

MOLECULAR PHYLOGENETICS OF THE BAT GENUS *SCOTOPHILUS* (CHIROPTERA: VESPERTILIONIDAE): PERSPECTIVES FROM PATERNALLY AND MATERNALLY INHERITED GENOMES

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The genus *Scotophilus* is composed of 15 recognized species with 7 species distributed throughout sub-Saharan Africa, 4 distributed across southern and southeastern Asia, 3 endemic to Madagascar, and 1 endemic to Reunion Island. *Scotophilus* is plagued with problems in species definition, and systematic relationships among members of the genus are poorly understood. We used mitochondrial DNA (mtDNA) and Y-chromosome sequence data from 11 of the 15 recognized species, which represent the most comprehensive taxonomic coverage to date, to examine phylogenetic patterns within *Scotophilus*. All trees have *S. kuhlii* from Asia as the most basal species followed by *S. nux* from Africa. However, *S. heathii* from Asia is embedded within the other African *Scotophilus*, indicating a complex biogeography with multiple continental exchanges. Furthermore, the Malagasy taxa are most closely related to 2 different African species, suggesting independent colonizations of Madagascar from the continental mainland. In addition, African *S. dinganii* did not comprise a monophyletic group but exhibited at least 2 additional cryptic species based on high levels of genetic divergence in the cytochrome-*b* gene. The large-bodied *S. nigrita* is closely related to *S. dinganii* with a similar mtDNA haplotype but distinct *zfy* haplotype, suggesting a possible hybridization event in the most recent common ancestor that potentially represents a mitochondrial capture. Overall measures of interspecific genetic distances ranged from 4.2% to 19.2% for mtDNA data and 0.18% to 2.14% for Y-chromosome data, indicating that members of the genus *Scotophilus* are highly divergent from one another.

Key words: Chiroptera, cytochrome *b*, *Scotophilus*, Vespertilionidae, zinc finger Y (*zfy*)

Scotophilus occurs throughout sub-Saharan Africa, parts of southern and southeastern Asia, a majority of the Indomalayan Islands, Reunion Island, and Madagascar. The genus is in the family Vespertilionidae, subfamily Vespertilioninae, and tribe Scotophilini (Hofer and Van Den Bussche 2003). The type specimen of the genus (*S. kuhlii*) was 1st described by Leach (1821) based on a single immature specimen. However, the holotype still retained its deciduous milk teeth, which prompted debate on the validity of the genus. Nonetheless,

earlier zoologists were applying the name *Scotophilus* to almost every bat in the family Vespertilionidae with fewer than 38 teeth, resulting in taxonomic confusion (Dobson 1875).

Currently, 15 species of *Scotophilus* are recognized in the literature. Simmons (2005) recognized 12 species, including *S. borbonicus* (E. Geoffroy, 1803), *S. celebensis* Sody, 1928, *S. collinus* Sody, 1936, *S. dinganii* (A. Smith, 1833), *S. heathii* (Horsfield, 1831), *S. kuhlii* Leach, 1821, *S. leucogaster* (Cretzschmar, 1830), *S. nigrita* (Schreber, 1774), *S. nucella* Robbins, 1983, *S. nux* Thomas, 1904, *S. robustus* Milne-Edwards, 1881, and *S. viridis* (Peters, 1852). Grubb et al. (1998) recognized *S. nigritellus* de Winton, 1899, as distinct from *S. viridis* based on Koopman's (1984) treatment of this

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species and its uncertain taxonomic status. In addition, 2 Malagasy species have been recently described: *S. tandrefana* Goodman et al., 2005, and *S. marovaza* Goodman et al., 2006. Seven species occur in sub-Saharan Africa (*S. dinganii*, *S. leucogaster*, *S. nigrita*, *S. nigrnellus*, *S. nucella*, *S. nux*, and *S. viridis*), 3 are endemic to Madagascar (*S. robustus*, *S. tandrefana*, and *S. marovaza*), 1 is endemic to Reunion Island and is potentially extinct (*S. borbonicus*), 1 is endemic to the Indonesian island of Sulawesi (*S. celebensis*), 2 occur throughout India and Southeast Asia (*S. heathii* and *S. kuhlii*), and 1 occurs on the Indonesian islands of Java and Bali (*S. collinus*). Of the African species, *S. leucogaster* is the only one that has been recorded outside the continent, from extreme southwestern Saudi Arabia (Gaucher 1993) and Yemen (Al-Safadi 1991).

Members of *Scotophilus* have been shown to comprise a monophyletic group based on several lines of evidence. Several morphological features are diagnostic for the genus, including a poorly developed molar cusp pattern (Rosevear 1965), a single pair of large upper incisors with a dental formula of i 1/3, c 1/1, p 1/2, m 3/3, total 30 (Dobson 1875), and several distinct features of the baculum (Hill and Harrison 1987). Furthermore, Hill and Harrison (1987) concluded that *Scotophilus* and *Scotomanes* possess several bacular similarities and are sufficiently distinct from all other vespertilionines to warrant tribal status (Scotophilini). However, a recent study of family-wide vespertilionid phylogenetics by Hofer and Van Den Bussche (2003) based on mitochondrial 12S ribosomal RNA (rRNA), transfer RNA-valine (tRNA^{Val}), and 16S rRNA sequences did not find any support for a close relationship between *Scotomanes* and *Scotophilus*. They recovered a monophyletic *Scotophilus* and the level of divergence from other vespertilionines was sufficient to recognize it as the sole member of the tribe Scotophilini. However, their study did not specifically examine relationships within the genus because only 7 of 15 species were used and these were only represented by single specimens.

The objectives of our study were to infer a molecular phylogeny for the genus *Scotophilus* based on cytochrome-*b* and nuclear zinc finger Y (*zfy*) sequence data, investigate the level of concordance between a mitochondrial and a Y-chromosome data set, examine phylogeographic patterns in *Scotophilus*, and investigate the taxonomy and systematics of the genus.

MATERIALS AND METHODS

Specimens examined.—In this study, 137 specimens were examined representing 11 currently recognized species of *Scotophilus* from 10 countries and more than 30 geographic localities (Appendix I). The total number of specimens of each species is as follows: 56 *S. dinganii*, 20 *S. viridis*, 8 *S. nigrnellus*, 9 *S. nux*, 2 *S. leucogaster*, 32 *S. kuhlii*, 4 *S. heathii*, 2 *S. robustus*, 1 *S. tandrefana*, 2 *S. marovaza*, and 1 *S. nigrita*. All specimens were included in the cytochrome-*b* data set. The *zfy* gene is located on the Y chromosome and is only found in

males. Of the 137 specimens examined, 68 were males and of these 49 were included in the *zfy* data set, representing 8 currently recognized species of *Scotophilus*. The remaining 19 male samples either did not amplify or the sequencing reaction was unsuccessful. In the combined data set, 49 specimens were included, representing 8 currently recognized species of *Scotophilus*. Outgroup taxa include *Myotis welwitschii* and *Eptesicus serotinus* for the cytochrome-*b* data set, *E. serotinus* and *Myotis tricolor* for the Y-chromosome data set, and *E. serotinus* for the combined data set. Outgroup taxa were chosen because they represent bats of the family Vespertilionidae that share close relationships to *Scotophilus*, which has been shown to be monophyletic (Hofer and Van Den Bussche 2003), and because sequences or tissues were readily available. All animal-handling protocols were in accordance with guidelines of the American Society of Mammalogists (Gannon et al. 2007).

