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Morphological and genetic evolution in eastern populations of the *Macrhybopsis aestivalis* complex (Cypriniformes: Cyprinidae), with the descriptions of four new species

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Abstract

For many years the North American cyprinid fish *Macrhybopsis aestivalis* (common name: Speckled Chub) was regarded as a single widespread and morphologically variable species, occurring in rivers throughout much of the Mississippi Valley and geographically adjacent eastern Gulf slope drainages, west to the Rio Grande basin in Texas, New Mexico, and Mexico. Eisenhour (1997) completed a morphological study of western populations of the Speckled Chub, the results of which appeared thereafter in published form (Eisenhour 1999, 2004). He demonstrated the existence of five valid species west of the Mississippi River (*aestivalis, marconis, australis, tetranema, hyostoma*), of which the name *aestivalis* was shown to be restricted to the population occurring in the Rio Grande and the geographically adjacent Rio San Fernando system, in northeastern Mexico. Eisenhour (2004) considered populations throughout the middle Mississippi Valley and its major tributaries to be a single morphologically variable species (*hyostoma*), and he also indicated that populations of *Macrhybopsis* from eastern Gulf slope drainages may represent a complex of species. Genetic confirmation of Eisenhour's conclusions regarding western species appeared in the publication by Underwood *et al.* (2003), who also showed that western populations of *M. hyostoma*, as presently recognized, are genetically much more complex than previously considered.

Meanwhile, the present authors were involved in a companion study of eastern populations of *Macrhybopsis*, for which a genetic summary of the eastern Gulf coast species was published by Mayden & Powers (2004). Based on their findings, four species were recognized from southeastern drainages (identified as species A–D), although no formal tax-

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onomic descriptions were included. Their genetic data, in combination with meristic, morphometric and other morphological data presented herein, form the basis for a revised classification of eastern *Macrhybopsis* populations, including formal descriptions of the four new species from eastern Gulf coast drainages.

Key words: Cyprinidae, *Macrhybopsis,* new species, eastern Gulf slope drainages, genetics, morphology, biogeography

Introduction

The *Macryhybopsis aestivalis* complex is a group of wide-ranging eastern North American cyprinid fishes, typically inhabiting large streams and rivers and characterized by a distinctive suite of morphological characters, the most obvious of which is one or two pairs of well-developed maxillary barbels.

The complex had its genesis with Girard's (1856) description of *Gobio aestivalis* from the Rio San Juan, a tributary of the Rio Grande in Nuevo Leon, Mexico. This was followed by descriptions of *Ceratichthys sterletus* Cope *in* Cope & Yarrow 1875, from the upper Rio Grande, New Mexico; *Nocomis hyostomus* Gilbert 1884, from the White River, Indiana; *Hybopsis aestivalis marconis* Jordan & Gilbert 1886, from the Rio San Marcos, Texas; *Hybopsis tetranemus* Gilbert 1886, from the upper Arkansas River drainage, Kansas; and *Extrarius australis* Hubbs & Ortenburger 1929, from the Red River in Oklahoma. All were subsequently accorded species recognition, although *sterletus* was briefly relegated to the synonymy of *aestivalis* by Jordan & Gilbert (1886: 8). Considering the extensive geographical distribution of the complex, together with its wide morphological diversity, it is surprising that these are the only names that have, until now at least, been formally proposed for the included species.

Hubbs & Ortenburger (1929: 23–28) tentatively regarded all as valid species, but noted that several morphological features, particularly development of the maxillary barbels (including both size and number), eye size, body pigmentation, and degree of squamation, have strong ecological correlations; with populations living in more turbid environments tending to have better developed barbels, smaller eyes, more pallid bodies, and scales notably reduced in size and distribution. They also cited examples of variation in barbel development among individuals within the same population and occasionally in the same individual. Based on this, they placed all members of the *aestivalis* complex in the genus *Extrarius*, which had earlier been proposed by Jordan (1919) for sole reception of the four-barbeled form, *tetranemus*. Hubbs & Ortenburger (1929) also suggested that *Extrarius* might be most closely related to *Macrhybopsis* Cockerell & Allison 1909. Jordan *et al.* (1930: 138–139) recognized both of the last two genera, with *Extrarius* retained for the sole reception of *tetranemus*. No justification was given for this arrangement. Bailey (1951: 192) downgraded *Extrarius* and *Macrhybopsis*, together with several other genera, to subgenera of the greatly expanded genus *Hybopsis*, which was characterized exclusively by the shared presence of one or two pairs of maxillary barbels in all included species. Although certain subgenera (e.g., *Couesius, Nocomis*) were shortly thereafter reelevated to genus, generic assignment of the *aestivalis* complex remained unchanged until *Extrarius* was restored to a genus by Mayden (1989). Coburn & Cavender (1992), in their phylogenetic treatment of North American cyprinid genera, placed *Extrarius* in the expanded genus *Macrhybopsis*, an assignment accepted by Mayden *et al.* (1992) and which is generally followed today.

Although Hubbs & Ortenburger (1929) did not change the existing *status quo* regarding species recognition, they planted the "seed" that ultimately led to downgrading of all nominal forms within the complex to subspecies of *Extrarius aestivalis* by their comment: “The evidence afforded by the material now preserved in various museums (admittedly insufficient) in fact suggests that a fairly complete gradation may eventually be found, connecting races or forms having a single barbel of moderate length with those exhibiting two long ones, on each side.” We have been unable to find a specific publication in which this suggestion was actually formalized. The first indication of such a change appeared in Greene's (1935: 79) publication on Wisconsin fishes, under the account of *Extrarius aestivalis hyostomus*. Greene provided no accompanying documentation, but stated: "The very interesting relationships of *E. aestivalis* subspecies are partially set forth by Hubbs & Ortenburger (1929: 23–25)." This was further evidenced by Kuhne's (1939: 49) listing of *Extrarius aestivalis hyostomus* from Tennessee and the mid-south, Hubbs' (1940b: 5) reference to *Extrarius aestivalis sterletus* in his paper on fishes from the Big Bend region of Texas, Gerking’s (1945: 51) inclusion of *E. aestivalis hyostomus* in his study of Indiana fishes, and Trautman’s (1957: 310–311) reference to *Hybopsis aestivalis hyostoma* in the first edition of his book on Ohio fishes.

Hubbs (1940a, 1941) published two papers providing additional details and examples of the effects of environment and nutrition on fish morphology and evolution. Although the genus *Extrarius* was mentioned only briefly, it is clear from the contents of these papers that Hubbs continued to accept his earlier conclusion that the morphological differences seen in widespread populations of this genus are the end product of widely diverse environmental conditions, and that further study would reveal many examples of parallel ecological and morphological clines. To what extent such morphological differences might be genetically fixed remained unresolved, although continued recognition of subspecies within the *aestivalis* complex (Johnson 1942; Hubbs 1945, 1946; Eddy & Surber 1947; Trautman 1957, 1981; Cross 1967; and other workers) indicated tacit agreement that some level of genetic differentiation was involved.

Moore (1950: 82–85) provided further evidence of modifications in cutaneous sense organs of *Extrarius* and other genera of barbeled minnows living in muddy waters of the Great Plains. This study was later reviewed by Metcalf (1966: 108–110). Davis & Miller (1967) and Reno (1969) broadened this investigation to include development of the brain and cephalic lateral-line systems, respectively.

An extreme interpretation of the relationship of environment to taxonomy is found in Bailey's (1956: 333–334) discussion of the cyprinid fishes *Hybognathus nuchalis* and *Hybognathus placitus*. He expressed the opinion that the smaller eye and smaller (thus more numerous) scales characteristic of the latter species are a direct result of increased water turbidity and are not genetically fixed. Although morphological variation in the *aestivalis* complex was not discussed in Bailey's work, one may assume that had such a discussion appeared his conclusions would have been the same. Bailey & Allum (1962: 71–75, pl. 1) later modified these earlier ideas regarding *Hybognathus* and restored *placitus* to a species, based on the findings by Niazi & Moore (1962) outlining differences in morphology of the basioccipital bone.

Despite the above-cited references and occasional allusion elsewhere in the earlier literature to morphological variation in *Extrarius* (or *Hybopsis*) *aestivalis*, the only early taxonomic analysis of the complex appears to be Higgins' (1977) unpublished thesis. From a taxonomic standpoint, Higgins chose to accept the *status quo*, and there is no indication that he considered reelevation to species of any of the other above-mentioned members of the *aestivalis* complex.

Although it seemed logical that ambient environment has been a factor in shaping body morphologies of different members of the *Macrhybopsis aestivalis* complex, ichthyologists found it increasingly difficult to accept the prevailing opinion that these distinctive populations were mere ecophenotypes, or at best subspecies. First, morphological variation in *M. aestivalis* exceeded that of other eastern North American species of Cyprinidae, and was equaled or exceeded only in widely disjunct and geographically isolated populations of certain species in western North America, most notably *Rhinichthys osculus* (Oakey *et al.* 2004). In addition, little evidence had been presented to support the concept of morphological clines among populations of the *M. aestivalis* complex, the one notable exception relating to populations of *M. aestivalis* in the Rio Grande basin (Eisenhour 2004). To the contrary, examination of material from recent collections appeared to reinforce morphological integrities of the various populations, and in several instances revealed sympatry (or syntopy) of morphologically distinct taxa, with no apparent evidence of gene interchange.

The scenario described above has resulted in several recent independent studies aimed at resolution of the *Macrhybopsis aestivalis* problem. The first, which involved morphological variation in populations west of the Mississippi River utilizing univariate and multivariate analyses, was by Eisenhour (1997). Formal publication of this work appeared in two subsequent papers (Eisenhour 1999, 2004), although the taxonomic conclusions had earlier been anticipated by Gilbert (1998). Eisenhour (2004) accorded species recognition to all but one described member of the complex (*M. aestivalis, M. marconis, M. australis, M. tetranema*, *M. hyostoma*), with *sterletus* being relegated to the synonymy of *M. aestivalis*. He found the taxonomy of *M. hyostoma* to be especially complex west of the Mississippi River, where a high degree of morphological variation is exhibited, involving combinations of meristics, morphometry, and pigmentation. Most notably, some western populations have two pairs of barbels, a condition never observed in specimens of *M. hyostoma* from the east. In addition, specimens from more southerly western drainages tend to have more pallid bodies, smaller eyes, higher scale counts, and longer barbels. All of these features suggest some degree of ecophenotypic variation, and would appear to lend support to Hubbs & Ortenburger's (1929) ideas. Earlier investigators were uncertain of the status of these western populations. Breukelman (1940) assigned the manuscript name "*sesqualis*" to Kansas specimens, presumably on the advice of Carl Hubbs. Davis & Miller (1967) restricted the name *hyostoma* to populations east of the Mississippi River, leaving most of those to the west unassigned to subspecies. Eisenhour ultimately concluded that, despite demonstrably wide variability, the name *hyostoma* is applicable to most western populations, as well as to all eastern populations living in, and north of, the Tennessee River drainage. His conclusions have been reinforced by the findings of Luttrell *et al.* (1999) and Underwood *et al.* (2003), which involve ecological and genetic interactions between *M. hyostoma* and several western species in the complex. The above publications were followed by Mayden & Powers (2004), which involved an analysis of eastern members of the *Macrhybopsis aestivalis* complex based on information derived from assessment of levels of variation in 25 nuclear gene loci, using allozyme electrophoresis. These genetic data are extremely useful for testing lineage independence of taxa and examining species relationships.

