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# Comparative biogeography reveals differences in population genetic structure of five species of stream fishes

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The distribution of genetic variation in Texas stream fishes has been shaped by a complex mix of historical and anthropogenic factors. Although Texas was not glaciated during the Pleistocene, the rise in sea level following this epoch isolated formerly connected drainages. More recently, the construction of dams, modifications of stream systems, and the release of commercially raised fish have influenced the patterns of genetic diversity. To examine how these different factors have impacted Texas stream fishes, we compared the genetic structure of five species of fish spanning two families and inhabiting two adjacent drainages: *Lepomis megalotis*, *Lepomis cyanellus*, *Cyprinella lutrensis*, *Cyprinella venusta*, and *Campostoma anomalum*. Our analyses of the mitochondrial D-Loop show that genetic patterns differ strongly across species. A phylogeographical split between the Brazos and Trinity drainages was seen in conspecific populations of *Lepomis* species and is probably the result of the historical separation of these river systems. In contrast, contemporary ecological and anthropogenic factors, such as the desiccation of streams during summer, and the translocation of bait fish, appear to have a stronger influence on the genetic patterns in the remaining species. The contrasting results demonstrate the importance of using a multi-species, comparative approach for landscape genetic studies as single species patterns may not be representative of others and thus may obscure differential effects of historical versus recent events as well as natural versus anthropogenic forces. By comparing closely related species that differ in their life history and economic importance it may be possible to disentangle the relative roles of historical, intrinsic, and anthropogenic influences on different organisms within a region.  *Biological Journal of the Linnean Society*, 2012, ••, ••–••.

ADDITIONAL KEYWORDS: anthropogenic disturbance – freshwater – genetic diversity – habitat connectivity – mitochondrial DNA – phylogeography.

## INTRODUCTION

The partitioning of intraspecific genetic variation is expected to be consistent with historical geomorphology (Avise, 2000). However, the differential response of species to external influences can complicate interspecific predictions (Berendzen, Gamble & Simons, 2008). By comparing ecologically different sympatric species of varying degrees of relatedness, it is possible to infer broad-scale geographical patterns despite the masking of historical signal by more recent extrinsic factors such as anthropogenic disturbance. Likewise, a comparative approach can reveal the impact that anthropogenic disturbance has on modern populations. Thus a comparative approach can identify regional evolutionary forces, specific intrinsic reactions to similar selective pressures among different taxonomic groups, and the differential response

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to anthropogenic disturbance (Tibbets & Dowling, 1996).

Multiple factors influence the biogeographical patterns of North American stream fishes. Geological and climatic factors such as Pleistocene glaciation, river capture events, and changing flow regimes impact all of the species living in a given area (Richardson & Gold, 1995; Waters & Nordt, 1995; Kreiser, Mitton & Woodling, 2001). In contrast, intrinsic factors such as life-history characteristics, demographic history, and response to habitat attributes are not expected to be reflected in all the species in a given area (Neville, Dunham & Peacock, 2006; Dionne *et al*., 2008; Haponski *et al*., 2009). Anthropogenic factors can both partition genetic variation through the construction of barriers to migration (Reid *et al*., 2008; Beneteau, Mandrak & Heath, 2009) and eliminate population structure through human-mediated dispersal (van Houdt *et al*., 2005; Cegelski *et al*., 2006; Clemento *et al*., 2009). The interaction of these factors can make it difficult to interpret biogeographical patterns of a region.

Previous genetic studies of central Texas stream fishes have revealed a variety of geographical patterns and barriers to dispersal across species (King, Zimmerman & Beitinger, 1985; Ashbaugh, Echelle & Echelle, 1994; Richardson & Gold, 1995; Kristmundsdóttir & Gold, 1996; Kreiser *et al*., 2001). Many of these authors made broad inferences about regional biogeographical history and the underlying forces influencing fish populations based on single species patterns. Specific historical factors appear as strong forces in some single species studies, whereas the effects of these factors are not apparent in other species in the same area. Most of these biogeographical studies relate observed genetic patterns to historical stream connectivity, but recent factors such as human-mediated dispersal events and stocking of game fish may have strongly altered genetic signals of species in the region (Ray *et al*., 2012). By comparing closely related species that differ in their life history and economic importance it may be possible to disentangle the relative roles of historical, intrinsic, and anthropogenic influences on different organisms within a region.

Here, we compare the population structure of five common riverine fish species in two drainages in central Texas: *Lepomis megalotis* (Rafinesque, 1820) (longear sunfish), *Lepomis cyanellus* (Rafinesque, 1819) (green sunfish), *Cyprinella lutrensis* (Baird & Girard, 1853) (red shiner), *Cyprinella venusta* Girard, 1856 (blacktail shiner), and *Campostoma anomalum* (Rafinesque, 1820) (central stoneroller). We expected that genetic patterns of these species would generally reflect the geographical history of the region, including a clear split among the two major drainages and some degree of geographical substructure within each watershed. However, we also expected to discover differences in genetic structure due to more recent forces and the resulting species-specific responses.

Based on regional geography, life history, and differential anthropogenic use we expected to see several specific genetic signatures. In larger bodied species with small population sizes, higher trophic position, and low dispersal (genus *Lepomis*) we expected that genetic patterns would generally reflect the geographical history of the region (Kawamura *et al*., 2009). In smaller, highly abundant species of lower trophic position (genus *Cyprinella*) we expected that historical factors would play a reduced role in shaping contemporary population structure (Hubbs & Strawn, 1956; Schönhuth & Mayden, 2011). We expected genetic diversity in these species to be high, reducing the influence of genetic drift and limiting population divergence. In addition, *Cyprinella* species (especially *Cy. lutrensis*) are frequently moved and released by aquarists and fishermen (Herrington & DeVries, 2008). Hence, we anticipated that geographical structure would be further eroded by the anthropogenic dispersal of unrelated haplotypes similar to what has been shown in other species (Brunner, Douglas & Bernatchez, 1998). Finally, we expected to find a distinct pattern in the cyprinid *Campostoma anomalum* as this species is an obligate grazer less tolerant to harsh environmental conditions than the other studied species (Edwards, 1997). Previous studies have shown that *Ca. anomalum* population structure reflects source–sink dynamics depending on fluctuations in habitat quality and availability (Waits *et al*., 2008). We expected to see a similar pattern as this species frequently undergoes extinction and colonization cycles in seasonal pools in Texas summers, which can obscure historical signatures as a result of repeated bottlenecks and population expansions (Capone & Kushlan, 1991; Fritz, Tripe & Guy, 2002; Ostrand & Wilde, 2002; Stanley, Taylor & King, 2012). Using mitochondrial D-Loop sequences we investigated the differential influences of regional geographical history, intrinsic factors, and modern anthropogenic factors on the biogeographical patterns of species with varying life histories. In specific, we tested the following hypotheses: (1) genetic patterns will differ between genera, but will be rather similar when comparing congeneric species; (2) genetic patterns of *Lepomis* species reflect the historical connectivity of the region; (3) genetic diversity in *Cyprinella* species is high as a result of large population sizes and historical patterns have been altered by anthropogenic translocations; and (4) genetic diversity will be reduced in *Ca. anomalum* as a result of frequent bottlenecks and the genetic structure will reflect source–sink dynamics.

## MATERIALS AND METHODS

### STUDY SPECIES

We studied five species of fish that represent a range of body sizes, behaviours, and ecological requirements to gain a greater understanding of the biogeography of central Texas stream fishes. The two centrarchid species, *Lepomis megalotis* and *L. cyanellus*, are common in lower velocity pools (Thomas, Bonner & Whiteside, 2007). They migrate only short distances and have a home range of 30–60 m (Gerking, 1953). *Lepomis cyanellus* is known to out-compete other sunfish where they occur sympatrically, and may be more tolerant of harsh environmental conditions (Werner & Hall, 1977). *Cyprinella lutrensis* and *Cy. venusta* are co-distributed across much of Texas (Thomas *et al*., 2007), are common baitfish in the region, and are typically among the most abundant species present at a location (Pease *et al*., 2011). In addition, *Cy. lutrensis* is a popular aquarium species. *Campostoma anomalum* is an obligate grazer (Zimmerman, Merritt & Wooten, 1980) that mainly occurs in limestone streams of the Brazos and Trinity Basins in Texas. This species is less tolerant to siltation and high water temperatures than the other species in our study (Edwards, 1997).

