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Morphologically uniform bats *Hipposideros* aff. *ruber* (Hipposideridae) exhibit high mitochondrial genetic diversity in southeastern Senegal

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Two mitochondrial lineages of bats that are morphologically attributed to *Hipposideros ruber* have been shown to occur sympatrically in southeastern Senegal. We studied genetic diversity in these bats in the Niokolo Koba National Park using sequences of mitochondrial cytochrome *b* gene to determine the taxonomic status of the two genetic forms, and included skull morphology for comparison. Detailed multidimensional analysis of skull measurements indicated slight morphological differences between the two genetic forms. Exploration of peak frequency of the constant-frequency echolocation signals in a local population of *Hipposideros* aff. *ruber* was not available for both groups. Phylogenetic comparison with other available West African representatives of *H*. aff. *ruber* revealed paraphyletic relationship of the two Senegalese forms, with the less abundant form from Senegal forming a monophyletic group with that from Benin. Based on genetic divergence and sympatric occurrence, the two forms from Senegal might represent cryptic species. However, absence of nuclear gene flow between them is yet to be investigated to demonstrate their reproductive isolation.

Key words: cytochrome *b*, *Hipposideros caffer* complex, cryptic species, phylogeny

INTRODUCTION

Genetic differences in living organisms may precede phenotypic differences, making genetically distinct forms difficult or even impossible to detect by traditional morphological means (Yoder *et al.*, 2000; Jacobs *et al.*, 2006). Such forms are believed to belong to the same species until additional evidence shows that they represent independent evolutionary units. The existence of these so-called cryptic species (Mayr, 1996; Lincoln *et al.*, 1998) has been revealed for many taxonomic groups (Avise, 2004; Bickford *et al.*, 2007). Although careful examination of morphology, ecology or behaviour helps to discover cryptic species, molecular genetics has contributed enormously in the last two decades to discovering cryptic forms within traditionally recognised taxa (Avise, 2004; Bickford *et al.*, 2007).

The presence of cryptic species is a rather common phenomenon in bats, and molecular data play an important role in their recognition and formal systematic acknowledgement (Jones, 1997; Mayer and von Helversen, 2001; Baker and Bradley, 2006; Ibáñez *et al.*, 2006). Differences in call characteristics of echolocation signals have been likewise useful to recognize distinct forms deserving taxonomic recognition. The European vespertilionid *Pipistrellus pipistrellus*/*P. pygmaeus* complex is a classic example where differences in peak frequencies of search calls led to discovery of two distinct sonotypes, which have been subsequently confirmed by molecular methods to represent two species (Barratt *et al.*, 1997).

The genus *Hipposideros* Gray, 1831 represents the most speciose group within the Palaeotropical family of Old World leaf-nosed bats (Hipposideridae). It contains a high number of phonic types, some of which have been subsequently confirmed as new cryptic species by molecular analyses, e.g., *H. khakhouayensis* (Guillén-Servent and Francis, 2006) and *H. khasiana* (Thabah *et al.*, 2006) from Southeast Asia. Distinct phonic types exist also within African species of *Hipposideros*, although none or only limited molecular justification appeared to date to confirm their distinct taxonomic statuses. The first is the case of phonic types of African *H. commersoni* (E. Geoffroy, 1813), which were revealed by Pye (1972) and which have been recently raised to species rank as *H. gigas* (Wagner, 1845) and *H. vittatus* (Peters, 1852) (Simmons, 2005). The second example is the complex of forms pertaining to *H. caffer* (Sundevall, 1846). Currently recognised species of this complex, *H. caffer* and *H. ruber* (Noack, 1893), are generally assumed to differ in peak frequencies of their echolocation calls (Pye, 1972; Fenton, 1986; Heller, 1992; Jones *et al.*, 1993). However, the first study of the *H. caffer* complex using molecular genetic tools (Vallo *et al.*, 2008) showed that this group is composed of more than two evolutionary units that might represent separate species. As the metric characters traditionally used to distinguish between *H. caffer* and *H. ruber* overlap, importance of echolocation frequencies for taxonomy should be re-evaluated in relation to molecular phylogeny. Differences in echolocation parameters can be good indicators of cryptic species. Particularly with respect to social communication between conspecific individuals, differences in echolocation appear to be more valid for closely related *Hipposideros* bats than the concept of adaptation to feeding on hearing insects (Barratt *et al.*, 1997; Jones *et al*., 1997; Bogdanowicz *et al.*, 1999; Guillén-Servent *et al*., 2000; Sedlock and Weyandt, 2009).

