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A Comparative Study of Isolation in Headwater Fishes

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A COMPARATIVE STUDY OF ISOLATION IN HEADWATER FISHES

by

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ABSTRACT

A COMPARATIVE STUDY OF ISOLATION IN HEADWATER FISHES

by Bjorn Victor Schmidt

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Headwater resident fishes may be prone to a high rate of population fragmentation within river networks because large streams have habitat conditions outside of their preferred ecological niche and may limit gene flow in the dendritic ecological network. To investigate patterns of population structure, asymmetrical gene flow, and influences on genetic distance and isolation from connecting habitat pathways, species specific ecological traits, and basin scale characteristics, a multi-species, multi-regional study was performed. Six headwater species of fish from four taxonomic groupings were sampled for genetic material in three regions of paired neighbor drainages and then genotyped for eight microsatellite loci.

All species were found to have a nested hierarchical population structure relating to regional and geographical structure of drainages. There were also differences in rates of fragmentation across the species and regions studied, with *Fundulus olivaceus* and the Lower Mississippi River having the lowest rates. Most of the headwater species were found to have patterns with the majority of drainages supporting asymmetrical upstream gene flow along the main stem of the networks. Five of the species were found to have significant Isolation by Distance, and four of the species were found to have significant Isolation by Resistance due to large streams. The reservoir in the Pearl River was found to not significantly increase genetic distance, while the reservoir in the Little Red River significantly increased genetic distance. Headwater specialization and a combination of

opportunistic strategy and periodic strategy life history traits were found to increase isolation rates across species. The amount of available habitat within drainages and the shape of the drainage were found to have the most influence on genetic distance patterns at large scales.

This study shows that natural fragmentation of populations within networks is common across different species of headwater fishes, and is related to specific ecological characteristics of those species and regional characteristics of the drainage network. This project contributes to the understanding of how habitat preference within dendritic networks influences genetic population structure and provides a background rate of fragmentation in common headwater species that can be used for comparison with threatened or endangered species.

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DEDICATION

Luck affects everything; let your hook always be cast; in the stream where you least expect it, there will be a fish. – Ovid

For my son Andrew, may you always have the courage to cast your hook in life.

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LIST OF ABBREVIATIONS

Θ	Mutation Scaled Population Size
A	The average number of alleles per locus
AIC	Akaike Information Criteria
ANCOVA	Analysis of Covariance
AR	Arkansas
BB	Big Black River
BL	Black River
BP	Bayou Pierre
BSF	Basin Shape Factor
D	Nei's Unbiased Genetic Distance
DA	Total Cumulative Drainage Area
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleotides
DVM	Death Valley Model of Gene Flow
ESS	Effective Sample Size
F_{ST}	Fixation Index
GCP	Gulf Coastal Plain Region
H_e	Expected Heterozygosity
H_o	Observed Heterozygosity
HWE	Hardy-Weinberg Equilibrium
IBD	Isolation by Distance
IBDM	Isolation by Distance Model of Gene Flow

IBR	Isolation by Resistance
IR	Within Drainage Isolation Ratio
K	The number of unique genetic populations
LD	Linkage Disequilibrium
LMR	Lower Mississippi River Region
M	Mutation Scaled Migration Rate
MCMC	Monte Carlo Markov Chain
MMRR	Multiple Matrix Regression with Randomization
MS	Mississippi
MW	Middle White River
NA	The total number of alleles across all loci
NHD	National Hydrography Dataset
PCR	Polymerase Chain Reaction
PG	Pascagoula River
PR	Pearl River
SED	High Salt Buffer Solution
SHM	Stream Hierarchy Model of Gene Flow
ULO	Upper Limit of Occurrence
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
USM	The University of Southern Mississippi
WH	White River Region

CHAPTER I – COMPARATIVE HIERARCHICAL POPULATION STUCTURE OF HEADWATER FISHES

Introduction

In many stream systems, fish species distributions tend to respond to abiotic conditions predicted by a linear ecological gradient of stream size (Vannote et al. 1980). However, certain aspects of the landscape or effects of a dendritic river system can cause deviations from predicted abiotic conditions of this linear ecological gradient. In particular, habitat patch distribution, stochastic disturbance events, and hierarchical arrangement patterns of the network can all contribute to deviations from predicted habitats of this linear stream size gradient across a landscape (Benda et al. 2004). Habitats within dendritic stream networks are nested hierarchically across the following scales: microhabitat associations, stream reaches, streams, subcatchments, and drainage basins (Lowe et al. 2006). Because movement between habitats is limited to within the dendritic network for obligate aquatic species such as fish, this hierarchical arrangement of habitats can lead to a likewise hierarchical pattern of genetic differentiation that can also be influenced by spatial arrangement of network branches (streams) and nodes (confluences) (Lowe et al. 2006; Grant et al. 2007). A hierarchical population structure pattern in river networks appears to be common and has been found in both large-bodied stream fishes with an increased dispersal ability (Castric et al. 2001; Whiteley et al. 2006; Vähä et al. 2007; Warnock et al. 2010; Harris et al. 2014) and small-bodied stream fishes with a more limited dispersal ability (Austin et al. 2011; Brauer et al. 2013; Ginson et al. 2015). Therefore, for many stream fishes, population genetic structure patterns may best fit a hierarchical island model of gene flow, with populations within neighborhoods

exchanging more genes than populations between neighborhoods (Slatkin & Voelm 1991).

Headwater fish residents, or species that have all or part of their ecological niche on the small stream end of the linear ecological gradient, represent an interesting group for the study of hierarchical population structure patterns because they are confined to the upper branches of the dendritic system and therefore possibly more prone to increased isolation and fragmentation across a landscape. There are three categories of fish species that are located in headwater streams: headwater specialists that only use headwaters in a network, headwater generalists that use headwaters but can also use larger stream habitats, and temporary residents that temporarily use headwaters for a specific life history requirement such as during spawning or as nursery grounds (Meyer et al. 2007). Theoretically, headwater specialists are thought to be physiologically tolerant species capable of withstanding the harsh, variable abiotic conditions in headwaters (Rahel & Hubert 1991), efficient colonizers able to repopulate streams following local extirpations and/or efficient dispersers able to sustain separate populations across the landscape (Lohr & Fausch 1997). The likelihood of colonization events or gene flow between sites is influenced by both the dispersal ability of the species and the hierarchical, spatial arrangement of the drainage (Fagan 2002). However, little is known empirically about dispersal rates and population structure for many headwater resident fishes.

Headwaters streams comprise a large portion of the total area of most drainage basins (70-80%), and therefore tend to have large variability in habitat across streams due to regional differences and land use patterns across the drainage (Sidle et al. 2000; Jackson et al. 2001). In general, most headwater streams have a narrow width, increased

canopy cover, decreased primary productivity, and increased allochthonous carbon input (Vannote et al. 1980). These habitat characteristics differ substantially from larger, downstream sections of the network (Benda et al. 2004). Headwater species compositions of aquatic insects may be influenced primarily through local ecological conditions (niche dependent), while downstream species compositions are influenced more by regional dispersal patterns (Brown & Swan 2010). A similar pattern was found in fish from tropical river environments, where headwater species distributions were largely determined by environmental filters leading to clustering of functionally similar species, and downstream species distributions had increased overdispersion of functionally dissimilar groups (Carvalho & Tejerina-Garro 2014). In particular, water depth and dissolved oxygen concentration in headwaters could filter for species groups with appropriate physiological tolerances, while water depth and velocity parameters in headwaters could influence dispersal, colonization, and persistence patterns (Poff 1997; Súarez et al. 2007; Carvalho & Tejerina-Garro 2014). Additionally, Heinz et al. (2009) have shown through modeling that when ecological gradients are steep (such as between headwater and downstream habitats), dispersal rates and gene flow are decreased across the gradient.

Headwater specialists may be at an increased risk of extinction or local extirpations due to these processes affecting isolation and gene flow. In the southeastern United States, 25% of the headwater specialist species can be categorized as jeopardized (Etnier 1997; Meyer et al. 2007). In the midwestern United States, 50-64% of headwater species in the Maumee and Illinois rivers were found to be extirpated or declining in abundance (Karr et al. 1985). Threatened or endangered headwater residents tend to have

restricted ranges, lowered population sizes, and populations fragmented into distinct genetic clusters (Austin et al. 2011; Sterling et al. 2012). Studying the population structure patterns of common species with similar ecological traits to threatened species can help understand processes that impacted populations in the threatened species (Whiteley et al. 2006). Additionally, if "background" rates of isolation and fragmentation in common headwater species are known, then detected fragmentation of threatened headwater residents can be placed within a broader ecological context and possibly help guide management strategies.

Knowledge of how populations of headwater fishes are naturally fragmented and isolated could also be informative for how anthropogenic disturbances impact headwater streams. In the Appalachian region of the United States, coal and mineral mines have been found to have widespread negative effects on fish diversity and abundances of specific functional groups, including invertivores and lithophilic fishes, which are typically more numerous in headwater habitats (D'Ambrosio et al. 2009; Daniel et al. 2014). Additionally, across ecoregions in the study, 25-50% of the mines were found in small headwater streams (<100 km^2 drainage area), demonstrating that mining can be a significant stressor to headwater species in this region (Daniel et al. 2014). Other specific anthropogenic threats to headwater streams include channelization, water withdrawal, piping, impoundments, land development, and agricultural runoff (Freeman et al. 2007). New technologies and land use patterns can also form emerging threats to headwater streams. The recent trend of horizontal drilling and hydraulic fracturing in natural gas extraction can negatively impact headwater streams in shale basins, with negative effects, including an increase in sedimentation in nearby streams (Entrekin et al. 2011) This

siltation from these extraction methods can be a significant threat for lithophilic or turbidity sensitive headwater residents, such as *Etheostoma whipplei*, which was found to have lower reproductive success in streams impacted by hydraulic fracturing (Stearman et al. 2015).

A comparative approach in population genetics can be useful to examine how specific differences in ecological factors such as life history traits associated with dispersal affect structure patterns across a group of species of interest (Pauls et al. 2014). Simultaneously, regions can also be compared to examine differential landscape effects on population structure patterns for the different species of interest (Koch et al. 2005; Campbell et al. 2006). For this study, I examined six different common headwater resident fishes in three geographic regions. There have been multi-species comparative studies of population structure involving stream fishes (Zanatta & Wilson 2011; Husemann et al. 2012), including headwater species of fish (Tibbets & Dowling 1996; Turner and Robison 2006; Pilger et al. 2015), but to my knowledge no study has been done for multiple species of headwater fish across multiple geographic regions. For this study, I hypothesize that genetic structure patterns for headwater species will be influenced by the spatial structure of the river networks and by a shared occupancy for specific habitats along the linear stream size gradient. Because of the habitat differences between headwaters and large rivers that filter for certain ecological traits in headwater residents, and an inferred decrease in dispersal ability across larger streams in the network, I predict that headwater adapted species will have genetic populations arranged within nested groups reflecting the hierarchy of the stream networks and spatial arrangement of the geographic regions. Because headwater habitats select for

functionally similar species, I predict that patterns of isolation and hierarchically nested genetic populations will be similar across a broad range of phylogenetically distinct headwater resident fishes. Additionally, any regional landscape effects are predicted to affect these headwater residents in the same way, resulting in similar patterns across regions.

Methods

The species studied were *Fundulus olivaceus* (blackspotted topminnow), *Semotilus atromaculatus* (creek chub), *Erimyzon claviformis* (creek chubsucker), *Etheostoma artesiae* (redspot darter), *E. parvipinne* (goldstripe darter), and *E. whipplei* (redfin darter). These species vary in microhabitat preferences and specific life history traits, but all have at least part of their ecological niche in headwater streams, which was the criterion for selection in the study. Specific habitat preferences for these species are: *F. olivaceus* prefers stream margins and backwaters, *S.atromaculatus* prefers shallow and narrow streams without a high proportion of mud substrate, *E. claviformis* prefers muddy, slow moving streams, *E. artesiae* and *E. whipplei* prefer gravel and cobble substrates, and *E. parvipinne* prefers small forested streams with a large proportion of canopy cover (Meffe & Sheldon 1998; Smiley et al. 2005; Tyrone 2007; Schaefer et al. 2009; Stearman et al. 2015)

The regions studied were located in Mississippi and Arkansas, and were categorized as Gulf Coastal Plain (GCP; Pascagoula (PG: total cumulative drainage area (DA) = 24,700 km^2) and Pearl (PR: DA = 37,100 km^2); MS), Lower Mississippi River (LMR; Bayou Pierre (BP: DA = 2,800 km^2) and Big Black (BB: DA = 8,700 km^2); MS), and White River (WH; Little Red River (LR: DA = 5,200 km^2), Middle White River

(MW: DA = 36,100 km^2), and Black River (PR: DA = 22,100 km^2); AR) (Figure 1; DA determined through the National Hydrography Dataset Plus v. 2 (Horizon Systems)). Each region consisted of paired neighbor drainages that were each sampled for four headwater species. *Fundulus olivaceus, Semotilus atromaculatus*, and *Erimyzon oblongus*, were sampled from all 6 drainages across the three regions, while the three darter species were each only sample in one region (two paired drainages per species). *Etheostoma parvipinne* was sampled in the Gulf Coastal Plain region, *Etheostoma artesiae* was sampled in the Lower Mississippi River region, and *Etheostoma whipplei* was sampled in the White River region. For *E. whipplei* in the White River region, the sampled areas were the Little Red and the Middle White, while the other three species in this region were sampled in the Little Red and Black drainages. All sampling was done in Mississippi and Arkansas (i.e., no sampling was done in the Missouri portion of the Black drainage). Specific sample sites for each species are presented in Figures 2-7 and Appendix B.

Fifteen fish were sampled for each species at 6 locations in each drainage (36 total sampling locations for *F. olivaceus, S. atromaculatus,* and *E. claviformis,* and 12 total sampling locations for *E. artesiae, E. parvipinne,* and *E. whipplei*). Sampling was performed by backpack electrofishing and seining, and seining was exclusively used in areas of possible occurrence of non-target endangered species (*Etheostoma moorei* in the Little Red and *E. rubrum* in Bayou Pierre). Sampling in Mississippi occurred from 2010-2011, and sampling in Arkansas occurred in 2012. Fin clips were performed in the field and preserved in either a saturated salt buffer solution (SED) or 100% ethanol. A total of 540 fin clips were collected per species for *F. olivaceus, S. atromaculatus,* and *E.*

claviformis, and a total of 180 fin clips were collected per species for *E. artesiae*, *E. parvipinne,* and *E. whipplei*.

DNA was extracted using the DNAeasy Tissue Kit (QIAGEN Inc., Valencia, CA). Eight microsatellite loci were amplified via the polymerase chain reaction (PCR) for each species (Table 1). Amplifications were done in a 12.5 µl reaction containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.01% gelatin, 1.5 mM $MgCl_2$, 200 µM dNTPs, 0.188 units of *Taq* polymerase (Promega), 0.3 µM of M13 tailed forward primer (Boutin-Ganache et al. 2001), 0.3 µM of the reverse primer, 0.1 µM of the M13 labeled primer (LI-COR), 20-100 ng of the template DNA and water to reach the final volume. PCR cycling conditions were: an initial denaturing step at 94 °C for 2 minutes, followed by 35 cycles of 94 °C for 30 seconds, 56°C for 1 minute, then 72 °C for 1 minute. A final elongation step at 72 °C for 10 minutes was then performed. Microsatellite alleles were visualized on acrylamide gels using a LI-COR 4300 DNA Analysis system and scored using Gene ImagIR v. 4.03 (Scanalytics Inc. 2001).

The program GENEPOP v. 4.2 (Raymound & Rousset, 1995) was used to perform exact tests for deviations from Hardy-Weinberg equilibrium (HWE) per sampling location and linkage disequilibrium (LD) per locus. Sequential Bonferroni corrections were used to adjust significance values of tests with multiple comparisons (Rice 1989). The program Micro-checker v. 2.2 was used to investigate the presence of null alleles per locus. Loci that were identified as having widespread, significant deviations from HWE or LD or being identified as likely having null alleles were removed from the dataset prior to further analysis.

The total number of alleles per population, the average number of alleles per loci, and observed and expected heterozygosities were determined using the program GenAlEx v. 6.5 for both sample sites and drainages (combining all sample sites in a drainage) (Peakall & Smouse 2006). Pairwise F_{ST} values between sites within drainages and between sites within regions were also determined using GenAlEx v. 6.5.

The Bayesian inference based program STRUCTURE v. 2.3.4 (Pritchard et al. 2000) was used to probabilistically determine the best number of distinct genetic clusters (K) in the dataset. I used a hierarchical approach similar to the methods used by Vaha et al. (2007) and Harris et al. (2014). In this method, the best number of clusters was determined for the whole dataset for each species. Each unique genetic cluster was then run in the program independently to look for further finer-scale population structure. Sample locations were assigned to clusters for the next round of analysis based on their population q scores (assignment went to the highest q score). This was repeated until a K of 1 was achieved, or groupings corresponded to individual sample locations. Larger datasets (540 individuals for *F. olivaceus*, *S. atromaculatus*, and *E. claviformis*) were run with a burn-in length of 5,000,000 followed by a Monte Carlo Markov Chain (MCMC) length of 500,000 for assumed k values of 1 to 20, with 20 iterations per each assumed K value. All other analyses (180 individuals or less) were run with a burn-in length of 500,000 followed by a MCMC length of 150,000 for assumed K values of the number of sample locations in the dataset + 2, with 20 iterations per each assumed K value. The admixture model was selected and sampling locations were used as prior information (Hubisz et al. 2009). The program Structure Harvester (Earl and vonHoldt 2012) was used to collate the data and examine delta K values based on the Evanno et al. (2005)

method, which were used to determine the best number of clusters during each round of analysis. Panmixia (K of 1) was determined by having flat mean likelihood profiles across the different values of K and having low delta K values (< 2). Individual and population q scores were averaged across replicates for the best value of K using the program CLUMPP (Jakobsson and Rosenberg 2007) and subsequently visualized with the program Distruct (Rosenberg 2004). The average population q scores were summarized for each hierarchy level in the analysis for each study species. For comparisons of fine-scale population structure between species and regions, an *ad hoc* statistic called the fragmentation ratio was used. This simple ratio was defined as the number of unique genetic clusters for each species within regions divided by the number of sample locations in that region (or maximum possible number of unique genetic clusters).

Results

Two loci for *E. claviformis* (Mohu-Lav229 and Ce146) showed widespread significant deviations from HWE, and one of those two (Mohu-Lav229) also showed a possible presence of null alleles. Therefore, these two loci were not used in further analyses (analyses were done with 6 loci for *E. claviformis*). All of the other loci used in the study did not show widespread, significant deviations from HWE, deviations from LD, or possible presence of null alleles.

The mean number of alleles per locus (A) across sample sites for *F. olivaceus* was 9.34, and the average observed heterozygosity (H_o) was 0.685 (Table 2). Genetic diversity (A) was similar across drainages for *F. olivaceus*, and the Little Red River had a reduced level of observed heterozygosity (0.546) compared with the average from the

other five drainages (0.709) (Table 3). The mean number of alleles per locus across sample sites for *S. atromaculatus* was 5.56, and the average observed heterozygosity was 0.543 (Table 4). The Pearl River had a large reduction in genetic diversity (A = 5.13) and observed heterozygosity (H_o = 0.290) compared with average from the other five drainages (A = 13.075; H_o = 0.594) for *S. atromaculatus* (Table 5). For *E. claviformis*, the mean number of alleles per locus across sample sites was 7.83 and the average observed heterozygosity was 0.688 (Table 6). Genetic diversity (A) was similar across drainages for *E. claviformis*, and the Little Red River had a reduced level of observed heterozygosity (0.586) compared with the average from the other 5 drainages (0.709) (Table 7). For *E. artesiae*, the mean number of alleles per locus across sample sites was 8.77 and the average observed heterozygosity was 0.689 (Table 8). Genetic diversity (A) was similar across the two drainages for *E. artesiae*, and the Big Black had a reduced level of observed heterozygosity (0.623) compared with the observed heterozygosity in Bayou Pierre (0.760) (Table 9). For *E. parvipinne*, the mean number of alleles per locus across sample sites was 9.85, and the average observed heterozygosity was 0.687 (Table 10). Genetic diversity (A) and observed heterozygosity was similar across the two drainages for *E. parvipinne* (Table 11). For *E. whipplei*, the mean number of alleles per locus across sample sites was 7.73, and the average observed heterozygosity was 0.651 (Table 12). The Middle White drainage had reduced genetic diversity (A = 14.13) and observed heterozygosity (H_o = 0.562) compared to the Little Red drainage (A = 18.38; H_o = 0.742) for *E. whipplei*, which was a pattern driven by low values in upstream sites along the Middle White (MW1-3, MW 6), with downstream sites (MW4 and MW5) having values similar to the Little Red sites (Figure 7, Table 12, Table 13).

The highest within drainage pairwise F_{ST} values for *F. olivaceus* were found in the Little Red drainage (average = 0.099), and the highest between drainage pairwise F_{ST} values within regions were found in the WH region (average = 0.148) (Tables 14-16, Table 26). The lowest within drainage pairwise F_{ST} values for *F. olivaceus* were found in the Bayou Pierre drainage (average = 0.028), and the lowest between drainage pairwise F_{ST} values within regions were found in the LMR region (average = 0.070) (Tables 14-16, Table 26). The highest within drainage pairwise F_{ST} values for *S. atromaculatus* were found in the Pascagoula drainage (average = 0.236), and the highest between drainage pairwise F_{ST} values within regions were found in the GCP region (average = 0.370) (Tables 17-19, Table 26). The high within drainage values for the Pascagoula were driven by very high pairwise values for matches between PG4 and PG 5 (Chickasawhay River) with other sites in the drainage, and these values were similar to matches for other sites in the drainage (PG1-3, PG 6) with sites in the Pearl (Table 18). The lowest within drainage pairwise F_{ST} values for *S. atromaculatus* were found in the Big Black drainage (average = 0.048), and the lowest between drainage pairwise F_{ST} values within regions were found in the LMR region (average = 0.080) (Tables 17-19, Table 26). The highest within drainage pairwise F_{ST} values for *E. claviformis* were found in the Little Red drainage (average = 0.092) and Pascagoula drainage (average = 0.091), and the highest between drainage pairwise F_{ST} values within regions were found in the WH region (average = 0.123) (Tables 20-22, Table 26). The lowest within drainage pairwise F_{ST} values for *E. claviformis* were found in the Big Black drainage (average = 0.040) and the Bayou Pierre drainage (average = 0.041), and the lowest between drainage pairwise F_{ST} values within regions were found in the LMR region (average = 0.051) (Tables 20-22, Table 26).

Within drainage pairwise F_{ST} values for *E. artesiae* were similar for the two study drainages, with slightly lower values in Bayou Pierre (average = 0.055; BB average = 0.068) (Table 23, Table 26). Within drainage pairwise F_{ST} values for *E. parvipinne* were similar for the two study drainages, with slightly lower values in the Pascagoula drainage (average = 0.065; PR average = 0.079) (Table 24, Table 26). Within drainage pairwise F_{ST} values for *E. whipplei* were similar for the two study drainages (LR average = 0.062; MW average = 0.069) (Table 25, Table 26).

All species showed hierarchical population structure. For each drainage throughout the three regions, there were two or three hierarchy levels (average = 2.5) found for *F. olivaceus* (Figure 8), three or four levels (average =3.67) for *S. atromaculatus* (Figures 9-10), and two or three levels (average = 2.83) for *E. claviformis* (Figure 11). For the darter species, which were each sampled in a single region, the hierarchical population structure consisted of two levels for *E. artesiae* (Figure 12), two levels for *E. parvipinne* (Figure 13), and three levels for *E. whipplei* (Figure 14). *Semotilus atromaculatus* had the highest average population q scores across the hierarchy levels, and also was the only species to have 4 levels of analyses (Table 27). *Fundulis olivaceus* had the lowest average population q scores across all rounds of analysis, and *E. parvipinne* had the lowest score for a single round of analysis (0.7945 = 1st level) (Table 27).

Most of the clustering in the upper hierarchical levels corresponded to drainage and region divisions. For all three species that were sampled in the three regions, drainages in the GCP and WH regions tended to separate out independently in the first level of STRUCTURE analysis (with the exception being the Black drainage for *F.*

olivaceus, which grouped with the LMR drainages). On the other hand, drainages in the LMR region (Bayou Pierre and Big Black) did not separate from each other until the second level in the hierarchical analysis for all three species. For the species only sampled in one region, *E. artesiae* and *E. whipplei* had drainage based clusters for the first round of analysis, while *E. parvipinne* had a more complex cluster arrangement for the first round.

Although most sites clustered into their own respective drainages, some sample locations initially were grouped with other drainages during the upper two levels of the hierarchical analyses. These were PG4 and PG5 for *S. atromaculatus* (grouped with the Pearl drainage), PG1 for *E. claviformis* (grouped with Bayou Pierre drainage), PG6 for *E. claviformis* (grouped with the Pearl), PR3 for *E. claviformis* (grouped with the Pascagoula drainage), PG5 for *E. parvipinne* (grouped with the Pearl drainage), and MW4 and MW5 for *E. whipplei* (both grouped with the Little Red drainage). The majority of the sites (6/8) that were grouped with other drainages than their own were from the GCP region: Pascagoula (5 sites) and Pearl (1 site). Most (5/6) of these GCP sites were grouped with the neighbor drainage in the same region, and one site was grouped with a drainage in another region. Additionally, the four Pascagoula sites grouped with the Pearl were in tributary systems to the Chickasawhay River on the eastern side of the drainage. *Fundulus olivaceus* was the only species that was sampled in the GCP region that did not have a collection site that initially grouped with a separate drainage.

Patterns of genetic clustering were mostly consistent across drainages (number of clusters within 2) for *S. atromaculatus*, *E. claviformis*, *E. artesiae*, *E. parvipinne*, and *E.*

whipplei, with larger deviations from the pattern (cluster difference greater than 2) occurring in *F. olivaceus* in the BP, BB, and PR drainages (Table 28). The GCP and WH regions contained a similar, high number of genetic clusters across species compared with the LMR region, which contained a lower number of clusters across species (Table 28). There were two main groupings that were present when comparing fragmentation ratios of the six species (Table 29). *Fundulus olivaceus* and the redfin darter species, *E. artesiae* and *E. whipplei*, had lower fragmentation ratios, representing fewer fine scale genetic clusters and can be placed in a lower fragmentation group. *Semotilus atromaculatus* and *E. claviformis* had moderately high fragmentation ratios when averaged across all three regions. *Etheostoma parvipinne* had the highest fragmentation ratio among the species, but this value was comparable to *S. atromaculatus* and *E. claviformis* values in the same region. Therefore, *S. atromaculatus, E. claviformis,* and *E. parvipinne* can be placed together in a higher fragmentation group.

There were also strong regional effects on fragmentation patterns at the finest-scale of population structure. The highest fragmentation ratios for the three species that were sampled across all three regions were found in: for *F. olivaceus,* the White River, for *S. atromaculatus,* the Gulf Coastal Plain and White River, and for *E. claviformis*, the Gulf Coastal Plain and White River. Regional effects seemed to influence species based on group assignment above. For the low fragmentation group (*F. olivaceus* and the redfin darters, *E. artesiae* and *E. whipplei*), the WH region produced the highest fragmentation rates (although the high fragmentation drainage was different in *F. olivaceus* and *E whipplei* in the region (Table 28)), and the LMR region and GCP region (*F. olivaceus* only) produced lower fragmentation rates. Regional effects were slightly different for the

high fragmentation group (*S. atromaculatus*, *E. claviformis*, and *E. parvipinne*). For this group, the highest fragmentation rates were in the GCP (only region for *E. parvipinne*) and WH regions, while the LMR region had the lowest fragmentation rates. There were some reversals in fragmentation ratio ranking from regional effects among the three species sampled across all regions. For the WH and GCP region, *S. atromacualtus* and *E. claviformis* had higher fragmentation ratios than *F. olivaceus*. However, in the LMR region, there was a reversal with *F. olivaceus* having higher fragmentation ratios than both *S. atromaculatus* and *E. claviformis* (which had the two lowest fragmentation ratios across all species-region pairings). When averaged across all species, the Lower Misssissippi River region had much lower fragmentation ratios than the White River and Gulf Coastal Plain (Table 30). Additionally, there were only three cases of drainage wide panmixia (no sub-structure within the drainage), and all three cases occurred in the LMR region: Bayou Pierre for *F. olivaceus* and Big Black for *S. atromaculatus* and *E. claviformis* (Figures 8-10).

Discussion

Most of the data showed hierarchical nesting of populations into regions and drainages at the upper levels of structure, and patterns of within drainage structure were generally species dependent, and therefore the results support the hypothesis for the study. These results also support the prediction that drainage networks promote hierarchical nesting of populations for headwater species across a landscape. Although all of the headwater species showed hierarchically nested population structure, there were also clear differences between groups of species and regional effects on those groups, and therefore predictions that species and regions would have similar responses were not

supported. Although headwater habitats may act as a filter for specific ecological characteristics and life history traits, there must be other factors that influence population structure and gene flow for these species of fish that promote these differences. For the low fragmentation group, *F. olivaceus* and the redfin darters, *E. artesiae* and *E. whipplei*, there may be some ecological traits related to dispersal ability that help mitigate isolating effects of headwater habitats. Likewise, for the high fragmentation group, *S. atromaculatus*, *E. claviformis*, and *E. parvipinne*, there may be some ecological traits that promote isolation and limit dispersal ability.

One likely influence on these groupings is niche breadth along the linear stream size gradient. *Fundulus olivaceus* is the least obligate headwater fish among the species studied and can be found in both headwaters and larger tributaries of the sample drainages. Additionally, their specific microhabitat is stream margins, which will vary less as stream size increases compared to the specific microhabitats of the other species. *Etheostoma artesiae* and *E. whipplei*, are headwater and small stream specialist species, while *S. atromaculatus*, *E. claviformis*, and *E. parvipinne* are headwater specialist species (Ross 2001). Therefore, these patterns of fragmentation could be the result of the increased dispersal ability across medium to large streams in the drainage for *F. olivaceus*, *E. artesiae*, and *E. whipplei*, in comparison to *E. parvipinne*, *S. atromaculatus*, and *E. claviformis* due to differences in niche breadth and tolerance of conditions in larger streams. This increased dispersal ability would lower divergence rates between populations and lead to decreased fragmentation rates across a region.

Regional effects on the fragmentation rates of species tended to be in the same direction, with LMR having decreased fragmentation rates and WH and GCP having

increased fragmentation rates, with specifics depending upon group assignment into the low or high fragmentation group. The drainages differ in various parameters such as size, shape, slope, soil type, land cover, and other variables that could influence dispersal and population structure patterns (e.g. Huey et al. 2008; Cook et al. 2011; Waters et al. 2015). These variables could act in a combined manner to produce these regional effects on population structure. Lower fragmentation ratios in the LMR region could be influenced by those two drainages being the smallest and third smallest in the dataset. This region also produced lower pairwise F_{ST} values, and Bayou Pierre commonly had the lowest within drainage pairwise F_{ST} values. The Little Red has the second smallest drainage area, but was associated with high fragmentation ratios, which could be influenced by the reservoir in the drainage or the increased slope associated with the higher elevation differences in the drainage. The Little Red was also associated with reduced heterozygosity in *F. olivaceus* and *E. claviformis*, suggesting possible reductions in population size in the drainage, which could also contribute to the higher fragmentation rates. A large reduction in genetic diversity and observed heterozygosity was also seen in the Pearl drainage for *S. atromaculatus*, indicating the probability of reduced population sizes for this species in the Pearl, which could be a historical pattern (founder effect) or a current pattern (possible reservoir effect or limited available habitat across the drainage).

Although general trends were similar across species, supporting the hypothesis that different headwater species would be affected by regional differences in similar directions, there were also some slight differences in responses by the species. The reversal in the fragmentation ratio ranking for *S. atromaculatus* and *E. claviformis* in the Lower Mississippi River region indicates that the magnitude of the regional effects on

these two species was larger than the effects on *F. olivaceus*. Both *S. atromaculatus* and *E. claviformis* have larger maximum lengths compared with the other species, use pool microhabitats as adults and run habitats for juveniles (Ross 2001). Some combination of these similar ecological traits combined with the regional fragmentation limiting effects of the LMR region could be responsible for these patterns.

The WH region and the GCP region had similar, high fragmentation rates in the data and both drainages produced the highest rates of within drainage pairwise F_{ST} values across regions. These regions also both contained patterns of sites grouping with other drainages at higher hierarchical levels of STRUCTURE analysis. Sample sites grouping with other drainages at these higher levels indicates a stronger fragmentation effect within the drainage and indicates more within drainage isolation for those sites with lower rates of gene flow to other within drainage sites. Additionally, the repeated pattern of sites in the Chickasawhay River (eastern) portion of the Pascagoula drainage grouping with sites in the Pearl drainage across three different headwater species suggests a possible Pearl origin for some populations in this portion of the drainage combined with low gene flow to other regions in the drainage. Because there are multiple species involved, historical stream capture from the Upper Pearl to the Chickasawhay region seems the most likely scenario, although further investigations would be needed for confirmation. In particular, the pairwise F_{ST} values for *S. atromaculatus* between "normal" sites in the Pascagoula with the two Chickasawhay sites that grouped with the Pearl were very similar to pairwise F_{ST} values between the "normal" Pascagoula sites and sites in the Pearl, supporting a possible Pearl origin for the two Chickasawhay sites. The grouping of MW4 and MW5 for *E. whipplei* with the Little Red drainage is consistent

with the spatial arrangement of the stream network, as the confluence of the tributary system for these two sites is near the mouth of the Little Red while the other sites in the MW are separated from the Little Red by much longer distances along the White River (Figure 7). This grouping is also supported by patterns of pairwise F_{ST}, genetic diversity, and observed heterozygosity (Table 12, Table 25).

Historical patterns of gene flow and drainage colonization could also be an influence in the dataset. Phylogeographic information is lacking for most of these species, but analyses for *F. olivaceus* indicate a low rate of range wide structure using markers designed for examining longer evolutionary time scale differences (Duvernell et al. 2013). This type of pattern is consistent with a recent range-wide expansion and colonization of drainages, which could influence trends seen in the microsatellite markers investigating more recent structure patterns. If *F. olivaceus* has a shorter residence time in these drainages compared with the other species, then the time for divergence for microsatellite loci would be reduced, which could impact observed fragmentation rates. Therefore, *F. olivaceus* having a lower fragmentation rate in these regions may be a combined effect of the ecological traits for the species and longer term historical effects of residence time in the drainages. If a stream capture event did occur between the Pearl and the Pascagoula, a lack of a Pearl origin in the Chickasawhay region for *F. olivaceus* could also be attributed to recent colonization of the drainages if the stream capture event occurred prior to such colonization. It is likely that each species has their own unique historical colonization pattern and residence time in the drainages, which could also impact their fragmentation results as well.

This study demonstrates that nested, hierarchical population structure is common across a phylogenetically diverse group of headwater fishes, with upper levels of structure following regional and drainage differences and lower levels of structure following specific regions within drainages. This pattern can be useful for understanding background rates of fragmentation in common species as a comparison for threatened or endangered headwater species. This study also demonstrates that general trends from regional effects are similar across most headwater fishes studied. Patterns tended to be split across two groups, a low fragmentation group and a high fragmentation group, showing that while headwater species may be ecologically similar in order to reside in headwaters, there are still differences in ecological traits and life history traits that can influence gene flow and dispersal across the study groups. Additionally, these differences likely interact with regional effects in complicated ways to change the magnitude of effects on fragmentation patterns. Historical patterns of residence time and drainage colonization can also be different across species and add more complexity for observed patterns.

Table 1

Microsatellite loci used for each species in the study

Species	Microsatellite Loci
F. olivaceus	Fno56[a], Fno91[a], Fno112[a], Fno261[a] Fh-6[b], Fh-20[b], Fh-B101[b], Fh-B103[b]
S. atromaculatus	Sat403[c,] Sat406[c], Sat407[c], Sat409[c] Sat411[c], Sat412[c], Sat413[c], Sat414[c]
E. claviformis	Mohu-Lav229[d], Mohu-Lav294[d], Mohu-Lav336[d], US4[e] US6[e], Ce13s[f], Ce52[f], Ce146[f]
E. artesiae	Eca6[g], Eca11[g], Eca37[g], Eca49[g] Eca70[g], Esc18[h], Esc26[h], Esc132[h]
E. whipplei	Eca6[g], Eca11[g], Eca37[g], Eca49[g] Eca70[g], Esc18[h], Esc26[h], Esc132[h]
E. parvipinne	Eca11[g], Eca44[g], Eca46[g], Eca71[g] Esc18[h], Esc26[h], Esc132[h], EOsD107[i]

Letters associated with loci correspond to being developed for the following species and reference: a = *Fundulus notatus* (Feldheim et al. 2014), b = *Fundulus heteroclitus* (Adams et al. 2005), c = *Semotilus atromaculatus* (Skalski & Grose 2006), d = *Moxostoma hubbsi* (Lippé et al. 2004), e = *Catostomus ardens* (Cardall et al. 2007), f = *Cycleptus elongatus* (Bessert et al. 2007), g = *Etheostoma caeruleum* (Tonnis 2006), h = *Etheostoma scotti* (Gabel et al. 2008), i = *Etheostoma osburni* (Switzer et al. 2008).

Table 2

Genetic diversity and heterozygosity for F. olivaceus across sites

Site	NA	A	H_o	H_e
BP1	85	10.625	0.745	0.818
BP2	85	10.625	0.700	0.814
BP3	100	12.5	0.717	0.856
BP4	90	11.25	0.692	0.812
BP5	84	10.5	0.683	0.818
BP6	81	10.125	0.713	0.768
BB1	60	7.5	0.642	0.694
BB2	68	8.5	0.633	0.611
BB3	86	10.75	0.657	0.668
BB4	62	7.75	0.702	0.678
BB5	77	9.625	0.709	0.700
BB6	72	9	0.713	0.730
PG1	75	9.375	0.775	0.730
PG2	74	9.25	0.692	0.680
PG3	79	9.875	0.658	0.685
PG4	74	9.25	0.750	0.712
PG5	91	11.375	0.775	0.691
PG6	88	11	0.692	0.642
PR1	72	9	0.640	0.691
PR2	73	9.125	0.658	0.662
PR3	93	11.625	0.748	0.807
PR4	100	12.5	0.775	0.820
PR5	78	9.75	0.725	0.684
PR6	81	10.125	0.683	0.692
BL1	78	9.75	0.808	0.768
BL2	66	8.25	0.725	0.748
BL3	75	9.375	0.713	0.777
BL4	55	6.875	0.801	0.699
BL5	80	10	0.716	0.773
BL6	71	8.875	0.622	0.733

Table 2 (continued).

Site	NA	A	H_o	H_e
LR1	51	6.375	0.554	0.573
LR2	46	5.75	0.525	0.571
LR3	48	6	0.508	0.449
LR4	63	7.875	0.617	0.634
LR5	58	7.25	0.546	0.597
LR6	71	8.875	0.633	0.749
Average	74.72	9.340	0.685	0.709

NA = Total number of alleles across all loci. A = The average number of alleles per locus. H_o = Observed heterozygosity. H_e = expected heterozygosity. Sample site codes are (LMR): BP = Bayou Pierre, BB = Big Black, (GCP): PG = Pascagoula, PR = Pearl, (WH): BL = Black, and LR = Little Red. Each site was analyzed independently (15 individuals per site). Average values are for all 36 sample locations.

Table 3

Genetic diversity and heterozygosity for F. olivaceus across drainages

Drainage	NA	A	H_o	H_e
BP	171	21.375	0.708	0.853
BB	164	20.5	0.675	0.738
PG	169	21.125	0.724	0.757
PR	169	21.125	0.705	0.785
BL	155	19.375	0.732	0.832
LR	164	20.5	0.565	0.698
Average	165.33	20.667	0.685	0.777

NA = Total number of alleles across all loci. A = The average number of alleles per locus. H_o = Observed heterozygosity. H_e = expected heterozygosity. Sample site codes are (LMR): BP = Bayou Pierre, BB = Big Black, (GCP): PG = Pascagoula, PR = Pearl, (WH): BL = Black, and LR = Little Red. All sample locations in a drainage were combined into a single population for analysis (90 individuals per drainage). Average values are for the 6 drainages.

Table 4

Genetic diversity and heterozygosity for S. atromaculatus across sites

Site	NA	A	H_o	H_e
BP1	62	7.750	0.650	0.720
BP2	51	6.375	0.628	0.674
BP3	52	6.500	0.708	0.727
BP4	67	8.375	0.700	0.725
BP5	56	7.000	0.517	0.594
BP6	56	7.000	0.633	0.706
BB1	47	5.875	0.593	0.617
BB2	57	7.125	0.675	0.717
BB3	67	8.375	0.715	0.715
BB4	59	7.375	0.760	0.733
BB5	53	6.625	0.589	0.696
BB6	54	6.750	0.623	0.710
PG1	48	6.000	0.608	0.615
PG2	39	4.875	0.567	0.540
PG3	33	4.125	0.467	0.473
PG4	33	4.125	0.402	0.436
PG5	30	3.750	0.345	0.405
PG6	45	5.625	0.617	0.550
PR1	18	2.250	0.250	0.253
PR2	29	3.625	0.346	0.316
PR3	22	2.750	0.367	0.352
PR4	19	2.375	0.242	0.283
PR5	21	2.625	0.250	0.254
PR6	22	2.750	0.283	0.291
BL1	59	7.375	0.678	0.731
BL2	51	6.375	0.625	0.676
BL3	59	7.375	0.725	0.734
BL4	59	7.375	0.675	0.699
BL5	47	5.875	0.483	0.645
BL6	50	6.250	0.555	0.660

Table 4 (continued).

Site	NA	A	H_o	H_e
LR1	37	4.625	0.573	0.571
LR2	37	4.625	0.536	0.553
LR3	39	4.875	0.508	0.627
LR4	37	4.625	0.608	0.584
LR5	40	5.000	0.517	0.569
LR6	46	5.750	0.525	0.632
Average	44.47	5.559	0.543	0.577

NA = Total number of alleles across all loci. A = The average number of alleles per locus. H_o = Observed heterozygosity. H_e = expected heterozygosity. Sample site codes are (LMR): BP = Bayou Pierre, BB = Big Black, (GCP): PG = Pascagoula, PR = Pearl, (WH): BL = Black, and LR = Little Red. Each site was analyzed independently (15 individuals per site). Average values are for all 36 sample locations.

Table 5

Genetic diversity and heterozygosity for S. atromaculatus across drainages

Drainage	NA	A	H_o	H_e
BP	117	14.625	0.638	0.765
BB	104	13.000	0.661	0.758
PG	96	12.000	0.502	0.752
PR	41	5.125	0.290	0.368
BL	113	14.125	0.624	0.780
LR	93	11.625	0.546	0.715
Average	94	11.75	0.544	0.690

NA = Total number of alleles across all loci. A = The average number of alleles per locus. H_o = Observed heterozygosity. H_e = expected heterozygosity. Sample site codes are (LMR): BP = Bayou Pierre, BB = Big Black, (GCP): PG = Pascagoula, PR = Pearl, (WH): BL = Black, and LR = Little Red. All sample locations in a drainage were combined into a single population for analysis (90 individuals per drainage). Average values are for the 6 drainages.

Table 6

Genetic diversity and heterozygosity for E. claviformis across sites

Site	NA	A	H_o	H_e
BP1	46	7.667	0.686	0.774
BP2	53	8.833	0.733	0.812
BP3	51	8.500	0.678	0.774
BP4	31	5.167	0.713	0.692
BP5	49	8.167	0.622	0.794
BP6	54	9.000	0.687	0.778
BB1	48	8.000	0.778	0.796
BB2	48	8.000	0.800	0.774
BB3	52	8.667	0.842	0.811
BB4	57	9.500	0.798	0.816
BB5	42	7.000	0.800	0.752
BB6	51	8.500	0.756	0.805
PG1	48	8.000	0.633	0.757
PG2	67	11.167	0.689	0.842
PG3	47	7.833	0.572	0.745
PG4	54	9.000	0.644	0.811
PG5	54	9.000	0.684	0.777
PG6	44	7.333	0.700	0.737
PR1	48	8.000	0.800	0.804
PR2	46	7.667	0.683	0.776
PR3	59	9.833	0.712	0.801
PR4	55	9.167	0.644	0.783
PR5	47	7.833	0.742	0.748
PR6	58	9.667	0.667	0.810
BL1	36	6.000	0.667	0.676
BL2	39	6.500	0.689	0.723
BL3	45	7.500	0.756	0.723
BL4	35	5.833	0.600	0.615
BL5	33	5.500	0.656	0.649
BL6	38	6.333	0.833	0.779

Table 6 (continued).

Site	NA	A	H_o	H_e
LR1	34	5.667	0.456	0.530
LR2	37	6.167	0.533	0.590
LR3	33	5.500	0.571	0.588
LR4	61	10.167	0.644	0.757
LR5	52	8.667	0.731	0.705
LR6	39	6.500	0.581	0.668
Average	62.63	7.829	0.688	0.744

NA = Total number of alleles across all loci. A = The average number of alleles per locus. H_o = Observed heterozygosity. H_e = expected heterozygosity. Sample site codes are (LMR): BP = Bayou Pierre, BB = Big Black, (GCP): PG = Pascagoula, PR = Pearl, (WH): BL = Black, and LR = Little Red. Each site was analyzed independently (15 individuals per site). Average values are for all 36 sample locations.

Table 7

Genetic diversity and heterozygosity for E. claviformis across drainages

Drainage	NA	A	H_o	H_e
BP	90	15.000	0.688	0.828
BB	81	13.500	0.796	0.847
PG	111	18.500	0.654	0.904
PR	97	16.167	0.708	0.877
BL	84	14.000	0.700	0.796
LR	95	15.833	0.586	0.748
Average	93	15.500	0.689	0.833

NA = Total number of alleles across all loci. A = The average number of alleles per locus. H_o = Observed heterozygosity. H_e = expected heterozygosity. Sample site codes are (LMR): BP = Bayou Pierre, BB = Big Black, (GCP): PG = Pascagoula, PR = Pearl, (WH): BL = Black, and LR = Little Red. All sample locations in a drainage were combined into a single population for analysis (90 individuals per drainage). Average values are for the 6 drainages.

Table 8

Genetic diversity and heterozygosity for E. artesiae across sites

Site	NA	A	H_o	H_e
BP1	76	9.500	0.795	0.805
BP2	86	10.750	0.867	0.843
BP3	53	6.625	0.643	0.689
BP4	84	10.500	0.775	0.806
BP5	78	9.750	0.791	0.819
BP6	78	9.750	0.679	0.790
BB1	52	6.500	0.473	0.728
BB2	61	7.625	0.518	0.637
BB3	60	7.500	0.579	0.725
BB4	75	9.375	0.664	0.735
BB5	66	8.250	0.723	0.721
BB6	73	9.125	0.758	0.737
Average	70.17	8.771	0.689	0.753

NA = Total number of alleles across all loci. A = The average number of alleles per locus. H_o = Observed heterozygosity. H_e = expected heterozygosity. Sample site codes are (LMR): BP = Bayou Pierre and BB = Big Black. Each site was analyzed independently (15 individuals per site). Average values are for all 12 sample locations.

Table 9

Genetic diversity and heterozygosity for E. artesiae across drainages

Drainage	NA	A	H_o	H_e
BP	153	19.125	0.760	0.868
BB	159	19.875	0.623	0.799
Average	156	19.500	0.691	0.833

NA = Total number of alleles across all loci. A = The average number of alleles per locus. H_o = Observed heterozygosity. H_e = expected heterozygosity. Sample site codes are (LMR): BP = Bayou Pierre and BB = Big Black. All sample locations in a drainage were combined into a single population for analysis (90 individuals per drainage). Average values are for the 2 drainages.

Table 10

Genetic diversity and heterozygosity for E. parvipinne across sites

Site	NA	A	H_o	H_e
PG1	89	11.125	0.697	0.834
PG2	88	11.000	0.741	0.847
PG3	92	11.500	0.758	0.861
PG4	74	9.250	0.618	0.834
PG5	69	8.625	0.605	0.800
PG6	82	10.250	0.602	0.844
PR1	72	9.000	0.746	0.789
PR2	87	10.875	0.681	0.845
PR3	76	9.500	0.703	0.773
PR4	60	7.500	0.753	0.785
PR5	77	9.625	0.613	0.788
PR6	80	10.000	0.725	0.829
Average	78.83	9.854	0.687	0.819

NA = Total number of alleles across all loci. A = The average number of alleles per locus. H_o = Observed heterozygosity. H_e = expected heterozygosity. Sample site codes are (GCP): PG = Pascagoula and PR = Pearl. Each site was analyzed independently (15 individuals per site). Average values are for all 12 sample locations.

Table 11

Genetic diversity and heterozygosity for E. parvipinne across drainages

Drainage	NA	A	H_o	H_e
PG	191	23.875	0.673	0.934
PR	169	21.125	0.703	0.914
Average	180	22.500	0.688	0.924

NA = Total number of alleles across all loci. A = The average number of alleles per locus. H_o = Observed heterozygosity. H_e = expected heterozygosity. Sample site codes are (GCP): PG = Pascagoula and PR = Pearl. All sample locations in a drainage were combined into a single population for analysis (90 individuals per drainage). Average values are for the 2 drainages.

Table 12

Genetic diversity and heterozygosity for E. whipplei across sites

Site	NA	A	H_o	H_e
MW1	29	3.625	0.517	0.499
MW2	49	6.125	0.475	0.532
MW3	52	6.500	0.567	0.577
MW4	69	8.625	0.667	0.715
MW5	68	8.500	0.708	0.768
MW6	47	5.875	0.434	0.486
LR1	52	6.500	0.583	0.599
LR2	77	9.625	0.817	0.753
LR3	68	8.500	0.792	0.749
LR4	80	10.000	0.742	0.809
LR5	84	10.500	0.792	0.809
LR6	67	8.375	0.725	0.798
Average	61.83	7.729	0.651	0.675

NA = Total number of alleles across all loci. A = The average number of alleles per locus. H_o = Observed heterozygosity. H_e = expected heterozygosity. Sample site codes are (WH): MW = Middle White and LR = Little Red. Each site was analyzed independently (15 individuals per site). Average values are for all 12 sample locations.

Table 13

Genetic diversity and heterozygosity for E. whipplei across drainages

Drainage	NA	A	H_o	H_e
MW	113	14.125	0.562	0.668
LR	147	18.375	0.742	0.833
Average	130	16.250	0.652	0.751

NA = Total number of alleles across all loci. A = The average number of alleles per locus. H_o = Observed heterozygosity. H_e = expected heterozygosity. Sample site codes are (WH): MW = Middle White and LR = Little Red. All sample locations in a drainage were combined into a single population for analysis (90 individuals per drainage). Average values are for the 2 drainages.

Table 14

Pairwise F_{ST} values for F. olivaceus in the LMR region

	BP1	BP2	BP3	BP4	BP5	BP6	BB1	BB2	BB3	BB4	BB5	BB6
BP1	0.000											
BP2	0.024	0.000										
BP3	0.022	0.024	0.000									
BP4	0.022	0.020	0.028	0.000								
BP5	0.035	0.023	0.035	0.029	0.000							
BP6	0.030	0.027	0.037	0.036	0.034	0.000						
BB1	0.065	0.063	0.065	0.072	0.065	0.057	0.000					
BB2	0.113	0.097	0.099	0.125	0.102	0.079	0.071	0.000				
BB3	0.082	0.068	0.073	0.087	0.071	0.050	0.051	0.039	0.000			
BB4	0.076	0.066	0.063	0.081	0.067	0.049	0.057	0.051	0.029	0.000		
BB5	0.068	0.056	0.062	0.074	0.058	0.036	0.053	0.040	0.032	0.036	0.000	
BB6	0.053	0.057	0.056	0.065	0.059	0.036	0.057	0.065	0.043	0.049	0.037	0.000

LMR = Lower Mississippi River. BP = Bayou Pierre. BB = Big Black.

Table 15

Pairwise F_{ST} values for F. olivaceus in the GCP region

	PG1	PG2	PG3	PG4	PG5	PG6	PR1	PR2	PR3	PR4	PR5	PR6
PG1	0.000											
PG2	0.029	0.000										
PG3	0.077	0.099	0.000									
PG4	0.063	0.087	0.026	0.000								
PG5	0.035	0.047	0.073	0.061	0.000							
PG6	0.087	0.113	0.063	0.058	0.040	0.000						
PR1	0.141	0.172	0.129	0.116	0.171	0.160	0.000					
PR2	0.106	0.138	0.110	0.097	0.146	0.143	0.064	0.000				
PR3	0.054	0.070	0.088	0.070	0.087	0.123	0.070	0.050	0.000			
PR4	0.077	0.104	0.093	0.075	0.104	0.115	0.050	0.047	0.033	0.000		
PR5	0.095	0.133	0.110	0.100	0.138	0.144	0.069	0.030	0.050	0.040	0.000	
PR6	0.135	0.178	0.123	0.107	0.168	0.147	0.041	0.052	0.074	0.043	0.045	0.000

GCP = Gulf Coastal Plain. PG = Pascagoula. PR = Pearl.

Table 16

Pairwise F_{ST} values for F. olivaceus in the WH region

	BL1	BL2	BL3	BL4	BL5	BL6	LR1	LR2	LR3	LR4	LR5	LR6
BL1	0.000											
BL2	0.057	0.000										
BL3	0.054	0.049	0.000									
BL4	0.075	0.092	0.077	0.000								
BL5	0.034	0.048	0.040	0.085	0.000							
BL6	0.046	0.073	0.074	0.105	0.053	0.000						
LR1	0.160	0.162	0.156	0.183	0.141	0.164	0.000					
LR2	0.151	0.175	0.150	0.181	0.136	0.171	0.058	0.000				
LR3	0.189	0.229	0.204	0.247	0.188	0.222	0.097	0.126	0.000			
LR4	0.103	0.134	0.139	0.194	0.114	0.093	0.112	0.136	0.137	0.000		
LR5	0.123	0.164	0.125	0.172	0.120	0.135	0.105	0.107	0.106	0.094	0.000	
LR6	0.066	0.099	0.079	0.106	0.060	0.078	0.082	0.084	0.114	0.076	0.055	0.000

WH = White River. BL = Black. LR = Little Red.

Table 17

Pairwise F_{ST} values for S. atromaculatus in the LMR region

	BP1	BP2	BP3	BP4	BP5	BP6	BB1	BB2	BB3	BB4	BB5	BB6
BP1	0.000											
BP2	0.052	0.000										
BP3	0.061	0.058	0.000									
BP4	0.026	0.053	0.065	0.000								
BP5	0.069	0.051	0.097	0.073	0.000							
BP6	0.053	0.059	0.082	0.050	0.052	0.000						
BB1	0.068	0.126	0.109	0.085	0.145	0.117	0.000					
BB2	0.053	0.086	0.084	0.059	0.117	0.091	0.054	0.000				
BB3	0.044	0.078	0.082	0.046	0.069	0.067	0.056	0.028	0.000			
BB4	0.046	0.076	0.070	0.047	0.088	0.076	0.050	0.037	0.027	0.000		
BB5	0.059	0.082	0.087	0.052	0.099	0.078	0.067	0.054	0.043	0.039	0.000	
BB6	0.065	0.080	0.076	0.059	0.109	0.095	0.084	0.058	0.050	0.036	0.041	0.000

LMR = Lower Mississippi River. BP = Bayou Pierre. BB = Big Black.

Table 18

Pairwise F_{ST} values for S. atromaculatus in the GCP region

	PG1	PG2	PG3	PG4	PG5	PG6	PR1	PR2	PR3	PR4	PR5	PR6
PG1	0.000											
PG2	0.086	0.000										
PG3	0.133	0.055	0.000									
PG4	0.310	0.358	0.384	0.000								
PG5	0.348	0.364	0.399	0.136	0.000							
PG6	0.070	0.076	0.107	0.336	0.382	0.000						
PR1	0.401	0.444	0.496	0.302	0.286	0.451	0.000					
PR2	0.358	0.413	0.456	0.240	0.252	0.406	0.082	0.000				
PR3	0.338	0.386	0.439	0.275	0.258	0.361	0.202	0.167	0.000			
PR4	0.351	0.417	0.471	0.299	0.267	0.409	0.135	0.129	0.105	0.000		
PR5	0.381	0.447	0.501	0.305	0.281	0.433	0.130	0.098	0.130	0.101	0.000	
PR6	0.373	0.419	0.466	0.283	0.227	0.426	0.116	0.082	0.151	0.085	0.056	0.000

GCP = Gulf Coastal Plain. PG = Pascagoula. PR = Pearl.

Table 19

Pairwise F_{ST} values for S. atromaculatus in the WH region

	BL1	BL2	BL3	BL4	BL5	BL6	LR1	LR2	LR3	LR4	LR5	LR6
BL1	0.000											
BL2	0.038	0.000										
BL3	0.068	0.070	0.000									
BL4	0.061	0.069	0.060	0.000								
BL5	0.096	0.078	0.085	0.078	0.000							
BL6	0.045	0.050	0.068	0.083	0.107	0.000						
LR1	0.105	0.118	0.132	0.117	0.116	0.135	0.000					
LR2	0.102	0.126	0.138	0.106	0.143	0.140	0.086	0.000				
LR3	0.103	0.099	0.120	0.091	0.106	0.125	0.130	0.113	0.000			
LR4	0.127	0.147	0.099	0.138	0.143	0.158	0.158	0.165	0.147	0.000		
LR5	0.097	0.117	0.114	0.108	0.124	0.137	0.093	0.087	0.134	0.123	0.000	
LR6	0.096	0.090	0.106	0.084	0.090	0.117	0.113	0.108	0.033	0.119	0.102	0.000

WH = White River. BL = Black. LR = Little Red.

Table 20

Pairwise F_{ST} values for E. claviformis in the LMR region

	BP1	BP2	BP3	BP4	BP5	BP6	BB1	BB2	BB3	BB4	BB5	BB6
BP1	0.000											
BP2	0.033	0.000										
BP3	0.049	0.029	0.000									
BP4	0.049	0.052	0.051	0.000								
BP5	0.042	0.034	0.043	0.069	0.000							
BP6	0.035	0.019	0.028	0.047	0.037	0.000						
BB1	0.056	0.040	0.046	0.081	0.048	0.055	0.000					
BB2	0.045	0.031	0.046	0.062	0.047	0.036	0.051	0.000				
BB3	0.051	0.041	0.050	0.070	0.059	0.046	0.031	0.054	0.000			
BB4	0.049	0.042	0.056	0.062	0.054	0.043	0.034	0.041	0.030	0.000		
BB5	0.057	0.053	0.060	0.088	0.053	0.061	0.035	0.046	0.047	0.049	0.000	
BB6	0.044	0.027	0.036	0.055	0.051	0.032	0.029	0.040	0.031	0.035	0.048	0.000

LMR = Lower Mississippi River. BP = Bayou Pierre. BB = Big Black.

Table 21

Pairwise F_{ST} values for E. claviformis in the GCP region

	PG1	PG2	PG3	PG4	PG5	PG6	PR1	PR2	PR3	PR4	PR5	PR6
PG1	0.000											
PG2	0.098	0.000										
PG3	0.123	0.037	0.000									
PG4	0.092	0.047	0.072	0.000								
PG5	0.114	0.054	0.092	0.067	0.000							
PG6	0.121	0.097	0.123	0.110	0.118	0.000						
PR1	0.100	0.074	0.102	0.070	0.080	0.108	0.000					
PR2	0.115	0.077	0.108	0.082	0.088	0.084	0.058	0.000				
PR3	0.092	0.053	0.065	0.055	0.079	0.117	0.071	0.092	0.000			
PR4	0.105	0.086	0.112	0.072	0.099	0.114	0.045	0.064	0.072	0.000		
PR5	0.096	0.088	0.119	0.083	0.090	0.117	0.067	0.063	0.088	0.060	0.000	
PR6	0.104	0.071	0.099	0.066	0.070	0.100	0.046	0.066	0.061	0.052	0.061	0.000

GCP = Gulf Coastal Plain. PG = Pascagoula. PR = Pearl.

Table 22

Pairwise F_{ST} values for E. claviformis in the WH region

	BL1	BL2	BL3	BL4	BL5	BL6	LR1	LR2	LR3	LR4	LR5	LR6
BL1	0.000											
BL2	0.082	0.000										
BL3	0.123	0.076	0.000									
BL4	0.146	0.086	0.070	0.000								
BL5	0.074	0.062	0.098	0.074	0.000							
BL6	0.082	0.060	0.061	0.088	0.069	0.000						
LR1	0.157	0.165	0.168	0.206	0.154	0.145	0.000					
LR2	0.130	0.144	0.144	0.190	0.130	0.130	0.107	0.000				
LR3	0.146	0.166	0.148	0.209	0.159	0.129	0.108	0.124	0.000			
LR4	0.077	0.086	0.086	0.127	0.086	0.065	0.069	0.067	0.077	0.000		
LR5	0.078	0.064	0.098	0.123	0.090	0.085	0.120	0.104	0.125	0.056	0.000	
LR6	0.104	0.082	0.087	0.113	0.089	0.079	0.106	0.098	0.121	0.055	0.041	0.000

WH = White River. BL = Black. LR = Little Red.

Table 23

Pairwise F_{ST} values for E. artesiae in the LMR region

	BP1	BP2	BP3	BP4	BP5	BP6	BB1	BB2	BB3	BB4	BB5	BB6
BP1	0.000											
BP2	0.032	0.000										
BP3	0.067	0.061	0.000									
BP4	0.026	0.035	0.059	0.000								
BP5	0.040	0.025	0.077	0.048	0.000							
BP6	0.065	0.059	0.104	0.066	0.063	0.000						
BB1	0.088	0.074	0.115	0.085	0.082	0.097	0.000					
BB2	0.117	0.106	0.142	0.100	0.114	0.126	0.099	0.000				
BB3	0.083	0.075	0.115	0.078	0.089	0.098	0.060	0.072	0.000			
BB4	0.094	0.084	0.128	0.091	0.091	0.098	0.055	0.078	0.049	0.000		
BB5	0.077	0.072	0.108	0.069	0.078	0.097	0.063	0.053	0.043	0.073	0.000	
BB6	0.072	0.062	0.093	0.064	0.066	0.099	0.085	0.082	0.060	0.088	0.054	0.000

LMR = Lower Mississippi River. BP = Bayou Pierre. BB = Big Black.

Table 24

Pairwise F_{ST} values for E. parvipinne in the GCP region

	PG1	PG2	PG3	PG4	PG5	PG6	PR1	PR2	PR3	PR4	PR5	PR6
PG1	0.000											
PG2	0.060	0.000										
PG3	0.067	0.057	0.000									
PG4	0.070	0.057	0.054	0.000								
PG5	0.076	0.080	0.061	0.086	0.000							
PG6	0.067	0.057	0.054	0.067	0.067	0.000						
PR1	0.071	0.078	0.069	0.079	0.087	0.075	0.000					
PR2	0.061	0.060	0.056	0.070	0.070	0.057	0.066	0.000				
PR3	0.085	0.087	0.063	0.097	0.069	0.085	0.087	0.061	0.000			
PR4	0.070	0.070	0.077	0.087	0.082	0.077	0.089	0.071	0.106	0.000		
PR5	0.072	0.075	0.072	0.081	0.073	0.065	0.092	0.081	0.085	0.088	0.000	
PR6	0.057	0.070	0.062	0.073	0.076	0.058	0.076	0.067	0.091	0.051	0.070	0.000

GCP = Gulf Coastal Plain. PG = Pascagoula. PR = Pearl.

Table 25

Pairwise F_{ST} values for E. whipplei in the WH region

	MW1	MW2	MW3	MW4	MW5	MW6	LR1	LR2	LR3	LR4	LR5	LR6
MW1	0.000											
MW2	0.100	0.000										
MW3	0.075	0.039	0.000									
MW4	0.064	0.058	0.039	0.000								
MW5	0.092	0.084	0.066	0.022	0.000							
MW6	0.117	0.060	0.051	0.069	0.094	0.000						
LR1	0.153	0.136	0.100	0.088	0.082	0.151	0.000					
LR2	0.120	0.077	0.079	0.048	0.043	0.115	0.061	0.000				
LR3	0.153	0.143	0.131	0.081	0.062	0.184	0.120	0.066	0.000			
LR4	0.104	0.088	0.077	0.032	0.027	0.116	0.085	0.035	0.049	0.000		
LR5	0.145	0.101	0.109	0.059	0.045	0.146	0.120	0.043	0.058	0.032	0.000	
LR6	0.152	0.113	0.110	0.067	0.044	0.146	0.092	0.044	0.054	0.035	0.028	0.000

WH = White River. MW = Middle White. LR = Little Red.

Table 26

Average pairwise F_{ST} values within drainages and between drainages in regions

Species	BP	BB	LMR	PG	PR	GCP	BL/MW	LR	WH
F. olivaceus	0.028	0.047	0.070	0.064	0.050	0.119	0.064	0.099	0.148
S. atromaculatus	0.060	0.048	0.080	0.236	0.118	0.370	0.070	0.114	0.117
E. claviformis	0.041	0.040	0.051	0.091	0.064	0.090	0.084	0.092	0.123
E. artesiae	0.055	0.068	0.092						
E. parvipinne				0.065	0.079	0.073			
E. whipplei							0.069	0.062	0.101

LMR = Lower Mississippi River region: BP = Bayou Pierre and BB = Big Black. GCP = Gulf Coastal Plain Region: PG = Pascagoula and PR = Pearl. WH = White River region: BL = Black, MW = Middle White, and LR = Little Red. *E. whipplei* was sampled in the Middle White (includes the Black), other species were sampled only within the Black drainage. Values are averages for pairwise values between sample sites within drainages (drainage columns) and between sample sites between drainages in the same region (region columns)

Table 27

Average population q scores for the species at different hierarchy levels of STRUCTURE analysis

Species	Hierarchy level	Average population q score
F. olivaceus	1	0.8397
S. atromaculatus	1	0.9911
E. claviformis	1	0.8846
E. artesiae	1	0.9601
E. parvipinne	1	0.7945
E. whipplei	1	0.8953
F. olivaceus	2	0.8357
S. atromaculatus	2	0.9434
E. claviformis	2	0.8852
E. artesiae	2	0.8647
E. parvipinne	2	0.9546
E. whipplei	2	0.8639
F. olivaceus	3	0.879
S. atromaculatus	3	0.9109
E. claviformis	3	0.9029
E. whipplei	3	0.9617
S. atromaculatus	4	0.9465

Population q scores were averaged across all analyses at that hierarchy level. Hierarchy level one corresponds to the full dataset, and numbers are added with sequential hierarchy rounds of analysis.

Table 28

The number of fine-scale, unique genetic populations identified by STRUCTURE across species and drainages

Species	Total	LMR BP	LMR BB	GCP PG	GCP PR	WH BL/MW	WH LR
F. olivaceus	19	1	5	3	2	2	6
S. atromaculatus	24	4	1	5	5	4	5
E. claviformis	25	2	1	5	6	6	5
E. artesiae	6	4	2				
E. parvipinne	11			6	5		
E. whipplei	7					4	3
Drainage Totals		11	9	19	18	16	19

LMR = Lower Mississippi River Region (BP = Bayou Pierre and BB = Big Black). GCP = Gulf Coastal Plain Region (PG = Pascagoula and PR = Pearl). WH = White River Region (BL = Black, MW = Middle White, and LR = Little Red,). *E. whipplei* was sampled in the Middle White (includes the Black), other species were sampled only within the Black drainage.

Table 29

Fragmentation ratios for the six study species

Region	Species	Fragmentation Ratio
LMR	*F. olivaceus*	0.5
	S. atromaculatus	0.42
	E. claviformis	0.25
	E. artesiae	0.5
GCP	*F. olivaceus*	0.42
	S. atromaculatus	0.83
	E. claviformis	0.92
	E. parvipinne	0.92
WH	*F. olivaceus*	0.67
	S. atromaculatus	0.75
	E. claviformis	0.92
	E. whipplei	0.58
All Regions	*F. olivaceus*	0.53
	S. atromaculatus	0.67
	E. claviformis	0.69
* 12 sites	*E. artesiae**	0.5
	*E. parvipinne**	0.58
	*E. whipplei**	0.92

Fragmentation Ratio = the number of unique genetic clusters in a region divided by the number of sample sites in the region.

Table 30

Fragmentation ratio averages for all species in a region

Region	Average Fragmentation Ratio
Lower Mississippi River	0.42
Gulf Coastal Plain	0.77
White River	0.73

Fragmentation Ratio = the number of unique genetic clusters in a region divided by the number of sample sites in the region.

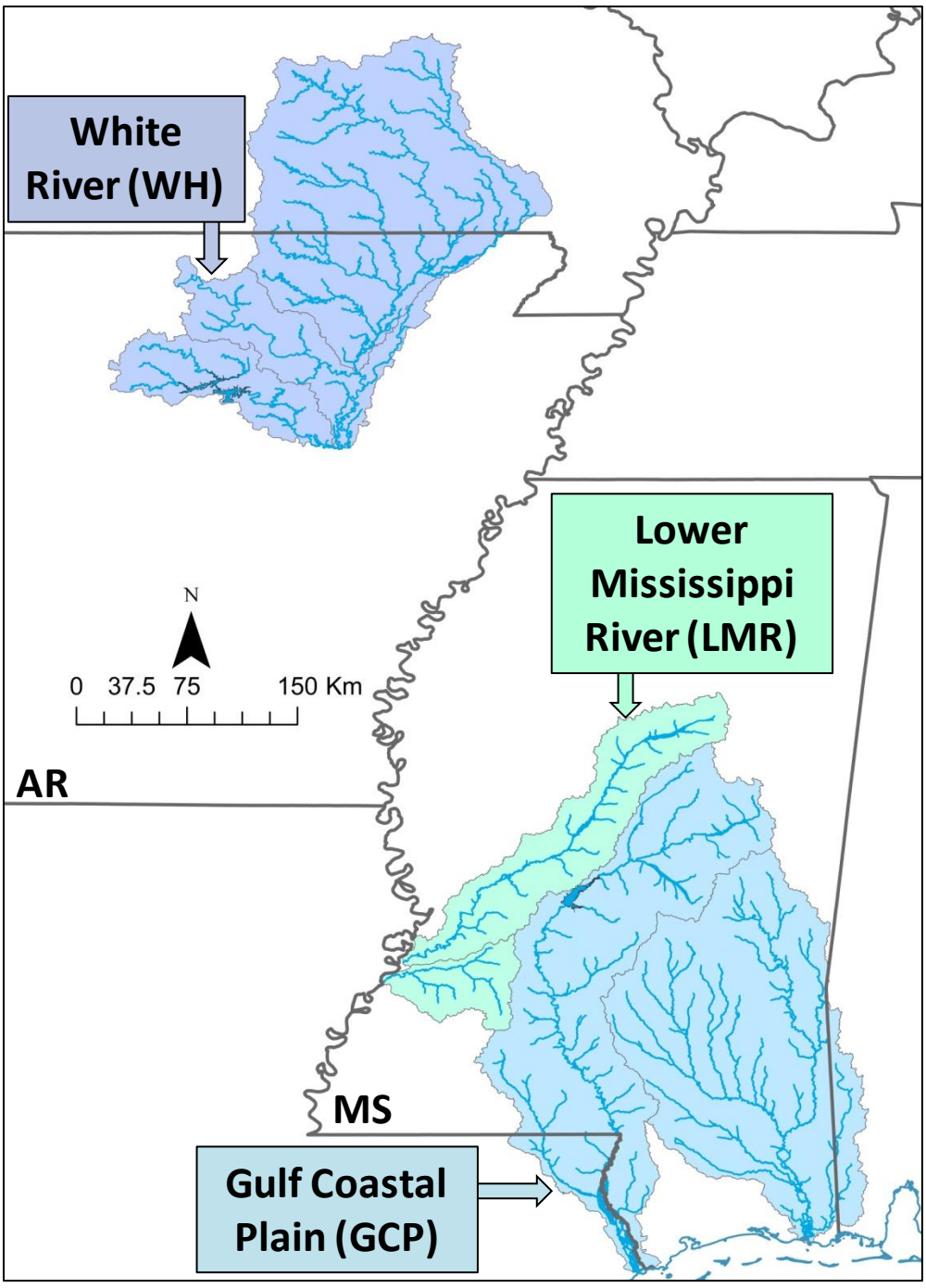

Figure 1. The three geographical regions for the study.

The LMR and WH regions are connected by the Mississippi River. The two GCP drainages each flow independently into the Gulf of Mexico.

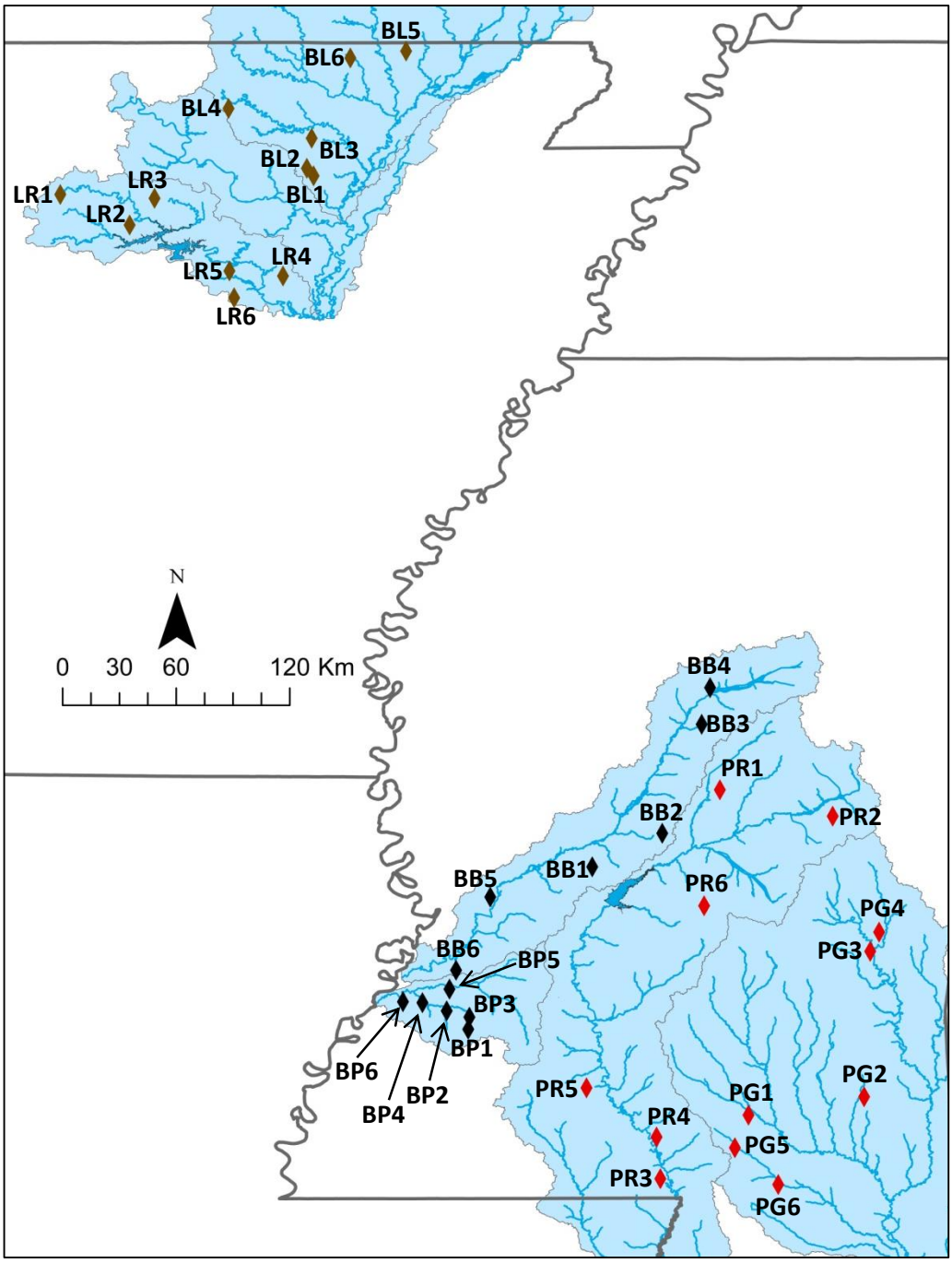

Figure 2. Sample locations for *F. olivaceus*.

Drainage codes are (LMR – black diamonds): BP = Bayou Pierre, BB = Big Black, (GCP – red diamonds): PG = Pascagoula, PR = Pearl, (WH – brown diamonds): BL = Black, and LR = Little Red. Sample site numbers are species specific (specific locations in Appendix B).

