Phylogenetics, biogeography and co-adaptation between a Batesian model (*Micrurus fulvius*) and mimic (*Lampropeltis triangulum*)

by

Michael Friedman

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11/7/08	Rob DeSalle
Date	Chair of Examining Committee
11/7/08	Laurel A. Eckhardt
Date	Executive Officer

Chris Raxworthy
Janis Roze
Robert Rockwell
John Wahlert
Supervisory Committee

The City University of New York

Abstract

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Advisor: Dr. Rob DeSalle

The coral snake, *Micrurus fulvius* (Elapidae), and the scarlet king snake, *Lampropeltis triangulum* (Colubridae), members of a Batesian mimicry complex in the southeastern and southern United States were subjects of a study of model-mimic co-evolution. Inferring co-evolution required discovery of distinct terminals within each group, for posterior phylogenetic inference and analysis of co-variation of color patterns among sympatric populations of each species. In addition, haplotype and population differentiation were assessed. Also, hypotheses regarding selection on color patterns were tested. The latter included model-mimic tracking, environmental effects and frequency dependence of mimetic color pattern variation. Population Aggregation Analysis failed to diagnose terminals for either group. Haplotype and population genetic analyses showed both groups to be largely panmictic, with some north/south differentiation. Morphometric analyses of means and variations in proportions of color pattern components failed to reveal geographic tracking by mimics of models, but manifested possible independent geographic or environmental effects on each of the species. Frequency-dependent selection was falsified for color pattern variation in mimics.

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I. General Introduction

In 1862, H.W. Bates hypothesized that certain noxious insects warn off predators with aposematic coloration, and that other, inoffensive insects derive protection by mimicking the coloration of the noxious species (Bates, 1862). A century and a half of laboratory and field research confirmed Bates' hypothesis for a wide variety of organisms and warning modalities, ranging from flatworms that apparently mimic venomous snakes through complex systems of butterfly mimicry and snake mimicry, including visual, kinesthetic and auditory modalities (Pough, 1988; Greene and McDiarmid, 2005). In 1879, Fritz Mueller formulated a related hypothesis covering mimicry complexes in which both organisms were, to varying degrees, toxic, which was thenceforth-designated "Muellerian mimicry."

With respect to Batesian mimicry systems, much subsequent research has sought to address questions relating to the behavioral ecology and evolution of mimicry systems, especially regarding the origin and subsequent evolution of the respective model and mimic warning signals (Waldbauer, 1988; Joron and Mallet, 1998). What was initially conceived as relatively straightforward purifying selection, causing mimics to converge on models, has proven to be more complex. Authors have employed field research, phylogenetics and mathematical modeling -- particularly game theory -- to investigate hypothesized arms races between models and mimics (Gavrilets and Hastings, 1998; Ceccarelli and Crozier, 2006), the causes of mimic variation, "imperfection" or polymorphism,

(Joron and Mallet, 1998; Edmunds, 2000; Johnstone, 2002; Harper and Pfennig, 2007a), and perception and memory and/or innate avoidance in predators (Chittka and Osorio, 2007; Pfennig, 2001).

The two snakes selected for this study, the nonvenomous scarlet king snake (*Lampropeltis triangulum elapsoides*) and the venomous eastern coral snake (*Micrurus fulvius fulvius*) form a Batesian mimicry complex, together with the non-venomous scarlet snakes (*Cemophora coccinea*) [Figure 1-1], all found in the southeastern United States. Although some authors (Grobman, 1978) questioned

Figure 1-1. Members of a Batesian mimicry complex in the southern United States. Top left, *Micrurus fulvius*; middle, *Lampropeltis triangulum*; bottom right, *Cemophora coccinea elapsoides.* Photos by M. Friedman.

the existence of Batesian mimicry among these snakes, repeated experimental data confirmed the selective advantage of the aposematic coloration in encounters with predators. A recent study by Pfennig, et al. (2001) confirmed that

aposematic coloration increases fitness of *L. triangulum* where these are sympatric with *M. fulvius*, as compared with allopatric mimics. Another study by Harper and Pfennig (2007b) showed that color patterns among mimics tend to break down where models are not present, although allopatric mimetic patterns may be maintained by gene flow.

Mimic and model color patterns are concordant where they live in sympatry. Various authors have commented on the remarkable parallels in color pattern between Micruruine elapids and members of the *L. triangulum* complex, together with other mimics, across the overall distribution of these taxa (Greene and McDiarmid, 2005). However, this agreement is not perfect: mimic subspecies appear to be polymorphic in some areas where multiple models are present (Greene and McDiarmid, 2005), and even among the scarlet king snakes, *Lampropeltis triangulum elapsoides*, the mimicry is not perfect. In general, scarlet king snakes in the United States have a different sequence of bands than their models, possibly due to developmental constraints. In addition, mimics show considerable variation in the width of their red, black and yellow bands (Williams, 1988). Geographic groupings of *M. fulvius* also show regional variation (Roze, 1996). Which, again, raises the question of the modality of evolutionary interaction between the *M. fulvius* and *L. triangulum*. Specifically, do the two co-evolve? Does mutual selection actually drive an “arms race”? Is there a frequency-dependent effect? Is there environmental influence?

The phylogenetics of *L. triangulum* and *M. fulvius* pose challenges to systematists, more so in the case of *L. triangulum*. Phylogenetic inference and taxonomic classification of the genus *Lampropeltis*, and *L. triangulum*, in particular, have proven difficult. This is largely due to the nominal species' (*L. triangulum*) extremely wide distribution (Canada to Ecuador) and a phylogenetic character assessment made difficult by homogeneity. Recent debates have addressed the species status of *L. triangulum*, its phylogenetic relationships with other members of the genus *Lampropeltis* and the status of various subspecies, including *L. t. elapsoides* (Burbrink and Lawson, 2007; Harper and Pfennig, 2007b). All 25 subspecies of the nominal species *L. triangulum* are currently under revision (Burbrink, 2008). *L. t. elapsoides*, itself, is intriguing from a taxonomic standpoint. Phylogenies based on mitochondrial DNA group this southeastern U.S. snake apart from other conspecifics (Harper and Pfennig, 2007b). It is sympatric with three other subspecies, and allegedly interbreeds with at least one of them, *L. t. triangulum*, but only in part of their joint distribution. Elsewhere they behave like valid, separate biological species (Williams, 1988).

While the genus *Micrurus* doesn't pose the problems of being a single nominal species, there has not been a great deal of phylogenetic work on the taxon and there are still questions regarding the relationships between the three genera that comprise the Micrurines, as well as the pattern of descent within the genus

Micrurus. Within *Micrurus fulvius*, there are 5 subspecies whose phylogenetic status awaits clarification (Roze, 1996). While one of these subspecies, *Micrurus fulvius fulvius*, is sympatric with *L. t. elapsoides*, there have been efforts in the past to recognize a second subspecies in southern Florida (Roze, 1996).

Phylogenetic resolution of terminals within these two taxa would allow me to employ phylogenetic tools to test hypotheses of co-evolution, driven by Batesian mimicry (Brooks and McLennan, 1991; Brower, 1996; Ceccarelli and Crozier, 2006). Through its use of nested hierarchies of homologies, systematics permits the formulation and testing of hypotheses of evolutionary relationships among organisms. The patterns of relationship derived from systematic analyses then enable us to test hypotheses concerning causal historical processes. The methods of phylogenetic systematics can be fruitfully applied to investigating origins and causal processes in the evolution of behaviors, ecotypes and ecological associations (Brooks and McLennan, 1991). Thus, I believe that phylogenetic systematics is an appropriate framework to study the evolution of mimicry.

This study will test the hypothesis of co-evolution between *L. t. elaposides* and *M. fulvius fulvius*. Co-evolution between model and mimics, driven by the mimetic association itself, will be supported if the two groups show significant geographic color pattern co-variation and phylogenetic congruence (cf. Brooks and

McLennan, 1991; Ronquist, 2003). Hypotheses of co-evolution may be evaluated by mapping color pattern co-variation on reconciled phylogenies for the mimic and the model, similar to the way in which species are mapped on area cladograms in vicariant biogeography (Brooks and McLennan, 1991). The specific methodology of this study is:

1. Test the hypothesis that geographically variant populations of the two nominal species constitute terminal taxa and explore the genetic variation between these populations, employing mitochondrial DNA to perform Population Aggregation Analysis (Davis and Nixon, 1992) In addition, haplotype analysis (Bermingham and Avise, 1986) and AMOVA (Excoffier, et al., 1992) will be performed, in order to ascertain population differentiation and phylogeographic structure, which can also be tested against color pattern variation in the two species.

2. Color pattern correlations will be assessed among sympatric populations of *M. fulvius* and *L. triangulum*.

3. Evaluate hypotheses of co-evolution among mimics and models by comparing patterns of co-adaptation and co-speciation.

II. DNA extraction from formalin-fixed tissues

II.A. Introduction:

With many organisms, large numbers of specimens that can yield high quality DNA for research are simply unavailable. Formalin-fixed tissues found in museum collections represent a potentially bountiful source of specimens. However, practice, and the literature suggest that formalin preserved specimens pose a serious methodological challenge and may not be useful at all for DNA sequence analysis. A recent meeting convened at the National Academy of Sciences discussed in detail these problems with formalin-preserved specimens in the context of DNA barcoding (Tang, 2006).

DNA isolation from most formalinized tissues is problematic because formalin cross-links DNA and cellular proteins and unbuffered formalin solutions may also degrade DNA (Gilbert, et al., 2007). Such effects of formalin can impede the Polymerase Chain Reaction (PCR) and either cause failure in PCR or reduce available sequence length of amplified products. Several methods have been used to extract formalin-fixed DNA, with varying degrees of success (Tang, 2006; Gilbert, et al., 2007; Schander and Halanych, 2003). The NAS study recommended a more detailed examination of the factors involved in success and failure of DNA isolation procedures.

Consequently, the impact of formalin preservation on tissue type in two species

of snake - *Micrurus fulvius* and *Lampropeltis triangulum elapsoides* – was assayed. These two species have different behavioral ecologies and anatomies. *Micrurus* belongs to the family Elapidae, and relies on highly toxic venom to subdue its prey and protect itself. *Lampropeltis* is a member of the Colubridae and is a powerful constrictor. Intercostal musculature is employed by *Lampropeltis* to kill its prey. On the other hand, *Micrurus* neither utilizes these muscles for predation, nor for rapid motion. Differential muscular development should entail differential mitochondrial development and abundance and perhaps result in differing degrees of DNA isolation efficacy in formalin preserved specimens.

II.B. Materials and Methods:

Specimens: Out of a total of 138 specimens of *Lampropeltis triangulum elapsoides* and 112 specimens of *Micrurus fulvius* that were sampled for this study, 95 *Lampropeltis* and 81 *Micrurus* were obtained on loan or as tissue samples from various institutions and listed as formalin preserved. Specimens were obtained in the field and from individual and institutional sources. Table 2-1 provides a breakdown of sources of tissues, including formalin-fixed specimens, for each species. Institutional samples consisted of integumentary or organ tissue samples or loans of intact specimens, all of which are currently preserved in ethanol. Approximately 5 cm sections of inter costal muscle tissue was excised from each of the intact specimens. All tissue samples were stored in 95% ethanol

at 4°C.

Table 2-1. Sources of formalin-fixed tissues.

Source	Lampropeltis triangulum	Micrurus fulvius	Tissue Storage
Field Collection	12	1	Cryo, buffer
Individual donation	14	15	Dessication, EtOH, buffer
Institution:			
Auburn University Museum	19	20	Formalin, EtOH
California Academy of Science			
Field Museum of Natural History	2	4	SED buffer, EtOH
University of Georgia Museum of Natural History	9	8	Formalin, EtOH
Illinois Natural History Survey	10	5	Formalin, EtOH
Harvard Museum of Comparative Zoology			
University of Florida	11	4	Formalin, EtOH
University of Michigan Museum of Zoology	7	12	Formalin, EtOH
Smithsonian Institution			
Carnegie Museum of Natural History	8	9	SED, EtOH
	11	5	Formalin, EtOH
	20	23	Form, EtOH
	19	5	Formalin, EtOH
Totals	142	111	

DNA Extraction: The present study examines the efficacy of a method derived from Shedlock, et al (1997; devised by Cathy Dayton of the U.S. Fish and Wildlife Service (www.dmacc.edu/instructors/cmeckerman/formalin/dayton_protocol.doc)). The Dayton and Shedlock approaches are members of the family of "soak methods"

that rely on prolonged soaking in buffers, reportedly to disassociate formalin from tissues (Eckerman and Walsh, 1997).

Following the Dayton protocol, 20 mg of tissue was blotted dry and cross-linked in a Stratolinker (Stratagene Cloning Systems, La Jolla, CA), for 15 seconds, after the samples were suspended in 10 ml of a Glycine-Tris-EDTA (GTE) buffer, consisting of 100 mM glycine, 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA. The buffer solution was prepared in 1000 ml aliquots. Tissue samples were soaked in the GTE solution for a total of 72 hours, during which time the solution was entirely replaced three times after which there was no further agitation. The supernatant was then drained and the tissue was dried at 55° C until all visible liquid had evaporated, then cut into smaller pieces with a sterile razor blade and placed in 1.5 ml microcentrifuge tubes. Subsequent tissue processing employed the Gentra (now QIAGEN) Puregene Tissue Kit (QIAGEN, Valencia, CA), which is essentially an alcohol/salt extraction method employing glycogen to coalesce the precipitated DNA. All DNA extracts were stored at -4° C.

PCR: The nicotinamide adenine dinucleotide dehydrogenase subunit 4 (ND4 gene) mitochondrial gene region was chosen for the phylogenetic component of this study because it reliably reflects evolutionary history (Russo, et al., 1996) and it rapidly evolves, making it suitable for resolving relationships at the

population-species boundary (Cracraft and Helm-Bychowski, 1991). In particular, an 891 base pair (bp) fragment of mtDNA was used, including a 697 bp portion of the 3' end of the ND4 gene, and a 194 bp section of three transfer ribonucleic acid (tRNA) genes (tRNAHis, tRNASer, tRNALeu) using primers known as ND4 (CACCTATGACTACCAAAAGCTCATGTAGAAGC) and Leu (TGGTGCAAATCCAAGTAAAAGTAATG) (Rodríguez-Robles and De Jesus-Escobar, 1999). These primers have a proven track record with all squamates and, in fact, were initially designed for *Xenopus laevis*, so they were employed for amplification in both *Lampropeltis* and *Micrurus*.

In addition, due to the degraded nature of the DNA found in formalin-fixed tissues, sets of primers were designed bracketing overlapping 150-250 b.p. segments within a 561 b.p. section of the ND4-tRNA gene region, using the software package AMPLIFY 3.1 (http://engels.genetics.wisc.edu/amplify) (See Table 2-2). Designations refer to the starting position of the primer on a *Pituophis*

Table 2-2. Primers Used for Amplification of Formalin-Fixed Tissues

Primer Designation	Nucleotides	G-C%	Tm °C	Annealing T °C
F8_ND4_MDF	GCAGGCTCCATAGTACTAGC	55.0	54.6	50.4
R215_ND4_MDF	ATTGCGGCAATTACTARGCC	47.5	54.7	
F140_ND4_MDF	AACCTGCCTCCAACAAACG	52.6	61.1	56.0
R308_ND4_MDF	GGCTAAACAGAATAGTGCAGAGG	47.8	59.5	
F239_ND4_MDF	AAGTTTATCAGGAGCCATAGCC	45.5	58.8	55.0
R395_ND4_MDF	CCACCAAGTTGTGAGTATTGG	47.6	58.0	
F342_ND4_MDF	ATTGCCCACGGATTTACTTC	45.0	58.9	56.6
R476_ND4_MDF	CATCAGTTGAATAATGAGGATGC	39.1	58.6	
F361_ND4_MDF	CCTTACACGAGGATTCCACAA	47.6	60.0	55.0
R519_ND4_MDF	TGCTGTAATGAGTATTGATAGTCCAA	34.6	59.2	

melanoleucus (Genbank Accession Number AF141117) reference sequence. The same primer pairs were optimized and used for both *M. fulvius* and *L.*

triangulum.

During all phases of the experiments reported on in this paper we enforced common lab practices to either prevent or detect contamination. These practices include invalidation of an experiment if negative (water) controls showed positive PCR products, invalidation of an experiment where positive controls did not show amplification and standard PCR approaches to avoid contamination. From those valid PCR reactions that were sequenced, we also rejected any sequences that when BLASTed to the NCBI database gave hits to species other than *L. triangulum elapsoides* or *Micrurus fulvius*.

As a first step, optimum PCR annealing temperatures were determined for each primer pair. An Eppendorff Mastercycler ep (Eppendorf North America, Westbury, NY) gradient thermocycler was used for this purpose. A ±0.5°C gradient was applied to the Tm for each primer pair across 6 samples of each of 6 specimens (non-formalin-fixed) that had previously been successfully amplified. Optimum annealing temperatures are also listed for each primer pair in Table 2-2. A second preliminary step was to test the primer pairs with formalin-fixed specimens. For this purpose, the AMNH Herpetology Collection provided five specimens of *L. triangulum triangulum* (Accession Numbers AMNH 91963, 16427, 93665, 3730 and 62832) with which three primer pairs were initially tested.

All Polymerase Chain Reactions were carried out with Eppendorff gradient thermocyclers. PuReTaq Ready-To-Go PCR beads (Amersham Biosciences, UK) were used to amplify all formalin-fixed samples. The beads were placed in 21 ul of water, to which 1 ul of each of the primers were added, together with 2 ul of template. The PCR cycle was ramped up at 96°C for 2 minutes, followed by 35-40 cycles of denaturation at 94°C for 30 seconds, annealing at the appropriate temperature for 30 seconds and extension at 72°C for 30 seconds, followed by a final extension at 72°C for 7 minutes, after which the reaction was held at 4°C. To give a very rough idea of the relative success of amplification with the selected primer pairs, electrophoresis of the PCR product yielded 32 bands out of 113 samples for primer pair 8/215; 31 out of 174 samples for 140/308; 43 out of 174 for 239/395; 99 out of 133 for 342/476 and 116 out of 168 for 361/519.

Sequencing: PCR product was initially purified using the ethanol-isopropanol method. In later runs, a Biomek FXP Laboratory Automation Workstation robot (Beckman-Coulter, Inc., Fullerton, CA) was used for PCR product purification. Cycle sequencing was carried out on the Eppendorff thermocycler, with a 94°C ramp-up for 5 minutes, followed by 40 cycles of melting at 94°C for 15 seconds, annealing at 50°C for 15 seconds and elongation at 60°C for 4 minutes13. The reaction mixture consisted of 1 ul of extender buffer, 1.0 ul of primer, 0.5 ul of Big Dye (Applied Biosystems, Inc., Foster City, CA) and from 1 to 5 ul of the cleaned

PCR product, depending on the intensity of the band it generated. The 5 ul reaction mixture was completed with distilled water. Cycle sequencing clean-up was carried out using the alcohol precipitation method (Lehn, 2005). Sequencing was done on an Applied Biosystems 3730/3730x DNA Analyzer.

Data analysis: Sequences were initially aligned and edited using SEQUENCHER 4.6 for Mac (Gene Codes Corporation, Ann Arbor, MI). Additional alignment and editing was carried out with MacClade 4.08 for OSX (Sinauer Associates, Sunderland, MA).

II.C. Results

Out of approximately 2,000 sequencing reactions from all tissue sources, successful amplification from 97 *Lampropeltis* and 27 *Micrurus* sequences were obtained. Another 64 *Micrurus* sequences were obtained from formalin-fixed tissues, but, when submitted to BLASTsearches, these latter *Micrurus* sequences aligned to other snake species, including *Lampropeltis triangulum*. These positive *Micrurus* amplifications were deemed the product of contamination. Table 2-3 lists the number and percentage of successful reactions by PCR primer pair or

Table 2-3. Successfully sequenced DNA fragments from Formalin-Fixed Tissues

DNA Fragment	# Sequences, *L. triangulum*	# Sequences *M. fulvius*
8-215	47	1
140-308	12	0
239-395	50	2
342-476	70	10
361-519	63	0
Total	242	13

ND4 gene fragment. The *L. triangulum* DNA specimens, with success rates varying from 13% to 74%, clearly are more efficient at producing positive PCR products for all possible PCR fragments than the *Micrurus* samples, whose success rates ranged from 0% to 12%. Table 2-4 shows the number and percentage of successfully sequenced *Micrurus* and *Lampropeltis* specimens by institution. There is no apparent relationship between institution and success rate.

Table 2-4. Successfully sequenced formalin-fixed specimens by institution of origin.

Institution	Total *Lampropeltis*	*Lampropeltis* Sequenced	*Lampropeltis* Success rate	Total *Micrurus*	*Micrurus* Sequenced	*Micrurus* Success rate
Auburn University Museum	19	14	74%	20	1	5%
Field Museum of Natural History	9	7	78%	8	1	13%
University of Georgia Museum of Natural History	10	6	60%	5	1	20%
Illinois Natural History Survey	4	0	0%	4	0	0%
Harvard Museum of Comparative Zoology	7	6	86%	12	3	25%
University of Michigan Museum of Zoology	9	4	44%	4	1	25%
Smithsonian Institution	20	10	50%	23	1	4%
Carnegie Museum of Natural History	19	14	74%	5	0	0%
Totals	95	61	64%	81	8	10%

One of the PCR fragments showed an unusually low success rate for both species (fragment 140-308). Given that this primer pair should produce an intermediate length product, length of the product most likely is not the cause of the low success rate of this primer pair. The low success rate of this primer pair may be the result of the primers not being as efficient as others that were

compared. The other four fragments were amplified at a relatively high rate for the *L. triangulum* DNA samples and at low rates for most of the *Micrurus* DNA samples. One primer pair in particular, performed slightly better for *Micrurus* than the other primer pairs for this species (342-476). This PCR fragment is the smallest expected product at 136 base pairs and suggests that design of PCR primers to produce smaller fragments might allow for more of the *Micrurus* DNA samples to be successfully amplified. The success rate of the *L. triangulum* DNA samples is about twenty fold higher than in the *Micrurus* samples.

II.D. Discussion

We suggest that the great disparity between successful amplifications obtained for formalin preserved *Micrurus* and *Lampropeltis* specimens is most likely due to the inherent differences in tissue makeup of the musculature of these two species. When dissecting out the intercostal muscle tissue from the two species, the tissue in the venomous *M. fulvius* was noticeably more diffuse than the denser musculature apparent in the constrictor *L. triangulum*. The implication is that the denser muscle tissue of the constricting colubrid may contain a greater number (or virtual number) of mitochondria - and more mtDNA - than the more diffuse tissue in the inter-costal region of the venomous elapid. Although there have apparently been no relevant comparative studies on these organisms, the amount of both mitochondria and mitochondrial DNA is known to vary between

cell types and within cell types at varying metabolic levels (Robin and Wong, 1988; Kazakova and Markosian, 1966; Behrens and Himms-Hagen, 1977).

We can rule out primer specificity as a source of the disparity of amplification quality. Attempts were made to design *Micrurus*-specific primers, but these were discarded when they failed to produce amplification or sequences of reasonable length and quality PCR products. On the other hand, the ND4 primers easily amplified (non formalin-fixed) *Micrurus* template DNA, although the proportion of sequences amplified was lower (data not shown). Differential post-formalin treatment can be ruled out as a factor in the disparity of success of amplification of the two species in this study, because specimens were shipped together in alcohol-saturated gauze and, in some cases, stored together in 90% ethanol.

In summary, the Dayton method appears capable of producing robust but differential results with formalin-fixed tissues. Careful consideration should be given to the type of tissue to be sampled in light of the biology of the organisms when formalin-preserved tissues are used. In the current study, tissue from dense muscle tissue preserved in formalin performed almost 20 times better than tissues from muscle fiber depauperate tissues. The following recommendations with respect to formalin preserved specimens made in previous studies should be reiterated: 1) the amplification of short sequences is recommended for situations in which DNA degradation is likely, as is the case for formalin-fixed, archival

tissues; 2) serious precautions must be taken to avoid contamination of samples at every level of processing, as is true with any archival tissue, including formalin preserved tissues.

III. Molecular phylogenetics and phylogeography

III.A.Introduction

This project started out as an effort to understand model-mimic co-evolution within a phylogenetic paradigm. Among the problems to which it succumbed was the lack of phylogenetic resolution within the two groups under study, *Lampropeltis triangulum elapsoides* and *Micrurus fulvius fulvius*. It was, perhaps, overly optimistic to seek to identify terminal taxa and infer a phylogenetic hypothesis among such geographically and taxonomically restricted organisms. The justifications were that a) the taxonomy of the entire nominal species *Lampropeltis triangulum* requires – and is currently under – revision (Burbrink, 2008) and it has been suggested that *Micrurus fulvius fulvius* may consist of at least one other diagnostically distinct grouping (Roze, 1996), and b) populations of diverse organisms in the Southeastern United States show species and population-level divisions that apparently coincide with specific biogeographic vicariant barriers and phylogeographic breaks (Bermingham and Avise, 1986; Mayden, 1988; Avise, 1996; Roman, et al., 1998; Walker and Avise, 1998; Burbrink, et al., 2000; Weisrock and Janzen, 2000; Liu, et al., 2005; Pauly, et al., 2007).

The nominal species *Lampropeltis triangulum* has long needed taxonomic revision. Consisting of 25 subspecies distributed from southern Canada to the Andes range in Ecuador and Colombia, this ‘species’ includes robust snakes

close to 2 m. in length, such as *Lampropeltis triangulum hondurensis*, as well as the small, fossorial subspecies *L. triangulum elapsoides*, one of the subjects of this study. Gouping these animals together as conspecifics has provoked skepticism for a number of years (Greene, 2001). The most recent, thorough, revision of *L. triangulum* grouped and classified subspecies by color pattern (Williams, 1988). Beyond criticisms of the subspecies concept (Burbrink, et al., 2000; Manier, 2004), the use of color patterns been questioned as an adequate criterion for taxonomic grouping, particularly among the mimetic *Lampropeltis* (Rodriguez-Robles, et al., 1999; Bryson, Jr., et al., 2007). The aposematic models for most of these snakes, Micrurine elapids, share a widespread, if more southerly, distribution and the same general color pattern of alternating red, black and yellow or white bands. In both groups, color patterns have been shown to be plastic and regionally polymorphic (McDiarmid and Greene, 2005; Bryson, Jr., et al., 2007).

Phylogenetic systematic studies of Colubrids, in general, and groups close to *Lampropeltis*, in particular, were long seen as problematic, in good part due to the lack of diagnosable morphological characters for these snakes (Keogh, 1996). Keogh (1996) conducted a study of the entire Lampropeltini tribe that relied on just 17 characters, some of which were discretized morphometric characters (scale counts). Several earlier investigations had been carried out using protein electrophoresis to elaborate phylogenies for Colubrids and some

genera within the Lampropeltines (George and Dessauer, 1970; Lawson and Dessauer, 1981; Dowling and Maxson, 1990), but until relatively recently, molecular genetic techniques had not been applied to the phylogenetic study of either the species *L. triangulum* or its genus.

Rodríguez-Robles and de Jesús-Escobar (1999) used both parsimony and maximum likelihood approaches to conduct a molecular phylogenetics analysis of the Lampropeltinine snakes, using the ND4 mitochondrial gene and neighboring tRNA-coding sequences. Their study mapped behavioral-ecological attributes and ancestral areas on the resulting phylogenies, and proposed a biogeographic hypothesis for the lampropeltinines – that their ancestral area was western North America. This result was built-upon and supported by a later study by Burbrink and Lawson (2007). Rodríguez-Robles, et al. (1999) also assessed the phylogeography of the California mountain kingsnake (*Lampropeltis zonata*), using the same gene and approach. Rodríguez-Robles and de Jesús-Escobar (2000) also carried out a taxonomic revision of the lampropeltinine *Pituophis melanoleucus* species complex, finding solid phylogenetic support for assigning species status to three members of that group. Of some relevance to the present study, while Keogh (1996) found *Lampropeltis* to be monophyletic, both Dowling and Maxson (1990) and Rodríguez-Robles and de Jesús-Escobar (1999) showed *Lampropeltis* to be paraphyletic. In the first two studies, *L. triangulum* was shown as a derived species, while it wasn't present in the latter study.

Fetzner, Jr. (2001) constructed a phylogeny for all 25 subspecies of *L. triangulum*, together with *Lampropeltis alterna*, *L. calligaster, L. getula, L. mexicana, L. pyromelana, L. ruthveni, L. triangulum* and *L.zonata*, using the ND4 gene. He found that the nominal species was, in fact, polymorphic with respect to related species. This was supported by Bryson, et al. (2007), who found that *L. triangulum* is polyphyletic with respect to the related *L. Mexicana* complex. What is interesting, according to Fetzner Jr.'s phylogeny, is that *L. t. elapsoides* groups apart from other conspecifics in a polytomous arrangement. Two specimens of *L. t. temporalis* group with it in a clade with 100% bootstrap support. *L. t. temporalis* has been held to be an intergrade between *L. t. triangulum* and *L. t. elapsoides* (Williams, 1988), and, in fact, the other specimens of *L. t. temporalis* group with *L. t. triangulum*. The clade containing *L. t. elapsoides*, together with other members of the *L. triangulum* complex, also contains *L. zonata, L. Mexicana* and *L. pyromelana*. Sister to this clade is another that contains other members of the *L. triangulum* complex (including *L. t. triangulum), L. getula* and *L. alterna,* together with the genus *Stilosoma*.

L. t. elapsoides is found on the Atlantic coastal plain and Piedmont from Virginia south through Florida and west, following the coastal plain and Piedmont to the Mississippi River in Louisiana. It extends north along the Tennessee River Valley into central Tennessee, and along the Cumberland Plateau into Kentucky (Williams, 1988). The scarlet kingsnake is the smallest member of the *L.*

triangulum complex, reaching no more than about 0.5 m. It is a largely fossorial snake, usually encountered under loose bark, logs and other debris, and emerging only at night and after heavy rains. Its principal habitat is southern pine and mixed scrub on sandy, well-drained soils, and is much more rarely encountered in deciduous wood, although it has apparently adapted to this type of habitat in marginal areas where it is sympatric with *L. t. triangulum. L. t. elapsoides* is morphologically distinct from its neighboring conspecifics and clearly adapted to its fossorial existence: its head is indistinct from its neck and it has a narrow snout and underslung mandible. In addition, *L. t. elapsoides* has experienced a reduction or loss of head scalation present in other members of the *triangulum* complex, particularly the loreal scales (Williams, 1988).

One aspect of the population genetics of this organism, which has bearing on its taxonomy, origin and biogeography, is that it apparently behaves like a "ring species" (cf. Moritz, et al., 1992). Along the Atlantic coastal plain, *L. t. elapsoides* apparently intergrades with *L. t. triangulum* where the two are sympatric. Such intergrades, as noted were formerly considered a separate subspecies, *L. t. temporalis* (Williams, 1988; Armstrong, et al., 2001). Curving around the Appalachians in the south, *L. t. elapsoides* extends north into areas of northern Georgia and Alabama, as well as central Tennessee and Kentucky, where the two conspecifics are also found in sympatry, but do not interbreed (Williams, 1988). *L. t. elapsoides* also coexists from western Kentucky and northwestern

Tennessee through northern Mississippi with *L. t. sypsila* and in Louisiana with *L. t. amaura*. Williams (1988) affirmed that *L. t. elapsoides* intergrades with *L.t. sypsila* and may intergrade with *L. t. amaura*, but more recently Armstrong, et al. (2001) presented evidence that, at least in western Kentucky, the two constitute distinct sympatric populations.

Turning to *Micrurus fulvius*, the status of this species is more well-defined, both taxonomically and geographically. The Micrurine elapids consist of three genera, *Leptomicrurus, Micruroides* and *Micrurus*, encompassing 124 species distributed throughout the Americas, with the exception of Canada and Chile. With a few exceptions, aposematic combinations of red, black and yellow or white bands characterize members of these genera. *Micrurus fulvius*, itself, is a widespread species, found from southeastern North Carolina, along the Atlantic coastal plain south through the Florida peninsula and onto the Florida keys, then west, as far north as southern Arkansas, through eastern Texas, south to central Mexico (Roze, 1996).

The subspecies analyzed in this study, *Micrurus fulvius fulvius,* was assigned its current taxonomic status by Schmidt (1928), who distinguished this subspecies from *M. fulvius barbouri*, found in southernmost Florida, on the basis of a lack of black tipping on the red dorsal scales in the latter. *M. fulvius fulvius* is the southeastern and southern subspecies of *M. fulvius*, extending west to the

eastern tip of Louisiana. It is separated from its conspecifics by a gap that is at least 100 miles wide around the Mississippi River (Roze, 1996).

There have been a number of higher-level phylogenetic assessments of the Elapidae, encompassing the Micrurines, and fewer examining relationships within the coral snakes. Using 16S rDNA and cytochrome b mtDNA, Keogh (1998) and Slowinski and Keogh (2000) inferred a close relationship between the Micrurines and the Asian coral snakes (*Calliophis*). In a study using Cytochrome b and ND4 mitochondrial genes and the c-mos nuclear gene, Castoe, et al. (2007) found robust support for a clade containing the Asian (*Calliophis* and *Sinomicrurus*) and American coralsnakes and confirmed monophyly for the Micruruines. Slowinski (1995), using allozymes and morphological data, examined the phylogenetics of the three Micrurine genera, determining that *Micrurus* was apparently paraphyletic without the inclusion of *Leptomicrurus* within *Micrurus*, which he recommended. While this study included only 18 species, it showed a derived sister relationship between *M. fulvius* and the Central American *M. alleni* within a polytomous group of Mexican and Central American species, which in turn is nested within a South American group. Castoe, et al's (2007) maximum parsimony phylogeny showed a polyphyletic Micrurine grouping, while their maximum likelihood and Bayesian phylogenies showed a monophyletic grouping of American coral snakes. The MP, ML and Bayesian phylogenies also presented *M. fulvius* as basal to the two other *Micrurus* and the single

Leptomicrurus included in their study, although that configuration only enjoyed significant bootstrap support on the ML tree and even greater nodal support expressed as the Posterior Probability in their Bayesian analysis. As in Slowinski's (1995) study, their data showed *Micrurus* to be paraphyletic with respect to *Leptomicrurus*.

There have been few biogeographic or phylogeographic studies directly concerning either *Lampropeltis triangulum* or *Micrurus fulvius*. Rodríguez-Robles and de Jesús-Escobar (1999) used area cladograms to infer a probable western north American origin for the Lampropeltini tribe, which includes *Lampropeltis* and closely related colubrid taxa, while Burbrink and Lawson (2007) used four mitochondrial and one nuclear gene by way of parsimony, maximum likelihood and Bayesian phylogenetic reconstructions to estimate that this tribe originated in the Oriental zoogeographic region and then dispersed over the Beringian land bridge to North America by the early Miocene. This appears to be concordant with the fossil record: the earliest fossils of this group appear in north America in the Middle Miocene (Holman, 2000), *Lampropeltis* among them. The earliest fossils of *L. triangulum* in north America appear in the Late Miocene in Nebraska, with other, later fossils found throughout the current north American distribution of the species. There does not appear to be any reference to *L. triangulum elapsoides* in the fossil record. *Micrurus* also appeared among Nebraska fossils in the Middle Miocene (Holman, 2000), which is north of their current distribution.

Holman (2000) attributes a South American origin to the coral snakes, based on his interpretation of the fossil evidence. The earliest possible *M. fulvius* appeared in Florida in the Early Pleistocene, with more unequivocal finds dating back to the Middle to Late Pleistocene (Holman, 2000), in Texas and Florida.

However, there has arisen a substantial body of biogeographic and phylogeographic literature concerning the flora and fauna of the southeastern and southern regions of the United States, where these two species are found. While the two fields share a focus on reconstructing the evolutionary history of organisms and shedding light on the geological history of the areas they inhabit, biogeography makes use of the methods and results of phylogenetic systematics and thus relies on species-level designations of terminals to infer area histories, pinpoint areas of endemism and identify common evolutionary events (Mayden, 1988). Mayden considered phylogenetic hypotheses to be the prerequisite to testing biogeographic relationships. Hennig (1966) considered species to be the smallest unit of phylogenetic analysis.

Phylogeography, on the other hand, is focused on the geographic relationship of genealogical lineages or haplotypes at the intraspecific level. As the cladistic methods it employs require bifurcation and divergence among the units of analysis, phylogeography usually employs mtDNA or other non-recombining genetic elements (Rodriguez-Robles, et al., 1999). In this, it forms a distinct sub-

branch of population genetics, whose goal is defined by Hartl and Clark (1997) as, “to understand the forces that have an impact on levels of genetic variation.”

Biogeographic and phylogeographic studies have identified a number of putative common tracts separated by geological or ecological barriers to gene flow, which, in conjunction with geological and climatic change, have produced common allopatric speciation events among organisms. Studies of a wide variety of taxa point to several such barriers (or tracts -- what is a barrier to a terrestrial organism is a tract for an aquatic organism) to gene flow. To cite some of the barriers and studies, these include the Mississippi River (rat snakes- Burbrink, et al., 2000), the Mobile Bay-Tombigbee River system (North American racers [snakes]-Burbrink, et al., 2007b), the Appalachicola-Flint-Chattahoochee River System (pocket gophers-Avise, 1979; tulip trees-Sewell, et al., 1996; flatwoods salamander-Pauly, et al., 2007), the Appalachian-Blue Ridge Mountains (slider turtle-Avise, et al., 1992; tiger salamanders-Church, et al., 2003) and associated Fall Line (flatwoods salamander-Pauly, et al., 2007), the Florida Central Highlands (dwarf siren salamander-Liu, et al., 2006) and the Savannah River system (Soltis, et al., 2006; trilling chorus frog-Lemmon, et al., 2007). Mayden (1988), Avise (1996), Walker and Avise (1998) and Soltis, et al. (2006) summarize and assess many of the relevant biogeographic and phylogeographic studies to date. However, Soltis, et al. (2006) also point out that many species do not conform to these biogeographic tracts and caution against over-interpretation

of common patterns, since events at different times may give rise to similar phylogeographic patterns, a phenomenon they call “pseudocongruence.” Burbrink (2008) noted that *L. triangulum* conforms to the phylogeographic breaks seen in *Pantherophis*, based on ongoing research by him and one of his students.

The geological and climatological phenomena that have been cited as affecting southeastern and southern flora and fauna through isolation events include inundations of coastal and low-lying areas during inter-glacial periods (Neill, 1957; Blaney, 1971; Marshall, et al., 2000; Liu, et al., 2006), together with intrusions of seawater and expansion of other river drainage systems (Pauly, et al., 2007); increased aridity in the south during glacial periods (Clark, et al., 1999; Trapnell, et al., 2007; Moler, 2008) and isolation of pockets of more mesic environments; advances of ice sheets and associated cooling (and drying) throughout much of the south leading to glacial ‘refugia’ (Jackson, et al., 2000; Peters, et al., 2005; Trapnell, et al., 2007; Fontanella, et al., 2008).

These historical events leave footprints in the phylogeographic patterns of populations and the biogeographic patterns or area cladograms of species. Biogeography relies on allopatric speciation events which leave tracks in congruent phylogenies of species (Brooks and McLennan, 1991; Humphries and Parenti, 1998). These phylogenies can be reconciled and area cladograms

constructed which test hypotheses of common evolutionary history in the same way that species cladograms test hypotheses of common ancestry (Mayden, 1988; Humphries and Parenti, 1998). The methods devised to test area relationships have also been employed to test relationships between gene trees and species trees, hosts and parasites and co-evolving species (Brooks and McLennan, 1991; Page, 1993; Charleston and Perkins, 2003; Ronquist, 2003).

Early studies of the biogeography of the southern United States (and more generally) focused on the effects of glaciation, sometimes in combination with other climatological, ecological or geological features, on species and subspecies distribution, identifying areas that offered suitable habitats to organisms displaced by glacial maxima as “refugia.” Blair (1958), for example, proposed that cooling during the Wisconsin glaciation displaced many warm-adapted organisms into refugia in Florida and Mexico, and brought a more northern flora and fauna south. Allopatric speciation ensued, followed by range expansion in the wake of the Laurentide retreat, which allowed flora and fauna to expand in appropriate habitats up to geographic or ecological limits, such as the Mississisippi River or the western edge of the eastern deciduous forest.

Glacial advance and retreat can have various effects on the distribution of organisms. Most obviously, they are correlated with changes in the global climate. It has been estimated that the last glacial advance (LGM -- last glacial

maxima) may have lowered temperatures and decreased precipitation across the eastern United States by as much as 15-20° for January temperatures and 10° for July temperatures and 40mm less rainfall, as compared with modern conditions (Jackson, et al., 2000). Consequently, vegetation typical of the boreal forest was found at least as far south as northwestern Georgia, and possibly up to the Gulf coast of Florida. At the same time, southern vegetation types seem to have withdrawn to the south. Of particular relevance to this study is the finding that the southern pine forest -- the favored habitat of *Lampropeltis triangulum elapsoides* -- appears to have been pushed back to peninsular Florida during the LGM (Delcourt and Delcourt, 1980; Watts and Stuiver, 1980; Jackson, 2000). Both (or all three -- including the shift in favored habitats) of these factors may have influenced the extent of possible refugia, with lowered temperatures displacing organisms south and increased aridity restricting some to more mesic environments and further fragmenting populations (Clark, et al., 2003; Trapnell, et al, 2007; Moler, 2008). However, some authors warn against overestimating the influence of refugia on species assemblages, citing less haplotype congruence across taxa than might be expected if many species shared the same refugium (Loehle, 2007; Soltis, et al., 2006).

Glaciation and glacial retreat also brought with them large-scale fluctuations in sea level, with the global sea level up to 120 meters lower than present during the LGM and corresponding increases during the interglacials. These sea level

changes led to inundations of coastal areas and embayment and expansion of river basins during glacial maxima (Bermingham and Avise, 1986; Pauly, et al., 2007) and exposure of wider expanses of continental shelf during interglacials. Such glacial embayment is thought to have enlarged the Apalachicola-Flint watershed greatly and formed a major vicariant barrier between the Gulf of Mexico and the Appalachians (Neill, 1957; Burbrink, 2000; Pauly, et al., 2007). At various times during past interglacial periods much of Florida (though apparently not during the Pleistocene -- Moler, 2008) was submerged, possibly forming several larger or smaller islands, and fragmenting populations of organisms (Neill, 1957; Bermingham and Avise, 1986).

III.B. Materials and Methods

1. Specimens

Out of a total of 138 specimens of *Lampropeltis triangulum elapsoides* and 112 specimens of *Micrurus fulvius* that were sampled for this study, 95 *Lampropeltis* and 81 *Micrurus* were obtained on loan or as tissue samples from various institutions and listed as formalin preserved. Specimens were obtained in the field and from individual and institutional sources. Appendix 1 provides specimen data, including institutional tissue sources for each species. *M. fulvius* specimens were obtained from Arkansas (3), South Carolina (12), Georgia (10), Florida (60), Alabama (21), Mississippi (1) and Louisiana (5). *L. t. elapsoides* specimens were obtained from North Carolina (5), South Carolina (19), Georgia (11), Florida (40),

Alabama (20), Mississippi (8) and Louisiana (10). Figure 3-1 maps specimen localities.

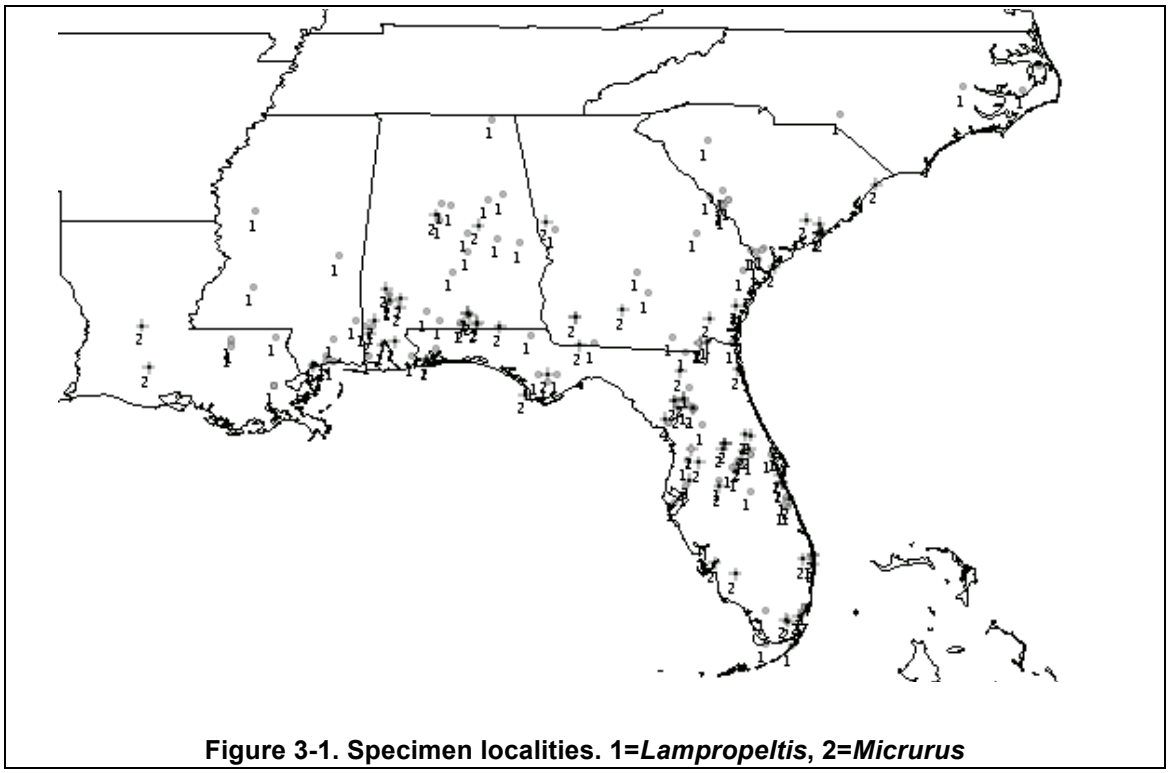


Figure 3-1. Specimen localities. 1=*Lampropeltis*, 2=*Micrurus*

Individual donations consisted of shed skins (8), entire dessicated road-kills (2) and integumentary or liver tissue samples in ethanol or tissue buffer. Tail-clippings were excised from field-collected animals. The resulting lesions were subsequently treated with a topical antiseptic and the animals were released. Tail-clippings were removed from *Micrurus* after maneuvering the animals into a transparent plastic tube, slightly wider than the diameter of the animals. With the animals unable to turn around, tissue samples and longitudinal measurements could be made without risk. Duplicate samples of these were transported in cryogenic dry-shippers and lysis buffer provided by the Ambrose Monell Cryocollection (AMCC) at AMNH (http://research.amnh.org/amcc/), and

subsequently accessioned in AMCC (Accession Numbers AMNH 106134-44, 50).

Institutional specimens consisted of integumentary or organ tissue samples or loans of intact animals, all of which are currently preserved in ethanol. Of the whole specimens, I excised approximately 5 cm sections of intercostal muscle tissue, which were stored in 95% ethanol. I also photographed all snakes captured in the field and 139 institutional specimens. Field photography was accomplished using a 3.1 megapixel Kodak DC 4800 digital camera with a 28-84 mm zoom. Lab photography was conducted with a Microptics ML-1000 Digital Imaging System (Microptics, Inc., Palmyra, VA). All photographs were taken with a metric scale for comparison. All specimens photographed in the lab were placed on a neutral, light colored background. I measured snout-vent and tail lengths for most of the whole specimens, by running a string along the length of the animals and then measuring the string with a meter stick. Bands were counted posterior to the head and anterior to the vent.

2. DNA Extraction

For all specimens except those stored at some point in formalin, DNA extraction was conducted using a Qiagen DNeasy Blood and Tissue Kit (using the Purification of Total DNA from Animal Tissues -- Spin-Column Protocol) (QIAGEN, Valencia, CA), an alcohol/detergent/salt extraction method. In the case

of shed skins, the protocol for fresh tissues was modified by increasing the amounts of proteinase k and tissue buffer 2-3-fold (Takacs, 2003). After the initial precipitation, reagents were used at normal levels.

Following the Dayton protocol, I blotted dry and cross-linked 20 mg of tissue in a Stratolinker (Stratagene Cloning Systems, La Jolla, CA), for 15 seconds, after which I suspended the samples in 10 ml of a Glycine-Tris-EDTA (GTE) buffer, consisting of 100 mM glycine, 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA. I prepared the buffer solution in 1000 ml aliquots. Tissue samples were soaked in the GTE solution for a total of 72 hours, during which time the solution was entirely replaced three times. There was no further agitation. The supernatant was then drained and the tissue was dried at 55° C until all visible liquid had evaporated, then cut into smaller pieces with a sterile razor blade and placed in 1.5 ml microcentrifuge tubes. Subsequent tissue processing employed the Gentra (now QIAGEN) Puregene Tissue Kit (QIAGEN, Valencia, CA), which is another alcohol/detergent/salt extraction method employing glycogen to coalesce the precipitated DNA. All DNA extracts were stored at -4° C.

3. PCR

The nicotinamide adenine dinucleotide dehydrogenase subunit 4 (ND4 gene) mitochondrial gene region was chosen for the phylogenetic component of this study because it reliably reflects evolutionary history (Russo, et al., 1996) and it

rapidly evolves, making it suitable for resolving relationships at the population-species boundary (Cracraft and Helm-Bychowski, 1991). I used an 891 base pair (bp) fragment of the ND4 gene, including a 697 bp portion of the 3' end of the ND4 gene, and a 194 bp section of three transfer ribonucleic acid (tRNA) genes (tRNAHis, tRNASer, tRNALeu) using primers known as ND4 (CACCTATGACTACCAAAAGCTCATGTAGAAGC) and Leu (TGGTGCAAATCCAAGTAAAAGTAATG) (Rodríguez-Robles and De Jesus-Escobar, 1999). These primers have a proven track record with all squamates and, in fact, were initially designed for *Xenopus laevis*, so they were employed for amplification in both *Lampropeltis* and *Micrurus*.

In addition, due to the degraded nature of the DNA found in formalin-fixed tissues, I designed sets of primers bracketing overlapping 150-250 b.p. segments within a 561 b.p. section of the ND4-tRNA gene region, using the software package AMPLIFY 3.1 (http://engels.genetics.wisc.edu/amplify) (See Table 2-2). Designations refer to the starting position of the primer on a *Pituophis melanoleucus* (Genbank Accession Number AF141117) reference sequence. The same primer pairs were used for both *M. fulvius* and *L. triangulum*.

For the ND4/Leu primer pair, PCRs were carried out with Perkin-Elmer 9600 and Eppendorff Mastercycler ep (Eppendorf North America, Westbury, NY) gradient thermocyclers. Initially, a 50 ul reaction mix was prepared, consisting of 5 ul PCR

buffer, 5 ul dNTP, 0.1 ul of each primer, 5 ul of BSA 0.1 ul of Taq polymerase, 33.7 ul of water and 1 ul of template DNA. Subsequently, puReTaq Ready-To-Go PCR beads (Amersham Biosciences, UK) were substituted for the Taq "cocktail." The beads come in strips of 1.5 ul tubes. To each bead was added 21 ul of water, 1 ul of each of the primers and 2 ul of template DNA. The denaturation was ramped up at 96°C for 2 minutes, followed by 35-40 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds and extension at 72°C for 30 seconds, followed by a final extension at 72°C for 7 minutes, after which the reaction was held at 4°C.

As a first step, I determined optimum PCR annealing temperatures for each primer pair. The Eppendorff gradient thermocycler was used for this purpose. A ±0.5°C gradient was applied to the Tm for each primer pair across 6 samples of each of 6 specimens (non-formalin-fixed) that had previously been successfully amplified. The optimum annealing temperatures so determined, are also listed for each primer pair in Table 2-2. A second preliminary step was to test the primer pairs with formalin-fixed specimens. For this purpose, the AMNH Herpetology Collection provided five specimens of *L. triangulum (triangulum)* (Accession Numbers AMNH 91963, 16427, 93665, 3730 and 62832) with which three primer pairs were initially tested. This initial assay yielded PCR product on from three to all five specimens for distinct fragments.

All Polymerase Chain Reactions on formalinized tissues were carried out with Eppendorff gradient thermocyclers. PuReTaq Ready-To-Go PCR beads were used to amplify all formalin-fixed samples. The PCR cycle was ramped up at 96°C for 2 minutes, followed by 35-40 cycles of denaturation at 94°C for 30 seconds, annealing at the appropriate temperature for 30 seconds and extension at 72°C for 30 seconds, followed by a final extension at 72°C for 7 minutes, after which the reaction was held at 4°C. Electrophoresis of the PCR product yielded 32 bands out of 113 samples for primer pair 8/215; 31 out of 174 samples for 140/308; 43 out of 174 for 239/395; 99 out of 133 for 342/476 and 116 out of 168 for 361/519.

4. Sequencing

PCR product was initially purified using the ethanol-isopropanol method (Lehn, 2005). In later runs, a Biomek FXP Laboratory Automation Workstation robot (Beckman-Coulter, Inc., Fullerton, CA) was used for PCR product purification. Cycle sequencing was carried out on the Eppendorff thermocycler, with a 94°C ramp-up for 5 minutes, followed by 40 cycles of melting at 94°C for 15 seconds, annealing at 50°C for 15 seconds and elongation at 60°C for 4 minutes (Lehn, 2005). The reaction mixture consisted of 1 ul of extender buffer, 1.0 ul of primer, 0.5 ul of Big Dye (Applied Biosystems, Inc., Foster City, CA) and from 1 to 5 ul of the cleaned PCR product, depending on the intensity of the band it generated. The 5 ul reaction mixture was completed with distilled water. Cycle sequencing

clean-up was carried out using the alcohol precipitation method (lehn, 2005). Sequencing was done on an Applied Biosystems 3730/3730x DNA Analyzer (Applied Biosystems, Inc., Foster City, CA).

Sequences were initially aligned and edited using SEQUENCHER 4.6 for Mac (Gene Codes Corporation, Ann Arbor, MI). Additional alignment and editing was carried out with MacClade 4.08 for OSX (Maddison and Maddison, 1992).

Out of approximately 2,000 sequencing reactions from all tissue sources, 97 Lampropeltis and 27 *Micrurus* sequences were obtained. Another 64 *Micrurus* sequences were obtained from formalin-fixed tissues, but, when submitted to the NCBI online Basic Local Alignment Search Tool (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi), these aligned to other species, including *Lampropeltis triangulum*, and were not used in the analysis. Of the *Lampropeltis* sequences obtained, 81 were from formalin-fixed tissues. Of the *Micrurus* sequences 11 were from formalin-fixed specimens. However, these sequences consisted of one or more fragments, often not overlapping. Character statistics from MaClade showed that 61% of the samples were missing at least half of the possible 862 nucleotides available in this contig and 26% were missing more than 80% of available sites. The *Lampropeltis* DNA specimens clearly are more efficient at producing positive PCR products for all possible PCR fragments ranging from 13% success to 74% success. The *Micrurus* samples

showed much lower success rates ranging from 0% to 12% success. For the full sequence (809 b.p. for *Lampropeltis* and 811 b.p. for *Micrurus*) 51.91% and 53% of the matrices were missing data, respectively.

Missing data may contribute to ambiguity and increase the number of most parsimonious trees in a phylogenetic analysis (Wiens, 1998, Wiens, 2003, Kearney, 2002). It has a more immediate practical effect in population genetics programs, such as DNAsp and Arlequin. In these programs, every nucleotide site containing missing data is discounted. Given the large amount of missing data for both species in my sequence sets, I assayed several different approaches to reducing missing data.

5. Phylogenetic Analysis

Phylogenetic analysis of taxa requires non-reticulating terminals. According to Hennig (1966), a phylogenetic analysis has the goal of inferring hierarchic descent relationships among species, which are the smallest inclusive units suitable for a phylogenetic analysis. This is so because species are the smallest sets of groups of organisms that do not show reticulate relationships. Arguably, the species concept that is most compatible with phylogenetic systematics is, appropriately, the Phylogenetic Species Concept (PSC) (Cracraft, 1989; Nixon and Wheeler, 1990; Luckow, 1995; Davis, 1997; Goldstein and Desalle, 2000). Nixon and Wheeler defined a phylogenetic species as “the smallest aggregation

of populations (sexual) or lineages (asexual) diagnosable by a unique combination of character states in comparable individuals." If a phylogenetic species is an appropriate terminal for phylogenetic analysis, then it must be independently determined prior to the analysis (Davis and Nixon, 1992). In agreement with the PSC, Population Aggregation Analysis (PAA) is the character-based diagnostic procedure that facilitates species delineation (Davis and Nixon, 1992; Goldstein and Desalle, 2000). Populations of sexually reproducing organisms that are suspected of constituting phylogenetic species are examined for characters or suites of characters that are unique and shared by all members of the population, but not by any members of other populations (Davis and Nixon, 1992; Birstein, et al., 1998; Goldstein and Desalle, 2000). It is iterative process, in that populations are added to the analysis until the largest grouping of organisms sharing the unique characters is identified. PAA was conducted using MacClade. Separate populations were inferred, based on the biogeographic and phylogeographic literature, to occur within the following areas: 1) east or west of the Appalachicola-Flint-Chattahoochee River system, 2) east or west of the coastal plain (bordered by the Piedmont and the Fall Line) on the Atlantic, or north or south on the Gulf Coast, 3) east or west of the Mississippi (although the Mississippi was regarded as the distribution limit for the two species, in most cases), 4) east or west of the Mobile-Tombigbee River system, and 5) north or south of the Savannah River system. Initially, sequences were obtained from Florida and South Carolina for *L. t. elapsoides*. These were aligned

in Sequencher and the contig opened in MacClade under the match first and color by states settings to allow for easy visualization. Sequences were grouped by hypothesized population, and visually examined for characters or suites of characters that uniquely defined each population. As more sequences were added, these were further grouped by population and diagnostic characters were examined to determine if they were still inclusive of their respective populations. As other putative populations were added, additional characters were investigated as possible diagnostics for their populations.

Although no legitimate terminals were identified, all sequences were used to construct gene trees using parsimony criteria. Phylogenetic analyses were conducted with PAUP ver. 4.0b10, WINCLADA ver. 1.00.08 (Nixon, 1992) or TNT ver. 1.1. (Goloboff, et. al, 2008). In all cases, heuristic searches were conducted, holding 500, 1,000 or 10,000 trees, respectively, at 100 replications each. In all cases the TBR search algorithm was used. In all cases of multiple most parsimonious trees, a strict consensus was found. In all cases, bootstrap support for nodes was calculated.

For *Lampropeltis triangulum elapsoides*, phylogenetic analyses using Maximum Parsimony were conducted with 97 specimens on a 809 b.p. contig with *L. getula, L. t. campbelli* and *L. t. triangulum* as outgroups. A shorter section of 12 complete sequences comprising a 638 b.p. contig was used to assess higher-

level phylogenetic relationships among the genus *Lampropeltis*. This included *Pantherophis vulpinus* (Genbank DQ902306) as outgroup (Burbrink and Lawson, 2007), and included *Lampropeltis calligaster* (DQ902311)*, L. getula* (AY739629)*, L. zonata* (AF138762)*, L. alterna* (AY739636)*, L. ruthveni* (AY739642)*, L. Mexicana* (AY739639)*, L.triangulum campbelli* (AY739638)*, L.t. conanti* (AY739643)*, L. pyromelana* (AY497313), *L. t. gentilis* (AY739632) and *L.t. triangulum.*

For *Micrurus fulvius fulvius*, phylogenetic analyses using parsimony were conducted using an 811 b.p. contig with *Naja naja* (AY713378), *Bungarus fasciatus* (U49297), *Pseudonaja affinis* (DQ098446), *Micrurus mipartitus* (EF137406), *M. lemniscatus* (AF228439), *M. surinamensis* (AF228444), *M. altirostris* (AF228429), *M. brasiliensis* (AF228427), *M. frontalis* (AF228425), *M. corallinus* (AF228424) and 32 specimens of *M. fulvius*, including 2 Genbank sequences of unknown locality (EF137405 and U49298). This contig, with all taxa and *N. naja, B. fasciatus and P. affinis* as outgroups, was used for higher level phylogenetic analysis. It was also used for analysis of the *M. fulvius* ingroup, with *M. mipartitus* and *M. corallinus* as outgroups. In addition, parsimony analysis was conducted on a 686 b.p. contig, consisting of 7 complete sequences plus *M. mipartitus* and *M. corallinus* as outgroups.

6. Phylogeographic Analysis

The “Fuse Redundant Taxa” feature in MacClade was used to reduce the high computational load among largely homogeneous sequences and the proportion of missing data. This command fuses all taxa whose sequences contain identical nucleotides. Given the greatly varying fragment length of the available sequences, this provided a parsimonious rough estimate of the minimum number of possible haplotypes, since a greater number of complete sequences could have introduced more, but not less, variability into the sample. The resulting sequences were submitted to WINCLADA, using the same parsimony search parameters and procedure as the phylogenetic analyses. In the case of *L. triangulum elapsoides*, the original 97 sequences reduced to 35 and the missing data load reduced to 34.18%. In the case of *M. fulvius*, the 30 sequences reduced to 12, with a reduction in missing data to 32.5%.

Parsimony analysis of these merged sequences resulted in large numbers of equally parsimonious trees and poorly resolved consensuses. Allowing that such consensus trees represent information losses and, so, poor summaries of the available data (Carpenter, 1988), I utilized successive weighting, an a posteriori weighting procedure relying on the number of change steps each character must undergo on a given tree (Farris, 1969; Farris, 1984). Farris (1984) summarized the reasons for preferring this approach: “the characters of a study are often treated in practice as if they all provided equally cogent evidence on phylogenetic relationship. No one supposes, however, that characters in general all deserve

the same weight—that they all yield equally strong evidence." Characters that exhibit greater degrees of consistency or "cladistic reliability" are considered stronger evidence based on the most parsimonious assessment and are weighted accordingly using consistency, rescaled consistency or retention indices (Kitching, et al., 1997). Average character reliability is determined based on the initial cladograms, and these values are used to reweight the characters, which are then subject to another round of cladistic analysis. The resulting indices are again used to weight the character matrices, which are again submitted for analysis. This procedure is repeated until successive cladograms are stable, as measured by tree length. Successive weighting was carried out using TNT. Sequences from the "fused" data set were submitted under the heuristic search settings as above. The results were then reweighted by using the command-line subroutine "rewt.run ri," which uses the cumulative retention index to calculate the character weights. This data was successively reweighted and resubmitted for heuristic search until tree lengths stabilized. The reweighted data was then submitted for bootstrap analysis to assess character support.

Haplotypes were generated in two ways with roughly commensurate results. By setting the alignment parameters in Sequencher at 100% match and 100% overlap, all identical sequences were joined in a contig, which constituted the haplotype. Using the "Merge Redundant Taxa" feature in MacClade, as above, identical sequence segments can be fused to form haplotypes. With missing

data, this procedure can be adjusted to assemble more or less conservative haplotype estimates, by checking a feature that will “consider taxa redundant even if states are not identical, as long as a resolution of missing or uncertain data could make them identical.” This feature was checked in all cases.

For *L. t. elapsoides*, 3 subsets of the ND4 contig were generated, in an effort to prioritizing either numbers of taxa (specimens) or numbers of nucleotides. The first was a 595 base pair segment consisting of 12 specimens and the outgroup *Lampropeltis getula*. This section produced 7 haplotypes. The second consisted of 244 consecutive base pairs from 44 specimens of *L.t. elapsoides* and three outgroups: *L. getula, L. t. campbelli*, and *L. t. triangulum*. The 244 b.p. section produced 10 haplotypes. In addition, the 638 b.p. contig used previously for phylogenetic analyses was subsequently used to generate haplotypes, which contained somewhat more missing data than the 595 b.p. contig, and the same number of informative sites, but was used to assess divergence times. It generated the same 7 haplotypes as the 595 b.p. fragment.

For *M. fulvius*, two additional treatments were used to generate haplotypes. The 686 b.p. contig, with 11 specimens, produced three haplotypes. A 101 b.p. contig, consisting of 26 sequences, produced 5 haplotypes.

All sequences used to generate haplotypes plus the haplotypes themselves were then submitted for maximum parsimony analysis, as above.

