PHYLOGEOGRAPHY OF THE AMERICAN GREEN TREEFROG SPECIES GROUP

by

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ABSTRACT

PHYLOGEOGRAPHY OF THE AMERICAN GREEN TREEFROG SPECIES GROUP

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The southeastern United States is an important temperate biodiversity hotspot, housing a multitude of endemic species and multiple large rivers fragmenting their ranges. To determine if the Riverine Barriers Hypothesis explains population divergence in this region, this study utilizes of a combination of phylogeographic methods and ecological niche modeling to investigate the role of rivers in genetic dispersal. Intra-and interspecific phylogenies for the hylid treefrogs *Dryophytes cinereus, D. gratiosus* and *D. squirellus* were estimated using nuclear and mitochondrial DNA sequences to identify relationships and genetic variation within these species and test for population structure, especially with respect to potential riverine barriers. Ecological niche models were then constructed to identify potential environmental factors affecting population structure. The results of the phylogeographic studies indicate that the Mississippi River could be a barrier to gene flow within *D. cinereus*; however, two samples from the western clade appeared on the eastern side of the river, indicating a limited gene exchange or colonization. Ecological niche models revealed little ecological niche variation between the two clades of *D. cinereus* which supports the presence of a geographic barrier to gene flow in a relatively homogeneous environment. However, the individuals belonging to clades associated with opposing sides of the river suggest that the river might not serve as an absolute barrier to dispersal. Evidence of hybridization between *D. cinereus* and *D. gratiosus* based on the phylogenies indicates that the ultimate cause of the variation in *D. cinereus* might not be entirely geographic. This study provides new insight into a long-debated question regarding the presence of reproductive character displacement in *D. cinereus* versus the possibility that intraspecific variation resulted from isolation during historic climate fluctuations, and it enlightens previous studies that indicate sex-biased hybridization in this group.

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Chapter 1: Introduction to the project

1.1 Introduction

Understanding the processes underlying the origin and maintenance of biodiversity is critical for many fields within biology, including speciation and conservation biology (Amano & Sutherland, 2013). The loss of biodiversity can impact agriculture, waste management, tourism, medicine, and can have devastating effects on ecological systems (Pimentel et al., 1997; Wu & David, 2002). Currently, the threats facing biodiversity are more numerous and severe than we have resources to combat, thereby placing immense importance on understanding how biodiversity evolves to focus conservation efforts to regions in which the most results can be attained (Gaston, 2000; Myers et al., 2000).

The effects of geography and factors such as climate on biodiversity are reflected in population genetic patterns (Avise, 1994; Aivse, 2009; Hickerson et al., 2010b). Patterns of biodiversity have been well studied in tropical and temperate biodiversity hotspots (Gascon et al., 2000; Voelker et al., 2013); however, broad-scale contributors to diversity are still debated heavily (Pontarp & Wiens, 2016; Ricklefs, 2003). Numerous studies investigate how to interpret these patterns to understand how biodiversity evolved and is maintained (Queiroz at al, 2014; Wilcove et al., 1998). Continuing to study the mechanisms underlying biodiversity in both tropical and temperate hotspots (Gaston, 2000) contributes to a broader understanding of how biodiversity is generated at a time when the Earth is facing the sixth major extinction event in its history.

Since biodiversity is neither an equally distributed nor randomly occurring phenomenon, emphasis should be placed on identifying and interpreting these patterns and the underlying processes (Noss et al., 2015). Researchers are working to develop methods to conserve biodiversity based on additional insights into these patterns (Myers et al., 2000). Myers (1988) published a set of standards for identifying imperiled forest biodiversity hotspots and proposed eleven regions of particular importance. By the year 2000, revisions and expansions of his criteria resulted in the recognition of 25 terrestrial hotspots with the basis being levels of endemism (Myers et al., 2000; Myers, 2003). Continuous revision of these criteria and qualifying hotspots lead to additional increases, and by 2015, 35 regions were declared as terrestrial hotspots of concern for conservation (Marchese, 2015). Since this update, the southeastern United States has been added to this list because of its high levels of endemism in terms of plants, amphibians, reptiles, and freshwater fishes (Noss et al., 2015).

Many mechanisms for the origin and persistence of biodiversity have been proposed (Schneider et al., 1999). Classically, Alfred Russel Wallace observed that rivers serve as many range boundaries for many species and hypothesized that rivers act as barriers for species dispersal, promoting differentiation on either side (Wallace, 1852). If rivers do serve as barriers to dispersal, population genetic structure should be associated with major rivers (Link et al., 2015; Voelker et al., 2013). This is referred to as the Riverine Barriers Hypothesis, tested in many groups of animals to understand biodiversity in the South American tropics (Link et al., 2015; Moraes et al., 2016; Patton et al., 1994), in the tropical forests of Africa (Eriksson et al., 2004; Voelker et al., 2013), and in the Asian tropics (Jalil et al., 2008) using a variety of animals to understand its impacts across a range of taxa. This hypothesis has also been studied in the southeastern United States with the Apalachicola and the Mississippi Rivers using skinks (Jackson & Austin, 2010), watersnakes (Makowsky et al., 2010), ratsnakes (Burbrink et al., 2000) skunks (Barton & Wisely, 2012), fence lizards (Pounds & Jackson 1981), pocket gophers (Soto-Centeno et al., 2013), and salamanders (Herman & Bouzat, 2016). Many of these studies show these rivers to serve as important geographic barriers to gene dispersal, but other studies indicate otherwise. For example, perhaps not surprisingly, the river otter shows no population structure associated with the Mississippi River (Latch et al. 2008). Additionally, a comparative study using both the North American bullfrog and the spring peeper frog did not find evidence that the Mississippi River serves as a geographic barrier to gene flow (Austin et al., 2004). Thus, the importance of rivers as barriers to gene flow, and potential drivers of biodiversity, varies greatly in multiple lineages of organisms.

1.2 The study system

Southeastern United States

The southeastern United States is a temperate biodiversity hotspot (Hickerson et al., 2010b; Lydeard & Mayden, 1995b; Noss et al., 2015). This region supports high species richness and endemism of animals such as unionid mussels (McCullagh et al., 2002), plethodontid salamanders (Milanovich et al., 2010), freshwater fishes, snails, and turtles (Lydeard & Mayden, 1995b). It is also the location with the highest levels of temperate amphibian diversity (Hof et al., 2011). Research that focuses on determinants of species richness and endemism in the southeastern United States can provide insight into broader determinants of biodiversity (Donovan et al., 2000; Lydeard & Mayden, 1995b; Rissler & Smith, 2010).

During the late Tertiary (specifically the Miocene and Pliocene epochs), the southeastern United States experienced several important geological events including dramatic fluctuations in sea level, a renewed uplift of the Appalachian mountains following their erosion during the Mesozoic (Cleaves, 1989), and dramatic changes in river systems (Avise & Walker, 1998; Lemmon et al., 2007). More recently, in the Quaternary (the Pleistocene and Holocene epochs), this region experienced dramatic changes in climate (Lemmon et al., 2007). While many debate the effects these events have had on species diversity (Avise & Walker, 1998; Burbrink et al., 2008; Lemmon et al., 2007), many investigators agree that the southeastern United States is an important temperate biodiversity hotspot (Lemmon et al., 2007; Lydeard & Mayden, 1995a; Rissler & Smith, 2010). Intraspecific genetic studies in regions with rich geological and climatic histories can reveal the influences that these potentially vicariant factors had in determining patterns of gene flow and divergence (Lemmon et al., 2007; Rissler et al., 2006; Wiens, 2004b).

Several major geographical features proposed to be barriers promoting diversity span this hotspot. The Mississippi River Valley and the Apalachicola River represent boundaries for the distributions of many species (Conant & Collins, 1998) and could be filters for dispersal in small terrestrial vertebrates (Makowsky et al., 2010; Pauly et al., 2007). Alternatively, these rivers may not serve as barriers to dispersal, and geographic variation within lineages could represent the effects of other historic events (Austin et al., 2004; Petit et al., 2003; Zeisset & Beebee 2008).

Dryophytes cinereus species group

Amphibians are valuable organisms for study of the Riverine Barriers Hypothesis because of their dependence on water for survival (Colwell, 2000; Gascon et al., 1998; Gascon et al., 2000; Zeisset & Beebee, 2008). If the Riverine Barriers Hypothesis can be generalized to all organisms, animals dependent upon water for survival as well as animals who find large bodies of waters perilous should fit the predictions of the hypothesis (Colwell, 2000). However, if rivers are not barriers to animals with an affinity to water, alternative explanations must be sought.

Approximately 87% of known species of amphibians belong to the species rich group Anura, i.e., frogs (Duellman & Trueb, 1994), and knowledge of this group grows rapidly as new species are discovered and described (Duellman et al., 2016; Faivovich et al., 2005; Mendelson, 2011). One highly speciouse lineage of anurans is the family Hylidae with approximately 676 species (Duellman et al., 2016). Hylids are an oft-studied group for biodiversity, systematic, evolutionary, ecological, and behavioral research and prove valuable in analyzing and understanding patterns of species richness (Wiens et al., 2006, Zeisset & Beebee, 2008).

Recently, the family Hylidae underwent major taxonomic revisions (Duellman et al., 2016). Previously recognized as approximately 870 species in one family (Faivovich et al., 2005), the Hylidae are now considered 934 species in the unranked taxon Arboranae (from the Latin words for tree and frogs) containing three families, Hylidae, Phyllomedusidae, and Pelodryadidae. In this new classification, the family Hylidae contains 676 species placed in seven subfamilies and 49 genera (Duellman et al., 2016). The taxonomic revisions are based entirely on molecular data, and morphological characters were not explored for these revisions (although much of the original taxonomy was morphologically-based). The investigators reconstructed a molecular-based phylogeny using 503 taxa and 16,128 aligned sites for a total of 19 nuclear and mitochondrial genes (Duellman et al., 2016).

Nineteen recognized species of hylids occur in the southeastern United States, including eight in the genus *Dryophytes* (previously *Hyla;* Conant & Collins, 1998; Duellman et al., 2016). The *Dryophytes cinereus* species group contains three species (*Dryophytes cinereus*, *D. gratiosus*, and *D. squirellus*) that inhabit the region (Figure 1.1.; Conant & Collins, 1998; Faivovich et al., 2005). Since its original description in 1799, multiple proposed synonyms for *Dryophytes cinereus* include several accounts of this species along the east coast (Frost, 2016). One species, since synonymized under *D. cinereus*, (*Hyla semifasciata*) was described from a locality near Houston, Texas (Leidy et al., 1856). This description differentiates these Texas samples from those further east based on morphological variation, particularly the size and length of the lateral stripe extending down their body and legs. A longer white stripe (supposedly) extends the length of the body in the Texas samples in comparison to those from the eastern part of the range (Leidy et al., 1856). More recent investigations into the geographic variation of the white stripe (Aresco, 1996) did not recover statistically significant results; however, the specimens in this study did not include samples from populations west of Alabama, east of the Mississippi River. The lack of variation described within this region is unsurprising based on its limited geographic extent. Furthermore, the Aresco study did not differentiate between males and females, nor did it give approximate ages for the specimens. For these reasons, caution should be exercised when interpreting the significance of results obtained regarding snout-urostyle length (SUL) and tibio-fibula length (TFL) between the northern Alabama populations and the other four populations in southern Alabama, central Alabama, the Florida panhandle, and South Carolina. While these results might vary depending upon the age and sex of the specimens, the length of the white stripe likely would not, and Aresco found no significant differences in the length of the stripe. Currently, the eastern and western members of this species are considered to be continuous. However, studies have not addressed broad scale geographic variation in this species group.

Members of the *Dryophytes cinereus* group are small, morphologically conservative, insectivorous frogs that are primarily arboreal (Freed, 1980; Jameson & Richmond, 1971; Wygoda, 1988). They breed in small, often temporary ponds and bodies of water near permanent water sources (Garton & Brandon, 1975). This relatively young and broadly sympatric lineage spans much of the southeastern United States, with *D. cinereus* extending into Texas, including the DFW area. Collectively, the group crosses many proposed phylogeographic barriers (Figure 1.2.; Blair, 1958b; Conant & Collins, 1998; Holman, 2003; Makowsky et al., 2010; Swenson & Howard, 2005; Wiens, 2007). Documentation of hybridization between *Dryophytes gratiosus* and *D. cinereus* includes locations in Alabama, Georgia, and Mississippi (Fortman & Altig, 1974; Gerhardt, 1974b; Gunzburger, 2005; Lamb & Avise, 1986; Mecham, 1960; Schlefer et al., 1986).

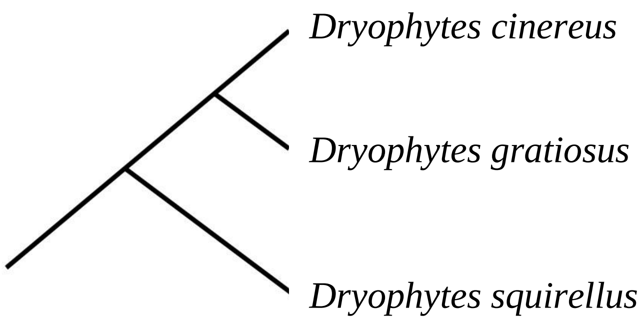

Figure 1.1. Phylogenetic relationships of the members of the *Dryophytes cinereus* species group (Faivovich et al., 2005).

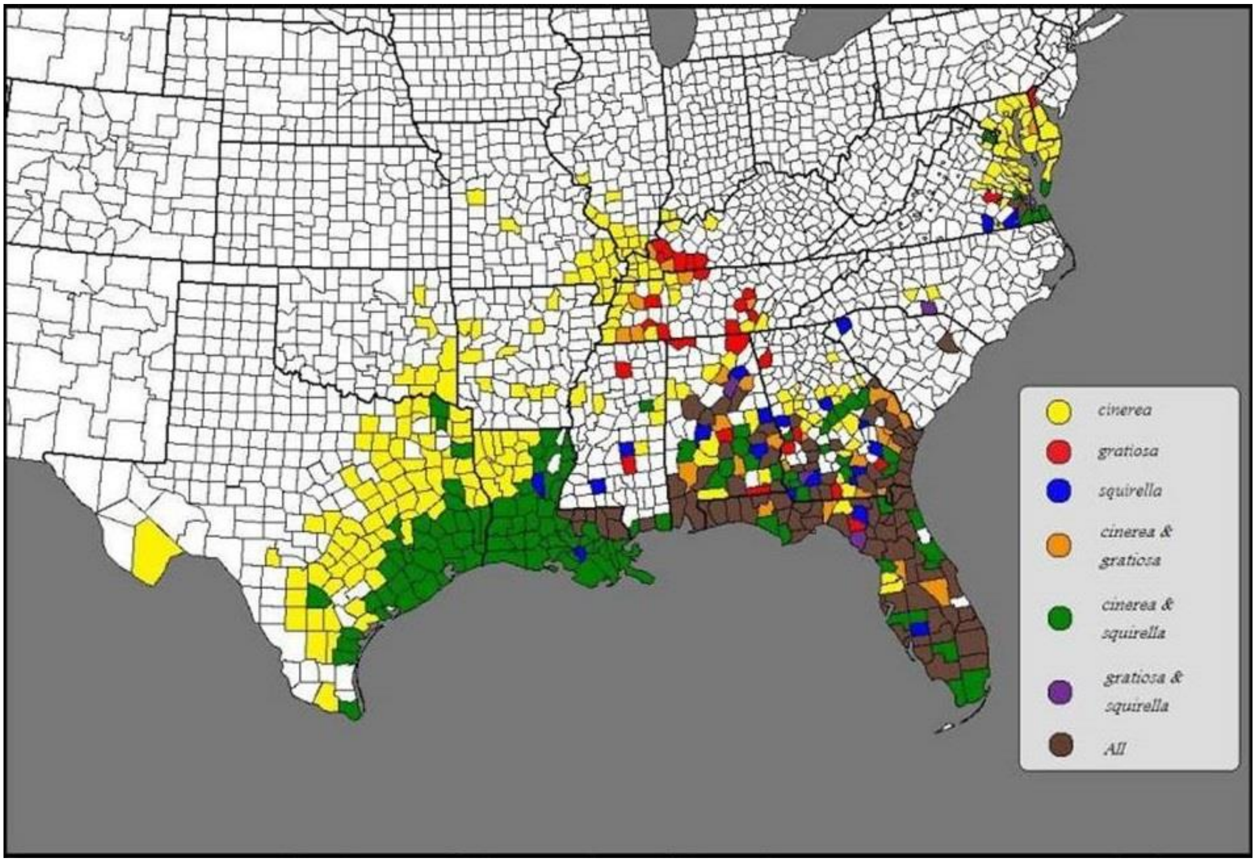

Figure 1.2. Distribution map for members of the *Dryophytes cinereus* species group. Counties shown in the map are based on museum records compiled by the National Amphibian Atlas (National Amphibian Atlas, 2014b). .

Throughout their range, members of the *Dryophytes cinereus* species are often abundant in a variety of habitats (Garton & Brandon, 1975; Gunzburger, 2006; Stebbins & Cohen, 1995; Wright, 1931). They all breed in small permanent bodies of water or in ephemeral pools, and all three species, where sympatric, often use the same locations for reproduction (Leips & Travis, 1994). *Dryophytes cinereus* is the only member of the species group and of the genus in the United States that breeds in permanent bodies of water with large predatory fishes as well (Leips et al., 2000). Their tadpoles develop in 28- 45 days depending on environmental conditions (Duellman & Trueb, 1994; Leips & Travis, 1994). All three species have a long breeding period, which begins as early as March and continues to as late as September until the weather becomes too cool (Funderburg, 1953; Gunzburger, 2006; Webb, 1965).

Females locate conspecific males for mating by following their calls (Duellman & Trueb, 1994). While the call of *D. squirellus* is notably different from the other two species in this group, *D. cinereus* and *D. gratiosus* resemble each other (Blair, 1958a). In areas of sympatry, which is most of the range of *D. gratiosus*, character displacement has been proposed regarding their mating calls and female preference, though not all of these results are significant, and results conflict between studies (Asquith et al., 1988; Höbel & Gerhardt, 2003; Oldham & Gerhardt, 1975). In the field, calls from *D. gratiosus* and *D. cinereus* are difficult to differentiate, especially when they are not calling together. At breeding sites, the location of calling males is different between the two species; males of *D. cinereus* call from vegetation along the perimeters of pools of water, and males of *D. gratiosus* call while floating in the center (Gerhardt et al., 1980). Males of *D. cinereus* attempt to mate with almost any incoming female, hypothesized to be the source of the hybridization (Gerhardt et al., 1980). Hybrids between these two species have been documented throughout their range for at least fifty years based on morphology (Gunzburger, 2005), restriction site analysis of mitochondrial DNA, and allozymes (Lamb & Avise, 1986).

In this study, I used the *Dryophytes cinereus* species group, which spans extensive distributions crossing many rivers of varying sizes, in order to investigate population genetic structure, hybridization, and the potential effects of rivers as barriers to intraspecific gene flow. I reconstructed intra- and interspecific molecular-based phylogenies using mitochondrial and nuclear DNA and investigated potential population structure associated with riverine barriers. I also generated ecological niche models to determine if ecological overlap occurred between clades identified by phylogenetic analysis. I used the resulting distributional predictions to identify whether a simple barrier (e.g., river) separated them or if other factors underlie intraspecific population structure.

Chapter 2: Phylogeography

2.1 Introduction

Phylogeography is an interdisciplinary science that has risen in popularity since the 1990s, largely as a result of increasing efficiency and cost effectiveness of generating genetic data that can be used to analyze intraspecific phylogeny, population structure, and their relationships to geographic and other environmental variables (Hickerson et al., 2010b). These methods have been used in a wide variety of studies to uncover cryptic species (Bryson Jr. et al., 2010), to estimate the strength of barriers to gene flow (Link et al., 2015), and to provide insight into speciation patterns (Avise & Walker, 1998; Avise, 2009).

In this study, I investigate the phylogeography of the *Dryophytes cinereus* species group across their range within the southeastern United States with a particular focus on the role of potential riverine barriers and other environmental variables. This has much broader implications in terms of elucidating patterns and determinants of biodiversity and genetic diversity in this region, in addition to understanding patterns of biodiversity globally.

2.2 Materials and Methods

Sampling

Specimens were collected in the field at night by identifying species by call and then following the call to the individual or by searching suitable habitats by day and collecting individuals while they were basking or hiding. Live specimens were placed in disposable plastic food containers and then in a cooler maintained at a temperature below 10°C. Specimens were maintained in these conditions until euthanized, either in the lab or in the field when necessary. Road-killed specimens were also collected and used in molecular studies if they were physically identifiable at the species level.

I collected a total of 153 specimens of *Dryophytes cinereus* and *D. squirellus* from fifty-four localities: 121 *D. cinereus* (thirty-seven localities) and thirty-two *D. squirellus* (twelve localities). I did not collect any specimens of *D. gratiosus* myself despite extensive effort, due in part to their shy nature; it is the least frequently encountered of these three species and is restricted to a smaller geographical range (Brandt, 1936). Additionally, it breeds almost entirely in temporary bodies of water, and such locations are difficult to predict and locate in the field (Blouin, 1990; Gunzburger, 2005). I heard many calling during my fieldwork, but they were on protected lands, such as state and national parks, where I was not permitted to collect.

For *Dryophytes cinereus*, I also received three samples from Jeffrey Streicher for three localities and one sample from the California Academy of Sciences from another locality. Additionally, five *D. cinereus* submissions for the genes that I used were available on GenBank, representing five more localities. For *D. squirellus*, I received four samples from the Museum of Vertebrate Zoology (University of California at Berkeley) from two localities and retrieved four sets of sequences for the genes of interest from GenBank representing four additional localities. Tissue samples for *D. gratiosus* were obtained from the California Academy of Sciences and the Museum of Vertebrate Zoology. From the California Academy of Sciences, I received seven tissues from three localities and from the Museum of Vertebrate Zoology I received seven specimens from two localities. Additionally, sequences for three individuals from two localities were available on GenBank. One of the GenBank samples for *D. gratiosus* was from the pet trade and had no locality data. I included it in my analyses to help increase the number of samples for phylogenetic analysis and assessment of intraspecific variation, but not for analysis of geographical barriers. For many of the GenBank samples, not all individuals had all the sequences I used in my analysis; therefore, I only used them for reconstruction of individual gene trees and gene-specific variation. As many samples as possible were used for each of the phylogenetic analyses.

Overall, I had 134 individual *D. cinereus* from forty-six localities, forty *D. squirellus* from eighteen localities, and 18 *D. gratiosus* from seven localities. When sampling, I focused my efforts on opposite sides of major rivers, especially the Mississippi and Apalachicola, as these are the two dominant rivers proposed as barriers to dispersal of multiple species (Makowsky et al., 2010; Swenson & Howard, 2005). All collections were done following my IACUC protocol A13.016. Permission to collect was obtained from all relevant state agencies. Specimens were deposited in the Amphibian and Reptile Diversity Research Center at the University of Texas at Arlington.

Outgroup samples to root the trees were selected using a hierarchical outgroup strategy with multiple species of hylids (*Hyla meridionalis, H. orientalis, H. arborea, Dryophytes japonicus, D. eximius, D. versicolor*) and the more distantly related *Osteopilus septentrionalis*. Sequences for *O. septentrionalis* and two of the *D. versicolor* were from samples obtained in the field and genetic material for them was collected and prepared using the same methods as for *D. cinereus, D. squirellus*, and *D. gratiosus*. The rest of the outgroup sequences were obtained from GenBank.

All samples and their localities are listed in Table 2.1, and maps of the localities are shown in Figures 2.1, 2.2, and 2.3. Samples from GenBank and their accession numbers are shown in Table 2.2.

Table 2.1. Specimens used in molecular analysis. Legend: LSUMZ = Louisiana State University Museum of Natural History (the former Museum of Zoology); MVZ = Museum of Vertebrate Zoology; SIH = S.I. Hoegg; TNHC = Texas Natural History Collection; CAS = California Academy of Sciences; JWS = Jeffrey W. Streicher; PNP = Paul N Pasichnyk; AMNHA = American Museum of Natural History – Amphibians

Sample ID	Taxon	State	County	GPS N	GPS W	Tissue or GenBank
LSUMZ 48181	*Dryophytes cinereus*	LA	Jefferson	29.690	90.100	GenBank
MVZ 145385	*Dryophytes cinereus*	TX	Travis	30.267	97.743	GenBank
SIH-06	*Dryophytes cinereus*	N/A	N/A	N/A	N/A	GenBank
TNHC 61054	*Dryophytes cinereus*	TX	Jasper	30.940	94.040	GenBank
CAS 223871	*Dryophytes cinereus*	MD	Prince Georges	38.737	76.720	Tissue
JWS 357	*Dryophytes cinereus*	TX	Smith	32.333	95.368	Tissue
JWS 474	*Dryophytes cinereus*	TX	Angelina	31.279	94.500	Tissue
JWS T007	*Dryophytes cinereus*	VA	Chesapeake	36.598	76.382	Tissue
PNP 003	*Dryophytes cinereus*	TX	Harrison	32.735	94.117	Tissue
PNP 010	*Dryophytes cinereus*	TX	Tarrant	32.793	97.106	Tissue
PNP 011	*Dryophytes cinereus*	TX	Tarrant	32.793	97.106	Tissue
PNP 014	*Dryophytes cinereus*	TX	Tarrant	32.793	97.106	Tissue
PNP 015	*Dryophytes cinereus*	TX	Grayson	33.806	96.576	Tissue
PNP 018	*Dryophytes cinereus*	OK	Choctaw	34.037	95.376	Tissue
PNP 019	*Dryophytes cinereus*	OK	Choctaw	34.037	95.376	Tissue
PNP 020	*Dryophytes cinereus*	OK	Choctaw	34.037	95.376	Tissue
PNP 021	*Dryophytes cinereus*	OK	Choctaw	34.037	95.376	Tissue
PNP 025	*Dryophytes cinereus*	TX	Liberty	30.145	94.641	Tissue
PNP 026	*Dryophytes cinereus*	TX	Liberty	30.145	94.641	Tissue
PNP 027	*Dryophytes cinereus*	TX	Liberty	30.145	94.641	Tissue
PNP 029	*Dryophytes cinereus*	TX	Liberty	30.145	96.641	Tissue
PNP 030	*Dryophytes cinereus*	TX	Matagorda	28.839	95.853	Tissue
PNP 031	*Dryophytes cinereus*	TX	Matagorda	28.839	95.853	Tissue
PNP 034	*Dryophytes cinereus*	TX	Bastrop	30.110	97.323	Tissue
PNP 035	*Dryophytes cinereus*	TX	Bastrop	30.110	97.323	Tissue

Table 2.1 - continued

PNP 047	*Dryophytes cinereus*	FL	Sarasota	27.327	82.443	Tissue
PNP 051	*Dryophytes cinereus*	FL	Sarasota	27.327	82.443	Tissue
PNP 052	*Dryophytes cinereus*	FL	Sarasota	27.327	82.443	Tissue
PNP 053	*Dryophytes cinereus*	FL	Sarasota	27.327	82.443	Tissue
PNP 054	*Dryophytes cinereus*	FL	Sarasota	27.327	82.443	Tissue
PNP 055	*Dryophytes cinereus*	FL	Sarasota	27.327	82.443	Tissue
PNP 056	*Dryophytes cinereus*	FL	Sarasota	27.327	82.443	Tissue
PNP 057	*Dryophytes cinereus*	FL	Sarasota	27.327	82.443	Tissue
PNP 058	*Dryophytes cinereus*	FL	Sarasota	27.327	82.443	Tissue
PNP 059	*Dryophytes cinereus*	FL	Sarasota	27.327	82.443	Tissue
PNP 060	*Dryophytes cinereus*	FL	Sarasota	27.327	82.443	Tissue
PNP 061	*Dryophytes cinereus*	FL	Sarasota	27.327	82.443	Tissue
PNP 063	*Dryophytes cinereus*	FL	Sarasota	27.253	82.291	Tissue
PNP 070	*Dryophytes cinereus*	FL	Okeechobee	27.269	80.830	Tissue
PNP 071	*Dryophytes cinereus*	FL	Okeechobee	27.269	80.830	Tissue
PNP 072	*Dryophytes cinereus*	FL	Okeechobee	27.269	80.830	Tissue
PNP 073	*Dryophytes cinereus*	FL	Okeechobee	27.269	80.830	Tissue
PNP 074	*Dryophytes cinereus*	FL	Okeechobee	27.269	80.830	Tissue
PNP 075	*Dryophytes cinereus*	FL	Okeechobee	27.269	80.830	Tissue
PNP 076	*Dryophytes cinereus*	FL	Okeechobee	27.269	80.830	Tissue
PNP 077	*Dryophytes cinereus*	FL	Okeechobee	27.269	80.830	Tissue
PNP 078	*Dryophytes cinereus*	FL	Okeechobee	27.269	80.830	Tissue
PNP 079	*Dryophytes cinereus*	FL	Okeechobee	27.269	80.830	Tissue
PNP 084	*Dryophytes cinereus*	FL	Lake	28.744	81.768	Tissue
PNP 085	*Dryophytes cinereus*	FL	Alachua	29.506	82.102	Tissue
PNP 088	*Dryophytes cinereus*	FL	Marion	29.385	81.968	Tissue
PNP 097	*Dryophytes cinereus*	AL	Baldwin	30.272	87.655	Tissue
PNP 098	*Dryophytes cinereus*	AL	Baldwin	30.272	87.655	Tissue

Table 2.1 – Continued

PNP 099	*Dryophytes cinereus*	AL	Baldwin	30.272	87.655	Tissue
PNP 100	*Dryophytes cinereus*	AL	Baldwin	30.272	87.655	Tissue
PNP 102	*Dryophytes cinereus*	AL	Baldwin	30.273	87.571	Tissue
PNP 103	*Dryophytes cinereus*	AL	Escamida	30.319	87.395	Tissue
PNP 104	*Dryophytes cinereus*	AL	Escamida	30.319	87.395	Tissue
PNP 105	*Dryophytes cinereus*	AL	Escamida	30.319	87.395	Tissue
PNP 106	*Dryophytes cinereus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 107	*Dryophytes cinereus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 108	*Dryophytes cinereus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 109	*Dryophytes cinereus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 110	*Dryophytes cinereus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 111	*Dryophytes cinereus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 112	*Dryophytes cinereus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 113	*Dryophytes cinereus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 114	*Dryophytes cinereus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 115	*Dryophytes cinereus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 116	*Dryophytes cinereus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 117	*Dryophytes cinereus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 118	*Dryophytes cinereus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 119	*Dryophytes cinereus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 120	*Dryophytes cinereus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 121	*Dryophytes cinereus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 122	*Dryophytes cinereus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 126	*Dryophytes cinereus*	LA	St. Martin	30.342	91.722	Tissue
PNP 127	*Dryophytes cinereus*	LA	St. Martin	30.342	91.722	Tissue
PNP 131	*Dryophytes cinereus*	LA	Tangipahoa	30.504	90.518	Tissue
PNP 132	*Dryophytes cinereus*	LA	Tangipahoa	30.504	90.518	Tissue
PNP 133	*Dryophytes cinereus*	LA	Tangipahoa	30.504	90.518	Tissue
PNP 134	*Dryophytes cinereus*	LA	Tangipahoa	30.504	90.518	Tissue
PNP 135	*Dryophytes cinereus*	LA	Tangipahoa	30.504	90.518	Tissue

Table 2.1 – Continued

PNP 136	*Dryophytes cinereus*	LA	Tangipahoa	30.504	90.518	Tissue
PNP 137	*Dryophytes cinereus*	LA	Tangipahoa	30.504	90.518	Tissue
PNP 138	*Dryophytes cinereus*	LA	Tangipahoa	30.504	90.518	Tissue
PNP 139	*Dryophytes cinereus*	LA	Tangipahoa	30.504	90.518	Tissue
PNP 140	*Dryophytes cinereus*	LA	Tangipahoa	30.504	90.518	Tissue
PNP 141	*Dryophytes cinereus*	LA	Tangipahoa	30.504	90.518	Tissue
PNP 142	*Dryophytes cinereus*	LA	Tangipahoa	30.504	90.518	Tissue
PNP 143	*Dryophytes cinereus*	LA	Tangipahoa	30.504	90.518	Tissue
PNP 144	*Dryophytes cinereus*	LA	Tangipahoa	30.504	90.518	Tissue
PNP 153	*Dryophytes cinereus*	TX	Tarrant	32.805	97.098	Tissue
PNP 154	*Dryophytes cinereus*	TX	Tarrant	32.805	97.098	Tissue
PNP 155	*Dryophytes cinereus*	TX	Tarrant	32.805	97.098	Tissue
PNP 156	*Dryophytes cinereus*	TX	Tarrant	32.805	97.098	Tissue
PNP 157	*Dryophytes cinereus*	TX	Tarrant	32.805	97.098	Tissue
PNP 158	*Dryophytes cinereus*	TX	Tarrant	32.805	97.098	Tissue
PNP 159	*Dryophytes cinereus*	TX	Tarrant	32.805	97.098	Tissue
PNP 160	*Dryophytes cinereus*	TX	Tarrant	32.805	97.098	Tissue
PNP 163	*Dryophytes cinereus*	TX	Tarrant	32.730	97.401	Tissue
PNP 164	*Dryophytes cinereus*	TX	Tarrant	32.730	97.401	Tissue
PNP 165	*Dryophytes cinereus*	TX	Tarrant	32.866	97.077	Tissue
PNP 166	*Dryophytes cinereus*	TX	Tarrant	32.866	97.079	Tissue
PNP 167	*Dryophytes cinereus*	TX	Tarrant	32.918	97.303	Tissue
PNP 168	*Dryophytes cinereus*	TX	Tarrant	32.918	97.303	Tissue
PNP 169	*Dryophytes cinereus*	TX	Tarrant	32.918	97.303	Tissue
PNP 173	*Dryophytes cinereus*	TX	Tarrant	32.918	97.303	Tissue
PNP 174	*Dryophytes cinereus*	TX	Tarrant	32.918	97.303	Tissue
PNP 175	*Dryophytes cinereus*	LA	Bossier	32.547	93.478	Tissue
PNP 176	*Dryophytes cinereus*	LA	Bossier	32.547	93.478	Tissue
PNP 177	*Dryophytes cinereus*	FL	Walton	30.484	86.280	Tissue
PNP 178	*Dryophytes cinereus*	FL	Walton	30.484	86.280	Tissue

Table 2.1 – Continued

PNP 179	*Dryophytes cinereus*	FL	Washington	30.771	85.540	Tissue
PNP 180	*Dryophytes cinereus*	FL	Washington	30.771	85.540	Tissue
PNP 182	*Dryophytes cinereus*	GA	Baker	31.253	84.527	Tissue
PNP 183	*Dryophytes cinereus*	GA	Baker	31.253	84.527	Tissue
PNP 186	*Dryophytes cinereus*	GA	Muscogee	32.546	84.884	Tissue
PNP 187	*Dryophytes cinereus*	GA	Muscogee	32.543	84.872	Tissue
PNP 188	*Dryophytes cinereus*	GA	Putnam	33.320	83.411	Tissue
PNP 189	*Dryophytes cinereus*	GA	Putnam	33.320	83.411	Tissue
PNP 190	*Dryophytes cinereus*	GA	Greene	33.421	83.263	Tissue
PNP 191	*Dryophytes cinereus*	GA	Floyd	34.269	85.172	Tissue
PNP 192	*Dryophytes cinereus*	GA	Floyd	34.272	85.189	Tissue
PNP 193	*Dryophytes cinereus*	AL	St. Clair	33.874	86.269	Tissue
PNP 197	*Dryophytes cinereus*	Miss	Newton	32.336	89.291	Tissue
PNP 198	*Dryophytes cinereus*	Miss	Warren	32.365	90.718	Tissue
PNP 199	*Dryophytes cinereus*	Miss	Warren	32.365	90.718	Tissue
AMNH A168404	*Dryophytes gratiosus*	N/A	N/A	N/A	N/A	GenBank
AMNH A181719	*Dryophytes gratiosus*	FL	Marion	29.385	81.968	GenBank
LSUMZ H15929	*Dryophytes gratiosus*	LA	St. Tammany	30.440	89.780	GenBank
CAS 169394	*Dryophytes gratiosus*	FL	Sumter	28.718	82.236	Tissue
CAS 169395	*Dryophytes gratiosus*	FL	Sumter	28.718	82.236	Tissue
CAS 169396	*Dryophytes gratiosus*	FL	Sumter	28.718	82.236	Tissue
CAS 175122	*Dryophytes gratiosus*	FL	Citrus	28.699	82.452	Tissue
CAS 175123	*Dryophytes gratiosus*	FL	Citrus	28.699	82.452	Tissue
CAS 175125	*Dryophytes gratiosus*	FL	Hernando	28.564	82.363	Tissue
CAS 175126	*Dryophytes gratiosus*	FL	Hernando	28.564	82.363	Tissue
MVZ 137619	*Dryophytes gratiosus*	GA	Jenkins	32.809	82.129	Tissue
MVZ 137620	*Dryophytes gratiosus*	GA	Jenkins	32.809	82.129	Tissue
MVZ 145405	*Dryophytes gratiosus*	GA	Jenkins	32.809	82.129	Tissue
MVZ 145406	*Dryophytes gratiosus*	GA	Jenkins	32.809	82.129	Tissue

Table 2.1 – Continued

MVZ 161962	*Dryophytes gratiosus*	NC	Wake	35.851	78.421	Tissue
MVZ 161963	*Dryophytes gratiosus*	NC	Wake	35.851	78.421	Tissue
MVZ 161964	*Dryophytes gratiosus*	NC	Wake	35.851	78.421	Tissue
AMNH A168427	*Dryophytes squirellus*	FL	Alachua	29.506	82.102	GenBank
LSUMZ 48185	*Dryophytes squirellus*	LA	Jefferson	29.690	90.100	GenBank
LSUMZ H487	*Dryophytes squirellus*	LA	East Baton Rouge	30.620	91.160	GenBank
MVZ 145422 (G)	*Dryophytes squirellus*	NC	Carteret	34.748	77.013	GenBank
MVZ 145422	*Dryophytes squirellus*	NC	Carteret	34.748	77.013	Tissue
MVZ 145423	*Dryophytes squirellus*	NC	Carteret	34.748	77.013	Tissue
MVZ 145430	*Dryophytes squirellus*	NC	Brunswick	33.921	78.021	Tissue
MVZ 145431	*Dryophytes squirellus*	NC	Brunswick	33.921	78.021	Tissue
PNP 028	*Dryophytes squirellus*	TX	Liberty	30.116	94.644	Tissue
PNP 036	*Dryophytes squirellus*	FL	Miami-Dade	25.608	80.547	Tissue
PNP 062	*Dryophytes squirellus*	FL	Sarasota	27.253	82.291	Tissue
PNP 086	*Dryophytes squirellus*	FL	Marion	29.385	81.968	Tissue
PNP 087	*Dryophytes squirellus*	FL	Marion	29.385	81.968	Tissue
PNP 089	*Dryophytes squirellus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 090	*Dryophytes squirellus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 091	*Dryophytes squirellus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 092	*Dryophytes squirellus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 093	*Dryophytes squirellus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 094	*Dryophytes squirellus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 095	*Dryophytes squirellus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 096	*Dryophytes squirellus*	LA	Calcasieu	30.290	93.192	Tissue
PNP 101	*Dryophytes squirellus*	AL	Baldwin	30.531	87.901	Tissue
PNP 123	*Dryophytes squirellus*	LA	St. Martin	30.342	91.722	Tissue
PNP 124	*Dryophytes squirellus*	LA	St. Martin	30.342	91.722	Tissue
PNP 125	*Dryophytes squirellus*	LA	St. Martin	30.342	91.722	Tissue
PNP 129	*Dryophytes squirellus*	LA	Livingston	30.382	90.648	Tissue
PNP 145	*Dryophytes squirellus*	LA	Livingston	30.478	90.749	Tissue

Table 2.1 – Continued

PNP 146	*Dryophytes squirellus*	LA	Livingston	30.478	90.749	Tissue
PNP 147	*Dryophytes squirellus*	LA	Livingston	30.478	90.749	Tissue
PNP 148	*Dryophytes squirellus*	LA	Livingston	30.478	90.749	Tissue
PNP 149	*Dryophytes squirellus*	LA	Livingston	30.478	90.749	Tissue
PNP 150	*Dryophytes squirellus*	LA	Livingston	30.478	90.749	Tissue
PNP 151	*Dryophytes squirellus*	LA	Livingston	30.478	90.749	Tissue
PNP 152	*Dryophytes squirellus*	LA	Livingston	30.478	90.749	Tissue
PNP 181	*Dryophytes squirellus*	GA	Baker	31.278	84.278	Tissue
PNP 184	*Dryophytes squirellus*	GA	Baker	31.344	84.571	Tissue
PNP 185	*Dryophytes squirellus*	GA	Baker	31.344	84.571	Tissue
PNP 194	*Dryophytes squirellus*	Miss	Newton	32.341	89.138	Tissue
PNP 195	*Dryophytes squirellus*	Miss	Newton	32.341	89.138	Tissue
PNP 196	*Dryophytes squirellus*	Miss	Newton	32.341	89.138	Tissue
PNP 040	*Osteopilus septentrionalis*	FL	Collier	26.294	81.812	Tissue
Hyla_meridionalis	*Hyla meridionalis*	N/A	N/A	N/A	N/A	GenBank
Hyla_orientalis	*Hyla orientalis*	N/A	N/A	N/A	N/A	GenBank
Hyla_arborea	*Hyla arborea*	N/A	N/A	N/A	N/A	GenBank
Dryophytes japonicus	*Dryophytes japonicus*	N/A	N/A	N/A	N/A	GenBank
Dryophytes eximius	*Dryophytes eximius*	N/A	N/A	N/A	N/A	GenBank
UMFS5545	*Dryophytes versicolor*	N/A	N/A	N/A	N/A	GenBank
PNP 023	*Dryophtes versicolor*	TX	Wise	33.061	97.799	Tissue
PNP 024	*Dryophytes versicolor*	TX	Wise	33.061	97.799	Tissue

Table 2.2. Sequences used in molecular analysis obtained from GenBank. Legend: LSUMZ = Louisiana State University Museum of Natural History (the former Museum of Zoology); MVZ = Museum of Vertebrate Zoology; SIH = S.I. Hoegg; TNHC = Texas Natural History Collection; CAS = California Academy of Sciences; JWS = Jeffrey W. Streicher; PNP = Paul N Pasichnyk; AMNHA = American Museum of Natural History – Amphibians

Sample ID	**Taxon**	**12S**	**16S**	**RAG-1**
X86238	*Dryophytes cinereus*	X86238	N/A	N/A
LSUMZ 48181	*Dryophytes cinereus*	DQ830810	DQ830810	N/A
MVZ 145385	*Dryophytes cinereus*	KF794110	KF794110	N/A
TNHC 61054	*Dryophytes cinereus*	AY680271	AY680271	N/A
SIH 06	*Dryophytes cinereus*	N/A	AY330892	AY323766
AMNH A168404	*Dryophytes gratiosus*	AY843630	AY843630	N/A
LSUMZ H15929	*Dryophytes gratiosus*	EF566966	EF566966	N/A
AMNH A181719	*Dryophytes gratiosus*	GQ374899	GQ374903	N/A
AMNH A168427	*Dryophytes squirellus*	AY843678	AY843678	N/A
LSUMZ 48185	*Dryophytes squirellus*	AY819378	AY819510	N/A
LSUMZ H487	*Dryophytes squirellus*	EF566965	EF566965	N/A
MVZ 145422	*Dryophytes squirellus*	N/A	N/A	FJ227074
Dryophytes arenicolor	*Dryophytes arenicolor*	JN830892	JN830892	N/A
Dryophytes eximius	*Dryophytes eximius*	JN830879	JN830879	GU989060
Dryophytes femoralis	*Dryophytes femoralis*	AY843627	AY843627	FJ227060
Dryophytes japonicas	*Dryophytes japonicas*	KP742620	DQ055821	FJ227068
Hyla meridionalis	*Hyla meridionalis*	FJ882756	FJ882756	FJ227085
Hyla orientalis	*Hyla orientalis*	KP742635	KP742754	FJ227102
TNHC 61055	*Dryophytes versicolor*	EF566951	EF566951	N/A
UMFS 5545	*Dryophytes versicolor*	AY843682	AY843682	N/A

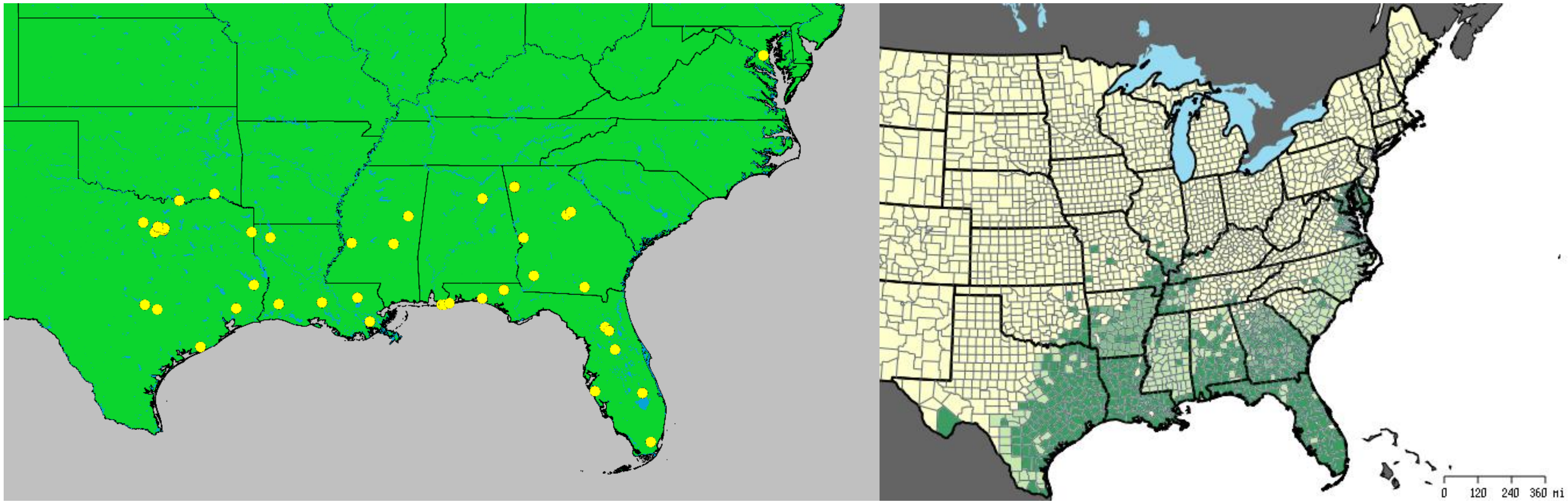

Figure 2.1. (Left) Map of the locations from which *Dryophytes cinereus* samples were obtained. (Right) Map of the range of *Dryophytes cinereus* based on museum records and published accounts. Darker green represents museum records, lighter green represents presumed presence, and cream-colored represents no known occurrence. Range map was obtained from the National Amphibian Atlas (National Amphibian Atlas, 2014b).