Our field work in the Niokolo Koba National Park (NKNP), Senegal, yielded a large number of leaf-nosed bats tentatively identified as *H. ruber*. These bats represent a distinct phylogenetic lineage restricted to West Africa, ranging from Senegal to Benin ('lineage D' of Vallo *et al.*, 2008), and are probably not closely related to *H. ruber* s. str., which was described from Tanzania. Although samples of *Hipposderos* aff. *ruber* bats from NKNP showed no obvious morphological differences, in the subsample used by Vallo *et al.* (2008), one haplotype significantly differed from the main mitochondrial lineage of the Senegalese population. This interesting intrapopulational pattern inspired us to investigate phylogenetic relationships in *H.* aff. *ruber* from Senegal using sequences of the mitochondrial gene for cytochrome *b* (cyt*b*). We studied genetic diversity of populations in the NKNP to evaluate the relevance of two sympatric mitochondrial lineages for the systematics of the *H. caffer* complex. We further analysed sequences of male-specific nuclear zinc finger region on the Y chromosome (*zfy* — Page *et al.*, 1987) in the two mitochondrial lineages to obtain independent evidence for reproductive separation. Additionally, we used skull morphometrics in relation to patterns revealed on the genetic level. As the divergent haplotype within Senegalese *H.* aff. *ruber* came from the village of Dar Salam at the northwestern border of the NKNP, we also explored echolocation calls in the local population to determine whether calls differ in correspondence to genetic lineages. We hypothesised that possible differences in echolocation frequencies and morphology would support presence of cryptic species suggested by genetic divergence.

Materials and Methods

Sampling

Bats were netted at six localities in the NKNP, SE Senegal, between 2004 and 2007 (Fig. 1 and Appendix). Captured specimens were weighted and external measurements were recorded. A subset of 52 specimens was collected and preserved in ethanol for further study. Tissue samples (spleen) were taken from collected specimens. Skulls were extracted from ethanol-preserved vouchers for morphological analysis. All biological material including voucher specimens was deposited at the Institute of Vertebrate Biology of the Academy of Sciences, Brno, Czech Republic. For comparison, we included additional samples and GenBank sequences of West African *H.* aff. *ruber* from Benin, Ghana, and Ivory Coast (Lim *et al.*, 2007; Vallo *et al.*, 2008), and another hipposiderid bat, *Asellia tridens*, as an outgroup (Benda and Vallo, 2009 — see Appendix).

Morphological Analysis

One external and 15 skull dimensions of collected specimens were measured following Benda and Vallo (2009) and using mechanical callipers with a precision of 0.02 mm: LAt = forearm length, LCr = greatest length of skull including premaxillae, LOc = occipito-canine length, LCc = condylo-canine length, LaZ = zygomatic width, LaI = width of interorbital constriction, LaInf = rostral width between foramina infraorbitalia, LaN = neurocranium width, LaM = mastoid width, ANc = height of neurocranium, LBT = largest horizontal length of tympanic bulla, CC = width across upper canines at crowns, M^3M^3 = width across third upper molars, CM^3 = length of upper tooth-row from front of canine to back of third molar, LMd = condylar length of mandible, ACo = height of coronoid

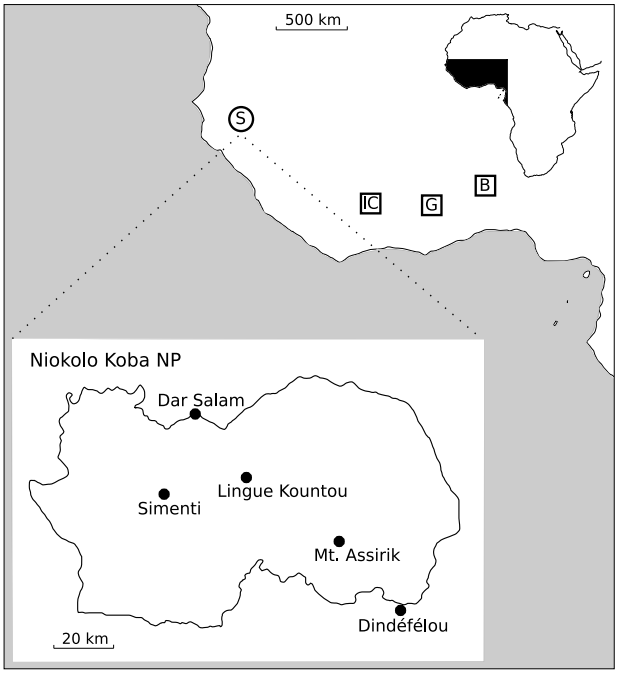

FIG. 1. Distribution of localities sampled in Niokolo Koba NP, southeastern Senegal, and of all West African populations sampled; Senegal (circle; S), and Ivory Coast, Ghana, and Benin (squares; IC, G, B, respectively)

process, CM_3 = length of lower tooth-row from front of canine to back of third molar. Analysis of variance and canonical discriminant analysis using raw data values were employed to reveal morphological differences in our dataset. Statistical analyses were performed using Statistica 6.0 software (StatSoft, Tulsa, OK, USA).