In addition, for subsequent calculation of divergence times, all haplotypes and constituent sequences were submitted to MEGA, ver. 4.0.2. (Tamura, et al., 2007). Data was coded as vertebrate, mitochondrial, protein-coding sequences. Data was used to generate networks under both Minimum Evolution and UPGMA settings. Parameters for ME and UPGMA phylogenetic reconstruction were by default: all sites included, transitions and transversions included, all codon positions included and assuming homogeneous patterns among lineages. The only alteration made was to conduct Tajima's relative rate test within MEGA to test for rate homogeneity among lineages. For that test, all positions and all changes were accepted by default. In no case did ingroup lineages show rate heterogeneity ($P>>0.05$). On the other hand, the outgroups did show rate heterogeneity in one case, that of the 686 b.p. sequence of *M. fulvius*, using *M. mipartitus* and *M. frontalis* as outgroups. In this case, The $\chi 2$ test statistic was 5.57 ($P = 0.01829$ with 1 degree[s] of freedom), rejecting the null hypothesis of equal rates between lineages. As a result, for this data set, the sequences were submitted to PHYML (Guindon, et al., 2005), which provided an estimated Gamma shape parameter of 1.1, which was used in MEGA. The resulting phylogenies were linearized under the assumption of equal evolutionary rates in all lineages.

7. Diversity Indices, Population Structure and Demographics

For this component, all sequence data was first converted to a nexus format in MacClade. It was then imported into DNAsp, ver. 4.10.9 (Rosas J., et al., 2006). DNAsp was used to code haplotypes and write script for Arlequin, ver. 3.1 (Excoffier L., 2005) and provide measures of genetic diversity and demographics. For *L. t. elapsoides*, the 595 b.p. and 244 b.p. (both with minimal or no missing data) sequence sections were imported into DNAsp and coded as haploid mitochondrial DNA. For *M. f. fulvius*, the 688 and 101 b.p. fragments were imported into DNAsp and similarly treated.

Populations were defined in DNAsp using the “Define Sequence Sets” function. Populations were defined by ecoregion using the Oak Ridge National Laboratory Geoecology Data Base (Olson, et al., 1980), vicariant zone, and by geographic distance, in the form of groups created by cluster analysis (see Appendix 1 for cluster groupings). Vicariant zones were coded as follows:

Barrier	Designation
Appalachicola River	E=1, W=2
Coastal Plain	E(Atlantic Coast) or S(Gulf Coast)=1, W(Atlantic coast) or N(Gulf Coast)=2
Mississippi	E=1, W=2

Mobile Basin	E=1, W=2
Savannah river	N=1, S=2

So, for example, a specimen from Miami, Florida would be coded 11112. Cluster analysis made use of Ward's (Ward, 1963) method of clustering, which involves minimizing the Sum of Squares between pairs of clusters that are formed at each step in the algorithm (Dupont, et al., 2001). Various combinations of these criteria were also employed, with a view to balancing sample size per population and numbers of populations.

For the 595 b.p. *L. t. elapsoides* sequences, a set of 10 clusters was used to group data. These were then further assembled by vicariant zone or ecological region. In some cases specimens were shifted to accomodate vicariant breaks. Further grouping was conducted in Arlequin. The 244 b.p. segment, with more samples, was clustered in 10 and 15 groups by distance, and then further grouped by vicariant zone and ecoregion. The 688 b.p. fragment of *M. fulvius* had too low a sample size to pursue further population genetic analysis, although sequence and haplotype diversity data was generated by DNAsp. The 101 b.p. sample was clustered in 10 groups and divided by ecoregion and vicariant zone. All data was then exported in Arlequin format, which saved a haplotype file and a project file for each data set. When the data was exported, DNAsp provided a listing of the number of sequences, the number of sites, minus those with missing

data, the number of haplotypes and haplotype diversity. The “Polymorphic sites” analysis provided details about variable, non-variable and parsimony-informative sites. Analysis of “DNA Polymorphism” provided measures nucleotide diversity (π) and variance of π. The “Gene Flow and Genetic Differentiation” option, with alignment gaps excluded, was used to calculate estimates of gene flow (Nm), based on Gst and mismatch Fst values. The option “Fu’s and Li’s (and other) Tests,” with all mutations considered, was used to calculate Fu’s Fs statistic, which gives an estimate of recent population expansion, contraction or stasis. “Tajima’s Test” provided a measure of Tajima’s D value, another measure of population expansion. Finally, the “Population Size Changes” analysis provided an estimate of Tau (τ), the relative time in generations since population expansion (Fontanella, et al., 2008), with either constant or changing population size. As Arlequin was used to calculate the Tau value, needed to estimate population expansion times, the DNAsp estimate of Tau was used only for sequence set Mf688, which could not be analyzed in Arlequin, due to low sample size.

Each data set was then submitted to Arlequin. Populations as hypothesized above were treated in two ways with Arlequin’s “Group” feature. Either they were clumped together in a single group or they were placed in several groups, based on geographic region. Arlequin’s AMOVA feature was invoked under the Standard AMOVA format, with settings for 1,000 permutations and calculating

conventional F statistics in order to investigate population differentiation. In addition, in order to identify which, if any pairs of populations were significantly different, pairwise Fst values were calculated under the population comparisons function, with parameters set at 1000 permutations at a significance level of 0.05. Finally, "Mismatch Distribution Analysis" was used to "estimate parameters of spatial expansion." The Tau value estimated by Arlequin in this way was used to calculate mean and population-specific Tau values for calculation of mean expansion times. Upper and lower values for the expansion time were calculated from the 5% and 95% quintile Tau values. These calculations were based on the formula $\mathbf{T=\tau/2\mu}$, where T is the date of expansion of the population or the lineage in question and μ is the product of the mutation rate, the generation time and the sequence length (Peters, et al., 2005; Fontanella, et. al., 2008; Fontanella, 2008).

The mutation rate employed was based on Zamudio and Greene's (1997) and Calsbeek, et al.'s (2003) estimated reptilian mtDNA rates of 0.47%-1.79%/My, and Calsbeek, et al.'s (2003) estimated mean of 0.854%/My. In their study of the bushmaster, *Lachesis*, Zamudio and Greene (1997) estimated a "reptilian mtDNA evolutionary rate," based on 5 studies meeting the criteria of equivalent mass to *Lachesis* and divergence of at least 5 million years. The rate estimates were from 0.47% to 1.32% per million years. However, neither *L. t. elapsoides*, nor *M. fulvius* fully meet Zamudio and Greene's criteria, since *Lachesis* is a very heavy-bodied snake, which can mass up to 5 kg. In contrast, a large *L. t. elapsoides*

might mass 2% of that, and a *M. fulvius*, perhaps 10%. Zamudio and Greene warned that evolutionary rate varies according to metabolic rate and body size, differing between endotherms and ectotherms, and shows an inverse correlation with body mass. Calsbeek, et al. (2003) use a modified version of Zamudio and Greene's rates, from 0.47% to 1.79%, with an average of 0.854%. Those were the parameters used in this study, although they are problematic, both for Zamudio and Greene's reason, and because Calsbeek, et al., don't state how they arrived at their modification. Given the smaller size of the two taxa in this study, a better rate estimate might well lie at the upper end of Calsbeek, et al's estimate, or even the upper end of the1%-2%/mya rate for cytochrome b in the small to medium sized lizards of the genus *Anolis* (Thorpe, et al., 2005).

8. Divergence Times

Divergence times for lineages were estimated using the program MEGA, ver. 4.0.2. As noted previously, MEGA will provide a divergence estimate in conjunction with distance-related methods of tree construction, such as nearest neighbor-joining, UPGMA and Minimum Evolution (ME). ME was initially chosen, as a form of statistically-based parsimony (Swofford, et al., 1996). ME searches for the tree with the minimum sum of branch lengths (Rzhetsky and Nei, 1992). The clustering UPGMA method was subsequently added, as it is apparently based on the simple procedure of grouping progressively more distant taxa. This embodies the assumption that evolutionary rates in all lineages are the same

(Huelsenbeck, 1995). All sites with missing data or gaps were removed from the dataset. Parameters for tree construction were as noted above. Evolutionary distances were calculated using Maximum Composite Likelihood and are in units of numbers of substitutions per site. Molecular clock rates were based on Zamudio and Greene's (1997), Calsbeek, et al.'s (2003) and Thorpe's (2005) substitution rates in units of millions of years.

Molecular clock rates may also be calibrated using the fossil record, given the existence of resolved, well-supported phylogenetic hypotheses. Fossils can provide parameters for the emergence of species (Burbrink, 2007). The latest (inside) date for the emergence of an organism may be verified through the discovery of recognized members of that species. The earliest fossil evidence of a sister group in a clade can also be used to place latest possible dates on the divergence of the two species. Successively more distant sister groups in monophyletic groupings can be used to place outer brackets on the species of interest. Unfortunately, snake skeletal material is delicate, loosely articulated and easily dispersed and, thus, the fossil record, particularly for smaller snakes, is sketchy. No fossil remains of scarlet king snakes, per se, have been recorded in the literature. Fossils of *Lampropeltis triangulum* have been recorded, dating back to the late Miocene (~5-12 mya) (Holman, 2000). The earliest members of this species found in the southeastern United States date from the early Pleistocene (~1.9-0.9 mya) in Florida (Holman, 2000). Given the evident

paraphyly of the nominal species *L. triangulum* with respect to the other members of the genus, however, use of these fossils to calibrate a molecular clock for L. t. elapsoides might be problematic, and was eschewed in favor of substitution rate estimates. Nevertheless, the Florida fossil record for *L. triangulum* concords roughly with divergence estimates for *L.t. elapsoides*. An outer bracket estimate for the divergence of *L. t. elapsoides* may well be provided by the earliest fossils for recognizable members of the genus *Lampropeltis*, dating from the middle Miocene (~12-16 mya) (Holman, 2000). The species coinciding with this fossil record is listed as indeterminate, however, so it cannot be used to date a particular outgroup. The oldest fossils identified as *L. getula* date to the late Miocene (~5-9 mya) (Holman, 2000), although given the lack of resolution within the genus, this may not be a useful parameter for *L. triangulum elapsoides*. The earliest fossils identified as *Micrurus fulvius*, date back to the middle to late Pleistocene in Texas (~900,000 - 400,000 years ago) and possibly to the early Pleistocene in Florida (~1.9 – 0.9 mya) (Holman, 2000). The oldest unequivocal Florida (and southeastern) specimen dated to the late Pleistocene (150,000 – 10,000 years ago) (Holman, 2000). The oldest fossil remains of a (undetermined) member of the genus in the U.S. dates to the middle Miocene (12 – 16 mya) (Holman, 2000). Again, in the case of *Micrurus*, fossil evidence was not used to calibrate the molecular clock, given the dearth of specimens. It was, however, used to check the estimated divergence times calculated on the basis of evolutionary rates (Zamudio and Greene, 1997; Calsbeek, 2003).

III.C. Results

For the ensuing discussion, specimen clusters, vicariant zones, ecozones and haplotype designations are provided, in Appendices 2-4.

III.C.1. Phylogenetic Hypotheses

1.a. Higher Level Phylogenies

Higher-level phylogenetic analyses of available full ND4 sequences seemed to confirm that the species *Lampropeltis triangulum* was, in reality, a polyphyletic grouping. This assessment was conducted with seven congeners and three other members of the *triangulum* group, together with 12 specimens of *L. t. elapsoides*. *Pantherophis vulpinus* was used as the outgroup (Burbrink, 2007b). Of the 638 base pair section of the ND4 gene employed, 448 were constant, 110 were parsimony-informative and 80 were variable, but not informative. The resulting strict consensus of 64 most parsimonious trees, is shown in Figure 3-2. The total length of this tree was 172, and the Consistency and Retention Indices were both 0.75. As may be seen, *Lampropeltis triangulum elapsoides* shows an indeterminate relationship to other well-supported and resolved clades containing other members of the genus interspersed with other members of the nominal species *Lampropeltis triangulum*. It should be noted that using the full 889 base pair sequences available from Genbank and either with or without successive weighting *L. calligaster* is placed as the basal group, although the node only

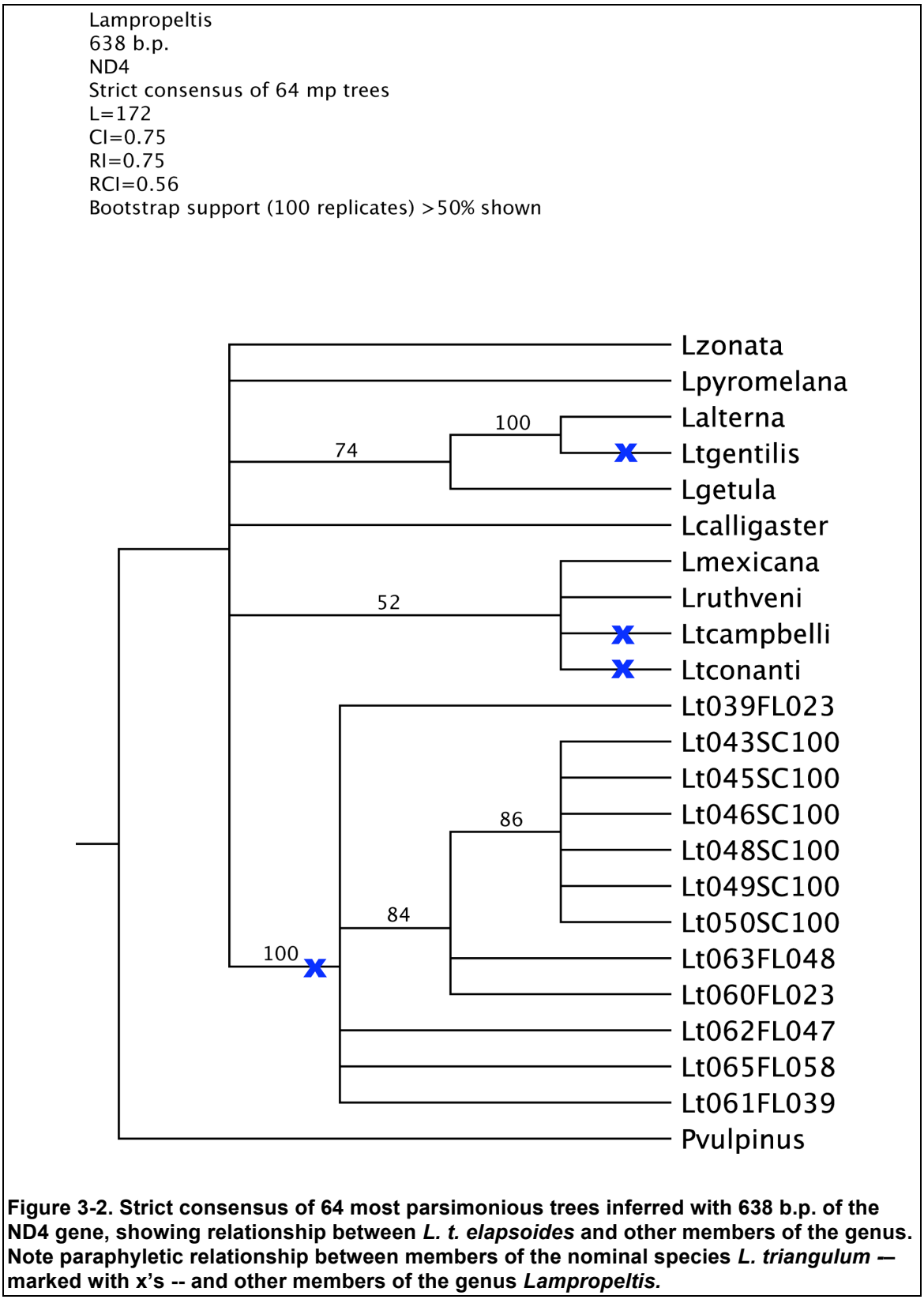


Figure 3-2. Strict consensus of 64 most parsimonious trees inferred with 638 b.p. of the ND4 gene, showing relationship between *L. t. elapsoides* and other members of the genus. Note paraphyletic relationship between members of the nominal species *L. triangulum* — marked with x's -- and other members of the genus *Lampropeltis.*

receives 30% bootstrap support. Successive weighting also somewhat enhances resolution of some of the outer clades. At the same time, resolution is lost among the shorter, more fragmentary *L.t. elapsoides* sequences. The polyphyly of *Lampropeltis triangulum* remains unchanged with successive weighting. The available specimen of *L. t. triangulum* was not initially included in this analysis because it was incomplete, consisting of 460 base pairs, but when it is included it reduces the resolution of the consensus tree: apparently, by weakly grouping with the *L. getula* clade, it forces the latter species into a polytomy with other congeners.

For *Micrurus f. fulvius*, as noted above, the full 30 specimens for which sequences were obtained were included in the analysis, together with seven congeners and three additional Elapid outgroups (given the uncertain relationships within the Elapidae). This analysis was conducted with an 813 base pair sequence consisting of the ND4 gene and neighboring tRNA sequences. The consensus of 1,000 most parsimonious trees is shown in Figure 3-3. With outgroups included, 489 characters were constant, 107 were variable, but uninformative, and 215 were parsimony-informative, but also 32.5% of the nucleotides were missing. The *M. fulvius* specimens formed a polytomy to which *M. corallinus* was basal. *M. fulvius* and its sister species *M. corallinus*, appear to be the most derived members of the *Micrurus* clade. Basal to these two are a group consisting of South American and Central American species.

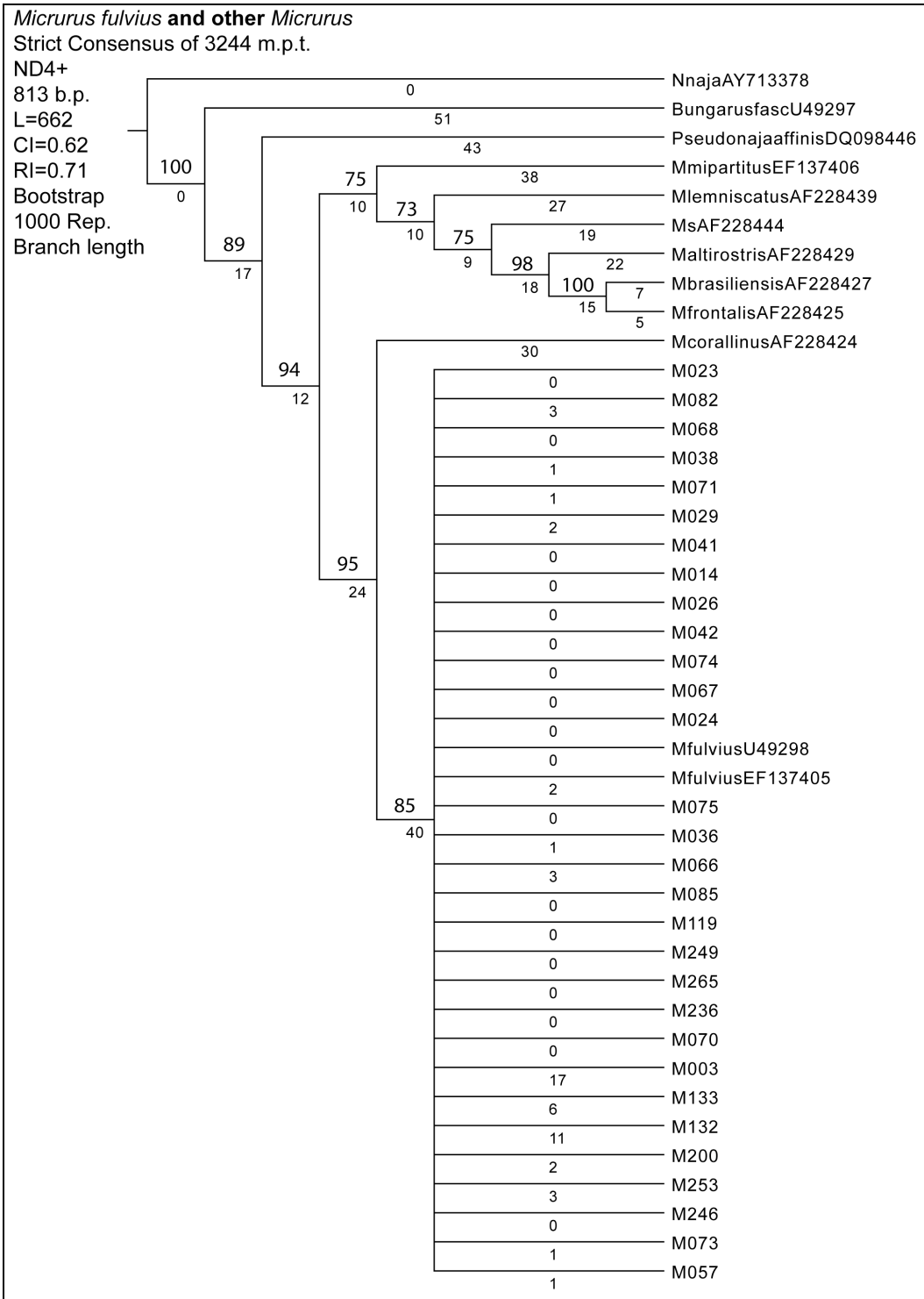


Figure 3-3. Strict consensus tree for 32 *Micrurus fulvius* and other *Micrurus* sequences consisting of 813 base pairs of ND4 sequence. Two sequences (U49298 and EF137405) were provided by Genbank.

1.b. Phylogenetic Hypotheses

The apparent paraphyly of the *L. triangulum* group, the presence of major geographic barriers in the southeastern and southern United States and the initial phylogenetic signal presented by the Aiken, South Carolina group of *elapsoides* suggested the possibility that unique diagnostic characters could be found for *L.t. elapsoides* that would permit the use of Population Aggregation Analysis to test for cladogenesis within this taxon. Suggestions that distinct populations of *Micrurus fulvius* could be further distinguished taxonomically, also raised the possibility that terminal taxa could be identified within this group. However, this method failed to find diagnosable characters or suites of characters for either taxon. Full sequences for both taxa are shown in Appendix 5 and 6.

1.c. Phylogeography and Population Genetics

Given the lack of geographic structure at the phylogenetic level, and the weak phylogenetic signal, the next step was to test for phylogeographic structuring.

The set of 100 sequences (97 *L.t. elapsoides* and 3 outgroups, *L. getula, L. t. campbelli* and *L.t. triangulum*) bracketed a total length of 809 base pairs, consisting of 713 constant characters, 36 variable, uninformative sites and 60 parsimony-informative sites. The strict consensus tree had a total length of 247, with Ci=0.37 and Ri=0.38 (Figure 3-4). The consensus contained 4 internal clades. One clade consisted of an Alabama specimen as sister group to a

Lampropeltis t. elapsoides
809 base pairs
ND4+
Strict consensus of 10,000 m.p.t.
L=247
CI=0.37
RI=0.38
Branch length above branches
Bootstrap support >50% below branches
(1,000 replicates)

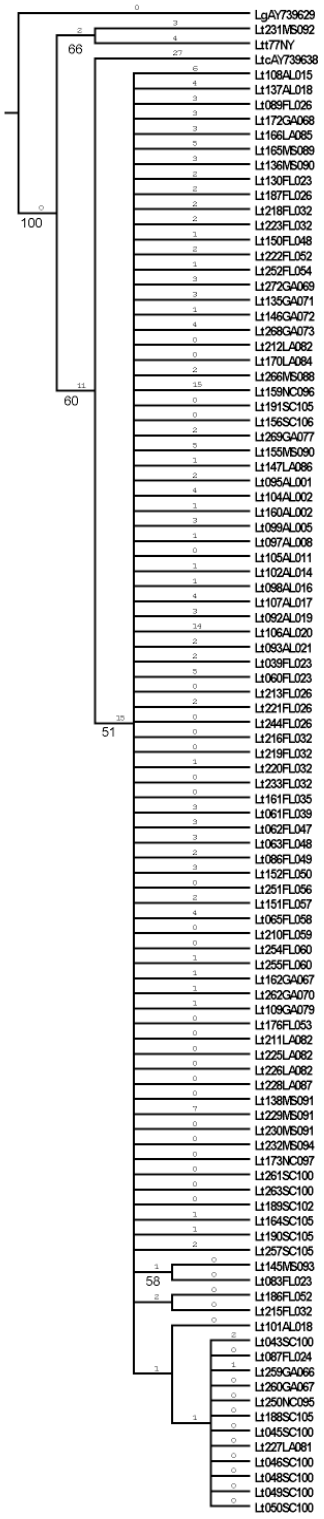


Figure 3-4. Strict consensus tree for 97 *Lampropeltis triangulum elapsoides* specimens used in this study.

polytomy of eastern seaboard specimens from Florida up through North Carolina and one Louisiana specimen. This included the previously observed Aiken, South Carolina group. The other two groups were one dyad of Florida specimens and another of a Florida and Mississippi specimen. A specimen from northwestern Mississippi (Lt231) groups together with *L. t. triangulum*.

These sequences were fused using the "merge redundant taxa" function in MaClade. Given the greatly varying fragment length of the available sequences, this provided a parsimonious rough estimate of the minimum number of possible haplotypes, while reducing the amount of missing data and the computational load due to character redundancy. The resulting 35 sequences (32 *L.t. elapsoides* and 2 outgroups), consisted of 713 constant characters, 37 parsimony-uninformative, variable characters, and 59 parsimony informative sites (including outgroups), with 34.18% missing nucleotides. The 32 merged sequences represent a rough **minimum** estimate of haplotypes, since a greater number of complete sequences would have introduced more, but not less, variability into the sample. That implies that distinct haplotypes were present in at least a third of these samples. Both the unweighted strict consensus and the successively reweighted consensus trees are shown in Figure 3-5. Again, the salient structure in these slightly more resolved trees was a clade of “haplotypes” containing the eastern Coastal Plain specimens, including the Aiken group, plus two specimens from Louisiana. Another divergent pair of “haplotypes” included

Georgia specimens, plus adjacent Alabama and Florida specimens. In addition, a terminal consisting of a North Carolina and an Alabama specimen resolved as basal to the rest of the *L.t. elapsoides* (save the lone specimen that continued to group with *L.t.. triangulum*). When successively weighted, these sequences produced a consensus tree with an additional two terminals, from Mississippi and Alabama, which were nested within the NC-AL terminal and sister to the rest of the *L.t. elapsoides*.

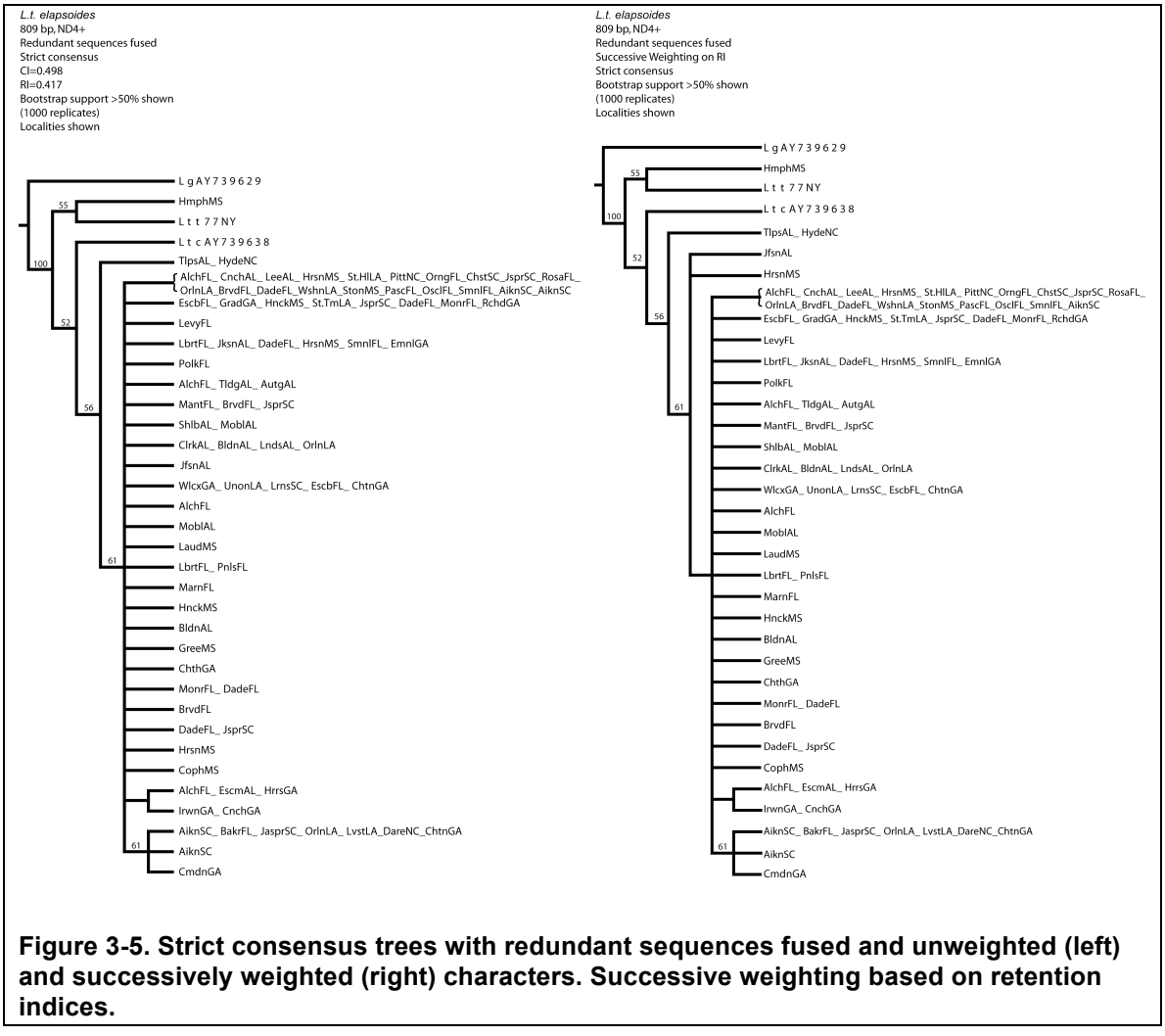


Figure 3-5. Strict consensus trees with redundant sequences fused and unweighted (left) and successively weighted (right) characters. Successive weighting based on retention indices.

The 595 base pair sequence (designated L595), including *L. getula* as an outgroup sequence, consisted of 528 constant characters, 9 parsimony-informative characters and 58 variable, but uninformative sites. Phylogenetic analysis based on parsimony generated two most parsimonious trees, whose strict consensus, with L=70, Ci=0.95 and Ri=0.90, is shown in Appendix 7. DNAsp reported haplotype diversity (Hd) for the 595 base pair fragment at 0.773, with a variance of 0.01628, based on 7 recognized haplotypes. With 7 haplotypes out of 12 specimens, distinct haplotypes were present in 58% of these specimens, which represented a limited sample size and area: 6 specimens from a restricted area in north and central Florida, plus 6 specimens from Aiken, South Carolina. All of the Aiken specimens grouped as one haplotype and the remaining specimens constituted individual haplotypes. These haplotypes are shown in parenthesis in Appendix 7. The haplotypes are also shown in Figure 3-6 as a rooted haplotype tree, with terminals plotted on a map of the region. The haplotype sequences contained 7 parsimony informative sites when including the outgroup and only 5 without the outgroup, shown in Appendix 8. This consensus haplotype tree is partially resolved, with two clades and two singletons. One clade contained the ubiquitous Aiken sequences. The remaining sequences were all from Florida. The two parsimony trees (Appendix 9) differed in relative positions (basal or derived) of haplotypes 2 (Aiken, SC) and 6 (eastern panhandle), and 1 (northern highlands) and 5 (northern highlands). Other, non-

parsimony treatments of this data show greater resolution, favoring one or the other of these configurations (see below, under “divergence”). All of these

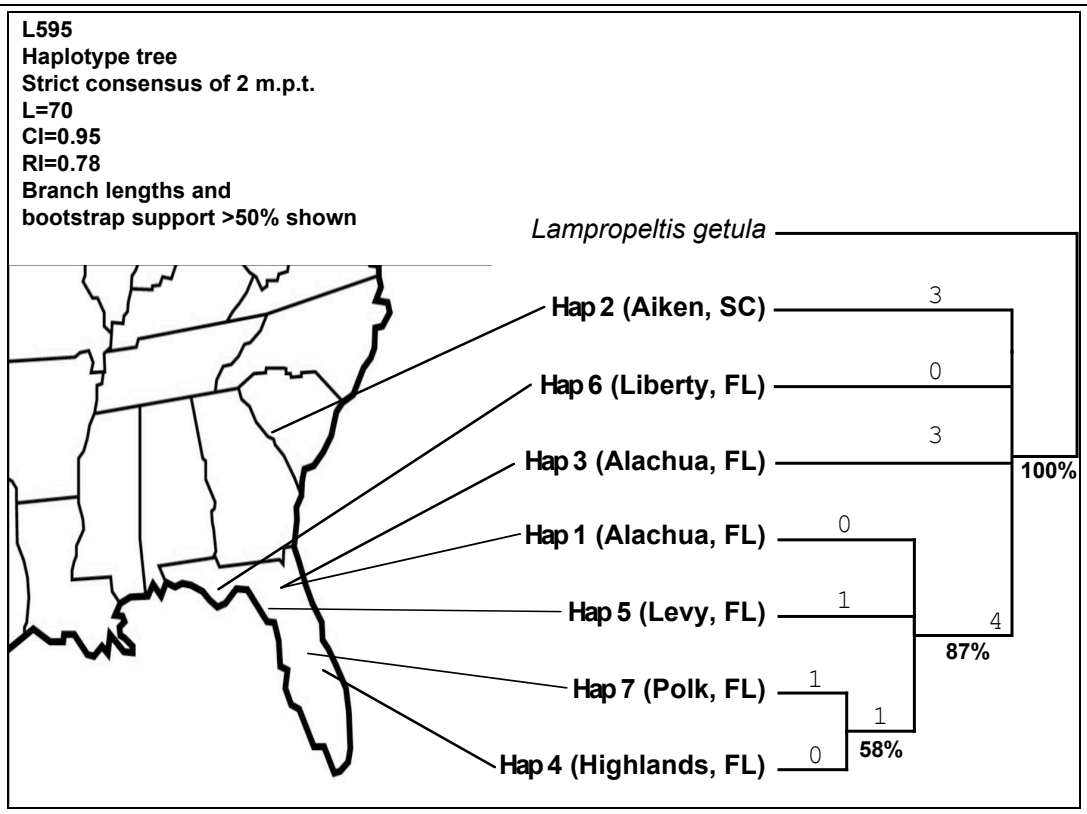


Figure 3-6. Strict consensus of 2 most parsimonious haplotype trees inferred from 12 x 595 bp ND4 sequences for *Lampropeltis triangulum elapsoides.*

treatments seem to agree upon a derived pair of haplotypes from the east-central Florida lowlands.

In addition, the 638 base pair sequence set, comprising the same specimens as the 595 base pair set, also generated the same 7 haplotypes as the 595 base pair set. This set was used because of the additional information content present in the 43 additional nucleotides. However, three of the sequences were missing considerable sections of those 43 nucleotides, so evaluation of these haplotypes

by way of DNAsp or ARLEQUIN was not conducted and data on haplotype diversity is unavailable. The Maximum Parsimony consensus tree was identical to the 595 base pair tree. These haplotypes were employed for divergence time estimates (see Appendix 10).

The 244 base pair sequence (designated L244) contained 205 constant characters, 23 parsimony-informative characters and 16 variable, but uninformative sites, including outgroup sequences *L. getula, L.t. triangulum* and *L.t. campbelli*. The strict consensus, with L=70, Ci=0.58 and Ri=0.57, is shown in Appendix 11. DNAsp reported haplotype diversity (Hd) for the 244 base pair fragment was 0.4038, based on 4 recognized haplotypes, since that program discounts all missing sites. However, Sequencher was used to identify 10 haplotypes, meaning that distinct haplotypes were present among at least 23% of these specimens. These 10 haplotype sequences, are shown in Appendix 12 and shown as a consensus haplotype tree plotted on a map of the southern U.S. in Figure 3-7. The haplotype tree, shown rooted with an outgroup (*L. getula*) for polarity, contained 9 informative characters with the outgroup, and only 4 without it. The gene tree was largely a polytomy, although the specimen from north-western Mississippi that grouped with *L.t. triangulum* in more complete sequence sets, appeared as the sister terminal to the remaining *L.t. elapsoides,* and an

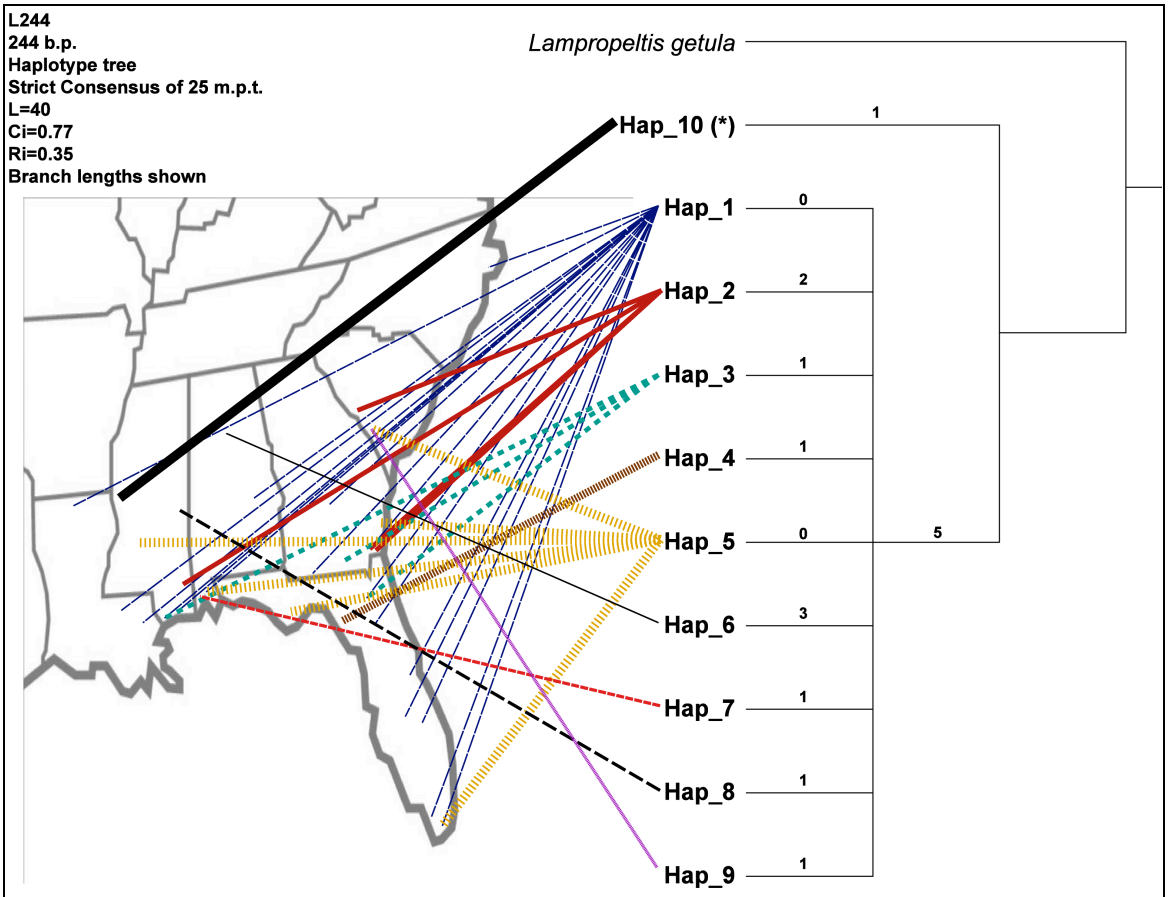


Figure 3-7. Haplotype consensus tree inferred from 44 *Lampropeltis triangulum* sequences, 244 b.p.

internal clade appeared containing the Aiken group, but also specimens from southern Georgia and southeastern Mississippi. The haplotype tree showed no geographic structure, with haplotype 1 representing 19 specimens, haplotype 2 representing 9 specimens, haplotype 3 representing 3 specimens, haplotype 5 representing 7 specimens and the remaining haplotypes representing individual specimens.

The same procedure was utilized for the coral snakes as was employed for the scarlet kings. A 789 base pair ND4+ contig (subset of the 811 b.p. used for the

full sequence set) was processed under the “merge redundant taxa” command in MacClade and the 30 *Micrurus fulvius* terminals were reduced to the 12 shown in Figure 3-8. The “haplotypes” so formed, shown in Appendix 13, contained 10 parsimony-informative sites and 21 singleton sites, but also 32.5% missing data. One terminal consisted of 18 widespread specimens, one contained 3 wide-ranging Florida specimens, one consisted of a pair from northern peninsular Florida and central Georgia, while the rest represented individuals from various parts of Florida and one from Louisiana. Parsimony analysis gave 13 most parsimonious trees, of which the strict consensus had a length of 218, Ci=0.90 and Ri=0.56. A single specimen from the central highlands of Florida resolved as basal to the remaining terminals, although bootstrap support was <50%. Successive weighting produced the same consensus tree.

In addition, only 7 specimens were fully represented by the 811 base pair sequences. When these were used to generate a phylogeny using Maximum Parsimony, a single southwestern Florida (Collier County) specimen resolved as basal to a polytomy consisting of the remaining specimens, all from Florida.

The 688 b.p. segment consisted of 10 specimens, plus one outgroup (*M. partitus*). It contained 580 constant characters, 105 variable, uninformative characters and only 3 parsimony-informative characters. The strict consensus, with Ci=0.99 and Ri=0.67, is shown in Figure 3-9. The consensus tree was

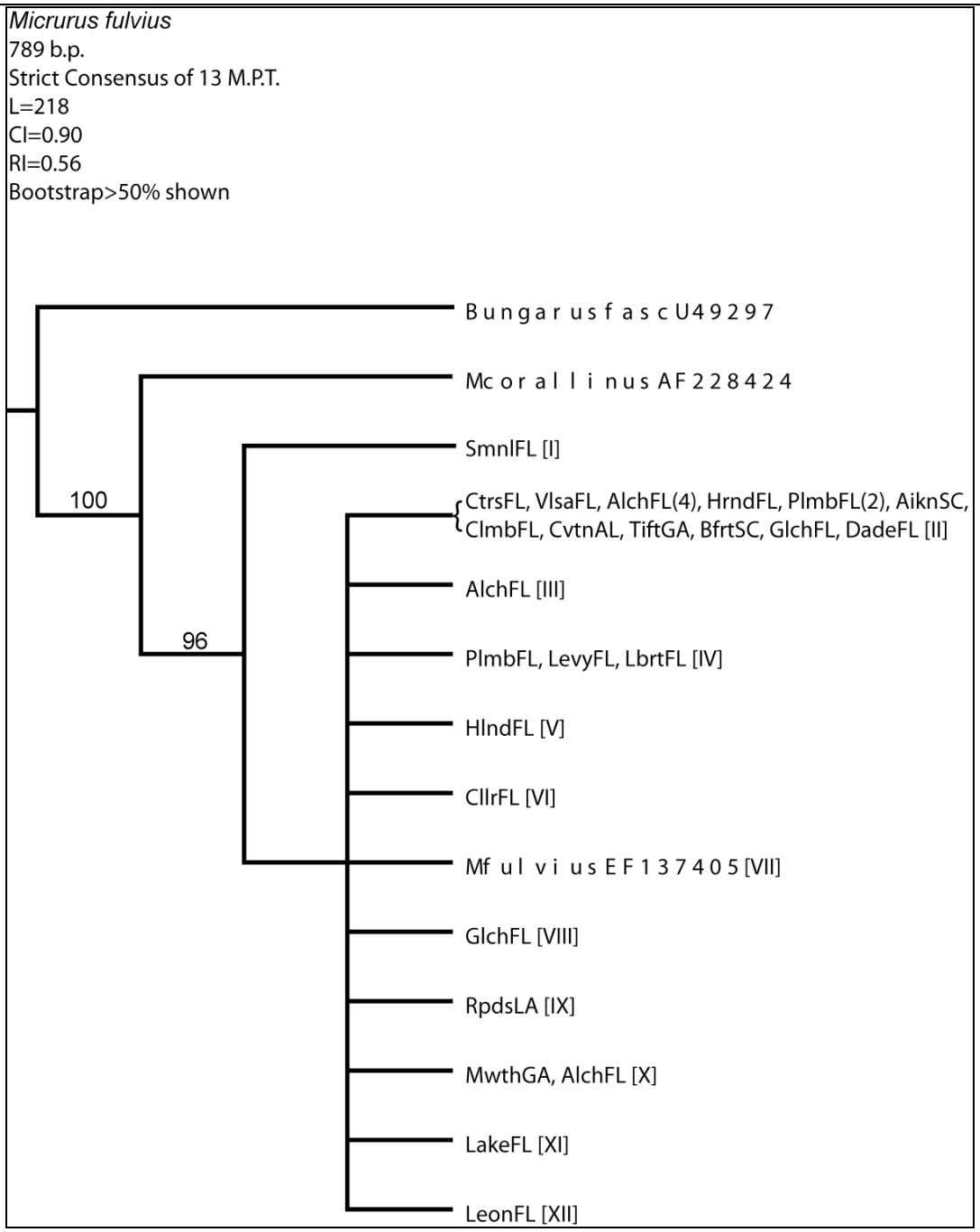


Figure 3-8. Strict consensus tree of merged sequences for *M. fulvius*. Haplotype designations are shown in parentheses. The tree was inferred from 26 sequences (EF134075 provided by Genbank) of varying length with a maximum of 789 base pairs.

unresolved, except for the Collier County specimen, from the Great Cypress Swamp, which is sister to the remaining specimens. This basal specimen had 86% bootstrap support (at 1000 replicates). This sequence segment generated

three haplotypes, shown in parentheses in Figure 3-9, one corresponding to the lone southwestern Florida specimen, one other representing a southeastern Florida specimen (Palm Beach) and the other representing widely-distributed specimens from Florida and a lone South Carolina specimen. Successive weighting produced the same consensus. The 101 base pair segment consisted of 26 terminals, including 2 outgroups (*B. fasciatus and M. corallinus*). It contained 14 parsimony informative sites, when including outgroups, but 7 informative sites and 5 singleton sites among *M. fulvius,* alone. The strict consensus tree, shown in Appendix 14, had a length of 42, Ci=0.88 and Ri=0.82. In this case, a clade consisting of 3 specimens from northern Florida, southern Georgia and central Louisiana was sister to the remaining *M. fulvius*. The Louisiana specimen was basal to the Florida/Georgia pair. This sequence set produced 5 haplotypes, whose sequences are shown in Appendix 15. DNAsp reported haplotype diversity (Hd) for the 101 base pair fragment (effectively 98 sites, with 3 sites eliminated due to missing data) was 0.292, based on 3 recognized haplotypes. However, the five haplotypes obtained via MaClade for these 21 specimens meant that distinct haplotypes were present in 24% of these specimens. The haplotype designated “Haplotype 1” represented the northern Florida/southern Georgia basal pair, Haplotype 2 represented widespread specimens from Florida, Georgia, Alabama and South Carolina, Haplotype 3 represented the basal Louisiana specimen, Haplotype 4 represented Genbank specimen EF137405, of unknown locality, and Haplotype 5 represented the

southwest Florida (Collier County) specimen. Parsimony analysis of these haplotypes produced a single haplotype tree with Ci=0.79 and Ri=0.76, shown in

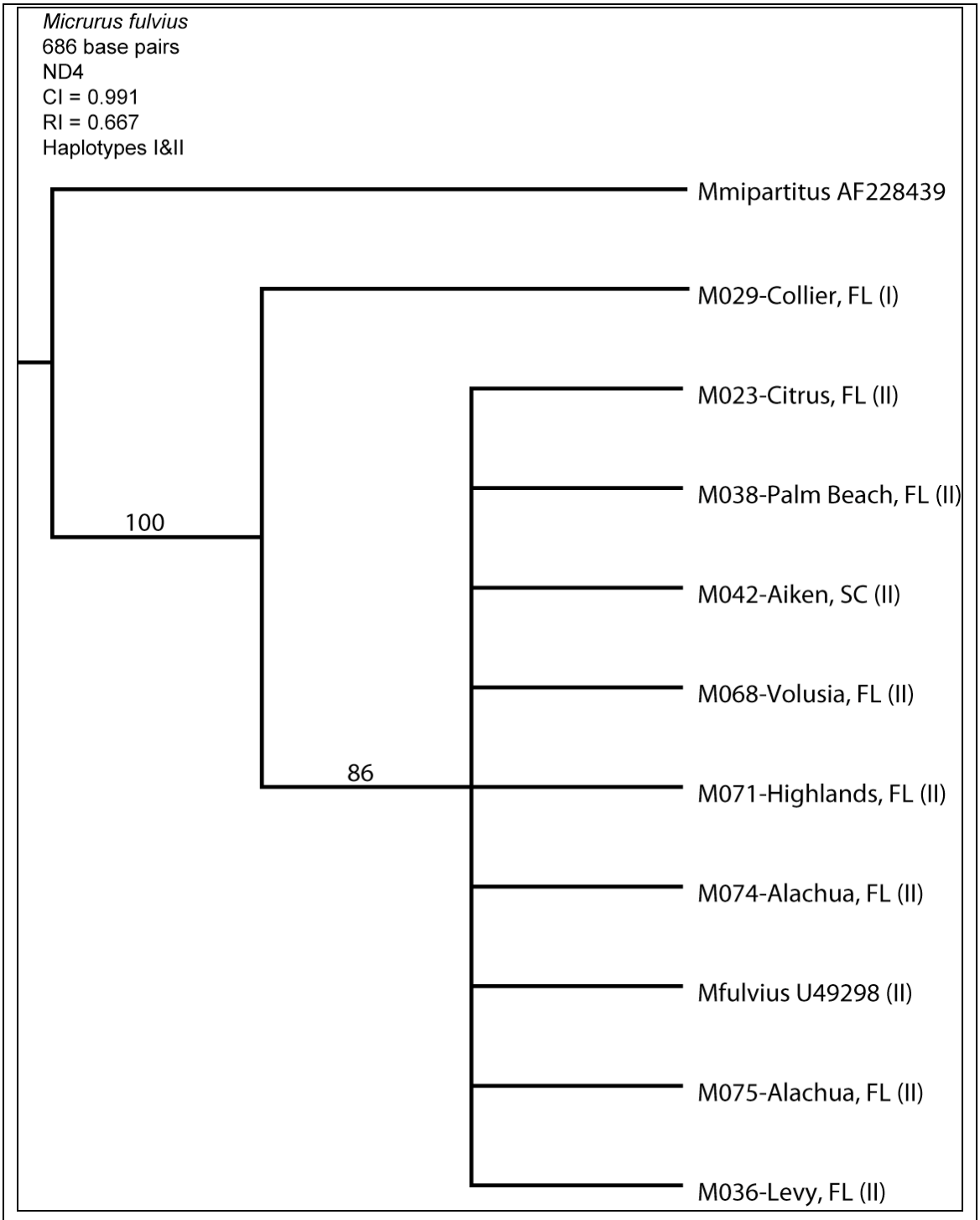


Figure 3-9. Strict consensus of 10 x 686 b.p. sequences of *M. fulvius*, showing haplotype designations.

Figure 3-10. In this tree, Haplotype 1 grouped with Haplotype 3 as a sister clade to the remaining specimens, with Haplotype 5 basal to Haplotypes 2 and 4.

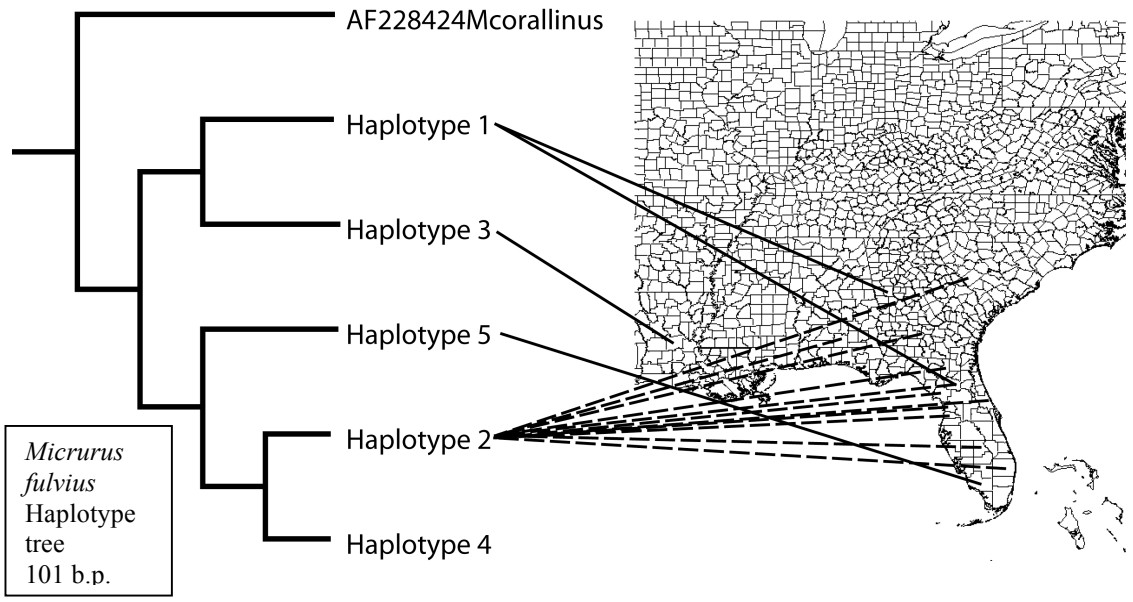


Figure 3-10. Single most parsimonious tree for haplotypes generated from 17 x 101 b.p. sequences from *M. fulvius.*

1.d. Diversity Indices, Population Structure and Demographics

Summary diversity and demographic data is shown in Table 3-1. The nucleotide

Table 3-1. Diversity and demographic data for *L. triangulum* and *M. fulvius* sequences. Calculations of population expansion time based on the formula T=τ/2μ, where μ is adjusted for generation time. Upper and lower τ (Tau) intervals are set according to upper and lower 95% values. Mean substitution rate used is 0.854%/my (see text).

Lineage	Tajima's D	Fu's F	Hd	Pi	K	Nm (Gst)	Nm (mismatch Fst)		Mean Tau	Lower Tau	Upper Tau	Rate of evolution / million years	Mean Time (yrs) Since Expansion	Lower estimate	Upper Estimate
L244 (15 clusters)	-0.8206	-1.3005	0.4038	0.00208	0.424	0.74		0.71	0.56881	0.04387	0.80919	8.54E-09	27297.28952	2105.328829	38833.16697
L244_w15_11112_13 (Atl. FL/GA)	na	na	na	na	na	na	na		3.631	0.19336	5.52148	8.54E-09	174252.3131	9279.379583	264976.7728
L244_w15_11112_7 (FL n. highlands)	na	na	na	na	na	na	na		0.764	0	1.80664	8.54E-09	36664.49111	0	86700.96364
L244_w15_21122_4 (w. Of Mobile)	na	na	na	na	na	na	na		3	0.57031	3.5	8.54E-09	143970.5148	27369.27477	167965.6006
L595	0.4008	-0.1712	0.773	0.00792	4.712	0.88		0.24	2.13721	0.71338	23.38721	8.54E-09	42060.29953	14039.32065	460260.3664
L595 ward2 (FL n. highlands)	na	na	na	na	na	na	na		8.54883	0.2207	93.54883	8.54E-09	168241.0013	4343.376695	1841041.269
M686	-1.23716	0.856	0.286	0.00084	0.571	0.82		0.83	3.691			8.54E-09	79099.81793	0	0
M384-485	0.77907	3.126	0.292	0.02041	2.000	-14.32		2.92	2.73177	0.91732	4.35417	8.54E-09	395890.3355	132938.7623	631009.8662

diversity (π) for the 595 base pair sequences was 0.00792, while the average pairwise nucleotide differences (k) was 4.712. Both Tajima's D and Fu's F_s statistic were insignificant (D=0.4008 and F_s = -0.1712) for this fragment, both indicating no recent range expansion. Gene flow estimates for the 595 b.p. fragment ranged from Nm=0.88 (Gst=0.36129) for haplotypes to 0.16 for sequences (γst=0.76235), with a calculation of gene flow from mismatch distribution weighing in at Nm=0.24 (Fst=0.67797). Ages of population geographic expansion were based on Zamudio and Greene's (1997) and Calsbeek, et al. (2003) estimated reptilian mtDNA rates of 0.47%-1.79%/My and

Calsbeek, et al.'s (2003) estimated mean of 0.854%/My. However, if we accept Zamudio's warning that evolutionary rates inversely correlate with body mass and that her calculations were geared to a 3-5 kg snake (*Lachesis*), a better estimate for the much smaller *L. t. elapsoides* and *M. fulvius* would lie at the upper end of those rates or within the 1%-2%/My calculated by Thorpe, et al. (2005) for small lizards. A mean Tau value of 2.13721 was calculated for this fragment, for an estimate of expansion time for the entire population of approximately 42,000 years, with lower and upper estimates of 14,000 and 460,000 years, respectively. With a 2% substitution rate, that range becomes 6,000-20,000 years, with an average of 18,000 years. For each of the designated populations, only one had a non-zero Tau value. A cluster of 3 haplotypes from the northern peninsular Florida "northern highlands," with a π of 5.333 (as compared to the other clusters'

values of 1.000, 0 and 0), generated a Tau value of 8.54883 (Tau (qt 2.5%)=0.22070, Tau (qt 97.5%)= 93.54883), which gave an estimated expansion time of 168,241 years, but ranging anywhere from 4,343 to 1,841,041 years.

AMOVA suggested non-random distribution of variation for populations based on the 10 distance-based clusters, of which 4 were present in this data set. The F_{st} value was 0.59829 ($P<0.01$), with 60% of the variation among populations and 40% within populations. Most of this variation appears to be due to the effects of a South Carolina-Georgia cluster (containing the Aiken group) and an eastern Florida panhandle-Georgia group, with population F_{st} values of 0.75214, each. When pairwise F_{st} values are taken into account, the only significant values are between the Aiken, SC grouping (designated 10-7) and the northern highlands Florida grouping (10-2) (F_{st}= 0.67568, $P<0.01$), and between the former and a Florida eastern central lowlands grouping (10-6) (F_{st} = 0.80645, $P<0.05$). AMOVA data is shown for *L. t. elapsoides* and *M. f. fulvius* in Table 3-2. Pairwise Fst values for this sequence segment are shown in Table 3-3, with significant values marked with an asterisk.

When the four "populations" were grouped by vicariant zones (north and south of the Savannah River), the F_{st} value increased slightly to 0.6250 ($P<0.01$), with the variation between zones accounting for 28% of variation, that among populations for 34% of variation and within-population variation representing 38% of the total.

Table 3-2. AMOVA calculated for populations based on geographic distance, Oak Ridge National Laboratories-designated Ecozones and vicariant zones. Fst's calculated from haplotype frequencies.

Fragment	Population Descriptions	Source of Variation	df	Percentage of Variation	Fst	p
M101	Clusters (Ward 10)	Among populations	6	34.54	0.34536	0.40469
		Within populations	11	65.46		
M101	Vicariant Zones	Among populations	3	64.44	0.64444	0.32356
		Within populations	14	35.56		
M101	E/W of Appalachicola	Among populations	1	47.97	0.47967	0.06354
		Within populations	16	52.03		
M101	Ecozones (Oak Ridge)	Among populations	2	60.80	0.60795	0.11926
		Within populations	15	39.20		
L244	Clusters (Ward 15)	Among populations	12	48.22	0.48221	0.00000
		Within populations	31	51.78		
L244	Clusters (Ward 15) x Vicariant Zones	Among groups	5	9.05	0.47778	0.00098
		Among populations	10	38.73		
		Within populations	28	52.22		
L244	Ecozone x Vicariant Zone x Clusters (Ward 15)	Among groups	3	11.86	0.56299	0.00098
		Among populations	13	44.43		
		Within populations	26	43.70		
L244	Clusters (Ward 15) x Savannah River	Among groups	1	43.06	0.61943	0.00293
		Among populations	13	18.89		
		Within populations	28	38.06		
L595	Clusters (Ward 10)	Among populations	3	59.83	0.59829	0.00000
		Within populations	8	40.17		
L595 (Pairwise difference)	Clusters (Ward 10)	Among populations	3	75.81	0.75805	0.00000
		Within populations	8	24.19		
L595	Clusters (Ward 10) x Savannah River	Among groups	1	28.41	0.62500	0.00000
		Among populations	2	34.09		
		Within populations	8	37.50		

The nucleotide diversity (π) across *L. t. elapsoides* haplotypes for the 244 b.p. fragment was 0.00208±.00005 (P=0.05), while the average number of nucleotide differences between pairs (k) was 0.424. Tajima's D was insignificantly negative (-0.8206) for this fragment and Fu's F_s statistic was also slightly negative and not significant (-1.3005), both indicating no recent range expansion. Gene flow estimates (Nm) for this fragment ranged from 0.74 (Gst=0.40302) for haplotypes to 0.38 (γst=0.57114) for sequences. Calculations of gene flow based on mismatch distribution yielded Nm=0.71 (Fst: 0.41304). A mean Tau value of 0.56881 was calculated for this fragment, for an estimate of expansion time for the population of approximately 27,297 years, with lower and upper estimates of 2,105 and 38,833 years, respectively. It should be noted that the 244 b.p. fragment has 1/3 of the nucleotide diversity, 1/10 of the pairwise differences and a little more than half of the haplotype diversity of the 595 b.p. fragment (in spite of being a larger sample set), and, consequently, does not adequately sample the ND4 gene.

Table 3-3. Population pairwise Fst's for *L. triangulum*. Populations are four of ten groups of specimens (595 base pairs) clustered by geographic distance.

Cluster	10-2	10-6	10-7	10-9
10-2	0.00000			
10-6	0.00000	0.00000		
10-7	0.67568*	0.80645*	0.00000	
10-9	0.00000	0.00000	1.00000	0.00000

* Significant at p=0.05

AMOVA for populations defined in terms of geographic distance (15 clusters, of which these specimens represented 12) suggests non-random distribution of

genetic variation (F_{st}=0.48221, P<0.01), with 48% of variation distributed among populations, and 52% within populations. All of the significant variation between populations occurred between cluster one, which contained the Aiken, SC specimens, and the remaining groups (pairwise F_{st} values were significant at P=0.05 for 6 comparisons). See Table 3-4 for pairwise Fst values for this sequence fragment.

Table 3-4. Population pairwise Fst's for a 244 b.p. ND4 fragment for *L. triangulum*. Populations are13 of 16 groups of specimens clustered by geographic distance. MW represents specimens found west of the Mississippi River.

	15-01	15-03	15-04	15-06	15-05	15-07	15-08	15-09	15-10	15-12	15-13	15-14	MW
15-01	0.000												
15-03	1.000*	0.000											
15-04	0.836*	-0.109	0.000										
15-06	1.000	0.000	-0.318	0.000									
15-05	1.000	0.000	-0.318	0.000	0.000								
15-07	0.804*	0.000	-0.045	-0.263	-0.263	0.000							
15-08	1.000	0.000	-1.000	0.000	0.000	-1.000	0.000						
15-09	1.000*	0.000	-0.109	0.000	0.000	0.000	0.000	0.000					
15-10	0.804*	0.000	-0.045	-0.263	-0.263	-0.143	-1.000	0.000	0.000				
15-12	1.000	0.000	-1.000	0.000	0.000	-1.000	0.000	0.000	-1.000	0.000			
15-13	0.442	0.333	0.158	0.111	0.111	0.067	-0.333	0.333	0.067	-0.333	0.000		
15-14	1.000*	0.000	-0.175	0.000	0.000	-0.091	0.000	0.000	-0.091	0.000	0.250	0.000	
MW	1.000	0.000	-1.000	0.000	0.000	-1.000	0.000	0.000	-1.000	0.000	-0.333	0.000	0.000

When the 244 b.p. fragment was further grouped by vicariant zone, genetic variation was significantly non-randomly distributed (F_{st}=0.47778, P<0.01), with 9% of the variation between vicariant zones, 39% of the variation between populations and 52% of the variation within populations. Similarly, significant F_{st} values were obtained when grouping populations (clusters) by ecozone (F_{st}=0.56299, P<0.01), with variation among ecozones accounting for 12%, within clusters 44% and within populations 44% of variation. Significant F_{st} values were also obtained when clusters were grouped east and west of the Appalachicola River, with F_{st}=0.45805 (P<0.01). In this case, -7% of variation could be attributed to the regions, themselves, while the variation between and within populations

was 53% and 54%, respectively. The most salient F_{st} value was obtained when grouping populations north and south of the Savannah River. In this case, F_{st}=0.61943 (P<0.01), with 43% of variation attributable to region, 19% due to population differences and 38% to variation within populations. Other combinations of region and cluster were insignificant.

