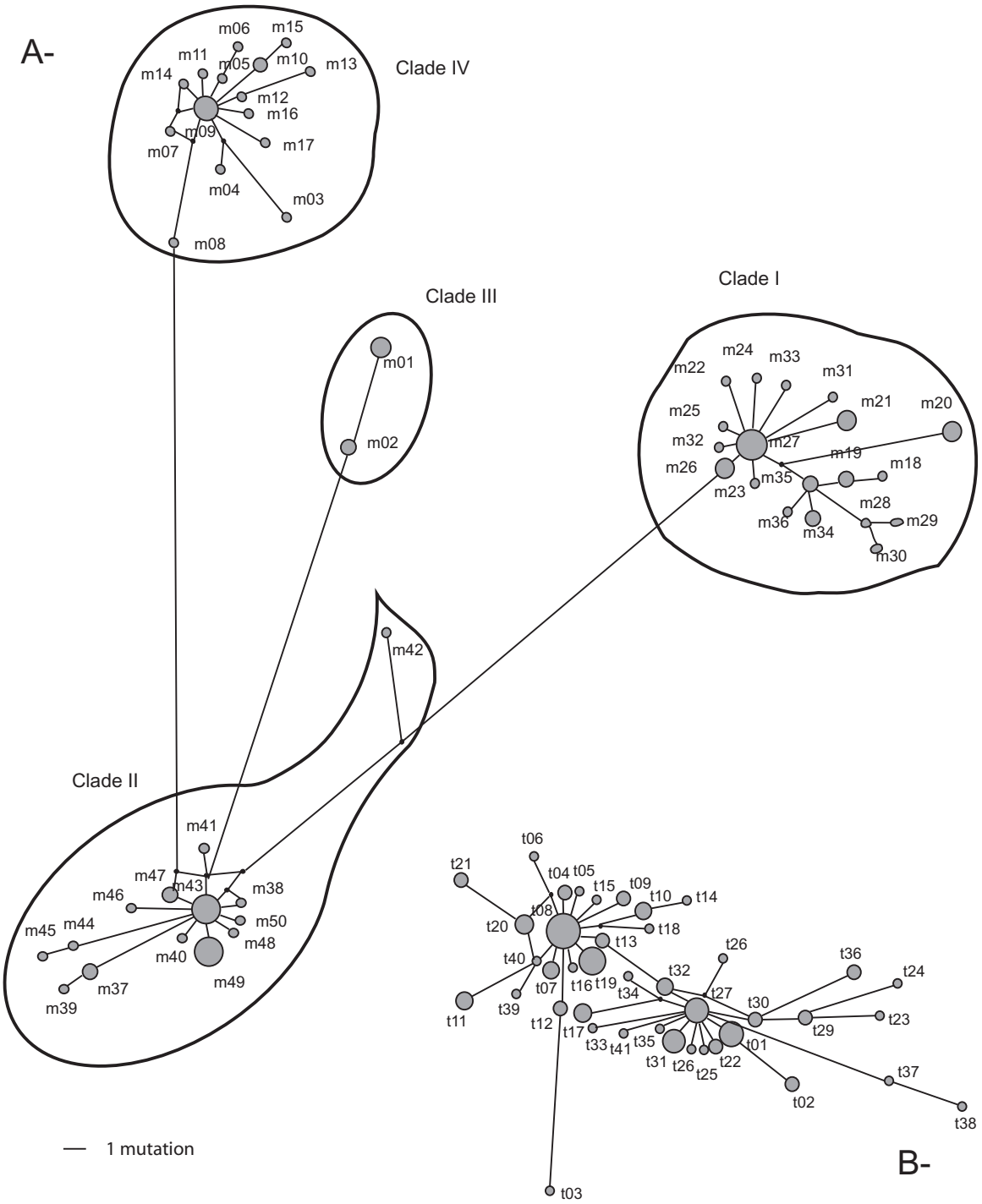

Figure 6. Minimum spanning network of *Praomys misonnei* (A) and *Praomys tullbergi* (B) cytochrome *b* haplotypes. Circle sizes are proportional to the number of similar haplotypes observed in the data set. Branch lengths are proportional to the number of mutations between haplotypes. See Appendix S1 for the haplotype designations.

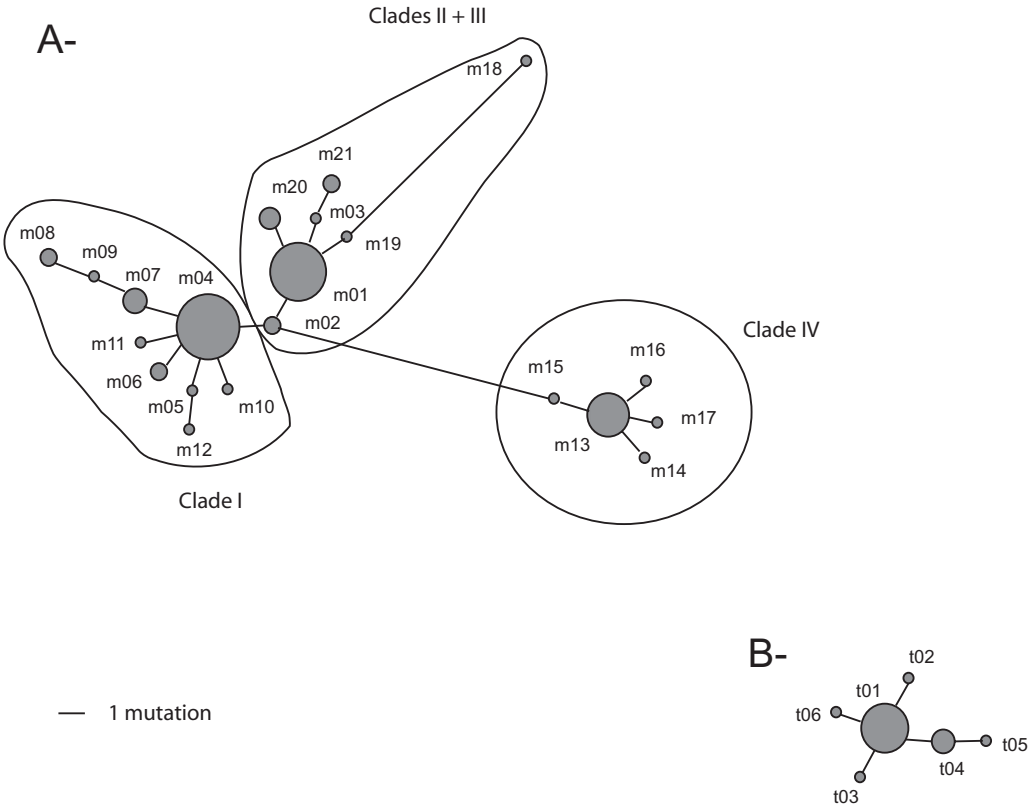

Figure 7. Minimum spanning network of *Praomys misonnei* (A) and *Praomys tullbergi* (B) *16S* haplotypes. Circle sizes are proportional to the number of similar haplotypes observed in the data set. Branch lengths are proportional to the number of mutations between haplotypes. See Appendix S1 for the haplotype designations.

