

Evolution of the *Cyprinella lutrensis* Species-Complex. II. Systematics and Biogeography of the Edwards Plateau Shiner, *Cyprinella lepida*

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We examined geographic samples of the Edward's Plateau shiner, *Cyprinella lepida*, for restriction site variation of mitochondrial (mt)DNA. Sixteen restriction endonucleases were used to assay mtDNAs from 32 individuals of *C. lepida* sampled from the three rivers (Nueces, Frio, and Sabinal) of the Nueces River basin. Maximum-parsimony analysis using *Cyprinella lutrensis* as the outgroup and *Cyprinella venusta* as the root revealed that *C. lepida* in the Frio and Sabinal rivers is distinct from *C. lepida* in the Nueces River. A tree generated from a matrix of genetic distance using the Fitch-Margoliash method also revealed the same two distinct lineages of *Cyprinella* within the Nueces River basin. Percentage nucleotide sequence divergence estimates (\pm SE) between pairwise comparisons of the two evolutionary lineages of *Cyprinella* in the Nueces River basin and *C. lutrensis* were as follows: 8.36 ± 0.01 (*Cyprinella* in the Frio and Sabinal rivers vs *C. lutrensis*), 9.04 ± 0.03 (*Cyprinella* in the Frio and Sabinal rivers vs *Cyprinella* in the Nueces River), and 9.52 ± 0.03 (*Cyprinella* in the Nueces River vs *C. lutrensis*). Assuming mtDNA mutation-rate equivalency within *Cyprinella*, the two lineages (species) of *Cyprinella* in the Nueces River basin could have evolved nearly instantaneously in geological time. The estimates of mtDNA nucleotide sequence divergence, in concert with geographic considerations and the assumption that the genus *Cyprinella* originated north of the Coastal Plain, are consistent with the hypothesis that the ancestor(s) to the lineages of *Cyprinella* in the Nueces River basin entered the western (Gulf) Coastal Plain preglacially and from the northwest.

THE *Cyprinella lutrensis* species-complex is a putatively monophyletic assemblage of 10 or so species of North American cyprinid fishes inhabiting the western Mississippi basin, the American southwest, and northern Mexico (Mayden, 1989). The nominal species, *C. lutrensis*, is widespread and occurs in at least three physiographic provinces: the Great Plains, Central Lowlands, and Coastal Plain. Gibbs (1957) hypothesized that the ancestor(s) to *C. lutrensis* and the *C. lutrensis* species-complex evolved from an upland form that dispersed southward into the Central Lowlands and western Coastal Plain. Metcalf (1966) hypothesized that *C. lutrensis* occurred in the "Ancient Plains Stream," a preglacial drainage in North America that extended from north-central Nebraska through southeastern Oklahoma. This suggests that the ancestor(s) to *C. lutrensis* and the *C. lutrensis* complex may have dispersed to the Gulf Coastal Plain during the Tertiary. However, renewed uplift of the Rocky Mountains during the late Tertiary caused eastward-flowing Great Plains streams to leave extensive deposits of gravel, sand, and clay alluvium in the lower Central Plains (Sheldon, 1979), making regional geologic change and potential routes of fish dis-

persal southward from the Great Plains during the late Miocene-Pliocene difficult to identify.

Two biogeographic hypotheses of stream capture or flows have been proposed that could account for dispersal of the ancestor(s) to the *C. lutrensis* species-complex south to the Coastal Plain. The first involves connections of an ancient Great Plains stream east and south to what is now the Red River in eastern Oklahoma, followed by connections to the Gulf of Mexico (Gulf) through the Trinity or Brazos rivers in present-day east Texas (Connor and Suttkus, 1986). Dispersal west to the mouths of southeasterly flowing streams along the western Coastal Plain might then have occurred along what is now coastal Texas during a time when the saline waters of the Gulf had receded toward the continental shelf (Belcher, 1975). This hypothesis is compatible with dispersal of *C. lutrensis* (or its ancestor) to the western Coastal Plain during mid- to late Pleistocene: a connection between the Red and Brazos rivers has been dated to 0.6 Myr ago (Swineford et al., 1955); and the waters of the Gulf did not recede toward the continental shelf until periods of glacial maxima (Belcher, 1975). The second hypothesis involves connections in the western

Great Plains among an ancient Great Plains stream, western tributaries of the present-day Arkansas and Canadian rivers, headwaters of an ancient Rio Grande drainage, and eastward-flowing streams of the Llano Estacado. Geological information known for this region (Thomas, 1972; Belcher, 1975) could place the timing of this scenario of events to the Miocene-Pliocene border.

