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Population Structure of Central Stoneroller (Campostoma anomalum) on the Ozark Plateau in Arkansas and Missouri

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ABSTRACT

Stream fishes on the Ozark Plateau have been influenced both by historic events (i.e. vicariance versus dispersal) and ecological forces (i.e. flow regime). To examine the role of these processes, genetic structure of Central Stoneroller (*Campostoma anomalum*), an ecologically important omnivorous minnow with a broad distribution and elevated abundance, was evaluated across populations in the White River drainage of the Ozark Plateau in Arkansas and Missouri. Fin clips of five to eight individuals were taken at each of 20 sites (N=138 individuals; average=6.9), selected so as to represent two different flow regimes: intermittently flashy (N=10 sites; N=73 individuals; average=7.3/ site) and groundwater flashy (N=10 sites; N=65 individuals; average=6.5/ site). Two mitochondrial DNA genes (ATPase 6 and ATPase 8) were sequenced across 842 base pairs to provide comparative data. Genetic diversity within and among sites and flow regimes was evaluated using an analysis of molecular variance (AMOVA), and a Mantel test was conducted to compare genetic versus stream distance to test for isolation by distance patterns. Genetic structure was further explored through visualization with a haplotype network and a maximum likelihood analysis, where *Notropis stramineus* served (Sand Shiner) as outgroup. Potential historic population expansion within either flow regime was examined with a mismatch distribution. Based on the extent of genetic variability among populations, the analyses indicated that flow regime had no significant effect on genetic structure in the study species. Most sites appeared panmictic with individuals dispersing freely across the study area, at least historically, while three sites appear to be somewhat isolated. The haplotype network and ML-tree demonstrated that individuals are genetically similar. There is also a historic signal of population expansion in either flow regime. However, the presence of a rare lineage 7% diverged from Central Stoneroller indicates that diversity in the White River is more complex than originally thought, and that further studies are needed to delimit the distribution, define the species, and determine if co-occurring lineages are hybridizing.

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INTRODUCTION

Aquatic environments in North America are becoming increasingly impacted by modifications to instream flow (i.e. damming, channelization) and by abrupt changes in riparian land use (i.e. agriculture, logging, and urbanization; Blann et al. 2009). In the United States, approximately 31% of the surface area is classified as cropland, 3% is considered urban (Brown et al. 2005), and unaltered freshwater systems no longer exist (Naiman et al. 1995). Furthermore, only 42 rivers in the United States remain free flowing and are deemed as high quality lotic habitat (Benke and Cushing 2005, and references therein).

The extensive stream modifications have equally extensive effects. For example, excess logging depletes riparian zones, alters energy inputs, and increases bank erosion, sediment transport, and water turbidity (Gregory 2006). Impervious surfaces found in urban areas promote excessive runoff and this, in turn, elevates waste input and nutrient loading of aquatic ecosystems, often extending far beyond urban limits (Alexander et al. 2008; Haggard and Bartsch 2009). The freshwater fauna is adversely affected by these anthropogenic changes in that species have historically adapted to specific environmental conditions that have now been radically altered. In fact, sediment loading, increased temperatures, pollution, and hypoxia have reduced species richness and population sizes of fish in more than half of the rivers in the United States (National Research Council 1992). Extinction rates for freshwater organisms will soon exceed those for terrestrial and coastal faunas, particularly as development continues unabated (Argent et al. 2003). Thus, freshwater ecosystems are deteriorating, often too quickly for easily wrought solutions (Blann et al. 2009).

The Ozark Plateau of central North America retains some of the most pristine aquatic environments on the continent (Robison and Buchanan 1988). However, as above, these watersheds are increasingly impacted by changes in land use, primarily large-scale agricultural and industrial livestock

production, that in turn impact the diverse ichthyofauna of the region. Riparian modifications such as deforestation or channelization adversely affect stream morphology and flow, and induce a more homogeneous habitat and concomitantly reduce species richness (Wiens 2002). Elevated nitrogen levels that stem from livestock maintenance in these watersheds have also degraded many regional streams (Haggard et al. 2003). Organic waste enters the water column by seeping easily through the porous Mississippian karst limestone that forms the landscape of the region (Bonett and Chippindale 2004). The decomposition of this waste demands high levels of oxygen, depleting dissolved oxygen in regional streams and exerting undue stress on fish populations (Babcock et al. 1981; Dauwalter et al. 2008). Clearly, land use changes can have devastating effects on aquatic ecosystems, and are of particular concern in watersheds with unique and endemic fish communities.

Streams are characterized by the variability and predictability of flow, as modulated by climate, vegetation, and surrounding terrain (Poff et al. 1997). Flow regimes also influence the composition of freshwater fish communities and other biota by creating niches that promote particular life history stages and strategies (Olden 2009; Crow et al. 2013). Flow levels also correspond to the greatest variability found among freshwater fish communities (Poff 1995). Flow regime is associated with phenotypic variation and genetic diversity in fishes, with higher flows corresponding to steady cruise-swimming phenotypes, while lower flows (i.e. pools and lakes) favor burst-swimming performance (Langerhans 2008). Flow regulations from impoundments often provoke morphological responses in stream fishes, and Haas et al. (2010) noted that body shape of *Cyprinella venusta* (Blacktail Shiner) differed significantly between riverine and reservoir populations. Population genetic responses also adjust according to temporal changes in stream intermittency, with the greatest genetic similarities occurring between intermittent and subsequent flowing events (Rutledge et al. 1990). This effect could be the result of a population bottleneck during intermittency, with a reduced gene pool of colonizers for subsequent flowing conditions. Since biota are adapted to natural hydrographs (Naiman et al. 1995),

studying phylogeography of freshwater fishes in the context of flow variation offers an exceptional opportunity to evaluate how habitat, dispersal ability, and life history shaped the historic population demography of a species. Given that dispersal of fishes in riverine systems is linear, using habitat as a baseline is much more interpretable for freshwater fishes, particularly when compared to terrestrial ecosystems (Kanno et al. 2011). Consequently, an association between habitat and phylogeographic patterns in a species should be apparent.

Abiotic factors are important indicators of assemblage and population structure in stream fishes. Species richness decreases in intermittent streams but increases within perennial streams as dispersal pathways become more connected (Warfe et al. 2013). *Campostoma anomalum* (Central Stoneroller), an ecologically important and widespread freshwater minnow, exhibited distinct population structure in the Great Miami River Basin (Ohio), with genetic diversity decreasing in lower order sections as environmental stressors increased (Silbiger et al. 1998). Genetic structure also reflected a source-sink dynamic, with populations in streams with high habitat quality providing emigrants to populations in habitats with lower quality (Waits et al. 2008). Density of *C. anomalum* was negatively correlated with depth, and positively correlated with flood frequency, intensity, canopy openness, and temperature (Smith 2008). These components may correlate with abundance and thus would possibly be a response to stream primary production. Faster growth and a shift to more opportunistic reproductive strategies was also observed in *C. anomalum* inhabiting stream segments with greater environmental variation, as opposed to larger downstream segments with more stable flows (Spranza and Stanley 2000). Stanley et al. (2012) considered *C. anomalum* to be a fluvial specialist and an indicator species for connected riffle–pool habitat in central Texas.

Clearly, studies have documented how flow regimes contribute to community structure of freshwater fishes, and how abiotic factors affect the movement and abundance of *C. anomalum*, but few studies have evaluated the effects of flow regime on the genetic population structure of this

species. The objective of the current study was to evaluate potential effects of two different flow regimes on genetic diversity within and among populations of *C. anomalum*. Two flow regimes were selected: groundwater flashy (the most common regime found in Ozark streams; hereon referred to as groundwater) and intermittent flashy (a headwater stream component found throughout the Ozark region; hereon referred to as intermittent). Groundwater streams are characterized by a stable base flow and seldom dry completely, but water levels can vary throughout the year (Leasure et al. 2014). Intermittent streams frequently have running surface water across reaches throughout the year, with flows occasionally suspended (Poff et al. 1997; Leasure et al. 2014). Fewer species occur at sites with intermittent flow, as compared to those with more stable flows (Warfe et al. 2013), and assemblages in intermittent reaches usually comprise a subset of species found in perennial reaches, suggesting that community composition is indeed impacted by flow.

OBJECTIVES

The goal of this study was to calculate the genetic structure of *C. anomalum* in the White River of the Ozark Highlands, and to determine if statistical differences exist among populations found in groundwater versus intermittent flashy flow regimes. A second goal was to identify potential underlying processes that shape the observed genetic patterns, so as to provide guidance for management aimed at conserving the diversity of stream fishes in Arkansas. The following questions were thus explored: (1) Are observed patterns the result of historic processes, such as glaciation and subsequent stream capture? Or are they the result of more contemporary processes, and thus reflective of population bottlenecks and founder effects that stem from sharp variance in flow regime? Similarly, are intermittent streams recolonized by individuals from groundwater streams? Alternatively, does genetic structure reflect an isolation-by-distance model, where genetic divergence increases simply as a function of distance between sites?