The current study concerns eastern trans-Mississippi populations of the complex, with emphasis on those from eastern Gulf slope drainages. Taxonomy of these morphologically divergent populations utilizes morphometric and meristic data, combined with genetic information in Mayden & Powers (2004). We conclude from these data that five eastern species exist, of which one (*M. hyostoma*) ranges from the Tennessee River north to the Ohio River drainage. In contrast to populations referred to that species west of the Mississippi River, eastern populations of *M. hyostoma* appear to show limited morphological or pigmentary variation. The remaining four species (all described as new) are confined to the eastern Gulf slope, including the Pearl and Pascagoula drainages and Lake Ponchartrain (*M. tomellerii*), the Mobile Bay basin (*M. boschungi* and *M. etnieri*), and three independent river drainages of southeastern Alabama and the Florida panhandle (*M. pallida*) (Figs. 1–2). Based on these combined studies, we now conclude that the entire *Macrhybopsis aestivalis* complex comprises nine valid species. It may be speculated that further studies on the complex, centering on genetic and morphological investigations of *M. hyostoma* throughout its range, could reveal additional nameworthy taxa.

Our interpretation of the evolutionary and distributional history of the *Macrhybopsis aestivalis* complex is based on available taxonomic, genetic, and biogeographic information derived primarily from co-distributed species for which phylogenetic relationships have been investigated. Although the entire complex is evaluated, much of the discussion involves species from the eastern Gulf slope and *Macrhybopsis hyostoma*, which is the most wide-ranging species and the one likely central to the group's remaining taxonomic complexities. Included is a summary of that species' morphological variation, considered relative to its evolution and distribution, and particularly as it relates to isolation and differentiation of the four eastern Gulf slope congeners described herein. Morphological information on *M. hyostoma* populations west of the Mississippi River is taken largely from Eisenhour (1997, 2004). Other important sources of information relevant to *Macrhybopsis* diversity include Eisenhour (1999), Luttrell *et al.* (1999), and Underwood *et al.* (2003), and genetic information derived from Mayden & Powers (2004). This discussion also relies on historical river systems and geology, and history of sea level changes and accompanying drainage patterns. However, the phylogenetic relationships of species of *Macrhybopsis* and variation within *M. hyostoma* warrants additional study with more thorough geographic sampling and characters.

Methods and materials

Meristic characters were evaluated following methods described by Hubbs & Lagler (1947, 2004). Characters include pharyngeal teeth, total vertebrae, anal rays, pectoral rays, pelvic rays, lateral-line scales, body circumferential scales (total and above and below lateral line), predorsal scales, and caudal peduncle scales (total and above and below lateral line). Data were collected from a total of 806 specimens.

Morphometric variation was assessed by standard and truss measurements, generally following Hubbs & Lagler (1947, 2004) and Humphries *et al.* (1981). The former authors (Hubbs & Lagler 1947: 8, fig. 2) included a topographic figure of a soft-rayed fish in their book on Great Lakes fishes. This figure remained unchanged in subsequent editions of the book, the last of which was recently published in revised form (Hubbs & Lagler 2004: 29, fig. 3). In this figure the anterior juncture of both the dorsal and anal fin to the body was termed the "origin," but for the paired pectoral and pelvic fins terminology for this specific area remained undefined. There was no accompanying discussion in the text. In other literature sources, "origin" has been consistently used as defined above, and a different term ("insertion") has been used to denote the anterior juncture of the paired fins (i.e., pectoral and pelvic) to the body (e.g., Trautman 1981: 58–59; Robison & Buchanan 1988: 494–495; Sublette *et al.*

1990: 357–358; Boschung & Mayden 2004: 47). The latter distinction has not always been uniformly adopted, however, and in some cases the two terms have been used interchangeably (Jenkins & Burkhead 1994: 1042, 1044; Mettee *et al.* 1996: 805).

Pertinence of the above discussion relates to six measurements employed in the present study, for which one or both termini involve the posterior juncture of a fin with the body. Since we were unaware of a specific defining term for this location, we opted to employ the word "insertion," although this may conflict with earlier uses of this term in some ichthyological literature. For ease of reference, pertinent measurements are italicized in the following list.

Thirty-three standard and truss measurements were recorded to the nearest 0.1 mm from 247 specimens, using a dissecting scope and dial calipers. These include standard length, snout length, orbital diameter, bony interorbital width, caudal peduncle depth, mouth gape, upper jaw length, maxillary barbel length, dorsal-fin base, anal-fin base, predorsal length, prepelvic length, dorsal origin to anal origin, dorsal origin to *anal insertion*, dorsal origin to pelvic origin, *dorsal insertion* to pelvic origin, *dorsal insertion* to anal origin, *dorsal insertion* to *anal insertion*, *dorsal insertion* to upper caudal peduncle, *dorsal insertion* to lower caudal peduncle, pectoral origin to dorsal origin, pectoral origin to pelvic origin, pectoral origin to branchiostegal junction, pelvic origin to anal origin, pelvic origin to branchiostegal junction, *anal insertion* to upper caudal peduncle, *anal insertion* to lower caudal peduncle, pectoral-fin length, pelvic-fin length, depressed dorsal-fin length, depressed anal-fin length, tip of snout to top of gill slit, tip of snout to branchiostegal junction.

Statistical analyses included sheared principal components analysis (sPCA) of raw mensural data (D. L. Swofford, SAS program for computing sheared PCA, unpubl., 1984, privately distributed). Sexes were analyzed separately and in combination. Scatterplots of second and third sheared principal components for all five species were examined for divergence and geographic trends. To eliminate the influence of species not readily diagnosed by meristic, pigmentary or gross morphological characters, sPCA was conducted separately on specimens from the Mobile Bay basin below the Fall Line (*Macrhybopsis boschungi*) and from Gulf Coast drainages between Mobile Bay and the Mississippi River (*Macrhybopsis tomellerii*).

For the discrete character analysis employed in PAUP (Swofford 1993), the locus was considered the character and combinations of alleles within a taxon represented character states. Character states were coded for unique combinations of alleles using all alleles (Mayden & Matson 1992). Phylogenies were generated using Fitch parsimony (unordered) and generalized parsimony (Swofford & Olsen 1990) accomplished through the use of stepmatrices constructed for each locus (Mabee & Humphries 1993). Stepmatrices were calculated using a customized C program (Mayden *et al.* unpubl. data). Stepmatrices used are presented in Mayden & Powers (2004). *Macrhybopsis storeriana* was the outgroup in this study.

Methods for allozyme electrophoresis and data analysis follow those described by Wood & Mayden (1992), Mayden & Matson (1992), Wood *et al.* (2002), and Mayden & Powers (2004). Buffer systems were provided in Mayden & Matson (1992). For the data analysis BIOSYS-1 (Swofford & Selander 1981) and PAUP* (Swofford 1993) were employed in the population genetic and phylogenetic analyses. Genetic distances included Edwards and Cavalli-Szforza Edwards Chord, Prevosti and Rogers, Cavalli-Szforza Edwards Arc and Modified Rogers. Generalized and Fitch parsimony were used for discrete coded characters representing different allelic combinations (Mayden & Matson 1992).

Illustrations of the five species (Figs. 1A–E) are identical to those appearing in Plates 20D and 21A–D of *The Fishes of Alabama* (Boschung & Mayden 2004), except that *Macrhybopsis pallida* (Fig. 1D [=Plate 21C in Alabama book]) has been slightly modified, at the authors' request, in order to better distinguish the highly diagnostic anterior maxillary barbel.

Synonomic references appearing in the species account of *Macrhybopsis hyostoma* are limited to publications involving populations within our present area of coverage (i.e., east of the Mississippi River).

Specimens from which meristic and morphometric data were taken were included among the type material or otherwise referred to in the text are from the following museum collections: University of Alabama (UAIC); Florida Museum of Natural History, University of Florida (UF); Museum of Zoology, University of Michigan (UMMZ), Tulane University (TU), University of Kansas (KU), University of Tennessee (UT), Illinois Natural History Survey (INHS), National Museum of Natural History (formerly United States National Museum) (USNM), Academy of Natural Sciences of Philadelphia (ANSP). In addition, selected confirmed museum records for *Macrhybopsis etnieri* from the Auburn University Museum (AUM) were employed for purposes of plotting distributions.

A

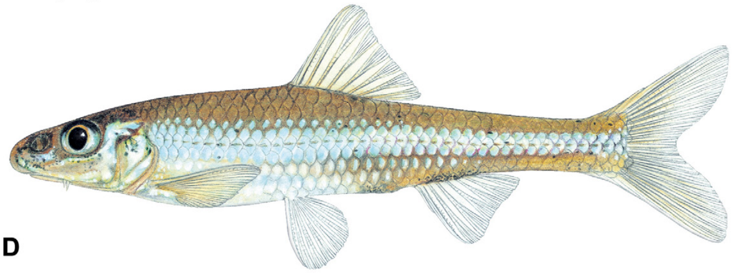
Macrhybopsis hyostoma

B

Macrhybopsis boschungi

C

Macrhybopsis etnieri



D

Macrhybopsis pallida

E

Macrhybopsis tomellerii

FIGURE 1. Species of *Macrhybopsis aestivalis* species complex from eastern North America. A) *Macrhybopsis hyostoma*, UAIC 11060.03, Female, 34 mm SL, Alabama, Limestone County, Elk River, 28 September 1994. B) *Macrhybopsis boschungi*, UAIC 10845.03, Female, 50 mm SL, Alabama, Dallas County, Cahaba River, 12 July 1993. C) *Macrhybopsis etnieri*, UAIC 11053.01, Female, 44 mm SL, Alabama, Bibb County, Cahaba River, 24 June 1994. D) *Macrhybopsis pallida*, UAIC 10855.04, Female, 36 mm SL, Alabama, Escambia County, Conecuh River, 15 July 1993. E) *Macrhybopsis tomellerii*, UAIC 11364.03, Female, 51 mm SL, Mississippi, Covington County, Pascagoula River drainage, 18 February 1994.

