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SYSTEMATICS OF ISOLATED POPULATIONS OF SHORT-TAILED SHREWS (SORICIDAE: *BLARINA*) IN TEXAS

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Two isolated and taxonomically unassigned populations of short-tailed shrews (*Blarina*) exist in Texas, 1 in the Lost Pines region including Bastrop County and 1 at Aransas National Wildlife Refuge on the Gulf Coast. Molecular and morphological methods were used to determine the systematic status of the 2 disjunct Texas populations. Multivariate analyses of size-influenced cranial measurements were unsuccessful in identifying specimens from these populations at the species level. Phylogenetic analyses of the mitochondrial cytochrome-*b* gene revealed that the samples from disjunct Texas populations form a monophyletic sister clade to *B. hylophaga* from Kansas and Nebraska; there is little divergence between the 2 Texas populations. Genetic divergence between Texas and Kansas–Nebraska *B. hylophaga* is comparable to taxonomically recognized east–west divisions within *B. brevicauda* and *B. carolinensis*. Therefore, the name *Blarina hylophaga plumbea*, which originally was applied to the Aransas County population, also should include the Bastrop County population.

Key words: Aransas, *Blarina*, Lost Pines, short-tailed shrew, Texas

Short-tailed shrews in the genus *Blarina* are endemic to eastern North America. Three species have been described: the northern short-tailed shrew (*Blarina brevicauda*), Elliot's short-tailed shrew (*Blarina hylophaga*), and the southern short-tailed shrew (*Blarina carolinensis*—Nowak 1999). Of these 3 species, 2 occur in Texas: *B. carolinensis* inhabits the eastern one-third of Texas, and *B. hylophaga* has been found in northeastern Texas (Fig. 1). Two isolated populations of *Blarina* have been discovered in Texas that remain taxonomically unassigned.

In 1941, 2 short-tailed shrews were discovered at Aransas National Wildlife Refuge, Aransas County, Texas. The shrews were 400 km southwest of the known distribution of any congeners, and morphologically unique enough to warrant recognition as a new subspecies, *Blarina brevicauda plumbea* (Davis 1941). A later study that examined 7 shrews collected at Aransas National Wildlife Refuge reported that these individuals were morphologically distinct from *B. carolinensis* in eastern Texas, but no comparison was made to *B. hylophaga* (Schmidly and Brown 1979). Later it was determined that shrews from Aransas were similar to *B. hylophaga* from Oklahoma rather than to *B. carolinensis* from eastern Texas (George et al. 1981). This population now is known as *B. hylophaga plumbea*.

In 1989, another isolated population of *Blarina* was discovered at Bastrop State Park, Bastrop County, Texas (Dixon et al. 1989, 1990). Of 4 specimens collected, 3 were identified as *B. hylophaga* and 1 as *B. carolinensis* based on a suite of cranial measurements (Baumgardner et al. 1992). The Bastrop County population exists within the Lost Pines region, approximately 130 km west of the known range of *B. carolinensis* in Texas. This region is characterized by stands of loblolly pine (*Pinus taeda*) on sandy soils, and is the westernmost outpost of these pines in Texas. Although Bastrop County is located in the Blackland Prairie region of Texas (Diamond et al. 1987), the vegetation is similar to that of the Piney Woods of eastern Texas.

Systematics and taxonomy of *Blarina* historically have been based on morphology. Initially, only 1 species was recognized in the genus, *B. brevicauda*, with the subspecies *B. b. carolinensis* and *B. b. hylophaga* elevated to specific status in the early 1970s (Genoways and Choate 1972; Handley 1971) and 1980s (George et al. 1981), respectively. Karyotypes (George et al. 1982) and molecular analyses (Brant and Ortí 2002, 2003) have redefined the systematics of this taxon. However, the isolated populations of *Blarina* found in central and coastal Texas have not been reexamined with modern methods.

Indeed, species-level identification based on morphological characters is not likely to be straightforward in this case. The 2 species (*B. carolinensis* and *B. hylophaga*) are remarkably