Table 2. BEAST estimates of time to most recent common ancestor (TMRCA, in Mya) for the main *Praomys* clades identified in the phylogenetic analyses

	Cyt *b*		*16S*	
	Mean	Range	Mean	Range
P. misonnei, *P. rostratus* and *P. tullbergil*	2.343	1.532–3.256		
P. tullbergi	0.311	0.158–0.486	0.428	0.081–0.999
P. misonnei	0.942	0.586–1.336	1.273	0.290–2.688
Clades I, II, and III	0.712	0.434–1.019	0.586	0.149–1.241
Clades II and III	0.569	0.307–0.849	0.303	0.063–0.659
Clade II	0.375	0.196–0.581		
Clade I	0.218	0.114–0.343	0.353	0.090–0.731
Clade IV	0.177	0.084–0.300	0.396	0.076–0.922
Clade III	0.097	0.036–0.166		

The mean and range (95% confidence intervals derived from the Bayesian proability distribution) over two runs are given. The *16S* estimate of the TMRCA of clades II and III was calculated without haplotype m02.

Our molecular analyses identified three or four major geographical clades within *P. misonnei*, whereas no genetic structure was found in *P. tullbergi*. Two additional discriminant analyses were performed on a larger data set to test for possible geographical morphometric variation within *P. misonnei*. For these analyses both genetically and nongenetically identified specimens were considered (see the Material and methods section).

Table 3. Forward stepwise discriminant function analyses, used to assess morphometric differences between operational taxonomic units of *Praomys* species

	1st discriminant analysis		2nd discriminant analysis		3rd discriminant analysis	
	CV1	CV2	CV1	CV2	CV1	CV2
Variables	Correlations		Correlations		Correlations	
PRCO	0.131	0.458	0.192	–0.097	0.028	–0.138
HEBA	0.184	0.446	0.232	–0.114	Excluded	Excluded
HEPA	Excluded	Excluded	0.246	–0.092	0.063	–0.150
PAFL	0.352	0.186	0.504	–0.050	0.258	–0.526
DIA1	0.163	0.362	0.212	–0.200	–0.035	–0.218
DIA2	Excluded	Excluded	0.211	–0.202	Excluded	Excluded
INTE	Excluded	Excluded	0.127	–0.149	–0.081	–0.224
ZYGO	0.181	0.511	0.235	–0.187	–0.011	–0.212
PALA	–0.166	0.121	0.104	0.222	0.253	–0.326
UPTE	0.095	–0.089	0.127	0.062	0.127	–0.054
UPDA	–0.074	0.100	Excluded	Excluded	Excluded	Excluded
M1BR	–0.148	0.007	–0.184	–0.358	–0.393	–0.028
ZYPL	0.345	0.574	0.319	–0.198	0.026	–0.317
BNAS	Excluded	Excluded	0.299	–0.141	0.051	–0.049
LNAS	–0.018	0.493	0.106	0.058	Excluded	Excluded
LOTE	Excluded	Excluded	0.077	0.095	0.128	–0.091
BULL	0.026	0.270	0.171	0.035	0.136	–0.417
BRCA	–0.103	0.408	0.050	–0.020	0.017	–0.305
DINC	0.285	0.240	0.319	–0.001	0.179	–0.106
ROHE	0.389	0.396	0.446	0.051	0.281	–0.106
ROBR	0.083	0.187	0.100	–0.093	Excluded	Excluded

First analysis: *P. misonnei* males, *P. misonnei* females, *P. tullbergi* males, and *P. tullbergi* females.
Second analysis: *P. misonnei* from West Africa, *P. misonnei* from West Central Africa, *P. misonnei* from Central and East Africa, *P. tullbergi* males, and *P. tullbergi* females.
Third analysis: *P. misonnei* from West Africa, *P. misonnei* from West Central Africa, *P. misonnei* from Central and East Africa.
See the Material and methods section for definitions of the variables.

We first compared six OTUs of *P. misonnei* (West Africa males, 41 individuals; West Africa females, 37 individuals; West Central Africa males, 42 individuals; West Central Africa females, 29 individuals; Central + East Africa males, 18 individuals; Central + East Africa females, 18 individuals) and two OTUs of *P. tullbergi* (males, 41 individuals; females, 37 individuals). Because of the small sample size, we were not able to include the OTU from Nigeria (clade III). After a stepwise forward discriminant analysis, 20 variables were retained (Table 3). We found that 48% of the total variation is expressed by the first canonical variate, whereas 24% of the total variance is explained by the second canonical variate (Fig. 9). The first canonical variate discriminates male plus female *P. tullbergi* (high canonical scores) from the 'West Central Africa males and females' and 'Central + East Africa males and females' *P. misonnei* OTUs (low canonical scores). The *P. misonnei* OTUs 'West Africa males' and 'West Africa females' tend to have intermediate canonical scores on the first axis (Fig. 9). The factor structure matrix indicates that PAFL, ROHE, ZYPL, and DINC show the highest correlation with the first canonical axis (Table 3). Nearly all variables are positively correlated with this axis. The second canonical variate tends to discriminate the two *P. misonnei* OTUs 'West Africa males' and 'West Africa females' (high canonical scores) from all other OTUs (low canonical scores). The factor structure matrix indicates that M1BR is the most negatively correlated variable on the second canonical variate, and that PALA is the most positively correlated variable on this axis. When the two first axes are considered, sexual dimorphism seems low in *P. tullbergi* and in the three geographical regions for *P. misonnei*. However, when all seven axes are considered, sexual dimorphism is slightly significant for the Central + East African *P. misonnei*

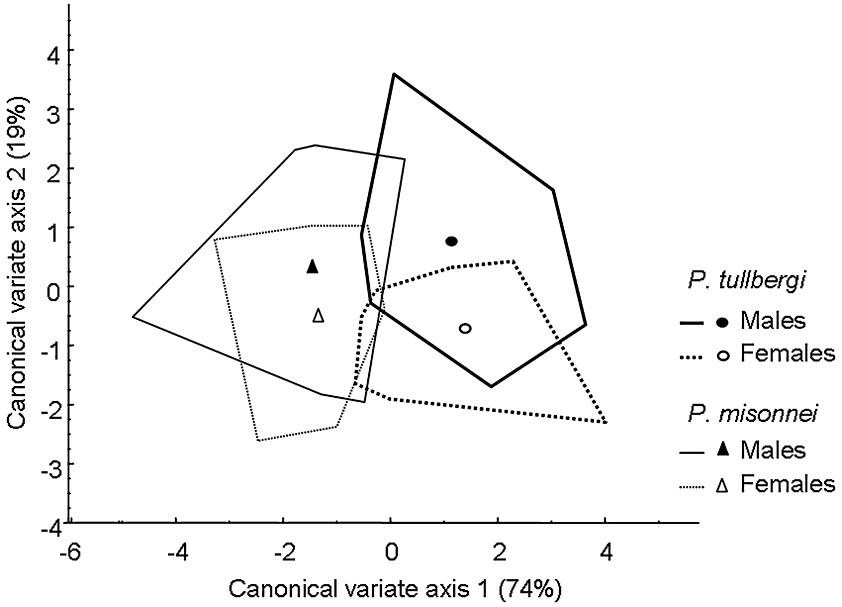

Figure 8. Canonical variate axes 1 and 2 resulting from a canonical variate analysis of *Praomys misonnei* (males and females) and *Praomys tullbergi* (males and females). Group centroids (symbols) and extreme limits of each scatter plot of points are indicated. Only specimens previously identified by molecular analyses were included in this analysis.

