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Systematics of the Blacktail Shiner (*Cyprinella venusta*) Inferred from Analysis of Mitochondrial DNA

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We used mitochondrial DNA (mtDNA) restriction-site analysis to study systematics and biogeography of the blacktail shiner, *Cyprinella venusta*. MtDNA restriction sites were assayed from 20 populations of *C. venusta* collected from across its geographic range. Maximum-parsimony and neighbor-joining methods revealed four major, mtDNA-based phylogeographic clades (groups): these were termed Chocktawhatchee, Apalachicola, Mobile, and Western. The Western clade contained four distinct mtDNA lineages: Pearl-Pascagoula, Mississippi, East Texas, and West Texas. MtDNA phylogeographic subdivision within *C. venusta* is not strictly concordant with geographic subdivisions (ranges) of the three nominal subspecies (*C. v. venusta*, *C. v. cercostigma*, and *C. v. stigmatura*). MtDNA clades within *C. venusta* exhibited an east to west pattern of divergence across the Gulf Coastal Plain, with phylogenetic relationships among the clades inferred to be (Chocktawhatchee (Apalachicola (Mobile, Western))). Observed phylogeographic discontinuities involving the Apalachicola and Mobile clades corresponded to previously hypothesized zones of vicariance. Taxonomic revision of blacktail shiners may be warranted.

THE blacktail shiner, *Cyprinella venusta*, is a cyprinid fish distributed throughout the south-central and southeastern United States. Its range extends eastward from the Rio Grande River in western Texas to the Suwannee River in northern Florida, northward to the Mississippi Embayment of southern Illinois (Page and Burr, 1991). The species was described by Girard (1856) based on specimens taken from the Sabinal River in the Edwards Plateau region of west Texas. Subsequent to Girard's description, populations of *C. venusta* have been referred to by at least 10 different taxonomic names (Gibbs, 1957a, 1957b). Included are *C. cercostigma*, *Photogenis stigmaturus*, and *P. eurystomus* (Gibbs, 1957b). Bailey et al. (1954) recognized a single taxon (*C. venusta*) but suggested that a study of geographic variation in *C. venusta* might yield subspecific designations. Gibbs (1957a, 1957b) reviewed morphological and meristic variation

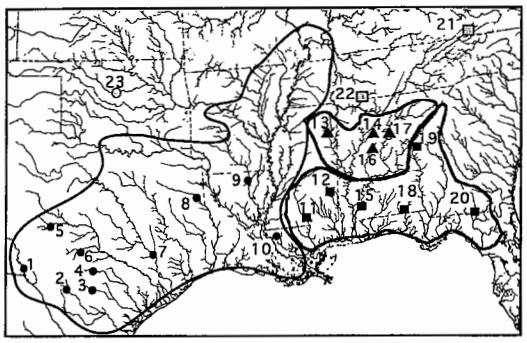

Fig. 1. Hypothesized distribution of the three subspecies of *Cyprinella venusta*. Subspecies ranges follow Gibbs (1957b) and Gilbert and Burgess (1979). Circles indicate samples from the hypothesized range of *C. v. venusta*; squares indicate samples from the hypothesized range of *C. v. cercostigma*; and triangles indicate samples from the hypothesized range of *C. v. stigmatura*. Samples of *C. venusta* (1–20) and outgroups *C. galactura* (21–22) and *C. lutrensis* (23) were obtained from the following river drainages (see Material Examined for collection localities): 1, Pecos; 2, Frio; 3, Guadalupe; 4, San Antonio; 5, Concho; 6, South Llano; 7, lower Brazos; 8, Sabine; 9, Ouachita; 10, lower Mississippi; 11, Pearl; 12, Pascagoula; 13, Tombigbee; 14, Black Warrior; 15, lower Alabama; 16, Cahaba; 17, Coosa; 18, Chocktawhatchee; 19, Chattahoochee; 20, Suwannee; 21, upper Tennessee; 22, middle Tennessee; and 23, Arkansas.

in *C. venusta* and recognized three subspecies: *C. v. venusta*, from the Mississippi River basin west to the Rio Grande River; *C. v. cercostigma*, from (and within) the Mississippi River basin east to the Suwannee River in Florida; and *C. v. stigmatura*, from the upper Alabama and Tombigbee river systems, primarily above the Fall Line in Alabama and Georgia (Fig. 1).

