EVOLUTIONARY RELATIONSHIPS IN SOME NORTHERN GROUPS OF THE DIRECT-DEVELOPING FROG GENUS *CRAUGASTOR* (ANURA: CRAUGASTORIDAE)

by

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ABSTRACT

EVOLUTIONARY RELATIONSHIPS IN SOME NORTHERN GROUPS OF THE DIRECT-DEVELOPING FROG GENUS *CRAUGASTOR* (ANURA: CRAUGASTORIDAE)

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Comparing phylogeographic patterns among vertebrates can give insight into the landscape and ecological determinants of phylogenetic diversity. However, in certain taxonomic groups (e.g., birds and mammals) natural history traits can be more influential in structuring phylogeographic patterns than historical interactions with the landscape. In contrast, groups like anurans (6132+ species) with poor dispersal capability and strict ecological requirements often display patterns of genetic diversification consonant with the geological and climatic characteristics of a landscape. The patterns resulting from these interactions can be used to elucidate the temporal dynamics of ecological differentiation, historical biogeography, and morphological evolution. The study of anurans is also timely since they have recently experienced globally distributed declines in biodiversity and are a conservation priority. A large proportion of remaining anuran diversity in the New World tropics is dominated by direct-developing frogs of the mega-diverse group Terrarana. In the northern Neotropics, frogs of the genus *Craugastor* are by far the most abundant terraranan group. Although they are relatively common and species rich, the phylogeographic relationships within many *Craugastor* lineages are not well characterized. Herein I examine phylogeography in several poorly known *Craugastor* groups found north of the Nicaraguan depression using mitochondrial and nuclear DNA. Specifically, I examined (1) phylogenetic relationships in the genus *Craugastor*, (2) the subgenus *Hylactophryne* (*C. augusti* and *C. bocourti* species series), (3) the *C. mexicanus* species series, and (4) the *C. rhodopis* species series. Using this research, I address several longstanding nomenclatural issues and identify several hitherto unnamed lineages. I then use these data to discuss ecological diversification, nucleotide substitution rate variation, and biogeography. Collectively, my data indicate that the *Craugastor* groups I examined are extremely diverse and an important faunal component of Mexico and northern Central America. Given this diversity, I suspect that the patterns of molecular diversity observed in northern *Craugastor* will play an important future role in understanding the evolution of biodiversity across this dynamic region.

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CHAPTER 1

INTRODUCTION

Many historical processes are thought to be involved in the evolutionary diversification of lineages. Some of the more salient determinants of relatedness in terrestrial organisms are related to interactions with the landscape. The study of patterns and explanatory phenomena related to genetic variation across physical landscapes often requires a multifaceted examination of phylogenetic levels that is collectively termed phylogeography (Avise, 2000). These examinations temporally span population (i.e., population genetics) to interspecific (i.e., molecular systematics) levels of relatedness. As a researcher, I am primarily interested in synthesizing traditional elements of systematics (i.e., museum science, taxonomy, morphology, etc.) with our rapidly advancing understanding of speciation genomics. During this pursuit I have found that large molecular datasets employing population level sampling across several levels of relatedness are particularly helpful in not only determining phylogenetic relationships, but also for characterizing generalized patterns of organismal evolution. In this dissertation I discuss a series of studies on frogs in the genus *Craugastor* from throughout the United States, Mexico, and northern Central America. Using DNA sequences I examined these amphibians across several evolutionary tiers in order to comment on phylogenetic relationships and general evolutionary patterns.

Anurans (frogs and toads) are a notable vertebrate group because they are the largest order of living amphibian (6132 species; http://www.amphibiaweb.org/). Sadly, these diverse amphibians have experienced recent global declines to the point where many researchers consider the last three decades a mass extinction event (McCallum, 2007). This loss in diversity is discouraging given the ideal research models that anurans provide for several biological research disciplines. In particular, frogs and toads are considered one of the better vertebrate models (relative to groups like birds and mammals) for detecting historical interactions with the landscape given their typically poor dispersal abilities and strict ecological requirements. These natural history traits often allow for the detection of genetic signatures related to anthropogenic processes like urbanization (Hitchings and Beebee, 1997: Noël et al., 2007). During my earlier graduate studies at George Mason University, I became fascinated with an enormous radiation of direct-developing frogs known as *Eleutherodactyus* (700+ species). In the seven years since that time, this massive group was divided into several smaller genera, and most species that I have conducted research on were placed in the Middle American genus *Craugastor*. While several researchers (notably Andrew J. Crawford, Matthew P. Heinicke, and S. Blair Hedges) have studied molecular variation in *Craugastor,* there is a notable lack of information concerning the diversity of this group in the United States, Mexico, and northern Central America. Thus, (1) the limited availability of molecular information on northern *Craugastor* and (2) unparalleled access to tissue samples at the UTA Amphibian and Reptile Diversity Research Center motivated my investigation of several northern *Craugastor* groups. In order to establish a relevant framework for these investigations, I begin this dissertation with a chapter that reviews the genus *Craugastor*.

1.1 History of the genus *Craugastor*

Direct-developing frogs in the genus *Craugastor* (Anura: Craugastoridae) occur in a variety of habitats from the southwestern United States to northern South America. These anurans belong to a massive taxonomic group known as Terrarana which contains former members of the genus *Eleutherodactylus* (Hedges et al., 2008). Within this group, *Craugastor* has been consistently recognized as a monophyletic Middle American lineage based initially on morphology (Lynch, 1986) and later molecules (Crawford and Smith, 2005; Heinicke et al., 2007; Hedges et al., 2008; Pyron and Wiens, 2011). Recently, *Craugastor* (and the sister genus *Haddadus*) were placed within a monophyletic family called Craugastoridae by Hedges et al. (2008). While the actual content of this family is still being debated (see Pyron and Wiens, 2011), the monophyly of *Craugastor* and *Haddadus* is not controversial. Owing to high levels of diversity (115+ species; Frost, 2012) and rampant morphological homoplasy, recognizing natural groups within *Craugastor* has been a challenging endeavor. The genus has been divided into several categories of closely related species that are either referred to as ‘species groups’ or ‘species series’. However, the organizational schemes suggested by different authors vary substantially (e.g., Savage, 1987; Lynch, 2000). This nomenclatural instability has continued into recent examinations with several groupings identified in the first molecular investigation of *Craugastor* (Crawford and Smith, 2005) being modified based on nomenclatural priority (Hedges et al., 2008).

Herein, I use the nomenclature of Hedges et al. (2008), but composite phylogenetic relationships from several studies (Crawford and Smith, 2005; Heinicke et al., 2007). In this system the genus *Craugastor* is divided into three subgenera (*Campbellius, Craugastor* and *Hylactophryne*), which collectively contain eight species series (Fig. 1.1 and 1.2). Some of these species series are further divided into several species groups (Hedges et al., 2008). There are relatively few molecular studies that have focused on relationships within species groups, species series, or species that occur north of the Nicaraguan depression. In contrast, there are several studies that have investigated molecular variation in lower Central American groups of *Craugastor*. Specifically, phylogeographic examinations have been conducted on most of the *C. podiciferus* species group (in the *C. rhodopis* species series; Crawford 2003a; Crawford 2003b, Streicher, 2007; Streicher et al., 2009) and members of the *C. fitzingeri* species series (Crawford et al., 2007). These studies have been used to discuss several evolutionary phenomena including patterns of ecological divergence (Crawford et al., 2007), discuss rates of nucleotide evolution (Crawford, 2003a), and identify putative vicariance hypotheses (Streicher et al., 2009). Given the insight that these studies have offered outside of identifying phylogenetic relationships, more detailed investigations of other *Craugastor* groups have the potential to identify study systems of broad interest and relevance to biologists.

There are eight *Craugastor* species series within the three subgenera proposed by Hedges et al. (2008). The subgenus *Campbellius* contains a single species series, the

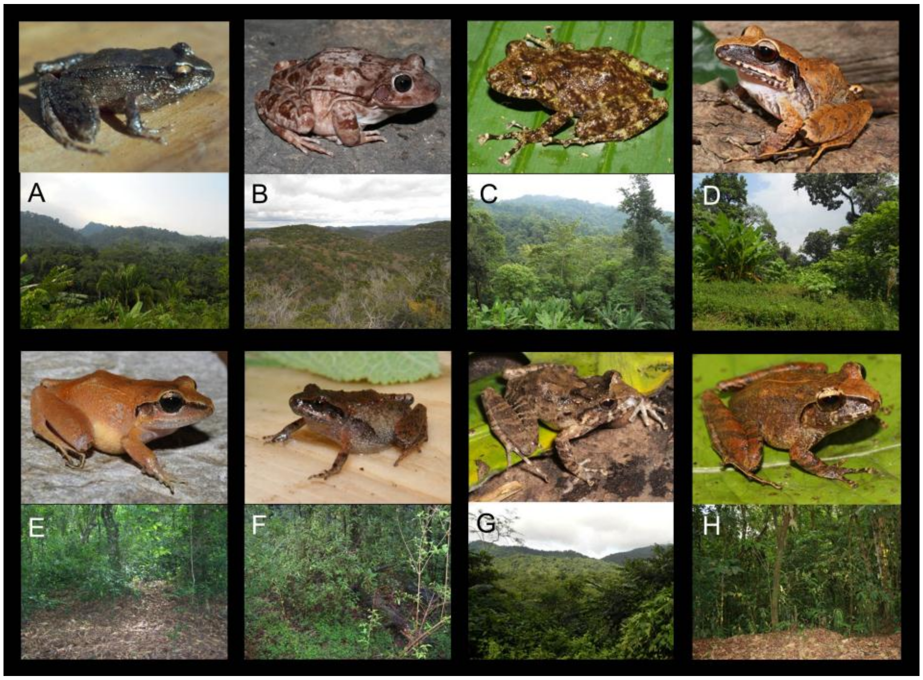

Figure 1.1. Representatives of the eight *Craugastor* species series along with images of habitat associated with each. (A) *milesi* series, *Craugastor* (*Campbellius*) *daryi* from Guatemala (photograph: Jonathan A. Campbell); (B) *augusti* series, *Craugastor* (*Hylactophryne*) *augusti* from Texas, USA; (C) *bocourti* series, *Craugastor* (*Hylactophryne*) *nefrens* from Izabal, Guatemala; (D) *laticeps* series, *Craugastor* (*Craugastor*) *laticeps* from Izabal, Guatemala; (E) *rhodopis* series, *Craugastor* (*Craugastor*) *loki* from Oaxaca, Mexico; (F) *mexicanus* series, *Craugastor* (*Craugastor*) aff. *mexicanus* from Estado de México, Mexico; (G) *punctariolous* series, *Craugastor* (*Craugastor*) *rupinius* (photograph: Eric N. Smith) from Suchitepéquez, Guatemala; and (H) *fitzingeri* series, *Craugastor* (*Craugastor*) aff. *angelicus* from San Vito, Costa Rica.

milesi species series, and is restricted to northern Central America in the countries of Guatemala, Honduras, and Mexico. The *C. milesi* species series is a conservation priority because they experienced putative declines to the point where, until recently (Kolby and McCranie, 2009), they were thought to be extinct. The subgenus *Hylactophryne* is the northernmost ranging *Craugastor* (into the southwestern United States) and contains two species series, the *C. augusti* species series and the *C. bocourti* species series. These species series are restricted to northern Central America, Mexico, and the United States and are examined in detail in the following chapter. The subgenus *Craugastor* is by far the most diverse group with a total of five species series that range from northern South America northward to Mexico. The content of this subgenus is as follows: the *C. mexicanus* species series, *C. laticeps* species series, *C. punctariolus* species series, *C. fitzingeri* species series, and *C. rhodopis* species series. Two of these species series that occur in the Mexico and northern Central America (*mexicanus* and *rhodopis*) are the topics of chapters 3 and 4 respectively.

There have been several molecular phylogenetics studies of the genus *Craugastor* (Crawford and Smith, 2005; Heinicke et al., 2007; Hedges et al., 2008; Pyron and Wiens, 2011). However, all of these studies have focused on species level sampling. In this chapter I describe a large cluster analysis on all available *Craugastor* DNA sequences derived from a fragment of mitochondrial DNA (mtDNA). This genic region is part of a ribosomal subunit cluster that is often used as a genetic barcode in amphibians (Vences et al., 2005). I acquired data for this gene fragment from 319 frogs using a combination of direct DNA sequencing in the Smith lab at UTA and data acquisition from online resources (e.g., GenBank). The goal of generating this "barcode tree" was to infer general patterns of diversification for each of the *Craugastor* species series given fairly extensive geographic sampling. I also chose to conduct this analysis to test the validity of the species series proposed by Hedges et al. (2008). Fortunately, I was able to include all currently recognized *Craugastor* species series in this sampling and used two individuals from the *milesi* species series, 34 individuals from the *augusti* species series, 56 individuals from the *bocourti* species series, 45 individuals from the *punctarioulus* species series, six individuals from the *fitzingeri* species series, 59 individuals from the *mexicanus* species series, 112 individuals from the *rhodopis* species series, and five individuals form the *laticeps* species series.

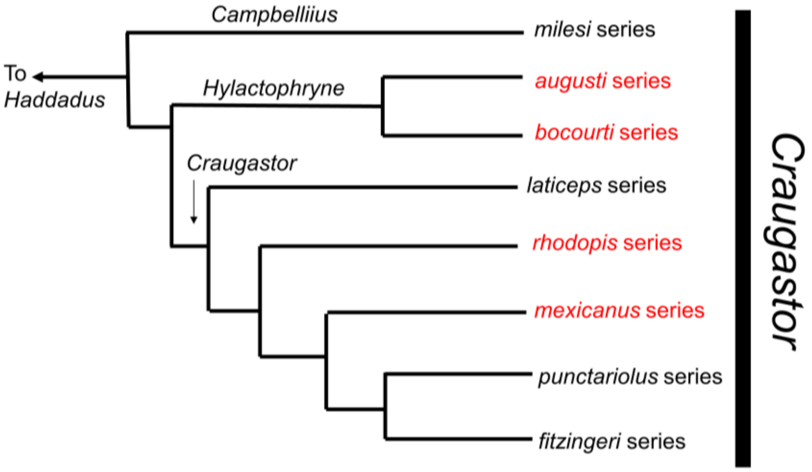

Figure 1.2. Phylogenetic relationships among species series in the genus *Craugastor* as proposed by Hedges et al. (2008) and Crawford and Smith (2005). Species series that are examined in this dissertation are highlighted in red.

1.2 Methods

During the course of my dissertation work I acquired *Craugastor* samples from field expeditions in the United States (Texas), Mexico, Guatemala, Costa Rica, and Ecuador. I also acquired a large tissue library from my collaborators. Specifically, Eric N. Smith, Jonathan A. Campbell, Josiah H. Townsend, James R. McCranie, Andrew J. Crawford, Sean M. Rovito, Manuel E. Acevedo, Uri O. García-Vásquez, Luis Canseco-

Márquez, Ronnie Garcia, John Malone, Jorge Ferrari-Castro, Carlos Vásquez Almazán, and Thomas J. Devitt provided a large proportion of the tissue samples analyzed below. I received laboratory assistance from Jacobo Reyes-Velasco and Ruben U. Tovar while generating data for the *C. mexicanus* species series and *C. rhodopis* species series segments of this analysis. Tissue samples were stored in 100% ethanol or an SDS-based lysis buffer. All animals were handled and euthanized according the UTA IACUC protocol A08.25 to C. Franklin, J. Campbell, E. Smith, and J. Streicher.

Genomic DNA was isolated from liver or muscle tissue using a Qiagen DNeasy kit (Qiagen®, Valencia, California, USA). I amplified a fragment of the mtDNA 12S ribosomal subunit gene (12S) using the frequently employed primer set of 12SF (5' AAA CTG GGA TTA GAT ACC CCA CTA T 3') and 12SR (5' ACA CAC CGC CCG TCA CCC TC 3'). Each PCR reaction had a total volume of 25 µL which included 8.5µL of DEPC water, 2µL of primers (10 µM concentration), and 12.5µL of GreenTaq Master Mix (Promega, Madison, Wisconsin, USA). I used a standard thermal cycling profile that included 40 cycles of 95 C denaturation, 50 C annealing, and 72 elongation plus an additional 5 sec extension after each elongation (see Streicher *et al.*, 2009). DNA isolates and PCR products were visualized on a 1–2% agarose gels. Reactions were cleaned using either AMPure magnetic beads (Agencourt$^{®}$, Bioscience, Beverly, Massachusetts, USA) or ExoSap-IT (USB/Affymetrix, Santa Clara, California, USA). Cycle sequencing reactions used a BigDye® terminator kit (Applied Biosystems [ABI], Foster City, California, USA) and the resulting samples were analyzed with an ABI 3130xl genetic analyzer at the UTA Genomics Core Facility. Resulting chromatograms were edited and aligned in the program Sequencher 4.1 (GeneCodes, Ann Arbor, Michigan, USA). A cluster analysis was conducted tree in the program PAUP* 4.0 (Swofford, 2002) using a distance criterion to build a neighbor joining tree. This tree was then visualized using the program FigTree (Rambaut, 2007). To obtain descriptive statistics on maximum within-species series and between-species series sequence divergence (SD), I calculated pairwise genetic distances (uncorrected "*p*" distances) using MEGA 5.0 (Tamura et al. 2007).

I augmented my molecular sampling by obtaining 12S data from the online NCBI genetic resource library GenBank. The sequences I downloaded originated primarily from three prior studies: Frost et al. (2006), Hedges et al. (2008), Streicher et al. (2009). Voucher information and a full list of the 12S sequences I generated and downloaded can be viewed in Appendix A.

1.3 Results

In total, the *Craugastor* 12S alignment was 548 base pairs (bp) in length with 311 variable sites and 264 parsimony informative sites. With the exception of a small approximately 60 bp region at the 3' end of the alignment, which likely corresponds to a variable loop region, the alignment was unambiguous. To characterize the influence of the ambiguously aligned 60 bp loop region, I constructed neighbor joining trees that both included and excluded this region. In terms of cluster pattern I found that the two datasets produced highly similar results. Given these findings, I chose to present the distance analysis generated from the complete alignment (Fig. 1.3), as there are clearly identifiable motifs in the loop region that, while not homologous, contain phylogentic information.

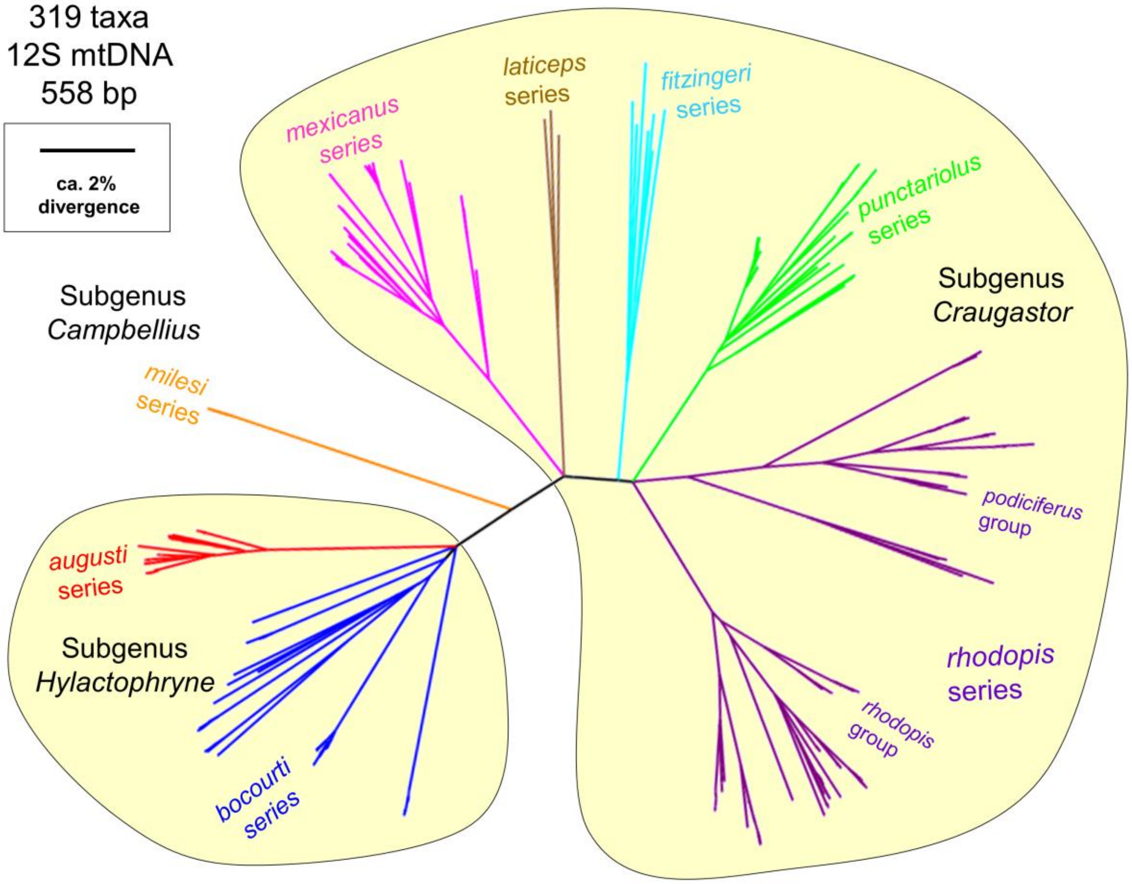

Figure 1.3. Unrooted neighbor joining phylogram depicting generalized mitochondrial relationships among frogs of the genus *Craugastor*. Note all three subgenera and all eight species series of Hedges et al. (2008) are supported by this cluster analysis. Scale bar is equivalent to approximately 2% sequence divergence (uncorrected "*p*"). See Appendix A for a list of taxa used in this analysis.