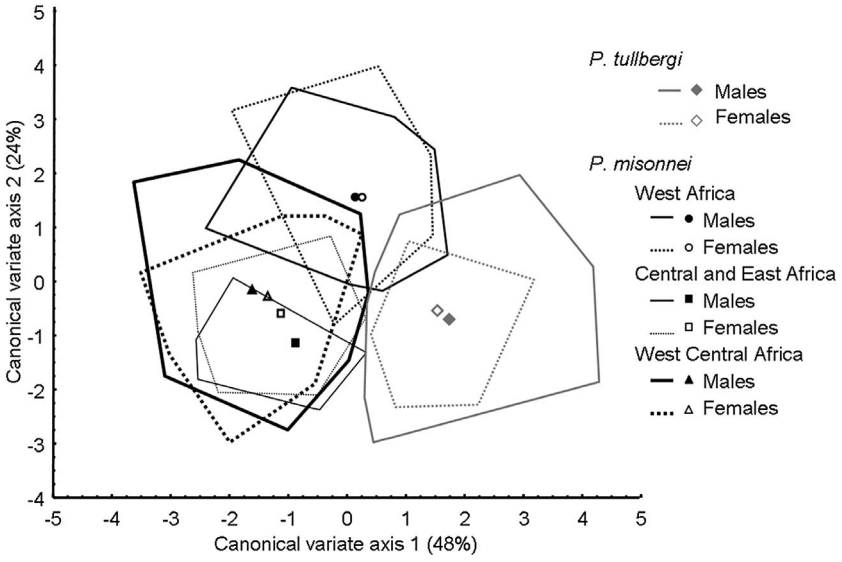

Figure 9. Canonical variate axes 1 and 2 resulting from a canonical variate analysis comparing three operational taxonomic units (OTUs) of *Praomys misonnei* (West Africa, West Central Africa, and Central + East Africa), and two OTUs of *Praomys tullbergi* (males and females). The group centroids (symbols) and extreme limits of each scatter plot of points are indicated.

($P = 0.042$), and for the West African *P. misonnei* ($P = 0.017$).

When only the six OTUs of *P. misonnei* (West Africa males and females; West Central Africa males and females; Central + East Africa males and females) are entered into the stepwise forward discriminant analysis, 16 variables are retained (Table 3). We found that 53% of the total variation is expressed by the first canonical variate, whereas 27% of the total variance is explained by the second canonical variate (Fig. 10). The first canonical variate tends to discriminate the OTUs from West Central and Central + East Africa (low canonical scores) from the OTUs 'West Africa males and females' (high canonical scores). The factor

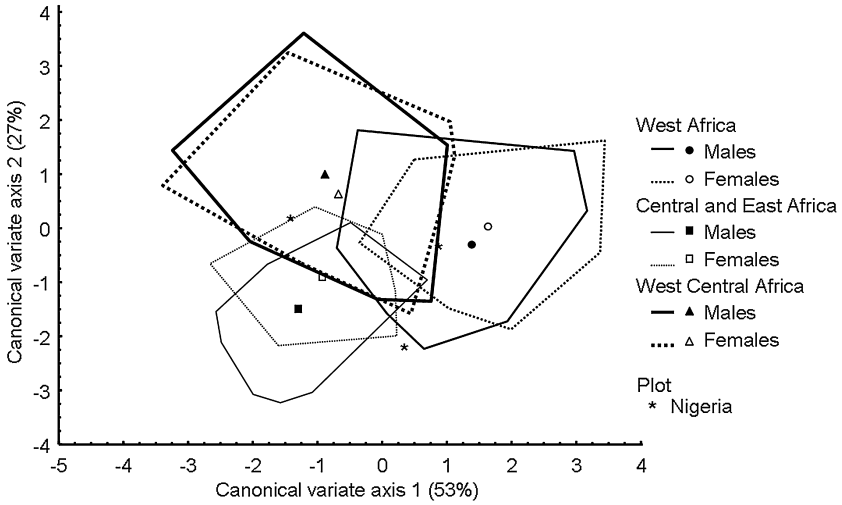

Figure 10. Canonical variate axes 1 and 2 resulting from a canonical variate analysis comparing the three operational taxonomic units (OTUs) of *Praomys misonnei* (West Africa, West Central Africa, and Central + East Africa). Two individuals from Nigeria were plotted on the graph. The group centroids (symbols) and extreme limits of each scatter plot of points are indicated.

structure matrix indicates that ROHE, PALA, PAFL, and M1BR show the highest correlation with the first canonical axis. ROHE, PALA, and PAFL are positively correlated with this axis; M1BR is negatively correlated with this axis (Table 3). On the second canonical variate there is a gradient of increasing canonical scores between the OTUs 'Central + East Africa', 'West Africa', and 'West Central Africa'. The factor structure matrix indicates that PAFL, BULL, PALA, ZYPL, and BRCA show the highest correlation with the second canonical axis. All variables are negatively correlated with this axis (Table 3). The scattergram of the first and second canonical variate axes tends to separate the OTUs from each geographical region, but there is still an overlap. The percentage of correct classification for each OTU varies from 81% (OTU 'West Africa') to 85% (OTU 'Central + East Africa'). Three adult skulls of *P. misonnei* from Nigeria could be measured, and were plotted on the scattergram of the two first canonical variates. One of them falls within the OTUs 'West Central Africa males and females', one falls within the OTUs 'West Africa males and females', and the last one falls outside the range of the six studied OTUs. Taking into account the five axes of the discriminant analysis, no significant sexual dimorphism is recorded for the West Central African populations of *P. misonnei* ($P = 0.104$). Sexual dimorphism is significant for the East and Central African population of *P. misonnei* ($P = 0.027$), and for the West African populations of *P. misonnei* ($P = 0.008$). The axis of the third canonical variate (14% of the total variance) discriminates males (high canonical scores) from females (low canonical scores). Nearly all variables are positively correlated with this third canonical axis (data not shown), indicating a size axis: most values of cranial and dental measurements are larger in males than in females.

