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Phylogeography and predicted distribution of African-Arabian and Malagasy populations of giant mastiff bats, *Otomops* spp. (Chiroptera: Molossidae)

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Otomops martiensseni is sparsely distributed throughout sub-Saharan Africa and southwestern Arabia (Yemen). *Otomops madagascariensis* from the dry portions of Madagascar is widely recognised to be a distinct species. Based on mitochondrial DNA sequences of the cytochrome *b* gene (1,004 base pairs; $n = 50$) and the control region (D-loop, 290 base pairs; $n = 52$), two Oriental outgroup species (*O. wroughtoni* and *O.* cf. *formosus*) formed a monophyletic clade that was the sister group to the Afro-Malagasy taxa, composed of *O. martiensseni* and *O. madagascariensis*. Within the Afro-Malagasy clade, we discovered three well-supported but genetically similar clades (inter-clade genetic distances of 3.4–4.4%) from 1) north-eastern Africa and Arabia, 2) African mainland except northeast Africa, and 3) Madagascar. Taken together, haplotype networks, estimated divergence times, regional species richness and historical demographic data tentatively suggested dispersal from Asia to Africa and Madagascar. To understand ecological determinants of phylogeographic, biogeographic and genetic structure, we assessed the potential distribution of *O. martiensseni* throughout sub-Saharan Africa with ecological niche modelling (MaxEnt) based on known point localities ($n = 60$). The species is predicted to occur mainly in woodlands and forests and in areas of rough topography. Continuity of suitable habitats supported our inferred high levels of continental gene flow (relatively low genetic distances), and suggested that factors other than habitat suitability have resulted in the observed phylogeographic structure (e.g., seasonal mass migrations of insects that might be tracked by these bats). Based on a Bayesian relaxed clock approach and two fossil calibration dates, we estimated that African and Oriental clades diverged at 4.2 Mya, Malagasy and African clades at 1.5 Mya, and African clades 1 and 2 at 1.2 Mya. Integrating phylogenetic, phylogeographic, population genetic and ecological approaches holds promise for a better understanding of biodiversity patterns and evolutionary processes.

Key words: *Otomops*, mitochondrial DNA, phylogeography, Africa, Madagascar, ecological niche modelling

INTRODUCTION

The Palaeotropical genus *Otomops* Thomas, 1913 currently comprises seven species of large-bodied (25–50 g — Mutere, 1973) molossid bats. Five species of Oriental origin have very restricted distributions in southern India, Cambodia, Java, Alor Island (Indonesia), the Philippines, and Papua New Guinea, suggesting an Oriental centre of origin for the genus. The two Afro-Malagasy species are widely but sparsely distributed in Africa and Madagascar (Peterson *et al*., 1995; Simmons, 2005). *Otomops martiensseni* (Matschie, 1897) occurs on the Arabian Peninsula (Yemen) and the African mainland from Ethiopia and Eritrea in the north, to Ivory Coast in the west and Durban, South Africa in the south. *Otomops madagascariensis* Dorst, 1953 is only known from the drier regions of Madagascar

(Goodman *et al*., 2005). These high-flying bats have seldom been captured in mist nets and the vast majority of distributional information derives from day-roost sites. In Madagascar, these sites are typically associated with caves in areas of exposed sedimentary rock. In northeast and east-central Africa, *O. martiensseni* colonies frequently occupy caves in volcanic hills and mountains (such as the lava tunnels on Mt. Suswa in Kenya), largely coinciding with the entire length of the Great African Rift Valley from Eritrea and Djibouti in the north to the Zambezi Escarpment of northern Zimbabwe in the south. The genus is conspicuously absent from the central Congo Basin (with scattered records in Angola, Central African Republic, and the Democratic Republic of the Congo along the periphery of the basin), but is present in low-lying areas of west Africa (Ivory Coast) and the Durban metropolitan region and surroundings on the east coast of South Africa.

Chubb (1917) described *O. icarus* Chubb, 1917 from Durban, South Africa, as a species distinct from *O. martiensseni*. While this view is not currently upheld by most authors (Harrison, 1957; Meester *et al*., 1986; Koopman, 1993; Bronner *et al*., 2003; Simmons, 2005), populations from east Africa (*martiensseni*) and South Africa (*icarus*) have been shown to be distinct in cytochrome *b* and D-loop mitochondrial sequences, albeit at a low sequence divergence (Kimura 2-parameter-corrected) of 2.5% for cytochrome *b* (Lamb *et al*., 2006). However, nuclear markers (PCR-RAPDs) showed very high genetic similarities between individuals from Kenya and South Africa (Lamb *et al*., 2006).

In east Africa, *O. martiensseni* has usually been found in colonies of hundreds to tens of thousands of individuals roosting in caves (Mutere, 1973; Kock *et al*., 2005), where apparent substantial declines in populations have been used to justify the IUCN listing as 'Near Threatened' (Mickleburgh *et al*., 2004; but see Kock *et al*., 2005, for a contrasting view). Around Durban, colonies are smaller and individuals roost in buildings. Elsewhere, for example in central, east, and west Africa, they are known to roost in hollow trees (Decher *et al*., 1997; Fenton *et al*., 2002). In Madagascar, except for one case discussed below, *O. madagascariensis* is unknown from synanthropic settings and most cave colonies are less than 70 individuals (Andriafidison *et al*., 2007; SMG, unpublished data). Roosts in Durban typically comprise up to 30 individuals, one adult male and one to 10+ adult females with young, suggesting a harem social structure (Richardson and Taylor, 1995; Taylor, 1998, 2000; Fenton *et al*., 2002). In South Africa, due to its restricted distribution, *O. martiensseni* has recently been assigned a 'Vulnerable' IUCN rating (Friedmann and Daly, 2004).

Our study has five objectives. The first was to delimit Afro-Malagasy species of *Otomops* based on evidence from the mitochondrial cytochrome *b* and the D-loop regions of samples from 20 localities. Specifically, our first aim is to test the validity and distributional limits of the described forms, *martiensseni*, *icarus*, and *madagascariensis*. Mitochondrial DNA sequences have contributed significantly towards delimiting species and revealing cryptic species diversity in bats. A recent study of the mitochondrial ND1 gene increased the known diversity of western Palaearctic bats from 46 to 54 in an apparently well-studied fauna (Mayer *et al*., 2007). This includes the description of cryptic lineages within five European vespertilionid species found on the Iberian Peninsula (Ibáñez *et al*., 2006) and new species recognized within *Myotis mystacinus* (Kuhl, 1817), *Plecotus austriacus* (Fischer 1829) (Mayer and von Helversen, 2001) and the *Pipistrellus pipistrellus-pygmaeus* complex within the Mediterranean Region (Hulva *et al*., 2004). Similarly, DNA sequences have led to increases in recognized species diversity in numerous New World bats including, for example in the genus *Carollia* Gray, 1838 (Baker and Bradley, 2006).

Our second aim was to compare and contrast genetic structure and demographic history of Afro-Malagasy *Otomops* populations from regions known to have diverse roosting habitats and possibly different breeding systems. To this end we estimated population genetic and demographic population history parameters separately for three genetically defined clades from mainland Africa and Madagascar. Understanding patterns and causes of underlying genetic structure is critical to predicting modes of speciation, past historical events (e.g., bottlenecks which result in reduced heterozygosity), possible evolutionary trajectories of diverging species, and the presence of 'evolutionary significant units' (ESU) or unique gene pools, which merit formal conservation protection (Moritz, 1994; Brown and Houlden, 2000).

Thirdly, we attempted to infer historical patterns of colonization of *Otomops* from Asia, Africa and Madagascar. Since population genetic data can illuminate alternative dispersal hypotheses (Russell *et al.*, 2007), we combined phylogenetic, phylogeographic, and population genetic data to investigate

patterns of colonization of *Otomops* from Madagascar and Africa in the light of available evidence, which suggests a predominant pattern of dispersal from the African mainland to Madagascar but not vice versa (Eger and Mitchell, 2003; Ratrimomanarivo *et al*., 2007; Russell *et al*., 2007).

Fourthly, we used a Bayesian approach, with relaxed clock model, to date nodes using fossil calibrations of earliest *Mops* divergence in the mid-Miocene (11.2–16.4 Mya — McKenna and Bell, 1997; Jones *et al*., 2005) and earliest molossid divergence in Africa (17.5–18.0 Mya — Arroyo-Cabrales *et al*., 2002).

Fifthly and finally, we examined phylogeographic and population genetic patterns in the light of current ecological and biogeographical data. On the one hand, morphological capability of *Otomops* for long distance dispersal (Rydell and Yalden, 1997), and the likelihood that this species is capable of long-distance migration (Kock *et al*., 2005), would lead us to predict the absence of deep phylogeographic structure at continental and local scales. On the other hand, scattered distribution and paucity of collecting records indicates a seemingly rare species complex for which we might hypothesize some degree of geographical isolation and phylogeographic differentiation, yet such a situation may be an artefact of the difficulty of catching these animals or access to roosting sites. Using the MaxEnt program, we modelled the potential distribution of *Otomops* throughout sub-Saharan Africa under current environmental conditions to assess if patterns of phylogeographic structure and potential distribution are concordant. Such integration of ecological and phylogeographic approaches has rarely been attempted previously (but see Rissler *et al*., 2006; Peterson and Nyári, 2007; Raxworthy *et al*., 2007), but should be further explored since current biogeographic patterns are the result of both ecological and historical processes. Our study models only current ecology but it is possible with advanced climatic models to predict past climatic effects such as Pleistocene refugia (Peterson and Nyári, 2007).

