THE PHYLOGEOGRAPHY OF THE SHORT-TAILED SHREWS

(GENUS *BLARINA*) OF SOUTHEAST TENNESSEE

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Understanding the distributions of organisms is key to deciphering their biogeography. Shrews of the genus *Blarina* are some of the most common and abundant mammals in this region. Two species are found in southeast Tennessee: *Blarina brevicauda* and *Blarina carolinensis*. To clarify their geographic ranges, *Blarina* vouchers were collected throughout the study area and mitochondrial DNA cytochrome *b* genes were isolated and sequenced. I collected and compared 53 DNA sequences from shrews throughout southeast Tennessee and southwest North Carolina to 101 samples obtained from Genbank. Results indicate *Blarina brevicauda* is found in areas north and west of the Tennessee River and *Blarina carolinensis* is found in most areas south and east of the Tennessee River. *B. brevicauda* specimens fell into a monophyletic *B. brevicauda* clade, resolving with Genbank sequence data into haplotypes classified as either ‘Appalachian’ or ‘East-Central’. *B. carolinensis* specimens were monophyletic, resolving into an ‘Eastern’ haplotype.
DEDICATION

For his tireless work in conservation of some of the most beautiful landscape in the southeast, I would like to dedicate this research to Jim Brown, former director of the Tennessee River Gorge Trust.
ACKNOWLEDGEMENTS

I would like to sincerely thank Dr. Tim Gaudin and Dr. Joey Shaw for serving as co-committee chairs and Dr. Thomas Wilson for serving on my committee. I was extremely lucky to have such a knowledgeable and patient triumvirate to guide me during my studies. I would also like to thank Dr. Stylianos Chatzimanolis for lending a computer to run data analysis and Dr. Margaret Kovach for her advice and seemingly endless supply of supplemental chemicals. Numerous undergraduate and graduate students assisted with the trapping and preparation of shrews, and for that I am extremely grateful. I thank the staff of the Department of Biological and Environmental Sciences at UTC for their time and resources. I would like to thank the Provost because my research was partially funded by the UTC Provost Student Research Award. To all of the private citizens, land managers, and governing bodies that granted me permits and access to land in order to collect data, I thank you. Finally I thank my wife, Sarah, for her constant love and friendship.
# TABLE OF CONTENTS

DEDICATION ........................................................................................................ iv

ACKNOWLEDGEMENTS .................................................................................. v

LIST OF TABLES ............................................................................................... vii

LIST OF FIGURES ............................................................................................ ix

CHAPTER

I. INTRODUCTION .......................................................................................... 1

II. THE PHYLOGEOGRAPHY OF SHORT-TAILED SHREWS
(GENUS BLARINA) OF SOUTHEAST TENNESSEE ........................................... 4

  Introduction ..................................................................................................... 4
  Historical Ranges ......................................................................................... 7
  Current Ranges ............................................................................................ 8
  The Role of Genetic Data in Determining Species Ranges ......................... 9
  Methods ....................................................................................................... 11

Results ............................................................................................................. 14

  Genetic Identification of *Blarina* species ................................................... 14
  Phylogenetic Inference ............................................................................... 15
  Molecular Clock $T_{MRCA}$ Estimates .......................................................... 16
  Population Structure of *Blarina* ................................................................. 17
  *Blarina carolinensis* ................................................................................... 17
  *Blarina brevicauda* .................................................................................. 18

Discussion ....................................................................................................... 19

  Historical Distribution vs Current Ranges .................................................. 19
  Phylogeography of *Blarina carolinensis* and *Blarina brevicauda* ............ 22
  The Role of Tennessee’s Physiographic Variation on Species’ Ranges .......... 26
  Conclusions ................................................................................................. 28

  vi
III. CONCLUSIONS AND FUTURE RESEARCH ......................................................... 36

REFERENCES ........................................................................................................... 39

APPENDIX

A. PHYSIOGRAPHIC PROVINCES OF SOUTHEAST TENNESSEE ................. 44

B. SAMPLING LOCALITIES FOR SHORT-TAILED SHREWS ...................... 46

C. MEASUREMENTS OF EXTERNAL MORPHOLOGY OF CAPTURED SHREWS ............................................................................................. 48

VITA ........................................................................................................................... 51
LIST OF TABLES

1  Ages of most recent common ancestors for the nodes on the phylogenetic tree from Figure 2. Age estimates are in millions of years and represent the mean of three replications. Asterisks represent nodes calibrated using fossil data ...........................................................................................................17

2  Fu’s $F_S$ for *Blarina carolinensis*. $F_S$ for the Western haplotype indicates range expansion ..................................................................................................................18

3  Results for AMOVA analysis of *Blarina brevicauda* haplotypes. All $F_S$ values indicate recent population expansion. $F_{ST}$ comparisons show strong support for the definition of each clade as a population........................................19
LIST OF FIGURES

1  Location of collecting localities and *Blarina* species distributions in southeast Tennessee.................................................................15

2  Phylogenetic tree for *Blarina* based on cytochrome *b* gene sequences from 53 specimens from southeast Tennessee and 101 *Blarina* and *Cryptotis* (outgroup) GenBank sequences..............................................................................................................29

3  Distribution of *Blarina carolinensis* haplotypes in southeast Tennessee (A) and the eastern United States (B) .........................................................30

4  Haplotype distributions of *Blarina brevicauda* in southeast Tennessee (A) and the eastern United States (B) .................................................................31
LIST OF SYMBOLS

° degrees
< less than
LIST OF ABBREVIATIONS

B.  \textit{Blarina}

DNA  deoxyribonucleic acid

PCR  polymerase chain reaction

GIS  geographic information systems

mm  millimeter

FN  fundamental number

NALMA  North American land mammal age

ybp  years before present

\( \mu \)L  microliters

C  Celsius

dNTPs  deoxynucleotide triphosphates

s  seconds

MCMC  Markov chain Monte Carlo

TS  transition

TV  transversion

\( T_{MRCA} \)  time to most recent common ancestor

mya  million years ago

g  grams

TRGT  Tennessee River Gorge Trust
CHAPTER I

INTRODUCTION

How plants and animals came to occupy their current ranges is arguably one of the most studied questions in biology. An important aspect to this query is first determining the boundaries of species. One of the first to recognize geographic patterns of animal and plant distributions and describe major zoological regions still recognized today was Philip Lutley Sclater (1858). Other scientists like Alfred Russel Wallace (1863) advanced the science of biogeography, understanding the importance of studying all of nature. Species distributions are continuously changing; therefore it is important to continuously monitor expansion and reduction of species ranges.

One common, important group of small mammals in North America is the short-tailed shrew genus *Blarina*. The northern short-tailed shrew (*B. brevicauda*) and the southern short-tailed shrew (*B. carolinensis*) are both found in southeast Tennessee and appear very similar morphometrically. However, the exact ranges of the two species remain incompletely understood (French, 1981; Braun & Kennedy, 1983; Jeffries, 1999; Webster et al., 2011). My master’s thesis research has been devoted to clarifying the geographic distributions of these two species with the help of mitochondrial DNA sequence data.

While at the University of Tennessee at Chattanooga I developed a project to examine the distributions of *Blarina* within the areas surrounding Chattanooga. Dr. Tim Gaudin introduced the research to me in my first semester at UTC. Under his tutelage, I learned how to trap for the
target species and how to prepare voucher specimens for the UTC Natural History Museum.

During the fall semester of 2010, I began collecting liver, kidney, and muscle tissues from all *Blarina* specimens that were processed by Dr. Gaudin’s Mammalogy class, to determine which tissues would be best for mitochondrial DNA extractions and PCR.

