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Ecology and Genetics of Lungless Salamanders (Family Plethodontidae) in the Gulf Coastal Plain

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ECOLOGY AND GENETICS OF LUNGLESS SALAMANDERS (FAMILY PLETHODONTIDAE) IN THE GULF COASTAL PLAIN

by

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ABSTRACT

ECOLOGY AND GENETICS OF LUNGLESS SALAMANDERS (FAMILY PLETHODONTIDAE) IN THE GULF COASTAL PLAIN

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During the last half century, lungless salamanders (Family Plethodontidae) have been the subject of numerous studies in the fields of ecology and genetics. While most works have focused on the species-rich Eastern Highlands region, there has been a recent shift towards plethodontid assemblages within the Coastal Plain. The research presented herein applies hierarchical occupancy models and both mitochondrial and nuclear genes to address questions pertinent to the biology and conservation of plethodontids within the Gulf Coastal Plain. The results of a multi-species Bayesian single-season occupancy model indicated that two environmental gradients, upstream drainage area and stream drying, influenced the probability of occurrence for multiple species of stream-breeding plethodontids. Further, species varied in their responses to these gradients. A second model was used to ask whether asymmetric interactions also influenced occurrence for three species of brook salamanders (Genus *Eurycea*). More specifically, the model tested whether the southern two-lined salamander (*E. cirrigera*) might act as the dominant predator and or competitor to either the three-lined (*E. guttolineata*) or dwarf (*E. quadridigitata*) salamanders. The results of this second model suggested that environmental gradients likely work in tandem with negative interactions to shape the distribution of *E. guttolienata* within the Gulf Coastal Plain. Like hierarchical occupancy models, genetic tools are also shedding light on complex relationships among and within

species of lungless salamanders. This research investigated phylogeographic patterns within a wide-ranging species of plethodontid, the spotted dusky salamander (*Desmognathus conanti*). Sequence data revealed that there were geographically discrete, deeply divergent mitochondrial lineages within *D. conanti* which may be the result of isolation brought about by fluctuating sea levels during the late Miocene through the Pleistocene. Data from six rapidly mutating microsatellite markers indicated that there had been recent gene flow across some of these lineages in the southern Gulf Coastal Plain. However, these data also suggest that a northern lineage may have remained distinct. The relationships described and occurrence probabilities estimated by the aforementioned models, in combination with conclusions from analyses of genetic data, improve our ability to conserve regional plethodontid biodiversity within this unique physiographic province.

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2015 and the Gulf Coast Research Lab in 2014 and 2015. Over the course of this experience, I have learned that a successful PhD is the culmination of a personal passion surrounded and bolstered by a supportive, engaged community. The former cannot persist without the latter.

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DEDICATION

To Brandon C. Morris, my husband and closest friend, who has marveled, endured, and celebrated by my side throughout this process, and with whom I am excited to share my next adventure. I could not have hoped for a better teammate. To my parents, Brian K. and Laura J. Lamb, and my sister, Jessica K. L. Jones, each of which has cheered for me loudly and without fail. I could not have asked for better role models. To my grandmother, Ellen G. Lamb, and grandfather, William S. Lamb, whose tenacity and compassion I hope to live up to.

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Abstract

There are large gaps in our knowledge of the ecology of species and populations of stream-breeding plethodontid salamanders in the Gulf Coastal Plain. Data describing where these salamanders are likely to occur along environmental gradients, as well as their likelihood of being detected, will be useful in preventing and managing amphibian declines. This study uses presence/absence data from leaf litter bag surveys and a hierarchical Bayesian multi-species single-season occupancy model to estimate the occurrence of five species of plethodontids in small to medium headwater streams and tributaries in the Gulf Coastal Plain. Average detection probabilities across species were high (range = 0.420 – 0.939) and unaffected by sampling covariates specific to survey methods in this study. Estimates of occurrence probabilities differed substantially between species (range = 0.093 – 0.707) and were influenced by the size of the upstream drainage area of a site, as well as by the maximum proportion of the stream reach that dried during the summer. The effect of each gradient on occupancy differed across species of salamanders. These results demonstrate that hierarchical multi-species models successfully estimate occupancy parameters for both rare and common stream-breeding plethodontids. The resulting models clarify how species are distributed within stream networks, and they provide baseline values that will be useful in evaluating the conservation statuses of plethodontid species within lotic systems in the Gulf Coastal Plain.

Introduction

Lungless salamanders (Family Plethodontidae) comprise a significant proportion of the vertebrate biomass within a variety of temperate ecosystems (Burton & Likens [1975]; but see Semlitsch, O'Donnell & Thompson [2014]), and they play important roles in energy and nutrient cycling within these systems (Davic & Welsh Jr. 2004; Best & Welsh 2014). The ecology and natural history of plethodontid salamanders have been the focus of numerous studies (see Hairston [1949] and Wells [2010] for a review), but the majority have involved species or populations in the Appalachians or Piedmont, rather than the Gulf Coastal Plain (Means 2000). The Gulf Coastal Plain is a physiographic province with a unique history, topography, and suite of climates and habitats (Kirkman, Brown & Leopold 2007). The environmental gradients that shape species occurrence, or the importance of any particular gradient, may differ among these provinces. In light of ongoing amphibian declines (Stuart et al. 2004), including the enigmatic decline of some species within the Gulf Coastal Plain (Means & Travis 2007; Maerz et al. 2015), it is imperative that we collect baseline data describing where species are likely to occur, as well as at what frequency we might expect to encounter populations within an area. These data will enable us to detect, monitor, and possibly prevent declines of plethodontids in the future.

Hierarchical occupancy models quantify relationships between the occurrence of a species and environmental covariates while simultaneously accounting for imperfect detection (MacKenzie et al. 2002; MacKenzie *et al*. 2006; Royle & Dorazio 2008). They are increasingly being applied towards the ecology and conservation of a myriad of amphibian species, including anurans (Pellet and Schmidt 2005; Walls et al. 2011;

Waddle et al. 2012; Lehtinen and Witter 2014) and caudates (Bailey et al. 2004a; Bailey et al. 2004b; Grant et al. 2009; Walls et al. 2013). These models are powerful tools when used with amphibians for which detection is usually imperfect (MacKenzie et al. 2002) due to the influence of sampling conditions (e.g., humidity, temperature [Walls et al. 2011; Waddle et al. 2012]) or study design (Bailey et al. 2004b; Walls et al. 2013; Lehtinen & Witter 2014; Grant, Wiewel & Rice 2014). Failing to incorporate detection probabilities can result in false absences which contribute to an inaccurate understanding of species distributions and associations (MacKenzie et al. 2002; MacKenzie 2006; Royle & Dorazio 2008).

This study used hierarchical occupancy models to investigate the effects of three environmental gradients, including stream size (Means 2000; Waldron, Dodd & Corser 2003), topography (Means 2000; Marshall & Camp 2006), and stream impermanence, on stream-breeding salamander occupancy in headwater streams in the Gulf Coastal Plain. Each of these gradients has been described in the literature as an important factor affecting the occurrence of stream-breeding plethodontids in the greater Coastal Plain (Means 2000), but the hypothesized relationships between each species and gradient have not been explicitly tested. Although sometimes correlated, these environmental gradients can vary independently of one another, and the impact of each on occupancy probabilities should be considered separately.

There are a variety of ecological factors that change along the stream size gradient, such as water temperature and the composition of the fish community, which could affect the occupancy of stream-breeding plethodontids (Vannote et al. 1980). Plethodontids persist at sites containing fishes capable of consuming larval and

metamorphosed individuals (e.g., *Lepomis* [Petranka 1983; Wells 2010]*;* pers. obs.) but salamanders may mitigate this predation pressure by occupying smaller streams in which predator gape-size is limited (Vannote et al. 1980). Temperature affects many important physiological processes across amphibian taxa (Wells 2010), and recent work with stream-breeding plethodontids in mid-Atlantic drainages indicates that for some species of plethodontids the probability of occurrence increases with decreasing average water temperatures (Grant et al. 2014). These factors, as well as others that vary along this gradient, may act in a complex, synergistic fashion to shape species occurrence within a drainage. Stream size may be a holistic metric by which we can estimate occupancy in the Gulf Coastal Plain.

Plethodontid species diversity is highest at intermediate elevations where the climate is cool and wet (Kozak & Wiens 2010, 2012), and the shape of the landscape through which a stream flows may determine how species are organized within the catchment. The Coastal Plain lacks the extreme relief seen elsewhere (e.g., Appalachians), but it does contain relatively steep hills, bluffs, and deep ravines (Kirkman et al. 2007) in which conditions can substantially differ from those in flat bottomland habitats (e.g., temperature, humidity, rate of flow). Populations of stream-breeding plethodontids within the Gulf Coastal Plain may be relegated to specific habitats along this topographic gradient if the species is physiologically constrained by its recent evolutionary history (e.g., it may only persist in cool seeps in ravines if it has recently diverged from a montane-adapted species and is restricted by a low thermal maximum) (Bernardo & Spotila 2006; Kozak & Wiens 2010, 2012). Competitive exclusion may also play a role in the distribution of species along this gradient, either in the arrangement of

species when moving perpendicularly away from the stream (e.g., Hairston 1949, 1986), or in their distribution between steep, headwater origins and swampy downstream habitats (e.g., Means 1975).

Fewer studies ask how the third gradient, stream impermanence, affects plethodontid occupancy (though see discussions in Bruce 1982 & 2005). Larval periods among biphasic species in the Gulf Coastal Plain range from four months to more than two years in duration, and there is considerable intraspecific variation in this trait (Dundee & Rossman 1989; Petranka 1998; Bruce 2005). Ephemeral streams and streams that only partially dry are common in the Gulf Coastal Plain and are occupied by some species of plethodontids (e.g., dwarf salamander, [*Eurycea quadridigitata*], three-lined salamander [*E. guttolineata*], and the southern dusky salamander [*Desmognathus auriculatus*] [Petranka 1998; Bruce 2005]). However, we do not understand how occupancy probabilities change for these species along this drying gradient. Occupancy of these habitats may be precluded or limited by metamorphic parameters (e.g., developmental rate) or other physiological restrictions for species derived from lineages that more recently occupied stable stream habitats (e.g., *Desmognathus*) (Bruce 2005). Although adult salamanders that survive periods of low water levels can buffer a population from local extirpation, these populations can only persist for a limited amount of time in the absence of any recruitment (Price, Browne & Dorcas 2012).

This study used Bayesian methods and a hierarchical multi-species single-season model (Kéry & Royle 2008; Royle & Dorazio 2008; Waddle et al. 2013) to estimate salamander occupancy in small to medium headwater streams and tributaries in the Gulf Coastal Plain. This strategy allows us to fit a model using numerous parameters for

multiple, ecologically similar species treated as random effects (Link et al. 2002; MacKenzie 2006). This type of multi-species model is more precise when quantifying occupancy probabilities for rare species (Kéry & Royle 2008; Waddle et al. 2013; Walls et al. 2011).

Methods

Study area and site selection

I selected 60 sites along two habitat gradients, stream size (i.e., wet-width and drainage area) and surrounding topography, in an effort to represent the diversity of small to medium 1st and 2nd order (Strahler 1964) stream habitats present in the Pascagoula River Drainage. Sites were a 50 m long reach of stream and, if in the same stream, separated by at least 100 m of stream length. This distance likely prevented individuals from moving between sites over the duration of this study (Cecala, Price & Dorcas 2009; Wells 2010). Streams were located in the Bienville National Forest (6 streams), De Soto National Forest (17 streams in the De Soto district, 7 in the Chickasawhay district), and in the Ward Bayou Wildlife Management Area (2 streams) in Mississippi, USA.

Data collection

A subset of sites were sampled between May and July of 2012 and the remainder between May and July of 2013. Each site was sampled 3 times. I used leaf-litter bags (hereafter litter bags) to detect both larval and metamorphosed salamanders in streams (Pauley & Little 1998; Waldron et al. 2003). Five litter bags, separated by 10 m, were deployed at each site, for a total of 300 bags. Litter bags were made from a double layered 70 x 70 cm square of plastic wildlife netting with pores 1.5 cm in diameter (Waldron et al. 2003) and were filled with leaf litter from stream banks *in situ.* I sunk

bags using wood or gravel and used mason line to secure each bag to the stream bank. To check a litter bag, I quickly lifted it from the water while sweeping a dip net beneath it and then placed the litter bag in a large plastic container (Waldron et al. 2003; Mattfeldt & Grant 2007). After checking the dip net for salamanders, I poured water from the stream over the bag until it was submerged and then agitated the bag for 60 seconds to dislodge salamanders. I then poured the contents of the container through the dip net. This process of submerging and agitating the litter bag was repeated until it failed to dislodge any salamanders for two consecutive attempts. All salamanders were identified to species, measured (i.e., total length and snout-to-vent-length), sexed (if possible), and released in the stream close to the litter bag in which they were found, except for a small number of individuals collected for use in other studies.

At each site I collected habitat data describing the three gradients of interest: stream size, surrounding topography, and stream impermanence. Various types of measurements have been used to describe stream size across studies (e.g., wet-width [Waldron et al. 2003], drainage area [Snodgrass et al. 2007], Strahler stream order [Strahler 1964; Means 2000]). These data may differ in terms of their biological relevance. I recorded stream size using two metrics, wet-width and upstream drainage area (ha). The wetted-width of the stream was measured to the nearest 1 cm at distances of 5, 15, 25, 35, and 45 meters along each 50 meter site during each sampling occasion. The average of these data for each site constitute the width covariate (hereafter Width). I used the U.S. Geological Survey National Hydrography Dataset (NHD) and ArcGIS to estimate upstream drainage area (hereafter DA). Sites within the same stream have the same value for DA because they were too close to differ appreciably in this metric. I used

topographic maps to estimate DA for streams that were too small to be included in the NHD. A clinometer was used to measure the slope (% slope) of the streamside habitat along a 10 m line perpendicular to the stream on each side of the bank at meters 0, 25, and 50 within each site. These data were then averaged for each site (hereafter Topography). Sites included in this study dried either completely, partially, or never during the course of data collection. The NHD categorizes streams as intermittent or permanent, but these categories may be too imprecise to be biologically relevant. I estimated stream impermanence by quantifying the maximum proportion of the stream that dried during the sampling season. Three equally spaced depth measurements were taken across the wetted-width of the stream at meters 5, 15, 25, 35, and 45 within a site, giving a total of 15 depth measurements per site for each of the three sampling occasions. I calculated the proportion of points equaling zero during each sampling occasion and used the maximum of these three values to describe stream impermanence (Dry).

I used three sampling covariates that I hypothesized could influence detection probability as a consequence of the choice of survey method (i.e., litter bags), including litter bag submergence, sampling date, and the type and proportion of in-stream cover present within the stream. Waldron et al. (2003) note that the number of metamorphosed salamanders caught in litter bags is negatively correlated with the proportion of the bag that is submerged beneath the surface of the water, suggesting that adults of some species may not utilize the entirety of the stream channel. This possibility, combined with the sampling period (i.e., summer), could result in lower detection probabilities for species with shorter larval periods (e.g., some species of brook salamanders [Genus *Eurycea*] and dusky salamanders [Genus *Desmognathus*] [Petranka 1998; JYL unpubl. data]). With this

in mind, I estimated litter bag submergence for each bag to the nearest 25% prior to checking the bag for salamanders and then averaged these percentages for each sampling occasion and site (hereafter Submerge). To control for the effect of the time of year, I also included the number of days since May 1 as a detection covariate (hereafter Day).

Waldron et al. (2003) suggest that the availability of natural cover within the stream is negatively correlated with the likelihood that salamanders would utilize litter bags (i.e., lower densities in bags due to greater availability of suitable refugia elsewhere). Anecdotal evidence from the Gulf Coastal Plain suggests that streams containing more in-stream cover generally support greater densities of plethodontid larvae, increasing the detectability of this life stage. I quantified the amount and type of in-stream cover available to salamanders using five equally spaced, 4 m wide belt transects crossing the stream. Within these transects, I visually assessed, to the nearest 1, 5, or 10%, the area covered by bare substrate, leaf-litter, woody debris, aquatic vegetation, and roots. Totals for a transect could sum to more than 100% because in-stream cover can describe three-dimensional structure. The average proportions of each type of in-stream cover from belt transects were calculated for each site. These data were then used in a principal components analysis of covariates, and site scores along the first principal axis were used in the model (hereafter Cover).

Data analysis

The model herein estimates probability of occurrence for five species of stream-breeding plethodontid salamanders. This type of hierarchical occupancy model uses a detection history from repeat visits (y = 0, 1) to estimate occurrence (z), detection probabilities (p), and covariate-responses for each species (i). Occurrence is a latent

variable estimated using the probability of occurrence (Ψ). There are two possible outcomes when a species is not detected across sampling occasions at a site (k), either it does not occur at the site ($z_{ik} = 0$) and therefore was not available for detection, or it occurs at the site ($z_{ik} = 1$), but researchers failed to observe it (MacKenzie 2006). Similar models allow for the presence of hypothetically undetected species across sites (Kéry & Royle 2008), but I have chosen to structure this model such that the total number of species is known (Waddle et al. 2013).

Site and sampling covariates are used to separately model Ψ and p, respectively, through application of the logit link function, and the effect size for site (*ß*) and sampling (α) parameters are estimated for each species (Royle & Dorazio 2008). I used four covariates to model Ψ, including Width, DA, Topography, and Dry. Data for each covariate were centered and scaled. Table 1.1 lists the *a priori* hypotheses for how each of the five species might respond to each of the four site covariates. These hypotheses are based on relationships described in the literature (e.g., Petranka 1998; Means 2000; Waldron et al. 2003), as well as on personal observations of stream-breeding plethodontids in the Gulf Coastal Plain. I expected to encounter these species of stream-breeding plethodontids based on a pilot study completed by JYL in streams in the Pascagoula River Drainage. I included three covariates to model p, including Submerge, Day, and Cover. Submerge and Day were centered and scaled. All statistics and ordinations were completed in the programming language R (R Core Team 2014)

I used Bayesian analysis with uninformative priors to estimate model parameters. Priors for occupancy and detection probabilities were distributed uniform from 0 to 1. Priors for the effect(s) of covariates were distributed normally with a mean of 0 and

variance equaling 10. Kuo and Mallick (1998) variable selection was incorporated into the model. This method of model selection uses a binary inclusion parameter multiplied against each covariate to determine whether that covariate should be included in the final model (Royle & Dorazio 2008; O'Hara & Sillanpää 2009). If the covariate improves the fit of the model the posterior distribution of the inclusion parameter for that covariate will have a mean closer to 1. All values of inclusion parameters were binomially distributed on 0.5 with a variance equaling 1. This multi-species model was fit using the Markov chain Monte Carlo (MCMC) method in WinBUGS (ver. 1.4.3) (Spiegelhalter et al. 2003). WinBUGS was called from R using the package R2WinBUGS (Sturtz, Ligges & Gelman 2005). I used three parallel MCMC chains 10,000 iterations in length with a burn-in length of 5,000 and a thinning rate of 10. Markov chain convergence was assessed using R-hat, a potential scale reduction factor (Gelman & Shirley 2011). I report the mean values and 95% Bayesian credible intervals of the posterior distributions for those parameters (covariates) that were maintained in the final model after Kuo and Mallick (1998) selection.

Results

I captured 2,065 larval, metamorphosing, and transformed salamanders belonging to 5 different species of plethodontid salamanders in litter bags (Table 1.2). The only spotted dusky salamanders (*Desmognathus conanti*) detected during this study were metamorphosed individuals, but larvae and metamorphosing or transformed individuals of each other species were captured in litter bags. Southern two lined salamanders (*Eurycea cirrigera*) were detected in 60%, three lined salamanders (*E. guttolineata*) in 30%, and *E. quadridigitata* in 13% of the total of 180 sampling visits across all 60 sites.

Desmognathus conanti and the southern red salamander (*Pseudotriton ruber vioscai*) were detected in ca. 6% of the total visits. The only non-plethodontid salamander detected using this method during this study was the lesser siren (*Siren intermedia*), of which two adults were caught at two sites in the Chickasawhay district.

Study sites were all in relatively small streams in terms of both wetted-widths (mean = 186.09 cm; SD = 90.28 cm) and upstream drainage areas (mean = 513 ha; SD = 471 ha). Seven sampled streams similar in size were too small to be included in the NHD, and I used topographic maps to estimate the upstream drainage areas of these streams to be 38 ha, a value that is half that of the smallest sampled stream in this study included in the NHD. Many streams flowed through flat, or only gently sloping, topographies, but some moved through very steep terrain (mean = 6.10 % slope; SD = 11.91 % slope). The majority of sites contained water throughout the summer, or 10% or less of their reach dried (mean = 0.13 maximum proportion dry; SD = 0.292). Four sites dried completely during the second sampling occasion, three of which remained dry for the remainder of the study. These sites, as well as those sites at which all litter bags were lost due to heavy rain events or tampering, have detection histories including "not applicable" across all species for that sampling occasion. These missing response data (i.e., NA values) are estimated by WinBUGS (Kéry 2010).

Estimated mean detection probabilities (mean p ± SD) ranged from 0.420 ± 0.129 to 0.939 ± 0.027 (Table 1.3) and were lowest for *E. quadridigitata,* for which the third greatest number of individuals were caught (n = 120) (Table 1.2). The 95% Bayesian credible intervals (95% BCI) varied greatly among species and were widest for *D. conanti* and *P. ruber vioscai* (Table 1.3). None of the three sampling covariates (i.e.,

Submerge, Day, Cover) were retained in the model after Kuo and Mallick (1998) variable selection and the BCIs for these covariates overlapped zero, indicating that they did not account for any appreciable variation in detection probabilities. Unlike that of either of the other sampling covariates, the mean value for the posterior distribution of the inclusion parameter for Cover approached significance (Kuo & Mallick 1998). In the principal components analysis of in-stream cover, the first principal component explained close to 75% of the total variation and organized sites along an axis from greater amounts of bare substrate to those with greater amounts of any type of cover. To test a simpler hypothesis regarding in-stream cover (i.e., whether the proportions of leaf-litter and bare substrate alone would significantly influence p), I re-ran the hierarchical multi-species model using the primary axis from a second principal components analysis in which the only data included were those describing the average proportions of bare substrate and leaf-litter. Redefining Cover in this way did not affect variable selection or the model results in any way.

Minimum occupancy, defined as the proportion of sampled sites at which the species was detected at least once, ranged from 0.08 to 0.65 (Table 1.4). Estimates of the mean finite probability of occurrence (i.e., across sampled sites) (mean $\Psi \pm$ SD) ranged from 0.093 ± 0.018 to 0.707 ± 0.062, and the 95% BCI was greatest for *E. quadridigitata* (Table 1.4). Two site covariates, DA and Dry (Table 1.5), were retained in the model after Kuo and Mallick (1998) variable selection.

Estimates of the DA effect β parameter were positive for *E. cirrigera* and negative for *P. ruber vioscai* (Table 1.5), and the 95% BCI for these species did not overlap zero. These results indicate a significant effect of drainage area on Ψ for these salamanders,

with *E. cirrigera* occurring in reaches further downstream that have larger upstream drainage areas, and *P. ruber vioscai* occupying sites closer to the stream origin in reaches with smaller upstream drainage areas (Figure 1.1). Values for the 95% BCI overlapped zero for each of the three other species.

The effect of stream impermanence (Dry) on Ψ was significant for each of the three species of brook salamanders (Genus *Eurycea*) (Table 1.3). The Dry effect was negative for *E. cirrigera*, suggesting that *E. cirrigera* is more likely to occur at lotic sites in which less of the reach dries (Figure 1.2). The Dry effect on Ψ was positive for both *E. guttolineata* and *E. quadridigitata*, indicating that they tend to occupy streams more prone to drying (Figure 1.2). The effect of stream impermanence on the average probability of occurrence differed among these species of *Eurycea*. The average Ψ for *E. quadridigitata* increases gradually from permanent surface water to reaches in which half of the stream dries, whereas Ψ for *E. guttolineata* approaches 1.0 much more quickly. There was a steep decrease in average Ψ for *E. cirrigera* across the wetter portion of the stream drying gradient. Although the 95% BCIs overlap 0 for both *D. conanti* and *P. ruber vioscai*, Dry had an overall negative effect on Ψ for these salamanders (Table 1.3), both of which were infrequently captured (Table 1.2).

Discussion

The modeling results indicate that two gradients, stream size and impermanence, affect stream-breeding salamander occupancy in the Gulf Coastal Plain and that their effects are not identical across species. They further suggest that methods used to quantify stream size may not be equally informative (i.e., width vs. upstream drainage area) and that patterns along certain gradients (i.e., topography) may instead be the result

of species associations with a different but frequently correlated gradient (i.e., stream size). This model identified a significant, negative relationship between upstream drainage area and either finite or average Ψ for *P. ruber vioscai*, but not for *D. conanti*. *Desmognathus conanti* was infrequently encountered in this study and the 95% BCI for estimates of p were wider for this species than for any other. This suggests that the model lacked precision, possibly due to unmodeled variability in detection probabilities which affect the model's ability to identify covariate effects if the number of sampling occasions is small (MacKenzie et al. 2002; MacKenzie 2006). I suspect that future studies incorporating a greater number and diversity of sites will strengthen the overall negative trend in the 95% BCI for the effects of DA on the occurrence of *D. conanti*.