My distance analysis of the mtDNA 12S gene recovered eight distinct clusters of *Craugastor*. These clusters correspond completely to the species series proposed by Hedges et al. (2008). Within my current sampling, one species series in particular (the

C. rhodopis species series) appears to contain substantially more diversity at the 12S locus than is observed in the other *Craugastor* species series. In the neighbor-joining tree, the *Craugastor rhodopis* species series has two cl early identifiable clusters that correspond to the northern and southern species groups (*C. rhodopis* and *C. podiciferus* groups of Hedges et al. [2008]) respectively.

All species series contained fairly deep levels of inter-series sequence divergence (> 9% uncorrected "*p*"; Table 1.1). The species series with the highest levels of intra-series divergence was the *C. rhodopis* species series (8.3%) followed by the *C. laticeps* species series (6.8%) and the *C. fitzingeri* species series (6.5%). Despite fairly thorough geographic and phylogenetic sampling, the *C. mexicanus* species series and *C. augusti* species series had relatively low levels of intra-series divergence (2.7 and 3%, respectively).

Table 1.1 Estimates of average between and within (bolded along diagonal) *Craugastor* species series sequence divergence (uncorrected "*p*") of 12S mitochondrial DNA.

	milesi	*augusti*	*bocourti*	*mexicanus*	*fitzingeri*	*laticeps*	*rhodopis*
milesi	**0.007**						
augusti	0.140	**0.030**					
bocourti	0.114	0.085	**0.059**				
mexicanus	0.109	0.119	0.096	**0.027**			
fitzingeri	0.104	0.115	0.088	0.094	**0.065**		
laticeps	0.156	0.103	0.100	0.116	0.121	**0.068**	
rhodopis	0.132	0.121	0.103	0.116	0.101	0.129	**0.083**
punctariolous	0.108	0.123	0.108	0.103	0.105	0.136	0.111

1.4 Discussion and Conclusions

The genus *Craugastor* contains massive amounts of diversity that are comparable to that observed in many of the other large groups within Terrarana (see Heinicke et al., 2007; Fig. 1.3). Given these levels of diversity, I predict that in addition to the hitherto unrecognized lineages I discuss in the following chapters, many new species will be described. Encouragingly, my extensive sampling of the genus *Craugastor* appears to be completely congruent with the nomenclatural system proposed by Hedges et al. (2008). Although my cluster analysis (Fig. 1.3) contained substantial sampling biases towards the groups discussed later in this dissertation (and the *C. punctariolus* species series), the levels of intra-species sequence divergence (Table 1.1) indicate that the phylogenetic depth of coverage was comparable in many groups despite my sampling issues. If future examinations reveal similar levels of genomic divergence in the nuclear genomes of the *C. rhodopis* species group and the *C. podiciferus* species group taxa, it may be advantageous to elevate each of these groups to 'species series'. Additionally, future comparisons that include more of the *C. milesi* species series taxa (I was only able to include *C. daryi* and *C. milesi*) would be helpful in identifying the extant levels of diversity in this imperiled (Kolby and McCranie, 2009) branch of the *Craugastor* evolutionary tree.

CHAPTER 2

THE SUBGENUS *HYLACTOPHRYNE*

The subgenus *Hylactophryne* contains some of the most distinctive *Craugastor* species in terms of adult body size and breeding vocalization. The subgenus comprises (1) the *C. augusti* species series and (2) the *C. bocourti* species series. Several species in this subgenus inhabit some of the more impressive geologic formations of North America (e.g., cenotes of the Yucatan peninsula [*C. yucatanensis*], limestone caves of the Edwards Plateau [*C. augusti*], and caves of the Sierra Madre Oriental [*C. decoratus*]). All previous molecular studies indicate that the subgenus is monophyletic (Crawford and Smith, 2005; Heinicke et al., 2007; Hedges et al., 2008).

The *C. augusti* species series is known colloquially as barking frogs and includes two species: *C. augusti* and *C. tarahumaraensis* (Fig. 2.1). These frog species are distributed across much of mainland Mexico and the southwestern United States (Zweifel, 1956). The *Craugastor augusti* species series includes the northernmost ranging components of Craugastoridae and is the only *Craugastor* that has managed to colonize a temperate biome. Across their expansive geographic distribution, barking frogs have conserved body geometries, but vary widely in color pattern, integumentary characteristics, and breeding vocalization (JWS unpublished data; Zweifel, 1956; Goldberg et al., 2004). Previous examinations of mtDNA suggest that this group may be polytypic (Goldberg et al., 2004), however, these authors sampled mostly within the United States.

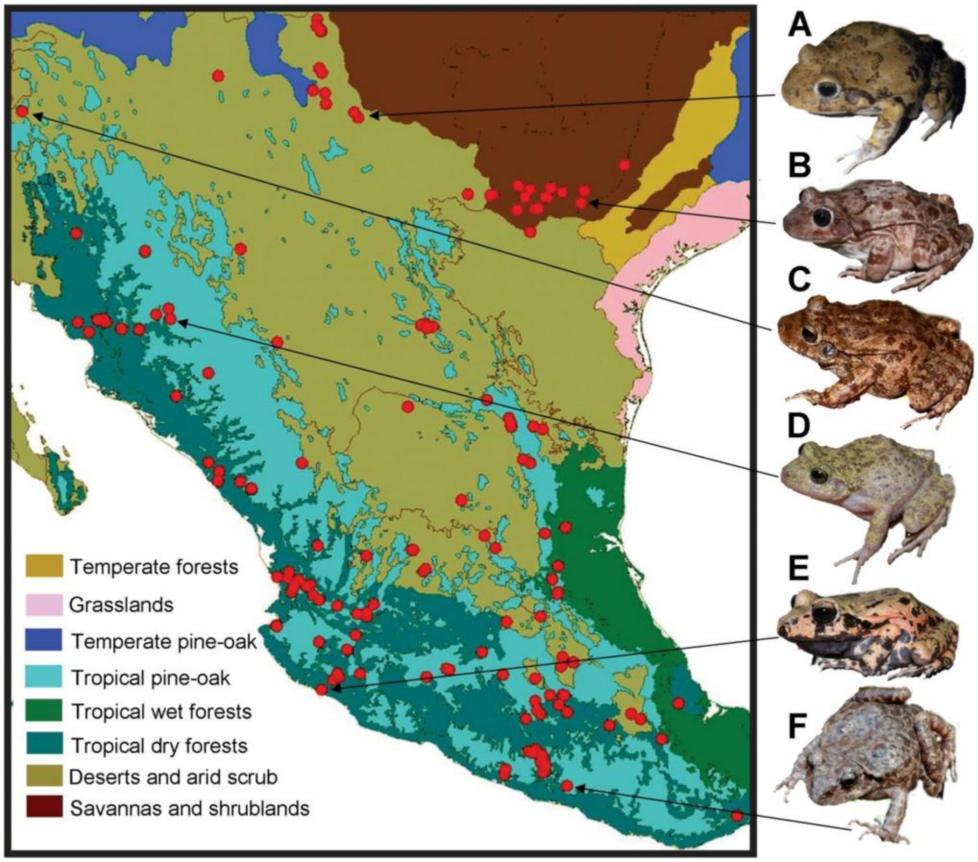

Figure 2.1. Representatives of the *Craugastor augusti* species series, the barking frogs. Localities (red dots) indicated in the map correspond to museum geo-referenced distributional data collected from natural history collections in the United States and Mexico (O. Flores-Villela, unpublished data). Ecoregion layers and designations were obtained from the World Wildlife Fund (Olson et al., 2001). Specimens pictured and their respective letters (except for C) correspond to molecular clades depicted in Appendix D.

The second division within *Hylactophryne* is the *C. bocourti* species series (Fig. 2.2). These frogs are also known as rain frogs and are easily distinguished from other northern *Craugastor* by the presence of large expanded pads on fingers III and IV (Campbell et al., 1989). They also have much smaller adult body sizes than their sister taxa in the *C. augusti* species series. Members of this group represent the remainder of the *Eleutherodactylus alfredi* group discussed by previous authors (Lynch, 1966,; Lynch, 1967; Campbell et al., 1989; Smith, 2005). The series is distributed from Mexico (as far north as southern Tamaulipas) eastward to central Guatemala and northwestern Honduras. This species series contains 19 species: *C. alfredi*, *C. batrachylus, C. bocourti*, *C. campbelli*, *C. cyanocthebius*, *C. decoratus*, *C. galacticorhinus*, *C. glaucus*, *C. guerreroensis*, *C. megalotympanum*, *C. nefrens*, *C. polymniae*, *C. silvicola*, *C. spatulatus*, *C. stuarti*, *C. taylori*, *C. uno*. *C. xucanebi*, and *C. yucatanensis*. One of the major operational criteria used to differentiate these species has been the identification of subtle morphological variation. This variation includes morphological characteristics like palmar and plantar tubercle depth (Smith, 2005), color pattern (Canseco-Márquez and Smith, 2004), and toe pad width (McCranie and Smith, 2005). Recently, we examined molecular variation (mtDNA and nDNA) across the range of the enigmatic *C. bocourti* species series member *C. uno* (Streicher et al., 2011; Appendix C). We found that despite the presence of discrete morphological groups (including characters like skin texture, color pattern, and character ratios to SVL) and large gaps between their respective geographic distributions, populations of *C. uno* in the Sierra Madre del Sur of Mexico display very little genetic divergence from one another. Thus, from the perspective of genomic divergence our *C. uno* study calls into question the validity of several *C. bocourti* species series taxa that have been defined solely on the basis of morphology.

Figure 2.2. Representative taxa in the *Craugastor bocourti* species series (unless otherwise noted photographs by Eric N. Smith). Clockwise from top left: (1) *C. campbelli* from Izabal, Guatemala, (2) *C.* aff. *decoratus* from Hidalgo, Mexico, (3) *C.* aff. *decoratus* from Queretaro, Mexico (photograph: Thomas J. Devitt), (4) *C. xucanebi* from Guatemala, (5) *C. nefrens* from Izabal, Guatemala, and (6) *C. uno* from Guerrero, Mexico.

In this chapter, I conducted an extensive molecular examination of the subgenus *Hylactophryne* using a sampling strategy that included most major species. Additionally, with the aid of collaborators I was able to obtain fairly extensive geographical sampling within many of the species I examined. These data were used to test assumptions related to (1) the validity of several species in the *C. bocourti* species series, (2) the polytypic species concept associated with the *C. augusti* species series, and (3) the relative rate of diversification in this northern group of direct-developing frogs.

2.1 Methods

I examined mitochondrial and nuclear DNA in 31 *C. augusti* species series individuals and 54 *C. bocourti* species series individuals. A complete list of specimen vouchers, locality information, and GenBank numbers is given in Appendices A and B. I used the distantly related species *C. milesi* as an outgroup. I sequenced a 460 bp segment of the mtDNA 12S ribosomal subunit gene (12S) and 350, 573 and 493 bp fragments of the nDNA rhodopsin (exon 1; Rho), recombination activating protein 1 (RAG-1) and tyrosinase precursor (Tyr) genes respectively. Laboratory methods for DNA isolation and PCR amplification followed those described in the previous chapter for 12S. Protocols for nDNA loci used the primer sequences Rhod.ma (5' AAC GGA ACA GAA GGY CC 3') and Rhod.md (5' GTA GCG AAG AAR CCT TC 3') for Rho (Vences et al., 2003), Tyr1C (5'GGC AGA GGA WCR TGC CAA GAT GT 3') and Tyr1G (5' TGC TGG GCR TCT CTC CAR TCC CA 3') for Tyr and R182 (5' GCC ATA ACT GCT GGA GCA TYA T 3') and R270 (5' AGY AGA TGT TGC CTG GGT CTT C 3') for RAG-1 (Hedges et al., 2008), along with the touchdown thermal cycling protocols used by Streicher et al. (2009). For nDNA loci I assumed that a site was heterozygous if equal chromatogram peaks were present for both bases (Hare and Palumbi, 1999). To assess general patterns of relatedness and geographic variation, I concatenated the four loci prior to phylogenetic tree-building. I used the program MacClade 3.0 (Maddison and Maddison, 2002) to construct a concatenated alignment.

While concatenation of multiple mtDNA and nDNA loci (i.e., the supermatrix approach) is controversial because it is theoretically at odds with how genomes evolve and are inherited (see Edwards, 2008), this approach typically yields results consistent with existing taxonomies and is still considered a useful analytical tool in molecular systematics (Pyron and Wiens, 2011). I constructed phylogenetic trees using the program MEGA 5 (Tamura et al., 2011). I used several phylogenetic criteria to generate trees including Maximum Likelihood (ML), Maximum Parsimony (MP), and Minimum Evolution (ME). In ML analyses I employed the GTR+I+G model of nucleotide evolution (for reasons discussed in Pyron and Weins, 2011) with five discrete gamma categories and used a partial deletion criterion with a 95% cutoff to deal with missing data and gaps. For MP analysis I used a close-neighbor-interchange on random trees searching methodology (Nei and Kumar, 2000). In distance analyses (ME) I used uncorrected *p*-distances and complete deletion for missing character data. In each phylogenetic analysis I estimated branch support from 1000 bootstrap pseudoreplicates. I considered a node to be well supported if it received support value higher than 70% (Hillis and Bull, 1993).

2.2 Results

My final concatenated alignment contained a total of 2064 base pairs (bp). This included 500 bp of 12S, 652 bp of Rag-1, 350 bp of Rho, and 559 bp of Tyr. Of these 2064 bp, 404 were variable and 289 were parsimony informative. In terms of missing data, the sampling for each of these loci was as follows: 86 taxa (12S), 76 taxa (Rag-1), 73 taxa (Rho), and 52 taxa (Tyr). As in previous examinations of the subgenus

Hylactophryne, my phylogentic reconstruction recovered well defined *C. augusti* species series and *C. bocourti* species series clades (Appendix D). Within the *Hylactophryne* species series I observed several instances of potentially invalid taxa in the form of shared mtDNA and nDNA haplotypes. These include *C. tarahumaraensis* in the *C. augusti* species series, *C. stuarti*, *C. nefrens*, and *C. cyanocthebius* in the *C. bocourti* species series. Within the *C. bocourti* species series, I found strong nodal support for several clades that contain the following five species: *C. bocourti*, *C. alfredi*, *C. xucanebi*, *C. campbelli*, and *C. uno*. Additionally, there was substantial variation in the DNA sequences obtained from individuals referable to *C. decoratus* which may indicate, as previously suspected (E. Smith, pers. comm.) that this taxon may contain multiple species. Two specimens (JAC 21604 and JAC 21615) collected in the southern Sierra Madre Oriental/northern Sierra Madre del Sur that my collaborators and I have been unable to confidently assign to a nomen were found to be distinct relative to the other *C. bocourti* species series members.

2.3 Discussion

The analyses presented in this chapter continue to support the reciprocal monophyly of the *C. augusti* species series and the *C. bocourti* species series within the subgenus *Hylactophryne*. Interestingly, I found that while the *C. augusti* species series inhabits a wider range of habitats than the *C. bocourti* species series, it displays relatively low levels of within species series sequence divergence. One interpretation is that the *C. augusti* species series is relatively young. Since these taxa are found in an ecologically diverse array of habitats ranging from deserts to tropical forests yet display little mitochondrial or nuclear sequence divergence, it is likely that the *C. augusti* species series only recently expanded to its present distribution. This distributional pattern may be related to the use of subterranean refugia during cold and dry periods and requires further investigation. Given the ecological diversity observed throughout the distribution of these taxa (Fig. 2.1), there is also potential for localized ecological adaptation which may explain the well-supported sub-structuring observed in the mtDNA and nDNA data generated for the *C. augusti* species series (Appendix D).

Within the *C. bocourti* species series, my analyses recovered many of the subgroups recognized by Lynch (1966). In particular the *decoratus* subgroup (listed as *C.* aff. *decoratus* in Appendix D) contains several distinctive lineages and should be explored further. As we speculated in our *C. uno* manuscript (Streicher et al., 2011; Appendix C), I found that several southern members of the *C. bocourti* species series appear to be invalid. The molecular phylogeny suggests that there are three clades inhabiting Guatemala and Honduras. The first of these is *C. bocourti*, a very distinctive large-bodied species that is unlikely to be confused with other *Craugastor* from the region. The second is *C. xucanebi*, a smaller species distributed around the Guatemalan Plateau. The third is *C. campbelli*, which is similar in gestalt to *C. xucanebi*, but distributed in southern Guatemala and northern Honduras. If future investigations support the patterns presented here, then the taxon *C. stuarti* will become a junior synonym of *C. xucanebi*, and the taxa *C. nefrens* and *C. cyanocthebius* will likely become junior synonyms of *C. campbelli*.

In summary, the molecular phylogeny presented in this chapter provides a comparative framework for (1) revising taxonomy within *Hylactophryne* and (2) exploring topics related to diversification rates and ecological specialization in species radiations. Species radiations that occur during a brief period of geologic time are known from throughout the Tree of Life, arising from processes that include ecological adaptation (Givnish and Systsma 2000) or long-range dispersal events (Van Bocxlaer et al. 2010). Radiations resulting from ecological niche divergence likely occur because of either competition driving diversifying selection (Schluter 1994) or key innovations that allow for major shifts in life history, physiological capacity, or phenotypic plasticity (von Dohlen and Moran 2000; Roelants et al. 2011). Thus, understanding the interactions between ecological setting and the physiological characteristics of species is important for identifying putative mechanisms of lineage diversification, especially for very recent episodes of adaptive divergence (Losos and Mahler 2010). Given that the *C. augusti* species series and the *C. bocourti* species series are sister groups yet display disparate patterns of phylogeographic diversity in sympatry, *Hylactophryne* represents an ideal system with which to explore adaptive divergence in a terrestrial vertebrate.

CHAPTER 3

THE *CRAUGASTOR MEXICANUS* SPECIES SERIES

Frogs in the subgenus *Craugastor* are mostly leaf-litter inhabitants that display ecologies not terribly dissimilar from insects. Among fieldworkers, these frogs are often referred to as ‘dirt frogs’ because they are considered to (1) be common as dirt or (2) look like dirt (owing to a brown [though variable] dorsal coloration). Dirt frogs are often the most abundant member of the local leaf litter herpetofauna (Scott, 1976), with populations often numbering in the millions (Lieberman, 1986). However, even these common frogs have apparently succumbed to recent amphibian declines (Crawford et al., 2010). In central and southern Mexico, one of the most abundant dirt frog groups is the *C. mexicanus* species series. Members of the *Craugastor mexicanus* species series are often confused for one another because they are small and nondescript yet possess phenotypically polymorphic color patterns and skin textures. Further these species often occur in sympatry with members of the phylogenetically distant *C. rhodopis* species series (see Chapter 4 and Fig. 1.3) which share several ecological and morphological characteristics with *C. mexicanus* species series frogs. Unlike the *C. rhodopis* species series taxa discussed in the following chapter, the *C. mexicanus* species series typically has been underrepresented in molecular examinations of the genus *Craugastor*. This is unfortunate because studies that have used *C. mexicanus* species series taxa have identified novel mitochondrial gene arrangements and increased nucleotide substitution rates relative to other *Craugastor* (e.g., Crawford and Smith, 2005). These frogs are also of interest because they are the only *Craugastor* species series that is endemic to a single country; Mexico (although there are some anecdotal reports that the species *C. montanus* may enter Guatemala). Currently, the group is thought to contain seven species: *C. hobartsmithi, C. mexicanus, C. montanus, C. occidentalis, C. omiltemanus, C. pygmaeus*, and *C. saltator* (Hedges et al., 2008). However, in terms of morphology, many of these species are either poorly defined or exhibit overlapping variation with their putative relatives (pers. obs.). For reasons discussed below and in the next chapter, I recommend the removal of *C. occidentalis* from the *C. mexicanus* species series (this taxon is more appropriately placed within the *C. rhodopis* species group of the *C. rhodopis* species series).

To better understand the levels of diversity that exist within the *C. mexicanus* species series, I sequenced a mitochondrial gene fragment (12S) from 58 taxa sampled from the Mexican states of Jalisco, Puebla, Estado de México, Hidalgo, Guerrero, and Oaxaca. These data were generated to assist in a taxonomic revision of the group that I am currently conducting with Eric N. Smith. To inform our review, the molecular data generated for this chapter were used to (1) identify the number of matrilineal lineages within the *C. mexicanus* species series and (2) determine several characteristics of the species series including phylogenetic depth and levels of phylogeographic variation.

Figure 3.1. Representatives of major matrilineal lineages in the *Craugastor mexicanus* species series (clade designations [A-G] correspond to those depicted in Appendix E): (A) Clade 1 from Talpa de Allende, Jalisco, Mexico (Field ID JAC 30722); (B) JAC 21230 *Craugastor* sp. B from Oaxaca; (C) *Craugastor* sp. C (JAC 21873) from Oaxaca; *Craugastor* sp. D (JAC 27244) from Avandaro, Estado de México; (E) *Craugastor* sp. E from Guerrero (JAC 22117); (F) *C. pygmaeus* (JAC 30818) from Matias Romero Oaxaca; (G) *C. mexicanus* (JAC 21289) from Oaxaca.