My second semester at UTC was mostly spent in the genetics lab of Dr. Joey Shaw. I was instructed how to extract DNA from the collected tissue samples and run PCRs. I also attempted to extract DNA from museum specimens. By using museum skins and skeletons, I would have been able to theoretically increase the sample size of *Blarina* without spending valuable time in the field setting traps. I attempted these extractions five different times following the procedures from Asher and Hofreiter (2006). These procedures proved ineffective in extracting usable amounts of DNA and were therefore abandoned. I then focused my efforts on the tissues already collected from the *Blarina* specimens the previous semester.

Muscle tissues proved to be best for DNA extraction. In the first wave of extraction and PCR attempts, I successful extracted DNA and amplified the cytochrome *b* gene from 13 of 16 (81.25%) muscle tissues. Amplifications only worked in 6 of 16 (37.5%) liver tissues and 4 of 16 (25%) kidney tissues. From that point forward, I only collected muscle and kidney tissues.

At this point, I was able to construct a preliminary phylogeny and learn how to use complex phylogenetic computer software programs like PAUP, BEAST, Sequencher, and MacClade. The preliminary phylogeny supported results obtained by Brant and Orti (2002) and enabled me to develop a more focused plan for collecting shrews from southeast Tennessee. My committee and I agreed on a plan to collect at least three shrews from each of a variety of new localities in the region. These shrews, along with others collected previously by Dr. Gaudin, could provide significant clarification of *Blarina* species ranges in southeast Tennessee.
detailed account of this research is provided in the following chapter. Conclusions and suggestions for future research can be found in the ultimate chapter.
CHAPTER II

THE PHYLOGEOGRAPHY OF SHORT-TAILED SHREWS (GENUS BLARINA) OF SOUTHEAST TENNESSEE

This chapter is a lightly revised manuscript of the same name to be submitted to the Journal of Mammalogy in April 2013 by Timothy Gaudin, Joey Shaw, and myself. My use of ‘we’ throughout the chapter refers to these co-authors and myself. My contributions include (1) development and implementation of the project from the core ideas introduced to me by my co-authors, (2) the fieldwork with the assistance of Timothy Gaudin and numerous UTC undergraduates, (3) the lab work, (4) the data analyses, and (5) the large majority of the writing.

Introduction

One of the most commonly studied characteristics of a species is the geographic range that it occupies. Historically scientists relied mostly on observational and capture data to draw broad generalities about species’ ranges, but today new techniques are available to more clearly define these ranges. Genetically based phylogenies can provide scientists with greatly refined borders of species ranges, even for species that are morphologically and ecologically similar (Avise et al., 1987). Advanced software packages such as BEAST v1.6.1 (Drummond & Rambaut, 2007) use varying statistical probabilities to allow reliable recovery of phylogenies within or among species. Similar advancements in geographic information systems (GIS) provide easier mapping and tracking of species’ borders that are in constant flux. The practice of
using phylogenetic data to infer historical and current geographic ranges is known as phylogeography. It was initially used to provide range data on a large spatial scale. Since then, new tools and methods have arisen to more clearly establish geographic ranges on smaller spatial scales (Postma & Noordwijk, 2005). This includes the analysis of mitochondrial DNA data to examine intraspecific populations and distributions (Avise et al., 1987). There has been especially great interest in the role geographic barriers play in the restriction or broadening of a species’ range.

One region of interest to phylogeographers is the southeastern United States, particularly areas of middle and eastern Tennessee. The Tennessee River and four physiographic provinces traverse middle and eastern Tennessee (see Appendix A), making the landscape full of physical barriers for small terrestrial organisms. In a recent review of phylogeographic studies covering a broad range of organisms throughout the eastern U.S., Soltis et al. (2006) found a large concentration of phylogeographic breaks in this region. The authors were unsure of the significance of this result and stated that the area should be investigated to determine how the landscape influences species distributions.

The present study will investigate the ranges of short-tailed shrews (genus Blarina Gray, 1823) in southeast Tennessee using phylogeographic inference. Blarina species are some of the most common, most abundant terrestrial small mammals in the eastern United States. They are found in a wide variety of habitats, including grasslands, woodlands, scrub, and wetlands (Choate et al., 1994). Blarina can be distinguished from other shrews by their short tails, dentition, and uniform slate gray pelage. Blarina have one falciform incisor, five unicusps, one premolar and three molars in the upper toothrow and one procumbent incisor, one unicuspid, one premolar and three molars in the lower toothrow, for a total of thirty-two teeth (Choate, 1968).
Some members of the genus *Sorex* share this dental formula, but *Blarina* can be differentiated by their very reduced upper unicuspid three and four, most evident in lateral view. Members of *Sorex* also have much longer tails than those of *Blarina*. Shrews of the genus *Cryptotis* have similarly reduced tails, but have only four unicuspid on the upper toothrow and a brown dorsum with a whitish venter (Hall, 1981). The various species of *Blarina* are also known for their toxic saliva and echolocation abilities, both of which are very rare traits among mammals (Churchfield, 1990). The genus is comprised of four species: *Blarina brevicauda*, the Northern short-tailed shrew found throughout much of northeastern North America, *Blarina carolinensis*, the Southern short-tailed shrew found throughout the southeastern United States, *Blarina hylophaga*, Elliot’s short-tailed shrew found in the midwestern United States from southern Iowa and Nebraska to north Lousiana and Texas, and *Blarina shermani*, Sherman’s short-tailed shrew found in Lee and Collier counties of south Florida (Benedict *et al*., 2006; Choate *et al*., 1994; Wilson & Reeder, 2005). *Blarina brevicauda* and *Blarina carolinensis* are the only species from the genus found in the area pertinent to this study.

Because of their similar overall appearance, the easiest way to distinguish the two species of *Blarina* in this study area is by size. *B. brevicauda* has a snout to tail length normally >100mm, whereas in *B. carolinensis* it is normally <90mm. Moreover, *B. brevicauda* has an occipito-premaxillary length >20.5mm, whereas in *B. carolinensis* the length is <19mm (George *et al*., 1986; Choate *et al*., 1994). However, many specimens captured in southeast Tennessee have intermediate measurements, complicating species-level identification. Therefore, the most reliable way to separate the two species is via karyotype. *B. brevicauda* has an FN value of 48 (2n=48-50) and *B. carolinensis* has an FN range of 41-45 (2n=31-46) (George *et al*., 1982; Qumsiyeh *et al*., 1999).
**Historical Ranges**

Based on fossil evidence, *Blarina brevicauda* and *Blarina carolinensis* are thought to have once been sympatric throughout the southeast. *B. brevicauda* sized fossils first appear in the fossil record in the late Pliocene and date to the Blancan NALMA (North American Land Mammal Age; Kurten & Anderson, 1980; Jones *et al*., 1984). *B. carolinensis* appears later and is found in the same fossil deposits as *B. brevicauda* at Ladd’s Quarry and Skidaway Island in Georgia, Peccary Cave in Arkansas, and Cumberland Cave in Maryland. The specimens from Cumberland Cave are thought to be from the early Pleistocene (mid-Irvingtonian NALMA; Kurten & Anderson, 1980; Jones *et al*., 1984). Those from Peccary Cave and Skidaway Island date to the late Pleistocene (Rancholabrean NALMA; Kurten & Anderson, 1980; Jones *et al*., 1984). Fossils from Ladd’s Quarry date to the early Holocene (Jones *et al*., 1984; Hulbert & Pratt, 1998). Jones *et al.* (1984) suggest that *B. carolinensis* first emerged as a separate species due to chromosomal rearrangements that occurred when *B. brevicauda* was divided into two groups in the early Irvingtonian NALMA, *B. brevicauda* being restricted by moisture to forests of the northeast and *B. carolinensis* to coastal marshlands of the south. For the period of the Wisconsinan glaciation (Rancholabrean), Jones *et al.* (1984) drew a boundary between the two species through southern Alabama, Georgia and South Carolina and suggested a northward range expansion of *B. carolinensis* and corresponding retreat of *B. brevicauda* as climate continentality increased in the early Holocene. Tennessee fossil data are consistent with this hypothesis. *B. brevicauda* is identified from several Pleistocene faunas of Tennessee (Corgan and Breitburg, 1996) including one from Lookout Mountain (Gaudin *et al*., 1998; Jeffries, 1999), but *B. carolinensis* is only known from Cheek Bend Cave in middle Tennessee, dating from 5000 ybp.
and younger (Klippel & Parmalee, 1982) and from an undated but likely Holocene age site in Hamilton County, TN (OHS Cave; Gaudin et al., 2011).