The effects of stream impermanence on estimates of Ψ in this study are not necessarily surprising given the natural histories of these five species of plethodontids. Although the 95% BCIs overlap 0 for *P. ruber vioscai*, the interval has a clear negative trend, which suggests that this salamander requires access to greater amounts of surface water for most of the year, as is the case for *E. cirrigera.* These modeling results align with previous expectations, which were based both on the duration of larval periods for these species, as well as on the natural histories of metamorphosed individuals. Larval periods for both *E. cirrigera* (up to 2 -3 years [Dundee & Rossman 1989; Mount 1975] and *P. ruber vioscai* (up to 3.5 years [Petranka 1998]) are lengthy. The larval period of *D. conanti* can range from approximately six (Dundee & Rossman 1989; unpubl. data) to as many as 13 months (Mount 1975), and the 95% BCI for this species also had a negative, though not statistically significant, skew. *Desmognathus conanti* is a semi-aquatic species frequently found within a few meters of small streams or seepage waters in the Gulf

Coastal Plain (pers. obs.), and abundances for many *Desmognathus* along Appalachian streams are highest within 15 m of the water's edge (Crawford & Semlitsch 2007). This close association with aquatic habitats, combined with a larval period of moderate length, may result in a negative relationship between *D. conanti* and stream impermanence in future studies incorporating a greater number of sites. However, Price *et al.* (2012) have demonstrated that other species of *Desmognathus* occupy semi-permanent streams and can survive varying severities of drought. Consequently, it is also feasible that subsequent work will demonstrate that this gradient has no effect on Ψ for *D. conanti.*

Estimates of Ψ for both *E. guttolineata* and *E. quadridigitata* indicated that these species were more likely to occur at sites wherein a greater proportion of the stream dries. These species are capable of successful recruitment in ephemeral sites due to their shortened larval periods, which are typically less than one year for *E. guttolineata* (Bruce 1982), and can be as little as three or four months for some populations of both species (Bruce 1970; Dundee & Rossman 1989). Still, this capacity does not prevent them from also occupying habitats with more permanent surface waters, hence my *a-priori* predictions. I hypothesize that the direction of the effect of stream impermanence on estimates of Ψ for both *E. guttolineata* and *E. quadridigitata* may in part be a response to negative interactions (e.g., predation and or competition) with other species of stream-breeding caudates during either or both life history stages (i.e., among larval or metamorphosed individuals) (Morin 1983; Bruce 2008). *Eurycea cirrigera* may preferentially inhabit reaches within headwater streams with larger upstream drainage in order to reduce similar pressures.

This model does not include species interactions. Hierarchical species interaction models have been developed but their applications are limited in the number of species they can include (e.g., < 4 species [MacKenzie, Bailey & Nichols 2004]) and in the types of relationships that can be estimated (e.g., Waddle et al. 2010). A strength of the multi-species model employed here is that the treatment of species as random effects allows for data from frequently encountered species to be used to estimate parameters for less common species (i.e., "shrinkage" [Walls et al. 2011]), such as *P. ruber vioscai* and *D. conanti* in the case of this research. It is unlikely that other modeling configurations would be sensitive enough to detect effects in these species.

Litter bags (Pauley & Little 1998; Waldron et al. 2003) were the only sampling method employed during this study, and the detection model confirmed that this is a useful method for capturing species of stream-breeding plethodontids in the Gulf Coastal Plain. As in other studies, litter bags successfully detected rare species like *P. ruber* (Waldron et al. 2003; Mattfeldt & Grant 2007; Mackey et al. 2010; Table 1.2). Kuo & Mallick (1998) variable selection demonstrates that the ability of this sampling method to detect species was not a function of bag submergence, sampling date, or the prevalence of in-stream cover within a site. Further, the estimated values for p across species in this model are substantially larger than they are in other studies that use litter bags to sample stream-breeding plethodontids (e.g., Mattfeldt & Grant, 2007). This may be a consequence of different analytical approaches (i.e., Bayesian vs. information criterion analyses), but the choice of bag size and method of agitating the bags could also have contributed to increased detection probabilities. Still, future studies comparing sampling methods in the Gulf Coastal Plain are warranted. Although perhaps more effective at

removing a greater number of individual salamanders, the method of checking bags presented here was time consuming (i.e., in this study, the maximum number of agitations for a single bag was 11). I also encountered some of the same drawbacks as did Mattfeldt & Grant (2007) (e.g., occasional bag loss and 2 incidental captures and fatalities of snakes).

The modeling results illustrate that beta-diversity of stream-breeding plethodontids in headwater streams in the Gulf Coastal Plain is shaped by both upstream drainage area and the availability of surface water during the summer months. Lotic sites with varying hydrologies (i.e., duration of surface flow or inundation) may increase the overall species diversity of plethodontids in the Gulf Coastal Plain, and the sensitivity of this gradient to watershed development (Allan 2004) and climate change (Brooks 2009) could alter long term probabilities of occupancy for certain species. Streams included in this study occurred on National Forests or Wildlife Management Areas and were selected in an effort to reduce the effect of anthropogenic disturbance. Consequently, the occupancy estimates produced should serve as baseline values against which probabilities of occurrence in disturbed sites within the same physiographic province can be compared. Subsequent efforts should include multi-year studies across a larger number of sites to further clarify patterns for *Desmognathus*, as well as estimate the effects of hydrology on long term occupancy and dynamic parameters (e.g., rates of colonization and extinction [Royle & Kéry 2007; Walls *et al.* 2011]) for stream-breeding plethodontids in the Gulf Coastal Plain.

Table 1.1

A-priori hypotheses regarding the probability of occurrence (Ψ) and environmental gradients.

Species	Stream size	Stream impermanence	Topography
Spotted dusky salamander (*Desmognathus conanti*)	–	–	+
Two-lined salamander (*Eurycea cirrigera*)	–	–	0
Three-lined salamander (*E. guttolineata)*	0	0	0
Dwarf salamander complex (*E. quadridigitata*)	0	0	0
Southern red salamander (*Pseudotriton ruber vioscai*)	–	–	+

Note: Negative signs (−) mark relationships for which the probability of occurrence (Ψ) is predicted to decrease as the value of the covariate increases. Positive signs (+) mark relationships for which Ψ is predicted to increase as the value of the covariate increases. Zeros indicate that there is no predicted relationship between this species and the covariate.

Table 1.2

Count of plethodontid salamanders caught in leaf litter bags across 60 sites.

Species	Larvae	Transformed	Total
Spotted dusky salamander (*Desmognathus conanti*)	0	17	17
Two-lined salamander (*Eurycea cirrigera*)	1637	105	1742
Three-lined salamander (*Eurycea guttolienata*)	67	100	167
Dwarf salamander complex (*Eurycea quadridigitata*)	85	35	120
Southern red salamander (*Pseudotriton ruber vioscai*)	17	2	19
Total	1806	169	2,065

Note: Transformed individuals include those near the completion of metamorphosis.

Table 1.3

Estimated detection probabilities and 95% Bayesian credible intervals (BCI) for stream-breeding plethodontids.

Species	p (SD)	Lower 95% BCI	Upper 95% BCI
Spotted dusky salamander (*Desmognathus conanti*)	0.464 (0.173)	0.123	0.763
Two-lined salamander (*Eurycea cirrigera*)	0.939 (0.027)	0.879	0.984
Three-lined salamander (*Eurycea guttolienata*)	0.459 (0.061)	0.346	0.576
Dwarf salamander complex (*Eurycea quadridigitata*)	0.420 (0.129)	0.163	0.649
Southern red salamander (*Pseudotriton ruber vioscai*)	0.624 (0.135)	0.342	0.865

Table 1.4

Summary of occurrence modeling for stream-breeding plethodontids.

Species	Minimum Occupancy	FS Ψ (SD)	Lower 95% BCI	Upper 95% BCI
Spotted dusky salamander (*Desmognathus conanti*)	0.10	0.134 (0.054)	0.100	0.283
Two-lined salamander (*Eurycea cirrigera*)	0.65	0.656 (0.008)	0.650	0.667
Three-lined salamander (*E. guttolineata)*	0.58	0.707 (0.062)	0.617	0.850
Dwarf salamander complex (*E. quadridigitata*)	0.27	0.382 (0.130)	0.267	0.767
Southern red salamander (*Pseudotriton ruber vioscai*)	0.08	0.093 (0.018)	0.083	0.150

Note: Minimum occupancy is defined as the proportion of sites at which the species was detected at least once. FS Psi (Ψ) is the finite sample occupancy probability, the probability of occurrence of that species across our sampling sites from the posterior distribution. The lower and upper bounds of the 95% Bayesian Credible Interval (BCI) are given.

Table 1.5

Estimates with 95% Bayesian credible intervals (BCI) of the logit-scale ß for the effect of upstream drainage area (ha) and stream impermanence (maximum proportion of the stream that dried) on the probability of occurrence (Ψ) for each species.

Species	ß DA	ß Dry
Spotted dusky salamander (*Desmognathus conanti*)	-0.995 (-2.795 – 0.702)	-2.860 (-8.187 – 0.354)
Two-lined salamander (*Eurycea cirrigera*)	2.613 (1.012 – 5.871) *	-1.938 (-3.957 – -0.513) *
Three-lined salamander (*Eurycea guttolienata*)	-0.774 (-1.886 – 0.523)	2.153 (0.240 – 6.220) *
Dwarf salamander complex (*Eurycea quadridigitata*)	-0.080 (-1.288 – 0.850)	1.197 (0.054 – 4.492) *
Southern red salamander (*Pseudotriton ruber vioscai*)	-2.326 (-5.377 – -0.331) *	-2.516 (-7.868 – 0.397)

Note: Drainage area is indicated with "DA," and impermanence with "Dry." Lower and upper BCIs are given in parentheses; significant effects that do not overlap 0 are indicated with an asterisk..

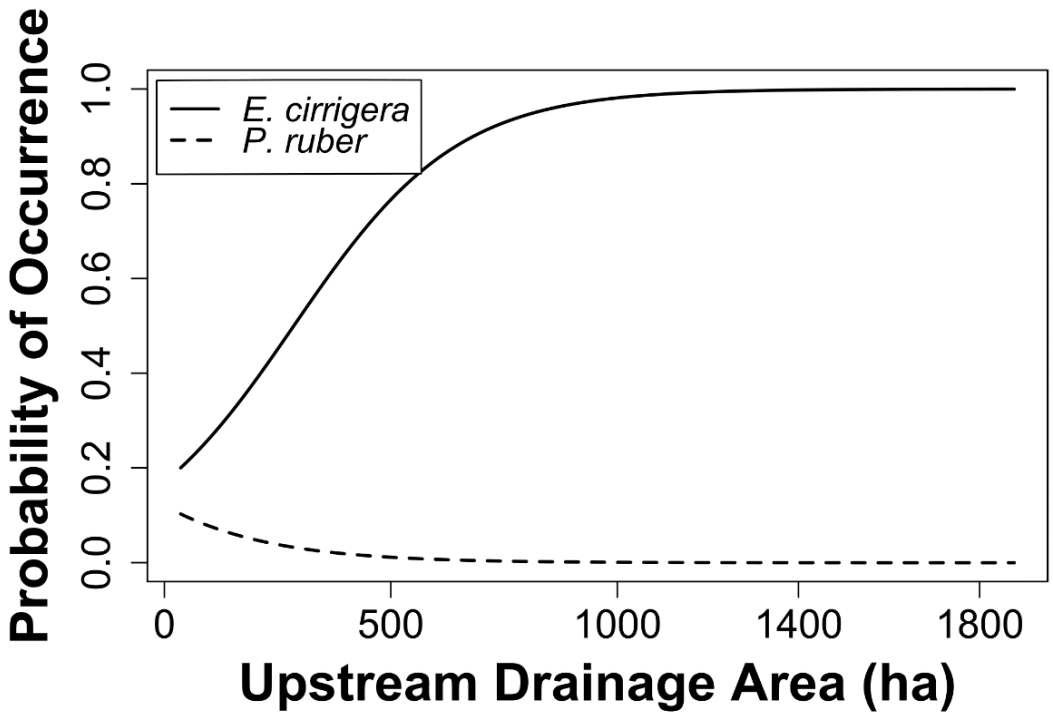

Figure 1.1. Effect of upstream drainage area (ha) on the average probability of occurrence for *E. cirrigera* and *P. ruber vioscai*.

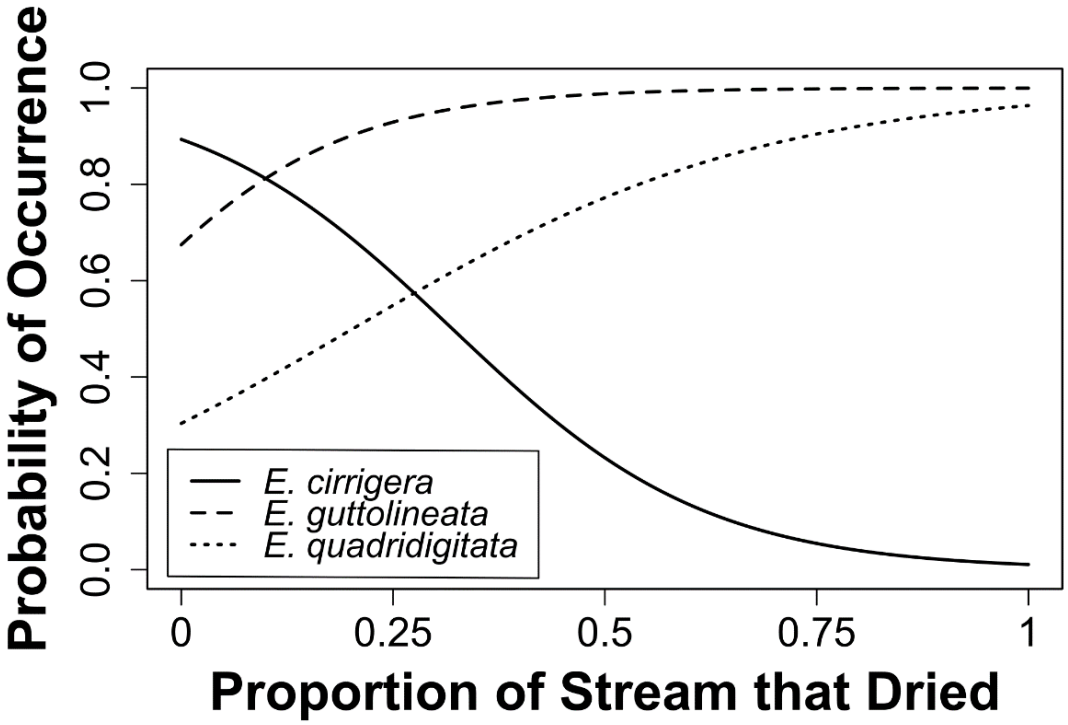

Figure 1.2. Effect of stream impermanence (maximum proportion of the stream that dried) on the average probability of occurrence for three species of *Eurycea*.

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CHAPTER II – ASYMMETRIC INTERACTIONS AMONG BROOK SALAMANDERS IN THE GULF COASTAL PLAIN

Abstract

Environmental gradients and species interactions influence the structure of assemblages of lungless salamanders (Family Plethodontidae), and it is likely that these associations will differ among regions due to unique combinations of species and habitats. Multiple species of brook salamanders occur syntopically within the Gulf Coastal Plain (i.e., *Eurycea cirrigera, E. guttolineata,* and the *E. quadridigitata* complex). These species share similar diets but differ in larval size and the duration of their larval periods with *E. cirrigera* attaining the largest sizes as larvae. I hypothesize that the presence of *E. cirrigera* could affect the occurrence of *E. guttolineata* and *E. quadridigitata* through interference competition and or intraguild predation during the larval period. I applied a hierarchical Bayesian occupancy model to presence-absence data for these species from across 60 sites in South Mississippi to determine whether the presence of the hypothesized dominant species (*E. cirrigera*) affected the probabilities of occurrence and detection of either of the two subordinate species (*E. guttolineata* and *E. quadridigitata*). This model also included stream permanence and drainage area as covariates for occupancy. Modeling results indicated that the presence of *E. cirrigera* has a significant, negative effect on the probability of occurrence of *E. guttolineata*, but no effect on the occurrence of *E. quadridigitata,* or on the probability of detecting either species. These salamanders respond differently to stream permanence, and future work should include both field and mesocosm studies to disentangle the effects of species interactions and environmental gradients.

Introduction

Environmental gradients and interactions among species work in concert to shape the distributions of taxa through space and time. When, where, and how both factors affect local patterns of occurrence is not only ecologically interesting but also important for the conservation and management of regional biodiversity. Interactions between species, including negative interactions such as competition and predation, may bias species occurrence along environmental gradients. As a result, we may underestimate the ability of a species to colonize new habitats or fail to accurately predict species responses to management actions. These relationships can be difficult to disentangle, particularly when species are detected imperfectly, which is the case for many amphibians (MacKenzie et al. 2002, Mazerolle et al. 2007). Hierarchical occupancy models enable us to account for imperfect detection when estimating species occurrence probabilities across environmental gradients (MacKenzie et al. 2002, MacKenzie 2006, Royle and Dorazio 2008). Newer models have recently been developed that also incorporate species interactions when estimating occurrence and detection probabilities (MacKenzie et al. 2004, Waddle et al. 2010, Miller et al. 2012).

These hierarchical interaction models may be particularly useful for species of lungless salamanders (Family Plethodontidae). Negative interactions among plethodontids include intraguild predation (e.g., spring salamander [*Gyrinophilus porphyriticus*], blackbelly salamander [*Desmognathus quadramaculatus*]) (Petranka 1998) and competition or agonistic behaviors. Both competition and predation may be mitigated by niche displacement (e.g., occupying different tributaries within a drainage [Means 1975, Camp et al. 2013]; segregation perpendicular to the stream edge [Hairston

1949a, 1986, Keen 1982, Rissler et al. 2004, Grover 2009]). These interactions, and their influence on species occurrence, may be more complex for biphasic plethodontids than for those that do not have an aquatic larval phase (Bruce 2008). Work within ephemeral wetlands containing newts (Family Salamandridae) and mole salamanders (Family Ambystomatidae) has demonstrated that the pressure from predation and competition occurring between and within species varies with the developmental stages that are involved (i.e., egg, larvae, adult) (Morin 1983). Intraguild predation has been documented among larval plethodontids, and, as in terrestrial interactions, size matters (Resetarits 1991, Gustafson 1993, 1994, Beachy 1993, 1994). Evidence for competition among larval plethodontid salamanders is less clear. Most larval plethodontids are generalists that consume a wide variety of invertebrates (Lannoo 2005, Wells 2010). There can be a great deal of overlap in the size, quantity, and type of prey consumed by different size classes of larvae (Petranka 1984). Some studies suggest that both predation and interference competition explain differences in survival and growth rates among subordinate species (Gustafson 1993, 1994). Others indicate that growth is not influenced by the presence of similarly sized conspecifics or heterospecifics, despite the unnaturally high densities used to test interaction hypotheses (Beachy 1994). As is the case with metamorphosed *Desmognathus,* larval plethodontids could reduce the effect of predation and or competition by occupying different microhabitats (e.g., discriminating by substrate size) or stream reaches, or by reducing their activity levels (Resetarits 1991, Gustafson 1994).

Brook salamanders (Genus *Eurycea*) are often the most frequently encountered plethodontids along many headwater streams in the Gulf Coastal Plain (see Chapter 1),

but we know very little about interactions among these species. Three or more species of *Eurycea* are syntopic in streams within this region, including the southern two-lined salamander (*E. cirrigera*), three-lined salamander (*E. guttolineata*), and the dwarf salamander (*E. quadridigitata* complex [Lamb and Beamer 2012]). Findings in Chapter 1 suggest that occupancy by these species in small headwater streams is affected by environmental gradients, but I also suspect that species interactions might have played a role in species occurrence. Unpublished abundance data from the previous study led to the suspicion that *E. cirrigera* might negatively impact *E. guttolineata* and or *E. quadridigitata*. These species pairs were detected together at ca. 30% and 12% of sites, respectively, and the number of larvae and recent metamorphs in leaf litter bags (Waldron et al. 2003) for *E. guttolineata* (mean = 1.65 per bag, range = 1 – 6) or *E. quadridigitata* (mean = 2.13 per bag, range = 1 – 11) were highest at sites where *E. cirrigera* were not detected.

Predation or competition among metamorphosed individuals of these species may be unlikely. Aggressive behaviors appear to vary widely within *Eurycea*. Species within the two-lined salamander complex may exhibit territoriality (e.g., northern two-lined salamander, *E. bislineata* [Grant 1955]), but more recent work suggests that these salamanders do not defend discrete territories and exhibit mate-guarding behaviors instead (e.g., dark-sided salamander, *Eurycea aquatica* [Deitloff et al. 2014]). *Eurycea cirrigera*, which is generally less robust than *E. aquatica*, has not exhibited either behavior in laboratory trials (Deitloff et al. 2014). Similarly, neither aggression nor interference competition has been observed among male *E. guttolineata* (Jaeger 1988),

and even less attention has been paid to negative interactions involving the *E. quadridigitata* complex (Bonett and Chippindale 2005).

If intraguild predation, aggression, and or competition occurs among *E. cirrigera*, *E. guttolineata,* and *E. quadridigitata*, it may be more likely to take place among larvae due to differences in the larval life histories of these species. Populations of *E. cirrigera* in South Mississippi likely have a larval period of 1 – 2 years (JYL pers. obs., Petranka 1984, Dundee and Rossman 1989). The larval period of *E. guttolineata* is often less than one year (Petranka [1984], but see Bruce [1982] for an exception), and larvae in the *E. quadridigitata* complex can metamorphose after less than 6 months (Petranka 1998). One consequence of this difference in the durations of the larval period is that individuals of *E. cirrigera* have the opportunity to grow to larger sizes than do either of the other two species. In 2012 and 2013, JYL measured a total of 1,789 larval *Eurycea* from across multiple sites in the Pascagoula River Drainage in South Mississippi. The maximum size observed for non-metamorphosing larval *E. cirrigera* (n = 1637; snout-to-vent length [SVL] = 35 mm) was much larger than that reached by either *E. guttolineata* (n = 67; SVL = 25 mm) or *E. quadridigitata* (n = 85; SVL = 20 mm). A second consequence of differences in larval life histories is that larger larval *E. cirrigera* (i.e., > 25 mm SVL) can, and do, occur in streams at the same time as do much smaller larvae of *E. guttolineata* and *E. quadridigitata*. For example, larval *E. cirrigera* measured 10 – 34 mm SVL (N = 185), *E. guttolineata* measured 13 – 25 mm SVL (N = 39), and larval *E. quadridigitata* measured 9 – 19 mm SVL (N = 45) (JYL unpubl. data) across sites in the Pascagoula River Drainage in May 2013. The size discrepancy between larval *E. cirrigera* and other *Eurycea* in streams in the Pascagoula River, as well as likely

elsewhere within the Gulf Coastal Plain, is comparatively as large as is that between larval plethodontids used in mesocosm studies that test for intraguild predation (Gustafson 1993, Beachy 1993). Although predation of smaller *Eurycea* by *E. cirrigera* has not been demonstrated in manipulative studies, larval *E. cirrigera* are able to consume smaller, larval mole salamanders (Genus *Ambystoma*) where they co-occur (Petranka 1984, Pauley and Watson 2005), and aggressive behavior has been documented among larvae of a related species (i.e., Blue Ridge two-lined salamander, *E. wilderae* [Wiltenmuth 1997]). Similarly, differences in size-class can result in non-lethal, negative interactions due to the threat of predation or as a consequence of agonistic behaviors (i.e., reduction in activity levels or avoidance by subordinates) (Rudolf 2006). Competitive interactions within or between size classes among larval *Eurycea* are feasible because all three species overlap to some degree in terms of their invertebrate prey items (Petranka 1984, Bonett and Chippindale 2005, Pauley and Watson 2005, Ryan and Douthitt 2005), and these larvae can be found in similar aquatic microhabitats within this region.

I used the single-season, multi-species, hierarchical Bayesian asymmetric interaction model described in Waddle et al. (2010) to ask whether environmental variables and negative species interactions affected the occurrence and detection of three species of brook salamanders in the Gulf Coastal Plain. Specifically, this model tested the hypothesis that *E. cirrigera* acts as the dominant species, and that its presence decreases the probabilities of occupancy and detection for both *E. guttolineata* and *E. quadridigitata*. The asymmetry of the model is reflected in that the reverse is not true for *E. cirrigera.* The results of models in Chapter 1 demonstrated that upstream drainage area influenced occupancy for *E. cirrigera* and that stream impermanence affected occupancy

probabilities across these species of *Eurycea*. Consequently, both of these significant environmental gradients were incorporated into this species interaction model

Methods

This interaction model uses a subset of the data originally analyzed with the hierarchical, Bayesian, multi-species model used in Chapter 1. The dataset to which the current model is applied includes the detection and non-detection data for three species of *Eurycea*, *E. cirrigera*, *E. guttolineata*, and *E. quadridigitata*, from across 60 sites in the Pascagoula River Drainage in South Mississippi. Each site was sampled three times during either Summer 2012 or 2013. This model also incorporates data describing covariates that the previous model indicated were biologically relevant, upstream drainage area ("DA") and stream impermanence ("Dry").

The structure of Waddle et al.'s (2010) model specifies an asymmetry between a dominant and one or more subordinate species. The occurrence (z), or occupancy state, of the subordinate species (Species A) at a site is determined by the occupancy state of the dominant species (Species B) at that site, but the reverse is not true. One example of this hypothetical relationship can be seen in predator-prey dynamics when the predator is a generalist. The presence of the predator may decrease the mean occurrence of a species of prey, but the mean occurrence of the predator is independent of the presence of that particular species of prey (Waddle et al. 2010).

Three parameters are used to model the interrelated occupancy states of subordinate Species A and dominant Species B (i.e., z^A and z^B):

1. the probability of occurrence of dominant Species B = $\Psi^B = Pr(z^B = 1)$,

2. the probability of occurrence of subordinate Species A, given the presence of Species B = $\Psi^{A|B} = Pr(z^A = 1 \mid z^B = 1)$,
3. and the probability of occurrence of subordinate Species A, in the absence of Species B, where a lowercase "b" is used to denote absence = $\Psi^{A|b} = Pr(z^A = 1 \mid z^B = 0)$.