Materials and Methods

Samples

Otomops samples were obtained from Yemen, sub-Saharan Africa (Burundi, Ethiopia, Ivory Coast, Kenya, Tanzania, Zimbabwe and South Africa), the drier portions of Madagascar, Cambodia (*O. wroughtoni*) and the Philippines (*O.* cf. *formosus* — Appendix). South African samples included those from residential areas within the Durban metropolitan region and on the adjacent north and south coastlines of eastern South Africa (Ballito, Durban, Pinetown, Silverglen, Scottburgh). For the cytochrome *b* study, the following molossid outgroups were sequenced in our Durban laboratory: *Tadarida fulminans* (Thomas, 1903), *Mops midas* (Sundevall, 1843), *M. condylurus* (A. Smith, 1833) and *M. leucostigma* G. M. Allen, 1918. Sequences for *Pipistrellus abramus* (Temminck, 1838) were taken from the NCBI GenBank (No. NC005436). As D-loop sequences were used for haplotype and population genetic analysis and not analysed phylogenetically, we did not use the specific outgroups.

DNA Sequencing and Analysis

Genetic variation of *Otomops* was investigated using mitochondrial cytochrome *b* (n = 50) and D-loop sequencing (n = 52). *Otomops* DNA was isolated, using a DNeasy® DNA isolation kit (Qiagen), from liver, heart, kidney or muscle tissues previously stored in 80% ethanol or EDTA. Cytochrome *b* trees were further rooted on both molossid (*Tadarida fulminans*, *Mops midas*, *M. condylurus*, *M. leucostigma*) and non-molossid (*Pipistrellus abramus*) outgroups.

PCR amplifications for all samples except *O. wroughtoni* and *O.* cf. *formosus* were performed in the laboratory of JL in South Africa, whilst DNA from the two Asian samples was amplified and sequenced in the laboratory of WB. For the majority of the samples, the cytochrome *b* gene was PCR-amplified (Saiki *et al*., 1988) as two overlapping double-stranded fragments using the following primer pairs: L 14723 (5'-ACC AATGCAATGAAAAATCATCGTT-3') and H 15553 (5'-TAGGCAAATAGGAAATATCATTCTGGT-3'); L15146 (5'-CATGAGGACAAATATCATTCTGAG-3') and H15915 (5'-TCTCCATTTCTGGTTTACAAGAC-3') (Irwin *et al*., 1991). In order obtain a complete sequence, primers L14723 and L46RC (5'-CTCAG AAAG ATATTTG TCCTCATG-3'), as well as H53RC (5'-AC CAGAATGATATTTCCTATTTGCC-TA-3') and H15915, were used to obtain additional data on the first and last ≈400 bp of sequence, respectively. Amplifications with these primers were performed in 25 µl volumes. Each reaction contained 0.8 µl sterile water, 2.5 µl 10 X reaction buffer (Super-Therm), 4 µl 25 mM $MgCl_2$ (Super-Therm), 0.5 µl 10 mM deoxynucleoside-triphosphate mixture (dNTPs) (Roche Diagnostics), 0.2 µl Taq polymerase (5 U/µl) (Super-Therm) and 4 µl of each primer (6 µM) (forward and reverse) per reaction. The thermal cycling parameters used were as follows: 94°C for 4 min; 36 cycles of (94°C for 40 s, 50°C for 45 s and 72°C for 40 s); 72°C for 10 min. Target fragments were purified from excised gel bands using a QIAquick® Gel Extraction Kit (QIAGEN Inc.) and sequenced at Inqababiotec, Pretoria, South Africa.

For the Asian taxa, amplification and sequencing of the entire cytochrome *b* gene was carried out using primers: rhin1L (5'-ATGACATGAAAAATCACCGTTGTA-3') and Nyc2H (5'-GTTTACAAGACCRGKGTAATKDAT-3'). Additional primers Oto420L (5'-CTGAGGAGCAACAGTCATCA-3'), Oto751L (5'-GGAGACCCCGACAATTACAC-3'), Oto1029H (5'-TAC TGGTTGGCCTCCRATTC-3') and Nyc4H (5'-ACDGARAA DCCVCCTCARATTCA-3') were used for sequencing, which was carried out with a CEQ8000 sequencer (Beckman-Coulter).

Amplification of sequences from the Asian taxa was performed in 50µl total volume containing 3–5 µl template, 0.2 µM of each primer, 5× amplification buffer (250 mM KCl, 7.5 mM

$MgCl_2$, 50 mM Tris, pH 8.4), 1 mM of each dNTP [Sigma-Aldrich, Germany]) and 2U REDTaq Polymerase (Sigma-Aldrich, Germany). The thermal cycling parameters used were as follows: 95°C for 2 min; 35 cycles of (94°C for 15 s, 50°C for 20 s and 72°C for 1 min); 72°C for 3 min.

The D-loop regions of all samples were amplified as a single fragment using primers P (5'-TCCTACCATCAGCACCCAAAGC-3') and E (5'-CCTGAAGTAGGAACCAGATG-3') (Wilkinson and Chapman, 1991). Amplifications were performed as described above for cytochrome *b*. The thermal cycling parameters used were as follows: 95°C for 2 min; 40 cycles of (95°C for 60 s, 55°C for 90 s and 72°C for 120 s); 72°C for 7 min. Target fragments were purified from excised gel bands using a QIAquick® Gel Extraction Kit (QIAGEN Inc.) and sequenced at Inqababiotec, Pretoria, South Africa. For the two Asian samples, sequencing, was carried out in a CEQ8000 sequencer (Beckman-Coulter).

All fragments were sequenced in both directions. Sequences were deposited in GenBank (Appendix). Sequences were aligned using the CLUSTAL W option (Thompson *et al*., 1994) of the BioEdit program (Version 5.0.9 for Windows 95/98/NT) and by visual inspection. Sequences were trimmed to a common length of 1,004 nucleotides (nt) for the cytochrome *b* gene and 290 nt for the D-loop. In all analyses, indels were treated as a fifth character, rather than as missing data.

We used MODELTEST 3.7 (Posada and Crandall, 1998) to determine that the cytochrome *b* sequences best fit a model of (GTR+I+G). This model specifies unequal base frequencies and a gamma-distributed mutation rate with shape parameter $\alpha = 1.1064$. The proportion of invariant sites was 0.470. Subsequent analyses were carried out using the assumptions of this model and parameter values specified.

Phylogenetic and Phylogeographic Analysis

We used a Bayesian likelihood analysis to look for phylogenetic signal within our *Otomops* cytochrome *b* sample (MrBayes version 3.0b4; Huelsenbeck and Ronquist, 2001). For each analysis, we ran four Markov chains for 5 million generations each, sampling every 100 chains. The chains were heated with the temperature scaling factor T = 0.02. We discarded the first 25,000 trees as burn-in, and constructed a 50% majority rule consensus tree from the remaining trees. We identified three *Otomops* clades based on the architecture of these trees (see below), and calculated GTR genetic distances between these clades and outgroups in PAUP for the cytochrome *b* data (Swofford, 1998).

In some intraspecific analyses, a hierarchical tree may be inappropriate for representing relationships among haplotypes because the period of time over which the samples have evolved is so short that ancestral and descendant haplotypes exist (Posada and Crandall, 2001; Kratysberg *et al*., 2004). In such instances, a haplotype network is more appropriate to illustrate relationships among the sampled haplotypes by using multiple pathways to reflect possible homoplasy or reverse mutations. Since our D-loop data showed greater variability than for cytochrome *b*, we used this dataset to construct a haplotype network using the TCS computer programme (Clement *et al*., 2000).

Analysis of Molecular Variance

In order to test for significant molecular variance structure between the three Afro-Malagasy clades defined by phylogenetic analyses, the sampled populations were subdivided into groups ('south-east-central-west', $n = 9$ populations; 'north-east', $n = 3$ populations; Madagascar, $n = 4$ populations), and the cytochrome *b* dataset ($n = 1{,}004$ nt) was analysed by hierarchical Analysis of Molecular Variance (AMOVA) using the program Arlequin 3.01 (Excoffier *et al*., 2005). Fixation indices were calculated (for individuals, populations and groups) in a conventional fashion and their significance tested using a non-parametric permutation approach described in Excoffier *et al*. (1992), consisting of permuting haplotypes, individuals or populations, among individuals, populations or groups of populations. After each permutation round, all statistics were recomputed to obtain their null distributions.

Population Genetic Analysis

Because of its higher variability, the D-loop dataset was also used for population genetic analyses; these were performed separately for each of the three genetically-defined groups. Following Rogers and Harpending (1992) and Russell *et al*. (2005), we used haplotype (h) and nucleotide (π) diversity values, neutrality tests (Fu's 1997 F_s and Fu and Li's 1993 D* and F*), and mismatch distribution analysis (distribution of observed pairwise nucleotide differences) to estimate whether each population group was stationary or had undergone an historical population expansion. High h with low π, a unimodal pairwise difference distribution, significant F_s but non-significant D* and F*, and a high ratio of number of variable sites (S) to average number of pairwise differences (d) (S/d), are indicators of an historical population expansion event (Russell *et al*., 2005 and references therein). These analyses were carried out with DnaSP version 4.0 (Rozas *et al*., 2003). Based on the distribution of pairwise nucleotide differences, the time since expansion, tau (τ), could be calculated in mutational units. Although males and females may reach sexual maturity in one year (Mutere, 1973), data from captive bats (E. J. Richardson and W. White, personal communication) indicate an estimated average generation time of approximately two years for the species. Given this generation time and two independent estimates of D-loop mutation rates based on divergence rates per million years of 17.3% ($\mu = 1.73 \times 10^{-7}$ per generation) and 33% ($\mu = 3.3 \times 10^{-7}$ per generation) (Rogers and Harpending, 1992), this approach allowed approximation of the absolute time of expansion, using the formula $\tau = 2u$ t, where u was calculated as the product of the mutation rate (μ: mutations per site per generation) and sequence length (290 bp), and t was the time (in generations) since expansion.

