

SYSTEMATICS OF LOCALLY ENDEMIC POPULATIONS OF
SHORT-TAILED SHREWS, *BLARINA* (INSECTIVORA:
SORICIDAE), IN BASTROP AND ARANSAS
COUNTIES, TEXAS

THESIS

Presented to the Graduate Council of Texas State University – San Marcos
in Partial Fulfillment of the Requirements

For the Degree

Master of SCIENCE

By

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ABSTRACT

SYSTEMATICS OF LOCALLY ENDEMIC POPULATIONS OF SHORT-TAILED
SHREWS, *BLARINA* (INSECTIVORA: SORICIDAE), IN BASTROP
AND ARANSAS COUNTIES, TEXAS

by

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Two isolated populations of short-tailed shrews exist in Texas; one in the Lost Pines region including Bastrop County and one at Aransas National Wildlife Refuge on the Gulf Coast. Fossil evidence suggests that two species of short-tailed shrew once were widespread in central Texas; *Blarina hylophaga* now inhabits Nebraska, Kansas, Oklahoma and Montague County, Texas, and *Blarina carolinensis* inhabits the Southeastern United States through the eastern third of Texas. Molecular and morphological methods were used to determine the systematic status of the two disjunct Texas populations. In morphological analyses, nine cranial measurements were analyzed using principal components analysis (PCA), and it was determined that Texas specimens were intermediate between the smaller *B. carolinensis* and larger *B. hylophaga*.

Multivariate analysis of variance (MANOVA) determined that the nine cranial measures could be used to differentiate among three groups: *B. carolinensis*, *B. hylophaga*, and Texas *Blarina* (Pillai's Trace= 1.23, $P < 0.001$). Phylogenetic analyses of the mitochondrial cytochrome *b* gene for three specimens from Aransas County and 20 specimens from Bastrop County revealed that the Texas groups are sister to *B. hylophaga* from Kansas and Nebraska. Based on the available evidence, subspecies status is warranted for the Texas short-tailed shrews. Because the Aransas population previously had been designated *Blarina hylophaga plumbea*, it is recommended that this subspecies now include the Bastrop County population as well. Biogeographic hypotheses are examined with respect to Texas as a Pleistocene refugium and these disjunct populations as relictual isolates. Areas in Texas inhabited by short-tailed shrews may harbor other locally endemic taxa; these areas should be examined closely as they may represent high value habitat for conservation efforts.

INTRODUCTION

During the Late Pleistocene glacial maximum, approximately 18,000 years ago, glaciers covered northern North America (Pielou 1991). Fossil evidence suggests that the southern United States were much colder at that time and supported boreal flora and fauna (e.g. Larson et al. 1972). The southern states provided refugia from the extreme cold of periglacial environments, but as the climate warmed and the ice sheets in northern North America receded, many animals and plants expanded their ranges northward. These former refugia still harbor relictual populations of species that have shifted their ranges northward. For example, *Neotoma floridana smalli* in the Florida Keys (Hayes and Harrison 1992) and *Microtus pinetorum* in Texas (Lundelius 1967) are considered to be relictual populations because they are small isolated populations that remain in the Pleistocene refugium that is no longer a primary part of the species' range. Both of these states also harbor unique populations of short-tailed shrews (*Blarina*); in Florida, the now extirpated subspecies *Blarina carolinensis shermani* is hypothesized to have been a relictual isolate of the northernmost species of short-tailed shrew, *B. brevicauda* (Genoways and Choate 1998). In Texas, one isolated population of short-tailed shrew in Aransas County has been classified as *B. hylophaga plumbea*, also belonging to a species whose distribution is further north.

Short-tailed shrews in the genus *Blarina* are endemic to eastern North America.

Three species have been described using morphological and molecular characters:

Blarina brevicauda is the northern short-tailed shrew, *Blarina hylophaga* is Elliot's short-tailed shrew, and *Blarina carolinensis* is the southern short-tailed shrew (Nowak 1999).

Blarina brevicauda is the largest of the short-tailed shrews, and can be found from the Plains States through New England and southeastern Canada. *Blarina carolinensis* inhabits the southeastern United States. *Blarina hylophaga* is found in the south-central section of the country in Oklahoma, Nebraska, Missouri, and Arkansas and on the Oklahoma border in Montague County in Texas (Figure 1). These shrews are known for their voracious appetites and venomous saliva; *Blarina* is one of four genera of mammals known to be venomous, three of which are members of Order Insectivora (Vaughan et al. 2000). These small mammals may be found in diverse habitats, including grasslands, bottomland forest, and upland woods; their diet includes invertebrates, small vertebrates, and some plant matter (Schmidly 1983).

Systematics and taxonomy of *Blarina* has been primarily based on morphology. Initially, only one species was recognized in the genus, *Blarina 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 have redefined the systematics of this taxon (Brant and Ortí 2002, 2003). However, questions remain regarding the isolated populations of *Blarina* found in central and coastal Texas.

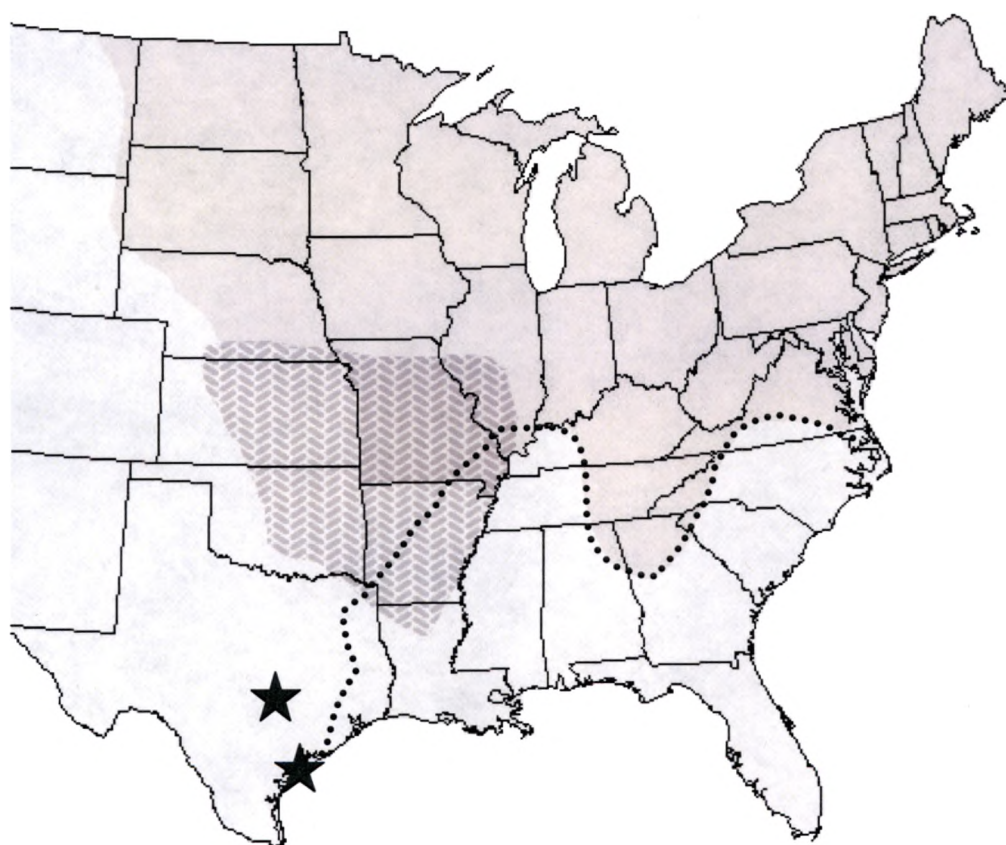


FIGURE 1. Distribution of three species of short-tailed shrew, *Blarina*, in the eastern United States (modified from George et al. 1982). Stars represent two isolated populations of *Blarina* in Texas. *Blarina brevicauda* is in grey, *B. hylophaga* is patterned, and the range of *B. carolinensis* is below the dotted line.

In 1941, two short-tailed shrews were discovered at the Aransas National Wildlife Refuge, Aransas County, Texas. The shrews were 400 kilometers southwest of the known range of any congeners, and morphologically unique enough to warrant recognition as a new subspecies, *Blarina hylophaga plumbea* (Davis 1941). A later study using seven shrews collected at Aransas NWR reported that these individuals were morphologically distinct from *Blarina carolinensis* in East Texas, but no comparison was made to *B. hylophaga* (Schmidly and Brown 1979). Later it was found that shrews from Aransas NWR were more similar to *B. hylophaga* from Oklahoma than *B. carolinensis* from East Texas (George et al. 1981). In 1994, *B. hylophaga plumbea* was evaluated as a candidate for listing as a federally endangered subspecies, but it subsequently was rejected due in part to a lack of taxonomic clarity (Beattie 1994).

In 1989, another isolated population of *Blarina* was discovered at Bastrop State Park, Bastrop County, Texas (Dixon et al. 1989; Dixon et al. 1990). Of four specimens collected, three were identified as *B. hylophaga* and one as *B. carolinensis* based on a suite of cranial measurements (Baumgardner et al. 1992). This population exists within the Lost Pines region, approximately 130 kilometers west of the range of *B. carolinensis*. 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), it bears more resemblance to the Piney Woods of East Texas.

The two Texas populations are separated by large geographic distances. There are no museum records that would indicate any continuity between them, nor connecting

either to other populations of *Blarina*. It is difficult to assess the factors that limit species ranges, and short-tailed shrews present a particularly challenging case because they are found in a variety of habitats. Although they are habitat generalists, *Blarina* do prefer areas that are mesic but not saturated (Genoways and Choate 1998). These shrews are semi-fossorial and inhabit areas with pliable soil or ground cover, allowing construction of burrows and runways (Genoways and Choate 1998). Bastrop and Aransas counties both have sandy soils and considerable ground cover in unburned areas. While mammal-trapping efforts are largely undocumented, major universities in Texas consistently engage in widespread trapping that would likely have uncovered evidence of a continuous, contemporary Texas *Blarina* population.

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. In Travis County, central Texas, the most recent record is a specimen dated at approximately 1,015 years before present and tentatively identified as *B. carolinensis* (Table 1 and Figure 2) (Jones et al. 1984; Lundelius 1967). Lundelius (1986) also noted a possible *B. carolinensis* from Mac's Cave in Travis County dated at 600 years before present. 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 two disjunct populations that remain in Texas may be relictual isolates, although it also has been hypothesized that shrews may have arrived at Aransas via dispersal rather than being Pleistocene inhabitants of the area.

TABLE 1. Localities from which fossils of *Blarina hylophaga* and *B. carolinensis* have been reported in Texas; species identification based on 26 dental and dentary measurements in comparison to modern specimens (Jones et al. 1984). All dates are noted in years before present.

Location	County	Date or Period	Species
Barton Springs	Travis	1,015 +/- 150	<i>B. carolinensis</i>
Cave Without a Name	Kendall	10,900 +/- 190	<i>B. carolinensis</i>
Felton Cave	Sutton	7,770 +/- 130	<i>B. carolinensis</i>
Friesenhahn	Bexar	Wisconsinan	<i>B. carolinensis</i>
Hall's Cave	Kerr	Holocene	<i>B. carolinensis</i>
Klein Cave	Kerr	7,683 +/- 643	<i>B. carolinensis</i> and <i>B. hylophaga</i>
Longhorn Cavern	Burnet	Late Wisconsinan (~10,500)	<i>B. carolinensis</i>
Schulze Cave	Edwards	9,680 +/- 700	<i>B. hylophaga</i>
Miller's Cave	Llano	3,008 +/- 410 and 7,200 +/- 300	<i>B. carolinensis</i> and <i>B. hylophaga</i>

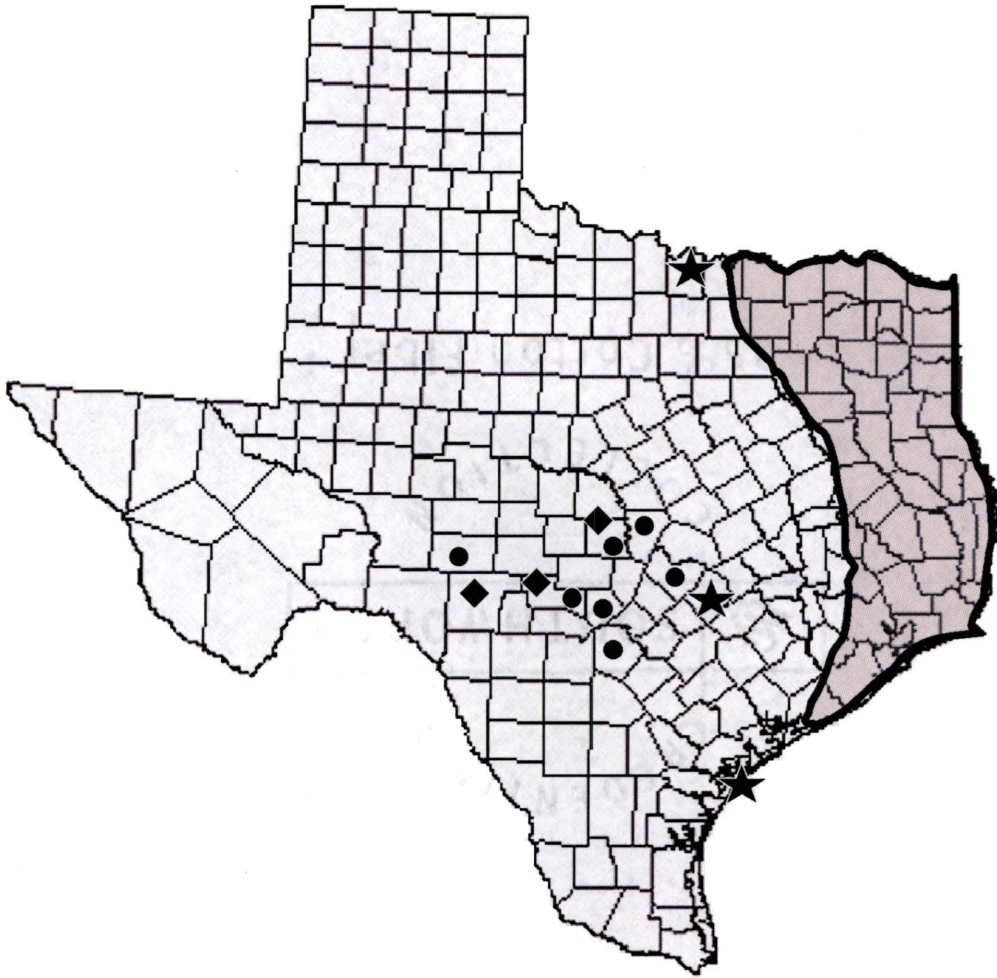


FIGURE 2. Distribution of fossil and extant short-tailed shrews (*Blarina*) in Texas.

Shaded region in East Texas represents current range of *B. carolinensis*; stars represent extant populations of *B. hylophaga*. Solid circles represent fossil *B. carolinensis* and diamonds represent fossil *B. hylophaga*.

(Schmidly and Brown 1979). Based on the fossil evidence (Table 1), it is possible that the isolation of these populations occurred between 1,000 and 5,000 years ago. Species-level identification based on morphological characters is not necessarily straightforward. These two species are remarkably similar, and in fact clinal variation in size may result in overlap between small *B. hylophaga* from the southern portion of their range with large *B. carolinensis* from their northern range; where the ranges of the two 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 one 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 both *B. hylophaga* and *B. carolinensis*. Additionally, due to clinal variation in size, disjunct populations may be 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 isolated populations of either taxon.

A technique that offered a potential solution to the shrew identification problem was karyotyping. *Blarina* exhibit considerable polymorphism with respect to chromosome fusions, and evidence for Robertsonian fans has been noted in *B. carolinensis* (Qumsiyeh et al. 1999; Qumsiyeh et al. 1997). Fundamental numbers (FN) for each species are distinct, with the largest variation within *B. carolinensis* (FN= 44, 45,

or 52); *B. hylophaga* has a fundamental number of 60 or 61 for the one locality sampled (George et al. 1982). Another technique useful in identifying morphologically indistinct organisms is DNA sequencing, which is becoming more readily available. Whereas karyotyping requires tissue from live specimens, DNA sequencing can be performed on stored tissue or even museum skins. DNA also provides more information for phylogenetic analyses as well as population genetics and individual identification, obviating the need for karyotyping for identification purposes. Studies contrasting chromosomal and mitochondrial DNA evolution in the *Sorex araneus* group, which exhibits considerable intraspecific variation in chromosome arrangements, have shown that homoplasy in karyologic data can obscure evolutionary relationships (Taberlet et al. 1994).

Taxonomic revisions of the genus *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 due to size overlap between *B. hylophaga* and *B. carolinensis*. The study by George et al. (1981) reaffirmed the taxonomic status of the Aransas subspecies *Blarina 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 three species of *Blarina*. The sequences from their study were

available on the GenBank database, providing baseline *Blarina* sequences for comparison of novel Texas *Blarina* sequences.

Phylogenetic analyses of mitochondrial DNA and quantitative morphological techniques were chosen to resolve this taxonomic problem. Both Davis (1941) and Dixon et al. (1989) recognized that the respective isolated populations differed from *B. carolinensis* in East Texas. However, the taxonomic status remains unresolved for these populations. With the availability of museum specimens for morphological analyses and the recent publication of a molecular phylogeny for *Blarina*, the data were available to help resolve questions regarding the two disjunct Texas populations.

The objective of this study was to resolve the systematic status of the two isolated populations of short-tailed shrew in Texas using morphological and molecular techniques. Additionally, biogeographical hypotheses were explored in an attempt to explain the current distribution of short-tailed shrews in Texas.

MATERIALS AND METHODS

Specimen Collection

Specimens were handled according to directives of TSU IACUC permit #KUMJTX_02.

Trapping for the Bastrop County population occurred in conjunction with concurrent herpetofaunal survey work on the Griffith League Ranch, which comprises approximately 5,000 acres, under Texas Parks and Wildlife permit SPR-0102-191 and U.S. Fish and Wildlife Service permit TE039544-1. Over 100 pitfall traps were placed along 23 drift fence arrays in grasslands, pine-oak woodlands, and oak-juniper woodlands and checked daily from 2001 through 2003. Thirteen Y-shaped arrays were created using three lines of drift fence 50 feet in length radiating from a central point, with 5-gallon buckets buried at the center and each terminus. Five linear arrays were set in grassland with buckets every 100 feet; two lines were 400 feet and three lines were 500 feet in length. One linear array in a pine forest was 100 feet in length with two terminal buckets and two internal buckets spaced 25 feet apart. Four linear arrays were placed in a grassland in a rectangular formation, two 100 feet in length with two terminal buckets and one central bucket, and two 150 feet in length with four evenly spaced buckets. Six arrays were added in the spring of 2003 that lacked drift fence; each consisted of four buckets arranged linearly along a 150 foot transect. These traps were checked daily from

June 26 through July 31, 2003. Trapping in Aransas County took place at the Aransas National Wildlife Refuge (ANWR), comprising approximately 54,000 acres, under permit number 03-013. Twelve pitfall traps were placed along three 100-foot drift fence arrays located in or near oak mottes. Four 50-meter lines, each with two terminal pitfall traps, in use for an ANWR herpetofauna survey were monitored for shrews ten days of every month during 2003.

Voucher specimens were taken as necessary, prepared by Richard W. Manning and deposited at the Texas Tech University collection. Skeletal muscle, organs, and/or blood were catalogued and stored at -80°C .

Specimens collected and prepared by previous researchers also were utilized in this analysis. Jim Yantis collected several specimens in Houston and Anderson counties in east Texas. Texas Cooperative Wildlife Collection (TCWC) housed several specimens and allowed us access to them (Appendix I), including the removal of skin samples from museum study skins. Samples from museum skins were taken with utmost care to preserve the integrity of the skin and to prevent contamination among specimens. Skin samples were approximately 5 mm^2 taken from the area around the incision made during the preparation of the skin. Scissors were flame-sterilized and gloves were changed before each skin clip.

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, as established by Choate (1972) included, as seen in Figure 3:

- Occipitopremaxillary length
- Length P4-M3
- Cranial breadth
- Breadth of zygomatic plate
- Maxillary breadth
- Interorbital breadth
- Length of mandible
- Height of mandible
- 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 using species as the independent variable and the nine cranial characters as response variables to determine if there were differences between species. After performing MANOVA, individual analysis of variance (ANOVA) tests (Quinn and Keough 2002) were performed for each cranial measure using the same *a priori* groups as in the MANOVA. Because of the increase in Type I error associated with multiple tests, the Bonferroni procedure was used to adjust the significance level (Quinn and Keough 2002). The resulting alpha level, adjusted for nine tests, was 0.0055.

S-Plus 6.1TM software was used for all morphological analyses.



FIGURE 3. Nine cranial measurements used in this study, as in Choate (1972).