The joint occupancy models for these species can be represented using the following Bernoulli (Bern) processes, which directly ties the occupancy state of Species A to that of Species B:

$$\text{(Dominant) Species B:} \quad z^B \mid \Psi^B \sim \text{Bern}(\Psi^B)$$

$$\text{(Subordinate) Species A:} \quad z^A \mid z^B, \Psi^{A|B}, \Psi^{A|b} \sim \text{Bern}(z^B * \Psi^{A|B} + [1 - z^B] * \Psi^{A|b})$$

Species observations in the field (y), also known as detection histories, are distributed Bernoulli and depend on the occupancy state of that species at that site, as well as on its probability of being detected (p). We use the following to model the detection history of Species B:

$$\text{(Dominant) Species B:} \quad y^B \mid z^B, p^B \sim \text{Bern}(z^B * p^B)$$

According to this equation, if Species B is truly absent (i.e., $z^B = 0$), then $y^B = 0$ with a probability of 1. If Species B is present (i.e., $z^B = 1$), then it is detected with a probability of p^B during each sampling occasion (Waddle et al. 2010). The asymmetry of the model dictates that this parameter, p^B, is not contingent upon the occupancy state of subordinate Species A.

Two different parameters are used to model the probability of detection of the subordinate Species A during a single observation at a site:

1. the probability of detecting Species A, given that both species are present = $p^{A|B}$ = $Pr(y^A = 1 \mid z^A = 1, z^B = 1)$,
2. and the probability of detecting Species A, given that dominant Species B is absent = $p^{A|b} = Pr(y^A = 1 \mid z^A = 1, z^B = 0)$.

It is possible to parameterize the Waddle et al. (2010) model such that the detection of the subordinate species is not contingent upon the occurrence of the dominant species. However, in this scenario, it is feasible that the presence of the hypothesized dominant species, *E. cirrigera*, could affect the detection of either of the subordinate species, *E. guttolineata* and or *E. quadridigitata*, by causing them to reduce their activity levels and or seek out different microhabitats (e.g., Resetarits 1991, Gustafson 1993). The detection history for Species A is modeled as follows:

(Subordinate) Species A: $y^A \mid z^A, z^B, p^{A|B}, p^{A|b} \sim Bern(z^A \{z^B * p^{A|B} + [1 - z^B] * p^{A|b}\})$

In this equation, if Species A is absent (i.e., $z^A = 0$), then $y^A = 0$ with a probability of 1. Alternatively, if Species A is present (i.e., $z^A = 1$), then it is detected with a probability of $p^{A|B}$ in the presence of Species B, and of $p^{A|b}$ in the absence of Species B (Waddle et al. 2010).

Each of the Ψ and p parameters can be modeled using environmental covariates, and the logit function can be used to link data describing these covariates to both parameters for each species. For this model, let i reference the sample location (i = 1, … 60), and *j* the sampling occasion or visit (j = 1, … 3). In this model, DA and Dry are used as covariates for occupancy for the hypothesized dominant Species B, *E. cirrigera*:

(Dominant) Species B (*Eurycea cirrigera*) $\text{logit}(\Psi_i^B) = \beta_0^B + \beta_1^B Dry_i + \beta_2^B DA_i$

Where β_0^B is the intercept, and β_1^B and β_2^B are effect parameters for Dry and DA, respectively. Data describing each covariate were centered to have a mean of 0 and then scaled in R.

I hypothesize that both of the subordinate species, *E. guttolineata* and *E. quadridigitata*, will have the same relationship with *E. cirrigera* (i.e., Ψ and p will both be affected by the occupancy state of *E. cirrigera*). The only environmental covariate applied to occupancy for each of the subordinate species is Dry:

For both subordinate species (*E. guttolineata* and *E. quadridigitata*)

$$\text{logit}(\Psi_i^A) = \beta_{0B}^A * z_i^B + \beta_{0b}^A * (1 - z_i^B) + \beta_1^A Dry_i$$

Where β_{0B}^A is the effect parameter for occurrence in the presence of the dominant species (*E. cirrigera*), β_{0b}^A is the effect parameter for occurrence of the subordinate species in the absence of the dominant species, and β_1^A is the effect parameter for Dry for the subordinate species.

No environmental covariates are used to model detection for dominant Species B, or for either subordinate species. This simplifies the detection model for *E. cirrigera*, which will not require the use of the logit function:

$$p_{ij}^B = z^B * p^B$$

This model maintains a constant probability of detection across sites and sampling occasions for *E. cirrigera.* This is a reasonable assumption given that the probability of detecting *E. cirrigera* was close to 1.00 in the modeling results from Chapter 1. Detection probabilities for the subordinate species were contingent upon the occupancy state of the dominant species, and were allowed to vary among sampling occasions. Essentially, the

occupancy state of the predator is treated somewhat like an environmental covariate, and the logit function is needed:

$$\text{logit}(p_{ij}^{A}) = \alpha_{0B}^{A} * z_i^{B} + \alpha_{0b}^{A} * (1 - z_i^{B})$$

One difference between the model presented here and that in Waddle et al. (2010) is that, due to the continuous nature of the environmental covariates of interest, I am unable to test for an interaction effect of the presence of the dominant species and either covariate on occupancy or detection. As a result, this model cannot distinguish finer ecological points such as whether the effect of the presence of *E. cirrigera* on occupancy by *E. guttolineata* and *E. quadridigitata* is magnified in more permanent sites. The same limitation is true for this model's estimates of detection.

I used Bayesian analysis with flat priors to estimate model parameters. Priors for overall occupancy and detection probabilities were distributed uniform from 0 to 1. Priors for the effects of environmental covariates for *E. cirrigera* were distributed normally with a mean of 0 and variance equaling 0.001. Those for the subordinate species were distributed normally with a mean of 0 and variance equaling 0.01. Due to the simplicity of the model, no method of variable or model selection was used. This species interaction model was fit using the Markov chain Monte Carlo (MCMC) method in winBUGS (ver 1.4.3) (Spiegelhalter et al. 2003). WinBUGS was called from R using the package R2WinBUGS (Sturtz et al. 2005). I used three parallel MCMC chains 30,000 in length with a burn-in length of 5,000 and a thinning rate of 10. Markov chain convergence was assessed using R-hat, a potential scale reduction factor (Gelman and Shirley 2011). I report the mean values and 95% Bayesian credible intervals (BCI) of the posterior distributions for the parameters of interest.

Results

Minimum occupancy, which is defined as the proportion of sampled sites at which the species was detected at least once, was 0.65, 0.58, and 0.27 for *Eurycea cirrigera*, *E. guttolineata,* and *E. quadridigitata,* respectively. All three species of brook salamander were detected at 4 sites, and only at 3 sites did I fail to detect any species of *Eurycea.* *Eurycea guttolineata* and *E. cirrigera* were detected together at 18 sites, whereas *E. guttolineata* was detected in the absence of *E. cirrigera* at 17 sites (Table 2.1). Similarly, *E. quadridigitata* and *E. cirrigera* were detected together at 7 sites, and *E. quadridigitata* was detected independently of *E. cirrigera* at 9 sites (Table 2.1). The mean SVL for larval *E. guttolineata* caught in litter bags was 19.60 ± 1.49 mm (N = 67; range = 12 – 25 mm), whereas that for larval *E. quadridigitata* was 15.33 ± 2.25 mm (N = 85; range = 9 – 20 mm). I define large larvae of *E. cirrigera* as those individuals that are greater than or equal to 25 mm SVL. These larvae are likely in their second year of growth and may have the greatest degree of overlap in streams containing other species of *Eurycea* in terms of phenology. This large size class of *E. cirrigera* was present in at least 74% of the 39 occupied sites during the summer months (N = 221; mean = 28.08 ± 2.65 mm SVL; range = 25 – 35 mm).

Unlike the multi-species model in Chapter 1, this species interaction model does not allow for "shrinkage," whereby data from one species informs the posterior probability estimates of other, ecologically similar species (Walls et al. 2011). Consequently, estimates between the two models are not numerically identical, but they are very similar. *Eurycea cirrigera* and *E. guttolineata* occupied ca. 65% and 63% of sites, respectively, whereas *E. quadridigitata* only occupied approximately 30.6% of sites

(Table 2.2). Average detection probabilities were all greater than 0.5, with *E. cirrigera* demonstrating a detection probability close to 1.0 (Table 2.3).

The results of this interaction model nearly mirror the multi-species model in terms of the relationships between each species and the environmental covariates DA and Dry (Table 2.4). *Eurycea cirrigera* is more likely to occur at sites with larger upstream drainage areas, and at sites that are more permanent. Alternatively, even when species interactions are used to model occurrence, both *E. guttolineata* and *E. quadridigitata* are still more likely to occupy sites in which a greater proportion of the stream dries during the summer months. The 95% BCI slightly overlaps zero for the effect of Dry on Ψ, but the interval has an overall strong, negative trend (Table 2.4). I suspect that this difference in the results between the current model and that in Chapter 1 is due to the smaller data set fit by the species interaction model, and that stream impermanence is still an important predictor of occupancy for *E. guttolineata*.

To test the null hypothesis that the occupancy state of *E. cirrigera* had no effect on that of either *E. guttolineata* or *E. quadridigitata*, I estimated the average conditional probability of occupancy for each subordinate species both in the presence ($\Psi^{A|B}$) and in the absence ($\Psi^{A|b}$) of the hypothesized dominant species. I then compared the distributions of these two conditional posterior probabilities (i.e., subtracting the distribution of $\Psi^{A|b}$ from that of $\Psi^{A|B}$) for each hypothesized subordinate species. The distribution of differences for *E. guttolineata* was negative and the 95% BCI did not overlap zero (Table 2.5). This modeling result indicates that the presences of *E. cirrigera* decreases the probability of occupancy by *E. guttolienata* across sites. The overall distribution of differences for *E. quadridigitata* was also negative, but the 95% BCI

overlapped zero (Table 2.5). The odds ratio describes the magnitude of the effect that the presence of the hypothesized dominant species has on the probability of occupancy of the hypothesized subordinate species. The odds ratio for *E. guttolineata* suggests that this species is 1.44 times more likely to occur in the absence of *E. cirrigera* than in its presence (Figure 2.1).

Discussion

The modeling results indicate that stream impermanence has a strong, positive effect on the occurrence of both *E. guttolineata* and *E. quadridigitata* even after I incorporate the co-occurrence of a hypothesized dominant species, *E. cirrigera*. *Eurycea guttolineata* and *E. quadridigitata* differ in their responses to the presence of *E. cirrigera*. Conditional occupancy probabilities for *E. guttolineata* were slightly, but significantly, larger when *E. cirrigera* was absent ($\Psi^{A|b} = 0.877$) compared to when it was present ($\Psi^{A|B} = 0.628$). Contrastingly, the effect of *E. cirrigera* on the occurrence of *E. quadridigitata*, though generally negative ($\Psi^{A|B} = 0.242$; $\Psi^{A|b} = 0.414$), was not significant. The narrow 95% BCI for the differences in the distributions of the conditional probabilities for both of the hypothesized subordinate species indicate that this interaction model had high precision. This fact, combined with the high average detection probabilities across species (range = 0.552 – 0.928), suggests that the modeling results are not biased (Waddle et al. 2010). Still, the results for *E. quadridigitata* should be interpreted with a degree of caution given the generally low number of sites at which *E. quadridigitata* was detected with *E. cirrigera* (N = 7). Future field studies should endeavor to incorporate a larger number of sites across the stream impermanence gradient.

Previous work demonstrates that larval plethodontid populations in small headwater streams are regulated by resource availability (Johnson and Wallace 2005, Bruce 2008), and mesocosm experiments suggest that intraguild predation also plays a role (Resetarits 1991, Gustafson 1993, 1994, Beachy 1994, 1997, Bruce 2008). Johnson and Wallace (2005) studied the effects of litter-exclusion on a population of *E. wilderae* in North Carolina and found that larvae in the exclusion treatment experienced reduced growth and exhibited overall lower densities and total biomass. They attributed these effects to changes in prey quality or larval activity (i.e., increased hatchling drift downstream due to lack of appropriate prey and or low cover availability). Small headwater streams are "bottom-up" systems in which productivity is driven by allochthonous inputs and their subsequent effects on the aquatic invertebrate community (Vannote et al. 1980). The high larval densities that can occur in these streams may result in competition within and among size-classes.

Determining whether competition, predation, or a combination of the two is responsible for the proposed relationship between *E. guttolineata* and *E. cirrigera* is beyond the scope of this model (Waddle et al. 2010). However, the size of the effect of *E. cirrigera* on the occurrence of *E. guttolineata* was relatively small (i.e., odds ratio = 1.44) compared to that for a known predator, the Cuban tree frog (*Osteopilus septentrionalis*), and two native species of tree frogs (i.e., green [*Hyla cinerea*] and squirrel [*H. squirella*] tree frogs; odds ratios of 9.0 and 15.7, respectively) (Waddle et al. 2010). Large odds ratios would reflect strong competition or predation. Therefore, I posit that the weak yet significant interaction identified by these modeling results more likely represents a low level of competition or aggression between *E. cirrigera* and *E. guttolineata*, rather than a

predator-prey relationship. The maximum number of larval *E. cirrigera* in a single litter bag (N = 29 larvae; mean SVL = 16.3 ± 5.3 mm; range = 11 – 32 mm SVL) was nearly five times that of the maximum number of *E. guttolineata* in any bag across sites (N = 6). Larval *E. cirrigera* and *E. guttolienata* were found together in a total of 77 litter bags across the 18 sites where these species were detected together (Table 2.1). Simple linear regression models tested in R suggested that the number of larval *E. cirrigera* in a litter bag did not have a statistically significant effect on the number of larval *E. guttolienata* within bags. That said, the relationship between the number of larvae detected in litter bags and larval densities in the stream has not been established, and this study was not designed to estimate or compare raw abundance data. The presence of *E. cirrigera* appears to have the strongest effect on the occurrence of *E. guttolineata* at sites where less than ¼ of the stream dried during the summer months (Figure 2.2), but more complex models are required to determine if there is an interaction between these co-variates. *Eurycea guttolineata* may preferentially inhabit streams that are prone to more severe drying in an effort to avoid the loss in fitness that may be associated with streams in which *E. cirrigera* occur and are abundant.

These modeling results propose that there is an asymmetric interaction between *E. cirrigera* and *E. guttolineata* wherein the former dominates the latter. Both mesocosm experiments and *in situ* removal or exclusion experiments (e.g., Johnson and Wallace 2005) in which species composition, density, and size-classes are manipulated across relevant environmental gradients should be used to thoroughly test this hypothesized relationship. Another potentially important variable to consider in future species interaction models involving plethodontids in the Gulf Coastal Plain is both predation by

and competition with native species of fish (Ennen et al. 2016), such as darters (Family Percidae), madtoms (Family Ictaluridae), sunfish and bass (Family Centrarchidae), and piscivorous minnows (Family Cyprinidae). Streams in the Gulf Coastal Plain are very rarely fishless, and representatives from each of these families have either been dip netted or removed from litter bags across many sites in this study. Differences in the gape-size of species across the river continuum (Vannote et al. 1980) may dictate whether the interaction with larval or metamorphosed plethodontids is predatory or competitive in nature.

Table 2.1

Minimum number of sites at which pairs of species of Eurycea were detected together as well as independent of congeners.

	E. cirrigera	*E. guttolineata*	*E. quadridigitata*
Eurycea cirrigera	18	14	3
Eurycea guttolineata	-	9	8
Eurycea quadridigitata	-	-	1

Note: Numbers along the diagonal represent the minimum number of sites at which a species was detected when no other *Eurycea* were detected. Numbers above the diagonal represent the minimum number of sites where only those two species were detected together. All three species of *Eurycea* were detected together at 4 sites. *Eurycea* were not detected at only three out of the total 60 sites.

Table 2.2

Estimates of average occupancy and 95% Bayesian credible intervals (BCI) for three species of Eurycea.

Species	Avg. Ψ (SD)	Lower 95% BCI	Upper 95% BCI
Southern two-lined salamander (*Eurycea cirrigera*)	0.646 (0.320)	0.001	1.00
Three-lined salamander (*Eurycea guttolineata*)	0.631 (0.218)	0.322	1.00
Dwarf salamander complex (*Eurycea quadridigitata*)	0.306 (0.205)	0.091	0.898

Note: Avg. Ψ is the occupancy probability across all potential sites. Standard deviations are given in parentheses.

Table 2.3

Estimated detection probabilities and 95% Bayesian credible intervals (BCI) for three species of Eurycea.

Species	p (SD)	Lower 95% BCI	Upper 95% BCI
Southern two-lined salamander (*Eurycea cirrigera*)	0.928 (0.024)	0.875	0.968
Three-lined salamander (*Eurycea guttolineata*)	0.552 (0.042)	0.502	0.656
Dwarf salamander complex (*Eurycea quadridigitata*)	0.579 (0.062)	0.503	0.715

Note: Standard deviations are given in parentheses.

Table 2.4

Estimates with 95% Bayesian credible intervals (BCI) of the logit-scale ß for the effect of upstream drainage area (ha) and stream impermanence (maximum proportion of the stream that dried) on the probability of occurrence (Ψ) across three species of Eurycea.

Species	ß DA	ß Dry
Two-lined salamander (*Eurycea cirrigera*)	2.687 (1.232– 4.599) *	-2.327 (-4.249 – -0.828) *
Three-lined salamander (*Eurycea guttolienata*)	NA	1.507 (-0.032 – 4.133)
Dwarf salamander complex (*Eurycea quadridigitata*)	NA	0.738 (0.027 – 1.911) *

Note: Drainage area is indicated with "DA," and impermanence with "Dry." DA was not included as a covariate for either *E. guttolineata* or *E. quadridigitata*. Lower and upper BCIs are given in parentheses; significant effects that do not overlap 0 are indicated with an asterisk.

Table 2.5

Effect of the presence of the hypothesized dominant species, E. cirrigera, on the probabilities of occupancy of each of the subordinate species.

Species	Mean $\Psi^{A\|B} - \Psi^{A\|b}$	Lower 95% CI	Upper 95% CI
Three-line salamander (*Eurycea guttolineata*)	-0.248	-0.498	-0.010
Dwarf salamander complex (*E. quadridigitata*)	-0.171	-0.456	0.097

Note: Bayesian 95% credible intervals (CI) are given. "Mean $\Psi^{A|B} - \Psi^{A|b}$" represented the distribution of differences between the conditional posterior probabilities for the subordinate species in the presence ($\Psi^{A|B}$) and in the absence ($\Psi^{A|B}$) of the hypothesized dominant species.

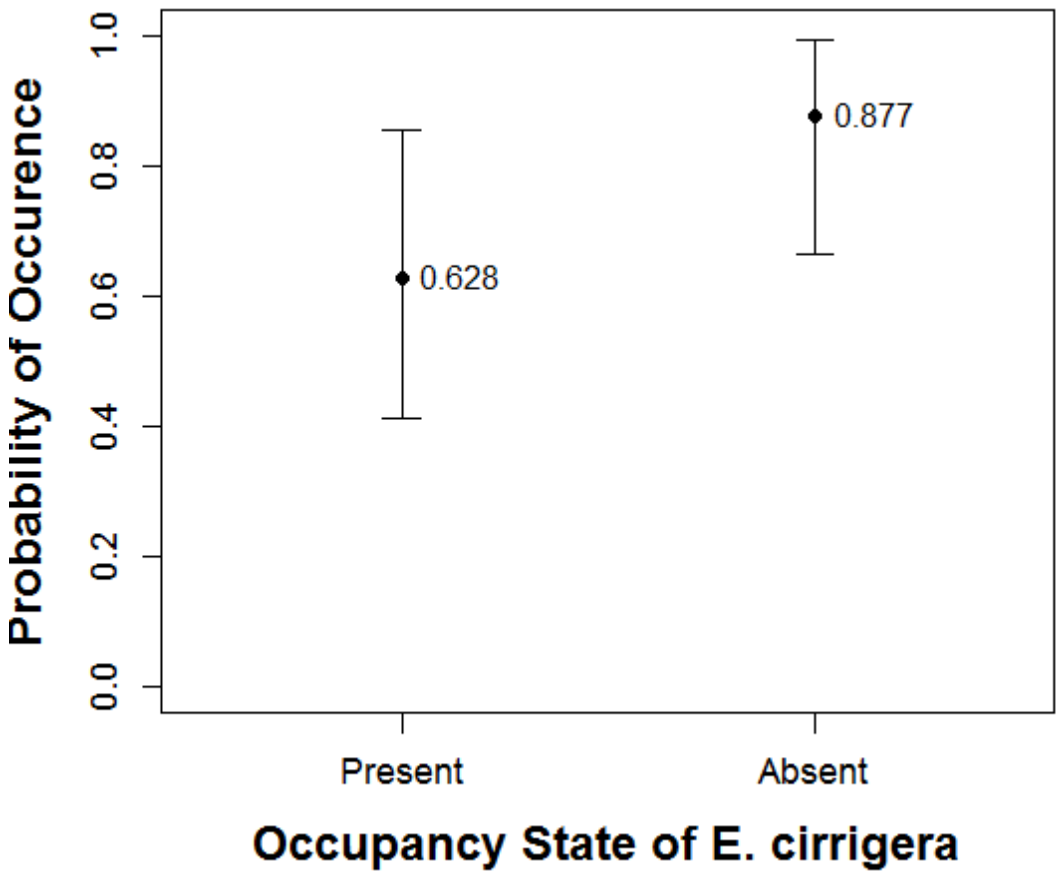

Figure 2.1. Magnitude of the difference in the posterior distributions of Ψ for *Eurycea guttolineata* in the presence and absence of the hypothesized dominant species, *E. cirrigera*.

Intervals represent 95% Bayesian Credible Intervals and mean values for the posterior probability distributions are depicted.

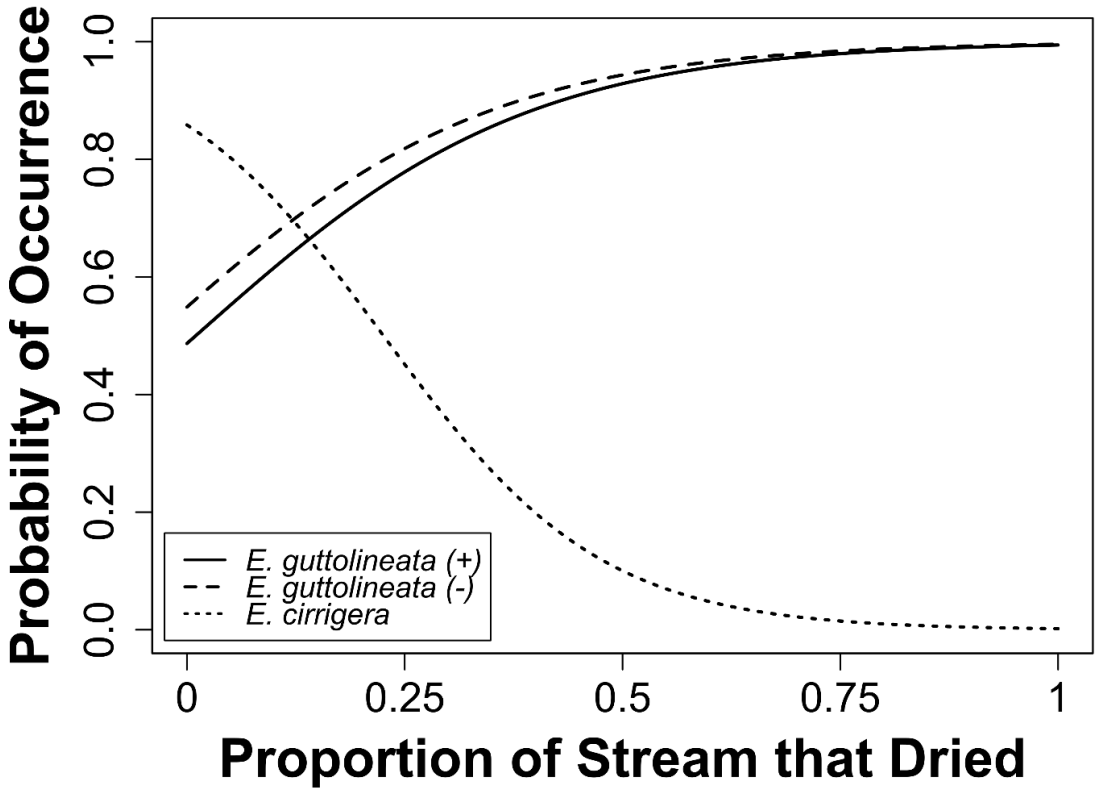

Figure 2.2. Estimated relationship between *E. cirrigera* and *E. guttolineata* and stream impermanence across the sites sampled.

The solid line plots the probability of occurrence of *E. guttolineata* when *E. cirrigera* is present and the wide-dashed line for when *E. cirrigera* is absent.

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CHAPTER III PHYLOGEOGRAPHY OF THE WIDE RANGING SPOTTED DUSKY SALAMANDER (*DESMOGNATHUS CONANTI*)

Abstract

Biodiversity, both in terms of the number of species as well as their genetic diversity, has been underappreciated in the Coastal Plain. This region has experienced a complicated history of fluctuating sea levels, which were responsible for the isolation of lineages as well as their subsequent dispersal. Previous work suggests that a wide-ranging species of plethodontid, the spotted dusky salamander (*Desmognathus conanti*), may contain several evolutionarily independent lineages. The current study provides increased geographic breadth and depth of sampling of mitochondrial sequence data across the Gulf Coastal Plain to examine the distribution of these lineages. I sequenced a 531 base pair portion of the cytochrome oxidase 1 gene from across multiple sites in the Gulf Coastal Plain. These novel sequences, combined with those provided by others, resulted in a total of 151 samples of *D. conanti* distributed across 59 sites in the southeastern U.S. I used these data in Bayesian and Maximum Likelihood phylogenetic analyses, statistical parsimony network analyses, as well as in analyses of molecular variance to determine the evolutionary relationships among mitochondrial clades and to make inferences regarding the underlying forces that may have shaped these lineages. I examined more recent geographic structure in the western portion of the range of *D. conanti* by genotyping a total of 291 individuals from 13 sites at six microsatellite loci. I applied a hierarchical Bayesian clustering approach to determine whether current genetic structure reflected historic divisions, as well as what factors might be affecting ongoing gene flow within *D. conanti*. The results of this study indicate that deeply divergent mitochondrial

clades were initially isolated by sea level fluctuations during the Miocene and Pliocene and that further substructure may have resulted from similar vicariance events during the Pleistocene. The microsatellite data identify three genetic groups within *D. conanti*, a northern population that corresponds with *D. conanti* (*sensu stricto*) and two southern populations that are the product of more recent gene flow across historic mitochondrial lineages, all of which have been influenced to some degree by modern drainage structure. This work further emphasizes the importance of applying multiple molecular markers in phylogeographic studies.