The focus of this study is the Edwards Plateau shiner, *Cyprinella lepida*, a *C. lutrensis* complex-species restricted to the Nueces River basin of south-central Texas. The species has been the source of some taxonomic confusion. Girard (1856) originally described *C. lepida*, but Jordan and Evermann (1896) later synonymized it with *Notropis bubalinus*. Hubbs (1954) resurrected *C. lepida*, and it was considered a distinct species for many years (Hubbs, 1956; Bailey et al., 1960; Lytle, 1972). However, C. Hubbs (Texas Parks and Wildlife Department, 1972, unpubl.), effectively synonymized *C. lepida* with *C. lutrensis* by omission of *C. lepida* from a checklist of Texas fishes. Recent morphological studies by Matthews (1987) and Mayden (1989), however, have indicated that *C. lepida* is a distinct and valid species. We focused the study on *C. lepida* because it is restricted to the three rivers of the Nueces River basin: the Nueces, Frio, and Sabinal. The spring-fed headwaters of all three rivers originate above the Balcones Escarpment along the southern portion of the Edwards Plateau, and all three rivers drain south-southeast into the western Coastal Plain where they ultimately join and empty into the Gulf.

Because *C. lutrensis* does not occur in the Nueces River basin, the divergence of *C. lepida* and its subsequent isolation in the Nueces River basin could provide an important clue regarding dispersal of *C. lutrensis* or its ancestor(s) into the western Coastal Plain. To examine divergence of *C. lepida* from *C. lutrensis*, we employed restriction site analysis of mitochondrial (mt)DNA. Mitochondrial (mt)DNA has proven to be an informative and powerful tool in studies focused on systematics, taxonomy, and phylogeography (Avisé et al., 1987; Moritz et al., 1987; Avisé, 1992). In particular, mtDNA has been useful in investigations of recent patterns of regional endemism where informative phylogenetic lineages are likely the result of a once widespread ancestral species or species complex (Avisé et al., 1987; MacNeil and Strobeck, 1987; Lamb et al., 1989). The utility of mtDNA in this respect is its genetic haploidy and maternal mode of transmission, both of which can lead to rapid geographic sorting of lineages in the absence of gene flow (Avisé, 1989; Neigel and

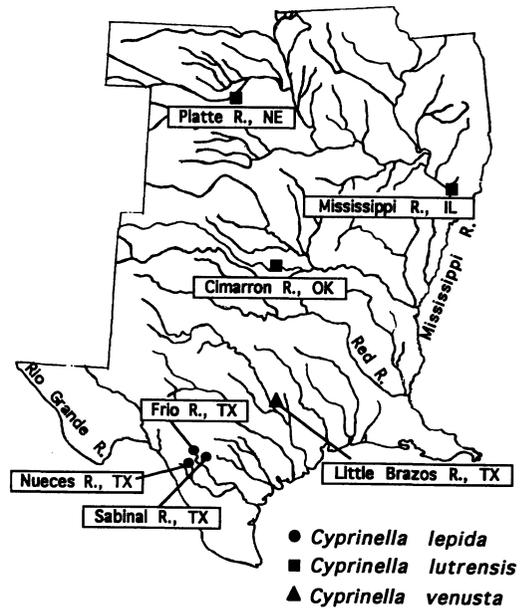


Fig. 1. Sample localities of *Cyprinella* examined in the study.

Avisé, 1986). The isolation of populations by some extrinsic barrier, given a sufficient period of time, will result in the extinction of lineages and result in monophyletic assemblages. The maternal mode of transmission and effective absence of genetic recombination also allow for straightforward interpretation of mtDNA phylogenies.

MATERIALS AND METHODS

All individuals examined in the study were collected by seine in 1987 and 1988. Samples representing *C. lepida* were obtained from the Nueces ($n = 10$), Frio ($n = 10$), and Sabinal rivers ($n = 12$). Samples of *C. lutrensis* were obtained from the Cimarron ($n = 11$), Platte ($n = 12$), and Mississippi ($n = 11$) rivers. One sample of *C. venusta* ($n = 10$) was obtained from the Little Brazos River. Individual specimens were immediately frozen whole in liquid nitrogen for transport to the laboratory where they were stored at -80°C . A map of sample localities is shown in Figure 1.