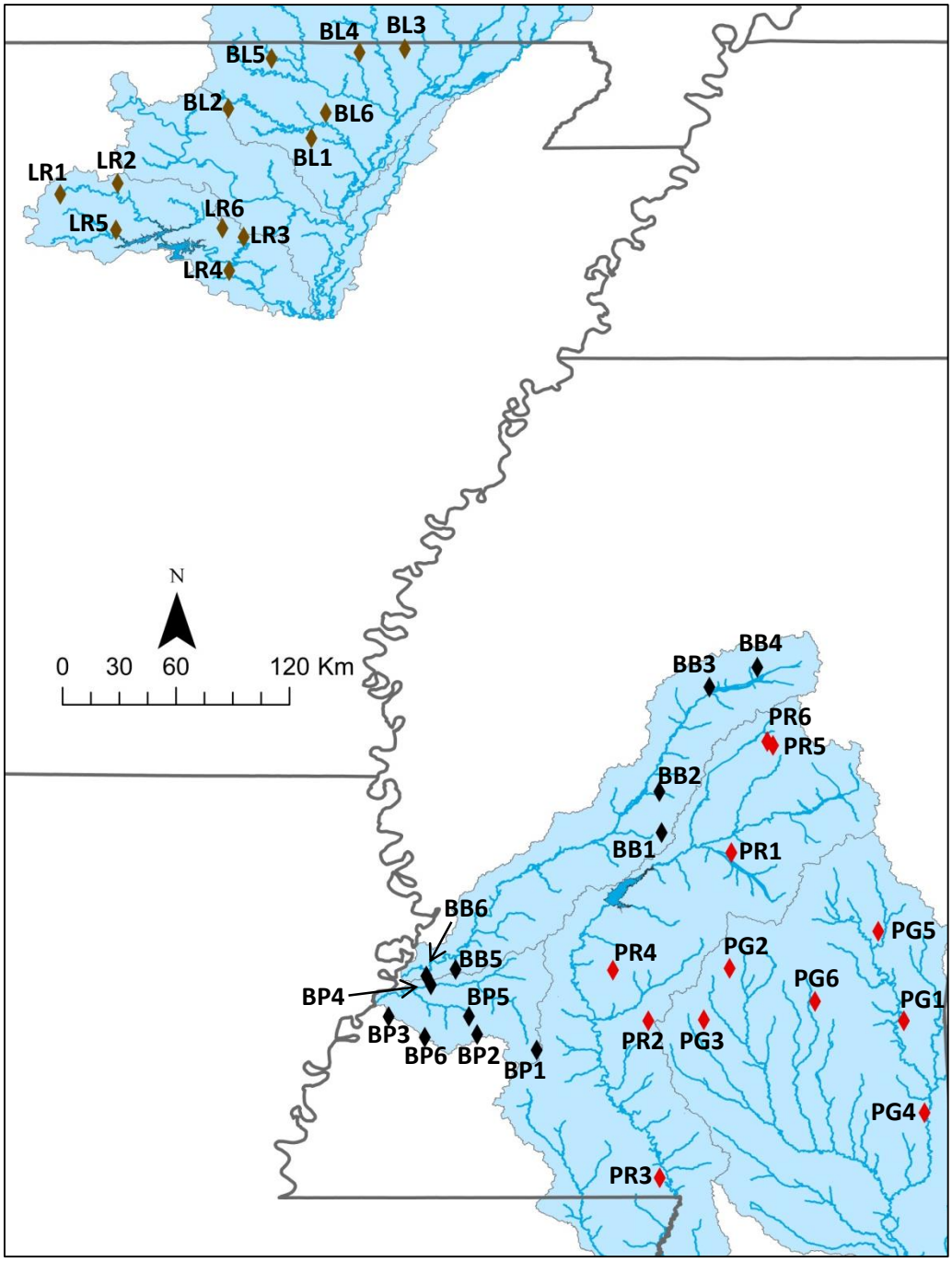

Figure 3. Sample locations for *S. atromaculatus*.

Drainage codes are (LMR – black diamonds): BP = Bayou Pierre, BB = Big Black, (GCP – red diamonds): PG = Pascagoula, PR = Pearl, (WH – brown diamonds): BL = Black, and LR = Little Red. Sample site numbers are species specific (specific locations in Appendix B).

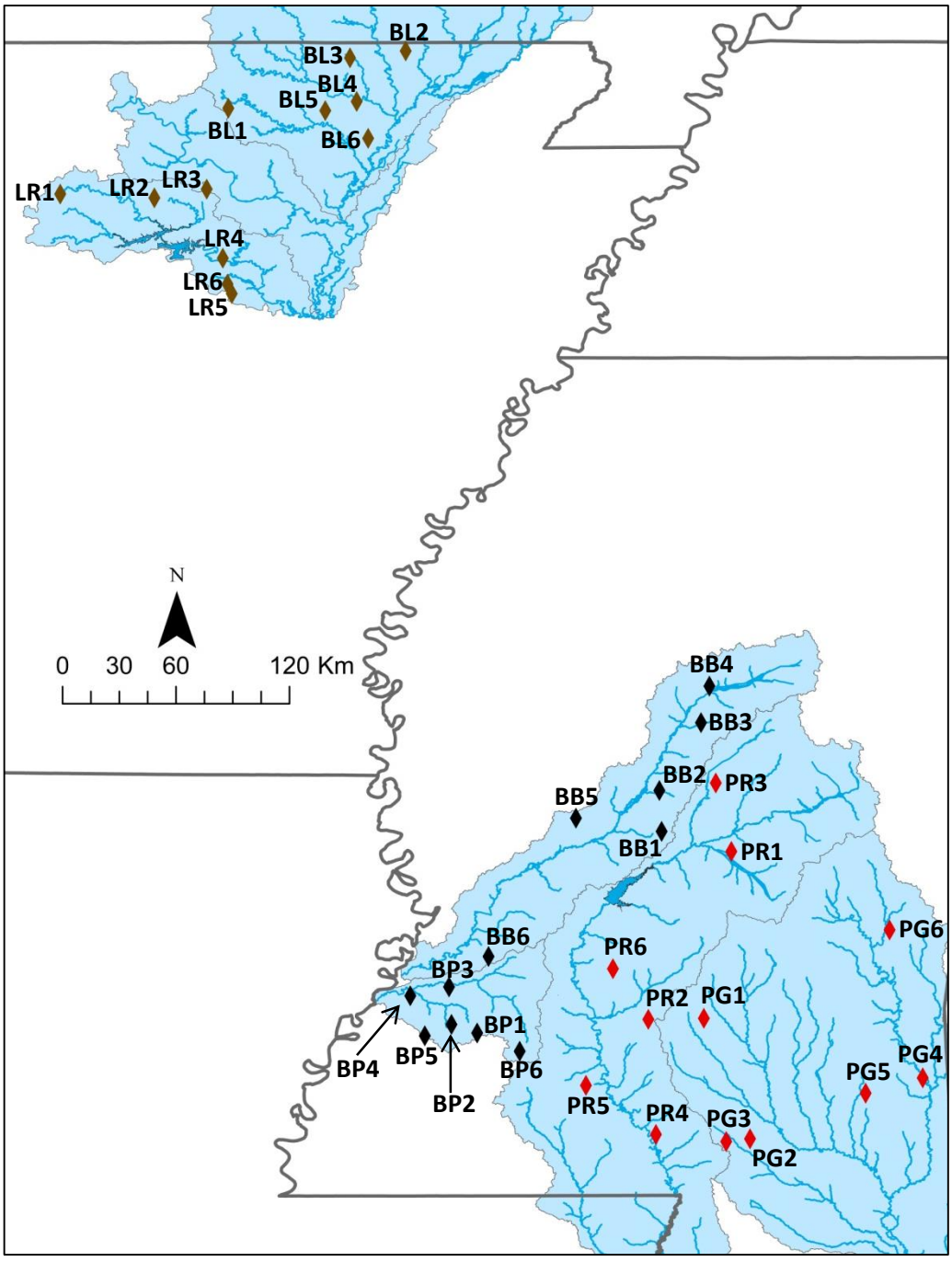

Figure 4. Sample locations for *E. claviformis*.

Drainage codes are (LMR – black diamonds): BP = Bayou Pierre, BB = Big Black, (GCP – red diamonds): PG = Pascagoula, PR = Pearl, (WH – brown diamonds): BL = Black, and LR = Little Red. Sample site numbers are species specific (specific locations in Appendix B).

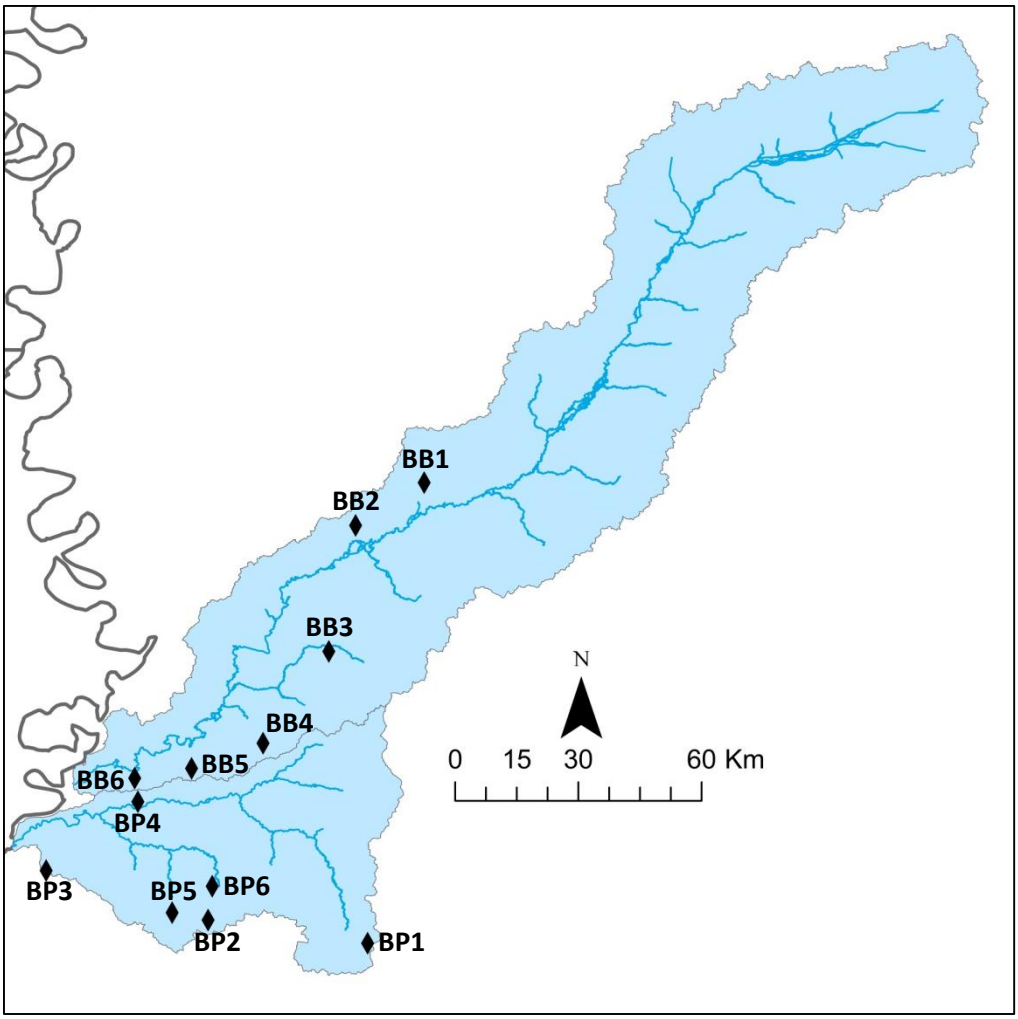

Figure 5. Sample locations for *E. artesiae.*

Drainage codes are (LMR – black diamonds): BP = Bayou Pierre and BB = Big Black. Sample site numbers are species specific (specific locations in Appendix B). *Etheostoma artesiae* does not occur in the upper regions of the Big Black drainage.

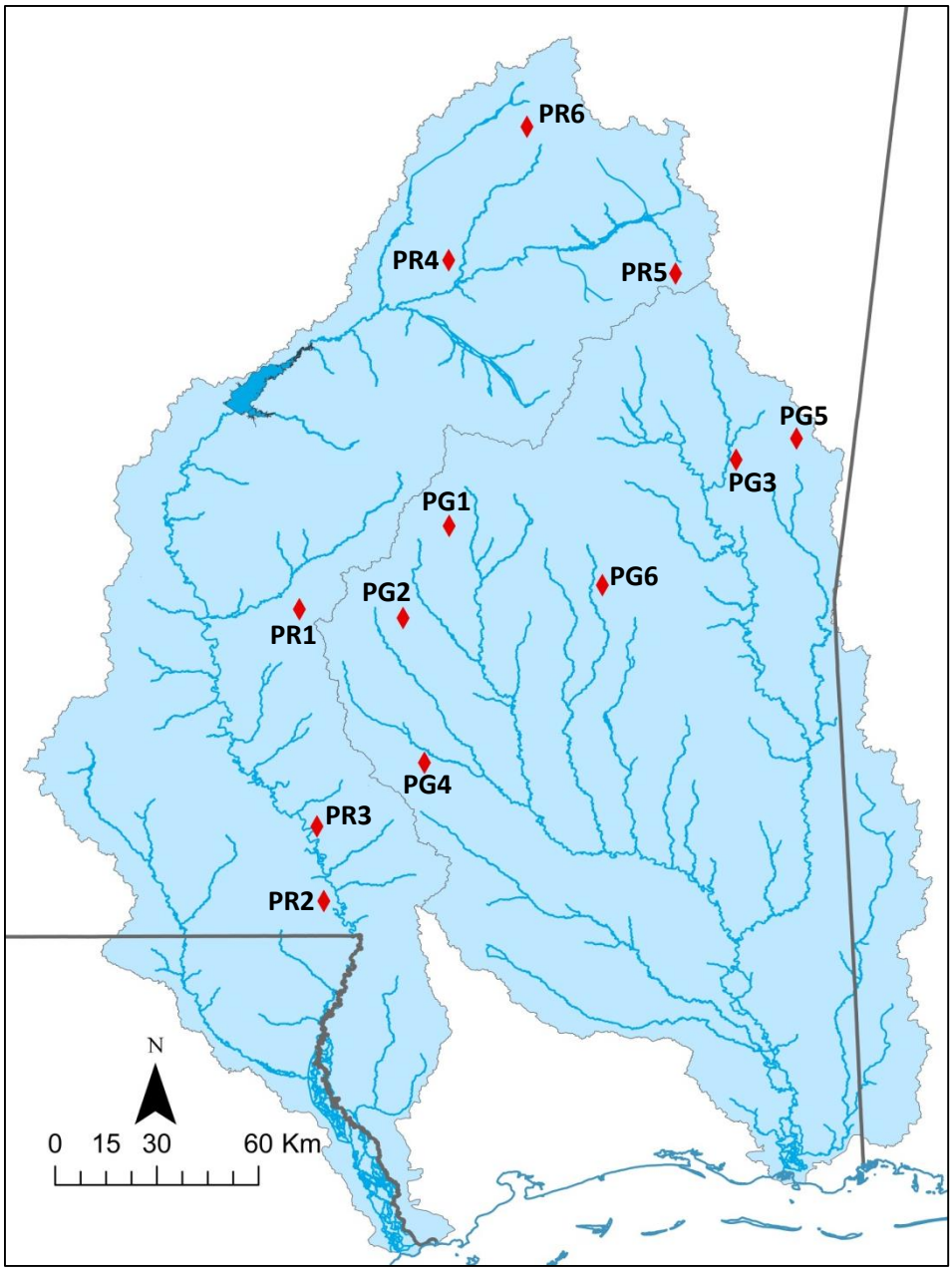

Figure 6. Sample locations for *E. parvipinne*.

Drainage codes are (GCP – red diamonds): PG = Pascagoula and PR = Pearl. Sample site numbers are species specific (specific locations in Appendix B).

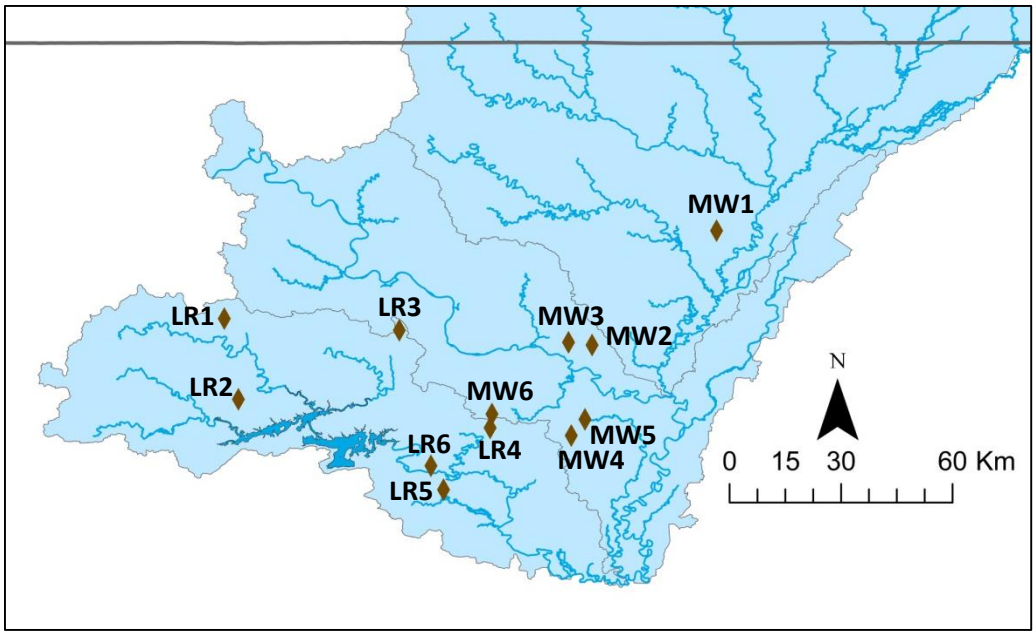

Figure 7. Sample locations for *E. whipplei*.

Drainage codes are (WH – brown diamonds): MW = Middle White (includes the Black drainage) and LR = Little Red. Sample site numbers are species specific (specific locations in Appendix B).

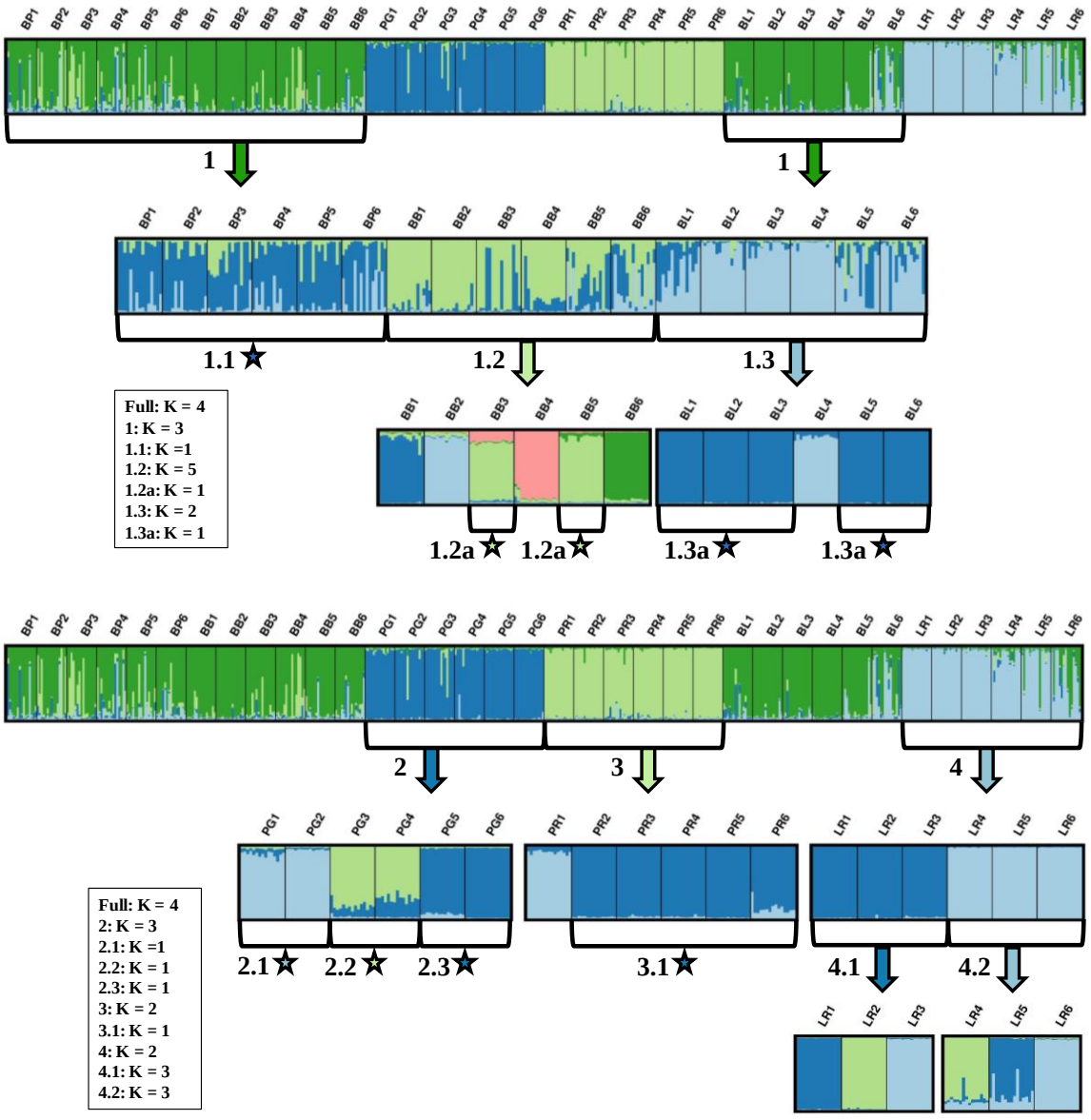

Figure 8. Hierarchical STRUCTURE analyses for *F. olivaceus*.

Vertical bars are individuals and colors are proportions of genotypes assigned to clusters by the program. Separations between bar graphs, numbers, and letters represent different analyses at that hierarchy level. Sample site codes are (LMR): BP = Bayou Pierre, BB = Big Black, (GCP): PG = Pascagoula, PR = Pearl, (WH): BL = Black, and LR = Little Red. Sample site numbers are species specific. Arrows indicate sample site inclusion for next hierarchy level analysis. Stars indicate groups that were identified as panmictic (K of 1). Plots used for determination of K for each analysis are in Appendix C.

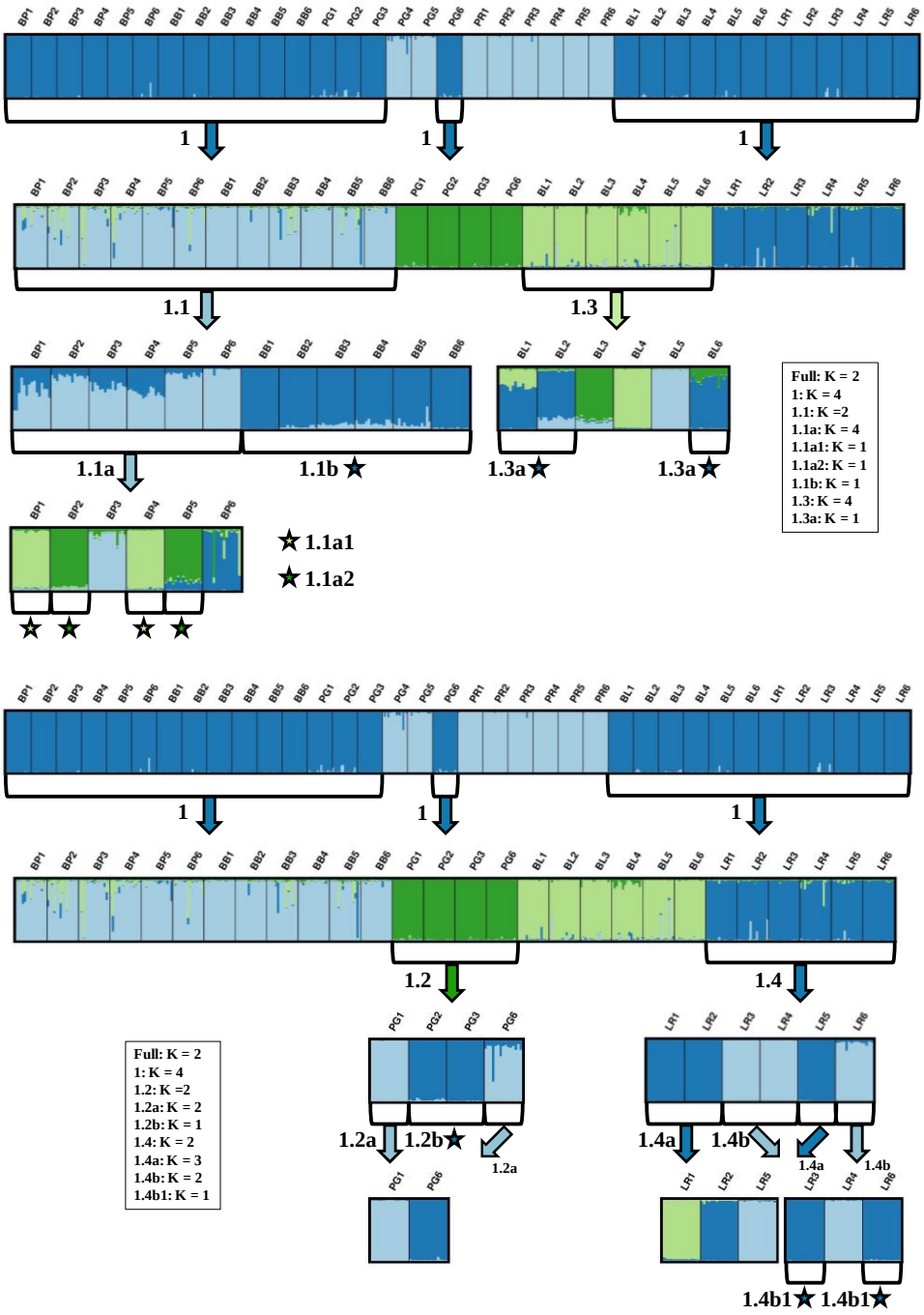

Figure 9. Hierarchical STRUCTURE analyses for *S. atromaculatus* (group one).

Vertical bars are individuals and colors are proportions of genotypes assigned to clusters by the program. Separations between bar graphs, numbers, and letters represent different analyses at that hierarchy level. Sample site codes are (LMR): BP = Bayou Pierre, BB = Big Black, (GCP): PG = Pascagoula, PR = Pearl, (WH): BL = Black, and LR = Little Red. Sample site numbers are species specific. Arrows indicate sample site inclusion for next hierarchy level. Stars indicate groups that were identified as panmictic (K of 1). Plots used for determination of K for each analysis are in Appendix C.

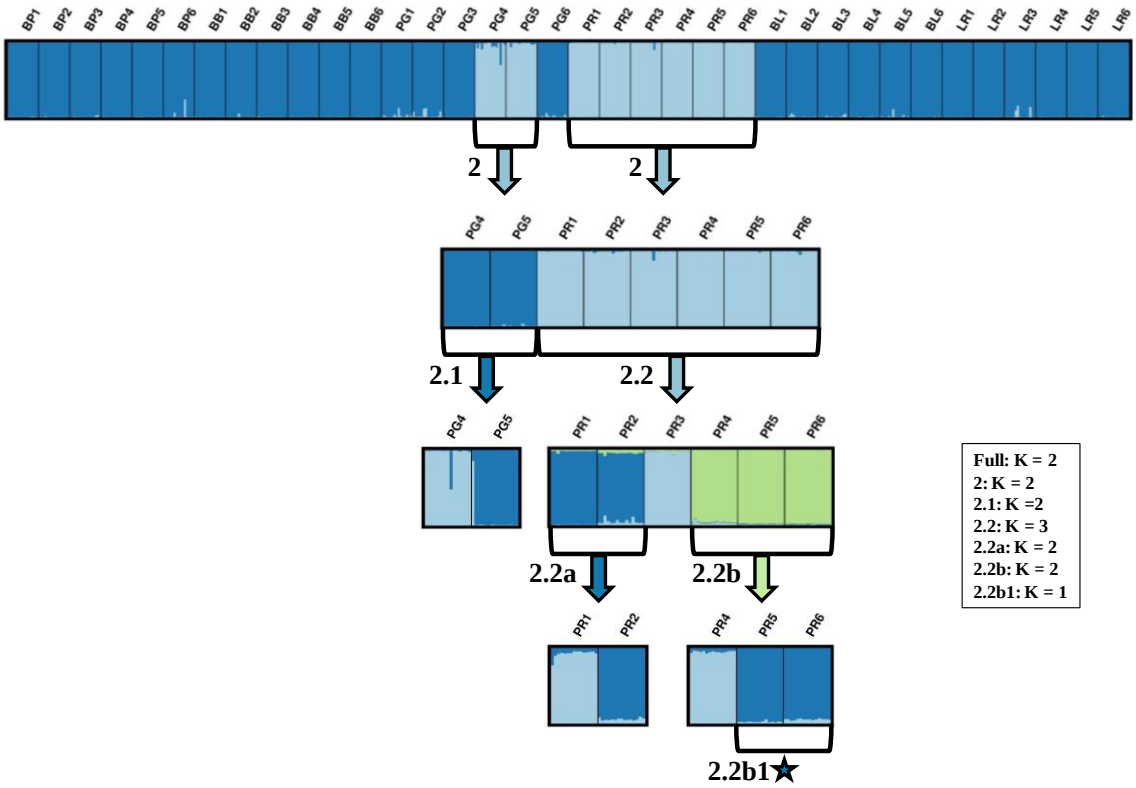

Figure 10. Hierarchical STRUCTURE analyses for *S. atromaculatus* (group two).

Vertical bars are individuals and colors are proportions of genotypes assigned to clusters by the program. Separations between bar graphs, numbers, and letters represent different analyses at that hierarchy level. Sample site codes are (LMR): BP = Bayou Pierre, BB = Big Black, (GCP): PG = Pascagoula, PR = Pearl, (WH): BL = Black, and LR = Little Red. Sample site numbers are species specific. Arrows indicate sample site inclusion for next hierarchy level. Stars indicate groups that were identified as panmictic (K of 1). Plots used for determination of K for each analysis are in Appendix C.

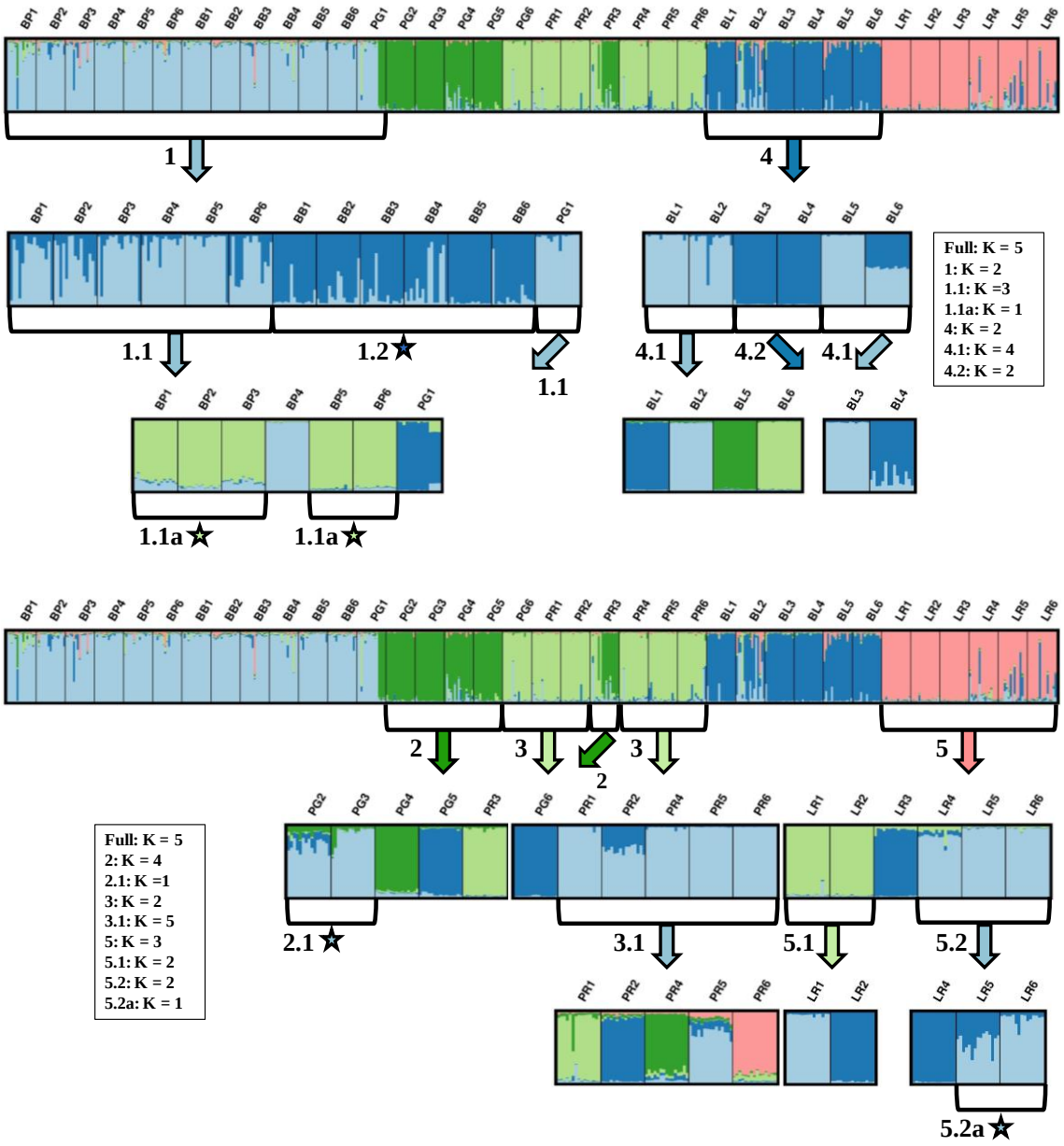

Figure 11. Hierarchical STRUCTURE analyses for *E. claviformis*.

Vertical bars are individuals and colors are proportions of genotypes assigned to clusters by the program. Separations between bar graphs, numbers, and letters represent different analyses at that hierarchy level. Sample site codes are (LMR): BP = Bayou Pierre, BB = Big Black, (GCP): PG = Pascagoula, PR = Pearl, (WH): BL = Black, and LR = Little Red. Sample site numbers are species specific. Arrows indicate sample site inclusion for next hierarchy level. Stars indicate groups that were identified as panmictic (K of 1). Plots used for determination of K for each analysis are in Appendix C.

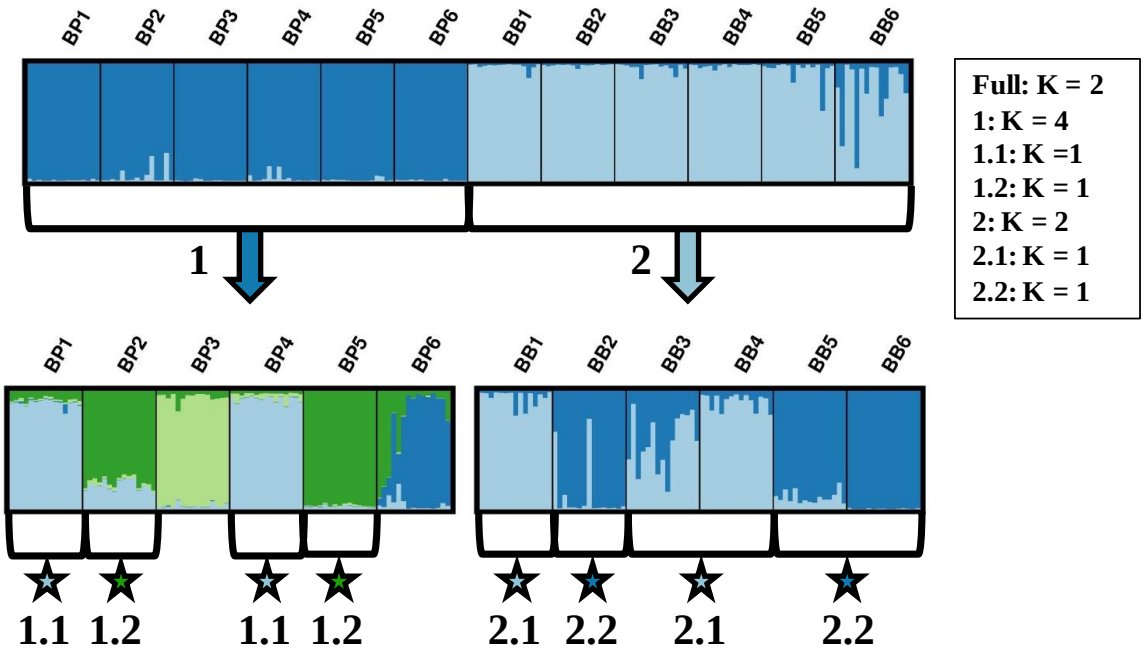

Figure 12. Hierarchical STRUCTURE analyses for *E. artesiae*.

Vertical bars are individuals and colors are proportions of genotypes assigned to clusters by the program. Separations between bar graphs, numbers, and letters represent different analyses at that hierarchy level. Sample site codes are (LMR): BP = Bayou Pierre and BB = Big Black. Sample site numbers are species specific. Arrows indicate sample site inclusion for next hierarchy level. Stars indicate groups that were identified as panmictic (K of 1). Plots used for determination of K for each analysis are in Appendix C.

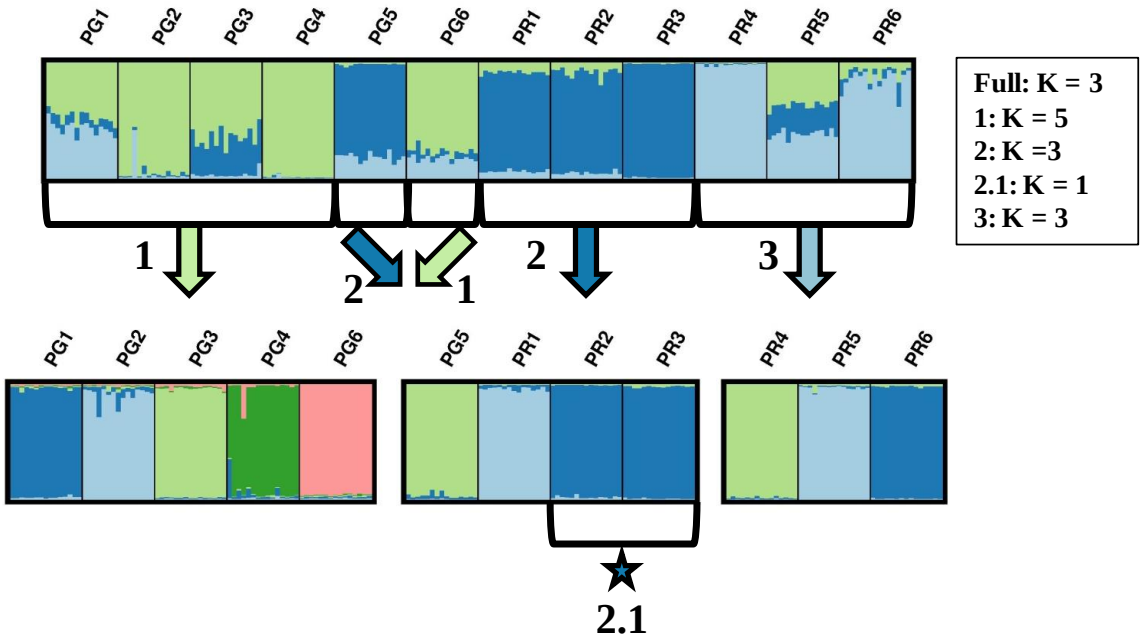

Figure 13. Hierarchical STRUCTURE analyses for *E. parvipinne*.

Hierarchical Structure analyses for *E. parvipinne*. Vertical bars are individuals and colors are proportions of genotypes assigned to clusters by the program. Separations between bar graphs, numbers, and letters represent different analyses at that hierarchy level. Sample site codes are (GCP): PG = Pascagoula and PR = Pearl. Sample site numbers are species specific. Arrows indicate sample site inclusion for next hierarchy level. Stars indicate groups that were identified as panmictic (K of 1). Plots used for determination of K for each analysis are in Appendix C.

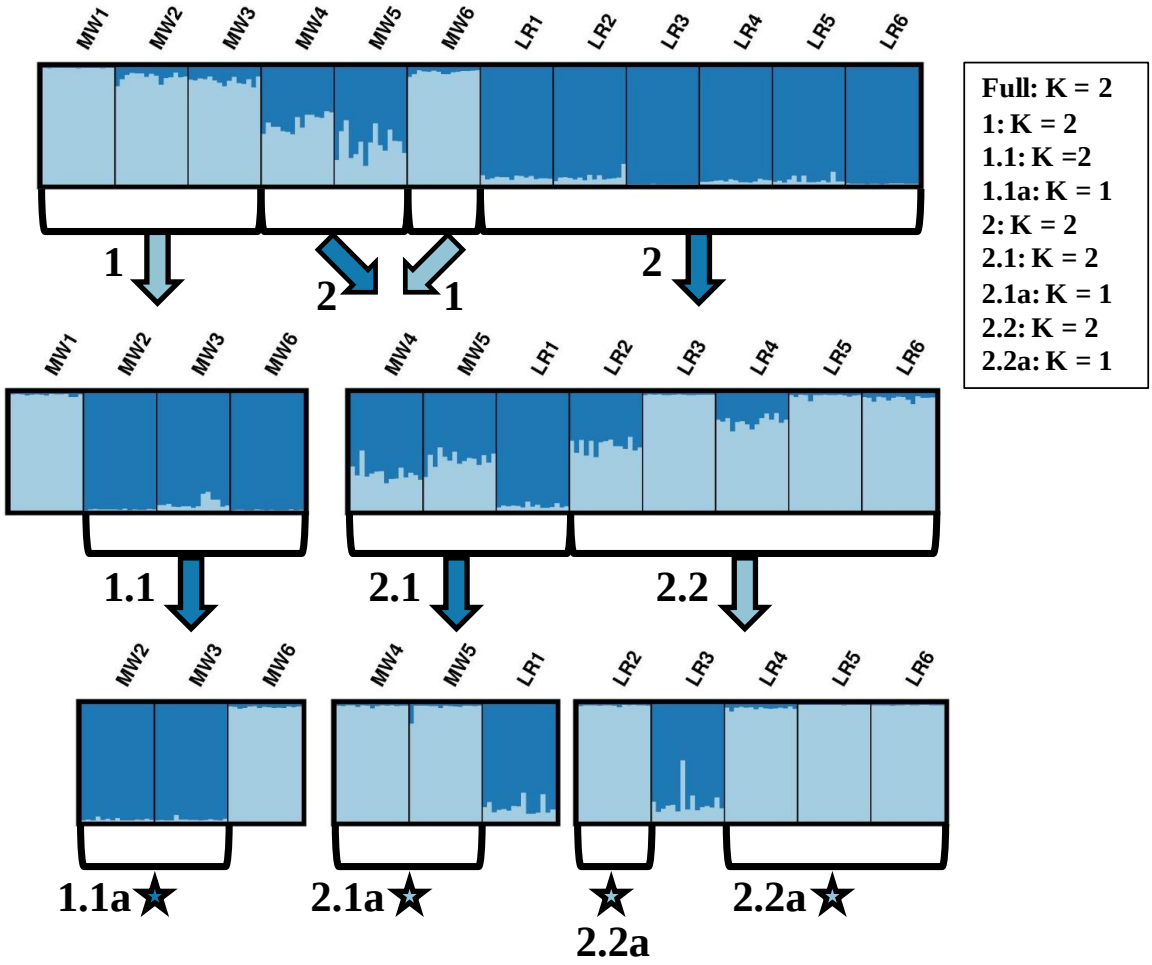

Figure 14. Hierarchical STRUCTURE analyses for *E. whipplei*.

Vertical bars are individuals and colors are proportions of genotypes assigned to clusters by the program. Separations between bar graphs, numbers, and letters represent different analyses at that hierarchy level. Sample site codes are (WH): MW = Middle White and LR = Little Red. Sample site numbers are species specific. Arrows indicate sample site inclusion for next hierarchy level. Stars indicate groups that were identified as panmictic (K of 1). Plots used for determination of K for each analysis are in Appendix C.

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CHAPTER II – DIRECTIONAL GENE FLOW BETWEEN HEADWATER SYSTEMS ALONG THE MAIN STEM OF A RIVER NETWORK

Introduction

Dendritic river networks offer interesting environments for the study of dispersal patterns because for purely aquatic organisms, such as fish, movement and associated dispersal rates are constrained by the dendritic network spatial arrangement. Two features of dendritic networks are that they have habitats structured hierarchically from headwaters to large rivers and that they have unidirectional flow of water down slope (Benda et al. 2004; Lowe et al. 2006). Habitat patches for species in dendritic ecological networks are shaped and influenced by the specific branching nature of the network, and broadly there are two types of categories over which linear patches of habitat can be present: branches (streams) and nodes (confluences) (Grant et al. 2007). Unidirectional flow of water often influences movement capabilities in the network and causes directional bias in dispersal, which in turn can influence connectivity between patches created from the hierarchical dendritic structure (Rodriguez-Iturbe et al. 2009).

Recent theoretical and simulation studies have predicted influences of the spatial configuration and unidirectional flow of water in dendritic river networks on directional dispersal, and its effects on genetic diversity and metapopulation connectivity (Morrisey & Kerckhove 2009; Altermatt 2013; Paz-Vinas & Blanchet 2015; Thomaz et al. 2016). Genetic diversity (allelic richness) should be highest in core populations in a dendritic river network, which are the populations at or directly below confluences in the middle sections of occupied habitat in the network (Paz-Vinas & Blanchet 2015). This pattern emerges in cases of asymmetrical dispersal in river networks, and specifically cases with

increased dispersal in the downstream direction and decreased dispersal in the upstream direction due to combined effects of slope, gravity, and water discharge. In this type of situation, headwater populations will have higher net emigration than immigration and lower population sizes, which would increase effects of genetic drift causing increased frequency of fixation of alleles (Morrisey & Kerckhove 2009). Although headwater populations in this situation would be genetically depauperate, they would contribute rare alleles to downstream populations through confluences, increasing the genetic diversity in the core populations of a dendritic river network (Altermatt 2013; Paz-Vinas & Blanchet 2015). Thomaz et al. (2016) found in models that included asymmetrical downstream dispersal that genetic diversity of populations in downstream sections was three times higher than genetic diversity of populations in headwaters, supporting this pattern. Recent empirical studies have also detected downstream biased movement and gene flow in stream fishes. Lamphere & Blum (2012) have demonstrated asymmetrical downstream biased gene flow and decreasing upstream genetic diversity along a continuous, linear range of habitats for *Cottus bairdi*, a small benthic fish. Likewise, Vøllestad et al. (2012) showed that the majority of movement observed for *Salmo trutta* occurred as juveniles in a downstream direction.

Headwater specialist fish species represent an interesting system for the examination of asymmetrical dispersal because of the unique constraints on dispersal placed on them by their ecological traits and the hierarchical structuring of the network. By definition, these species reach their highest abundances in small streams of the network and have lower abundances and occupancy rates in larger streams (Meyer et al. 2007). They may have specific ecological traits which make them more resistant to the

harsh, variable abiotic conditions in headwaters, which can act as an ecological filter preventing colonization or causing increased rates of extirpation for other species (Rahel & Hubert 1991; Carvalho & Tejerina-Garro 2014). Because their abundance decreases with stream size, headwater specialist populations across a network may best be represented as occurring in isolated patches of optimal habitat (small tributary systems) separated from each other by unsuitable habitat (large tributary streams and main stem rivers) (Winemiller et al., 2010). Therefore, asymmetrical movement in a downstream direction could result in individuals being pushed out of their preferred habitat. Theoretical models indicate that in cases where individuals tend to be pushed out of their headwater niche by unidirectional flow, persistence of populations in those habitats is dependent upon the ability to invade upstream habitats (Lutscher et al., 2010). Metapopulation modeling also showed a longer time to extinction in large and complex (more branching) dendritic networks with upstream biased dispersal (Campbell Grant 2011). Therefore, selection pressure may exist in headwater fish species for upstream-biased dispersal to maintain their populations in their preferred niche and to counteract downstream displacement from flow. This type of pattern would be analogous to the colonization cycle theory of aquatic insects, where emerged headwater specialized insects are hypothesized to fly upstream to oviposit to counter downstream displacement from drift and maintain occupancy in their preferred niche (Müller 1954; Griffith et al. 1998).

Certain ecological life history traits of headwater specialist species seem adaptive for limiting downstream displacement that would support this prediction. Headwater species tend to have a larger egg size and a reduced body size, which may be adaptive for retention of individuals in headwater patches (Turner & Trexler 1998; Knouft & Page

2003; Turner & Robison 2006). Larger egg sizes have been correlated with decreased duration of larval drift in darters (Paine 1984). Larval drift represents passive downstream displacement for fish, and decreasing drift duration would reduce the risk of being pushed out of the preferred habitat. Decreasing duration would also reduce exposure to large flow events, which can significantly displace larval fish into downstream habitats (Harvey 1987).

If displacement out of preferred headwater habitats did occur, such as through high flow events influencing adults or larval fish, then individuals would need to travel either upstream or downstream along the tributary river or main stem to find the next suitable small tributary system to return to headwater habitats. Such dispersal events are likely rare and occur over larger scales than within single tributary systems, and therefore genetic inferences may be more suited for investigations into these events than traditional ecological methods (Lowe & Allendorf 2010). Examination of directional bias of dispersal along the main stem for headwater fish species can lead to better understanding of processes maintaining those species in headwaters in the presence of unidirectional flow away from headwaters, and can lead to inferences for how ecological traits influence directionality of asymmetrical dispersal in dendritic river networks. My hypothesis for this study is that directionality of gene flow along main stems in river networks will be influenced by habitat preference within dendritic networks. I predict that headwater specialist species will have adaptive upstream bias in dispersal reflective of a directional bias for maintenance of populations in headwaters or for recolonization of extirpated sites, and that this asymmetrical dispersal preference will be evident when examining

gene flow between headwater patches of habitat separated by main stem and large tributary rivers.

Methods

Study areas, collection site locations, study species, DNA extraction, and microsatellite amplification and visualization were the same as reported in Chapter I.

The program Migrate v. 3.64 (Beerli & Felsenstein 2001) was used to compare models of gene flow between populations for each independent species-drainage pairing in the dataset. The program can use Bayesian based inference and coalescent theory to simultaneously estimate Θ (mutation scaled effective population size) and M (mutation scaled migration rate between populations) under different gene flow model scenarios. Through the use of thermodynamic integration, log marginal likelihood values for different gene flow models can be converted into Bayes factors allowing for model comparison and selection of the most likely model among the scenarios analyzed (Beerli & Palczewski 2010). Pre-defined populations for the models were derived from the finest-scale population clusters as determined by hierarchical Structure v. 2.3.4 (Pritchard et al., 2000) analysis as reported in Chapter I. For cases where Structure analysis indicated panmixia (K of 1) for the whole drainage (three cases in the dataset: Big Black for *S. atromaculatus*, Big Black for *E. claviformis*, and Bayou Pierre for *F. olivaceus*), populations for Migrate analyses were created based on geographical and tributary concordance.

Five different gene flow models were compared for each species-drainage pairing. The null model of panmixia (model 1) was used for comparison and was created by combining all 6 drainage populations into one large population and estimating Θ with the

program. Two models with symmetric geneflow (proceeding upstream and downstream along the main stem) were used: 2) full model, where all populations are allowed to have gene flow with all other populations, ignoring dendritic network structure (Island Model) (Wright 1931) and 3) symmetric stepping stone model, where populations only have symmetric gene flow with their nearest neighbor along the main stem or tributary rivers, allowing dendritic structure to dictate gene flow, but with gene flow occurring in both upstream and downstream directions along the main stem (Kimura & Weiss 1964). The last two models had the same configuration as the symmetric stepping stone model, but only allowed asymmetric gene flow (one direction along the main stem) and were: 4) upstream model, with gene flow occurring between nearest neighbors of the main stem and tributary rivers proceeding upstream along the main stem and 5) downstream model, with gene flow occurring between nearest neighbors of the main stem and tributary rivers proceeding downstream along the main stem. In some population arrangements, the full model (2) and the symmetric stepping stone model (3) were the same, and only four models were compared in those instances. Estimates for Θ were not varied across models (every population had an estimate for Θ in every model), and therefore the only differences in the models were either population combinations for estimates of M and/or directionalities for estimates of M. Two sites of *S. atromaculatus* in the Chickasawhay River of the Pascagoula originally grouped with the Pearl drainage in hierarchical structure analysis and had a high rate of genetic distance to other sites in the drainage ($F_{ST} \approx 0.4$ to other sites in the Pascagoula) (Chapter I). Constructions of coalescent genealogies in Migrate failed in finding roots for trees in models 3-5 with inclusion of these populations, and model failure was likely caused by allele frequencies in these

populations being very different from their nearest neighbors. Therefore these populations were treated as isolated populations within the drainage, and because Migrate analysis needs all populations in the model to have gene flow to one other population, they were removed from the models, and only four populations in the Pascagoula were analyzed for *S. atromaculatus*.

For stepping stone based models (3-5), gene flow was inferred as being between the nearest neighbor tributary systems for those populations. For example: in the downstream model, gene flow would move down the tributary system from the collection site, enter the main stem, move down the main stem to the nearest neighbor tributary system in the study, and then move upstream within that tributary system to the collection site in that system. Main stem assignment for the network was determined by comparing total cumulative drainage area of branches at confluences, with the main stem being assigned to the larger branch, with total cumulative drainage area being determined from the NHDplus v. 2 dataset (Horizon Systems) in ArcGIS. For cases where total cumulative drainage area of branches at a junction were similar in size (difference of < 100 km^2), then models 3-5 allowed gene flow simultaneously up or down both branches from the junction (i.e., both branches were treated as equivalent "main stems" for gene flow). If more than one population occurred in the same tributary system, then gene flow in models 3-5 proceeded along the tributary main stem in a secondary fashion compared with the network main stem. In such cases, the downstream site along the tributary main stem was defined as being the site for gene flow along the network main stem to other tributary systems, and the upstream site along the tributary main stem only had gene flow with the downstream site along the tributary main stem. Two of the drainages had

reservoirs between populations: Greer's Ferry Lake in the Little Red River (built in 1964) and Ross Barnett Reservoir in the Pearl River (built in 1963). For the models, gene flow was allowed to pass through these reservoirs along historical main stem channels, and therefore inferences for these drainages are of historical gene flow and do not reflect current constraints on gene flow imposed by these reservoirs.

The mutation model selected for Migrate was the Brownian motion model which was the most appropriate among the choices for microsatellite data because it approximates a stepwise pattern of mutation, which would be expected in microsatellites, and also runs faster than a discrete stepwise ladder model. Original genealogies were derived from a UPGMA tree, and original estimates of Θ and M at the start of the Markov-Chain Monte Carlo sampling were derived from F_{ST} calculations. Prior distributions for Θ and M were uniform, and values were species specific and set through fine tuning of the program using simple migration models looking for bounding of upper limits for parameters and unimodality and normality of parameter distributions. Metropolis-Hastings sampling was used on proposal distributions of estimates. The number of recorded steps in the chain was 50,000, with a prior burn-in of 10,000 steps and a sampling increment of recording trees every 100 steps. Static heating (thermodynamic integration) was used with 4 chains at the following temperatures: 1.0, 1.5, 3.0, and 1,000,000.0. Bezier approximation scores across all loci were used as log marginal likelihood scores, which were converted into log Bayes factors for model comparison (Beerli & Palczewski 2010). Convergence for analyses was assessed through examining parameter distributions for unimodality and ESS scores above 1000 for all parameters.

Results

The panmixia, full model, and symmetric stepping stone model were never selected as the most likely scenario across all species studied. There was variability in selection of the upstream and downstream models across the species and drainage pairings, with some repeated patterns. The downstream model was selected as the most likely scenario in four drainages for *F. olivaceus*, and the upstream model was selected in two drainages (Table 31). For *S. atromaculatus* (Table 32) and *E. claviformis* (Table 33), the upstream model was selected in four drainages and the downstream model was selected in two drainages. For the darter species, *E. artesiae* (Table 34), *E. parvipinne* (Table 35), and *E whipplei* (Table 36), the upstream model was the only model selected, with selection in two drainages per species. Model selection probability among the considered scenarios in all cases was greater than 99.99%. Populations and gene flow directionality for the selected models are mapped as follows: *F. olivaceus* (Figures 15-20), *S. atromaculatus* (Figures 21-26), *E. claviformis* (Figures 27-32), *E. artesiae* (Figures (33-34), *E. parvipinne* (Figures 35-36), and *E. whipplei* (Figures 37-38).

Discussion

The hypothesis that habitat preference within dendritic networks will influence directionality of gene flow was supported by the results. These results also support the prediction that headwater specialized species of fish will exhibit asymmetrical upstream dispersal through the analysis of directionality of possibly rare, long distance gene flow events along main stems in the river network for 5 out of 6 of the species studied. *Fundulus olivaceus* had the majority (67%) of its drainages with asymmetrical downstream gene flow, which is a pattern assumed for more generalist fish species due to

influences of the unidirectional flow of water (Morrisey & Kerckhove 2009; Thomaz et al. 2016). Hierarchical structure analyses also found that among these headwater residents, *F. olivaceus* had a lower fragmentation rate and hierarchically nested structure (Chapter I). These two results indicate that *F. olivaceus* could be considered more of a tributary generalist compared with the other species in the study that may have varying degrees of headwater specialization. Hybrid contact zone studies of *F. olivaceus* and *F. notatus* have shown a pattern of *F. olivaceus* individuals being present in main stem locations below confluences with tributaries containing other *F.olivaceus* individuals, including the Pearl drainage, which was shared with this study, and variability and repetition of this pattern was dependent upon specific drainages and the spatial structure of the contact zone (Schaefer et al. 2011). Such spatial arrangement of individuals could suggest a downstream dispersal into the main stem from tributary populations, which would be in agreement with these results for asymmetric downstream gene flow into and through main stem habitats. Furthermore, the specific microhabitat for *F. olivaceus* is stream margins, and this habitat is expected to not vary as much with an increase in stream size compared with other headwater specialized microhabitats. Therefore, if stream margin habitats along main stems are still suitable habitats, there may be less of a selection pressure on the species for upstream dispersal compared to the other headwater species studied.

Patterns of directional dispersal were similar for *S. atromaculatus* and *E. claviformis*, with both species exhibiting overall upstream bias (67%), but also having 33% of the study drainages with downstream directed gene flow. These species also had very similar fragmentation rates across these drainages in the hierarchical structure

analysis (Chapter I), and both of these results suggest that dispersal patterns and degree of headwater specialization may be similar for these two species. None of the darters exhibited downstream-directed gene flow, suggesting that upstream-directed dispersal is selected for the most in this group among the headwater species studied.

Ecological based movement studies have not been performed on all of these species, however there have been previous studies for *F. olivaceus* and *S. atromacualtus*. A mark recapture study on *F. olivaceus* in a tributary showed that most individuals did not move extensively, with average distance moved being 0.9 m per day (Alldredge et al., 2011). A mark recapture study for *S. atromaculatus* showed that they had similar movements, with median net distance moved being 50 m (1.19 m per day) and maximum distance moved being 550 m over a six week interval (Belica & Rahel 2008). However, another mark-recapture study done for *S. atromaculatus* over a wider range of sampled pools (5 km of stream), detected a much higher maximum distance travelled of 4678 m over two month sampling intervals, indicating a greater ability for dispersal in a network than previous studies on *S. atromaculatus* (Walker & Adams 2014). A meta-analysis of fish movement studies found that among the groups studied here, Fundulidae (includes *Fundulus*) had the least movement with mean movement distance of mobile fish around 10 m, followed by Percidae (includes *Etheostoma*) (≈ 200m), followed by Cyprinidae (includes *Semotilus*) (≈ 1000 m), followed by Catostomidae (includes *Erimyzon*)(>10,000 m) (Radinger & Wolter 2014). A positive relationship was also found between distance moved and stream size (stream order, width, and discharge) and body size (Radinger & Wolter 2014). Body size for the species in this study generally agrees with the order of mean movement distances for families presented above, with *F. olivaceus* and the

Etheostoma species being smaller than *S. atromaculatus* and *E. claviformis*, and therefore dispersal capabilities for these study species likely are in the same order as the families presented above (Ross 2001). However, because less movement was found in headwater streams in the meta-analysis, the dispersal capabilities of these specific members of those families are likely lower than the values listed. These trends indicate that most movements for these species are probably local, and gene flow along the main stem such as that inferred through this study could be likely rare events.

Upstream bias in directionality for *S. atromaculatus* has previously been demonstrated across smaller spatial scales including Ohio (Stork & Mormot 1981), North Carolina (Hall 1972), and Arkansas (Walker & Adams 2014). Boizard et al. (2009) found in Québec, Canada that *S. atromaculatus* invaded areas upstream of impassable barriers through interconnections between drainage systems, suggesting an active upstream bias of dispersal and colonization through a population genetic study. *Semotilus atromaculatus* was also documented as one of the first species to colonize restored sections of a stream that were previously channelized (Moore & Lamberti 2003). This evidence combined with the results of this study seem to indicate a pattern for this species, with upstream biased dispersal possibly due to maintenance and colonization of their preferred ecological niche in the presence of downstream flow.

There was also the possibility of a regional effect in this study. For both *F. olivaceus* and *E. claviformis*, dispersal directionality switched in the Lower Mississippi River region (Bayou Pierre and Big Black). This reversal in pattern highlights the complex interactions between local habitat conditions influenced by regional factors and dispersal and movement. A recent study on *F. olivaceus* shows that localized movements

are tightly connected to spatial arrangement of habitats (Clark & Schaefer 2016). There could be some regional effects on habitats that also influence rare long distance movements for these two species in these drainages that reverses their dispersal pattern in relation to the other regions studied.

Although these results suggest that upstream biased dispersal along main stem habitats is more often found across drainages for headwater specialist species and downstream biased dispersal is more often found across drainages in tributary generalists, further confirmation is likely necessary from more fine-scale population studies (linear movement within a tributary system) for *F. olivaceus*, *E. claviformis*, *E. artesiae*, *E. parvipinne*, and *E. whipplei*. Additionally, ecologically based methods such as mark-recapture studies with distance moved and directionality may be necessary for confirmation of dispersal patterns for *E. claviformis*, *E. artesiae*, *E. parvipinne*, and *E. whipplei*. These types of studies would help confirm overall implications found from examining rare long distance movement and see if they were consistent with more local and regional movements. Given the above reservations, this study demonstrates a trend for upstream dispersal in headwater specialist fish from rare dispersal events along the main stem, which could possibly indicate a bias for upstream dispersal for maintenance of populations in headwaters or for colonization of new streams. These results show the influence of specific ecological constraints of niche selection on dispersal dynamics in dendritic river networks with unidirectional flow, and how theoretical paradigms of gene flow in dendritic river networks with predicted downstream biased dispersal may need to be modified when considering cases of headwater specialization.

Table 31

Migration model comparisons for F. olivaceus using log Bayes Factors

Drainage	Migration Model	Log Bayes Factor	Model Probability
Bayou Pierre	panmictic	10452.08	<0.0001
	full = sym. stepping stone	136665	<0.0001
	upstream*	**0**	**>0.9999**
	downstream	553295.1	<0.0001
Big Black	panmictic	3909.54	<0.0001
	full	305112	<0.0001
	sym. stepping stone	195027	<0.0001
	upstream*	**0**	**>0.9999**
	downstream	585609.3	<0.0001
Pascagoula	panmictic	10394.26	<0.0001
	full = sym. stepping stone	274412.8	<0.0001
	upstream	1403.62	<0.0001
	downstream*	**0**	**>0.9999**
Pearl	panmictic	1425.46	<0.0001
	full = sym. stepping stone	94118.5	<0.0001
	upstream	58848.56	<0.0001
	downstream*	**0**	**>0.9999**
Black	panmictic	2702.54	<0.0001
	full = sym. stepping stone	74710.02	<0.0001
	upstream	84.48	<0.0001
	downstream*	**0**	**>0.9999**
Little Red	panmictic	21387.84	<0.0001
	full	522015.3	<0.0001
	sym. stepping stone	149738.2	<0.0001
	upstream	404572.5	<0.0001
	downstream*	**0**	**>0.9999**

sym. = symmetrical; * indicates the model selected for that drainage by the analysis.

Table 32

Migration model comparisons for S. atromaculatus using log Bayes Factors

Drainage	Migration Model	Log Bayes Factor	Model Probability
Bayou Pierre	panmictic	22702.98	<0.0001
	full	248210.9	<0.0001
	sym. stepping stone	147775.3	<0.0001
	upstream*	**0**	**>0.9999**
	downstream	652426	<0.0001
Big Black	panmictic	738.46	<0.0001
	full	214435.5	<0.0001
	sym. stepping stone	51880.8	<0.0001
	upstream*	**0**	**>0.9999**
	downstream	1453.38	<0.0001
Pascagoula	panmictic	6714.98	<0.0001
	full	76373.08	<0.0001
	sym. stepping stone	52638.5	<0.0001
	upstream	1192.96	<0.0001
	downstream*	**0**	**>0.9999**
Pearl	panmictic	4619.1	<0.0001
	full	54674.96	<0.0001
	sym. stepping stone	33147.36	<0.0001
	upstream*	**0**	**>0.9999**
	downstream	758.42	<0.0001
Black	panmictic	12487.32	<0.0001
	full	263434.8	<0.0001
	sym. stepping stone	436193.2	<0.0001
	upstream	8286.04	<0.0001
	downstream*	**0**	**>0.9999**
Little Red	panmictic	30409.5	<0.0001
	full	362880.9	<0.0001
	sym. stepping stone	137645.8	<0.0001
	upstream*	**0**	**>0.9999**
	downstream	251548	<0.0001

sym. = symmetrical; * indicates the model selected for that drainage by the analysis.

Table 33

Migration model comparisons for E. claviformis using log Bayes Factors

Drainage	Migration Model	Log Bayes Factor	Model Probability
Bayou Pierre	panmictic	2979.74	<0.0001
	full = sym. stepping stone	98796.72	<0.0001
	upstream	64434.34	<0.0001
	downstream*	**0**	**>0.9999**
Big Black	panmictic	7621.88	<0.0001
	full	105910.4	<0.0001
	sym. stepping stone	128121.3	<0.0001
	upstream	1443.54	<0.0001
	downstream*	**0**	**>0.9999**
Pascagoula	panmictic	21601.92	<0.0001
	full	279341.2	<0.0001
	sym. stepping stone	152159.1	<0.0001
	upstream*	**0**	**>0.9999**
	downstream	285697.4	<0.0001
Pearl	panmictic	1990.12	<0.0001
	full	203985.5	<0.0001
	sym. stepping stone	19061.6	<0.0001
	upstream*	**0**	**>0.9999**
	downstream	42416.34	<0.0001
Black	panmictic	4595.26	<0.0001
	full	220328.4	<0.0001
	sym. stepping stone	103822.5	<0.0001
	upstream*	**0**	**>0.9999**
	downstream	478903.8	<0.0001
Little Red	panmictic	9892.74	<0.0001
	full	340823.7	<0.0001
	sym. stepping stone	53792.22	<0.0001
	upstream*	**0**	**>0.9999**
	downstream	1512553	<0.0001

sym. = symmetrical; * indicates the model selected for that drainage by the analysis.

Table 34

Migration model comparisons for E. artesiae using log Bayes Factors

Drainage	Migration Model	Log Bayes Factor	Model Probability
Bayou Pierre	panmictic	2258.6	<0.0001
	full	274195.9	<0.0001
	sym. stepping stone	263136.5	<0.0001
	upstream*	**0**	**>0.9999**
	downstream	1646883	<0.0001
Big Black	panmictic	12934.88	<0.0001
	full = sym. stepping stone	79981.86	<0.0001
	upstream*	**0**	**>0.9999**
	downstream	15383.8	<0.0001

sym. = symmetrical; * indicates the model selected for that drainage by the analysis.

Table 35

Migration model comparisons for E. parvipinne using log Bayes Factors

Drainage	Migration Model	Log Bayes Factor	Model Probability
Pascagoula	panmictic	1771.26	<0.0001
	full	438746.8	<0.0001
	sym. stepping stone	44033.34	<0.0001
	upstream*	**0**	**>0.9999**
	downstream	564280.7	<0.0001
Pearl	panmictic	7967.36	<0.0001
	full	444039.6	<0.0001
	sym. stepping stone	81095.2	<0.0001
	upstream*	**0**	**>0.9999**
	downstream	604077.7	<0.0001

sym. = symmetrical; * indicates the model selected for that drainage by the analysis.

Table 36

Migration model comparisons for E. whipplei using log Bayes Factors

Drainage	Migration Model	Log Bayes Factor	Model Probability
Black	panmictic	7576.1	<0.0001
	full	299374.4	<0.0001
	sym. stepping stone	164190	<0.0001
	upstream*	**0**	**>0.9999**
	downstream	59928.3	<0.0001
Little Red	panmictic	8093.8	<0.0001
	full = sym. stepping stone	132760.2	<0.0001
	upstream*	**0**	**>0.9999**
	downstream	1173868	<0.0001

sym. = symmetrical; * indicates the model selected for that drainage by the analysis.

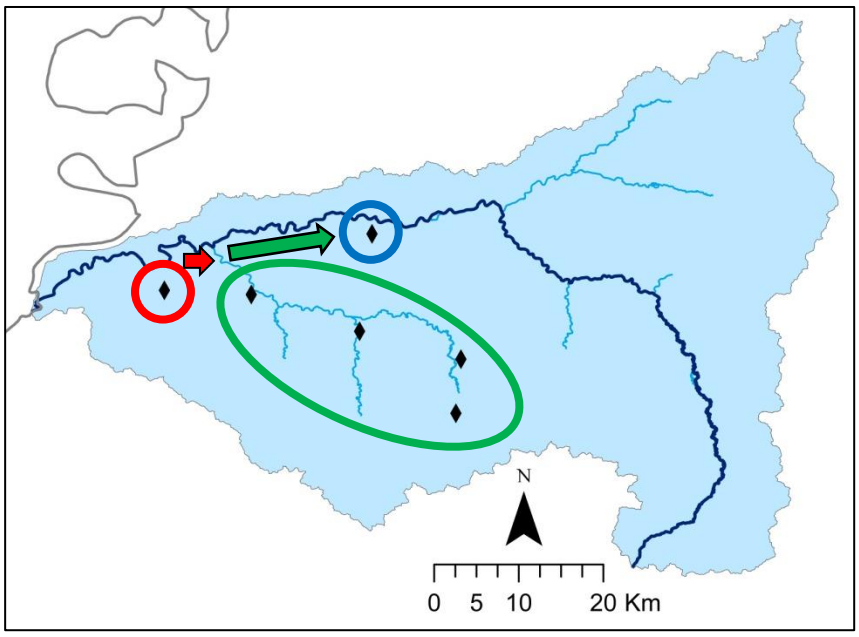

Figure 15. The most likely model (upstream) for *F. olivaceus* in Bayou Pierre.

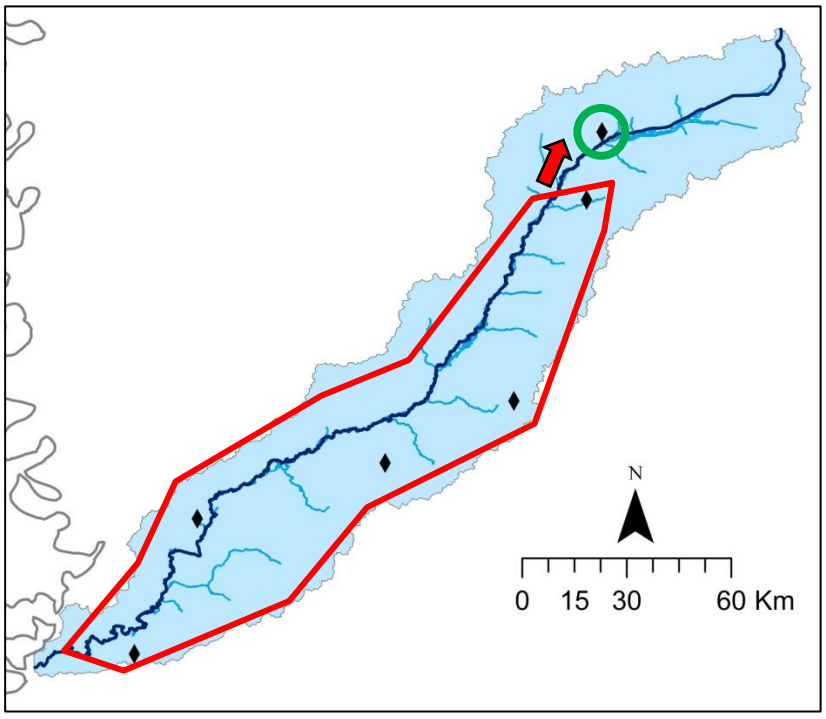

Figure 16. The most likely model (upstream) for *F. olivaceus* in the Big Black River.

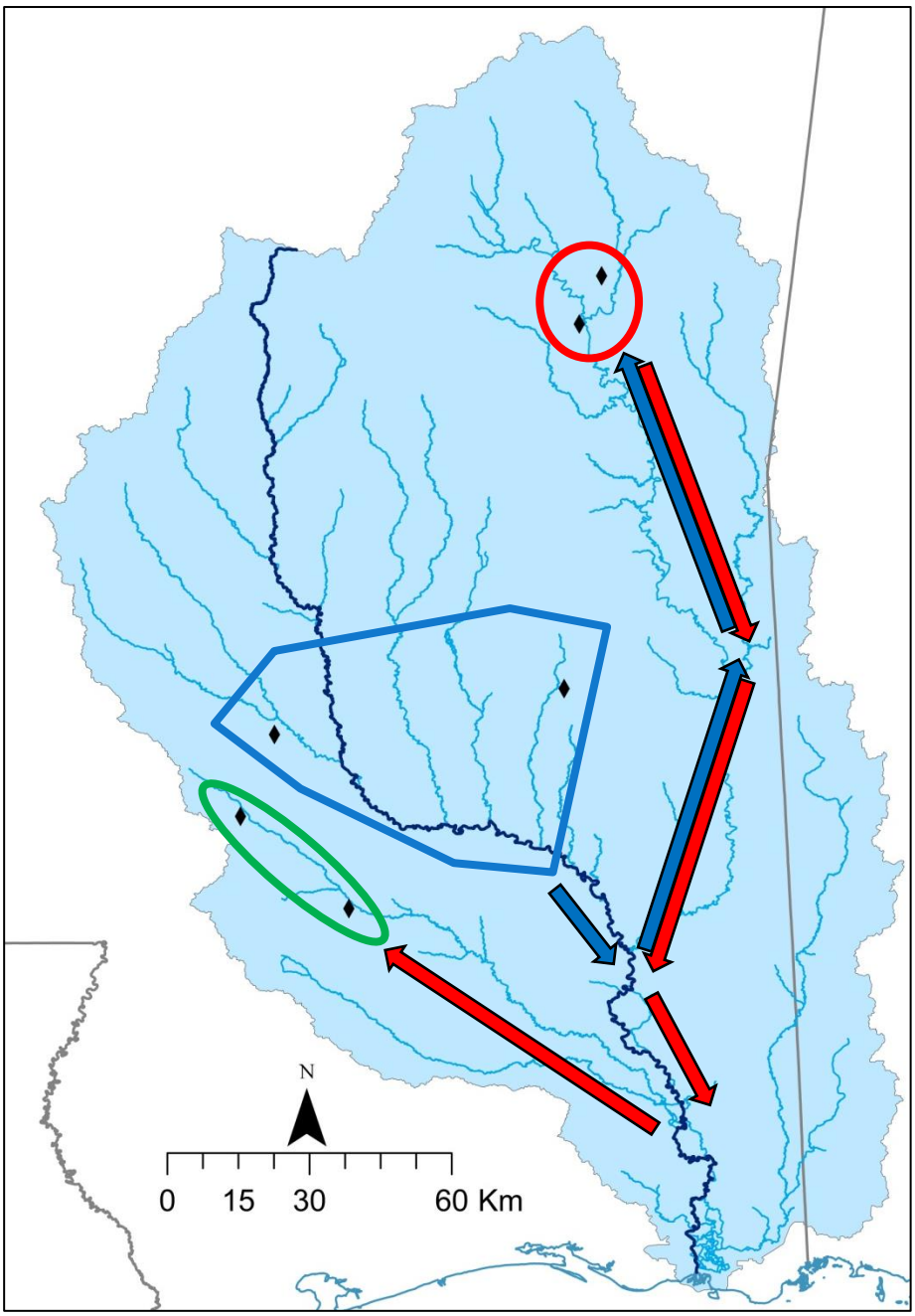

Figure 17. The most likely model (downstream) for *F. olivaceus* in the Pascagoula River.

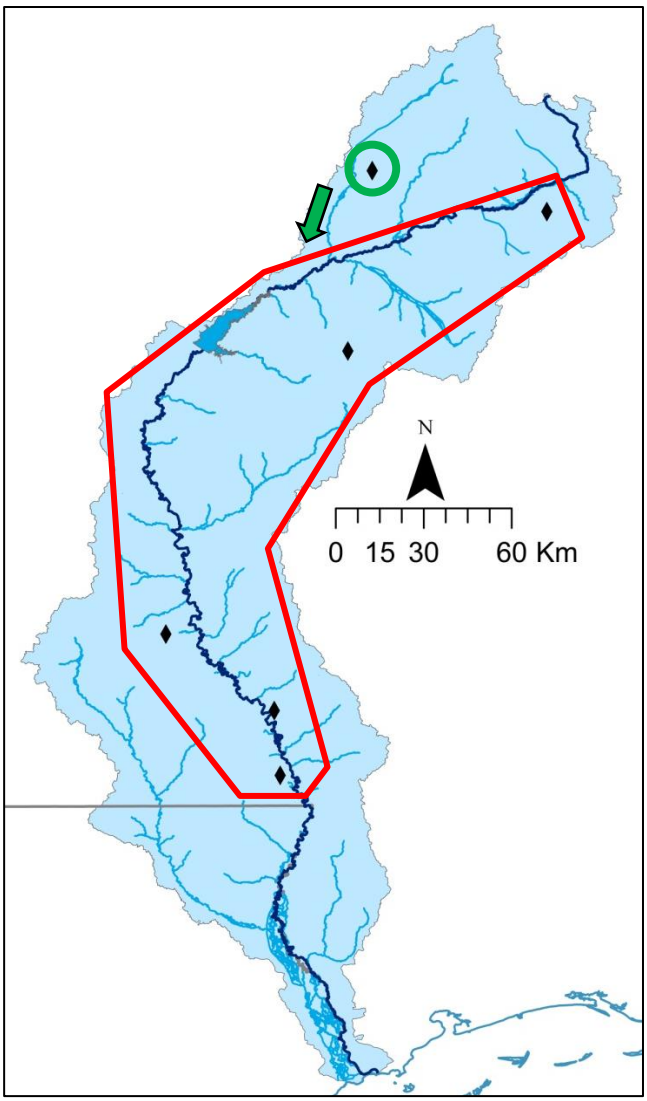

Figure 18. The most likely model (downstream) for *F. olivaceus* in the Pearl River.