Dating

Our cytochrome *b* data were used to estimate nodal dates. Recent studies have attempted to calibrate molecular dates of deeper (family-level) nodes of the chiropteran phylogeny based on a robust higher phylogeny and compilation of data from earliest fossil occurrences of bats (Jones *et al*., 2005; Teeling *et al*., 2005). Fossil calibrations of bat molecular phylogenies have also been attempted at the intrageneric level, e.g., in *Myotis* where a cytochrome *b* divergence rate of 4% per Myr (mutation rate of 0.02 substitutions/site/Myr) was applied based on the divergence of two species (Ruedi and Mayer, 2001; Hulva *et al*., 2004). In our study, the 'mid-Miocene' fossil date (11.2–16.4 Mya — Böhme, 2003; given as 13.8 Mya by Jones *et al*., 2005) for the first fossil occurrence of *Mops* (McKenna and Bell,

1997) was available to calibrate the earliest cytochrome *b* sequence divergence (13.8%; see Table 1) for the three *Mops* species included in this study (*M. midas*, *M. condylurus* and *M. leucostigma*), giving a divergence rate of 1%. Additionally, using the date of the oldest known African molossid (17.5–18.0 Mya; *Tadarida rusingae* — Arroyo-Cabrales *et al*., 2002) and the deepest divergence between the three African molossid genera sampled in this study (Table 1: 19.0–20.0% obtained between *M. leucostigma* and *O. wroughtoni*), we again obtain a divergence rate of just over 1% per million years (mutation rate of 0.005 subsitutions/site/Myr). If however, one adopts the deepest estimated crown divergence date for all molossids (35–38 Mya; Jones *et al*., 2005), the divergence rate decreases to just over 0.5% (mutation rate 0.0025 substitutions/site/Myr). These two rates (0.0025 and 0.005) are an order of magnitude lower than the 0.036 third codon substitutions/site/Myr for Chiroptera (n = 222 species) estimated by Nabholz *et al*. (2008), probably due to faster-evolving third codon position (neutral) rates, possible saturation in our data and non-correspondence of available fossil dates. The median third codon chiropteran rate of 0.036 obtained by Nabolz *et al*. (2008) is closer to the value of 0.02 obtained for *Myotis*. The latter estimate was based on all substitutions (as in our study); thus, we chose the value of 0.02 as a fixed mean rate for Bayesian dating analyses as described below.

Before using simple models to date nodes based on the molecular clock (as was common until recently), one should test the assumption of a global clock for a given dataset. Using the program PAUP version 4.0b10, and the cytochrome *b* dataset, likelihood scores were calculated for a pruned 13-taxon ML tree (all molossid species and two or three haplotypes for each Afro-Malagasy lineage), in which the strict molecular clock was enforced (-ln L = 3719.00) and not enforced (-ln L = 3704.90). Applying the Likelihood Ratio Test, with 11 degrees of freedom (number of taxa minus two), we obtained a chi-square test statistic of 28.20, which significantly ($P < 0.01$) rejected the molecular clock assumption. To overcome this problem, we used a Bayesian approach with an uncorrelated relaxed clock model assuming that branch-specific rates followed a log-normal distribution, which gives more biologically reasonable results compared to the exponential model (A. Rambaut, personal communication). Bayesian analysis (Markov Chain Monte Carlo [mcmc] algorithm, with length 5,000,000, sampled every 1,000 iterations with burn-in of 100,000) was used to estimate mean and 95% confidence limits of nodal dates assuming the relaxed clock model described above, the GTR+I+G substitution model (based on the results of MODELTEST 3.7) and a mean mutation rate of 0.02 substitutions/site/Myr. The analysis was achieved using the program BEAST v. 1.4 (Drummond and Rambaut, 2006a) in conjunction with the programs BEAUti v.1.4 (Drummond and Rambaut, 2006*b*) and Tracer v. 1.3 (Rambaut and Drummond, 2005).

Ecological Niche Modelling

Georeferenced distribution records (precision of 0.001 decimal degrees) of *O. martiensseni* (n = 60, data set available from JF — Fig. 3) were used to predict its potential distribution across sub-Saharan Africa south of 20°N. To avoid confounding influences due to phylogeny and geographical outliers, both the Arabian Peninsula (Yemen: 1 record) and Madagascar (14 records) were excluded from modelling. We employed the recently developed MaxEnt algorithm (version 2.3; Phillips *et al*., 2006) that has been shown to perform particularly well with presence-only data (Elith *et al*., 2006). Sixteen continuous environmental variables were used as predictors in the final model, including topography (SRTM30: log contrast [mean of maximum-minimum values], calculated with a rectangular 9 * 9 km moving window analysis), climate (WORLDCLIM version 1.4 [Hijmans *et al*., 2005]: mean maximum temperature in April [$T_{max}4$], August [$T_{max}8$], October [$T_{max}10$], December [$T_{max}12$], mean minimum temperature in April [$T_{min}4$], August [$T_{min}8$], mean precipitation in April [Prec4], August [Prec8], December [Prec12], mean annual precipitation [Bio12], precipitation seasonality [Bio15]) and land cover (MODIS Vegetation Continuous Fields [=VCF], collection 3 [Hansen *et al*., 2003]: VCF% tree cover and VCF% bare ground; Africa mosaic SPOT VGT data 2000 [Mayaux *et al*., 2004]: unclassified Near Infra-Red-spectral channel, and unclassified Red-spectral channel). The environmental data were set to a spatial grid resolution of 30 arc seconds (≈ 1 km) and aligned with the digital elevation model (SRMT30) using GIS (ArcView 3.2a with Spatial Analyst 2.0a extension). The MaxEnt model was run with all distribution records (100% training), the regularization multiplier was set to 2.5 to account for statistical over fitting given the relatively large number of predictor variables, and maximum number of iterations was set to 1,000; other MaxEnt settings were kept unchanged. Model performance was assessed with proportion of presences correctly classified (sensitivity), proportion of absences correctly classified (specificity), and discrimination ability (area under the curve [AUC] of a receiver operating characteristic [ROC] plot). Since MaxEnt produces a continuous probability (ranging from 0 to 100% predicted area), we transformed the continuous model output to a map representing probabilities above three pre-defined thresholds: a) 10 percentile training presence, b) equal training sensitivity and specificity, and c) minimum training sensitivity plus specificity. The contribution of each explanatory variable to model performance was evaluated with a jackknife procedure implemented in MaxEnt, where variables are successively omitted and then used in isolation to measure their relative as well as absolute contribution to the model.

Results

Phylogenetic Inference

Bayesian analysis of 1,004 nucleotides of the mitochondrial cytochrome *b* gene yielded a tree in which *Otomops* taxa studied formed a monophyletic clade (posterior probability [pp] 0.75 — Fig. 1). This clade was separated from the molossid outgroups (*Tadarida fulminans*, *Mops midas*, *M. leucostigma* and *M. condylurus*) by an average GTR-corrected genetic distance of 17.8%, and from the vespertilionid outgroup (*Pipistrellus abramus*) by 24.3% (Table 1). Neighbour-joining and maximum parsimony analysis yielded trees with congruent topology (data not shown).

Within the *Otomops* clade, the Oriental species, *O.* cf. *formosus* and *O. wroughtoni*, showed relatively deeper divergence (GTR-corrected cytochrome *b*

distance, 6.6%) than that between lineages within the Afro-Malagasy clade (GTR-corrected cytochrome *b* distance, 3.1 to 4.9%). The average genetic distances between the Afro-Malagasy clade and *O. wroughtoni* and *O.* cf. *formosus* were 11.2% and 10.6%, respectively (see Table 1 for between-groups genetic distances). The Afro-Malagasy *Otomops* clade was subdivided into two reciprocally-monophyletic lineages comprising all samples from Africa plus Yemen (pp 0.96) and Madagascar (pp 1.00), respectively. The mean genetic distance separating African from Malagasy lineages was 4.4%. The African lineage was further split into two well-supported (pp 1.00), reciprocally monophyletic sister-lineages comprising samples from 1) north-east Africa (Ethiopia, Kenya) and Yemen (hereafter, Clade 1) and 2) mainland sub-Saharan Africa (South Africa, Zimbabwe, Tanzania, Burundi, Ivory Coast), excluding north-east Africa and Yemen (Clade 2). A mean genetic distance of 3.4% separated these lineages from each other.

Haplotype Network

The Afro-Malagasy *Otomops* sample of 50 individuals comprised 32 D-loop haplotypes, with a haplotype diversity (*h*) of 0.986 and a nucleotide diversity (π) per site of 0.106. Most haplotypes (21) comprised only one individual, whilst the rest of the haplotypes comprised between two and five individuals. Statistical parsimony analysis of D-loop data yielded partial networks from the three previously mentioned geographical regions when set at the 95% connection limit. A single network including all *Otomops* samples was formed when the connection limit was fixed at 100 mutational steps (Fig. 2). Overall, haplotypes separated into the three groups defined previously by cytochrome *b* data. Thirty-two mutational steps separated South African samples (Pinetown1 and 4) from Madagascar (Toliara14). Clade 2 (Ivory Coast) was connected to Clade 1 (Kenya7) by 30 mutational steps. At this limit, no connection was evident between the Malagasy network and the Clade 1 network. The Oriental taxa appear closer to the Malagasy (51 mutational steps) group than the south-east-central-west Africa (Clade 2) group (57 mutational steps).