Gibbs (1957b), in a precladistic analysis, hypothesized that either *C. v. venusta* or *C. v. cercostigma* was most closely related to the ancestral form and that *C. v. stigmatura* may have evolved from an ancestor that became isolated and diverged during high-sea levels in the Pleistocene. He hypothesized that the ancestor(s) to *C. venusta* radiated from a Mississippi River "hub," reached the Gulf Coastal Plain, and then dispersed (and evolved) into *C. v. venusta* to the west and *C. v. cercostigma* to the east. Presumably, *C. v. stigmatura* then evolved from a *C. v. cercostigma*-like ancestor. Gibbs (1957a, 1957b) also suggested that *C. spiloptera* was the closest relative to *C. venusta*. Alternatively, Mayden (1989) hypothesized that *C. venusta* was sister to *C. galactura* on the basis of nine derived osteological characters. Conner and Suttkus (1986) placed *C. venusta* in a group of "residual" species hypothesized to have existed preglacially in the Gulf Coastal Plain. Preglacial drainage patterns of this area are thought to be quite different from present (Conner and Suttkus, 1986; Robison, 1986; Starnes and Etnier, 1986) and are summarized in Mayden (1988). Briefly, the major preglacial drainages in the southeastern United States were the Teays-Mississippi drainage, the Mobile Bay drainage, the Chattahoochee River, and the Appalachian River. The existence of the Appalachian River and hypothesized connection(s) between the precursor(s) of the upper Tennessee River system and the eastern Gulf Coastal Plain have been debated (Starnes and Etnier, 1986; Mayden, 1987, 1988).

In this study, we employed analysis of restriction-endonuclease-site variation of mtDNA to infer a hypothesis of phylogenetic relationships among geographically based, mtDNA lineages of *C. venusta*; examine patterns of mtDNA divergence within and among geographic samples from the range of the three nominal subspecies of *C. venusta*; and utilize the mtDNA-based phylogeny to examine historical biogeography of this species. The use of mtDNA restriction sites as characters in phylogenetic inference has been reviewed extensively (Avise et al., 1987; Moritz et al., 1987; Avise, 1989), and mtDNA restriction sites have proven effective in phylogenetic analysis of closely related North American cyprinids (Dowling et al., 1992; Richardson and Gold, 1995a; Schmidt et al., 1994).

Materials and Methods

Specimens of *C. venusta* were collected by seine from 20 naturally occurring populations throughout its range (Fig. 1). Samples from two populations of *C. galactura* and one population of *C. lutrensis* were used as outgroups. Collection localities, dispositions of voucher specimens, and number of individuals assayed from each population are given in Material Examined. Eight to 13 individuals from each population were frozen in liquid nitrogen, returned to the laboratory, and stored at −80 C.

Procedures for mtDNA extraction, precipitation, and storage follow those described in Gold and Richardson (1991). Fifteen 6-base restriction endonucleases were used to digest 1.0–1.5 μg of DNA in 40 μl reactions following manufacturer's specifications. The enzymes were

*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. Procedures for agarose gel electrophoresis, transfer to nylon membranes, Southern hybridization, and autoradiography follow those described in Gold and Richardson (1991). The probe used was an intact mtDNA molecule from *C. lutrensis* cloned into bacteriophage lambda by using EMBL arms. Fragments of mtDNA were sized by fitting migration distances to a least-squares regression line of lambda DNA-*Hin*dIII fragment migration distances. All sites for each restriction enzyme were mapped using single and double digestions (Brown and Vinograd, 1974). Polymerase chain reaction (PCR) was used to amplify various regions of the mtDNA molecule to localize restriction sites either by single digestions with restriction enzymes, or as gel-purified, ^{32}P-labeled, mtDNA probes hybridized to genomic blots. Locations of PCR-amplified fragments used in mapping are given in Kristmundsdóttir (1994) and may be obtained upon request from the second author. PCR reactions were carried out using 0.5–1.0 units of *Taq* DNA polymerase per 50 μl reaction (Engelke et al., 1990). Reaction profiles were optimized for each set of primers but generally were within the following specifications: 30 cycles of denaturation at 94 C for 1 min, annealing at 45–47C for 1–2 min, and extension at 72 C for 1.5–2 min. A final extension was at 72 C for 5 min, followed by cooling and storage of samples at 10 C. Orientation of mtDNA restriction sites on a mtDNA gene map and homology testing of comigrating DNA fragments were confirmed by using specific PCR amplification products as probes in Southern hybridizations.

Relationships among populations of *C. venusta* were inferred through maximum-parsimony (MP) analysis of restriction site presence/absence matrices and neighbor-joining (NJ) analysis of pairwise matrices of nucleotide sequence divergence. Both analyses employed *C. galactura*, the putative sister species to *C. venusta* (Mayden, 1989), and *C. lutrensis*, a member of the *C. lutrensis* clade (Mayden, 1989), as outgroups. The GENERATE program in the Restriction Enzyme Analysis Package (REAP) of McElroy et al. (1992) was used to construct a presence/absence restriction-site matrix for composite mtDNA genotypes (haplotypes). The restriction-site matrix for individual haplotypes is given in Kristmundsdóttir (1994) and may be obtained upon request from the second author.