**Current Ranges**

The two species are now considered by most experts to be parapatric, although some authors suggest small areas of sympatry (Jones et al., 1984; Benedict, 1999). Whereas the geographic ranges of the species have been described throughout much of Tennessee (French, 1981; Braun & Kennedy, 1983; Webster et al., 2011), the species’ ranges in southeast Tennessee remain unclear.

Several workers have investigated geographic ranges of the species of *Blarina* in the midsouth region and some general aspects of their distribution have been agreed upon. *B. brevicauda* is known from the Blue Ridge Mountains of Georgia, Tennessee, and the Carolinas as well as the piedmont of northern Georgia; there are also several isolated populations from the coastal plain of west Georgia and eastern Alabama (Braun & Kennedy, 1983; French 1981; George et al., 1986; Mengak et al., 1987; Webster et al., 2011). The range of *B. carolinensis* extends through the piedmont and coastal plain of the Carolinas, the coastal plains of Georgia, Alabama, and Mississippi, and north through the Mississippi River Valley of western Tennessee and Kentucky (George et al., 1982; Braun & Kennedy, 1983; Mengak et al., 1987). However, in middle and eastern Tennessee the ranges of the two species are not unambiguously resolved. Braun and Kennedy (1983) used discriminant function analysis of skull morphometric data to recognize *B. brevicauda* in central Tennessee. In contrast, French (1981) reported that the *Blarina* specimens of central Tennessee were intermediate in size between the two species and therefore could not be confidently assigned to either species, but were more like that of *B.*
carolinensis. Because of the intermediate sizes of individuals from the Cumberland Plateau, Webster et al. (2011) recognized a new sub-species of *B. brevicauda* from this region that they called *B. b. cumberlandensis*.

None of the latter three studies extensively sampled from the Tennessee Valley area of southeast Tennessee, a likely zone of contact between the two species. Only one unpublished study (Jeffries, 1999) conducted an intensive survey of *Blarina* distribution in southeast Tennessee. The study used morphometric analysis of cranial measurement data to separate *B. brevicauda* from *B. carolinensis* based on size and shape differentials. Results indicated that *B. brevicauda* is found throughout the piedmont and Blue Ridge Mountains of northern Georgia and the Cumberland Plateau and Blue Ridge Mountains of middle and easternmost Tennessee. *B. carolinensis* was found to be restricted to the Valley and Ridge system south of Knoxville in Tennessee, and in northwest Georgia and northeast Alabama. The southern species was also assigned to specimens from Sand and Lookout Mountains in Alabama and Georgia, respectively. Many specimens from the Ridge and Valley, Cumberland Plateau, and Unaka Mountains physiographic regions could not be confidently identified as either *B. brevicauda* or *B. carolinensis*. These specimens had intermediate morphology and were placed in one species based on size, and the other based on shape.

**The Role of Genetic Data in Determining Species Ranges**

Recent phylogenetic studies of *Blarina* have clarified specific level differences over a broad geographic area of eastern North America. Brant and Orti (2002, 2003) used mitochondrial DNA sequence data from the cytochrome *b* gene to examine the phylogeny of *Blarina* species and determine their ranges throughout the eastern United States. They found genetically distinct
groups within _B. brevicauda_ and _B. carolinensis_ that could be tied to geographic barriers. A similar analysis conducted at a smaller spatial scale should allow for a better understanding of the phylogeography of _Blarina_ across the varied terrain and multiple physiographic provinces in southeast Tennessee. Our aim was to sample heavily from this region and to integrate our new data into the framework of Brant and Orti (2002, 2003). We felt the use of mitochondrial DNA sequence data should help clarify biogeographic discrepancies among previous morphometric studies (French, 1981; Braun & Kennedy, 1983; Jeffries, 1999; Webster _et al._, 2011).

A phylogeographic analysis of _Blarina_ in the region might also illuminate regional dispersal patterns and population history. Global warming has been proposed as an influence on the expansion of geographic ranges of warm-climate mammal species. Recent publications (Keller _et al._, 2003; Chen _et al._, 2011; Eichler & Gaudin, 2011) seem to confirm the recent movement of various small mammal species to higher elevation or to more northerly geographic areas. This study may provide evidence that _B. carolinensis_ is slowly moving into areas formerly occupied by _B. brevicauda_ or displacing _B. brevicauda_, constricting the range of the northern species. Fossil evidence suggests _B. carolinensis_ recently migrated to Hamilton County, Tennessee since fossil shrews from Pleistocene deposits were identified as _B. brevicauda_ (Gaudin _et al._, 1998; Jeffries, 1999) and Holocene fossils were identified as _B. carolinensis_ (Gaudin _et al._, 1998, 2011; Jeffries, 1999). A genetic analysis of current distributions in conjunction with this fossil data could possibly clarify if _B. carolinensis_ is now occupying areas evacuated by _B. brevicauda_ or if _B. carolinensis_ is displacing _B. brevicauda_.

The ultimate goal of this research is to establish the geographic ranges of _Blarina_ species in southeast Tennessee using phylogeographic inference, especially in the areas surrounding Chattanooga and the Tennessee River Valley. Evaluating relatedness among _Blarina_ individuals
in southeast Tennessee should prove valuable in exploring the question of sympatry vs. parapatry, in evaluating the gene flow and introgression within each species, and in determining the history of dispersal within the genus. Clarifying the historical biogeography of this important, abundant genus of small insectivorous mammals will ultimately be of interest not only to other phylogeographers, but also to anyone with an interest in the taxonomic diversity, ecology, conservation, and evolution of North American mammals.

Methods

Shrews were collected from various localities in southeast Tennessee and southwest North Carolina. To ensure thorough coverage of the area, samples were collected from 13 localities that extend from the southern Unaka Mountains of North Carolina in the east to the eastern Highland Rim of Tennessee in the west (See Appendix 2). This transect covered various physiographic regions including the southern Unaka mountains, the Valley and Ridge, the Cumberland Plateau, and the Eastern Highland Rim (Luther, 1977).