Population Genetics

Using D-loop data, genetic structure and demographic histories were examined separately for the three defined groups. Populations from Madagascar

TABLE 1. Net between-groups genetic distances for *Otomops* samples and outgroups based on cytochrome *b* sequences. GTR-corrected distances are represented below the diagonal, and p-distances above the diagonal

	Taxon	1	2	3	4	5	6	7	8	9	10	11
[1]	*Otomops* S-E-C-W Africa		0.016	0.020	0.030	0.100	0.096	0.140	0.142	0.152	0.148	0.199
[2]	*Otomops* Ivory Coast	0.021		0.033	0.047	0.103	0.097	0.144	0.147	0.153	0.149	0.204
[3]	*Otomops* N-E Africa	0.031	0.037		0.034	0.102	0.095	0.148	0.144	0.154	0.153	0.203
[4]	*Otomops madagascariensis*	0.038	0.049	0.044		0.100	0.096	0.149	0.149	0.157	0.151	0.203
[5]	*Otomops wroughtoni*	0.110	0.116	0.111	0.113		0.062	0.156	0.170	0.168	0.169	0.210
[6]	*Otomops* cf. *formosus*	0.105	0.108	0.106	0.105	0.066		0.148	0.164	0.164	0.160	0.210
[7]	*Mops midas*	0.158	0.162	0.169	0.171	0.178	0.168		0.144	0.123	0.118	0.228
[8]	*Tadarida fulminans*	0.161	0.166	0.164	0.170	0.197	0.190	0.163		0.156	0.155	0.222
[9]	*Mops condylurus*	0.173	0.173	0.175	0.180	0.194	0.189	0.138	0.177		0.029	0.241
[10]	*Mops leucostigma*	0.169	0.169	0.175	0.172	0.196	0.183	0.132	0.176	0.030		0.244
[11]	*Pipistrellus abramus*	0.235	0.240	0.241	0.240	0.250	0.250	0.285	0.274	0.295	0.300	

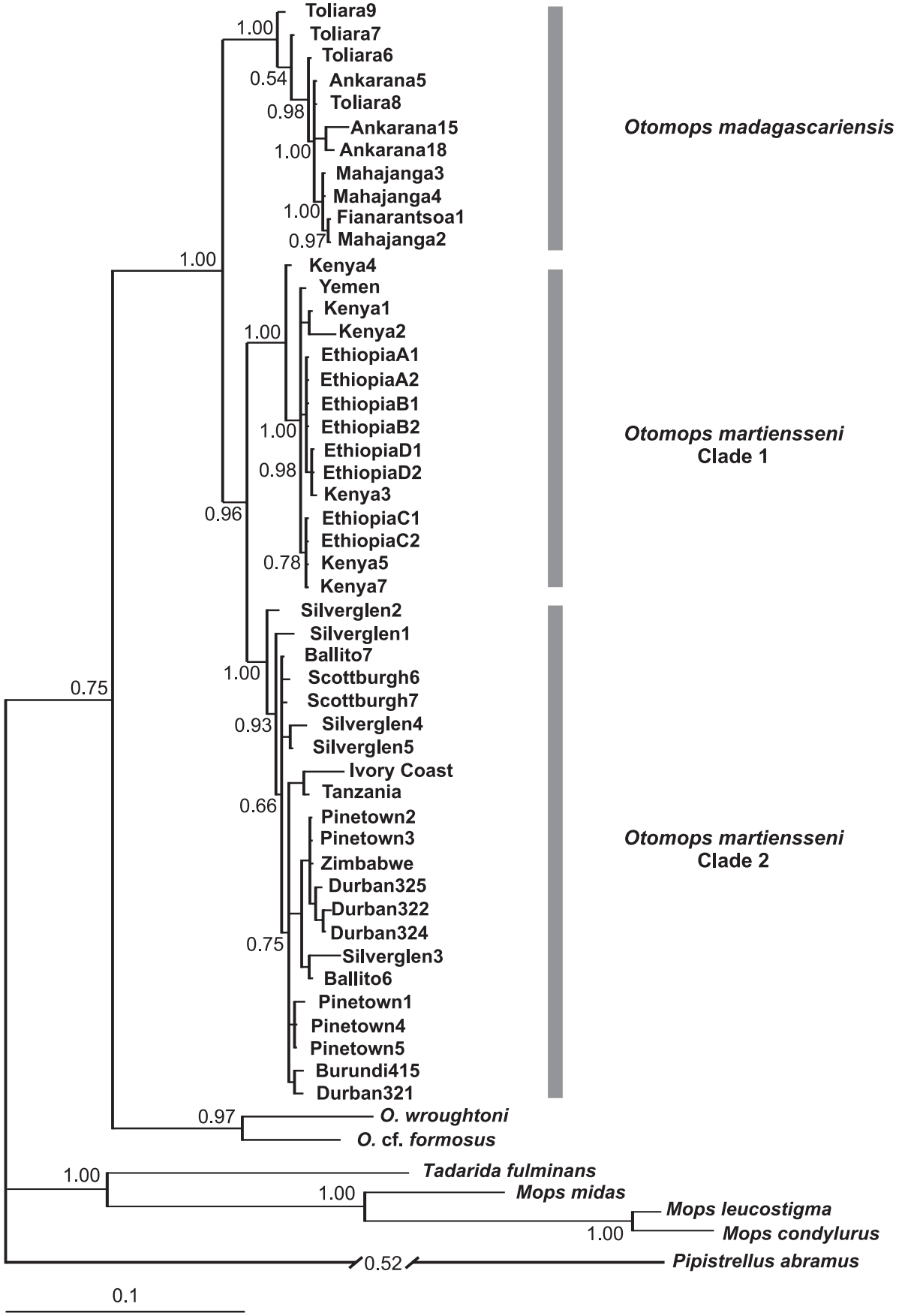

FIG. 1. Bayesian phylogram of cytochrome *b* data (1,004 nucleotides). See Appendix for explanation of locality information

closely fitted the expectations of a model of exponential demographic expansion, e.g., a unimodal mismatch distribution (Fig. 4), low nucleotide diversity but high haplotype diversity, and significant F_S (Table 2). On the other hand, the two mainland groups from Africa and Arabia, whilst meeting certain expectations, showed clearly multimodal mismatch distributions. Using D-loop divergence rates of 17.3% and 33%, we obtained a date for the Madagascar population expansion of between 27,400 and 52,200 yr BP (Table 2).

Analysis of Molecular Variance

AMOVA of cytochrome *b* data revealed significant variance among the three Afro-Malagasy clades (F_{CT} = 0.032; P < 0.01), whilst variance due to populations within groups was not significant at the 1% level, although it was at the 5% level (F_{SC} = 0.06, P = 0.04 — Table 3). Molecular variance was largely dominated by within-population (individual) variance, which explained 91.0% of the total (Table 3). The significant (P < 0.05) variance between populations within groups was a result of the variation in the south-east-central-west clade (Clade 2). In addition, AMOVAs were calculated for each of the groups separately to test for significance of molecular population variance (F_{ST} values). In the case of Madagascar and north-east Africa (Clade 1), F_{ST} values were non-significant while the south-east-central-west clade (Clade 2) was statistically significant at the 5% level (F_{ST} = 0.060, P = 0.03) due to divergence of the Ivory Coast population.

Dating

Based on cytochrome *b*, in spite of the reasonably high confidence limits for the relaxed clock

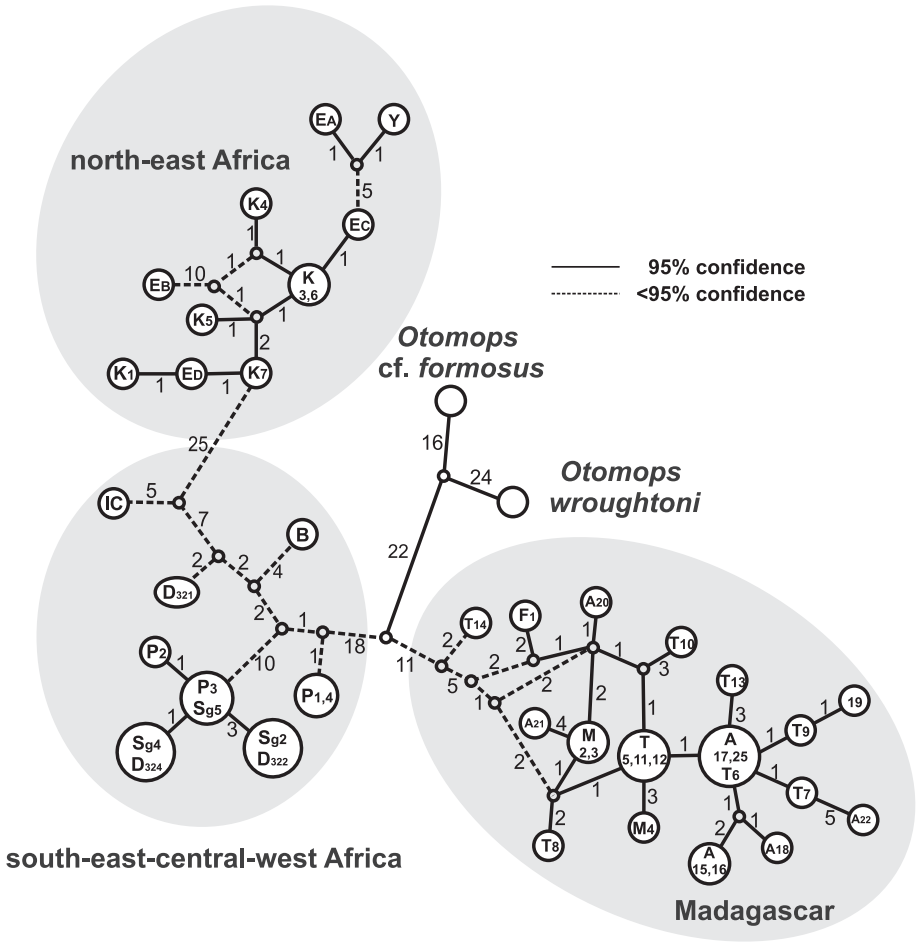

FIG. 2. Statistical parsimony network of D-loop (290 nucleotides) data. Figures next to connections indicate numbers of mutational steps between haplotypes. Locality codes as follows: Y = Yemen, E = Ethiopia, K = Kenya, IC = Ivory Coast, B = Burundi, P = Pinetown, D = Durban, S = Silverglen, A(15–18) = Ankarana, A(20–25) = Antsiranana, F = Fianarantsoa, M = Mahajanga, T = Toliara. See Appendix for further explanation of locality information

