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# CRYPTIC SPECIES IN AN INSECTIVOROUS BAT, *SCOTOPHILUS DINGANII*

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In recent years many cryptic bat species have been unmasked by differences in their echolocation calls. The yellow house bat (*Scotophilus dinganii*) is 1 of 3 species of *Scotophilus* currently described in southern Africa and is distinguished from the other 2 species by its size and yellow venter. Here we use genetic, morphological, and echolocation call data to show the existence of a cryptic species. We found that *S. dinganii* consists of 2 forms, one that uses a peak echolocation frequency of 44 kHz and the other a peak frequency of 33 kHz. Both forms have yellow venters. The 44-kHz phonic type is up to 15% smaller than the 33-kHz phonic type and differed genetically by an average cytochrome-*b* (*Cytb*) sequence divergence of 3.3%. Furthermore, combined phylogenetic analyses of *Cytb* and control region sequences indicate that the 2 phonic types are reciprocally monophyletic, suggesting that they are sibling species.

Key words: bats, cryptic species, echolocation, mitochondrial DNA, morphology, *Scotophilus dinganii*, *Scotophilus viridis*

The speciation process involves ecological, geographical, and behavioral barriers that act in concert or separately (Yoder et al. 2000) to produce the phenotypic and genetic divergence that characterizes speciation. A consequence of this is that phenotypic and genetic divergence may not begin at the same time and may not proceed at the same rate. This can lead to the existence of cryptic species in which there is marked genetic divergence but relatively little phenotypic divergence or vice versa. For example, morphologically similar common ravens (*Corvus*) in North America (Omland et al. 2000), mouse lemurs (*Microcebus*) in Madagascar (Yoder et al. 2000), and various species of bats in Europe (*Plecotus*—Kiefer et al. 2002; Mayer and von Helversen 2001a; *Myotis*—Mayer and von Helversen 2001a; von Helversen et al. 2001) have been shown to be genetically distinct. On the other hand, 2 bat species (*Eptesicus serotinus* and *Eptesicus nilssonii*) that are morphologically very different were found to be genetically very similar (Mayer and von Helverson 2001b). Thus, species recognition requires a range of phenotypic, phylogenetic, and biological data.

In recent years some cryptic bat species have been unmasked by differences in their echolocation calls. For example, *Pipistrellus pipistrellus* has been shown to consist of 2 distinct species, *P. pipistrellus* and *P. pygmaeus*, on the basis of differences in the frequency of their echolocation calls despite marked similarity in their morphology (Jones and van Parijs 1993). Examination of genetic (Barratt et al. 1997; Hulva et al. 2004; Mayer and von Helversen 2001a) and ecological data later confirmed the existence of 2 distinct species (Barlow 1997). Similarly, differences in echolocation frequency in the high-duty–cycle insectivorous bat species *Rhinolophus philippinensis* have been used to identify genetically distinct morphs (Kingston and Rossiter 2004). Thus, selection on sensory systems may initiate the speciation process and can provide clues to the identification of cryptic species.

The insectivorous bat genus *Scotophilus* (family Vespertilionidae) occurs in southern and southeastern Asia and sub-Saharan Africa (Schlitter et al. 1980). Currently, 4 species are recognized in Africa, *S. leucogaster*, *S. nigrita*, *S. dinganii*, and *S. viridis* (Simmons 2005). Only the latter 3 occur in southern Africa, with *S. nigrita* (forearm length $>$ 70 mm) the largest and least common, known from only 3 localities, Botswana, eastern Zimbabwe, and Mozambique (Taylor 2000). Both *S. dinganii* (forearm length 51–58 mm) and *S. viridis* (forearm length 44–52 mm) are common in South Africa, with *S. dinganii* occurring sympatrically with *S. viridis* throughout the latter's range (Taylor 2000). These 2 species are usually distinguished in the field by forearm length and color of the venter, which is yellow in the larger *S. dinganii* and white, gray, or dull brown in the smaller *S. viridis* (Schlitter et al. 1980; Taylor 2000). However, Fenton and Rautenbach (1998)

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noticed 2 color variants of the smaller form in Kruger National Park and north-central Zimbabwe, one with whitish venters and the other with yellow venters. They thus hypothesized that there may be a 4th species of *Scotophilus* in southern Africa, a cryptic form smaller than *S. dinganii* but with a yellow venter. In support of Fenton and Rautenbach's (1998) hypothesis, we captured a large and a small form, both with yellow venters, at St. Lucia Wetland Park in KwaZulu–Natal Province in April 2003. When we flew them in a flight room, the 2 forms used peak echolocation frequencies that differed by an average of 10 kHz, suggesting the existence of a cryptic species. Thus, among the medium-sized to small *Scotophilus* in southern Africa, there may be 3 rather than 2 species: *S. dinganii*, which is medium sized, has a yellow venter, and echolocates at 33 kHz, *S. viridis*, which is smaller with a white, gray, or brown venter and echolocates at 40 kHz, and an as yet undescribed species that is small with a yellow venter and echolocates at 44 kHz.

Our aim in this study was to investigate the existence of a cryptic species by collecting genetic, morphological, and echolocation call data on the 2 yellow-bellied forms of *Scotophilus* from different sites in South Africa and Zambia.

## Materials and Methods

*Study sites*.—Bats were captured using mist nets in South Africa at St. Lucia Wetland Park (28°22′S, 32°25′E) in KwaZulu–Natal Province, and at Skukuza (24°59′S, 31°35′E) in Kruger National Park. In Zambia, bats were captured at Leopard's Hill Farm in Lusaka (15°30′S, 28°15′E), Bruce Miller's Farm (18°37′S, 27°00′E) near Choma, and Mike Fisher's Farm (12°56′S, 28°16′E) near Kitwe. All research reported here was done in a humane way, in accordance with guidelines of the American Society of Mammalogists (Animal Care and Use Committee 1998), and was approved by the Animal Experimentation Committee of the University of Cape Town.

*Echolocation*.—We recorded the echolocation calls of bats captured in mist nets as we released them back into the same habitat in which they were caught. We also recorded the calls of some of these individuals, before release, while flying them in a portable flight room ($10 \times 3 \times 2$ m). Bats recorded while being released were followed for as long as possible after release to ensure that search-phase calls were recorded (O'Farrell et al. 1999). In both recording situations, the high-frequency output of the Pettersson D980 bat detector (Pettersson Elektronik AB, Uppsala, Sweden) was recorded directly onto a Dell Latitude C810 notebook computer (Dell Computer [Pty.] Ltd., Bryanston, South Africa). We analyzed these recordings using BatSound Pro software (version 3.20, Pettersson Elektronik AB) on the same computer using a sampling rate of 44,100 Hz (16 bits, mono) and a threshold of 15. We measured peak frequency of the dominant harmonic and bandwidth at 20 dB below peak frequency from the FFT power spectrum (size 1024). A Hanning window was used to eliminate effects of background noise. We measured call duration and interpulse interval from the oscillogram. We measured a single high-quality search-phase call, selected on the basis of the signal to noise ratio, from each bat to avoid pseudoreplication and, in the case of hand-released bats, to ensure that we only analyze search-phase calls and not handheld calls or calls immediately after release.

We used 2-factor analysis of variance (ANOVA) with Tukey tests to compare echolocation calls between different sizes of yellow-bellied bats (bats with forearm lengths $<$ 52 mm versus bats with forearm lengths $>$ 52 mm) recorded as they were released, and between calls recorded from some bats from the same 2 groups flown in the flight room.

*Tissue sampling and outgroup*.—We collected 3-mm-diameter tail punches from live bats or flight muscle tissue (where vouchers were taken) from *S. dinganii* from each site in South Africa and Zambia. Additional tissues from *S. dinganii* and *S. viridis* were provided by the Transvaal Museum, W. White, and F. Cotterill (Appendix I). *Scotophilus heathii*, an Asian *Scotophilus*, was chosen as the outgroup. Cytochrome-*b* (*Cytb*) data for this species were downloaded from GenBank (AF376831—Ruedi and Mayer 2001).

*DNA extraction, amplification, sequencing, and phylogenetic analysis*.—Total genomic DNA was extracted from tissue preserved in 95% ethanol using a standard phenol–chloroform extraction procedure (Sambrook et al. 1989). A 604-base pari (bp) fragment of the mitochondrial *Cytb* gene was amplified using primers L14724 and H15275 (Irwin et al. 1991). Primers C and E from Wilkinson and Chapman (1991) were used to amplify approximately 1,000 bp of the control region. Polymerase chain reactions were performed as described by Jacobs et al. (2004). The ends of sequences were trimmed to facilitate comparisons among all individuals sequenced. A total of 534 bp (*Cytb*) and 391 bp (control region) were aligned using Clustal X (Thompson et al. 1997). All unique sequences have been deposited in GenBank (accession numbers AY754059–AY754095; Appendix I).