Occipito-premaxillary length (a), P4-M3 (b), cranial breadth (c), breadth of zygomatic plate (d), maxillary breadth (e), interorbital breadth (f), length of mandible (g), height of mandible (h), and articular breadth (i).

DNA Sequencing

Cytochrome *b* from the mitochondrial genome was used in all analyses because baseline data for large-scale sampling of United States *Blarina* exclusive of Texas were readily available from GenBank. Although 500 bases from the 16S gene also were available for *Blarina*, that gene was excluded from these analyses because of perceived ambiguities regarding its alignment and phylogenetic analysis (Springer and Douzery 1996). The cytochrome *b* gene encodes a protein which spans the inner matrix, inner membrane, and outer intermembrane area in the mitochondrion, and acts as a component of complex III of the mitochondrial oxidative phosphorylation system (Griffiths 1997; Irwin et al. 1991). This gene has been widely utilized in systematic studies (e.g. Bradley and Baker 2001; Johns and Avise 1998; Voelker and Edwards 1998; Yoder et al. 1996). Although cytochrome *b* is useful in assessing phylogenies, potential pitfalls include differential evolutionary pressures based on location in the membrane (Griffiths 1997), insertions of the gene into the nuclear genome (Mirol et al. 2000; Mundy et al. 2000), and rate heterogeneity among taxa (Spradling et al. 2001).

The QiagenTM DNeasy kit was used to extract genomic DNA from skeletal muscle samples. The polymerase chain reaction (PCR) was used to amplify fragments of the mitochondrial genome. Amplification of the cytochrome *b* gene was in a 50 μ l reaction using 10 μ l *Taq* buffer (0.3 M TRIS, 0.0175 M MgCl₂, and 0.075 M (NH₄)₂SO₄, pH 8.5), 0.5 μ l DMSO, 0.5 μ l dNTP's (2.5 mM dATP, dCTP, dGTP, and dTTP), 0.5 μ l (10 mM) of each primer, 0.25 μ l *Taq* polymerase, and 0.5 μ l tDNA. The cytochrome *b* gene was sequenced for all samples. The following primers were used in PCR and sequencing: cytochrome *b*, L14724: 5'-CGAAGCTTGATAGAAAAACCATCGTTG-3' and H15915:

5'-AACTGCAGTCATCTCCGGTTTACAAGAC-3' (Irwin et al. 1991); plus an internal sequencing primer, cytbR1: 5'-GCTTCGTTGTTTGGAGGT-3' (Brant and Ortí 2002), and novel internal sequencing primers shrewCBF1: 5'-YTATTTTCTCCAGACTTACTAGGAGACCC-3' (where Y is C or T), and shrewCBR3: 5'-CCTCATGGAAGGACATACCCTATAAAGGCAGT-3'. The GeneAmp® PCR System 9700 performed denaturation at 94° C for 1 min followed by 35 cycles of 94° C (for 1 min), 50° C (for 30 s), and 72° C (for 1 min), and then a final extension of 72° C for 5 minutes. Results of PCR were visualized on a 1% agarose gel. The Marligen™ Rapid PCR Purification System was used for PCR cleanup. Clean PCR product was cycle sequenced in a 9 µl reaction using 0.5 µl primer, 3 µl Big Dye version 3.0, 2- 4 µl clean PCR product (depending on concentration) and 1.5- 3.5 µl water (depending on concentration of PCR product). GeneAmp® PCR System 9700 was used to perform 25 cycles of 96° C for 30 s, 50° C for 1 min, and 60° C for 4 min. Cycle sequence products were cleaned using 700 µl Sephadex solution (0.0625 g/ml) in a Centri-sep column (Princeton Separations). The ABI Prism 377 XL DNA sequencer assayed clean cycle sequence products using a 6% polyacrylamide gel.

Precautions were taken to avoid contamination when extracting, amplifying, and sequencing DNA from TCWC museum skins. Protocols for ancient DNA extraction included bleaching all laboratory bench surfaces, flame sterilizing metal utensils, UV sterilizing consumables such as tubes and micropipettor tips as well as pipettors, and including a negative control reaction for the extraction and subsequent PCR reactions. Extraction was performed using standard phenol-chloroform protocols rather than a kit. Tissue was incubated for 48 hours at 55 °C after adding 500 µl STE buffer, 25 µl

20%SDS, and 25 μ l of 20 mg/ml proteinase K. After two purifications in 500 μ l of PCI solution (25 phenol: 24 chloroform: 1 isoamyl alcohol) and two purifications in 500 μ l chloroform, tDNA was precipitated in 0.1 volume of 2M NaCl and 2.5 volumes of 99% ethanol. The tDNA pellet was dried 24 hours later and re-suspended in ddH₂O.

Amplification was performed using primers cyt**b**L as the forward primer and shrewCBR3 as the reverse primer for a total of 395 bases. Cycle sequencing was performed as previously described with ancient DNA protocols enforced.

Sequences were aligned using Sequencher™ 4.1. Sequences were compared with 38 previously aligned sequences obtained from GenBank, as published in Brant and Ortí (2002).

Phylogenetic Analyses

Thirty-four *Blarina*, two *Cryptotis parva* and two *Sorex cinereus* cytochrome *b* sequences from GenBank (accession numbers AF395449-86) were aligned with 23 Bastrop/Aransas sequences and exported as a NEXUS file to PAUP* 4.0b10 (Swofford 1999). *Sorex cinereus* and *Cryptotis parva* were designated as paraphyletic outgroups. Individuals were coded according to geographic location and GenBank accession number (Figure 4, Table 2). Morphological characters were excluded from phylogenetic analyses because of continuous variation in cranial measurements that could not be coded for analysis in an objective manner; see Poe and Wiens (2000) and Zelditch et al. (2000) for a full review.

Saturation was estimated by graphing the uncorrected-p distance on the x-axis as a measure of time since divergence against absolute number of transitions and

TABLE 2. Collection localities and abbreviations for *Blarina* specimens from Brant and Ortí (2002). Map codes correspond to locations in Figure 4. Individual abbreviations are noted as used in all phylogenetic analyses. Two-letter abbreviations used for all U.S. states. B.bre= *Blarina brevicauda*, B.car= *Blarina carolinensis*, B.hyl= *Blarina hylophaga*.

Species	County and State	Map Code	Individual Number
<i>B. brevicauda</i>	Lancaster, NB	8	64.B.bre.NB
	Dixon, NB	9	65.B.bre.NB
	Valley, NB	1	61.B.bre.NB
	Wooster, OH	10	69.B.bre.OH
	Wooster, OH	10	72.B.bre.OH
	Manitoba, Canada	12	62.B.bre.Manitoba
	Manitoba, Canada	12	63.B.bre.Manitoba
	Allamakee, IA	11	66.B.bre.IA
	Trigg, KY	14	67.B.bre.KY
	Trigg, KY	14	68.B.bre.KY
	Grafton, NH	13	70.B.bre.NH
	Grafton, NH	13	71.B.bre.NH
	James City, VA	15	73.B.bre.VA
	James City, VA	15	74.B.bre.VA
<i>B. hylophaga</i>	Nuckolls, NB	3	75.B.hyl.NB
	Nuckolls, NB	3	80.B.hyl.NB
	Lincoln, NB	2	81.B.hyl.NB
	Lincoln, NB	2	78.B.hyl.NB
	Richardson, NB	4	79.B.hyl.NB
	Richardson, NB	4	76.B.hyl.NB
	McPherson, KS	5	77.B.hyl.KS
	Montgomery, KS	6	82.B.hyl.KS
<i>B. carolinensis</i>	GA	22	50.B.car.GA
	GA	22	49.B.car.GA
	Vernon, LA	18	57.B.car.LA
	Webster, LA	17	55.B.car.LA
	Polk, AR	19	59.B.car.AR
	Polk, AR	19	56.B.car.AR
	Highlands, FL	20	53.B.car.FL
	Highlands, FL	20	54.B.car.FL
	Lancaster, VA	23	51.B.car.VA
	Lancaster, VA	23	52.B.car.VA
	Jackson, IL	21	60.B.car.IL
	Jackson, IL	21	58.B.car.IL

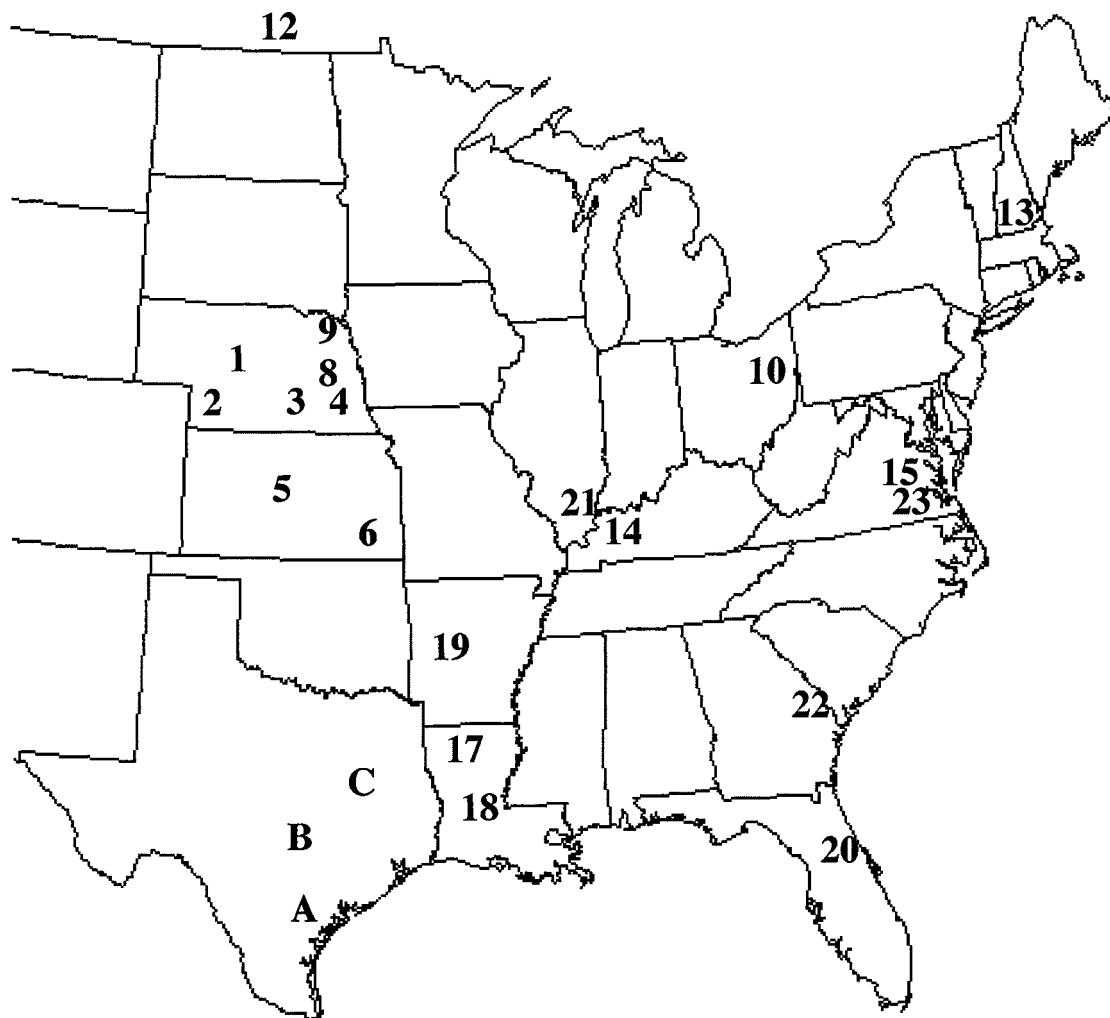


FIGURE 4. Sampling locations for this study and Brant and Ortí (2002). Numbers represent trapping locations by county as in Table 2. In Texas, A is Aransas County, B is Bastrop County, and C approximates Houston and Anderson counties where trapping occurred for this study (Appendix I).

transversions separately on the y-axis (Griffiths 1997). Saturation curves were created for the entire gene as well as for each codon position within the intermembrane, matrix, and transmembrane regions as in Griffiths (1997). Additionally, a partition homogeneity test with 100 repetitions was conducted to ensure that the three regions were suitable for uniform analyses. Neighbor joining (NJ) analysis was performed using HKY distances and bootstrapped using 1,000 pseudoreplicates. Maximum parsimony (MP) analysis was performed using a heuristic search with tree-bisection reconnection (TBR) branch swapping; gaps were treated as missing. The dataset was bootstrapped using 1,000 pseudoreplicates each with 5 random addition replicates. Weighted parsimony was performed similarly, but with transversions (TV) and transitions (TI) weighted based on estimation of the TI/TV ratio using maximum likelihood and codon positions weighted based on number of changes at each position. A bootstrap similar to that of MP analysis was performed on the weighted dataset.

Modeltest 3.06 (Posada and Crandall 1998) was used to determine the nucleotide substitution model that best fit the data. This model with the parameters given by Modeltest was used in a maximum likelihood (ML) analysis using a full heuristic search. Bayesian analyses were performed using MrBayes (v. 3.0, Huelsenbeck and Ronquist 2001) with 1,000,000 generations of four Monte Carlo Markov chains (MCMC) sampled every 1,000 generations. Only one outgroup is allowed by the software, so *Sorex cinereus* AF395485 was specified as the outgroup; however, *Cryptotis parva* was retained in the analysis.

The “Evaluate Random Trees” option in PAUP* (Swofford 1999) was used to create a frequency distribution of random trees and generate the g_1 statistic. This statistic is a measure of skewness that indicates the amount of phylogenetic signal in a dataset (Huelsenbeck 1991). A random dataset would have a normal distribution of random trees and a positive g_1 statistic, whereas a dataset with signal will produce a left-skewed distribution of random trees with a negative value for g_1 (Huelsenbeck 1991).

Each novel sequence and the compiled data set was 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 (nuclear introns) was exposed prior to the final analyses.

RESULTS

Specimen Collection

Three fresh specimens were collected from traps placed on the Aransas Wildlife Refuge (Appendix I). Twenty-seven specimens were collected on Griffith League Ranch in Bastrop County, and one (MF8057) was collected at Schulz Ranch in southern Bastrop County (Appendix I). Additional morphological and molecular data were obtained from preserved specimens at the Texas Cooperative Wildlife Collection (Appendix I).

Morphological Analyses

Most cranial measures were linearly correlated, with Pearson's correlation coefficient (r) ranging from 0.29 to 0.84 (Table 3). PCA ($n= 52$) recovered nine principal components, the first of which (PC1) explained 77.7% of the variance and had an eigenvalue of 0.97. The second component (PC2) was retained for use in the ordinal plot (Figure 5) 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 occipito-premaxillary length which had a loading of 0.708. Remaining principal components were not included in further analyses; all had eigenvalues less than 0.25 and explained less than 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

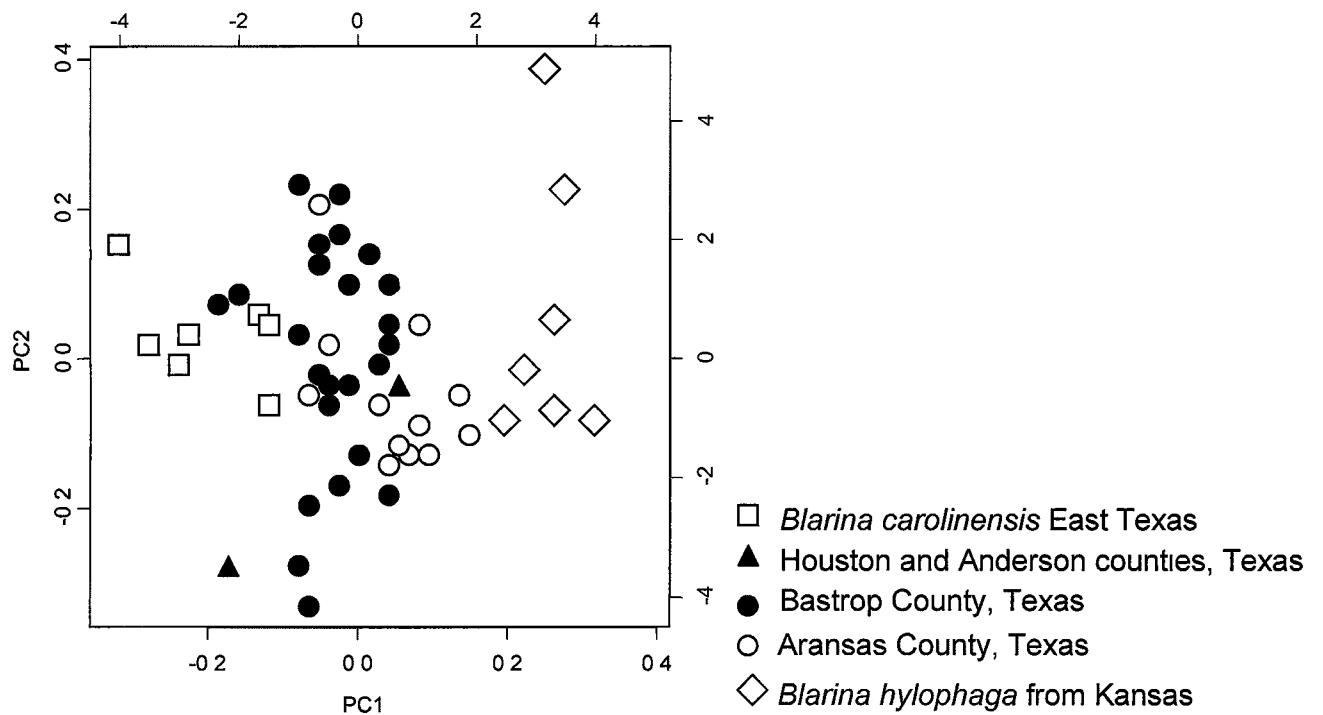


FIGURE 5. Ordinal plot of the first two principal components in the analysis of nine cranial measurements for *Blarina* ($n=52$). All nine cranial characters, as used in Choate (1972), were positively correlated with PC1. Symbols differ for geographic regions as indicated in key.

intermediate between the two species on PC1, although there was overlap between two Bastrop specimens and *B. carolinensis*.

MANOVA was used to differentiate among three groups: TCWC *B. carolinensis* and TCWC 51797 (previously identified as *B. carolinensis*), *B. hylophaga* from Kansas, and Texas *Blarina*; the Pillai's trace test statistic was 1.22837 ($P < 0.001$). In univariate tests of the nine cranial measures, two were non-significant: zygomatic plate breadth ($P = 0.792$) and interorbital breadth ($P = 0.009$).

DNA Sequencing

Cytochrome *b* sequences 1140 nucleotides in length were obtained for three *Blarina* specimens from Aransas NWR and 20 specimens from Bastrop County. The Texas populations do not share any haplotypes with *Blarina hylophaga* from Kansas and Nebraska (Brant and Ortí 2002). Four distinct haplotypes were present, one unique to Aransas NWR (Haplotype A) and three in the Bastrop population (Haplotypes B1, B2, and B3). Haplotype A was unique to the three Aransas 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 one individual, MF 9158, and differed from Haplotype B2 by only one nucleotide (see Appendix 2). Non-synonymous mutations resulted in differing proteins, with changes at amino acid positions 23, 25, 189, and 327 (Table 4). Amino acid 25 is located in the matrix domain of the cytochrome *b* protein, which normally includes mostly polar amino acids; amino acid 189 is located in the transmembrane domain in Helix D, which usually contains hydrophobic amino acids (Griffiths 1997). Partial cytochrome *b* sequence was obtained for the paratype of *B.*

hylophaga plumbea, TCWC#1542; the first 400 bases of cytochrome *b* were sequenced successfully and found to be identical to Haplotype B1.

The sequences obtained are assumed to be mtDNA rather than nuclear pseudogenes from mtDNA sequence (also known as numts) based on the following characteristics as outlined by Zhang and Hewitt (1996): 1) PCR amplification consistently produced only one band, 2) No sequence ambiguities or background bands persisted, 3) No unexpected insertions, deletions, or stop codons occurred, 4) Nucleotide sequences were not radically different from those expected, and 5) Phylogenetic analyses did not yield an unusual or contradictory tree topology.

Phylogenetic Analyses

The complete alignment consisted of 1140 nucleotides of cytochrome *b* for 38 taxa from GenBank, three Aransas specimens, and 20 Bastrop specimens. The treelength distribution for 10,000 randomly generated trees was skewed left (Figure 6) with a g_1 of -0.389, therefore these data are significantly more structured than random data ($P < 0.01$) (Hillis and Huelsenbeck 1992). There was no significant difference found among the intermembrane, matrix, and transmembrane regions of the gene in the partition homogeneity test ($P = 0.50$). Base frequencies were equivalent at first positions ($\chi^2 = 1.5$, $P > 0.5$) but did not conform to a 1:1:1:1 ratio for position 2 ($\chi^2 = 65.64$, $P < 0.01$) or position 3 ($\chi^2 = 240.6$, $P < 0.01$) (Table 5).

