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Phylogeography Of Northern Populations Of The Black-Tailed Rattlesnake (crotalus Molossus Baird And Girard, 1853) With The Revalidation Of C. Ornatus Hallowell, 1854

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PHYLOGEOGRAPHY OF NORTHERN POPULATIONS OF THE BLACK-
TAILED RATTLESNAKE (*CROTALUS MOLOSSUS* BAIRD AND GIRARD,
1853) WITH THE REVALIDATION OF *C. ORNATUS* HALLOWELL, 1854

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Acting Dean of the Graduate School

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by

Christopher G. Anderson

2011

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by

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THESIS

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ABSTRACT

The black-tailed rattlesnake (*Crotalus molossus* Baird and Girard, 1853) is a wide-ranging species complex with representatives from the southwestern United States through the highlands of central and southern Mexico. The systematics of this group has received little attention and in the past six decades only two taxonomic revisions have been proposed (the transfer of *C. basiliscus oaxacus* to *C. molossus* and the elevation of *C. m. estebanensis* to full species). However, a recent revision of the Neotropical rattlesnakes (*C. durissus* and *C. simus*) recovered a polyphyletic *C. molossus*. Sequenced data were obtained from three mitochondrial genes (ATPases 8 and 6, cyt *b* and ND4) and three nuclear genes (*c-mos*, EXPH5 and RAG1) to examine phylogenetic relationships among *C. molossus* lineages and the closely related species *C. basiliscus* and *C. totonacus*. Results recovered strong support for two deeply divergent and polyphyletic northern *C. molossus* lineages that are morphologically distinct. Biogeographical patterns and divergence dates inferred from mitochondrial data suggest the diversification of *C. molossus* is the result of a Neogene vicariance event associated with uplift of the Sierra Madre Occidental. Population-level analyses suggested that major lineages of *C. molossus* have been stable since their initial divergence. On the basis of morphological and molecular evidence I propose that the synonym *Crotalus ornatus* Hallowell 1854 be resurrected and applied to populations in the Chihuahuan Desert and central Texas. This thesis represents the most complete phylogenetic analysis of any member of the *C. molossus* group and demonstrates discordance between the contemporary taxonomy of the group and its true diversity.

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CHAPTER 1: GENERAL INTRODUCTION

1.1 INTRODUCTION TO THE FAMILY VIPERIDAE

The Viperidae is a diverse and highly derived group united by the solenoglyphous condition, in which a large canaliculated fang is attached to a highly kinetic maxillary bone that is used for the delivery of venom. Vipers have been the focus of a plethora of research spanning the disciplines of ecology, physiology, systematics, functional morphology, behavior, development and medicine (e.g., Scheut and Grober 2000; Greenbaum 2004; Meik and Pires-daSilva 2009). The functional morphology of the viperid venom apparatus has received a great deal of attention by the scientific community (e.g., Kardong 1982; Vonk et al. 2008). Venom components have garnered much attention among medical researchers, particularly in the treatment of envenomation in humans and potential pharmaceutical applications, making the Viperidae an extremely important taxon in medicine. Norris (2004) and Warrell (2004) provided extensive discussions of medical implications in North America and South America, respectively.

Systematics of the Viperidae has been the focus of many previous studies, and higher-level relationships are relatively well understood. Monophyly of the vipers is unchallenged and their phylogenetic position near the base of the Colubroidea (the superfamily including the Xenodontidae, Pareatidae, Viperidae, Elapidae and all historical and current members of the Colubridae) has been supported by several recent molecular studies (Kelly et al. 2003; Wiens et al. 2008; Zaher et al. 2009; Pyron et al. 2011). Since their divergence from the remaining Colubroidea in the early to mid- Paleogene (67–38 million years ago [mya]) (Wüster et al. 2008;

Vidal et al. 2009), the vipers diversified into ~295 extant species in three subfamilies: the Viperinae (true vipers, 13 genera), Azemiopinae (Feae's viper, one monotypic genus) and the Crotalinae (pitvipers, 30 genera). Relationships between the subfamilies are widely accepted and supported by morphological and molecular evidence (Campbell and Lamar 2004). Previously, Azemiopinae and Causinae (the African genus *Causus*) were presumed to be basal viperids (Liem et al. 1971; Groombridge 1986). Consequently, several authors have erroneously assigned *Causus* as an outgroup in studies focused on the Viperinae (e.g., Herman et al. 1999; Lenk et al. 2001). However, subsequent molecular analyses have shown *Causus* to be nested within the Viperinae (Wüster et al. 2008; Pyron et al. 2011), and Azemiopinae was identified as the sister group to the Crotalinae, a relationship first suggested by Cadle (1992) and later supported by molecular data (Kelly et al. 2003; Wiens et al. 2008; Wüster et al. 2008; Pyron et al. 2011). In spite of recent advances in viperid systematics, the geographical origin of the Viperidae remains undefined, but molecular and biogeographical data suggest an Asian origin for the family (Wüster et al. 2008).

The Viperidae is a wide-ranging family with representatives occupying a variety of ecological niches throughout tropical and temperate regions worldwide, exclusive of many oceanic islands (e.g., the Hawaiian and Galapagos islands) and the landmasses south of Wallace's line (i.e., southeast Indonesia, Australia and New Zealand). The Viperinae (~88 species) is widespread through most of Africa, Europe, the Middle East, India and islands of the Sunda Shelf in Southeast Asia. Azemiopinae is a monotypic taxon represented by *Azemiops feae* from southern China and adjacent portions of Vietnam and Myanmar. The ~206 species in the Crotalinae are distributed through Indonesia, Asia and the Americas, with one species (*Gloydius halys*) present in southeastern Europe. Only the Crotalinae were able to invade the New World.

The exceptional diversity of pitvipers in the Americas is the result of a single species, a member of the lineage that gave rise to the Asian genus *Gloydus*, which entered North America through Beringia (Malhotra et al. 2010). A specific date for this interchange is unclear. However, the most recent estimates suggest that pitvipers entered the Americas in the late Oligocene or early Miocene (14–27 mya) (Wüster et al. 2008; Castoe et al. 2009), a date that closely follows the invasion of the New World by the Lampropeltini snakes (Burbrink and Lawson 2007). Global cooling beginning at the Eocene-Oligocene boundary transformed the Beringian climate, reducing the wide subtropical forest to a narrow band of boreal forest by the mid-Miocene (Sanmartin et al. 2001). This cooling would have effectively stopped the exchange of squamate groups through the Bering Land Bridge. Fossils representing *Agkistrodon*, *Crotalus* and *Sistrurus* appear in central North America by the mid- to late Miocene (~9 mya), and fossils of unidentified viperid species are present in early Miocene deposits from Nebraska (Parmley and Holman 2007). Additionally, molecular data suggest that most New World pitviper genera diverged by the mid-Miocene (Castoe et al. 2009; Wüster et al. 2008). In the Americas, pitvipers underwent a massive radiation, resulting in 14 genera and ~117 species occurring throughout southern North America and the Neotropics. One of these groups, the rattlesnakes (*Crotalus* and *Sistrurus*), has experienced an extensive diversification in temperate North America (Campbell and Lamar 2004).

1.2 INTRODUCTION TO *CROTALUS*

Thirty-five species of *Crotalus* (Alvarado-Diaz and Campbell 2004; Wüster et al. 2005; Quijada-Mascareñas and Wüster 2006; Douglas et al. 2007; Beaman and Hayes 2008; Campbell and Flores-Villela 2008) occupy a range from southern Canada through the continental United

States to western Costa Rica and South America east of the Andes (Campbell and Lamar 2004). The phylogenetic position of the rattlesnakes within the Viperidae is stable, with the genus *Agkistrodon* consistently placed as sister to the rattlesnakes, and monophyly of the group is supported by both morphological and molecular evidence (e.g., Campbell and Lamar 2004; Castoe and Parkinson 2006; Wüster et al. 2008; Meik and Pires-daSilva 2009). The rattlesnakes are distinguished from other New World pitvipers by several synapomorphies, including: (1) lack of a nasal pore; (2) position of the loreal pit below the naso-ocular line; and (3) the rattle. The rattle and structures associated with it are sufficient to unite all members of *Crotalus* and *Sistrurus* in a monophyletic group (Campbell and Lamar 2004). Meik and Pires-daSilva (2009) separated rattlesnakes from other New World pitvipers by the numbers of caudal and precloacal vertebrae. In rattlesnakes, incorporation of caudal vertebrae into the style (the fused caudal vertebrae associated with the rattle and rattle matrix) results in a trend toward fewer caudal vertebrae and a shorter tail. Unfortunately, character states of the style do not appear to be useful in addressing relationships between rattlesnake species (Meik and Pires-daSilva 2009).

Previously, the presence of nine large head plates (absent in *Crotalus*) was thought to be a synapomorphy distinguishing the members of *Sistrurus* from *Crotalus* (Campbell and Lamar 2004). However, molecular analyses by Murphy et al. (2002) indicated that *Sistrurus ravus* (a taxon with nine large head plates) is better placed at the base of the *Crotalus* tree, and these authors subsequently suggested a taxonomic revision to reflect this. Campbell and Lamar (2004) upheld this reclassification, but noted the uncertainty surrounding the actual position of *C. ravus* within *Crotalus*. This taxonomic revision was not unexpected, as the monophyly of *Sistrurus* (with respect to *ravus*) had been questioned on previous occasions (e.g., Foote and McMahon 1977; Minton 1992). As is often the case in snake classification, hemipenis morphology is a

good diagnostic tool for distinguishing *Crotalus* from *Sistrurus*. In *Crotalus*, the hemipenes are usually slightly longer (8–12 subcaudals in length when everted versus 5–10 in *Sistrurus*) and with few exceptions, basal spines abruptly transition to calyces, which are small pockets between retiform ridges on the hemipenis. In the case of *Sistrurus*, basal spines grade into calyces (Campbell and Lamar 2004).

1.3 BIOGEOGRAPHY OF *CROTALUS*

It is likely that after its introduction to the New World, the ancestor of the American pitvipers would have rapidly expanded its range through southern North America and Central America. The initial divergence in this region resulted in a Central American bothropoid group (the ancestor of *Atropoides*, *Bothriechis*, *Bothriopsis*, *Bothrocophias*, *Bothropoides*, *Bothrops*, *Cerrophidion* and *Porthidium*) and a North American/Mexican lineage (the ancestor of *Agkistrodon*, *Crotalus*, *Lachesis*, *Ophryacus* and *Sistrurus*). Shortly after this initial divergence, the North American lineage split, resulting in an *Ophryacus-Lachesis* group in southern Mexico and Central America, and an *Agkistrodon*-like lineage in Mexico. In the early Miocene, this *Agkistrodon*-like lineage underwent a divergence between populations in the Mexican lowlands and the Mexican plateau, which resulted in the divergence of the rattlesnakes (*Crotalus* and *Sistrurus*) from *Agkistrodon* (Gutberlet and Harvey 2004). It should be noted here that a recent study by Jaden et al. (2011) erected the genus *Mixcoatlus* to remedy the paraphyly of *Agkistrodon*, *Cerrophidion* and *Ophryacus* caused by the poorly known *A. browni*. This revision to the taxonomy of Middle American pitvipers places *A. browni*, *C. barbouri* and *O. melanurus* in the genus *Mixcoatlus*, which is sister to the now monotypic *Ophryacus undulatus* (Jadin et al. 2011).

The genus *Crotalus* reaches its greatest diversity in the vicinity of the Mexican plateau and adjacent mountain ranges. This region of central Mexico is generally viewed as the origin of the rattlesnakes (e.g., Murphy et al. 2002; Campbell and Lamar 2004; Place and Abramson 2004), a hypothesis supported by the basal position of many of the species groups that currently occupy the region, including *C. polystictus*, *C. ravus* and *C. triseriatus* (Klauber 1972; Murphy et al. 2002; Castoe and Parkinson 2006). The earliest rattlesnake fossils from the Miocene of Nebraska (Parmley and Holman 2007) and recent molecular data (Kubatco et al. 2011) suggest that the genus *Sistrurus* diverged and expanded into the Great Plains by the mid-Miocene (9–13 mya), and many of the major species groups within *Crotalus* would have been present by the mid- to late Miocene (Douglas et al. 2006; Wüster et al. 2008; Castoe et al. 2009).

1.4 SYSTEMATICS AND TAXONOMY OF *CROTALUS*

From a taxonomic standpoint, *Crotalus* has been relatively stable with only one recent attempt to reclassify the genus. Hoser (2009a) split *Crotalus* into eight genera (*Crotalus*, three synonyms of *Crotalus* and four new genera) based on his interpretation of a handful of contributions to rattlesnake systematics, particularly from the work of Murphy et al. (2002) and Campbell and Lamar (2004). This review, and Hoser's recent work in general, has garnered a great deal of scrutiny from snake taxonomists. Many criticized his arguments for reclassification, and some have questioned the validity of his publication outlet, *The Australian Journal of Herpetology* (AJH). In response to Hoser's review of the cobras (Hoser 2009b), Wallach et al. (2009) provided a detailed account of AJH and the complicated procedure involved in acquiring a printed version; this ultimately led the authors to the conclusion that the publication does not meet the requirements set forth by the International Code of Zoological Nomenclature, making

the names published therein unavailable. However, it should be noted that a long-standing rivalry (outlined in Hoser 2009c) seems to exist between Hoser and a number of snake taxonomists, including at least one of the coauthors of Wallach et al. (2009). This seems to have begun with a review of the elapid genus *Acanthophis* (Hoser 1998), in which five new species and one new subspecies were described, one of which (*A. wellsi*) is now considered valid. Subsequently, Wüster et al. (2001), Williams et al. (2006) and Wallach et al. (2009) detailed serious flaws in Hoser's methods, including scant evidence in support of reclassification and poor species diagnoses. The arguments for reclassification of the rattlesnakes as presented by Hoser (2009a) do not seem to demonstrate a comprehensive understanding of phylogenetics or a thorough investigation of the existing literature. For example, Hoser assigned the genus *Aechmophrys* to a group comprised of *Crotalus cerastes*, *C. intermedius*, *C. polystictus*, *C. pricei*, *C. tancitarensis*, *C. transversus* and *C. willardi*. Murphy et al. (2002), the study on which most of Hoser's conclusions seem to be based, only recognized this grouping in the preferred phylogeny after accounting for morphological evidence, and failed to recover this group in any of their unweighted parsimony analyses. Moreover, studies that inferred relationships differing from the preferred phylogeny of Murphy et al. (2002) seem to have been ignored (e.g., Parkinson 1999; Parkinson et al. 2002; Castoe and Parkinson 2006).

While not fully resolved, there has been little contention regarding phylogenetic relationships within *Crotalus*. By and large, similar clades and topologies are recognized across multiple morphological and molecular studies (e.g., Klauber 1972; Murphy et al. 2002; Campbell and Lamar 2004). There are, however, some notable exceptions between the two most complete phylogenetic treatments of the rattlesnakes (i.e., Castoe and Parkinson 2006; Murphy et al. 2002). For example, Castoe and Parkinson (2006) identified *C. polystictus* as the basal taxon in

Crotalus, not *C. ravus* (previously *Sistrurus ravus*) as presented in the preferred phylogeny of Murphy et al. (2002). Additional discrepancies exist between these two studies, notably the placement of *C. adamanteus*, *C. enyo*, *C. horridus* and *C. willardi*. In both cases, the authors used the same mitochondrial genes to examine relationships between representatives of the same 27 rattlesnake species, but the two studies implemented different approaches. Murphy et al. (2002) only sought to address relationships among the rattlesnakes, whereas Castoe and Parkinson (2006) examined relationships between 116 Old and New World pitviper species. Murphy et al. (2002) examined one additional gene (resulting in 2.9 kb versus 2.3 kb) with maximum parsimony (MP) analyses, whereas Castoe and Parkinson (2006) implemented MP and Bayesian (partitioned and unpartitioned) analyses. In their MP analysis, Castoe and Parkinson (2006) recovered weak support for clades within *Crotalus* and inferred *Agkistrodon* as the sister to *Lachesis* and *Ophryacus* that, together, form a clade that is sister to the rattlesnakes. The partitioned Bayesian analysis of the latter study recovered a phylogeny more typical of relationships proposed by previous investigators, with *Agkistrodon* inferred as sister to the rattlesnakes, albeit with low support. To date, no study has implemented nuclear markers to address large-scale relationships within the genus *Crotalus*. However, recent studies focusing on *Sistrurus* (Gibbs and Diaz 2010; Kubatko et al. 2011) have identified a number of nuclear markers that seem to be useful in identifying species-level divergence in *Sistrurus* and possible other North American pitviper taxa. Furthermore, there has yet to be a phylogenetic analysis that includes all members of the genus *Crotalus*. Paucity of specimens, unfamiliarity with their natural history, and the recent discovery of one species have prevented the inclusion of the long-tailed rattlesnakes (*C. ericsmithi*, *C. lannomi* and *C. stejnegeri*) in any recent phylogenetic analysis of the genus. Most contention regarding *Crotalus* systematics is associated with fine-

scale problems concerning species complexes, and many distinct species may still be obscured by outdated subspecies taxonomy.