3.1 Methods

The methods used in generating a matrilineal phylogeny for the *C. mexicanus* species series largely followed those outlined in the previous chapters. Specifically, this included those laboratory and analytical methods described for the mtDNA 12S datasets. Maximum likelihood (ML) analyses employed a GTR+I+G model of nucleotide substitution. I also used distance (minimum evolution; ME) and maximum parsimony (MP) criteria to reconstruct phylogenetic trees and 1000 bootstrap pseudoreplicates to infer nodal support. I used two *C. rhodopis* species series (*Craugastor*. aff. *loki* [ENS 10371 and ENS 10391]) taxa as outgroups in this examination. A full list of voucher specimens, locality information, and GenBank accession numbers can be found in Appendix A.

In this chapter and the following chapter, I use exclusively mitochondrial data to infer patterns of diversity and relatedness. As discussed previously, current trends in molecular systematics indicate that multilocus mtDNA and nDNA datasets are favorable to those datasets that utilize only one of these genomic templates (Edwards, 2008). However, for sexually reproducing species with relatively deep mtDNA divergence levels, phylogenetic reconstructions are typically congruent with species trees derived from multiple genetic loci (Avise and Walker, 1999; Hebert et al., 2003). As such, I used patterns observed at deeper levels of mtDNA divergence to assess the accuracy of previous classification schemes used for the species series discussed in this chapter and chapter four.

3.2 Results

The 12S sequence data generated for the *C. mexicanus* species series resulted in a 444 base pair alignment for 60 taxa. Of the 444 bp, 134 were variable and 117 were parsimony informative. All three evolutionary criteria (ML, ME, MP) used to obtain support measures were largely concordant in their recovery of several major matrilineal groups within the *C. mexicanus* species series (Appendix E). My sampling of the *C. mexicanus* species series was monophyletic relative to the two outgroup taxa. Geographically, most of the matrilineal diversity in the *C. mexicanus* species series occurs in the states of Guerrero and Oaxaca. In these states, there are multiple matrilineal haplogroups (clades B, D, E, F, and G; Appendix E) that vary in the size of their distribution. Notably, two of these clades (F and G) are widespread, with representatives also occurring in the states of Puebla, Hidalgo, and Veracruz.

3.3 Discussion

The matrilineal phylogeny for the *C. mexicanus* species series reveals several interesting patterns that more or less confirm our previous suspicions that the group is fairly diverse and in a present state of taxonomic disarray. One may note that I have neglected to specifically identify many taxa featured in Appendix E outside of those clades I refer to as *C.* aff. *pygmaeus* and *C.* aff. *mexicanus*. The decision to not include nomenclatural designations in this chapter is based on my experience with examining type specimens of the *C. mexicanus* species series. I have now examined most of the type material for *C. mexicanus* species series and *C. rhodopis* taxa. The types I have examined are housed at the Field Museum of Natural History (Chicago, Illinois, USA), Illinois Natural History Survey (Champaign-Urbana, Illinois, USA), Smithsonian Institute Natural History Museum (Washington D.C., USA), and the British Museum of Natural History (London, England, UK). Collectively, this encompasses most of the named specimens in these groups. Discouragingly, I have encountered several issues that are well known (and described previously by researchers Jay M. Savage and John D. Lynch [see Savage and Emerson, 1970; Lynch, 2000]) related to morphological homoplasy and inconsistent ontogenetic sampling that at present impede my ability to match specimens in the molecular dataset with types. In summary, while the current sampling surely includes lineages referable to *C. hobartsmithi, C. mexicanus C. montanus, C. omiltemanus, C. pygmaeus*, and *C. saltator*, the matching of molecular identity with taxonomic identity in the group awaits a future and more thorough morphological analysis.

There are, however, several fascinating patterns that I can discuss using the analysis presented here. Perhaps the most salient of these is a striking case of elevational segregation between clades G and F of the matrilineal phylogeny (Fig. 3.2). In this system at approximately 2000 m there is a phylogeographic break where one clade occurs above and the other below this elevational boundary. Additionally, a cursory examination of morphology in these clades has revealed that the lower elevation clade is comprised of individuals with small adult body sizes (males 10.83–11.23 mm, females 14.54–16.59 mm) and the higher elevation clade is comprised of individuals with larger adult body sizes (males 18.68–25.23 mm, females 22.12–36.13 mm).

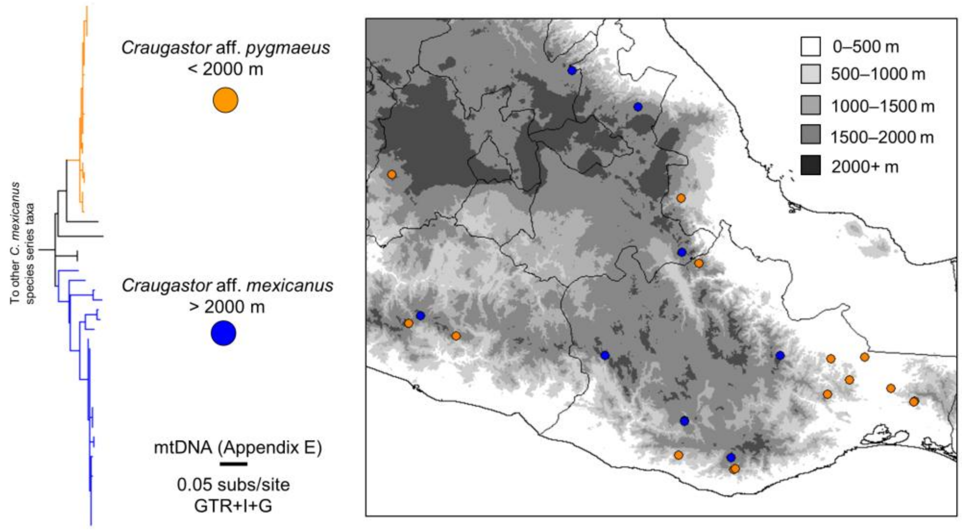

Figure 3.2. Evidence for the occurrence of elevational segregation in the *Craugastor mexicanus* species series of central Mexico.

This pattern warrants additional exploration but I speculate it may be primarily related to either physiological adaptation or direct competition. I suspect these processes because we have (1) observed the same pattern of larger body sizes at higher elevations in *C. podiciferus* from Isthmian Central America (unpublished data; Streicher et al., 2009) and (2) at lower elevations, *C. mexicanus* species series taxa share habitat with the much larger *C. rhodopis* species series taxon *C. loki*, whereas at higher elevations in the Sierra Madre del Sur and Sierra Madre Oriental they are the predominant leaf litter *Craugastor*.

Based on the molecular results presented here, I also have been reexamining morphology in the *C. mexicanus* species series. Three key characteristics that seem to have diagnostic value (at least relative to the syntopic *C. rhodopis* species series taxa *C. rhodopis*, *C. loki*, and *C. occidentalis*) are a speckled venter, reduced vomerine teeth, and black mesentery around the testes (as described by Lynch [2000]). However, at present these observations are based on limited sample sizes and mostly anecdotal. The future addition of nDNA to this dataset would greatly aid inference capabilities regarding the distinction of unsorted ancestral haplotypes from well sorted/defined lineages. Once nuclear data are available, a future coalescent based multilocus (mtDNA and nDNA) approach would be an ideal method for delimiting species within this diverse and morphologically perplexing group.

CHAPTER 4

THE *CRAUGASTOR RHODOPIS* SPECIES SERIES

Among dirt frogs that inhabit Mexico and Central America, the *Craugastor rhodopis* species series is perhaps the most abundant and frequently encountered. Relative to other *Craugastor*, this species series has been exceptionally well characterized using molecular methods (Miyamoto, 1983; Crawford, 2003a; Crawford 2003b; Chen, 2005; Streicher et al., 2009). Molecules have been particularly useful diagnostic tools because of the morphological polymorphism that occurs in most species (Lynch, 1966; Savage and Emerson, 1970; Savage, 2002; also see Figs 4.1–4.3). Although these molecular studies have offered much insight into the evolution of these frogs, they have been phylogenetically biased towards the predominantly lower Central American segment of the *C. rhodopis* species series, the *C. podiciferus* group. The *C. podiciferus* group contains eight species (*C. bransfordii*, *C. jota*, *C. lauraster*, *C. persimilis*, *C. podiciferus*, *C. polyptychus*, *C. stejnegerianus*, and *C. underwoodi*) that are distributed from eastern Honduras to western Panama. In contrast, the northern segment of the *C. rhodopis* species series, the *C. rhodopis* group, contains only two species (*C. loki* and *C. rhodopis*) and is distributed from Mexico southward through parts of Guatemala, Belize, El Salvador, and Honduras. The *C. rhodopis* group is known to contain substantially more diversity than current taxonomy would suggest (see Crawford & Smith, 2005; Hedges *et al*., 2008), although a thoroughly sampled molecular analysis has yet to be performed on this group. In addition to the phenotypic polymorphism observed in *C. loki* and *C. rhodopis*, the syntopic presence of an ecologically and morphologically similar *Craugastor* assemblage in Mexico (the *C. mexicanus* species series; Chapter 3), coupled with sexual size dimorphism in both groups, has produced a plethora of (typically invalid) species descriptions (Lynch, 2000).

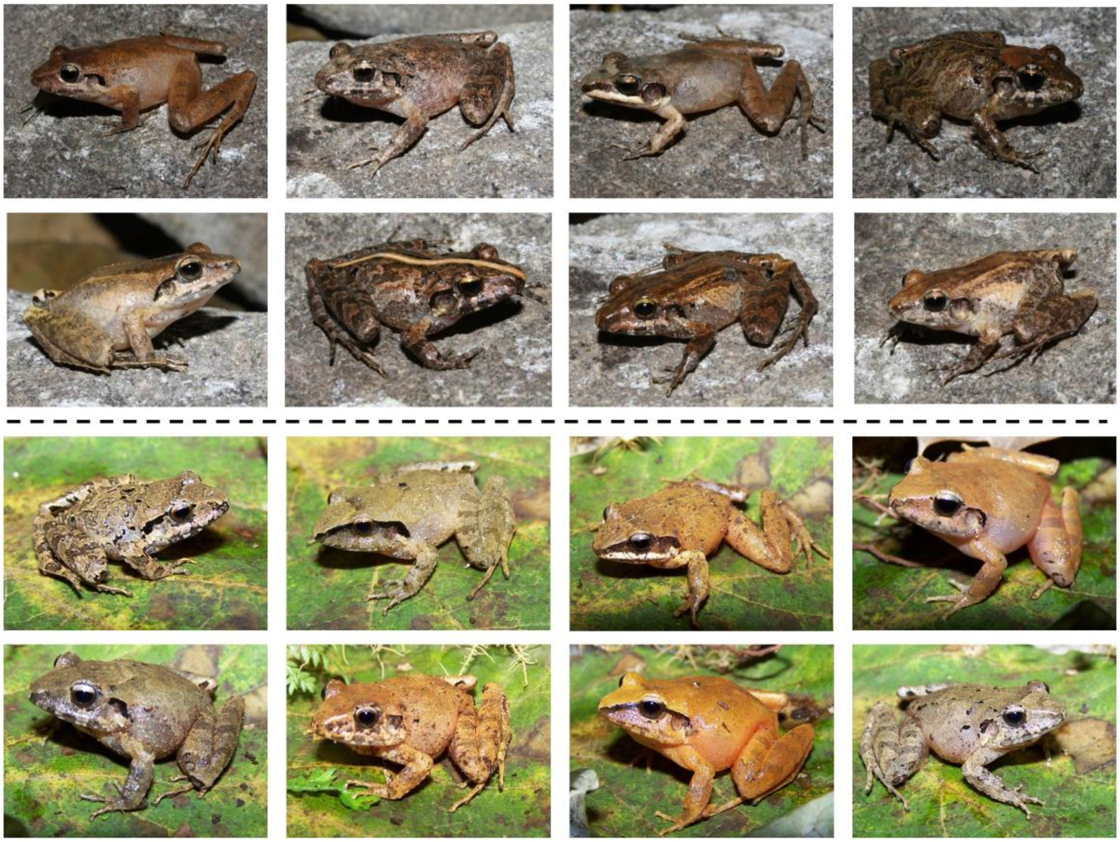

Figure 4.1. Population level color pattern variation in *Craugastor rhodopis* from near Banderilla, Veracruz, Mexico (above dotted line), and from near Tlanchinol, Hidalgo, Mexico (below dotted line).

Figure 4.2. Population level color pattern variation in *Craugastor* aff. *loki* from near Matías Romero, Oaxaca, Mexico (left of dotted line) and from near El Chupadero, Suchitepéquez, Guatemala (right of dotted line; photographs by Eric N. Smith).

Figure 4.3. Population level color pattern variation in *Craugastor occidentalis* from the Mexican states of Colima, Jalisco, Nayarit, Sinaloa, and Guerrero (some photographs by Eric N. Smith and Paulino Ponce-Campos).

In the current chapter I investigated phylogenetic and phylogeographic relationships in the *C. rhodopis* species group using a fragment of mitochondrial DNA. Based on the examination of type material (discussed in Chapter 3) I have included the taxa *C. occidentalis*, *C. loki*, and *C. rhodopis* in my concept of this group. Owing to the efforts of my many collaborators (specifically Eric N. Smith, Jonathan A. Campbell, Paulino Ponce-Campos, Uri O. García-Vásquez, and Eli B. Greenbaum) I was able to include samples from across three countries in this analysis (Mexico, Guatemala, and El Salvador; Fig. 4.4).

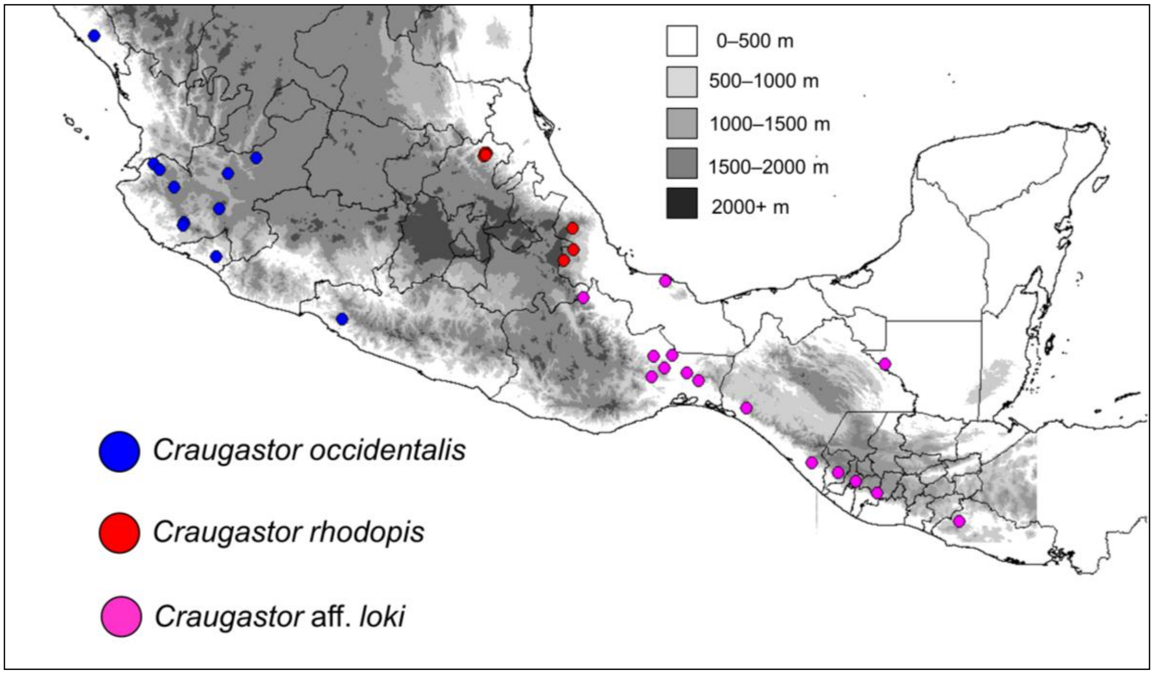

Figure 4.4. Geographic distribution of sampling for the construction of a matrilineal phylogeny for the *Craugastor rhodopis* species group. Taxonomic designation of samples follows the phylogenetic relationships observed in Appendix F.

As with the *C. mexicanus* species series (Chapter 3), I am presently engaged in a systematic revision of the *C. rhodopis* species group. Thus, the data I present below have been generated to inform nomenclatural decision making and assess phylogenetic diversity within this group.

4.1 Methods

The methods I used to sequence, align, and analyze 12S in the *C. rhodopis* species series were identical to those I have described previously (Chapters 1–3). I used several previously generated *C. podiciferus* species group mtDNA sequence as outgroups. In total I used 11 outgroup samples that included the taxa *C. podiciferus*, *Craugastor* sp. A (Streicher et al., 2009), and *C. underwoodi*. I also used two members of the *C. mexicanus* species series as distantly related outgroups (JAC 30722 and JAC 27244). A complete list of specimen vouchers, locality information, and GenBank accession numbers can be found in Appendix A.

To characterize color pattern traits and polymorphism in different matrilineal lineages, I examined 216 museum specimens. This sampling included individuals originating from 15 different populations of *C. rhodopis* species series taxa. I scored for the presence or absence of 11 color traits that are easily observed in preserved specimens. I documented the presence or absence of a (1) canthal mask, (2) supratympanic mask, (3) dorsolateral stripe, (4) mid-dorsal stripe, (5) cloacal blotch, (6) dorsal flecking, (7) interocular blotch, (8) lip barring, (9) lip barring, (10) knee blotch, and (11) fused mid-dorsal dot on each of these specimens. The specimens I examined are listed in Appendix G.

4.2 Results

The generation of 12S data in the *C. rhodopis* species series resulted in a 479 bp alignment. This alignment contained 210 variable characters and 169 parsimony informative characters. The resulting matrilineal phylogeny of this alignment produced some interesting results regarding putative relationships in the *C. rhodopis* species series (Appendix F). First, these analyses revealed that, as was suspected from previous morphological analyses, *C. occidentalis* (presently included in the *C. mexicanus* species series) is deeply nested with the *C. rhodopis* species series and sister to a monophyletic group containing taxa referable to *C. loki* and *C. rhodopis*. Second, as observed in my larger analysis of 12S (Chapter 1), the *C. rhodopis* species group and the *C. podiciferus* species group of Hedges et al. (2008) are well defined. Finally, there are two *C. loki* haplotypes (UOGV 385 and UOGV 370) that are highly divergent from those haplotypes observed in individuals collected syntopically (at least in the case of UOGV 370 from Veracruz). Because of these anomalous haplotypes I have chosen to refer to *C. loki* (as defined by Lynch [2000]) as *C.* aff. *loki* for the duration of this chapter.

Based on the phylogenetic reconstruction in Appendix F, my examination of color pattern polymorphism included three matrilineal lineages: *C.* aff. *loki* (eight populations; n = 97), *Craugastor* sp. 1 (one population; n = 22), and *C. rhodopis* (six populations; n =97). I found that, with few exceptions, the characters I examined occurred with similar frequencies across all three lineages (Fig. 4.5.)

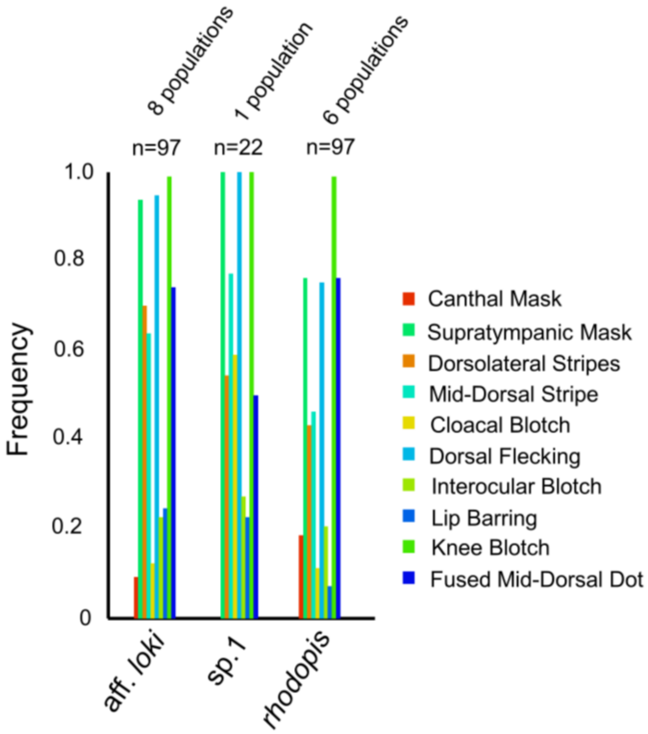

Figure 4.5. Frequency of select color pattern traits across three of the *Craugastor rhodopis* species group matrilineal lineages.

4.3 Discussion

As proposed by Lynch (2000) it appears that within the *C. rhodopis* species group there is a highland species (*C. rhodopis*) and a lowland species (*C. loki*). However, I did not find any evidence of *C. rhodopis* occurring outside of the Sierra Madre Oriental as Lynch (2000) suggests. One of the more interesting results of the mtDNA analysis of the *C. rhodopis* species group is the discovery of two highly divergent haplotypes from *C. loki* in Tabasco (UOGV 385) and Veracruz (UOGV 370), respectively. These haplotypes are interesting because the frogs (while both subadult individuals) are morphologically similar (at least in gross gestalt) to other specimens I have examined from these areas. However, in the cluster analysis performed in Chapter

1 (Fig. 1.3), the specimen UOGV 385 clustered within the *C. punctariolus* species series. The specimen UOGV 370 was collected from Volcán San Martín in Veracruz (the type locality of *C. loki* and its junior synonym *C. sanmartinensis*). I have examined the types of both *C. loki* (UIMNH 67057) and *C. sanmartinensis* (UIMNH 67058) and while they are difficult to distinguish from one another, they are diagnosable in gestalt from other *C. loki* found at lower elevations in the region. Thus, it is possible that they represent a *Craugastor* lineage endemic to Volcán San Martín. In summary, the divergent *C. loki* haplotypes may be explained by either (1) a valid *C. loki* (UOGV 370) and misidentified subadult *C. rupinius* (UOGV 385), or (2) a single widespread *C. loki* that possesses several low frequency ancient mitochondrial haplotypes that have been maintained by the enormous population sizes of *C loki* (i.e., genetic drift should have an extremely limited effect which would allow for old and divergent haplotypes to persist).