### SAMPLING

The Brazos and Trinity River systems are two of the largest drainage basins in the south-western USA (Fig. 1). River and tributary stream channels as well as the surrounding landscape have been subjected to numerous natural and human alterations (Zeug & Winemiller, 2008; Pease *et al*., 2011), including the construction of dams and reservoirs, improved low water crossings, and channelization. These rivers

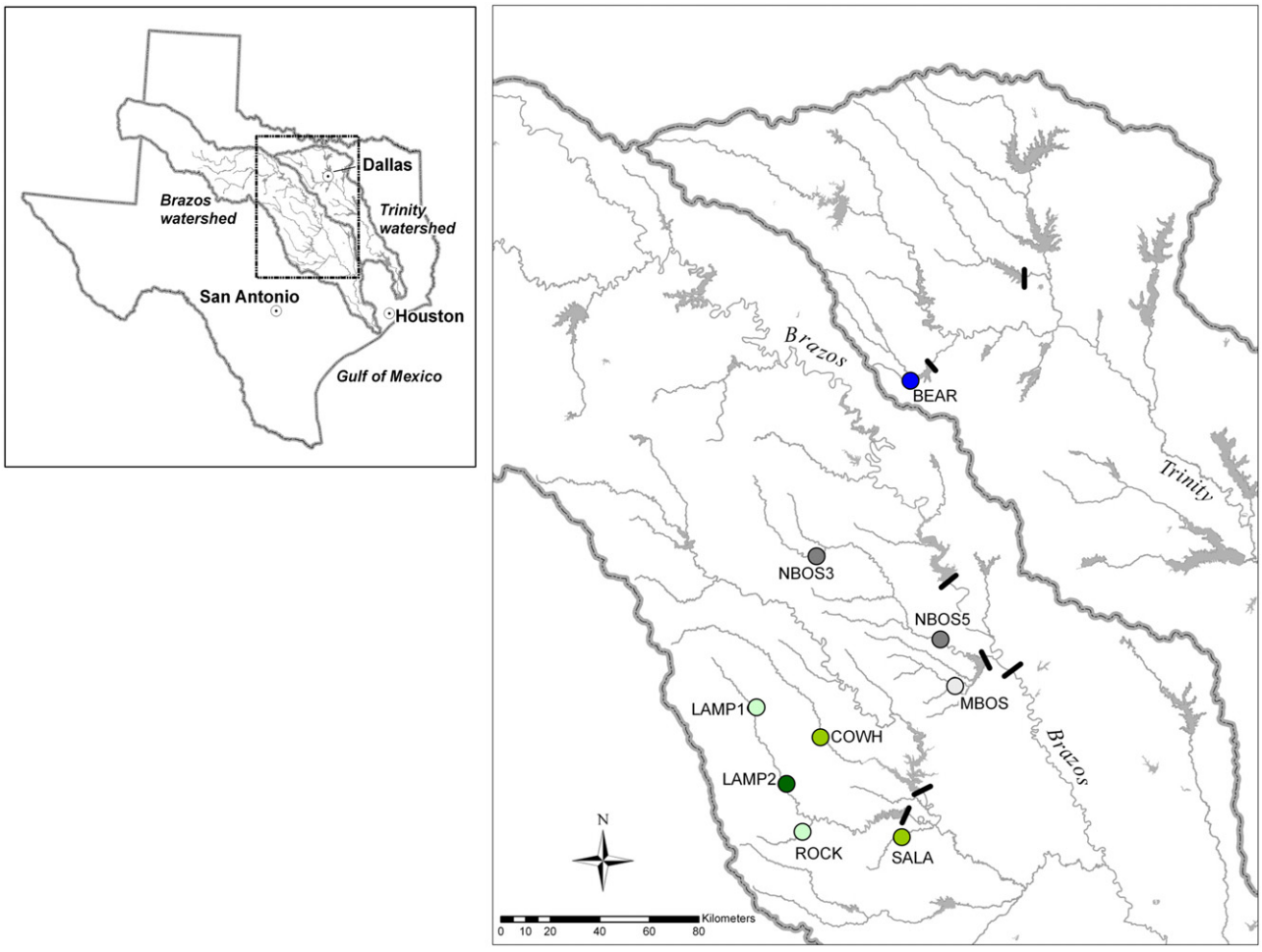


**Figure 1.** Map of Texas showing the location of the sampling region; and expanded view of the sampling region. The locations of sampling sites is indicated with colored circles (Trinity – blue, Bosq1 – dark grey, Bosq2 – light grey, Little1 – dark green, Little2 – medium green, Little3 – light green); black bars indicate the locations of dams.

**Table 1.** Sampling locations; map code names identified below are from previous studies of the same locations (Pease *et al.*, 2011)

| Site | Pease *et al.* (2011) | Site ID | Drainage | Latitude (°N) | Longitude (°W) |
|---|---|---|---|---|---|
| Bear Creek | BEAR | Trinity | Trinity | 32.59442 | 97.51018 |
| Rocky Creek | ROCK | Little3 | Brazos | 30.94494 | 97.99117 |
| Middle Bosque River | MBOS | Bosq2 | Brazos | 31.50748 | 97.35624 |
| North Bosque River | NBOS3 | Bosq1 | Brazos | 31.97692 | 98.03974 |
| North Bosque River | NBOS5 | Bosq1 | Brazos | 31.63760 | 97.36640 |
| Cowhouse Creek | COWH | Little2 | Brazos | 31.28327 | 97.88241 |
| Salado Creek | SALA | Little2 | Brazos | 30.91275 | 97.60105 |
| Lampasas River | LAMP2 | Little1 | Brazos | 31.37802 | 98.18063 |
| Lampasas River | LAMP1 | Little3 | Brazos | 31.11558 | 98.05432 |

follow roughly parallel paths to the Gulf of Mexico and were probably last connected during low sea levels of the Pliocene 2.5 Mya (Richardson & Gold, 1995).

Within the Brazos River, the Little and Bosque Rivers have been fragmented in the last 80 years due to dam and reservoir construction. Waco Dam and Lake Waco were built in 1930, creating a barrier between the Upper Bosque Rivers and the Little River system. Belton Dam and Lake were completed in 1954, separating Cowhouse Creek from the remaining sections of the Little River drainage thereby adding an additional barrier between Cowhouse Creek and the Bosque River. In 1968, Stillhouse Dam and Lake were constructed, separating the Lampasas River and Rocky Creek from the remaining portions of the Little River drainage. Sampling locations used in this study correspond to sites from several large-scale fish community studies (Table 1; Pease *et al.*, 2011; Stanley *et al.*, 2012).

All species were sampled from five sites within the Brazos River drainage and one site from the Trinity River drainage (Fig. 1, Table 1). Between 12 and 20 specimens were sampled for each species at each location (Table 2). Some sites within the Brazos River drainage yielded small sample sizes of certain species ($n < 10$) and hence they were replaced by similar local sites within the same catchment. Specimens were collected using a backpack electrofisher (Smith-Root Model LR-24) and seine nets ($4.6 \times 1.8$ m or $1.8 \times 1.8$ m). Fish were identified in the field and stored at –20 °C until further processing.

## Molecular analyses

Genomic DNA was extracted from fin tissue using the Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's protocol for tissue samples.

For amplification and sequencing of the mitochondrial D-Loop, two primer pairs were designed using Primer3 (Rozen & Skaletsky, 2000). For *Ca. anomalum*, *L. megalotis*, and *L. cyanellus*, primers CR-F and CR-R + M13-41 (Appendix 1) were designed using the complete mitochondrial (mtDNA) sequences of 23 species of fish obtained from GenBank. These primers were designed to allow the amplification of the D-Loop for a wide variety of taxa and should be suitable for most fish species. An M13-41 adaptor was added to the reverse primer to allow for sequencing with M13 universal primers. Although the above mentioned primers worked for some shiner samples, amplification success was low and specific new primers were designed. Primers for *Cy. venusta* and *Cy. lutrensis*, ShinerCR-F and ShinerCR-R, were designed using GenBank sequences from *Cy. lutrensis* and *Cy. spiloptera*. Both primer pairs were designed to be located in the tRNA-regions surrounding the D-Loop (tRNA-proline and tRNA-phenylalanine). All primer sequences used in this study are given in Appendix 1.

The D-Loop has proven to be a high utility marker; it is part of the mitochondrial genome and hence has a smaller effective population size compared with nuclear markers due to the maternal inheritance of mtDNA. Within the vertebrate mitochondrial genome the D-Loop is the fastest evolving section (Cui, Liu & Chu, 2010) and therefore is suitable to analyse population structure in fish (Iervolino, de Resende & Hilsdorf, 2010).

PCR was performed using the following set-up: 36.6 μL of distilled $H_2O$, 6 μL of 10× PCR buffer (reaction concentration 1×), 4.8 μL of dNTP mixture (0.2 μM each), 0.6 μL of DyNAzyme™ DNA Polymerase (1.2 U, Finnzymes, Vantaa, Finland), 3 μL of each primer (0.5 μM, Integrated DNA technologies, Coralville, IA, USA) and 6 μL of DNA template adding up to a total volume of 60 μL. Amplification conditions