Details of genomic DNA extraction, precipitation, and storage may be found in Gold and Richardson (1991). Sixteen restriction endonucleases were used to digest mtDNA molecules according to manufacturer's specifications: *Apa*I, *Bcl*I, *Bgl*II, *Bst*EII, *Eco*RI, *Eco*RV, *Hpa*I, *Kpn*I, *Nco*I, *Nsi*I, *Pst*I, *Pvu*II, *Sac*I, *Sac*II, *Sca*I, and *Xho*I. Methods of agarose electrophoresis,

transfer to nylon membranes, hybridization, and autoradiography may be found in Gold and Richardson (1991). Hybridization employed an intact mtDNA molecule from *C. lutrensis* labeled with (32 P)dCTP (New England Nuclear, sp. act. = 3000 Ci/mM) by random priming (Feinberg and Volgelstein, 1984). A description of the mtDNA probe used may be found in Richardson and Gold (1991). MtDNA fragments were sized by fitting migration distances to a least-squares regression line of lambda DNA-*Hind*III fragment migration-distances. Homology of fragments was tested by side-by-side comparisons, when necessary. Relative map positions of all restriction sites were determined by double-digestion (Sambrook et al., 1989).

A restriction-site presence/absence matrix for individual mtDNA composite genotypes (haplotypes) was constructed using the GENERATE program in the Restriction Enzyme Analysis Package (REAP) of McElroy et al. (1992). Maximum-parsimony analysis of mtDNA haplotypes ("gene tree" analysis) and of individual sample localities (samples) employed the Phylogenetic Analysis Using Parsimony (PAUP) package of Swofford (1991). When samples were used as operational units, restriction sites were scored as present (code 1) if they occurred at a sample locality or absent (code 0) if they did not. All autapomorphic and symplesiomorphic characters were removed before PAUP runs using the REDUCE program in REAP. Bootstrapping (Felsenstein, 1985) was used to assess reproducibility of phylogenetic hypotheses obtained from maximum parsimony and employed algorithms in PAUP. All trees produced using maximum-parsimony were rooted with *C. venusta*, a species placed by Maiden (1989) in the clade sister to the *C. lutrensis* species-complex. Estimates of nucleotide sequence divergence among haplotypes were calculated after Nei and Tajima (1981) using the DSE program in REAP. The interhaplotype (distance) matrix was used to calculate an interpopulational (intersample) nucleotide sequence divergence matrix based on haplotype frequencies (Nei, 1987). Trees were generated from haplotype and sample distance matrices using the Fitch-Margoliash method and the FITCH program in the Phylogeny Inference Package (PHYLIP) of Felsenstein (1992). This method relaxes the assumption of rate homogeneity along branches of a phylogenetic tree (Fitch and Margoliash, 1967).

RESULTS

Single digestions with 16 restriction endonucleases yielded 142 unique restriction sites

among 72 individuals assayed (avg. = 57 sites/individual). The mean genome size of complete single digestions was 16.7 ± 0.2 kilobases (kb). One individual of *C. lutrensis* from the Cimarron River was found to possess a tandem duplication of approximately 3.6 kb (Richardson and Gold, 1991). No evidence of site or length heteroplasmy was observed. Digestion patterns of the 16 enzymes revealed 32 composite mtDNA genotypes (haplotypes) among the 72 individuals (Table 1). Only two haplotypes were found in more than one sample: haplotype 9 was found in *Cyprinella* from the Frio and Sabinal rivers, and haplotype 14 was found in *C. lutrensis* from the Cimarron and Mississippi rivers.

Maximum-parsimony analysis of mtDNA haplotypes generated 36 equally parsimonious "gene" trees of 143 steps. Bootstrap analysis (500 replicates, 50% majority rule) produced a tree identical to the strict consensus tree (Fig. 2). Four clades within the ingroup had bootstrap values of 95% or greater. These included the following: (1) five haplotypes representing *C. lutrensis* from the Platte River; (2) 12 haplotypes representing all individuals of *C. lutrensis*; (3) four haplotypes representing *Cyprinella* from the Nueces River; and (4) seven haplotypes representing *Cyprinella* from the Frio and Sabinal rivers. Relationships among *C. lutrensis* and the clades of *Cyprinella* from the Nueces River and from the Frio and Sabinal rivers were not resolved at $\geq 50\%$ bootstrapping. The two clades of *Cyprinella* from the Nueces River basin differed from each other by a minimum of 43 restriction sites and from the *C. lutrensis* by an average of 47.9 restriction sites (Fig. 3). Almost identical maximum-parsimony trees were obtained when haplotypes were combined to form operational units comprised of samples. We also carried out maximum-parsimony analyses using Dollo parsimony criteria (Farris, 1977) to assess effects of weighting restriction-site gains over losses. The major features (i.e., clades reproduced by $\geq 95\%$ bootstrapping) generated using maximum-parsimony analysis with unordered character states (Fig. 2) remained identical when using Dollo parsimony.