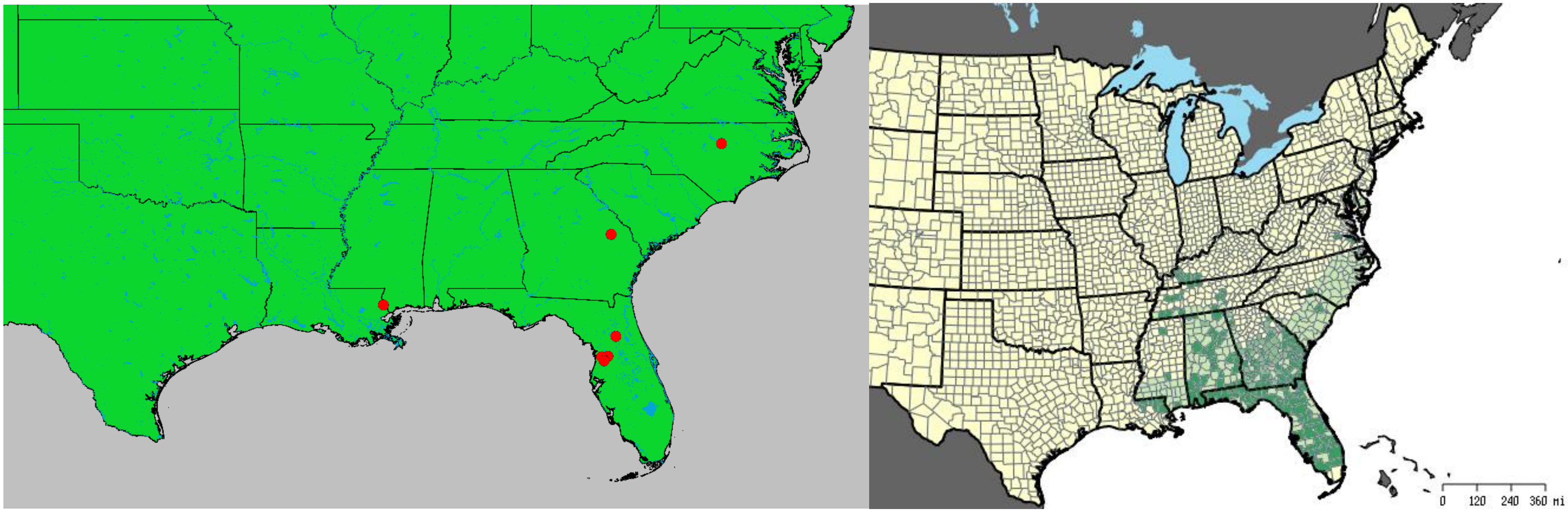

Figure 2.2. (Left) Map of the locations from which *Dryophytes gratiosus* samples were obtained. (Right) Map of the range of *Dryophytes gratiosus* based on museum records and published accounts. Darker green represents museum records, lighter green represents presumed presence, and cream-colored represents no known occurrence. Range map was obtained from the National Amphibian Atlas (National Amphibian Atlas, 2014a).

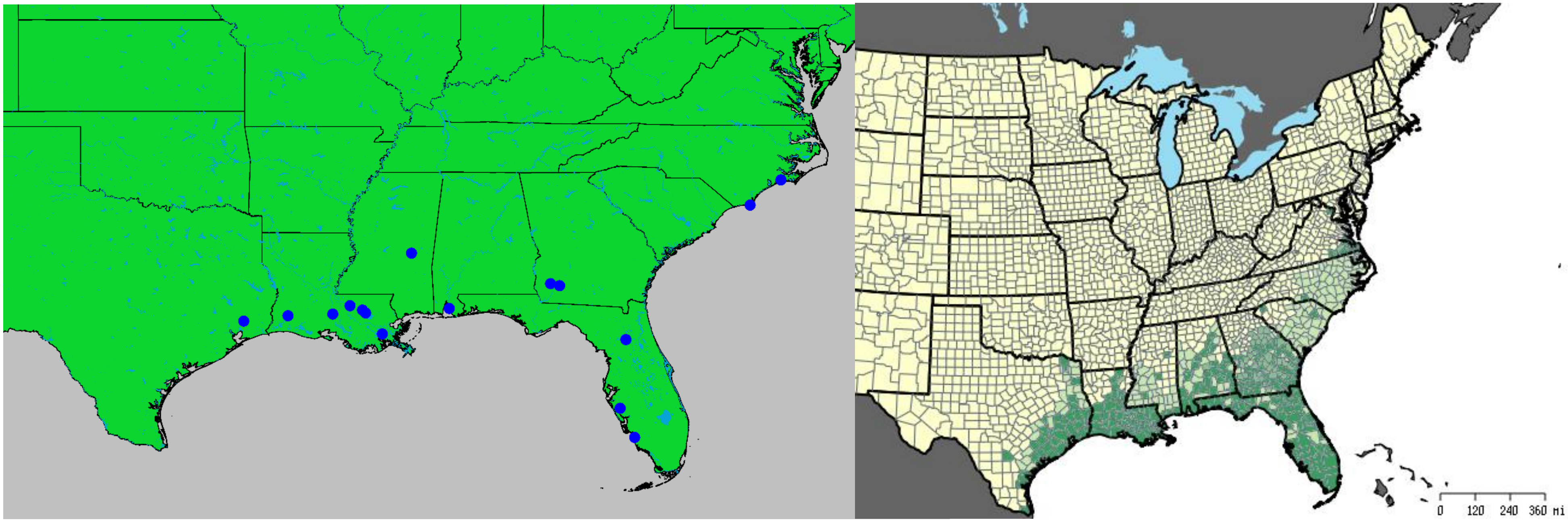

Figure 2.3. (Left) Map of the locations from which *Dryophytes squirellus* samples were obtained. (Right) Map of the range of *Dryophytes squirellus* based on museum records and published accounts. Darker green represents museum records, lighter green represents presumed presence, and cream-colored represents no known occurrence. Range map was obtained from the National Amphibian Atlas (National Amphibian Atlas, 2014c).

DNA extraction

Individuals were euthanized by placing approximately one mL of liquid Benzocaine (20%) on their head or venter. After a minimum of five minutes while no heartbeat was detected, tissue samples were collected, and the specimens were fixed in 10% formalin and subsequently maintained in 70% ethanol. Samples of either liver or thigh muscle tissue were taken from each specimen and either put into DNA lysis buffer (1M TrisBase, 0.5M NaCl, 0.5M EDTA, 10% SDS) or 100% ethanol and stored at -80° C.

Whole genomic DNA was extracted from the tissues using a Qiagen DNeasy Blood & Tissue Kit. PCR amplifications were carried out in 10µL to 20µL volumes using Promega GoTaq Flexi or Maxima Hot Start Master Mix (Tables 2.3 & 2.4). The mitochondrial data comprised fragments of 12S and 16S ribosomal RNA (rRNA), and the nuclear data represent a portion of the Recombination Activating Gene 1 (RAG-1). The primers used for these fragments were either obtained from the literature (Wiens et al., 2007; Ye et al., 2012) and in one case modified for this species group, or designed by me using NCBI's Primer-BLAST. Primer sequences are shown in Table 2.5.

Table 2.3. PCR recipe using Promega GoTaq Flexi

Chemical	% of Reaction
Water	0.385
PCR Buffer	0.200
25mM Mg	0.080
dNTPs 2mM	0.100
f-Primer 10uM	0.060
r-Primer 10uM	0.060
BSA	0.010
Promega GoTaq Flexi	0.005
DNA <0.5ug/50ul	0.100
Total	1

Table 2.4. PCR recipe using Maxima Hot Start Master Mix

Chemical	% of Reaction
Water	0.280
f-Primer 10uM	0.060
r-Primer 10uM	0.060
Maxima Hot Start Master Mix	0.500
DNA <0.5ug/50ul	0.100
Total	1.000

Table 2.5. Primers used in generating sequence data

Locus Name	Primer Name	Primer Sequence (5'-3')	Source
RAG-1	Amp-RAG-1 F	AGCTGCAGYCARTACCAYAARATGTA	Wiens et al. 2007
	Hemi-RAG-1 R	CTCTGCAGCATTTCCAATGTCAC	Wiens et al. 2007
16S	16SF-PNP3	CTGTTTAYCAAAAACATCGCC	Modified from Wiens et al. 2007
	16SR-PNP3	AACTCAGATCACGTAGGG	This study
12S	12SF-PNP3	GTTACGACTTGCCTCTTCTGT	This study
	L1091	AAACTGGGATTAGATACCCCACTAT	Wiens et al. 2007

Samples amplified for RAG-1 were subjected to an initial denaturation at 94°C for 120s, 20 cycles of denaturation at 94°C for 30s, annealing at 55°C for 30s, and extension at 72°C for 60s, 6 cycles of denaturation at 94°C for 30s, annealing at 54°C for 30s, and extension at 72°C for 60s, 6 cycles of denaturation at 94°C for 30s, annealing at 52°C for 30s, and extension at 72°C for 60s, and 20 cycles of denaturation at 94°C for 30s, annealing at 50°C for 30s, and extension at 72°C for 60s. A final extension followed this protocol at 72°C for 600s, after which the reactions were held at 4°C until they were retrieved and placed in a refrigerator for storage at approximately 4°C. Samples amplified for 12S or 16S were subjected to an initial denaturation at 95°C for 120s, 35 cycles of denaturation at 95°C, annealing at 50°C for 30s, and extension at 72°C for 280s, followed by a final extension at 72°C for 600s, after which the samples were held at 4°C until they were retrieved and placed in a refrigerator at approximately 4°C (Tables 2.6 & 2.7). All amplification reactions included a negative control to ensure no contamination occurred.

Table 2.6. Thermal cycler protocol for the fragment RAG-1

RAG-1 PCR Protocol															
Temp (°C)	94	94	55	72	94	54	72	94	52	72	94	50	72	72	4
Time (s)	120	30	30	60	30	30	60	30	30	60	30	30	60	600	∞
Cycles	1		20			6			6			20		1	

Table 2.7. Thermal cycler protocol for the fragments 12S and 16S

Mitochondrial PCR Protocol						
Temp (°C)	95	95	50	72	72	4
Time (s)	120	30	30	280	600	∞
Cycles	1		35		1	

After amplification, success of the reactions was determined qualitatively using a 1% TAE-agarose gel with ethidium bromide to visualize the DNA. After amplification, PCR reactions were cleaned using the USB ExoSAP-IT reagent by adding 0.1µL of the reagent mixed with 0.9µL of water per 10µL reaction and then heated to 37°C for 30 minutes to remove primers and dNTPs, then 80°C for 15 minutes to denature the enzymes. PCR products were then sequenced using a Big Dye Terminator v3.1 cycle sequencing kit in 10µL reactions (Tables 2.8 & 2.9). These reactions were subjected to an initial denaturation at 96°C for 180s followed by 24 cycles of a denaturation at 96°C for 10s, annealing at 50°C for 15s, and extension at 60°C for 240s, after which the samples were held at 4°C until they were retrieved. Then, the DNA was precipitated by adding 2µL 5M NaOAc (sodium acetate) with 40 µL 100% ethanol and placing them at -20°C for 20 minutes. After this, reactions were centrifuged at 1500RPM for 45 minutes. The solution was then dumped out of the reaction wells, and the sequencing plate was heated to 50°C for 2 minutes to evaporate any remaining ethanol in the reaction wells. The DNA was then rehydrated in 10 µL HiDi formamide diluted 1:1 with nuclease free water and heated to 95°C for 3 minutes and sequenced using an Applied Biosystems 3130xl Genetic Analyzer at the Genomics Core Facility at the University of Texas at Arlington.

Table 2.8. Recipe for sequencing reaction

Chemical	% of Reaction
Buffer	0.175
Water	0.575
Big Dye	0.040
Primer	0.060
DNA	0.150
Total	1.000

Table 2.9. Thermal cycler protocol for the Big Dye sequencing reaction

Big Dye Protocol					
Temp (°C)	96	96	50	60	4
Time (s)	180	10	15	240	∞
Cycles	1	24			1

Sequences were then edited and assembled using Sequencher v4.5 (Gene Codes Corp) and aligned using the ClustalW algorithm (Larkin et al., 2007) using default parameters, visually inspected, and trimmed using MEGA v6.06 (Tamura et al., 2013). Concatenation of the sequences was done manually using Microsoft Excel and Notepad. The mitochondrial dataset included 333bp of 12S rRNA and 380bp 16S rRNA, and the nuclear dataset included 677bp of RAG-1. The amount of genetic data generated in this study is comparable to that used in many other phylogeographic studies, and the sequence regions used in this study have been widely used in amphibian phylogeny and phylogeography (e.g., Gvoždík et al., 2010; Nicolas et al., 2015; Rissler & Apodaca, 2007; Wiens et al., 2007).

Genetic analysis

Several samples were removed for which sequence quality was poor before performing the following analyses. Phylogenetic analyses were performed using RaxML-HPC2 on XSEDE v8.2.8 (Stamatakis, 2014) on the CIPRES gateway (Miller et al., 2011). Maximum-likelihood (ML) analysis was conducted using 100 ML tree searches and 10,000 bootstrap replicates for node support values on the best scoring topology. This process was repeated for the mitochondrial, nuclear, and concatenated (mitochondrial and nuclear) datasets.

Bayesian analysis was performed using MrBayes on XSEDE v3.2.6 (Ronquist et al., 2012) on the CIPRES gateway (Miller et al., 2011) and the 64bit MrBayes for Windows v3.2.6 (Ronquist et al., 2012). Searches used default (flat) priors. Markov Chain Monte Carlo (MCMC) searches were conducted for 10,000,000 generations, sampling every 500 generations. Three heated chains and one cold chain were used. Stationarity was determined using TRACER v1.6 (Rambaut et al., 2014). *BEAST (Drummond et al., 2012) was then used to perform coalescent species tree analysis on the concatenated mitochondrial and nuclear dataset. The same priors and MCMC criteria were used as in the Bayesian tree searches.

For all phylogenetic analyses, the first 25% of the trees were discarded as burnin. Each analysis was conducted for the mitochondrial, nuclear, and concatenated datasets. The data were partitioned by gene (RAG-1, 12S, and 16S), gene location (mitochondria, nuclear), and the RAG-1 gene was also partitioned by codon position. The model of sequence evolution used was the GTR + I + G determined in Paup/MrModeltest 2.3 as being the top performing model (Nylander, 2004). All trees were visualized and edited using the program FigTree v1.4.2 (Rambaut, 2007).

Estimates of divergence times were performed using BEAST v 2.4.3 (Bouckaert et al., 2014). The data were partitioned by gene (RAG-1, 12S, and 16S), gene location (mitochondria, nucleus), and the RAG-1 gene was also partitioned by codon position. This analysis was performed twice using both the GTR + G and HKY models of sequence evolution. Both models were used to check for consistency between models and the possibility that the GTR model was over-parameterizing the data. GTR + I + G was not used in this analysis as it is not recommended by the developers of BEAST 2 (Bouckaert et al., 2014). The dates were calibrated using fossil records (Holman, 2003). There were three fossil records for *Dryophytes cinereus,* two from Florida and one from Kansas, all dating from 0.012 to 13.6 million years ago (mya). There were two accounts for *D. squirellus* from Kansas and Nebraska which dated from 10.3 to 13.6 mya. Four fossil accounts exist for *D. gratiosus* from Alabama, Florida, Kansas, and Nebraska which dated 0.012 to 13.6 mya. No fossil records exist for hypothesized ancestors of these species; extinct hylid frogs from this region belong to other clades (Holman, 2003). The European clade was dated with *Hyla europaea* which only has one account ranging from 23.03 to 28.4 mya (Holman, 2003). MCMC searches were conducted for 150,000,000 generations, sampling every 15,000 generations. Stationarity was determined in the program TRACER v1.6 (Rambaut et al., 2014). The target tree was then produced using TreeAnnotator provided with BEAST v2.4.3 (Bouckaert et al., 2014). The first 25% of the trees were discarded as burnin, and the tree was then visualized and edited in Figtree v1.4.2 (Rambaut, 2007).

Haplotype networks (*D. cinereus* only) were constructed using the mitochondrial and nuclear datasets with the median-joining (MJ) algorithm (Bandelt et al., 1999) using the program PopArt (Population Analysis with Reticulate Trees; Leigh & Bryant, 2015) and default settings. Each sequence was geotagged to organize the haplotype network by location. The networks were viewed and organized in PopArt.

To explore population structure and gene flow, DnaSP (Librado & Rozas, 2009) was used to calculate the number of segregating sites, number of haplotypes, nucleotide diversity (π), Watterson's estimator (θ), and the fixation index (F_{ST}) for populations identified by the phylogeographic analyses. Unphased sequences used for phylogenetic analysis was entered into DnaSP and phased before it was used to perform the calculations. The calculations were performed using default settings on the mitochondrial, nuclear, and concatenated datasets.

Pairwise uncorrected nucleotide differences were calculated for each of the sequence alignments (RAG-1, 16S, 12S) using the Maximum Composite Likelihood model (Tamura et al., 2004) in MEGA6 (Tamura et al., 2013). There were 70 RAG-1 nucleotide sequences with a total of 677 nucleotides per sequence, 117 16S nucleotide sequences with a total of 380 nucleotides per sequence, and 102 12S nucleotide sequences with a total of 333 nucleotides per sequence. These matrices were created to identify sequences that had high levels of divergence and might need to be analyzed more critically.

2.3 Results

Pairwise nucleotide differences are shown in Appendix 1 (Tables A.1.1-A.1.3). These differences were used to identify specimens that were outliers (and for which sequence quality was poor), exhibiting divergence well above realistic values. Outstanding specimens were removed from the alignments before running the analyses.

While the results based on nuclear and mitochondrial datasets separately differed, both Bayesian and ML analyses recovered similar topologies based on these loci individually and in combination (Figures 2.4 – 2.9). Additionally, the *BEAST species tree using the concatenated dataset recovered relationships among the three species consistent with those previously hypothesized with good support values (Figure 2.10). Individual gene trees can be found in

Appendix 1 (Figures A.1.1.-A.1.6). I found similar divergence time estimates for the species used in this study and the results from other studies. Divergence estimates within the species of *Dryophytes cinereus* revealed considerable amounts of time separates the eastern and western clades.

Across all analyses, *Dryophytes cinereus* grouped into two clades representing the eastern and western extents of their range. When examining the mitochondrial and nuclear datasets separately, interesting patterns emerge. In the mitochondrial tree, *D. cinereus* showed relatively little variation within the western and eastern clades, with some structure within each clade. The support values for the two clades were strong, and in Bayesian analyses, posterior probabilities (BPP) for the eastern clade and western clade were 100.0% and 88.9%, respectively. Maximum likelihood (ML) non-parametric bootstrap values for these nodes indicate similar results, and the support values for the nodes were 79.0% for the eastern clade and 76.0% for the western clade. These bootstrap values indicate good support for these clades (Hillis & Bull, 1993). Each method of analysis revealed that structure within the groups was the same.

Mitochondrial analysis within *Dryophytes gratiosus* strongly supported all identified individuals as members of one lineage (BPP = 100.0%; ML bootstrap = 100.0%). Within *D. gratiosus*, strong support separated four samples with surprising levels of divergence (but within this clade); this should be interpreted with caution (BPP = 100.0%; ML bootstrap = 92.0%). These museum tissues were collected in the 1970's, and physical examination of these specimens will be conducted once the loan request has been approved.

Dryophytes squirellus exhibited little mitochondrial variation except in the case of two sequences for samples obtained from museum loans (BPP = 100.0%; ML bootstrap = 100.0%). I obtained these loans at the same time as the *D. gratiosus* loans from the same museum. The tissues for both of these species were collected within a year of each other at different locations along the east coast in the late 1970's. All other *D. squirellus* sequences obtained were from specimens that I collected. The individuals exhibiting this pattern came from North Carolina, and while no previously identified phylogeographic barriers separated these specimens, there appears to be substantial divergence within this species.

While all of the mitochondrial analyses recover the previously hypothesized relationships among the three species, the nuclear tree did not. First, within *Dryophytes cinereus*, all of the eastern samples revealed a closer relationship to each other and to *D. gratiosus* than to western samples of *D. cinereus* (BPP = 98.2%; ML bootstrap= 65.0%). While the ML analysis did not reveal strong support for this relationship, neither analysis supported an individual *D. gratiosus* lineage (BPP = 100.0%; ML bootstrap = 100.0%).

Dryophytes squirellus did not show population structure between samples from the north or south in the nuclear dataset, as was recovered in the mitochondrial dataset. However, the samples that grouped as a more northern lineage did display more divergence in the nuclear tree than the other samples. The vouchers for these samples need to be physically inspected to ensure accurate identification, and additional samples and loci would confirm whether or not this pattern persists.

Unsurprisingly, concatenated gene trees for all specimens using Bayesian and ML methods recovered similar results to each other, as the mitochondrial and nuclear trees separately were almost identical. These trees for the concatenated dataset did indicate a division between samples from the eastern and western extents of the distributions of *Dryophytes cinereus* (eastern: BPP = 98.2%; ML bootstrap = 78.0%; western: BPP = 100.0%; ML bootstrap = 76%). However, the analysis of the concatenated dataset obscured the relationship revealed in the nuclear dataset grouping *D. gratiosus* within the eastern clade of *D. cinereus* (BPP = 100.0%; ML bootstrap = 100.0%).

Divergence time estimates (Figures 2.11-2.12) recovered similar results using the GTR and HKY models of nucleotide substitution. These analyses report that over 6 million years separate the eastern and western clades of *Dryophytes cinereus,* approximately 10 million years separate *D. gratiosus* from *D. cinereus,* and approximately 12 million years separate this sister species from *D. squirellus*. Results of analyses showing 95% highest posterior densities (HPD) are provided in Appendix 1 (Figure A.1.7.-A.1.8.).

Based on PopArt the mitochondrial and nuclear sequences separately for *Dryophytes cinereus* were sorted into five clusters (Figures 2.13-2.14; Tables 2.10-2.11). These clusters are organized by color that represent the geographic locations of the samples. In the networks, the sizes of the circles represent the proportion of samples sharing the same haplotype. Lines connecting the haplotypes indicate the sample's most closely related haplotype based on number of inferred substitutions. Between these five clusters, two major groups corresponded to the eastern and western clades found in the phylogenetic analyses. These relationships were recovered in both the nuclear and mitochondrial haplotype networks. All individual samples grouped into either the eastern and western clades, consistent with geographic location with the exception of a small group of western haplotypes that occur in a limited area on the east side of the Mississippi River (Figure 2.15).

Fixation indices based on the nuclear data suggest surprising levels of gene flow between the eastern *Dryophytes cinereus* and *D. gratiosus*. However, the concatenated mitochondrial-nuclear data set suggest a long history of isolation (F_{ST} = 0.80943), reflecting a strong influence of the mitochondrial data, as the mitochondrial data set alone did not indicate gene flow between the eastern and western clades of *D. cinereus* (F_{ST} = 0.72047). However, as in the phylogenetic analyses, the nuclear data set gave different results. No lineages show substantial evidence of gene flow except eastern *D. cinereus* and *D. gratiosus* (F_{ST} = 0.15558). This reveals that gene flow between eastern *D. cinereus* and *D. gratiosus* likely is greater than between what is currently considered the same species (*D. cinereus*) on opposite sides of the Mississippi River. The distributions of the eastern clade of *D. cinereus* and *D. gratiosus* overlap for the entirety of the range of *D. gratiosus*.

Indices of DNA polymorphism (based on analyses using DnaSP) are shown in Table 2.15. Polymorphism indices show low levels of nucleotide diversity across all groups in the nuclear dataset, as expected for a conserved gene like RAG-1. However, *Dryophytes gratiosus* and the eastern clade of *D. cinereus* display higher levels of nucleotide diversity for this locus (π = 0.00556; π = 0. 00878, respectively) compared to the western clade of *D. cinereus* or *D. squirellus* (π = 0.00051; π = 0.00295, respectively). The mitochondrial data set does not reveal this pattern for *D. gratiosus* and the eastern clade of *D. cinereus* (π = 0.01582; π = 0.00430, respectively) compared to *D. squirellus* or the western clade of *D. cinereus* (π = 0.01394; π = 0.00232, respectively). Furthermore, few segregating sites exist for RAG-1 except in the eastern clade of *D. cinereus*. Calculations reveal 27 segregating sites in this group compared to three found in the western clade.

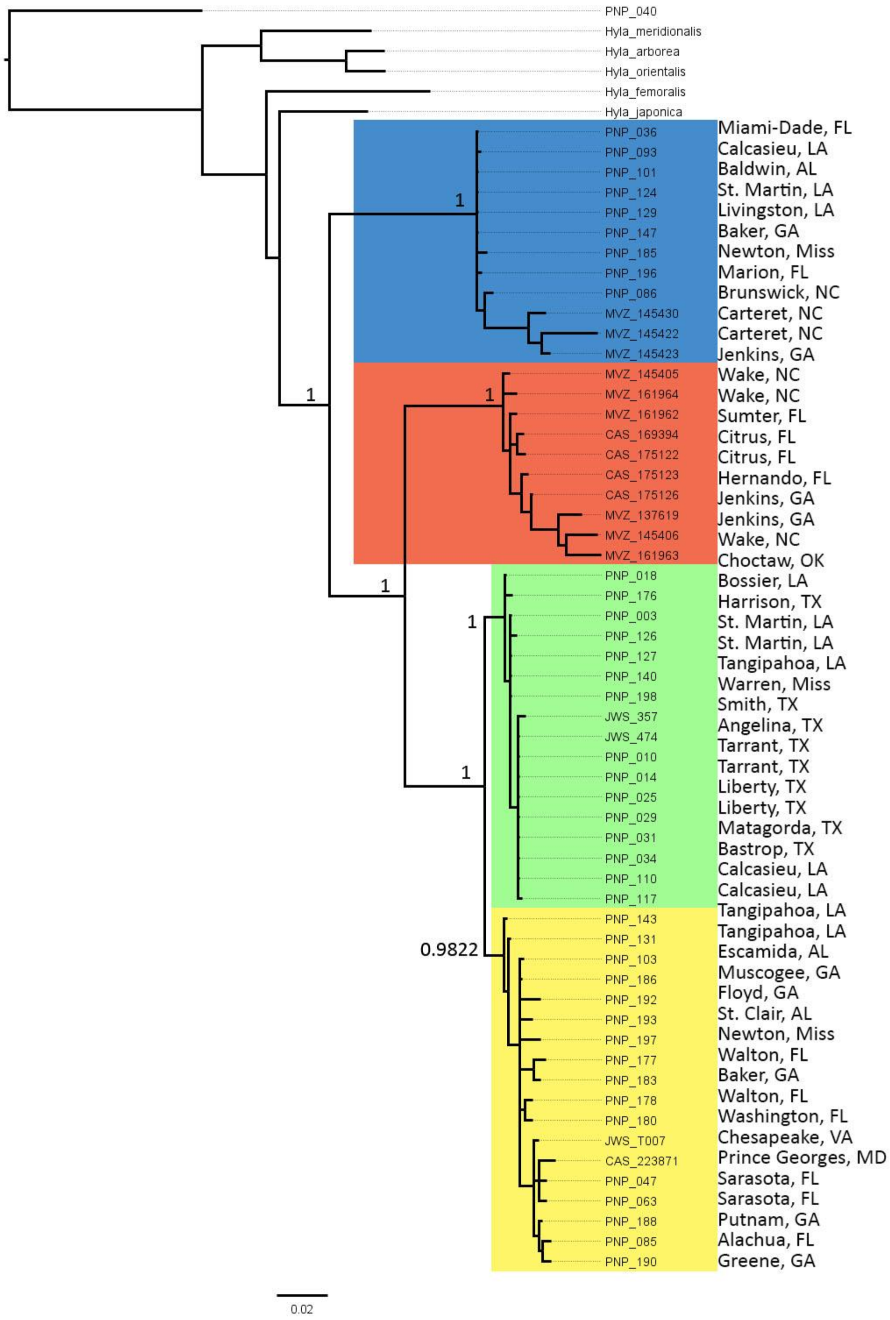

Figure 2.4. Bayesian phylogenetic tree of the *Dryophytes cinereus* species group. Data for this analysis include all loci sampled (mitochondrial and nuclear). Values on the branches represent Bayesian posterior probabilities. Specimens highlighted in blue represent members of *D. squirellus*, specimens in red represent *D. gratiosus*, *D. cinereus* is divided into two clades, the western clade in green and the eastern clade in yellow.

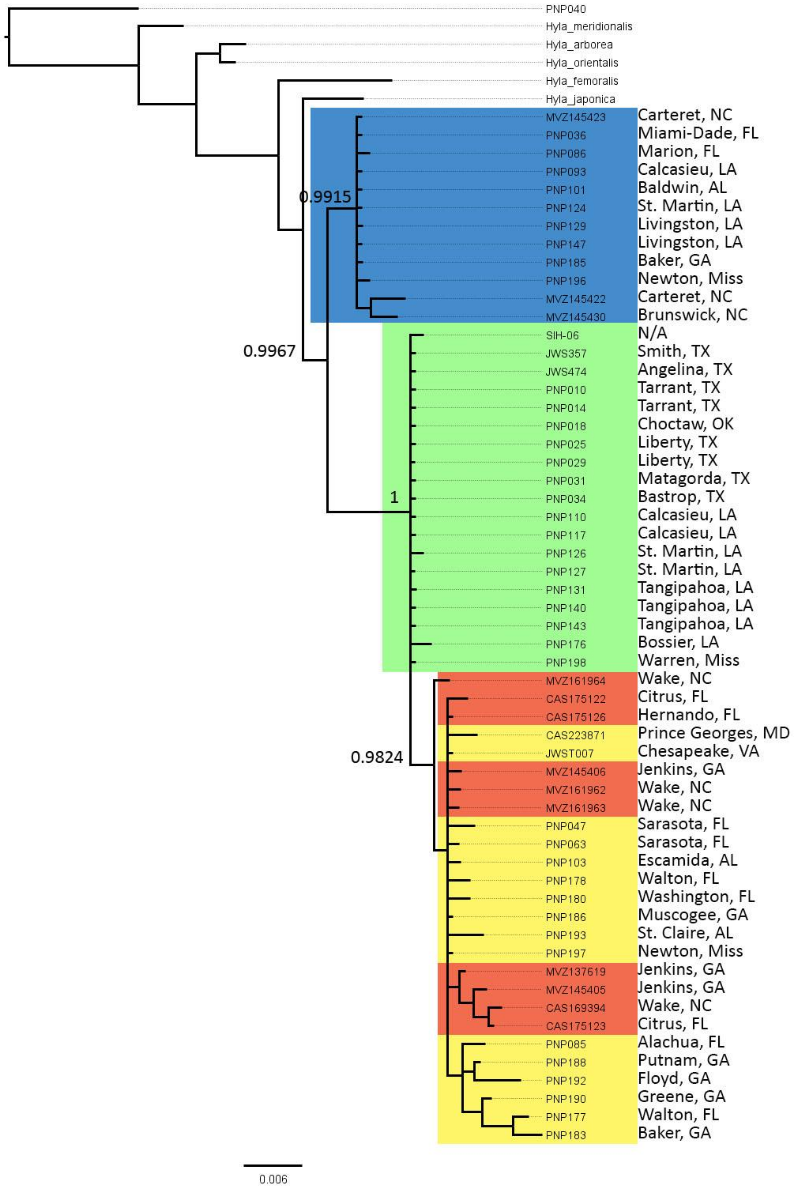

Figure 2.5. Bayesian phylogenetic tree of the *Dryophytes cinereus* species group. Data for this analysis include only RAG-1 (nuclear). Values on the nodes represent Bayesian posterior probabilities. Specimens highlighted in blue represent members of *D. squirellus*, specimens in red bars are from *D. gratiosus*, specimens in yellow bars are from the eastern clade of *D. cinereus*, and specimens from the western clade of *D. cinereus* are in green.

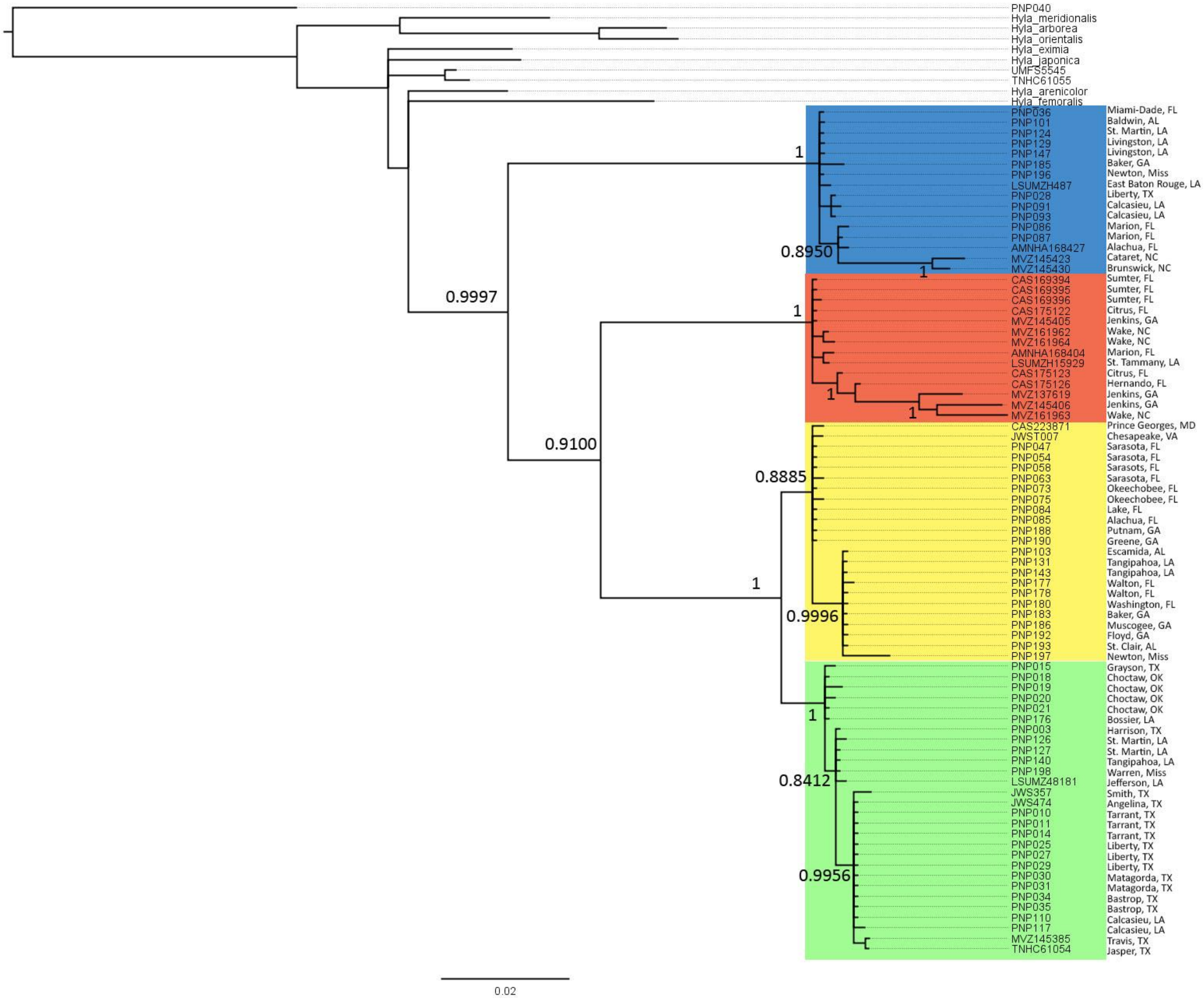

Figure 2.6. Bayesian phylogenetic tree of the *Dryophytes cinereus* species group. Data for this analysis include all mitochondrial loci sampled (12S and 16S). Values on the nodes represent Bayesian posterior probabilities. Specimens highlighted in blue represent members of *D. squirellus*, specimens in red represent *D. gratiosus*, and specimens from *D. cinereus* are divided into two clades, the eastern clade in yellow and the western clade in green.

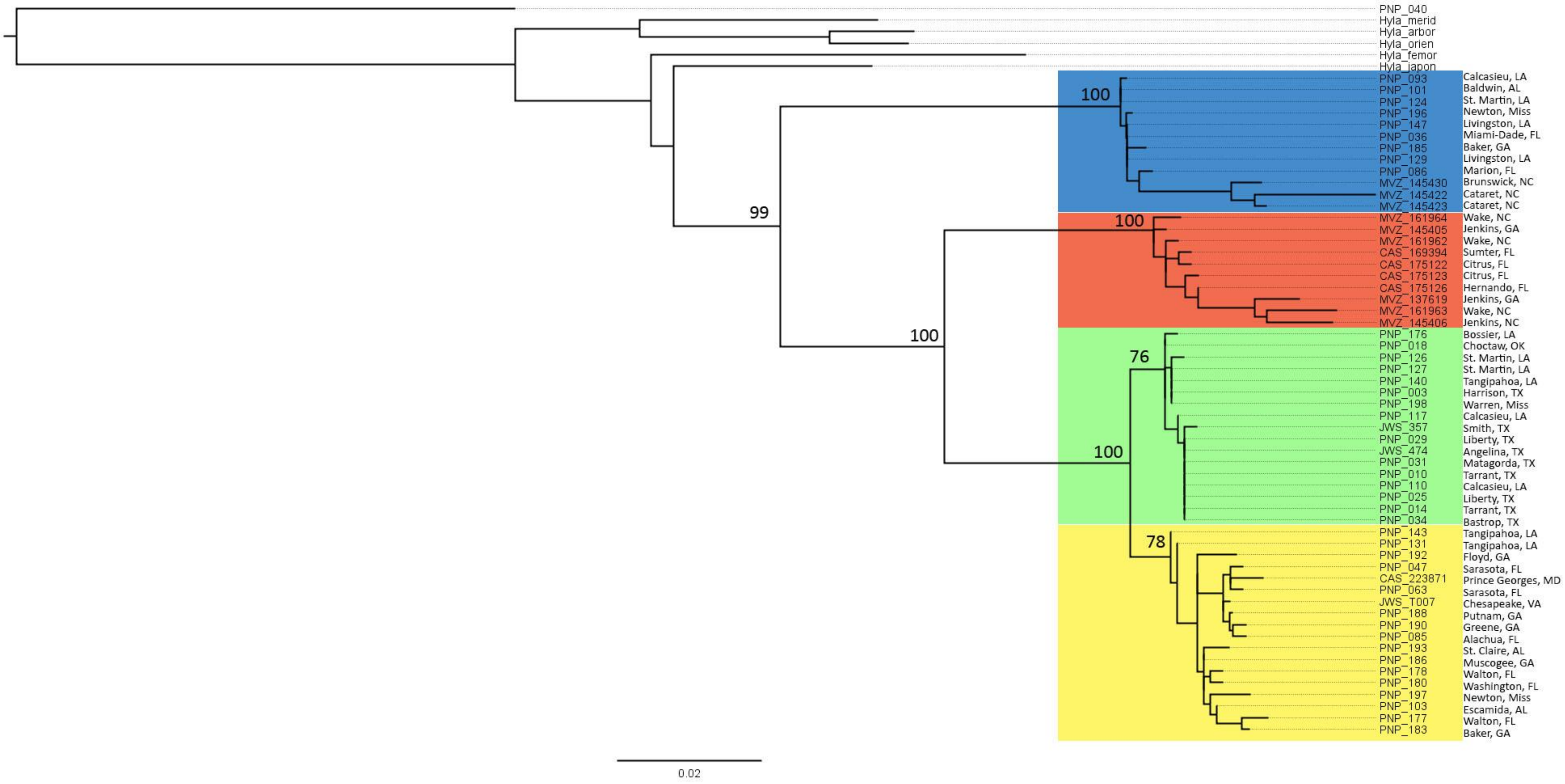

Figure 2.7. Maximum likelihood phylogenetic tree of the *Dryophytes cinereus* species group. Data for this analysis include all loci sampled (mitochondrial and nuclear). Values on the branches represent maximum likelihood nonparametric bootstrap percentages. Specimens highlighted in blue represent members of *D. squirellus*, specimens in red represent *D. gratiosus*, *D. cinereus* is divided into two clades, the western clade in green and the eastern clade in yellow.

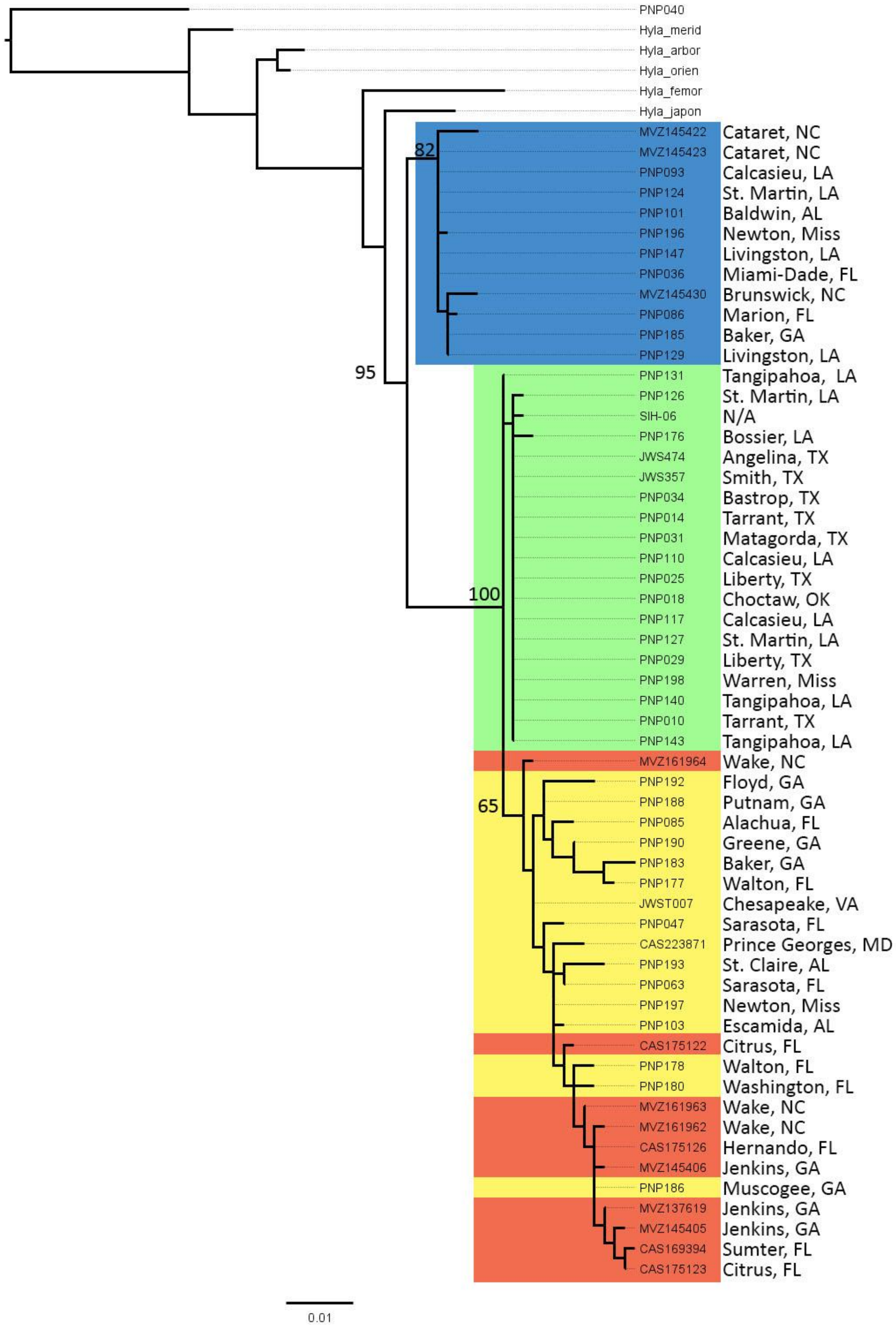

Figure 2.8. Maximum likelihood phylogenetic tree of the *Dryophytes cinereus* species group. Data for this analysis include only RAG-1 (nuclear). Values on the nodes represent maximum likelihood nonparametric bootstrap percentages. Specimens highlighted in blue represent members of *D. squirellus*, specimens in red bars are from *D. gratiosus*, specimens in yellow bars are from the eastern clade of *D. cinereus*, and specimens from the western clade of *D. cinereus* are in green.

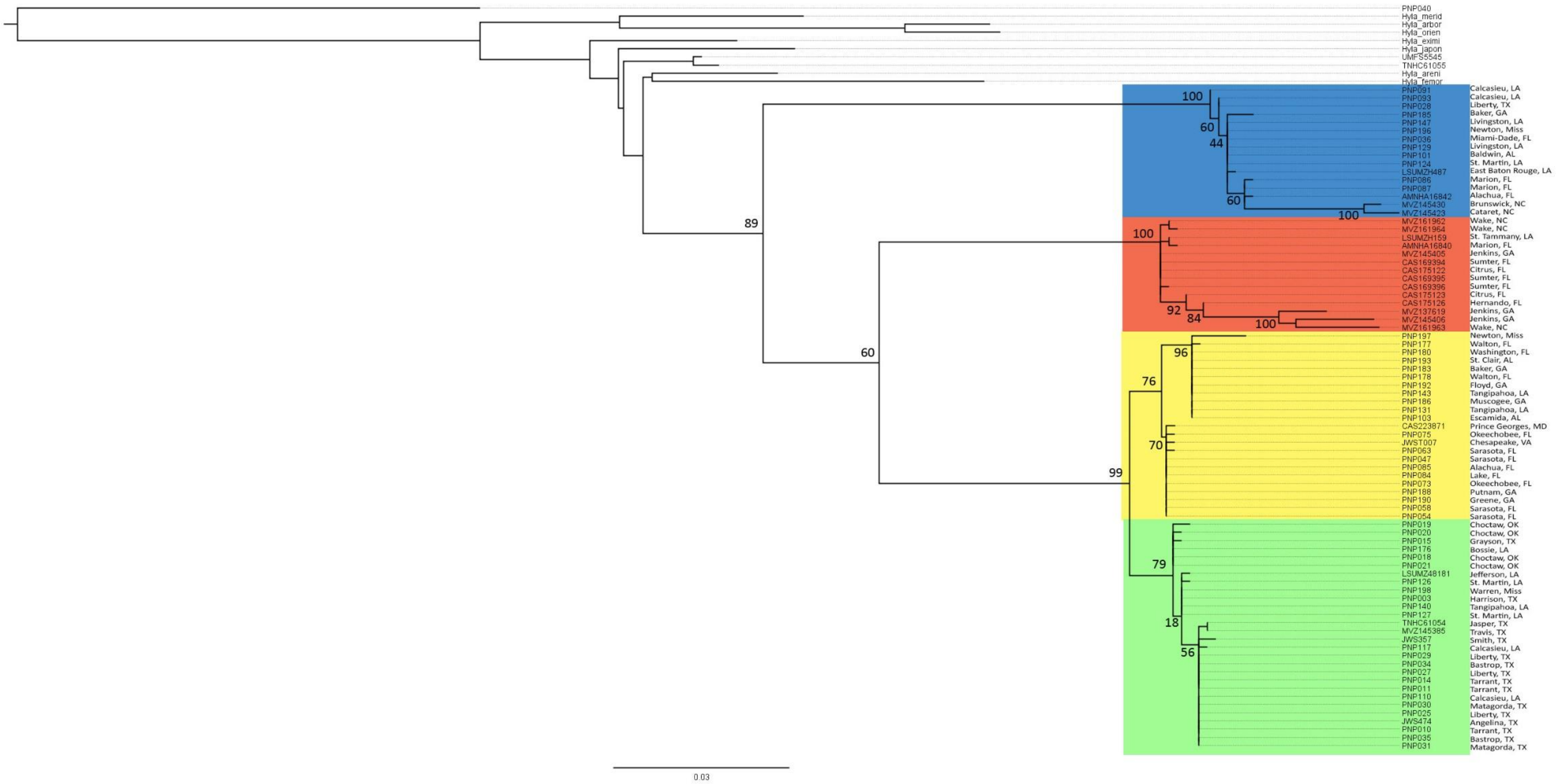

Figure 2.9. Maximum likelihood phylogenetic tree of the *Dryophytes cinereus* species group. Data for this analysis include all mitochondrial loci sampled (12S and 16S). Values on the branches represent maximum likelihood nonparametric bootstrap percentages. Specimens highlighted in blue represent members of *D. squirellus*, specimens in red represent *D. gratiosus*, and specimens from *D. cinereus* are divided into two clades, the eastern clade in yellow and the western clade in green.