The application of modern molecular techniques has demonstrated deep divergences within species complexes (e.g., Pook et al. 2000; Ashton and de Queiroz 2001; Douglas et al. 2002) and in some cases, identified them as paraphyletic or polyphyletic (e.g., Wüster et al. 2005). Amid the recent push to eliminate the outdated subspecies classification (Collins 1991), several large species complexes within *Crotalus* have received a great deal of attention. A review of *C. mitchellii* by Douglas et al. (2007) resulted in the authors arguing for the elevation of *C. mitchellii stephansi* to species status. Concurrent phylogeographic analyses of *C. viridis* (Pook et al. 2000; Ashton and de Queiroz 2001; Douglas et al. 2002) resulted in the reclassification of the *C. viridis* species complex into three species (Beaman and Hays 2008) (seven species are recognized by Collins and Taggart [2009]). Additionally, taxonomic arguments by Campbell and Lamar (2004), Savage et al. (2005) and Quijada-Mascareñas and Wüster (2006), and phylogeographic evidence presented by Wüster et al. (2005) have resulted in the elevation of four lineages of the Neotropical rattlesnake (*C. durissus*) to species status. The latter study also identified incongruence between the taxonomy and phylogenetic relationships of the black-tailed rattlesnake (*C. molossus*), suggesting the group likely represents a species complex.

1.5 THE BLACK-TAILED RATTLESNAKE

Crotalus molossus Baird and Girard 1853, commonly known as the black-tailed rattlesnake, is a wide-ranging complex of four subspecies (Fig. 1.5.1). The southernmost taxon *C. m. oaxacus* Gloyd 1948 (Campbell and Lamar 1989) occurs in Oaxaca and southeastern

Puebla, Mexico, *C. m. nigrescens* Gloyd 1936 ranges from Michoacán and northern Puebla through much of central Mexico as far north as west-central Chihuahua, *C. m. estebanensis* Klauber 1949 (elevated to a full species by Grismer 1999) occurs only on Isla San Esteban in the Gulf of California, and *C. m. molossus* Gloyd 1936 ranges from the Mexican states of Sonora,

Figure 1.5.1 Geographic distribution of the *Crotalus molossus* group. Modified from a map by Campbell and Lamar (2004).

Chihuahua and Coahuila to central Texas, New Mexico and Arizona (Campbell and Lamar 2004). Throughout this wide geographic range, the black-tailed rattlesnake is a habitat generalist with a strong affinity for rocky terrain, and is often found in or near canyons, cliffs or talus slopes. Elevation records range from near sea level to 2,930 meters (m). Plant associations vary from pine-oak and tropical dry forests to mesquite grassland, and occasionally creosote dominated deserts (Degenhardt et al. 1996; Werler and Dixon 2000; Campbell and Lamar 1989, 2004).

In general, *Crotalus molossus* is a medium-sized rattlesnake with adults averaging about 100 cm in total length (Degenhardt et al. 1996; Campbell and Lamar 2004). Ventral scales number 164–199 in males and 168–201 in females; subcaudals range from 21–30 in males and 16–26 in females. Dorsal scale rows are usually 25 or 27 at midbody. Usually, there are 2–4 loreals, two internasals, two prefrontals, 2–3 preoculars, five postoculars, 2–5 intersupraoculars, 6–10 prefoveals, 13–20 supralabials and 14–21 infralabials (Klauber 1972; Degenhardt et al. 1996; Campbell and Lamar 2004). Campbell and Lamar (2004) discussed the apparent north-south clinal variation within the species, with northern snakes tending to have a light ground color, often with grays, greens and yellows, whereas darker browns and olives commonly appear in the coloration of southern snakes. The dark brown or black rhombic dorsal blotches seem to be more variable in northern snakes, ranging from 20–43, versus 24–34 in southern populations. In nearly all cases, average scale counts for southern snakes are lower than those of northern snakes (Campbell and Lamar 2004).

This moderately sized rattlesnake reaches sexual maturity at about 70 cm (females). Observations of copulation are reported from late summer and fall. Ovulation occurs the following spring, and 3–16 (average of seven) live young are born in July or August. Neonate *C.*

molossus measure 23–31 cm in length and weigh 11–28 grams (Degenhardt et al. 1996; Werler and Dixon 2000).

In the United States, the northern black-tailed rattlesnake is mostly restricted to mountainous regions within the Sonoran and Chihuahuan deserts south of the Colorado Plateau. The species ranges as far north as the Grand Canyon in Arizona, and from there its range extends south through most of Sonora, Mexico. From Sonora and Arizona, *C. molossus* is present through the northern Sierra Madre Occidental and the disjunct mountain ranges between the Sierra Madre and Rocky Mountains in southwestern New Mexico. In the Chihuahuan Desert its distribution follows the mountain ranges along the Rio Grande valley upstream to southern Sandoval County northwest of Albuquerque, New Mexico, and extends southeast through the Trans Pecos region of Texas. A substantial portion of the Edwards Plateau of south-central Texas is also occupied by the species (Degenhardt et al. 1996; Werler and Dixon 2000; Campbell and Lamar 2004). This south-central Texas population is apparently disjunct from the remaining population in Texas (based on range maps provided in Werler and Dixon [2000] and Campbell and Lamar [2004]). In Mexico, *C. m. molossus* occupies most of Sonora, northern and eastern Chihuahua and central Coahuila. There is a substantial intrusion of *C. m. nigrescens* into the northern Sierra Madre Occidental of western Chihuahua (Campbell and Lamar 2004).

The black-tailed rattlesnake is abundant throughout the southern ranges of the Rocky Mountains and the northern ranges of the Sierra Madre Occidental, but is generally absent from the lower valleys between these ranges (Degenhardt et al. 1996). The northern Chihuahuan desert is characterized by rocky mountain ranges often surpassing 2000 m that are separated by arid planes and basins. Here, desert scrubland communities generally occur below 1064 m, and desert grassland communities occupy elevations up to 1580 m, with montane forests occurring at

high elevations of north facing mountain slopes (Werler and Dixon 2000). Annual precipitation ranges from 75 millimeters (mm) at low elevation to 400 mm at high elevations, with the majority of this precipitation tending to fall during the North American monsoon season (Morafka, 1977; Campbell and Lamar 2004) from late June through September, with peak precipitation in late July and August (NOAA 2004). Through much of this region, *C. molossus* is confined to semi-montane and montane habitats, generally occurring in rocky foothills and canyons as well as the mountains that are associated with these features (Degenhardt et al. 1996; Werler and Dixon 2000). At the northwestern border of the Chihuahuan Desert is the Cochise Filter Barrier, a narrow desert corridor that allows biotic exchange between the Sonoran and Chihuahuan ecosystems, when adequate climatic conditions persist. Approximately 4,000 years ago, this region was transformed into the desert grassland conditions that persist now. However, during the Wisconsin glacial maximum (14,000–20,000 years ago), this area experienced a vertical displacement of plant communities as great as 600 m, allowing the contemporary mesic forest communities present at high elevations to dominate lower elevations of the area (Morafka 1977). Similar expansions of forest communities likely occurred in association with other glaciation events and may have permitted contact between eastern and western populations of *C. molossus*.

1.6 SYSTEMATICS OF THE BLACK-TAILED RATTLESNAKE

Previous research of *Crotalus molossus* has focused on ecology (Beck 1995; Greene et al. 2002), physiology (Beaupre 1993) and toxinology (Chen and Real 1997), but systematics of the species has received comparatively little attention. Moreover, general phylogenetic studies of rattlesnakes have either not addressed species closely allied with *C. molossus* (e.g., Pook et al.

2000; Ashton and de Queiroz 2001; Douglas et al. 2006, 2007; Castoe et al. 2007), or have focused on large-scale relationships between many taxa (e.g., Murphy et al. 2002; Castoe and Parkinson 2006).

Several hypotheses regarding the phylogenetic position of the black-tailed rattlesnake have been suggested. Gloyd (1940) recognized a group that included *Crotalus basiliscus*, *C. durissus*, *C. horridus* and *C. molossus*. A well-regarded morphological study by Klauber (1972) identified *C. m. oaxacus* as a subspecies of the Mexican west-coast rattlesnake (*C. basiliscus*), and placed *C. molossus* as sister to the timber rattlesnake (*C. horridus*) based on the shared presence of a vestigial left lung. Murphy et al. (2002) inferred *C. basiliscus* and *C. molossus* as sister taxa based on five mitochondrial genes. Campbell and Lamar (2004) presented a consensus phylogeny for the American pitvipers, grouping *C. basiliscus* and *C. molossus* in a clade with the Neotropical rattlesnakes (*C. durissus*, *C. simus* and *C. totonacus*) and *C. enyo*; this conclusion was largely based on the results of Murphy et al. (2002). The latest available phylogeny that includes most of the recognized members of *Crotalus* (Castoe and Parkinson 2006) infers a similar relationship between *C. basiliscus*, *C. enyo*, *C. molossus* and the Neotropical rattlesnakes. To date, no thorough phylogenetic study of any member of the *C. molossus* complex has been completed.

One recent contribution (Wüster et al. 2005) to the systematics of the black-tailed rattlesnake came from a review of the Neotropical rattlesnakes (*Crotalus durissus* and *C. simus*), a species complex closely related to *C. molossus*. This study incorporated several *C. molossus* samples from the three mainland subspecies. The results of the two-gene analysis recovered a weakly supported, paraphyletic *C. molossus* species complex, and moreover, the nominate subspecies (*C. m. molossus*) was recovered as polyphyletic with very low support (Fig. 1.6.1). A

well-supported clade including *C. basiliscus*, *C. totonacus* and the three mainland subspecies of *C. molossus* were placed as sister to the Neotropical group (*C. durissus* and *C. simus*). Within this clade, *C. m. nigrescens* is inferred as the basal member of the *C. molossus* group (*C. m. molossus*, *C. m. nigrescens*, *C. m. oaxacus* and *C. estebanensis*). A subsequent divergence resulted in a *C. totonacus*/eastern *C. m. molossus* clade assigned as sister to a *C. m. oaxacus* and *C. basiliscus*/western *C. m. molossus* clade. Both phylogenies presented by Wüster et al. (2005) display similar topologies and similarly weak support between eastern and western populations of *C. m. molossus*, and their relationships to *C. basiliscus* and *C. totonacus*.

Figure 1.6.1 Phylogenetic relationships among lineages of the *Crotalus molossus* group, simplified from Wüster et al. (2005). Support values at nodes are Bremer support/maximum parsimony bootstrap/maximum likelihood bootstrap/Bayesian posterior probability. Dashes indicate no support.

1.7 RESEARCH FOCUS AND OBJECTIVES

The discipline of phylogeography utilizes phylogenetic signals and the contemporary geographic distributions of lineages to investigate the evolutionary history of a taxon. Many components, and certainly the concepts important in phylogeography, have been present in systematic biology (in historical biogeography and later in cladistics, phylogenetics and population genetics) since its inception. However, the science of phylogeography was not formally defined until Avise et al. (1987) employed a combination of phylogenetic and population genetics methods to explain intraspecific geographic patterns presented in case studies of several taxa occurring in the southeastern United States. Phylogeographic techniques have since been applied at various taxonomic scales to infer historical population demographics, historical biogeographical patterns (i.e., vicariance and dispersal), cryptic species and conservation priorities. The use of Colubroidean snakes as models to test phylogeographic hypotheses has deepened our understanding of the respective taxa, identified hidden diversity and provided insight into the evolutionary drivers that shape the biota of ecoregions (e.g., Wüster et al. 2005; Burbrink et al. 2008; Castoe et al. 2009; Pook et al. 2009; Bryson et al. 2011).

The intent of this thesis is to build on the baseline data for *Crotalus molossus* provided by Wüster et al. (2005) and examine members of the *C. molossus* group from a phylogeographic perspective. The specific objectives of this study are to: (1) elucidate the phylogenetic relationship between the two northern *C. molossus* lineages identified by Wüster et al. (2005); (2) identify biogeographic patterns among northern populations of *C. molossus*; (3) determine the extent of genetic variation among populations; (4) estimate divergence dates between the major lineages in the *C. molossus* group; (5) identify discrete morphological characters; and (6)

investigate the taxonomy of the *C. molossus* group and suggest revisions to reflect the true diversity of the group.

CHAPTER 2: MATERIALS AND METHODS

2.1 SPECIMEN ACQUISITION

A total of 31 blood and tissue samples representing 21 localities throughout the range of *Crotalus molossus* in the United States and an additional 13 outgroup samples were obtained from the Laboratory for Environmental Biology of the University of Texas at El Paso (UTEP). The University of Texas at Arlington Department of Biology (UTA) provided samples of *C. m nigrescens* from Durango, Mexico. Additional sequence data for *C. totonacus* and *C. basiliscus* were downloaded from GenBank and are indicated in Appendix I. Blood and tissue samples collected for this study were preserved in 95% ethanol, whereas whole specimens were preserved in 10% buffered formalin and maintained for long-term storage in 70% ethanol.

2.2 LABORATORY PROTOCOLS

Genomic DNA was extracted from blood and tissue samples using the Qiagen DNeasy Blood and Tissue Kit (Valencia, CA). Approximately 25 mg of tissue was removed for extraction and placed in a 1.5 ml microcentrifuge tube. If stored in ethanol, the samples were soaked in deionized water for 90 minutes (min) at 0.2°C to allow the ethanol to diffuse out of the tissue. The samples were then allowed to dry at 0.2°C for at least one hour. Tissue samples were digested in a solution of 180 μ l of buffer ATL and 20 μ l of Proteinase K and incubated at 55°C for 30 min before vortexing for 10–20 seconds (s) and left to incubate overnight. Next, 200 μ l of buffer AL was added to each microcentrifuge tube. Each sample was then vortexed for 10–20 s and allowed to incubate at 70°C for 10 min, after which, 200 μ l of 100% ethanol was added and

the samples were vortexed again for 10–20 s. Blood samples were treated differently—approximately 20 μ l of 95% ethanol containing blood was transferred to a microcentrifuge tube containing 20 μ l of Proteinase K, 200 μ l of Buffer AL was added and these samples were vortexed for 10–20 s. The samples were then incubated at 55°C for 10 min and 200 μ l of 100% ethanol was added. The entire solution containing digested blood or tissue was transferred into a Qiagen spin column and centrifuged at 8000 revolutions per minute (rpm) for 1 min; the solution that passed through the filter of the spin column was discarded. Next, 500 μ l of Buffer AW1 was added and the samples were again centrifuged at 8000 rpm for 1 min. The fluid was discarded and 500 μ l of Buffer AW2 was added; samples were centrifuged at 14,000 rpm for 3 min and the fluid was discarded. Finally, 200 μ l of Buffer AE was added and samples were centrifuged at 8000 rpm for 1 min. This step was repeated twice and the fluid was deposited in pre-labeled microcentrifuge tubes following each repetition. The 400 μ l of solution retained from the final step was used for Polymerase Chain Reaction (PCR).

Three mitochondrial (mtDNA) sequences were amplified using primers specified in Table 2.2.1. Mitochondrial genes cytochrome *b* (cyt *b*), NADH dehydrogenase subunit 4 (ND4) and ATP synthase subunits 8 and 6 (ATPases 8 and 6), were selected because of their utility in previous studies that investigated systematics of the Viperidae (e.g., Arévalo et al. 1994; Pook et al. 2000; Douglas et al. 2002, 2006; Pook et al. 2009). ATPases 8 and 6 exist on a contiguous mitochondrial sequence and a reading frame shift allows the 5' end of ATPase 6 to slightly overlap the 3' end of ATPase 8 (Dovc and Hecht 1995; Lovette et al. 1999; Joseph et al. 2004). Because ATPases 8 and 6 occur as a continuous sequence, they were treated as a single gene. Unlinked nuclear loci have an evolutionary history different from that of mitochondrial genes. Consequently, analysis of nDNA can recover phylogenetic patterns that are incongruent with

mitochondrial gene trees, making nuclear loci a useful tool for identifying introgression and incomplete lineage sorting (Wiens et al. 2010). For this reason, three nuclear genes were included. The genes recombination activating protein-1 (RAG1, Wiens et al. 2008), oocyte maturation factor (*c-mos*, Lawson et al. 2005) and exophilin 5 (EXPH5, Portik et al. 2010, 2011) were chosen for their use in fine-scale phylogenetic analyses and potential usefulness in addressing phylogeographic relationships. Amplification was completed using a denaturation temperature of 95°C (initial denaturation period of 2 min, subsequent steps were 35 s in duration), annealing at 50°C for 35 s, and extension at 72°C for 95 s with 4 s added to the extension per cycle for 32 (ND4 and *cyt b*) and 34 cycles (nDNA genes). Amplification of ATPases 8 and 6 was accomplished in 34 cycles using similar cycle parameters, except that the annealing temperature was 46°C for a duration of 35 s. Amplicons from PCR were visualized with 1.5% agarose gel electrophoresis, and samples were purified following standard protocols specified for Agencourt AMPure XP (Beckman Coulter, Brea, CA).

Table 2.2.1 List of primers used for amplification and sequencing of each fragment.

Gene	Primer Name	Source	Primer Sequence (5' to 3')
ND4	ND4	Arévalo et al. 1994	CACCTATGACTACCAAAAGCTCATGTAGAAGC
	HIS1276	Pook et al. 2009	TTCTATCACTTGGATTTCACCA
<i>cyt b</i>	703Bot	Pook et al. 2000	TCAAACATCTCAACCTGATGAAA
	MVZ16	Pook et al. 2000	GGCAAATAGGAAGTATCATTCTG
ATPases 8 and 6	L8331	Douglas et al. 2002	AAAGCRTYRGCCTTTTAAGC
	H9236	Douglas et al. 2002	GTTAGTGGTCAKGGGCTTGGRTC
	8.3	Douglas et al. 2002	TGATAKGCRGTGTGCTTGCTG
RAG1	RAG1f1a	Wiens et al. 2008	CAGCTGYAGCCARTACCATAAAAT
	RAG1r2	Wiens et al. 2008	CTTCTAGCAAAATTTCCATTCAT
<i>c-mos</i>	S77	Lawson et al. 2005	CATGGACTGGGATCAGTTATG
	S78	Lawson et al. 2005	CCTTGGGTGTGATTTTCTCACCT
EXPH5	EXPH5 F1	Portik et al. 2010, 2011	AATAAACTKGCAGCTATGTACAAAACAAGTC
	EXPH5 R1	Portik et al. 2010, 2011	AAYCGCCCTTCTGTGAGTGACCTCT

Sequencing of forward and reverse strands of PCR products was executed with an ABI 3700xl capillary DNA sequencer at the UTEP DNA Core Facility. Chromatograph data obtained from sample contigs were interpreted in the program SeqMan (Swindell and Plasterer 1997). The generally conservative nature of the genes used in this study allowed preliminary alignment to be done by eye and the program MacClade v4.08 (Maddison and Maddison 2000) was used to translate sequences to amino acids to ensure accuracy of the reading frame and to confirm that stop codons were not present in the data. GenBank accession numbers for sequence data used in this study are provided in Appendix I.