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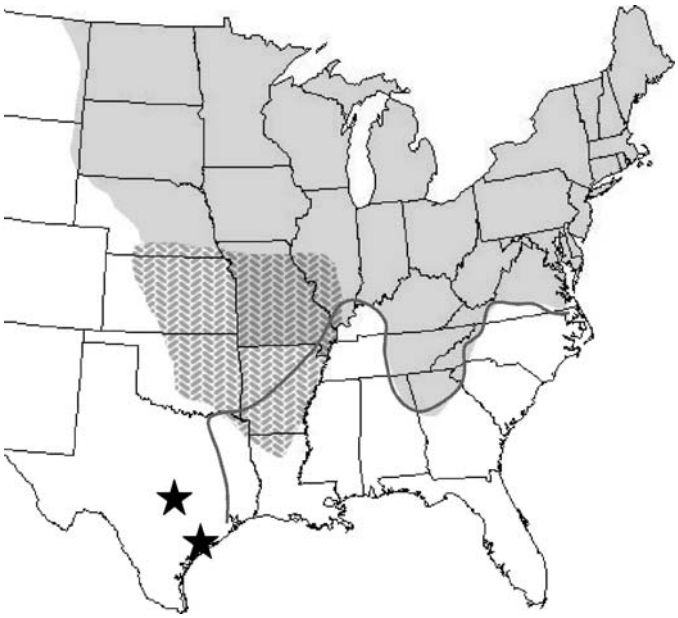

FIG. 1.—Distribution of 3 species of short-tailed shrew (*Blarina*) in the eastern United States (modified from George et al. 1982). Stars represent 2 isolated unassigned populations of *Blarina* in Texas. Gray indicates range of *B. brevicauda*, pattern indicates range of *B. hylophaga*, and the range of *B. carolinensis* is south and east of the dotted line.

similar, and clinal variation in size results in overlap between small *B. hylophaga* from the southern part of their range with large northern *B. carolinensis*; where the ranges of the 2 species overlap, however, they are morphologically distinct (George et al. 1981). In fact, Schmidly and Brown (1979) stated that shrews from what is now delineated as *B. carolinensis* were "a southward extension of the cline [*B. hylophaga*]," and Stangl and Carr (1997) stated that range limits established by previous studies enabled "workers in Texas and Oklahoma to assign their specimens of *Blarina* to 1 species or the other based solely on geographic grounds." Thus, identification of *Blarina* within their respective ranges is possible, but isolated populations present a problem in that they may overlap morphologically with either *B. hylophaga* or *B. carolinensis*. Additionally, because of clinal variation in size, disjunct populations may be morphologically smaller or larger than the typical population. Because morphological characters used in previous studies of *Blarina* are size-related (e.g., Choate 1972), this overlap in size is a confounding factor when identifying these isolated populations.

Taxonomic revisions of *Blarina* and particularly *B. hylophaga*, which was recognized as a species only recently (George et al. 1981), have caused confusion in the literature over the identity of Texas short-tailed shrews. Studies performed to date on short-tailed shrews in Bastrop and Aransas counties, Texas, have utilized cranial and external morphological characters that are ambiguous because of size overlap between *B. hylophaga* and *B. carolinensis*. George et al. (1981) reaffirmed the taxonomic status of the Aransas subspecies *B. hylophaga plumbea*, but the tentative identification of both *B. hylophaga* and *B. carolinensis* in Bastrop County by Baumgardner et al. (1992) cast doubt on the classification of Texas *Blarina*. It seems that in this case, additional characters are necessary to support or refute morphological data. Brant and Ortí (2002) used mitochondrial DNA sequences to resolve evolutionary relationships among the 3 species of *Blarina*. The sequences from their study were available in the GenBank database, providing a range-wide collection of *Blarina* sequences for comparison with the isolated Texas populations of *Blarina*.

The objectives of this study were to resolve the systematics of the Texas short-tailed shrews and to explore biogeographical hypotheses. The systematic status of the 2 isolated populations of short-tailed shrew in Texas was examined by using morphological and molecular techniques. Additionally, biogeographical hypotheses to explain the current distribution of short-tailed shrews in Texas were tested.

MATERIALS AND METHODS

Short-tailed shrews were trapped in pitfall traps (18.93 liter) on 400-m linear drift fences or 100-m-radius Y-shaped drift fence arrays in heterogeneous habitats in 2 study sites. At the Griffith League Ranch in Bastrop County, Texas, 100 pitfall traps were checked daily from 2001 through 2003. At the Aransas National Wildlife Refuge in Aransas County, Texas, 12 pitfall traps were placed along three 30-m drift fences in or near oak stands; these traps were checked in summer and autumn of 2003. Trapping methods followed guidelines established by the American Society of Mammalogists (Animal Care and Use Committee 1998). Tissue or blood was collected and stored in 95% ethanol at −80°C. Historical and recent specimens held in the Texas Cooperative Wildlife Collection (TCWC) also were used for morphological and molecular analyses (Appendix I). All specimens collected and prepared were deposited in the mammal collection at Texas Tech University Natural Science Research Laboratory in Lubbock, Texas (TTU 100794-100823; see Appendix I).