***Hypotheses Under Test*.---** The following hypotheses were explored to address these questions:

$H_0$1: Genetic variability among populations of *C. anomalum* differs between groundwater and intermittent flashy flows.

H_A1: Genetic variability among populations of *C. anomalum* does not differ between groundwater and intermittent flashy flows.

$H_0$2: Population structure of *C. anomalum* does not differ between groundwater and intermittent streams.

H_A2: Populations structure of *C. anomalum* differs between groundwater and intermittent streams.

$H_0$3: Population structure of *C. anomalum* in tributaries of the White river is the result of isolation-by-distance processes.

H_A3: Population structure of *C. anomalum* in tributaries of the White river is not driven by geographic distances among sites.

H_04: Populations of *C. anomalum* in groundwater streams have undergone recent expansion.

H_A4: Populations of *C anomalum* in groundwater streams have not undergone recent expansion.

H_05: Populations of *C. anomalum* in intermittent streams have undergone recent expansion.

H_A5: Populations of *C. anomalum* in intermittent streams have not undergone recent expansion.

MATERIALS AND METHODS

The native fish community in the Ozarks comprises more than 175 species, 19 of which are endemic to the area (Petersen 1998). Some are of economic value while others are important due to their ecological roles and sensitivities to environmental disturbances. Consequently, such species are essential for ecosystem services and serve a critical function as mediators of habitat quality (Robison and Buchanan 1988).

The Study Species.--- Central Stoneroller (*C. anomalum*) is an abundant, widespread, sexually dimorphic, and omnivorous minnow that feeds primarily on algae (Cyprinidae; Matthews 1987). Breeding males develop tubercles on the anterodorsal region of the head and bright breeding colors on fins. The species is relatively vagile and inhabits both pools and riffles, but prefers cool, flowing water as found in gravel-bottomed headwater streams across the mid-western and eastern USA (Robison and Buchanan 1988). The abundance, widespread distribution, and ecological importance of this minnow make it an appropriate study species to gauge environmental and anthropogenic impacts on fishes in headwater streams (Landis 2009).

Sampling Methods.--- During summer 2014, 600 individuals of *C. anomalum* were collected at 20 sites in the White River drainage of the Ozark Plateau (northwest Arkansas and southern Missouri; Figure 1). Sites generally consisted of bedrock-to-pebble substrates with pool-riffle morphologies, and represented two different flow regimes: groundwater flashy and intermittent flashy (Table 1). Fishes were collected using a Smith-Root, Inc. LR-24 Electrofisher (200V) in stream reaches approximately 100 meters in length. Fin clips were sampled from each individual, placed in 95% ethanol, and stored at -20°C.

DNA Extraction, Amplification, and Sequencing.--- Genomic DNA was extracted from five to eight fin clips/ site at each of twenty sites (N=138 individuals; average=6.9), using the Qiagen DNeasy kit (QIAGEN Corporation, Maryland, USA) following manufacturer's instructions. Individuals were selected from each flow regime: intermittent (N=10 sites; N=73 individuals; average=7.3/ site) and groundwater (N=10 sites; N=65 individuals; average=6.5/ site). Extracted DNA was used as template in polymerase chain reaction (PCR) to amplify two mitochondrial (mt)DNA genes (ATPase 6 and ATPase 8) using conditions specified in Douglas et al. (2003). Amplification success was confirmed using a 1.5% agarose gel stained with GelGreen and visualized with a UV transluminator. Amplicons were cleaned using an Exonuclease 1/Shrimp Alkaline Phosphatase incubation prior to sequencing. Each sample was sequenced in forward and reverse direction using BigDye v3.1 terminators (Applied Biosystems, Inc). Sequences were resolved on an ABI 3730 automated sequencer at the W.M. Keck Center, University of Illinois-Urbana/Champaign.

Sequence Comparisons.--- Sequences were manually edited using Sequencher (version 4.1; Genecodes Corporation, Ann Arbor, Michigan, USA) and aligned using Mesquite (Maddison and Maddison 2015). Haplotypes were compiled from raw sequence data. Genetic diversity within and among sites was explored using DNASP (version 5.1, Rozas et al. 2003), with the following parameters calculated: number of polymorphic sites, haplotype diversity (*h*; a measure of the number and frequency of haplotypes in a population), nucleotide diversity (π; a measure of divergence among haplotypes in a population), average number of pairwise differences (k), and with Tajima's D (Tajima 1989) and Fu's F-statistic (Fu 1997) derived as a check for neutrality of sequence evolution, as opposed to selection. Divergence between lineages was calculated via p-distance in MEGA5 (Tamura et al. 2001).

Population Genetic Diversity and Mismatch Distributions.--- The distribution of genetic diversity among sites and flow regimes, as well as drainages (defined in Table 1), was characterized using pairwise F_{ST} values (derived from 16,000 permutations) in an AMOVA (Analysis of Molecular Variance) as

implemented in Arlequin (version 3.5, Excoffier et al. 2005). Significance was determined using Bonferroni corrected *p*-values. The association between genetic divergence (pairwise F_{ST}), flow regime, and geographic distance was estimated using a Mantel test (Mantel 1967) conducted using program *ecodist* in the R package (Goslee and Urban 2007). Geographic distances were computed as pairwise distances (in km) between all sites using QGIS (version 2.0.1, Quantum GIS Development Team 2013) and used to test for isolation-by-distance patterns. The correlation between genetic and geographic distances was further analyzed with a linear regression model in R (version 3.1.3, R Core Team 2014).

Mismatch distribution analyses (MDA; Rogers and Harpending 1992) were conducted in DNASP to test for population expansion in the study species, where mismatch is defined as the number of nucleotide differences between all pairs of individuals. Expansion leaves a relatively indelible molecular mark for a long period because populations converge but slowly to new equilibria. Hence, a large initial expansion will obscure for a considerable temporal period any effects of subsequent minor expansions. Conversely, if a population should reduce in size, it will quickly converge to a new equilibrium, thus making a reduction signature more difficult to detect (Rogers 1995). The R_2 statistic (Ramos-Onsins and Rozas 2002) was employed to assess statistical significance of MDAs.

Phylogenetic Analysis and Haplotype Network.--- The input dataset for phylogenetic analyses was composed of unique haplotypes. Models of sequence evolution were determined by jModelTest (version 2.1.6, Darriba et al. 2012), and choices were established by Akaike information criterion (AIC; Posada and Buckley 2004). The best-fitting model served as input for a maximum likelihood (ML) analysis (Felsenstein 1981) to infer an evolutionary tree, with *Notropis stramineus* as outgroup. Bootstrap tests for nodal support were conducted using ML in MEGA5 (Tamura et al. 2001). Bootstrap values above 70% were considered confident (Hillis and Bull 1993). A minimum spanning tree of haplotypes was constructed using TCS (version 1.21, Clement et al. 2005).

RESULTS

DNA Sequencing and Sequence Comparisons.--- Sequencing of 147 *C. anomalum* yielded 842 base pairs (bp) of the complete ATPase 6 and ATPase 8 genes with no insertions or deletions. The 35 unique haplotypes clustered into two distinct lineages (A and B), separated from one another at approximately 6.8 % (± 0.009 SD) sequence divergence. Lineage A was represented by 138 individuals, while lineage B was only detected in nine.

Across all 138 sequences in lineage A, 32 unique haplotypes were detected. Of the 842 sites, 3.7% were polymorphic, with 55% of these being parsimony informative (2% of total). Haplotype diversity was high (h = 0.895 ± 0.015 SD), while nucleotide diversity was low (π = 0.003 ± 0.000 SD) with an average number of 2.8 nucleotide differences (k) between sequences. Tajima's D-statistic and Fu's F-statistic were negative and not significant, indicating neutral sequence evolution (i.e., no selection; Table 2).

Lineage B showed less genetic diversity with only three haplotypes detected across nine individuals (Table 3). Only 0.4% of sites were polymorphic and none parsimony informative. Haplotype diversity, nucleotide diversity, and k were low (h = 0.417 ± 0.191 SD, π < 0.001 ± 0.000 SD, k = 0.7), indicating low divergence among haplotypes. Geographic distribution of haplotypes also appeared random (Table 3). Subsequent analyses focused only on lineage A.

Of the 32 haplotypes in lineage A (Appendix B), 20 were unique to a particular site, while 12 were shared among sites (Table 4). Haplotype distribution reflected phylogeographic structure in that three shared haplotypes only occurred in drainage A and four occurred in drainages B and D. Four common haplotypes occurred in both drainages, and constituted 59% of the individuals. The most common haplotype (detected in 24% of individuals) occurred in 15 of the 20 sites (Table 4).