The two sets of data suggest that there is differentiation between the northern population (Aiken group) of *L.t. elapsoides*, on the one hand, and northern and eastern Florida populations, on the other, with additional differentiation on the Florida peninsula and panhandle. This pattern should be taken with caution, given the limited sampling and the backdrop of limited genetic variation and geographic structure, overall, leading to a hypothesis that *L. t. elapsoides* may still constitute a single population across its range, with post-Pleistocene expansion from one or more refugia in which some differentiation occurred.

Small sample size/sequence length limited the possibility for conducting population genetic analysis of *M. fulvius*, but some data was obtained for the two fragments assessed previously. The nucleotide diversity (π) across *M. fulvius* haplotypes for the 101 b.p. fragment was 0.02041±0.00177 (P=0.05), while the average number of nucleotide differences between pairs (k) was 2.000. Tajima's D and Fu's F_s statistic (-0.77907 and 3.126, respectively) were both close to 0 and insignificant for this fragment, indicating no recent range expansion. Gene

flow (Nm) estimates ranged from -14.32 (Gst) to 2.92 (Fst). A mean Tau value of 2.73177 was calculated for this fragment, for an estimate of expansion time for the population of approximately 396,000 years, with lower and upper estimates of 133,000 and 631,000 years, respectively. However, as with *L. t. elapsoides*, there were large discrepancies in the values for nucleotide diversity and pairwise differences between the 101 b.p. fragment and the 686 base pair fragment. In this case, the short fragment's nucleotide diversity was over an order of magnitude larger than the large fragment, and the number of pairwise differences among the sequences in the short fragment was four times higher than among the longer fragment.

AMOVA for populations designated according to distance (10 clusters, of which these specimens represented 9) did not falsify random distribution of genetic variation: although the Fst value was high, it failed to achieve significance. When the specimens were grouped by vicariant zone, again, the Fst was quite high (0.64444), but not significant. When specimens were grouped east or west of the Apalachicola River, structured distribution of genetic variation achieved near significance, at P=0.06354±0.00824, with an Fst value of 0.47967.

For the 686 base pair section, only 7 *M. f. fulvius* specimens were present. All, except 1, were from Florida and two haplotypes were recognized, with Hd=0.286. Nucleotide diversity (π) was 0.00084±0.00004. The average number of pairwise

differences in nucleotides (k) was 0.571. Both Tajima's D and Lu's F_s values were insignificant (D= -1.23716, F_s=0.856). Although low sample size precluded AMOVA, DNAsp estimated Fst at 0.0000 and Gst at 0.37824. Gene flow, as expressed as Nm, was 0.82, based on Gst values, and 0.83, based on mismatch Fst values. Insufficient sampling also prevented calculation of Tau values in ARLEQUIN for this sequence, although DNAsp estimated Tau at 3.691, without upper and lower values, for an estimated time of expansion of some 79,000 years.

1.e. Divergence Times

Average and upper and lower estimated divergence times for each taxon and its lineages, calculated with MEGA using both Minimum Evolution and UPGMA approaches, are shown in Table 3-5.

Using the 638 base pair segment and all Lampropeltines, and given the substitution rate of 0.854% per million years (0.47%, 1.79%), *L. t. elapsoides* appears to have parted ways with its congeners about 5.560 million years ago (2.653-10.102 mya). UPGMA gives roughly the same estimate as ME, about5.476 million years ago (2.612-9.949 mya). This same substitution rate would set the basal split within the kingsnakes (*Lampropeltis*), subtending *L. calligaster* from other members of the genus, at some 6.489 million years ago (UPGMA gives the same estimate). The earliest fossils of this genus found in

Table 3-5. Estimated times to most recent common ancestors (TMRCA) for sequence segments for both *L. triangulum* and *M. fulvius*, both for individual sequences and haplotypes. Nodes refer to trees shown in Figures 3-11 and 3-12, and Appendices 16-26.

Fragment	ME Tree Node	Divergence Time (million years) (upper, lower estimates*) ME	UPGMA Tree Node	Divergence Time (million years) (upper, lower estimates*) UPGMA
L809 merged	*L. t. triangulum-L.t. 231+L. t. elapsoides*	1.686 (0.804, 3.063)	*L. t. triangulum-L. t. elapsoides*	1.356 (0.647, 2.464)
	Lt231- L. t. elapsoides	0.995 (0.475, 1.807)	*L. t. triangulum-Lt231*	0.701 (0.334, 1.273)
	1	0.767 (0.366, 1.393)	1	0.769 (0.366, 1.393)
	2	0.304 (0.145, 0.552)	2	0.528 (0.252, 0.960)
	3	0.299 (0.143, 0.543)	3	0.290 (0.139, 0.528)
	4	0.053 (0.025, 0.097)	4	0.284 (0.135, 0.516)
			5	0.276 (0.132, 0.502)
			6	0.242 (0.115, 0.439)
L638	*P. vulpinus-Lampropeltis*	9.260 (4.418, 16.826)	*P. vulpinus-Lampropeltis*	9.260 (4.418, 16.826)
	2	5.560 (2.653, 10.102)	3	5.476 (2.612, 9.949)
	7	0.760 (0.363, 1.381)	9	0.760 (0.363, 1.381)
	8	0.447 (0.213, 0.813)	10	0.554 (0.265, 1.007)
			11	0.297 (0.142, 0.540)
	9	0.297 (0.142, 0.540)		
	10	0.198 (0.094, 0.359)	12	0.198 (0.094, 0.359)
L638 (haplotypes)	1	0.697 (0.333, 1.267)	1	0.697 (0.333, 1.267)
	2	0.447 (0.213, 0.812)	2	0.447 (0.213, 0.812)
	3	0.297 (0.142, 0.540)	3	0.297 (0.142, 0.540)
	4	0.198 (0.094, 0.359)	4	0.198 (0.094, 0.359)
	5	0.099 (0.047, 0.179)	5	0.099 (0.047, 0.179)
	6	0.099 (0.047, 0.179)	6	0.099 (0.047, 0.179)
L595	1	0.760 (0.362, 1.380)	1	0.760 (0.363, 1.381)
	2	0.447 (0.213, 0.812)	2	0.554 (0.265, 1.007)
			3	0.297 (0.142, 0.540)
	3	0.297 (0.142, 0.540)		
	4	0.198 (0.094, 0.359)	4	0.198 (0.094, 0.359)
	5,6	0.099 (0.047, 0.179)	5,6	0.099 (0.047, 0.179)
	7	0 (0, 0)	7	0 (0, 0)
L595 (haplotypes)	1	0.680 (0.324, 1.236)	1	0.697 (0.332, 1.266)
	2	0.647 (0.308, 1.175)	2	0.447 (0.213, 0.812)
	3	0.297 (0.162, 0.539)	3	0.297 (0.142, 0.539)
	4	0.197 (0.094, 0.359)	4	0.197 (0.094, 0.359)
	5, 6	0.099 (0.047, 0.179)	5, 6	0.099 (0.047, 0.179)
L244	1	1.548 (0.738, 2.813)	1	1.548 (0.738, 2.813)
	2	0.356 (0.647, 0.170)	2	0.305 (0.145, 0.554)
	3	0.069 (0.033, 0.125)	3	0.297 (0.142, 0.539)
	4	0	4	0.288 (0.138, 0.524)
			5	0
			6	0
L244 (haplotypes)	1	2.157 (1.030, 3.919)	1	2.157 (1.029, 3.919)
	2	1.047 (0.499, 1.902)	2	1.047 (0.499, 1.902)
	3	0.682 (0.325, 1.239)	3	0.781 (0.373, 1.419)
	4	0.281 (0.134, 0.510)	4	0.634 (0.303, 1.152)
	5	0.281 (0.134, 0.510)	5	0.374 (0.178, 0.679)
	6	0	6	0.336 (0.160, 0.611)
	7	0	7	0.280 (0.134, 0.508)
			8	0.280 (0.134, 0.508)

Table 3-5 (Continued).

M686	Ingroup-outgroup	48.631 (23.202, 88.363)	Ingroup-outgroup	48.631 (23.202, 88.363)
	1	0.090 (0.043, 0.163)	1	0.090 (0.043, 0.163)
	2	0	2	0
M686 (haplotypes)	1	0.214 (0.102, 0.388)	1	0.214 (0.102, 0.388)
	2	0.085 (0.041, 0.155)	2	0.085 (0.041, 0.155)
M101	Ingroup-outgroup	4.712 (2.248, 8.562)	Ingroup-outgroup	5.552 (2.649, 10.088)
	1	2.662 (1.270, 4.836)	1	2.662 (1.270, 4.836)
	2	1.064 (0.508, 1.934)	2	1.064 (0.508, 1.934)
	3	0.020 (0.010, 0.036)	3	0.340 (0.162, 0.617)
			4	0
	4	0	5	0
M101 (haplotypes)	1	2.942 (1.403, 5.345)	1	2.942 (1.403, 5.345)
	2	1.570 (0.749, 2.853)	2	1.570 (0.749, 2.853)
	3	0.303 (0.144, 0.550)	3	0.303 (0.144, 0.550)

North America date from middle Miocene (~12-16 mya) Nebraska, Saskachewan and North Dakota (Holman, 2000). The earliest fossils of *L. getula* and *L. triangulum* found originated in late Miocene (~5-12 mya) Nebraska, while the earliest *L. calligaster* fossils found, dated from late Pliocene Nebraska (~2-3 mya) (Holman, 2000). *L.t. elapsoides* appears to have experienced its own divergence 0.760 mya (between 0.363 and 1.381 mya), which places it roughly within the Pastonian interglacial period (0.8-0.6 mya) of the middle Pleistocene. UPGMA estimates the same timing. Within the *L. t. elapsoides*, the basal divergence time for a split between the putative Aiken group from a Florida group is calculated at 0.447 million years ago (from 0.213 - 0.813 mya). The split between northern and central Florida specimens occurred some 0.198 million years ago (from 0.094 - 0.359 mya). The Minimum Evolution and UPGMA trees are shown, with average divergence times, in Appendices 16 and 17.

The 595 base pair fragment also gave an ME divergence time for *L.t. elapsoides* specimens of 0.760 million years (1.380 mya-0.362 mya). Within this group, the split between the South Carolina lineage and Florida lineages also took place some 0.447 mya, and differentiation among Florida genotypes took place about 0.198 mya. The divergence times for haplotypes within the 595 b.p. sequence set were also calculated by Minimum Evolution and UPGMA procedures, and are shown in Figure 3-11. The estimated average for the divergence time between the Florida north highlands and central lowlands haplotypes was 197,000 years (between 94,000-359,000 years) and for the two haplotypes within each region, about 99,000 years, placing them in the Illinoian and Wisconsin glacial periods, respectively.

The divergence between another of these north Florida haplotypes and an eastern panhandle haplotype took place some 297,000 years ago (539,000-142,000 years ago) (possibly in the Yarmouth interglacial). And the basal divergence occurred 680,000 years ago (324,000-1,236,000 years ago) (possibly in the Pastonian interglacial period or the preceding Kansan glacial). However, the Minimum Evolution approach places the Aiken haplotype as basal to the other haplotypes in contrast to the lineage tree, in which the Aiken specimens are derived from Florida lineages. In the UPGMA analysis, the Aiken group is sister to the eastern Florida panhandle haplotype and both are derived from a north Florida haplotype, while the position of the highland and lowland haplotypes

remains the same with both methods. In the UPGMA tree, the Aiken-Florida split occurred 0.297 mya (0.142-0.539 mya), while the divergence time between the

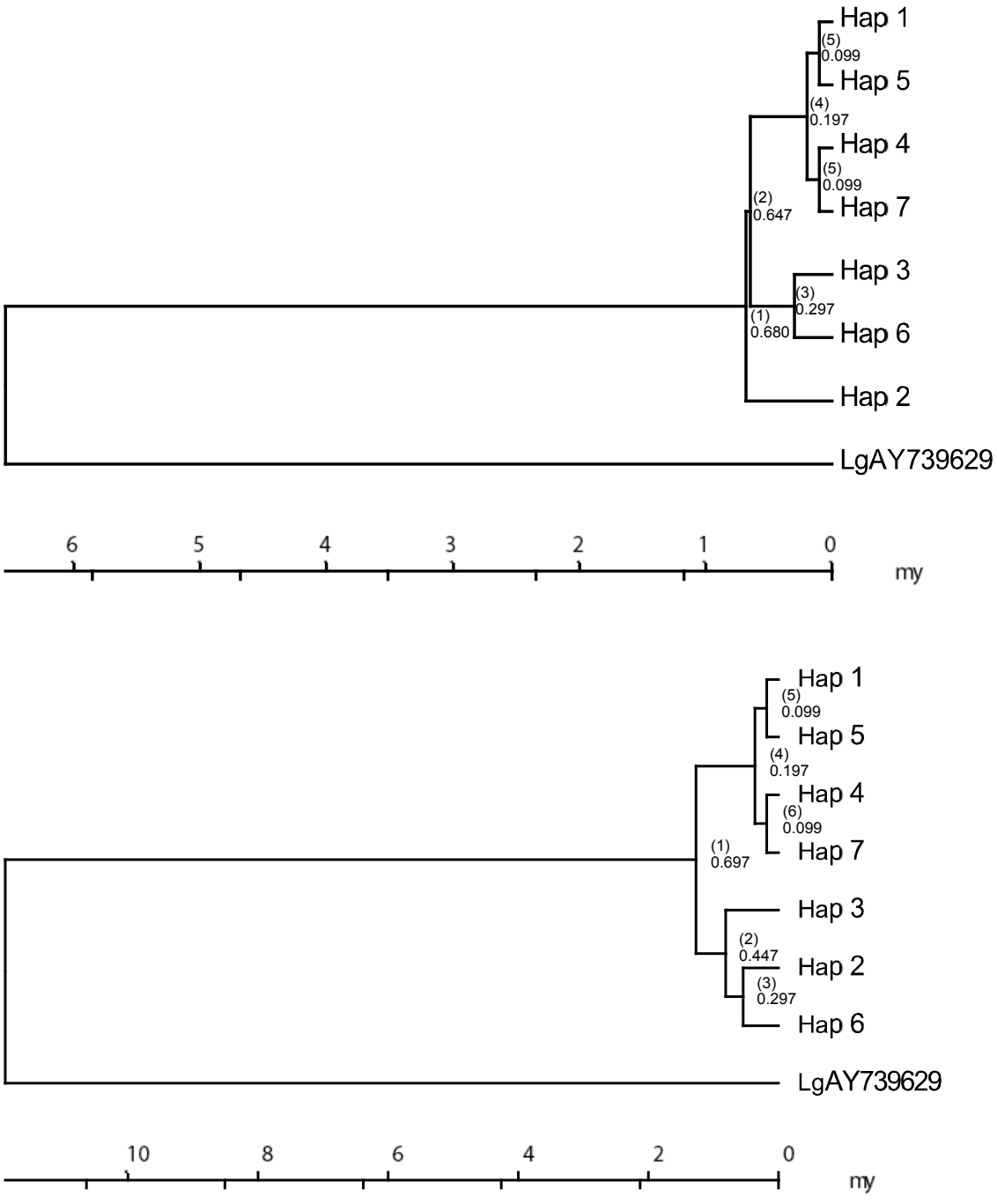


Figure 3-11. Minimum Evolution tree (above) and UPGMA tree (below) showing estimated divergence times since Most Recent Common Ancestors at nodes for 595 base pair haplotypes of *Lampropeltis triangulum.* Calculations based on substitution rate of 0.854%/my (see text). Numbers in parentheses refer to nodes listed in Table 3-5.

Florida north highlands and central lowlands haplotypes was 0.197 million years (between 0.094 and 0.359 million years) and for the two haplotypes within each region, about 0.99 million years. In both trees, the only node with significant bootstrap support is that subtending the north-central Florida haplotypes.

The 244 base pair fragment gave a divergence time less than half that of the longer fragments: 0.356 million years (0.647-0.170 mya), with the split between the lineage containing the Aiken group (plus a Georgia and a Mississippi specimen) and most other specimens taking place some 0.288 mya. The Minimum Evolution and UPGMA trees are shown in Appendices 18 and 19. The corresponding haplotype trees are shown in Appendices 20 and 21.

Finally, the 809 base pair "merged" sequence set was analyzed to estimate a possible divergence time between *L. t. triangulum,* the Mississippi *L. t. elapsoides* specimen that grouped with it (Lt231), and the remaining *L. t. elapsoides.* Minimum Evolution and UPGMA generated two different tree topologies and divergence estimates at these respective nodes. The ME tree shows a divergence between *L. t. triangulum* and the haplotype represented by specimen Lt231 at 1.686 mya, then another divergence between the latter and the rest of the *L. t. elapsoides* 0.995 mya. The UPGMA calculation shows a divergence between a binary clade containing *L. t. triangulum* and specimen Lt231, and the remaining *L. t. elapsoides* 1.356 mya. "Haplotype" Lt231 and *L. t.*

t. then diverged 0.701 mya. Coalescence of *L. t. elapsoides* haplotypes then occurs 0.767 and 0.769 mya in the ME and UPGMA trees, respectively (Appendices 22 and 23).

Among the *Micrurus fulvius*, all estimates should be considered with an even greater degree of caution due to very low sample size and missing data. For the 686 base pair fragment, shown in Figure 3-12 (the ME and UPGMA trees were the same), the estimated divergence time for 6 specimens, all from Florida, except a South Carolina specimen, from a basal southwestern Florida specimen was 90,000 years. The haplotype tree indicated a divergence between the southwest Florida haplotype and its derived haplotypes 214,000 years ago (if valid, possibly in the Yarmouth interglacial), and a further divergence between a southeastern Florida haplotype and the remaining north and central Florida and South Carolina haplotype 85,000 years ago (the Wisconsin glaciation).

For the 101 b.p. fragment, the ME and UPGMA trees shown, respectively, in Appendices 24 and 25, common ancestry occurred some 2.662 million years ago. An undifferentiated grouping of 18 specimens from Florida, South Carolina, Georgia and Alabama shared a common ancestor 0.020 mya. A clade representing a specimen from central Louisiana and a pair from central Georgia and northern Florida diverged 1.064 million years ago. The latter two specimens shared a common ancestor very recently (0 mya). In turn, *M.fulvius* and

M.corallinus shared a common ancestor 4.712 mya. According to both ME and UPGMA estimates, shown in Appendix 26, the basal divergence of haplotypes

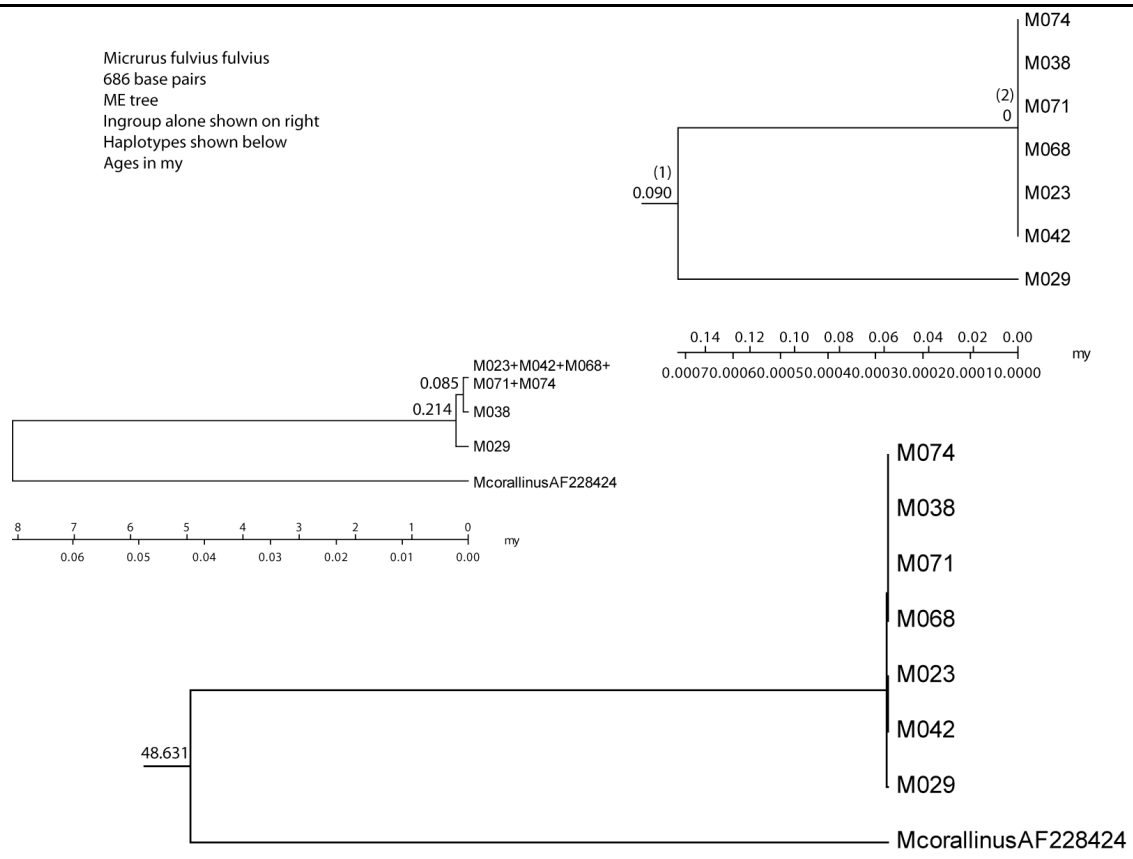


Figure 3-12. Minimum Evolution trees showing estimated TMRCA at nodes for a 686 base pair ND4 segment for *Micrurus fulvius*. The bottom tree shows individual specimens with outgroup, top right shows the ingroup with nodes indicated in Table 3-5 shown in parentheses and left depicts the haplotype tree.

occurred some 2.942 million years ago, which exceeds the earliest known fossils of *Micrurus* found in Florida by more than a million years (Holman, 2000). The resulting two branches consisted of one clade containing a central Louisiana haplotype – based on what is ostensibly a separate subspecies -- and a Georgia-Florida haplotype, while the other clade contained a widespread Atlantic and Gulf coastal plain haplotype together with the southwestern Florida haplotype. The former pair of haplotypes diverged some 1.570 mya. According to this data, the

widespread haplotype diverged from the southwestern Florida haplotype some 303,000 years ago (compare this with 90,000 years for the 686 base pair fragment). By comparison, the earliest fossils of *Micrurus* in the United States are from middle Miocene Nebraska (~15 mya) (Holman, 2000). The earliest unambiguous *M. fulvius* fossils are from middle to late Pleistocene Texas and late Pleistocene Florida (~0.012 mya). Fossils of more ambiguous identity (only *M. fulvius* is currently found in Florida) were found in early Pleistocene (1.8 mya) deposits in Florida.

III.D. Discussion

1. Higher Level Phylogenies

As with other recent work on the *L. triangulum group,* this analysis also indicated that it is paraphyletic (Fetzner, Jr., 2000; Harper, 2006; Burbrink, 2008). For example, a clade with 74% bootstrap support contains *L. getula* as outgroup to a 100% supported clade consisting of *L. t. gentilis* and *L. alterna*. All of these studies point to the need for a thorough assessment of the genus *Lampropeltis*, making use of a variety of molecular and morphological characters. Application of PAA to assess the subspecies (Williams, 1988) for possible species status prior to conducting phylogenetic analysis, would strengthen the latter by providing demonstrably non-reticulating terminal taxa for the analysis (Nixon and Davis, 1992).

There has been considerable debate over the origin of the New World Micrurine elapids (Roze, 1996), with several authors espousing a North American origin via the Siberian land bridge (Roze, 1996), and others favoring a South American, Gondwanan origin (Savage, 1982; Holman, 2000). Castoe, et al.'s (2007) phylogenies with the western U.S. and Mexican *Micruruoides* basal to the remaining Micruruines, would appear to support a North American origin for the group. The gene tree analyzed in this study suggests a southern derivation for *Micrurus* (although not necessarily for all Micruruines, as it does not include the sister genera, *Micruroides* and *Leptomicrurus)*. *Micrurus fulvius* here appears as the most derived member of the group, with the widespread South American *L. corallinus* as its outgroup. The remaining species considered in this analysis range widely through South America and up through Central America.

2. Ingroup Phylogenetics and Biogeography

When the first complete *Lampropeltis triangulum elapsoides* sequences were assembled, consisting of 12 specimens from northern and central Florida and Aiken, South Carolina, it appeared that there might be diagnostically distinct northern and southern terminal taxa. There were three nucleotide sites, at positions 169, 409 and 433, that fit the criteria for PAA and set the group of specimens from Aiken, South Carolina, apart from the Florida specimens. However, as further specimens were added, the three diagnostic alleles were found to occur in specimens from other geographic regions. Unfortunately, the

fragmentary and limited nature of the available sequences prevented a search for additional diagnostic characters and suites of characters. Thus, the available data falsified a phylogenetic and, so, a vicariant biogeographic hypothesis for an ingroup comprised of terminal taxa within the nominal *L. t. elapsoides*.

In the case of *Micrurus fulvius fulvius*, there were never sufficient samples to adequately test a phylogenetic hypothesis. An initial sample consisting of 7 specimens from Florida was homogeneous, except for a single specimen from southwestern Florida that might have been a candidate for PAA had further samples been available. The “neotropical” region in the southern extreme of Florida has been posited as a distinct floral and faunal zone (Neill, 1957). Additional sampling appeared to falsify a phylogenetic hypothesis for distinct populations of *M. f. fulvius*, although sample size and sequence length were extremely limited. Particularly in the case of this snake, further sampling from the southern extreme of Florida is needed to assess the relationship between an alleged south Florida subspecies *M. f. barbouri* and *M. f. fulvius*. In addition, although it was not within the scope of this study, PAA might be fruitfully applied to revise the relationship between the 5 recognized subspecies of *M. fulvius* occurring across its distribution from Florida down through central Mexico.

The falsification of phylogenetic hypotheses for these two groups of sympatric model and mimic organisms also implies the falsification of a co-evolutionary

hypothesis, *sensu* Page (1994) or Brooks and McLennan (1991). It would seem that the taxonomic and geographic scope of this study were too narrow. The proposed methodology might be fruitfully applied to clarifying the relationship between *L.t. elapsoides* and some of its con- sub-specific neighbors, a relationship which bears some resemblance to the “ring” process of incipient speciation observed in several taxa. Hindsight proposes a protocol starting with assessment of *Micrurus fulvius* subspecies and their sympatric *L. triangulum* subspecies as putative terminal taxa.

3. Phylogeography and Evolutionary Demographics

3.a. *Lampropeltis triangulum elapsoides*

With respect to *L. t. elapsoides*, the most complete data set, composed of the “merged” taxa, shows a large proportion of haplotypes, but little, if any, geographic structure. Two twigs in a vast polytomy may reveal derived haplotypes along the Gulf coastal plain and haplotypes to the north, including the Aiken group, but the latter haplotypes also grouped specimens from Louisiana, elsewhere in South Carolina, North Carolina, Florida and Georgia. However, all of the additional sequences in this group were fragments, no more than 250 base pairs in length, so although the parsimonious solution is to accept their homology with the Aiken group, it is a conservative estimate, in that any nucleotide differences in the missing sequences would differentiate the specimens from the Aiken group. While several haplotypes grouped two or more specimens from the

same vicariant region, these were all unresolved with respect to each other. There were also a large number of uninformative, singleton “haplotypes” within this polytomy.

Turning to more complete sequences sets with fewer specimens the story is somewhat different. Some geographic structure is evident for haplotypes generated for the 638 base pair and 595 base pair sequences, which include most of the ND4 gene, but are limited to haplotypes from three Florida regions and one South Carolina region. These collectively show a pattern of haplotype differentiation from the northern Florida highlands to the central Florida Atlantic lowlands, and possibly a northern derivation from Florida haplotypes. This pattern is evident in all three approaches to evolutionary reconstruction employed. In addition, further differentiation is observed with respect to the Aiken, South Carolina, haplotype in both Minimum Evolution and UPGMA trees. It may be noted that the Aiken haplotype can either be basal to all haplotypes, basal to a pair of Florida haplotypes or derived from Florida haplotypes. Parsimony analysis reduces this to a basal polytomy with a resolved Florida derivative.

Among the 244 base pair haplotypes, there is no further resolution: the northern Mississippi specimen is basal to the remaining specimens and an Aiken plus Mississippi plus Georgia haplotype is derived from the main clade.

Finally, there were significant Fst values, using both 595 b.p. and 244 b.p. fragments, indicating geographic structure at the population genetic level. When the Fst values for the larger fragment are teased apart by pairwise relationships or by framing populations by hypothesized vicariant zone, the significant differentiation seems to involve the Aiken specimens, northern Florida highland specimens and eastern Florida coastal lowland specimens, and possibly the Appalachicola region of the panhandle (with only 1 sample present). For the smaller fragment, we can add significant differences between the Aiken group and several other regions.

The generally low nucleotide diversity and lack of resolution in full gene and haplotype trees, resulting in a "shallow" phylogeny and lack of geographic structure, would seem to indicate that *L. t. elapsoides* constitutes a single population. At the same time, the relatively large number of private alleles and high Fst values would seem to rule out high rates of gene flow. Nm values seem to show a low migration rate. Given the low vagility[1] of these snakes, the known geographic barriers (not to mention modern, human-created barriers) and the geological history of the area, it seems unlikely that the observed genetic uniformity and lack of resolution are due to high rates of gene flow.

[1] Fontanella (2008) noted that the ring-necked snake, which is similar in size and habits to *L. t. elapsoides,* has "limited dispersal abilities," and a home range limited to 70 meters in diameter.

Another plausible explanation consistent with these results and the geological history of the area, might be a relatively recent range expansion from Pleistocene refugia in Florida and possibly elsewhere along the southern Atlantic and Gulf coastal plain. This is consistent with the relatively large number of haplotypes with little sub-structuring and the low nucleotide diversity. It is also consistent with the geographic structuring seen in Florida, but not elsewhere. Fontanella, et al. (2008) found a similar situation with respect to regional lineages of the widespread ring-necked snake (*Diadophis punctatus*). Walker, et al. (1998), found a similar "shallow phylogeny" for the widespread snapping turtle (*Chelydra serpentina*), and suggested that a historical population bottleneck followed by a recent range expansion would explain their data. As noted above, during the Wisconsin glaciation *L. t. elapsoides'* favored ecological and climatic regime was reduced to the Florida Peninsula the (Schmidtling, 2007; Jackson, et al., 2000). Following the retreat of this glacier, the current southern pine forest spread throughout the southeast, and with it, possibly, *L. t. elapsoides*. The timing for this scenario falls within the broad bounds of the estimated expansion time for specimens (460,000-14,000 years (down to 6,000 years for the 2%/My rate estimate) for the 595 bp fragment and 39,000-2,000 for the 244 b.p. fragment). Possibly on the negative side of the ledger, Tajima's D and Fu's Fs values are low and fail to support a scenario of recent population expansion. Further, divergence times for *L. t. elapsoides* as a whole, and subgroups therein, predate the Wisconsin glaciation. The average divergence time for the entire *L. t.*

elapsoides population, determined with the 638 b.p. and 595 b.p. haplotypes, is about 700,000 years (with the 2%/My rate estimate, this comes down to 300,000 years). Although these divergence times were based on a limited set of sequences from a restricted part of their distribution, the greater amount of nucleotide diversity present in a wider selection of samples would seem to push the coalescence dates even further back.

It would seem that haplotype differentiation took place prior to the indicated range expansion, possibly facilitated by the previous four waves of glaciation and interglacial periods. Differentiation among Florida haplotypes might have occurred during interglacial inundations of the peninsula or during arid glacial epochs, may have become fragmented in mesic environments. Divergence time is too uncertain to provide a more precise scenario. Following the retreat of the Laurentide sheet, then, these haplotypes expanded outward to their current range, where the process of lineage sorting is underway. The placement of the Aiken group is problematic in determining the location of refugia. If it is derived from Florida haplotypes, then this hypothesis is consistent with a Florida refugium. It may have originated in Florida, then spread north. If it is basal, it may indicate the presence, at some point during the post-coalescent period, of an additional refugium on the Atlantic coastal plain. Similarly, the basal placement of some haplotypes on the “merged” tree might indicate Gulf coast refugia for populations of *L. t. elapsoides*.

Some of the more enigmatic aspects of *L.t. elapsoides'* phylogeography might provide clues to its evolutionary history and origin. For example:

1) The observation (Williams, 1978) observation that *L. t. elapsoides* hybridizes with *L. t. triangulum* in areas of sympatry on the Atlantic coastal plain, but not in areas of sympatry in the central Piedmont and that in those areas of the Piedmont where the two subspecies coexist, *L. t. elapsoides* has actually moved out from its traditional southern pine woods habitat into hardwood forest, inhabited by *L. t. triangulum*. Is this a case of incipient speciation of *L.t. elapsoides*? Does this imply recently renewed contact in the west, but long-term contact in the east, perhaps in Pleistocene refugia? Long-term separation and divergence might have permitted the evolution of reproductive isolation mechanisms, while continued contact might have permitted continued gene flow. Or vice versa? Could the west be an area of long-term contact and isolation via competitive exclusion, sort of an incipient sympatric speciation? Meanwhile, could the east be the arena for secondary contact? Again, Population Aggregation Analysis and a resolved phylogeny based on total evidence for the *triangulum* group could help clarify this issue.

2) The anomalous situation of *L. t. elapsoides* specimen Mf 231 from Humphreys County, northwestern Mississippi, a 474 base pair fragment of ND4, which

consistently groups with *L. t. triangulum*. Currently, *L.t. elapsoides* is sympatric with *L.t. sypsila,* not *L.t. triangulum,* in that area. Is this evidence of gene flow? A relic of past contact or even a shared ancestor? Or sampling error?

It should be noted that hybridization even between distinct species within the Lampropeltinini is not unknown.

3.b. *Micrurus fulvius fulvius*

Micrurus provided even poorer quality DNA and fewer samples than *Lampropeltis*, further reducing the scope and reliability of analyses. The low sample size, missing data and fragmentary sequences give a very limited scope to phylogenetic analysis and render population genetics almost impossible, since both are affected by missing data and the latter, in particular, is affected by low numbers of samples – in terms of both taxa and sequences. Moreover, with coarse-grained sampling, the ability to marshal data to hypothesize biologically meaningful populations is absent. Nevertheless, with the available data, such as it is, we might sketch out the bare outlines of an approach.

For *M. fulvius*, the fullest sequence set (consisting of 686 b.p. and 7 specimens) indicated possible well-supported (86%) basal resolution in southern Florida with a lone specimen from Collier County, Florida (Mf29), as basal to a polytomy from

Florida. The fullest set of specimens (with 101 base pairs and 21 specimens) produced a polytomy with an outgroup, a single specimen from central Louisiana (Mf3 -- according to the literature, *M. f. tenere*) with a pair from central Georgia (Mf133) and northern Florida (Mf132). The latter pair resolved as sister to the Louisiana specimen, without significant bootstrap support. In neither the "merged," full sequence set, nor the 101 b.p. sequences, did the southern Florida specimen resolve apart from the remaining Florida specimens, although it did so in the 101 b.p. haplotype tree. While AMOVA values are not significant, structure was indicated east and west of the Apalachicola River. This should not be taken as solid evidence of a phylogeographic break, however, as only one specimen was sampled west of the Apalachicola River.

Low nucleotide diversity and widespread haplotypes would seem to indicate another panmictic population. The sparse available phylogenetic and haplotype data suggests that there may be some differentiation of Florida populations within the broader ecological and geographic zones in Florida, but assessment will require further sampling. However, in the fullest sequence set and the haplotype network distilled from the fullest set of secimens, Mf29 emerges repeatedly as sister to most of the Florida specimens. A population of *M. f. fulvius* found at the southernmost end of the Florida peninsula has been thought to represent a distinct subspecies (*M. f. barbouri*) or even species. All of the remaining Florida samples grouped as a single haplotype, which, together with the specimen Mf29,

diverged from the Louisiana and a Georgia-northern highland Florida pair, which may point to an east-west phylogeographic split.

Average population expansion times for *M. fulvius* ranged from 79,000 to 396,000 years, while the mean divergence estimate was 90,000 years for the 686 b.p. fragment (214,000 years for haplotype coalescence), while the estimate for the 101 b.p. fragment was about 2.662 million years. The latter also indicated that the Louisiana, Georgia and Florida specimens diverged 1.064 million years ago, and the Florida specimens diverged 20,000 years ago. In the absence of other evidence, these dates place the most recent common ancestor anywhere from middle Pleistocene to the late Miocene.

While the low nucleotide diversity, lack of phylogenetic resolution and widespread haplotypes may indicate that *M. f. fulvius* constitutes a single population, the lack of data makes it harder to make this case. The opposite is true, as well: even though Fst values were high, they were not significant, primarily due to low sample size. Meanwhile the population expansion time estimate may be real or artifactual, but if real, it doesn't square with a post-Pleistocene expansion explanation of the observed genetic pattern. The evidence -- scanty as it is -- of east-west structuring also runs counter to a panmictic or recently expanded population. The observed pattern appears to have developed by no later than the

mid Pleistocene, and possibly as far back as the late Miocene (the 101 b.p. haplotypes indicate the basal divergence from 1.4 to 5.35 mya).

One may speculate on the role of glacial advance-retreat and subsequent modification in the Mississippi and Tombigbee-Mobile River systems and climatic variables in the initial differentiation of eastern and western haplotypes. This scenario assumes a western common ancestry for *M. f. fulvius.* Later glacial advance may have facilitated the repeated vicariant divergence of eastern from western haplotypes. A western origin squares with the location of the earliest *Micrurus* fossils and the current range of *Micrurus fulvius*. An alternative scenario depends on the hypothesis that neotropical forms arrived in the southern Florida peninsula during periods of low sea level, when either a land bridge or a series of islands connected Florida to South America. That scenario would mesh with the most parsimonious optimization of ancestral states in the phylogenetic tree, a Florida ancestral state for *M. f. fulvius*, with subsequent migration and vicariant divergence toward the west., and with a close relationship between *M. fulvius* and *M. corallinus*.

The differentiation within Florida haplotypes, particularly the basal divergence between a southwestern haplotype and the remaining Florida haplotypes, falls roughly within the time-frame and spatial coordinates for an initial separation resulting from the submergence of parts of the Florida peninsula, and the

formation of one or more large islands, such as intermittently occurred during the early Pliocene period up through the Pleistocene, from 5 million years ago (Fontanella, 2008). Although *M. f. fulvius* currently seems to occupy a broader array of habitats than *L. t. elapsoides*, subsequent differentiation may have resulted from the separation of mesic pockets within the more arid periods of glacial advance, as well as embayments or isolation due to further sea-level fluctuations.

IV. Color pattern morphometrics, co-adaptation and mimicry

IV.A.Introduction

Aposematic coral snakes (*Micrurus fulvius*) and scarlet king snakes (*Lampropeltis triangulum elapsoides*) in the southeastern United States have long been held to exemplify Batesian mimicry, in which a harmless mimic possesses a color pattern closely similar to that of a noxious model in order to deceive and deter predators (Wallace, 1871; Greene and McDiarmid, 2005). Frequently, these – aposematic -- color patterns have evolved to stand out and alert predators to the noxious characteristics of the model organism. This phenomenon has been shown to occur in a wide variety of organisms, principally among invertebrates, but also among fish, amphibians, birds, reptiles and mammals (Pough, 1988). Moreover, this defensive mimicry is expressed in perhaps as many sensory modalities as there are senses. Pough (1988) indicates that due to our own sensory predilections, humans might not even recognize these cases of mimicry, or the scope of mimetism among organisms demonstrating visual mimicry. This might explain, in part, some of what constitutes apparently "imperfect" mimicry. In addition, various organisms with toxic or noxious defenses may also evolve convergent aposematic color patterns, which enable them to spread the learning process among predators, and thus enhance the impact of the warning colors. Various organisms have also evolved mimetic characteristics that enable them to deceive prey, rather than predators. These include predatory or ectoparasitic blennies that mimic cleaner wrasses

(Cote, 2003), which enables them to approach prey, or various avian brood parasites, whose eggs resemble those of their hosts (Pough, 1988).

The evolution of aposematic coloration, itself, poses a dilemma for biologists, because the first organisms of their population to possess outstanding coloration would be expected to face strong selective pressure and have a poor chance of survival and reproduction. Several authors (Mappes, et al., 2005; Marples, et al, 2005; Sherrat, 2002) have hypothesized that predators' avoidance of novel food items ("neophobia") and dietary conservatism may give incipient aposematic organisms a chance to reach a critical population size where learning and reinforcement can kick in. Another paradox arises in the case of extremely noxious or toxic organisms, whose bite, sting or ingestion is lethal to predators, and thus forestalls the learning apparently necessary for predator recognition of aposematic coloration. Various solutions to this problem were proposed, including limitations in venom delivery by smaller coral snakes, a range of reactions to venom by predators, the dissuasive effects of rear-fanged Muellerian mimics of coral snakes, social or empathic learning by predators, and innate avoidance of coral snake patterns (Greene and McDiarmid, 1981; Pough, 1988; Greene and McDiarmid, 2005). The latter has since been confirmed in experimental studies with naïve and wild predators and painted and clay coral snake models (Smith, 1975; Greene and McDiarmid, 1981; Pough, 1986; Brodie, 1993; Brodie and Janzen, 1995). In fact, Pough (1986) suggested that the severe

toxicity of venomous serpents is what selected for innate avoidance and is what sets mimicry complexes involving these models apart from other Batesian mimicry complexes.

Batesian mimicry poses a similar problem. A mimic evolving the aposematic coloration of a model must pass through an intermediate phase, which would render it vulnerable to predators. Authors have proposed similar solutions to this paradox, including predator aversion to unfamiliar and attraction to more familiar prey, pre-adaptation, peak shift in predatory discrimination, kin selection, genetic drift and the shifting balance concept may have favored the evolution of novel, mimetic patterns (Mallet and Joron, 1999).

The dynamic of model-mimic evolution has generated considerable controversy. Under the classical model of Batesian mimicry, there was a selective advantage for both models and Batesian mimics to converge on a particular color pattern (Joron and Mallet, 1998; Caley and Schluter, 2003). Variants would get picked off, giving rise to purifying selection. In addition, this is a frequency-dependent selection model, since mimics would have to be rare compared to models in order for predators to come to associate and continue to associate aposematic coloration with an unpleasant encounter (Joron and Mallet, 1998). However, the very success of mimics posed a threat to the models. The more predators encountered and successfully attacked mimics, the greater would be the

selective pressure against models with the same color pattern. This would give rise to an evolutionary chase or "arms race" between models and mimics (Dawkins and Krebs, 1979). The degree to which models would be adversely affected by mimics was related to the relative frequency of models and mimics in the population (Joron and Mallet, 1998).

Dawkins and Krebs (1979) drew on earlier suggestions and codified the concept of the co-evolutionary “arms race” between or among species, by which adaptation in one calls forth counter-adaptation in predator, prey or competitor. One conclusion they drew was that the side in this “arms race” that faces the strongest selection pressure will predominate in that race. However, even earlier, population geneticist R.A. Fisher had applied this concept to Batesian mimicry complexes, noting that while close resemblance to the aposematic model favored the mimic, it harmed the model by leaving open the possibility that the model would be mistaken for a mimic by a predator. In turn, this would create a selective pressure favoring models that were distinct from their mimics. An evolutionary chase would ensue (Gavrilets and Hastings, 1998).

Researchers have since debated the applicability of this model to mimicry systems. Joron and Mallet (1998) considered the co-evolutionary arms race to be an unlikely explanation for model-mimic evolutionary dynamics, first, due to the intense purifying, frequency-dependent selection on the model, which would tend

to eliminate deviants from the favored color pattern. Second, they pointed to the normally greater abundance of models than mimics, which implied that the selective pressure from mimics would be less than the pressure from other models. This latter assumption is questionable in many cases (Greene and McDiarmid, 2005) and may well be patchy in most cases (Mappes, et al, 2005). Ceccarelli and Crozier (2006) conducted a study of the evolutionary dynamics of the Batesian mimicry complex consisting of ants and their salticid spider mimics and found that the phylogenetic patterns that emerged were characteristic of adaptive radiation by the mimic, rather than model-mimic co-speciation.

Alternately, very much in line with Dawkins and Krebs (1979), some researchers have assayed population genetic models that indicate that a cyclic co-evolutionary chase may ensue if the interaction between model and mimic species is stronger than intraspecific interactions (Gavrilets, 1997; Gavrilets and Hastings, 1998; Kopp and Gavrilets, 2006; Norrstrom, et al, 2006). There is a dearth of evidence for the “arms race” in the realm of Batesian mimicry, as noted by Gavrilets and Hastings (1998). Joron and Mallet (1998) cite one case where it was applied as a putative explanation for polymorphism in the butterfly *Danaus chrysippus*. On the other hand, the concept has been applied fruitfully to analyze host-parasite co-evolution (Brooks and McLennan, 1991; Page, 1994; Hafner and Page, 1995).

It would appear that such a co-evolutionary chase might also be a reasonable explanation of the close similarity of the Micrurine elapids and their mimics, even down to regional polymorphisms, behavioral traits and ontogenetic changes in color pattern. For example, in southern Costa Rica, the normally banded juvenile *Micrurus alleni* becomes jet black as it matures. The sympatric *L. triangulum* undergoes the same change (Greene and McDiarmid, 2005). Greene and McDiarmid (2005) cite the example of a Costa Rican population of *M. mipartitus* that possesses polymorphic red or white bands. Leenders, et al (1996) attributed this polymorphism to an attempted evolutionary escape from Batesian mimics. Greene and McDiarmid (2005) note that a putative mimic of this snake from 20 km away shows the same polymorphism.

Based on the large body of work utilizing a phylogenetic approach to analyze host-parasite co-evolution, the present study initially sought to evaluate an arms race hypothesis of model-mimic co-evolution between *L. triangulum* and *M. fulvius.* Co-evolutionary theory (Brooks and McLennan, 1991; Ceccarelli and Crozier, 2006) predicted that such a process would involve phylogenetic congruence between co-speciating taxa and co-adaptation in color patterns among sympatric models and mimics. Although the full hypothesis couldn't be tested on the two species chosen, as terminal taxa could not be defined within each group, the prediction of co-adaptation – that is, a pattern of mimics tracking models in sympatric geographic populations – was tested as a first hypothesis.

In the southeastern U.S., coral snakes and scarlet king snakes are not identical. The band colors themselves differ in order, giving rise to the popular sayings that distinguish them, “red touches yellow, you’re a dead fellow, red touches black, you’re okay, Jack.” This difference in arrangement may reflect ontogenetic differences in pattern formation between the two species. Observations on specimens in this study seem to indicate that in *M. fulvius* band formation departs from coalescence of black stippling, while in *L. triangulum*, spreading patches of black appear to produce new bands. Thus, genetic architecture may impose limitations on similarity between the two species (Meyer, 2006).

However, within the mimetic semblance itself, there are varying degrees of perfection and differing amounts of variation in mimic color patterns across the distribution of the two species. This is a phenomenon common to Batesian mimicry systems in general and it appears to contradict the standard depiction of purifying selection of models and mimics (Sherrat, 2002). A considerable body of work has developed on the related issues of mimetic imperfection, mimetic variation and mimetic polymorphism. Within the limited geographic and taxonomic scale of the current study, the first two are issues of immediate interest.

Pough (1988) suggested that anomalous imperfect mimicry may, in part, be due to our sensory limitations. Organisms with different sensory modalities and parameters may find that mimics resemble models much more closely than we can perceive. Another explanation for mimetic imperfection may be the severe toxicity of some models, which would allow for more imperfect mimics to evolve under the "umbrella" of their protection, as is the case with the coral snakes and other putative venomous snake models (Pough, 1988; Edmunds, 2000; Caley and Schluter, 2003; Greene and McDiarmid, 2005). Edmunds (2000) summarized several possible additional reasons for this phenomenon and noted the interplay between mimetic perfection and relative abundance of models and mimics. Beyond the explanations noted above, these included the effect of movement on color pattern, the transitional effect of progress toward greater (or less) mimetic perfection, selection for predator "confusion" just long enough for mimics to escape, and mimetic "generalization" allowing mimics to combine patterns of several models over a broad geographic expanse.

In recent years, several authors have attempted to address these issues, using Game Theory and other mathematical modeling approaches embodying various assumptions to generate testable hypotheses, as well as organismal studies in the field or laboratory. Much of this literature draws on the robust work on both Batesian and Muellerian mimicry done in entomology, particularly among hoverflies (wasp and bee mimics) and butterflies (mimicking other butterflies).

Edmunds (2000) generated a model based on multiple models and mimics living in non-overlapping patches at somewhat arbitrary densities to examine his hypothesis that mimetic generalization across multiple models could give rise to common and widespread imperfect mimics. For purposes of this study, this approach may not be appropriate because it seemed to ignore the differing bases for predator aversion to noxious models. Innate aversion develops on a longer time-scale than learned aversion. In addition, this mimicry complex includes only one model, although Edmunds' approach may be appropriate on a larger geographic and taxonomic scale.

Sherrat (2002) used a signal-detection model to examine the evolution of mimetic imperfection. He used his approach to test Edmunds' hypotheses and to explore other possible explanations. He hoped to delineate what population densities would generate imperfect mimicry, what proportions of models and mimics would exist at equilibrium, and (in light of Edmunds' model) what degree of similarity could be expected of a generalist mimic of models differing in number and noxiousness. His relevant conclusion was that imperfect mimicry could be expected to evolve in single-model systems, due to a nonlinear relationship between predator attack rates and model-mimic similarity. Mutations conferring improved similarity will be favored up to a range of values. When the mimic reaches a "valley" where further improvement is selectively neutral, other

selective factors may assert themselves, such as environmental or reproductive requirements. Sherratt found that the proportion of mimics to models that could be supported before the mimetic system breaks down is directly proportional to the cost of attacking a model/benefit of attacking a mimic. His model predicted that the higher the mimic-to-model ratio, the more similar the mimic must be to the model to gain protection, and the greater the cost of attacking a model, the less perfect the mimicry need be. This model may be applicable, although it, too, seems to abstract from the specific forms of predator psychology and sensory modes. In this case, the cost would seem to be so high and the predator attack rate so low, that, all else being equal, the model would seem to predict an extremely broad phenotypic valley for mimics of *M. fulvius.* This may be the case for *L. triangulum*, and even more so for other sympatric banded snakes, such as *Cemophora coccinea* that may derive a measure of protection from their resemblance to *Micrurus.*

Holloway, et al. (2002) used an experimental approach to study the relationship between mimetic imperfection and variation in hoverflies and the social wasps they mimicked. Three hypotheses were tested. First, that mimics were imperfect because they were in the process of evolving toward perfection. In that case, they predicted, directional selection would tend to progressively reduce variation. Second, that imperfect mimics had evolved as closely as they could, due to biological constraints. This would lead, via normalizing selection, to across-the-

board low levels of variation. Third, more abundant mimics (possibly due to enhanced food supply) would show higher levels of variation and relaxed selection as mimicry broke down. Fourth, the high toxicity of the social wasps would lead to predator aversion toward any mimics that resembled the models, as well as the models (or Muellerian mimics) themselves. This would lead to a relaxed normalizing selection on color pattern (as contrasted with classical aposematism and Batesian mimicry) and the highest variation among closest mimics and decreasing variation among more imperfect mimics, which had either evolved as far as possible (constraints) or were in the process of evolving. Holloway notes that the latter hypothesis was supported by the data, although not at significant levels.

Holen and Johnstone (2004) used evolutionary game theory and signal detection theory to predict the evolutionary outcomes in aggressive and Batesian mimicry systems, when improved mimicry entails fitness costs to the mimics. The model is based on two important diagnostic parameters: mimetic load and incentive for deception. The former represents a selection pressure of mimics on predators. It varies with the predator's encounter rate with mimics, and so the relative abundance of mimics with respect to models, and the benefit to be derived from these encounters (as compared with the cost of attacking defended models). It varies inversely with the noxiousness of the model. The incentive for deception is the extent to which the fitness of mimics depends on their ability to fool predators.

It varies with the rate at which mimics encounter predators and the ease of capture of mimics. Holen and Johnstone's model predicts inaccurate Batesian mimicry at low mimetic loads (as would be the case for highly venomous coral snakes and secretive milk snakes). As mimetic load increases mimetic accuracy increases, as predators become more discrminating. Mimetic perfection is constrained by fitness costs. At equilibrium, the perfection of mimicry will increase if the incentive for deception increases. At high mimetic loads (for example, high abundance of mimics with respect to models or less toxic models), a low incentive for deception (the mimics are hard to capture or are secretive) should produce inaccurate mimics. At high or very high mimetic loads, a high incentive for deception might produce mimetic dimorphism or inaccuracy, respectively. The authors discuss additional outcomes, including those resulting from enhanced predator discrimination and conspicuous aposematic coloration. They summarize their results as follows:

1) Batesian mimics that are very common and/or copy very weakly defended models should evolve inaccurate mimicry or mimetic polymorphism, and 2) Batesian mimics with high mimetic loads high incentives for deception should evolve mimetic polymorphism. Although some of the predictions seem relevant to this study, it would be difficult to apply this model in this study without knowing the encounter rate between *L. triangulum* and predators and without quantitative measures of the terms contained in the two concepts of incentive for deception and mimetic load for this species. As a basis for their models, the authors

assume that color patterns evolve on an evolutionary timescale, while predators' response evolves on a behavioral timescale. Therefore, the model may not be applicable where predators develop an innate response to aposematic coloration (Holen, 2008).

The most relevant research to date on the evolutionary dynamics of *M. fulvius* and *L. triangulum* for the present study has come out of David Pfennig's laboratory (Pfennig, et al, 2001; Harper and Pfennig, 2007; Pfennig, et al, 2007). Harper and Pfennig (2007) sought to investigate the effects of frequency-dependent selection on mimics. They predicted that where models are rare relative to mimics, the latter should be under strong selection to conform to the former's aposematic coloration. Thus, departure from a mean color pattern and excess variation would be selected against where models were scarce. Since *M. fulvius* has a more restricted range than *L. triangulum,* Harper and Pfennig hypothesized that this might occur on the margins of *M. fulvius*' distribution, where the ratio of models to mimics would be low ('edge sympatry'). On the other hand, they predicted that where models are relatively abundant, mimics were under relaxed selection and might deviate more from the aposematic pattern, particularly as other selection costs and benefits assert themselves. Thus, in areas central to the sympatric co-distribution of the species ('deep sympatry'), increased variation of mimics from models and mean differences might be expected. On the face of it, this appears to be a reasonable approach, more so if

one takes into account the presence of an additional “poor” mimic, *Cemophora coccinea,* in “deep sympatry” with the models. Nevertheless, Harper and Pfennig’s study has several conceptual and methodological problems. As noted for previous studies, it doesn’t account for the differing timescales for innate and learned aversion and how this might effect selection on color patterns over a relatively small geographic range. Second, the generalization that margins equal low model/mimic and deep equals high model/mimic might not be valid, given the patchiness of distribution of most organisms and the patchiness that may be involved in origin and maintenance of aposematism and mimicry (Mappes, et al, 2005). Moreover, the authors’ sampling was restricted to a few counties in southern North Carolina and a few counties in northern Florida, which may not adequately sample model to mimic ratios among populations. Nevertheless, their proposal was reasonable and concrete. Given available data in the present study, it provided the second hypothesis tested in this study.

Sherrat (2002) and Holen and Johnstone (2004) raised the possibility that within a generally mimetic arrangement, other selective forces may assert themselves. One such possibility is the ectotherm’s need for thermoregulation. Various authors have discussed thermoregulation as a possible selective force favoring dark or light coloration, and have sought correlations between thermal regime and needs, latitude and dermal melanin. For example, Tanaka (2006) examined thermoregulation in striped versus melanistic morphs of the rat snake *Elaphe*

quadrivirgata. He could not find any appreciable thermoregulatory advantage accruing to the melanistic individuals, as predicted, but he concluded that melanistic snakes have potential thermal advantages.

Williams (2007) conducted a wide-ranging study of color pattern in bumblebees and found a counterintuitive inverse relationship between melanization and latitude, at least between lower and middle latitudes, in which the darkest bees were found in the lowest latitudes. He concluded that there was potentially an unspecified thermoregulatory fitness component to these bees' color patterns, together with aposematism and crypticism.

Clusella Trullas, et al. (2007) conducted a thorough analysis of evidence for and against thermoregulation in ectotherms. She noted the complexity of trying to draw a linear correlation between melanin content and thermoregulation and oversimplistic selection schemes favoring melanin content. She then analysed the underlying assumptions behind these claims. Clusella Trullas concluded that: 1) there is evidence that melanistic diurnal species generally inhabit cooler regions than lighter organisms, 2) there is evidence, particularly among insects, for fitness advantages for melanistic individuals in cooler climates, 3) there is a lack of evidence to make the case for a melanin-body-size trade-off, and 4) there is some evidence for coadaptation between color pattern, physiology and behavior. One point she makes, with reference to the melanin-body-size issue,

might have relevance to Williams (2007) observation and to the apparent inverse correlation in amounts of black in *M. fulvius* and the much smaller and more secretive *L. triangulum elapsoides*. That is, that smaller individuals show less "thermal intertia": melanistic individuals in smaller animals heat up more quickly, dissipate heat quickly via convection and make temperature adjustments with greater ease than lighter organisms. Thus a quick emergence in the early morning followed by hiding during the heat of the day might be sufficient for small, melanistic, desert or equatorial species.

Finally, Nussear, et al. (2000) examined the biophysical aspects of the relationship between color pattern and thermoregulation. They noted that reflection, absorption and emission determine the thermal budget of a body, and showed that melanin content and visible color do not correlate well with the actual thermal budget.

Given unexplained variation in the color patterns of *L. triangulum* and *M. fulvius* and the possibility that environmental factors may play a role in selection in these species if selection for aposematism is relaxed, the final hypothesis tested in this study was that color pattern is influenced by those forces, particularly by average ambient temperature. This would lead to the prediction that mean proportions of dark coloration should show a correlation with one or more environmental factors and should differ among ecological zones.

IV.B. Materials and Methods

Photographs of 137 specimens (73 *Lampropeltis* and 64 *Micrurus*) were analyzed using the program Image J ver. 1.36b (Rasband, 1997-2008). This program allows the user to measure the length or the area of an image, among many other features, using the program's tracing or area tools. Prior to analysis, images were calibrated by repeatedly (10x) tracing the same 1 cm length of the metric ruler in the photograph and calculating the average number of pixels in the path. This figure was then used to define the number of pixels/cm in the "Set Scale" feature of the program. This allowed me to calculate the approximate error of my measurements, at least linear measurements, although any error may have been compounded in area measurements (for photos taken with the Kodak camera in the field, x=0.999403751, s.d.= 0.007450123, n=11; for photos taken with the Microptics system, x=1.001428926, s.d.= 0.00639323, n=11). The exceptions were a small number of snakes in which a ruler was not included in the photograph. In those cases, there was no scalar dimension to the measurements. They were simply the number of pixels in the image.

Measurements consisted of the area of each band starting with the first red band behind the head in *L. triangulum* and the first yellow band behind the head in *M. fulvius* up to the last band before the vent in both species. An area measure was chosen over the simpler longitudinal measure (along the dorsum) due to the

contorted state of museum specimens. In many of these, the dorsal midline was not visible or indistinguishable in the photographs. In addition, since the width of each band varied around the circumference of the snake's body, the area was deemed a more reliable measure of the extent of each color. Each band was expressed as a proportion of the entire triad red-yellow-black-yellow in coral snakes and red-black-yellow-black in scarlet kings. The average proportion and its standard deviation were calculated for each color in in each triad in each snake. Since the data consisted of proportions, the data from the few photos lacking metric scales was simply the proportion of pixels to pixels, and was commensurate. Measurement data is provided in Appendix 3.

JMP ver. 4.0.2 (SAS Institute, 1989-2000) and StatView ver. 5.0 (SAS Institute, 1992-1998) statistical software packages were used to conduct all analyses. Color pattern data distribution (both untransformed and log transformed) was examined and descriptive statistics generated for each color pattern component for each of the two species using the distribution function of JMP. The Fit Y by X function in JMP was used to conduct a t-test for significant differences in each component of the color patterns for each species by sex. This was meant to be a rough indicator of a possible confounder, since I was unable to sex all specimens (*M. fulvius,* n=30; *L. t. elapsoides,* n=42). In JMP, the Multivariate function was used to calculate and show correlations between the colors for each species. Within the Distribution function, the Shapiro-Wilk Test was used to test goodness

of fit for normal distribution and the KSL test was used to test for goodness-of-fit for lognormal distributions.

The geographic subunits employed in this study were Oak Ridge Laboratories-designated Ecoregions (Olson, et al., 2003), EPA-designated Level IV Ecoregions (Omernik, 1986), vicariant zones and clusters based on geographic distance.

Vicariant zones were generated as a consensus of phylogeographic and biogeographic studies of a large number of different organisms in the southeastern United States. The geographic barriers were identified as the Savannah River drainage, Appalachicola River drainage, Appalachian-Blue Ridge Mountains and Piedmont, and Mobile River drainage, with the Mississippi River as a western boundary. Areas divided by these barriers were numerically coded.

In order to group specimens by geographic proximity, clusters were generated in JMP using the Clustering function under the hierarchical and standardized data settings. The Ward method was applied, which groups data points by minimizing variance between them, and is regarded as efficient and conservative (Ward, 1963; Morey, 1983). The grouping variables were latitude and longitude. These were separated by species and labeled by county or specimen number. The

number of clusters was set at 10 or 25. Based on these clusters, on vicariant zones or on ecoregions, JMP's Tables function was used to generate summary tables of color pattern statistics stratified by species and grouped by geographic subunit for subsequent analyses.

To test the first hypothesis, Analysis of Variance was performed with JMP using the Fit Y by X procedure for color pattern components by species for each geographic unit in order to test for significant differences among regions. In addition, ANOVA was employed in JMP in order to assess fits of color pattern to longitudes and latitudes for each species as a whole and by sex.

In StatView, the Correlations and Regressions functions were used to generate correlation matrices, perform correlation z tests and produce bivariate regression plots based on specimen summary data sheets grouped by geographic unit. As some of the color pattern components (black and red) did not follow a normal distribution, but did not show significant difference from a lognormal distribution, while other components (yellow and black+red) did not significantly deviate from a normal distribution, correlations were tested using both parametric and nonparametric methods. For the latter, the Spearman correlation was calculated using the non-parametric test function in StatView.

To test the second hypothesis, correlations were conducted with StatView as noted above, or with JMP, using the Multivariate function. The original complete specimen data (not summary data) was tested against latitude, longitude, annual number of growing days and annual rainfall. Coordinate data was obtained using Google Earth, based on locality data provided, where such data didn't include coordinates. County-level growing season and rainfall data was obtained from the Oak Ridge National Laboratory GEOECOLOGY Database, together with the previously noted Ecoregion data Oak Ridge National Laboratory (Olson, et al. 2003).

In order to test the third hypothesis, I employed the sampling methodology used by Pfennig, et al. (2007). I gathered specimen data from 16 institutions for a total of 571 specimens (including those I had sampled for tissues and morphometric data) of *M. fulvius* (338) and *L. triangulum elapsoides* (232). Pfennig, et al. (2007) used this data to calculate relative population frequencies for the models and mimics for several counties in North Carolina where the two co-exist sympatrically as an example of "edge sympatry," where the ratio of models to mimics is low, and several counties in the state of Florida as examples of "deep sympatry," where the ratio is high.

Given the problem of low sample sizes for specific localities, specimens were grouped in clusters, as explained previously. Rather than assume *a priori* that

reported edges of the more limited *Micrurus* distribution automatically represent areas of a low model to mimic ratio, I determined the ratio for the resulting clusters. The use of museum samples to estimate population parameters is problematic, since they do not necessarily represent a random sample of populations, but rather depend on specific events, such as real estate development or the presence of herpetologists. An example of this was provided by Roze (1996) for the genus *Micrurus*. The imposition of a bounty on *Micrurus* in Seminole County, Florida, in the 1930s, led to the slaughter of thousands of snakes, many of which ended up in museum collections. Nevertheless, in the absence of mark and recapture studies of these two species, there does not appear to be any other way to make such population estimates.

Unpaired t-tests for significant differences between the two species' mean color pattern values in each of the clusters were conducted with JMP. In addition, Levene's Test for equality of variance was also implemented in JMP to test the predicted variance differences between the two species in areas of high versus low model to mimic ratios.