Morphological analyses.—External measurements were taken when the condition of the specimen allowed; these included total length, tail length, hind-foot length, and ear length. Cranial measurements were taken as established by Choate (1972): occipitopremaxillary length, length of P4–M3, cranial breadth, breadth of zygomatic plate, maxillary breadth, interorbital breadth, length and height of mandible, and articular breadth. Principal components analysis (PCA—Quinn and Keough 2002) using a covariance matrix was used to determine whether statistical differences exist between these populations of *Blarina* and specimens from other parts of the United States. Multivariate analysis of variance (MANOVA—Quinn and Keough 2002) was performed by using species as the independent variable and the 9 cranial characters as response variables to determine if there were differences between species.

DNA sequencing.—The Qiagen DNeasy kit (Valencia, California) was used to extract genomic DNA from skeletal muscle and blood samples. Amplification of the cytochrome-*b* gene was performed in a 50-µl reaction using 10 µl of Taq buffer (0.3 M Tris, 0.0175 M $MgCl_2$, and 0.075 M $(NH_4)_2SO_4$, pH 8.5), 0.5 µl of dimethylsulfoxide, 0.5 µl deoxynucleoside triphosphates (2.5 mM each), 0.5 µl of each primer (10 µM), 0.25 µl of Taq polymerase, and 0.5 µl of tDNA. The following primers were used in polymerase chain reaction (PCR) and bidirectionally sequencing cytochrome *b*: L14724 and H15915 (Irwin et al. 1991); an internal sequencing primer, cytbR1 (Brant

and Ortí 2002), and novel internal sequencing primers shrewCBF1: 5′-YTATTTTCTCCAGACTTACTAGGAGACCC-3′ (where Y is C or T), and shrewCBR3: 5′-CCTCATGGAAGGACATACCCTATAAA GGCAGT-3′. The thermal profile consisted of denaturation at 94°C for 1 min followed by 35 cycles of 94°C (1 min), 50°C (30 s), and 72°C (1 min), and then a final extension of 72°C for 5 min. PCR products were purified and sequenced with PCR and internal primers by using Big Dye version 3.0 (Applied Biosystems, Foster City, California) according to the manufacturer's specifications.

Phylogenetic analyses.—Sequences were aligned by using Sequencher 4.1 software (Gene Codes Corporation, Ann Arbor, Michigan). Novel sequences were aligned with 38 sequences obtained from GenBank, as published in Brant and Ortí (GenBank accession numbers AF395449–86; 2002). Thirty-four cytochrome-*b* sequences from *Blarina* representing all 3 species, 2 for *Cryptotis parva*, and 2 for *Sorex cinereus* from GenBank were aligned with 23 sequences from Bastrop–Aransas specimens and exported as a NEXUS file to PAUP* 4.0b10 (Swofford 2002). *S. cinereus* and *C. parva* were designated as monophyletic outgroups. Individuals were coded according to geographic location and GenBank accession number.

Neighbor-joining analysis (Saitou and Nei 1987) was performed by using HKY distances (based on Modeltest results; see "Results" section) and bootstrapped by using 10,000 pseudoreplicates. Maximum-parsimony analysis was performed by using a heuristic search with tree-bisection-reconnection branch swapping. The data set was bootstrapped by using 1,000 pseudoreplicates each with 5 random addition replicates (Felsenstein 1985).

MrModeltest 2.0 (http://www.csit.fsu.edu/~nylander/), a modified version of Modeltest 3.5 (Posada and Crandall 1998), was used to determine the nucleotide substitution model that best fit the data. The model and parameters given by MrModeltest were used in a maximum-likelihood analysis by using a full heuristic search, with starting trees for branch-swapping obtained by using neighbor joining. A bootstrap maximum-likelihood analysis was performed with 1,000 pseudoreplicates. Bayesian analyses were performed by using MrBayes (v. 3.0—Huelsenbeck and Ronquist 2001) with 1,000,000 generations of 4 Monte Carlo Markov chains sampled every 1,000 generations. As recommended in the MrModeltest output, the parameters estimated by MrModeltest were not included in the commands for MrBayes; instead, the general model was outlined in the commands. Only the last 500 of the 1,000 trees generated were used to generate the Bayesian consensus topology, enforcing a burn-in of 500,000 generations.

Each novel sequence and the compiled data set were examined thoroughly, including translation to amino acid sequence and substitution pattern, to ensure the integrity of subsequent analyses. Likewise, such scrutiny ensured that any erroneous sequence contaminant whether exogenous (PCR contamination) or endogenous (pseudogenes) was exposed before the final analyses.