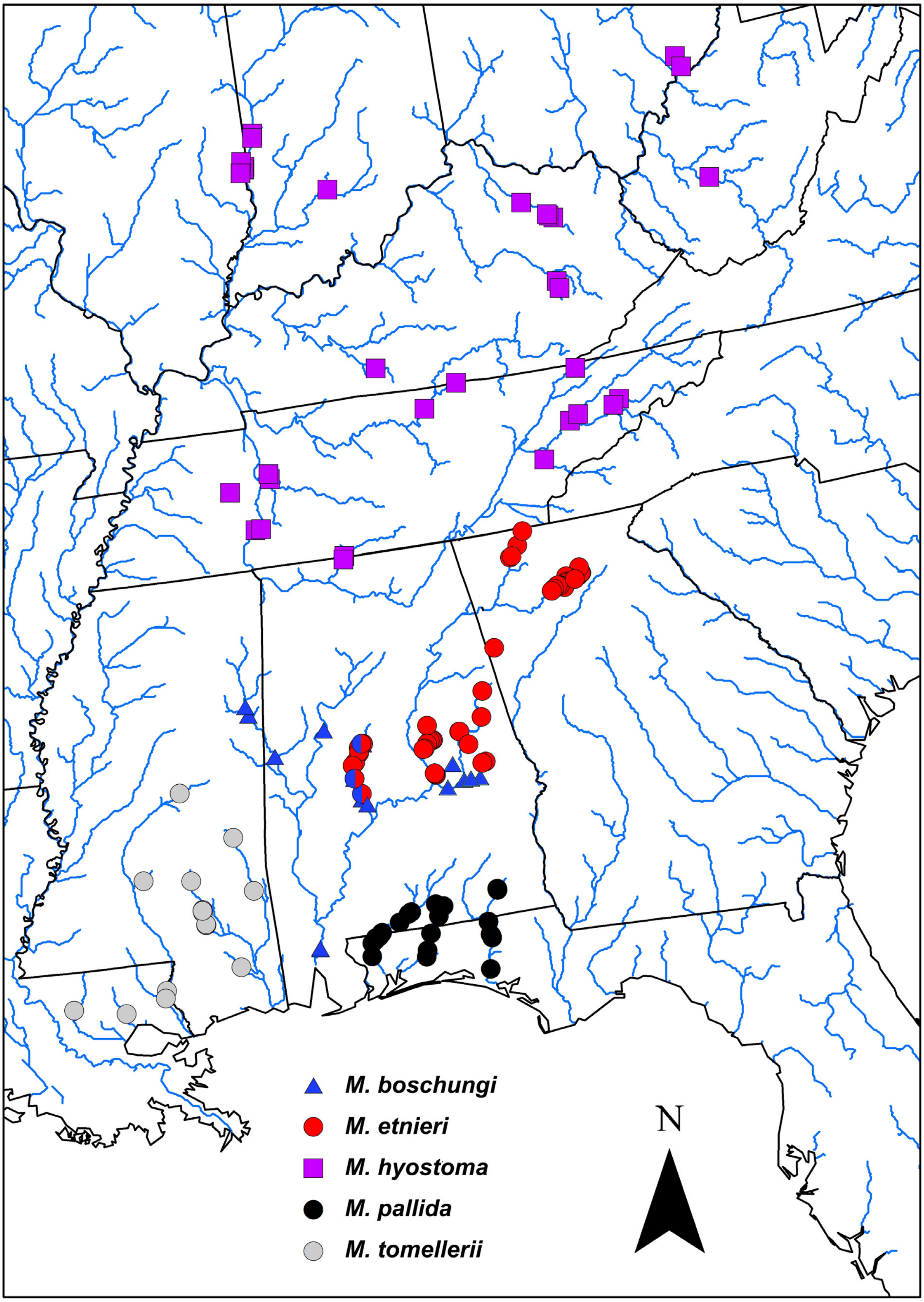


FIGURE 2. Distribution of southeastern species of *Macrhybopsis* east of the Mississippi River. Individual species are identified by symbol and color. Circles with half blue and half red represent locations where *M. boschungi* and *M. etnieri* occur syntopically.

Genus *Macrhybopsis*

Macrhybopsis Cockerell & Allison 1909: 162 (as subgenus of *Hybopsis*; type species *Gobio gelidus* Girard 1856, by original designation).

Extrarius Jordan 1919: 342 (type species *Hybopsis tetranemus* Gilbert 1886, by original designation [also monotypic]).

Macrhybopsis was proposed by Cockerell & Allison (1909), based on aspects of scale morphology. Although poorly founded, the genus received recognition because the type species (*gelida*) is characterized most prominently by a single pair of maxillary barbels, and was accordingly placed by Hubbs & Ortenburger (1929: 25) in the "*Hybopsis* complex" of North American minnows. Jordan (1919) meanwhile had erected a new genus, *Extrarius*, for reception of a single species (*tetranema*) distinguished by the consistent presence of a second pair of maxillary barbels. Hubbs & Ortenburger (1929), in their description of another new and closely related four-barbeled species (*australis*), determined that these two species bear a close relationship to another widespread two-barbeled species then known as *Hybopsis aestivalis*. They accordingly referred all three species to *Extrarius*, in which genus they remained until it was submerged by Bailey (1951: 192) into the broad genus *Hybopsis*, an artificial assemblage of North American cyprinids for which all included species possess one or two pairs of maxillary barbels. This arrangement remained largely unchanged until *Extrarius* was restored to a genus by Mayden (1989), to include the *aestivalis* species complex. Mayden (1989) also accorded recognition to *Macrhybopsis*, which comprised a clade including *M. storeriana, M. gelida*, and *M. meeki*; and considered it most closely related to the monophyletic genus *Extrarius.*

Coburn & Cavender (1992: 349), in their phylogenetic treatment of North American cyprinid genera, included 16 North American genera in a group termed by them the "chub clade," within which nine (including *Macrhybopsis*) were included in the so-called "exoglossin clade." *Extrarius aestivalis* was formally included within *Macrhybopsis* (Coburn & Cavender 1992: 354) based on six shared synapomorphies, including an enlarged nasal capsule; metapterygoid with an enlarged prong for insertion of the adductor palatine muscle; urohyal with an eroded horizontal lamina and a serrate vertical lamina; an elongated hyoid bar; a short triangular basihyal; and elongated branchiostegal rays. This assignment was accepted by Mayden *et al.* (1992), and is generally followed today.

As presently conceived *Macrhybopsis* comprises 12 species, including *M. storeriana* (Kirtland), *M. gelida* (Girard), *M. meeki* (Jordan & Evermann), and the nine species now recognized in the *Macrhybopsis aestivalis* species complex. The following generic diagnosis is copied verbatim from Boschung & Mayden (2004: 206): Body elongate, relatively slender; head flattened ventrally, snout conical to blunt. One or two barbels at corner of each jaw. Lateral scale rows 34 to 50; predorsal scales 14 to 24. Anal rays 7 or 8; pectoral rays 13 to 18. Pharyngeal teeth 1,4-4,1 or 4-4, or combinations thereof. Nuptial tubercles on pectoral fins. Enlarged nasal capsule. Elongated branchiostegal rays. Coloration dusky to silvery, with no chromatic pigments.

In species of the genus, tastebuds and lateral-line neuromasts are exceedingly abundant, and the eyes range from quite small (in *M. gelida* and *M. meeki*) to what could be described as "normal" in size. These senses working together account for their ability to occupy both clear and turbid waters (Branson 1963, 1975; Davis & Miller 1967; Reno 1969).

Systematic accounts

Macrhybopsis hyostoma (Gilbert 1884)

Shoal Chub

Figs. 1A, 2; Table 1

Nocomis hyostomus.—Gilbert 1884: 203 (original description; East Fork of White River, at Bedford, Lawrence County, Indiana). Böhlke 1953: 34 (types in Stanford University collection). Gilbert 1998: 93–94 (in type catalogue of North American Cyprinidae; syntypes in USNM and CAS). Eschmeyer 1998: 754 (in world catalogue of fishes; location of types).

?*Hybopsis montanus.*—Meek 1885: 526–527 (original description; "Upper Missouri River basin"; identification uncertain).

Hybopsis (*Erinemus*) *hyostomus.*—Jordan 1885: 29 (in list).

Hybopsis hyostomus.—Woolman 1892: 258, 284–285, 287 (Kentucky records). Meek 1895: 137 (Nebraska and Ohio records). Forbes & Richardson 1920: 163 (Illinois distribution).

Hybopsis aestivalis.—Branson 1963: 215–217, 219 (olfactory apparatus morphology). Branson 1975: 109–111, figs. 1–2 (olfactory lamellae formation). Clay 1975: 138–139 (key; description; Kentucky distribution). Higgins 1977: 1–43, fig. 4 (in part; geographic variation in morphology). Wallace 1980: 180 (in part; brief account; distribution map of *M. aestivalis* complex). Gammon *et al.* 1991: 145 (Sugar Creek, Indiana). Gammon & Gammon 1993: 77 (extirpated from Eel River, Indiana).

Hybopsis (Erimystax) hyostomus.—Jordan & Evermann 1896: 315–316 (key; description; distribution).

Hybopsis hyostoma.—Evermann & Hildebrand 1916: 445 (Clinch and Tennessee rivers records). Evermann 1918: 321, 346, 367 (Kentucky and Tennessee records).

Extrarius hyostomus.—Hubbs & Ortenburger 1929: 23 (included in *Extrarius*).

Macrhybopsis aestivalis.—Jordan *et al.* 1930: 138 (in part; list). Coburn & Cavender 1986: 1 (in part; phylogeny; included in *Macrhybopsis*). Robins *et al.* 1991: 21, 76 (in part; in list). Coburn & Cavender 1992: 332 (in part; phylogeny of cyprinid genera). Mayden *et al.* 1992: 836 (in part; in list of North American fishes). Dimmick 1993: 174, 176, 178, 181–182 (phylogeny of barbeled cyprinids). Etnier & Starnes 1994: 20, 192–194 (in part; description; Tennessee localities mapped; morphological variation). Stauffer *et al.* 1995: 15, 55, 102–103 (general account; key; West Virginia distribution mapped). Mettee *et al.* 1996: 218–219 (in part; general account; photograph [upper figure only]; Alabama localities mapped).

Extrarius aestivalis.—Gerking 1945: 51 (general account; Indiana distribution mapped). Dimmick 1988: 73, 75, 78, fig. 2a (ultrastructure of barbels). Mayden 1989: 14–15, fig. 9h (in part; phylogeny; *Extrarius* elevated to genus). Carney *et al.* 1992: 205–206 (Tippecanoe River, Indiana). Page & Burr 1991: 106, pl. 9, map 118 (in part; description; distribution).

Extrarius aestivalis hyostomus.—Gerking 1945: 51 (distribution in Indiana).

Hybopsis aestivalis hyostoma.—Trautman 1957: 87, 310–311 (key; general account; Ohio distribution; status). Davis & Miller 1967: 7, 10, 14, 16–18, fig. 13 (brain morphology). Douglas 1974: 100 (subspecies in Louisiana). Clay 1975: 139 (subspecies in Kentucky). Higgins 1977: 13–14, 42, figs. 5–7 (geographic variation in morphology). Trautman 1981: 93, 288–290, fig. 47 (key; general account; Ohio distribution; status). Wallace 1980: 180 (list of subspecies). Burr & Warren 1986: 371 (brief account; subspecies in Kentucky; Kentucky distribution mapped).

Hybopsis (Extrarius) aestivalis.—Davis & Miller 1967: 4–9, 15–18, 32–36 (in part; brain morphology). Reno 1969: 738, 740–743, 746–748, 753, 762–763, 765–766, 770; figs. 2, 26 (in part; variation and morphology of sensory pore system). Jenkins & Lachner 1971: 4, 6–9, 12 (scale morphology; vertebral counts). Burr & Warren 1986: 89, 371 (in part; habitat; Kentucky distribution mapped).

Macrhybopsis aestivalis hyostomus.—Etnier & Starnes 1994: 193 (subspecies in Tennessee and Cumberland rivers).

Macrhybopsis aestivalis hyostoma.—Boschung & Mayden 2004: 108–109, pl. 20D (general account; illustration; distribution in Alabama).

Macrhybopsis hyostoma.—Nelson *et al.* 2004: 73 (in part; in North American fish checklist; elevated to species). Page *et al.* 2013: 73 (in part; in North American checklist).