MP analysis employed Version 3.0s of the Phylogenetic Analysis Using Parsimony (PAUP) program (Swofford, 1991, unpubl.). Bootstrapping (Felsenstein, 1985) was used to assess reproduceability of trees in 10 separate runs of 100 replicates each. The DSE program in REAP was used to generate estimates of nucleotide sequence divergence among mtDNA haplotypes (Nei and Tajima, 1981), and the interhaplotype distance matrix was used to generate an interpopulational nucleotide sequence divergence matrix based on haplotype frequencies (Nei, 1987). Nucleotide sequence divergence estimates were corrected for variation within samples. NJ analysis of the interpopulational matrix employed the Neighbor program in Version 3.4 of the Phylogenetic Inference Package (PHYLIP; Felsenstein, 1991, unpubl.). The interpopulational distance matrix also was clustered using the unweighted pair-group method using arithmetic averages, UPGMA (Sneath and Sokal, 1973). MtDNA evolutionary-rate heterogeneity, suggested by different topologies among MP, NJ, and UPGMA analyses, was tested using the method of Beverley and Wilson (1984).

Results

Single digestions with 15 restriction enzymes were used to survey restriction-site variation among a total of 211 individuals. These included 8–11 individuals of *C. venusta* from each of 20 populations and 7–11 individuals each of *C. galactura* and *C. lutrensis*. The mtDNA molecule of *C. venusta* and outgroups was estimated to be 16,700 ± 200 basepairs (bp). A total of 162 restriction sites, with an average of 52 sites per individual, and 83 mtDNA haplotypes were identified among the three species. This included 131 restriction sites and 76 haplotypes in *C. venusta*. A listing of all restriction sites and their map location, restriction-fragment patterns for individual haplotypes, and frequencies of haplotypes within each population are given in Kristmundsdóttir (1994) and may be obtained upon request from the second author.

We were concerned initially that hybridization between *C. venusta* and *C. lutrensis* (Gilbert, 1961) might confound data analysis, particularly in areas where the two species are sympatric. However, early results from this and a related study in our laboratory (involving geographic variation of mtDNA in *C. lutrensis*) indicated that individuals of *C. venusta* and *C. lutrensis*, sampled from areas where the two are (or are not) sympatric, differed in mtDNA nucleotide

sequence by 10–13%. This difference is fairly substantial, in that restriction fragment patterns for almost all of the restriction enzymes used differ trenchantly between the two species. We now have surveyed nearly 200 individuals of *C. venusta* (this paper) and well over 500 individuals of *C. lutrensis* (Richardson and Gold, 1995a, 1995b, unpubl.) and only found three instances where individuals were identified in the field as either *C. venusta* or *C. lutrensis* but which possessed a mtDNA phenotype of the opposite species. Because the mtDNAs of the two species are so easily discerned, hybridization between the two is not a confounding problem in this study.

MP analysis of the haplotype matrix employed 132 phylogenetically informative restriction sites and generated 3900 shortest trees, each of 289 steps. A strict consensus tree (not shown) was used to identify cohesive groupings (clades) of mtDNA haplotypes that could be employed as operational taxonomic units (OTUs) in further phylogenetic analysis. Two distinct mtDNA lineages were found among individuals sampled from each of four localities: lower Mississippi River, Louisiana; lower Alabama River, Alabama; lower Brazos River, Texas; and Frio River, Texas. MtDNA haplotypes from each of these four localities were separated into two OTUs, 1 and 2, where OTU 2 contained fewer haplotypes. Populations from each of the remaining localities were treated as separate OTUs, yielding a total of 26 OTUs for all samples of *C. venusta* and the two outgroup species. The haplotype restriction-site matrix was then used to create a locality-based, restriction-site matrix for these OTUs, where a site was scored as present if it occurred in any individual sampled from a population (OTU), or absent if it did not (Dowling et al., 1992). The resultant matrix of 26 OTUs was then used in further phylogenetic analysis. Collapsing a haplotype matrix in this way has the effect of emphasizing restriction-site gains over restriction-site losses. This can reduce potential homoplasy, because restriction-site losses are expected to be more common than restriction-site gains and to not necessarily reflect identical base-pair substitutions.