Analysis of Echolocation Calls

Echolocation calls of individuals captured at Dar Salam were recorded in time expansion (10×) and heterodyne mode with a bat-detector Pettersson D240x (Pettersson Elektronik AB, Uppsala, Sweden; sampling rate 307 kHz) with built-in microphone linked to a Sony MiniDisc MZ. Sound recordings were saved in uncompressed digital format (.wav). Animals were flown individually in a volary made of mosquito net (2 × 2 × 2 m). Echolocation calls were recorded when bats were flying towards the microphone only, thus compensation for the Doppler effect should have the same rate in the small constant-sized volary. Peak frequency of the constant frequency (CF) component of calls with maximum energy derived from power spectra was extracted from time-expanded (10×) sequences of approx. 1 s (FFT size 512 samples, Hanning window) with Bat-Sound 3.31 software (Pettersson Elektronik AB). We measured peak frequencies of four consecutive, randomly selected calls from the sonograms of each individual and took their average as the final value.

DNA Processing

Total genomic DNA was extracted from ethanol-preserved tissue with DNeasy Tissue Kit (Qiagen, Halden, Germany) according to the manufacturer's protocol. Complete cyt*b* gene was amplified using universal primers L14724 and H15915 (Irwin *et al.*, 1991). Each PCR reaction contained 0.8 μM of each primer, 0.2 mM dNTP, 1U of HotMaster Taq DNA polymerase with 5 μl of corresponding 10× buffer (Eppendorf, Hamburg, Germany), and 2–5 μl of extracted DNA in 50 μl volume. Initial denaturation at 94°C for 3 min was followed by 35 cycles of denaturation for 40 s at 94°C, annealing for 40 s at 50°C, and extension for 90 s at 65°C, with final extension at 65°C for 5 min. Partial sequences of *zfy* gene were amplified using primers 33X5YF and LGL 331 (Trujillo *et al.*, 2009) and the same PCR protocol. The resulting PCR products were purified with QIAquick PCR Purification Kit (Qiagen) and sequenced commercially (Macrogen, Seoul, Korea) with the same primers using Big-Dye Terminator sequencing chemistry (Applied Biosystems, Foster City, CA, USA) on ABI 3730xl sequencer. Sequences were assembled and edited in Sequencher 4.6 (Gene Codes, Ann Arbor, MI, USA). Sequences were submitted to GenBank with accession numbers HQ343240–HQ343266 (Appendix).

Phylogenetic Analysis

Sequences of *Hipposideros* aff. *ruber* from Senegal were aligned in BioEdit 7.0 (Hall, 1999). Polymorphism within the Senegalese sequence dataset was assessed using DnaSP 4.0 (Rozas *et al.*, 2003) and a median-joining network of cyt*b* sequences was constructed in Network 4.2 (Fluxus Technology, Clare, UK). Based on this initial analysis, the cyt*b* dataset was reduced to haplotypes representing main haplogroups to facilitate reconstruction of phylogenetic trees and additional sequences including the outgroup were added for subsequent analyses (Appendix).

Phylogenetic trees were reconstructed in PAUP* 4.10b (Sinauer Associates, Sunderland, MA, USA) using maximum parsimony (MP) and maximum likelihood (ML) methods. In both methods, tree space was heuristically searched with tree bisection-reconnection swapping algorithm on 100 random sequence additions. Hasegawa-Kishino-Yano evolutionary model with gamma-distributed among-site rate variation (HKY85 + Γ — Hasegawa *et al.*, 1985; Yang, 1996) was used in ML analysis. This five-parameter model was suggested by the program Modeltest 3.7 (Posada and Crandall, 1998) as the 3rd best model under the Akaike Information Criterion (AIC), and was chosen in preference to two more complex models with eight parameters in order to reduce variance in parameter estimates. Reliability of branching pattern was assessed by bootstrapping using 1000 replicates in both analyses. Phylogeny was further estimated using Bayesian inference in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) with the same model. We used two independent simultaneous Metropolis-coupled MCMC runs of four chains running for 10^6 generations, sampled every 100th generation, starting from random trees. The first 2,500 sampled trees were discarded as burn-in. A 50% majority rule consensus tree was constructed from remaining trees with posterior probabilities representing confidence estimates of topology. Templeton test (Templeton, 1983) and Shimodaira-Hasegawa test (SH test — Shimodaira and Hasegawa, 1999) with RELL re-sampling algorithm and 1,000 bootstrap replicates were used to compare tree topologies. Sequence divergences were based on pairwise Kimura two-parameter genetic distances (K2P — Kimura, 1980).

Results

In the Senegalese dataset, 29 haplotypes of cyt*b* (1,140 bp) were identified among 52 analysed individuals, resulting in high haplotype diversity (H = 0.966, SD = 0.01). As most haplotypes were closely related, nucleotide diversity was low (π = 0.009, SD = 0.002). Median-joining network showed diversification into two groups within Senegalese *H.* aff. *ruber*, denoted here with respect to the original lineage D by Vallo *et al.* (2008) as D1 and D2 (Fig. 2). Group D1 comprised 27 haplotypes representing 47 specimens. Group D2 was formed by two haplotypes from five specimens. Groups D1 and D2 differed by 2.4–3.6% K2P-distance. Genetic structure was not related to distribution of sampling sites, and bats of groups D1 and D2 occurred syntopically at Dar Salam, Simenti and Lingue Kountou (Appendix). Sequences of *zfy* (1,316 bp) from four specimens of group D1 and from two specimens of group D2 were identical.