The above literature citations, other than *Hybopsis montanus*, are limited to eastern populations of *Macrhybopsis hyostoma* from within the area of geographical coverage defined in the present paper.

Despite its broad geographic range and demonstrated morphological variability, the nomenclature of *Macrhybopsis hyostoma*, as presently recognized, has remained remarkably stable, with no junior synonyms as yet having been identified (Gilbert 1998: 28).

A list of non-type material examined during the present study, comprising 43 lots and 409 total specimens, appears in Appendix 1.

Status of *Hybopsis montanus*. The identity of *Hybopsis montanus* is uncertain (Gilbert 1998: 116; Eisenhour 2004: 31). Meek (1885: 526–527) indicated the description to have been based on three specimens, supposedly from the "upper Missouri River basin," having 4-4 pharyngeal teeth (slightly hooked), a pair of well developed maxillary barbels, 37 lateral-line scales, 14 predorsal scales, and six anal rays (last character obviously erroneous), a slender and elongated body, body silvery (without spots), and dorsal fin situated directly above pelvic fins. Other than anal-ray count and lack of spots on the body, this is an accurate description of most populations of *Macrhybopsis hyostoma*, which does not occur in the upper Missouri River basin or any closely adjacent area (Wallace 1980: 180). The three specimens now in the type jar (USNM 36882) all have 1,4-4,1 pharyngeal teeth and are clearly identifiable as *Notropis dorsalis* (Gilbert 1998: 116; Eisenhour 2004: 31), a species absent from the upper Missouri basin (Gilbert & Burgess 1980a: 260). A substitution of specimens has obviously occurred at some time in the past, and the precise status of *Hybopsis montanus* will remain a mystery.

Syntypes. A total of 40 syntypes of *Nocomis hyostomus* were identified by Gilbert (1998: 93–94) and Eisenhour (2004: 31). All were collected at the type locality (White River, near Bedford, Lawrence County, Indiana) and are included in the following three museum lots (measurements in standard length): USNM 34980 (6, 44.0–47.4), CAS-SU 888 (30, 40.7–49.9) and CAS 58626 (4, 40.1–45.3). Böhlke (1953: 34) erroneously listed USNM 34980 as the "holotype"

Table 1. Variation of selected meristic characters in eastern species of *Macrhybopsis*. Incom. refers to number of incomplete scale rows.

	Lateral-line scales							Body-circumferential scales (above and between lateral lines)							Body-circumferential scales (below and between lateral lines)								
	35	36	37	38	39	No.	Mean	9	10	11	12	13	No.	Mean	incom.	9	10	11	12	13	14	No.	x
Macrhybopsis hyostoma	2	12	36	16	11	77	37.29	2	9	66	3		80	10.88	14			6	28	26	6	66	12.48
Macrhybopsis boschungi	9	38	26	9	2	84	36.49		2	73	8	1	84	11.10	73				4	7		11	12.64
Macrhybopsis tomellerii	12	33	33	5		83	36.37		1	68	14		83	11.16	83							---	-------
Macrhybopsis etnieri		16	24	37	10	87	37.47	32	26	25	4		87	10.01	1	1	17	31	33	4		87	11.25
Macrhybopsis pallida	5	15	36	21	8	85	37.14	1	21	48	11	2	83	10.90	76		2	2	3			7	11.14

	Total body circumferential scales (including lateral-line scales)												Predorsal scales								
	Incom.	20	21	22	23	24	25	26	27	28	No.	Mean	14	15	16	17	18	19	20	No.	Mean
Macrhybopsis hyostoma	14			1	2	7	26	21	8	1	56	25.39	14	21	23	20	5	2		85	15.85
Macrhybopsis boschungi	73						4	3	3	1	11	26.09	3	13	32	16	16	4		84	16.49
Macrhybopsis tomellerii	83										----	------		1	12	33	27	8	2	83	17.42
Macrhybopsis etnieri	1	1	12	17	19	15	15	6	1		87	23.26	4	17	29	21	9	2		87	16.24
Macrhybopsis pallida	75					4	4				8	24.50	4	14	23	21	16	5		83	16.55

	Pectoral-fin rays (both sides combined)								Pelvic-fin rays (both sides combined)				
	12	13	14	15	16	17	No.	Mean	7	8	9	No.	Mean
Macrhybopsis hyostoma		1	18	114	37		170	15.70	3	163		166	7.98
Macrhybopsis boschungi			15	118	25		158	15.06	1	157		158	7.99
Macrhybopsis tomellerii			16	97	52	1	166	15.23	7	159		166	7.96
Macrhybopsis etnieri	1	21	84	46			152	14.15	7	162	1	170	7.96
Macrhybopsis pallida		27	80	53	4		164	14.24	1	167		168	7.99

	Anal-fin rays						Dorsal-fin rays				Total Vertebrae							
	6	7	8	9	No.	Mean	7	8	No.	Mean	34	35	36	37	38	39	No.	Mean
Macrhybopsis hyostoma		4	106	6	116	8.02		85	85	8.00			8	40	27	2	77	37.30
Macrhybopsis boschungi		6	142		148	7.96		89	59	8.00	1	9	79	14			103	36.03
Macrhybopsis tomellerii		10	114	1	125	7.93		90	90	8.00	1	26	55	17			99	35.89
Macrhybopsis etnieri		8	182		190	7.96	1	104	105	7.99				23	121	40	184	38.09
Macrhybopsis pallida	2	171	2		175	7.00		84	84	8.00		10	53	13			76	36.04

	Pharyngeal teeth			
	4-4	0,4-4,1	1,4-4,0	1,4-4,1
Macrhybopsis hyostoma	25			
Macrhybopsis boschungi	28			
Machybopsis tomellerii	25			
Macrhybopsis etnieri	2	5	8	56
Macrhybopsis pallida	25			

Diagnosis. The following diagnosis is based solely on populations occurring east of the Mississippi River (comprising a total of 43 lots and 409 specimens), the geographic limits of which extend from the Tennessee River drainage north to the Ohio River drainage (Fig. 2, Appendix 1). Those from the Ohio River drainage include material from the states of West Virginia, Kentucky, Ohio and Indiana west to the Wabash River system, which (in its lower reaches) forms the boundary between Indiana and Illinois. An expanded diagnosis of *Macrhybopsis hyostoma* appears in Eisenhour's (2004: 31–34) study, which is largely confined to populations west of the Mississippi River.

A member of the *Macrhybopsis aestivalis* complex, as defined in the generic diagnosis. Characterized by a single pair of moderately long maxillary barbels, 4-4 pharyngeal teeth, eight anal rays, anal opening closer to anal-fin origin than pelvic-fin origin (ca. 70 percent of intervening distance), and prominent dark melanophores on upper two-thirds of body (Fig. 1A) (also see Smith 1979: 75; Trautman 1981: fig. 47; Boschung & Mayden 2004: pl. 20D). Among other eastern species of the complex, the first four characters are shared with *M. boschungi* and *M. tomellerii*, which differ most notably in having the body melanophores much reduced in size (Figs. 1B, 1E). Other important diagnostic characters for *M. hyostoma* include a complete bridge of scales across belly (two to five scale rows deep), the bridge incomplete or absent in about 20 percent of specimens examined; genital papillae extremely reduced; dorsal-fin origin situated very slightly posterior to imaginary line extending upward from pelvic fin origins; and pectoral fins in both sexes relatively short, rounded to bluntly pointed, never sharply pointed in adult males.

The two remaining eastern members of the complex (*M. etnieri* and *M. pallida*) differ from *M. hyostoma* in having the anal opening located midway between the origins of the pelvic and anal fins. Additional diagnostic differences include 1,4-4,1 pharyngeal teeth in *M. etnieri* and a combination of two pairs of maxillary barbels and seven anal rays in *M. pallida*.

Description. Characters listed in the diagnosis are not repeated here, unless additional clarification is required. Variation in meristic characters is presented in Table 1.

Dorsal-fin rays 8; anal-fin rays usually 8 (range 7–9); pectoral-fin rays usually 15 (range 14–16); pelvic-fin rays usually 8 (occasionally 7); lateral-line scales modally 37 (range 35–39); predorsal scales irregularly distributed and poorly defined, numbering 15–17 (range 14–19); body-circumferential scale rows above and between lateral lines usually 11 (range 9–12); body-circumferential scale rows below and between lateral lines usually 12–13 (range 11–14), the scales occasionally incomplete or absent from middle of belly; total caudal-peduncle scale rows uniformly 12 (five scale rows above and below lateral lines on each side of caudal peduncle); total vertebrae 37–38 (range 36–39); scales present and well developed in area between pelvic and anal fins; anal and dorsal fins usually angular and slightly falcate, the anteriormost rays (when depressed) extending about same distance posteriorly as posteriormost rays; head somewhat rounded and moderately flattened ventrally; mouth inferior and horizontal, not as wide as head; lips moderately fleshy, not thickened posteriorly; eyes oval in shape and relatively large, the diameter less than snout length; snout moderately long, blunt and rounded, its length more than length of orbit; genital papillae poorly developed as small conical or cylindrical extensions in both sexes; four or five rudimentary gill rakers on upper limb of outer (anteriormost) gill arch, these usually absent from lower limb but with four or five tiny gill rakers occasionally present; pharyngeal teeth short, thin, and hooked, with little or no grinding surface; nuptial tubercles present in membrane immediately posterior to first pectoral-fin ray; pectoral-fin rays 2–10 thickened in nuptial males and containing large, conical, recurved uniserial tubercles; tiny tubercles sometimes present on anteriormost rays of dorsal and pelvic fins in nuptial males.

Specimens in life without chromatic pigmentation; translucent pale green or gray on upper two-thirds of body, silvery white on lower third of body, including belly; predorsal stripe and postdorsal stripe usually absent, but occasionally present as a thin faint line; scales on upper side of body occasionally margined with a thin line of pigment; pigment on scales on upper two-thirds of body either evenly distributed over scale or slightly more concentrated on posterior half of scale; tiny melanophores on body often most densely concentrated along mid-side of body, where they may form a faint lateral stripe that is particularly prominent on posterior third of body.

Females attain a larger size than males, the largest female examined 61.3 mm SL (UT 55.1511) from Barren River, Warren County, Kentucky; the largest male recorded 53.6 mm SL (SIUC 7317) from Green River, Hart County, Kentucky (Eisenhour 1997).

Comments. As presently conceived, *Macrhybopsis hyostoma* is the most wide-ranging and morphologically variable member of the *M. aestivalis* complex. Based partly on its broad and centralized distribution, Eisenhour

(1997) presumed *M. hyostoma* to be the group's ancestral species. This situation remains complex and uncertain, however, and additional genetic study is required.

Among other eastern species, it most closely resembles *Macrhybopsis boschungi* and *M. tomellerii*, with which it shares a number of meristic and morphometric characters listed in the preceding diagnosis. However, genetic data, as discussed elsewhere in this paper, do not indicate an intimate relationship with either of those species.

Distribution. *Macrhybopsis hyostoma* is distributed, east of the Mississippi River, from the Tennessee River drainage of Alabama north to the Ohio River drainage in Illinois east to West Virginia (Fig. 2). The distribution of populations currently referred to this species from the Mississippi River westward are discussed elsewhere in this paper.

Habitat. Eastern populations of *Macrhybopsis hyostoma* inhabit large rivers and lowermost sections of the their major tributaries, in areas ranging from turbid to relatively clear water, a moderate to strong current, and a bottom of sand and gravel.