**Table 2.** Summary of statistical analyses calculated in DnaSP

| Species | Trinity | Bosq1 | Bosq2 | Little 1 | Little 2 | Little 3 | Total |
|---|---|---|---|---|---|---|---|
| *Lepomis cyanellus* (bp = 889) | | | | | | | |
| *N* | 16 | 14 | 7 | 15 | 15 | 13 | 80 |
| *K* | 2.97 | 2.70 | 2.48 | 2.32 | 2.51 | 2 | 3.75 |
| *S* | 10 | 8 | 8 | 6 | 6 | 10 | 24 |
| *H* | 5 | 6 | 3 | 3 | 5 | 5 | 20 (25) |
| *Hd* | 0.45 | 0.74 | 0.67 | 0.65 | 0.78 | 0.69 | 0.89 |
| π | 0.00334 | 0.00355 | 0.00279 | 0.00262 | 0.00290 | 0.00225 | 0.00492 |
| *Lepomis megalotis* (bp = 910) | | | | | | | |
| *N* | 15 | 20 | 19 | 19 | 19 | 20 | 112 |
| *K* | 1.92 | 6.94 | 2.02 | 0.959 | 0.39 | 0.2 | 8.77 |
| *S* | 8 | 33 | 7 | 5 | 2 | 2 | 45 |
| *H* | 8 | 8 | 6 | 7 | 3 | 3 | 30 (30) |
| *Hd* | 0.84 | 0.85 | 0.81 | 0.67 | 0.37 | 0.20 | 0.85 |
| π | 0.00214 | 0.00776 | 0.00226 | 0.00107 | 0.00043 | 0.00022 | 0.00985 |
| *Cyprinella lutrensis* (bp = 1018) | | | | | | | |
| *N* | 15 | 14 | 16 | 16 | 15 | 12 | 88 |
| *K* | 37.30 | 10.86 | 4.52 | 20.73 | 14.50 | 42.39 | 26.55 |
| *S* | 105 | 64 | 16 | 87 | 84 | 83 | 141 |
| *H* | 14 | 8 | 9 | 11 | 12 | 9 | 42 (63) |
| *Hd* | 0.99 | 0.91 | 0.87 | 0.95 | 0.95 | 0.94 | 0.95 |
| π | 0.03752 | 0.01080 | 0.00449 | 0.02067 | 0.01444 | 0.04273 | 0.02703 |
| *Cyprinella venusta* (bp = 950) | | | | | | | |
| *N* | 16 | 15 | 15 | 15 | 16 | 15 | 92 |
| *K* | 3.45 | 2.86 | 3.28 | 2.31 | 3.33 | 2.13 | 3.11 |
| *S* | 11 | 11 | 11 | 8 | 11 | 6 | 21 |
| *H* | 12 | 11 | 12 | 7 | 11 | 6 | 38 (41) |
| *Hd* | 0.97 | 0.95 | 0.96 | 0.80 | 0.94 | 0.82 | 0.95 |
| π | 0.00364 | 0.00301 | 0.00346 | 0.00243 | 0.00349 | 0.00225 | 0.00329 |
| *Campostoma anomalum* (bp = 1026) | | | | | | | |
| *N* | 15 | 15 | 16 | 16 | 15 | 16 | 93 |
| *K* | 3.39 | 0.93 | 4.14 | 1.13 | 0.533 | 2.06 | 3.13 |
| *S* | 19 | 4 | 28 | 6 | 1 | 5 | 27 |
| *H* | 4 | 5 | 6 | 4 | 2 | 5 | 18 (18) |
| *Hd* | 0.47 | 0.73 | 0.68 | 0.58 | 0.53 | 0.73 | 0.80 |
| π | 0.00330 | 0.00091 | 0.00404 | 0.00110 | 0.00052 | 0.00201 | 0.00305 |

(*N* = sample size, *K* = average number of nucleotide differences, *S* = number of polymorphic sites, *H* = number of haplotypes, *Hd* = haplotype diversity, π = nucleotide diversity). The parenthetical value for total haplotypes indicates the number of haplotypes present when gaps are considered.

were as follows: 94 °C for 3 min, followed by 30 cycles of 94 °C for 1 min denaturation, 58–61 °C 1 min annealing (*L. megalotis* and *L. cyanellus*, *Ca. anomalum*: 58–60 °C; *Cy. lutrensis*, *Cy. venusta*: 61 °C) and 72 °C for 2 min elongation, with a final elongation step at 72 °C for 10 min.

PCR products were visualized on a 1% agarose gel stained with Gel Red (0.1×, Biotium, Hayward, CA, USA) and purified using Solid-phase Reversible Immobilization (SPRI; DeAngelis, Wang & Hawkins, 1995) with carboxylated magnetic beads (Bangs Laboratories, Fishers, IN, USA), and a 96-Ring SPRIplate (Agencourt, Beverly, MA, USA). The purified PCR products were sequenced at the Yale Sequencing Facility (New Haven, CT, USA). Sequences were deposited in GenBank (Appendix 2).

## Sequence analysis

Sequences were inspected, trimmed, and aligned using Geneious 5.0.3 (Drummond *et al.*, 2011). Measures of genetic diversity including the average number of nucleotide differences (*K*), the number

**Table 3.** Estimates of θ as proxy for $N_e$ calculated with migrate-N

| | *Lepomis cyanellus* | *Lepomis megalotis* | *Cyprinella lutrensis* | *Cyprinella venusta* | *Campostoma anomalum* |
|---|---|---|---|---|---|
| θ(Trinity) | 0.00116 | 0.00099 | 0.01120 | 0.01058 | 0.01208 |
| θ(Little1) | 0.00190 | 0.00100 | 0.01066 | 0.00251 | 0.00123 |
| θ(Little2) | 0.00122 | 0.00042 | 0.01873 | 0.00325 | 0.00028 |
| θ(Little3) | 0.00712 | 0.00041 | 0.00408 | 0.00108 | 0.01185 |
| θ(Bosq1) | 0.00910 | 0.00394 | 0.01011 | 0.01303 | 0.00124 |
| θ(Bosq2) | 0.00120 | 0.00157 | 0.00857 | 0.03348 | 0.00759 |
| Mean (±stdw) | 0.00362 (±0.00354) | 0.00139 (±0.00132) | 0.01056 (±0.00476) | 0.01066 (±0.01216) | 0.00571 (±0.00550) |

of polymorphic sites (*S*), the number of haplotypes excluding gaps, the number of haplotypes including gaps, haplotype diversity (*Hd*), and nucleotide diversity (π) were calculated in DNASP (Librado & Rozas, 2009) and are given in Table 2. Tajima's *D*-test was performed in MEGA v.5 (Tamura *et al*., 2011). Maps illustrating the distribution of haplotypes across populations were constructed for all species (Appendix 3). All haplotypes, including those delineated by only indels, are displayed on maps.

Haplotype networks were created using the median joining approach at the default conditions in NETWORK 4.6.1.0 (Bandelt, Forster & Rohl, 1999). We used a Bayesian approach to construct unrooted trees in MRBAYES v.3.1.2 to show the relationships of haplotypes (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Akaike's information criterion (AIC) as implemented in MRMODELTEST 3.04 (Nylander, 2004) was used to determine which substitution models best fitted our data. For *Cy. lutrensis*, *Cy. venusta*, and *L. megalotis*, the best model was GTR + I + G, for *Ca. anomalum* GTR + I, and for *L. cyanellus* HKY + I. Bayesian analyses were run for 10 million generations, sampled every thousand generations, with a burn-in of 1000 samples (10%). The outputs were checked for convergence in TRACER v1.5 (Rambaut & Drummond, 2009) and displayed using FIGTREE 1.3.1 (Rambaut, 2011). The trees are given in Appendix 4.

Estimates of θ were generated in MIGRATE-N v.3.2.16 (Beerli & Palczewski, 2010; Table 3). Default settings were used for most variables but transition/transversion ratios were calculated in MEGA v.5. An estimate of $\theta = 2N_f\mu$ ($N_f$ = female effective population size, μ = mutation rate) was used as a is a proxy for female effective population size. The generation times of the studied species are similar (McElroy *et al*., 2003; COSEWIC, 2005; Froese & Pauly, 2012), but the mutation rate of D-Loops in these species is unknown. Although this means that population size cannot be separated from the mutation rate using θ, we assume similar mutational rates across our study species as has been done previously (Bernatchez & Wilson, 1998). Based on this assumption, large differences in θ should primarily reflect variation in female effective population size.

The IBD web service 3.16 (Jensen, Bohonak & Kelley, 2005) was used to calculate $\Phi_{st}$ values and to test isolation by distance models using Mantel tests. Two isolation by distance models were tested for each species: (1) linear distance (LD) and (2) stream distance (SD). Linear distances were calculated using GPS coordinates of sampling locations and the online application gpsvisualizer (http://www.gpsvisualizer.com). Stream distances were computed as pairwise distances along the stream network in ArcGIS. Models were run under 30 000 randomizations with the Kimura two-parameter correction, gaps were treated as transitions, and missing data was not considered (Table 4).

## RESULTS

The total number of samples per species ranged from 80 to 112 individuals (Table 2), with 12–20 samples per site (with the exception of one site, Bosq2 for *L. cyanellus*, where difficulty in sequencing resulted in a sample size of seven). Length of alignments varied among species and ranged from 889 to 1026 bp. GenBank accession numbers for these sequences can be found in Appendix 2.