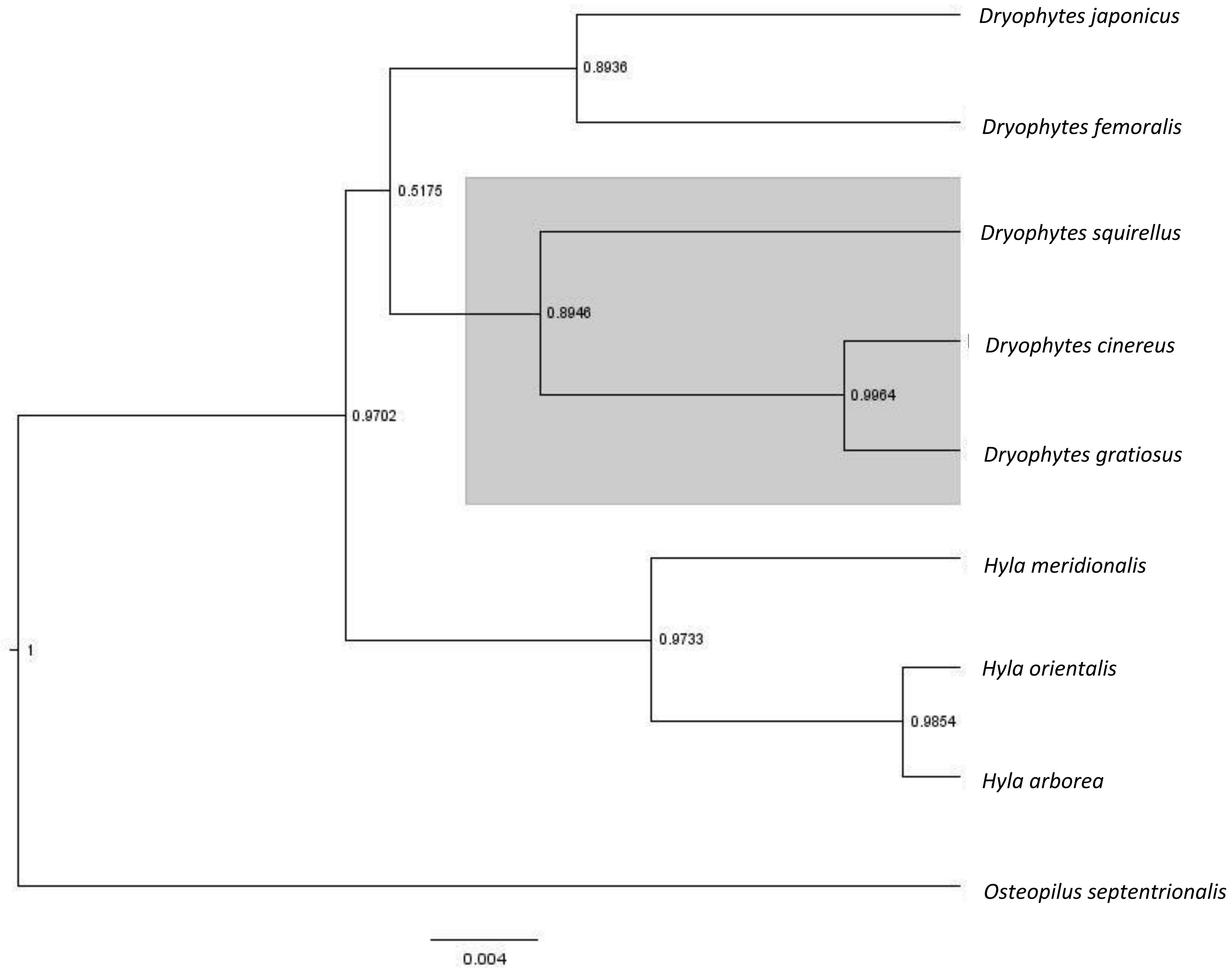

Figure 2.10. *BEAST coalescent tree based on the concatenated dataset (12S, 16S, and RAG-1). Node values represent Bayesian posterior probabilities. The grey box represents the species from the *Dryophytes cinereus* species group.

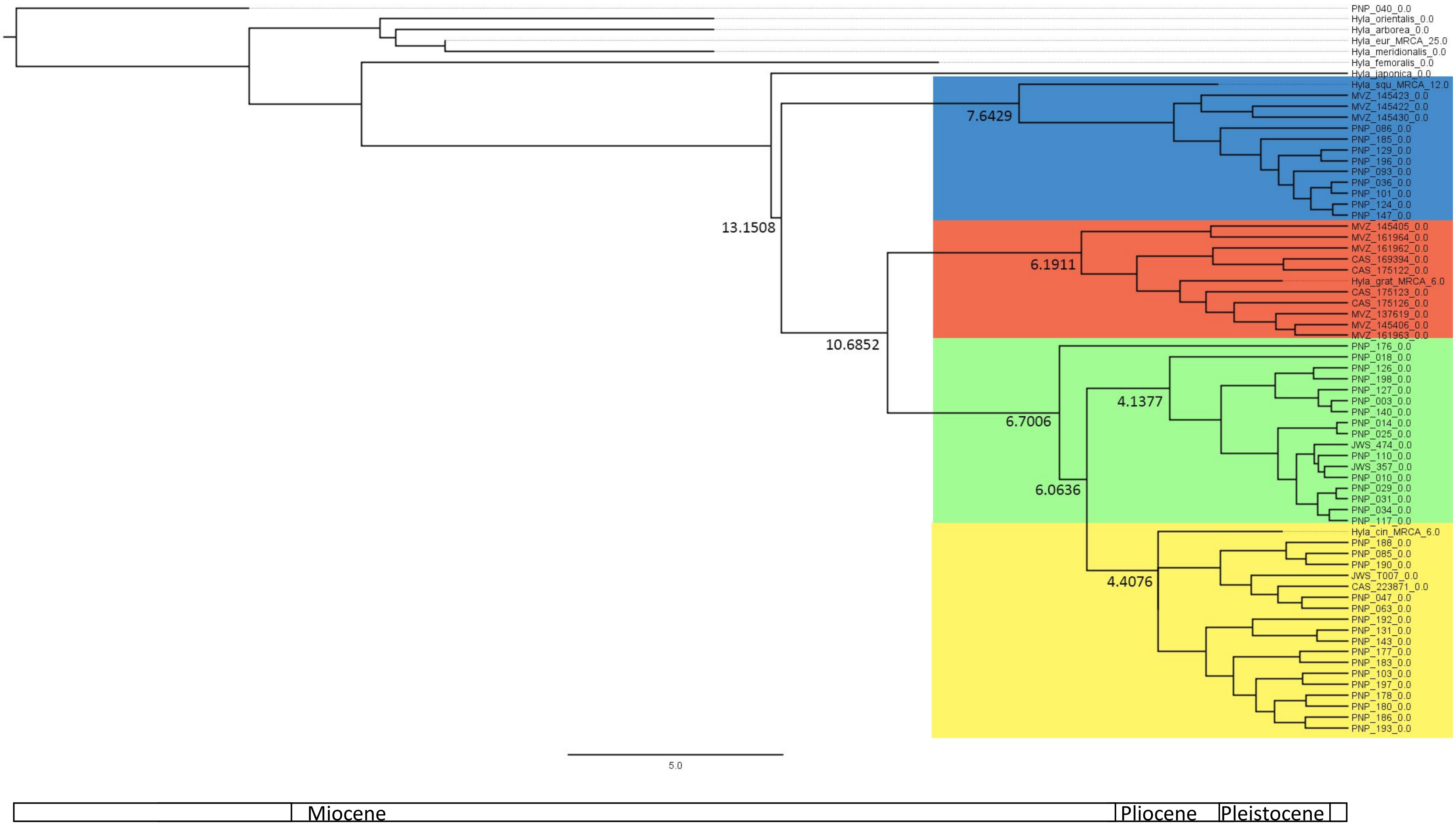

Figure 2.11. Divergence estimates for the *Dryophytes cinereus* species group using the HKY model of nucleotide substitution. Data for this tree and time estimates include all loci sampled (12S, 16S, and RAG-1). Values on the branches represent million years before present. Specimens highlighted in blue represent members of *D. squirellus*, specimens in red represent *D. gratiosus*, and specimens from *Dryophytes cinereus* are divided into two clades, the eastern clade in yellow and the western clade in green. The scale bar represents millions of years before present.

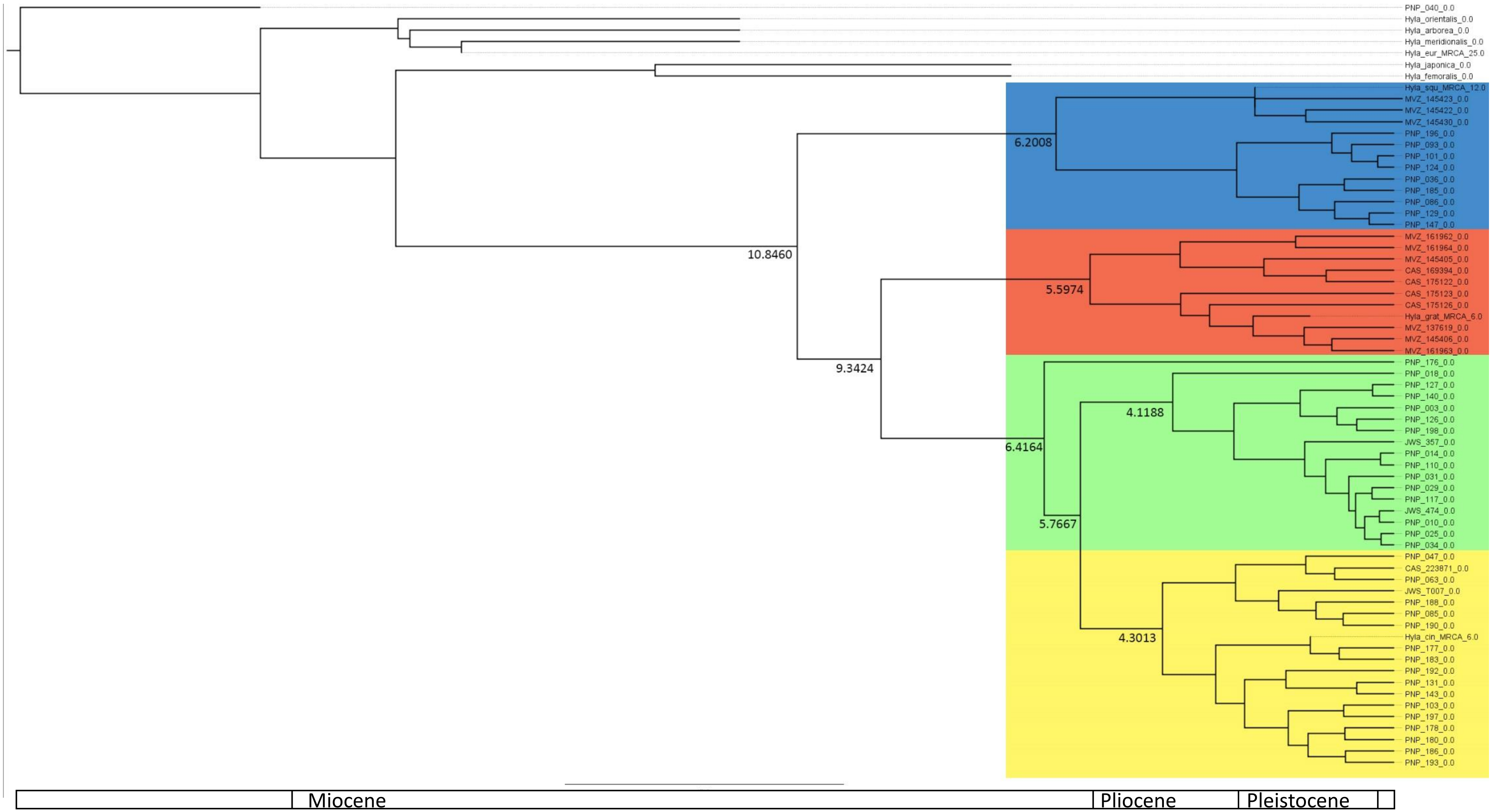

Figure 2.12. Divergence estimates for the *Dryophytes cinereus* species group using the GTR + G model of nucleotide substitution. Data for this tree and time estimates include all loci sampled (12S, 16S, and RAG-1). Values on the branches represent million years before present. Specimens highlighted in blue represent members of *D. squirellus*, specimens in red represent *D. gratiosus*, and specimens from *D. cinereus* are divided into two clades, the eastern clade in yellow and the western clade in green. The scale bar represents millions of years before present

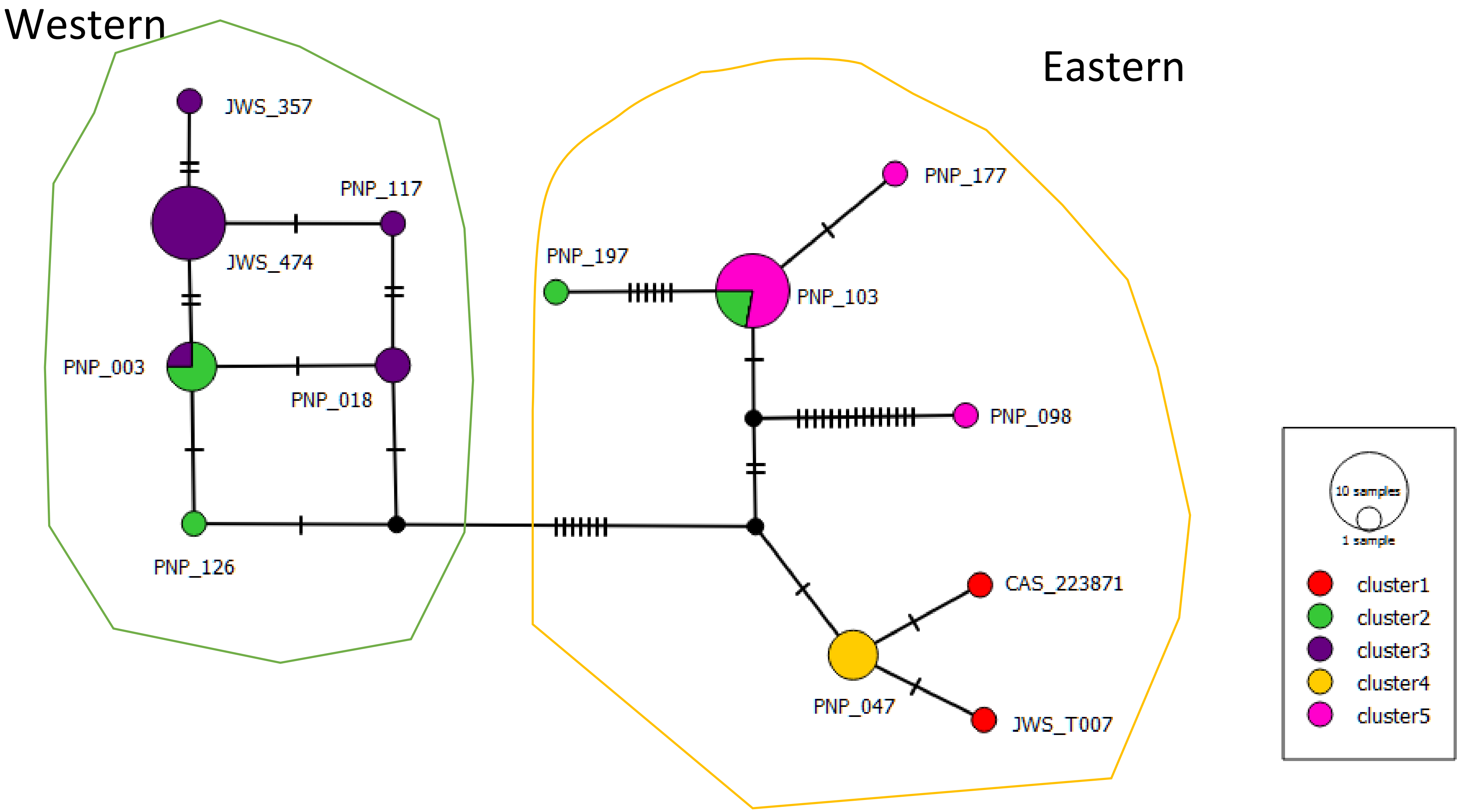

Figure 2.13. Mitochondrial haplotype network for *Dryophytes cinereus* created in PopArt. The circles represent shared haplotypes, and the five colors represent locations based on proximity to each other; Red (cluster 1) = samples from the north Atlantic coast; green (cluster 2) = samples from Louisiana; purple (cluster 3) = samples from Texas, Oklahoma, and eastern Louisiana; yellow (cluster 4) = samples from central and peninsular Florida and southern Georgia; pink (cluster 5) = samples from east of the Mississippi to Florida and northern Georgia.

Table 2.10. List of specimens included in each cluster of mitochondrial haplotypes from PopArt

Cluster	Sample	State	County	GPS N	GPS W
1	CAS 223871	MD	Prince Georges	38.737	76.720
	JWS T007	VA	Chesapeake	36.598	76.382
2	PNP 126	LA	St. Martin	30.342	91.722
	PNP 127	LA	St. Martin	30.342	91.722
	PNP 131	LA	Tangipahoa	30.504	90.518
	PNP 140	LA	Tangipahoa	30.504	90.518
	PNP 143	LA	Tangipahoa	30.504	90.518
	PNP 197	Miss	Newton	32.336	89.291
	PNP198	Miss	Warren	32.365	90.718
3	JWS 357	TX	Smith	32.333	95.368
	JWS 474	TX	Angelina	31.278	94.500
	PNP 003	TX	Harrison	32.735	94.117
	PNP 010	TX	Tarrant	32.793	97.106
	PNP 014	TX	Tarrant	32.793	97.106
	PNP 018	OK	Choctaw	34.037	95.376
	PNP 025	TX	Liberty	30.145	94.641
	PNP 027	TX	Liberty	30.145	94.641
	PNP 029	TX	Liberty	30.116	94.644
	PNP 031	TX	Matagorda	28.839	95.853
	PNP 034	TX	Bastrop	30.110	97.323
	PNP 110	LA	Calcasieu	30.290	93.192
	PNP 117	LA	Calcasieu	30.290	93.192
	PNP 176	LA	Bossier	32.547	93.478
4	PNP 047	FL	Sarasota	27.327	82.443
	PNP 085	FL	Alachua	29.506	82.102
	PNP 188	GA	Putnam	33.320	83.411
	PNP 190	GA	Greene	33.421	83.263
5	PNP 098	AL	Baldwin	30.272	87.655
	PNP 103	AL	Escamida	30.319	87.395
	PNP 177	FL	Walton	30.484	86.280
	PNP 178	FL	Walton	30.484	86.280
	PNP 180	FL	Washington	30.771	85.540
	PNP 183	GA	Baker	31.253	84.527
	PNP 186	GA	Muscogee	32.546	84.884
	PNP 192	GA	Floyd	34.272	85.189
	PNP 193	AL	St. Clair	33.874	86.269

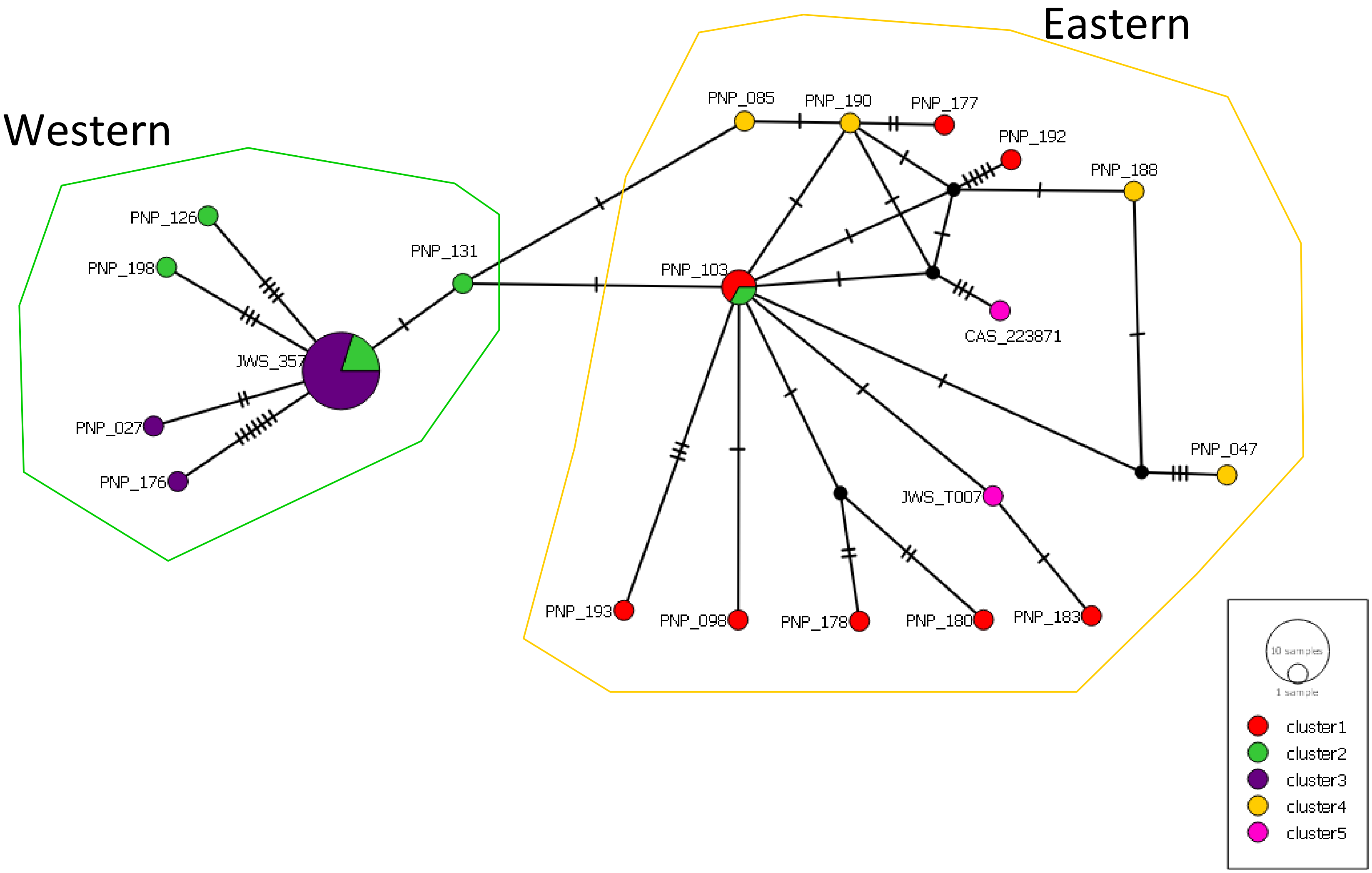

Figure 2.14. Mitochondrial haplotype network for *Dryophytes cinereus* created in PopArt. The circles represent shared haplotypes, and the five colors represent locations based on proximity to each other; Red (cluster 1) = samples from east of the Mississippi to Florida and northern Georgia; green (cluster 2) = samples from Louisiana; purple (cluster 3) = samples from Texas, Oklahoma, and eastern Louisiana; yellow (cluster 4) = samples from central and peninsular Florida and southern Georgia; pink (cluster 5) = samples from the north Atlantic coast..

Table 2.11. List of specimens included in each cluster of nuclear haplotypes from PopArt

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	PNP 103	AL	Escamida	30.319	87.395
	PNP 177	FL	Walton	30.484	86.280
	PNP 178	FL	Walton	30.484	86.280
	PNP 180	FL	Washington	30.771	85.540
	PNP 183	GA	Baker	31.253	84.527
	PNP 186	GA	Muscogee	32.546	84.884
	PNP 192	GA	Floyd	34.272	85.189
	PNP 193	AL	St. Clair	33.874	86.269
2	PNP 126	LA	St. Martin	30.342	91.722
	PNP 127	LA	St. Martin	30.342	91.722
	PNP 131	LA	Tangipahoa	30.504	90.518
	PNP 140	LA	Tangipahoa	30.504	90.518
	PNP 143	LA	Tangipahoa	30.504	90.518
	PNP 197	Miss	Newton	32.336	89.291
	PNP198	Miss	Warren	32.365	90.718
3	JWS 357	TX	Smith	32.333	95.368
	JWS 474	TX	Angelina	31.278	94.500
	PNP 003	TX	Harrison	32.735	94.117
	PNP 010	TX	Tarrant	32.793	97.106
	PNP 014	TX	Tarrant	32.793	97.106
	PNP 018	OK	Choctaw	34.037	95.376
	PNP 025	TX	Liberty	30.145	94.641
	PNP 027	TX	Liberty	30.145	94.641
	PNP 029	TX	Liberty	30.116	94.644
	PNP 031	TX	Matagorda	28.839	95.853
	PNP 034	TX	Bastrop	30.110	97.323
	PNP 110	LA	Calcasieu	30.290	93.192
	PNP 117	LA	Calcasieu	30.290	93.192
	PNP 176	LA	Bossier	32.547	93.478
4	PNP 047	FL	Sarasota	27.327	82.443
	PNP 085	FL	Alachua	29.506	82.102
	PNP 188	GA	Putnam	33.320	83.411
	PNP 190	GA	Greene	33.421	83.263
5	CAS 223871	MD	Prince Georges	38.737	76.720
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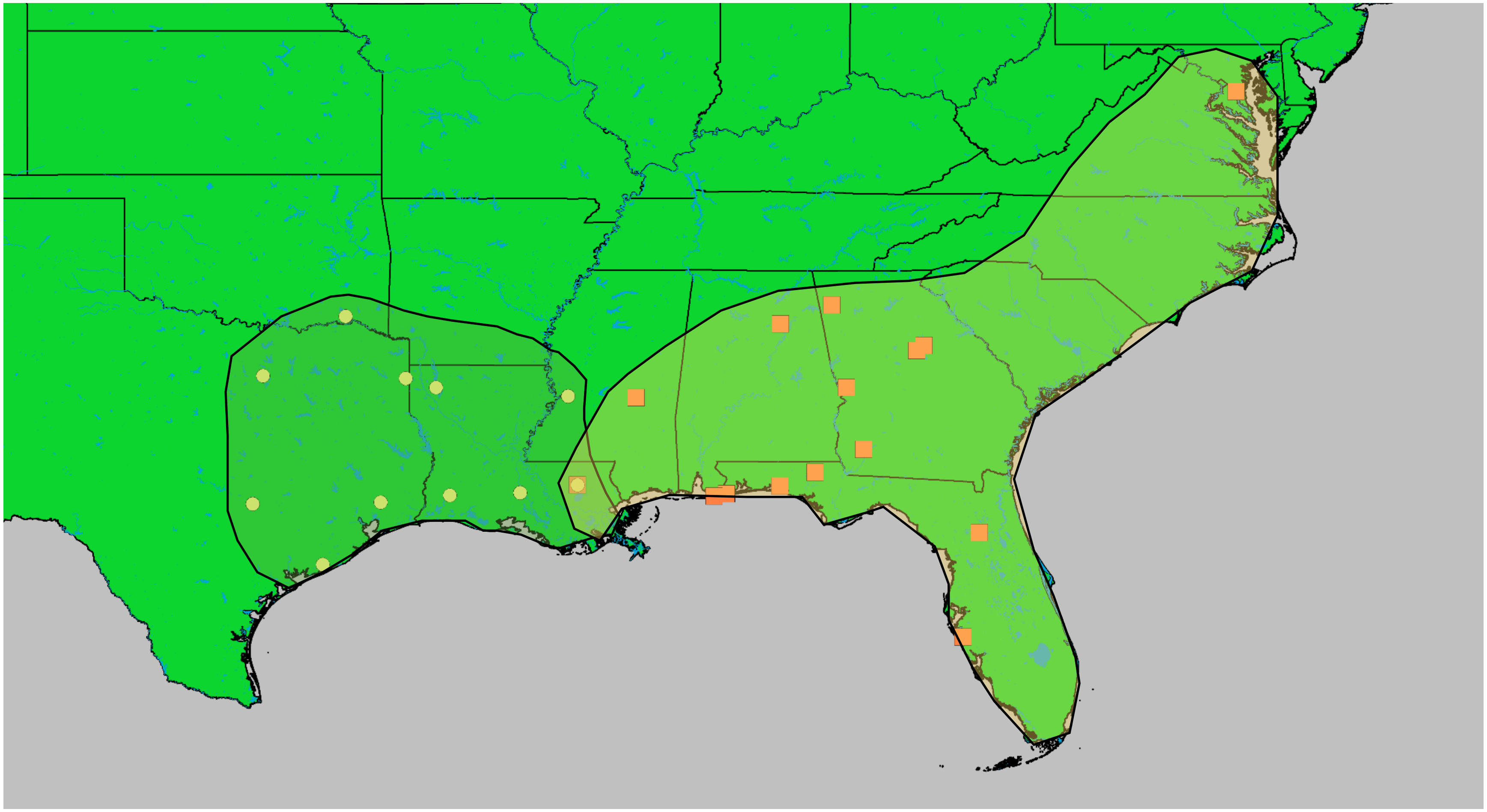

Figure 2.15. All *Dryophytes cinereus* samples included in the western and eastern clades. Samples that were phylogenetically associated with the western clade are represented by yellow circles, and all the samples from the eastern clade are represented by orange squares.

Table 2.12. F_{ST} between populations using the mitochondrial dataset displayed below the diagonal. Mean pairwise distances displayed above the diagonal.

	Dryophytes cinereus (East)	*Dryophytes cinereus* (West)	*Dryophytes gratiosus*	*Dryophytes squirellus*
Dryophytes cinereus (East)	-	0.013	0.060	0.065
Dryophytes cinereus (West)	0.80943	-	0.062	0.067
Dryophytes gratiosus	0.87460	0.89078	-	0.080
Dryophytes squirellus	0.88901	0.90675	0.85669	-

Table 2.13. F_{ST} between populations using the nuclear dataset displayed below the diagonal. Mean pairwise distances displayed above the diagonal.

	Dryophytes cinereus (East)	*Dryophytes cinereus* (West)	*Dryophytes gratiosus*	*Dryophytes squirellus*
Dryophytes cinereus (East)	-	0.006	0.005	0.020
Dryophytes cinereus (West)	0.55887	-	0.007	0.019
Dryophytes gratiosus	0.15558	0.64154	-	0.020
Dryophytes squirellus	0.77893	0.91690	0.89283	-

Table 2.14. F_{ST} between populations using the concatenated dataset displayed below the diagonal. Mean pairwise distances displayed above the diagonal.

	Dryophytes cinereus (East)	*Dryophytes cinereus* (West)	*Dryophytes gratiosus*	*Dryophytes squirellus*
Dryophytes cinereus (East)	-	0.011	0.038	0.047
Dryophytes cinereus (West)	0.72047	-	0.039	0.047
Dryophytes gratiosus	0.80973	0.86866	-	0.055
Dryophytes squirellus	0.86350	0.90863	0.85166	-

Table 2.15. Indices of genetic polymorphism using phased sequence data. Legend: n = number of samples included in the group; S = number of segregating sites; π = nucleotide diversity; θ = Watterson estimator (4Nμ); mito = mitochondrial; nuc = nuclear; con = concatenated (mitochondrial and nuclear)

	n	S (mito-713bp)	S (nuc-677bp)	Haplotypes (mito)	Haplotypes (nuc)	π (mito)	π (nuc)	π (con)	θ (mito)	θ (nuc)	θ (con)
Dryophytes squirellus	24	35	9	7	7	0.01394	0.00295	0.00857	0.01324	0.00356	0.00851
Dryophytes gratiosus	20	37	12	8	14	0.01582	0.00556	0.01080	0.01473	0.00500	0.00997
Dryophytes cinereus (all)	70	27	30	13	23	0.01092	0.00716	0.00907	0.00797	0.00920	0.00857
Dryophytes cinereus (East)	36	14	27	7	20	0.00430	0.00878	0.00650	0.00479	0.00962	0.00715
Dryophytes cinereus (West)	34	6	3	6	3	0.00232	0.00051	0.00143	0.00208	0.00108	0.00159

2.4 Discussion

Surprisingly, the Apalachicola River did not appear to be a phylogeographic barrier for any of the species used in this study even though it has been proposed as a barrier to the dispersal of other species (e.g., Makowsky et al., 2010; Means, 1977; Swenson & Howard, 2005). However, some of the other animals used in phylogeographic studies in this region, such as small reptiles (Makowsky et al., 2010; Fontanella et al., 2008; Soltis et al., 2006), skunks (Barton & Wisely, 2012), and pocket gophers (Soto-Centeno et al., 2013), might not share the same affinity for water that amphibians do, and older smaller rivers might be a stronger barrier for them, such as the Apalachicola River.

Dryophytes squirellus revealed no phylogeographic breaks around the Mississippi River; though it did show distant separation between samples from North Carolina and the Gulf Coastal region (BPP = 100.0%; ML bootstrap = 99.0% for each clade). The inclusion of additional samples from northern Georgia and South Carolina could help to investigate this unexpected pattern in the future. During my trip to South Carolina, I visited five sites but did not hear *D. squirellus* calling at night nor did I find them during my collections by day. These regions support lower abundance of these species, and possibly I was not looking in the optimal locations (National Amphibian Atlas, 2014c). Also, since the DNA from tissue samples from the museums showed considerable divergence from the rest of the group, PCR amplification and sequences need to be repeated to eliminate the possibility of contamination, and the specimens need to be physically examined to ensure that they were correctly identified. Additionally, this study did not indicate gene flow between *D. squirellus* and other species used in this study.

The lack of variation in *Dryophytes squirellus* on either side of the Mississippi River does not correspond with other studies that suggest its strength as a phylogeographic barrier.

However, the behavior of this species explains these patterns. This small frog spends most of its life hiding in trees unless it descends to forage or breed (Pope, 1919). Heavy rainstorms blow individuals out of trees, which has earned it the common name “rain frog” (Taylor et al., 2007), thereby associating migrations of this species with storms and summer rains (Wright, 1931). During these events, the Mississippi River could distribute animals blown from the trees to either side, promoting continuity across the river.

Phylogenetic analysis consistently separated the eastern and western clades of *Dryophytes cinereus* but indicated a close relationship using mitochondrial and concatenated mitochondrial and nuclear data (i.e., most analyses indicated that they form a well-supported monophyletic group). This vicariance corresponds to the Mississippi River; although two samples from the western clade appeared east of the Mississippi River. This could be due to the fact that the amphibians breed in water, and tadpoles may, on extremely rare occasions, survive the rigors and dangers of traversing major river courses (or the adults may occasionally be able to cross). Amphibians are often known to be philopatric (Blaustein et al., 1984; Heemeyer & Lannoo, 2012; Lin et al., 2012), and these animals might return to their original sites to breed, keeping the two lineages separated even if limited trans-riverine exchange is possible. Additionally, of the three species included in this study, *D. cinereus* is the only species with breeding site preferences that might include localities like the Mississippi River (Oldham & Gerhardt, 1975), and two of the samples of *D. cinereus* collected for this study were found breeding in the Colorado River in Texas, indicating that potentially these frogs also breed in locations like the Mississippi River. However, not much is known regarding whether *D. cinereus* shows philopatric preferences for breeding sites, and the likelihood that this complex situation occurs seems low. More simply, the two individuals caught on the eastern side of the Mississippi

River could have migrated there, or may represent relicts of a vicariance event that separated most of the western clade from the eastern clade. Gerhardt (1974a; 1974b; 1978; Gerhardt et al., 1980; Höbel & Gerhardt, 2003; Oldham & Gerhardt 1975) proposed based on call data that these animals display reproductive character displacement in areas of sympatry with *D. gratiosus*. He had no genetic data, and Lisa Barrow (Florida State University, pers. comm.) very recently found, based on a very large nuclear data set, that *D. cinereus* from the same small area on the east side of the Mississippi River in which I found frogs that genetically match members of the western clade were extremely divergent from other members of the eastern clade (she did not examine specimens from the western side of the river). *Dryophytes gratiosus* does not cross the Mississippi River nor does it extend north along the river, near the collection site of one of the *D. cinereus* samples belonging to the western clade.

The most striking results phylogeographically concern *Dryophytes gratiosus* and the eastern clade of *D. cinereus*. Documentation of hybrids between these two species dates as far back as 60 years (Gerhardt et al., 1980; Gunzburger, 2005; Lamb & Avise, 1986; Mecham, 1960; Toews & Brelsford, 2012). Since Lamb and Avise (1986), no genetic studies investigating this hybridization appear in the literature. Their study using restriction site analysis of mitochondrial DNA and allozymes found evidence for nuclear introgression without mitochondrial introgression between members of *D. gratiosus* and *D. cinereus*. Since, many studies documenting this hybridization's effects on female preference, vocalization, morphology, and behavior do appear (Gerhardt et al., 1980; Gunzburger, 2005; Höbel & Gerhardt, 2003). The nuclear phylogeny based on my study did not distinguish between these two species while mitochondrial phylogenies consistently separate *D. gratiosus* from the eastern *D. cinereus*. Moderate F_{ST} values for the nuclear dataset ($F_{ST} = 0.16$) support these findings, indicating high levels of gene flow between these two groups, consistent with behavioral and morphological studies. Comparatively, some human populations exhibit fixation indices around 0.10 (Elhaik, 2012). Between these frog populations, mitochondrial F_{ST} values of the western and eastern clades of *D. cinereus* ($F_{ST} = 0.81$) were comparable to the value between *D. gratiosus* and the eastern clade of *D. cinereus* ($F_{ST} = 0.87$). Nuclear F_{ST} values between these same populations did not recover the same pattern (*D. gratiosus* and eastern *D. cinereus* = 0.16; western and eastern *D. cinereus* = 0.56). The discordance between the nuclear and mitochondrial results could be consistent with gene flow between *D. gratiosus* and the eastern *D. cinereus,* which according to behavioral observations by Höbel and Gerhardt (2003) and Lamb and Avise (1986) appear to be biased toward male *D. cinereus* mating with female *D. gratiosus*. However, the nuclear gene utilized in this study is a conserved protein coding gene, and little variation is present between species. While conservation of this gene is expected, higher fixation indices between the other species used in these analyses indicate that the pattern observed is relatively low. Conversely, the nuclear data strongly separated *D. gratiosus* and the eastern *D. cinereus* from the western clade of *D. cinereus.* However, most studies investigating hybridization using population genetic methods and F_{ST} do so with more loci than in this study. These results indicate that to more fully understand the type, extent, and implications of this hybridization requires additional investigation using more nuclear genes.

The results from this study do concur with the previous genetic evaluation of *Dryophytes cinereus/D. gratiosus* hybridization patterns using mitochondrial and allozyme data (Lamb & Avise, 1986). In both this study and Lamb & Avise (1986), mitochondrial DNA was found to be highly divergent between these groups, yet nuclear data showed relative genetic uniformity. Potentially, the behavior of these species explains these patterns. These species have overlapping preferences for breeding locations (Oldham & Gerhardt, 1975), and many breeding sites for these species have been anthropogenically disturbed (e.g. mowing along water's edges), disrupting the species' natural segregation at breeding sites where *D. gratiosus* calls from the center of bodies of water and *D. cinereus* calls from vegetation along its perimeter. The relative abundance of *D. cinereus* is higher than that of *D. gratiosus* (Schlefer et al., 1986), and *D. cinereus* males exhibit satellite behavior at breeding locations where they are especially abundant (Lamb & Avise, 1986). Even though *D. gratiosus* females do not exhibit preference towards the calls of *D. cinereus* males, any satellite males present in abundance around these breeding sites can intercept incoming *D. gratiosus* females attracted to conspecific males in deeper waters (Oldham & Gerhardt 1975). Furthermore, *D. gratiosus* are not reported to exhibit satellite behavior (Lamb & Avise 1986). Given these behavioral observations, it is likely that at breeding locations where *D. gratiosus* and eastern *D. cinereus* are present, satellite *D. cinereus* males may mate with incoming *D. gratiosus* females. Possibly, human disturbance at breeding locations of these species contributes to this hybridization (Lamb & Avise, 1986). By removing the vegetation elevating *D. cinereus* at the edges of breeding sites, they may more easily encounter the incoming females of *D. gratiosus* which move at ground level (Höbel & Gerhardt, 2003). However, the range-wide patterns of variation indicate that hybridization between these species occurred long before anthropogenic disturbance of their breeding locations (Mecham, 1960).

Divergence estimates between these species indicate millions of years separate the eastern and western clades of *Dryophytes cinereus*. Possibly, populations of *D. cinereus* separated during the climate fluctuations in the early Miocene and late Pliocene evolved separate behaviors that distinguish them upon secondary contact. These species could have expanded after glaciation to either side of the Mississippi River. Southern Texas and peninsular Florida likely provided refugia for many species whose postglacial expansion extended toward the Mississippi River (Swenson & Howard, 2005). Populations of *D. cinereus* from Florida coming back into contact with *D. gratiosus* (if the two became separated into distinct geographic areas) could have favored evolution of more distinct calls, perhaps via reinforcement and/or character displacement. Nevertheless, overlap of breeding habitat preferences in a more continuous environment during the Holocene may still have allowed limited gene flow to occur between these species. Conversely, populations isolated in Texas refugia may have evolved slightly different calls than its eastern relatives.

Perhaps the most likely explanation for the combination of deep genetic divergences on either side of the Mississippi found independently by me and Barrow et al. (with an apparently small extension of the western clade to the eastern side of the river) is that two species are present within what is currently considered *Dryophytes cinereus*. This is extremely consistent with the call variation identified by Höbel & Gerhardt (2003), which tracks the same east-west split, with the same small extension of western call types to the eastern side of the river in an area where the neither the "eastern" call type of *D. cinereus,* nor *D. gratiosus,* appear to occur. Taxonomic implications of these results are explored more fully later, in the Conclusions and Future Directions section.

This study is useful in that it provides additional support to the results found by Lamb & Avise (1986) that sex biased hybridization is occurring between these two species and provides a broader collection of samples reinforcing Mecham’s (1960) suggestion that this hybridization is not an isolated phenomenon. It also provides insight into rivers’ strength (specifically the Mississippi) as phylogeographic barriers to gene dispersal in this group of amphibians, perhaps having led to the occurrence of two species within what currently is considered *Dryophytes* *cinereus*. This study corroborates the conclusions of Austin et al. (2004) that rivers are not necessarily absolute barriers to gene flow for anurans, although further investigation into the cause of the *D. cinereus* split is needed. However, potential environmental differences on either side of the river could explain that the genetic variation observed is reflective of different ecological preferences between the two groups.

Chapter 3: Ecological Niche Modeling

3.1 Introduction

Ecological niche modelling is a useful tool to biologists (Wiens & Graham, 2005). Using niche models, researchers predicted invasive species' potential distributions (Peterson, 2003), outbreaks of diseases caused by harmful bacteria (Blackburn et al., 2007), investigated climate change's effects on species distributions (Pearson & Dawson, 2003), reconstructed species distributions in historic conditions (Waltari et al., 2007), and inferred how geographic barriers are affecting current species' distributions (Graham et al., 2004). When combining the data from phylogenetics and the environmental information from niche models, researchers improve their understanding of whether or not barriers to gene flow exist or if ecological differences restrict species dispersal.

Using climate data to estimate ecological divergence within and among species enlightens patterns in the distributions of species (Hickerson et al., 2010a; Peterson & Kluza, 2005; Rissler & Apodaca, 2007; Wiens, 2004a; Wiens et al., 2010). Incorporating climate layers and presence data for species generate robust predictive maps of its distribution (Phillips & Dudík, 2008; Rissler & Apodaca, 2007). Environmental differences between distributions strengthen the knowledge of previously identified phylogeographic clades, determining if the populations or species are ecologically divergent (Raxworthy et al., 2007). These ecological divergences between closely related populations help evaluate the role of ecological divergence in speciation (Graham et al., 2004; Wiens & Graham, 2005). If niches are similar in allopatry, but divergent in sympatry, it indicates allopatric speciation associated with vicariance; if the opposite is true, it can indicate sympatric speciation caused by behavioral isolation (Wiens & Graham, 2005).

Two primary means of performing ecological niche models use the programs Maxent and Desktop GARP (Pereira, 2002; Phillips & Dudík, 2008). These widely used programs recover similar results and produce robust predictions of species distributions (Anderson & Gonzalez Jr, 2011; Peterson et al., 2007; Warren & Seifert, 2011). Investigators debate which of these programs more accurately transfers, or predicts, species distributions based on a subset of input data. If a model over-predicts the distribution of the input samples, this can mislead the researcher to believe that a physical or behavioral barrier limits the range of the species instead of ecological differences prohibiting expansion (Peterson et al., 2007). This study employs both methods to reduce the possibility of over-predicting the distributions of the clades used in the analysis.

3.2 Materials and Methods

GPS coordinates for all collected specimens were measured using a Garmin eTrex Legend at the site where the specimen was secured. These coordinates were maintained with species identifiers in Microsoft Excel and organized into clades based on the results from Chapter Two. Current Bioclim data layers of the finest available resolution (representative of 1960 – 1990) were downloaded from the WorldClim database (Hijmans et al., 2005) in addition to an altitude layer downloaded directly through ESRI (ESRI & Components, 2001). Frequently used in species distribution models, these layers represent annual trends in temperature and precipitation and seem to capture species’ preferences well (Table 3.1; Warren & Seifert, 2011). These layers were then imported to ArcGIS, and one layer was selected and trimmed manually to adequately encompass the range of the species used in the study. This layer was then used as a template layer to trim the other 18 Bioclim layers. The resultant 19 identically sized Bioclim layers were then stored in a directory referenced by a .csv file.

Table 3.1. Summary of the 19 BioClim variables used in Ecological Niche Modeling

Layer Name	Variable
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Range (mean of monthly * (max temp – min temp))
BIO3	Isothermality (BIO2/BIO7)*100
BIO4	Temperature Seasonality (standard deviation *100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range (BIO5-BIO6)
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

Maxent was used to perform the ecological niche modeling (Phillips & Dudík, 2008). The model was executed with default settings but modified to include 10,000 bootstrap repetitions, using 25% of the points to evaluate the performance of the model.

In addition to Maxent, GARP generated ecological niche models using the same input data executed on the mMWeb servers using their environmental layers (Qiao et al., 2012). Unaltered North American Bioclim layers maintained on the mMWeb servers were used to project the distributions. The program operated using default settings with 1,000 iterations to give more stable results (Pereira, 2002).