Results

Morphological analyses.—Most cranial measures were linearly correlated, with Pearson's correlation coefficient (r) ranging from 0.29 to 0.84. PCA ($n = 52$) recovered 9 principal components, the 1st of which (PC1) explained 77.7% of the variance and had an eigenvalue of 0.97. The 2nd component (PC2) was retained for use in the ordinal plot (Fig. 2) but only explained 7.9% of the variation and had an eigenvalue of 0.31. All cranial characters had positive loadings on PC1, the largest being occipitopremaxillary length, which had a loading of 0.708. Remaining principal components were not included in further analyses; all had eigenvalues < 0.25 and explained $<5\%$ of the variation. On PC1, *B. carolinensis* and *B. hylophaga* were distinguishable, with *B. carolinensis* having lower scores on that component. Specimens from Texas were intermediate between the 2 species on PC1, although there was overlap between 2 Bastrop specimens and *B. carolinensis*.

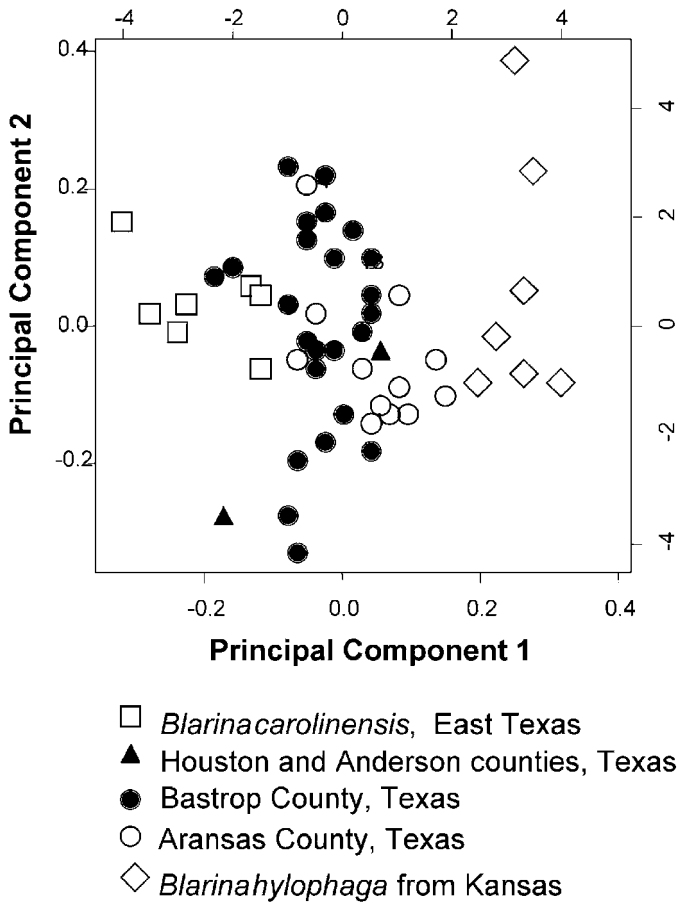

Fig. 2.—Ordinal plot of the first 2 principal components in the analysis of 9 cranial measurements for *Blarina* ($n = 52$). All 9 cranial characters, as used in Choate (1972), were positively correlated with PC1. Symbols differ for geographic regions as indicated in key.

Multivariate analysis of variance (MANOVA) was used to differentiate among 3 groups: *B. carolinensis*, including specimen TCWC 51797 (previously identified as *B. carolinensis*—Baumgardner et al. 1992), *B. hylophaga* from Kansas, and *Blarina* from Texas. The Pillai trace test statistic was 1.228 ($P < 0.001$), indicating that centroids for these 3 groups are significantly different (Quinn and Keough 2002).

DNA sequencing.—Cytochrome-*b* sequences 1,140 nucleotides in length were obtained for 3 specimens of *Blarina* from Aransas National Wildlife Refuge and 20 specimens from Bastrop County (GenBank Accession numbers AY546659–81). The Texas populations do not share any haplotypes with *B. hylophaga* from Kansas and Nebraska (Brant and Ortí 2002) Five distinct haplotypes were present, 1 unique to Aransas (haplotype A) and 4 in the Bastrop population (haplotypes S, B1, B2, and B3). Haplotype A was unique to the 3 Aransas