TABLE 4. Amino acids for five haplotypes in two isolated populations of *Blarina* in Texas compared to GenBank sequences from all three species of *Blarina* (see Table 2) from Brant and Ortí (2002). Haplotype B1 was the predominant haplotype in samples from Bastrop County ($n=14$), with Haplotype B2 ($n=4$) less common, and Haplotype B3 found in only one individual. Haplotype A is the only haplotype found in the Aransas County samples. Haplotype S was only present in the individual from southern Bastrop County.

	A.A. 23	A.A. 25	A.A. 189	A.A. 327
Haplotype B1	Alanine	Serine	Isoleucine	Isoleucine
Haplotypes B2, B3	Alanine	Alanine	Isoleucine	Isoleucine
Haplotype A	Alanine	Serine	Valine	Isoleucine
Haplotype S	Threonine	Serine	Isoleucine	Valine
<i>Blarina hylophaga</i>	Alanine	Serine	Isoleucine	Isoleucine
<i>Blarina carolinensis</i>	Alanine	Serine	Isoleucine	Isoleucine
<i>Blarina brevicauda</i>	Alanine	Serine	Isoleucine	Isoleucine

TABLE 5. Mean base frequencies for all *Blarina* individuals in this study as well as outgroups for each codon position over the entire cytochrome *b* gene.

	A	C	G	T
Codon Position 1	0.26596	0.26096	0.22433	0.24875
Codon Position 2	0.19741	0.23756	0.14467	0.42036
Codon Position 3	0.38871	0.35490	0.04418	0.21221

Saturation of base substitutions does occur in the cytochrome *b* data set between ingroup and outgroup (Figure 7). Saturation curves produced for each codon position within the three domains of the membrane protein showed that saturation does not occur at any codon position in any protein region within ingroup taxa, but ingroup-outgroup saturation is present in third position transitions of all three regions of the protein as well as first codon position transitions of the transmembrane region (Appendix 3). There is 0.5-0.6% divergence in cytochrome *b* between the two Texas lineages and 1.2-2.2% between Texas and other populations of *B. hylophaga* (Table 6).

TABLE 6. Percent difference between and within species of *Blarina*, calculated over all 1140 bases of cytochrome *b*. *Blarina carolinensis*, *B. hylophaga*, and *B. brevicauda* sequences from Brant and Ortí (2002), Texas *Blarina* sequences from Bastrop and Aransas counties.

	<i>B. carolinensis</i>	<i>B. hylophaga</i>	Texas <i>Blarina</i>	<i>B. brevicauda</i>
<i>B. carolinensis</i>	0.09- 3.9%			
<i>B. hylophaga</i>	6.1- 11.1%	0.1- 0.97%		
Texas <i>Blarina</i>	5.7- 6.6%	1.2-2.5%	0.1- 0.6%	
<i>B. brevicauda</i>	6.5- 8.2%	8.6- 10%	8.3-9.0%	0.1- 2.9%

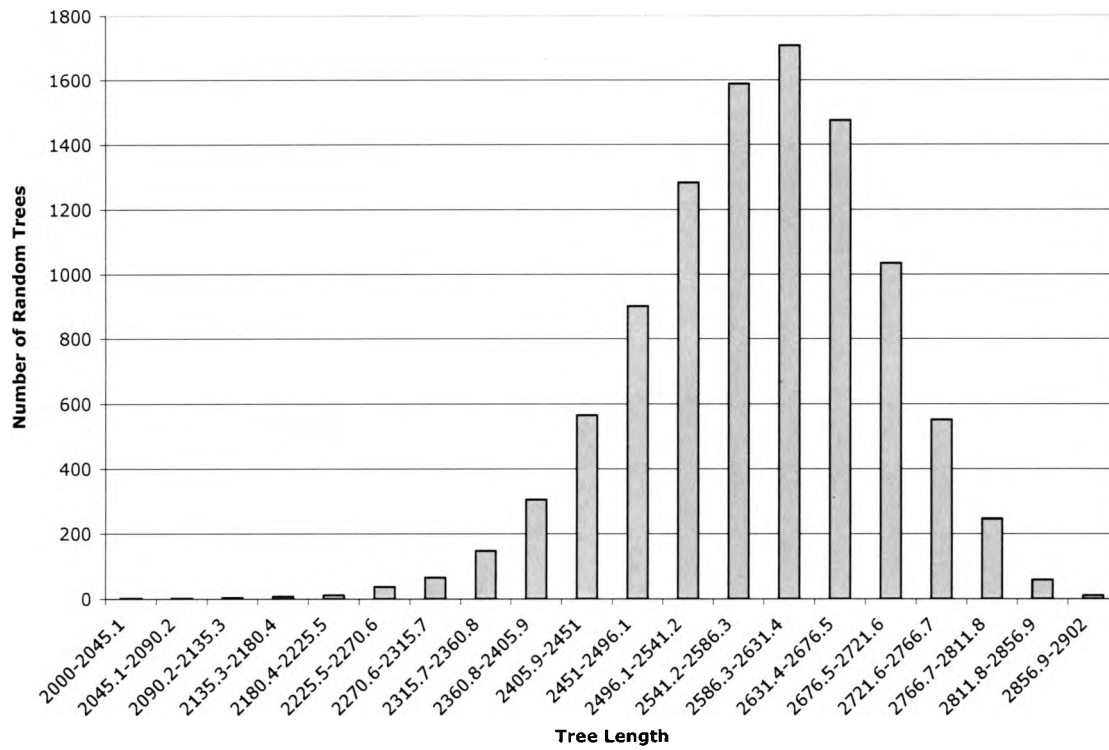


FIGURE 6. Distribution of 10,000 randomly generated trees from the *Blarina* data set of 1140 nucleotides of cytochrome *b* for 61 taxa. Trees were generated using parsimony criteria. The g_1 statistic, a measure of skewness and phylogenetic signal, was -0.389.

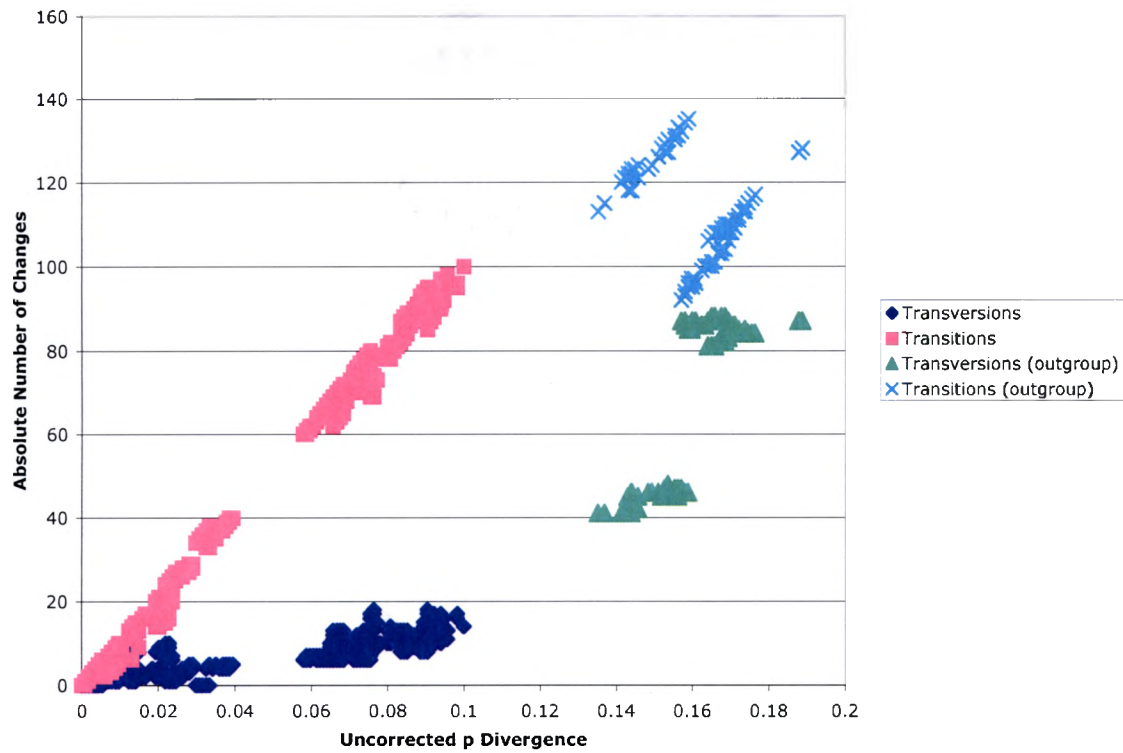


FIGURE 7. Saturation curve for the complete cytochrome *b* gene for the *Blarina* data set, constructed using uncorrected p distance as a measure of time on the X-axis and absolute number of transitions and transversion on the Y-axis. Transitions and transversions labeled separately for ingroup and outgroup. Saturation does not occur within the ingroup but does occur between ingroup and outgroup transitions.

Parsimony analyses of the full data set with all characters equally weighted resulted in 18 equally parsimonious trees with tree length 565, retention index (RI) of 0.941 and consistency index (CI) of 0.724 (Figure 8). Of the molecular characters, 801 were constant and 322 of the variable sites were parsimony informative. The MP topology supports three monophyletic species of *Blarina* with *B. brevicauda* basal within the genus, and Texas *Blarina* sister to *B. hylophaga*. The NJ bootstrap topology agrees with MP at the interspecific level; the Texas clade is sister to Kansas/Nebraska *B. hylophaga* (Figure 9). Weighted parsimony was performed with differential weights for each codon position based on the number of changes for each position in the unweighted MP topology (Figure 8): codon position 1= 0.1125, position 2= 1, and position 3= 0.019, transversions were weighted at 5 times the transversion weight based on the TI/TV ratio from the ModelTest 3.06 analysis. The resulting topology had a polytomy at the interspecific level and a sister relationship between Texas *Blarina* and Kansas/Nebraska *B. hylophaga* (Figure 10).

Maximum likelihood analysis was performed using the parameters given by Modeltest 3.06, which were nucleotide frequencies of A=0.3118, C= 0.2912, T= 0.2779, and G= 0.1191; TI/TV ratio of 5.2241; proportion of invariable sites equal to zero; and a gamma distribution parameter of 0.1813, which cumulatively indicated a high number of practically invariable sites (Nei and Kumar 2000). The resulting topology supported a monophyletic *B. hylophaga* within the Texas *Blarina*, and *B. hylophaga* basal within the genus (Figure 11).

The 50% majority-rule consensus tree for Bayesian analysis was calculated using 500 trees (Figure 12), and the resulting topology supported a sister relationship between *B. brevicauda* and *B. carolinensis*. Also, the Texas clade was sister to *B. hylophaga* with 97% posterior probability. The proportion of trees supporting a particular clade is considered to be the Bayesian posterior probability for that clade (Wilcox et al. 2002). Bayesian support values are considered less conservative than bootstrap and possibly closer estimates of phylogenetic accuracy, given that the correct model of evolution is in use (Wilcox et al. 2002).

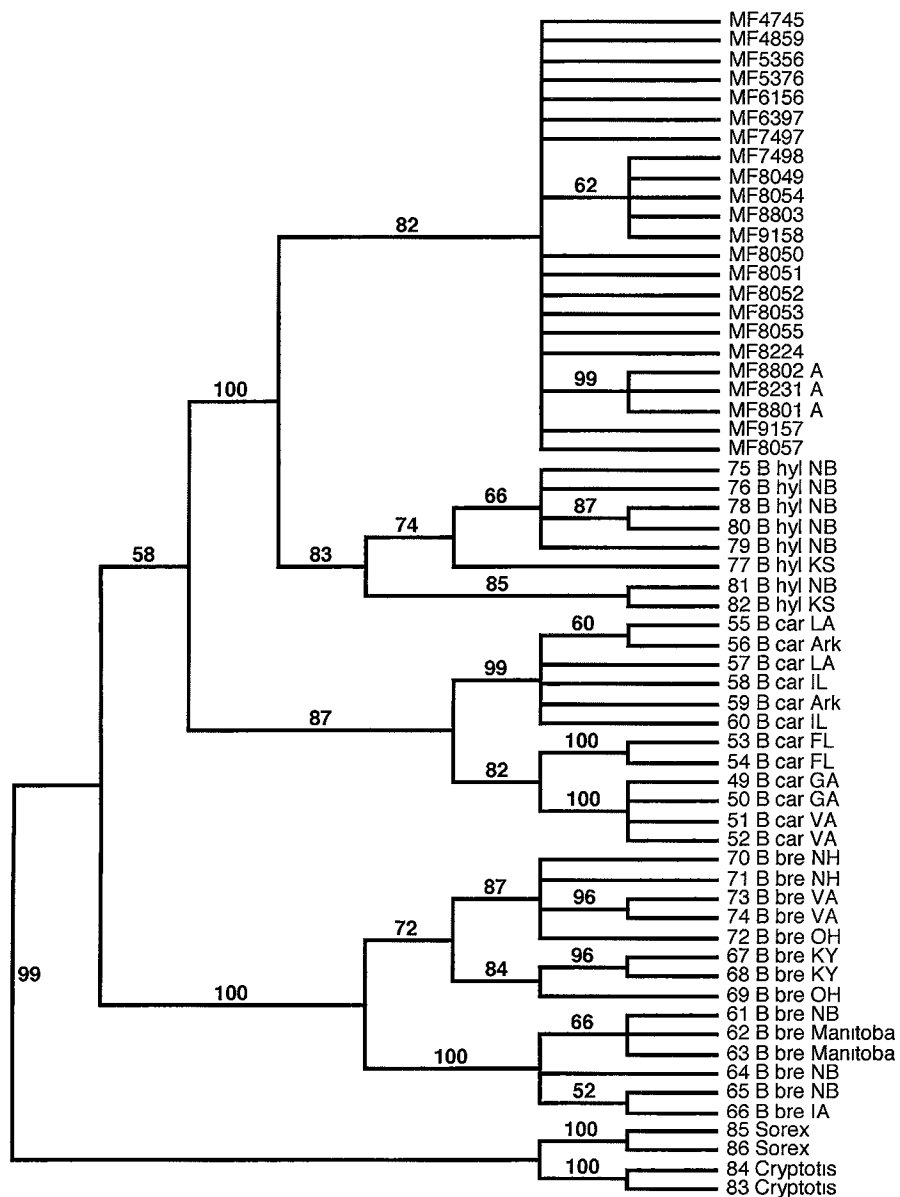


FIGURE 8. Bootstrap consensus tree for cytochrome *b* in three species of *Blarina* and Texas specimens using unweighted parsimony with bootstrap support values on respective branches. Three species of *Blarina* abbreviated as in Table 2, MF numbers are assigned to all Bastrop and Aransas county specimens, the latter with an “A” appended. *Cryptotis* and *Sorex* were used as outgroups.

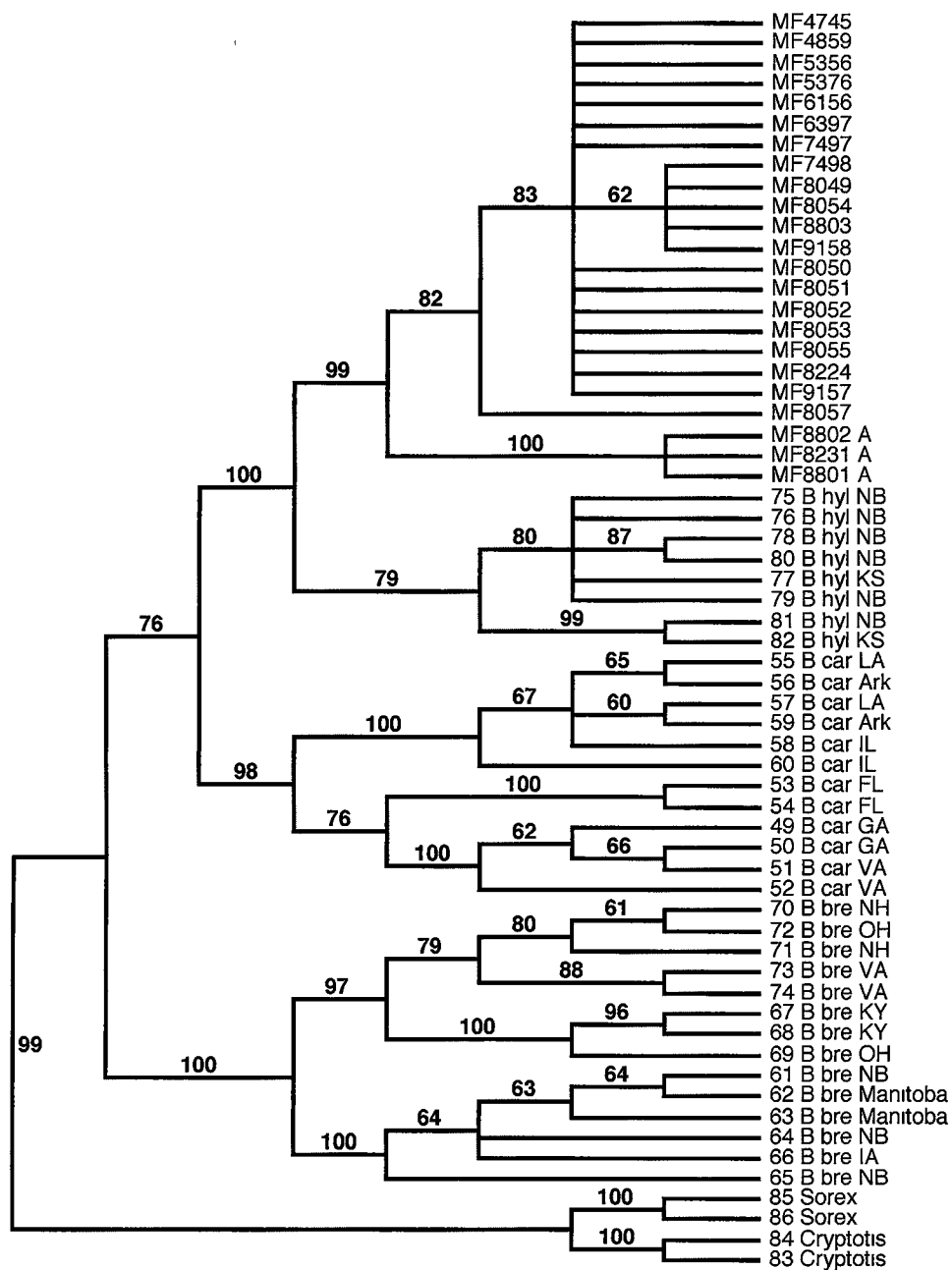


FIGURE 9. Neighbor joining consensus tree with bootstrap values on respective branches created using HKY nucleotide substitution model. *Sorex* and *Cryptotis* are outgroups, three species of *Blarina* labeled as in Table 2; MF numbers for Texas specimens as in Appendix I, all from Bastrop except for three with an “A” appended. Tree created using 1140 bases of cytochrome *b*.

FIGURE 10. Consensus tree with bootstrap values on respective branches for weighted parsimony in which codon position 1 was weighted 0.1125, position 2 at 1, and position 3 at 0.019. Transversions weighted at five times the weight of transitions. *Sorex* and *Cryptotis* are outgroups, three species of *Blarina* labeled as in Table 2; MF numbers for Texas specimens as in Appendix I, all from Bastrop except for three with an “A” appended. Tree created using 1140 bases of cytochrome *b*.