*Data analysis*.—Base composition was estimated using MEGA v2.1 (http://www.megasoftware.net). The *Cytb* and control region data sets were analyzed separately as well as in a combined matrix after testing for incongruence using the incongruence length difference test implemented in PAUP* v4b10 (Swofford 2002). This test was implemented under parsimony with 10 random addition sequences of taxa and 100 replicates to generate the null distribution (Farris et al. 1995). Phylogenetic analyses were executed in PAUP* v4b10 (Swofford 2002) using maximum parsimony and maximum likelihood. Bayesian analysis was performed in MrBayes version 3.0b4 (Huelsenbeck and Ronquist 2001).

Base frequency stationarity was evaluated using a chi-square test implemented in PAUP with uninformative characters excluded (Waddell et al. 1999). Trees were generated using equal weighting and the heuristic search option with tree-bisection-reconnection branch swapping and stepwise addition of taxa using 1,000 random sequence addition replicates with 1 tree retained per stepwise addition replicate. Nodal support for the maximum-parsimony analyses for all 3 data sets (i.e., *Cytb*, control region, and the 2 combined) was assessed from 1,000 nonparametric bootstrap replicates (full heuristic search; 2 random stepwise addition of taxa).

The most likely nucleotide substitution model was estimated using MODELTEST v 3.06 (Posada and Crandall 1998) in combination with the hierarchical likelihood-ratio test for maximum-likelihood analyses. Tree searches were performed using 10 random stepwise additions of taxa and tree-bisection-reconnection branch swapping. No swapping algorithm was used to bootstrap the maximum-likelihood trees because of the computing time required. Three independent Bayesian runs with different random starting trees were performed to ensure convergence on the same topology. Bayesian analysis was implanted as described by Matthee et al. (2004). Nodes that received $\geq$70% bootstrap support and $\geq$0.95 Bayesian posterior probability were considered well supported.

The software program zt (Bonnet and Van de Peer 2002) was used to test the null hypothesis that genetic divergence values were independent of geographic distances between collection localities using a simple Mantel test. The significance of the test statistic was evaluated by comparing the observed value with a reference

distribution obtained by an exact permutation procedure with the number of permutations equal to the factorial of the matrix size.

*Color of the ventral pelage*.—The venters of all specimens used in the genetic and morphological analyses (see below) were examined under a fluorescent light and categorized as either yellow (slight yellowish wash to canary yellow) or light brown (no yellow present). We also examined skins of *S. viridis* and *S. dinganii* from the Northern Flagship Institute (Pretoria) and the Amathole Museum (King Williams Town).

*Skull morphology*.—We investigated morphological differences among *S. dinganii* and between different forms of *S. dinganii* and *S. viridis* by comparing skull measurements taken using dial calipers under a Leica Zoom 2000 microscope (Buffalo, New York) at 12× magnification. We measured skulls from voucher specimens of *S. dinganii* captured at St. Lucia Wetland Park (KwaZulu–Natal Province, South Africa), and Leopard's Hill Farm and Bruce Miller's Farm (Zambia), as well as from museum specimens of *S. dinganii* collected in Kruger National Park and KwaZulu–Natal Province of South Africa (museum voucher numbers obtainable from the corresponding author). Five specimens of *Scotophilus* collected by F. Cotterill from another site in Zambia, Dandro Park (16°9′S, 26°3′E) on Kafue Flats also were included.

We used forward stepwise discriminant function analysis (DFA) on the skull measurements classifying captured specimens and the 5 collected by F. Cotterill, on the basis of their echolocation calls, or their DNA sequences, or venter color, or all 3. We used museum specimen labels and venter color to classify museum specimens.

*External morphology*.—The external morphology of specimens of *S. dinganii* for which we also had DNA sequences were compared. We measured body mass, forearm length, head length (from posterior edge of the occiput to tip of the snout), wing area, and wingspan from live bats and calculated wing loading, aspect ratio, and wing-tip shape index as described in Schoeman and Jacobs (2003). We used forward stepwise DFA on the above parameters for those individuals for which we had DNA sequences. All statistics were calculated in Statistica 6.1 (StatSoft, Inc., Tulsa, Oklahoma).

## Results

We captured 43 *Scotophilus*, all with yellow venters (Appendix I). These represent large and small forms of *Scotophilus* and were captured at all of the sites visited, often in the same net. All of the bats were caught in the 1st hour after dusk. In addition, the 13 specimens of *S. dinganii* obtained from the Northern Flagship Institution in South Africa (11), F. Cotterill (1), and W. White (1) all had yellow venters. The 2 specimens of *S. viridis*, 1 each from the Northern Flagship Institution and F. Cotterill, both had light brown venters. The ventral pelage of 51 skins of *S. dinganii* examined at the Amathole Museum (King Williams Town, South Africa) ranged in color from a slight yellowish wash to canary yellow. All of these specimens were collected in the Eastern Province of South Africa. The ventral pelage of 18 skins of *S. viridis* examined at the same museum ranged from gray to light brown. There was no trace of yellow on the ventral pelage of these bats. Two of these specimens were collected in Kruger National Park, 2 from Zambia, and 14 from Namibia. Furthermore, on the basis of differences in color of the venter, we were able to correctly assign (confirmed by DNA sequences) the 5 specimens obtained from F. Cotterill (Appendix I) to either *S. dinganii*–44 kHz (see below) or *S. viridis*.

*Echolocation*.—Peak echolocation frequency and forearm lengths divided the bats into 2 groups. The larger yellow-bellied *Scotophilus* with a forearm length $> 52$ mm had a lower mean peak echolocation frequency (mean $\pm$ *SD* $= 33.4 \pm 1.4$ kHz, range $= 31.0$–$35.8$ kHz, $n = 24$) in free flight than the smaller yellow-bellied *Scotophilus* with a forearm $< 52$ mm (frequency $44.3 \pm 0.9$ kHz, range $= 43.2$–$45.3$ kHz, $n = 4$; ANOVA, $F = 188.0$, *d.f.* $= 1, 31$, $P < 0.001$; Tukey test, $P < 0.001$). Although both forms increased their peak echolocation frequency in the flight room, the smaller bats had a mean peak frequency ($48.1 \pm 2.0$, range $= 45.6$–$50.9$ kHz, $n = 4$) that was still about 10 kHz higher than that used by the larger bats ($38.9 \pm 0.7$ kHz, range $= 38.5$–$39.4$ kHz, $n = 2$; ANOVA, $F = 86.8$, *d.f.* $= 1, 31$, $P = 0.0001$; Tukey test, $P < 0.001$). Peak frequency within the large bats did not differ significantly between Zambia ($33.0 \pm 1.4$ kHz) and Kruger National Park ($34.1 \pm 1.2$ kHz; *t*-test $= 1.7$, *d.f.* $= 20$, $P > 0.06$). Thus, 2 distinct phonic groups were recorded in yellow-bellied bats—a group of smaller bats (forearm $< 52$ mm) that echolocated at about 44 kHz (called henceforth *S. dinganii*–44 kHz) and a group of larger bats (forearm $> 52$ mm) that echolocated at about 33 kHz (*S. dinganii*–33 kHz).

*Genetic analyses*.—Genetic analysis included 56 specimens of *S. dinganii* and 2 individuals of *S. viridis* (Appendix I), resulting in a data set comprising 534 aligned nucleotides for 59 taxa when *Cytb* sequence data for the outgroup, *S. heathii*, was included. The control region was amplified for 52 specimens of *S. dinganii* and 1 *S. viridis* (the specimen from South Africa), resulting in an alignment length of 391 nucleotides for 53 taxa. Amplification of this region from 5 samples for which *Cytb* data were obtained (KZF1, GAU3, KNP3, KNP9, and FWC5053—see Appendix I) was unsuccessful despite multiple attempts at amplification. No control region sequence data were available on GenBank for *S. heathii*. The alignment for *Cytb* for all taxa contained 108 variable characters of which 70 were parsimony informative. The control region data set was characterized by 97 variable characters of which 47 were parsimony-informative characters.