Data collection.—Tissue samples used for genetic analysis included heart, kidney, or liver, or a combination of these. Total genomic DNA was extracted by established protocols (Maniatis et al. 1982). An approximately 1,260-base pair (bp) segment of the mitochondrial DNA (mtDNA) was amplified via the polymerase chain reaction (Saiki et al. 1988) utilizing primers LGL 765F and LGL 766R that amplify the entire cytochrome-*b* gene (Bickham et al. 1995, 2004). To isolate the Y-chromosome segment of interest, approximately 2,200 bp of DNA was amplified with primers 33X5YF (5'-GCA GCA GCT TAT GGT AAG TGA-3') and LGL 331 (Cathey et al. 1998). Amplifications were conducted on a GeneAmp PCR System 2700 thermal cycler (Applied Biosystems, Foster City, California) as follows: a hot start of 3 min at 95°C followed by 32 cycles of 95°C for 45 s of denaturing, 50°C for 30 s of annealing, and 70°C for 2.5 min of extension, with a final extension of 70°C for 5 min. Amplification reactions consisted of the following: 0.1–0.5 µg of genomic DNA; 5 µl of 10× PCR buffer (0.1 M Tris-HCl, pH 8.5, 0.025 M MgCl₂, 0.5 M KCl), 5 µl of 8 mM deoxynucleoside triphosphate mix (2 mM deoxyadenosine triphosphate, deoxythymidine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate, in 0.1 M Tris-HCl, pH 7.9), 1 µl of a 10-mM solution of each primer (LGL 765F and LGL 766R for cytochrome *b* and 33X5YF and LGL 331 for *zfy*), and 2.5 units AmpliTaq *Taq* polymerase (Applied Biosystems), and brought to a volume of 50 µl with deionized water. A Qiagen PCR purification kit (Qiagen Inc., Valencia, California) was used to clean the amplified fragments and prepare the templates for sequencing. Cycle sequencing utilized ABI Prism BigDye Terminators version 2.0 and version 3.0 (Applied Biosystems) and sequencing reactions were conducted on a GeneAmp PCR System 2700 thermal cycler as follows: 25 cycles of 96°C for 30 s of denaturing, 50°C for 15 s of annealing, and 60°C for 4 min of extension. Sequencing primers for the entire (1,140-bp) cytochrome *b* were LGL 765F, LGL 766R, and 388F (5'-GGY TAT GTT CTY CCA TGA GG-3'), an internal primer designed for complete bidirectional sequencing. Sequencing primers for *zfy* included 33X5YF, 33X6R (5'-CCC TCA CCT

GTT TGG TAY TGC-3'), 33X6F (5'-RGC AGT ACC AAA CAG GTG AGG-3'), LGL 331, Scot *zfy* 495F (5'-TAG GTA CAT GGA CTT TCA GC-3'), and Scot *zfy* 1470F (5'-TTA GGT GAT AAT TCT GAC GG-3'). Sequence reactions were then purified using Sephadex (Sigma-Aldrich, St. Louis, Missouri) spin columns, dehydrated in a vacuum centrifuge, and frozen until sequence visualization and collection on an ABI 377 automated sequencer (Applied Biosystems). Raw sequences were automatically analyzed using Sequencing Analysis Software (Applied Biosystems).

Sequences were aligned by eye and ambiguities were corrected in Sequencher version 4.1 (Gene Codes Corporation, Ann Arbor, Michigan) for the cytochrome-*b* sequences through comparison of the electropherograms. The *zfy* sequences were submitted to Clustal X (Thompson et al. 1997) for alignment utilizing the default gap-cost ratio (15.00:6.66) and again with a gap-cost ratio of 5:4. The use of 2 gap-cost ratios allowed for verification of the placement of insertions-deletions (indels) in the alignment of all *zfy* sequences. The resultant alignments were refined by eye and ambiguities corrected in Sequencher version 4.1.

Data analysis.—A neighbor-joining tree based on Kimura 2-parameter distances was constructed for both the cytochrome-*b* and the *zfy* data sets to allow for the determination of unique haplotypes in each of the 2 data sets. All subsequent phylogenetic analyses involved data matrices composed of a single representative of each respective haplotype for each of the 2 data sets.

A maximum-parsimony analysis was performed on each data set and the combined data matrix in PAUP* version 4.0b4a (Swofford 1999). A branch-and-bound option was used for the Y-chromosome data set and the heuristic search option was used for the cytochrome-*b* data set with tree-bisection-reconnection branch swapping. Starting trees were obtained via stepwise addition. Data were polarized via the outgroup method, and the outgroup taxa were chosen based on the study of Hofer and Van Den Bussche (2003). Phylogenetically informative characters were unordered and equally weighted with gaps treated as missing data. Stability of clades was examined by bootstrap analysis (Felsenstein 1985), which consisted of 1,000 pseudoreplicates with resampling of all characters, heuristic searching, and tree-bisection-reconnection branch swapping.

The best-fit maximum-likelihood (ML) model for each data set was determined using MODELTEST version 3.06 (Posada and Crandall 1998). MODELTEST helps choose the model of DNA substitution that best fits the data set through hierarchical hypothesis testing with the use of likelihood ratio tests and the Akaike information criterion (Akaike 1974). ML analysis of each data set and the combined data matrix was performed in PAUP*. The ML tree was constructed using a heuristic search. Bootstrap analysis for all data sets consisted of 100 pseudoreplicates with resampling of all characters, fast-heuristic searching, and tree-bisection-reconnection branch swapping.

A Bayesian analysis under the best-fit model was performed using MRBAYES (Huelsenbeck and Ronquist 2001). This

program utilizes Markov chain Monte Carlo methods for the Bayesian inference of phylogeny and is based on the posterior probability of a phylogenetic tree given an observed aligned matrix of DNA sequence data. The Bayesian analysis was implemented for 1×10^6 generations with 1 cold and 3 incrementally heated Markov chains, random starting trees for each chain, trees sampled every 10 generations, and the analysis repeated 3 independent times to insure convergence of the chains to the same posterior probability distribution and that the likelihoods reached stable values (Huelsenbeck et al. 2002) for each data set. Values for model parameters were not defined a priori in the analysis but were treated as unknown variables with uniform priors in each Bayesian analysis (Leaché and Reeder 2002).

RESULTS

Amplification, sequencing, and alignment.—The entire 1,140 bp of cytochrome *b* were sequenced for all samples amplified except for 3 samples where the 1st several base pairs were unreadable (FMNH 166186, first 24 bp unreadable; ROM 110843, first 30 bp unreadable; TK 33266, first 8 bp unreadable). These unreadable bases were coded as unknown in the data matrix. In the alignment, there were no indels, the start codon was ATG, and the stop codon was AGA for all samples except for those samples in clade 9 (Fig. 1), which was AGG. Five hundred seven base pairs were variable and of these, 431 bp were potentially parsimony informative. A neighbor-joining tree identified 88 unique cytochrome-*b* haplotypes, which were used for subsequent phylogenetic analysis.

A total of 51 individuals were sequenced for the *zfy* gene, including 2 outgroup taxa. The total number of base pairs sequenced per individual ranged from 1,597 bp to 2,100 bp and averaged 1,963 bp. Ambiguous bases at the beginning and end of the sequence were coded as missing. The default gap-cost ratio (15.00:6.66) in Clustal X and a gap-cost ratio of 5:4 resulted in identical alignments. The aligned data matrix included 12 indels ranging in size from 1-bp insertions and deletions to a 152-bp insertion in *S. leucogaster* (SP 10136 and SP 10137). Indels unique to *S. kuhlii* contained 4 deletions ranging from 1 bp to 17 bp and 3 insertions ranging from 1 bp to 7 bp. Indels shared by *S. kuhlii* and *S. nux* included a 3-bp and a 31-bp insertion. A 50-bp deletion was unique to *S. nux*. A 152-bp insertion was unique to *S. leucogaster* and a 2-bp deletion was unique to *S. viridis*. The indels were coded as missing data and accounted for 276 characters in the total *zfy* data matrix of 2,283 aligned characters, of which 1,997 characters were constant and 132 characters were potentially parsimony informative. Excluding the outgroup taxa, 93 total characters were variable, with 76 of these characters being potentially parsimony informative. Construction of a neighbor-joining tree identified 20 unique *zfy* haplotypes.

