PHYLOGEOGRAPHY OF *CRYPTOTIS PARVA* IN THE UNITED STATES USING MORPHOMETRICS AND POPULATION GENETICS

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ABSTRACT

The least shrew (*Cryptotis parva*) is a short-tailed shrew (Insectivora: Soricidae) whose distribution encompasses the central and eastern United States from New Mexico and Wyoming eastward to the Atlantic coast and northward to Michigan, Southern Ontario, and New York. Traditional taxonomy recognizes five subspecies of least shrew in the United States: *C. p. parva*, *C. p. harlani*, *C. p. elasson*, *C. p. floridana*, and *C. p. berlandieri*. Most of these taxa, however, were named on the basis of morphological characters in relatively few specimens, and the validity of some designations has been questioned. The current study used morphological cranial characters in conjunction with molecular techniques to do the first thorough revision of the genus in the United States in about 100 years. Seven cranial measurements were used to perform multivariate statistics on 1020 specimens to elucidate geographic patterns in morphology. Additionally, three genetic markers (cytochrome-b, barcode, and Apolipoprotein-B) were used to infer genetic relationships using Bayesian, maximum likelihood, and maximum parsimony methods. Results indicate that two genetically distinct, sympatric species of least shrew exist in the United States, *Cryptotis parva* and *Cryptotis floridana*, and that both species show evidence of phenotypic plasticity throughout their ranges. Furthermore, within *C. parva* there is no evidence for the validity of *C. p. harlani* or *C. p. elasson*.

ACKNOWLEDGMENTS

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INTRODUCTION

Shrews belong to a very speciose family, Soricidae, comprised of over 250 currently recognized species. Members of this family span Africa, Eurasia, and the Americas. The least shrew, *Cryptotis parva*, is a short-tailed, small shrew in the subfamily Soricinae, and is the only shrew to have penetrated into South America (Churchfield, 1990). It averages about 70-92 mm in total length, with a 13-26 mm tail, 9-13 mm hind foot, and weighs around 4.0 g (Webster et al., 1985). Its dense, short pelage is brownish to grayish in color with a paler underside, its tail is bicolored, and its eyes and ears are small. The pointed snout contains 30 pigmented teeth with bilobed incisors, four unicuspids, and a W-shaped ectoloph pattern on the fourth premolar and molars. The fourth unicuspid in *Cryptotis parva* is minute and usually not visible in a lateral view. This unique dentition distinguishes *Cryptotis* from other North American short-tailed shrews (genus *Blarina*). Finally, as is the case with all shrews, *Cryptotis parva* lacks zygomatic arches and auditory bullae (Hall, 1981; Whitaker, 1974).

The least shrew is the only species of *Cryptotis* in the United States. Its geographic distribution (Fig. 1) ranges throughout the Southwest eastward to the East Coast, southward to Florida, and northward to southern Ontario, New York, and Connecticut (Hall, 1981). Newly discovered populations of *C. parva* in Wyoming (Marquardt et al., 2006) and New Mexico (Hafner and Shuster, 1996) indicate a recent westward range expansion. Five subspecies currently are recognized (Merriam, 1895; Hall, 1981; Whitaker and Hamilton, 1998). The subspecies were first described in the mid-1800s to the mid-1900s based primarily on cranial and dental characteristics as well as variations in pelage coloration. The subspecies *C. p. parva* is found throughout most of the range described above; it is moderate in size. *C. p. floridana*, which is the largest subspecies in external and cranial measurements, is restricted to peninsular Florida and southern parts of Georgia. *C. p. berlandieri* is similar to *C. p. parva* in size, but it has been described as having noticeably larger teeth that vary to a subtle degree in their orientation

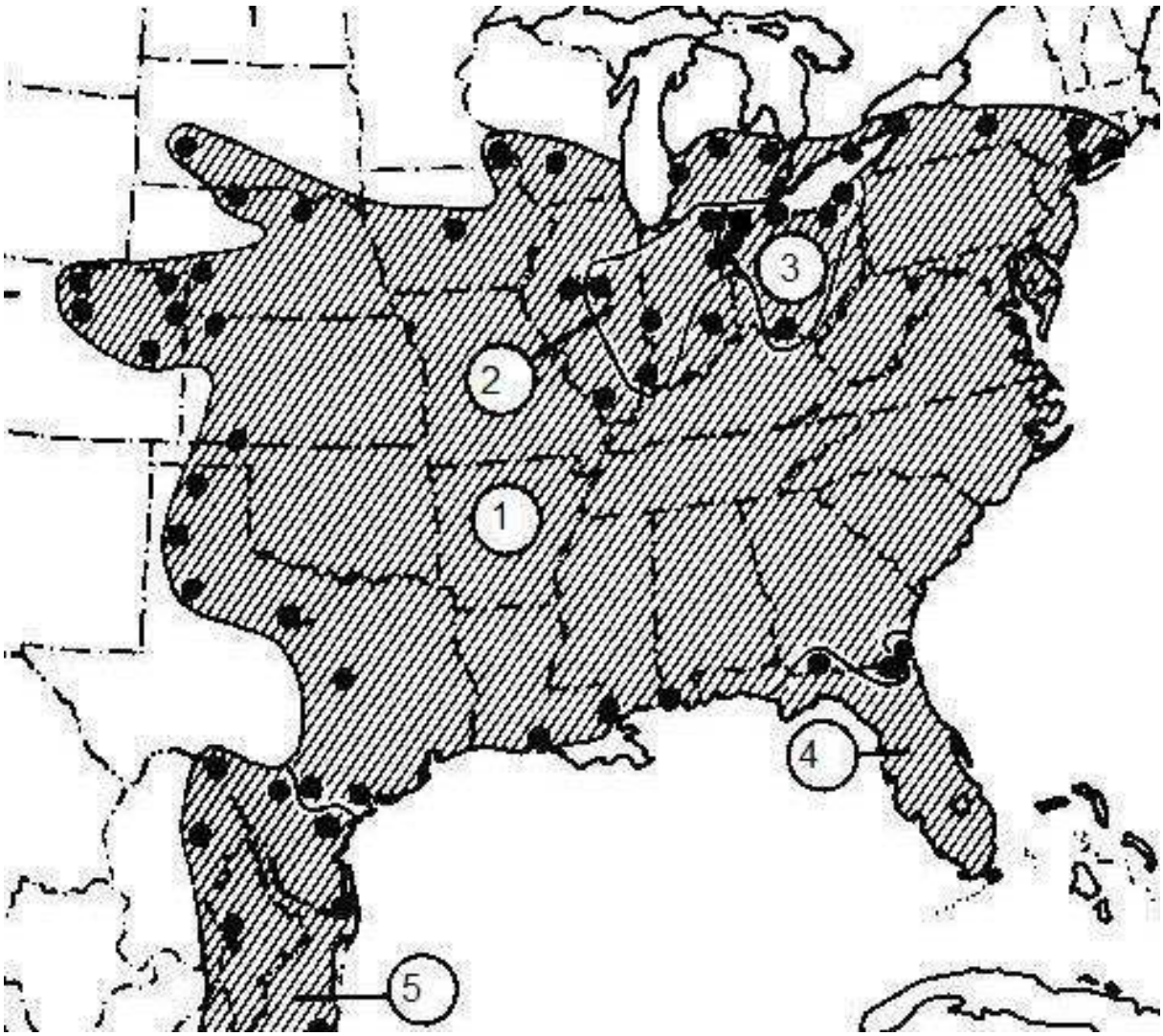

Fig. 1: Traditional taxonomic distribution of *Cryptotis parva* and its subspecies in the United States. (1=*C. p. parva*, 2=*C. p. harlani*, 3=*C. p. elasson*, 4=*C. p. floridana*, 5=*C. p. berlandieri*) Map modified from Hall (1981). Dots represent marginal records; exact locations and citations can be found in Hall & Kelson (1959) and Hall (1981).

(Baird, 1859); it inhabits the Rio Grande Valley southward into northern Mexico. *C. p. elasson* of Ohio and *C. p. harlani* of Indiana and parts of Illinois are smaller than *C. p. parva* (Bole and Moulthroup, 1942). Prior to these descriptions there was much taxonomic confusion, and, in fact, members of the genus *Cryptotis* were included in the genus *Blarina* (Whitaker, 1974).

Bole and Moulthroup (1942) considered a specimen of *Cryptotis harlani* from New Harmony, Indiana, to be an intergrade between *C. p. elasson* and *C. p. parva*, noting that, although it was similar in size to *C. p. parva*, its pelage was darker with a hint of gray (like that of *C. p. elasson*). It is noteworthy that only 12 specimens of *C. p. harlani* from Illinois and Indiana were examined by Bole and Moulthrop (1942), and of these, nine were taken from owl pellets, so the skulls could not be compared in their entirety. Pelage color can be useful in identifying variation in some organisms; however, in most shrew species this character is highly variable (Choate, 1970). In most parts of the range, least shrews exhibit seasonal variation in pelage color, displaying a darker hue in the winter than in the summer (Jackson, 1961; Lyon, 1936). Also, juvenile shrews have been reported to be slightly darker than adults (Jackson, 1961), and museum specimens often become foxed and pigmentation changes over time (Lowery, 1974; Handley and Varn, 1994).

In the last three decades the validity of *C. p. harlani* has been doubted. In Illinois, *C. p. harlani* was thought to occupy the eastern part of the state, whereas *C. p. parva* was thought to occupy the western and southern parts of the state. Mumford and Whitaker (1982) declined to differentiate specimens from Indiana as a separate subspecies other than *C. p. parva*, claiming more investigation was needed to clarify the issue. Hoffmeister (2002) performed canonical variate and discriminant function analyses on five groups of *Cryptotis* from eastern Nebraska (*C. p. parva*), western Illinois (*C. p. parva*), eastern Illinois (*C. p. harlani*), southern Illinois (*C. p. parva*), and western Indiana (*C. p. harlani*). This study revealed no significant difference in cranial characteristics between any of these groups. Based on these results, Hoffmeister (2002) concluded that *Cryptotis parva harlani* was not a distinct subspecies.

Least shrews from Florida are larger in size when compared to specimens of *Cryptotis parva* throughout the remainder of its geographic range, and currently these populations are designated as the

subspecies *C. p. floridana* (Whitaker, 1974; Hall, 1981). There is some question, however, as to exactly how different least shrews in Florida and parts of southern Georgia are when the species as a whole is compared on a larger geographic scale, and taxonomic designation has fluctuated between subspecific and specific levels over the years. Baird (1857) noted obvious differences between an individual from Indian River, Florida and those from other locations in the geographic distribution of *Cryptotis*. Whitaker and Hamilton (1998) agreed with Handley and Varn (1994) that specimens in southeastern Georgia and Florida are also darker and have longer tails than *Cryptotis parva* from sites located farther north. Handley and Varn (1994) agreed with the conclusion of Merriam (1895) that these organisms were specifically distinct, with specimens from southeastern Georgia and peninsular Florida comprising *Cryptotis floridana*.

Large sizes of *C. parva* have been observed in animals along the Atlantic Coast as well as in Florida, leading some investigators to believe that the range of *C. p. floridana* is not restricted solely to Florida. Handley and Varn (1994) compared samples from southern Florida, northern Florida, coastal South Carolina, coastal North Carolina, and Raleigh, North Carolina (the latter used to represent typical *C. p. parva* in size similar to that at the type locality in Blair, Nebraska). They noted that specimens demonstrate clinal decreases in size from south to north and that they often appear larger near the coastal regions of North and South Carolina. In the specimens they examined there was a 10.2% decrease in total length from southern Florida to coastal North Carolina (indicating that even within Florida organisms are biggest in the southern part of the state), but from coastal North Carolina to Raleigh (a much shorter geographic distance) there was a 12.3% decrease in total size. Also, the tail length in specimens from Florida northward along the coast to North Carolina was 25% of their total length, but those of Raleigh specimens averaged 21%. These findings led to the conclusion that specimens from coastal North Carolina and coastal South Carolina represent the same taxon found Florida. This interpretation finds support from Baird (1857), who first suggested that least shrew specimens from South Carolina constituted the same population as those in Florida.

Preliminary evidence (Hutchinson, 2007) from statistical analyses of cranial characteristics suggests that specimens of *C. parva* from the Outer Banks of North Carolina are noticeably and abruptly larger than those existing on the adjacent mainland in Dare and Hyde counties. The possibility exists that these specimens may represent an undescribed subspecies or *C. p. floridana*. Further investigation into these specimens is warranted to better identify their taxonomic status.

Hall (1981) last provided the distributional limits of the five subspecies of *Cryptotis parva*, but his interpretation was based in large part on the revision done by Merriam (1895) almost 100 years earlier, which was based on morphological characteristics of relatively few specimens. Changes in morphology are assumed to reflect changes in genetic structure (Avise, 2004), so taxonomy based on morphology is often useful when delineating genera or species. However, this method alone often falls short when used to infer population dynamics within a species that may not have had time for the morphological changes to reflect the molecular changes. Modern molecular techniques can be used in conjunction with traditional morphometrics to gain a better understanding of the phylogeography of closely related taxa.

Mitochondrial DNA is useful for studying evolutionary events on the intraspecific level because of its high rate of evolutionary substitutions and maternal inheritance (Kocher et al., 1989; Avise, 2004). Cytochrome-b (cyt-b) and cytochrome oxidase I (COI) are two mitochondrial markers that have been shown to be appropriate for resolving relationships over the last 20 MY and have been widely used to analyze animal sequences (Harrison, 1989; Irwin et al., 1991; Peppers and Bradley, 2000; Shinohara et al., 2003; Avise, 2004; Blois and Arbogast, 2006). Nuclear DNA evolves at a much slower rate than mitochondrial DNA and, therefore, is useful for resolving relationships at higher taxonomic levels. The Apolipoprotein-B (ApoB) marker from the nuclear genome will be used in conjunction with mitochondrial DNA to provide multiple lines of evidence for the genetic relationships within *Cryptotis* of the United States. The ApoB gene was chosen in this study because of the availability of published sequences of three species of least shrews, including one sequence of *Cryptotis parva*.

This study will use both morphology and molecular data to infer the phylogenetic relationships of *Cryptotis parva* throughout its range in the United States. Specifically, the following null hypotheses will be tested:

1) Samples of *Cryptotis parva parva*, *Cryptotis parva elasson*, *Cryptotis parva harlani*, *Cryptotis parva floridana*, and *Cryptotis parva berlandieri* are not significantly different from one another in their morphometrics or genetics.
2) Specimens along the East Coast of the United States are not significantly larger than those further inland.
3) The population of *Cryptotis parva* on the Outer Banks of North Carolina is not distinct from those on the adjacent mainland in their morphometrics or genetics.

METHODS

Morphology

A total of 1700 specimens of *Cryptotis parva* from 18 museums were examined for morphological analysis (Appendix I). Materials examined from these specimens included combinations of skins, skulls, and complete skeletons. External measurements (total length, tail length, hind foot length, and weight) as well as any additional information were recorded from specimen tags. Furthermore, seven cranial measurements were taken with digital calipers to the nearest 0.1 mm from individuals (n=1020) whose condition allowed for the complete suite of measurements to be taken. These measurements included: greatest length of skull (GLS), occipital-premaxillary length (OPL), interorbital breadth (IB), greatest cranial breadth (GCB), width across molars (WM), palatine length (PL), and the distance from the fourth premolar to third molar (P4-M3). These measurements were selected because they have proved useful in determining shrew relationships by other investigators (Bole and Moulthrop, 1942; Choate, 1970; Genoways and Choate, 1972; Moncrief et al., 1982; Woodman and Timm, 2000). For definitions of measurements see Table 1 and Fig. 2.

Table 1: Definition of cranial characteristics measured on 1020 specimens of *Cryptotis parva*. The numbers correlate with those in Figure 2.

Number	Abbreviation	Measurement	Definition
1.	**GLS**	Greatest Length of Skull	Greatest distance from the posterior-most projection of the occipital to the anterior-most projection of the upper incisors
2.	**OPL**	Occipital-Premaxillary Length	Distance from the posterior-most projection of the exoccipital condyle to the anterior-most projection of the premaxillae
3.	**IB**	Interorbital Breadth	Least distance across the orbits measured perpendicular to the longitudinal axis of the cranium
4.	**GCB**	Greatest Cranial Breadth	Greatest mastoidal breadth measured perpendicular to the longitudinal axis of the cranium
5.	**WM**	Width Across Molars	Greatest distance across the palate between the labial-most projections of the upper molars
6.	**PL**	Palatal Length	Greatest distance from the anterior-most point of the upper incisors to the hind edge of the bony palate
7.	**P4-M3**	Fourth Premolar to Third Molar Length	Greatest distance from the anterior-most projection of the upper premolar to the posterior-most projection of the upper third molar

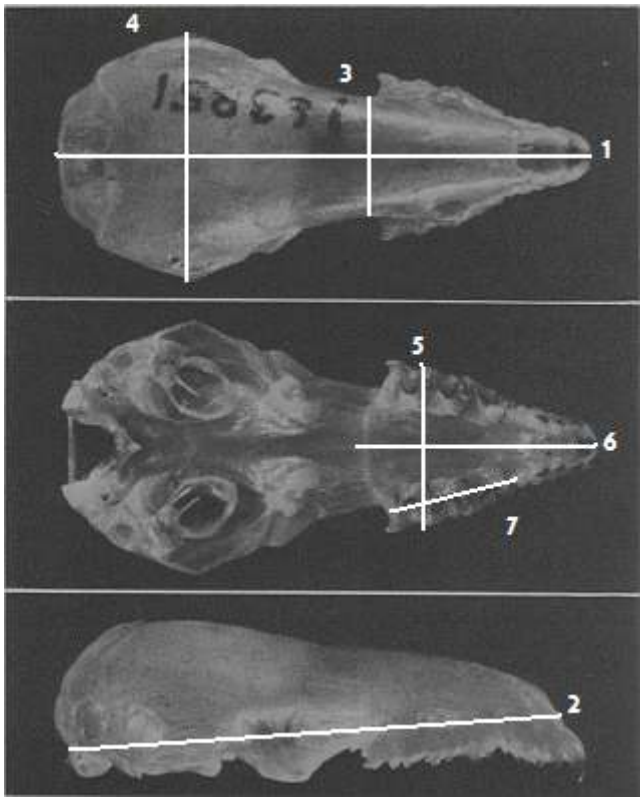

Fig. 2: Measurements taken from 1020 specimens of *Cryptotis parva*. The numbers correlate with the definitions given in Table 1. (Image modified from Whitaker, 1974)

Because external measurements were taken by many individuals, and are therefore highly variable, only cranial measurements taken personally were used in statistical analyses. Individuals whose cranial sutures were not fused were considered to be juveniles and were excluded from analyses. Individuals for which cranial measurements were able to be taken were grouped into 67 operational taxonomic units (OTUs) based on geographic location, being careful not to cross any current taxonomic designations, major physiographic provinces, or major geographic boundaries (Fig. 3).