TABLE 2. Diversity and neutrality statistics based on 290 nucleotides of the mitochondrial D-loop

Parameter	South-east-central-west Africa	North-east Africa	Madagascar	Expectation#
Nucleotide diversity (π)	0.03856	0.03015	0.01959	Low
Haplotype diversity (h)	0.939	0.952	0.968	High
Expansion coefficient (S/d)	2.90	3.34	5.87	High
Fu and Li's (1993) F*	0.43886	0.72155	-1.55917	NS
Fu and Li's (1993) D*	0.45708	0.91022	-1.29140	NS
Fu's (1997) F_s	0.005	-0.509	-7.523*	Significant
Raggedness (rg)	0.0611	0.0434	0.0144	
Mismatch distribution	Multimodal	Multimodal	Unimodal	Unimodal
Tau (τ)	4.767	4.382	2.621	
Time since expansion (yr BP)			27,390–52,240 yr†	

— Expected trends for a model of demographic population expansion (Hull and Girman, 2005)
* — $P < 0.001$
† — Value obtained from formula $\tau = 2ut$, following Rogers and Harpending (1992), where u was the product of mutation rate (μ) per generation (two rates for the D-loop were used from Rogers and Harpending, 1992: 17.3% divergence per million years, or $\mu = 1.73 \times 10^{-7}$ mutations per site per generation, and 33.0% divergence or 3.3 mutations $\times 10^{-7}$) multiplied by sequence length (290 bp) and t was the time (in generations) since expansion (generation time taken as two years)

model estimates, our data suggest that African and Oriental *Otomops* lineages diverged around 4.2 (2.8–6.1) Myr, Malagasy and African lineages split around 1.5 (0.9–2.2) Myr, whilst the south-east-central-west (Clade 2) and north-east (Clade 1) clades split around 1.2 (0.7–1.8) Myr. The two Oriental species diverged 1.9 (1.1–3.2) Myr (Table 4).

Potential Distribution and Habitat Suitability

The algorithm converged after 620 iterations with a regularized training gain of 1.633. Model performance as assessed by the area under the curve (AUC) was very high (0.927) and thus had a strong discriminatory power. Threshold a) gave a value of 7.553 and resulted in a fractional predicted area of 31.4% (training omission rate: 8.5%), threshold b) had a value of 20.764 and resulted in a fractional predicted area of 16.4% (training omission rate: 16.9%), and threshold c) had a value of 50.888 with a fractional predicted area of 4.5% (training omission rate: 27.1%). The two environmental variables with the highest explanatory power when used in isolation were mean maximum temperature in October (Tmax10) and topographic roughness (log STRM30 contrast), i.e. these variables contained the most useful information by themselves. The environmental variables that decreased the overall model most when omitted were two land cover data sets (SPOT VGT unclassified Red-spectral channel and VCF% tree cover), which therefore had the most information that was not present in the other variables. The MaxEnt model (Fig. 3) predicted a potential distribution for *O. martiensseni* largely coinciding with mesic to humid savannas, woodlands, and forests, particularly in areas of rough topography (e.g., Ethiopian Highlands, Albertine Rift, Eastern Arc Mountains, and volcanoes between Kenya and Tanzania). The rainforest region of the central Congo Basin received moderate to very low suitability scores. According to the model, two regions showed patchy and therefore fragmented habitat suitability:

TABLE 3. Results of Analysis of Molecular Variance (AMOVA). Fixation indices for the combined sample (a) were as follows: $F_{SC} = 0.060$, $F_{ST} = 0.090$, $F_{CT} = 0.032$. Based on 10,100 permutations, all these values were significant ($P < 0.01$). Fixation index for Clade 2 (b) was as follows: $F_{ST} = 0.061$ ($P = 0.03$)

Source of variation	*d.f.*	Sum of squares	Variance components	Variance explained (%)
a) All populations				
Among groups	2	1.672	0.0162 Va	3.2
Among populations within groups	13	6.936	0.0291	5.8
Within populations	32	14.600	0.4566 Vb	91.0
Total	47	23.208	0.5016 Vc	
b) Clade 2 (S-E-C-W Africa)				
Among populations	8	4.309	0.0301 Va	6.0
Within populations	13	6.100	0.4692 Vb	94.0
Total	21	10.409	0.4993 Vc	

1) low to moderate suitability from east Nigeria to southwest Sudan, interrupting predicted ranges in west and east Africa, and 2) moderate suitability between Malawi and north Mozambique in the north and south Mozambique and South Africa in the south, separating predicted ranges in east and southern Africa.

Discussion

Taxonomy of Afro-Malagasy Otomops

Three reciprocally-monophyletic groups are discernable from phylogenetic (Fig. 1) and phylogeographic (Fig. 2) analyses. Results from cytochrome *b* and D-loop sequences are congruent, lending further support to this conclusion. The lineage from Madagascar is referable to *O. madagascariensis*. Morphological divergence (Peterson *et al*., 1995) and geographical isolation provide substantial evidence for recognition of this species. On the other hand, Clade 2 (south-east-central-west Africa) includes localities that are close to the type localities of both *martiensseni* (Magrotto Plantation, west of Tanga, at the foot of the East Usambara Mountains, Tanzania — Matschie, 1897) and *icarus* (Durban, South Africa); hence, *icarus* is the junior synonym of *martiensseni*. If the two African clades represent distinct species, then Clade 1 (north-east Africa + Yemen) would constitute an undescribed taxon. Pending a detailed morphological and morphometric assessment of Afro-Malagasy *Otomops* involving much broader geographic representation (L. Richards, unpublished data), the two African-Arabian lineages are provisionally flagged as evolutionarily significant units (ESU), but are maintained under the name *O. martiensseni*. Although the Clade1-Clade2 divergence of 3.1% (Table 1; excluding comparisons with Ivory Coast) falls below the arbitrary 5% cytochrome *b* divergence threshold for distinct species suggested by Baker and Bradley (2006), it is only slightly below the minimum divergence of 3.3 % reported by them for sister species of bats, based on data from 10 studies, which did not include any test cases within the family Molossidae. There are examples of molossid sister species