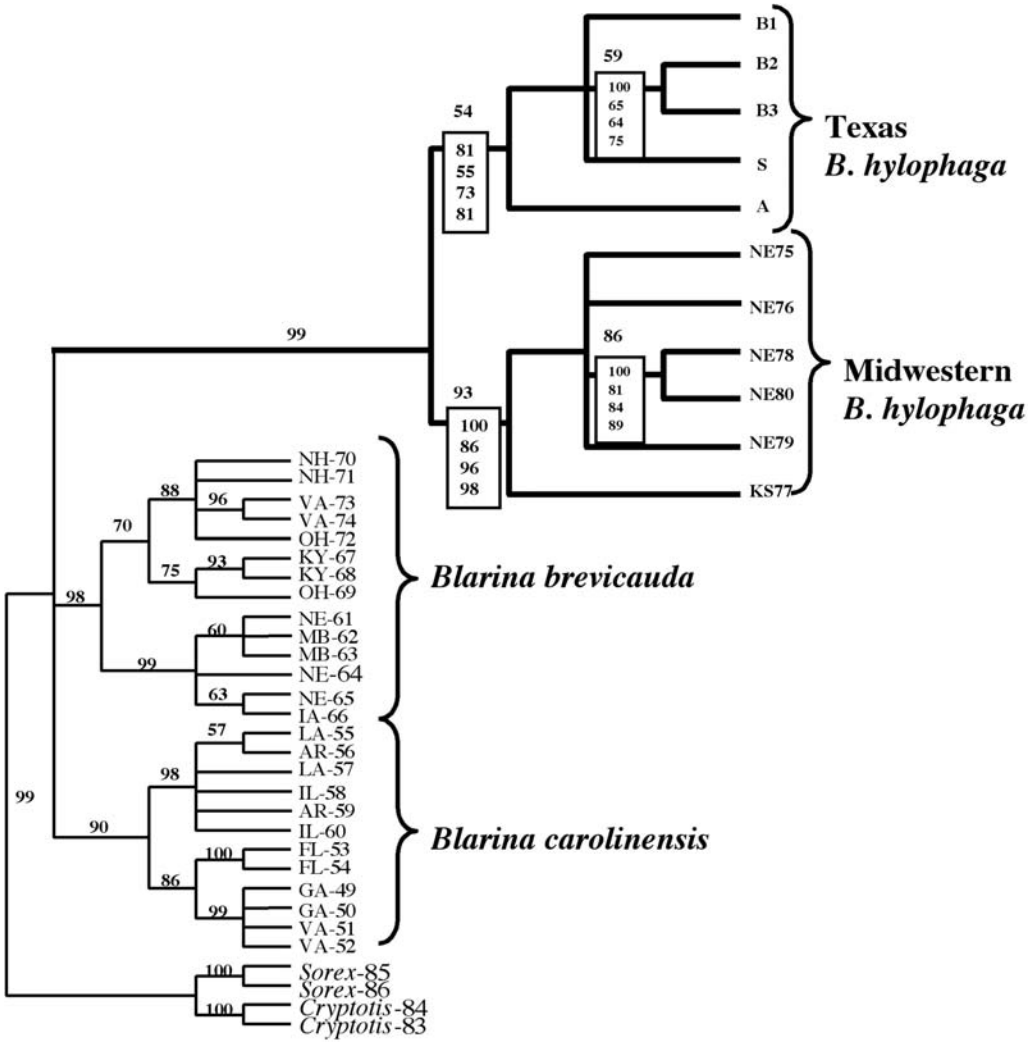

FIG. 3.—Consensus tree created with 330 bootstrap pseudoreplicates of maximum-likelihood analysis with the complete data set for *Blarina*. Bootstrap support values are noted above respective branches. Branch support values within the boxes below the branches from top to bottom are Bayesian, maximum likelihood, maximum parsimony, and neighbor joining. Individuals sequenced in this study are labeled by haplotype, with A being the Aransas haplotype and all others originating in Bastrop County, and all other taxa are labeled with the 2-letter abbreviation for the state (AR = Arkansas, FL = Florida, GA = Georgia, IL = Illinois, IA = Iowa, KS = Kansas, KY = Kentucky, LA = Louisiana, NE = Nebraska, NH = New Hampshire, OH = Ohio, TX = Texas, and VA = Virginia) or Canadian province (MB = Manitoba) of origin and the last 2 digits of the unique GenBank accession number.

individuals. Haplotype B1 was the predominant haplotype in Bastrop County ($n = 14$) and haplotype B2 was less common ($n = 4$). Haplotype B3 was present in only 1 individual, and differed from haplotype B2 by only 1 nucleotide. Haplotype S was unique to an individual captured in southern Bastrop County.

Phylogenetic analyses.—Of the sequences downloaded from GenBank as baseline data, 2 sequences of *B. hylophaga* (GenBank accession numbers AF395481 and AF395482) contained stretches of anomalous sequence at the 5′ and 3′ ends as well as an internal stop codon in 1 instance. These flaws are likely due to the lack of bidirectional sequencing and inadequate examination of sequences before publication by Brant and Ortí (2002). These sequences were not used in our phylogenetic analyses because of the poor quality; however, other sequences generated by Brant and Ortí were acceptable and used for comparison against sequences generated in our study.