The average number of nucleotide differences (*K*), the number of polymorphic sites (*S*), the number of haplotypes, haplotype diversity (*Hd*), and nucleotide diversity (π) were calculated for all species for all sites and for the total data sets (Table 2). Haplotype diversity was similar across congeneric species, with *Cyprinella* species exhibiting very high diversity (0.95 for both *Cy. lutrensis* and *Cy. venusta*). *Lepomis* species had moderate haplotype diversity (0.89 for *L. cyanellus* and 0.85 for *L. megalotis*), while *Ca. anomalum* had the lowest overall haplotype diversity (0.80). Nucleotide diversity (π) ranged between 0.02703 and 0.00305, with *Cy. lutrensis*

**Table 4.** Results of test for isolation by distance using a stream distance and a linear distance model performed with the IbD web service; values in bold are significant ($P < 0.05$)

| Species | Model | $Z$ | $r^2$ | $P$ |
|---|---|---|---|---|
| *Lepomis cyanellus* | Stream distance | 5039.8188 | 0.423 | **0.0098** |
| | Linear distance | 579.7911 | 0.163 | 0.1043 |
| *Lepomis megalotis* | Stream distance | 8284.9804 | 0.584 | **0.0400** |
| | Linear distance | 983.2947 | 0.492 | **0.0290** |
| *Cyprinella lutrensis* | Stream distance | 2782.1661 | 0.405 | **0.0280** |
| | Linear distance | 301.0348 | 0.350 | **0.0387** |
| *Cyprinella venusta* | Stream distance | 698.4734 | 0.0119 | 0.6817 |
| | Linear distance | 94.0834 | 0.0000 | 0.4790 |
| *Campostoma anomalum* | Stream distance | 3162.6515 | 0.0273 | 0.5688 |
| | Linear distance | 492.8249 | 0.003954 | 0.4234 |

and *L. megalotis* exhibiting the highest values and *Ca. anomalum* and *Cy. venusta* having the lowest diversity. Tajima's *D* tests were performed for all species to identify signatures of selection or population expansion. Only *Ca. anomalum* exhibited a significant value ($D = -1.86$; $P < 0.01$).

Pairwise $\Phi_{st}$ values varied among species with most estimates being significantly different from zero (Appendix 3). In the least differentiated species, *Cy. venusta*, 47% of pairwise comparisons indicated that populations are genetically divergent. In contrast, all *Cy. lutrensis* comparisons yielded significant $\Phi_{st}$ values. *Lepomis megalotis*, *L. cyanellus* and *Ca. anomalum* had large numbers of significant $\Phi_{st}$ values (87, 93 and 80%, respectively).

The estimates of θ obtained from migrate-N analyses indicate different population sizes for all species (Table 3). Estimates for *Cyprinella* species are highest (~0.0106) while *Lepomis* species had values almost an order of magnitude smaller (*L. cyanellus*, 0.0036; *L. megalotis*, 0.0014). The estimates for *Campostoma* were intermediate (0.0057). This suggests that *Lepomis* species have the smallest population sizes, followed by *Campostoma*, while shiners have the largest population sizes.

The results of Mantel tests differed across the study species (Table 4). *Lepomis megalotis* (SD: $r^2 = 0.584$, $P = 0.04$; LD: $r^2 = 0.492$, $P = 0.029$) and *Cy. lutrensis* (SD: $r^2 = 0.405$, $P = 0.028$; LD: $r^2 = 0.35$, $P = 0.0387$) showed a significant correlation between geographical distance and genetic distance. The comparison between genetic distance and stream distance, but not linear distance, was significant in *L. cyanellus* (SD: $r^2 = 0.423$, $P = 0.0098$; LD: $r^2 = 0.163$, $P = 0.104$). Other comparisons yielded non-significant correlations.

Species haplotype networks vary in complexity (Fig. 2A–E). The networks generated for sunfish are of intermediate complexity (Fig. 2A, B) with several common ($n > 10$) and moderately common ($n > 4$) haplotypes. Geographical structure is evident in the *L. megalotis* network, with haplotypes from the Trinity River appearing strongly divergent. Less geographical structure is evident in *L. cyanellus*. However, in this species most individuals collected at the Trinity River location share a single haplotype that is divergent from the haplotypes found in the Brazos River drainage. The networks for the shiner species exhibit the most complex structure, where haplotypes are numerous, diverse, and show no apparent geographical structure (Fig. 2C, D). When gaps are considered, no haplotypes are shared in *Cy. lutrensis*, and their geographical distribution has no apparent pattern. Three subnetworks and one highly divergent haplotype are present, separated by large numbers of unsampled haplotypes. The haplotype network of *Cy. venusta* is similarly complex, but lacks any large mutational branches. *Campostoma anomalum* has the simplest network with two abundant haplotypes prevailing (Fig. 2E). However, strong geographical structure is not apparent and two highly divergent haplotypes are present. In summary, three different degrees of complexity were found across the species sampled. The analyses yielded the simplest network for *Ca. anomalum*, while the networks for the two species of *Lepomis* were of intermediate complexity. The networks generated for the *Cyprinella* species were most complex with many rare interconnected haplotypes. Geographical structure was only apparent in the two sunfish species.

## DISCUSSION

Combinations of historical, anthropogenic, and species-specific processes are known to affect the population structure of species (Faber, Rybka &

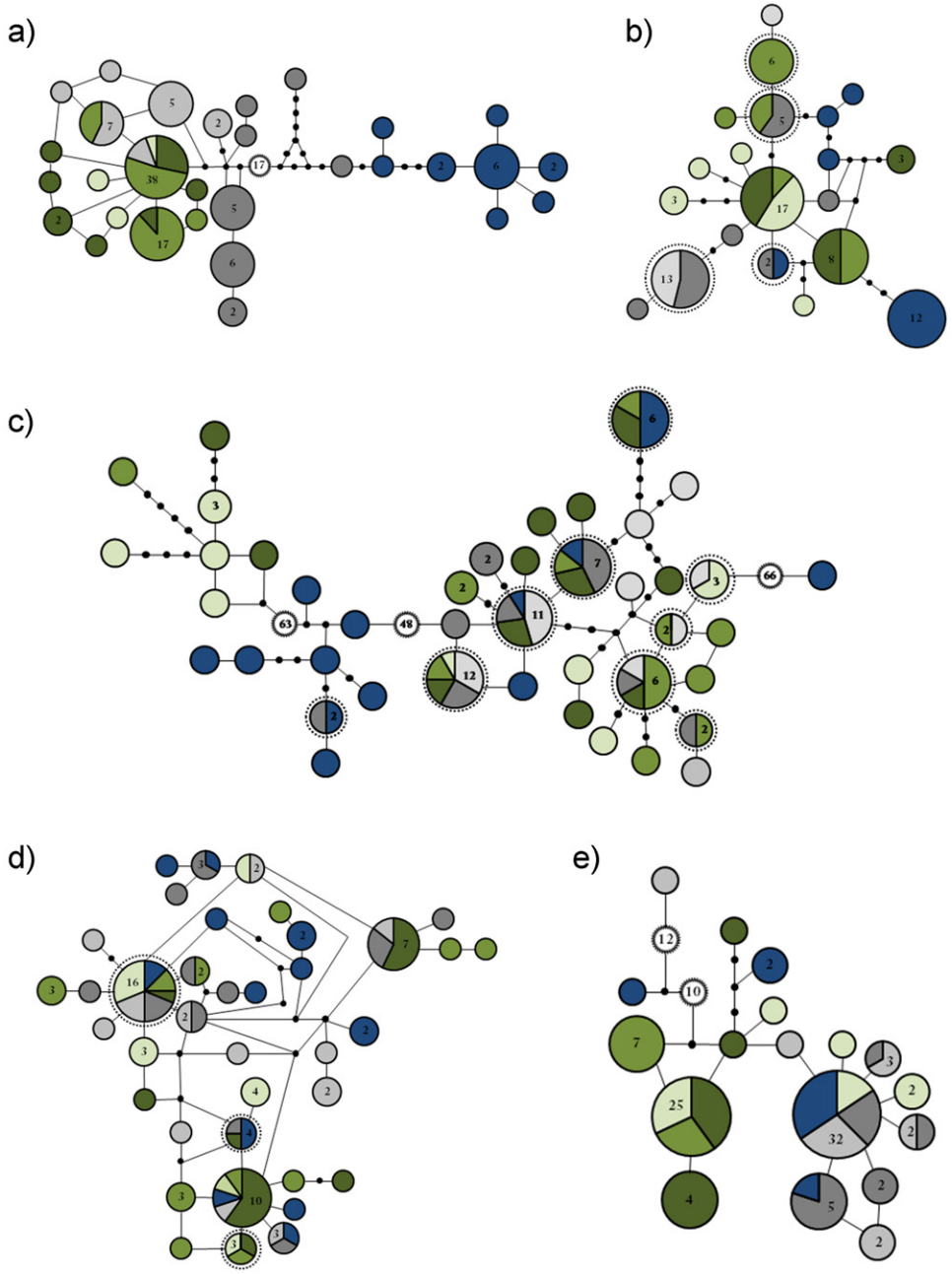


**Figure 2.** Haplotype networks for a) *Lepomis megalotis* (N=112), b) *Lepomis cyanellus* (N=80), c) Cyprinella lutrensis (N=88), d) *Cyprinella venusta* (N=92), and e) *Campostoma anomalum* (N=93); colors indicate the sampling location of each haplotype and match those used in Figure 1. Numbers in circles represent the number of individuals the respective haplotype was found in, black small circles represent unsampled haplotypes, numbers in white circles indicate the number of unsampled haplotypes dividing subnetworks or individuals from each other, dotted lines around haplotype circles indicate that some individuals with this haplotype had an insertion/deletion event.

White, 2009; Stepien *et al.*, 2009). It is often difficult to distinguish the impact of each of these factors based solely on observed patterns of genetic differentiation. Through the use of comparative methods we examined the role of these factors in five central Texas stream fishes.