Population Genetic Structure---The haplotype network reflected a starburst pattern with most haplotypes separated by a single nucleotide from the four common haplotypes. A hierarchical analysis of genetic variation (AMOVA) by flow regime indicated some level of isolation among sites, with 84% of genetic variation occurring within sites (p = 0.00), 15% between sites within flow regimes (p = 0.00), and only 1% between flow regimes (p =0.19; Table 5a). An analysis of genetic variation in drainages indicated similar results in that 83% of variation occurred within sites (p = 0.00), 9% between sites within drainages (p = 0.02), and 7% between drainages (p = 0.10; Table 5b). A pairwise distance matrix of F_{ST} values (Table 6) reflects additional but subtle genetic structure among sites. Three sites (i.e., Minks Creek, Spider Creek, and West Fork) located in intermittent streams at the headwaters of the White River, diverged from 3-6 other sites, all of which were geographically distant. The Mantel test (r = 0.11, P = 0.12) and the regression analysis (R^2 = 0.055, P < 0.01) revealed a minor relationship between genetic divergence and geographic distance (Figure 3).

Haplotype Network, Mismatch Distribution, and Phylogenetic Analysis.--- The starburst arrangement of haplotypes (Fig. 2) supports a hypothesis of rapid clade expansion and thus may mask subtle genetic structure induced by flow regime. The MDA analysis revealed similar patterns with regard to historical changes in population size and indicated that *C. anomalum* populations in the study area trend towards expansion (i.e., a relatively unimodal Poisson distribution was derived for observed vs. expected curves; Fig. 4). However, the R_2 statistic (R_2 = 0.043, P = 0.064) revealed a non-significant MDA analysis, suggesting that the population has not undergone substantial expansion and/or that expansion may be relatively recent. Model selection identified Tamura-Nei as the best model of sequence evolution for the two genes. The ML tree (Fig. 5) was rooted at *N. stramineus* and depicts two well-differentiated clades (lineage A and B).

DISCUSSION

The Study Species.--- *Campostoma anomalum* extends from central to eastern United States (Matthews 1987; Burr 1980), with a possible range expansion into the Hudson River (Schmidt et al. 2007). It is extremely abundant in small to medium sized streams composed of rocky substrate, and is recognized as being ecologically tolerant, a factor that promotes its numerical dominance in many fish communities (Matthews 1987). Several studies have attempted to resolve the taxonomy of the genus (Buth and Burr 1978; Cashner et al. 2010), yet a definite resolution has not been reached (see below). Three (of five) recognized lineages (i.e., *C. a. pullum, C. a. anomalum,* and *C. a. michauxi*) are geographically distinct and in fact should be elevated to species rank (Blum et al. 2008; Cashner et al. 2010).

Campostoma anomalum has been the focus of several important ecological and phylogenetic studies in midwestern North America. For example, ecosystem-level studies have assessed the impact of the species on algal cover in streams, thus evaluating its direct impacts on nutrient uptake and release (Matthews et al. 1987; Power et al. 1988). Notable population studies revealed significant structure in response to various environmental factors, including stream order and land use changes (Husemann et al. 2012; Waits et al. 2008; Silbiger et al. 1998). Other studies of *C. anomalum* (Blum et al. 2012) found that linearized F_{ST} distances correspond significantly with geographic distance, suggesting the involvement of isolation-by-distance in the geographic structuring of populations.

Hypotheses Relating to Stream Flow.--- This study measured the phylogeographic relationships among populations of *C. anomalum* within the White River drainage of the Ozark Mountains. The hypotheses being tested were in the context of whether population structure and genetic variability of *C. anomalum* differed according to stream location and flow characteristics (i.e., groundwater flashy vs. intermittent flashy). Also of interest was the extent that isolation-by-distance occurred with regard to population

differentiation, and whether populations in either flow regime (as above) have experienced recent expansions.

The results of this study rejected the hypothesis that populations of *C. anomalum* from groundwater flashy and intermittent flashy streams differed in their genetic structure and levels of variability. The AMOVA results demonstrated that most variation occurs within individual sites (F_{ST}), whereas the least amount occurred between flow regimes (F_{CT}). Although this would indicate connectedness between sites, there was some level of isolation between sites within flow regimes (F_{SC}). This is expected as gene flow would be inhibited both by distance as well as by intervening unfavorable habitat (i.e. lakes and dams). Similar results were found when analyzing genetic variability between drainages. Most of the variation occurred within sites and some occurred between sites within drainages, as expected. There was no significant variation between drainages, however. The AMOVA results are consistent with the pairwise F_{ST} analysis indicating that flow regime did not significantly impact the distribution of genetic variability in *C. anomalum*. Although three of the intermittent sites showed significant divergence from other more geographically distant populations, it is likely that these three sites are swaying the level of variation between sites within flow regime and drainages to be significant.

Previous Studies of Population Structure and Ecology in C. anomalum.--- This outcome is inconsistent with previous phylogenetic studies of flow and population structure in *C. anomalum*. For example, Silbiger et al. (1998) measured genetic diversity of *C. anomalum* in the Great Miami River Basin (Ohio) using random amplified polymorphic DNA (RAPD) and found that genetic diversity was positively correlated with stream order, such that the least amount of diversity occurred in lower order and less stable streams. Similarly, populations most vulnerable to stressors were those found in more isolated streams (Silbiger et al. 1998), a situation that could eventually diminish genetic variability and elevate

inbreeding and genetic drift (Wright 1931), particularly if additional stressors were encountered. Waits et al. (2008) found that streams in more developed urban areas of southwestern Ohio not only exhibited populations of *C. anomalum* with less genetic diversity, but the observed haplotypes were actually just a subset of those found in less urbanized streams. Put simply, populations of *C. anomalum* in higher quality habitats acted as a source for those populations in lower quality habitat (i.e., source-sink dynamics of population structure). In all cases, significant structure was detected, and stream characteristics were a major determining factor.

The contrasting results between this and previous studies could be explained by the resolution of the molecular marker used to detect population structure. Mitochondrial DNA can be insufficient in detecting fine scale structure, as geographically isolated populations can often be linked through ancestral polymorphisms (Avise 2000). This means that although populations have been potentially separated for thousands of years, they still retain a high probability of shared haplotypes, in that not enough time has elapsed for haplotypes to become differentiated within each.

Horne et al. (2008) advises against hastily interpreting genetic structure provided by a single molecular marker as being weak, in that a marker with more contemporary resolution may detect finer scaled structure not detectable by the resolution of the more coarse marker. It would thus be beneficial to re-evaluate these hypotheses using faster evolving molecular markers, such as highly variable microsatellite DNA (Li et al. 2002). Such markers would have a much higher resolution than mtDNA in detecting intraspecific genetic structure. Given the current results, *C. anomalum* within the White River drainage is viewed as a panmictic species, with the relatively pristine habitat of the Ozark Plateau offering minimal barriers to its gene flow. This interpretation is also supported by the presence of shared haplotypes in *C. anomalum* that extend across distant localities. Again, these patterns can be further validated by a comparative phylogeographic study using numerous species, or alternatively, through the use of microsatellite DNA markers in a single study species (Horne et al. 2008).

Husemann et al. (2012) validated the importance of using multiple species as a comparative approach to determine the underlying processes influencing landscape genetics. Their comparative biogeographic study evaluated five study species, including *C. anomalum*, with the mitochondrial D-loop as a molecular marker. All study species demonstrated contrasting responses to historical and anthropogenic forces. Specifically, *C. anomalum* exhibited signs of frequent population bottlenecks and subsequent expansions during the dry season, making the impacts of historical events much more difficult to interpret. A single study species, especially one such as *C. anomalum* that is ecologically tolerant, may not be a good assay for the elucidation of general patterns across biogeographic scales. Thus, an investigation employing multiple species that share a distribution similar to that of *C. anomalum* in the White River drainage would be a much better evaluator of freshwater fish population structure in the Ozark region.

Isolation by Distance, Haplotype Networks, and Mismatch Distributions.--- The hypothesis that isolation by distance promotes genetic structure was rejected, given a non-significant Mantel result and evidence from linear regression that highlighted the scant population structure in *C. anomalum*. These results, much like the AMOVA analysis, indicate negligible barriers to gene flow in the study species. In addition, the non-significant result of the Mantel test in the initial analysis precluded the use of partial Mantel tests in further dissecting the influence of flow regime on population genetic structure.

Results from the haplotype network, molecular diversity analyses, and the mismatch distribution collaboratively emphasize the fact that populations of *C. anomalum* in the White River have undergone recent expansion. The starburst pattern displayed by the haplotype network, concomitant with high values for *h* (haplotype diversity) and low values for π (average pairwise nucleotide differences), demonstrate a rapid increase in the number of rare but closely related haplotypes (Grant and Bowen 1998), again suggesting a population expansion for *C. anomalum* in the White River

drainage. The more common haplotypes located in the central part of the network, with multiple connections outward to more rare haplotypes, suggest that the central, common haplotypes are more ancestral, and provide the basis from which the rarer haplotypes then emerge, differing only by one or a few base pairs (Grant and Bowen 1998).