Next, using the Regression and Correlation features in StatView, I carried out regression and correlation analyses of means, mean differences and standard deviations of summary color pattern components for each species against model to mimic ratio by cluster and EPA ecoregion. Untransformed and log-transformed

data was used, as noted above. In addition, when assessing correlations, I used both total data (grouped in clusters) and the summary data tables generated in JMP. The former was used to examine correlations between mean color values and population ratios, while the latter was used to examine correlations between cluster means, cluster standard deviations of the means, cluster mean differences between models and mimics and the difference between standard deviations of models and mimics by cluster.

IV.C. Results

Data for all specimens of the two taxa represented in this part of the study are presented in Appendix 3. For *L. t. elapsoides*, 72 specimens were included in this study of color pattern For *M. f. fulvius*, 66 specimens were included. Sex was determined for 21 female and 21 male *L. t. elapsoides*, and 13 female and 12 male *M. f. fulvius*. An additional 6 *L. t. elapsoides* and 1 *M. fulvius* were tentatively sexed, but identified as juveniles and excluded from those figures.

As the box plots shown in Figure 4-1 demonstrate, sexual dimorphism occurs at significant levels (p=0.05) for *L. t. elapsoides*, but not *M. fulvius*, and for both red and black, but not yellow, bands. For the scarlet kingsnakes, black represented 42.6% in females and 49.6% in males (t-value=-2.476, 40 d.f., P=0.0176), while red assumed 42.1% in females and 33.8% in males (t=2.472, 40 d.f., P=0.0178).

Thus, sex is a possible confounder in analyses involving black and red bands. No correction was made for sex in the ensuing analyses.

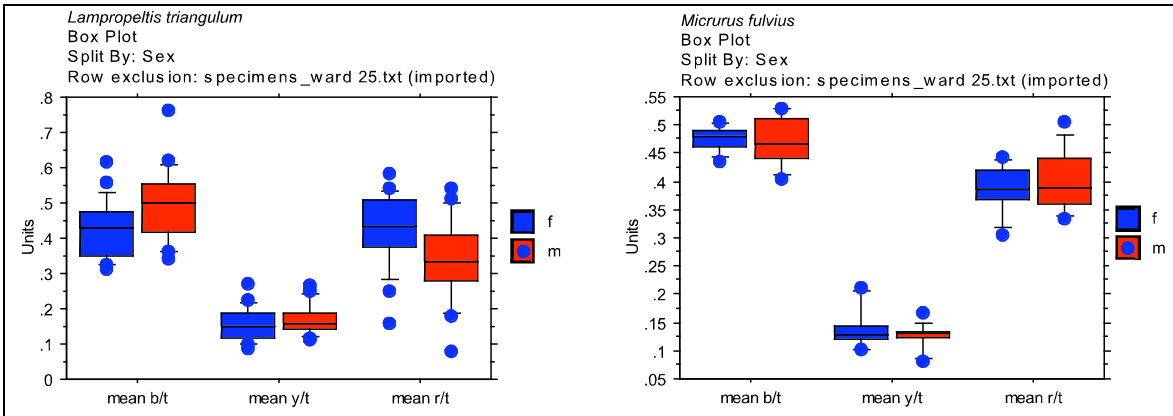


Figure 4-1. Sexual dimorphism is significant (p=0.018) in proportions of red and black coloring in *Lampropeltis triangulum elapsoides*, but not in *Micrurus fulvius.* In each pair of boxes, the female is on the left.

As percentages of each triad of colors, black represented 46.1% in all *L. t. elapsoides* and 48.4% in *M. fulvius*. Yellow represented 15.5% and 12.4%, respectively. And red was 38.6% and 39.3% of each triad for the two species.

It should be noted that there was a strong inverse correlation between proportions of red and black in both species (*L. triangulum*: r=-0.925, p<0.0010; *M. fulvius*: r=-0.831, p<0.001). However, the correlation between black and yellow was not significant in either snake, and that between red and yellow was lower, but significant in both species (*L. triangulum*: r=-0.471, p<0.0001; r=-0.342, p=0.0054). Thus, apparently as the color pattern develops, red and black bands develop at each other's expense, with yellow seeming to maintain a stable proportion (Figure 4-2).

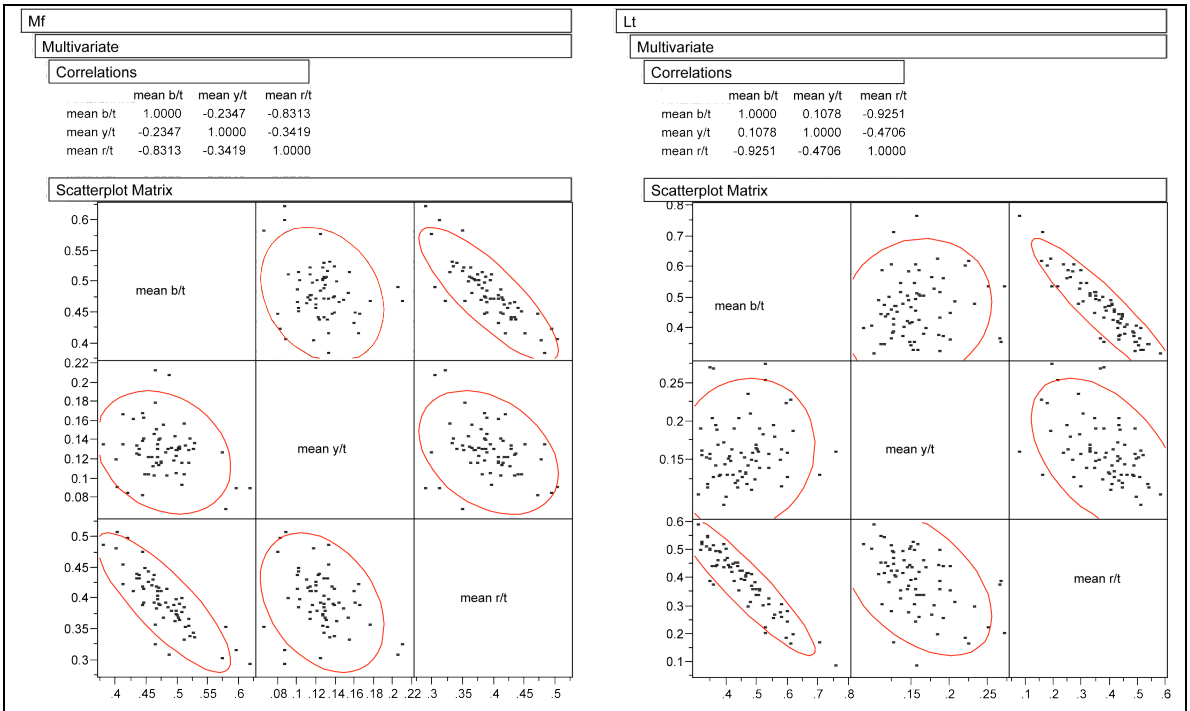


Figure 4-2. Proportions of red and black coloration are strongly and inversely correlated in *Micrurus fulvius* (left) and *Lampropeltis triangulum elapsoides* (right), as shown in the bottom left and top right squares in each chart. Red and yellow show less of a correlation, although still significant. Black and yellow do not show a significant correlation. The implication is that black and red are not independent of each other and together may constitute a unit.

Variation for all three colors, as expressed in standard deviations, was greater for *L. t. elapsoides* than *M. fulvius*, as may be seen in the box plots in Figure 4-3. Variation in the proportion of black was 0.090984 and 0.05057233, respectively. Yellow varied by 0.0320472 and 0.02365107, respectively. Red varied by 0.09186261 in *L. t.*, versus 0.04569683 in *M. f.* It should be noted that yellow varied least within each species.

Finally, for *L. triangulum* color pattern data, the Shapiro-Wilk test rejected (p=0.05) the null hypothesis of normal distribution for black (W=0.954345, p=0.0291) and yellow (W=0.916464, p<0.0001), while failing to reject normality

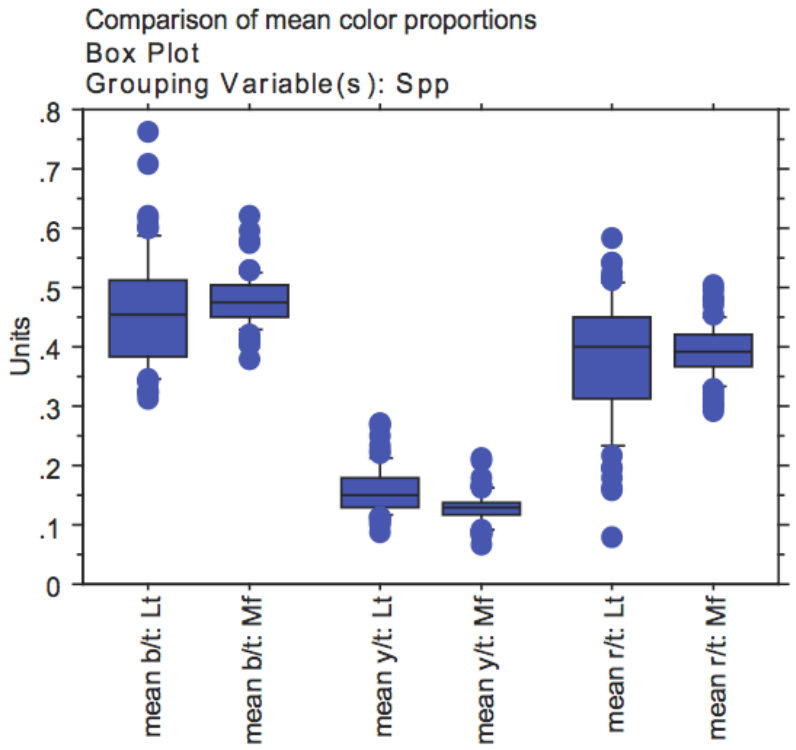


Figure 4-3. *L. t. elapsoides* and *M. fulvius,* on the left and right, respectively, in each pair of box plots, do not show significantly different mean proportions of red (right) or black (left), but do present significantly different mean amounts of yellow, among all specimens examined. Note the greater amount of variation among the mimetic *L. triangulum.*

for red. For *M. fulvius*, normal distribution could not be rejected for black or red, but yellow did not appear to fit a normal distribution (W=0.952832, p=0.0359). However, a KSL test indicated that for *Lampropeltis*, log-normality was rejected for red bands (D=0.135999, p=0.01), but not for black or yellow. In *Micrurus*, log-normality was rejected only for yellow (D=0.110647, p=0.0498). Therefore, subsequent results will be presented in parametric, log-transformed and non-parametric form.

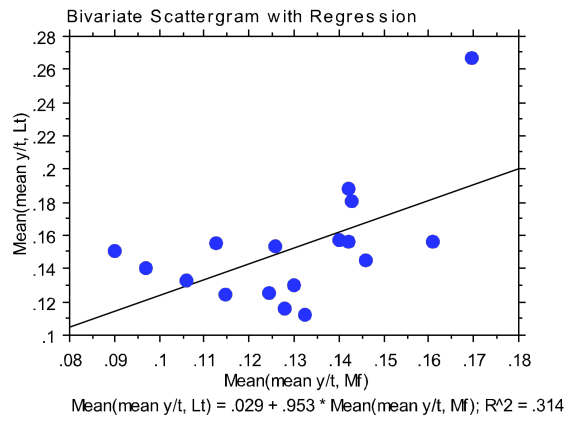
Hypothesis I: Arms Race Hypothesis: Color Pattern Correlations Between *Lampropeltis triangulum* and *Micrurus fulvius*

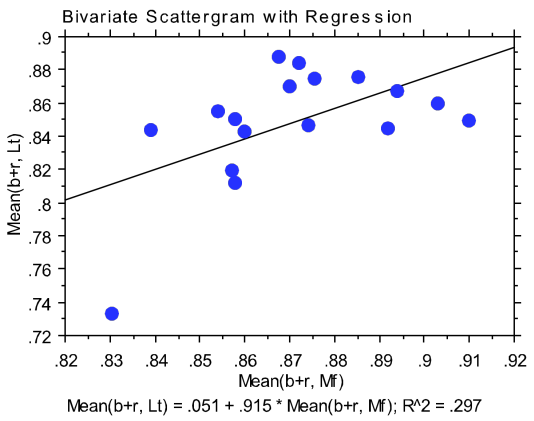
For the totality of specimens, there was no significant difference in proportions of black or red between the two species, but there was a significant difference in the amount of yellow (mean difference=0.031, 134 d.f., t=5.214, p<0.0001) and for "dark" bands, black+red (mean difference=0.03, 134 d.f., t=5.042, p<0.0001), see Table 4-1 and Figure 4-3. When examined by vicariant zone or ecozone (Oak

Table 4-1. Unpaired means comparison for *L. triangulum* and *M. fulvius* (hypothesized difference=0).

Measurement	Mean Diff.	DF	t-value	P-value	95% lower	95% upper	
mean b/t	-0.019	134	-1.448	0.1498	-0.044	0.007	
log mean b/t	-0.056	134	-2.04	0.0433	-0.109	-0.002	
mean y/t	0.031	134	5.214	<.0001	0.019	0.042	
log mean y/t	0.208	134	5.38	<.0001	0.132	0.285	
mean r/t	-0.011	134	-0.784	0.4344	-0.04	0.017	
log mean r/t	-0.072	134	-1.58	0.1165	-0.162	0.018	
mean (b/t+r/t)	-0.03	134	-5.042	<.0001	-0.042	-0.018	
log mean (b+r)	0.036	134	-4.98	<.0001	-0.05	-0.021	

Ridge designated), there were no significant (with p= 0.05) correlations for color pattern, although this was possibly attributable to the small number of data points (4 vicariant zones and 3 ecozones). When the finer-grained EPA ecoregion designations were used, with 17 data points (but fewer specimens per point), no significant correlation was determined for red or black, but the amount of yellow in the two species showed a significant correlation (r=0.560, p=0.0178; r_{log}=0.476, p=0.0524), as did the combined black and red (r=0.545, p=0.0222; r_{log}= 0.558, p=0.0183; rho=0.4828, p=0.0496) (see Figure 4-4). Finally, for





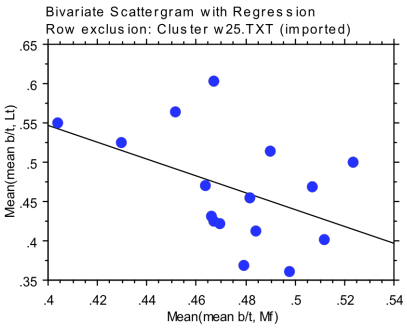
Correlation Coefficient
Hypothesized Correlation = 0

	Correlation	Count	Z-Value	P-Value	95% Lower	95% Upper
Mean(mean y/t, Lt), Mean(mean y/t, Mf)	.560	17	2.369	.0178	.109	.820
Mean(log (b+r), Lt), Mean(log (b+r), Mf)	.558	17	2.359	.0183	.106	.819
Mean(b+r, Lt), Mean(b+r, Mf)	.545	17	2.287	.0222	.087	.813

Figure 4-4. When specimens were grouped by Environmental Protection Agency-designated Ecozones, the proportions dark (red and black) and light (yellow) coloration in the two species showed a significant correlation.

clusters of specimens separated by geographic distance, contrary to the ecoregions, there was a significant inverse correlation between the two species in amount of red (r=-0.533, p=0.0323; r_{log}=-0.576, p=0.0179; rho=-0.506, p=0.0501), a nearly significant inverse correlation in amount of black (r=-0.454, p= 0.0773; r_{log}=-0.465, p=0.0693; rho=-0.500, p=0.0528) and no significant correlation in the respective proportions of yellow (see Figure 4-5), nor of black+red.

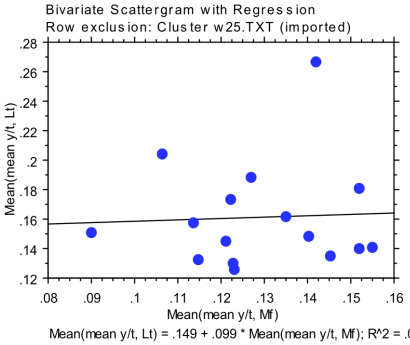
When the two species were tested for correlation in amounts of variance (expressed in standard deviations), no significant values were shown for clusters, EPA ecoregions, Oak Ridge ecozones or vicariant zones.



Mean(mean b/t, Lt) = .972 - 1.063 * Mean(mean b/t, Mf); R^2 = .206

Correlation Coefficient
Hypothesized Correlation = 0
Row exclusion: Cluster w25.TXT (imported)

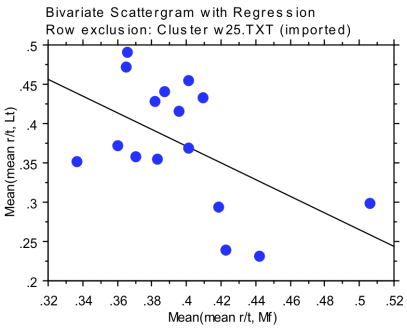
	Correlation	Count	Z-Value	P-Value	95% Lower	95% Upper
Mean(mean b/t, Lt), Mean(mean b/t, Mf)	-.454	16	-1.766	.0773	-.775	.054



Mean(mean y/t, Lt) = .149 + .099 * Mean(mean y/t, Mf); R^2 = .003

Correlation Coefficient
Hypothesized Correlation = 0
Row exclusion: Cluster w25.TXT (imported)

	Correlation	Count	Z-Value	P-Value	95% Lower	95% Upper
Mean(mean y/t, Lt), Mean(mean y/t, Mf)	.050	16	.181	.8562	-.457	.533



Mean(mean r/t, Lt) = .797 - 1.064 * Mean(mean r/t, Mf); R^2 = .284

Correlation Coefficient
Hypothesized Correlation = 0
Row exclusion: Cluster w25.TXT (imported)

	Correlation	Count	Z-Value	P-Value	95% Lower	95% Upper
Mean(mean r/t, Lt), Mean(mean r/t, Mf)	-.533	16	-2.141	.0323	-.814	-.050

Figure 4-5. When grouped in clusters based on geographic distance, *Lampropeltis triangulum elapsoides* and *Micrurus fulvius* were significantly and inversely correlated in proportions of red coloration, nearly significant in black and not significant for yellow.

Finally, as red and black were inversely related to each other and relatively independent of yellow, the two darker colors were summed and the summed values (b+r) and their logarithms (log[b+r]), as well as the log(stdev[b+r]) were tested for correlation. No significant correlation was found for these composite variables between the two species for distance-based clusters. However, significant correlations were found for EPA ecoregions, with r=0.545 (p=0.0222), r_{log}=0.558 (p=0.0183), and rho=0.483, p=0.0534). This is to be expected, as there was also significant correlation for yellow -- the two colors combined would tend to vary as an inverse with yellow.

Hypothesis 2: Color pattern correlates with geographic/environmental/climatological variables

Temperature and rainfall data for specimens is provided in Appendix 27. Proportions of black, yellow and red all showed moderate, but significant or nearly significant (at p=0.05), degrees of correlation with latitude in *L. t. elapsoides*, with black and yellow showing a negative correlation with latitude (see Table 4-2). The combined dark bands (b+r) were significantly correlated with latitude for this species. *M. fulvius* showed no significant correlation between color pattern and latitude, although, when the dark bands were combined, correlation achieved near significance.

Table 4-2. Parametric, log-transformed and non-parametric correlation coefficients and significance levels for proportional areas of each color and environmental and geographic variables for *L. triangulum* and *M. fulvius.*

Species	Correlation	Pearson		Log-transform		Spearman	
		r	p	r	p	rho	p
L. t.	Black x latitude	-0.253	0.0317	-0.244	0.0386	-0.149	ns
L. t.	Yellow x latitude	-0.387	0.0007	-0.363	0.0016	-0.290	0.0145
L. t.	Red x latitude	0.368	0.0013	0.391	0.0006	0.238	0.0446
L. t.	(Black + Red) x Latitude	0.375	0.0011	0.378	0.0009	0.257	0.0301
M.f.	Black x latitude	0.159	ns	0.151	ns	0.160	ns
M.f.	Yellow x latitude	-0.220	ns	-0.202	ns	-0.204	ns
M.f.	Red x latitude	-0.019	ns	-0.022	ns	0.007	ns
M.f.	(Black + Red) x Latitude	0.236	0.0601	0.238	0.058	0.235	0.0619
L. t.	Black x longitude	-0.321	0.058	-0.303	0.0093	-0.354	0.0028
L. t.	Yellow x longitude	-0.139	ns	-0.128	ns	-0.180	ns
L. t.	Red x longitude	0.341	0.0032	0.355	0.002	0.358	0.0026
L. t.	(Black + Red) x Longitude	0.147	ns	0.147	ns	0.199	ns
M.f.	Black x longitude	0.436	0.0003	0.427	0.0004	0.366	0.0037
M.f.	Yellow x longitude	-0.053	ns	-0.058	ns	-0.028	ns
M.f.	Red x longitude	-0.402	0.0009	-0.406	0.0008	-0.395	0.0017
M.f.	(Black + Red) x Longitude	0.037	ns	0.038	ns	-0.058	ns
L. t.	Black x Growing days	0.237	0.0452	0.224	0.058	0.219	0.0651
L. t.	Yellow x Growing days	0.369	0.0013	0.351	0.0023	0.313	0.0083
L. t.	Red x Growing days	-0.349	0.0025	-0.358	0.0018	-0.290	0.0144
L. t.	(Black + Red) x Growing days	-0.364	0.0015	-0.366	0.0014	-0.289	0.0148
M.f.	Black x Growing days	-0.143	ns	-0.140	ns	-0.148	ns
M.f.	Yellow x Growing days	0.154	ns	0.115	ns	0.035	ns
M.f.	Red x Growing days	0.048	ns	0.045	ns	0.080	ns
M.f.	(Black + Red) x Growing days	-0.158	ns	-0.163	ns	-0.04	ns
L. t.	Black x Annual rainfall	0.003	ns	0.018	ns	0.052	ns
L. t.	Yellow x Annual rainfall	0.196	ns	0.189	ns	0.230	0.0526
L. t.	Red x Annual rainfall	-0.076	ns	-0.064	ns	-0.112	ns
L. t.	(Black + Red) x Annual rainfall	-0.191	ns	-0.192	ns	-0.216	0.0688
M.f.	Black x Annual rainfall	0.333	0.0068	0.312	0.0116	0.109	ns
M.f.	Yellow x Annual rainfall	-0.024	ns	-0.052	ns	-0.147	ns
M.f.	Red x Annual rainfall	-0.317	0.0103	-0.326	0.0082	-0.219	0.0827
M.f.	(Black + Red) x Annual Rainfall	0.01	ns	0.008	ns	-0.172	ns

On the other hand, both species showed significant or near-significant (at p=0.05), and contrary patterns of correlation between longitude and black and red bands, but not for yellow. That is, there was a negative correlation for black in *Lampropeltis* and a positive correlation in *Micrurus,* with the opposite occurring

for red. For both, when the dark colors were summed, significant correlation disappeared.

There is also significant, if moderate, correlation between the length of the growing season (a surrogate for temperature regime), and coloration in *L. t. elapsoides*. The yellow bands show the strongest correlation, followed by black bands, while red shows an inverse correlation. The combined dark bands are significantly negatively correlated with the length of the growing season. For *Micrurus*, there was no significant correlation with this variable.

Contrary to what was observed for the length of the growing season, there was no significant correlation between annual rainfall and color pattern for *L. triangulum*, although the Spearman correlation coefficient (rho) achieved near significance for yellow and (thus) the dark colors. However, for *Micrurus*, there was significant correlation between annual rainfall and black and red (negative correlation). There was no significant correlation for the two colors combined. For scattergrams of correlations with environmental/geographic variables, see Appendices 28-31.

Finally, these environmental variables' correlation with color pattern variation in the two species was assessed using clusters to group values, by calculating standard deviations for color pattern and environmental variables and then

calculating correlation coefficients between these values. Significant correlation (p=0.05) was found between latitude and variation in black coloration only for *M. fulvius*, (linear: r=0.651, n=11, p=0.028; log: r=0.654, p=0.0269; Spearman: ns). Significant correlation values were noted for longitude and variation in black coloration for both *M. fulvius* (linear: r=0.631, p=0.0355; log: ns; Spearman: rho=0.6760, p=0.0021) and *L. triangulum* (linear: ns; log: r=-0.614, n=13, p=0.0239; Spearman: rho=-0.5989, p=0.0306). Significant values were also obtained between longitude and variation in red coloring for *L. triangulum* (linear: r=-0.636, p=0.0176; log: r=-0.649, p=0.0144; Spearman: rho=-0.6319, p=0.0205). Significant or nearly significant correlation was also found between the length of the growing season and variation in black coloration in *M. fulvius* (linear: r=-0.601, p=0.0496; log: p=-0.0582, r=0.0597; Spearman: ns) and yellow coloration (linear: ns; log: ns; Spearman: rho=0.633, p=0.0284) and black+red coloration (Spearman: rho=0.6 and p=0.0378) in *L. triangulum*.

Hypothesis 3: Color pattern in *Lampropeltis triangulum elapsoides* is subject to frequency-dependent selection, predicting greater variation in color pattern among mimics where the ratio of models to mimics is higher.

Figure 4-6 shows the dendrograms of specimens clustered by geographic distance through the Ward method. Appendix 27 also lists the ratios of models to

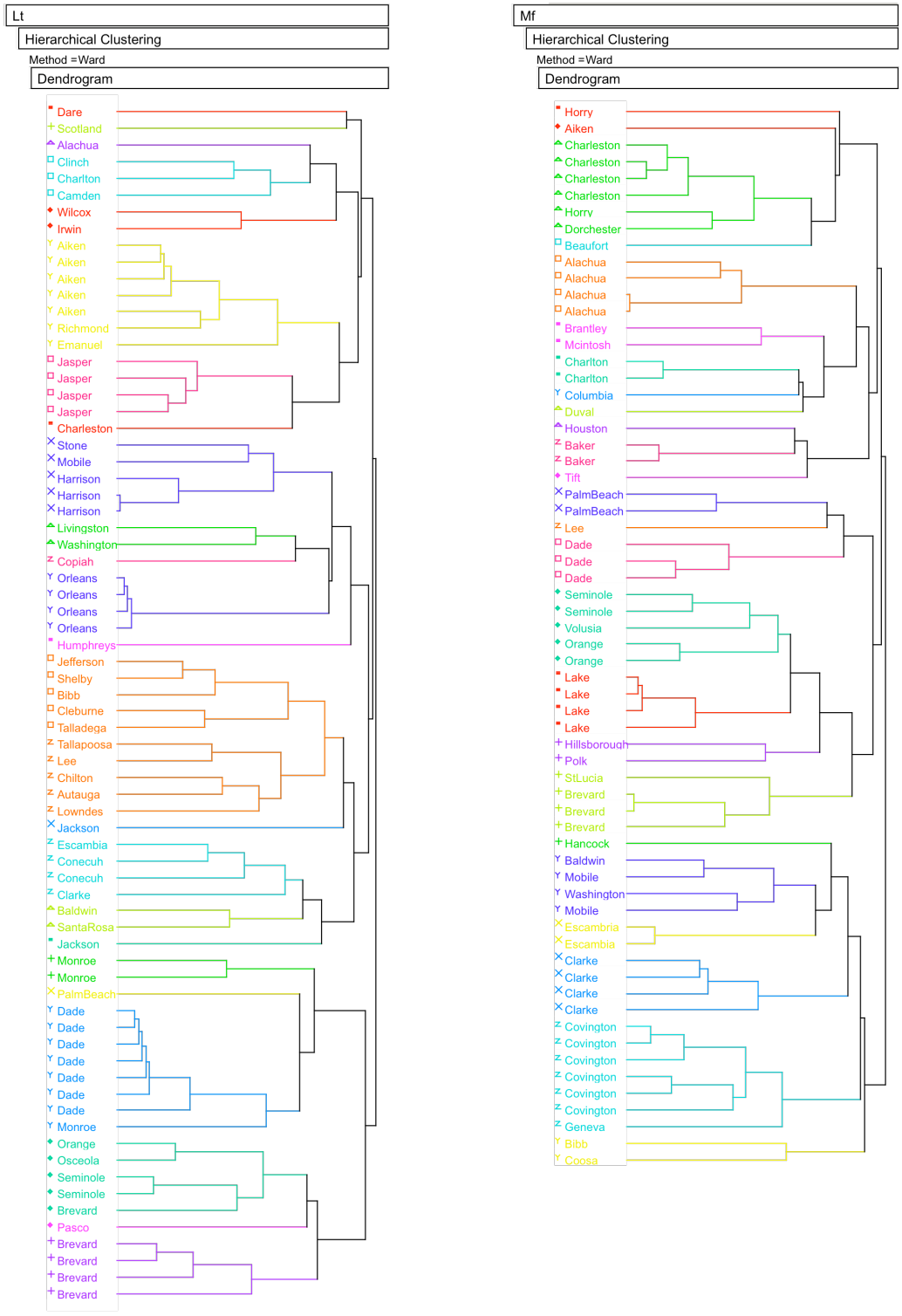


Figure 4-6. Coordinates were used to group specimens by geographic distance using the Ward method of Cluster Analysis, which groups items according to their variation from a central location.

mimics per cluster. Overall, ratios varied from 0 to 7.0, although available specimens did not represent all clusters. The *M.f./L.t.* ratio was significantly correlated (negatively) with latitude (r=-0.510, p=0.0083), but not with longitude.

As shown in the table in Appendix 32, significant or near significant differences between the two snakes with respect to various elements of the mean color pattern values were found in 4 clusters. In a cluster from southern Florida, presenting a model/mimic ratio of 1.4:1, the specimens differed in mean amounts of red, black and black+red. In another cluster from western Alabama, with a ratio of 1.1:1, they differed in amounts of red and black. In another, from northern peninsular Florida and adjacent Georgia, with a ratio of 2.5:1, they varied in black alone. In a fourth, also with a ratio of 1.1:1, from the Gulf Coastal plain from western panhandle Florida through eastern Alabama, they differed at near significance in the combined black+red measure. However, it is notable that in none of these cases were significant differences associated with the highest model to mimic ratios, but rather with the intermediate range.

Levene's Test for unequal variance found no significant differences for variances for combined black+red coloration in specimens grouped in 25 clusters, in which low sample size was a pervasive problem. When specimens were grouped more inclusively in 10 clusters, again, no significant differences were found, although specimens clustered around Aiken, South Carolina, were significant at P<0.10

with F=3.2504. Finally, when specimens were grouped by vicariant zone, models and mimics found on the eastern coastal plain, south of the Savannah River were found to present significantly different variances, with F=11.2962 and P=0.0014. For black coloration, alone, again, Levene found no significant differences for the specimens grouped in 25 clusters, with the same proviso regarding low sample size. However, for the smaller grouping of 10 clusters, a significant difference in variance was found between specimens of the two taxa from northern Florida (model/mimic=2.48717949) and eastern peninsular Florida (model/mimic=3.21212121), with F=10.9111 and 11.3192, respectively, and P<0.01 in both cases. These two clusters had among the highest model to mimic ratios, but results were not fully consistent, as a southern Florida cluster, presenting a model/mimic ratio of 2.15, did not present a significant difference in variances for the two species.

Means and standard deviations of color pattern data for both species, as well as the difference in mean values between the two species were correlated against model to mimic ratios for the groupings based on 25 clusters. For mimics (*L. triangulum)*, mean proportions of black correlated positively (r=0.507, p=0.0178; r(log)=0.503, p=0.0189; and rho=0.441, p=0.0488) and – to a lesser degree -- red bands negatively (r=-0.465, p=0.0328; r(log)=0.431, p=0.502; rho=-0.0357, p=ns) with the frequency of models to mimics (see Table 4-3). No significant correlation was found between variation in any dimension of color pattern for

models or mimics and the relative proportions of models and mimics, nor between the differences between means and the *Mf*/*Lt* ratio.

Table 4-3. Mean proportional areas of red and black banding in *Lampropeltis triangulum elapsoides* showed significant correlation with the frequency ratio of models:mimics calculated by geographic area (25 clusters). However, it should be noted that, contrary to frequency-dependent predictions, no significant correlations were seen for either variation in color pattern or absolute differences in mimic and model mean color patterns and model:mimic ratios.

Correlation Coefficient										
Hypothesized Correlation = 0		Linear			Log Transform			Spearman		
	Count	r	Z-Value	P-Value	r	Z-Value	P-Value	rho	Z-Value	P-Value
Mf/Lt, Lt, Mean(b/t, Lt)	21	0.507	2.369	0.0178	0.503	2.348	0.0189	0.441	1.97	0.0488
Mf/Lt, Lt, Mean(b/t, Mf)	18	-0.223	-0.88	ns	-0.221	-0.87	ns	-0.17	-0.702	ns
Mf/Lt, Lt, Mean(y/t, Lt)	21	0.063	0.267	ns	0.096	0.409	ns	0.016	0.073	ns
Mf/Lt, Lt, Mean(y/t, Mf)	18	0.141	0.55	ns	0.13	0.507	ns	0.141	0.583	ns
Mf/Lt, Lt, Mean(r/t, Lt)	21	-0.465	-2.134	0.0328	-0.431	-1.958	0.0502	-0.357	-1.595	ns
Mf/Lt, Lt, Mean(r/t, Mf)	18	0.122	0.474	ns	0.139	0.541	ns	0.165	0.681	ns
Mf/Lt, Lt, Std Dev(b/t, Lt)	13	0.226	0.726	ns	0.238	0.767	ns	0.157	0.7	ns
Mf/Lt, Lt, Std Dev(b/t, Mf)	11	-0.189	-0.541	ns	-0.155	-0.442	ns	-0.141	-0.583	ns
Mf/Lt, Lt, Std Dev(y/t, Lt)	13	-0.109	-0.347	ns	-0.061	-0.195	ns	0.159	0.552	ns
Mf/Lt, Lt, Std Dev(y/t, Mf)	11	-0.192	-0.549	ns	-0.257	-0.745	ns	-0.264	-0.834	ns
Mf/Lt, Lt, Std Dev(r/t, Lt)	13	-0.13	-0.415	ns	-0.063	-0.199	ns	-0.137	-0.476	ns
Mf/Lt, Lt, Std Dev(r/t, Mf)	11	-0.052	-0.147	ns	-0.332	-0.977	ns	-0.373	-1.179	ns
Mf/Lt, Lt, Mean(b+r, Lt)	21	0.031	0.13	ns	0.035	0.149	ns	-0.022	-0.076	ns
Mf/Lt, Lt, Mean(b+r, Mf)	18	-0.11	-0.427	ns	-0.113	-0.439	ns	0.091	0.287	ns
Mf/Lt, Lt, Std Dev(b+r, Lt)	13	-0.132	-0.419	ns	-0.076	-0.24	ns	-0.137	-0.476	ns
Mf/Lt, Lt, Std Dev(b+r, Mf)	11	-0.22	-0.633	ns	-0.295	-0.858	ns	-0.373	-1.179	ns
Mf/Lt, abs[b(*Mf*) - b(*Lt*)]	16	0.138	0.501	ns	0.059	0.213	ns	-0.022	-0.085	ns
Mf/Lt, abs[y(*Mf*) - y(*Lt*)]	16	0.003	0.012	ns	0.195	0.714	ns	0.24	0.929	ns
Mf/Lt, abs[r(*Mf*) - r(*Lt*)]	16	0.261	0.965	ns	0.344	1.293	ns	0.243	0.94	ns
Mf/Lt, Diff Mf and Lt (mean [b+r])	16	-0.053	-0.193	ns	0.04	0.127	ns	-0.046	-0.177	ns

IV.D. Discussion

It is widely accepted that *L. triangulum* is a Batesian mimic of *M. fulvius* (Greene and McDiarmid, 1981; Pough, 1986; Greene and McDiarmid, 2005; Harper and Pfennig, 2007). Under Batesian mimicry, edible organisms take advantage of the aposematic coloration of noxious models by evolving ever-closer resemblance to them (Joron and Mallet, 1998; Greene and McDiarmid, 2005), although Batesian mimicry is not limited to visual aspects of organisms (Pough, 1986).

Under the classical description of aposematic coloration and Batesian mimicry, purifying selection should drive models and their mimics toward a consistent

"pure" pattern, recognizable to predators (Endler and Greenwood, 1988). The first part of this seems to be borne out by the relative lack of variation found among *M. fulvius* in this study. This relationship should hold while mimics are rare relative to models, and to a greater degree the more toxic the model (Pough, 1986; Sherrat, 2002, Holloway, et al., 2002). However, if mimics become too common relative to models, they -- and the models -- should lose protection, as predators learn that some of the supposed noxious prey is, in fact, edible. However, Pough (1986) suggested that coral snakes, due to their extremely high toxicity, might have selected for an innate, rather than a learned, aversion in predators.

Gilbert (2005), studying mimicry between hoverflies and wasps, observed that the more noxious the model, the more abundant the mimics it could support. Holloway, et al. (2002), in their study of Muellerian mimicry complexes consisting of hoverflies and wasps, analyzed the relationship between relaxed selection, mimetic imperfection and variation. They predicted four possible relationships between mimetic imperfection and mimic variation. First, a positive correlation might exist through the process of normalizing selection if mimics were in a process of perfecting their mimicry. This is probably not the case in the *M. fulvius/L. triangulum* complex, as variation in *L. triangulum* color pattern shows no correlation with mimetic perfection, expressed as the absolute difference between mean color patterns for the two species. A second relationship might

obtain if mimics had evolved as close to perfection as they could, given available genetic variation (or developmental constraints). In this case, they predicted low levels of correlation between mimetic imperfection and color pattern variation. Again, while there are apparently developmental differences in the color patterns of *L. triangulum* and *M. fulvius*, this possibility doesn't seem to hold, here, as, in some locations, the patterns are statistically indistinguishable. A third possibility, pertains to the specific case of their wasp-hoverfly system, in which human-induced habitat changes (increasing numbers of aphid prey) led to increased mimic abundance and relaxed selection in some perfect mimics, producing increased variance. Their fourth prediction was that sheer toxicity of wasp stings would lead predators to avoid even imperfect mimics. Good Batesian mimics could have higher levels of variation and still be protected, while poor mimics would show lower levels of variation. They found that the fourth prediction more closely corresponds with multi-species mimicry complexes among wasps and hoverflies. They were referring to separate mimic species, but this might translate into population differences within species. Given the high toxicity and relative scarcity (or simply secretiveness) of *M. fulvius* and *L. triangulum*, respectively, the fourth prediction would seem pertinent to this study, and, again, is borne out by the general observation of higher variation among mimics than models.

Sherratt (2002) used a modeling approach based on signal detection theory and optimum foraging to test the effects of noxiousness of model and population

density of models and mimics and various costs of enhanced mimicry or imperfect mimicry. Sherratt affirmed that imperfect mimicry should evolve in single model systems, because of a non-linear relationship between model-mimic similarity and optimal predator attack rates on mimics, leaving space for a wide range of mimic phenotypes around the optimal model phenotype. Adding to Holloway, et al.'s (2002) observations, Sherratt predicted that if model is noxious or the mimic relatively scarce or hard to find, mimetic imperfection would result. In addition, once mimics reach a phenotypic plateau where further improvement is selectively neutral, other factors, including mate recognition and thermoregulation, might become countervailing selective forces to further improvement in mimetic pattern. Sherratt also postulated that the ratio of models to mimics that could be sustained by the system was directly proportional to the cost-benefit ratio (cost of attacking models vs. "profitability" of attacking mimics). He stated that as mimics become more relatively abundant, and the cost-benefit ratio decreases, the more perfect the mimicry would need to be to confer protection. As will be seen below, in the discussion of the Harper, et. al. (2007), hypothesis, the results of this study are ambiguous in this regard. Where mimics are most abundant relative to models, the closest means would be expected, while among sympatric populations where models were more relatively abundant, means would be expected to diverge more. The highest mean differences were, in fact, found in areas of intermediate model:mimic frequencies. However, it should be reiterated that *L.. elapsoides* is highly secretive.

On the other hand, where mimics become common, they may be subject to diversifying, density-dependent selection, developing polymorphism, latching on to other models, where these are present, and other selective forces (Holen and Johnstone, 2004; Holloway, et al., 2002), which should increase variation in color patterns. Holen and Johnstone (2004), using game and signal detection models in which they attempted to integrate fitness costs of enhanced identity with aposematic models, predicted increased variation in Batesian mimics when the latter are common and/or the model is weakly defended. This contrasts with other studies that predicted increased mimetic perfection and diminished variation when mimics are abundant relative to models (Sherratt, 2002; Harper, et al., 2007).

IV.D.1. Arms-Race Hypothesis

The classical model also gives rise to the so-called arms-race hypothesis. Under this model, selection favors those mimics that are as similar to models as possible, while favoring models that can be distinguished from their mimics by predators. The result is a “pursuit” by mimics of sympatric models (Dawkins and Krebs, 1979; Gavrilets, 1997). Gavrilets (1997) provided a model that predicted that the “arms race” should prevail when the model has a “stronger incentive to win” or greater genetic variation in pattern than the mimic. In fact, it may be predicted that these correlations would track biogeographic differentiation

between populations and speciation, perhaps even driving the latter through the process of co-evolution. Joron and Mallet (1988) considered the 'arms race' model unlikely due to the more rapid evolutionary rate of mimics than models and the strong purifying selection experienced by the model. They also predicted that co-evolution would be limited, particularly where the model is more common than the mimic, because the effect of the mimic on the model would be less than that of the model on the mimic. In fact, at least one study, conducted on spider mimics of ants (Ceccarelli and Crozier, 2007) found that the phylogenetic pattern of the mimics, rather than indicating co-evolution with models, pointed to adaptive radiation as the mechanism responsible for evolution of mimetic patterns.

In fact, the data in the present study would also appear to refute the arms-race hypothesis, at least at the geographic and taxonomic level tested. There were no direct positive correlations based on geographic location in any aspect of color pattern between the models and mimics. There was a significant correlation for combined black and red between the two species by ecozone, which may involve tracking by the mimic (see below), but these are not strictly geographic. In fact for black and red bands, taken separately, there was a negative correlation for the two species by geographic location. This would suggest that either some other factor or factors are at work modifying the existing pattern, or another model of selection is at work. The fact that major elements of the color patterns of *L. triangulum* and *M. fulvius* appear to correlate by ecoregions -- which are not

distributed geographically, but do have a historical biogeographic matrix (distributions of geographic barriers) – would seem to indicate that either environmental factors or historical factors have played a role in color pattern distribution.

IV.D.2. Selection By Environmental Factors

Crypsis and environmental variables are known to play a role in color pattern selection (Williams, 2006; Tanaka, 2007; Malhotra and Thorpe, 2000; Clusella Trullas, et al, 2007).

One possibility, thermoregulation, represents a consideration in selection for color pattern in ectothermic organisms. In particular, the amount of melanin in the skins of reptiles has been shown to correlate with climate (Clusella Trullas, et al., 2007), although this may not be related to thermoregulation (Nussear, et. al., 2000).

There is a correlation between dark coloration (black+red) and latitude for both models and mimics, more significantly so for mimics. Within this, though, we find an inverse correlation between black and latitude for the mimics, which is counterintuitive, as black would seem to be the biologically significant thermoregulatory color. On a much larger scale and across species, Williams

(2007) also found the darkest bumblebees in his survey at lower latitudes. The models show no significant correlation between black band area and latitude.

Average ambient temperature, as expressed as annual number of growing days also seems to correlate significantly with mean combined black and red in the mimics. All color components show significant or near significant correlation with average temperature in the mimics, although, again, the amount of black shows a paradoxical increase with number of growing days. There were no such correlations for models. With respect to the latter, however, Clusella Trullas, et al. (2007) noted that smaller animals have a lower thermal inertia, which may allow smaller snakes with more melanin to warm up quickly during a brief window in the morning in hotter climates.

In the two species under study, evidence with respect to selection for thermoregulation is equivocal and it appears unlikely that color pattern variation is explained by thermoregulatory considerations.

If thermoregulation were a selective factor of equal weight for models and mimics, however, black coloration in the larger *M. fulvius* might be expected to show a greater clinal variation than the much smaller *L. triangulum elapsoides* (Clusellas Trullas, et al., 2007), due to their greater thermal inertia. However, such is not the case.

In addition, *L. t. elapsoides* "is a burrowing form, nocturnal in its surface activity, or occasionally diurnal after heavy rains" (Williams, 1988). Thus, solar radiation is not likely to be a major means of thermoregulation in this animal.

Finally, Nussear, et al. (2000) noted that the visible color of an animal does not necessarily correlate with either absorptance or emittance of solar radiation either in the visible spectrum or at the longer-wavelength, infrared part of the spectrum.

In order to adequately assess the role of thermoregulation and its association with color pattern, measurement of actual melanin content and the skin absortance, emittance and reflectance among both species should be determined. One additional factor that was not taken into account, but should also be assessed, is the amount of black stippling that appears within the red bands for the two species.

Since the color pattern of *M. fulvius* doesn't seem affected by thermoregulatory constraints with regard to average ambient temperature, other possibilities should be considered. One possibility is color matching (Gibbons and Lillywhite, 1981). For this possibility to be tested, soil types and spectral properties of characteristic vegetation and other ground cover should be surveyed.

Finally, unexpected correlations were observed between amount of red (negative for *M. fulvius*) and black (negative for *L. triangulum*) and longitude for both models and mimics, and between annual rainfall and black and red in models, but not mimics, although the combined black+red color measure and yellow attained near significant correlations with rainfall in the mimics. The correlations between the two species' colors and longitude was inverse. There is no correlation between the model/mimic ratio and longitude. There is, however, a correlation between rainfall and longitude ($r=0.457$, $p<0.0001$). Malhotra and Thorpe (2000) also found a correlation between rainfall and color pattern in *Anolis* lizards, which they attributed to biogeography.

It's conceivable that the observed correlation between color and longitude might represent clinal variation, based on gene flow between eastern and western sections of *L. triangulum*'s distribution, each of which might, in turn, be influenced by allopatric *Micrurus* populations. *L.t. elapsoides* extends further west than *M. fulvius* – up to the Mississippi River. A 100 mile gap separates *M. fulvius fulvius* from its western conspecific, *M. fulvius tenere* (Roze, 1996). At the Mississippi, *L.t. elapsoides* encounters its western conspecifics *L.t. amaura* and *L.t. sypsila,* with which it is suspected to intergrade (Williams, 1988; Armstrong, et al., 2001). It is not known what proportion of their color patterns consist of red and black bands. Molecular data from this study points to the possibility of a single panmictic population of *L. triangulum* in organisms with low vagility, which does

not generally favor an explanation based on gene flow, but rather *in situ* variation. This hypothesis could be tested with further sampling of intermediate subpopulations of both species, and color pattern measurements of populations west of the Mississippi River of the two species.

The observed correlation between *M. fulvius* and *L. triangulum* for combined black and red bands by ecozone, as well as the correlation shown by both with latitude, may indicate that an environmental factor(s) helps shape the colors and pattern of at least one of these species. Two possibilities are suggested. Either both may be under selection by such factors, or models may respond to such a factor, and are then tracked by mimics.

However, this tracking may be conditioned by the somewhat different habits of the two species. *M. fulvius* is diurnal or crepuscular, *L. t. elapsoides* is said to be largely fossorial, only emerging at night or when it is overcast. While Exnerova, et al. (2006) and Hinman, et al. (1997) affirmed the importance of specific colors for aposematism (red, in the former, black in the latter), they dealt with diurnal or phosphorescent organisms. However, under light-poor conditions, red and black might be indistinguishable, leaving an impression of darker and lighter bands. Thus, a visual predator engaging with either of the two species under those lighting conditions would encounter animals with roughly similar proportions of dark and light bands. Light-dark visual contrasts are used by crepuscular and

nocturnal organisms, including horned beetles (Thery, et al., 2008) and butterfly fish (Neudecker, 1989), for intra- and inter-specific communication. Aposematically-patterned crepuscular tree-frogs apparently derived protection from their patterns, although it was unknown if this was due to aposematism or crypsis, or how their crepuscular or nocturnal predators identified the noxious frogs and distinguished them from non-toxic frogs (Robertson and Robertson, 2008). As Endler and Greenwood (1988) noted, predators differing in terms of foraging time may have radically different senses of visual perception, and so may exercise different selective pressures on their prey's color pattern.

IV.D.3. Frequency-dependent Selection

Contrary to the density-dependent, diversifying selection predicted by Holen and Johnstone (2004), Harper and Pfennig (2007) hypothesized that, in fact, where models were common relative to mimics, predators would avoid mimics with varying degrees of similarity with models, while the presence of a low ratio of models to mimics would lead predators to remember and avoid only those mimics that most closely resembled the models, a frequency-dependent purifying selection. Harper and Pfennig et al.'s, (2007) methodology was employed to test this hypothesis. Institutional specimen data for *L. t. elapsoides* and *M. fulvius* was used to calculate relative population frequencies for the models and mimics, which were expressed as a ratio of models/mimics. The authors then chose several counties in North Carolina where the two species coexist as an example

of “edge sympatry,” where the ratio of models to mimics is low, and several counties in the state of Florida as exemplars of “deep sympatry,” where the ratio is high.

All institutional specimens listed were grouped with cluster analysis under the “Ward method,” which groups items by distance based on variation in their distribution. Rather than assume a priori that reported edges of the more limited *Micrurus* distribution necessarily represent areas of a low model to mimic ratio, ratios were calculated for the resulting clusters. The use of museum samples to estimate population parameters is problematic, since they do not represent a random sample of populations, but rather depend on locality-specific events, such as real estate development or the presence of herpetologists. A clear example of this was provided by Janis Roze (1996), who pointed out that the imposition of a bounty on *M. fulvius* in Seminole County, Florida, in the 1930s, led to the slaughter of thousands of snakes, many of which ended up in institutional collections. Nevertheless, in the absence of mark and recapture studies of these two species, there is no other way to make demographic estimates.

Harper and Pfennig (2007), observed significant differences in the mean color patterns of the populations of *L. triangulum* and *M. fulvius* in Florida, as compared to no significant difference in North Carolina, and determined from this

that mimicry was subject to frequency-dependent selection. However, the present study found that differences in means were apparently independent of the ratio of models to mimics. The cluster with the highest model to mimic ratio showed no significant difference in mean color pattern values, while the clusters with the most significant differences had intermediate model to mimic ratios. None of the clusters showed consistency with respect to differences in means for all three colors in the aposematic "triad."

Harper and Pfennig (2007) then tested their sympatric "populations" of models and mimics for differences in variation in "edge" versus "deep" sympatry. The results of this study, with respect to differences in variation for areas of high versus low model/mimic ratios, the data is more equivocal. Although there was no significant difference in combined red and black coloration between models and mimics for any of the "populations" identified by cluster analysis, irrespective of model/mimic frequency, there did appear to be significant variation differences ($F=11.2962$, $P=0.0014$) between models and mimics grouped by biogeographic criteria south of the Savannah River on the eastern coastal plain, which roughly corresponds with Harper and Pfennig's (2007) findings. This broad region also showed the highest average model to mimic ratio (2.3853211). Furthermore, significant differences in variation were found for black coloration between *M. fulvius* and *L. triangulum* in some clusters where high model/mimic occurred. However, this pattern was not completely consistent, as other high frequency

areas did not show significant differences and one low frequency area showed near significant differences. What is required is a larger number of samples from more westerly “sub-populations.”

Parametric and non-parametric correlation analyses were then carried out between model to mimic frequencies and means, mean differences and standard deviations for components of color pattern and their logarithms for all clusters.

In addition to proportions of red, black and yellow, the combination black+red was also tested. The inverse proportionality of these colors and the relative independence of yellow with regard to the two individual colors red and black seemed to indicate that the three colors were a developmental unit, in which the proportions of red or black could only vary at the expense of the other: the red and black constituting a “dark” subunit, and the yellow, a light subunit. The argument used by Harper and Pfennig (2007), for choosing to use red and black, because they constitute “independent” units, is questionable.

While the mean amount of red and/or black in mimics correlated with the frequency, the amount of variation did not. Harper and Pfennig’s (2007) model predicted increasing variation with increasing proportion of models to mimics. In addition, the absolute value of difference in means showed no correlation with the frequency, as might be predicted, also apparently falsifying this hypothesis.

The correlation between mean black or red and frequency may well reflect some geographic or environmental patterning, as the ratio of models to mimics shows a latitudinal gradient. Thus, Harper and Pfennig's observation, based on two geographic locations, one in Florida and the other in North Carolina, might well reflect a geographic, rather than a frequency-dependent, effect. This correlation might be better assessed (given more thorough sampling) using factor analysis or Principal Component Analysis to tease apart the respective causes of variation.

On balance, there does appear to be some support for the frequency-dependent selection model, particularly in the enhanced mimic variation in some areas in which model/mimic ratios are high. However, the lack of correlation between these ratios and the amount of variance, together with correlations between dark coloration and ecoregion, latitude or growing season, among mimics, indicate that superimposed or intertwined with the mimetic pattern -- perhaps the spatially and temporally predominant selective force -- are various other selective forces, including thermoregulation, that may come to the fore. Furthermore, it is possible that both density dependent and frequency dependent selection is in effect at differing population densities of the two species, depending on the frequency of encounters with predators (Holen and Johnstone, 2004). This frequency is patchy, and so the effect on color pattern might also be expected to be patchy (Mappes, et al., 2005).

V. Conclusion

V.A. Formalinized Tissue Extraction

1. The Dayton method (Eckerman & Walsch, 1997) successfully extracted mitochondrial DNA from formalin-fixed intercostal muscle tissues.

2. Success rates differed between the two species, *Lampropeltis triangulum* and *Micrurus fulvius.* This difference was probably attributable to the differing tissue composition between the species. *L. triangulum* employs the intercostal musculature to constrict its prey, beyond its general uses in all vertebrates. The musculature is visibly denser and richer in muscle fiber. *M. fulvius* employs these muscles for general movement and respiration, and the tissue is visibly thinner and more diffuse than in *L. triangulum.* Differing density of myocytes and energy requirements of the tissues presumably translates into differing mitochondrial density in these tissues. The larger number of mitochondria present in *L. triangulum* would translate into a greater amount of recoverable mtDNA, which might be significant given the destructive impact of formalin-fixation on DNA. Therefore, efficient use of archival materials for DNA extraction would entail not only anatomical considerations, but consideration of the behavioral physiology and ecology of the particular organism, as well.

3. In this study, the protocols employed for extraction and amplification of DNA from formalinized tissues were unable to recover the entire 891 base pair ND4

sequence. However, fragments bracketed by internal primers ranging in length from 136-207 base pairs attained varying degrees of success, with the smallest fragment yielding the best result, independent of the G-C content in these primers. These results suggest that careful design of primer pairs bracketing compact DNA segments might provide good results with archival samples.

V.B. Molecular Phylogenetics and Phylogeography

1. No evidence was found for the existence of distinct species among *Lampropeltis triangulum elapsoides* or *Micrurus fulvius fulvius.* Populations were specifically diagnosed using Population Aggregation Analysis under the Phylogenetic Species Concept (Nixon & Wheeler, 1990), but apparent panmixis and continued gene flow among geographic regions fail to support species-level divisions under the Biological Species Concept (Futuyma, 1998), as well.

2. Some evidence was found supporting the contention that the *L. triangulum* group is polyphyletic in that members of this group form clades with other species in the genus *Lampropeltis.*

3. By and large, neither *L. t. elapsoides,* nor *M. f. fulvius* evinced clearly defined geographic structure with respect to ND4 haplotypes, although some hierarchic structure was discernable in the former with respect to the northern highlands and central coastal lowlands regions of Florida. In the latter, there was no solid

evidence for structure: an unresolved group of Florida haplotypes was derived from a haplotype from southern Florida, but all were based on single individuals.

4. In terms of population differentiation as expressed in Fst values, *L. t. elapsoides* manifested statistically significant between-group versus within-group variation with respect to populations in South Carolina, the Florida northern highlands and Florida Atlantic coast central lowlands. No statistically significant differentiation was observed for *M. f. fulvius.*

5. The above suggests that one or more previously isolated populations, at least of *L. t. elapsoides*, experienced a relatively recent range expansion. Isolation may have been due to Pleistocene glaciation and restriction of ancestral population(s) in refugia in Florida and perhaps on the Atlantic Coastal Plain in South Carolina. Estimated timing for a population expansion is roughly concordant with such a scenario. In the case of *M. f. fulvius*, limited sampling makes speculation even on this scale difficult.

6. Sample sizes were low and sampling of populations spotty for both species, but particularly for *M. fulvius*. This was further complicated by incomplete and low-quality sequence data.

7. A finer-grained sampling of both groups is recommended, particularly along the under-sampled western and northern parts of the distribution of the two subspecies, as well as southern Florida (particularly the Keys) and the areas that appear to show some differentiation. Improved sampling might permit discernment of distinct populations of the two groups.

8. Additional DNA sequences, both mitochondrial and nuclear, with similar mutation rate characteristics should be employed for phylogenetic and population genetic analyses.

9. I would also recommend the use of Population Aggregation Analysis as a rigorous diagnostic tool to test for species-level divergence among the previously identified subspecies of *L. triangulum* and *M. fulvius*, where this is made possible by clearly defined allopatric populations. This would be particularly useful to assess the status of *L. t. triangulum, L. t. amaura, L.t. sypsila* and *L. t. elapsoides*. This might be applied not only among allopatric geographic populations of the snakes, but extending the concept of population, to ostensibly sympatric groupings of these organisms, groupings stratified at a finer level by ecological association. Such associations might be delineated by Ecological Niche Modeling (Waltari, et al. 2007; Raxworthy 2008).

V.C. Color Pattern Morphometrics, Model/Mimic Co-adaptation

1. The hypothesis of model-mimic co-evolution (*sensu* Brooks and McLennan 1991) could not be tested, given the lack of phylogenetic hypotheses for the two taxa.

2. For all specimens across the joint distribution of the two subspecies, given aposematism and mimicry, color pattern in the mimics (*L. t. elapsoides*) seems to follow the principle that “close enough is good enough.” The amount of red and black coloration is not statistically distinguishable the two species, but the amount of yellow is.

3. Given an inverse correlation between red and black coloration, particularly in *L. t. elapsoides*, an apparent ontogenetic linkage between the two colors, and the behavioral ecology of this snake, it is recommended that analysis of model-mimic co-adaptation focus on the unit red+black or on yellow.

4. There did not appear to be any tracking of the model by the mimic, predicted by the arms race model of model-mimic co-evolution, as might be indicated by co-varying means or standard deviations among sympatric groups of the two species. On the contrary, the mean amounts of red and black showed an *inverse* correlation between the two species.

5. There may have been some degree of environmental influence on color pattern, since elements of color pattern showed significant correlation with latitude for *L .t. elapsoides*, longitude for both, length of growing season for *L. t. elapsoides* and annual rainfall for *Micrurus.* What the relationship might be is hard to discern, since some correlations were reversed in the two species and black bands showed a positive correlation with length of growing season in *L. t. elapsoides*, contrary to expectation. The amount of red+black, which might be construed as a dark unit, showed a significant negative correlation in this snake with length of growing season, as might be expected if thermoregulation were involved in color pattern selection. Finally, the two species showed correlated red+black and yellow means when ecozones were compared. This data is inconclusive, but merits further research. It would be worthwhile developing more comprehensive environmental data sets for sympatric distribution of the two species. In addition, Factor Analysis might permit various environmental, geographic and co-adaptive effects to be disentangled from each other.

6. Evidence presented here, generally, does not support the hypothesis of frequency dependent selection put forward by Harper, et al. (2007). Contrary to prediction, neither color pattern variance in mimics nor the difference in mean color patterns between models and mimics showed a correlation with the model/mimic frequency. However, this study did find significant differences in variance between the two species in some, but not all, areas where model to

mimic ratios were high, as predicted by Harper, et al.'s (2007) model. Sampling density and sample size were problems in this aspect of the study. Further sampling is needed to fill in the gaps in this study.

V.D. Final Conclusion

The Model-mimic co-evolution is in principle a testable hypothesis that could shed light on evolutionary processes in general. Various methods for approaching co-evolving associations of species have been proposed and implemented in studies of vicariant biogeography (*cf.* Wiley, 1988; Mayden, 1988; Page, 1988), hosts and parasites (*cf.* Page and Hafner, 1996) and other associations (*cf.* Brooks and McLennan, 1991; Page and Charleston, 1998), including model-mimic systems (*cf.* Ceccarelli and Crozier, 2006). Methodological philosophies employed vary from population genetics to phylogenetic systematics, but the latter is a particularly powerful tool for assessing co-speciation. Tools such as Brooks Parsimony Analysis (Brooks, 1991), Tree Reconciliation (Page and Charleston, 1998) and Jungles (Charleston, 1998) have proven to be powerful (if controversial) aids in assessing co-evolutionary associations.

Under the principle of parsimony, if the phylogenies of two sympatric associate taxa show congruent branching patterns, a common biological or biogeographic cause is indicated. In the case of host-parasite co-speciation, this is fairly

straightforward, since the parasitic relationship would appear to be a powerful, if not predominant, selective pressure on the parasite (and in some cases on the host, as well). The host, in a sense, IS the environment for the parasite.

Other types of co-evolution may not be quite so straightforward. In the case of models and mimics, the degree of selection involved in the mimetic association may depend on model, mimic and predator population density. All of these, in turn, are often patchy in time and space. Other selective pressures may come to the fore, including reproductive needs, thermoregulation and structural or ontogenetic limitations. From a co-speciation perspective, a speciation event for a model may not be matched by a similar event for a mimic, in the same way that parasites are pulled into divergence with their hosts. From a co-adaptation point of view, the complex of selective pressures can produce varying degrees of mimetic imperfection or even the collapse of the mimetic association and the replacement of aposematic coloration with cryptic coloration, for example.

Even given a unilateral selection regime for mimicry, implicating mimicry, itself, in the co-divergence may be difficult. Whatever happens to be the focal character of the mimetic association, whether ant-like body form or color pattern or vocalization or scent, this must map to both model and mimic cladograms, which must be reconcilable around this homologue, much as areas reconcile species phylogenies in vicariant biogeography. Where color pattern is the mimetic factor,

this poses the difficulty that such a character is not often discrete among a taxon, but rather is a continuous morphometric character. A method of homologizing means or variances of the trait on a reconciled tree must be employed. Two possible paths are those used by Baker, et al. (2001) and Baker and Wilkinson (2003) to show evolutionary correlations between eye-stalk allometry in sexually dimorphic Diopsid flies and Huey and Bennett (1987) to assess co-adaptation of two behavioral-physiological traits -- preferred temperature and optimal performance temperature – in lizards.

What seems to be crucial for both co-speciation and co-adaptation are, 1) a sufficient density or toxicity of noxious models, 2) a sufficient intensity of predation, and 3) a high degree of consensus recognition and avoidance by predators on models and mimics exercising purifying selection on both. A study using the methodology of phylogenetic systematic would seem to be particularly applicable where these conditions obtain. And such a methodology might help identify areas where they don't.

From a methodological point of view, what is crucial for testing hypotheses of co-evolution (as Mayden (1988) pointed out for vicariant biogeography) are the existence of phylogenies for the respective taxa. While the present study was plagued by a number of problems, in principle, the main problem was perhaps the geographic and taxonomic scale chosen for the investigation, which did not

permit phylogenetic or biogeographic analyses to be undertaken. The goals of this study might be realized as envisioned across the joint distribution of the Micruruine Elapids and specific higher taxa that include mimics, which include *Lampropeltis, Pliocercus, Erythrolamprus,* and others) from the southern United States to Argentina. The following considerations may assist in choosing an appropriate area:

i. The smallest area should encompass reasonably complete monophyletic clades (at least 3-taxon statements) for models and mimics. Incomplete sampling will fail to find existing congruence. Currently, there is a lack of published phylogenetic work on both *Micrurus* and the *Lampropeltis triangulum* complex, but they are under study at present. The more geographically restricted clades containing mimics, such as *Pliocercus* or *Erythrolamprus* (although the latter are Muellerian, rather than Batesian mimics) may also provide fruitful material for a co-evolutionary study of models and mimics, but again, phylogenetic inference within these genera is lacking.

ii. Consideration should be given to the smallest area containing more than three hypothesized species of both models and mimics under the Biological Species Concept. Phylogenies can be inferred concomitantly. Islands adjacent to mainland sites, both containing mimicry complexes, may be worth closer scrutiny.

iii. A study could also examine the smallest area containing three or more geographically-bounded and biologically distinct populations of a mimetic species and a similar number of model populations (possibly each a distinct morph), again, with groups of islands where populations may differentiate in isolation, being good candidate areas.