Recently a series of molecular studies have reported a similar phylogeographic pattern in amphibian and reptile species that have distributions traversing the longitudinal axis of the Trans-Mexican Volcanic Belt in Mexico (e.g., Devitt, 2006; Bryson et al., 2011; Greenbaum et al., 2011; Cox et al., In Press). The patterns that were recovered for *C. occidentalis* (another species with a distribution spanning the Trans-Mexican Volcanic Belt; Fig. 4.4) are consistent with the patterns reported by the other studies. Collectively, these studies indicate that the biotic communities of western Mexico were probably well established prior to the orogeny of the Trans-Mexican Volcanic Belt and that this recent addition to the Mexican landscape has acted as a barrier (or in some cases a filter barrier) to dispersal.

Ultimately, the taxonomic fate of many of the *C. rhodopis* species series taxa awaits a complimentary nDNA dataset. However, I feel that the mitochondrial and phenotypic polymorphism patterns discussed in this Chapter are an exciting prelude to future reconnaissance for several reasons: (1) as in some of the *C. mexicanus* species series lineages (Chapter 3; Fig. 3.3) elevational distribution appears to play a critical role in the phylogenetic structuring of the most derived *C. rhodopis* species group lineages (*C. rhodopis* and *C. loki*); (2) recordings of breeding vocalizations from *C. occidentalis* that occur south of the Trans-Mexican Volcanic Belt are loud and bird-like, and starkly contrast the description of a 'faint' call that has been reported elsewhere for the species (Hedges et al., 2008) indicating that the two *C. occidentalis* clades (Fig. 4.5) may represent distinct species; and (3) as long suspected and shown by previous molecular work in Isthmian Central American *Craugastor* (Crawford et al., 2003a; Streicher et al., 2009), color pattern polymorphism in this group is not related to phylogenetic structuring (Fig. 4.5) and is likely produced by an ecological phenomenon like balancing selection from avian predation (see Poulin et al., 2001).

APPENDIX A

SPECIMENS EXAMINED

Taxon	Voucher	Locality	Elevation (m)	Chapter	Reference	12S
***augusti* species series**						
C. augusti	JWS 251	USA: Texas: Bandera County: FM 337 between Medina and Vanderpool	664	One and Two	This study	JX001730
C. augusti	JWS 253	USA: Texas: Real County: RR 336 between Leakey and Prade Ranch	671	One and Two	This study	JX001722
C. augusti	JWS 277	USA: Texas: Real County: RR 337 between Camp Wood and Leakey	684	One and Two	This study	JX001731
C. augusti	TJD 770	Mexico: Guerrero: 15.9 km NW Atoyac de Álvarez on road to El Paraíso	762	One and Two	This study	JX001745
C. augusti	TJD 777	Mexico: Guerrero: 6.6 km W of Mazatlán on road to Tejocote	1838	One and Two	This study	JX001743
C. augusti	TJD 830	Mexico: Puebla: Carretera Izucar de Matamoros a Tlapanala	1357	One and Two	This study	JX001744
C. augusti	TJD 847	Mexico: Guerrero: 1.8 km E of MX 95 libre on road to Acahuizotla	918	One and Two	This study	JX001748
C. augusti	TJD 883	Mexico: Nayarit: Road to San Blas from MEX 15	44	One and Two	This study	JX001748
C. augusti	UTA A-57707 (JAC 23344)	Mexico: Jalisco: Carretera Santa María de Las Angeles-Bolaños	1602	One and Two	This study	JX001717
C. augusti	UTA A-57708 (JAC 23347)	Mexico: Jalisco: Carretera Santa María de Las Angeles-Bolaños	1602	One and Two	This study	JX001718
C. augusti	JAC 23345	Mexico: Jalisco: Carretera Santa María de Las Angeles-Bolaños	1602	One and Two	This study	JX001716
C. augusti	JAC 23346	Mexico: Jalisco: Carretera Santa María de Las Angeles-Bolaños	1602	One and Two	This study	JX001721
C. augusti	JAC 23544	Mexico: Nayarit: Carretera Tepic-Las Varas	824	One and Two	This study	JX001736
C. augusti	JAC 23564	Mexico: Nayarit: Ceboruco: Carretera Jala-Cerro	2137	One and Two	This study	JX001737
C. augusti	UTA A-59477 (JAC 24786)	Mexico: Michoacán: MEX 120	1030	One and Two	This study	JX001720
C. augusti	UTA A-54930 (JRM 4651)	Mexico: Guerrero: 71 KM E Chilpancingo along road to Tlapa	N/A	One and Two	This study	JX001739
C. augusti	JWS 284	USA: Texas: Edwards County: RR 335 between HWY 41 and Vance	605	One and Two	This study	JX001714
C. augusti	JWS 292	USA: Texas: Edwards County: RR 335 between HWY 41 and Vance	599	One and Two	This study	JX001732

Taxon	Voucher	Locality	Elevation (m)	Chapter	Reference	12S
C. augusti	JWS 294	USA: Texas: Edwards County: US 377 between Rocksprings and US 277	631	One and Two	This study	JX001734
C. augusti	JWS 295	USA: Texas: Edwards County: US 377 between Rocksprings and US 277	579	One and Two	This study	JX001729
C. augusti	JWS 296	USA: Texas: Val Verde County: US 377 between Carta Valley and US 277	573	One and Two	This study	JX001733
C. augusti	JAC 28298	Mexico: Colima: Agua Fria close to Minatitlan off HWY 98	790	One and Two	This study	JX001726
C. augusti	JAC 30056	Mexico: Colima: Road from Comala to Minatitlan	741	One and Two	This study	JX001719
C. augusti	JAC 30105	Mexico: Colima: Road from Comala to Minatitlan: side road to El Terrero	1108	One and Two	This study	JX001724
C. augusti	JAC 30106	Mexico: Colima: Road from Comala to Minatitlan: side road to El Terrero	1108	One and Two	This study	JX001715
C. augusti	JAC 30107	Mexico: Colima: Road from Comala to Minatitlan: side road to El Terrero	1108	One and Two	This study	JX001723
C. augusti	JAC 30108	Mexico: Colima: Road from Comala to Minatitlan: side road to El Terrero	1108	One and Two	This study	JX001725
C. augusti	JMM 151	USA: Texas: Reeves County	N/A	One and Two	This study	JX001728
C. augusti	JMM 152	USA: Texas: Pecos County	N/A	One and Two	This study	JX001727
C. augusti	JAC 30527	Mexico: Jalisco: near Volcán de Tequila	N/A	One and Two	This study	JX001738
C. augusti	JAC 8191	Mexico: Jalisco: 2.4 KM NW of Tapalpa	N/A	One	Darst and Cannatella, 2004	AY326011
C. augusti	UAZ catalogued	Mexico: Sonora: Alamos	N/A	One	Frost et al., 2006	DQ283271
C. tarahumaraensis	JAC 29189	Mexico: Chihuahua: HWY 24 between Guadalupe y Calvo and Badiraguato	2524	One and Two	This study	JX001735
***bocourti* species series**						
C. aff. *decoratus*	JAC 22728	Mexico: Oaxaca: Ejido Clemencia	1246	One and Two	This study	JX001706
C. aff. *decoratus*	JAC 22727	Mexico: Oaxaca: Ejido Clemencia	1246	One and Two	This study	JX001710
C. aff. *decoratus*	SMR 1327	Mexico: San Luis Potosí: Curva de la Iglesia, Ahuacatlan	1193	One and Two	This study	JX001747
C. aff. *decoratus*	JAC 26066	Mexico: Hidalgo: Municipio Tlanchinol	1475	One and Two	This study	JX001708

Taxon	Voucher	Locality	Elevation (m)	Chapter	Reference	12S
C. aff. *decoratus*	JRM 4770	Mexico: Oaxaca: HWY 175, 7.5 m S Vista Hermosa	1890	One and Two	This study	JX001712
C. alfredi	JAC 24288	Mexico: Veracruz: Municipio Angelica	500	One and Two	This study	JX001669
C. alfredi	ENEPI 6852 (ENS 10031)	Mexico: Oaxaca: Jacatepec	240	One and Two	This study	JX001688
C. alfredi	JAC 21987	Mexico: Guerrero: Autopista Acapulco-Tierra Colorado	265	One and Two	This study	JX001674
C. alfredi	JAC 21987**	Mexico: Veracruz: Municipio Coedoba: Cruz de los Naranjos	1100	One	Frost et al., 2006	DQ283318
C. bocourti	UTA A-51220 (ENS 8060)	Guatemala: Quiché: Uspantán	2070	One and Two	This study	JX001713
C. bocourti	UTA A-51245 (ENS 8245)	Guatemala: Quiché: Uspantán	1550	One and Two	This study	JX001711
C. bocourti	UTA A-55235	Guatemala: Baja Verapaz: Parulhá: Biotopo del Quetzal	N/A	One and Two	Heinicke et al., 2007	EF493713
C. campbelli	UTA A-55228 (ENS 7069)	Guatemala: Izabal: Montañas del Mico	900	One and Two	This study	JX001703
C. campbelli	UTA A-53048 (JAC 20552)	Guatemala: Izabal: Sierra de Santa Cruz	830	One and Two	This study	JX001693
C. campbelli	UTA A-53034 (JAC 20572)	Guatemala: Izabal: Sierra de Santa Cruz	830	One and Two	This study	JX001705
C. campbelli	UTA A-53035 (ENS 10239)	Guatemala: Izabal: Puerto Barrios	860	One and Two	This study	JX001702
C. cyanocthebius	TS1	Honduras: Cusuco National Park	N/A	One and Two	This study	JX001740
C. cyanocthebius	TS2	Honduras: Cusuco National Park	N/A	One and Two	This study	JX001749
C. cyanocthebius	TS3	Honduras: Cusuco National Park	N/A	One and Two	This study	JX001746
C. cyanocthebius	TS4	Honduras: Cusuco National Park	N/A	One and Two	This study	JX001742
C. nefrens	UTA A-51370 (ENS 7841)	Guatemala Izabal: Sierra de Caral	890	One and Two	This study	JX001707

Taxon	Voucher	Locality	Elevation (m)	Chapter	Reference	12S
Craugastor sp.	JAC 21604	Mexico: Oaxaca: Sierra Mixe	1466	One and Two	This study	JX001700
Craugastor sp.	JAC 21615	Mexico: Oaxaca: Sierra Mixe	N/A	One and Two	This study	JX001701
Craugastor sp.	SBH 2008	Mexico	N/A	One	Hedges et al., 2008	EU186703
C. stuarti	UTA A-51371 (ENS 7940)	Guatemala: Huehuetenango: La Democracia	2070	One and Two	This study	JX001697
C. stuarti	UTA A-51373 (ENS 7943)	Guatemala: Huehuetenango: La Democracia	2085	One and Two	This study	JX001676
C. stuarti	UTA A-51374 (ENS 7946)	Guatemala: Huehuetenango: La Democracia	1985	One and Two	This study	JX001692
C. stuarti	JAC 24518	Mexico: Chiapas: 13 KM NW Pueblo Nuevo on HWY 195	1750	One and Two	This study	JX001675
C. uno	ENEPI 6433 (ENS 9496)	Mexico: Guerrero: Sierra Madre del Sur	1870	One and Two	Streicher et al., 2011	GU576485
C. uno	ENEPI 6431 (ENS 9494)	Mexico: Guerrero: Sierra Madre del Sur	1870	One and Two	Streicher et al., 2011	GU576484
C. uno	UTA A-59250 (JAC 21377)	Mexico: Oaxaca: Carretera San Jose Pacifico-Candelaria	1550	One and Two	Streicher et al., 2011	GU576482
C. uno	UTA A-59252 (JAC 25673)	Mexico: Guerrero: Sierra Madre de Malinal-Tepec	2295	One and Two	Streicher et al., 2011	GU576486
C. xucanebi	UTA A-51361 (ENS 7804)	Guatemala: Alta Verapaz: KM 209 Carretera Guatemala-Cobán	1600	One and Two	This study	JX001670
C. xucanebi	ENS 8853	Guatemala: Huehuetenango: Barillas	1305	One and Two	This study	JX001704
C. xucanebi	ENS 8890	Guatemala: Huehuetenango: Barillas	1335	One and Two	This study	JX001699
C. xucanebi	ENS 8891	Guatemala: Huehuetenango: Barillas	1275	One and Two	This study	JX001673
C. xucanebi	ENS 9000	Guatemala: Huehuetenango: Barillas	1230	One and Two	This study	JX001668
C. xucanebi	ENS 8999	Guatemala: Huehuetenango: Barillas	1230	One and Two	This study	JX001698

Taxon	Voucher	Locality	Elevation (m)	Chapter	Reference	12S
C. xucanebi	ENS 9001	Guatemala: Huehuetenango: Barillas	1230	One and Two	This study	JX001690
C. xucanebi	UTA A-51363 (ENS 8100)	Guatemala: Quiché: Uspantán	1940	One and Two	This study	JX001685
C. xucanebi	UTA A-51364 (ENS 8117)	Guatemala: Quiché: Uspantán	1635	One and Two	This study	JX001696
C. xucanebi	MEA 3384	Guatemala: Sacatepéquez: Santa Lucia		One and Two	This study	JX001689
C. xucanebi	UTA A-51368 (JAC 19267)	Guatemala: Huehuetenango: Sierra de los Cuchumatanes	960	One and Two	This study	JX001686
C. xucanebi	UTA A-51367 (JAC 19266)	Guatemala: Huehuetenango: Sierra de los Cuchumatanes	975	One and Two	This study	JX001687
C. xucanebi	UTA A-51369 (JAC 19316)	Guatemala: Huehuetenango: Sierra de los Cuchumatanes	995	One and Two	This study	JX001684
C. xucanebi	JAC 20480	Guatemala: Alta Verapaz: Finca San Juan	1285-1380	One and Two	This study	JX001681
C. xucanebi	JAC 20481	Guatemala: Alta Verapaz: Finca San Juan	1285-1380	One and Two	This study	JX001682
C. xucanebi	JAC 20482	Guatemala: Alta Verapaz: Finca San Juan	1285-1380	One and Two	This study	JX001683
C. xucanebi	JAC 20483	Guatemala: Alta Verapaz: Finca San Juan	1285-1380	One and Two	This study	JX001677
C. xucanebi	JAC 20484	Guatemala: Alta Verapaz: Finca San Juan	1285-1380	One and Two	This study	JX001678
C. xucanebi	JAC 20485	Guatemala: Alta Verapaz: Finca San Juan	1285-1380	One and Two	This study	JX001680
C. xucanebi	JAC 20486	Guatemala: Alta Verapaz: Finca San Juan	1285-1380	One and Two	This study	JX001679
C. xucanebi	JAC 19442	Guatemala: Huehuetenango: road between La Trinidad and Finca San Francisco	1660	One and Two	This study	JX001691
C. xucanebi	MEA 2024	Guatemala		One and Two	This study	JX001672
C. xucanebi	ENS 8852	Guatemala: Huehuetenango: Barillas	1255	One and Two	This study	JX001694

Taxon	Voucher	Locality	Elevation (m)	Chapter	Reference	12S
***fitzingeri* species series**						
C. andi	MVZ 207254	Costa Rica: Puntarenas: Monteverde	N/A	One	Hedges et al., 2008	EU186687
C. crassidigitus	MVZ 207248	Costa Rica: Puntarenas: Pension Quetzal: Monteverde	N/A	One	Hedges et al., 2008	EU186733
C. emcelae	AMNH 124468	Panama: Chiriquí	N/A	One	Hedges et al., 2008	EU186738
C. fitzingeri	DMH 86-112	Costa Rica: Limon: Estacion Experimental La Lola	N/A	One	Darst and Cannatella, 2004	AY326001
C. longirostris	KU 177803	Ecuador: Pichincha: Santo Domingo	N/A	One	Heinicke et al., 2007	EF493395
C. longirostris	FMNH 257678	Panama: Chiriquí	1100	One	Streicher et al., 2009	EF562328
C. longirostris	FMNH 257561	Panama: Chiriquí	1100	One	Streicher et al., 2009	EF562327
C. melanostictus	MVZ 203856	Costa Rica: Cartago: Trail from Refugio Nacional Tapanti to Tres de Junio	N/A	One	Hedges et al., 2008	EU186683
***laticeps* species series**						
C. aff. *laticeps*	FN 252316	Honduras: Santa Barbara: Buena Vista	1440	One	This study	JX002024
C. aff. *laticeps*	MVZ 143299	Guatemala: Huehuetenango: Barillas	N/A	One	Hedges et al., 2008	EU186731
C. lineatus	MVZ 143301	Guatemala: Huehuetenango: Barillas	N/A	One	Hedges et al., 2008	EU186732
***mexicanus* species series**						
C. aff. *mexicanus*	UTA A-55240 (ENS 9561)	Mexico: Guerrero: Sierra Madre del Sur	2075	One and Three	This study	JX001967
C. aff. *mexicanus*	UTA A-55232 (ENS 9633)	Mexico: Oaxaca: San Andres Chicahuastla	2325	One and Three	This study	JX001986
C. aff. *mexicanus*	JAC 21162	Mexico: Oaxaca: Carretera Coconales-Zacatepec	2425	One and Three	This study	JX001964

Taxon	Voucher	Locality	Elevation (m)	Chapter	Reference	12S
C. aff. *mexicanus*	JAC 21176	Mexico: Oaxaca: Carretera Coconales-Zacatepec	1625	One and Three	This study	JX001946
C. aff. *mexicanus*	JAC 21148	Mexico: Oaxaca: along road above Totontepec	2355	One and Three	This study	JX001959
C. aff. *mexicanus*	JAC 21809	Mexico: Oaxaca: Hwy 175, 10.3 miles S San Miguel Suchixtepec	2044	One and Three	This study	JX001981
C. aff. *mexicanus*	JAC 21613	Mexico: Oaxaca: Sierra Mixe	1650	One and Three	This study	JX001977
C. aff. *mexicanus*	JAC 21572	Mexico: Oaxaca: Sierra Mixe	1650	One and Three	This study	JX001976
C. aff. *mexicanus*	JAC 21431	Mexico: Oaxaca: Portillo del Rayo	1550	One and Three	This study	JX001966
C. aff. *mexicanus*	JAC 21573	Mexico: Oaxaca: Sierra Mixe	1650	One and Three	This study	JX001994
C. aff. *mexicanus*	JAC 21768	Mexico: Oaxaca: 2.9 miles NE Sola de Vega	1554	One and Three	This study	JX001998
C. aff. *mexicanus*	JAC 21781	Mexico: Oaxaca: 2.9 miles NE Sola de Vega	1554	One and Three	This study	None
C. aff. *mexicanus*	JAC 21819	Mexico: Oaxaca: Carretera Sola de Vega-Juquila	1876	One and Three	This study	JX001999
C. aff. *mexicanus*	JAC 21722	Mexico: Oaxaca: 2.9 miles NE Sola de Vega	1554	One and Three	This study	None
C. aff. *mexicanus*	JAC 21409	Mexico: Oaxaca: San Felipe Cieneguilla	2700	One and Three	This study	JX001996
C. aff. *mexicanus*	JAC 21230	Mexico: Oaxaca: Sierra Madre del Sur	1550	One and Three	This study	JX001991
C. aff. *mexicanus*	JAC 21846	Mexico: Oaxaca: Carretera Sola de Vega-Juquila	1953	One and Three	This study	JX001979
C. aff. *mexicanus*	JAC 21837	Mexico: Oaxaca: Carretera Sola de Vega-Juquila	1953	One and Three	This study	JX001975
C. aff. *mexicanus*	JAC 21950	Mexico: Oaxaca: Municipio Putla de Guerrero	2481	One and Three	This study	JX001995
C. aff. *mexicanus*	JAC 22778	Mexico: Oaxaca: Sierra Mazateca	2566	One and Three	This study	JX001993
C. aff. *mexicanus*	UTA A-56579 (JAC 22389)	Mexico: Puebla: Sierra Negra	2382	One and Three	This study	JX001982
C. aff. *mexicanus*	JAC 23171	Mexico: Oaxaca: Municipio San Miguel Chimalapa	1265	One and Three	This study	JX001980
C. aff. *mexicanus*	JAC 21289	Mexico: Oaxaca: Sierra Miahuatlán	3015	One and Three	This study	JX001978
C. aff. *mexicanus*	ISZ 229	Mexico: Puebla: Tlatlauquitepec	N/A	One and Three	This study	JX001955