Our first hypothesis was supported by the obtained data. The species in our study have markedly different genetic structures across our study region, indicating differential responses to similar historical or anthropogenic influences. Nevertheless, some broad generalities can be observed when comparing conge-

neric species within our data. The two *Lepomis* species show evidence of a genetic split among the Trinity and Brazos drainages. The split among drainages is most evident in *L. megalotis*, where both the geographical distribution of haplotypes (Appendix 3a) and the haplotype network (Fig. 2A) clearly differentiate Brazos populations from the population in the Trinity River system. Furthermore, the North Bosque River (Bosq1) also appears isolated, having a tight grouping of distinct haplotypes within the network, none of which are shared with other sampling locations. Only within the Little River system and the Middle Bosque River (Bosq2) does extensive sharing of haplotypes occur. Isolation of the Trinity and Brazos Rivers is also evident in *L. cyanellus* (Fig. 2B, Appendix 3b). In *L. cyanellus*, however, we see a different pattern within the Brazos River systems, with the North Bosque River (Bosq1) sharing haplotypes with the Little River system, as opposed to the Middle Bosque River (Bosq2). Furthermore, green sunfish from the Trinity site are found at different positions in the network and do not form a single distinct group as found in *L. megalotis*. One haplotype is even shared between Trinity and Bosque sites. Both *Lepomis* species show significant patterns of isolation by stream distance, which supports our second hypothesis and is expected considering their relatively smaller effective female population sizes (Table 3), low migration rates (Gerking, 1953), and the relatively strong philopatry of most sunfish species (Gerking, 1953). The isolation by distance results, combined with the geographical distribution of haplotypes, indicate that biogeographical patterns within this genus are largely shaped by historical patterns of stream connectivity.

The two shiner (*Cyprinella*) species have similar genetic patterns compared with each other, although markedly different from those observed in the genus *Lepomis*. *Cyprinella venusta* and *Cy. lutrensis* have high genetic diversity with many site-specific haplotypes (Table 2, Fig. 2C, D, Appendix 3c, d). This is not surprising considering their large effective population sizes in the region (Table 3; Pease *et al*., 2011) and tolerance of stagnant disconnected pools during summer months (Stanley *et al*., 2012). Hence, large stable populations have probably contributed to the high levels of observed genetic diversity and its maintenance in shiner species (Frankham, 1996). While large effective population sizes may have contributed to the high genetic diversity of shiners, anthropogenic factors also may have influenced the geographical distribution of haplotypes as predicted by our third hypothesis.

In *Cy. venusta*, haplotypes are shared across all sampling locations resulting in no discernible geographical pattern even between the historically isolated Trinity and Brazos drainages (Fig. 2D, Appendix 3d). However, all sampled haplotypes are closely related with no major mutational gaps in the dataset. In *Cy. lutrensis*, genetic diversity is higher and when gaps are considered no haplotypes are shared between locations (Fig. 2C, Appendix 3c). In addition, large mutational gaps are present in the dataset, suggesting the presence of some historical component. A central haplotype grouping within the network is distinctly separated from other groups and predominantly comprises Trinity River samples. A small grouping of haplotypes found only in the Little River system is also distinctly isolated from the rest of the dataset. However, the majority of haplotypes are contained in a complex network of closely related haplotypes that represents all sampling locations. The presence of multiple, highly divergent haplotype groups at any given location, as seen in *Cy. lutrensis*, is consistent with anthropogenic dispersal of non-native, commercially distributed lineages. As common baitfish and aquarium species, *Cy. lutrensis* has a history of invading, hybridizing, and outcompeting congeners when introduced (DeVivo, 1995). Furthermore, red shiners appear to increase in abundance in response to anthropogenic disturbances in the region, particularly in response to effluent discharge and sedimentation (Pease *et al*., 2011).

In summary, both shiner species probably maintain high genetic diversity through a combination of large, stable populations and anthropogenic translocation of specimens via aquarium and bait trade. Anthropogenic dispersal of non-native alleles has probably led to the distortion of natural population structure and erased much of the signatures of historical processes.

Our last hypothesis was also supported by our data. *Campostoma anomalum* exhibited the lowest genetic variation of all species in this study. The estimates of effective female population size were intermediate between the shiners and the sunfish (Table 3). The stoneroller was the only species with a significant Tajima's $D$ value ($D = -1.86$; $P < 0.01$). Such a value is typically indicative of either selective pressure or recent demographic expansion, the latter of which is consistent with the documented source–sink dynamics associated with *Ca. anomalum* (Waits *et al*., 2008). Problematically, Tajima's $D$ tests can be skewed by the presence of long branches and our dataset contains two highly divergent, rare haplotypes (Fig. 2E, Appendix 4e). Further sampling of *Campostoma* might help to determine whether these haplotypes are truly rare or are simply an artefact of undersampling.

Among our sampled species, *Ca. anomalum* is the least tolerant of harsh conditions, generally preferring flowing cool water (Edwards, 1997). Consequently, populations of this species are often

extirpated during summer months and recolonize during favourable hydrological conditions, a pattern that could result in the presence of apparently rare divergent haplotypes through the loss of intermediate haplotypes during rapid population declines. Geographically, we see little evidence of separation among drainages within this species, with haplotypes shared between the Trinity and Brazos rivers as well as across most sites (Fig. 2E, Appendix 3e, 4e). Whereas in *Cyprinella* we believe that pattern of shared haplotypes across isolated drainages may be the result of anthropogenic translocation, the same is unlikely for *Campostoma* as its sensitivity to disturbance and handling make it a poor bait species and we can find no evidence to indicate that the species is common in the aquarium trade.

## CONCLUSIONS

Biogeographical studies typically focus on one or few closely related species. This limited scope can distort broader biogeographical patterns by the extrapolation of species-specific responses to environmental and anthropogenic events. Here we try to overcome these limitations by examining five species, representing three genera from two families, collected from similar locations and using a common genetic marker. This approach allows us to distinguish common biogeographical patterns and species-specific responses, thus providing insights that cannot be derived from single species analyses. Using this method, we have documented the retention of historical patterns in larger, less abundant fish while identifying the impacts of modern ecological and anthropogenic factors on smaller, generally more abundant, and/or commercially important species. Such contrasting results both indicate the need for appropriate taxon sampling to address the biogeographical question being investigated and highlight the differential responses to anthropogenic forces by ecologically diverse species.

This study exemplifies the importance of coordinating choices of study species with questions of interest. The studied cyprinid species would be unsuitable for analysing historical patterns in this region, yet may make good models for questions related to more recent factors such as anthropogenic disturbance. In contrast, centrarchid species would be more suitable to investigate historical connectivity patterns. While some findings of our study are consistent with historical climatic and geological events, it is clear that the biogeographical patterns in this region are shaped by a complex mixture of extrinsic historical, modern ecological, and anthropogenic factors in addition to intrinsic characteristics of species. Future landscape and conservation genetic studies will profit from using interspecies comparisons, which provide a more complete picture of the relative roles that historical and contemporary extrinsic and intrinsic factors play in shaping the population structure of stream fish.

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## APPENDIX 1

Primers used in this study and GenBank accession numbers from sequences used for primer design

| Primer | Sequence | Referenced GenBank accession numbers |
|---|---|---|
| CR-F | 5′GGATTTTAACCCYCACCMCT3′ | NC_009859, NC_009865, NC_009063, NC_010957, NC_009869, NC_003195, NC_009857, NC_009873, NC_010958, NC_009864, NC_009860, NC_009851, NC_009867, NC_009858, NC_009863, NC_009874, NC_009852, NC_004409, NC_009866, NC_009870, NC_009854, NC_009868, NC_008106 |
| CR-R + M13-41 | 5′CGCCAGGGTTTTCCCAGTCACGAC TTCTAGGGCTCATCTTAACATCTTC3′ | |
| ShinerCR-F | 5′CTCCCRCCCCYGGCTCCCAA′3 | NC_008643, AB070206, NC_008103 |
| ShinerCR-R | 5′TGCATGCGGAGCTTTCTAGGGC′3 | |