A single classification analysis of variance (F-test, significance level 0.05) was used to test for significant geographic variation among the OTUs using the GLM procedure in SAS (v9.1), and a Tukey's HSD posteriori pairwise test was used to determine which OTUs differed significantly. Principal components analysis was performed by deriving a product-moment correlation matrix from variance-standardized character means for each OTU, extracting eigenvectors, and generating a two dimension plot of OTUs. Additionally, the MEANS, CLUSTER, and TREE procedures were used in SAS (v9.1) to generate means for each measurement of each OTU, cluster the means hierarchically, and create a phenogram based on the clusters, respectively. Finally, coordinates for each individual were acquired from the MANIS database, when possible, or interpreted from Google Earth (v5.1) for analyses of clinal variation using standard correlation and regression calculations derived from Excel between cranial size and latitude and longitude. Furthermore, the coordinates were put into ArcGIS (v9.3.1) to interpolate measurements for parts of the range where specimens were either not available or too badly damaged to measure.

Genetics

Fresh tissue samples were obtained from New Hanover and Brunswick counties of North Carolina (n=3), frozen tissue collections at the Natural History Museum at UNCW (n=7), and loan requests made to frozen tissue banks around the country (n=10). However, these individuals neither encompassed the scope of the distribution of *Cryptotis parva* in the United States, nor allowed for

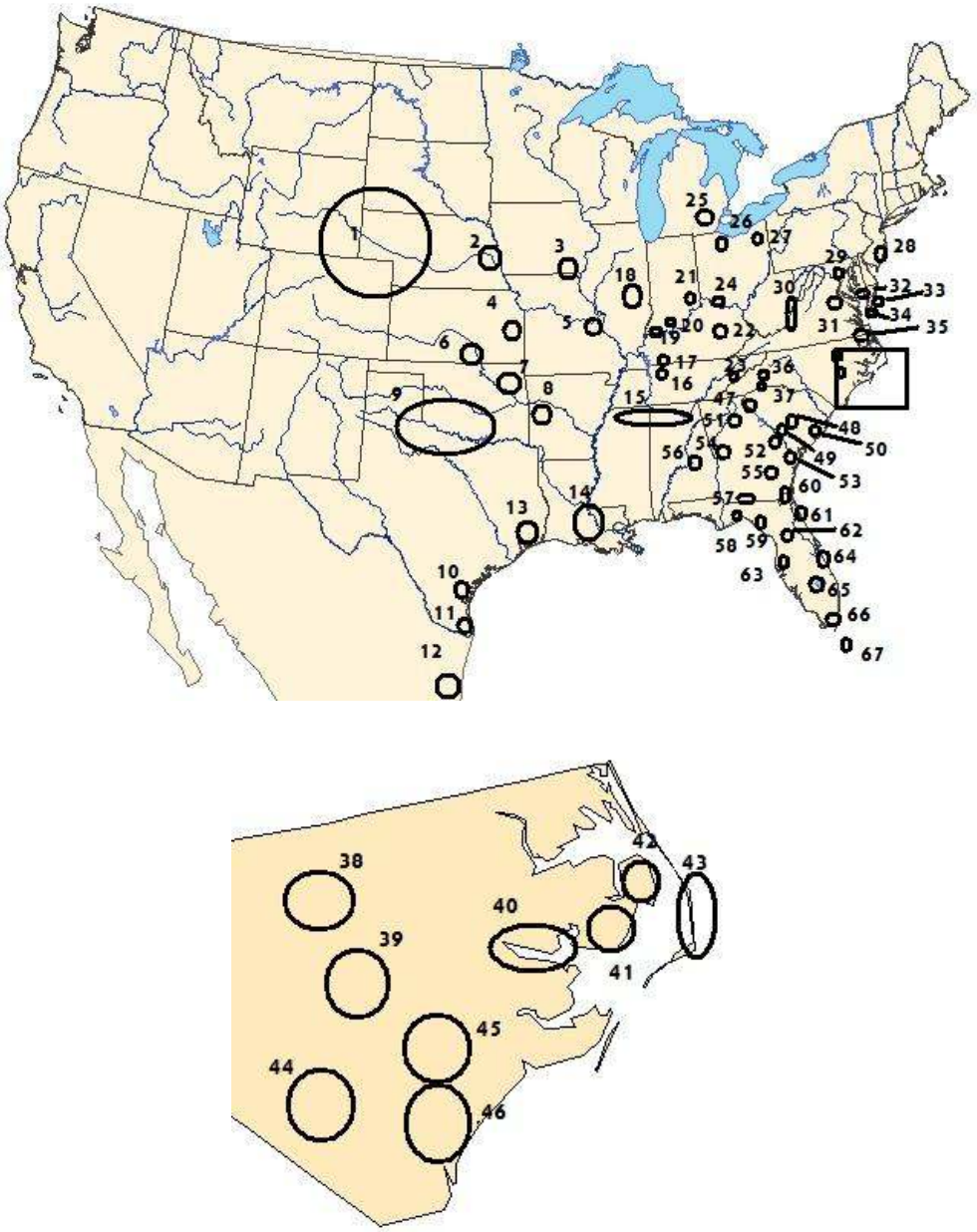

Fig. 3: Locations of 67 OTUs in the United States and Mexico.

extensive sample size. Fresh tissues were supplemented using toes from prepared specimens at the UNCW Natural History Museum (n=13) and using soft tissue that remained on museum skulls after the cleaning process was complete, referred to as residual tissue (n=46). Finally, 11 sequences were obtained from the GenBank database, including outgroup sequences of *Blarina brevicauda*, *B. carolinensis*, *C. magna*, *C. goldmani*, and *C. mexicana* for cytochrome-b and *C. magna* and *C. goldmani* for ApoB. A complete list of source museums, tissue type, markers amplified, and collection locations are in Appendix II.

Tissues were extracted according to the protocol of the Animal Extraction Kit from MOBIO Laboratories (Carlsbad, CA). Following extraction, PCR inhibitors were removed using the MOBIO PowerClean Kit.

A 218 bp fragment of cytochrome-b was amplified for 79 individuals using primers 950F/15915, 950F/1118R, 1021F/15915 (Irwin et al., 1991). A 143 bp fragment of cytochrome oxidase I was amplified for 21 fresh tissue extractions using primers COIF/COIR, and a 210 bp fragment of Apolipoprotein-B was amplified for 14 fresh tissue extractions using ApoF/ApoR (Table 2). Due to the low success rate for COI and ApoB, these markers were used only to corroborate any patterns observed in the cyt-b data. PCR amplification was performed following the protocol of GoTaq Green Master Mix from Promega Corp. (Madison, WI). Thermocycler conditions were set at 40 cycles of 95°C for 2min, 95°C for 50s, 50°C for 50s, and 72°C for 40s, with an extension at 72°C for 5min at the end of the PCR. PCR products were first visualized on a 2% agarose gel electrophoresed for 20min and soaked in ethidium bromide for 15min. Products were then purified according to the ExoSAP protocol from USB Corporation (Cleveland, OH) before final sequencing was outsourced to Macrogen Inc. (Seoul, Korea). Sequences were aligned with *Sequencher 4.8* and refined by eye, and the possibility of pseudogenes in the dataset was rejected by the lack of stop codons and the presence of expected proportions of bases in the sequences (i.e. low GC content in mitochondrial DNA).

To apply the appropriate substitution model to the alignment of the three markers, JModelTest version 0.1.1 (Guindon and Gascuel, 2003; Posada, 2008.) was used. This software analyzes 88 nucleotide substitution models of increasing complexity and uses a likelihood ratio test based on the AIC

Table 2: Primers used to amplify mitochondrial and nuclear markers.

Primer	Sequence (5´-3´)
950F	TCYAAACAACGAAGYATAATA
1021F	AGGACARCCCGTCGAACAYCC
1118R	TCRARTAGGCTTGTGATTGG
COIF	CCGYTGAYTATTYTCTACYAACCAC
COIR	GAAAATTATRACRAATGCGTGRGC
ApoF	TGAGAAAGTCAGAGACCAGGC
ApoR	ACAGAGAAGCCAGAACCCAGG

criterion to find the best-fit model, including appropriate gamma distributions for modeling rate heterogeneity across sites and percentage of invariant sites for modeling the unchanging portion of the data. The hypothesis of recent population growth was tested using Tajima's D, Fu's Fs, and mismatch distribution tests in Arlequin v3.1 (Excoffier et al., 2005) using the appropriate gamma and base frequencies obtained from jModelTest. Additionally, genetic diversity indices of Theta-pi and Theta-S were obtained. Analysis of Molecular Variance (AMOVA) was run based on the genetic structure obtained from phylogenetic analyses, using clades that grouped together with posterior and bootstrap probabilities greater than 50%. Haplotype genealogies were estimated using minimum spanning network analyses in TCS version 1.21 (Clement et al., 2000), which calculates the level of divergence for connections that have a maximum parsimony probability greater than 0.95 (Templeton et al., 1992).

Inference of phylogeny was done using Bayesian methods for the cytochrome-b marker in BEAST v1.4.8 and its accompanied program Tree Annotator v1.5.3. Bayesian analyses use

predetermined priors to search the set of trees with the best topologies and combination of parameters (Felsenstein, 2004). For this study, all priors were left at the default values except for clock calibrations. Assigning *Blarina brevicauda* and *Blarina carolinensis* as a monophyletic outgroup, the lower and upper bounds of the split between *Cryptotis* and *Blarina* were set at 9 MYA and 15 MYA, respectively, based on the ages of the oldest modern *Cryptotis* fossil and the oldest *Adeloblarina* fossil (Harris, 1998). *Cryptotis goldmani*, *C. mexicana*, and *C. magna* were also assigned to be included in order to better calibrate the bounds. Three independent MCMC analyses were run for 80 million iterations with a burn-in of 10%. Each analysis was viewed using Tracer v1.4, appropriate ESS values were verified (>200), and convergence between runs was checked. The three analyses were then combined to create one uniform tree using Tree Annotator v1.5.3, and the tree was viewed and formatted using FigTree v1.3. PAUP* version 4.0 (Swofford, 2003) was used to calculate bootstrap values using 1000 replicates, likelihood optimality criterion, neighbor-joining search method, and the best-fit model results from jModelTest. These values were then superimposed onto the Bayesian gene tree created for cytochrome-b. Maximum likelihood trees were generated in PAUP* for the nuclear Apolipoprotein-B and the mitochondrial COI genes according to the parameters determined in jModelTest. Additionally, bootstrap values were generated for both markers using both likelihood and parsimony optimality criteria.

RESULTS

Morphology

Ninety percent of the variation among the 67 OTUs is accounted for on the first two principal components (Table 3). Principal component I (PC I) explains 83% and principal component II (PC II) explains 7% of the overall variation in the data set. All seven cranial characteristics load positive on PC I and have approximately the same loadings (Table 3). Therefore, PC I reflects size. Only interorbital breadth (IB) and greatest cranial breadth (GCB) load positively on PC II and eigenvector loadings indicate that IB, GCB, and palatal length (PL) have the most influence, with the latter loading negatively. Therefore PC II represents shape, indicating an inverse trend between cranial and interorbital width on

one hand and palatal length on the other along this factor. Width across molars (WM) also loads negatively on PC II, indicating that more robust animals do not necessarily become more robust in toothrow characteristics.

Table 3: Eigenvector loadings on principal components I & II for seven measurements in *C. parva*.

Variable	PCI	PCII
GLS	0.41	-0.09
OPL	0.40	-0.07
IB	0.35	0.58
GCB	0.35	0.55
WM	0.37	-0.11
PL	0.37	-0.51
P4_M3	0.39	-0.26
Total variation explained (%)	83	7

When PCI and PC II are plotted against one another (Fig. 4), four groups of OTUs are revealed. A general pattern of increasing size from inland populations to coastal populations, with the largest individuals residing in Florida, is apparent. Group I has the most negative PC I values. This group includes OTUs from the remainder of the species range. This group is formed from inland OTUs with only two exceptions, OTU 46 from New Hanover County, North Carolina and OTU 41 from Hyde County, North Carolina.

Group II is made up of only three OTUS (8, 10, and 49) from the general areas of Logan County, Arkansas, Aransas County, Texas, and Aiken County, South Carolina. Least shrews from these OTUs are moderate in overall size (PC I), but they have negative values on PC II and are characterized by having narrow cranial and interorbital regions but relatively long palates. No geographic coordination between these groups is apparent, however.

Group III is primarily formed from coastal OTUs (13, 33, 34, 42, 43, 50, 55, and 57) as well as two OTUs in Florida (63 and 65). These OTUs are smaller than those in Group IV in overall size (PC I) and they have less variation in shape (PC II). The largest group (Group IV) consists of eight OTUs from

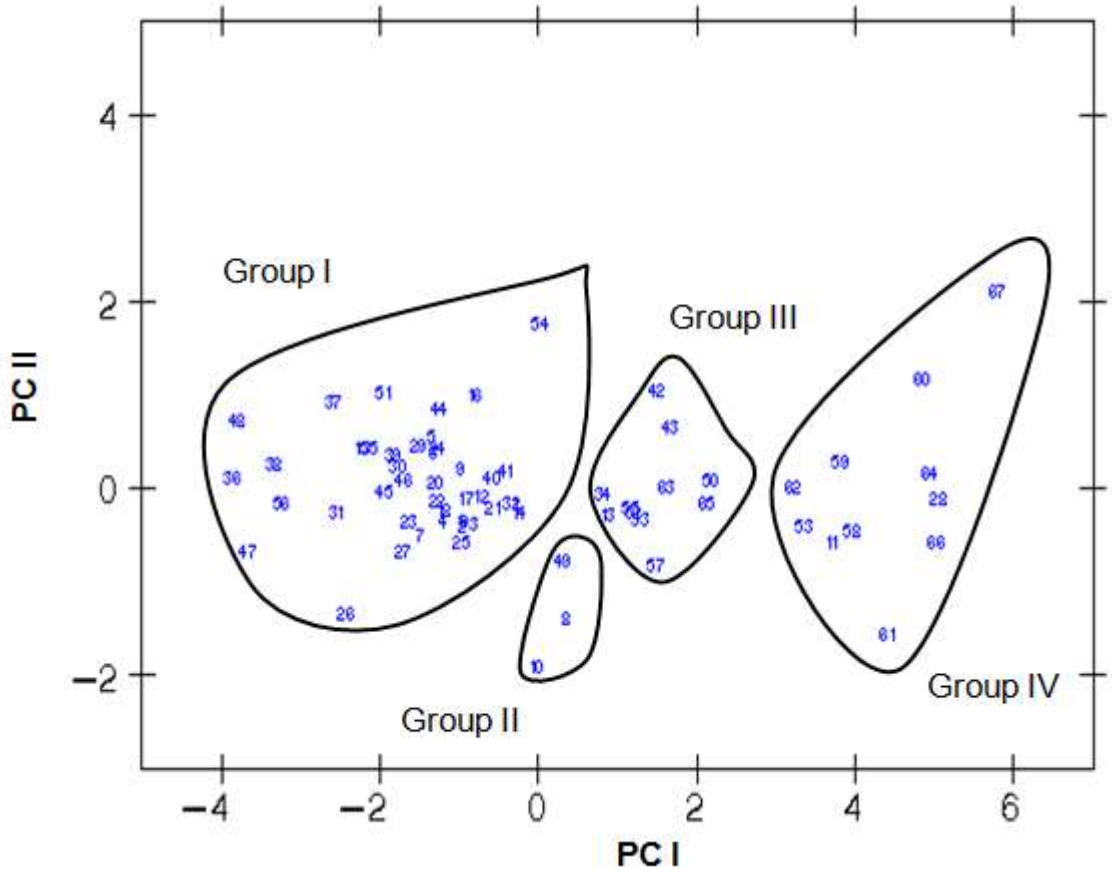

Fig. 4: Principal component analysis for the means of 67 OTUs of *Cryptotis parva*. Principal Component I (Prin1) represents overall size and Principal Component II (Prin2) represents an inverse relationship between interorbital and cranial breadth on one hand and palatal length on the other.

Florida (OTUs 58, 59, 60, 61, 62, 64, 66, and 67) as well as OTU 28 from New Jersey, OTU 11 from Hidalgo County, Texas, and OTU 53 from Liberty County, Georgia, which are all coastal locations. These OTUs are strongly positive on PC I, but their PC II loadings range from strongly positive (OTU 67, Key Largo, Florida) to strongly negative (OTU 61, St. Johns County, Florida), indicating large variation in cranial robustness and palatal length.

It is important to note that the group assemblages produced by this PCA do not correlate geographically with the distributions of *C. p. elasson* or *C. p. harlani* whose type localities are included in OTU 26 and OTU 19, respectively. OTU 19 is neither unique along PC I or PC II which coincides with the results found by Hoffmeister (2002). Also, the type locality for *C. p. berlandieri* (OTU 12) is nested well within Group I, and does not appear unique according to this analysis. OTU 26 is not unique with respect to Group I along PC I, but it is the most negative from that group along PC II. However, it is not the most negative given data from other groups, meaning that while individuals in this area may be on the slender side, they are not the most slender individuals when considering the entire range under investigation. OTUs 42 and 43 represent individuals from the Outer Banks, North Carolina and the adjacent mainland in Dare County, North Carolina. Both fall out within in Group III on the positive side on the PC I axis.

A phenogram (Fig. 5) created using the seven cranial characteristics analyzed supports the trend seen in the principal components analysis. Two major clades are apparent in the phenogram, which loosely correspond to Group IV and Groups I, II, and III in the PCA. Although there are a few exceptions such as OTUs 52, 55, and 8, which include individuals around the areas of Burke and Ben Hill counties in Georgia and Logan County in Arkansas, the biggest animals group together into a clade consisting exclusively of OTUs from Florida and other coastal regions. OTUs 18-21 represent individuals of *C. p. harlani* from Indiana and Illinois, and OTUs 24, 26, and 27 represent individuals of *C. p. elasson*. Note that these OTUs do not group together in any notable pattern.