Examination of visual morphological key characters

According to Lecompte *et al.* (2002a, 2005), *P. misonnei* and *P. tullbergi* can be distinguished on the shape of the interorbital constriction, and on the size and shape of the t3 cusp of the M^1. However, in the description of *P. misonnei*, Van der Straeten & Dieterlen (1987: 4) stated that the 'interorbital constriction and the t3 on M^1 are as in *P. tullbergi*'. After a careful examination of all the skulls of these two species for which the species identification was confirmed by molecular data, it appears that these two characters can no longer be used to differentiate these two species. The interorbital constriction is gradual in both species, and its shape varies within each species. In some individuals it is amphora-shaped, in others it is more bold in the middle of the frontals. The t3 on M^1 is not isolated in most specimens of both species. In the remaining specimens it is hardly isolated. This last character state seems to be more frequent in some populations than in others.

According to Lecompte *et al.* (2002a, 2005), several other characters can be used to differentiate *P. tullbergi* from *P. misonnei*. After a careful examination of all skulls for which the species identification was confirmed by molecular data, it appears that none of

the characters proposed by Lecompte *et al.* (2002a) and Lecompte (2003) can be used to differentiate these two species.

The size of the tympanic hook, the characteristics of the posterior limit and edge of the acoustic meatus, and the size and shape of the labial cingular conule on M_2 were found to be highly variable within each species. The t3 cusp on M^3 was absent in all specimens of *P. tullbergi* and in nearly all specimens of *P. misonnei*. We compared the ratio of molar row length/maximal length of the skull between sexes and species (ANOVA and Scheffé's post hoc tests; Scheffé, 1959). For a given sex, no significant difference was found between species ($P > 0.05$). Within species, a significant difference was obtained between males and females ($P < 0.05$): the ratio was greater than 0.150 in females (0.155 for *P. misonnei* and 0.153 for *P. tullbergi*), and was smaller than 0.150 in males (0.149 for *P. misonnei* and 0.148 for *P. tullbergi*).

Because these eight characters cannot be used to discriminate these two species, we then carefully compared the skulls of these two species in order to find discrete characters that could be used to discriminate them. However, we could not find any characters that could reliably distinguish the two taxa.

DISCUSSION

Taxonomic implications

Our analyses show that *P. misonnei* and *P. tullbergi* are clearly differentiated based on cytochrome *b* and *16S* data, and that they represent two distinct species. These results confirm those previously obtained by Nicolas *et al.* (2005) and Lecompte *et al.* (2002b, 2005). Owing to our broad geographic sampling, we are able to determine the precise distributional range of each species. Contrary to prior suggestions (e.g. Lecompte *et al.*, 2001; Musser & Carleton, 2005), the geographical distribution of *P. tullbergi* is relatively limited: it occurs in West Africa from eastern Guinea to western Ghana (west of the Volta River), through the Ivory Coast. *Praomys misonnei* is widely distributed from Ghana (east of the Volta River) to Kenya, through Togo, Benin, Nigeria, Cameroon, Central African Republic, Gabon, Democratic Republic of Congo, Kenya, and Uganda. *Praomys misonnei* and *P. tullbergi* are genetically and morphologically close to *P. rostratus* (this study; Nicolas *et al.*, 2005). This last species is endemic to West Africa, were it occurs in Guinea, south-eastern Senegal, southern Mali, Liberia, and Ivory Coast (V. Nicolas, unpubl. data).

Our morphometrical analyses show that *P. tullbergi* and *P. misonnei* cannot be easily separated craniometrically: 10% of individuals are misclassified in our discriminant analysis based on molecularly identified specimens. However, several cranial measurements (ROHE, ZYPL, PAFL, and DINC) are significantly lower in *P. misonnei* than in *P. tullbergi* (Table 3; Fig. 8; Appendix S2). Van der Straeten & Dieterlen (1987) also recorded significantly lower ROHE, ZYPL, and PAFL in *P. misonnei* than in *P. tullbergi*, but did not consider DINC to be an important character for species discrimination. According to these authors, UPTE, UPDA, M1BR, LOTE, BULL, and BRCA are important for the discrimination between these two species, and are significantly smaller in *P. tullbergi* than in *P. misonnei*. In our study, these variables do not, or only weakly, contribute to the discrimination between species (Table 3). The discrepancies between our results and those of Van der Straeten & Dieterlen (1987) might be explained by differences in the geographical sampling area. Indeed, contrary to Van der Straeten & Verheyen (1981), who only measured *P. misonnei* specimens from the type locality (Irangi, Democratic Republic of Congo), we included in our study specimens from numerous localities from most of the geographical range of this species. Our protocol allowed us to take into account interpopulation variability, and therefore to select morphometrical characters that could be used for the discrimination between species on a relatively wide geographical scale. Geographical morphometric variability is high in *P. misonnei*. Several skull measurements (PAFL, ROHE, and DINC) of the western populations (from eastern Ghana, Togo, and Benin) of *P. misonnei* are intermediate between those of *P. tullbergi* and other populations of *P. misonnei* (Central + East Africa and West Central Africa; Table 3; Fig. 9; Appendix S2). This result could explain why all *P. misonnei* specimens from the Western African region are considered in the literature as belonging to *P. tullbergi* (e.g. Lecompte *et al.*, 2002a; Musser & Carleton, 2005). Several other measurements were found to be significantly greater (PALA) or smaller (M1BR) in western populations of *P. misonnei* than in West Central or Central–Eastern populations of the same species (Table 3; Fig. 10; Appendix S2). Moreover, PAFL, BULL, PALA, ZYPL, and BRCA were smaller in West Central African populations of *P. misonnei* than in Central–Eastern ones. These data indicate that morphometrical geographical variation is high in this species, which probably explains why its taxonomy and geographical distribution is the subject of much debate. Skulls and mandible shapes are known to be capable of ecophenotypic response during the lifetime of the individual animal (Rychlik, Ramalhinho & Polly, 2006). These structures covary with many environmental factors, such as latitude, temperature, altitude, and precipitation. Owing to the large geographical range of *P. misonnei*, and associated

changes in geoclimatic factors, it is therefore not surprising to find morphometrical differences between populations from distinct geographic regions.

In the first discriminant analyses, we did not find significant sexual dimorphism in *P. misonnei*, whereas morphometric sexual dimorphism was recorded in *P. tullbergi*. In *P. tullbergi*, most cranial and dental measurements were larger in males than in females. These results are congruent with those obtained by Van der Straeten & Dieterlen (1987) for *P. misonnei*, and by Akpatou *et al.* (2007) and Van der Straeten & Verheyen (1981) for *P. tullbergi*. However, in the second discriminant analyses, we did not find significant sexual dimorphism in *P. tullbergi*, whereas morphometric sexual dimorphism was recorded in some populations of *P. misonnei*. Once again, when sexual dimorphism was recorded, males tended to be bigger than females for nearly all of the measurements. Additional analyses on larger samples are necessary to draw final conclusions. Male-biased sexual dimorphism in body size is common among many species of mammals, and can be the result of a number of factors, such as sexual selection, mating strategy, seasonal food availability, and population densities (Isaac, 2005). Seasonal food availability influences sexual dimorphism because it affects the length of the breeding season and polygyny potential. Likewise, population density influences resource availability, and evidence suggests that food scarcity differentially constrains the growth of the sexes. Karubian & Swaddle (2001) have suggested that these influences should be examined on a taxon-by-taxon basis. Moreover, Krackow, Schmidt & Elepfandt (2003) found that sexual dimorphism in adult weight exerts correlated effects of blastocyst development, which can cause sex-ratio shifts as a result of interactions with the timing of uterine responsiveness. Unfortunately, data on the ecology, sex-ratio, and mating strategies of *P. tullbergi* and *P. misonnei* are still too scarce to try to infer the determinants of sexual dimorphism in the two species, and to test for possible regional differences.