The final alignment for this study consisted of 1,140 nucleotides of cytochrome *b* for 36 taxa from the previous study by Brant and Ortí (2002—GenBank accession numbers AF395449–80 and AF395483–86) and 5 Texas haplotypes produced in the current study. Phylogenetic analysis of this data set with all methods (neighbor joining, maximum parsimony, maximum likelihood, and Bayesian) confirmed that the Texas shrews form a monophyletic group with Kansas–Nebraska *B. hylophaga* (Fig. 3). Having established the monophyly of the group of interest, *B. hylophaga*, we removed extraneous sequences in order to focus attention on this group. This species-level data set for *B. hylophaga* consisted of Texas

shrews, *B. hylophaga* (GenBank numbers AF395475–80), and 2 individual *B. brevicauda* retained as outgroup. This approach also eliminated the saturation that was shown to occur between *Blarina* and the paraphyletic outgroups of *Sorex* and *Cryptotis* (see Brant and Ortí 2002; Fig. 2).

Parsimony analysis of all sequences of *Blarina* with characters equally weighted resulted in 36 equally parsimonious trees with tree length of 566, retention index (RI) of 0.925, and consistency index (CI) of 0.737. Of the molecular characters, 799 were constant and 323 of the variable sites were parsimony informative. The maximum-parsimony topology supported 3 monophyletic species of *Blarina*. In the genus, *B. brevicauda* was basal with *B. hylophaga* and *B. carolinensis* being sister clades with 60% bootstrap support. Maximum-likelihood analysis of the data set of *Blarina* was performed by using the HKY+G model (Hasegawa et al. 1985) as specified by both Modeltest 3.5 (Posada and Crandall 1998) and MrModeltest 2.0 (http://www.csit.fsu.edu/~nylander/). This model specified nucleotide frequencies of A = 0.3104, C = 0.2914, T = 0.2778, and G = 0.1204; transition to transversion ratio of 5.1146; proportion of invariable sites equal to zero; and a gamma distribution parameter of 0.1826, which cumulatively indicated a high number of practically invariable sites (Nei and Kumar 2000). The resulting topology supported *B. hylophaga* basal within the genus. The 50% majority-rule consensus tree constructed by using 500 trees produced in Bayesian analysis also supported *B. hylophaga* basal within the genus.

For the species-level data set of *B. hylophaga*, there were 998 constant characters and 99 parsimony-informative characters. Six most-parsimonious trees were found with a length of 156, CI of 0.936, and RI of 0.937. The divergence (uncorrected *p*) within the Texas *Blarina* was 0.09–0.97%; between Texas and other populations of *B. hylophaga*, divergence was 1.3–2.6%. For the species-level data set of *B. hylophaga*, maximum likelihood was performed by using parameters as given by the likelihood-ratio test in MrModeltest, fitting the HKY+G nucleotide substitution model: base frequencies A = 0.2799, C = 0.2902, G = 0.1412, T = 0.2887; transition to transversion ratio of 6.4201; proportion of invariable sites equal to zero; and gamma of 0.3484. Exact values for gamma and base frequencies were not included in the Bayesian analysis; instead, basic information about the model was included.

Discussion

Morphology.—Analyses performed here describe the 2 Texas populations as intermediate in size between *B. hylophaga* and *B. carolinensis*. Because all of the cranial measures were positively correlated, PC1 can be considered a size component in the PCA (Flessa and Bray 1977; Rohlf and Bookstein 1987), and PC2 and subsequent principal components can be considered size-free shape components (Humphries et al. 1981). Thus, these shrews vary in size, with *B. carolinensis* being the smallest, Texas *B. hylophaga* intermediate, and *B. hylophaga* the largest of the groups in the analysis. The shape component PC2 accounts for little variation (7.9%) in cranial morphology and the 3 groups in this comparison have considerable overlap on that axis (Fig. 2). This is congruent with previous studies using PCA to differentiate species of *Blarina* (Genoways and Choate 1972).

One individual short-tailed shrew (specimen TCWC 51209) captured in Bastrop County previously had been identified as *B. carolinensis*, and indeed grouped with that species in the morphological analyses. However, as previously mentioned, the characters used to identify these shrews are purely size-based, and this individual most likely was a young adult based on degree of ankylosis and lack of tooth wear. This example illustrates one of the pitfalls of using size-based characters to identify animals; individuals that are not fully grown will be classified as the smaller of the 2 species. Another example can be seen in our TCWC eastern Texas specimens; 1 from northeastern Texas fits with the Texas *B. hylophaga* on the ordinal plot (Fig. 2). Although *B. hylophaga* has not previously been reported from these counties, it is difficult to say if these specimens are unusually large *B. carolinensis* or an unrecognized population of *B. hylophaga*.