Etymology. The species name *hyostoma* is derived from the words *hyo* (=hog) and *stoma* (=mouth), in reference to the underlying position of the mouth relative to the projecting snout.

Macrhybopsis boschungi Gilbert & Mayden sp. nov.

Mobile Chub
Figs. 1B, 2; Table 1

Hybopsis hyostomus.—Gilbert 1891: 155, 157 (in part; records from Tuscaloosa, Alabama).
Hybopsis aestivalis.—Cook 1959: 129–130 (in part; general account; considered very rare in Mississippi).
Extrarius aestivalis.—Mayden 1989: 14 (in part; chub clade; phylogeny based on cranial osteology).
Extrarius sp. cf *aestivalis*.—Boschung 1989: 50 (Tombigbee River distribution map; diagnoses of two undescribed species in Mobile Bay basin; ecological notes).
Macrhybopsis aestivalis.—Boschung 1992: 52 (in part; Alabama). Mettee *et al.* 1996: 218–219 (in part; distribution map, description and account of species complex in Alabama).
Macrhybopsis sp. ("Mobile chub").—Eisenhour 2004: 37, 47 (in part; phylogenetic tree involving other members of genus).
Macrhybopsis sp. cf *M. aestivalis* "A" (Gulf Chub).—Boschung & Mayden 2004: 209, plate 21A (general account [refers in part to *Macrhybopsis tomellerii*]; color illustration).

Holotype. UF 175766 (*ex* UT 44.2312), 57.0 mm SL female, Cahaba River, at U.S. highway 80 bridge, 16 km W of Selma, Dallas Co., Alabama, D. A. Etnier and class, 17 May 1981.

Paratypes. The following paratypes, comprising 34 lots and 617 total specimens, are listed here in abbreviated fashion by state, river system, and county, followed by museum catalogue number and numbers of specimens. Complete locality data appear in Appendix 1.

Catalogue numbers accompanied by an asterisk (*) denote lots originally containing specimens of both *Macrhybopsis boschungi* and *M. etnieri,* where the species were sampled syntopically.

Alabama: Cahaba River: (Bibb Co.) UAIC 1443.06 (1), UF 116293 (1)* (mixed lot also includes four *M. etnieri* [UF 15434]); UAIC uncat. (2)* (mixed lot also includes four *M. etnieri* [UAIC 7198.02]); UMMZ 250267 (2)* (mixed lot includes 28 *M. etnieri* [UMMZ 250266]); (Dallas Co.) UT 44.2312 (76 originally, now 41), UF 175767 (5), USNM 437193 (5), UMMZ 250263 (5), ANSP 200789 (5), TU 204138 (5), MCZ 171826 (5), KU 41378 (5) (preceding seven lots of paratopotypes all *ex* UT 44.2312); UAIC 7188.01 (20), UAIC 7189.04 (62), UAIC 10845.03 (1 [illustrated female specimen, fig. 1B; *ex* Boschung & Mayden 2004: pl. 21A]); (Perry Co.) UAIC 962.06 (5), UAIC 1437.19 (2), UAIC 5819.01 (1), UAIC 6430.04 (1), UAIC uncat. (5)* (mixed lot also includes 18 *M. etnieri* [UAIC 6791.03]), UAIC uncat. (2)* (mixed lot also includes seven *M. etnieri* [UAIC 6799.02]), UAIC uncat. (20)* (mixed lot also includes ten *M. etnieri* [UAIC 7194.03]).

Coosa River: (Elmore Co.) UF 116296 (5).

Tallapoosa River: (Macon Co.) UAIC 1516.04 (2), UT 44.1949 (2), UF 116295 (13); (Montgomery Co.) UF 116297 (3).

Tombigbee River: (Pickens Co.) UAIC 4330.03 (192); (Tuscaloosa Co.) USNM 36715 (3), USNM 43531 (18).

Alabama River: (Mobile Co.) UF 20859 (16), UF 20862 (5).

Mississippi: Tombigbee River: (Clay Co.) UF 28985 (7); (Lowndes Co.) UT 33.1204 (150).

Diagnosis. A species in the *Macrhybopsis aestivalis* complex, as described in the generic diagnosis.

Macrhybopsis boschungi (Fig. 1B) resembles the allopatrically distributed *M. tomellerii* (Fig. 1E), with which it shares a lightly spotted body; a single pair of moderately long maxillary barbels; 4-4 pharyngeal teeth; eight anal rays; anal opening distinctly closer to anal-fin origin than pelvic-fin origin (ca. 70 percent of intervening distance); genital papillae extremely reduced; and similar scale counts (usually 36–37 lateral-line scales and 11 rows of scales above and between the lateral lines on opposite sides of body) (Table 1).

The above two species are distinguished by consistent differences in head morphology, most notably a longer snout in *M. boschungi* (which exceeds the postorbital length) versus a shorter snout in *M. tomellerii,* the length of which equals the postorbital length). A second difference (not readily evident from gross examination or in poorly preserved specimens) may be expressed as an imaginary line extending upward from the angle formed by juncture of the lachrymal groove and posterior flap of snout, which in *M. boschungi* runs forward of the nares but in *M. tomellerii* intersects the anterior margin of the nares.

M. boschungi and *M. tomellerii* together bear a superficial resemblance to eastern populations of *M. hyostoma* (Fig. 1A), from which they differ most obviously in having a less heavily spotted body.

M. boschungi differs from *M. etnieri* (Fig. 1C) in pharyngeal-tooth count (4-4 vs. 1,4-4,1); position of anal opening about two-thirds (70 percent) of distance between pelvic and anal-fin origins (vs. midway); dorsal-fin positioned directly above pelvic fins (vs. distinctly posterior); a less heavily spotted body; and longer maxillary barbels.

M. boschungi differs from *M. pallida* (Fig. 1D) in having a single pair of maxillary barbels (vs. two pairs); eight anal rays (vs. seven); position of anal opening about two-thirds of distance between pelvic and anal-fin origins (vs. midway); a slightly more heavly spotted body. (vs. essentially pallid); and a greater average body size, as discussed in the individual species accounts.

Description. Characters listed in the Diagnosis are not repeated here, unless additional clarification is required. Variation in meristic characters is presented in Table 1.

Dorsal rays 8; anal rays 8 (rarely 7); pectoral rays usually 15 (occasionally 14 or 16); pelvic rays usually 8 (rarely 7); lateral-line scales usually 36–37 (range 35 to 39); body-circumferential scale rows above and between lateral lines on opposite sides of body usually 11, occasionally 12 (range 10 to 13); scales sometimes present on belly preceding anal fin, more often incomplete or absent (about 75 percent of time); total body-circumferential scale rows (when complete) 25 to 28; predorsal scales irregularly distributed and poorly defined, usually numbering 15 to 18 (rarely 14 or 19); total vertebrae usually 36, sometimes 35 or 37 (very rarely 34).

Dorsal fin angular and slightly falcate, the anteriormost rays (when depressed) extending about same distance posteriorly as posteriormost rays; head moderately rounded dorsally and moderately flattened ventrally; mouth inferior and horizontal, its width about 60 percent of head width; lips moderately fleshy, not thickened posteriorly; eyes oval in shape and relatively large, the diameter about 60 percent of preorbital distance.

A small percentage of specimens of *M. boschungi* examined (11 of 84 [13 percent]) possessed a complete bridge of scales across the belly, a character never observed in any of the 83 individuals examined of *M. tomellerii*. In addition, *M. boschungi* exhibits a slight downward shift in predorsal-scale counts (Table 1).

Females attain a larger maximum size than males. The largest specimen examined (UF 175766 [the holotype]), is a 57.0 mm SL female from the Cahaba River, Dallas County, Alabama, 17 May 1981. The largest male examined, 47.2 mm SL (UT 44.2312), is from the same collection.

Comments. Similarities and relationships of *Macrhybopsis boschungi* and other species, especially the morphologically similar *M. tomellerii,* are discussed in the account of *M. tomellerii*.

Distribution. *Macrhybopsis boschungi* is endemic to the lower Mobile basin of Alabama and northeastern Mississippi, where its upstream range limits are sharply delineated by the Fall Line (Fig. 2). As discussed subsequently, it is replaced above the Fall Line by *Macrhybopsis etnieri*, to which it is not intimately related.

Habitat. *Macrhybopsis boschungi* inhabits the larger, moderately clear to turbid rivers and the lowermost parts of their major tributaries below the Fall Line in the Mobile Bay basin. In such areas water currents are moderate to strong and the bottom is comprised of a combination of gravel, sand and silt.

Conservation Status. Although this species has occasionally been collected in large numbers, ongoing stream modifications and habitat alteration throughout the lower Mobile Bay basin do not bode well for its future. In particular, construction of the Tenn-Tom waterway, which has changed the original free-flowing Tombigbee River into a series of standing pools, has served to eliminate this species from a major portion of its original geographic range. This species should be closely monitored throughout remaining parts of its range.

Etymology. Named for the late Dr. Herbert T. Boschung, Emeritus Professor of Biology at the University of Alabama, for his many contributions over the years to southeastern ichthyology in general and the state of Alabama in particular, including co-authorship of *Fishes of Alabama* (Boschung & Mayden 2004).

It should be noted here that the vernacular name "Mobile Chub" used here differs from the name "Gulf Chub" applied by Boschung & Mayden (2004: 209; plate 21A). Considering its geographical distribution, we consider the former name to be more appropriate for the species.

Macrhybopsis etnieri Gilbert & Mayden sp. nov.

Coosa Chub
Figs. 1C, 2; Table 1

Hybopsis hyostomus.—Gilbert 1891: 155, 157 (in part; record from Oxford, Alabama). Scott 1951: 36–37 (summary of fish sampling from Coosa River, near Childersburg Alabama; reference to "*Nocomis hyostomus*" believed to refer to *Macrhybopsis etnieri*).

Hybopsis aestivalis.— Smith-Vaniz 1968: 40 (in part; general account; Alabama). Dahlberg & Scott 1971: 16, 59 (Georgia records). Stiles & Etnier 1971: 14–16 (annotated list of fishes from Conasauga River system, Tennessee and Georgia). Yerger 1978: 12–13 (photograph of specimen from Cahaba River; account refers to Florida populations of *Macrhybopsis pallida*). Wallace 1980: 180 (in part; brief account; distribution map of *M. aestivalis* complex).

Extrarius sp. cf. *aestivalis.*—Boschung 1989: 50 (diagnostic characters distinguishing it from other undescribed congener in Mobile Bay basin).

Macrhybopsis aestivalis.—Boschung 1992: 52 (in part; partial synonymy for Alabama; general account). Etnier & Starnes 1994: 192–194, pl. 59 (in part; general account; morphological variation; localities mapped for Conasauga River in Tennessee). Mettee *et al.* 1996: 218–219 (in part; general account; localities mapped for entire *M. aestivalis* complex in Alabama and from Mobile Bay basin in Georgia).

Macrhybopsis sp. cf. *M. aestivalis* "B"—Boschung & Mayden 2004: 208–209; plate 21B (characters; relationships; distribution; spot-distribution map for Alabama [in part]; colored illustration).