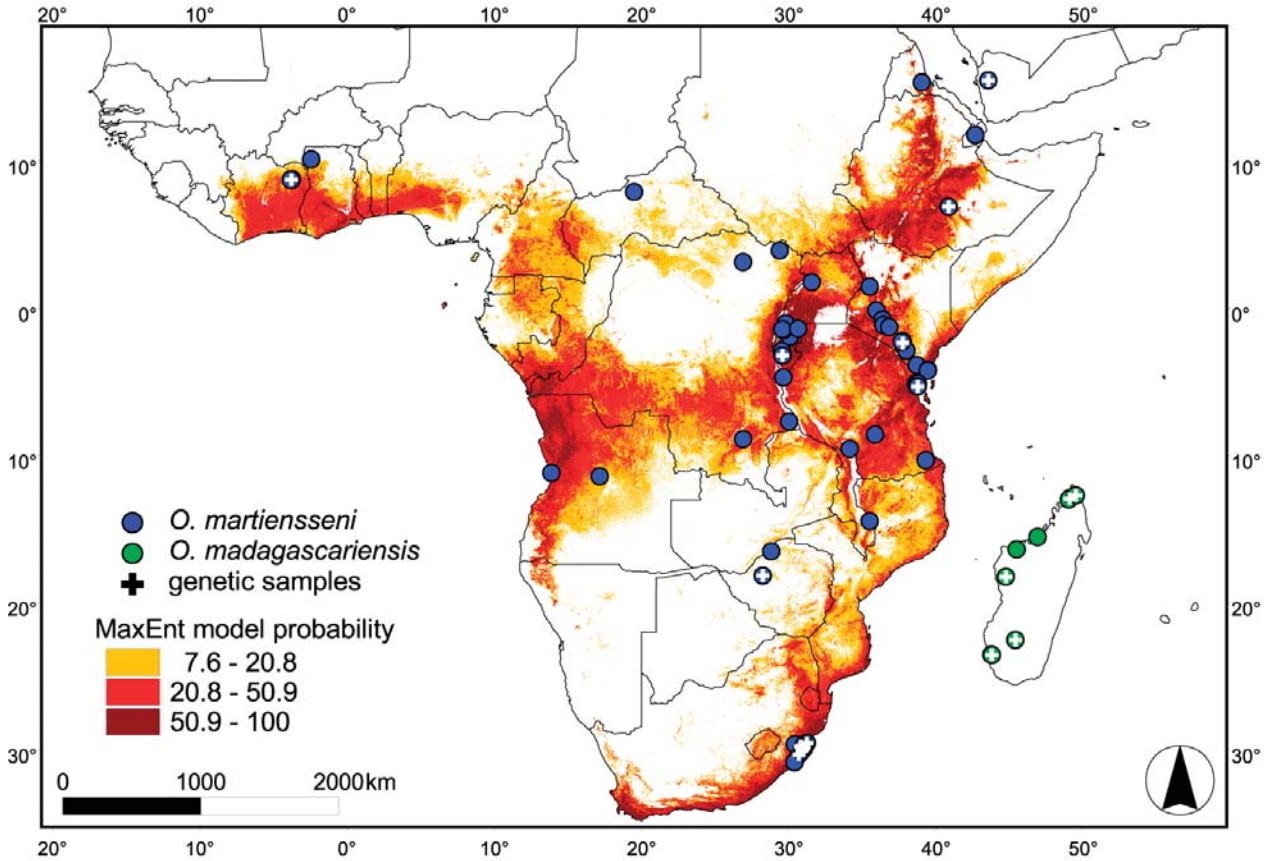

Fig. 3. Modelled potential distribution (MaxEnt) of *Otomops* based on known records of occurrence. Localities sampled for molecular study are indicated with crosses. Note that the Arabian Peninsula and Madagascar were excluded from ecological niche modelling. See text for explanation of different thresholds and distribution probabilities

separated by smaller genetic distances than this 3.3%. For example, the cytochrome *b* genetic distance between *Mops leucostigma* and *M. condylurus* is 2.5% (Ratrimonanarivo *et al*., In press). Other well-defined bat species have also been reported to be genetically very similar, as shown for four pairs of western Palaearctic vespertilionid bat species (< 2.5% of divergence for the mitochondrial ND1 gene, which evolves at a rate similar to cytochrome *b* — Mayer *et al*., 2007).

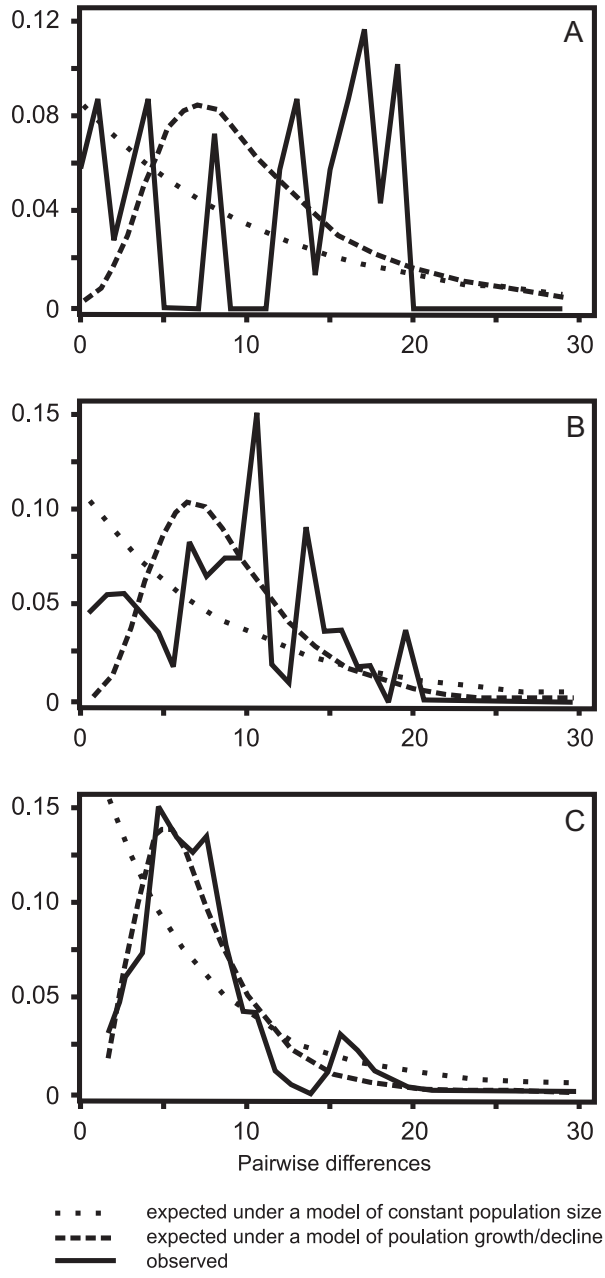

FIG. 4. Mismatch distributions for three phylogenetic groups of *Otomops* based on 1,004 nucleotides of the mitochondrial cytochrome *b* gene (A: south-east-central-west Africa; B: north-east Africa; C: Madagascar)

Phylogeny, Phylogeography and Dispersal of Afro-Malagasy Otomops

Bayesian and other phylogenetic approaches support the monophyly of the four species of *Otomops* studied here, the reciprocal monophyly of the Oriental and Afro-Malagasy species-groups and of the three lineages within the Afro-Malagasy clade. The Oriental species appear to be older, separated by larger genetic distances (cytochrome *b*, GTR-corrected divergence = 6.6%) than the three Afro-Malagasy lineages (3.1–4.4% excluding Ivory Coast, mean 3.8%; Table 1). Although only one west African population (Ivory Coast) was sampled, it is clear that, in spite of its phylogenetic affinity to south-central-east African populations (Clade 2), it is somewhat unique (divergence from other Clade 2 members from south-central-east Africa = 2.1%) and may represent a distinct lineage. However, more data are required to disentangle phylogeographic relationships within the south-east-central-west clade, where genetic sampling of populations from Angola, northern Democratic Republic of the Congo, and the Central African Republic would be particularly critical.

Since the Malagasy lineage is genetically largely equidistant between the south-east-central-west (Clade 1) and north-east (Clade 2) lineages from the mainland, it is difficult to establish the pattern of dispersal and speciation within the Afro-Malagasy clade. If one assumes an Asian origin for the genus (based on higher species richness and older divergences at least for the two species sampled), then the D-loop network indicates a scenario whereby the Oriental ancestor dispersed independently to Madagascar and South Africa, followed by northwards dispersal from South Africa. Another possibility is that Madagascar was occupied first from the Oriental Region, and that one or two subsequent independent colonisations took place, giving rise to the two African clades.

Prevailing easterly (Trade; throughout year) and strong south-easterly (Monsoon; during the austral winter) winds between Madagascar and lesser islands and the African mainland (Jury, 2003) could have aided the dispersal to Africa from Madagascar by fast and high-flying bats such as *Otomops*. Easterly trade winds affect southern Madagascar and southern Africa and could plausibly explain the origin of the south-east-central-west lineage from Madagascar, whilst the strong south-easterly Monsoon winds blow over northern Madagascar in austral winter towards the coast of Ethiopia and

TABLE 4. Estimate absolute ages of nodes (Bayesian analysis – BEAST program) for uncorrelated relaxed clock models with log-normal distribution of branch rates, and fixed mean mutation rate of 0.02 substitution/site/Myr

Node	Relaxed lognormal molecular clock		
	Myr	95% confidence limits	
		Lower	Upper
Otomops	4.20	2.76	6.07
Oriental *Otomops*	1.92	1.06	3.15
Africa + Madagascar	1.51	0.94	2.25
Africa (Clades 1 & 2)	1.16	0.68	1.77
Mops spp.	4.59	2.45	7.00
Tadarida-Mops-Otomops	9.78	6.66	14.70

could account for the origin of the north-east clade.

Recent analyses of the biogeographic affinities of the Malagasy bat fauna indicate that the majority of taxa are of Afrotropical origin (Eger and Mitchell, 2003). More recent work indicates that several species groups colonized the island on different occasions and from different directions. An excellent example can be found in pipistrelloid bats; Bates *et al*. (2006) described a new species of *Pipistrellus* from Madagascar that has close affinity to Oriental species, while other members of this group occurring on the island are of Afrotropical origin. Other genera such as *Emballonura* are rather speciose on western Pacific Ocean islands and southeastern Asia and have their western limit on Madagascar (Goodman *et al*., 2006). Hence, although more limited than from Africa, there are clear examples of bats colonizing Madagascar from Oriental origins, which is in parallel with one scenario presented here for *Otomops*.

Population Genetics and Demographic History

Based on our analyses of the three lineages of *Otomops* occurring in the Afro-Malagasy region, only the lineage from Madagascar shows evidence of exponential demographic expansion. Based on the τ-value obtained from mismatch coefficients, this expansion was estimated to have occurred some 27,000–52,000 years BP. While few data are available on life history traits of *O. madagascariensis*, including dispersal and seasonal movements, a few observations can be made. Bat fossils recovered from breccia deposits in the karst system of Anjohibe (Samonds, 2007), at the northern end of the Mahajanga Province and 400 km from Bemaraha, have been dated to a range from about 80,000–10,000 years ago, using uranium series ($^{230}Th/^{234}U$). A wide assortment of bat taxa was recovered from the deposits, which did not include *O. madagascariensis*, even though it currently inhabits this cave system (Goodman *et al*., 2005). This information can be construed as corroboration of this species' recent colonization of the Anjohibe Cave system. If this is indeed correct, it would support the hypothesis of recent or ongoing range expansion in this species, as found in the genetic data. During the late Quaternary, there was very significant climate change in Madagascar (see Burney *et al*., 2004 and citations therein), which might be associated with range expansion in this taxon, as has been shown for bats in the Holarctic region (Ruedi and Castella, 2003).