3.3 Results

Predictive distribution maps from the Maxent and GARP analyses can be found in figures 3.1-3.4. The squares on the Maxent results map represent samples used to train the models, and the purple squares indicate the samples used to test the models. Overall, the programs predicted the actual distributions of the species well. Both programs produced similar results, and the areas of high probability for finding the specimens were almost identical. However, over-predictions of the ranges of each respective clade indicate a barrier separates them. Additional runs using different numbers of bootstrap replicates and different percentages of test locations are located in Appendix 2 (Figures A.2.1.-A.2.3.). However, altering these parameters did little to change the prediction maps, and all the predicted distributions of the clades overlapped with each other.

The area under the receiver operating characteristics (ROC) curve (AUC) evaluated the performance of the Maxent predictions. For the eastern clade, these values were 0.932 for the training data and 0.962 for the test data, meaning that the models perform better than random predictions (0.5 means random; 1.0 is the maximum possible value). For the western clade, the AUC for training data was 0.962 and 0.983 for the test data. These values are significantly better than random performance of the models ($P < 0.05$).

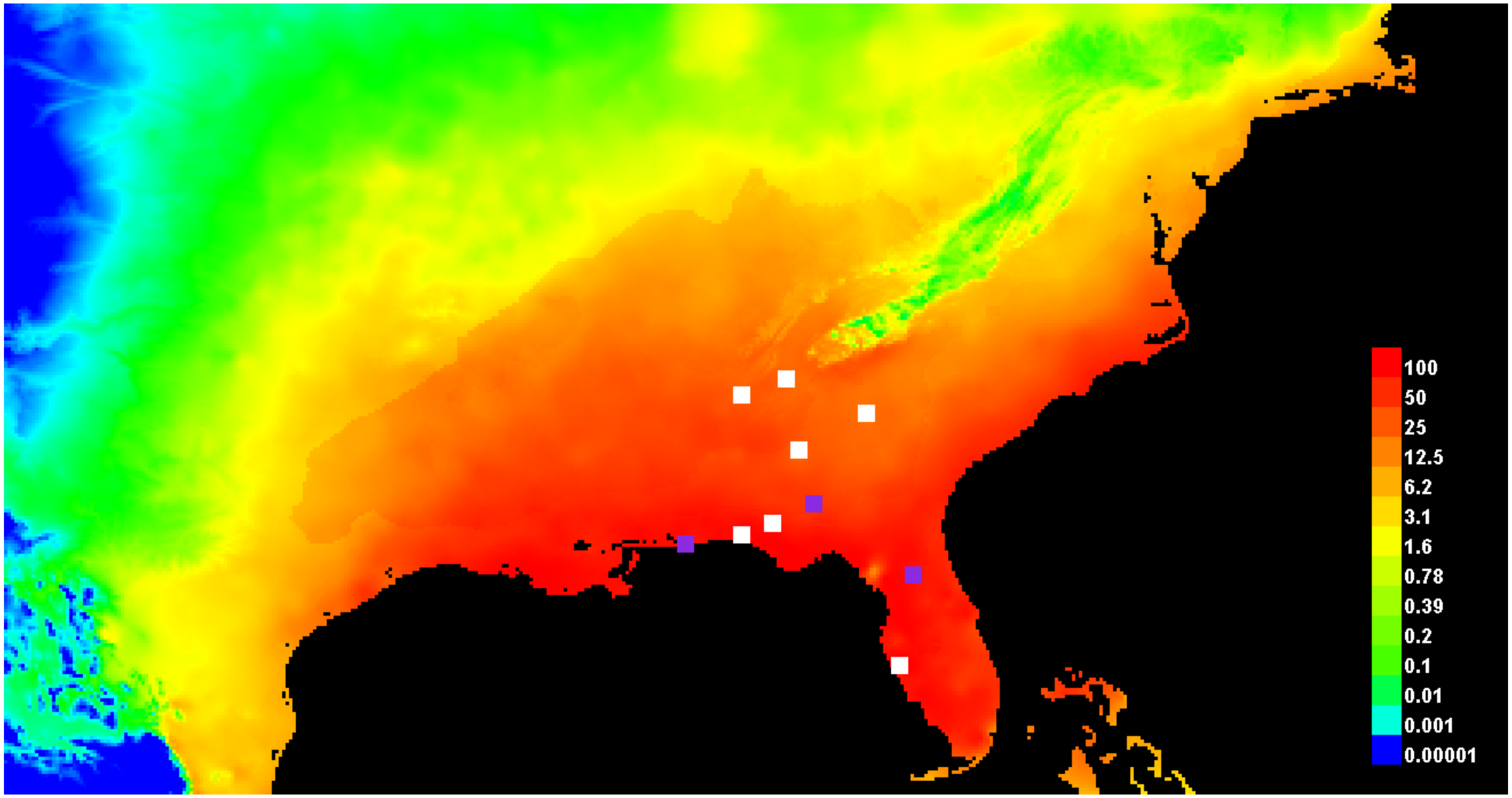

Figure 3.1. Predictive map of the eastern clade of *Dryophytes cinereus* using the program Maxent. Model was executed using 10,000 bootstraps and 25% of the samples to test the model. White squares on the map are the presence localities used for training, and purple squares indicate the samples used to test the models. Warmer colors indicate a higher probability for predicting the presence of the species. AUC for training data = 0.932; AUC for test data = 0.962

Figure 3.2. Predictive map of the eastern clade of *Dryophytes cinereus* using the program Desktop GARP. Model was executed using default values on the server mMWeb. Brighter colors indicate a higher probability for predicting the presence of the species.

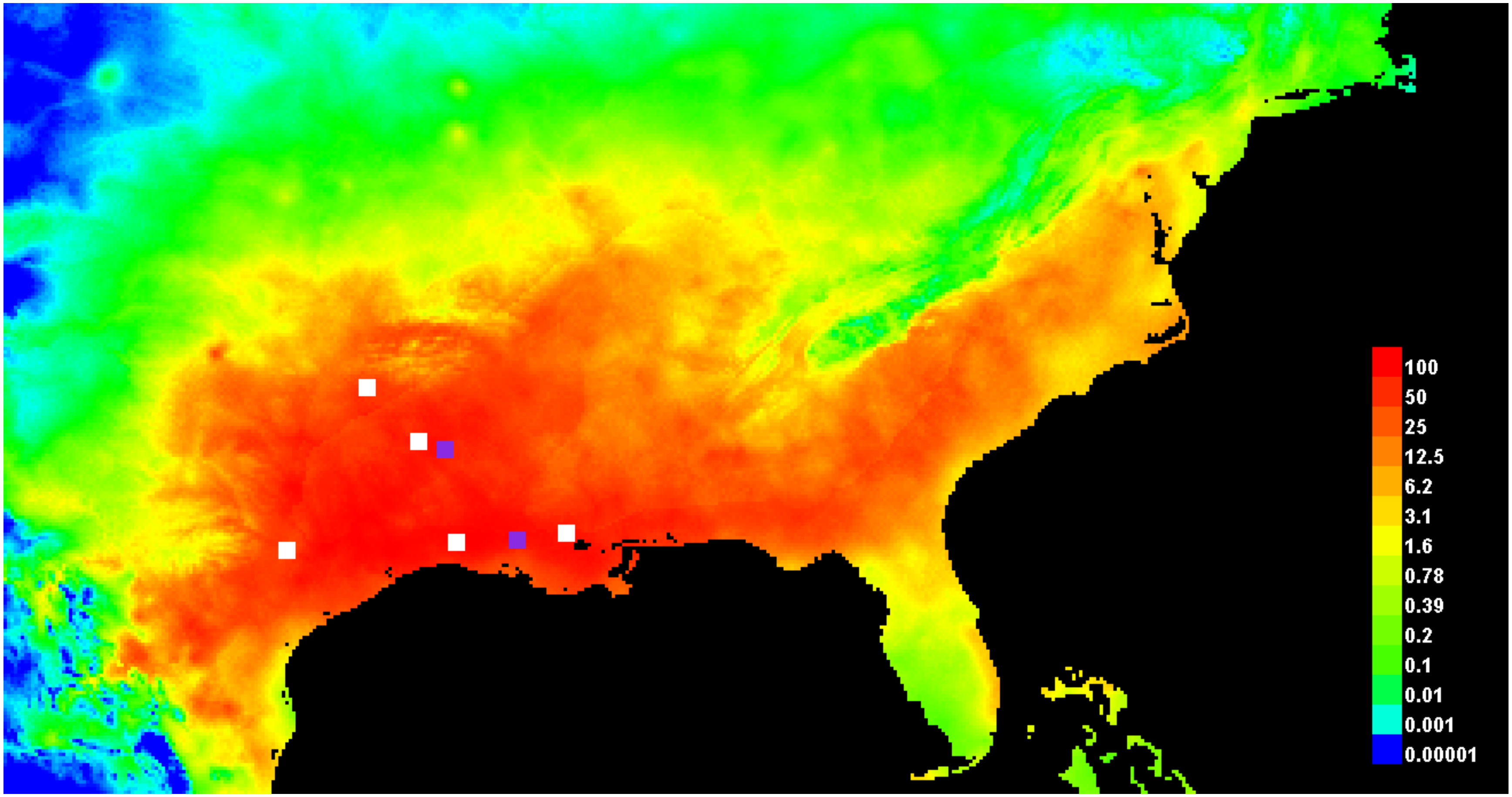

Figure 3.3. Predictive map of the western clade of *Dryophytes cinereus* using the program Maxent. Model was executed using 10,000 bootstraps and used 25% of the samples to test the model. White squares on the map are the presence localities used for training, and purple squares indicate the samples used to test the models. Warmer colors indicate a higher probability for predicting the presence of the species. AUC for training data = 0.962; AUC for training data = 0.983

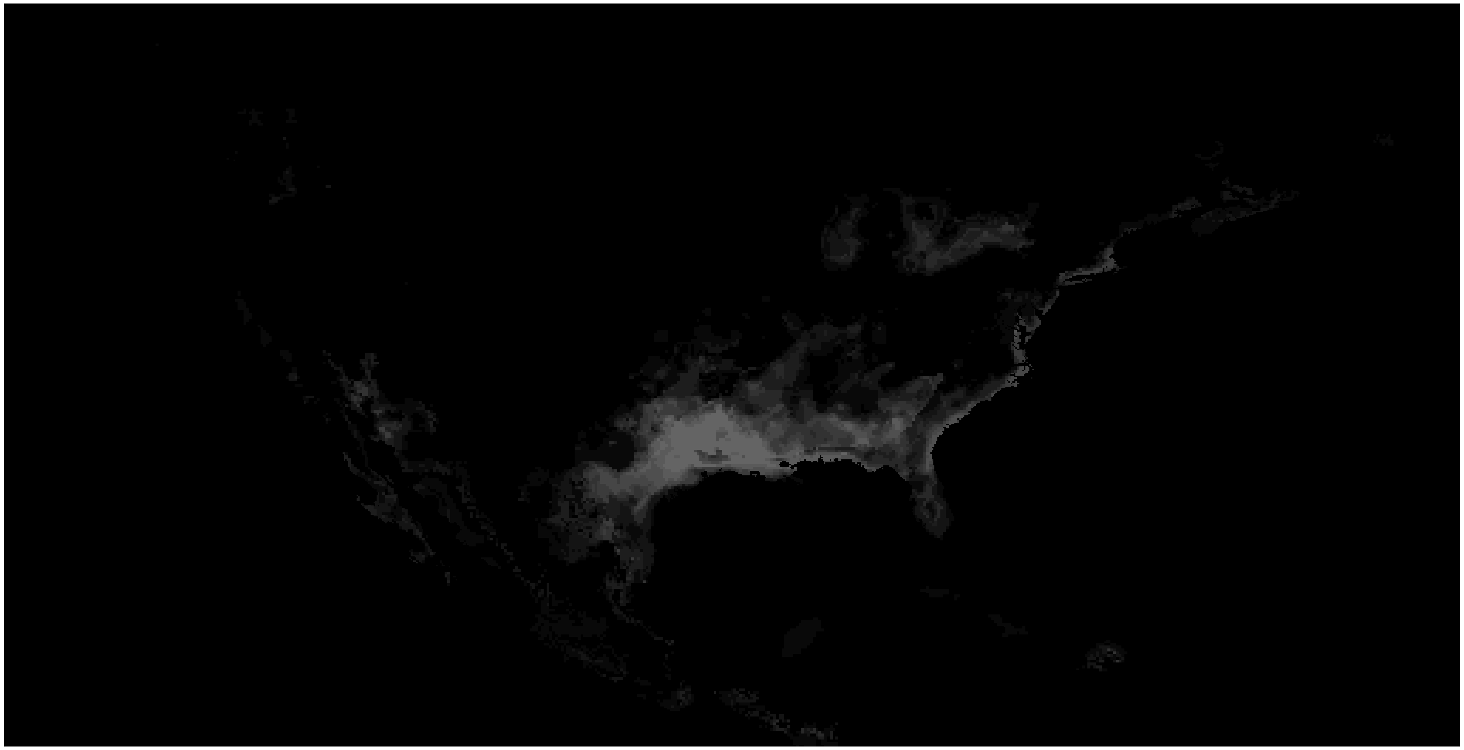

Figure 3.4. Predictive map of the western clade of *Dryophytes cinereus* using the program Desktop GARP. Model was executed using default values on the server mMWeb. Brighter colors indicate a higher probability for predicting the presence of the species.

3.4 Discussion

In the results of the Maxent and GARP analyses of the species' distributions, apparently few differences exist between the eastern and western clades' niche envelopes, as much of theses distributions overlap one another. The Maxent models appeared to provide less overlap than the GARP models, as predicted in the literature (Peterson et al., 2007), and the tests of the models showed good support for the predicted distributions. If the distributions excluded each other, the genetic differences between the clades could be explained by the evolution of different environmental preferences (Peterson, 2003). However, considerable over-prediction of the clades' observed ranges and diverse environmental conditions across the large distributions indicate a lack of ecological differences between the clades (Makowsky et al., 2010). While overlap between these two groups' niche models indicate homogeneity of the two groups' preferences, there are expanses of each clades' ranges with lower probabilities of finding the other. Possibly, enough variation between these two regions exist, but the models do not capture the specific variables responsible for the isolation of each group.

More likely, a behavioral or geographic explanation for the differences between these clades exists. Since this is a relatively young species, the overlap of the distribution maps is indicative that the animals in the two clades have not evolved different niche preferences (Wiens & Graham, 2005). *Dryophytes cinereus* exhibits character displacement in a slightly exaggerated mating call in areas of sympatry with *D. gratiosus*, (Höbel & Gerhardt, 2003). Several previous studies have investigated call variation and female preference for calls in this species group without always showing significant results but consistently finding differences between the groups (Gerhardt, 1974b; Gerhardt, 1978; Gerhardt et al., 1980; Oldham & Gerhardt, 1975). This character displacement could be a behavioral explanation for the genetic variation observed in this group, which reflects in the distributions of the species, as *D. gratiosus* extends no further than the Mississippi River.

Geographically, the Mississippi River separates these two clades. However, this river provides a questionable barrier between these two clades, as specimens collected from the eastern side of the river included specimens genetically closer to the western clade. Without obvious ecological distinction between these two clades, identifying the driving force of this genetic divergence becomes more difficult, and since the Mississippi River is an obvious structure, designating it as the barrier to dispersal in this group provides a simple solution. While speciation by character displacement is a more complicated and hotly debated process, it more closely aligns with the distributions of these two groups, as does the possibility of a cryptic species radiating from its refugial zone.

Chapter 4: Conclusions and Future Directions

The population structure in *Dryophytes cinereus* requires a biological explanation regarding its cause. This study indicates that the Riverine Barriers Hypothesis might not be the sole explanation for this variation. Neither *D. cinereus* nor *D. squirellus* appear to be affected by the Apalachicola River, a proposed geographic barrier for many species. *Dryophytes cinereus* does appear to be largely but not absolutely split genetically by the Mississippi River, but a few anomalies in the dataset present problems when interpreting the results.

First, two specimens collected from the eastern side of the Mississippi River belonged to the western genetic clade. Niche models for the clades of *D. cinereus* on either sides of the river did not reveal environmental differences for the eastern and western clades (Figures 3.1, 3.3), which could indicate that a geographic barrier contributed to this variation. However, if the river is a barrier, the ecological similarity on either side of the river does not explain why these two samples do not contribute to gene flow. The likelihood of a situation occurring in which these two samples are isolated events captured by this study seem low. While these could be rare anomalies, more likely this river is not a complete barrier for this species, but that some other behavioral or historical explanation coincides geographically with the Mississippi River.

Seeking alternative explanations for this variation should exclude the river as its cause. The existence of *Dryophytes gratiosus* provides an alternative explanation for the two western samples collected on the eastern side as this closely aligns with their range (Figures 2.2, 2.4). Since *D. cinereus* is proposed to exhibit reproductive character displacement in areas of sympatry with *D. gratiosus* (Gerhardt, 1974a; Höbel & Gerhardt, 2003; Oldham & Gerhardt, 1975), such behavior could lead to reproductive isolation of populations of *D. cinereus* (Gerhardt, 2013; Pfennig & Ryan, 2006). The discordance between the mitochondrial and nuclear signal in *D. gratiosus* and the sympatric eastern clade of *D. cinereus* supports this behavioral explanation for the genetic variation as well.

Behavioral preferences between these species also may explain these patterns. Considering the similarities in breeding location preferences and behaviors as explained in Chapter Two (Lamb & Avise, 1986; Rhymer & Simberloff, 1996; Toews & Brelsford, 2012), these species do interact at breeding sites. However, if this genetic variation in *Dryophytes cinereus* indicates character displacement caused by areas of sympatry with *D. gratiosus*, it presents an interesting argument against the Riverine Barriers Hypothesis. Assuming the Mississippi River is a geographic barrier preventing *D. gratiosus* from expanding westward, the character displacement is indirectly affected by this riverine barrier. Without the presence of *D. gratiosus* on the western side of the Mississippi River, one cannot definitively state that the river caused the geographic break. *D. gratiosus* exhibits different behaviors than the other two species in this group, which could explain the river's influence on its distribution patterns. They burrow and hide more frequently (Neill, 1952) and do not breed in permanent bodies of water, like *D. cinereus* (Gunzburger, 2005). Also, due to its large size and its preference to burrow instead of hide in trees, *D. gratiosus* is not affected by heavy rainstorms like *D. squirellus* (Taylor et al., 2007; Wright, 1931). For *D. gratiosus,* it seems likely that the Mississippi River, which marks the westernmost extent of its range, is the reason it does not extend further. However, additional studies would need to be performed to assess whether the river affects the range of *D. gratiosus.*

Reproductive character displacement in anuran populations has been proposed as drivers for intraspecific variation (Lemmon, 2009; Höbel & Gerhardt, 2003; Gerhardt, 2013; Richards-Zawacki & Cummings, 2011). Given the propensity for *Dryophytes cinereus* and *D. gratiosus* to hybridize in areas of sympatry, this is a viable explanation in this group as well. The high levels of nuclear gene flow found in this study provide interesting evidence supporting this hypothesis, and this needs to be investigated further. Since this study only used one conserved nuclear gene, a low mutation rate could explain the lack of variation between these species. This could be resolved using a multi-locus approach with next generation sequencing techniques to identify more polymorphisms and estimate types of hybridization (Davey & Blaxter 2010; Hohenlohe et al., 2011).

Alternatively, a historical explanation should also be considered. The assertion that reproductive character displacement caused the divergence between the eastern and western clades assumes that *Dryophytes cinereus* is one continuous species. Estimated divergence times between *D. gratiosus* and *D. cinereus* place their separation around 10 mya (Bryson et al., 2010; Duellman et al., 2016), and this study corroborates these findings. The dramatic fluctuations in river systems in the southeastern United States and the climactic changes during these times reasonably explain their divergence (Lemmon 2009; Swenson & Howard, 2005). Other studies support this pattern, and while the Mississippi River does not currently provide a geographic barrier to the dispersal of anuran species, evidence may indicate that this river played a historic role in the formation of other species (Austin et al., 2004; Swenson & Howard, 2005). The much wider Mississippi River around 5 – 10 mya could isolate these species, and continued fluctuations in climate, sea level, and river systems spanning the southeastern United States for the following millions of years could initiate the isolation of the eastern and western clades of *D. cinereus*.

If *Dryophytes cinereus* is two distinct lineages originating during the climatic fluctuations and changing sea levels from the Pliocene, the variations in call structure and morphological evidence used to support character displacement may reflect the occurrence of two distinct species. Then, upon secondary contact, these behavioral preferences exaggerate the characteristics evolved during periods of isolation. Previously, specimens from the vicinity of Houston, Texas, were described as *Hyla semifasciata,* was identified in (Leidy et al., 1856), and the basis for its distinction from the eastern clade was morphological differences between them. This was later synonymized with the earlier-described *D. cinereus* (Schneider, 1799). However, amphibians commonly exhibit morphological conservatism, and in the southeastern United States, many of the amphibians are morphologically conservative and thus exhibit few differences between species (Hillis et al., 1983; Stuart et al., 2006). Sometimes, minor morphological differences thought to be uninformative do, in the light of modern molecular data, prove to be consistent with the presence of distinct species rather than variation within a species. Furthermore, many cryptic species have been discovered in this region, making this a likely scenario (Hillis et al., 1983; Lemmon et al., 2007). Lisa Barrow and colleagues estimated over 2 million years' divergence between the eastern and western clades of *D. cinereus* (with their highly divergent samples corresponding to samples from just east of the Mississippi in the same areas where I found frogs with "western" genetic composition) (Barrow et al., 2016). Although only two samples were collected by her on the eastern side of the river representing the western clade of *D. cinereus* that I identified, the correspondence between the results of our completely independent studies is striking. Further, the geographic genetic variation that we found corresponds extremely closely with call variation identified by Höbel & Gerhardt (2003). This study with fewer loci but more samples from the western clade than Barrow et al. (2016) found that approximately 6 million years separated the two groups. While divergence estimates calibrated with fossils from the North American hylids can show varying results (Soltis et al., 2006), both studies indicate that considerable differences exist between these two clades.

Estimates of this variation between 2.4 and 6 mya would correspond to dramatic changes in river systems and sea levels in the United States, and these have been driving factors in many speciation processes (Barrow et al., 2016; Austin et al., 2004). After these two lineages diverged genetically and behaviorally, secondary contact between them could make them appear as a continuous lineage, but they would still be separated behaviorally in areas of sympatry. Mitochondrial and nuclear data indicate that the eastern and western clades are distinct lineages, and the considerable overlap in the niche models without the presence of the genetic haplotypes could indicate competitive exclusion between these ecologically similar species. If further investigation indicates that these are two distinct lineages, naming the western clade *Dryophytes semifasciatus* honors its original description in 1856.

Compared to *Dryophytes cinereus, D. squirellus* is a much smaller frog, which does not breed in large permanent bodies of water containing predatory fish. Yet, this species does not show any variation in population structure on either side of the Mississippi River. This lack of population structure can also be explained behaviorally as its migratory patterns are strongly affected by rains (Taylor et al., 2007; Wright, 1931). Similarly, the Spring Peeper, another comparably sized treefrog inhabiting this region, does not show patterns concordant with the Riverine Barriers Hypothesis (Austin et al., 2004). The agreement between these studies indicate that riverine barriers' influence on anuran populations might not be as strong as in other groups of animals.

Future directions for this study include obtaining more samples of both *Dryophytes cinereus* and *D. gratiosus* to be sequenced for additional loci to understand the extent of hybridization in this group. While the amount of genetic material used in this study is standard for phylogenetic and phylogeographic studies, it lacks the amount of data to sufficiently investigate hybridization patterns. The low F_{ST} values between the eastern clade of *D. cinereus* and *D. gratiosus* cannot be thoroughly addressed using one nuclear gene; although it does provide interesting directions for future studies. The use of next gen sequencing methods, for example RAD-seq, would be ideal to investigate this relationship with additional samples (Davey & Blaxter 2010; Hohenlohe et al., 2011). Additionally, the long branch lengths and outstanding sequence divergences found for some of the specimens in the phylogeographic study need to be investigated further. These samples need to be re-amplified and sequenced to verify the results as they could alter the topologies recovered in the phylogenetic trees. The museum specimens also need to be physically obtained to verify accurate identification of these specimens. Furthermore, using calling males to focus collection efforts introduces the possibility of having more males in the dataset than females, thus making the signals from directional introgression difficult to detect. Additional samples of both males and females would be needed to investigate patterns in nuclear and mitochondrial gene flow between these groups. Obtaining naturally occurring hybrids would also be useful in continuing to investigate these questions. However, considering that success of hybrids in reproduction and survivorship is lower than non-hybrids, and their presence in the wild is much lower, obtaining these samples would be difficult using traditional collecting methods (Höbel & Gerhardt, 2003).

Increasing in frequency, the discovery of cryptic species reveals that temperate regions support more species diversity than previously considered. This study identified that while some groups might span large ranges and exhibit little genetic variation, other closely related species span the same region and reveal surprising patterns of diversification. *Dryophytes squirellus* exhibits low geographic variation in the loci used in this study, and the Riverine Barriers Hypothesis does not appear to predict geographic breaks within this species. In regards to the other two species in this group, the Riverine Barriers Hypothesis potentially explains some of the variation. While *D. cinereus* exhibits geographic variation near the Mississippi River, the influence of the river directly on this species is debatable. The presence of individuals on the opposite side of the river than their closest relatives suggests that the river poses little resistance to dispersal. However, a solution must exist explaining the presence of geographic variation in *D. cinereus*. To better understand the processes underlying this pattern, additional investigations must be executed. The results of this study support the hypothesis proposed by Lamb & Avise (1986) regarding the occurrence of hybridization in this group. While this study did not investigate the mechanisms behind behavioral isolation, it provides a genetic platform to continue addressing the questions posed by Gerhardt (Höbel & Gerhardt, 2003). The conflicting results from this study further illustrate the complexity of biodiversity and the plethora of potential factors contributing to its evolution. While individual studies estimate the effects of isolated variables on diversification, few things certainly define biodiversity. As our understanding of biodiversity becomes increasingly more complex, the importance of conserving biodiversity becomes increasingly more important, as losses are unrecoverable with unpredictable impacts on other species

Appendix 1.

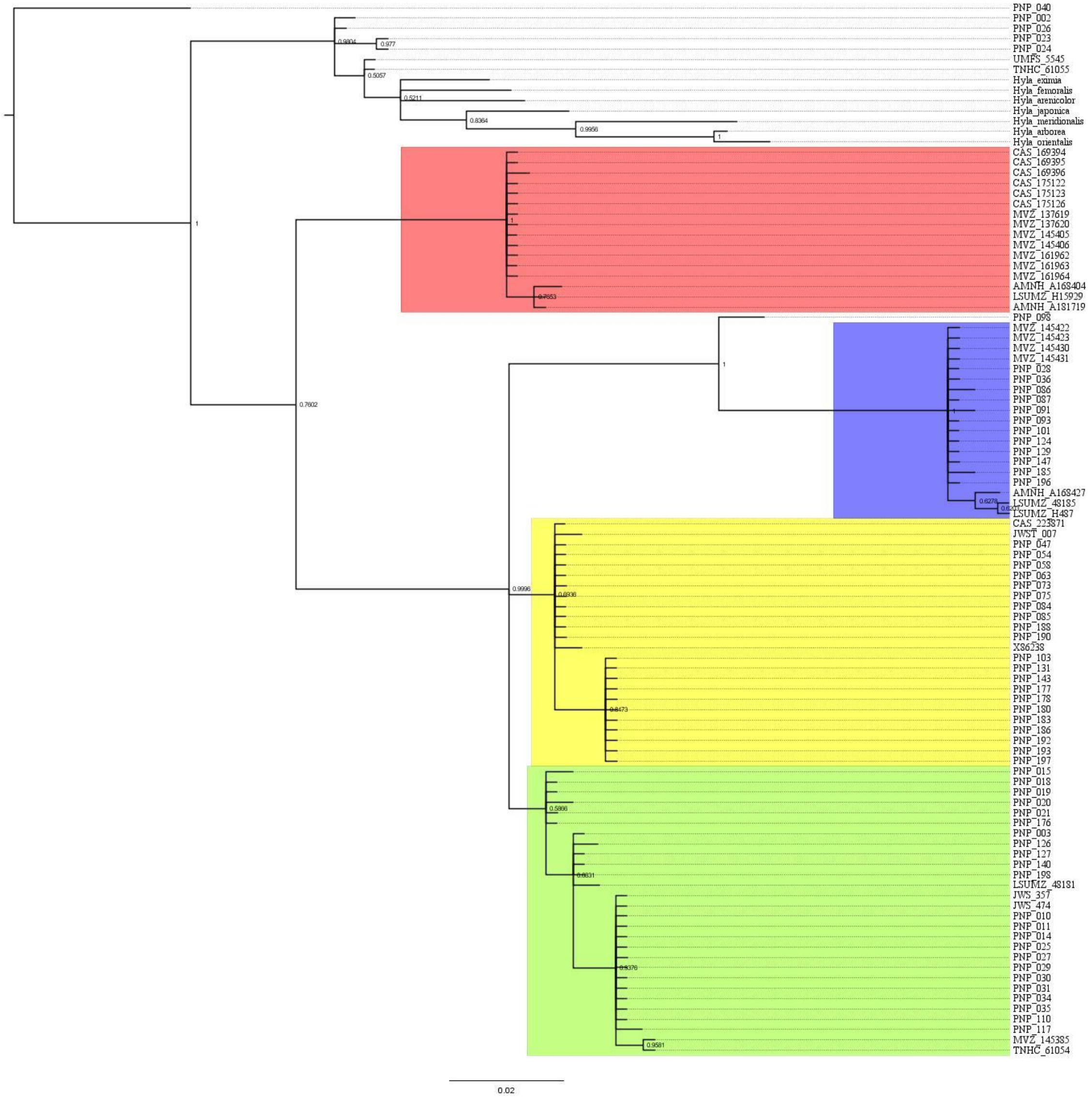

Figure A.1.1. Bayesian tree using the mitochondrial gene 12S. Numbers at the nodes represent Bayesian posterior probabilities. Samples highlighted in red are *Dryophytes gratiosus*, samples in blue are *Dryophytes squirellus*, samples in yellow are from the eastern clade of *Dryophytes cinereus*, and samples in green are from the western clade of *Dryophytes cinereus*.

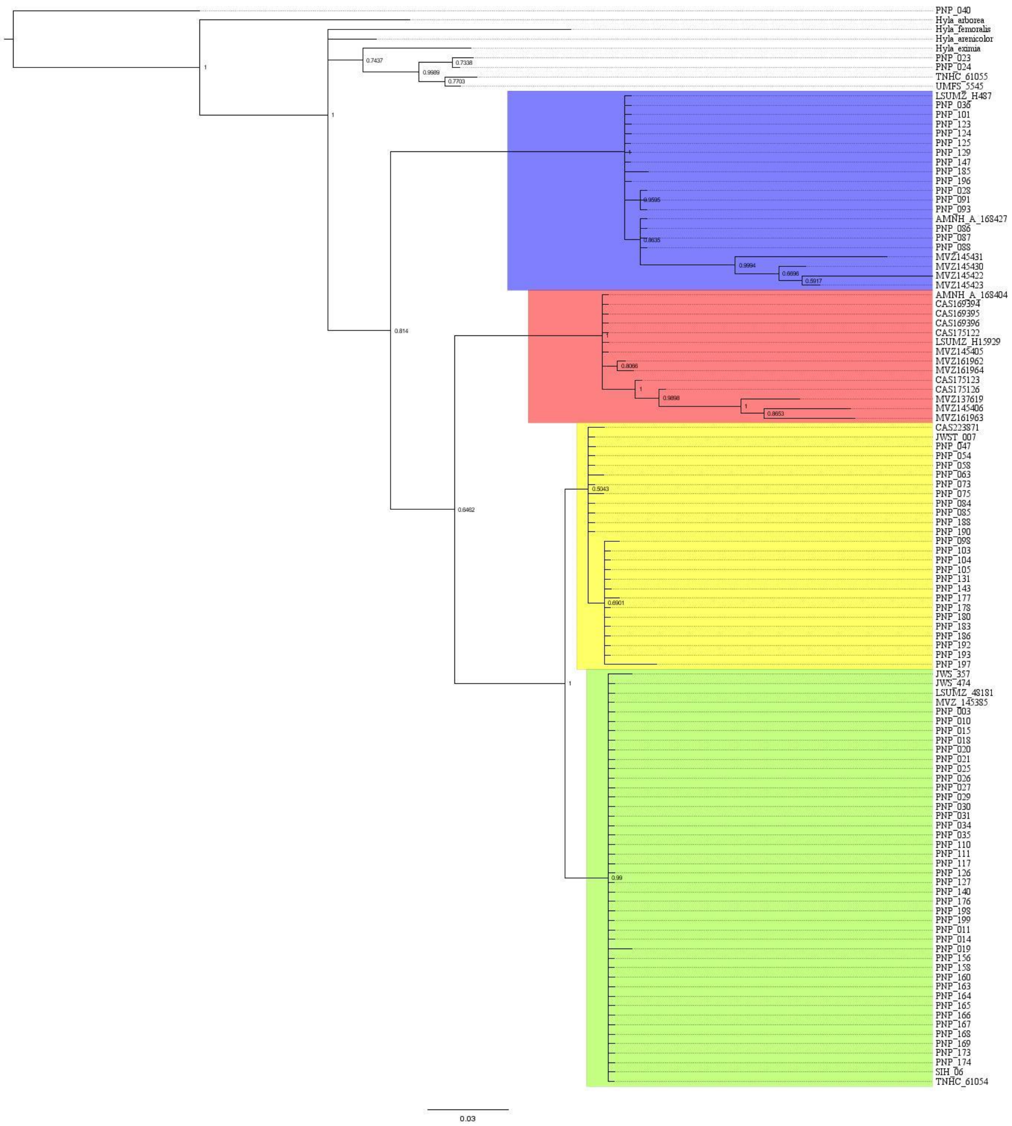

Figure A.1.2. Bayesian tree using the mitochondrial gene 16S. Numbers at the nodes represent Bayesian posterior probabilities. Samples highlighted in red are *Dryophytes gratiosus*, samples in blue are *Dryophytes squirellus*, samples in yellow are from the eastern clade of *Dryophytes cinereus*, and samples in green are from the western clade of *Dryophytes cinereus.*

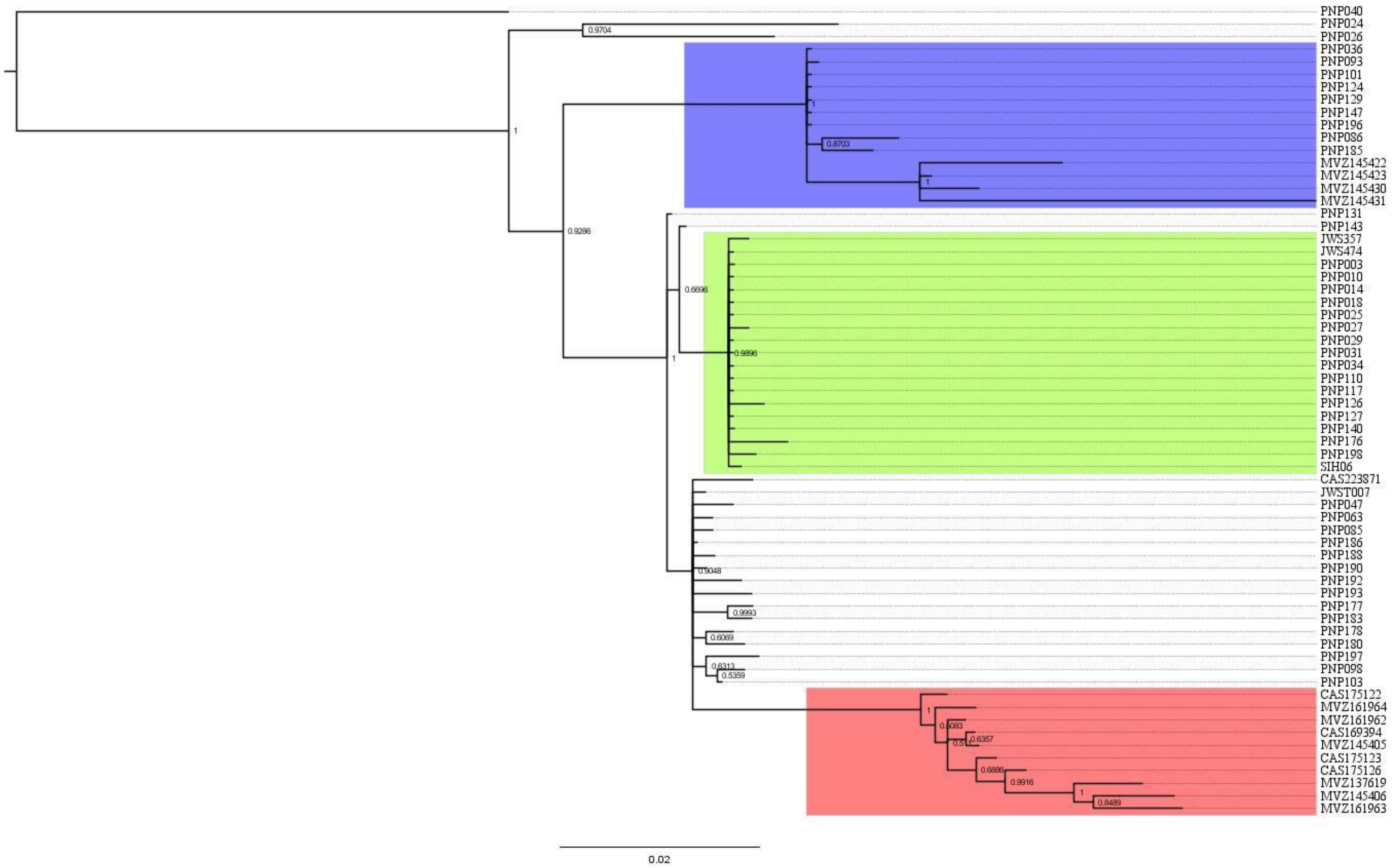

Figure A.1.3. Bayesian tree using the nuclear gene RAG-1 and the mitochondrial gene 16S. Numbers at the nodes represent Bayesian posterior probabilities. Samples highlighted in red are *Dryophytes gratiosus*, samples in blue are *Dryophytes squirellus*, samples that aren't highlighted are from the eastern clade of *Dryophytes cinereus*, and samples in green are from the western clade of *Dryophytes cinereus.*

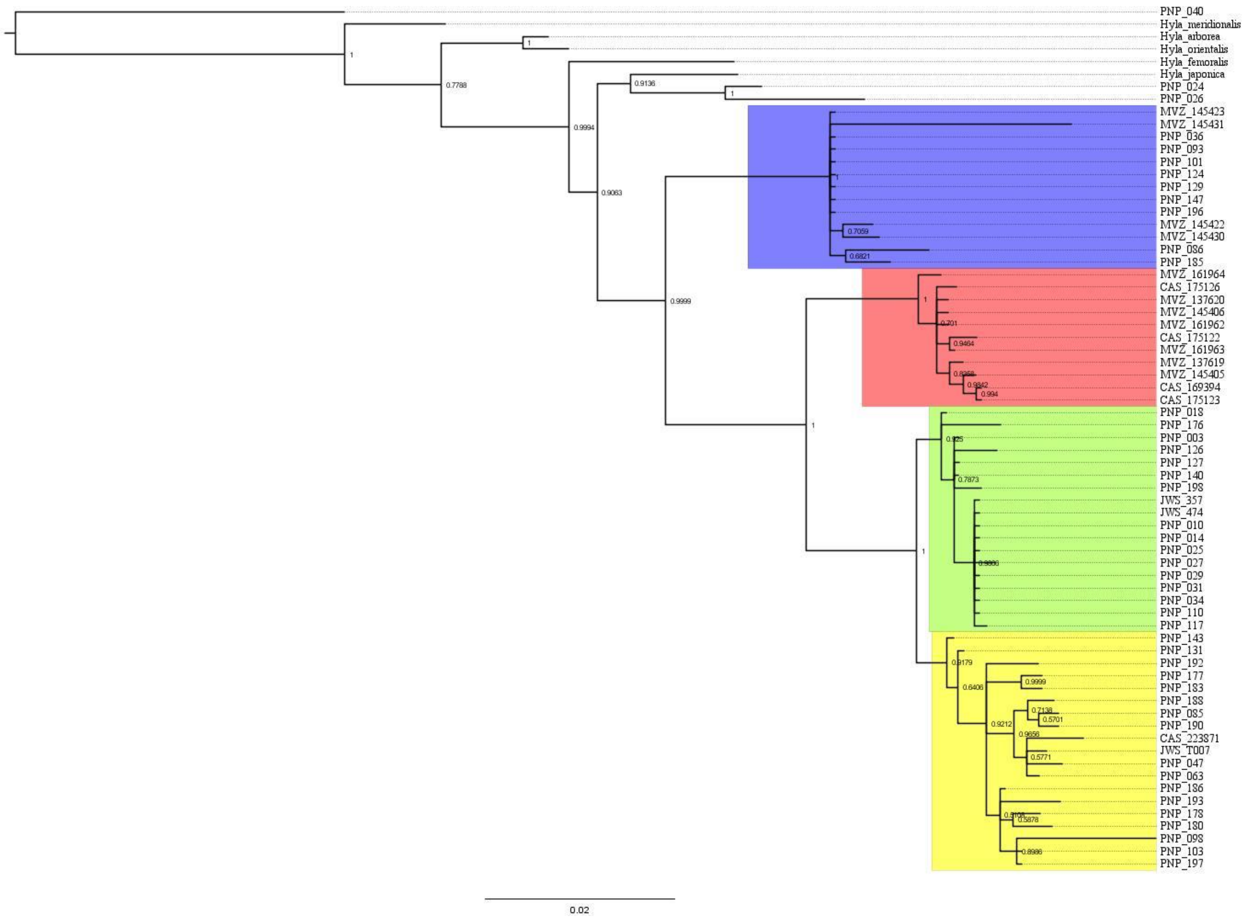

Figure A.1.4. Bayesian tree using the nuclear gene RAG-1 and the mitochondrial gene 12S. Numbers at the nodes represent Bayesian posterior probabilities. Samples highlighted in red are *Dryophytes gratiosus*, samples in blue are *Dryophytes squirellus*, samples in yellow are from the eastern clade of *Dryophytes cinereus*, and samples in green are from the western clade of *Dryophytes cinereus.*

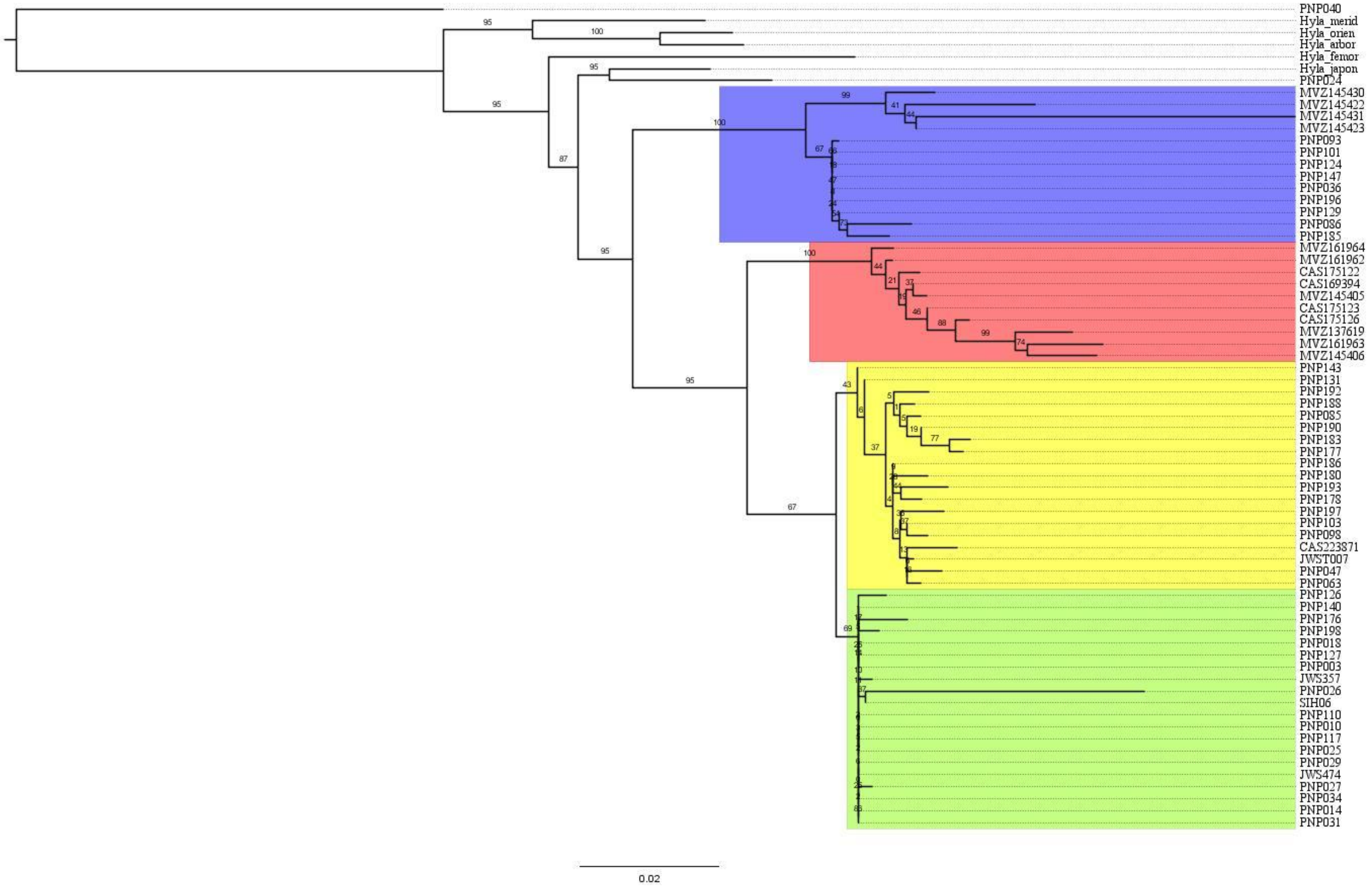

Figure A.1.5. Maximum likelihood tree using the nuclear gene RAG-1 and the mitochondrial gene 16S. Numbers on the branches represent ML scores. Samples highlighted in red are *Dryophytes gratiosus*, samples in blue are *Dryophytes squirellus*, samples in yellow are from the eastern clade of *Dryophytes cinereus*, and samples in green are from the western clade of *Dryophytes cinereus*

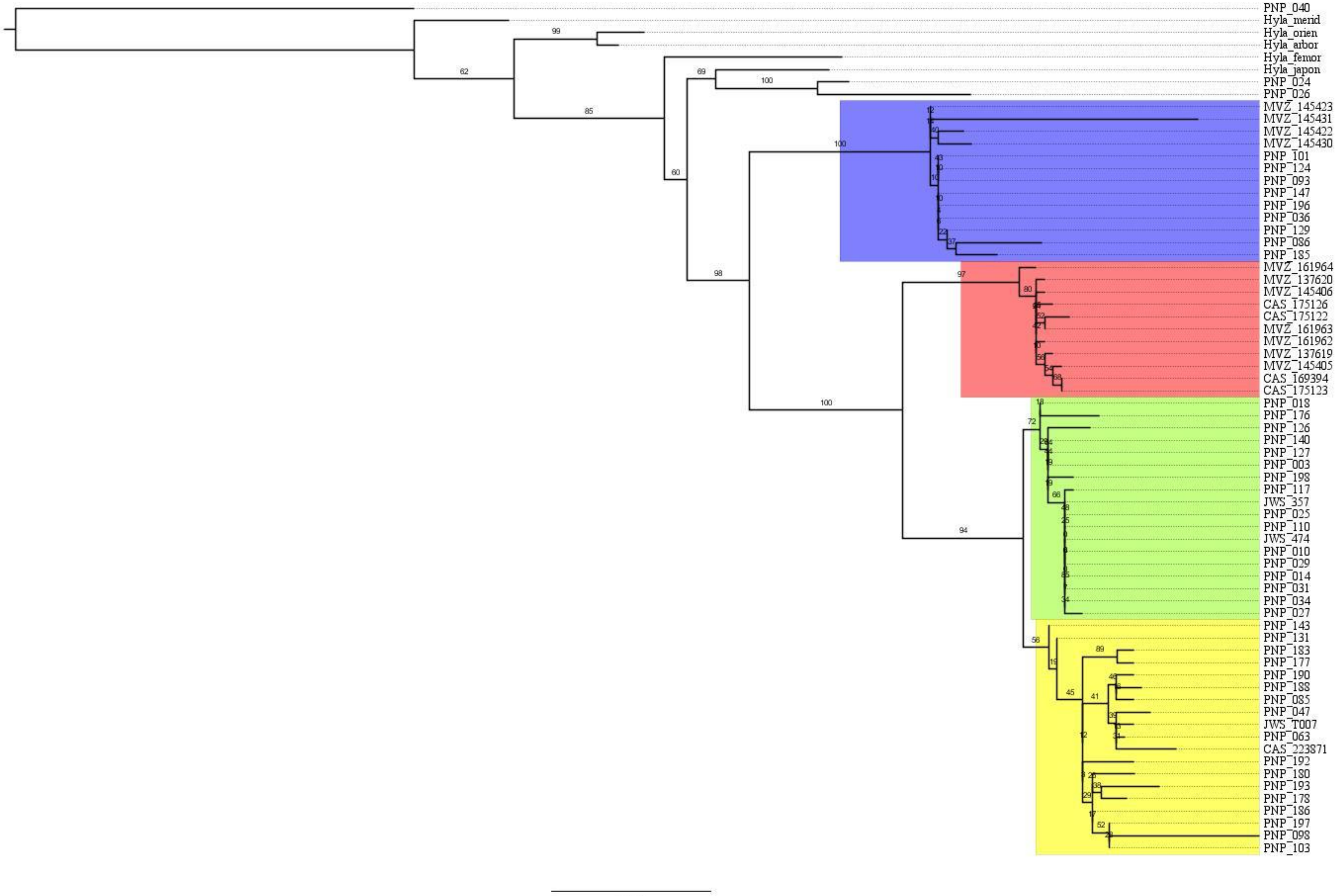

Figure A.1.6. Maximum likelihood tree using the nuclear gene RAG-1 and the mitochondrial gene 12S. Numbers on the branches represent ML scores. Samples highlighted in red are *Dryophytes gratiosus*, samples in blue are *Dryophytes squirellus*, samples in yellow are from the eastern clade of *Dryophytes cinereus*, and samples in green are from the western clade of *Dryophytes cinereus*

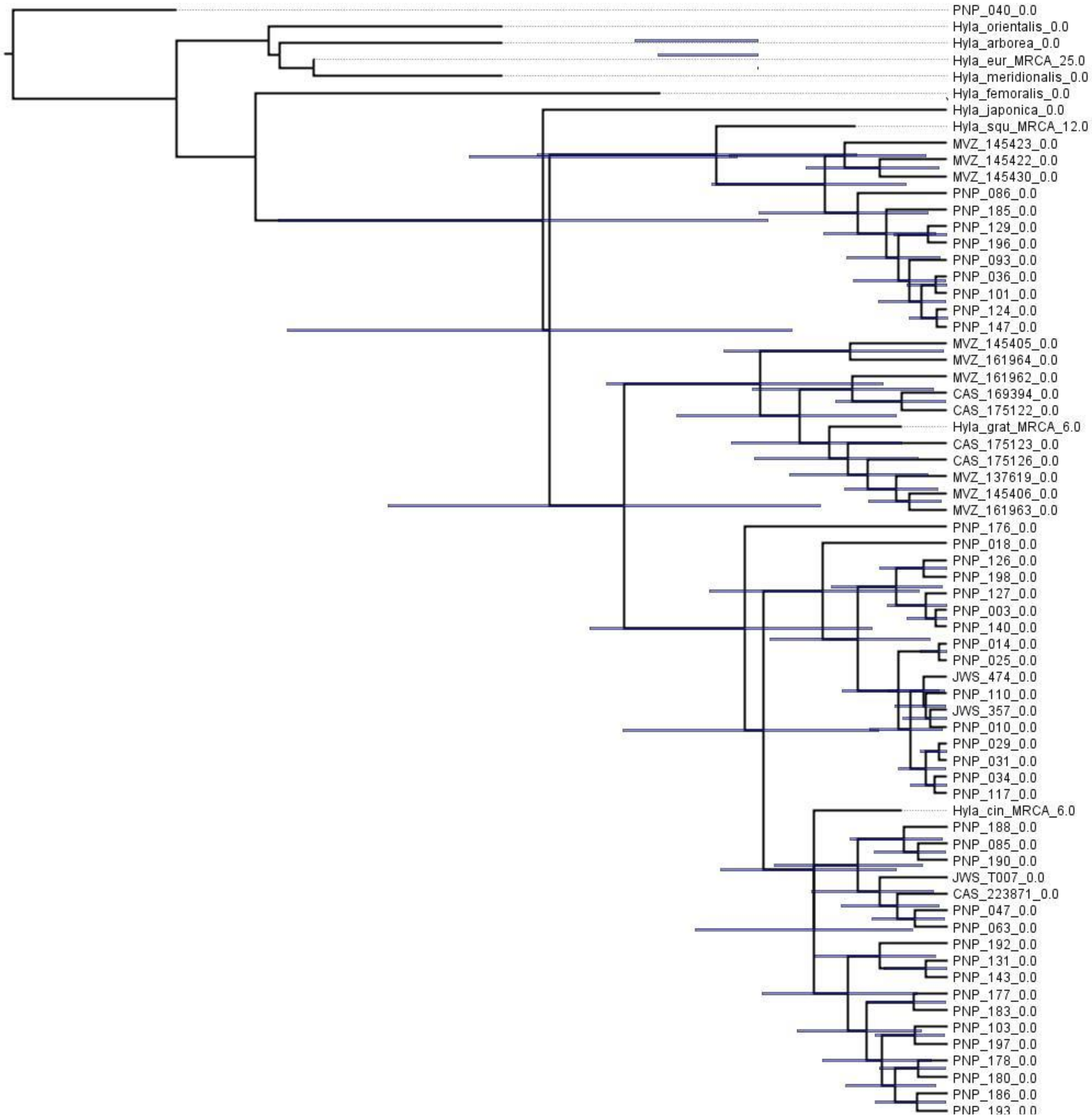

Figure A.1.7. Divergence estimates for the *Dryophytes cinereus* species group using the HKY model of nucleotide substitution. Data for this tree includes all loci sampled (12S, 16S, and RAG-1). Bars represent 95% highest posterior densities (HPD).