Data-set congruence.—An incongruence length difference test (Farris et al. 1994) as implemented in PAUP* as the

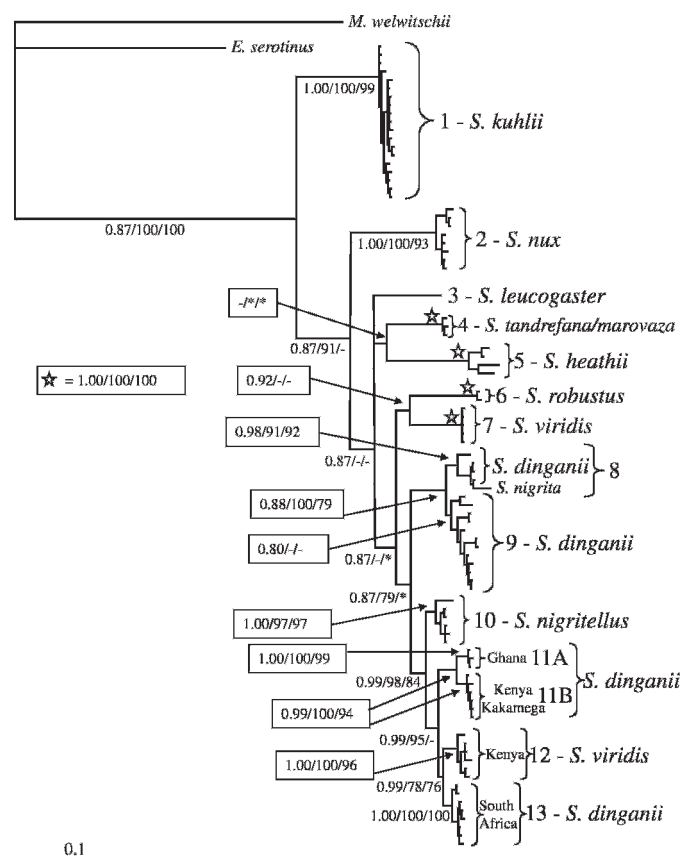


FIG. 1.—Bayesian phylogram from analysis of cytochrome-*b* gene sequences (1,140 base pairs). *Myotis welwitschii* and *Eptesicus serotinus* were designated as outgroup taxa. Numbers in boxes represent node support (Bayesian posterior probabilities/parsimony bootstrap percentages/maximum-likelihood bootstrap percentages). All nodes shown are supported by posterior probability or bootstrap values > 50 or 0.50 in at least 1 of the analyses. Support values are only shown for those nodes supported by posterior probability or bootstrap percent ≥ 70 (0.70 Bayesian posterior probability). Values designated with $(-)$ are support values > 50 but < 70 (0.50 and 0.70 for Bayesian posterior probability) and those designated $(*)$ are nodes with support values < 50 and indicate collapsed nodes in that particular analysis. Clades mentioned in the text are numbered 1–13.

partition homogeneity test was conducted to compare the 2 data sets. The incongruence length difference test resulted in a test statistic of $P = 0.24$, thus failing to support the null hypothesis that the 2 data sets are incongruent. Therefore, the *cytochrome-b* and the *zfy* data sets were combined for phylogenetic analysis. Of the 49 specimens included in the combined data set, 42 had unique sequences. The combined data matrix included 3,364 total characters (including all indels), of which 2,712 characters were constant, 190 characters were uninformative, and 462 characters were potentially parsimony informative.

Phylogenetic analyses.—Phylogenetic analyses of the cytochrome-*b*, *zfy*, and combined data sets using parsimony, likelihood, and Bayesian methods resulted in similar topologies with the only differences being in the resolution and support of the ancestral nodes. Only the Bayesian phylogram with proportional branch lengths is presented with Bayesian posterior probabilities, maximum-parsimony bootstrap support values, and ML bootstrap support values displayed.

An analysis of maximum parsimony of the cytochrome-*b* data resulted in 768 equally parsimonious trees of 1,554 steps. One of the maximum-parsimony trees had the following description: consistency index (CI) = 0.4408, homoplasy index (HI) = 0.5592, rescaled consistency index (RC) = 0.3882, and retention index (RI) = 0.8807. The program MODELTEST selected the GTR + Γ + I model as the best fit of nucleotide substitution for the cytochrome-*b* data set. The observed nucleotide frequencies were A = 0.3247, C = 0.2798, G = 0.1080, and T = 0.28750. The gamma shape parameter was set to α = 1.4748 and the assumed proportion of invariable sites was set to 0.4861. The ML tree had a log-likelihood score of -8,687.99. The 3 independent Bayesian analyses converged on stable posterior probability values after a burn-in time of 100,000 generations. The phylogeny inferred has a log-likelihood score of -8,758.16.

Pairwise genetic distance measures are presented in Table 1 for the identified clades based on the cytochrome-*b* data. Genetic distances ranged from 4.2% to 19.2% between and from 0% to 10.1% within previously identified species.

TABLE 1.—Pairwise genetic distances (Kimura 2-parameter) expressed as percentages within (diagonal) and between clades of the cytochrome-*b* phylogeny for the genus *Scotophilus*. *Scotophilus nigrita* is designated as *Sn* and is within clade 8.

Clade	1	2	3	4	5	6	7	8	S_n	9	10	11A	11B	12	13
1	0.8														
2	17.4	1.4													
3	17.0	14.6	0												
4	16.8	14.4	12.7	0.6											
5	17.5	16.7	14.4	13.6	0.4										
6	19.2	16.2	13.4	12.8	14.6	0.5									
7	16.2	15.0	13.5	12.6	16.0	10.4	0.1								
8	17.4	13.9	14.0	12.2	15.2	12.3	11.2	1.2							
S_n	17.8	14.5	15.3	13.3	15.9	12.9	9.5	3.3	0						
9	17.5	14.3	13.1	12.6	14.9	12.0	11.4	4.9	2.4	2.0					
10	16.4	14.3	12.4	12.3	15.3	11.2	10.0	8.7	11.4	8.3	1.1				
11A	17.3	14.7	13.1	12.2	14.7	11.3	11.3	10.1	10.7	9.7	6.3	0.3			
11B	17.1	14.6	12.7	12.4	14.5	11.9	11.0	9.3	9.8	9.7	6.1	2.8	0.2		
12	17.3	14.3	12.6	13.0	14.2	12.1	11.5	9.8	10.4	9.7	5.8	5.8	5.5	1.2	
13	18.0	14.5	12.0	12.5	14.3	11.4	11.0	9.4	10.1	9.3	5.3	5.5	5.2	4.2	0.6

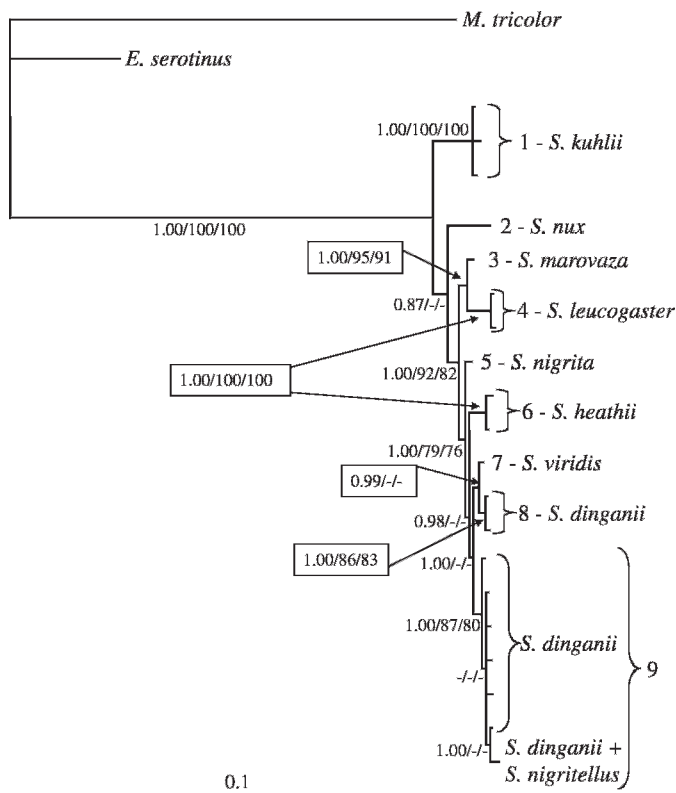


FIG. 2.—Bayesian phylogram from analysis of partial sequences of the *zfy* gene (2,283 base pairs). *Myotis tricolor* and *Eptesicus serotinus* were designated as outgroups. Node support is designated as in Fig. 1. Clades mentioned in the text are numbered 1–9.