All seven variables measured display significant ($p<0.0001$) geographic variation. The subsequent posterior test (data available from author upon request) revealed that GLS and OPL exhibit

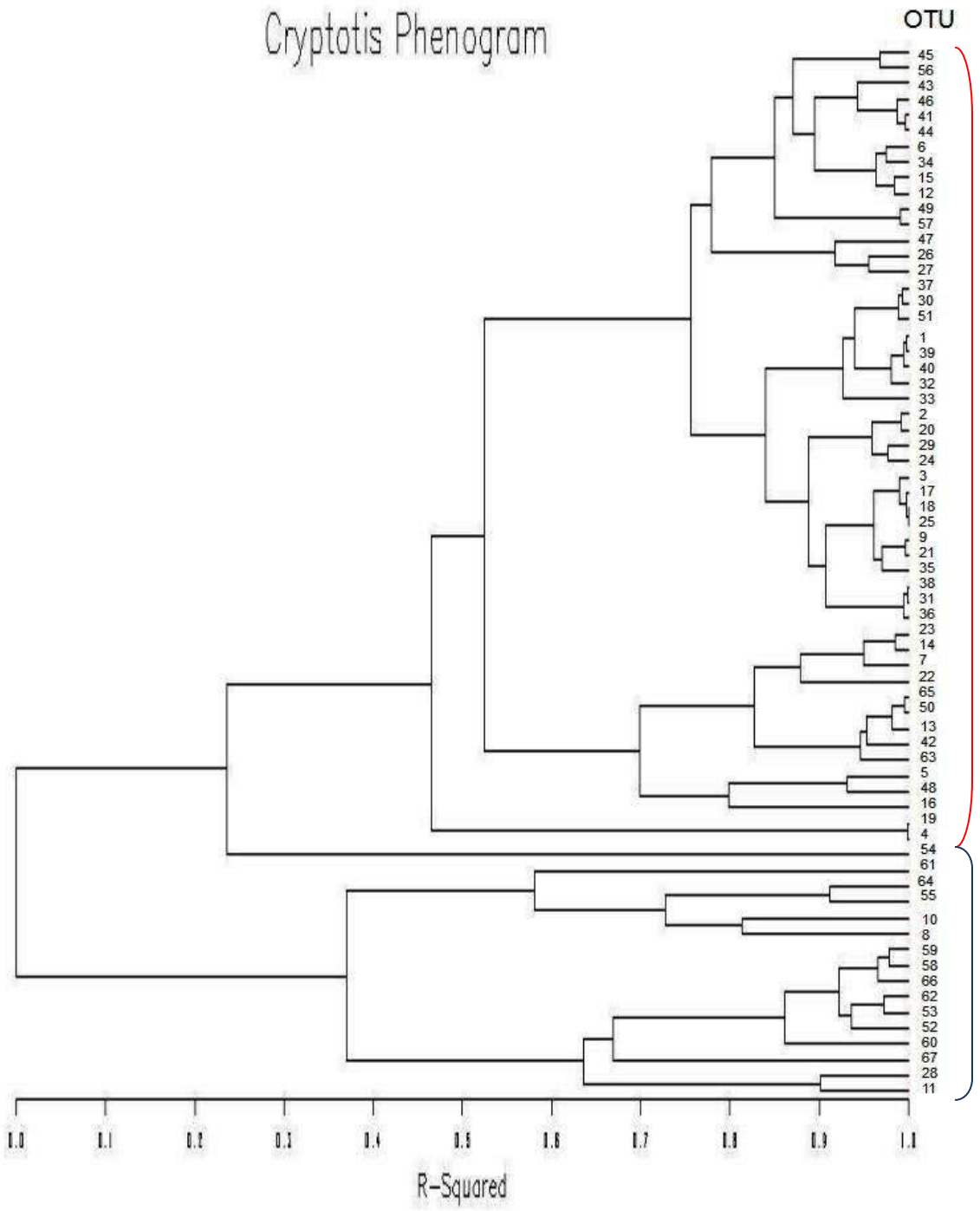

Fig. 5: Phenogram based on a cluster analysis of seven cranial characters. The OTUs bracketed in blue loosely correlate to Group IV from the principal components analysis while those bracketed in red loosely correlated to Groups I, II, & III.

the same pattern. Generally speaking, OTUs in Florida, Texas, and along the Atlantic Coast were not significantly different from one another, but were significantly different from those in the remainder of the range. There was no apparent geographic signal in IB or GCB except in extreme parts of the range (northwest and southern Florida). Relatively few significant differences were evident in WM, PL, and the distance from the fourth premolar to the third molar (P4-M3) measurements, and those OTUs that were significant in these variables showed little geographic pattern. Rather, patches of larger and smaller individuals reside more or less randomly throughout the range. However, even when there is a pocket of smaller individuals, for example, the difference is clinal. In all measurements OTUs in Florida were largest. In no measurement were specimens from the type locality of *C. p. elasson* (OTU 26) and those from the type locality of *C. p. harlani* (OTU 19) significantly different from one another. For comparative purposes *C. p. berlandieri* is represented by specimens from Tamaulipas, Mexico (OTU 12), the type locality. These specimens were significantly smaller than those from OTUs 67 (GLS, OPL, and GCB), 66 (GLS and OPL), 64 (GLS and OPL), 62 (GLS and GCB), and 60, 59, 58, and 28 (GLS), but not significantly different from *C. p. parva* in Texas or elsewhere in its range. OTU 16 (Cheatham County, Tennessee) and OTU 27 (representing Lake County, Ohio, which is an area near the individual used for molecular analyses from Portage County, Ohio) were both similar to specimens from Florida (OTUs 58, 59, 60, 62, 64, and 67) and New Jersey (OTU 28) and significantly different from all other OTUs in the measurement of GLS. However, while they were not significantly different from Florida samples, they were smaller by an average of 1.45 mm (OTU 16) and 1.67 mm (OTU 27). No other geographic variation was present in any other measurements for these two OTUS except for OPL and GCB where they were only different from the largest of the samples (Dade Co. and Key Largo, Fl and New Jersey). Furthermore, specimens from OTU 42 (mainland of Dare County, North Carolina) and OTU 43 (Outer Banks of Dare County, North Carolina) were not significantly different from one another in any measurement.

To further investigate whether larger sized shrews in Florida and along the coast were a result of an abrupt increase or a clinal increase in response to geography, size was plotted against latitude and

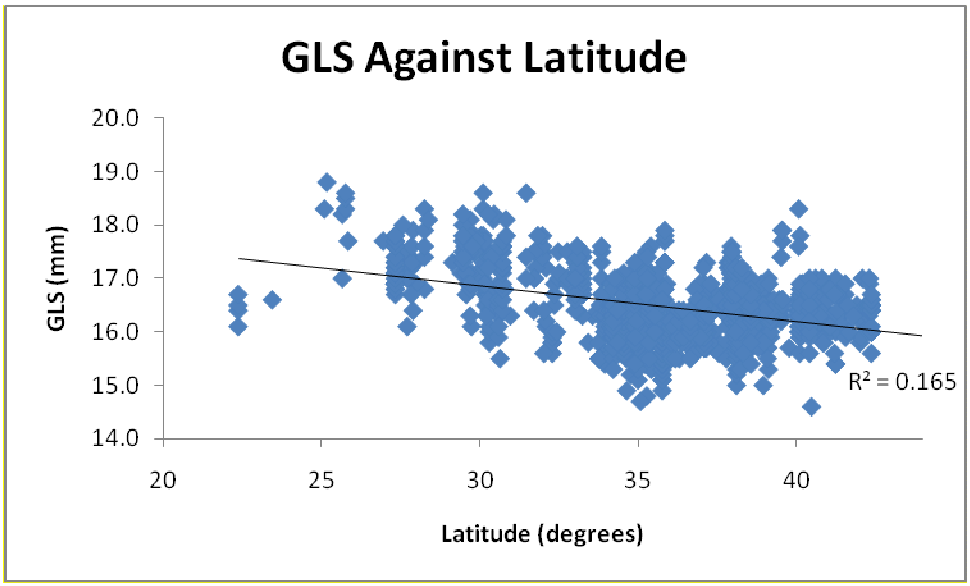

Fig. 6: GLS plotted against latitude for 1020 specimens of *Cryptotis parva*.

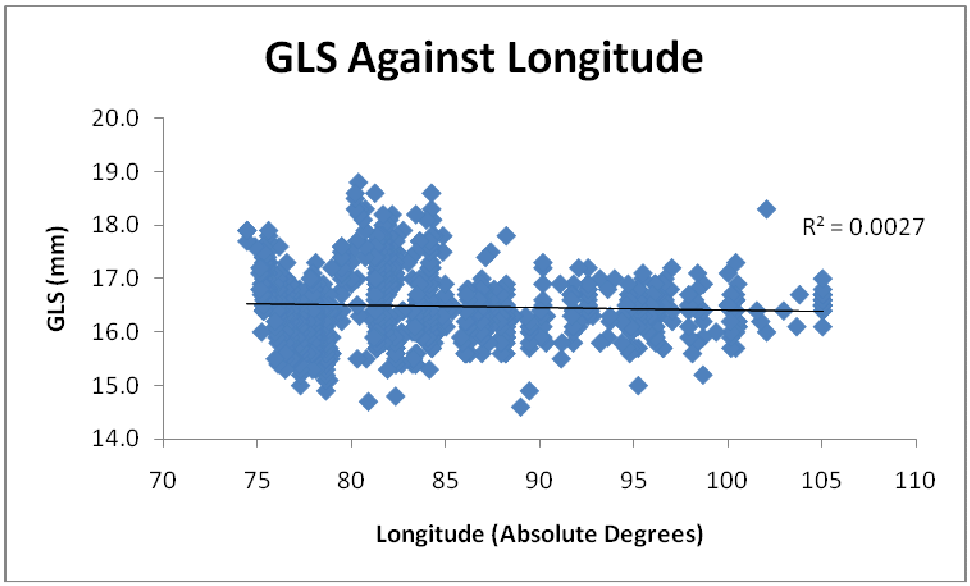

Fig. 7: GLS plotted against longitude for 1020 specimens of *Cryptotis parva*.

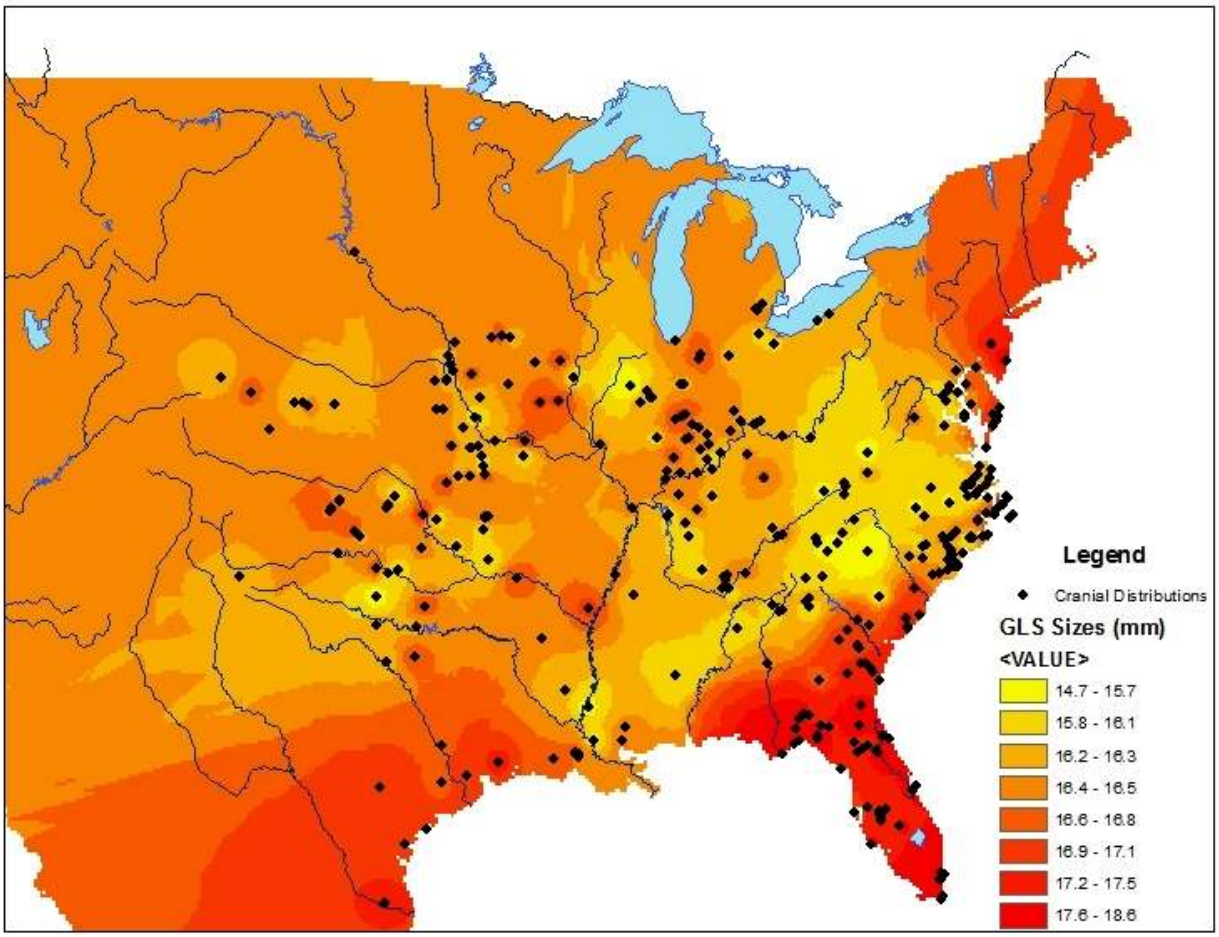

Fig. 8: Interpolated GLS measurements from 1020 specimens using ArcGIS. Individual specimen locations indicated by a black dot.

longitude and correlation analyses were performed (Fig. 6 and Fig. 7). Cranial sizes for the entire species range were also interpolated in ArcMap version 9.3.1 (ESRI Inc.) using the spatial analyst tool, the inverse distance weighted method, and the cranial sizes of the 1020 individuals measured (Fig. 8). Because GLS was determined to express the most variation in the ANOVA, it was used as a proxy for overall cranial size in these analyses. There is a significant negative correlation (-0.41, $p<0.0001$) between latitude and GLS, however the R^2 value is only 0.165. Most of this correlation lies between the latitudes of 25° and 30° which roughly correspond to the latitudes of Florida and southern Georgia. There is no significant correlation between longitude and GLS ($p=0.07$). In the scatterplot, however, increased sizes around the longitudes that correspond with the East Coast are apparent. These results indicate that *Cryptotis* does exhibit an abrupt increase in size in the southern parts of its range. The map of interpolated distances nicely displays the results found in the morphological analyses. Using the inverse distance weighted method cells without data are assigned values based on cells with data. The program assumes closer cells should be weighted heavier than cells further away. One caveat of the method, however, is that it is sensitive to cells with only one data point represented and displays them with a tight circle of color. Also, the model interpolates for the entire area in question so the color gradient extends beyond the bounds of the species range.

Genetics

Cytochrome-b

Amplification of the cytochrome-b fragment successfully yielded clean sequences for 79 *Cryptotis parva* samples: 20 fresh samples, 13 toe tissue samples, and 46 samples of residual cranial tissue, spanning the species range in the United States (Appendix II). The best fit model of nucleotide substitution according to the AIC criterion was TIM1+G. This model was used for all analyses in PAUP* software (Swofford, 2003), however, it is not available in BEAST software and for that reason the second best model of HKY+G was used to construct a phylogeny of cytochrome-b. Data was partitioned by

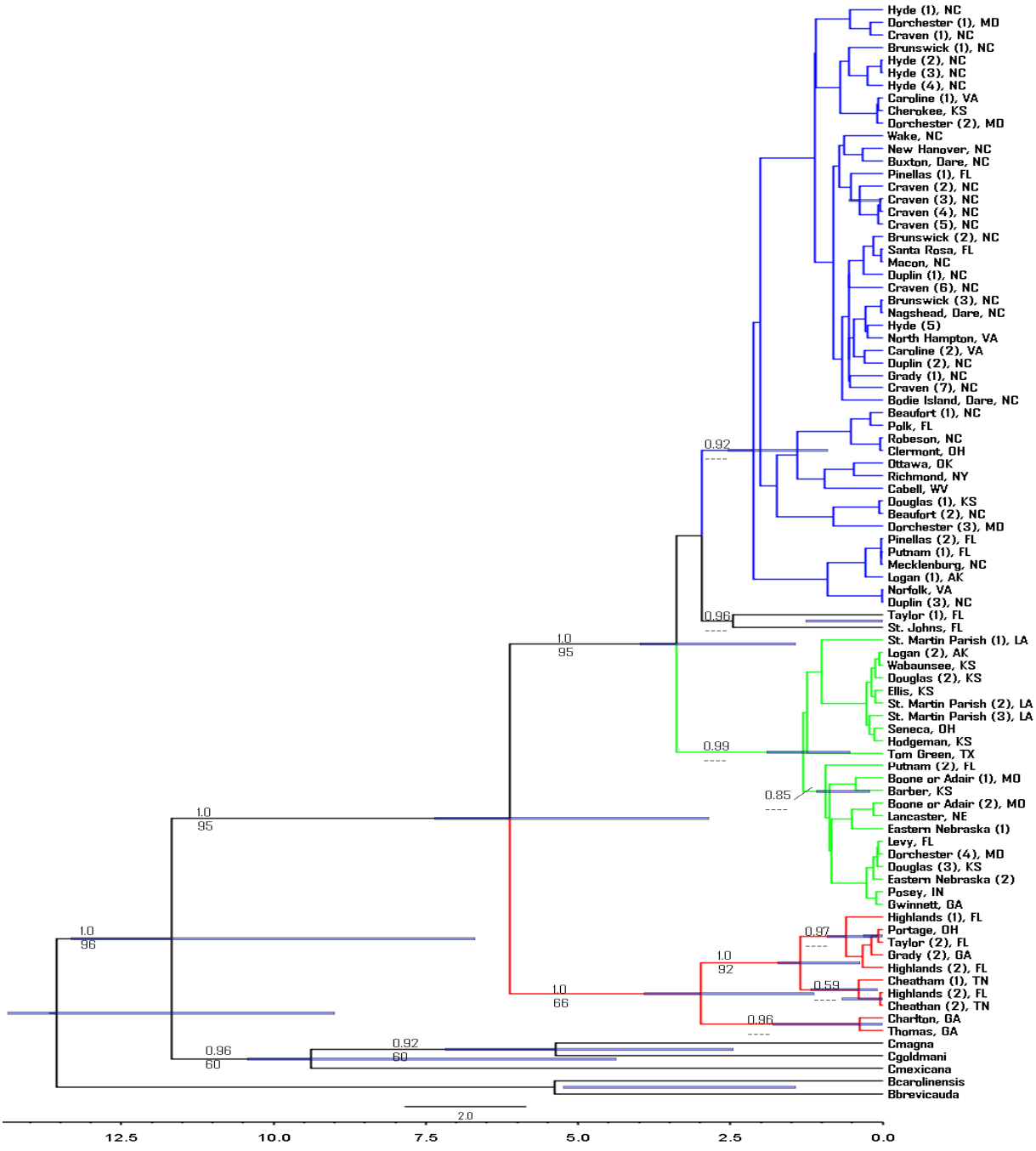

Fig. 9: Bayesian phylogenetic tree based on a 218 bp fragment of the cytochrome-b. Fifty percent consensus tree created from the results of three runs that concurrently estimated phylogeny, mutation parameters, and branch lengths. Time scale is in million years before present. Red indicates the Floridana Group and blue and green branches are part of the Parva Group. Green branches indicate the Parva East population and blue branches indicate the Parva West population. Posterior probabilities of >0.50 are displayed above branches and bootstrap probabilities >50% are displayed below. If a branch does not have bootstrap support, values are replaced with dashes. The black lines are the individuals from St. Johns County, Florida and Taylor County, Florida that are loosely referred to as the Parva South Population.

codon position and a Yule Speciation event was determined to have the lowest posteriors and highest Bayes factors. Bootstrap values were obtained using the distance criterion and a neighbor-joining search method. To calculate bootstrap values using PAUP* (Swofford, 2003) the following parameters of the optimized model (T1M1+G) were used: alpha=0.189, A=0.34, C=0.27, G=0.11 and T=0.28.