The biogeographic literature can also provide guidelines for choosing an appropriate area for a study. Knowledge of phylogeographic breaks, vicariant zones and areas of endemism for other species may provide clues as to possible speciation patterns among models and mimics, although vicariant speciation then becomes a possible confounder in the co-evolutionary relationship of models and mimics.

With these considerations in mind, the region from southern Mexico down through Brazil and Bolivia, at least, contains a large concentration of identified Micrurines and an array of mimic taxa (Roze, 1996; Williams, 1988). Given the geological history of the Central American isthmus, its fairly recent origin and the potential for organisms to differentiate through dispersal and mimicry, that area might provide a useful laboratory for testing hypotheses of co-evolution.

Appendices

Appendix 1. Specimen Localities and accession numbers.

Specimen #	Spp	Inst	State	County	Latitude	Longitude	Sequenced
5	Lt	UF121375	FL	IndianRiver	27.7	80.58	
18	Lt	CAS214401	FL	Gulf	30.13	85.16	
24	Lt	CAS207260	FL	Citrus	28.75	82.3	
39	Lt	MF39	FL	Alachua	29.51	82.27	x
43	Lt	AMNH106135	SC	Aiken	33.34	81.75	x
44	Lt	AMNH106136	SC	Aiken	33.34	81.75	
45	Lt	AMNH106137	SC	Aiken	33.34	81.75	x
46	Lt	AMNH106138	SC	Aiken	33.34	81.75	x
47	Lt	AMNH106139	SC	Aiken	33.34	81.75	
48	Lt	AMNH106140	SC	Aiken	33.39	81.62	x
49	Lt	AMNH106141	SC	Aiken	33.34	81.75	x
50	Lt	AMNH106142	SC	Aiken	33.2	81.71	x
52	Lt	AMNH106144	SC	Aiken	33.34	81.75	
59	Lt	UF114326	FL	Liberty			
60	Lt	UF122783	FL	Alachua	29.51	82.28	x
61	Lt	UF124059	FL	Highlands	27.63	81.33	x
62	Lt	UF128553	FL	Levy	29.22	82.76	x
63	Lt	UF128357	FL	Liberty	30.23	84.89	x
64	Lt	UF131770	FL	Levy	29.25	82.73	
65	Lt	UF131771	FL	Polk	28.34	81.8	x
76	Lt	MF76	FL	Brevard	28.66	80.85	
78	Lt	MF78	FL	Hernando	28.55	82.39	
79	Lt	MF79	FL	Manatee	27.59	82.43	
80	Lt	MF80	FL	Liberty	30.15	84.96	
81	Lt	MF81	FL	Liberty	30.15	84.96	
83	Lt	MF83	FL	Alachua	29.5	82.43	x
84	Lt	MF84	FL	Bradford	29.9	82.36	
86	Lt	MF86	FL	Manatee	27.54	82.48	x
87	Lt	MF87	FL	Baker	30.57	82.43	x
89	Lt	MF89	FL	Brevard	27.87	80.52	x
90	Lt	Lte90	AL	Conecuh	31.11	86.59	
91	Lt	AUM18479	FL	Jackson	30.88	85.3	
92	Lt	AUM33635	AL	Shelby	33.29	86.77	x
93	Lt	AUM16610	AL	Talladega	33.41	86.09	x
94	Lt	AUM16609	AL	Cleburne	33.49	85.81	
95	Lt	AUM28	AL	Autauga	32.43	86.45	x
96	Lt	AUM21014	AL	Clarke	31.65	87.92	
97	Lt	AUM4224	AL	Conecuh	31.31	87.24	x
98	Lt	AUM2061	AL	Lee	32.6	85.49	
99	Lt	AUM21094	AL	Clarke	31.3	87.89	x
100	Lt	AUM22384	AL	Chilton	32.78	86.47	x
101	Lt	AUM15833	AL	Mobile	31.03	88.38	x
102	Lt	AUM33174	AL	Jackson	34.89	86	x
103	Lt	AUM2467	AL	Bibb	33.03	86.97	
104	Lt	AUM4250	AL	Baldwin	30.48	87.49	x
105	Lt	AUM20002	AL	Escambia	31.16	86.97	x
106	Lt	AUM2050	AL	Tallapoosa	32.68	85.91	x
107	Lt	AUM23454	AL	Lowndes	32.05	86.75	x
108	Lt	AUM06100	AL	Jefferson	33.32	86.95	x
109	Lt	AUM11517	GA	Wilcox	32.06	83.31	x

Appendix 1 (Continued)

130	Lt	AMNH127785	FL	Alachua	29.49	82.27	x
135	Lt	FMNH8092	GA	Grady	30.73	84.13	x
136	Lt	FMNH2017	MS	Hancock	30.31	89.33	x
137	Lt	FMNH944	AL	Mobile	30.7	88.2	x
138	Lt	FMNH95181	MS	Harrison	30.4	89.1	x
139	Lt	FMNH21592	MS	Harrison	30.41	88.93	
140	Lt	FMNH21591	MS	Harrison	30.41	88.93	
145	Lt	FMNH194566	MS	Lauderdale	32.37	88.54	x
146	Lt	FMNH240439	GA	Harris	32.86	84.85	x
147	Lt	FMNH240471	LA	Union			x
150	Lt	Lt150	FL	Liberty	30.02	84.98	x
151	Lt	CMNH2056	FL	Pinellas	27.77	82.64	x
152	Lt	CMNH2086	FL	Marion	29.2	82.1	x
153	Lt	CMNH7173	NC	Wake	35.78	78.64	
155	Lt	CMNH7249	MS	Hancock	30.31	89.33	x
156	Lt	CMNH9559	SC	Laurens	34.52	82.02	x
159	Lt	CMNH15096	NC	Hyde	35.44	76.17	x
160	Lt	CMNH19806	AL	Baldwin	30.7	87.7	x
161	Lt	CMNH19840	FL	Escambia	30.47	87.21	x
162	Lt	CMNH27787	GA	Charlton	30.77	82.01	x
163	Lt	CMNH35152	FL	Alachua	29.51	82.1	
164	Lt	CMNH66591	SC	Jasper	32.43	81.01	x
165	Lt	CMNH72027	MS	Greene	31.16	88.56	x
166	Lt	CMNH91822	LA	StTammany	30.78	90.84	x
167	Lt	CMNH91865	LA	StHelena	30.78	90.84	
168	Lt	CMNH91867	LA	StHelena	30.78	90.84	
169	Lt	CMNH91903	LA	StHelena	30.8	90.84	
170	Lt	CMNH91986	LA	StHelena	30.8	90.84	x
172	Lt	CMNH116867	GA	Chatham	32.07	81.36	x
173	Lt	CMNH136856	NC	Pitt	35.5	77.25	x
174	Lt	INHS4467	FL	PalmBeach	26.71	80.07	
175	Lt	INHS10477	FL	Monroe	25.09	80.45	
176	Lt	INHS10478	FL	Orange	28.41	81.56	
177	Lt	INHS10479	FL	Alachua	29.59	82.39	
186	Lt	USNMNH204238	FL	Monroe	25.14	80.93	x
187	Lt	USNMNH210070	FL	Brevard	28.72	80.74	x
188	Lt	USNMNH218904	SC	Jasper	32.47	80.99	x
189	Lt	USNMNH307604	SC	Charleston	32.78	79.93	
190	Lt	USNMNH330074	SC	Jasper	32.46	81.19	x
191	Lt	USNMNH330075	SC	Jasper	32.43	81.11	x
204	Lt	USNMNH12928	LA	Orleans	29.95	90.08	
210	Lt	USNMNH10743	FL	SantaRosa	30.63	87.04	
211	Lt	USNMNH12927	LA	Orleans	29.95	90.08	
212	Lt	USNMNH12928	LA	Orleans	29.95	90.08	x
213	Lt	USNMNH13644	FL	Brevard	28.29	80.67	
214	Lt	USNMNH18030	FL	Brevard	27.71	80.6	
215	Lt	USNMNH26303	FL	Dade	25.83	80.19	x
216	Lt	USNMNH28251	FL	Dade	25.83	80.19	
217	Lt	USNMNH28910	FL	Dade	25.83	80.19	
218	Lt	USNMNH30945	FL	Dade	25.83	80.19	x
219	Lt	USNMNH36566	FL	Dade	25.83	80.19	
220	Lt	USNMNH38160	FL	Dade	25.83	80.19	
221	Lt	USNMNH42127	FL	Brevard	28.13	80.63	x
222	Lt	USNMNH85323	FL	Monroe	25.75	80.94	x

Appendix 1 (Continued)

223	Lt	USNMNH85324	FL	Dade	25.73	80.24	
225	Lt	UMMZ52253	LA	Orleans	29.95	90.08	
226	Lt	UMMZ84446	LA	Orleans	29.95	90.08	
227	Lt	UMMZ133468	LA	Livingston	30.65	90.87	x
228	Lt	UMMZ230350	LA	Washington	30.84	90.04	
229	Lt	UMMZ76805	MS	Harrison	30.4	88.97	x
230	Lt	UMMZ76806	MS	Harrison	30.4	88.97	
231	Lt	UMMZ86697	MS	Humphreys	33.19	90.4	x
232	Lt	UMMZ117524	MS	Stone	30.81	88.97	
233	Lt	UMMZ117525	MS	Harrison	30.42	89.09	x
244	Lt	MCZ6799	FL	Brevard	28.13	80.63	
250	Lt	MCZ129307	NC	Dare	35.89	75.85	x
251	Lt	MCZ150093	FL	Pasco	28.06	82.41	x
252	Lt	MCZ166232	FL	Osceola	28.33	81.47	x
254	Lt	MCZ168515	FL	Seminole	28.65	81.2	x
255	Lt	MCZ170332	FL	Seminole	28.65	81.2	x
257	Lt	MCZ177904	SC	Jasper	32.29	81.08	x
259	Lt	UGMNH6092	GA	Camden	30.72	81.55	x
260	Lt	UGMNH19562	GA	Charlton	30.72	82.16	x
261	Lt	UGMNH45918	SC	Aiken	33.57	81.72	
262	Lt	UGMNH6096	GA	Emanuel	32.79	82.22	
263	Lt	UGMNH7162	SC	Aiken	33.28	81.73	
266	Lt	UGMNH45917	MS	Copiah	31.78	90.45	x
268	Lt	UGMNH6090	GA	Irwin	31.66	83.12	x
269	Lt	UGMNH6089	GA	Richmond	33.48	81.98	x
270	Lt	UGMNH6095	NC	Scotland	34.99	79.55	
272	Lt	UGMNH19356	GA	Clinch	30.85	82.67	x
3	Mf		LA	Rapides			x
12	Mf	CAS195959	FL	Hernando	28.55	82.39	
14	Mf	CAS178659	FL	Hernando	28.51	82.17	x
22	Mf	CAS208639	FL	Broward	26.27	80.25	
23	Mf	CAS208638	FL	Citrus	28.75	82.3	x
26	Mf	MF26	FL	PalmBeach	26.79	80.29	x
28	Mf	MF28	FL	Alachua	29.5	82.42	
29	Mf	MF29	FL	Collier	26.45	81.41	x
30	Mf	MF30	FL	Alachua	29.5	82.43	
36	Mf	AMNH149969	FL	Levy	28.84	82.69	x
37	Mf	MF37	FL	Alachua	29.51	82.45	
38	Mf	MF38	FL	PalmBeach	26.8	80.07	x
41	Mf	MF41	FL	Alachua	29.52	82.22	x
42	Mf	AMNH106134	SC	Aiken	33.28	81.73	x
57	Mf	AMNH106150	FL	Liberty	30.16	84.99	x
58	Mf	MF58	FL	PalmBeach	26.64	80.44	
66	Mf	UF92555	FL	Gilchrist	29.62	82.65	x
67	Mf	UF123797	FL	Alachua	29.82	82.59	x
68	Mf	UF124690	FL	Volusia	29.03	81.31	x
69	Mf	UF126072	FL	Alachua	29.64	82.37	
70	Mf	UF112929	FL	Gilchrist	29.64	82.61	x
71	Mf	UF118833	FL	Highlands	27.59	81.39	x
72	Mf	UF117317	FL	Jefferson	30.16	83.58	
73	Mf	UF117318	FL	Leon	30.34	84.64	x
74	Mf	UF129770	FL	Alachua	29.62	82.35	x
75	Mf	MF75	FL	Alachua	29.5	82.42	x
82	Mf	MF82	FL	Alachua	29.8	82.29	x

Appendix 1 (Continued)

85	Mf	MF85	FL	Columbia	30.25	82.52	x
88	Mf	MF88	FL	Alachua	29.5	82.43	
110	Mf	AUM153	AL	Coffee	31.33	85.85	
111	Mf	AUM18485_54	AL	Houston	30.69	84.38	
112	Mf	AUM20781	AL	Clarke	31.4	87.73	
113	Mf	AUM23221	AL	Clarke	31.53	87.91	
114	Mf	AUM13735	AL	Clarke	31.55	87.71	
115	Mf	AUM859	AL	Geneva	30.99	85.94	
116	Mf	AUM21647	AL	Covington	31.09	86.54	
117	Mf	AUM18258	AL	Covington	31.13	86.29	
118	Mf	AUM18232	AL	Baldwin	30.76	87.82	
119	Mf	AUM31164	AL	Covington	31.09	86.3	x
120	Mf	AUM20780	AL	Bibb	33.12	87.06	
121	Mf	AUM23225	AL	Houston	31.22	85.31	
122	Mf	AUM20988	AL	Washington	31.15	88.2	
123	Mf	AUM06612	AL	Covington	31.3	86.48	
124	Mf	AUM05834	AL	Covington	31.3	86.48	
125	Mf	AUM23041	AL	Coosa	32.93	86.26	
126	Mf	AUM06385	AL	Geneva	31.04	85.87	
127	Mf	AUM20779	AL	Clarke	31.75	87.98	
128	Mf	AUM24285	AL	Mobile	30.89	88.29	
129	Mf	AUM21149	AL	Covington	31.25	86.43	
131	Mf	MF131	FL	Seminole	28.69	81.14	
132	Mf	MF132	FL	Alachua	29.5	82.42	x
133	Mf	FMNH240508	GA	Meriwether	33.04	84.68	x
134	Mf	FMNH240401	GA	Charlton	30.72	82.07	
143	Mf	FMNH37708	FL	Monroe	25.08	80.46	
157	Mf	CMNH9857	SC	Lexington	33.86	81.45	
158	Mf	CMNH9864	SC	Lexington	33.88	81.46	
171	Mf	CMNH92190	SC	Beaufort	32.43	80.64	
178	Mf	INHS10501	FL	Alachua	29.51	82.55	
179	Mf	INHS10502	FL	Alachua	29.51	82.28	
180	Mf	INHS10503	FL	Alachua	29.71	82.46	
181	Mf	INHS10504	FL	Alachua	29.71	82.46	
182	Mf	USNMNH142093	FL	Dade	25.59	80.55	
183	Mf	USNMNH8783	FL	Escambia	30.42	87.22	
184	Mf	USNMNH7776	FL	St.Joseph's Isl.	30.18	89.42	
185	Mf	USNMNH60559	FL	Polk	28.06	81.78	
192	Mf	USNMNH1120	SC	Charleston	32.79	79.99	
194	Mf	USNMNH13651	FL	Brevard	28.29	80.67	
195	Mf	USNMNH24356	FL	Lake	28.85	81.68	
196	Mf	USNMNH44517	FL	PalmBeach	26.62	80.06	
197	Mf	USNMNH44554	MS	Hancock	30.31	89.33	
198	Mf	USNMNH48342	SC	Charleston	32.8	79.89	
199	Mf	USNMNH48344	SC	Charleston	32.8	79.89	
200	Mf	USNMNH69672	FL	Lake	28.85	81.68	x
201	Mf	USNMNH75240	FL	Lake	28.85	81.68	
202	Mf	USNMNH81100	FL	Lake	28.77	81.73	
203	Mf	USNMNH81985	FL	Orange	28.54	81.38	
205	Mf	USNMNH130156	GA	Charlton	30.73	82.15	
206	Mf	USNMNH210105	FL	Columbia	30.21	82.52	
207	Mf	USNMNH260848	FL	Volusia	29.02	81.21	
208	Mf	USNMNH307424	FL	Seminole	28.75	81.23	
209	Mf	USNMNH310885	FL	Duval	30.24	81.44	

Appendix 1 (Continued)

224	Mf	USNMNH142094	FL	Dade	25.56	80.5	
236	Mf	UMMZ65627	SC	Beaufort	32.15	80.76	x
237	Mf	UMMZ94934	SC	Horry	33.69	78.88	
238	Mf	UMMZ178030	SC	Horry	32.96	79.93	
239	Mf	MCZ131	FL	Escambia	30.42	87.22	
240	Mf	MCZ132	SC	Charleston	32.78	79.93	
241	Mf	MCZ181	AL	Mobile	30.69	88.05	
242	Mf	MCZ6660	FL	Brevard	28.13	80.63	
243	Mf	MCZ6703	FL	Hillsborough	28.19	82.36	
245	Mf	MCZ6834	FL	Brevard	28.13	80.63	
246	Mf	MCZ46748	FL	Dade	25.66	80.32	x
247	Mf	MCZ53948	FL	StLucie	27.82	80.48	
248	Mf	MCZ54018	FL	Lee	26.64	81.87	
249	Mf	MCZ121235	FL	PalmBeach	26.8	80.06	x
253	Mf	MCZ166907	FL	Seminole	28.73	81.34	x
256	Mf	MCZ170335	FL	Orange	28.49	81.44	
258	Mf	UGMNH6557	GA	Baker	31.21	84.47	
264	Mf	UGMNH6554	GA	Mcintosh	31.43	81.48	
265	Mf	UGMNH6550	GA	Tift	31.34	83.59	x
267	Mf	UGMNH6556	GA	Baker	31.21	84.47	

Appendix 2. Specimen groupings by cluster (geographic distance), ecozone (Oak Ridge) and vicariant zone.

Spec. #	Spp	Inst	Ecozone (Oak Ridge)	Vicariant zone	Cluster (25)	Cluster (15)	Cluster (10)
5	Lt	UF121375	2311	11112	21	5	6
18	Lt	CAS214401	2311	11112	2	3	9
24	Lt	CAS207260	2311	11112	14	12	6
39	Lt	MF39	2311	11112	3	7	2
43	Lt	AMNH106135	2311	11111	1	1	7
44	Lt	AMNH106136	2311	11111	1	1	7
45	Lt	AMNH106137	2311	11111	1	1	7
46	Lt	AMNH106138	2311	11111	1	1	7
47	Lt	AMNH106139	2311	11111	1	1	7
48	Lt	AMNH106140	2311	11111	1	1	7
49	Lt	AMNH106141	2311	11111	1	1	7
50	Lt	AMNH106142	2311	11111	1	1	7
52	Lt	AMNH106144	2311	11111	1	1	7
59	Lt	UF114326	2311	11112		3	
60	Lt	UF122783	2311	11112	3	7	2
61	Lt	UF124059	2311	11112	6	5	6
62	Lt	UF128553	2311	11112	23	7	2
63	Lt	UF128357	2311	11112	2	3	9
64	Lt	UF131770	2311	11112	23	7	2
65	Lt	UF131771	2311	11112	14	12	6
76	Lt	MF76	2311	11112	14	12	6
78	Lt	MF78	2311	11112	14	12	6
79	Lt	MF79	2311	11112	10	15	6
80	Lt	MF80	2311	11112	2	3	9
81	Lt	MF81	2311	11112	2	3	9
83	Lt	MF83	2311	11112	23	7	2

Appendix 2 (Continued)

84	Lt	MF84	2311	11112	23	7	2
86	Lt	MF86	2311	11112	10	15	6
87	Lt	MF87	2311	11112	12	13	2
89	Lt	MF89	2311	11112	21	5	6
90	Lt	Lte90	2311	21112	9	6	8
91	Lt	AUM18479	2311	21112	18	6	9
92	Lt	AUM33635	2320	21112	8	10	5
93	Lt	AUM16610	2320	22112	8	10	5
94	Lt	AUM16609	2320	22112	8	10	5
95	Lt	AUM28	2320	21112	8	10	5
96	Lt	AUM21014	2320	21112	19	6	8
97	Lt	AUM4224	2311	21112	9	6	8
98	Lt	AUM2061	2320	21112	8	10	5
99	Lt	AUM21094	2320	21112	19	6	8
100	Lt	AUM22384	2320	21112	8	10	5
101	Lt	AUM15833	2311	21122	19	4	8
102	Lt	AUM33174	2211	22112	24	10	5
103	Lt	AUM2467	2320	21112	8	10	5
104	Lt	AUM4250	2311	21112	9	6	8
105	Lt	AUM20002	2311	21112	9	6	8
106	Lt	AUM2050	2320	21112	8	10	5
107	Lt	AUM23454	2320	21112	8	6	5
108	Lt	AUM06100	2320	22112	8	10	5
109	Lt	AUM11517	2311	11112	18	3	9
130	Lt	AMNH127785	2311	11112	3	7	2
135	Lt	FMNH8092	2311	11112	18	3	9
136	Lt	FMNH2017	2311	21122	11	4	3
137	Lt	FMNH944	2311	21122	19	4	8
138	Lt	FMNH95181	2311	21122	11	4	3
139	Lt	FMNH21592	2311	21122	11	4	3
140	Lt	FMNH21591	2311	21122	11	4	3
145	Lt	FMNH194566	2320	21122	16	10	5
146	Lt	FMNH240439	2320	22112	8	10	5
147	Lt	FMNH240471	2320	22222			
150	Lt	Lt150	2311	11112	2	3	9
151	Lt	CMNH2056	2311	11112	10	15	6
152	Lt	CMNH2086	2311	11112	3	7	2
153	Lt	CMNH7173	2320	12111	22	8	1
155	Lt	CMNH7249	2311	21122	11	4	3
156	Lt	CMNH9559	2320	12111	1	1	7
159	Lt	CMNH15096	2320	11111	7	8	1
160	Lt	CMNH19806	2311	21112	19	6	8
161	Lt	CMNH19840	2311	21112	9	6	8
162	Lt	CMNH27787	2311	11112	12	13	2
163	Lt	CMNH35152	2311	11112	3	7	2
164	Lt	CMNH66591	2311	11111	13	14	7
165	Lt	CMNH72027	2311	21122	19	4	8
166	Lt	CMNH91822	2311	21122	5	4	3
167	Lt	CMNH91865	2320	21122	5	4	3
168	Lt	CMNH91867	2320	21122	5	4	3

Appendix 2 (Continued)

169	Lt	CMNH91903	2320	21122	5	4	3
170	Lt	CMNH91986	2320	21122	5	4	3
172	Lt	CMNH116867	2311	11112	13	14	7
173	Lt	CMNH136856	2320	11111	7	8	1
174	Lt	INHS4467	4110	11112	20	9	4
175	Lt	INHS10477	4110	11112	4	9	4
176	Lt	INHS10478	2311	11112	14	12	6
177	Lt	INHS10479	2311	11112	23	7	2
186	Lt	USNMNH204238	4110	11112	4	9	4
187	Lt	USNMNH210070	2311	11112	14	12	6
188	Lt	USNMNH218904	2311	11111	13	14	7
189	Lt	USNMNH307604	2320	11111	25	14	7
190	Lt	USNMNH330074	2311	11111	13	14	7
191	Lt	USNMNH330075	2311	11111	13	14	7
204	Lt	USNMNH12928	2312	21122	5	4	3
210	Lt	USNMNH10743	2311	21112	9	6	8
211	Lt	USNMNH12927	2312	21122	5	4	3
212	Lt	USNMNH12928	2312	21122	5	4	3
213	Lt	USNMNH13644	2311	11112	21	5	6
214	Lt	USNMNH18030	2311	11112	21	5	6
215	Lt	USNMNH26303	4110	11112	4	9	4
216	Lt	USNMNH28251	4110	11112	4	9	4
217	Lt	USNMNH28910	4110	11112	4	9	4
218	Lt	USNMNH30945	4110	11112	4	9	4
219	Lt	USNMNH36566	4110	11112	4	9	4
220	Lt	USNMNH38160	4110	11112	4	9	4
221	Lt	USNMNH42127	2311	11112	21	5	6
222	Lt	USNMNH85323	4110	11112	17	9	4
223	Lt	USNMNH85324	4110	11112	4	9	4
225	Lt	UMMZ52253	2312	21122	5	4	3
226	Lt	UMMZ84446	2312	21122	5	4	3
227	Lt	UMMZ133468	2311	21122	5	4	3
228	Lt	UMMZ230350	2311	21122	5	4	3
229	Lt	UMMZ76805	2311	21122	11	4	3
230	Lt	UMMZ76806	2311	21122	11	4	3
231	Lt	UMMZ86697	2312	21122	16	10	5
232	Lt	UMMZ117524	2311	21122	11	4	3
233	Lt	UMMZ117525	2311	21122	11	4	3
244	Lt	MCZ6799	2311	11112	21	5	6
250	Lt	MCZ129307	2320	11111	7	8	1
251	Lt	MCZ150093	2311	11112	10	15	6
252	Lt	MCZ166232	2311	11112	14	12	6
254	Lt	MCZ168515	2311	11112	14	12	6
255	Lt	MCZ170332	2311	11112	14	12	6
257	Lt	MCZ177904	2311	11111	13	14	7
259	Lt	UGMNH6092	2311	11112	12	13	2

Appendix 2 (Continued)

260	Lt	UGMNH19562	2311	11112	12	13	2
261	Lt	UGMNH45918	2311	11111	1	1	7
262	Lt	UGMNH6096	2311	11112	1	1	7
263	Lt	UGMNH7162	2311	11111	1	1	7
266	Lt	UGMNH45917	2320	21122	16	4	5
268	Lt	UGMNH6090	2311	11112	18	3	9
269	Lt	UGMNH6089	2311	11112	1	1	7
270	Lt	UGMNH6095	2320	11111	22	8	1
272	Lt	UGMNH19356	2311	11112	12	13	2
3	Mf			22222			
12	Mf	CAS195959	2311	11112	14	12	6
14	Mf	CAS178659	2311	11112	14	12	6
22	Mf	CAS208639	4110	11112	20	9	4
23	Mf	CAS208638	2311	11112	14	12	6
26	Mf	MF26	4110	11112	20	9	4
28	Mf	MF28	2311	11112	23	7	2
29	Mf	MF29	4110	11112	17	9	4
30	Mf	MF30	2311	11112	23	7	2
36	Mf	AMNH149969	2311	11112	14	12	6
37	Mf	MF37	2311	11112	23	7	2
38	Mf	MF38	4110	11112	20	9	4
41	Mf	MF41	2311	11112	3	7	2
42	Mf	AMNH106134	2311	11111	1	1	7
57	Mf	AMNH106150	2311	11112	2	3	9
58	Mf	MF58	4110	11112	20	9	4
66	Mf	UF92555	2311	11112	23	7	2
67	Mf	UF123797	2311	11112	23	7	2
68	Mf	UF124690	2311	11112	14	12	6
69	Mf	UF126072	2311	11112	23	7	2
70	Mf	UF112929	2311	11112	23	7	2
71	Mf	UF118833	2311	11112	6	5	6
72	Mf	UF117317	2311	11112	23	7	2
73	Mf	UF117318	2311	11112	18	3	9
74	Mf	UF129770	2311	11112	23	7	2
75	Mf	MF75	2311	11112	23	7	2
82	Mf	MF82	2311	11112	23	7	2
85	Mf	MF85	2311	11112	23	7	2
88	Mf	MF88	2311	11112	23	7	2
110	Mf	AUM153	2320	21112	9	6	8
111	Mf	AUM18485_54	2311	21112	18	3	9
112	Mf	AUM20781	2320	21112	19	6	8
113	Mf	AUM23221	2320	21112	19	6	8
114	Mf	AUM13735	2320	21112	19	6	8
115	Mf	AUM859	2311	21112	9	6	8
116	Mf	AUM21647	2311	21112	9	6	8
117	Mf	AUM18258	2311	21112	9	6	8
118	Mf	AUM18232	2311	21112	19	6	8
119	Mf	AUM31164	2311	21112	9	6	8
120	Mf	AUM20780	2320	21112	8	10	5
121	Mf	AUM23225	2311	21112	18	6	9

Appendix 2 (Continued)

122	Mf	AUM20988	2311	21122	19	4	8
123	Mf	AUM06612	2311	21112	9	6	8
124	Mf	AUM05834	2311	21112	9	6	8
125	Mf	AUM23041	2320	21112	8	10	5
126	Mf	AUM06385	2311	21112	9	6	8
127	Mf	AUM20779	2320	21112	19	6	8
128	Mf	AUM24285	2311	21122	19	4	8
129	Mf	AUM21149	2311	21112	9	6	8
131	Mf	MF131	2311	11112	14	12	6
132	Mf	MF132	2311	11112	23	7	2
133	Mf	FMNH240508	2320	22112	8	10	5
134	Mf	FMNH240401	2311	11112	12	13	2
143	Mf	FMNH37708	4110	11112	4	9	4
157	Mf	CMNH9857	2320	11111	1	1	7
158	Mf	CMNH9864	2320	11111	1	1	7
171	Mf	CMNH92190	2320	11111	13	14	7
178	Mf	INHS10501	2311	11112	23	7	2
179	Mf	INHS10502	2311	11112	3	7	2
180	Mf	INHS10503	2311	11112	23	7	2
181	Mf	INHS10504	2311	11112	23	7	2
182	Mf	USNMNH142093	4110	11112	4	9	4
183	Mf	USNMNH8783	2311	21112	9	6	8
184	Mf	USNMNH7776		11112	11	4	3
185	Mf	USNMNH60559	2311	11112	14	15	6
192	Mf	USNMNH1120	2320	11111	25	14	7
194	Mf	USNMNH13651	2311	11112	21	5	6
195	Mf	USNMNH24356	2311	11112	14	12	6
196	Mf	USNMNH44517	4110	11112	20	9	4
197	Mf	USNMNH44554	2311	21122	11	4	3
198	Mf	USNMNH48342	2320	11111	25	14	7
199	Mf	USNMNH48344	2320	11111	25	14	7
200	Mf	USNMNH69672	2311	11112	14	12	6
201	Mf	USNMNH75240	2311	11112	14	12	6
202	Mf	USNMNH81100	2311	11112	14	12	6
203	Mf	USNMNH81985	2311	11112	14	12	6
205	Mf	USNMNH130156	2311	11112	12	13	2
206	Mf	USNMNH210105	2311	11112	23	7	2
207	Mf	USNMNH260848	2311	11112	14	12	6
208	Mf	USNMNH307424	2311	11112	14	12	6
209	Mf	USNMNH310885	2311	11112	12	13	2
224	Mf	USNMNH142094	4110	11112	4	9	4
236	Mf	UMMZ65627	2320	11111	13	14	7
237	Mf	UMMZ94934	2320	11111	15	8	1
238	Mf	UMMZ178030	2320	11111	25	14	7
239	Mf	MCZ131	2311	21112	9	6	8
240	Mf	MCZ132	2320	11111	25	14	7
241	Mf	MCZ181	2311	21122	19	6	8
242	Mf	MCZ6660	2311	11112	21	5	6
243	Mf	MCZ6703	2311	11112	10	15	6

Appendix 2 (Continued)

245	Mf	MCZ6834	2311	11112	21	5	6
246	Mf	MCZ46748	4110	11112	4	9	4
247	Mf	MCZ53948	2311	11112	21	5	6
248	Mf	MCZ54018	2311	11112	17	9	4
249	Mf	MCZ121235	4110	11112	20	9	4
253	Mf	MCZ166907	2311	11112	14	12	6
256	Mf	MCZ170335	2311	11112	14	12	6
258	Mf	UGMNH6557	2311	21112	18	3	9
264	Mf	UGMNH6554	2311	11112	12	13	2
265	Mf	UGMNH6550	2311	11112	18	3	9
267	Mf	UGMNH6556	2311	21112	18	3	9

Appendix 3. Color pattern measurements (average proportions of each color), sex and grouping by Environmental Protection Agency-designated Ecozones.

Spec #	Accession	Spp	Sex	Ecozone (EPA)	mean b/t	stdev b/t	mean y/t	stdev y/t	mean r/t	stdev r/t
43	AMNH106135	Lt	m	65c	0.620	0.104	0.185	0.036	0.194	0.096
44	AMNH106136	Lt	m?	65c	0.487	0.048	0.147	0.025	0.366	0.066
45	AMNH106137	Lt	f	65c	0.434	0.056	0.118	0.019	0.448	0.069
90	Lte90avg	Lt	f	65f	0.345	0.029	0.111	0.016	0.543	0.040
91	AUM18479	Lt	f	65g	0.455	0.038	0.126	0.016	0.419	0.041
92	AUM33635	Lt	m	67h	0.451	0.037	0.156	0.014	0.393	0.048
93	AUM16610	Lt		67f	0.385	0.034	0.128	0.011	0.486	0.044
94	AUM16609	Lt	f	45d	0.472	0.049	0.117	0.013	0.411	0.059
95	AUM28	Lt	f	65i	0.415	0.050	0.152	0.023	0.433	0.057
96	AUM21014	Lt	f	65q	0.430	0.051	0.188	0.022	0.381	0.040
97	AUM4224	Lt	f	65f	0.341	0.019	0.150	0.015	0.509	0.029
98	AUM2061	Lt	f	45b	0.441	0.066	0.121	0.022	0.438	0.071
100	AUM22384	Lt		45a	0.467	0.042	0.132	0.013	0.401	0.053
101	AUM15833	Lt	f	65f	0.508	0.056	0.159	0.036	0.333	0.074
102	AUM33174	Lt		68c	0.395	0.046	0.171	0.017	0.434	0.058
103	AUM2467	Lt	m	67f	0.491	0.031	0.157	0.017	0.353	0.043
104	AUM4250	Lt	m	75a	0.468	0.045	0.129	0.013	0.402	0.045
105	AUM20002	Lt	f	65f	0.395	0.048	0.090	0.013	0.515	0.058
106	AUM2050	Lt		45a	0.381	0.045	0.127	0.011	0.492	0.047
107	AUM23454	Lt	m	65d	0.344	0.027	0.116	0.011	0.540	0.033
108	AUM06100	Lt	m	67g	0.401	0.046	0.143	0.024	0.456	0.061
109	AUM11517	Lt	m	65p	0.362	0.040	0.126	0.018	0.512	0.053
174	INHS4467	Lt	m	76c	0.525	0.040	0.181	0.013	0.294	0.048
175	INHS10477	Lt	m	76d	0.761	0.053	0.158	0.030	0.081	0.065
176	INHS10478	Lt	f	75d	0.446	0.028	0.202	0.016	0.352	0.028
177	INHS10479	Lt	m	75d	0.514	0.017	0.188	0.018	0.355	0.053
186	USNMNH204238	Lt	m	76d	0.600	0.056	0.221	0.018	0.179	0.062
187	USNMNH210070	Lt	m	75d	0.500	0.040	0.167	0.020	0.332	0.055
188	USNMNH218904	Lt	f	63h	0.314	0.026	0.102	0.016	0.584	0.030
189	USNMNH307604	Lt	m	75j	0.603	0.069	0.158	0.019	0.240	0.083
190	USNMNH330074	Lt	m	75i	0.473	0.047	0.233	0.023	0.294	0.058
191	USNMNH330075	Lt	f	63h	0.402	0.083	0.100	0.029	0.499	0.096
210	USNMNH10743	Lt	juv_m	75a	0.462	0.031	0.151	0.014	0.387	0.039
211	USNMNH12927	Lt	f	73k	0.349	0.021	0.269	0.039	0.382	0.055
212	USNMNH12928	Lt		73k	0.428	0.037	0.129	0.020	0.443	0.051
213	USNMNH13644	Lt	juv_f	75d	0.319	0.048	0.188	0.038	0.493	0.041
214	USNMNH18030	Lt	juv_m	75d	0.513	0.080	0.138	0.024	0.350	0.075

Appendix 3 (Continued)

215	USNMNH26303	Lt	juv_f	76d	0.531	0.042	0.272	0.047	0.197	0.022
216	USNMNH28251	Lt	f	76d	0.615	0.073	0.226	0.045	0.159	0.076
217	USNMNH28910	Lt	f	76d	0.558	0.064	0.193	0.031	0.249	0.070
218	USNMNH30945	Lt	juv_f	76d	0.584	0.065	0.161	0.016	0.255	0.071
219	USNMNH36566	Lt	m	76d	0.531	0.082	0.251	0.052	0.218	0.097
220	USNMNH38160	Lt	f	76d	0.483	0.039	0.208	0.010	0.309	0.041
221	USNMNH42127	Lt	juv_f	75d	0.408	0.015	0.128	0.027	0.464	0.035
222	USNMNH85323	Lt	m	76a	0.369	0.033	0.141	0.009	0.491	0.035
223	USNMNH85324	Lt	f	76d	0.410	0.027	0.149	0.012	0.441	0.036
225	UMMZ52253	Lt		73k	0.476	0.044	0.140	0.082	0.413	0.044
226	UMMZ84446	Lt		73k	0.360	0.033	0.202	0.016	0.438	0.042
227	UMMZ133468	Lt		74d	0.350	0.033	0.156	0.023	0.494	0.050
228	UMMZ230350	Lt		65f	0.422	0.042	0.161	0.032	0.417	0.067
229	UMMZ76805	Lt		75k	0.582	0.087	0.148	0.015	0.270	0.096
230	UMMZ76806	Lt		75k	0.378	0.033	0.167	0.018	0.455	0.045
231	UMMZ86697	Lt		73a	0.372	0.031	0.187	0.029	0.442	0.049
232	UMMZ117524	Lt		65f	0.497	0.032	0.123	0.025	0.380	0.049
233	UMMZ117525	Lt		75a	0.543	0.049	0.156	0.018	0.302	0.063
244	MCZ6799	Lt	f	75d	0.485	0.040	0.108	0.013	0.407	0.049
250	MCZ129307	Lt	f	63c	0.324	0.044	0.161	0.027	0.516	0.045
251	MCZ150093	Lt	m	75b	0.362	0.027	0.267	0.019	0.372	0.040
252	MCZ166232	Lt		75d	0.448	0.051	0.132	0.015	0.420	0.065
254	MCZ168515	Lt		75c	0.369	0.033	0.193	0.032	0.438	0.055
255	MCZ170332	Lt	m	75c	0.585	0.074	0.113	0.013	0.302	0.071
257	MCZ177904	Lt		63h	0.499	0.041	0.146	0.010	0.355	0.046
259	UGMNH06092	Lt	f	75j	0.323	0.038	0.154	0.026	0.524	0.060
260	UGMNH19562	Lt		75g	0.601	0.068	0.125	0.014	0.274	0.078
261	UGMNH45918	Lt		65c	0.560	0.031	0.178	0.023	0.262	0.038
262	UGMNH06096	Lt		65p	0.503	0.082	0.162	0.026	0.335	0.105
263	UGMNH07162	Lt	m	65c	0.542	0.071	0.138	0.013	0.320	0.078
266	UGMNH45917	Lt		74c	0.350	0.043	0.150	0.028	0.500	0.067
268	UGMNH6090	Lt	m	65l	0.420	0.045	0.146	0.021	0.434	0.048
269	UGMNH06089	Lt		65c	0.707	0.073	0.129	0.033	0.164	0.073
270	UGMNH6095	Lt	m	65c	0.503	0.077	0.163	0.028	0.335	0.074
272	UGMNH19356	Lt		75e	0.441	0.048	0.112	0.019	0.447	0.062
42	AMNH106134	Mf	m	65c	0.404	0.057	0.090	0.017	0.506	0.054
111	AUM18485_54	Mf	f	65g	0.488	0.038	0.129	0.016	0.384	0.047
112	AUM20781	Mf		65f	0.440	0.059	0.123	0.014	0.436	0.064
113	AUM23221	Mf	m	65q	0.530	0.078	0.135	0.019	0.335	0.071
114	AUM13735	Mf		65q	0.448	0.032	0.162	0.012	0.391	0.023
116	AUM21647	Mf	m	65g	0.529	0.063	0.129	0.016	0.343	0.076
117	AUM18258	Mf		65g	0.520	0.021	0.128	0.009	0.352	0.024
118	AUM18232	Mf		65f	0.469	0.037	0.134	0.012	0.397	0.041
119	AUM31164	Mf		65g	0.450	0.044	0.102	0.010	0.448	0.046
120	AUM20780	Mf		67h	0.431	0.041	0.161	0.021	0.409	0.038
122	AUM20988	Mf		65f	0.472	0.032	0.139	0.009	0.389	0.031
123	AUM06612	Mf		65f	0.598	0.016	0.088	0.009	0.314	0.017
124	AUM05834	Mf		65f	0.462	0.044	0.111	0.022	0.427	0.028
125	AUM23041	Mf	m	45a	0.504	0.041	0.130	0.013	0.366	0.038
126	AUM06385	Mf		65g	0.513	0.036	0.156	0.018	0.331	0.044
127	AUM20779	Mf		65q	0.492	0.034	0.130	0.013	0.378	0.033
128	AUM24285	Mf		65f	0.581	0.060	0.067	0.016	0.352	0.053
129	AUM21149	Mf		65f	0.514	0.049	0.102	0.021	0.384	0.051
178	INHS10501	Mf		75c	0.470	0.048	0.129	0.017	0.401	0.053
179	INHS10502	Mf		75d	0.440	0.032	0.129	0.011	0.431	0.033
180	INHS10503	Mf		75c	0.506	0.042	0.131	0.021	0.362	0.058

Appendix 3 (Continued)

181	INHS10504	Mf		75c	0.468	0.059	0.115	0.012	0.417	0.059
182	USNMNH142093	Mf	m	76a	0.446	0.034	0.081	0.016	0.472	0.036
183	USNMNH8783	Mf	m	75a	0.445	0.034	0.167	0.013	0.388	0.039
184	USNMNH7776	Mf	f		0.348	0.033	0.146	0.011	0.506	0.032
185	USNMNH60559	Mf	f	75b	0.466	0.116	0.211	0.145	0.322	0.084
192	USNMNH1120	Mf		75j	0.476	0.039	0.115	0.034	0.409	0.052
193	USNMNH1142	Mf		63h	0.500	0.066	0.104	0.013	0.396	0.071
194	USNMNH13651	Mf	f	75d	0.488	0.023	0.207	0.021	0.305	0.024
195	USNMNH24356	Mf	juv.f	75c	0.461	0.039	0.121	0.021	0.419	0.039
196	USNMNH44517	Mf		76c	0.414	0.053	0.166	0.017	0.420	0.059
197	USNMNH44554	Mf		75k	0.523	0.054	0.140	0.025	0.336	0.058
198	USNMNH48342	Mf	f	75j	0.505	0.114	0.130	0.028	0.388	0.093
199	USNMNH48344	Mf		75j	0.521	0.038	0.115	0.020	0.364	0.047
200	USNMNH69672	Mf	f	75c	0.474	0.047	0.140	0.017	0.386	0.045
201	USNMNH75240	Mf	m	75c	0.495	0.024	0.118	0.013	0.387	0.028
202	USNMNH81100	Mf		75c	0.403	0.030	0.119	0.016	0.478	0.031
203	USNMNH81985	Mf		75c	0.501	0.064	0.122	0.018	0.377	0.068
204	USNMNH130154	Mf		75f	0.468	0.038	0.150	0.016	0.382	0.041
205	USNMNH130156	Mf		75g	0.509	0.047	0.115	0.028	0.377	0.062
206	USNMNH210105	Mf	m	75e	0.516	0.030	0.132	0.014	0.352	0.034
207	USNMNH260848	Mf	m	75d	0.437	0.040	0.132	0.019	0.431	0.042
208	USNMNH307424	Mf	m	75d	0.484	0.043	0.126	0.015	0.390	0.037
209	USNMNH310885	Mf	m	75f	0.447	0.032	0.140	0.011	0.412	0.039
224	USNMNH142094	Mf		76a	0.472	0.060	0.112	0.022	0.415	0.065
234	UMMZ84444	Mf		34	0.385	0.038	0.159	0.017	0.455	0.043
235	UMMZ92843	Mf		35e	0.371	0.037	0.135	0.029	0.494	0.031
236	UMMZ65627	Mf		75j	0.470	0.050	0.121	0.025	0.409	0.067
237	UMMZ94934	Mf		63h	0.465	0.051	0.145	0.023	0.390	0.069
238	UMMZ178030	Mf		63h	0.381	0.042	0.134	0.015	0.485	0.044
239	MCZ131avg	Mf		75a	0.575	0.043	0.125	0.020	0.300	0.046
240	MCZ132	Mf		75j	0.421	0.043	0.083	0.020	0.496	0.049
241	MCZ181avg	Mf		75i	0.621	0.045	0.088	0.017	0.291	0.044
242	MCZ6660	Mf		75d	0.454	0.031	0.121	0.021	0.425	0.038
243	MCZ6703	Mf		75b	0.498	0.019	0.142	0.010	0.360	0.019
245	MCZ6834	Mf	f	75d	0.455	0.056	0.103	0.018	0.442	0.067
246	MCZ46748	Mf	f	76c	0.437	0.025	0.125	0.023	0.438	0.040
247	MCZ53948	Mf		75d	0.467	0.029	0.178	0.020	0.356	0.043
248	MCZ54018	Mf	f	75b	0.479	0.038	0.155	0.018	0.366	0.043
249	MCZ121235	Mf	f	76c	0.445	0.040	0.138	0.019	0.416	0.050
253	MCZ166907	Mf	m	75c	0.414	0.035	0.134	0.033	0.452	0.064
256	MCZ170335	Mf	f	75c	0.502	0.059	0.129	0.023	0.370	0.068
258	UGMNH06557	Mf	f	65g	0.501	0.046	0.128	0.030	0.371	0.055
264	UGMNH06554	Mf		75f	0.509	0.052	0.092	0.020	0.399	0.063
265	UGMNH6550	Mf	f	65f	0.464	0.105	0.101	0.024	0.435	0.125
267	UGMNH06556	Mf	f	65g	0.485	0.037	0.101	0.016	0.414	0.043
271	UGMNH37090	Mf		75f	0.475	0.030	0.116	0.021	0.409	0.042

Appendix 4. Haplotype designations.

Spec. #	Spp	Inst	Lat	Lon	Hap L595	Hap L244	Hap M101
39	Lt	MF39	29.51	82.27	1	1	
43	Lt	AMNH106135	33.34	81.75	2	2	
45	Lt	AMNH106137	33.34	81.75	2	2	
46	Lt	AMNH106138	33.34	81.75	2	2	
48	Lt	AMNH106140	33.39	81.62	2	2	
49	Lt	AMNH106141	33.34	81.75	2	2	
50	Lt	AMNH106142	33.2	81.71	2	2	

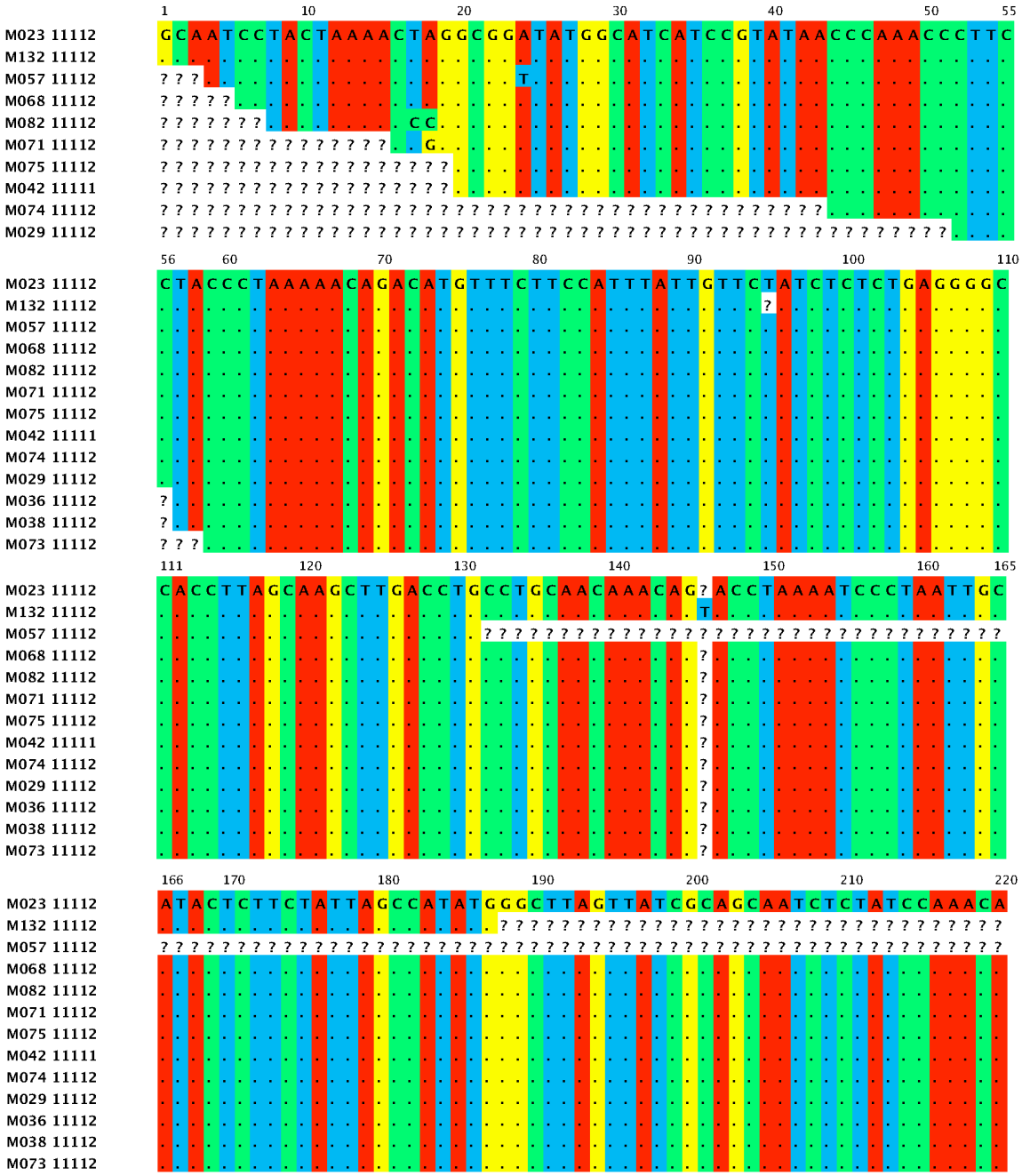
Appendix 4 (Continued)

60	Lt	UF122783	29.51	82.28	3	3	
61	Lt	UF124059	27.63	81.33	4	1	
62	Lt	UF128553	29.22	82.76	5	4	
63	Lt	UF128357	30.23	84.89	6	5	
65	Lt	UF131771	28.34	81.8	7	1	
104	Lt	AUM4250	30.48	87.49		5	
106	Lt	AUM2050	32.68	85.91		1	
108	Lt	AUM06100	33.32	86.95		6	
109	Lt	AUM11517	32.06	83.31		1	
135	Lt	FMNH8092	30.73	84.13		1	
136	Lt	FMNH2017	30.31	89.33		11	
137	Lt	FMNH944	30.7	88.2		7	
138	Lt	FMNH95181	30.4	89.1		1	
145	Lt	FMNH194566	32.37	88.54		8	
147	Lt	FMNH240471				1	
150	Lt	Lt150	30.02	84.98		5	
152	Lt	CMNH2086	29.2	82.1		1	
155	Lt	CMNH7249	30.31	89.33		3	
159	Lt	CMNH15096	35.44	76.17		1	
160	Lt	CMNH19806	30.7	87.7		5	
162	Lt	CMNH27787	30.77	82.01		1	
164	Lt	CMNH66591	32.43	81.01		9	
165	Lt	CMNH72027	31.16	88.56		2	
166	Lt	CMNH91822	30.78	90.84		1	
170	Lt	CMNH91986	30.8	90.84		1	
172	Lt	CMNH116867	32.07	81.36		5	
186	Lt	USNMNH204238	25.14	80.93		1	
215	Lt	USNMNH26303	25.83	80.19		1	
218	Lt	USNMNH30945	25.83	80.19		1	
220	Lt	USNMNH38160	25.83	80.19		5	
221	Lt	USNMNH42127	28.13	80.63		1	
231	Lt	UMMZ86697	33.19	90.4		10	
257	Lt	MCZ177904	32.29	81.08		5	
259	Lt	UGMNH6092	30.72	81.55		12	
260	Lt	UGMNH19562	30.72	82.16		13	
266	Lt	UGMNH45917	31.78	90.45		5	
272	Lt	UGMNH19356	30.85	82.67		14	
3	Mf						3
14	Mf	CAS178659	28.51	82.17			2
23	Mf	CAS208638	28.75	82.3			2
26	Mf	MF26	26.79	80.29			2
29	Mf	MF29	26.45	81.41			5
36	Mf	AMNH149969	28.84	82.69			2
38	Mf	MF38	26.8	80.07			2
42	Mf	AMNH106134	33.28	81.73			2
68	Mf	UF124690	29.03	81.31			2
71	Mf	UF118833	27.59	81.39			2
74	Mf	UF129770	29.62	82.35			2
75	Mf	MF75	29.5	82.42			2
82	Mf	MF82	29.8	82.29			2
85	Mf	MF85	30.25	82.52			2
119	Mf	AUM31164	31.09	86.3			2
132	Mf	MF132	29.5	82.42			1
133	Mf	FMNH240508	33.04	84.68			1

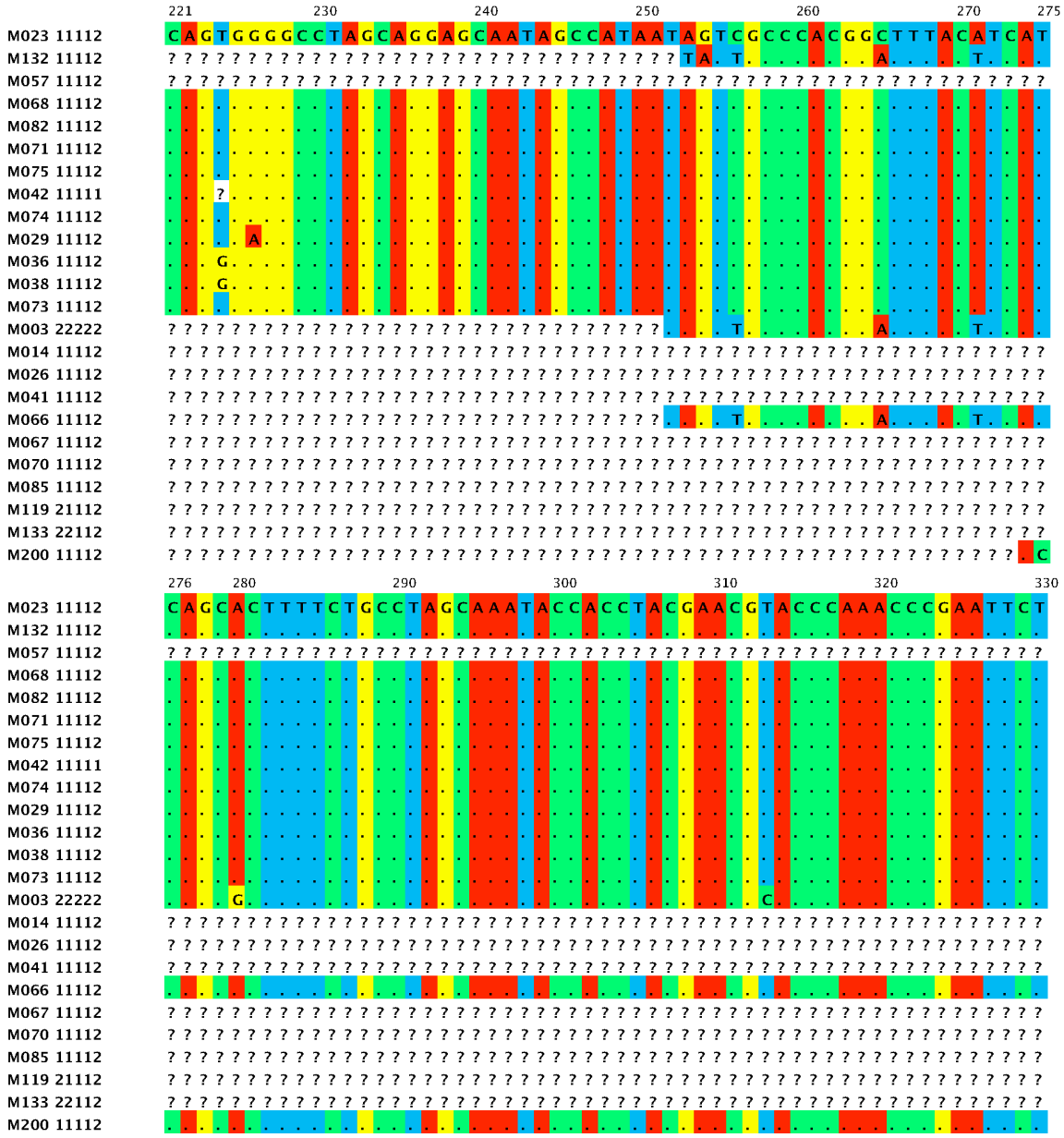
Appendix 4 (Continued)

249	Mf	MCZ121235	26.8	80.06			2
265	Mf	UGMNH6550	31.34	83.59			2

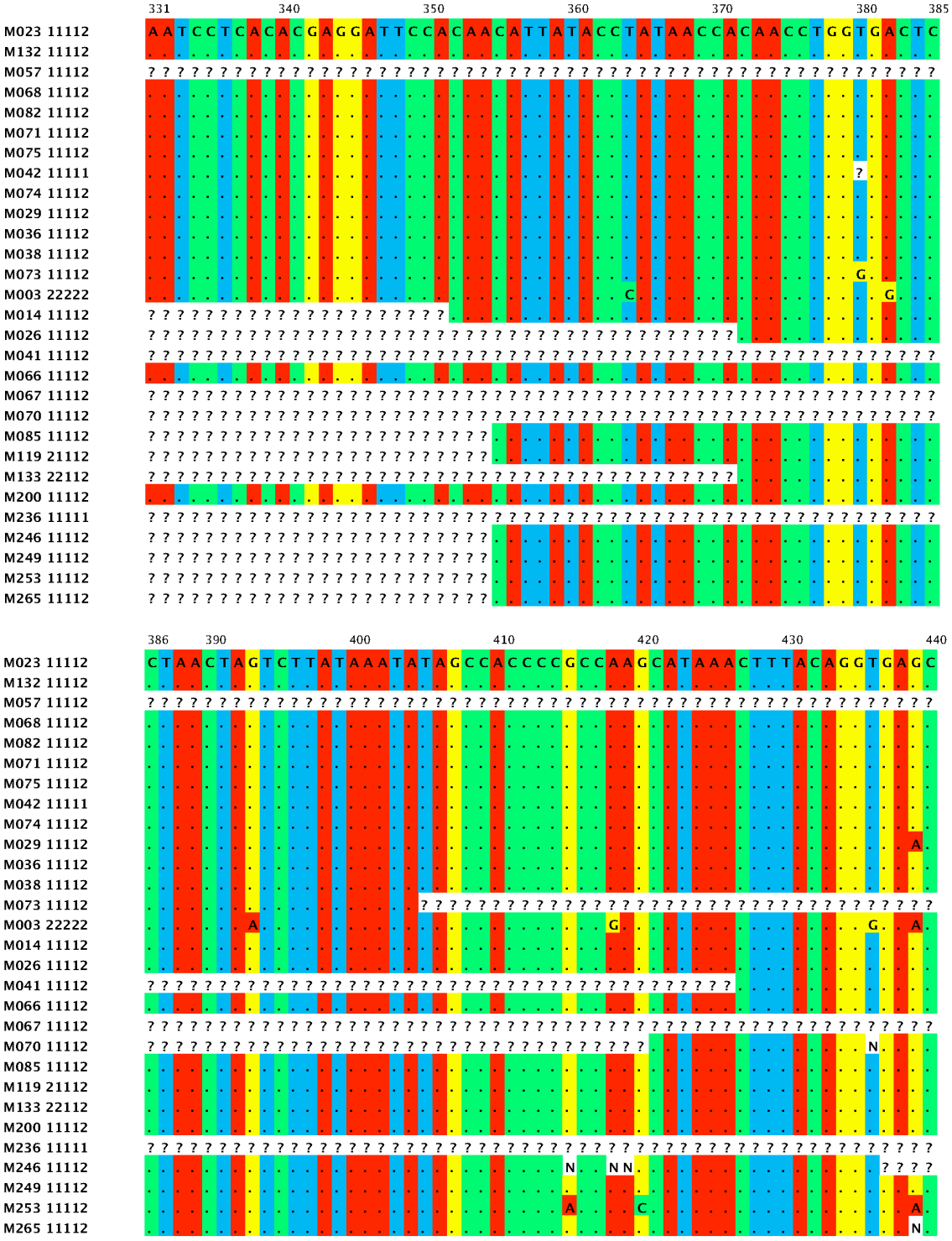
Appendix 5. *Micrurus fulvius* ND4 sequences.