Until recently, there was no evidence of *O. madagascariensis* from the central highlands of Madagascar, other than at Isalo at the western edge of this zone or its occurrence in a synanthropic setting. However, in February 2007 a sub-adult female was captured in a building in a residential neighbourhood of Antananarivo. Whether this individual represents a local resident population or a dispersing individual is unknown. However, this record demonstrates that current information on the distribution of *O. madagascariensis* is incomplete and further fieldwork is needed.

Determinants of Phylogeographic and Population Genetic Structure

In general, genetic structure has been mostly documented in bats with low dispersal capabilities (Worthington-Wilmer *et al*., 1994; Burland *et al*., 1999), but not typically in more wide-ranging species (McCracken *et al*., 1994; Webb and Tidemann, 1996; Russell *et al*., 2005). Wing shape and predicted flight capability suggest that *O. martiensseni* is capable of long-range dispersal (Long, 1995; Rydell and Yalden, 1997) and we predicted little or no phylogeographic structure at local or continental scales. However, social factors such as high female philopatry can promote genetic structure in maternally

inherited mtDNA of mobile species such as bats and other mammals (e.g., McCracken, 1987; Chesser, 1991; Miller-Butterworth *et al*., 2003; Ruedi and Castella, 2003). Thus, postulated stable harem formation in Durban colonies (Fenton *et al*., 2002) could encourage female philopatry, leading to structuring at the local level in maternally-inherited mitochondrial sequences but not in biparentally-inherited nuclear genes.

Notwithstanding the fact that some large populations (e.g., from cave colonies in Kenya and Ethiopia) were undersampled, the current data for both mitochondrial sequences of *Otomops* do not show any evidence for strong female philopatry, e.g., clustering of 'matrilines' according to known colonies (e.g., in Durban, east Africa or Madagascar where specific samples are from colonies). Rather, most individuals have unique haplotypes, and haplotypes do not group genetically according to specific colonies. However, more complete sampling of individuals from known colonies, and the use of additional markers such as microsatellites, is clearly required before we can make any conclusions about harem structure.

Little information is available on the group composition of *O. madagascariensis*. In a cave near Antsalova, 15 individuals of this species were captured with a hand net at a single day-roost site, of which 10 (66%) were adult females (SMG, unpublished data). At another nearby site, one roosting colony consisted of 57 pregnant females and five adult males (Andriafidison *et al*., 2007). In a cave near Sarodrano, 11 individuals of this species were captured during the dusk exit, seven (63%) of which were females (SMG, unpublished data). Hence, social groupings may show parallels to the African species discussed above.

What is surprising is the genetic association between the Durban population and geographically widely separated populations from Ivory Coast (5,700 km), Burundi (3,000 km), Tanzania (2,800 km) and Zimbabwe (1,300 km). These genetic data, combined with scattered records of *O. martiensseni* throughout Africa and from Yemen, and the largely uninterrupted habitat suggested by the MaxEnt model (Fig. 3) indicate a population with continuous distribution and/or one capable of large-scale migrations (as suggested by Mutere, 1973; Kock *et al*., 2005 based on observations of seasonal and periodic cave evacuations in large Kenyan breeding colonies). Information on wing morphology and diet reflects the fact that these bats fly high and potentially long distances in search of airborne prey (mostly moths — Fenton and Griffin, 1997; Rydell and Yalden, 1997). It is possible that seasonal migrations of these large, fast and high-flying insectivorous bats may be triggered by documented insect migrations (Pedgley *et al*., 1995), which track movements of the Inter-Tropical Convergence Zone (ITCZ), in much the same way as *Tadarida brasiliensis* (Geoffroy, 1824) has been documented to exploit mass migrations of breeding insects from Mexico to the southern United States (Lee and McCracken, 2005). Supporting such a scenario in Africa, seasonal migrations and mass outbreaks have been well documented in the African armyworm *Spodoptera exempta* (Lepidoptera: Noctuidae — Tucker, 1984, 1994; Wilson and Gatehouse, 1993). The movements of the ITCZ and associated insect biomass from central to southern (and northern) Africa could possibly explain the high degree of relatedness between haplotypes from Burundi, Tanzania, Zimbabwe and South Africa, and their relative genetic distinctiveness from north-east lineage haplotypes, suggesting migratory behaviour or regular long-range dispersal of *O. martiensseni*.

If this is linked to insect migration, we would expect opposite patterns of population movements in the northern and southern hemisphere. Apart from the Ivory Coast sample that is phylogenetically linked to the south-east-central-west lineage, all other samples of this clade come from the southern hemisphere (Burundi, Tanzania, Zimbabwe, South Africa). The north-east lineage (Yemen, Ethiopia, Kenya), on the other hand, is found in the northern hemisphere. If we suppose that the south-east-central lineage is tracking insect phenology and migration with an austral cycle (migrating from the equator southwards) and the north-east lineage is tracking insect phenology with a boreal cycle (migrating from the equator northwards), there might be sufficient geographical isolation leading to the observed phylogeographic pattern, despite the fact that both lineages are found in geographic proximity around the equator. Hockey (2000) presented an illuminating synthesis of intra-African migratory behaviour of birds, showing that it is mainly the insectivorous birds feeding on aerial insects (such as swift, swallows and nightjars) that follow insect phenology from tropical to more temperate latitudes with the onset of the wet seasons. According to this observation, we would expect high-flying bats such as molossids to show a similar pattern.

The potential distribution model did not reveal any apparent gap in habitat suitability between records belonging to the north-east lineage and

south-east-central-west lineage, hence suggesting that factors other than habitat suitability under current climatic conditions have resulted in the observed phylogeographic structure. Possibly, past climatic fluctuations were more important and might have resulted in the phylogeographic structure of continental populations of *O. martiensseni* observed today. Our record from Ivory Coast represents the first for the country and the second for west Africa. Decher *et al*. (1997) speculated that the single specimen from Ghana might have been a disoriented vagrant. However, *O. martiensseni* was regularly captured with elevated mist nets during recent fieldwork in Comoé National Park, Ivory Coast (JF, unpublished data). It is very likely that the lack of records within several regions, which were predicted to offer suitable habitat by the distribution model, is mainly due to inappropriate sampling techniques or a lack of surveys in these regions. According to the distribution model, there is a high probability that *O. martiensseni* should occur in large parts of west Africa (from south-west Ivory Coast to south-central Nigeria), throughout large parts of the Ethiopian Highlands, and throughout a broad region in southern Democratic Republic of the Congo and north-west Angola. The distribution model also predicts a narrow strip of very high habitat suitability along the southeastern coast of South Africa down to the Cape Region. Further fieldwork in these areas is required to validate and refine the actual distribution of *O. martiensseni*, particularly in the light of pronounced phylogeographic structure within its distribution range.

Since phylogeographic structure may result from both current and historical processes, it is important to consider past climates. Past bottlenecks can also influence current genetic structures; in *Myotis myotis* (Borkhausen, 1797) from Europe, genetic structure varied geographically due to the historical effects of the Last Glacial Maximum (Ruedi and Castella, 2003). It is widely acknowledged that Africa underwent a drastic aridification around 1 Mya (deMenocal 2004), which has been invoked to explain vicariance events affecting montane-adapted taxa in the 'Montane Circle' of Africa (e.g., Bowie *et al*., 2006; Carleton *et al*., 2006). Since this date coincides with the estimated date (0.7–1.8 Myr) of the genetic divergence between Clade 1 (which extends along the Albertine or Western Rift and into the Eastern Arc Mountains of Tanzania as far north as the Usambara Massifs) and Clade 2 (from the Kenyan Rift northwards, including the Ethiopian Rift), it is plausible to assume that aridification could have led to population contractions due to low food availability, and restriction of populations occupying distinct mountain ranges associated with the Rift Valley and Eastern Arc Mountains. Indeed, in studies on a montane forest passerine, using molecular data and coalescence approaches, Bowie *et al*. (2006) identified vicariance events separating Albertine Rift from Kenyan Rift populations, and Eastern Arc Mountains from the Kenyan Rift populations. Estimated dates for these events coincided very closely with the range of dates estimated for divergence of Clades 1 and 2 (0.7–1.8 Myr — Table 4).

Why should profound aridification result in vicariance of African Montane Circle populations but not to the same extent between far-flung localities from Durban, Ivory Coast and Tanzania belonging to Clade 1? *Otomops martiensseni* is clearly a species capable of long-range dispersal. Perhaps more importantly, individuals show adaptability in being able to exploit diverse roosting situations, such as tree hollows in lowland rain forest habitats and even man-made sites such as buildings in the metropolis of Durban, South Africa. These life history traits must be important in maintaining gene flow where alternative (non-cavernicolous) roosts were available in abundance. On the other hand, in higher altitude, often treeless mountainous habitats associated with the Rift Valley of east Africa, it is possible that past aridification and/or colder temperatures may have played a vital role in local extinctions of the relative few and sparsely located *Otomops* cave colonies, perhaps forcing them (through low temperatures or low food availability) to move into lower valleys where fewer cave roosts were available.

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Appendix.