Four clades emerge in the resulting cytochrome-b gene tree of *Cryptotis parva* (Fig. 9), which do not support the traditional systematic distinctions of *C. p. parva*, *C. p. elasson*, *C. p. harlani*, and *C. p. floridana*. Most major clades described in the tree are strongly supported by posterior probabilities >0.90 and bootstrap values >50%. The first of these clades (referred to herein as Floridana Group) to emerge (6.1 MYA) consists of three individuals from Highlands County, Florida, two individuals from Cheatham County, Tennessee, and one individual each from Thomas, Charlton, and Grady counties in Georgia as well as Portage County, Ohio and Taylor County, Florida. Bayesian and bootstrap support is strong (posterior probability=1.0, bootstrap=66) for the monophyly of the Floridana Group to the exclusion of the remainder of the individuals sampled (referred to as the Parva Group). Structuring within the Floridana Group began around 3.0 MYA, producing one clade beginning with individuals from Charlton and Thomas counties in Georgia (posterior probability=0.95) and a second clade comprised of the remaining specimens (posterior probability=1; bootstrap=92). Structure within the Parva Group began around 3.4 MYA when three clades emerge. The first radiation to occur within the Parva Group forms what will be referred to as the Parva West Population which consists primarily of individuals from the western United States (Arkansas, Louisiana, Kansas, Missouri, Texas, Nebraska, and Indiana) with the exception of five individuals from Ohio, Maryland, Georgia, and Florida. Bayesian support is strong (posterior probability=1.0) and there is relatively high bootstrap support (85%) for the monophyly of the Parva West Population. The next radiation within the Parva Group occurred approximately 3.0 MYA and produced two clades. This node, however, has neither Bayesian nor bootstrap support. This is due to the instability of the first clade to emerge consisting of only two individuals, one from St. Johns County, Florida and one from Taylor County, Florida. Uncertainty exists if these two individuals should form their own clade (Parva South Population) or belong to the Parva East Population. The last clade to

emerge from the Parva Group will be referred to as the Parva East Population, which primarily contains individuals from the eastern United States (Maryland, North Carolina, West Virginia, Virginia, New York, and Ohio) except for four individuals from Arkansas, Florida, Oklahoma, and Georgia. The monophyly of the Parva East and Parva West Populations is strongly supported with posterior probabilities of 0.92 and 0.99, respectively; however, bootstrap support is weak for both clades. Structuring within these two clades began roughly 1.3 MYA (Parva West) and 2.1 MYA (Parva East), but no definitive geographic patterns emerge. Support for terminal nodes of both the Parva Group and the Floridana Group are lacking, indicating quick population growth and expansion. The nucleotide substitution rate in this analysis was 0.89subs/site/MY. This rate seems low when considering the high metabolic rate of least shrews, but it is consistent with data found in other studies (Fumagalli et al., 1999; Brant and Ortí, 2002).

To further investigate population dynamics, the data was initially analyzed without predetermined population structure. A mismatch distribution was performed on the traditional *C. parva* (Fig. 10) which showed three major peaks and possibly a fourth smaller peak, indicating that the data set represents multiple populations. Subsequently, structure was enforced in the analyses performed based on the three main clades (Floridana Group, Parva West, and Parva East) determined in the Bayesian phylogeny. The individuals from St. Johns and Taylor counties, in Florida, were excluded from these analyses due to the small sample size and low support of the clade. Phylogenetically, neither individual clearly was placed among the other well-defined clades. The clade representing Charlton and Thomas counties in Georgia may represent a unique population within the Floridana Group; however, because of small sample size these individuals were analyzed together with the rest of the Floridana Group. Mismatch distributions performed according to the structure recognized in the Bayesian analysis (Fig. 11-13) show signals of expansion in each group and population. Fu's Fs and Tajima's D values significantly support expansion in Parva West and Parva East populations. For both populations Fu's Fs is largely negative and highly significant ($p<0.0001$). Tajima's D is significantly negative (-1.37, $p<0.0001$) for Parva East, but is not significant for Parva West (1.54, $p<0.524$). The mismatch distribution of the Floridana Group either

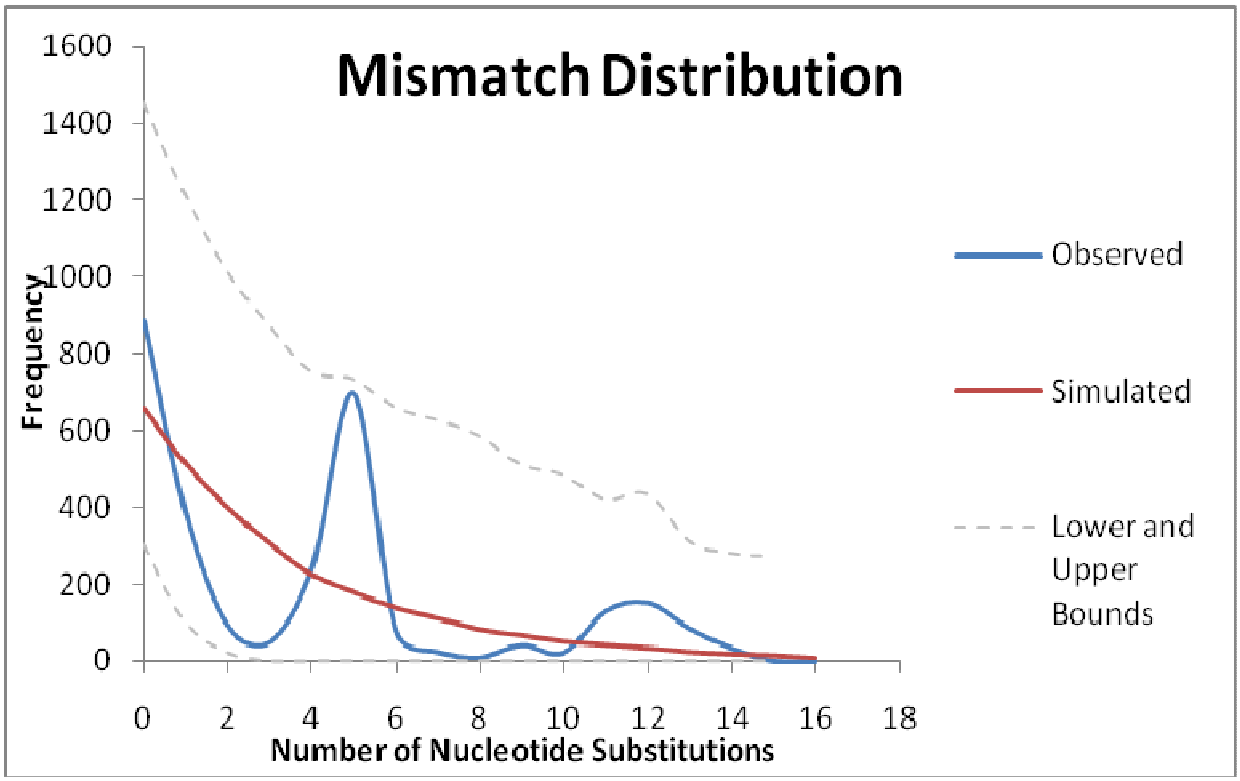

Fig. 10: Mismatch distribution of all samples for which cytochrome-b was amplified. Also shown are simulated values under constant size (red), including the 5% and 95% bounds.

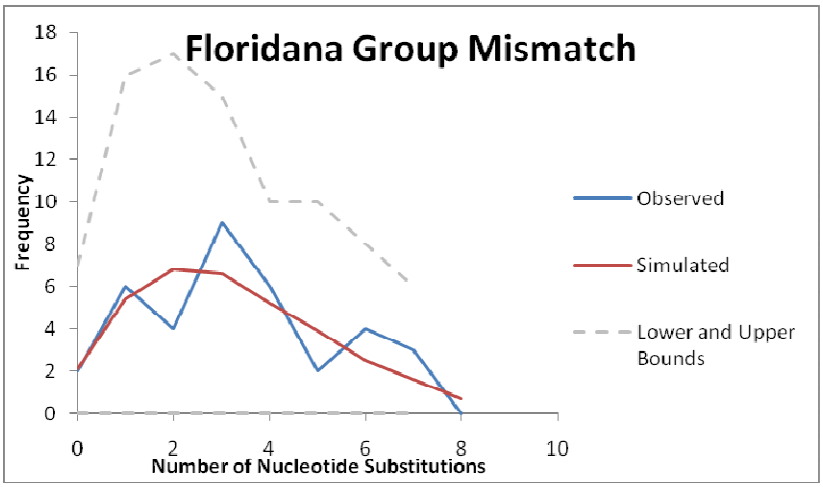

Fig. 11: Mismatch distribution frequency for the Floridana Group

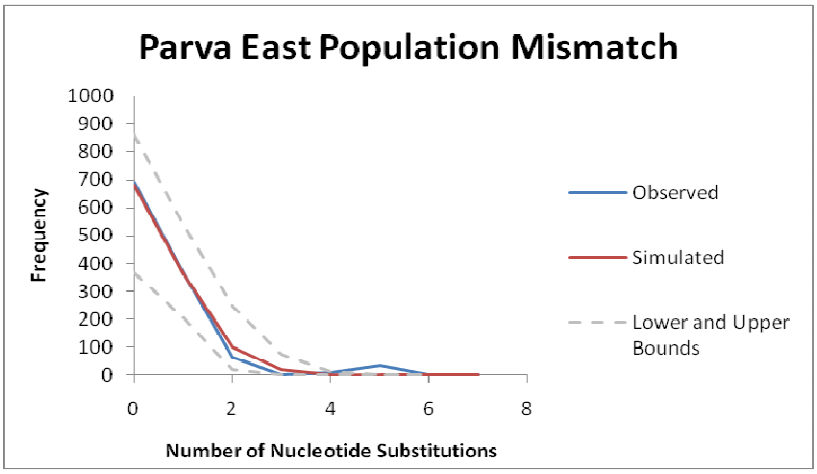

Fig. 12: Mismatch distribution frequency for the Parva East Popualtion.

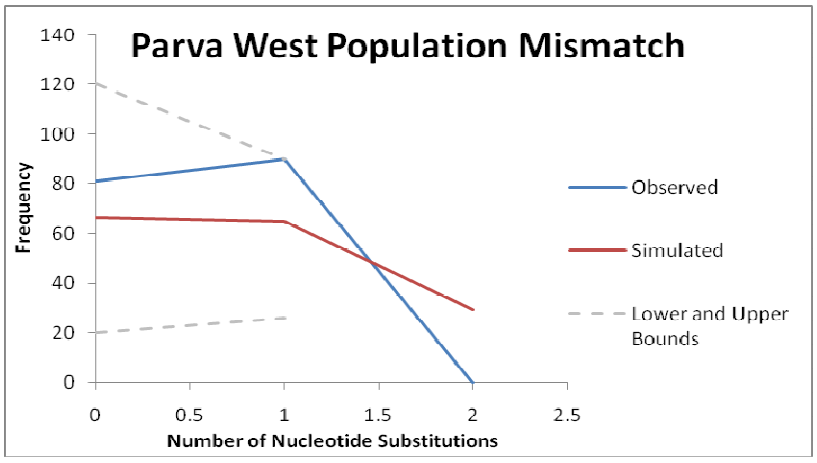

Fig. 13: Mismatch distribution frequency for the Parva West Population.

represents a single expansion that is not sampled efficiently to display a smooth peak, or represents three peaks. Also, some conflict exists in the Fu's Fs and Tajima's D values for this group. Tajima's D value for this group indicates significant contraction in the Floridana Group (0.03, $p<0.015$) while Fu's Fs strongly indicates expansion (-6.46, $p<0.0001$). It is possible that the conflict in this group may indicate additional structure within the Floridana Group.

AMOVA results supported the separation of the Floridana Group from that of the Parva Group as well as the separation of the Parva West Population from the Parva East Population. Variation among groups (Floridana Group and Parva Group) accounted for 76.35% of the total variation in the data set. The variation among populations within groups (Parva West Population and Parva East Population) accounted for 19.81% of the total variation. Only 3.83% of the variation was explained by the relationships within populations. Theta-S (1.35, 0.29) and Theta-pi (0.63, 0.53) diversity indices for the Parva East and Parva West populations, respectively, indicate slightly more diversity in Parva East. The most diversity, however, is found in the Floridana Group (Theta-S = 3.31, Theta-pi = 3.33).

When these data are compared to those from a network analysis (Fig. 14) it is clear that these expansions correlate to what was identified in the phylogeny as the Floridana Group, followed by the Parva West Population and Parva East Population. The results of the network analysis show that haplotypes connected by fewer than five nucleotide substitutions are connected with a parsimony value of >0.95. All cytochrome-b haplotypes were connected in one network. Network analysis considers the most frequent haplotype to be the oldest and is indicated by a rectangle. Haplotypes are connected by lines in the genealogy and the open circles between the lines represent missing haplotypes that are either locally extinct or unsampled in the analysis. The deepest structure in this analysis is apparent in distinguishing the Floridana Group (red circles) from other haplotypes, followed by separating the Parva West (green circles) from Parva East haplotypes (blue circles). Consistent with the mismatch distribution, the Parva East haplotypes display a fairly recent expansion with haplotypes generally one substitution removed from the most frequent haplotype. The gray circle represents the two individuals from St. Johns

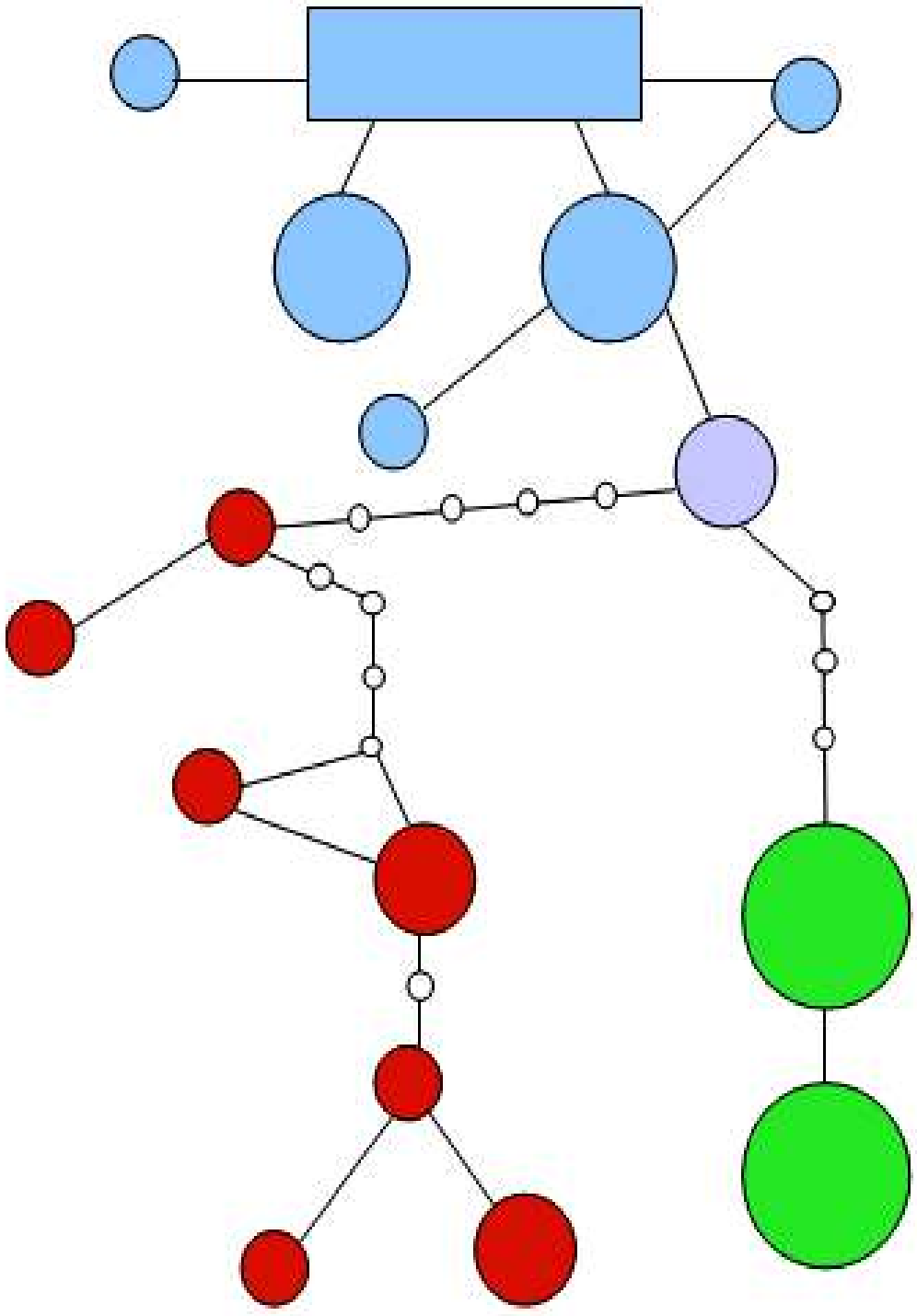

Fig. 14: Network analysis of least shrew cytochrome-b sequences. Rectangle indicates the oldest, most frequent haplotype, and sizes of circles indicate frequency of haplotype. Open circles indicate missing haplotypes. Colors correspond to clades described in Bayesian analysis: Red = Floridana Group, Green = Parva West, Blue=Parva East, Grey=Parva South.

and Taylor counties in Florida that grouped at the base of the Parva East clade in the phylogeny, but with little statistical support. In agreement with the Bayesian and ML analyses, these individuals identify more closely with the Parva East group (separated by fewer missing haplotypes). Network analyses indicate that this haplotype bridges the three clades, a pattern not observed from phylogenetic analysis.