Base frequencies did not deviate from stationarity across lineages for either of the mitochondrial data sets (*Cytb*: $\chi^2 = 74.66$, *d.f.* $= 174$, $P = 1.00$; control region: $\chi^2 = 47.0$, *d.f.* $= 156$, $P = 1.00$). The result of the incongruence length difference test was not significant ($P = 0.33$), indicating that the 2 respective genes exhibit less intergenic incongruence than 2 random partitions of a homogeneous data set.

Independent gene analyses resulted in largely congruent topologies and given the nonsignificant result of the incongruence length difference test, the data were combined. Parsimony analysis of the combined data resulted in 384 maximum-parsimony trees and the strict consensus is presented in Fig. 1. Although the topologies recovered by Bayesian and maximum-likelihood analyses of the combined data set were slightly different, all well-supported nodes in the maximum-parsimony tree (bootstrap $> 70\%$) were congruent across the different methods of phylogenetic analyses.

Regardless of the method of phylogenetic analysis or data set used, *S. dinganii*–44 kHz forms a highly supported

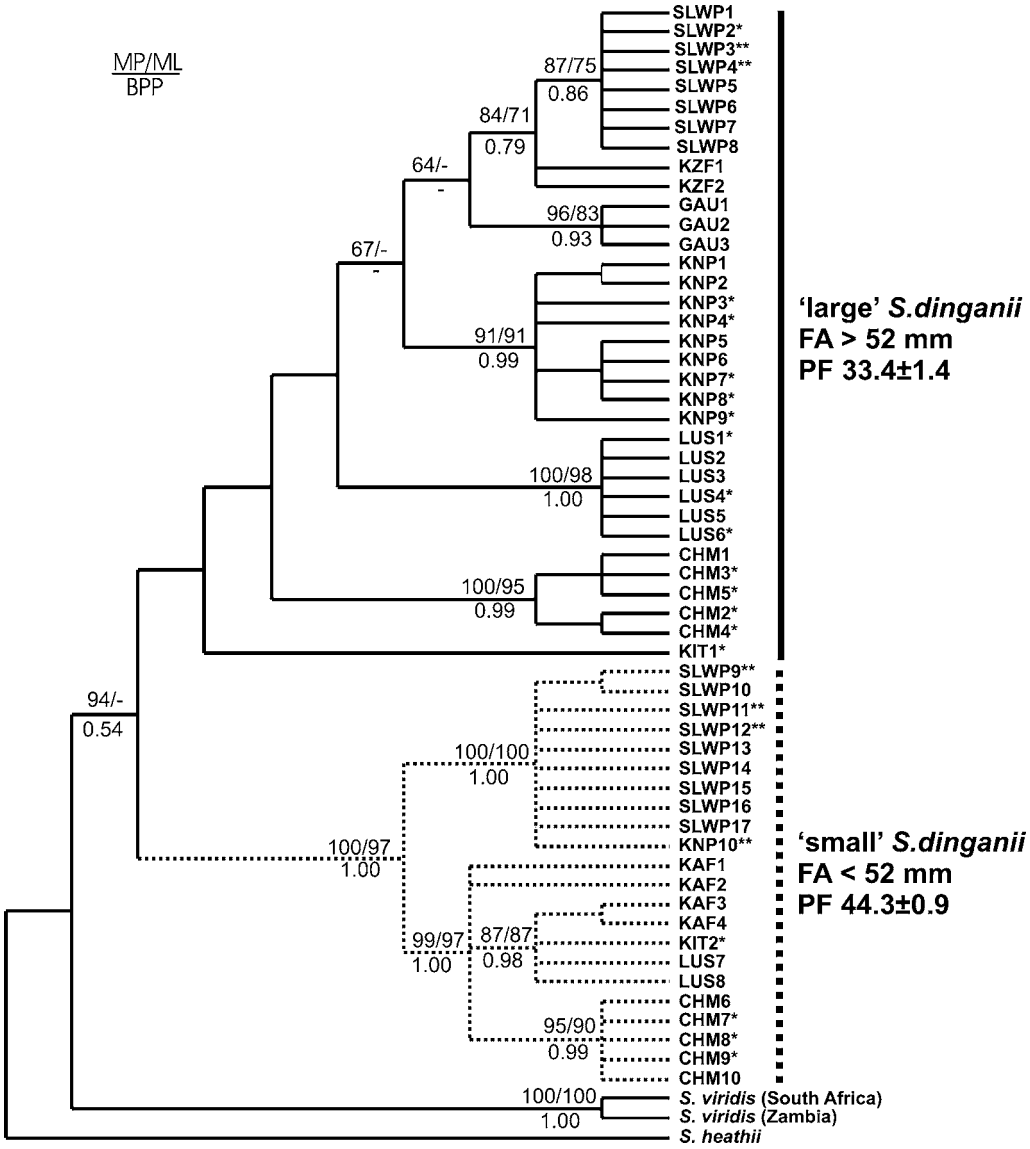


**FIG. 1.**—Strict consensus of 384 equally parsimonious trees recovered from analysis of combined cytochrome-*b* and control region sequence data for *Scotophilus dinganii* and *S. viridis* (tree length = 194, consistency index = 0.69, retention index = 0.94). Maximum-parsimony (MP) and maximum-likelihood (ML) bootstrap values > 50% are indicated above nodes; Bayesian posterior probability (BPP) values > 0.50 are indicated below nodes. SLWP = St. Lucia Wetland Park, KZF = Kranzkloof Nature Reserve, GAU = Gauteng, KNP = Kruger National Park, LUS = Lusaka, CHM = Choma, KIT = Kitwe, FA = forearm, PF = peak frequency. Specimens for which hand-released echolocation calls were recorded are indicated by a single asterisk; specimens for which only flight room calls were recorded are indicated by a double asterisk.

monophyletic group (Fig. 1). Both forms of *S. dinganii* appear to be reciprocally monophyletic, although bootstrap support for the *S. dinganii*–33 kHz clade was low in all analyses. *S. viridis* clearly forms a separate lineage from *S. dinganii*–44 kHz, to which it is morphologically similar. Both forms of *S. dinganii* clustered together in several lineages corresponding to sampling locality, with the Zambian specimens probably basal to the South African forms (Fig. 1).

Sequence divergence between the 2 forms of *S. dinganii* ranged from 2.4% to 3.9% (average 3.3%) for *Cytb* and 6% to 8.2% (average 6.8%) for the more rapidly evolving control region. Within *S. dinganii*–44 kHz, *Cytb* divergence values among individuals ranged from 0% to 1.5% (average 0.8%) compared to a range of 0–3% for *S. dinganii*–33 kHz. Control region divergence within *S. dinganii*–44 kHz ranged from 0% to 4.1% (average 2.2%) compared to a range of 0–4.3% (average 2.4%) for *S. dinganii*–33 kHz. Distance of the 2 forms of *S. dinganii* to *S. viridis* ranged from 7.9% to 9.4% (average 8.6%) for *Cytb* and 14.9% to 18% (average 16.4%) for control region sequences. The *Cytb* sequence of *S. heathii* was on average 11.4% divergent from the ingroup *Scotophilus* sequences (range 10.9–13.5%).

Mantel test results indicated significant isolation by distance for both forms of *S. dinganii* when control region divergence values were used (large form: $r = 0.625$, $P = 0.001$; small form: $r = 0.88$, $P = 0.02$). When *Cytb* divergence values were tested,

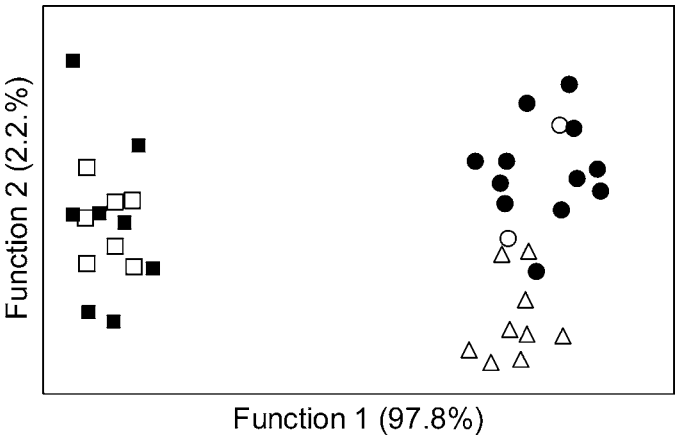


**FIG. 2.**—Plot of canonical scores from forward stepwise discriminant function analysis on skull parameters of *Scotophilus* species (Appendix II). Squares = *S. dinganii*–33 kHz, triangles = *S. dinganii*–44 kHz, and circles = *S. viridis*. Open symbols indicate specimens classified by DNA sequences.

there was significant isolation by distance for *S. dinganii*–33 kHz ($r = 0.45$, $P = 0.02$) but not for *S. dinganii*–44 kHz ($r = 0.91$, $P = 0.056$). When both data sets were pooled, there was significant isolation by distance for both the large ($r = 0.62$, $P = 0.009$) and small ($r = 0.90$, $P = 0.025$) forms.