## APPENDIX 2

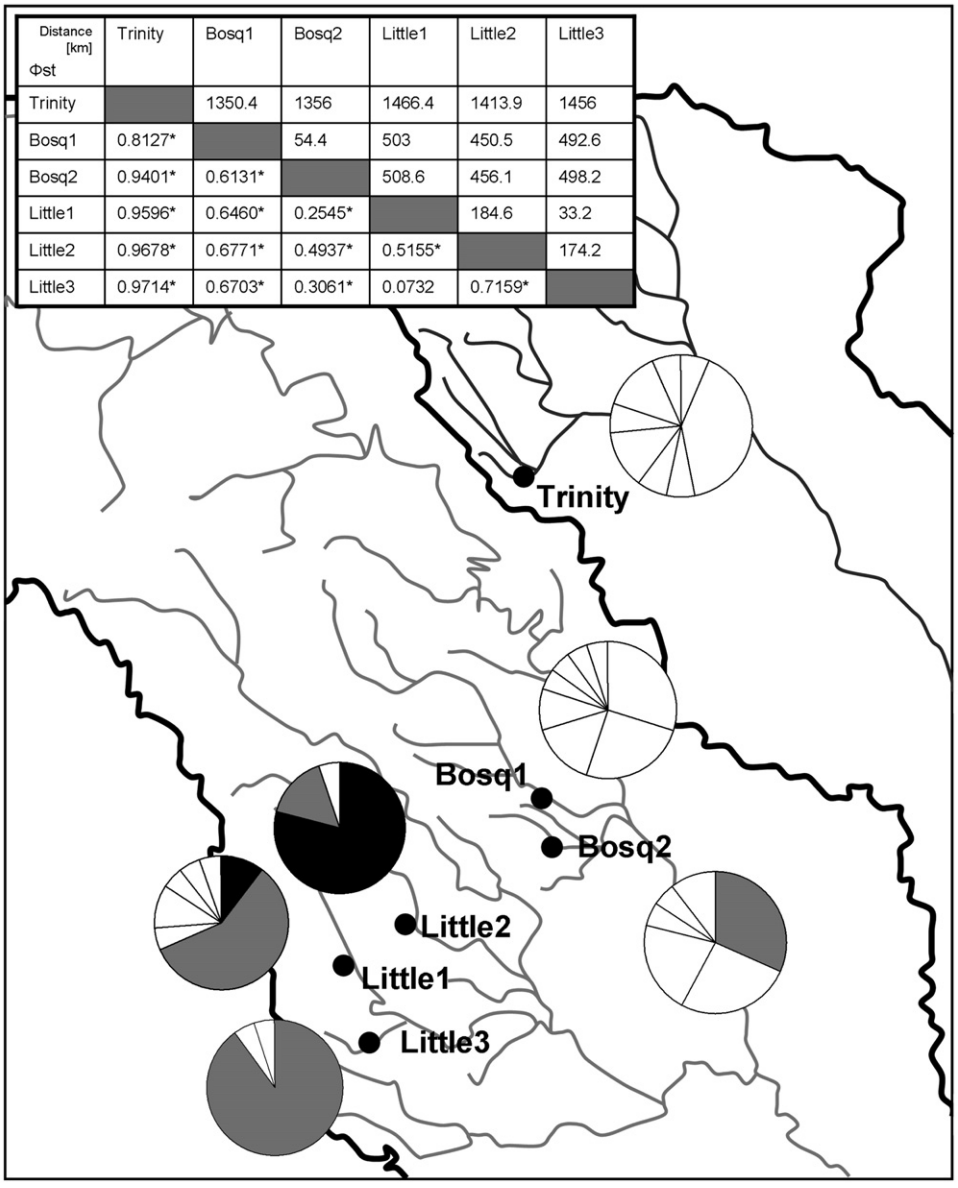
GenBank accession numbers

| Species | Location | GenBank accession numbers |
|---|---|---|
| *Lepomis megalotis* | Trinity | JN832386–JN832400 |
| | Bosq1 | JN832458–JN832477 |
| | Bosq2 | JN832439–JN832457 |
| | Little 1 | JN832420–JN832438 |
| | Little 2 | JN832401–JN832419 |
| | Little 3 | JN832478–JN832497 |
| *Lepomis cyanellus* | Trinity | JN832370–JN832385 |
| | Bosq1 | JN832343–JN832356 |
| | Bosq2 | JN832336–JN832342 |
| | Little 1 | JN832321–JN832335 |
| | Little 2 | JN832306–JN832320 |
| | Little 3 | JN832357–JN832369 |
| *Cyprinella lutrensis* | Trinity | JN832126–JN832140 |
| | Bosq1 | JN832200–JN832213 |
| | Bosq2 | JN832184–JN832199 |
| | Little 1 | JN832156–JN832171 |
| | Little 2 | JN832141–JN832155 |
| | Little 3 | JN832172–JN832183 |
| *Cyprinella venusta* | Trinity | JN832214–JN832229 |
| | Bosq1 | JN832276–JN832290 |
| | Bosq2 | JN832261–JN832275 |
| | Little 1 | JN832246–JN832260 |
| | Little 2 | JN832230–JN832245 |
| | Little 3 | JN832291–JN832305 |
| *Campostoma anomalum* | Trinity | JN832033–JN832047 |
| | Bosq1 | JN832080–JN832094 |
| | Bosq2 | JN832064–JN832079 |
| | Little 1 | JN832048–JN832063 |
| | Little 2 | JN832111–JN832125 |
| | Little 3 | JN832095–JN832110 |

## APPENDIX 3

Maps of the distribution of haplotypes. Circles represent the distribution of haplotypes. White segments represent haplotypes unique to a single sample location and colored segments representing haplotypes shared across sample sites. Stream distance and Φst values are displayed in the upper left corner. Φst values with asterisks indicate significant values ($p < 0.05$) obtained from 30,000 permutations.

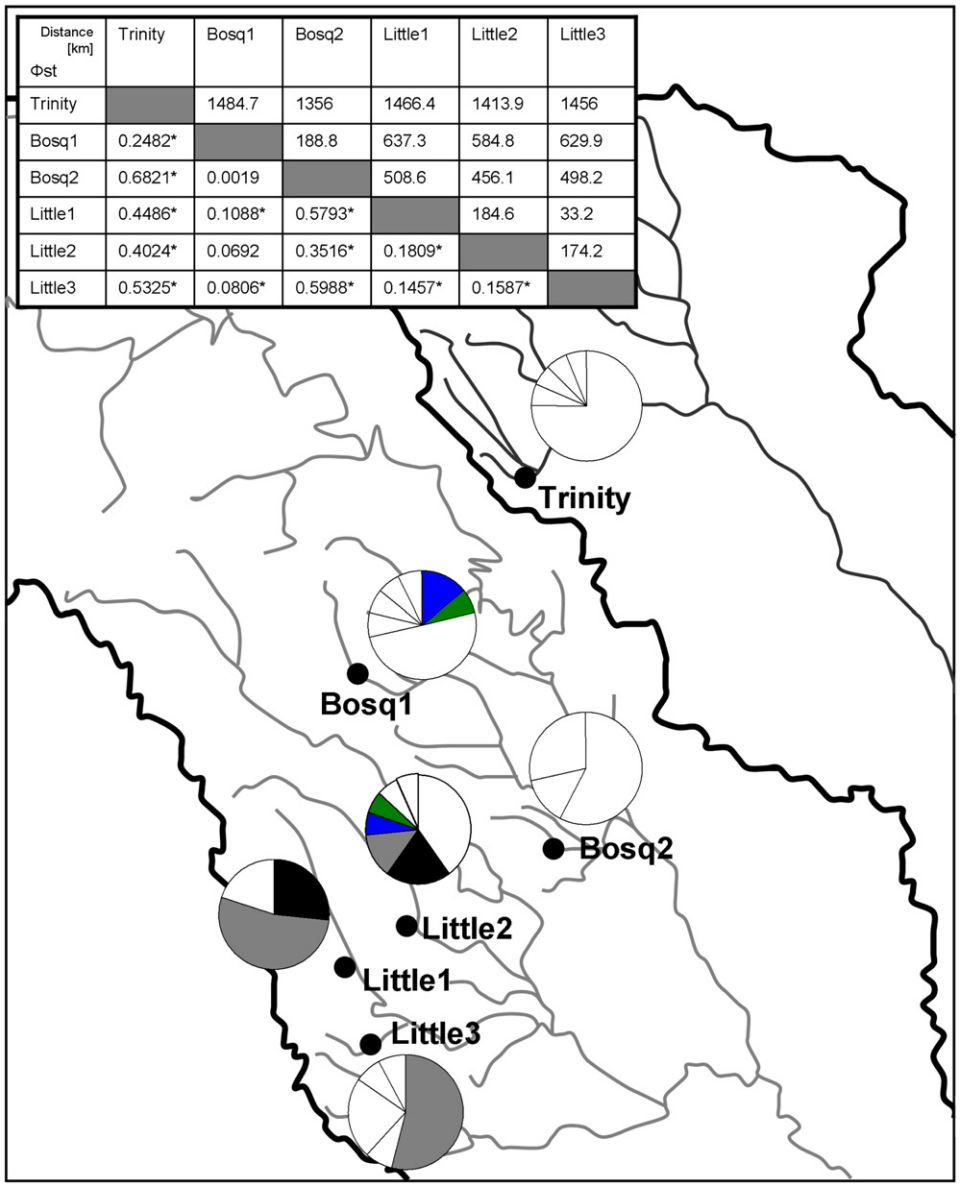
a) *L. megalotis*




| Distance [km] / Φst | Trinity | Bosq1 | Bosq2 | Little1 | Little2 | Little3 |
|---|---|---|---|---|---|---|
| Trinity | | 1350.4 | 1356 | 1466.4 | 1413.9 | 1456 |
| Bosq1 | 0.8127* | | 54.4 | 503 | 450.5 | 492.6 |
| Bosq2 | 0.9401* | 0.6131* | | 508.6 | 456.1 | 498.2 |
| Little1 | 0.9596* | 0.6460* | 0.2545* | | 184.6 | 33.2 |
| Little2 | 0.9678* | 0.6771* | 0.4937* | 0.5155* | | 174.2 |
| Little3 | 0.9714* | 0.6703* | 0.3061* | 0.0732 | 0.7159* | |