Introduction

Amphibian declines have been recorded across the globe (Stuart et al. 2004, Wake and Vredenburg 2008) and species within the most diverse family of salamanders, Family Plethodontidae, are among those that have been affected (Highton 2005, Rovito et al. 2009, Graham et al. 2010, Maerz et al. 2015). Disease, climate change, and habitat degradation have each been implicated in amphibian declines (Wake and Vredenburg 2008), and new global threats, such as the recently described chytrid fungus *Batrachochytrium salamandrivorans* (Martel et al. 2013, 2014), will likely continue to surface. Occupancy and detectability modeling will be useful in documenting and describing natural and aberrant fluctuations in amphibian populations (MacKenzie et al. 2002, Mazerolle et al. 2007), but the long-term survival of amphibian species, and therefore the preservation of regional biodiversity, will also depend on our understanding of historic and current patterns of population connectivity (i.e., gene flow) and genetic diversity across the landscape (Semlitsch 2002, Avise 2004, Beebee 2005). It will be impossible to comprehend the full extent and impact of amphibian declines without

knowing exactly what we may be losing. Phylogeographic studies can uncover lineages within a species that, due to their genetic and or ecological divergence, may be sufficiently unique as to warrant independent management and conservation considerations. Though species delimitation is not necessarily an intent of this field of study, it can often be a consequence, particularly in the case of cryptic species, which are not an uncommon phenomenon among plethodontids (Highton 2000, Bernardo 2011).

The southeastern United States is known for having high biodiversity across many taxa found on the North American continent (e.g., inland freshwater fishes [Matamoros et al. 2015]; caudates [Wake and Vredenburg 2008]; woody flora [Kirkman et al. 2007]). However, the biodiversity and endemism of terrestrial and freshwater taxa within a major physiographic province in this region, the North American Coastal Plain, has historically been underappreciated (Noss et al. 2015), particularly when considering the Gulf Coastal Plain (GCP) (Lydeard and Mayden 1995). The flora and fauna of the GCP have been shaped by an interesting geologic history. The GCP has never been glaciated therefore populations have had more time to accrue genetic differences. Large fluctuations in sea level caused by glacial cycles from the Miocene through the Pleistocene created barriers, and, in an alternating fashion, potential routes of dispersal across and or between major rivers (Saucier 1994, Soltis et al. 2006, Noss et al. 2015). For some taxa in the GCP, Glacial minima lead to the formation of marine embayments which resulted in speciation on either side of river drainages (e.g., flatwoods salamander complex, *Ambystoma cingulatum* and *A. bishopi* [Pauly et al. 2007]). In other cases, isolation and diversification occurred among freshwater taxa that were restricted to individual drainages by suitable habitats, which were receding upstream in the face of encroaching

brackish waters (e.g., map and sawback turtles, Genus *Graptemys* [Lindeman and Rhodin 2013]). During sea level minima, large rivers that may currently be impassible to headwater or terrestrial taxa were entrenched, which could have facilitated dispersal into adjoining systems further downstream (e.g., *Etheostoma caeruleum* [Ray et al. 2006]; Swift et al. 1986, Saucier 1994) or across river boundaries. Relatively few studies emphasize the phylogeography of species of plethodontids within the GCP, but those that have suggest that historic (Kozak et al. 2006, Herman and Bouzat 2016, Folt et al. 2016) as well as modern river boundaries (Herman and Bouzat 2016) have shaped genetic lineages.

The distribution of the spotted dusky salamander (*Desmognathus conanti*) spans major physiographic features that act as genetic breaks within and between other taxa. The range of this species is expansive compared to that of close relatives in the Appalachians (e.g., Santeetlah dusky salamander [*Desmognathus santeetlah*]) and the Gulf Coastal Plain (e.g., Apalachicola dusky salamander [*D. apalachicolae*]) (see Lannoo [2005] for range maps), and the identity and monophyly of *D. conanti* have been questioned by multiple authors (Karlin and Guttman 1986, Bonett 2002, Kozak et al. 2005, Beamer and Lamb 2008). *Desmognathus conanti* was first described by Rossman (1958) as a subspecies of the northern dusky salamander (*D. fuscus*) based on populations from Illinois and western Kentucky. Endeavors to disentangle the phylogenetic relationships among desmognathines have to this point involved allozyme studies (Karlin and Guttman 1986, Bonett 2002) and mitochondrial DNA sequencing (Kozak et al. 2005, Beamer and Lamb 2008). These works highlight three important areas containing different lineages of *D. conanti*, including the Lower Tennessee River Drainage, rivers

draining the GCP, and those in the Atlantic Coastal Plain (ACP) (Karlin and Guttman 1986, Bonett 2002, Kozak et al. 2005, Beamer and Lamb 2008). Most of these studies have sampled infrequently in the GCP or in rivers that are part of the Lower Mississippi River Valley. Consequently, the geographic extent of each lineage within *D. conanti* is poorly defined.

I used a finer scale sampling approach, both in terms of the number of sites and the number of individuals sampled, and two types of molecular markers, mitochondrial sequence data and six microsatellite loci (Lamb et al. 2015), to ascertain whether there was substantial genetic structure among populations of *D. conanti* (sensu lato; SL) in the Gulf Coastal Plain. Microsatellites are neutral and highly variable short tandem repeats of often two to four base pairs within the nuclear genome, and they have a wide variety of applications in ecology and conservation genetics (Selkoe and Toonen 2006). The different rates of mutation and modes of inheritance of these two types of molecular markers (i.e., uni- versus biparental) allowed me to account for historic or ongoing gene flow among lineages. My goals were to 1) describe the distribution of and evolutionary relationships among historic lineages, 2) identify the likely factors responsible for shaping these lineages, 3) determine whether the same genetic structure was consistent across datasets, and 4) identify more recent barriers to gene flow between populations.

Methods

Study species and sampling

Populations attributed to *Desmognathus conanti* (SL) can be found throughout the majority of Louisiana, Mississippi, Alabama, and Tennessee, in the western panhandle of Florida, the northern half of Georgia, and in parts of Arkansas and South Carolina

(Means and Bonett 2005). This is a semi-aquatic salamander that is frequently associated with seepage habitat, be it along ravine type, low order streams (Strahler 1964) (Valentine 1963, Means 2000, 2005, Jensen et al. 2008, Graham et al. 2010) or in swampy bottomlands and floodplain pools (pers. obs.; Means 1974, Jensen et al. 2008). Although neither adults nor larvae likely make long distance movements, some in-stream and limited over-land dispersal (e.g., between interlacing headwater streams) is possible (Grant et al. 2009, Miller et al. 2015). Dispersal through or along floodplains between streams may also be feasible, though there may be agonistic interactions with lowland desmognathines (e.g., Means 1974).

Between July 2011 and January 2016, I collected tail-tip tissue and a limited number of vouchers from populations of *Desmognathus conanti* (SL) from across multiple drainages in Louisiana and Mississippi. I attempted to collect tissue from at minimum three to five individuals per site. I also undertook more thorough sampling and repeat visits to select sites in an effort to collect a sufficient number of samples for microsatellite genotyping. Though not the focus of this study, I collected tissue from populations of the southern dusky salamander (*D.* cf. *auriculatus*, see discussion in Beamer and Lamb [2008]) to include in phylogenetic analyses. Salamanders were primarily caught by hand, but I also used dipnetting and leaf litter bags (Waldron et al. 2003). The data that I collected were supplemented with mitochondrial sequences from published (Beamer and Lamb [2008] via GenBank) and unpublished sources. Don Shepard (Central Arkansas University), and Joseph Bernardo, Tony Hibbitts, and Gary Voelker (Texas A&M University; Hibbitts et al. 2015) graciously shared sequences for multiple species of *Desmognathus*. I also obtained tissue samples from the Louisiana

State University Museum of Natural Science Collection of Genetic Resources, the Mississippi Museum of Natural Science, and D. B. Means (Coastal Plains Institute) (Table 3.1). Sequences for *D. conanti* (SL) came from a total of 59 sites (Figure 3.1). Geographic coordinates were approximated using county data for those donated sequences that lacked more specific locality information.

Molecular methods

I extracted genomic DNA from tail tip tissue using the Blood & Tissue DNEasy* Kit (Qiagen Group, Valencia, CA). I used the polymerase chain reaction (PCR) and primers published by Beamer and Lamb (2008) (forward 5' CGGCCACTTTACCYRTGATAATYACTCG 3'; reverse 5' GTATTAAGATTTCGGTCTGTTAGAAGTAT 3') to amplify a ca. 550 base pair segment of the mitochondrial gene cytochrome oxidase 1 (*cox1*) from a subset of samples. PCRs were performed with a total volume of 25 µL containing 0.5 µL template DNA, 0.1 µL *Taq* polymerase, 0.75 µL of each primer, 2 µL each of 25 mM magnesium chloride and 200 µM dNTPs, 2.5 µL of NEB buffer, and 16.4 µL of nuclease free water. Amplification was performed as follows: 1 cycle at 95˚C for 1 min.; 30 cycles of 95˚C, 50˚C, and then 72˚C for 1 min. each; 1 cycle at 72˚C for 3 min. Amplified *cox1* DNA was cleaned by adding 0.25 microliters each of Shrimp Alkaline Phosphatase and Exonuclease 1 (USB ®), heating samples to 37 ˚C for 15 min., and finally to 85˚C for 15 min. to denature the enzymes. Cleaned samples were sent to Eurofins Scientific © for sequencing. Using these methods, I obtained sequences for between 1 and 9 individuals per locality. The total number of individuals per species that I sequenced for this study included 121 individuals of *D. conanti* (SL), 16 individuals of *D.* cf. *auriculatus*, 3

individuals of *D. apalachicolae,* and 3 individuals of *D. auriculatus* (*sensu stricto;* SS). The final *cox1* dataset, including sequences borrowed from other sources, contains a total of 151 samples of *D. conanti* (SL), as well as 96 sequences from 15 other described species of *Desmognathus* (Table 3.1; Beamer and Lamb [2008]).

For the microsatellite dataset, I genotyped five to 73 individuals from across 13 localities (Figure 3.2) for six polymorphic loci, resulting in a total of 291 samples. The six loci used, *Dcon05*, *Dcon12*, *Dcon14*, *Dcon21*, *Dcon36*, and *Dcon40*, were originally characterized in Lamb et al. (2015). PCRs were performed and allele sizes were scored as described in Lamb et al. (2015), but published conditions did not consistently amplify across all individuals or populations. Increasing the concentration of $MgCl_2$ to 3 mM and the amount of DNA template used, and/or decreasing the annealing temperature to 54˚C, resolved most of these issues.

Mitochondrial DNA data analyses

I edited, aligned, and checked sequences for stop codons in Sequencher ™ ver. 5.1 and used the program TCS 1.21 (Clement et al. 2000) to identify identical *cox1* haplotypes. To depict the distribution of genetic diversity across the landscape, I calculated haplotype and nucleotide diversity in Arlequin ver. 3.5.2.2 (Excoffier and Lischer 2010) using all *D. conanti* (SL) sequences and partitioning by major river drainages. Some drainages contained only one site or few sites with limited sample sizes. Where this was the case I combined sites and drainages in to regionally appropriate groups (e.g., Lower MS River Drainages group, East of Mobile group).

The dataset of unique *cox1* haplotypes was used to construct maximum-likelihood and Bayesian phylogenies. Maximum-likelihood (ML) analysis was performed

in MEGA 6.06 (Tamura et al. 2013) and the appropriate evolutionary model for the unpartitioned dataset was determined in jModelTest 2.1.7 (Darriba et al. 2012) using Akaike's Information Criterion (AIC) (Akaike 1973). I constructed the ML phylogeny using an initial neighbor joining/Bio NJ tree and an heuristic, subtree-pruning-regrafting search method. Branch support values were estimated using 1000 bootstrap replicates. To complete a Bayesian analysis, I used MrBayes 3.2.5 (Ronquist et al. 2012) and partitioned the dataset according to codon position. I used Mesquite (Maddison and Maddison 2015) and jModelTest with AIC to determine the appropriate evolutionary model for each nucleotide position within codons. Two, independent Monte Carlo Markov Chain (MCMC) analyses were run in MrBayes using four simultaneous chains with a length of 5,000,000 generations and sampling frequency of every 100 generations. I used split standard deviation values to determine whether convergence had occurred (<0.01), and discarded all sampled trees prior to convergence. Posterior probability support values were calculated for post burn-in topologies in MrBayes using the sump and sumt commands. The analysis resulted in a 50% consensus tree, which was viewed in the program FigTree (Rambaut and Drummond 2009). *Desmognathus aeneus* was used to root the tree in both analyses (Titus and Larson 1996, Rissler and Taylor 2003). I used MEGA to calculate the net average pairwise distances (p-distances) between well-supported clades (posterior probabilities ≥ 0.95) and subclades (10,000 bootstrap replications), as well as a general poikilothermic mitochondrial DNA clock (i.e., 0.5 – 1.3% sequence divergence per million years [Ma]) (Hardy et al. 2002) to coarsely estimate divergence times. Haplotype and nucleotide diversities for each clade and subclade were calculated using Arlequin.

I used TCS 1.21 (Clement et al. 2002) to construct statistical parsimony networks using haplotypes of *D. conanti* (SL) from sites within the region in which my sampling was the most thorough (i.e., Lower Tennessee River, Lower Mississippi River Valley, and Gulf Coast drainages). Bifurcating phylogenies may not be capable of resolving relationships among recently diverged intra- or interspecific lineages due to multifurcation or hybridization (Posada and Crandall 2001). Network analyses may be a more appropriate choice when attempting to determine relationships under these circumstances. Statistical parsimony networks determine the maximum number of mutational steps that can occur between haplotypes before the probability of multiple substitutions at a given site is greater than 5% (Templeton et al. 1992, Clement et al. 2000, Chen et al. 2010). The 95% parsimony criterion has been proposed as a metric that can be used to identify unique species by grouping haplotypes in to unlinked networks and thus it may be useful in delineating candidate or cryptic species (e.g., Hart and Sunday 2007, Chen et al. 2010, Young et al. 2013). Others warn that the 95% parsimony criterion may identify diverging intraspecific genetic lineages, but that isolated networks would persist at lower parsimony criteria (e.g., 90%) if a unique species status was warranted (Centeno-Cuadros et al. 2009). I visualized networks identified by analyses in TCS 1.21 in PopART (Leigh 2016).

To determine what physiographic features may have shaped genetic lineages within *D. conanti* (SL), I conducted an analysis of molecular variance (hereafter AMOVA) (Excoffier et al. 1992) as implemented in Arlequin. I tested six models based on *a priori* hypotheses regarding potentially biologically relevant barriers to movement. Pairwise distances were used, and the significance of each model was determined using

1,000 permutations in Arlequin. Two models placed populations of *D. conanti* (SL) in to two groups (i.e., K=2), one that uses the Mississippi River as a partition (Model 1; Soltis et al. 2006) and another that groups populations into two provinces defined by their freshwater fish assemblages (i.e., Central Gulf Coastal Plains and the Atlantic-Floridian provinces as outlined in Matamoros et al. [2015]) (Model 2). These fish faunal provinces primarily correspond with GCP and ACP drainages, respectively, except that the Atlantic-Floridian province extends to the eastern boundary of the Mobile River basin, thus testing one variation on the east-west discontinuity often seen among taxa in the southeastern USA (Soltis et al. 2006, Matamoros et al. 2015). Model 3 uses the Mississippi River and Eastern Continental Divide to partition populations of *D. conanti* (SL) in to three groups. The Eastern Continental Divide has contributed to genetic structure within other species of *Desmognathus* (e.g., *Desmognathus marmoratus* [Voss et al. 1995, Jones 2006]).

Models 4 and 5 further subdivide localities by partitioning them according to drainage structure, the former creating six groups delineated by the hydrologic units proposed by Seaber et al. (1987). Model 5 organizes populations in to 15 groups that generally correspond with modern river drainages, though some drainages are pooled because they contained fewer samples per site. The river groups for Model 5 are as follows: ACP drainages (i.e., Altamaha and Savannah Rivers), Neches, Sabine, Red, Ouachita, Big Black, Yazoo, Lower Mississippi River drainages (i.e., Homochitto River and smaller drainages feeding in to the Lower Mississippi), Pontchartrain drainages (i.e., Amite and Tangipahoa Rivers), Pearl, Pascagoula, Mobile, GCP rivers east of the Mobile (e.g., Escambia, Yellow, and Choctawhatchee Rivers), Lower Tennessee River drainages

(e.g., Bear Creek and Pickwick Lake), and the Upper Tennessee River. I also tested partitions that primarily corresponded with the U.S. Environmental Protection Agency/U.S. Geological Survey level three ecoregions (i.e., Southcentral Plains, Mississippi Valley Loess Plains, and Southeastern Plains; [U.S. E.P.A. 2003]) but chose to pool samples north of the Fall Line due to fewer total samples in those areas (Model 6, K=4).

I also completed a spatial analysis of molecular variance (SAMOVA) wherein group structure was not predefined as it is by the *a priori* AMOVA models tested in Arlequin. In a SAMOVA, the number of groups tested is determined by the user (i.e., K=1 …. N) and the program draws from both genetic and coordinate datasets to create geographically homogenous and maximally differentiated groups (i.e., maximizing the amount of genetic variance explained by groups; Φ_{CT}) at each value of K. I tested values of K from 1 to 18 in the program SAMOVA 2.0 (Dupanloup et al. 2002) with a pairwise distance matrix, 100 simulated annealing processes, and 20,000 permutations.

Microsatellite data analyses

I used the program Arlequin to calculate the average observed (H_O) and expected heterozygosities (H_E), and to determine whether the assumptions of Hardy-Weinberg equilibrium (HWE) and Linkage Disequilibrium (LD) were met for each locus within each population. Analyses for HWE used Markov chains 1,000,000 steps in length with burn-ins of 100,000 steps, and LD was assessed with 10,000 permutations of the dataset. I used the program ML-NullFreq (Kalinowski and Taper 2006) to check for the presence of null alleles using 10,000 randomizations. Population pairwise genetic differentiation (pairwise F_{ST}; 1,000 permutations) and global F-statistics (10,000 permutations) across

five loci and populations were calculated in Arlequin, as were inbreeding coefficients (F_{IS}) for each population (10,000 permutations). The significance of p-values was determined after adjusting α for multiple comparisons using the Bonferroni method wherever applicable.

The program STRUCTURE uses Bayesian inference and MCMC methods to cluster individuals in to discrete genetic populations that are in linkage equilibrium and HWE (Pritchard et al. 2000). When sites are sampled unevenly (i.e., different numbers of individuals), or when suspected hierarchical groups are not equally represented by the sampling distribution, STRUCTURE and the associated ad-hoc evaluators (e.g., ΔK [Evanno et al. 2005]) may incorrectly determine the number of genetic groups (K) (Puechmaille 2016). To address this potential issue, I completed analyses in STRUCTURE using two versions of the dataset, one using the full dataset (291 samples) and another using a subsample of the data. In the subsampled dataset (199 samples), I randomly excluded individuals from sites with larger sample sizes until the maximum number of individuals at any site was 20 (Puechmaille 2016). Both datasets were analyzed in STRUCTURE ver. 2.3.4 with a burn-in period of 50,000 and a sampling period of 100,000. Individuals were allowed to have mixed ancestry and sampling location was used to inform the prior distribution (Hubisz et al. 2009). I tested values of K from 1 to 16 with 20 iterations of each value and examined the mean log-likelihood and ΔK scores (Evanno et al. 2005) for each value to determine the appropriate K, as well as whether any hierarchical grouping of populations was evident (Pritchard et al. 2000). STRUCTURE HARVESTER ver. 0.6.94 was used to summarize the results of the STRUCTURE runs and calculate ΔK (Earl and vonHoldt 2012). CLUMPP 1.1.2

(Jakobsson and Rosenberg 2007) was used to align and average replicates for relevant values of K and then these results were visualized in DISTRUCT 1.1 (Rosenberg 2004).

Isolation by distance (IBD) is a phenomenon wherein differences in the allele frequencies between populations are correlated with geographic distance, and has been observed among plethodontids at much smaller distance intervals than those that occur in this study (Cabe et al. 2007, Miller et al. 2015). To test for IBD, I performed a simple Mantel test (Legendre and Legendre 1998) and permuted a linearized (Rousset 1997) pairwise-population F_{ST} matrix, the original matrix having been completed in Arlequin, against a geographic distance matrix in kilometers (km) using 10,000 permutations. I visually compared differences in allele frequencies among populations with a principal coordinates analysis using the population-pairwise F_{ST} matrix. These analyses were completed in R ver. 3.2.3 (R Core Team 2014) using the packages vegan (Oksanen et al. 2016) and fossil (Vavrek 2011). All plots were created in SigmaPlot ver. 12.5.

Results

Mitochondrial lineages

Alignment and editing resulted in a final sequence length of 531 base pairs, with a total of 198 parsimony informative sites. I did not detect any stop codons within the open reading frame of these sequences. This dataset included 78 unique sequences (i.e. haplotypes) among the 151 sequences belonging to *D. conanti* (SL). An average of 3 haplotypes were detected per site, but this number ranged from 1 to 9 (maximum at Site #67 Ward Bayou, Pascagoula River Drainage). When the dataset was partitioned by major river drainages, haplotype diversity, defined as the number of haplotypes divided by the total number of sequenced individuals, averaged 0.65 ± 0.25 standard deviations

(SD) (range = 0.25 – 1). The average nucleotide diversity across all drainages was 0.017 ± 0.017 SD (range = 0.0009 – 0.034) (Table 3.2). The Red, Pearl, and Mobile River Drainages had high haplotype and nucleotide diversities. Although haplotype diversity in the Pascagoula River Drainage (0.49) was noticeably lower than in the aforementioned Red and GCP Drainages, nucleotide diversity was comparatively high (0.026 ± 0.013 SD) in part due to the presence of the distinct "Dark Ward" haplotype (haplotype label "conanti_Pasca_1"; Table 3.1) at the Ward Bayou site (Site #67; Figure 3.1). Within the Pascagoula River, both Ward Bayou and Black Creek exhibited high haplotype diversities, but haplotypes were more similar among sites in the latter than in the former. Ward Bayou was only represented by one site, but it had the second highest nucleotide diversity (0.038 ± 0.021 SD) of any tributary or major drainage across the dataset (Table 3.2).

The ML (log likelihood = -6933.57) and Bayesian (25,251 post-burn-in trees sampled; marginal likelihood = -7061.33) phylogenies recovered many of the same, well-supported clades (i.e., bootstrap support values ≥ 85, Bayesian posterior probabilities [BPP] ≥ 0.95). The topologies of these trees were also similar in that there were multiple polytomies, even at deeper nodes. For these reasons I have chosen to focus on the Bayesian 50% consensus phylogeny (Figure 3.3). The Bayesian analysis presented here recovered many of the same clades as did Beamer and Lamb (2008). Neither their study, nor that of Hibbitts et al. (2015) included haplotypes belonging to populations of *D.* cf. *auriculatus* from West of the Mobile Drainage. These populations from Mississippi and Louisiana formed a reciprocally monophyletic clade within a much larger clade containing multiple other species of *Desmognathus* but excluding the topotypic clade for

D. auriculatus (SS) (Figure 3.3). This study did not recover a strong clade that contained all *D. conanti* (SL) haplotypes and *D. santeetlah* (Kozak et al. 2005, Beamer and Lamb 2008, Hibbitts et al. 2015). Instead, the most inclusive group that contained the greatest number of *D. conanti* (SL) haplotypes had only moderate Bayesian support (BPP = 0.92) and poor ML support (bootstrap value = 26). Removing haplotypes unique to this study (e.g., *D.* cf. *auriculatus* from MS and LA, Dark Ward; Table 3.1) resulted in the recovery of a clade containing *D. conanti* (SL) and *D. santeetlah*.

Significant genetic structure was apparent within *D. conanti* (SL) in this study. Analyses identified 6 major clades with BPPs ≥ 0.95, four of which occurred in GCP, Lower Tennessee River, and Lower Mississippi River Drainages, and two of which occurred in the ACP (Figure 3.3 and Figure 3.4). Although the relationships among these major clades of *D. conanti* (SL) remain uncertain due to polytomies within the tree, the geographic distributions of these clades nevertheless demonstrate some of the same patterns found in previous studies. Lower Tennessee River sites were genetically distinct from those in the Upper Tennessee River (Bonett 2002) as well as from those in the ACP (Kozak et al. 2005, Beamer and Lamb 2008). The most widely distributed clade in this study ranged across the Lower Tennessee River, parts of the Lower Mississippi River Valley, and into multiple GCP Drainages (Karlin and Guttman 1986, Beamer and Lamb 2008) (here the Eastern clade). Other sites further South in the Lower Mississippi River Valley and Pontchartrain Drainages, as well as a single site on the western edge of the Pearl River Drainage (i.e., Site #52 in the Bogue Lusa Creek; Figure 3.1) formed a distinct clade (Kozak et al. 2005, Beamer and Lamb 2008) (here the Central clade) (Figure 3.3 and Figure 3.4).