Based on the cytochrome-*b* data, monophyly of the entire genus is highly supported but basal relationships within *Scotophilus* are poorly resolved (Fig. 1). The Bayesian analysis produced a phylogeny that has more resolution than either the maximum-parsimony or ML bootstrap consensus trees, including weak branch support for a *S. heathii* + *S. tandrefana*/*marovaza* sister-group relationship. However, all analyses identified a close phylogenetic relationship and genetic similarity (0.6% sequence divergence; Table 1) between *S. tandrefana* and *S. marovaza*. Similarly, *S. nigrita* groups within and is most similar (1.2% sequence divergence) to a population of *S. dinganii* from Ethiopia. Furthermore, *S. dinganii* and *S. viridis* are not each reciprocally monophyletic clades.

Phylogenetic analysis of the *zfy* data set using parsimony resulted in 2 equally parsimonious trees of 315 steps. Both maximum-parsimony trees had the following description: CI = 0.9651, HI = 0.0349, RC = 0.9239, and RI = 0.9574. A bootstrap consensus tree was calculated based on 1,000 pseudoreplicates with resampling of 2,283 characters. The HKY + Γ model was selected as the best-fit model of nucleotide substitution for the *zfy* data set. The ML analysis had nucleotide frequencies of A = 0.28890, C = 0.18020, G = 0.19920, and T = 0.33170. The gamma shape parameter was $\alpha = 1.1055$ and the transition:transversion ratio was 1.7937. The ML analysis resulted in an ML tree with a log-likelihood score of -4,745.99. The Bayesian analysis of the *zfy* data under the HKY + Γ model of nucleotide substitution produced a phylogeny (Fig. 2) with strong posterior probability support. The 3 independent analyses converged on stable posterior probability values after a burn-in time of 30,000 generations. The phylogeny inferred had a log-likelihood score of -4,768.58.

Pairwise genetic distance measures of the *zfy* data ranged from 0.18% to 2.14% between clades and from 0% to 0.16% within clades (Table 2). These values of *zfy* divergence are similar to those reported by Wallner et al. (2003) for *Equus* species and by Lawson and Hewitt (2002) for several sheep and goat species. Genetic distances between *Scotophilus* and the outgroup taxa for the *zfy* data averaged 16.64%. The absolute number of nucleotide changes ranged from 1 to 41 changes within *Scotophilus* and from 115 to 178 changes between *Scotophilus* and the outgroup taxa.

The *zfy* tree has a highly supported monophyletic *Scotophilus*, as did the cytochrome-*b* tree, but better support of more basal relationships. For example, *S. marovaza* is sister to *S. leucogaster*, which is sister to a clade consisting of *S. nigrita*, *S. heathii*, *S. viridis*, *S. dinganii*, and *S. nigritellus* (Fig. 2). An interesting result was a nearly identical haplotype for *S. nigritellus* and an individual of *S. dinganii*.

Phylogenetic analysis of the combined cytochrome-*b* and *zfy* data sets using parsimony resulted in 14 equally parsimonious trees at 1,305 steps. The tree description for 1 of the 14 trees is as follows: CI = 0.5824, HI = 0.4176, RC = 0.4989, and RI = 0.8567. Bootstrap support values over 70% are labeled on the appropriate nodes (Fig. 3). The program MODELTEST selected the GTR + Γ + I model as the best-fit

TABLE 2.—Pairwise genetic distances (Kimura 2-parameter) expressed as percentages within (diagonal) and between clades of the *zfy* phylogeny (Fig. 2) for *Scotophilus*.

Clade	1	2	3	4	5	6	7	8	9
1	0.10								
2	2.04	0							
3	1.67	1.27	0						
4	2.14	1.74	0.66	0.05					
5	1.71	1.33	0.50	0.89	0				
6	1.93	1.75	0.82	1.30	0.61	0.10			
7	1.67	1.43	0.56	1.03	0.28	0.56	0		
8	1.86	1.40	0.66	1.08	0.33	0.66	0.18	0.05	
9	1.81	1.40	0.73	1.18	0.42	0.73	0.37	0.43	0.16

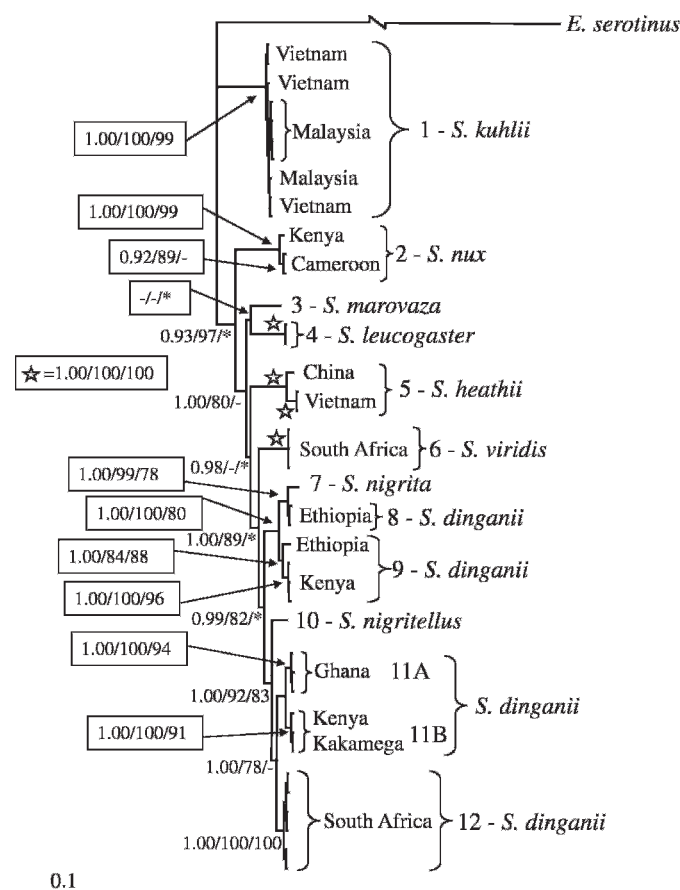


FIG. 3.—Bayesian phylogram from analysis of the combined cytochrome-*b* and *zfy* gene data sets. *Eptesicus serotinus* was designated as the outgroup taxon. Node support is designated as in Fig. 1. Clades mentioned in the text are numbered 1–12.

model of nucleotide substitution for the combined data set. ML analysis had nucleotide frequencies of A = 0.29740, C = 0.21630, G = 0.17080, and T = 0.31550. The gamma shape parameter was $\alpha = 0.5120$ and the assumed proportion of invariable sites was 0.6417. The ML analysis resulted in a tree with a log-likelihood score of $-11,030.59$.

The Bayesian analysis of the combined data matrix under the GTR + Γ + I model of nucleotide substitution produced a phylogeny (Fig. 3) that recovered the same clades as the maximum-parsimony and ML inferred phylogenies but with greater resolution at the ancestral nodes. The 3 independent analyses converged on stable posterior probability values after a burn-in time of 30,000 generations. The phylogeny inferred has a log-likelihood score of $-11,065.86$. The topology is very similar to that recovered using only the cytochrome-*b* data set with the exception that there is weak support for a sister-group relationship between *S. marovaza* and *S. leucogaster*, which was recovered in the *zfy* tree. In the cytochrome-*b* phylogeny, there is a weak sister-group relationship between a clade consisting of *S. marovaza* and *S. tandrefana*, and *S. heathii* (Fig. 1). The combined phylogeny shows *S. kuhlii* as the most basal species of *Scotophilus* and the African *Scotophilus* comprise a monophyletic clade with the inclusion of Asian *S. heathii*.

DISCUSSION

Phylogenies produced by the cytochrome-*b* data set resulted in 13 relatively well-supported clades (Fig. 1) with substantial genetic diversity between them (Table 1). Phylogenies based on the *zfy* data set with a reduced number of specimens resulted in 9 well-supported clades (Fig. 2), and had greater resolution of ancestral nodes than the cytochrome-*b* phylogenies. The phylogenies based on the combined data set (Fig. 3) had better support for identified clades, and increased resolution of the ancestral nodes.