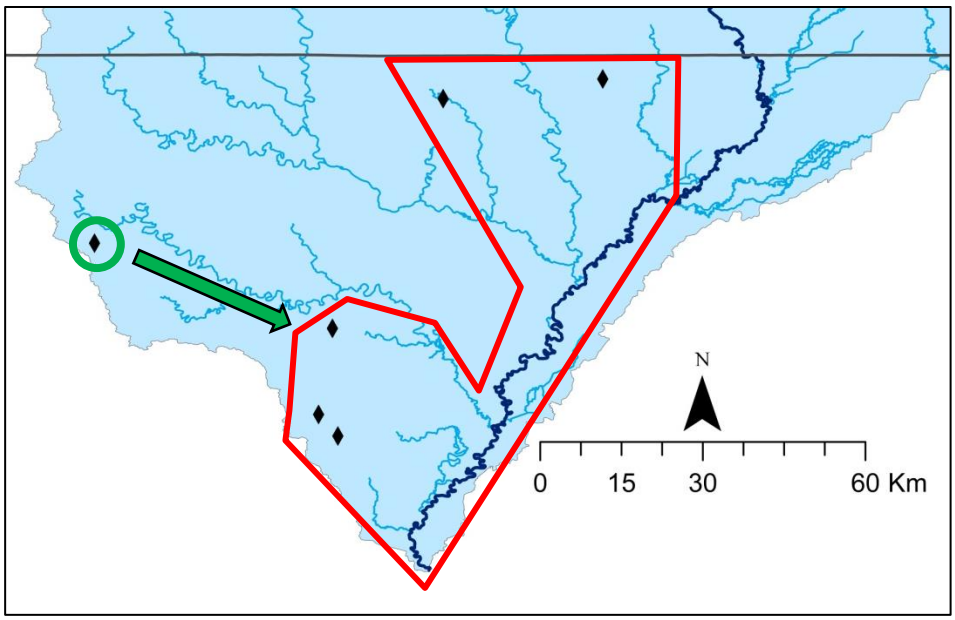

Figure 19. The most likely model (downstream) for *F. olivaceus* in the Black River.

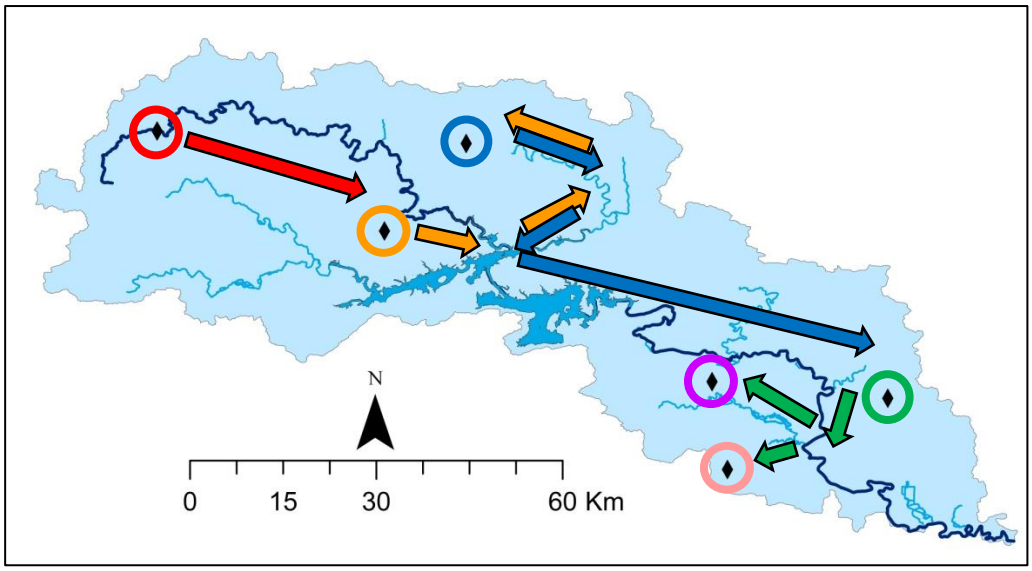

Figure 20. The most likely model (downstream) for *F. olivaceus* in the Little Red River.

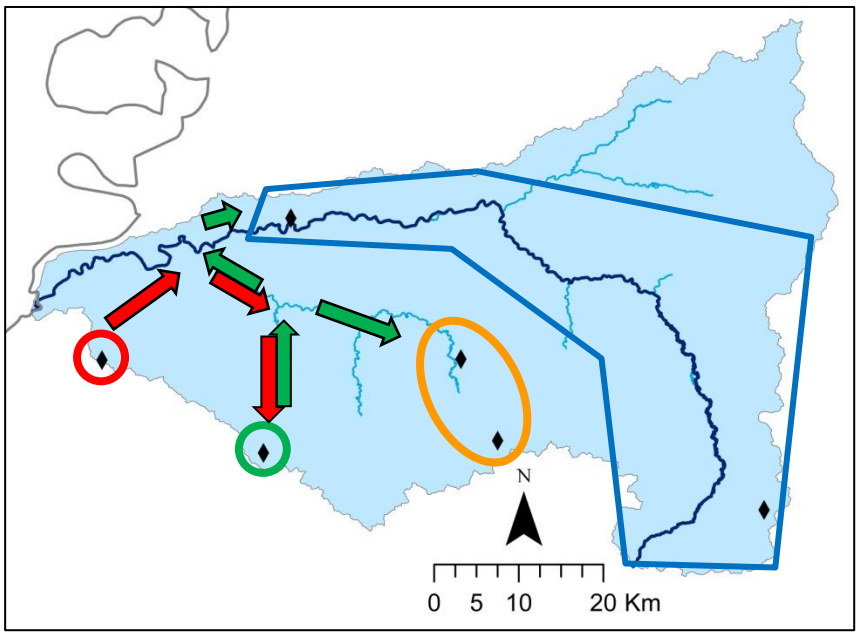

Figure 21. The most likely model (upstream) for *S. atromaculatus* in Bayou Pierre.

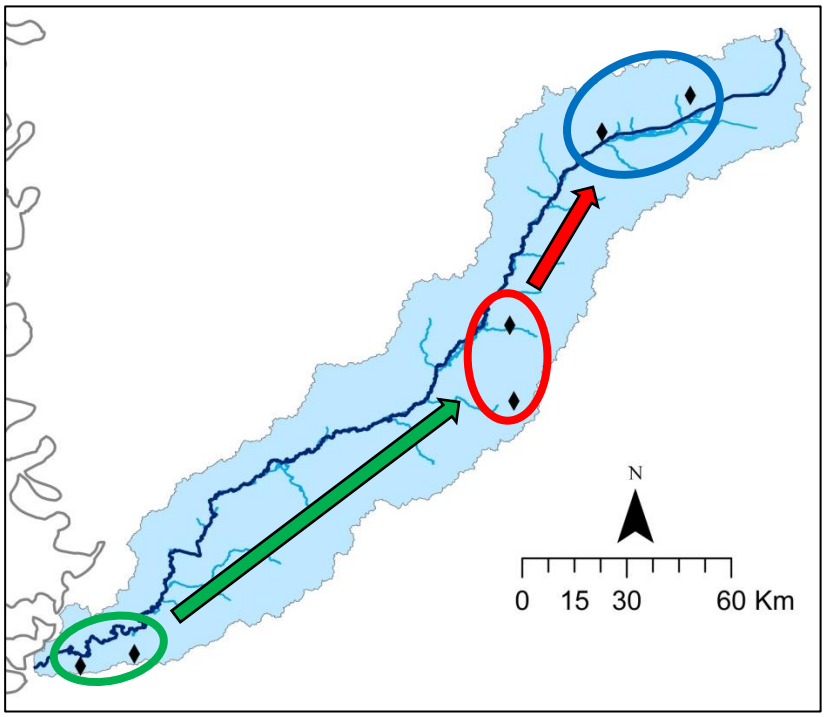

Figure 22. The most likely model (upstream) for *S. atromaculatus* in the Big Black River.

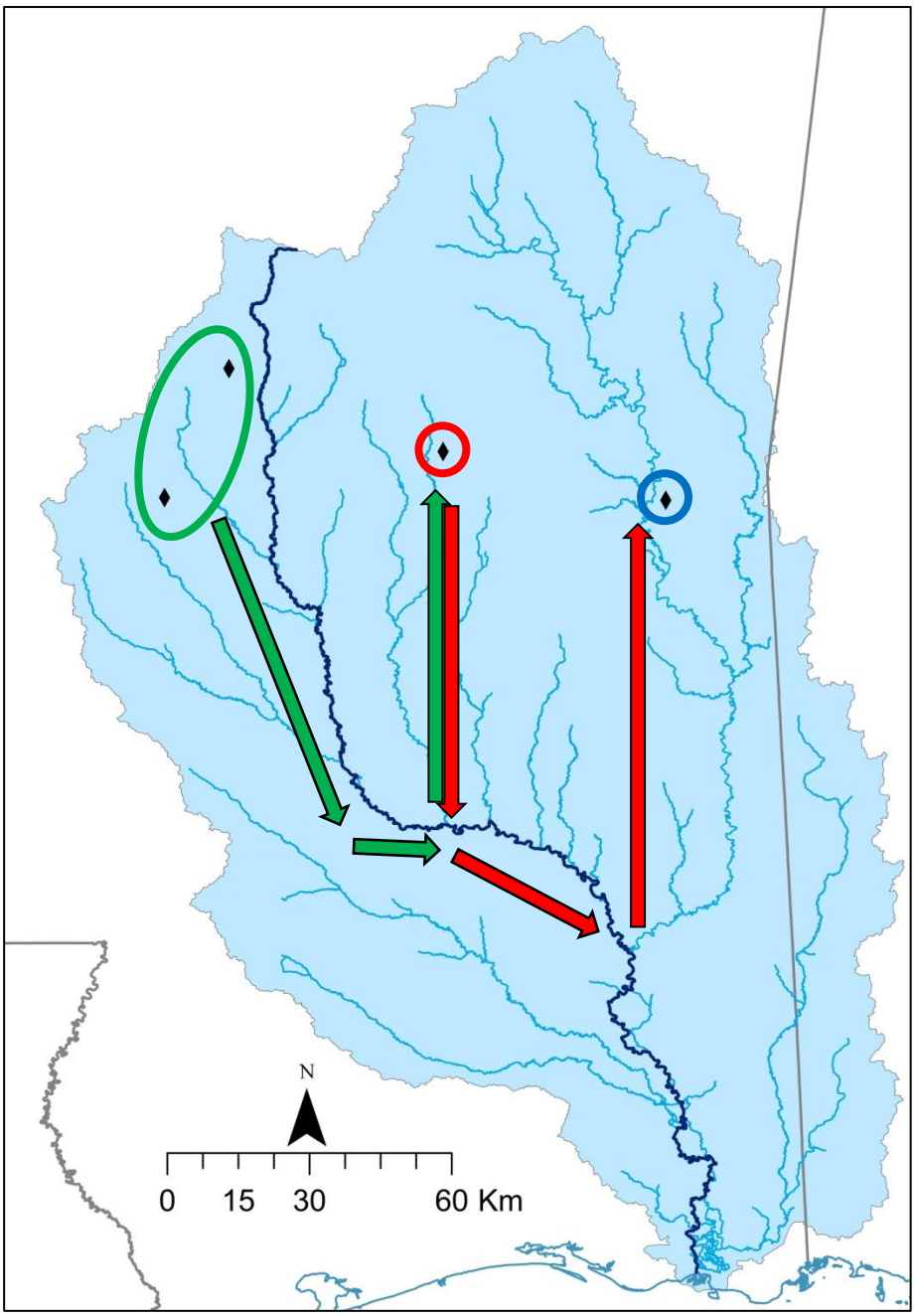

Figure 23. The most likely model (downstream) for *S. atromaculatus* in the Pascagoula River.

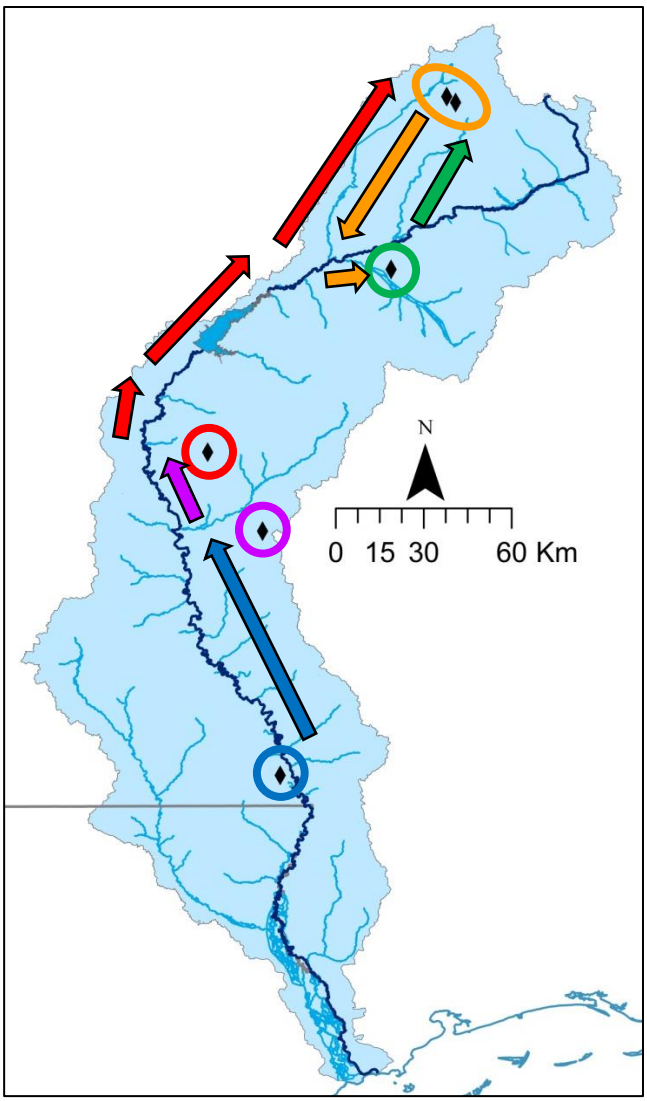

Figure 24. The most likely model (upstream) for *S. atromaculatus* in the Pearl River.

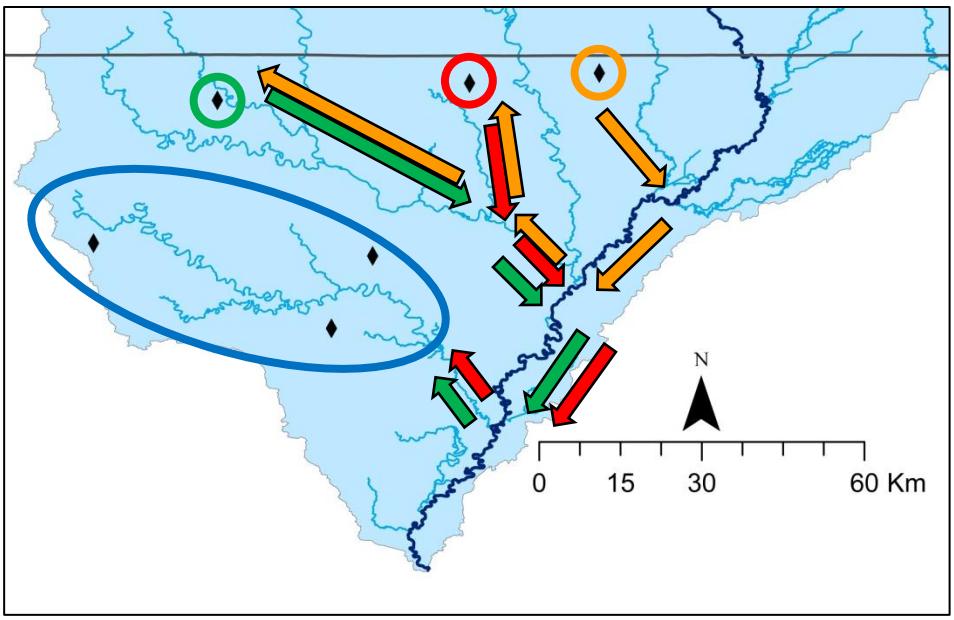

Figure 25. The most likely model (downstream) for *S. atromaculatus* in the Black River.

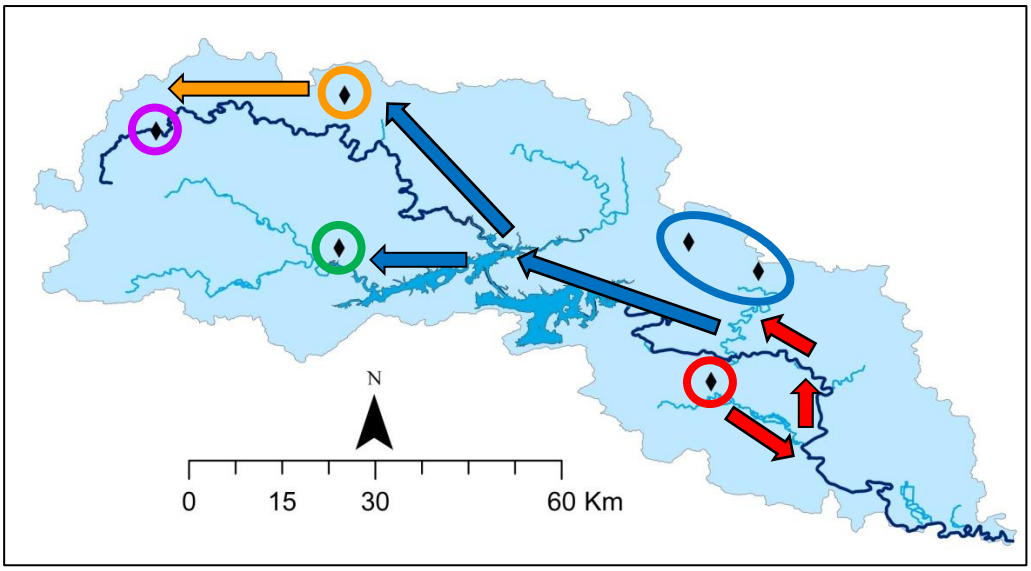

Figure 26. The most likely model (upstream) for *S. atromaculatus* in the Little Red River.

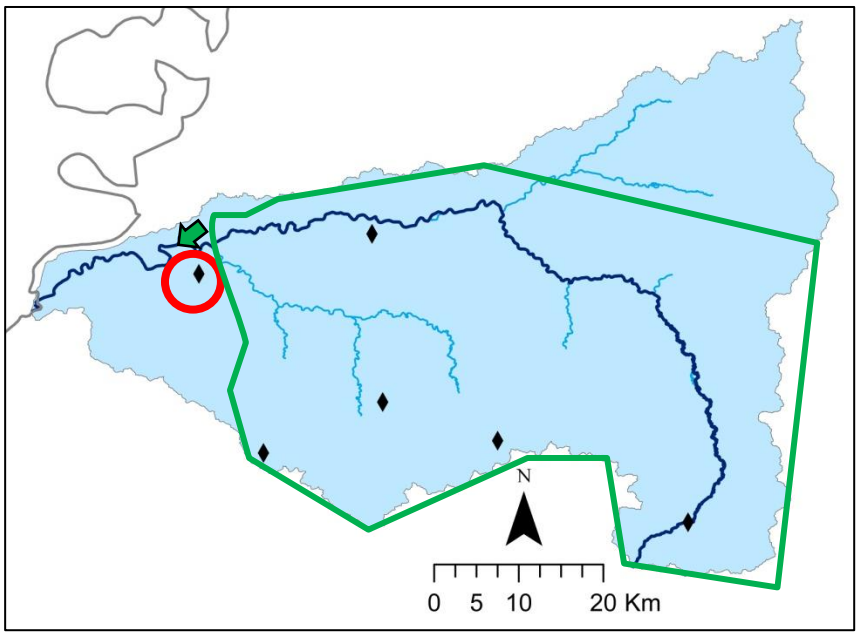

Figure 27. The most likely model (downstream) for *E. claviformis* in Bayou Pierre.

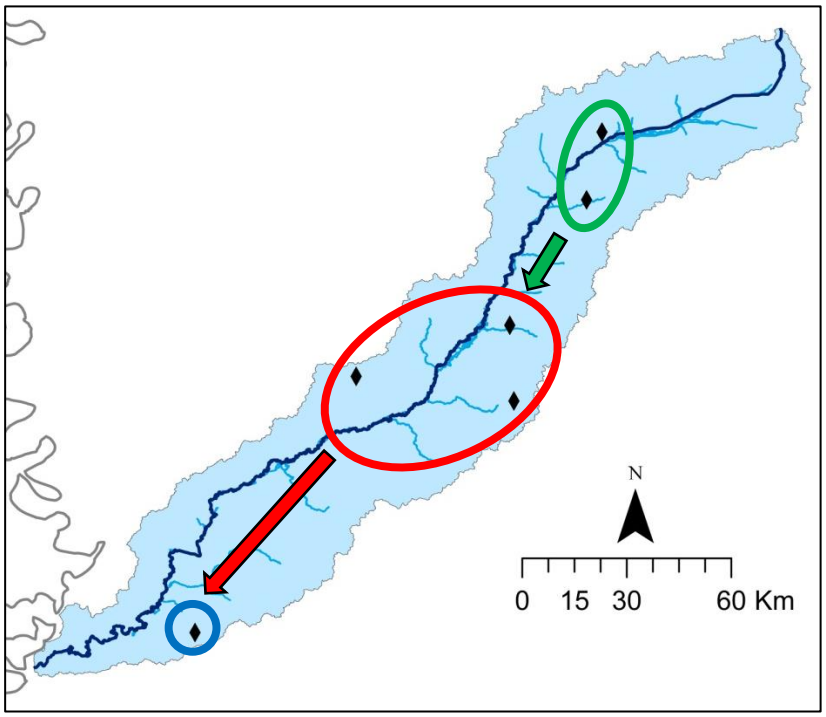

Figure 28. The most likely model (downstream) for *E. claviformis* in the Big Black River.

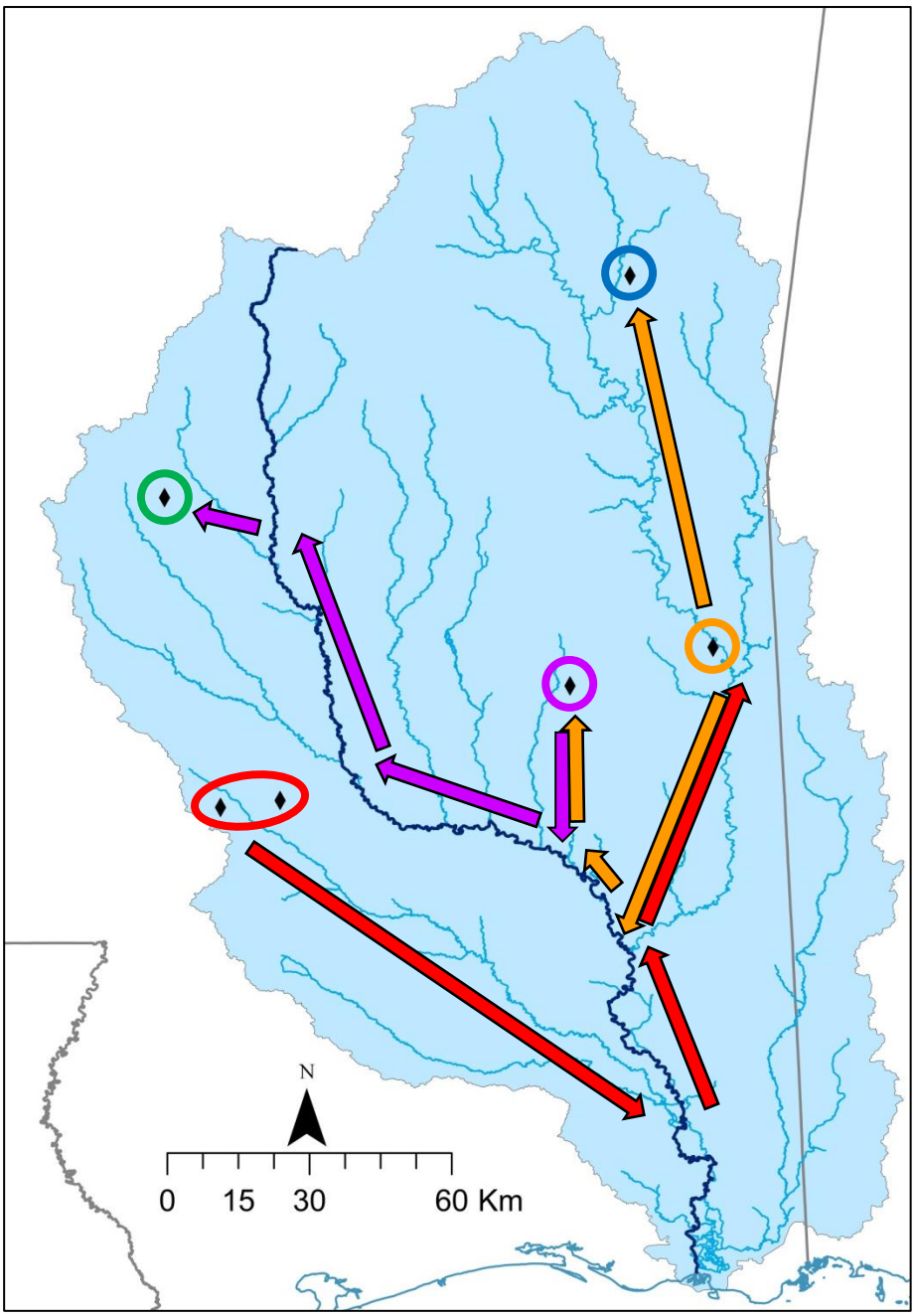

Figure 29. The most likely model (upstream) for *E. claviformis* in the Pascagoula River.

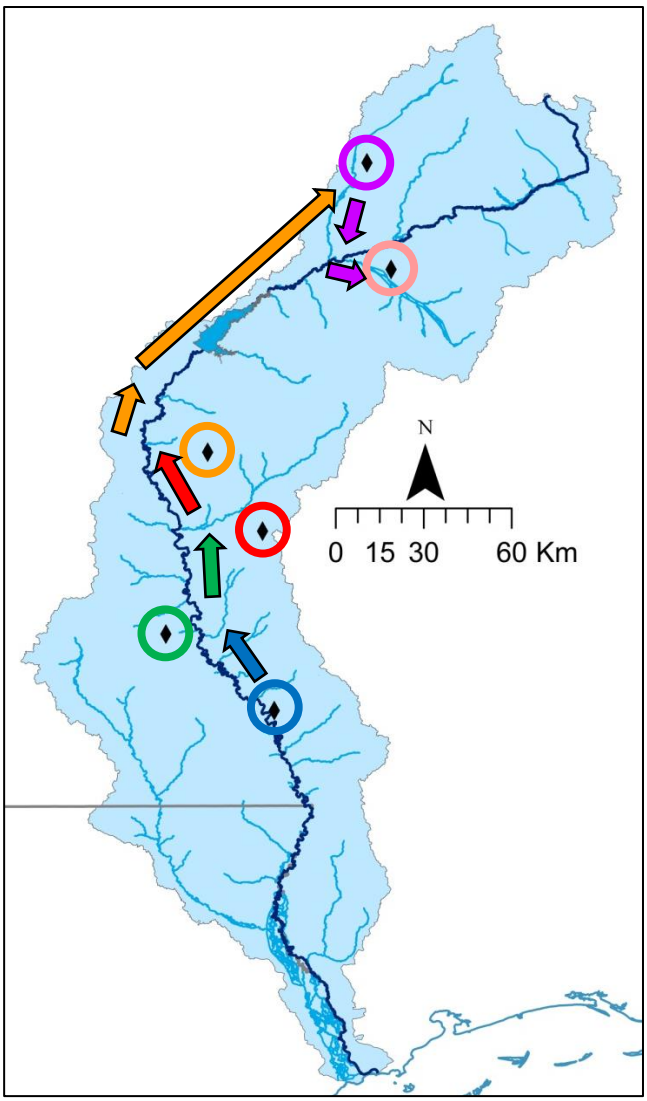

Figure 30. The most likely model (upstream) for *E. claviformis* in the Pearl River.

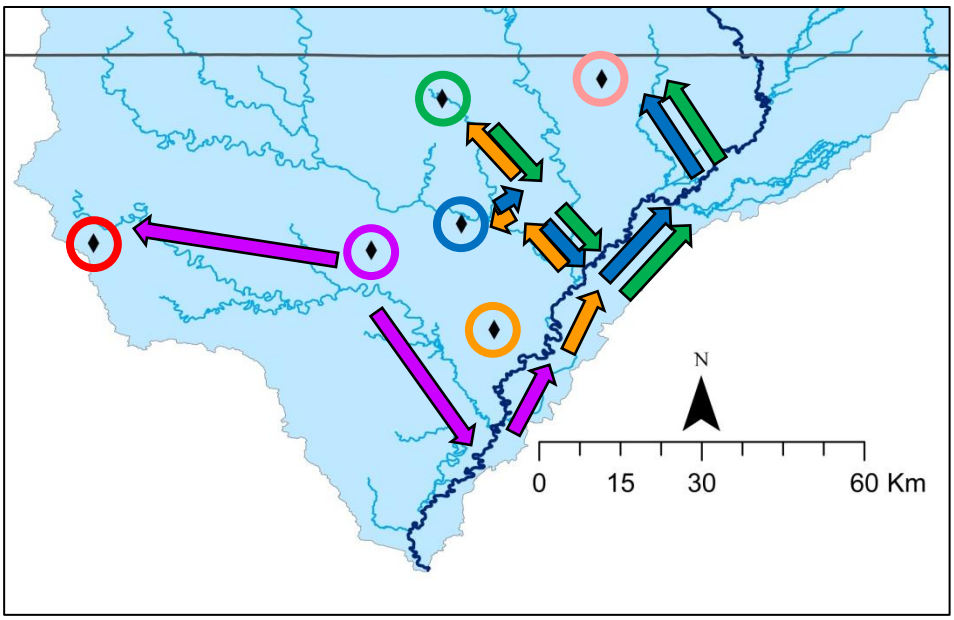

Figure 31. The most likely model (upstream) for *E. claviformis* in the Black River.

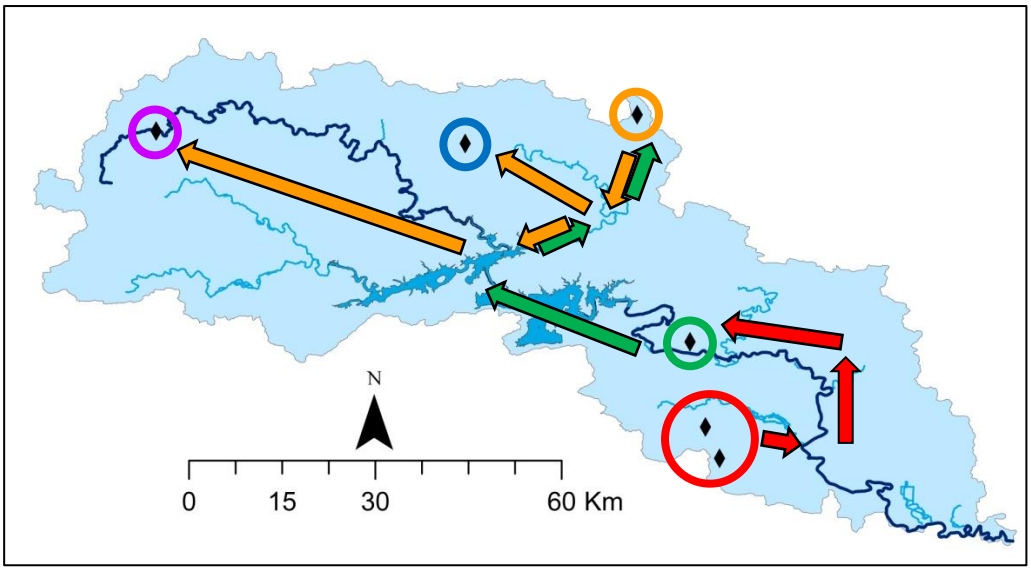

Figure 32. The most likely model (upstream) for *E. claviformis* in the Little Red River.

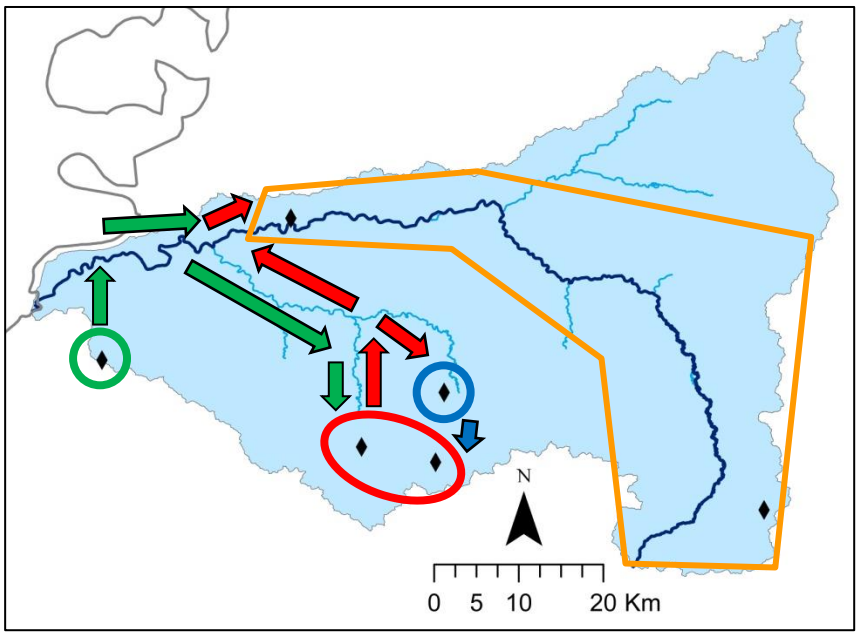

Figure 33. The most likely model (upstream) for *E. artesiae* in Bayou Pierre.

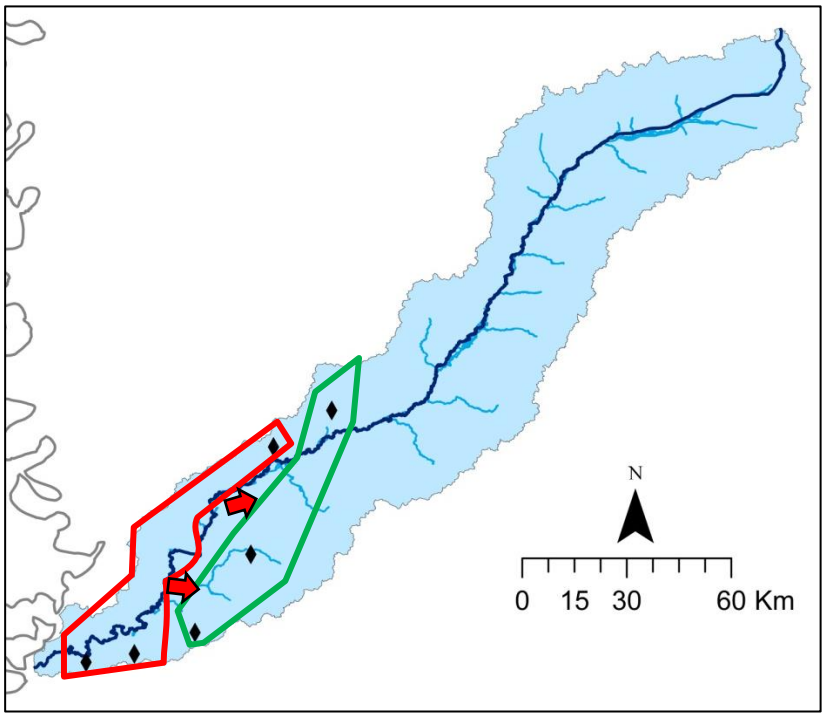

Figure 34. The most likely model (upstream) for *E. artesiae* in the Big Black River.

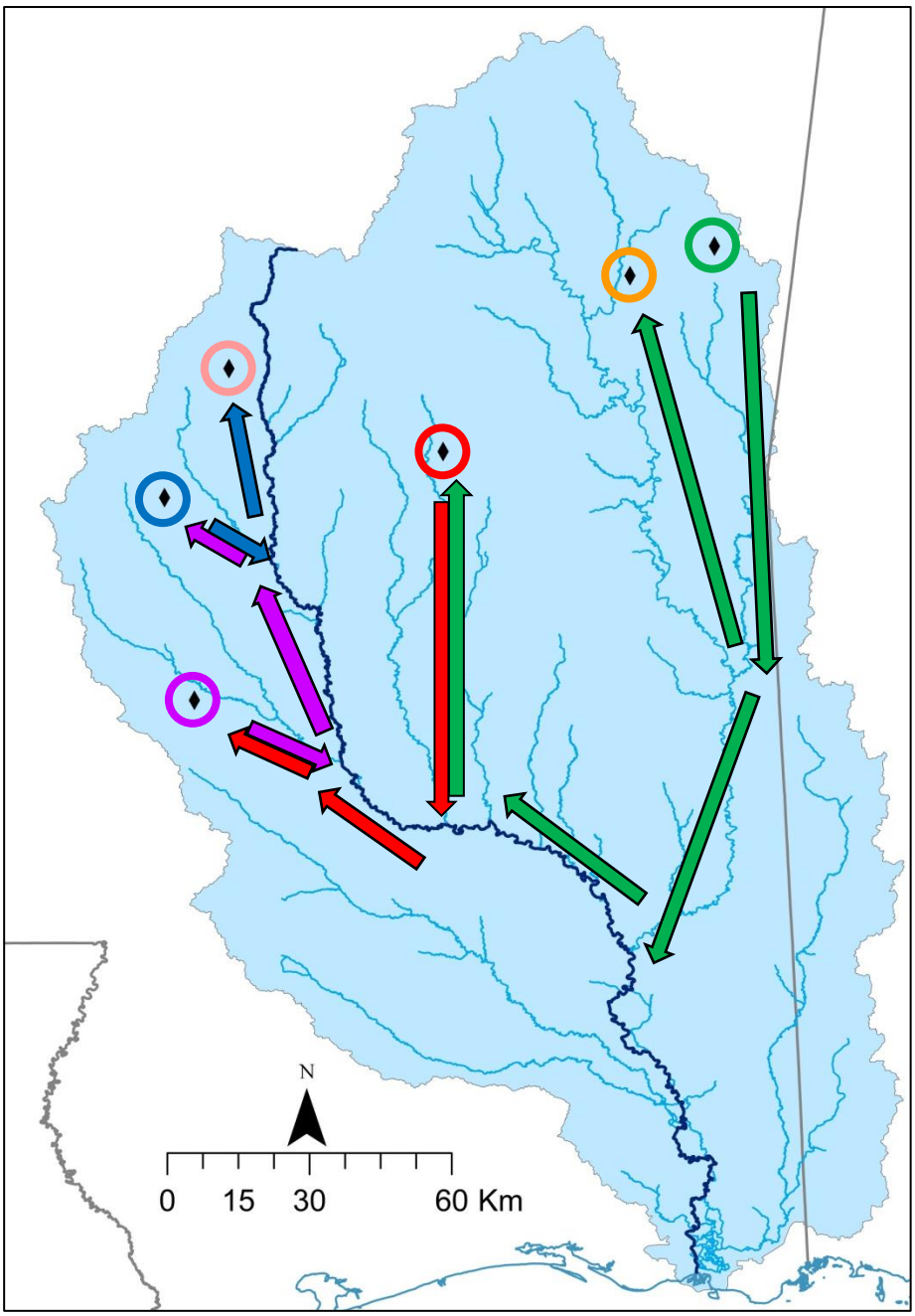

Figure 35. The most likely model (upstream) for *E. parvipinne* in the Pascagoula River.

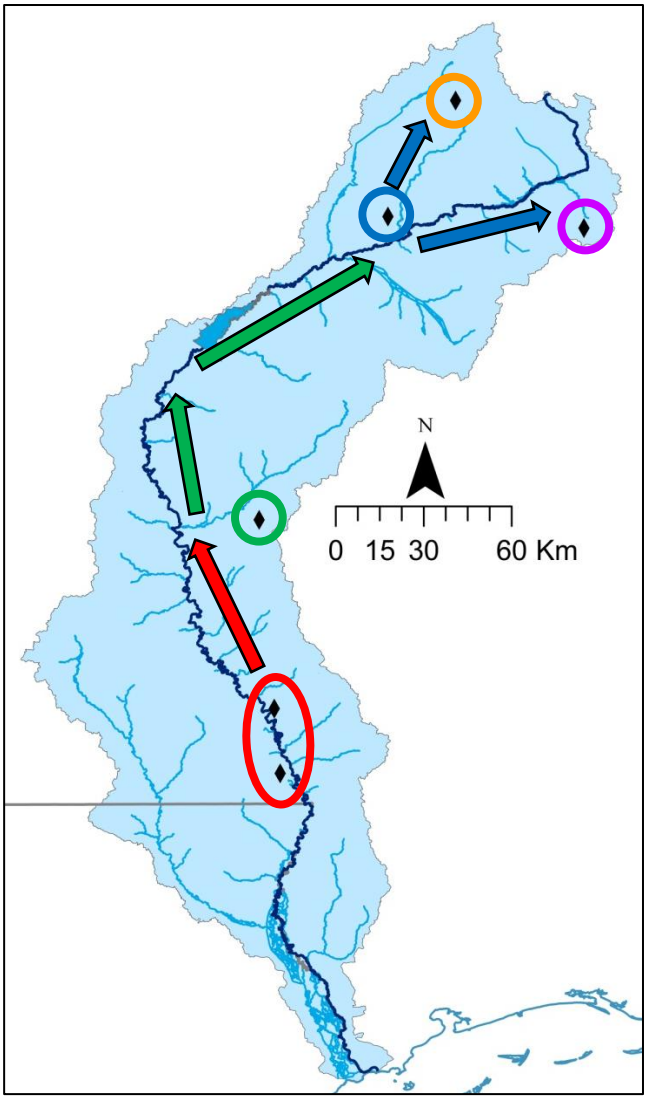

Figure 36. The most likely model (upstream) for *E. parvipinne* in the Pearl River.

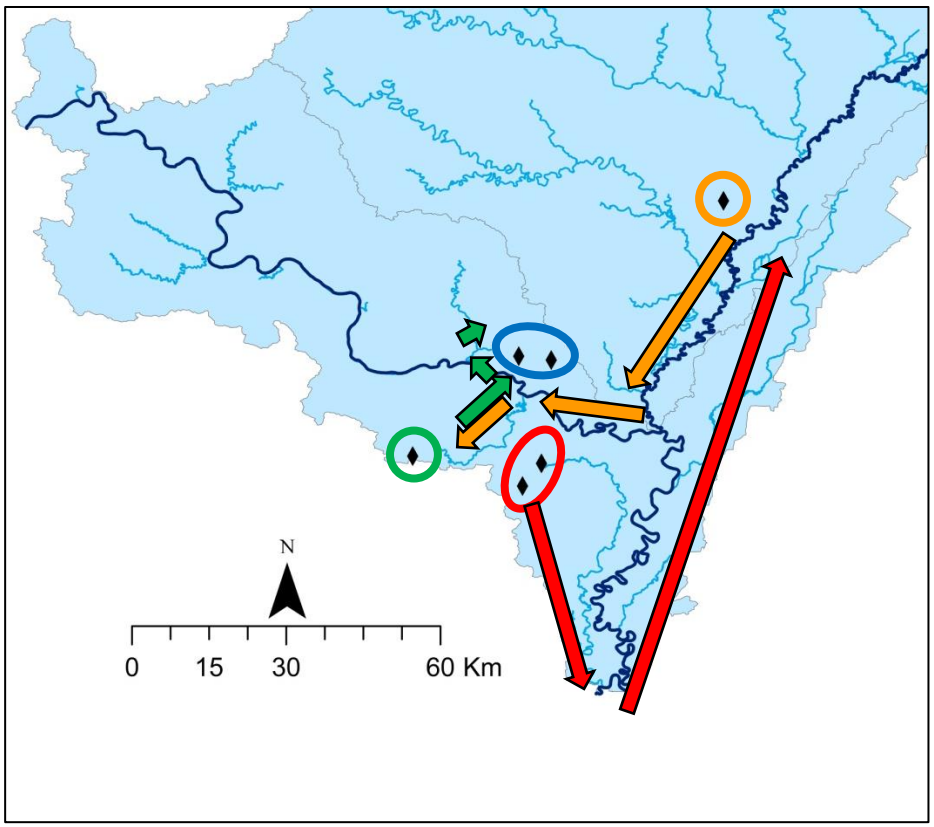

Figure 37. The most likely model (upstream) for *E. whipplei* in the Middle White River.

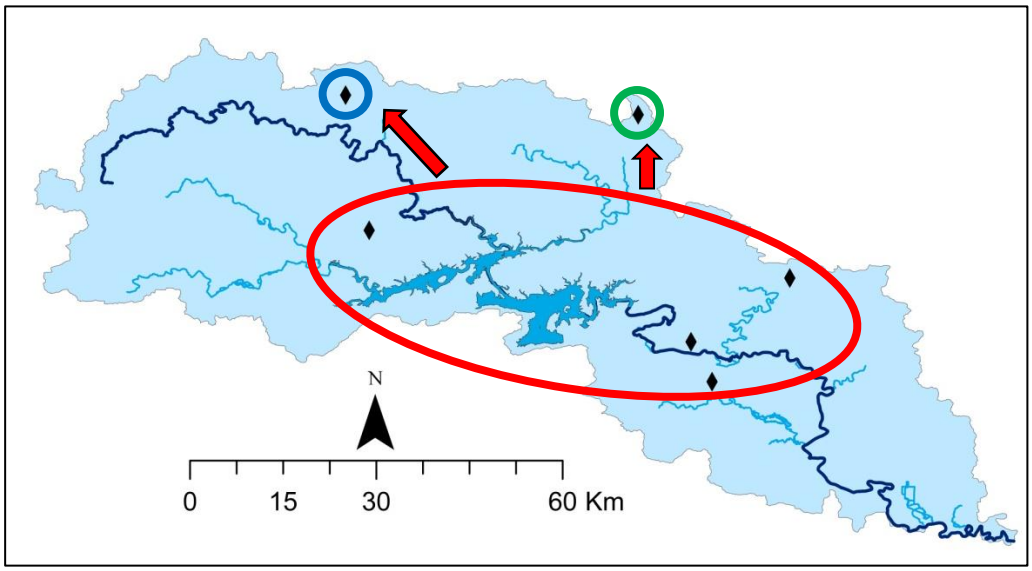

Figure 38. The most likely model (upstream) for *E. whipplei* in the Little Red River.

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CHAPTER III – ISOLATION BY DISTANCE AND ISOLATION BY CONNECTING PATHWAY RESISTANCE FOR HEADWATER FISHES

Introduction

Isolation by Distance (IBD) is a pattern where there is a positive relationship between geographic distance and genetic distance, and occurs in situations where dispersal is more frequent across shorter distances, and more distant populations experience lower rates of gene flow (Wright 1943; Slatkin 1993). IBD has been documented across many systems and species, including stream networks involving headwater resident fishes (Kanno et al. 2011; Sterling et al. 2012; Earnest et al. 2014). Under IBD, genetic drift has a greater influence than dispersal for distant populations, which allows for genetic divergence, and other processes that reduce dispersal could also increase genetic divergence through the same mechanisms (Rousset 1997; Wang & Bradburd 2014).

Recently, new techniques and interpretations for the effects of environmental heterogeneity on dispersal and gene flow have been developed. McRae (2006) introduced the concept of Isolation by Resistance (IBR) to examine the influence of different habitats or environmental effects along connecting areas on gene flow. This concept compares gene flow to electrical conductance, and features of the landscape connecting populations can act as resistance to gene flow in a similar manner as resistors for conductance in an electrical circuit. IBR was developed to study organisms able to disperse in a two dimensional landscape and uses circuit theory with predictions that multiple pathways and wider areas of suitable habitat along pathways will increase gene flow (McRae & Beier 2007). When designing resistance surfaces, one can use occurrence

data for range boundaries and expert opinion on parameter selection, creating either a univariate or multivariate model for parameters affecting resistance (Spear et al. 2010). Assigning specific resistance values to different habitats or weighting resistance values for different parameters in a multivariate model have been considered the most difficult aspect of creating resistance surfaces in an IBR framework (Spear et al. 2010).

Obligate aquatic organisms in dendritic stream networks offer some advantages for studying the resistance of connecting environments on gene flow. Pathways for movement between populations must usually follow the hierarchical, branching structure of the dendritic river network and therefore dispersal is generally constrained to a known path (Rodriguez-Iturbe et al. 2009). Additionally, habitat differences along a linear ecological gradient of stream size ranging from headwaters to large rivers are generally consistent and predictable across river networks (Vannote et al. 1980). A multitude of environmental parameters can be correlated with stream size, including channel width, channel depth, temperature, riparian shading, slope, and substrate composition among others (Rice et al. 2008). In particular, headwaters and small streams (1^{st} to 3^{rd} order streams) will have very different local environmental conditions than larger streams in the network based on these multiple parameters. If these variables correlated with stream size impact dispersal, then dendritic stream networks are advantageous for studying environmental resistance to gene flow because stream size can be used as a surrogate for multivariate environmental change along a well-known and predictable gradient, and movement pathways between populations are constrained and known as well from the stream network arrangement.

Fish distributions within drainages are often influenced by both local habitat conditions as predicted by the linear stream size gradient, and spatial position of the stream within the network (Smith & Kraft 2005; Hitt & Angermeier 2011; Yan et al. 2011). The influence of spatial position within the network on species distributions highlights the importance of population connectivity on structuring fish assemblages. Population connectivity in stream networks depends on both the physical habitats of the connecting stream habitats and the specific life history traits of the organism studied (Öckinger et al. 2010). Fishes with specific life history traits adapted for small, headwater streams may have less connectivity and dispersal across connecting pathways with larger streams due to the large environmental differences associated with stream size. This potential for a reduction in connectivity is evident in a study of fish distribution patterns that showed abrupt changes in species compositions at headwater and main stem junctions (highly adventitious streams) in Kansas, and a more continuous change along main stem and tributary axes (Thornbrugh & Gido 2009). Specific ecological traits adapted for headwater conditions may reduce survivability in larger streams, which could act as barriers to dispersal and reduce connectivity in the network. One headwater species of fish was found to have decreased survival rates with increasing depth and increased survival rates with increasing stream gradient (Kanno et al. 2014). Because depth increases with increasing stream size, and gradient decreases with increasing stream size, larger streams in the network would have reduced survivability for this fish and might negatively impact the ability to disperse across those habitats (Bisson & Montgomery 1996). Genetic studies on headwater fishes have corroborated the theory that larger

streams in river networks can form barriers to gene flow and promote isolation within smaller tributary catchments (Turner & Robison 2006; Hollingsworth & Near 2009).

Predictions for connectivity and gene flow can be made for certain organisms and connecting pathways in streams. Hughes et al. (2013) used life history traits and connecting habitat characteristics of published genetic studies in rivers to predict models of gene flow for different aquatic organisms and found a high proportion (73%) of the study results for fishes conforming to their a priori predicted model for those situations. In particular, three of the models used in their study may be of relevance to headwater adapted fishes. The Stream Hierarchy Model (SHM) predicts that gene flow will occur within hierarchically nested patterns of the drainage (i.e., network spatial structure will influence connectivity, with populations in the same tributary system having increased dispersal and populations separated in different tributary systems having decreased dispersal) (Meffe & Vrijenhoek 1998; Hughes et al. 2009). In the SHM for headwater fishes, IBD may be present, but populations with larger rivers separating them are predicted to have increased genetic distance compared with populations with the same river distance and separated by a pathway with smaller streams. The Isolation by Distance Model (IBDM) predicts a steady rise in genetic distance with an increase in stream distance (larger river habitats would not increase genetic distance in the above scenario) (Wright 1943; Slatkin 1993). The Death Valley Model (DVM) predicts low rates of dispersal between populations, with isolation occurring for a substantial time, allowing for high population genetic divergence and no patterns of IBD (Meffe & Vrijenhoek 1998; Hughes et al. 2009). Hughes et al. (2013) predicted headwater ("upland") species to have patterns of gene flow reflective of the SHM, with larger

downstream rivers limiting gene flow and decreasing connectivity. The DVM may also be found in headwater species with strict breeding requirements, with larger rivers in the network forming an even stronger barrier to gene flow (Fluker et al. 2014).

Although large rivers may form a "soft" barrier to gene flow for headwater species because they don't physically restrict movements and the barrier effect is due to ecological differences in habitat, many river networks also include "hard" barriers to gene flow that are impassable, preventing movement either in one direction (waterfalls, culverts with plunge pools) or both (dams) (Meeuwig et al. 2010). Dams also create lentic reservoir habitat that replaces former lotic river habitat that acted as ecological corridors for connectivity between tributary systems, and therefore stream populations above dams that are separated by reservoirs can have reduced connectivity and increased isolation (Falke & Gido 2006; Yan et al. 2011). This increase in isolation has been found to reduce genetic diversity in populations above reservoirs (Yamamoto et al. 2004; Skalski et al. 2008; Horreo et al. 2011; Franssen 2012) and increase genetic distance in populations separated by lentic habitat and/or dams (Yamamoto et al. 2004; Dehais et al. 2010; Hudman & Gido 2013).

The goal of this study is to examine IBD and IBR patterns for different species of headwater fish across multiple drainages. IBR could be driven by both larger streams in the network and dams and lentic, reservoir habitats. The hypothesis being addressed is that there will be a relationship between pairwise geographic distance and pairwise resistance values on genetic distance for populations of headwater species of fish. I predict that headwater populations will have significant IBD, and that larger streams in the network will be barriers to gene flow, increasing genetic distance with a resulting

pattern as predicted by the SHM. I also predict that dams and reservoir habitats will be barriers to gene flow, increasing pairwise genetic divergence of populations that have pathways through those features.

Methods

Study areas, collection site locations, study species, DNA extraction, and microsatellite amplification and visualization were the same as reported in Chapter I. The Pearl River drainage and the Little Red River drainage were used for analyses of dams and reservoirs on gene flow. In the Pearl River, dam construction near Jackson, MS completed in 1963 created the Ross Barnett Reservoir, which is the state's largest drinking water source with an area of 134 km^2 and an upstream drainage area of 7,900 km^2 (Figure 39) (Zhang and Liu, 2013). In the Little Red River, dam construction near Heber Springs, AR completed in 1964 created Greers Ferry Lake, with an area of around 164 km^2 and an upstream drainage area of 2,970 km^2 (Figure 40) (De Lanois & Green 2011; Magoulick & Lynch 2015).

Genetic distance was determined using pairwise F_{ST} values between populations, and these values were assessed independently for each drainage of species occurrence (2-6 drainages per species) using GenAlEx v. 6.5 (Peakall & Smouse 2006). Interpretations for gene flow using F_{ST} require assumptions from Wright's Island Model, which are likely not found in most systems (Marko & Hart 2011). However, F_{ST} has proven to be robust to violations of these assumptions across many different systems, and there is an ease of interpretation of it as a metric of gene flow over other such metrics, which is why it was used in analyses of IBD and IBR (Neigel 2002; Whitlock 2011). Prior to analysis, F_{ST} values were converted into $F_{ST}/(1 - F_{ST})$, which is traditionally used for gene flow

along a linear habitat such as streams (Rousset 1997). Geographical distance was determined through connecting pathway lengths in the stream network using the NHD plus v. 2 dataset (Horizon Systems) (Figure 41). Pairwise genetic distance and geographical distance values for individual drainages were combined into a single matrix for each species. Because they are sister species that are thought to have similar life history traits and responses to IBD and IBR patterns in their respective drainages of occurrence, *Etheostoma artesiae* and *E. whipplei* were combined into a single redfin darter group matrix for both analyses to increase statistical power. Values were standardized (converted into Z-scores) for ease of interpreting regression coefficients. Matrix regressions between pairwise $F_{ST}/(1 - F_{ST})$ and pairwise river distance were performed using a Multiple Matrix Regression with Randomization (MMRR) procedure in R (R Core Team 2013) to determine IBD patterns for each group (Wang 2013).

Three different influences of stream size along the connecting pathway were evaluated for IBR patterns (Figure 40). In all three cases, stream size was determined using total cumulative drainage area (DA) using the NHD plus v.2 dataset. Average stream size for the entire connecting path was determined by doing a weighted average of DA, with values being the DA upstream of confluences along the path and the weighting being the length of segments between confluences. Maximum stream size encountered was determined by finding the highest DA along the connecting path. Large stream habitat influence was assessed by calculating the length of large habitats along the connecting pathway. Large habitats were a priori defined as having DAs greater than 2000 km^2 (this value corresponds to river habitat conditions that would be very different than those in small streams and therefore best represented possible barriers to gene flow)

or being lentic reservoir habitat. Only main stem habitats and major tributaries of the study drainages had DAs greater than 2000 km^2, reinforcing the use of this value as large habitat conditions in the network. These variables were assessed independently for each drainage, and then combined into a single matrix per species or group. Values were standardized (converted into Z-scores) for ease of interpreting regression coefficients. Matrix regressions between pairwise $F_{ST}/(1 - F_{ST})$ and the three pairwise stream size variables were performed using a MMRR procedure in R to determine IBR patterns for each group. Alpha values for significance levels were adjusted using the False Discovery Rate (FDR) method with a false discovery rate of 0.05 for simple linear regressions (Benjamini & Hochberg 1995). FDR procedures were done independently for each variable due to probable correlations between variables causing non-independence between tests across the four variables. If there were significant IBD patterns and IBR patterns after FDR corrections, then all significant variable matrices were combined in a full MMRR analysis to determine relative strengths of IBD and IBR effects on genetic differentiation.

To assess the influence of dams and reservoir habitats as barriers to gene flow, Nei's unbiased genetic distance (D) was calculated for each pairwise population grouping in drainages with reservoirs (Pearl and Little Red) and their unimpounded neighbor drainages (Pascagoula and Black/Middle White) using GenAlEx v. 6.5 (Nei 1978; Peakall & Smouse 2006). The unbiased form of Nei's D corrects for small sample sizes and was chosen as a distance metric because 15 individuals were used per site in the study. In order to increase statistical power, all species data was combined into one dataset for analysis. Pairwise comparisons were grouped into those that had reservoirs

and dams along connecting pathways and those that had river connections. A preliminary permuted Analysis of Covariance (ANCOVA) was performed on only the river connection sites per region (Pearl and Pascagoula; Little Red and Black/Middle White), with the response variable being D, the covariate being river distance (to control for IBD patterns), and the independent variable being a categorical drainage value, in order to determine if patterns of genetic distance were similar for river connected sites in both drainages in the region. If there was no difference, pairwise river connected sites for impounded drainages were combined with pairwise river connected sites in non-impounded drainages to increase sample size and comparative power against reservoir and dam connected sites. A permuted ANCOVA was then performed independently on the two full regional datasets, with the dependent variable being D, the covariate being river distance, and the independent variable being a categorical assignment for reservoir and dam pathway pairings and river pathway pairings. A weighted effect coding was used for categorical assignment based on sample size of the two groups. All ANCOVAs were performed in R (R Core Team 2013).

Results

Within drainage pairwise F_{ST} values were similar and substantial across small spatial scales (within single drainages) across the headwater species, with the exception of *S. atromaculatus*, which was different from the other species by having very high pairwise F_{ST} values from two sites in the Pascagoula drainage that gave the species a higher maximum and a higher variability (Table 37). Without these specific pairwise comparisons for *S. atromaculatus*, the values were more similar to the other groups (max

= 0.202; mean = 0.083; sd = 0.037). *Etheostoma parvipinne* had the highest minimum F_{ST} values and the least variability in the dataset.

Fundulus olivaceus had a significant IBD pattern (Figure 42) and no significant IBR pattern for the three stream size variables chosen. *Semotilus atromaculatus* had a significant IBD pattern (Figure 43) and a significant large habitat IBR effect (Figure 44). When these two variables were combined in a multiple regression, only the large habitat variable (IBR) was significant (Figure 45). *Erimyzon claviformis* had a significant IBD pattern (Figure 46), a positive trend of maximum stream size IBR that was not significant after FDR correction of alpha values (Figure 47), and a significant large habitat IBR pattern (Figure 48). When the two significant variables were combined in a multiple regression, only river distance (IBD) was significant (Figure 49). The redfin darter group had a significant IBD pattern (Figure 50) and a significant large habitat IBR pattern (Figure 51). When these two variables were combined in a multiple regression, only the large habitat variable (IBR) was significant (Figure 52). *Etheostoma parvipinne* did not have a significant IBD pattern or a significant IBR pattern for the three stream size variables chosen.

For both regions analyzed for reservoir and dam effects, there was no significant difference in genetic distance patterns for river connected sites in the impounded drainage (Pearl and Little Red), and the non-impounded drainages (Pascagoula and Black/Middle White), and therefore these river connected pairings were added to the impounded drainage river connected pairings for analysis. For the Pearl and Pascagoula drainages, there was no difference in genetic distance for river connected pairings and reservoir and dam connected pairings (Figure 53). For the Little Red and Black/Middle White

drainages, pairwise connections crossing a reservoir or dam had significantly higher genetic distances than pairwise connections crossing rivers (Figure 54).

Discussion

The hypothesis that pairwise genetic distance would be related to pairwise geographic and resistance values was supported for all of the species except for *E. parvipinne*. There were differences in IBD and IBR patterns for the different groups of headwater fishes, confirming with expectations from different presented models of gene flow, and therefore the prediction that all headwater species fit the SHM was not supported. Four of the five groups studied had significant IBD patterns, and *E. parvipinne* did not have a significant IBD pattern, suggesting a possible difference in regional migration-drift equilibrium conditions for the species. A significant IBD pattern can suggest a regional migration-drift equilibrium, while the lack of a significant IBD pattern can suggest that drift is more important than gene flow in the region and that populations are largely isolated from one another (Hutchison & Templeton 1999). Furthermore, in the absence of migration-drift equilibrium, gene flow interpretation from pairwise F_{ST} values may be inaccurate, and actual population divergence for *E. parvipinne* may be larger than that estimated based on F_{ST} (Marko & Hart, 2011). Hierarchical population clustering also found a high fragmentation rate for *E. parvipinne* in the region (92% of sampled populations were unique genetic clusters), also suggesting a pattern of isolated populations with little gene flow (Chapter I). Therefore, *E. parvipinne* appears to fit the DVM of gene flow, with no significant IBD pattern and isolated populations in the study region.

Fundulus olivaceus had a significant IBD pattern, but did not have a significant IBR pattern, and therefore also did not fit predictions of the SHM. Instead, *F. olivaceus* best fits predictions of the IBDM of gene flow, with no effect from larger streams in the network clustering genetically similar groups in tributary systems. *Fundulus olivaceus* also had lower fragmentation rates across the regions compared to the other species, and downstream biased dispersal, indicating that it is less headwater specialized compared with the other headwater resident species which could explain a lack of fit with the SHM since large habitats may not influence them in the same way as the other headwater species (Chapters I and II).

Semotilus atromaculatus, E. claviformis, and the redfin darter group fit predictions of the SHM for headwater fish with significant IBD patterns and significant stream size IBR associations with genetic distance. Two of these groups, *S. atromaculatus* and the redfin darter group, had identical patterns, with large habitat IBR having the most influence on genetic distance, and river distance (IBD) had the most influence on genetic distance for *E. claviformis*. Therefore, large stream habitats are slightly less of a barrier to dispersal in *E. claviformis*, allowing for distance to have the largest effect, while for *S. atromaculatus* and the redfin darter group, large stream habitats are more of a barrier to dispersal and genetically structure populations more than distance.

These results are similar to a comparative study of three fish species that occur in headwater reaches in an arid stream system of New Mexico (Pilger et al. 2015). Two of the species (*Tiaroga cobitis* and *Meda fulgida*) were thought to possibly fit the SHM, while *Gila nigra* had more restricted gene flow analogous to the pattern observed for *E.*

parvipinne, which I have referred to as the DVM (Pilger et al. (2015) referred to this pattern as a modified Headwater Model (Hughes et al. 2009)). Another comparative study of headwater (spring and seep) adapted fishes found a similar variability in influence of connecting habitats, and the species (*Etheostoma boshungi*) with specialization in breeding habitat and breeding migration had limited dispersal across larger rivers in the drainage while the other spring headwater species (*E. tuscumbia*) did not seem to be influenced in the same manner (Fluker et al. 2014).

A possible reason for these results is that there are some combinations of life history traits and habitat preferences between these groups of species that shape their dispersal capabilities and IBD and IBR patterns. *Etheostoma parvipinne* has been found to have a high association of abundance with increased canopy cover, increased percent sandy substrate, and increased water velocity in headwater streams of Mississippi (Smiley et al. 2005). Larger streams in the network tend to have decreased canopy cover, decreased sand and increased silt, and decreased water velocity, so these specific habitat associations may contribute to the high isolation rates of *E. parvipinne* in the network through decreased gene flow across unsuitable habitat. *Fundulus olivaceus* is most common along stream margins and backwater areas, where it is tends to be associated with structure (Schaefer et al. 2009). These types of habitat requirements are not expected to vary much with an increase in stream size compared with the requirements for the other species in the study, and therefore larger streams may not disrupt their preferred habitats in the same manner as the other species, leading to an absence of IBR patterns. In North Carolina blackwater streams, *Erimyzon oblongus* (the sister species for *E. claviformis* which likely has similar habitat associations) was found to be associated with

deep, wide, slow, and muddy stream reaches, while *S. atromaculatus* was found to be associated with shallow and narrow stream reaches with an absence of mud (Meffe and Sheldon 1988). Again, these specific habitat associations for *E. claviformis* are likely to vary less with increasing stream size as larger streams will tend to be deeper, wider, have slower water velocities, and have a greater percentage of silt and mud, and therefore *E. claviformis* may be able to disperse across larger streams more readily than *S. atromaculatus*, which has habitat associations that will tend to be disrupted with increased stream size. *Etheostoma whipplei* in Arkansas was found to be associated with gravel and cobble substrates, and *Etheostoma artesiae* was also found to be associated with gravel and cobble substrates in Louisiana (Tyrone 2007; Stearman et al. 2015). Additionally, both species have had negative population impacts resulting from increased siltation, which suggests that larger streams in the network (which would likely have less gravel, cobble, and increased silt) may form barriers to dispersal, explaining the fit for the SHM for this group (Williams et al. 2005; Tyrone 2007; Stearman et al. 2015). Again, these results are similar to another study of headwater species of fish in an arid stream system, where large rivers were thought to be barriers to gene flow for *T. cobitis* due to a decrease in preferred habitat (Pilger et al. 2015).

There was a difference in reservoir effects on genetic distance for all study species across the two regions with reservoirs, so the prediction that dams and reservoirs would be barriers to gene flow was supported in the Little Red and not supported in the Pearl. Similar to my results for the Pearl drainage, other studies have also found limited effects of dams on population connectivity and genetic distance. Branco et al. (2012) found no effects of dams on fish species distributions in Portugal, and Clemento et al.

(2009) found no significant genetic differentiation between above dam and below dam populations of *Oncorhynchus mykiss* in three drainages of California.

Because dam construction in the Pearl (1963) and Little Red (1964) finished within one year of each other, a similar number of generations for the study fish in the two regions has occurred allowing for genetic differences to build from IBR effects of the reservoirs, so differences in reservoir effects of the two systems are not likely due to time of impoundment. There is a large difference between the two systems in dendritic connected habitat above the reservoir, which may have an influence on the observed genetic patterns. The Pearl River has a larger drainage area above the reservoir (7,900 km^2), with four major subdrainages (Yockanookany River, Lobutcha Creek, Tuscolameta Creek, and the Upper Pearl River) joining together into a main stem before entering the reservoir, maintaining dendritic, lotic connections between these subdrainages (Figure 39). The Little Red River has a comparatively smaller drainage area above the reservoir (2,970 km^2) and also has four major subdrainages, but there are three systems that independently flow into the reservoir and are therefore separated by lentic habitat: 1) South Fork Little Red River (also contains Archey Creek), 2) Middle Fork Little Red River, and 3) Beech Fork (Figure 40). If populations above reservoirs are able to support a large effective population size, then the effects of genetic drift would be mitigated and there would be less differentiation between populations separated by the lentic habitats, even with dispersal limitation across reservoir habitats and dams (Hudman and Gido, 2013). Differences in response to dams in the network for the headwater species in these two drainages may be due to a larger connected dendritic system in the Pearl drainage, with four spatially nested tributary systems allowing for larger population sizes and

mitigating effects of genetic drift. Because the dendritic system above Greers Ferry Lake has been fragmented into three disjunct branches, population sizes for the species in these branches may have declined more quickly, allowing barrier effects of the reservoir and dam to become evident. The effects from this fragmentation of the Upper Little Red River have been well documented for the endangered, endemic darter, *Etheostoma moorei*, which is completely confined to tributary systems above the dam. Prolonged drought and timber harvesting has negatively affected the region after dam construction, leading to an 80% decline in abundance for *E. moorei* (Wine et al. 2001; Johnson et al. 2006). It is likely that the four headwater species studied in the region were also negatively impacted by drought, timber harvesting, and the fragmented river network preventing dispersal and rescue effects, and this could be why there is an increased barrier effect in this drainage due to the effects of genetic drift on small populations.

These results show that different headwater species follow predictions set by different models of gene flow for IBD and IBR patterns, and the specific models of gene flow may be associated with specific habitat associations for the species and the changes in those habitat availabilities along a linear stream size gradient. The results also show that genetic isolating effects of reservoirs and dams may occur more rapidly in drainages lacking lotic, dendritic connections for upstream tributary systems that have also undergone drought and land use impacts. Together these results highlight the complex interactions between life history traits, habitat associations, dendritic network spatial structure, and dam construction can have on the genetic structure for headwater adapted fishes.

Table 37

Summary statistics for within drainage pairwise F_{ST} values for the different species and groups

Species or Group	minimum	maximum	average	standard dev.
F. olivaceus	0.02	0.137	0.059	0.028
S. atromaculatus	0.026	0.399	0.108	0.087
E. claviformis	0.019	0.146	0.069	0.03
redfin darter group	0.022	0.12	0.063	0.024
E. parvipinne	0.051	0.106	0.072	0.014

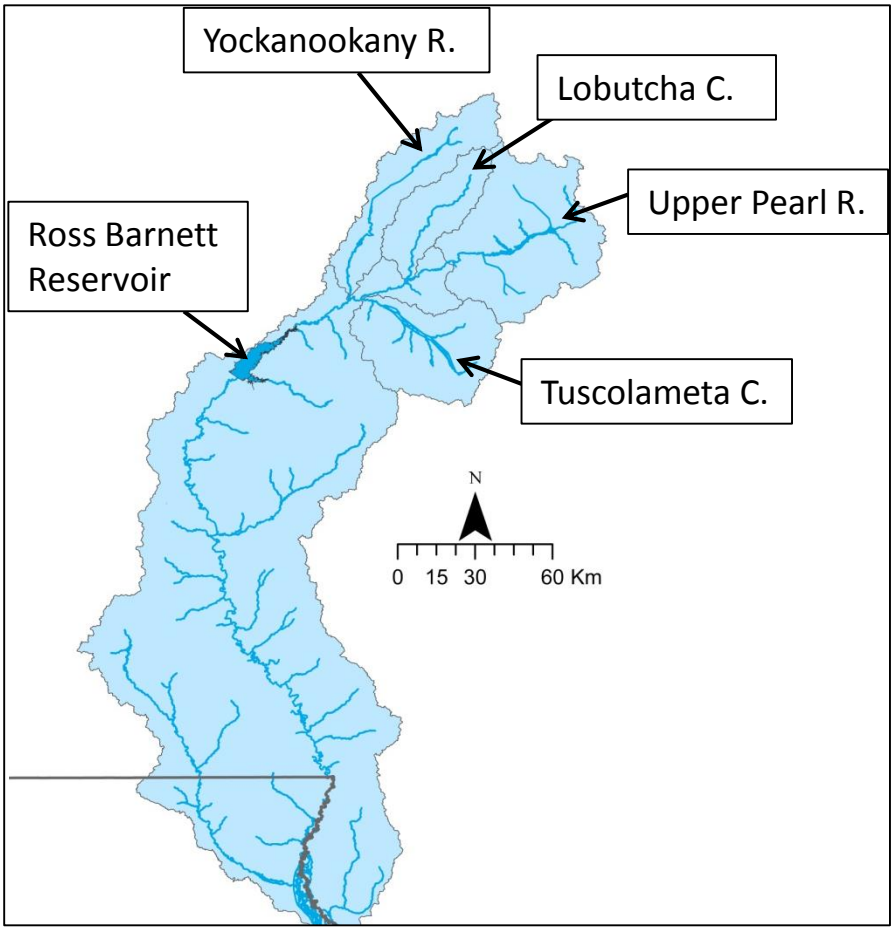

Figure 39. The Pearl River drainage, showing the locations of Ross Barnett Reservoir and upstream tributary networks.

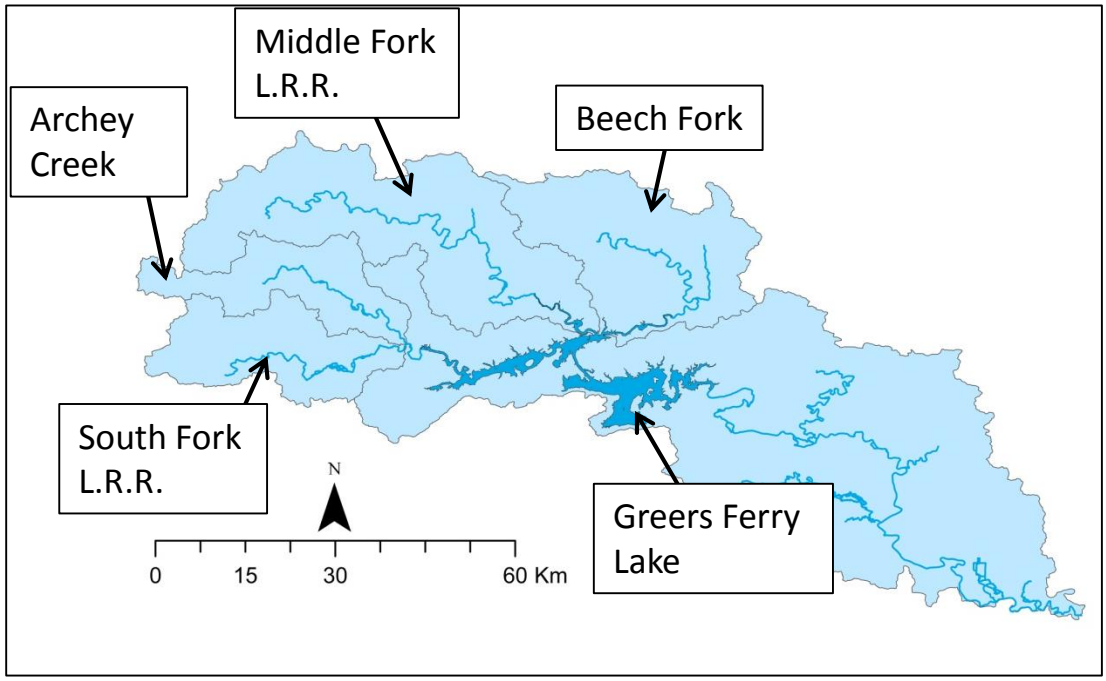

Figure 40. The Litter Red River drainage, showing the locations of Greers Ferry Lake and upstream tributary networks.

L.R.R. = Little Red River

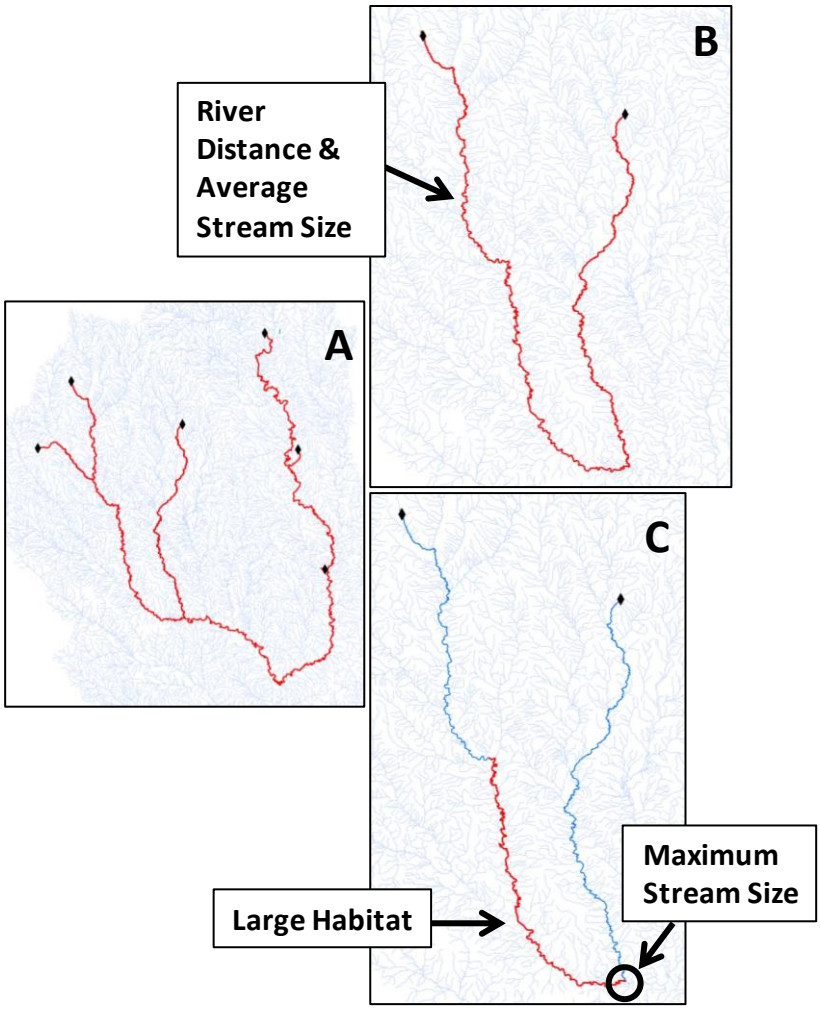

Figure 41. Methods for obtaining IBD and IBR variables in the study.