2.3 PHYLOGENETIC INFERENCE

Sequence data were analyzed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) methods in PAUP* 4.0b (Swofford 2002), RAxML v7.2.6 (Stamatakis 2006) and MrBayes v3.1 (Ronquist and Huelsenbeck 2003), respectively. The MP analyses were conducted using a heuristic search algorithm with 25 random-addition replicates, accelerated character transformation and tree bisection-reconnection branch swapping. Zero-length branches were collapsed to polytomies. Node support was assessed with 1,000 nonparametric bootstrap pseudoreplicates (Felsenstein 1985). Maximum-likelihood (ML) analyses of single-gene and combined datasets were performed with the GTRGAMMA model in RAxML v7.2.6 (Stamatakis 2006). Datasets were partitioned by codon position in single gene datasets, and by gene and codon position in combined gene datasets. ML analyses were initiated with random starting trees and utilized the rapid hill-climbing algorithm (Stamatakis et al. 2007). Support for clades inferred by analyses was assessed with the rapid bootstrap algorithm with 1,000 replicates (Stamatakis et al. 2008).

Bayesian inference (BI) was conducted in MrBayes v3.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) for single-gene and combined datasets. Prior to BI analyses, the most appropriate evolutionary model for each codon position was inferred using the Akaike information criterion (AIC) implemented in the program jModelTest (Posada 2008). Prior to Bayesian inference, the datasets were partitioned by codon position (single gene) or by gene and codon position (combined datasets) and the evolutionary model inferred by jModelTest was specified for each partition (Brandley et al. 2005). Lemmon and Moriarty (2004) demonstrated that choosing an under parameterized substitution model could strongly bias estimations of branch length, posterior probabilities and other model specifications important for correct phylogenetic inference. Likewise, use of an over parameterized model will result in imprecise estimations of branch lengths. Therefore, when the best model suggested by jModelTest could not be implemented in MrBayes, the next most complex model was chosen. This method seems to be the best compromise for model selection, as the implementation of a model with more complex parameters seems to result in a less biased phylogenetic inference than would be obtained from a less complex model. Probability distributions were assessed with parallel Markov Chain Monte Carlo (MCMC) searches in which four Markov chains were run with default priors, a temperature of 0.05, and initiated from randomly generated starting trees. Two MCMC searches were run for 10,000,000 generations to ensure that independent runs had converged on the same topology and trees were sampled every 1,000 generations. Are we there yet? (AWTY) (Nylander et al. 2008) was used to verify that convergence had been reached. As outlined by Brandley et al. (2011), the “cumulative” command in AWTY was implemented to construct the posterior probability plots that were examined for stationarity of split frequencies. This procedure was repeated at least four times to reduce the probability that local optima were

influencing the results. Phylogenies inferred from BI and ML analyses were visualized in FigTree v1.3.1 (Rambaut and Drummond 2010).

2.4 GENETIC DIVERGENCE AND MONOPHYLY TESTS

In order to examine genetic diversity within and among lineages of the *Crotalus molossus* group, specimens were assigned to groups based on their position in the mtDNA phylogeny. Groups specified for analysis were restricted to geographically distinct and well-supported clades. Sequence divergence was estimated within and among these groups of *C. molossus* by using uncorrected pairwise (p) distances calculated in MEGA v5.0 (Tamura et al. 2007). Monophyly of clades recovered in phylogenetic analyses was tested using the Shimodaira-Hasegawa (SH) and approximately unbiased (AU) tests implemented in CONSEL v0.1i (Shimodaira and Hasegawa 2001; Shimodaira 2002). This was accomplished by constraining the phylogeny of *C. molossus* to be monophyletic; likelihood scores were then calculated in RAxML and values from the constraint tree were evaluated against those of the preferred tree using CONSEL.

2.5 DIVERGENCE DATING

Divergence dates were estimated from mitochondrial datasets using a Bayesian relaxed molecular clock in the program BEAST v1.6.1 (Drummond and Rambaut 2007). This method infers divergence dates based on known node constraints (e.g., fossils, mutation rate, or the formation of a biogeographic barrier) specified prior to analysis (Drummond and Rambaut 2007), while allowing substitution rates to vary among branches (Drummond et al. 2006).

Divergence dates were estimated with a Yule process speciation tree model, uncorrelated lognormal clock and node constraints obtained from the fossil record with lognormal distributions. Tree models were linked for analyses, but substitution and clock models remained unlinked. Preliminary analyses using site models specified for Bayesian analyses resulted in ESS values < 200. Consequently, the model parameters GTR+G and GTR+I+G were specified for *cyt b* and ND4, respectively. MCMC analyses were run four times each for 20,000,000 generations, with samples retained every 1,000 generations to ensure effective sample sizes greater than 200 in each run. The four independent runs were combined in LogCombiner v1.6.1 (Drummond and Rambaut 2007) for analysis in Tracer v1.5 (<http://tree.bio.ed.ac.uk/software/tracer/>) to confirm stationarity, convergence and stability of MCMC runs.

Two fossil calibrations were used, including the minimum ages of *Agkistrodon contortrix* and the genus *Sistrurus* estimated from fossil evidence presented by Holman (2000) and Parmley and Holman (2007), respectively. Sequence data for four outgroup taxa, *A. contortrix*, *A. piscivorus*, *S. catenatus* and *S. miliarius*, were obtained from Genbank for use as clock calibration references (Appendix I). Previously used calibration constraints specified by Bryson et al. (2011) were used for *Agkistrodon* and *Sistrurus*. The minimum age of *A. contortrix* was constrained with a zero offset of 6 mya, a lognormal mean of 0.01 and a lognormal standard deviation of 0.42, placing its origin in the middle Hemphillian North American Land Mammal Age (NALMA). The minimum age of *Sistrurus* was constrained with a zero offset of 8 mya, a lognormal mean of 0.01 and a standard deviation of 0.76, placing the age of *Sistrurus* at the beginning of the Clarendonian NALMA.

2.6 POPULATION AND GEOGRAPHIC STRUCTURE ANALYSIS

Haplotype median-joining networks were constructed using the program NETWORK v4.600 (<http://www.fluxus-engineering.com>; Bandelt et al. 1999). Given the high degree of divergence and strong support for clades, separate haplotype networks were constructed for the eastern and western *C. m. molossus* lineages with the mtDNA dataset (weights = 10 and $\epsilon = 0$). Genetic diversity within and among geographical clades recovered by phylogenetic inferences was calculated for the mtDNA dataset and individual nDNA genes in ARLEQUIN v3.5 (Excoffier and Lischer 2010). In addition, ARLEQUIN was used to conduct a hierarchical analysis of molecular variance (AMOVA, Excoffier et al. 1992). AMOVA was used to infer the most probable configuration of geographical clades by analyzing various combinations of 2–7 groups that corresponded to the geographical hierarchy recovered in phylogenetic analyses. The most parsimonious combinations of geographical clades were those that maximized variation among groups (F_{CT}) and were statistically significant (Zhang et al. 2010). Population expansion was assessed in ARLEQUIN with Fu's F_s and Tajima's D tests of neutrality (Fu 1997) and mismatch distributions estimates (for clades with adequate sampling). Mismatch distributions test for sudden population expansions based on a raggedness index calculated for observed data points around a simulated curve. Rogers and Harpending (1992) demonstrated that under an infinite sites model, the mismatch distribution exhibited by populations that have undergone recent expansion will be smooth or unimodal, whereas populations that are stable will exhibit a ragged or multimodal distribution (Harpending 1994; Harpending et al. 1998).

2.7 MORPHOLOGICAL ANALYSIS

The morphological assessment consisted of six *C. totonacus*, two *C. basiliscus* and 103 specimens from the *Crotalus molossus* group (99 *C. molossus*, and type material of *C. molossus*, *C. ornatus*, *C. m. oaxacus* and *C. m. estebanensis*) representing populations from throughout the known geographic range of *Crotalus molossus*. Localities and collection data for examined specimens are presented in Appendix II. Each specimen was assigned to one of five lineages (eastern *C. m. molossus*, western *C. m. molossus*, *C. m. nigrescens*, *C. basiliscus* or *C. totonacus*) based on results inferred from phylogenetic analyses, collection data associated with the specimen (e.g., locality and preliminary identification by the collector) and diagnostic characters for previously recognized taxa in the *C. molossus* group (Gloyd 1936, 1948; Klauber 1948, 1972).

Characters examined herein were chosen from previous morphological investigations of Colubroidean snakes (Devitt et al. 2008; LaDuc and Johnson 2003), and were identified as either highly variable in the *C. molossus* group (Klauber 1972; Campbell and Lamar 1989, 2004), or used in the diagnosis of *C. m. nigrescens* by Gloyd (1948). All mensural data were collected with a digital caliper under a dissecting microscope and rounded to the nearest 0.1 mm. Snout–vent (SVL) and tail length (TL) were measured with a metric ruler and rounded to the nearest 1.0 mm. The type specimens of *C. molossus* and *C. ornatus* are preserved as skins with the head and tail attached. Consequently, SVL was estimated based on ratios of head length to SVL and TL to SVL for these specimens. Because of damage to some specimens (including type material), it was not possible to collect a complete dataset for some individuals. Therefore, mensural, meristic and qualitative characters were assessed on the right side of the head only.

Data recorded from each specimen included 21 mensural, 16 meristic and two qualitative characters: SVL; TL; head length (HL); head width (HW)—measured at the widest point of the head; naso-ocular distance (NOD); interocular distance (ID); internasal distance (INAS); rostral scale height (RH); rostral scale width (RW); mental scale height (MH); mental scale width (MW); chin shield length (CSL); chin shield width (CSW); upper preocular scale height (UPH); upper preocular scale length (UPL); lower preocular scale height (LPH); lower preocular scale length (LPL); prefrontal scale length (PFL); prefrontal scale width (PFW); supraocular scale length (SOCL); supraocular scale width (SOCW). Meristic data included: number of ventral scales (VENT)—following the method proposed by Dowling (1951); subcaudals (SCDL); internasals (INAS); prefrontals (PFRO); loreals (LOR); intersupraoculars (ISO)—assessed as the fewest number of scales between supraoculars posterior to the frontals; supralabials (SUPRA); infralabials (INFRA); foveals (FOV)—lacunals; subfoveals (SFOV); prefoveals (PFOV); number of dorsal blotches (DBLOT); number of fused dorsal blotches (FDBLOT)—counted as the number of dorsal blotches connected by at least one scale row; dorsal scale rows counted at the neck, midbody and one head length anterior to the vent (DSRN, DSRM and DSRV, respectively). Qualitative characters were postrostral scale (POSTROST)—presence or absence, and contact (present or absent) between the prenasal and first supralabial (PRE-SUP).

Mean, standard deviation and range of meristic and mensural characters were calculated for each group, and a pairwise comparison of means (ANOVA) was conducted. To eliminate the effect of size, an analysis of covariance (ANCOVA) with snout–vent length as the covariate was conducted on the mensural dataset. Principal components analyses (PCA), conducted in Minitab 17 (Minitab® Statistical Software, State College, PA), were used to identify patterns of variation in the data that may be useful in assigning specimens to the geographical lineages identified in

phylogenetic analyses. All analyses used the covariance matrix. To eliminate the effects of sexual dimorphism and size on mensural characters, PC analyses were conducted separately on adult male and female datasets using the residuals obtained from the ANCOVA. The meristic dataset was assessed with PC analyses of raw data. Sexually dimorphic characters (VENT and SCDL) did not influence the delineation of taxonomic groups, and the meristic dataset was not partitioned by sex.

CHAPTER 3: RESULTS

3.1 PHYLOGENETIC ANALYSIS

A total of three mtDNA genes (ND4 = 678 base pairs [bp]; cyt *b* = 728 bp; ATPases 8 and 6 = 637 bp) were analyzed for 31 samples of *Crotalus molossus*. Because some samples were degraded, only 23 *C. molossus* samples were amplified for three nDNA genes (RAG1 = 926 bp; c-mos = 570 bp; EXPH5 = 770 bp). Samples of *C. m. nigrescens* failed to amplify for all nDNA genes and only sequences of cyt *b* and ND4 for *C. totonacus* and *C. basiliscus* were available from GenBank. In spite of the large amount of missing data, these taxa were included in analyses of the six-gene dataset (ND4; cyt *b*; ATPases 8 and 6; RAG1; c-mos; EXPH5). Although this may be cause for concern, the placement of taxa that are missing a significant portion of sequence data can be accurately inferred in a phylogeny, given an adequate number of informative characters (Wiens 2003; Pyron et al. 2011).

Nucleotide substitution models selected by jModelTest and alternative models implemented in BI analyses are presented in Table 3.1.1. The topologies of trees generated from MP, ML and BI analyses of individual mtDNA genes were mostly congruent. The only discrepancies were between weakly supported nodes and some rearrangements of the outgroup taxa in MP analyses. However, analyses of single nuclear genes recovered extensive incongruence among gene trees. Trees recovered from separate nDNA analyses were examined in detail, on a node-by-node basis; this revealed low support for incongruent nodes among phylogenies. This suggested that the incongruence among gene trees was weak and that combining these datasets, which reflect slightly different evolutionary histories, may result in a more accurate phylogenetic estimation (Wiens 1998). Consequently, various combinations of

the data were analyzed (mtDNA dataset [ND4; *cyt b*; ATPases 8 and 6], a nDNA dataset [c-mos; EXPH5; RAG1] and a combined six-gene dataset).

Table 3.1.1 Model parameters specified by jModelTest and alternative models implemented in Bayesian inference analyses.

Gene Codon Position	Model Specified by jModelTest	Model Implemented in MrBayes
ATPases 8 and 6	HKY+G	
Codon 1	TIM2+I+G	HKY+I+G
Codon 2	TPM3uf+I+G	HKY+I+G
Codon 3	TPM1uf+I+G	HKY+I+G
<i>cyt b</i>	TIM2+G	
Codon 1	TIM2ef+I+G	K80+I+G
Codon 2	TIM1+I+G	GTR+I
Codon 3	HKY+I	HKY+I
ND4	TIM3+I+G	
Codon 1	GTR+I+G	GTR+I+G
Codon 2	TPM2uf+I	HKY+I
Codon 3	TVM3ef+I+G	HKY+G
c-mos	GTR+I+G	
Codon 1	HKY	HKY
Codon 2	TVM+G	GTR+G
Codon 3	TrN+I	HKY+I
EXPH5	TPM1uf+I+G	
Codon 1	TIM1+I	GTR+I
Codon 2	TrN+I	HKY+I
Codon 3	K80+I+G	K80+I+G
RAG1	TPM1uf+I+G	
Codon 1	F81	F81
Codon 2	F81	F81
Codon 3	TPM3uf+I	GTR+I

The MP analysis of the mtDNA dataset recovered six most parsimonious trees with 1,864 steps (774 variable sites, 591 parsimony-informative sites, consistency index [CI] of 0.518 and a

retention index [RI] of 0.761). Parsimony analysis of the six-gene dataset found four most parsimonious trees with 1,580 steps (518 of the 759 variable sites were informative, CI = 0.585 and RI = 0.760). Tree topologies presented for the mtDNA and six-gene datasets (Figs. 3.1.1 and 3.1.2, respectively) are the best scoring trees found by RAxML ($-\ln L = -10756.53$ and $-\ln L = -12699.08$, respectively). Posterior probability plots constructed with AWTY indicated that convergence of Bayesian MCMC searches was reached relatively quickly in all analyses (~20% of generations), so a conservative 25% of sampled generations were discarded and posterior probabilities were estimated from the remaining post burn-in trees.

In the mtDNA and six-gene datasets (Figs. 3.1.1 and 3.1.2, respectively), *Crotalus molossus nigrescens* was recovered at the base of a well-supported, monophyletic clade including *C. basiliscus*, *C. totonacus* and *C. m. molossus*. However, the latter taxon was recovered as paraphyletic with deeply divergent eastern and western *C. molossus* lineages, with strong support from both the mtDNA and six-gene datasets. The geographic distribution of clades within the divergent eastern and western *C. m. molossus* lineages is presented in Figure 3.1.3. Within the western lineage, the Madrean clade is inferred with strong support, and is geographically restricted to the northern extensions of the Sierra Madre Occidental, the Madrean Archipelago, and southern terminal ranges of the Rocky Mountains in southwestern New Mexico and southeastern Arizona. A second well-supported group within the western *C. m. molossus* lineage (the Sonoran clade) was recovered from populations in west-central Arizona. In the eastern *C. m. molossus* lineage, a well-supported basal divergence separates a clade in the western Chihuahuan Desert (the Chihuahuan clade) from two closely related clades occurring in the Big Bend region and the Stockton and Edwards plateaus (Big Bend and Stockton clades,

Figure 3.1.1 Phylogeny inferred from the combined mtDNA dataset (ATPases 8 and 6, cyt *b* and ND4); node labels are MP bootstrap/ML bootstrap/Bayesian posterior probability. Samples representing the currently recognized *Crotalus molossus* group are in bold text. Colored bars and abbreviations correspond to five geographically defined clades: Sonoran (Son), Madrean (Mad), Chihuahuan (Chih), Big Bend (Bigbend) and Stockton (Stock).