Locality and specimen details and Genbank accession numbers of sampled individuals of *Otomops* and outgroups. RSA — Republic of South Africa; DM — Durban Natural Science Museum; FMNH — Field Museum of Natural History, Chicago; HZM — Harrison Zoological Museum, Sevenoaks; NMP — National Museum of the Czech Republic, Prague; NMK — National Museums of Kenya, Nairobi; ROM — Royal Ontario Museum, Toronto; SMF — Senckenberg Museum, Frankfurt a. M.; SMG — Université d'Antananarivo, Département de Biologie Animale, Antananarivo (uncatalogued specimen from SMG); Pb: collectors' numbers of Petr Benda (not accessioned in a museum)

Species	Geographic origin	Co-ordinates	Museum no.	Sample label	Sex	GenBank #	
						Cytochrome *b*	D-loop
Otomops martiensseni s.l.	RSA: Ballito, 20 km N of Durban	29.533°S, 31.211°E	N/A	Ballito6	?	EF216411	
– ” –	– ” –	– ” – – ” –	N/A	Ballito7	?	EF216412	
– ” –	RSA: 473 Silverglen Dve, Chatsworth, Durban	29.928°S, 30.900°E	N/A	Silverglen1	?	EF216406	
– ” –	– ” –	– ” – – ” –	N/A	Silverglen2	?	EF216407	EF216451
– ” –	– ” –	– ” – – ” –	N/A	Silverglen3	?	EF216408	
– ” –	– ” –	– ” – – ” –	N/A	Silverglen4	?	EF216409	EF216452
– ” –	– ” –	– ” – – ” –	N/A	Silverglen5	?	EF216410	EF216453
– ” –	RSA: 31 Ann Arbour Rd, Scottburgh, 25 km S of Durban	30.300°S, 30.745°E	N/A	Scottburgh6	♀	EF216418	
– ” –	– ” –	– ” – – ” –	N/A	Scottburgh7	♂	EF216419	
– ” –	RSA: Ocean View Farm, Park Rynie, 30 km S of Durban	30.339°S, 30.731°E	DM 8031	Durban324	♀	EF216426	EF216446
– ” –	– ” –	– ” – – ” –	DM 8032	Durban323	♂	EF216427	
– ” –	RSA: Brynderyn Flats, Morningside, Durban	29.864°S, 31.040°E	DM 7909	Durban321	♂	EF216424	EF216444
– ” –	RSA: Kingsway School, Amanzimtoti, Durban	30.039°S, 30.894°E	DM 7914	Durban322	♂	EF216425	EF216445
– ” –	RSA: 8 Buys Rd, Pinetown, Durban	29.757°S, 30.639°E	DM 8421	Pinetown1	♀	EF216413	EF216447
– ” –	– ” –	– ” – – ” –	N/A	Pinetown2	♀	EF216414	EF216448
– ” –	– ” –	– ” – – ” –	N/A	Pinetown3	♀	EF216415	EF216449
– ” –	– ” –	– ” – – ” –	N/A	Pinetown4	♀	EF216416	EF216450
– ” –	– ” –	– ” – – ” –	N/A	Pinetown5	♂	EF216417	
– ” –	Ethiopia: S of Omar Caves, 40 km west Ginir, Bale Province	6.90°N, 0.850°E	NMP 91203	EthiopiaA1	♀	EF216429	EF216461
– ” –	– ” –	– ” – – ” –	NMP 91203	EthiopiaA2	?	EF216430	EF216462
– ” –	– ” –	– ” – – ” –	Pb2512	EthiopiaB1	♀	EF216431	EF216463
– ” –	– ” –	– ” – – ” –	Pb2512	EthiopiaB2	?	EF216432	EF216464
– ” –	– ” –	– ” – – ” –	NMP 91202	EthiopiaC1	♀	EF216433	EF216465
– ” –	– ” –	– ” – – ” –	NMP 91202	EthiopiaC2	?	EF216434	EF216466
– ” –	– ” –	– ” – – ” –	NMP 91201	EthiopiaD1	♀	EF216435	EF216467
– ” –	– ” –	– ” – – ” –	NMP 91201	EthiopiaD2	?	EF216436	EF216468
– ” –	Kenya: Ithundu Caves, Chyulu Hills, Kiboko, Makuena District	2.358°S, 7.717°E	NMK 15462	Kenya1	♂	EF216428	EF216455
– ” –	– ” –	– ” – – ” –	NMK 15461	Kenya2	♀	EF216438	

APPENDIX. Continued

Species	Geographic origin	Co-ordinates	Museum no.	Sample label	Sex	GenBank #	
						Cytochrome *b*	D-loop
Otomops martiensseni s.l.	Kenya: Ithundu Caves, Chyulu Hills, Kiboko, Makuena District	2.358°S, 7.717°E	NMK 15464	Kenya3	♀	EF216439	EF216456
– ” –	– ” –	– ” – – ” –	NMK 15463	Kenya4	♂	EF216440	EF216457
– ” –	– ” –	– ” – – ” –	NMK 15459	Kenya5	♂	EF216441	EF216458
– ” –	– ” –	– ” – – ” –	NMK 15465	Kenya6	♂		EF216459
– ” –	– ” –	– ” – – ” –	NMK 15460	Kenya7	♀	EF216442	EF216460
– ” –	Burundi: 2.3 km N, 0.7 km W Teza, Kibira	3.200°S, 0.55°E	FMNH 137633	Burundi415	♀	EF216423	EF216443
– ” –	Zimbabwe: Hostes Nicholle Institute, Sengwa	18.167°S, 28.217°E	ROM 83979	Zimbabwe	♀	EF216421	
– ” –	Yemen: Hud Sawa Cave, Al-Mawheet	15.483°N, 43.533°E	SMF 87650	Yemen	♂?	EF216437	EF216469
– ” –	Ivory Coast: Comoé NP	8.715°N, 3.797°W	SMF 92049	Ivory Coast	♂	EF216420	EF216454
– ” –	Tanzania: Tongwe F.R, Tanga, Muheza District	5.317°S, 38.733°E	SMF 79542	Tanzania	♀	EF216422	
Otomops madagascariensis	Madagascar: Parc National de Bemaraha, Province de Mahajanga	18.245°S, 44.717°E	FMNH 169667	Mahajanga2	♀	EF216373	EF216384
– ” –	– ” –	– ” – – ” –	FMNH 169694	Mahajanga3	♂	EF216374	EF216385
– ” –	– ” –	– ” – – ” –	FMNH 169695	Mahajanga4	♂	EF216375	EF216386
– ” –	Madagascar: Parc National de Isalo, Province de Fianarantsoa	22.540°S, 45.380°E	FMNH 166073	Fianarantsoa1	♀	EF216372	EF216383
– ” –	– ” –	– ” – – ” –	SMG 10996	Fianarantsoa2	♀		EF216401
– ” –	Madagascar: Grotte d’Ambanila, Province de Toliara	23.540°S, 43.746°E	FMNH 172938	Toliara5	♀	EF216376	EF216387
– ” –	– ” –	– ” – – ” –	FMNH 172940	Toliara6	♂	EF216377	EF216388
– ” –	– ” –	– ” – – ” –	FMNH 172934	Toliara7	♂	EF216378	EF216389
– ” –	– ” –	– ” – – ” –	FMNH 172936	Toliara12	♂		EF216394
– ” –	– ” –	– ” – – ” –	FMNH 172942	Toliara13	♂		EF216395
– ” –	Madagascar: Grotte de Bishihiko, Province de Toliara	23.549°S, 43.767°E	FMNH 172944	Toliara8	♀	EF216379	EF216390
– ” –	– ” –	– ” – – ” –	FMNH 172948	Toliara9	♂	EF216380	EF216391
– ” –	– ” –	– ” – – ” –	FMNH 172951	Toliara10	♂		EF216392
– ” –	– ” –	– ” – – ” –	FMNH 172953	Toliara11	♀		EF216393
– ” –	– ” –	– ” – – ” –	FMNH 172947	Toliara14	♂		EF216396
– ” –	Madagascar: Réserve Spéciale d’Ankarana, Province d’Antsiranana	12.942°S, 49.055°E	FMNH 176354	Ankarana15	♀	EF216381	EF216397
– ” –	– ” –	– ” – – ” –	FMNH 176355	Ankarana16	♂		EF216398
– ” –	– ” –	– ” – – ” –	FMNH 176356	Ankarana17	♂		EF216399
– ” –	– ” –	– ” – – ” –	FMNH 176357	Ankarana18	♂	EF216382	EF216400
– ” –	Madagascar: Réserve Spéciale d’Analamerana, Province d’Antsiranana	12.712°S, 49.474°E	FMNH 178849	Antsiranana20	♀		EF216402

APPENDIX. Continued

Species	Geographic origin	Co-ordinates	Museum no.	Sample label	Sex	GenBank #	
						Cytochrome *b*	D-loop
Otomops madagascariensis	Madagascar: Réserve Spéciale d'Analamerana, Province d'Antsiranana	12.712°S, 49.474°E	FMNH 178850	Antsiranana21	♀		EF216403
– ” –	– ” –	– ” – – ” –	FMNH 178851	Antsiranana22	♀		EF216404
– ” –	– ” –	– ” – – ” –	FMNH 178852	Antsiranana25	♂		EF216405
Otomops wroughtoni	Cambodia: Chhep District, Preah Vihear Province	13.59°N, 105°16°E	HZM 3.33440	*Otomops wroughtoni*	♂	EF504251	EF504253
Otomops cf. *formosus*	Philippines: Barangay Balbalasang, Kalinga Province, Luzon Is	17.458°S, 121.0683°E	FMNH 167240	*Otomops* cf. *formosus*	♂	EF504252	EF504254
Tadarida fulminans	Madagascar					EU760911	
Mops midas	Madagascar					EF474049	
Mops leucostigma	Madagascar					EF474029	
Mops condylurus	KwaZulu-Natal, South Africa					EF474030	
Pipistrellus abramus						NC005436	