In all analyses, several phylogenetic observations are supported. The Asian *S. kuhlii* is found to be the most basal species. African members of *Scotophilus* form a paraphyletic group with the inclusion of *S. heathii* from Asia, which suggests several exchanges between these 2 continents. *S. nux* is the most basal African species. *S. nigritellus* is distinct from *S. viridis*, as suggested by Grubb et al. (1998). *S. viridis* is not monophyletic because it is found in 2 divergent clades; specimens from Kenya group with *S. dinganii* from South Africa, and specimens of *S. viridis* from South Africa group with *S. robustus* from Madagascar. These relationships could not be corroborated with the *zfy* data set because there were no males of *S. robustus* and no males of *S. viridis* from Kenya available for study. The Bayesian analysis of the *zfy* data support a sister-group relationship between *S. viridis* from South Africa and *S. dinganii* from Ethiopia, but there was no support of this relationship in the ML and parsimony analyses (Fig. 2). Individuals identified as *S. dinganii* are found in 4 clades (Fig. 1, clades 8, 9, 11, and 13). Specimens from the Kakamega Forest of Kenya, coastal Ghana, and northeastern South Africa share a close relationship (Fig. 1, clades 11A, 11B, and 13) with inclusion of *S. viridis* from Kenya (Fig. 1, clade 12). This large clade is sister to a grouping of Ethiopian *S. dinganii* (Fig. 1, clades 8 and 9) and *S. nigrita*.

The higher-level relationships among species of the genus remain unclear. There is weak statistical support for nodes joining several species and the branch lengths are small, suggesting a rapid radiation of *Scotophilus*, or phylogenetically noisy data. There is saturation in the cytochrome-*b* data set (Fig. 4), which explains the more poorly supported basal relationships in comparison to the *zfy* tree. Nonetheless, these results corroborate the distant relationship between the 2 Indomalayan species as reported by Hoofer and Van Den Bussche (2003) based on 12S rRNA, 16S rRNA, and tRNA^{Val} data.

The inferred phylogenies provide molecular support for species previously identified through morphological methods and recognized in the literature, as well as support for cryptic species of *Scotophilus*. However, the inferred phylogenies do not support the recently described species from Madagascar: *S. marovaza* (Goodman et al. 2005) and *S. tandrefana* (Goodman et al. 2006). The cytochrome-*b* phylogeny groups them (Fig. 1, clade 4) with a genetic divergence of 0.6%, a level indicative of within-species divergence (Baker and Bradley 2006; Bradley and Baker 2001). This indicates that either the cytochrome-*b* data are not representative of the evolutionary history of these 2 taxa or that the speciation event that split them may be fairly

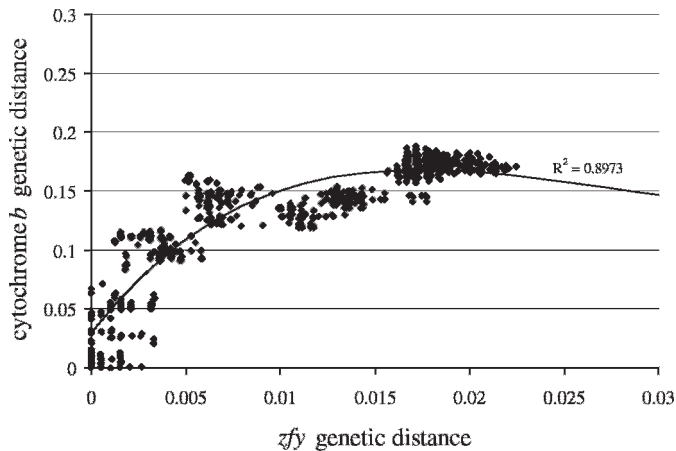


FIG. 4.—Plot of pairwise comparisons of genetic distance based on the cytochrome-*b* gene and the region of the *zfy* gene sequenced in this study. The scales present on the x and y axes account for an approximately 10-fold difference in mutation rates between the cytochrome-*b* gene and the *zfy* gene as can be seen in the linear portion of the curve. At around 12–15% divergence, the cytochrome-*b* gene becomes saturated, whereas the *zfy* gene has not yet reached saturation. This saturation is a potential cause of the loss of resolution at the basal nodes in the cytochrome-*b* phylogeny for *Scotophilus*.

recent and not yet reflected in the cytochrome-*b* gene. Unfortunately, we could not obtain sequences from the *zfy* gene to test these alternative hypotheses.

Clade 8 (Fig. 1) includes an individual identified as *S. nigrita* that clusters with *S. dinganii* from Ethiopia. The large-bodied African species *S. nigrita*, represented by SP 5505 from Kenya, is most similar to an Ethiopian *S. dinganii* (1.2% sequence divergence) based on mtDNA but a more distant relationship to all *S. dinganii* based on *zfy* (Fig. 2). The size difference between *S. nigrita* and *S. dinganii* make the 2 species easily identifiable. This association suggests that *S. nigrita* shares a cytochrome-*b* haplotype that is similar to those found in Ethiopian *S. dinganii*. This provides some evidence for a potential mitochondrial capture event in the history of this species possibly due to a hybridization event with an *S. dinganii*-like ancestor. The occurrence of a similar introgressive hybridization has been reported in North American deer (Cathey et al., 1998). Unfortunately, the sample size of *S. nigrita* available for this study is the single individual sequenced. Additional specimens of *S. nigrita* are needed to further investigate this evolutionary scenario.

Robbins et al. (1985) noted that southern African *S. viridis* were slightly larger and exhibited greater size variation than other *S. viridis* in their study. Individuals that were identified as *S. viridis* occur in divergent clades of the cytochrome-*b* tree (11% sequence difference), suggesting the occurrence of 2 species. *S. viridis* was described originally based on material collected in coastal Mozambique. South African individuals identified as *S. viridis* group weakly with *S. robustus* from Madagascar. Individuals identified as *S. viridis* from Kenya are sister to *S. dinganii* from South Africa. This implies that either this sample of *S. viridis* from Kenya is *S. dinganii* or it is a new species of *Scotophilus*. Preliminary examination of

voucher material suggests that this group is not *S. dinganii* but rather has superficial morphological similarities to *S. viridis*. *S. viridis* from Kenya also differs from *S. dinganii* from South Africa by 4.2% sequence divergence, a value that is within the documented range of sequence divergence for closely related species (Baker and Bradley 2006; Bradley and Baker 2001). Therefore, this population from Kenya most likely represents a new species that has not been described morphologically. All specimens within this clade were females so no *zfy* data are available for comparison to the cytochrome-*b* data.

The well-supported monophyletic clade of *S. nigrnellus* and >5% sequence divergence of cytochrome *b* from other individuals merit recognition from *S. viridis* and other species, as suggested by Grubb et al. (1998). However, an individual identified as *S. nigrnellus* (SP 11111) has a nearly identical *zfy* sequence to that of an individual identified as *S. dinganii* from Ghana (SP 10180). This specimen is morphologically similar to the other *S. nigrnellus* in terms of ventral pelage coloration, which suggests that there is incomplete lineage sorting.

Individuals identified as *S. dinganii* appear in several divergent cytochrome-*b* lineages, and may represent cryptic species, such as that reported by Jacobs et al. (2006). The individuals from Ethiopia and Kenya, excluding the Kakamega Forest specimens, have an average sequence divergence of 4.9% (Fig. 1, clades 8 and 9). Well-supported monophyletic clades of *S. dinganii* from coastal Ghana and the Kakamega Forest of Kenya differ from each other by 2.8% sequence divergence (Fig. 1, clades 11A and 11B) but differ from *S. dinganii* from Ethiopia and Kenya, excluding the Kakamega Forest specimens, by a mean sequence divergence of 9.7% and from *S. dinganii* from South Africa by 5.5%.

The Malagasy *Scotophilus* appear to have multiple origins from Africa because *S. robustus* and the clade containing *S. tandrefana* and *S. marovaza* do not share a sister-group relationship in the cytochrome-*b* tree. In continental Africa, there appear to be at least 10 species of *Scotophilus*, including *S. dinganii* (southern Africa), *S. dinganii* (eastern Africa), *S. dinganii* (Ghana to Kakamega Forest, Kenya), *S. viridis* (southern Africa), *S. viridis* (eastern Africa), *S. nigrnellus*, *S. nux*, *S. leucogaster*, *S. nucella*, and *S. nigrita*. However, our molecular study did not include 4 of the 15 currently recognized species (*S. nucella* from Africa, *S. borbonicus* from Reunion Island, *S. celebensis* from Sulawesi, and *S. collinus* from Indonesia). Therefore, no supported conclusion can be made as to an Asian or African origin of *Scotophilus*. Continued investigation is needed to fully understand the origins of the genus and to elucidate the phylogenetic relationships present. Nonetheless, our study suggests that *S. heathii* represents an invasion of Asia from Africa because it is well embedded in the African clade. One member of the African *Scotophilus*, *S. leucogaster*, occurs in Yemen and Saudi Arabia, possibly indicating a route between Africa and Asia used by a *Scotophilus*-like ancestor.