Taxon	Voucher	Locality	Elevation (m)	Chapter	Reference	12S
C. aff. *mexicanus*	JAC 24290	Mexico: Veracruz: Municipio Angelica	500	One and Three	This study	JX001965
C. aff. *pygmaeus*	JAC 21148	Mexico: Oaxaca: near Ayutla	1900	One and Three	This study	None
C. aff. *pygmaeus*	JAC 21373	Mexico: Oaxaca: Carretera San Jose Pacifico-Candelaria	550	One and Three	This study	JX001950
C. aff. *pygmaeus*	JAC 21883	Mexico: Oaxaca: Sierra Madre del Sur	668	One and Three	This study	JX001972
C. aff. *pygmaeus*	JAC 21859	Mexico: Oaxaca: San Gabriel Mixtepec	710	One and Three	This study	JX001990
C. aff. *pygmaeus*	JAC 22881	Mexico: Oaxaca: Carretera Santa María Chimalapa-Lazaro	250	One and Three	This study	JX001948
C. aff. *pygmaeus*	JAC 22883	Mexico: Oaxaca: Carretera Santa María Chimalapa-Lazaro	250	One and Three	This study	None
C. aff. *pygmaeus*	JAC 22882	Mexico: Oaxaca: Carretera Santa María Chimalapa-Lazaro	250	One and Three	This study	JX001947
C. aff. *pygmaeus*	JAC 22896	Mexico: Oaxaca: Carretera Santa María Chimalapa-Lazaro	250	One and Three	This study	None
C. aff. *pygmaeus*	JAC 22950	Mexico: Oaxaca: Carretera Santa María Guienagati-Lachidola	1135	One and Three	This study	JX001951
C. aff. *pygmaeus*	JAC 22767	Mexico: Oaxaca: Sierra Mazateca: Carretera Puerto Soledad	1221	One and Three	This study	JX001963
C. aff. *pygmaeus*	JAC 22957	Mexico: Oaxaca: Carretera Santa María Guienagati-Lachidola	1135	One and Three	This study	JX001969
C. aff. *pygmaeus*	UTA A-57976 (JAC 22929)	Mexico: Oaxaca: Carretera Santa María Guienagati-Lachidola	1175	One and Three	This study	JX001954
C. aff. *pygmaeus*	JAC 22189	Mexico: Guerrero: Grutas de Juxtlahuaca	931	One and Three	This study	JX001971
C. aff. *pygmaeus*	JAC 23153	Mexico: Oaxaca: Municipio San Miguel Chimalapa	1345	One and Three	This study	JX001973
C. aff. *pygmaeus*	JAC 23034	Mexico: Oaxaca: Carretera to El Progreso after intersection with MEX 185	300	One and Three	This study	JX001949
C. aff. *pygmaeus*	JAC 23033	Mexico: Oaxaca: Carretera to El Progreso after intersection with MEX 185	300	One and Three	This study	JX001968
C. aff. *pygmaeus*	JAC 30818	Mexico: Oaxaca: Road from Santo Domingo Petapa to Loma Santa Cruz	560	One and Three	This study	JX001962
C. aff. *pygmaeus*	JAC 30855	Mexico: Oaxaca: Road from Santo Domingo Petapa to Loma Santa Cruz	560	One and Three	This study	JX001989
C. aff. *pygmaeus*	LCM 1209	Mexico: Guerrero: Chilpancingo: Agua de Obispo	N/A	One and Three	This study	JX001957

Taxon	**Voucher**	**Locality**	**Elevation (m)**	**Chapter**	**Reference**	**12S**
C. aff. *pygmaeus*	IDF 59	Mexico: Guerrero: Chilpancingo: Acahuizotla	N/A	One and Three	This study	JX001988
C. aff. *pygmaeus*	JAC 24289	Mexico: Veracruz: Municipio Angelica	500	One and Three	This study	JX001961
Craugastor sp.	UTA A-55246 (ENS 9595)	Mexico: Oaxaca: Sierra Madre del Sur: Rio Salado	1245	One and Three	This study	JX001987
Craugastor sp.	JAC 22117	Mexico: Guerrero: between Yetla and Vuella del Sur	1825	One and Three	This study	JX001983
Craugastor sp.	JAC 30720	Mexico: Jalisco: Road between Talpa de Allende and El Cuale	1771	One and Three	This study	JX001985
Craugastor sp.	JAC 30722	Mexico: Jalisco: Road between Talpa de Allende and El Cuale	1771	One and Three	This study	JX001984
Craugastor sp.	JAC 27244	Mexico: México: Road from Avandaro to El Manzano E of Cerro Gordo	2264	One and Three	This study	JX001952
Craugastor sp.	JAC 21873	Mexico: Oaxaca: Sierra Madre del Sur	1051	One and Three	This study	JX001970
Craugastor sp.	JAC 21885	Mexico: Oaxaca: Sierra Madre del Sur	668	One and Three	This study	JX001958
***milesi* species series**						
C. daryi	UTA A-57940	Guatemala: Baja Verapaz: Parulhá: Biotopo del Quetzal	N/A	One	Heinicke et al., 2007	EF493531
C. milesi	MEA 622	Guatemala: Alta Verapaz: Chisec	N/A	One and Two	This study	JX001695
***punctariolus* species series**						
C. berkenbuschii	JAC 22873	Mexico: Oaxaca		One	This study	JX002010
C. berkenbuschii	JAC 22889	Mexico: Oaxaca		One	This study	JX002013
C. berkenbuschii	JAC 22885	Mexico: Oaxaca		One	This study	JX002012
C. berkenbuschii	JAC 22789	Mexico: Oaxaca		One	This study	JX002011
C. aff. *laevissimus*	JHT 2529	Honduras: Comayagua: Parque Nacional Montaña de Comayagua, Qurbrada El Gavilán	1150	One	This study	JX002023

Taxon	Voucher	Locality	Elevation (m)	Chapter	Reference	12S
C. aff. *laevissimus*	JHT 2539	Honduras: Comayagua: Parque Nacional Montaña de Comayagua, above Río Negro	1200	One	This study	JX002035
C. aff. *laevissimus*	JHT 2978	Honduras: Cortes: Parque Nacional Cerro Azul Meámbar , Los Pinos Centro de Visitantes	750	One	This study	JX002032
C. aff. *laevissimus*	JHT 2993	Honduras: Cortes: Parque Nacional Pico Bonito, Rio Cangrejal	200	One	This study	JX002019
C. aff. *laevissimus*	JHT 3000	Honduras: Santa Barbara: Parque Nacional Montaña de Santa Barbara, Las Quebradas	1450	One	This study	JX002022
C. aff. *laevissimus*	JHT 3004	Honduras: Santa Barbara: Compañia Agrícola Paradise (former Plowden Finca)	700	One	This study	JX002000
C. aff. *laevissimus*	N556	Nicaragua: Jinotega: Reserva Natural Cerro Kilambé: Campamento Oedipina	1625	One	This study	JX002031
C. aff. *laevissimus*	N639	Nicaragua: Region Autonoma Atlantico Norte: Parque Nacional Saslaya	1300	One	This study	JX002021
C. aff. *laevissimus*	N950	Nicaragua: Jinotega: Reserva Natural Cerro Kilambé: El Chiflon	1540	One	This study	JX002025
C. aff. *laevissimus*	FN 253320	Honduras: Santa Barbara: Buena Vista	1440	One	This study	JX002001
C. aff. *laevissimus*	FN 252814	Honduras: Cortes: Buenos Aires	1000	One	This study	JX002002
C. aff. *laevissimus*	FN 253065	Honduras: Olancho: Talgua Arriba	N/A	One	This study	JX002005
C. aff. *laevissimus*	FN 213154	Honduras: Olancho: Piedra Blanca	N/A	One	This study	JX002018
C. aff. *laevissimus*	FN 253575	Honduras: Copan: San Isidro	1050	One	This study	JX002008
C. aff. *laevissimus*	FN 253656	Honduras: Santa Barabara: El Cedral	1700	One	This study	JX002006
C. aff. *rugulosus*	JHT 2489	Honduras: Cortes: Parque Nacional Cerro Azul Meámbar , Los Pinos Centro de Visitantes	750	One	This study	JX002026
C. aff. *rugulosus*	JHT 2510	Honduras: Cortes: Parque Nacional Cerro Azul Meámbar , Los Pinos Centro de Visitantes	750	One	This study	JX002033
C. aff. *rugulosus*	FN 212610	Honduras: Intibuca: M. Mixcure	1800	One	This study	JX002030
C. aff. *rugulosus*	FN 213838	Honduras: Cortes: Los Pinos	750	One	This study	JX002003
C. aff. *rugulosus*	MVZ 207279	Costa Rica: Guanacaste: Quebrada Floricita: Volcán Cacao	N/A	One	Hedges et al., 2008	EU186680
C. angelicus	MVZ 149762	Costa Rica: Heredia: Chompipe vicinity of Volcán Barba	N/A	One	Hedges et al., 2008	EU186681
C. aurilegulus	C007	Honduras: Atlantida: PN Pico Bonito, Quebrada de Oro	972	One	This study	JX002028

Taxon	Voucher	Locality	Elevation (m)	Chapter	Reference	12S
C. aurilegulus	C023	Honduras: Atlantida: PN Pico Bonito, Cangrejal	90	One	This study	JX002036
C. aurilegulus	JHT 2779	Honduras: Yoro: Parque Nacional Pico Pijol, road above El Porvenir de Morazan	1450	One	This study	JX002034
C. aurilegulus	FN 252588	Honduras: Olancho: Q. Chilantro	360	One	This study	JX002004
C. aurilegulus	FN 252530	Honduras: Atlantida: near Santa Ana	30	One	This study	JX002007
C. charadra	JHT 1813	Honduras: Cortes: El Paraiso Valley	115	One	This study	JX002027
C. charadra	FN 252466	Honduras: Santa Barbara: Rio Liston	405	One	This study	JX002020
C. emleni	FN 252319	Honduras: Francisco Morazan: Los Golondrinos	1600	One	This study	JX002029
C. megacephalus	MVZ 207243	Costa Rica: Guacaste: Volcán Cacao	N/A	One	Hedges et al., 2008	EU186688
C. obesus	AMNH 124540	Panama: Chiriquí	N/A	One	Hedges et al., 2008	EU186737
C. punctariolus	SIUC 7066	Panama: Cocle: Parque Nacional El Cope	N/A	One	Frost et al., 2006	DQ283168
C. ranoides	USNM FS-195393	Panama: Isla Escudo de Veraguas, West Point	N/A	One	Frost et al., 2006	DQ283105
C. rupinius	KU 289861	El Salvador: Usulutan: Cerro del Tigre	N/A	One	Hedges et al., 2008	EU186669
C. rupinius	JAC 23091	Mexico: Chiapas	N/A	One	This study	JX002015
C. rupinius	JAC 23090	Mexico: Chiapas	N/A	One	This study	JX002014
C. sandersoni	UTA A-49803	Guatemala: Izabal: Sierra de Santa Cruz	N/A	One	Heinicke et al., 2007	EF493712
C. vocalis	JAC 23603	Mexico: Nayarit	N/A	One	This study	JX002016
C. vocalis	JAC 23602	Mexico: Nayarit	N/A	One	This study	JX002017
***rhodopis* species series**						
C. aff. *loki*	UTA A-54821 (ENS 10371)	Mexico: Veracruz: Sierra de Las Tuxtlas: Volcán San Martín	755	One and Four	This study	JX002101

Taxon	Voucher	Locality	Elevation (m)	Chapter	Reference	12S
C. aff. *loki*	UTA A-54822 (ENS 10376)	Mexico: Veracruz: Sierra de Las Tuxtlas: Volcán San Martín	755	One and Four	This study	JX002100
C. aff. *loki*	MEA 1948	Guatemala: Quetzaltenango: Volcán Santa Maria	1600	One and Four	This study	JX002078
C. aff. *loki*	UTA A-56525 (JAC 23071)	Mexico: Chiapas	45	One and Four	This study	JX002044
C. aff. *loki*	UTA A-56524 (JAC 23070)	Mexico: Chiapas	45	One and Four	This study	JX002069
C. aff. *loki*	UTA A-56594 (JAC 23035)	Mexico: Oaxaca: Carretera to El Progreso after intersection with MEX 185	300	One and Four	This study	JX002084
C. aff. *loki*	JAC 19864	Guatemala: San Marcos: San Rafael Pie de la Cuesta	1590	One and Four	This study	JX002086
C. aff. *loki*	UTA A-56562 (JAC 23134)	Mexico: Oaxaca: Carretera Santa María Guienagati-Lachidola	1170	One and Four	This study	JX002065
C. aff. *loki*	UTA A-56532 (JAC 22856)	Mexico: Chiapas: Carretera Tonala-Costa Rica: La Sepultura	197	One and Four	This study	JX002091
C. aff. *loki*	UTA A-56533 (JAC 22857)	Mexico: Chiapas: Carretera Tonala-Costa Rica: La Sepultura	197	One and Four	This study	JX002090
C. aff. *loki*	JAC 22721	Mexico: Oaxaca: El Mirador Municipio Santa María Chicholla	1058	One and Four	This study	JX002053
C. aff. *loki*	UTA A-56590 (JAC 22621)	Mexico: Veracruz: Sierra de Las Tuxtlas: Volcán San Martín		One and Four	This study	JX002055
C. aff. *loki*	JAC 21744	Mexico: Oaxaca: N of Palomares	80	One and Four	This study	JX002093
C. aff. *loki*	UTA A-56561 (JAC 22896)	Mexico: Oaxaca: Carretera Santa María Chimalapa-Lazaro	250	One and Four	This study	JX001974
C. aff. *loki*	UTA A-56536 (JAC 23107)	Mexico: Oaxaca: Camino Niltepec-El Palmar	340	One and Four	This study	JX002097
C. aff. *loki*	JAC 30854	Mexico: Oaxaca: Road between San Juan Mazatlan and La Mixtequita	633	One and Four	This study	JX002073

Taxon	Voucher	Locality	Elevation (m)	Chapter	Reference	12S
C. aff. *loki*	JAC 30820	Mexico: Oaxaca: Road from Santo Domingo Petapa to Loma Santa Cruz	560	One and Four	This study	JX002043
C. aff. *loki*	UOGV 370	Mexico: Veracruz: Ejido Xagapan: Volcán San Martín: Los Tuxtlas	N/A	One and Four	This study	JX002057
C. aff. *loki*	ENS 13395	Guatemala: Suchitepequez: Eco Lodge Los Tarrales	760	One and Four	This study	JX002099
C. aff. *loki*	ENS 13396	Guatemala: Suchitepequez: Eco Lodge Los Tarrales	760	One and Four	This study	JX002038
C. aff. *loki*	ENS 13392	Guatemala: Suchitepequez: Eco Lodge Los Tarrales	760	One and Four	This study	JX002074
C. aff. *loki*	ENS 13391	Guatemala: Suchitepequez: Eco Lodge Los Tarrales	760	One and Four	This study	JX002079
C. aff. *loki*	ENS 13394	Guatemala: Suchitepequez: Eco Lodge Los Tarrales	760	One and Four	This study	JX002092
C. aff. *loki*	ENS 13393	Guatemala: Suchitepequez: Eco Lodge Los Tarrales	760	One and Four	This study	JX002085
C. aff. *loki*	GAR 626	Guatemala: Peten: La Libertad: Parque Nacional Sierra de Lacuadon	N/A	One and Four	This study	JX002098
C. aff. *loki*	UTA A-55245 (GAR 400)	Guatemala: Peten: La Libertad: Parque Nacional Sierra de Lacuadon	N/A	One and Four	This study	JX002071
C. aff. *loki*	EBG 192	El Salvador: Santa Ana	800	One and Four	This study	JX002066
C. aff. *loki*	EBG 194	El Salvador: Santa Ana	800	One and Four	This study	JX002054
C. aff. *loki**	UOGV 385*	Mexico: Tabasco: Huimanguillo: Cerro Las Flores, Antenas PEMEX	N/A	One and Four	This study	JX002063
C. aff. *podiciferus*	UTA A-52449	Costa Rica: Puntarenas	1520	One and Four	Streicher et al. 2009	EF562312
C. aff. *podiciferus*	FMNH 257757	Costa Rica: Puntarenas: Coto Brus	1410	One and Four	Streicher et al. 2009	EF562293
C. aff. *podiciferus*	FMNH 257755	Costa Rica: Puntarenas: Coto Brus	1410	One and Four	Streicher et al. 2009	EF562289
C. aff. *podiciferus*	FMNH 257756	Costa Rica: Puntarenas: Coto Brus	1410	One and Four	Streicher et al. 2009	EF562290
C. aff. *podiciferus*	FMNH 257653	Costa Rica: Puntarenas: Coto Brus	1410	One and Four	Streicher et al. 2009	EF562292
C. aff. *podiciferus*	MVZ 164825	Costa Rica: Heredia	2100	One and Four	Streicher et al. 2009	EF562303
C. aff. *podiciferus*	UCR 17441	Costa Rica: Heredia	2000	One and Four	Streicher et al. 2009	EF562299

Taxon	Voucher	Locality	Elevation (m)	Chapter	Reference	12S
C. aff. *podiciferus*	UCR 17439	Costa Rica: Heredia	2000	One and Four	Streicher et al. 2009	EF562298
C. aff. *podiciferus*	UCR 18062 (FB 4307)	Costa Rica: Heredia	1900	One and Four	Streicher et al. 2009	EF562302
C. aff. *podiciferus*	MVZ FC-13463	Costa Rica: Heredia: Chompipe vicinity of Volcán Barba		One	Heinicke et al., 2007	EF493360
C. aff. *podiciferus*	FMNH 257669	Costa Rica: Puntarenas	1500	One	Streicher et al., 2009	EF562320
C. aff. *podiciferus*	FMNH 257672	Costa Rica: Puntarenas	1500	One	Streicher et al., 2009	EF562318
C. aff. *podiciferus*	UCR 16355	Costa Rica: Heredia	1500	One	Streicher et al., 2009	EF562316
C. aff. *podiciferus*	FMNH 257671	Costa Rica: Puntarenas	1500	One	Streicher et al., 2009	EF562314
C. aff. *podiciferus*	UCR 17469	Costa Rica: Heredia	2000	One	Streicher et al., 2009	EF562310
C. aff. *podiciferus*	UCR 16356	Costa Rica: San Jose	1940	One	Streicher et al., 2009	EF562308
C. aff. *podiciferus*	UCR 16357	Costa Rica: San Jose	1600	One	Streicher et al., 2009	EF562306
C. aff. *podiciferus*	FMNH 257595	Costa Rica: Cartago	1600	One	Streicher et al., 2009	EF562304
C. aff. *podiciferus*	UCR 17442	Costa Rica: Heredia	2000	One	Streicher et al., 2009	EF562300
C. aff. *podiciferus*	UCR 16360	Costa Rica: San Jose	1313	One	Streicher et al., 2009	EF562296
C. aff. *podiciferus*	FMNH 257550	Costa Rica: Puntarenas: Coto Brus	1350	One	Streicher et al., 2009	EF562294
C. aff. *podiciferus*	FMNH 257652	Costa Rica: Puntarenas: Coto Brus	1350	One	Streicher et al., 2009	EF562288
C. aff. *podiciferus*	UCR 16361	Costa Rica: Alajuela	1930	One	Streicher et al., 2009	EF562321
C. aff. *podiciferus*	MVZ 149813	Costa Rica: Puntarenas	1500	One	Streicher et al., 2009	EF562319
C. aff. *podiciferus*	FMNH 257670	Costa Rica: Puntarenas	1500	One	Streicher et al., 2009	EF562317
C. aff. *podiciferus*	UCR 16354	Costa Rica: Heredia	1500	One	Streicher et al., 2009	EF562315
C. aff. *podiciferus*	UCR 16353	Costa Rica: Heredia	1500	One	Streicher et al., 2009	EF562313

Taxon	Voucher	Locality	Elevation (m)	Chapter	Reference	12S
C. aff. *podiciferus*	FMNH 257673	Costa Rica: Puntarenas	1500	One	Streicher et al., 2009	EF562311
C. aff. *podiciferus*	UCR 17462	Costa Rica: Heredia	2000	One	Streicher et al., 2009	EF562309
C. aff. *podiciferus*	UCR 16358	Costa Rica: San Jose	1600	One	Streicher et al., 2009	EF562307
C. aff. *podiciferus*	FMNH 257596	Costa Rica: Cartago	1600	One	Streicher et al., 2009	EF562305
C. aff. *podiciferus*	UCR 17443	Costa Rica: Heredia	2000	One	Streicher et al., 2009	EF562301
C. aff. *podiciferus*	UCR 16359	Costa Rica: San Jose	1313	One	Streicher et al., 2009	EF562297
C. aff. *podiciferus*	FMNH 257758	Costa Rica: Puntarenas	1410	One	Streicher et al., 2009	EF562295
C. aff. *podiciferus*	FMNH 257651	Costa Rica: Puntarenas	1350	One	Streicher et al., 2009	EF562291
C. bransfordii	MVUP 1875	Panamá: Bocas del Toro	50	One	Streicher et al., 2009	EF562324
C. bransfordii	AMNH A-124398	Panamá		One	Heinicke et al., 2007	EF493822
C. occidentalis	JAC 30503	Mexico: Colima: Road from HWY 54 to Ixtlahuacan	221	One and Four	This study	JX002039
C. occidentalis	JAC 27289	Mexico: Guerrero: HWY 134 from Ixtapa to Cd. Altamirano	473	One and Four	This study	JX002088
C. occidentalis	JAC 30502	Mexico: Colima: Road from HWY 54 to Ixtlahuacan	221	One and Four	This study	JX002040
C. occidentalis	JAC 30595	Mexico: Sinaloa: HWY 40 between Villa Union and Concordia	162	One and Four	This study	JX002070
C. occidentalis	JAC 28622	Mexico: Jalisco: Roads between Sayula and Zacoalco de Torres, and Tapalpa	2238	One and Four	This study	JX002048
C. occidentalis	JAC 23901	Mexico: Jalisco: Puerto Las Mazos	1119	One and Four	This study	JX002061
C. occidentalis	JAC 23799	Mexico: Jalisco: Carretera Las Palmas-La Estancia	557	One and Four	This study	JX002087
C. occidentalis	UTA A-60772 (JAC 23694)	Mexico: Jalisco: Carretera La Estancia-La Mascota	1957	One and Four	This study	JX002089
C. occidentalis	UTA A-59511 (JAC 23699)	Mexico: Jalisco: Carretera La Estancia-La Mascota	1957	One and Four	This study	JX002046