Pitfall traps, Sherman traps, and museum snap traps were used to capture the shrews. These traps were set among woody debris in a variety of habitats including forests, forest edges, power line cuts, and mountain balds. A minimum of three *Blarina* specimens was collected from every locality. All procedures followed the guidelines set forth by the American Society of Mammalogists for the capture, handling, and euthanasia of small mammals species (Sikes *et al.*, 2011). Liver, kidney, and muscle tissues were collected for genetic analysis and stored at -20°C. Most specimens were also prepared as skin, skull, and skeleton vouchers, and are now archived at the UTC Natural History Museum along with all tissue samples used. These procedures were approved by the UTC IACUC (#0911CC-01).
DNA was extracted using a DNEasy Blood and Tissue kit (Qiagen, Valencia, CA USA). We amplified a 1051 base pair region of mitochondrial cytochrome b DNA by polymerase chain reaction (PCR) using primers H15915 (5’-AACTGCAGTCATCTCCGG TTTACAAGAC-3’) and L14724 (5’-CGAAGCTTGATAGAAAAACCATCGTTG-3’). The PCR mixture contained 16.375 µL water, 2.5 µL buffer, 2 µL mixed dNTPs, 1 µL 3mM MgCl2, 0.5 µL BSA, 0.25 µL of each primer, 0.125 µL taq polymerase and 2 µL of DNA for a total of 25 µL reactions. Conditions for amplification comprised an initial denaturing step for 1 minute at 94°C followed by 30 thermal cycles of 94°C (for 45 s), 50°C (for 55 s), and 72°C (for 55 s) for melting, annealing, and extension respectively. A final extension step of 72°C was conducted for 1 min 30 s (Brandt & Orti, 2002). PCR product was visualized using gel electrophoresis. PCR was then purified with Exosap and sequenced in the forward and reverse directions using Big Dye terminator procedures by the University of Tennessee at Knoxville Molecular Biology Research Facility. Sequence data was then analyzed using the computer program Sequencher 4.7 to ensure correct nucleotide identification and MacClade 4.08 to align the sequences for tree building. Generated sequence data was compared to other genetic data for Blarina archived in the GenBank database (Brant & Orti 2002, 2003). Sequences for each newly collected individual were submitted to NCBI GenBank (Accession numbers XXXXXXX-XXXXXXXX).

Maximum parsimony and Bayesian phylogenetic trees were created to identify shrews to species and analyze the population structure of Blarina. PAUP 4.0b10 was used to complete maximum parsimony analysis by heuristic searches starting with stepwise addition and replicated 15 times. The initial tree was estimated at random. Because Blarina exhibits high transition (TS) and transversion (TV) saturation at the third codon position (Brant & Orti, 2002) we down weighted the third codon informative value. In PAUP, we partitioned the characters into first,
second, and third codon positions and then weighted the third codon according to PAUP’s rescaled consistency index. Tree-bisection-reconnection was used for branch swapping and 50% majority rule consensus was used for tree building. A bootstrap analysis of 500 replicates was used to measure statistical support for the resulting phylogenetic tree. We used BEAST 1.6.1 to construct a phylogenetic tree using Bayesian analysis (Drummond & Rambaut, 2007). BEAST uses a Markov chain Monte Carlo (MCMC) to estimate equilibrium distributions and is improved as the number of steps increases. Posterior probabilities were calculated from $10^7$ iterations after the first $10^5$ iterations were discarded as burn-in. We used the HKY+I+G model of DNA substitution (Hasegawa et al., 1985; Yang, 1993; Gu et al., 1995). We constrained the third codon position in order to limit saturation effects of TS and TV by partitioning into three codons and weighting the third as 0.5 rather than 1.0. TreeAnnotator 1.6.1 was used to create a maximum clade credibility tree from 10,001 trees after a burn-in of 1,000 trees. The program FigTree 1.3.1 was used to visualize the tree. For both parsimony and Bayesian analyses, we used Cryptotis parva as the outgroup (George, 1986).

A molecular clock analysis was completed using BEAST 1.6.1 to measure the time to most recent common ancestor ($T_{MRCA}$) of all phylogroups. A Bayesian skyline plot was used as the demographic model (Drummond et al., 2005). This model allows estimation of genealogy, nucleotide rate substitution, and demographic parameters based on the dataset. To calibrate the clock, we used Jones et al. (1984) fossil data to estimate divergence times of the Blarina – Cryptotis division (2.2mya) and the B. brevicauda – B. carolinensis division (1.8mya). We ran the analysis for $10^7$ iterations and discarded the first $10^5$ iterations as burn-in. Results were visualized using the program Tracer 1.6.1. We repeated this process three times in an attempt to
minimize age ranges for $T_{MRCA}$, and we report the mean $T_{MRCA}$ and 95% credibility intervals (similar to 95% confidence intervals).

SAMOVA version 1.0 was used for geographical analyses of monophyletic groups. SAMOVA combines spatial latitude and longitude data with an analysis of molecular variance (AMOVA) to determine population structure (Dupanloup et al., 2002). This analysis was completed for *Blarina brevicauda* and *Blarina carolinensis* independently. We assumed 3 geographical distributions for *B. brevicauda* and 2 geographical distributions for *B. carolinensis*. We evaluated population growth of each phylogroup using Fu’s $F_S$ statistic (Fu, 1997) and determined the extent of genetic diversity among phylogroups by calculating F-statistics in Arlequin 3.11 under the null hypothesis that there is no diversity among groups. Maps of *Blarina* distributions were created using ArcMap10 (ESRI, 2011).

**Results**

*Genetic Identification of Blarina Species*

We were able to successfully extract and sequence 1051 nucleotide base pairs for fifty-three *Blarina* specimens. Thirty-two of these shrews were genetically identified as *Blarina brevicauda* and twenty-one were identified as *Blarina carolinensis*. All shrews caught on the northern and western sides of the Tennessee River were identified as *B. brevicauda*. Shrews caught in the southern Unaka Mountains in North Carolina were also identified as *B. brevicauda*. Shrews throughout the Valley and Ridge and parts of the Cumberland Plateau on the south side of the Tennessee River were identified as *B. carolinensis*. One specimen from the southern side of the river Cash House locality in the Tennessee River Gorge of the Cumberland Plateau was identified as *B. carolinensis* and two other specimens from the same site were identified as *B.
brevicauda. A map of the distributions of the two species (Figure 1) and measurements and locality data (Appendix C) are provided.

Figure 1 Location of collecting localities and Blarina species distributions in southeast Tennessee. *B. brevicauda* was genetically identified from sites represented by black circles and *B. carolinensis* was genetically identified from sites with black triangles. The TRGT Cash House site, represented by the black star, contained one shrew identified as *B. carolinensis* and two shrews identified as *B. brevicauda*. To visualize the divisions within each species, refer to Figures 3 and 4.

**Phylogenetic Inference**

Both Bayesian and parsimony phylogenetic analyses resulted in a tree similar to that of Brant and Orti (2002). We found strong support for a monophyletic group of *Blarina brevicauda* and *Blarina carolinensis*, with *Blarina hylophaga* the basal, sister species for the genus. From *B. brevicauda* we found support for clades previously discovered by Brant and Orti (2003) and named ‘Western’, ‘East Central’, and ‘Appalachian’ haplotypes. The Western clade was
comprised exclusively of GenBank sequences. Of the specimens we collected, 25 *B. brevicauda* specimens from southeast Tennessee and southwest North Carolina resolved with the Appalachian clade. The other 7 *B. brevicauda* specimens resolved with the East Central haplotype. Bayesian posterior probabilities also supported a division within the East Central haplotype into what we have designated ‘East Central – North’ and ‘East Central – South’ groups. *B. brevicauda* from southeast Tennessee resolved with GenBank sequences from Kentucky into the ‘East Central – South’ clade. *B. brevicauda* GenBank sequences from Ohio, Indiana, and Wisconsin made up the ‘East Central – North’ clade. This division within the Eastern *B. brevicauda* clade was not supported by parsimony analysis, not was it supported by Brant and Orti (2003). *B. carolinensis* was also split into ‘Eastern’ and ‘Western’ clades. All *B. carolinensis* from southeast Tennessee resolved within the Western clade, aligning with *B. carolinensis* GenBank sequences from Louisiana, Arkansas, and Illinois. All of these groups can be seen in the tree, redrawn from FigTree output, in Figure 2 below.