MP analysis of the locality-based, restriction-site matrix of 26 OTUs generated 14 shortest trees of 197 steps each (CI = 0.461, RI = 0.717). The strict-consensus tree (Fig. 2) and the strict-consensus tree of haplotypes (not shown) had the same topology, indicating that collapsing the haplotype matrix into a locality-based matrix did not affect phylogenetic inference. Seven mtDNA lineages within *C. venusta* appear fairly well resolved. For ease of identification, each lineage was assigned a regional designation (OTUs in parentheses): Chocktawhatchee (Chocktawhatchee), Apalachicola (Suwannee and Chattahoochee), Mobile (Coosa, Cahaba, Black Warrior, Alabama1, and Tombigbee), Pearl-Pascagoula (Alabama2, Pascagoula, Pearl, and Mississippi2), Mississippi (Mississippi1 and Ouachita), East Texas (Sabine and Brazos2), and West Texas (Frio2, Brazos1, South Llano, Concho, San Antonio, Guadalupe, and Pecos). Frio1 did not fall into a cohesive grouping with other OTUs, occurring in an unresolved polychotomy with the Pearl-Pascagoula, Mississippi, East Texas, and West Texas lineages. Sister-group relationships between Pascagoula and Alabama2 and between Mississippi2 and Pearl (both in the Pearl-Pascagoula lineage) and between Ouachita and Mississippi1 (Mississippi lineage) were well supported in MP analysis (Fig. 2).

MP analysis grouped the seven mtDNA lineages into four major clades (referred to hereafter as Chocktawhatchee, Apalachicola, Mobile, and Western), each of which was supported by >50% bootstrapping (Fig. 2). The Western clade comprised an unresolved polychotomy that included the Pearl-Pascagoula, Mississippi, East Texas, and West Texas mtDNA lineages. Based on outgroup comparison, the Chocktawhatchee clade is basal and sister to all remaining clades. Inferred phylogenetic relationships among the major clades were (Chocktawhatchee (Apalachicola ((Mobile, Western)))).

The tree generated by NJ analysis of a nucleotide sequence divergence (distance) matrix of the 26 OTUs was virtually identical to the MP consensus tree, except that relationships in the Mobile and Western groups were "resolved" into a nested series (Fig. 3). In both MP and NJ trees, the pattern of nested sister relationships among major mtDNA clades (groups) and within the Western clade (group) essentially followed an east to west geographic pattern across the Gulf Coastal Plain (Fig. 4).

The UPGMA phenogram (not shown) differed from the MP- and NJ-generated trees primarily in reversing placement of the Chocktawhatchee and Apalachicola clades (groups). This indicated that the Apalachicola clade (group) was the most divergent in mtDNA nucleotide sequence within *C. venusta* and suggested the possibility of mtDNA evolutionary-rate heterogeneity in one or both of the clades (groups). Relative rate tests (after Beverley and Wilson, 1984) revealed that accumulated mtDNA sequence divergence along the branch defining

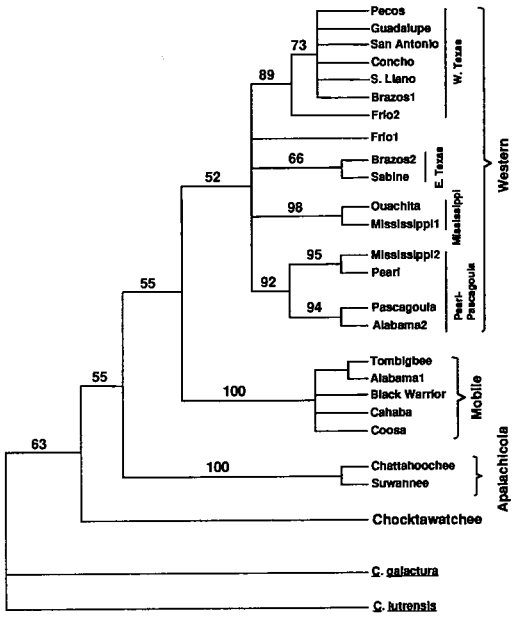

Fig. 2. Strict-consensus tree generated from maximum-parsimony analysis of 24, locality-based OTUs of *Cyprinella venusta*. *Cyprinella galactura* was used to polarize restriction-site characters among samples of *C. venusta*, and the tree was rooted with *C. lutrensis*. Numbers on branches indicate the proportion of times (from 1000 replicates) that a node was distinguished in bootstrap analysis. Branch lengths are not proportional to the number of character-state changes along each lineage.