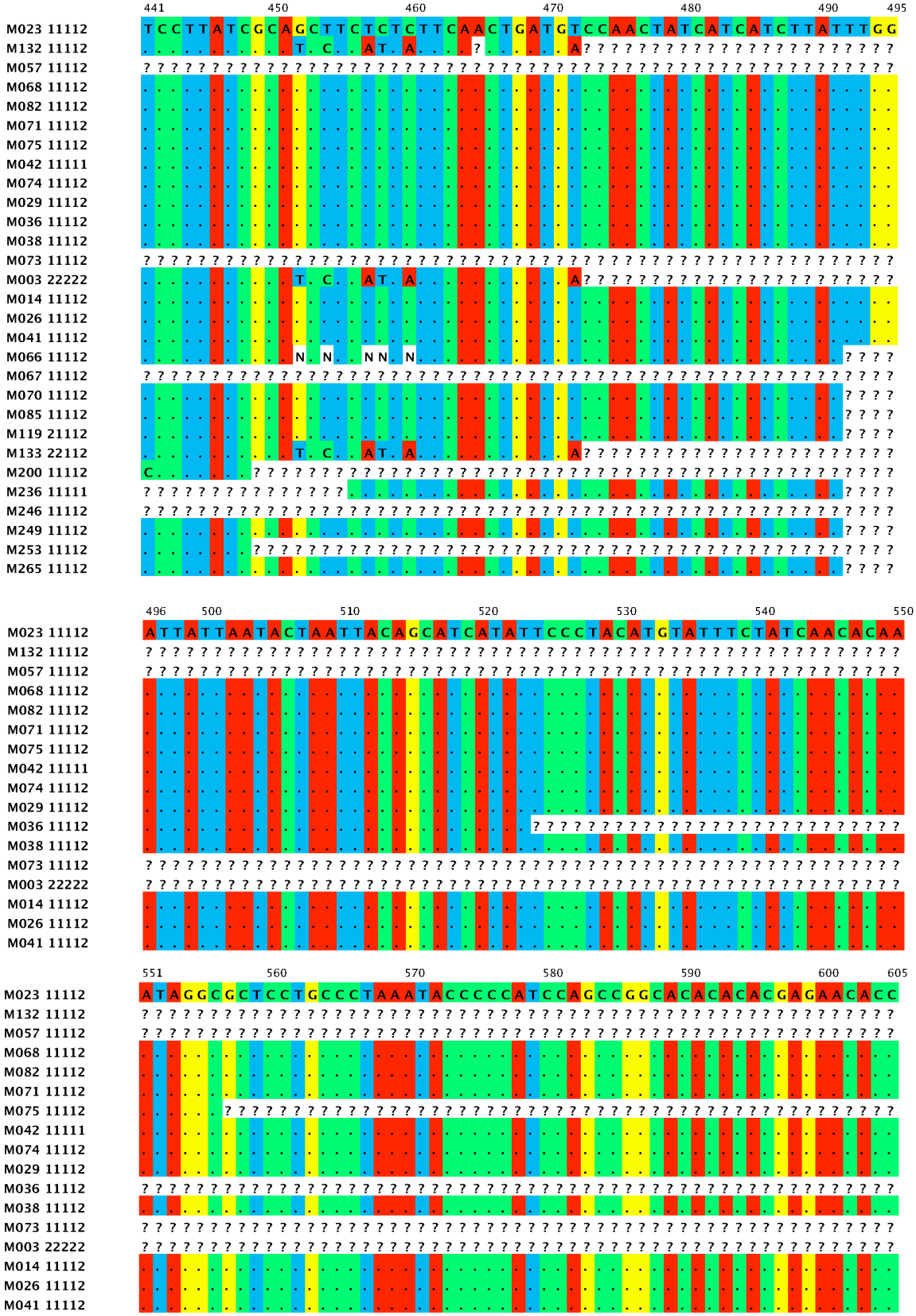
Appendix 5 (Continued)



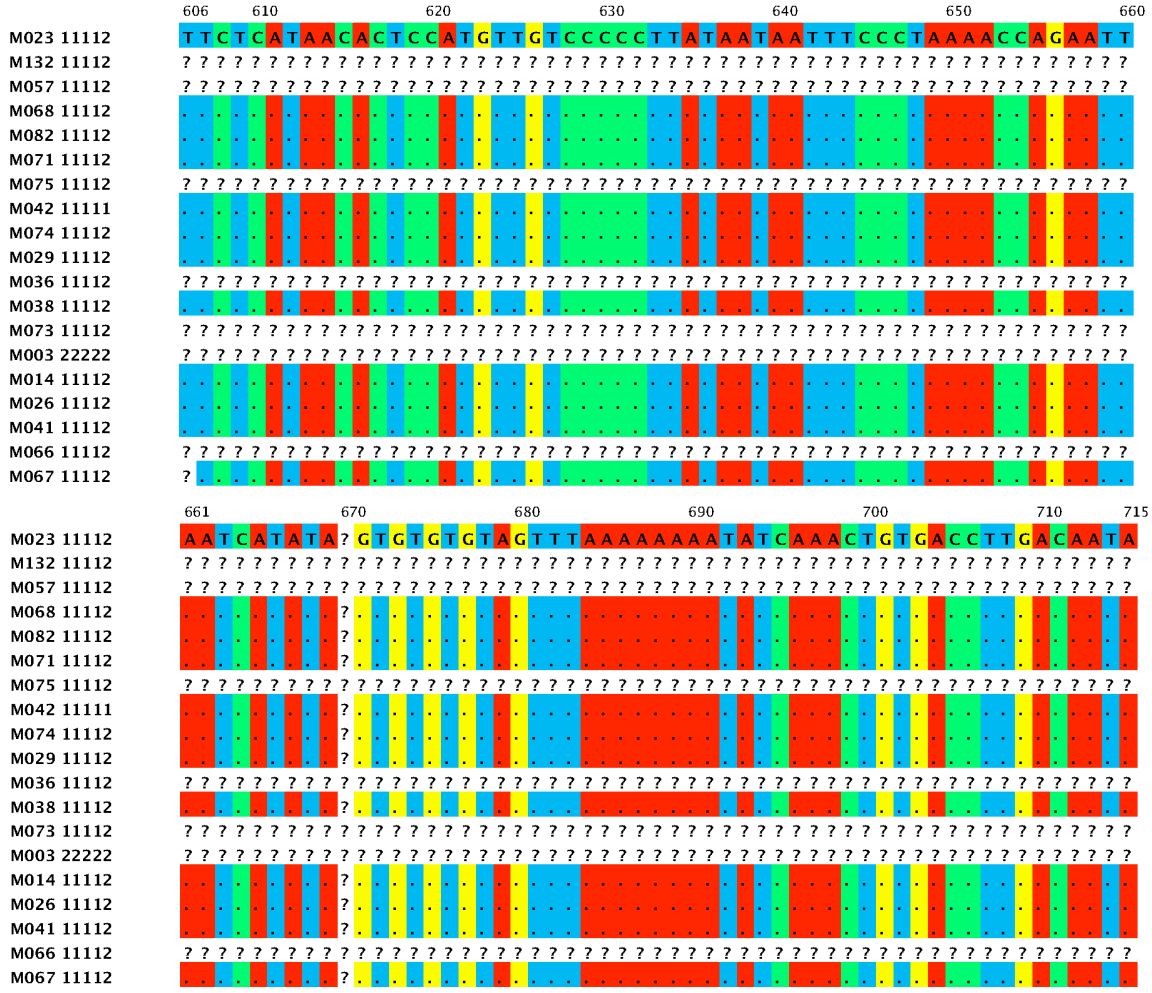
Appendix 5 (Continued)



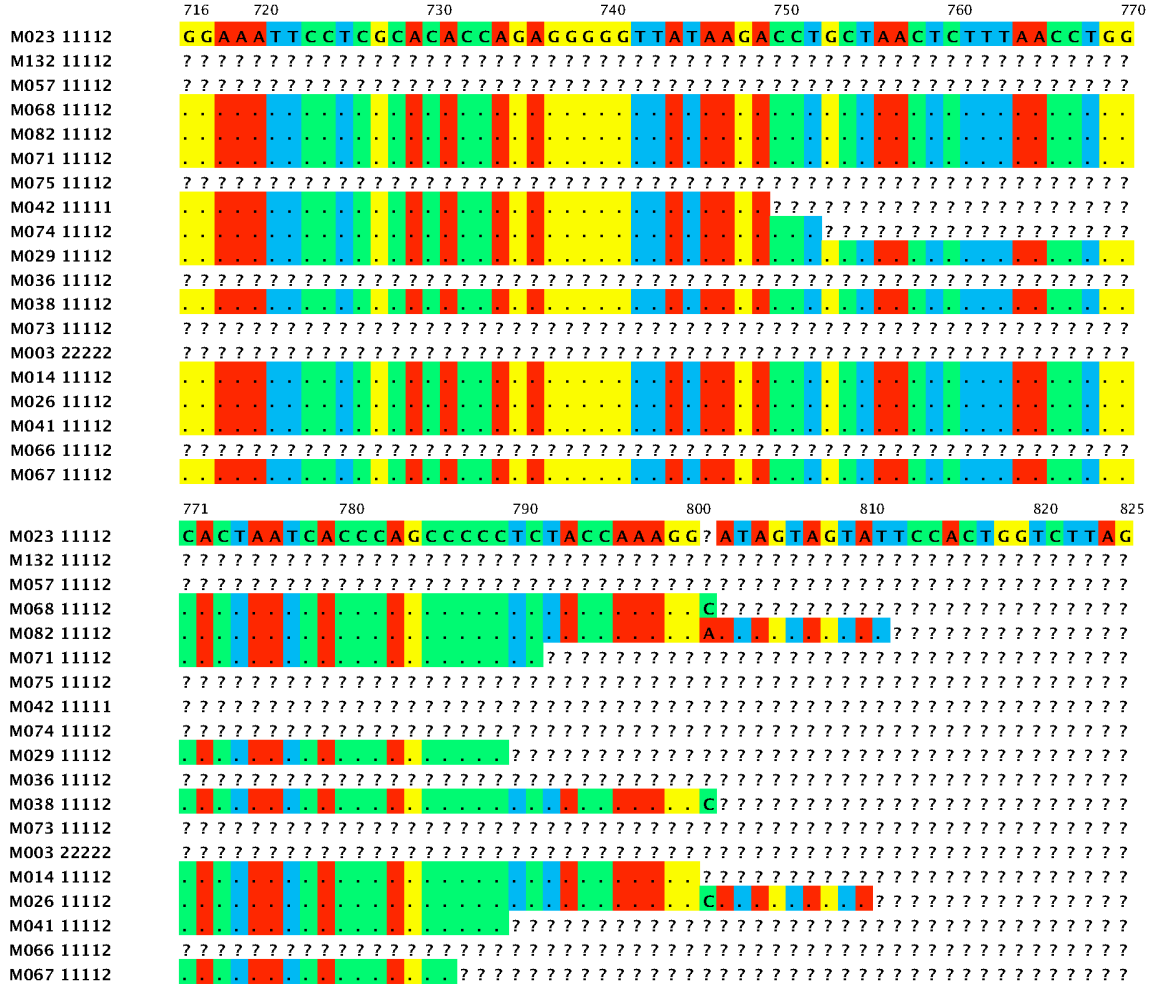
Appendix 5 (Continued)



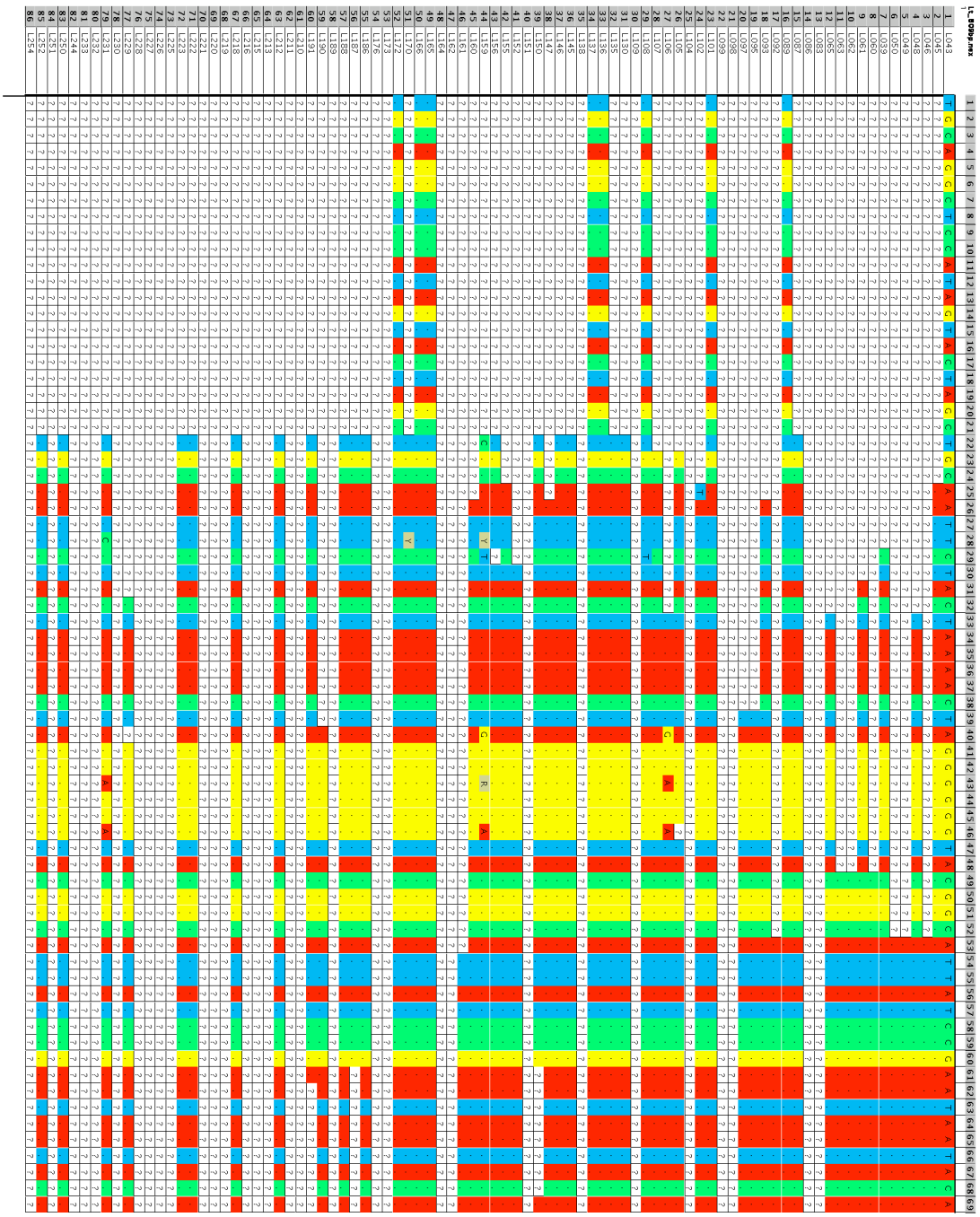
Appendix 5 (Continued)



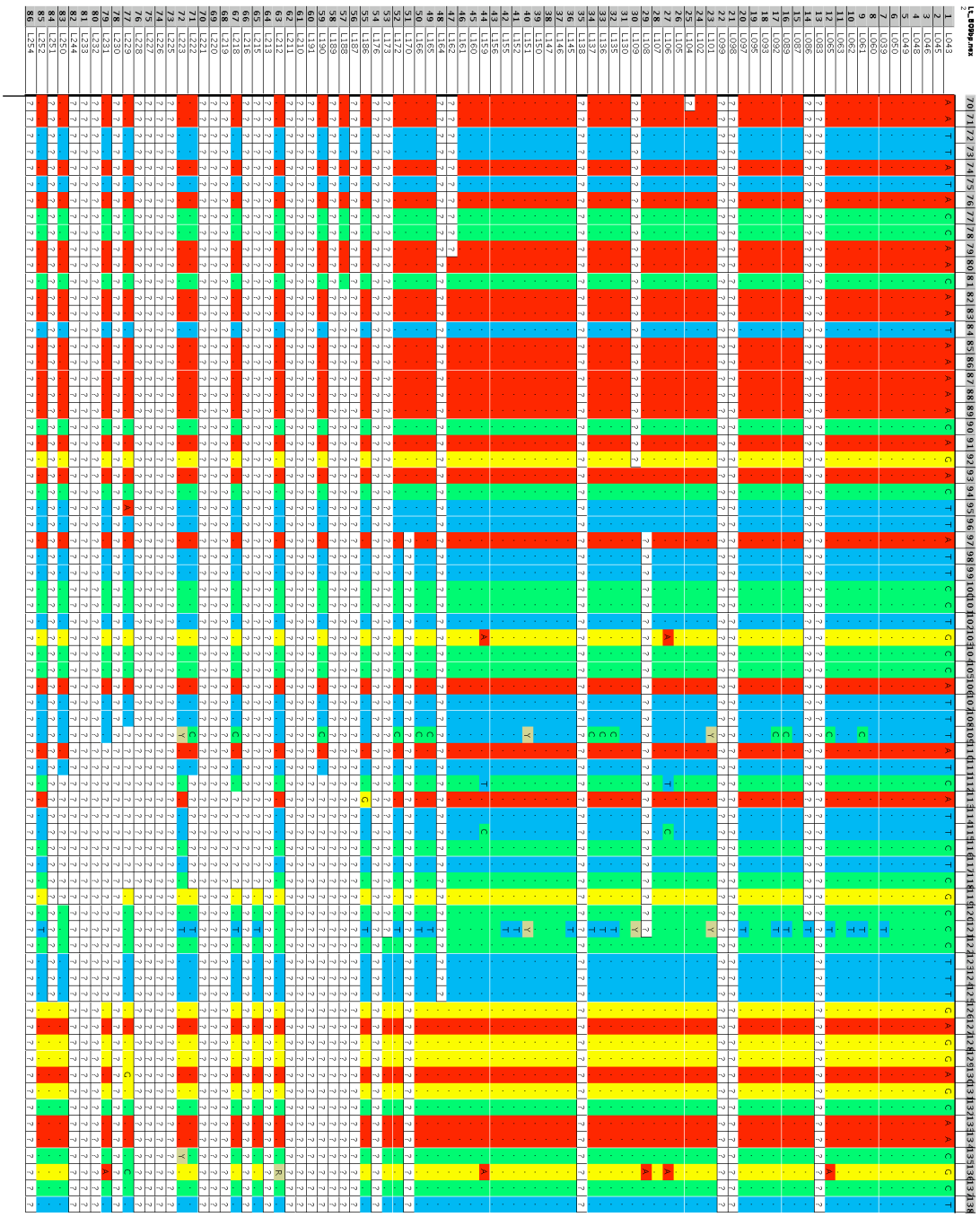
Appendix 5 (Continued)



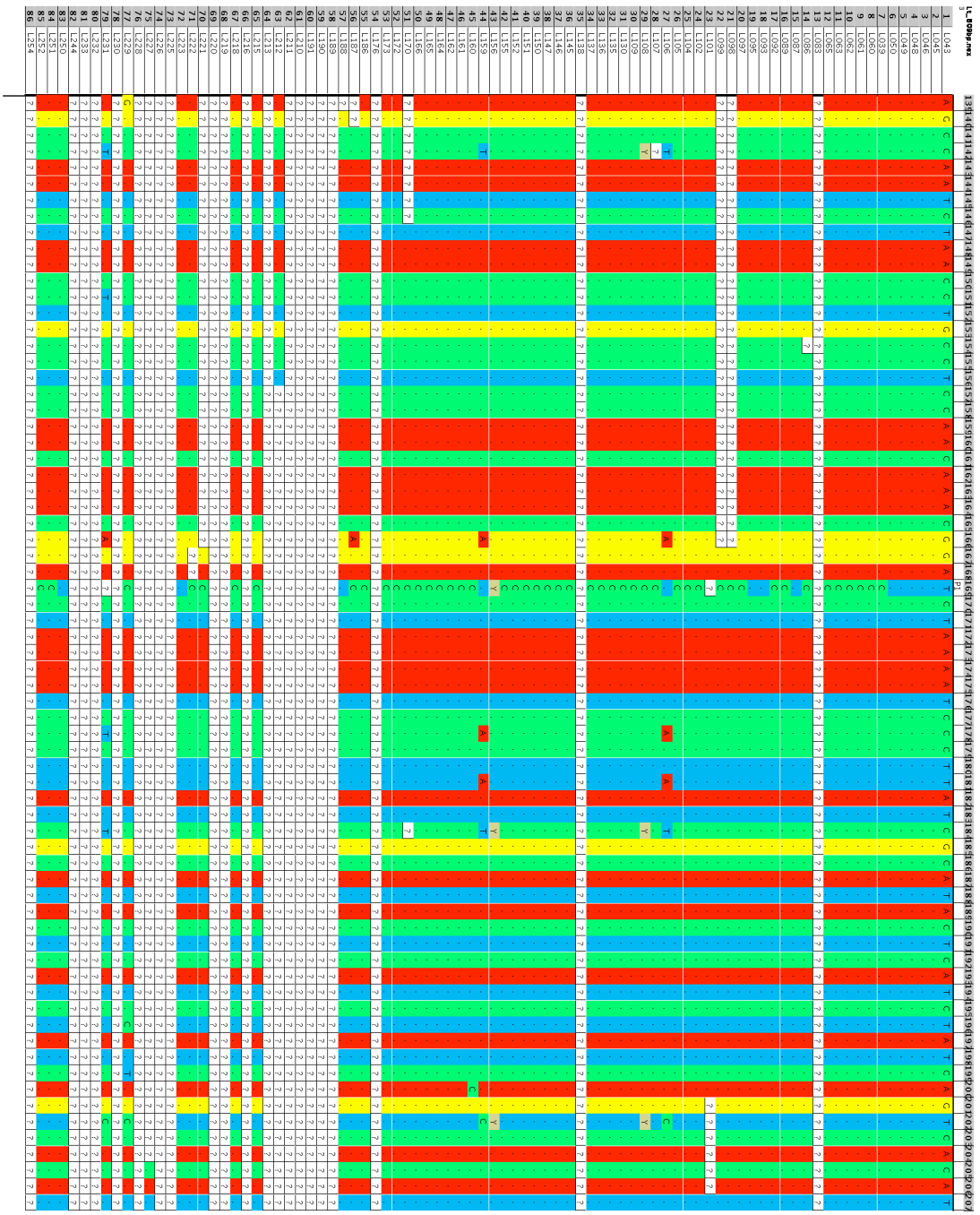
Appendix 6. *Lampropeltis triangulum* ND4 Sequences.



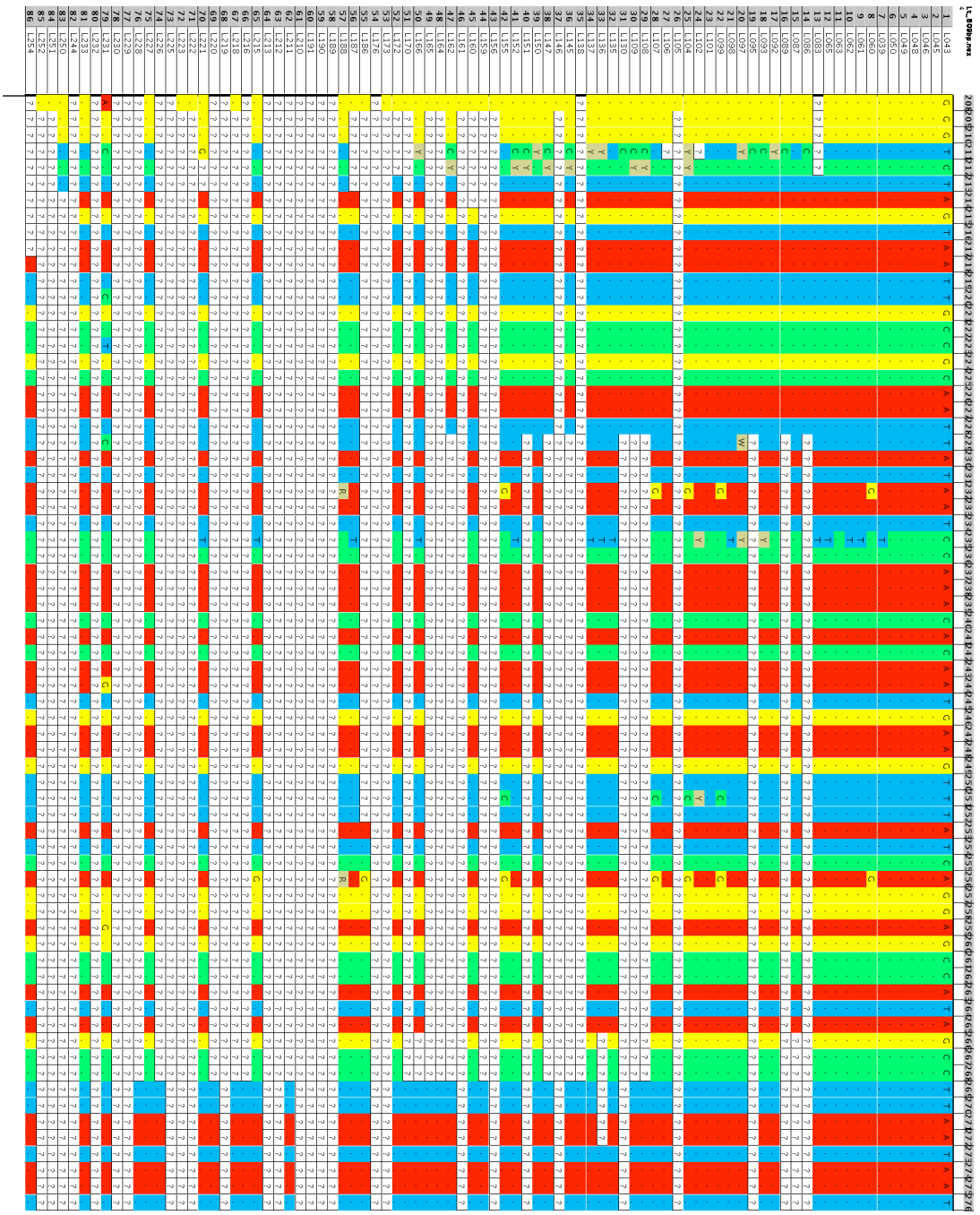
Appendix 6 (Continued)



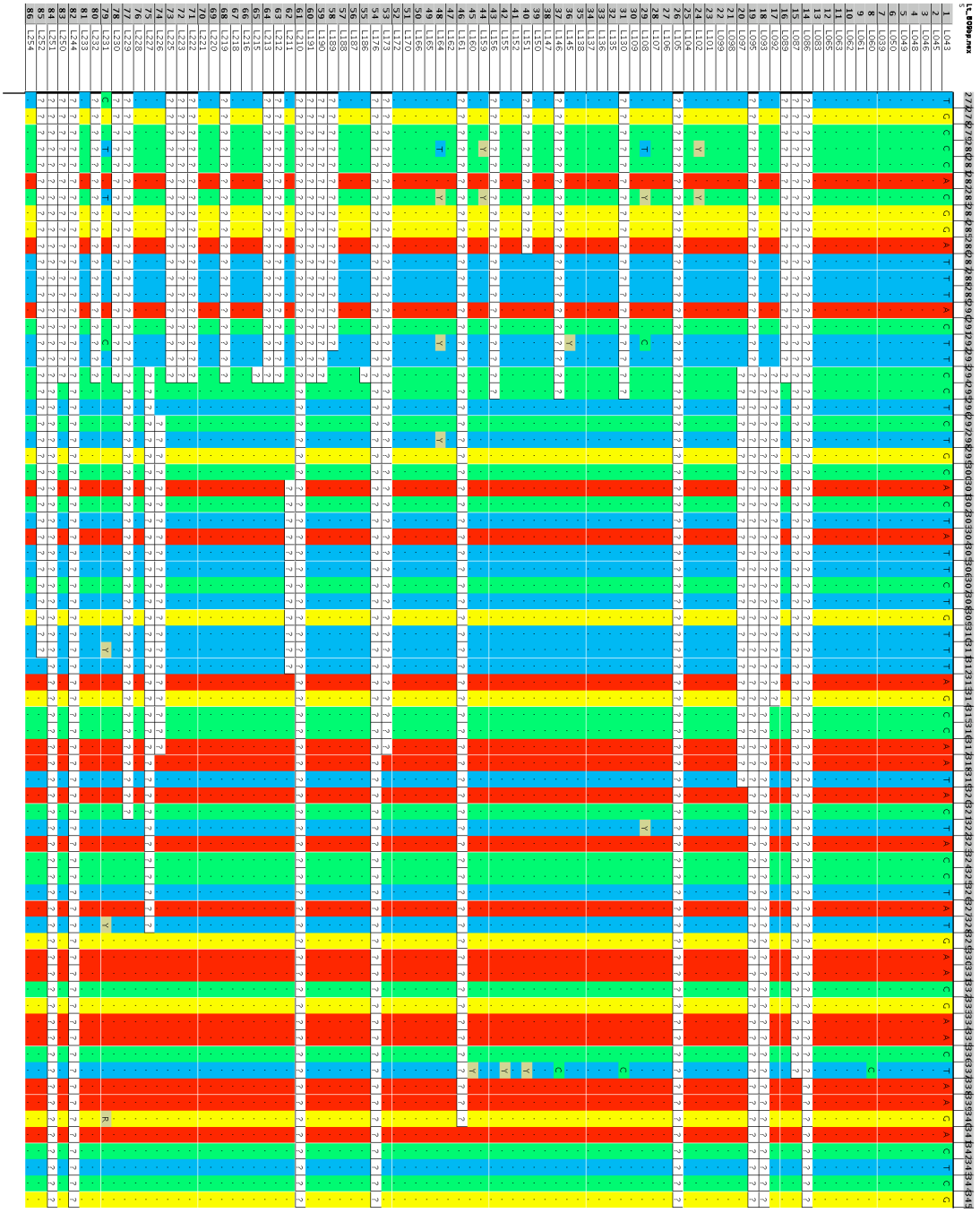
Appendix 6 (Continued)



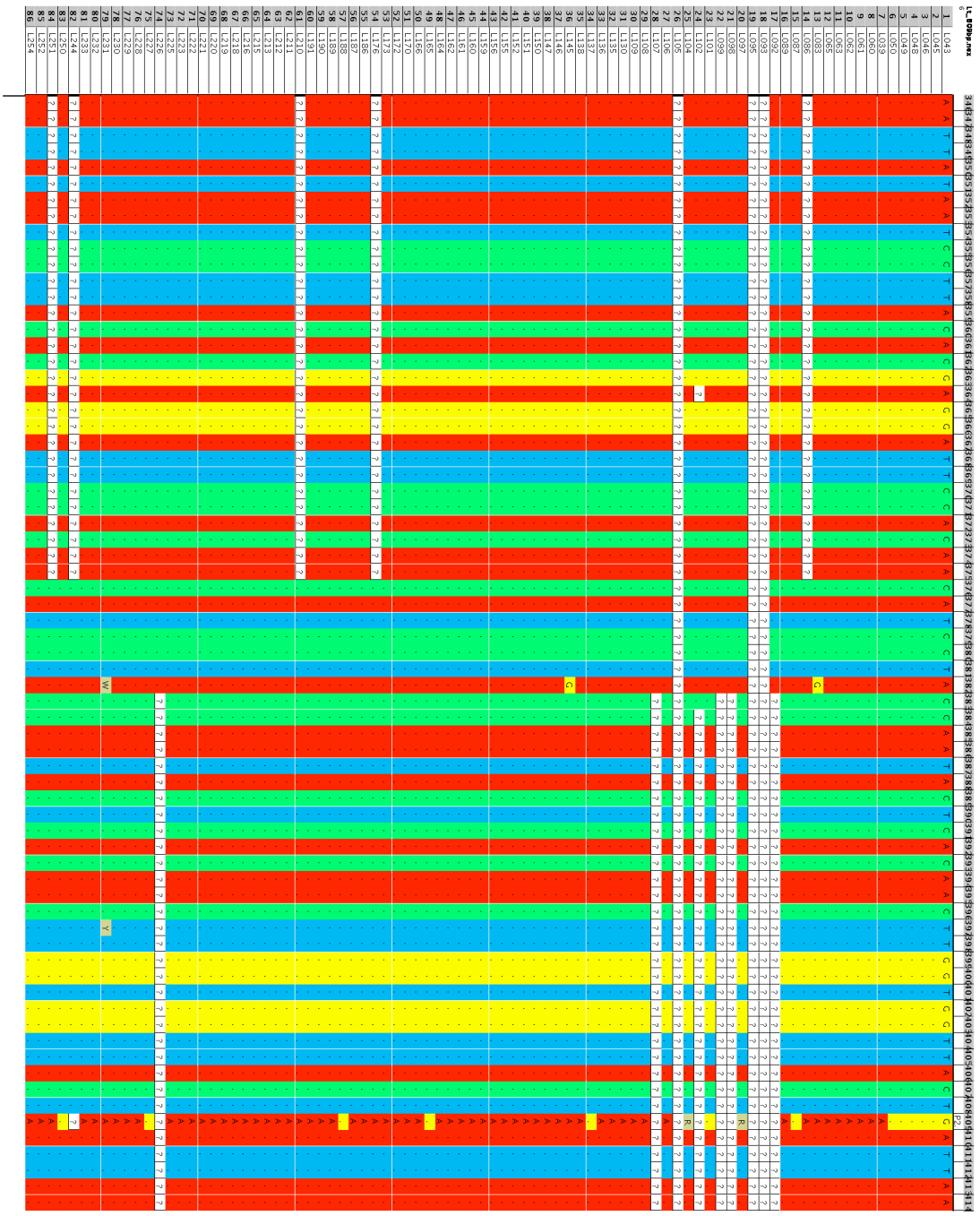
Appendix 6 (Continued)



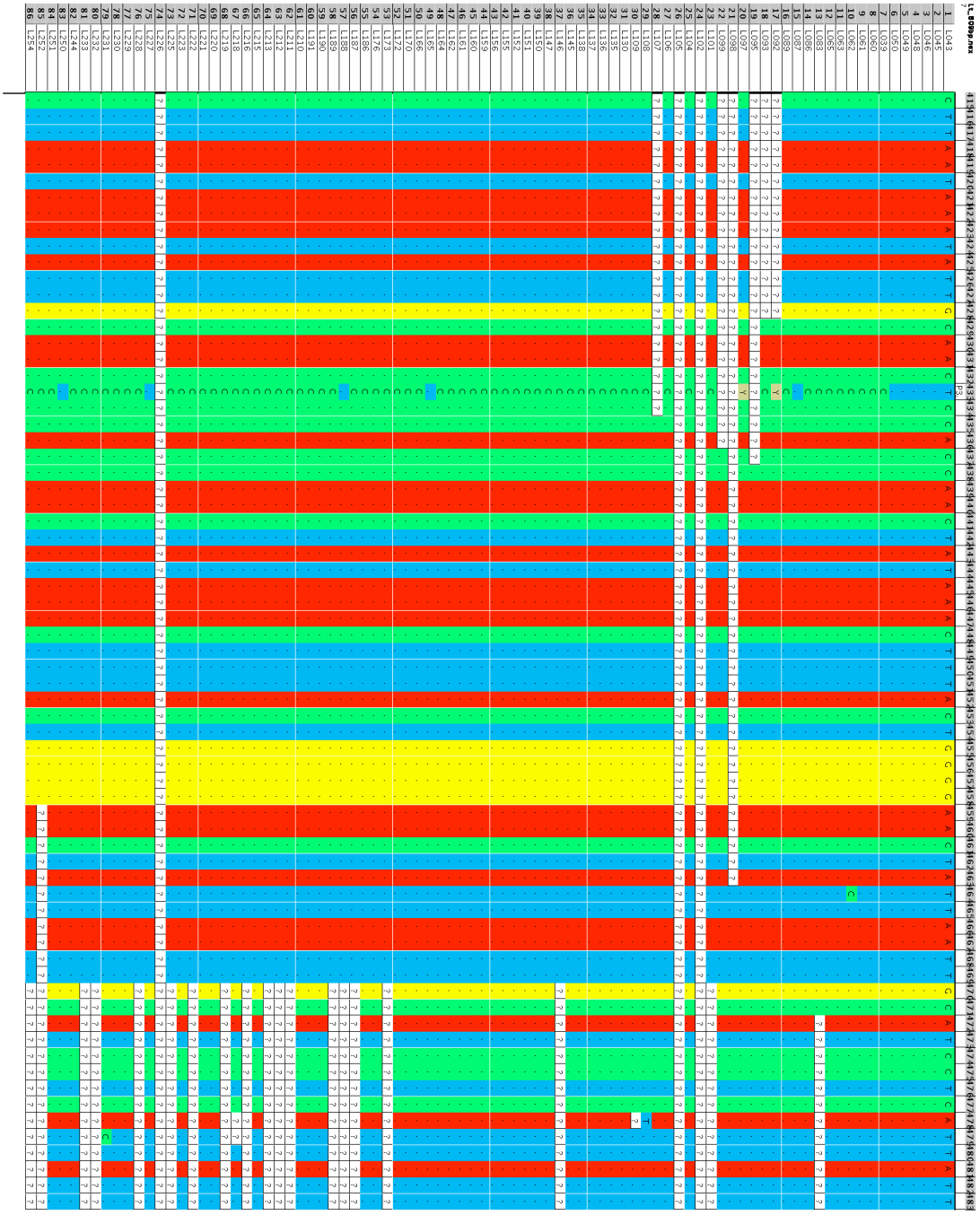
Appendix 6 (Continued)



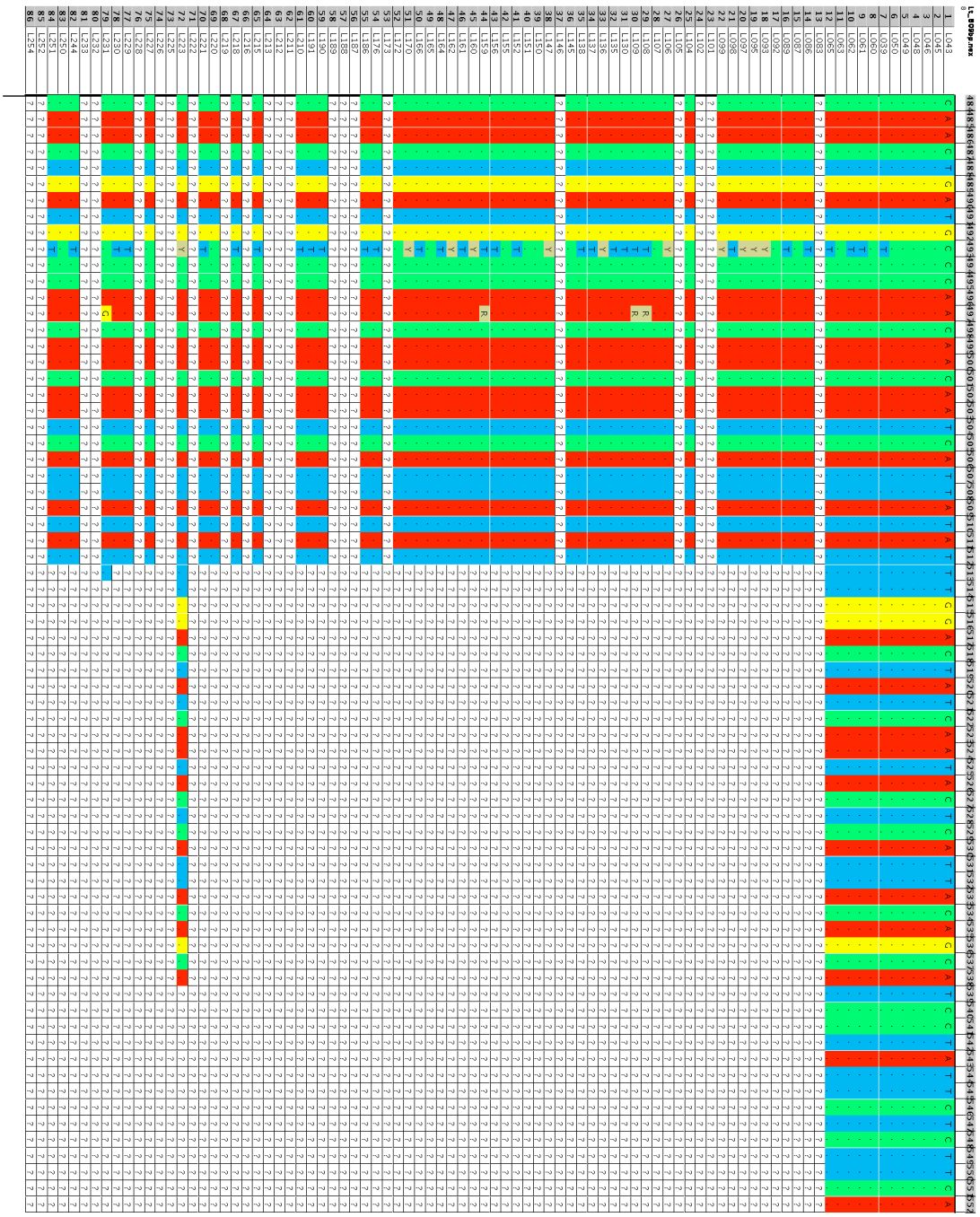
Appendix 6 (Continued)



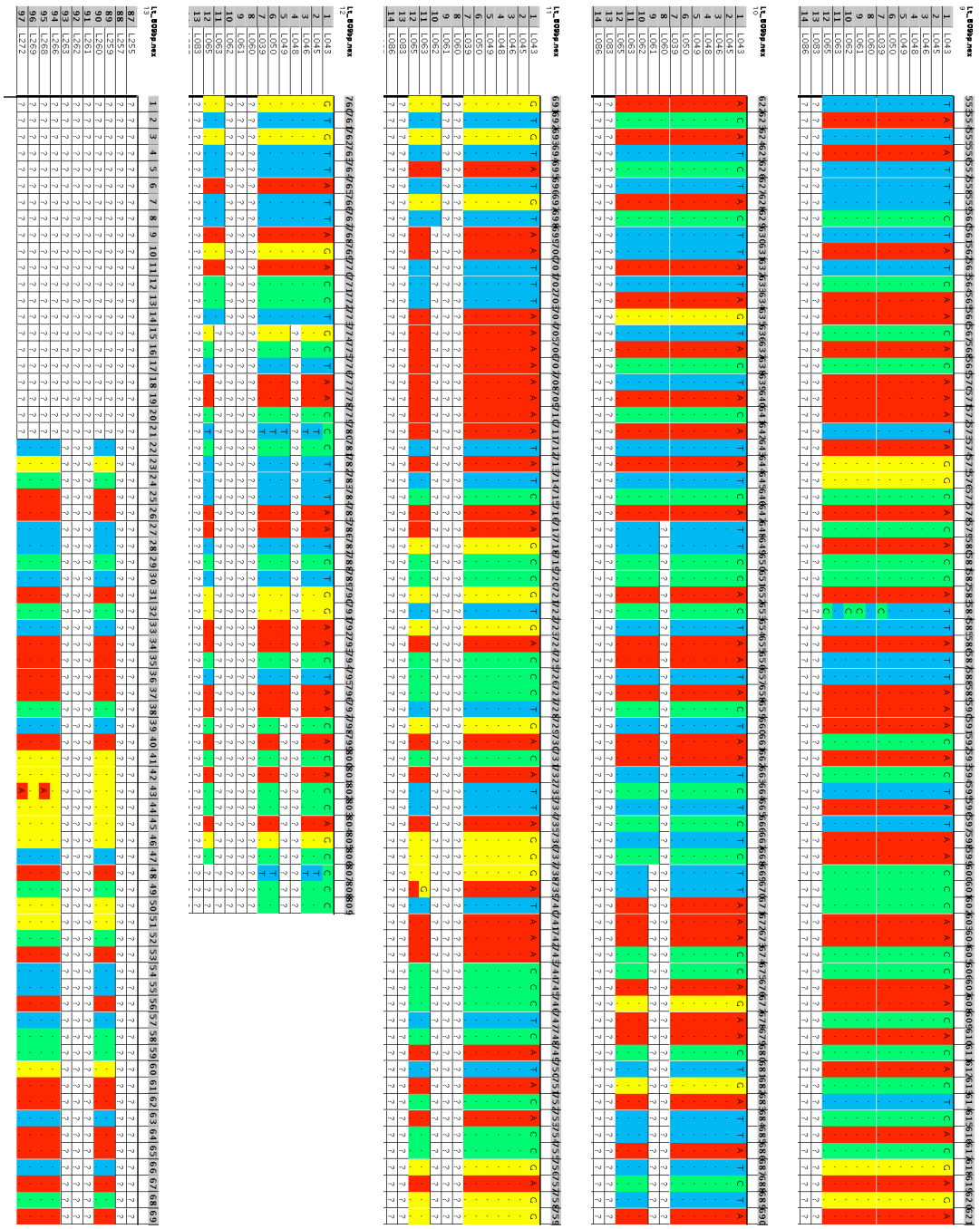
Appendix 6 (Continued)



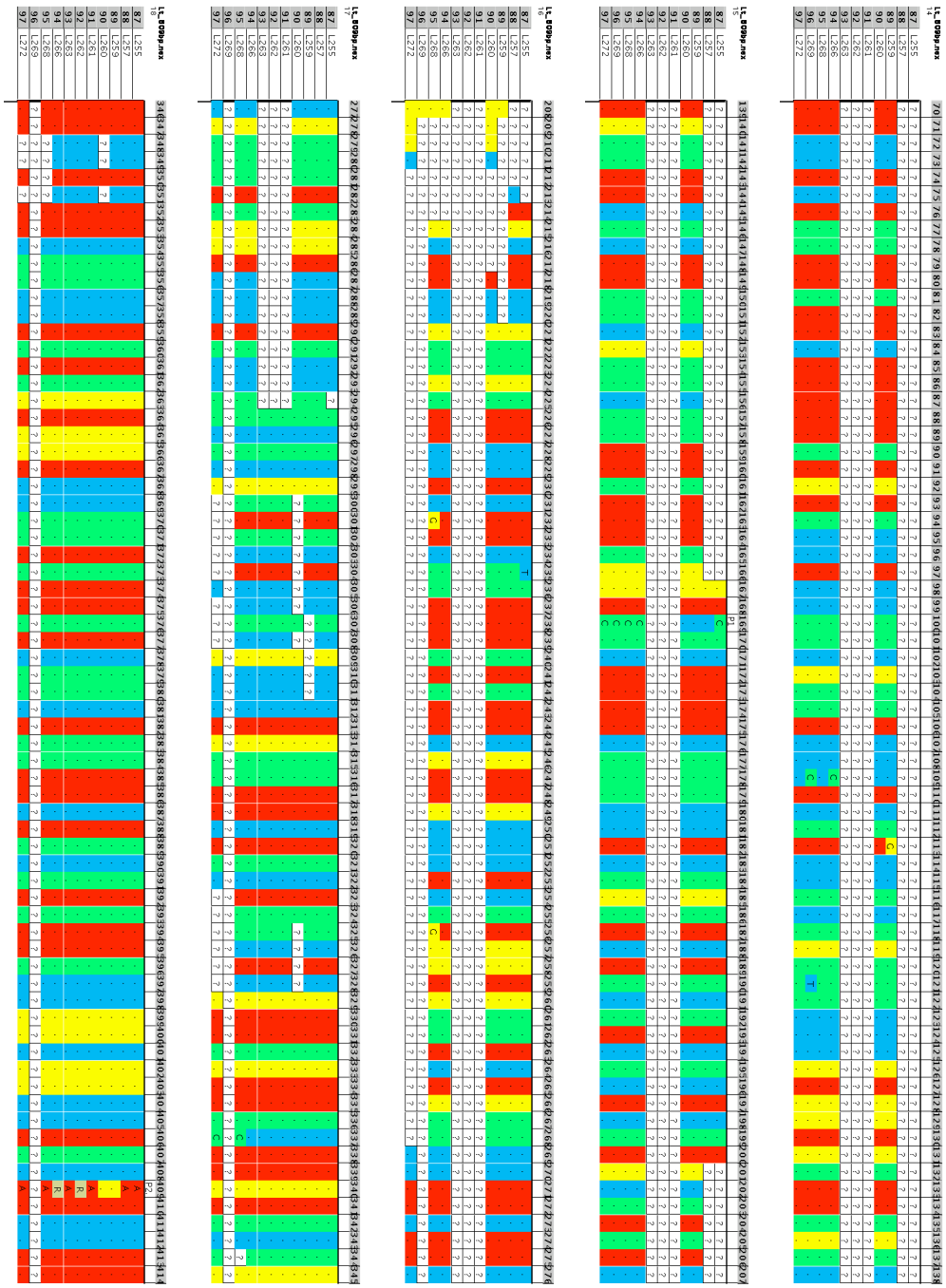
Appendix 6 (Continued)



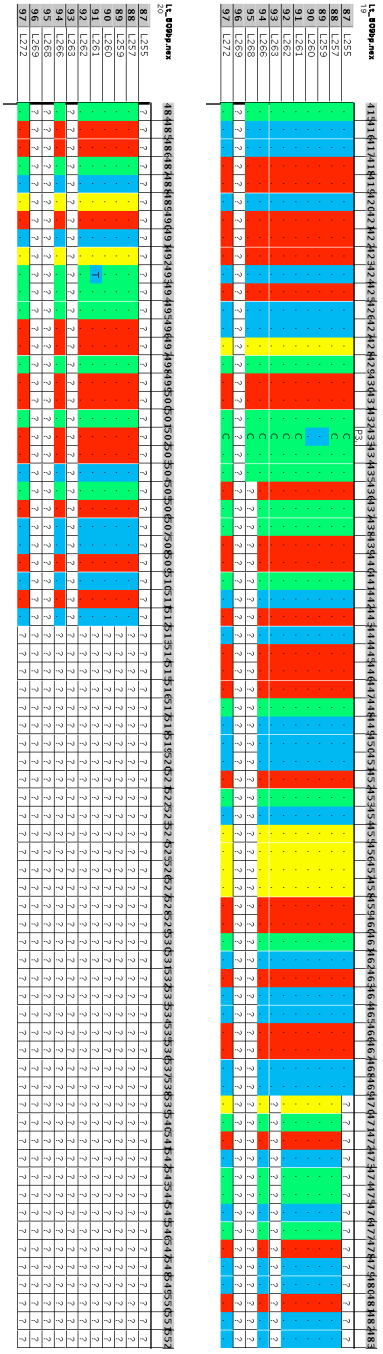
Appendix 6 (Continued)



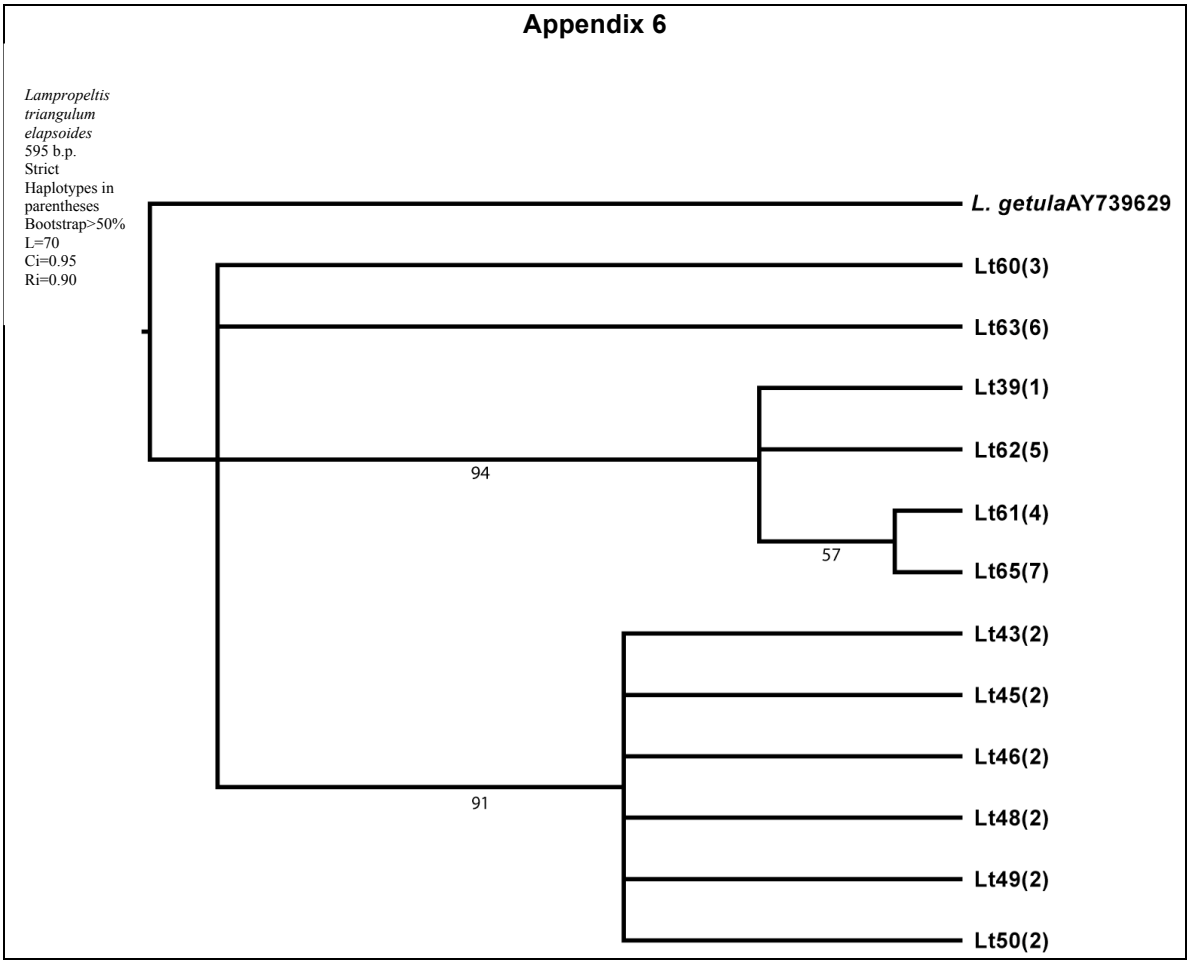
Appendix 6 (Continued)



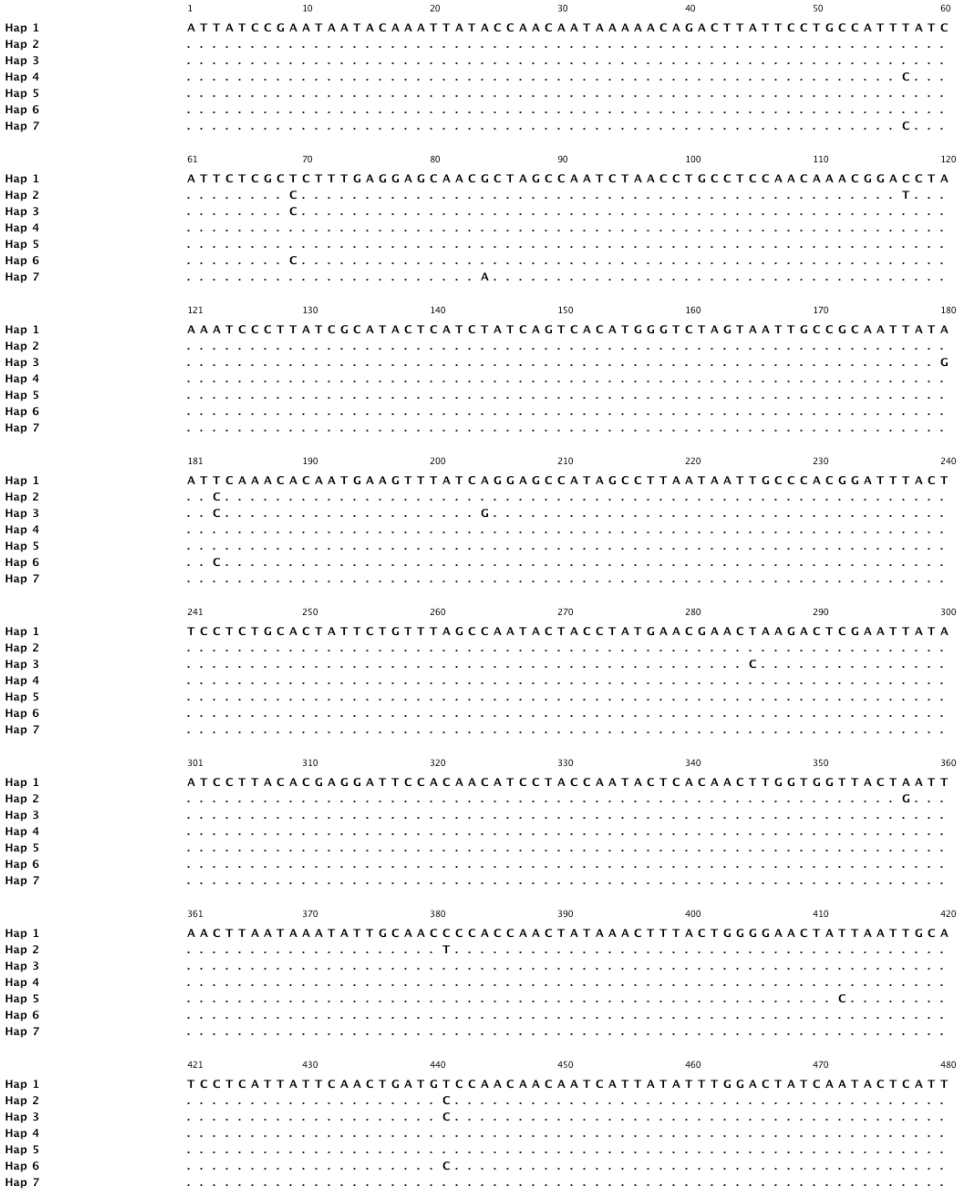
Appendix 6 (Continued)



Appendix 6

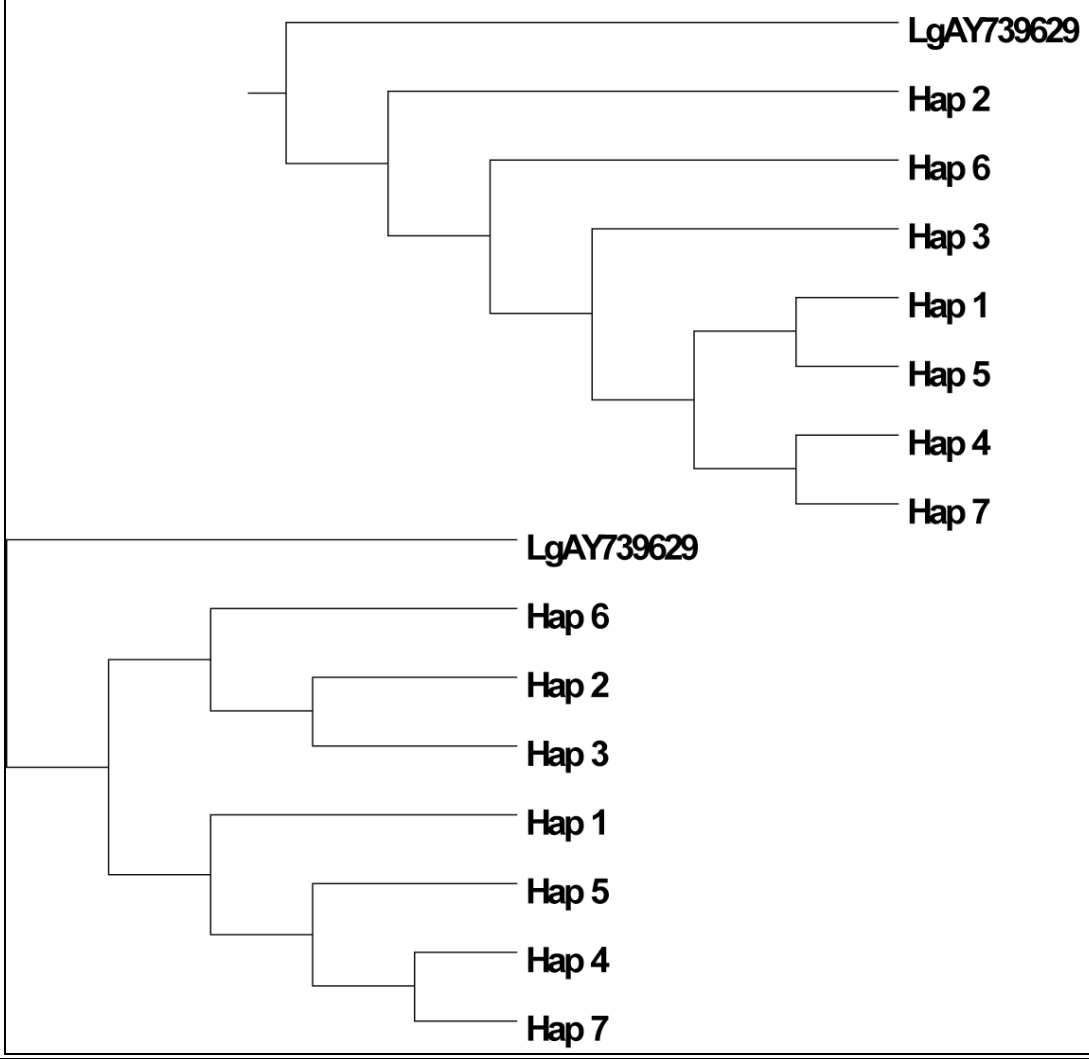

Lampropeltis triangulum elapsoides
595 b.p.
Strict
Haplotypes in parentheses
Bootstrap>50%
L=70
Ci=0.95
Ri=0.90
*L. getula*AY739629
Lt60(3)
Lt63(6)
Lt39(1)
Lt62(5)
94
Lt61(4)
57
Lt65(7)
Lt43(2)
Lt45(2)
Lt46(2)
91
Lt48(2)
Lt49(2)
Lt50(2)

Appendix 7


1 10 20 30 40 50 60
Hap 1 ATTATCCGAATAATACAAATTATACCAACAATAAAAACAGACTTATTCCTGCCATTTATC
Hap 2 . . . . . .
Hap 3 . . . . . .
Hap 4 . . . . . C . . .
Hap 5 . . . . . .
Hap 6 . . . . . .
Hap 7 . . . . . C . . .
61 70 80 90 100 110 120
Hap 1 ATTCTCGCTCTTTGAGGAGCAACGCTAGCCAATCTAACCTGCCTCCAACAAACGGACCTA
Hap 2 . . . . . C . . . . . T . . .
Hap 3 . . . . . C . . . . . .
Hap 4 . . . . . .
Hap 5 . . . . . .
Hap 6 . . . . . C . . . . . .
Hap 7 . . . . . A . . . . . .
121 130 140 150 160 170 180
Hap 1 AAATCCCTTATCGCATACTCATCTATCAGTCACATGGGTCTAGTAATTGCCGCAATTATA
Hap 2 . . . . . .
Hap 3 . . . . . G
Hap 4 . . . . . .
Hap 5 . . . . . .
Hap 6 . . . . . .
Hap 7 . . . . . .
181 190 200 210 220 230 240
Hap 1 ATTCAAACACAATGAAGTTTATCAGGAGCCATAGCCTTAATAATTGCCCACGGATTTACT
Hap 2 . . C . . . . . .
Hap 3 . . C . . . . . G . . . . . .
Hap 4 . . . . . .
Hap 5 . . . . . .
Hap 6 . . C . . . . . .
Hap 7 . . . . . .
241 250 260 270 280 290 300
Hap 1 TCCTCTGCACTATTCTGTTTAGCCAATACTACCTATGAACGAACTAAGACTCGAATTATA
Hap 2 . . . . . .
Hap 3 . . . . . C . . . . . .
Hap 4 . . . . . .
Hap 5 . . . . . .
Hap 6 . . . . . .
Hap 7 . . . . . .
301 310 320 330 340 350 360
Hap 1 ATCCTTACACGAGGATTCCACAACATCCTACCAATACTCACAACTTGGTGGTTACTAATT
Hap 2 . . . . . G . . .
Hap 3 . . . . . .
Hap 4 . . . . . .
Hap 5 . . . . . .
Hap 6 . . . . . .
Hap 7 . . . . . .
361 370 380 390 400 410 420
Hap 1 AACTTAATAAATATTGCAACCCCACCAACTATAAACTTTACTGGGGAACTATTAATTGCA
Hap 2 . . . . . T . . . . . .
Hap 3 . . . . . .
Hap 4 . . . . . .
Hap 5 . . . . . C . . . . . .
Hap 6 . . . . . .
Hap 7 . . . . . .
421 430 440 450 460 470 480
Hap 1 TCCTCATTATTCAACTGATGTCCAACAACAATCATTATATTTGGACTATCAATACTCATT
Hap 2 . . . . . C . . . . . .
Hap 3 . . . . . C . . . . . .
Hap 4 . . . . . .
Hap 5 . . . . . .
Hap 6 . . . . . C . . . . . .
Hap 7 . . . . . .