*Skull morphology*.—In the DFAs on skull parameters (Appendix II), we classified individuals of the small form of *Scotophilus* as *S. dinganii*–44 kHz if they had a yellow venter and *S. viridis* if they had a gray or brown venter. Large *Scotophilus* with yellow venters were classified as *S. dinganii*–33 kHz.

The 3 groups were significantly separated by DFAs on 6 skull parameters (greatest skull length, dentary length, postorbital width, length of upper and lower toothrows, and length of upper molars; Wilks' lambda 0.01, $F = 16.6$, *d.f.* = 26, 48, $P < 0.0001$; Fig. 2; Table 1). *S. dinganii* (44 kHz) and *S. viridis* were separated from *S. dinganii*–33 kHz along function 1 (Fig. 2). *S. dinganii* (44 kHz) also was separated from *S. viridis* on function 2, although there was some overlap (Fig. 2). All of our initial classifications of bats into the 3 groups were upheld by DFAs with the exception of 2 individuals, one classified by us and the museum as *S. viridis* and the other classified by us as *S. dinganii*–44 kHz. However, because the identities of both of these individuals were confirmed by DNA sequences, we interpret this result as an indication of morphological overlap (Fig. 2) between *S. dinganii*–44 kHz and *S. viridis* rather than instances of misidentification. Our reclassification of a museum specimen from *S. viridis* to *S. dinganii*–44 kHz on the basis of its yellow venter was upheld by DFA.

Specimens for which we also had DNA sequence data were correctly assigned to their respective groups (Fig. 2). We are therefore confident in our identification of *S. dinganii*–33 kHz, and *S. viridis* corresponds to groups similarly labeled by previous workers. What we are calling *S. dinganii*–44 kHz is therefore an undescribed form.

These analyses are supported by multivariate ANOVA with post hoc Tukey tests on the 6 parameters extracted by DFAs.

**TABLE 1.**—Results of discriminant function analysis (DFA) on skull parameters. Only the results of those parameters that contributed significantly to the DFA model are shown.

| | Function 1 | Function 2 | Wilks' λ | F to remove | *P* |
|---|---|---|---|---|---|
| Dentary toothrow length | −1.198 | −0.622 | 0.016 | 7.601 | <0.002 |
| Skull length | −0.756 | −1.287 | 0.016 | 6.668 | <0.005 |
| Postorbital width | 0.913 | 0.269 | 0.015 | 6.568 | <0.005 |
| Maxillary toothrow length | 1.988 | 0.633 | 0.015 | 5.765 | <0.009 |
| Length of maxillary molars M1−3 | −0.838 | 0.193 | 0.014 | 4.936 | <0.016 |
| Dentary length | −0.677 | 0.635 | 0.013 | 4.072 | <0.030 |
| Eigenvalue | 47.033 | 1.078 | | | |
| Cumulative % | 97.8 | 100 | | | |
| Wilks' λ | 0.010 | 0.481 | | | |
| $\chi^2$ | 138.1 | 21.9 | | | |
| *d.f.* | 26 | 12 | | | |
| *P* | <0.0001 | <0.038 | | | |

Significant differences (ANOVA, $F = 26.5$, *d.f.* = 12, 62, $P < 0.001$) were found between the small *S. dinganii*–44 kHz and the large *S. dinganii*–33 kHz and between *S. dinganii*–33 kHz and *S. viridis* (Appendix II). Only postorbital skull width was not significantly different among the 3 groups (Tukey test, $P > 0.3$). The measurements for the remaining 5 parameters for the large *S. dinganii*–33 kHz were larger than those for the small *S. dinganii*–44 kHz and *S. viridis* (Tukey test, $P < 0.001$). No significant differences were found in the measurements for all 6 parameters between the small *S. dinganii*–44 kHz and *S. viridis* (Appendix II).

*External morphology*.—The 5 groups of *S. dinganii* identified by DNA sequences were significantly separated by DFAs on 5 morphological parameters (Wilks' lambda 0.04, $F = 9.4$, *d.f.* = 20, 113, $P < 0.0001$; Table 2; Fig. 3). Aspect ratio was the only variable that did not contribute significantly to the explained variance (Table 3).

The above interpretation is supported by multivariate ANOVA comparing *S. dinganii*–33 kHz and *S. dinganii*–44 kHz from Zambia (3 sites combined), St. Lucia Wetland Park, and Kruger National Park (*S. dinganii*–33 kHz only). Differences were found among sites ($F = 9.2$, *d.f.* = 20, 90, $P < 0.03$), but not between sexes ($F = 2.5$, *d.f.* = 5, 27, $P > 0.06$), and the interaction between sex and clade was not significant ($F = 1.3$, *d.f.* = 20, 90, $P > 0.2$).

At all sites *S. dinganii*–44 kHz was smaller than *S. dinganii*–33 kHz, with shorter forearms and heads (Tukey test, $P < 0.001$ in all cases; Table 2). In Zambia and St. Lucia Wetland Park, no significant differences were found in any of the wing parameters (Tukey test, $P > 0.1$ in all cases), with the exception of wing loading, between *S. dinganii*–44 kHz and their *S. dinganii*–33 kHz counterparts. Wing loading was higher in *S. dinganii*–44 kHz from St. Lucia Wetland Park than in *S. dinganii*–33 kHz from the same site. The forearm lengths of *S. dinganii*–33 kHz and *S. dinganii*–44 kHz did not overlap at either site (Table 2). Similarly, although most of the wing parameters of the 2 forms overlapped appreciably, size parameters overlapped only slightly (Table 2). Only Zambia and St. Lucia Wetland Park were compared here because we caught only 1 *S. dinganii*–44 kHz at Kruger National Park.

**TABLE 2.**—Morphological parameters for *Scotophilus dinganii*–44 kHz (small) and *S. dinganii*–33 kHz (large).

| | Zambia | | | | St. Lucia | | | | Kruger National Park | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Small, 44 kHz ($n = 8$) | | Large, 33 kHz ($n = 11$) | | Small, 44 kHz ($n = 9$) | | Large, 33 kHz ($n = 7$) | | Large, 33 kHz ($n = 7$) | |
| | $\bar{X} \pm SD$ | Range | $\bar{X} \pm SD$ | Range | $\bar{X} \pm SD$ | Range | $\bar{X} \pm SD$ | Range | $\bar{X} \pm SD$ | Range |
| Forearm length (cm) | 4.9 ± 0.2 | 4.6–5.2 | 5.7 ± 0.2 | 5.4–6.1 | 4.7 ± 0.1 | 4.6–4.9 | 5.3 ± 0.1 | 5.2–5.4 | 5.3 ± 0.1 | 5.1–5.4 |
| Mass (g) | 27.9 ± 6.1 | 21.0–37.0 | 33.4 ± 8.0 | 21.5–47.0 | 28.0 ± 3.1 | 25.0–34.0 | 26.6 ± 2.8 | 23.0–30.5 | 24.9 ± 1.7 | 21.5–28.0 |
| Total length (cm) | 12.4 ± 0.3 | 12.0–12.8 | 13.9 ± 0.4 | 12.9–14.3 | 11.7 ± 0.3 | 11.3–12.3 | 12.9 ± 0.2 | 12.6–13.2 | 12.8 ± 0.2 | 12.6–13.2 |
| Head length (cm) | 2.2 ± 0.1 | 2.1–2.2 | 2.5 ± 0.1 | 2.4–2.6 | 2.2 ± 0.1 | 2.1–2.4 | 2.4 ± 0.1 | 2.2–2.5 | 2.4 ± 0.04 | 2.3–2.4 |
| Wingspan (cm) | 31.1 ± 1.4 | 30.2–34.1 | 36.2 ± 1.5 | 32.8–38.5 | 30.5 ± 0.8 | 29.7–32.6 | 34.7 ± 1.1 | 32.8–36.3 | 33.2 ± 0.8 | 31.9–34.3 |
| Wing area ($cm^2$) | 178.2 ± 13.0 | 153.3–199.8 | 220.5 ± 13.3 | 187.0–234.5 | 160.3 ± 5.9 | 153.7–170.7 | 198.3 ± 14.3 | 186.4–229.2 | 192.1 ± 9.1 | 177.4–205.4 |
| Wing loading ($Nm^{-2}$) | 15.3 ± 3.1 | 11.9–20.8 | 14.7 ± 2.9 | 10.9–19.7 | 17.1 ± 1.7 | 15.3–20.0 | 13.1 ± 1.1 | 11.4–15.1 | 12.8 ± 1.2 | 10.6–13.8 |
| Aspect ratio | 5.8 ± 0.3 | 5.3–6.0 | 6.0 ± 0.2 | 5.7–6.3 | 5.8 ± 0.2 | 5.5–6.2 | 6.1 ± 0.5 | 5.4–6.5 | 5.7 ± 0.2 | 5.4–6.0 |
| Wing-tip shape index | 0.9 ± 0.2 | 0.7–1.2 | 0.6 ± 0.04 | 0.6–0.7 | 0.6 ± 0.05 | 0.6–0.7 | 0.6 ± 0.1 | 0.6–0.7 | 0.6 ± 0.1 | 0.4–0.7 |