Figure 3.1.2 Phylogeny inferred from the six-gene dataset (ATPases 8 and 6, cyt *b*, ND4, c-mos, EXPH5 and RAG1); node labels are MP bootstrap/ML bootstrap/Bayesian posterior probability. Samples representing the currently recognized *Crotalus molossus* group are in bold text. Colored bars and abbreviations correspond to five geographically defined clades: Sonoran (Son), Madrean (Mad), Chihuahuan (Chih), Big Bend (Bigbend) and Stockton (Stock).

Figure 3.1.3 Sampling localities and geographically defined clades recovered in haplotype and phylogenetic analyses of *Crotalus molossus*. Gray shading depicts the general distribution of *C. m. molossus*; modified from a map by Campbell and Lamar (2004).

Figure 3.1.4 Phylogenetic trees inferred from single nDNA datasets for c-mos, EXPH5 and RAG1. Samples representing the currently recognized *Crotalus molossus* group are in bold text. Abbreviations correspond to five geographically defined clades recovered within *C. m. molossus*: Sonoran (Son), Madrean (Mad), Chihuahuan (Chih), Big Bend (Bigbend) and Stockton (Stock). The western rattlesnake group refers to *C. cerberus*, *C. oreganus* and *C. viridis*.

respectively). Strongly supported sister relationships between *C. totonacus* and the eastern *C. molossus* lineage, and *C. basiliscus* and the western *C. m. molossus* lineage were recovered in BI analyses, but MP and ML analyses recovered weak to moderate support for these sister relationships (< 78%).

Single-gene analyses of nDNA datasets resulted in greatly dissimilar topologies among gene trees (Fig. 3.1.4). No single nuclear gene was able to provide resolution among populations of *Crotalus molossus*. Inference of the *c-mos* gene recovered eastern and western *C. m. molossus* lineages in a monophyletic group that is sister to *C. horridus*. Although a monophyletic western *C. m. molossus* lineage was recovered in analyses of EXPH5, its position was ambiguous with respect to outgroup taxa. In the absence of *C. basiliscus* and *C. totonacus*, analysis of the combined nDNA dataset (not shown) recovered a monophyletic *C. molossus* with weak MP and ML support and moderate Bayesian posterior probability support (23% MP, 64% ML, 0.94 BI), and the position of a monophyletic western lineage of *C. m. molossus* was unresolved. Despite the lack of resolution in the combined nDNA dataset, results of the mtDNA and six-gene datasets are nearly identical, which may be the result of bias caused by the more rapidly evolving and hence more variable mitochondrial genes (Wiens et al. 2010).

3.2 GENETIC DIVERGENCE AND HYPOTHESIS TESTS

In order to examine genetic diversity among populations of *Crotalus molossus*, specimens were assigned to groups based on well-supported, geographically distinct clades indicated in Figures 3.1.1 and 3.1.2. Uncorrected *p*-distances (Table 3.2.1) within and among clades of *C. molossus* are provided for the mtDNA dataset. The greatest divergence among lineages in the *C. molossus* group was between *C. m. nigrescens* and the Sonoran clade (9.2%).

Distances ranging from 7.6–7.9% were observed between eastern and western *C. m. molossus* lineages. Divergence between the western lineage and *C. basiliscus* ranged from 7.3–8.1%, and a genetic divergence of 7.1–7.2% was recovered between *C. totonacus* and the eastern lineage. Among clades within the eastern lineage, divergences ranged from 0.5% between the Big Bend and Stockton clades to 1.2% between the Bigbend/Stockton and Chihuahuan clades. Divergence between the Sonoran and Madrean clades of the western lineage was 3.6%.

All analyses of the mtDNA and six-gene datasets recovered a paraphyletic *Crotalus m. molossus*. In hypothesis tests, monophyly of this taxon was rejected for both the mtDNA (AU $P = 0.0004$; SH $P = 0$) and six-gene (AU $P = 0.00005$; SH $P = 0$) datasets.

Table 3.2.1 Uncorrected p -distances within and among groups of *Crotalus molossus*, which are calculated from the combined mtDNA dataset. Six lineages within the *C. molossus* group (the Chihuahuan clade [Chi], the Big Bend clade [BB], the Stockton clade [Stock], the Madrean clade [Mad], the Sonoran clade [Son.], *C. m. nigrescens* [Cmn]), *C. basiliscus* (Cbas) and *C. totonacus* (Ctot) were examined.

	Chi.	BB	Stock	Mad	Son	Cmn	Cbas	Ctot
Chi.	0.003							
BB	0.012	0.002						
Stock	0.012	0.005	0.002					
Mad	0.076	0.077	0.076	0.007				
Son	0.079	0.078	0.079	0.036	0.022			
Cmn	0.078	0.081	0.080	0.085	0.092	0.002		
Cbas	0.078	0.077	0.075	0.073	0.081	0.086	NA	
Ctot	0.072	0.071	0.071	0.085	0.085	0.090	0.081	0.003

3.3 Population and geographic structure analysis

Estimates of genetic diversity and neutrality tests within populations are presented in Table 3.3.1. The haplotype diversity ($h = 1.0$) and nucleotide diversity ($\pi = 0.0516$) among all

individuals of *C. basiliscus*, *C. totonacus* and all populations of *C. molossus* suggests that very little gene flow occurred between populations, and that the geographically defined clades identified herein likely evolved in allopatry. In the AMOVA (Table 3.3.2), the greatest genetic variance among groups ($F_{CT} = 0.860$, $P < 0.05$) was found with seven groups ([*nigrescens*] [*basiliscus*] [Madrean] [Sonoran] [*totonacus*] [Chihuahuan] [Bigbend, Stockton]). This is congruent with the well-supported clades recovered in the phylogenetic analyses (Figs. 3.1.1 and 3.1.2).

Table 3.3.1 Genetic diversity estimates and neutrality tests within populations of *Crotalus basiliscus*, *C. totonacus* and the *C. molossus* group. n = sample size; π = nucleotide diversity; h = haplotype diversity; κ = mean number of pairwise differences.

Clade	n	π	h	κ	Fu's F_s	P -value	Tajima's D	P -value
Chihuahuan	9	0.0031	1.0	5.53	-4.535	0.004	-0.296	0.417
Big Bend	4	0.0008	1.0	1.67	-2.181	0.018	0.168	0.741
Stockton	6	0.0026	1.0	5.33	-2.035	0.058	0.648	0.762
Madrean	6	0.0062	1.0	12.20	-0.661	0.216	-0.990	0.196
Sonoran	2	0.0211	1.0	43.00	3.761	0.599	—	—
<i>nigrescens</i>	2	0.0029	1.0	6.00	1.792	0.511	—	—
<i>totonacus</i>	2	0.0045	1.0	6.00	-0.661	0.216	—	—
<i>basiliscus</i>	1	—	—	—	—	—	—	—
Total	32	0.0516	1.0	68.80	-6.306	0.015	0.175	0.648

Table 3.3.2 Hierarchical analysis of molecular variance among lineages in the *Crotalus molossus* group. (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

Groups	F _{ST}	F _{SC}	F _{CT}	% among populations	% within populations
All populations	0.923	—	—	92.30	7.70
2 Groups [<i>nigrescens</i>] [<i>basiliscus</i> , <i>totonacus</i> , Chihuahuan, Bigbend, Stockton, Madrean, Sonoran]	0.945***	0.917***	0.336	33.59	5.48
2 Groups [<i>nigrescens</i> , <i>totonacus</i> , Chihuahuan, Bigbend, Stockton, Madrean, Sonoran] [<i>basiliscus</i>]	0.941***	0.921***	0.250	25.01	5.92
2 Groups [<i>nigrescens</i> , <i>basiliscus</i> , Chihuahuan, Bigbend, Stockton, Madrean, Sonoran] [<i>totonacus</i>]	0.942***	0.919***	0.281	28.06	5.85
3 Groups [<i>nigrescens</i>] [Madrean, Sonoran, <i>basiliscus</i>] [<i>totonacus</i> , Chihuahuan, Bigbend, Stockton]	0.941***	0.861***	0.578***	57.81	5.88
3 Groups [<i>nigrescens</i>] [Madrean, Sonoran, <i>basiliscus</i> , <i>totonacus</i>] [Chihuahuan, Bigbend, Stockton]	0.939***	0.855***	0.581**	58.06	6.09
3 Groups [<i>nigrescens</i> , <i>basiliscus</i>] [Madrean, Sonoran, <i>totonacus</i>] [Chihuahuan, Bigbend, Stockton]	0.938***	0.857***	0.564*	56.42	6.21
3 Groups [<i>nigrescens</i> , <i>basiliscus</i> , <i>totonacus</i>] [Madrean, Sonoran] [Chihuahuan, Bigbend, Stockton]	0.937***	0.842***	0.606**	60.62	6.22
4 Groups [<i>nigrescens</i>] [<i>basiliscus</i>] [Madrean, Sonoran] [<i>totonacus</i> , Chihuahuan, Bigbend, Stockton]	0.942***	0.841***	0.637**	63.67	5.79
4 Groups [<i>nigrescens</i>] [<i>basiliscus</i> , Madrean, Sonoran] [<i>totonacus</i>] [Chihuahuan, Bigbend, Stockton]	0.941***	0.794***	0.711**	71.11	5.96
4 Groups [<i>nigrescens</i>] [<i>basiliscus</i> , <i>totonacus</i>] [Madrean, Sonoran] [Chihuahuan, Bigbend, Stockton]	0.940***	0.789***	0.714***	71.44	6.03
5 Groups [<i>nigrescens</i>] [<i>basiliscus</i>] [<i>totonacus</i>] [Madrean, Sonoran] [Chihuahuan, Bigbend, Stockton]	0.941***	0.721***	0.790**	78.96	5.88
5 Groups [<i>nigrescens</i>] [<i>basiliscus</i> , Madrean, Sonoran] [<i>totonacus</i>] [Chihuahuan] [Bigbend, Stockton]	0.928***	0.830***	0.573*	57.34	7.24
6 Groups [<i>nigrescens</i>] [<i>basiliscus</i>] [Madrean, Sonoran] [<i>totonacus</i>] [Chihuahuan] [Bigbend, Stockton]	0.928***	0.725***	0.737**	73.72	7.22
6 Groups [<i>nigrescens</i>] [<i>basiliscus</i>] [Madrean] [Sonoran] [<i>totonacus</i>] [Chihuahuan, Bigbend, Stockton]	0.940***	0.652***	0.828*	82.85	5.97
7 Groups [<i>nigrescens</i>] [<i>basiliscus</i>] [Madrean] [Sonoran] [<i>totonacus</i>] [Chihuahuan] [Bigbend, Stockton]	0.927***	0.478***	0.860*	85.89	7.32

Mismatch distributions were generated from well-sampled clades with the mtDNA dataset including the Madrean clade (western lineage) and the three clades from the eastern lineage. Mismatch distributions were also generated with c-mos, EXPH5 and RAG1 datasets for clades that had sufficient sample sizes (Fig. 3.3.1). The mtDNA dataset recovered multimodal distributions and non-significant raggedness indices for all four clades, indicating that the populations are stable or slowly declining (Rogers and Harpending 1992). Non-significant Fu's F_s tests ($P > 0.02$) (Table 3.3.1) for the Madrean, Sonoran and Stockton clades also support a hypothesis of selective neutrality and population stability. However, the significant Fu's F_s tests recovered from the Chihuahuan and Big Bend clades ($P = 0.004$ and 0.018 , respectively) suggest recent demographic expansion. This is also reflected in the lower (although not significant) raggedness indices recovered for these groups.

Haplotype networks constructed for the eastern and western *C. m. molossus* lineages (Fig. 3.3.2) identified geographic variation within and among clades. In the eastern lineage, the three clades identified in phylogenetic analyses (Chihuahuan, Bigbend and Stockton) are separated by 25 and 11 mutations, respectively. One sample in the Chihuahuan clade (Chih 2) is separated from the remaining group by 101 mutations, but this result is a consequence of missing data. Two additional samples (Chih 6 and Chih 7) are separated from the remaining clade members by 79 mutations. These two samples, taken from Luna and Sierra Counties in New Mexico, may represent another geographically distinct clade. However, phylogenetic inferences did not indicate marked divergence from the Chihuahuan clade. The western lineage is separated from the eastern by 142 mutations. Within the western lineage, the Sonoran and Madrean clades are separated by 49 mutations. In some cases, the large number of mutations observed between

Figure 3.3.1 Mismatch distributions computed from mitochondrial (ATPases 8 and 6, *cyt b*, and ND4), *c-mos*, EXPH5 and RAG1 datasets for the well-sampled Chihuahuan, Big Bend, Stockton and Madrean clades. Sample sizes were not large enough to calculate mismatch distributions for the Big Bend clade (*c-mos* and RAG1) or Chihuahuan clade (EXPH5). Dashed lines indicate the observed distribution of pairwise differences and solid lines illustrate the expected distribution.

Figure 3.3.2 Haplotype networks for eastern (A) and western (B) lineages of *Crotalus m. molossus*, calculated from the mitochondrial dataset (ATPases 8 and 6, *cyt b* and ND4) in the program Network.

haplotypes within well-supported lineages is not evident in phylogenetic inferences and seems to correspond to missing sequence data (e.g., Bigbend 1, Chih 2, 6 and 7).

3.4 DIVERGENCE DATING

A mitochondrial dataset consisting of *cyt b* and ND4 was used to estimate divergence dates in BEAST; data from ATPases 8 and 6 were not included in dating analyses because sequences were not available for *Crotalus basiliscus* or *C. totonacus*. Estimated divergence dates (Fig. 3.4.1) suggest a basal diversification within the *C. m. molossus* group likely beginning in the Hemphillian NALMA of the late Miocene ~5.1 mya (95% Highest Posterior Density = 3.30–7.42 mya), and all clades were present by the mid Pliocene. Mean age for the basal divergence between eastern and western *C. m. molossus* lineages (including their respective sister taxa) is estimated at ~4.63 mya (95% HPD = 2.90–6.67 mya). Subsequent divergence between *C. basiliscus* and the western *C. m. molossus* lineage occurred during the early Pliocene ~4.02 mya (95% HPD = 2.51–5.94 mya). The initial divergence among western *C. m. molossus* clades occurred near the Pliocene-Pleistocene boundary ~1.86 mya (95% HPD = 1.03–2.94 mya). Divergence between *C. totonacus* and the eastern lineage of *C. m. molossus* occurred in the early Pliocene (~3.77 mya; 95% HPD 2.25–5.64 mya). However, the basal divergence among clades in the eastern lineage did not occur until the mid-Pleistocene ~0.73 mya (95% HPD = 0.39–1.17 mya). Geographically, this divergence corresponds to a valley running from southeastern Otero County, New Mexico through south-central Presidio County, Texas.

Figure 3.4.1 Cladogram with estimated divergence times for the major lineages within the *Crotalus molossus* group. Values at nodes are mean divergence dates and bars represent 95% highest posterior densities; open circles indicate calibration points. Samples representing the currently recognized *Crotalus molossus* group are in bold text. Abbreviations correspond to five geographically defined clades: Sonoran (Son), Madrean (Mad), Chihuahuan (Chih), Big Bend (Bigbend) and Stockton (Stock).

3.5 MORPHOLOGICAL ANALYSES

Univariate statistics (descriptive statistics, ANOVA and ANCOVA) of morphometric data (Table 3.5.1) did not identify any mensural characters that were significantly different between eastern and western *Crotalus m. molossus* lineages. However, univariate analysis of the meristic data indicated several characters that might be diagnostic when differentiating between eastern and western *C. m. molossus* lineages (Table 3.5.2). Informative meristic characters are DBLOT, FDBLOT, DSRV, INAS, PFOV and LOR ($P < 0.05$). Statistically significant differences between eastern and western clades were not observed in any of the qualitative characters; 6 of 101 *C. m. molossus* (one eastern *C. m. molossus*, three western *C. m. molossus* and two *C. m. nigrescens*) specimens examined lacked a postrostral (POSTROST) scale, and 12 of 101 (two eastern *C. m. molossus*, four western *C. m. molossus* and six *C. m. nigrescens*) exhibited contact between the first supralabial and prenasal (PRE-SUP).

Eigenvalues and principal component (PC) loadings for the first three principal components recorded from PCA of morphometric and meristic datasets are presented in Tables 3.5.3 and 3.5.4, respectively. Principal components analyses of regressed morphometric data did not indicate any differences among lineages of the *C. molossus* group (Fig. 3.5.1). Loadings from the latter analysis indicated that PC1 is a representation of tail length (TL), and PC2 and PC3 are the influence of head length (HL) and head width (HW) in both males and females. Principal component analyses of raw meristic data indicated morphologically distinct eastern and western *C. m. molossus* lineages (Fig. 3.5.2). Examination of PC loadings obtained from the raw dataset indicated that PC1 reflects the number of ventral scales (VENT), and PC2 reflects the overall number of dorsal blotches (DBLOT) and the extent of fusion in the dorsal pattern (FDBLOT). The placement of the type specimen of *C. molossus* (USNM 485) in score plots for

Table 3.5.1 Descriptive statistics and *P*-values obtained from one-way ANOVA and ANCOVA of regressed meristic data for the eastern and western *C. m. molossus* lineages.