Fitch-Margoliash trees generated from matrices of genetic distance among haplotypes and among samples yielded topologies essentially identical to trees generated using maximum parsimony. In the tree of samples (Fig. 4), *Cyprinella* from the Nueces River, and from the Frio and Sabinal rivers, appear (slightly) more similar to one another than to the clade of *C. lutrensis* samples. The branch uniting *Cyprinella* from the Frio and Sabinal rivers to *Cyprinella*

TABLE 1. GEOGRAPHIC DISTRIBUTION OF 32 MITOCHONDRIAL DNA HAPLOTYPES.

MtDNA haplotype ^a	Nueces River drainage			<i>Cyprinella lutrensis</i>			<i>Cyprinella venusta</i>
	Nueces River	Frio River	Sabinal River	Cimarron River	Platte River	Mississippi River	
1	4	—	—	—	—	—	—
2	3	—	—	—	—	—	—
3	2	—	—	—	—	—	—
4	1	—	—	—	—	—	—
5	—	2	—	—	—	—	—
6	—	4	—	—	—	—	—
7	—	1	—	—	—	—	—
8	—	1	—	—	—	—	—
9	—	1	11	—	—	—	—
10	—	1	—	—	—	—	—
11	—	—	1	—	—	—	—
14	—	—	—	7	—	7	—
15	—	—	—	1	—	—	—
16	—	—	—	1	—	—	—
17	—	—	—	2	—	—	—
18	—	—	—	—	—	—	1
19	—	—	—	—	—	—	1
20	—	—	—	—	—	—	1
21	—	—	—	—	—	—	1
22	—	—	—	—	—	—	1
23	—	—	—	—	—	—	1
24	—	—	—	—	—	—	1
25	—	—	—	—	—	—	1
26	—	—	—	—	—	—	2
28	—	—	—	—	—	2	—
30	—	—	—	—	—	1	—
36	—	—	—	—	—	1	—
45	—	—	—	—	1	—	—
46	—	—	—	—	8	—	—
47	—	—	—	—	1	—	—
48	—	—	—	—	1	—	—
49	—	—	—	—	1	—	—
Total	10	10	12	11	12	11	10

^a MtDNA fragment patterns (and sites) for each restriction enzyme may be obtained from the first author.

from the Nueces River, however, is very short. Branch lengths of each of the three higher-level groups (i.e., *Cyprinella* from the Nueces River, *Cyprinella* from the Frio and Sabinal rivers, and *C. lutrensis*) are appreciably longer. Nucleotide sequence divergence (in percent, \pm SE) between pairwise comparisons of the three higher-level clades are 9.04 ± 0.03 (Frio-Sabinal *Cyprinella* vs Nueces *Cyprinella*), 8.36 ± 0.01 (Frio-Sabinal *Cyprinella* vs *C. lutrensis*), and 9.52 ± 0.03 (Nueces *Cyprinella* vs *C. lutrensis*).

DISCUSSION

The distribution of mtDNA haplotypes among samples of *Cyprinella* from the Nueces River basin clearly indicate the occurrence of two mtDNA assemblages. MtDNA haplotypes of *Cy-*

prinella from the Frio and Sabinal rivers differed from one another by only a few restriction sites and by an average nucleotide sequence divergence of 0.46%. MtDNA haplotypes of *Cyprinella* from the Frio and Sabinal rivers collectively, however, differed from those of *Cyprinella* from the Nueces River by a minimum of 43 restriction sites and by an average nucleotide sequence divergence of 9.04%. Even when nucleotide sequence divergence estimates were corrected for intrapopulation variation, the difference between *Cyprinella* from the Frio and Sabinal rivers and *Cyprinella* from the Nueces River could not be attributed to elevated within-sample (intrapopulation) variation. Based on the genetic divergence between the two mtDNA assemblages, we hypothesize that *Cyprinella* in the Frio and Sabinal rivers and in the

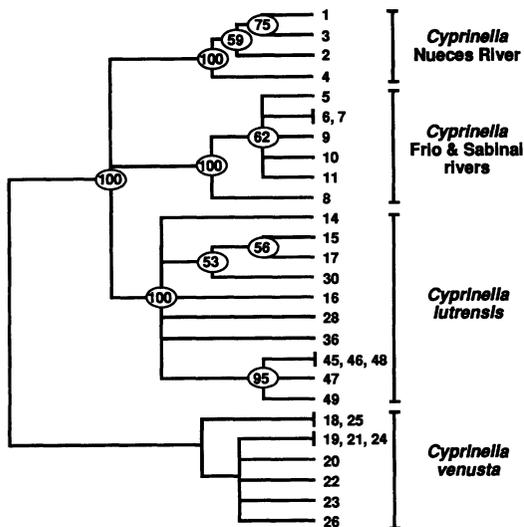


Fig. 2. Strict consensus tree of mtDNA haplotypes produced by maximum-parsimony analysis. Numbers at nodes indicate the proportion of times (from 500 replicates) that a group was distinguished in bootstrap analysis.