## DISCUSSION

Large forearm size, yellow venter, and the similarity of both its skull morphology and DNA sequences to museum specimens of *S. dinganii* identify our 33-kHz phonic type as *S. dinganii*. Its peak frequency also matches that reported for *S. dinganii* (Taylor 1999, 2000). Unfortunately, we were unable to obtain the type specimen of *S. dinganii* for comparison.

Examination of the genetic, morphological, and echolocation data presented here suggests that the 44-kHz phonic type is a cryptic species. It is nevertheless possible that the 44-kHz phonic type is either *S. viridis* or the southern African subspecies of *S. leucogaster* (Robbins et al. 1985). *S. viridis* has been described as having some yellow in its ventral pelage (Robbins et al. 1985) and our comparison between *S. viridis* and the 44-kHz phonic type shows them to be morphologically similar. In fact, in the absence of our echolocation and genetic data, we would have concluded that only 2 forms were represented in our Fig. 2. However, the large sequence divergence from *S. viridis* and the phylogenetic affinity of the 44-kHz phonic type with *S. dinganii* suggest that it is a sister taxon of *S. dinganii* rather than *S. viridis*. The echolocation peak frequency used by *S. viridis* (40 kHz—Fenton and Bell 1981) also differs from that of the 44-kHz phonic type, albeit by only 4 kHz. Furthermore, we were able to correctly classify *S. viridis* and the 44-kHz phonic type on the basis of the yellow venter pelage of the latter. The statement that the ventral pelage of *S. viridis* ranges in color from light brown to yellow (Robbins et al. 1985) is probably due to the inclusion of the 44-kHz phonic type with *S. viridis*, on the basis of similar morphology.

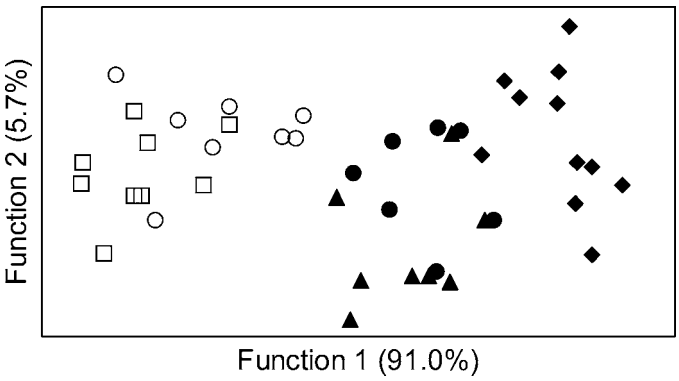


**FIG. 3.**—Plot of canonical scores from forward stepwise discriminant function analysis on external morphological parameters for *Scotophilus* species. Open symbols are *S. dinganii*–44 kHz and solid symbols are *S. dinganii*–33 kHz. Locations: for 44-kHz form—open circles, Zambia; open squares, St. Lucia Wetland Park; for 33-kHz form—solid circles, Kruger National Park; solid triangles, St. Lucia Wetland Park; solid diamonds, Zambia.

It is unlikely that the 44-kHz phonic type is *S. leucogaster* because the latter does not have the yellow venter whereas the former does. Simmons (2005) also restricts *S. leucogaster* to eastern Africa. Furthermore, *S. leucogaster* is not a sister taxon to *S. dinganii* (Hoofer and Van Den Bussche 2003), whereas examination of our genetic data suggests that the 44-kHz phonic type is.

Thus, the existence of a cryptic species is supported by genetic, morphological, and echolocation data. The low sequence divergence between the 2 phonic types of *S. dinganii* suggests either a recent divergence between these 2 lineages or a decreased rate of mitochondrial DNA evolution in this particular clade. Unfortunately, no fossil calibration points are available for *Scotophilus*; therefore, no acceptable estimates of divergence times are possible. However, the morphological differences between the 2 phonic types of *S. dinganii* are of the same magnitude as that between *S. dinganii*–33 kHz and *S. viridis*, a species recognized as distinct (Schlitter et al. 1980; this study).

Although a gene tree is not necessarily the same as a species tree (Nichols 2001), and the phylogenetic pattern we observe may simply reflect random lineage sorting from a polymorphic ancestor (Harrison 1998), multiple lines of evidence (echolocation, morphology, and mitochondrial DNA data) suggest that the small form is indeed a separate evolutionary lineage. The geographical partitioning of lineages in both lineages of *S. dinganii* and the overall significant isolation-by-distance results reflect restricted gene flow between geographic localities. Differences in size and echolocation probably reflect difference in ecology because there is a strong correlation between morphology and echolocation on the one hand and foraging habitat and diet on the other (Findley and Wilson 1982; Norberg and Rayner 1987; Schnitzler and Kalko 2001).

**TABLE 3.**—The results of discriminant function analysis on 5 external morphological parameters.

| | Forearm length | Head length | Wing loading | Wing-tip shape index | Aspect ratio | Eigenvalue | Cumulative % | Wilks' λ | $\chi^2$ | *d.f.* | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Function 1 | 0.661 | 0.708 | −0.207 | −0.284 | 0.185 | 10.147 | 91.0 | 0.039 | 119.7 | 20 | <0.0001 |
| Function 2 | 0.387 | −0.256 | 0.564 | −0.767 | −0.423 | 0.637 | 96.7 | 0.438 | 30.5 | 12 | <0.002 |
| Function 3 | 0.108 | −0.173 | −0.728 | 0.024 | −0.668 | 0.236 | 98.8 | 0.717 | 12.3 | 6 | <0.06 |
| Function 4 | −0.422 | 0.593 | 0.446 | 0.066 | −0.460 | 0.128 | 100.0 | 0.886 | 4.5 | 2 | >0.1 |
| Wilks' λ | 0.070 | 0.071 | 0.053 | 0.053 | 0.048 | | | | | | |
| F-remove | 6.550 | 6.890 | 2.909 | 2.988 | 1.948 | | | | | | |
| *P* | <0.001 | <0.001 | <0.04 | <0.04 | >0.12 | | | | | | |

The sympatric distribution of *S. dinganii* (see also Fenton and Rautenbach 1998) and the 44-kHz phonic type raises the possibility that the divergence occurred in sympatry. However, ecological differentiation (disruptive selection by intraspecific competition) and assortative mating, or at least assortative roosting behavior (e.g., Jones and van Parijs 1993), remain to be demonstrated in the 2 forms of *S. dinganii*. Furthermore, as was the case with the *P. pipistrellus*–*P. pygmaeus* complex (Hulva et al. 2004), much more needs to be known about the population structure and geographic distribution of the 2 cryptic *Scotophilus* species before the mode of speciation can be determined. Lastly, forearm length, head length, total body length, and peak echolocation frequency are good characters to distinguish between *S. dinganii* and the 44-kHz phonic type in the field, whereas the yellow venter distinguishes *S. dinganii* and the 44-kHz phonic type from *S. viridis* and *S. leucogaster*.

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## LITERATURE CITED

ANIMAL CARE AND USE COMMITTEE. 1998. Guidelines for the capture, handling, and care of mammals as approved by the American Society of Mammalogists. Journal of Mammalogy 79:1416–1431.

BARLOW, K. E. 1997. The diets of two phonic types of the bat *Pipistrellus pipistrellus* in Britain. Journal of Zoology (London) 243:597–609.