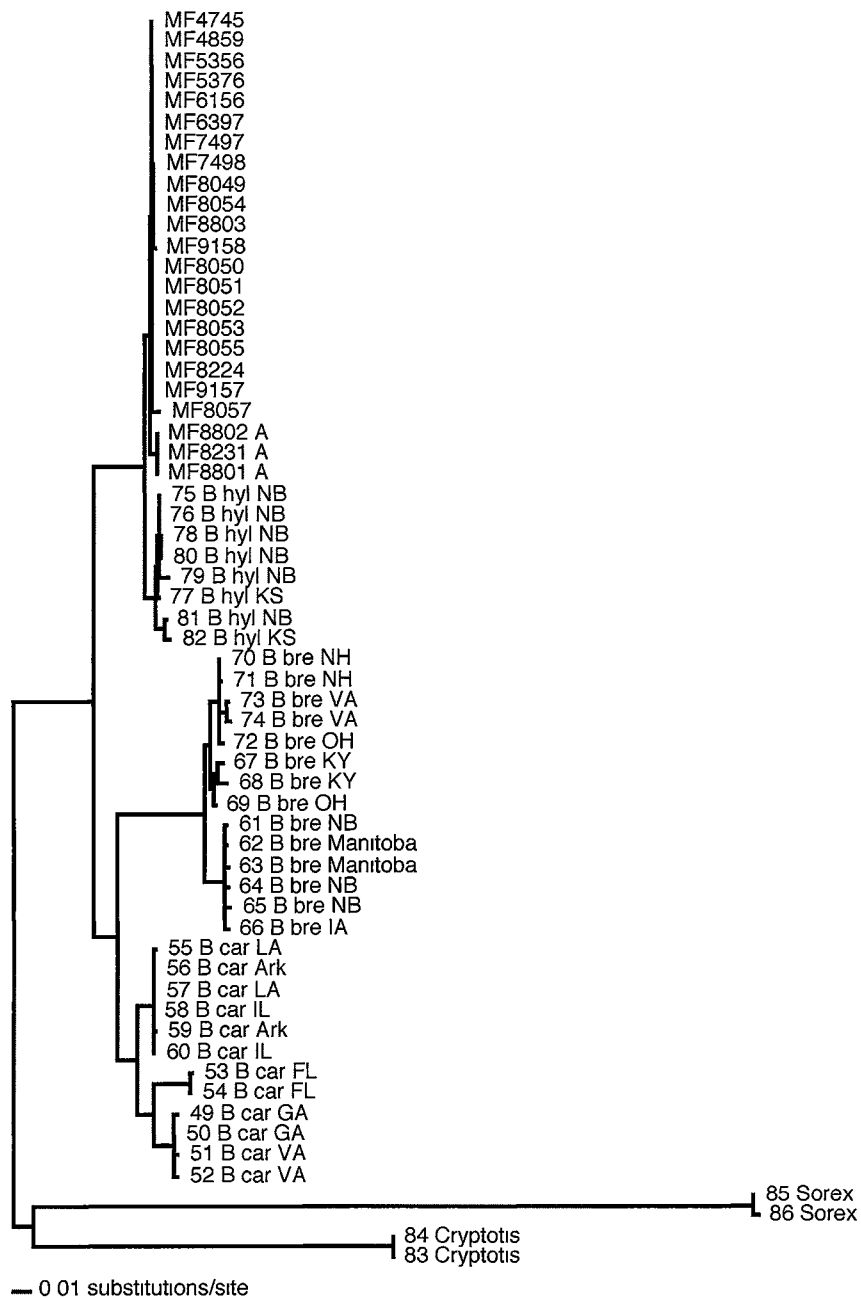


FIGURE 11. Maximum likelihood topology created from the *Blarina* cytochrome *b* data set of 1140 bases for 55 individuals using the HKY+G model of nucleotide substitution with gamma of 0.1813, Ti/Tv ratio of 5.2241, proportion of invariable sites equal to zero, and base frequencies of A=0.3118, C=0.2912, G=0.1191, and T=0.2779. Branch lengths proportional to number of changes; see key directly below figure.

FIGURE 12. 50% Majority-rule consensus tree from 500 trees produced after 1,000,000 generations of four Monte Carlo Markov Chains in MrBayes v.3.0. Bayesian posterior probabilities are noted on respective branches. *Sorex* and *Cryptotis* are outgroups; abbreviations for individuals follows Table 2 except for Texas specimens designated by MF numbers, Aransas specimens with an “A” appended.

DISCUSSION

Morphology

Analyses performed here support the two Texas populations being 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 PC 2 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 three groups in this comparison have considerable overlap on that axis (Figure 5). This is congruent with previous studies using PCA to differentiate species of *Blarina* (Genoways and Choate 1972).

One individual short-tailed shrew (TCWC# 51209) captured in Bastrop County previously had been identified as *B. carolinensis*, and indeed fit 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 the pitfalls of using size-based characters to identify animals; individuals that are not fully

grown will be classified as the smaller of the species. Another example can be found in the East Texas specimens from Houston and Anderson counties, one of which fits in with the Texas *B. hylophaga* on the ordinal plot (Figure 5). 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 additional population of *B. hylophaga*.

Clinal variation in size has been documented in previous studies of *Blarina* (e.g. George et al. 1981; Jones and Findley 1954), and the Texas shrews being smaller than northern populations of *B. hylophaga* supports those studies. This clinal variation correlates with Bergmann's Rule, which states that within a taxon, animals at higher latitudes are larger than those closer to the equator (Brown and Lomolino 1998). However, factors other than latitude also affect body size. Because the *Blarina* in Central Texas inhabit an island-like fragment of pine forest and the coastal population is equally insular by analogy, it is possible that their body sizes have changed due to differences in predators, competitors, or other niche factors from the main population. Vertebrate species that colonize islands often exhibit size differences from their mainland ancestors (Case 1978). There is no consistent trend for insectivores to be larger or smaller on islands (Lomolino 1985), so there is no prediction for these shrews to be larger or smaller than the analogous "mainland" shrew population means.

Because morphological differences among species of *Blarina* are primarily size-based, however, island effects should be a consideration when observing trends in body size. Additionally, as there is no size-free shape component to differentiate between species of *Blarina*, morphology is not useful to identify shrews in geographically isolated populations. For example, one key to the identification of soricids differentiates between

B. hylophaga and *B. carolinensis* based on size, stating that animals less than 100 millimeters (mm) in total length, with condylobasal length of less than 20 mm and weighing less than 10 grams are *B. carolinensis* (Choate et al. 1994). Few of the Texas *B. hylophaga* would be correctly identified using this key, as they are small enough to be incorrectly identified as *B. carolinensis*.

Molecular Phylogenetics

The use of mitochondrial DNA in phylogenetic studies is widespread and, despite controversy, been well established as an effective tool to examine evolutionary histories of taxa. Because the mitochondrial genome is uniparentally inherited and haploid, its effective population size (N_e) is 1/4 that of nuclear markers (Moore 1995). While this may enable early detection of speciation, ancestral polymorphisms in incipient species may result in misleading patterns in gene trees (Neigel and Avise 1986). Conversely, speciation may be detected early based on phenotypic differences between incipient species in which mtDNA divergence cannot be detected (Nice and Shapiro 1999). It is essential to be aware of these possibilities and to be cautious when making conclusions based primarily on mtDNA.

Cytochrome *b* frequently is used in studies of molecular phylogenetics of animals (Adachi and Hasegawa 1996). Evolutionary pressures differ within this gene by membrane region, codon position, and amino acid. Based on the saturation curves produced for the codon positions within each transmembrane region (Appendix 3), down-weighting third positions throughout the gene will result in a loss of significant amounts of unsaturated positions that could otherwise be informative. Hence, weighted parsimony

may result in a loss of phylogenetic signal in this gene. Additionally, when creating saturation curves it is essential to consider saturation within the ingroup and between the ingroup and outgroup separately.

DNA was successfully extracted and sequenced from two museum skins, one of which was the paratype for the subspecies *B. hylophaga plumbea*. This individual was collected at the Aransas NWR in 1941 by Davis (1941). The 400 bases of cytochrome *b* sequenced for this individual are identical to Haplotype B1, the predominant haplotype in Bastrop County. Phylogenetic analyses using cytochrome *b* unambiguously place the paratype of *Blarina hylophaga plumbea* in the *B. hylophaga* clade. These analyses should allay any doubts regarding the phylogenetic placement of this isolated population.

The Texas populations of short-tailed shrews form a monophyletic sister group to other U.S. populations of *Blarina hylophaga*; within Texas, Bastrop and Aransas harbor unique clades. Many of the molecular analyses were in agreement in the placement of the Texas clade as a sister group to *B. hylophaga*. Maximum likelihood supported an alternate hypothesis of a sister relationship between Aransas and the Kansas/Nebraska *B. hylophaga*, with the remainder of Texas *Blarina* sister to that group; this topology is probably an artifact of anomalies in the Kansas/Nebraska GenBank sequences, and is in fact unsupported by any synapomorphies. This can be assessed by observing the length of the terminal branches of *B. hylophaga* in comparison to *B. carolinensis*. There was no overlap in haplotypes between Texas and Kansas/Nebraska *B. hylophaga*. Because sampling was limited at Aransas NWR, it is possible that other haplotypes are present in the population but were not found in this study.

Results differed among analyses with respect to the relationships of the three species of *Blarina*. Previous morphological studies have supported the (*B. brevicauda* (*B. carolinensis*, *B. hylophaga*)) topology (Jones et al. 1984; Stangl and Carr 1997), but the recent molecular phylogeny by Brant and Ortí (2002) supports a (*B. hylophaga* (*B. brevicauda*, *B. carolinensis*)) topology. In this study, the former relationship was supported in neighbor joining and parsimony analysis, and the latter in Bayesian and ML analyses. Weighted parsimony did not resolve the interspecific relationship. Support for either of the resolved relationships was generally low and consisted of synonymous third and first codon position changes, many of which had low CI values for the topology in question, indicating homoplasy. Other genes may be successful in recovering any interspecific structure, or morphology can be used as additional evidence to confirm *B. brevicauda* as a basal species within *Blarina*.

Biogeography and Areas of Endemism

These shrews may represent relictual isolates of *B. hylophaga*'s Pleistocene range in Texas. Aransas and Bastrop counties do not have similar floras, but must share some characteristics that make them hospitable to short-tailed shrews. Similarities between Aransas and Bastrop counties that could make both suitable as shrew habitat include deep sandy soils, ancient trees, deep leaf litter, abundance of prey, and suitable thermal climate. While other areas of sandy soil exist in Texas, such as the coastal sand plain in South Texas, lack of leaf litter or other factors may prohibit shrews from inhabiting the area. It also is possible that *Blarina* do not occupy all suitable habitats in Texas, and

other circumstances have narrowed their distribution. Examining patterns of distribution in other species may aid in understanding the pattern of short-tailed shrew distributions in Texas.

Many small mammals inhabited Texas in the Pleistocene and may aid in understanding the pattern of distribution in *Blarina hylophaga* in Texas. Other small mammals with ranges extending southward from the midwest include the thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*) and the woodland vole (*Microtus pinetorum*), both of which were reported at Aransas NWR by Davis (1941). Small mammals having Pleistocene-era fossils in Texas whose current ranges no longer include Texas or include only relictual populations include two additional species of vole (*Microtus pennsylvanicus* and *M. ochrogaster*), the southern bog lemming *Synaptomys cooperi*, the ermine *Mustela erminea*, and the cinereus shrew *Sorex cinereus* (Lundelius 1967). The common pattern among these animals is a northward-shifting range, probably a consequence of warming temperatures over time. Some of these taxa, such as *Microtus*, may be more tolerant of warm weather and therefore able to maintain isolated populations in Texas, especially in favorable habitats.

The Lost Pines area in Central Texas is an area characterized by stands of loblolly pine (*Pinus taeda*) on sandy soils. This area is superficially similar to the Piney Woods of East 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. Animals such as flying squirrels (*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. An endemic insect, the Texas long-lipped beetle (*Telegeusis texensis*), recently was described from specimens collected in the Lost Pines (Taber and Fleenor 2003).

These animals inhabit a unique ecosystem that 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). This evidence strongly suggests that the Lost Pines region is a remnant of a more widespread pine forest.

Aransas National Wildlife Refuge also harbors unique fauna; it is most well known as the wintering grounds for the endangered whooping crane (*Grus americana*). The rare Texas scarlet snake, *Cemophora lineri*, also has been found on the refuge. Both the Lost Pines and Aransas NWR may represent areas of local endemism for many taxa. Both areas have been scrutinized as endangered species habitat, but also should be examined at the ecosystem level because of their unique nature.

Taxonomy

While the debate over species concepts continues, some model must be applied in order to define species taxonomically. Often a combinatorial approach is necessary, using several data types to confirm the status of a taxon; for example, Wiens and Penkrot (2002) proposed an approach using DNA and morphological data to delimit species. In the case of these isolated populations of *Blarina*, however, morphological characters can

be nebulous or even misleading. Characters such as nuclear DNA or a karyotype may be useful to support mtDNA phylogenies in this case. In an instance involving isolated populations of pocket gophers which only had mtDNA evidence available, a “molecular-phenetics species concept” was implemented, using known differences of closely related taxa to create a standard to identify potential species-level differences (Demastes et al. 2002). This model is similar to the “cytochrome-*b* species concept” (Bradley and Baker 2001), but with the caveat that other types of evidence should be applied as well.

The Biological Species Concept (BSC) (Mayr 1942), Genealogical Species Concept (GSC) (Mishler and Donoghue 1982), and Phylogenetic Species Concept (PSC) (Cracraft 1983; Nelson and Platnick 1981) have been discussed philosophically to a tremendous extent. It is difficult to decide which concept is appropriate to apply, so it is necessary to consider the applicability of each concept to this research, which involves two allopatric populations of morphologically indistinguishable animals with differences in molecular traits.

The BSC defines species as “groups of interbreeding natural populations that are reproductively isolated from other such groups” (Mayr 1969). Observations of interbreeding are difficult to obtain for many if not most species, and for allopatric populations these observations could only take place in captive organisms. For Texas *Blarina*, it is unknown whether breeding with Oklahoma, Kansas, or Nebraska *Blarina* would be successful; therefore, no evidence is available to support or refute classification of Texas *Blarina* under this concept.

Employment of the phenetic measure of percent divergence as a criterion to define species may be misleading when considering the differential rate of evolution

among taxa (Spradling et al. 2001) that may be affected by body size, thermal habit, and metabolic rate (Martin and Palumbi 1993; Rand 1994). Oxygen radicals cause damage to mtDNA, increasing rate of evolution in the mitochondrial genome (Richter et al. 1988); shrews exhibit extremely high oxygen consumption per gram body mass, *Blarina brevicauda* basal metabolic rates being over 150% of the expected value (Churchfield 1990). Therefore intra- and inter-species sequence divergences cannot be standardized among taxa; however, it remains reasonable to use a within-taxon standard to gauge differences in that taxon. Both *B. carolinensis* and *B. brevicauda* have intraspecific divisions that are attributable to the Mississippi River; distances between those east-west clades average 3.3% and 2.5%, respectively (Table 6). The genetic distance between Texas *B. hylophaga* and the northern population of that species ranges from 1.2-2.2%, and the genetic distance between the Aransas and Bastrop populations is 0.5%. This evidence along with the monophyly of this group is sufficient evidence to classify the Texas *B. hylophaga* as a subspecies, *Blarina hylophaga plumbea* (Davis 1941) under the GSC.

The PSC defines species as “the smallest aggregation of ... populations... diagnosable by a unique combination of character states” (Wheeler and Platnick 2000). Under the strictest interpretation of this concept, every haplotype in this study would constitute a separate species; there is no room for intraspecific classification under the PSC. Certainly both Aransas and Bastrop populations would warrant species status, since each population does have a unique combination of character states. Widespread use of the PSC by taxonomists would undoubtedly lead to significant increase in the number of recognized species, if only by elevating subspecies to species.

The designation of subspecies is a taxonomic tradition that has been brought into question under several species concepts. A subspecies can be defined as “a recognizably different population...that occupies a different geographic area from other populations of the same species” (Futuyma 1998); however, problems arise when attempting to differentiate between a subspecies and a population, which is “a group of conspecific organisms that occupy a...well defined geographic region and exhibit reproductive continuity from generation to generation” (Futuyma 1998). Many subspecies are recognized as morphologically different ecotypes; this use is perpetuated in field guides. It has been noted that recognition of a subspecies requires as much evidence as for a species, with the additional assumptions that the lineage may reconnect and interbreed with the main lineage; although it is convenient to designate subspecies in collections and field guides, it is difficult to justify a subspecies concept (Frost et al. 1992). One potential utility of subspecies lies in conservation of locally endemic subspecies which may be protected under the Endangered Species Act; such geographic variants of a species are important components of biodiversity (O'Brien and Mayr 1991). From the perspective of traditional recognition of subspecies as geographic variants or “a genetically distinct set of populations with a discrete range” (Brown and Lomolino 1998), the short-tailed shrews in Aransas and Bastrop counties can be recognized as a subspecies.

The two isolated populations of *Blarina* in Texas certainly warrant subspecies recognition, and possibly species recognition under strict interpretation of the PSC (Cracraft 1983). The Texas populations appear to be monophyletic (*sensu* Hennig 1966) in that they are more closely related to each other than to other lineages and they appear to have descended from a common ancestor. Therefore, based on geographic isolation

and genetic similarity, it is justifiable to separate them into a single Texas endemic subspecies: *Blarina hylophaga plumbea*.

Parallels can be drawn between the Texas and Florida subspecies of short-tailed shrews. Two subspecies 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 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 be determined using DNA, but the opportunity to conserve this unique population was lost.

The description of the endemic Texas subspecies *Blarina hylophaga plumbea* should be followed with population estimates and characterization of the ecology of these populations. The isolated populations in Aransas and Bastrop counties, Texas, should be monitored so that they do not meet the same fate as *B. c. shermani*.

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APPENDIX 1

Tissue sample and voucher information for individuals of the genus *Blarina* collected for this study or used in morphological analyses. All individuals in the Michael Forstner (MF) tissue catalog are identified with their unique catalog number. Location, collector, and external measurements may be noted; if cytochrome *b* was successfully sequenced for that individual, haplotype is noted (B1, B2, and B3 from Bastrop County, A from Aransas County, and S for one individual from southern Bastrop County—see Results). For individuals belonging to the Texas Cooperative Wildlife Collection (TCWC) and used only for morphological analyses, TCWC number, collection date, sex, and location are noted.

MF#	Collector #	Haplotype	Voucher	Sex
MF# 4745	GL 101; RWM 3686	B1	Skin, skull	
MF# 4859	GL 75; RWM 3683	B1	Skin, skull	
MF# 5356	RWM 3691 GL275	B1	Skin, skull	M
MF# 5375	RWM3693 G374		Skin, skull	F
MF# 5376	RWM3692 G373	B1	Skin, skull	F
MF# 6156	RWM 3694	B1	Skeleton only	U
MF# 6397	RWM 3689; GL300		Skin, skull	M
MF# 7497	RWM 3700	B1	Skin, skull	M
MF# 7498	RWM 3701	B2	Skin, skull	F
MF# 8049	RWM 3711	B2	Skin, skull	F
MF# 8050	RWM 3712	B1	Skin, skull	F
MF# 8051	RWM 3713	B1	Skin, skull	F
MF# 8052	RWM 3714	B1	Skin, skull	M
MF# 8053	RWM 3716	B1	Skin, skull	M
MF# 8054	RWM 3717	B2	Skin, skull	F
MF# 8055	RWM 3718	B1	Skin, skull	F
MF# 8057	RWM 3715		Skin, skull	M
MF# 8224	RWM 3720	B1	Skin, skull	F
MF# 8225	RWM 3721		Skin, skull	M
MF# 8226	RWM 3722		Skin, skull	F
MF# 8227	RWM 3723		Skeleton	
MF# 8228	RWM 3724		Skeleton	
MF# 8230	RWM 3675		Skin, skull	
MF# 8231	RWM 3725	A		F
MF# 8741			Released alive	U
MF# 8801	RWM 3726	A	Skin, skull	U
MF# 8802	RWM 3727	A	Skin, skull	M
MF# 8803	RWM 3728	B2	Skin, skull	M
MF#9156	RWM#3729		Skel. only	F
MF#9157	RWM#3730	B1	Skin, skull	Juv M
MF#9158	RWM#3731	B3	Skin, skull	M
MF#9159	RWM#3732		Skel. only	U
MF#9238	Yantis			
MF#9239	Yantis			
MF#9240	Yantis			

MF#	Collector #	Haplotype	Voucher	Sex
MF#9241	Yantis			
MF#9242	Yantis			
MF#9243	Yantis			
MF#9268	TCWC#1541		Skin, skull	F
MF#9269	TCWC#1542	B1	Skin, skull	M
MF#9270	TCWC#30395		Skin, skull	F
MF#9271	TCWC#30396		Skin, skull	F
MF#9272	TCWC#31833		Skin, skeleton	F
MF#9273	TCWC#31834		Skin, skeleton	F
MF#9274	TCWC#31835		Skin, skeleton	F
MF#9275	TCWC#31836		Skin, skeleton	F
MF#9276	TCWC#31837		Skin, skull	F
	TCWC#51207		Skull, alcohol specimen	U
	TCWC#51208		Skin, Skull	M
	TCWC#51209		Skeleton	U
	TCWC#51797		Skin, skeleton	F
	TCWC#27628			F
	TCWC#33360			F
	TCWC#34970			F
	TCWC#34971			F
	TCWC#33359			F
	TCWC#33361			F
	TCWC#50138			M
	TCWC#50143			F
	TCWC#50131			F
	TCWC#50132			M
	TCWC#50133			M
	TCWC#50134			F
	TCWC#33351			M
	TCWC#34952			M
	TCWC#34956			F
	TCWC#33337			F
	TCWC#33355			F