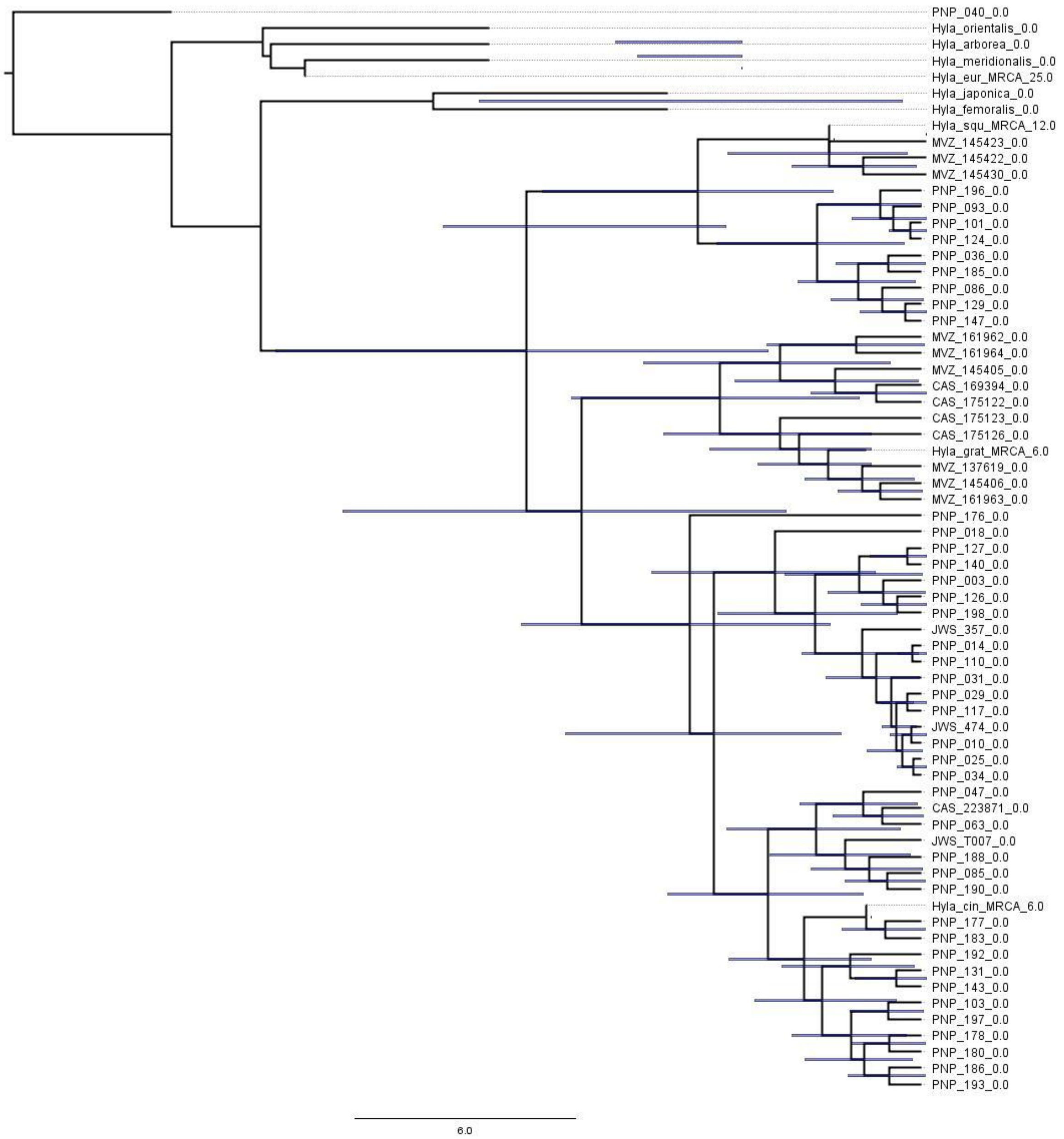

Figure A.1.8. Divergence estimates for the *Dryophytes cinereus* species group using the GTR model of nucleotide substitution. Data for this tree includes all loci sampled (12S, 16S, and RAG-1). Bars represent 95% highest posterior densities (HPD).

Table A.1.1. Pairwise nucleotide differences in nuclear RAG-1 sequence. Cells highlighted in blue represent samples from Dryophytes *squirellus*, cells highlighted in red represent samples from *D. gratiosus*, cells highlighted in yellow represent samples from the eastern clade of *D. cinereus*, and cells highlighted in green represent samples from the western clade of *D. cinereus*.

		1	2	3	4	5	6	7	8	9	10	11	12
1	MVZ145422												
2	MVZ145423	0.006											
3	MVZ145430	0.010	0.007										
4	MVZ145431	0.050	0.044	0.054									
5	PNP036	0.006	0.000	0.006	0.046								
6	PNP093	0.006	0.000	0.007	0.044	0.000							
7	PNP101	0.006	0.000	0.007	0.046	0.000	0.000						
8	PNP124	0.006	0.000	0.007	0.046	0.000	0.000	0.000					
9	PNP129	0.007	0.001	0.006	0.047	0.000	0.001	0.001	0.001				
10	PNP147	0.006	0.000	0.006	0.046	0.000	0.000	0.000	0.000	0.000			
11	PNP196	0.006	0.000	0.004	0.044	0.000	0.000	0.000	0.000	0.000	0.000		
12	PNP086	0.023	0.017	0.020	0.058	0.015	0.015	0.017	0.017	0.015	0.015	0.014	
13	PNP185	0.017	0.011	0.015	0.054	0.009	0.007	0.011	0.011	0.009	0.009	0.009	0.018
14	CAS169394	0.029	0.026	0.034	0.069	0.024	0.026	0.026	0.026	0.027	0.026	0.023	0.044
15	CAS175122	0.032	0.029	0.033	0.074	0.026	0.029	0.029	0.029	0.027	0.027	0.024	0.043
16	CAS175123	0.027	0.024	0.032	0.067	0.023	0.024	0.024	0.024	0.026	0.024	0.021	0.042
17	CAS175126	0.029	0.026	0.033	0.071	0.024	0.026	0.026	0.026	0.027	0.026	0.023	0.043
18	MVZ137619	0.029	0.026	0.033	0.070	0.024	0.026	0.026	0.026	0.027	0.026	0.023	0.043
19	MVZ137620	0.021	0.018	0.026	0.062	0.017	0.018	0.018	0.018	0.020	0.018	0.015	0.035
20	MVZ145405	0.026	0.023	0.030	0.066	0.021	0.023	0.023	0.023	0.024	0.023	0.020	0.040
21	MVZ145406	0.024	0.021	0.029	0.066	0.020	0.021	0.021	0.021	0.023	0.021	0.018	0.039
22	MVZ161962	0.027	0.024	0.032	0.069	0.023	0.024	0.024	0.024	0.026	0.024	0.021	0.042
23	MVZ161963	0.027	0.024	0.032	0.069	0.023	0.024	0.024	0.024	0.026	0.024	0.021	0.042
24	MVZ161964	0.026	0.023	0.027	0.067	0.020	0.023	0.023	0.023	0.021	0.021	0.018	0.037
25	CAS223871	0.037	0.035	0.038	0.081	0.032	0.035	0.035	0.035	0.033	0.034	0.031	0.050
26	JWST007	0.027	0.024	0.029	0.068	0.021	0.024	0.024	0.024	0.023	0.023	0.020	0.039
27	PNP047	0.034	0.029	0.032	0.071	0.027	0.031	0.030	0.030	0.029	0.029	0.026	0.045
28	PNP063	0.026	0.023	0.027	0.067	0.020	0.023	0.023	0.023	0.021	0.021	0.018	0.037
29	PNP103	0.030	0.027	0.032	0.072	0.024	0.027	0.027	0.027	0.026	0.026	0.023	0.042
30	PNP178	0.033	0.030	0.035	0.072	0.027	0.029	0.030	0.030	0.029	0.029	0.026	0.043

Table A.1.1. Continued

		1	2	3	4	5	6	7	8	9	10	11	12
31	PNP180	0.035	0.032	0.037	0.076	0.029	0.032	0.032	0.032	0.030	0.030	0.027	0.047
32	PNP186	0.024	0.021	0.027	0.066	0.020	0.021	0.021	0.021	0.021	0.021	0.018	0.037
33	PNP193	0.032	0.029	0.034	0.071	0.026	0.027	0.029	0.029	0.027	0.027	0.024	0.042
34	PNP197	0.029	0.026	0.030	0.071	0.023	0.026	0.026	0.026	0.024	0.024	0.021	0.040
35	PNP098	0.032	0.029	0.035	0.072	0.027	0.029	0.029	0.029	0.029	0.029	0.026	0.045
36	PNP085	0.028	0.024	0.029	0.066	0.021	0.024	0.024	0.024	0.023	0.023	0.020	0.037
37	PNP188	0.031	0.027	0.032	0.071	0.024	0.027	0.027	0.027	0.026	0.026	0.023	0.042
38	PNP192	0.035	0.032	0.037	0.078	0.029	0.032	0.032	0.032	0.030	0.030	0.027	0.047
39	PNP190	0.027	0.024	0.032	0.069	0.023	0.024	0.024	0.024	0.026	0.024	0.021	0.042
40	PNP177	0.034	0.030	0.037	0.076	0.029	0.030	0.030	0.030	0.030	0.030	0.027	0.047
41	PNP183	0.030	0.027	0.034	0.072	0.026	0.027	0.027	0.027	0.027	0.027	0.024	0.043
42	PNP131	0.021	0.018	0.026	0.062	0.017	0.018	0.018	0.018	0.020	0.018	0.015	0.035
43	JWS357	0.023	0.020	0.027	0.064	0.018	0.020	0.020	0.020	0.021	0.020	0.017	0.037
44	JWS474	0.023	0.020	0.027	0.064	0.018	0.020	0.020	0.020	0.021	0.020	0.017	0.037
45	PNP003	0.023	0.020	0.027	0.064	0.018	0.020	0.020	0.020	0.021	0.020	0.017	0.037
46	PNP010	0.023	0.020	0.027	0.064	0.018	0.020	0.020	0.020	0.021	0.020	0.017	0.037
47	PNP014	0.023	0.020	0.027	0.064	0.018	0.020	0.020	0.020	0.021	0.020	0.017	0.037
48	PNP018	0.023	0.020	0.027	0.064	0.018	0.020	0.020	0.020	0.021	0.020	0.017	0.037
49	PNP025	0.023	0.020	0.027	0.064	0.018	0.020	0.020	0.020	0.021	0.020	0.017	0.037
50	PNP027	0.026	0.023	0.031	0.067	0.022	0.023	0.023	0.023	0.025	0.023	0.022	0.041
51	PNP029	0.023	0.020	0.027	0.064	0.018	0.020	0.020	0.020	0.021	0.020	0.017	0.037
52	PNP031	0.023	0.020	0.027	0.064	0.018	0.020	0.020	0.020	0.021	0.020	0.017	0.037
53	PNP034	0.021	0.018	0.026	0.063	0.017	0.018	0.018	0.018	0.020	0.018	0.017	0.036
54	PNP110	0.023	0.020	0.027	0.064	0.018	0.020	0.020	0.020	0.021	0.020	0.017	0.037
55	PNP117	0.023	0.020	0.027	0.064	0.018	0.020	0.020	0.020	0.021	0.020	0.017	0.037
56	PNP126	0.029	0.026	0.034	0.071	0.024	0.026	0.026	0.026	0.027	0.026	0.023	0.044
57	PNP127	0.023	0.020	0.027	0.064	0.018	0.020	0.020	0.020	0.021	0.020	0.017	0.037
58	PNP140	0.023	0.020	0.027	0.064	0.018	0.020	0.020	0.020	0.021	0.020	0.017	0.037
59	PNP143	0.023	0.020	0.027	0.064	0.018	0.020	0.020	0.020	0.021	0.020	0.017	0.037
60	PNP176	0.034	0.030	0.038	0.076	0.029	0.031	0.030	0.030	0.032	0.030	0.028	0.048

Table A.1.1. Continued

		1	2	3	4	5	6	7	8	9	10	11	12
61	PNP198	0.027	0.024	0.032	0.069	0.023	0.024	0.024	0.024	0.026	0.024	0.021	0.040
62	SIH06	0.024	0.021	0.029	0.066	0.020	0.021	0.021	0.021	0.023	0.021	0.018	0.039
63	*Hyla_arborea*	0.037	0.030	0.038	0.073	0.027	0.030	0.030	0.030	0.032	0.030	0.029	0.048
64	*Hyla_femoralis*	0.035	0.030	0.038	0.079	0.029	0.030	0.030	0.030	0.032	0.030	0.029	0.048
65	*Hyla_japonica*	0.024	0.018	0.026	0.066	0.017	0.018	0.018	0.018	0.020	0.018	0.017	0.035
66	*Hyla_orientalis*	0.038	0.032	0.040	0.078	0.029	0.032	0.032	0.032	0.033	0.032	0.031	0.050
67	*Hyla_meridionalis*	0.048	0.041	0.046	0.086	0.038	0.041	0.041	0.041	0.043	0.041	0.040	0.060
68	Hyla versicolor	0.032	0.029	0.034	0.072	0.026	0.029	0.029	0.029	0.027	0.027	0.026	0.044
69	Osteopilus septentrionalis	0.104	0.097	0.102	0.139	0.095	0.097	0.097	0.097	0.098	0.097	0.096	0.118

Table A.1.1. Continued

		13	14	15	16	17	18	19	20	21	22	23	24
1	MVZ145422												
2	MVZ145423												
3	MVZ145430												
4	MVZ145431												
5	PNP036												
6	PNP093												
7	PNP101												
8	PNP124												
9	PNP129												
10	PNP147												
11	PNP196												
12	PNP086												
13	PNP185												
14	CAS169394	0.037											
15	CAS175122	0.037	0.006										
16	CAS175123	0.035	0.000	0.006									
17	CAS175126	0.037	0.007	0.009	0.006								
18	MVZ137619	0.037	0.004	0.009	0.004	0.006							
19	MVZ137620	0.029	0.006	0.007	0.004	0.004	0.004						
20	MVZ145405	0.034	0.003	0.010	0.003	0.007	0.004	0.004					
21	MVZ145406	0.032	0.006	0.007	0.004	0.004	0.004	0.003	0.006				
22	MVZ161962	0.035	0.006	0.007	0.004	0.004	0.004	0.003	0.006	0.003			
23	MVZ161963	0.035	0.004	0.004	0.004	0.004	0.004	0.003	0.006	0.003	0.003		
24	MVZ161964	0.031	0.010	0.009	0.009	0.009	0.009	0.004	0.006	0.006	0.007	0.007	
25	CAS223871	0.043	0.017	0.012	0.015	0.015	0.015	0.014	0.017	0.014	0.013	0.013	0.015
26	JWST007	0.032	0.006	0.004	0.006	0.006	0.004	0.004	0.006	0.004	0.004	0.004	0.004
27	PNP047	0.039	0.014	0.010	0.014	0.014	0.012	0.011	0.012	0.011	0.012	0.012	0.010
28	PNP063	0.031	0.009	0.003	0.007	0.007	0.007	0.004	0.006	0.006	0.006	0.004	0.003
29	PNP103	0.035	0.009	0.004	0.007	0.007	0.007	0.006	0.009	0.006	0.006	0.006	0.007
30	PNP178	0.037	0.010	0.007	0.010	0.010	0.010	0.009	0.012	0.009	0.009	0.006	0.010

Table A.1.1. Continued

		13	14	15	16	17	18	19	20	21	22	23	24
31	PNP180	0.040	0.012	0.009	0.012	0.012	0.012	0.010	0.013	0.010	0.010	0.007	0.012
32	PNP186	0.031	0.003	0.004	0.003	0.003	0.001	0.001	0.003	0.001	0.001	0.001	0.004
33	PNP193	0.035	0.012	0.015	0.012	0.012	0.012	0.012	0.010	0.013	0.013	0.013	0.013
34	PNP197	0.034	0.007	0.003	0.006	0.006	0.006	0.004	0.007	0.004	0.004	0.004	0.006
35	PNP098	0.038	0.004	0.007	0.004	0.009	0.006	0.007	0.004	0.007	0.007	0.007	0.010
36	PNP085	0.031	0.012	0.011	0.011	0.011	0.011	0.008	0.011	0.008	0.009	0.009	0.007
37	PNP188	0.035	0.012	0.010	0.011	0.010	0.010	0.007	0.010	0.007	0.009	0.009	0.007
38	PNP192	0.040	0.017	0.015	0.015	0.015	0.015	0.012	0.015	0.012	0.013	0.013	0.012
39	PNP190	0.035	0.009	0.010	0.007	0.007	0.007	0.004	0.007	0.004	0.006	0.006	0.007
40	PNP177	0.040	0.011	0.013	0.011	0.012	0.009	0.010	0.010	0.010	0.010	0.010	0.012
41	PNP183	0.037	0.010	0.013	0.010	0.013	0.010	0.010	0.010	0.010	0.012	0.012	0.012
42	PNP131	0.029	0.009	0.010	0.007	0.007	0.007	0.003	0.004	0.004	0.006	0.006	0.004
43	JWS357	0.031	0.009	0.010	0.007	0.007	0.007	0.004	0.006	0.004	0.006	0.006	0.006
44	JWS474	0.031	0.010	0.012	0.009	0.009	0.009	0.004	0.006	0.006	0.007	0.007	0.006
45	PNP003	0.031	0.010	0.012	0.009	0.009	0.009	0.004	0.006	0.006	0.007	0.007	0.006
46	PNP010	0.031	0.010	0.012	0.009	0.009	0.009	0.004	0.006	0.006	0.007	0.007	0.006
47	PNP014	0.031	0.010	0.012	0.009	0.009	0.009	0.004	0.006	0.006	0.007	0.007	0.006
48	PNP018	0.031	0.010	0.012	0.009	0.009	0.009	0.004	0.006	0.006	0.007	0.007	0.006
49	PNP025	0.031	0.010	0.012	0.009	0.009	0.009	0.004	0.006	0.006	0.007	0.007	0.006
50	PNP027	0.035	0.015	0.017	0.014	0.014	0.014	0.009	0.012	0.011	0.012	0.012	0.012
51	PNP029	0.031	0.010	0.012	0.009	0.009	0.009	0.004	0.006	0.006	0.007	0.007	0.006
52	PNP031	0.031	0.010	0.012	0.009	0.009	0.009	0.004	0.006	0.006	0.007	0.007	0.006
53	PNP034	0.029	0.010	0.012	0.009	0.009	0.009	0.004	0.006	0.004	0.007	0.007	0.006
54	PNP110	0.031	0.010	0.012	0.009	0.009	0.009	0.004	0.006	0.006	0.007	0.007	0.006
55	PNP117	0.031	0.010	0.012	0.009	0.009	0.009	0.004	0.006	0.006	0.007	0.007	0.006
56	PNP126	0.037	0.017	0.018	0.015	0.012	0.015	0.011	0.012	0.012	0.013	0.013	0.012
57	PNP127	0.031	0.010	0.012	0.009	0.009	0.009	0.004	0.006	0.006	0.007	0.007	0.006
58	PNP140	0.031	0.010	0.012	0.009	0.009	0.009	0.004	0.006	0.006	0.007	0.007	0.006
59	PNP143	0.031	0.010	0.012	0.009	0.009	0.009	0.004	0.006	0.006	0.007	0.007	0.006
60	PNP176	0.042	0.021	0.023	0.020	0.020	0.020	0.015	0.017	0.017	0.018	0.018	0.017

Table A.1.1. Continued

		13	14	15	16	17	18	19	20	21	22	23	24
61	PNP198	0.032	0.015	0.016	0.014	0.010	0.013	0.009	0.010	0.010	0.012	0.012	0.010
62	SIH06	0.032	0.012	0.013	0.010	0.010	0.010	0.006	0.007	0.007	0.007	0.009	0.007
63	*Hyla_arborea*	0.042	0.046	0.048	0.046	0.048	0.048	0.040	0.045	0.045	0.046	0.043	0.041
64	*Hyla_femoralis*	0.042	0.041	0.044	0.042	0.044	0.044	0.038	0.041	0.041	0.043	0.041	0.041
65	*Hyla_japonica*	0.029	0.035	0.038	0.034	0.035	0.035	0.027	0.032	0.032	0.033	0.033	0.032
66	*Hyla_orientalis*	0.043	0.048	0.049	0.048	0.049	0.049	0.041	0.046	0.046	0.048	0.044	0.043
67	*Hyla_meridionalis*	0.053	0.056	0.056	0.056	0.056	0.056	0.048	0.053	0.053	0.054	0.051	0.049
68	*Hyla versicolor*	0.037	0.043	0.043	0.042	0.043	0.043	0.035	0.040	0.040	0.040	0.041	0.037
69	*Osteopilus septentrionalis*	0.108	0.102	0.103	0.102	0.105	0.105	0.097	0.100	0.102	0.103	0.100	0.100

Table A.1.1. Continued

		25	26	27	28	29	30	31	32	33	34	35	36
1	MVZ145422												
2	MVZ145423												
3	MVZ145430												
4	MVZ145431												
5	PNP036												
6	PNP093												
7	PNP101												
8	PNP124												
9	PNP129												
10	PNP147												
11	PNP196												
12	PNP086												
13	PNP185												
14	CAS169394												
15	CAS175122												
16	CAS175123												
17	CAS175126												
18	MVZ137619												
19	MVZ137620												
20	MVZ145405												
21	MVZ145406												
22	MVZ161962												
23	MVZ161963												
24	MVZ161964												
25	CAS223871												
26	JWST007	0.011											
27	PNP047	0.017	0.008										
28	PNP063	0.011	0.003	0.007									
29	PNP103	0.010	0.001	0.009	0.003								
30	PNP178	0.017	0.007	0.015	0.007	0.009							

Table A.1.1. Continued

		25	26	27	28	29	30	31	32	33	34	35	36
31	PNP180	0.017	0.007	0.017	0.009	0.010	0.010						
32	PNP186	0.011	0.001	0.009	0.003	0.001	0.006	0.007					
33	PNP193	0.021	0.011	0.020	0.010	0.013	0.013	0.018	0.007				
34	PNP197	0.009	0.001	0.007	0.001	0.000	0.007	0.009	0.001	0.012			
35	PNP098	0.014	0.003	0.011	0.006	0.003	0.012	0.013	0.003	0.013	0.003		
36	PNP085	0.014	0.005	0.012	0.008	0.009	0.011	0.009	0.006	0.017	0.008	0.012	
37	PNP188	0.014	0.006	0.009	0.007	0.009	0.012	0.010	0.006	0.018	0.007	0.012	0.006
38	PNP192	0.020	0.011	0.017	0.012	0.013	0.017	0.018	0.011	0.023	0.012	0.017	0.012
39	PNP190	0.015	0.006	0.012	0.007	0.009	0.012	0.013	0.004	0.018	0.007	0.010	0.006
40	PNP177	0.018	0.008	0.015	0.012	0.009	0.015	0.017	0.006	0.017	0.009	0.009	0.011
41	PNP183	0.020	0.004	0.014	0.010	0.009	0.016	0.018	0.006	0.015	0.009	0.009	0.014
42	PNP131	0.016	0.006	0.012	0.004	0.009	0.012	0.013	0.004	0.013	0.007	0.010	0.006
43	JWS357	0.017	0.006	0.012	0.006	0.009	0.012	0.013	0.004	0.015	0.007	0.010	0.006
44	JWS474	0.018	0.007	0.013	0.006	0.010	0.013	0.015	0.006	0.015	0.009	0.012	0.008
45	PNP003	0.018	0.007	0.013	0.006	0.010	0.013	0.015	0.006	0.015	0.009	0.012	0.008
46	PNP010	0.018	0.007	0.013	0.006	0.010	0.013	0.015	0.006	0.015	0.009	0.012	0.008
47	PNP014	0.018	0.007	0.014	0.006	0.010	0.013	0.015	0.006	0.015	0.009	0.012	0.008
48	PNP018	0.018	0.007	0.013	0.006	0.010	0.013	0.015	0.006	0.015	0.009	0.012	0.008
49	PNP025	0.018	0.007	0.013	0.006	0.010	0.013	0.015	0.006	0.015	0.009	0.012	0.008
50	PNP027	0.022	0.014	0.020	0.012	0.015	0.017	0.020	0.011	0.020	0.014	0.017	0.014
51	PNP029	0.018	0.007	0.013	0.006	0.010	0.013	0.015	0.006	0.015	0.009	0.012	0.008
52	PNP031	0.018	0.007	0.014	0.006	0.010	0.013	0.015	0.006	0.015	0.009	0.012	0.008
53	PNP034	0.018	0.007	0.014	0.006	0.010	0.013	0.015	0.006	0.015	0.009	0.012	0.008
54	PNP110	0.018	0.007	0.014	0.006	0.010	0.013	0.015	0.006	0.015	0.009	0.012	0.008
55	PNP117	0.018	0.007	0.013	0.006	0.010	0.013	0.015	0.006	0.015	0.009	0.012	0.008
56	PNP126	0.024	0.014	0.018	0.012	0.016	0.020	0.021	0.012	0.018	0.015	0.018	0.014
57	PNP127	0.018	0.007	0.013	0.006	0.010	0.013	0.015	0.006	0.015	0.009	0.012	0.008
58	PNP140	0.018	0.007	0.014	0.006	0.010	0.013	0.015	0.006	0.015	0.009	0.012	0.008
59	PNP143	0.018	0.007	0.013	0.006	0.010	0.013	0.015	0.006	0.015	0.009	0.012	0.008
60	PNP176	0.029	0.018	0.024	0.017	0.021	0.024	0.026	0.017	0.026	0.020	0.023	0.018

Table A.1.1. Continued

		25	26	27	28	29	30	31	32	33	34	35	36
61	PNP198	0.023	0.012	0.018	0.010	0.015	0.018	0.020	0.010	0.020	0.013	0.016	0.012
62	SIH06	0.020	0.009	0.015	0.007	0.012	0.015	0.017	0.007	0.017	0.010	0.013	0.009
63	*Hyla_arborea*	0.057	0.046	0.053	0.043	0.049	0.049	0.051	0.043	0.051	0.048	0.051	0.047
64	*Hyla_femoralis*	0.054	0.042	0.050	0.040	0.043	0.048	0.049	0.038	0.048	0.043	0.045	0.043
65	*Hyla_japonica*	0.045	0.034	0.040	0.032	0.036	0.040	0.041	0.031	0.038	0.035	0.038	0.034
66	*Hyla_orientalis*	0.059	0.048	0.054	0.045	0.051	0.051	0.053	0.045	0.053	0.049	0.052	0.048
67	*Hyla_meridionalis*	0.066	0.055	0.054	0.051	0.057	0.057	0.059	0.053	0.062	0.056	0.059	0.055
68	*Hyla versicolor*	0.048	0.039	0.045	0.037	0.041	0.045	0.046	0.037	0.043	0.040	0.045	0.037
69	*Osteopilus septentrionalis*	0.117	0.103	0.108	0.100	0.103	0.107	0.109	0.101	0.111	0.104	0.103	0.103

Table A.1.1. Continued

		37	38	39	40	41	42	43	44	45	46	47	48
31	PNP180												
32	PNP186												
33	PNP193												
34	PNP197												
35	PNP098												
36	PNP085												
37	PNP188												
38	PNP192	0.010											
39	PNP190	0.007	0.012										
40	PNP177	0.012	0.017	0.006									
41	PNP183	0.014	0.018	0.009	0.006								
42	PNP131	0.009	0.013	0.006	0.012	0.012							
43	JWS357	0.009	0.010	0.004	0.010	0.010	0.001						
44	JWS474	0.010	0.012	0.007	0.013	0.013	0.001	0.000					
45	PNP003	0.010	0.012	0.007	0.013	0.013	0.001	0.000	0.000				
46	PNP010	0.010	0.012	0.007	0.013	0.013	0.001	0.000	0.000	0.000			
47	PNP014	0.010	0.012	0.007	0.014	0.013	0.001	0.000	0.000	0.000	0.000		
48	PNP018	0.010	0.012	0.007	0.013	0.013	0.001	0.000	0.000	0.000	0.000	0.000	
49	PNP025	0.010	0.012	0.007	0.013	0.013	0.001	0.000	0.000	0.000	0.000	0.000	0.000
50	PNP027	0.017	0.018	0.014	0.020	0.020	0.008	0.006	0.006	0.006	0.006	0.006	0.006
51	PNP029	0.010	0.012	0.007	0.013	0.013	0.001	0.000	0.000	0.000	0.000	0.000	0.000
52	PNP031	0.010	0.012	0.007	0.014	0.013	0.001	0.000	0.000	0.000	0.000	0.000	0.000
53	PNP034	0.011	0.012	0.007	0.014	0.013	0.001	0.000	0.000	0.000	0.000	0.000	0.000
54	PNP110	0.010	0.012	0.007	0.013	0.013	0.001	0.000	0.000	0.000	0.000	0.000	0.000
55	PNP117	0.010	0.012	0.007	0.013	0.013	0.001	0.000	0.000	0.000	0.000	0.000	0.000
56	PNP126	0.015	0.018	0.014	0.020	0.020	0.007	0.006	0.006	0.006	0.006	0.006	0.006
57	PNP127	0.010	0.012	0.007	0.013	0.013	0.001	0.000	0.000	0.000	0.000	0.000	0.000
58	PNP140	0.010	0.012	0.007	0.013	0.013	0.001	0.000	0.000	0.000	0.000	0.000	0.000
59	PNP143	0.010	0.012	0.007	0.013	0.013	0.001	0.000	0.000	0.000	0.000	0.000	0.000
60	PNP176	0.021	0.023	0.018	0.024	0.024	0.012	0.011	0.010	0.010	0.010	0.011	0.010

Table A.1.1. Continued

		37	38	39	40	41	42	43	44	45	46	47	48
61	PNP198	0.015	0.017	0.012	0.018	0.018	0.006	0.004	0.004	0.004	0.004	0.004	0.004
62	SIH06	0.012	0.014	0.009	0.015	0.015	0.003	0.001	0.001	0.001	0.001	0.001	0.001
63	*Hyla_arborea*	0.049	0.054	0.046	0.053	0.049	0.040	0.041	0.041	0.041	0.041	0.041	0.041
64	*Hyla_femoralis*	0.046	0.051	0.043	0.046	0.043	0.037	0.038	0.038	0.038	0.038	0.038	0.038
65	*Hyla_japonica*	0.037	0.040	0.034	0.040	0.037	0.027	0.027	0.027	0.027	0.027	0.027	0.027
66	*Hyla_orientalis*	0.051	0.056	0.048	0.054	0.051	0.041	0.043	0.043	0.043	0.043	0.043	0.043
67	*Hyla_meridionalis*	0.053	0.062	0.054	0.058	0.057	0.048	0.050	0.049	0.049	0.049	0.050	0.049
68	*Hyla versicolor*	0.038	0.043	0.040	0.045	0.043	0.035	0.037	0.037	0.037	0.037	0.037	0.037
69	*Osteopilus septentrionalis*	0.106	0.113	0.100	0.101	0.100	0.097	0.097	0.098	0.098	0.098	0.099	0.098

Table A.1.1. Continued

		49	50	51	52	53	54	55	56	57	58	59	60
31	PNP180												
32	PNP186												
33	PNP193												
34	PNP197												
35	PNP098												
36	PNP085												
37	PNP188												
38	PNP192												
39	PNP190												
40	PNP177												
41	PNP183												
42	PNP131												
43	JWS357												
44	JWS474												
45	PNP003												
46	PNP010												
47	PNP014												
48	PNP018												
49	PNP025												
50	PNP027	0.006											
51	PNP029	0.000	0.006										
52	PNP031	0.000	0.006	0.000									
53	PNP034	0.000	0.006	0.000	0.000								
54	PNP110	0.000	0.006	0.000	0.000	0.000							
55	PNP117	0.000	0.006	0.000	0.000	0.000	0.000						
56	PNP126	0.006	0.012	0.006	0.006	0.006	0.006	0.006					
57	PNP127	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.006				
58	PNP140	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.006	0.000			
59	PNP143	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000		
60	PNP176	0.010	0.017	0.010	0.011	0.011	0.010	0.010	0.017	0.010	0.010	0.010	

Table A.1.1. Continued

		49	50	51	52	53	54	55	56	57	58	59	60
61	PNP198	0.004	0.011	0.004	0.004	0.004	0.004	0.004	0.011	0.004	0.004	0.004	0.015
62	SIH06	0.001	0.008	0.001	0.001	0.001	0.001	0.001	0.007	0.001	0.001	0.001	0.012
63	*Hyla_arborea*	0.041	0.047	0.041	0.041	0.041	0.041	0.041	0.048	0.041	0.041	0.041	0.053
64	*Hyla_femoralis*	0.038	0.044	0.038	0.038	0.038	0.038	0.038	0.045	0.038	0.038	0.038	0.050
65	*Hyla_japonica*	0.027	0.033	0.027	0.027	0.027	0.027	0.027	0.034	0.027	0.027	0.027	0.038
66	*Hyla_orientalis*	0.043	0.049	0.043	0.043	0.043	0.043	0.043	0.049	0.043	0.043	0.043	0.054
67	*Hyla_meridionalis*	0.049	0.054	0.049	0.050	0.050	0.049	0.049	0.054	0.049	0.049	0.049	0.061
68	*Hyla versicolor*	0.037	0.041	0.037	0.037	0.037	0.037	0.037	0.043	0.037	0.037	0.037	0.048
70	*Osteopilus septentrionalis*	0.098	0.105	0.098	0.099	0.099	0.099	0.098	0.106	0.098	0.099	0.098	0.111

Table A.1.1. Continued

		61	62	63	64	65	66	67	68	69
61	PNP198									
62	SIH06	0.006								
63	*Hyla_arborea*	0.046	0.043							
64	*Hyla_femoralis*	0.043	0.040	0.038						
65	*Hyla_japonica*	0.032	0.029	0.033	0.030					
66	*Hyla_orientalis*	0.048	0.045	0.007	0.040	0.035				
67	*Hyla_meridionalis*	0.054	0.051	0.023	0.049	0.038	0.021			
68	*Hyla versicolor*	0.042	0.037	0.043	0.043	0.023	0.041	0.051		
70	*Osteopilus septentrionalis*	0.104	0.099	0.091	0.102	0.093	0.090	0.079	0.104	

Table A.1.2. Pairwise nucleotide differences in mitochondrial 16S sequences. Cells highlighted in blue represent samples from Dryophytes *squirellus*, cells highlighted in red represent samples from *D. gratiosus*, cells highlighted in yellow represent samples from the eastern clade of *D. cinereus*, and cells highlighted in green represent samples from the western clade of *D. cinereus*.