A mismatch distribution analysis revealed a unimodal pattern consistent with population expansion as well (Rogers and Harpending 1992). A lack of significance with the R_2 statistic, like that for the Fu's F statistic, may indicate a relatively weak signal that cannot be recognized statistically. Alternatively, the sample size may be too small in that it hindered the computation of historic changes in population size (Douglas et al. 2006). It is also possible that these populations experienced a strong selective sweep, as the two events are often indistinguishable from one another, particularly when these analytical methods are employed. A selective sweep yields a haplotype pattern similar to that found when neutral variants of phenotypically advantageous alleles accumulate in the population (Brookfield 2001).

Cryptic Variation in C. anomalum.--- An unexpected result was the identification of two lineages separated by approximately 7% sequence divergence. Lineage B likely represents a second and more cryptic taxon, potentially a second species that co-occurs with lineage A. Although found at multiple locations, it is also very rare in the White River, comprising only 6% of total individuals evaluated. More studies are needed to determine the actual distribution of this lineage and whether it is a separate species. *Campostoma spadiceum* (the Highland Shiner; Cashner et al. 2010) is a newly described species near to but not confirmed from the current collecting sites. Cashner et al. (2010) note the following: "To the north, within Arkansas, there is a distinct difference in taxa found in south-draining tributaries of the Arkansas River (all *C. spadiceum* so far as we know), and north or east-draining tributaries of the White

River [all *C. a. pullum* or *C. oligolepis* (Largescale Stoneroller)]" (p. 305). Given this information, it would be unusual if indeed Lineage B was actually *C. spadiceum*.

Alternatively, Lineage B is the result of a vicariant event that separated isolated populations from other *C. anomalum* and started the speciation process (Horne et al. 2008). A third explanation would be the occurrence of hybridization between two syntopic species, a phenomenon noted by numerous studies within this region. For example, Rakocinski (1980) found evidence of limited hybridization and introgression between *C. oligolepis* and *C. anomalum,* where sympatric in the Ozark Plateau. Another account noted an instance of heterogeneric spawning between *C. anomalum* and *Nocomis leptocephalus* (Maurakis and Woolcott 1998). Furthermore, Bossu and Near (2009) indicated an occurrence of introgression between *Etheostoma spp*. in the Ozark Plateau region. Again, additional studies are required to determine if the two lineages are hybridizing or simply co-occurring.

It is possible that hybridization has occurred among several species in the Ozarks, given the paleogeographic history of the region (Appendix A). Historically, it was relatively untouched by impacts of Pleistocene glaciation, and given this, the fauna has had a longer temporal period to diversify and subsequently colonize previously glaciated regions. Therefore, the Ozark region served as a glacial refuge and was habitable for a much longer period, thus increasing the probability of diversification or hybridization.

Additional analyses are needed to delimit the observed genetic diversity and phylogeographic history of *C. anomalum* so as to test for hybridization events. Several studies comparing gene trees derived from two types of markers concluded that using one marker to investigate hybridization would have prompted misleading interpretations (Shaw 2002; Bachtrog et al. 2006). If lineage B is indeed the result of hybridization between *C. oligolepis* and *C. anomalum*, as Rakocinski (1980) has observed, perhaps conservation efforts should go towards identifying and maintaining the diversity and viability of *C. oligolepis* populations on the Ozark Plateau. Due to stricter habitat requirements and its relative

numerical paucity, this lineage is clearly more vulnerable to extinction than the more abundant *C. anomalum* (Burr and Smith 1976).

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TABLES

Table 1: Overview of 147 *Campostoma anomalum* specimens used to determine population structure across 20 sites in the White River drainage on the Ozark Plateau in Arkansas (AR) and Missouri (MO). Sampling sites represent two flow regimes and are listed alphabetically; geographic distribution of sites is provided Figure 1. Listed for each site are: Site = locality acronym; Flow = flow regime, with GW = groundwater flashy, and IM = intermittent flashy; Drainage A-J; N = number of specimens; Locality = stream name, and locality information with state, county and latitude (N) / longitude (W).

Site	Flow	Drainage	N	Locality	State	County	Latitude (N)	Longitude (W)
BRUS	GW	A	8	Brush Creek	AR	Washington	36.131211	93.948083
CLCR	GW	B	8	Clear Creek	AR	Boone	36.122016	92.903423
COWS	GW	C	8	Cowskin Creek	MO	Douglas	36.965145	92.726148
HOGC	GW	B	8	Hog Creek	AR	Boone	36.151714	92.960925
HUZZ	GW	B	6	Huzzah Creek	AR	Boone	36.231834	92.990015
LIBE	GW	C	8	Little Beaver	MO	Taney	36.799673	92.908690
SUGA	GW	B	5	Sugar Orchard	AR	Boone	36.291167	92.919110
SULO	GW	D	5	Sugar Loaf Creek	AR	Boone	36.387254	92.970669
SWAN	GW	C	8	Swan Creek	MO	Taney	36.700713	93.097324
SYLA	GW	E	8	Sylamore Creek	AR	Stone	35.994070	92.210800
CHER	IM	A	7	Cherry Creek	AR	Madison	35.989033	93.827983
FLAT	IM	F	8	Flatrock Creek	AR	Newton	36.075829	93.103605
LONG	IM	G	7	Long Creek	AR	Boone	36.292399	93.282247
MAST	IM	A	8	Mast Creek	AR	Washington	35.853857	94.004442
MINK	IM	H	7	Minks Creek	AR	Madison	35.893545	93.582264
OSAG	IM	I	8	Osage Creek	AR	Boone	36.163822	93.353172
SPID	IM	J	8	Spider Creek	AR	Carroll	36.441026	93.841951
TOWN	IM	A	8	Town Branch	AR	Washington	36.044794	94.176363
WEFO	IM	A	8	West Fork	AR	Washington	35.848494	94.105501
WHAR	IM	A	6	Wharton Creek	AR	Madison	36.021875	93.631034

Table 2: Molecular diversity in 138 *Campostoma anomalum* representing lineage A and sequenced across 842 base pairs encompassing the entire ATPase 8 and 6 genes. Samples were collected from 20 sites in the White River drainage, Ozark Plateau, Arkansas and Missouri. Site information is provided in Table 1. Listed for each site are: Site = locality acronym; N = sample size; H = number of haplotypes; h = haplotype diversity (SE = standard error); π = nucleotide diversity (SE = standard error); k = average number of nucleotide differences (SE = standard error); as well as Tajima's D and Fu's F statistics. Detailed locality information is provided in Table 1.

	Lineage A								
Site	**N**	**H**	***h***	**SE**	**π**	**SE**	**k**	**Tajima's D**	**Fu's F**
BRUS	5	4	0.90	0.16	0.0036	0.0007	3.0	0.29	-0.33
CLCR	8	6	0.90	0.11	0.0032	0.0006	2.7	-0.64	-1.88
COWS	7	4	0.81	0.13	0.0022	0.0005	1.8	-0.56	-0.32
HOGC	8	5	0.90	0.10	0.0023	0.0006	2.0	-0.70	-1.19
HUZZ	6	5	0.90	0.12	0.0030	0.0008	2.5	-1.01	-1.62
LIBE	8	3	0.70	0.01	0.0020	0.0004	1.6	1.73	1.16
SUGA	5	5	1.00	0.13	0.0036	0.0005	3.0	-0.75	-2.24
SULO	5	4	0.90	0.16	0.0024	0.0007	2.0	0.70	-1.01
SWAN	8	3	0.50	0.20	0.0022	0.0010	1.9	-0.92	1.41
SYLA	5	5	1.00	0.13	0.0036	0.0008	3.0	0.29	-2.24
CHER	7	5	0.91	0.10	0.0041	0.0008	3.4	0.26	-0.44
FLAT	8	5	0.90	0.10	0.0023	0.0005	1.9	0.00	-1.23
LONG	6	3	0.80	0.12	0.0032	0.0005	2.7	1.22	1.57
MAST	8	5	0.90	0.09	0.0042	0.0007	3.5	0.71	0.06
MINK	7	3	0.50	0.20	0.0009	0.0004	0.8	-0.27	-0.44
OSAG	8	5	0.80	0.20	0.0025	0.0005	2.1	0.50	-1.00
SPID	8	4	0.80	0.11	0.0025	0.0008	2.1	-0.49	0.23
TOWN	8	6	0.90	0.11	0.0031	0.0006	2.6	-0.10	-1.91
WEFO	8	4	0.80	0.11	0.0021	0.0008	1.8	-1.06	-0.07
WHAR	5	4	0.90	0.16	0.0045	0.0011	3.8	0.91	0.05
	138								

Table 3: Distribution of mitochondrial DNA haplotypes representing Lineage B (B1-B3) in *Campostoma anomalum* and derived from sequencing 842 base pairs encompassing the entire ATPase 8 and 6 gene. Samples were collected from 20 sites in the White River drainage, Ozark Plateau, Arkansas and Missouri. Listed for each site are: Site = locality acronym; N = number of samples; H = total number of haplotypes; U = number of unique haplotypes. Detailed locality information is provided in Table 1.