Evolutionary divergence estimates (i.e., net average p-distances) between Bayesian clades of *D. conanti* (SL) averaged 6.19% and ranged between 3.5 – 9.90% (Table 3.3A). The net average p-distance subtracts the mean within group genetic distance from the average between group distances, thereby providing a conservative estimate for evolutionary divergence compared to uncorrected p-distances. The p-distances reported herein between clades of *D. conanti* (SL) are considerably larger than are those between sister species for many vertebrates (e.g., 1 – 3% [Avise and Walker 1999]), and many values are comparable to those between some species of *Desmognathus.* Uncorrected p-distances between two sister species of dusky salamanders, the Blue Ridge (*D. orestes*) and the Allegheny Mountain (*D. ochrophaeus*) duskies, averaged 6.22% (Tilley et al. 2008), and those for the closely related dwarf black-bellied (*D. folkertsi*) and black-bellied dusky salamanders (*D. quadramaculatus*) averaged 4.29% (Wooten et al. 2010).

Further substructure was also apparent within many of the major clades of *D. conanti* (SL) (Figure 3.3) observed in this study. Within-clade p-distances averaged 2.83% and ranged between 1.90% (Central) and 4.10% (South Central 2) for major clades (Table 3.3A). When haplotypes were organized and compared according to subclades the average within-clade p-distance was 2.02% and ranged from 0.30% to 4.10% (Table 3.3B). The Central clade contained two subclades, Central 1 and Central 2 (Figure 3.3 and Figure 3.4), with BPPs $\geq$ 0.95 and average p-distances of 2.00% (Table 3.3B). Interestingly, the Central 1 subclade exhibited the lowest haplotype and nucleotide diversities (Table 3.4), which may be indicative of a more recent expansion in to the smaller tributaries feeding the Lower Mississippi River Drainage. The Eastern clade,

which had the second highest overall nucleotide diversity among the major clades (0.028 ± 0.014 SD; Table 3.4), contained three well-supported subclades, Northern, Upper, and Lower (moving from north to south) (Figure 3.3 and Figure 3.4), with between-clade p-distances that averaged 2.13% (Table 3.3B). The Northern subclade contains a haplotype from Beamer and Lamb (2008) collected from western Kentucky, near to the topotype locality for *D. conanti* (SS) (Rossman 1958). This subclade, and thus *D. conanti* (SS), penetrates at least as far in to the GCP as the Lower Yazoo, Lower Big Black, and upper Tombigbee Rivers (Figure 3.4). Despite the wide range covered by both the Northern and Lower subclades, haplotypes within each were more similar to one another than were haplotypes within the South Central 2 clade (nucleotide diversity = 0.025 ± 0.016 SD [Table 3.4]; intra-clade p-distances = 4.10% [Table 3.3A]). South Central 2 included two localities, one in the Red River (Site #3) and one in the Ouachita (Site #5), and I suspect that there is further structure within this lineage that my limited sampling in that area was unable to capture.

River drainages exhibiting particularly high nucleotide diversities (Table 3.2) contained multiple major clades and or subclades (e.g., Red, Pearl, Pascagoula, and Mobile Rivers) (Figure 3.4). Although there are some drainages in which clades overlapped, I only detected more than one lineage at two sites, Ward Bayou (Site #67; Dark Ward and Lower subclade) and the second in the Noxubee River (Site #48; Upper and Lower subclades) (Figure 3.3 and Figure 3.4). Many other *D. conanti* (SL) were sampled at Ward Bayou, but only one of the nine sequenced samples, and none of the other 45 individuals screened via restriction fragment length polymorphisms, exhibited the Dark Ward haplotype (unpublished data). The Noxubee River site marks the point

where the spring-fed, headwater origins for two separate tributaries leading in to the Noxubee, Panther Creek to the West and Jones Creek to the East, are separated by a gravel road along the hilltop (Figure 3.4). A total of five individuals were sequenced from this site, two from Panther Creek and three from Jones Creek. Individuals on either side of the road belonged to separate subclades, those from Panther Creek to the Lower subclade, and those from Jones Creek to the Upper subclade (Figure 3.3 and Figure 3.4). Although there were noticeable microhabitat differences on either side of the road these differences were not consistent across sites within each subclade.

The same clades and subclades with strong support in Bayesian and ML analyses were also borne out in the network analyses but the polytomies present in the Bayesian and ML trees were not resolved. TCS 1.21 identified a total of 13 networks differentiated by ≥ 10 mutational steps in the GCP when the 95% statistical parsimony probability criteria (SPP) was used. At this SPP the analysis was dividing Bayesian clades into networks consisting of only one or two sites (e.g., haplotypes at Site #47 were an independent network). Consequently, I suspect that, at least for this vertebrate, isolated networks at 95% SPP represent intraspecific genetic structure rather than species level differentiation (Centeno-Cuadros et al. 2009). The independent haplotype networks formed by TCS 1.21 corresponded with Bayesian subclades and then major clades of *D. conanti* (SL) when I tested progressively less stringent SPPs. When SPP was 91% there were a total of 10 networks, including four singletons (i.e., unconnected haplotypes), separated by ≥ 14 mutational steps (Figure 3.5). The networks matched the following major Bayesian clades: South Central 1, South Central 2, Central, as well as the subclades Northern, Upper, and Lower. The four singletons included Dark Ward, a

haplotype from the Neches River in Texas (Site #64), another from the Red River in Louisiana (Site #3), and a haplotype from the Choctawhatchee River in Florida (Site #32) (Figure 3.5). At a SPP of 90%, the Northern, Upper, and Lower networks formed a single network, bringing the total number of networks in the GCP to 7 (isolated by ≥15 mutational steps). The shortest connection between the Northern and Lower subclades occurred between haplotypes in the Lower Big Black and Lower Yazoo Rivers (Sites #44 & 45) and haplotypes at a site in the Lower Pearl (Site #25) (Figure 3.1). There were two different shortest paths between the Upper and Lower networks, one between haplotypes from the upper Pascagoula (Sites #61 and #68) and Black Creek, and a second between the same upper Pascagoula sites and sites in the Bogue Chitto River in the Pearl River Drainage (Figure 3.1).

The best AMOVA model tested was Model 5, which divided sites in to major river drainages and explained slightly more than 50% of the genetic variance among sites. However, each of the SAMOVA models was ranked higher than any of the *a priori* models (Table 3.5). The SAMOVA K = 3 model (Figure 3.6) had the largest change in the amount of variance explained by groups ($\Delta\Phi_{CT}$ = 0.429; genetic variance explained = 51.55%). However, the best SAMOVA model, which was associated with the third largest value for $\Delta\Phi_{CT}$ and was the first model for which the amount of variance explained by groups surpassed that of the variance among sites within groups, was the K = 8 model ($\Delta\Phi_{CT}$ = 0.028; genetic variance explained = 67.48) (Table 3.5 and Figure 3.6). Most of the same lineages apparent in the Bayesian and network analyses are repeated in the eight groups identified by this model, with the following exceptions: 1) the Neches site (Site #64) and 2) the Noxubee site (Site #48) are placed in what is otherwise a group

containing sites with Lower subclade haplotypes, and 3) SAMOVA formed a group containing all ACP sites, as well as the single site in the Upper Tennessee River drainage (Site #39). The K = 7 model had a higher value for $\Delta\Phi_{CT}$ ($\Delta\Phi_{CT}$ = 0.045), but was not substantially different from the K = 8 model. SAMOVA did not consistently place the Neches site within the same group across the tested levels of K. The analysis began forming groups containing only a single site at higher values for K.

Genetic groups identified by microsatellite loci

One locus, *Dcon12*, violated HWE across multiple sites (N = 6). Two others, *Dcon05* and *Dcon18* were not in HWE at one site per locus. Each of the six loci exhibited significant LD in at least one of the 13 sites, but this was almost always at sites with small sample sizes ($n \leq 12$), and the same loci were not consistently paired (Table 3.6 and Table 3.7). ML-Null detected an excess of homozygotes, and therefore potentially the presence of null-alleles, within *Dcon12* in five populations, as well as in *Dcon21* in three populations (Sites #8 – 10) and *Dcon05* in one population (Site #26). *Dcon21* was monomorphic at Sites #9 and 10, and only two allele sizes were detected at Site #8. Nearly half of the individuals at Site #26 did not amplify at *Dcon05*. Whether this was due to issues with PCR conditions or the presence of null alleles is currently uncertain. ML-Null did not detect scoring errors or large allele dropout at any sites. Average H_O within populations was high (mean = 0.7444, range = 0.8694 – 0.6349) (Table 3.7). I found indications of inbreeding within nine of the 13 sites genotyped ($p < 0.05$), and the average F_{IS} calculated across all populations and five loci was 0.0630 and statistically significant. Population specific F_{IS} values ranged from 0.0020 (Site #49 in the Upper Pearl River) to 0.2043 (Site#22 in the Leaf River). Removing *Dcon12* from the dataset

did not affect the overall outcome of preliminary analyses in STRUCTURE, therefore I retained *Dcon12* in the full and subsampled dataset.

Sites are genetically differentiated from one another (weighted average F_{ST} = 0.1134; p-value = 0.00), but not all population pairwise F_{ST} values were significant (Table 3.8). The non-significant values generally correspond with pairs that include sites with the highest percentages of missing data across the five loci used (e.g., Site #26) and those with the smallest sample sizes (Table 3.7). However, some sites, such as those in the Lower Leaf River, are relatively close to one another (i.e., < 1 Km) (Figure 3.2). Allele frequencies at the northernmost sites differ substantially from those in the Pascagoula and Pearl River drainages, whereas allele frequencies in the Homochitto River are more similar to others in the southern GCP (Figure 3.7). The average distance between sites was ca. 237 Km and ranged between 0.61 and 470 Km. The simple Mantel test indicated that there was significant correlation between geographic and genetic distance matrices (Mantel's r = 0.6182, p = 0.0010, r^2 = 0.3822). However, some sites separated by shorter distances (i.e., <100 km) had pairwise F_{ST} values that were comparatively as high or higher than those between sites at either extreme of the sampled range (Figure 3.8). Consequently, other factors are also influencing genetic structure within the microsatellite dataset at this scale.

Sites included in the microsatellite dataset belong within the Central and Eastern major clades. Individuals from at least two sites were genotyped for each of the three Eastern subclades (i.e., Sites #8 – 10 and 12 for Northern; Sites #49 and 61 for Upper; Sites #7, 21, 22, and 67 for Lower), as well as for the Central 1 clade (Sites #26 and 27) (Figures 2 and 4). Although Site #67 in Ward Bayou is included in this microsatellite

dataset, the Dark Ward individual is not. Given the reciprocal monophyly and discrete distributions of these mitochondrial clades, I expected to detect an initial division that represented major clades at K = 2, as well as indication of further subdivision representative of subclades at K = 4. I also expected that the largest value for K would correspond with the total number of sites (K = 13). These expectations were partially supported by the results of analyses in STRUCTURE.

STRUCTURE identified similar hierarchical groupings in both the full and subsampled datasets. There was strong North-South break followed by the distinction two populations among individuals in southern sites (Figure 3.9 and Figure 3.10). The North-South division was particularly apparent in the subsampled dataset, for which there was a large peak in ΔK at K = 2, followed by much smaller peaks at larger values of K (Figure 3.10). One of the southern groups at K = 3 contained individuals from the Pearl and Lower Leaf, and the second contained individuals from the Upper Leaf, Lower Pascagoula, and Homochitto Rivers (Figure 3.9). There was a slight difference in the total number of genetic groups identified in the full (K = 8) and subsampled (K = 7) datasets (Figure 3.10), but populations generally corresponded with sites (Figure 3.9). There were three drainages in which this was not the case in the full dataset. Individuals from sites in the Lower Tennessee River (Sites #8 – 10) were grouped into a single population, as were individuals from sites in the Lower Leaf River (Sites #7, 21, and 22) and those from the Homochitto River (Sites #26 and 27) (Figure 3.9A). Individuals from those sites were grouped in the same manner at K = 7 in the subsampled dataset, but STRUCTURE also placed individuals from the upper Pearl with those in the Lower Pascagoula River (Figure 3.9B). There was a peak in the value for ΔK in both analyses at K = 10 (Figure 3.10), but

the additional populations only contributed to a greater degree of admixture across individuals and were not informative (e.g., individuals at sites in the Lower Leaf River in the full dataset [Figure 3.9A], and across multiple sites in the subsampled dataset [Figure 3.9B]).

Discussion

This study confirmed the presence of multiple divergent mitochondrial clades within *D. conanti* (SL), the origins of which may best be explained by recent geologic history such as the major changes in sea level occurred across the late Oligocene, late Miocene, and Pliocene (Swift et al. 1986). These sea level fluctuations likely facilitated the dispersal of many plethodontid clades out of the Eastern Highlands both across and within drainages, as well as the subsequent isolation and diversification of lineages (Martin et al. 2016). Analyses by Martin et al. (2016) suggest that the oldest dispersal of plethodontids out of the Eastern Highlands and into the Interior Highlands involved the ancestors of Interior Highlands *Eurycea* (ca. 28.9 Ma). Another old dispersal involved the ancestors of the Ouachita dusky salamander (*D. brimleyorum*), which diverged from other *Desmognathus* ca. 17.4 – 14.7 Ma (Martin et al. 2016). The Dark Ward haplotype likely represents one of the deepest divergences among lineages of *D. conanti* (SL) within the GCP that was sampled during the course of this study (ca. 10.20% - 8.10% sequence divergence [Table 3.3B] and divergence times of ca. 20.4 – 6.0 Ma [Table 3.9]). Multiple attempts to locate other individuals within this lineage have been unsuccessful, despite encountering many other plethodontids during each survey of Site #67, and Dark Ward may represent an infrequently occurring, highly restricted lineage. Coarse estimates of divergence times suggest that most of the major clades within *D. conanti* (SL) became

isolated during the latter half of the Miocene or in the early Pliocene (≥ 3.5 Ma; Table 3.9) and may have also coincided with fluctuating sea levels.

The possibility for ongoing declines in the western portions of the range of *D. conanti* (SL) (Beamer and Lamb 2008, Hibbitts et al. 2015), combined with the fact that this region contains unique mitochondrial lineages, highlights the need for more thorough sampling within and across drainages that are west of the Mississippi River. The current range of South Central 1 across the Neches, Sabine, and Red Rivers may be the result of expansions within the last ca. 2.6 Ma to 10,000 years (Saucier 1994). There are similarly distributed lineages within other aquatic (e.g., blackstripe topminnow, *Fundulus notatus* [Duvernell et al. 2013]) and terrestrial taxa (e.g., common ground skink, *Scincella lateralis* [Jackson and Austin 2010]), as well as indications of unique lineages within the Neches River (Duvernell et al. 2013). I did not collect large numbers of samples from either the South Central 1 or South Central 2 clades, therefore individuals were not genotyped across microsatellite loci as part of this study. However, rapidly mutating, polymorphic markers need to be applied to these populations to better understand their phylogenetic and phylogeographic history, as well as patterns in ongoing gene flow and levels of genetic diversity.

More recent fluctuations in sea level during the Late Pliocene and Pleistocene, along with shifting connections among modern GCP and Lower Mississippi River drainages (Saucier 1994), are likely responsible for the distributions of subclades within the Central and Eastern clades. The arc of the Northern subclade across the Lower Tennessee, Upper Tombigbee, Yazoo, and Big Black rivers is reminiscent of the ranges and inferred dispersal patterns for northern and Eastern Highland associated stream taxa

(e.g., northern hogsucker [*Hypentelium nigricans*; Berendzen et al. 2003], rainbow darter [*Etheostoma caeruleum*; Ray et al. 2006], and multiple species of madtom catfishes [*Noturus* spp.; Egge 2007]). Understanding the distributions of the Upper and Lower mitochondrial lineages is more difficult due to limited sampling in Alabama. Mitochondrial lineages within other amphibians (Newman and Rissler 2011) as well as in some reptiles (Jackson and Austin 2010) demonstrate a shared history between the Tombigbee, Pearl, and Lower Pascagoula Rivers. The Upper lineage of *D. conanti* (SL) might be found elsewhere within the Noxubee and Tombigbee Drainages. If this is the case, then its occurrence in the upper Pascagoula could represent a southwestward projection either via movement through intermediary aquatic habitats, close headwater seeps, and or stream capture events. Based on endemism in other GCP fauna (e.g., Ennen et al. 2010), it is also feasible that the Upper lineage evolved within the Pascagoula River Drainage during a period of sea level maxima, and that it expanded outward in to the Pearl and Noxubee Rivers. Under this scenario, increased connectivity between GCP drainages, perhaps due to the eastward shift in the positions of major channels or the westward movement of river mouths in the Late Pliocene-Pleistocene (Swift et al. 1986) may have resulted in the expansion of the Lower lineage across Lower Pascagoula River.

There is likely further historic, genetic structure within the Lower subclade that the current dataset is unable to capture. The Lower subclade of *D. conanti* (SL) spans major rivers in the GCP that separate intraspecific lineages as well as closely related taxa (Soltis et al. 2006, Lemmon et al. 2007, Gamble et al. 2008, Jackson and Austin 2010, Newman and Rissler 2011). This study detected a well-supported, narrowly distributed clade within the Lower subclade that occurred across three sites in the Lower

Chickasawhay, Lower Pascagoula, and Lower Mobile River Drainages (Site #20, 67, and 31, respectively) (Figure 3.3). The main stem of the Pascagoula River divides coastal distributions of clades in *S. lateralis* (Jackson and Austin 2010) and future investigations with *D. conanti* (SL) should attempt to more thoroughly sample within the Chickasawhay River to determine the extent to which the main stem of the Pascagoula and its major tributaries (i.e., Leaf and Chickasawhay Rivers) have contributed to historic genetic structure.

I propose that *D. conanti* (SL) contains lineages that, in the least, qualify as evolutionarily significant units (ESU), but that, upon further investigation, may warrant recognition as independent species. Definitions for ESUs vary in the weight that they place on delineating criteria (i.e., reproductive isolation and evolutionary legacy [Waples 1991], reciprocal monophyly [Moritz 1994, 2002], ecological and genetic exchangeability [Crandall et al. 2000]). Despite their differences, the overarching theme across concepts is to preserve current and future biodiversity, and the application of multiple criteria will allow us to better accomplish this goal (Fraser and Bernatchez 2001). Due to the limitations of the current data both west of the Mississippi River as well as within the ACP, the remainder of this discussion focuses on *D. conanti* (SL) occurring in the Lower Tennessee River Drainage and the GCP.

The Northern subclade is the most differentiated unit within this region and meets the expectations for many of the various definitions for an ESU. The results of this study demonstrate that this group was historically isolated and suggest that there may be little to no current gene flow between it and other populations within the GCP. The Northern clade appears to be the same as the *D. conanti* "clade D" in Kozak et al. (2005), which

extends along drainages in western Tennessee but does not pervade the upper reaches of the Tennessee Drainage, nor those drainages in the ACP. This Northern clade may have split more recently (ca. 4.0 – 1.5 Ma) from other mitochondrial clades within *D. conanti* (SL), but speciation events during the Pliocene and Pleistocene are not uncommon among vertebrates (Avise et al. 1998). Unpublished data (this author) suggest that the Northern clade may have diverged from southern populations both in terms of habitat specificity (i.e., occupying higher declivity streams dominated by different substrates) and morphology (i.e., larger adult body sizes). Testing the hypothesis that the Northern clade has remained distinct from populations further south, and is the only group to which the epithet *conanti* should apply, will require a larger dataset that includes corroborating phylogenies based on sequence data from other genes, as well as ecological and morphological comparisons across drainages.

Delimiting ESUs among southern sites is more challenging. Mitochondrial sequence divergence between the Central subclades and any of the nearby Eastern clades ranged between 4.40 and 5.10%, and in the field I noted that populations in the Homochitto River exhibited more yellow pigmentation along their sides than was typical for populations in either the Pascagoula or Pearl Rivers. Narrowly distributed endemics are present within Lower Mississippi River Drainages (e.g., bayou darter [*Etheostoma rubrum*]) and in Pontchartrain Drainages (e.g., broadstripe topminnow [*Fundulus euryzonus*]) (Ross 2001), and this, combined with the mitochondrial data, led to the hypothesis that individuals from the Homochitto River would form a well differentiated population within the microsatellite dataset.

Analyses with the microsatellite data identified two southern populations of *D. conanti* (SL) but the distributions of these populations and the nature of any isolating barriers were unclear due to the few number of sites genotyped. Current barriers to gene flow may not correspond with the main stems of large rivers in the southern GCP. For example, the Pearl River does not appear to have isolated populations on either side of its channel. However, there may be aspects within drainages that serve to isolate these more broadly distributed genetic groups (i.e., the distinction between Upper and Lower Leaf River sites within the Pascagoula River). Nevertheless, the approximate edge or zone of overlap between these two southern populations does not correspond with that for the Central and Eastern clades (i.e., Bogue Chitto River). Nor does it correspond with the divide between Upper and Lower subclades. Consequently, it appears that the Central, Lower, and Upper mitochondrial clades did not diverge to a degree that prevented gene flow upon secondary contact, and that these mitochondrial lineages may represent failed incipient species (Tilley et al. 2013). Hybridization among lineages of plethodontids, be they recognized species or intraspecific clades, is not uncommon (Tilley 1988, Highton 2000, Tilley et al. 2013), and the results of this study reiterate the need for applying multiple markers to phylogeographic and phylogenetic studies.

Desmognathus conanti (SL) exhibits a high degree of IBD (Mantel's r = 0.6182; p = 0.0010) and individuals are likely isolated across shorter distances than those between the majority of sites in this study. Distances ranging from 0.9 to 19.7 Km (F_{ST} = 0.027 – 0.405) (Miller et al. 2015) in forested habitat and 2.5 to 48 Km (F_{ST} = 0.08 – 0.51) in urban environments (Munshi-South et al. 2013) have led to significant differentiation among populations of *D. fuscus*. There were three drainages in this study in which sites

were separated by moderate geographic distances (< 20 km), including the Lower Tennessee River Drainage (#8 – 10), Lower Leaf River (#7, 21, 22), and in the Homochitto River (#26 and 27). Site #10 was not significantly differentiated from either Site #8 or 9, which, given the distance between #8 and 10 (Figure 3.2), may be a result of the low number of samples at the latter site (N = 5). Similarly, sites #26 and 27 are also separated by a substantial distance and are not genetically differentiated, but I again suspect that this is due to small sample sizes at Site #27, as well as the effect of missing data for Site #26 (i.e., *Dcon05*). Sites in the Lower Leaf River are connected by much shorter geographic distances, small creeks, as well as a shared floodplain, and I suspect that it is more feasible for there to have been gene flow among these sites within the recent past. As seen in studies with other desmognathines (Apodaca et al. 2012) F_{IS} values were positive and significant across most sites, indicating an excess of homozygotes and the possibility for either inbreeding or within-site genetic substructure (Allendorf and Luikart 2007). For some of the sites in this study, individuals were sampled from within a stretch of stream ca. 25 – 50 m in length (e.g., Site #12 and 49), whereas at others individuals were sampled from across a larger distance (i.e., ca. 1 Km of continuous habitat for Site #67). It is feasible that relatedness among individuals varies across sites, and that this may be contributing to significant F_{IS} values, but fine scale genetic structure within populations of *D. conanti* (SL) is beyond the scope of the present work.

This work verifies that there is significant genetic diversity within a plethodontid salamander occurring across much of the GCP. Phylogeographic patterns within *D. conanti* (SL) are likely a consequence of vicariance and dispersal events facilitated by

shifting sea levels and drainage connections within the Coastal Plain. Mitochondrial sequence data not only confirm that *D. conanti* (SL) contains substantial genetic structure (Karlin and Guttman 1986, Bonett 2002, Kozak et al. 2005, Beamer and Lamb 2008), but also that the *sensu stricto* lineage of *D. conanti* can be found within drainages in the GCP. The mitochondrial and microsatellite datasets identify likely ESUs within *D. conanti* (SL) and emphasize the importance of applying multiple markers to phylogeographic inquiries. Future endeavors will focus on testing the degree to which *D. conanti* (SS) has remained isolated from other populations in the GCP by applying microsatellites to individuals from sampled sites elsewhere within the Yazoo and Big Black Rivers. I will also attempt to elucidate the boundaries between southern populations by genotyping individuals across a longitudinal transect of sampled sites in Louisiana and Mississippi.

Table 3.1

Sample locality and haplotype data

ID#	Species	Site #	State	County	Drainage (Tributary)	Haplotype	Source
319	conanti	5	Louisiana	Catahoula	Ouachita (Big Creek)	conanti_Ouachita_1	JYL
320	conanti	5	Louisiana	Catahoula	Ouachita (Big Creek)	conanti_Ouachita_1	JYL
323	conanti	5	Louisiana	Catahoula	Ouachita (Big Creek)	conanti_Ouachita_1	JYL
324	conanti	5	Louisiana	Catahoula	Ouachita (Big Creek)	conanti_Ouachita_2	JYL
EU311709	conanti	36	Georgia	Wayne	Altamaha	conanti_EU311709	*A*
EU311710	conanti	36	Georgia	Wayne	Altamaha	conanti_EU311710	*A*
415	conanti	44	Mississippi	Warren	Big Black	conanti_BBlack_1	JYL
426	conanti	44	Mississippi	Warren	Big Black	conanti_BBlack_1	JYL
416	conanti	44	Mississippi	Warren	Big Black	conanti_BBlack_Yazoo_1	JYL
417	conanti	44	Mississippi	Warren	Big Black	conanti_BBlack_Yazoo_1	JYL
427	conanti	44	Mississippi	Warren	Big Black	conanti_BBlack_Yazoo_1	JYL
EU311684	conanti	32	Florida	Washington	Choctawhatchee	conanti_EU311684	*A*

Table 3.1 (continued).