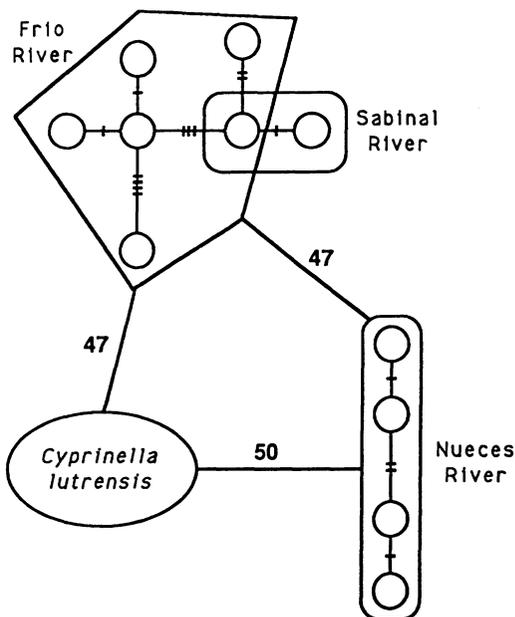


Fig. 3. Minimum-length parsimony network separating haplotypes from the Nueces River basin. Hatch marks represent gains/losses of restriction sites. Numbers on branches between pairwise comparisons of *Cyprinella* represent the average number of restriction site changes among haplotypes.

Nueces River are distinct species. Operationally, separate species designation for the evolutionary lineages of *Cyprinella* in the Frio and Sabinal rivers vs *Cyprinella* in the Nueces River is reinforced by comparison with *C. lutrensis*. Both lineages of *Cyprinella* in the Nueces River basin are roughly equidistant from *C. lutrensis* in total number of restriction-site differences and in average (corrected) nucleotide sequence divergence.

Taxonomy and systematics.—Our hypothesis that *Cyprinella* from the Frio and Sabinal rivers represent a distinct species from *Cyprinella* in the Nueces River poses a nomenclatorial problem. Girard (1856) originally described *C. lepida* from material taken in the Frio River. The description by Girard, however, was somewhat incomplete, and the type specimens were ultimately lost (Hubbs, 1954). Subsequently, *C. lepida* was considered a valid species by several authors (e.g., Hubbs, 1956; Lytle, 1972) until Hubbs (Texas Parks and Wildlife Department, 1972, unpubl.) effectively synonymized *C. lepida* with *C. lutrensis* by omitting *C. lepida* from a checklist of Texas fishes. Matthews (1987) examined meristic, morphometric, and life-color variation among 105 populations of *C. lutrensis*, including *Cyprinella* from the Nueces River basin. He concluded that *Cyprinella* from the Nueces River basin warranted species status based on distinctive nuptial coloration in males and extreme

conditions (relative to *C. lutrensis*) in 11–18 measurement and 3–4 meristic characters. Nearly all *Cyprinella* from the Nueces River basin examined by Matthews, however, were from the Nueces River. He did examine four specimens of *Cyprinella* from the Frio River and noted that principal component analysis placed these four specimens well outside the cluster that included *Cyprinella* from the Nueces River and that the four specimens differed in male nuptial coloration from *Cyprinella* in the Nueces River. Maiden (1989) examined several mor-

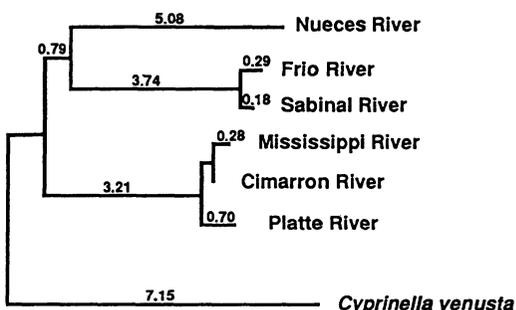


Fig. 4. Distance tree generated by the Fitch-Margoliash procedure. Branch lengths to nodes and terminal taxa represent output from the FITCH program in PHYLIP ($\times 100$).

phological (including osteological) characters among several species of *Cyprinella* and found *C. lepida* to be significantly distinct from *C. lutrensis*. All of the *C. lepida* material examined by Mayden were from the Nueces River.