Character	Lineage	Mean (millimeters)	SE Mean	StDev	<i>P</i>-value (ANOVA; ANCOVA)
SVL	Eastern	778.2	26.0	151.5	0.663; —
	Western	794.7	24.4	117.0	
TL	Eastern	56.67	2.38	13.67	0.342; 0.402
	Western	60.05	2.49	11.94	
HL	Eastern	40.50	1.20	6.995	0.598; 0.721
	Western	41.41	1.10	5.289	
HW	Eastern	30.997	1.15	6.706	0.886; 0.534
	Western	31.230	1.01	4.825	
NOD	Eastern	6.421	0.195	1.137	0.712; 0.948
	Western	6.526	0.191	0.914	
ID	Eastern	13.574	0.428	2.494	0.579; 0.926
	Western	13.917	0.399	1.915	
INAS	Eastern	6.785	0.201	1.173	0.506; 0.680
	Western	6.983	0.199	0.954	
RH	Eastern	4.421	0.157	0.917	0.820; 0.798
	Western	4.470	0.116	0.556	
RW	Eastern	4.747	0.146	0.852	0.129; 0.046
	Western	5.078	0.146	0.701	
MH	Eastern	3.662	0.134	0.779	0.289; 0.319
	Western	3.857	0.099	0.474	
MW	Eastern	5.147	0.187	1.088	0.247; 0.228
	Western	5.452	0.155	0.743	
CSL	Eastern	8.091	0.304	1.772	0.794; 0.448
	Western	7.983	0.227	1.089	
CSW	Eastern	3.429	0.150	0.877	0.542; 0.777
	Western	3.552	0.097	0.467	
UPH	Eastern	2.768	0.097	0.565	0.503; 0.128
	Western	2.678	0.074	0.353	
UPL	Eastern	4.509	0.156	0.912	0.788; 0.329
	Western	4.448	0.146	0.702	
LPH	Eastern	1.303	0.055	0.323	0.093; 0.030
	Western	1.452	0.067	0.323	
LPL	Eastern	5.082	0.175	1.019	0.707; 1.000
	Western	5.174	0.140	0.672	
PFL	Eastern	3.915	0.137	0.800	0.359; 0.376
	Western	4.091	0.113	0.540	
PFW	Eastern	5.212	0.192	1.118	0.300; 0.313
	Western	5.500	0.178	0.855	
SOCL	Eastern	8.847	0.263	1.532	0.843; 0.752
	Western	8.926	0.286	1.373	
SOCW	Eastern	4.927	0.165	0.960	0.970; 0.314
	Western	4.917	0.169	0.809	

Table 3.5.2 Descriptive statistics and *P*-values obtained from one-way ANOVA of meristic data for the eastern and western *C. m. molossus* lineages.

Character	Lineage	Mean	SE Mean	StDev	<i>P</i> -value
VENT*	Eastern	189.47	0.588	3.86	0.284
	Western	190.49	0.754	4.71	
SCDL*	Eastern	23.279	0.370	2.423	0.407
	Western	22.795	0.455	2.839	
DBLOT	Eastern	28.762	0.399	2.583	0.000
	Western	31.977	0.454	2.980	
FDBLOT	Eastern	8.405	0.279	1.809	0.000
	Western	1.907	0.413	2.706	
DSRN	Eastern	30.786	0.179	1.159	0.244
	Western	31.140	0.241	1.582	
DSRM	Eastern	26.929	0.110	0.712	0.817
	Western	26.977	0.174	1.144	
DSRV	Eastern	20.405	0.128	0.828	0.036
	Western	20.814	0.142	0.932	
PFRO	Eastern	2.143	0.073	0.4722	0.856
	Western	2.163	0.081	0.5314	
INAS	Eastern	2.333	0.126	0.816	0.025
	Western	2.930	0.227	1.486	
ISO	Eastern	5.571	0.153	0.991	0.223
	Western	5.256	0.205	1.347	
SUPRA	Eastern	17.167	0.148	0.961	0.400
	Western	17.372	0.191	1.254	
INFRA	Eastern	17.286	0.129	0.835	0.425
	Western	17.442	0.161	1.053	
SFOV	Eastern	0.833	0.076	0.4897	0.054
	Western	0.605	0.089	0.5831	
PFOV	Eastern	7.595	0.271	1.754	0.000
	Western	9.465	0.300	1.968	
LOR	Eastern	3.333	0.179	1.162	0.016
	Western	4.023	0.214	1.406	

* Sexually dimorphic characters, mean VENT female = 192.00 (eastern), 193.67 (western); mean VENT male = 187.27 (eastern), 188.56 (western). Mean SCDL female = 20.783 (eastern), 20.167 (western); mean SCDL male = 25.182 (eastern), 24.556 (western).

Figure 3.5.1 Score plots of first and second principal components obtained from PCA of morphometric data regressed against SVL for female (A) and male (B) specimens representing *Crotalus m. molossus* (eastern and western lineages), *C. m. nigrescens* and *C. tototacus*.

Figure 3.5.2 Score plots of first and second principal components obtained from PCA of raw meristic data representing *Crotalus m. molossus* (eastern and western lineages), *C. m. nigrescens*, *C. totonacus*, and type specimens of *C. ornatus* and *C. molossus*.

both the raw and standardized (not shown) datasets suggests that this specimen was collected from the western lineage.

Table 3.5.3 Eigenvalues and PC loadings obtained from a PCA of morphometric data for female and male specimens representing *Crotalus m. molossus* (eastern and western lineages), *C. m. nigrescens* and *C. totonacus*.

	<u>Females</u>			<u>Males</u>		
	PC1	PC2	PC3	PC1	PC2	PC3
Eigenvalue	25.418	11.142	2.168	43.334	19.454	2.393
Proportion	0.614	0.269	0.052	0.614	0.276	0.034
TL	0.959	0.260	-0.072	0.982	-0.048	-0.171
HL	0.217	-0.559	0.620	0.110	0.662	0.465
HW	0.124	-0.631	-0.738	-0.094	0.650	-0.721
NOD	0.047	-0.085	0.066	0.035	0.125	0.104
ID	0.044	-0.253	0.112	0.055	0.185	0.293
INAS	0.035	-0.122	0.103	0.022	0.076	0.099
RH	0.037	-0.085	0.086	0.032	0.061	0.176
RW	0.006	-0.074	0.039	0.031	0.063	0.118
MH	0.005	-0.152	0.038	-0.007	0.065	-0.033
MW	0.029	-0.120	0.102	0.024	0.065	0.009
CSL	0.027	-0.114	0.084	0.075	0.045	0.091
CSW	0.044	-0.102	-0.009	0.008	0.027	-0.030
UPH	0.006	-0.178	0.014	0.016	0.175	0.159
UPL	0.022	-0.050	0.042	0.030	0.062	0.151
LPH	0.046	-0.058	0.020	0.024	0.090	-0.003
LPL	0.033	-0.015	0.039	0.005	0.041	0.024
PFL	0.041	-0.096	0.029	0.020	0.081	0.044
PFW	0.017	-0.018	-0.016	0.002	0.020	0.034
SOCL	0.024	-0.072	0.014	0.013	0.074	0.054
SOCW	0.032	-0.102	0.070	0.013	0.082	0.138

Table 3.5.4 Eigenvalues and PC loadings obtained from a PCA of raw meristic data from specimens representing *Crotalus m. molossus* (eastern and western lineages), *C. m. nigrescens* and *C. totonacus*.

	PC1	PC2	PC3
Eigenvalue	34.843	17.881	10.681
Proportion	0.400	0.205	0.123
DBLOT	0.135	0.396	0.630
FDBLOT	-0.001	-0.891	0.232
DSRN	0.110	-0.051	-0.100
DSRM	0.072	-0.036	-0.026
DSRV	0.047	0.023	-0.063
VENT	0.964	-0.068	0.050
SCDL	-0.124	-0.024	0.678
PFRO	0.008	0.000	-0.014
INAS	0.060	0.055	-0.063
ISO	0.038	-0.067	-0.084
SUPRA	0.037	-0.050	-0.074
INFRA	0.023	-0.022	-0.103
SFOV	0.000	-0.017	-0.021
PFOV	0.079	0.159	-0.191
LOR	0.065	0.057	-0.090

3.6 TAXONOMY OF *CROTALUS MOLOSSUS*

The eastern lineage of *Crotalus m. molossus* inferred by Wüster et al. (2005) and the present study is morphologically and genetically distinct from the western lineage. The phylogenetic analyses of this study recovered strongly supported, reciprocally monophyletic eastern and western *C. m. molossus* lineages. The uncorrected pairwise mtDNA divergence calculated between eastern and western lineages (7.6–7.9%) is comparable to or surpasses values reported between currently recognized rattlesnake species (e.g., *C. mitchellii* and *C. stephansi*, 5.2–7.3% [Douglas et al. 2007]; *C. viridis* and *C. oreganus*, 3.5–5.6% [Pook et al. 2000]; *C. atrox* and *C. ruber*, 4.61% [Castoe et al. 2007]). Monophyly of the *C. molossus* group was rejected by AU and SH tests. Population-level analyses indicated that the eastern and western lineages are separated by 142 mutations, and mismatch distributions suggest that these clades are stable and have not been in contact since their divergence in the early Pliocene. Univariate statistical analyses identified distinct morphological character states between the lineages (dorsal blotches and the extent of fusion between anterior dorsal blotches, dorsal scale rows anterior to the vent, internasals, prefoveals and loreals), and PC analyses of meristic data clearly delineated distinct eastern and western lineages. Given these substantial differences between the eastern and western lineages of *C. m. molossus*, I recognize each of them as a distinct species, and resurrect the name *Crotalus ornatus* Hallowell 1854 for the eastern lineage. Under the Evolutionary Species Concept (Simpson 1961; Wiley 1978) and its more recent incarnation, the General Lineage Concept (de Queiroz 1998, 1999), a species is a lineage with an evolutionary path that is distinct from other lineages. Furthermore, the General Lineage Concept simplifies species delineation by removing the prerequisites (e.g., morphological distinctness, reproductive

isolation or ecological divergence) that had to be met under previous species concepts, and only requires that a species be evolving separately from other lineages.

3.7 TAXONOMY OF *CROTALUS MOLOSSUS SENSU STRICTO*

Baird and Girard (1853) described *Crotalus molossus* from USNM 485, an adult male collected from Fort Webster, Santa Rita Del Cobre mine, New Mexico. This locality is about 4.5 km northeast of the town of Bayard in Grant Co. New Mexico, and seems to be at or near the biogeographical boundary that differentiates the eastern and western *C. m. molossus* lineages. Specimens collected from within 75 km north and 35 km west of this locality have been unequivocally identified as members of the western lineage. Two samples, one collected from ~62 km northeast and the other ~60 km southeast of the type locality, were recovered within the eastern lineage. Interestingly, a specimen collected in the town of Bayard (UTEP 20155) may be a hybrid—it was recovered in analyses of the mtDNA and six-gene datasets as *C. atrox*, whereas analyses of the combined nDNA dataset recovered this specimen in the Madrean clade with moderate support (80% MP; 67% ML; 0.92 BI). The description of the holotype provided by Baird and Girard (1853) reported a ground color of “roll sulfur.” Examination of live specimens, color photos in the UTEP collection and published color plates (Campbell and Lamar 1989, 2004; Beaman and Hayse 2008) suggest that a ground color of pale to bright yellow is typical for the Madrean clade. However, Baird and Girard (1853) noted extensive fusion of anterior dorsal blotches, a character state common in the eastern lineage. Baird and Girard (1853) reported the following scale counts for the holotype: two frontals, four prefrontals, two internasals, five interoculabials, 18 supralabials, 17 infralabials and 29 dorsal scale rows at midbody. These are consistent with the values that I recorded from USNM 485. In addition, I recorded 33 and 22

dorsal scale rows at the neck and anterior to the vent, respectively, 188 ventrals, 26 subcaudals, six intersupraoculars, no subfoveals, 13 prefoveals and five loreals. This specimen is preserved as a skin with the head and tail attached and is in relatively good condition. However, the color pattern has faded somewhat and only 22 dorsal blotches (five of them fused) are visible before the extent of fading obscures the color pattern at about the 135th ventral. The following measurements were recorded; tail length = 62 mm, head length = 43.1 mm, head width = 35.6 mm. Baird and Girard (1853) did not report measurements in their description and an accurate SVL could not be measured from the skin. However, an estimated SVL of ~77–83 cm for the holotype was obtained from mean values for ratios of head length to SVL and tail length to SVL for male specimens of *C. m. molossus* used in this study. The SVL of the holotype in life would have been slightly larger as fluid preserved specimens are known to experience distortion and shrinkage during preservation (Vervust et al. 2009). Three character states place USNM 485 in the western lineage, including DSRV (22), PFOV (13) and LOR (5). The presence of five fused dorsal blotches (FDBLOT) is high compared to the mean of 1.91 for the western lineage, yet this value is well within the variance of 7.32. Based on inference of nDNA data obtained from UTEP 20155 collected near the type locality, the morphological data collected from the type specimen, and the description of the specimen in life provided by Baird and Girard (1853), I conclude that USNM 485 was collected from a population within the Madrean clade of the western *C. m. molossus* lineage.

3.8 REDISCUSSION OF *CROTALUS ORNATUS*

Holotype.—An adult female (USNM 486 [Fig. 3.8.1]) collected by A. Heermann, from a locality “near Pecos River, N. W. Texas.” The specimen was collected during the survey of a

Figure 3.8.1 Head and body of the holotype of *Crotalus ornatus*, USNM 486.

railroad route to the Pacific, led by Lieutenant Parke, U.S. Top Engineer (collection date unknown).

Diagnosis.—*Crotalus ornatus* (Fig. 3.8.2) can be distinguished from most congeners by having: (1) a medium sized SVL (adults 70–100 cm, rarely to 130 cm SVL); (2) two large, triangular internasal scales; (3) two large prefrontals; (4) two large frontal scales; (5) 6–10 prefoveals; (6) 2–4 loreals; (7) two preoculars; (8) five postoculars; (9) 2–7 intersupraoculars; (10) 16–18 supralabials; (11) 14–21 infralabials; (12) 23–29 dorsal scale rows at midbody; (13) 164–205 ventrals; (14) 16–30 subcaudals (16–26 in females and 21–30 males); (15) internasal-prefrontal region and supraoculars black or dark brown; (16) dark pigmentation of dorsal pattern extends from nape to occipital and parietal regions; (17) 22–33 (mean = 29) rhomboid dorsal body blotches that often coalesce with lateral blotches at midbody to form bands or chevrons (dorsal blotches of some specimens may become diffuse posteriorly, represented by barely distinct or absent bands anterior to vent); (18) light medial spots longer than wide on anterior portion of body, becoming broader at midbody separating dorsal blotches; (19) medial spots often become indistinguishable from ground color on posterior $\frac{1}{4}$ of body; (20) two white or light grey, irregular paravertebral spots present within each dorsal blotch, usually separated by 1–6 dorsal scales rows on anterior portion of body, but darken slightly and grow to extend across midline of dorsum to fill interior of dorsal blotches on posterior $\frac{1}{2}$ of body; (21) tail black or dark brown with faint cross-bands sometimes visible; (22) proximal rattle segment black or dark brown (Tables 3.5.1 and 3.5.2).

Comparisons.—*Crotalus ornatus* is unique among *C. molossus* lineages in having 4–11 prefoveals; 2–7 loreals; a ground color of gray, olive-green, olive-brown, or tan; 22–33 rhombic dorsal blotches, each separated by a light gray, white or pale yellow medial spot; and anterior 6–

Figure 3.8.2 Representative specimens of *Crotalus ornatus* and *C. molossus* in life: (A) *C. ornatus*, Hueco Mountains, El Paso Co., Texas; (B) *C. ornatus*, Davis Mountains, Jeff Davis Co., Texas (CGA 06); (C) *C. ornatus*, Terlingua, Brewster Co., Texas; (D) *C. molossus sensu stricto*, Santa Rita Mountains, Santa Cruz Co., Arizona; (E) side profile of head of *C. ornatus*, Chisos Mountains, Big Bend National Park, Brewster Co., Texas; (F) side profile of head of *C. molossus sensu stricto*, Santa Rita Mountains, Santa Cruz Co., Arizona. With the exception of CGA 06, the photographed animals were not collected.

10 dorsal blotches connected by 1–3 scale rows, often resembling paravertebral stripes or a thick dorsal stripe surrounding light medial spots. *Crotalus ornatus* most closely resembles *C. molossus sensu stricto*, but can be distinguished from the latter taxon by its lower average scale counts ($P < 0.001$) for prefoveals (8 vs. 10) and loreals (3 vs. 4), fewer dorsal blotches (29 vs. 32) and a dorsal blotch pattern where the first 6–10 dorsal blotches are almost always connected (dorsal blotches are almost always separated by a light medial spot in *C. molossus sensu stricto*). *Crotalus ornatus* can be distinguished from *C. m. nigrescens* and *C. m. oaxacus* by having a higher number of dorsal scale rows at midbody (27 vs. 25), a higher number of ventrals (up to 205 in females vs. 187 in *C. m. nigrescens* and fewer in *C. m. oaxacus* [Klauber 1972]), dark pigmentation on the head restricted to the internasal-prefrontal area, with thin (1 scale row wide), sometimes ornate lines on the occipital and parietal regions (extensive pigmentation often covers most of the head of the latter two lineages), and light medial and paravertebral spots in the dorsal pattern (usually absent in the latter two taxa). In *C. m. oaxacus* it is common for adults to retain black and white tail rings common in juveniles of the *C. molossus* group (Campbell and Lamar 1989, 2004), but this character does not persist to adulthood in *C. ornatus*.