EU311677	conanti	29	Alabama	Butler	Escambia	conanti_EU311677	*A*
EU311679	conanti	33	Florida	Santa Rosa	Escambia	conanti_EU311679	*A*
EU311671	conanti	1	Louisiana	West Feliciana	Lower MS (Bayou Sara)	conanti_LowerMS_Homo	*A*
121	conanti	28	Mississippi	Wilkinson	Lower MS (Clark Creek)	conanti_LowerMS_Homo	JYL
122	conanti	28	Mississippi	Wilkinson	Lower MS (Clark Creek)	conanti_LowerMS_Homo	JYL
295	conanti	26	Mississippi	Franklin	Lower MS (Homochitto)	conanti_Homo_2	JYL
292	conanti	26	Mississippi	Franklin	Lower MS (Homochitto)	conanti_LowerMS_Homo	JYL

Table 3.1 (continued).

293	conanti	26	Mississippi	Franklin	Lower MS (Homochitto)	conanti_LowerMS_Homo	JYL
305	conanti	27	Mississippi	Franklin	Lower MS (Homochitto)	conanti_Homo_1	JYL
297	conanti	27	Mississippi	Franklin	Lower MS (Homochitto)	conanti_LowerMS_Homo	JYL
298	conanti	27	Mississippi	Franklin	Lower MS (Homochitto)	conanti_LowerMS_Homo	JYL
299	conanti	27	Mississippi	Franklin	Lower MS (Homochitto)	conanti_LowerMS_Homo	JYL
304	conanti	27	Mississippi	Franklin	Lower MS (Homochitto)	conanti_LowerMS_Homo	JYL

Table 3.1 (continued).

2361	conanti	58	Mississippi	Wilkinson County	Lower MS (Homochitto)	conanti_Homo_3	LSUMZ
1939	conanti	51	Louisiana	East Feliciana Parish	Lower MS (Karr Creek)	conanti_LowerMS_Homo	LSUMZ
307	conanti	4	Louisiana	West Feliciana	Lower MS (Thompson Creek)	conanti_LowerMS_Homo	JYL
308	conanti	4	Louisiana	West Feliciana	Lower MS (Thompson Creek)	conanti_LowerMS_Homo	JYL
309	conanti	4	Louisiana	West Feliciana	Lower MS (Thompson Creek)	conanti_LowerMS_Homo	JYL
EU311667	conanti	40	Kentucky	Livingston	Lower Tennessee	conanti_EU311667	*A*
203	conanti	9	Mississippi	Tishomingo	Lower Tennessee (Bear Creek)	conanti_Tenness_2	JYL

Table 3.1 (continued).

204	conanti	9	Mississippi	Tishomingo	Lower Tennessee (Bear Creek)	conanti_Tenness_2	JYL
190	conanti	10	Mississippi	Tishomingo	Lower Tennessee (Bear Creek)	conanti_Tenneess_3	JYL
199	conanti	10	Mississippi	Tishomingo	Lower Tennessee (Bear Creek)	conanti_Tenness_2	JYL
186	conanti	8	Mississippi	Tishomingo	Lower Tennessee (Pickwick Lake)	conanti_Tenness_1	JYL
187	conanti	8	Mississippi	Tishomingo	Lower Tennessee (Pickwick Lake)	conanti_Tenness_1	JYL
EU311678	conanti	31	Alabama	Baldwin	Mobile	conanti_EU311678	*A*
EU311712	conanti	30	Alabama	Lawrence	Mobile	conanti_Tenneess_3	*A*
94726	conanti	64	Texas	Tyler	Neches	conanti_94726	*B*

Table 3.1 (continued).

EU311672	conanti	68	Mississippi	Jasper	Pascagoula	conanti_EU311672	*A*
EU311685	conanti	68	Mississippi	Jasper	Pascagoula	conanti_EU311685	*A*
ASU23806	conanti	68	Mississippi	Jasper	Pascagoula	conanti_EU311685	D. Shepard
262	conanti	23	Mississippi	Forrest	Pascagoula (Black Creek)	conanti_BlkCrk_4	JYL
327	conanti	41	Mississippi	Lamar	Pascagoula (Black Creek)	conanti_BlkCrk_2	JYL
328	conanti	41	Mississippi	Lamar	Pascagoula (Black Creek)	conanti_BlkCrk_3	JYL
19	conanti	59	Mississippi	Perry	Pascagoula (Black Creek)	conanti_Pearl_BlkCrk_1	JYL
22	conanti	59	Mississippi	Perry	Pascagoula (Black Creek)	conanti_Pearl_BlkCrk_1	JYL
123	conanti	60	Mississippi	Perry	Pascagoula (Black Creek)	conanti_BlkCrk_1	JYL
124	conanti	60	Mississippi	Perry	Pascagoula (Black Creek)	conanti_BlkCrk_1	JYL
5	conanti	63	Mississippi	Perry	Pascagoula (Black Creek)	conanti_BlkCrk_5	JYL

Table 3.1 (continued).

8	conanti	63	Mississippi	Perry	Pascagoula (Black Creek)	conanti_BlkCrk_6	JYL
171	conanti	11	Mississippi	Lauderdale	Pascagoula (Chickasawhay)	conanti_Chick_3	JYL
174	conanti	11	Mississippi	Lauderdale	Pascagoula (Chickasawhay)	conanti_Chick_3	JYL
100	conanti	20	Mississippi	Wayne	Pascagoula (Chickasawhay)	conanti_Chick_1	JYL
110	conanti	20	Mississippi	Wayne	Pascagoula (Chickasawhay)	conanti_Chick_2	JYL
112	conanti	20	Mississippi	Wayne	Pascagoula (Chickasawhay)	conanti_Chick_2	JYL
7	conanti	7	Mississippi	Forrest	Pascagoula (Leaf)	conanti_Leaf_Pasca_1	JYL
10	conanti	7	Mississippi	Forrest	Pascagoula (Leaf)	conanti_Leaf_Pasca_1	JYL
280	conanti	21	Mississippi	Forrest	Pascagoula (Leaf)	conanti_Leaf_2	JYL
274	conanti	21	Mississippi	Forrest	Pascagoula (Leaf)	conanti_Leaf_Pasca_1	JYL
275	conanti	21	Mississippi	Forrest	Pascagoula (Leaf)	conanti_Leaf_Pasca_1	JYL
278	conanti	21	Mississippi	Forrest	Pascagoula (Leaf)	conanti_Leaf_Pasca_1	JYL

Table 3.1 (continued).

279	conanti	21	Mississippi	Forrest	Pascagoula (Leaf)	conanti_Leaf_Pasca_1	JYL
38	conanti	22	Mississippi	Forrest	Pascagoula (Leaf)	conanti_Leaf_Pasca_1	JYL
238	conanti	24	Mississippi	Simpson	Pascagoula (Leaf)	conanti_Leaf_3	JYL
239	conanti	24	Mississippi	Simpson	Pascagoula (Leaf)	conanti_Leaf_3	JYL
240	conanti	24	Mississippi	Simpson	Pascagoula (Leaf)	conanti_Leaf_3	JYL
241	conanti	24	Mississippi	Simpson	Pascagoula (Leaf)	conanti_Leaf_3	JYL
383	conanti	61	Mississippi	Jones	Pascagoula (Leaf)	conanti_Leaf_1	JYL
385	conanti	61	Mississippi	Jones	Pascagoula (Leaf)	conanti_Leaf_1	JYL
386	conanti	61	Mississippi	Jones	Pascagoula (Leaf)	conanti_Leaf_1	JYL
401	conanti	61	Mississippi	Jones	Pascagoula (Leaf)	conanti_Leaf_1	JYL
405	conanti	61	Mississippi	Jones	Pascagoula (Leaf)	conanti_Leaf_1	JYL
78	conanti	67	Mississippi	Jackson	Pascagoula (Ward Bayou)	conanti_Pasca_1	JYL
43	conanti	67	Mississippi	Jackson	Pascagoula (Ward Bayou)	conanti_Leaf_Pasca_1	JYL

Table 3.1 (continued).

50	conanti	67	Mississippi	Jackson	Pascagoula (Ward Bayou)	conanti_Leaf_Pasca_1	JYL
89	conanti	67	Mississippi	Jackson	Pascagoula (Ward Bayou)	conanti_Pasca_2	JYL
119	conanti	67	Mississippi	Jackson	Pascagoula (Ward Bayou)	conanti_Pasca_3	JYL
55	conanti	67	Mississippi	Jackson	Pascagoula (Ward Bayou)	conanti_Pasca_3	JYL
87	conanti	67	Mississippi	Jackson	Pascagoula (Ward Bayou)	conanti_Pasca_4	JYL
90	conanti	67	Mississippi	Jackson	Pascagoula (Ward Bayou)	conanti_Pasca_4	JYL
95	conanti	67	Mississippi	Jackson	Pascagoula (Ward Bayou)	conanti_Pasca_5	JYL
116	conanti	15	Louisiana	Washington	Pearl (Bogue Chitto)	conanti_Pearl_6	JYL
117	conanti	15	Louisiana	Washington	Pearl (Bogue Chitto)	conanti_Pearl_6	JYL
18065	conanti	52	Louisiana	Washington Parish	Pearl (Bogue Lusa Creek)	conanti_Pearl_10	LSUMZ
18034	conanti	52	Louisiana	Washington Parish	Pearl (Bogue Lusa Creek)	conanti_Pearl_7	LSUMZ

Table 3.1 (continued).

18066	conanti	52	Louisiana	Washington Parish	Pearl (Bogue Lusa Creek)	conanti_Pearl_7	LSUMZ
18090	conanti	52	Louisiana	Washington Parish	Pearl (Bogue Lusa Creek)	conanti_Pearl_8	LSUMZ
18035	conanti	52	Louisiana	Washington Parish	Pearl (Bogue Lusa Creek)	conanti_Pearl_9	LSUMZ
EU311673	conanti	2	Louisiana	Washington	Pearl (Lower Pearl)	conanti_EU311673	*A*
136	conanti	25	Mississippi	Marion	Pearl (Lower Pearl)	conanti_Pearl_1	JYL
147	conanti	25	Mississippi	Marion	Pearl (Lower Pearl)	conanti_Pearl_1	JYL
149	conanti	25	Mississippi	Marion	Pearl (Lower Pearl)	conanti_Pearl_2	JYL
148	conanti	25	Mississippi	Marion	Pearl (Lower Pearl)	conanti_Pearl_5	JYL
131	conanti	25	Mississippi	Marion	Pearl (Lower Pearl)	conanti_Pearl_BlkCrk_1	JYL
442	conanti	49	Mississippi	Scott	Pearl (Pelahatchie Creek)	conanti_Pearl_3	JYL

Table 3.1 (continued).

443	conanti	49	Mississippi	Scott	Pearl (Pelahatchie Creek)	conanti_Pearl_3	JYL
460	conanti	49	Mississippi	Scott	Pearl (Pelahatchie Creek)	conanti_Pearl_3	JYL
462	conanti	49	Mississippi	Scott	Pearl (Pelahatchie Creek)	conanti_Pearl_3	JYL
461	conanti	49	Mississippi	Scott	Pearl (Pelahatchie Creek)	conanti_Pearl_4	JYL
EU311674	conanti	16	Mississippi	Amite	Pontchartrain (Amite)	conanti_EU311674	*A*
20527	conanti	54	Louisiana	Tangipahoa Parish	Pontchartrain (Tangipahoa)	conanti_Tangi_1	LSUMZ
20528	conanti	54	Louisiana	Tangipahoa Parish	Pontchartrain (Tangipahoa)	conanti_Tangi_1	LSUMZ
20529	conanti	54	Louisiana	Tangipahoa Parish	Pontchartrain (Tangipahoa)	conanti_Tangi_1	LSUMZ
20530	conanti	54	Louisiana	Tangipahoa Parish	Pontchartrain (Tangipahoa)	conanti_Tangi_1	LSUMZ

Table 3.1 (continued).

318	conanti	6	Louisiana	Rapides	Red (Brown Creek)	conanti_Red_1	JYL
313	conanti	6	Louisiana	Rapides	Red (Brown Creek)	conanti_Red_2	JYL
314	conanti	6	Louisiana	Rapides	Red (Brown Creek)	conanti_Red_3	JYL
317	conanti	6	Louisiana	Rapides	Red (Brown Creek)	conanti_Red_3	JYL
20700	conanti	56	Louisiana	Natchitoches Parish	Red (Chaplin Lake)	conanti_Red_4	LSUMZ
20701	conanti	56	Louisiana	Natchitoches Parish	Red (Chaplin Lake)	conanti_Red_4	LSUMZ
EU311699	conanti	3	Louisiana	Grant	Red (Grant Parish)	conanti_EU311699	*A*
18126	conanti	53	Louisiana	Natchitoches Parish	Red (Kisatchie Bayou)	conanti_Red_5	LSUMZ
TJH2756	conanti	65	Texas	Newton	Sabine	conanti_TJH2756	*B*
TJH3263	conanti	65	Texas	Newton	Sabine	conanti_TJH2756	*B*
TJH3264	conanti	65	Texas	Newton	Sabine	conanti_TJH2756	*B*
TJH3265	conanti	65	Texas	Newton	Sabine	conanti_TJH2756	*B*

Table 3.1 (continued).

TJH2757	conanti	65	Texas	Newton	Sabine	conanti_TJH2757	*B*
TJH2758	conanti	65	Texas	Newton	Sabine	conanti_TJH2757	*B*
TJH3262	conanti	65	Texas	Newton	Sabine	conanti_TJH3262	*B*
TJH3266	conanti	66	Texas	Sabine	Sabine	conanti_TJH3266	*B*
TJH3269	conanti	66	Texas	Sabine	Sabine	conanti_TJH3269	*B*
TJH3270	conanti	66	Texas	Sabine	Sabine	conanti_TJH3270	*B*
EU311651	conanti	35	Georgia	Effingham	Savannah	conanti_EU311651	*A*
TJR2470	conanti	37	Georgia	Richmond	Savannah	conanti_TJR2470	D. Shepard
EU311668	conanti	38	South Carolina	Barnwell	Savannah	conanti_EU311668	*A*
463	conanti	50	Mississippi	Prentiss	Tombigbee (Caveness Branch)	conanti_Tenness_2	JYL
432	conanti	48	Mississippi	Winston	Tombigbee (Noxubee)	conanti_Noxubee_1	JYL
433	conanti	48	Mississippi	Winston	Tombigbee (Noxubee)	conanti_Noxubee_1	JYL
434	conanti	48	Mississippi	Winston	Tombigbee (Noxubee)	conanti_Noxubee_2	JYL
435	conanti	48	Mississippi	Winston	Tombigbee (Noxubee)	conanti_Noxubee_2	JYL

Table 3.1 (continued).

436	conanti	48	Mississippi	Winston	Tombigbee (Noxubee)	conanti_Noxubee_2	JYL
1907	conanti	57	Mississippi	Winston County	Tombigbee (Noxubee)	conanti_Noxubee_3	LSUMZ
EU311698	conanti	39	North Carolina	Henderson	Upper Tennessee	conanti_EU311698	*A*
429	conanti	47	Mississippi	Carrol	Yazoo (Little Sand Creek)	conanti_Yazoo_2	JYL
431	conanti	47	Mississippi	Carrol	Yazoo (Little Sand Creek)	conanti_Yazoo_2	JYL
428	conanti	47	Mississippi	Carrol	Yazoo (Little Sand Creek)	conanti_Yazoo_3	JYL
430	conanti	47	Mississippi	Carrol	Yazoo (Little Sand Creek)	conanti_Yazoo_3	JYL
218	conanti	12	Mississippi	Union	Yazoo (Little Tallahatchie)	conanti_Yazoo_5	JYL
219	conanti	12	Mississippi	Union	Yazoo (Little Tallahatchie)	conanti_Yazoo_5	JYL

Table 3.1 (continued).

418	conanti	45	Mississippi	Warren	Yazoo (Lower Yazoo)	conanti_BBlack_Yazoo_1	JYL
419	conanti	45	Mississippi	Warren	Yazoo (Lower Yazoo)	conanti_BBlack_Yazoo_1	JYL
420	conanti	45	Mississippi	Warren	Yazoo (Lower Yazoo)	conanti_BBlack_Yazoo_1	JYL
421	conanti	45	Mississippi	Warren	Yazoo (Lower Yazoo)	conanti_BBlack_Yazoo_1	JYL
422	conanti	46	Mississippi	Warren	Yazoo (Lower Yazoo)	conanti_Yazoo_1	JYL
423	conanti	46	Mississippi	Warren	Yazoo (Lower Yazoo)	conanti_Yazoo_1	JYL
424	conanti	46	Mississippi	Warren	Yazoo (Lower Yazoo)	conanti_Yazoo_1	JYL
425	conanti	46	Mississippi	Warren	Yazoo (Lower Yazoo)	conanti_Yazoo_4	JYL
266	conanti	34	Florida	Santa Rosa	Yellow	conanti_Yellow_1	JYL
267	conanti	34	Florida	Santa Rosa	Yellow	conanti_Yellow_1	JYL
271	apalachicolae	NA	Florida	Liberty	Apalachicola	apalachicolae_ Apalachie_1	JYL
272	apalachicolae	NA	Florida	Liberty	Apalachicola	apalachicolae_ Apalachie_2	JYL
273	apalachicolae	NA	Florida	Liberty	Apalachicola	apalachicolae_ Apalachie_3	JYL

Table 3.1 (continued).

BTL238	apalachicolae	NA	Florida	Liberty	Apalachicola	apalachicolae_BTL238	D. Shepard
BTL239	auriculatus	NA	Florida	Wakulla	-	auriculatus_BTL239	D. Shepard
268	auriculatus	NA	Florida	Wakula	Ochlockonee	auriculatus_ Ochlockonee_1	JYL
269	auriculatus	NA	Florida	Wakula	Ochlockonee	auriculatus_ Ochlockonee_1	JYL
270	auriculatus	NA	Florida	Wakula	Ochlockonee	auriculatus_ Ochlockonee_2	JYL
DBS2394	brimleyorum	NA	Arkansas	Ouachita	-	brimleyorum_DBS2394	D. Shepard
FC11578	brimleyorum	NA	Arkansas	LeFlore	-	brimleyorum_FC11578	D. Shepard
KJI1153	brimleyorum	NA	Arkansas	Ouachita	-	brimleyorum_KJI1153	D. Shepard
KJI1160	brimleyorum	NA	Arkansas	Ouachita	-	brimleyorum_KJI1160	D. Shepard
RMB2201	brimleyorum	NA	Arkansas	Polk	-	brimleyorum_RMB2201	D. Shepard
RMB2327	brimleyorum	NA	Arkansas	Nevada	-	brimleyorum_RMB2327	D. Shepard
169	cf. auriculatus	NA	Mississippi	Lamar	Pascagoula (Black Creek)	cf.auriculatus_ Pearl_BlkCrk_1	JYL
170	cf. auriculatus	NA	Mississippi	Lamar	Pascagoula (Black Creek)	cf.auriculatus_ Pearl_BlkCrk_1	JYL

Table 3.1 (continued).

256	cf. auriculatus	NA	Mississippi	Forrest	Pascagoula (Black Creek)	cf.auriculatus_BlkCrk_1	JYL
260	cf. auriculatus	NA	Mississippi	Forrest	Pascagoula (Black Creek)	cf.auriculatus_ Pearl_BlkCrk_2	JYL
265	cf. auriculatus	NA	Mississippi	Forrest	Pascagoula (Black Creek)	cf.auriculatus_ Pearl_BlkCrk_2	JYL
160	cf. auriculatus	NA	Mississippi	Wayne	Pascagoula (Chickasawhay)	cf.auriculatus_Chick_1	JYL
437	cf. auriculatus	NA	Mississippi	Madison	Pearl	cf.auriculatus_ Pearl_BlkCrk_1	JYL
438	cf. auriculatus	NA	Mississippi	Madison	Pearl	cf.auriculatus_ Pearl_BlkCrk_1	JYL
439	cf. auriculatus	NA	Mississippi	Madison	Pearl	cf.auriculatus_ Pearl_BlkCrk_1	JYL
441	cf. auriculatus	NA	Mississippi	Madison	Pearl	cf.auriculatus_ Pearl_BlkCrk_1	JYL
472	cf. auriculatus	NA	Mississippi	Neshoba	Pearl (Nanih Waiya Creek)	cf.auriculatus_Pearl_2	JYL
473	cf. auriculatus	NA	Mississippi	Neshoba	Pearl (Nanih Waiya Creek)	cf.auriculatus_Pearl_2	JYL
474	cf. auriculatus	NA	Mississippi	Neshoba	Pearl (Nanih Waiya Creek)	cf.auriculatus_ Pearl_BlkCrk_2	JYL

Table 3.1 (continued).

475	cf. auriculatus	NA	Mississippi	Neshoba	Pearl (Nanih Waiya Creek)	cf.auriculatus_ Pearl_BlkCrk_2	JYL
351	cf. auriculatus	NA	Louisiana	St. Tammany	Pearl (Talisheek Creek)	cf.auriculatus_Pearl_1	JYL
352	cf. auriculatus	NA	Louisiana	St. Tammany	Pearl (Talisheek Creek)	cf.auriculatus_ Pearl_BlkCrk_1	JYL
FC13580	fuscus	NA	North Carolina	Duplin	-	fuscus_FC13580	D. Shepard

Note: All sites from which sequences of *D. conanti* (SL) were obtained are listed. Any sequences loaned to JYL are also indicated. Accession#/ID#s correspond with unique identification numbers from GenBank, the donating source, or JYL's field notes. Haplotype names correspond with those on the tree. *A* notes sequences from Beamer and Lamb (2008). Those sequences from Beamer and Lamb (2008) belonging to species of *Desmognathus* other than *D. conanti* (SL) are not included in this Table. *B* notes sequences donated from Tony Hibbtts and Gary Voelker used in Hibbitts et al. (2015). A minus indicates that the information was not available.

Table 3.2

Mitochondrial haplotype diversity statistics partitioned by river drainage

River drainages & subdivisions	Nsites	Ns	Nh	Nucleotide diversity
Atlantic Coastal Plain	4	5	5	0.059 ± 0.036
Altamaha River	1	2	2	0.002 ± 0.003
Savannah River	3	3	3	0.058 ± 0.044
Neches	1	1	1	NA
Sabine	2	10	6	0.005 ± 0.003
Sabine Co., TX	1	3	3	0.005 ± 0.005
Newton Co., TX	1	7	3	0.004 ± 0.003
Red	4	8	6	0.028 ± 0.016
Chaplin Lake	1	2	1	NA
Kisatchie Bayou	1	1	1	NA
Brown Creek	1	4	3	0.013 ± 0.01
Grant Parish, LA	1	1	1	NA
Ouachita	1	4	2	0.001 ± 0.001
Big Black	1	5	2	0.002 ± 0.002
Yazoo	4	14	6	0.013 ± 0.007
Lower Yazoo River	2	8	3	0.002 ± 0.002
Upper Yazoo River	1	6	3	0.015 ± 0.009
Lower Mississippi	7	16	4	0.004 ± 0.003
Homochitto River	3	9	4	0.007 ± 0.004
Smaller Rivers	4	7	1	NA
Pontchartrain	2	5	2	0.005 ± 0.004
Amite River	1	1	1	NA
Tangipahoa River	1	4	1	NA
Pearl	5	18	12	0.034 ± 0.018
Bogue Chitto River	1	2	1	NA
Bogue Lusa River	1	5	4	0.006 ± 0.004
Lower Pearl River	2	6	5	0.004 ± 0.003
Pelahatchie Creek	1	5	2	0.001 ± 0.001
Pascagoula	14	43	21	0.026 ± 0.013
Ward Bayou	1	9	6	0.038 ± 0.021
Black Creek	5	9	7	0.007 ± 0.005
Leaf River	5	17	4	0.019 ± 0.01
Chickasawhay River	2	5	3	0.024 ± 0.016
Jasper Co., MS	1	3	2	0.001 ± 0.002
Mobile	5	9	5	0.031 ± 0.017

Table 3.2 (continued).

Lower Mobile	1	1	1	NA
Noxubee River	2	6	3	0.021 ± 0.013
Tombigbee River	1	1	1	NA
Upper Mobile	1	1	1	NA
East of Mobile	4	5	4	0.021 ± 0.014
Escambia River	2	2	2	0.009 ± 0.01
Yellow River	1	2	1	NA
Choctawhatchee	1	1	1	NA
Lower Tennessee	4	7	4	0.011 ± 0.007
Bear Creek	2	4	2	0.001 ± 0.001
Pickwick Lake	1	2	1	NA
Livingston Co.	1	1	1	NA
Upper Tennessee	1	1	1	NA

Note: Major river drainages, or grouped rivers, are indicated in bold font. Where specific locality data were not published or furnished, sites were generalized to the county or parish in which they occurred. Nsites = number of sites, Ns = number of sequences, Nh = number of haplotypes. Nucleotide diversity is given with standard deviations.

Table 3.3

A. Estimates of net evolutionary divergence between major Bayesian clades

Clades	SC1	SC2	Central	Eastern	DW	ACP1	ACP2	UTN
SC1	2.10%	0.007	0.008	0.007	0.012	0.009	0.01	0.01
SC2	3.80%	4.10%	0.007	0.007	0.012	0.01	0.009	0.009
Central	5.30%	4.20%	1.90%	0.007	0.012	0.01	0.008	0.01
Eastern	4.40%	3.50%	3.50%	2.90%	0.011	0.009	0.008	0.009
DW	9.60%	9.90%	9.40%	8.00%	NA	0.012	0.012	0.013
ACP1	5.80%	6.70%	6.00%	5.40%	8.30%	2.60%	0.009	0.01
ACP2	6.30%	5.70%	4.50%	4.10%	8.10%	4.90%	3.40%	0.009
UTN	7.00%	6.10%	6.80%	5.40%	9.60%	5.80%	5.20%	NA

Table 3.3 (continued).