MF#	Location
MF# 4745	Griffith League Ranch
MF# 4859	Griffith League Ranch
MF# 5356	Griffith League Ranch
MF# 5375	Griffith League Ranch
MF# 5376	Griffith League Ranch
MF# 6156	Griffith League Ranch
MF# 6397	Griffith League Ranch
MF# 7497	Griffith League Ranch
MF# 7498	Griffith League Ranch 10-2
MF# 8049	Line 14, Griffith League
MF# 8050	Bucket 5-1, Griffith League
MF# 8051	Line 13, Griffith League
MF# 8052	Bucket 12-2, Griffith League
MF# 8053	Griffith League
MF# 8054	Griffith League
MF# 8055	Griffith League
MF# 8057	10 miles west of Smithville
MF# 8224	Griffith League Ranch; 16-1
MF# 8225	Griffith League Ranch; 16-E
MF# 8226	Griffith League Ranch
MF# 8227	# 8
MF# 8228	Griffith League Ranch
MF# 8230	Aransas National Wildlife Refuge
MF# 8231	Aransas National Wildlife Refuge
MF# 8741	Griffith League bucket B-S
MF# 8801	Aransas NWR, Stinson's trap in U-20
MF# 8802	Aransas NWR, trap in open motte by rocky spot
MF# 8803	Griffith League Ranch trap 14-1
MF#9156	Griffith League Ranch 12-3
MF#9157	Griffith League Ranch A-W
MF#9158	Griffith League Ranch V20 bucket 1
MF#9159	Griffith League Ranch
MF#9238	
MF#9239	14 mi. E. center of Palestine. 31 deg. 41' N, 95 deg. 23' W
MF#9240	

MF#	Location
MF#9241	12 mi. NNW center of Ratcliff, 31deg.33' N, 95deg 9' W
MF#9242	12 mi NE center of Crockett, 31 deg 24' N, 95 deg 17' W
MF#9243	20 mi SE center of Palestine 31 deg 39' N, 95 deg 19' W
MF#9268	Aransas National Wildlife Refuge
MF#9269	Aransas Refuge, near Dagger Point
MF#9270	Aransas National Wildlife Refuge
MF#9271	Aransas National Wildlife Refuge
MF#9272	Aransas National Wildlife Refuge
MF#9273	Aransas National Wildlife Refuge
MF#9274	Aransas National Wildlife Refuge
MF#9275	Aransas National Wildlife Refuge
MF#9276	Aransas National Wildlife Refuge
TCWC#51207	ca 2 mi. E Bastrop (county seat), 30deg7'N, 97deg16'W
TCWC#51208	ca 2 mi. E Bastrop (county seat), 30deg7'N, 97deg16'W
TCWC#51209	ca 2 mi. E Bastrop (county seat), 30deg7'N, 97deg16'W
TCWC#51797	ca 2 mi. E Bastrop (county seat), 30deg7'N, 97deg16'W
TCWC#27628	
TCWC#33360	
TCWC#34970	
TCWC#34971	
TCWC#33359	
TCWC#33361	
TCWC#50138	
TCWC#50143	
TCWC#50131	
TCWC#50132	
TCWC#50133	
TCWC#50134	
TCWC#33351	
TCWC#34952	
TCWC#34956	
TCWC#33337	
TCWC#33355	

MF#	County	State	Collection date	Tissue type
MF# 4745	Bastrop	TX	4/22/01	skeletal muscle
MF# 4859	Bastrop	TX	4/13/01	skeletal muscle
MF# 5356	Bastrop	TX	5/25/01	skeletal muscle
MF# 5375	Bastrop	TX	7/2/01	skeletal muscle
MF# 5376	Bastrop	TX	7/2/01	skeletal muscle
MF# 6156	Bastrop	TX	7/10/01	skeletal muscle
MF# 6397	Bastrop	TX	5/7/01	skeletal muscle
MF# 7497	Bastrop	TX	7/10/02	Heart and skeletal muscle
MF# 7498	Bastrop	TX	7/10/02	Heart and skeletal muscle
MF# 8049	Bastrop	TX	1/11/03	Skeletal muscle and heart
MF# 8050	Bastrop	TX	1/27/03	Skeletal muscle and heart
MF# 8051	Bastrop	TX	1/11/03	Skeletal muscle and heart
MF# 8052	Bastrop	TX	1/26/03	Skeletal muscle and heart
MF# 8053	Bastrop	TX	unknown	Skeletal muscle and heart
MF# 8054	Bastrop	TX	unknown	Skeletal muscle and heart
MF# 8055	Bastrop	TX	unknown	Skeletal muscle and heart
MF# 8057	Bastrop	TX	2/2/03	no eyelids; skel muscle, heart
MF# 8224	Bastrop	TX	5/12/03	heart and sk. muscle
MF# 8225	Bastrop	TX	6/4/03	heart and sk. muscle
MF# 8226	Bastrop	TX	6/4/03	heart and sk. muscle
MF# 8227	?	?	?	heart and sk. muscle
MF# 8228	Bastrop	TX	5/18/03	heart and sk. muscle
MF# 8230	Aransas	TX	12/3/00	Vertebrae off study specimen
MF# 8231	Aransas	TX	6/12/03	Heart and skel muscle; 1st array
MF# 8741	Bastrop	TX	6/18/03	blood
MF# 8801	Aransas	TX	6/13/03	Muscle
MF# 8802	Aransas	TX	6/25/03	Muscle, heart, liver
MF# 8803	Bastrop	TX	7/4/03	Muscle, heart, liver
MF#9156	Bastrop	TX	5/21/03	Muscle, heart, liver
MF#9157	Bastrop	TX	6/14/03	Muscle, heart, liver
MF#9158	Bastrop	TX	6/21/03	Muscle, heart, liver
MF#9159	Bastrop	TX		Muscle, heart, liver
MF#9238				tooth
MF#9239	Anderson	TX	6/13/02	Dried skin from skull

MF#9240				Alcoholic specimen skin/ muscle sample
MF#9241	Houston	TX	6/5/02	Alcoholic specimen skin/ muscle sample
MF#9242	Houston	TX	5/19/02	Alcoholic specimen skin/ muscle sample
MF#9243	Anderson	TX	6/6/02	
MF#9268	Aransas	TX	1/13/41	Museum skin clip
MF#9269	Aransas	TX	3/15/41	Museum skin clip
MF#9270	Aransas	TX	1/23/76	Museum skin clip
MF#9271	Aransas	TX	1/24/76	Museum skin clip
MF#9272	Aransas	TX	5/24/76	Museum skin clip
MF#9273	Aransas	TX	5/22/76	Museum skin clip
MF#9274	Aransas	TX	7/25/76	Museum skin clip
MF#9275	Aransas	TX	6/18/76	Museum skin clip
MF#9276	Aransas	TX	6/18/76	Museum skin clip
TCWC#51207	Bastrop	TX	3/25/89	
TCWC#51208	Bastrop	TX	3/27/90	
TCWC#51209	Bastrop	TX	7/25/90	
TCWC#51797	Bastrop	TX	Jun-91	
TCWC#27628	Newton	TX	4/10/74	
TCWC#33360	Tyler	TX	1/12/78	
TCWC#34970	Tyler	TX	1/6/79	
TCWC#34971	Tyler	TX	1/7/79	
TCWC#33359	Tyler	TX	1/12/78	
TCWC#33361	Tyler	TX	2/11/78	
TCWC#50138	Geary	KS	6/24/86	
TCWC#50143	Geary	KS	6/30/86	
TCWC#50131	Geary	KS	6/23/86	
TCWC#50132	Geary	KS	6/23/86	
TCWC#50133	Geary	KS	6/23/86	
TCWC#50134	Geary	KS	6/23/86	
TCWC#33351	Tyler	TX	1/5/78	
TCWC#34952	Hardin	TX	1/28/79	
TCWC#34956	Hardin	TX	1/28/79	
TCWC#33337	Hardin	TX	1/12/78	
TCWC#33355	Tyler	TX	1/7/78	

MF#	Collector	Total L.	Tail L.	HF	Weight
MF# 4745	unknown				
MF# 4859	unknown				
MF# 5356	unknown	94	20	12	8.2
MF# 5375	unknown	80	20	11	6.1
MF# 5376	unknown	92	21	12	7.4
MF# 6156	unknown	90	21	12	6.2
MF# 6397	unknown	90	20	12	8
MF# 7497	unknown	88	18	11	8
MF# 7498	unknown	94	21	11	7.5
MF# 8049	unknown				
MF# 8050	unknown				6.9 g
MF# 8051	unknown				5.7 g
MF# 8052	unknown				10 g
MF# 8053	unknown				
MF# 8054	unknown				
MF# 8055	unknown				
MF# 8057	TR Simpson				
MF# 8224	Todd Swannack	84	17	12	
MF# 8225	Todd Swannack	91	18	12	
MF# 8226	Todd Swannack	80	17	12	
MF# 8227	Todd Swannack	90	21	13	
MF# 8228	Todd Swannack				
MF# 8230	Richard W, Manning				
MF# 8231	S. Morris	88	18	13	
MF# 8741	S. Morris				
MF# 8801	S.Morris				
MF# 8802	S. Morris	83	18	12	
MF# 8803	T.Swannack	83	18	11	
MF#9156	S. Morris	86	19	13	6.2
MF#9157	S. Morris	80	17	12	5.4
MF#9158	S. Morris	89	18	12	5.6
MF#9159	S. Morris				
MF#9238	Jim Yantis				
MF#9239	Jim Yantis				

MF#	Collector	Total L.	Tail L.	HF	Weight
MF#9240	Jim Yantis				
MF#9241	Jim Yantis				
MF#9242	Jim Yantis				
MF#9243	Jim Yantis				
MF#9268	Stevenson, J.				
MF#9269	Davis, W.B.				
MF#9270	R.A. Sparks				
MF#9271	R.A. Sparks				
MF#9272	W. Brown				
MF#9273	W. Brown				
MF#9274	W. Brown				
MF#9275	W. Brown				
MF#9276	R.A. Sparks				
TCWC#51207	J.R. Dixon and J. Godwin				
TCWC#51208	N. Dronen and Scarbrough				
TCWC#51209	N. Dronen and Scarbrough				
TCWC#51797	G. Baumgardner				
TCWC#27628					
TCWC#33360					
TCWC#34970					
TCWC#34971					
TCWC#33359					
TCWC#33361					
TCWC#50138					
TCWC#50143					
TCWC#50131					
TCWC#50132					
TCWC#50133					
TCWC#50134					
TCWC#33351					
TCWC#34952					
TCWC#34956					
TCWC#33337					
TCWC#33355					

APPENDIX 2

Final alignment of 1140 nucleotides of cytochrome *b* for 61 individuals used in phylogenetic analyses in this study. Rows are taxa, columns are nucleotide positions as numbered in column headings. Specimens from Texas have unique numbers as catalogued in the Michael Forstner (MF) tissue catalog (Appendix 1). Thirty-eight sequences downloaded from GenBank are designated using the last two digits of the GenBank accession number, an abbreviated species name (B.hyl= *Blarina hylophaga*, B.car= *B. carolinensis*, B.bre= *B. brevicauda*, Cryptotis= *Cryptotis parva*, Sorex= *Sorex cinereus*), and either a two-letter state code for U.S. specimens or a complete province name for Canadian specimens.

	10	20	30	40	50
MF4745	ATGACAAATATCCGAAAA	ACTCACCCACTAATAAAA	ATCATCAACAGCTC		
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A					
MF8231.A					
MF8801.A					
MF8803					
MF9157					
MF8057					
MF9158					
75.B.hyl.NB			C.....		
76.B.hyl.NB			C.....		
78.B.hyl.NB			C.....		
80.B.hyl.NB			C.....		
81.B.hyl.NB			C.....		
82.B.hyl.KS			C.....		
77.B.hyl.KS			C.....		
79.B.hyl.NB			C.....		TG..
70.B.bre.NH		C.....		C.....	T.....
71.B.bre.NH		C.....		C.....	T.....
73.B.bre.VA		C.....		C.....	T.....
74.B.bre.VA		C.....		C.....	T.....
67.B.bre.KY		T..C.....	C.....	C.....	T..?
69.B.bre.OH		C.....		C.....	T.....
68.B.bre.KY		T..C.....		C.....	T.....
72.B.bre.OH		C.....		C.....	T.....
61.B.bre.NB			G.....	C.....	T.....
62.B.bre.Manitoba			G.....	C.....	T.....
63.B.bre.Manitoba			G.....	C.....	T.....
64.B.bre.NB			G.....	C.....	T.....
65.B.bre.NB			G.....	C.....	T.....
66.B.bre.IA			G.....	C.....	T.....
55.B.car.LA				C.....	
56.B.car.Ark				C.....	
57.B.car.LA				C.....	
58.B.car.IL				C.....	
53.B.car.FL				C.....	
54.B.car.FL				C.....	
59.B.car.Ark				C.....	
60.B.car.IL				C.....	
49.B.car.GA				C.....	
50.B.car.GA				C.....	
51.B.car.VA				C.....	
52.B.car.VA				C.....	
85.Sorex		CC.A.....	C.....	CT.....	T..T.....
86.Sorex		CC.A.....	C.....	CT.....	T..T.....
84.Cryptotis		C.....	C.....	T.....	T.....
83.Cryptotis		C.....	C.....	T.....	T.....

	60	70	80	90	100
MF4745	ATTTATTGACCTACCCGCACCATCCAATATTTTCATCGTGATGAAACTTTG				
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498	G.....				
MF8049	G.....				
MF8050					
MF8051					
MF8052					
MF8053					
MF8054	G.....				
MF8055					
MF8224					
MF8802.A					
MF8231.A					
MF8801.A					
MF8803	G.....				
MF9157					
MF8057	A.....				
MF9158	G.....				
75.B.hyl.NB					
76.B.hyl.NB					
78.B.hyl.NB					
80.B.hyl.NB					
81.B.hyl.NB					
82.B.hyl.KS					
77.B.hyl.KS					
79.B.hyl.NB					
70.B.bre.NH	C.....A.....				
71.B.bre.NH	C.....A.....				
73.B.bre.VA	C.....A.....				
74.B.bre.VA	C.....A.....				
67.B.bre.KY	C.....A.....				
69.B.bre.OH	C.....A.....				
68.B.bre.KY	C.....A.....				
72.B.bre.OH	C.....A.G.....				
61.B.bre.NB	C.....C.....C.....				
62.B.bre.Manitoba	C.....C.....C.....				
63.B.bre.Manitoba	C.....C.....C.....				
64.B.bre.NB	C.....C.....				
65.B.bre.NB	C.....C.....				
66.B.bre.IA	C.C.....C.....				
55.B.car.LA	A.....T.....				
56.B.car.Ark	A.....T.....				
57.B.car.LA	A.....T.....				
58.B.car.IL	A.....T.....				
53.B.car.FL	C.....A.....T.....				
54.B.car.FL	C.....A.....T.....				
59.B.car.Ark	A.....T.....				
60.B.car.IL	A.....T.....				
49.B.car.GA	C.....A.....T.....				
50.B.car.GA	C.....A.....T.....				
51.B.car.VA	C.....A.....T.....				
52.B.car.VA	C.....A.....T.....				
85.Sorex	TT...T...T...T...C.....A.....T...C.				
86.Sorex	TT...T...T...T...C.....A.....T...C.				
84.Cryptotis	T...A.C.....C.C.....A.....T...C.				
83.Cryptotis	T...A.C.....C.C.....A.....T...C.				

	110	120	130	140	150
MF4745	GCTCCCTATTAGGCATTTGCCTAATCATTC	CAAATCCTAACAGGCCTATTC			
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A					
MF8231.A					
MF8801.A					
MF8803					
MF9157					
MF8057					
MF9158					
75.B.hyl.NB	C.....				
76.B.hyl.NB	C.....				
78.B.hyl.NB	C.....				
80.B.hyl.NB	C.....				
81.B.hyl.NB	C.....				
82.B.hyl.KS	C.....				
77.B.hyl.KS	C.....				
79.B.hyl.NB	C.....				
70.B.bre.NH	GC.....	T.....	C.....	G.....	T.....
71.B.bre.NH	GC.....	T.....	C.....	G.....	T.....
73.B.bre.VA	GC.....	T.....	C.....	G.....	T.....
74.B.bre.VA	GC.....	T.....	C.....	G.....	T.....
67.B.bre.KY	GC.....	T.....			T.....
69.B.bre.OH	GC.....	T.....			T.....
68.B.bre.KY	GC.....	T.....			T.....
72.B.bre.OH	GC.....	T.....	C.....	G.....	T.....
61.B.bre.NB	GC.....	G.....	T.....		T.....
62.B.bre.Manitoba	GC.....	G.....	T.....		T.....
63.B.bre.Manitoba	GC.....	G.....	T.....		T.....
64.B.bre.NB	GC.....	G.....	T.....		T.....
65.B.bre.NB	GC.....	G.....	T.....		T.....
66.B.bre.IA	GC.....	G.....	T.....		T.....
55.B.car.LA	C.....	T.....		T.....	G.....
56.B.car.Ark	C.....	T.....		T.....	G.....
57.B.car.LA	C.....	T.....		T.....	G.....
58.B.car.IL	C.....	T.....		T.....	G.....
53.B.car.FL	T.....			T.....	
54.B.car.FL	T.....			T.....	
59.B.car.Ark	C.....	T.....		T.....	G.....
60.B.car.IL	C.....	T.....		T.....	G.....
49.B.car.GA		T.....	G.....		T.....
50.B.car.GA		T.....		T.....	
51.B.car.VA		T.....		T.....	
52.B.car.VA		T.....		T.....	
85.Sorex	T.....	TC.....	TG.....	T.....	G.....
86.Sorex	T.....	TC.....	TG.....	T.....	G.....
84.Cryptotis	TT.....	C.....	T.....	T.....	C.....
83.Cryptotis	TT.....	C.....	T.....	T.....	C.....

	160	170	180	190	200
MF4745	CTAGCCATACATTACACATCAGACACAGTAACTGCCTTTTCATCTGTCAC				
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A				C.	
MF8231.A				C.	
MF8801.A				C.	
MF8803					
MF9157					
MF8057					
MF9158					
75.B.hyl.NB					
76.B.hyl.NB					
78.B.hyl.NB					
80.B.hyl.NB					
81.B.hyl.NB	Y.....				
82.B.hyl.KS	Y.....				
77.B.hyl.KS					
79.B.hyl.NB					
70.B.bre.NH	T.....C.....				
71.B.bre.NH	T.....C.....				
73.B.bre.VA	T.....C.....				
74.B.bre.VA	T.....C.....				
67.B.bre.KY	T.....C.....				
69.B.bre.OH	T.....C.....				
68.B.bre.KY	T.....CAC.....				
72.B.bre.OH	T.....C.....				
61.B.bre.NB	T.....C.....				
62.B.bre.Manitoba	T.....C.....				
63.B.bre.Manitoba	T.....C.....				
64.B.bre.NB	T.....C.....				
65.B.bre.NB	T.....CT.....				
66.B.bre.IA	T.....C.....				
55.B.car.LA	G.....T.....G.....A.....				
56.B.car.Ark	G.....T.....G.....A.....				
57.B.car.LA	G.....T.....G.....A.....				
58.B.car.IL	G.....T.....G.....A.....				
53.B.car.FL	C.....A.....				
54.B.car.FL	C.....A.....				
59.B.car.Ark	G.....T.....G.....A.....				
60.B.car.IL	G.....T.....G.....A.....				
49.B.car.GA	G.....T.....G.....A.....C.....				
50.B.car.GA	G.....T.....G.....A.....C.....				
51.B.car.VA	G.....T.....G.....A.....C.....				
52.B.car.VA	G.....T.....G.....A.....C.....				
85.Sorex	A.....C.....T.....G.....A.....C.....C.....A.....				
86.Sorex	A.....C.....T.....G.....A.....C.....C.....A.....				
84.Cryptotis	T.....T.....A.....C.....T.....C.....A.....				
83.Cryptotis	T.....T.....A.....C.....T.....C.....A.....				

	210	220	230	240	250
MF4745	CCATATTTGCCGAGACGTTAATTACGGTTGACTGATCCGCTATCTACACG				
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A	C.....C.....				
MF8231.A	C.....C.....				
MF8801.A	C.....C.....				
MF8803					
MF9157					
MF8057					
MF9158					
75.B.hyl.NB	C.....				
76.B.hyl.NB	C.....				
78.B.hyl.NB	C.....				
80.B.hyl.NB	C.....				
81.B.hyl.NB					
82.B.hyl.KS	C.....				
77.B.hyl.KS	C.....				
79.B.hyl.NB	C.....				
70.B.bre.NH	...C.....G....C.....A.....				
71.B.bre.NH	...C.....G....C.....A.....				
73.B.bre.VA	...C.....G....C.....A.....				
74.B.bre.VA	...C.....G....C.....A.....				
67.B.bre.KY	...C.....C.....A.....				
69.B.bre.OH	...C.....C.....A.....				
68.B.bre.KY	T..C.....C.....A.....T...				
72.B.bre.OH	...C.....G....C.....A.....				
61.B.bre.NB	...C.....C.....C....A.....				
62.B.bre.Manitoba	...C.....C.....C....A.....				
63.B.bre.Manitoba	...C.....C.....C....A.....				
64.B.bre.NB	...C.....C.....C....A.....				
65.B.bre.NB	...C.....C.....C....A.....				
66.B.bre.IA	...C.....C.....C....A.....				
55.B.car.LA	...C.....C....G.....				
56.B.car.Ark	...C.....C....G.....				
57.B.car.LA	...C.....C....G.....				
58.B.car.IL	...C.....C....G.....				
53.B.car.FL	C....G.....G....				
54.B.car.FL	C....G.....G....				
59.B.car.Ark	...C.....C....G.....				
60.B.car.IL	...C.....C....G.....				
49.B.car.GA	C.....G....				
50.B.car.GA	C.....G....				
51.B.car.VA	C.....G....				
52.B.car.VA	C.....G....				
85.Sorex	A..C....T.....A..C....A....A.....C..T..T.				
86.Sorex	A..C....T.....A..C....A....A.....C..T..T.				
84.Cryptotis	C.....A....T.....A....A..T.....				
83.Cryptotis	C.....A....T.....A....A..T.....				