The nomenclatorial problem is that Girard described *C. lepida* from the Frio River, whereas nearly all morphological data used to define *C. lepida* are from an apparently distinct species of *Cyprinella* in the Nueces River. Based on priority of description, one alternative would be to retain *C. lepida* for *Cyprinella* from the Frio and Sabinal rivers and refer to *Cyprinella* from the Nueces River as an undescribed species. However, because complete descriptive morphological data are available only for *Cyprinella* from the Nueces River, a second alternative would be to retain *C. lepida* for *Cyprinella* from the Nueces River and formally describe *Cyprinella* from the Frio and Sabinal rivers. For this paper, we choose the first alternative and refer hereafter to *Cyprinella* from the Frio and Sabinal rivers as *C. lepida* and use *C. sp. (Nueces)* to refer to *Cyprinella* from the Nueces River.

Using mtDNA restriction-site data, neither maximum parsimony nor Fitch-Margoliash analysis of genetic distances were able to adequately resolve relationships among *C. lepida*, *C. sp. (Nueces)*, and *C. lutrensis*. Very possibly, additional molecular characters or a thorough analysis of morphological-character variation in *Cyprinella* from the Frio and Sabinal rivers may help resolve the trichotomy. It also is possible that resolving the trichotomy may prove extremely difficult. Assuming mtDNA mutation-rate equivalency across the three lineages, the essentially identical nucleotide-sequence-divergence estimates among the three lineages suggest each may have originated near-instantaneously in geological time. A similar hypothesis has been proposed for unresolved polychotomies in other organismal groups where molecular dating suggests that lineages could have originated at approximately the same time (Hixson and Brown, 1986; Gatesy et al., 1992).

Biogeography.—The evolutionary origin and biogeography of *C. lepida*, *C. sp. (Nueces)*, and other members of the *C. lutrensis* species-complex is related both to dispersal/vicariance of the ancestor(s) to *C. lutrensis* and the *C. lutrensis* species-complex, and to the historical geography of the western Coastal Plain. With respect to the latter, many of the present-day geologic features that characterize central-west Texas were already formed at the Miocene-Pliocene border. The Balcones Fault, a large underground fault zone that runs east then north

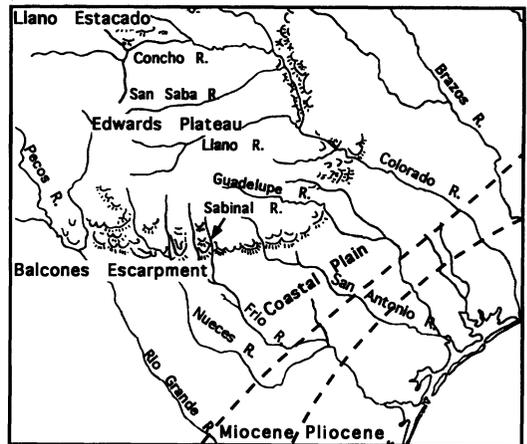


Fig. 5. Map showing many details of the present-day geology of the area surrounding the Nueces River basin. Dashed lines represent location of coast line during periods of sea level rise corresponding to the Miocene and Pliocene.

through central Texas and is part of the much larger Ouachita foldbelt (Nicholas and Rozendal, 1984), had shattered by mid-Miocene (Walton, 1990), resulting in subduction of the underlying sedimentary limestone to the south and southeast and elevation to the north and west. This resulted in what is now called the Balcones Escarpment, with the Edwards Plateau to the north and west and the western Coastal Plain to the south and east. It was during and following this faulting that the canyons of the Nueces, Frio, and Sabinal rivers were cut (Walton, 1990). To the south, much of the western Coastal Plain below the Edwards Plateau was inundated by the Gulf of Mexico during the Miocene and Pliocene (Belcher, 1975). The waters of the Gulf gradually receded to present-day levels by the end of the Pliocene, although minor fluctuations continued to persist throughout the Quaternary (Belcher, 1975; Swift et al., 1986). The late Tertiary also was a period of volcanic activity in west Texas that was associated, in part, with renewed uplift of the Rocky Mountains (Sheldon, 1979). Rivers flowing to the east from newly uplifted areas deposited extensive amounts of sand, gravel, and clay, forming what is referred to as the Llano Estacado or staked plains north of the Edwards Plateau (Sheldon, 1979). A map showing many details of the present-day geology of the region is shown in Figure 5.