		1	2	3	4	5	6	7	8	9	10	11
1	LSUMZ_H487											
2	PNP_036	0.000										
3	PNP_101	0.000	0.000									
4	PNP_123	0.000	0.000	0.000								
5	PNP_124	0.000	0.000	0.000	0.000							
6	PNP_125	0.000	0.000	0.000	0.000	0.000						
7	PNP_129	0.000	0.000	0.000	0.000	0.000	0.000					
8	PNP_147	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
9	PNP_185	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005			
10	PNP_196	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005		
11	PNP_028	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.008	0.003	
12	PNP_091	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.008	0.003	0.000
13	PNP_093	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.008	0.003	0.000
14	AMNH_A_168427	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.008	0.003	0.005
15	PNP_086	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.008	0.003	0.005
16	PNP_087	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.008	0.003	0.005
17	PNP_088	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.008	0.003	0.005
18	MVZ145422	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.078	0.075	0.078
19	MVZ145423	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.047	0.044	0.047
20	MVZ145430	0.039	0.039	0.039	0.039	0.039	0.039	0.039	0.039	0.042	0.039	0.042
21	MVZ145431	0.067	0.067	0.067	0.067	0.067	0.067	0.067	0.067	0.074	0.067	0.070
22	AMNH_A_168404	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.110
23	CAS169394	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.108	0.107	0.111
24	CAS169395	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.108	0.107	0.111
25	CAS169396	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.108	0.107	0.111
26	CAS175122	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.108	0.107	0.111
27	LSUMZ_H15929	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.110
28	MVZ145405	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.108	0.107	0.111
29	MVZ161962	0.111	0.111	0.111	0.111	0.111	0.111	0.111	0.111	0.111	0.111	0.114
30	MVZ161964	0.114	0.114	0.114	0.114	0.114	0.114	0.114	0.114	0.114	0.114	0.117

Table A.1.2. Continued

		1	2	3	4	5	6	7	8	9	10	11
31	CAS175123	0.111	0.111	0.111	0.111	0.111	0.111	0.111	0.111	0.111	0.111	0.114
32	CAS175126	0.111	0.111	0.111	0.111	0.111	0.111	0.111	0.111	0.111	0.111	0.114
33	MVZ137619	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.158	0.157	0.160
34	MVZ145406	0.149	0.149	0.149	0.149	0.149	0.149	0.149	0.149	0.149	0.149	0.152
35	MVZ161963	0.173	0.173	0.173	0.173	0.173	0.173	0.173	0.173	0.173	0.173	0.176
36	CAS223871	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.099	0.092	0.095
37	JWST_007	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.095	0.089	0.092
38	PNP_047	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.095	0.089	0.092
39	PNP_054	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.095	0.089	0.092
40	PNP_058	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.095	0.089	0.092
41	PNP_063	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.099	0.092	0.095
42	PNP_073	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.095	0.089	0.092
43	PNP_075	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.099	0.092	0.095
44	PNP_084	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.095	0.089	0.092
45	PNP_085	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.095	0.089	0.092
46	PNP_188	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.095	0.089	0.092
47	PNP_190	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.095	0.089	0.092
48	PNP_098	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.096	0.089	0.092
49	PNP_103	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.092	0.086	0.089
50	PNP_104	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.092	0.086	0.089
51	PNP_105	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.092	0.086	0.089
52	PNP_131	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.092	0.086	0.089
53	PNP_143	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.092	0.086	0.089
54	PNP_177	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.096	0.089	0.092
55	PNP_178	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.092	0.086	0.089
56	PNP_180	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.092	0.086	0.089
57	PNP_183	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.092	0.086	0.089
58	PNP_186	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.092	0.086	0.089
59	PNP_192	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.092	0.086	0.089
60	PNP_193	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.092	0.086	0.089

Table A.1.2. Continued

		1	2	3	4	5	6	7	8	9	10	11
61	PNP_197	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.103	0.096	0.099
62	JWS_357	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.109	0.103	0.106
63	JWS_474	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
64	LSUMZ_48181	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
65	MVZ_145385	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
66	PNP_003	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
67	PNP_010	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
68	PNP_015	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
69	PNP_018	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
70	PNP_020	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
71	PNP_021	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
72	PNP_025	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
73	PNP_026	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
74	PNP_027	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
75	PNP_029	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
76	PNP_030	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
77	PNP_031	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
78	PNP_034	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
79	PNP_035	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
80	PNP_110	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
81	PNP_111	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
82	PNP_117	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
83	PNP_126	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
84	PNP_127	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
85	PNP_140	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
86	PNP_176	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
87	PNP_198	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
88	PNP_199	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
89	PNP011	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
90	PNP014	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099

Table A.1.2. Continued

		1	2	3	4	5	6	7	8	9	10	11
91	PNP019	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.106	0.100	0.103
92	PNP156	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
93	PNP158	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
94	PNP160	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
95	PNP163	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
96	PNP164	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
97	PNP165	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
98	PNP166	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
99	PNP167	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
100	PNP168	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
101	PNP169	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
102	PNP173	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
103	PNP174	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
104	SIH_06	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
105	TNHC_61054	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.102	0.096	0.099
106	*Hyla versicolor*	0.093	0.093	0.093	0.093	0.093	0.093	0.093	0.093	0.094	0.093	0.096
107	*Hyla versicolor*	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.103
108	*Hyla versicolor*	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.097	0.096	0.100
109	*Hyla versicolor*	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.106
110	*Hyla_meridionalis*	0.141	0.141	0.141	0.141	0.141	0.141	0.141	0.141	0.141	0.141	0.144
111	*Hyla_orientalis*	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.153
112	*Hyla_japonica*	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.103
113	*Hyla_eximia*	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.116
114	*Hyla_arenicolor*	0.099	0.099	0.099	0.099	0.099	0.099	0.099	0.099	0.099	0.099	0.102
115	*Hyla_femoralis*	0.137	0.137	0.137	0.137	0.137	0.137	0.137	0.137	0.138	0.137	0.140
116	*Hyla_arborea*	0.146	0.146	0.146	0.146	0.146	0.146	0.146	0.146	0.147	0.146	0.150
117	*Osteopilus septentrionalis*	0.197	0.197	0.197	0.197	0.197	0.197	0.197	0.197	0.198	0.197	0.201

Table A.1.2. Continued

		12	13	14	15	16	17	18	19	20	21	22
1	LSUMZ_H487											
2	PNP_036											
3	PNP_101											
4	PNP_123											
5	PNP_124											
6	PNP_125											
7	PNP_129											
8	PNP_147											
9	PNP_185											
10	PNP_196											
11	PNP_028											
12	PNP_091											
13	PNP_093	0.000										
14	AMNH_A_168427	0.005	0.005									
15	PNP_086	0.005	0.005	0.000								
16	PNP_087	0.005	0.005	0.000	0.000							
17	PNP_088	0.005	0.005	0.000	0.000	0.000						
18	MVZ145422	0.078	0.078	0.071	0.071	0.071	0.071					
19	MVZ145423	0.047	0.047	0.041	0.041	0.041	0.041	0.041				
20	MVZ145430	0.042	0.042	0.036	0.036	0.036	0.036	0.045	0.016			
21	MVZ145431	0.070	0.070	0.064	0.064	0.064	0.064	0.080	0.049	0.055		
22	AMNH_A_168404	0.110	0.110	0.107	0.107	0.107	0.107	0.091	0.117	0.108	0.141	
23	CAS169394	0.111	0.111	0.108	0.108	0.108	0.108	0.092	0.118	0.108	0.141	0.000
24	CAS169395	0.111	0.111	0.108	0.108	0.108	0.108	0.092	0.118	0.108	0.141	0.000
25	CAS169396	0.111	0.111	0.108	0.108	0.108	0.108	0.092	0.118	0.108	0.141	0.000
26	CAS175122	0.111	0.111	0.108	0.108	0.108	0.108	0.092	0.118	0.108	0.141	0.000
27	LSUMZ_H15929	0.110	0.110	0.107	0.107	0.107	0.107	0.091	0.117	0.108	0.141	0.000
28	MVZ145405	0.111	0.111	0.108	0.108	0.108	0.108	0.092	0.118	0.108	0.141	0.000
29	MVZ161962	0.114	0.114	0.111	0.111	0.111	0.111	0.095	0.121	0.111	0.141	0.003
30	MVZ161964	0.117	0.117	0.114	0.114	0.114	0.114	0.098	0.118	0.108	0.138	0.005

Table A.1.2. Continued

		12	13	14	15	16	17	18	19	20	21	22
31	CAS175123	0.114	0.114	0.111	0.111	0.111	0.111	0.092	0.118	0.108	0.142	0.008
32	CAS175126	0.114	0.114	0.111	0.111	0.111	0.111	0.092	0.112	0.102	0.135	0.013
33	MVZ137619	0.160	0.160	0.157	0.157	0.157	0.157	0.098	0.122	0.130	0.143	0.051
34	MVZ145406	0.152	0.152	0.149	0.149	0.149	0.149	0.098	0.115	0.112	0.127	0.060
35	MVZ161963	0.176	0.176	0.173	0.173	0.173	0.173	0.105	0.137	0.145	0.131	0.066
36	CAS223871	0.095	0.095	0.096	0.096	0.096	0.096	0.133	0.122	0.116	0.139	0.080
37	JWST_007	0.092	0.092	0.092	0.092	0.092	0.092	0.130	0.118	0.112	0.136	0.077
38	PNP_047	0.092	0.092	0.092	0.092	0.092	0.092	0.130	0.118	0.112	0.136	0.077
39	PNP_054	0.092	0.092	0.092	0.092	0.092	0.092	0.130	0.118	0.112	0.136	0.077
40	PNP_058	0.092	0.092	0.092	0.092	0.092	0.092	0.130	0.118	0.112	0.136	0.077
41	PNP_063	0.095	0.095	0.096	0.096	0.096	0.096	0.133	0.122	0.116	0.139	0.080
42	PNP_073	0.092	0.092	0.092	0.092	0.092	0.092	0.130	0.118	0.112	0.136	0.077
43	PNP_075	0.095	0.095	0.096	0.096	0.096	0.096	0.133	0.122	0.116	0.139	0.080
44	PNP_084	0.092	0.092	0.092	0.092	0.092	0.092	0.130	0.118	0.112	0.136	0.077
45	PNP_085	0.092	0.092	0.092	0.092	0.092	0.092	0.130	0.118	0.112	0.136	0.077
46	PNP_188	0.092	0.092	0.092	0.092	0.092	0.092	0.130	0.118	0.112	0.136	0.077
47	PNP_190	0.092	0.092	0.092	0.092	0.092	0.092	0.130	0.118	0.112	0.136	0.077
48	PNP_098	0.092	0.092	0.093	0.093	0.093	0.093	0.129	0.125	0.119	0.143	0.077
49	PNP_103	0.089	0.089	0.089	0.089	0.089	0.089	0.133	0.121	0.115	0.139	0.080
50	PNP_104	0.089	0.089	0.089	0.089	0.089	0.089	0.133	0.121	0.115	0.139	0.080
51	PNP_105	0.089	0.089	0.089	0.089	0.089	0.089	0.133	0.121	0.115	0.139	0.080
52	PNP_131	0.089	0.089	0.089	0.089	0.089	0.089	0.133	0.121	0.115	0.139	0.080
53	PNP_143	0.089	0.089	0.089	0.089	0.089	0.089	0.133	0.121	0.115	0.139	0.080
54	PNP_177	0.092	0.092	0.093	0.093	0.093	0.093	0.137	0.125	0.119	0.143	0.083
55	PNP_178	0.089	0.089	0.089	0.089	0.089	0.089	0.133	0.121	0.115	0.139	0.080
56	PNP_180	0.089	0.089	0.089	0.089	0.089	0.089	0.133	0.121	0.115	0.139	0.080
57	PNP_183	0.089	0.089	0.089	0.089	0.089	0.089	0.133	0.121	0.115	0.139	0.080
58	PNP_186	0.089	0.089	0.089	0.089	0.089	0.089	0.133	0.121	0.115	0.139	0.080
59	PNP_192	0.089	0.089	0.089	0.089	0.089	0.089	0.133	0.121	0.115	0.139	0.080
60	PNP_193	0.089	0.089	0.089	0.089	0.089	0.089	0.133	0.121	0.115	0.139	0.080

Table A.1.2. Continued

		12	13	14	15	16	17	18	19	20	21	22
61	PNP_197	0.099	0.099	0.100	0.100	0.100	0.100	0.141	0.133	0.126	0.151	0.096
62	JWS_357	0.106	0.106	0.106	0.106	0.106	0.106	0.145	0.133	0.127	0.151	0.087
63	JWS_474	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
64	LSUMZ_48181	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
65	MVZ_145385	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
66	PNP_003	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
67	PNP_010	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
68	PNP_015	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
69	PNP_018	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
70	PNP_020	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
71	PNP_021	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
72	PNP_025	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
73	PNP_026	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
74	PNP_027	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
75	PNP_029	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
76	PNP_030	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
77	PNP_031	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
78	PNP_034	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
79	PNP_035	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
80	PNP_110	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
81	PNP_111	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
82	PNP_117	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
83	PNP_126	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
84	PNP_127	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
85	PNP_140	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
86	PNP_176	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
87	PNP_198	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
88	PNP_199	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
89	PNP011	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
90	PNP014	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080

Table A.1.2. Continued

		12	13	14	15	16	17	18	19	20	21	22
91	PNP019	0.103	0.103	0.103	0.103	0.103	0.103	0.142	0.130	0.124	0.148	0.084
92	PNP156	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
93	PNP158	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
94	PNP160	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
95	PNP163	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
96	PNP164	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
97	PNP165	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
98	PNP166	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
99	PNP167	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
100	PNP168	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
101	PNP169	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
102	PNP173	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
103	PNP174	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
104	SIH_06	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
105	TNHC_61054	0.099	0.099	0.099	0.099	0.099	0.099	0.137	0.125	0.119	0.143	0.080
106	*Hyla versicolor*	0.096	0.096	0.097	0.097	0.097	0.097	0.130	0.123	0.113	0.151	0.081
107	*Hyla versicolor*	0.103	0.103	0.104	0.104	0.104	0.104	0.126	0.130	0.120	0.158	0.078
108	*Hyla versicolor*	0.100	0.100	0.100	0.100	0.100	0.100	0.126	0.120	0.110	0.151	0.087
109	*Hyla versicolor*	0.106	0.106	0.106	0.106	0.106	0.106	0.126	0.126	0.117	0.157	0.087
110	*Hyla_meridionalis*	0.144	0.144	0.141	0.141	0.141	0.141	0.165	0.159	0.155	0.186	0.113
111	*Hyla_orientalis*	0.153	0.153	0.154	0.154	0.154	0.154	0.177	0.184	0.180	0.209	0.137
112	*Hyla_japonica*	0.103	0.103	0.104	0.104	0.104	0.104	0.130	0.130	0.120	0.158	0.074
113	*Hyla_eximia*	0.116	0.116	0.113	0.113	0.113	0.113	0.139	0.136	0.130	0.162	0.084
114	*Hyla_arenicolor*	0.102	0.102	0.103	0.103	0.103	0.103	0.136	0.129	0.119	0.153	0.081
115	*Hyla_femoralis*	0.140	0.140	0.141	0.141	0.141	0.141	0.178	0.162	0.156	0.185	0.130
116	*Hyla_arborea*	0.150	0.150	0.150	0.150	0.150	0.150	0.169	0.176	0.172	0.193	0.133
117	Osteopilus septentrionalis	0.201	0.201	0.202	0.202	0.202	0.202	0.246	0.230	0.230	0.255	0.194

Table A.1.2. Continued

		23	24	25	26	27	28	29	30	31	32	33
1	LSUMZ_H487											
2	PNP_036											
3	PNP_101											
4	PNP_123											
5	PNP_124											
6	PNP_125											
7	PNP_129											
8	PNP_147											
9	PNP_185											
10	PNP_196											
11	PNP_028											
12	PNP_091											
13	PNP_093											
14	AMNH_A_168427											
15	PNP_086											
16	PNP_087											
17	PNP_088											
18	MVZ145422											
19	MVZ145423											
20	MVZ145430											
21	MVZ145431											
22	AMNH_A_168404											
23	CAS169394											
24	CAS169395	0.000										
25	CAS169396	0.000	0.000									
26	CAS175122	0.000	0.000	0.000								
27	LSUMZ_H15929	0.000	0.000	0.000	0.000							
28	MVZ145405	0.000	0.000	0.000	0.000	0.000						
29	MVZ161962	0.003	0.003	0.003	0.003	0.003	0.003					
30	MVZ161964	0.005	0.005	0.005	0.005	0.005	0.005	0.003				

Table A.1.2. Continued

		23	24	25	26	27	28	29	30	31	32	33
31	CAS175123	0.008	0.008	0.008	0.008	0.008	0.008	0.011	0.013			
32	CAS175126	0.013	0.013	0.013	0.013	0.013	0.013	0.016	0.013	0.005		
33	MVZ137619	0.051	0.051	0.051	0.051	0.051	0.051	0.048	0.045	0.045	0.039	
34	MVZ145406	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.057	0.054	0.048	0.045
35	MVZ161963	0.066	0.066	0.066	0.066	0.066	0.066	0.066	0.063	0.060	0.054	0.045
36	CAS223871	0.080	0.080	0.080	0.080	0.080	0.080	0.083	0.086	0.090	0.096	0.140
37	JWST_007	0.077	0.077	0.077	0.077	0.077	0.077	0.080	0.083	0.086	0.092	0.136
38	PNP_047	0.077	0.077	0.077	0.077	0.077	0.077	0.080	0.083	0.086	0.092	0.136
39	PNP_054	0.077	0.077	0.077	0.077	0.077	0.077	0.080	0.083	0.086	0.092	0.136
40	PNP_058	0.077	0.077	0.077	0.077	0.077	0.077	0.080	0.083	0.086	0.092	0.136
41	PNP_063	0.080	0.080	0.080	0.080	0.080	0.080	0.083	0.086	0.090	0.096	0.140
42	PNP_073	0.077	0.077	0.077	0.077	0.077	0.077	0.080	0.083	0.086	0.092	0.136
43	PNP_075	0.080	0.080	0.080	0.080	0.080	0.080	0.083	0.086	0.090	0.096	0.140
44	PNP_084	0.077	0.077	0.077	0.077	0.077	0.077	0.080	0.083	0.086	0.092	0.136
45	PNP_085	0.077	0.077	0.077	0.077	0.077	0.077	0.080	0.083	0.086	0.092	0.136
46	PNP_188	0.077	0.077	0.077	0.077	0.077	0.077	0.080	0.083	0.086	0.092	0.136
47	PNP_190	0.077	0.077	0.077	0.077	0.077	0.077	0.080	0.083	0.086	0.092	0.136
48	PNP_098	0.077	0.077	0.077	0.077	0.077	0.077	0.080	0.083	0.086	0.092	0.136
49	PNP_103	0.080	0.080	0.080	0.080	0.080	0.080	0.083	0.086	0.089	0.095	0.139
50	PNP_104	0.080	0.080	0.080	0.080	0.080	0.080	0.083	0.086	0.089	0.095	0.139
51	PNP_105	0.080	0.080	0.080	0.080	0.080	0.080	0.083	0.086	0.089	0.095	0.139
52	PNP_131	0.080	0.080	0.080	0.080	0.080	0.080	0.083	0.086	0.089	0.095	0.139
53	PNP_143	0.080	0.080	0.080	0.080	0.080	0.080	0.083	0.086	0.089	0.095	0.139
54	PNP_177	0.083	0.083	0.083	0.083	0.083	0.083	0.086	0.089	0.093	0.099	0.143
55	PNP_178	0.080	0.080	0.080	0.080	0.080	0.080	0.083	0.086	0.089	0.095	0.139
56	PNP_180	0.080	0.080	0.080	0.080	0.080	0.080	0.083	0.086	0.089	0.095	0.139
57	PNP_183	0.080	0.080	0.080	0.080	0.080	0.080	0.083	0.086	0.089	0.095	0.139
58	PNP_186	0.080	0.080	0.080	0.080	0.080	0.080	0.083	0.086	0.089	0.095	0.139
59	PNP_192	0.080	0.080	0.080	0.080	0.080	0.080	0.083	0.086	0.089	0.095	0.139
60	PNP_193	0.080	0.080	0.080	0.080	0.080	0.080	0.083	0.086	0.089	0.095	0.139

Table A.1.2. Continued

		23	24	25	26	27	28	29	30	31	32	33
61	PNP_197	0.097	0.097	0.097	0.097	0.096	0.097	0.100	0.103	0.103	0.109	0.155
62	JWS_357	0.088	0.088	0.088	0.088	0.087	0.088	0.091	0.094	0.097	0.103	0.148
63	JWS_474	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
64	LSUMZ_48181	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
65	MVZ_145385	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
66	PNP_003	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
67	PNP_010	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
68	PNP_015	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
69	PNP_018	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
70	PNP_020	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
71	PNP_021	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
72	PNP_025	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
73	PNP_026	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
74	PNP_027	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
75	PNP_029	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
76	PNP_030	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
77	PNP_031	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
78	PNP_034	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
79	PNP_035	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
80	PNP_110	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
81	PNP_111	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
82	PNP_117	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
83	PNP_126	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
84	PNP_127	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
85	PNP_140	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
86	PNP_176	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
87	PNP_198	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
88	PNP_199	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
89	PNP011	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
90	PNP014	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140

Table A.2. Continued

		23	24	25	26	27	28	29	30	31	32	33
91	PNP019	0.084	0.084	0.084	0.084	0.084	0.084	0.088	0.091	0.094	0.100	0.145
92	PNP156	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
93	PNP158	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
94	PNP160	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
95	PNP163	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
96	PNP164	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
97	PNP165	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
98	PNP166	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
99	PNP167	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
100	PNP168	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
101	PNP169	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
102	PNP173	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
103	PNP174	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
104	SIH_06	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
105	TNHC_61054	0.081	0.081	0.081	0.081	0.080	0.081	0.084	0.087	0.090	0.096	0.140
106	*Hyla versicolor*	0.081	0.081	0.081	0.081	0.081	0.081	0.084	0.087	0.091	0.097	0.141
107	*Hyla versicolor*	0.078	0.078	0.078	0.078	0.078	0.078	0.081	0.084	0.088	0.094	0.138
108	*Hyla versicolor*	0.087	0.087	0.087	0.087	0.087	0.087	0.084	0.087	0.096	0.102	0.141
109	*Hyla versicolor*	0.087	0.087	0.087	0.087	0.087	0.087	0.084	0.087	0.096	0.102	0.141
110	*Hyla_meridionalis*	0.114	0.114	0.114	0.114	0.113	0.114	0.110	0.114	0.124	0.130	0.160
111	*Hyla_orientalis*	0.137	0.137	0.137	0.137	0.137	0.137	0.137	0.141	0.148	0.148	0.188
112	*Hyla_japonica*	0.075	0.075	0.075	0.075	0.074	0.075	0.078	0.081	0.084	0.090	0.134
113	*Hyla_eximia*	0.084	0.084	0.084	0.084	0.084	0.084	0.081	0.084	0.093	0.100	0.134
114	*Hyla_arenicolor*	0.081	0.081	0.081	0.081	0.081	0.081	0.078	0.081	0.090	0.096	0.134
115	*Hyla_femoralis*	0.130	0.130	0.130	0.130	0.130	0.130	0.133	0.137	0.134	0.140	0.173
116	*Hyla_arborea*	0.133	0.133	0.133	0.133	0.133	0.133	0.133	0.137	0.144	0.151	0.191
117	*Osteopilus septentrionalis*	0.191	0.191	0.191	0.191	0.194	0.191	0.191	0.195	0.202	0.210	0.246

Table A.1.2. Continued

		34	35	36	37	38	39	40	41	42	43	44
31	CAS175123											
32	CAS175126											
33	MVZ137619											
34	MVZ145406											
35	MVZ161963	0.051										
36	CAS223871	0.129	0.151									
37	JWST_007	0.125	0.148	0.003								
38	PNP_047	0.125	0.148	0.003	0.000							
39	PNP_054	0.125	0.148	0.003	0.000	0.000						
40	PNP_058	0.125	0.148	0.003	0.000	0.000	0.000					
41	PNP_063	0.129	0.151	0.005	0.003	0.003	0.003	0.003				
42	PNP_073	0.125	0.148	0.003	0.000	0.000	0.000	0.000	0.003			
43	PNP_075	0.129	0.151	0.005	0.003	0.003	0.003	0.003	0.005	0.003		
44	PNP_084	0.125	0.148	0.003	0.000	0.000	0.000	0.000	0.003	0.000	0.003	
45	PNP_085	0.125	0.148	0.003	0.000	0.000	0.000	0.000	0.003	0.000	0.003	0.000
46	PNP_188	0.125	0.148	0.003	0.000	0.000	0.000	0.000	0.003	0.000	0.003	0.000
47	PNP_190	0.125	0.148	0.003	0.000	0.000	0.000	0.000	0.003	0.000	0.003	0.000
48	PNP_098	0.125	0.147	0.008	0.005	0.005	0.005	0.005	0.008	0.005	0.008	0.005
49	PNP_103	0.128	0.151	0.005	0.003	0.003	0.003	0.003	0.005	0.003	0.005	0.003
50	PNP_104	0.128	0.151	0.005	0.003	0.003	0.003	0.003	0.005	0.003	0.005	0.003
51	PNP_105	0.128	0.151	0.005	0.003	0.003	0.003	0.003	0.005	0.003	0.005	0.003
52	PNP_131	0.128	0.151	0.005	0.003	0.003	0.003	0.003	0.005	0.003	0.005	0.003
53	PNP_143	0.128	0.151	0.005	0.003	0.003	0.003	0.003	0.005	0.003	0.005	0.003
54	PNP_177	0.132	0.155	0.008	0.005	0.005	0.005	0.005	0.008	0.005	0.008	0.005
55	PNP_178	0.128	0.151	0.005	0.003	0.003	0.003	0.003	0.005	0.003	0.005	0.003
56	PNP_180	0.128	0.151	0.005	0.003	0.003	0.003	0.003	0.005	0.003	0.005	0.003
57	PNP_183	0.128	0.151	0.005	0.003	0.003	0.003	0.003	0.005	0.003	0.005	0.003
58	PNP_186	0.128	0.151	0.005	0.003	0.003	0.003	0.003	0.005	0.003	0.005	0.003
59	PNP_192	0.128	0.151	0.005	0.003	0.003	0.003	0.003	0.005	0.003	0.005	0.003
60	PNP_193	0.128	0.151	0.005	0.003	0.003	0.003	0.003	0.005	0.003	0.005	0.003

Table A.1.2. Continued

		34	35	36	37	38	39	40	41	42	43	44
61	PNP_197	0.144	0.164	0.019	0.016	0.016	0.016	0.016	0.019	0.016	0.019	0.016
62	JWS_357	0.137	0.160	0.022	0.019	0.019	0.019	0.019	0.022	0.019	0.022	0.019
63	JWS_474	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
64	LSUMZ_48181	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
65	MVZ_145385	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
66	PNP_003	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
67	PNP_010	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
68	PNP_015	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
69	PNP_018	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
70	PNP_020	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
71	PNP_021	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
72	PNP_025	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
73	PNP_026	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
74	PNP_027	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
75	PNP_029	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
76	PNP_030	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
77	PNP_031	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
78	PNP_034	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
79	PNP_035	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
80	PNP_110	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
81	PNP_111	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
82	PNP_117	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
83	PNP_126	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
84	PNP_127	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
85	PNP_140	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
86	PNP_176	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
87	PNP_198	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
88	PNP_199	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
89	PNP011	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
90	PNP014	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013

Table A.1.2. Continued

		34	35	36	37	38	39	40	41	42	43	44
91	PNP019	0.134	0.157	0.022	0.019	0.019	0.019	0.019	0.022	0.019	0.022	0.019
92	PNP156	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
93	PNP158	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
94	PNP160	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
95	PNP163	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
96	PNP164	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
97	PNP165	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
98	PNP166	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
99	PNP167	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
100	PNP168	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
101	PNP169	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
102	PNP173	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
103	PNP174	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
104	SIH_06	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
105	TNHC_61054	0.129	0.152	0.016	0.013	0.013	0.013	0.013	0.016	0.013	0.016	0.013
106	*Hyla versicolor*	0.134	0.153	0.083	0.080	0.080	0.080	0.080	0.083	0.080	0.083	0.080
107	*Hyla versicolor*	0.130	0.150	0.089	0.086	0.086	0.086	0.086	0.089	0.086	0.089	0.086
108	*Hyla versicolor*	0.130	0.156	0.089	0.086	0.086	0.086	0.086	0.089	0.086	0.089	0.086
109	*Hyla versicolor*	0.130	0.156	0.089	0.086	0.086	0.086	0.086	0.089	0.086	0.089	0.086
110	*Hyla_meridionalis*	0.155	0.180	0.132	0.128	0.128	0.128	0.128	0.132	0.128	0.132	0.128
111	*Hyla_orientalis*	0.174	0.200	0.142	0.138	0.138	0.138	0.138	0.141	0.138	0.142	0.138
112	*Hyla_japonica*	0.130	0.149	0.098	0.095	0.095	0.095	0.095	0.098	0.095	0.098	0.095
113	*Hyla_eximia*	0.130	0.153	0.094	0.090	0.090	0.090	0.090	0.093	0.090	0.094	0.090
114	*Hyla_arenicolor*	0.130	0.149	0.074	0.071	0.071	0.071	0.071	0.074	0.071	0.074	0.071
115	*Hyla_femoralis*	0.180	0.194	0.119	0.115	0.115	0.115	0.115	0.119	0.115	0.119	0.115
116	*Hyla_arborea*	0.172	0.195	0.148	0.144	0.144	0.144	0.144	0.148	0.144	0.148	0.144
117	Osteopilus septentrionalis	0.240	0.260	0.189	0.184	0.184	0.184	0.184	0.180	0.184	0.189	0.184

Table A.1.2. Continued

		45	46	47	48	49	50	51	52	53	54	55
31	CAS175123											
32	CAS175126											
33	MVZ137619											
34	MVZ145406											
35	MVZ161963											
36	CAS223871											
37	JWST_007											
38	PNP_047											
39	PNP_054											
40	PNP_058											
41	PNP_063											
42	PNP_073											
43	PNP_075											
44	PNP_084											
45	PNP_085											
46	PNP_188	0.000										
47	PNP_190	0.000	0.000									
48	PNP_098	0.005	0.005	0.005								
49	PNP_103	0.003	0.003	0.003	0.003							
50	PNP_104	0.003	0.003	0.003	0.003	0.000						
51	PNP_105	0.003	0.003	0.003	0.003	0.000	0.000					
52	PNP_131	0.003	0.003	0.003	0.003	0.000	0.000	0.000				
53	PNP_143	0.003	0.003	0.003	0.003	0.000	0.000	0.000	0.000			
54	PNP_177	0.005	0.005	0.005	0.005	0.003	0.003	0.003	0.003	0.003		
55	PNP_178	0.003	0.003	0.003	0.003	0.000	0.000	0.000	0.000	0.000	0.003	
56	PNP_180	0.003	0.003	0.003	0.003	0.000	0.000	0.000	0.000	0.000	0.003	0.000
57	PNP_183	0.003	0.003	0.003	0.003	0.000	0.000	0.000	0.000	0.000	0.003	0.000
58	PNP_186	0.003	0.003	0.003	0.003	0.000	0.000	0.000	0.000	0.000	0.003	0.000
59	PNP_192	0.003	0.003	0.003	0.003	0.000	0.000	0.000	0.000	0.000	0.003	0.000
60	PNP_193	0.003	0.003	0.003	0.003	0.000	0.000	0.000	0.000	0.000	0.003	0.000

Table A.1.2. Continued

		45	46	47	48	49	50	51	52	53	54	55
61	PNP_197	0.016	0.016	0.016	0.016	0.014	0.014	0.014	0.014	0.014	0.016	0.014
62	JWS_357	0.019	0.019	0.019	0.025	0.022	0.022	0.022	0.022	0.022	0.025	0.022
63	JWS_474	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
64	LSUMZ_48181	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
65	MVZ_145385	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
66	PNP_003	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
67	PNP_010	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
68	PNP_015	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
69	PNP_018	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
70	PNP_020	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
71	PNP_021	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
72	PNP_025	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
73	PNP_026	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
74	PNP_027	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
75	PNP_029	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
76	PNP_030	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
77	PNP_031	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
78	PNP_034	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
79	PNP_035	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
80	PNP_110	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
81	PNP_111	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
82	PNP_117	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
83	PNP_126	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
84	PNP_127	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
85	PNP_140	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
86	PNP_176	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
87	PNP_198	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
88	PNP_199	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
89	PNP011	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
90	PNP014	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016

Table A.1.2. Continued

		45	46	47	48	49	50	51	52	53	54	55
91	PNP019	0.019	0.019	0.019	0.025	0.022	0.022	0.022	0.022	0.022	0.025	0.022
92	PNP156	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
93	PNP158	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
94	PNP160	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
95	PNP163	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
96	PNP164	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
97	PNP165	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
98	PNP166	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
99	PNP167	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
100	PNP168	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
101	PNP169	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
102	PNP173	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
103	PNP174	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
104	SIH_06	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
105	TNHC_61054	0.013	0.013	0.013	0.019	0.016	0.016	0.016	0.016	0.016	0.019	0.016
106	*Hyla versicolor*	0.080	0.080	0.080	0.079	0.083	0.083	0.083	0.083	0.083	0.086	0.083
107	*Hyla versicolor*	0.086	0.086	0.086	0.086	0.089	0.089	0.089	0.089	0.089	0.092	0.089
108	*Hyla versicolor*	0.086	0.086	0.086	0.085	0.089	0.089	0.089	0.089	0.089	0.092	0.089
109	*Hyla versicolor*	0.086	0.086	0.086	0.085	0.089	0.089	0.089	0.089	0.089	0.092	0.089
110	*Hyla_meridionalis*	0.128	0.128	0.128	0.128	0.131	0.131	0.131	0.131	0.131	0.135	0.131
111	*Hyla_orientalis*	0.138	0.138	0.138	0.131	0.135	0.135	0.135	0.135	0.135	0.138	0.135
112	*Hyla_japonica*	0.095	0.095	0.095	0.095	0.098	0.098	0.098	0.098	0.098	0.101	0.098
113	*Hyla_eximia*	0.090	0.090	0.090	0.090	0.093	0.093	0.093	0.093	0.093	0.097	0.093
114	*Hyla_arenicolor*	0.071	0.071	0.071	0.077	0.074	0.074	0.074	0.074	0.074	0.077	0.074
115	*Hyla_femoralis*	0.115	0.115	0.115	0.122	0.119	0.119	0.119	0.119	0.119	0.122	0.119
116	*Hyla_arborea*	0.144	0.144	0.144	0.137	0.141	0.141	0.141	0.141	0.141	0.145	0.141
117	*Osteopilus septentrionalis*	0.184	0.184	0.184	0.184	0.188	0.188	0.188	0.188	0.188	0.192	0.188

Table A.1.2. Continued

		56	57	58	59	60	61	62	63	64	65	66
31	CAS175123											
32	CAS175126											
33	MVZ137619											
34	MVZ145406											
35	MVZ161963											
36	CAS223871											
37	JWST_007											
38	PNP_047											
39	PNP_054											
40	PNP_058											
41	PNP_063											
42	PNP_073											
43	PNP_075											
44	PNP_084											
45	PNP_085											
46	PNP_188											
47	PNP_190											
48	PNP_098											
49	PNP_103											
50	PNP_104											
51	PNP_105											
52	PNP_131											
53	PNP_143											
54	PNP_177											
55	PNP_178											
56	PNP_180											
57	PNP_183	0.000										
58	PNP_186	0.000	0.000									
59	PNP_192	0.000	0.000	0.000								
60	PNP_193	0.000	0.000	0.000	0.000							

Table A.1.2. Continued

		56	57	58	59	60	61	62	63	64	65	66
61	PNP_197	0.014	0.014	0.014	0.014	0.014						
62	JWS_357	0.022	0.022	0.022	0.022	0.022	0.036					
63	JWS_474	0.016	0.016	0.016	0.016	0.016	0.030	0.005				
64	LSUMZ_48181	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000			
65	MVZ_145385	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000		
66	PNP_003	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	
67	PNP_010	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
68	PNP_015	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
69	PNP_018	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
70	PNP_020	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
71	PNP_021	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
72	PNP_025	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
73	PNP_026	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
74	PNP_027	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
75	PNP_029	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
76	PNP_030	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
77	PNP_031	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
78	PNP_034	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
79	PNP_035	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
80	PNP_110	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
81	PNP_111	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
82	PNP_117	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
83	PNP_126	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
84	PNP_127	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
85	PNP_140	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
86	PNP_176	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
87	PNP_198	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
88	PNP_199	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
89	PNP011	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
90	PNP014	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000

Table A.1.2. Continued

		56	57	58	59	60	61	62	63	64	65	66
91	PNP019	0.022	0.022	0.022	0.022	0.022	0.036	0.011	0.005	0.005	0.005	0.005
92	PNP156	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
93	PNP158	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
94	PNP160	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
95	PNP163	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
96	PNP164	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
97	PNP165	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
98	PNP166	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
99	PNP167	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
100	PNP168	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
101	PNP169	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
102	PNP173	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
103	PNP174	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
104	SIH_06	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
105	TNHC_61054	0.016	0.016	0.016	0.016	0.016	0.030	0.005	0.000	0.000	0.000	0.000
106	*Hyla versicolor*	0.083	0.083	0.083	0.083	0.083	0.099	0.090	0.083	0.083	0.083	0.083
107	*Hyla versicolor*	0.089	0.089	0.089	0.089	0.089	0.106	0.097	0.090	0.090	0.090	0.090
108	*Hyla versicolor*	0.089	0.089	0.089	0.089	0.089	0.105	0.102	0.095	0.095	0.095	0.095
109	*Hyla versicolor*	0.089	0.089	0.089	0.089	0.089	0.105	0.102	0.095	0.095	0.095	0.095
110	*Hyla_meridionalis*	0.131	0.131	0.131	0.131	0.131	0.150	0.133	0.125	0.125	0.125	0.125
111	*Hyla_orientalis*	0.135	0.135	0.135	0.135	0.135	0.154	0.136	0.128	0.128	0.128	0.128
112	*Hyla_japonica*	0.098	0.098	0.098	0.098	0.098	0.112	0.106	0.099	0.099	0.099	0.099
113	*Hyla_eximia*	0.093	0.093	0.093	0.093	0.093	0.110	0.114	0.106	0.106	0.106	0.106
114	*Hyla_arenicolor*	0.074	0.074	0.074	0.074	0.074	0.090	0.081	0.074	0.074	0.074	0.074
115	*Hyla_femoralis*	0.119	0.119	0.119	0.119	0.119	0.137	0.123	0.116	0.116	0.116	0.116
116	*Hyla_arborea*	0.141	0.141	0.141	0.141	0.141	0.160	0.150	0.142	0.142	0.142	0.142
117	*Osteopilus septentrionalis*	0.188	0.188	0.188	0.188	0.188	0.210	0.198	0.189	0.189	0.189	0.189

Table A.1.2. Continued

		67	68	69	70	71	72	73	74	75	76	77
61	PNP_197											
62	JWS_357											
63	JWS_474											
64	LSUMZ_48181											
65	MVZ_145385											
66	PNP_003											
67	PNP_010											
68	PNP_015	0.000										
69	PNP_018	0.000	0.000									
70	PNP_020	0.000	0.000	0.000								
71	PNP_021	0.000	0.000	0.000	0.000							
72	PNP_025	0.000	0.000	0.000	0.000	0.000						
73	PNP_026	0.000	0.000	0.000	0.000	0.000	0.000					
74	PNP_027	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
75	PNP_029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
76	PNP_030	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
77	PNP_031	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
78	PNP_034	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
79	PNP_035	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
80	PNP_110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
81	PNP_111	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
82	PNP_117	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
83	PNP_126	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
84	PNP_127	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
85	PNP_140	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
86	PNP_176	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
87	PNP_198	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
88	PNP_199	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
89	PNP011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
90	PNP014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table A.1.2. Continued

		67	68	69	70	71	72	73	74	75	76	77
91	PNP019	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
92	PNP156	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
93	PNP158	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
94	PNP160	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
95	PNP163	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
96	PNP164	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
97	PNP165	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
98	PNP166	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
99	PNP167	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100	PNP168	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
101	PNP169	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
102	PNP173	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
103	PNP174	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
104	SIH_06	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
105	TNHC_61054	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
106	TNHC_61055	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
107	UMFS_5545	0.090	0.090	0.090	0.090	0.090	0.090	0.090	0.090	0.090	0.090	0.090
108	PNP_023	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095
109	PNP_024	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095
110	*Hyla_meridionalis*	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
111	*Hyla_orientalis*	0.128	0.128	0.128	0.128	0.128	0.128	0.128	0.128	0.128	0.128	0.128
112	*Hyla_japonica*	0.099	0.099	0.099	0.099	0.099	0.099	0.099	0.099	0.099	0.099	0.099
113	*Hyla_eximia*	0.106	0.106	0.106	0.106	0.106	0.106	0.106	0.106	0.106	0.106	0.106
114	*Hyla_arenicolor*	0.074	0.074	0.074	0.074	0.074	0.074	0.074	0.074	0.074	0.074	0.074
115	*Hyla_femoralis*	0.116	0.116	0.116	0.116	0.116	0.116	0.116	0.116	0.116	0.116	0.116
116	*Hyla_arborea*	0.142	0.142	0.142	0.142	0.142	0.142	0.142	0.142	0.142	0.142	0.142
117	PNP_040	0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.189

Table A.1.2. Continued

		78	79	80	81	82	83	84	85	86	87	88
61	PNP_197											
62	JWS_357											
63	JWS_474											
64	LSUMZ_48181											
65	MVZ_145385											
66	PNP_003											
67	PNP_010											
68	PNP_015											
69	PNP_018											
70	PNP_020											
71	PNP_021											
72	PNP_025											
73	PNP_026											
74	PNP_027											
75	PNP_029											
76	PNP_030											
77	PNP_031											
78	PNP_034											
79	PNP_035	0.000										
80	PNP_110	0.000	0.000									
81	PNP_111	0.000	0.000	0.000								
82	PNP_117	0.000	0.000	0.000	0.000							
83	PNP_126	0.000	0.000	0.000	0.000	0.000						
84	PNP_127	0.000	0.000	0.000	0.000	0.000	0.000					
85	PNP_140	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
86	PNP_176	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
87	PNP_198	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
88	PNP_199	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
89	PNP011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
90	PNP014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table A.1.2. Continued		78	79	80	81	82	83	84	85	86	87	88
91	PNP019	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
92	PNP156	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
93	PNP158	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
94	PNP160	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
95	PNP163	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
96	PNP164	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
97	PNP165	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
98	PNP166	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
99	PNP167	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100	PNP168	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
101	PNP169	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
102	PNP173	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
103	PNP174	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
104	SIH_06	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
105	TNHC_61054	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
106	*Hyla versicolor*	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
107	*Hyla versicolor*	0.090	0.090	0.090	0.090	0.090	0.090	0.090	0.090	0.090	0.090	0.090
108	*Hyla versicolor*	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095
109	*Hyla versicolor*	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095
110	*Hyla_meridionalis*	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
111	*Hyla_orientalis*	0.128	0.128	0.128	0.128	0.128	0.128	0.128	0.128	0.128	0.128	0.128
112	*Hyla_japonica*	0.099	0.099	0.099	0.099	0.099	0.099	0.099	0.099	0.099	0.099	0.099
113	*Hyla_eximia*	0.106	0.106	0.106	0.106	0.106	0.106	0.106	0.106	0.106	0.106	0.106
114	*Hyla_arenicolor*	0.074	0.074	0.074	0.074	0.074	0.074	0.074	0.074	0.074	0.074	0.074
115	*Hyla_femoralis*	0.116	0.116	0.116	0.116	0.116	0.116	0.116	0.116	0.116	0.116	0.116
116	*Hyla_arborea*	0.142	0.142	0.142	0.142	0.142	0.142	0.142	0.142	0.142	0.142	0.142
117	*Osteopilus septentrionalis*	0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.189

Table A.1.2. Continued

		89	90	91	92	93	94	95	96	97	98	99
61	PNP_197											
62	JWS_357											
63	JWS_474											
64	LSUMZ_48181											
65	MVZ_145385											
66	PNP_003											
67	PNP_010											
68	PNP_015											
69	PNP_018											
70	PNP_020											
71	PNP_021											
72	PNP_025											
73	PNP_026											
74	PNP_027											
75	PNP_029											
76	PNP_030											
77	PNP_031											
78	PNP_034											
79	PNP_035											
80	PNP_110											
81	PNP_111											
82	PNP_117											
83	PNP_126											
84	PNP_127											
85	PNP_140											
86	PNP_176											
87	PNP_198											
88	PNP_199											
89	PNP011											
90	PNP014	0.000										

Table A.1.2. Continued

		89	90	91	92	93	94	95	96	97	98	99
91	PNP019	0.005	0.005									
92	PNP156	0.000	0.000	0.005								
93	PNP158	0.000	0.000	0.005	0.000							
94	PNP160	0.000	0.000	0.005	0.000	0.000						
95	PNP163	0.000	0.000	0.005	0.000	0.000	0.000					
96	PNP164	0.000	0.000	0.005	0.000	0.000	0.000	0.000				
97	PNP165	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000			
98	PNP166	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000		
99	PNP167	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
100	PNP168	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
101	PNP169	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
102	PNP173	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
103	PNP174	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
104	SIH_06	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
105	TNHC_61054	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
106	*Hyla versicolor*	0.083	0.083	0.087	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
107	*Hyla versicolor*	0.090	0.090	0.094	0.090	0.090	0.090	0.090	0.090	0.090	0.090	0.090
108	*Hyla versicolor*	0.095	0.095	0.099	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095
109	*Hyla versicolor*	0.095	0.095	0.099	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095
110	*Hyla_meridionalis*	0.125	0.125	0.130	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
111	*Hyla_orientalis*	0.128	0.128	0.133	0.128	0.128	0.128	0.128	0.128	0.128	0.128	0.128
112	*Hyla_japonica*	0.099	0.099	0.103	0.099	0.099	0.099	0.099	0.099	0.099	0.099	0.099
113	*Hyla_eximia*	0.106	0.106	0.110	0.106	0.106	0.106	0.106	0.106	0.106	0.106	0.106
114	*Hyla_arenicolor*	0.074	0.074	0.081	0.074	0.074	0.074	0.074	0.074	0.074	0.074	0.074
115	*Hyla_femoralis*	0.116	0.116	0.120	0.116	0.116	0.116	0.116	0.116	0.116	0.116	0.116
116	*Hyla_arborea*	0.142	0.142	0.147	0.142	0.142	0.142	0.142	0.142	0.142	0.142	0.142
117	*Osteopilus septentrionalis*	0.189	0.189	0.195	0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.189

Table A.1.2. Continued

		100	101	102	103	104	105	106	107	108	109	110
91	PNP019											
92	PNP156											
93	PNP158											
94	PNP160											
95	PNP163											
96	PNP164											
97	PNP165											
98	PNP166											
99	PNP167											
100	PNP168											
101	PNP169	0.000										
102	PNP173	0.000	0.000									
103	PNP174	0.000	0.000	0.000								
104	SIH_06	0.000	0.000	0.000	0.000							
105	TNHC_61054	0.000	0.000	0.000	0.000	0.000						
106	*Hyla versicolor*	0.083	0.083	0.083	0.083	0.083	0.083					
107	*Hyla versicolor*	0.090	0.090	0.090	0.090	0.090	0.090	0.011				
108	*Hyla versicolor*	0.095	0.095	0.095	0.095	0.095	0.095	0.024	0.019			
109	*Hyla versicolor*	0.095	0.095	0.095	0.095	0.095	0.095	0.019	0.013	0.005		
110	*Hyla_meridionalis*	0.125	0.125	0.125	0.125	0.125	0.125	0.102	0.096	0.090	0.096	
111	*Hyla_orientalis*	0.128	0.128	0.128	0.128	0.128	0.128	0.123	0.117	0.123	0.123	0.089
112	*Hyla_japonica*	0.099	0.099	0.099	0.099	0.099	0.099	0.053	0.048	0.053	0.053	0.096
113	*Hyla_eximia*	0.106	0.106	0.106	0.106	0.106	0.106	0.059	0.054	0.048	0.048	0.091
114	*Hyla_arenicolor*	0.074	0.074	0.074	0.074	0.074	0.074	0.050	0.045	0.044	0.044	0.099
115	*Hyla_femoralis*	0.116	0.116	0.116	0.116	0.116	0.116	0.100	0.097	0.103	0.103	0.147
116	*Hyla_arborea*	0.142	0.142	0.142	0.142	0.142	0.142	0.120	0.114	0.119	0.119	0.095
117	*Osteopilus septentrionalis*	0.189	0.189	0.189	0.189	0.189	0.189	0.157	0.169	0.164	0.168	0.172

Table A.1.2. Continued

		111	112	113	114	115	116	117
91	PNP019							
92	PNP156							
93	PNP158							
94	PNP160							
95	PNP163							
96	PNP164							
97	PNP165							
98	PNP166							
99	PNP167							
100	PNP168							
101	PNP169							
102	PNP173							
103	PNP174							
104	SIH_06							
105	TNHC_61054							
106	*Hyla versicolor*							
107	*Hyla versicolor*							
108	*Hyla versicolor*							
109	*Hyla versicolor*							
110	*Hyla_meridionalis*							
111	*Hyla_orientalis*							
112	*Hyla_japonica*	0.134						
113	*Hyla_eximia*	0.132	0.060					
114	*Hyla_arenicolor*	0.113	0.047	0.054				
115	*Hyla_femoralis*	0.138	0.106	0.114	0.083			
116	*Hyla_arborea*	0.044	0.130	0.129	0.113	0.148		
117	Osteopilus septentrionalis	0.180	0.182	0.177	0.179	0.204	0.179	

Table A.1.3. Pairwise nucleotide differences in 12S mitochondrial sequences. Cells highlighted in blue represent samples from Dryophytes *squirellus*, cells highlighted in red represent samples from *D. gratiosus*, cells highlighted in yellow represent samples from the eastern clade of *D. cinereus*, and cells highlighted in green represent samples from the western clade of *D. cinereus*.