	260	270	280	290	300
MF4745	CAAACGGCGCATCCATATTTTTCATCTGCTTATTTCTACACGTCGGACGA				
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A					
MF8231.A					
MF8801.A					
MF8803					
MF9157					
MF8057				T	
MF9158					
75.B.hyl.NB			C.G		
76.B.hyl.NB			C.G		
78.B.hyl.NB			C.G		
80.B.hyl.NB			C.G		
81.B.hyl.NB			C.G		
82.B.hyl.KS			C.G		
77.B.hyl.KS			C.G		
79.B.hyl.NB			C.G		
70.B.bre.NH	...T.....A....C..T.....C.....				
71.B.bre.NH	...T.....A....C..T.....C.....				
73.B.bre.VA	...T.....A....C..T.....C.....				
74.B.bre.VA	...T.....A....C..T.....C.....				
67.B.bre.KY	...T.....A....C..T.....C.....				
69.B.bre.OH	...T.....A....C..T.....C.....				
68.B.bre.KY	...T.....A....C..T.....C.....				
72.B.bre.OH	...T.....A....C..T.....C.....				
61.B.bre.NB	...T.....A....C..T.....C.....				
62.B.bre.Manitoba	...T.....A....C..T.....C.....				
63.B.bre.Manitoba	...T.....A....C..T.....C.....				
64.B.bre.NB	...T.....A....C..T.....C.....				
65.B.bre.NB	...T.....A....C..T.....C.....				
66.B.bre.IA	...T.....A....C..T.....C.....				
55.B.car.LA	G..C.....				
56.B.car.Ark	G..C.....				
57.B.car.LA	G..C.....				
58.B.car.IL	G..C.....				
53.B.car.FL	G..C.....				
54.B.car.FL	G..C.....				
59.B.car.Ark	G..C.....				
60.B.car.IL	G..C.....				
49.B.car.GA	C.....				
50.B.car.GA	C.....				
51.B.car.VA	C.....A.....				
52.B.car.VA	C.....				
85.Sorex	...T..T.....C..T..T..C...C..T.....				
86.Sorex	...T..T.....C..T..T..C...C..T.....				
84.Cryptotis	A.....G..C.....G..T..T.....				
83.Cryptotis	A.....G..C.....G..T..T.....				

	310	320	330	340	350
MF4745	GGTCTTTACTACGGATCCTATATATTCTAGAGACATGAAACATTGGTGT				
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A					
MF8231.A					
MF8801.A					
MF8803					
MF9157					
MF8057					
MF9158					
75.B.hyl.NB	A.....				
76.B.hyl.NB	A.....				
78.B.hyl.NB	G.A.....				
80.B.hyl.NB	G.A.....				
81.B.hyl.NB	A.....T.....				
82.B.hyl.KS	A.....T.....				
77.B.hyl.KS	A.....				
79.B.hyl.NB	A.....				
70.B.bre.NH	..C.....G.....C.....CT...A.....				
71.B.bre.NH	..C.....G.....C.....CT...A.....				
73.B.bre.VA	..C.....G.....C.....CT...A.....				
74.B.bre.VA	..C.....G.....C.....CT...A.....				
67.B.bre.KY	..C.....G.....C.....CT...A.....				
69.B.bre.OH	..C.....G.....C.....CT...A.....				
68.B.bre.KY	..C.....G.....C.....CT...A.....				
72.B.bre.OH	..C.....G.....C.....CT...A.....				
61.B.bre.NB	..C.....G.....C.....CA...A.....				
62.B.bre.Manitoba	..C.....G.....C.....CT...A.....				
63.B.bre.Manitoba	..C.....G.....C.....CT...A.....				
64.B.bre.NB	..C.....G.....C.....CT...A.....				
65.B.bre.NB	..C.....G.....C.....CT...A.....				
66.B.bre.IA	..C.....G.....C.....CT...A.....				
55.B.car.LA	..C.....G.....C.....CT.....				
56.B.car.Ark	..C.....G.....C.....CT.....				
57.B.car.LA	..C.....G.....C.....CTC.....				
58.B.car.IL	..C.....G.....C.....CT.....				
53.B.car.FL	..C.....G.....C.....T.G.....				
54.B.car.FL	..C.....G.....C.....T.G.....				
59.B.car.Ark	..C.....G.....C.....CT.....				
60.B.car.IL	..C.....G.....C.....CT.....				
49.B.car.GA	..C.....C.....CT.G.....				
50.B.car.GA	..C.....C.....CT.G.....				
51.B.car.VA	..C.....C.....CT.G.....				
52.B.car.VA	..C.....C.....CT.G.....				
85.Sorex	C..T.....A.....C.....A.....C..A..				
86.Sorex	C..T.....A.....C.....A.....C..A..				
84.Cryptotis	..C..A.....A.....A.....T.....G..				
83.Cryptotis	..C..A.....A.....A.....T.....G..				

	360	370	380	390	400
MF4745	CCTGCTACTATTTGCAGTTATAGCGACTGCCTTTATAGGGTATGTCCTCC				
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A					
MF8231.A					
MF8801.A					
MF8803					
MF9157					
MF8057					
MF9158					
75.B.hyl.NB					
76.B.hyl.NB					
78.B.hyl.NB					
80.B.hyl.NB					
81.B.hyl.NB					
82.B.hyl.KS					
77.B.hyl.KS					
79.B.hyl.NB					
70.B.bre.NH					T.
71.B.bre.NH					T.
73.B.bre.VA					T.
74.B.bre.VA					T.
67.B.bre.KY					T.
69.B.bre.OH					T.
68.B.bre.KY	G.....				T.
72.B.bre.OH					T.
61.B.bre.NB					T.
62.B.bre.Manitoba					T.
63.B.bre.Manitoba					T.
64.B.bre.NB	...A.....				T.
65.B.bre.NB					T.
66.B.bre.IA					T.
55.B.car.LA		T.....		C.....	T.
56.B.car.Ark		T.....		C.....	T.
57.B.car.LA		T.....		C.....	T.
58.B.car.IL		T.....		C.....	T.
53.B.car.FL		C.....C.....	T.....	C.....	T.
54.B.car.FL		C.....C.....	T.....	C.....	T.
59.B.car.Ark		T.....		C.....	T.
60.B.car.IL		T.....		C.....	T.
49.B.car.GA	...A.....	T.....		C.....	T.
50.B.car.GA	...A.....	T.....		C.....	T.
51.B.car.VA	...A.....	T.....		C.....	T.
52.B.car.VA	...A.....	C.....		C.....	T.
85.Sorex	A..A...T...C....A...A.....			C..A..T.	
86.Sorex	A..A...T...C....A...A.....			C..A..T.	
84.Cryptotis	.T.A..CT...C....A...T.....			A..C.....	
83.Cryptotis	.T.A..CT...C....A...T.....			A..C.....	

	410	420	430	440	450
MF4745	CATGAGGACAAATGTCATTCTGAGGTGCCACAGTCATTACCAACCTACTC				
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A					
MF8231.A					
MF8801.A					
MF8803					
MF9157					
MF8057					
MF9158					
75.B.hyl.NB					G..
76.B.hyl.NB					G..
78.B.hyl.NB					G..
80.B.hyl.NB					G..
81.B.hyl.NB					G..
82.B.hyl.KS					G..
77.B.hyl.KS					G..
79.B.hyl.NB					G..
70.B.bre.NH			T..	C..T..T..	
71.B.bre.NH			T..	C..T..T..	
73.B.bre.VA			T..	C..T..T..	
74.B.bre.VA			T..	C..T..T..	
67.B.bre.KY			T..	C..T..T..	
69.B.bre.OH			T..	C..T..T..	
68.B.bre.KY			T..	C..T..T..	
72.B.bre.OH			T..	C..T..T..	
61.B.bre.NB				C..T..T..	
62.B.bre.Manitoba				C..T..T..	
63.B.bre.Manitoba				C..T..T..	
64.B.bre.NB				C..T..T..	
65.B.bre.NB				C..T..T..	
66.B.bre.IA				C..T..T..	
55.B.car.LA				C..	
56.B.car.Ark				C..	
57.B.car.LA				C..	
58.B.car.IL				C..	
53.B.car.FL				C..	
54.B.car.FL				C..	
59.B.car.Ark				C..	
60.B.car.IL				C..	
49.B.car.GA				C..	
50.B.car.GA				C..	
51.B.car.VA				C..	
52.B.car.VA				C..	
85.Sorex		T..	A..A..	A..T..A	
86.Sorex		T..	A..A..	A..T..A	
84.Cryptotis		A..T..	C..T..T..T..	TT..	
83.Cryptotis		A..T..	C..T..T..T..	TT..	

	460	470	480	490	500
MF4745	TCAGCCATCCCTTATATTGGATCCGACCTTGTCCAATGAATCTGAGGTGG				
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A					G.....
MF8231.A					G.....
MF8801.A					G.....
MF8803					
MF9157					
MF8057					
MF9158					
75.B.hyl.NB					G.....
76.B.hyl.NB					G.....
78.B.hyl.NB					G.....
80.B.hyl.NB					G.....
81.B.hyl.NB			T.....		G.....
82.B.hyl.KS			T.....		G..C..
77.B.hyl.KS					G.....
79.B.hyl.NB					G.....
70.B.bre.NH	T....C..C.....	T.....	T.....		
71.B.bre.NH	T....C..C.....	T.....	T.....		
73.B.bre.VA	T....C..C.....	T.....	T.....		
74.B.bre.VA	T....C..C.....	T.....	T.....		
67.B.bre.KY	T....C.....	T.....	T.....		
69.B.bre.OH	T....C.....	T.....	T.....		
68.B.bre.KY	T....C.....	T.....	T.....		
72.B.bre.OH	T....C..C.....	T.....	T.....		
61.B.bre.NB	T....C.....	T.....	T.....		
62.B.bre.Manitoba	T....C.....	T.....	T.....		
63.B.bre.Manitoba	T....C.....	T.....	T.....		C..
64.B.bre.NB	T....C.....	T.....	T.....		
65.B.bre.NB	T....C.....	T.....	T.....		
66.B.bre.IA	T....C.....	T.....	T.....		
55.B.car.LA		T.....	T.....		G..C..
56.B.car.Ark		T.....	T.....		G..C..
57.B.car.LA		T.....	T.....		G..C..
58.B.car.IL		T.....	T.....		G..C..
53.B.car.FL	T.....	T.....	T.....		C..
54.B.car.FL	T.....	T.....	T.....		C..
59.B.car.Ark		T.....	T.....		G..C..
60.B.car.IL		T.....	T.....		G..C..
49.B.car.GA		T.....	T.....		
50.B.car.GA		T.....	T.....		C..
51.B.car.VA		T.....	T.....		C..
52.B.car.VA		T.....	T.....		C..
85.Sorex	A....C..C.....	T..A..	T.A..AG		
86.Sorex	A....C..C.....	T..A..	T.A..AG		
84.Cryptotis	A.....	T.....	T.A..T		
83.Cryptotis	A.....	T.....	T.A..T		

	510	520	530	540	550
MF4745	ATTCTCAGTTGACAAAGCAACTCTTACCCGATTCTTCGCCTTCCACTTCA				
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A				T	
MF8231.A				T	
MF8801.A				T	
MF8803					
MF9157					
MF8057					
MF9158					
75.B.hyl.NB					
76.B.hyl.NB					
78.B.hyl.NB					
80.B.hyl.NB					
81.B.hyl.NB		T			T
82.B.hyl.KS		T			T
77.B.hyl.KS					
79.B.hyl.NB					
70.B.bre.NH	..T.....	C	C		
71.B.bre.NH	..T.....	C	C		
73.B.bre.VA	..T.....	C	C		
74.B.bre.VA	..T.....	C	C		
67.B.bre.KY	G..T.....	C	C		T
69.B.bre.OH	..T.....	C	C		T
68.B.bre.KY	G..T.....	C	C		T
72.B.bre.OH	..T.....	C	C		
61.B.bre.NB	..T....C.....	C			
62.B.bre.Manitoba	..T....C.....	C			
63.B.bre.Manitoba	..T....C.....	C			
64.B.bre.NB	..T....C.....	C			
65.B.bre.NB	..T....C.....	C			
66.B.bre.IA	..T....C.....	C			
55.B.car.LA	G.....			T	
56.B.car.Ark	G.....				
57.B.car.LA	G.....				
58.B.car.IL	G.....				
53.B.car.FL	G.....		T		T
54.B.car.FL	G.....		T		T
59.B.car.Ark	G.....				
60.B.car.IL	G.....				
49.B.car.GA	G.....	C		T	T
50.B.car.GA	G.....	C		T	T
51.B.car.VA	G.....	C		T	T
52.B.car.VA	G.....	C		T	T
85.Sorex		T	A	C	C
86.Sorex		T	A	C	C
84.Cryptotis	C..T....	A		C	T
83.Cryptotis	C..T....	A		C	T

	560	570	580	590	600
MF4745	TTCTTCCCTTTGTAATTGCTGCACTAGCCGGAGTACACCTCCTTTTCCTC				
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A	G.....				
MF8231.A	G.....				
MF8801.A	G.....				
MF8803					
MF9157					
MF8057					
MF9158					
75.B.hyl.NB	...C.....				
76.B.hyl.NB	...C.....				
78.B.hyl.NB	...C.....				
80.B.hyl.NB	...C.....				
81.B.hyl.NB					
82.B.hyl.KS					
77.B.hyl.KS	...C.....				
79.B.hyl.NB	...C.....T.....				
70.B.bre.NH	T..C.....C.....C.....G.....				
71.B.bre.NH	T..C.....C.....C.....G.....				
73.B.bre.VA	T..C.....C.....C.....G.....				
74.B.bre.VA	T..C.....C.....C.....G.....				
67.B.bre.KY	...C..T..C.....C.....C.....G.....				
69.B.bre.OH	T..C.....C.....C.....G.....A				
68.B.bre.KY	T..C.....C.....C.....G.....				
72.B.bre.OH	T..C.....C.....C.....A.....				
61.B.bre.NB	T..C.....C.....C.....G.....G.....				
62.B.bre.Manitoba	.C.....T..C.....C.....C.....G.....G.....				
63.B.bre.Manitoba	T..C.....C.....C.....G.....G.....				
64.B.bre.NB	T..C.....C.....C.....G.....G.....				
65.B.bre.NB	T..C.....C.....C.....G.....G.....				
66.B.bre.IA	T..C.....C.....C.....G.....G.....				
55.B.car.LA	C.....C.....				
56.B.car.Ark	C.....C.....				
57.B.car.LA	C.....C.....				
58.B.car.IL	C.....C.....				
53.B.car.FL	C.....G..C.....A.A				
54.B.car.FL	C.....G..C.....A				
59.B.car.Ark	CA.....A.....C.....				
60.B.car.IL	C.....C.....				
49.B.car.GA	C.....C.....				
50.B.car.GA	C.....C.....				
51.B.car.VA	C.....C.....				
52.B.car.VA	C.....C.....				
85.Sorex	.CT.G.....CA.T..C.....C.....A..C..G.....A....G				
86.Sorex	.CT.G.....CA.T..C.....C.....A..C..G.....A....G				
84.Cryptotis	...C..A.....C.....C..C.....C..T..T....T...				
83.Cryptotis	...C..A.....C.....C..C.....C..T..T....T...				

	610	620	630	640	650
MF4745	CACGAAACAGGCTCAAACAACCCATCTGGACTATCATCAGACGCTGACAA				
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A					
MF8231.A					
MF8801.A					
MF8803					
MF9157					
MF8057					
MF9158					
75.B.hyl.NB				G	
76.B.hyl.NB				G	
78.B.hyl.NB				G	
80.B.hyl.NB				G	
81.B.hyl.NB				G	
82.B.hyl.KS				G	
77.B.hyl.KS				G	
79.B.hyl.NB				G	
70.B.bre.NH	A.....T.....				
71.B.bre.NH	A.....T.....				
73.B.bre.VA	A.....T.....				
74.B.bre.VA	A.....T.....				
67.B.bre.KY	A.....T.....C.....				
69.B.bre.OH	A.....T.....C.....				
68.B.bre.KY	A.....T.....C.....				
72.B.bre.OH	A.....T.....				
61.B.bre.NB	A.....T.....				
62.B.bre.Manitoba	A.....T.....				
63.B.bre.Manitoba	A.....T.....				
64.B.bre.NB	A.....T.....				
65.B.bre.NB	A.....T.....				
66.B.bre.IA	A.....T.....				
55.B.car.LA	G.....T.....G.....				
56.B.car.Ark	G.....T.....G.....				
57.B.car.LA	G.....T.....G.....				
58.B.car.IL	G.....T.....G.....				
53.B.car.FL	T.....G.....G.....				
54.B.car.FL	T.....G.....G.....				
59.B.car.Ark	G.....T.....G.....				
60.B.car.IL	G.....T.....G.....				
49.B.car.GA	T.G.....G.....				
50.B.car.GA	T.G.....G.....				
51.B.car.VA	T.G.....G.....				
52.B.car.VA	T.G.....G.....				
85.Sorex	T..A.....GT.....A.....				
86.Sorex	T..A.....GT.....A.....				
84.Cryptotis	T.....A.....GC.....C..T..				
83.Cryptotis	T.....A.....GC.....C..T..				

	660	670	680	690	700
MF4745	AATTCCATTCCACCCATACTATAACAATTAAAGACATCCTAGGAGTACTCA				
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A					
MF8231.A					
MF8801.A					
MF8803					
MF9157					
MF8057					
MF9158					
75.B.hyl.NB	G.....				
76.B.hyl.NB	G.....				
78.B.hyl.NB	G.....				
80.B.hyl.NB	G.....				
81.B.hyl.NB	T.....				
82.B.hyl.KS	T.....				
77.B.hyl.KS					
79.B.hyl.NB	G.....				
70.B.bre.NH	G.....T.....C.....C...T.				
71.B.bre.NH	G.....T.....C.....C...T.				
73.B.bre.VA	T.....C.....C...T.				
74.B.bre.VA	T.....C.....C...T.				
67.B.bre.KY	G.....T.....C.....C...T.				
69.B.bre.OH	G.....T.....C.....C...T.				
68.B.bre.KY	G.....T.....C.....C...T.				
72.B.bre.OH	G.....T.....C.....C...T.				
61.B.bre.NB	G.....G...T.....C.....C...T.				
62.B.bre.Manitoba	G.....G...T.....C.....C...T.				
63.B.bre.Manitoba	G.....G...T.....C.....C...T.				
64.B.bre.NB	G.....G...T.....C.....C...T.				
65.B.bre.NB	G.....T.....C.....C...T.				
66.B.bre.IA	G.....G...T.....C.....C...T.				
55.B.car.LA	T.....T.....T.....T.				
56.B.car.Ark	T.....T.....T.....T.				
57.B.car.LA	T.....T.....T.....T.				
58.B.car.IL	T.....T.....T.....T.				
53.B.car.FL	T.....T.....T.....T.				
54.B.car.FL	T.....T.....T.....T.				
59.B.car.Ark	T.....T.....T.....T.				
60.B.car.IL	T.....T.....T.....T.				
49.B.car.GA	T.....T.....T.....T.				
50.B.car.GA	T.....T.....T.....T.				
51.B.car.VA	T.C.....T.....T.				
52.B.car.VA	T.....T.....T.....T.				
85.Sorex	C.....C.....T.....C...C				
86.Sorex	C.....C.....T.....C...C				
84.Cryptotis	T.....T.T.....A.T..TC				
83.Cryptotis	T.....T.T.....A.T..TC				

	710	720	730	740	750
MF4745	TCTTGATCCTAGTACTA	ACATGCCTAGTACTAT	TTTTCTCCAGACTTACTA		
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A					
MF8231.A					
MF8801.A					
MF8803					
MF9157					
MF8057					
MF9158					
75.B.hyl.NB					
76.B.hyl.NB					
78.B.hyl.NB				C.....	
80.B.hyl.NB				C.....	
81.B.hyl.NB					
82.B.hyl.KS					C.....
77.B.hyl.KS					
79.B.hyl.NB					
70.B.bre.NH	C.....		G.....	C.....	C.....
71.B.bre.NH	C.....		G.....	C.....	C.....
73.B.bre.VA	C.....		G.....	C.....	C.....
74.B.bre.VA	C.....		G.....	C.....	C.....
67.B.bre.KY	C.....		G.....	C.....	C.....
69.B.bre.OH	C.....		G.....	C.....	C.....
68.B.bre.KY	C.....		G.....	C.....	C.....
72.B.bre.OH	C.....		G.....	C.....	C.....
61.B.bre.NB	C.....		G.....	C.....	C.....
62.B.bre.Manitoba	C.....		G.....	C.....	C.....
63.B.bre.Manitoba	C.....		G.....	C.....	C.....
64.B.bre.NB	C.....		G.....	C.....	C.....
65.B.bre.NB	C.....		G.....	C.....	C.....
66.B.bre.IA	C.....		G.....	C.....	C.....
55.B.car.LA	C.....				C..T..
56.B.car.Ark	C.....				C..T..
57.B.car.LA	C.....				C..T..
58.B.car.IL	C.....				C..T..
53.B.car.FL	C.....	G.....	G.....		C..T.G
54.B.car.FL	C.....	G.....	G.....		C..T.G
59.B.car.Ark	C.....				C..T..
60.B.car.IL	C.....				C..T..
49.B.car.GA	C.....		G.....	G.....	C..T..
50.B.car.GA	C.....		G.....	G.....	C..T..
51.B.car.VA	C.....		G.....	G.....	C..T..
52.B.car.VA	C.....		G.....	G.....	C..T..
85.Sorex	AC.C..TT.....	C.....	C.T.....	CT....C..C..T..T.....	
86.Sorex	AC.C..TT.....	C.....	C.T.....	CT....C..C..T..T.....	
84.Cryptotis	C.A.....		T.....	C.....	
83.Cryptotis	C.A.....		T.....	C.....	