				Lineage B		
Site	**N**	**H**	**U**	**B1**	**B2**	**B3**
BRUS	3	1	0	3	-	-
CLCR	0	-	-	-	-	-
COWS	1	1	0	1	-	-
HOGC	0	-	-	-	-	-
HUZZ	0	-	-	-	-	-
LIBE	0	-	-	-	-	-
SUGA	0	-	-	-	-	-
SULO	0	-	-	-	-	-
SWAN	0	-	-	-	-	-
SYLA	3	2	1	2	-	1
CHER	0	-	-	-	-	-
FLAT	0	-	-	-	-	-
LONG	1	1	1	-	1	-
MAST	0	-	-	-	-	-
MINK	0	-	-	-	-	-
OSAG	0	-	-	-	-	-
SPID	0	-	-	-	-	-
TOWN	0	-	-	-	-	-
WEFO	0	-	-	-	-	-
WHAR	1	1	0	1	-	-
Total	**9**	**3**	**2**	**7**	**1**	**1**

Table 4: Distribution of mitochondrial DNA haplotypes representing lineage A (1-32) in 138 *Campostoma anomalum* and derived from sequencing 842 base pairs encompassing the entire ATPase 8 and 6 genes. Samples were collected from 20 sites in the White River drainage, Ozark Plateau, Arkansas and Missouri. Detailed locality information is provided in Table 1.

	Haplotypes Lineage A																															
Site	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**
BRUS	1	2	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CLCR	-	-	-	1	-	-	1	1	1	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
COWS	-	-	-	2	-	-	-	-	-	-	1	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HOGC	-	-	-	1	-	-	2	1	-	-	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HUZZ	-	-	-	-	-	-	1	-	2	1	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LIBE	-	3	-	-	-	-	-	-	-	-	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SUGA	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-
SULO	-	2	-	1	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SWAN	-	-	-	6	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
SYLA	-	-	-	1	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-
CHER	1	2	-	1	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FLAT	-	-	-	2	-	-	-	-	-	-	2	2	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LONG	-	2	-	2	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MAST	2	1	-	2	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MINK	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OSAG	-	-	-	4	-	-	-	-	-	-	1	1	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-
SPID	-	-	-	-	-	-	-	-	-	-	1	-	-	3	-	-	-	-	-	-	3	1	-	-	-	-	-	-	-	-	-	-
TOWN	-	-	-	1	-	1	-	-	-	-	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-
WEFO	-	1	-	3	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
WHAR	-	1	-	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Total	**4**	**14**	**1**	**33**	**5**	**8**	**5**	**2**	**5**	**2**	**22**	**12**	**1**	**4**	**1**	**1**	**1**	**1**	**1**	**1**	**3**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**

Table 5: Hierarchical Analysis of Molecular Variance (AMOVA) in 138 *Campostoma anomalum* representing lineage A and sequenced across 842 base pairs encompassing the entire ATPase 8 and 6 genes. Samples were collected from 20 sites in the White River drainage, Ozark Plateau, Arkansas and Missouri. Listed are variance (*V*) and haplotypic correlation at corresponding level (*F*). Also listed is probability (*p*) of a more extreme variance component than that observed. (a) Analysis of genetic variation of sites grouped by flow regime; (b) analysis of genetic variation of sites grouped by drainage (defined in Table 1).

a

Source of variation	d.f.	Sum of squares	Variance Component			Fixation indices		
				V	%	*F*	Value	*p*
Among flow regime	1	4.13	Va	0.022	1.53	F_{CT}	0.02	0.19
Among sites within flow regime	18	46.77	Vb	0.206	14.68	F_{SC}	0.15	0.00
Within sites	118	139.08	Vc	1.179	83.79	F_{ST}	0.16	0.00

b

Source of variation	d.f.	Sum of squares	Variance Component			Fixation indices		
				V	%	*F*	Value	*p*
Among drainages	9	30.08	Va	0.099	7.09	F_{CT}	0.07	0.10
Among sites within drainages	10	20.86	Vb	0.132	9.38	F_{SC}	0.10	0.02
Within sites	118	138.48	Vc	1.174	83.53	F_{ST}	0.16	0.00

Table 6: Pairwise F_{ST} distance matrix derived from 138 *Campostoma anomalum* of lineage A sequenced across 842 base pairs encompassing the entire ATPase 8 and 6 genes. Samples were collected from 20 sites in the White River drainage, Ozark Plateau, Arkansas and Missouri. Detailed locality information is provided in Table 1. *F*st values are listed below diagonal and corresponding significant *p*-values (Bonferroni corrected) are above diagonal. Statistically significant values ($p < 0.002$) are in **bold**.

	BRUS	CLCR	COWS	HOGC	HUZZ	LIBE	SUGA	SULO	SWAN	SYLA	CHER	FLAT	LONG	MAST	MINK	OSAG	SPID	TOWN	WEFO	WHAR
BRUS															*					
CLCR	0.00														*					
COWS	0.01														*				*	
HOGC	0.13	0.00	0.08												*				*	
HUZZ	0.00	0.00	0.00	0.04											*					
LIBE	0.00	0.05	0.18	0.10	0.05										*				*	
SUGA	0.63	0.00	0.10	0.00	0.00	0.02									*				*	
SULO	0.00	0.10	0.12	0.25	0.00	0.08	0.15													
SWAN	0.11	0.17	0.13	0.33	0.20	0.40	0.35	0.21									*			
SYLA	0.00	0.00	0.00	0.02	0.00	0.15	0.01	0.12	0.05											
CHER	0.00	0.13	0.14	0.23	0.05	0.06	0.16	0.00	0.19	0.12										
FLAT	0.05	0.00	0.00	0.00	0.02	0.11	0.00	0.20	0.20	0.00	0.18									
LONG	0.00	0.00	0.00	0.07	0.00	0.00	0.04	0.00	0.07	0.00	0.00	0.00								
MAST	0.00	0.03	0.02	0.14	0.02	0.14	0.13	0.00	0.00	0.00	0.00	0.05	0.00				*			
MINK	0.42	0.42	0.44	**0.58**	**0.48**	**0.67**	**0.59**	0.53	0.07	0.33	0.44	0.49	0.40	0.22			*			*
OSAG	0.08	0.01	0.01	0.14	0.10	0.28	0.19	0.20	0.00	0.00	0.19	0.01	0.01	0.00	0.24					
SPID	0.24	0.13	0.29	0.12	0.23	0.15	0.02	0.34	**0.47**	0.16	0.30	0.12	0.20	**0.27**	**0.67**	0.30			*	
TOWN	0.03	0.00	0.00	0.00	0.03	0.14	0.05	0.16	0.10	0.00	0.15	0.00	0.00	0.00	0.35	0.00	0.18			
WEFO	0.24	0.31	0.28	0.46	0.33	**0.52**	**0.46**	0.33	0.00	0.20	0.27	0.35	0.23	0.05	0.10	0.14	**0.57**	0.22		
WHAR	0.00	0.18	0.19	0.31	0.10	0.18	0.22	0.00	0.18	0.14	0.00	0.25	0.00	0.00	**0.42**	0.21	0.38	0.18	0.23	