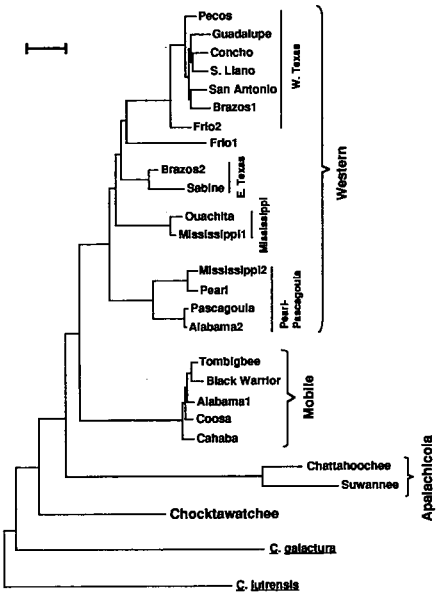

Fig. 3. Tree generated by neighbor-joining method. Branch lengths to nodes and terminal taxa represent output from the Neighbor program in PHYLIP. Bar represents 1% difference in mtDNA nucleotide sequence.

the Apalachicola clade (group) was almost twice that of any other branch, including that leading to the Chocktawhatchee clade or group (Table 1). Relative rate tests utilizing single OTUs (data not shown) indicated that accumulated mtDNA sequence divergence along the branch leading to the Suwannee mtDNA lineage was considerably greater than that leading to the Chattahoochee mtDNA lineage.

Discussion

MtDNA phylogeographic subdivision within *C. venusta* is not strictly concordant with geographic subdivision (ranges) of the three subspecies of *C. venusta* defined by Gibbs (1957b) on the basis of external morphology. Briefly, the range of *C. v. cercostigma* was hypothesized to extend from the Suwannee River in Florida westward to the Mississippi River basin. In our study, three of four major, mtDNA-based clades were identified within this range: Chocktawhatchee (Chocktawhatchee mtDNA lineage), Apalachicola (Suwannee and Chattahoochee mtDNA lineages), and Western (Pearl-Pascagoula mtDNA lineage). The range of *C. v. stigmatura* was hypothesized to encompass the upper Alabama and Tombigbee drainages, primarily above the Fall Line in Alabama and Georgia. Haplotypes from all individuals sampled above the Fall Line, and all but one haplotype from individuals sampled at the lower Alabama River locality, which is below the Fall Line in Alabama, were included in the Mobile clade. The Mobile clade thus corresponds geographically to *C. v. stigmatura*, and Gibbs's

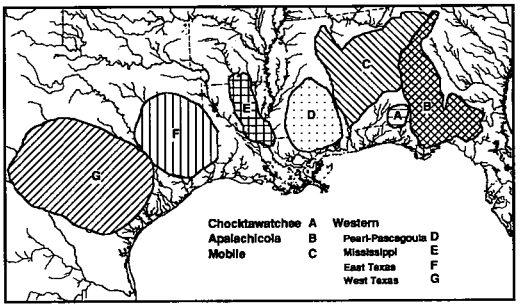

Fig. 4. Geographic distribution of major mtDNA-based clades and lineages within *Cyprinella venusta*.

TABLE 1. RELATIVE RATE TESTS OF MTDNA NUCLEOTIDE SEQUENCE DIVERGENCE (DISTANCE) AMONG CLADES OF *Cyprinella venusta*. Values *a* and *b* indicate amount of sequence divergence accumulated in lineages A and B, respectively, since divergence from a common ancestor. If rates of mtDNA evolution are similar, the ratio (R) of values *a* and *b* should approximate unity.

Outgroup	Ingroup* A	Ingroup* B	*a*	*b*	Ratio†
C. lutrensis and *C. galactura*	Chocktawhatchee	Apalachicola	0.033	0.070	2.121
		Mobile	0.035	0.041	1.171
		Western	0.032	0.040	1.250
Chocktawhatchee	Apalachicola	Mobile	0.059	0.031	1.903
		Western	0.065	0.033	1.970
Chocktawhatchee	Mobile	Western	0.030	0.027	1.111
Apalachicola	Mobile	Western	0.024	0.033	1.375

* Values of *a* and *b* were estimated separately for all possible relative rate comparisons of single OTUs and then averaged.
† The ratio of values *a* and *b* was inverted (if necessary) to yield a value ≥ unity.

(1957b) hypothesis of a cohesive group of *C. venusta* primarily above the Fall Line appears to be corroborated. Finally, three mtDNA lineages (Mississippi, East Texas, and West Texas) were identified from within the range hypothesized to circumscribe *C. v. venusta*. These three mtDNA lineages, however, were included in a multichotomy with the Pearl-Pascagoula mtDNA lineage (thus forming the Western clade). The Pearl-Pascagoula mtDNA lineage occurs within the range proposed for *C. v. cercostigma*.