BARRATT, E. M., R. DEAVILLE, T. M. BURLAND, M. W. BRUFORD, G. JONES, P. A. RACEY, AND R. K. WAYNE. 1997. DNA answers the call of pipistrelle bat species. Nature 387:138–139.

BONNET, E., AND Y. VAN DE PEER. 2002. zt: a software tool for simple and partial Mantel tests. Journal of Statistical Software 7:1–12.

FARRIS, J. S., M. KALLERSJO, A. G. KLUGE, AND C. BULT. 1995. Constructing a significance test for incongruence. Systematic Biology 44:570–572.

FENTON, M. B., AND G. P. BELL. 1981. Recognition of species of insectivorous bats by their echolocation calls. Journal of Mammalogy 62:233–243.

FENTON, M. B., AND I. L. RAUTENBACH. 1998. Impacts of ignorance and human and elephant populations on the conservation of bats in African woodlands. Pp. 261–269 in Bat biology and conservation (T. H. Kunz and P. A. Racey, eds.). Smithsonian Institution Press, Washington, D.C.

FINDLEY, J. S., AND D. E. WILSON. 1982. Echolocation, insect hearing, and feeding ecology of insectivorous bats. Pp. 243–260 in Ecology of bats (T. H. Kunz, ed.). Plenum Press, New York.

FREEMAN, P. W. 1981. Correspondence of food habits and morphology in insectivorous bats. Journal of Mammalogy 62:166–173.

HARRISON, R. G. 1998. Linking evolutionary patterns and processes: the relevance of species concepts for the study of speciation. Pp. 19–31 in Endless forms: species and speciation (D. J. Howard and S. H. Berlocher, eds.). Oxford University Press, New York.

HOOFER, S. R., AND R. A. VAN DEN BUSSCHE. 2003. Molecular phylogenetics of the chiropteran family Vespertilionidae. Acta Chiropterologica 5:1–63.

HUELSENBECK, J. P., AND F. RONQUIST. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.

HULVA, P., I. HORÁČEK, P. P. STRELKOV, AND P. BENDA. 2004. Molecular architecture of *Pipistrellus pipistrellus*/*Pipistrellus pygmaeus* complex (Chiroptera: Vespertilionidae): further cryptic species and Mediterranean origin of the divergence. Molecular Phylogenetics and Evolution 32:1023–1035.

IRWIN, D. M., T. D. KOCHER, AND A. C. WILSON. 1991. Evolution of the cytochrome *b* gene of mammals. Journal of Molecular Evolution 32:128–144.

JACOBS, D. S. 1996. Morphological divergence in an insular bat, *Lasiurus cinereus semotus*. Functional Ecology 10:622–630.

JACOBS, D. S., G. N. EICK, E. J. RICHARDSON, AND P. TAYLOR. 2004. Genetic similarity amongst phenotypically diverse little free-tailed bats, *Chaerephon pumilus*. Acta Chiropterologica 6:13–21.

JONES, G., AND S. M. VAN PARIJS. 1993. Bimodal echolocation in pipistrelle bats: are cryptic species present? Proceedings of the Royal Society of London, B. Biological Sciences 251:119–125.

KIEFER, A., F. MAYER, J. KOSUCH, O. VON HELVERSEN, AND M. VEITH. 2002. Conflicting molecular phylogenies of European long-eared bats (*Plecotus*) can be explained by cryptic diversity. Molecular Phylogenetics and Evolution 25:557–566.

KINGSTON, T., AND S. J. ROSSITER. 2004. Harmonic hopping in Wallacea's bats. Nature 429:654–657.

MATTHEE, C. A., B. JANSEN VAN VUUREN, D. BELL, AND T. J. ROBINSON. 2004. A molecular supermatrix of the rabbits and hares (Leporidae) allows for the identification of five intercontinental exchanges during the Miocene. Systematic Biology 53:433–447.

MAYER, F., AND O. VON HELVERSEN. 2001a. Sympatric distribution of two cryptic bat species across Europe. Biological Journal of the Linnean Society 74:365–374.

MAYER, F., AND O. VON HELVERSEN. 2001b. Cryptic diversity in European bats. Proceedings of the Royal Society of London, B. Biological Sciences 268:1825–1832.

NICHOLS, R. 2001. Gene trees and species trees are not the same. Trends in Ecology and Evolution 16:358–364.

NORBERG, U. M., AND J. M. V. RAYNER. 1987. Ecological morphology and flight in bats (Mammalia: Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. Philosophical Transactions of the Royal Society of London, B. Biological Sciences 316:335–427.

O'FARRELL, M. J., B. W. MILLER, AND W. L. GANNON. 1999. Qualitative identification of free-flying bats using the Anabat detector. Journal of Mammalogy 80:11–23.

OMLAND, K. E., C. L. TARR, W. I. BOARMAN, J. M. MARZLUFF, AND R. C. FLEISCHER. 2000. Cryptic genetic variation and paraphyly in ravens. Proceedings of the Royal Society of London, B. Biological Sciences 267:2475–2482.

POSADA, D., AND K. A. CRANDALL. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14:818–819.

ROBBINS, C. B., F. DE VREE, AND V. VAN CAKENBERGHE. 1985. A systematic revision of the African bat genus *Scotophilus* (Vespertilionidae). Musee Royal de l'Afrique Centrale Tervuren, Belgique 246:51–84.

RUEDI, M., AND F. MAYER. 2001. Molecular systematics of bats of the genus *Myotis* (Vespertilionidae) suggests deterministic ecomorphological convergences. Molecular Phylogenetics and Evolution 21:436–448.

SAMBROOK, J., E. F. FRITSCH, AND T. MANIATIS. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

SCHLITTER, D. A., I. L. RAUTENBACH, AND D. A. WOLHUTER. 1980. Karyotypes and morphometrics of two species of *Scotophilus* in South Africa (Mammalia: Vespertilionidae). Annals of the Transvaal Museum 32:231–239.

SCHNITZLER, H.-U., AND E. K. V. KALKO. 2001. Echolocation by insect-eating bats. BioScience 51:557–569.

SCHOEMAN, M. C., AND D. S. JACOBS. 2003. Support for the allotonic frequency hypothesis in an insectivorous bat community. Oecologia 134:154–162.

SIMMONS, N. 2005. Order Chiroptera. Pp. 312–529 in Mammal species of the world: a taxonomic and geographic reference (D. E. Wilson and D. M. Reeder, eds.). 3rd ed. Johns Hopkins University Press, Baltimore, Maryland.

SWOFFORD, D. L. 2002. PAUP* 4.0. Phylogenetic analysis using parsimony (*and other methods). Beta version 4.0b4a. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

TAYLOR, P. J. 1999. Echolocation calls of twenty South African bat species. South African Journal of Zoology 33:114–124.

TAYLOR, P. J. 2000. Bats of southern Africa. University of Natal Press, Scottsville, South Africa.

THOMPSON, J. D., T. J. GIBSON, F. PLEWNIAK, F. JEANMOUGIN, AND D. G. HIGGINS. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25:4876–4882.

VON HELVERSEN, O., K.-G. HELLER, F. MAYER, AND A. NEMETH. 2001. Cryptic mammalian species: a new species of whiskered bat (*Myotis alcathoe* n. sp.) in Europe. Naturwissenschaften 88:217–223.

WADDELL, P. J., Y. CAO, J. HAUF, AND M. HASEGAWA. 1999. Using novel phylogenetic methods to evaluate mammalian mtDNA, including amino acid–invariant sites—LogDet plus site stripping, to detect internal conflicts in the data, with special reference to the positions of hedgehog, armadillo, and elephant. Systematic Biology 48:31–53.

WILKINSON, G. S., AND A. M. CHAPMAN. 1991. Length and sequence variation in evening bat D-loop mtDNA. Genetics 128:607–617.

YODER, A. D., R. M. RASOLOARISON, S. M. GOODMAN, J. A. IRWIN, S. ATSALIS, M. J. RAVOSA, AND J. H. GANZHORN. 2000. Remarkable species diversity in Malagasy mouse lemurs (Primates, *Microcebus*). Proceedings of the National Academy of Sciences USA 97:1325–1330.