FIGURES

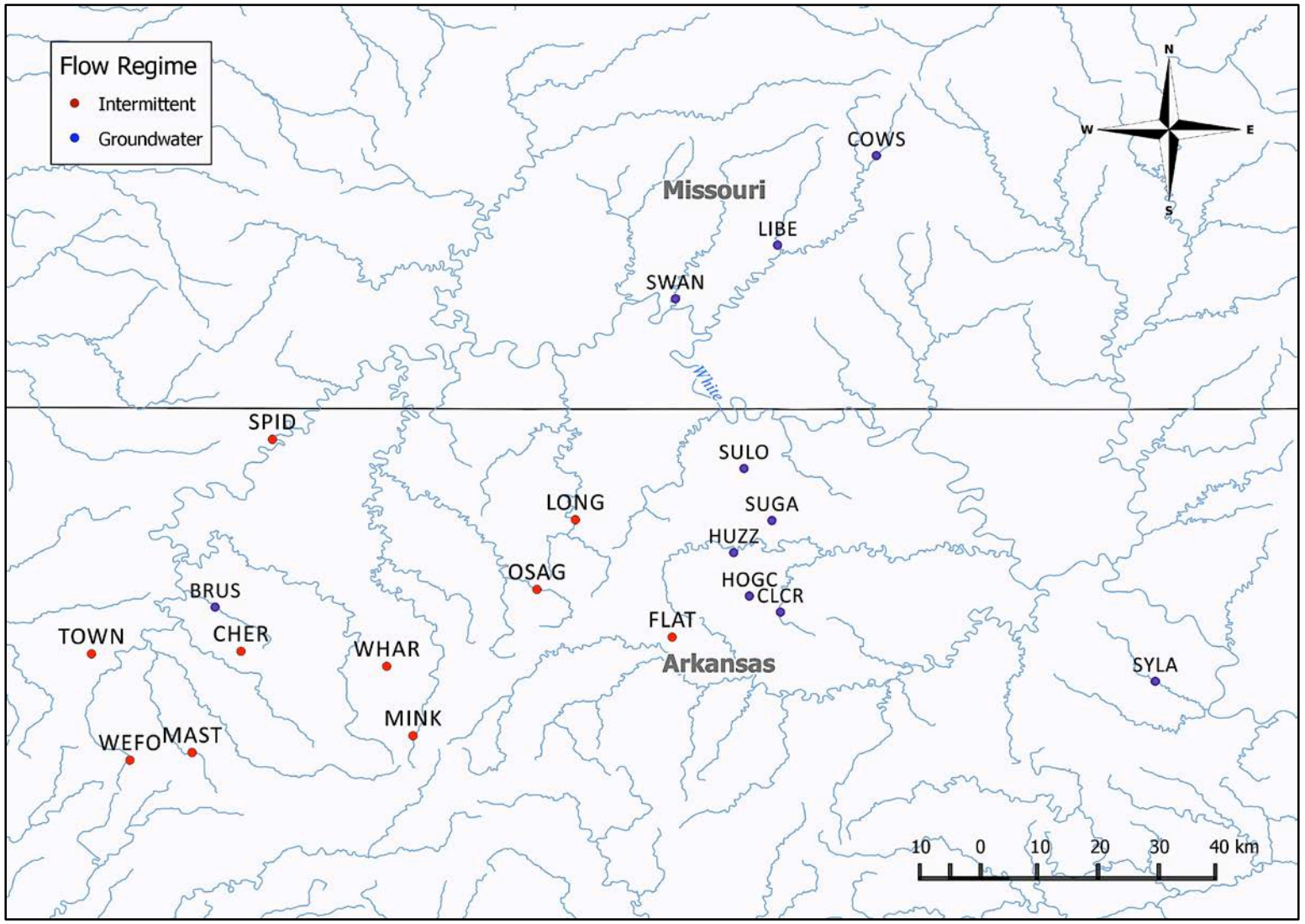

Figure 1: Map of sample sites in the White River drainage of the Ozark Plateau, AR/ MO. Red dots indicate locations that have intermittent flashy flow regimes, whereas blue dots signify sites that have groundwater flows. Detailed locality information is provided in Table 1.

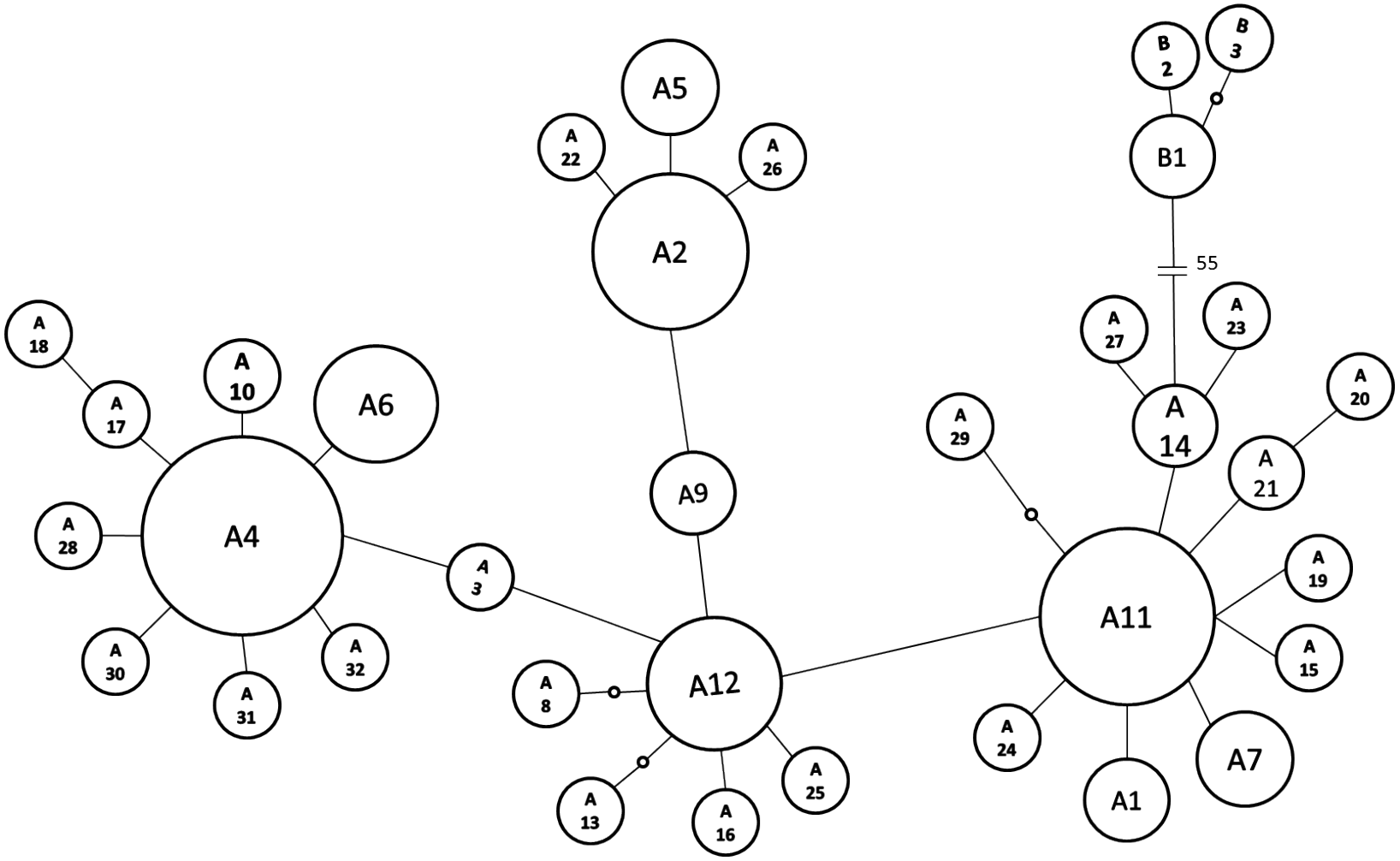

Figure 2: Haplotype network depicting distribution of the 35 *Campostoma anomalum* haplotypes representing lineage A and B from the White River drainage of the Ozark Plateau, AR/ MO, and derived from mitochondrial DNA ATPase 8 and 6. Numbers within circles refer to haplotype names (defined in Table 3 and Table 4). Size of circle is proportional to the number of individuals representing each. Haplotype 11 was identified as the ancestral haplotype from which others derived. Circles without numbers are haplotypes predicted to occur but were not observed.