We recorded echolocation calls of 16 specimens from Dar Salam. Variation in peak frequencies within the four measured consecutive calls of individual bats was very low (around 0.1 kHz). Given the low intra-individual variation in peak frequency and similar recording conditions in the constant-sized volary leading to similar recording bias (i.e., equal compensation for the Doppler effect), we compared average peak frequencies among individuals. Peak frequencies ranged from 130 kHz to 139 kHz (Appendix). Genetic results showed that all of the recorded specimens belonged to group D1, hence precluding a comparison with group D2 as originally expected. Frequencies were related to sex, as males called at significantly higher frequencies (134–139 kHz, n = 8) than females (130–135 kHz, n = 8 — Mann-Whitney test, U = 4, P < 0.01).

Univariate analysis of forearm length and skull morphometrics did not reveal distinct morphotypes. Measurements of the two haplogroups D1 and D2 mostly overlapped (Table 1); they differed only in CM^3 (ANOVA, F = 5.81, *d.f.* = 48, P < 0.05) and slightly in ANc (ANOVA, F = 3.78, *d.f.* = 48, P = 0.058). Males and females of groups D1 and D2 did not differ in size. Canonical discriminant analysis of skull dimension revealed LCo, LCc, LaZ, LaI, CC, ANc, CM^3 and CM_3 as the most important variables distinguishing between groups D1 and D2 along the 1st canonical axis (CV1), which explained 71.0% of variance (Fig. 3).

Phylogenetic relationships were reconstructed among 11 cyt*b* sequences of *H.* aff. *ruber*: six from Senegal (four from haplogroup D1 and two from haplogroup D2), two from Ivory Coast, two from Ghana, and one from Benin. Heuristic search under MP criterion yielded a single best tree 269 steps long (Fig. 4) showing five well-supported (bootstrap support ≥ 70%, Bayesian posterior probability ≥ 0.95) phylogenetic lineages, which corresponded to the respective geographic origin of samples, and included the two distinct haplogroups from Senegal: Senegal D1, Senegal D2, Benin, Ghana, and Ivory Coast. Genetic divergences among the five lineages ranged 2.0–5.4% (Table 2). Divergences within the lineages except those from Senegal were small (up to 0.1%). Monophyletic relationship was supported between lineages Senegal D2 and Benin, and between lineages Ivory Coast and Ghana, respectively. Senegal D1 was placed as sister lineage to a clade

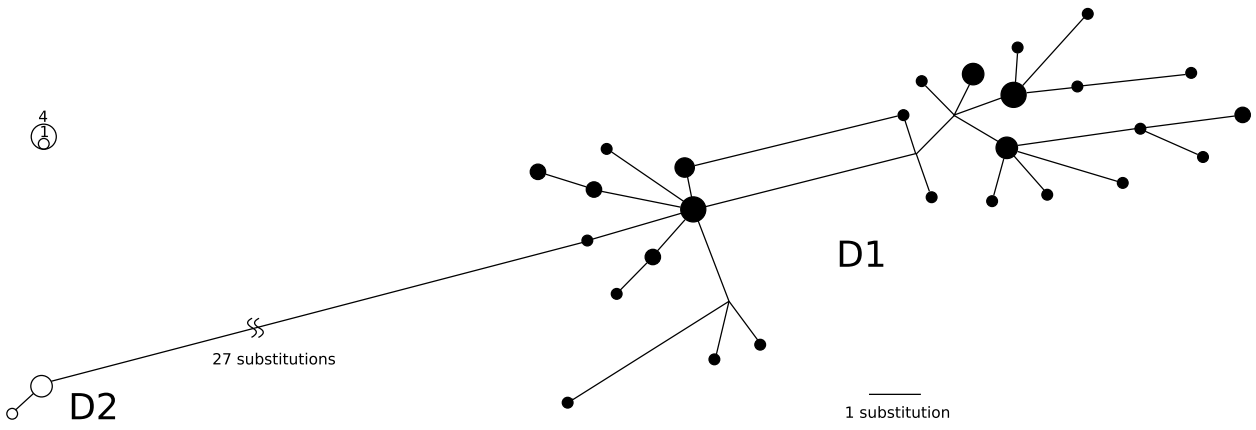

Fig. 2. Median-joining network of 29 haplotypes found in the population of NKNP. Size of nodes is proportional to frequency of particular haplotypes. Black and white circles denote haplogroups D1 and D2, respectively, and this scheme is consistent with Figs. 3 and 4

TABLE 1. Forearm length and skull measurements (in mm) of the examined specimens. See Materials and Methods for abbreviations of measurements