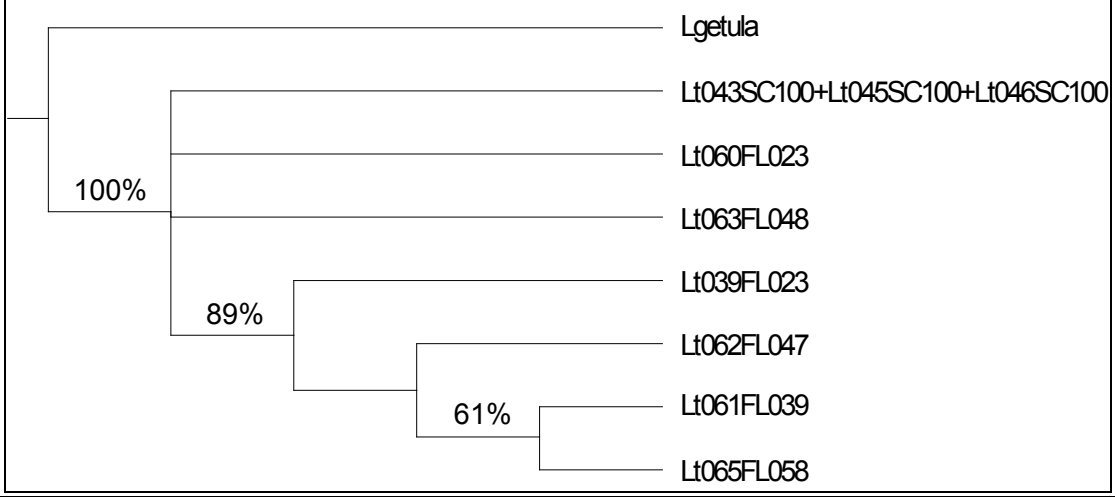
Appendix 8

Lampropeltis triangulum elapsoides
Haplotypes
2 most parsimonious trees
595 b.p.
L=70
Ci=0.95
Ri=0.78

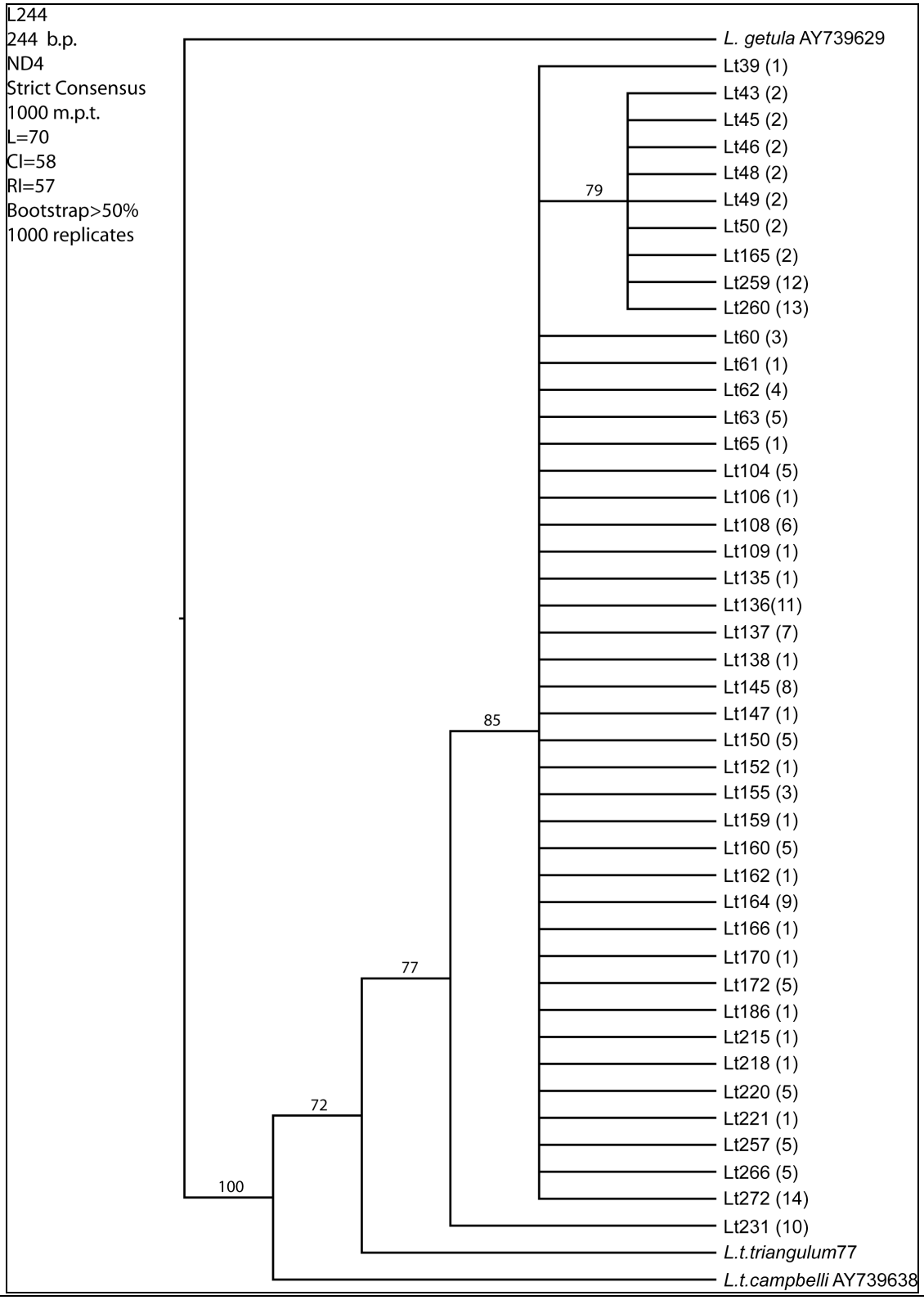


Appendix 9

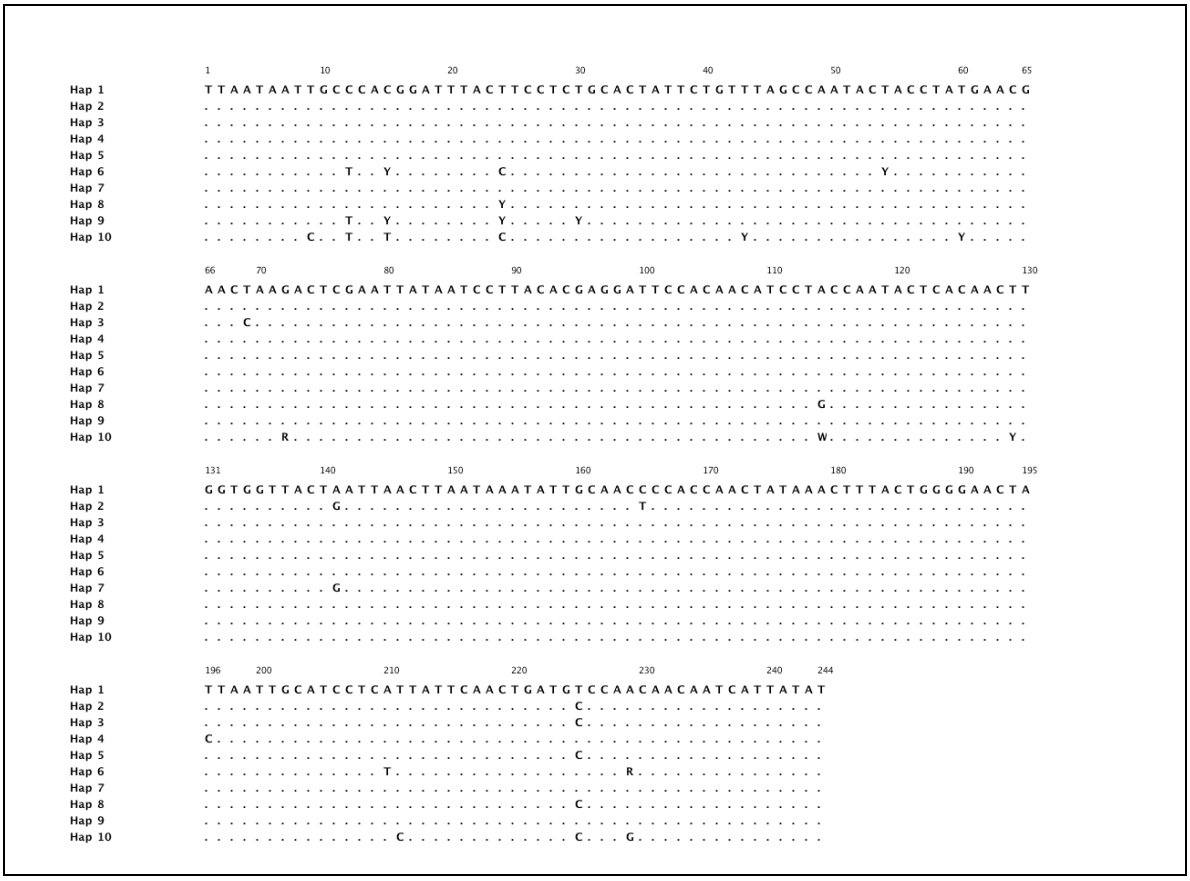
L. t. elapsoides
638 b.p.
haplotypes
Maximum Parsimony
Strict consensus
bootstrap >50% shown
L=74
CI=0.95
RI=0.78



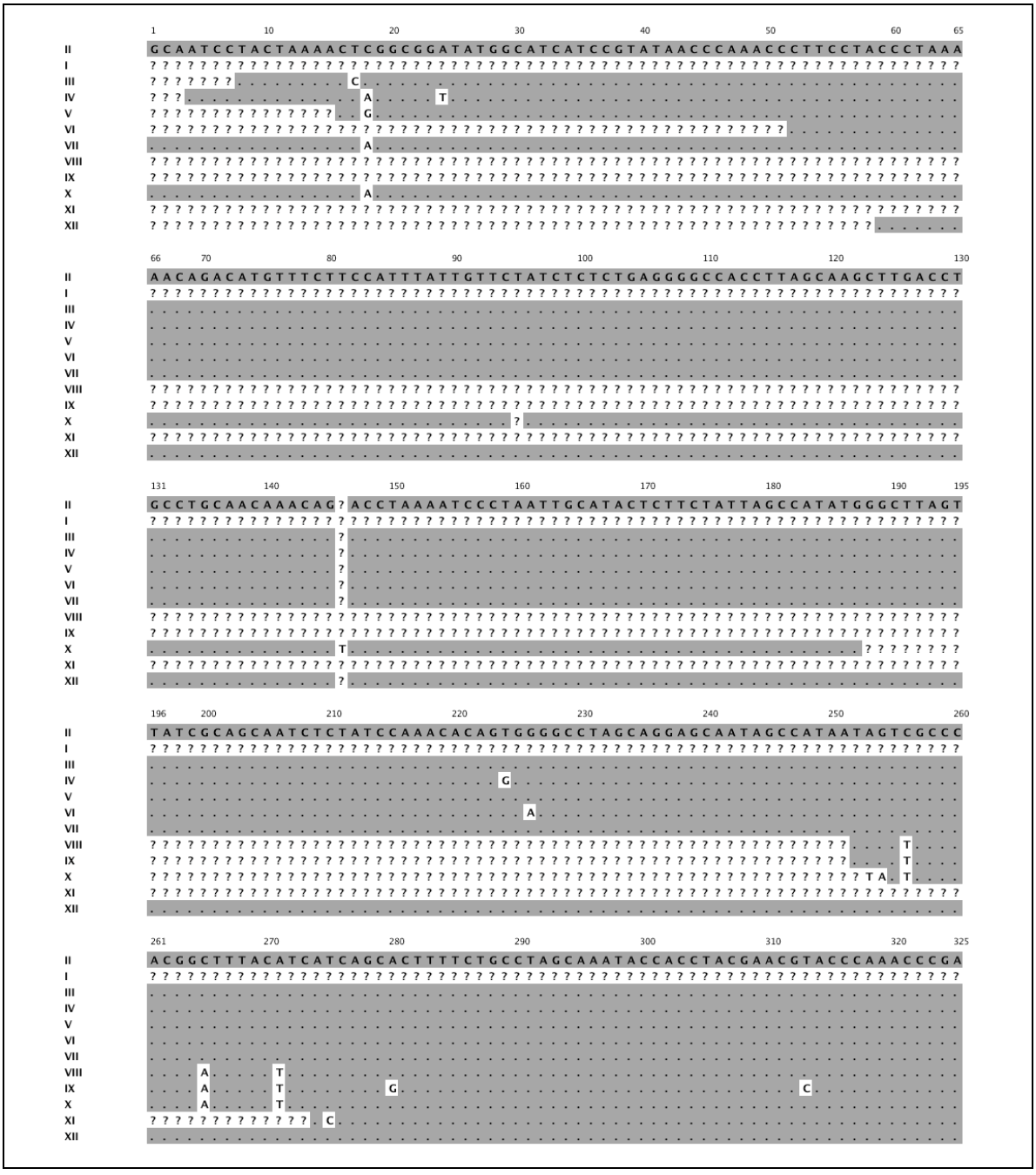
Appendix 10


L244
244 b.p.
ND4
Strict Consensus
1000 m.p.t.
L=70
CI=58
RI=57
Bootstrap>50%
1000 replicates
L. getula AY739629
Lt39 (1)
Lt43 (2)
Lt45 (2)
Lt46 (2)
Lt48 (2)
Lt49 (2)
Lt50 (2)
Lt165 (2)
Lt259 (12)
Lt260 (13)
Lt60 (3)
Lt61 (1)
Lt62 (4)
Lt63 (5)
Lt65 (1)
Lt104 (5)
Lt106 (1)
Lt108 (6)
Lt109 (1)
Lt135 (1)
Lt136(11)
Lt137 (7)
Lt138 (1)
Lt145 (8)
Lt147 (1)
Lt150 (5)
Lt152 (1)
Lt155 (3)
Lt159 (1)
Lt160 (5)
Lt162 (1)
Lt164 (9)
Lt166 (1)
Lt170 (1)
Lt172 (5)
Lt186 (1)
Lt215 (1)
Lt218 (1)
Lt220 (5)
Lt221 (1)
Lt257 (5)
Lt266 (5)
Lt272 (14)
Lt231 (10)
L.t.triangulum77
L.t.campbelli AY739638
79
85
77
72
100

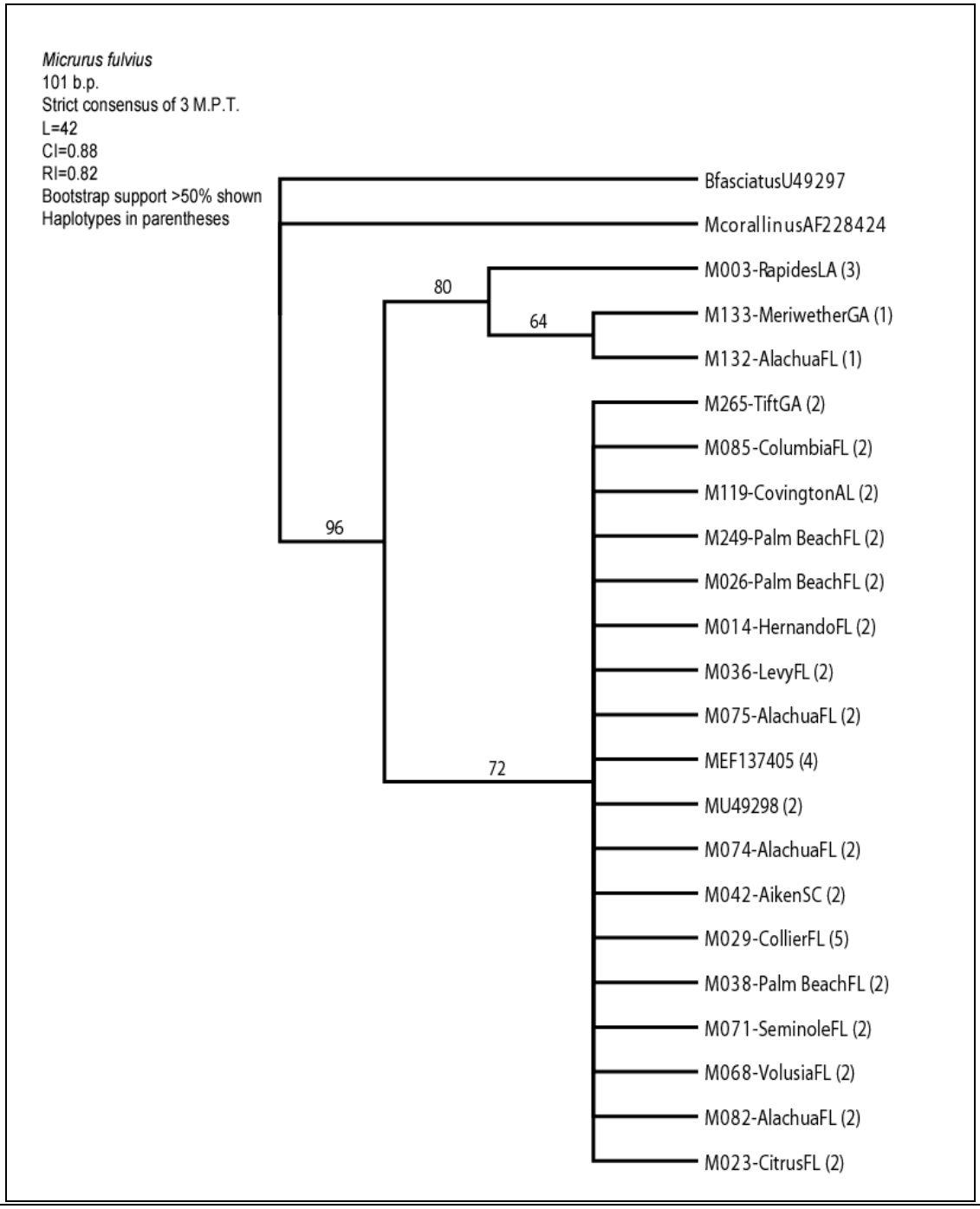
Appendix 11


1 10 20 30 40 50 60 65
Hap 1 T T A A T A A T T G C C C A C G G A T T T A C T T C C T C T G C A C T A T T C T G T T T A G C C A A T A C T A C C T A T G A A C G
Hap 2 . . . . . .
Hap 3 . . . . . .
Hap 4 . . . . . .
Hap 5 . . . . . .
Hap 6 . . . . . T . . Y . . . . . C . . . . . Y . . . . . .
Hap 7 . . . . . .
Hap 8 . . . . . Y . . . . . .
Hap 9 . . . . . T . . Y . . . . . Y . . . . . Y . . . . . .
Hap 10 . . . . . C . . T . . T . . . . . C . . . . . Y . . . . . Y . . . . .
66 70 80 90 100 110 120 130
Hap 1 A A C T A A G A C T C G A A T T A T A A T C C T T A C A C G A G G A T T C C A C A A C A T C C T A C C A A T A C T C A C A A C T T
Hap 2 . . . . . .
Hap 3 . . . C . . . . . .
Hap 4 . . . . . .
Hap 5 . . . . . .
Hap 6 . . . . . .
Hap 7 . . . . . .
Hap 8 . . . . . G . . . . . .
Hap 9 . . . . . .
Hap 10 . . . . . R . . . . . W . . . . . Y .
131 140 150 160 170 180 190 195
Hap 1 G G T G G T T A C T A A T T A A C T T A A T A A A T A T T G C A A C C C C A C C A A C T A T A A A C T T T A C T G G G G A A C T A
Hap 2 . . . . . G . . . . . T . . . . . .
Hap 3 . . . . . .
Hap 4 . . . . . .
Hap 5 . . . . . .
Hap 6 . . . . . .
Hap 7 . . . . . G . . . . . .
Hap 8 . . . . . .
Hap 9 . . . . . .
Hap 10 . . . . . .
196 200 210 220 230 240 244
Hap 1 T T A A T T G C A T C C T C A T T A T T C A A C T G A T G T C C A A C A A C A A T C A T T A T A T
Hap 2 . . . . . C . . . . . .
Hap 3 . . . . . C . . . . . .
Hap 4 C . . . . . .
Hap 5 . . . . . C . . . . . .
Hap 6 . . . . . T . . . . . R . . . . . .
Hap 7 . . . . . .
Hap 8 . . . . . C . . . . . .
Hap 9 . . . . . .
Hap 10 . . . . . C . . . . . C . . G . . . . . .

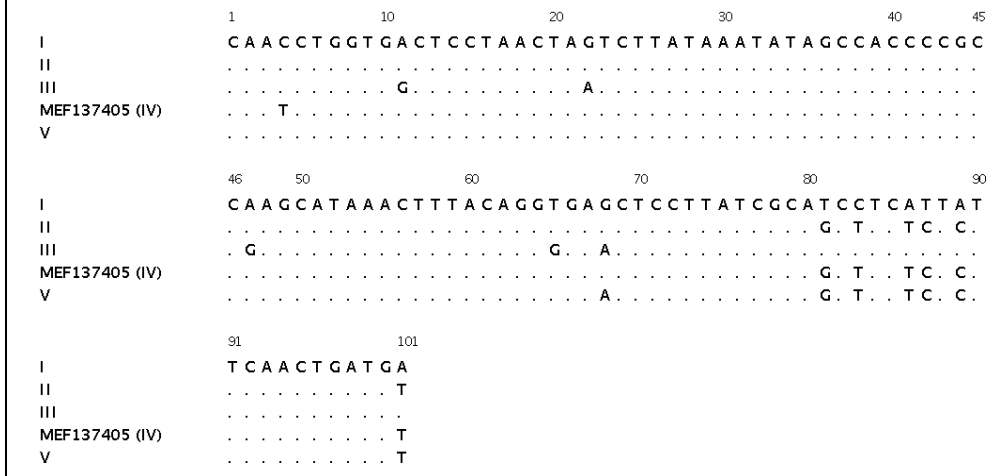
Appendix 12


1 10 20 30 40 50 60 65
II GCAATCCTACTAAAACTCGGCGGATATGGCATCATCCGTATAACCCAAACCCTTCCTACCCTAAA
I ??
III ??????.........C...
IV ???..............A.....T...
V ???????????????..G...
VI ???
VII ....A...
VIII ???
IX ???
X ....A...
XI ???
XII ???
66 70 80 90 100 110 120 130
II AACAGACATGTTTCTTCCATTTATTGTTCTATCTCTCTGAGGGGCCACCTTAGCAAGCTTGACCT
I ??
III ....
IV ....
V ....
VI ....
VII ....
VIII ???
IX ???
X ....?..
XI ???
XII ....
131 140 150 160 170 180 190 195
II GCCTGCAACAAACAG?ACCTAAAATCCCTAATTGCATACTCTTCTATTAGCCATATGGGCTTAGT
I ???
III ....?..
IV ....?..
V ....?..
VI ....?..
VII ....?..
VIII ???
IX ???
X ....T.....................................????????
XI ???
XII ....?..
196 200 210 220 230 240 250 260
II TATCGCAGCAATCTCTATCCAAACACAGTGGGGCCTAGCAGGAGCAATAGCCATAATAGTCGCCC
I ???
III ....
IV ....G..
V ....
VI ....A...
VII ....
VIII ??T.....
IX ??T.....
X ??TA.T.....
XI ???
XII ....
261 270 280 290 300 310 320 325
II ACGGCTTTACATCATCAGCACTTTTCTGCCTAGCAAATACCACCTACGAACGTACCCAAACCCGA
I ???
III ....
IV ....
V ....
VI ....
VII ....
VIII ....A.....T...
IX ....A.....T.........G.....................................C.....
X ....A.....T...
XI ???????????????.C...
XII ....

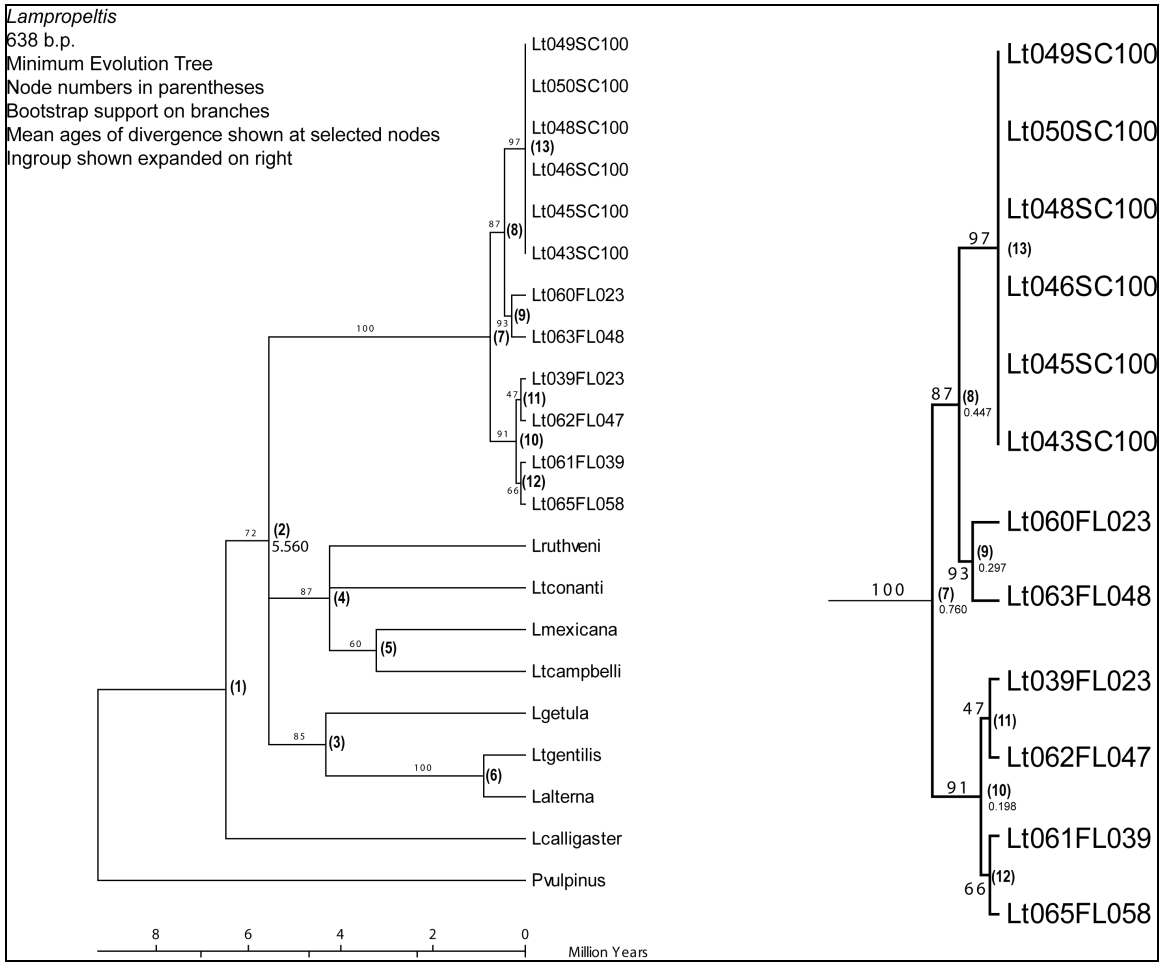
Appendix 13


Micrurus fulvius
101 b.p.
Strict consensus of 3 M.P.T.
L=42
CI=0.88
RI=0.82
Bootstrap support >50% shown
Haplotypes in parentheses
BfasciatusU49297
McorallinusAF228424
M003-RapidesLA (3)
M133-MeriwetherGA (1)
M132-AlachuaFL (1)
M265-TiftGA (2)
M085-ColumbiaFL (2)
M119-CovingtonAL (2)
M249-Palm BeachFL (2)
M026-Palm BeachFL (2)
M014-HernandoFL (2)
M036-LevyFL (2)
M075-AlachuaFL (2)
MEF137405 (4)
MU49298 (2)
M074-AlachuaFL (2)
M042-AikenSC (2)
M029-CollierFL (5)
M038-Palm BeachFL (2)
M071-SeminoleFL (2)
M068-VolusiaFL (2)
M082-AlachuaFL (2)
M023-CitrusFL (2)
80
64
96
72

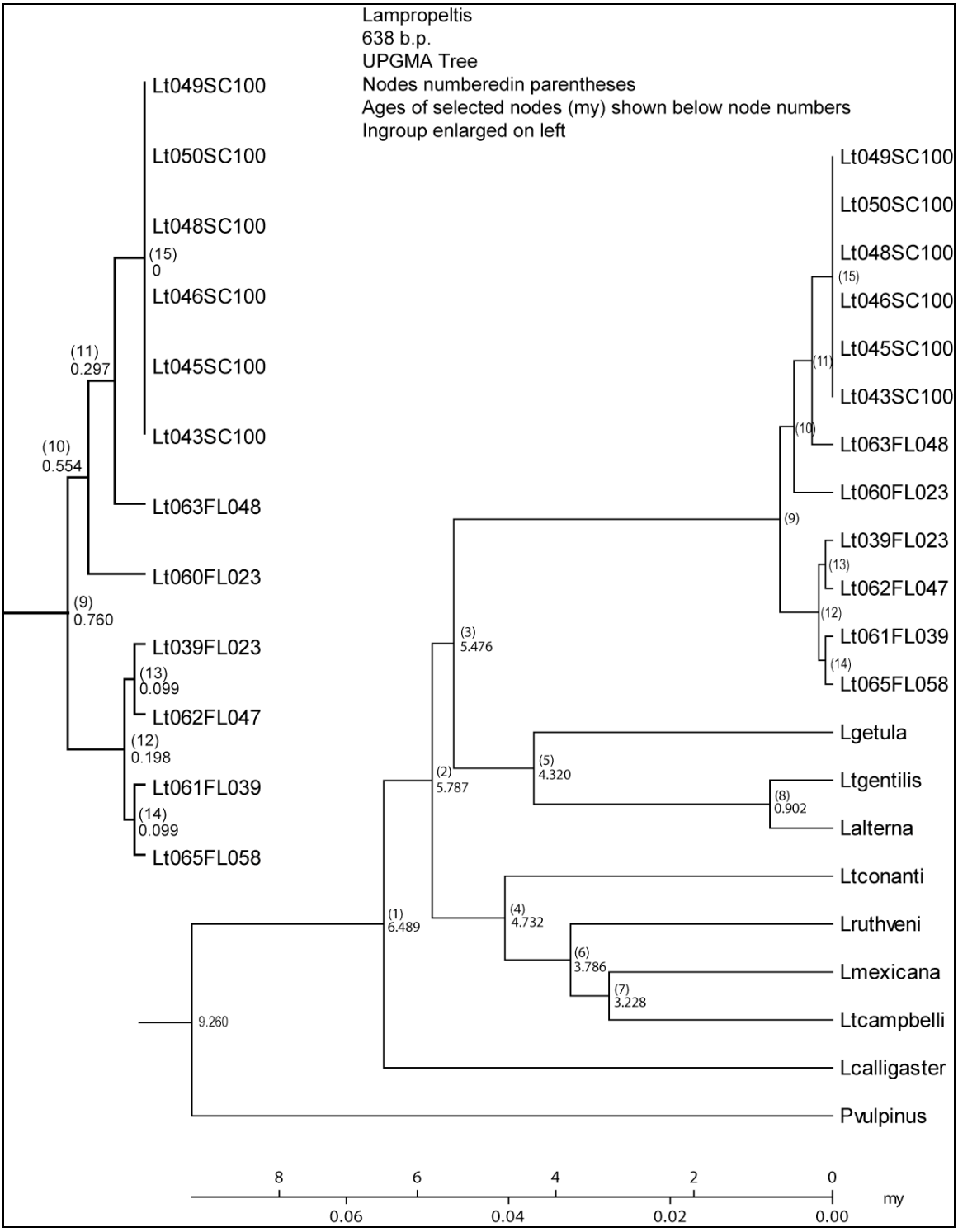
Appendix 14


1 10 20 30 40 45
I C A A C C T G G T G A C T C C T A A C T A G T C T T A T A A A T A T A G C C A C C C C G C
II . . . . . .
III . . . . . G . . . . . A . . . . . .
MEF137405 (IV) . . . T . . . . . .
V . . . . . .
46 50 60 70 80 90
I C A A G C A T A A A C T T T A C A G G T G A G C T C C T T A T C G C A T C C T C A T T A T
II . . . . . G . T . . T C . C .
III . G . . . . . G . . A . . . . . .
MEF137405 (IV) . . . . . G . T . . T C . C .
V . . . . . A . . . . . G . T . . T C . C .
91 101
I T C A A C T G A T G A
II . . . . . T
III . . . . . .
MEF137405 (IV) . . . . . T
V . . . . . T

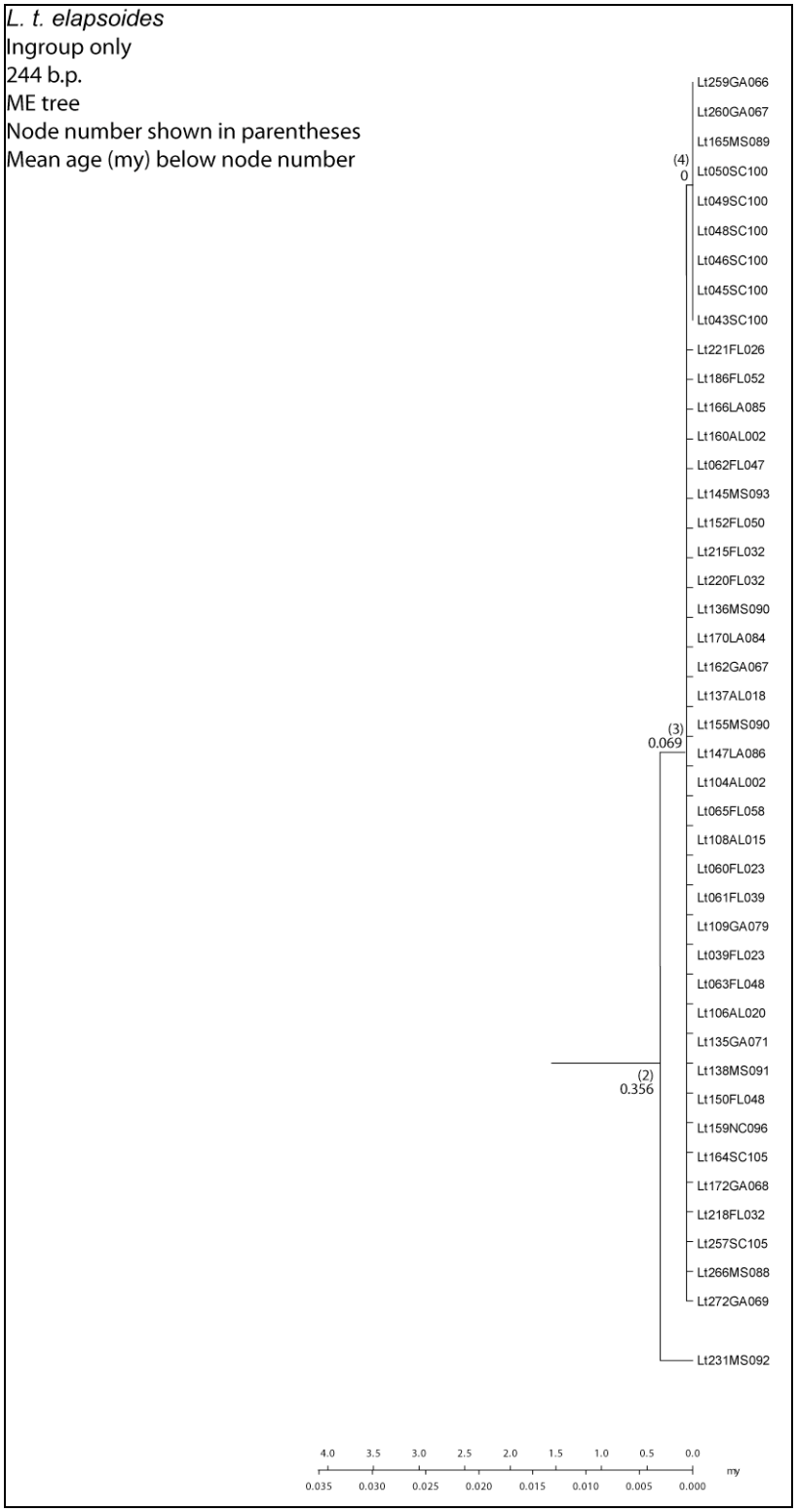
Appendix 15


Lampropeltis
638 b.p.
Minimum Evolution Tree
Node numbers in parentheses
Bootstrap support on branches
Mean ages of divergence shown at selected nodes
Ingroup shown expanded on right
Lt049SC100
Lt050SC100
Lt048SC100
(13)
Lt046SC100
Lt045SC100
(8)
Lt043SC100
Lt060FL023
(9)
(7)
Lt063FL048
Lt039FL023
(11)
Lt062FL047
(10)
Lt061FL039
(12)
Lt065FL058
(2)
5.560
Lruthveni
Ltconanti
(4)
Lmexicana
(5)
Ltcampbelli
(1)
Lgetula
(3)
Ltgentilis
(6)
Lalterna
Lcalligaster
Pvulpinus
8
6
4
2
0
Million Years
0.447
0.297
0.760
0.198

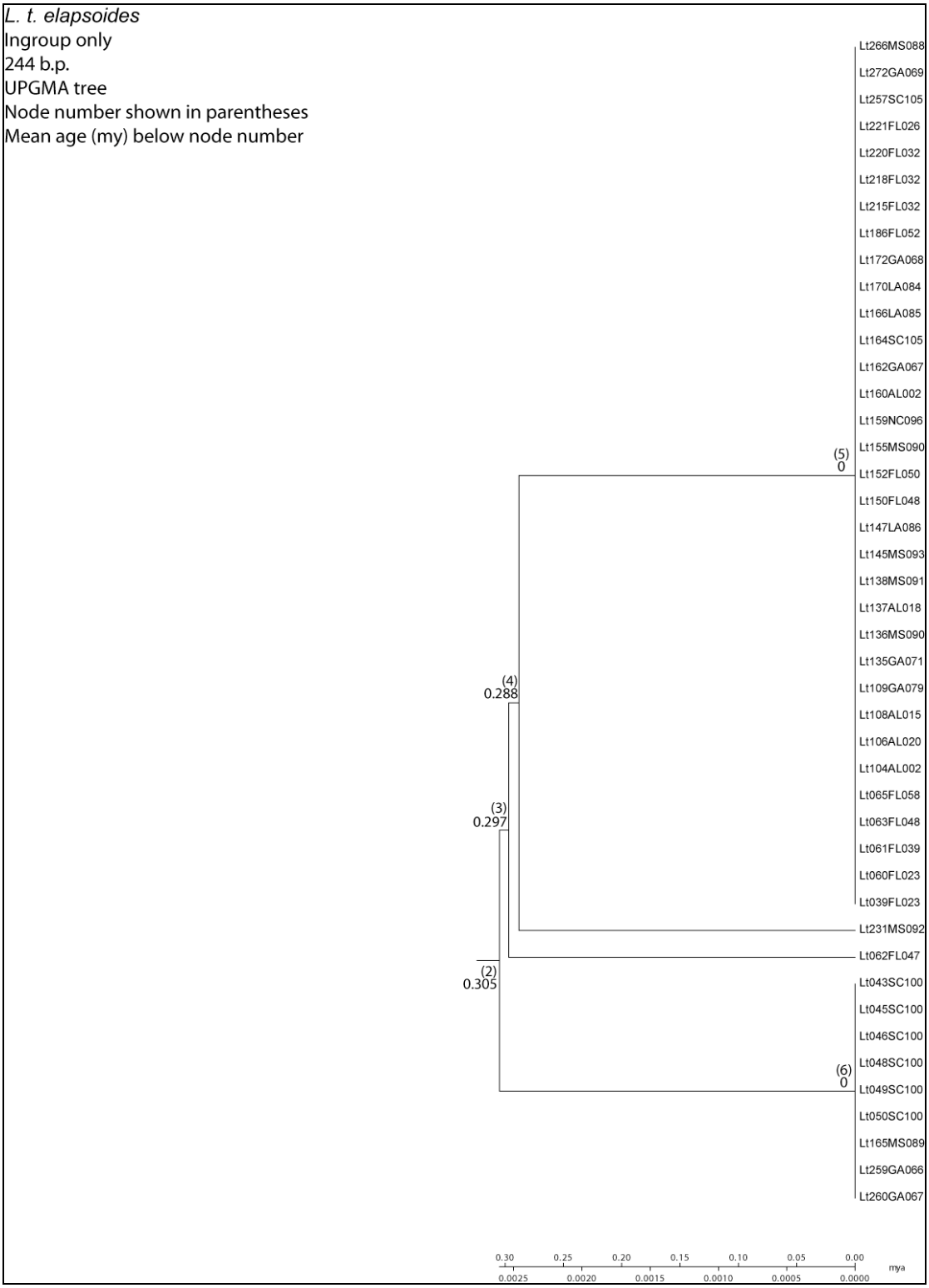
Appendix 16


Lampropeltis
638 b.p.
UPGMA Tree
Nodes numberedin parentheses
Ages of selected nodes (my) shown below node numbers
Ingroup enlarged on left
Lt049SC100
Lt050SC100
Lt048SC100
(15)
0
Lt046SC100
(11)
0.297
Lt045SC100
Lt043SC100
(10)
0.554
Lt063FL048
Lt060FL023
(9)
0.760
Lt039FL023
(13)
0.099
Lt062FL047
(12)
0.198
Lt061FL039
(14)
0.099
Lt065FL058
9.260
(1)
6.489
(2)
5.787
(3)
5.476
(4)
4.732
(5)
4.320
(6)
3.786
(7)
3.228
(8)
0.902
Lgetula
Ltgentilis
Lalterna
Ltconanti
Lruthveni
Lmexicana
Ltcampbelli
Lcalligaster
Pvulpinus
8
6
4
2
0
my
0.06
0.04
0.02
0.00

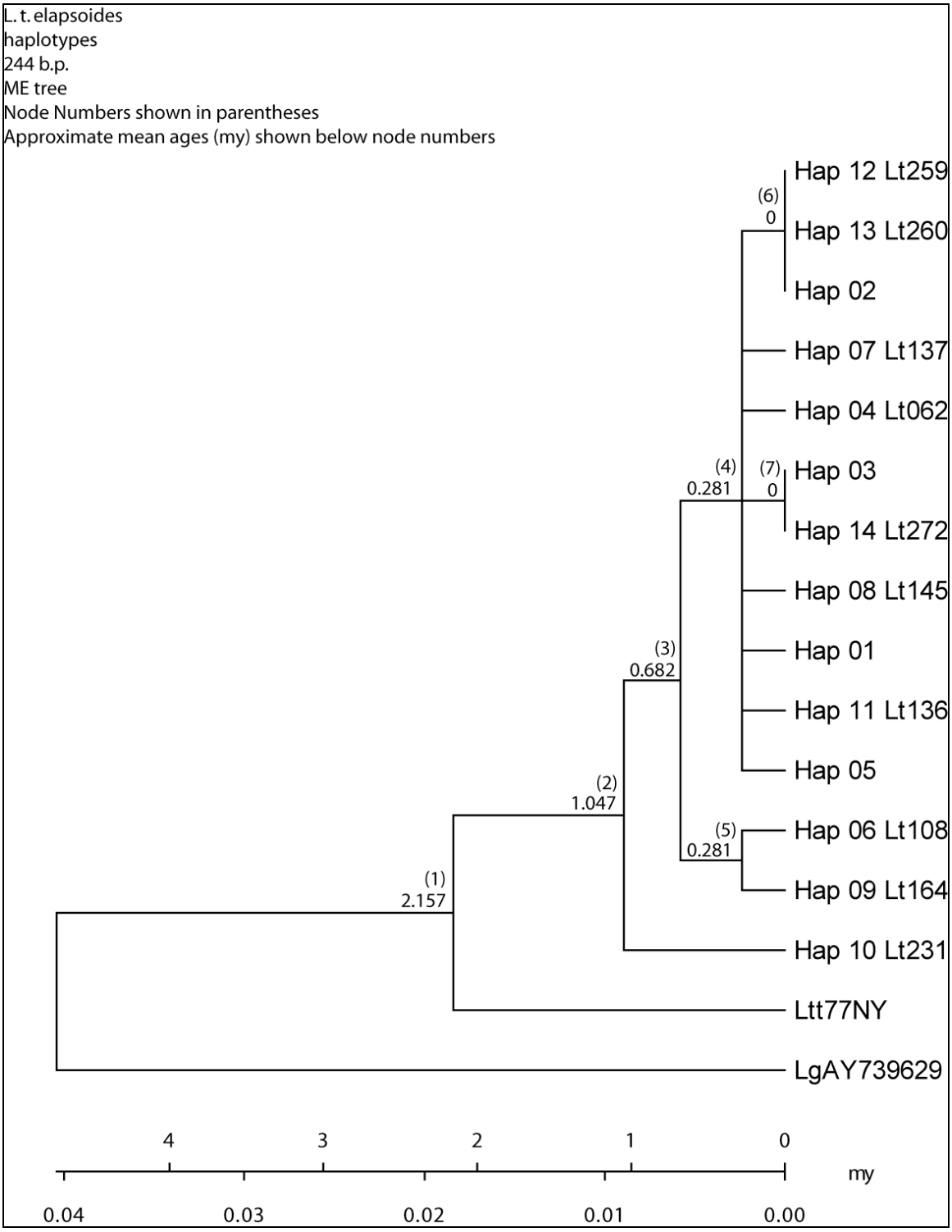
Appendix 17


L. t. elapsoides
Ingroup only
244 b.p.
ME tree
Node number shown in parentheses
Mean age (my) below node number
Lt259GA066
Lt260GA067
Lt165MS089
(4)
0
Lt050SC100
Lt049SC100
Lt048SC100
Lt046SC100
Lt045SC100
Lt043SC100
Lt221FL026
Lt186FL052
Lt166LA085
Lt160AL002
Lt062FL047
Lt145MS093
Lt152FL050
Lt215FL032
Lt220FL032
Lt136MS090
Lt170LA084
Lt162GA067
Lt137AL018
Lt155MS090
(3)
0.069
Lt147LA086
Lt104AL002
Lt065FL058
Lt108AL015
Lt060FL023
Lt061FL039
Lt109GA079
Lt039FL023
Lt063FL048
Lt106AL020
Lt135GA071
Lt138MS091
(2)
0.356
Lt150FL048
Lt159NC096
Lt164SC105
Lt172GA068
Lt218FL032
Lt257SC105
Lt266MS088
Lt272GA069
Lt231MS092
4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
my
0.035 0.030 0.025 0.020 0.015 0.010 0.005 0.000

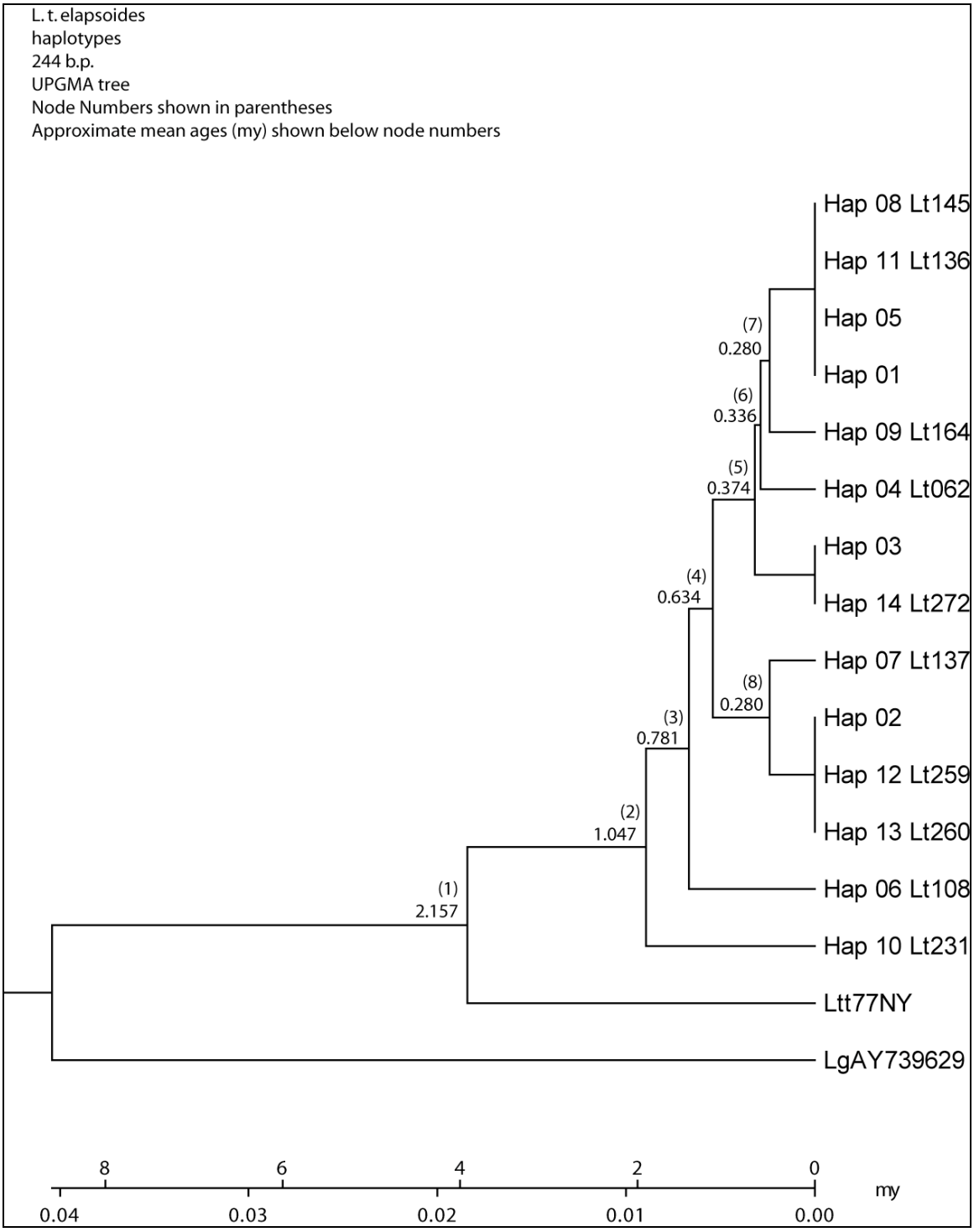
Appendix 18


L. t. elapsoides
Ingroup only
244 b.p.
UPGMA tree
Node number shown in parentheses
Mean age (my) below node number
Lt266MS088
Lt272GA069
Lt257SC105
Lt221FL026
Lt220FL032
Lt218FL032
Lt215FL032
Lt186FL052
Lt172GA068
Lt170LA084
Lt166LA085
Lt164SC105
Lt162GA067
Lt160AL002
Lt159NC096
Lt155MS090
(5)
0
Lt152FL050
Lt150FL048
Lt147LA086
Lt145MS093
Lt138MS091
Lt137AL018
Lt136MS090
Lt135GA071
(4)
0.288
Lt109GA079
Lt108AL015
Lt106AL020
Lt104AL002
Lt065FL058
(3)
0.297
Lt063FL048
Lt061FL039
Lt060FL023
Lt039FL023
Lt231MS092
Lt062FL047
(2)
0.305
Lt043SC100
Lt045SC100
Lt046SC100
Lt048SC100
(6)
0
Lt049SC100
Lt050SC100
Lt165MS089
Lt259GA066
Lt260GA067
0.30 0.25 0.20 0.15 0.10 0.05 0.00
0.0025 0.0020 0.0015 0.0010 0.0005 0.0000
mya

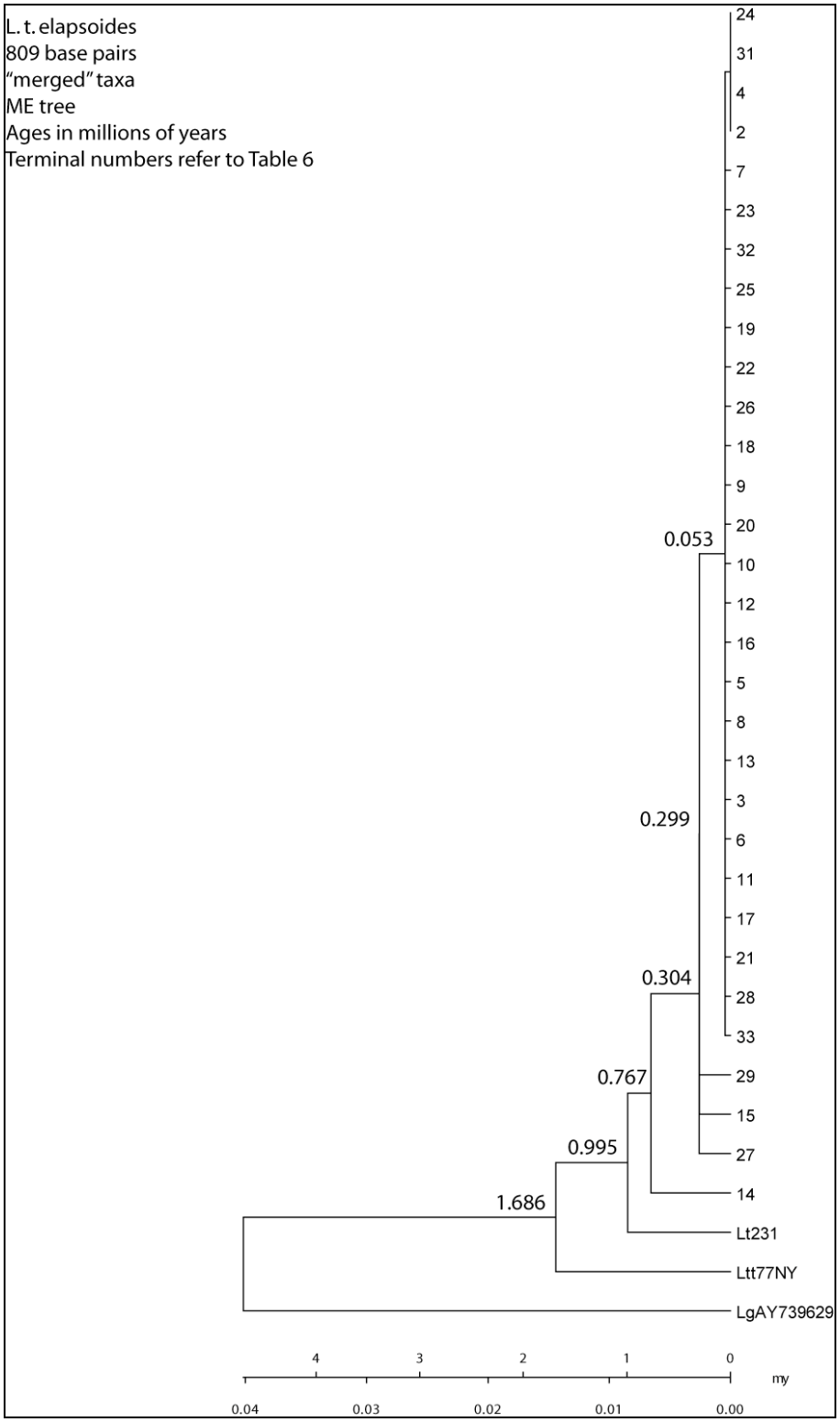
Appendix 19


L. t. elapsoides
haplotypes
244 b.p.
ME tree
Node Numbers shown in parentheses
Approximate mean ages (my) shown below node numbers
Hap 12 Lt259
(6)
0
Hap 13 Lt260
Hap 02
Hap 07 Lt137
Hap 04 Lt062
(4)
0.281
(7)
0
Hap 03
Hap 14 Lt272
Hap 08 Lt145
(3)
0.682
Hap 01
Hap 11 Lt136
Hap 05
(2)
1.047
(5)
0.281
Hap 06 Lt108
(1)
2.157
Hap 09 Lt164
Hap 10 Lt231
Ltt77NY
LgAY739629
4
3
2
1
0
my
0.04
0.03
0.02
0.01
0.00

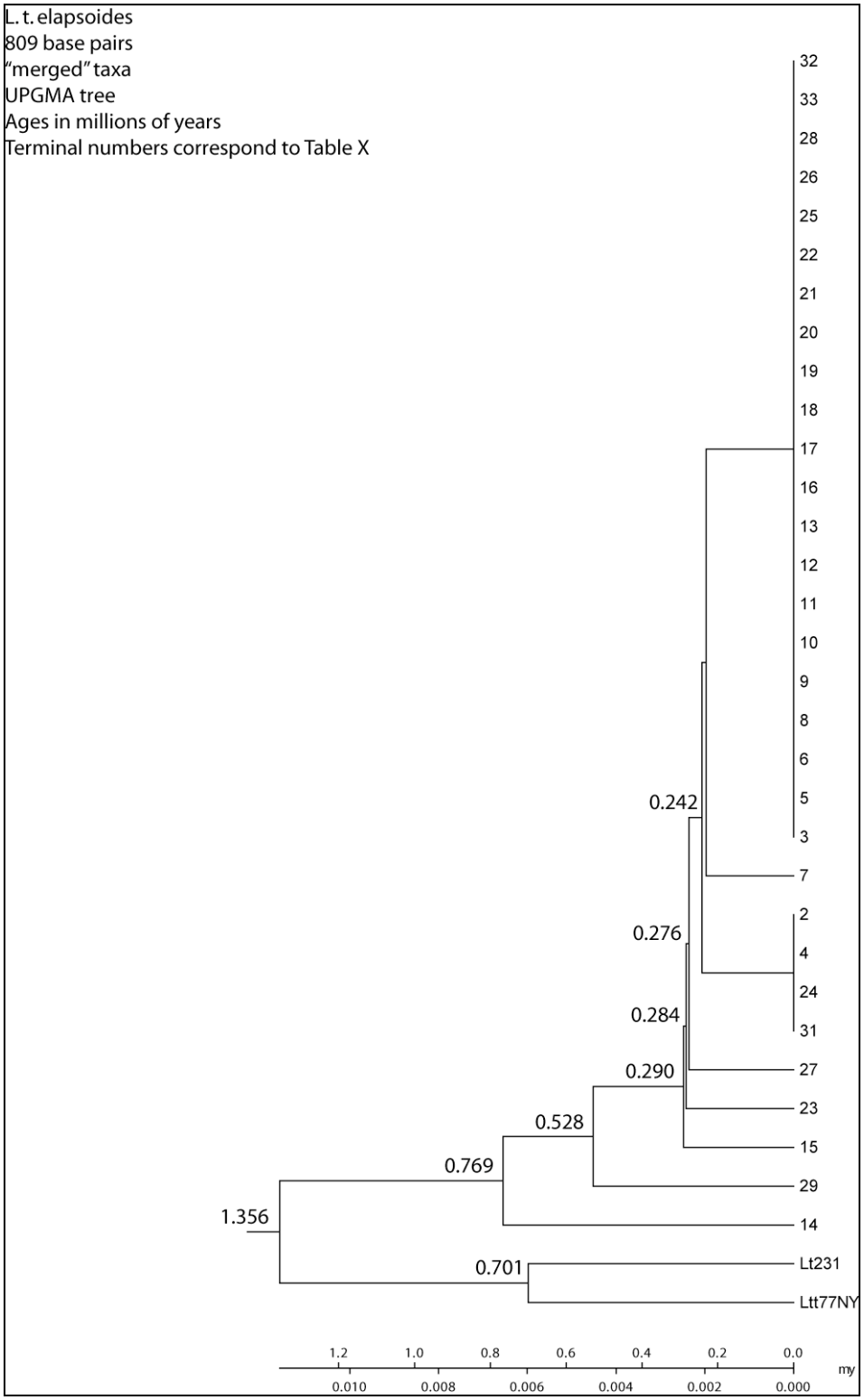
Appendix 20


L. t. elapsoides
haplotypes
244 b.p.
UPGMA tree
Node Numbers shown in parentheses
Approximate mean ages (my) shown below node numbers
Hap 08 Lt145
Hap 11 Lt136
Hap 05
Hap 01
Hap 09 Lt164
Hap 04 Lt062
Hap 03
Hap 14 Lt272
Hap 07 Lt137
Hap 02
Hap 12 Lt259
Hap 13 Lt260
Hap 06 Lt108
Hap 10 Lt231
Ltt77NY
LgAY739629
(7)
0.280
(6)
0.336
(5)
0.374
(4)
0.634
(8)
0.280
(3)
0.781
(2)
1.047
(1)
2.157
8
6
4
2
0
my
0.04
0.03
0.02
0.01
0.00

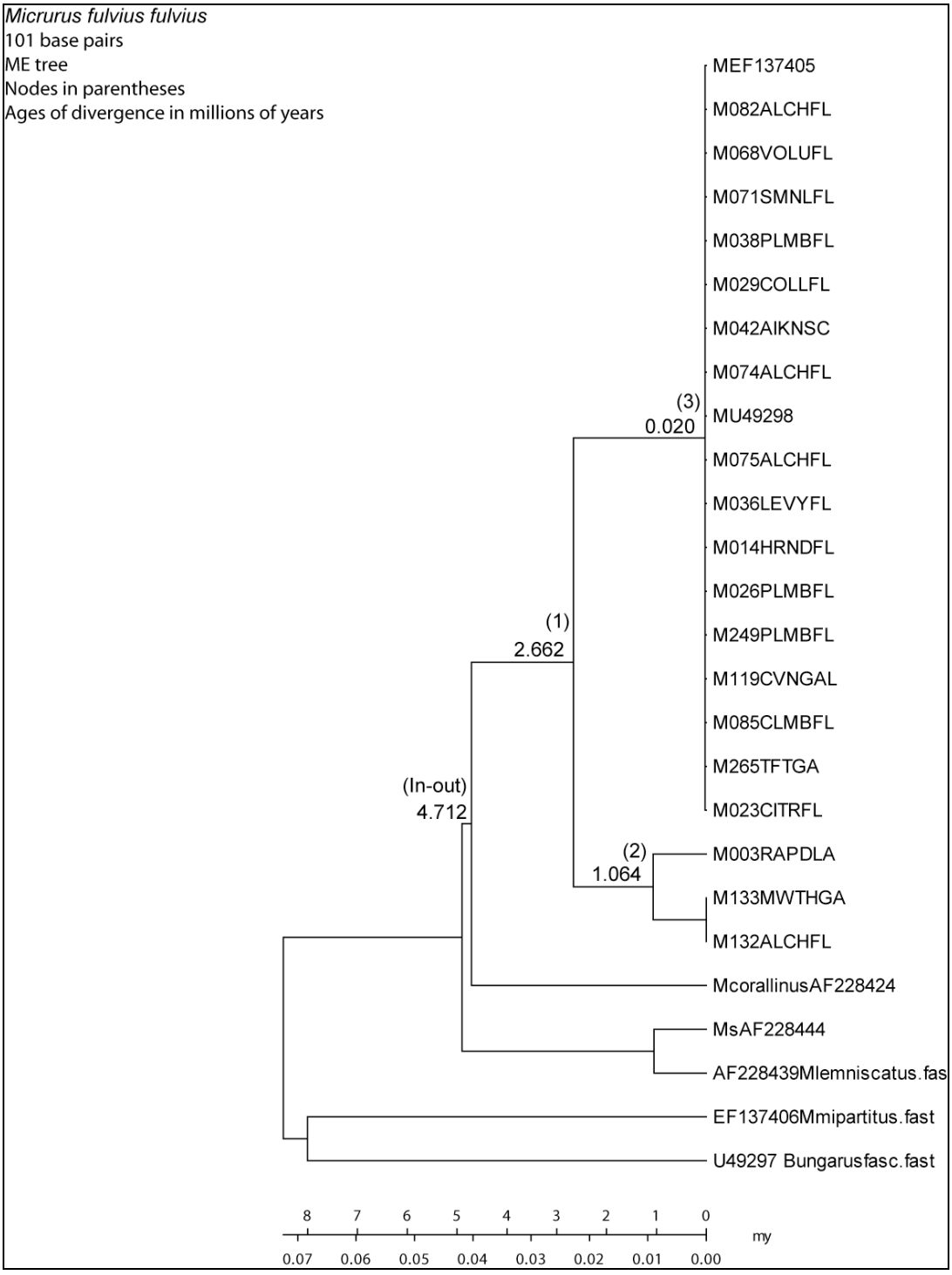
Appendix 21


L. t. elapsoides
809 base pairs
"merged" taxa
ME tree
Ages in millions of years
Terminal numbers refer to Table 6
24
31
4
2
7
23
32
25
19
22
26
18
9
20
0.053
10
12
16
5
8
13
3
0.299
6
11
17
21
0.304
28
33
0.767
29
15
0.995
27
1.686
14
Lt231
Ltt77NY
LgAY739629
4
3
2
1
0
my
0.04
0.03
0.02
0.01
0.00

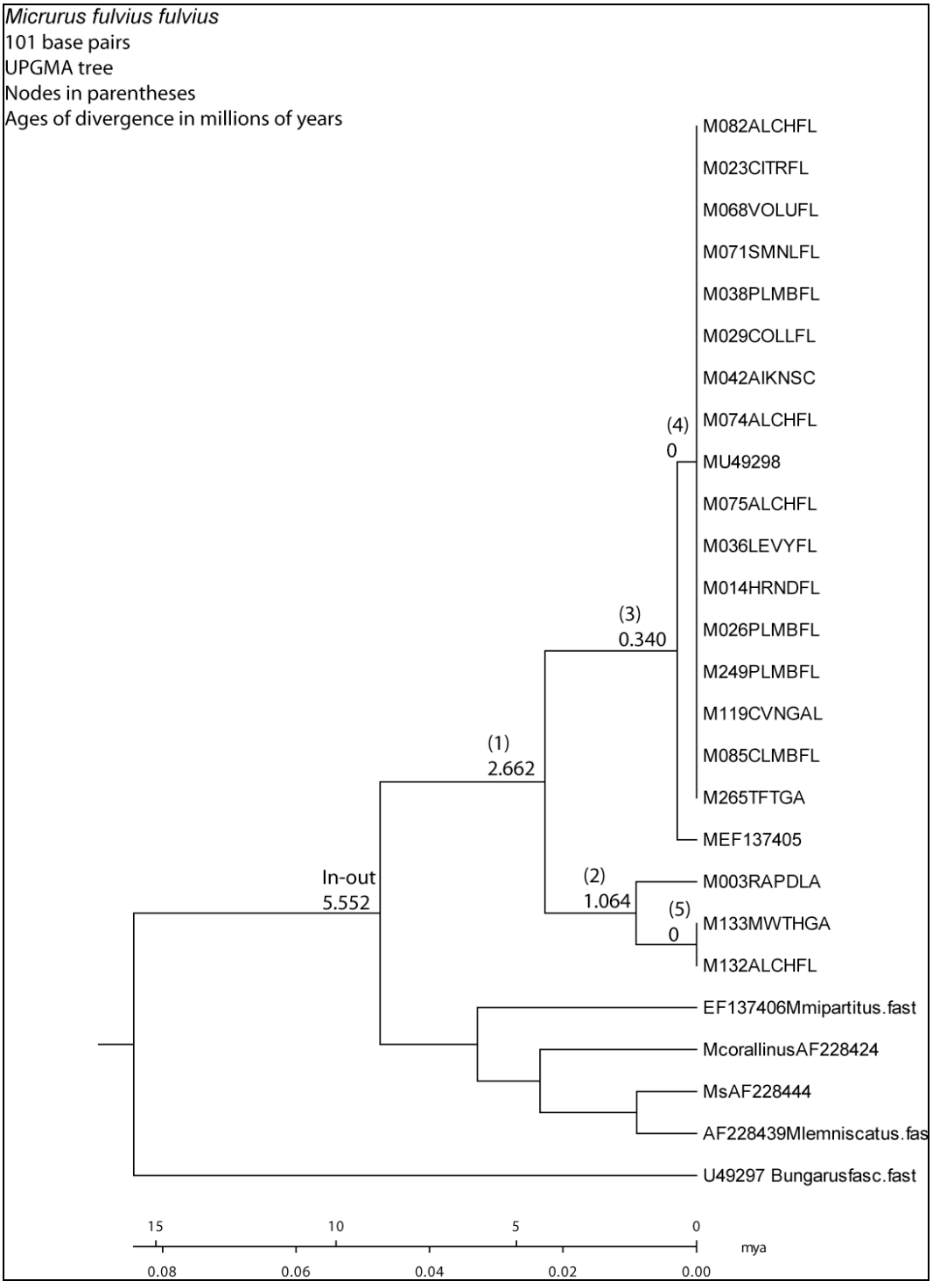
Appendix 22


L. t. elapsoides
809 base pairs
"merged" taxa
UPGMA tree
Ages in millions of years
Terminal numbers correspond to Table X
32
33
28
26
25
22
21
20
19
18
17
16
13
12
11
10
9
8
6
5
3
7
2
4
24
31
27
23
15
29
14
Lt231
Ltt77NY
0.242
0.276
0.284
0.290
0.528
0.769
1.356
0.701
1.2
1.0
0.8
0.6
0.4
0.2
0.0
my
0.010
0.008
0.006
0.004
0.002
0.000