	760	770	780	790	800
MF4745	GGAGACCCAGACAATTATACACCAGCCAACCCCTAAACACGCCTCCCCA				
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A					
MF8231.A					
MF8801.A					
MF8803					
MF9157					
MF8057					
MF9158					
75.B.hyl.NB					
76.B.hyl.NB					
78.B.hyl.NB					
80.B.hyl.NB					
81.B.hyl.NB					
82.B.hyl.KS			T.	T.	
77.B.hyl.KS	C.....				
79.B.hyl.NB					
70.B.bre.NH	G.....		T.T.	C.A.	
71.B.bre.NH	G.....		T.T.	C.A.	
73.B.bre.VA	G.....		T.T.	C.A.	
74.B.bre.VA	G.....		T.T.	C.A.	
67.B.bre.KY	G.....		T.T.	C.A.	
69.B.bre.OH	G.....		T.T.	C.A.	
68.B.bre.KY	G.....G.....		T.T.	C.A.	
72.B.bre.OH	G.....		T.T.	C.A.	
61.B.bre.NB	G.....		T.T.	C.A.	
62.B.bre.Manitoba	G.....		T.T.	C.A.	
63.B.bre.Manitoba	G.....		T.T.	C.A.	
64.B.bre.NB	G.....		T.T.	C.A.	
65.B.bre.NB	G.....		T.T.	C.A.	
66.B.bre.IA	G.....		T.T.	C.A.	
55.B.car.LA			T.	C.A.	
56.B.car.Ark			T.	C.A.	
57.B.car.LA			T.	C.A.	
58.B.car.IL			T.	C.A.	
53.B.car.FL	C.....			C.G.	
54.B.car.FL	C.....			C.G.	
59.B.car.Ark			T.	C.A.	
60.B.car.IL			T.	C.A.	
49.B.car.GA	G.....			C.G.T.	
50.B.car.GA	G.....			C.G.T.	
51.B.car.VA	G.....			C.G.T.	
52.B.car.VA	G.....			C.G.T.	
85.Sorex	..C...G.....	C.....	T.	A.A.....	
86.Sorex	..C.....	C.....	T.	A.A.....	
84.Cryptotis	T.....		T.T.	T.T.A.A.....	
83.Cryptotis	T.....		T.T.	T.T.A.A.....	

	810	820	830	840	850
MF4745	CATTAAGCCAGAATGATATTTTCTATTTGCCTACGCCATTCTGCGATCCA				
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A					
MF8231.A					
MF8801.A					
MF8803					
MF9157					
MF8057					T.
MF9158					
75.B.hyl.NB					
76.B.hyl.NB					
78.B.hyl.NB					
80.B.hyl.NB					
81.B.hyl.NB					
82.B.hyl.KS					
77.B.hyl.KS					
79.B.hyl.NB					
70.B.bre.NH	A.....C...T.....T.....C..A.....T.				
71.B.bre.NH	A.....C...T.....T.....C..A.....T.				
73.B.bre.VA	A.....C...T.....T.....C..A.....T.				
74.B.bre.VA	A.....C...T.....T.....C..A.....T.				
67.B.bre.KY	A.....C...T.....T.....C..A.....T.				
69.B.bre.OH	A.....C...T.....T.....C..A.....T.				
68.B.bre.KY	A.....C...T.....T.....C..A.....T.				
72.B.bre.OH	A.....C...T.....T.....C..A.....T.				
61.B.bre.NB	...C..A.....C...T.....T.....A.....T.				
62.B.bre.Manitoba	...C..A.....C...T.....T.....A.....T.				
63.B.bre.Manitoba	...C..A.....C...T.....T.....A.....T.				
64.B.bre.NB	...C..A.....C...T.....T.....A.....T.				
65.B.bre.NB	...C..A.....C...T.....T.....A.....T.				
66.B.bre.IA	...C..A.....C...T.....T.....A.....T.				
55.B.car.LA	T.....C..A.....T.				
56.B.car.Ark	T.....C..A.....T.				
57.B.car.LA	T.....C..A.....T.				
58.B.car.IL	T.....C..A.....T.				
53.B.car.FL	C..A.....T.				
54.B.car.FL	C..A.....T.				
59.B.car.Ark	T.....C..A.....T.				
60.B.car.IL	T.....C..A.....T.				
49.B.car.GA	A.....C..A.....T.				
50.B.car.GA	A.....C..A.....T.				
51.B.car.VA	A.....C..A.....T.				
52.B.car.VA	A.....C..A.....T.				
85.Sorex	A..T.....C.....T.....C.....A.				
86.Sorex	A..T.....C.....T.....C.....A.				
84.Cryptotis	T.....G.....C..C.....C..T.....T.....A.....T.				
83.Cryptotis	T.....G.....C..C.....C..T.....T.....A.....T.				

	860	870	880	890	900
MF4745	TCCCTAATAAAATTAGGGGGAGTACTAGCCCTAGTCCTATCTATCTCATT				
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A					
MF8231.A					
MF8801.A					
MF8803					
MF9157					
MF8057					
MF9158					
75.B.hyl.NB	A.....				C
76.B.hyl.NB	A.....				C
78.B.hyl.NB	A.....				C
80.B.hyl.NB	A.....				C
81.B.hyl.NB	G.....A.....				C
82.B.hyl.KS	G.....A.....				C
77.B.hyl.KS	A.....				C
79.B.hyl.NB	A.....				C
70.B.bre.NH	...C.....A.....G..T.....			C.....	C
71.B.bre.NH	...C.....A.....T.....			C.....	C
73.B.bre.VA	...C.....A.....G..T.....			C.....	C
74.B.bre.VA	...C.....A.....G..T.....			C.....	C
67.B.bre.KY	...C.....A.....G..T.....G..C.....				
69.B.bre.OH	...C.....A.....G..T.....G..C.....				
68.B.bre.KY	...C.....A.....G..T.....G..C.....				
72.B.bre.OH	...C.....A.....G..T.....C.....			C.....	C
61.B.bre.NB	...C.....A..G..G..G..T.....G..C.....				
62.B.bre.Manitoba	...C.....A..G..G..G..T.....G..C.....				
63.B.bre.Manitoba	...C.....A..G..G..G..T.....G..C.....				
64.B.bre.NB	...C.....A..G..G..G..T.....G..C.....				
65.B.bre.NB	...C.....A..G..G..G..T.....G..C.....				
66.B.bre.IA	...C.....A..G..G..G..T.....G..C.....				
55.B.car.LA	...C....G....A....G..C.....				
56.B.car.Ark	...C....G....A....G..C.....				
57.B.car.LA	...C....G....A....C.....				
58.B.car.IL	...C....G....A....C.....				
53.B.car.FL	...C..C.....A.....C.....				C
54.B.car.FL	...C..C.....A.....C.....				C
59.B.car.Ark	...C....G....A.....C.....				
60.B.car.IL	...C....G....A.....C.....				C
49.B.car.GA	...C.....A.....C.....				
50.B.car.GA	...C.....A.....C.....				
51.B.car.VA	...C.....A.....C.....				
52.B.car.VA	...C.....A.....C.....				
85.Sorex	C.....T.....C.....T.....A.....AG.C				
86.Sorex	C.....T.....C.....T.....A.....AG.C				
84.Cryptotis	.T..C..C.....T.....C.....A...				
83.Cryptotis	.T..C..C.....T.....C.....A...				

	910	920	930	940	950
MF4745	TTAGCCTTTATCCCCCTTCTCCACACCTCCAAACAACGAAGTATAATATT				
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A					
MF8231.A					
MF8801.A					
MF8803					
MF9157					
MF8057					
MF9158	T.....				
75.B.hyl.NB					
76.B.hyl.NB					
78.B.hyl.NB					
80.B.hyl.NB					
81.B.hyl.NB					
82.B.hyl.KS					
77.B.hyl.KS					
79.B.hyl.NB					
70.B.bre.NH	C.....C..G....				
71.B.bre.NH	C.....C..G....				
73.B.bre.VA	C.....C.....C..G....				
74.B.bre.VA	C.....A.....C..G....				
67.B.bre.KY	C.....C..G....				
69.B.bre.OH	C.....C..G....				
68.B.bre.KY	C.....C..G....				
72.B.bre.OH	C.....C..G....				
61.B.bre.NB	C.....C..G....				
62.B.bre.Manitoba	C.....C..G....				
63.B.bre.Manitoba	C.....C..G....				
64.B.bre.NB	C.....A.....C..G....				
65.B.bre.NB	C.....C.....C..G....				
66.B.bre.IA	C.....C..G....				
55.B.car.LA	C.....T.....C..G....				
56.B.car.Ark	C.....T.....C..G....				
57.B.car.LA	C.....T.....C..G....				
58.B.car.IL	C.....T.....C..G....				
53.B.car.FL	C.....C.....C..G....				
54.B.car.FL	C.....C.....C..G....				
59.B.car.Ark	C.....T.....C..G....				
60.B.car.IL	C.....T.....C..G....				
49.B.car.GA	C.....C..G....				
50.B.car.GA	C.....C..G....				
51.B.car.VA	C.....C..G....				
52.B.car.VA	C..G....				
85.Sorex	C...AG.AG...T.C..T...A.....				
86.Sorex	C...AG.AG...T.C..T...A.....				
84.Cryptotis	C...T...T...T.A..T...T.....C.....				
83.Cryptotis	C...T...T...T.A..T...T.....C.....				

	960	970	980	990	1000
MF4745	TCGCCCATT	CAGTCAATG	CTTATTTGA	ATCCTAGTAG	CAGATCTCCTTA
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A					
MF8231.A					
MF8801.A					
MF8803					
MF9157					
MF8057			G.....	G.....	
MF9158					
75.B.hyl.NB					C.....
76.B.hyl.NB					C.....
78.B.hyl.NB					C.....
80.B.hyl.NB					C.....
81.B.hyl.NB					C.....
82.B.hyl.KS					C.....
77.B.hyl.KS					C.....
79.B.hyl.NB	...AAA...				C.....
70.B.bre.NH	C.....	C...G...C...	C.....	C.....	C.....
71.B.bre.NH	C.....	C...G...C...	C.....	C.....	C.....
73.B.bre.VA	C.....	CA...G...C...	C.....	C.....	C.....
74.B.bre.VA	C.....	CA...G...C...	C.....	C.....	C.....
67.B.bre.KY	C.....	C...G...C...	C.....	C.....	C.....
69.B.bre.OH	C.....	C...G...C...	C.....	C.....	C.....
68.B.bre.KY	C.....	C...G...C...	C.....	C.....	C.....
72.B.bre.OH	C.....	C...G...C...	C.....	C.....	C.....
61.B.bre.NB	C.....	C...G...C...	C...G.....	C.....	C.....
62.B.bre.Manitoba	C.....	C...G...C...	C...G.....	C.....	C.....
63.B.bre.Manitoba	C.....	C...G...C...	C...G.....	C.....	C.....
64.B.bre.NB	C.....	C...G...C...	C...G.....	C.....	C.....
65.B.bre.NB	C.....	C...G...C...	C...G.....	C.....	C.....
66.B.bre.IA	C.....	C...G...C...	C...G.....	C.....	C.....
55.B.car.IA	C.....	C.....	C.....	T.....	
56.B.car.Ark	C.....	C.....	C.....	T.....	
57.B.car.LA	C.....	C.....	C.....	T.....	
58.B.car.IL	C.....	C.....	C.....	T.....	
53.B.car.FL	C.....	C.....	C.....	T.....	C.....
54.B.car.FL	C.....	C.....	C.....	T.....	C.....
59.B.car.Ark	C.....	C.....	C.....	T.....	
60.B.car.IL	C.....	C.....	C.....	T.....	
49.B.car.GA	C.....	C.....	C.....	T.....	G...C.....
50.B.car.GA	C.....	C.....	C.....	T.....	G...C.....
51.B.car.VA	C.....	C.....	C.....	T.....	G...C.....
52.B.car.VA	C.....	C.....	C.....	T.....	G...C.....
85.Sorex	C.....	T...C.....	C.....	C.....	A...C.....
86.Sorex	C.....	T...C.....	C.....	C.....	A...C.....
84.Cryptotis	...A....	A...C.....	C.....	TT.....	T...C.....
83.Cryptotis	...A....	A...C.....	C.....	TT.....	T...C.....

	1010	1020	1030	1040	1050
MF4745	CACTAACATGAATCGGAGGACAACCCGTCGAACACCCATT	CATCATCAT			
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A					
MF8231.A					
MF8801.A					
MF8803					
MF9157					
MF8057					
MF9158					
75.B.hyl.NB					
76.B.hyl.NB					
78.B.hyl.NB					
80.B.hyl.NB					
81.B.hyl.NB					
82.B.hyl.KS					
77.B.hyl.KS					
79.B.hyl.NB					
70.B.bre.NH			T.....	T.....	T.....
71.B.bre.NH			T.....	T.....	T.....
73.B.bre.VA			T.....	T.....	T.....
74.B.bre.VA			T.....	T.....	T.....
67.B.bre.KY			T.....	T.....	T.....
69.B.bre.OH			T.....	T.....	T.....
68.B.bre.KY			T.....	T.....	T.....
72.B.bre.OH			T.....	T.....	T.....
61.B.bre.NB				T.....	T.....
62.B.bre.Manitoba				T.....	T.....
63.B.bre.Manitoba				T.....	T.....
64.B.bre.NB				T.....	T.....
65.B.bre.NB				T.....	T.....
66.B.bre.IA				T.....	T.....
55.B.car.IA				T.....	
56.B.car.Ark				T.....	
57.B.car.IA				T.....	
58.B.car.IL				T.....	
53.B.car.FL					C
54.B.car.FL					C
59.B.car.Ark				T.....	
60.B.car.IL				T.....	
49.B.car.GA				T.....	
50.B.car.GA				T.....	
51.B.car.VA				T.....	
52.B.car.VA				T.....	
85.Sorex	...T.....	T.C.....	A.T.....	T.....	T.T.T..
86.Sorex	...T.....	T.C.....	A.T.....	T.....	T.T.T..C
84.Cryptotis	.T.....		G.....		T.....
83.Cryptotis	.T.....		G.....		T.....

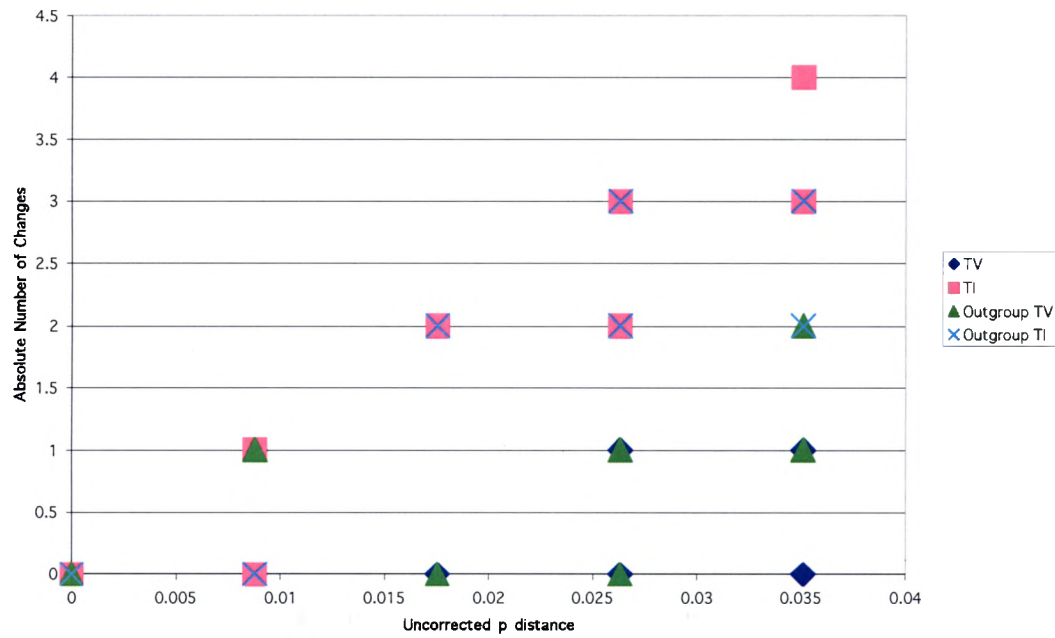
	1060	1070	1080	1090	1100
MF4745	GGACAATTAGCTTCTATCTTATATTTCTCCTACTTCTAGTCATTATACC				
MF4859					
MF5356					
MF5376					
MF6156					
MF6397					
MF7497					
MF7498					
MF8049					
MF8050					
MF8051					
MF8052					
MF8053					
MF8054					
MF8055					
MF8224					
MF8802.A					
MF8231.A					
MF8801.A					
MF8803					
MF9157					
MF8057					
MF9158					
75.B.hyl.NB					
76.B.hyl.NB					
78.B.hyl.NB					
80.B.hyl.NB					
81.B.hyl.NB					
82.B.hyl.KS					
77.B.hyl.KS					
79.B.hyl.NB				T	
70.B.bre.NH	C.....		T.....CT.....	C.....	
71.B.bre.NH	C.....		T.....CT.....	C.....	
73.B.bre.VA	C.....		T.....CT.....	C..G..	
74.B.bre.VA	C.....		T.....CT.....	C..G..	
67.B.bre.KY	C.....		T.....CT.....	C.....	
69.B.bre.OH	C.....		T.....CT.....	C.....	
68.B.bre.KY	C.....		T.....CT.....	C.....	
72.B.bre.OH	C.....		T.....CT.....	C.....	
61.B.bre.NB	C.....		T.....T.....	C.....	
62.B.bre.Manitoba	C.....		T.....T.....	C.....	
63.B.bre.Manitoba	C.....		T.....T.....	C.....	
64.B.bre.NB	C.....		T.....T.....	C.....	
65.B.bre.NB	C.....		T.....T.....	C.....	
66.B.bre.IA	C.....C.....		T.....T.....	C.....	
55.B.car.LA	C.....		T.....	C.....	
56.B.car.Ark	C.....		T.....	C.....	
57.B.car.LA	C.....		T.....	C.....	
58.B.car.IL	C.....		T.....	C.....	
53.B.car.FL	C.....C.....		T.....	C.....	
54.B.car.FL	C.....C.....		T.....	C.....	
59.B.car.Ark	C.....		T.....	C.....	
60.B.car.IL	C.....		T.....	C.....	
49.B.car.GA	..G.....C.....C.....		T.....	C.....	
50.B.car.GA	..G.....C.....C.....		T.....	C.....	
51.B.car.VA	..G.....C.....C.....		T.....	C.....	
52.B.car.VA	..G.....C.....C.....		T.....	C.....	
85.Sorex	A..A.....		T..A.C.....	AC.C.....	
86.Sorex	A..A.....		T..A.C.....	AC.C.....	
84.Cryptotis	..T..GC...A..C.....G..C..TA.AT....		CT....T.....		
83.Cryptotis	..T..GC...A..C.....G..C..TA.AT....		CT....T.....		