A: All connecting pathways for a single species drainage pairing. B: River distance (IBD) and average stream size (DA) (IBR) were calculated along the whole network connecting pathway. C: Large habitat (IBR) was calculated as the distance along the pathway with DA greater than 2000 km^2 and maximum stream size (IBR) was the largest DA encountered along the pathway.

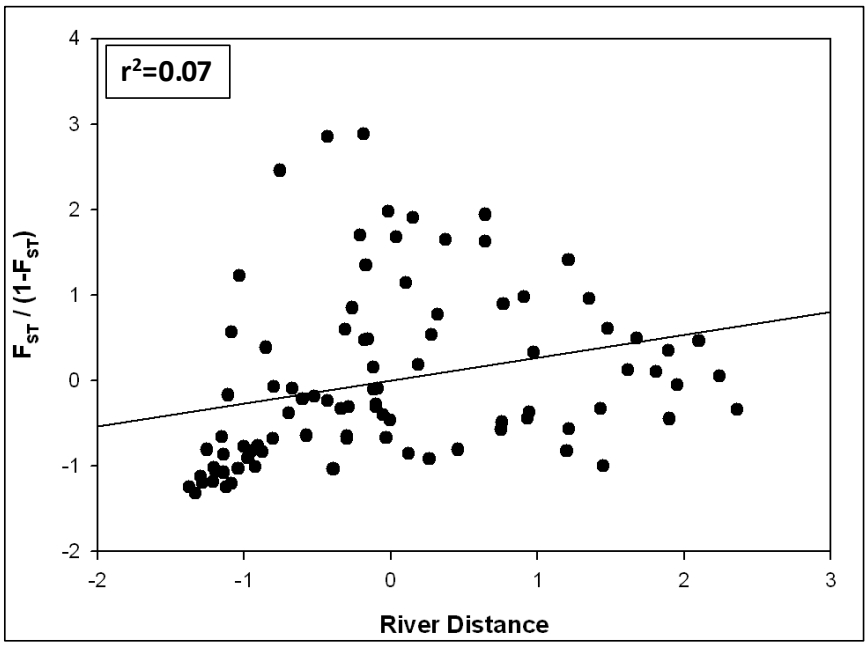

Figure 42. Within drainage river distance (IBD) pattern for *F. olivaceus*.

(p = 0.04*, coef = 0.267). * significant following FDR adjustment of alpha levels.

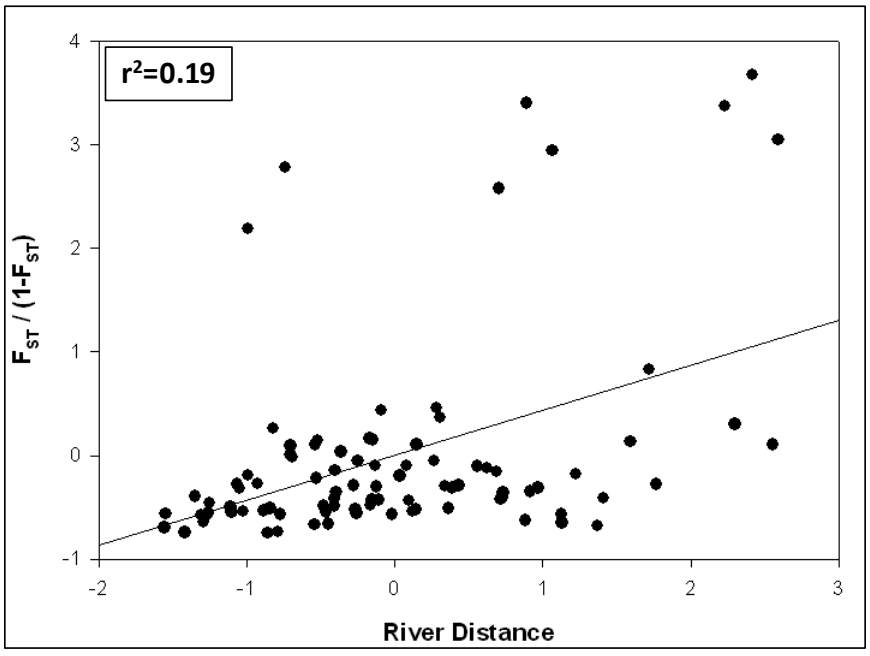

Figure 43. Within drainage river distance (IBD) pattern for *S. atromaculatus*.

(p = 0.003*, coef = 0.435). * significant following FDR adjustment of alpha levels.

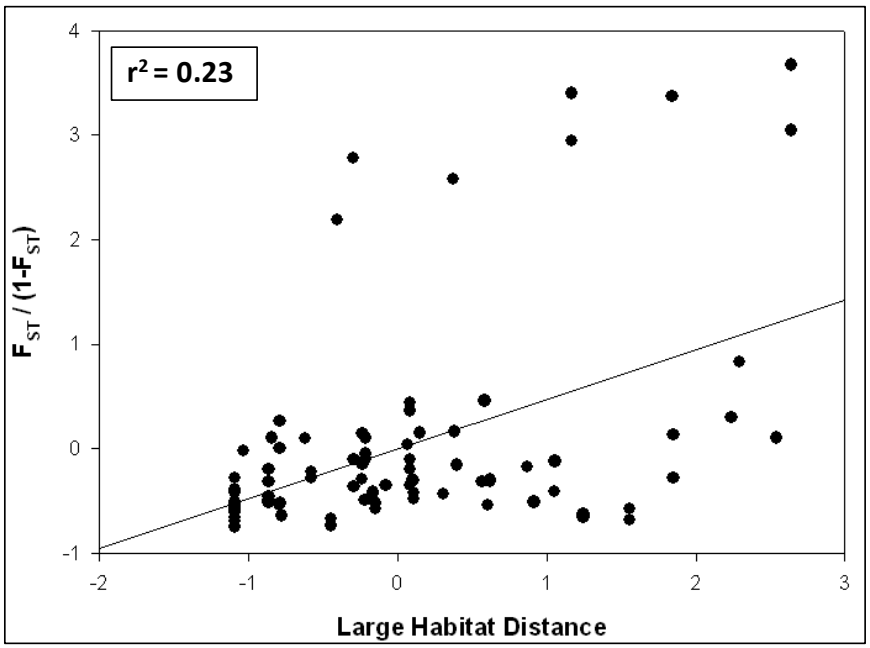

Figure 44. Within drainage large habitat (IBR) pattern for *S. atromaculatus*.

(p = 0.004*, coef = 0.474). * significant following FDR adjustment of alpha levels.

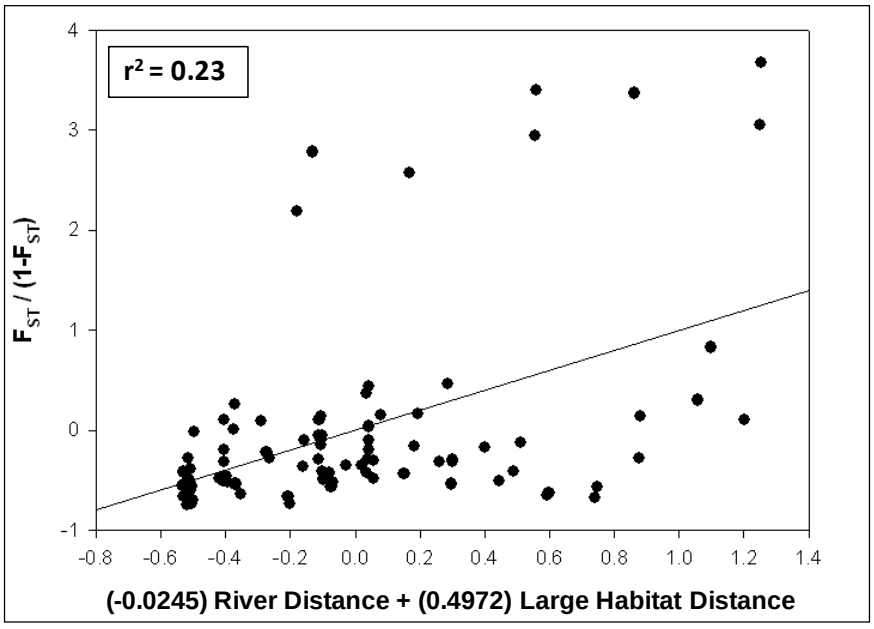

Figure 45. Multiple regression analysis for *S. atromaculatus*.

river distance (IBD): p = 0.924, coef = -0.025; large habitat (IBR): p = 0.006, coef = 0.497.

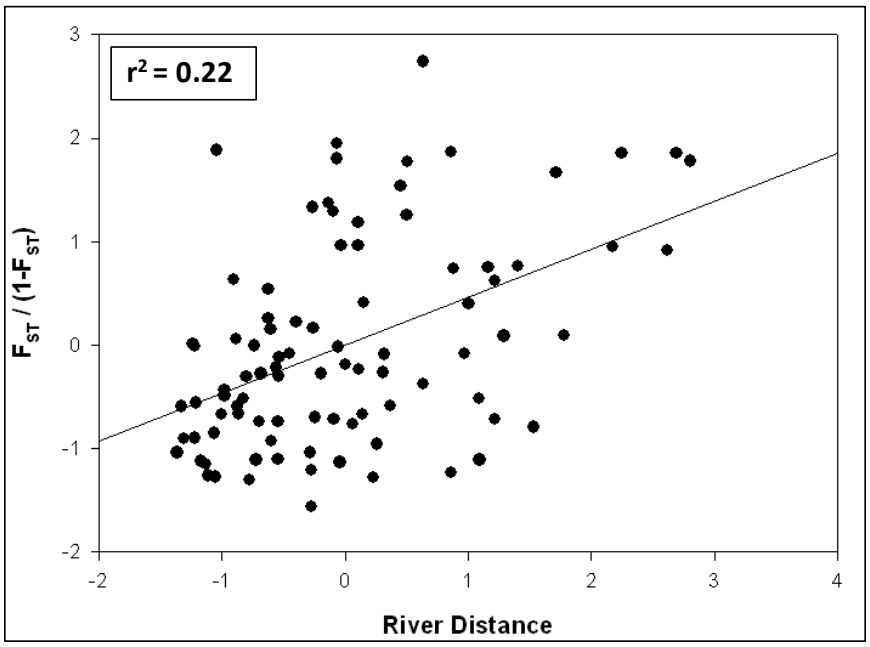

Figure 46. Within drainage river distance (IBD) pattern for *E. claviformis*.

(p = 0.005*, coef = 0.464) * significant following FDR adjustment of alpha levels.

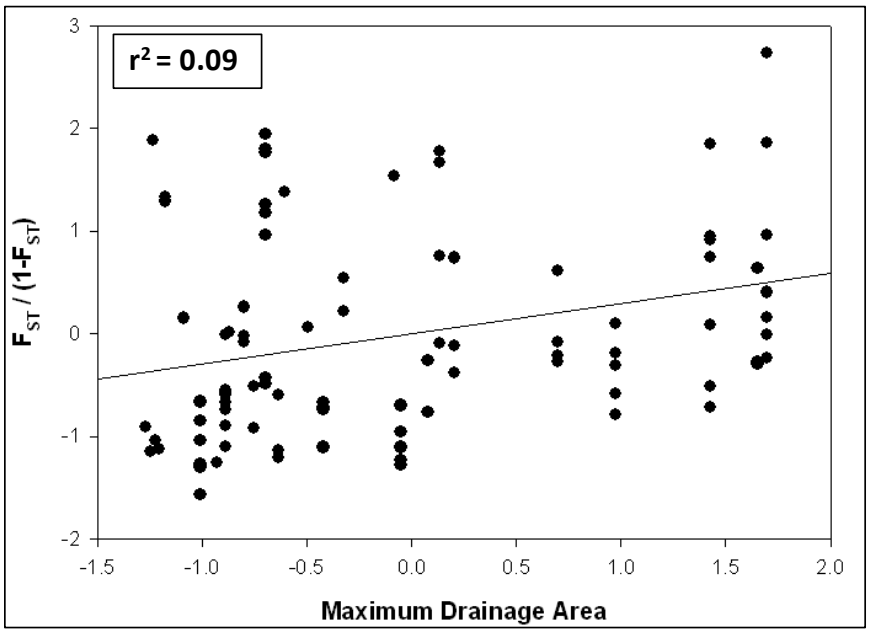

Figure 47. Within drainage maximum stream size (IBR) pattern for *E. claviformis*.

(p = 0.036, coef = 0.294). The trend was not significant following FDR adjustment of alpha levels.

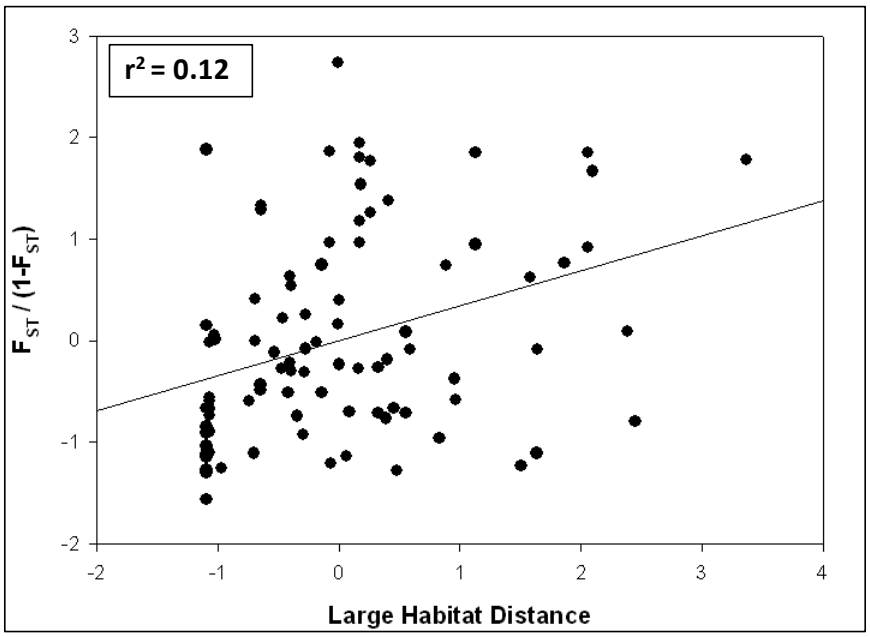

Figure 48. Within drainage large habitat (IBR) pattern for *E. claviformis*.

(p = 0.009*, coef = 0.348). * significant following FDR adjustment of alpha levels.

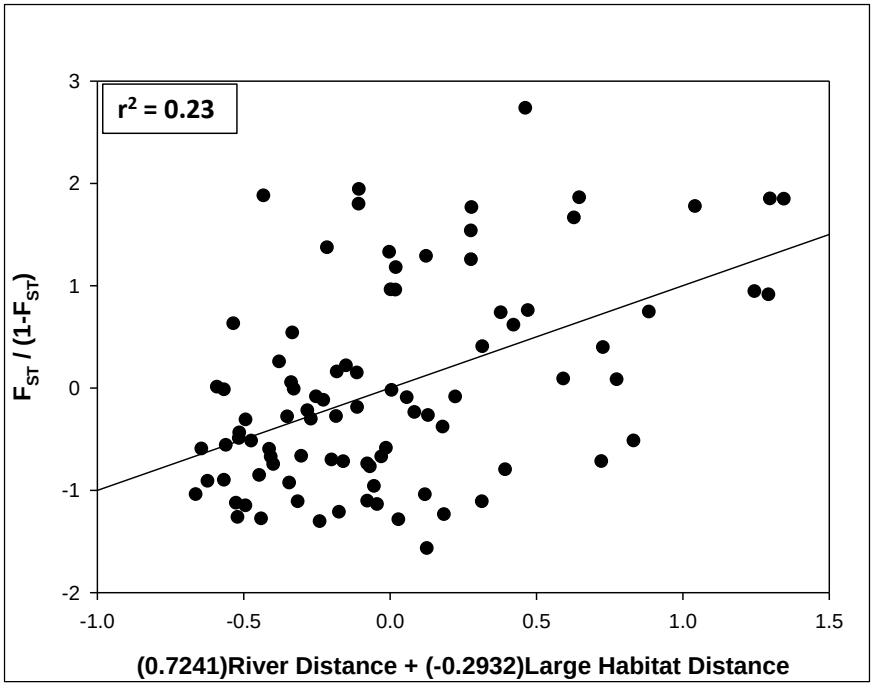

Figure 49. Multiple regression analysis for *E. claviformis*.

within drainage river distance (IBD): p = 0.013, coef = 0.724; large habitat (IBR): p = 0.228, coef = -0.293.

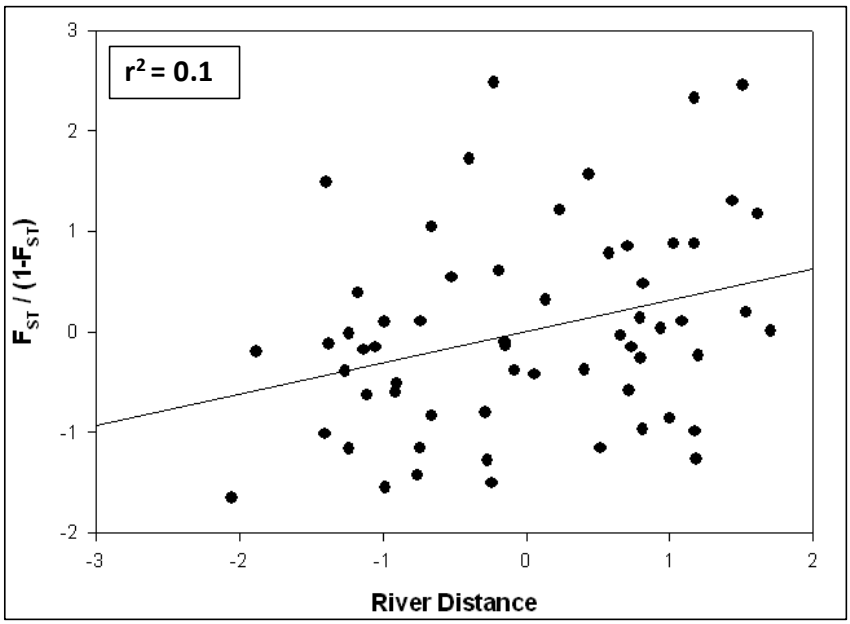

Figure 50. Within drainage river distance (IBD) pattern for the redfin darter group (*E. artesiae* and *E. whipplei*).

(p = 0.037*, coef = 0.318). * significant following FDR adjustment of alpha levels.

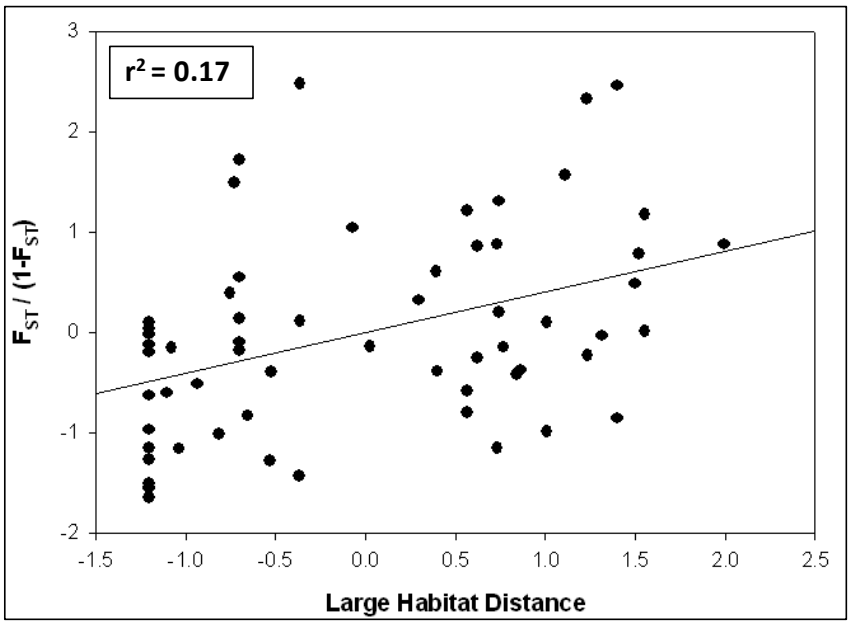

Figure 51. Within drainage large habitat (IBR) pattern for the redfin darter group (*E. artesiae* and *E. whipplei*).

(p = 0.013*, coef = 0.410). * significant following FDR adjustment of alpha levels.

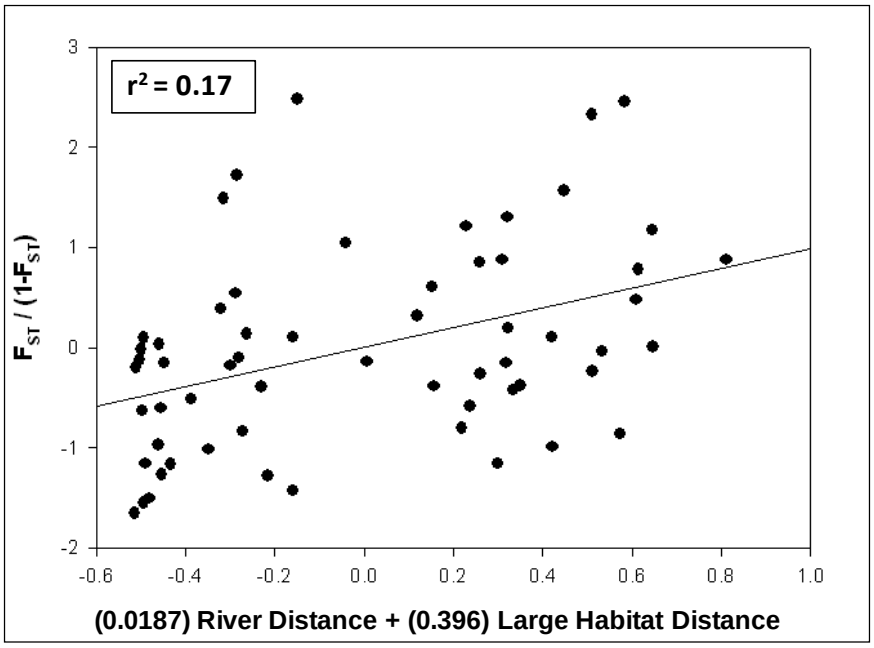

Figure 52. Multiple regression analysis for the redfin darter group (*E. artesiae* and *E. whipplei*).

within drainage river distance (IBD): p = 0.928, coef = 0.019; large habitat (IBR): p = 0.031, coef = 0.396.

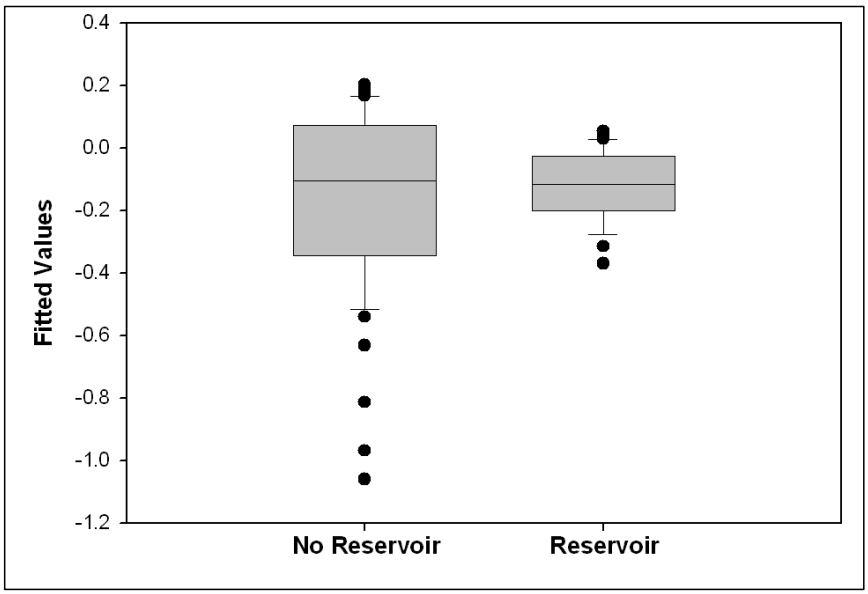

Figure 53. Reservoir effect on pairwise genetic distances for all four species in the Pearl and Pascagoula Rivers.

Fitted values = pairwise genetic distance (D) adjusted by pairwise river distance from ANCOVA analysis. reservoir category: $p = 0.416$, partial $eta^2 = 0.007$; covariate pairwise river distance: $p = 0.005$, partial $eta^2 = 0.07$.

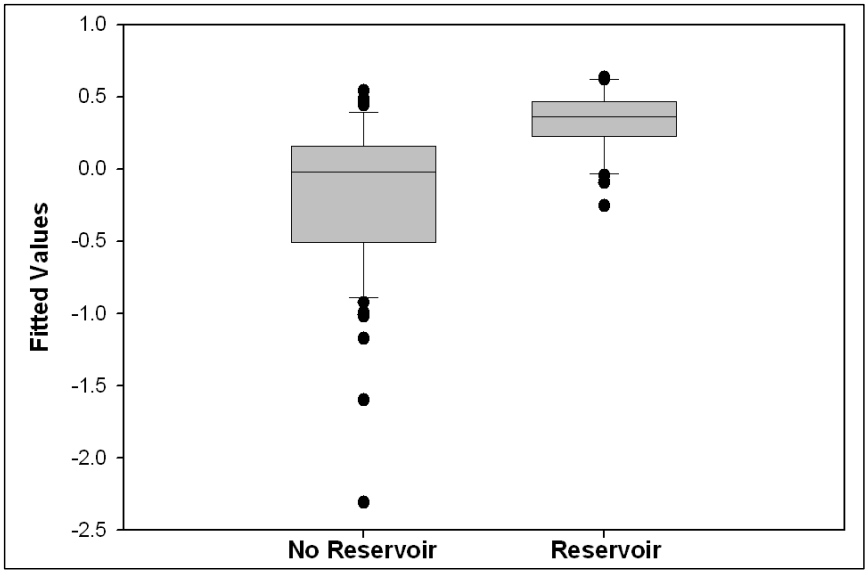

Figure 54. Reservoir effect on pairwise genetic distances for all four species in the Little Red, Black, and Middle White Rivers.

Fitted values = pairwise genetic distance (D) adjusted by pairwise river distance from ANCOVA analysis. reservoir category: $p = 0.033$, partial $eta^2 = 0.026$; covariate pairwise river distance: $p < 0.001$, partial $eta^2 = 0.198$.

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CHAPTER IV – THE INFLUENCE OF HABITAT SPECIALIZATION AND LIFE HISTORY TRAITS ON ISOLATION RATES OF HEADWATER FISHES

Introduction

A multiple species approach within the same landscape for population genetics studies can identify species related factors that affect population connectivity and gene flow patterns, because historical landscape and regional effects may be similar for sympatric species (Bohonak 1999). Population connectivity through gene flow depends on both the physical habitats connecting populations and the specific life history traits that influence dispersal patterns of the species of study (Öckinger et al. 2010). Therefore, in a multi-species comparative study, life history traits associated with dispersal are likely to cause differences in genetic structure patterns (Pelc et al. 2009; Goldberg & Waits 2010; Kelly & Palumbi 2010; Burns et al. 2014).

Because of complex behavioral interactions between dispersal behavior and population size, life history traits that affect both fecundity and survivability are thought to influence patterns of gene flow (Waples 1987). Comparative analyses revealed fecundity related life history traits of clutch size and egg size to have the most influence on gene flow patterns in darters, with decreased clutch size and increased egg size being associated with decreased rates of gene flow (Turner et al. 1996; Turner & Trexler 1998). A large meta-analysis across multiple families of freshwater fish found a positive association between body size and movement, and therefore larger maximum body size may increase dispersal and gene flow (Radinger & Wolter 2014). Studies of macroinvertebrates and salamanders found that having obligate or mostly obligate aquatic dispersal led to greater genetic differentiation between populations in stream networks

when compared with species that were capable of terrestrial or flight-mediated dispersal (Zickovich & Bohonak 2007; Steele et al. 2009; Alp et al. 2012). Husemann et al. (2012) used body size, trophic position (and associated population sizes in streams), and tolerance to harsh environments to make predictions about the genetic structure of two centrarchids and three cyprinids in Texas, and these were supported by their results, with lower inferred population sizes and lower tolerance to harsh environments leading to increased population structuring.

Headwaters are considered harsh environments with high environmental variability, and abiotic conditions in headwaters may filter for species compositions with requisite ecological and life history traits (Rahel & Hubert 1991; Poff 1997; Carvalho & Tejerina-Garro 2014). The River Habitat Template theory predicts that in temporally variable systems, such as headwater streams, species will have resistance and resilience adaptive traits such as smaller body size, higher fecundity, and shorter reproductive cycles (Townsend & Hildrew 1994). Winemiller (1989) and Winemiller & Rose (1992) devised a theoretical triangle of life history trait sets for freshwater fishes, grouping species into either periodic, opportunistic, or equilibrium reproductive strategies based on temporal stability and environmental heterogeneity as well as trade-offs associated with specific traits. The periodic strategy is predicted to have a larger body size, a later age of maturation, a longer life span, a higher fecundity, and a shorter breeding season, and would be adaptive for predictable, seasonal variability in flows associated with spawning (Winemiller 1989; Winemiller & Rose 1992). The opportunistic strategy is predicted to have a smaller body size, an earlier age of maturation, a shorter life span, a lower fecundity, and a smaller egg size, and would be adaptive for unpredictable environments

with large variations in flow (Winemiller 1989; Winemiller & Rose 1992). The equilibrium strategy is predicted to have a larger body size, a longer breeding season, and larger eggs, and would be adaptive in stable, predictable environments with regular flow patterns (Winemiller 1989; Winemiller & Rose 1992). Because of the high rate of variability in flow and environmental conditions in many headwaters, most headwater species may be predicted to have an opportunistic strategy with colonization adaptive life history traits. If flows in headwaters are more stable, such as in perennial spring-fed systems, then they may be expected to have traits representative of an equilibrium strategy. Because of the variability in headwater habitats across a landscape, different headwater species may have different strategies to exploit their specific stream and habitat preference, and generalizations based solely on stream size are hard to make. Pease et al. (2012) did not find a relationship between breeding strategy and linear position within the catchment of their study, highlighting the complicated nature of matching life history trait predictions based on flow variability with stream size. Specific life history traits that have been shown through observation to be associated with headwater residency include a smaller body size, earlier age of maturity, and a shorter life span, which match the predictions of an opportunistic strategy (Schlosser 1990). Across a large set of taxonomically diverse North American freshwater fish, Mims et al. (2010) found a strong agreement with sets of life history variables in the context of tradeoffs predicted for these three strategies, and among the groups being compared in this study, *Fundulidae*, *Etheostoma*, and chubs in *Cyprinidae* had a high association with traits representing an opportunistic strategy, while *Catostomidae* was mostly associated with a periodic strategy (large bodied, late maturing, long life span, high fecundity).

Habitat specificity has also been shown to be associated with population structure and gene flow, including studies of headwater resident fishes. For a comparison of two salmonids, the species with increased spawning habitat specificity, a more complex mating behavior, and smaller population sizes was found to have increased genetic distance between populations (Whiteley et al. 2004). Turner & Robison (2006) compared gene flow in two headwater specialized species (*Noturus taylori* and *Etheostoma pallididorsum*) to one that occupied a broader range of habitats (*E. radiosum*) and found that the more headwater specialized species had greater genetic differentiation between populations. For two different sets of sister species pairings of darters with differing degree of specialization (one in streams and one in spring habitats), the spring associated darter had higher genetic structure than the stream associated darter in both comparisons (Fluker 2011). Fluker et al. (2014) also compared two sister species of darters that used spring and seep habitats and found that the species with a migratory breeding strategy and specialized breeding habitat in seeps had increased population divergence and structure than the species that permanently occupied spring headwater habitats. Tibbets & Dowling (1996) compared two habitat specialists that have populations in headwater reaches (*Tiarogo cobitis* and *Meda fulgida*) with a habitat generalist (*Agosia chrysogaster*), and found higher isolation and fragmentation rates in the habitat specialists. Pilger et al. (2015) compared the same two habitat specialists with another species that occurs in headwaters (*Gila nigra*) across a finer scale. Levels of headwater specialization may be different for these three species, as *G. nigra* occurred in less main stem sites (one) than *T. cobitis* (four) and *M. fulgida* (three), and *G. nigra* also had the most isolation and genetic differentiation between populations in the study (Pilger et al. 2015). The agreement of the

results of these studies suggest that increased specialization of habitat can lead to higher isolation rates for species in stream networks, including specialization in headwater habitats.

Variability in population structure patterns and rates of isolation have been found across a taxonomically diverse group of headwater adapted species. Chapter I showed that there were different patterns of hierarchical structure and different fragmentation and isolation rates. Additionally, Chapter III showed that there were different responses to pairwise river distance and large rivers as resistance mechanisms to gene flow in the stream network for these same headwater species, leading to gene flow patterns conforming to predictions from three different models of gene flow. The goal of this chapter is to examine these differences in the context of differing habitat specialization and life history traits. The hypothesis being tested is that habitat specialization and life history traits are related to within-drainage genetic isolation patterns for headwater fishes. I predict that increased headwater specialization will lead to increased rates of isolation. I also predict that life history traits associated with an opportunistic strategy (smaller body size, earlier age of maturation, decreased longevity, decreased fecundity, and shorter reproductive season) will be positively correlated with higher isolation rates across these species because this set of traits will indicate a preference for variable headwater streams, which would influence population size and other dynamics associated with dispersal and gene flow.

Methods

Study areas, collection site locations, study species, DNA extraction, and microsatellite amplification and visualization were the same as reported in Chapter I.

Nei's unbiased genetic distance (D) was calculated for each pairwise population grouping using GenAlEx v. 6.5 (Nei 1978; Peakall & Smouse 2006). The unbiased form of Nei's D corrects for small sample sizes and was chosen as a distance metric because 15 individuals were used per site in the study. Previous studies have shown differing historical and regional effects on genetic distance patterns across multiple species (Husemann et al. 2012). To mitigate these influences and to also mitigate differences based on characteristics such as heterozygosities of specific microsatellite loci across the different species, an *ad hoc* within drainage isolation ratio (IR) statistic was employed for a "standardization" purpose on genetic distance. This statistic was made possible because of the paired neighbor drainage design, and uses assumptions that historical effects were similar for each pair of drainages because of the shared geography and geological history of the regions. Each IR was calculated independently for each sample site and was the average pairwise D for that site when paired with other sites in the same drainage (5 values) divided by the average pairwise D for that site when paired with sites in the neighbor drainage (6 values). An increase in isolation ratio means an increase in isolation and decreased gene flow and connectivity for that site within the drainage, and values close to one indicate similar patterns of gene flow within the drainage as compared to gene flow between neighbor drainages (high within drainage isolation for that site).

The degree of headwater specialization was determined by analyzing presence data for patterns of niche breadth along a linear stream size gradient. Museum collections

with open access (fishnet.org) were used along with the USM Fish Collection to find presence localities for the six species of headwater fish. Only collection records in the study drainages were used (six drainages for *F. olivaceus*, *S. atromaculatus*, and *E. claviformis* and two drainages for *E. artesiae*, *E. parvipinne*, and *E. whipplei*). To increase resolution and predictive power for stream size preference, collection localities for the sister species *E. artesiae* and *E. parvipinne* were combined into a redfin darter group for analysis, as they occupied similar habitats in their respective regions (Tyrone 2007; Stearman et al. 2015). After removing duplicate localities, each site of occurrence was mapped to a stream segment in ArcGIS, and the total cumulative drainage area (DA) for that segment was assigned to the locality using the NHD plus v 2 dataset (Horizon Systems). Because of the possibility of collections in large rivers being rare for the species, a statistic called the upper limit of occurrence (ULO) was used in place of maximum stream size. This statistic takes into account the dispersion of the dataset of stream sizes and was the mean DA of occurrence plus two standard deviations (equivalent to 95% of species occurrences being below that DA for a normal distribution of stream sizes). ULO was used as a surrogate for niche breadth along a stream size gradient, with species that had higher values being found more often in larger streams. For summary comparison, the percentage of collection sites that were in headwaters (defined as having a DA ranging from 0-50 km^2) was also determined for each species to see restrictive headwater spatial patterns.

When comparing life history traits among different groups, it is important to account for historical, phylogenetic constraints on those traits based on the lineage of the particular species of study (Duminil 2007). By placing traits within a phylogenetic

perspective, one can examine how traits have varied or may be adaptive for specific reproductive strategies or environments. Life history traits for the study species were obtained from the Fishtraits Database, which is an online repository of life history traits for 809 species found in the United States compiled from reports in the literature (Frimpong & Angermeier 2009). Specific values used in the study were ones that may be associated with Winemiller & Rose's (1992) breeding strategies and included maximum reported length, age at maturation, longevity, maximum reported fecundity, and length of breeding season. To provide a phylogenetic context, these values were also obtained for closely related groups for each study species. Species in the same genus were used as the phylogenetic grouping when the number of congeneric species in the database was high (>20), and this occurred for *Fundulus* (n = 25 species) and *Etheostoma* (n = 109 species). Recent phylogenetic hypotheses were used for group formation through additions of sister clade genera for *S. atromaculatus* and *E. claviformis*, and additions of sister genera proceeded until sample size of the group was > 20 species. For *S. atromaculatus*, after *Semotilus,* the immediate sister genera *Hemitremia* and *Couesius* were added (Creek Chub clade), followed by the slightly less related genera *Snyderichthys, Lepidomeda*, and *Meda* (Plagopterin clade), followed by the slightly less related *Acrocheilus* and *Gila* (western clade) (n = 24 species) (based on phylogenetic hypothesis of Simons & Mayden 1997). For *E. claviformis*, after *Erimyzon*, the immediate sister genus *Minytrema* was added (Erimyzonini clade), followed by the slightly less related genera *Xyrauchen*, *Chasmistes*, *Catostomus*, and *Deltistes* (Catostomini clade) (n = 26 species) (based on the phylogenetic hypothesis of Chen & Mayden 2012). For each phylogenetic group, life history traits were converted into Z-scores based on the average and standard deviation

values for that group. Deviations from phyologenetic averages were interpreted as adaptations for different reproductive strategies within constraints imposed by evolutionary history.

Prior to analysis, isolation rates and ULO values were log(x+1) transformed and then standardized (converted into Z-scores). Linear regressions were performed singularly between independent variables (ULO and phylogenetic Z-scores of life history traits) and isolation ratios. If multiple independent variables were significant, then they were run in a multiple linear regression with a full model with variable order based on significance values of independent linear regressions. Only variables that had data for all of the study species were used in the full model. Retention of variables was then determined through a stepwise, backwards selection process based on Akaike Information Criteria (AIC) scores. A final multiple linear regression was then performed for the retained variables. All regressions were performed with permutations due to possible non-independence effects from pairwise data generating isolation ratios, and all data analysis was performed in R (R Core Team 2013).

Results

For creation of ULO values, a total of 1018 localities were used for *F. olivaceus* (Figure 55), 99 localities for *S. atromaculatus* (Figure 56), 110 localities for *E. claviformis* (Figure 57), 167 localities for the redfin darter group (*E. artesiae* and *E. whipplei*) (Figure 58), and 53 localities for *E. parvipinne* (Figure 59). *Etheostoma parvipinne* had the lowest ULO value, which was followed by a grouping of *S. atromaculatus*, the redfin darter group, and *E. claviformis*, which was followed by *F. olivaceus* with the highest value (Table 38). *Semotilus atromaculatus*, *E. claviformis*, and

E. parvipinne had the highest restriction to headwater streams among collection sites (>80% occurrence rate). The redfin darter group had a qualitatively lower headwater restriction (56% occurrence rate), but did not have a comparatively higher ULO value indicating an affinity for both headwaters and small streams. *Fundulus olivaceus* had the lowest headwater restriction (44%) and the highest ULO value, indicating occurrence in a wider range of stream sizes compared with the other species.

For raw data values, *S. atromaculatus* and *E. claviformis* were similar in the dataset with a larger maximum size, a later onset of maturation, increased longevity, and a much larger total fecundity (Table 39). The remaining four species were similar in total size, age at maturation, longevity, and fecundity. *Fundulis olivaceus* had a much longer breeding season than the other five species, and the breeding season lengths of the two larger fishes were similar to the other smaller bodied fishes. When examining relationships to phylogenetic averages for these species, age related traits appeared to conform to predictions of the opportunistic strategy of reproduction (Table 40). All of the species had an earlier onset of reproduction than the average for their groups, and five of the species had decreased longevity (data was not available for *E. parvipinne*). Values were mixed for total length, with *Erimyzon claviformis* being small for its group and *S. atromaculatus*, *E. artesiae* and *E. whipplei* being large for their respective groups (opportunistic expectations were a smaller body size). *Erimyzon claviformis* had higher than average fecundity, and *F. olivaceus* and *S. atromaculatus* had lower than average fecundity (opportunistic expectations were for lower fecundity). *Semotilus atromaculatus*, *E. claviformis*, and *E. parvipinne* had lower than average breeding season lengths, while *F. olivaceus* had a much higher than average breeding season length (short

breeding season is predicted for the periodic strategy and a long breeding season is predicted for the equilibrium strategy).

A significant negative relationship was found between ULO value and isolation ratio across the study species, indicating that isolation increases as the ULO value decreases (Figure 60). A weak, significant negative relationship was found between Z-scores of total length and isolation ratio, indicating that as body sizes decreased isolation increased, but the predictive power of the relationship was very small (Figure 61). A significant relationship was found between Z-scores for age of sexual maturation and isolation, indicating that earlier reproduction within the phylogeny was associated with increased isolation (Figure 62). A similar negative relationship was found between Z-scores of longevity and isolation ratio, indicating that life-history changes that lead to a decreased lifespan within the phylogenetic group are associated with increased isolation (Figure 63). A significant positive relationship was found between Z-scores of fecundity and isolation ratio, indicating that increased fecundity within phylogenetic groupings are correlated with increased isolation (Figure 64). A significant negative relationship was found between Z-scores of breeding season length and isolation ratio, indicating that decreased breeding season within phylogenetic groups are associated with increased isolation (Figure 65).

Stepwise, backwards model selection using AIC values lead to a final model with three retained variables: ULO, Z-scores for age of maturation, and Z-scores for breeding season length, indicating that these three variables had the best predictive power for isolation ratios in a multiple linear regression framework (Figure 66). Among these variables, ULO and Z-scores for age of maturation had very similar, large effect sizes in

the model, indicating that they had the most influence, and Z-scores for breeding season length had a substantial but lower effect size and influence in the model than the other variables. From structuring of variables by the model, *E. parvipinne* had the highest isolation ratios, followed by *E. claviformis,* followed by *E. artesiae* and *E. whipplei*, followed by *S. atromaculatus* and *F. olivaceus*.

Discussion

Patterns of occupancy and headwater specialization through the ULO values across these species perfectly matched patterns of isolation by distance, isolation by resistance by large streams in the network, and assigned models of gene flow for these species (Chapter III). *Fundulus olivaceus* was assigned the Isolation by Distance Model of gene flow with no effects of large rivers on genetic structure (Chapter III, Wright 1943; Slatkin 1993; Hughes et al. 2009). They also had the highest ULO values and lowest restriction to headwaters, and therefore tended to occupy larger streams in the network as well as headwaters. Based on these results, this species could be classified as a tributary generalist that also has a relatively high frequency of occurrence in headwaters (≈45%). *Erimyzon claviformis* was assigned the Stream Hierarchy Model of gene flow and showed significant isolation by resistance from large rivers, but isolation by distance had a stronger influence on genetic distance (Chapter III, Meffe & Vrijenhoek 1998; Hughes et al. 2009). *Erimyzon claviformis* had a high restriction to headwaters (≈85%) and the second highest ULO values behind *F. olivaceus*. Therefore, populations may be genetically structured in headwaters, but they have some tolerance to and occupancy in medium to large size stream habitats in the watershed, and those streams are not as strong of a barrier to gene flow as compared with species in the study with lower upper limits of

occurrence. Both *S. atromaculatus* and the redfin darter group were assigned a Stream Hierarchy Model of gene flow with large streams being barriers to dispersal, increasing genetic distance (Chapter III, Meffe & Vrijenhoek 1998; Hughes et al. 2009). These species had lower upper limits of dispersal than *E. claviformis*, further supporting that large rivers are barriers to gene flow through unsuitable habitat conditions that preclude occupancy. Although they had a similar response in upper limits of occurrence, they had different patterns of headwater restriction, with *S. atromaculatus* (≈85%) being more restricted than the redfin darter group (≈55%), and therefore the redfin darter group could be categorized as a headwater and small stream specialist instead of a true headwater specialist. *Etheostoma parvipinne* was assigned the Death Valley Model of gene flow with isolated populations that had no significant relationships with distance or large rivers (Chapter III, Meffe & Vrijenhoek 1998; Hughes et al. 2009). They also had very small ULO values and a high rate of headwater restriction (≈80%), further corroborating a pattern of isolation in headwaters and non-occupancy in medium to large streams in the drainage. The three species with headwater restrictions above 80% (*S. atromaculatus, E. claviformis*, and *E. parvipinne*) could be categorized as true headwater specialists, but they differ in the range of sizes of streams of occasional occurrence and therefore the degree of headwater specialization.

The hypothesis that habitat specialization and life history traits would be associated with isolation rates for the study species was supported. The prediction that increased headwater specialization across species would increase within drainage isolation rates of sites was also supported by the data. These results were consistent with other studies that found increased genetic distance and population structuring with

increasing rates of specialization (Tibbets & Dowling 1996; Whiteley et al. 2004; Turner & Robison 2006; Fluker et al. 2014). The prediction that opportunistic strategy adapted traits would be associated with increased isolation was partly supported by the various life history regressions, depending upon the life history trait used. Both age of maturation and longevity followed predictions, with opportunistic strategy traits (low for both) being associated with increased isolation. These traits indicate adaptation for highly variable systems, and a possible link with genetic isolation could include a lower population size in these systems due to increased variability. Body size also followed predictions (smaller body size for opportunistic strategy), although the relationship was very weak. These opportunistic strategy traits (smaller body size and earlier reproduction) may be an adaptive response to fragmented populations. An intraspecific study of brook trout found that fragmented populations in isolated streams had local adaptations for smaller body size and earlier reproduction, and these adaptations can shift the demographics of the population younger, allowing for increased reproductive output, and possibly reduce local extirpation risk through high recruitment (Letcher et al. 2007). Relationships between fecundity and breeding season length with isolation did not conform to predictions from the opportunistic strategy (low fecundity leading to higher isolation and no relationship with isolation for breeding season length). Instead, increased isolation was associated with traits seemingly adapted for the periodic strategy, with increased fecundity and decreased breeding season being associated with increased isolation.

The best predictive model had increased headwater specialization, one opportunistic strategy trait (decreased age of maturation), and one periodic strategy trait (decreased breeding season length) associated with an increase in genetic isolation of

sites within a drainage. This mixed effect from different reproductive strategies may be common across freshwater fish, as most species are unlikely to align perfectly with all of the reproductive strategy predictions, and from examining Z-score traits, none of these species aligned perfectly with the opportunistic strategy. Other studies have observed this as well, with some species occupying an intermediate space along the theoretical triangle of reproductive strategies (Mims & Olden 2012). The specific traits indicative of increased isolation among these species indicate adaptations for habitats that are highly variable in flow regime, but also have some predictable flows associated with spawning. A possible scenario that could represent these seemingly disparate patterns is found in streams that are subject to drought conditions. If there is high variability year to year in drought severity, then selection might favor earlier onset of reproduction, because populations with a later onset would not be able to recover as quickly after droughts. At the same time if in the same streams there were predictable higher flows most years during certain months, selection might favor intense reproduction timed with those flows. All of the species in this study that had > 80 % restriction in headwaters from museum localities also had negative Z-score values for breeding season length and negative Z-score values for age at maturation, which would fit hypothetical predictions related to drought-prone streams with seasonal flows outlined above.

This study shows how the ecology of species, specifically habitat specialization in headwaters, an earlier age of maturation, and a reduced breeding season, promotes natural isolation in watersheds for multiple fish species. In doing so, it adds to a growing pattern in the literature of increased genetic isolation with increased habitat specialization in dendritic stream environments. It also shows increased genetic isolation related to

some opportunistic strategy life history traits and some periodic strategy life history traits, indicating mixed effects on gene flow from associated sets of life history traits. Additionally, many population genetic studies are done on threatened or imperiled species, often with populations in headwaters, where multiple natural and anthropogenic factors have already caused lowered population sizes and isolation prior to the study (Johnson 2009; Slack et al. 2010; Fluker et al. 2014; Pilger et al. 2015; Sterling et al. 2015). By using common species headwater species such as in this study, a background pattern of isolation for headwater species can be established for comparison to more threatened taxa (Whiteley et al. 2006). Results from this multi-species approach can attribute a process (headwater specialization or adaptation to variable habitat with seasonal flows) that promotes natural fragmentation and isolation, and this context may help understand historical processes that helped promote isolation before the addition of other stressors for threatened small stream fishes.

Table 38

Upper limit of occurrence values and percentage of occurrences in headwaters for the study species

Species or Group	Upper Limit of Occurrence (km^2)	Headwater (0-50 km^2) Percentage
E. parvipinne	370	0.8
S. atromaculatus	1950	0.87
redfin darter group	2638	0.56
E. claviformis	3434	0.86
F. olivaceus	6986	0.44

upper limit of occurrence = mean total cumulative drainage area of occurrences from museum records + 2 standard deviations; *E. artesiae* and *E. whipplei* were analyzed together as the redfin darter group.

Table 39

Life history trait summary of the study species

Species	Total Length	Age at Maturation	Longevity	Fecundity	Season Length
F. olivaceus	9.7	1	3	239	6.5
S. atromaculatus	30	2	5	7157	1.5
E. claviformis	36	2	5.5	83013	2.25
*E. artesiae**	9	1	3	400	3
*E. whipplei**	9	1	3	400	3
E. parvipinne	7.5	1	NA	400	1.5

Values came for the FishTraits Database (Frimpong & Angermeier 2009). Total length = cm. Age at Maturation and Longevity = years. Fecundity = maximum reported. Season length = number of months for reproductive season. * traits in the database were combined for these species. NA = not available.

Table 40

Life history trait Z-scores within respective phylogenetic groups

Species	Total Length	Age at Maturation	Longevity	Fecundity	Season Length
F. olivaceus	-0.15	-0.17	-0.26	-0.36	1.6
S. atromaculatus	0.31	-0.22	-0.42	-0.29	-0.64
E. claviformis	-0.72	-1.2	-0.73	0.59	-0.56
*E. artesiae**	0.78	-0.51	-0.09	0.02	0.12
*E. whipplei**	0.78	-0.51	-0.09	0.02	0.12
E. parvipinne	0.04	-0.51	NA	0.02	-0.65

Raw values for phylogenetic groups came from the FishTraits database (Frimpong & Angermeier 2009). Z-score values indicate the number of standard deviations from the group average. Negative values indicate traits that are lower than average and positive values indicate traits that are higher than average in the respective phlyogenetic groups. * traits in the database were combined for these species. NA = not available.

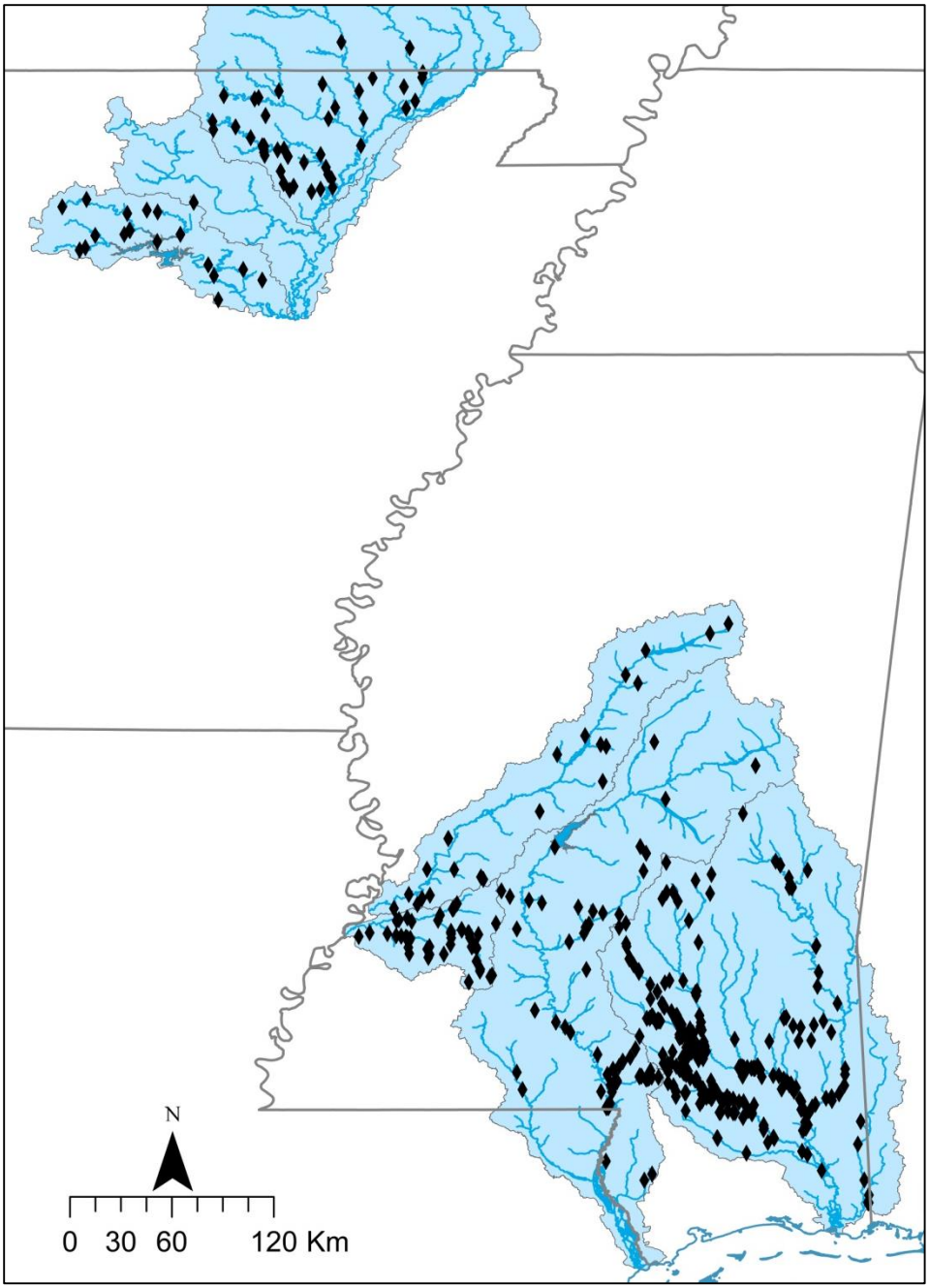

Figure 55. Museum collection localities for *F. olivaceus* in the study drainages.

(n = 1018).

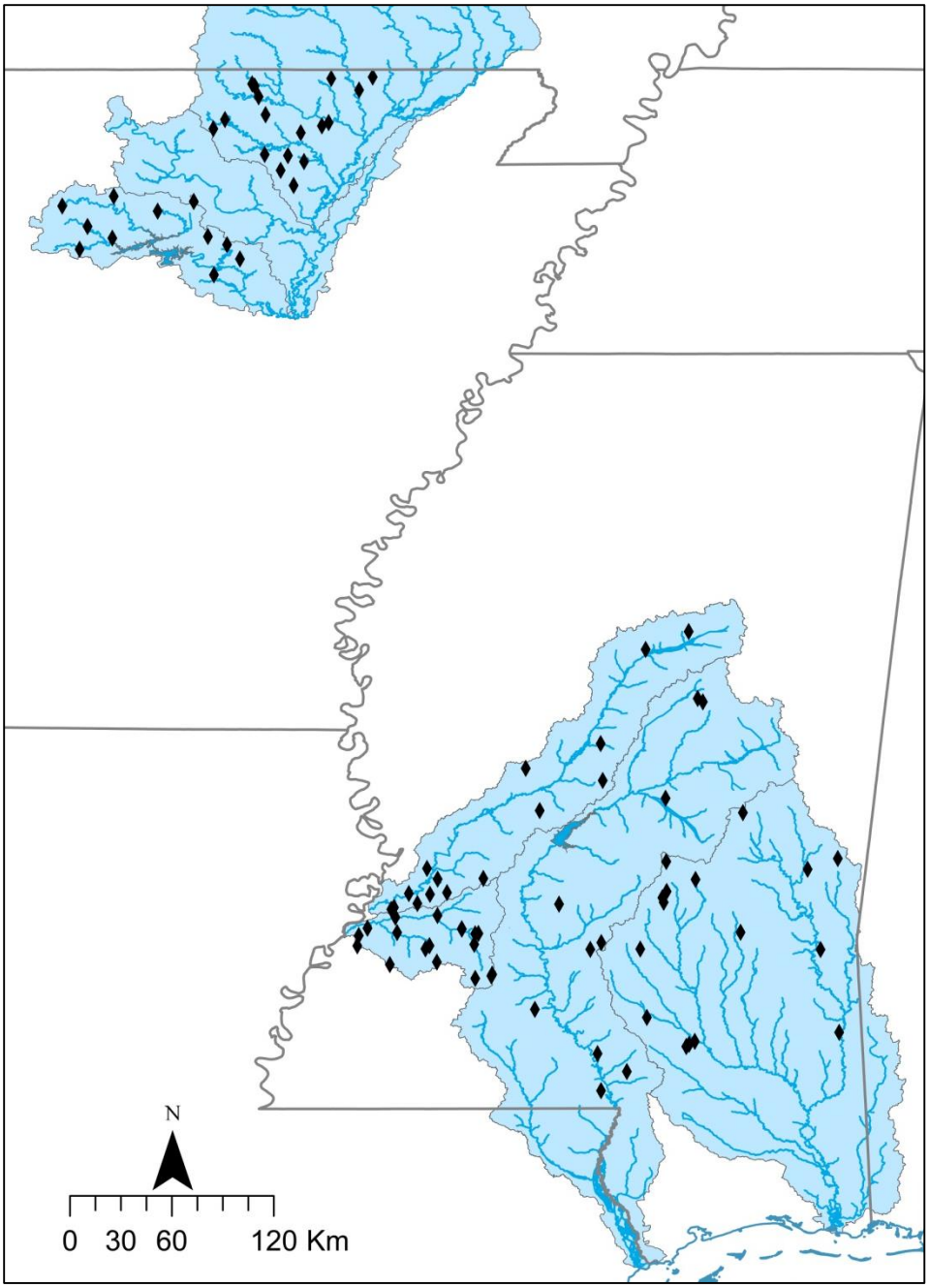

Figure 56. Museum collection localities for *S. atromaculatus* in the study drainages.

(n = 99)

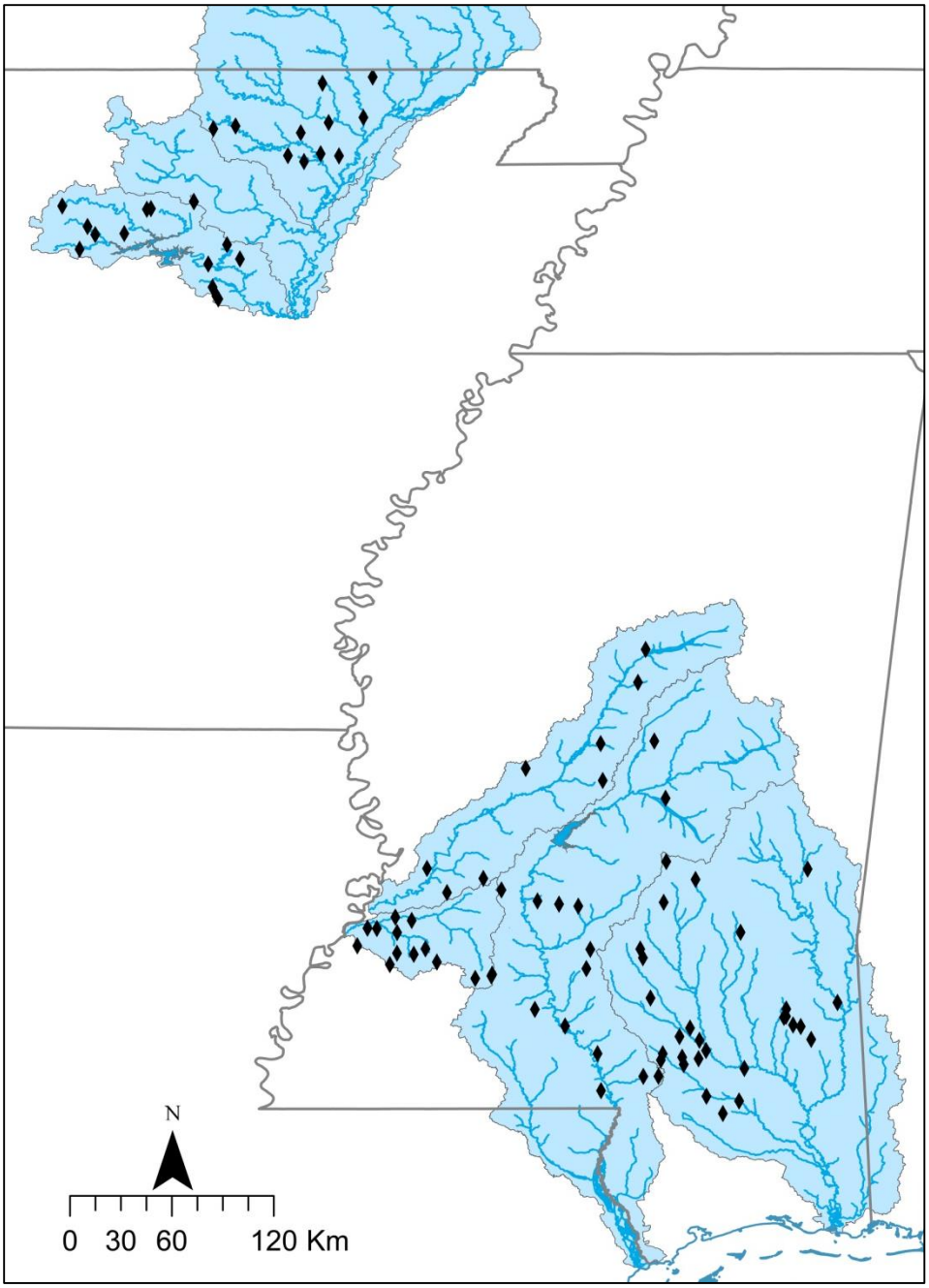

Figure 57. Museum collection localities for *E. claviformis* in the study drainages.

(n = 110).

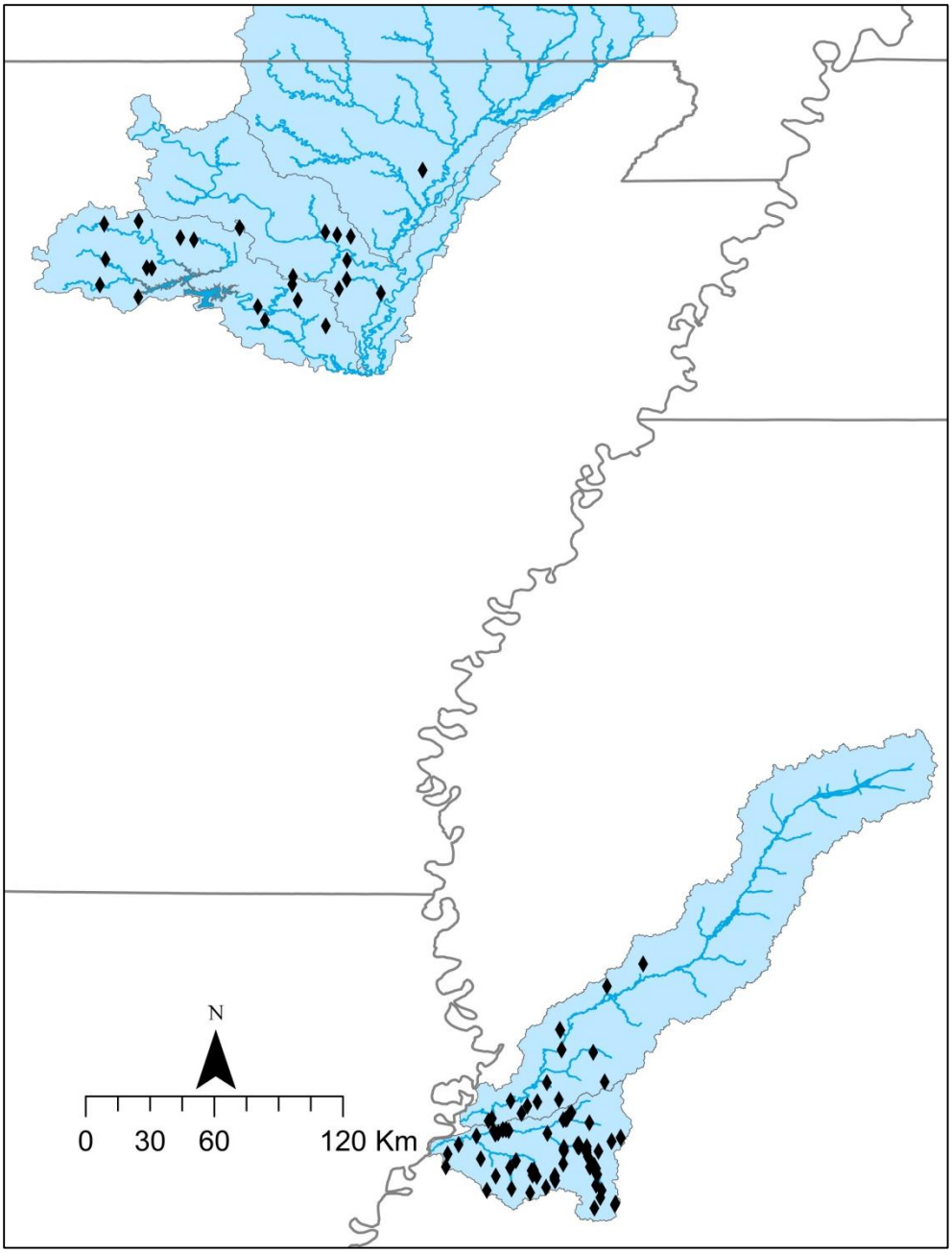

Figure 58. Museum collection localities for the redfin darter group, *E. artesiae* (MS) and *E. whipplei* (AR), in the study drainages.

(n = 167)

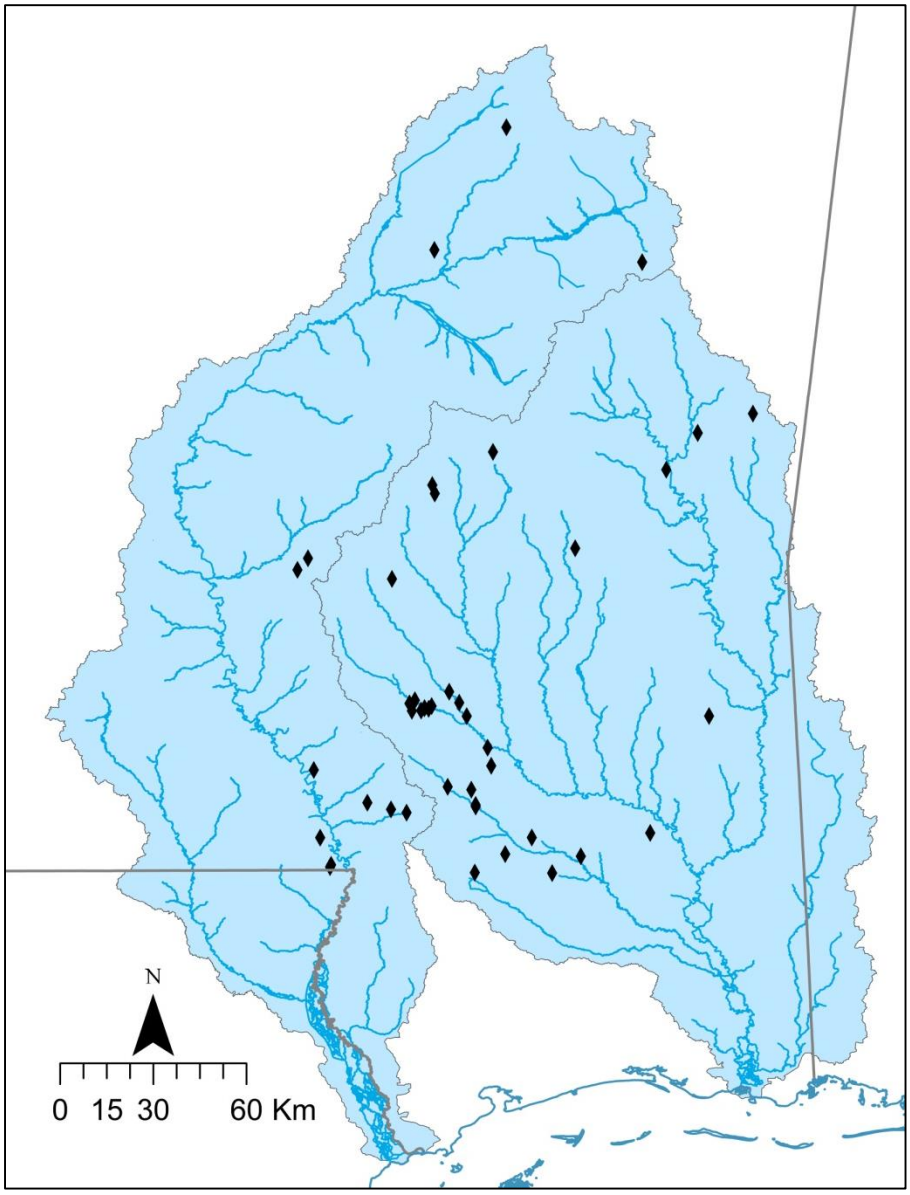

Figure 59. Museum collection localities for *E. parvipinne* in the study drainages.

(n = 53).

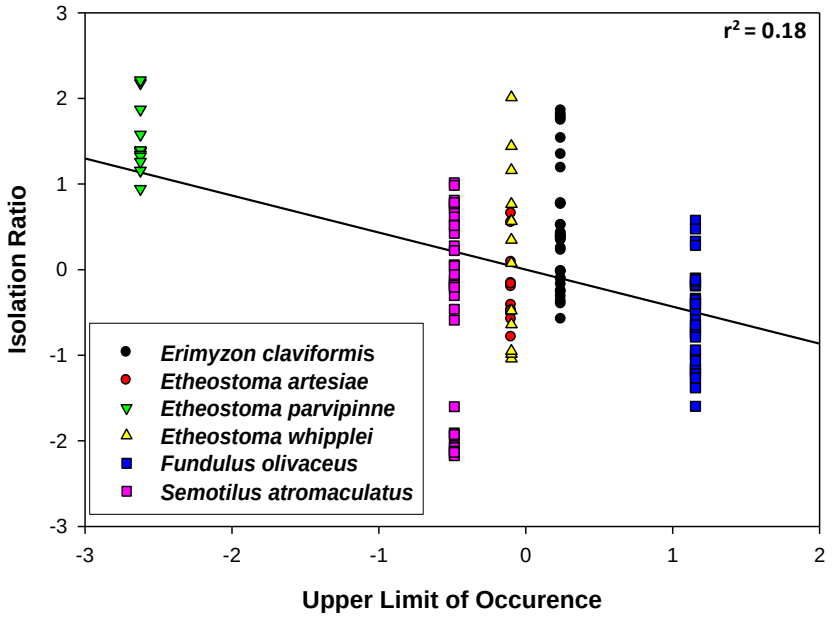

Figure 60. Linear regression between upper limit of occurrence and isolation ratio.

($p < 0.00001$, coef = -0.433).

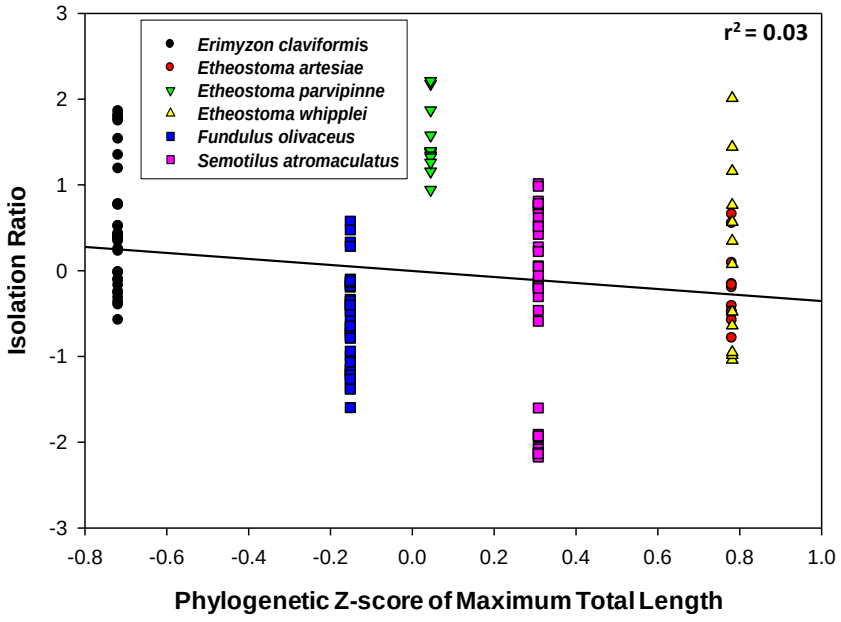

Figure 61. Linear regression between maximum total length Z-score and isolation ratio.

($p = 0.028$, coef = -0.351).

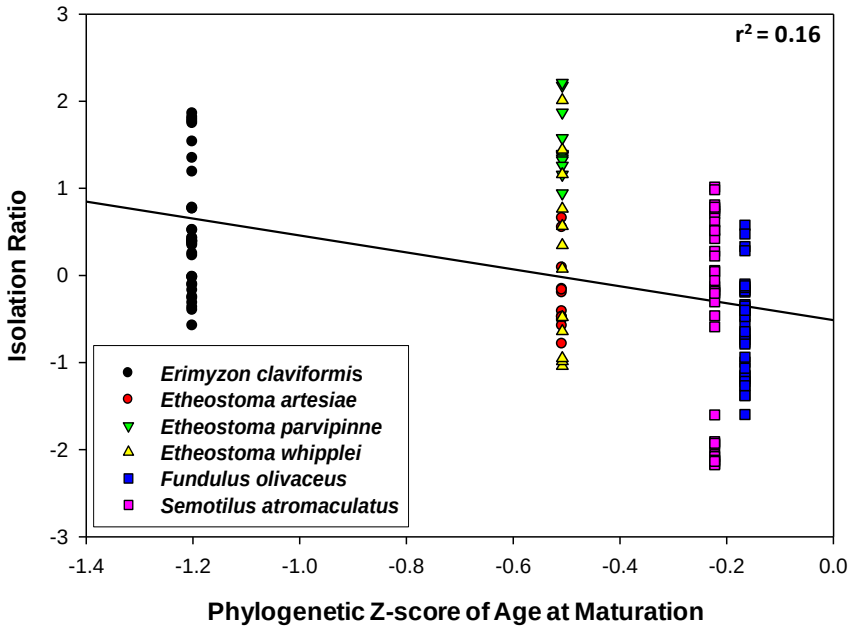

Figure 62. Linear regression between age of maturation Z-score and isolation ratio.

($p < 0.00001$, coef = -0.972).

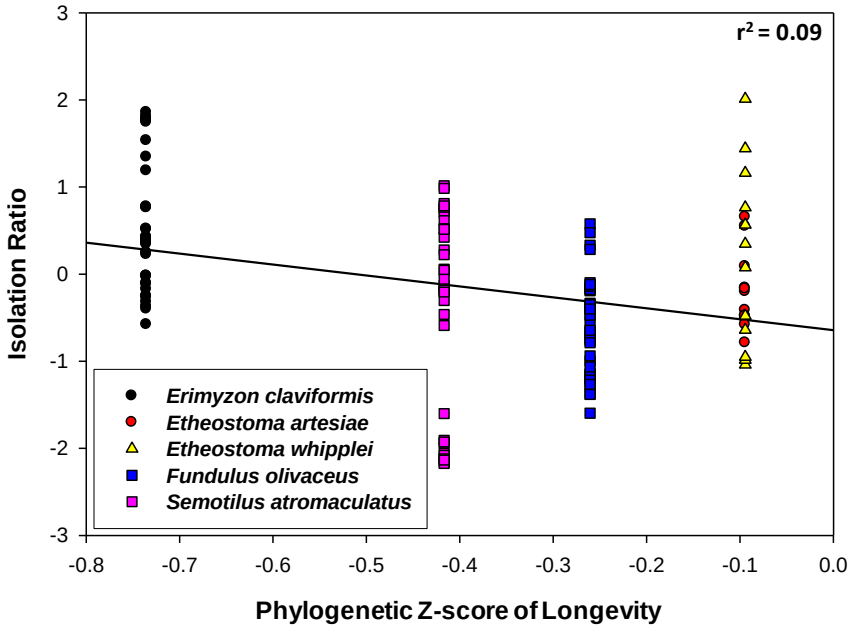

Figure 63. Linear regression between longevity Z-score and isolation ratio.

($p < 0.00001$, coef = -1.254). Longevity data was not available for *E. parvipinne*, and they were not included in this regression).