Measurements	Haplogroup D1					Haplogroup D2				
	n	$\bar{x}$	Min	Max	SD	*n*	$\bar{x}$	Min	Max	SD
LAt	47	47.88	44.20	49.80	1.100	4	47.15	46.30	48.30	0.780
LCr	45	18.92	18.27	19.62	0.298	5	18.77	18.28	19.11	0.338
LOc	45	18.68	17.78	19.23	0.294	5	18.56	18.14	18.86	0.264
LCc	45	16.34	15.83	16.83	0.238	5	16.23	16.03	16.37	0.149
LaZ	45	10.51	10.02	10.86	0.222	5	10.57	10.28	10.75	0.195
LaI	45	2.88	2.59	3.09	0.120	5	2.82	2.60	2.98	0.143
LaInf	45	5.07	4.81	5.42	0.114	5	5.06	4.98	5.18	0.088
LaN	45	8.31	7.75	8.68	0.176	5	8.27	7.85	8.73	0.370
LaM	45	9.89	9.52	10.28	0.154	5	9.80	9.69	9.97	0.121
ANc	45	5.85	5.29	6.38	0.223	5	6.07	5.80	6.67	0.353
CC	45	4.98	4.73	5.24	0.134	5	4.93	4.75	5.06	0.138
M^3M^3	45	7.15	6.68	7.42	0.159	5	7.11	6.82	7.25	0.168
CM^3	45	7.08	6.78	7.29	0.123	5	6.95	6.84	7.05	0.076
LMd	45	12.38	11.82	12.74	0.197	5	12.41	12.31	12.51	0.091
ACo	45	3.04	2.74	3.31	0.142	5	3.03	2.93	3.23	0.117
CM_3	45	7.65	7.37	7.93	0.138	5	7.62	7.55	7.68	0.048
LBT	45	3.36	3.07	3.55	0.092	5	3.34	3.28	3.48	0.080

Senegal D2 + Benin, but the statistical support for this group was low. ML tree (-ln*L* = 2741.72205) and Bayesian consensus tree showed the same supported groups as the MP tree: Senegal D2 + Benin and Ivory Coast + Ghana, and their supported sister relationship. However, they also revealed a rather unclear pattern of paraphyletic haplotypes belonging to the group Senegal D1 placed basally in the tree. Thus, both monophyly of Senegal D1 and its phylogenetic position considering the monophyly were tested using Templeton and SH tests. Constrained ML tree with forced monophyly of Senegal D1 (-ln*L* = 2743.04876) did not differ significantly from the original ML tree (Templeton test: diff. length = 4, z = -1.1547, P = 0.2482; SH test: diff. -ln*L* = 1.32671, P = 0.168) and monophyly of Senegal D1 thus could not be rejected. Position of other lineages in the constrained tree was otherwise

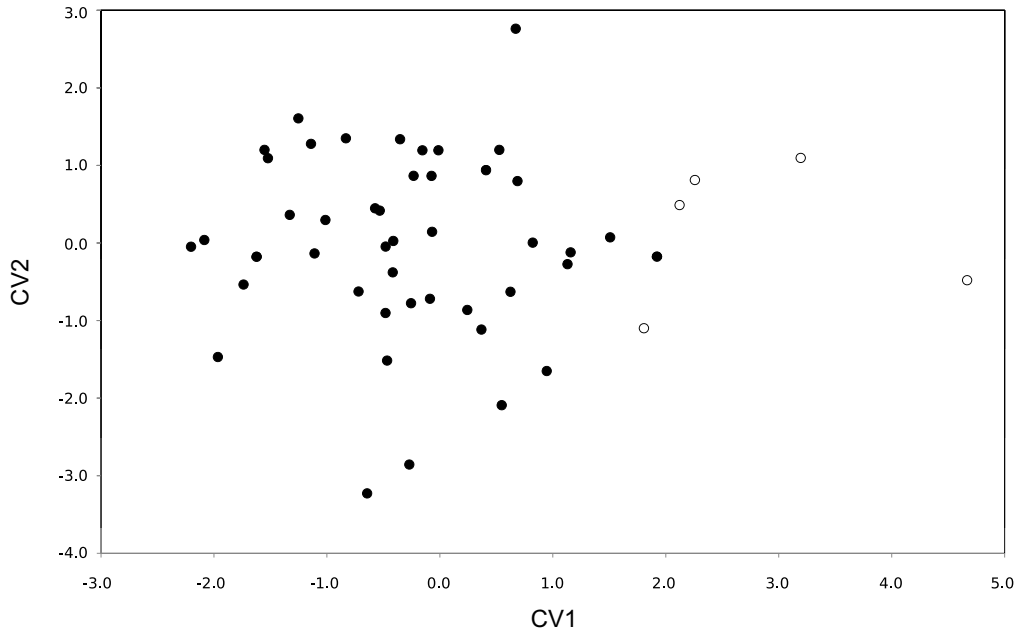

FIG. 3. Plot of main canonical variables CV1 and CV2 from canonical discriminant analysis of 15 skull measurements. Symbols as in Fig. 2

identical to the original ML tree. Although ML analysis favoured sister relationship between Senegal D2 + Benin and Ivory Coast + Ghana (73% bootstrap support) in contrast to MP analysis that pointed towards sister relationship of Senegal D1 to Senegal D2 + Benin (67% bootstrap support), these two phylogenetic hypotheses did not differ from each other significantly (Templeton test: diff. length = 3, z = -0.9045, P = 0.37; SH test: diff. -ln$L$ = 0.71613, P = 0.29). Consequently, mutual positions of the lineages Senegal D1, Senegal D2 + Benin and Ivory Coast + Ghana remained unresolved.