_Molecular Clock $T_{MRCA}$ Estimates_

Age estimates and 95% credibility intervals for haplotypes from the phylogenetic tree in Figure 2 are reported below (Table 2). The large ranges of the credibility intervals are likely due, in part, to inexact estimations of nucleotide substitution rates. The cytochrome $b$ sequence is known to evolve at different rates in different species (Irwin et al., 1991) and therefore the dynamic model used was deemed appropriate in the absence of empirically determined evolutionary rates for _Blarina_ cytochrome $b$. 
Table 1

Ages of most recent common ancestors for the nodes on the phylogenetic tree from Figure 2. Age estimates are in millions of years and represent the mean of three replications. Asterisks represent nodes calibrated using fossil data.

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>T_{MRCA} Mean (Lower limit–Upper limit) (Millions of years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptotis sp.</td>
<td>Blarina sp.</td>
<td>2.588 (2.134–3.054)*</td>
</tr>
<tr>
<td>B. hylophaga</td>
<td>B. carolinensis &amp; B. brevicauda</td>
<td>1.995 (1.603–2.361)</td>
</tr>
<tr>
<td>B. carolinensis</td>
<td>B. brevicauda</td>
<td>1.667 (1.334–2.029)*</td>
</tr>
<tr>
<td>B. carolinensis ‘Eastern’</td>
<td>B. carolinensis ‘Western’</td>
<td>0.882 (0.4713–1.3869)</td>
</tr>
<tr>
<td>B. brevicauda ‘East Central’ &amp; B. brevicauda ‘Appalachian’</td>
<td>B. brevicauda ‘Western’</td>
<td>0.488 (0.2305–0.7773)</td>
</tr>
<tr>
<td>B. brevicauda ‘East Central’</td>
<td>B. brevicauda ‘Appalachian’</td>
<td>0.2905 (0.1484–0.4606)</td>
</tr>
</tbody>
</table>

Population structure of Blarina

*Blarina carolinensis* – Results of the SAMOVA analysis support a separation of *B. carolinensis* into the aforementioned Eastern and Western haplotypes. Results indicate a longitudinal separation of the *B. carolinensis* Eastern group from the *B. carolinensis* Western group. The Eastern group seems to be comprised of populations confined to the Atlantic Coastal Plain and lowlands of Virginia, Georgia, and Florida whereas shrews from southeast Tennessee and westward to the Gulf Coastal Plain of Louisiana, Arkansas, and southern Illinois resolve with the Western phylogroup. Fu’s $F_S$ was calculated (Table 3) and showed population expansion of the Western, but not Eastern, group. The genetic variance between the two groups was statistically significant, as $F_{ST} = 0.74264$, $P << 0.01$. Small sample size of the Eastern phylogroup of *B. carolinensis* (n=6) may skew these results.
Table 2

AMOVA $F_{ST}$ and Fu’s $F_S$ for haplotypes of *Blarina carolinensis*. $F_S$ for the Western haplotype indicates range expansion.

<table>
<thead>
<tr>
<th></th>
<th>Eastern group</th>
<th>Western group</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{ST}$ between groups</td>
<td>0.74264, $P&lt;&lt;&lt;0.01$</td>
<td></td>
</tr>
<tr>
<td>Fu’s $F_S$</td>
<td>0.13189, $P=0.323$</td>
<td>-17.95013, $P&lt;&lt;&lt;0.001$</td>
</tr>
</tbody>
</table>

*B. brevicauda* – The West, East Central, and Appalachian haplotypes of *B. brevicauda* were also supported by the SAMOVA analysis. I identified a longitudinal separation of the West *B. brevicauda* phylogroup from the East Central and Appalachian phylogroups. This separation correlated with the Mississippi River, a barrier to gene flow between the groups (Brant & Orti, 2002). Separation was also seen between the East Central and Appalachian clades. The geographic division between the two groups is likely a diagonal separation extending along the eastern edge of the Cumberland Plateau in the south and the western edge of the Allegheny Plateau in the north. SAMOVA did not clearly separate the groups, possibly because multiple populations in Southeast Tennessee and Central Ohio contained at least one *B. brevicauda* specimen that resolved in the East Central group and others that resolved in the Appalachian group, or vice versa. Table 3 shows Fu’s $F_S$ values for the three groups. All indicate population expansion, and the variance among the groups was significant.
Table 3

Results for AMOVA analysis of *Blarina brevicauda* haplotypes. All $F_S$ values indicate recent population expansion. $F_{ST}$ comparisons show strong support for the definition of each clade as a population.

<table>
<thead>
<tr>
<th></th>
<th>Western</th>
<th>East Central</th>
<th>Appalachian</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F</em>&lt;sub&gt;S&lt;/sub&gt;</td>
<td>-4.91497 (P=0.008)</td>
<td>-7.65528 (P&lt;0.001)</td>
<td>-24.81204 (P=0.012)</td>
</tr>
<tr>
<td>$F_{ST}$ x Western</td>
<td>***</td>
<td>0.65436 (P&lt;0.001)</td>
<td>0.71195 (P&lt;0.001)</td>
</tr>
<tr>
<td>$F_{ST}$ x East Central</td>
<td>0.65436 (P&lt;0.001)</td>
<td>***</td>
<td>0.34654 (P&lt;0.001)</td>
</tr>
<tr>
<td>$F_{ST}$ x Appalachian</td>
<td>0.71195 (P&lt;0.001)</td>
<td>0.34654 (P&lt;0.001)</td>
<td>***</td>
</tr>
</tbody>
</table>

**Discussion**

*Historical Distribution vs Current Ranges*

Previous morphometric studies were unclear as to which species of *Blarina* was found in Middle and East Tennessee (French, 1981; Braun & Kennedy, 1983; Jeffries, 1999; Webster *et al*., 2011). Based on the data collected from this study, *Blarina carolinensis* can be found on the southern and eastern sides of the Tennessee River in southeast Tennessee and *Blarina brevicauda* can be found on the northern and western sides of the Tennessee River in southeast Tennessee and at high elevations in the southern Unaka Mountains of North Carolina. This genetic evidence does not agree with previous studies that describe *B. brevicauda* as occurring throughout east Tennessee (Braun & Kennedy, 1983) or that identify a possible subspecies of *B. brevicauda* in the Cumberland Plateau (Webster *et al*., 2011). The northern range expansion of *B. carolinensis* into the Valley and Ridge system of East Tennessee is likely contained by the Tennessee River as it crosses the region from east to west near Knoxville, TN, based on previous morphometric results (French, 1981; Jeffries, 1999). Both species were found in one location on
the southern banks of the Tennessee River in the Tennessee River Gorge just to the west of Chattanooga. This likely indicates a zone of sympatry that might extend throughout much of the Tennessee River Gorge. Pleistocene aged *B. brevicauda* remains have been collected from Lookout Mountain (Gaudin *et al*., 1998) in Tennessee, the Valley and Ridge of northwest Georgia, and the coastal plain in southeast Georgia (Hulbert & Pratt, 1998). Similarly aged *B. carolinensis* remains were found from the Georgia sites. Today, only *B. carolinensis* is known from these locales in Georgia, whereas *B. brevicauda* is not. Further sampling efforts in the gorge could help elucidate how far this zone extends and if other *B. brevicauda* individuals occupy other areas on the southern banks of the Tennessee River.