Apolipoprotein and Cytochrome Oxidase I

The GTR model was used with base frequencies of: A=0.38, C=0.20, G=0.13, T=0.29, acquired from jModelTest, to generate a maximum likelihood gene tree (Fig. 15) for a 210 bp fragment of Apolipoprotein-B for 14 *Cryptotis parva*, one *Cryptotis magna*, one *Cryptotis goldmani*, and one *Blarina brevicauda* assigned as outgroup (Appendix II). A full heuristic search with TBR branch swapping was performed to generate the tree. A heuristic search was also used to generate bootstrap values from 1000 replicates using the likelihood criterion, and a fast-heuristic search of 1000 replicates generated the bootstrap values according to the parsimony criterion. Two individuals from Highlands County, Florida group together to the exclusion of all other *Cryptotis* with bootstrap value of 60 for both likelihood and parsimony criterion. The remainder of the *Cryptotis* sampled from the United States generated a likelihood bootstrap probability of 52; there is no reportable parsimony value. Finally, an individual from Tom Green County, Texas (Genbank) and an individual sequenced from Missouri are grouped together with likelihood and parsimony bootstrap values of 60 and 58, respectively. The only difference between the ML tree shown and one generated from MP is that the individual from Craven County, North Carolina does not fall out with the individuals from western states as it does here. Although bootstrap values are low and there is high amount of polytomy in this gene tree, the general structure seen in the cytochrome-b data is reflected in the Apolipoprotein-B data as well. The colors of the terminal labels in Fig. 15 correspond to the clades from which these individuals were found in the cytochrome-b data. The two individuals from Florida clearly represent a group of their own. Also, the same general pattern of an eastern and western clade of *Cryptotis*, with the western clade originating from the eastern clade, is seen in the nuclear data. The discrepancy of the placement of one individual from Craven County, NC could

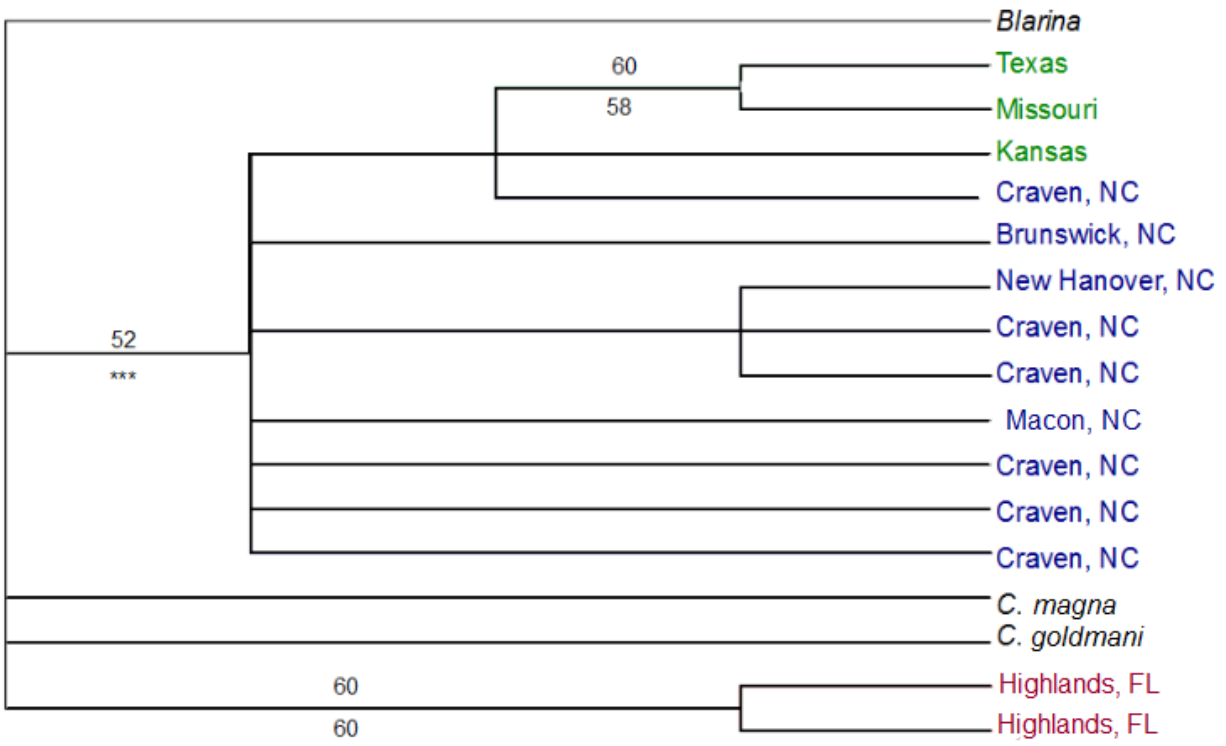

Fig 15: Maximum likelihood tree of a 210 bp fragment of ApoB. Bootstrap probabilities generated from the likelihood criterion are placed above the line and those generated from the parsimony criterion are found below the line. The colors correlate to the colored clades in the cytochrome-b gene tree.

be explained by the slower rate of evolution in the nuclear genome compared to that of mitochondrial genome or to differential lineage sorting (Avise, 2004).

The K80 (K2P) model with a gamma value of 0.07 and ti/tv=8.61 was used to create a maximum likelihood tree for a 143 bp fragment of the mitochondrial COI gene for 20 individuals of *Cryptotis parva* (Fig. 16). A full heuristic search with TBR branch swapping was performed to generate the ML tree. Also, bootstrap values were generated using fast-heuristic searches for both likelihood and parsimony criteria at 1000 replicates. Unfortunately, at the time of this publication there were no published barcode sequences for any short-tailed shrews. Furthermore, alignment was unsuccessful using the barcode sequences published for *Crocidura* and *Sorex* specimens. For that reason, the maximum likelihood tree described is midpoint rooted. Amplification of the barcode in specimens representing the Floridana Group was also unsuccessful. Nonetheless, the phylogeny and bootstrap values offer strong support for the structure of the Parva East and the Parva West Populations, although there is overlap between these groups. Most of the North Carolina specimens grouped together with bootstrap values of 98 (likelihood) and 72 (parsimony). Contrary to expectations, however, one specimen from Brunswick County does fall outside of this group, and one specimen from Wabaunsee, Kansas (which would be expected to fall within the Parva West population) falls within the Parva East population. There is no bootstrap support for the placement of the specimen from Brunswick County, however, indicating that this sample needs to be confirmed (which could not be done) or Brunswick County needs to be better sampled.

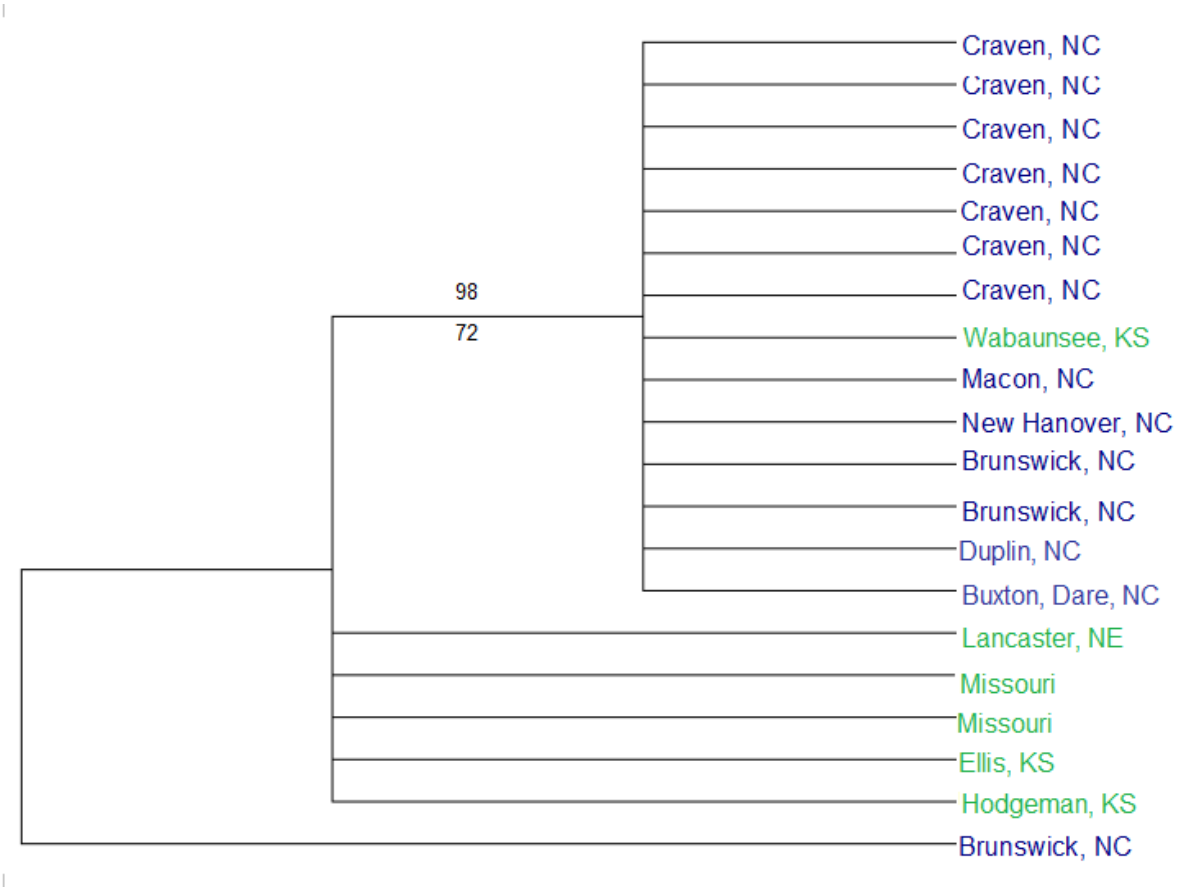

Fig. 16: Maximum likelihood tree of a 143 bp fragment of the cytochrome oxidase I of the mitochondria. Bootstrap probabilities generated from the likelihood criterion are placed above the line and those generated from the parsimony criterion are found below the line. The colors correlate to the colored clades in the cytochrome-b gene tree.

Discussion

Taxonomy and Systematics

Neither molecular nor morphological analyses support subspecific designation of *Cryptotis parva elasson* in Ohio. In the cytochrome-b phylogeny a sample from northern Ohio groups with the Floridana Group while two others, one of which is from the type locality of *C. p. elasson*, groups with the Parva group. In neither group do these individuals break off into their own clade. In fact, in the Parva Group the two samples from Ohio are not even assigned to the same population. Morphologically, all OTUs representing Ohio samples are statistically small in size when compared with samples from Florida and other coastal areas, whether they genetically fall into the Floridana Group or the Parva Group. However, for the most part they are not statistically significantly different from other *Cryptotis* in surrounding states.

The case is the same with regards to *Cryptotis parva harlani*. The individual genetically analyzed was assigned to the same clade as others from Ohio within the Parva Group. Admittedly this race was not well represented in the molecular analysis. However, in the morphological analyses *C. p. harlani* was represented by three separate areas of Indiana (OTUs 11, 12, and 54), one of which includes the type locality for the subspecies, as well as one area of Illinois (OTU 53). These results agree with those of Hoffmeister (2002) showing that specimens from Indiana are the same morphologically as specimens from Ohio. Furthermore, the null hypotheses (specimens from Ohio and Indiana are not significantly different from one another nor from other *Cryptotis parva*) could not be rejected. Therefore, there is no validity to the distinct taxonomic status traditionally given to specimens of *Cryptotis* in these regions and both should be referred to as *Cryptotis parva parva*.

Cryptotis parva berlandieri was represented in the morphological aspect of this study by two OTUs (11 and 12). Neither principal components analysis nor analysis of variance supported the subspecific designation given to these individuals, as one would expect if the designation were valid. It would appear, based on these data, that the individuals collected from these locations are actually members of *C. p. parva*. These results coincide with the findings of Raun (1965) who found there was

just as much variation in cranial characters within *C. p. parva* in Texas as between *C. p. parva* and *C. p. berlandieri*. It is possible that *C. p. berlandieri* does not rightfully exist, or that the distribution line of the subspecies actually exists somewhere further south in Mexico and that the individuals sampled are integrades between the two subspecies. Unfortunately lack of sampling within what is currently recognized as *C. p. berlandieri*, small sample size (n=1) for OTU 11, and lack of molecular data make determination of taxonomic status with any reasonable amount of certainty impossible. For this reason, the null hypothesis that the subspecies *C. p. berlandieri* is not significantly different from *C. p. parva* cannot be supported or rejected and the traditional status of this shrew should be maintained until more data become available.

Regarding populations of *Cryptotis parva* observed on the Outer Banks of North Carolina, there is morphological evidence suggesting that these individuals are large, as is demonstrated by the interpolated GLS measurements. However, so are the individuals on the mainland of Dare County, and the difference in size observed is not statistically significant. Taxonomic uniqueness of Outer Banks populations is not supported by molecular evidence either. Individuals from the Outer Banks are identical genetically to other individuals within the Parva East Population, which means that the null hypothesis that shrews from the Outer Banks and shrews from the mainland of North Carolina are not significantly different cannot be rejected.

Molecular analyses of both mitochondrial cyt-b and nuclear Apo-B data show that *Cryptotis* in Florida (but not all Florida *Cryptotis*) is unique enough to be elevated to specific taxonomic designation and be referred to as *Cryptotis floridana*. The *C. floridana* representatives form a reciprocally monophyletic clade that has long branch lengths representing genetic distances comparable to that seen between *C. goldmani* and *C. mexicana* (Fig. 9). Not only does the clade presented in the phylogenies represent individuals from Florida (Highlands and Taylor counties), but it also includes individuals from Grady, Thomas, and Charlton counties in Georgia, as well as Cheatham County, Tennessee, and Portage County, Ohio. This suggests that this species once had a much larger distribution that has since been fragmented, perhaps in response to competition from *C. parva*. What also becomes clear from the

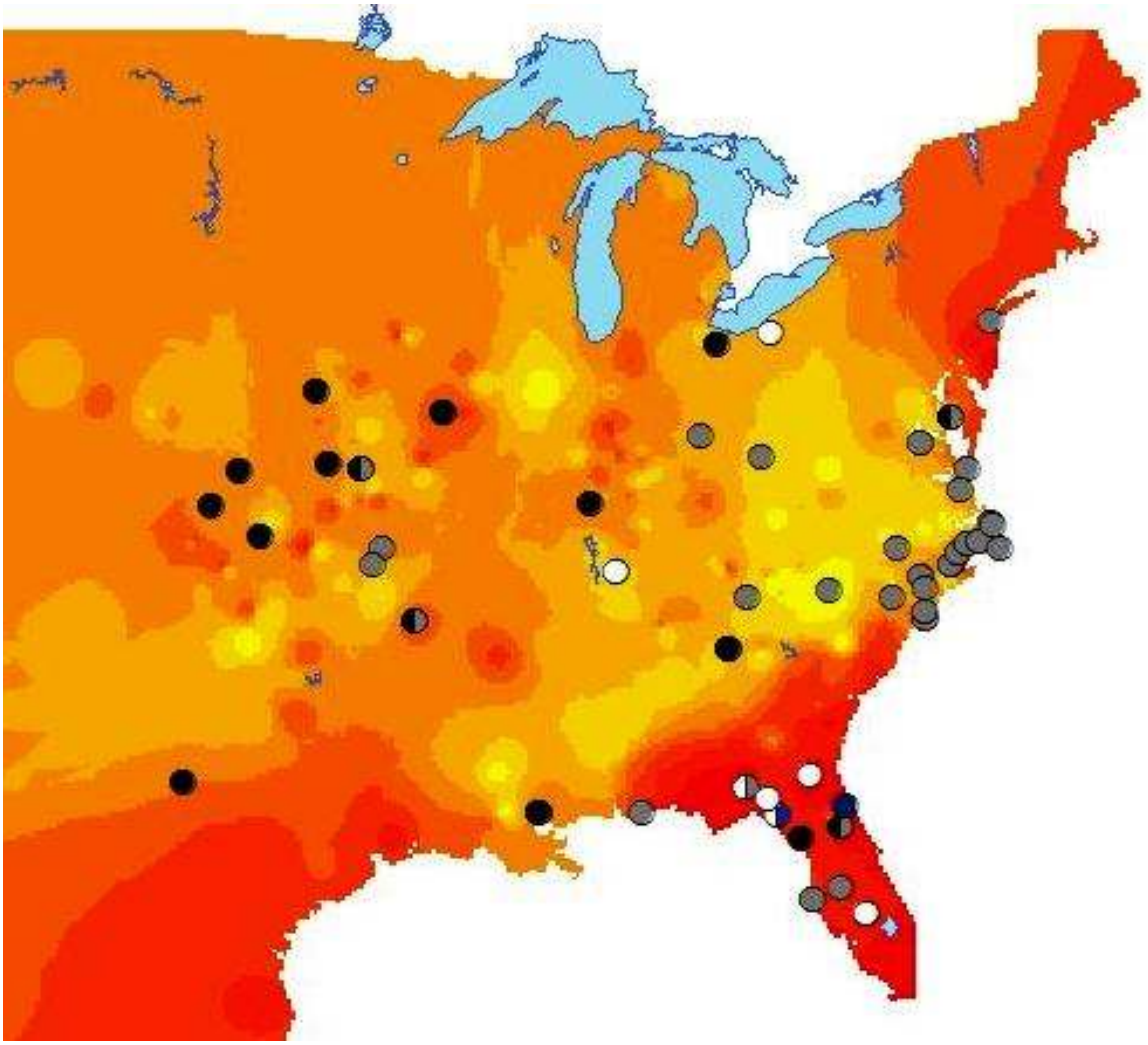

Fig. 17: Geographic distribution of molecular sequences overlaid onto the interpolated GLS sizes. Black=*C. parva* west, Gray=*C. parva* east, Blue=*C. parva* (undetermined clade, Parva South), White=*C. floridana*.

molecular data is that both species (*C. floridana* and *C. parva*, and both east and west Parva Populations) occupy Florida (Fig. 17). Additionally, both species were found in Grady County, Georgia and Taylor County, Florida.

Revised Distribution

Cryptotis parva appears to contain only two subspecies. The range of *C. p. berlandieri* was not altered in this study, pending molecular analyses of the group. *C. p. parva*, on the other hand, has a range that spans the majority of the United States from mid-Texas, northern New Mexico (Owen and Hamilton, 1986), mid-eastern Colorado (Siemer et al., 2006), and southern Wyoming (Marquardt et al., 2006) east to the Atlantic Coast, and from southern Texas, Georgia, and Florida north to South Dakota, Michigan, and New England (Fig. 18). Apparently, however, the species has been locally extirpated from Michigan and northern Ohio (Philip Myers, personal communication). The youngest specimen examined for this study from Michigan or northern Ohio was collected in 1957. Therefore, without more rigorous sampling, the exact bounds of the northern limits of *Cryptotis parva* are unknown. Two clades within this species emerged in the analysis of two mitochondrial markers (cyt-b and COI), one nuclear marker (Apo-B), and analyses of population genetics (Fig. 17). The Parva West clade consists mostly of samples from states west of the Mississippi while the Parva East clade consists mostly of samples east of the Appalachian Mountains. These two clades appear to have a large zone of intergradation between these two landmarks. The pattern of genetic differentiation marked by the Mississippi River and the Appalachian Mountains can be seen in other short-tailed shrews of the genus *Blarina* (Brant and Ortí, 2002; Brant and Ortí, 2003). *Neotoma floridana* also was found by Hayes and Harrison (1992) to be a species made up of a western phylogroup and a northeastern phylogroup.