Based on the phylogenies inferred from mtDNA, *zfy*, and the combined data set, there potentially are an additional 3 species of *Scotophilus* (Table 3), including 2 previously identified as *S. dinganii* and 1 previously identified as *S. viridis*. The 2 *S.*

TABLE 3.—The 18 species of *Scotophilus* recognized in this study. A plus sign (+) indicates support and a minus sign (–) indicates a lack of support based on morphology, mitochondrial DNA (mtDNA), or Y-chromosome DNA (zfy).

Species	Morphology	mtDNA	zfy
<i>S. nigrita</i> (Schreber, 1774)	+	–	+
<i>S. borbonicus</i> (E. Geoffroy, 1803)	+	No data	No data
<i>S. kuhlii</i> Leach, 1821	+	+	+
<i>S. leucogaster</i> (Cretzschmar, 1830)	+	+	+
<i>S. heathii</i> (Horsfield, 1831)	+	+	+
<i>S. dinganii</i> (A. Smith, 1833)	+	+	+
<i>S. viridis</i> (Peters, 1852)	+	+	+
<i>S. robustus</i> Milne-Edwards, 1881	+	+	No data
<i>S. nigritellus</i> de Winton, 1899	+	+	No data
<i>S. nux</i> Thomas, 1904	+	+	+
<i>S. celebensis</i> Sody, 1928	+	No data	No data
<i>S. collinus</i> Sody, 1936	+	No data	No data
<i>S. nucella</i> Robbins, 1983	+	No data	No data
<i>S. tandrefana</i> Goodman et al., 2005	+	+	+
<i>S. marovaza</i> Goodman et al., 2006	+	–	No data
<i>S. dinganii</i> -like (eastern Africa)	No data	+	+
<i>S. dinganii</i> -like (Ghana to Kakamega)	No data	+	+
<i>S. viridis</i> -like (eastern Africa)	No data	+	No data

dinganii-like species include eastern populations from Ethiopia and Kenyan localities other than the Kakamega Forest specimens and western populations from Ghana to the Kakamega Forest of Kenya. The *S. viridis*-like species is from eastern Africa. We agree with Simmons (2005) and Robbins et al. (1985) in restricting *S. borbonicus* to Reunion Island and Madagascar. These potentially new species and other taxa not present in this study (*S. celebensis*, *S. collinus*, *S. borbonicus*, and *S. nucella*) result in a total of 18 species of *Scotophilus*.

There is a growing literature on cryptic species and their identification based on genetic data, including *S. dinganii* in South Africa (Jacobs et al. 2006). Other examples exist for the Onychophora (Trewick 1998), birds (Baker et al. 1995), and mammals (Kingston et al. 2001; Olson et al. 2004). The use of mtDNA to define species was investigated by Bradley and Baker (2001) in relation to mammals and used as a test of the genetic species concept. The use of mtDNA in conjunction with nuclear DNA markers allows for a more robust definition of species based on nucleotide sequences. The results of Bradley and Baker (2001) indicated that cytochrome-*b* genetic distance values between 2% and 11% were indicative of probable species and that distances above 11% were indicative of species recognition. In the genus *Scotophilus*, known morphological species differ from other known morphological species by 4% to more than 16% sequence divergence.

The 3 putative new species identified in this study should be validated with morphology or other ecological data, or both. Jacobs et al. (2006) report sympatric species of *S. dinganii*-like bats in southern Africa based on cytochrome-*b* sequence data (3.4% sequence divergence) and echolocation frequency data (peak echolocation frequencies of 44 kHz for one and 33 kHz for the other). This result has implications for taxonomy and systematic of *Scotophilus*, including the suggestion that call frequency data may be a useful character for separating species in

this genus, and that the widespread *S. dinganii* may be a complex of several closely related species that deserves further inquiry.

This study has resulted in several testable hypotheses and questions regarding the systematics of the genus *Scotophilus*. For example, is *S. nigrita* a species that has hybridized with an *S. dinganii*-like ancestor? How many cryptic species of *S. dinganii*-like bats occur in sub-Saharan Africa? The origin of Malagasy *Scotophilus* needs to be further examined with an increased taxonomic representation. Also, a thorough survey of the widespread Asian species (*S. kuhlii* and *S. heathii*) is needed to characterize these taxa within the context of systematics and taxonomy of *Scotophilus*.

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APPENDIX I

Specimens examined including GenBank accession numbers for cytochrome-*b* and partial *zfy* sequences used in the inference of a molecular phylogeny for the bat genus *Scotophilus*.

Species	Tissue ID	Voucher ID	Country: locality	Sex	Cytochrome <i>b</i>	GenBank accession
<i>M. welwitschii</i>	GenBank	GenBank	South Africa: Transvaal	—	AF376874	—
<i>M. tricolor</i>	SP 13200	CM 114030	Ethiopia	♂	—	EU751022
<i>E. serotinus</i>	AK 10826	AK 10826	Azerbaijan: Gobustan: Historical Reserve	♂	EU751000	EU751001
<i>S. dinganii</i>	SP 5454	CM 102245	Kenya: Coast Province: Taita District	♀	EU750962	—
<i>S. dinganii</i>	SP 5453	CM 102244	Kenya: Coast Province: Taita District	♀	EU750961	—
<i>S. dinganii</i>	SP 5078	CM 102252	Kenya: Western Province: Kakamega District	♀	EU750982	—
<i>S. dinganii</i>	SP 5368	DAS 8625	Kenya: Rift Valley Province: West Pokot District	♀	EU750959	—
<i>S. dinganii</i>	SP 5052	CM 102251	Kenya: Western Province: Kakamega District	♀	EU750985	—
<i>S. dinganii</i>	SP 5452	DAS 8740	Kenya: Coast Province: Taita District	♀	EU750961	—
<i>S. dinganii</i>	SP 5451	DAS 8739	Kenya: Coast Province: Taita District	♀	EU750966	—
<i>S. dinganii</i>	SP 5051	CM 102253	Kenya: Western Province: Kakamega District	♂	EU750980	EU751005
<i>S. dinganii</i>	SP 5077	DAS 8217	Kenya: Western Province: Kakamega District	♂	EU750981	EU751006
<i>S. dinganii</i>	SP 5082	CM 102253	Kenya: Western Province: Kakamega District	♂	EU750981	EU751004
<i>S. dinganii</i>	SP 5084	CM 102255	Kenya: Western Province: Kakamega District	♂	EU750981	EU751004
<i>S. dinganii</i>	SP 5370	CM 102247	Kenya: Rift Valley Province: West Pokot District	♂	EU750968	EU751002
<i>S. dinganii</i>	SP 5386	CM 102249	Kenya: Rift Valley Province: West Pokot District	♂	EU750966	EU751002
<i>S. dinganii</i>	TK 33149	CM 98050	Kenya: Rift Valley Province: Nakuru District	♂	EU750970	EU751002
<i>S. dinganii</i>	TK 33395	CM 98057	Kenya: Eastern Province: Machakos District	♂	EU750964	—
<i>S. dinganii</i>	TK 33534	CM 98051	Kenya: Western Province: Kakamega District	♂	EU750984	—
<i>S. dinganii</i>	SP 5083	CM 102254	Kenya: Western Province: Kakamega District	♀	EU750970	—
<i>S. dinganii</i>	TK 33142	Kim Nelson 216	Kenya: Rift Valley Province: Nakuru District	♀	EU750969	—
<i>S. dinganii</i>	TK 33141	Kim Nelson 215	Kenya: Rift Valley Province: Nakuru District	♀	EU750970	—
<i>S. dinganii</i>	SP 5385	CM 102248	Kenya: Rift Valley Province: West Pokot District	♀	EU750961	—
<i>S. dinganii</i>	SP 5369	CM 102246	Kenya: Rift Valley Province: West Pokot District	♀	EU750967	—
<i>S. dinganii</i>	TK 33359	CM 98054	Kenya: Eastern Province: Machakos District	♀	EU750965	—
<i>S. dinganii</i>	TK 33360	CM 98045	Kenya: Eastern Province: Machakos District	♀	EU750965	—
<i>S. dinganii</i>	TK 33361	CM 98044	Kenya: Eastern Province: Machakos District	♀	EU750965	—
<i>S. dinganii</i>	TK 33535	CM 98052	Kenya: Western Province: Kakamega District	♀	EU750981	—
<i>S. dinganii</i>	TK 33536	CM 98053	Kenya: Western Province: Kakamega District	♀	EU750983	—
<i>S. dinganii</i>	TK 33140	CM 98048	Kenya: Rift Valley Province: Nakuru District	♀	EU750981	—
<i>S. dinganii</i>	TK 33189	CM 98043	Kenya: Coast Province: Kwale District	♀	EU750963	—
<i>S. dinganii</i>	SP 13027	CM 114043	Ethiopia: Gondar Province	♀	EU750954	—
<i>S. dinganii</i>	AK 21213	DAS 10292	Ethiopia: Oromiya Region: Dogy River Bridge	♂	EU750956	—
<i>S. dinganii</i>	AK 21234	DAS 10309	Ethiopia: Oromiya Region: Dogy River Bridge	♀	EU750956	—
<i>S. dinganii</i>	AK 21215	RGT 19	Ethiopia: Oromiya Region: Dogy River Bridge	♀	EU750956	—
<i>S. dinganii</i>	AK 21235	DAS 10310	Ethiopia: Oromiya Region: Dogy River Bridge	♂	EU750956	EU751002
<i>S. dinganii</i>	AK 21214	DAS 10293	Ethiopia: Oromiya Region: Dogy River Bridge	♀	EU750956	—
<i>S. dinganii</i>	AK 21259	DAS 10329	Ethiopia: Oromiya Region: Dogy River Bridge	♀	EU750957	—
<i>S. dinganii</i>	AK 21223	DAS 10300	Ethiopia: Oromiya Region: Dogy River Bridge	♂	EU750958	EU751003
<i>S. dinganii</i>	AK 21163	RGT 07	Ethiopia: Gambela Region: Bishen Waca Lake	♂	EU750960	EU751002
<i>S. dinganii</i>	TM 38151	TM 38151	South Africa	♀	EU750996	—
<i>S. dinganii</i>	TM 37655	TM 37655	South Africa: Transvaal Province: Pafuri District	♂	EU750996	EU751007
<i>S. dinganii</i>	TM 37656	TM 37656	South Africa: Transvaal Province: Pafuri District	♂	EU750994	—