TABLE 2. Pairwise genetic divergences among phylogenetic lineages of West African *H.* aff. *ruber*. K2P — Kimura two-parameter distance (%)

K2P	Senegal D2	Senegal D1	Benin	Ivory Coast
Senegal D1	2.6–3.6			
Benin	2.0–2.1	3.2–4.1		
Ivory Coast	5.1–5.2	4.0–4.3	5.3–5.4	
Ghana	4.4–4.7	4.5–5.1	5.2–5.3	2.8–3.0

DISCUSSION

Analysis of mitochondrial DNA sequences of *H.* aff. *ruber* revealed two distinct genetic lineages with sequence divergence of 2.4–3.6% co-occurring in the Niokolo Koba NP, southeastern Senegal. Only five specimens from our collection belonged to lineage D2. Haplotypes of both lineages were present at three localities in the NKNP. Absence of the lineage D2 at two other localities may have been due to low sample size, although habitat preferences or competition between the two lineages may also be a relevant explanation. As previous results indicated existence of both lineages at Dar Salam (Vallo *et al.*, 2008), we compared echolocation calls in the local population. The differences in call frequencies might indicate presence of cryptic species (Jones, 1997; Mayer and von Helversen, 2001). Our results showed peak echolocation frequencies of CF calls to range 130–139 kHz, which is fairly similar to the combined range of cryptic forms of *H. bicolor* (128.0–144.5 kHz — Kingston *et al.*, 2001) and *H. ridley* (65–72 kHz — Francis *et al.*, 1999). However, genetic analysis revealed that all 16 individuals, in which we successfully recorded and analysed echolocation frequencies, belonged to the more abundant lineage D1. Therefore, the original hypothesis of congruence between echolocation and genetic diversity in Senegalese *H.* aff. *ruber* could

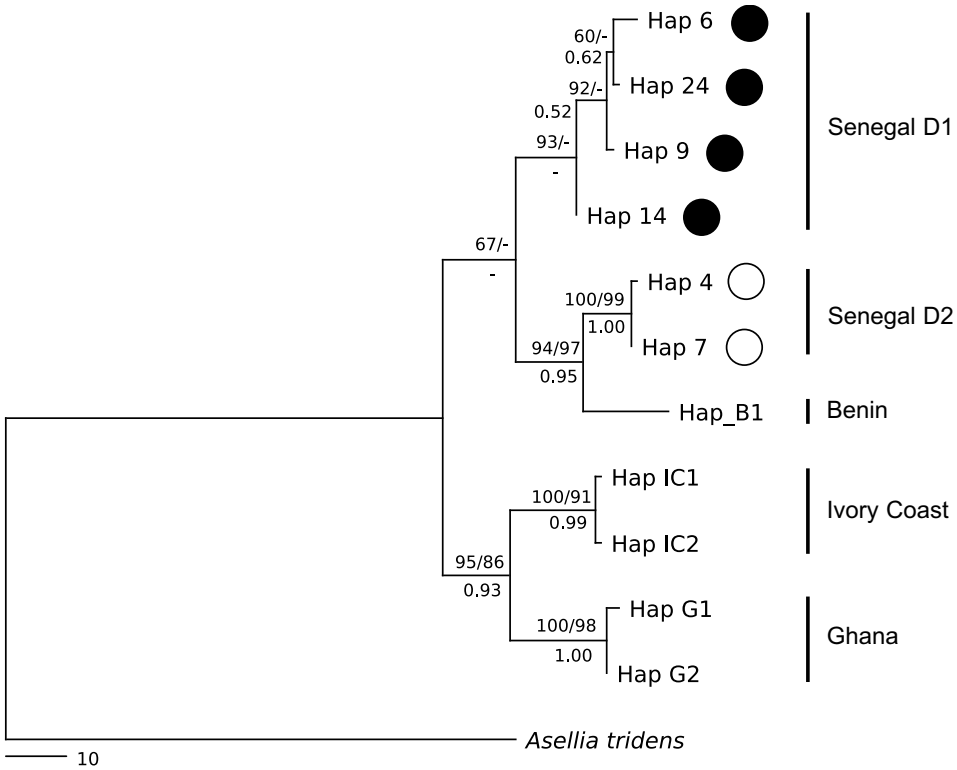

FIG. 4. Maximum parsimony tree depicting phylogenetic relationships in West African *H.* aff. *ruber*. Bootstrap support for MP and ML ≥ 70% (above branches) and posterior probabilities of BA ≥ 0.95 (below braches) are considered significant. Symbols as in Fig. 2

not be tested. Distribution of echolocation frequencies within lineage D1 was related to sex, with males calling at significantly higher frequencies than females. Similar relationship of echolocation frequency and sex has been shown in *H. ruber* from Equatorial Guinea (Guillén-Servent *et al.*, 2000). The frequency range of 130–139 kHz found in the bat population from Dar Salam should be regarded as intraspecific variation.