B. Estimates of net evolutionary divergence between Bayesian clades and subclades)

Clades	SC1	SC2	C1	Lower	Upper	Northern	DW	ACP1	C2	ACP2	UTN
SC1	2.10%	0.007	0.009	0.008	0.008	0.008	0.012	0.009	0.009	0.009	0.01
SC2	3.80%	4.10%	0.008	0.007	0.008	0.007	0.013	0.01	0.007	0.009	0.01
C1	5.60%	4.70%	0.30%	0.008	0.009	0.009	0.013	0.01	0.005	0.009	0.011
Lower	4.90%	4.00%	4.40%	2.00%	0.005	0.005	0.012	0.01	0.008	0.009	0.009
Upper	5.10%	4.50%	5.10%	2.00%	0.80%	0.006	0.012	0.01	0.008	0.008	0.01
Northern	4.80%	3.90%	4.60%	2.00%	2.40%	1.60%	0.011	0.01	0.008	0.009	0.01
DW	9.60%	9.90%	10.20%	8.80%	8.90%	7.80%	NA	0.012	0.012	0.012	0.013
ACP1	5.80%	6.70%	6.80%	5.80%	6.20%	6.50%	8.30%	2.60%	0.01	0.009	0.009
C2	5.80%	4.60%	2.00%	4.50%	4.80%	4.60%	9.70%	6.30%	1.30%	0.009	0.011
ACP2	6.30%	5.70%	5.20%	4.60%	4.60%	4.70%	8.10%	4.90%	4.90%	3.40%	0.009
UTN	7.00%	6.10%	7.70%	6.00%	6.00%	6.10%	9.60%	5.80%	7.10%	5.20%	NA

Note: Net average p-distances, accounting for average within-group distances, are given as percentages. Inter-clade comparisons are below, and standard error estimates above, the diagonal. Intra-clade comparisons are given along the diagonal. Table 3A compares major Bayesian clades, and Table 3B major Bayesian clades and sub-clades, where appropriate. SC1 = South Central 1; SC2 = South Central 2; C1 = Central 1; DW = Dark Ward; C2 = Central 2; UTN = Upper Tennessee River.

Table 3.4

Mitochondrial haplotype diversity statistics partitioned by Bayesian clades with posterior probabilities ≥0.95

Clades	Nseq	Nh	Nucleotide diversity
Dark Ward	1	1	NA
Upper Tennessee River	1	1	NA
ACP 1	3	3	0.026 ± 0.02
ACP 2	2	2	0.034 ± 0.035
South Central 1	18	12	0.018 ± 0.01
South Central 2	5	3	0.025 ± 0.016
Central	26	10	0.016 ± 0.009
Central 1	15	3	0.001 ± 0.001
Central 2	11	7	0.013 ± 0.007
Eastern	95	46	0.028 ± 0.014
Northern	28	11	0.013 ± 0.007
Upper	22	8	0.008 ± 0.004
Lower	45	27	0.017 ± 0.009

Note: Nseq= number of sequences, Nh=number of haplotypes. Nucleotide diversity is given with standard deviations.

Table 3.5

Results of the six AMOVA models and three SAMOVA models showing how % variance is partitioned among groups, within groups, and within populations of D. conanti (SL)

Models/K	% variance among groups	% variance among populations within groups	% variance within populations
AMOVA			
1. Mississippi River	40.77	50.61	8.62
2. Biogeographic provinces (K=2)	10.3	78.48	11.22
3. Mississippi River & Eastern Continental Divide	42.82	48.45	8.73
4. Hydrologic units (K=6)	34.02	54.95	11.03
5. Major river drainages (K=15)	50.59	28.24	21.17
6. Level 3 Ecoregions (K=4)	36.17	52.8	11.03
SAMOVA			
3	51.55	39.58	8.87
7	64.64	24.5	10.86
8 *	67.48	21.61	10.91

Note: The proposed best model is indicated with an asterisk. Pairwise distances were used for both AMOVA and SAMOVA analyses. The significance of each AMOVA model was determined using 1,000 permutations in Arlequin ver. 3.5.2.2. Values of K from 1 to 18 were tested in SAMOVA 2.0 with 100 simulated annealing processes and 20,000 permutations, followed by 1,000 permutations.

Table 3.6

Significant linkage disequilibrium across loci and sampling sites after Bonferroni correction

	Locus					
Site #	*Dcon05*	*Dcon12*	*Dcon14*	*Dcon18*	*Dcon21*	*Dcon36*
7			A	A		
8						
9	A	A				
10						
12	B	A	B			A
21		AB	A			B
22		A		A	AB	
25			A			A
26						
27	A	A				
49						
61						
67						

Note: Loci in disequilibrium are indicated by letter pairs. *Dcon12* contains the greatest number of pairings. Significance was determined for each population using a Bonferroni adjusted $\alpha = 0.0034$.

Table 3.7

Summary allelic information for each of the 13 locations genotyped across six microsatellite loci

Site	N	N_A	Mean H_O	Mean H_E	F_{IS}	% Missing Data
7 Lower Leaf.	25	10	0.8059	0.8579	0.0600	16
8 Upper TN.	8	7	0.7232	0.8111	0.1142 *	12
9 Upper TN	12	9	0.6556	0.7495	0.1313 *	9
10 Upper TN.	5	5	0.6917	0.7525	0.0879	17
12 Upper Yazoo.	23	12	0.7552	0.8243	0.0858 *	10
21 Lower Leaf.	12	8	0.7866	0.8436	0.0718	10
22 Lower Leaf.	12	7	0.6838	0.8495	0.2043 *	22
25 Lower Pearl.	38	16	0.8694	0.9172	0.0529 *	9
26 Homochitto.	26	12	0.7183	0.8760	0.1858 *	22
27 Homochitto.	10	7	0.7706	0.8600	0.1089 *	7
49 Upper Pearl.	21	7	0.7928	0.7942	0.0020	36
61 Upper Leaf.	26	7	0.6349	0.7684	0.1774 *	15
67 Lower Pascagoula.	73	12	0.7890	0.8519	0.0744 *	7
	Mean	9	0.7444	0.8274	0.1044	

Note: Observed (H_O) and expected (H_E) heterozygosity were estimated using exact tests for each site and locus with Markov chains 1,000,000 steps in length and with a burn in of 100,000 steps. Fixation indices (F_{IS}) were determined for each population using 10,000 permutations, and the mean given is calculated from these values. Significant F_{IS} are indicated with an asterisk. Both analyses were performed in Arlequin ver. 3.5.2.2. % Missing Data indicates the number of genotypes missing from across loci and individuals for that population and was calculated in GenAlEx. N=number of individuals. N_A=mean number of alleles as calculated in GenAlEx..

Table 3.8

Pairwise population F_{ST} values by site as calculated in Arlequin ver. 3.5.2.2

	8	9	10	12	49	61	21	22	7	25	67	27
9	0.0861	0										
10	0.0310	0.0158	0									
12	0.1580	0.2177	0.1680	0								
49	0.1734	0.2132	0.2117	0.1748	0							
61	0.1732	0.2293	0.1793	0.1791	0.1297	0						
21	0.1471	0.2046	0.1278	0.1578	0.0505	0.1273	0					
22	0.1661	0.1951	0.1763	0.1183	0.0812	0.1043	-0.0102	0				
7	0.1168	0.1802	0.1198	0.1181	0.0496	0.0937	0.0085	-0.0032	0			
25	0.1035	0.1413	0.0996	0.1071	0.0526	0.0948	0.0606	0.0367	0.0343	0		
67	0.1456	0.1898	0.1378	0.1589	0.0651	0.0883	0.0918	0.0447	0.0527	0.0747	0	
27	0.1341	0.1982	0.1442	0.1022	0.09120	0.0720	0.0639	0.0257	0.0406	0.0600	0.0624	0
26	0.0949	0.1748	0.1371	0.0660	0.1084	0.0596	0.0416	0.0040	0.0290	0.0097	0.0353	-0.0182

Note: The data were permuted 1,000 times. All values are significant after Bonferroni correction ($p < 0.000641$) except those underlined. The locus *Dcon12* is excluded.

Table 3.9

Estimates of divergence time in millions of years based on the poikilothermic mitochondrial DNA clock (Hardy et al. 2002)

Clades	SC1	SC2	C1	Lower	Upper	N	DW	ACP1	C2	ACP2
SC2	7.6-2.9									
C1	11.2-4.3	9.4-3.6								
Lower	9.8-3.8	8.0-3.1	8.8-3.4							
Upper	10.2-3.9	9.0-3.5	10.2-3.9	4.0-1.5						
N	9.6-3.7	7.8-3.0	9.2-3.5	4.0-1.5	4.8-1.8					
DW	19.2-7.4	19.8-7.6	20.4-7.8	17.6-6.8	17.8-6.8	15.6-6.0				
ACP1	11.6-4.5	13.4-5.2	13.6-5.2	11.6-4.5	12.4-4.8	13.0-5.0	16.6-6.4			
C2	11.6-4.5	9.2-3.5	4.0-1.5	9.0-3.5	9.6-3.7	9.2-3.5	19.4-7.5	12.6-4.8		
ACP2	12.6-4.8	11.4-4.4	10.4-4.0	9.2-3.5	9.2-3.5	9.4-3.6	16.2-6.2	9.8-3.8	9.8-3.8	
UTN	14.0-5.4	12.2-4.7	15.4-5.9	12.0-4.6	12.0-4.6	12.2-4.7	19.2-7.4	11.6-4.5	14.2-5.5	10.4-4.0

Note: Calculated assuming an evolutionary rate of 0.5 – 1.3% sequence divergence per million years. SC1 = South Central 1; SC2 = South Central 2; C1 = Central 1; N = Northern; DW = Dark Ward; C2 = Central 2; UTN = Upper Tennessee River.

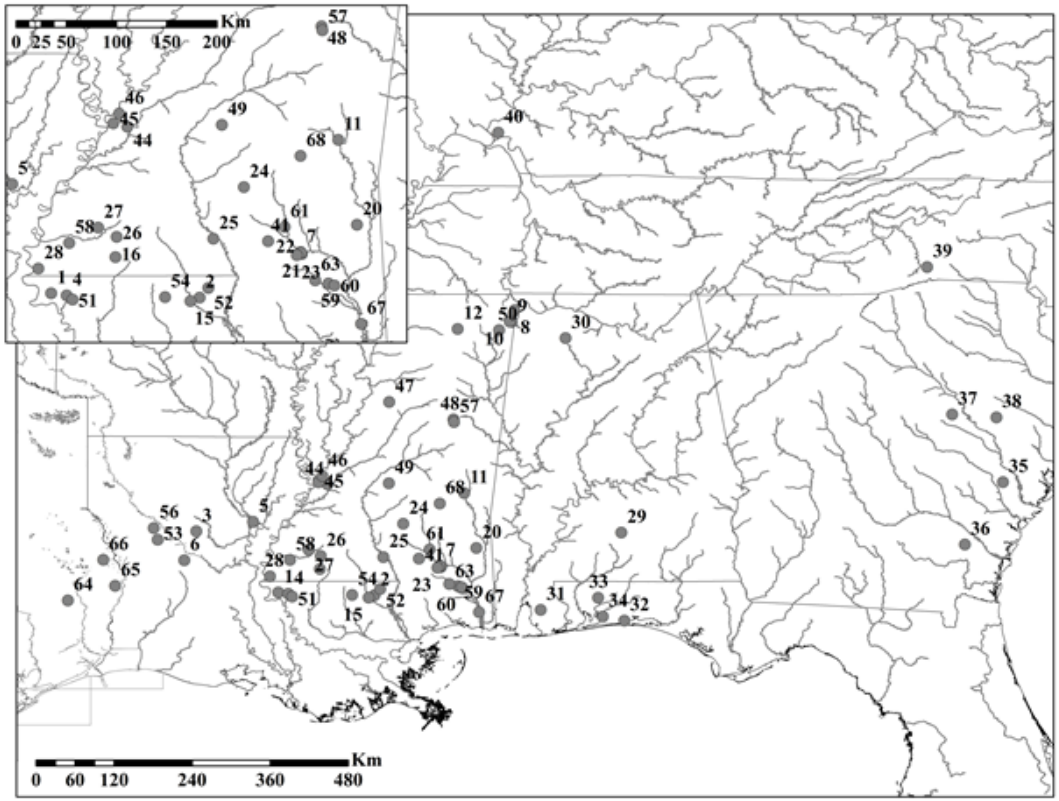

Figure 3.1. Sample sites for *D. conanti* (SL).

Any site from which sequences of *D. conanti* (SL) were obtained are included. Some sequences obtained from other sources lacked specific information, therefore GPS coordinates were approximated using county identity. Site numbers, which are not continuous, correspond with those in Table 1. The inset further focuses on the Lower Mississippi River Drainage and the Gulf Coastal Plain Drainage.

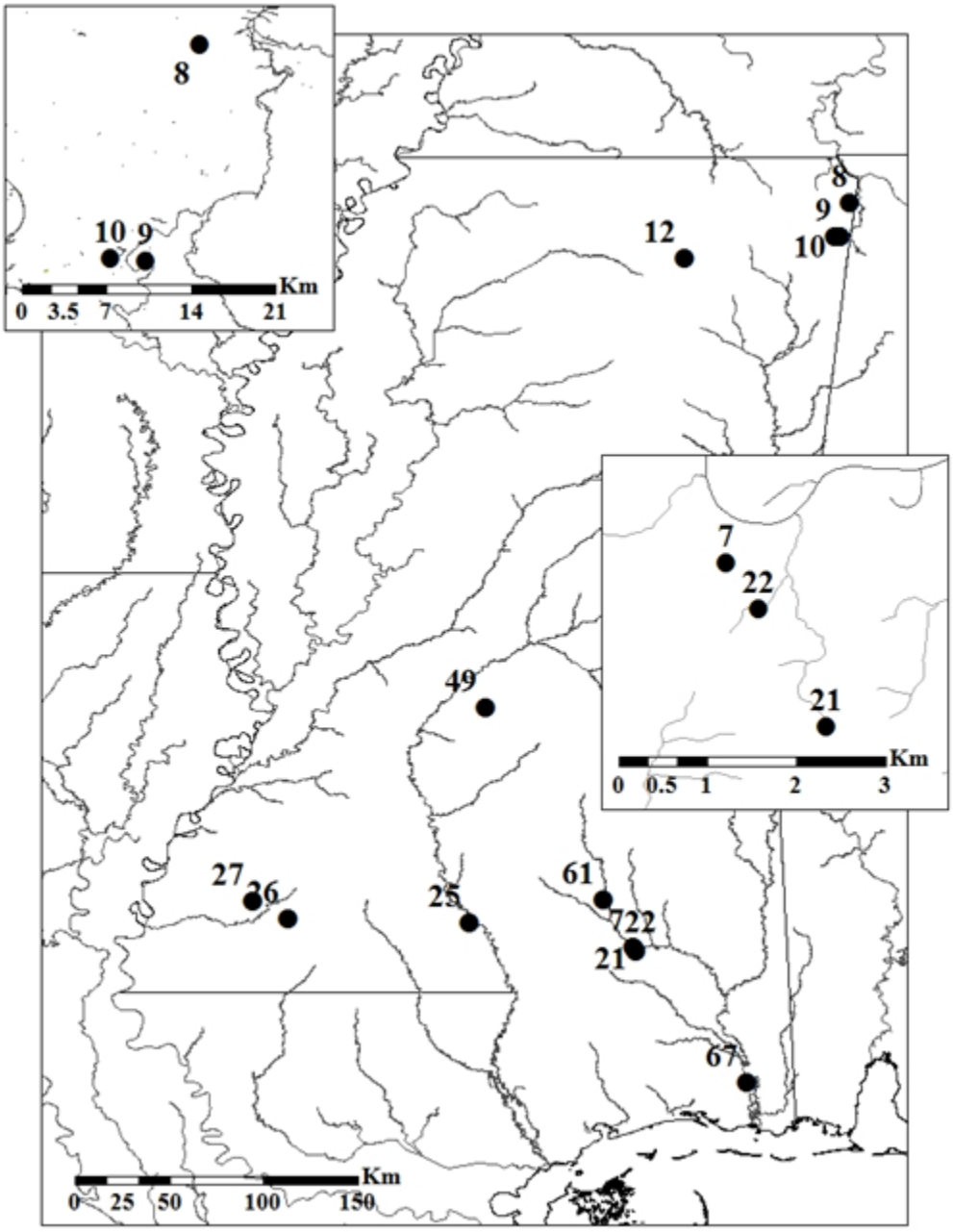

Figure 3.2. Sites at which populations of *D. conanti* (SL) were genotyped for six microsatellite loci.

Site numbers are the same as those in Table 1, as well as in Figure 1. The uppermost inset focuses on sites in the Lower Tennessee River Drainage and the lower inset on Ragland Hills, an area of relief along the Leaf River in the Pascagoula River Drainage.

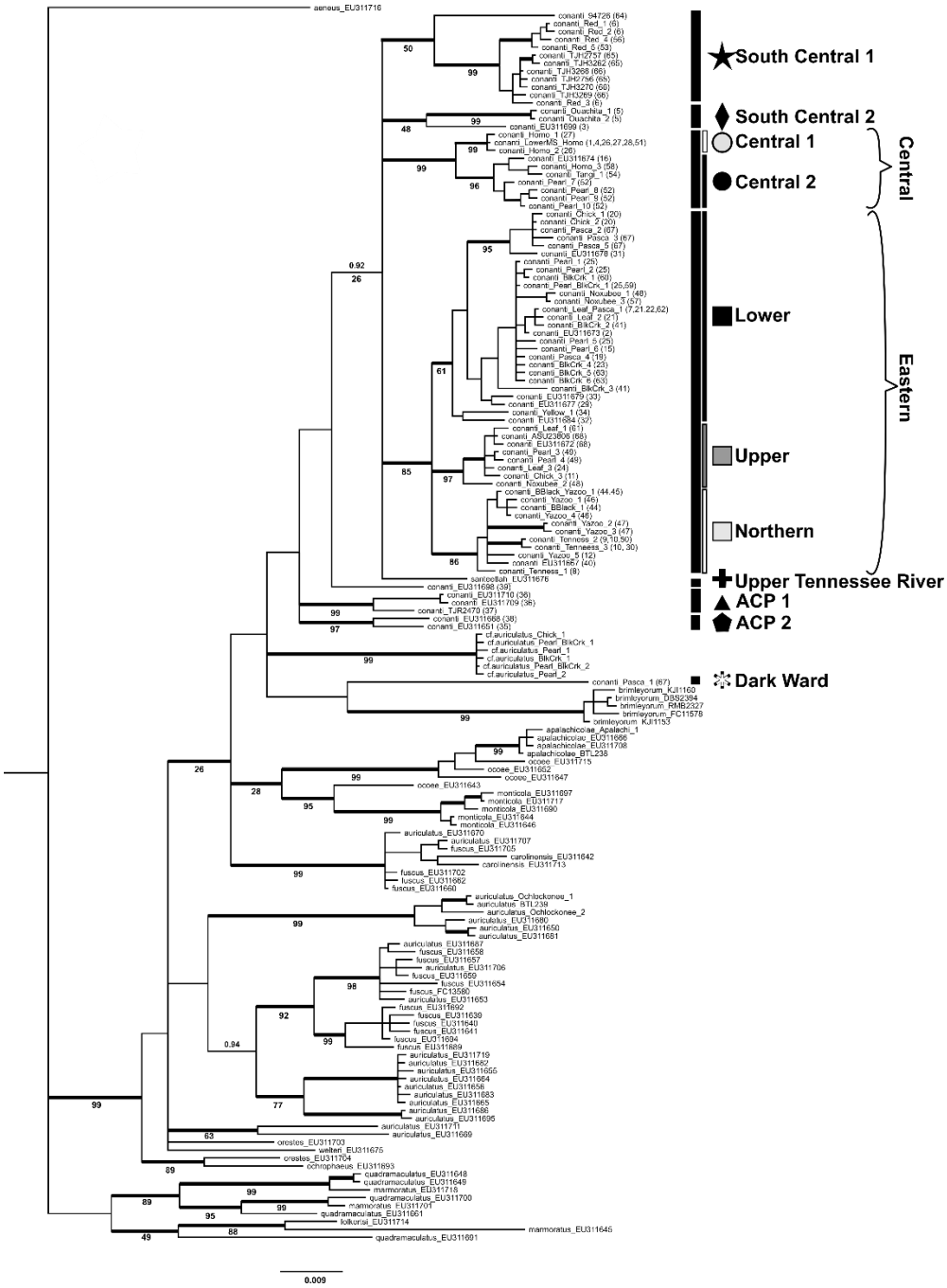

Figure 3.3. Bayesian 50% majority-rule consensus phylograms of the *cox1* dataset for *Desmognathus*.

Posterior probabilities are based on 25,251 post-burn-in trees which had an average marginal likelihood score of -7,061.33. Probabilities ≥0.95 are indicated by thick branches, and probabilities >0.90 for deeper nodes are noted. Bootstrap support values are given below branches for larger clades with probabilities ≥0.95. *Desmognathus aeneus* was used to root the tree. Symbols correspond with those on the map in Figure 4. Major (thick bars) and minor clades (thin bars) are identified to the right of the phylogram.

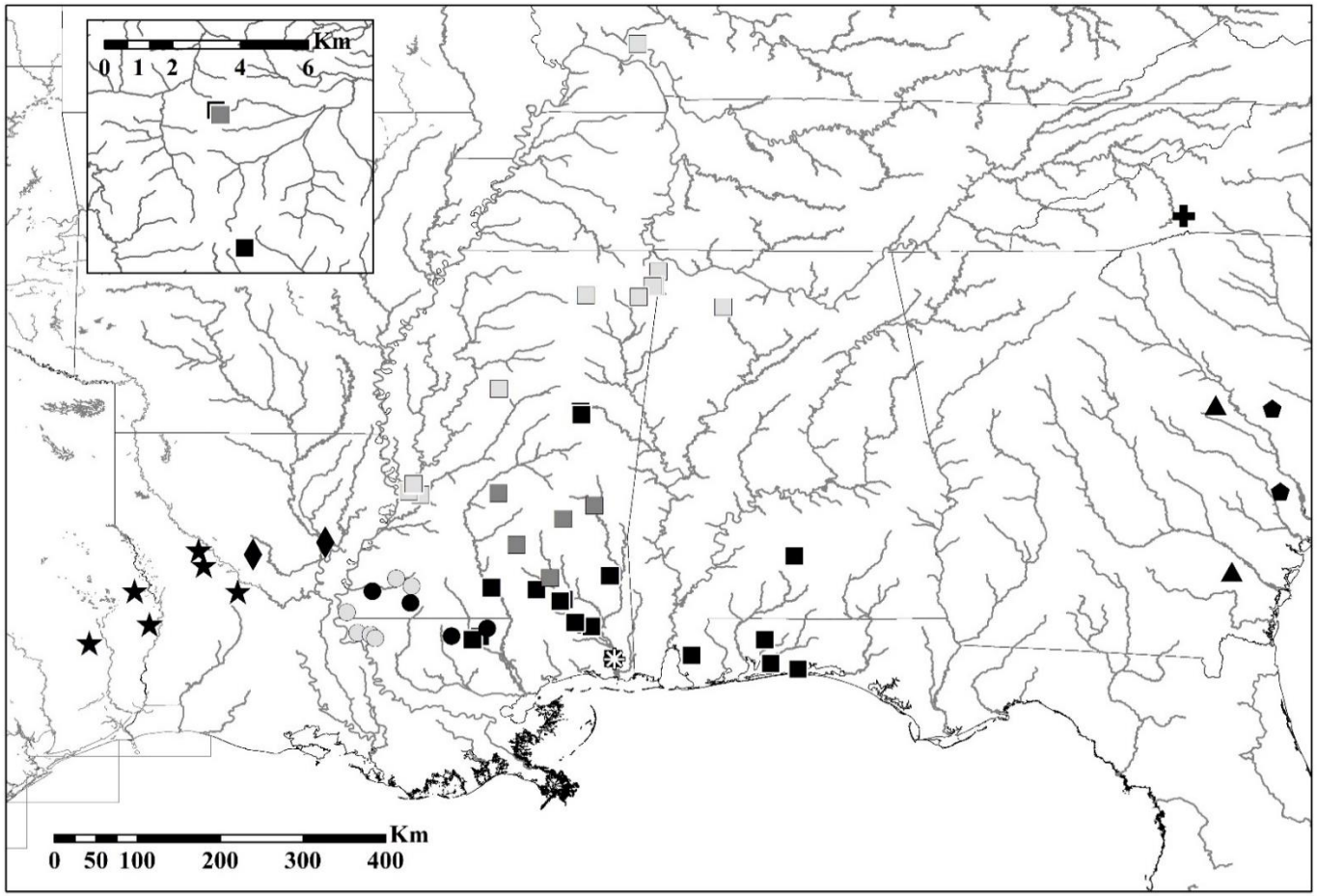

Figure 3.4. Distribution of major Bayesian clades and subclades across sampling sites for *D. conanti* (SL).

Symbols correspond with those opposite clades in Figure 3. The inset depicts sites 48 (northern) and 57 (southern). Two distinct lineages were found at both Site #48 (Lower and Upper; Noxubee River) and #67 (Lower and Dark Ward; Ward Bayou.