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## APPENDIX I

Captured bats and museum specimens used in genetic and phenotypic analyses. Specimens for which vouchers were taken are indicated by the prefix TM (lodged in the Northern Flagship Institution). The code used in analyses is the code we assigned each specimen to make identification of the locality from which the specimens were collected easier (see the key to abbreviations in the footnote[a]).

| Species | Specimen number | Collection locality | Source of material | Forearm length (cm) | Code used in analyses | GenBank accession no. (cytochrome *b*) | GenBank accession no. (control region) |
|---|---|---|---|---|---|---|---|
| *Scotophilus dinganii* | P84 | Kranzkloof Nature Reserve, KZN, SA | Teresa Kearney | 5.3 | KZF1 | AY754063 | |
| | P85 | Kranzkloof Nature Reserve, KZN, SA | Teresa Kearney | 5.3 | KZF2 | AY754063 | AY754079 |
| | TM47501 | Bruce Miller Hunting Lodge Reservoir, Choma, Zambia | DSJ, UCT, SA | 5.7 | CHM1 | AY754066 | AY754083 |
| | DSJZM72 | Bruce Miller Hunting Lodge Reservoir, Choma, Zambia | DSJ, UCT, SA | 5.5 | CHM2 | AY754067 | AY754084 |
| | DSJZM73 | Bruce Miller Hunting Lodge Reservoir, Choma, Zambia | DSJ, UCT, SA | 5.6 | CHM3 | AY754066 | AY754083 |
| | DSJZM37 | Bruce Miller Pond, Choma, Zambia | DSJ, UCT, SA | 5.5 | CHM4 | AY754067 | AY754084 |
| | DSJZM30 | Bruce Miller Pond, Choma, Zambia | DSJ, UCT, SA | 5.6 | CHM5 | AY754066 | AY754083 |
| | DSJZM16 | Leopard Hill Farm, Lusaka, Zambia | DSJ, UCT, SA | 5.7 | LUS1 | AY754065 | AY754082 |

**APPENDIX 1.**—Continued.

| Species | Specimen number | Collection locality | Source of material | Forearm length (cm) | Code used in analyses | GenBank accession no. (cytochrome *b*) | GenBank accession no. (control region) |
|---|---|---|---|---|---|---|---|
| | DSJZM17 | Leopard Hill Farm, Lusaka, Zambia | DSJ, UCT, SA | 5.6 | LUS2 | AY754065 | AY754082 |
| | TM47502 | Leopard Hill Farm, Lusaka, Zambia | DSJ, UCT, SA | 5.5 | LUS3 | AY754065 | AY754082 |
| | DSJZM21 | Leopard Hill Farm, Lusaka, Zambia | DSJ, UCT, SA | 5.7 | LUS4 | AY754065 | AY754082 |
| | DSJZM22 | Leopard Hill Farm, Lusaka, Zambia | DSJ, UCT, SA | 5.9 | LUS5 | AY754065 | AY754082 |
| | TM47500 | Leopard Hill Farm, Lusaka, Zambia | DSJ, UCT, SA | 5.4 | LUS6 | AY754065 | AY754082 |
| | DSJZM86 | Mike Fisher Farm, Kitwe, Zambia | DSJ, UCT, SA | 6.1 | KIT1 | AY754068 | AY754085 |
| | TM39624 | Pilgrims Rest Camp, KNP, SA | Northern Flagship Institution | 5.1 | KNP1 | AY754060 | AY754077 |
| | TM39625 | Pilgrims Rest Camp, KNP, SA | Northern Flagship Institution | 5.1 | KNP2 | AY754060 | See KNP1 |
| | DSJKP1 | Skukuza, KNP, South Africa | DSJ, UCT, SA | 5.4 | KNP3 | AY754064 | |
| | DSJKP4 | Skukuza, KNP, South Africa | DSJ, UCT, SA | 5.1 | KNP4 | AY754064 | AY754080 |
| | TM47496 | Skukuza, KNP, South Africa | DSJ, UCT, SA | 5.1 | KNP5 | AY754064 | AY754081 |
| | TM47497 | Skukuza, KNP, South Africa | DSJ, UCT, SA | 5.3 | KNP6 | AY754064 | AY754081 |
| | DSJKP18 | Skukuza, KNP, South Africa | DSJ, UCT, SA | 5.4 | KNP7 | AY754064 | AY754081 |
| | DSJKP19 | Skukuza, KNP, South Africa | DSJ, UCT, SA | 5.3 | KNP8 | AY754064 | AY754081 |
| | 23.12.02Sd1(AED) | White River, Mpumalanga, SA | DSJ, UCT, SA | 5.4 | KNP9 | AY754064 | |
| | WW2004/01 | Crocodile Farm, KZN, SA | Wendy White | 5.2 | SLWP1 | AY754059 | AY754076 |
| | WW2004/02 | Sugarloaf Camp, KZN, SA | Wendy White | 5.4 | SLWP2 | AY754059 | AY754076 |
| | DSJ54 | Sugarloaf Camp, KZN, SA | DSJ, UCT, SA | 5.3 | SLWP3 | AY754059 | AY754076 |
| | DSJ76 | Sugarloaf Camp, KZN, SA | DSJ, UCT, SA | 4.9 | SLWP4 | AY754059 | AY754076 |
| | DSJ85 | Sugarloaf Camp, KZN, SA | DSJ, UCT, SA | 5.4 | SLWP5 | AY754059 | AY754076 |
| | DSJ86 | Sugarloaf Camp, KZN, SA | DSJ, UCT, SA | 5.4 | SLWP6 | AY754059 | AY754076 |
| | DSJ87 | Sugarloaf Camp, KZN, SA | DSJ, UCT, SA | 5.2 | SLWP7 | AY754059 | AY754076 |
| | TM47495 | Sugarloaf Camp, KZN, SA | DSJ, UCT, SA | 5.4 | SLWP8 | AY754059 | AY754076 |
| | TM46657 | Cullinan, Gauteng, SA | Northern Flagship Institution | 5.4 | GAU1 | AY754061 | AY754078 |
| | TM46906 | Tswaing Crater, Gauteng SA | Northern Flagship Institution | 5.7 | GAU2 | AY754062 | AY754078 |
| | TM46905 | Tswaing Crater, Gauteng, SA | Northern Flagship Institution | 5.8 | GAU3 | AY754061 | |
| | DSJZM57 | Bruce Miller Masuku Lodge Dam, Choma, Zambia | DSJ, UCT, SA | 5.1 | CHM6 | AY754073 | AY754093 |
| | DSJZM58 | Bruce Miller Masuku Lodge Dam, Choma, Zambia | DSJ, UCT, SA | 5.0 | CHM7 | AY754073 | AY754093 |
| | DSJZM66 | Bruce Miller Camp, Choma, Zambia | DSJ, UCT, SA | 4.7 | CHM8 | AY754073 | AY754093 |
| | DSJZM74 | Bruce Miller Hunting Lodge Reservoir, Choma, Zambia | DSJ, UCT, SA | 5.2 | CHM9 | AY754073 | AY754093 |
| | TM47509 | Bruce Miller Hunting Lodge Reservoir, Choma, Zambia | DSJ, UCT, SA | 4.8 | CHM10 | AY754073 | AY754094 |
| | TM47508 | Dendro Park, Kafue Flats, Zambia | F.W.C. Cotterill | 4.6 | KAF1 | AY754070 | AY754089 |
| | TM47505 | Dendro Park, Kafue Flats, Zambia | F.W.C. Cotterill | 4.9 | KAF2 | AY754071 | AY754090 |
| | TM47506 | Dendro Park, Kafue Flats, Zambia | F.W.C. Cotterill | 4.9 | KAF3 | AY754072 | AY754091 |
| | TM47507 | Dendro Park, Kafue Flats, Zambia | F.W.C. Cotterill | 4.7 | KAF4 | AY754072 | AY754091 |
| | TM47510 | Leopard Hill Farm, Lusaka, Zambia | DSJ, UCT, SA | 5.1 | LUS7 | AY754072 | AY754092 |
| | TM47511 | Leopard Hill Farm, Lusaka, Zambia | DSJ, UCT, SA | 4.6 | LUS8 | AY754072 | AY754092 |
| | DSJZM111 | Mike Fisher Dam, Kitwe, Zambia | DSJ, UCT, SA | 5.0 | KIT2 | AY754072 | AY754092 |
| | TM47504 | Skukuza, KNP, SA | DSJ, UCT, SA | 4.8 | KNP10 | AY754069 | AY754087 |