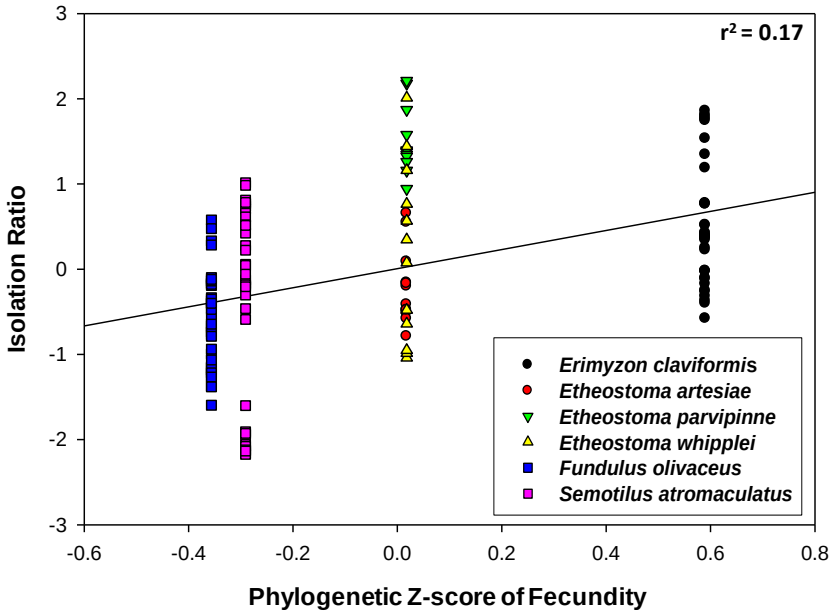

Figure 64. Linear regression between maximum reported fecundity Z-score and isolation ratio.

($p < 0.00001$, coef = 1.121).

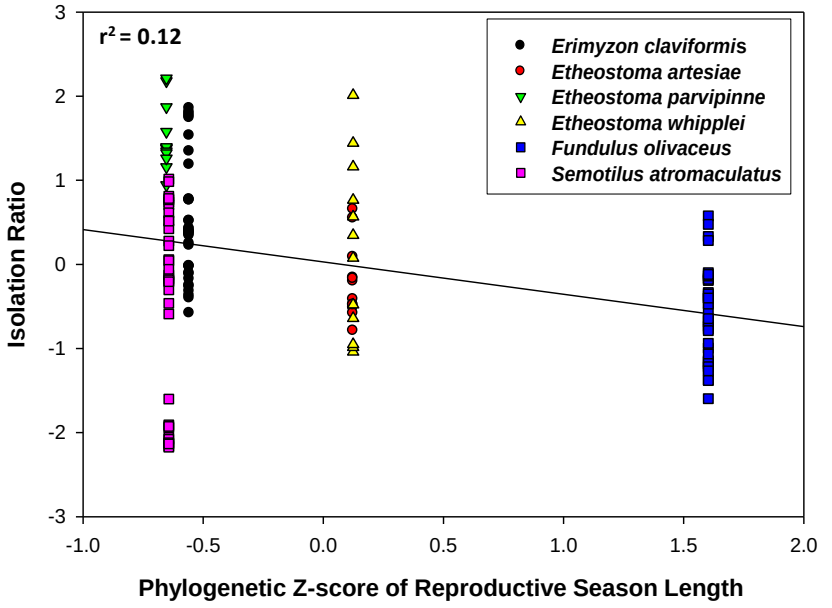

Figure 65. Linear regression between length of reproductive season Z-score and isolation ratio.

($p < 0.00001$, coef = -0.384).

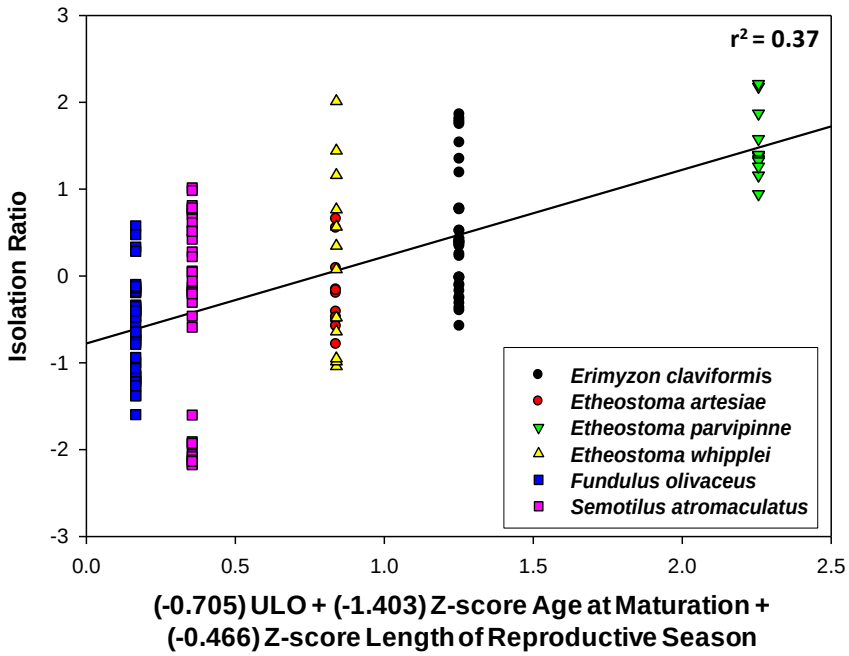

Figure 66. Multiple linear regression for the study species.

upper limit of occurrence: $p < 0.00001$, coef = -0.705, partial $eta^2 = 0.24$); age of maturation Z-scores: $p < 0.00001$, coef = -1.403, partial $eta^2 = 0.23$; length of reproductive season Z-scores: $p < 0.00001$, coef = -0.466, partial $eta^2 = 0.06$.

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CHAPTER V – THE INFLUENCE OF BASIN SCALE CHARACTERISTICS ON THE GENETIC STRUCTURE OF HEADWATER FISHES

Introduction

A major goal of landscape genetics studies has been to identify features in a landscape that influence population genetic patterns through the restriction or facilitation of gene flow (Manel et al. 2003). Recently, landscape genetics studies have focused on multiple spatial scale patterns, and found that different landscape effects can influence gene flow at different spatial scales (Mitsui et al. 2010; Rasic & Keyghobadi 2012; Manel & Holderegger 2013). Recent concepts of riverine ecology have also emphasized the importance of spatial scales and the connections between them, leading to a holistic view of river systems as dendritic ecological networks with connections across multiple scales influencing patterns that affect aquatic organisms that reside in them (Fausch et al. 2002; Benda et al. 2004; Lowe et al. 2006a; Grant et al. 2007). An ecologically important component for dendritic networks is patterns of water flow (magnitude, frequency, timing, duration, and variability of flow events), and these patterns are driven by climactic (precipitation) processes and regional geologic and environmental (soil type, topography, vegetation, and land use) processes (Poff et al. 1997). Many factors that regulate population demographics such as habitat availability, growth, mortality, and dispersal distance and frequency are in turn regulated and influenced by flow dynamics of the system (Railsback et al. 2003; Anderson et al. 2006). Therefore regional differences that impact the flow regimes of drainages have the potential for causing differing effects on population dynamics and gene flow for aquatic species in those systems.

Specific characteristics that influence the flow regime of a basin include drainage size, drainage shape, and drainage density. Drainage basin size influences the overall size of flows in downstream sections of the network, and size and shape interact to influence hydrograph behavior following precipitation events (Strahler 1964; Fisher et al. 2007). For narrow, trellis shaped drainages (elongate drainages with little branching in tributaries (Figure 67)), runoff from precipitation events flow into the main stem at the same time at multiple points along the main stem, with the result being that storm discharge does not magnify in the main stem, leading to a lower peak discharge when compared with wider, heart or fan shaped drainages (Ward & Trimble 2004). In wider, heart shaped drainages (Figure 68), there is a lag time for storm discharge to enter the main stem as it flows through more complex dendritic branching, and this lag time can lead to synchronicity of timing for high flows at confluences, creating a much higher peak discharge in the main stem (Ward & Trimble 2004). Regional equations for estimating peak discharge are routinely developed for predictive purposes, and one developed for Arkansas using drainage size, elevation, and a drainage shape factor shows that 100 year peak flow events in trellis shaped drainages would only be around 1/3 of the volume of a comparable perfectly square drainage (Hodge & Tasker 1995; Ward & Trimble 2004). Drainage density is the measurement of the number of unique water bearing streams within the basin, and is influenced by landscape topography, susceptibility of the landscape to generate run-off following precipitation, and slope stability (resistance to erosion) in the region (Horton 1945; Tucker & Bras 1998). An increase in drainage density will also tend to increase peak flow volumes for the basin (Chorley & Morgan 1962). Large variations from normal to peak flow can influence fish

populations through indirect effects, such as habitat alterations, influences on nutrient cycling, or alteration of food availability, and direct effects, such as increased mortality of early life stages (Schlosser 1990). Peak flow events alter habitats in fluvial systems through greatly increased transport rates of sediment, substrate, and woody debris, and peak flows are known to alter and shape ecologically relevant habitats such as riffle-pool sequences and sandbars (Poff et al. 1997). Differences in population genetic patterns for the freshwater fish, *Neosilurus hyrtlii*, in two systems in Australia were attributed to differences in flow regime, with the more hydrologically variable system having lower genetic diversity (Huey et al. 2008).

Stream slope has been found across multiple taxa to influence population genetic structuring in river networks, possibly through increased barriers to upstream dispersal because of flow and gravity effects. Slope has been found to have an isolating effect in salamanders (Lowe et al. 2006b), frogs (Richards-Zawacki 2009), shrimp (Cook et al. 2007) and fish (Caldera and Bolnick 2008; Cook et al. 2011; Kanno et al. 2011). Slope along stream connections was found to increase genetic differentiation between populations of lake dwelling threespine sticklebacks, *Gasterosteus aculeatus*, within a Canadian watershed (Caldera & Bolnick 2008). In a study of the northern trout gudgeon, *Mogurnda mogurnda*, in Australia, slope was found to be the most important driver for genetic distance in the system (Cook et al. 2011). Genetic differentiation was also found to increase between populations of a headwater resident fish, brook trout, *Salvelinus fontinalis*, that were separated by streams with a high slope (Kanno et al. 2011).

Other studies linking drainage characteristics with population genetic patterns include effects of drainage density and geological differences. Increased complexity

(number of tributaries and hierarchical nesting) was found to increase genetic differentiation between populations of chum salmon, *Oncorhynchus keta*, in Alaska (Olsen et al. 2008). Genetic simulation models show that an increase in drainage density alone can increase genetic differentiation between populations up to seven times higher (Thomaz et al. 2016). A comparative approach was used in New Zealand across two drainages with differing underlying geologies, and found that the geological formation that promoted deep incision in the main stem with bedrock substrates promoted higher genetic differentiation between populations than the geological formation that had gravel substrates in the main stem for several species of galaxiid fishes, *Galaxias*, that use gravel microhabitats (Waters et al. 2015).

Another basin scale feature that could influence population genetic patterns in dendritic networks is the amount of available habitat for the species of interest. Models of dispersal show that as available habitat across a landscape decreases, dispersal rates also decrease, which would increase genetic differentiation between populations (Travis & Dytham 1999). The amount of available habitat has been shown to be a factor for the genetic structuring of populations in fragmented systems (Wood & Pullin 2002). Studies have also shown a link between available habitat and genetic variation for stream fishes, with decreasing available habitat resulting in a loss of genetic diversity (Heath et al. 2001; Whiteley et al. 2010).

Populations of different species of headwater fish have been shown to have a nested hierarchical population structure, with different regions producing different isolation and fragmentation patterns (Chapter I). The goal of this study was to investigate the effect of basin scale level characteristics on genetic differentiation of headwater

fishes. The specific hypothesis being tested was that these basin scale characteristics would be related to genetic distance between populations in the drainage. I predict that as available habitat across the basin decreases, genetic difference between populations will increase. I also predict that drainage basins with higher slopes will have increased population divergence. Additionally, basin scale parameters that promote variability in flows, including higher peak flows, such as increased basin size, wider basin shapes, and increased drainage density are predicted to increase genetic differences between populations.

Methods

Study areas, collection site locations, study species, DNA extraction, and microsatellite amplification and visualization were the same as reported in Chapter I.

Nei's unbiased genetic distance (D) was calculated for each pairwise population grouping using GenAlEx v. 6.5 (Nei 1978; Peakall & Smouse 2006). The unbiased form of Nei's D corrects for small sample sizes and was chosen as a distance metric because 15 individuals were used per site in the study. The average within drainage D was calculated for each sample site for all species-drainage pairings. To investigate effects across all species, all species data was pooled for analyses, and the average within drainage pairwise genetic distance at each sample location was used as the response variable.

Various basin characteristics were calculated using the NHD plus v. 2 dataset (Horizon systems) in ArcGIS. Drainage basin size was determined through main stem length values for all of the basins. Main stem length was calculated as the distance moving upstream, starting from the mouth of the drainage and consistently moving up the

larger tributary (determined by cumulative drainage area) at each confluence. Drainage slope patterns were estimated by using the average slope along the entire main stem, which was determined as a weighted average, with segment length between confluences being the weighting factor. Drainage density was calculated as the total length of all streams in the basin divided by the cumulative total drainage area at the mouth of the basin (Horton 1945). Basin Shape Factor (BSF) was used to measure shape characteristics and was calculated as the square of the length of the main stem divided by the total cumulative drainage area at the mouth of the drainage (Horton 1932; Jena & Tiwari 2006). Larger BSF values indicate more elongate, trellis shaped drainages, while smaller values indicate wider, heart, or pear shaped drainages.

A previous analysis used museum record localities to generate stream size preference distributions for these headwater species (Chapter IV). An upper limit of occurrence value was created that was the mean cumulative drainage area across all sampled localities in these drainages plus two standard deviations (equivalent to around 95% of occurrences below the drainage area of that value). This upper limit of occurrence value was used for each species-drainage pairing to determine a percent available habitat value, determined by the percentage of streams in the drainage that were below this upper limit of occurrence value. Percentage was determined linearly, equal to the total linear sum of streams in the drainage below the upper limit of occurrence drainage area value divided by the total linear sum of all streams in the drainage.

Linear regression analyses were performed between these basin characteristics and average within drainage genetic distance. Because there was a known Isolation by Distance (IBD) pattern present in the data (Chapter III), average pairwise river distance

was included in multiple linear regression analysis as a covariate with each independent variable to control for distance effects between sites. All variables were log (x+1) transformed and then standardized (converted into Z-scores) before analysis to allow direct comparisons of coefficient values from the regressions. If multiple variables were significant, they were combined in a full model with average river distance as a covariate, with order of variables dependent upon significance values. Retention of variables from the full model was then determined through a stepwise, backwards selection process based on Akaike Information Criteria (AIC) scores. A final multiple linear regression analysis was then performed on the remaining variables. All regression analyses were performed with permutations due to possible non-independence effects involving averages of pairwise data, and all analyses were performed in R (R Core Team 2013).

Results

There were three more elongate shaped drainages (Big Black, Little Red, and Pearl) with higher BSF values and four wider shaped drainages (Pascagoula, Black, Bayou Pierre, and Middle White) with lower BSF values (Table 41; Figure 69). The Pearl River had the longest main stem length and Bayou Pierre had the shortest, with other drainages grouping in the middle. There was a high drainage density grouping (Big Black, Bayou Pierre, Pascagoula, and Black) and a low drainage density grouping (Little Red, Middle White, and Pearl). The highest average main stem slopes were in Arkansas (Little Red and Black) and Bayou Pierre (which was the smallest main stem river), and the lowest main stem slopes were associated with the larger rivers (Pascagoula, Big Black, Pearl, and Middle White). Percent available habitat was species-drainage pairing specific and values ranged between 90 and 100 percent.

There was a significant pattern of IBD in the dataset, justifying the inclusion of average river distance as a covariate in the analyses of other variables (Figure 70). There was a significant negative relationship between percent available habitat for species-drainage pairings and average genetic distance after controlling for IBD, showing that as percent available habitat decreased genetic distance increased (Figure 71). There was a significant negative relationship between basin shape factors and average genetic distance after controlling for IBD, showing that as drainage shape factors decreased and drainages became wider genetic distance increased (Figure 72). There was no relationship between main stem length and average genetic distance after controlling for IBD (Figure 73) and no relationship between drainage density and average genetic distance after controlling for IBD (Figure 74). There was a weakly significant positive relationship between average main stem slope and average genetic distance after controlling for IBD, showing that increased main stem slope was associated with increased genetic distance (Figure 75).

The backwards, stepwise selection method resulted in three variables being retained in the final multiple linear regression model with average genetic distance: average river distance, percent available habitat, and BSF (average main stem slope was eliminated) (Figure 76). Basins were generally grouped by region in the model, with Bayou Pierre and Big Black having lower average genetic distance, Black, Little Red, and Middle White having intermediate genetic distance, and Pascagoula and Pearl having larger genetic distance. The Gulf Coastal Plain drainages (Pascagoula and Pearl) showed the greatest variability within the model either from species or site effects. Among the three independent variables, percent available habitat had the largest effect on average

genetic distance ($p < 0.001$, coef = -0.318, partial $eta^2 = 0.095$), followed by average river distance which had a slightly smaller effect ($p < 0.001$, coef = 0.302, partial $eta^2 = 0.090$), followed by BSF which was smaller in effect size than the other two variables, but still had influence on genetic distance in the model ($p = 0.009$, coef = -0.233, partial $eta^2 = 0.066$).

Discussion

The prediction that an increase in slope would also increase genetic distance for these headwater species was weakly supported in the dataset in that there was a marginally significant positive relationship. Although it was not a very strong significant pattern for this study, the effects of slope on increasing genetic differences among dendritic populations is well documented (Caldera & Bolnick 2008; Cook et al. 2011; Kanno et al. 2011). The predictions that an increase in main stem length and drainage density would increase genetic distance were not supported by the data. It is possible that the large variability in the dataset through pooling multiple species in the same analysis only allowed the strongest trends in the data to be significant, especially because of the high variability seen in the Pascagoula and Pearl drainages in average genetic distances across the different species. One complication that was not addressed in the study was that each species could have their own unique history of gene flow and timing of colonization for each drainage, which could produce extra variability in the dataset. Other comparative studies have found that different species had different historical effects in the same drainage, influencing the observed patterns of genetic differentiation (Husemann et al. 2012; Fluker et al. 2015). It is possible that the large variation in genetic distance patterns observed in the Gulf Coastal Plain drainages (Pascagoula and Pearl)

may in part be due to different historical effects for the different species, however that line of analysis was outside of the scope of the study and a greater number of populations specifically from that area would likely be needed to assess historical effects independently for the four species sampled there.

The prediction that wider basin shapes would lead to increased genetic differentiation between sample sites was supported by the data. This pattern could possibly be influenced through the known connection between drainage shape and peak flow volumes. Larger peak flow volumes could disrupt populations through direct mortality, or larger shifts in habitat with increased rates of substrate transport (Schlosser 1990; Poff et al. 1997). Additionally, during large peak flows, the main stem can influence tributaries through rising water at the mouth of the tributaries, with high water backing up into the tributary over a range of meters to several kilometers (Beckmann et al. 2005). This process can change substrates of affected reaches in the tributary to become more similar to main stem substrates, and small fish have also shown to be protected from high flow effects of the main stems with increasing habitat complexity and distance upstream from the mouth of the tributary (Mérigoux & Ponton 1999; Beckmann et al. 2005). Any of these processes could influence population demographics or dispersal patterns for these headwater and tributary resident fishes, leading to the observed correlation between drainage shape and genetic distance.

The observed pattern between basin shape and genetic differentiation of populations could also be related to patterns of significant confluence effects in the network. Significant confluences (when two similar sized streams come together at a junction) have the greatest effects on creating and modifying different habitats within the

river network (Benda et al. 2004a). The number of significant confluences in a network is also directly related to the shape of the drainage, with wider drainages (low BSF) having a higher number of significant confluences throughout the whole network due to increased dendritic branching than elongate, narrow drainages (high BSF) (Benda et al. 2004a). Effects on the main stem from significant confluences that may be relevant to headwater fish dispersal are a wider channel, increased fine substrate, deeper pools, higher gradient, and a higher frequency of flow disturbance (Benda et al. 2004b). Predators also have been shown to increase in the main stem below tributary confluences, which could influence dispersal patterns (Kiffney et al. 2006). Another function of basin shape is that wider drainages will tend to have larger tributaries and larger main stems and therefore habitats in both of these branches may impact dispersal of headwater adapted fishes (Benda et al. 2004a).

The prediction that percent available habitat will negatively correlate with genetic distance between populations was supported, and available habitat had the largest influence on genetic distance in the combined model. Available habitat was based solely on stream size, which is likely an inaccurate measure of the actual available habitat for these species, as many of these species tend to be associated with certain microhabitats that vary across a spatial scale due to regional differences such as soil type and land use. However, available habitat from stream size is still significant and the strongest predictor of genetic distance in the models, indicating that it matches the broad patterns of habitat availability for the drainage. Species that prefer headwaters in river networks are confined to the upper branches of the dendritic ecological network, and when percent available habitat (small streams) decreases on a basin scale, it means that there is an

increased percentage of streams with drainage areas above the upper limit of occurrence in the network separating these upper branch systems (Grant et al. 2007). This reduction in network wide available habitat could decrease local population sizes due to decreased stream area, and could limit dispersal between available habitats across larger streams, and both processes would lead to increased genetic distance.

This study attempted to show that large basin scale parameters can influence local differences in genetic distance across a broad range of taxonomically diverse headwater fishes. Both percent available habitat within the network and basin shape had strong significant effects on genetic distance across all of the species. Multiple processes could be drivers for these observed patterns, and identifying specific processes may be difficult because of correlated effects, particularly for basin shape, which has effects on tributary and main stem size, the number of significant confluences, and peak flow volumes. Although the role of basin shape in structuring the physical habitat and hydrology of a river network is well known, this study marks a novel assessment of basin shape on the genetic structuring of populations of headwater species. This study also shows that local dynamics and genetic population connectivity for headwater fishes within drainage basins can be influenced by patterns across the whole drainage network.

Table 41

Basin scale characteristics for the study drainages

Drainage	Basin Shape Factor	Main Stem Length (km)	Drainage Density	Average Main Stem Slope
Big Black	7.59	438	1.22	0.0003
Little Red	3.99	284	0.91	0.0018
Pearl	3.81	746	0.65	0.0002
Pascagoula	2.84	414	1.04	0.0004
Black	2.67	472	1.04	0.0009
Bayou Pierre	2.4	153	1.19	0.0008
Middle White	1.64	331	0.8	0.0002

Black, Little Red, and Middle White are in Arkansas (White River), Bayou Pierre and Big Black are in Mississippi (Lower Mississippi River), and Pascagoula and Pearl are also in Mississippi (Gulf Coastal Plain). Higher basin shape factors indicate elongate and narrow drainages, while lower numbers indicate wide, heart shaped drainages. A higher drainage density value indicates more streams within the basin.

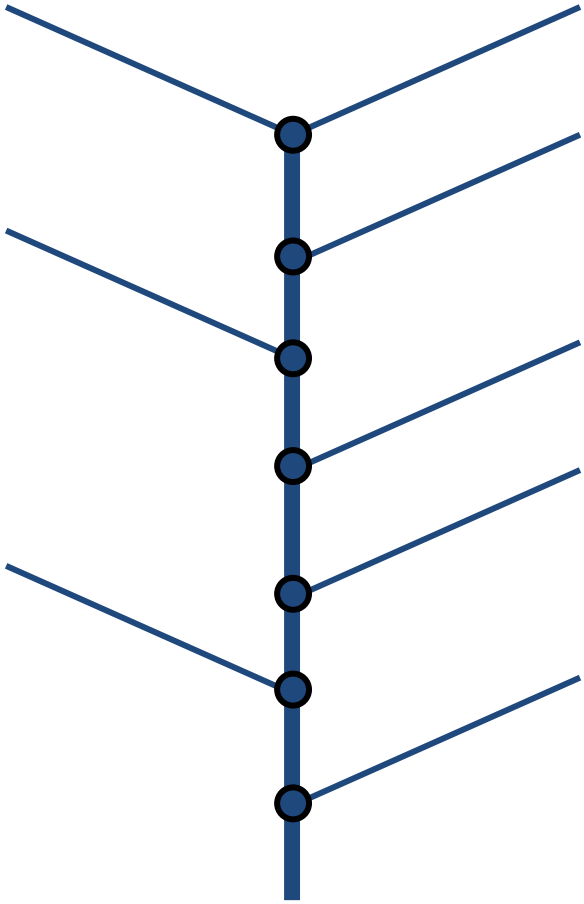

Figure 67. An elongate river network with seven confluences.

There is a low amount of dendritic branching within tributaries in trellis networks, leading to smaller tributary sizes and decreased peak flows.

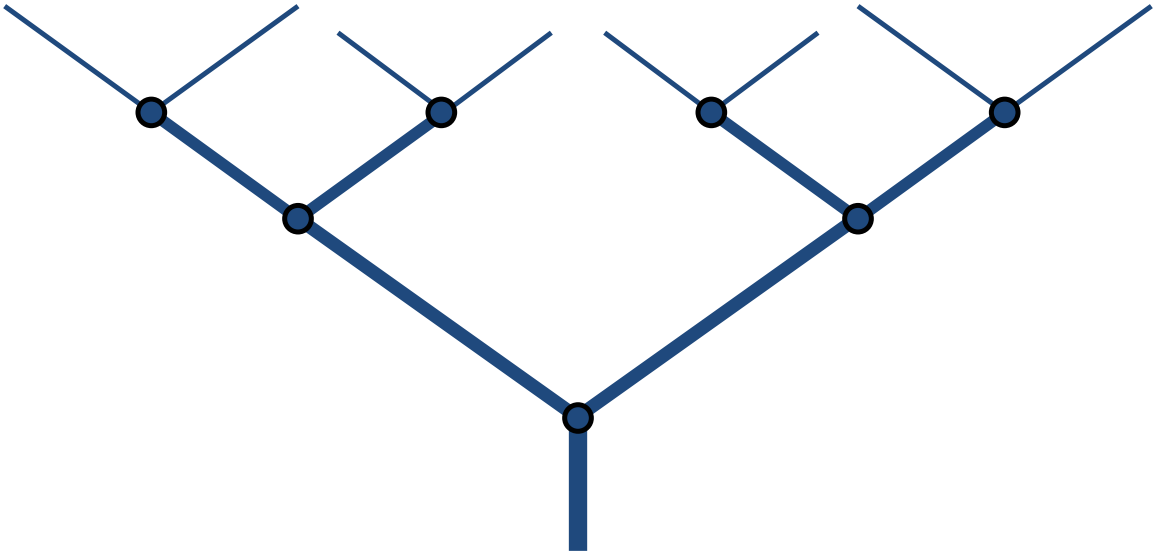

Figure 68. A wide, heart shaped river network with seven confluences.

There is a high amount of dendritic branching within tributaries in heart shaped networks, leading to larger tributary sizes and increased peak flows.

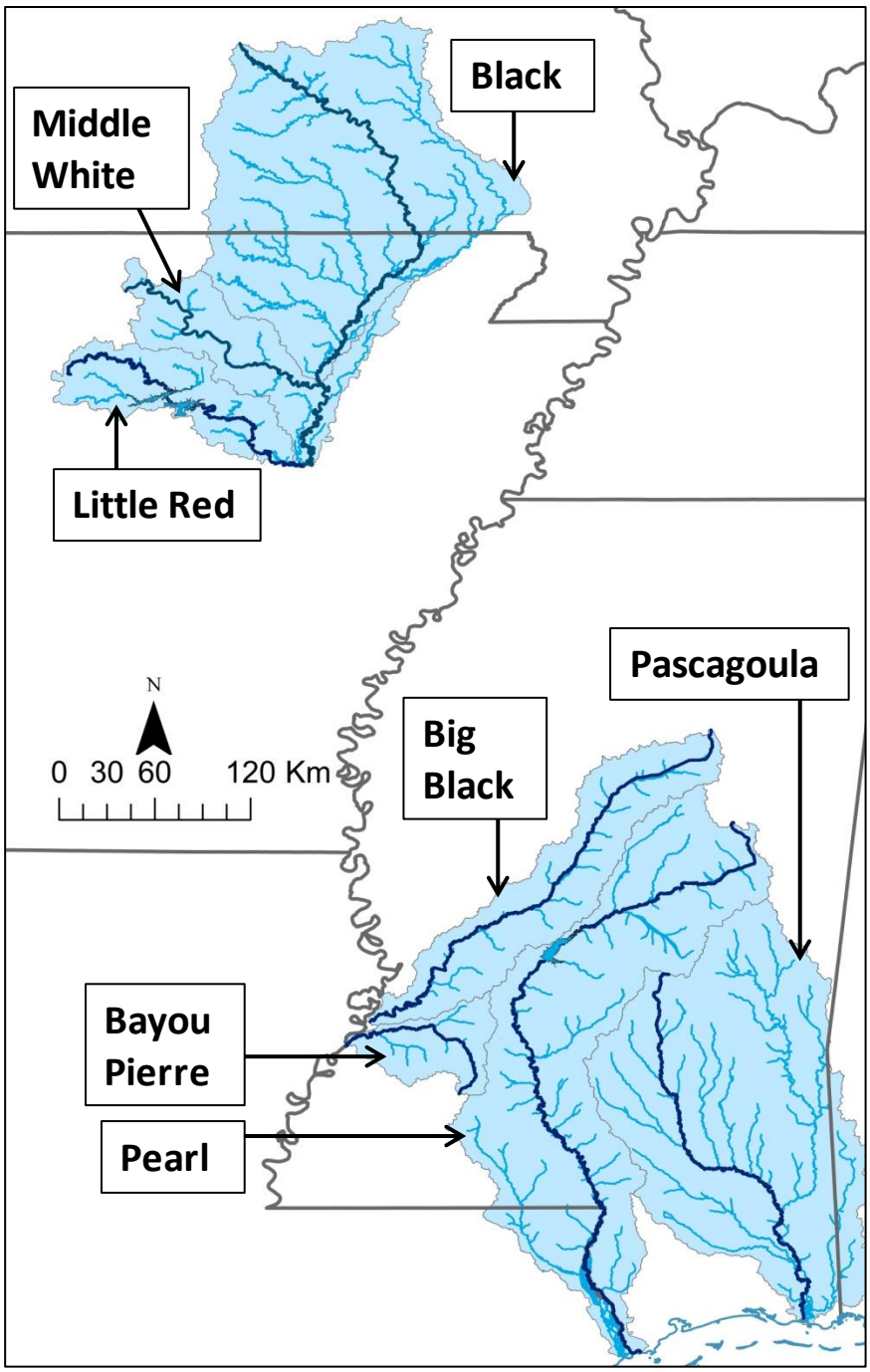

Figure 69. Map of the different study drainages with main stems (dark blue).

The Black River is included as a tributary within the Middle White drainage for analyses.

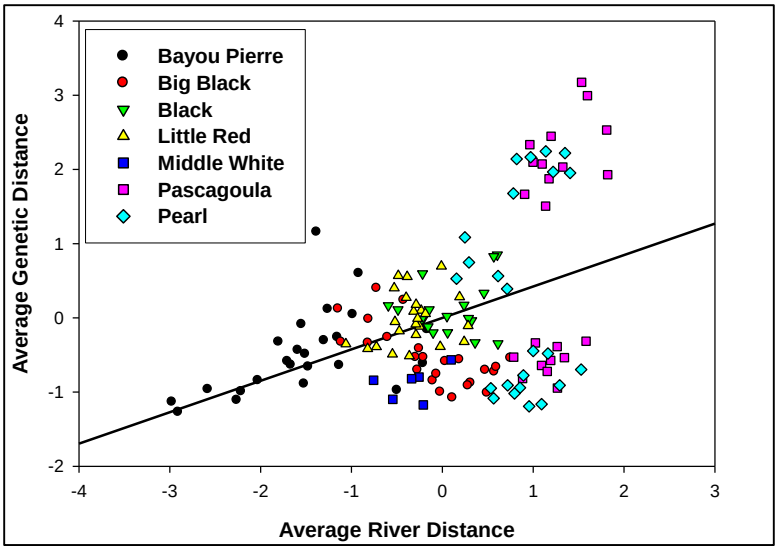

Figure 70. Linear regression between average river distance and average genetic distance between sites within drainages.

($r^2 = 0.17$, $p < 0.00001$, coef = 0.423).

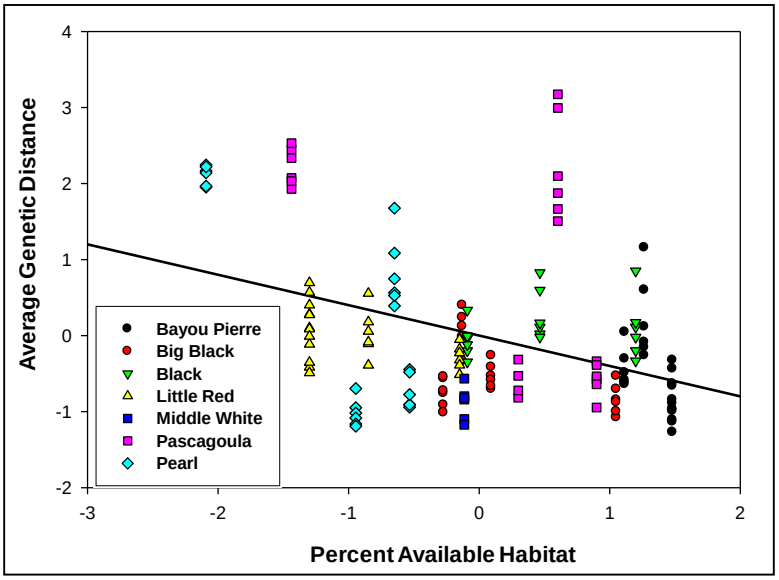

Figure 71. Linear relationship between percent available habitat and average genetic distance.

percent available habiat = percentage of streams below the upper limit of occurrence. When run with average river distance in a multiple linear regression, there was a significant result ($r^2 = 0.22$; covariate - average river distance: $p < 0.00001$, coef = 0.303, partial $eta^2 = 0.085$; percent available habitat: $p < 0.001$, coef = -0.259, partial $eta^2 = 0.064$).

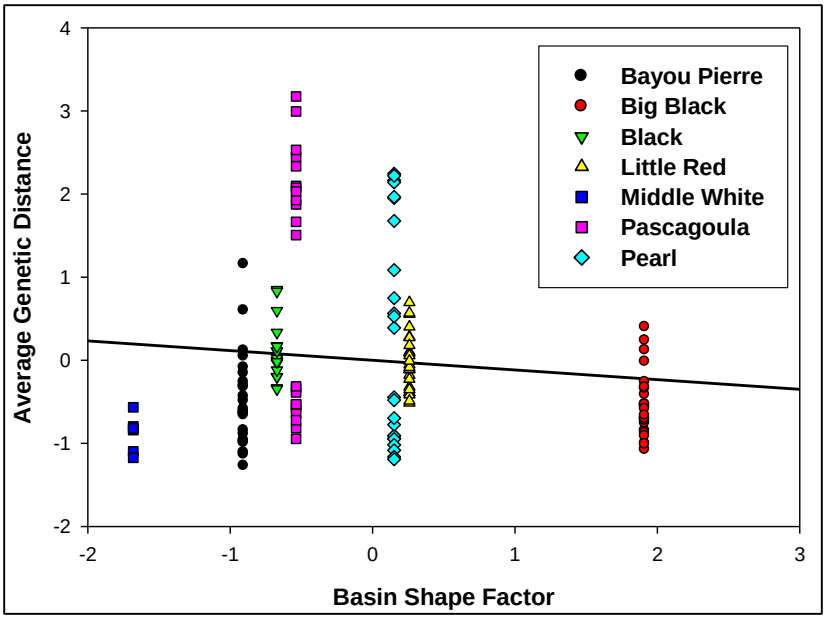

Figure 72. Linear relationship between basin shape factor and average genetic distance.

When run with average river distance in a multiple linear regression, there was a significant result ($r^2 = 0.2$; covariate - average river distance: $p < 0.00001$, coef = 0.443, partial $eta^2 = 0.196$; basin shape factor: $p < 0.026$, coef = -0.169, partial $eta^2 = 0.034$).

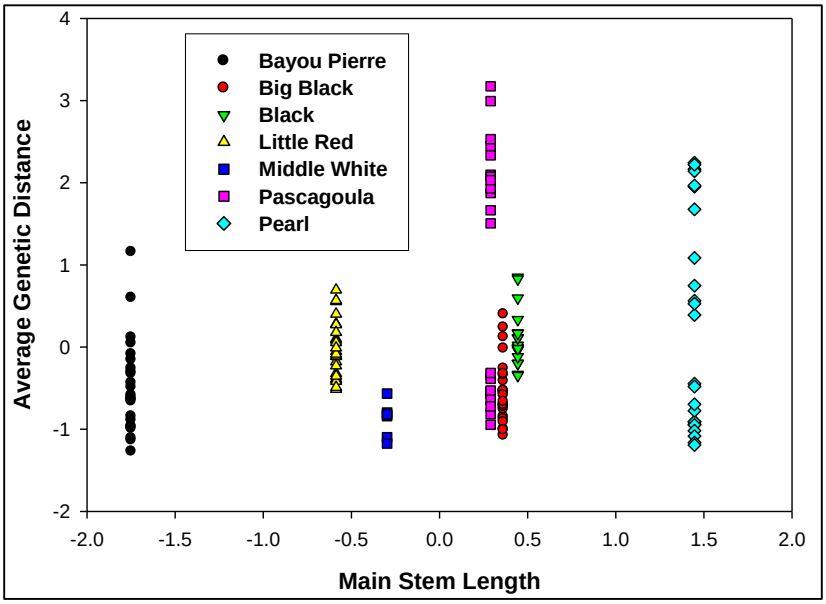

Figure 73. Linear relationship between main stem length and average genetic distance.

When run with average river distance in a multiple linear regression, there was not a significant result ($r^2 = 0.18$; covariate - average river distance: $p < 0.00001$, coef = 0.567; main stem length: $p < 0.225$, coef = -0.19).

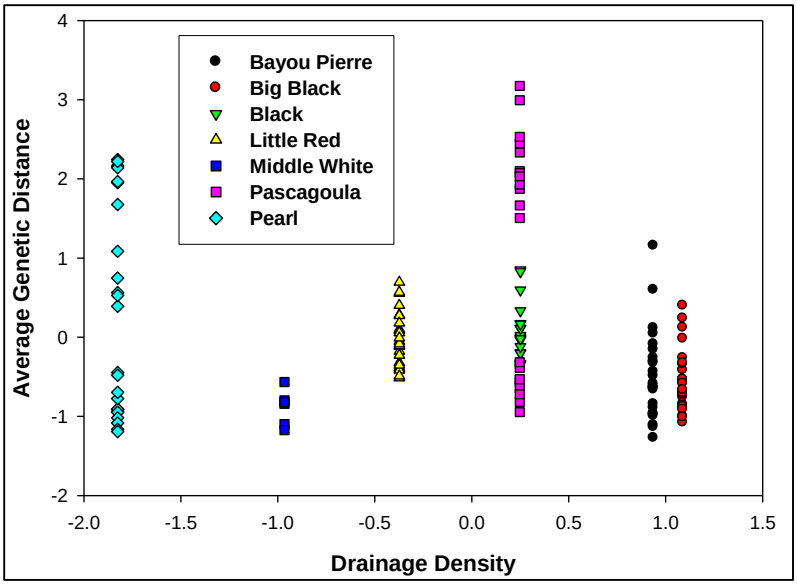

Figure 74. Linear relationship between drainage density and average genetic distance.

When run with average river distance in a multiple linear regression, there was not a significant result ($r^2 = 0.17$; covariate - average river distance: $p < 0.00001$, coef = 0.426 ; drainage density: $p < 0.823$, coef = 0.006).

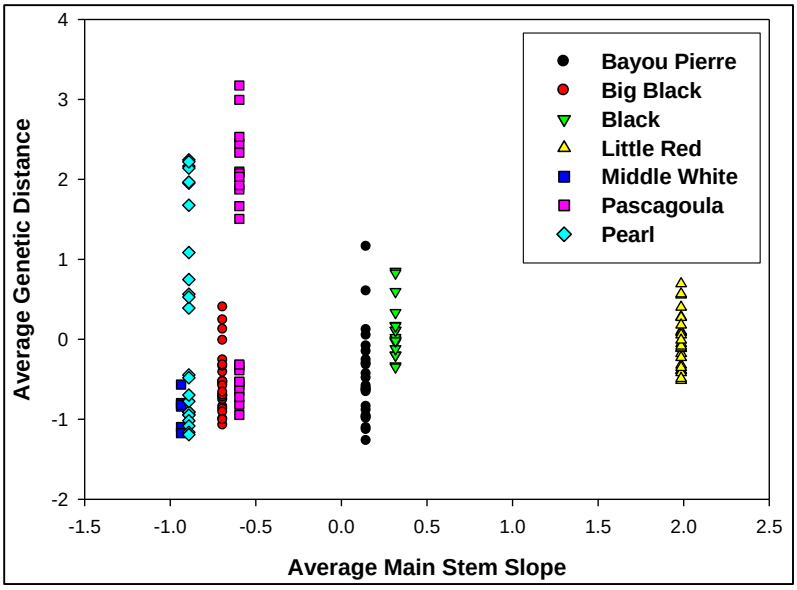

Figure 75. Linear relationship between average main stem slope and average genetic distance.

When run with average river distance in a multiple linear regression, there was a weak, significant result ($r^2 = 0.18$; covariate - average river distance: $p < 0.00001$, coef = 0.467, partial $eta^2 = 0.19$; average main stem slope: $p < 0.05$, coef = 0.12, partial $eta^2 = 0.015$).

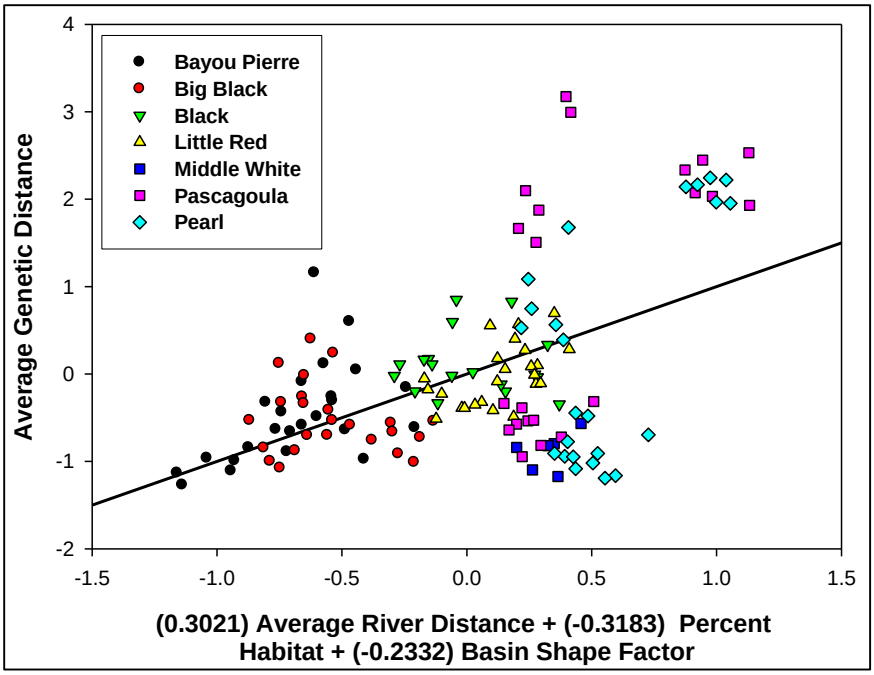

Figure 76. Multiple linear regression for the study drainages.

$r^2 = 0.267$; average river distance: $p < 0.00001$, coef = 0.302, partial $eta^2 = 0.090$; percent available habitat $p < 0.00001$, coef = -0.318, partial $eta^2 = 0.095$; basin shape factor: $p = 0.009$, coef = -0.233, partial $eta^2 = 0.066$).

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APPENDIX A – IACUC Approval Letter

THE UNIVERSITY OF SOUTHERN MISSISSIPPI

Institutional Animal Care and Use Committee

118 College Drive #5147
Hattiesburg, MS 39406-0001
Phone: 601.266.4063
Fax: 601.266.4377

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:	**13041102**
PROJECT TITLE:	**An experimental study of convergent evolution and species fusion in replicate Fundulus hybrid zones**
PROPOSED PROJECT DATES:	**April 2013 – September 2015**
PROJECT TYPE:	**New**
PRINCIPAL INVESTIGATOR(S):	**Jake Schaefer**
DEPARTMENT:	**Biological Sciences**
FUNDING AGENCY/SPONSOR:	**NSF-Pending**
IACUC COMMITTEE ACTION:	**Designated Member Review**
PROTOCOL EXPIRATON DATE:	**September 30, 2015**

Jodie M. Jawor, Ph.D.
IACUC Chair

4/23/2013
Date

APPENDIX B – Sample Localities

Table A1.

Sample localities for F. olivaceus

Site	Latitude	Longitude	Shared
BP1	31.80588	-90.74060	
BP2	31.89313	-90.84230	
BP3	31.86323	-90.73460	a
BP4	31.93203	-90.95740	
BP5	31.99662	-90.82882	b
BP6	31.93653	-91.05098	
BB1	32.57790	-90.15160	
BB2	32.73725	-89.81795	c
BB3	33.25620	-89.63120	d
BB4	33.43048	-89.59058	e
BB5	32.43413	-90.63540	
BB6	32.08645	-90.79875	f
PG1	31.39792	-89.40880	
PG2	31.48447	-88.85939	
PG3	32.17572	-88.83041	
PG4	32.26770	-88.73382	g
PG5	31.24202	-89.47285	
PG6	31.06637	-89.26833	
PR1	32.94574	-89.54503	h
PR2	32.82064	-89.00888	
PR3	31.09600	-89.82700	i
PR4	31.29202	-89.84538	j
PR5	31.52619	-90.17697	k
PR6	32.39247	-89.61933	
BL1	35.86520	-91.47443	
BL2	35.90117	-91.50655	
BL3	36.04397	-91.48343	l
BL4	36.18589	-91.87805	m
BL5	36.45953	-91.03497	n
BL6	36.42647	-91.30052	o

Table A1(continued).

Site	Latitude	Longitude	Shared
LR1	35.77700	-92.67767	p
LR2	35.63142	-92.34930	
LR3	35.75948	-92.22990	q
LR4	35.38945	-91.61933	
LR5	35.41295	-91.87480	r
LR6	35.28520	-91.85205	

Shared letters correspond to sites where more than one species was collected (sites also appear in sample localities for other species).

Table A2.

Sample localities for S. atromaculatus

Site	Latitude	Longitude	Shared
BP1	31.70308	-90.40698	s
BP2	31.77652	-90.69558	t
BP3	31.8625	-91.11607	u
BP4	32.01398	-90.91527	v
BP5	31.86323	-90.7346	a
BP6	31.76345	-90.9443	w
BB1	32.73725	-89.81795	c
BB2	32.93258	-89.82903	x
BB3	33.43048	-89.59058	e
BB4	33.52518	-89.36327	
BB5	32.08645	-90.79875	f
BB6	32.05528	-90.93677	
PG1	31.8429	-88.66542	
PG2	32.09261	-89.49531	y
PG3	31.84528	-89.61922	z
PG4	31.40454	-88.56712	
PG5	32.2677	-88.73382	g
PG6	31.93405	-89.08855	aa

Table 41 (continued).

Site	Latitude	Longitude	Shared
PR1	32.64319	-89.48528	bb
PR2	31.84370	-89.88368	cc
PR3	31.09600	-89.82700	i
PR4	32.08305	-90.04935	dd
PR5	33.15318	-89.28787	ee
PR6	33.17122	-89.31563	
BL1	36.04397	-91.48343	l
BL2	36.18589	-91.87805	m
BL3	36.46813	-91.03912	
BL4	36.45160	-91.25433	
BL5	36.42307	-91.67285	
BL6	36.16445	-91.41525	
LR1	35.77700	-92.67767	p
LR2	35.82868	-92.40498	ff
LR3	35.57222	-91.80577	
LR4	35.41295	-91.87480	r
LR5	35.60680	-92.41258	
LR6	35.61578	-91.90662	

Shared letters correspond to sites where more than one species was collected (sites also appear in sample localities for other species).

Table A3.

Sample localities for E. claviformis

Site	Latitude	Longitude	Shared
BP1	31.77652	-90.69558	t
BP2	31.81748	-90.81780	
BP3	31.99662	-90.82882	b
BP4	31.95415	-91.01310	
BP5	31.76345	-90.94430	w
BP6	31.68973	-90.49267	

Table 41 (continued).

Site	Latitude	Longitude	Shared
BB1	32.73725	-89.81795	c
BB2	32.93258	-89.82903	x
BB3	33.25620	-89.63120	d
BB4	33.43048	-89.59058	e
BB5	32.80152	-90.22557	
BB6	32.14417	-90.64180	gg
PG1	31.84528	-89.61922	z
PG2	31.27267	-89.39787	
PG3	31.25803	-89.51040	
PG4	31.56209	-88.57555	
PG5	31.48954	-88.84840	
PG6	32.26871	-88.73385	hh
PR1	32.64319	-89.48528	bb
PR2	31.84370	-89.88368	cc
PR3	32.94574	-89.54503	h
PR4	31.29202	-89.84538	j
PR5	31.52619	-90.17697	k
PR6	32.08305	-90.04935	dd
BL1	36.18589	-91.87805	m
BL2	36.45953	-91.03497	n
BL3	36.42647	-91.30052	o
BL4	36.21837	-91.26800	
BL5	36.17343	-91.41722	
BL6	36.04237	-91.21328	ii
LR1	35.77700	-92.67767	p
LR2	35.75948	-92.22990	q
LR3	35.80017	-91.98058	jj
LR4	35.46985	-91.90485	kk
LR5	35.30190	-91.86200	
LR6	35.34733	-91.88213	

Shared letters correspond to sites where more than one species was collected (sites also appear in sample localities for other species).

Table A4.

Sample localities for E. artesiae

Site	Latitude	Longitude	Shared
BP1	31.70308	-90.40698	s
BP2	31.75390	-90.76224	
BP3	31.86250	-91.11607	u
BP4	32.01398	-90.91527	v
BP5	31.76987	-90.84025	
BP6	31.82760	-90.75237	
BB1	32.71337	-90.28835	
BB2	32.61950	-90.43983	
BB3	32.34273	-90.49747	
BB4	32.14417	-90.64180	gg
BB5	32.08645	-90.79875	f
BB6	32.06513	-90.92279	

Shared letters correspond to sites where more than one species was collected (sites also appear in sample localities for other species).

Table A5.

Sample localities for E. parvipinne

Site	Latitude	Longitude	Shared
PG1	32.09261	-89.49531	y
PG2	31.84528	-89.61922	z
PG3	32.26871	-88.73385	hh
PG4	31.46340	-89.56164	
PG5	32.32425	-88.57397	
PG6	31.93405	-89.08855	aa
PR1	31.87142	-89.89279	
PR2	31.09600	-89.82700	i
PR3	31.29202	-89.84538	j
PR4	32.79817	-89.49642	
PR5	32.76267	-88.89478	
PR6	33.15318	-89.28787	ee

Shared letters correspond to sites where more than one species was collected (sites also appear in sample localities for other species).

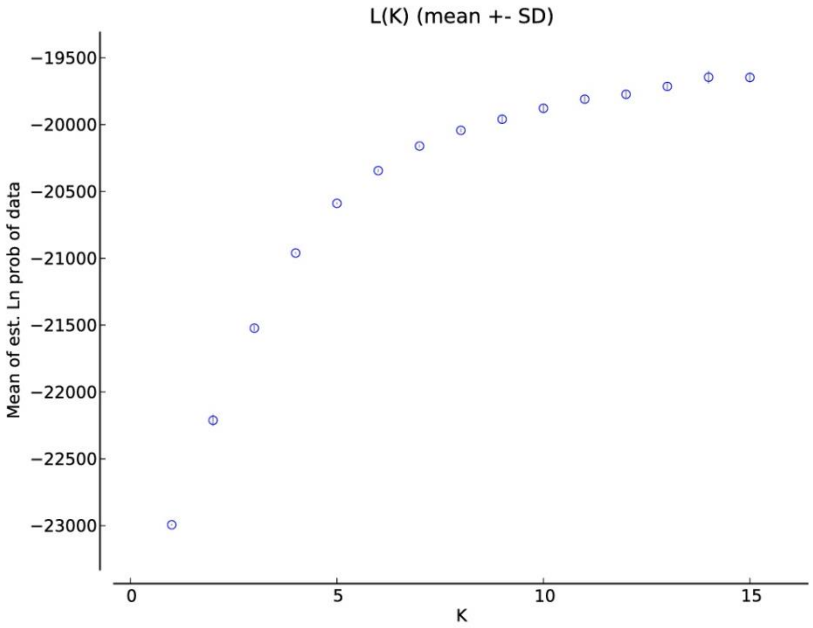
Table A6.

Sample localities for E. whipplei

Site	Latitude	Longitude	Shared
MW1	36.04237	-91.21328	ii
MW2	35.76433	-91.51462	
MW3	35.77133	-91.57168	
MW4	35.54443	-91.56430	
MW5	35.58398	-91.53147	
MW6	35.59670	-91.75720	
LR1	35.82868	-92.40498	ff
LR2	35.63260	-92.37105	
LR3	35.80017	-91.98058	jj
LR4	35.56358	-91.76057	
LR5	35.41295	-91.87480	r
LR6	35.46985	-91.90485	kk

Shared letters correspond to sites where more than one species was collected (sites also appear in sample localities for other species).

APPENDIX C – STRUCTURE Analyses K plots



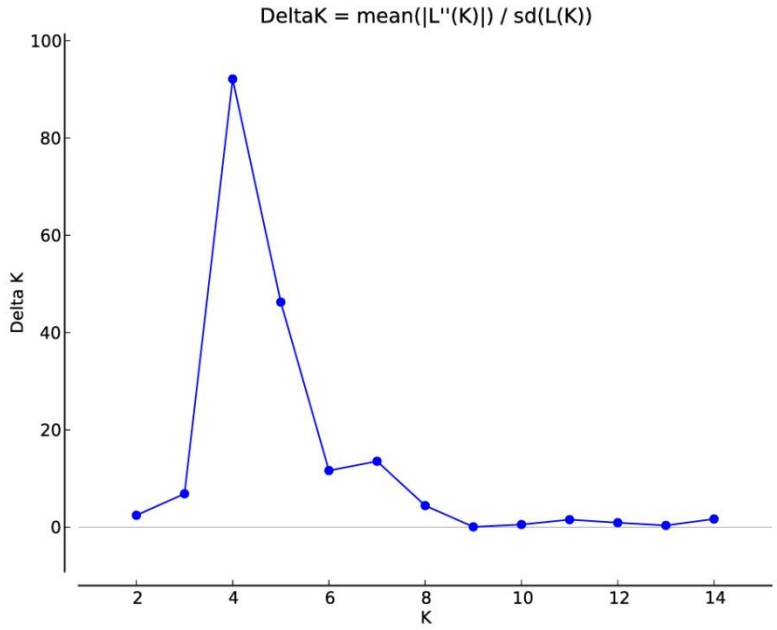


Figure A1. Mean likelihood and Delta K values for *F. olivaceus*: Full Dataset.

K of 4 was chosen.

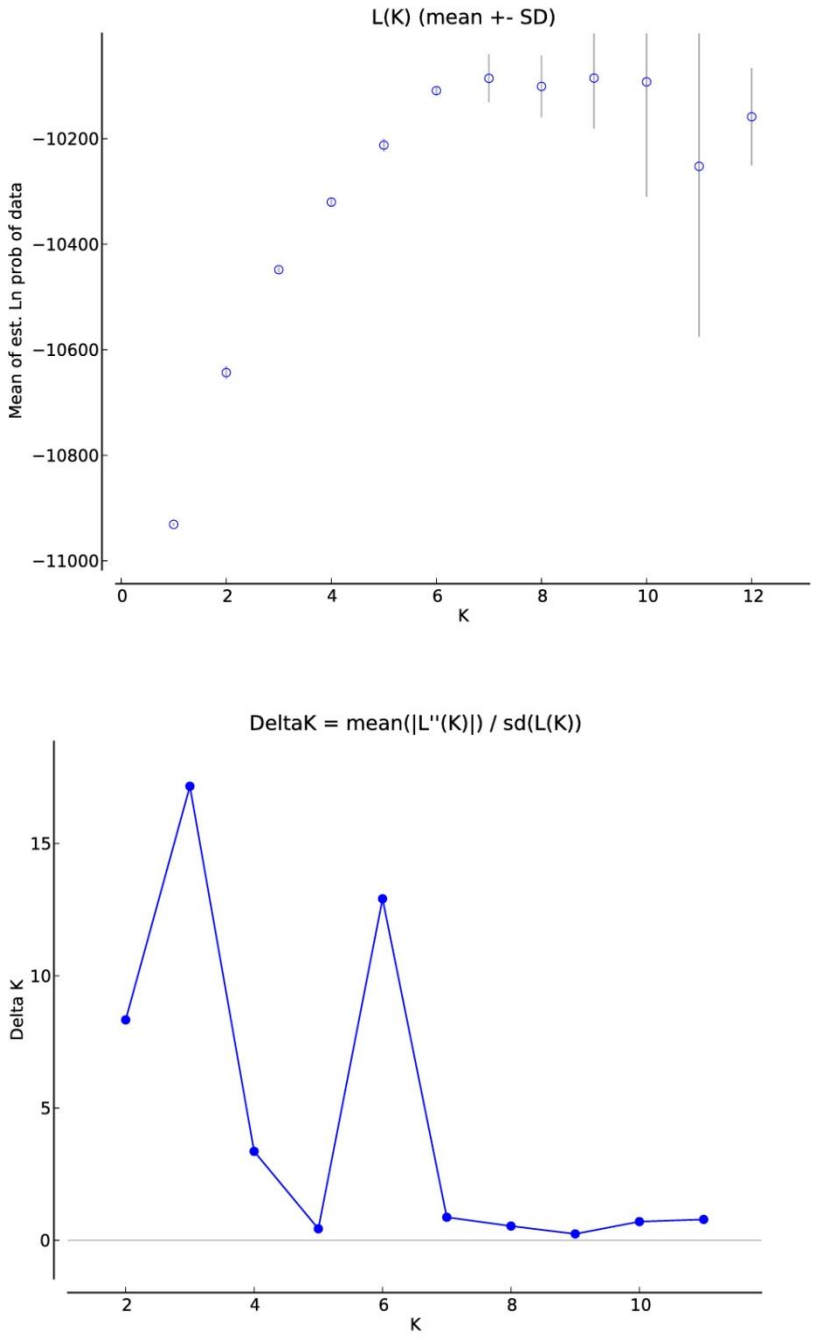


Figure A2. Mean likelihood and Delta K values for *F. olivaceus*: Group 1.

K of 3 was chosen.

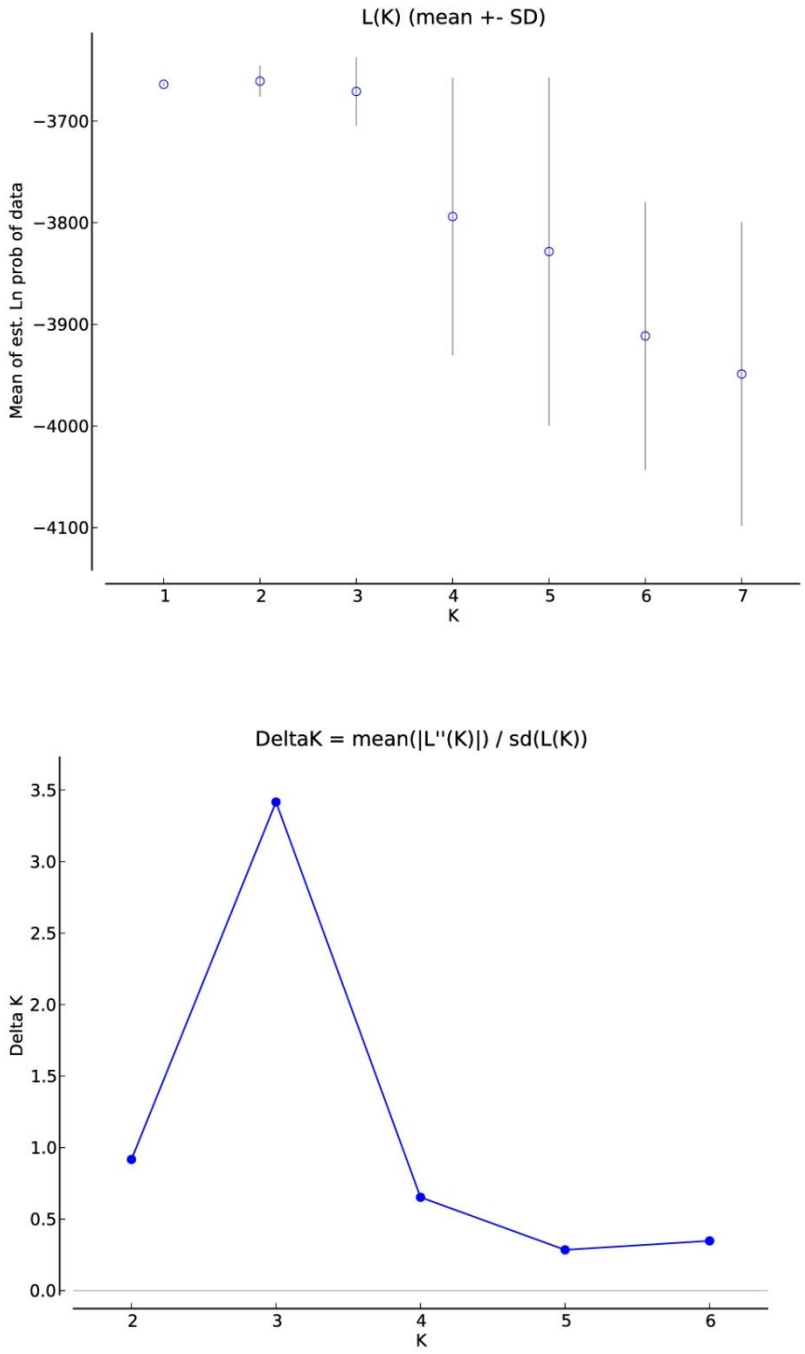


Figure A3. Mean likelihood and Delta K values for *F. olivaceus*: Group 1.1.

K of 1 was chosen (flat likelihood profile).

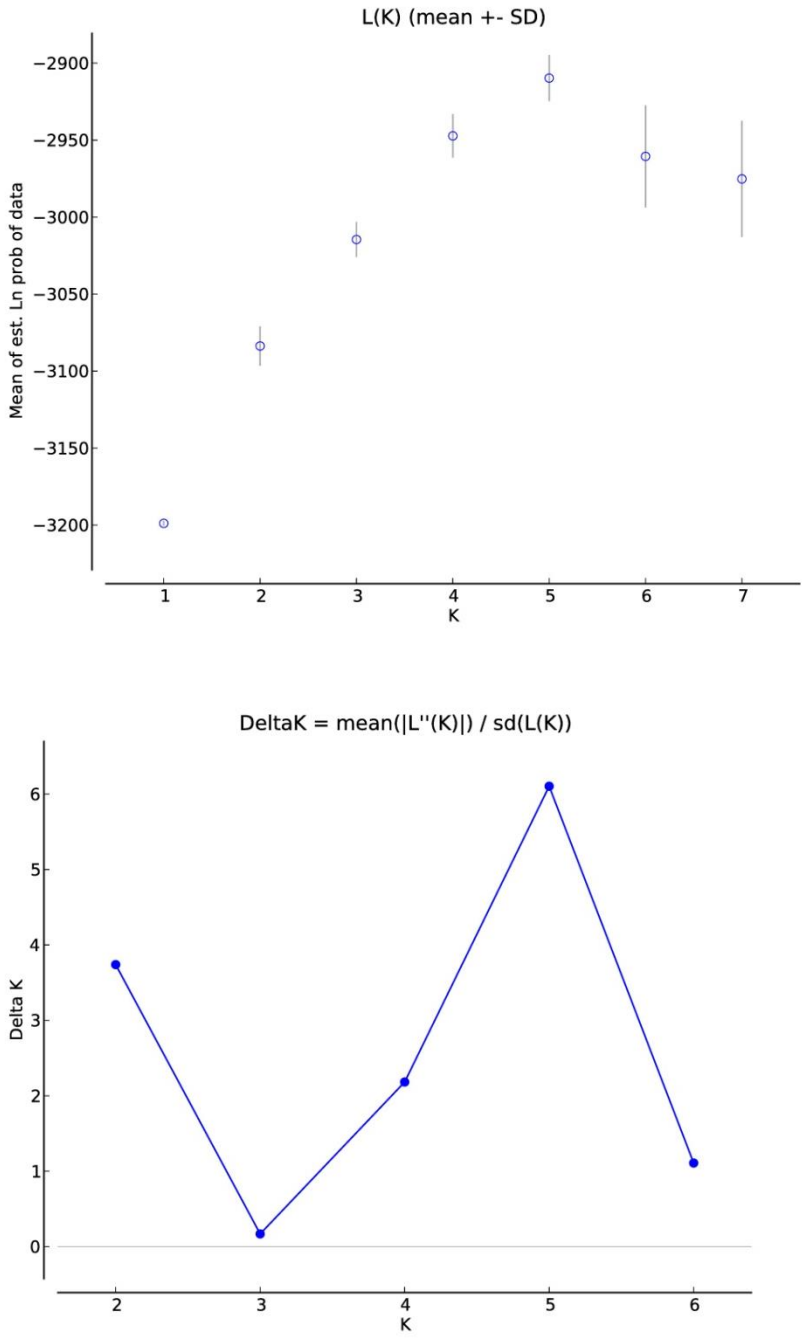


Figure A4. Mean likelihood and Delta K values for *F. olivaceus*: Group 1.2.

K of 5 was chosen.

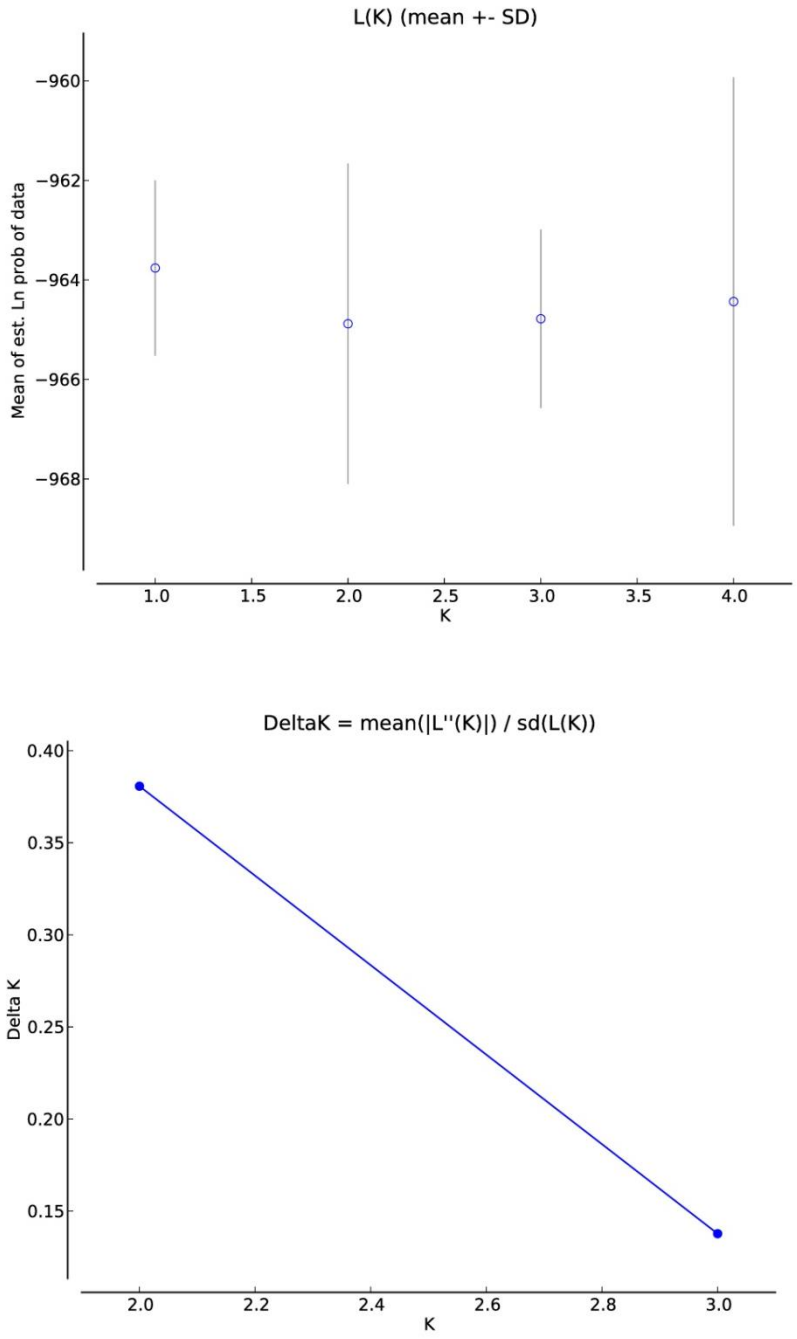


Figure A5. Mean likelihood and Delta K values for *F. olivaceus*: Group 1.2a.

K of 1 was chosen (Delta K < 2 and flat likelihood profile).

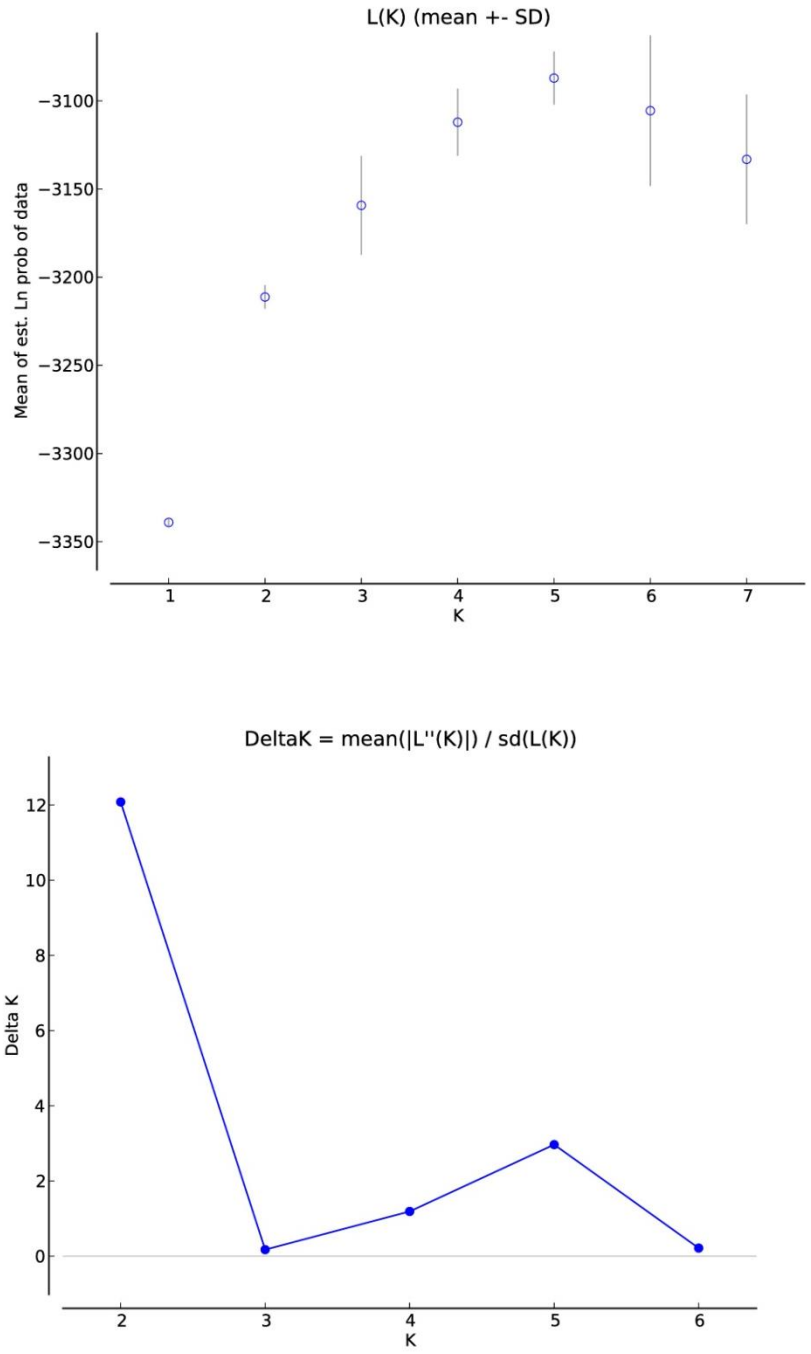


Figure A6. Mean likelihood and Delta K values for *F. olivaceus*: Group 1.3.

K of 2 was chosen.

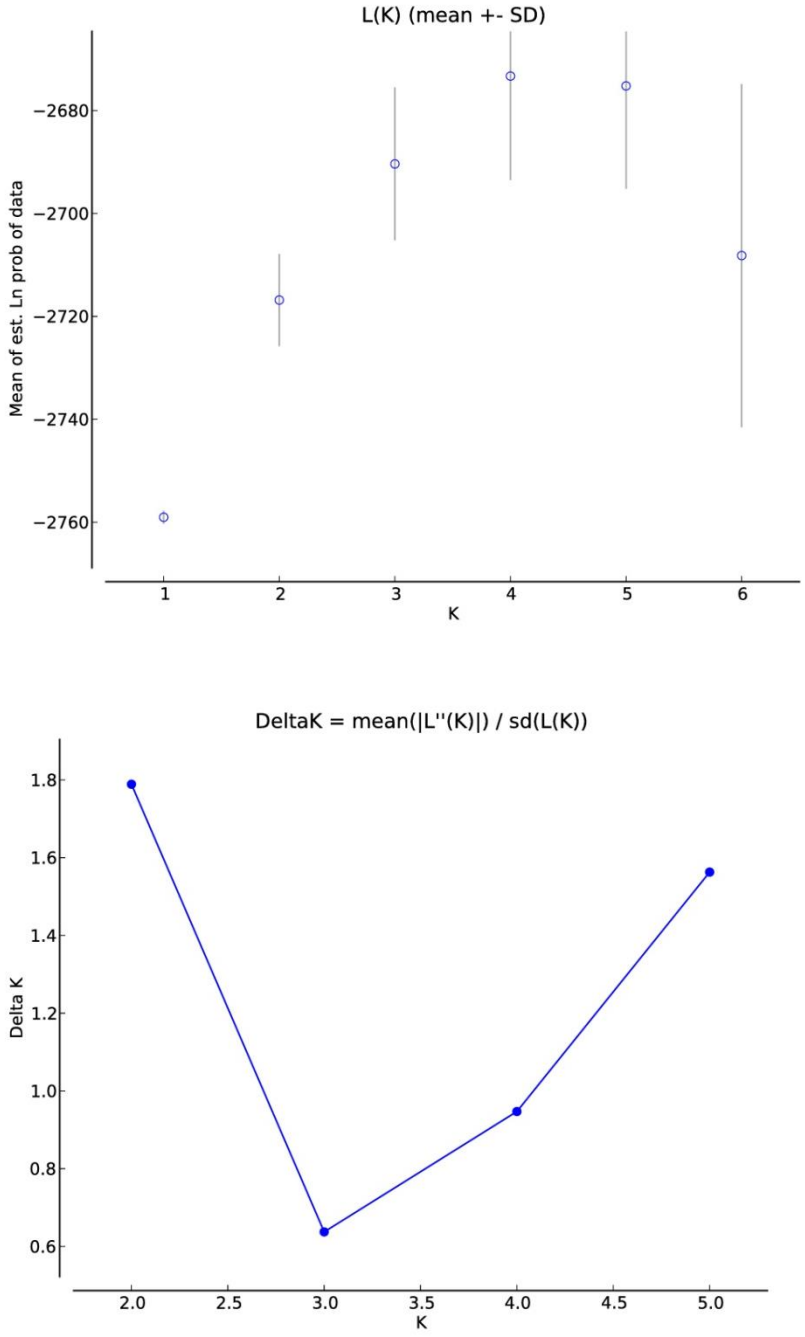


Figure A7. Mean likelihood and Delta K values for *F. olivaceus*: Group 1.3a.

K of 1 was chosen (Delta K < 2).