Cryptotis floridana most likely occupies southern Florida and parts of southern Georgia including Grady, Thomas, and Charlton counties. It is uncertain if these two populations are connected along the eastern coast of Florida or have been completely isolated. Isolated populations of *C. floridana* are also present in Cheatham Counties, Tennessee and Portage County, Ohio. The specimen from Portage County

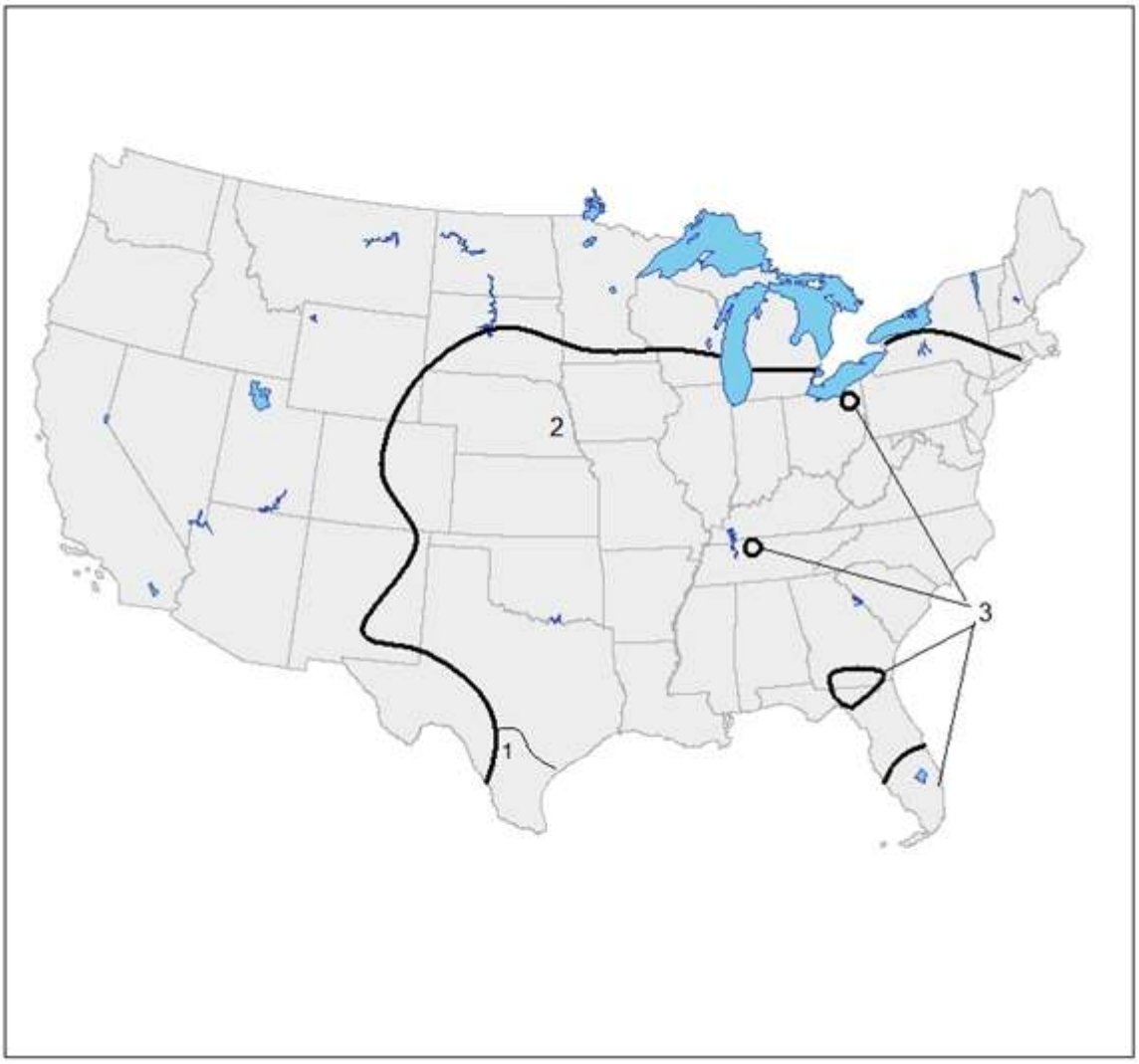

Fig. 18: Revised distribution of *Cryptotis parva* and *Cryptotis floridana* in the United States. Distributions are derived from the specimen localities resulting from an exhaustive search of museum databases and recent literature. 1=*C. p. berlandieri*, 2=*C. p. parva*, and 3=*C. floridana*. Mapping coordinate system is GCS_North_American_1983.

was collected in 1940 so, again, it is uncertain if this population persists or has been extirpated. The revised distributions described are subject to change with more intense sampling in priority areas.

Phenotypic Plasticity and Bergmann's Rule

One reason that the distinction between species within Florida has gone unobserved for so long could be explained by the concept of phenotypic plasticity. The morphological data from multiple statistical tests suggest specimens retain moderate sizes in the majority of the genus' range in the United States, but are large in Florida and near the Atlantic Coast, regardless of which species, or clade within species (in the case of *Cryptotis parva*) to which they belong. This trend does not appear to be a clinal increase in size from west to east or north to south, but rather an abrupt increase. Both races of *C. parva* are found in Florida, and, both races are larger in Florida than in the remainder of the range. This pattern occurs with specimens of *Cryptotis floridana* as well. Those that are found in Florida are large in size, and those found in isolated pockets of Tennessee (corresponds to OTU 16) and Ohio (OTU 27) are small. This explains the incongruence between this study and past studies that have been based purely on phenotypic characters. Cases of phenotypic plasticity demonstrate the importance of using molecular data in conjunction with morphological data in describing species and assessing evolutionary classifications. Phenotypic characters can often evolve independently in response to environmental factors, which has important ecological implications but can confuse phylogenetic relationships (Avise, 2004).

Cryptotis floridana could represent a cryptic species or a sibling species. Molecular techniques have been very effective in identifying similar cases of cryptic structure in both invertebrates and vertebrates (Avise, 2000; Peppers and Bradly, 2000; Herbert et al., 2004; Olson et al., 2004; Stuart et al., 2006), including in other genera as species of shrews (Basset et al., 2006; Dubey et al., 2007). It is possible, however, that this is not a cryptic species, but that there is a morphological difference between the two species that has remained unnoticed. Woodman et al. (2003) and Woodman and Morgan (2005) have proposed morphological differences in the osteology of the humerus and forefeet, respectively, in species of *Cryptotis*. Furthermore, the difference between the two species could be due to habitat

partitioning or different ecological requirements. Specimens of *Cryptotis* that are caught in the wild are usually associated with primary successional habitats such as marshes, meadows, fields, and prairies, but they have also been collected from mature sand pine scrubs and mesic flatwoods. Kale (1972) reports a series of almost 200 *Cryptotis* caught in a mature oak forest in Indian River, Florida (close to the type locality of *C. floridana*) during a small mammal census studying the relationships of mosquitoes and their hosts.

Bergmann's rule states that animals in colder climates and higher latitudes will generally have larger body sizes than those of the same species in warmer climates in order to maintain normal body temperatures. The data collected in this study suggest that *Cryptotis parva* and *Cryptotis floridana* are exceptions to this rule. Correlation analyses indicate that size negatively correlated with latitude, and that the organisms are largest in Florida and other coastal locations. Ashton et al. (2000) performed a meta-analysis of body length data available for 110 species of mammals. Although most of the studies provided support for Bergmann's rule, some exceptions were observed. The kangaroo rat (*Dipodomys*) did not conform to Bergmann's rule and it was suggested by Best (1981) that seasonality, not temperature, affected body size. Voles also did not conform to the rule, presumably because of a negative relationship of temperature and food availability. Finally, two species of weasels were found to conflict with the rule, possibly because of predator/prey interactions with voles (Ashton et al., 2000). Meiri and Dayan (2003) performed another review including 149 mammalian species. They found that while Bergmann's rule can be used as a generalized pattern in ecology, it is sensitive to mass measurements rather than linear measurements. In their study they found that animals in the smallest weight class (4-50g), which included insectivores and rodents, there was no observable validity in the rule. Freckleton et al. (2003) extended the original dataset of Ashton et al. to include body mass and came to the same conclusions as Meiri and Dayan (2003) that smaller mammals are less affected by Bergmann's rule than larger mammals.

Biogeography

Molecular dating of this data indicates that the split between *Blarina* and *Cryptotis* happened 13.6 MYA. The isolation and subsequent speciation of *Cryptotis parva* and *Cryptotis floridana* (6.1 MYA) coincides with other lines of evidence suggesting that speciation is often a pre-Pleistocene phenomenon for many taxa (Zink and Slowinski, 1995; Demboski and Cook, 2001; Avise et al., 2009). An increase in number of haplotypes around 0.7-0.1 MYA indicates an expansion event suggesting that there was an out-of-Florida dispersal trend after a period of isolation and subsequent speciation.

All haplotypes within the eastern and western lineages of *Cryptotis parva* are similar, indicating a more recent expansion event in these groups. The two radiations of *C. parva* suggest two source populations which became isolated about 1.7 MYA towards the beginning of the latest glacial event. Brant and Ortí (2003) noticed a similar pattern in specimens of *Blarina brevicauda* and postulated that the increased water levels of the Mississippi River during interglacial periods would have prevented easy dispersal of eastern and western isolates across the Mississippi River Valley. Most of the radiation within the two lineages of *Cryptotis parva* is concentrated between 1 MYA and 0.2 MYA (mid-late Pleistocene) and is marked by high haplotype diversity and low nucleotide divergence, which is consistent with structuring formed by fluctuating glaciations events. The eastern clade began moving west and south out of a refugia that may have existed somewhere on the East Coast while the western clade began moving east and south from a refugia possibly existing in the Southwestern United States (Estill and Cruzan, 2001; Sorrie and Weakley 2001). Exact locations of glacial refugia are uncertain, but studies hypothesize a southwestern refugia having occurred somewhere around northern Texas, Oklahoma, and southern Kansas (Jones et al., 1984). At the same time that the two lineages of *Cryptotis parva* were moving towards the interior of the country, *Cryptotis floridana* was dispersing from Florida and as the two lineages of *Cryptotis parva* began to converge, the competition pressure may have suppressed *C. floridana* into isolated pockets.

CONCLUSIONS

Conservation

Arguably, the two lineages of *Cryptotis parva* may fall into the definition of management unit (MU) given by Moritz (1994) because of the significant divergence between the two clades, however, all indications produced by population level analyses suggest that both clades within *Cryptotis parva* are expanding and maintaining genetic diversity. Furthermore, evidence suggests that this species has a large geographic distribution that is expanding in the northwest of its range. Therefore, it is more prudent to focus conservation efforts on its sister species, *Cryptotis floridana*.

Even though some molecular analyses such as the mismatch distribution suggest that *Cryptotis floridana*, as a population, is historically expanding, it is clear that the species is currently undergoing severe habitat fragmentation and isolation, specifically in northern Ohio and Tennessee. It is possible that despite the habitat fragmentation, *C. floridana* so far has managed to maintain genetic diversity within the populations, possibly due to periods of water level decline during glaciations cycles that may have allowed more connectivity. Additionally, the species represents an evolutionarily significant unit that is reciprocally monophyletic to *C. parva* in mitochondrial and possibly nuclear genes (Moritz, 1994), and for this reason the species merits conservation. Further study of the isolated pockets of this species may yield information on what environmental variables allow this species to thrive in these areas. That information can then be used to model where other pockets may exist, and determine if there is a possibility of other relict populations in other parts of the United States. The data obtained from such a modeling study could then be applied to ecosystem based approaches of the conservation of *Cryptotis floridana*. If this management technique is applied to Florida, for example, the unique ecology that allows two species of *Cryptotis* to coexist would be focused on in its entirety which would positively benefit other Florida biota. Several species have been found to have unique races that exist only in Florida (Soltis, 2006; Avise et al., 2009) as evidenced by a typical zone of hybridization or intergredation along the midsection of the panhandle. Prioritizing conservation of the entire Florida ecology would serve to conserve two species and three genetic clades of least shrews.

Future Focus

The inconclusive placement of the two individuals from St. Johns and Taylor counties in Florida may be the result of inadequate sampling in this region. Sampling efforts in the future should be prioritized to areas between these locations and well established haplotypes. It is also necessary to focus sampling efforts to areas in and around Portage County, Ohio, and Cheatham County, Tennessee, in order to quantify the frequency and distribution of *Cryptotis floridana* in these areas.

Furthermore, sampling efforts need to be prioritized in southern Georgia and Florida. Specifically, there is potentially a second population in Florida as evidenced by the strong support for the large split between individuals from Charlton and Thomas counties in Georgia from the remainder of the *C. floridana*. Furthermore, the branch lengths between these potential *C. floridana* populations is comparable to that seen separating the *C. parva* east and *C. parva* west clades. More individuals from this area are needed to confirm this relationship and to further investigate the dynamics within that population.

Because much of the starting material for molecular analyses was highly degraded and in low concentrations, only a small fragment of each marker was amplified. It is possible that if the entire mitochondrial and/or nuclear genomes were sequenced, the phylogenetic signal within the genus would be improved. With sampling more clearly directed, resources can be used to acquire fresh tissue that will permit the amplification of larger gene fragments.

Finally, it is important to expand the search of morphological characters that might reflect the genetic differences observed between *C. parva* and *C. floridana* as well as between the two genetic clades within *C. parva*. Additionally, it would be useful to test the extent of phenotypic plasticity observed in cranial characters of the least shrew experimentally.

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Appendix I

Canada

Ontario (n=2). *Norfolk County*: Longpoint (1 ROM, 1 USNM).

United States

Arkansas (n=11). *Benton County*: 9 mi N Bentonville (1 KU). *Desha County*: White River National Wildlife Refuge (1 NMNH). *Logan County*: 11 mi W New Blaine, jct SR 197 & SR 22 (2 CU). *Ouachita County*: Camden (1 NMNH). *Washington County*: 3 mi SE Winslow (1 KU); 1.5 mi S Winslow (5 KU).

Alabama (n=22). *Clarke County*: Jackson (1 NMNH). *Clay County*: Talladega National Forest (1 CU). *Dekalb County*: Mentone, Lookout Mountain (2 AMNH). *Jackson County*: Hogwood Ridge, 1.4 mi S Tennessee border (1 NCSM); 0.5 mi N Money Hollow, 0.75 mi E Rt 79, Jack Gap (4 NCSM); Woodville (1 NMNH). *Lee County*: Auburn (1 KU). *Marshall County*: Cane Creek, 6 mi N Oleander (1 NMNH). *Mobile County*: Alabama Port (1 NMNH). *Montgomery County*: Barachias (7 NMNH). *Talapaloosa County*: HOBE, Horseshoe Bend (1 NMNH). *County Unknown*: Sand Mountain (1 NMNH).

Colorado (n=20). *Larimer County*: 1.8 mi N, 0.5 mi E Fort Collins, Willox Lane (15 MSB). *Kit Carson County*: 4 mi E Flagler (1 KU). *Morgan County*: 2 mi N, 2.5 mi W Fort Morgan (2 KU). *Yuma County*: 1 mi E Laird (2 KU).

Connecticut (n=1). *Middlesex County*: Westbrook Salt Marsh (1 AMNH).

Delaware (n=3). *New Castle County*: Delaware City (2 NMNH); 1.5 mi N Odessa (1 NMNH).

Florida (n=185). *Alachua County*: South Gainesville, Paynes Prairie, Hailes Landing (3 UMMZ); 6 mi E Gainesville (4 UMMZ); 5 mi NW Hawthorn (3UMMZ); La Crosse (3 NMNH). *Baker County*: Glen St Mary (4 NMNH); Osceola National Forest, Junction 1-10, FSR 263 (1 FLMNH). *Bradford County*: exact location unknown (2 FLMNH). *Brevard County*: Canaveral (1 NMNH); Floridana Beach (1 NCSM); Georgiana (1 NMNH); Grant (1 ROM); Oak Lodge (2 MCZ); Oak Lodge, E

Penn Opp Micco (12 MCZ). *Charlotte County*: Englewood (3 FMNH). *Colombia County*: Lake City (1 NMNH); Osceola National Forest (1 FLMNH). *Dade County*: Hialeah (1 KU); WSW Kendall (1 KU); 4 mi W Kendall (1 KU); Miami, 108 St (3 KU). *Dixie County*: Oldtown (1 NMNH). *Duval County*: TIMU, Thomas Creek (1 UNCW). *Escambia County*: Pensacola (1 NMNH). *Franklin County*: 8 mi SSE Panacea, Alligator Point (2 NMNH). *Hardee County*: 4 mi N, 4 mi E Duetee (1 KU); 0.5 mi SE Fort Green Springs (1 KU); 1 mi S Fort Green Springs (1 KU); 2 mi N, 1 mi E Fort Green Springs (1 KU). *Highlands County*: Archbold Biological Station (1 FMNH, 1 FLMNH); Archbold Biological Station, 8 mi S Lack Placid (8 FLMNH); 8 mi E Archbold Biological Station, Buck Island Ranch (3 FMNH); Avon Park (3 NCSM); Gould Unit (1 FLMNH); Hicoria, Archbold Biological Station (1 FMNH); Highlands Hammock State Park (1 FMNH); 4 mi N Lake Placid, near Lake Francis (9 FLMNH); Royce Unit (2 FLMNH); SR 70, 7.5 mi Hwy 27 (1 FMNH); Exact location unknown (1 FMNH). *Hillsborough County*: 2 mi N, 2 mi E Fort Lonesome (1 KU); 1 mi N Picnic (1 KU); 1 mi N, 4 mi W Picnic (1 KU). *Lake County*: Clermont, 0.5 mi NW Citrus Tower on US 27 (1 FLMNH). *Leon County*: AKE Iamonia, Tall Timbers Inc. (1 GMNH); 3 km W Tallahassee (1 LSU). *Levy County*: 18 mi SW Otter Creek (1 FLMNH); salt marsh on Wacosassa Bay (1 FLMNH). *Madison County*: Ellaville, vicinity of Ellaville (1 FLMNH); Madison (5 NMNH). *Marion County*: McIntosh (1 NMNH); Ocala National Forest (1 GMNH). *Monroe County*: Key Largo (2 KU). *Pinellas* County: exact location unknown (3 FLMNH). *Polk County*: Arbuckle State Forest (1 FMNH); 6 mi SW Bartow (2 KU); 6 mi S, 1 mi W Bartow (1 KU); 0.5 mi S, 2 mi W Brewster (1 KU); 1.5 mi S, 3 mi E Brewster (1 KU); 2 mi S Brewster (1 KU); Flaming Arrow Boy Scout Camp (1 FMNH); 1 mi S, 7 mi W Fort Meade (1 KU); 15 mi SW Fort Meade (1 KU); Lakeland (1 NCSM); Lakeland, Orange Park Study Area (1 NCSM); Orange Park (1 NCSM). *Putnam County*: 3 mi E Melrose, Smith Lake Sandhill, Ordway Preserve (3 FLMNH); Ordway Preserve (9 FLMNH); Rodman (6 FLMNH); Welaka (2 FLMNH); Welaka Preserve (1 FLMNH); 1 mi S Welaka, University of Florida Conservation Reserve (5 UMMZ). *Santa Rosa County*: Girl Scout on S Shore of Pensacola (1 FMNH). *St Johns County*: south end of Anastasia Island, 15 mi S St Augustine (1 AMNH); Carterville (3 MCZ); Summer Haven (2 MCZ); Tolomata River, St Johns Rd

158 (1 FLMNH). *Taylor County*: 4 mi NNW Perry (2 AMNH, 1 KU); 4 mi NNW Perry, Blanham Farm (1 AMNH); 4 mi SW Perry (1 AMNH, 1 KU); 5 mi N Salem, Hwy 19/98 (1 UNCW). *Volusia*: Enterprise (1 FMNH). *Wakulla County*: St Marks National Wildlife Refuge (1 FMNH, 2 NMNH); St Marks National Wildlife Refuge, Panacea Unit (4 NMNH); St Marks National Wildlife Refuge, St Marks Unit (9 NMNH). *Walton County*: 3 mi W Freeport on Hwy 20 (1 FLMNH). *Washington County*: Chipley (1 KU). *County Unknown*: exact location unknown (1 FMNH).