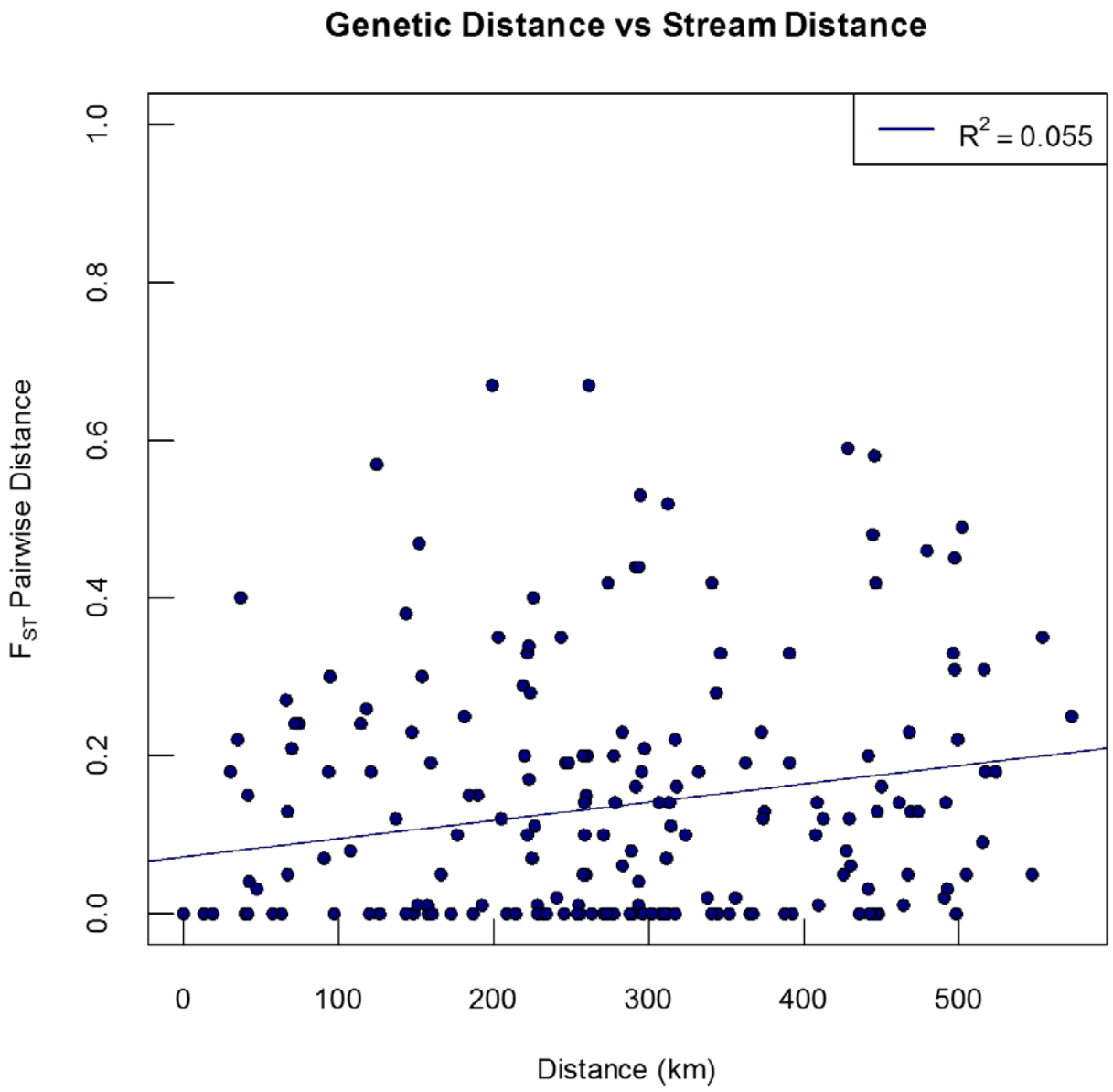

Figure 3: Linear regression depicting the relationship between genetic distances and stream distances for *Campostoma anomalum* haplotypes representing lineage A from the White River drainage of the Ozark Plateau, AR/ MO. DNA data are derived from mitochondrial DNA ATPase 8 and 6.

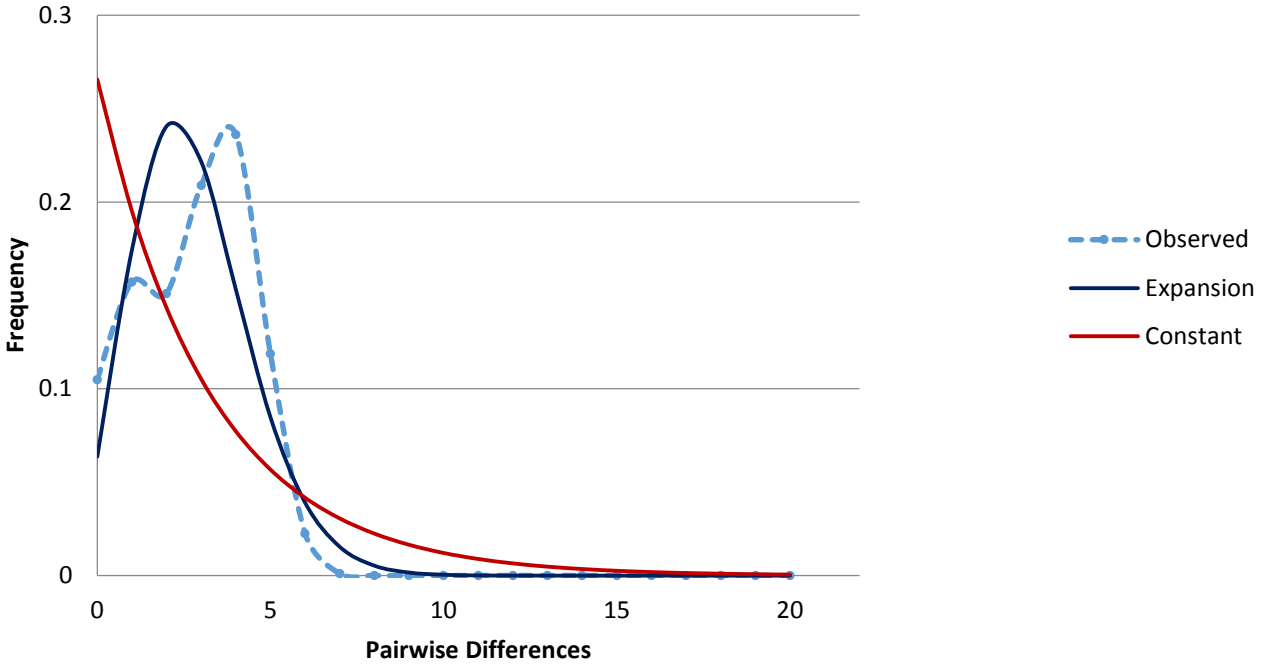

Figure 4: Mismatch distribution for *Campostoma anomalum* haplotypes representing lineage A from the White River drainage of the Ozark Plateau, AR/ MO. DNA data are derived from mitochondrial DNA ATPase 8 and 6. Solid blue line is the expected distribution found under a model of population expansion. Solid red line shows the expected distribution under a model of constant population size. Dotted blue line shows the observed distribution.

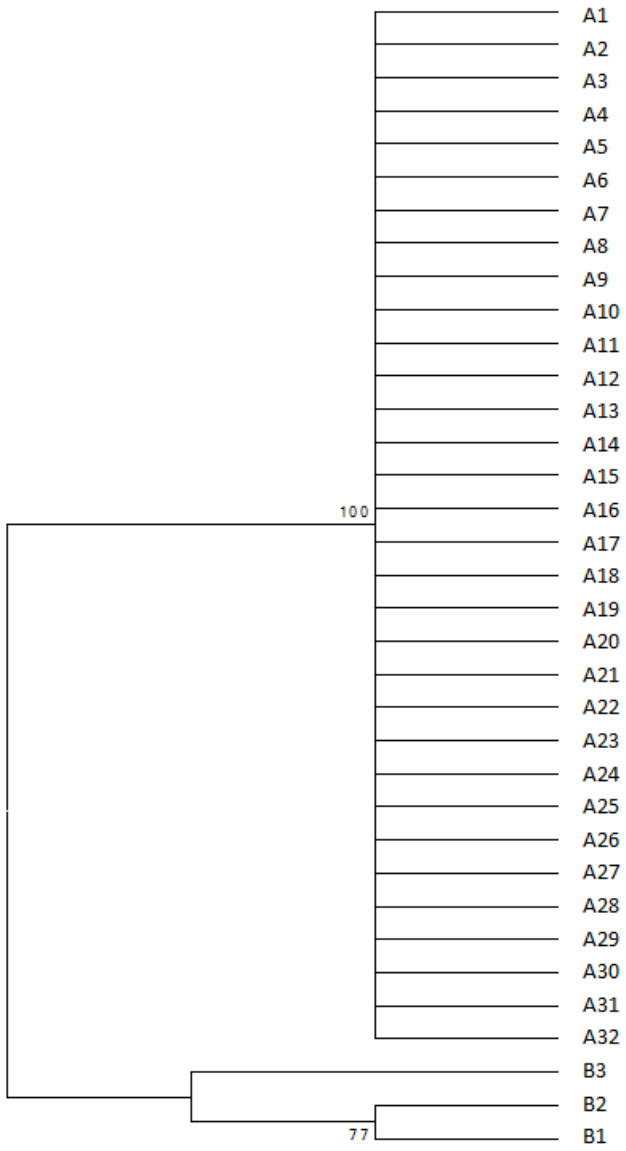

Figure 5: Maximum likelihood phylogenetic tree with the highest log likelihood (-1617.6989) based on the Tamura-Nei model is depicted for 35 *Campostoma anomalum* haplotypes from the White River drainage of the Ozark Plateau, AR/ MO, as derived from mitochondrial DNA ATPase 8 and ATPase 6. Numbers adjacent to nodes indicate % bootstrap values from 1000 replications. Outgroup (*Notropis stramineus*) is not shown. Haplotype names are labeled as per Table 4 for Lineage A and Table 3 for Lineage B.

APPENDICES

Appendix A

Biogeographic History of the Central Highlands

The Interior Highlands of central North America harbors a broad spectrum of biodiversity, and the underlying processes that shaped the biota in this region must be identified so as to conserve and protect it. The area comprises the Ouachita and Ozark highlands and is primarily recognized for the diversity of its freshwater fauna, with many taxa endemic to it alone (Bonett and Chippindale 2004). This diversity is a product of geological diversity of the region and its unique drainage history. Over millions of years, fish distributions expanded or contracted as a result of dispersal and vicariance, with geological separation promoting insularity (Hoagstrom and Berry 2006). It is thought that the Central Highlands (including the Ozark, Ouachita, and Eastern highlands) were once connected during the late Pliocene (c. 2.6 MYA) and only separated following Pleistocene glaciations, when developing lowlands intervened (Thornbury 1965, Berendzen et al. 2010). The Nearctic was covered with ice north of the Missouri River and west of the Mississippi River, leaving the Interior Highlands as a refugium for biota. During repeated glacial advances and retreats, the Mississppi River floodplain was formed, separating the Interior Highlands from the Eastern Highlands (Figure 6). In additon, fluctuating sea levels and the formation of the Arkansas River floodplain resulted in the separation of the Ozark Plateau and Ouachita Mountains (Berendzen et al. 2010).