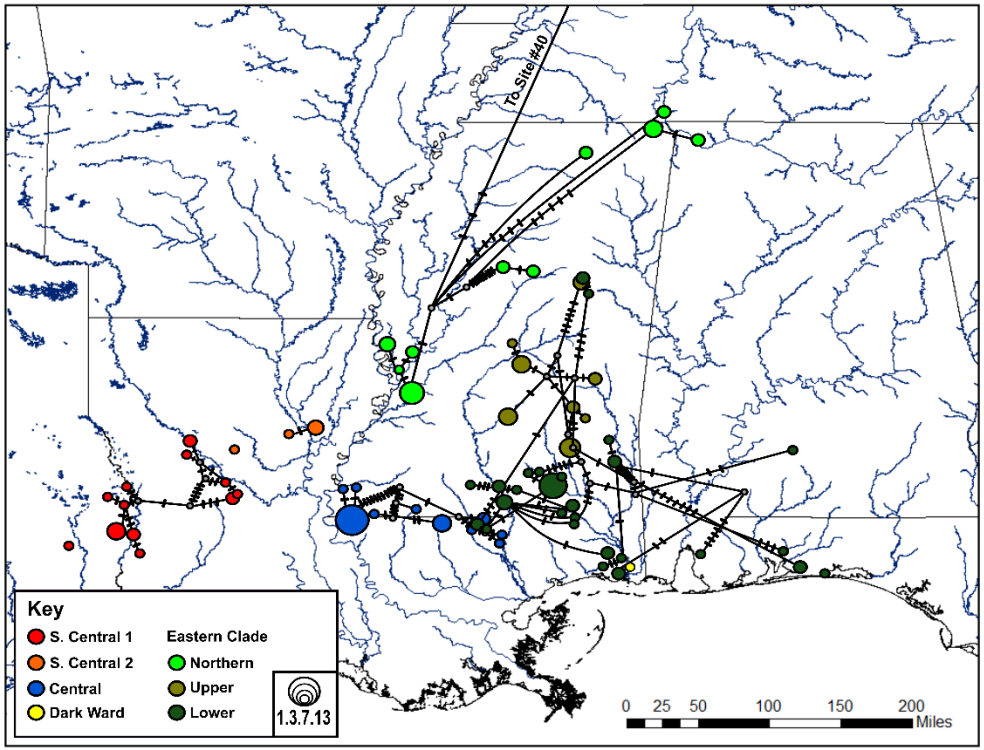

Figure 3.5. Statistical parsimony networks for haplotypes of *D. conanti* (SL) within the Lower Tennessee, Lower Mississippi River, and Gulf Coastal Plains Drainages roughly aligned by sample site on a drainage map.

Networks were generated in TCS 1.21 using a 91% statistical parsimony probability. Colors correspond with Bayesian clades. Depicted are 10 networks, including 4 singletons. Unconnected networks and singletons are separated by ≥14 mutational steps. Hash marks represent single mutations and small gray circles represent transitional haplotypes that were not sampled. Circle size corresponds with the number of sequences for that particular haplotype across the dataset.

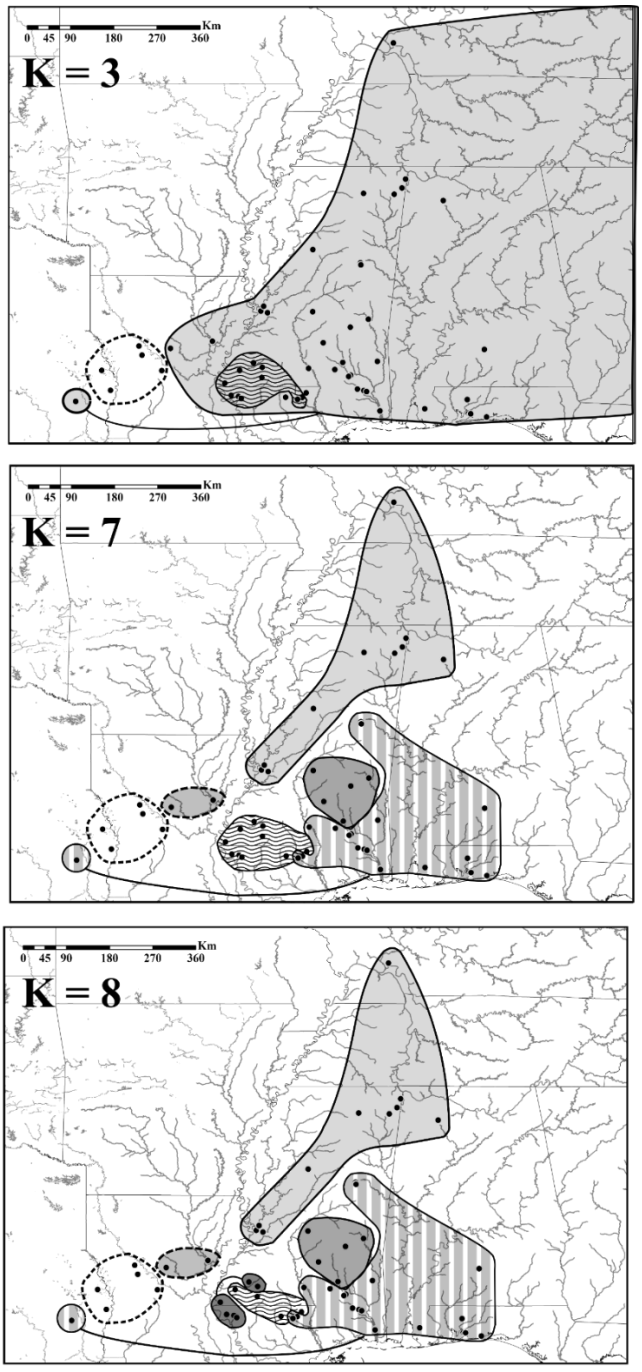

Figure 3.6. A selection of SAMOVA model results.

Panels depict those groups identified by SAMOVA models wherein K = 3, K = 7, and K = 8. These models had high $\Delta\Phi_{CT}$ scores and are explained in the text. The four Atlantic Coastal Plain sites and Upper Tennessee River sites are not depicted but are part of the largest group at K = 3, and form a single group in the K = 7 and K = 8 models.

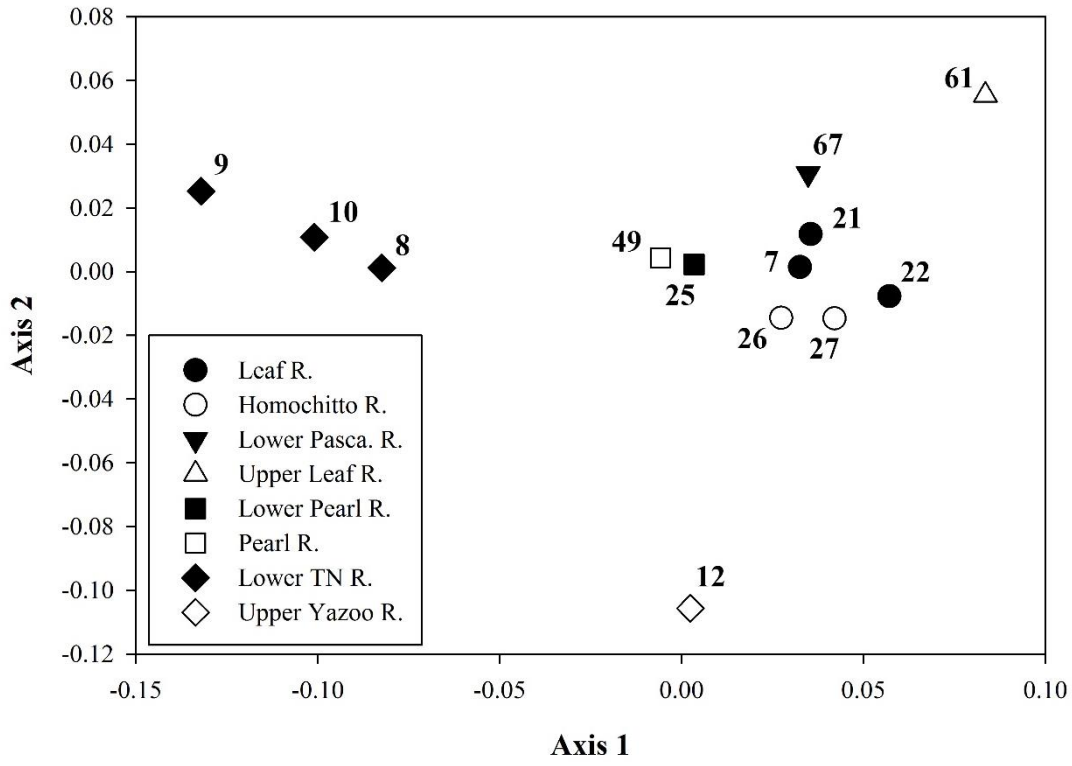

Figure 3.7. Principal coordinates analysis (PCoA) of pairwise population F_{ST} values between sites as calculated in Arlequin ver. 3.5.2.2.

Sites are numbered and described by drainage divisions. The PCoA explains a total of 79.98% of the variation among sites (Axis 1 = 61.82%, Axis 2 = 6.93%).

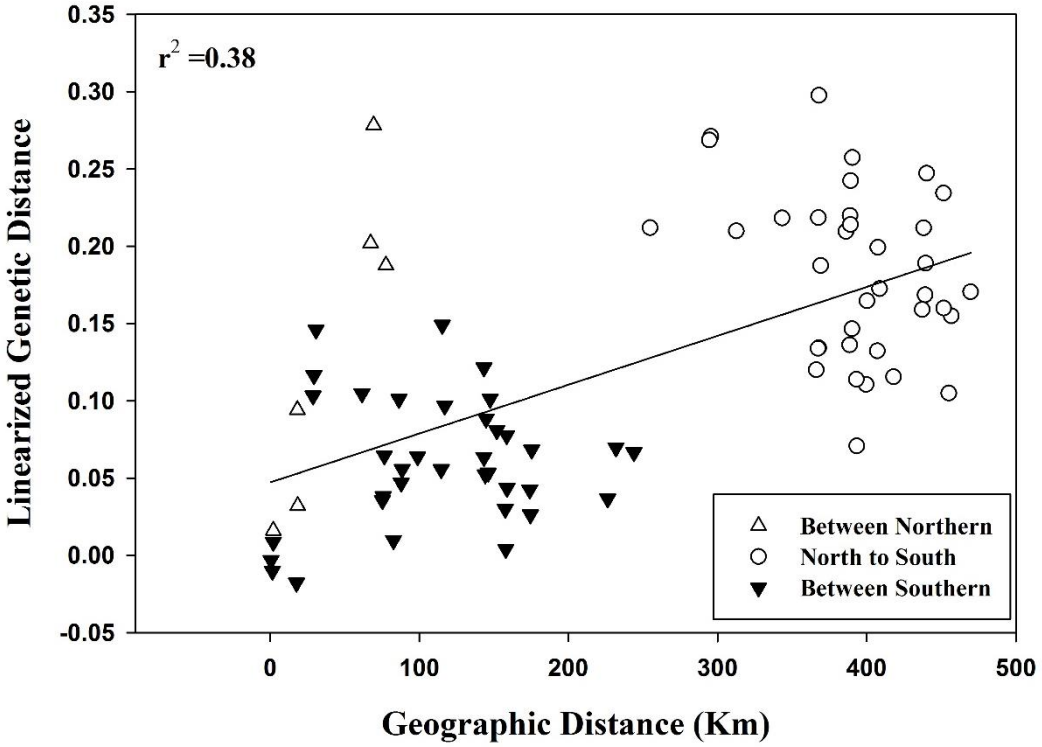

Figure 3.8. Linearized genetic distance (Rousset 1997) plotted against the geographic distance matrix.

Points are coded according to the type of site pairings. The trend line represents the significant correlation between the matrices according to the Mantel test ($r = 0.6182$; $p = 0.0010$).

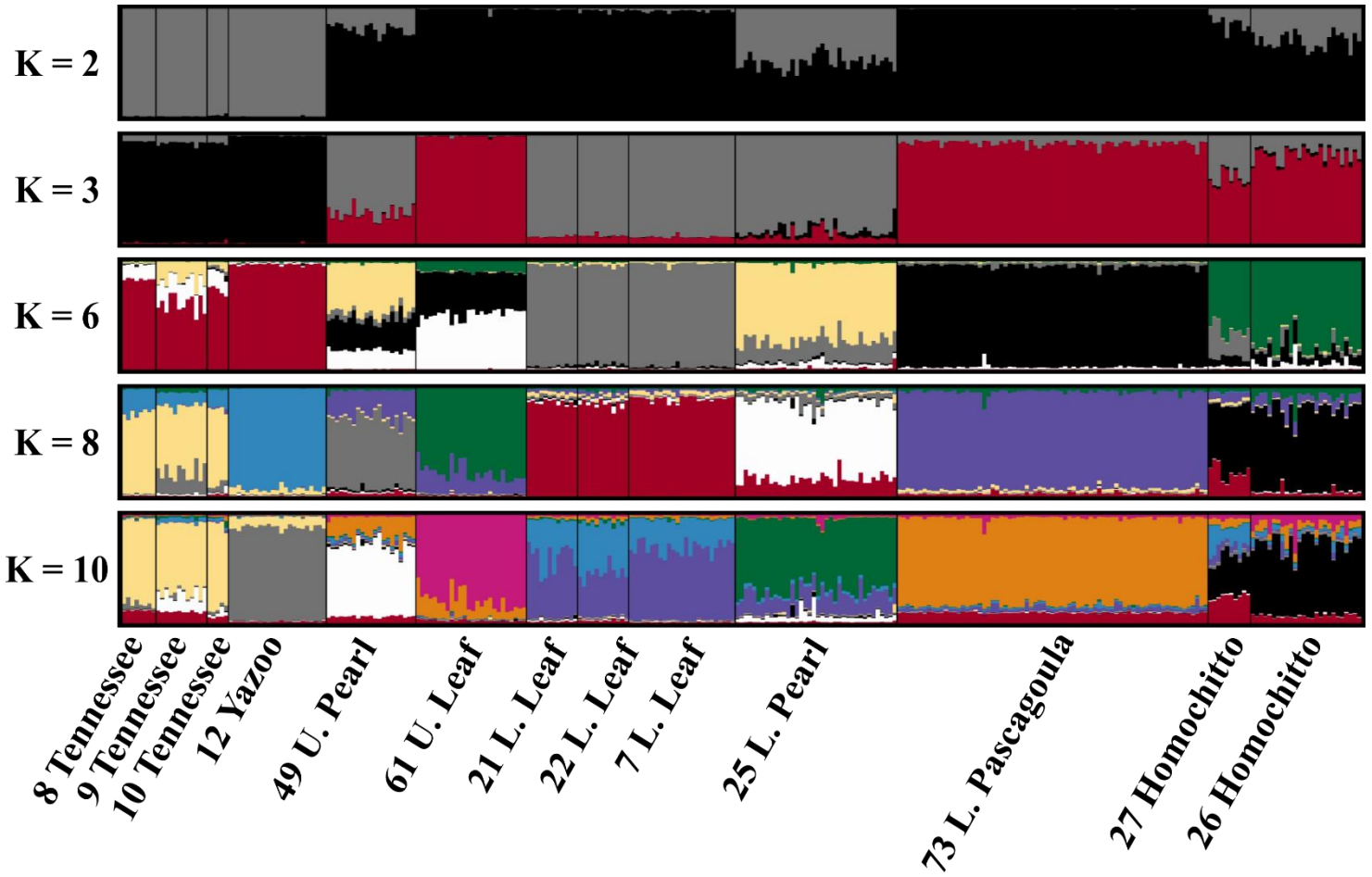

Figure 3.9. Bayesian clustering of individuals in STRUCTURE genotyped across six microsatellite loci using the full dataset (N = 291 individuals).

Black lines separate sample sites which are identified by number and drainage. Colors indicate different genetic groups.

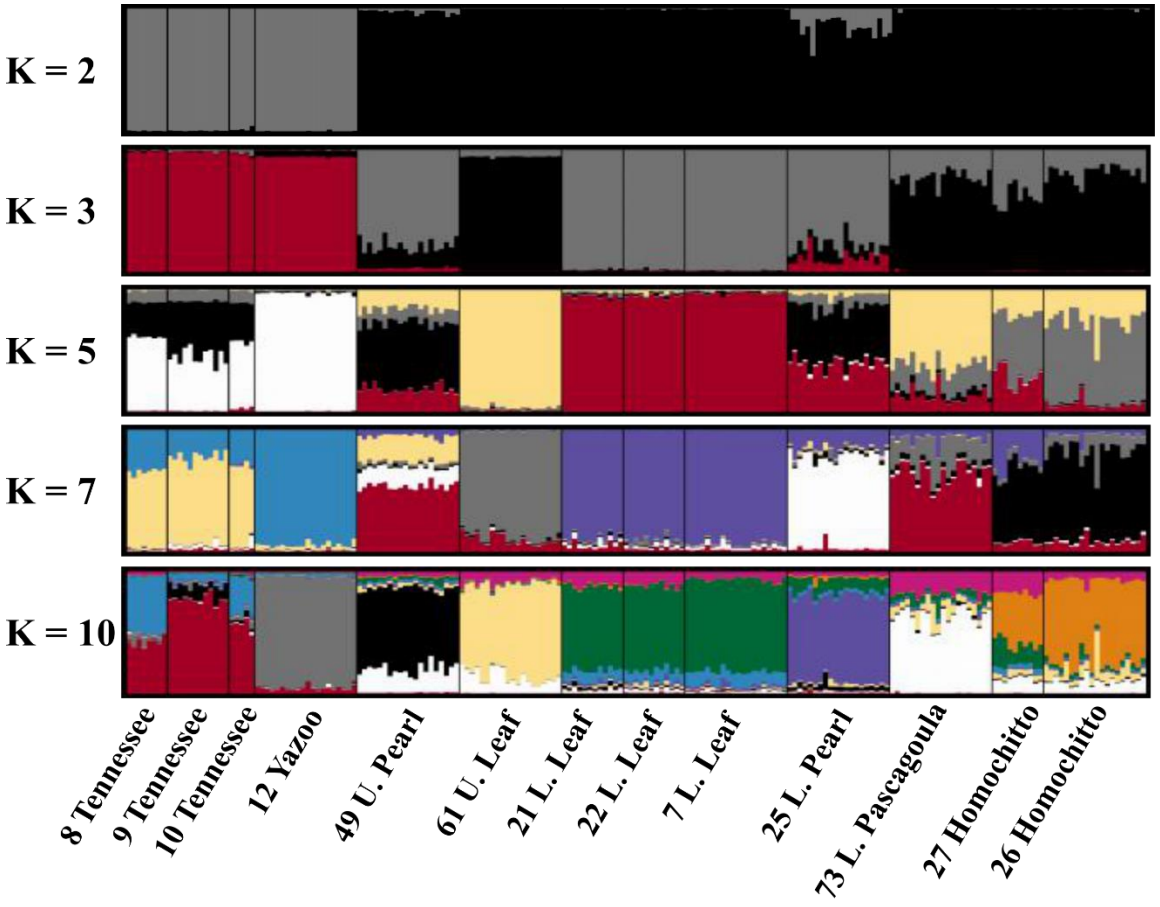

Figure 3.10. Bayesian clustering of individuals in STRUCTURE genotyped across six microsatellite loci using the subsampled dataset (N = 199 individuals.

Black lines separate sample sites which are identified by number and drainage. Colors indicate different genetic groups.

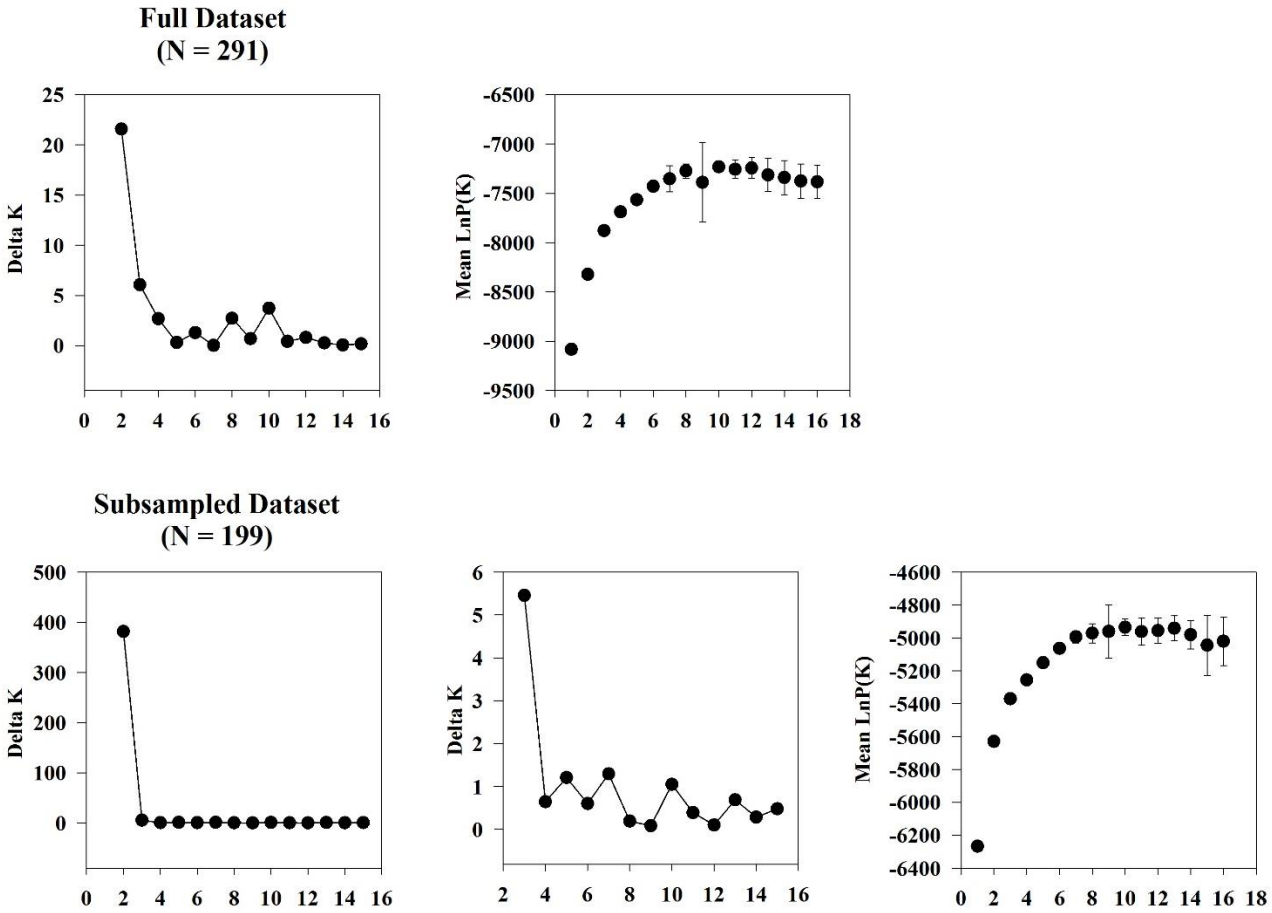

Figure 3.11. STRUCTURE HARVESTER ver. 0.6.94 results depicting values for ΔK (Evanno et al. 2005) and the mean log likelihood of K for the full and subsampled datasets.

Standard deviation estimates are provided for the mean log likelihoods, Mean LnP(K). The second Delta K plot for the subsampled dataset focuses on values of K from 2 – 16)

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APPENDIX A – INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE PROTOCOLS

The University of Southern Mississippi
Institutional Animal Care and Use Committee
118 College Drive #5147
Hattiesburg, MS 39406-0001
Tel: 601.266.6820
Fax: 601.266.5509
www.usm.edu/spa/policies/animals

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
NOTICE OF COMMITTEE ACTION

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the three year approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes (see attached) should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

PROTOCOL NUMBER: **11061301**
PROJECT TITLE: **Habitat Associations of Plethodontid Salamander Communities in Southern Mississippi**
PROPOSED PROJECT DATES: **06/15/2011 to 06/15/2014**
PROJECT TYPE: **New Project**
PRINCIPAL INVESTIGATOR(S): **Carl Qualls, Ph.D.**
COLLEGE/DIVISION: **College of Science & Technology**
DEPARTMENT: **Biological Sciences**
FUNDING AGENCY/SPONSOR: **N/A**
IACUC COMMITTEE ACTION: **Designated Reviewer Approval**
PROTOCOL EXPIRATION DATE: **09/30/2013**

Jodie M. Jawor, Ph.D.
IACUC Chair

6/15/11
Date

Figure A1. IACUC protocol number 11061301.

THE UNIVERSITY OF
SOUTHERN MISSISSIPPI.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
118 College Drive #5116 | Hattiesburg, MS 39406-0001
Phone: 601.266.4063 | Fax: 601.266.4377 | iacuc@usm.edu | www.usm.edu/iacuc

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE NOTICE OF COMMITTEE ACTION

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the three year approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes (see attached) should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

PROTOCOL NUMBER:	**13101702**
PROJECT TITLE:	**General Amphibian Collection and Housing**
PROPOSED PROJECT DATES:	**9/2013 – 9/ 2015**
PROJECT TYPE:	**New**
PRINCIPAL INVESTIGATOR(S):	**Carl P. Qualls**
DEPARTMENT:	**Biological Science**
FUNDING AGENCY/SPONSOR:	**NSF Graduate Research Fellowship Program**
IACUC COMMITTEE ACTION:	**Full Committee Approval**
PROTOCOL EXPIRATON DATE:	**September 30, 2015**

Frank Moore, Ph.D.
IACUC Chair

Date 10-23-13

Figure A2. IACUC protocol number 13101702.

THE UNIVERSITY OF
SOUTHERN MISSISSIPPI

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

118 College Drive #5116 | Hattiesburg, MS 39406-0001
Phone: 601.266.6791 | Fax: 601.266.4377 | iacuc@usm.edu | www.usm.edu/iacuc

NOTICE OF COMMITTEE ACTION

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

PROTOCOL NUMBER:	**15101508 (Replaces 13101702)**
PROJECT TITLE:	**Amphibian Collection & Housing**
PROPOSED PROJECT DATES:	**10/2015 - 09/2018**
PROJECT TYPE:	**Renewal**
PRINCIPAL INVESTIGATOR(S):	**Carl Qualls**
DEPARTMENT:	**Biological Sciences**
FUNDING AGENCY/SPONSOR:	**N/A**
IACUC COMMITTEE ACTION:	**Full Committee Approval**
PROTOCOL EXPIRATON DATE:	**September 30, 2018**

Frank Moore, PhD
IACUC Chair

10/01/2015
Date

Figure A3. IACUC protocol number 15101508.