Crotalus ornatus is most easily distinguished from *C. estebanensis* by geographic distribution; the former is widespread throughout most of the Chihuahuan Desert, whereas the latter is an insular endemic in the Gulf of California. Morphologically, *C. ornatus* differs from *C. estebanensis* in having relatively dark dorsal blotches that create a dorsal pattern that boldly contrasts with the ground color (dorsal blotches are paler and the pattern moderately contrasting with the ground color in *C. estebanensis*), and dark pigmentation on the head in the internasal-prefrontal area of *C. ornatus* lacks dark coloration in the prefrontal-internasal area (absent in *C. estebanensis*) (Grismer 1999; Campbell and Lamar 2004). Klauber (1949) noted longitudinal and

transverse compression of the rattle of *C. estebanensis* as a character unique among members of the *C. molossus* group, which includes *C. ornatus*.

Crotalus ornatus differs from *C. totonacus* in being smaller (rarely to 125 cm vs. commonly exceeding 150 cm), in having a larger number of intersupraoculars (5 vs. 1 or 2), prefoveals (8 vs. 3 or 4), supralabials (17 vs. 14) and infralabials (17 vs. 15), in having an anterior dorsal pattern of interconnected but usually distinct dorsal blotches (paravertebral stripes [usually without distinct blotches] on the neck transition into an intricate pattern on the head in *C. totonacus*), and by the lack of a vertebral ridge (relatively well developed in *C. totonacus*) (Campbell and Lamar 2004).

Description of the holotype.—Rostral about as high as wide; internasals (2) large and triangular, in contact medially, anterior medial portion contacts apex of rostral; prefrontals (2) large and roughly square, in contact medially, anterior edge broadly contacts prenasal and partially contacts postnasal scale; frontals (2) large and roughly square, anterior in broad contact with prefrontals, mostly in contact medially but separated anteriorly by a single small scale, posterior border rounded and followed by intersupraoculars (7); prenasal large and rectangular, upper edge in broad contact with internasal and partial contact with postnasal, anterior portion broadly contacts rostral; postnasal irregular, surrounds upper, lower and posterior borders of nostril; loreals (3) prevent contact between postnasal and preoculars; postsupraloreal (canthal) roughly square and in contact with supraloreal, upper preocular, supraocular (see remarks) and prefrontal; supraloreal round, in contact with postsupraloreal, upper and lower preoculars, infraloreal, postrostral and prefrontal; infraloreal irregular, in contact with supraloreal, lower preocular, upper lacunal, two upper prefoveals and postnasal; preoculars (2) in contact with supraocular, loreals and upper lacunal (see remarks); prefoveals (9), the largest anterior scale is

in broad contact with rostral and together with second prefoveal prevents contact between prenasal and first supralabial; lacunals (3) are partially visible on exterior margin of loreal pit; supralabials (18) separated from prenasal, postnasal and infraloreal by prefoveals, fourth (and possibly fifth) in contact with lower lacunal scale; 17 infralabials; dorsal scale rows are 33/27/20; ventrals are 194 and subcaudals are 19.

Measurements of the holotype.—The holotype is an adult female; Hallowell (1854) reported the following measurements: body length = 41 inches (in.) (~104 cm); tail length = 2 in. (~5.1 cm); head length = 1.75 in. (~45 mm); and head width = 1.5 in. (~38 mm). Reexamination of the specimen recovered the following measurements: tail length = 4.8 cm; head length = 46.1 mm; head width = 32.4 mm (see remarks); and interocular distance = 14.2 mm. Because the specimen is preserved as a skin with head and tail attached, SVL could not be accurately measured (see remarks).

Coloration of the holotype.—Dorsum of head grayish and beige with brown and tan appearing in internasal-prefrontal area with some brown flecking on scales of the anterior occipital region; dorsum of body gray with 24 brown, rhomboid dorsal blotches, seven (anterior) connected by one or more scale rows; medial spots off white, anterior spots roughly as long as wide, but becoming wider at midbody; paravertebral spots gray, distinct on anterior of body but expand and connect dorsally to fill interior of dorsal blotches by midbody; tail and proximal rattle segment black; gular area lacking dark pigmentation but stained beige or yellowish; venter mostly off white or yellow with slight gray mottling at midbody, which becomes more extensive posteriorly; ventral surface of tail dark gray.

Distribution.—With the exception of populations occupying the Edward's Plateau of central Texas, the northern Chihuahuan Desert defines the geographical distribution of *Crotalus*

ornatus. Meristic analyses of specimens from northern Mexico suggest that *C. ornatus* is present as far south as central Coahuila, Mexico in the mountains of the Cuatro Ciénegas Basin. The southern border for the lineage is unclear with available data. However, records for *C. m. nigrescens* (Klauber 1972; Campbell and Lamar 2004) suggest that the distribution of *C. ornatus* likely terminates at a boundary in southern Coahuila. Numerous authors have suggested a biogeographical barrier in northern Durango and southern Coahuila to explain divergences between species in the northern and southern Chihuahuan Desert (e.g., Baker 1956; Peterson 1976; Hafner et al. 2008). In this region, the Río Nazas flows from its source in the Sierra Madre Occidental of north-central Durango and terminates in the Laguna de Mayrán (Conant 1963), part of an arid bolson known as the Parras Basin that transects southern Coahuila (Krutak 1967). The Parras Basin is bordered by extensions of the Sierra Madre Oriental to the south and the Coahuila Peninsula and Coahuila Marginal Folded Belt to the north (Krutak 1967). Baker (1956) suggested that the Southern Coahuila Filter Barrier is defined by the Río Nazas, Parras Basin and the mountain ranges south of the basin. The arid bolson of this barrier would present a formidable obstacle for any montane or semi-montane species, and is likely the southern boundary for *C. ornatus*. However, montane corridors may allow contact with *C. m. nigrescens* in southeastern Coahuila and western Nuevo León. Gloyd (1940) suggested a zone of intergradation near Galeana, Nuevo León, Mexico, which is well outside of the currently recognized range of the northern black-tailed rattlesnake.

The geographic boundaries of *Crotalus ornatus* in the Mexican state of Chihuahua are less clear. In the absence of a biogeographic barrier in this area, it may be informative to discuss the distribution of *C. ornatus* with respect to *C. m. nigrescens*. Tanner (1985) suggested that *C. m. molossus* occurs through most of Chihuahua, and that a wide area of intergradation with *C. m.*

nigrescens occurs in northern Durango and southern Chihuahua. Possible *C. m. molossus* x *nigrescens* hybrids reported from northeastern Durango near the town of Pedriceña (Fouquette and Rossman 1963) and southwest Chihuahua, near Samachique (Gloyd 1940) seem to support this. Conversely, Klauber (1972) indicated the presence of *C. m. nigrescens* in central Chihuahua and proposed a zone of intergradation along the entire southern boundary of *C. m. molossus*. Likewise, Campbell and Lamar (2004) indicated a substantial incursion of *C. m. nigrescens* into west-central Chihuahua, but did not discuss introgression. Three specimens, (UTA R 17837–38 and UTEP 3052) seem to extend the range of *C. m. nigrescens* to the Sierra del Nido, approximately 75 km northwest of Ciudad Chihuahua. The Sierra Madre Occidental and Oriental are well known corridors for dispersal from southern Mexico, and the presence of *C. m. nigrescens* in the Sierra Madre of central Chihuahua is not unexpected. Unfortunately, the lack of taxonomic attention received by the *C. molossus* group has resulted in a failure to recognize distinct species and a subsequent lack of resolution with regard to geographic distribution of distinct lineages. Further sampling through central and southern Chihuahua is needed to accurately define the geographic distributions of lineages within the *C. molossus* group.

Extensive sampling from localities throughout the range of the black-tailed rattlesnake in the United States has allowed a much clearer biogeographical picture of *Crotalus ornatus* north of Mexico. The mountain ranges associated with the southeastern Colorado Plateau in New Mexico represent the northwestern limits of the lineage. From there, it is distributed throughout the mountain ranges of southern New Mexico and western Texas. In the United States, *C. ornatus* occupies two of the biotic provinces recognized by Blair (1949), the Chihuahuan and the Balconian. The Chihuahuan province is characterized by the basin-and-range topography that is

typical of the northern Chihuahuan Desert, and encompasses south-central New Mexico, Texas (west of the Pecos River), eastern Chihuahua and central Coahuila, Mexico. The Edwards Plateau or Balcones Escarpment of central Texas defines the Balconian province (Werler and Dixon 2000). The eastern boundary of the species extends out of the Chihuahuan province to include the limestone canyons of the southern and eastern Edwards Plateau (Werler and Dixon 2000; Campbell and Lamar 2004). Populations on the Edwards Plateau are apparently disjunct from the populations of the adjacent Stockton Plateau and Chihuahuan province (Werler and Dixon 2000; Campbell and Lamar 2004). Werler and Dixon (2000) illustrated this disjunction in Texas populations between the Devils River of central Val Verde County and the vicinity of Rock Springs in eastern Edwards County, a distance of approximately 75 km.

Remarks.—Hallowell (1854) provided a vague account of the collection locality as “near Pecos River, N. W. Texas” for the holotype. However, PC analysis of meristic data placed the holotype near specimens from the Beach and Baylor Mountains of Culberson Co., Texas. This could fit the general location given by Hallowell. McDiarmid et al. (1999) cited the locality as “between El Paso and San Antonio, Pecos River, Texas”; this differs from the original description and restricts the locality to somewhere on the Pecos River in Texas as opposed to the subjective reference of proximity to the Pecos River given by Hallowell. If USNM 486 were collected from a locality on the Pecos River, the type locality could be restricted to five counties (Crane, Crocket, Pecos, Terrell and Val Verde), which lie within the distribution of *C. ornatus* and border the Pecos River in Texas (Werler and Dixon 2000; Campbell and Lamar 2004).

The specimen is preserved as a skin with the head and tail attached, and the overall condition of the specimen is fair. However, several characters could not be confidently assessed. Examination of the dorsal pattern revealed 20 dorsal blotches before the pattern was lost to

fading on the posterior $\frac{1}{4}$ of the body. The original description reported 17 blotches, but this did not include the seven fused anterior dorsal blotches. Damage to scales on the head prevented accurate measurement of upper and lower preoculars, supraoculars and prefrontals. Damage to the supraoculars makes it difficult to determine if the upper loreal makes partial contact with the frontal. Because of distortion of the head and skin around the eye, the postoculars, suboculars and interoculabials could not be assessed. The condition of the head suggests that it has been laterally compressed or had become dehydrated after preservation, causing disproportionate lateral shrinkage. Given the method of preservation of the specimen and the measurements reported by Hallowell (1854), a confident measurement of SVL could not be obtained. However, estimates of SVL (based on ratios of TL to SVL and HL to SVL) range from 74–100 cm for the specimen. The 26 cm discrepancy among estimates seems to be the result of the specimen's exceptionally short tail, only 4.9% of the SVL calculated from the measurements provided in Hallowell (1854) (5.2% if Hallowell's measurement of body length included the tail) compared to 6.5% calculated from specimens examined in the present study. Based on my examination of the specimen, I am confident in designating the specimen as an adult female with a SVL greater than 80 cm.

CHAPTER 4: DISCUSSION

4.1 PHYLOGENY AND TAXONOMIC STATUS OF *CROTALUS MOLOSSUS*

To date, this thesis represents the most comprehensive phylogenetic analysis of any member of the *Crotalus molossus* group. Five major lineages associated with the *C. molossus* group were recovered (*C. m. nigrescens*, *C. basiliscus*, *C. totonacus*, eastern *C. m. molossus* [recognized herein as *C. ornatus*] and western *C. m. molossus* [*C. molossus sensu stricto*]). *Crotalus ornatus* is confined to the vicinity of the northern Chihuahuan desert, occupying a region from south-central New Mexico through south-central Texas and adjacent Mexican states, whereas *C. molossus sensu stricto* occupies a range that is roughly equivalent to the Sonoran Desert east of the Colorado River. Despite the presence of both lineages in the southern ranges of the Rocky Mountains and the Madrean Archipelago, these two lineages are not sister taxa and do not seem to have been in contact with one another since their initial divergence 4.63 mya. The phylogenetic patterns recovered herein corroborate the polyphyly of *C. m. molossus* recovered by Wüster et al. (2005).

The well-supported and geographically defined lineages inferred herein demonstrate discordance between the current taxonomy of the *Crotalus molossus* group and its true diversity. Since the initial description of *C. molossus* by Baird and Girard (1853), only two authors have attempted to reclassify the species. Garman (1884) attempted to reclassify *C. molossus* as a variant of *C. basiliscus*, and Boulenger (1896) assigned the name *C. terrificus* to a group of rattlesnakes (*C. basiliscus*, *C. durissus* and *C. molossus*); most recently the epithet *terrificus* has been associated with a lineage of *C. durissus* (*C. d. terrificus*, Laurenti 1768) occurring in southern and western Brazil, Bolivia, extreme southeastern Peru, Paraguay, Uruguay and

Argentina (Campbell and Lamar 2004). Only one species (*C. ornatus*, Hallowell 1854) has been synonymized with *C. molossus*. Hallowell (1854) described *Crotalus ornatus* from USNM 486, a specimen collected near the Pecos River in Texas. Hallowell gives no further locality data, but any locality in the state of Texas will unequivocally lie within the distribution of the eastern lineage of *C. m. molossus* inferred in this study. Additionally, Hallowell's description is consistent with character states exhibited by the eastern lineage of *C. m. molossus*.

I prefer not to formally propose reclassification of the lineages *C. m. nigrescens* and *C. m. oaxacus*, as no specimens of the latter and only two specimens of the former lineage were available for molecular analyses. However, *C. m. nigrescens* is consistently inferred as the basal member of a group that includes *C. basiliscus*, *C. totonacus* and the remaining *C. molossus* group. It is logical that the subspecific epithet *nigrescens* will be elevated to full species and applied to all representatives of *C. molossus* occurring south of ~25° N latitude. Additionally, some morphological overlap exists between specimens designated as *C. m. nigrescens*, *C. molossus sensu stricto* and *C. ornatus*. However, this overlap may be the result of failure to identify intergrades or properly assign specimens. In his description of *C. m. nigrescens*, Gloyd (1936) discussed a number of characters (not examined in the present study) that seem to hold true for this lineage, including head coloration and the extent of dark pigmentation anterior to the vent. The true phylogenetic position of *C. m. oaxacus* was not examined in the present study and remains poorly understood. Wüster et al. (2005) recovered *C. m. oaxacus* as sister to a clade containing *C. basiliscus* and *C. molossus sensu stricto* with weak support, and Campbell and Lamar (1989) attributed previous taxonomic confusion to plesiomorphic characters that *C. m. oaxacus* shares with *C. basiliscus*. Extensive sampling throughout the range of *C. molossus sensu lato* is needed to clearly define geographical boundaries and assess its diversity.

4.2 BIOGEOGRAPHY OF *CROTALUS MOLOSSUS* AND *CROTALUS ORNATUS*

Phylogenetic analyses of the mtDNA and six-gene datasets have revealed biogeographic structure, with three well-supported clades in the eastern lineage (Fig. 3.1.1). The two specimens representing the apparently disjunct Edwards Plateau population (UTEP 17515 and 17520) are grouped with strong support in all analyses, but they are nested within the Stockton clade, which is strongly supported as the sister group to the Big Bend clade. Within the Big Bend clade, specimens from the western and northern Big Bend region (northern Brewster Co., central Presidio Co. and Jeff Davis Co., Texas) are grouped with strong support. A genetic divergence of 1.2% corresponding to an estimated divergence date of 0.73 mya was identified between specimens from the Bigbend clade and those occurring at the Indio Mountains Research Station (IMRS), a lower elevation extension of the Eagle Mountains in extreme southeastern Hudspeth Co., Texas. This suggests the area between these localities may represent a previously unaddressed barrier between populations of montane and semi-montane species. IMRS is separated from the Bigbend specimens by a swath of desert grassland stretching from the New Mexico border near Dell City, Texas through the towns of Van Horn and Marfa, Texas to the south. South of Van Horn, in western Jeff Davis Co. and north-central Presidio Co., the grassland becomes a wide valley bordered by the Sierra Vieja and Van Horn mountains to the west. East of these mountain ranges, the arid grassland (the Marfa Plains) gains slightly in elevation over 30 km, before reaching the Davis Mountains. The Marfa Plains continue southeast from the town of Marfa, slightly past the Brewster County line. While not particularly low in elevation, the Marfa Plains are 400–600 m lower than the adjacent mountain ranges and offer no obvious refuge for dispersing montane species. In central Presidio County, a chain of

disjunct mountain ranges funnels the arid grassland into a few narrow corridors that connect to the Presidio and Redford Bolsons. These adjoining bolsons form a low valley more than 25 km wide, which follows the Rio Grande for about 100 km from the town of Candelaria, Texas to the foothills south of the Bofecillos Mountains (Mraz and Keller 1980). Together, the Marfa Plains and Presidio Bolson seem to represent a recent biogeographic barrier for *C. ornatus*.

Crotalus molossus sensu stricto seems to reach its southern boundary near the Yaqui River (at about 28° N latitude) in the state of Sonora, Mexico. From there, the lineage occurs through the Sonoran Desert. Specimens examined from Isla Tiburón, off the central coast of Sonora, Mexico, are characteristic of specimens inferred in the western lineage by phylogenetic analyses. Morphological and molecular data indicate that populations from the Sierra San Luis of northwestern Chihuahua, Mexico and the Madrean Archipelago of southwestern New Mexico represent the eastern boundary of the lineage. Presumably, *C. molossus sensu stricto* is present through the Sierra Madre Occidental south of the Sierra San Luis from extreme western Chihuahua through most of Sonora, Mexico.