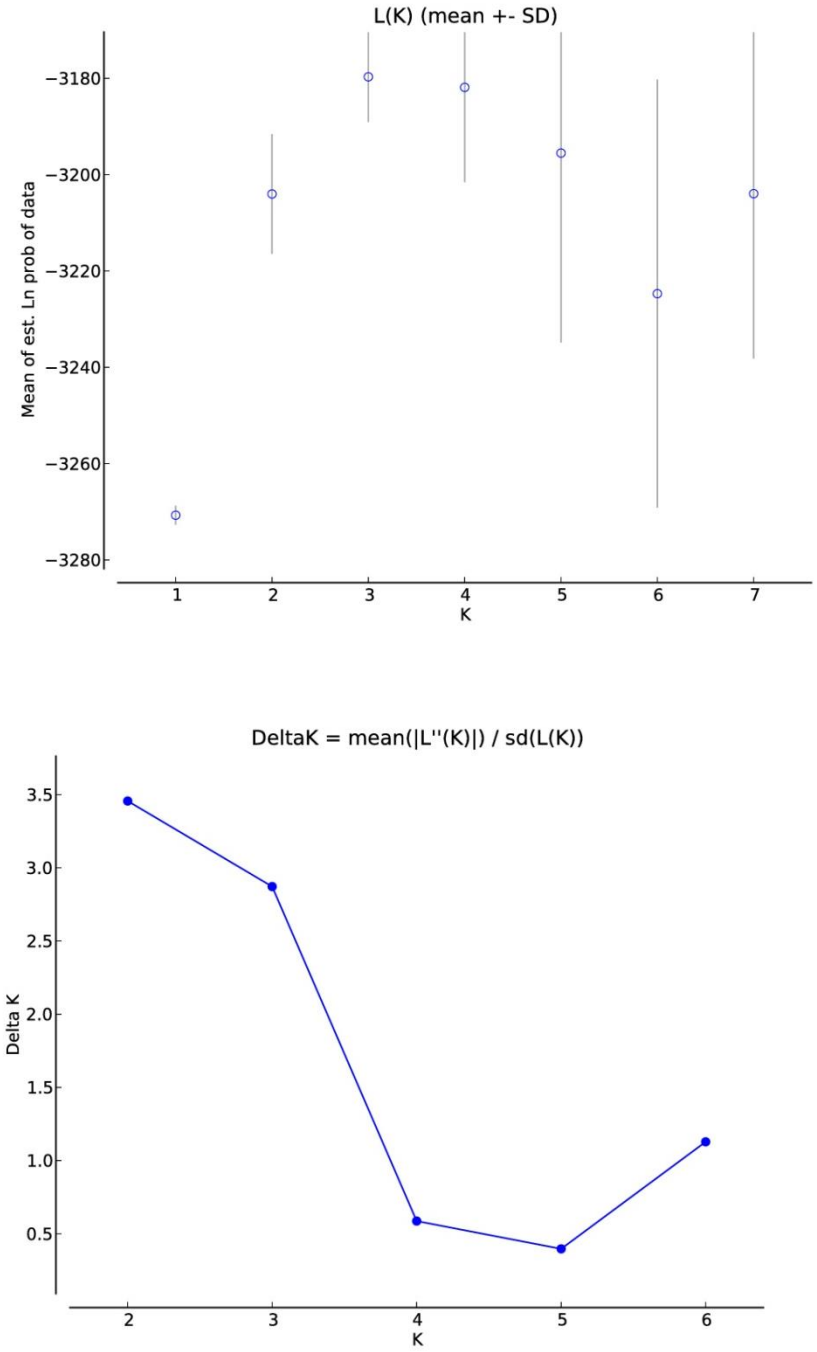


Figure A8. Mean likelihood and Delta K values for *F. olivaceus*: Group 2.

K of 3 was chosen. A K of 3 was chosen over a K of 2 because they had comparable scores, and a K of 3 resulted in cleaner groupings.

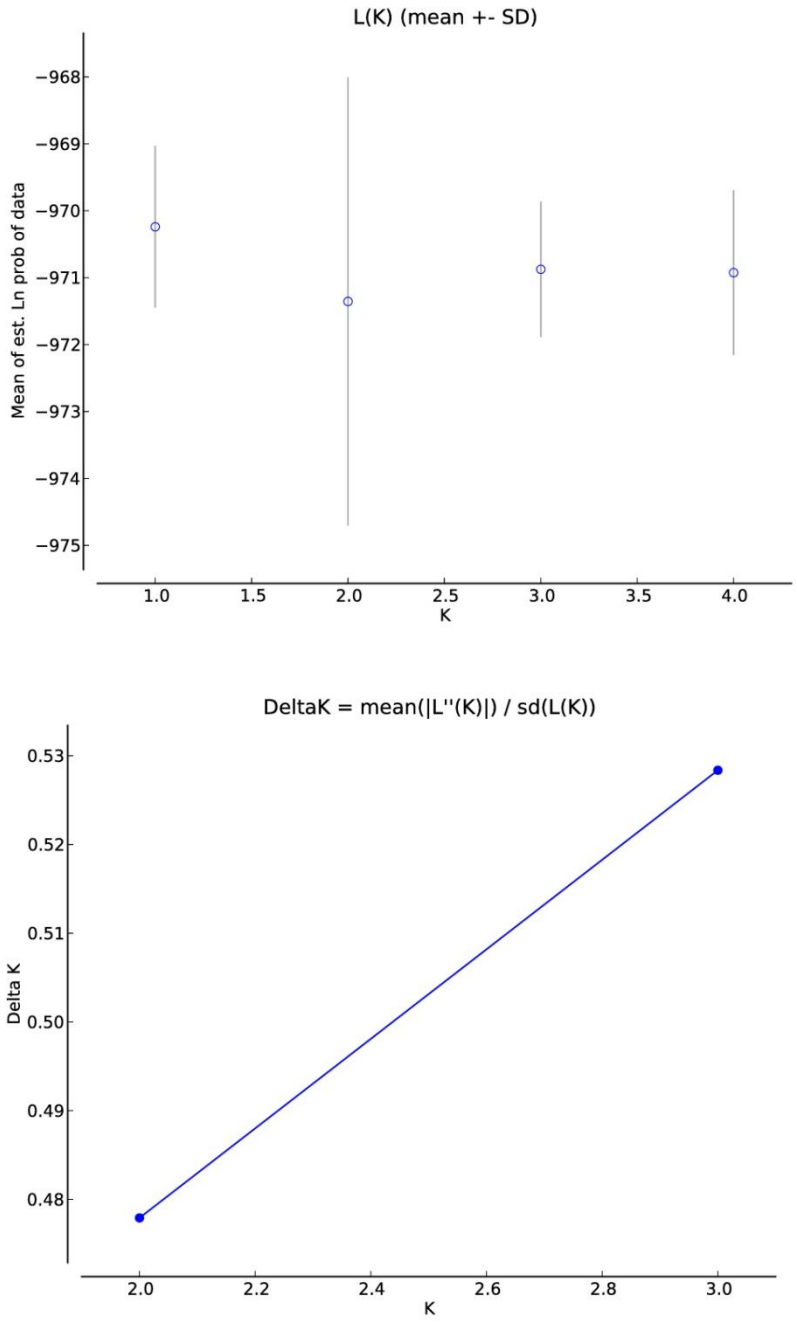


Figure A9. Mean likelihood and Delta K values for *F. olivaceus*: Group 2.1.

K of 1 was chosen (Delta K < 2 and flat likelihood profile).

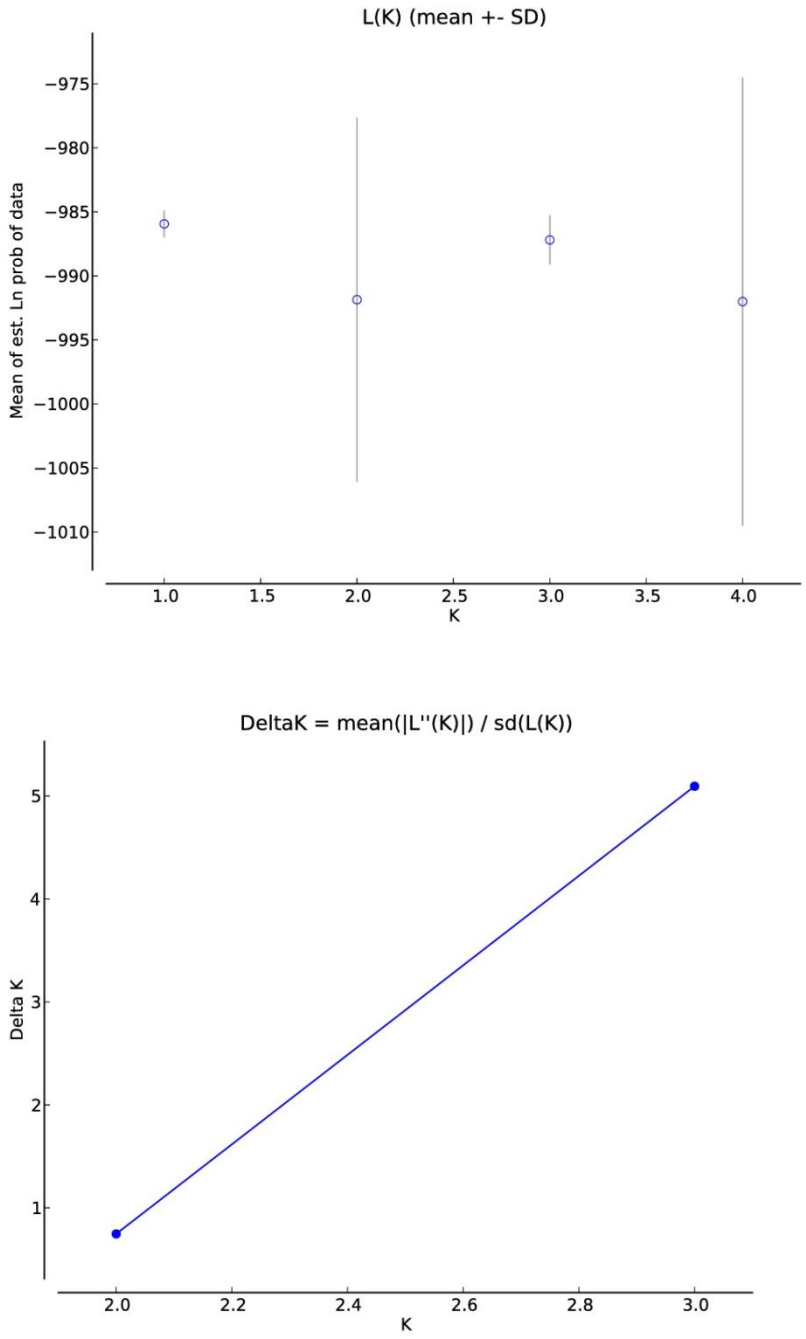


Figure A10. Mean likelihood and Delta K values for *F. olivaceus*: Group 2.2.

K of 1 was chosen (flat likelihood profile).

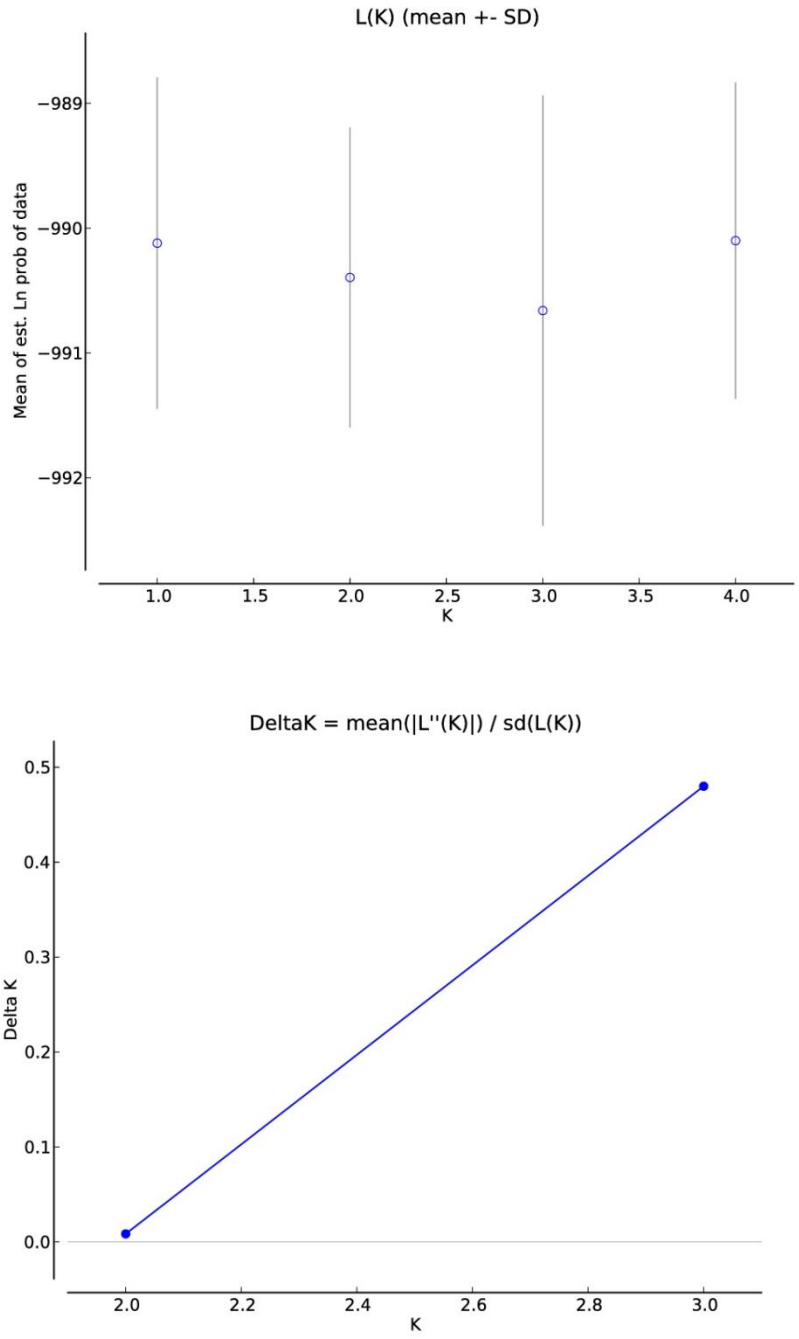


Figure A11. Mean likelihood and Delta K values for *F. olivaceus*: Group 2.3.

K of 1 was chosen (Delta K < 2 and flat likelihood profile).

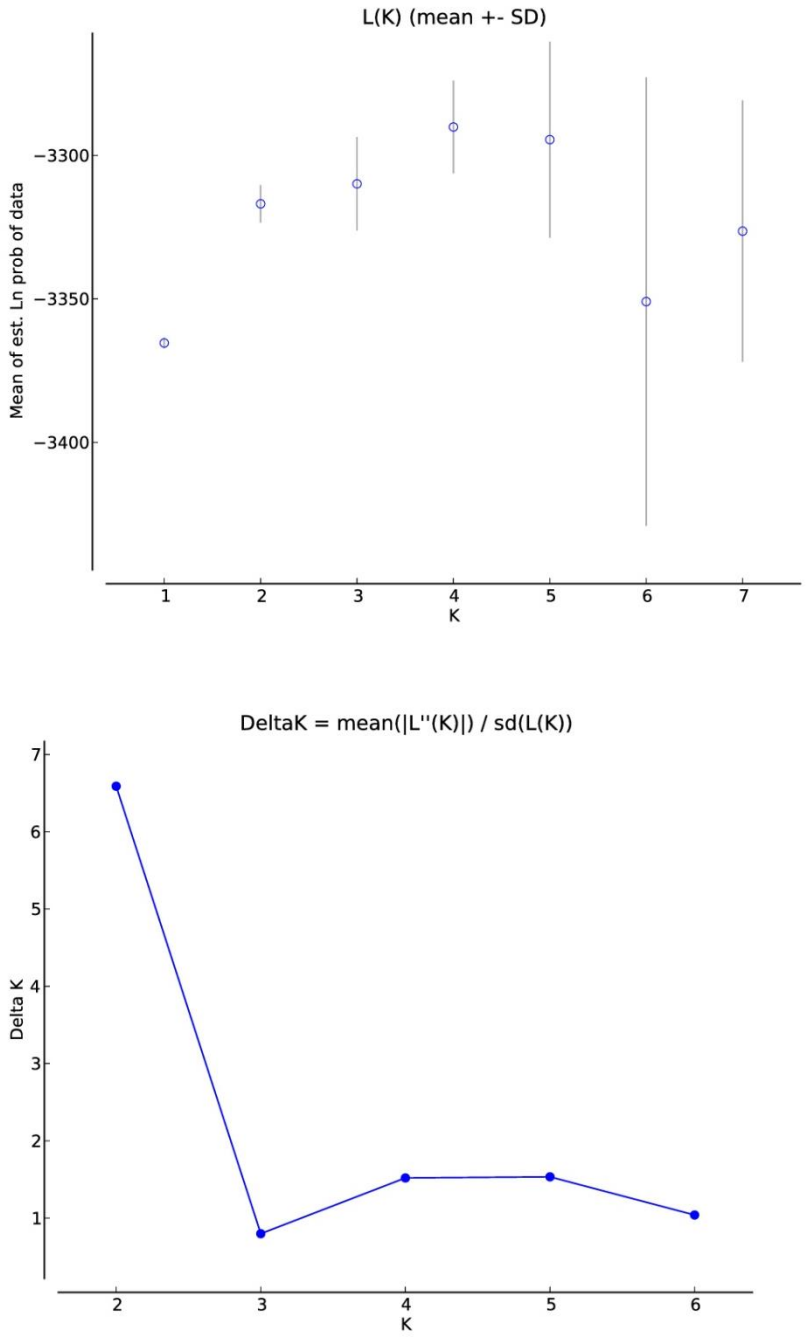


Figure A12. Mean likelihood and Delta K values for *F. olivaceus*: Group 3.

K of 2 was chosen.

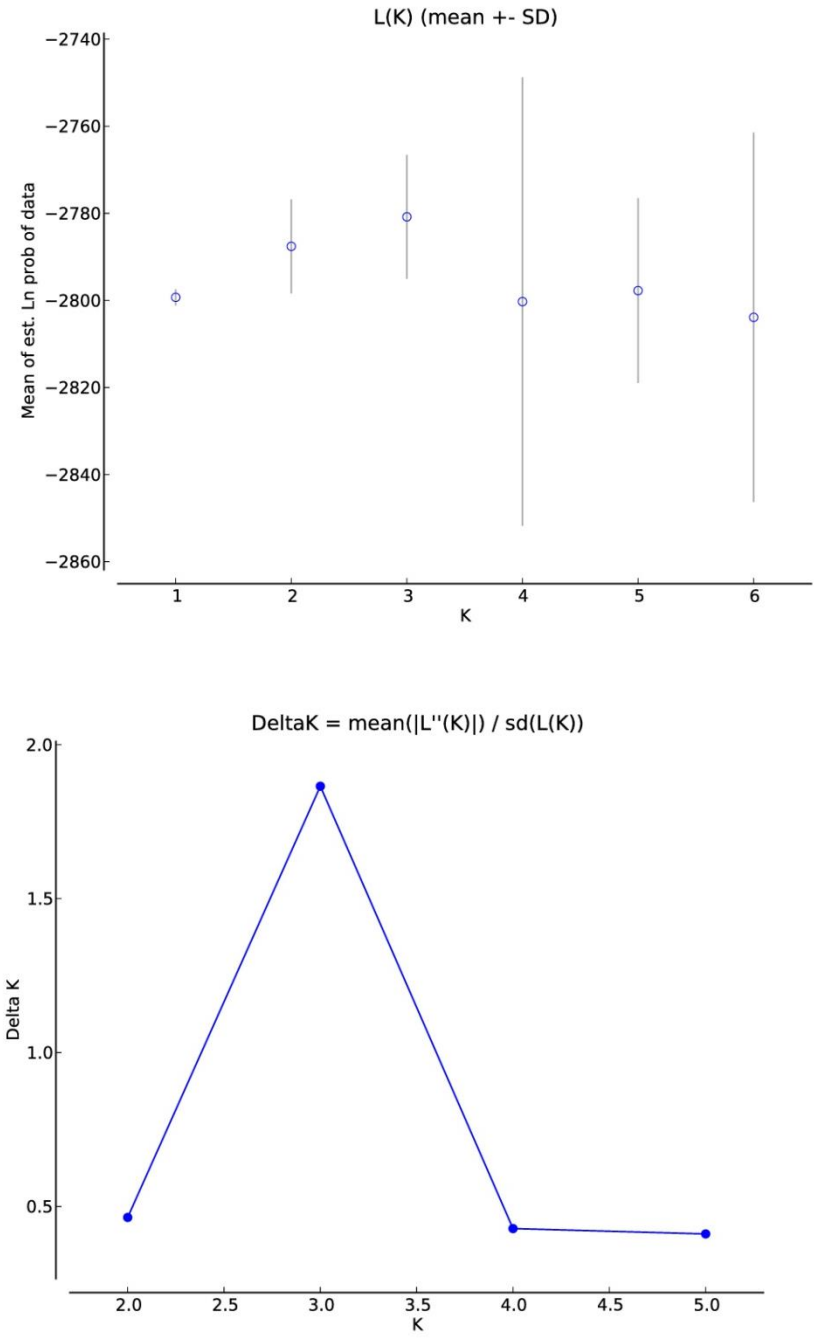


Figure A13. Mean likelihood and Delta K values for *F. olivaceus*: Group 3.1.

K of 1 was chosen (Delta K < 2 and flat likelihood profile).

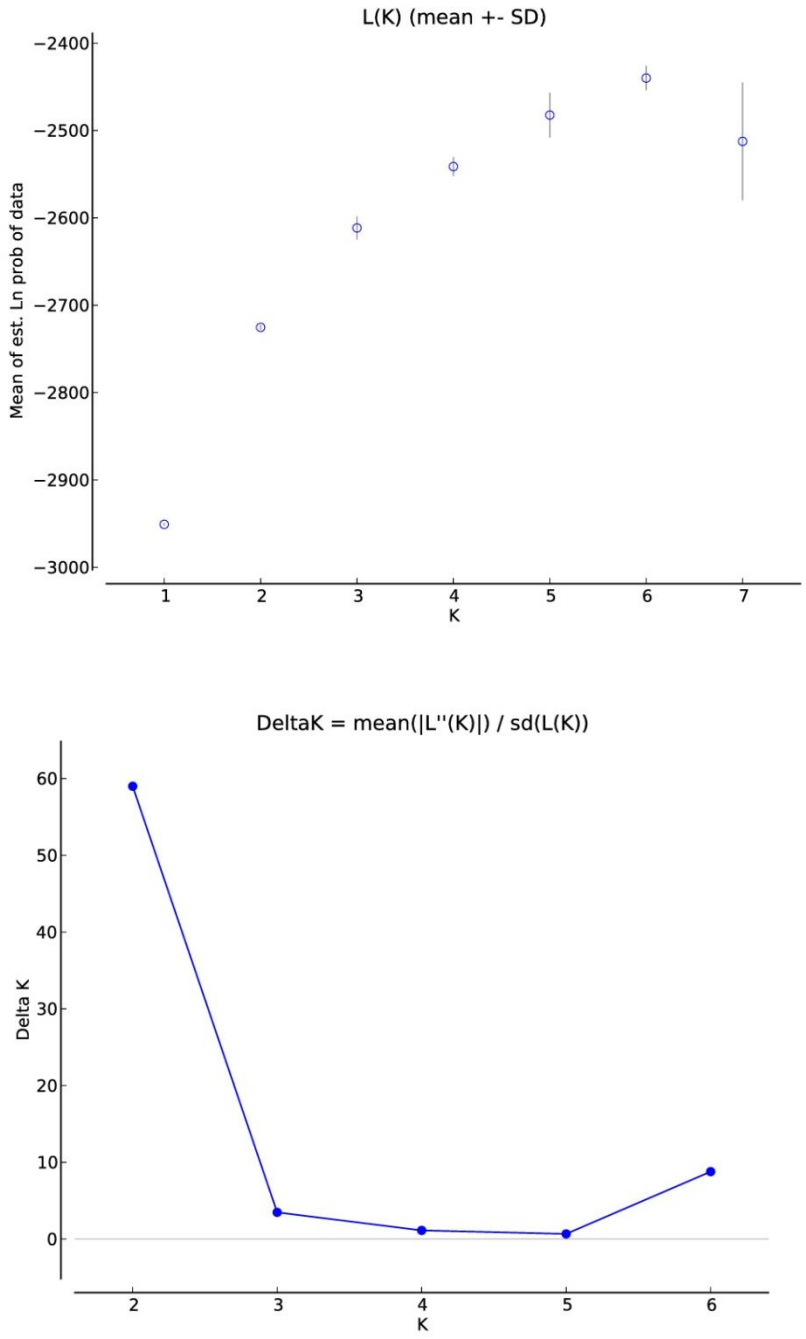


Figure A14. Mean likelihood and Delta K values for *F. olivaceus*: Group 4.

K of 2 was chosen.

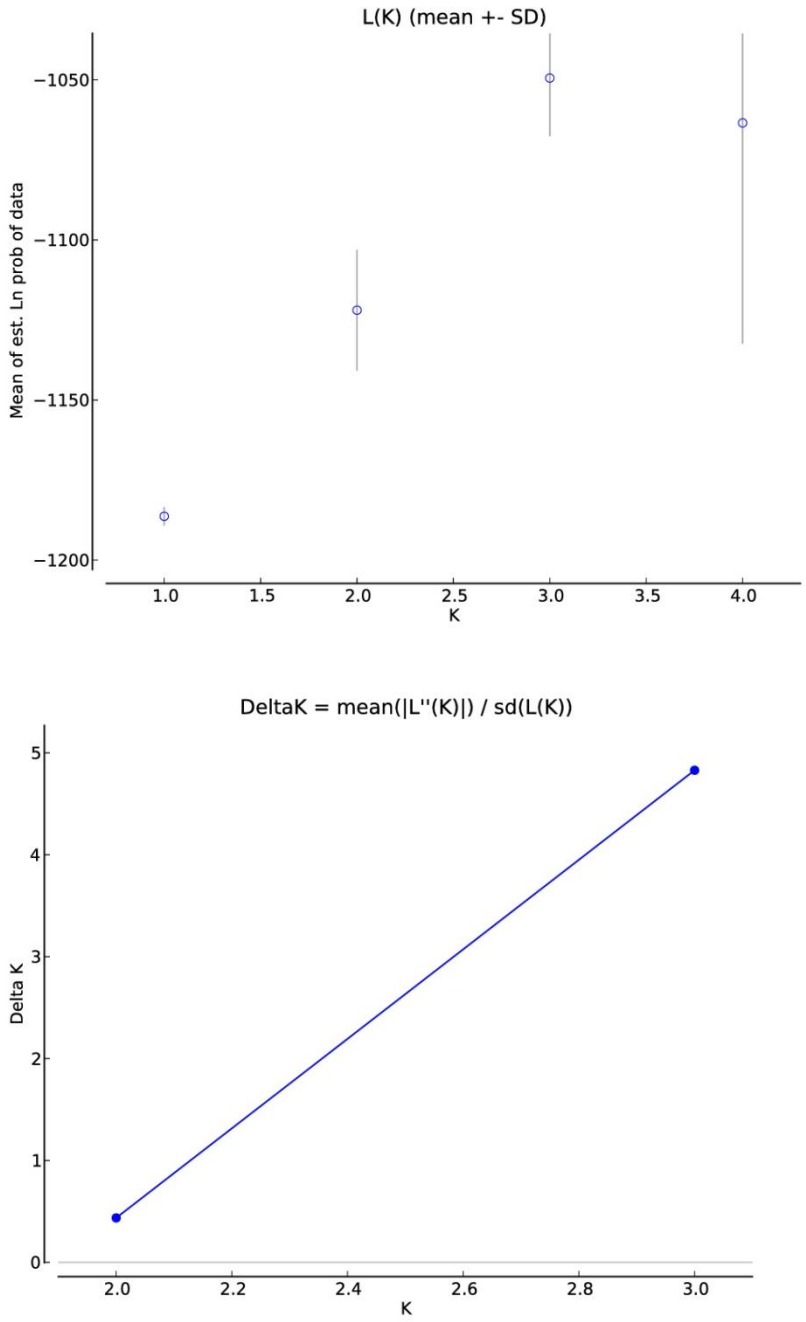


Figure A15. Mean likelihood and Delta K values for *F. olivaceus*: Group 4.1.

K of 3 was chosen.

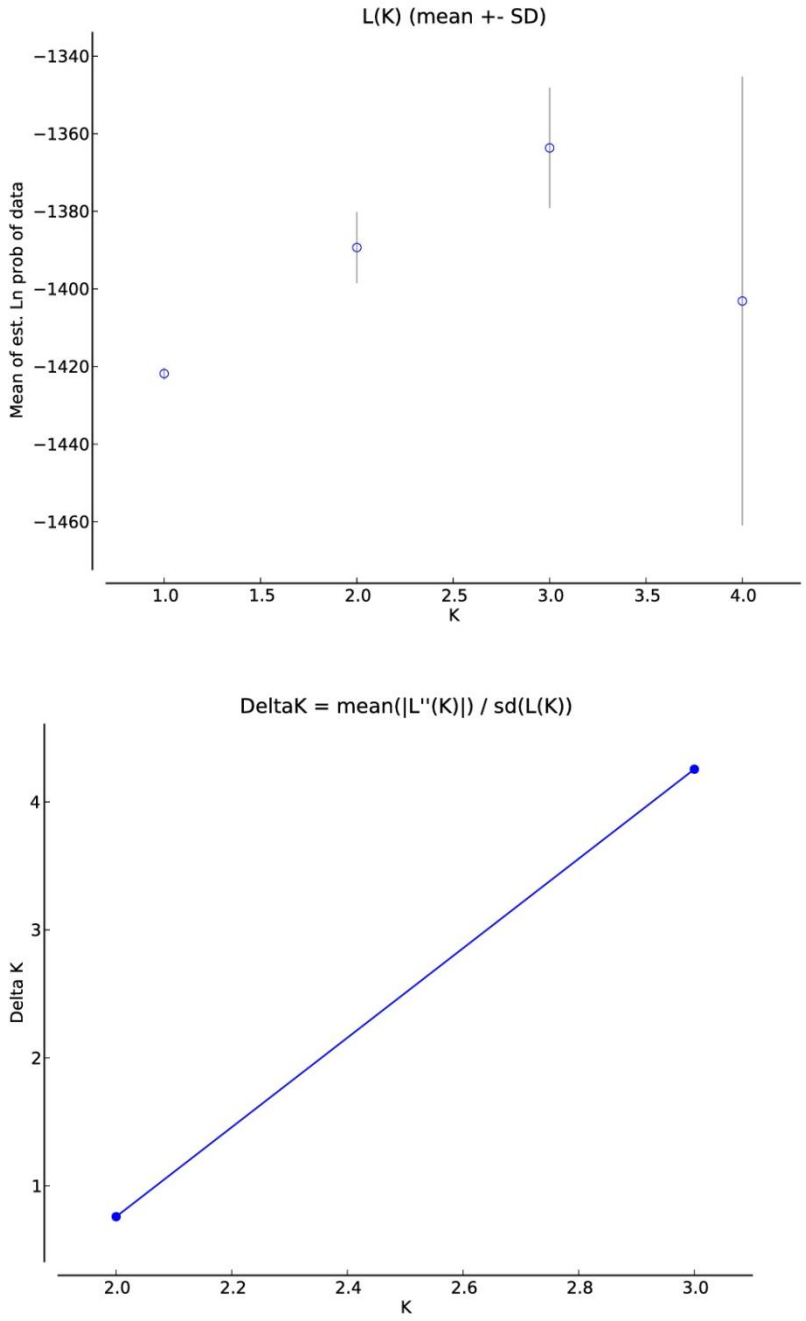


Figure A16. Mean likelihood and Delta K values for *F. olivaceus*: Group 4.2.

K of 3 was chosen.

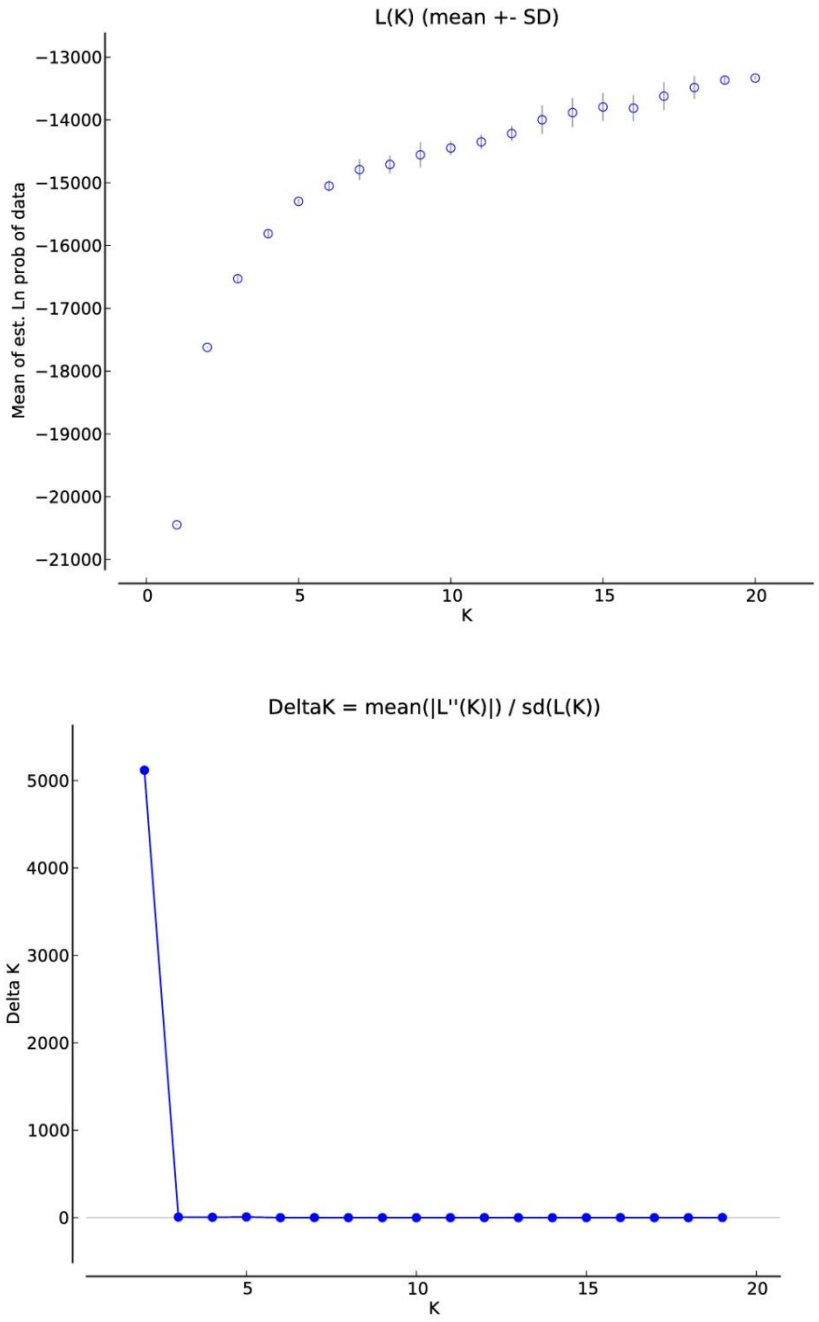


Figure A17. Mean likelihood and Delta K values for *S. atromaculatus*: Full Dataset.

K of 2 was chosen.

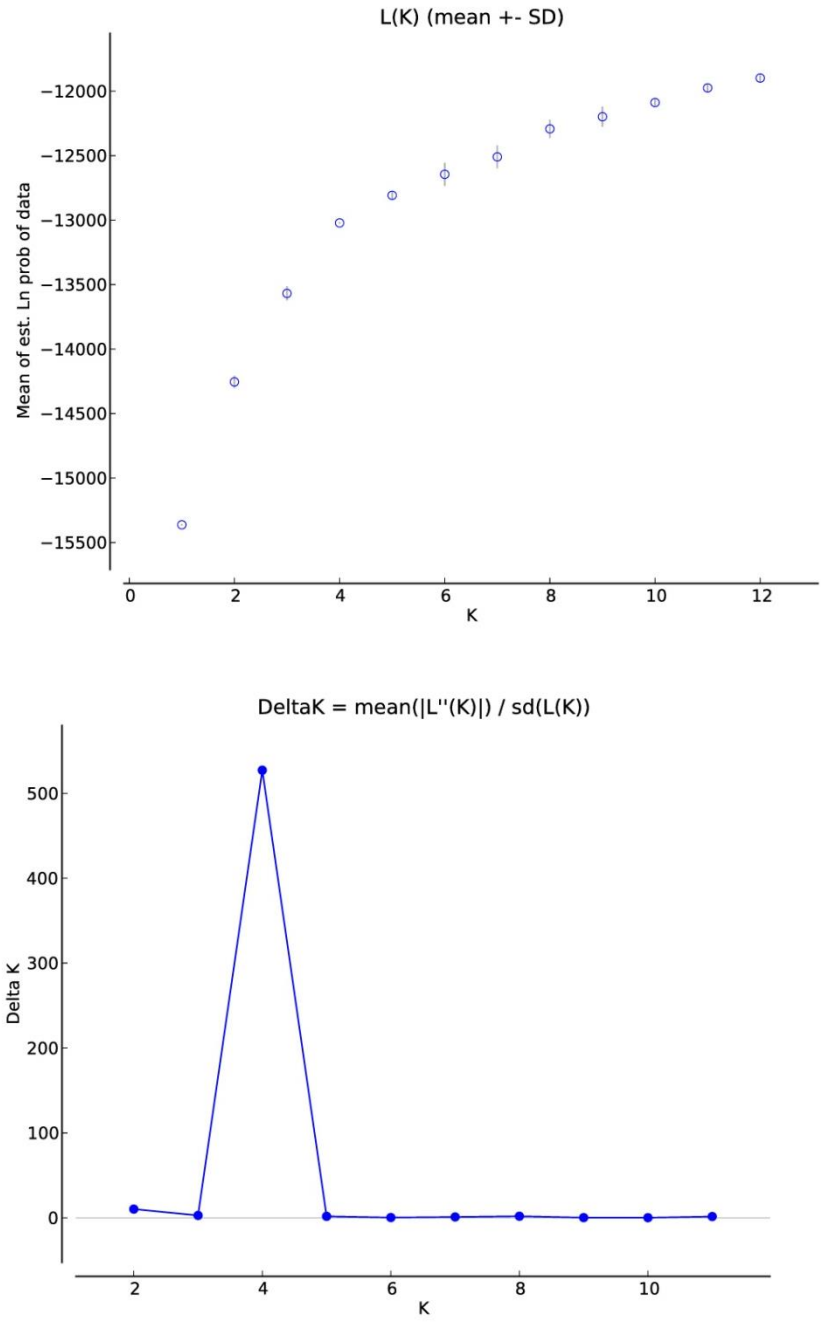


Figure A18. Mean likelihood and Delta K values for *S. atromaculatus*: Group 1.

K of 4 was chosen.

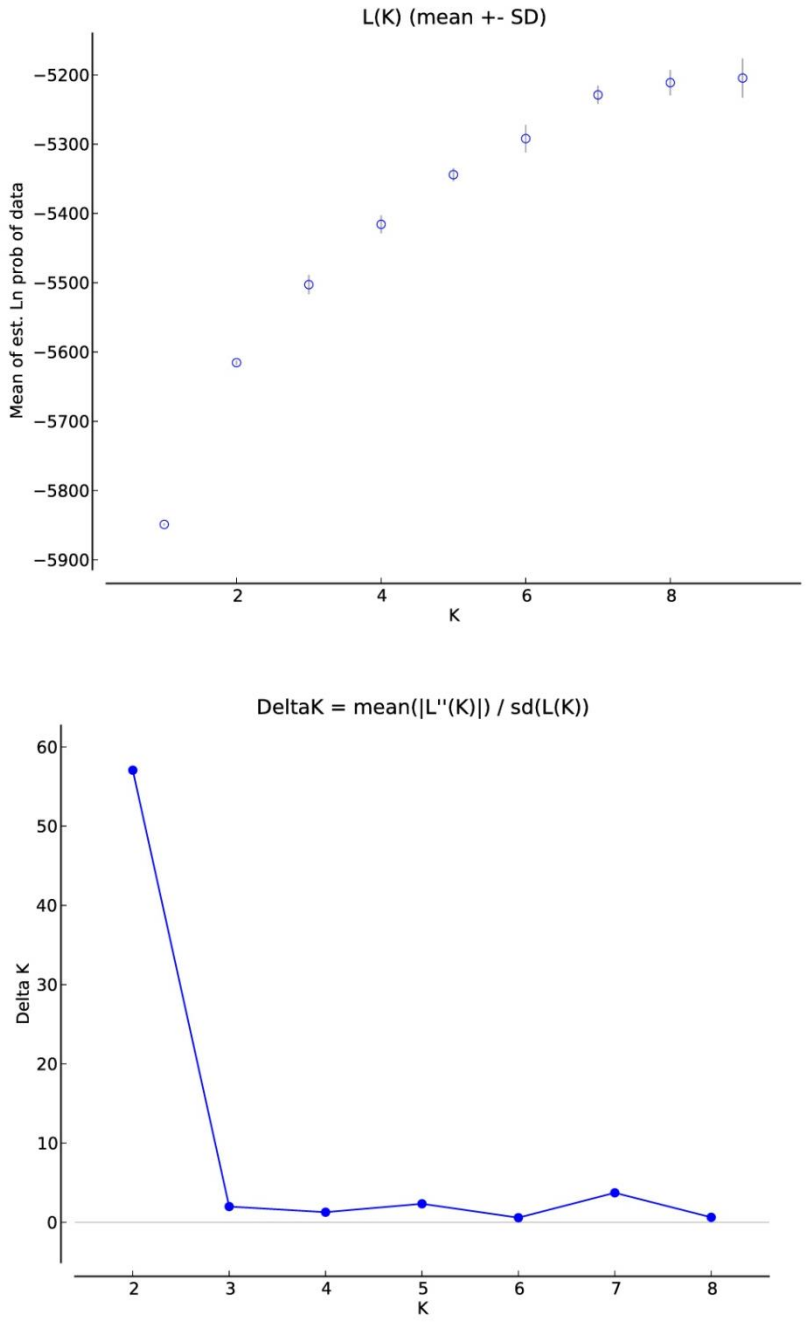


Figure A19. Mean likelihood and Delta K values for *S. atromaculatus*: Group 1.1.

K of 2 was chosen.

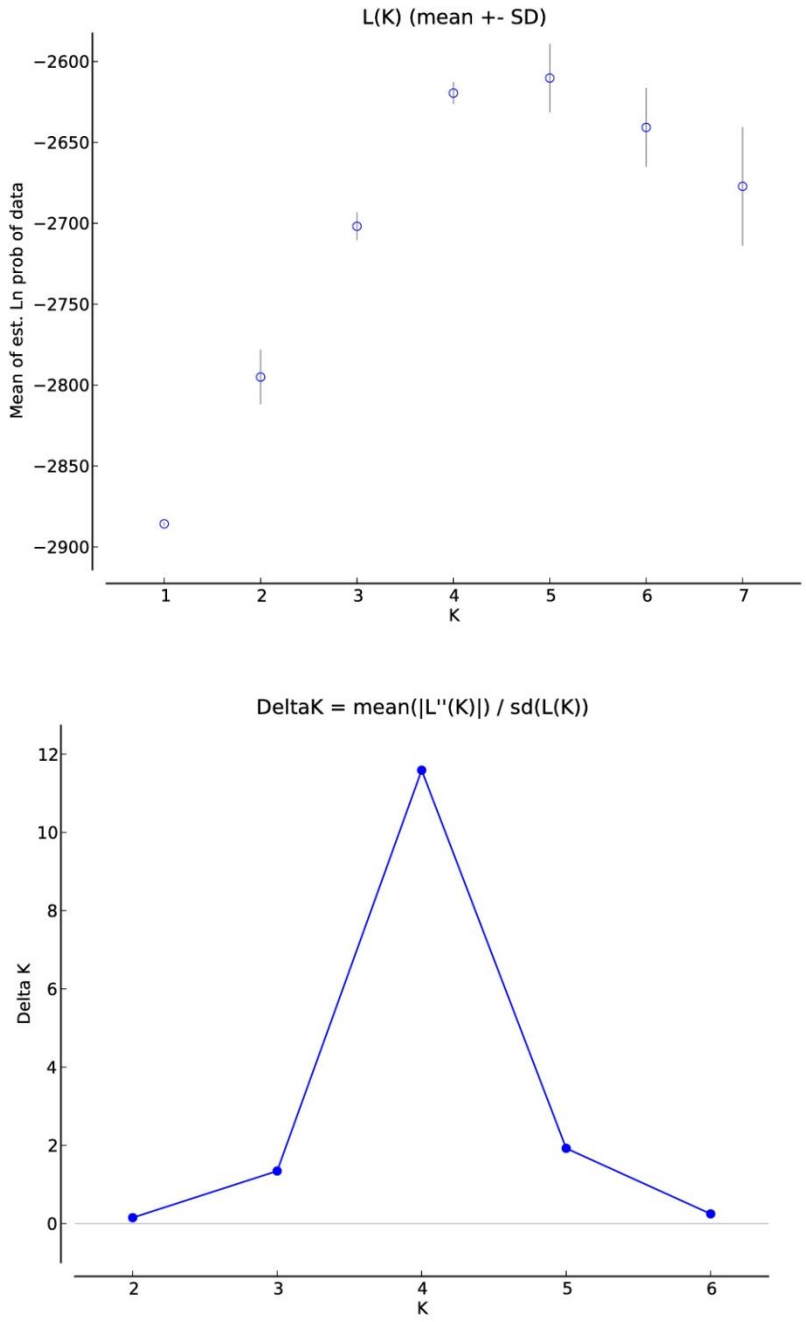


Figure A20. Mean likelihood and Delta K values for *S. atromaculatus*: Group 1.1a.

K of 4 was chosen.

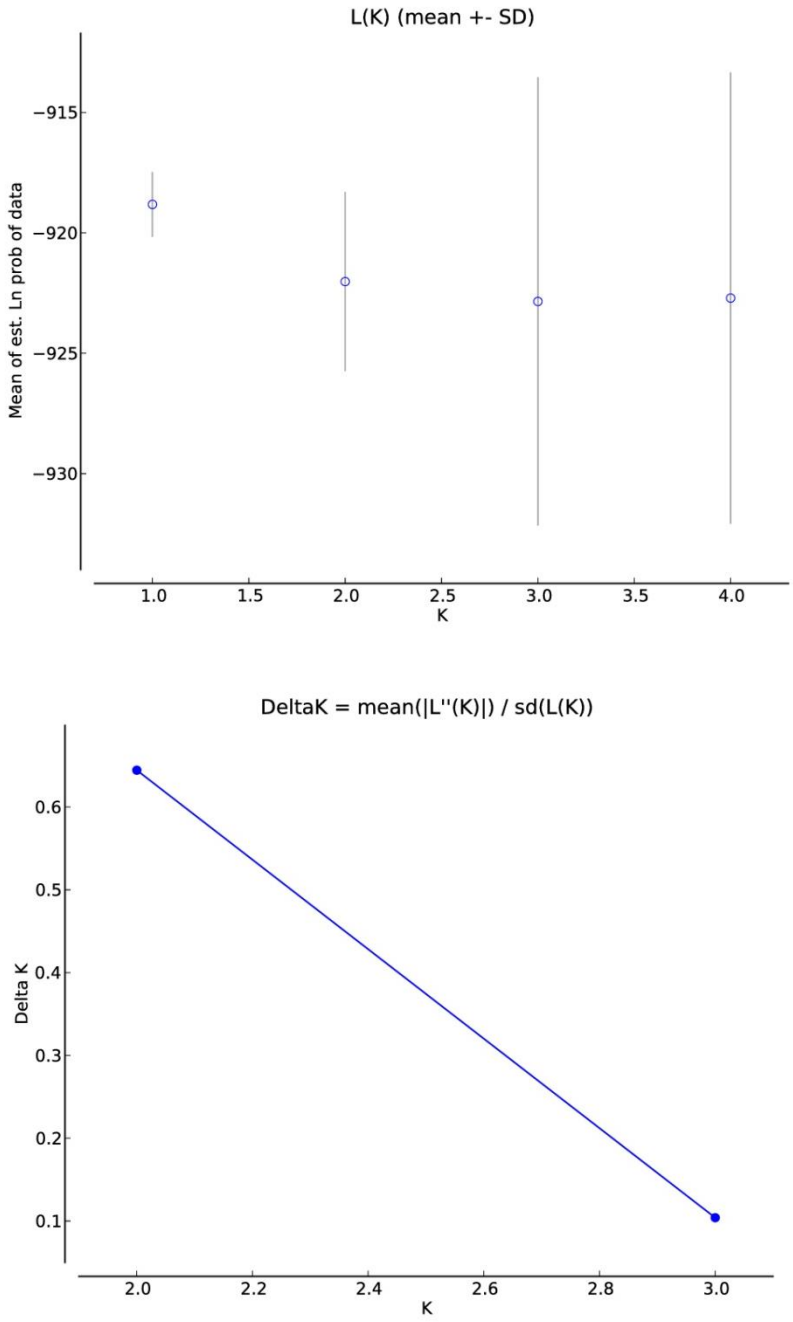


Figure A21. Mean likelihood and Delta K values for *S. atromaculatus*: Group 1.1a1.

K of 1 was chosen (Delta K < 2 and flat likelihood profile).

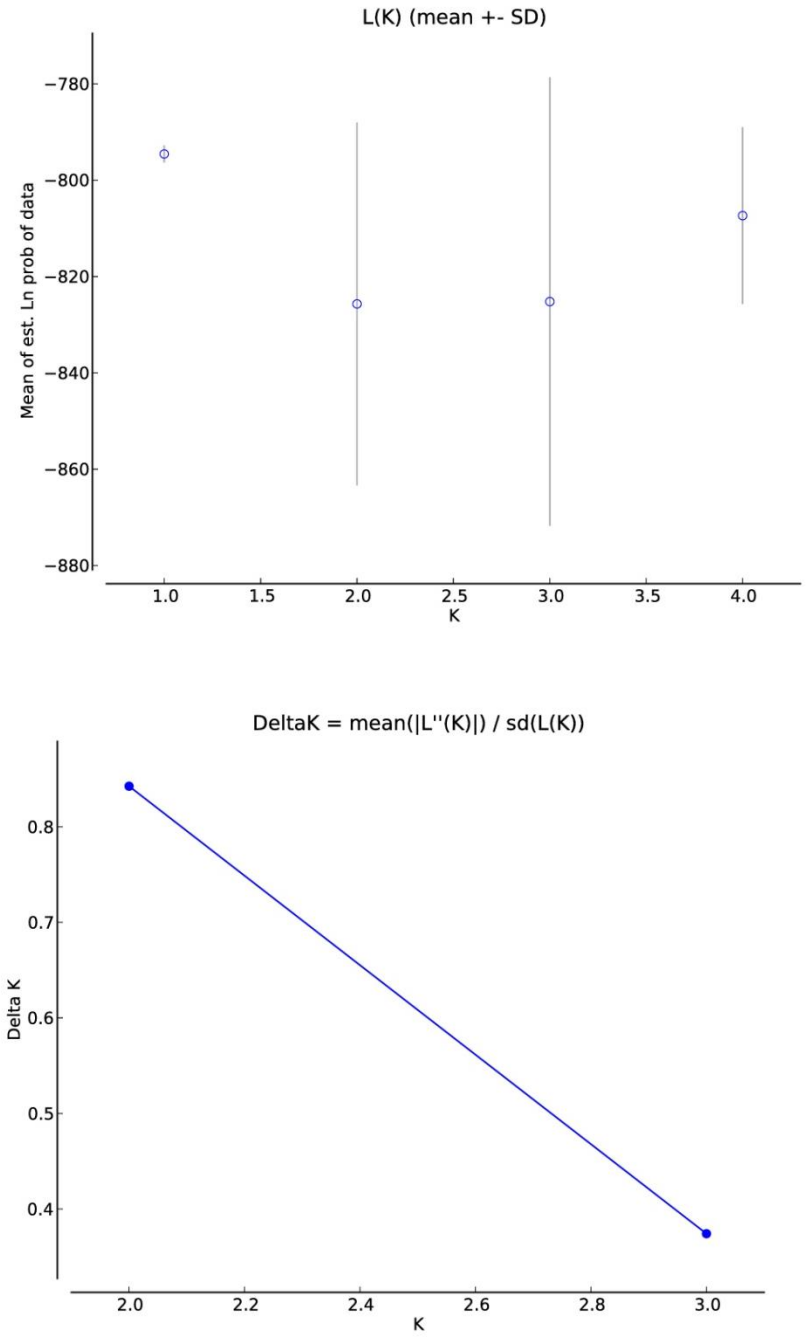


Figure A22. Mean likelihood and Delta K values for *S. atromaculatus*: Group 1.1a2.

K of 1 was chosen (Delta K < 2 and flat likelihood profile).

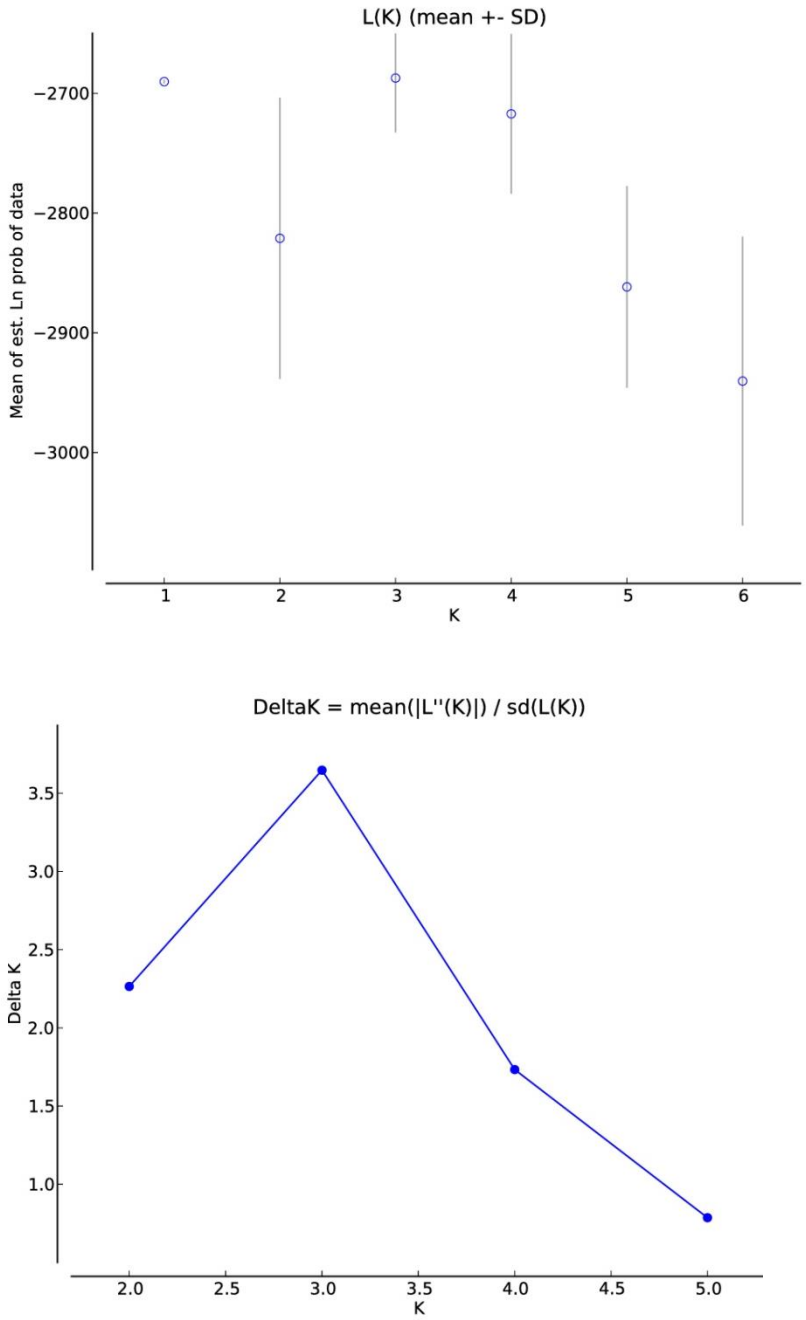


Figure A23. Mean likelihood and Delta K values for *S. atromaculatus*: Group 1.1b.

K of 1 was chosen (flat likelihood profile).

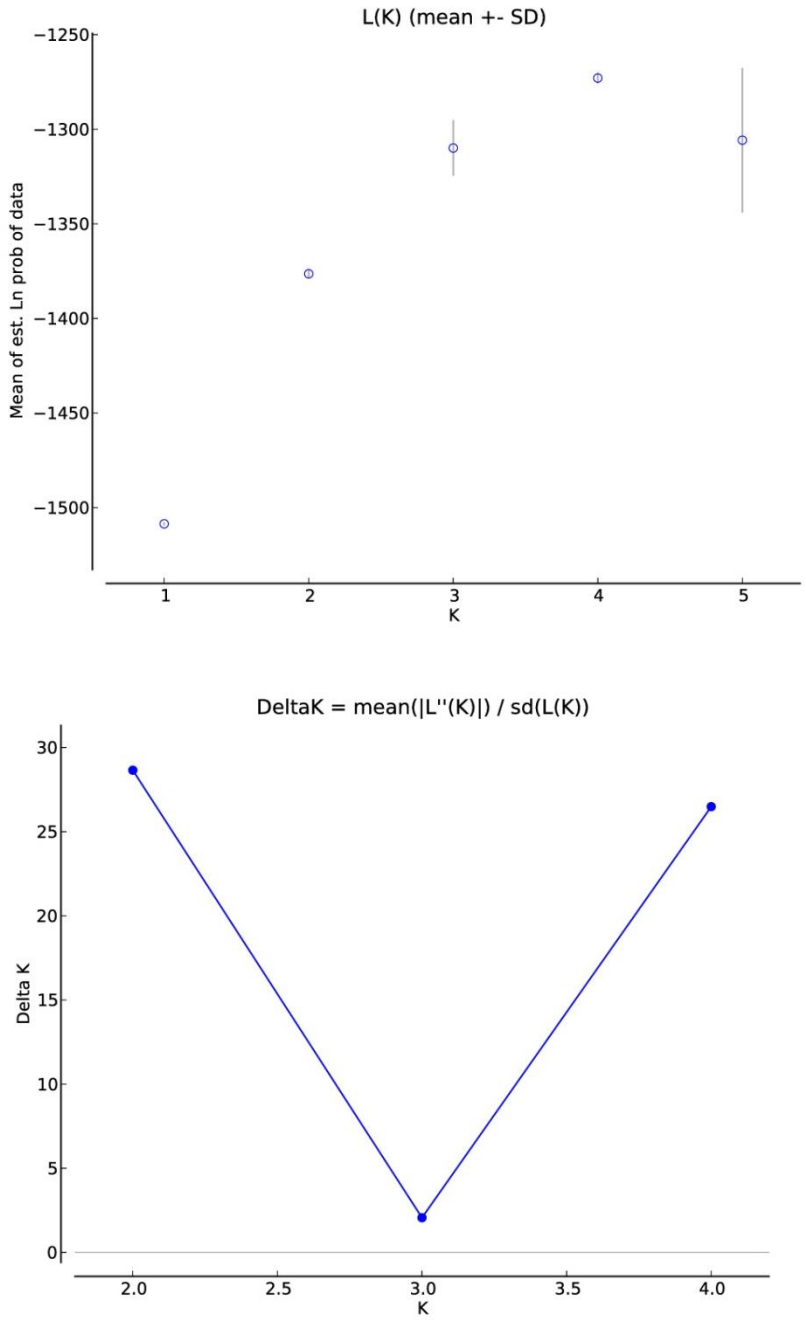


Figure A24. Mean likelihood and Delta K values for *S. atromaculatus*: Group 1.2.

K of 2 was chosen.

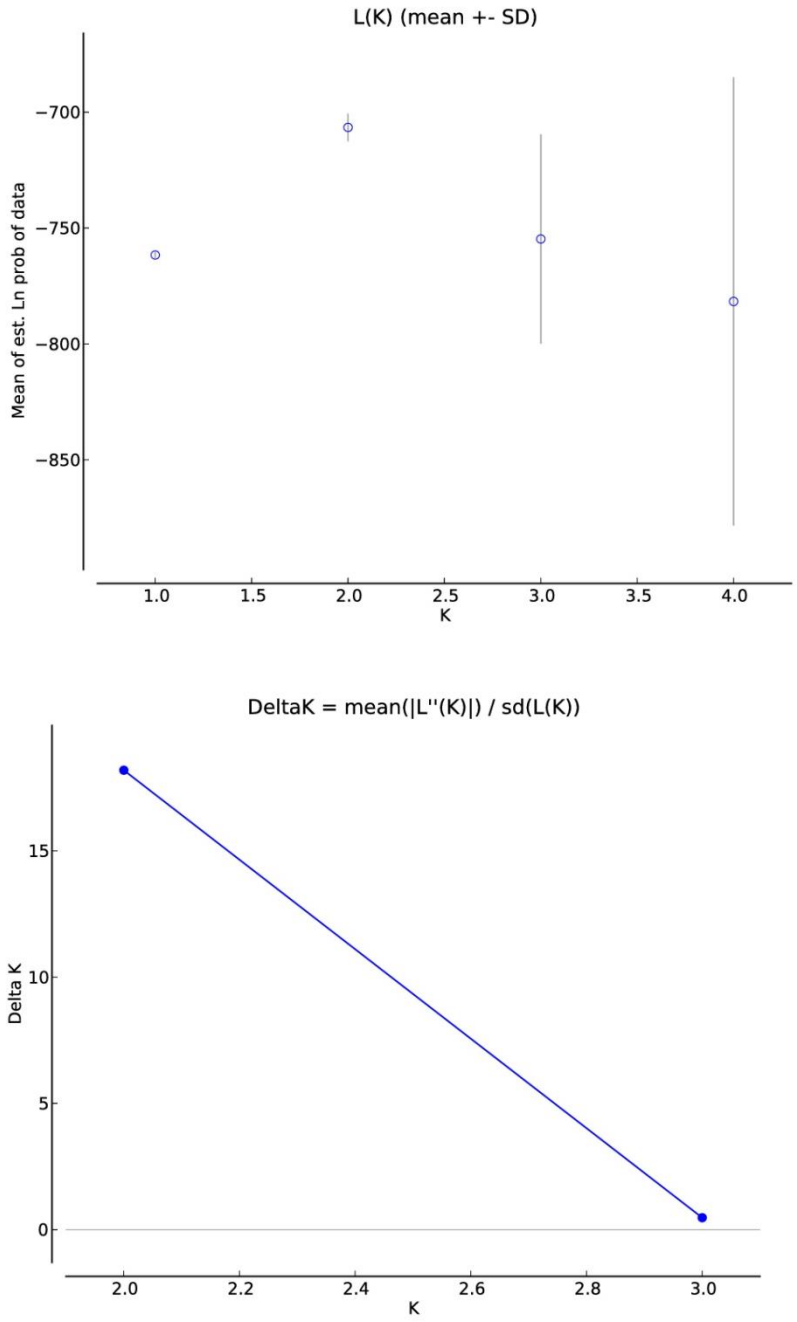


Figure A25. Mean likelihood and Delta K values for *S. atromaculatus*: Group 1.2a.

K of 2 was chosen.

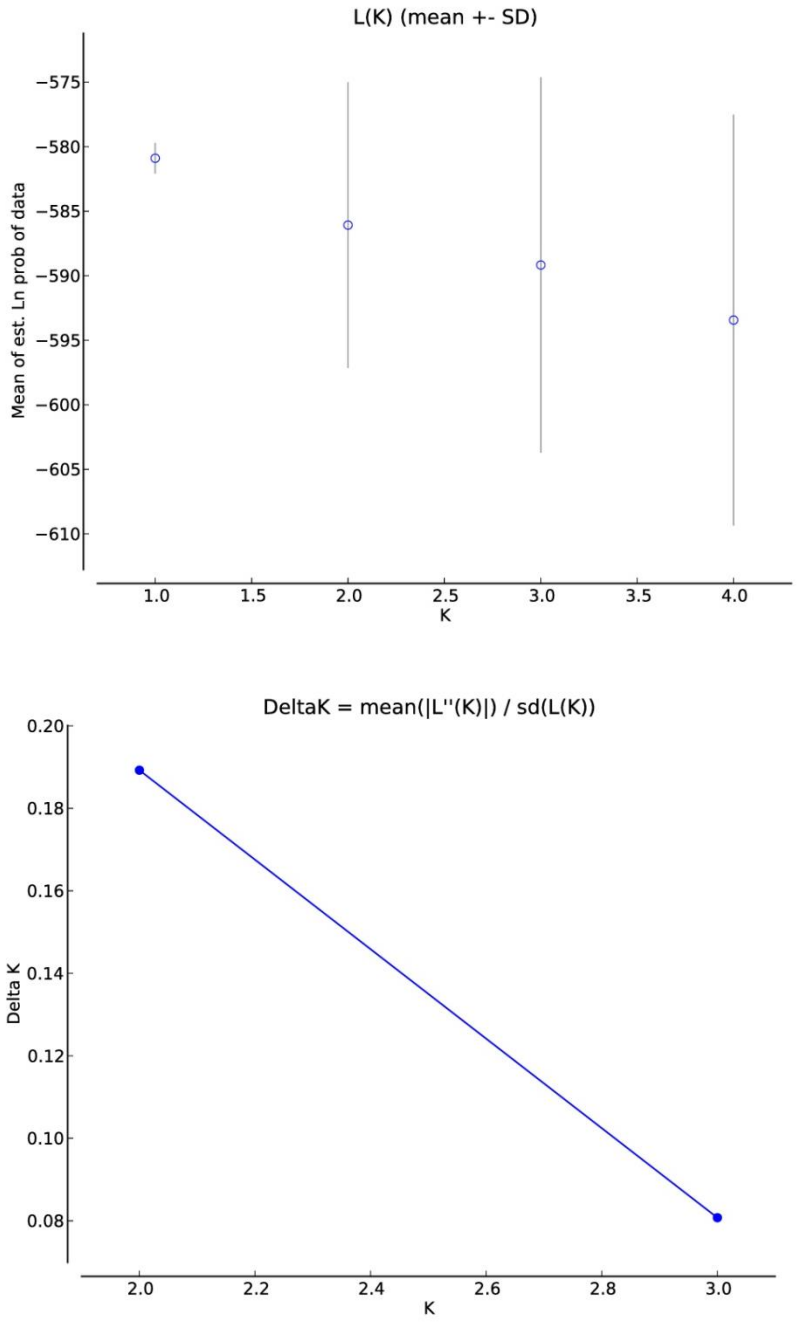


Figure A26. Mean likelihood and Delta K values for *S. atromaculatus*: Group 1.2b.

K of 1 was chosen (Delta K < 2 and flat likelihood profile).

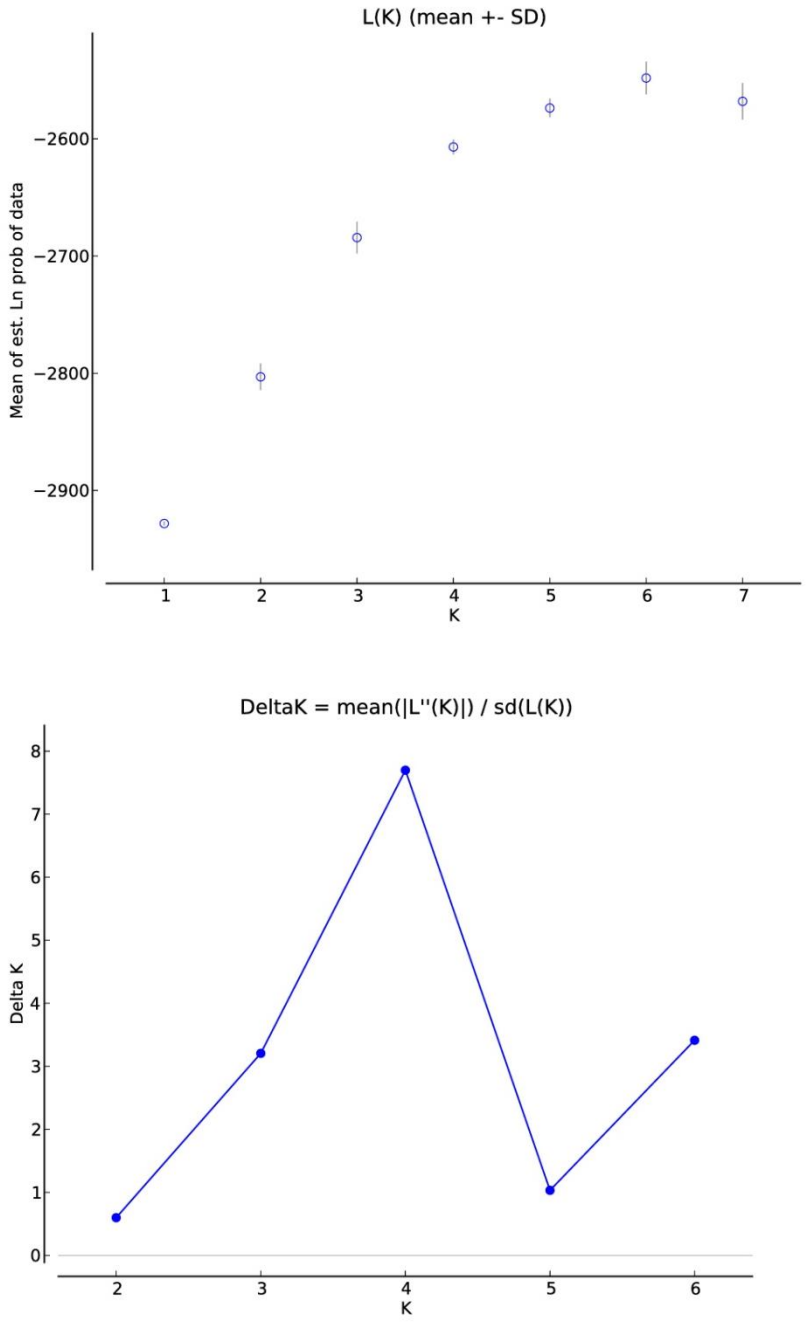


Figure A27. Mean likelihood and Delta K values for *S. atromaculatus*: Group 1.3.

K of 4 was chosen.

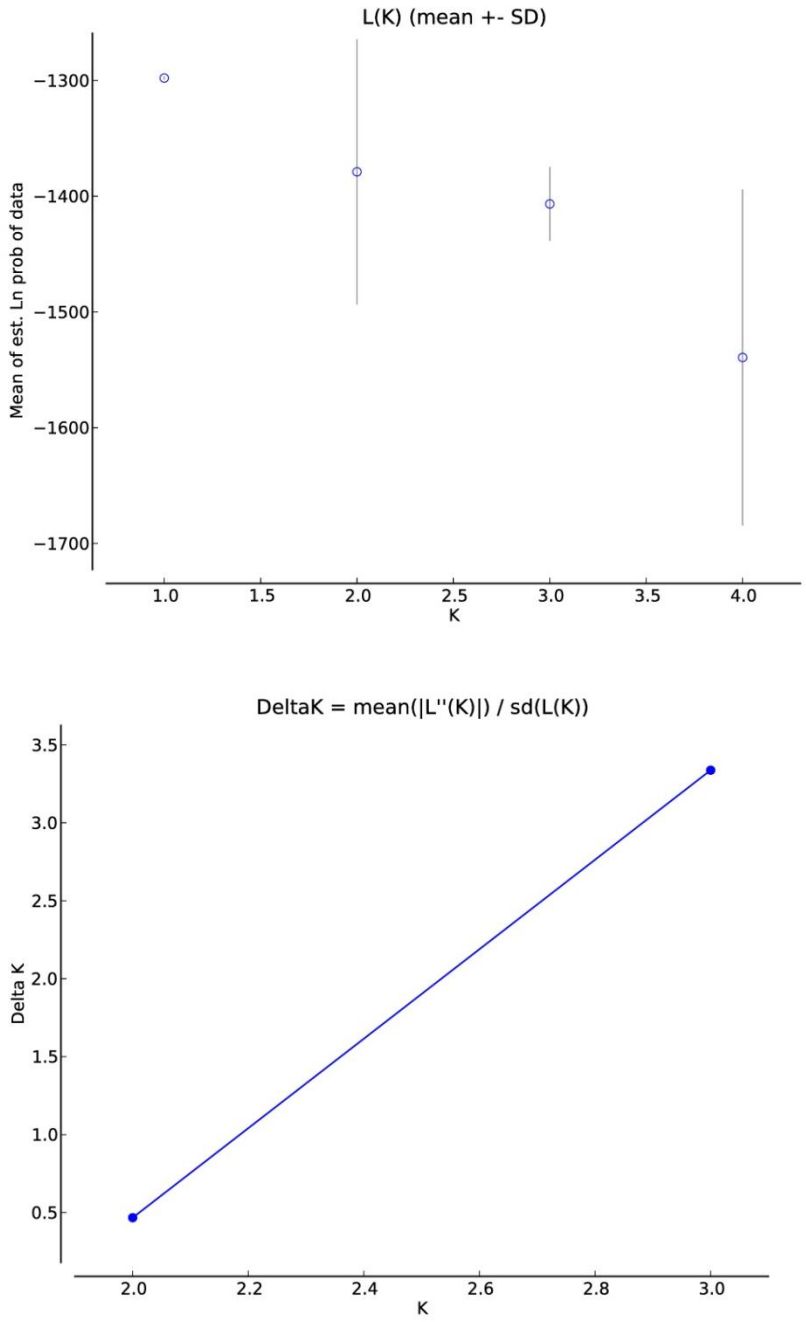


Figure A28. Mean likelihood and Delta K values for *S. atromaculatus*: Group 1.3a.

K of 1 was chosen (flat likelihood profile).

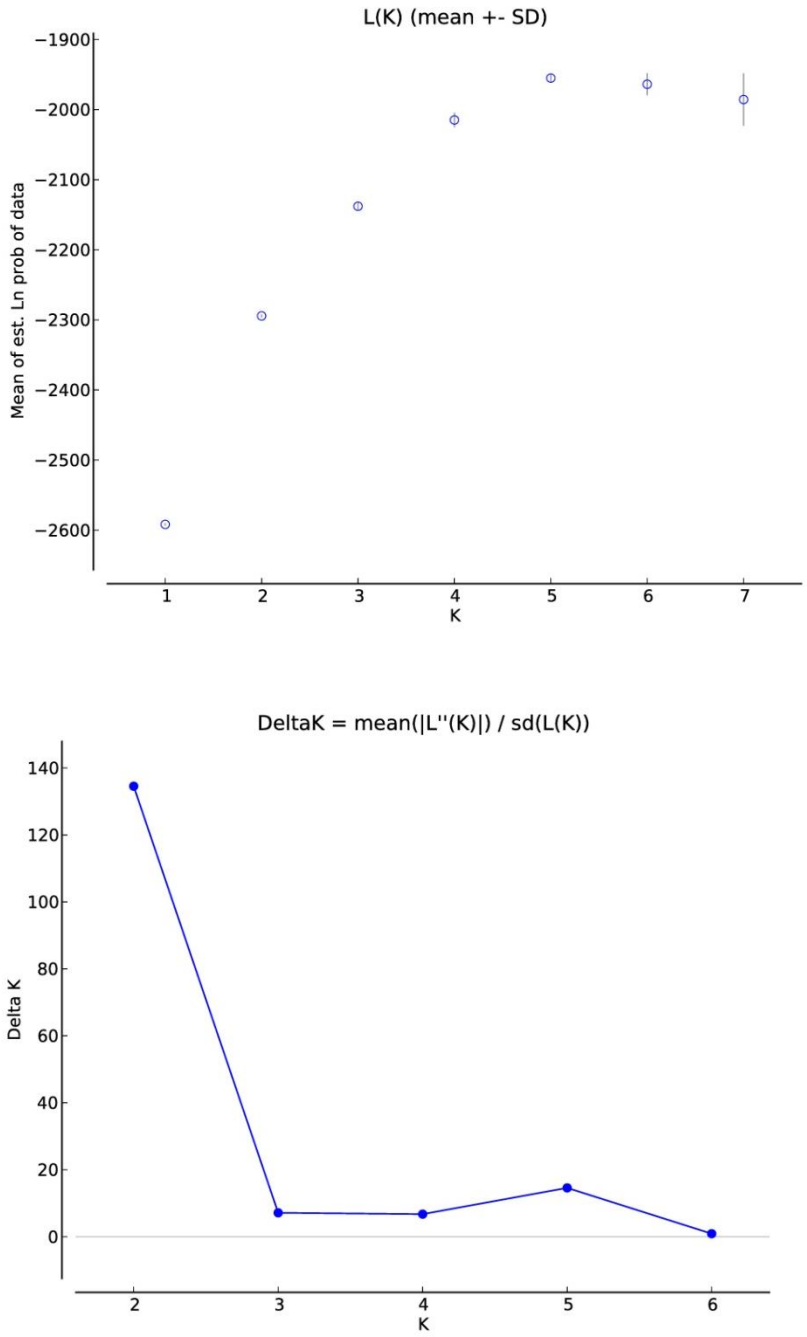


Figure A29. Mean likelihood and Delta K values for *S. atromaculatus*: Group 1.4.

K of 2 was chosen.