According to Lecompte *et al.* (2002a, 2005), several characters can be used to differentiate *P. tullbergi* from *P. misonnei*. However, none of them was found to be reliable for species identification in our study: they vary greatly within species, and some of them (e.g. ratio of molar row length/maximal length of the skull) reflect sexual dimorphism and not species differentiation. In conclusion, no discrete morphological character can be used to discriminate these two species.

Biogeographical implications

Praomys misonnei and *P. tullbergi* have an allopatric geographical distribution. *Praomys misonnei* is only found on the eastern side of the Volta River. *Praomys tullbergi* is only found on the western side of the Volta River (Fig. 4), where it co-occurs with *P. rostratus* in the Ivory Coast and Guinea. In our study, the phylogenetic relationships between these three species are unresolved. According to our results, the split between *P. tullbergi*, *P. rostratus*, and *P. misonnei* is estimated to have occurred approximatively 2.3 Mya (1.5–3.2 Mya). This estimate is older than the one obtained by Nicolas *et al.* (2005; *c*. 1.3 Mya). However, it is not surprising to find such a difference, as we have estimated the TMRCA, whereas Nicolas *et al.* estimated the time of divergence between populations. Moreover, whereas we used a relaxed molecular clock, these authors used a strict molecular clock. Undoubtedly, there is a large error associated with any divergence date estimate based on DNA sequence data, and 'divergence time estimates should be considered as temporal hypotheses amenable to future empirical testing' (Martinez-Solano *et al.*, 2006). However, used carefully, they provide useful phylogenetic information (Hewitt, 1996). The divergence between the three species of *Praomys* and between the three *P. misonnei* clades probably occurred during the Quaternary. In Africa, the Pleistocene was characterized by humid interglacial periods oscillating with arid glacial periods, and the associated expansion and contraction of forest habitats (deMenocal, 2004). These cyclic changes may have had strong influences on geographical patterns and levels of genetic differentiation among species and populations. According to Haffer's (1982) refuge theory, tropical rain forest was confined to specific core areas during arid Pleistocene periods. Assuming that the ecological preferences of *Praomys* spp. have not significantly changed during the last 2 Myr, these forest species may have become isolated into forest patches during arid periods, and may have experienced range expansion during more humid periods. Maley (1996) described such Pleistocene lowland forest refuge zones in equatorial Africa for the last arid phase (18 000 years BP). Core areas in equatorial Africa comprise the central part of the Congo–Zaïre depression, Cameroon/Gabon, eastern Ivory Coast/western Ghana, and Sierra Leone/Liberia. According to the theory of forest refugia, speciation/diversification occurred in refuge zones during unfavourable climatic conditions. The refuge hypothesis might well explain the diversification of West African *Praomys* species. *Praomys rostratus* and *P. tullbergi* could have become isolated into two West African forest refuge zones (the Sierra Leone/Liberia and the eastern Ivory Coast/western Ghana refuge, respectively) and *P. misonnei* might have become isolated in the Central African zone. More detailed phylogeographic analyses are necessary to test these hypotheses, and to infer a

possible centre of dispersion for each species. Therefore, it would be necessary to increase sample size per locality, to sequence more genes, and to sample the complete geographical range of *P. rostratus*. Within *P. misonnei*, three or four main geographical/molecular clades were identified. Two of them (clades I and IV) could correspond to appropriate refuge zones in Cameroon/Gabon and the Congo–Zaïre depression. Within *P. misonnei*, a high level of genetic variability and unresolved phylogeographic variation was observed in Benin, eastern Ghana, and Nigeria. No refuge zone was proposed by Maley (1996) in this area. In Benin and eastern Ghana the forest is only patchily distributed. This area represents the 'Dahomey gap region', where the Sudanian savannahs extend all the way to the sea, and interrupt the forest cover for approximately 200 km (Maley, 1996; Fig. 1). The Dahomey gap may be as old as 3 Myr (Robert & Chamley, 1987), and several phases of forest expansion and contraction occurred in this area during the Pleistocene (Dupont *et al.*, 2000; Salzmann & Hoelzmann, 2005). As *P. misonnei* is a forest-dependent species, it is not surprising to find a complex pattern of genetic variation in this region. Additional sampling would be useful to better understand the phylogeographic history of the species in this area.

Rivers may also provide barriers to distribution, leading to allopatric speciation (e.g. Colyn, Gautier-Hion & Verheyen, 1991 for forest mammals). During prolonged humid periods, waterways may have been wide enough to prevent mammal dispersal (Robbins, 1978). Alternatively, they may also have been sufficiently large during arid phases to stop the re-expansion of species from relict areas (Colyn *et al.*, 1991). In Africa, the basic hydrographic network has undergone considerable modification by successive phases of uplifts, and by subsidence from about the Miocene onwards (John, 1986). Although these changes were drastic in East Africa, in West Africa the pattern remained more stable because of the longer period of tectonic stability (John, 1986). However, hydrographic changes have been brought about by variations in climate, especially over the last million years of the Quaternary. These climatic variations have often been extreme, characterized by variations in rainfall and evaporation, causing the expansion, contraction, or even complete disappearance of river systems (John, 1986). The Volta River probably acted as an extrinsic geographic barrier to gene flow, reinforcing the differentiation between *P. tullbergi* and *P. misonnei*. Several other studies showed that the Volta River has acted as a barrier to gene flow in several other mammal species (e.g. Robbins, 1978; Bergmans, 1997). Unfortunately, precise palaeodrainage data are lacking for the Volta River. Our data show that the Volta River acts as an extrinsic geographic barrier to gene flow between *P. misonnei* and *P. tullbergi*. However, it is not clear whether the same role was played by this river in the past. Within *P. misonnei*, three main geographical/molecular clades were identified, and some of these clades were to some extent morphometrically different. A likely explanation for these major phylogeographic discontinuities is the presence of long-term extrinsic barriers to genetic exchange between regions. The Niger River and/or the Cross Rivers represent potential barriers to gene flow between clades I and III (Fig. 4). These rivers have also often been cited as factors limiting the distribution of other mammal species (Booth, 1958; Robbins, 1978; Maley, 1987, 2001). The Oubangui–Congo River may also have acted as a barrier to gene flow between clades I and IV (Fig. 4). However, additional sampling on each side of the Niger, Cross, and Oubangui rivers is necessary to tests these hypotheses.