Holotype. UF 90100 (female, 52.2 mm SL), from Etowah River, just off county road 76, 8.6 air km SE of center of Dawsonville, Dawson County, Georgia; Noel M. Burkhead and crew (field no. NMB 1281), 19 November 1991.

Paratypes. The following paratypes, comprising 71 lots and 513 total specimens, are listed in abbreviated fashion by state, river system, and county, followed by museum catalogue number and numbers of specimens. Complete locality data appear in Appendix 1.

Catalogue numbers accompanied by an asterisk (*) denote lots originally containing specimens of both *Macrhybopsis etnieri* and *M. boschungi,* where the species were sampled syntopically.

Alabama: Cahaba River: (Bibb Co.) UF 15434 (4)* [*ex* UAIC 1611.03], UF 116298 (12); UAIC 1611.03 (24)* [see UF 15434], UAIC 2029.04 (2), UAIC 4679.04 (1), UAIC 5584.01 (2), UAIC 6452.01 (12), UAIC 6788.03 (4), UAIC 6789.03 (36), UAIC 6797.03 (2), UAIC 7186.03 (9), UAIC 7187.03 (25), UAIC 7198.02 (7)*, UAIC 7702.03 (4), UAIC 11053.01 (1 [illustrated female specimen from Boschung & Mayden 2004: pl. 21B; present fig. 1C]); UMMZ 250266 (formerly UMMZ 171750) (28)*; INHS 76341 (5), INHS 76335 (3); (Perry Co.) UF 116294 (6); UAIC 6791.03 (18)*, UAIC 6798.03 (6), UAIC 6799.02 (7)*, UAIC 7191.03 (3), UAIC 7194.03 (10)*, UAIC 7197.02 (6), UAIC 7199.03 (4)*.

Tallapoosa River: (Chambers Co.) UF 116299 (6); (Lee Co.) UMMZ 111192 (1), UMMZ 111193 (5), UMMZ 111194 (12), UMMZ 142909 (1); (Randolph Co.) UT 44.2293 (3); (Tallapoosa Co.) UF 91617 (1); UT 44.2300 (1); UAIC 1040.03 (13).

Georgia: Coosa River: (Cherokee Co.) UF 86141 (3), UF 86274 (1), UF 86289 (12), UF 91296 (17), UF 91414 (1); UT 44.1891 (9), UT 44.1926 (6), UT 44.1937 (13), UT 44.1942 (3), UT 44.2230 (6), UT 44.2240 (2); (Dawson Co.) UF 15785 (2), UF 86162 (9), UF 86182 (1), UF 86203 (3), UF 86227 (9), UF 97284 (30), UF 237855 [*ex* UF 90100] (14 paratopotypes); UT 44.1934 (6); UMMZ 175589 (1); (Forsyth Co.) UF 86244 (59 originally, now 34), USNM 437193 (5), ANSP 200788 (5), MCZ 171825 (5), TU 204137 (5), KU 41377 (5) (preceding five series *ex* UF 86244); (Lumpkin Co.) UF 90116 (3).

Tallapoosa River: (Haralson Co.) UT 44.2794 (1).

Tennessee: Coosa River: (Bradley Co.) UF 42694 (1), UF 42743 (1); UT 44.303 (1), UT 44.484 (2), UT 44.1692 (2); (Polk Co.) UT 44.400 (1), UT 44.413 (11); USNM 230851 (1).

Diagnosis. A species of the *Macrhybopsis aestivalis* complex, as defined in the generic diagnosis. Distinguished from all other members in the complex in having 1,4–4,1 pharyngeal teeth; anal opening situated midway between pelvic and anal-fin origins (vs. 70 percent of distance in all other congeners except *Macrhybopsis pallida*); origin of dorsal fin situated distinctly posterior to imaginary line extending upward from pelvic-fin origins; belly anterior to pelvic fins usually with a complete bridge of scales (two to five scales deep), the bridge mostly obscured by overlying epidermal tissue about 20 percent of the time.

Other important diagnostic features include a single pair of short maxillary barbels; eight anal rays; upper two-thirds of body strongly pigmented with relatively large and prominent melanophores, interspersed with smaller melanophores (Fig. 1C); pectoral fins in both sexes short and moderately to strongly rounded, seldom if ever extending posterior to pelvic-fin origin; snout short and rounded, its length slightly longer than diameter of eye; tubercles on pectoral fins of nuptial males relatively well defined only in first row, those tubercles in subsequent rows tiny and poorly defined but apparently uniserial; genital papillae well developed.

Description.—Characters listed in the diagnosis are not repeated here, unless additional clarification is required. Variation in meristic characters is presented in Table 1.

Dorsal-fin rays 8; anal-fin rays 8, rarely 7; pectoral-fin rays usually 14–15 (range 12–15); pelvic-fin rays usually 8 (range 7–9); lateral-line scales usually 36–38 (occasionally 39); predorsal scales irregularly distributed and poorly defined, numbering 15–17 (rarely 18 or 19); body-circumferential scale rows above and between lateral lines on either side of body 9 to 11 (occasionally 12); body-circumferential scale rows below and between lateral lines on either side of body usually 10–12 (range 9–13); total caudal-peduncle scale rows uniformly 12 (five rows above and below lateral lines on either side of body); total vertebrae usually 38 (range 37–39), these averaging higher (often 39) in upper areas of Coosa River drainage in Georgia and Tennessee; pharyngeal teeth usually 1,4-4,1, the lesser tooth occasionally absent from one or both sides of pharyngeal arch; pharyngeal teeth short, thin, and hooked, with little or no grinding surface; anal and dorsal fins bluntly pointed to slightly falcate; head moderately rounded and moderately flattened ventrally; mouth inferior and horizontal, not as wide as head; lips moderately fleshy, not thickened posteriorly; eyes round and relatively small, their diameters slightly less than pre-orbital distance; pre-orbital and post-orbital distances approximately equal; four or five rudimentary gill rakers on upper limb of outer (anteriormost) gill arch, the rakers usually absent from lower limb of arch.

Specimens in life without chromatic pigmentation; translucent pale green or gray dorsally and silvery white ventrally; predorsal streak thin but usually present; postdorsal streak absent; thin line of pigment often present along margin of dorsolateral scales and sometimes on ventrolateral scales; lateral stripe on caudal peduncle poorly defined, fading anteriorly.

Females attain a larger size than males, the largest female (the holotype) examined 52.2 mm SL (UF 90100) from Etowah River, Dawson County, Georgia; the largest male examined 45.0 mm SL (UF 15434) from Cahaba River, Bibb County, Alabama.

Comments. *Macrhybopsis etnieri* is one of the most morphologically distinctive members of the *M. aestivalis* complex (Fig. 1C), most notably in having a unique pharyngeal-tooth count (usually 1,4-4,1), intermediate position of anus relative to pelvic and anal-fin origins, more posterior position of the dorsal fin relative to the pelvic fins, and very small nuptial tubercles on pectoral-fin rays of breeding males.

Macrhybopsis etnieri shares, with *M. pallida*, two characters unique to members of the *M. aestivalis* complex, namely an intermediate position of the anus relative to the origins of the pelvic and anal fins (versus over two-thirds of distance in other species), and well developed genital papillae (versus papillae lacking or extremely reduced). Eisenhour (2004: figs. 16–17) hypothesized a close relationship between the two species, presumably based largely on these two characters. Their respective geographic distributions raise doubts as to this proposed relationship, since biogeographically their ranges are quite disjunct and this relationship is not duplicated by any other known closely related pairs of fish species. This hypothesis also is not supported by genetic data presented in the present paper, which, in combination with the phylogenetic tree generated via Generalized Parsimony, shows *M. pallida* to have a very close relationship to the geographically adjacent, but morphologically dissimilar, *M. boschungi*. *M. etnieri*, in turn, appears to have a sister relationship to all remaining members of the *M. aestivalis* complex (see discussion on Relationships and Historical Biogeography).

Distribution. *Macrhybopsis etnieri* is restricted to upper sections of the Mobile Bay basin, above the Fall Line, including the Cahaba, Coosa and Tallapoosa river systems, in Alabama, northwestern Georgia, and extreme southeastern Tennessee (Fig. 2). It is replaced below the Fall Line by *Macrhybopsis boschungi*, to which it is not intimately related but with which it occurs sympatrically (or syntopically) in an approximately 40-km section of the middle Cahaba River system in central Alabama (Fig. 2). More detailed distributional information appears below.

Habitat. *Macrhybopsis etnieri* occurs in clear, moderately large flowing streams with a sand, gravel, or fine rubble bottom above the Fall Line in the Cahaba, Coosa and Tallapoosa river systems of Alabama, Georgia, and Tennessee. Collections are largely concentrated in three areas: the Etowah River (upper Coosa drainage) in northwestern Georgia; Hatchet Creek, in Coosa and Clay counties, Alabama; and the middle Cahaba River south of Birmingham, Alabama. Other collections are known from scattered localities, including the Choccolocco Creek watershed in Cleburne and Talladega counties, Alabama; the Tallapoosa River system of eastern Alabama and western Georgia; and the Conasauga River (upper Coosa drainage) in Tennnessee and Georgia. A large collection from the Coosa River, at Childersburg, Alabama, from which no voucher specimens appear to have been retained and which thus cannot be firmly documented (Scott 1951), is discussed below.

The comparatively large number of records for the Cahaba and Etowah systems, and from the Hatchet Creek watershed, is partly attributable to the heavier concentrations of fish collections from these three areas. The Cahaba River is in close proximity to Tuscaloosa, and has been sampled frequently over the years by ichthyologists from the University of Alabama; Hatchet Creek was visited frequently from 1978–1985 by personnel from nearby Auburn University; and the Etowah River was the focus of directed studies by personnel with the U.S. Geological Service, in Gainesville, Florida, from 1990–1994.

Since the Coosa River drainage in Alabama has been well sampled for fishes throughout the years, probably totaling over 700 collections (Boschung 1961; Mettee *et al.* 1996: 18; Boschung & Mayden 2004: 71, fig. 6.1), the scattered distribution and overall paucity of records of the Coosa Chub seems strange. One possible explanation is that populations of this species were once concentrated in the Coosa River proper, but have now disappeared following conversion of the Alabama portion of the river to a series of reservoirs (Boschung & Mayden 2004: 21). This hypothesis receives support from Scott (1951), who summarized results of a major pre-impoundment fisheries investigation, during July and August, 1949, of the Coosa River near Childersburg, in Talladega County. A wide variety of collecting techniques were employed, with smaller individuals being collected mostly with fish traps and liberal applications of rotenone. Heavy emphasis was placed on the larger and economically more important fishes, as evidenced from the summary table on page 37 of Scott's paper. Only three minnows were listed, which undoubtedly is a gross underrepresentation, given that at least 24 cyprinid species are known to occur in this portion of the Coosa River and its major tributaries (Boschung 1961; Mettee *et al.* 1996; Boschung & Mayden 2004). Surprisingly, one of the three cyprinids listed by Scott (1951: 37, table 6) was *Nocomis hyostomus* (referred to simply by the vernacular name "Chub"), which, based on site of collection (well above the Fall Line), almost certainly represents the species now recognized as *Macrhybopsis etnieri*. It is also significant that this species was indicated as being the most numerically dominant cyprinid encountered during the Childersburg operation. Unfortunately, no voucher specimens were apparently saved that would serve to provide positive verification.