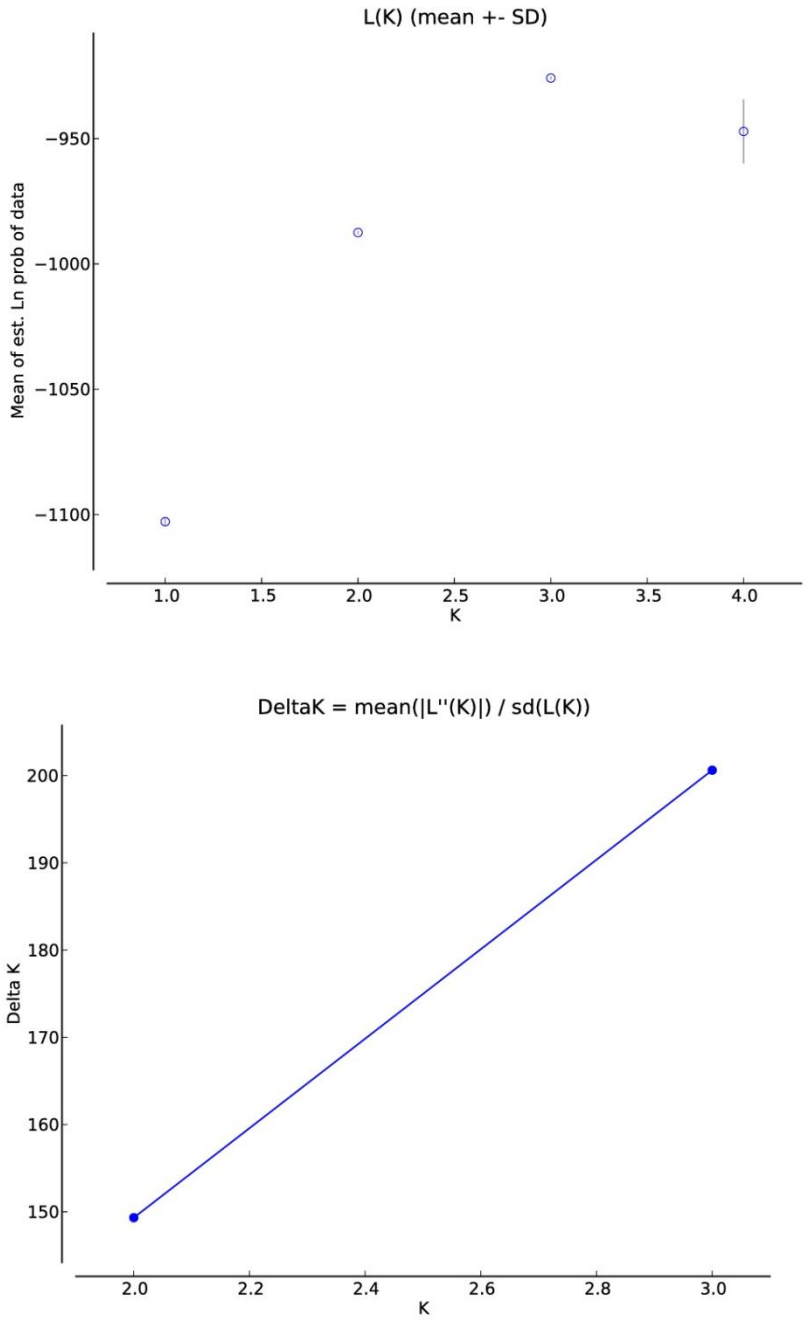


Figure A30. Mean likelihood and Delta K values for *S. atromaculatus*: Group 1.4a.

K of 3 was chosen.

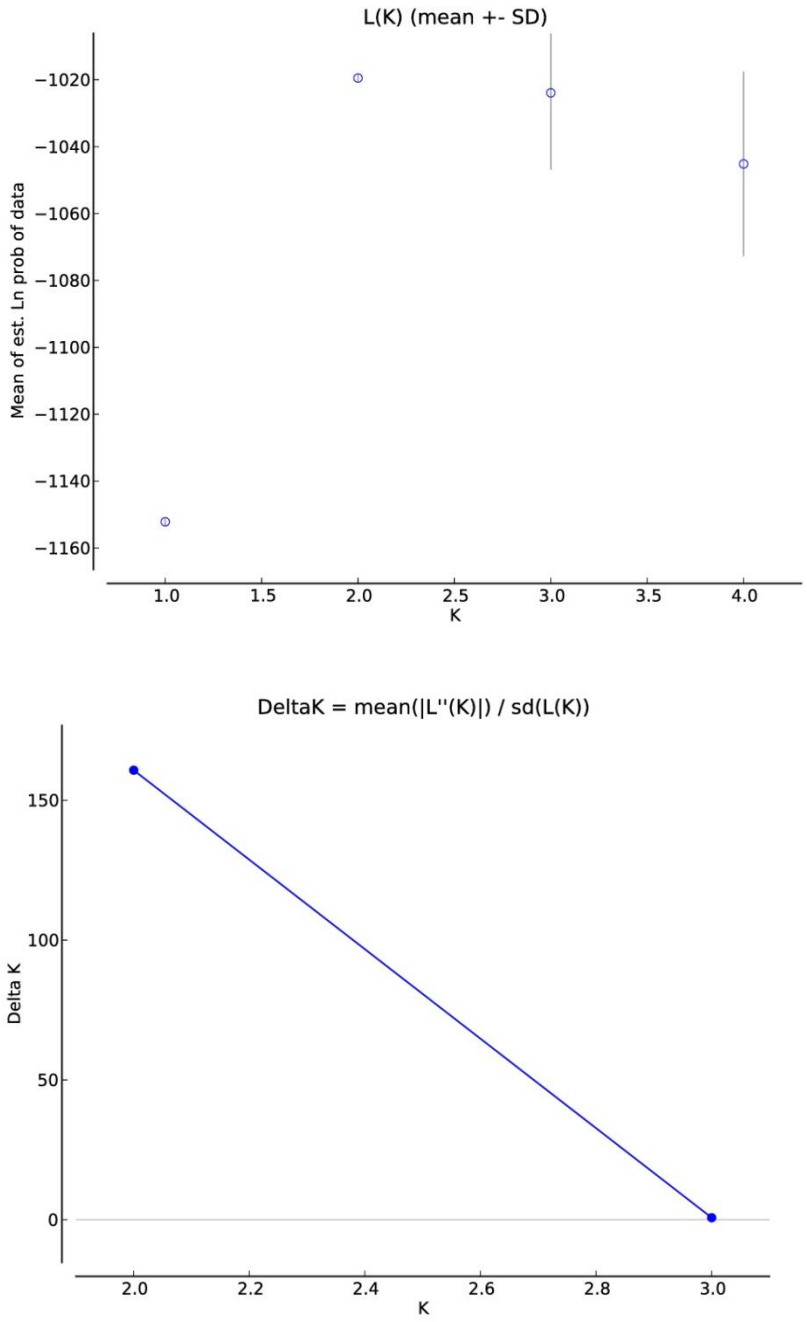


Figure A31. Mean likelihood and Delta K values for *S. atromaculatus*: Group 1.4b.

K of 2 was chosen.

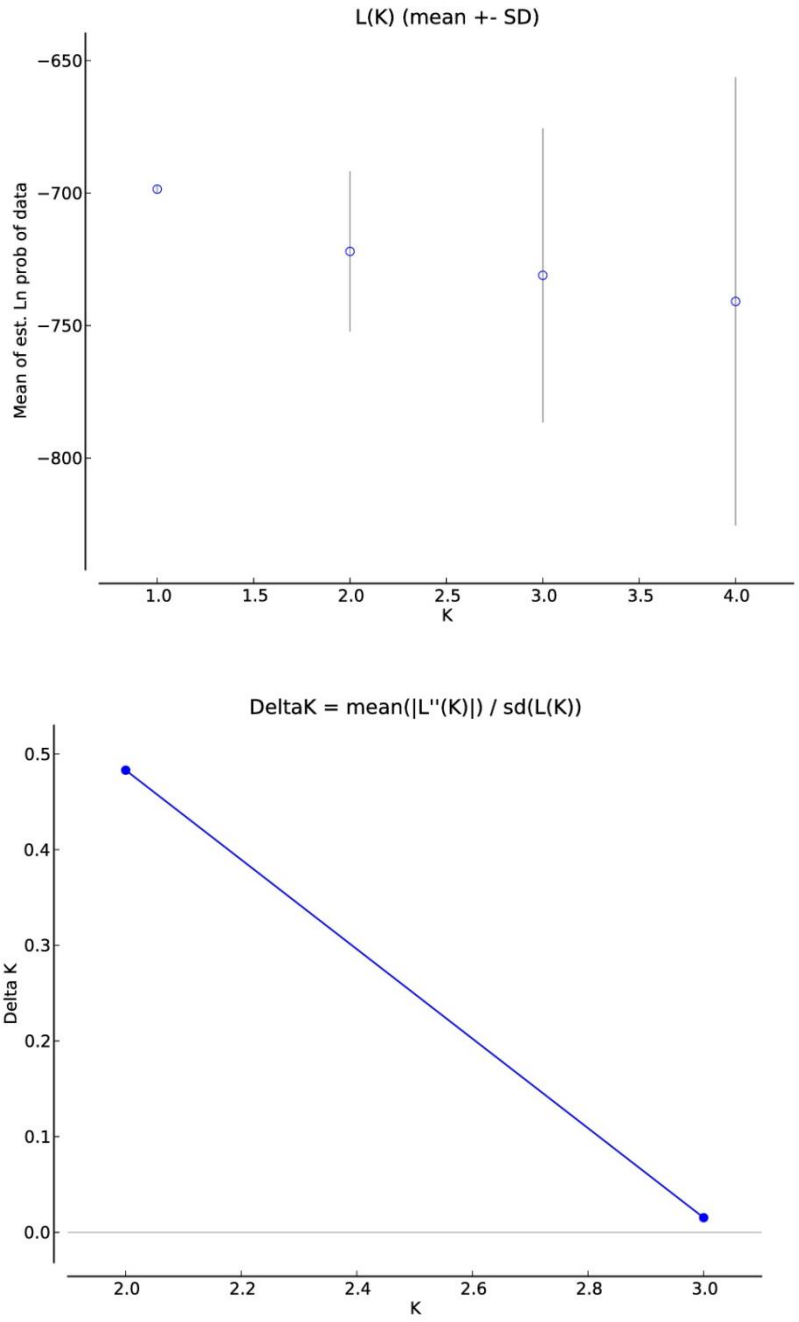


Figure A32. Mean likelihood and Delta K values for *S. atromaculatus*: Group 1.4b1.

K of 1 was chosen (Delta K < 2 and flat likelihood profile).

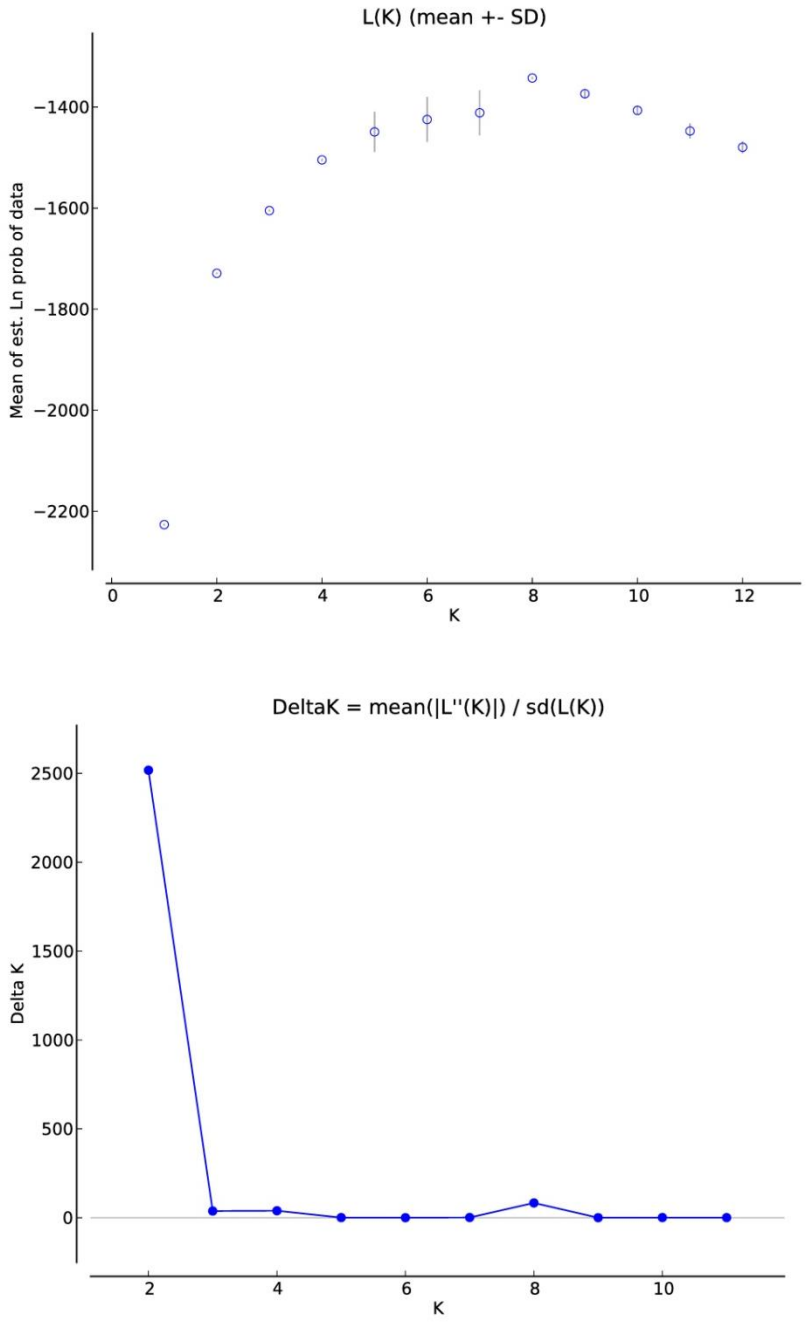


Figure A33. Mean likelihood and Delta K values for *S. atromaculatus*: Group 2.

K of 2 was chosen.

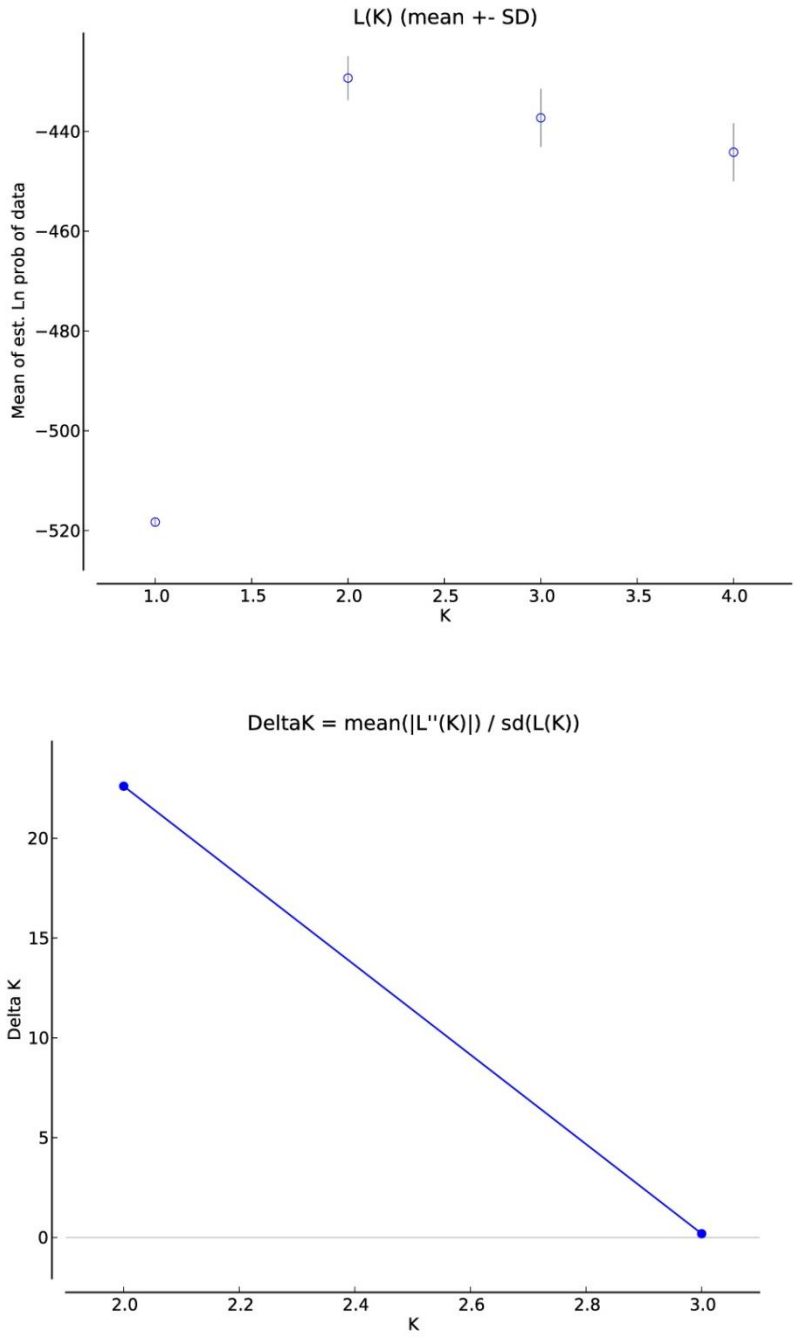


Figure A34. Mean likelihood and Delta K values for *S. atromaculatus*: Group 2.1.

K of 2 was chosen.

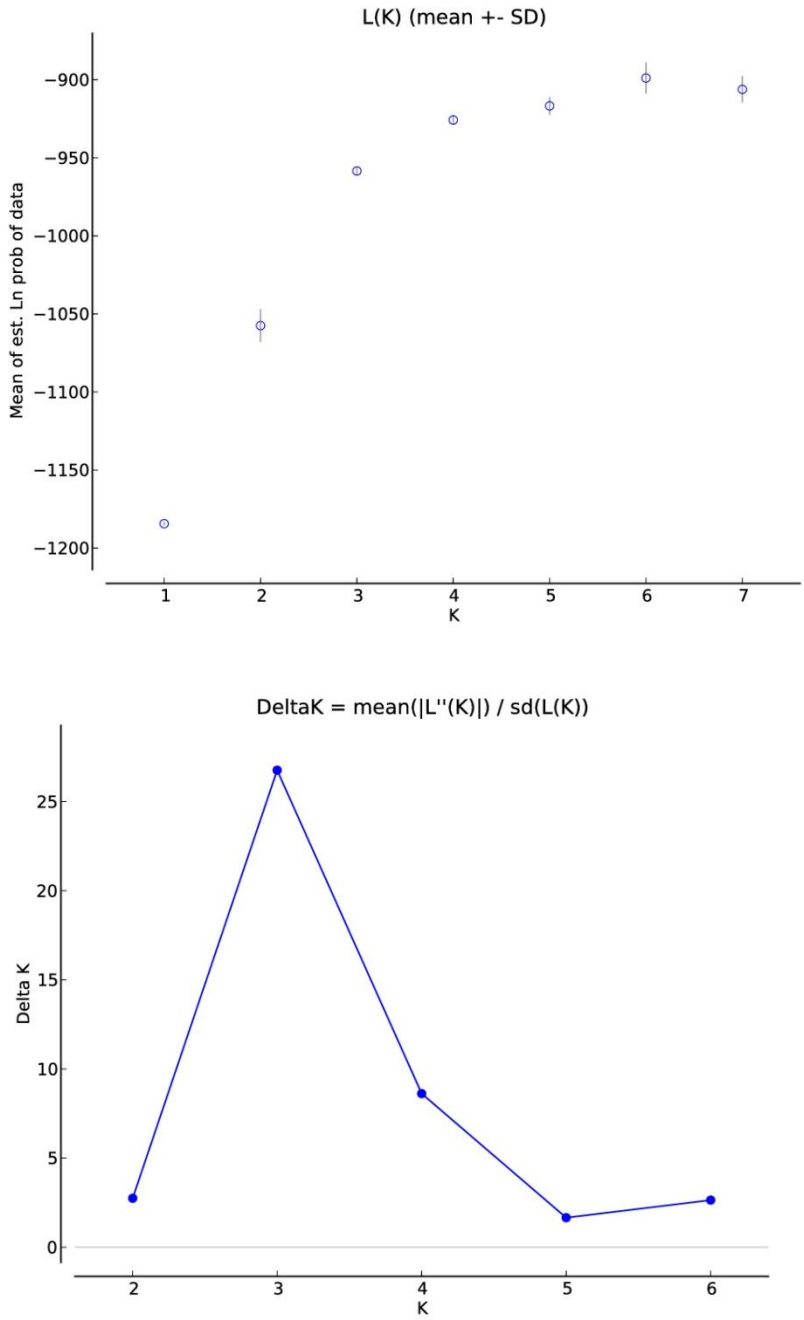


Figure A35. Mean likelihood and Delta K values for *S. atromaculatus*: Group 2.2.

K of 3 was chosen.

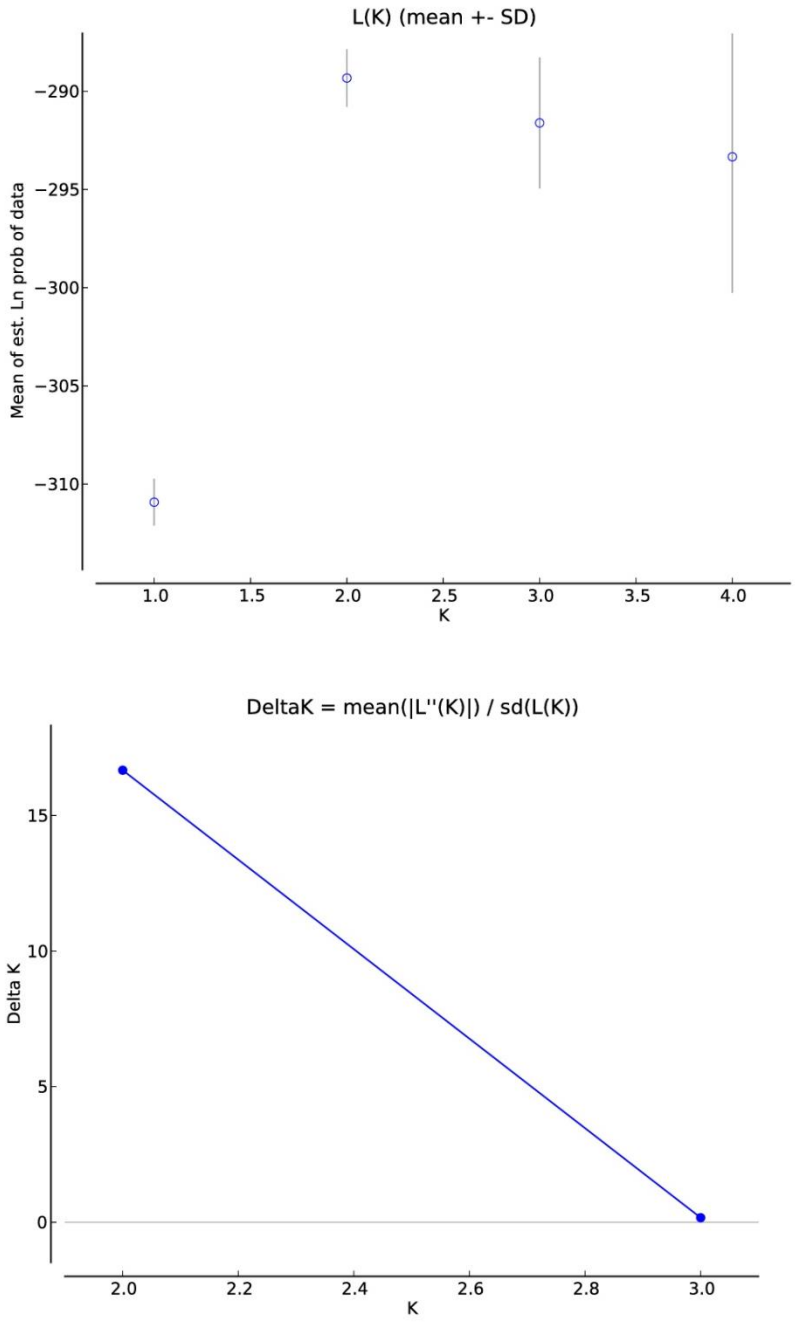


Figure A36. Mean likelihood and Delta K values for *S. atromaculatus*: Group 2.2a.

K of 2 was chosen.

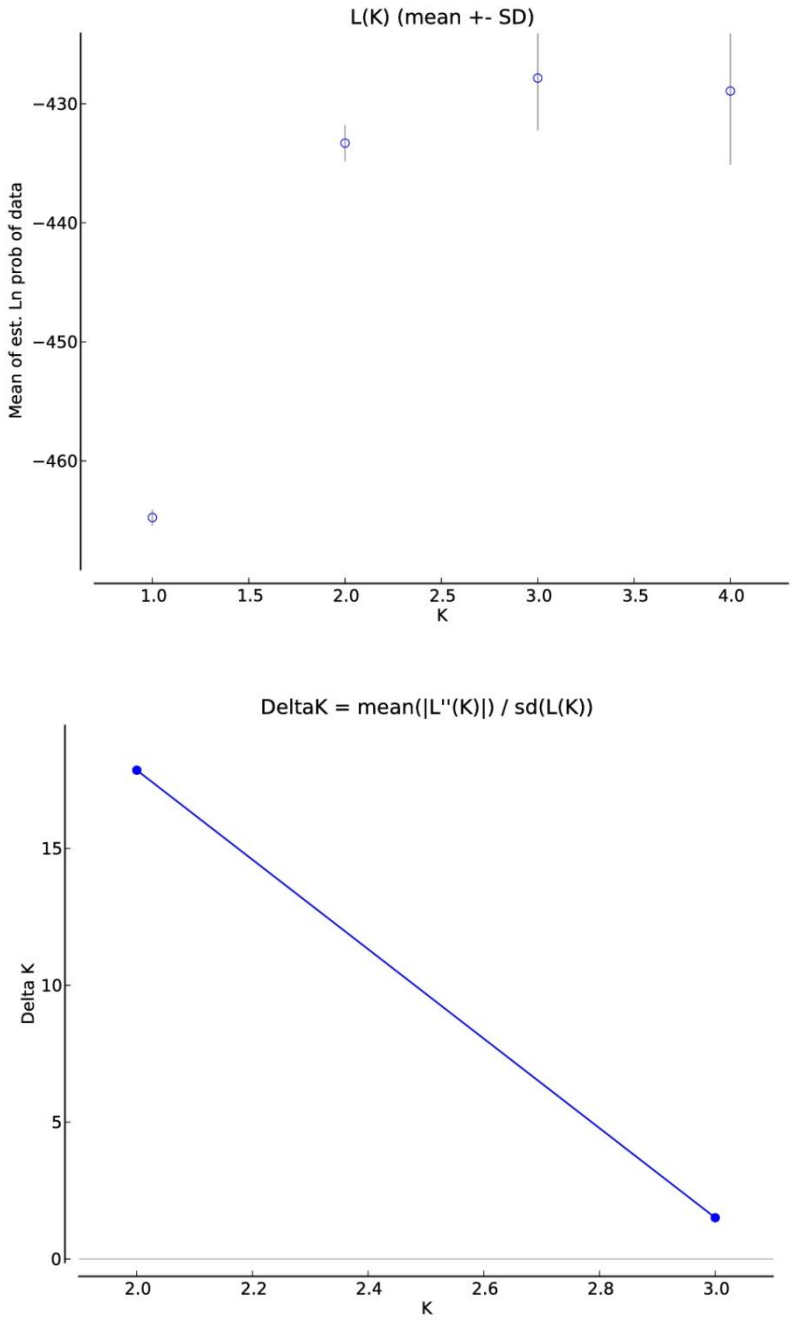


Figure A37. Mean likelihood and Delta K values for *S. atromaculatus*: Group 2.2b.

K of 2 was chosen.

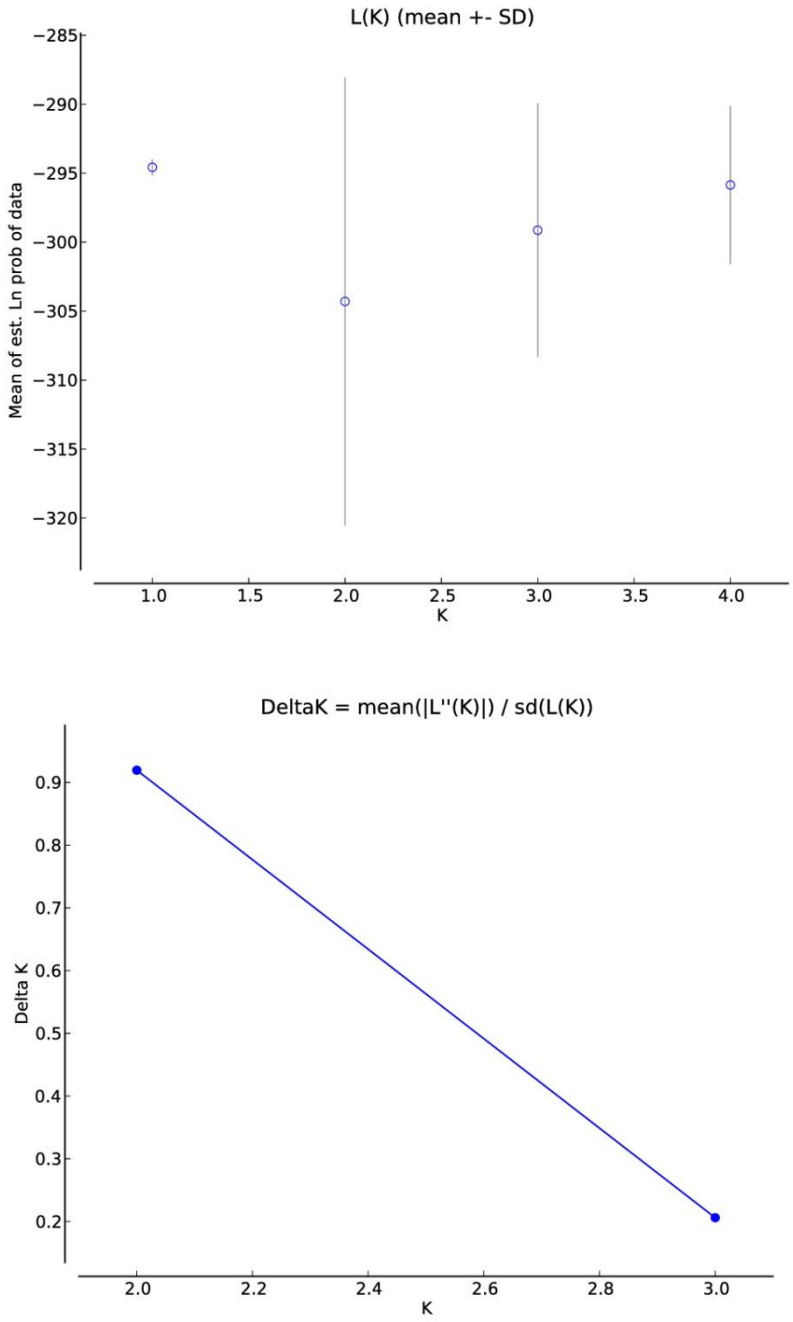


Figure A38. Mean likelihood and Delta K values for *S. atromaculatus*: Group 2.2b1.

K of 1 was chosen (Delta K < 2 and flat likelihood profile).

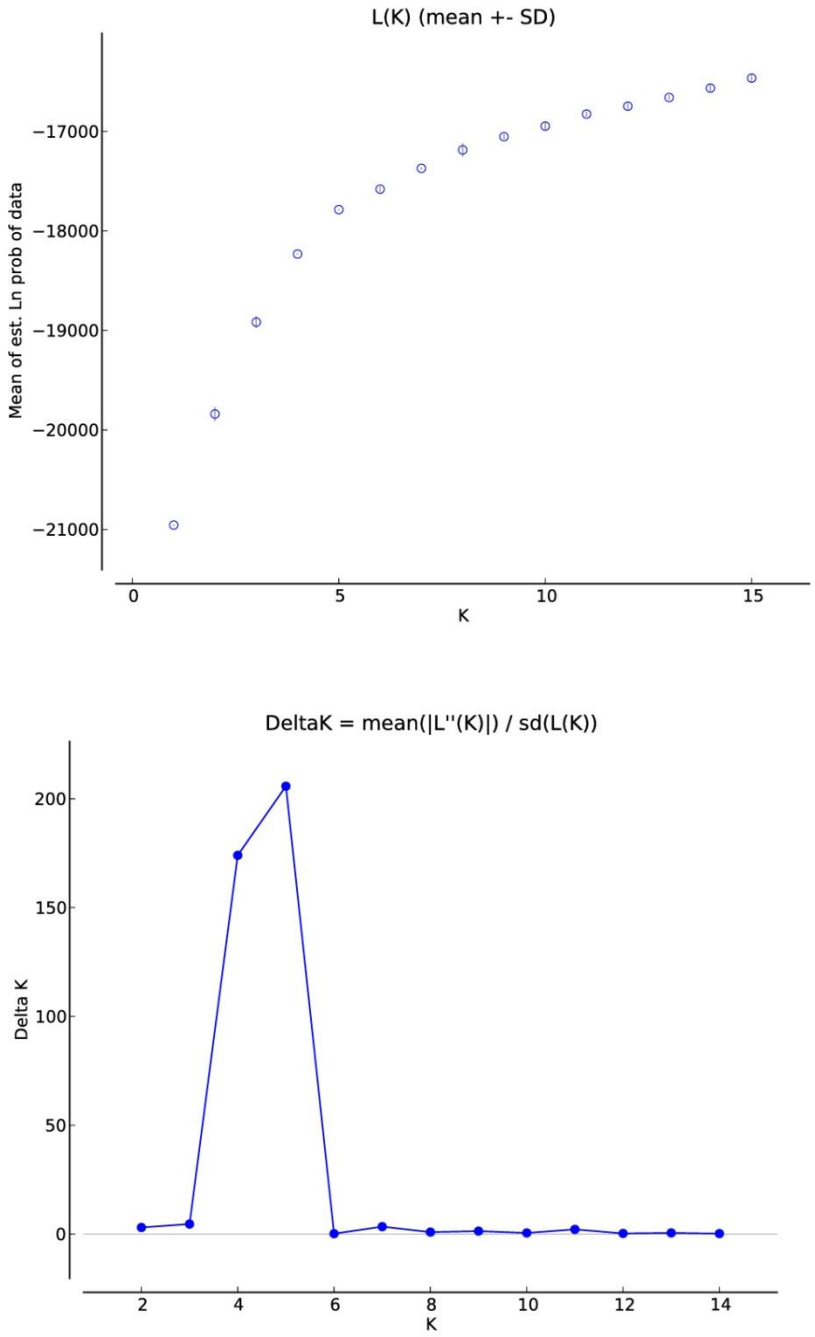


Figure A39. Mean likelihood and Delta K values for *E. claviformis*: Full Dataset.

K of 5 was chosen.

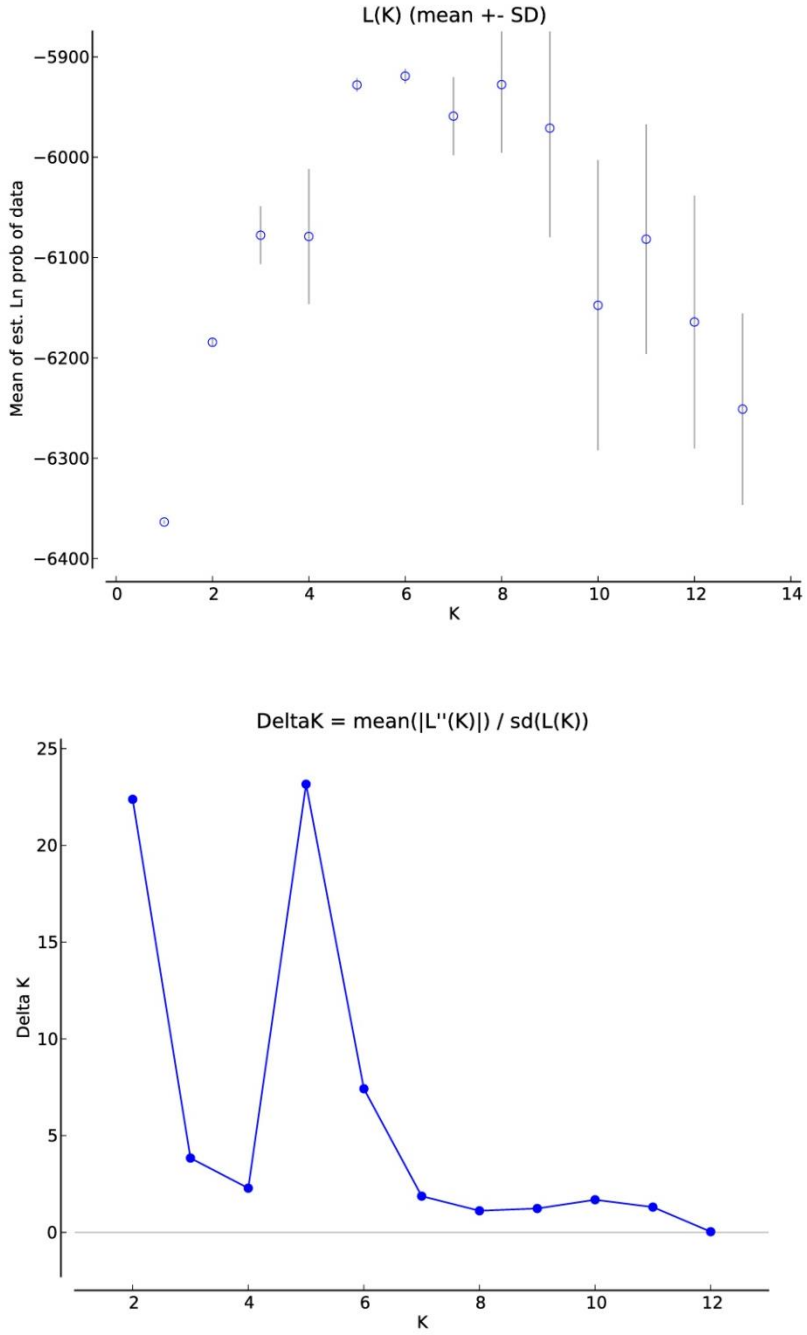


Figure A40. Mean likelihood and Delta K values for *E. claviformis*: Group 1.

K of 2 was chosen.

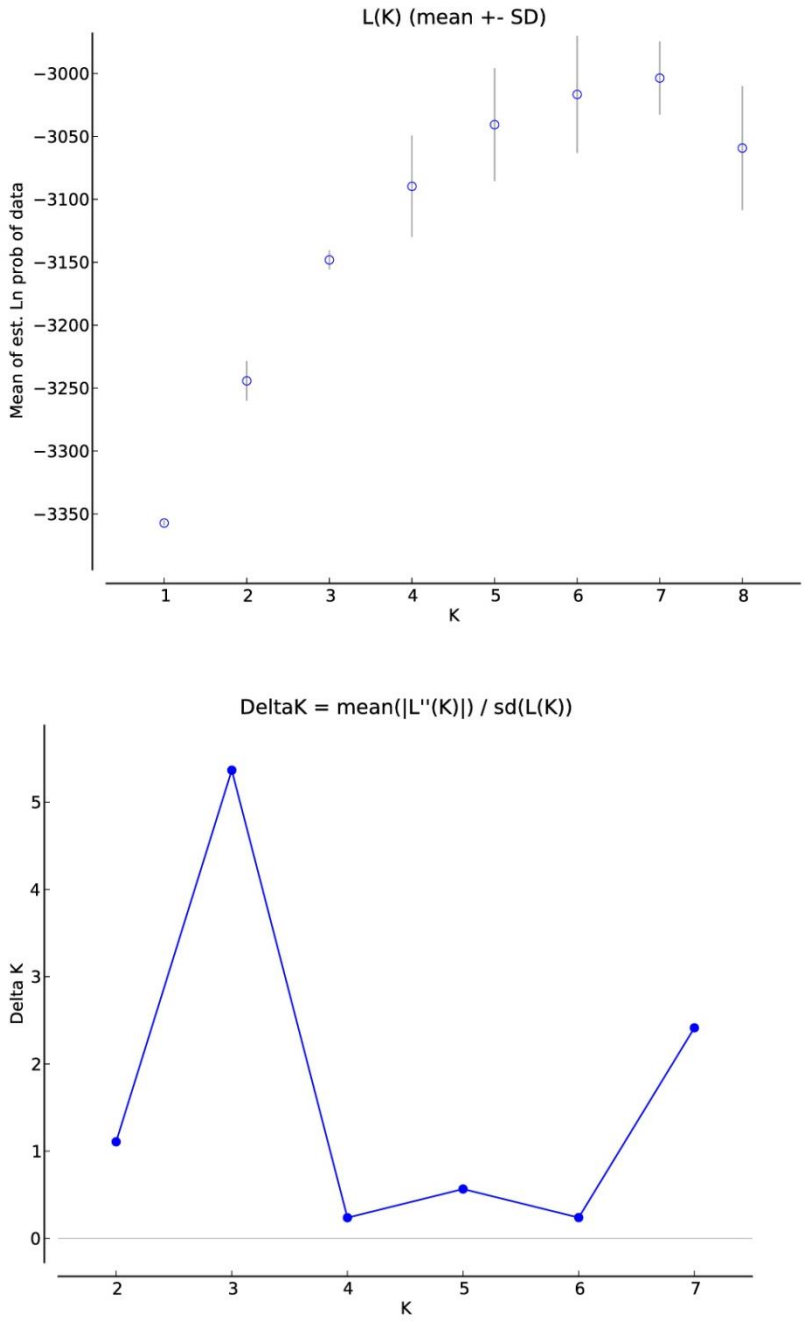


Figure A41. Mean likelihood and Delta K values for *E. claviformis*: Group 1.1.

K of 3 was chosen.

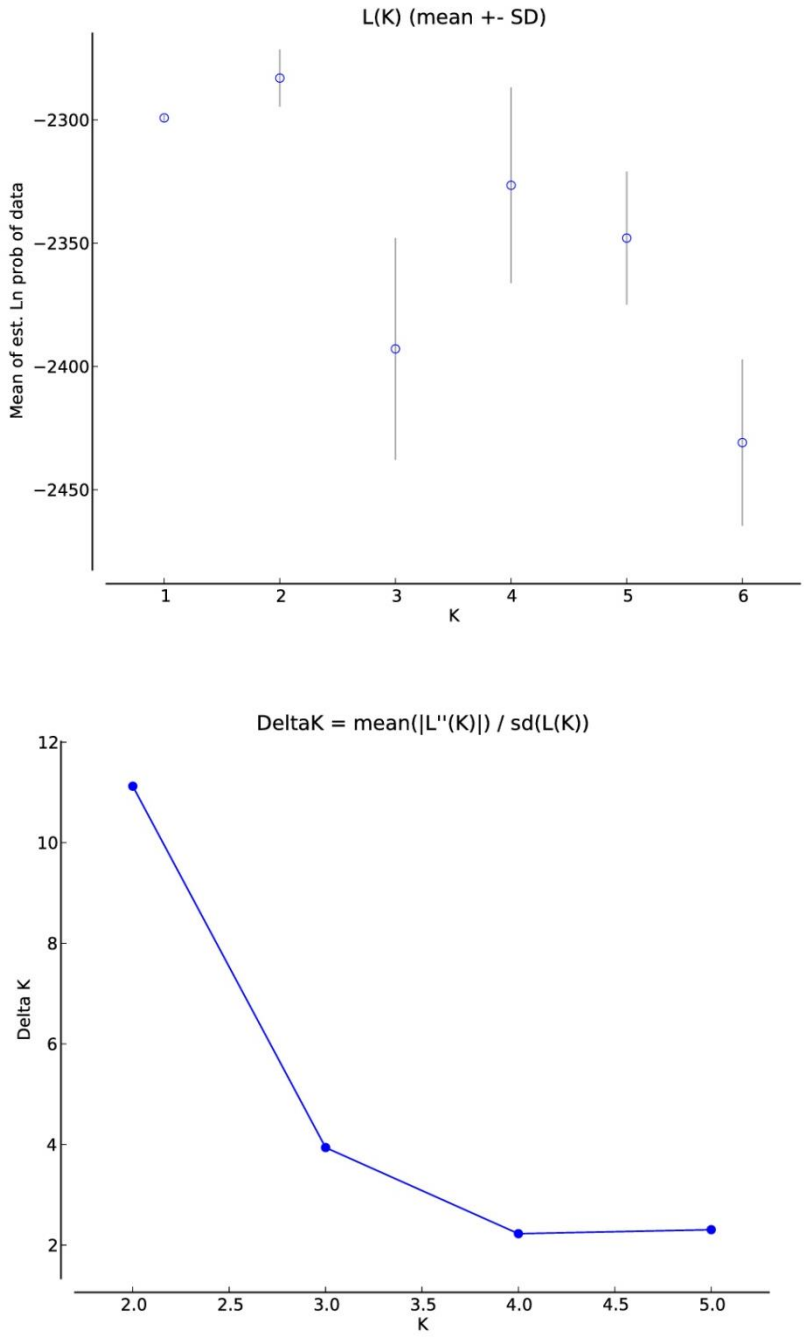


Figure A42. Mean likelihood and Delta K values for *E. claviformis*: Group 1.1a.

K of 1 was chosen (flat likelihood profile).

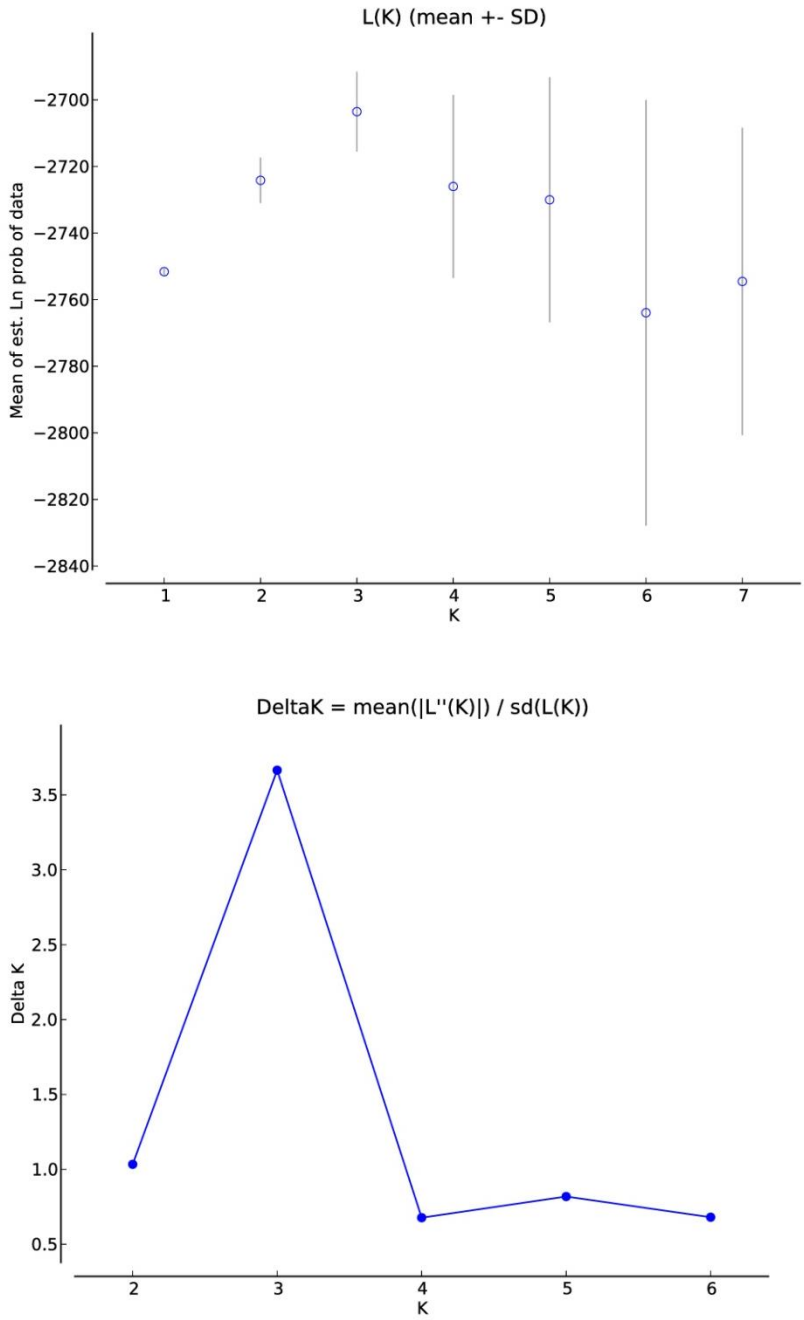


Figure A43. Mean likelihood and Delta K values for *E. claviformis*: Group 1.2.

K of 1 was chosen (low delta K and flat likelihood profile).

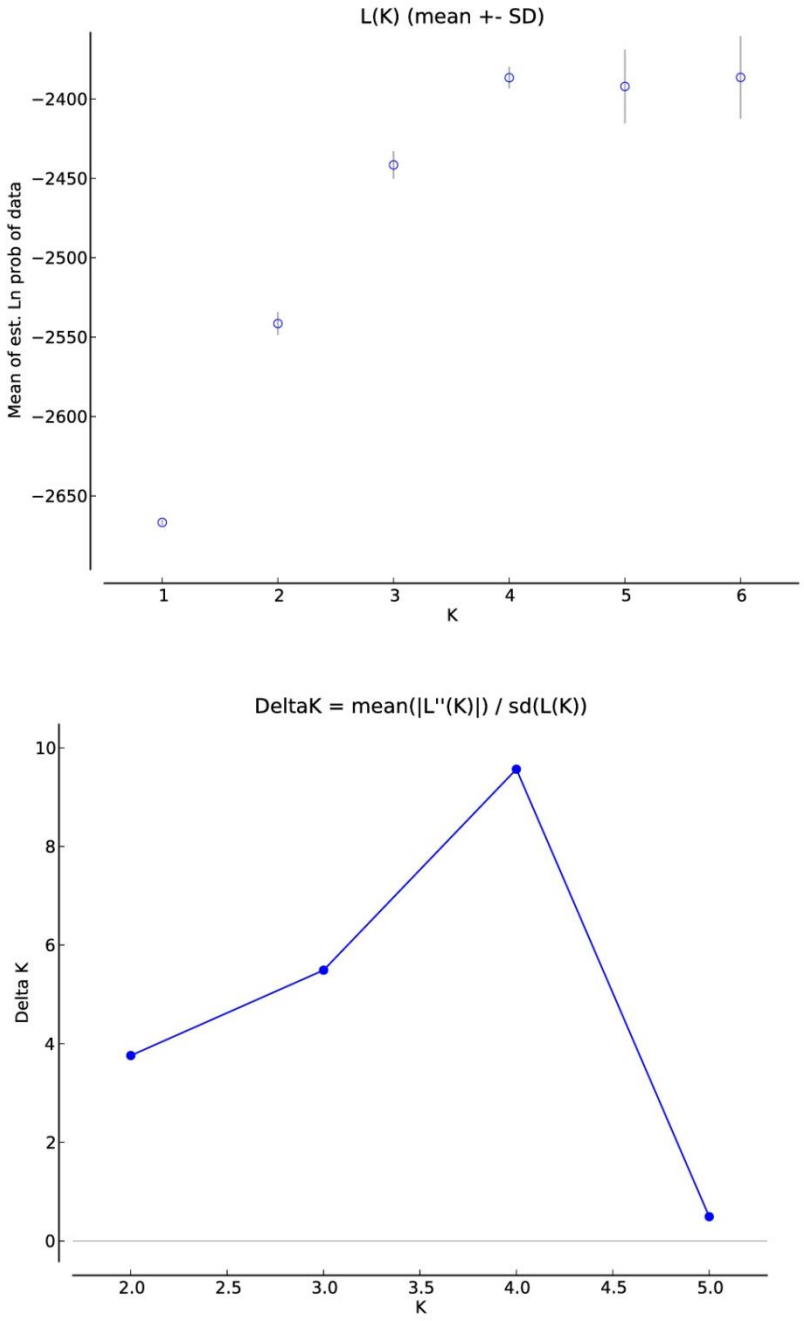


Figure A44. Mean likelihood and Delta K values for *E. claviformis*: Group 2.

K of 4 was chosen.

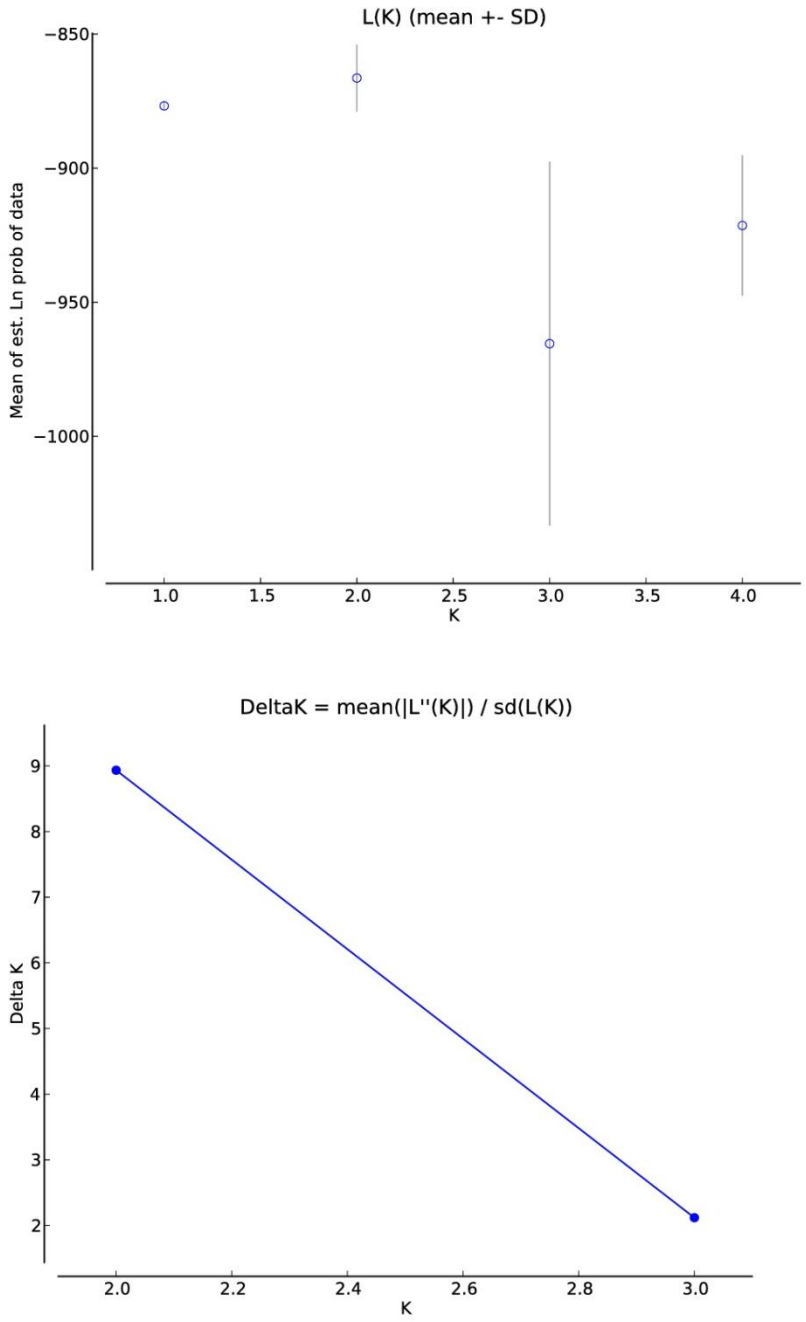


Figure A45. Mean likelihood and Delta K values for *E. claviformis*: Group 2.1.

K of 1 was chosen (flat likelihood profile).

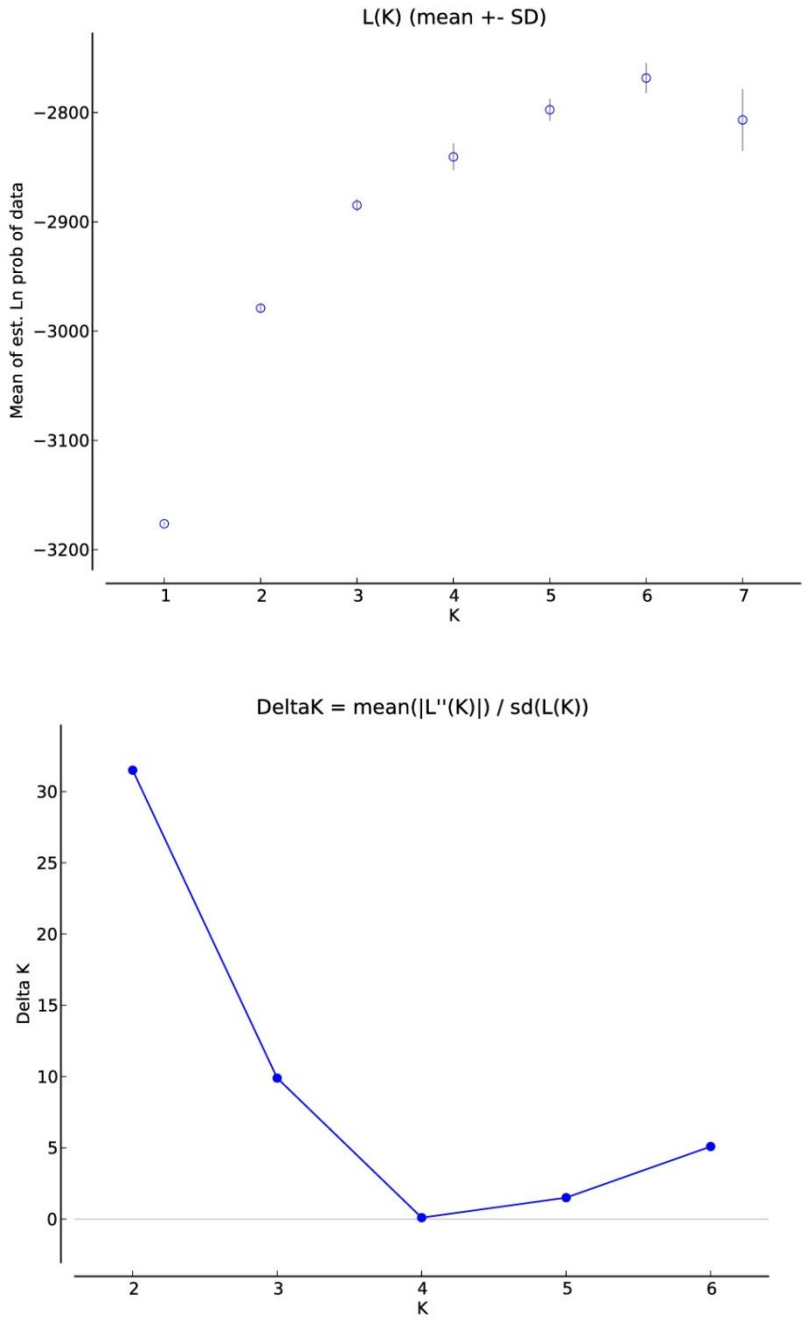


Figure A46. Mean likelihood and Delta K values for *E. claviformis*: Group 3.

K of 2 was chosen.

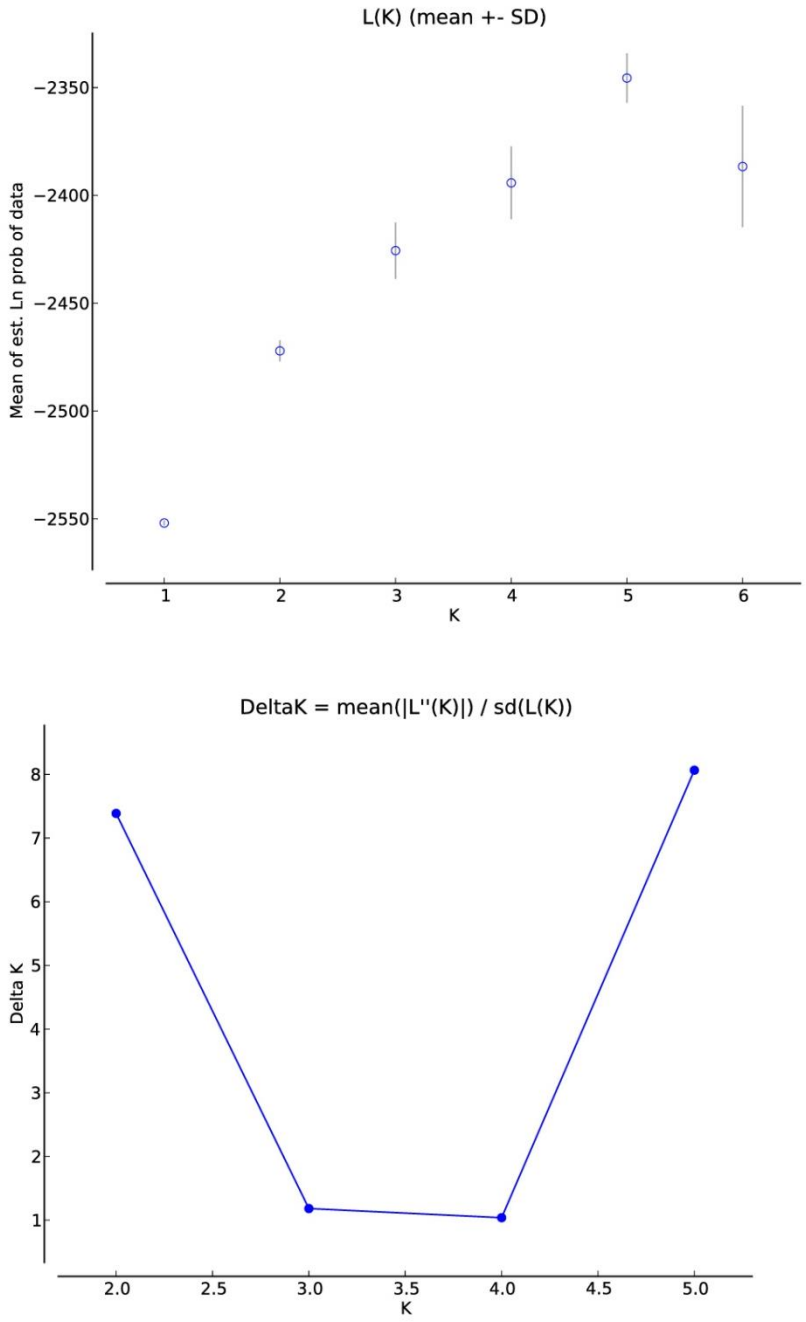


Figure A47. Mean likelihood and Delta K values for *E. claviformis*: Group 3.1.

K of 5 was chosen.

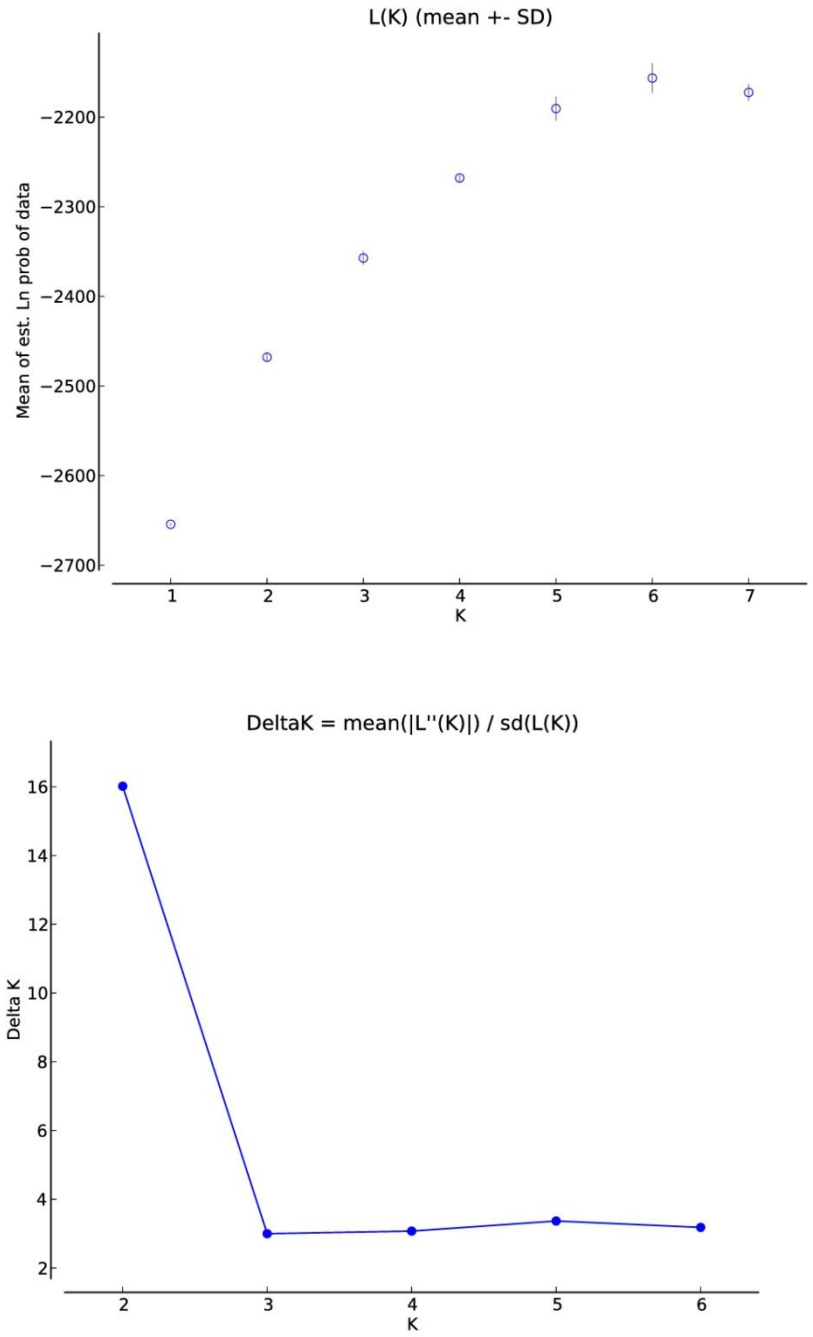


Figure A48. Mean likelihood and Delta K values for *E. claviformis*: Group 4.

K of 2 was chosen.

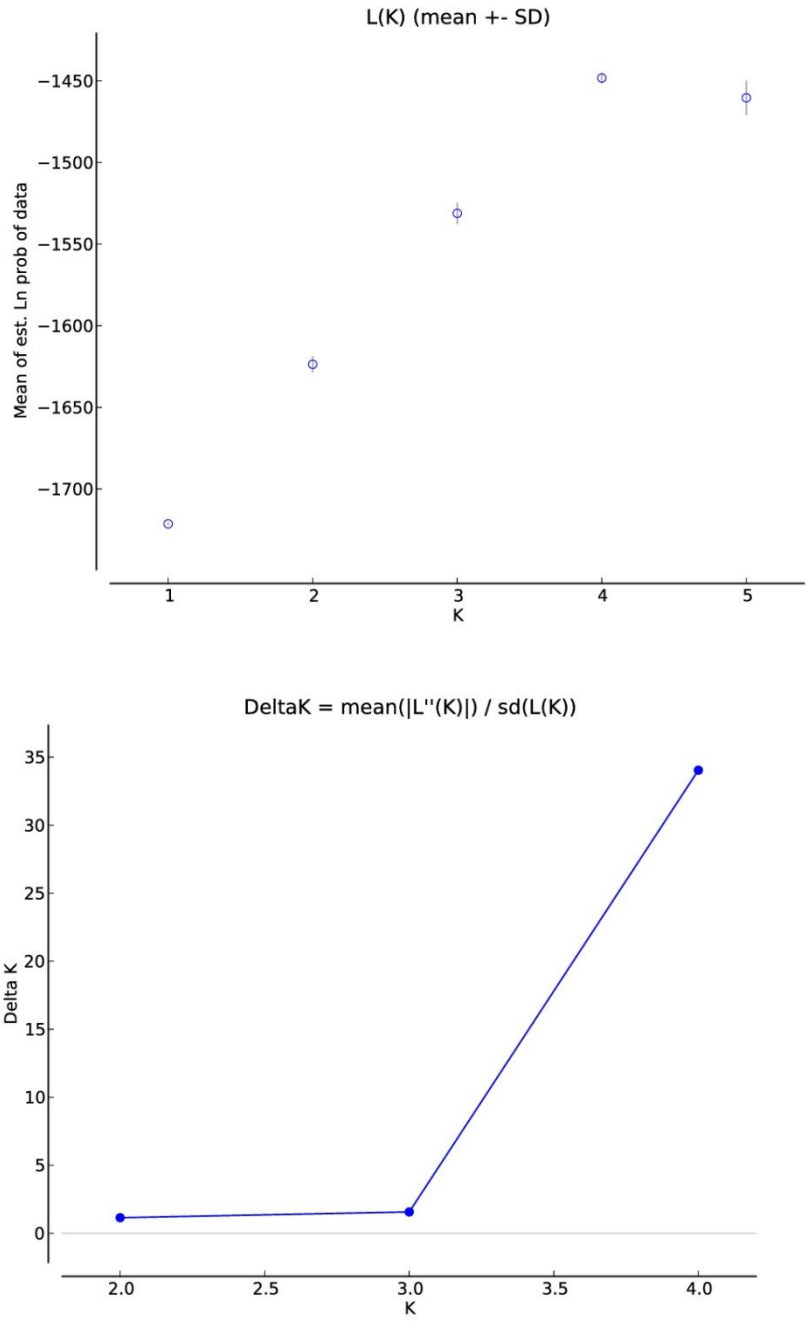


Figure A49. Mean likelihood and Delta K values for *E. claviformis*: Group 4.1.

K of 4 was chosen.

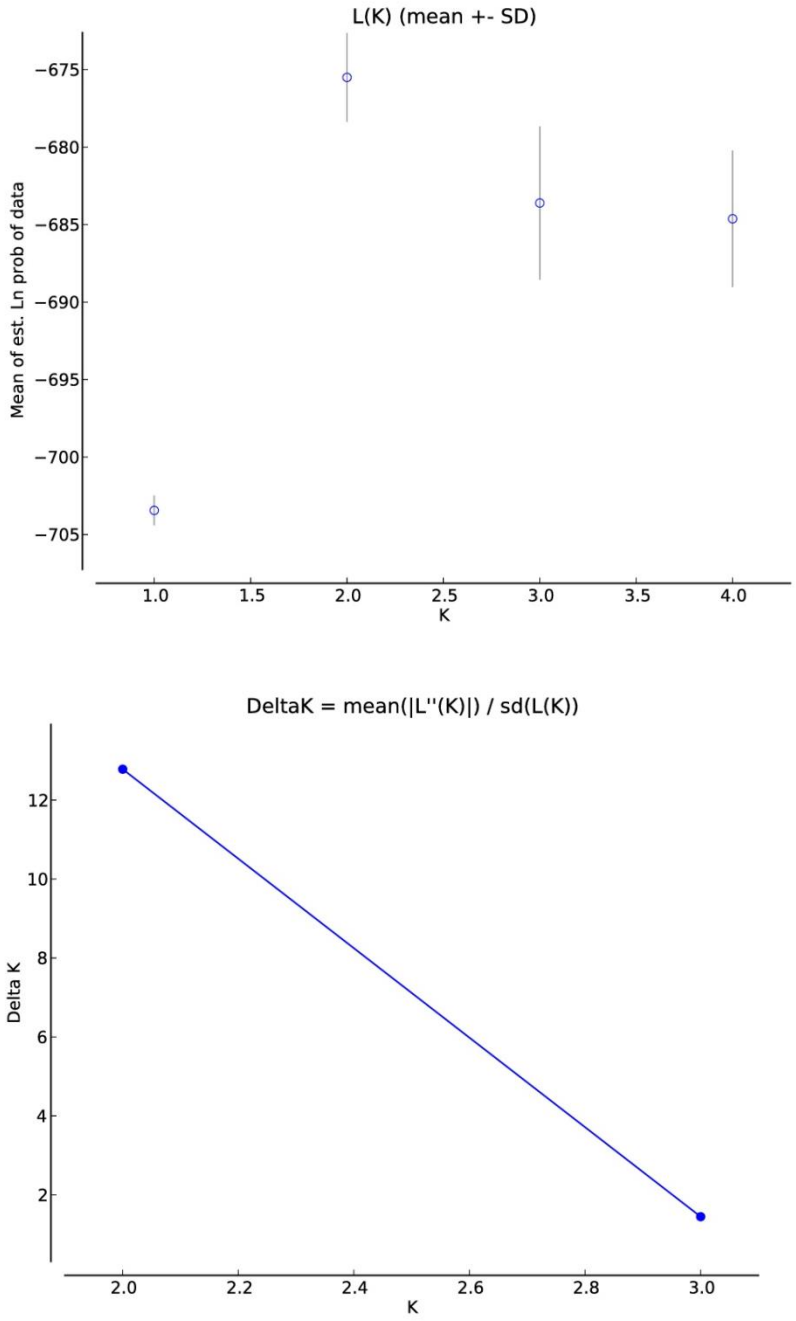


Figure A50. Mean likelihood and Delta K values for *E. claviformis*: Group 4.2.

K of 2 was chosen.

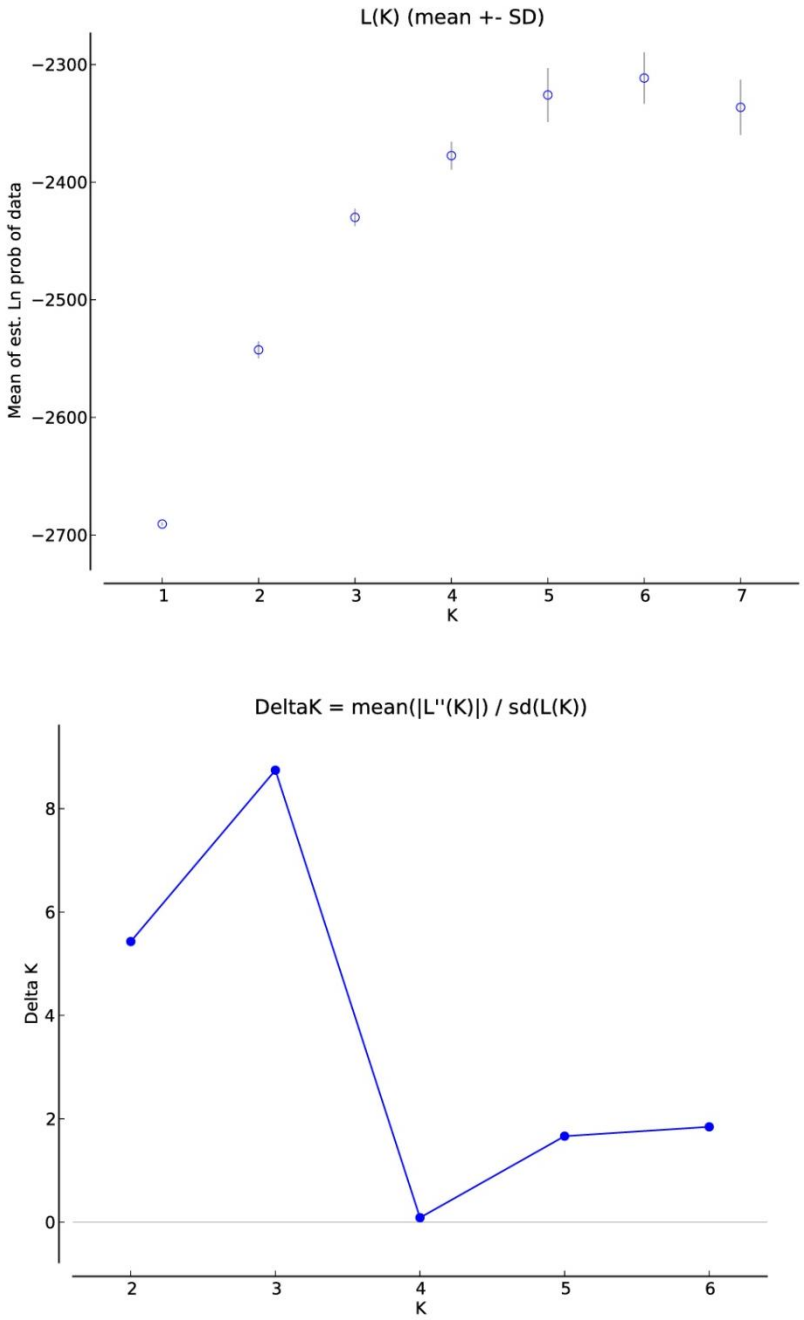


Figure A51. Mean likelihood and Delta K values for *E. claviformis*: Group 5.

K of 3 was chosen.

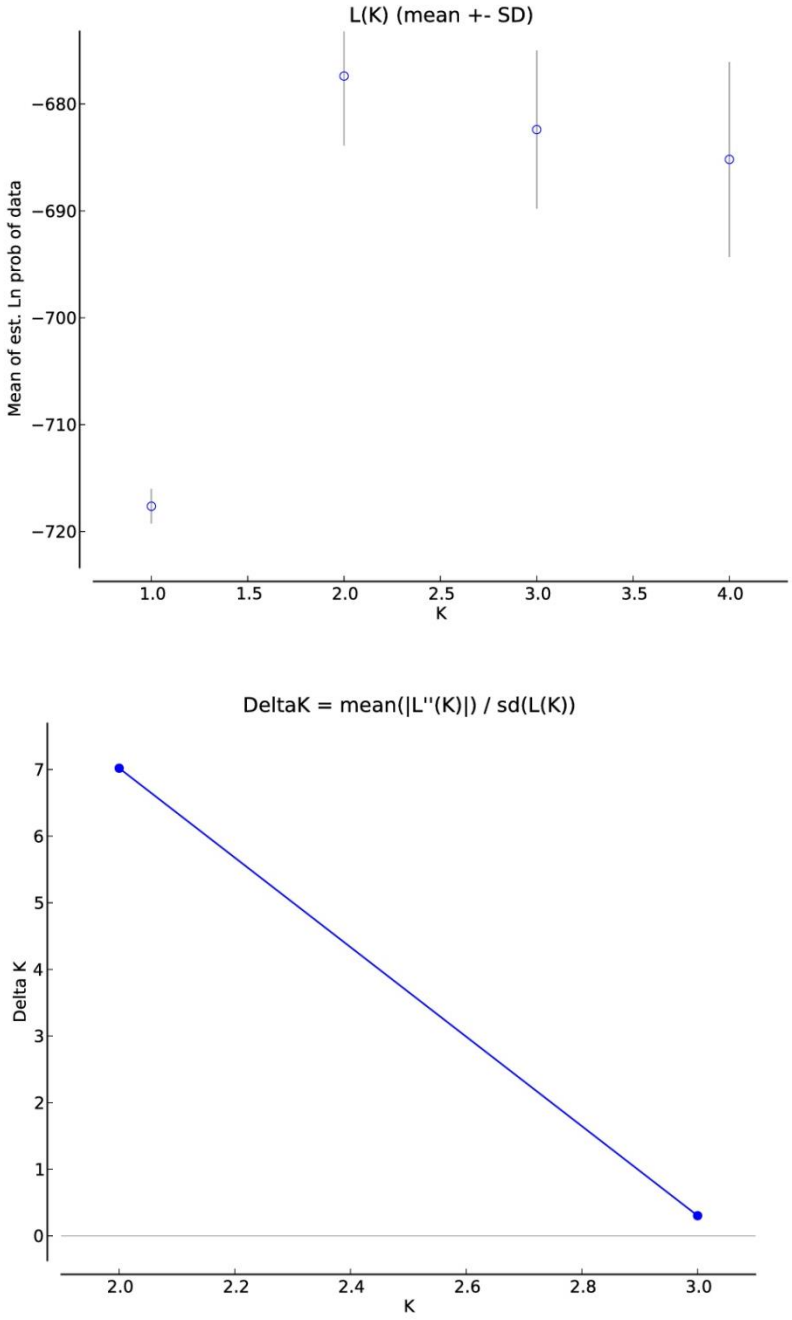


Figure A52. Mean likelihood and Delta K values for *E. claviformis*: Group 5.1.