Biogeography and areas of endemism.—The Bastrop and Aransas populations of *Blarina* are limited to small areas of a once-extensive range in Texas. Based on fossil evidence, short-tailed shrews were widespread in Texas for several thousand years. Because species-level identification using cranial morphology is problematic in *Blarina*, identification of fossils should be considered at the genus level only. Some of the oldest fossil records of *Blarina* in Texas date from approximately 10,900 years ago (Jones et al. 1984). In Travis County, central Texas, the most recent record is a specimen dated at approximately 1,015 years ago (Jones et al. 1984; Lundelius 1967). Lundelius (1986) also noted a short-tailed shrew from Mac's Cave in Travis County dated at 600 years ago. The changes in habitat associated with post-Pleistocene warming may have caused the short-tailed shrews to shift their ranges to the east and north to stay within more mesic habitats (Graham 1987; Lundelius 1967). The 2 disjunct populations that remain in Texas would then be relictual isolates, although it also has been hypothesized that shrews may have arrived on the Texas coast via dispersal rather than representing Pleistocene faunal remnants (Schmidly and Brown 1979). We support the hypothesis that these populations represent relictual isolates based on fossil evidence of the historical distribution as well as the unique ecosystems retained in both the Lost Pines and Aransas National Wildlife Refuge.

The Lost Pines area in Central Texas is characterized by stands of loblolly pine on sandy soils. This habitat is similar to the Piney Woods of eastern Texas, also an area containing loblolly pines on sandy soils. The Lost Pines hosts the westernmost distribution of these pines in Texas, as well as the fauna associated with them. Species such as southern flying squirrel (*Glaucomys volans*), pileated woodpecker (*Dryocopus pileatus*), and pine warbler (*Dendroica pinus*) have presumably disjunct populations in the Lost Pines. The endangered Houston toad (*Bufo houstonensis*) now is effectively restricted to the Lost Pines, although its distribution once included a much larger area of southeastern Texas. An endemic insect, the Texas long-lipped beetle (*Telegeusis texensis*), recently was

described from specimens collected in the Lost Pines (Taber and Fleenor 2003). The flora and fauna of the Lost Pines are not only unlike the surrounding Blackland Prairie, but different from Piney Woods of eastern Texas.

This unique ecosystem may be a relictual isolate or an outpost of pines created by dispersal. Fossil pollen evidence confirms that Bastrop County was the westernmost limit of the range of pine forests in Texas in late glacial and postglacial times (Larson et al. 1972), and that pines have been present for nearly 20,000 years (Bryant 1977). There are 2 competing hypotheses regarding the Pleistocene refugium of loblolly pines: a 1-refuge hypothesis posits that Florida was the sole refuge and the pines spread across the Southeast in postglacial times, and a 2-refuge hypothesis counters that Texas provided a 2nd refuge. Molecular data support the 2-refuge hypothesis, with eastern Texas and western Gulf populations of loblolly pine being putative descendants of the western refugium (Al-Rabab'ah and Williams 2004).

Aransas National Wildlife Refuge also harbors unique fauna; it is well known as the wintering ground for the endangered whooping crane (*Grus americana*). The rare Texas scarlet snake (*Cemophora lineri*) has been found on the refuge as well. Both the Lost Pines and Aransas National Wildlife Refuge may represent areas of local endemism for many taxa. Thus, *Blarina* is not unique as a Pliestocene faunal remnant in either of these Texas locations. Indeed, these patterns in species distributions are key in understanding the historical biogeography of short-tailed shrews.

An interesting parallel may exist within *Blarina* that would not only mirror the history of the Texas populations but also serve as a further cautionary tale. Two subspecies of short-tailed shrew inhabit Florida: *Blarina carolinensis peninsulae* and *B. carolinensis shermani*. Despite several concerted efforts, the latter has not been captured at the type locality in Ft. Myers since the initial type series was collected in 1955 (Layne 1992). Specimens collected in the 1980s are smaller than those in the series collected by Hamilton (1955), and are postulated as intergrades between *B. c. shermani* and *B. c. peninsulae*. Again, because size-based morphological characters are the basis of classification for these subspecies, clinal variation in size as well as other factors make it difficult to determine if this subspecies is a relictual isolate of *B. brevicauda* or a population of large *B. carolinensis*. Additionally, because material available for *B. c. shermani* is limited to museum specimens, karyotyping is not possible and DNA analyses have not yet been attempted. This subspecies was extirpated before it could be described; whether it was a relictual isolate of *B. brevicauda* or a unique population of *B. carolinensis* may yet be determined by using DNA, but the opportunity to conserve this unique population was lost. The isolated populations of short-tailed shrews in Aransas and Bastrop counties, Texas, should be monitored so that they do not face the same fate as *B. c. shermani*.