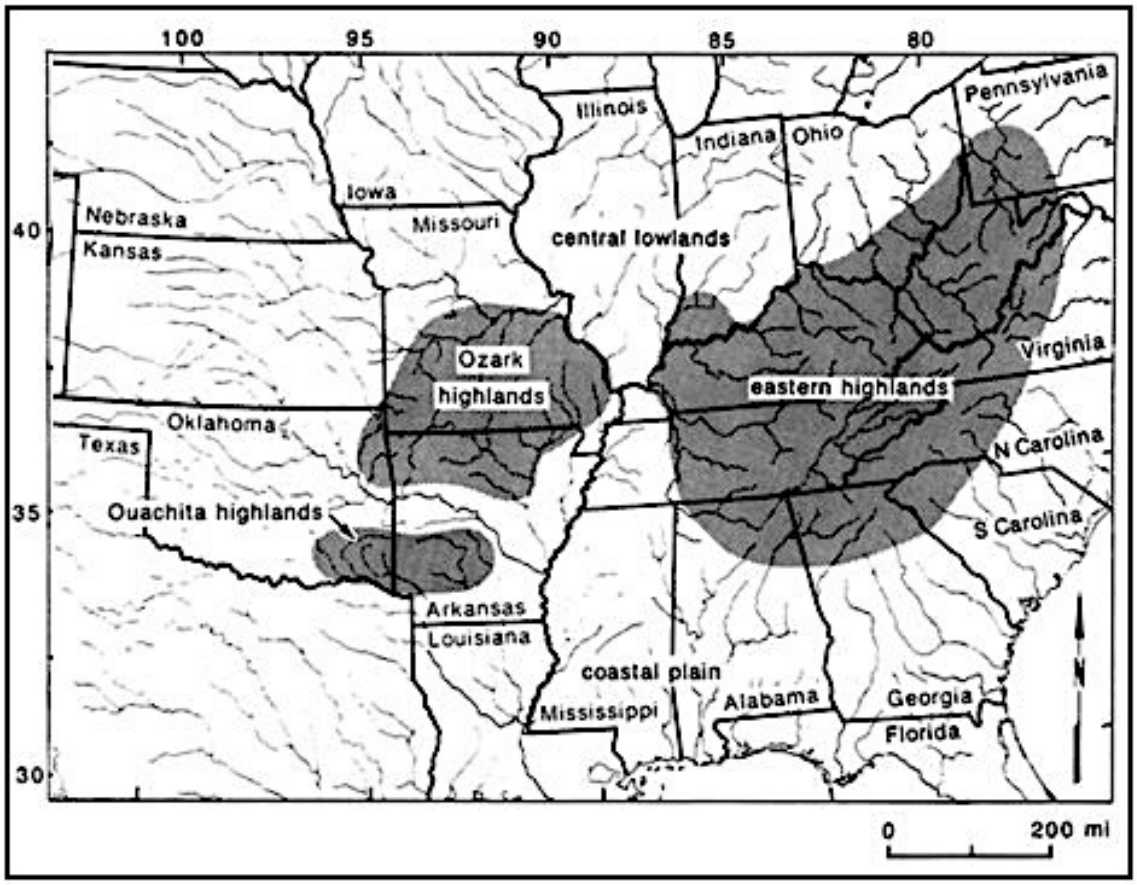

Figure 6– Major divisions of the Central Highlands region of North America (Mayden 1987)

Vicariance is built from geologic and phylogenetic evidence, and explains species distributions within the Central Highlands (Near et al. 2001). It suggests that the early separation of the Eastern and Interior highlands (11,000 years bp) similarly allowed their faunas to diverge as well, while the fauna within the Interior Highlands remained closely related (Berendzen et al. 2010). The close relationship between Ozarks and Ouachitas suggests that populations within these regions separated more recently as connectivities among river systems were altered by climate change associated with glaciation. Altered river connections, aslo known as stream capture, often resulted as drainages expanded, with component species subsequently introduced by dispersal into new areas (Berendzen et al. 2010). Presently, drainages in the Western Ozarks are relatively isolated from the rest of the region, being

drained only by the Spring and Elk rivers that flow into the Neosho River before joining the Arkansas River (Berendzen et al. 2010). Phylogeographic patterns derived for other fishes suggest the Neosho River was historically linked to the Osage River, while Spring and Elk rivers were connected to the White River (Berendzen et al. 2010).

Appendix B: Polymorphic sites in 32 haplotypes of lineage A of *Campostoma anomalum* and derived from sequencing 842 base pairs encompassing the entire ATPase 8 and 6 genes. Listed for each haplotype (A1-A32) are sites that were polymorphic with number indicating position of site. A = Adenine; G = Guanine; T = Thymine; C = Cytosine; "." indicates identical nucleotide.

	Lineage A - Polymorphic Sites																																
	48	**64**	**67**	**76**	**94**	**110**	**122**	**144**	**162**	**230**	**242**	**247**	**348**	**371**	**419**	**428**	**431**	**437**	**459**	**509**	**524**	**533**	**539**	**566**	**705**	**723**	**729**	**741**	**779**	**827**	**839**
A1	A	A	G	A	C	C	G	C	A	T	A	C	G	G	T	A	C	C	T	G	A	G	A	C	C	G	A	G	A	G	C
A2	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	C	.	.	.	G	.	.	.	.	.	.	.	.
A3	.	.	.	.	.	.	.	.	.	.	G	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A4	.	.	.	.	.	.	.	.	.	.	G	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T
A5	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	C	.	.	.	G	.	T	.	.	.	.	.	.
A6	.	.	.	G	.	.	.	.	.	.	G	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T
A7	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A8	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	A	G	.	.	.	.	.	.	.	.	.	.
A9	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.
A10	.	.	.	.	.	.	.	.	.	.	G	.	.	A	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T
A11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A12	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A13	.	.	.	T	.	.	T	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.
A15	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A16	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.
A17	.	.	.	.	.	.	.	.	.	.	G	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T
A18	.	.	.	.	.	.	.	.	G	.	G	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T
A19	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.
A20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	T
A21	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.
A22	.	.	.	.	.	.	.	.	.	C	G	.	.	.	.	.	.	.	C	.	.	.	G	.	.	.	.	.	.	.	.
A23	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.

Appendix B Cont.

	Lineage A - Polymorphic Sites																													
	48	**64**	**67**	**76**	**94**	**110**	**122**	**144**	**162**	**230**	**242**	**247**	**348**	**371**	**419**	**428**	**431**	**437**	**459**	**509**	**524**	**533**	**539**	**566**	**705**	**723**	**729**	**741**	**779**	**827**	**839**
A24	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A25	.	.	.	.	.	.	A	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A26	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	C	.	.	.	G	.	.	.	.	.	.	.	.
A27	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.
A28	.	.	.	.	.	.	.	.	.	.	G	.	.	A	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T
A29	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
A30	.	G	.	.	.	.	.	.	.	.	G	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T
A31	.	.	.	.	.	.	.	.	.	.	G	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	T
A32	.	.	.	.	.	.	.	.	.	.	G	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	T

Appendix C: Pairwise stream distances (km) for sampling localities in the White River drainage of the Ozark Plateau, AR/ MO. Sites are labeled by Locality ID as per Table 1. Geographic locations of sites are depicted in Figure 1.

	BRUS	CLCR	COWS	HOGC	HUZZ	LIBE	SUGA	SULO	SWAN	SYLA	CHER	FLAT	LONG	MAST	MINK	OSAG	SPID	TOWN	WEFO	WHAR
BRUS	0																			
CLCR	448	0																		
COWS	293	289	0																	
HOGC	447	13	288	0																
HUZZ	446	40	287	42	0															
LIBE	263	259	30	258	257	0														
SUGA	430	39	270	39	19	240	0													
SULO	296	176	137	181	187	107	184	0												
SWAN	226	222	67	221	220	37	203	70	0											
SYLA	392	148	307	157	172	277	151	204	166	0										
CHER	41	468	313	467	466	283	450	316	246	412	0									
FLAT	504	245	344	232	228	314	228	260	277	208	523	0								
LONG	233	312	157	311	310	126	293	160	90	255	253	367	0							
MAST	63	492	337	491	490	306	473	340	270	435	57	547	277	0						
MINK	273	446	291	445	444	261	428	294	224	390	293	501	225	317	0					
OSAG	427	409	254	408	407	223	390	257	187	352	248	464	192	272	114	0				
SPID	74	374	219	373	372	189	355	222	152	318	94	429	159	118	199	154	0			
TOWN	47	443	287	442	441	258	425	291	221	387	41	498	234	41	243	214	93	0		
WEFO	71	497	343	497	496	312	479	346	276	441	66	553	283	67	323	278	124	34	0	
WHAR	97	517	362	516	515	332	499	365	295	461	120	572	302	143	340	297	143	121	147	0