APPENDIX I.—Continued.

Species	Tissue ID	Voucher ID	Country: locality	Sex	GenBank accession	
					Cytochrome <i>b</i>	<i>zfy</i>
<i>S. dinganii</i>	TM 37668	TM 37668	South Africa: Transvaal Province: Pafuri District	♂	EU750994	EU751007
<i>S. dinganii</i>	TM 37669	TM 37669	South Africa: Transvaal Province: Pafuri District	♂	EU750996	EU751007
<i>S. dinganii</i>	SP 7731	CM 105747	South Africa: Transvaal Province: Farm Greefswald 37	♂	EU750999	EU751007
<i>S. dinganii</i>	SP 7732	CM 105748	South Africa: Transvaal Province: Farm Greefswald 37	♂	EU750993	EU751007
<i>S. dinganii</i>	SP 7755	CM 105749	South Africa: Transvaal Province: Farm Greefswald 37	♂	EU750992	EU751007
<i>S. dinganii</i>	SP 7789	CM 105750	South Africa: Transvaal Province: Farm Greefswald 37	♂	EU750998	EU751007
<i>S. dinganii</i>	TM 39625	TM 39625	South Africa: Mpumalanga Province: Satara	♀	EU750997	—
<i>S. dinganii</i>	TM 39624	TM 39624	South Africa: Mpumalanga Province: Satara	♂	EU750997	—
<i>S. dinganii</i>	F 52134	F 52134	South Africa: Kruger National Park	♀	EU750995	—
<i>S. dinganii</i>	SP 10179	CM 113641	Ghana: Greater Accra Region	♂	EU750977	EU751008
<i>S. dinganii</i>	SP 10180	CM 113642	Ghana: Greater Accra Region	♂	EU750978	EU751009
<i>S. dinganii</i>	SP 10181	CM 113643	Ghana: Greater Accra Region	♂	EU750979	EU751010
<i>S. leucogaster</i>	SP 10136	CM 113645	Ghana: Northern Region	♂	EU750940	EU751019
<i>S. leucogaster</i>	SP 10137	CM 113646	Ghana: Northern Region	♂	EU750940	EU751018
<i>S. nigrita</i>	SP 5505	CM 102256	Kenya: Coast Province: Taita District	♂	EU750955	EU751020
<i>S. nux</i>	SP 5053	CM 102257	Kenya: Western Province: Kakamega District	♂	EU750936	EU751017
<i>S. nux</i>	SP 5056	CM 102260	Kenya: Western Province: Kakamega District	♂	EU750936	EU751017
<i>S. nux</i>	TK 33485	DAS 7282	Kenya: Western Province: Kakamega District	♀	EU750938	—
<i>S. nux</i>	SP 5054	CM 102258	Kenya: Western Province: Kakamega District	♀	EU750939	—
<i>S. nux</i>	TK 33519	CM 98056	Kenya: Western Province: Kakamega District	♀	EU750936	—
<i>S. nux</i>	SP 5055	CM 102259	Kenya: Western Province: Kakamega District	♀	EU750937	—
<i>S. nux</i>	SP 10620	CM 108033	Cameroon: Southwest Province: Korup National Park	♂	EU750933	EU751017
<i>S. nux</i>	SP 10628	CM 108034	Cameroon: Southwest Province: Korup National Park	♂	EU750934	EU751017
<i>S. nux</i>	SP 10629	CM 108032	Cameroon: Southwest Province: Korup National Park	♀	EU750935	—
<i>S. nux</i>	SP 10571	CM 108035	Cameroon: Southwest Province: Baro	♀	EU750932	—
<i>S. nigritellus</i>	SP 11041	CM 113647	Ghana: Greater Accra Region	♀	EU750974	—
<i>S. nigritellus</i>	SP 11046	CM 113648	Ghana: Greater Accra Region	♂	EU750975	—
<i>S. nigritellus</i>	SP 11047	CM 113649	Ghana: Greater Accra Region	♀	EU750975	—
<i>S. nigritellus</i>	SP 11050	CM 113650	Ghana: Greater Accra Region	♂	EU750972	—
<i>S. nigritellus</i>	SP 11111	CM 113644	Ghana: Greater Accra Region	♂	EU750971	EU751009
<i>S. nigritellus</i>	SP 11112	CM 113651	Ghana: Greater Accra Region	♂	EU750973	—
<i>S. nigritellus</i>	SP 11113	CM 113652	Ghana: Greater Accra Region	♂	EU750976	—
<i>S. nigritellus</i>	SP 10161	CM 113653	Ghana: Greater Accra Region	♂	EU750975	—
<i>S. viridis</i>	TM 37671	TM 37671	South Africa: Transvaal: Pafuri District	♂	EU750949	EU751016
<i>S. viridis</i>	TM 37672	TM 37672	South Africa: Transvaal: Pafuri District	♂	EU750949	EU751016
<i>S. viridis</i>	TM 37675	TM 37675	South Africa: Transvaal: Pafuri District	♂	EU750952	EU751016
<i>S. viridis</i>	TM 37670	TM 37670	South Africa: Transvaal: Pafuri District	♀	EU750949	—
<i>S. viridis</i>	TM 37691	TM 37691	South Africa: Transvaal: Pafuri District	♀	EU750953	—
<i>S. viridis</i>	TK 33264	CM 98039	Kenya: Coast Province: Kwale District	♀	EU750989	—
<i>S. viridis</i>	TK 33265	DAS 7088	Kenya: Coast Province: Kwale District	♀	EU750988	—
<i>S. viridis</i>	TK 33266	CM 98040	Kenya: Coast Province: Kwale District	♀	EU750986	—
<i>S. viridis</i>	SP 5498	CM 102242	Kenya: Coast Province: Taita District	♀	EU750990	—
<i>S. viridis</i>	SP 5499	CM 102241	Kenya: Coast Province: Taita District	♀	EU750987	—
<i>S. viridis</i>	SP 5500	CM 102243	Kenya: Coast Province: Taita District	♀	EU750991	—

APPENDIX I.—Continued.