Molecular phylogenetics and taxonomy.—The Texas populations of short-tailed shrews form a monophyletic sister group to other United States populations of *B. hylophaga*; within Texas, both Bastrop and Aransas counties harbor unique clades. All molecular analyses were in agreement in the placement of the Texas clade as a sister group to *B. hylophaga* (Fig. 3). With regard to interspecific relationships, no single hypothesis is supported by a majority of the analyses; this lack of resolution may be the result of the exclusion of 16S rDNA previously used to resolve deeper phylogenetic nodes or to elimination of 2 anomalous sequences (Brant and Ortí 2002).

Brant and Ortí (2002) reported intraspecific divergences within *B. carolinensis* (3.4%) and *B. brevicauda* (2.5%) that correlated with east–west clades and recognized subspecies. Brant and Ortí (2002:171) remarked, "In contrast to [the other species of *Blarina*], *B. hylophaga* has no geographic structuring and a most restricted distributional range." Cytochrome-*b* sequences from their study show only 0.7% divergence within *B. hylophaga*; however, the difference between Kansas–Nebraska and Texas clades of this species ranges from 1.2% to 2.2%, a range on par with the 2 congeners. As in the other 2 species, a previously recognized subspecies correlates with the genetic divergence: the Texas subspecies, *B. hylophaga plumbea*. The molecular analyses presented here confirm the previous placement of the Aransas County population of short-tailed shrews within this subspecies, inclusive of the Bastrop County population of short-tailed shrews.

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APPENDIX I

Specimens examined.—The specimens examined are deposited in the Texas Cooperative Wildlife Collection at Texas A&M University (TCWC) and the collections of the Museum of Texas Tech University (TTU). Tissues are housed in the Michael R. J. Forstner tissue collection (MF) at Texas State University–San Marcos. Individuals characterized in molecular analyses are marked with an asterisk followed by their associated haplotype identification (A, B1, B2, B3, or S) and GenBank accession numbers (AY followed by a 6-digit number).

Blarina hylophaga.—Texas; Aransas County, Aransas National Wildlife Refuge (TCWC 1541, MF 9268; TCWC 1542, MF 9269; TCWC 30395, MF 9270; TCWC 30396, MF 9271; TCWC 31833, MF 9272; TCWC 31834, MF 9273; TCWC 31835, MF 9274; TCWC 31836, MF 9275; TCWC 31837, MF 9276; TTU 100815*, A, AY546676, MF 8231; TTU 100816*, A, AY546677, MF 8801; TTU 100817*, A, AY546675, MF 8802; TTU 100818*, B2, AY546678, MF 8803; TTU 100823, MF 8230). Bastrop County, 10 miles west of

Smithville (TTU 100806*, S, AY546680, MF 8057). Bastrop County, 5 miles north of Bastrop (TTU 100794*, B1, AY546660, MF 4859; TTU 100795*, B1, AY546659, MF 4745; TTU 100796*, B1, AY546661, MF 5356; TTU 100797*, B1, AY546662, MF 5376; TTU 100798; TTU 100799*, B1, AY546663, MF 6156; TTU 100800*, B1, AY546665, MF 7497; TTU 100801*, B2, AY546666, MF 7498; TTU 100802*, B2, AY546667, MF 8049; TTU 100803*, B1, AY546668, MF 8050; TTU 100804*, B1, AY546669, MF 8051; TTU 100805*, B1, AY546670, MF 8052; TTU 100807*, B1, AY546671, MF 8053; TTU 100808; TTU 100809*, B1, AY546673, MF 8055; TTU 100810*, B1, AY546674, MF 8224; TTU 100811, MF 8225; TTU 100812, MF 8226; TTU 100813, MF 8227; TTU 100814, MF 8228; TTU 100819, MF 9156; TTU 100820*, B1, AY546679, MF 9157; TTU 100821*, B3, AY546681, MF 9158; TTU 100822, MF 9159). Bastrop County, Bastrop State Park (TCWC 51207; TCWC 51208; TCWC 51209; TCWC 51797). Kansas; Geary County (TCWC 50138; TCWC 50143; TCWC 50131; TCWC 50132; TCWC 50133; TCWC 50134).

Blarina carolinensis.—Texas; Newton County (TCWC 27628). Tyler County (TCWC 33360; TCWC 34970; TCWC 34971; TCWC 33359; TCWC 33361; TCWC 33351; TCWC 33355). Hardin County (TCWC 34952; TCWC 34956; TCWC 33337).