Taxon	Voucher	Locality	Elevation (m)	Chapter	Reference	12S
C. occidentalis	UTA A-60767 (JAC 23781)	Mexico: Jalisco: Carretera entre Las Cruces y Atenguillo	1936	One and Four	This study	JX002058
C. occidentalis	JAC 23910	Mexico: Jalisco: Sierra de Manantlan: Puerto Las Mazos	1687	One and Four	This study	JX002049
C. occidentalis	UTA A-60779 (JAC 23940)	Mexico: Jalisco: Sierra de Manantlan: Puerto Las Mazos	1412	One and Four	This study	JX002060
C. occidentalis	UTA A-60781 (JAC 23947)	Mexico: Jalisco: Sierra de Manantlan: Puerto Las Mazos	1684	One and Four	This study	JX002064
C. occidentalis	L 197 397	Mexico: Jalisco	N/A	One and Four	This study	JX002050
C. occidentalis	PPC 8	Mexico: Jalisco	N/A	One and Four	This study	JX002052
C. occidentalis	PPC 10	Mexico: Jalisco	N/A	One and Four	This study	JX002047
C. occidentalis	PPC 11	Mexico: Jalisco	N/A	One and Four	This study	JX002062
C. occidentalis	PPC 12	Mexico: Jalisco	N/A	One and Four	This study	JX002051
C. occidentalis	PPC 13	Mexico: Jalisco	N/A	One and Four	This study	JX002041
C. occidentalis	PPC 14	Mexico: Jalisco	N/A	One and Four	This study	JX002068
C. occidentalis	PPC 15	Mexico: Jalisco	N/A	One and Four	This study	JX002045
C. rhodopis	JAC 22569	Mexico: Veracruz: Municipio La Perla: Metlac	1862	One and Four	This study	JX002059
C. rhodopis	JAC 29863	Mexico: Veracruz: HWY 140 West of Banderilla	1644	One and Four	This study	JX002072
C. rhodopis	JAC 29874	Mexico: Veracruz: HWY 140 West of Banderilla	1686	One and Four	This study	JX002067
C. rhodopis	JAC 29864	Mexico: Veracruz: HWY 140 West of Banderilla	1644	One and Four	This study	JX002042
C. rhodopis	JAC 29954	Mexico: Veracruz: Road between Totutla and Huatusco	1258	One and Four	This study	JX002075
C. rhodopis	JAC 26603	Mexico: Hidalgo: Carretera Federal 105 SW of Huejutla de Reyes	1486	One and Four	This study	JX002037
C. rhodopis	JAC 26043	Mexico: Hidalgo: Municipio Tlanchinol	1531	One and Four	This study	JX002081

Taxon	Voucher	Locality	Elevation (m)	Chapter	Reference	12S
C. rhodopis	JAC 26031	Mexico: Hidalgo: Municipio Tlanchinol	1538	One and Four	This study	JX002076
C. rhodopis	JAC 26602	Mexico: Hidalgo: Carretera Federal 105 SW of Huejutla de Reyes	1486	One and Four	This study	None
C. rhodopis	JAC 25901	Mexico: Hidalgo: Municipio Tlanchinol	1474	One and Four	This study	JX002082
C. rhodopis	JAC 25902	Mexico: Hidalgo: Municipio Tlanchinol	1474	One and Four	This study	JX002083
C. rhodopis	JAC 25941	Mexico: Hidalgo: Municipio Tlanchinol	1472	One and Four	This study	JX002080
C. rhodopis	JAC 25942	Mexico: Hidalgo: Municipio Tlanchinol	1472	One and Four	This study	JX002096
C. rhodopis	JAC 26042	Mexico: Hidalgo: Municipio Tlanchinol	1531	One and Four	This study	JX002094
C. rhodopis	JAC 26606	Mexico: Hidalgo: Municipio Tlanchinol	1486	One and Four	This study	JX002095
Craugastor sp.	AJC 890	Panama: Chiriquí	1663	One and Four	Streicher et al. 2009	EF562282
Craugastor sp.	MVUP 1720	Panama: Cocle	800	One	Streicher et al., 2009	EF562326
Craugastor sp.	FMNH 257562	Panama: Chiriquí	1100	One	Streicher et al., 2009	EF562286
Craugastor sp.	USNM 563039	Panama: Chiriquí	1663	One	Streicher et al., 2009	EF562284
Craugastor sp.	FMNH 257689	Panama: Chiriquí	1100	One	Streicher et al., 2009	EF562287
Craugastor sp.	USNM 563040	Panama: Chiriquí	1663	One	Streicher et al., 2009	EF562285
Craugastor sp.	MVUP 1875	Panama: Bocas del Toro	50	One	Streicher et al., 2009	EF562283
C. stejnegerianus	UCR 16332	Costa Rica: San Jose	900	One	Streicher et al., 2009	EF562325
C. underwoodi	USNM 561403	Costa Rica: Heredia	800	One and Four	Streicher et al. 2009	EF562323
C. underwoodi	UCR 16315	Costa Rica: Alajuela	960	One	Streicher et al., 2009	EF562322

*see text for explanation regarding potential misidentification

**this is a redundant *C. alfredi* sample acquired from GenBank

APPENDIX B

NUCLEAR DNA
GENBANK ACCESSION NUMBERS

Taxon	Voucher	Rag-1	Rho	Tyr
***augusti* species series**				
C. augusti	JWS 251	JX001751	JX001881	JX001932
C. augusti	JWS 253	JX001757	JX001882	JX001937
C. augusti	JWS 277	JX001755	JX001892	JX001936
C. augusti	TJD 770	JX001777	JX001869	None
C. augusti	TJD 777	JX001762	JX001866	None
C. augusti	TJD 830	JX001761	JX001865	None
C. augusti	TJD 847	JX001778	JX001867	None
C. augusti	TJD 883	None	None	None
C. augusti	UTA A-57707 (JAC 23344)	JX001764	JX001878	JX001929
C. augusti	UTA A-57708 (JAC 23347)	JX001763	JX001884	JX001924
C. augusti	JAC 23345	JX001765	JX001879	JX001931
C. augusti	JAC 23346	JX001766	JX001883	JX001930
C. augusti	JAC 23544	JX001773	JX001889	JX001928
C. augusti	JAC 23564	JX001760	JX001890	JX001933
C. augusti	UTA A-59477 (JAC 24786)	None	None	None
C. augusti	UTA A-54930 (JRM 4651)	JX001772	JX001880	JX001926
C. augusti	JWS 284	JX001753	JX001877	JX001940
C. augusti	JWS 292	JX001756	JX001876	JX001938
C. augusti	JWS 294	JX001758	JX001875	JX001944
C. augusti	JWS 295	JX001754	JX001874	JX001945
C. augusti	JWS 296	JX001759	JX001873	JX001934
C. augusti	JAC 28298	JX001779	JX001894	JX001939
C. augusti	JAC 30056	JX001752	JX001886	JX001923

Taxon	Voucher	Rag-1	Rho	Tyr
C. augusti	JAC 30105	JX001769	JX001893	JX001942
C. augusti	JAC 30106	JX001775	JX001888	JX001941
C. augusti	JAC 30107	JX001774	JX001885	JX001925
C. augusti	JAC 30108	JX001776	JX001895	JX001943
C. augusti	JMM 151	JX001767	None	JX001935
C. augusti	JMM 152	None	None	None
C. augusti	JAC 30527	JX001768	JX001887	JX001927
C. tarahumaraensis	JAC 29189	JX001771	JX001891	None
***bocourti* species series**				
C. aff. *decoratus*	JAC 22728	None	None	None
C. aff. *decoratus*	JAC 22727	None	None	None
C. aff. *decoratus*	SMR 1327	JX001799	None	None
C. aff. *decoratus*	JAC 26066	JX001797	JX001851	JX001907
C. aff. *decoratus*	JRM 4770	JX001798	JX001864	JX001906
C. alfredi	JAC 24288	JX001812	None	JX001908
C. alfredi	ENEPI 6852 (ENS 10031)	JX001805	JX001870	JX001901
C. alfredi	JAC 21987	None	JX001826	None
C. bocourti	UTA A-51220 (ENS 8060)	JX001813	JX001871	None
C. bocourti	UTA A-51245 (ENS 8245)	JX001814	JX001854	None
C. bocourti	GAR 181	JX001806	JX001824	None
C. campbelli	UTA A-55228 (ENS 7069)	JX001781	JX001856	None
C. campbelli	UTA A-53048 (JAC 20552)	JX001815	JX001853	JX001902
C. campbelli	UTA A-53034 (JAC 20572)	JX001793	JX001857	JX001909
C. campbelli	UTA A-53035 (ENS 10239)	JX001790	JX001852	JX001897

Taxon	Voucher	Rag-1	Rho	Tyr
C. cyanocthebius	TS1	JX001794	None	None
C. cyanocthebius	TS2	JX001800	None	None
C. cyanocthebius	TS3	JX001791	None	None
C. cyanocthebius	TS4	JX001780	None	None
C. nefrens	UTA A-51370 (ENS 7841)	JX001792	JX001825	JX001898
Craugastor sp.	JAC 21604	JX001795	JX001858	JX001905
Craugastor sp.	JAC 21615	JX001796	JX001833	JX001904
C. stuarti	UTA A-51371 (ENS 7940)	None	None	None
C. stuarti	UTA A-51373 (ENS 7943)	JX001810	JX001839	JX001912
C. stuarti	UTA A-51374 (ENS 7946)	None	JX001823	JX001911
C. stuarti	JAC 24518	JX001804	JX001844	JX001913
C. uno	ENEPI 6433 (ENS 9496)	GU576494	JX001860	GU576490
C. uno	ENEPI 6431 (ENS 9494)	None	JX001846	None
C. uno	UTA A-59250 (JAC 21377)	GU576496	JX001855	GU576492
C. uno	UTA A-59252 (JAC 25673)	GU576493	None	GU576488
C. xucanebi	UTA A-51361 (ENS 7804)	JX001819	JX001831	JX001915
C. xucanebi	ENS 8853	JX001788	JX001834	None
C. xucanebi	ENS 8890	JX001816	JX001872	None
C. xucanebi	ENS 8891	JX001809	JX001849	JX001922
C. xucanebi	ENS 9000	JX001817	JX001837	JX001918
C. xucanebi	ENS 8999	JX001750	JX001848	JX001921
C. xucanebi	ENS 9001	JX001821	JX001847	None

Taxon	Voucher	Rag-1	Rho	Tyr
C. xucanebi	UTA A-51363 (ENS 8100)	JX001783	JX001838	None
C. xucanebi	UTA A-51364 (ENS 8117)	JX001820	JX001840	None
C. xucanebi	MEA 3384	None	JX001842	JX001903
C. xucanebi	UTA A-51368 (JAC 19267)	JX001786	JX001841	JX001917
C. xucanebi	UTA A-51367 (JAC 19266)	JX001787	JX001845	JX001920
C. xucanebi	UTA A-51369 (JAC 19316)	JX001789	JX001832	JX001896
C. xucanebi	JAC 20480	JX001818	JX001835	JX001900
C. xucanebi	JAC 20481	JX001785	JX001828	None
C. xucanebi	JAC 20482	JX001803	JX001827	None
C. xucanebi	JAC 20483	JX001801	JX001829	JX001919
C. xucanebi	JAC 20484	JX001802	JX001830	JX001914
C. xucanebi	JAC 20485	JX001810	JX001863	None
C. xucanebi	JAC 20486	JX001784	JX001862	None
C. xucanebi	JAC 19442	JX001782	JX001861	JX001910
C. xucanebi	MEA 2024	JX001808	JX001843	None
C. xucanebi	ENS 8852	None	JX001850	JX001899

APPENDIX C

GENETIC AND MORPHOLOGICAL VARIATION IN *CRAUGASTOR UNO*

*This appendix was published as: Streicher, J.W., Meik, J.M., Smith, E.N., Campbell, J.A. 2011. Low levels of genetic diversity among morphologically distinct populations of an enigmatic montane frog from Mexico (*Craugastor uno*: Craugastoridae). Amphibia-Reptilia 32: 125–131.*

Abstract. *Craugastor uno* is a direct-developing species of frog endemic to high-elevation pine-oak forests of the Sierra Madre del Sur in Mexico. The species was described from a single female specimen collected in the state of Guerrero, but otherwise remains poorly known. It is listed as endangered by the IUCN and some authors have considered it extinct. A recent report expanding the range to include montane localities in the state of Oaxaca has been disputed. Herein we describe variation in a group of 7 *C. uno* specimens that includes males, females, and juveniles collected from 1972 to 2004. We compared individuals from localities in the states of Guerrero and Oaxaca using morphology and several genetic markers. Molecular analyses revealed *C. uno* exhibits little genetic variation at the loci sampled. Our findings suggest that while the Guerrero and Oaxaca populations have diverged only recently from one another, they appear to be presently isolated and may be distinguished by several distinct quantitative and qualitative morphological traits. Additionally, we report multilocus genetic evidence of this putative isolation in the form of regional segregation among haplotypes.

Keywords: Eleutherodactylus, Guerrero, Oaxaca, biogeography, 12S, Tyr, RAG-1

The highlands of southern Mexico that make up the Sierra Madre del Sur Pine-Oak Ecoregion are renowned for high levels of biodiversity (Olson et al., 2001; Casas-Andreu et al., 2004). Although this ecoregion is under significant pressure from human activities, new amphibian species continue to be discovered at a rather consistent rate (e.g., Meik et al., 2005, 2006; Campbell et al., 2009). However, many of these endemic species are represented by only small series of specimens, leaving many questions regarding their distributions and intraspecific variation unanswered.

Craugastor uno Savage (1984) is a poorly known species of direct-developing frog endemic to the Sierra Madre del Sur Pine-Oak Ecoregion. The species is thought to be endangered (Santos-Barrera and Canseco-Márquez, 2004) and is known from published reports of only 3 specimens (Savage, 1984; Smith and Chiszar, 2000; Hedges et al., 2008). The minimal literature discussing *C. uno* can be summarized as follows: Savage (1984) described the species based on a single specimen collected by J. A. Campbell from the Puerto del Gallo region of Guerrero in 1979. More recently, Smith and Chiszar (2000) reported a specimen from the municipality of Juquila in Oaxaca collected in 1972 by T. MacDougall. Some authors have since recognized the Oaxaca record (e.g., Casas-Andreu et al., 2004) while others disputed its validity (e.g., Santos-Barrera and Canseco-Márquez 2004). Additionally, Stuart et al. (2008) provided a conservation account for *C. uno* that mistakenly references a photograph of a frog belonging to the *C. mexicanus* species group (*sensu* Crawford and Smith, 2005). Ochoa-Ochoa et al. (2009) referred to *C. uno* as a ‘micro-endemic’ taxon and suggested that it may be extinct based on habitat loss and urban encroachment. Commentary on the phylogenetic placement of *C. uno* has been equally sparse with Lynch and Duellman (1997) assigning the species to *Craugastor* and Crawford and Smith (2005) hypothesizing a close relationship with members of the *C. alfredi* species group (including *C. bocourti*). Hedges et al. (2008) confirmed this relationship using DNA sequences derived from a topotypic specimen collected in 2002, and placed *C. uno* in their *C. bocourti* species series.

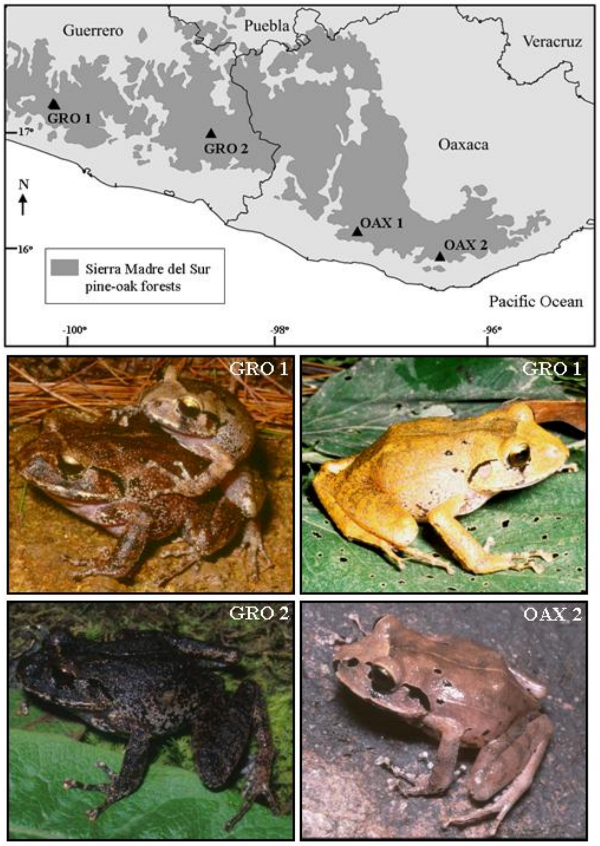

Figure C.1. Geographic origin of *Craugastor uno* specimens and in life voucher photographs. Photographs clockwise from top left: (1) an amplectant pair from near the type locality of Puerto del Gallo in Guerrero (UTA A-59557 & ENEPI 6433), (2) UTA A-59251 from Carretera Nueva Dehli-La Guitarra in Guerrero, (3) UTA A-59250 from Oaxaca, and (4) UTA A-59252 from Sierra Malinaltepec in Guerrero. Photographs by E. N. Smith.

Given (1) an endangered conservation status, (2) pressure from anthropogenic habitat modification in the Sierra Madre del Sur, and (3) the dispute regarding the validity of the Oaxacan record, a more thorough review is timely and appropriate for *C. uno*. We examined the majority of available *C. uno* specimens and report variation in 7 individuals from 3 putative populations across 5 localities (fig. 1). We compared these populations using 3 gene segments (1 mitochondrial [mtDNA], 2 nuclear [nDNA]) and several morphological characters.

The material examined for this study included 7 specimens (localities indicated in fig. 1): 3 females and 1 male from near the type-locality in Guerrero (GRO 1, including the female holotype), a female from a second Guerrero locality close to the Oaxaca border (GRO 2), a female from southwestern Oaxaca (OAX 1; Smith and Chiszar, 2000), and a juvenile male from south central Oaxaca (OAX 2). Additional voucher information, GenBank accession numbers, and locality information are listed in table 1.

We sequenced a 460 base pair (bp) segment of the mtDNA 12S ribosomal subunit gene (12S) and 573 and 493 bp fragments of the nDNA recombination activating protein 1 (RAG-1) and tyrosinase precursor (Tyr) genes, respectively. Laboratory methods for DNA isolation and PCR amplification followed those described by Streicher et al. (2009) including the use of primers 12SF and 12SR for 12S. Protocols for nDNA loci used the primer sequences Tyr1C and Tyr1G for Tyr and R182 and R270 for RAG-1 (Hedges et al., 2008), along with the touchdown thermal cycling protocols used by Streicher et al. (2009). Either AMPure magnetic beads (Agencourt®, Bioscience, Beverly, Massachusetts, USA) or ExoSap It (USB Corporation, Cleveland, Ohio, USA) were used to clean amplified fragments. Post PCR cleanup sequencing protocols were performed by SeqWright Inc. (Houston, Texas, USA; http://www.seqwright.com) or the UTA genomics core facility (Arlington, Texas, USA; http://gcf.uta.edu). For nDNA loci we assumed that a site was heterozygous if equal chromatogram peaks were present for both bases (Hare and Palumbi, 1999). Sequences were aligned using Sequencher 4.1 (GeneCodes, Ann Arbor, Michigan, USA), distance matrices were generated in PAUP* 4.0 (Swofford, 2002), and parsimony networks were created using the program TCS (Clement et al., 2000).

Morphometric analyses included the following 5 characters: snout–vent length (SVL), head length (HL), toe pad IV width (TP4), finger pad III width (FP3), and tibia length (TIB) with terminology following Duellman (2001) and Smith (2005). Data were analysed using SYSTAT 11 (Systat Software, Inc., Chicago, Illinois, USA). Gender in adult specimens was determined through dissection or the observation of amplectant behavior. The skull of UCM 56203 had been removed (post formalin preservation) and replaced with a cotton surrogate that retains the cranial skin in its natural position; thus, we estimated HL on this specimen by approximating the placement of the bones that define this measurement (i.e., from the posterior side of the quadratojugal to the premaxilla). One of the topotypic specimens (ENEPI 6433) was not available for morphological comparisons.

Table C.1. Voucher specimen and GenBank accession information for *Craugastor uno* specimens from Guerrero (GRO) and Oaxaca (OAX). An asterisk indicates sequences obtained from Hedges et al. (2008). UTA = University of Texas at Arlington, Arlington, Texas, USA; UCM = University of Colorado Museum, Boulder, Colorado, USA; ENEPI = Escuela Nacional de Estudios Profesionales, Iztacala, Universidad Nacional Autónoma de México, Mexico City, Mexico; ENS = Eric N. Smith personal field series; JAC = Jonathan A. Campbell personal field series; AMMC = Ambrose Monell Frozen Tissue Collection, American Museum of Natural History, New York, New York. USA.