A survey of museum fish collections has revealed only 13 confirmed Alabama collections of *Macrhybopsis etnieri* from the Alabama portion of the Coosa drainage, all housed at Auburn University (see Appendix 1 for detailed information). Of these, 11 are from Hatchet Creek, in Coosa and Clay counties, and the remaining two are from the Choccolocco Creek watershed in Talladega and Cleburne counties (see maps in Mettee *et al.* [1996: 218] and Boschung & Mayden [2004: 208]). Presence of *M. etnieri* at those localities may best be explained by those creeks' relatively large size, coupled with absence of dramatic alteration from large urban areas upstream.

The situation described for Alabama may be mirrored in Georgia as well. Considering the concentration of recent records from the Etowah River system in Georgia, it is noteworthy that *Macrhybopsis etnieri* appears not to have been encountered by David Starr Jordan in any of his collections from the upper Coosa drainage in that state (Jordan 1877; Jordan & Brayton 1878), none of which were from the Coosa River proper. From those authors' detailed descriptions of the streams sampled (most notably Silver and Rocky creeks, tributaries to the Etowah and Oostanaula rivers, respectively), the habitats sampled would appear entirely suitable for *Macrhybopsis etnieri*. The only other cyprinid species known from this region possessing a terminal maxillary barbel are *Rhinichthys* cf *atratulus* (confined to small streams), *Macrhybopsis storeriana* (a rare inhabitant of the mainstem Coosa River) (Mettee *et al.* 1996: 220), and *Hybopsis lineapunctata.* The last, which is the species most likely to be confused with *M. etnieri*, was reported by Jordan (1877: 328–330) under the name *Nocomis amblops* var. *winchelli,* and by Jordan & Brayton (1878: 53) as *Ceratichthys winchelli. Hybopsis lineapunctata* was described by Clemmer & Suttkus (1971), at which time all known pertinent material from the early Jordan collections was examined. Had any specimens of *M. etnieri* been present, they undoubtedly would have been noted. Likewise, no specimens were found among recently discovered remnants of the Jordan collections that had lain undetected at Butler University for well over a century, and which were analyzed by Gilbert (2009).

Based on the above evidence, it seems reasonable to conclude that *M. etnieri* has never been particularly common or uniformly distributed throughout the Coosa River basin.

Distributional interaction with *Macrhybopsis boschungi*. *Macrhybopsis etnieri* occurs sympatrically (or syntopically) with *M. boschungi* over an approximately 40-km section (airline distance) of the middle Cahaba River, from Sprott, in Perry County, upstream to about 10 km northeast of Centreville, in Bibb County. No samples of *Macrhybopsis* are known from farther upstream (Mettee *et al.* 1996: 218; Boschung & Mayden 2004: 208), although many fish collections have been obtained from upper reaches of the Cahaba River in Jefferson, Shelby, and St. Clair counties (Boschung & Mayden 2004: 71). The eight mixed collections available for study were taken from late May to early October during the years 1956–1984. None of these collections had individuals that were morphologically intermediate between *M. boschungi* and *M. etnieri*. In seven of the eight samples *M. etnieri* was the dominant form, collectively totaling 92 specimens compared to only 13 *M. boschungi*. The remaining mixed lot of 30 specimens included 20 *M. boschungi*.

Sixteen lots of fishes from within the zone of overlap in the Cahaba River comprised only *M. etnieri.* These varied in size from one to 39 specimens, with most (twelve of sixteen) including fewer than ten individuals. Six additional lots (all small) included only *M. boschungi,* each comprising from one to five specimens. To summarize, a total of 239 specimens of *M. etnieri* and 43 specimens of *M. boschungi* were included in collections from the middle Cahaba River in Bibb and Perry counties. This contrasts with the three lower Cahaba River collections from Dallas County (all west of Selma, about 29 air km south of the Sprott locality in Perry County), which comprise homogenous assemblages (total of 159 specimens) of *M. boschungi*.

Several conclusions can be drawn from the above. First, although the Fall Line provides a precise distributional separation for the two Mobile Basin species of *Macrhybopsis* in the Coosa and Tallapoosa drainages, this break is notably less sharp in the Cahaba River. Second, there is no evidence of gene interchange in any of the mixed samples from the last drainage. Third, within the area of sympatry *M. etnieri* appears to be the dominant form numerically, by a factor of around five to one. Fourth, there appears to be no obvious seasonal separation of the two species within the area of sympatry, mixed collections having been taken during May and June and from August to October. Although the absence of July samples might at first appear meaningful, it should be noted that only two very small samples (total of three specimens) were available from that month. Fifth, upstream distributions of both species in the Cahaba River appear to stop abruptly about 10 km northeast of Centreville. Finally, a complete distributional break between the two species in the Cahaba River occurs somewhere within the 29-km stretch between Sprott and Selma.

Conservation Status. *Macrhybopsis etnieri* has not been accorded any conservation status, probably because it has remained undescribed. Its scattered occurrence in several widely separated parts of the Coosa and Tallapoosa river systems is most likely a function of habitat preference. Other than the presumed collections by Scott (1951) in the mainsteam Coosa River, and three from Hatchet Creek in Coosa County, Alabama (AUM nos. 35067, 41781 and 58687), totaling 125, 43, and 37 specimens, respectively, all samples are of small to moderate size, with the vast majority comprising fewer than ten individuals. Although the species does not appear to be under any immediate threat, populations should continue to be monitored regularly.

Etymology. Named for Dr. David A. Etnier, Emeritus Professor of Zoology at the University of Tennessee, for his many contributions to southeastern ichthyology and aquatic biology, including co-authorship of the definitive book on the fishes of Tennessee.

Macrhybopsis pallida Gilbert & Mayden sp. nov.

Pallid Chub
Figs. 1D, 2; Table 1.

Hybopsis aestivalis.—Suttkus 1961: 233–234 (review of Mississippi fish book; first reported from Florida). Yerger & Suttkus 1962: 327 (additonal Florida records). Smith-Vaniz 1968: 40 (in part; Alabama). Mettee 1970: 11–12 (Choctawhatchee River drainage, Florida and Alabama). Yerger 1978: 12–13 (general account; listed as threatened in Florida; photograph is of *Macrhybopsis etnieri*). Wallace 1980: 180 (in part; brief account; distribution map of *M. aestivalis* complex).

Extrarius n. sp. cf *aestivalis.*—Gilbert & Yerger 1992: 133–137, fig. (general account; Florida distribution map; listed as rare in Florida).

Extrarius aestivalis.—Boschung 1992: 52 (in part; brief discussion of systematics; in list of Alabama fishes).

Macrhybopsis aestivalis.—Mettee *et al.* 1996: 218–219, lower fig. (in part; general account; localities mapped for all species of *M. aestivalis* complex in Alabama).

Macrhybopsis sp. cf. *M. aestivalis* "C".—Boschung & Mayden 2004: 209–210, pl. 21C (diagnosis; distribution; systematics; distribution map in part, Alabama).

Holotype. UF 73313, a 50.5 mm SL female from Choctawhatchee River, at U.S. highway 84 bridge, east of Clayhatchee, Dale and Houston counties, Alabama, T. C. Lewis and H. A. Beecher (field no. HAB 76), 10 January 1975.

Paratypes. The following paratypes, comprising 44 lots and 666 total specimens, are listed here in abbreviated fashion by state, county, and river drainage, followed by museum catalogue number and numbers of specimens. Complete locality data appear in Appendix 1.

Alabama: Escambia River: (Escambia Co.) UF 44666 (68), UF 73320 (10); TU 15948 (6), TU 81354 (9); UAIC 1823.03 (2), UAIC 10855.04 (1 [illustrated female from presumably larger series, *ex* Boschung & Mayden 2004: pl. 21C]).

Blackwater Bay [Yellow River system]: (Covington Co.) TU 73150 (1), TU 72958 (1); UAIC 4188.03 (1).

Choctawhatchee River: (Dale Co.) UF 73336 (29), UF 73488 (2); (Dale/ Houston cos.) UF 73468 (1); (Houston Co.) UAIC 3508.11 (14).

Florida: Escambia River: (Escambia Co.) UF 9333 (11), UF 53534 (2), UF 54366 (4), UF 130433 (8), UF 130470 (1), UF 138618 (2), UF 143879 (4), UF 150156 (1), UF 171841 (3), UF 171848 (9); (Escambia/Santa Rosa cos.) UF 75361 (2), UF 75441 (2), UF 172372 (8); (Santa Rosa Co.) UF 73352 (1), UF 73438 (15), UF 145534 (6), UF 145898 (36).

Blackwater Bay [Yellow River system]: (Okaloosa Co.) UF 57640 (5), TU 79682 (2), TU 82620 (5), TU 83078 (11), TU 101906 (4), TU 102299 (1).

Choctawhatchee River: (Holmes Co.) UF 55457 (21), UF 75477 (1), TU 20811 (28), UAIC 3126.13 (196 originally, now 166), USNM 437191 (5), UMMZ 250265 (5), ANSP 200787 (5), MCZ 171824 (5), KU 41376 (5), UT 44.13095 (5) (six series immediately preceding all *ex* UAIC 3126.13), UAIC 3195.06 (12) UAIC 4449.08 (4), TU 102794 (32); (Washington Co.) UAIC 3191.02 (84).

Diagnosis. A species of the *Macrhybopsis aestivalis* complex, as described in the generic diagnosis. *Macrhybopsis pallida* (Fig. 1D) is characterized by 4-4 pharyngeal teeth; seven anal rays; two pairs of prominent maxillary barbels; anal opening situated midway between pelvic and anal-fin origins; dorsal-fin origin situated directly above pelvic-fin origins; body largely devoid of pigment, the melanophores typically tiny~~;~~ genital papillae well developed.

Other diagnostic characters include a relatively elongate and slender body, with a relatively deep caudal peduncle, the depth of which may be as much as two-thirds the greatest depth of body; pectoral fins in both sexes usually long and pointed, often extending posteriorly to or past pelvic-fin origins; belly immediately anterior to pelvic fins lacking scales; nuptial tubercles on pectoral fins of breeding males uniserial.

Macrhybopsis pallida is one of the most morphologically distinctive members of the *M. aestivalis* complex. It is the only eastern species to possess two pairs of maxillary barbels and seven anal rays, and the intermediate position of the anal opening relative to the origins of the pelvic fins and anal fin is shared only with *M. etnieri* among the nine species comprising the entire species complex.

Description. Characters listed in the Diagnosis are not repeated here, unless additional clarification is required. Variation in meristic characters is presented in Table 1.