Finally, additional sampling in eastern Nigeria and western Cameroon would allow us to test the role of the Cameroon Volcanic Line (a succession of high volcanoes and plateaus that originated between 30 and 0.4 Mya; Cornacchia & Dars, 1983; Ubangoh, Pacca & Nyobe, 1998) as a potential barrier to gene flow between Nigerian (clade III) and Cameroonian (clade I) populations of *P. misonnei*.

CONCLUSION

Our study provides an example of the usefulness of mtDNA data to elucidate the limits between species and their geographical distribution. Our molecular results show that *P. misonnei* and *P. tullbergi* are two distinct species. These two species are cryptic, and no discrete morphological character or single metric measurement can be used to distinguish them. *Praomys misonnei* and *P. tullbergi* have allopatric geographical distributions: *P. misonnei* is only found on the eastern side of the Volta River, whereas *P. tullbergi* is only found on the western side of the Volta River. Although our study allows us to speculate on the role of Pleistocene forest refugia and fluvial systems in promoting speciation within the genus *Praomys*, more genes, localities (on both sides of the Niger, Cross, and Oubangui rivers), and specimens are necessary in order to reach final conclusions.

ACKNOWLEDGEMENTS

This work was supported by the 'Consortium National de Recheche en Génomique' (Evry, France), and the 'service de systématique moléculaire' of the Muséum National d'Histoire Naturelle (IFR 101, Paris, France). It is part of the agreement n°2005/67

between the Genoscope and the Muséum National d'Histoire Naturelle on the project 'Macrophylogeny of life' directed by Guillaume Lecointre. Molecular analyses were also conducted at the 'Centre Commun Marqueurs Moléculaires et Séquençage' (University of Rennes) and the Royal Belgian Institute of Natural Sciences (Brussels). Field studies were supported by: (1) EU-DGVIII Ecofac program 'Conservation et Utilisation Rationnelle des Ecosystèmes Forestiers en Afrique centrale http://www.ecofac.org'; (2) WWF Gabon; (3) EU-DGVIII Biofac program 'Origine et maintien de la biodiversité en Afrique centrale'; (4) PGRR-GFA Terra Systems (Ziama Forest, Guinea); (5) EU-INCO-DEV grant ICA4-CT2002-10050, VIZIER project LSHG-CT-2004-511960 'Nouvelles approches pour le traitement et le contrôle des fièvres hémorragiques en Afrique de l'Ouest'; (6) DWTC action 1 project 'Evaluating the effect of Pleistocene climate changes on speciation patterns in selected African vertebrates'; (7) PAMF Bénin, 'Projet d'Aménagement des Massifs Forestiers d'Agoua, des Monts Kouffé et de Wari-Maro'. Specimens from Uganda (Budongo Forest) and the Ituri Forest (DRC) were collected with support from the Marshall Field Fund (Field Museum of Natural History, Chicago) and the John D. and Catherine T. Macarthur Foundation (Chicago). We are grateful to all field collectors, particularly J. Kennis and P. Katuala.

REFERENCES

Akpatou B, Nicolas V, Pires D, N'Goran E, Colyn M. 2007. Morphometric differentiation between two murid rodents, *Praomys tullbergi* (Thomas, 1894) and *Praomys rostratus* (Miller, 1900), in West Africa. *Zootaxa* **1607:** 21–34.

Avise JC. 2000. *Phylogeography: the history and formation of species*. Cambridge: Harvard University Press.

Avise JC, Shapiro JF, Daniel SW, Aquadro CF, Lansman RA. 1983. Mitochondrial DNA differentiation during the speciation process in Peromyscus. *Molecular Biology and Evolution* **1:** 38–56.

Bandlet HJ, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16:** 37–48.

Bergmans W. 1997. Taxonomy and biogeography of African fruit bats (Mammalia, Megachiroptera). 5. The genera *Lissonycteris* Andersen, 1912, *Myonycteris* Matschie, 1899, and *Megaloglossus* Pagenstecher, 1885; general remarks and conclusions; annex: key to all species. *Beaufortia* **47:** 11–90.

Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* **22:** 148–155.

Booth AH. 1958. The Niger, the Volta and the Dahomey Gap as geographic barriers. *Evolution* **12:** 48–62.

Bonvicino CR, Lima JFS, Almeida FC. 2003. A new species of Calomys Waterhouse (Rodentia, Sigmodontinae-)from the Cerrado of Central Brazil. *Revista Brasileira de Zoologia* **20:** 301–307.

Carleton MD, Musser GG. 1984. Muroid Rodents. In: Aderson S, Jones KJ, eds. *Orders and Families of recent mammals of the world*. New York: John Wiley and Sons, 289–379.

Clement M, Posada D, Crandall K. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9:** 1657–1660.

Colyn M, Gautier-Hion A, Verheyen W. 1991. A re-appraisal of palaeoenvironmental history in Central Africa: evidence for a major fluvial refuge in the Zaire Basin. *Journal of Biogeography* **18:** 403–407.

Cornacchia M, Dars R. 1983. Un trait structural majeur du continent africain. Les linéaments centrafricains du Cameroun au golfe d'Aden. *Bulletin de la Société Géologique de France* **25:** 101–109.

Craul M, Zimmermann E, Rasoloharijaona S, Randrianambinina B, Radespiel U. 2007. Unexpected species diversity of Malagasy primates (*Lepilemur* spp.) in the same biogeographical zone: a morphological and molecular approach with the description of two new species. *BMC Evolutionary Biology* **7:** 83. doi: 10.1186/1471-2148-7-83.

Davis DHS. 1962. Distribution patterns of southern African Muridae, with notes on their fossil antecedents. *Annals of the cape Provincial Museum* **2:** 56–76.

Dayrat B. 2005. Towards integrative taxonomy. *Biological Journal of the Linnean Society* **85:** 407–415.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7:** 214.

Dupont LM, Jahns S, Marret F, Ning S. 2000. Vegetation change in equatorial West Africa: time-slices for the last 150 ka. *Palaeogeography, Palaeoclimatology, Palaeoecology* **155:** 95–122.

Ellerman JR. 1941. *The families and genera of living rodents. Volume II. Family Muridae*. London: British Museum (Natural History).

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52:** 696–704.

Guindon S, Lethiec F, Duroux P, Gascuel O. 2005. PHYML Online-a web server for fast maximum likelihood-based phylogenetic inference. *Nucleic Acids Research* **33** (Web Server Issue)**:** W557–9.

Haffer J. 1982. General aspects of the refuge theory. In: Prance GT, ed. *Biological diversification in the tropics*. New York: Columbia University Press, 6–24.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41:** 95–98.

Happold DCD. 1987. *The mammals of Nigeria*. Oxford: Clarendon Press.