Systematics and biogeography.—Gibbs (1957a, 1957b) hypothesized that the ancestor(s) to *C. venusta* dispersed to the Gulf Coastal Plain from the ancient Teays-Mississippi system and diverged into western (*C. v. venusta*) and eastern (*C. v. cercostigma*) forms. He further hypothesized that *C. v. stigmatura* evolved from the eastern (*C. v. cercostigma*) form during high-sea levels that occurred in the Pleistocene. This scenario suggests that the ancestral Mississippi River may have served as a route of dispersal of the ancestor to *C. venusta* and subsequently as a barrier during the vicariance of western (*C. v. venusta*) and eastern (*C. v. cercostigma*) blacktail shiners. However, basal mtDNA clades within *C. venusta* (i.e., Chocktawhatchee and Apalachicola) occur in the eastern Gulf Coastal Plain, suggesting that initial divergence within *C. venusta* occurred to the east and south of the Teays-Mississippi system and that the Mississippi River may not have served as a zone of vicariance for the earliest divergence in *C. venusta*.

Occurrence of basal mtDNA clades within *C. venusta* in the present-day eastern Gulf Coastal Plain supports the existence of one or more historical connections between the upper Tennessee River drainage and systems draining southward to the Gulf of Mexico. This follows in part from the presumed sister-group relationship (Mayden, 1989) between *C. venusta* and *C. galactura*, a species whose present-day distribution includes the upper Tennessee and Cumberland drainages (Gilbert and Burgess, 1979). According to this scenario, severance of one or more of these presumed connections could have precipitated divergence of *C. venusta*. Present distributions and hypothesized relationships among species or species groups in at least four families of freshwater fishes, i.e., Cyprinidae, Catastomidae, Cyprinodontidae, and Percidae (Mayden, 1987), support the hypothesis that one or more historical connections existed between the upper Tennessee River and drainages in the Gulf Coastal Plain that empty ultimately into the Gulf of Mexico. One hypothesized connection between the upper Tennessee and the upper Mobile Bay drainage has been termed the "Appalachian River" (references in Mayden, 1988:342) although, as noted by Swift et al. (1986) and Mayden (1987, 1988), there may have been at least two faunal exchanges between the upper Tennessee system and the eastern Gulf Coastal Plain. Mayden (1988) hypothesized that one connection may have existed during the early Tertiary; whereas a second (the Appalachian River) may have existed during the late Tertiary.

Based on an estimated (average) mtDNA nucleotide sequence divergence of 11.2% between *C. venusta* and *C. galactura*, severance of a presumed connection between an upper Tennessee River drainage and an eastern Gulf Coastal Plain drainage (and which could have given rise to *C. venusta*) would have been preglacial. Rates of

mtDNA evolution (nucleotide substitutions per 10^6 years) are estimated to average between 0.20% and 0.75% among poikilothermic vertebrates (Martin and Palumbi, 1993), and from 0.22–1.91% among bony fishes (Brown and Chapman, 1991; Martin and Palumbi, 1993; Bentzen et al., 1993). Assuming that mtDNA evolution in *Cyprinella* is similar, on average, to that in other bony fishes, the origin of *C. venusta* would clearly be preglacial. Interestingly, mtDNA sequence divergence between the North American cyprinids *Luxilus zonistius* (Apalachicola drainage) and *L. coccogenis* (upper Tennessee drainage) also is approximately 11% (Dowling et al., 1992). If rates of mtDNA sequence divergence in *Luxilus* are similar to those in *Cyprinella*, ancestors to both *C. venusta-C. galactura* and *L. zonistius-L. coccogenis* may have been affected by the same vicariant event.

Alternate scenarios to the above are that the ancestor to *C. venusta* entered the Gulf Coastal Plain from elsewhere (e.g., from the ancient Mississippi River) or that the genus *Cyprinella* evolved in the Gulf Coastal Plain, with *C. venusta* being a comparatively recent divergence. In both cases, divergence (vicariance) within *C. venusta* could still have occurred in an east to west direction across the Gulf Coastal Plain (as indicated by phylogenetic analysis of mtDNA variants), assuming that undifferentiated *C. venusta* occurred historically throughout much of its present-day range. Although there is no direct support for these alternatives, Starnes and Etnier (1986) have argued against the existence of the Appalachian River, noting the absence of direct geologic evidence supporting a connection between the upper Tennessee and upper Mobile Bay (Coosa River) systems. It also is worth noting that the hypothesis that *C. galactura* is sister to *C. venusta* is based on morphology (Mayden, 1989) and awaits further testing with other characters.