**APPENDIX 1.**—Continued.

| Species | Specimen number | Collection locality | Source of material | Forearm length (cm) | Code used in analyses | GenBank accession no. (cytochrome *b*) | GenBank accession no. (control region) |
|---|---|---|---|---|---|---|---|
| | DSJ51 | Sugarloaf Camp, KZN, SA | DSJ, UCT, SA | 4.8 | SLWP9 | AY754069 | AY754086 |
| | DSJ53 | Sugarloaf Camp, KZN, SA | DSJ, UCT, SA | 4.7 | SLWP10 | AY754069 | AY754088 |
| | DSJ55 | Sugarloaf Camp, KZN, SA | DSJ, UCT, SA | 4.6 | SLWP11 | AY754069 | AY754087 |
| | DSJ57 | Sugarloaf Camp, KZN, SA | DSJ, UCT, SA | 4.7 | SLWP12 | AY754069 | AY754087 |
| | DSJ73 | Sugarloaf Camp, KZN, SA | DSJ, UCT, SA | 4.6 | SLWP13 | AY754069 | AY754087 |
| | DSJ78 | Sugarloaf Camp, KZN, SA | DSJ, UCT, SA | 4.6 | SLWP14 | AY754069 | AY754087 |
| | DSJ80 | Sugarloaf Camp, KZN, SA | DSJ, UCT, SA | 4.6 | SLWP15 | AY754069 | AY754087 |
| | DSJ81 | Sugarloaf Camp, KZN, SA | DSJ, UCT, SA | 5.3 | SLWP16 | AY754069 | AY754087 |
| | DSJ52 | Sugarloaf Camp, KZN, SA | DSJ, UCT, SA | 4.8 | SLWP17 | AY754069 | AY754087 |
| *S. viridis* | TM39481 | Manditobe Dam, Limpopo Province, SA | Northern Flagship Institution | 5.0 | NA | AY754074 | AY754095 |
| | TM47503 | Dendro Park, Kafue Flats, Zambia | F. W. C. Cotterill | 5.1 | NA | AY754075 | |

[a] Abbreviations: CHM = Choma, DSJ = D. S. Jacobs, Gau = Gauteng, KAF = Kafue Flats, KIT = Kitwe, KNP = Kruger National Park, KZF = Kranzkloof Nature Reserve, KZN = KwaZulu Natal Province, LUS = Lusaka, NA = not applicable, SA = South Africa, SLWP = St. Lucia Wetland Park, UCT = University of Cape Town.

## APPENDIX II

Skull measurements (in mm) and scores on identification model of Schlitter et al. (1980) of 3 groups of *Scotophilus*. Unless otherwise indicated all measurements were taken as described by Freeman (1981) or Jacobs (1996).

| | *S. viridis* ($n = 14$) | | *S. dinganii*−44 kHz ($n = 9$) | | *S. dinganii*−33 kHz ($n = 16$) | |
|---|---|---|---|---|---|---|
| | $\bar{X} \pm SD$ | Range | $\bar{X} \pm SD$ | Range | $\bar{X} \pm SD$ | Range |
| Skull length | 17.4 ± 0.3 | 16.9−18.0 | 17.7 ± 0.4 | 17.0−18.2 | 20.1 ± 0.4 | 19.1−20.7 |
| Condylobasal length[a] | 15.2 ± 0.4 | 14.5−15.7 | 15.5 ± 0.3 | 15.0−15.8 | 17.3 ± 0.5 | 16.8−18.4 |
| Skull breadth | 8.9 ± 0.1 | 8.7−9.2 | 9.0 ± 0.2 | 8.7−9.3 | 9.7 ± 0.2 | 9.4−10.2 |
| Skull height[b] | 8.5 ± 0.4 | 8.0−9.8 | 8.4 ± 0.3 | 7.7−8.8 | 9.4 ± 0.4 | 8.8−10.6 |
| Skull height[c] | 7.7 ± 0.2 | 7.2−8.0 | 7.5 ± 0.3 | 6.9−7.9 | 8.5 ± 0.3 | 7.7−8.9 |
| Zygomatic breadth | 12.8 ± 0.3 | 12.2−13.2 | 12.7 ± 0.5 | 12.0−13.3 | 14.3 ± 0.7 | 3.2−14.8 |
| Postorbital width | 4.7 ± 0.2 | 4.3−5.0 | 4.7 ± 0.1 | 4.5−4.9 | 4.8 ± 0.2 | 4.4−5.2 |
| Width at upper canines | 6.3 ± 0.3 | 5.6−6.8 | 6.2 ± 0.1 | 6.0−6.4 | 7.2 ± 0.2 | 6.9−7.6 |
| Least palatal length | 5.8 ± 0.1 | 5.7−6.0 | 5.9 ± 0.4 | 5.7−6.7 | 6.7 ± 0.3 | 6.1−7.3 |
| Maxillary toothrow length | 6.2 ± 0.2 | 5.8−6.5 | 6.3 ± 0.1 | 6.0−6.5 | 7.2 ± 0.2 | 7.0−7.6 |
| Length of maxillary molars M1−3 | 3.9 ± 0.2 | 3.5−4.1 | 3.9 ± 0.1 | 3.7−4.1 | 4.5 ± 0.1 | 4.4−4.8 |
| Length of maxillary P4−M3 | 4.9 ± 0.1 | 4.7−5.1 | 5.0 ± 0.1 | 4.9−5.1 | 5.7 ± 0.2 | 5.4−6.0 |
| Crown breadth of M3−M3 | 8.2 ± 0.2 | 7.8−8.3 | 8.3 ± 0.2 | 8.0−8.6 | 9.3 ± 0.3 | 8.8−9.9 |
| Canine height | 3.3 ± 0.3 | 2.8−3.7 | 3.4 ± 0.2 | 3.1−3.6 | 4.2 ± 0.2 | 3.8−4.5 |
| Canine width | 1.1 ± 0.1 | 0.8−1.2 | 1.1 ± 0.1 | 1.0−1.3 | 1.3 ± 0.1 | 1.1−1.5 |
| Breadth of M2 | 2.0 ± 0.1 | 1.8−2.2 | 2.0 ± 0.1 | 1.8−2.1 | 2.3 ± 0.1 | 2.1−2.5 |
| Length of M2 | 1.6 ± 0.1 | 1.4−1.7 | 1.6 ± 0.1 | 1.5−1.6 | 1.8 ± 0.1 | 1.7−1.9 |
| Breadth of M3 | 2.0 ± 0.1 | 1.8−2.2 | 2.0 ± 0.1 | 1.8−2.1 | 2.3 ± 0.1 | 2.1−2.4 |
| Length of M3 | 0.8 ± 0.1 | 0.6−0.8 | 0.8 ± 0.1 | 0.7−0.9 | 0.8 ± 0.05 | 0.7−0.9 |
| Origin of masseter | 6.1 ± 0.2 | 5.7−6.4 | 6.2 ± 0.1 | 6.0−6.4 | 7.0 ± 0.3 | 6.5−7.7 |
| Insertion of masseter[d] | 3.2 ± 0.2 | 3.0−3.6 | 3.1 ± 0.1 | 3.0−3.3 | 3.74 ± 0.2 | 3.3−4.2 |
| Dentary length | 12.9 ± 0.3 | 12.4−13.3 | 12.6 ± 0.5 | 12.1−13.3 | 14.7 ± 0.3 | 14.0−15.0 |
| Dentary thickness | 2.1 ± 0.2 | 1.8−2.3 | 2.0 ± 0.1 | 1.9−2.2 | 2.4 ± 0.2 | 2.1−2.7 |
| Length of masseter muscle scar | 4.0 ± 0.3 | 3.5−4.4 | 4.3 ± 0.3 | 3.8−4.8 | 4.6 ± 0.2 | 4.2−5.0 |
| Coronoid height | 5.1 ± 0.2 | 4.7−5.4 | 5.0 ± 0.2 | 4.7−5.2 | 6.0 ± 0.2 | 5.6−6.5 |
| Dentary toothrow length | 6.7 ± 0.2 | 6.2−7.0 | 6.8 ± 0.1 | 6.8−7.0 | 7.9 ± 0.2 | 7.6−8.1 |
| Length of dentary m1−3 | 4.2 ± 0.2 | 3.8−4.5 | 4.3 ± 0.2 | 4.1−4.5 | 5.0 ± 0.2 | 4.6−5.3 |
| Score on model of Schlitter et al. 1980 | 6.6 ± 1.3 | 4.8−9.0 | 5.7 ± 1.4 | 4.0−6.9 | −3.9 ± 1.03 | −1.7 to −5.6 |

[a] From occipital condyle to anteriormost point of premaxillary bone.
[b] Height of skull from cochlea to top of sagittal crest.
[c] Height of skull from basiphenoid and basiocciptal bones to top of sagittal crest.
[d] Distance from anterior surface of the mandibular fossa to insertion of masseter muscle.