Species	Tissue ID	Voucher ID	Country: locality	Sex	GenBank accession	
					Cytochrome <i>b</i>	<i>zf</i>
<i>S. viridis</i>	F 52119	F 52119	South Africa: Kruger National Park	♀	EU750949	—
<i>S. viridis</i>	F 52120	F 52120	South Africa: Kruger National Park	♀	EU750949	—
<i>S. viridis</i>	F 52121	F 52121	South Africa: Kruger National Park	♀	EU750949	—
<i>S. viridis</i>	TM 37673	TM 37673	South Africa: Transvaal: Pafuri District	♀	EU750949	—
<i>S. viridis</i>	TM 37689	TM 37689	South Africa: Transvaal: Pafuri District	♀	EU750949	—
<i>S. viridis</i>	TM 37674	TM 37674	South Africa: Transvaal: Pafuri District	♀	EU750949	—
<i>S. viridis</i>	TM 38144	TM 38144	South Africa: Transvaal: Pafuri District	♀	EU750949	—
<i>S. viridis</i>	TM 38142	TM 38142	South Africa: Transvaal: Pafuri District	♀	EU750949	—
<i>S. viridis</i>	TM 38143	TM 38143	South Africa: Transvaal: Pafuri District	♂	EU750949	—
<i>S. viridis</i>	TM 38149	TM 38149	South Africa: Transvaal: Pafuri District	♂	EU750950	EU751016
<i>S. viridis</i>	TM 39482	TM 39482	South Africa	♂	EU750951	EU751016
<i>S. viridis</i>	TM 39481	TM 39481	South Africa: Northern Province: Punda Milia	♂	EU750951	—
<i>S. robustus</i>	SMG 10560	FMNH 166186	South Africa: Northern Province: Punda Milia	♀	EU750947	—
<i>S. robustus</i>	SMG 5930	FMNH 151939	Madagascar: Tsinoarivo Forest	♀	EU750948	—
<i>S. tandreana</i>	RBJ 161	UADBA 46923	Madagascar: Province de Mahajanga	♂	EU750941	—
<i>S. marovaza</i>	RBJ 215	UADBA 46965	Madagascar: Parc National d'Ankarafantsika	♀	EU750942	—
<i>S. marovaza</i>	SMG 14474	FMNH 184050	Madagascar: Province de Mahajanga, Marovaza	♂	EU750943	—
<i>S. heathii</i>	MVZ 176513	MVZ 176513	China: Yunnan Province	♂	EU750946	EU751021
<i>S. heathii</i>	MVZ 186416	MVZ 186416	Vietnam: Vinh Phu Province: Tam Dao	♂	EU750945	EU751012
<i>S. heathii</i>	MVZ 186412	MVZ 186412	Vietnam: Vinh Phu Province: Tam Dao	♂	EU750945	EU751012
<i>S. heathii</i>	F 42769	ROM 107786	Vietnam: Yok Don National Park	♀	EU750944	EU751011
<i>S. kuhlii</i>	AK 21476	No voucher	Malaysia: Kedah State: Jitra	♀	EU750915	—
<i>S. kuhlii</i>	AK 21477	No voucher	Malaysia: Kedah State: Jitra	♀	EU750916	—
<i>S. kuhlii</i>	AK 21478	No voucher	Malaysia: Kedah State: Jitra	♀	EU750915	—
<i>S. kuhlii</i>	AK 21479	No voucher	Malaysia: Kedah State: Jitra	♀	EU750917	—
<i>S. kuhlii</i>	AK 21480	No voucher	Malaysia: Kedah State: Jitra	♀	EU750916	—
<i>S. kuhlii</i>	AK 21481	No voucher	Malaysia: Kedah State: Jitra	♀	EU750918	—
<i>S. kuhlii</i>	AK 21482	No voucher	Malaysia: Kedah State: Jitra	♀	EU750919	—
<i>S. kuhlii</i>	AK 21483	No voucher	Malaysia: Kedah State: Jitra	♀	EU750915	—
<i>S. kuhlii</i>	AK 21484	No voucher	Malaysia: Kedah State: Jitra	♀	EU750920	—
<i>S. kuhlii</i>	AK 21485	No voucher	Malaysia: Kedah State: Jitra	♂	EU750921	—
<i>S. kuhlii</i>	AK 21486	No voucher	Malaysia: Kedah State: Jitra	♂	EU750916	—
<i>S. kuhlii</i>	AK 21487	No voucher	Malaysia: Kedah State: Jitra	♂	EU750915	—
<i>S. kuhlii</i>	AK 21488	No voucher	Malaysia: Kedah State: Jitra	♂	EU750915	EU751015
<i>S. kuhlii</i>	AK 21489	No voucher	Malaysia: Kedah State: Jitra	♂	EU750922	EU751015
<i>S. kuhlii</i>	AK 21490	No voucher	Malaysia: Kedah State: Jitra	♂	EU750915	EU751014
<i>S. kuhlii</i>	AK 21491	No voucher	Malaysia: Kedah State: Jitra	♂	EU750916	EU751015
<i>S. kuhlii</i>	AK 21492	No voucher	Malaysia: Kedah State: Jitra	♂	EU750920	—
<i>S. kuhlii</i>	AK 21493	No voucher	Malaysia: Kedah State: Jitra	♂	EU750917	EU751015
<i>S. kuhlii</i>	AK 21494	No voucher	Malaysia: Kedah State: Jitra	♂	EU750915	EU751015
<i>S. kuhlii</i>	F 44160	ROM 110835	Vietnam: Cat Tien	♀	EU750923	—

APPENDIX I.—Continued.

Species	Tissue ID	Voucher ID	Country: locality	Sex	GenBank accession	
					Cytochrome <i>b</i>	<i>zf</i> y
<i>S. kuhlii</i>	F 44162	ROM 110837	Vietnam: Cat Tien	♀	EU750924	—
<i>S. kuhlii</i>	F 44165	ROM 110840	Vietnam: Cat Tien	♂	EU750925	EU751013
<i>S. kuhlii</i>	F 44166	ROM 110841	Vietnam: Cat Tien	♂	EU750926	EU751015
<i>S. kuhlii</i>	F 44167	ROM 110842	Vietnam: Cat Tien	♂	EU750927	—
<i>S. kuhlii</i>	F 44168	ROM 110843	Vietnam: Cat Tien	♂	EU750928	EU751013
<i>S. kuhlii</i>	F 44282	ROM 110956	Vietnam: Soc Trang	♀	EU750929	—
<i>S. kuhlii</i>	F 44283	ROM 110957	Vietnam: Soc Trang	♀	EU750930	—
<i>S. kuhlii</i>	JLP 16928	MVZ 186418	Vietnam: Vinh Phu Province: Tam Dao	♂	EU750913	EU751015
<i>S. kuhlii</i>	JLP 16936	MVZ 186421	Vietnam: Vinh Phu Province: Tam Dao	♂	EU750931	EU751013
<i>S. kuhlii</i>	LRH 2945	LRH 2945	Philippines	♀	EU750914	—
<i>S. kuhlii</i>	EAR 1266	EAR 1266	Philippines	♂	EU750914	—
<i>S. kuhlii</i>	EAR 1371	EAR 1371	Philippines	♂	EU750914	—

Abbreviations for tissue ID and voucher specimen ID are: AK, DAS (> 10196), RGT = Texas Cooperative Wildlife Collection, College Station, Texas; CM, DAS, SP = Carnegie Museum of Natural History, Pittsburgh, Pennsylvania; FMNH, SMG, LRH, EAR = Field Museum of Natural History, Chicago, Illinois; MVZ, JLP = Museum of Vertebrate Zoology, Berkeley, California; ROM, F = Royal Ontario Museum, Toronto, Ontario, Canada; TK = Texas Tech University, Lubbock, Texas; TM = Transvaal Museum of Natural History, Pretoria, South Africa; UADBA, RBJ = Université d'Antananarivo, Département de Biologie Animale, Antananarivo, Madagascar.