Phylogenetic analysis of mtDNA variants indicated that initial divergence(s) within *C. venusta* involved populations in the Chocktawhatchee River (Chocktawhatchee clade) and the Chattahoochee and Suwannee rivers (Apalachicola clade). The Chattahoochee River is one of the oldest drainages in the region, having very likely been distinct during the Early Tertiary when the Coastal Plain was relatively narrow (Swift et al., 1986). Connections between the Chattahoochee and the Chocktawhatchee rivers may be quite old, because the Chattahoochee once flowed in the large valley now occupied by the Chocktawhatchee (Puri and Vernon, 1964, cited in Swift et al., 1986). Such connections may date to the Late Miocene when sea levels dropped to 80–100 m below current levels (Swift et al., 1986) and much of the present-day Gulf Coastal Plain was formed. Severance of the two rivers from one another, and from other drainages to the west, undoubtedly occurred by mid-Pliocene when sea levels rose to 50–80 m above present-day levels (Swift et al., 1986). The estimated mtDNA nucleotide sequence divergence of 7.3% between the Chocktawhatchee clade and the remaining clades of *C. venusta* is consistent with a preglacial divergence time that postdates the divergence of *C. venusta* and *C. galactura* (11.2% nucleotide sequence difference). This again assumes that mtDNA evolution in *Cyprinella* is, on average, similar to that in other bony fishes.

Divergence of the Apalachicola clade is confounded by apparent mtDNA rate acceleration, particularly in the Suwannee mtDNA lineage. Both the Chocktawhatchee and Apalachicola clades clearly represent early divergences within *C. venusta*, and both likely diverged preglacially, possibly in mid- to late Pliocene during a high-sea stand. Which divergence preceded the other in geological time, however, is problematic. The occurrence of a major phylogenetic discontinuity between the Apalachicola drainage and those to the west also has been documented in fish species from the families Amiidae, Centrarchidae, and Poeciliidae (Bermingham and Avise, 1986; Scribner and Avise, 1993).

Specimens of *C. venusta* belonging to the Mobile clade were collected primarily from localities in the upper Alabama and Tombigbee river systems, and only one collection from the Mobile basin drainage system (from the lower Alabama River) was made below the Fall Line. Even though all but one haplotype from the Little River was included in the Mobile clade, further study of *C. venusta* from the lower Alabama River system will be necessary to determine the geographic extent of the Mobile clade.

The four mtDNA lineages in the Western clade (Pearl-Pascagoula, Mississippi, East Texas, and West Texas) generally align either with known geomorphic features or described faunal provinces (Conner, 1977; Swift et al., 1986). MtDNA nucleotide sequence divergence among the four lineages ranged from 3.7–4.4%, suggesting that each could have diverged more-or-less simultaneously in geological time, an occurrence that could hinder or obscure inference of phylogenetic relationships (Hixson and Brown, 1986; Gatesy et al., 1992). Assuming again that mtDNA evolution in *Cyprinella* is, on average, similar to that in other bony fishes, divergence of lineages within the western clade

could have occurred during late Pliocene-Pleistocene times when any of several proposed high-sea stands (Swift et al., 1986) could have served as physical barriers and facilitated divergence. The proposed high-sea stands, alternatively, were followed by periods of low-sea stands where many presently independent Gulf Coast drainages presumably were connected on an exposed continental shelf (Conner and Suttkus, 1986). These hypothesized interdrainage connections during low-sea stands may explain the four instances where two phylogenetically distinct mtDNA groupings were found in the same drainage. In each case (i.e., lower Alabama River, lower Mississippi River, lower Brazos River, and Frio River), the distinct mtDNA groupings occurred in geographically adjacent mtDNA lineages, suggesting secondary admixture as the reason for co-occurrence.

Taxonomy.—Two questions raised by this study are whether any or all of the major clades within *C. venusta*, identified by mtDNA analysis, merit separate taxonomic status and whether the subspecies defined by Gibbs (1957b) merit revision, given the absence of complete correspondence geographically between clades identified from mtDNA analysis and subspecies defined by Gibbs (1957b). In general, support for taxonomic recognition could include monophyly of geographically cohesive samples, phenetic differentiation (whether based on DNA sequences or morphology), ecological or behavioral differentiation, and/or phylogeographic congruence with nominal taxa of other groups. Considering these criteria, the Chocktawhatchee, Apalachicola, Mobile, and Western clades may merit taxonomic recognition. First, all four appear to be monophyletic and to possess a suite of unique mtDNA character states. Second, considerable mtDNA sequence divergence is observed among the clades. The two most similar clades (Mobile and Western) differ in mtDNA sequence by 5.7%, a value that is commensurate with, or greater than, levels of mtDNA sequence divergence among other, closely related species of North American cyprinid fishes (Dowling and Brown, 1989; Smouse et al., 1991, Dowling et al., 1992). Third, although morphological and meristic character variation were not examined in this study, individuals were sampled from areas where differences in external morphology have been reported. Blacktail shiners from the upper Chattahoochee River (corresponding to the Apalachicola clade), for example, are sufficiently distinct morphologically to have been considered a separate species by Jordan (1877) and at least a race within *C. v. cercostigma* by Gibbs (1957b). In addition, blacktail shiners in the Mobile clade correspond geographically to *C. v. stigmatura*, a form that differs from other blacktail shiners in having a slender body and caudal peduncle and a high lateral-line scale count (Gibbs, 1957b).