The results from this study confirm that the range of *B. carolinensis* extends into the southeast of Tennessee, and fossil evidence suggests this is a recent, Holocene incursion (Klippel & Parmalee, 1982; Jeffries, 1999; Gaudin *et al*., 2011). Fossil evidence also indicates sympatry between the two species in Georgia throughout the Pleistocene, similar to the sympatry seen at one site in the Tennessee River Gorge. We were unable to determine whether *B. brevicauda* has emigrated from the Valley and Ridge area in southeast Tennessee due to changes in habitat and climate, or if *B. carolinensis* actively outcompeted *B. brevicauda* in the area. A more thorough examination of the fossil record, which should include extensive dating of fossils and the discovery of more fossils from new localities, would be needed to answer this question.

Areas directly northeast of Chattanooga on the eastern and western banks of the Tennessee River might show additional areas of sympatry between the species, similar to the TRGT Cash House site. We were unable to sample from this area due to a lack of trappable public land that did not succumb to seasonal flooding. The sympatry found at the TRGT Cash House site is peculiar. Since *B. brevicauda* was once found in the areas south of the Tennessee
River (Gaudin et al., 1998; Gaudin et al., 2011; Jeffries, 1999) and its range has since moved northward, these *B. brevicauda* specimens may represent some of the last *B. brevicauda* populations south of the Tennessee River. However, it is also possible these shrews crossed the river recently from the northern banks. *Blarina* have been documented swimming for brief periods (Fowle & Edwards, 1955), do not appear capable of traveling great distances across water (Getz & McGuire, 2008). That said, a local Tennessee River tributary, Suck Creek, is known to swell and create strong currents during heavy rain events. Suck Creek is located almost directly opposite of the TRGT Cash House site. The *B. brevicauda* specimens may have been “pushed” across the river during a flood event and subsequently captured. Determining the source population of these two specimens by genetic analysis was attempted by comparing mtDNA to sequences to those from the northern banks of the river, but results were inconclusive. Thus, we do not know if these individuals collected represent a stable resident population or are merely transients.

Since the two species seem to occupy the same area in at least part of their range a question regarding hybridization is raised. Identifying possible hybrids was beyond the scope of this study. Hybridization has been claimed to occur in sympatric zones between *Blarina hylophaga* and *Blarina brevicauda* in parts of the Midwest (Benedict, 1999). There, hybrids were identified using a combination of morphological characteristics and mitochondrial genetic markers; if a specimen’s size identified it as one species and mitochondrial DNA identified it as the other species, it was considered a hybrid. This designation of a hybrid might falsely identify specimens that are simply variable in size. Hybridization between *B. brevicauda* and *B. carolinensis* has not been studied. Because the diploid chromosome numbers of the two species are very different (*B. brevicauda* FN=48, 2n=48-50 and *B. carolinensis* FN=41-45, 2n=31-46),
reproduction between the two species would likely not result in viable offspring. Nevertheless, analysis of nuclear and mitochondrial DNA of *Blarina* in southeast Tennessee might be useful in investigating whether hybrids occur.

*Phylogeography of* Blarina carolinensis *and* Blarina brevicauda

One of the major goals of this study was to fill a gap in the data from previous studies of *B. brevicauda* and *B. carolinensis* biogeography in the southeastern U.S. (French, 1981; Braun & Kennedy, 1983; Jeffries, 1999; Brant & Orti, 2002, 2003; Webster *et al*., 2011). Phylogenetic analyses of sequence data generated from this study supports the phylogroups previously described by Brant and Orti (2002, 2003). *B. carolinensis* from the study area in this report resolves with shrews from Louisiana, Arkansas, and southern Illinois into a ‘Western’ clade (Figure 3). *B. brevicauda* from the study area resolve into either an ‘Appalachian’ clade with shrews from many northeastern states covered by the Appalachian mountains, or an ‘East central’ clade with shrews from the Midwestern states of Ohio, Indiana, Kentucky, and Wisconsin (Figure 4). Considering the relatively small home ranges of *Blarina* individuals and strong statistical phylogenetic support for each haplotype, it is no surprise that both species fill the requirements for a Category I phylogeographic hypothesis characterized by large mutational distances and spatial structuring of haplotypes (Avise, 2000). Many mtDNA surveys are consistent with Category I patterns.

Fossil evidence of *B. carolinensis* strongly suggests a recent range expansion into southeast Tennessee (Jeffries, 1999; Gaudin *et al*., 2011). Our results (Table 3) showed genetic support for expansion northward through the Valley and Ridge physiographic province of southeast Tennessee. Sampling throughout west and middle Tennessee might also provide
support for fossil evidence of recent expansion of *B. carolinensis* into Maury County, Tennessee (Klippel & Parmalee, 1982). Genetic data from this study strongly suggests shrews from the Tennessee Valley and Ridge are more genetically similar to *B. carolinensis* from the Gulf Coastal Plain and Mississippi River Valley in Louisiana, Arkansas, and southern Illinois. Other sequence data of specimens from the Atlantic Coastal regions of Georgia, Florida, and Virginia resolve into an Eastern clade. Allopatry of these two clades is suggested by SAMOVA analysis, although there is a considerable data gap in these geographic ranges throughout western Georgia and all of Alabama and Mississippi (Figure 3). Considering the general patterns of phylogeographic breaks in the eastern U.S., we suspect sampling through this area would show separation of the clades along the Apalachicola River or Tombigbee River (Soltis *et al*., 2006). Lack of *B. carolinensis* samples from Alabama, Mississippi, and western Georgia make it impossible to conclude if either of these common phylogeographic patterns is congruent to the Western and Eastern clades of *B. carolinensis*. Sea level and temperature changes in the Pliocene and Pleistocene have likely caused these patterns, suggesting repeated fragmentation and isolation of haplotypes (Scott & Upchurch, 1982; Riggs, 1983).

Genetic variance between the Eastern and Western clades of *B. carolinensis* was not surprising and indicates distinct lineage sorting that is most likely a product of separation by a major river like the Apalachicola or Tombigbee. Southeastern rivers have been identified as geographic barriers for haplotypes of many terrestrial organisms both small and large (*Avise et al.*, 1979; Hayes & Harrison, 1992; Ellsworth *et al*., 1994; Solis *et al*., 2006). The Western group was shown to have recently expanded, most likely moving into habitats once occupied by *B. brevicauda* during glacial periods. We estimated the most recent common ancestor of these two haplotypes to be approximately 882,000 years old (Table 2), but we felt the large range of
credibility intervals did not allow the association of this division with a specific glacial or interglacial period.

Brant and Orti (2002, 2003) discovered geographic separation of *B. brevicauda* haplotypes in the eastern United States confirmed in the present study, and described haplotype dispersal. The Western haplotype was separated from the Eastern (Appalachian and East Central) groups by the Mississippi River, a phylogeographic pattern not uncommon to terrestrial organisms (Al-Rabab’ah & Williams, 2002; Leache & Reader, 2002). Both the Western group and Eastern groups were found to have high haplotype diversity and low nucleotide diversity, indicating range expansion from refugia occupied during the glacial maximums of the Pleistocene. As glaciers melted, *B. brevicauda* moved northward from the southeast to its’ current range (Brant & Orti, 2003). This is supported by our Fu’s $F_S$ statistics for the *B. brevicauda* haplotypes. Although the fossil record of *Blarina* suggests a retreat of the southern range boundary, because Pleistocene aged fossils of both *B. brevicauda* and *B. carolinensis* were found throughout Georgia (Hulbert & Pratt, 1998), the overall ranges of the groups has increased northward in the present interglacial period. The division of the Eastern *B. brevicauda* clade into Appalachian and East Central haplotypes was strongly supported by SAMOVA analysis. Brant and Orti (2003) analyzed the two groups as one large Eastern phylogroup, but we evaluated the two clades individually since the genetic variance between them was significant.