Hewitt GM. 1996. Some genetic consequences of ice ages and their role in divergence and speciation. *Biological Journal of the Linnean Society* **58:** 247–276.

Honacki JH, Kinman KE, Koeppl JW. 1982. *Mammal species of the world: a taxonomic and geographic reference*. Lawrence, KS: Allen Press, Inc. and the Association of Systematics Collections.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17:** 754–755.

Huhndorf MH, Kerbis Peterhans JC, Loew SS. 2007. Comparative phylogeography of three endemic rodents from the Albertine Rift, east central Africa. *Molecular Ecology* **16:** 663–674.

Hutterer R, Dieterlen F, Nikolaus G. 1992. Small mammals from forest islands of eastern Nigeria and adjacent Cameroon, with systematical and biogeographical notes. *Bonner Zoologische Beitrage* **43:** 393–414.

Isaac JL. 2005. Potential causes and life-history consequences of sexual size dimorphism in mammals. *Mammal Review* **35:** 101–115.

Jacobs LL, Downs WR. 1994. The evolution of murine rodents in Asia. In: Tomida Y, Li C, Setogushi T, eds. *Rodents and Lagomorph families of Asian origin and diversification*. Tokyo: National Science Museum Monographs, 149–156.

Jansa SA, Barker FK, Heaney LR. 2006. The pattern and timing of diversification of Philippine endemic rodents: evidence from mitochondrial and nuclear gene sequences. *Systematic Biology* **55:** 73–88.

John DM. 1986. *The inland waters of tropical West Africa: an introduction and botanical review*. Stuttgart: Schweizerbart'sche Verlagsbuchhandlung.

Karubian J, Swaddle JP. 2001. Selection on females can create 'larger males'. *Proceedings of the Royal Society, Series B-Biological Sciences* **268:** 725–728.

Klecka WR. 1980. *Discriminant analysis*. London: Sage Publications.

Krackow S, Schmidt TA, Elepfandt A. 2003. Sexual growth dimorphism affects birth sex ratio in house mice. *Proceedings of Biological Sciences* **270:** 943–947.

Lavrenchenko LA, Verheyen WN, Verheyen E, Hulselmans J, Leirs H. 2007. Morphometric and genetic study of Ethiopian *Lophuromys flavopunctatus* Thomas, 1888 species complex with description of three new 70-chromosomal species (Muridae, Rodentia). *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique* **77:** 77–117.

Lecompte E. 2003. *Systématique et évolution du groupe Praomys (Rodentia, Murinae)*. Unpublished PhD Thesis, Muséum National d'Histoire Naturelle, Paris.

Lecompte E, Denys C, Granjon L. 2001. An identification key of the Praomys species (Rodentia: Muridae). In: Denys C, Granjon L, Poulet A, eds. *African small mammals. Proceeedings of the 8th International Symposium on African Small Mammals*. Paris: Colloques et Congrès – Editions de l' IRD, 127–139.

Lecompte E, Denys C, Granjon L. 2005. Confrontation of morphological and molecular data: the Praomys group (Rodentia, Murinae) as a case of adaptive convergences and morphological stasis. *Molecular Phylogenetics and Evolution* **37:** 899–919.

Lecompte E, Granjon L, Denys C. 2002a. The phylogeny of the *Praomys* complex (Rodentia: Muridae) and its phylogeographic implications. *Journal of Zoological Systematics and Evolutionary Research* **40:** 8–25.

Lecompte E, Granjon L, Kerbis Peterhans JC, Denys C. 2002b. Cytochrome b-based phylogeny of the *Praomys* group (Rodentia, Murinae): a new African radiation? *Comptes rendus Biologies* **325:** 1–14.

Lecompte E, Van der Straeten E, Petter F, Denys C. 1999. Caractéristiques cranio-dentaires de l'holotype de *Praomys* morio (Rodentia, muridae). *Mammalia* **63:** 530–534.

Maddison WP. 1997. Gene trees in species trees. *Systematic Biology* **46:** 523–536.

Maley J. 1987. Fragmentation de la forêt dense humide africaine et extension des biotopes montagnards au quaternaire récent: nouvelles données polliniques et chronologiques. Implications paléoclimatiques et biogéographiques. *Palaeoecology of Africa and the surrounding islands* **18:** 307–334.

Maley J. 1996. The African rain forest – main characteristics of changes in vegetation and climate from the Upper Cretaceous to the Quaternary. *Proceeding of the Royal Society of Edinburgh* **104B:** 31–73.

Maley J. 2001. The impact of arid phases on the African rain forest through geological history. In: Weber W, White L, Vedder A, Naughton-Treves L, eds. *African rain forest ecology and conservation*. New Haven, CT: Yale University Press, 68–87.

Martinez-Solano I, Teixeira J, Buckley D, Garcia-Paris M. 2006. Mitochondrial DNA phylogeography of *Lissotriton boscai* (Caudata, Salamandridae): evidence for old, multiple refugia in an Iberian endemic. *Molecular Ecology* **15:** 3375–3388.

Mayaux P, Bartholome E, Fritz S, Belward A. 2004. A new land-cover map of Africa for the year 2000. *Journal of Biogeography* **31:** 861–877.

deMenocal PB. 2004. African climate change and faunal evolution during the Pliocene-Pleistocene. *Earth and Planetary Science Letters* **220:** 3–24.

Michaux JR, Libois R, Filippucci MG. 2005. So close and so different: comparative phylogeography of two small mammal species, the yellow-necked fieldmouse (*Apodemus flavicollis*) and the woodmouse (*Apodemus sylvaticus*) in the Western Palearctic region. *Heredity* **94:** 52–63.

Misonne X. 1969. African and Indo-Australian Muridae: evolutionary trends. *Annales Musée Royal de l'Afrique Centrale, Tervuren, Belgique, serie IN-8, Sciences Zoologiques* **172:** 1–219.

Moore WS. 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evolution* **49:** 718–726.

Musser GG, Carleton MD. 2005. Superfamily Muroidea. In: Wilson DE, Reeder DM, eds. *Mammal species of the world: a taxonomic and geographic reference*. Baltimore, MD: The Johns Hopkins University Press, 894–1531.

Nicolas V, Quérouil S, Verheyen E, Verheyen W, Mboumba JF, Dillen M, Colyn M. 2006. Mitochondrial phylogeny of African wood mice, genus *Hylomyscus* (Roden-

tia, Muridae): implications for their taxonomy and biogeography. *Molecular Phylogenetics and Evolution* **38:** 779–793.

Nicolas V, Verheyen E, Verheyen W, Hulselmans J, Dillen M, Akpatou B, Dudu A, Wendelen W, Colyn M. 2005. Systematics of African lowland rainforest *Praomys* (Rodentia, Muridae) based on molecular and craniometrical data. *Zoological Journal of the Linnean Society* **145:** 549–553.