Voucher	**Sex**	**State**	**Locality**	**Locality ID**	**Elevation (m)**	**GPS (WGS 84 datum)**	**12S**	**Tyr**	**RAG-1**
UTA A-7984	♀	GRO	12.9 km SW Puerto de Gallo	GRO 1	2034 [1956]	[*ca.* 17.45907 N, 100.19562 W]	None	None	None
UTA A-59251 (Field ID JAC 22214; AMMC 118080)	♀	GRO	Carretera Nueva Dehli-La Guitarra	GRO 1	2020	17.46678 N, 100.19835 W	GU576483/EU186673*	EU186769*	EU186748*
UTA A-59252 (Field ID JAC 25673)	♀	GRO	Sierra de Malinaltepec: Carretera San Luis Acatlan-Tlapa de Comonfort	GRO 2	2295	17.17068 N, 98.68065 W	GU576486	GU576487/GU576488	GU576493
UTA A-59557 (Field ID ENS 9494)	♀	GRO	Carretera Puerto El Gallo-Nueva Dehli	GRO 1	1875	17.45611 N, 100.19780 W	GU576484	None	None
ENEPI 6433 (Field ID ENS 9496)	♂	GRO	Carretera Puerto El Gallo-Nueva Dehli	GRO 1	1870	17.45611 N, 100.19780 W	GU576484	GU576489/GU576490	GU576494
UCM 52603	♀	OAX	Near Santa Rosa Lachao, Juquila	OAX 1	1550 [1857]	[*ca.* 16.23500 N, 97.13917 W]	None	None	None
UTA A-59250 (Field ID JAC 21377)	♂, juv.	OAX	Carretera San Jose Pacífico-Candelaria Loxicha, Puerta del Sol	OAX 2	1550	15.97894 N, 96.51667 W	GU576482	GU576491/GU576492	GU57649/GU576494

DNA sequence divergence levels were relatively low between *C. uno* localities sampled for this study. The highest divergence values were 1.09, 1.01, and 1.41% (uncorrected "*p*" distances) for 12S, Tyr, and Rag-1, respectively. Phylogenetic analyses were consistent in that parsimony networks did not recover shared haplotypes among collection localities, but the relationships of regional haplotypes varied by locus (fig. 2). There were 4 haplotypes recovered for the mitochondrial gene fragment (12S). Additionally, we confirmed that the publically available *C. uno* 12S sequence (EU186673; Hedges et al., 2008) derived from UTA A-59251 was identical to the 12S sequence we generated for this individual. Several individuals were heterozygous (at a single site) for the nuclear loci used in this study. This produced 4 and 7 haplotypes for the RAG-1 and Tyr regions, respectively. The specimen from the Sierra de Malinaltepic (UTA A-59252) had 4 fewer nucleotides in its RAG-1 sequence than the other specimens. These indels were excluded from our parsimony analysis of RAG-1.

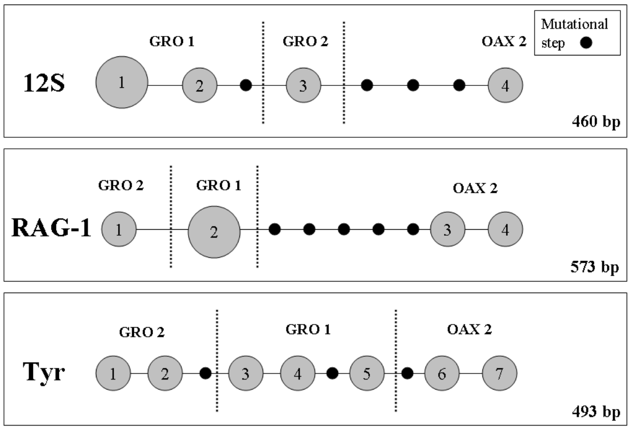

Figure C.2. Parsimony networks (95% plausible) for *Craugastor uno* mitochondrial 12S, and nuclear (Tyr and RAG-1) DNA sequences. See text for description of haplotypes by locus. Larger circles indicate that a haplotype was present in 2 individuals. Dotted line indicates separation between study localities (fig. 1; table 1).

In contrast to the low levels of genetic diversity, we found discernable morphological differences across populations when we generated ratios (to body size) for several characters (fig. 3). We also observed qualitative differences that seem to correspond to these populations. The most notable of these differences involved colour pattern and skin texture. Ground colour in life among Guerrero specimens was brown, gray, or orange, while specimens from Oaxaca were generally tan or pink (fig. 1). The orange and pink colouration appears gray in preservative. Both UCM 56203 and UTA A-59250 (OAX 1 and 2, respectively; fig. 1) possess dark blotches on the anterior and posterior sides of the external nares. This condition is absent in specimens from the type-locality (GRO 1; fig. 1) and is present only faintly on the anterior side in UTA A-59252 (GRO 2; fig. 1). Several colour characters were conserved across our sample. These included six black dorso-lateral blotches (three on each flank), a white mid-dorsal stripe, and a barred upper lip surface. With respect to skin texture, the dorsum is covered in similarly sized and uniformly distributed pustules in all topotypic specimens (GRO 1; fig. 1). In UTA A-59252 (GRO 2; fig. 1) these pustules appear to be much larger and more densely distributed, giving the skin a distinct rugosity. In contrast to the Guerrero specimens, both UCM 56203 and UTA A-59250 (OAX 1 and 2, respectively; fig. 1) possess small dorsal pustules giving the skin an almost smooth appearance.

An accurate species-level taxonomy for *Craugastor* has been hampered by extreme phenotypic polymorphisms that occur both within populations and among species (Savage and Emerson, 1970). Many traits that show extensive intrapopulation variation in other species of *Craugastor*, such as particular blotching patterns and dorsal skin texture (Savage, 2002), seem to be geographically consistent in *C. uno*. Whereas most members of the *C. bocourti* species series are slender in gestalt, *C. uno* is a robust frog that is superficially similar to barking frogs of the closely related *C. augusti* species group (Savage, 1984; Crawford and Smith, 2005). This robust gestalt was one of the few commonalities observed in available specimens. Although our sampling is limited, each population possessed unique morphological traits that have been previously important for species delimitation in the *C. bocourti* species series (Canseco-Márquez and Smith, 2004; Smith, 2005; McCranie and Smith, 2006).

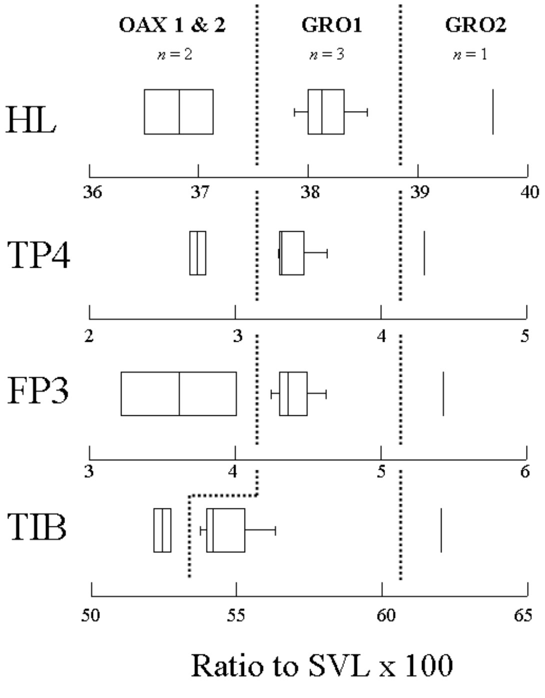

Figure C.3. Character ratios to snout–vent length (SVL) for a mixed sex group of adult and juvenile *Craugastor uno* (*n* = 6). HL = Head Length, TP4 = Toe pad IV width, FP3 = Finger pad III width, and TIB = Tibia length. Dotted line separates study localities (fig. 1; table 1).

Morphologically, there are three distinctive groups of *C. uno*: two in Guerrero (GRO1 and 2, respectively; fig. 1) and a third that includes both localities in Oaxaca (OAX 1 and 2; fig. 1). Given the small genetic distance between populations and our extremely limited sample size, for the time being we recommend that *C. uno* continue to be recognized as a single taxon. However, the morphological and genetic structure observed among populations suggests local divergence that may be indicative of recent speciation (figs. 2 and 3). Therefore, we recommend revisiting the species-level taxonomy once sample sizes are adequate to address gene flow among putative lineages.

All known specimens of *C. uno* were collected from mesic microhabitats of the Sierra Madre del Sur Pine-Oak Forest Ecoregion. These microhabitats are fragmented and restricted to higher elevations close to the Pacific Ocean. Thus, we assume the distribution of *C. uno* to be discontinuous. The minimal genetic distances among populations suggest that any localized isolation would have occurred very recently, likely no later than the Pleistocene (see Macey et al., 2001). This divergence estimate conflicts with the Miocene (or earlier) origins of the three tectono-stratigraphic terranes that dissect the Sierra Madre del Sur (Campa and Coney, 1983; Nieto-Samaniego et al., 2006). These terranes correspond with the inferred distributions of the three distinctive populations of *C. uno* and are demarcated by the Río Verde de Oaxaca drainage system (separating Oaxaca and Guerrero populations) and the Río Papagayo-Río Azul-Río Huacapa drainages (separating the Guerrero populations). An explanation for the clear association between these ancient terranes and populations that have been only recently isolated requires a vicariant event that is independent of the orogeny of the Sierra Madre del Sur. Vicariance models that invoke expansion and contraction of ecoregions, driven by recent climatic events (e.g., Savage, 2002; Guarnizo et al., 2009; Streicher et al., 2009), best explain the molecular results in context of the known geological history of this region.

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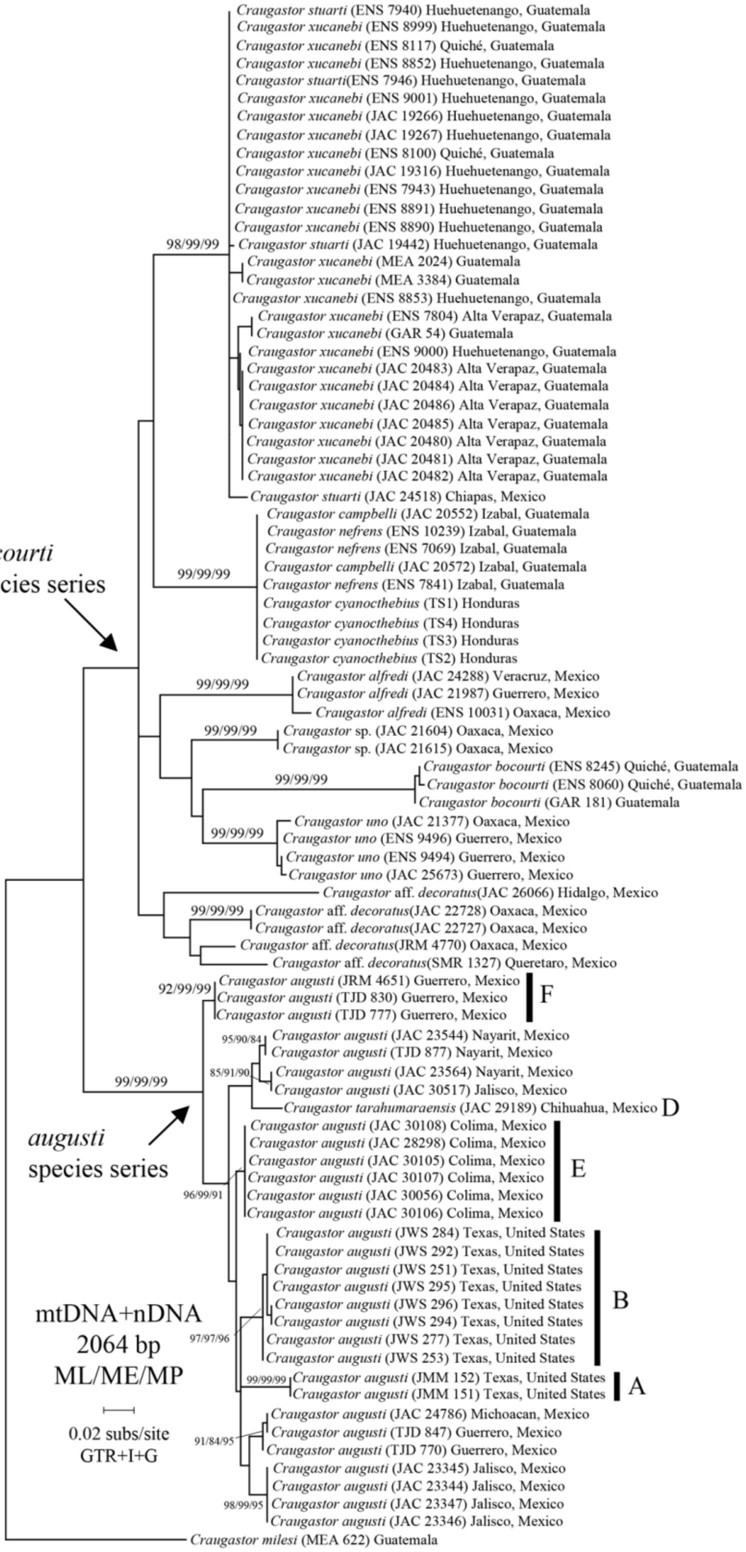
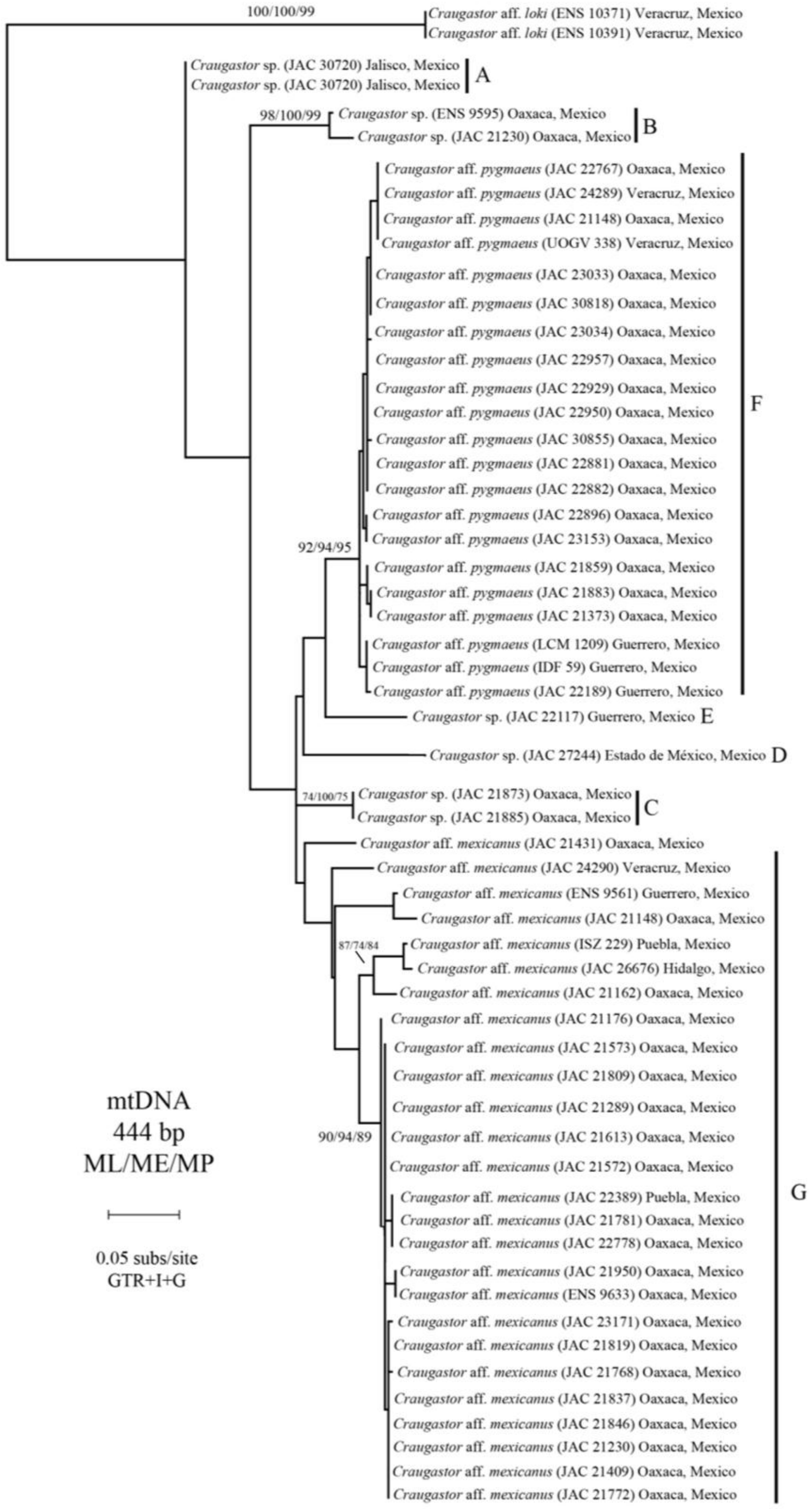
APPENDIX D

MAXIMUM LIKELIHOOD-BASED PHYLOGRAM DERIVED FROM MITOCHONDRIAL AND NUCLEAR DNA FOR MEMBERS OF THE SUBGENUS *HYLACTOPHRYNE*


Craugastor stuarti (ENS 7940) Huehuetenango, Guatemala
Craugastor xucanebi (ENS 8999) Huehuetenango, Guatemala
Craugastor xucanebi (ENS 8117) Quiché, Guatemala
Craugastor xucanebi (ENS 8852) Huehuetenango, Guatemala
Craugastor stuarti(ENS 7946) Huehuetenango, Guatemala
Craugastor xucanebi (ENS 9001) Huehuetenango, Guatemala
Craugastor xucanebi (JAC 19266) Huehuetenango, Guatemala
Craugastor xucanebi (JAC 19267) Huehuetenango, Guatemala
Craugastor xucanebi (ENS 8100) Quiché, Guatemala
Craugastor xucanebi (JAC 19316) Huehuetenango, Guatemala
Craugastor xucanebi (ENS 7943) Huehuetenango, Guatemala
Craugastor xucanebi (ENS 8891) Huehuetenango, Guatemala
Craugastor xucanebi (ENS 8890) Huehuetenango, Guatemala
98/99/99
Craugastor stuarti (JAC 19442) Huehuetenango, Guatemala
Craugastor xucanebi (MEA 2024) Guatemala
Craugastor xucanebi (MEA 3384) Guatemala
Craugastor xucanebi (ENS 8853) Huehuetenango, Guatemala
Craugastor xucanebi (ENS 7804) Alta Verapaz, Guatemala
Craugastor xucanebi (GAR 54) Guatemala
Craugastor xucanebi (ENS 9000) Huehuetenango, Guatemala
Craugastor xucanebi (JAC 20483) Alta Verapaz, Guatemala
Craugastor xucanebi (JAC 20484) Alta Verapaz, Guatemala
Craugastor xucanebi (JAC 20486) Alta Verapaz, Guatemala
Craugastor xucanebi (JAC 20485) Alta Verapaz, Guatemala
Craugastor xucanebi (JAC 20480) Alta Verapaz, Guatemala
Craugastor xucanebi (JAC 20481) Alta Verapaz, Guatemala
Craugastor xucanebi (JAC 20482) Alta Verapaz, Guatemala
Craugastor stuarti (JAC 24518) Chiapas, Mexico
Craugastor campbelli (JAC 20552) Izabal, Guatemala
Craugastor nefrens (ENS 10239) Izabal, Guatemala
bocourti
species series
Craugastor nefrens (ENS 7069) Izabal, Guatemala
Craugastor campbelli (JAC 20572) Izabal, Guatemala
99/99/99
Craugastor nefrens (ENS 7841) Izabal, Guatemala
Craugastor cyanocthebius (TS1) Honduras
Craugastor cyanocthebius (TS4) Honduras
Craugastor cyanocthebius (TS3) Honduras
Craugastor cyanocthebius (TS2) Honduras
99/99/99
Craugastor alfredi (JAC 24288) Veracruz, Mexico
Craugastor alfredi (JAC 21987) Guerrero, Mexico
Craugastor alfredi (ENS 10031) Oaxaca, Mexico
99/99/99
Craugastor sp. (JAC 21604) Oaxaca, Mexico
Craugastor sp. (JAC 21615) Oaxaca, Mexico
Craugastor bocourti (ENS 8245) Quiché, Guatemala
99/99/99
Craugastor bocourti (ENS 8060) Quiché, Guatemala
Craugastor bocourti (GAR 181) Guatemala
Craugastor uno (JAC 21377) Oaxaca, Mexico
99/99/99
Craugastor uno (ENS 9496) Guerrero, Mexico
Craugastor uno (ENS 9494) Guerrero, Mexico
Craugastor uno (JAC 25673) Guerrero, Mexico
Craugastor aff. decoratus(JAC 26066) Hidalgo, Mexico
99/99/99
Craugastor aff. decoratus(JAC 22728) Oaxaca, Mexico
Craugastor aff. decoratus(JAC 22727) Oaxaca, Mexico
Craugastor aff. decoratus(JRM 4770) Oaxaca, Mexico
Craugastor aff. decoratus(SMR 1327) Queretaro, Mexico
92/99/99
Craugastor augusti (JRM 4651) Guerrero, Mexico
Craugastor augusti (TJD 830) Guerrero, Mexico
Craugastor augusti (TJD 777) Guerrero, Mexico
F
95/90/84
Craugastor augusti (JAC 23544) Nayarit, Mexico
Craugastor augusti (TJD 877) Nayarit, Mexico
85/91/90
Craugastor augusti (JAC 23564) Nayarit, Mexico
99/99/99
Craugastor augusti (JAC 30517) Jalisco, Mexico
Craugastor tarahumaraensis (JAC 29189) Chihuahua, Mexico
D
Craugastor augusti (JAC 30108) Colima, Mexico
augusti
species series
Craugastor augusti (JAC 28298) Colima, Mexico
Craugastor augusti (JAC 30105) Colima, Mexico
Craugastor augusti (JAC 30107) Colima, Mexico
E
96/99/91
Craugastor augusti (JAC 30056) Colima, Mexico
Craugastor augusti (JAC 30106) Colima, Mexico
Craugastor augusti (JWS 284) Texas, United States
Craugastor augusti (JWS 292) Texas, United States
Craugastor augusti (JWS 251) Texas, United States
Craugastor augusti (JWS 295) Texas, United States
mtDNA+nDNA
2064 bp
ML/ME/MP
Craugastor augusti (JWS 296) Texas, United States
B
Craugastor augusti (JWS 294) Texas, United States
97/97/96
Craugastor augusti (JWS 277) Texas, United States
Craugastor augusti (JWS 253) Texas, United States
99/99/99
Craugastor augusti (JMM 152) Texas, United States
A
Craugastor augusti (JMM 151) Texas, United States
Craugastor augusti (JAC 24786) Michoacan, Mexico
0.02 subs/site
GTR+I+G
91/84/95
Craugastor augusti (TJD 847) Guerrero, Mexico
Craugastor augusti (TJD 770) Guerrero, Mexico
Craugastor augusti (JAC 23345) Jalisco, Mexico
Craugastor augusti (JAC 23344) Jalisco, Mexico
98/99/95
Craugastor augusti (JAC 23347) Jalisco, Mexico
Craugastor augusti (JAC 23346) Jalisco, Mexico
Craugastor milesi (MEA 622) Guatemala