Separation of *B. brevicauda* specimens from southeast Tennessee into different phylogroups was not expected. Most (25/32) *B. brevicauda* resolved with the Appalachian haplotype, but some (7/32) resolved with the East Central haplotype (Figure 4). The OSFSP site was the only locality where samples exclusively resolved into the East Central phylogroup (n=3; CC48, CC52, and CC102). Some *B. brevicauda* samples from the PCSF Bluff Point (n=2; CC13
and CC31), Spencer (n=1; CC38), and Dunlap (n=1; CC55) sites resolved with the East Central phylogroup while the majority of samples from each location resolved into the Appalachian phylogroup. GenBank sequences from Brant and Orti (2003) from Wooster, Ohio showed similar results, with one *B. brevicauda* specimen resolving with the East Central clade and others resolving with the Appalachian clade.

When studied independently of the Western clade, the East Central and Appalachian groups weakly represented a Category II phylogeographic hypothesis, with deep genetic differences between groups that have some area of sympatry (Avise, 2000). Etiology of the two groups is difficult to understand and may have occurred via different dispersal routes from southern Appalachian Mountain refugia (Brant & Orti, 2003). The results of the molecular clock analysis did not provide much insight into the historical causes of the current pattern of genetic diversity within *Blarina*. The age of the most recent common ancestor of the haplogroups could not be tightly constrained to a range less than 312,000 years (Table 2), a time much greater than the average glacial and interglacial cycle (Kurten & Anderson, 1980). The phylogeographic pattern of *B. brevicauda* is similar to that shown in salamanders from the genus *Ambystoma* (Church *et al*., 2003) and the black rat snake, *Elaphe obsoleta* (Burbrink *et al*., 2000). Additional samples from these mitochondrial DNA lineages along the Cumberland Plateau and Allegheny Plateau should be coupled with nuclear DNA sequence data to better understand the population structures and degree of hybridization between the two clades (Avise, 2000).

An interesting split was seen within the East Central group when Bayesian inference was employed. The East Central specimens from southeast Tennessee grouped with GenBank sequences from Kentucky to form a southern East Central group. The remaining East Central GenBank sequences from Wisconsin, Indiana, and Ohio formed a northern East Central
phylogroup. This division was not recovered in the maximum parsimony analysis. Although the groups were geographically distinct, we did not evaluate the groups using SAMOVA or AMOVA since they were not supported in both phylogenetic trees. The small sample size of the East Central northern group also discouraged further analysis in the present study. A more thorough collection of shrews throughout the East Central area may illustrate another population division within *B. brevicauda*.

*The Role of Tennessee’s Physiographic Variation on Species’ Ranges*

Considering the phylogeographic break densities reported by Soltis *et al.* (2006) and the congregation of *B. brevicauda* and *B. carolinensis* haplotypes in the study area, southeast Tennessee should be regarded as a biogeographic ‘hot spot’. We collected shrews from four physiographic provinces in the study area. *Blarina* from these regions resolved into three different haplotypes, two within *B. brevicauda* and one within *B. carolinensis*. Small terrestrial mammals may be prone to reduced and restricted gene flow when facing landscapes with many geographic features. This might explain the large amount of local genetic diversity in these species.

Within southeast Tennessee, *B. carolinensis* has expanded the northern edge of its range into the Valley and Ridge province, most likely moving in a northeastern direction from northern Alabama and Georgia. *B. carolinensis* has been excluded from the Cumberland Plateau by the Tennessee River, which runs along the western edge of the Valley and Ridge. At this time, it is unclear if the Tennessee River continues to exclude *B. carolinensis* from moving north into middle Tennessee from northern Alabama. *B. brevicauda* was found at elevation in the southern Unaka Mountains, but it may be possible that *B. carolinensis* will expand into this province from
the eastern edge of the Valley and Ridge since there is no river barrier. Global climate change has been linked to elevation changes and northern range expansions of small-bodied mammals in the area (Keller et al., 2003; Eichler & Gaudin, 2011). Future collection efforts could monitor these changes in *Blarina* species distributions by focusing collecting activities on the elevational transition from the eastern edge of the Valley and Ridge province into the western edge of the Unaka Mountains.

*B. brevicauda* was found north of the Tennessee River in the Cumberland Plateau and the Eastern Highland Rim and east of the Valley and Ridge province in the Unaka Mountains. The East Central and Appalachian haplotypes of *B. brevicauda* were found in the Cumberland Plateau. *B. brevicauda* from the Eastern Highland Rim resolved solely with the East Central haplotype, whereas *B. brevicauda* from the Unaka Mountains, Hixson, TRGT Cash House and Cedar Mountain resolved with the Appalachian group. The remaining localities that lie in the Cumberland Plateau indicate an area of sympatry for the two haplotypes. Currently, most trapping localities in the Cumberland Plateau are protected by conservation easements and land trusts, ensuring an almost pristine landscape for wildlife. Any disturbance to this habitat could greatly affect the population dynamics and gene flow within *B. brevicauda* and either provide more dispersal pathways for shrews in the forms of roads and bridges, or create a fragmented landscape that isolates the haplotypes (Johnston & Collinge, 2004; McKinney, 2006). Regardless of land use, it will be important to monitor and increase the collection of samples from these clades to further increase knowledge of the natural history of *B. brevicauda*.
Conclusions

The ranges of *B. brevicauda* and *B. carolinensis* in southeast Tennessee have been studied since the 1980’s. Previous research attempted to distinguish the two species by size using morphometric analysis. The present study clarified historical uncertainty concerning *Blarina* ranges using mitochondrial DNA sequence data. Major findings of this research includes (1) presence of *B. carolinensis* in the southern Valley and Ridge province, (2) identification of the Tennessee River as a dispersal barrier for *B. carolinensis*, and (3) the resolution of *Blarina* specimens in southeast Tennessee into three major haplotype clades within *B. brevicauda* and *B. carolinensis*. The variable geographic landscape of southeast Tennessee, consisting of four physiographic provinces, is proposed to be significant in explaining the high genetic diversity of *Blarina* in this region.
Figure 2 Phylogenetic tree for *Blarina* based on cytochrome *b* gene sequences from 53 specimens from southeast Tennessee and 101 *Blarina* and *Cryptotis* (outgroup) GenBank sequences. Bootstrap values based on maximum parsimony are shown above the branches and Bayesian posterior probability values are shown below the branches. The ‘n’ value under each species and haplotype are the number of Genbank sequences that make up that group, and is followed by the UTC ID of samples from southeast Tennessee (see Table 1).
Figure 3 Distribution of *Blarina carolinensis* haplotypes in southeast Tennessee (A) and the eastern United States (B). All *B. carolinensis* specimens from southeast Tennessee resolved with the Western haplotype, represented by solid circles. The western haplotype is represented by solid diamonds. Inferred range edges are drawn around each group using dashed lines. The gray area in B represents a paucity of genetic data from the region.
Fig. 4 Haplotype distributions of *Blarina brevicauda* in southeast Tennessee (A) and the eastern United States (B). East Central haplotypes are represented by squares, Appalachian haplotypes are represented by triangles, and localities containing both haplotypes are represented by circles. Inferred ranges are drawn using dashed lines. Arrows in A represent the boundary between *B. brevicauda* and *B. carolinensis*. The shaded region in B is a likely zone of sympatry between the two haplotypes and should be a focus for future research.
CHAPTER III
CONCLUSIONS AND FUTURE RESEARCH