Geographic structure is evident among populations of southeastern Arizona and southwestern New Mexico (Madrean clade) and west-central Arizona (Sonoran clade); the basal divergence among these clades occurred near the Pliocene-Pleistocene boundary ~1.86 mya and is represented by marked genetic divergence (3.6%). The estimated divergence date is consistent with the argument by Philips and Comus (2000), who suggested that patterns of diversity and endemism among Sonoran Desert plant assemblages indicated the formation of the Sonoran Desert at the Pliocene-Pleistocene boundary. Furthermore, this timescale is similar to that inferred by Douglas et al. (2010) for the basal diversification within the Gila monster (*Heloderma suspectum*). Niche conservatism and the likely origin of the *C. molossus* group in

the subtropical Sierra Madre Occidental (implied by the current distribution of the basal lineage of the group) of the late Miocene may have resulted in biogeographic patterns triggered by the onset of aridification in the Sonoran Desert at the Pliocene-Pleistocene boundary. However, only two samples from the Sonoran clade were included in this study, and there are no available samples representing populations between the Sonoran and Madrean clades. In contrast, the Madrean clade is well represented by samples from the mountains of southeastern Arizona and southwestern New Mexico. The basal divergence in this clade is estimated at the mid-Pleistocene (~0.31 mya), a common pattern within species occurring near the Cochise Filter Barrier (see examples below).

4.3 THE *CROTALUS MOLOSSUS* GROUP AT THE COCHISE FILTER BARRIER

A narrow desert corridor that allows the exchange of taxa between the Sonoran and Chihuahuan deserts defines the Cochise Filter Barrier (CFB). The specific physical barrier of the CFB is poorly defined and lacks any geographical features that could be construed as an impediment to dispersal. Consequently, the term is generally applied to the lower elevation passes over the Western Continental Divide in southwestern New Mexico (Riddle and Hafner 2006; Castoe et al. 2007; Pyron and Burbrink 2010). Some authors (e.g., Riddle and Hafner 2006; Wilson and Pitts 2010a) have treated the CFB as a region encompassing a relatively large area comprised of the Madrean Archipelago of southeastern Arizona and the Deming Plains, which is a swath of desert grassland extending from the Arizona-New Mexico border to the Rio Grande in southern New Mexico. This approach may be more appropriate given the prevalence of lineage spillover; approximately 33% of taxa examined by Pyron and Burbrink (2010) had representatives on both sides of the Western Continental Divide. Furthermore, many of the taxa

with divergences that could be associated with the CFB have ranges that are not constrained by the Western Continental Divide, but they remain in the vicinity of the CFB (e.g., Jaeger et al. 2005; Castoe et al. 2007; Mulcahy 2007).

In this region, *Crotalus ornatus* is present in the Cook's Range, the Mimbres Mountains, and the eastern slopes of the Black Range in south-central New Mexico. *Crotalus molossus sensu stricto* is present in the Madrean Archipelago of southwestern New Mexico, the Mogollon Mountains and the Pinos Altos Range north of the Deming Plains. The wide swaths of desert grassland that currently provide corridors between Sonoran and Chihuahuan habitats likely prevent dispersal between many of the mountain ranges occupied by *C. molossus* and *C. ornatus* in this region. However, mountain ranges that define the northern border of the Deming plains (Mogollon, Pinos Altos, Mimbres and Black Range) seem relatively well connected and might allow contact between lineages. In this region, the Mimbres River and western continental divide north of the Mimbres Valley seem to represent the only potential barrier that may explain the apparent lack of introgression between *C. ornatus* and *C. molossus*.

Following the last major glaciation of North America, the CFB and associated region began a period of aridification, resulting in a transformation from mesic woodlands to the modern desert grassland habitat (Morafka 1977). Similar climatic events have occurred across the CFB following each major North American glaciation and a number of taxa underwent recent divergences at the CFB that may be attributable to these events (e.g., *Callispepla*, Zink and Blackwell 1998; *Crotalus atrox*, Castoe et al. 2007; *Sceloporus magister* complex, Leaché and Mulcahy 2007; *Lampropeltis getula* complex, Pyron and Burbrink 2009). Presumably, these divergences are the result of aridification beginning at the Pliocene-Pleistocene boundary and subsequent Pleistocene glaciation events forced desert-adapted taxa into refugia on either side of

the CFB (Pyron and Burbrink 2010). This hypothesis fits desert-adapted species well and Pleistocene refugia are a likely explanation for post-Pliocene lineage divergence in the region. However, at least two recent studies have demonstrated deeper divergences and species boundaries at the CFB (e.g., *Hypsiglena torquata* species complex, Mulcahy 2008; *Dilophotopsis* sp., Wilson and Pitts 2010b). These relatively deep divergences are more likely the result of orogenic activity of the Sierra Madre Occidental and Rocky Mountains, which peaked in the mid- to late Miocene (Morafka 1977; Wilson and Pitts 2010a). Some authors have suggested that Neogene uplift of the area was minor and that the deep divergences reported for these taxa are the result of glaciation events occurring as early as 9 mya (see Wilson and Pitts 2010a and references therein). Regardless, the *C. molossus* group exhibits a deep divergence that seems to correspond well to the CFB and the Western Continental Divide. Indeed, the estimated mean divergence date of 4.63 mya between eastern and western *C. m. molossus* lineages (inclusive of their respective sister taxa *C. basiliscus* and *C. totonacus*) supports the idea of a Neogene vicariance event at the Sierra Madre Occidental.

4.4 INTROGRESSION AND INCOMPLETE LINEAGE SORTING

Hybridization is a common event among rattlesnakes, and *Crotalus molossus* is no exception. This study revealed a hybrid event between *C. atrox* and *C. molossus sensu stricto* (Figs. 3.1.1 and 3.1.2); the specimen (Mad 7, UTEP 20155) was collected in the town of Bayard, New Mexico and has no morphological characteristics distinguishing it as *C. atrox*. However, analyses of the mtDNA and six-gene datasets inferred this specimen as *C. atrox*, whereas analysis of the combined nDNA dataset recovered it as *C. molossus sensu stricto*. Several accounts describe hybridization between members of the *C. molossus* group and allies, including

C. m. molossus x *C. m. nigrescens* (Gloyd 1940; Fouquette and Rossman 1963; Tanner 1985), *C. molossus* x *C. basiliscus* and *C. molossus* x *C. totonacus* (Campbell and Lamar 2004). The black-tailed rattlesnake is a montane generalist and is largely considered to be a semi-montane species, but specimens have been recorded from sand dunes and creosote flats (Armstrong and Murphy 1979; Beck 1991), suggesting that members of the *C. molossus* group are capable of dispersing between populations, which are usually associated with mountains or foothills. At the CFB, there are no distinct features that would prevent contact between *C. molossus sensu stricto* and *C. ornatus*, and it would not be unexpected to find patterns of mitochondrial introgression similar to that reported between *Sceloporus cowlesi* and *S. tristichus* (Leaché 2009). These non-sister mtDNA lineages of the *S. undulatus* species group seem to occupy generally similar habitats in adjacent ranges. In this case, the latter author sampled a known hybrid zone and was able to demonstrate discordance between the mtDNA data and his multi-locus nuclear dataset that was attributed to introgression. Although there seems to be a propensity for introgression in the *C. molossus* group, as recorded herein between *C. atrox* and *C. molossus sensu stricto* and by previous studies, mtDNA introgression was not evident between my samples of *C. ornatus* and *C. molossus sensu stricto*.

Independent analyses of the nuclear loci included in this study revealed extensive incomplete lineage sorting in the genus *Crotalus*. In general, introgression and incomplete lineage sorting can be readily distinguished by the pattern of incongruence. In most cases of introgression, the incongruence among lineages occurs at geographic clade boundaries (e.g., Leaché 2009). Incongruence resulting from incomplete lineage sorting is usually stochastic with respect to phylogeographic and taxonomic boundaries (McGuire et al. 2007). Results inferred from the EXPH5 dataset (Fig. 3.1.4) illustrated random placement of *Crotalus* specimens,

indicating the presence of shared ancestral polymorphisms. This is not uncommon, especially among lineages that are weakly supported or have undergone rapid diversification (Wiens 2010a). In general, the New World Crotalinae is noted for its rapid diversification beginning 17–22 Ma, ultimately resulting in ~117 extant species (Castoe et al. 2009; Wüster et al. 2008); this presents a speciation interval roughly half that of the Viperidae as a whole (Wüster et al. 2008). The handful of studies that have utilized nDNA markers to investigate relationships among pitvipers have used introns to address large-scale relationships among Asian species and questions regarding the sister taxon to the American Crotalinae (e.g., Creer et al. 2003, 2005, 2006; Malhotra et al. 2010). These studies did not indicate incongruence among trees inferred from nuclear intron datasets. Recently Kubatko et al. (2011) employed a dataset of 18 nuclear introns to examine relationships within the genus *Sistrurus*; the authors recovered a weak phylogenetic signal (similar to the nuclear genes used herein) from some of the nuclear loci that they examined, whereas others provided a phylogenetic signal similar to mitochondrial markers. It is likely that the inherent selective pressures and rate heterogeneity associated with portions of the coding genes examined herein have not allowed lineage sorting among the rattlesnakes to reach completion.

4.5 CONCLUSIONS AND FUTURE DIRECTIONS

This study revealed that the diversity of *Crotalus molossus* has been underestimated for over 150 years. Five lineages were recovered in association with the *C. molossus* group. Three were previously assigned to *C. molossus* and one of these had no current taxonomic recognition. Diversification within the *C. molossus* group seems to be associated with the uplift of the Sierra Madre Occidental beginning in the late Miocene and continuing to the Pliocene. The resulting

lineages are well-supported in the inferred phylogenies and should be recognized as discrete taxonomic entities under the oldest available name. The western lineage should retain the present taxonomic designation *Crotalus molossus*. The previously synonymized name *Crotalus ornatus* is resurrected for the eastern lineage. Further study is needed for the currently recognized subspecies *C. m. nigrescens* and *C. m. oaxacus*.

The results of this study raise several questions regarding the diversity of the *Crotalus molossus* group. Future effort should be focused on defining the phylogeographic boundaries throughout Mexico. Moreover, sampling populations throughout Mexico for molecular analyses will open the door to answer questions that this study could not. For example, the phylogenetic relationship between *C. basiliscus*, *C. m. nigrescens* and *C. m. oaxacus* is still poorly defined. A greater sample size will provide better resolution for these relationships and will allow future researchers to investigate the evolutionary processes that have shaped the *C. molossus* group. In addition to phylogenetic work, an exhaustive analysis of morphometric, meristic, qualitative and hemipenial characters of the entire *C. molossus* group and its allies is needed.

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APPENDIX I

Samples used for genetic analyses in this study with voucher information, locality data, and GenBank accession numbers. All sequences are novel to the present study unless otherwise indicated. Institutional codes follow Leviton et al. (1985); additional voucher codes are as follows: CGA = C. G. Anderson field series; CLP = C. L. Parkinson field series; JAC = J. A. Campbell field series; Moody = S. M. Moody. IMRS = Indio Mountains Research Station. Complete locality data are provided in Appendix II.

Taxon and sample identifier	Voucher	Locality	GenBank Accession Numbers					
			cyt <i>b</i>	ND4	ATPases 8 and 6	c-mos	EXPH5	RAG1
<i>Agkistrodon contortrix</i>	Moody 338	USA, Ohio, Athens Co.	AY223612 ^a	AF156576 ^a	—	—	—	—
<i>Agkistrodon piscivorus</i>	CLP 30	USA, South Carolina	AY223610 ^a	AF156578 ^a	—	—	—	—
<i>Crotalus adamanteus</i>	UTEP 18472	USA, Florida, Baker Co.	JN620809	JN620959	JN620850	JN620891	JN620927	JN621000
<i>Crotalus atrox</i> 1	UTEP 19051	USA, Arizona, Maricopa Co.	JN620810	JN620960	JN620851	JN620892	JN620928	JN621001
<i>Crotalus atrox</i> 2	UTEP 19849	USA, Texas, Brewster Co.	JN620811	JN620961	JN620852	JN620893	JN620929	JN621002
<i>Crotalus basiliscus</i>	822*	Mexico, Nayarit, San Blas	AY704844 ^b	AY70489 ^b	—	—	—	—
<i>Crotalus cerastes</i>	UTEP 19278	USA, Arizona, La Paz Co.	JN620812	JN620962	JN620853	JN620894	JN620930	JN621003
<i>Crotalus cerberus</i>	UTEP 19166	USA, Arizona, Graham Co.	JN620813	JN620963	JN620854	JN620895	—	—
<i>Crotalus horridus</i>	UTEP 19288	USA, Texas, Montague Co.	JN620815	JN620965	JN620856	JN620897	JN620932	JN621004
<i>Crotalus lepidus</i>	UTEP 19237	USA, Texas, Presidio Co.	JN620816	JN620966	JN620857	JN620898	JN620933	JN621005
<i>Crotalus mitchellii</i>	UTEP 17628	USA, California, Imperial Co.	JN620817	JN620967	JN620858	JN620899	JN620934	JN621006
<i>Crotalus molossus</i> (Son 1)	UTEP 18857	USA, Arizona, Yavapai Co.	JN620843	JN620993	JN620884	JN620922	JN620954	JN621028
<i>Crotalus molossus</i> (Son 2)	UTEP 19091	USA, Arizona, Maricopa Co.	JN620845	JN620995	JN620886	JN620924	JN620956	JN621030

<i>Crotalus molossus</i> (Mad 1)	UTEP 18881	USA, Arizona, Santa Cruz Co., Canello Hills	JN620842	JN620992	JN620883	JN620921	JN620953	JN621027
<i>Crotalus molossus</i> (Mad 2)	UTEP 18777	USA, Arizona, Santa Cruz Co., Patagonia Mountains	JN620841	JN620991	JN620882	JN620920	JN620952	JN621026
<i>Crotalus molossus</i> (Mad 3)	UTEP 20156	USA, New Mexico, Grant Co., Big Burro Mountains	JN620826	JN620976	JN620867	JN620908	JN620942	JN621015
<i>Crotalus molossus</i> (Mad 4)	UTEP 18561	USA, New Mexico, Grant Co.	JN620837	JN620987	JN620878	JN620916	JN620948	JN621022
<i>Crotalus molossus</i> (Mad 5)	CGA 08	USA, New Mexico, Grant Co., Big Burro Mountains	JN620827	JN620977	JN620868	—	—	—
<i>Crotalus molossus</i> (Mad 6)	UTEP 19982	USA, New Mexico, Grant Co., Pinos Altos Range	JN620840	JN620990	JN620881	JN620919	JN620951	JN621025
<i>Crotalus molossus</i> (Mad 7)	UTEP 20155	USA, New Mexico, Grant Co., Bayard	JN620828	JN620978	JN620869	JN620909	JN620943	JN621016
<i>Crotalus molossus</i> (Chih 1)	UTEP 18677	USA, Texas, Hudspeth Co., IMRS	JN620820	JN620970	JN620861	JN620902	JN620937	JN621009
<i>Crotalus molossus</i> (Chih 2)	UTEP 14111	USA, Texas, Hudspeth Co., Quitman Mountains	JN620831	JN620981	JN620872	JN620911	—	—
<i>Crotalus molossus</i> (Chih 3)	UTEP 14761	USA, Texas, Hudspeth Co., IMRS	JN620835	JN620985	JN620876	—	—	—
<i>Crotalus molossus</i> (Chih 4)	UTEP 17491	USA, Texas, Hudspeth Co., IMRS	JN620821	JN620971	JN620862	JN620903	JN620938	JN621010
<i>Crotalus molossus</i> (Chih 5)	UTEP 14148	USA, Texas, Hudspeth Co., Hueco Mountains	JN620832	JN620982	JN620873	JN620912	—	JN621019
<i>Crotalus molossus</i> (Chih 6)	CGA 11	USA, New Mexico, Sierra Co., Kingston	JN620829	JN620979	JN620870	JN620910	JN620944	—
<i>Crotalus molossus</i> (Chih 7)	UTEP 16179	USA, New Mexico, Luna Co., Cooks Range	JN620836	JN620986	JN620877	JN620917	—	—
<i>Crotalus molossus</i> (Chih 8)	UTEP 18456	USA, Texas, El Paso Co., Franklin Mountains	JN620830	JN620980	JN620871	JN620914	JN620945	JN621018
<i>Crotalus molossus</i> (Chih 9)	UTEP 14763	USA, Texas, El Paso Co., Franklin Mountains	JN620834	JN620984	JN620875	JN620915	JN620947	JN621021
<i>Crotalus molossus</i> (Bigbend 1)	UTEP 19233	USA, Texas, Presidio Co., Shafter	JN620838	JN620988	JN620879	JN620917	JN620949	JN621023
<i>Crotalus molossus</i> (Bigbend 2)	CGA 06	USA, Texas, Jeff Davis Co., Davis Mountains	JN620825	JN620975	JN620866	JN620907	—	JN621014
<i>Crotalus molossus</i> (Bigbend 3)	UTEP 19788	USA, Texas, Brewster Co., Marathon	JN620839	JN620989	JN620880	JN620918	JN620950	JN621024
<i>Crotalus molossus</i> (Bigbend 4)	UTEP 18432	USA, Texas, Jeff Davis	JN620822	JN620972	JN620863	JN620904	JN620939	JN621011