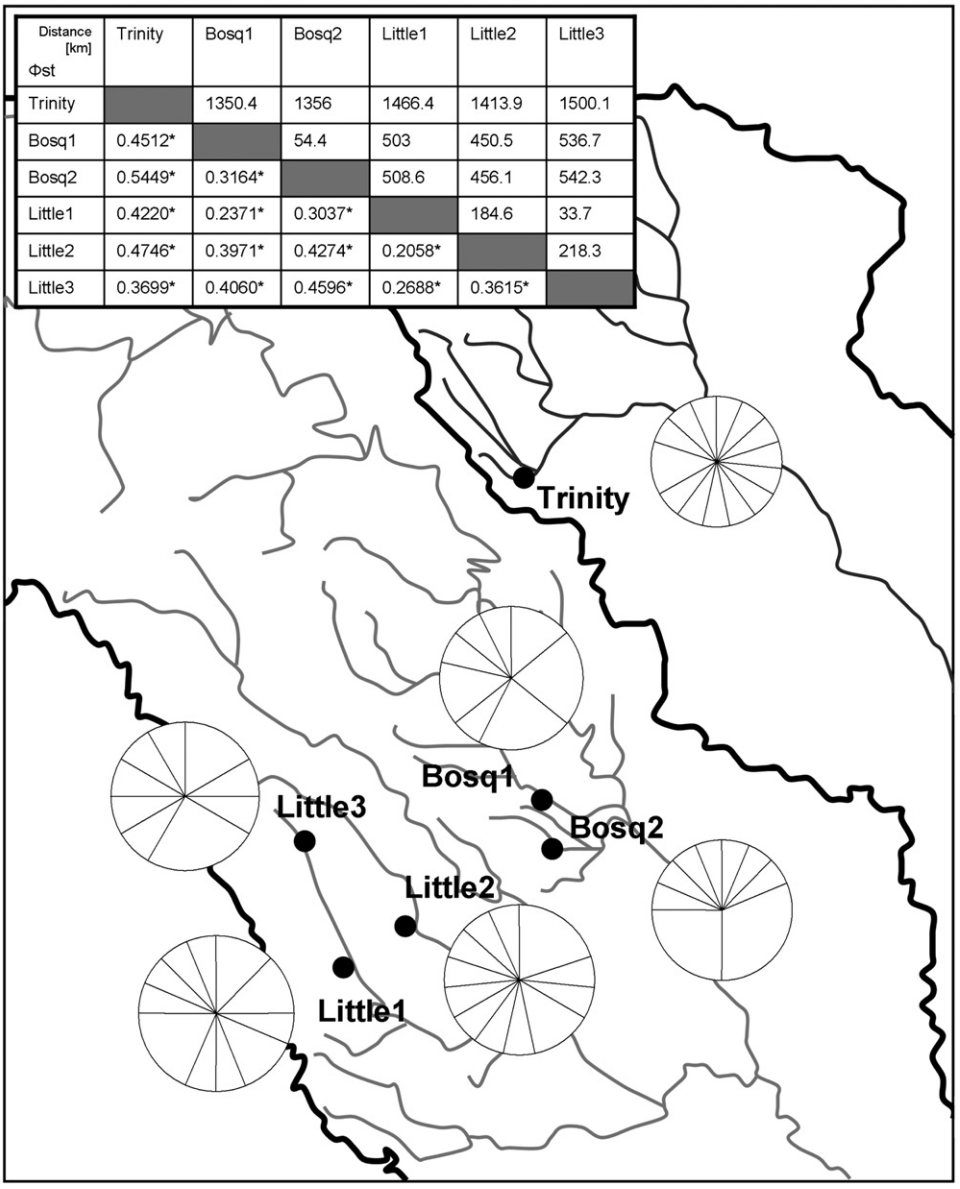
b) *L. cyanellus*

| Distance [km] Φst | Trinity | Bosq1 | Bosq2 | Little1 | Little2 | Little3 |
|---|---|---|---|---|---|---|
| Trinity | | 1484.7 | 1356 | 1466.4 | 1413.9 | 1456 |
| Bosq1 | 0.2482* | | 188.8 | 637.3 | 584.8 | 629.9 |
| Bosq2 | 0.6821* | 0.0019 | | 508.6 | 456.1 | 498.2 |
| Little1 | 0.4486* | 0.1088* | 0.5793* | | 184.6 | 33.2 |
| Little2 | 0.4024* | 0.0692 | 0.3516* | 0.1809* | | 174.2 |
| Little3 | 0.5325* | 0.0806* | 0.5988* | 0.1457* | 0.1587* | |



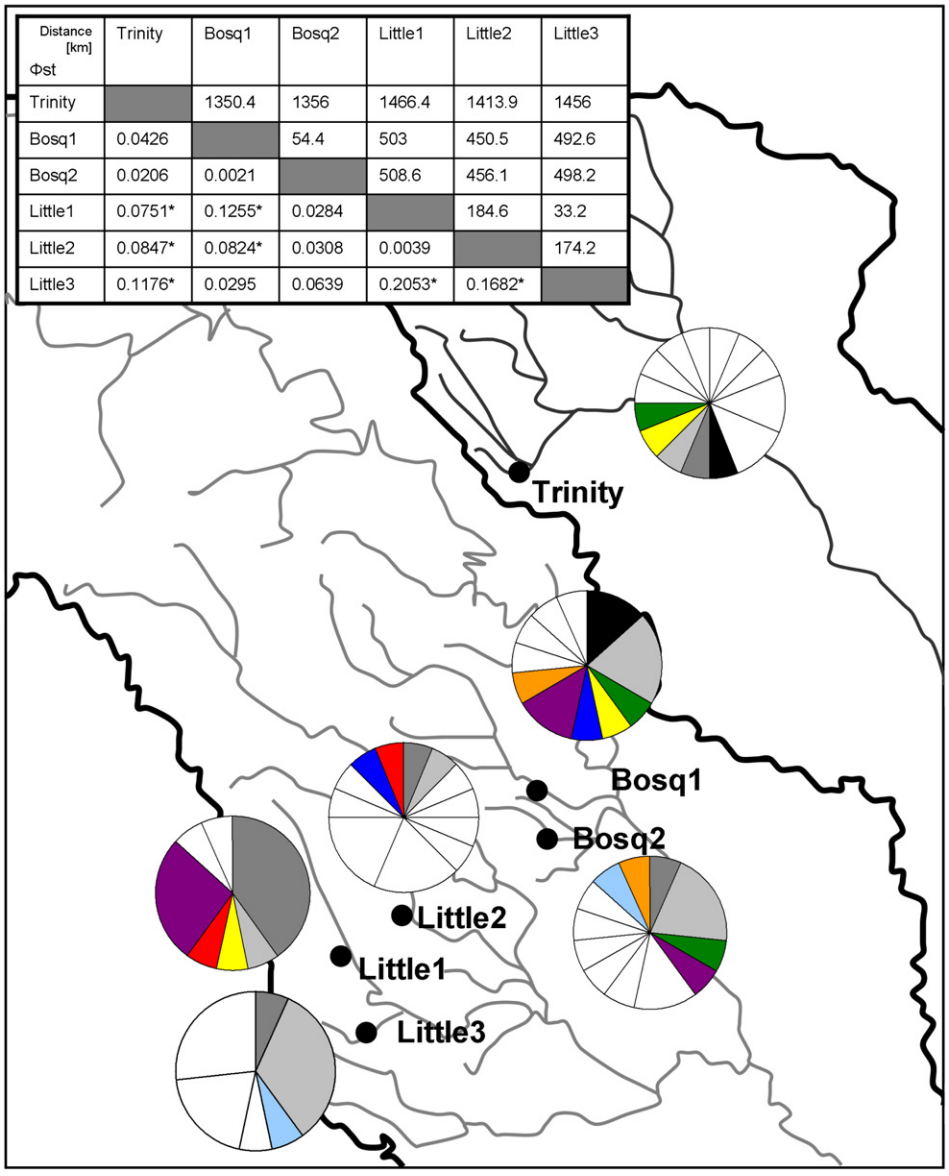
c) *C. lutrensis*

| Distance [km] Φst | Trinity | Bosq1 | Bosq2 | Little1 | Little2 | Little3 |
|---|---|---|---|---|---|---|
| Trinity | | 1350.4 | 1356 | 1466.4 | 1413.9 | 1500.1 |
| Bosq1 | 0.4512* | | 54.4 | 503 | 450.5 | 536.7 |
| Bosq2 | 0.5449* | 0.3164* | | 508.6 | 456.1 | 542.3 |
| Little1 | 0.4220* | 0.2371* | 0.3037* | | 184.6 | 33.7 |
| Little2 | 0.4746* | 0.3971* | 0.4274* | 0.2058* | | 218.3 |
| Little3 | 0.3699* | 0.4060* | 0.4596* | 0.2688* | 0.3615* | |