Morphological analysis showed only slight differences in skull dimensions. Overall, bats of the two lineages remain indistinguishable by morphological traits that are commonly used for species identification. Although the canonical discriminant analysis showed rather sufficient differences for separation of the lineages in some (mainly rostral) dimensions, the ranges of measured values overlap to such an extent that competent determination of taxa is not possible.

Given the values of sequence divergence and sympatric occurrence, the two lineages from Senegal could be considered separate species based on known limits of divergence between cryptic species (Baker and Bradley, 2006). Similar values of interspecific divergence (2.4–3.9%) have been found between cryptic forms of *Scotophilus dinganii* (A. Smith, 1833) from South Africa (Jacobs *et al.*, 2006). Furthermore, similar divergence (3.9–4.1%) was documented between two *Hipposideros* species from Southeast Asia, *H. khakhouayensis* and *H. rotalis* (Guillén-Servent and Francis, 2006). Sequence divergence values alone, however, have to be interpreted with caution when deciding on an appropriate taxonomic rank of genetically separate populations. Lausen *et al.* (2008) clearly showed that divergence values in mitochondrial sequences led to false classification of lineages within North American *Myotis lucifugus* as distinct species, as they discovered substantial nuclear gene flow among these lineages although mitochondrial sequence divergence ranged up to 5%. Similar mitochondrial differentiation not reflected in nuclear markers was found in *Myotis capaccinii* (Bilgin *et al.*, 2008). It is therefore important to document reproductive isolation of the two genetically distinct forms in Senegal to confirm their status as two species. Our use of a paternally inherited *zfy* gene as an independent phylogenetic marker to maternally inherited cyt*b* gene (Lim *et al.*, 2008; Trujillo *et al.*, 2009) did not provide a conclusive answer because all obtained *zfy* sequences were identical. On one hand, this uniformity may indicate extensive, male-mediated gene flow between haplogroups D1 and D2. On the other hand, it could also result from low mutation rate of this nuclear gene and a relatively recent split of both lineages. More informative estimation of relationships between the two Senegalese mitochondrial forms could be achieved using analysis of microsatellites, as this would detect gene flow between both lineages and thus provide necessary data for a taxonomic conclusion.

The basal node of phylogenetic tree remained unresolved suggesting a rapid radiation of the three basal lineages Senegal D1, Senegal D2 + Benin, and Ghana + Ivory Coast. Moreover, a sister relationship of the two Senegalese lineages was not supported and the lineage D2 turned out to be closely related to the lineage from Benin. According to this phylogenetic structure and considering geographical distribution of the lineages, the sympatric occurrence of the two lineages from Senegal, D1 and D2, may be explained as a secondary contact of two formerly isolated populations. The genetic distance, reaching over 5% among the sampled West African populations, further suggests that even lineages from Ghana and Ivory Coast might be regarded as cryptic species. Unlike the Senegalese lineages D1 and D2, these populations do not occur in sympatry and the genetic differences could be explained by the isolation by distance. On the other hand, conflict between geographic distance between sampled localities in Ghana and Benin (ca. 300 km), where the corresponding divergence exceeds 5%, and Senegal and Benin (ca. 2,000 km), with divergence only 2%, indicate a rather more complicated relationship. A broader sampling throughout West Africa would thus be needed to better understand the indicated phylogeographic pattern and resolve the question of cryptic diversity in *H.* aff. *ruber*.

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APPENDIX

List of specimens included in this paper. Echo — average peak frequency of CF echolocation call, Acc. number — accession number in the GenBank database