Finally, similar phylogeographic patterns are known for other species or species groups, including noncyprinids (Wiley and Mayden, 1985; Swift et al., 1986), and are consistent with hypothesized vicariant zones (Wiley and Mayden, 1985; Bermingham and Avise, 1986; Swift et al., 1986). Examples in cyprinids include the *Lythrurus roseipinnis* and the *Notropis* (*Hybopis*) *dorsalis* species groups. In the *L. roseipinnis* species group where *L. atrapiculus* occurs east of the Mobile basin in the Chocktawhatchee and Apalachicola drainages, *L. bellus* is found in upper Mobile basin drainages, and *L. roseipinnis* occurs in the lower Mobile basin drainage system and east to the Mississippi River (Snelson, 1972). In the *N.* (*Hybopsis*) *dorsalis* species group (Suttkus and Boschung, 1990; Coburn and Cavender, 1992; Wiley and Titus, 1992) where *N. ammophilus* occurs in the Mobile basin, populations of *N. longirostris*, found west of Mobile Bay to the Mississippi River, and east to the Apalachicola drainage, may represent cryptic species (Wiley and Titus, 1992), and *N. sabinae* is distributed from the Calcasieu River in Louisiana west to the San Jacinto River in east Texas (Gilbert, 1978).

Based on the above, we suggest that further study of blacktail shiners, with a focus on possible taxonomic revision, may be warranted. If so, the name *eurystoma* (Jordan, 1877), originally used for blacktail shiners from the upper Chattahoochee River, is available for the Apalachicola clade, as is the name *stigmatura* (Jordan, 1877) for the Mobile clade. The name *venusta* should be reserved for blacktail shiners occurring west of the Mobile basin, because *C. venusta* was described originally from specimens found in the Sabinal River, Texas (Girard, 1856).

Material Examined

Collection localities, drainages (in parentheses), number of specimens analyzed from each locality, and catalog numbers of voucher specimens [in brackets] are listed below. Voucher specimens for most collection localities were deposited in the Texas Cooperative Wildlife Collection (TCWC) at Texas A&M University. *Cyprinella venusta*—Pecos River (Pecos R.), Val Verde Co., Texas, 9; Frio River (Frio R.), Uvalde Co., Texas, 10 [TCWC 7290.01]; Guadalupe River (Guadalupe R.), Kerr Co., Texas, 10 [TCWC 7282.01]; Medina River (San Antonio

R.), Bandera Co., Texas, 10 [TCWC 7285.01]; South Concho River (Colorado R.), Tom Green Co., Texas, 9 [TCWC 7288.01]; South Fork Llano River (Colorado R.), Kimble Co., Texas, 10 [TCWC 7283.01]; Little Brazos River (lower Brazos R.), Burleson Co., Texas, 10; Sabine River (Sabine R.), Panola Co., Texas, 8; Ouachita River (Ouachita R.), Ouachita Par., Louisiana, 10 [TCWC 7281.01]; Thompson Creek (lower Mississippi R.), West Feliciana Par., Louisiana, 10; upper Little Creek (Pearl R.), Marion Co., Mississippi, 10 [TCWC 7277.01]; Red Creek (Pascagoula R.), Stone Co., Mississippi, 9 [TCWC 7285.01]; Chiwapa Creek (Tombigbee R.), Monroe Co., Mississippi, 10 [TCWC 7279.01]; tributary to Mulberry Fork (Black Warrior R.), Cullman Co., Alabama, 10 [TCWC 7287.01]; Little River (lower Alabama R.), Conecuh-Baldwin Co. line, Alabama, 9 [TCWC 7289.01]; Cahaba River (Cahaba R.), Jefferson Co., Alabama, 10 [TCWC 7286.01]; Choccolocco Creek (Coosa R.), Calhoun Co., Alabama, 10; Panther Creek (Chocktawhatchee R.), Houston Co., Alabama, 10 [TCWC 7278.01]; Centralhatchee Creek (Chattahoochee R.), Heard Co., Georgia, 8 [TCWC 7280.01]; Alapaha River (Suwannee R.), Echolz Co., Georgia, 11 [TCWC 7291.01]. *Cyprinella galactura*—North Fork of Holston River (upper Tennessee R.), Scott Co., Virginia, 3; Choats Creek (middle Tennessee R.), Giles Co., Tennessee, 4. *Cyprinella lutrensis*—Cimarron River (Arkansas R.), Kingfisher Co., Oklahoma, 11 [TCWC 7266.01].

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