K of 2 was chosen.

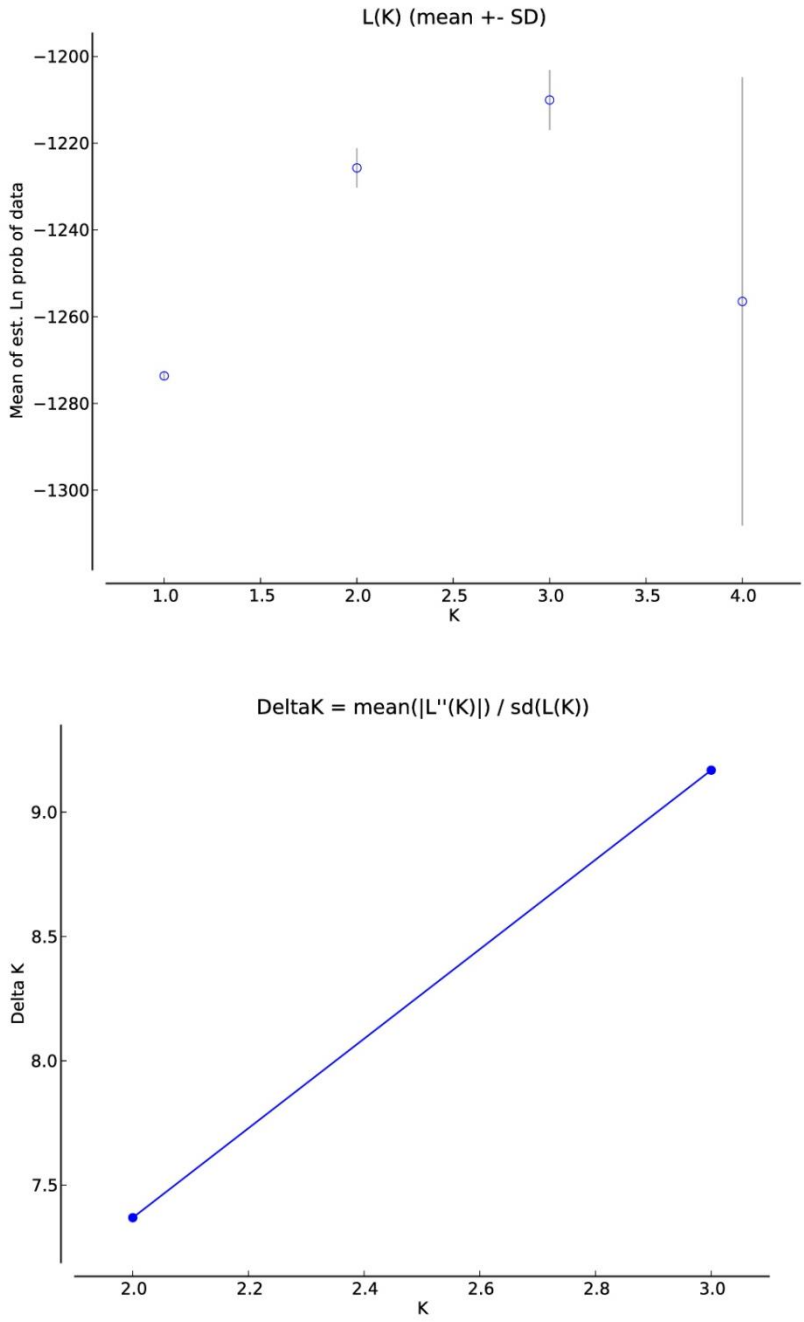


Figure A53. Mean likelihood and Delta K values for *E. claviformis*: Group 5.2.

K of 2 was chosen. A K of 2 was chosen over a K of 3 because they had comparable scores, and a K of 2 resulted in cleaner groupings.

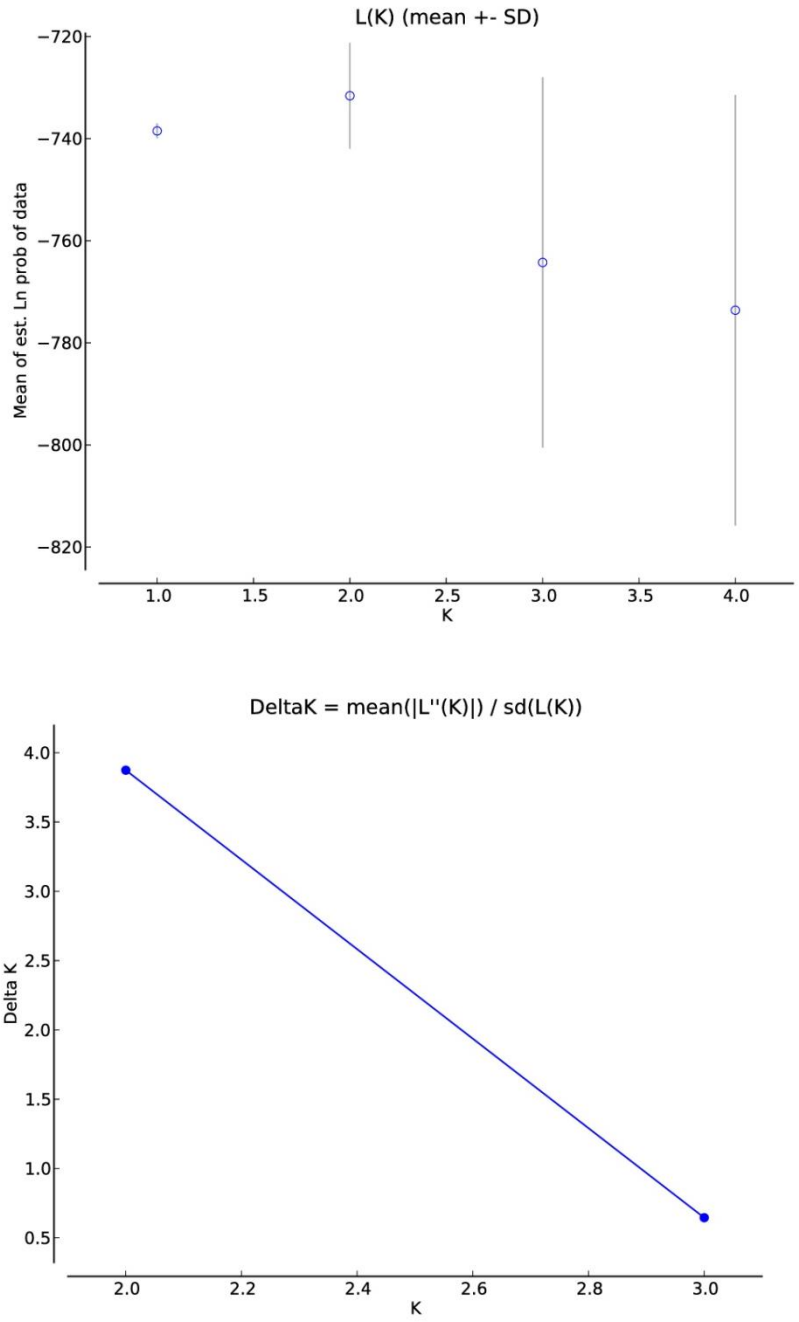


Figure A54. Mean likelihood and Delta K values for *E. claviformis*: Group 5.2a.

K of 1 was chosen (flat likelihood profile).

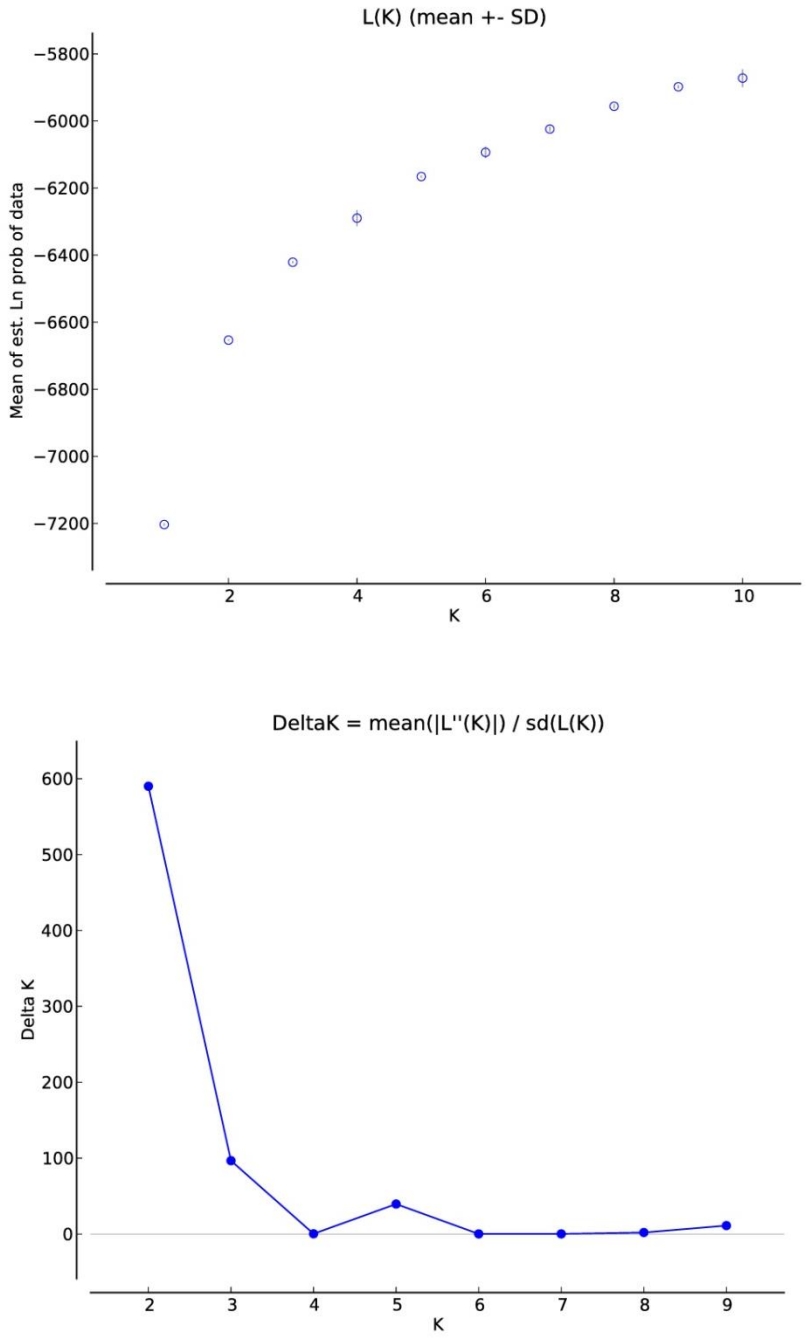


Figure A55. Mean likelihood and Delta K values for *E. artesiae*: Full Dataset.

K of 2 was chosen.

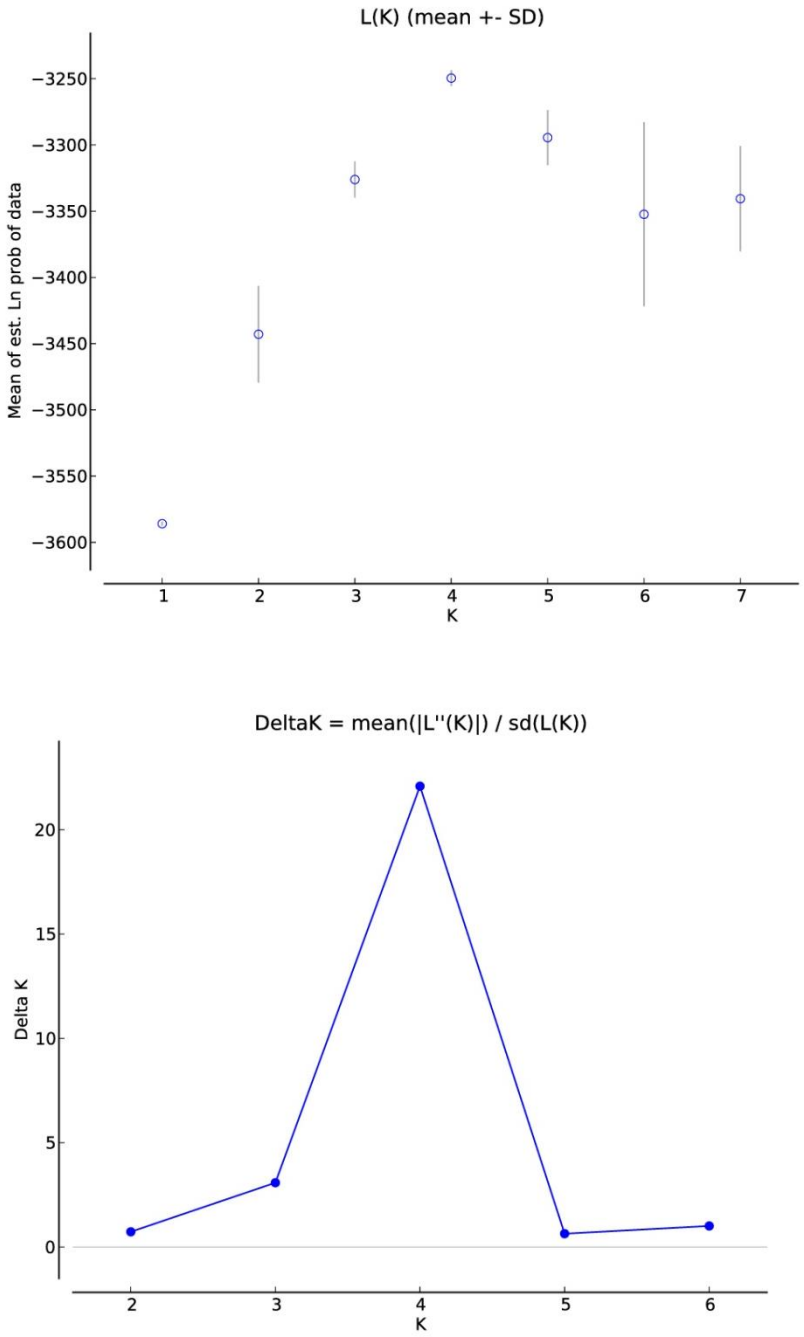


Figure A56. Mean likelihood and Delta K values for *E. artesiae*: Group 1.

K of 4 was chosen.

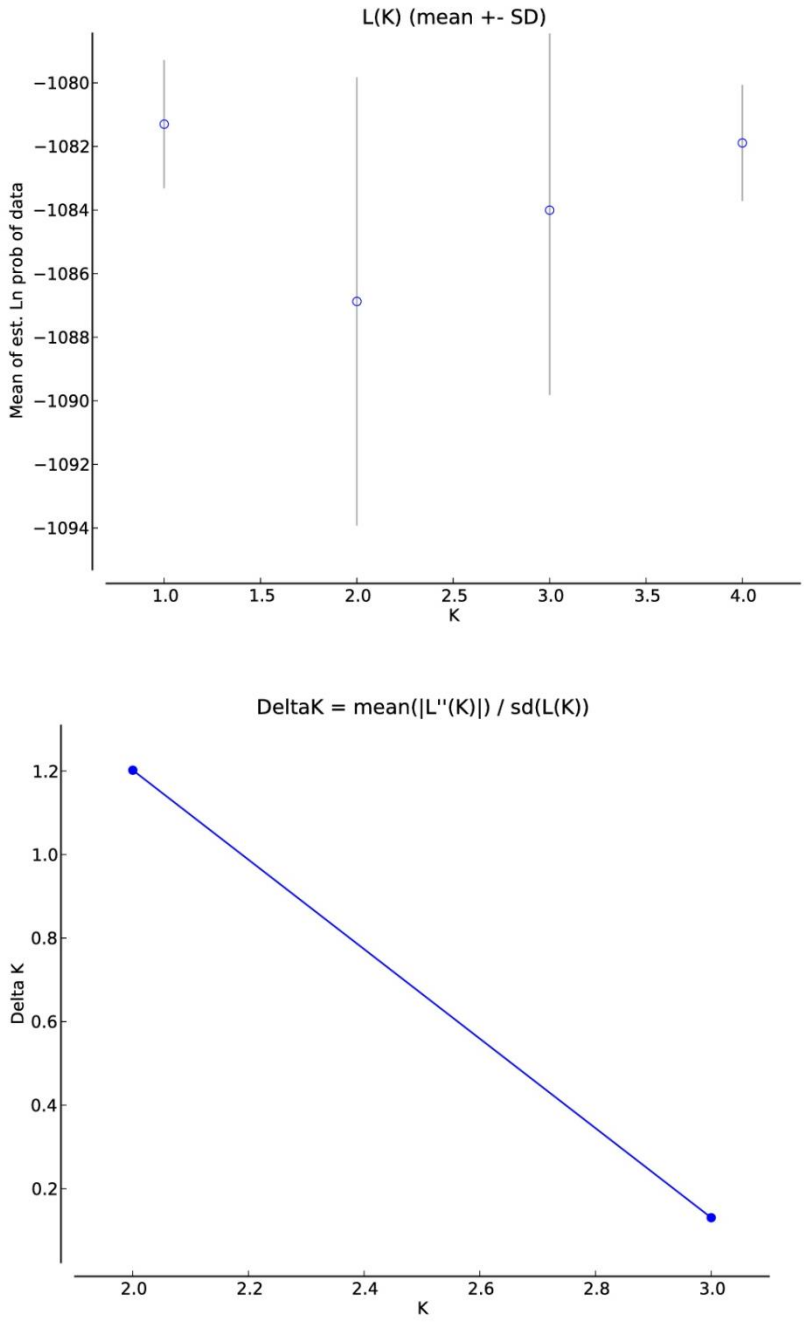


Figure A57. Mean likelihood and Delta K values for *E. artesiae*: Group 1.1.

K of 1 was chosen (Delta K < 2 and flat likelihood profile).

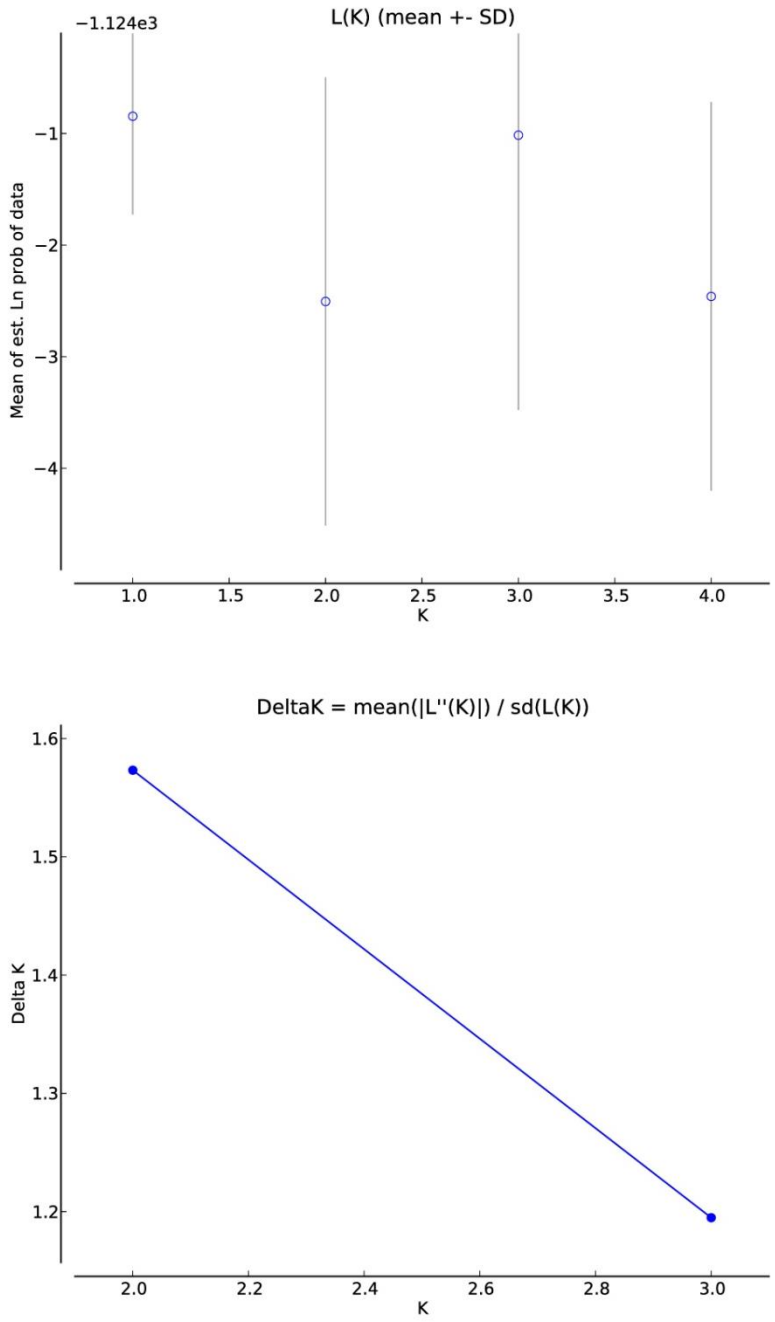


Figure A58. Mean likelihood and Delta K values for *E. artesiae*: Group 1.2.

K of 1 was chosen (Delta K < 2 and flat likelihood profile).

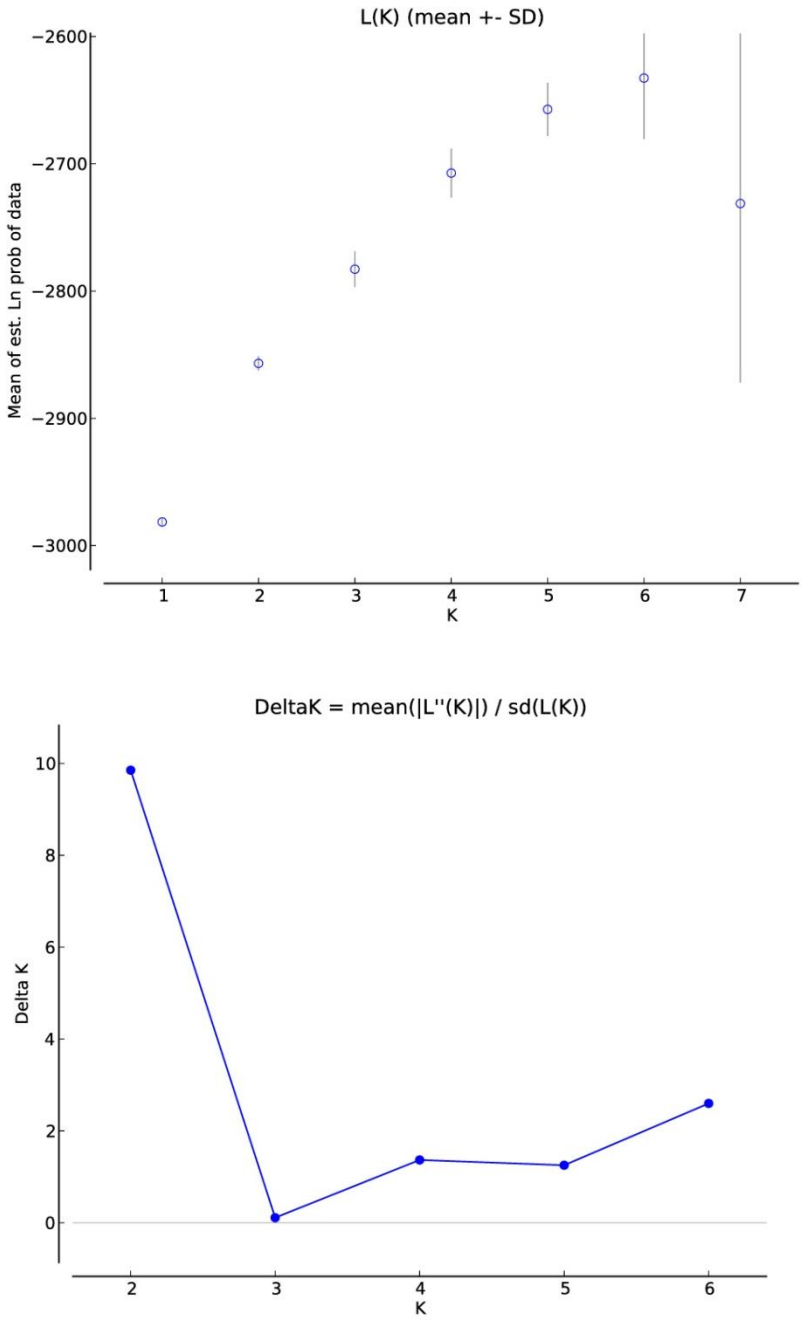
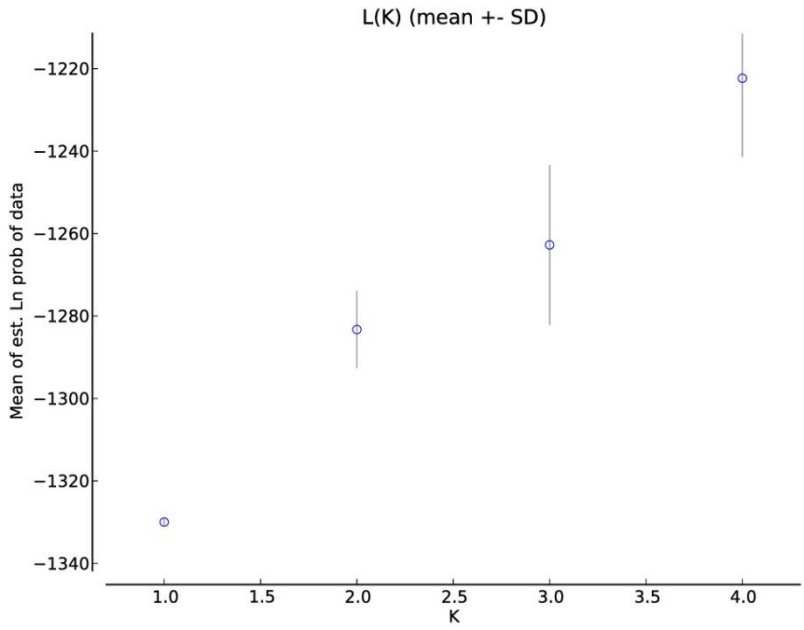


Figure A59. Mean likelihood and Delta K values for *E. artesiae*: Group 2.

K of 2 was chosen.



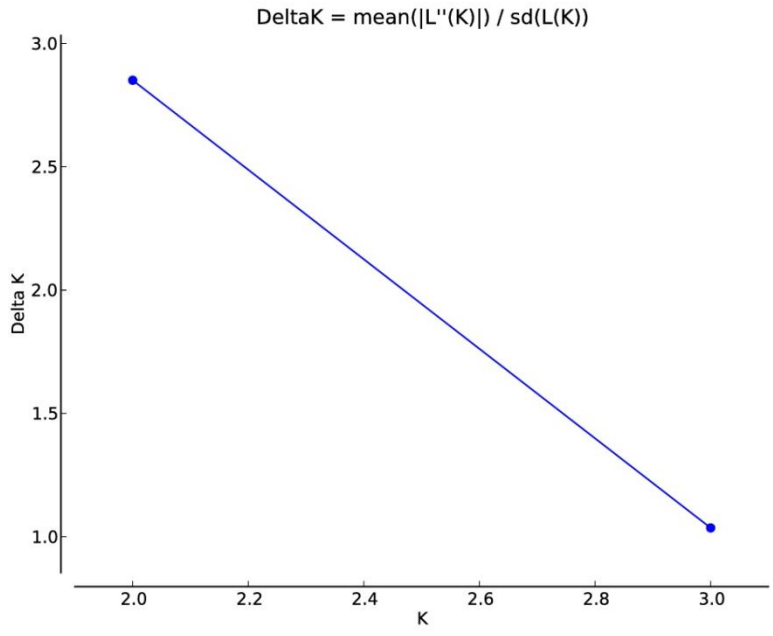


Figure A60. Mean likelihood and Delta K values for *E. artesiae*: Group 2.1.

K of 1 was chosen (low Delta K value and all sites with high admixture at K of 2; no individual q score for either group was > 0.6).

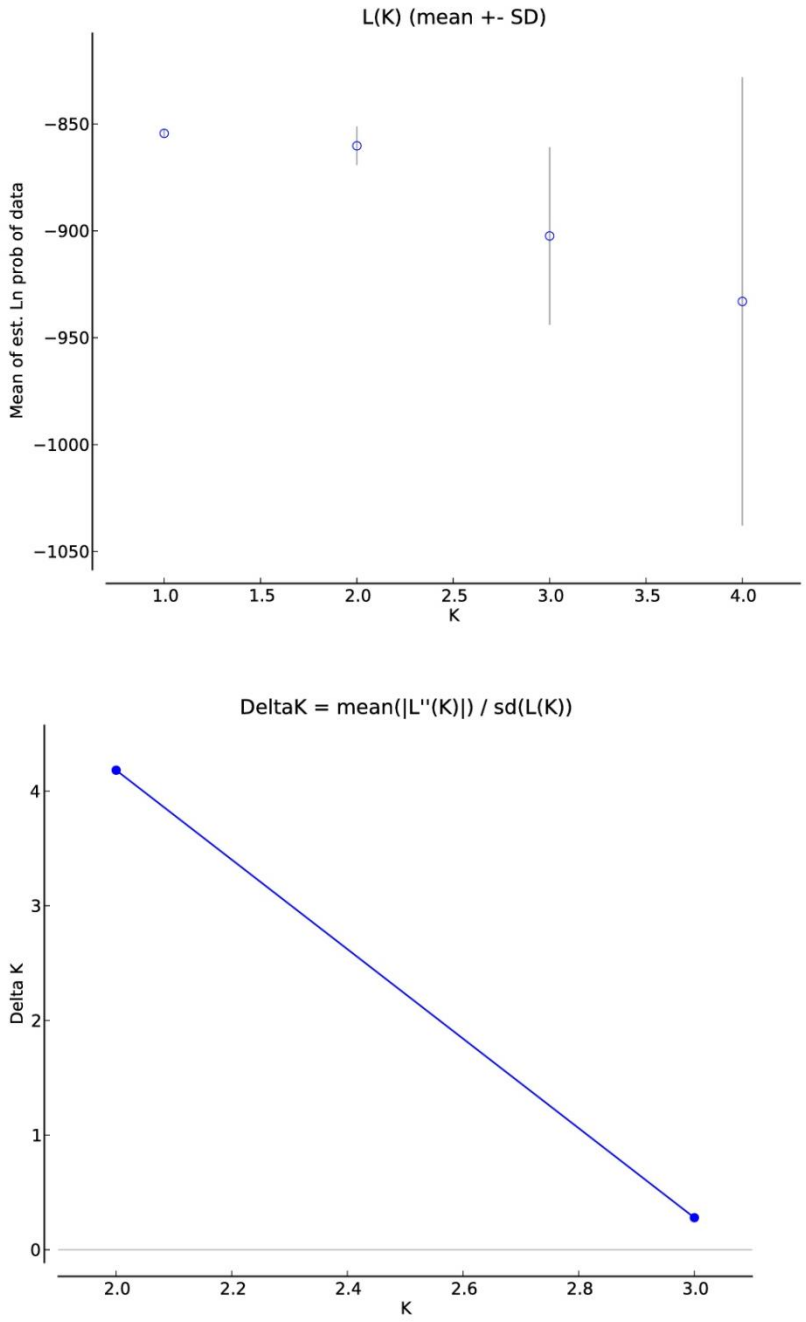


Figure A61. Mean likelihood and Delta K values for *E. artesiae*: Group 2.2.

K of 1 was chosen (low delta K and flat likelihood profile).

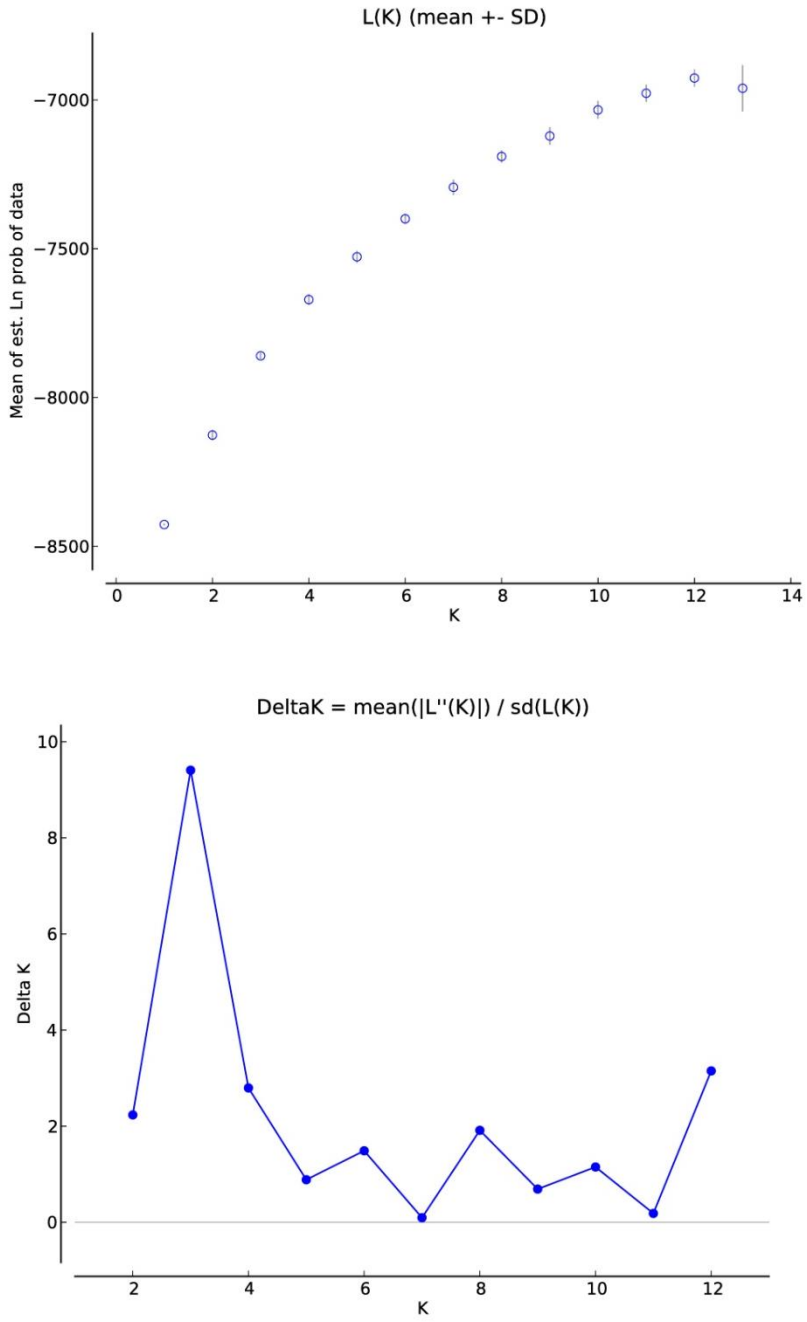


Figure A62. Mean likelihood and Delta K values for *E. parvipinne*: Full Dataset.

K of 3 was chosen.

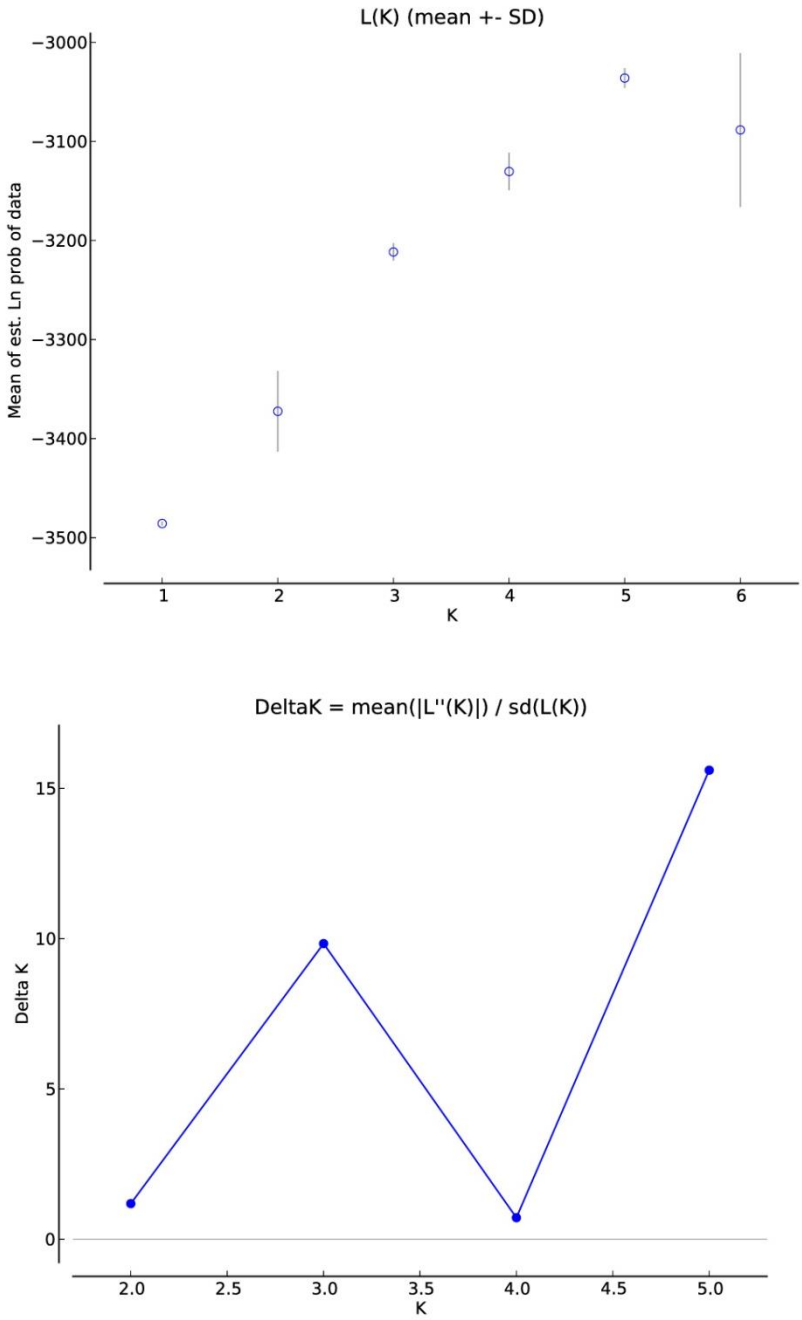


Figure A63. Mean likelihood and Delta K values for *E. parvipinne*: Group 1.

K of 5 was chosen.

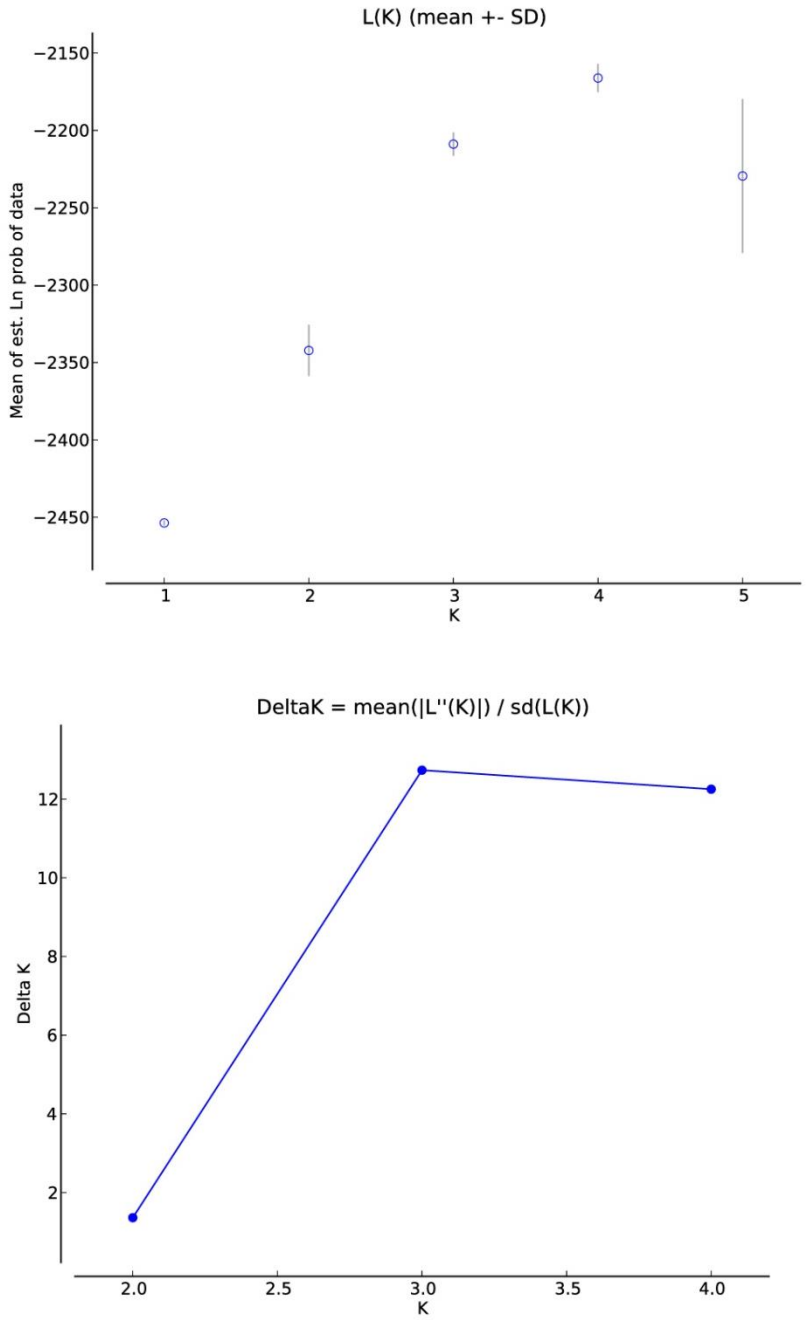


Figure A64. Mean likelihood and Delta K values for *E. parvipinne*: Group 2.

K of 3 was chosen.

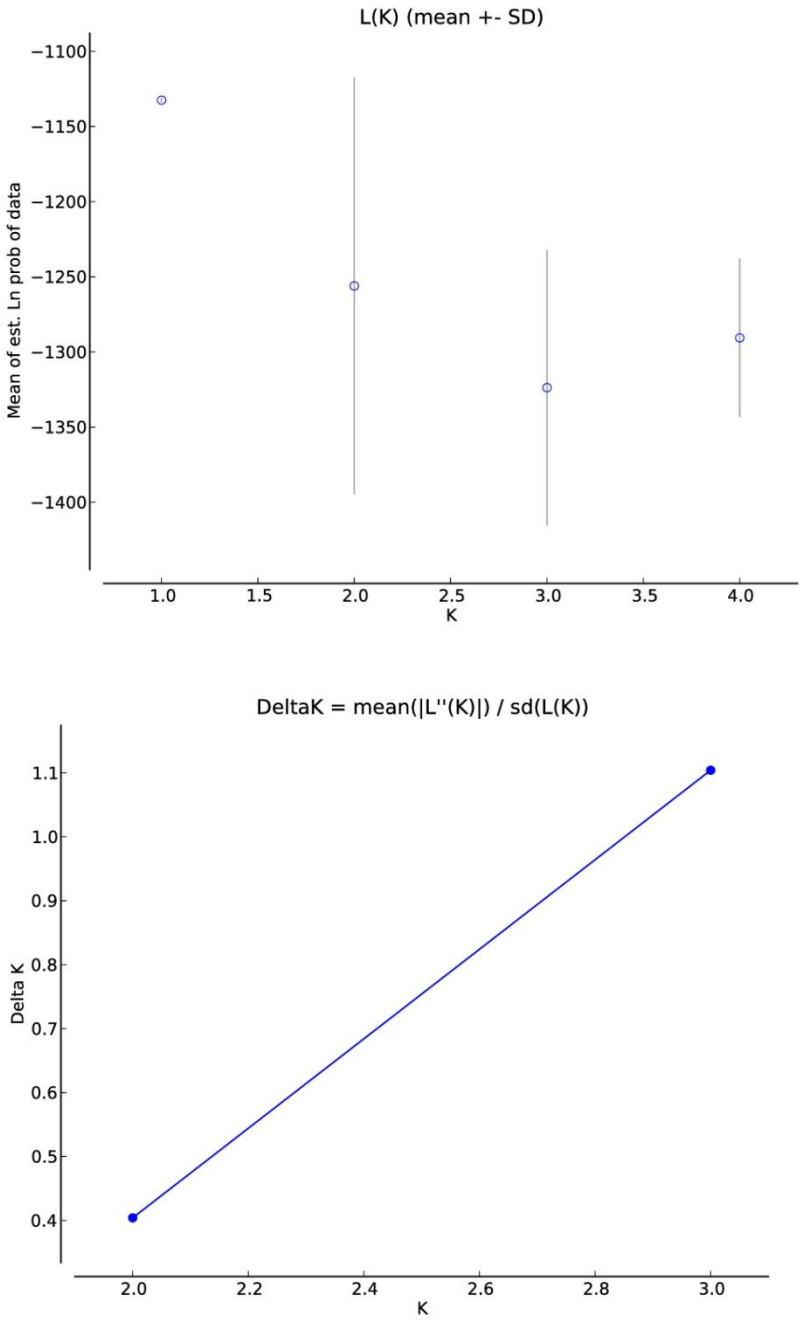


Figure A65. Mean likelihood and Delta K values for *E. parvipinne*: Group 2.1.

K of 1 was chosen (Delta K < 2 and flat likelihood profile).

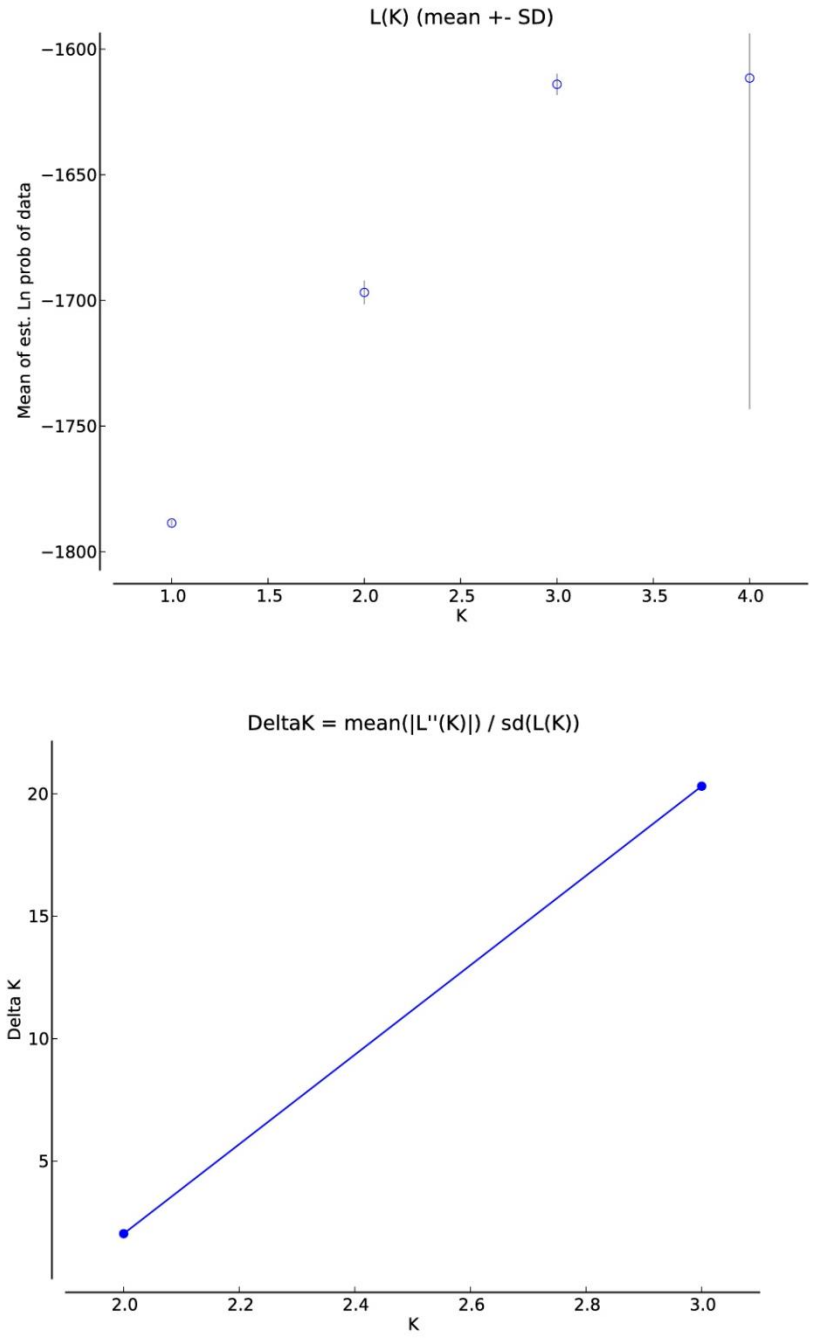


Figure A66. Mean likelihood and Delta K values for *E. parvipinne*: Group 3.

K of 3 was chosen.

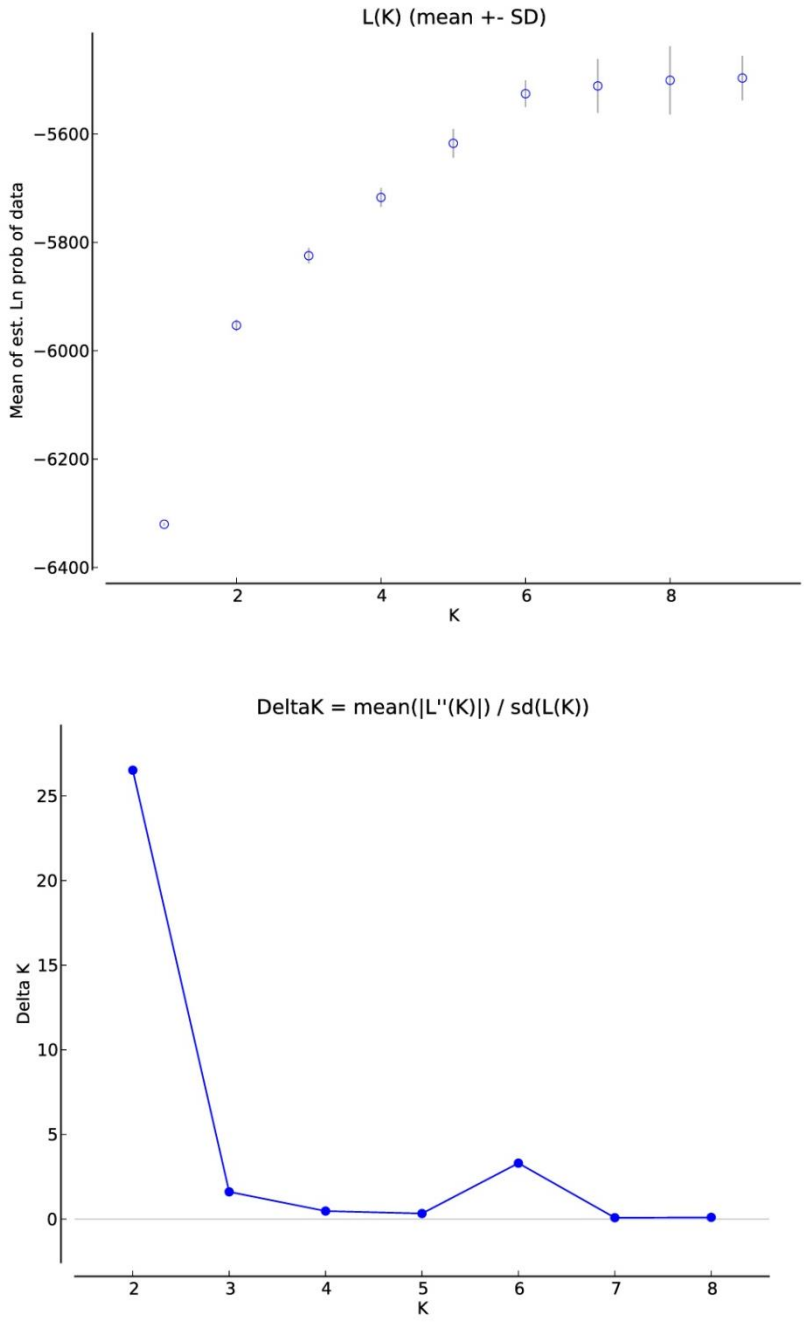


Figure A67. Mean likelihood and Delta K values for *E. whipplei*: Full Dataset.

K of 2 was chosen.

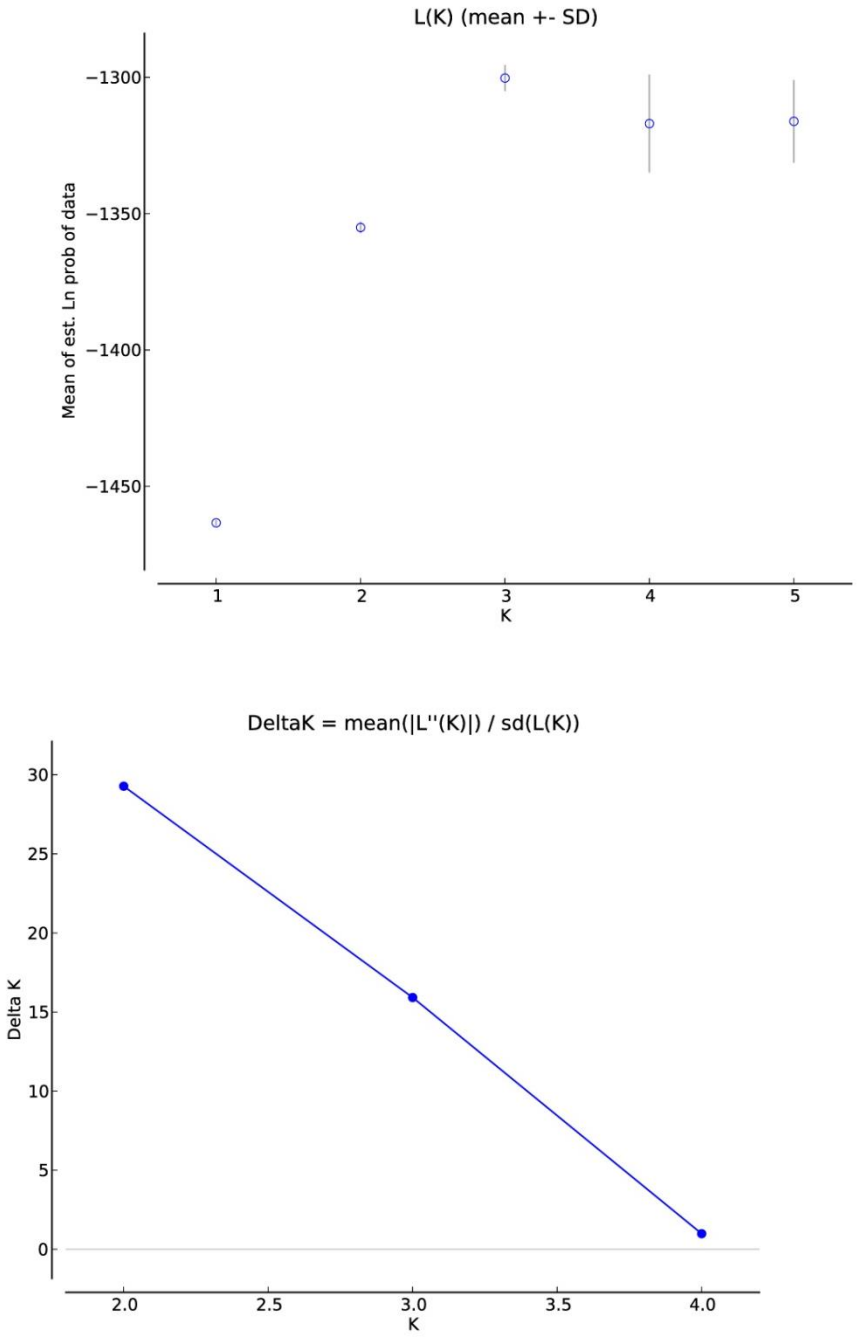


Figure A68. Mean likelihood and Delta K values for *E. whipplei*: Group 1.

K of 2 was chosen.

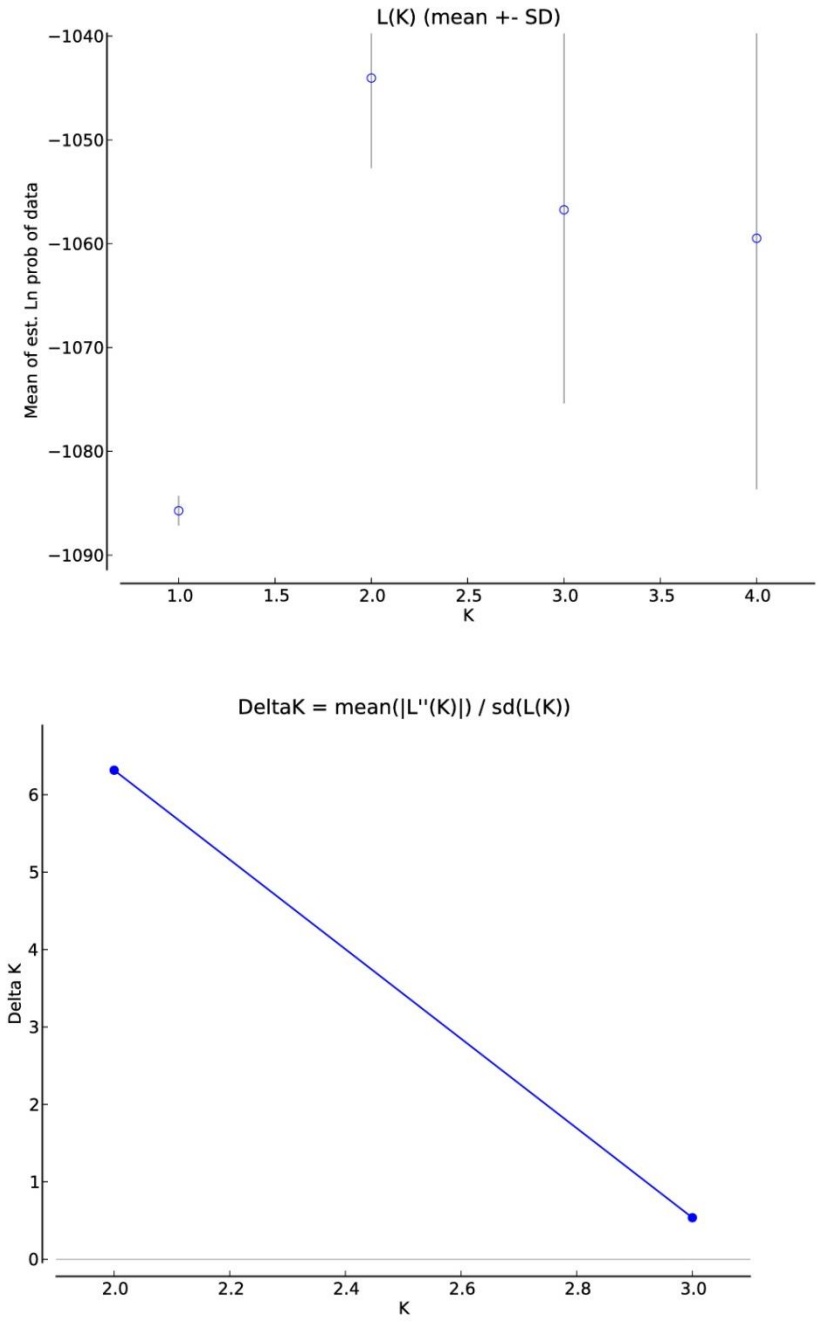


Figure A69. Mean likelihood and Delta K values for *E. whipplei*: Group 1.1.

K of 2 was chosen.

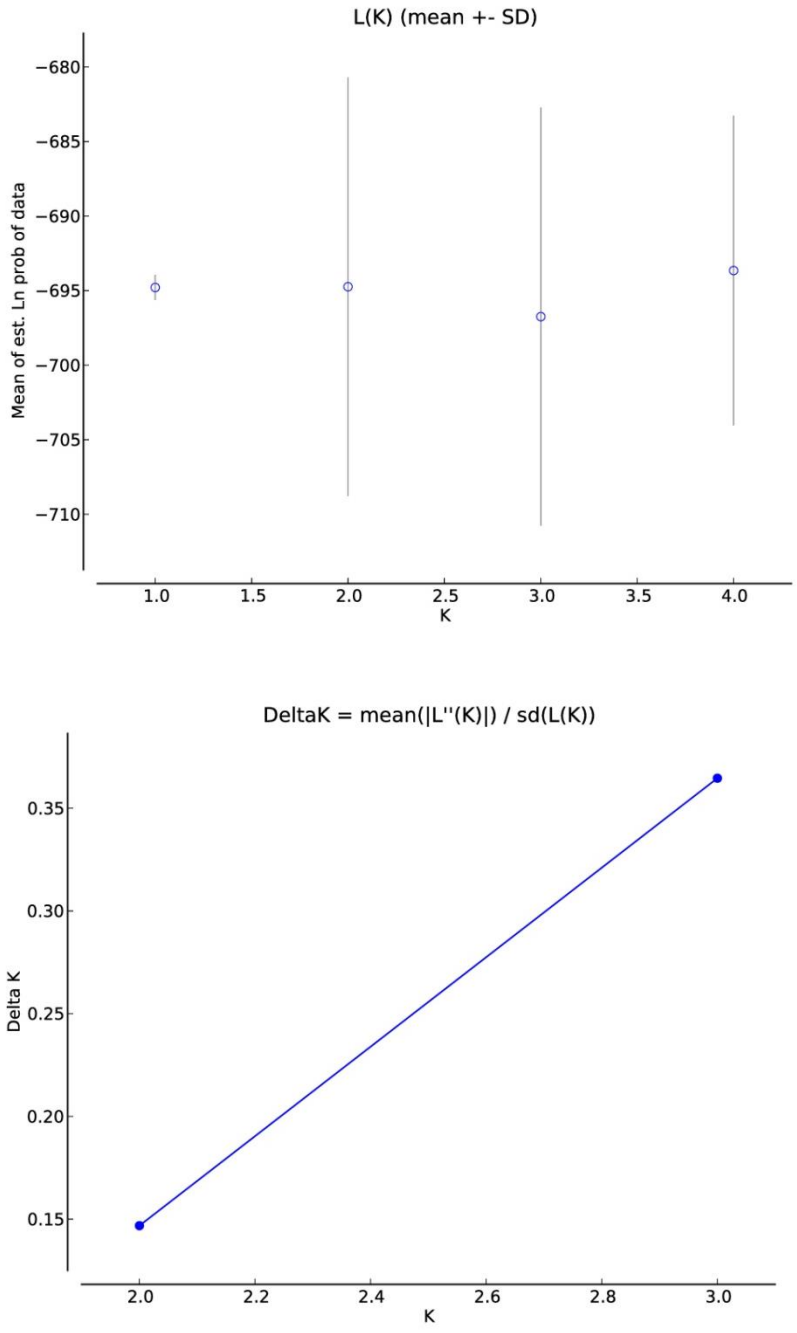


Figure A70. Mean likelihood and Delta K values for *E. whipplei*: Group 1.1a.

K of 1 was chosen (Delta K < 2 and flat likelihood profile).

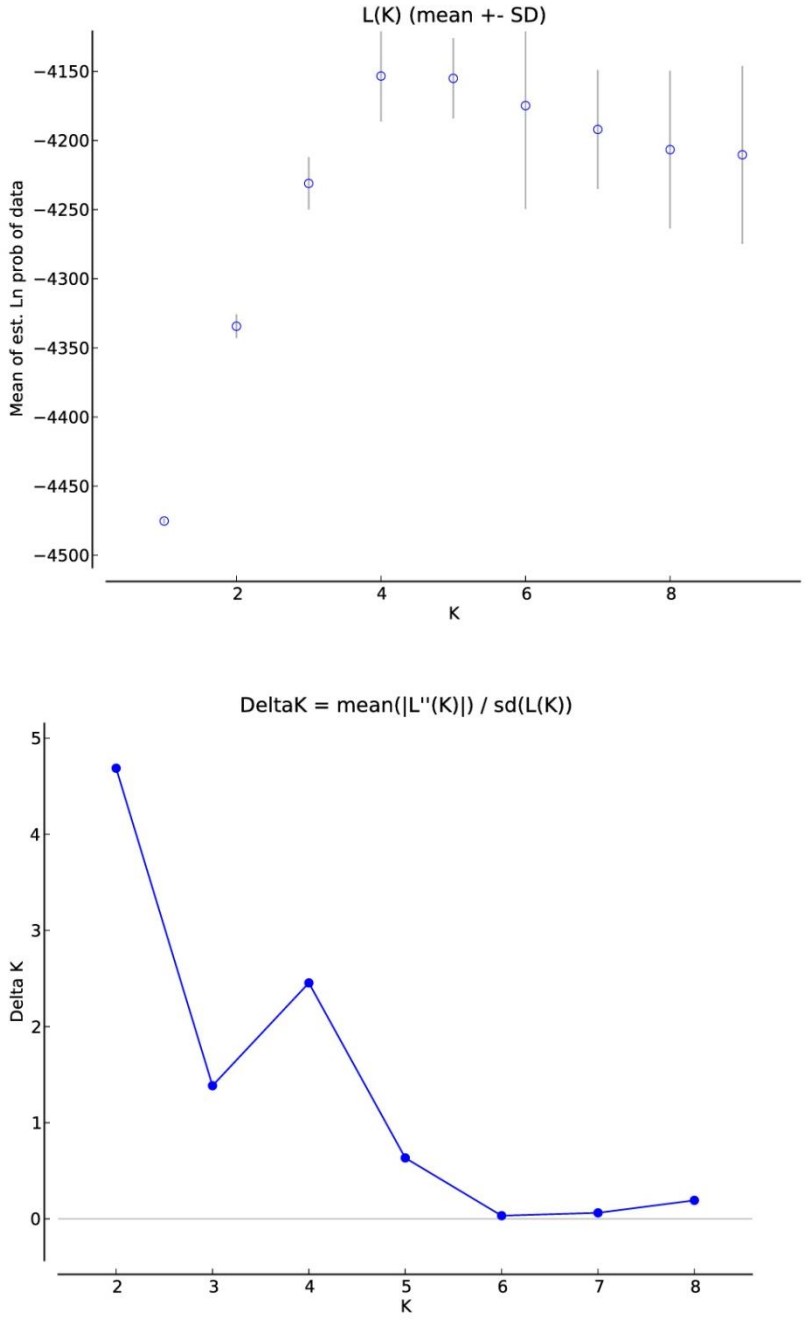


Figure A71. Mean likelihood and Delta K values for *E. whipplei*: Group 2.

K of 2 was chosen.

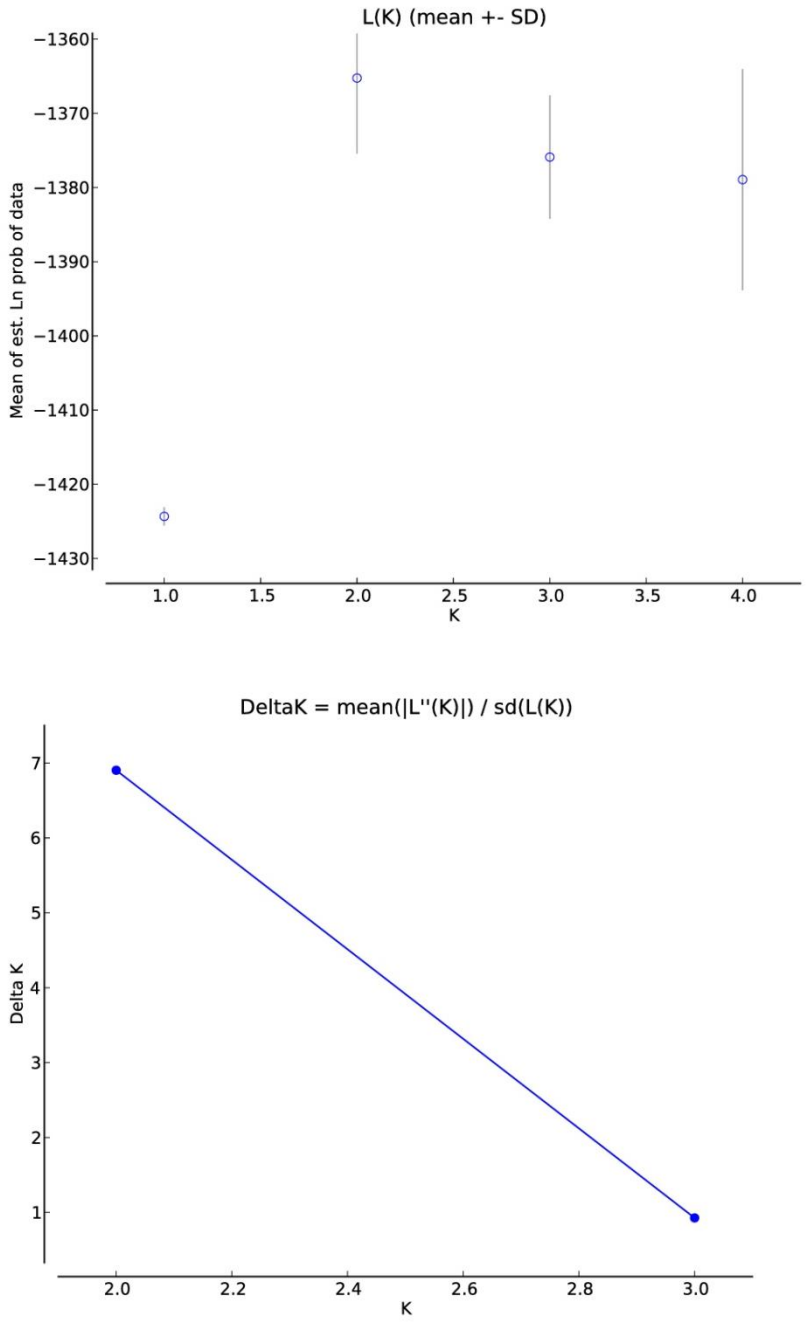


Figure A72. Mean likelihood and Delta K values for *E. whipplei*: Group 2.1.

K of 2 was chosen.

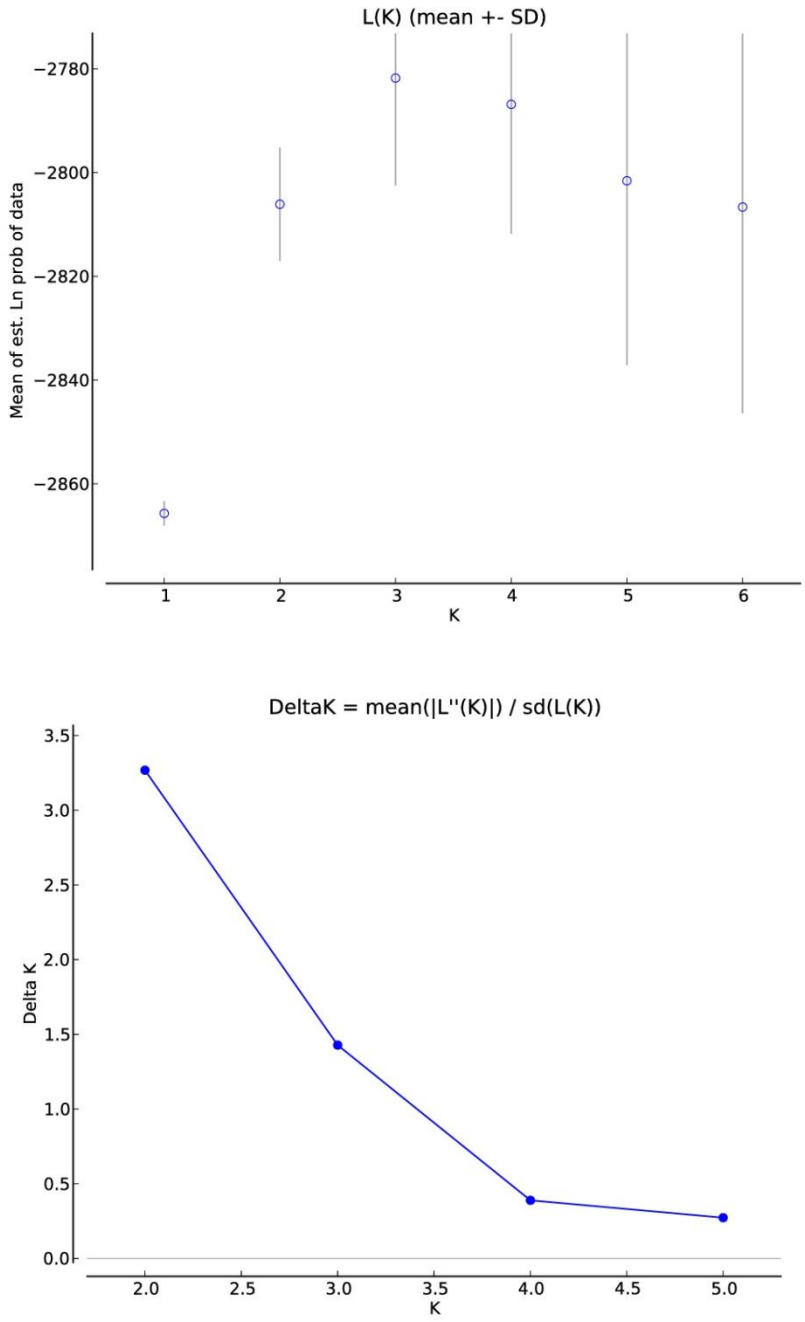


Figure A73. Mean likelihood and Delta K values for *E. whipplei*: Group 2.2.

K of 2 was chosen.

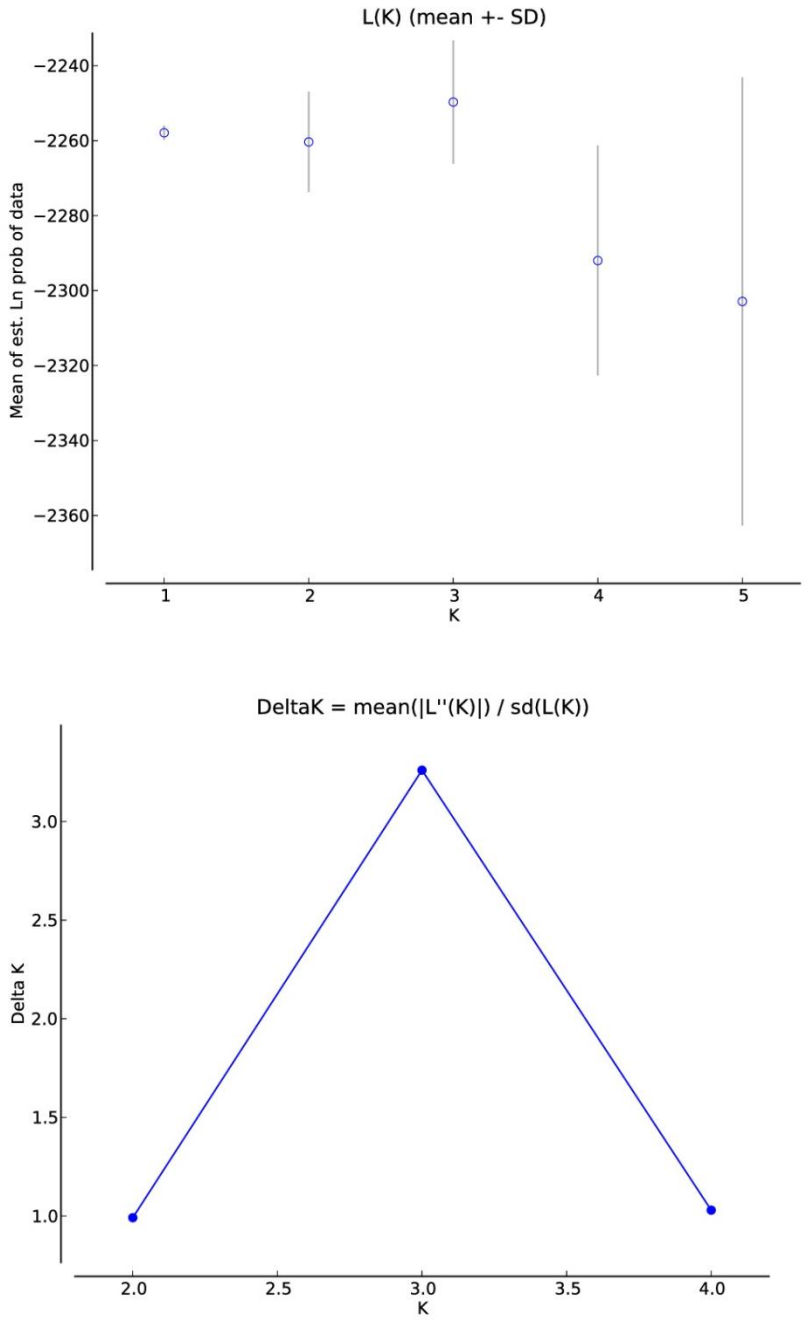


Figure A74. Mean likelihood and Delta K values for *E. whipplei*: Group 2.2a.

K of 1 was chosen (low delta K and flat likelihood profile