While completing my master’s research at the University of Tennessee at Chattanooga, I ultimately answered three questions about the distributions of Blarina species in southeast Tennessee. First, I was able to determine the species ranges within the area using mitochondrial DNA sequence data. I found that previous studies by French (1981) and Jeffries (1999) were accurate in their conclusions of ranges of *B. carolinensis* within the Valley and Ridge province of southeast Tennessee. *B. brevicauda* is found throughout the rest of the areas in southeast Tennessee, mostly north of the Tennessee River. Second, I was able to directly compare the sequences I generated to those from Brant and Orti (2002, 2003) and analyze how they resolved into the previously discovered population structures. *B. carolinensis* from southeast Tennessee resolved with shrews from Louisiana, Illinois, and Arkansas into a Western haplotype. *B. brevicauda* from southeast Tennessee resolved into either the Appalachian or East Central clades reported by Brant and Orti (2002, 2003). Third, I was able to compare the genetic diversity of the genus with the varied physiographic landscape of southeast Tennessee. The Valley and Ridge province has likely served as a low elevation corridor for northern expansion of *B. carolinensis* into southeast Tennessee. But, the species is likely contained to the west by the Tennessee River, given the two species are separated in the Tennessee River Gorge by the water barrier.

By collecting tissue samples from shrews over a broad geographic area, I was able to determine how some of the geographic features of southeast Tennessee contribute to species and
population distributions of small-bodied terrestrial organisms. I believe that I sufficiently sampled the area for the scope of my project, but there is room for improvement. Efforts could be made to increase the sample size by trapping new localities throughout the region, specifically areas directly north and south of the Tennessee River in northern Alabama and east and west of the river northeast of Chattanooga. Data from these areas would prove valuable in examining the extent to which the Tennessee River bounds *B. carolinensis*, preventing further range expansion.

Adding sample localities in the Cumberland Plateau and Eastern Highland Rim and further north into the plateau of Kentucky, West Virginia, and Ohio would contribute to the study of the East Central and Appalachian haplotypes, and to what extent the ranges of these clades of *B. brevicauda* overlap. Hybridization between these groups (and similarly between *B. brevicauda* and *B. carolinensis*) could also be examined using a combination of mitochondrial and nuclear DNA data. Mitochondrial DNA would enable researchers to trace maternal lineages of a species and nuclear DNA would be valuable for identifying actual hybridization between haplotypes or species.

It would also be interesting to see how an organism with a different dispersal mechanism is distributed throughout the study area. Examining the patterns across a wider variety of organisms might reveal a pattern and help explain the large number of phylogeographic breaks detected in the area by Soltis *et al.* (2006). Surveys in the Valley and Ridge might also reveal new species records or plants and animals in the Valley and Ridge physiographic province that are normally associated with the Gulf Coastal Plain of western Tennessee.

Given more time and resources, I would also have liked to collect samples from Alabama and Mississippi. There is a paucity of *Blarina* mitochondrial DNA data from this region. In order to determine the phylogeographic break between the Eastern and Western clades of *B.*
*carolinensis*, collections could be made on eastern and western sides of the Tombigbee and Apalachicola rivers. These two rivers have been identified as important biogeographic boundaries separating haplotypes in a variety of other organisms (Soltis *et al*., 2006). By collecting from these additional locations, it is likely that the location of the boundary between *B. carolinensis* haplotypes could be more accurately circumscribed.
REFERENCES


APPENDIX A

PHYSIOGRAPHIC PROVINCES OF

SOUTHEAST TENNESSEE
Appendix A. Physiographic provinces of southeast Tennessee. The following is a description of selected physiographic provinces of middle and east Tennessee. Data and descriptions are modified from Luther (1977) and Fullerton and Ray (1977).

<table>
<thead>
<tr>
<th>Province</th>
<th>Total Area</th>
<th>Elevation and Topography</th>
<th>Soil type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern Highland Rim</td>
<td>~6475km²</td>
<td>Average elevation of 305m; mostly level tableland cut by thin valleys</td>
<td>Formed primarily from limestone, chert, shale, and dolomite; strongly acidic</td>
<td>Part of the Highland Rim that surrounds the Central Basin in middle Tennessee averaging 40km in width and runs north-south across the state; covered mostly by deciduous forests with areas of dense cropland</td>
</tr>
<tr>
<td>Cumberland Plateau</td>
<td>~12950km²</td>
<td>Average elevation of 610m with some peaks higher than 1000m restricted to the northern portion of the plateau; tableland with extensive valleys, gorges, and escarpments</td>
<td>Shale, siltstone, clay, and limestone; loamy and acidic</td>
<td>Bordered by the Eastern Highland Rim to the west and the Valley and Ridge to the east; runs across the state diagonally and is 113km wide in the northwest and 80km wide in the southeast; covered mostly by deciduous forest</td>
</tr>
<tr>
<td>Valley and Ridge</td>
<td>~23000km²</td>
<td>Average elevation of 300m in the north and 230m in the south; mostly rolling upland divided by parallel ridges, valleys, and ravines</td>
<td>Limestone, shale, siltstone, sandstone, marble, and chert; highly acidic in the uplands but not acidic in valley floors</td>
<td>Lies between the Cumberland Plateau and Unaka Mountains and runs diagonally across the state from Chattanooga to the northeast Tennessee border; about 97km wide and covered by forests, cropland, and suburban development</td>
</tr>
<tr>
<td>Unaka Mountains</td>
<td>~4670km²</td>
<td>Average elevation of 900m but ranges from 300m to 2025m at Clingman’s Dome; rugged and mountainous with some sheltered coves</td>
<td>Igneous and metamorphic rock formations and outcrops; loamy, shallow soils</td>
<td>Easternmost province bordered by Valley and Ridge to the west; mostly forested with some suburban development</td>
</tr>
</tbody>
</table>
APPENDIX B

SAMPLING LOCALITIES FOR
SHORT-TAILED SHREWS
Appendix B. Sampling localities for short-tailed shrews. The following is information pertaining to the thirteen trapping localities proposed for this study.

<table>
<thead>
<tr>
<th>Name</th>
<th>County, State</th>
<th>GPS and Elevation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huckleberry Knob</td>
<td>Graham County, NC</td>
<td>35.3202, -83.9918</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1650m</td>
</tr>
<tr>
<td>Loudon</td>
<td>Loudon County, TN</td>
<td>35.692907, -84.432128</td>
</tr>
<tr>
<td></td>
<td></td>
<td>287m</td>
</tr>
<tr>
<td>McMinn</td>
<td>McMinn County, TN</td>
<td>35.38917, -84.7198</td>
</tr>
<tr>
<td></td>
<td></td>
<td>243m</td>
</tr>
<tr>
<td>Cleveland</td>
<td>Bradley County, TN</td>
<td>35.21185, -84.85197</td>
</tr>
<tr>
<td></td>
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APPENDIX C

MEASUREMENTS OF EXTERNAL MORPHOLOGY

OF CAPTURED SHREWS
Appendix C. Standard measurements of external morphology for short-tailed shrews collected from 13 trapping localities. Shrew species were identified using cytochrome b DNA sequence data.

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VITA

Casey Carpenter was born in Jackson, Tennessee on September 11, 1985. He grew up in Jackson and graduated from Jackson Christian School in 2004. He then attended the University of Georgia in Athens, GA where he majored in Genetics and received a Bachelor’s degree in 2008. After graduation from UGA, Casey taught English and Science to non-native English speakers in Bangkok, Thailand. He then traveled around the swamps of south Georgia and southern Boreal Forests of north New York to study bat roosting ecology. He completed his M.S. in Environmental Science from the University of Tennessee at Chattanooga in 2013.