	1110	1120	1130	1140
MF4745	AATCACAAGTCTATTTCGAAAACAATTATTAAAAATGAAGA			
MF4859				
MF5356				
MF5376				
MF6156				
MF6397				
MF7497				
MF7498				
MF8049				
MF8050				
MF8051				
MF8052				
MF8053				
MF8054				
MF8055				
MF8224				
MF8802.A				
MF8231.A				
MF8801.A				
MF8803				
MF9157				
MF8057				
MF9158				
75.B.hyl.NB				
76.B.hyl.NB				G
78.B.hyl.NB				G
80.B.hyl.NB				G
81.B.hyl.NB				G
82.B.hyl.KS				G
77.B.hyl.KS				G
79.B.hyl.NB				G
70.B.bre.NH	G.....		C.....	G
71.B.bre.NH	G.....		C.....	G
73.B.bre.VA	T.....		C.....	G
74.B.bre.VA	T.....		C.....	G
67.B.bre.KY	G.....	C.....	C.G.....	G
69.B.bre.OH	G.....		C.....	G
68.B.bre.KY	G.....	C.....	C.....	G
72.B.bre.OH	G.....		C.....	G
61.B.bre.NB	G.....		C.....	G
62.B.bre.Manitoba	G.....		C.....	G
63.B.bre.Manitoba	G.....		C.....	G
64.B.bre.NB	G.....		C.....	G
65.B.bre.NB	G.....		C.....	G
66.B.bre.IA	G.....		C.....	G
55.B.car.LA				G
56.B.car.Ark				G
57.B.car.LA				G
58.B.car.IL				G
53.B.car.FL			C.....	G
54.B.car.FL			C.....	G
59.B.car.Ark				G
60.B.car.IL				G
49.B.car.GA				G
50.B.car.GA				G
51.B.car.VA				G
52.B.car.VA				G
85.Sorex	T.....		T..CC.TC.....	G
86.Sorex	T.....		T..CC.TC.....	G
84.Cryptotis		C...C.T.....	T.....	SG
83.Cryptotis		C...C.T.....	T.....	G

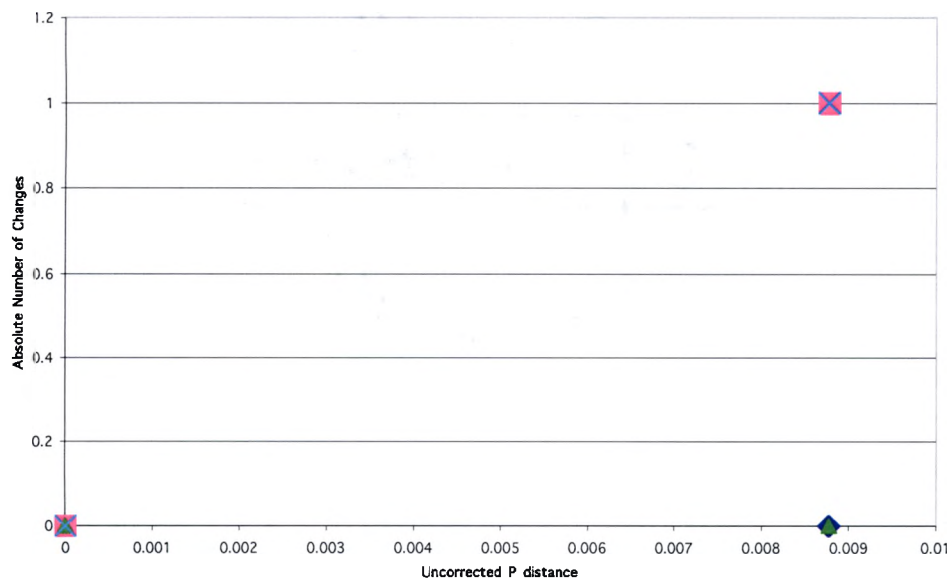
APPENDIX 3

Saturation curves for each codon position within each of three membrane regions of cytochrome *b* for the *Blarina* data set. *Sorex* and *Cryptotis* were the specified outgroups. Ingroup-outgroup saturation is present in third position transitions of all three regions of the protein as well as first codon position transitions of the transmembrane region. Symbols for ingroup and outgroup transitions (TI) and transversions (TV) are noted in the key.

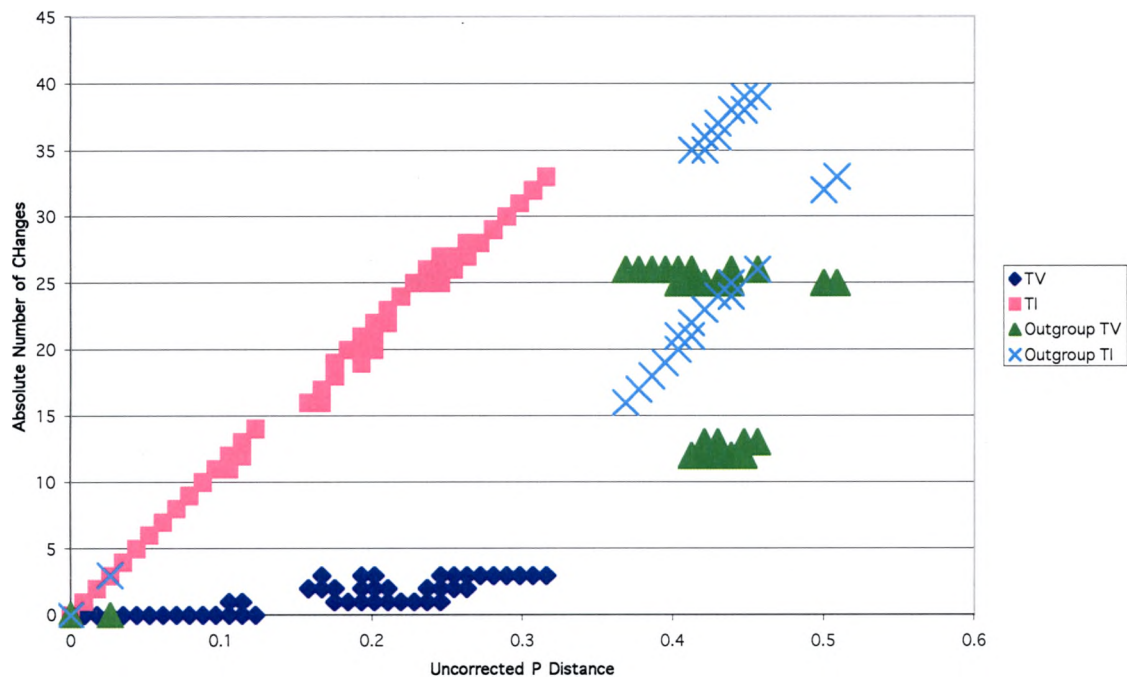
Intermembrane 1st Position



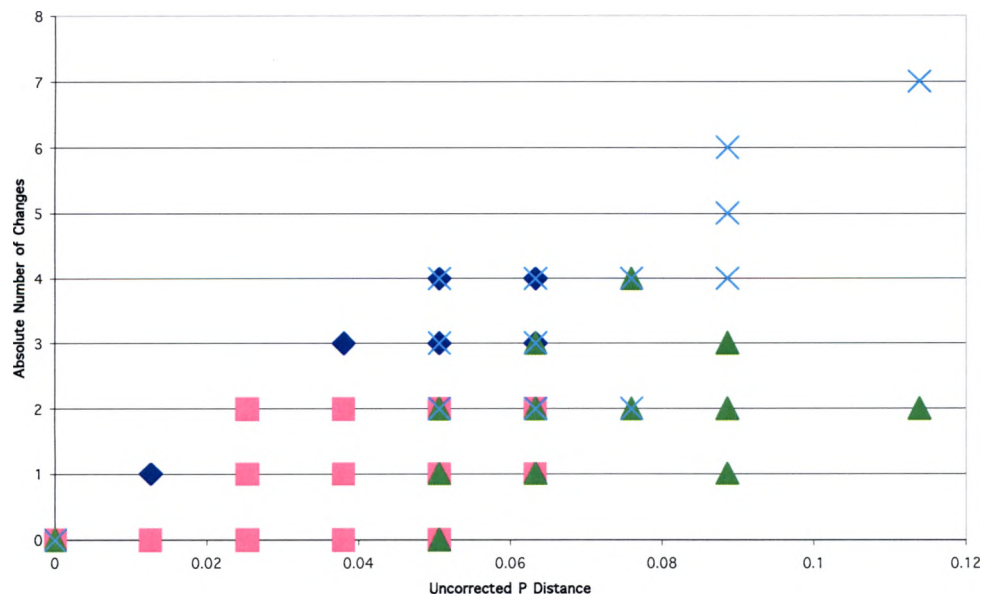
Intermembrane 2nd Position



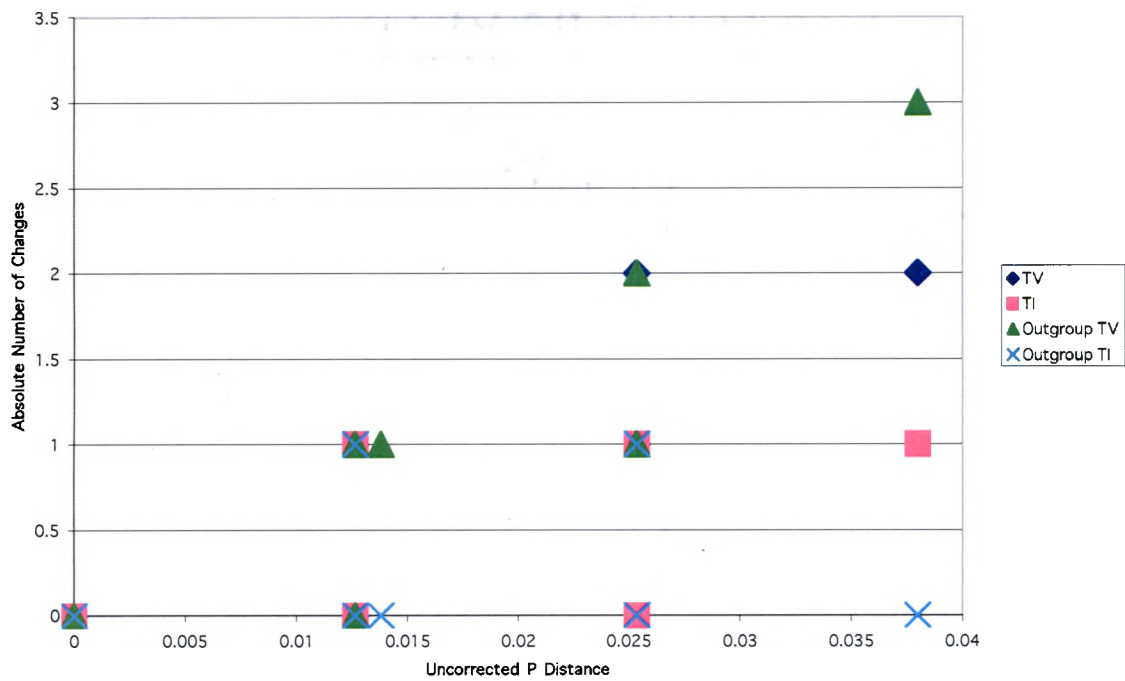
Intermembrane 3rd Position



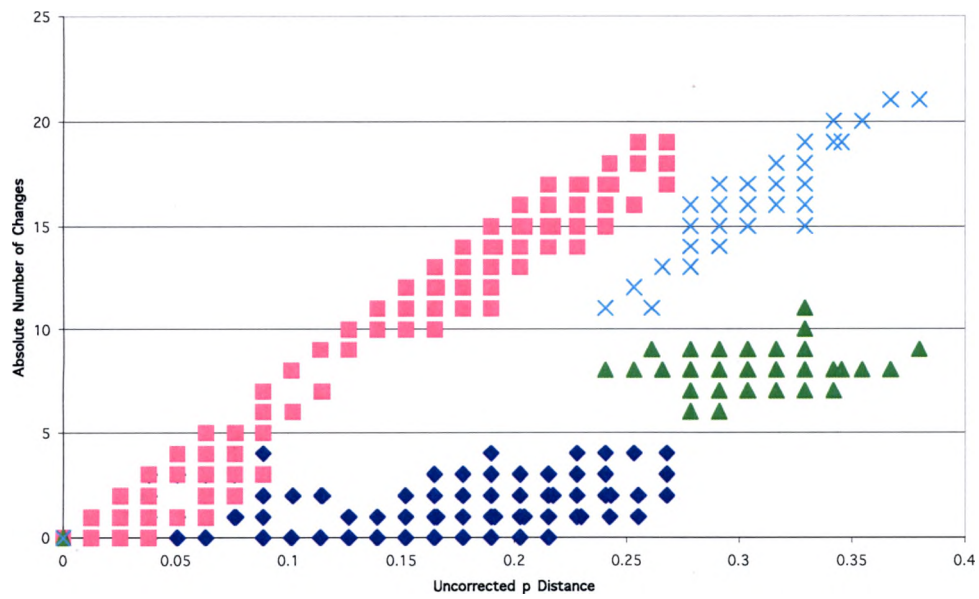
Matrix 1st Position

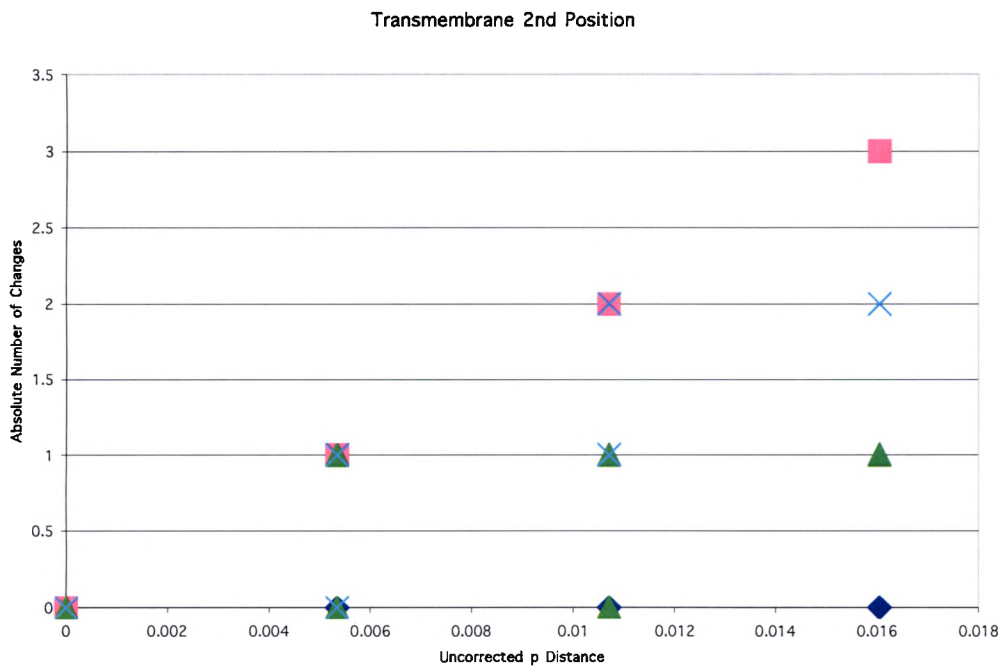
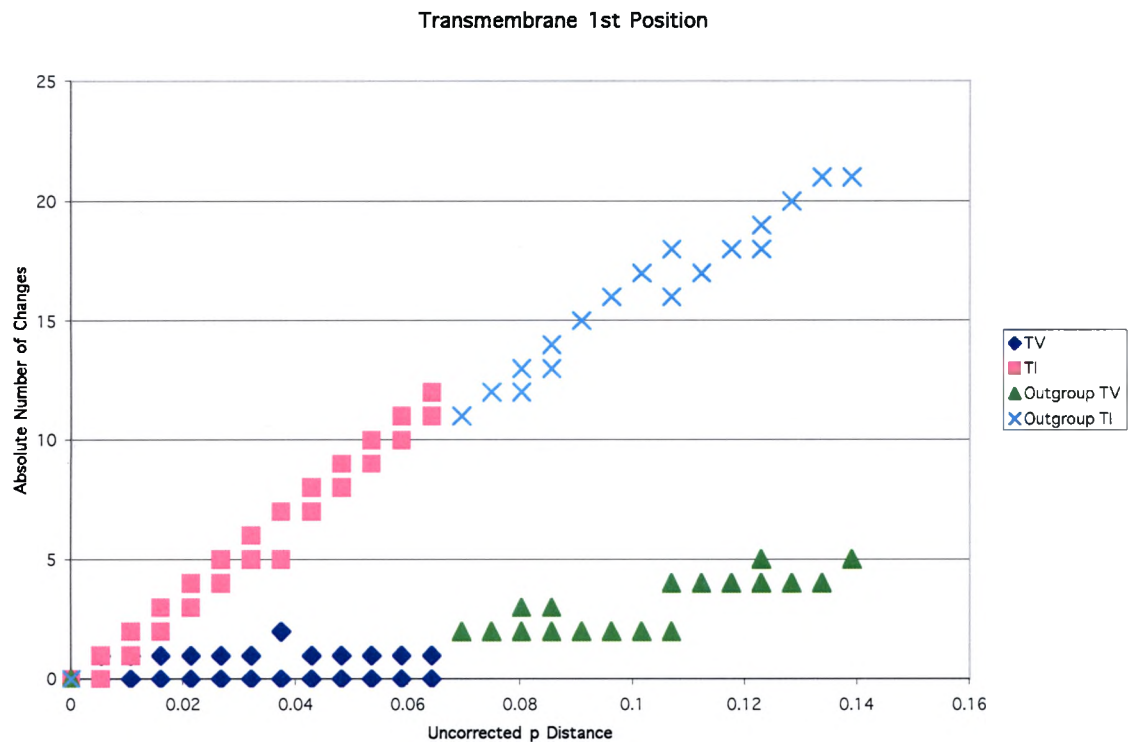


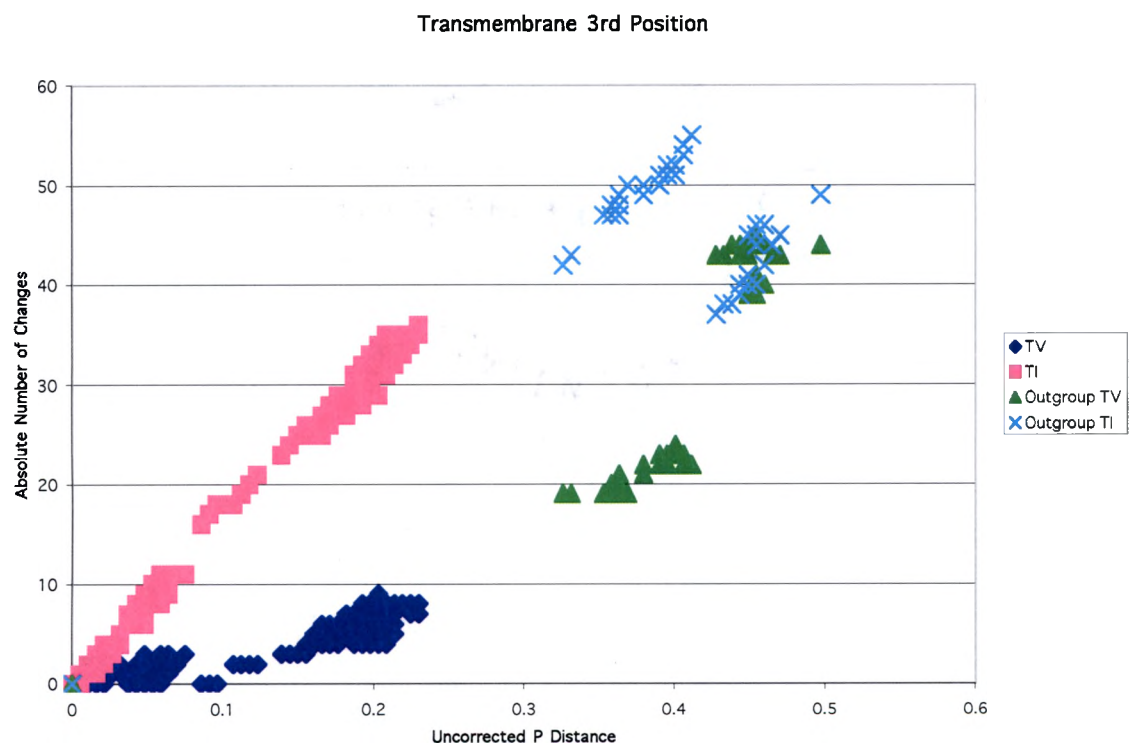
Matrix 2nd Position



Matrix 3rd Position







VITA

Susannah Reilly Morris was born in Houston, Texas, on 23 May 1975 to Judith McCullough Reilly and Brendan James Reilly. After graduating from Lamar High School in 1993, she attended the University of Texas at Austin and earned a Bachelor of Science in Biology in 1997. She enrolled as a graduate student in the Biology Department at Texas State University – San Marcos in Fall 2000. While at Texas State, she worked as an instructional assistant for Introductory Biology and Genetics, as a field technician at the Griffith League Ranch, and as a bird-banding intern for the Institute for Bird Populations.

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This thesis was typed by Susannah Morris.