Metcalf (1966) hypothesized that a preglacial Ancient Plains Stream flowed through both the Great Plains and Central Lowlands. This preglacial Plains Stream presumably drained streams

west of a divide near the Flint Hills in Kansas and included both the upper Platte River and several streams in the southern Great Plains. By the Pleistocene, the Plains Stream had unified several of the drainages of the Great Plains and diverted them southward (Cross et al., 1986). Assuming that the ancestor(s) to *C. lutrensis* and the *C. lutrensis* species-complex evolved from an upland form that evolved north of the western Coastal Plain (Gibbs, 1957), two important linkages of the Plains Stream that would have affected dispersal of these ancestor(s) southward are a Kansan transgression of the Red River followed by a connection with the present-day Trinity River, and a Kansan-Illinoian capture of a Brazos River tributary by the Red River, dated as occurring approximately 0.6 Mya (Connor and Suttkus, 1986; Swineford et al., 1955). Once in the Trinity and/or Brazos rivers, *C. lutrensis* (or its progenitors) easily could have moved downstream and ultimately reached a broadened coastal area that existed during glacial maxima when sea levels were extensively lowered (Connor and Suttkus, 1986). Given the virtual absence of evidence for lateral stream transfers among western Coastal Plain drainages (Connor and Suttkus, 1986), dispersal in the broadened coastal area west to the mouths of several southeasterly flowing streams (e.g., the ancestral Nueces River) likely would have been the primary route of progenitor(s) to *Cyprinella* in the Nueces River basin. Fluctuating sea levels during the Pleistocene (Swift et al., 1986) could have served to isolate *Cyprinella* in different drainages within the basin. This series of events strongly implicates mid- to late Pleistocene as the time when the lineages of *Cyprinella* evolved in the Nueces River basin.

A second possibility is that the ancestor(s) to *C. lutrensis* or the *C. lutrensis* species-complex could have entered the western Coastal Plain through connections in the western Great Plains. By the Tertiary, renewed uplift of the Rocky Mountains in what is now south-central Colorado created an ancestral Rio Grande-Pecos River that flowed southeasterly and could have emptied into the present-day lower Pecos River, the present-day Colorado River, or some other Gulf Coast drainage (Thomas, 1972; Belcher, 1975; Echelle and Echelle, 1978). Later, Miocene uplift of the Sangre de Cristo Mountains in northern New Mexico and Pliocene uplift of the Guadalupe Mountains in western Texas (Thomas, 1972; Belcher, 1975) would have severed connections between the Rio Grande River flowing south out of present-day New Mexico and streams and rivers in west Texas flowing southeasterly into the Gulf of Mexico. This could

have resulted in isolated populations of *C. lutrensis*-like fish north of the Nueces River basin. Subsequent stream captures could then have transferred *C. lutrensis*-like fish into the headwaters of streams draining the Nueces River basin. Integral in this scenario is the Ogallala Formation, a Tertiary mantle that covered the western Great Plains and extended into the Llano Estacado where the precursors to several Gulf Coast streams and rivers have their origins (Thornbury, 1965). Although patterns of potential stream capture across the Miocene-Pliocene Ogallala surface are difficult to reconstruct, the Ogallala was most certainly the primary aquifer in the western Plains, and its surface was relatively flat with little relief evident until the Balcones Escarpment below the central part of the Edwards Plateau. This series of events would significantly predate the Pleistocene and imply that lineages of *Cyprinella* in the Nueces River basin could have evolved preglacially, possibly during the late Miocene or early Pliocene.

Our inability to resolve phylogenetic relationships among *C. lepida*, *C. sp.* (Nueces), and *C. lutrensis*, and the near identity of nucleotide sequence divergence among the three lineages, suggests a vicariant event (or series of events) where connections among the various drainages were severed at roughly the same geological time. Assuming that the two lineages of *Cyprinella* in the Nueces River basin evolved within the same geological time frame, the estimates of approximately 9% mtDNA nucleotide sequence divergence among *C. lepida*, *C. sp.* (Nueces), and *C. lutrensis* clearly support the hypothesis that vicariance occurred preglacially. This argument is based primarily on a comparison of estimated rates of mtDNA sequence evolution required for divergence of *Cyprinella* in the Nueces River basin during the mid- to late Pleistocene versus divergence during the late Miocene-early Pliocene: divergence during the mid- to late Pleistocene (0.6–0.9 Myr ago) yields an mtDNA evolutionary rate of 10–15% per million years, whereas divergence during the late Miocene-early Pliocene (6.0–4.5 Myr ago) yields an mtDNA evolutionary rate of 1.5–2.0% per million years. The latter is far more consistent with rates of mtDNA evolution estimated in other poikilothermic vertebrates (Martin and Palumbi, 1993), where average rates appear to be in the range 0.20–0.75%, and in bony fishes, where estimated rates are 0.22% (*Alosa*; Bentzen et al., 1993), 0.50–0.90% (salmon; Martin and Palumbi, 1993), and 1.91% (*Fundulus*; Brown and Chapman, 1991).