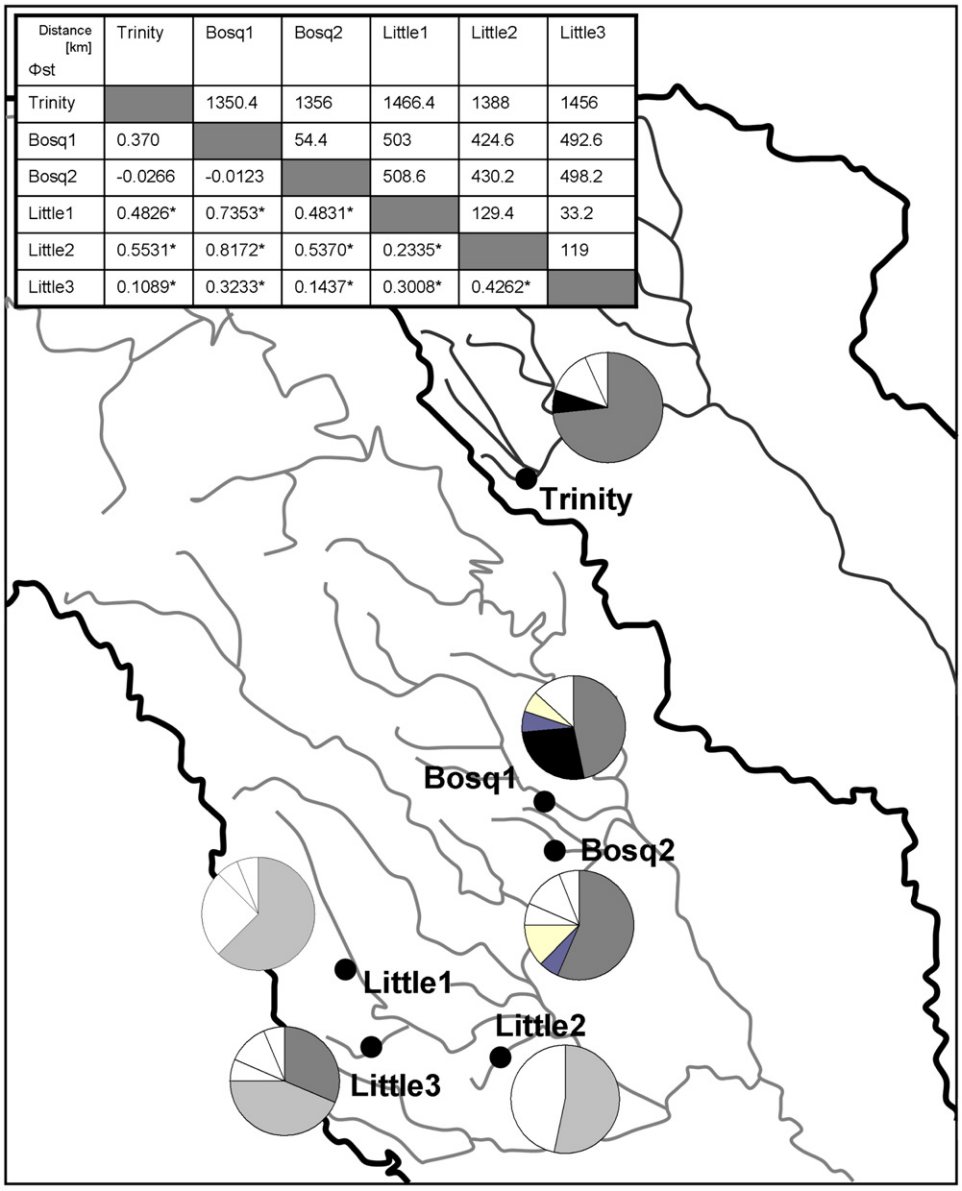
d) *C. venusta*

| Distance [km] Φst | Trinity | Bosq1 | Bosq2 | Little1 | Little2 | Little3 |
|---|---|---|---|---|---|---|
| Trinity | | 1350.4 | 1356 | 1466.4 | 1413.9 | 1456 |
| Bosq1 | 0.0426 | | 54.4 | 503 | 450.5 | 492.6 |
| Bosq2 | 0.0206 | 0.0021 | | 508.6 | 456.1 | 498.2 |
| Little1 | 0.0751* | 0.1255* | 0.0284 | | 184.6 | 33.2 |
| Little2 | 0.0847* | 0.0824* | 0.0308 | 0.0039 | | 174.2 |
| Little3 | 0.1176* | 0.0295 | 0.0639 | 0.2053* | 0.1682* | |



e) *C. anomalum*

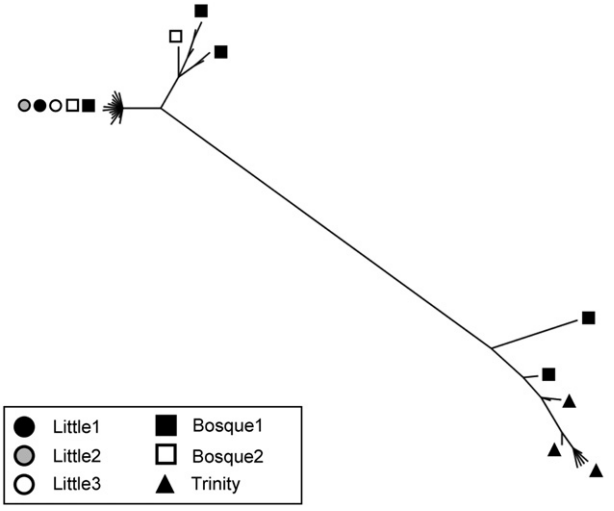
| Distance [km] Φst | Trinity | Bosq1 | Bosq2 | Little1 | Little2 | Little3 |
|---|---|---|---|---|---|---|
| Trinity | | 1350.4 | 1356 | 1466.4 | 1388 | 1456 |
| Bosq1 | 0.370 | | 54.4 | 503 | 424.6 | 492.6 |
| Bosq2 | -0.0266 | -0.0123 | | 508.6 | 430.2 | 498.2 |
| Little1 | 0.4826* | 0.7353* | 0.4831* | | 129.4 | 33.2 |
| Little2 | 0.5531* | 0.8172* | 0.5370* | 0.2335* | | 119 |
| Little3 | 0.1089* | 0.3233* | 0.1437* | 0.3008* | 0.4262* | |



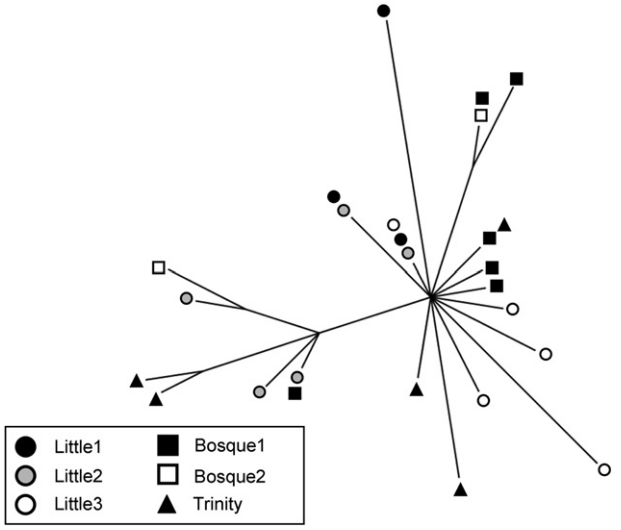
## APPENDIX 4

Bayesian trees generated from only unique haplotypes; locations where haplotypes were found are represented by symbols and are mapped on the branches of the trees

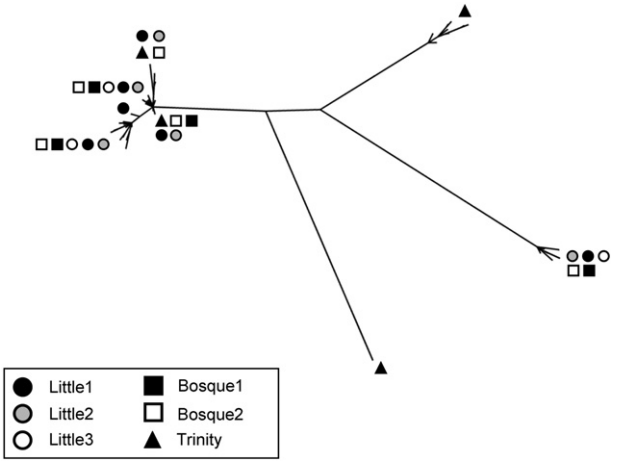
a) *L. megalotis*



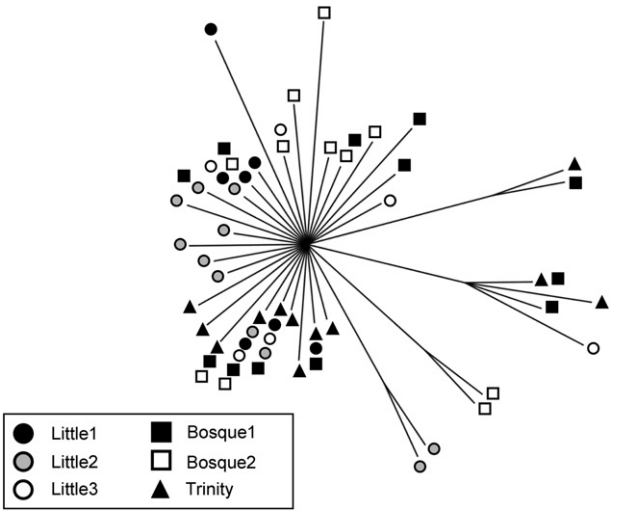

b) *L. cyanellus*




c) *C. lutrensis*




d) *C. venusta*




e) *C. anomalum*