Nicolas V, Wendelen W, Barrière P, Dudu A, Colyn M. 2008. Morphometrical variation in *Hylomyscus alleni* and *Hylomyscus stella* (Rodentia, Muridae), and description of a new species. *Journal of Mammalogy* **89:** 222–231.

Pamilo P, Nei M. 1988. Relationships between gene trees and species trees. *Molecular Biology and Evolution* **5:** 568–583.

Platnick NI. 1991. Patterns of biodiversity: tropical vs. temperate. *Journal of Natural History* **25:** 1083–1088.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* **14:** 817–818.

deQueiroz K. 1998. The general lineage concept of species, species criteria, and the process of speciation: a conceptual unification and terminological recommendations. In: Howardand DJ, Berlocher SH, eds. *Endless forms: species and speciation*. Oxford: Oxford University Press, 57–75.

Rambaut A, Drummond AJ. 2007. TRACER v1.4. Available at http://tree.bio.ed.ac.uk/software/tracer

Reeder DAM, Helgen KM, Wilson DE. 2007. Global trends and biases in new mammal species discoveries. *Museum of Texas Tech University* **269:** 1–35.

Ridley M. 2003. *Evolution*. Oxford: Blackwell.

Robbins CB. 1978. The Dahomey Gap. A reevaluation of its significance as a faunal barrier to West African high forest mammals. *Bulletin of the Carnegie Museum of Natural History* **6:** 168–174.

Robbins L, Choate JR, Robbins R. 1980. Nongeographic and interspecific variation in four species of *Hylomyscus* (Rodentia, Muridae) in southern Cameroon. *Annals of the Carnegie Museum* **49:** 31–48.

Robert C, Chamley H. 1987. Cenozoic evolution of continental humidity and paleoenvironment, deduced from kaolinite content of oceanic sediments. *Paleogeography Palaeoclimatology Palaeoecology* **60:** 171–187.

Ronquist F, Huelsenbeck JP. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Ronquist F, Huelsenbeck JP, Van der Mark P. 2005. *MrBayes 3.1 Manual*. Available at http://mrbayes.csit.fsu.edu/mb3.1_manual.pdf.

Rosevear DR. 1969. *The rodents of West Africa*. London: Trustees of the British Museum (Natural History).

Rychlik L, Ramalhinho G, Polly PD. 2006. Response to environmental factors and competition: skull, mandible and tooth shapes in Polish water shrews (*Neomys*, Soricidae, Mammalia). *Journal of Zoological Systematics and Evolutionary Research* **44:** 339–351.

Salzmann U, Hoelzmann P. 2005. The Dahomey gap: an abrupt climatically induced rain forest fragmentation in West Africa during the late Holocene. *Holocene* **15:** 190–199.

Scheffé H. 1959. *The analysis of variance*. New York: Wiley.

Shimodaira H, Hasegawa M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* **16:** 1114–1116.

Slatkin M, Maddison WP. 1989. A cladistic measure of gene flow inferred from the phylogenies of alleles. *Genetics* **123:** 603–613.

StatSoft Inc. 2001. *STATISTICA (data analysis software system), version 6*. Available at www.statsoft.com.

Sullivan F, Joyce P. 2005. Model selection in phylogenetics. *Annual Review of Ecology Evolution and Systematics* **36:** 445–466.

Ubangoh RS, Pacca IG, Nyobe JB. 1998. Palaeomagnetism of the continental sector of the Cameroon Volcanic Line, West Africa. *Geophysical Journal International* **135:** 362–374.

Van der Straeten E, Dieterlen F. 1987. Praomys misonnei, an new species of muridae from eastern Zaïre. *Stuttgarter Beiträge zur Naturkunde, ser A* **402:** 1–11.

Van der Straeten E, Dudu AM. 1990. Systematics and distribution of *Praomys* from the Masoko Forest Reserve (Zaire) with the description of a new species. In: Peters G, Hutterer R, eds. *Vertebrates in the tropics*. Bonn: Museum Alexander Koening, 73–83.

Van der Straeten E, Kerbis Peterhans JC. 1999. *Praomys degraaffi*, a new species of muridae (Mammalia) from central Africa. *South African Journal of Zoology* **34:** 80–90.

Van der Straeten E, Lecompte E, Denys C. 2003. *Praomys* petteri: une nouvelle espèce de Muridae africains (Mammalia, Rodentia). *Bonner Zoologische Beitrage* **4:** 329–345.

Van der Straeten E, Verheyen WN. 1981. Etude biométrique du genre *Praomys* en Côte d'Ivoire. *Bonner Zoologische Beiträge* **32:** 249–264.

Verheyen W, Bracke E. 1966. The influence of aging on the craniometrical characters of Praomys jacksoni (De Winton 1897). *Annales du Museum Royal d'Afrique Centrale* **144:** 91–101.

Verheyen WN, Hulselmans JLJ, Dierckx T, Colyn M, Leirs H, Verheyen E. 2003. A craniometric and genetic approach to the systematics of the genus *Dasymys* Peters, 1875, selection of a neotype and description of three new taxa (Rodentia, Muridae, Africa). *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique* **73:** 27–71.

Volobouev VT, Aniskin VM, Lecompte E, Ducroz JF. 2002. Patterns of karyotype evolution in complexes of sibling species within three genera of African murid rodents inferred from the comparison of cytogenetic and molecular data. *Cytogenetic Genome Research* **96:** 261–275.

Walsh PS, Metzger DA, Higuchi R. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR based typing from forensic material. *Biotechniques* **10:** 506–513.

Westheide W, Hass-Cordes E. 2001. Molecular taxonomy: description of a cryptic *Petitia* species (Polychaeta: Syllidae) from the island of Mahé (Seychelles, Indian Ocean) using RAPD markers and ITS2 sequences. *Journal of Zoological Systematics and Evolutionary Research* **39:** 103–111.

Winnepenninckx B, Backeljau T, De Wachter R. 1993. Extraction of high molecular weight DNA from molluscs. *Trends in Genetics* **9:** 407.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Included specimens of *Praomys misonnei* and *Praomys tullbergi*, with the following institutional abbreviations: FMNH, Field Museum of Natural History, Chicago, USA; MNHN, Museum National d'Histoire Naturelle, Paris, France; RMCA, Royal Museum for Central Africa, Tervuren, Belgium; SMFM, Senckenberg Museum Forschungsinstitut Senckenberg, Frankfurt, Germany; SMNS, Staatlisches Museum für Naturkunde, Stuttgart, Germany. Asterisks: determination of this specimen was confirmed by molecular data, but it was not included in our phylogenetic analysis (only a short sequence was available). Abbreviations: Pm, *P. misonnei*; Pt, *P. tullbergi*; W, West Africa; WC, West Central Africa; CE, Central and East Africa.

Appendix S2. Number of individuals, mean, range, and standard deviation of the 21 craniodental measurements included in the discriminant analysis. Results are given for each operational taxonomic unit (out). For the description of the measurements, see the Material and methods section. For the list of specimens included in each OTU, see Appendix S1.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.