Georgia (n=123). *Appling County*: 3 mi E Baxley (1 GMNH). *Berrian County*: 11 km E Nashville (1 NMNH). *Bulloch County*: Statesboro city limits (1 KU); 3 mi N Statesboro (2 KU); 5 mi N Statesboro (2 KU). *Ben Hill County*: Fitzgerald (2 GMNH). *Bryan County*: Fort Stewart, Hwy 144, 4 km W Hwy 67 jct (1 GMNH). *Burke County*: Di-Lane Plantation Wildlife Management Area (3 GMNH). *Camden County*: St Mary's (2 MCZ). *Charlton County*: Cowhouse Island, TT-14 Rd, Union Camp Land, Crew's Circle (1 GMNH); Okefenokee Swamp, Billy's Island (2 CU); Okefinokee Swamp, Race Pond, King's Canal (1 CU); 2 mi S Race Pond (1 AMNH). *Chatham County*: Skiddaway Island (1 MCZ). *Clarke County*: Athens (11 GMNH, 1 KU); Athens, College Station Road, Horseshoe Bend (1 GMNH); Athens, across river from UGA poultry (1 GMNH); Athens, Alps Rd field (1 GMNH); Athens, River Rd dump (1 GMNH); Athens, near Lake Kirota (2 GMNH); 1 mi SE Athens (3 GMNH); I-13 (1 GMNH); Sandy Creek (1 GMNH). *Cobb County*: Kennesaw Mountain (2 UNCW). *Dekalb County*: Atlanta, Panthersville Road, Georgia State University Property (1 GMNH); Clarkston, field at intersection of Stone Mt Freeway and Montreal Rd (2 GMNH). *Emanuel County*: Blundale, 14 km S Wadley, off FA Hwys 1 & 4 (1 GMNH). *Grady County*: Beachton (3 FLMNH, 5 NMNH); Beachton, Birdsong Plantation (4 NMNH); 4 mi S Beachton, Birdsong Plantation (5 NMNH); Beachton, Sherwood Plantation (11 NMNH). *Greene County*: Greensboro (1 GMNH). *Gwinnett County*: CHAT, Abbott's Bridge (2 UNCW). *Liberty County*: 0.7 mi S, 0.3 mi W of Canoochee River & Hwy 119 intersection (2 GMNH); Ft Stewart (4 KU); Riceboro, LeConte Plantation (4 NMNH); exact location unknown (1 NMNH). *McIntosh County*: Sapelo Island, various sites (7 GMNH). *Muscogee County*: Ft Benning, array 5 (3 GMNH). *Montgomery County*: Chatham (1 UMMZ); exact location unknown (8 MCZ). *Olgethorpe*

County: 10 mi SW Crawford (6 GMNH); Colbert, Angus Farm (1 GMNH). *Tift County*: 2 km E Tifton (2 GMNH). *Thomas County*: Boston (1 NMNH); Metcalf (1 FLMNH); Thomasville, Spring Hill (1 UMMZ).

Iowa (n=25). *Boone County*: Polly Creek (2 NMNH). *Decatur County*: Leon (2 NMNH). *Fremont County*: 3 mi S, 9 mi W Sidney (1 KU). *Lee County*: Montrase (1 NMNH); Washington (2 NMNH); Jefferson (3 NMNH). *Montgomery County*: Red Oak (5 NMNH). *Story County*: Ames (1 NMNH). *Cass County*: Atlantic (1 NMNH). *Des Moine County*: 2 mi W, 1 mi N West Burlington (1 KU). *Keokuk County*: Sigourney (1 KU). *Mahaska County*: 3.5 W Oskaloosa (3 KU). *Monona County*: 0.5 mi S, 2 mi W Moorhead (1 KU). *Van Buren County*: Lasey, Keosagua State Park (1 NMNH).

Illinois (n=37). *Alexander County*: Cache (1 FLMNH). *Champaign County*: SW Champaign (1 INHS); Fisher (1 INHS); Mayview (1 INHS); Phillips Tract (1 INHS); Seymour (2 INHS); Trelease Prairie (2 INHS); Trelease Woods (1 INHS); Urbana (6 INHS); 1.5 mi SW University of Illinois (1 INHS); 1.25 mi N Urbana (1 INHS); 2 mi S Urbana (1 INHS); 2.5 mi S Urbana (2 INHS); 3 mi E, 1 mi N Urbana (6 INHS); 4 mi E Urbana (1 INHS); 4 mi E Urbana, Mayview Prarie (1 INHS); 5 mi NE Urbana (3 INHS). *Dekalb County*: Cortland (1 NMNH). *Jasper County*: 7 mi SW Newton (1 MSB). *McClean County*: Bloomington, Archibuteo (1 NMNH). *Piatt County*: 5 mi W, 2.5 mi S Monticello Allerton Park (1 FLMNH). *Wabash County*: exact location unknown (1 NMNH).

Indiana (n=88). *Allen County*: 0.25 mi N New Haven (1 NMNH). *Benton County*: 8 mi N Harold, Otterbein (1 NMNH). *Clay County*: 1 mi W Brazil (2 NMNH). *Crawford County*: Wyandotte (1 NMNH). *Daviess County*: 4 mi E Odon (1 NMNH). *Dearborn*: Bright (1 NMNH). *Floyd County*: 6 mi N New Albany on Rt 111 (1 NMNH); 1 mi S St Joseph (1 NMNH). *Fountain County*: Attica (1 NMNH); 2 mi SE Wallace (1 INHS). *Franklin County*: Blooming Grove (1 NMNH). *Fulton County*: 0.5 mi W Rochester (1 NMNH). *Gibson County*: 1 mi S Patoka (1 NMNH). *Jackson County*: Kurtz (1 FMNH). *Jenmings County*: Crasley Fish and Game Area (1 NMNH). *Johnson County*: 0.25 mi W Edinburg (1 NMNH). *Knox County*: Bicknell (1 NMNH). *Marion County*: exact location unknown (1 NMNH). *Martin County*: Crane (1 NMNH); Crane, Naval Ammunition Depot (1 ROM). *Monroe*

County: 2 mi SE Bloomington (1 UMMZ). *Montgomery*: 3 mi S Waynetown (1 NMNH). *Orange County*: exact location unknown (1 NMNH). *Owen County*: 3 mi E Spencer, entrance to McCormic Creek State park (2 INHS). *Perry County*: Cannelton (1 NMNH). *Pike County*: 2 mi SE Coe, Purdue-Enos Study Area (1 NMNH); 2 mi N Spurgeon, Purdue-Enos Study Area (9 NMNH). *Porter County*: Exact location unknown (1 NMNH). *Posey County*: Hovey Lake (7 UMMZ); W Hovey Lake (1 UMMZ); exact location unknown (1 UMMZ). *Putnam County*: 0.5 mi W Pleasant Gardens (1 NMNH). *Ripley County*: Rexville (5 NMNH); 0.5 mi E Rexville (4 NMNH); 0.5 mi E, 0.25 mi S Rexville (1 NMNH). *Sullivan County*: exact location unknown (1 UMMZ). *Tippecanoe County*: west Lafayette (5 NMNH); 10 mi W Lafayette (7 NMNH); exact location unknown (3 NMNH). *Vigo County*: Terra Haute (1 NMNH); 1 mi S Terre Haute (3 INHS); 6 mi W Terre Haute (1 INHS). *Warrick County*: 5 mi N Newburgh (5 NMNH). *Washington County*: 3 mi N Smedley (4 NMNH).

Kansas (n=103). *Anderson County*: 1 mi N, 1.5 mi E Colony (1 KU); 3.7 mi S Garnett (1 KU); 2 mi S, 0.5 mi W Welda (1 KU). *Atchison County*: 1.5 mi S Muscotah (1 KU). *Barber County*: Plum Thicket, Sharon (1 KU); 3 mi N, 1 mi E Sharon (1 KU); 5 mi N, 0.5 mi E Sharon (1 KU); 5 mi N, 1.5 mi E Sharon (5 KU). *Butler County*: 1 mi N Towanda (1 KU). *Cherokee County*: 1 mi S Baxter Springs (1 KU); 2 mi N Baxter Springs (1 KU); 2 mi N, 0.5 mi W Baxter Springs (2 KU); 0.33 mi N, 6 mi E Baxter Springs (1 KU); 2 mi S, 1 mi E Galena (1 KU). *Coffey County*: 2.5 mi S Burlington (1 KU). *Cowley County*: 8.1 mi E Arkansas City (1 KU); 2 mi S, 0.5 mi W Udall (1 KU). *Doniphan County*: Geary (2 KU); 0.2 mi N Troy (1 KU). *Douglas County*: Lawrence (1 AMNH, 6 CU, 2 INHS, 1 UMMZ, 2 NMNH); 1 mi W Lawrence (3 MSB); 2 mi SW Lawrence (1 ROM); 1 mi E, 2 mi S Lawrence (1 FLMNH); 1 mi S, 8 mi W Lawrence (5 NMNH); 5 mi N Lawrence (4 NMNH); University of Kansas, Lawrence, Prairie Acre (1 FLMNH); exact location unknown (1 UMMZ). *Ellis County*: 11.25 mi N, 0.5 mi E Victoria (1 KK). *Greenwood County*: 0.25 mi E Hamilton (4 UMMZ). *Hodgeman County*: 0.5 mi S, 5 mi E Kalvesta (1 KK). *Jefferson County*: 14 km NE Lawrence (1 NMNH). *Kingman County*: quail farm (2 KU). *Leavenworth County*: Tom Searingen's House (4 KU). *Linn County*: 6 mi N Pleasanton (2 INHS). *Lyon County*: Emporia Bluestem Prairie Meadow (1 UMMZ). *Meade County*: 1.5 mi N

Fowler (9 KU); Meade State Park (1 INHS); Meade County State Lake, Old CCC barracks (6 KU); 1 mi SW Meade (1 KU); 8 mi S, 6 mi W Meade (1 KU); 17 mi S Meade (6 KU); 17 mi SW Meade (1 KU, 1 UMMZ). *Riley County*: exact location unknown (4 UMMZ). *Wabaunsee* County: 3 mi S, 3.7 mi E Alma (1 KK). *County Unknown*: eastern Kansas (2 KU); 1.5 mi SW Hayes (1 NMNH).

Kentucky (n=23). *Crittenden County*: Floodplain of Tradewater river, 13 air km W SR 120 and river (1 NMNH). *Edmonson County*: 1 mi S Brownsville (1 NMNH); 11 mi S Brownsville (1 NMNH). *Franklin County*: 2.2 km S Peaks Mill Rd & Holt Dr (1 NMNH). *Hopkins County*: 9 mi NE Madisonville (2 NMNH). *Kent County*: Trigg, 8 mi NNE Golden Pond (1 UMMZ). *Logan County*: 1.3 km W jct. SR 106 & US 431 (3 NMNH). *Meade County*: 2 mi SE Bradenburg Station (2 NMNH). *Ohio County*: 2 mi NE Hartford (1 NMNH). *Rockcastle County*: 6 mi S Berea (2 NMNH). *Trigg County*: Canton (8 NMNH).

Louisiana (n=42). *Acadia Parish*: Mermentau (12 FMNH). *Caldwell Parish*: Columbia (5 FMNH). *East Baton Rouge Parish*: Exact location unknown (1 UMMZ). *Saint Landry Parish*: 10 km S Opelousas (1 LSU). *Saint Martin Parish*: Butte La Rose (2 LSU); 8 km N Catahoula on outer W levee (1 LSU); Henderson (3 LSU); 1 km N Henderson (1 LSU); 2 km S Henderson, along levee (2 LSU); 6 km N Henderson on the levee (2 LSU); 14 km S Henderson (1 LSU); 2 km W Saint Martinville (8 LSU). *Washington Parish*: Hackley (3 FMNH).

Maryland (n=68). *Accomack County*: Chincoteague Island (1 NMNH). *Allegheny County*: 9 mi S Old Town (1 NMNH). *Anne Arundel County*: Annapolis, 4 mi W Broom Sage (1 NMNH); 2 mi N Annapolis (1 NMNH). *Baltimore County*: Baltimore (1 NCSM); Loch Raven Reservoir, east of Dulaney Valley Road (1 NMNH). *Calvert County*: Solomons (1 NMNH); 0.75 mi N Solomon Island (7 NMNH). *Charles County*: Port Tobacco (1 NMNH). *Dorchester County*: Cambridge (5 KU, 4 MCZ, 2 ROM, 3 UMMZ); Cambridge, Blackwater Refuge (1 NMNH). *Hartford County*: exact location unknown (1 NMNH). *Montgomery County*: Bethesda (1 NMNH). *Prince George's County*: Laurel (3 NMNH); Patuxent Research Refuge (6 NMNH); Oxon Hill (2 NMNH). *Queen Anne's County*: Parsons Island (1 NMNH). *Somerset County*: Irish Grove (1 NCSM). *Worcester County*: Assateague National Seashore

(6 KU); between Snow Hill & Assateague (1 NCSM); Chincoteague Bay (1 NMNH); Mills Island (4 NMNH); 4 mi S Ocean City, Assateague Island (2 NMNH); 5 mi S Ocean City, Assateague Island (5 NMNH); 15 mi S Ocean City, Assateague Island (1 NMNH); Pocomoke Cypress Swamp (1 NMNH); 5 mi SE Snow Hill, Chincoteague Bay (2 NMNH).

Michigan (n=37). *Lenawee County*: Adrian (1 NMNH). *Washtenaw County*: Ann Arbor (7 UMMZ); 5.5 mi SW Ann Arbor (1 UMMZ); 6 mi ENE Ann Arbor (6 UMMZ); Pittsfield (2 UMMZ); Portage Lake (2 UMMZ); 1 mi W Superior Twp (1 UMMZ); Whitmore Lake (16 UMMZ); Ypsilanti, Michigan State Normal (1 UMMZ).

Mississippi (n=15). *Adams County*: Washington (2 NMNH). *Kalamazoo County*: Vicksburg (4 NMNH). *Marshall County*: Wall Doxey State Park (9 KU).

Missouri (n=33). *Adair County*: 5 mi S Kirksville (2 NMNH); 5 mi N, 2 mi W Kirksville (11 FMNH); 6 mi N Kirksville (1 INHS). *Clay County*: Greenfield Village, NHC (1 KU). *Jackson County*: 4 mi NE Independence (1 KU); exact location unknown (1 UNCW). *Lewis County*: 8 mi N Lewiston, Deer Ridge Wildlife Area (3 KU). *Macon County*: 5 mi N, 1 mi W Macon, Atlanta Wildlife Area (1 KU); 8.5 mi N Macon, Atlanta Wildlife Area (1 KU). *Nodaway County*: 5 mi ENE Maryville (2 NMNH). *Pettis County*: Sedalia (1 NMNH); 4 mi NW Sedalia (1 KU). *Saline County*: Marshal (1 UMMZ). *Saint Charles County*: Portage des Sioux (3 NMNH). *County Unknown*: Exact location unknown (3 UNCW).

Nebraska (n=38). *Cass County*: 0.3 mi S, 2 mi W Weeping Water (1 KU); 0.4 mi N, 2 mi W Weeping Water (7 KU); 1 mi N, 2 mi W Weeping Water (2 KU); 2 mi N, 2 mi W Weeping Water (1 KU); 3 mi N, 2 mi W Weeping Water (1 KU). *Douglas County*: Omaha, 8240 Keystone Dr (2 KU). *Dundy County*: 2 mi SW Bendelman (1 KU); 5 mi N, 2 mi W Parks (1 KU). *Gage County*: 2 mi W, 1 mi S Barnston (1 KU). *Keith County*: 4 mi NNW Keystone (1 KU). *Lancaster County*: College View (1 FMNH); Lincoln (1 AMNH); West Lincoln (1 AMNH); West Lincoln, nr. Oak Creek (1 AMNH). *Pawnee County*: 8 mi W, 4 mi S Pawnee City (1 KU). *Red Willow County*: 8 mi S, 3 mi E McCook (1 KU). *Sarpy County*: Fort Crook (2 AMNH). *Washington County*: Blair (12 NMNH).

New Jersey (n=6). *Atlantic County*: 0.7 mi ESE Oceanville, Brigantine National Wildlife Refuge (4 NMNH). *Burlington County*: Burlington, near junction Wading River and Head's Creek (1 CU). *Ocean County*: ca. 6 mi SSE Tuckerton (1 NCSM).

New Mexico (n=3). *Quay County*: W side of Tucumcare Lake (1 MSB); 1.1 km SW Tucumcari Lake (1 MSB); 6.5 mi SW Tucumcari Airport (1 MSB).

New York: (N=5). *Orange County*: West Point (2 NMNH). *Richmond County*: Staten Island, Richmond (1 AMNH); Staten Island, New Dorp (1 AMNH). *Wayne County*: North Rose (1 NMNH).

North Carolina (n=479). *Alexander County*: 6 km NW Ellendale (1 UNCW). *Beaufort County*: Aurora (1 UNCW); Aurora Texas Gulf (4 UNCW); 4 km N Aurora (1 UNCW); 6 km N Aurora (14 UNCW); 3 km N South Creek. *Bertie County*: exact location unknown (2 UMMZ). *Bladen County*: Hwy 210 at Holly Creek (1 UNCW); 6 km ENE White Lake (1 NCSM). *Brunswick County*: 3 km SE Ash (2 UNCW); Belville (6 UNCW); Belville, 5.3 km W Wilmington (1 UNCW); Ev-henwood (3 UNCW); 5 km NE Freeland, SR 1335 (4 UNCW); Lincoln Plant (8 UNCW); near Rabontown (1 UNCW); 1 km N Rabontown (1 UNCW); 1 km NW Rabontown (1 UNCW); 2 km NW Rabontown (4 UNCW); 19 km WSW Shallotte (1 UNCW); 9 km W Wilmington (1 UNCW). *Buncombe County*: Enka (2 UMMZ); Leister (1 UNCW); Little Pisgah (1 NCSM); Weaverville (2 AMNH). *Burke County*: 5 km W Ramsey (3 UNCW). *Carteret County*: 10 km NE Beaufort (2 NMNH); Croatan National Forest (2 NCSM); 5 km ENE Harlowe (1 NCSM); 9 km S Merriman (5 NCSM); Morehead City, Bogue Island (2 NMNH). *Chowan County*: 2 km W Edenton, Hwy 17 (1 UNCW); 7 km SE Edenton (1 NCSM); 7 air km SE Edenton (2 NCSM); 14 km E Edenton, Sommerset Farm (1 NCSM); 9 km NE Maysville (1 NCSM); 9 km NW Valhalla (2 NCSM); exact location unknown (2 UNCW). *Columbus County*: 5 km NE Freeland, SR 1335 (2 UNCW). *Craven County*: Corner of Schull Road and Braxton Road (3 UNCW); End of Browns Farm Rd (8 UNCW); 2 km NNW Rhems (1 UNCW). *Cumberland County*: Gallberry Rd (1 UNCW); 2 km SSE Hope Mills (8 UNCW); 3 km S Vander, SR 2010 (8 UNCW). *Currituck County*: 1 km S Currituck (2 UMMZ); 5 km W Moyock (1 NCSM). *Dare County*: Bodie Island (13 UNCW); Bodie Island, S Nags Head (1 UNCW); Bodie Island, Oregon Inlet (1 UNCW); Buxton (1 NCSM, 2 UMMZ, 1