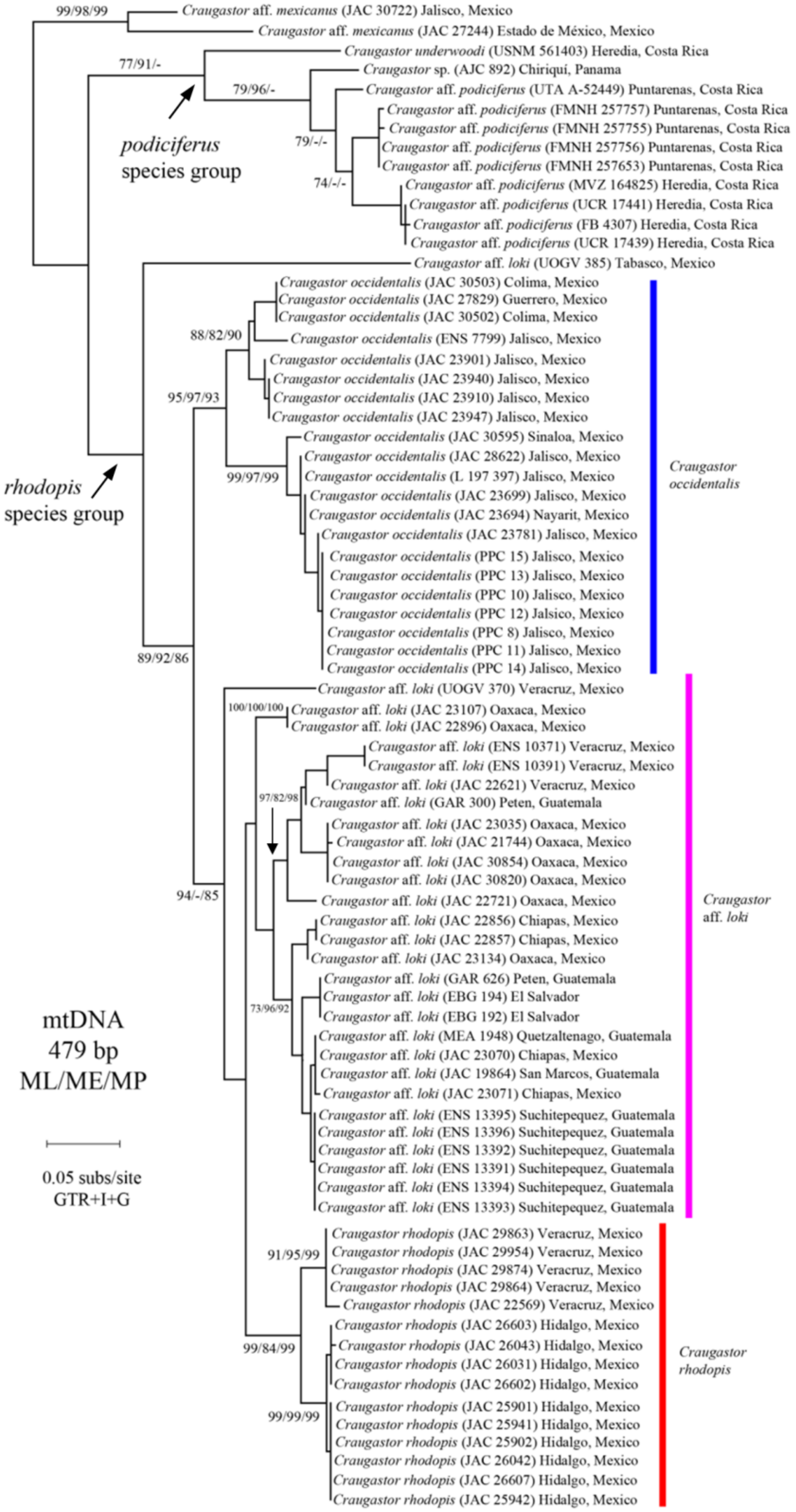
APPENDIX E

MAXIMUM LIKELIHOOD-BASED PHYLOGRAM DERIVED FROM MITOCHONDRIAL DNA FOR THE *CRAUGASTOR MEXICANUS* SPECIES SERIES


100/100/99
Craugastor aff. loki (ENS 10371) Veracruz, Mexico
Craugastor aff. loki (ENS 10391) Veracruz, Mexico
Craugastor sp. (JAC 30720) Jalisco, Mexico
Craugastor sp. (JAC 30720) Jalisco, Mexico
A
98/100/99
Craugastor sp. (ENS 9595) Oaxaca, Mexico
Craugastor sp. (JAC 21230) Oaxaca, Mexico
B
Craugastor aff. pygmaeus (JAC 22767) Oaxaca, Mexico
Craugastor aff. pygmaeus (JAC 24289) Veracruz, Mexico
Craugastor aff. pygmaeus (JAC 21148) Oaxaca, Mexico
Craugastor aff. pygmaeus (UOGV 338) Veracruz, Mexico
Craugastor aff. pygmaeus (JAC 23033) Oaxaca, Mexico
Craugastor aff. pygmaeus (JAC 30818) Oaxaca, Mexico
Craugastor aff. pygmaeus (JAC 23034) Oaxaca, Mexico
Craugastor aff. pygmaeus (JAC 22957) Oaxaca, Mexico
Craugastor aff. pygmaeus (JAC 22929) Oaxaca, Mexico
Craugastor aff. pygmaeus (JAC 22950) Oaxaca, Mexico
Craugastor aff. pygmaeus (JAC 30855) Oaxaca, Mexico
Craugastor aff. pygmaeus (JAC 22881) Oaxaca, Mexico
Craugastor aff. pygmaeus (JAC 22882) Oaxaca, Mexico
Craugastor aff. pygmaeus (JAC 22896) Oaxaca, Mexico
Craugastor aff. pygmaeus (JAC 23153) Oaxaca, Mexico
92/94/95
Craugastor aff. pygmaeus (JAC 21859) Oaxaca, Mexico
Craugastor aff. pygmaeus (JAC 21883) Oaxaca, Mexico
Craugastor aff. pygmaeus (JAC 21373) Oaxaca, Mexico
Craugastor aff. pygmaeus (LCM 1209) Guerrero, Mexico
Craugastor aff. pygmaeus (IDF 59) Guerrero, Mexico
Craugastor aff. pygmaeus (JAC 22189) Guerrero, Mexico
F
Craugastor sp. (JAC 22117) Guerrero, Mexico E
Craugastor sp. (JAC 27244) Estado de México, Mexico D
74/100/75
Craugastor sp. (JAC 21873) Oaxaca, Mexico
Craugastor sp. (JAC 21885) Oaxaca, Mexico
C
Craugastor aff. mexicanus (JAC 21431) Oaxaca, Mexico
Craugastor aff. mexicanus (JAC 24290) Veracruz, Mexico
Craugastor aff. mexicanus (ENS 9561) Guerrero, Mexico
Craugastor aff. mexicanus (JAC 21148) Oaxaca, Mexico
87/74/84
Craugastor aff. mexicanus (ISZ 229) Puebla, Mexico
Craugastor aff. mexicanus (JAC 26676) Hidalgo, Mexico
Craugastor aff. mexicanus (JAC 21162) Oaxaca, Mexico
Craugastor aff. mexicanus (JAC 21176) Oaxaca, Mexico
Craugastor aff. mexicanus (JAC 21573) Oaxaca, Mexico
Craugastor aff. mexicanus (JAC 21809) Oaxaca, Mexico
Craugastor aff. mexicanus (JAC 21289) Oaxaca, Mexico
90/94/89
Craugastor aff. mexicanus (JAC 21613) Oaxaca, Mexico
Craugastor aff. mexicanus (JAC 21572) Oaxaca, Mexico
Craugastor aff. mexicanus (JAC 22389) Puebla, Mexico
Craugastor aff. mexicanus (JAC 21781) Oaxaca, Mexico
Craugastor aff. mexicanus (JAC 22778) Oaxaca, Mexico
Craugastor aff. mexicanus (JAC 21950) Oaxaca, Mexico
Craugastor aff. mexicanus (ENS 9633) Oaxaca, Mexico
Craugastor aff. mexicanus (JAC 23171) Oaxaca, Mexico
Craugastor aff. mexicanus (JAC 21819) Oaxaca, Mexico
Craugastor aff. mexicanus (JAC 21768) Oaxaca, Mexico
Craugastor aff. mexicanus (JAC 21837) Oaxaca, Mexico
Craugastor aff. mexicanus (JAC 21846) Oaxaca, Mexico
Craugastor aff. mexicanus (JAC 21230) Oaxaca, Mexico
Craugastor aff. mexicanus (JAC 21409) Oaxaca, Mexico
Craugastor aff. mexicanus (JAC 21772) Oaxaca, Mexico
G
mtDNA
444 bp
ML/ME/MP
0.05 subs/site
GTR+I+G

APPENDIX F

MAXIMUM LIKELIHOOD-BASED PHYLOGRAM DERIVED FROM MITOCHONDRIAL DNA FOR THE *CRAUGASTOR RHODOPIS* SPECIES SERIES


99/98/99
Craugastor aff. *mexicanus* (JAC 30722) Jalisco, Mexico
Craugastor aff. *mexicanus* (JAC 27244) Estado de México, Mexico
77/91/-
Craugastor underwoodi (USNM 561403) Heredia, Costa Rica
Craugastor sp. (AJC 892) Chiriquí, Panama
79/96/-
Craugastor aff. *podiciferus* (UTA A-52449) Puntarenas, Costa Rica
Craugastor aff. *podiciferus* (FMNH 257757) Puntarenas, Costa Rica
Craugastor aff. *podiciferus* (FMNH 257755) Puntarenas, Costa Rica
79/-/-
Craugastor aff. *podiciferus* (FMNH 257756) Puntarenas, Costa Rica
podiciferus
species group
Craugastor aff. *podiciferus* (FMNH 257653) Puntarenas, Costa Rica
74/-/-
Craugastor aff. *podiciferus* (MVZ 164825) Heredia, Costa Rica
Craugastor aff. *podiciferus* (UCR 17441) Heredia, Costa Rica
Craugastor aff. *podiciferus* (FB 4307) Heredia, Costa Rica
Craugastor aff. *podiciferus* (UCR 17439) Heredia, Costa Rica
Craugastor aff. *loki* (UOGV 385) Tabasco, Mexico
Craugastor occidentalis (JAC 30503) Colima, Mexico
Craugastor occidentalis (JAC 27829) Guerrero, Mexico
Craugastor occidentalis (JAC 30502) Colima, Mexico
88/82/90
Craugastor occidentalis (ENS 7799) Jalisco, Mexico
Craugastor occidentalis (JAC 23901) Jalisco, Mexico
Craugastor occidentalis (JAC 23940) Jalisco, Mexico
95/97/93
Craugastor occidentalis (JAC 23910) Jalisco, Mexico
Craugastor occidentalis (JAC 23947) Jalisco, Mexico
Craugastor occidentalis (JAC 30595) Sinaloa, Mexico
Craugastor occidentalis (JAC 28622) Jalisco, Mexico
99/97/99
Craugastor occidentalis (L 197 397) Jalisco, Mexico
rhodopis
species group
Craugastor occidentalis (JAC 23699) Jalisco, Mexico
Craugastor occidentalis (JAC 23694) Nayarit, Mexico
Craugastor occidentalis (JAC 23781) Jalisco, Mexico
Craugastor occidentalis (PPC 15) Jalisco, Mexico
Craugastor occidentalis (PPC 13) Jalisco, Mexico
Craugastor occidentalis (PPC 10) Jalisco, Mexico
Craugastor occidentalis (PPC 12) Jalsico, Mexico
Craugastor occidentalis (PPC 8) Jalisco, Mexico
Craugastor occidentalis (PPC 11) Jalisco, Mexico
89/92/86
Craugastor occidentalis (PPC 14) Jalisco, Mexico
Craugastor
occidentalis
Craugastor aff. *loki* (UOGV 370) Veracruz, Mexico
100/100/100
Craugastor aff. *loki* (JAC 23107) Oaxaca, Mexico
Craugastor aff. *loki* (JAC 22896) Oaxaca, Mexico
Craugastor aff. *loki* (ENS 10371) Veracruz, Mexico
Craugastor aff. *loki* (ENS 10391) Veracruz, Mexico
Craugastor aff. *loki* (JAC 22621) Veracruz, Mexico
97/82/98
Craugastor aff. *loki* (GAR 300) Peten, Guatemala
Craugastor aff. *loki* (JAC 23035) Oaxaca, Mexico
Craugastor aff. *loki* (JAC 21744) Oaxaca, Mexico
Craugastor aff. *loki* (JAC 30854) Oaxaca, Mexico
Craugastor aff. *loki* (JAC 30820) Oaxaca, Mexico
94/-/85
Craugastor aff. *loki* (JAC 22721) Oaxaca, Mexico
Craugastor aff. *loki* (JAC 22856) Chiapas, Mexico
Craugastor aff. *loki* (JAC 22857) Chiapas, Mexico
Craugastor aff. *loki* (JAC 23134) Oaxaca, Mexico
Craugastor aff. *loki* (GAR 626) Peten, Guatemala
Craugastor aff. *loki* (EBG 194) El Salvador
73/96/92
Craugastor aff. *loki* (EBG 192) El Salvador
Craugastor aff. *loki* (MEA 1948) Quetzaltenago, Guatemala
Craugastor aff. *loki* (JAC 23070) Chiapas, Mexico
Craugastor aff. *loki* (JAC 19864) San Marcos, Guatemala
Craugastor aff. *loki* (JAC 23071) Chiapas, Mexico
Craugastor aff. *loki* (ENS 13395) Suchitepequez, Guatemala
Craugastor aff. *loki* (ENS 13396) Suchitepequez, Guatemala
Craugastor aff. *loki* (ENS 13392) Suchitepequez, Guatemala
Craugastor aff. *loki* (ENS 13391) Suchitepequez, Guatemala
Craugastor aff. *loki* (ENS 13394) Suchitepequez, Guatemala
Craugastor aff. *loki* (ENS 13393) Suchitepequez, Guatemala
Craugastor
aff. *loki*
mtDNA
479 bp
ML/ME/MP
0.05 subs/site
GTR+I+G
Craugastor rhodopis (JAC 29863) Veracruz, Mexico
91/95/99
Craugastor rhodopis (JAC 29954) Veracruz, Mexico
Craugastor rhodopis (JAC 29874) Veracruz, Mexico
Craugastor rhodopis (JAC 29864) Veracruz, Mexico
Craugastor rhodopis (JAC 22569) Veracruz, Mexico
Craugastor rhodopis (JAC 26603) Hidalgo, Mexico
99/84/99
Craugastor rhodopis (JAC 26043) Hidalgo, Mexico
Craugastor rhodopis (JAC 26031) Hidalgo, Mexico
Craugastor rhodopis (JAC 26602) Hidalgo, Mexico
Craugastor
rhodopis
Craugastor rhodopis (JAC 25901) Hidalgo, Mexico
99/99/99
Craugastor rhodopis (JAC 25941) Hidalgo, Mexico
Craugastor rhodopis (JAC 25902) Hidalgo, Mexico
Craugastor rhodopis (JAC 26042) Hidalgo, Mexico
Craugastor rhodopis (JAC 26607) Hidalgo, Mexico
Craugastor rhodopis (JAC 25942) Hidalgo, Mexico

APPENDIX G

CRAUGASTOR RHODOPIS SPECIES GROUP SPECIMENS EXAMINED FOR COLOR PATTERN

Field Museum of Natural History, Chicago, Illinois, USA

FMNH 70489, FMNH 70491, FMNH 70487, FMNH 70488, FMNH 70490, FMNH 70492, FMNH 70493, FMNH 70494, FMNH 20759, FMNH 113426, FMNH 125710, FMNH 123129, FMNH 124570, FMNH 123114, FMNH 125709, FMNH 20759, FMNH 123106, FMNH 123109, FMNH 113301, FMNH 190634, FMNH 123070, FMNH 123096, FMNH 111310, FMNH 123108, FMNH 94204, FMNH 113296, FMNH 20194, FMNH 123115, FMNH 123090, FMNH 108574, FMNH 123120, FMNH 125705, FMNH 20759, FMNH 125704, FMNH 123064, FMNH 123111, FMNH 123065, FMNH 125708, FMNH 123117, FMNH 126218, FMNH 94206, FMNH 124541, FMNH 123086, FMNH 126217, FMNH 94207, FMNH 125701, FMNH 110552, FMNH 125703, FMNH 125707, FMNH 124569, FMNH 124545, FMNH 20194, FMNH 20065, FMNH 70567, FMNH 70497, FMNH 70496, FMNH 70526, FMNH 70551, FMNH 70572, FMNH 70544, FMNH 70548, FMNH 70518, FMNH 70507, FMNH 70500, FMNH 70546, FMNH 70542, FMNH 70511, FMNH 70524, FMNH 70509, FMNH 70543, FMNH 70508, FMNH 70501

Illinois Natural History Survey, Champaign-Urbana, Illinois, USA

UIMNH 71423, UIMNH 14500, UIMNH 49210, UMNH 15965, UIMNH 14538, UIMNH 49212, UIMNH 49221, UMNH 49190, UIMNH 47992, UIMNH 14539, UIMNH 71424, UIMNH 71449, UIMNH 15849, UIMNH 15966, UIMNH 59855, UIMNH 49189, UIMNH 71442, UIMNH 71445, UIMNH 71430, UIMNH 15871, UIMNH 36868, UIMNH 71422, UIMNH 71448, UIMNH 49199, UIMNH 71469, UIMNH 15869, UIMNH 15935, UIMNH 49191, UIMNH 15881, UIMNH 71443, UIMNH 46314, UIMNH 14641, UIMNH 46329, UIMNH 46222, UIMNH 14622, UIMNH 46330, UIMNH 46216, UIMNH 14724, UIMNH 14619, UIMNH 14649, UIMNH 14714, UIMNH 14722, UIMNH 14600, UIMNH 14601, UIMNH 14634, UIMNH 14644, UIMNH 14636, UIMNH 14596, UIMNH 46360, UIMNH 14639, UIMNH 14710, UIMNH 46320, UIMNH 14588, UIMNH 56393, UIMNH 14673, UIMNH 56387, UIMNH 56388, UIMNH 57474, UIMNH 57359, UIMNH 57386, UIMNH 57396, UIMNH 57480, UIMNH 57370, UIMNH 57450, UIMNH 86608, UIMNH 57484, UIMNH 57481, UIMNH 57475, UIMNH 57485, UIMNH 57492, UIMNH 57487, UIMNH 57483, UIMNH 57490, UIMNH 57482

University of Kansas, Lawrence, Kansas, USA

KU 58684, KU 80898, KU 86810, KU 86804, KU 86805, KU 86788, KU 86795, KU 86809, KU 71088, KU 86796, KU 137357, KU 65988, KU 86800, KU 86806, KU 71089, KU 86811, KU 65987, KU 86813, KU 86803, KU 58673, KU 86802, KU 58682, KU 58680, KU 86807, KU 58683, KU 58674, KU 58681, KU 58678, KU 58685, KU 71087, KU 71086, KU 58675, KU 58676, KU 58679, KU 58660, KU 58645, KU 58664, KU 58641, KU 58650, KU 58665, KU 58652, KU 58655, KU 58636, KU 58633, KU 58642, KU 58646, KU 58654, KU 58667, KU 58668, KU 58658, KU 58672, KU 58663, KU 58670, KU 58649, KU 58634, KU 58662, KU 54072, KU 54073, KU 54075, KU 54079, KU 54076, KU 54070, KU 41947, KU 41944, KU 41928, KU 41946, KU 41941, KU 41953, KU 41930, KU 41945, KU 41929,KU 41949, KU 41934, KU 41955, KU 41950

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BIOGRAPHICAL INFORMATION

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