		1	2	3	4	5	6	7	8	9	10	11	12
1	MVZ145422												
2	MVZ145423	0.000											
3	MVZ145430	0.000	0.000										
4	MVZ145431	0.000	0.000	0.000									
5	PNP028	0.003	0.003	0.003	0.003								
6	PNP036	0.003	0.003	0.003	0.003	0.000							
7	PNP086	0.003	0.003	0.003	0.003	0.006	0.006						
8	PNP087	0.000	0.000	0.000	0.000	0.003	0.003	0.003					
9	PNP091	0.006	0.006	0.006	0.006	0.003	0.003	0.009	0.006				
10	PNP093	0.003	0.003	0.003	0.003	0.000	0.000	0.006	0.003	0.003			
11	PNP101	0.003	0.003	0.003	0.003	0.000	0.000	0.006	0.003	0.003	0.000		
12	PNP124	0.003	0.003	0.003	0.003	0.000	0.000	0.006	0.003	0.003	0.000	0.000	
13	PNP129	0.003	0.003	0.003	0.003	0.000	0.000	0.006	0.003	0.003	0.000	0.000	0.000
14	PNP147	0.003	0.003	0.003	0.003	0.000	0.000	0.006	0.003	0.003	0.000	0.000	0.000
15	PNP185	0.006	0.006	0.006	0.006	0.003	0.003	0.009	0.006	0.006	0.003	0.003	0.003
16	PNP196	0.003	0.003	0.003	0.003	0.000	0.000	0.006	0.003	0.003	0.000	0.000	0.000
17	AMNHA168427	0.003	0.003	0.003	0.003	0.006	0.006	0.006	0.003	0.009	0.006	0.006	0.006
18	LSUMZ48185	0.006	0.006	0.006	0.006	0.003	0.003	0.009	0.006	0.006	0.003	0.003	0.003
19	LSUMZH487	0.006	0.006	0.006	0.006	0.003	0.003	0.009	0.006	0.006	0.003	0.003	0.003
20	CAS169394	0.113	0.113	0.113	0.113	0.110	0.110	0.117	0.113	0.106	0.110	0.110	0.110
21	CAS169395	0.113	0.113	0.113	0.113	0.110	0.110	0.117	0.113	0.106	0.110	0.110	0.110
22	CAS169396	0.114	0.114	0.114	0.114	0.111	0.110	0.117	0.114	0.106	0.110	0.110	0.110
23	CAS175122	0.113	0.113	0.113	0.113	0.110	0.110	0.117	0.113	0.106	0.110	0.110	0.110
24	CAS175123	0.113	0.113	0.113	0.113	0.110	0.110	0.117	0.113	0.106	0.110	0.110	0.110
25	CAS175126	0.113	0.113	0.113	0.113	0.110	0.110	0.117	0.113	0.106	0.110	0.110	0.110
26	MVZ137619	0.113	0.113	0.113	0.113	0.110	0.110	0.117	0.113	0.106	0.110	0.110	0.110
27	MVZ137620	0.113	0.113	0.113	0.113	0.110	0.110	0.117	0.113	0.106	0.110	0.110	0.110
28	MVZ145405	0.113	0.113	0.113	0.113	0.110	0.110	0.117	0.113	0.106	0.110	0.110	0.110
29	MVZ145406	0.113	0.113	0.113	0.113	0.110	0.110	0.117	0.113	0.106	0.110	0.110	0.110
30	MVZ161962	0.113	0.113	0.113	0.113	0.110	0.110	0.117	0.113	0.106	0.110	0.110	0.110

Table A.1.3. Continued

		1	2	3	4	5	6	7	8	9	10	11	12
31	MVZ161963	0.113	0.113	0.113	0.113	0.110	0.110	0.117	0.113	0.106	0.110	0.110	0.110
32	MVZ161964	0.113	0.113	0.113	0.113	0.110	0.110	0.117	0.113	0.106	0.110	0.110	0.110
33	AMNHA168404	0.118	0.118	0.118	0.118	0.115	0.114	0.122	0.118	0.110	0.114	0.114	0.114
34	LSUMZH15929	0.117	0.117	0.117	0.117	0.114	0.114	0.121	0.117	0.110	0.114	0.114	0.114
35	AMNHA181719	0.117	0.117	0.117	0.117	0.114	0.114	0.121	0.117	0.110	0.114	0.114	0.114
36	CAS223871	0.086	0.086	0.086	0.086	0.083	0.082	0.089	0.086	0.079	0.082	0.082	0.082
37	JWST007	0.082	0.082	0.082	0.082	0.079	0.079	0.086	0.082	0.075	0.079	0.079	0.079
38	PNP047	0.086	0.086	0.086	0.086	0.083	0.082	0.089	0.086	0.079	0.082	0.082	0.082
39	PNP054	0.086	0.086	0.086	0.086	0.083	0.082	0.089	0.086	0.079	0.082	0.082	0.082
40	PNP058	0.086	0.086	0.086	0.086	0.083	0.082	0.089	0.086	0.079	0.082	0.082	0.082
41	PNP063	0.086	0.086	0.086	0.086	0.083	0.082	0.089	0.086	0.079	0.082	0.082	0.082
42	PNP073	0.086	0.086	0.086	0.086	0.083	0.082	0.089	0.086	0.079	0.082	0.082	0.082
43	PNP075	0.086	0.086	0.086	0.086	0.083	0.082	0.089	0.086	0.079	0.082	0.082	0.082
44	PNP084	0.086	0.086	0.086	0.086	0.083	0.082	0.089	0.086	0.079	0.082	0.082	0.082
45	PNP085	0.086	0.086	0.086	0.086	0.083	0.082	0.089	0.086	0.079	0.082	0.082	0.082
46	PNP188	0.086	0.086	0.086	0.086	0.083	0.082	0.089	0.086	0.079	0.082	0.082	0.082
47	PNP190	0.086	0.086	0.086	0.086	0.083	0.082	0.089	0.086	0.079	0.082	0.082	0.082
48	X86238	0.090	0.090	0.090	0.090	0.087	0.086	0.093	0.090	0.082	0.086	0.086	0.086
49	PNP103	0.097	0.097	0.097	0.097	0.094	0.093	0.100	0.097	0.089	0.093	0.093	0.093
50	PNP131	0.097	0.097	0.097	0.097	0.094	0.093	0.100	0.097	0.089	0.093	0.093	0.093
51	PNP143	0.097	0.097	0.097	0.097	0.094	0.093	0.100	0.097	0.089	0.093	0.093	0.093
52	PNP177	0.097	0.097	0.097	0.097	0.094	0.093	0.100	0.097	0.089	0.093	0.093	0.093
53	PNP178	0.097	0.097	0.097	0.097	0.094	0.093	0.100	0.097	0.089	0.093	0.093	0.093
54	PNP180	0.097	0.097	0.097	0.097	0.094	0.094	0.101	0.097	0.090	0.094	0.094	0.094
55	PNP183	0.097	0.097	0.097	0.097	0.094	0.093	0.100	0.097	0.089	0.093	0.093	0.093
56	PNP186	0.097	0.097	0.097	0.097	0.094	0.093	0.100	0.097	0.089	0.093	0.093	0.093
57	PNP192	0.097	0.097	0.097	0.097	0.094	0.093	0.100	0.097	0.089	0.093	0.093	0.093
58	PNP193	0.097	0.097	0.097	0.097	0.094	0.093	0.100	0.097	0.089	0.093	0.093	0.093
59	PNP197	0.097	0.097	0.097	0.097	0.094	0.093	0.100	0.097	0.089	0.093	0.093	0.093
60	PNP015	0.096	0.096	0.096	0.096	0.093	0.093	0.100	0.096	0.089	0.093	0.093	0.093

Table A.1.3. Continued

		1	2	3	4	5	6	7	8	9	10	11	12
61	PNP018	0.093	0.093	0.093	0.093	0.090	0.089	0.096	0.093	0.085	0.089	0.089	0.089
62	PNP019	0.093	0.093	0.093	0.093	0.090	0.089	0.096	0.093	0.085	0.089	0.089	0.089
63	PNP020	0.096	0.096	0.096	0.096	0.093	0.093	0.100	0.096	0.089	0.093	0.093	0.093
64	PNP021	0.093	0.093	0.093	0.093	0.090	0.089	0.096	0.093	0.085	0.089	0.089	0.089
65	PNP176	0.093	0.093	0.093	0.093	0.090	0.089	0.097	0.093	0.086	0.089	0.089	0.089
66	PNP003	0.096	0.096	0.096	0.096	0.093	0.093	0.100	0.096	0.089	0.093	0.093	0.093
67	PNP126	0.093	0.093	0.093	0.093	0.090	0.089	0.096	0.093	0.085	0.089	0.089	0.089
68	PNP127	0.096	0.096	0.096	0.096	0.093	0.093	0.100	0.096	0.089	0.093	0.093	0.093
69	PNP140	0.096	0.096	0.096	0.096	0.093	0.093	0.100	0.096	0.089	0.093	0.093	0.093
70	PNP198	0.096	0.096	0.096	0.096	0.093	0.093	0.100	0.096	0.089	0.093	0.093	0.093
71	LSUMZ48181	0.100	0.100	0.100	0.100	0.097	0.097	0.104	0.100	0.093	0.097	0.097	0.097
72	JWS_357	0.093	0.093	0.093	0.093	0.090	0.089	0.096	0.093	0.085	0.089	0.089	0.089
73	JWS474	0.093	0.093	0.093	0.093	0.090	0.089	0.096	0.093	0.085	0.089	0.089	0.089
74	PNP010	0.093	0.093	0.093	0.093	0.090	0.089	0.096	0.093	0.085	0.089	0.089	0.089
75	PNP011	0.093	0.093	0.093	0.093	0.090	0.089	0.096	0.093	0.085	0.089	0.089	0.089
76	PNP014	0.093	0.093	0.093	0.093	0.090	0.089	0.096	0.093	0.085	0.089	0.089	0.089
77	PNP025	0.093	0.093	0.093	0.093	0.090	0.089	0.096	0.093	0.085	0.089	0.089	0.089
78	PNP027	0.093	0.093	0.093	0.093	0.090	0.089	0.096	0.093	0.085	0.089	0.089	0.089
79	PNP029	0.093	0.093	0.093	0.093	0.090	0.089	0.096	0.093	0.085	0.089	0.089	0.089
80	PNP030	0.093	0.093	0.093	0.093	0.090	0.089	0.096	0.093	0.085	0.089	0.089	0.089
81	PNP031	0.093	0.093	0.093	0.093	0.090	0.089	0.096	0.093	0.085	0.089	0.089	0.089
82	PNP034	0.093	0.093	0.093	0.093	0.090	0.089	0.096	0.093	0.085	0.089	0.089	0.089
83	PNP035	0.093	0.093	0.093	0.093	0.090	0.089	0.096	0.093	0.085	0.089	0.089	0.089
84	PNP110	0.093	0.093	0.093	0.093	0.090	0.089	0.096	0.093	0.085	0.089	0.089	0.089
85	PNP117	0.089	0.089	0.089	0.089	0.086	0.085	0.093	0.089	0.082	0.085	0.085	0.085
86	MVZ145385	0.096	0.096	0.096	0.096	0.094	0.093	0.100	0.096	0.089	0.093	0.093	0.093
87	TNHC61054	0.096	0.096	0.096	0.096	0.094	0.093	0.100	0.096	0.089	0.093	0.093	0.093
88	PNP098	0.047	0.047	0.047	0.047	0.051	0.051	0.051	0.047	0.054	0.051	0.051	0.051
89	*Hyla versicolor*	0.078	0.078	0.078	0.078	0.082	0.082	0.082	0.078	0.078	0.082	0.082	0.082

Table A.1.3. Continued

		1	2	3	4	5	6	7	8	9	10	11	12
90	*Hyla versicolor*	0.068	0.068	0.068	0.068	0.072	0.072	0.072	0.068	0.068	0.072	0.072	0.072
91	*Hyla versicolor*	0.068	0.068	0.068	0.068	0.072	0.072	0.072	0.068	0.068	0.072	0.072	0.072
92	*Hyla versicolor*	0.075	0.075	0.075	0.075	0.079	0.078	0.079	0.075	0.075	0.078	0.078	0.078
93	*Hyla versicolor*	0.079	0.079	0.079	0.079	0.083	0.082	0.082	0.079	0.078	0.082	0.082	0.082
94	*Hyla versicolor*	0.079	0.079	0.079	0.079	0.083	0.082	0.082	0.079	0.078	0.082	0.082	0.082
95	*Hyla_arborea*	0.083	0.083	0.083	0.083	0.087	0.087	0.087	0.083	0.091	0.087	0.087	0.087
96	*Hyla_eximia*	0.076	0.076	0.076	0.076	0.080	0.079	0.079	0.076	0.075	0.079	0.079	0.079
97	*Hyla_femoralis*	0.090	0.090	0.090	0.090	0.094	0.093	0.094	0.090	0.089	0.093	0.093	0.093
98	*Hyla_arenicolor*	0.090	0.090	0.090	0.090	0.094	0.093	0.093	0.090	0.089	0.093	0.093	0.093
99	*Hyla_japonica*	0.104	0.104	0.104	0.104	0.108	0.108	0.108	0.104	0.104	0.108	0.108	0.108
100	*Hyla_orientalis*	0.080	0.080	0.080	0.080	0.084	0.083	0.084	0.080	0.087	0.083	0.083	0.083
101	*Hyla_meridionalis*	0.094	0.094	0.094	0.094	0.098	0.098	0.098	0.094	0.094	0.098	0.098	0.098
102	*Osteopilus septentrionalis*	0.129	0.129	0.129	0.129	0.134	0.133	0.133	0.129	0.129	0.133	0.133	0.133

Table A.1.3. Continued

		13	14	15	16	17	18	19	20	21	22	23	24
1	MVZ145422												
2	MVZ145423												
3	MVZ145430												
4	MVZ145431												
5	PNP028												
6	PNP036												
7	PNP086												
8	PNP087												
9	PNP091												
10	PNP093												
11	PNP101												
12	PNP124												
13	PNP129												
14	PNP147	0.000											
15	PNP185	0.003	0.003										
16	PNP196	0.000	0.000	0.003									
17	AMNHA168427	0.006	0.006	0.009	0.006								
18	LSUMZ48185	0.003	0.003	0.006	0.003	0.003							
19	LSUMZH487	0.003	0.003	0.006	0.003	0.003	0.000						
20	CAS169394	0.110	0.110	0.113	0.110	0.117	0.114	0.114					
21	CAS169395	0.110	0.110	0.113	0.110	0.117	0.114	0.114	0.000				
22	CAS169396	0.110	0.110	0.114	0.110	0.118	0.114	0.114	0.003	0.003			
23	CAS175122	0.110	0.110	0.113	0.110	0.117	0.114	0.114	0.000	0.000	0.003		
24	CAS175123	0.110	0.110	0.113	0.110	0.117	0.114	0.114	0.000	0.000	0.003	0.000	
25	CAS175126	0.110	0.110	0.113	0.110	0.117	0.114	0.114	0.000	0.000	0.003	0.000	0.000
26	MVZ137619	0.110	0.110	0.113	0.110	0.117	0.114	0.114	0.000	0.000	0.003	0.000	0.000
27	MVZ137620	0.110	0.110	0.113	0.110	0.117	0.114	0.114	0.000	0.000	0.003	0.000	0.000
28	MVZ145405	0.110	0.110	0.113	0.110	0.117	0.114	0.114	0.000	0.000	0.003	0.000	0.000
29	MVZ145406	0.110	0.110	0.113	0.110	0.117	0.114	0.114	0.000	0.000	0.003	0.000	0.000
30	MVZ161962	0.110	0.110	0.113	0.110	0.117	0.114	0.114	0.000	0.000	0.003	0.000	0.000

Table A.1.3. Continued

		13	14	15	16	17	18	19	20	21	22	23	24
31	MVZ161963	0.110	0.110	0.113	0.110	0.117	0.114	0.114	0.000	0.000	0.003	0.000	0.000
32	MVZ161964	0.110	0.110	0.113	0.110	0.117	0.114	0.114	0.000	0.000	0.003	0.000	0.000
33	AMNHA168404	0.114	0.114	0.118	0.114	0.114	0.110	0.110	0.006	0.006	0.003	0.006	0.006
34	LSUMZH15929	0.114	0.114	0.117	0.114	0.113	0.110	0.110	0.003	0.003	0.006	0.003	0.003
35	AMNHA181719	0.114	0.114	0.117	0.114	0.113	0.110	0.110	0.003	0.003	0.006	0.003	0.003
36	CAS223871	0.082	0.082	0.086	0.082	0.090	0.086	0.086	0.081	0.081	0.081	0.081	0.081
37	JWST007	0.079	0.079	0.083	0.079	0.086	0.083	0.083	0.084	0.084	0.084	0.084	0.084
38	PNP047	0.082	0.082	0.086	0.082	0.090	0.086	0.086	0.081	0.081	0.081	0.081	0.081
39	PNP054	0.082	0.082	0.086	0.082	0.090	0.086	0.086	0.081	0.081	0.081	0.081	0.081
40	PNP058	0.082	0.082	0.086	0.082	0.090	0.086	0.086	0.081	0.081	0.081	0.081	0.081
41	PNP063	0.082	0.082	0.086	0.082	0.090	0.086	0.086	0.081	0.081	0.081	0.081	0.081
42	PNP073	0.082	0.082	0.086	0.082	0.090	0.086	0.086	0.081	0.081	0.081	0.081	0.081
43	PNP075	0.082	0.082	0.086	0.082	0.090	0.086	0.086	0.081	0.081	0.081	0.081	0.081
44	PNP084	0.082	0.082	0.086	0.082	0.090	0.086	0.086	0.081	0.081	0.081	0.081	0.081
45	PNP085	0.082	0.082	0.086	0.082	0.090	0.086	0.086	0.081	0.081	0.081	0.081	0.081
46	PNP188	0.082	0.082	0.086	0.082	0.090	0.086	0.086	0.081	0.081	0.081	0.081	0.081
47	PNP190	0.082	0.082	0.086	0.082	0.090	0.086	0.086	0.081	0.081	0.081	0.081	0.081
48	X86238	0.086	0.086	0.090	0.086	0.086	0.082	0.082	0.084	0.084	0.085	0.084	0.084
49	PNP103	0.093	0.093	0.097	0.093	0.101	0.097	0.097	0.084	0.084	0.085	0.084	0.084
50	PNP131	0.093	0.093	0.097	0.093	0.101	0.097	0.097	0.084	0.084	0.085	0.084	0.084
51	PNP143	0.093	0.093	0.097	0.093	0.101	0.097	0.097	0.084	0.084	0.085	0.084	0.084
52	PNP177	0.093	0.093	0.097	0.093	0.101	0.097	0.097	0.084	0.084	0.085	0.084	0.084
53	PNP178	0.093	0.093	0.097	0.093	0.101	0.097	0.097	0.084	0.084	0.085	0.084	0.084
54	PNP180	0.094	0.094	0.098	0.094	0.101	0.098	0.098	0.085	0.085	0.085	0.085	0.085
55	PNP183	0.093	0.093	0.097	0.093	0.101	0.097	0.097	0.084	0.084	0.085	0.084	0.084
56	PNP186	0.093	0.093	0.097	0.093	0.101	0.097	0.097	0.084	0.084	0.085	0.084	0.084
57	PNP192	0.093	0.093	0.097	0.093	0.101	0.097	0.097	0.084	0.084	0.085	0.084	0.084
58	PNP193	0.093	0.093	0.097	0.093	0.101	0.097	0.097	0.084	0.084	0.085	0.084	0.084
59	PNP197	0.093	0.093	0.097	0.093	0.101	0.097	0.097	0.084	0.084	0.085	0.084	0.084
60	PNP015	0.093	0.093	0.096	0.093	0.100	0.096	0.096	0.087	0.087	0.088	0.087	0.087

Table A.1.3. Continued

		13	14	15	16	17	18	19	20	21	22	23	24
61	PNP018	0.089	0.089	0.093	0.089	0.096	0.093	0.093	0.084	0.084	0.084	0.084	0.084
62	PNP019	0.089	0.089	0.093	0.089	0.096	0.093	0.093	0.084	0.084	0.084	0.084	0.084
63	PNP020	0.093	0.093	0.096	0.093	0.100	0.096	0.096	0.087	0.087	0.088	0.087	0.087
64	PNP021	0.089	0.089	0.093	0.089	0.096	0.093	0.093	0.084	0.084	0.084	0.084	0.084
65	PNP176	0.089	0.089	0.093	0.089	0.097	0.093	0.093	0.084	0.084	0.085	0.084	0.084
66	PNP003	0.093	0.093	0.097	0.093	0.100	0.097	0.097	0.087	0.087	0.088	0.087	0.087
67	PNP126	0.089	0.089	0.093	0.089	0.096	0.093	0.093	0.084	0.084	0.084	0.084	0.084
68	PNP127	0.093	0.093	0.097	0.093	0.100	0.097	0.097	0.087	0.087	0.088	0.087	0.087
69	PNP140	0.093	0.093	0.097	0.093	0.100	0.097	0.097	0.087	0.087	0.088	0.087	0.087
70	PNP198	0.093	0.093	0.097	0.093	0.100	0.097	0.097	0.087	0.087	0.088	0.087	0.087
71	LSUMZ48181	0.097	0.097	0.101	0.097	0.096	0.093	0.093	0.091	0.091	0.092	0.091	0.091
72	JWS_357	0.089	0.089	0.093	0.089	0.096	0.093	0.093	0.087	0.087	0.088	0.087	0.087
73	JWS474	0.089	0.089	0.093	0.089	0.096	0.093	0.093	0.087	0.087	0.088	0.087	0.087
74	PNP010	0.089	0.089	0.093	0.089	0.096	0.093	0.093	0.087	0.087	0.088	0.087	0.087
75	PNP011	0.089	0.089	0.093	0.089	0.096	0.093	0.093	0.087	0.087	0.088	0.087	0.087
76	PNP014	0.089	0.089	0.093	0.089	0.096	0.093	0.093	0.087	0.087	0.088	0.087	0.087
77	PNP025	0.089	0.089	0.093	0.089	0.096	0.093	0.093	0.087	0.087	0.088	0.087	0.087
78	PNP027	0.089	0.089	0.093	0.089	0.096	0.093	0.093	0.087	0.087	0.088	0.087	0.087
79	PNP029	0.089	0.089	0.093	0.089	0.096	0.093	0.093	0.087	0.087	0.088	0.087	0.087
80	PNP030	0.089	0.089	0.093	0.089	0.096	0.093	0.093	0.087	0.087	0.088	0.087	0.087
81	PNP031	0.089	0.089	0.093	0.089	0.096	0.093	0.093	0.087	0.087	0.088	0.087	0.087
82	PNP034	0.089	0.089	0.093	0.089	0.096	0.093	0.093	0.087	0.087	0.088	0.087	0.087
83	PNP035	0.089	0.089	0.093	0.089	0.096	0.093	0.093	0.087	0.087	0.088	0.087	0.087
84	PNP110	0.089	0.089	0.093	0.089	0.096	0.093	0.093	0.087	0.087	0.088	0.087	0.087
85	PNP117	0.085	0.085	0.089	0.085	0.093	0.089	0.089	0.084	0.084	0.084	0.084	0.084
86	MVZ145385	0.093	0.093	0.097	0.093	0.093	0.089	0.089	0.091	0.091	0.092	0.091	0.091
87	TNHC61054	0.093	0.093	0.097	0.093	0.093	0.089	0.089	0.091	0.091	0.092	0.091	0.091
88	PNP098	0.051	0.051	0.054	0.051	0.051	0.054	0.054	0.114	0.114	0.114	0.114	0.114
89	*Hyla versicolor*	0.082	0.082	0.085	0.082	0.082	0.086	0.086	0.074	0.074	0.077	0.074	0.074

Table A.1.3. Continued

		13	14	15	16	17	18	19	20	21	22	23	24
90	*Hyla versicolor*	0.072	0.072	0.075	0.072	0.072	0.075	0.075	0.077	0.077	0.077	0.077	0.077
91	*Hyla versicolor*	0.072	0.072	0.075	0.072	0.072	0.075	0.075	0.077	0.077	0.077	0.077	0.077
92	*Hyla versicolor*	0.078	0.078	0.082	0.078	0.079	0.082	0.082	0.077	0.077	0.081	0.077	0.077
93	*Hyla versicolor*	0.082	0.082	0.086	0.082	0.075	0.078	0.078	0.081	0.081	0.084	0.081	0.081
94	*Hyla versicolor*	0.082	0.082	0.086	0.082	0.075	0.078	0.078	0.081	0.081	0.084	0.081	0.081
95	*Hyla_arborea*	0.087	0.087	0.090	0.087	0.079	0.083	0.083	0.107	0.107	0.103	0.107	0.107
96	*Hyla_eximia*	0.079	0.079	0.083	0.079	0.072	0.075	0.075	0.084	0.084	0.085	0.084	0.084
97	*Hyla_femoralis*	0.093	0.093	0.097	0.093	0.086	0.089	0.089	0.078	0.078	0.082	0.078	0.078
98	*Hyla_arenicolor*	0.093	0.093	0.097	0.093	0.086	0.089	0.089	0.081	0.081	0.085	0.081	0.081
99	*Hyla_japonica*	0.108	0.108	0.112	0.108	0.100	0.104	0.104	0.092	0.092	0.088	0.092	0.092
100	*Hyla_orientalis*	0.083	0.083	0.087	0.083	0.076	0.080	0.080	0.111	0.111	0.107	0.111	0.111
101	*Hyla_meridionalis*	0.098	0.098	0.102	0.098	0.090	0.094	0.094	0.114	0.114	0.110	0.114	0.114
102	*Osteopilus septentrionalis*	0.133	0.133	0.137	0.133	0.134	0.138	0.138	0.110	0.110	0.114	0.110	0.110

Table A.1.3. Continued

		25	26	27	28	29	30	31	32	33	34	35	36
1	MVZ145422												
2	MVZ145423												
3	MVZ145430												
4	MVZ145431												
5	PNP028												
6	PNP036												
7	PNP086												
8	PNP087												
9	PNP091												
10	PNP093												
11	PNP101												
12	PNP124												
13	PNP129												
14	PNP147												
15	PNP185												
16	PNP196												
17	AMNHA168427												
18	LSUMZ48185												
19	LSUMZH487												
20	CAS169394												
21	CAS169395												
22	CAS169396												
23	CAS175122												
24	CAS175123												
25	CAS175126												
26	MVZ137619	0.000											
27	MVZ137620	0.000	0.000										
28	MVZ145405	0.000	0.000	0.000									
29	MVZ145406	0.000	0.000	0.000	0.000								
30	MVZ161962	0.000	0.000	0.000	0.000	0.000							

Table A.1.3. Continued

		25	26	27	28	29	30	31	32	33	34	35	36
31	MVZ161963	0.000	0.000	0.000	0.000	0.000	0.000						
32	MVZ161964	0.000	0.000	0.000	0.000	0.000	0.000	0.000					
33	AMNHA168404	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006				
34	LSUMZH15929	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003			
35	AMNHA181719	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.000		
36	CAS223871	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.085	0.084	0.084	
37	JWST007	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.088	0.088	0.088	0.003
38	PNP047	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.085	0.084	0.084	0.000
39	PNP054	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.085	0.084	0.084	0.000
40	PNP058	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.085	0.084	0.084	0.000
41	PNP063	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.085	0.084	0.084	0.000
42	PNP073	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.085	0.084	0.084	0.000
43	PNP075	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.085	0.084	0.084	0.000
44	PNP084	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.085	0.084	0.084	0.000
45	PNP085	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.085	0.084	0.084	0.000
46	PNP188	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.085	0.084	0.084	0.000
47	PNP190	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.085	0.084	0.084	0.000
48	X86238	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.081	0.081	0.081	0.003
49	PNP103	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.089	0.088	0.088	0.009
50	PNP131	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.089	0.088	0.088	0.009
51	PNP143	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.089	0.088	0.088	0.009
52	PNP177	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.089	0.088	0.088	0.009
53	PNP178	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.089	0.088	0.088	0.009
54	PNP180	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.089	0.089	0.089	0.009
55	PNP183	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.089	0.088	0.088	0.009
56	PNP186	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.089	0.088	0.088	0.009
57	PNP192	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.089	0.088	0.088	0.009
58	PNP193	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.089	0.088	0.088	0.009
59	PNP197	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.089	0.088	0.088	0.009
60	PNP015	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.091	0.091	0.091	0.015

Table A.1.3. Continued

		25	26	27	28	29	30	31	32	33	34	35	36
61	PNP018	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.088	0.088	0.088	0.012
62	PNP019	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.088	0.088	0.088	0.012
63	PNP020	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.091	0.091	0.091	0.015
64	PNP021	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.088	0.088	0.088	0.012
65	PNP176	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.088	0.088	0.088	0.012
66	PNP003	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.092	0.091	0.091	0.015
67	PNP126	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.088	0.088	0.088	0.012
68	PNP127	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.092	0.091	0.091	0.015
69	PNP140	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.092	0.091	0.091	0.015
70	PNP198	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.092	0.091	0.091	0.015
71	LSUMZ48181	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.088	0.087	0.087	0.019
72	JWS_357	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.092	0.091	0.091	0.022
73	JWS474	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.092	0.091	0.091	0.022
74	PNP010	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.092	0.091	0.091	0.022
75	PNP011	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.092	0.091	0.091	0.022
76	PNP014	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.092	0.091	0.091	0.022
77	PNP025	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.092	0.091	0.091	0.022
78	PNP027	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.092	0.091	0.091	0.022
79	PNP029	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.092	0.091	0.091	0.022
80	PNP030	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.092	0.091	0.091	0.022
81	PNP031	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.092	0.091	0.091	0.022
82	PNP034	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.092	0.091	0.091	0.022
83	PNP035	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.092	0.091	0.091	0.022
84	PNP110	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.092	0.091	0.091	0.022
85	PNP117	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.088	0.088	0.088	0.018
86	MVZ145385	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.088	0.087	0.087	0.025
87	TNHC61054	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.088	0.087	0.087	0.025
88	PNP098	0.114	0.114	0.114	0.114	0.114	0.114	0.114	0.114	0.118	0.118	0.118	0.051
89	*Hyla versicolor*	0.074	0.074	0.074	0.074	0.074	0.074	0.074	0.074	0.081	0.077	0.077	0.088

Table A.1.3. Continued

		25	26	27	28	29	30	31	32	33	34	35	36
90	*Hyla versicolor*	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.081	0.081	0.081	0.084
91	*Hyla versicolor*	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.081	0.081	0.081	0.084
92	*Hyla versicolor*	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.084	0.081	0.081	0.091
93	*Hyla versicolor*	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.077	0.077	0.095
94	*Hyla versicolor*	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.077	0.077	0.095
95	*Hyla_arborea*	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.099	0.103	0.103	0.119
96	*Hyla_eximia*	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.081	0.081	0.081	0.089
97	*Hyla_femoralis*	0.078	0.078	0.078	0.078	0.078	0.078	0.078	0.078	0.078	0.074	0.074	0.103
98	*Hyla_arenicolor*	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.081	0.077	0.077	0.103
99	*Hyla_japonica*	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.084	0.088	0.088	0.106
100	*Hyla_orientalis*	0.111	0.111	0.111	0.111	0.111	0.111	0.111	0.111	0.103	0.107	0.107	0.116
101	*Hyla_meridionalis*	0.114	0.114	0.114	0.114	0.114	0.114	0.114	0.114	0.106	0.110	0.110	0.104
102	*Osteopilus septentrionalis*	0.110	0.110	0.110	0.110	0.110	0.110	0.110	0.110	0.118	0.114	0.114	0.113

Table A.1.3. Continued

		37	38	39	40	41	42	43	44	45	46	47	48
31	MVZ161963												
32	MVZ161964												
33	AMNHA168404												
34	LSUMZH15929												
35	AMNHA181719												
36	CAS223871												
37	JWST007												
38	PNP047	0.003											
39	PNP054	0.003	0.000										
40	PNP058	0.003	0.000	0.000									
41	PNP063	0.003	0.000	0.000	0.000								
42	PNP073	0.003	0.000	0.000	0.000	0.000							
43	PNP075	0.003	0.000	0.000	0.000	0.000	0.000						
44	PNP084	0.003	0.000	0.000	0.000	0.000	0.000	0.000					
45	PNP085	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
46	PNP188	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
47	PNP190	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
48	X86238	0.006	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	
49	PNP103	0.012	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.012
50	PNP131	0.012	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.012
51	PNP143	0.012	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.012
52	PNP177	0.012	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.012
53	PNP178	0.012	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.012
54	PNP180	0.012	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.012
55	PNP183	0.012	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.012
56	PNP186	0.012	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.012
57	PNP192	0.012	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.012
58	PNP193	0.012	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.012
59	PNP197	0.012	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.012
60	PNP015	0.018	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.018

Table A.1.3. Continued

		37	38	39	40	41	42	43	44	45	46	47	48
61	PNP018	0.015	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.015
62	PNP019	0.015	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.015
63	PNP020	0.018	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.018
64	PNP021	0.015	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.015
65	PNP176	0.015	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.015
66	PNP003	0.018	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.019
67	PNP126	0.015	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.015
68	PNP127	0.018	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.019
69	PNP140	0.018	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.019
70	PNP198	0.018	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.019
71	LSUMZ48181	0.022	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.015
72	JWS_357	0.025	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.025
73	JWS474	0.025	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.025
74	PNP010	0.025	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.025
75	PNP011	0.025	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.025
76	PNP014	0.025	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.025
77	PNP025	0.025	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.025
78	PNP027	0.025	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.025
79	PNP029	0.025	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.025
80	PNP030	0.025	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.025
81	PNP031	0.025	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.025
82	PNP034	0.025	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.025
83	PNP035	0.025	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.025
84	PNP110	0.025	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.025
85	PNP117	0.022	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.022
86	MVZ145385	0.028	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.022
87	TNHC61054	0.028	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.022
88	PNP098	0.055	0.051	0.051	0.051	0.051	0.051	0.051	0.051	0.051	0.051	0.051	0.055
89	*Hyla versicolor*	0.084	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.092

Table A.1.3. Continued

		37	38	39	40	41	42	43	44	45	46	47	48
90	*Hyla versicolor*	0.081	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.088
91	*Hyla versicolor*	0.081	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.088
92	*Hyla versicolor*	0.088	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.095
93	*Hyla versicolor*	0.092	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.091
94	*Hyla versicolor*	0.092	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.091
95	*Hyla_arborea*	0.116	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.115
96	*Hyla_eximia*	0.085	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.089	0.085
97	*Hyla_femoralis*	0.099	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.099
98	*Hyla_arenicolor*	0.099	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.099
99	*Hyla_japonica*	0.102	0.106	0.106	0.106	0.106	0.106	0.106	0.106	0.106	0.106	0.106	0.102
100	*Hyla_orientalis*	0.113	0.116	0.116	0.116	0.116	0.116	0.116	0.116	0.116	0.116	0.116	0.112
101	*Hyla_meridionalis*	0.108	0.104	0.104	0.104	0.104	0.104	0.104	0.104	0.104	0.104	0.104	0.100
102	*Osteopilus septentrionalis*	0.117	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.117

Table A.1.3. Continued

		49	50	51	52	53	54	55	56	57	58	59	60
31	MVZ161963												
32	MVZ161964												
33	AMNHA168404												
34	LSUMZH15929												
35	AMNHA181719												
36	CAS223871												
37	JWST007												
38	PNP047												
39	PNP054												
40	PNP058												
41	PNP063												
42	PNP073												
43	PNP075												
44	PNP084												
45	PNP085												
46	PNP188												
47	PNP190												
48	X86238												
49	PNP103												
50	PNP131	0.000											
51	PNP143	0.000	0.000										
52	PNP177	0.000	0.000	0.000									
53	PNP178	0.000	0.000	0.000	0.000								
54	PNP180	0.000	0.000	0.000	0.000	0.000							
55	PNP183	0.000	0.000	0.000	0.000	0.000	0.000						
56	PNP186	0.000	0.000	0.000	0.000	0.000	0.000	0.000					
57	PNP192	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
58	PNP193	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
59	PNP197	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
60	PNP015	0.018	0.018	0.018	0.018	0.018	0.019	0.018	0.018	0.018	0.018	0.018	

Table A.1.3. Continued

		49	50	51	52	53	54	55	56	57	58	59	60
61	PNP018	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.003
62	PNP019	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.003
63	PNP020	0.018	0.018	0.018	0.018	0.018	0.019	0.018	0.018	0.018	0.018	0.018	0.006
64	PNP021	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.003
65	PNP176	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.003
66	PNP003	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.006
67	PNP126	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.009
68	PNP127	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.006
69	PNP140	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.006
70	PNP198	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.006
71	LSUMZ48181	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.009
72	JWS_357	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.012
73	JWS474	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.012
74	PNP010	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.012
75	PNP011	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.012
76	PNP014	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.012
77	PNP025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.012
78	PNP027	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.012
79	PNP029	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.012
80	PNP030	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.012
81	PNP031	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.012
82	PNP034	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.012
83	PNP035	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.012
84	PNP110	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.012
85	PNP117	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.009
86	MVZ145385	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.015
87	TNHC61054	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.015
88	PNP098	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.055
89	*Hyla versicolor*	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.084

Table A.1.3. Continued

		49	50	51	52	53	54	55	56	57	58	59	60
90	*Hyla versicolor*	0.088	0.088	0.088	0.088	0.088	0.089	0.088	0.088	0.088	0.088	0.088	0.088
91	*Hyla versicolor*	0.088	0.088	0.088	0.088	0.088	0.089	0.088	0.088	0.088	0.088	0.088	0.088
92	*Hyla versicolor*	0.088	0.088	0.088	0.088	0.088	0.089	0.088	0.088	0.088	0.088	0.088	0.088
93	*Hyla versicolor*	0.092	0.092	0.092	0.092	0.092	0.093	0.092	0.092	0.092	0.092	0.092	0.092
94	*Hyla versicolor*	0.092	0.092	0.092	0.092	0.092	0.093	0.092	0.092	0.092	0.092	0.092	0.092
95	*Hyla_arborea*	0.116	0.116	0.116	0.116	0.116	0.117	0.116	0.116	0.116	0.116	0.116	0.115
96	*Hyla_eximia*	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.092
97	*Hyla_femoralis*	0.107	0.107	0.107	0.107	0.107	0.104	0.107	0.107	0.107	0.107	0.107	0.099
98	*Hyla_arenicolor*	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.110
99	*Hyla_japonica*	0.103	0.103	0.103	0.103	0.103	0.100	0.103	0.103	0.103	0.103	0.103	0.110
100	*Hyla_orientalis*	0.128	0.128	0.128	0.128	0.128	0.124	0.128	0.128	0.128	0.128	0.128	0.119
101	*Hyla_meridionalis*	0.101	0.101	0.101	0.101	0.101	0.098	0.101	0.101	0.101	0.101	0.101	0.107
102	*Osteopilus septentrionalis*	0.117	0.117	0.117	0.117	0.117	0.114	0.117	0.117	0.117	0.117	0.117	0.109

Table A.1.3. Continued

		61	62	63	64	65	66	67	68	69	70	71	72
61	PNP018												
62	PNP019	0.000											
63	PNP020	0.003	0.003										
64	PNP021	0.000	0.000	0.003									
65	PNP176	0.000	0.000	0.003	0.000								
66	PNP003	0.003	0.003	0.006	0.003	0.003							
67	PNP126	0.006	0.006	0.009	0.006	0.006	0.003						
68	PNP127	0.003	0.003	0.006	0.003	0.003	0.000	0.003					
69	PNP140	0.003	0.003	0.006	0.003	0.003	0.000	0.003	0.000				
70	PNP198	0.003	0.003	0.006	0.003	0.003	0.000	0.003	0.000	0.000			
71	LSUMZ48181	0.006	0.006	0.009	0.006	0.006	0.003	0.006	0.003	0.003	0.003		
72	JWS_357	0.009	0.009	0.012	0.009	0.009	0.006	0.009	0.006	0.006	0.006	0.009	
73	JWS474	0.009	0.009	0.012	0.009	0.009	0.006	0.009	0.006	0.006	0.006	0.009	0.000
74	PNP010	0.009	0.009	0.012	0.009	0.009	0.006	0.009	0.006	0.006	0.006	0.009	0.000
75	PNP011	0.009	0.009	0.012	0.009	0.009	0.006	0.009	0.006	0.006	0.006	0.009	0.000
76	PNP014	0.009	0.009	0.012	0.009	0.009	0.006	0.009	0.006	0.006	0.006	0.009	0.000
77	PNP025	0.009	0.009	0.012	0.009	0.009	0.006	0.009	0.006	0.006	0.006	0.009	0.000
78	PNP027	0.009	0.009	0.012	0.009	0.009	0.006	0.009	0.006	0.006	0.006	0.009	0.000
79	PNP029	0.009	0.009	0.012	0.009	0.009	0.006	0.009	0.006	0.006	0.006	0.009	0.000
80	PNP030	0.009	0.009	0.012	0.009	0.009	0.006	0.009	0.006	0.006	0.006	0.009	0.000
81	PNP031	0.009	0.009	0.012	0.009	0.009	0.006	0.009	0.006	0.006	0.006	0.009	0.000
82	PNP034	0.009	0.009	0.012	0.009	0.009	0.006	0.009	0.006	0.006	0.006	0.009	0.000
83	PNP035	0.009	0.009	0.012	0.009	0.009	0.006	0.009	0.006	0.006	0.006	0.009	0.000
84	PNP110	0.009	0.009	0.012	0.009	0.009	0.006	0.009	0.006	0.006	0.006	0.009	0.000
85	PNP117	0.006	0.006	0.009	0.006	0.006	0.009	0.012	0.009	0.009	0.009	0.012	0.003
86	MVZ145385	0.012	0.012	0.015	0.012	0.012	0.009	0.012	0.009	0.009	0.009	0.006	0.003
87	TNHC61054	0.012	0.012	0.015	0.012	0.012	0.009	0.012	0.009	0.009	0.009	0.006	0.003
88	PNP098	0.051	0.051	0.055	0.051	0.052	0.055	0.051	0.055	0.055	0.055	0.058	0.058
89	*Hyla versicolor*	0.081	0.081	0.084	0.081	0.081	0.085	0.081	0.085	0.085	0.085	0.088	0.085

Table A.1.3. Continued

		61	62	63	64	65	66	67	68	69	70	71	72
90	*Hyla versicolor*	0.084	0.084	0.088	0.084	0.085	0.088	0.084	0.088	0.088	0.088	0.092	0.088
91	*Hyla versicolor*	0.084	0.084	0.088	0.084	0.085	0.088	0.084	0.088	0.088	0.088	0.092	0.088
92	*Hyla versicolor*	0.084	0.084	0.088	0.084	0.085	0.088	0.084	0.088	0.088	0.088	0.092	0.088
93	*Hyla versicolor*	0.088	0.088	0.092	0.088	0.088	0.092	0.088	0.092	0.092	0.092	0.088	0.092
94	*Hyla versicolor*	0.088	0.088	0.092	0.088	0.088	0.092	0.088	0.092	0.092	0.092	0.088	0.092
95	*Hyla_arborea*	0.111	0.111	0.115	0.111	0.112	0.115	0.119	0.115	0.115	0.115	0.111	0.115
96	*Hyla_eximia*	0.089	0.089	0.092	0.089	0.089	0.092	0.089	0.092	0.092	0.092	0.088	0.092
97	*Hyla_femoralis*	0.095	0.095	0.099	0.095	0.096	0.095	0.099	0.095	0.095	0.095	0.091	0.095
98	*Hyla_arenicolor*	0.106	0.106	0.110	0.106	0.106	0.110	0.106	0.110	0.110	0.110	0.106	0.110
99	*Hyla_japonica*	0.107	0.107	0.110	0.107	0.107	0.110	0.107	0.110	0.110	0.110	0.106	0.110
100	*Hyla_orientalis*	0.115	0.115	0.119	0.115	0.116	0.119	0.123	0.119	0.119	0.119	0.115	0.119
101	*Hyla_meridionalis*	0.104	0.104	0.107	0.104	0.104	0.108	0.104	0.108	0.108	0.108	0.104	0.108
102	*Osteopilus septentrionalis*	0.106	0.106	0.109	0.106	0.106	0.110	0.106	0.110	0.110	0.110	0.114	0.106

Table A.1.3. Continued

		73	74	75	76	77	78	79	80	81	82	83	84
61	PNP018												
62	PNP019												
63	PNP020												
64	PNP021												
65	PNP176												
66	PNP003												
67	PNP126												
68	PNP127												
69	PNP140												
70	PNP198												
71	LSUMZ48181												
72	JWS_357												
73	JWS474												
74	PNP010	0.000											
75	PNP011	0.000	0.000										
76	PNP014	0.000	0.000	0.000									
77	PNP025	0.000	0.000	0.000	0.000								
78	PNP027	0.000	0.000	0.000	0.000	0.000							
79	PNP029	0.000	0.000	0.000	0.000	0.000	0.000						
80	PNP030	0.000	0.000	0.000	0.000	0.000	0.000	0.000					
81	PNP031	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
82	PNP034	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
83	PNP035	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
84	PNP110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
85	PNP117	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
86	MVZ145385	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
87	TNHC61054	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
88	PNP098	0.058	0.058	0.058	0.058	0.058	0.058	0.058	0.058	0.058	0.058	0.058	0.058
89	*Hyla versicolor*	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085

Table A.1.3. Continued

		73	74	75	76	77	78	79	80	81	82	83	84
90	*Hyla versicolor*	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.088
91	*Hyla versicolor*	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.088
92	*Hyla versicolor*	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.088
93	*Hyla versicolor*	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092
94	*Hyla versicolor*	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092
95	*Hyla_arborea*	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115
96	*Hyla_eximia*	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092
97	*Hyla_femoralis*	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095
98	*Hyla_arenicolor*	0.110	0.110	0.110	0.110	0.110	0.110	0.110	0.110	0.110	0.110	0.110	0.110
99	*Hyla_japonica*	0.110	0.110	0.110	0.110	0.110	0.110	0.110	0.110	0.110	0.110	0.110	0.110
100	*Hyla_orientalis*	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119
101	*Hyla_meridionalis*	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108
102	Osteopilus septentrionalis	0.106	0.106	0.106	0.106	0.106	0.106	0.106	0.106	0.106	0.106	0.106	0.106

Table A.1.3. Continued

		85	86	87	88	89	90	91	92	93	94	95	96
61	PNP018												
62	PNP019												
63	PNP020												
64	PNP021												
65	PNP176												
66	PNP003												
67	PNP126												
68	PNP127												
69	PNP140												
70	PNP198												
71	LSUMZ48181												
72	JWS_357												
73	JWS474												
74	PNP010												
75	PNP011												
76	PNP014												
77	PNP025												
78	PNP027												
79	PNP029												
80	PNP030												
81	PNP031												
82	PNP034												
83	PNP035												
84	PNP110												
85	PNP117												
86	MVZ145385	0.006											
87	TNHC61054	0.006	0.000										
88	PNP098	0.055	0.062	0.062									
89	*Hyla versicolor*	0.081	0.088	0.088	0.107								

Table A.1.3. Continued

		85	86	87	88	89	90	91	92	93	94	95	96
90	*Hyla versicolor*	0.084	0.092	0.092	0.104	0.009							
91	*Hyla versicolor*	0.084	0.092	0.092	0.104	0.009	0.000						
92	*Hyla versicolor*	0.084	0.092	0.092	0.104	0.003	0.006	0.006					
93	*Hyla versicolor*	0.088	0.088	0.088	0.108	0.006	0.009	0.009	0.003				
94	*Hyla versicolor*	0.088	0.088	0.088	0.108	0.006	0.009	0.009	0.003	0.000			
95	*Hyla_arborea*	0.111	0.111	0.111	0.109	0.058	0.058	0.058	0.055	0.051	0.051		
96	*Hyla_eximia*	0.089	0.088	0.088	0.108	0.028	0.019	0.019	0.025	0.022	0.022	0.058	
97	*Hyla_femoralis*	0.095	0.091	0.091	0.123	0.034	0.031	0.031	0.031	0.028	0.028	0.061	0.038
98	*Hyla_arenicolor*	0.106	0.106	0.106	0.119	0.035	0.031	0.031	0.031	0.028	0.028	0.068	0.038
99	*Hyla_japonica*	0.107	0.106	0.106	0.127	0.038	0.038	0.038	0.034	0.031	0.031	0.051	0.038
100	*Hyla_orientalis*	0.115	0.115	0.115	0.113	0.068	0.062	0.062	0.065	0.061	0.061	0.009	0.055
101	*Hyla_meridionalis*	0.104	0.104	0.104	0.117	0.064	0.064	0.064	0.061	0.057	0.057	0.054	0.050
102	*Osteopilus septentrionalis*	0.102	0.110	0.110	0.142	0.090	0.101	0.101	0.094	0.098	0.098	0.118	0.098

Table A.1.3. Continued

		97	98	99	100	101	102
90	*Hyla versicolor*						
91	*Hyla versicolor*						
92	*Hyla versicolor*						
93	*Hyla versicolor*						
94	*Hyla versicolor*						
95	*Hyla_arborea*						
96	*Hyla_eximia*						
97	*Hyla_femoralis*						
98	*Hyla_arenicolor*	0.041					
99	*Hyla_japonica*	0.048	0.048				
100	*Hyla_orientalis*	0.065	0.072	0.058			
101	*Hyla_meridionalis*	0.071	0.075	0.058	0.061		
102	*Osteopilus septentrionalis*	0.106	0.121	0.110	0.118	0.102	

Appendix 2:

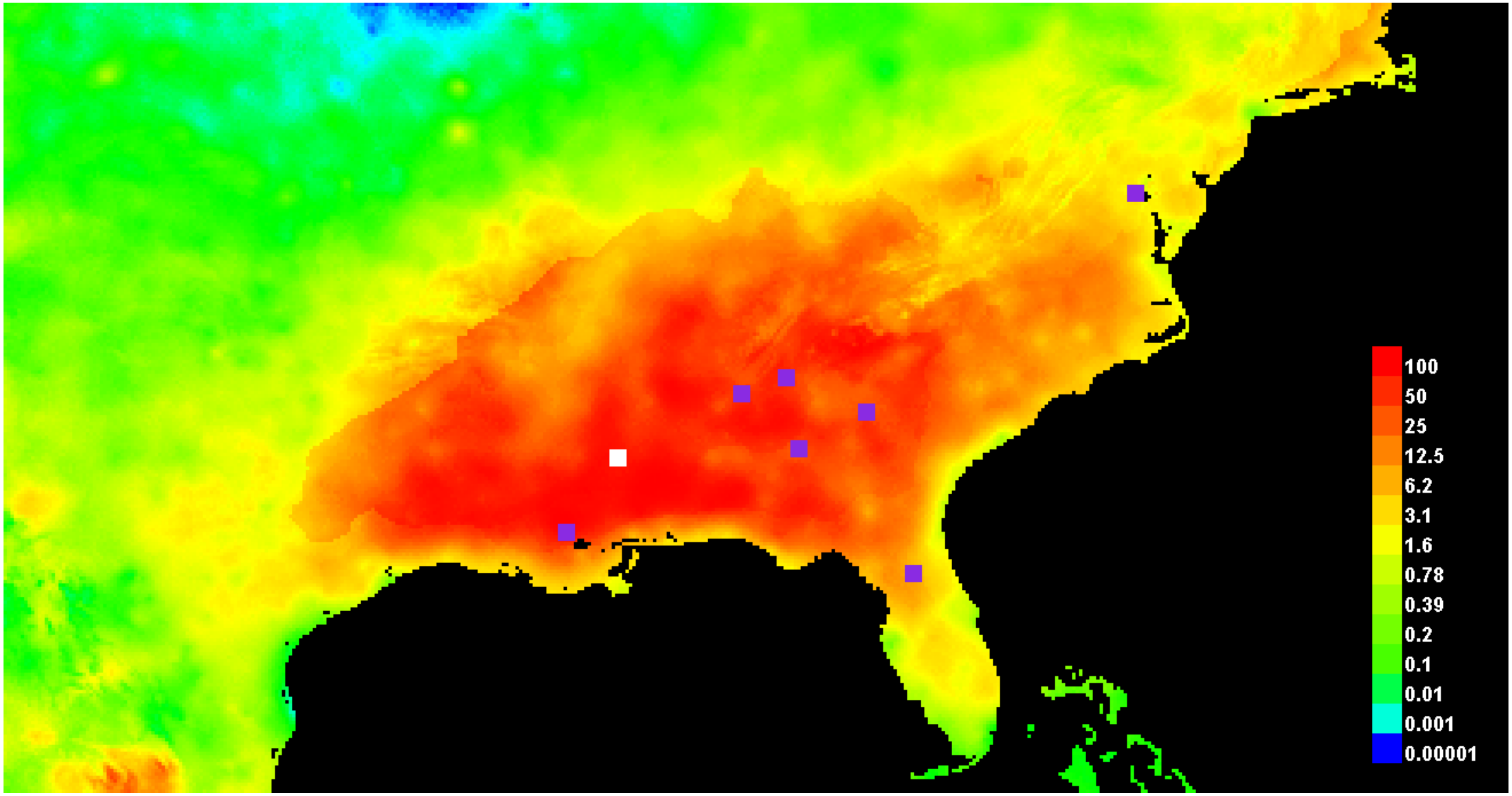

Figure A.2.1. Predictive map of the eastern clade of *Dryophytes cinereus* using the program Maxent. Model was built using 10,000 bootstraps and used 75% of the samples to test the model. White squares on the map are the presence localities used for training and purple squares indicate the samples used to test the models. Warmer colors indicate higher probability for predicting the location of species presence. AUC for training data = 0.950; AUC for test data = 0.827

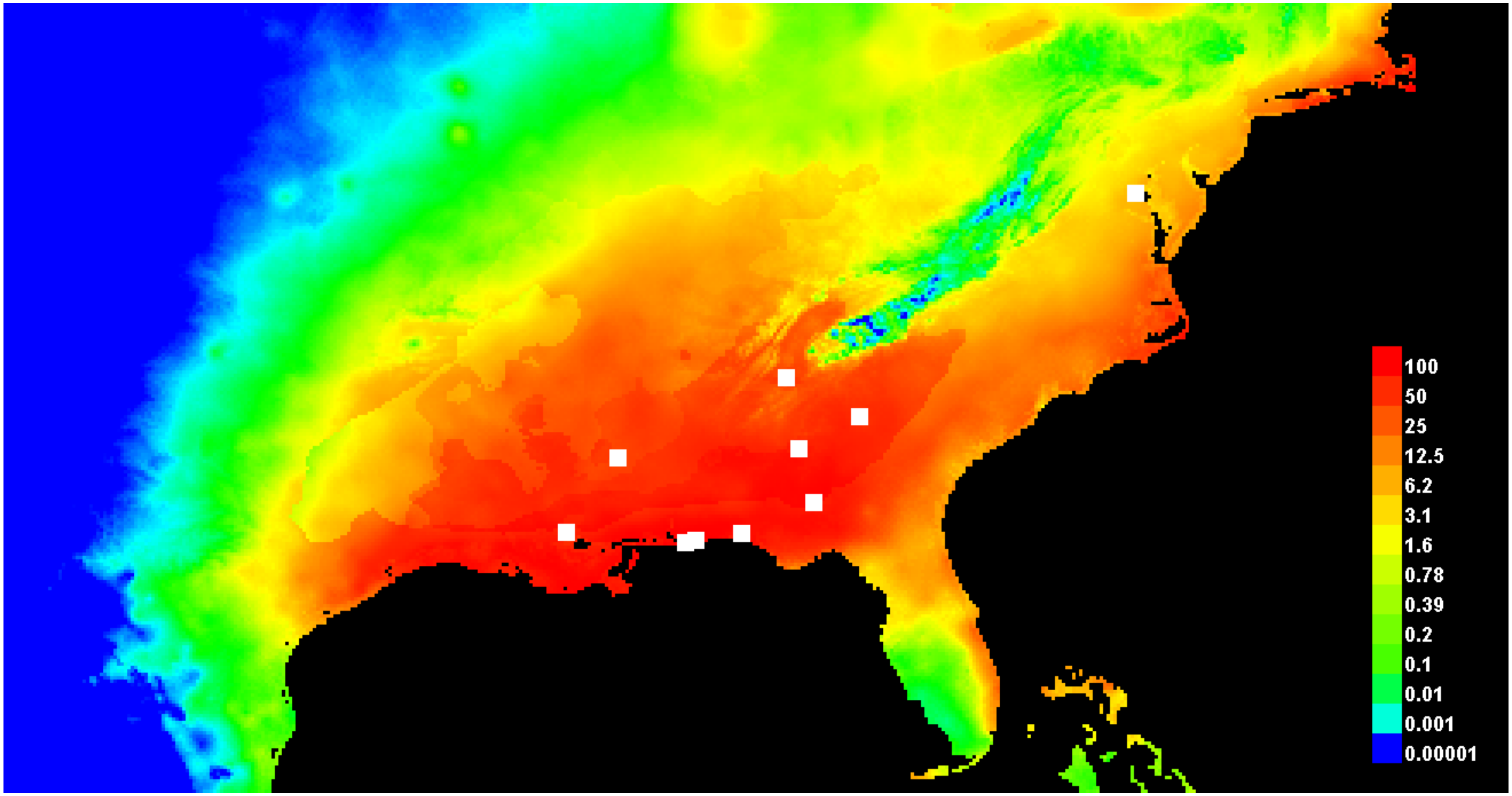

Figure A.2.2. Predictive map of the eastern clade of *Dryophytes cinereus* using the program Maxent. Model was built using 10,000 bootstraps and used the jackknife method to test the model. White squares on the map are the presence localities used for training. Warmer colors indicate higher probability for predicting the location of species presence. AUC for training data = 0.947

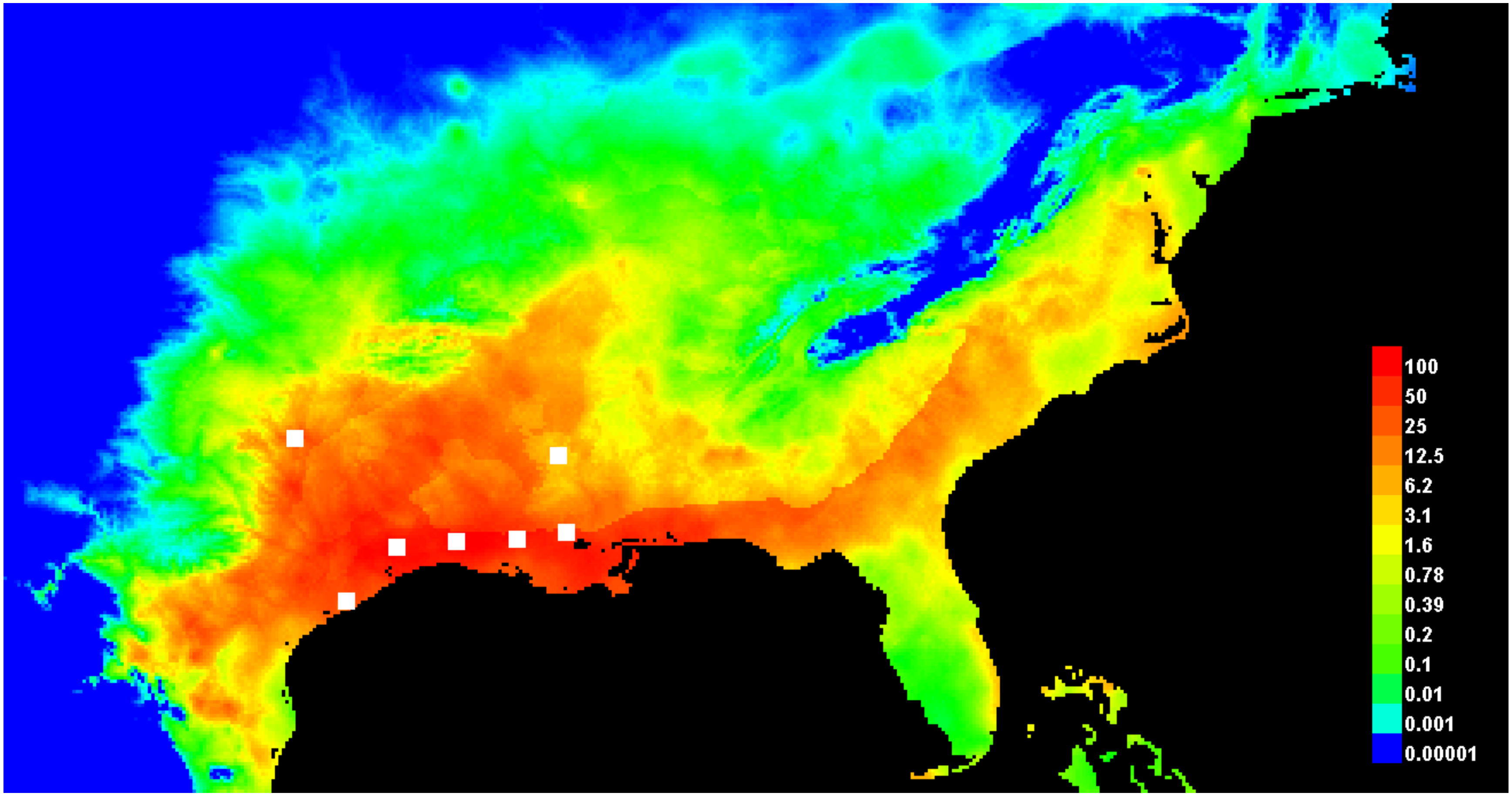

Figure A.2.3. Predictive map of the western clade of *Dryophytes cinereus* using the program Maxent. Model was built using 10,000 bootstraps and used the jackknife technique to test the model. White squares on the map are the presence localities used for training. Warmer colors indicate higher probability for predicting the location of species presence. AUC for training data = 0.974

References

Amano, T., & Sutherland, W. J. (2013). Four barriers to the global understanding of biodiversity conservation: wealth, language, geographical location and security. *Proceedings of the Royal Society of London B: Biological Sciences, 280*(1756). doi:10.1098/rspb.2012.2649

Anderson, R. P., & Gonzalez Jr, I. (2011). Species-specific tuning increases robustness to sampling bias in models of species distributions: An implementation with Maxent. *Ecological Modelling, 222*(15), 2796-2811. doi:http://dx.doi.org/10.1016/j.ecolmodel.2011.04.011

Aresco, M. (1996). Geographic variation in the morphology and lateral stripe of the green treefrog (*Hyla cinerea*) in the southeastern United States.*The American Midland Naturalist*, *135*(2), 293-298. doi:1. Retrieved from http://www.jstor.org.ezproxy.uta.edu/stable/2426712 doi:1

Asquith, A., Altig, R., & Zimba, P. (1988). Geographic variation in the mating call of the green treefrog *Hyla cinerea*. *The American Midland Naturalist, 119*(1), 101-110. doi:10.2307/2426058

Austin, J. D., Lougheed, S. C., & Boag, P. T. (2004). Discordant temporal and geographic patterns in maternal lineages of eastern north American frogs, *Rana catesbeiana* (Ranidae) and *Pseudacris crucifer* (Hylidae). *Molecular Phylogenetics and Evolution, 32*(3), 799-816.

Avise, J. C. (1994). *Molecular markers, natural history and evolution*. New York: Chapman & Hall.

Avise, J. C. (2009). Phylogeography: retrospect and prospect. *Journal of Biogeography, 36*.

Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., . . . Saunders, N. C. (1987). Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics, 18*(ArticleType: research-article / Full publication date: 1987 / Copyright © 1987 Annual Reviews), 489-522.

Avise, J. C., & Walker, D. (1998). Pleistocene phylogeographic effects on avian populations and the speciation process. *Proceedings of the Royal Society of London B: Biological Sciences, 265*.

Bandelt, H. J., Forster, P., & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution, 16*(1), 37-48.

Barrow, L, Soto-Centeno, J. Angel, Lemmon, Alan, & Lemmon, Emily. (2016). Targeted sampling and target capture: assessing phylogeographic conordance with genome-wide data. [Oral Presentation]. *Evolution.* 18 June. Available at: https://www.youtube.com/watch?v=P84n0nrqdiQ

Barton, H. D., & Wisely, S. M.. (2012) Phylogeography of striped skunks (*Mephitis mephitis)* in North America: Pleistocene dispersal and contemporary population structure. *Journal of Mammalogy, 93*(1), 38-51.

Blackburn, J. K., McNyset, K. M., Curtis, A., & Hugh-Jones, M. E. (2007). Modeling the geographic distribution of *Bacillus anthracis*, the causative agent of anthrax disease, for the contiguous United States using predictive ecologic niche modeling. *The American Journal of Tropical Medicine and Hygiene, 77*(6), 1103-1110.

Blair, W. F. (1958a). Call structure and species groups in U. S. treefrogs (Hyla). *The Southwestern Naturalist, 3*(1/4), 77-89. doi:10.2307/3669039

Blair, W. F. (1958b). Mating call in the speciation of anuran amphibians. *The American Naturalist*, *92*(862), 27-51.

Blaustein, A. R., O'Hara, R. K., & Olson, D. H. (1984). Kin preference behaviour is present after metamorphosis in *Rana cascadae* frogs. *Animal Behaviour*, *32*(2), 445-450. doi:http://dx.doi.org/10.1016/S0003-3472(84)80280-8

Blouin, M. S. (1990). Evolution of palatability differences between closely-related treefrogs. *Journal of Herpetology, 24*(3), 309-311. doi:10.2307/1564399

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C-H., Xie, D., Suchard, MA., Rambaut, A., & Drummond, A. J. (2014). BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, 10(4), e1003537. doi:10.1371/journal.pcbi.1003537

Brandt, B. B. (1936). The frogs and toads of eastern North Carolina. *Copeia, 1936*(4), 215-223. doi:10.2307/1436326

Bryson Jr., R. W., Nieto-Montes de Oca, A., Jaeger, J. R., & Riddle, B. R. (2010). Elucidation of cryptic diversity in a widespread nearctic treefrog reveals episodes of mitochondrial gene capture as frogs diversified across a dynamic landscape. *Evolution, 64*(8), 2315-2330.

Burbrink, F. T., Fontanella, F., Alexander Pyron, R., Guiher, T. J., & Jimenez, C. (2008). Phylogeography across a continent: the evolutionary and demographic history of the North American racer (Serpentes: Colubridae: *Coluber constrictor*). *Molecular Phylogenetics and Evolution, 47*(1), 274-288. doi:S1055-7903(07)00378-8 [pii] 10.1016/j.ympev.2007.10.020

Burbrink, F. T., Lawson, R., & Slowinski, J. B. (2000). Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. *Evolution*, *54*(6), 2107-2118.

Cleaves, E. T. (1989). Appalachian Piedmont landscapes from the Permian to the Holocene. *Geomorphology, 2*, 159-179.

Colwell, R. K. (2000). A barrier runs through it … or maybe just a river. *Proceedings of the National Academy of Sciences*, *97*(25), 13470-13472. doi:10.1073/pnas.250497697

Conant, R., & Collins, J. T. (1998). *A field guide to reptiles & amphibians : eastern and central North America* (3rd ed.). Boston: Houghton Mifflin.

Davey, J. W., & Blaxter, M. L. (2010). RADSeq: next-generation population genetics. *Briefings in functional genomics*, *9*(5-6), 416-423.

Donovan, M. F., Semlitsch, R. D., & Routman, E. J. (2000). Biogeography of the southeastern United States: a comparison of salamander phylogeographic studies. *Evolution*, *54*(4).

Drummond AJ, Suchard MA, Xie D & Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7 *Molecular Biology And Evolution 29*: 1969-1973.

Duellman, W. E., & Trueb, L. (1994). *Biology of amphibians*. Baltimore: Johns Hopkins University Press.

Duellman, W. E., Marion, A. B., & Hedges, S. B. (2016). Phylogenetics, classification, and biogeography of the treefrogs (Amphibia: Anura: Arboranae). *Zootaxa*, *4104*(1), 1-109.

Elhaik, E. (2012). Empirical distributions of F_{ST} from large-scale human polymorphism data. *PLoS ONE*, *7*(11).

Eriksson, J., Hohmann, G., Boesch, C., & Vigilant, L. (2004). Rivers influence the population genetic structure of bonobos (*Pan paniscus*). *Molecular Ecology, 13*(11), 3425-3435. doi:10.1111/j.1365-294X.2004.02332.x

ESRI, A., & Components, M. (2001). Environmental systems research institute. *California, USA*.

Faivovich, J., Haddad, C. F. B., Garcia, P. C. A., Frost, D. R., Campbell, J. A., & Wheeler, W. C. (2005). Systematic review of the frog family Hylidae, with special reference to Hylinae: phylogenetic analysis and taxonomic revision. *Bulletin of the American Museum of Natural History(294)*, 6-228.

Fontanella, F. M., Feldman, C. R., Siddall, M. E., & Burbrink, F. T. (2008). Phylogeography of *Diadophis punctatus*: extensive lineage diversity and repeated patterns of historical demography in a trans-continental snake. *Molecular Phylogenetics and Evolution, 46*(3), 1049-1070. doi:http://dx.doi.org/10.1016/j.ympev.2007.10.017

Fortman, J. R., & Altig, R. (1974). Characters of F1 hybrid frogs from six species of Hyla (Anura: Hylidae). *Herpetologica, 30*(3), 221-234.

Freed, A. N. (1980). Prey selection and feeding behavior of the green treefrog (*Hyla cinerea*). *Ecology, 61*(3), 461-465.

Frost, D. R. (2016). Amphibian species of the world: an online reference. Version 6.0. Electronic database. New York (NY): American Museum of Natural History. Available from: http://research.amnh.org/herpetology/amphibia/index.html (accessed 25 August 2016).

Funderburg, J. B. (1953). The Georgia tree frog, *Hyla gratiosa*, in North Carolina. *Herpetologica, 9*(4), 176-176.

Garton, J. S., & Brandon, R. A. (1975). Reproductive ecology of the green treefrog, *Hyla cinerea* in southern Illinois (Anura: Hylidae). *Herpetologica, 31*(2), 150-161.

Gascon, C., Lougheed, S. C., & Bogart, J. P. (1998). Patterns of genetic population differentiation in four species of Amazonian frogs: a test of the Riverine Barrier Hypothesis. *Biotropica, 30*(1), 104-119. doi:10.1111/j.1744-7429.1998.tb00373.x

Gascon, C., Malcolm, J. R., Patton, J. L., da Silva, M. N. F., Bogart, J. P., Lougheed, S. C., . . . Boag, P. T. (2000). Riverine barriers and the geographic distribution of Amazonian species. *Proceedings of the National Academy of Sciences, 97*(25), 13672-13677. doi:10.1073/pnas.230136397

Gaston, K. J. (2000). Global patterns in biodiversity. *Nature, 405*(6783), 220-227.

Gene Codes Corp. Sequencher (Version 4.5). Ann Arbor, MI USA.

Gerhardt, H. C. (1974a). Behavioral isolation of the tree frogs, *Hyla cinerea* and *Hyla andersonii*. *The American Midland Naturalist, 91*(2), 424-433. doi:1. Retrieved from http://www.jstor.org/stable/2424332 doi:1

Gerhardt, H. C. (1974b). The vocalizations of some hybrid treefrogs: acoustic and behavioral analyses. *Behaviour, 49*(1/2), 130-151.

Gerhardt, H. C. (1978). Mating call recognition in the green treefrog (*Hyla cinerea*): the significance of some fine-temporal properties. *Journal of Experimental Biology*, *74*(1), 59-73.

Gerhardt, H. C. (2013). Geographic variation in acoustic communication: reproductive character displacement and speciation. *Evolutionary Ecology Research*, *15*(6), 605-632.

Gerhardt, H. C., Sheldon, I. G., & Karlin, A. A. (1980). Natural hybrids between *Hyla cinerea* and *Hyla gratiosa*: morphology, vocalization and electrophoretic analysis. *Copeia, 1980*(4), 577-584.

Graham, C. H., Ron, S. R., Santos, J. C., Schneider, C. J., & Moritz, C. (2004). Integrating phylogenetics and environmental niche models to explore speciation mechanisms in Dendrobatid frogs. *Evolution, 58*(8), 1781-1793. doi:10.1111/j.0014-3820.2004.tb00461.x

Gunzburger, M. S. (2005). Differential predation on tadpoles influences the potential effects of hybridization between *Hyla cinerea* and *Hyla gratiosa*. *Journal of Herpetology, 39*(4), 682-687.

Gunzburger, M. S. (2006). Reproductive ecology of the green treefrog (*Hyla cinerea*) in northwestern Florida. *The American Midland Naturalist, 155*(2), 321-328. doi:10.1674/0003-0031(2006)155[321:REOTGT]2.0.CO;2

Gvoždík, V., Moravec, J., Klütsch, C., & Kotlík, P. (2010). Phylogeography of the Middle Eastern tree frogs (Hyla, Hylidae, Amphibia) as inferred from nuclear and mitochondrial DNA variation, with a description of a new species. *Molecular Phylogenetics and Evolution, 55*(3), 1146-1166. doi:http://dx.doi.org/10.1016/j.ympev.2010.03.015

Heemeyer, J. L., & Lannoo, M. J. (2012). Breeding migrations in crawfish frogs (*Lithobates areolatus*): long-distance movements, burrow philopatry, and mortality in a near-threatened species. *Copeia, 2012*(3), 440-450. doi:10.1643/CE-11-107

Herman, T. A., & Bouzat, J. L. (2015). Range-wide phylogeography of the four-toed salamander: out of Appalachia and into the glacial aftermath.*Journal of Biogeography*.

Hickerson, M. J., Carstens, B. C., Cavender-Bares, J., Crandall, K. A., Graham, C. H., Johnson, J. B., . . . Yoder, A. D. (2010a). Phylogeography's past, present, and future: 10 years after Avise, 2000. *Molecular Phylogenetics and Evolution, 54*(1), 291-301. doi:10.1016/j.ympev.2009.09.016

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology, 25*(15), 1965-1978. doi:10.1002/joc.1276

Hillis, D. M., Frost, J. S., & Wright, D. A. (1983). Phylogeny and biogeography of the *Rana pipiens* complex: a biochemical evaluation. *Systematic Biology,32*(2), 132-143.

Hillis, D. M., & Bull, J. J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic biology*, *42*(2), 182-192.

Höbel, G., & Gerhardt, H. C. (2003). Reproductive character displacement in the acoustic communication system of green tree frogs (*Hyla cinerea*). *Evolution*, *57*(4), 894-904.

Hof, C., Araujo, M. B., Jetz, W., & Rahbek, C. (2011). Additive threats from pathogens, climate and land-use change for global amphibian diversity. *Nature, 480*(7378), 516-519. doi:http://www.nature.com/nature/journal/v480/n7378/abs/nature10650.html#supplementary-information

Hohenlohe, P. A., Amish, S. J., Catchen, J. M., Allendorf, F. W., & Luikart, G. (2011). Next-generation RAD sequencing identifies thousands of SNPs for assessing hybridization between rainbow and westslope cutthroat trout.*Molecular ecology resources*, *11*(s1), 117-122.

Holman, J. A. (2003). *Fossil frogs and toads of North America*. Bloomington, IN: Indiana University Press.

Jackson, N. D., & Austin, C. C. (2010). The combined effects of rivers and refugia generate extreme cryptic fragmentation within the common ground skink (*Scincella lateralis*). *Evolution*, *64*(2), 409-428.

Jakob, S. S., Martinez-Meyer, E., & Blattner, F. R. (2009). Phylogeographic analyses and paleodistribution modeling indicate Pleistocene in situ survival of Hordeum species (*Poaceae*) in southern Patagonia without genetic or spatial restriction. *Molecular Biology and Evolution, 26*(4), 907-923. doi:DOI 10.1093/molbev/msp012

Jalil, M. F., Cable, J., Sinyor, J., Lackman-Ancrenaz, I., Ancrenaz, M., Bruford, M. W., & Goossens, B. (2008). Riverine effects on mitochondrial structure of Bornean orang-utans (*Pongo pygmaeus*) at two spatial scales. *Molecular Ecology, 17*(12), 2898-2909. doi:10.1111/j.1365-294X.2008.03793.x

Jameson, D. L., & Richmond, R. C. (1971). Parallelism and convergence in the evolution of size and shape in Holarctic Hyla. *Evolution, 25*(3), 497-508.

Joly, S., Stevens, M. I., & van Vuuren, B. J. (2007). Haplotype networks can be misleading in the presence of missing data. *Systematic biology*, *56*(5), 857-862.

Lamb, T., & Avise, J. C. (1986). Directional introgression of mitochondrial DNA in a hybrid population of tree frogs: The influence of mating behavior. *Proceedings of the National Academy of Sciences, 83*(8), 2526-2530.

Latch, E. K., Scognamillo, D. G., Fike, J. A., Chamberlain, M. J., & Rhodes, O. E. (2008). Deciphering ecological barriers to North American river otter (*Lontra canadensis*) gene flow in the Louisiana landscape. *Journal of Heredity, 99*(3), 265-274.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., . . . Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics, 23*(21), 2947-2948. doi:10.1093/bioinformatics/btm404

Leidy, J., Theodore C. Hilgard, Edw. Hallowell, Conrad, T., & John Cassin. (1856). December 30th; remarks on certain extinct species of fishes; notices of remains of extinct turtles of

New Jersey, collected by Prof. Cook, of the State Geological Survey, under the direction of Dr. W. Kitchell; synopsis of a new classification of the vegetable kingdom; note on the collection of reptiles from the neighborhood of San Antonio, Texas, recently presented to the Academy of Natural Sciences by Dr. A. Heermann; description of a new genus of Colubriform serpents from California; notices of extinct Vertebrata discovered by Dr. F. V. Hayden, during the expedition to the Sioux country under the command of Lieut. *Proceedings of the Academy of Natural Sciences of Philadelphia, 8,* 301-327. Retrieved from http://www.jstor.org/stable/4059166

Leigh, J. W., & Bryant, D. (2015). PopArt: full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, *6*(9), 1110-1116. doi:10.1111/2041-210X.12410

Leips, J., McManus, M. G., & Travis, J. (2000). Response of treefrog larvae to drying ponds; comparing temporary and permanent pond breeders. *Ecology, 81*(11), 2997-3008. doi:10.1890/0012-9658(2000)081[2997:ROTLTD]2.0.CO;2

Leips, J., & Travis, J. (1994). Metamorphic responses to changing food levels in two species of Hylid frogs. *Ecology, 75*(5), 1345-1356. doi:10.2307/1937459

Lemmon, E. M. (2009). Diversification of conspecific signals in sympatry: geographic overlap drives multidimensional reproductive character displacement in frogs. *Evolution, 63*(5), 1155-1170.

Lemmon, E. M., Lemmon, A. R., Cannatella, D. C., & Brown, J. (2007). Geological and climatic forces driving speciation in the continentally distributed trilling chorus frogs (Pseudacris). *Evolution, 61*(9), 2086-2103. doi:10.1111/j.1558-5646.2007.00181.x

Lemmon, E. M., Lemmon, A. R., Collins, J. T., Lee-Yaw, J. A., & Cannatella, D. C. (2007). Phylogeny-based delimitation of species boundaries and contact zones in the trilling chorus frogs (*Pseudacris*). *Molecular phylogenetics and evolution*, *44*(3), 1068-1082.

Librado, P., & Rozas, J. (2009). DnaSP v5. *Bioinformatics, 25*(11), 1451-1452. doi:10.1093/bioinformatics/btp187

Lin, H. D., Chen, Y. R., & Lin, S. M. (2012). Strict consistency between genetic and topographic landscapes of the brown tree frog (*Buergeria robusta*) in Taiwan. *Molecular Phylogenetics and Evolution, 62*(1), 251-262. doi:10.1016/j.ympev.2011.09.022

Link, A., Valencia, L. M., Céspedes, L. N., Duque, L. D., Cadena, C. D., & Di Fiore, A. (2015). Phylogeography of the critically endangered brown spider monkey (*Ateles hybridus*): testing the Riverine Barrier Hypothesis. *International Journal of Primatology, 36*(3), 530-547. doi:10.1007/s10764-015-9840-6

Lydeard, C., & Mayden, R. L. (1995a). A diverse and endangered aquatic ecosystem of the southeast United States. *Conservation Biology, 9*(4), 800-805. doi:10.1046/j.1523-1739.1995.09040800.x

Makowsky, R., Marshall, J. C., McVay, J., Chippindale, P. T., & Rissler, L. J. (2010). Phylogeographic analysis and environmental niche modeling of the plain-bellied watersnake (*Nerodia erythrogaster*) reveals low levels of genetic and ecological differentiation. *Molecular Phylogenetics and Evolution, 55*(3), 985-995. doi:10.1016/j.ympev.2010.03.012

Malnate, E. (1971). A catalog of primary types in the herpetological collections of the Academy of Natural Sciences, Philadelphia (ANSP). *Proceedings of the Academy of Natural*

Sciences of Philadelphia, 123, 345-375. Retrieved from http://www.jstor.org/stable/4064676

Marchese, C. (2015). Biodiversity hotspots: a shortcut for a more complicated concept. *Global Ecology and Conservation, 3*, 297-309. doi:http://dx.doi.org/10.1016/j.gecco.2014.12.008

McCullagh, W., Williams, J., McGregor, S., Pierson, J., & Lydeard, C. (2002). The Unionid (Bivalva) fauna of the Sipsey River in northwestern Alabama, an aquatic hotspot. *American Malacological Buletin., 17,* 1-15.

Means, D. B. (1977). Aspects of the significance to terrestrial vertebrates of the Apalachicola River drainage basin, Florida. *Florida Marine Research Publications*, *26*, 37-67.

Mecham, J. S. (1960). Introgressive hybridization between two southeastern treefrogs. *Evolution, 14*(4), 445-457.

Mendelson III, J. (2011). Shifted baselines, forensic taxonomy, and Rabbs' Fringe-limbed treefrog: the changing role of biologists in an era of amphibian declines and extinctions. *Herpetological Review, 42*(1), 21-25.

Milanovich, J. R., Peterman, W. E., Nibbelink, N. P., & Maerz, J. C. (2010). Projected loss of a salamander diversity hotspot as a consequence of projected global climate change. *PLoS ONE, 5*(8).

Miller, M. A., Pfeiffer, W., & Schwartz, T. (2011). The CIPRES science gateway: a community resource for phylogenetic analyses. *Proceedings of the 2011 TeraGrid Conference: Extreme Digital Discovery (41).*

Moraes, L. J. C. L., Pavan, D., Barros, M. C., & Ribas, C. C. (2016). The combined influence of riverine barriers and flooding gradients on biogeographical patterns for amphibians and squamates in south-eastern Amazonia. *Journal of Biogeography*. doi:10.1111/jbi.12756

Myers, N. (1988). Threatened biotas: "hot spots" in tropical forests. *Environmentalist, 8*(3), 187-208. doi:10.1007/bf02240252

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature, 403*(6772), 853-858. doi:http://www.nature.com/nature/journal/v403/n6772/suppinfo/403853a0_S1.html

Myers, N. (2003). Biodiversity hotspots revisited. *BioScience*, *53*(10), 916-917.

National Amphibian Atlas, U. (Cartographer). (2014a). Barking Treefrog (*Hyla gratiosa*). Retrieved from www.pwrc.usgs.gov/naa

National Amphibian Atlas, U. (Cartographer). (2014b). Green Treefrog (*Hyla cinerea*). Retrieved from www.pwrc.usgs.gov/naa

National Amphibian Atlas, U. (Cartographer). (2014c). Squirrel Treefrog (*Hyla squirella*). Retrieved from www.pwrc.usgs.gov/naa

Neill, W. T. (1952). Burrowing habits of *Hyla gratiosa. Copeia*, *1952*(3), 196-196.

Nicolas, V., Mataame, A., Crochet, P.-A., Geniez, P., & Ohler, A. (2015). Phylogeographic patterns in North African water frog *Pelophylax saharicus* (Anura: Ranidae). *Journal of Zoological Systematics and Evolutionary Research*, *53*(3), 239-248. doi:10.1111/jzs.12094

Noss, R. F., Platt, W. J., Sorrie, B. A., Weakley, A. S., Means, D. B., Costanza, J., & Peet, R. K. (2015). How global biodiversity hotspots may go unrecognized: lessons from the North American Coastal Plain. *Diversity and Distributions*, *21*(2), 236-244. doi:10.1111/ddi.12278

Nylander, J. A. A. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Oldham, R. S., & Gerhardt, H. C. (1975). Behavioral isolating mechanisms of the treefrogs *Hyla cinerea* and *H. gratiosa*. *Copeia, 1975*(2), 223-231. doi:10.2307/1442880

Patton, J. L., Maria Nazareth, F. D. S., & Malcolm, J. R. (1994). Gene genealogy and differentiation among arboreal spiny rats (Rodentia: Echimyidae) of the Amazon Basin: a test of the Riverine Barrier Hypothesis. *Evolution, 48*(4), 1314-1323. doi:10.2307/2410388

Pauly, G. B., Piskurek, O., & Shaffer, H. B. (2007). Phylogeographic concordance in the southeastern United States: the flatwoods salamander, *Ambystoma cingulatum,* as a test case. *Molecular Ecology, 16*(2), 415-429. doi:10.1111/j.1365-294X.2006.03149.x

Pearson, R. G., & Dawson, T. P. (2003). Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecology and Biogeography, 12*(5), 361-371. doi:10.1046/j.1466-822X.2003.00042.x

Pereira, R. S. (2002). Desktop Garp. *University of Kansas Biodiversity Research Center, Lawrence, Kansas*.

Peterson, A. (2003). Predicting the geography of species invasions via ecological niche modeling. *The Quarterly Review of Biology, 78*(4), 419-433. doi:10.1086/378926

Peterson, A. T., & Kluza, D. A. (2005). Ecological niche modeling as a new paradigm for large-scale investigations of diversity and distribution of birds. *USDA Forest Service General Technical Reports PSW-GTR-191.*

Peterson, A T., Papeş, M., & Eaton, M. (2007). Transferability and model evaluation in ecological niche modeling: a comparison of GARP and Maxent. *Ecography, 30*(4), 550-560. doi:10.1111/j.0906-7590.2007.05102.x

Petit, R. J., Aguinagalde, I., de Beaulieu, J. L., Bittkau, C., Brewer, S., Cheddadi, R., ... & Mohanty, A. (2003). Glacial refugia: hotspots but not melting pots of genetic diversity. *Science*, *300*(5625), 1563-1565.

Pfennig, K. S., & Ryan, M. J. (2006). Reproductive character displacement generates reproductive isolation among conspecific populations: an artificial neural network study. *Proceedings of the Royal Society of London B: Biological Sciences, 273*(1592), 1361-1368.

Phillips, S. J., & Dudík, M. (2008). Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography, 31*(2), 161-175. doi:10.1111/j.0906-7590.2008.5203.x

Pimentel, D., Wilson, C., McCullum, C., Huang, R., Dwen, P., Flack, J., . . . Cliff, B. (1997). Economic and environmental benefits of biodiversity: the annual economic and environmental benefits of biodiversity in the United Sates total approximately $300 billion. *BioScience, 47*, 747+.

Pontarp, M., & Wiens, J. J. (2016). The origin of species richness patterns along environmental gradients: uniting explanations based on time, diversification rate and carrying capacity. *Journal of Biogeography*.

Pope, P. H. (1919). Some notes on the amphibians of Houston, Texas.*Copeia*, (76), 93-98.

Pounds, J., & Jackson, J. (1981). Riverine barriers to gene flow and the differentiation of fence lizard populations. *Evolution, 35*(3), 516-528. doi:1. Retrieved from http://www.jstor.org/stable/2408199 doi:1

Qiao, H., Lin, C., Ji, L., & Jiang, Z. (2012). mMWeb-an online platform for employing multiple ecological niche modeling algorithms. *PLoS ONE, 7*(8), e43327.

Queiroz, C., Beilin, R., Folke, C., & Lindborg, R. (2014). Farmland abandonment: threat or opportunity for biodiversity conservation? A global review. *Frontiers in Ecology and the Environment, 12*(5), 288-296. doi:10.1890/120348

Rambaut, A. (2007). FigTree, a graphical viewer of phylogenetic trees (Version 1.4.2). Retrieved from http://tree.bio.ed.ac.uk/software/figtree/

Rambaut, A., Suchard, M. A., Xie, Z., & Drummond, A. (2014). Tracer (Version 1.6). Retrieved from http://beast.bio.ed.ac.uk/Tracer

Raxworthy, C. J., Ingram, C. M., Rabibisoa, N., & Pearson, R. G. (2007). Applications of ecological niche modeling for species delimitation: a review and empirical evaluation using day geckos (Phelsuma) from Madagascar. *Systematic Biology, 56*(6), 907-923. doi:10.1080/10635150701775111

Rhymer, J., & Simberloff, D. (1996). Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics, 27*, 83-109. Retrieved from http://www.jstor.org/stable/2097230

Richards-Zawacki, C. L., & Cummings, M. E. (2011). Intraspecific reproductive character displacement in a polymorphic poison dart frog, *Dendrobates pumilio*. *Evolution*, *65*(1), 259-267.

Ricklefs, R. E. (2004). A comprehensive framework for global patterns in biodiversity. *Ecology letters, 7*(1), 1-15.

Rissler, L. J., & Apodaca, J. J. (2007). Adding more ecology into species delimitation: Ecological niche models and phylogeography help define cryptic species in the black salamander (*Aneides flavipunctatus*). *Systematic Biology*, *56*(6), 924-942. doi:Doi 10.1080/10635150701703063

Rissler, L. J., Hijmans, R. J., Graham, C. H., Moritz, C., & Wake, D. B. (2006). Phylogeographic lineages and species comparisons in conservation analyses: a case study of California herpetofauna *The American Naturalist, 167*(5).

Rissler, L. J., & Smith, W. H. (2010). Mapping amphibian contact zones and phylogeographical break hotspots across the United States (Vol. 19). *Molecular Ecology*.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., . . . Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*. doi:10.1093/sysbio/sys029

Schlefer, E. K., Romano, M. A., Sheldon, I. G., & Ruth, S. B. (1986). Effects of twenty years of hybridization in a disturbed habitat on *Hyla cinerea* and *Hyla gratiosa*. *Journal of Herpetology*, *20*(2), 210-221.

Schneider, C. J., Smith, T. B., Larison, B., & Moritz, C. (1999). A test of alternative models of diversification in tropical rainforests: ecological gradients vs. rainforest refugia. *Proceedings of the National Academy of Sciences, 96*(24), 13869-13873. doi:10.1073/pnas.96.24.13869

Soltis, D. E., Morris, A. B., McLachlan, J. S., Manos, P. S., & Soltis, P. S. (2006). Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology, 15*(14), 4261-4293. doi:10.1111/j.1365-294X.2006.03061.x

Soto-Centeno, J. A., Barrow, L. N., Allen, J. M., & Reed, D. L. (2013). Reevaluation of a classic phylogeographic barrier: new techniques reveal the influence of microgeographic climate variation on population divergence. *Ecology and Evolution, 3*(6), 1603–1613. http://doi.org/10.1002/ece3.576

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*. doi:10.1093/bioinformatics/btu033

Stebbins, R. C., & Cohen, N. W. (1995). *A natural history of amphibians*. Princeton, N.J.: Princeton University Press.

Stuart, B. L., Inger, R. F., & Voris, H. K. (2006). High level of cryptic species diversity revealed by sympatric lineages of Southeast Asian forest frogs.*Biology letters*, *2*(3), 470-474.

Swenson, Nathan G., & Howard, Daniel J. (2005). Clustering of contact zones, hybrid zones, and phylogeographic breaks in North America. *The American Naturalist, 166*(5), 581-591.

Tamura K., Nei M., and Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* 101:11030-11035.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*. doi:10.1093/molbev/mst197

Taylor, R. C., Buchanan, B. W., & Dogerty, J. L. (2007). Sexual selection in the squirrel treefrog (*Hyla squirella*): the role of multimodal cue assessment in female choice. *Animal Behaviour*, *74*(6), 1753-1763.

Toews, D. P., & Brelsford, A. (2012). The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology, 21*(16), 3907-3930. doi:10.1111/j.1365-294X.2012.05664.x

Voelker, G., Marks, B. D., Kahindo, C., A'Genonga, U., Bapeamoni, F., Duffie, L. E., . . . Light, J. E. (2013). River barriers and cryptic biodiversity in an evolutionary museum. *Ecology and Evolution*, *3*(3), 536-545. doi:10.1002/ece3.482

Wallace, A. R. (1852). On the monkeys of the Amazon. *Annals And Magazine of Natural History, 14*, 451--454. doi:10.1080/037454809494374

Waltari, E., Hijmans, R. J., Peterson, A. T., Nyári, Á. S., Perkins, S. L., & Guralnick, R. P. (2007). Locating pleistocene refugia: comparing phylogeographic and ecological niche model predictions. *PLoS ONE, 2*(7), e563. doi:10.1371/journal.pone.0000563

Warren, D. L., & Seifert, S. N. (2011). Ecological niche modeling in Maxent: the importance of model complexity and the performance of model selection criteria. *Ecological Applications, 21*(2), 335-342. doi:10.1890/10-1171.1

Webb, R. G. (1965). Observations on breeding habits of the squirrel treefrog, *Hyla squirella*. *The American Midland Naturalist, 74*(2), 500-501. doi:10.2307/2423279

Wiens, J. J. (2004a). Speciation and ecology revisited: phylogenetic niche conservatism and the origin of species. *Evolution, 58*(1), 193-197.

Wiens, J. J. (2004b). What is speciation and how should we study it? *American Naturalist, 163*(6), 914-923. doi:10.1086/386552AN40077 [pii]

Wiens, J. J. (2007). Global patterns of diversification and species richness in amphibians. *The American Naturalist, 170*(2), S86-S106.

Wiens, J. J., Ackerly, D. D., Allen, A. P., Anacker, B. L., Buckley, L. B., Cornell, H. V., . . . Stephens, P. R. (2010). Niche conservatism as an emerging principle in ecology and conservation biology. *Ecology Letters, 13*(10), 1310-1324. doi:10.1111/j.1461-0248.2010.01515.x

Wiens, J. J., & Graham, C. H. (2005). Niche conservatism: integrating evolution, ecology, and conservation biology. *Annual Review of Ecology, Evolution, and Systematics,* *36*(ArticleType: research-article / Full publication date: 2005 / Copyright © 2005 Annual Reviews), 519-539.

Wiens, J. J., Graham, C. H., Moen, D. S., Smith, S. A., & Reeder, T. W. (2006). Evolutionary and ecological causes of the latitudinal diversity gradient in Hylid frogs: treefrog trees unearth the roots of high tropical diversity. *The American Naturalist, 168*(5).

Wiens, J. J., Kuczynski, C. A., Duellman, W. E., & Reeder, T. W. (2007). Loss and re-evolution of complex life cycles in marsupial frogs: does ancestral trait reconstruction mislead? *Evolution, 61*(8), 1886-1899. doi:10.1111/j.1558-5646.2007.00159.x

Wilcove, D. S., Rothstein, D., Dubow, J., Phillips, A., & Losos, E. (1998). Quantifying threats to imperiled species in the United States. *BioScience, 48*(8), 607-615. doi:10.2307/1313420

Wright, A. H. (1931). *Life-histories of the frogs of Okefinokee swamp, Georgia; North American Salientia (Anura) no.2*. New York,: The Macmillan company.

Wu, J., & David, J. L. (2002). A spatially explicit hierarchical approach to modeling complex ecological systems: theory and applications. *Ecological Modelling, 153*(1–2), 7-26. doi:http://dx.doi.org/10.1016/S0304-3800(01)00499-9

Wygoda, M. (1988). Adaptive control of water loss resistance in an arboreal frog. *Herpetologica, 44*(2), 251-257.

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., & Madden, T. L. (2012). Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics, 13*, 134. doi:10.1186/1471-2105-13-134

Zeisset, I., & Beebee, T. J. C. (2008). Amphibian phylogeography: a model for understanding historical aspects of species distributions. *Heredity, 101*(2), 109-119.

Biographical information

Paul Pasichnyk is a native Texan, born in Amarillo in 1986. His interest in nature began in his grandparents' backyard where his grandfather opened his life to a world underneath rocks. As a child, he had a failed entrepreneurial quest as a "lizard catcher," but that did not stop his interest in science and nature. With annual camping trips as a child and a family that encouraged curiosity, Paul continued to learn as much as he could about the world around him. Paul graduated from Bridgeport High School as salutatorian in 2005 and was accepted into Tarrant County College's Cornerstone Honors Program. He received his Associates degree in Humanities in the spring of 2007 and began his Bachelor's degree the following fall semester at the University of Texas at Arlington. Uncertain of his intentions for the future, he changed majors twice and decided to pursue a major in his original field of interest, Biology. During his last year, Paul met Dr. Charles M. Watson who opened his life to science in a more professional setting. In May 2010, Paul graduated from UTA with a Bachelor's degree in Biology and a minor in Spanish, and he reenrolled as a graduate student under Dr. Jonathan Campbell, whom he had met as an undergrad in Dr. Campbell's natural history course. At UTA, Paul was introduced to Dr. Paul Chippindale and his students who shared similar interests in biology, and he requested that Dr. Chippindale be his co-advisor. While a graduate student at UTA, Paul was offered an adjunct position at El Centro College in Dallas for the fall of 2014, which later led to a full time position that Paul accepted in the spring of 2015. Paul continued his work at UTA while teaching full time at El Centro.

Upon the completion of Paul's graduate work, he intends to continue to pursue his passion for teaching at El Centro, continuing his family's legacy as educators.