UNCW); 3 km WSW Buxton (1 NCSM); Cape Hatteras (1 AMNH, 1 UNCW); Little Kinnakeet (4 UNCW); Nagshead, Cape Hatteras (1 UNCW); Stumpy Point (1 NMNH); 13 km SW Stumpy Point (4 NMNH); 16 km SW Stumpy Point (1 NMNH); exact location unknown (1 NCSM, 1 UMMZ). *Duplin County*: 4 km ENE Magnolia, I-40 (8 UNCW); 4 km ESE Magnolia, I-40 (1 UNCW); 4 km W Magnolia, I-40 (2 UNCW); 2.5 km E Rose Hill, I-40 (3 UNCW); 5 km ESE Rose Hill, SR 1148 (1 UNCW); Wallace (2 UNCW); 4 km SSW Warsaw, I-40 (1 UNCW); 4 km W Warsaw, Hwy 24 (16 UNCW); 5 km S Warsaw, I-40 (5 UNCW); 5.5 km S Warsaw, I-40 (1 UNCW); 5 km S Watha, I-40 (1 UNCW). *Durham County*: Durham (1 NCSM); exact location unknown (1 NMNH). *Edgecombe County*: 6 km ESE Battleboro, Davenport Farm (2 NCSM); ca. Swift Creek, Davenport Farm (3 NCSM). *Forsyth County*: Bethbara (1 MVZ, 1 NCSM). *Gates County*: 6 km WSW Corapeake (1 NCSM); 4 km E Gatesville (1 NCSM). *Greene County*: 7 km S Snowhill, Hwy 258 (1 UNCW). *Guilford County*: Greensboro (3 UNCW). *Hertford County*: 2 km NW Akshoski (4 UNCW); mi S Winton off Rt 13 (1 NMNH); 5 km SE Winton, Hwy 45 (1 UNCW); exact location unknown (2 UNCW). *Hoke County*: 6 km W Raefod, Hwy 211 (1 UNCW); 12 km NE Raeford, Fort Bragg (1 NCSM); exact location unknown (1 NCSM). *Hyde County*: 9 km NW Engelhard (9 UNCW); 16 km N Engelhard (2 NMNH); 5 km W Lake Landing (7 NMNH); Lake Mattamusket National Wildlife Refuge (1 UNCW); 4 km N Scranton, Hwy 264 (6 UNCW); 5 km SW Woodington, SR 1925 (1 UNCW); exact location unknown (5 UNCW). *Jones County*: 6 km NE Maysville (6 NCSM); 11 km ESE Maysville (1 NCSM); exact location unknown (2 NCSM). *Mecklenburg County*: Pineville (1 GMNH, 1 UMMZ). *New Hanover County*: Carolina Beach (2 NCSM, 4 UNCW); 3 km SE Castle Hayne, I-40 (2 UNCW); 5 km SE Castle Hayne, I-40 (3 UNCW); Eagle Island (1 UNCW); Gordon Road exit, I-40 (1 UNCW); Ogden (1 UNCW); Wilmington (3 UNCW); 10 km N Wilmington (1 UNCW); Wrightsville (1 UNCW); Wrightsville Beach (3 UNCW). *Pasquotank County*: Elizabeth City (1 MVZ); Elizabeth City, Hwy 17 (3 UNCW); 8 km N Elizabeth City (2 UNCW). *Pender County*: 3 km NE Burgaw, I-40 (5 UNCW); 5.5 km SE Burgaw, I-40 (2 UNCW); 5.5 km SSE Burgaw, I-40 (2 UNCW); 7 km SE Burgaw, I-40 (17 UNCW); 8 km SSE Burgaw, I-40 (15 UNCW); 6 km N

Castle Hayne, I-40 (1 UNCW); 6 km NNE Castle Hayne, I-40 (2 UNCW); 10 km N Hampstead (1 UNCW); Holly Shelter Gamelands (10 UNCW); Lincoln Plant near Belville (6 UNCW); Moores Creek (7 UNCW); 5.5 km SE Wallace, I-40 (2 UNCW); 6.5 km SE Wallace, I-40 (1 UNCW); 2 km E Watha, I-40 (7 UNCW); 3 km E Willard, I-40 (9 UNCW). *Polk County*: 6 km NE Saluda, SR 1151(1 UNCW); 8 km NE Saluda, SR 1151 (3 UNCW); 8 km E Saluda, SR 1151 (6 UNCW). *Robeson County*: 6 km WSW Lumberton, SR 2503 (2 UNCW). *Rutherford County*: Scott Jenkins Bog (4 NCSM). *Sampson County*: 9 km NNW Delway (1 NCSM); 8 km E Faison (5 UNCW). *Scotland County*: 3 km SE Laurinburg, Hwy 24, 2 km from jct SR 1481 (6 NCSM); 8 km SW Laurinburg Hwy 15/401 (6 NCSM). *Stokes County*: 2 km NNE Dillard (1 NCSM). *Tyrrell County*: 16 km E Colombia, Hwy 64 (1 UNCW). *Union County*: 4 km NE center of Monroe (1 NCSM); exact location unknown (1 NCSM). *Wake County*: Raleigh (5 AMNH, 1 FMNH, 2 KU, 5 MCZ, 1 MVZ, 3 NCSM, 2 UMMZ; 26 NMNH); I-40 intersection with Hwy 64 (1 NCSM). *Washington County*: 1 km W Pleasant Grove, Hwy 64 (1 UNCW). *Wilkes County*: 6 km S Moravian Falls (2 UNCW); 7 km SSE Moravian Falls, Pores Knob (1 UNCW). *County unknown*: exact location unknown (1 NCSM).

Ohio (n=37). *Adams County*: Smoky Creek, Green Township (1 INHS). *Ashtabula County*: Geneva (2 UMMZ). *Clermont County*: 5 mi N Batavia (2 KU); Glen Este, Union Township (4 INHS); near Glen Este, Union Township (2 INHS); 4 mi E Goshen (1 INHS); Owensville (7 INHS). *Cuyahoga County*: Lyndhurst (1 UMMZ). *Hamilton County*: Cincinnati, California (1 INHS); Cincinnati, Delhi (1 INHS); Miami River Road (2 INHS). *Hancock County*: Bowling Green (1 AMNH). *Lake County*: Mentor, Wayside Gardens (1 UMMZ). *Lucas County*: nr Monclova (1 AMNH). *Mohoning County*: Ellsworth (1 NMNH). *Portage County*: Aurora Pond (1 UMMZ); Garrettsville (2 NMNH). *Seneca County*: Bettsville (6 UMMZ).

Oklahoma (n=34). *Adair County*: Stillwell, Boston Mountains (1 NMNH). *Canadian County*: El Reno (1 NMNH); 5.5 mi SE Hinton (1 KU). *Comanche County*: 3 N Cache (1 NMNH); Wichita Mountains Wildlife Refuge (6 NMNH). *Custer County*: 1.25 mi N, 0.75 mi W Weatherford (1 KU). *Harper County*: 3 mi N fort Supply (1 NMNH). *Murray County*: Sulfur, Rock Creek Camp Ground,

Platt National Park (1 KU). *Osage County*: 5.5 mi N, 4 mi E Shidler (1 KU); 12 mi N, 5 mi E Shidler, K. S. Adams Ranch (6 KU); *Ottawa County*: 2 mi S, 6 mi E Afton (3 KU). *Payne County*: Stillwater (2 UMMZ, 1 NMNH). *Pottawatomic County*: 1 mi SE Tecumsch (1 KU). *Rogers County*: Garnett (3 UMMZ); Exact location unknown (1 MSB). *Tulsa County*: Red Fork (1 NMNH). *Woodward County*: Woodward (1 NMNH); 2 S, 4.5 W Woodward (1 NMNH).

South Carolina (n=63). *Aiken County*: Savaannah River Plant, various sites (23 GMNH); Talatha (1 GMNH). *Anderson County*: 6.8 mi N Anderson (1 KU); *Barnwell County*: 33º0.25 N, 81º.25.50´W (7 GMNH). *Berkeley County*: 15.8 mi N Charleston (1 KU). *Charleston County*: near ACL, RR Station (2 GMNH); 3.5 mi W Charleston (1 KU); 15 mi S Charleston (1 KU); Lagaris Farm, St Andrews Parish (2 KU); McClellanville (6 NMNH); 3.4 km NE McClellanville (8 NMNH); Mount Pleasant (1 NMNH); Porchers Bluff (1 ROM); exact location unknown (1 ROM). *Darlington County*: Society Hill (1 NMNH). *Georgetown County*: Georgetown (1 NMNH). *Greenville County*: Hillsborough (1 NMNH). *Oconee County*: Tugaloo, Tugaloo Lake, 3.2 mi E int. Hwy 151 and US 441 (1 GMNH). *Orangeburg County*: 1 mi NW Orangeburg (1 ROM). *Richland County*: COSW (2 UNCW).

Tennessee (n=23). *Anderson County*: exact location unknown (1 NCSM). *Cheatham County*: 29 km W Nashville (3 LSU). *Giles County*: 6 mi E Pulaski (5 NMNH). *Knox County*: 5 mi SE Knoxville (1 KU); 10 mi W Knoxville (1 KU); 10 mi SW Knoxville (2 KU). *Lake County*: Reelfoot Lake (4 NMNH). *Montgomery County*: Clarksville (3 NMNH). *Shelby County*: 6 mi N Memphis (3 KU).

Texas (n=29). *Aransas County*: Aransas National Wildlife Refuge, North edge (2 MCZ). *Bexar County*: 1 mi E San Antonio, Highland (1 AMNH); 5 mi E San Antonio, U. S. 87 (1 KU). *Brazos County*: College Station (2 KU). *Colorado County*: Eagle Lake (1 KU). *Cooke County*: Gainsville (1 NMNH). *Denton County*: 4 mi WNW Justin (1 ROM). *Galveston County*: 0.5 mi N Lamarque (1 KU). *Hardin County*: 5.5 mi NW Sour Lake (3 MSB). *Harris County*: 1.7 mi S jct US Hwy 80 and Voss Road, west edge Houston (3 KU). *Hemphill County*: 1 mi N, 12 mi E Canadian, Lake Marvin (3 KU).

Hidalgo County: 7 mi S Alamo (1 MCZ). *Mitchell County*: Colorado City (2 NMNH). *Montagorde County*: 13 mi S, 8 mi W Bay City (1 NCSM). *Palo Pinto County*: 10 mi W Graford (2 MSB). *Randall County*: Buffalo Lake (1 MSB). *San Patricio*: Welder Wildlife Refuge, Tule Lake (1 MCZ); Welder Wildlife Refuge, Camp House (1 MCZ). *Wichita County*: 6.2 mi W Iowa Park (1 NMNH).

Virginia (n=117). *Accomack County*: Assateague Island (22 NMNH); Assateague Island, Chincoteague Refuge (2 NMNH); Assateague Island, Popes Island Coast Guard Station (3 NMNH); Chincoteague Refuge (6 NMNH); Chincoteague Island (1 NCSM, 4 NMNH); Wallops Island (12 NMNH). *Caroline County*: Fort APHill (31 UNCW). *Montgomery County*: 0.5 mi SW Blacksburg (1 UMMZ). *Norfolk County*: 9 mi S Portsmouth (1 NCSM). *Northampton County*: Oyster (7 AMNH). *Prince Williams County*: Manassas (1 NCSM). *Princess Anne's County*: 6.8 mi SE Pungo, Backbone National Wildlife Refuge (4 NMNH). *Russell County*: Roaring Springs Creek (1 UMMZ). *Smyth County*: Sugar Grove (1 UMMZ). *Tazewell County*: Burkes Garden (14 NMNH). *County Unknown*: coastal plain (1 NCSM); Dismal Swamp (1NMNH); Shenandoah National Park headquarters (3 NMNH); Yorktown National Monument (1 KU).

West Virginia (n=12). *Cabell County*: 0.5 mi S Cox Landing (2 KU). *Greenbrier County*: White Sulphur Springs (2 MCZ, 4 FMNH, 3 NMNH). *Kanawha County*: Charlestown (1 NMNH).

Mexico

Michoacan (n=2). 3 mi E Pátzcuaro (1 UMMZ); Sierra Baralosa, 1 hr 20 min (mule) NE Rancho Baralosa (1 UMMZ).

Tamaulipas (n=9). 1 mi S Altamira (8 KU); 10 mi W, 2 mi S Piedra, Sierra de Tamaulipas (1 UMMZ).

APPENDIX II

Information pertaining to the specimens used for molecular analyses. Not every individual was collected from an area where an OTU was designated for morphological analyses, however, the OTU number that most closely corresponds to the specimen location geographically is provided. Numbers in parentheses correlate to the order of multiple sequences from a single location in the Bayesian phylogeny. Also, in the material used column, RT=residual tissue and FT=fresh tissue.

Museum Number/Genbank Number	Location (County, State)	Material Used	Gene(s) Amplified	Associated OTU
UNCW 2657	Duplin (3), NC	Toe	Cyt-b	39
CU 16565	Logan (1), AK	RT	Cyt-b	8
GMNH 835	Mecklenburg, NC	RT	Cyt-b	36
FLMNH 26030	Pinellas (2), FL	RT	Cyt-b	63
KU 89175	Ottawa, OK	RT	Cyt-b	7
UNCW 4738	Hyde (1), NC	Toe	Cyt-b	41
UNCW 1297	Beaufort (1), NC	Toe	Cyt-b	40
CU 3747	Douglas (1), KS	RT	Cyt-b	4
NCSM 5619	Norfolk, VA	RT	Cyt-b	35
UNCW 8230	Caroline (1), VA	RT	Cyt-b	31
KU 153905	Clermont, OH	RT	Cyt-b	24
UNCW 5187	Hyde (4), NC	RT	Cyt-b	41
KU 14559	Cherokee, KS	RT	Cyt- b	7
KU 45465	Dorchester (3), MD	RT	Cyt-b	32
KU 45462	Dorchester (1), MD	RT	Cyt-b	32
UNCW 4072	Robeson, NC	Toe	Cyt-b	48
ANMH 30552	Richmond, NY	RT	Cyt-b	28
UNCW 17776	Brunswick (2), NC	FT	Cyt-b, COI, ApoB	46
FLMNH 26029	Pinellas (1), FL	RT	Cyt-b	63
UNCW 18936	Craven (3), NC	FT	Cyt-b, COI, ApoB	40
UNCW 18932	Craven (2), NC	FT	Cyt-b, COI, ApoB	40
UNCW 18935	Craven (5), NC	FT	Cyt-b, COI	40
UNCW 18933	Craven (4), NC	FT	Cyt-b, COI, ApoB	40
UNCW 5188	Hyde (5), NC	Toe	Cyt-b	41
FLMNH 5205	Grady (1), GA	RT	Cyt-b	57
UNCW 3319	Duplin (1), NC	RT	Cyt-b	39
UNCW 17777	Brunswick (3), NC	FT	Cyt-b, COI	46
UNCW 18311	New Hanover, NC	FT	Cyt-b, COI	46
UMMZ 54008	Wake, NC	RT	Cyt-b	38
AMNH 123992	North Hampton, VA	RT	Cyt-b	40
UNCW 18938/HBS 08-02*	Macon, NC	FT	Cyt-b, COI, ApoB	36
UNCW 18934	Craven (6), NC	FT	Cyt-b, COI, ApoB	40
UNCW 3318	Duplin (2), NC	Toe	Cyt-b, COI	39

UNCW 47	Dare, NC	Toe	Cyt-b	43
UNCW 11006	Caroline (2), VA	Toe	Cyt-b	31
UNCW 18937	Craven (7), NC	FT	Cyt-b, COI, ApoB	40
UNCW 1324	Dare, NC	Toe	Cyt-b	43
UNCW 1166	Dare, NC	Toe	Cyt-b	43
UNCW 18939	Craven (1), NC	FT	Cyt-b, COI	40
UNCW 4803	Hyde (3), NC	Toe	Cyt-b	41
UNCW 5129	Hyde (2), NC	Toe	Cyt-b	41
FLMNH 13115	Santa Rosa, FL	RT	Cyt-b	58
UNCW 18276	Brunswick (1), NC	RT	Cyt-b, COI	46
NCSM 2932	Polk, FL	RT	Cyt-b	63
KU 39058	Cabell, WV	RT	Cyt-b	30
ROM 17312	Dorchester (2), MD	RT	Cyt-b	32
UNCW 2357	Beaufort, NC	RT	Cyt-b	40
FLMNH 12663	St. Johns, FL	RT	Cyt-b	61
AMNH 131600	Taylor (1), FL	RT	Cyt-b	59
LSU 29802	St. Martin Parish (2), LA	RT	Cyt-b	14
CU 14774	Logan (2), AK	RT	Cyt-b	8
KU 1145	Ellis, KS	FT	Cyt-b, COI	6
LSU 29798	St. Martin Parish (1), LA	RT	Cyt-b	14
LSU 29803	St. Martin Parish (3), LA	RT	Cyt-b	14
UMMZ 122686	Seneca, Oh	RT	Cyt-b	26
KK 1582	Wabaunsee, KS	FT	Cyt-b, COI	4
CU 3745	Douglas (2), KS	RT	Cyt-b	4
KK 2146	Hodgeman, KS	FT	Cyt-b, ApoB, COI	6
UNCW 19450**	Boone or Adair (1), MO	FT	Cyt-b, COI	3
AF 395484	Eastern Nebraska (2)	Genbank	Cyt-b	2
UNCW 10088	Gwinnett, GA	Toe	Cyt-b	51
UMMZ 57264	Dorchester (4), MD	RT	Cyt-b	32
UNCW 19428	Lancaster, NE	FT	Cyt-b, COI	2
ROM 3603310019	Douglas (3), KS	RT	Cyt-b	4
AF 395483	Eastern Nebraska (1)	Genbank	Cyt-b	2
FLMNH 658	Putnam (1), FL	RT	Cyt-b	62
FLMNH 11	Levy, FL	RT	Cyt-b	62
UNCW 19451**	Boone or Adair, MO	FT	Cyt-b, COI, ApoB	3
UMMZ 65391	Posey, IN	RT	Cyt-b	19
KU 89175	Barber, KS	RT	Cyt-b	6
AB 175135	Tom Green, TX	Genbank	Cyt-b	9
UMMZ 6668	Putnam (2), FL	RT	Cyt-b	62
FLMNH 5206	Thomas, GA	RT	Cyt-b	57
CU 4038	Charlton, GA	RT	Cyt-b	61
FLMNH 31353	Highlands (2), FL	FT	Cyt-b, ApoB	65
UMMZ 122691	Portage, OH	RT	Cyt-b	27
UNCW 19473	Taylor (2), FL	RT	Cyt-b	59
FLMNH 31362	Highlands (1), FL	FT	Cyt-b, ApoB	65
AMNH 100199	Grady (2), GA	RT	Cyt-b	57
LSU 19446	Cheatham (2), TN	RT	Cyt-b	16
LSU 19444	Cheatham (1), TN	RT	Cyt-b	16

FLMNH 31363	Highlands (2), FL	FT	Cyt-b	65
UNCW 18941	Craven, NC	FT	ApoB	
DQ630196 (*Blarina*)	Michigan	Genbank	ApoB	NA
DQ630180	Tom Green, Texas	Genbank	ApoB	9
DQ630186 (*C. magna*)	Oaxaca, MX	Genbank	ApoB	NA
DQ630189 (*C. goldmani*)	Gurrero, MX	Genbank	ApoB	NA
AB175141 (*C. magna*)	NA	Genbank	Cyt-b	NA
AB175143 (*C. mexicana*)	NA	Genbank	Cyt-b	NA
AB175138 (*C. goldmani*)	NA	Genbank	Cyt-b	NA