Sample	Haplotype	Haplogroup	Echo (kHz)	Country	Locality	Acc. number	Source of sequence
IVB S8	Hap_1	D1	–	Senegal	Mt. Assirik	HQ343240	This study
IVB S95	Hap_9	D1	–	Senegal	Simenti	–	This study
IVB S112	Hap_2	D1	–	Senegal	Lingué Kountou	HQ343241	This study
IVB S119	Hap_3	D1	–	Senegal	Lingué Kountou	EU934478	Vallo *et al*. (2008)
IVB S139	Hap_6	D1	–	Senegal	Lingué Kountou	HQ343243	This study
IVB S218	Hap_8	D1	–	Senegal	Simenti	HQ343244	This study
IVB S253	Hap_6	D1	–	Senegal	Simenti	HQ343245	This study
IVB S272	Hap_9	D1	–	Senegal	Simenti	EU934481	Vallo *et al*. (2008)
IVB S273	Hap_10	D1	–	Senegal	Simenti	EU934482	Vallo *et al*. (2008)
IVB S275	Hap_11	D1	–	Senegal	Simenti	EU934483	Vallo *et al*. (2008)
IVB S278	Hap_5	D1	–	Senegal	Simenti	HQ343246	This study
IVB S280	Hap_12	D1	–	Senegal	Simenti	HQ343247	This study
IVB S283	Hap_13	D1	–	Senegal	Simenti	HQ343249	This study
IVB S285	Hap_14	D1	–	Senegal	Simenti	EU934484	Vallo *et al*. (2008)
IVB S290	Hap_15	D1	–	Senegal	Simenti	HQ343250	This study
IVB S291	Hap_11	D1	–	Senegal	Simenti	–	This study
IVB S341	Hap_16	D1	–	Senegal	Simenti	HQ343251	This study
IVB S342	Hap_9	D1	–	Senegal	Simenti	–	This study
IVB S362	Hap_17	D1	–	Senegal	Simenti	HQ343252	This study
IVB S695	Hap_18	D1	–	Senegal	Dar Salam	HQ343253	This study
IVB S701	Hap_14	D1	–	Senegal	Dar Salam	–	This study
IVB S702	Hap_19	D1	–	Senegal	Dar Salam	HQ343254	This study
IVB S803	Hap_5	D1	–	Senegal	Lingué Kountou	–	This study
IVB S819	Hap_3	D1	–	Senegal	Dindéfélou	–	This study
IVB S820	Hap_20	D1	–	Senegal	Dindéfélou	EU934485	Vallo *et al*. (2008)
IVB S821	Hap_21	D1	–	Senegal	Dindéfélou	HQ343255	This study
IVB S825	Hap_20	D1	–	Senegal	Dindéfélou	–	This study
IVB S899	Hap_5	D1	–	Senegal	Dar Salam	–	This study
IVB S900	Hap_22	D1	–	Senegal	Dar Salam	HQ343256	This study
IVB S1374	Hap_5	D1	–	Senegal	Dar Salam	EU934479	Vallo *et al*. (2008)
IVB S1377	Hap_27	D1	–	Senegal	Dar Salam	HQ343260	This study
IVB S1538	Hap_28	D1	138	Senegal	Dar Salam	HQ343261	This study
IVB S1539	Hap_11	D1	137	Senegal	Dar Salam	–	This study
IVB S1540	Hap_9	D1	134	Senegal	Dar Salam	–	This study
IVB S1541	Hap_14	D1	139	Senegal	Dar Salam	–	This study
IVB S1551	Hap_23	D1	132	Senegal	Dar Salam	HQ343262	This study

APPENDIX. Continued

Sample	Haplotype	Haplogroup	Echo (kHz)	Country	Locality	Acc. number	Source of sequence
IVB S1554	Hap_23	D1	136	Senegal	Dar Salam	–	This study
IVB S1555	Hap_24	D1	137	Senegal	Dar Salam	HQ343257	This study
IVB S1561	Hap_29	D1	133	Senegal	Dar Salam	HQ343263	This study
IVB S1654	Hap_24	D1	133	Senegal	Dar Salam	–	This study
IVB S1655	Hap_24	D1	136	Senegal	Dar Salam	–	This study
IVB S1657	Hap_25	D1	134	Senegal	Dar Salam	HQ343258	This study
IVB S1660	Hap_11	D1	131	Senegal	Dar Salam	–	This study
IVB S1662	Hap_5	D1	131	Senegal	Dar Salam	–	This study
IVB S1663	Hap_14	D1	135	Senegal	Dar Salam	–	This study
IVB S1664	Hap_14	D1	135	Senegal	Dar Salam	–	This study
IVB S1665	Hap_26	D1	130	Senegal	Dar Salam	HQ343259	This study
IVB S132	Hap_4	D2	–	Senegal	Lingué Kountou	HQ343242	This study
IVB S281	Hap_7	D2	–	Senegal	Simenti	HQ343248	This study
IVB S286	Hap_7	D2	–	Senegal	Simenti	–	This study
IVB S403	Hap_7	D2	–	Senegal	Dar Salam	–	This study
IVB S1400	Hap_7	D2	–	Senegal	Dar Salam	EU934480	Vallo *et al*. (2008)
NMP 91879	Hap_B1	–	–	Benin	Tagayé	EU934476	Vallo *et al*. (2008)
ROM 100516	Hap_IC1	–	–	Ivory Coast	Sibabli	EF584226	Lim *et al*. (2008)
ROM 100518	Hap_IC2	–	–	Ivory Coast	Sibabli	HQ343264	This study
IVB PV59	Hap_G1	–	–	Ghana	Buoyem	HQ343265	This study
IVB PV56	Hap_G2	–	–	Ghana	Buoyem	HQ343266	This study
	Asellia tridens	–	–	Egypt	–	FJ457617	Benda and Vallo (2009)