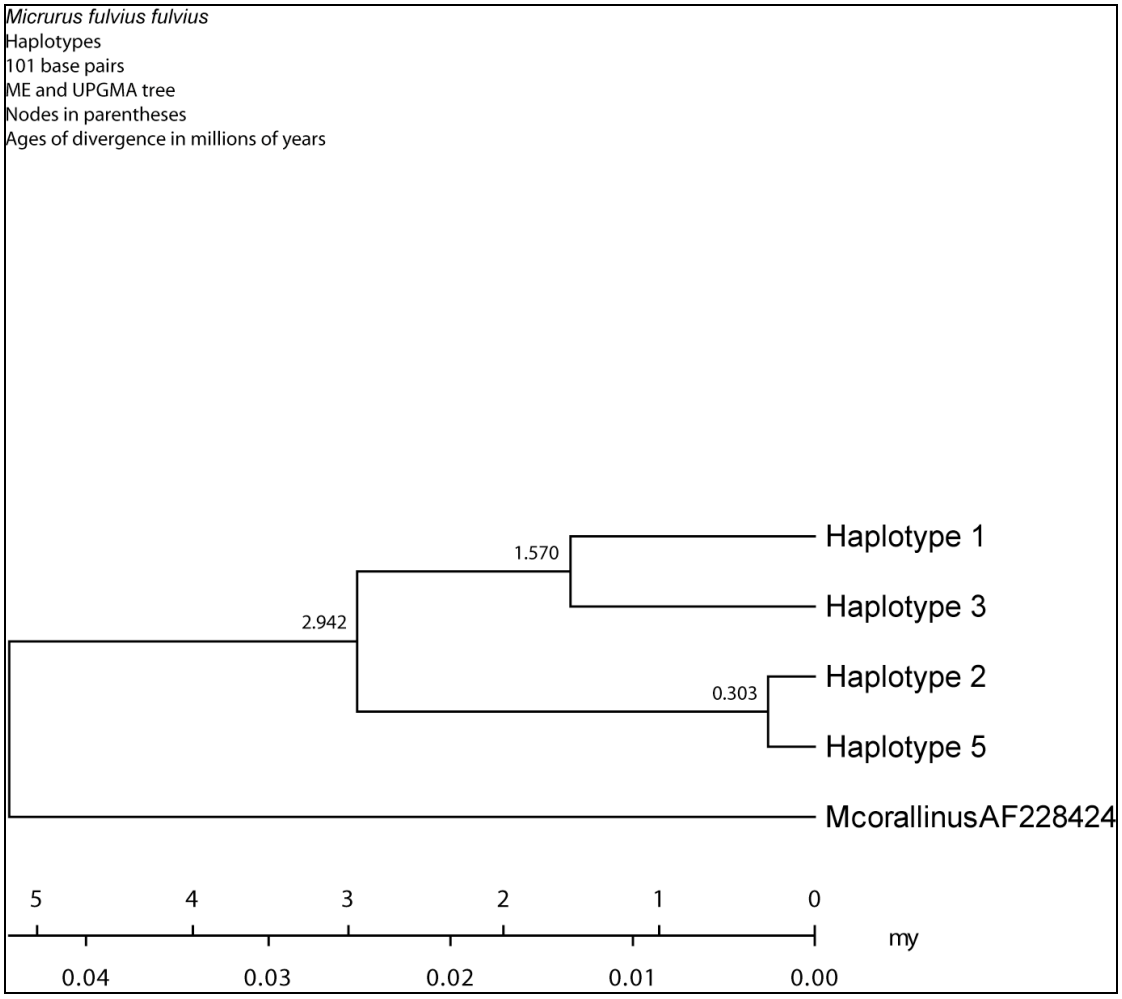
Appendix 23


Micrurus fulvius fulvius
101 base pairs
ME tree
Nodes in parentheses
Ages of divergence in millions of years
MEF137405
M082ALCHFL
M068VOLUFL
M071SMNLFL
M038PLMBFL
M029COLLFL
M042AIKNSC
M074ALCHFL
(3)
0.020
MU49298
M075ALCHFL
M036LEVYFL
M014HRNDFL
M026PLMBFL
(1)
2.662
M249PLMBFL
M119CVNGAL
M085CLMBFL
M265TFTGA
(In-out)
4.712
M023CITRFL
(2)
1.064
M003RAPDLA
M133MWTHGA
M132ALCHFL
McorallinusAF228424
MsAF228444
AF228439Mlemniscatus.fas
EF137406Mmipartitus.fast
U49297 Bungarusfasc.fast
8 7 6 5 4 3 2 1 0
my
0.07 0.06 0.05 0.04 0.03 0.02 0.01 0.00

Appendix 24


Micrurus fulvius fulvius
101 base pairs
UPGMA tree
Nodes in parentheses
Ages of divergence in millions of years
M082ALCHFL
M023CITRFL
M068VOLUFL
M071SMNLFL
M038PLMBFL
M029COLLFL
M042AIKNSC
(4)
0
M074ALCHFL
MU49298
M075ALCHFL
M036LEVYFL
M014HRNDFL
(3)
0.340
M026PLMBFL
M249PLMBFL
M119CVNGAL
(1)
2.662
M085CLMBFL
M265TFTGA
MEF137405
In-out
5.552
(2)
1.064
M003RAPDLA
(5)
0
M133MWTHGA
M132ALCHFL
EF137406Mmipartitus.fast
McorallinusAF228424
MsAF228444
AF228439Mlemniscatus.fas
U49297 Bungarusfasc.fast
15
10
5
0
mya
0.08
0.06
0.04
0.02
0.00

Appendix 25


Micrurus fulvius fulvius
Haplotypes
101 base pairs
ME and UPGMA tree
Nodes in parentheses
Ages of divergence in millions of years
1.570
2.942
0.303
Haplotype 1
Haplotype 3
Haplotype 2
Haplotype 5
McorallinusAF228424
5
4
3
2
1
0
my
0.04
0.03
0.02
0.01
0.00

Appendix 26

Spec. #	Spp	Inst	Cluster 25	Mf/Lt w25	Cluster 10	Mf/Lt W10	GROW_DAY	ANN_RAIN
43	Lt	AMNH106135	1	0.600000	7	0.611111	240	118.6
44	Lt	AMNH106136	1	0.600000	7	0.611111	240	118.6
45	Lt	AMNH106137	1	0.600000	7	0.611111	240	118.6
90	Lt	Lte90	9	1.105263	8	1.111111	250	146.3
91	Lt	AUM18479	18	2.500000	9	1.642857	255	142
92	Lt	AUM33635	8	0.454545	5	0.384615	220	138.3
93	Lt	AUM16610	8	0.454545	5	0.384615	220	132.7
94	Lt	AUM16609	8	0.454545	5	0.384615	214	133.8
95	Lt	AUM28	8	0.454545	5	0.384615	235	134.6
96	Lt	AUM21014	19	1.125000	8	1.111111	240	143.9
97	Lt	AUM4224	9	1.105263	8	1.111111	250	146.3
98	Lt	AUM2061	8	0.454545	5	0.384615	235	139.5
100	Lt	AUM22384	8	0.454545	5	0.384615	225	141.1
101	Lt	AUM15833	19	1.125000	8	1.111111	291	167.6
102	Lt	AUM33174	24	0.000000	5	0.384615	209	140.5
103	Lt	AUM2467	8	0.454545	5	0.384615	225	138.6
104	Lt	AUM4250	9	1.105263	8	1.111111	281	168.5
105	Lt	AUM20002	9	1.105263	8	1.111111	255	153.4
106	Lt	AUM2050	8	0.454545	5	0.384615	230	139.1
107	Lt	AUM23454	8	0.454545	5	0.384615	225	126.8
108	Lt	AUM06100	8	0.454545	5	0.384615	220	136.7
109	Lt	AUM11517	18	2.500000	9	1.642857	255	115.4
174	Lt	INHS4467	20	6.500000	4	2.150000	335	154.5
175	Lt	INHS10477	4	1.428571	4	2.150000	345	144.7
176	Lt	INHS10478	14	3.466667	6	3.212121	311	131.3
177	Lt	INHS10479	23	4.111111	2	2.487179	271	138.8
186	Lt	USNMNH204238	4	1.428571	4	2.150000	345	144.7
187	Lt	USNMNH210070	14	3.466667	6	3.212121	345	132
188	Lt	USNMNH218904	13	0.300000	7	0.611111	271	130.8
189	Lt	USNMNH307604	25	7.000000	7	0.611111	270	127.7
190	Lt	USNMNH330074	13	0.300000	7	0.611111	271	130.8
191	Lt	USNMNH330075	13	0.300000	7	0.611111	271	130.8
210	Lt	USNMNH10743	9	1.105263	8	1.111111	265	161.4
211	Lt	USNMNH12927	5	0.235294	3	0.285714	306	144.5
212	Lt	USNMNH12928	5	0.235294	3	0.285714	306	144.5
213	Lt	USNMNH13644	21	3.500000	6	3.212121	345	132
214	Lt	USNMNH18030	21	3.500000	6	3.212121	345	132
215	Lt	USNMNH26303	4	1.428571	4	2.150000	345	154
216	Lt	USNMNH28251	4	1.428571	4	2.150000	345	154
217	Lt	USNMNH28910	4	1.428571	4	2.150000	345	154
218	Lt	USNMNH30945	4	1.428571	4	2.150000	345	154
219	Lt	USNMNH36566	4	1.428571	4	2.150000	345	154
220	Lt	USNMNH38160	4	1.428571	4	2.150000	345	154
221	Lt	USNMNH42127	21	3.500000	6	3.212121	345	132
222	Lt	USNMNH85323	17	2.500000	4	2.150000	345	144.7
223	Lt	USNMNH85324	4	1.428571	4	2.150000	345	154
225	Lt	UMMZ52253	5	0.235294	3	0.285714	306	144.5
226	Lt	UMMZ84446	5	0.235294	3	0.285714	306	144.5
227	Lt	UMMZ133468	5	0.235294	3	0.285714	276	152.7
228	Lt	UMMZ230350	5	0.235294	3	0.285714	255	152.7
229	Lt	UMMZ76805	11	0.363636	3	0.285714	291	162.7
230	Lt	UMMZ76806	11	0.363636	3	0.285714	291	162.7
231	Lt	UMMZ86697	16	0.666667	10	4.750000	230	131.7

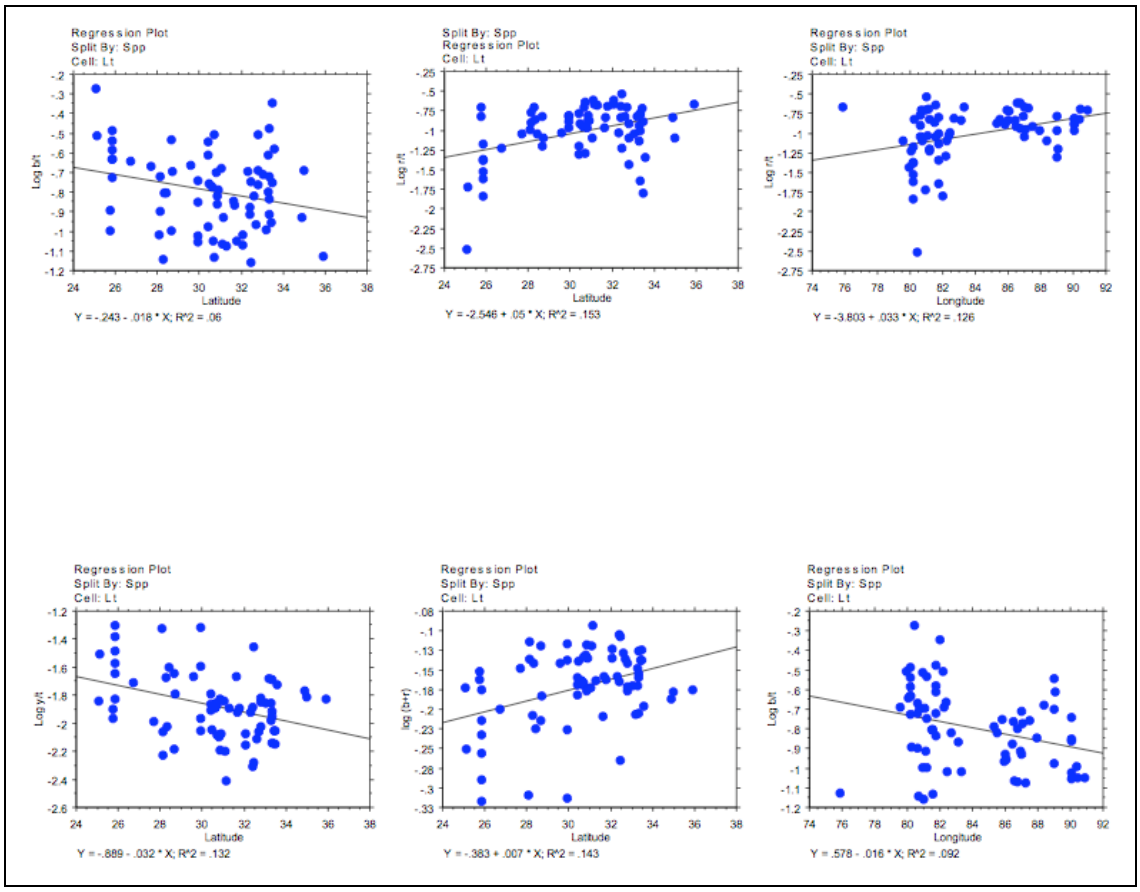
Appendix 27 (Continued)

232	Lt	UMMZ117524	11	0.363636	3	0.285714	270	166.5
233	Lt	UMMZ117525	11	0.363636	3	0.285714	291	162.7
244	Lt	MCZ6799	21	3.500000	6	3.212121	345	132
250	Lt	MCZ129307	7	0.000000	1	0.187500	265	134.9
251	Lt	MCZ150093	10	1.555556	6	3.212121	296	141.7
252	Lt	MCZ166232	14	3.466667	6	3.212121	316	130.7
254	Lt	MCZ168515	14	3.466667	6	3.212121	316	134.1
255	Lt	MCZ170332	14	3.466667	6	3.212121	316	134.1
257	Lt	MCZ177904	13	0.300000	7	0.611111	271	130.8
259	Lt	UGMNH6092	12	1.750000	2	2.487179	276	137
260	Lt	UGMNH19562	12	1.750000	2	2.487179	266	137.2
261	Lt	UGMNH45918	1	0.600000	7	0.611111	240	118.6
262	Lt	UGMNH6096	1	0.600000	7	0.611111	250	116.8
263	Lt	UGMNH7162	1	0.600000	7	0.611111	240	118.6
266	Lt	UGMNH45917	16	0.666667	10	4.750000	235	137.2
268	Lt	UGMNH6090	18	2.500000	9	1.642857	260	118.1
269	Lt	UGMNH6089	1	0.600000	7	0.611111	245	109
270	Lt	UGMNH6095	22	0.400000	1	0.187500	220	117.8
272	Lt	UGMNH19356	12	1.750000	2	2.487179	260	128.7
42	Mf	AMNH106134	1	0.600000	7	0.611111	240	118.6
111	Mf	AUM18485_54	18	2.500000	9	1.642857	255	132.8
112	Mf	AUM20781	19	1.125000	8	1.111111	240	143.9
113	Mf	AUM23221	19	1.125000	8	1.111111	240	143.9
114	Mf	AUM13735	19	1.125000	8	1.111111	240	143.9
116	Mf	AUM21647	9	1.105263	8	1.111111	260	149.2
117	Mf	AUM18258	9	1.105263	8	1.111111	260	149.2
118	Mf	AUM18232	19	1.125000	8	1.111111	281	168.5
119	Mf	AUM31164	9	1.105263	8	1.111111	260	149.2
120	Mf	AUM20780	8	0.454545	5	0.384615	225	138.6
122	Mf	AUM20988	19	1.125000	8	1.111111	245	146.6
123	Mf	AUM06612	9	1.105263	8	1.111111	260	149.2
124	Mf	AUM05834	9	1.105263	8	1.111111	260	149.2
125	Mf	AUM23041	8	0.454545	5	0.384615	225	133.8
126	Mf	AUM06385	9	1.105263	8	1.111111	255	140.4
127	Mf	AUM20779	19	1.125000	8	1.111111	240	143.9
128	Mf	AUM24285	19	1.125000	8	1.111111	291	167.6
129	Mf	AUM21149	9	1.105263	8	1.111111	260	149.2
178	Mf	INHS10501	23	4.111111	2	2.487179	271	138.8
179	Mf	INHS10502	3	2.090909	2	2.487179	271	138.8
180	Mf	INHS10503	23	4.111111	2	2.487179	271	138.8
181	Mf	INHS10504	23	4.111111	2	2.487179	271	138.8
182	Mf	USNMNH142093	4	1.428571	4	2.150000	345	154
183	Mf	USNMNH8783	9	1.105263	8	1.111111	270	165.6
185	Mf	USNMNH60559	14	3.466667	6	3.212121	311	135.3
192	Mf	USNMNH1120	25	7.000000	7	0.611111	270	127.7
193	Mf	USNMNH1142	25	7.000000	7	0.611111	255	126.9
194	Mf	USNMNH13651	21	3.500000	6	3.212121	345	132
195	Mf	USNMNH24356	14	3.466667	6	3.212121	306	133.6
196	Mf	USNMNH44517	20	6.500000	4	2.150000	335	154.5
197	Mf	USNMNH44554	11	0.363636	3	0.285714	281	158.7
198	Mf	USNMNH48342	25	7.000000	7	0.611111	270	127.7
199	Mf	USNMNH48344	25	7.000000	7	0.611111	270	127.7
200	Mf	USNMNH69672	14	3.466667	6	3.212121	306	133.6
201	Mf	USNMNH75240	14	3.466667	6	3.212121	306	133.6

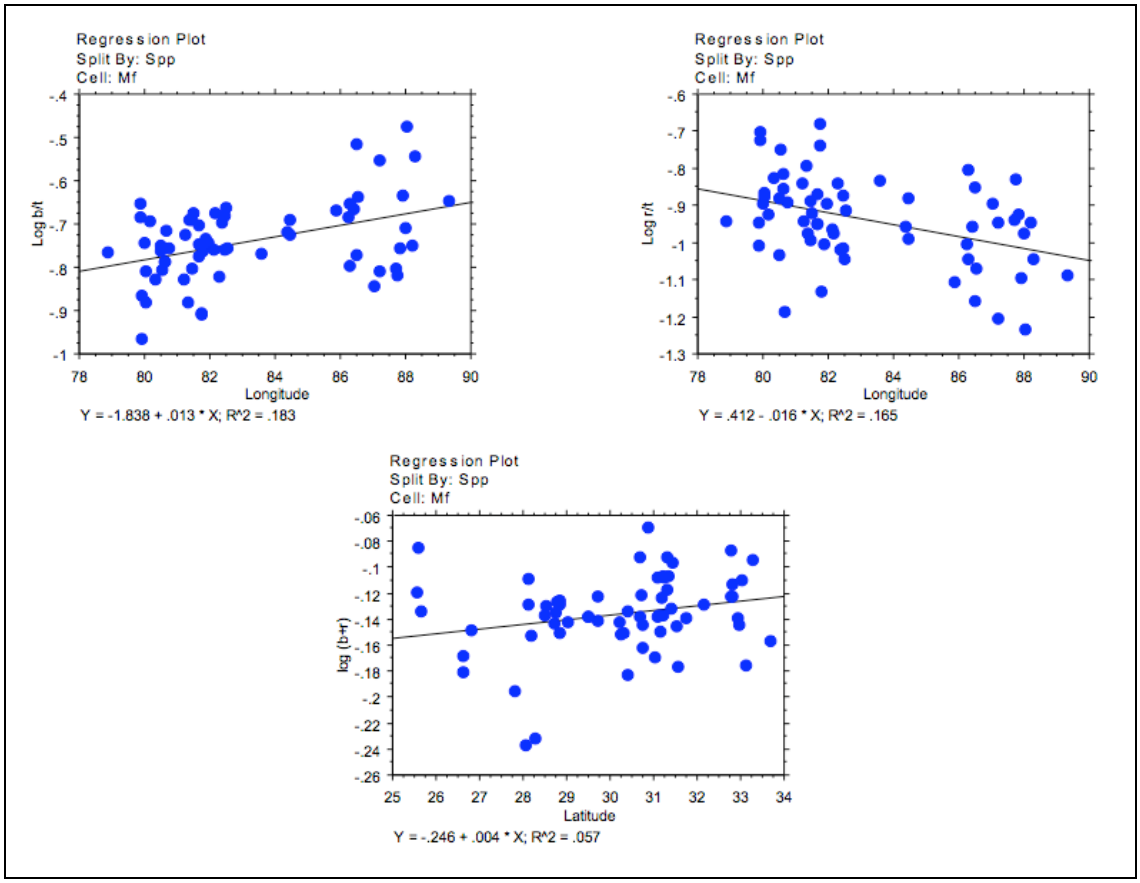
Appendix 27 (Continued)

202	Mf	USNMNH81100	14	3.466667	6	3.212121	306	133.6
203	Mf	USNMNH81985	14	3.466667	6	3.212121	311	131.3
204	Mf	USNMNH130154	12	1.750000	2	2.487179	266	137.2
205	Mf	USNMNH130156	12	1.750000	2	2.487179	266	137.2
206	Mf	USNMNH210105	23	4.111111	2	2.487179	260	137.8
207	Mf	USNMNH260848	14	3.466667	6	3.212121	306	136.1
208	Mf	USNMNH307424	14	3.466667	6	3.212121	316	134.1
209	Mf	USNMNH310885	12	1.750000	2	2.487179	281	139.6
224	Mf	USNMNH142094	4	1.428571	4	2.150000	345	154
236	Mf	UMMZ65627	13	0.300000	7	0.611111	276	125
237	Mf	UMMZ94934	15	0.333333	1	0.187500	245	130.5
238	Mf	UMMZ178030	25	7.000000	7	0.611111	245	130.5
239	Mf	MCZ131	9	1.105263	8	1.111111	270	165.6
240	Mf	MCZ132	25	7.000000	7	0.611111	270	127.7
241	Mf	MCZ181	19	1.125000	8	1.111111	291	167.6
242	Mf	MCZ6660	21	3.500000	6	3.212121	345	132
243	Mf	MCZ6703	10	1.555556	6	3.212121	321	132.5
245	Mf	MCZ6834	21	3.500000	6	3.212121	345	132
246	Mf	MCZ46748	4	1.428571	4	2.150000	345	154
247	Mf	MCZ53948	21	3.500000	6	3.212121	345	136.4
248	Mf	MCZ54018	17	2.500000	4	2.150000	340	137
249	Mf	MCZ121235	20	6.500000	4	2.150000	335	154.5
253	Mf	MCZ166907	14	3.466667	6	3.212121	316	134.1
256	Mf	MCZ170335	14	3.466667	6	3.212121	311	131.3
258	Mf	UGMNH6557	18	2.500000	9	1.642857	250	130.3
264	Mf	UGMNH6554	12	1.750000	2	2.487179	276	132
265	Mf	UGMNH6550	18	2.500000	9	1.642857	260	119.3
267	Mf	UGMNH6556	18	2.500000	9	1.642857	250	130.3
271	Mf	UGMNH37090	12	1.750000	2	2.487179	271	130.9

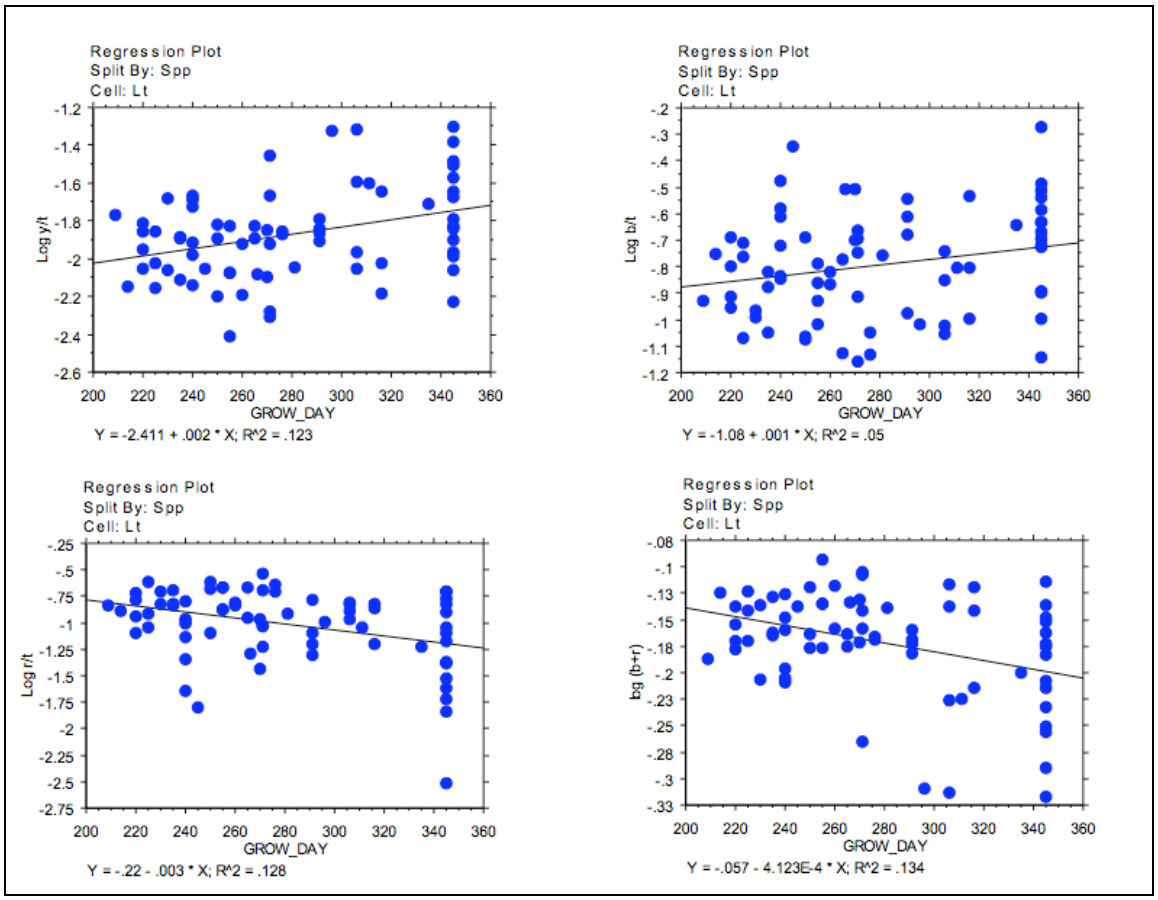
Appendix 27


Regression Plot
Split By: Spp
Cell: Lt
Log b/t
Latitude
Y = -.243 - .018 * X; R^2 = .06
Split By: Spp
Regression Plot
Cell: Lt
Log r/t
Latitude
Y = -2.546 + .05 * X; R^2 = .153
Regression Plot
Split By: Spp
Cell: Lt
Log r/t
Longitude
Y = -3.803 + .033 * X; R^2 = .126
Regression Plot
Split By: Spp
Cell: Lt
Log y/t
Latitude
Y = -.889 - .032 * X; R^2 = .132
Regression Plot
Split By: Spp
Cell: Lt
log (b+r)
Latitude
Y = -.383 + .007 * X; R^2 = .143
Regression Plot
Split By: Spp
Cell: Lt
Log b/t
Longitude
Y = .578 - .016 * X; R^2 = .092

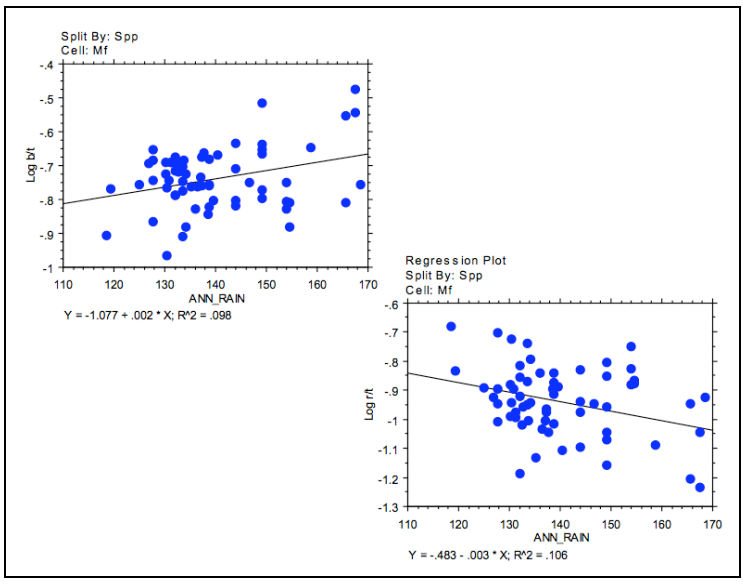
Appendix 28


Regression Plot
Split By: Spp
Cell: Mf
Log b/t
Longitude
Y = -1.838 + .013 * X; R^2 = .183
Regression Plot
Split By: Spp
Cell: Mf
Log r/t
Longitude
Y = .412 - .016 * X; R^2 = .165
Regression Plot
Split By: Spp
Cell: Mf
log (b+r)
Latitude
Y = -.246 + .004 * X; R^2 = .057

Appendix 29



Appendix 30



Appendix 31

Unpaired Means Comparison for mean b/t						
Grouping Variable: Spp						
Split By: Cluster W25						
Hypothesized Difference = 0						
	Mean Diff.	DF	t-Value	P-Value	95% Lower	95% Upper
Lt, Mf: 18	-0.072	5	-2.945	0.0321	-0.135	-0.009
Lt, Mf: 4	0.112	10	1.919	0.084	-0.018	0.242
Lt, Mf: 9	-0.11	12	-3.51	0.0043	-0.178	-0.042
Unpaired Means Comparison for Log b/t						
	Mean Diff.	DF	t-Value	P-Value	95% Lower	95% Upper
Lt, Mf: 18	-0.165	5	-2.789	0.0385	-0.317	-0.013
Lt, Mf: 4	0.209	10	2.03	0.0698	-0.02	0.438
Lt, Mf: 9	-0.246	12	-3.605	0.0036	-0.394	-0.097
Unpaired Means Comparison for mean y/t						
	Mean Diff.	DF	t-Value	P-Value	95% Lower	95% Upper
Lt, Mf: 19	0.051	8	2.223	0.0569	-0.002	0.105
Lt, Mf: 4	0.098	10	3.713	0.004	0.039	0.157
Unpaired Means Comparison for Log y/t						
	Mean Diff.	DF	t-Value	P-Value	95% Lower	95% Upper
Lt, Mf: 4	0.651	10	4.54	0.0011	0.331	0.97
Unpaired Means Comparison for mean r/t						
	Mean Diff.	DF	t-Value	P-Value	95% Lower	95% Upper
Lt, Mf: 4	-0.21	10	-3.426	0.0065	-0.347	-0.073
Lt, Mf: 9	0.106	12	3.277	0.0066	0.036	0.177
Unpaired Means Comparison for Log r/t						
	Mean Diff.	DF	t-Value	P-Value	95% Lower	95% Upper
Lt, Mf: 4	-0.734	10	-2.602	0.0264	-1.363	-0.105
Lt, Mf: 9	0.254	12	3.187	0.0078	0.08	0.428
Unpaired Means Comparison for b+r						
	Mean Diff.	DF	t-Value	P-Value	95% Lower	95% Upper
Lt, Mf: 19	-0.051	8	-2.223	0.0569	-0.105	0.002
Lt, Mf: 4	-0.098	10	-3.713	0.004	-0.157	-0.039
Unpaired Means Comparison for log (b+r)						
	Mean Diff.	DF	t-Value	P-Value	95% Lower	95% Upper
Lt, Mf: 19	-0.06	8	-2.292	0.0511	-0.12	3.65E-04
Lt, Mf: 4	-0.118	10	-3.541	0.0053	-0.191	-0.044

Bibliography

Armstrong, M. P., D. Frymire, and E. J. Zimmerer. 2001. Analysis of sympatric populations of *Lampropeltis triangulum sypsila* and *Lampropeltis triangulum elapsoides*, in western Kentucky and adjacent Tennessee with relation to the taxonomic status of the scarlet kingsnake. Journal of Herpetology 35:688-693.

Avise, J. C. 1996. Toward a regional conservation genetics perspective: phylogeography of faunas in the southeastern United States. Pages 431-470 *in* Conservation genetics: case histories from nature. (J. C. Avise, and J. L. Hamrick, eds.). Chapman & Hall, New York, Albany etc.

Avise, J. C., B. W. Bowen, T. Lamb, A. B. Meylan, and E. Bermingham. 1992. Mitochondrial DNA evolution at a turtle's pace: evidence for low genetic variability and reduced microevolutionary rate in the Testudines. Molecular Biology and Evolution 9:457-473.

Avise, J. C., C. Giblin-Davidson, J. Laerm, J. C. Patton, and R. A. Lansman. 1979. Mitochondrial DNA clones and matriarchal phylogeny within and among geographic populations of the pocket gopher, *Geomys pinetus*. Proceedings of the National Academy of Sciences of the United States of America 76:6694-6698.

Baker, R. H., and G. S. Wilkinson. 2001. Phylogenetic analysis of sexual dimorphism and eye-span allometry in stalk-eyed flies (Diopsidae). Evolution 55:1373-1385.

Baker, R. H., and G. S. Wilkinson. 2003. Phylogenetic analysis of correlation structure in stalk-eyed flies (Diasemopsis, Diopsidae). Evolution 57:87-103.

Bates, H. W. 2001. Contributions to an insect fauna of the Amazon valley: *Lepidoptera*: Heliconidae*in* Foundations of Tropical Forest Biology (R. L. Chazdon, and T. C. Whitmore, eds.). University of Chicago Press, Chicago.

Behrens, W. A., and J. Himms-Hagen. 1977. Alteration in skeletal muscle mitochondria of cold-acclimated rats: Association with enhanced metabolic response to noradrenaline. Journal of Bioenergetics and Biomembranes 9:41-63.

Bermingham, E., and J. C. Avise. 1986. Molecular zoogeography of freshwater

fishes in the southeastern United States. Genetics 113:939-965.

Birstein, V. J., P. Doukakis, B. Sorkin, and R. DeSalle. 1998. Population Aggregation Analysis of Three Caviar-Producing Species of Sturgeons and Implications for the Species Identification of Black Caviar. Conservation Biology 12:766-775.

Blair, W. F. 1958. Distributional patterns of vertebrates in the Southern United States in relation to past and present environments. Publ. Amer. Ass. Advanc. Sci. 51:433-468.

Blaney, R. M. 1971. An annotated check list and biogeographic analysis of the insular herpetofauna of the Apalachicola region, Florida. Herpetologica 27:406-430.

Brodie, E. D., III, . 1993. Differential avoidance of coral snake banded patterns by free-ranging avian predators in Costa Rica. Evolution 47:227-235.

Brodie, E. D. I., and F. J. Janzen. 1995. Experimental studies of coral snake mimicry: generalized avoidance of ringed snake patterns by free-ranging avian predators. Functional Ecology 9:186-190.

Brooks, D. R., and D. A. McLennan. 1991. Phylogeny, ecology, and behaviour: a research program in comparative biology. University of Chicago Press, Chicago.

Brower, A. V. Z. 1996. Parallel race formation and the evolution of mimicry in Heliconius butterflies: a phylogenetic hypothesis from mitochondrial DNA sequences. Evolution 50:195-221.

Brower, A. V. Z. 1996. Parallel race formation and the evolution of mimicry in Heliconius butterflies: a phylogenetic hypothesis from mitochondrial DNA sequences. Evolution 50:195-221.

Bryson, R. W., Jr., J. Pastorini, F. T. Burbrink, and M. R. J. Forstner. 2007. A phylogeny of the *Lampropeltis mexicana* complex (Serpentes: Colubridae) based on mitochondrial DNA sequences suggests evidence for species-level polyphyly within *Lampropeltis*. Molecular Phylogenetics and Evolution 43:674-684.

Burbrink, F. 2008. Personal communication.

Burbrink, F. T. 2001. Systematics of the eastern ratsnake complex (Elaphe Obsoleta). Herpetological Monographs 15:1-53.

Burbrink, F. T., F. Fontanella, R. A. Pyron, T. J. Guihera, and C. Jimeneza. 2007. Phylogeography across a continent: The evolutionary and demographic history of the North American racer (Serpentes: Colubridae: *Coluber constrictor*). Molecular Phylogenetics and Evolution 47:274-288.

Burbrink, F. T., and R. Lawson. 2007b. How and when did Old World ratsnakes disperse into the New World? Molecular Phylogenetics and Evolution 43:173-189.

Burbrink, F. T., R. Lawson, and J. B. Slowinski. 2000. Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): A critique of the subspecies concept. Evolution 54:2107-2118.

Caley, M. J., and D. Schluter. 2003. Predators favour mimicry in a tropical reef fish. Proceedings of the Royal Society of London. Series B. Biological Sciences 270:667-672.

Calsbeek, R., J. N. Thompson, and J. E. Richardson. 2003. Patterns of molecular evolution and diversification in a biodiversity hotspot: the California Floristic Province. Molecular Ecology 12:1021-1029.

Carpenter, J. 1988. Choosing among multiple equally parsimonious cladograms. Cladistics 4:291-296.

Castoe, T. A., E. N. Smith, R. M. Brown, and C. L. Parkinson. 2007. Higher-level phylogeny of Asian and American coralsnakes, their placement within the Elapidae (Squamata), and the systematic affinities of the enigmatic Asian coralsnake *Hemibungarus calligaster* Wiegmann, 1834. Zoological Journal of the Linnean Society 151:809-831.

Ceccarelli, F. S., and R. H. Crozier. 2006. Dynamics of the evolution of Batesian mimicry: molecular phylogenetic analysis of ant-mimicking *Myrmarachne* (Araneae: Salticidae) species and their ant models. European Society for Evolutionary Biology 20:286-295.

Charleston, M. A. 1998. Jungles: a new solution to the host/parasite phylogeny reconciliation problem. Mathematical Biosciences 149:191-223.

Charleston, M. A., and S. L. Perkins. 2003. Lizards, malaria, and jungles in the Caribbean. Pages 65-92 *in* Tangled trees: phylogeny, cospeciation, and coevolution. (R. D. M. Page, ed.) University of Chicago Press, Chicago & London.

Chittka, L., and D. Osorio. 2007. Cognitive dimensions of predator responses to imperfect mimicry. PLoS Biology 5:2754-2758.

Church, S. A., J. M. Kraus, J. C. Mitchell, D. R. Church, and D. R. Taylor. 2003. Evidence for multiple Pleistocene refugia in the postglacial expansion of the eastern tiger salamander, *Ambystoma tigrinum* tigrinum. Evolution 57:372-383.

Clark, A. M., B. W. Bowen, and L. C. Branch. 1999. Effects of natural habitat fragmentation on an endemic scrub lizard (*Sceloporus woodi*): An historical perspective based on mitochondrial DNA gene genealogy. Molecular Ecology 8:1093-1104.

Clark, A. M., P. E. Moler, E. E. Possardt, A. H. Savitzky, W. S. Brown, and B. W. Bowen. 2003. Phylogeography of the timber rattlesnake (*Crotalus horridus*) based on mtDNA sequences. Journal of Herpetology 37:145-154.

Clusella Trullas, S., J. H. van Wyk, and J. R. Spotila. 2007. Thermal melanism in ectotherms. Journal of Thermal Biology 32:235-245.

Cote, I. M. 2003. The cost of cleanerfish mimics to their models. Journal of Fish Biology 63:228-229.

Cracraft, J. 1989. Speciation and Its Ontology: The Empirical Consequences of Alternative Species Concepts for Understanding Patterns and Processes of Differentiation. Pages xiii, 679 p. *in* Speciation and its consequences (J. A. Endler, and D. Otte, eds.). Sinauer Associates, Sunderland, Mass.

Cracraft, J., and K. Helm-Bychowski. 1991. Parsimony and phylogenetic inference using DNA sequences: some methodological strategies. Pages 184–220 *in* Phylogenetic analysis of DNA sequences (M. M. Miyamoto, and J. Cracraft, eds.). Oxford University Press, Oxford.

Davis, J. I. 1997. Evolution, Evidence, and the Role of Species Concepts in Phylogenetics. SYSTEMATIC BOTANY 22:373-403.

Davis, J. I., and K. C. Nixon. 1992. Populations, genetic variation, and the delimitation of phylogenetic species. Systematic Biology 41:421-435.

Dawkins, R., and J. R. Krebs. 1979. Arms races between and within species. Proceedings of the Royal Society of London. Series B. Biological Sciences 205:489-511.

Delcourt, P. A., and H. R. Delcourt. 1981. Vegetation maps for eastern North America: 40,000 yr b.p. to the present. Pages viii, 263 p. *in* Geobotany II (R. C. Romans, ed.) Plenum, New York ; London.

Dowling, H. G., R. Highton, G. C. Maha, and L. R. Maxson. 1983. Biochemical evaluation of colubrid snake phylogeny. Journal of Zoology (London) 201:309-329.

Dowling, H. G., and L. R. Maxson. 1990. Genetic and taxonomic relations of the short-tailed snakes, genus *Stilosoma*. Journal of Zoology (London) 221:77-85.

Dupont, S., J. Mallefet, and Y. Dewael. 2001. Natural bioluminescence as a genetic marker for ophiuroid species. Belgian Journal of Zoology 131:89-94.

Eckerman, C. M., and E. J. Walsh. 1997. Breaking the formalin barrier: Development of molecular techniques for genetic analysis of museum specimens.

Edmunds, M. 2000. Why are there good and poor mimics? Biological Journal of the Linnean Society 70:459-466.

Edmunds, M., and Y. C. Golding. 1999. Diversity in mimicry. Trends in Ecology & Evolution 14:150.

Endler, J. A., and J. J. D. Greenwood. 1988. Frequency-Dependent Predation, Crypsis and Aposematic Coloration [and Discussion]. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences (1934-1990) 319:505-523.

Excoffier, L., L. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50.

Exnerova, A., K. Svadova, P. Stys, S. Barcalova, E. Landova, M. Prokopova, R. Fuchs, and R. Socha. 2006. Importance of colour in the reaction of passerine predators to aposematic prey: experiments with mutants of *Pyrrhocoris apterus* (Heteroptera). Biological Journal of the Linnean Society 88:143-153.

Farris, J. 1969. A successive approximations approach to character weighting. Systematic Zoology 18:374-385.

Farris, J. S., V. A. Funk, and N. I. Platnick. 1984. The logical basis of phylogenetic analysis. Conceptual Issues in Evolutionary Biology. MIT Press, Cambridge, MA:675–702.

Fetzner-Jr., J. W. 2001. Personal communication.

Fontanella, F. 2008. Personal communication.

Fontanella, F. M., C. R. Feldman, M. E. Siddall, and F. T. Burbrink. 2008. Phylogeography of *Diadophis punctatus*: extensive lineage diversity and repeated patterns of historical demography in a trans-continental snake. Molecular Phylogenetics and Evolution 46:1049-1070.

Futuyma, D. J. 1998. Evolutionary Biology, 3rd edition. Sinauer Associates, Inc., Sunderland.

Gavrilets, S. 1997. Coevolutionary chase in exploiter-victim systems with polygenic characters. Journal of Theoretical Biology 186:527-534.

Gavrilets, S., and A. Hastings. 1998. Coevolutionary chase in two-species systems with applications to mimicry. Journal of Theoretical Biology 191:415-427.

George, D. W., and H. C. Dessauer. 1970. Immunological correspondence of transferrins and the relationships of colubrid snakes. Comp. Biochem. Physiol. 33:617-627,2 pis.

Gibbons, J. R. H., and H. B. Lillywhite. 1981. Ecological segregation, color matching, and speciation in lizards of the *Amphibolurus decresii* species complex (Lacertilia: Agamidae). Ecology (Washington D C) 62:1573-1584.

Gilbert, F. 2005. The Evolution of Imperfect Mimicry. Insect Evolutionary Ecology: Proceedings of the Royal Entomological Society's 22nd Symposium.

Gilbert, M. T. P. 2007. The isolation of nucleic acids from fixed, paraffin-embedded tissues – which methods are useful, when? . PloS One 2:e537: 1-12.

Goldstein, P. Z., and R. DeSalle. 2000. Phylogenetic species, nested hierarchies, and character fixation. Cladistics 16:364-384.

Goloboff, P.A., J. S. Farris and K. C. Nixon. TNT, a free program for phylogenetic analysis. Cladistics 24:1–13

Greene, H. 2001. Personal communication.

Greene, H. W., and R. W. McDiarmid. 1981. Coral snake mimicry: Does it occur? Science 4513.

Greene, H. W., and R. W. McDiarmid. 2005. Wallace and Savage: heroes, theories and venomous snake mimicry. Pages 190-208 *in* Ecology and evolution in the tropics: a herpetological perspective. (M. A. Donnelly, B. I. Crother, C. Guyer, M. H. Wake, and M. E. White, eds.). University of Chicago Press, Chicago & London.

Grobman, A. B. 1978. An alternative solution to the coral snake mimicry problem (Reptilia, Serpentes, Elapidae). Journal of Herpetology 12:1-11.

Guindon, S., F. Lethiec, P. Duroux, and O. Gascuel. 2005. PHYML Online--a web server for fast maximum likelihood-based phylogenetic inference. Nucleic Acids Research 1:W557-9.

Hafner, M. S., and R. D. M. Page. 1995. Molecular phylogenies and host-parasite cospeciation: gophers and lice as a model system. Royal Society Philosophical Transactions Biological Sciences 349:77-83.

Harper, G. R., Jr., and D. W. Pfennig. 2007. Mimicry on the edge: why do mimics vary in resemblance to their model in different parts of their geographical range? Proceedings of the Royal Society Biological Sciences Series B 274:1955-1961.

Harper, G. R., Jr., and D. W. Pfennig. 2008. Selection overrides gene flow to break down maladaptive mimicry. Nature (London) 451:1103-1106.

Hartl, D. L., and A. G. Clark. 1997. Principles of population genetics. Third edition. Sinauer Associates, Inc., Sunderland, Massachusetts.

Hennig, W. 1966. Phylogenetic systematics. Univ. Illinois Press, Urbana.

Hinman, K. E., H. L. Throop, K. L. Adams, A. J. Dake, K. K. McLauchlan, and M. J. McKone. 1997. Predation by free-ranging birds on partial coral snake mimics: the importance of ring width and color. Evolution 51:1011-1014.

Holen, O. H. 2008. Personal communication.

Holen, O. H., and R. A. Johnstone. 2004. The evolution of mimicry under constraints. American Naturalist 164:598-613.

Holloway, G., F. Gilbert, and A. Brandt. 2002. The relationship between mimetic imperfection and phenotypic variation in insect colour patterns. Proceedings of the Royal Society Biological Sciences Series B 269:411-416.

Holman, J. A. 2000. Fossil snakes of North America: origin, evolution, distribution, paleoecology. Indiana University Press, Bloomington & Indianapolis.

Huelsenbeck, J. P. 1995. Performance of phylogenetic methods in simulation. Systematic Biology 44:17-48.

Huey, R. B., and A. F. Bennett. 1987. Phylogenetic studies of coadaptation: preferred temperatures versus optimal performance temperatures of lizards. Evolution 41:1098-1115.

Humphries, C. J., and L. R. Parenti. 1999. Cladistic biogeography : interpreting patterns of plant and animal distributions, 2nd edition. Oxford University Press, Oxford ; New York.

Jackson, S. T., R. S. Webb, K. H. Anderson, J. T. Overpeck, T. W. III, J. W. Williams, and B. C. S. Hansene. 2000. Vegetation and environment in Eastern North America during the Last Glacial Maximum. Quaternary Science Reviews 19:489-508.

Johnstone, R. A. 2002. The evolution of inaccurate mimics. Nature (London) 418:524-526.

Joron, M., and J. L. B. Mallet. 1998. Diversity in mimicry: paradox or paradigm? TREE 13:461-466.

Kazakova, T. B., and K. A. Markosian. 1966. Comparison of physicochemical properties of mitochondrial and nuclear deoxyribonucleic acid from rat liver cells. Nature, Lond. 211:79-80.

Kearney, M. 2002. Fragmentary taxa, missing data and ambiguity: Mistaken assumptions and conclusions. Systematic Biology 51:369-381.

Keogh, J. S. 1996. Evolution of the colubrid snake tribe Lampropeltini: a morphological perspective. Herpetologica 52:406-416.

Keogh, J. S. 1998. Molecular phylogeny of elapid snakes and a consideration of their biogeographic history. Biological Journal of the Linnean Society 63:177-203.

Kitching, I. J., P. L. Forey, C. J. Humphries, and D. M. Williams. 1998. Cladistics: The Theory and Practice of Parsimony Analysis. Oxford University Press, Oxford, New York, Tokyo.

Kopp, M., and S. Gavrilets. 2006. Multilocus genetics and the coevolution of quantitative traits. Evolution 60:1321-1336.

Lawson, R., and H.C. Dessauer. 1981. Electrophoretic evaluation of the colubrid genus *Elaphe* (Fitzinger). Isozyme Bull 14:83.

Leenders, T., G. Beckers, and H. Strijbosch. 1996. *Micrurus mipartitus* (NCN). Polymorphism. Herpetological Review 27:25.

Lehn, K. 2005. DeSalle Laboratory Manual. AMNH Sackler Institute for Comparative Genomics, NY.

Liu, F.-G. R., P. E. Moler, and M. M. Miyamoto. 2006. Phylogeography of the salamander genus *Pseudobranchus* in the southeastern United States. Molecular Phylogenetics and Evolution 39:149-159.

Loehle, C. 2007. Predicting Pleistocene climate from vegetation in North America. Climate of the Past 3:109-118.

Luckow, M. 1995. Species Concepts: Assumptions, Methods, and Applications. SYSTEMATIC BOTANY 20:589-605.

Malhotra, A., and R. S. Thorpe. 2000. The dynamics of natural selection and vicariance in the Dominican anole: patterns of within-island molecular and morphological divergence. Evolution 54:245-258.

Mallet, J., and M. Joron. 1999. Evolution of diversity in warning color and mimicry: Polymorphisms, shifting balance and speciation. Annual Review of Ecology and Systematics 30:201-233.

Manier, M. K. 2004. Geographic variation in the long-nosed snake, *Rhinocheilus lecontei* (Colubridae): beyond the subspecies debate. Biological Journal of the Linnean Society 83:65-85.

Mappes, J., N. Marples, and J. A. Endler. 2005. The complex business of survival by aposematism. Trends in Ecology and Evolution 20:598-603.

Marples, N. M., D. J. Kelly, and R. J. Thomas. 2005. Perspective: The evolution of warning coloration is not paradoxical. Evolution 59:933-940.

Marshall, S. D., and M. A. Deyrup. 2000. Biogeography and conservation biology of Florida'a *Geolycosa* wols spiders: threatened spiders in endangered ecosystems. Journal of Insect Conservation 4:11-21.

Mayden, R. L. 1988. Vicariance biogeography, parsimony, and evolution in North American freshwater fishes. Systematic Zoology 37:329-355.

Meyer, A. 2006. Repeating Patterns of Mimicry. PLoS Biol 4:e341.

Miyamoto, M. M., and J. Cracraft. 1991. Phylogenetic analysis of DNA sequences. Oxford University Press, New York.

Moler, P. 2008. Personal communication.

Morey, L. C., R. K. Blashfield, and H. A. Skinner. 1983. A Comparison of Cluster Analysis Techniques Withing a Sequential Validation Framework. Multivariate Behavioral Research 18:309 - 329.

Moriarty Lemmon, E., A. R. Lemmon, J. T. Collins, and D. C. Cannatella. 2008. A new North American chorus frog species (Amphibia: Hylidae: *Pseudacris*) from the south-central United States. Zootaxa 1675:1-30.

Moritz, C., C. J. Schneider, and D. B. Wake. 1992. Evolutionary relationships within the *Ensatina eschscholtzii* complex confirm the ring species interpretation. Systematic Biology 41:273-291.

Müller, F. 1879. Transactions of the Entomolgical Society of London:20-24.

NCBI. National Center for Biotechnology Information.

Neill, W. T. 1957. Historical biogeography of present-day Florida. Bull. Florida State Mus. Biol. Sci. 2:175-220.

Neudecker, S. 1989. Eye camouflage and false eyespots: chaetodontid responses to predators. Environmental Biology of Fishes 25:143-157.

Nixon, K. C. 2002. WinClada ver. 1.00.08. Published by the author, Ithaca, NY.

Nixon, K. C., and Q. D. Wheeler. 1990. An amplification of the phylogenetic species concept. Cladistics 6:211-223.

Norrstrom, N., W. M. Getz, and N. M. A. Holmgren. 2006. Coevolution of exploiter specialization and victim mimicry can be cyclic and saltational.

Evolutionary Bioinformatics Online 1:1-9.

Nussear, K. E., E. T. Simandle, and C. R. Tracy. 2000. Misconceptions about colour, infrared radiation, and energy exchange between animals and their environments. Herpetological Journal 10:119-122.

Olson, R. J., C. J. Emerson, M. K. Nungesser, Oak Ridge National Laboratory., and United States. Dept. of Energy. 1980. Geoecology : a county-level environmental data base for the conterminous United States. Oak Ridge National Laboratory, Oak Ridge.

Omernik, J. M., Association of American Geographers., and Corvallis Environmental Research Laboratory. 1986. Ecoregions of the conterminous United StatesCorvallis Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, OR.

Page, R. D. M. 1993. Genes, organisms, and areas: the problem of multiple lineages. Systematic Biology 42:77-84.

Page, R. D. M. 1994. Maps between trees and cladistic analysis of historical associations among genes, organisms and areas. Systematic Biology 43:58-77.

Page, R. D. M., and M. A. Charleston. 1998. Trees within trees: phylogeny and historical associations. Trends in Ecology & Evolution 13:356-359.

Page, R. D. M., and M. S. Hafner. 1996. Molecular phylogenies and host-parasite cospeciation: gophers and lice as a model system. Pages 255-270 *in* New Uses for New Phylogenies (P. H. Harvey, A. J. L. Brown, J. M. Smith, and S. Nee, eds.). Oxford University Press, Oxford, New York, Tokyo.

Pauly, G. B., O. Piskurek, and H. B. Shaffer. 2007. Phylogeographic concordance in the southeastern United States: the flatwoods salamander, *Ambystoma cingulatum*, as a test case. Molecular Ecology 16:415-429.

Peters, J. L., W. Gretes, and K. E. Omland. 2005. Late Pleistocene divergence between eastern and western populations of wood ducks (Aix sponsa) inferred by the 'isolation with migration' coalescent method. Molecular Ecology 14:3407-3418.

Pfennig, D. W., W. R. Harcombe, and K. S. Pfennig. 2001. Frequency-dependent Batesian mimicry. Nature (London) 410:323.

Pfennig, D. W., G. R. Harper, Jr., A. F. Brumo, W. R. Harcombe, and K. S.

Pfennig. 2007. Population differences in predation on Batesian mimics in allopatry with their model: selection against mimics is strongest when they are common. Behavioral Ecology and Sociobiology 61:505-511.

Pough, F. H. 1988. Mimicry of vertebrates: are the rules different? The American Naturalist 131 (supplement):S67-S102.

Rasband, W. S. 1997-2008. ImageJ. U. S. National Institutes of Health.

Robin, E. D., and R. Wong. 1988. Mitochondrial DNA molecules and virtual number of mitochondria per cell in mammalian cells. Journal of Cellular Physiology 136:507-513.

Rodriguez-Robles, J. A., and J. M. De Jesus-Escobar. 1999. Molecular systematics of New World lampropeltinine snakes (Colubridae): implications for biogeography and evolution of food habits. Biological Journal of the Linnean Society 68:355-385.

Rodriguez-Robles, J. A., and J. M. De Jesus-Escobar. 2000. Molecular systematics of New World gopher, bull, and pinesnakes (*Pituophis*: Colubridae), a transcontinental species complex. Molecular Phylogenetics and Evolution 14:35-50.

Rodriguez-Robles, J. A., D. F. Denardo, and R. E. Staub. 1999. Phylogeography of the California mountain kingsnake, *Lampropeltis zonata* (Colubridae). Molecular Ecology 8:1923-1934.

Roman, J., S. D. Santhuff, P. E. Moler, and B. W. Bowen. 1999. Population structure and cryptic evolutionary units in the alligator snapping turtle. Conservation Biology 13:135-142.

Ronquist, F. 2003. Parsimony analysis of co-evolving species associations. Pages 22-64 *in* Tangled Trees: Phylogeny, Cospeciation and Coevolution (R. D. M. Page, ed.) University of Chicago Press, Chicago and London.

Rosas, J., J. Sanchez-DelBarrio, X. Messeguer, and R. Rozas. 2003. DnaSP, DNA polymorphism analyses by the coalecent and other methods. Bioinformatics 19:2496-2497.

Roze, J. A. 1996. Coral snakes of the Americas: biology, identification, and venoms. Krieger Publishing Company, Malabar, Florida.

Russo, C. A. M., N. Takezaki, and M. Nei. 1996. Efficiencies of different genes and different tree-building methods in recovering a known vertebrate

phylogeny. Molecular Biology and Evolution 13:525-536.

Rzhetsky, A., and M. Nei. 1992. A simple method for estimating and testing minimum evolution trees. Molecular Biology and Evolution 9:945-967.

Rzhetsky, A., and M. Nei. 1993. Theoretical foundation of the minimum-evolution method of phylogenetic inference. Molecular Biology and Evolution 10:1073-1095.

Savage, J. M. 1982. The enigma of the Central American herpetofauna: dispersal or vicariance? Ann. Missouri Bot. Garden 69:464-547.

Schander, C., and K. M. Halanych. 2003. DNA, PCR and formalinized animal tissue: A short review and protocols. Organisms Diversity & Evolution 3:195-205.

Schmidt, K. P. 1928. Notes on American Coral Snakes. Bull. Antivenin Instit. Amer. Glenolden 2:pp. 63, 64.

Sewell, M. M., C. R. Parks, and M. W. Chase. 1996. Intraspecific chloroplast DNA variation and biogeography of North American *Liriodendron* L. (Magnoliaceae). Evolution 50:1147-1154.

Shedlock, A. M., M. G. Haygood, T. W. Pietsch, and P. Bentzen. 1997. Enhanced DNA extraction and PCR amplification of mitochondrial genes from formalin-fixed museum specimens. BioTechniques 22:394-400.

Sherratt, T. N. 2002. The evolution of imperfect mimicry. Behavioral Ecology 13:821.

Slowinski, J. B. 1995. A phylogenetic analysis of the New World coral snakes (Elapidae: Leptomicrurus, Micruroides, and Micrurus) based on allozymic and morphological characters. Journal of Herpetology 29:325-338.

Slowinski, J. B., and J. S. Keogh. 2000. Phylogenetic relationships of elapid snakes based on cytochrome b mtDNA sequences. Molecular Phylogenetics and Evolution 15:157-164.

Smith, S. M. 1975. Innate recognition of coral snake pattern by a possible avian predator. Science. 187:759-760.

Soltis, D. E., A. B. Morris, J. S. McLachlan, P. S. Manos, and P. S. Soltis. 2006. Comparative phylogeography of unglaciated eastern North America. Molecular Ecology 15:4261-4293.

Swofford, D. L., G. J. Olsen, P. J. Waddell, and D. M. Hillis. 1996. Phylogenetic Inference. Pages 407-514 *in* Molecular Systematics (D. M. Hillis, C. Moritz, and B. K. Mable, eds.). Sinauer Associates, Inc., Sunderland, MA.

Takacs, Z., 2003. Personal communication.

Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24:1596-1599.

Tanaka, K. 2005. Thermal aspects of melanistic and striped morphs of the snake *Elaphe quadrivirgata*. Zoological Science (Tokyo) 22:1173-1179.

Tang, E. P. Y. Year. Path to Effective Recovering of DNA from Formalin-Fixed Biological Samples in Natural History Collections: Workshop Summary *in*. National Academies Press.

Thery, M., S. Pincebourde, and F. Feer. 2008. Dusk light environment optimizes visual perception of conspecifics in a crepuscular horned beetle. Behavioral Ecology 19:627-634.

Thorpe, R., D. Leadbeater, and C. Pook. 2005. Molecular clocks and geological dates: cytochrome *b* of *Anolis extremus* substantially contradicts dating of Barbados emergence. Molecular Ecology 14:2087-2096.

Trapnell, D. W., J. P. Schmidt, P. F. Quintana-Ascencio, and J. L. Hamrick. 2007. Genetic Insights into the Biogeography of the Southeastern North American Endemic, *Ceratiola ericoides* (Empetraceae). Journal of Heredity 98:587-593.

Waldbauer, G. P. 1988. Aposematism and Batesian mimicry. Measuring mimetic advantage in natural habitats. Evolutionary Biology (New York) 22:227-259.

Walker, D., and J. C. Avise. 1998. Principles of phylogeography as illustrated by freshwater and terrestrial turtles in the southeastern United States. Annual Review of Ecology and Systematics 29:23-58.

Wallace, A. R. 1871. Contributions to the theory of natural selection. McMillan, London.

Waltari, E., R. J. Hijmans, A. Townsend Peterson, A. S. Nyari, S. L. Perkins, and R. P. Guralnick. 2007. Locating Pleistocene refugia: comparing

phylogeographic and ecological niche model predictions. PLoS ONE 2:1-11.

Ward, J. H. 1963. Hierarchical grouping to optimize an objective function. American Statistical Association Journal 56:236-244.

Watts, W. A., and M. Stuiver. 1980. Late Wisconsin climate of northern Florida and the origin of species-rich deciduous forest. Science 210:325-327.

Weisrock, D. W., and F. J. Janzen. 2000. Comparative molecular phylogeography of North American softshell turtles (*Apalone*): implications for regional and wide-scale historical evolutionary forces. Molecular Phylogenetics and Evolution 14:152-164.

Wiens, J. J. 1998. Does adding characters with missing data increase or decrease phylogenetic accuracy. Systematic Biology 47:625-640.

Wiens, J. J. 2003. Missing data, incomplete taxa and phylogenetic accuracy. Systematic Biology 52:528-538.

Wiley, E. O. 1988. Parsimony analysis and vicariance biogeography. Systematic Zoology 37:271-290.

Williams, K. L. 1988. Systematics and natural history of the American milk snake, *Lampropeltis triangulum*. Milwaukee Public Museum, Milwaukee.

Williams, P. 2007. The distribution of bumblebee colour patterns worldwide: possible significance for thermoregulation, crypsis, and warning mimicry. Biological Journal of the Linnean Society 92:97-118.

Zamudio, K. R., and H. W. Greene. 1997. Phylogeography of the bushmaster (*Lachesis muta*: Viperidae): implications for neotropical biogeography, systematics and conservation. Biological Journal of the Linnean Society 62:421-442.