		Co., Davis Mountains						
<i>Crotalus molossus</i> (Stock 1)	UTEP 19281	USA, Texas, Brewster Co., Christmas Mountains	JN620844	JN620994	JN620885	JN620923	JN620955	JN621029
<i>Crotalus molossus</i> (Stock 2)	UTEP 17520	USA, Texas, Gillespie Co., Willow City	JN620833	JN620983	JN620874	JN620913	JN620946	JN621020
<i>Crotalus molossus</i> (Stock 3)	UTEP 17515	USA, Texas, Hays Co.	JN620846	JN620996	JN620887	JN620925	JN620957	JN621031
<i>Crotalus molossus</i> (Stock 4)	CGA 03	USA, Texas, Val Verde Co., Loma Alta	JN620824	JN620974	JN620865	JN620906	JN620941	JN621013
<i>Crotalus molossus</i> (Stock 5)	UTEP 19994	USA, Texas, Terrell Co., Sanderson	JN620823	JN620973	JN620864	JN620905	JN620940	JN621012
<i>Crotalus molossus</i> (Stock 6)	UTEP 18415	USA, Texas, Pecos Co., Sheffield	JN620847	JN620997	JN620888	JN620926	JN620958	JN621032
<i>Crotalus molossus</i> (<i>nigrescens</i> 1)	JAC 29296	Mexico, Durango	JN620849	JN620999	JN620890	—	—	—
<i>Crotalus molossus</i> (<i>nigrescens</i> 2)	JAC 29233	Mexico, Durango	JN620848	JN620998	JN620889	—	—	—
<i>Crotalus oreganus</i> (<i>C. helleri</i>)	UTEP 17519	USA, California, San Diego Co.	JN620814	JN620964	JN620855	JN620896	JN620931	—
<i>Crotalus tigris</i>	UTEP 18442	USA, Arizona, Pima Co., Tucson Mountains	JN620818	JN620968	JN620859	JN620900	JN620935	JN621007
<i>Crotalus totonacus</i> (<i>Crotalus totonacus</i> 1)	SD*	Mexico, Tamaulipas	AY704837 ^b	AY704887 ^b	—	—	—	—
<i>Crotalus totonacus</i> (<i>Crotalus totonacus</i> 2)	3102*	Mexico, Queratero, Tomajo	AY704838 ^b	AY704888 ^b	—	—	—	—
<i>Crotalus viridis</i>	UTEP 19275	USA, New Mexico, Otero Co.	JN620819	JN620969	JN620860	JN620901	JN620936	JN621008
<i>Sistrurus catenatus</i> ^a	Moody 502	USA, Texas, Haskell Co.	AY223610 ^a	AY223648 ^a	—	—	—	—
<i>Sistrurus miliarius</i> ^a	UTA-Live collection	USA, Florida, Lee Co., Fort Meyers	AY223611 ^a	U41889 ^a	—	—	—	—

*DNA or tissue samples not associated with a collection from the study by Wüster et al. (2005).

^aGenBank sample from Parkinson et al. (2002).

^bGenBank sample from Wüster et al. (2005).

APPENDIX II

Specimens Examined

Institutional codes follow Leviton et al. (1985); CGA = C. G. Anderson field series, IMRS = Indio Mountains Research Station. Asterisks indicate specimens not included in morphological analyses.

Crotalus adamanteus—USA: Florida: Baker County: state HWY 127, 1.3 km N of state HWY 122, UTEP 18472*.

Crotalus atrox—USA: Arizona: Maricopa County: state HWY 71, 3.5 km NE of state HWY 60, 33.9757° N, 113.1410° W, 678 m, UTEP 19051*; Texas: Brewster County: FTM 2627, 4.8 km SE of US HWY 385, 29.6696° N, 103.1232° W, 780 m, UTEP 19849*.

Crotalus basiliscus—MEXICO: Michoacán: Sierra de Coalcomán, UTA R-6120. Sinaloa: Plomosas, UTA R-6071.

Crotalus cerastes—USA: Arizona: La Paz County: US HWY 95, 10 km NW of State HWY 72, at mile marker 138, 34.0703° N, 114.2597° W, UTEP 19278*.

Crotalus cerberus—USA: Arizona: Graham County: Pinaleno Mountains, state HWY 266, 1.4 km W of Stockton Pass, 32.5973° N, 109.8657° W, UTEP 19166*.

Crotalus estebanensis—MEXICO: Sonora: Isla San Esteban, USNM 64586 (paratype).

Crotalus oreganus—USA: California: San Diego County: Ko-Pah Mountains, vicinity of North McCain Valley Rd., N of Cottonwood BLM campground, 1350 m, UTEP 17519*.

Crotalus horridus—USA: Texas: Montague County: farm to market road 677, at Taovayas Indian Bridge on Red River, 33.9198° N, 97.5101° W, UTEP 19288*.

Crotalus lepidus—USA: Texas: Presidio County: Fuentes Ranch, ~4 km (by air) SE of Shafter, ~0.5 km (by air) WSW Ojo Chilicote, 29.7914° N, 104.2760° W, 1288 m, UTEP 19237*.

Crotalus mitchellii—USA: California: Imperial County: Black Mountain Rd, 5.5 km SE of state HWY 78, UTEP 17628*.

Crotalus ornatus—MEXICO: Coahuila: Sierra Encarnación, USNM 46507; Carbonero Canyon, Carmen Mountains, WNW of Múzquiz, USNM 103738; Cuatro Ciénegas (11 km SW of) USNM 159573; USA: New Mexico: Luna County: Cooke's Range, at mouth of Frying Pan Canyon, NW of Starvation Draw, detention dam No. 4, 1554 m, UTEP 16179; Florida Mountains, 32.08633° N, 107.61750° W, USNM 44496; Sierra County: Black Range, HWY 152, 12.6 km W of Hillsboro, near Kingston Ghost Town, 32.91904° N, 107.69004° W, 1843 m, CGA 11*; Socorro County: Questa Spring, in Red Rock Arroyo, San Mateo Mountains, Cibola National Forest, USNM 320318; Texas: Near Pecos River, USNM 486 (holotype); Brewster County: HWY 118, 17.5 km N of state HWY 170, 30.03086° N, 104.46840° W, 1581 m, UTEP 19281; Christmas

Mountains, Terlingua Ranch, west slope of White House Mountain, 29.43333° N, 103.39166° W, 1219 m, UTEP 11574; on US 90, 24.5 km WNW of US 385, 30.29703° N, 103.45428° W, 1419 m, UTEP 19788; 8 km N of Terlingua Ranch Resort headquarters, 29.51666° N, 103.38333° W, UTEP 11911; Culberson County: Beach Mountains, on state HWY 54, 13 km N of Van Horn, 31.14833° N, 104.81833° W, 1280 m, UTEP 11577; Baylor Mountains, on state HWY 54, 16 km N of Van Horn, 31.16633° N, 104.84383° W, UTEP 13799; on state HWY 54, 15 km N of Van Horn, 31.16000° N, 104.83666° W, UTEP 13026; Guadalupe Mountains, on McKittrick Canyon Rd., 0.8 km N of US 62/180, 31.94166° N, 104.71833° W, UTEP 8701; El Paso County: Franklin Mountains, residence at 4340 Loma Del Norte in town of El Paso, UTEP 18456*; Tom Mays Memorial Park, canyon to cottonwood springs, 31.91500° N, 106.50333° W, UTEP 2588; 3 km E of Red Rock Ranch, 31.87583° N, 106.55100° W, UTEP 14763; upper McKellegon canyon, on east facing slope, 31.83333° N, 106.48333° W, UTEP 12058; on Trans Mountain Road, 8 km W of junction with US 54, 31.88433° N, 106.50400° W, UTEP 11539; Tom Mays Park, 31.91666° N, 106.51666° W, UTEP 11540; Fusselman Canyon, 31.88333° N, 106.48333° W, UTEP 228; Hueco Mountains, 31.78333° N, 106.03333° W, UTEP 3670; 19.3 km ENE of Horizon City, in western foothills of Hueco Mountains, 31.7350° N, 106.0067° W, UTEP 3636; Gasline Road, Hueco Mountains, 31.7167° N 106.0167° W, UTEP 2456; Gillespie County: on Willow City Loop Rd., 6.6 km N of Willow City, 30.4415° N, 98.4985° W, UTEP 17520; Hays County: 8.0 km (by air) WSW of Wimberly, Rancho Cima, BSA Cockrell River Camp, 29.9542° N, 98.16944° W, UTEP 17515*; Hudspeth County: Eagle Mountains, Marine Ranch House, 30.9222° N, 105.0417° W, 1676 m, UTEP 1933; Wind Canyon, 1.6 km W of Eagle Mountain Ranch House, 30.905° N, 105.065° W, UTEP 1932; east slope of Eagle Bluff, 30.88333° N, 105.06666° W, 1981 m, UTEP 1931; Hueco Mountains, Hueco Ranch Rd, 0.64 km SSW of New Mexico state line, UTEP 14148*; Indio Mountains, N of IMRS headquarters, on road to Squaw Spring, 30.78333° N, 105.01666° W, UTEP 18677; 3 km NW of IMRS headquarters, 30.77833° N, 105.02833° W, 1265 m, UTEP 14761; Squaw Creek Canyon, 1 km SSW of Squaw Spring, 30.79166° N, 105.01666° W, 1219–1311 m, UTEP 11575; Squaw Creek Canyon near Squaw Spring, 30.79666° N, 105.01000° W, 1267 m, UTEP 12184; Purple Sage Mine Rd, 1.8 km SW of JCT with main ranch road, 30.76666° N, 105.02000° W, UTEP 17491; E of IMRS headquarters, 30.77666° N, 105.01500° W, UTEP 15359; Vicinity of IMRS headquarters, 30.78333° N, 105.01666° W, 1234 m, UTEP 15314; Quitman Mountains, 27.5 km NW of Indian Hot Springs, 30.97333° N, 105.49833° W, UTEP 9010; Quitman Pass Road, 31.06666° N, 105.46666° W, UTEP 14111; Jeff Davis County: Davis Mountains, on state HWY 118, 9.7 km N of junction with state HWY 166, 30.84316° N, 104.15083° W, UTEP 6423; on state HWY 118, 5.6 km N of junction with FTM 1837, 30.52150° N, 103.77066° W, UTEP 18432; Limpia Canyon, HWY 17, 29 km N of Fort Davis, 30.7733° N, 103.7531° W, 1270 m, CGA 06*; Pecos County: on FTM 2886, 27.4 km S of junction with I-10, 30.68366° N, 102.18950° W, UTEP 18415; Presidio County: Fuentes Ranch, 4 km SE of Shafter, 29.79266° N, 104.27883° W, 1228 m, UTEP 19233; Shafter, Fuentes Ranch, 29.79576° N, 104.24528° W, 1290 m, UTEP 19236; on state HWY 170, 39.3 km W of Lajitas, 29.35883° N, 104.08750° W UTEP 11362; Terrell County: Sanderson, residence near intersection of Blanco St. and railroad tracks, 30.13883° N, 102.39616° W, UTEP 19994; Val Verde County: Loma Alta, US 277, 1.6 km S of Loma Alta, 29.9044° N, 100.7706° W, CGA 03*.

Crotalus molossus—MEXICO: Chihuahua: Sierra del Nido, UTA R-17837–38; Arroyo Musteno, 2591 m, UTEP 3052; Between Guadalupe y Calvo and Parral, USNM 46486–87;

Distrito Federal: Mexico City, USNM 12724; Tlalpan, USNM 4653; Durango: 9.7 km SE of Llano Grande UTEP 3982; Nuevo León: UTA R-16147; Oaxaca: ~32 km NE of Cerro San Felipe, 2591 m, USNM 46467 (holotype [*C. m. oaxacus*]); San Luis Potosí: Mountains near Jesus Maria, 1829 m, USNM 46425; Sonora: Isla Tiburón, Tecomate, USNM 222084; Bahía Kino (16 km N and 2 km E of) USNM 240696; Sierra San Luis, UTA R-17839–43, UTA R-22457; San Luis Canyon, near Monument 66, Mexican Boundary, USNM 21050; Zapotitlán: Salinas, Puebla, UTA R-25854–56; USA: Arizona: Cochise County: Chiricahua Mountains, 37 km W of Apache, 31.46° N, 109.37° W, UTEP 5993; Canyon behind Fort Huachuca, USNM 17788; Forest Service Rd. 42, 3.2 km SW of Portal, USNM 574196; Gila County: UTA R-50685; Greenlee County: Blue River, Cooper Ranch, USNM 51896; Maracopa County: White Tank Mountains, 524 m, UTEP 11953; Forest road 19, near Bartlett Dam, 30.86021° N, 111.71193° W, UTEP 19091; Mazatzal Mountains, along Picadilla Creek, near Cline's Cabin, 1280 m, UTEP 13794; Pima County: Aravaca Road, 11.6 km E of state HWY 286, UTEP 17505; Santa Catalina Mountains, just off Catalina Highway, 4.7 km E of Mount Lemon Rd., 975 m, UTEP 13795; Forest Road 70, 8.1 mi SE of Continental, USNM 320314; Santa Cruz County: Atascosa Mountains, Forest road 39, 0.8 km E of Ruby, 31.4667° N, 111.2317° W, UTEP 15870; Canello Hills, HWY 83, 5 km S of Lyle Canyon Rd., UTEP 18731; state HWY 83, 1.5 km W of Canelo, 31.5548° N, 110.5019° W, 1533 m, UTEP 18881; Patagonia Mountains, Harshaw Rd., 5.5 km S of Harshaw Creek Rd., UTEP 16442; Kuncle Mountain, 31.49889° N, 110.70445° W, UTEP 18777; HWY 83, 1.13 km S of West Gates Rd., UTEP 16443; Yavapai County: state HWY 96, 43 km E of state HWY 97, 1000 m, UTEP 18857*; Fort Whipple, USNM 11879, USNM 253070; New Mexico: Catron County: 11.3 km S of Gila Cliff Dwellings, 33.12553° N, 108.26792° W, UTEP 3673; Nigrita River, Gila National Forest, USNM 44493; Grant County: US 180 between Silver City and Cliff, 20 km SE of Cliff, 32.82656° N, 108.50221° W, 1459 m, UTEP 20156; US 180 between Silver City and Cliff, 17 km SE of Cliff, 32.84103° N, 108.50952° W, 1459 m, CGA 08*; Fort Webster, Santa Rita Del Cobre mine, USNM 485 (holotype); Bayard, at junction of US 180 and Guinevan St., 32.75204° N, 108.13613° W, UTEP 20155; Big Burro Mountains, south rim of Sawmill Canyon, N of Forest Road 836, 32.56833° N, 108.47000° W, 1981 m, UTEP 11576; Mimbres Mountains, 3.2 km W of Emory Pass, 32.81666° N, 107.78333° W, UTEP 533; Pinos Altos Range, state HWY 35, 3.8 km E of state HWY 15, 33.03550° N, 108.18473° W, 1814 m, UTEP 19982; Gila Wilderness, N of Grapevine Campground, along Gila River, 33.23333° N, 108.20166° W, UTEP 18561; state HWY 35, 0.8 km E of mile marker 22, UTEP 14765; Redrock, USNM 44494; Hidalgo County: Alamo Hueco Mountains, U-Bar Ranch, Horse Canyon, 1646 m, UTEP 11062; U-Bar Ranch, Horse Canyon, 1646 m, UTEP 10756; Animas Mountains, Animas Peak, USNM 44492; Guadalupe Canyon, Monument 73, along Mexican Boundary, USNM 21049.

Crotalus tigris—USA: Arizona: Pima County: Tucson Mountains, foothills of Golden Gate Mountain, Kinney Rd, 3.5 km NW of State HWY 86, UTEP 18442*.

Crotalus totonacus—MEXICO: San Luis Potosí: 11.3 km N of Ciudad de Valles, KU 43569; Tamaulipas: 48 km S of Soto la Marina, north bridge at Santa Gertrudis, KU 157862; 21 km N of Soto la Marina at km Post 171, KU 174824; Soto la Marina, UTA R-7223–24; La Pesca, UTA R-7222.

Crotalus viridis—USA: New Mexico: Otero County: CR F001, 18 km (by road) NE Texas state line 32.07621° N, 105.74593° W, 1537 m, UTEP 19275*.

VITA

Christopher G. Anderson began his college career in 2003, completing a B.Sc. in biology at the University of Wisconsin-Stevens Point in December 2007. During his time at UWSP, he worked with the UWSP Herpetology Society and Dr. Erik Wild on various herpetology-related projects. Starting in 2004, Chris took an active role in the UWSP Herpetology Society where he held the offices of advertising coordinator, vivarium manager, vice-president and president. As an undergraduate, Chris received a UWSP student research grant to conduct a survey of reptile diversity in conjunction with a long-term survey of amphibian diversity at Reserva Amazonica, Peru led by Dr. Erik Wild and presented the results at the Joint Meeting of Ichthyologists and Herpetologists. Prior to graduation, he assisted with the curation of the UWSP Herpetology Collection. Chris began the pursuit of a Master of Science degree in biology under the guidance of Dr. Eli Greenbaum at the University of Texas at El Paso (January 2009). As a Masters student, Chris has served as a teaching assistant for Organismal Biology and a collections assistant in the Laboratory for Environmental Biology. He has received two UTEP Graduate School grants to support his research and has presented his research at the Southwestern Association of Naturalists Meeting, the Joint Meeting of Ichthyologists and Herpetologists, and the Chihuahuan Desert Conference. He plans to pursue a Ph.D. in evolutionary biology and would like to take a curatorial position at a museum where he can continue to make contributions to herpetology.

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This thesis/dissertation was typed by Christopher G. Anderson