Based on the above, we hypothesize that the

ancestor(s) of *Cyprinella* in the Nueces River basin arrived at the Edwards Plateau prior to the Pleistocene and subsequently gave rise to *C. lepida* and *C. sp.* (Nueces). The closest present-day watershed north of the headwaters of the Nueces, Frio, and Sabinal rivers is the Colorado River drainage, suggesting a sequence of ancient connections among western tributaries of a preglacial Great Plains stream, tributaries of the ancestral Rio Grande River, the ancestral Colorado River drainage, and the upper Nueces River basin. The present-day headwaters of several Colorado River tributaries (e.g., the Llano, San Saba, and Concho rivers) all occur in the south-central part of the Edwards Plateau just north of the Nueces River basin, and the present-day distance between the southernmost extension of the Llano River (the tributary of the Colorado River closest to the upper Nueces River basin) and the headwaters of the Nueces and Frio rivers is only 12 km and 40 km, respectively. Very possibly, the increased accumulation of sediments and debris resultant from the late Tertiary uplift of the Rocky Mountains and the vulcanism occurring in west Texas could have served to sever physical connections among drainages.

A preglacial divergence of *C. lepida* and *C. sp.* (Nueces) would appear to preclude the possibility that ancestors to *C. lepida* and *C. sp.* (Nueces) entered the western Coastal Plain from the Trinity or Brazos rivers in east Texas and then migrated to the mouth of the Nueces River along a broadened coastal plain. Most of the known connections between the Central Plains and Gulf Coast drainages were during the Pleistocene (Connor and Suttkus, 1986; Swineford et al., 1955), as was the broadened coastal plain. In addition, even if pre-Pleistocene faunal transfer occurred from the Great Plains to the Gulf via connections in east Texas, continued westward movement along the Gulf Coast would have been largely precluded by Miocene-Pliocene levels of the Gulf (Belcher, 1975), and there is virtually no direct evidence for stream captures among western Coastal Plain drainages (Connor and Suttkus, 1986).

There are two caveats to our biogeographic hypothesis regarding the origin of *Cyprinella* in the Nueces River basin. The first is our assumption that rates of mtDNA sequence evolution in *Cyprinella* are similar to those reported in other poikilothermic vertebrates. Variation in mtDNA evolutionary rate has been hypothesized for a number of vertebrates, including cyprinid fishes (Dowling and Brown, 1989; Kocher et al., 1989; Avise et al., 1992). However, for *Cyprinella* in the Nueces River basin to have

diverged during the mid-Pleistocene, the rate of mtDNA sequence evolution minimally would need to be increased 5–10 fold. Given that rates of mtDNA evolution in poikilotherms appear, on average, to be less than 1% per million years (Martin and Palumbi, 1993), we believe it possible that divergence of *Cyprinella* in the Nueces River basin may have occurred well before the Pliocene.

The second caveat regards our assumption (following Gibbs, 1957) that the ancestor(s) to *C. lutrensis* and the *C. lutrensis* species-complex evolved from an upland form that dispersed to the western Coastal Plain from the north. In a general way, our data are consistent with the hypothesis that *Cyprinella* in the Nueces River basin evolved by vicariance of a widespread population of *Cyprinella* that could have originated almost anywhere within the range currently occupied by notropin shiners. We believe this to be unlikely for two reasons. The first is the low incidence of endemism in the western Coastal Plain ichthyofauna and the extensive extralimital distributions of many of its species, both of which suggest that a large proportion of species (or their ancestors) evolved outside of the area (Connor and Suttkus, 1986). The second is that the genus *Luxilus*, the putative sister-group to *Cyprinella* (Mayden, 1989), is distributed well to the north of the Coastal Plain, suggesting that divergence of *Cyprinella* occurred outside (and to the north) of the Coastal Plain.

Conservation.—The apparent occurrence of two unique, endemic species of *Cyprinella* in the Nueces River basin has implications for conservation biology. Ground-water (underground aquifer) levels for much of south-central Texas have decreased substantially over the past decade, resulting in significantly reduced water-flow in spring-fed rivers such as the Sabinal, Frio, and Nueces (R. E. Edwards, pers. comm.). In addition, much of the land in the Nueces River basin is used for agriculture, and both overgrazing by cattle and possible stream pollution from pesticides and other chemicals pose serious problems for aquatic fauna (G. P. Garrett, pers. comm.). Recent and past efforts on our part to obtain additional specimens of *Cyprinella* from the Nueces, Frio, and Sabinal rivers suggest that population sizes have declined appreciably over the last 15–20 years. The decline in abundance is particularly evident in the Sabinal River where we have been unable to find *Cyprinella* except in the very headwaters in Lost Maples State Natural Area. Based on the above, we emphasize the need for conservation measures to protect both species.

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