Dorsal-fin rays 8; anal-fin rays usually 7 (rarely 6 or 8); pectoral-fin rays usually 14 and often 15 (range 13–16); pelvic-fin rays usually 8 (rarely 7); lateral-line scales usually 36–38 (range 35–39); predorsal scales usually 16–17, often 15–18 (range 14–19); body-circumferential scale rows above and between lateral lines usually 11, occasionally 10 or 12 (range 9–13); body-circumferential scale rows below and between lateral lines incomplete, the scales always missing from mid-belly area anterior to pelvic fins; total caudal-peduncle scale rows uniformly 12 (five scale rows above and below lateral lines on each side of caudal peduncle); total vertebrae usually 37, often 36 or 38; more posterior maxillary barbel longer and more prominent; dorsal fin bluntly pointed at tip; anal fin broadly rounded and never pointed at tip; pectoral fins pointed, sometimes extremely so, often extending posteriorly to or past pelvic-fin origins; head moderately rounded and moderately flattened ventrally; mouth inferior and horizontal, not as wide as head; lips moderately fleshy, not thickened posteriorly; eyes oval in shape and relatively large, the diameter nearly equal to distance from anterior margin of orbit to tip of snout; genital papillae well developed as conical or cylindrical extensions in both sexes; four or five rudimentary gill rakers on upper limb of outer (anteriormost) gill arch, the rakers usually absent from lower limb of arch; pharyngeal teeth short, thin, and hooked, with little or no grinding surface; nuptial tubercles present in membrane immediately posterior to first pectoral-fin ray in nuptial males; pectoral-fin rays 2–10 thickened in nuptial males, and containing large, conical, recurved uniserial tubercles; tiny tubercles sometimes present on rays of dorsal and pelvic fins of high nuptial males.

Females attain a significantly larger size than males. The largest specimen examined is a 51.5 mm SL female (UF 172372) from the Escambia River, at the state highway 4 bridge on the Escambia and Santa Rosa county line, collected on 14 May 1986. Only two other females of comparable size are known: the 50.5 mm holotype (UF 73313), collected on 10 January 1975, and another 50.5 mm specimen (UF 57640), collected on 6 June 1961. The largest male examined, 37.5 mm SL (TU 102299), was collected on 9 June 1977 from the Yellow River, Okaloosa County, Florida. Males with well developed nuptial tubercles have been seen as small as 28.5 mm SL (TU 101906).

Comments. *Macrhybopsis pallid*a does not bear a close physical resemblance to any other eastern members of the *M. aestivalis* species complex, and is unique among eastern species in possessing two pairs of maxillary barbels and seven anal rays, together with an overall pallid appearance, an unusually slender body coupled with a comparatively deep caudal peduncle, and an apparently smaller average body length.

*Macrhybopsis pallid*a shares with *Macrhybopsis australis* the combination of two pairs of maxillary barbels and seven anal rays. However, there is morphological and genetic evidence to indicate that these species are not closely related. *Macrhybopisis pallida* shares, along with *M. etnieri*, an intermediate position of the anus (situated midway between the origins of the pelvic and anal fins) and comparably developed genital papillae. These characters, which are unique among members of the *M. aestivalis* complex, undoubtedly were factors in Eisenhour's assumption of a close relationship between these two species (Eisenhour, 2004: figs. 16–17). Despite this, genetic data (discussed elsewhere in this paper) indicate unequivocally that *M. pallida* and *M. etnieri* are not intimately related, and that the former instead is sister to *M. boschungi*.

Distribution. *Macrhybopsis pallida* is endemic to the Escambia, Blackwater, and Choctawhatchee river drainages of southeastern Alabama and western panhandle Florida. This region is home to at least a dozen endemic fish species (some still undescribed) bearing a close phylogenetic relationship to species occurring in the adjacent Mobile Bay basin of Alabama and Mississippi (Clemmer 1971; Williams 1975; Swift *et al.* 1986; Suttkus & Bailey 1993; Mettee *et al.* 1996, Boschung & Mayden 2004; Pera & Armbruster 2006).

Habitat. *Macrhybopsis pallida* inhabits shallow unimpounded rivers of the western Florida panhandle and adjacent parts of Alabama. Bottoms of these streams are comprised of sand and gravel, combined with occasional small rubble, and water clarity ranges from relatively clear to moderately turbid. In recent years sections of these streams have become impacted by fine silt carried down from upstream, which is presumed to have had a negative impact on populations of this species.

Conservation status. *Macrhybopsis pallida* is an uncommon species, apparently the rarest of the five eastern members of the *M. aestivalis* complex. Although the total number of museum specimens examined (667) is substantial, this figure is misleading in many respects. Bailey *et al.* (1954) did not include the species in their analysis of Escambia River fishes in Alabama and Florida, which, combined with a small number of earlier samples, totaled approximately 30 fish collections available at that time. The first collection to include *M. pallida* appears to have been a series of 11 specimens (UF 9333), collected by William McLane in October, 1954, from the Escambia River near Pine Barren, Florida. Suttkus (1961) was the first to document the existence of the species, and Yerger & Suttkus (1962: 327) were first to record the species from Florida, although the McLane collection was not cited in either of those publications.

Beginning in the 1950's, fish collecting in eastern Gulf slope drainages of Alabama and Florida increased exponentially, and although the number of collections made from this region has not been precisely tabulated, this surely is now well into the hundreds. Considering this, the 44 total collections in which *Macrhybopsis pallida* has been included are not very many. Thirty of these collections comprise fewer than ten individuals, and of the total 667 specimens, 429 have come from seven collections. Breaking this down further, 381 specimens (all young-of-the-year individuals taken during the fall) are from only four collections (UAIC 3126.13, UAIC 3191.02, UF 44666, and UF 145898]).

Further evidence of the species' rarity is evidenced from intensive sampling efforts by United States Geological Service personnel, between 2001 and 2003, in the lower reaches of the Escambia River south of the Florida-Alabama state line. This project was for the specific purpose of determining the current status in Florida of six rare fish species occurring in the drainage, including *Macrhybopsis pallida*. Small-fish sampling was accomplished using 30-foot bag seines with fine-meshed cod ends, with sampling effort equally divided between day and night collections. Stream levels were very low throughout the sampling period, and collecting conditions were ideal. The three most common cyprinid species encountered were *Cyprinella* sp. cf *venusta* and *Notropis texanus* (4,000–5,000 total combined specimens), followed by *Hybopsis* sp. cf *winchelli* (ca. 500 total specimens). Although collected in far fewer numbers, the four dominant percid species occurring in the main-stem Escambia River (*Percina nigrofasciata, Percina austroperca, Percina vigil,* and *Ammocrypta bifascia*) were found in normal concentrations. Only 57 specimens of *Macrhybopsis pallida* (the largest 31.1 mm SL) were obtained in six collections, of which 36 were from one collection (UF 145898) on 30 September 2003. The combination of ideal collecting conditions, effective sampling gear, and relative abundance of other fish species with superficially similar ecological requirements, would lead to the expectation that *M. pallida* should have been found in far greater numbers than it actually was.

It is worth noting that shoal areas where seining collections were made during the 2001–2003 sampling period contained heavy silt loads, which conceivably could have negatively impacted a small, bottom-dwelling species such as *M. pallida*. If so, however, it might have been expected that other benthic species, especially one such as *Ammocrypta bifascia*, would have been equally affected. Also, this does not adequately explain the continued long-term rarity of *M. pallida,* at least during the past 60 years.

Etymology. The species name *pallida* is in reference to the generally pallid body pigmentation characteristic of this species.

Macrhybopsis tomellerii Gilbert & Mayden sp. nov.

Gulf Chub

Figs. 1E, 2; Table 1

Hybopsis aestivalis.—Cook 1959: 129–130 (in part; general account; considered very rare in Mississippi). Douglas 1974: 100–101 (in part; general account; Louisiana distribution map [Lake Pontchartrain and Pearl River drainage records only]; illustration is of *Macrhybopsis hyostoma*).

Extrarius aestivalis.—Mayden 1989: 14 (in part; chub clade; phylogeny based on cranial osteology).

Macrhybopsis aestivalis.—Ross 2001: 177–179 (in part; general account; Mississippi distribution map [Pearl and Pascagoula drainage records only]; photograph).

Macrhybopsis sp. "Mobile chub".—Eisenhour 2004: 37, 47 (in part; phylogenetic tree involving other members of genus).

Macrhybopsis sp. cf *M. aestivalis* "A" (Gulf Chub).—Boschung & Mayden 2004: 209 (general account [refers in part to *Macrhybopsis boschungi*]; color illustration).

Macrhybopsis sp. cf *aestivalis* (Coastal Chub).—Boschung & Mayden 2004: plate 21D (color illustration [also see preceding reference]).

Holotype. UF 28146 (47.1 mm SL female), Pearl River, ca. 0.8 km. south of Sunrise, on unmarked road, T11N, R9E, Secs. 25 and 30, Leake Co. Mississippi, Carter R. Gilbert *et al.,* 22 June 1980.

Paratypes. The following paratypes, comprising 24 lots and 567 total specimens (including nine paratopotypes), are listed here in abbreviated fashion by state, river system, and county, followed by museum catalogue number and numbers of specimens. Complete locality data appear in Appendix 1.

Louisiana: Pearl River: (Washington Psh.) TU 45061 (30, *ex* original lot of 192 specimens), TU 62182 (30, *ex* original lot of 2058 specimens).

Mississippi: Pearl River: (Leake Co.) UF 237859 (9) (paratopotypes, *ex* UF 28146). (Simpson Co.) TU 57271 (30, *ex* original lot of 141 specimens).

Pascagoula River: (Covington Co.) UAIC 11364.01 (1 [illustrated female specimen *ex* Boschung & Mayden 2004: pl. 21D]; present fig. 1E); (Forrest Co.) UAIC 6391.02 (9), UAIC 6392.02 (2), UAIC 6393.02 (5), UAIC 6412.02 (12), UAIC 6413.06 (6). (Jones Co.) INHS 79942 (6), INHS 76268 (81), TU 57445 (63), UT 44.1206 (159). (Lauderdale Co.) UT 44.2317 (20). (Smith Co.) TU 53791 (87 originally, now 57), UF 237864 (5), USNM 437190 (5), UMMZ 250264 (5), ANSP 200786 (5), MCZ 171824 (5), KU 41375 (5) (preceding six lots *ex* TU 53791). (Stone Co.) UAIC 893.02 (3). (Wayne Co.) TU 15395 (17).

Two additional lots (total of 21 specimens), not designated paratypes, from the Lake Pontchartrain drainage of Louisiana, are not included here but are listed in Appendix 1.

Diagnosis. A species in the *Macrhybopsis aestivalis* complex, as described in the generic diagnosis. *Macrhybopsis tomellerii* (Fig. 1E) resembles the allopatrically distributed *M. boschungi* (Fig. 1B), with which it shares a lightly spotted body; a single pair of moderately long maxillary barbels; 4-4 pharyngeal teeth; eight anal rays; anal opening closer to anal-fin origin than pelvic-fin origin (ca. 70 percent of intervening distance); and similar scale counts (usually 36–37 lateral-line scales and 11 rows of scales above and between the lateral lines on opposite sides of body) (Table 1).

The above two species are distinguished by consistent differences in head morphology, most notably a shorter snout in *M. tomellerii* (about equal to postorbital length) versus a longer snout in *M. boschungi,* the length of which exceeds the postorbital length. A second difference (not readily evident from gross examination or in poorly preserved specimens) may be expressed as an imaginary line extending upward from the angle formed by juncture of the lachrymal groove and posterior flap of snout, which in *M. tomellerii* intersects the anterior margin of the nares but in *M. boschungi* runs forward of the nares.

M. tomellerii and *M. boschungi* together bear a superficial resemblance to eastern populations of *M. hyostoma* (Fig. 1A), from which they differ most obviously in having a less heavily spotted body.

M. tomellerii differs from *M. etnieri* (Fig. 1C) in pharyngeal-tooth count (4-4 vs. 1,4-4,1); position of anal opening about two-thirds (70 percent) of distance beweeen pelvic and anal-fin origins (vs. midway); genital papillae extremely reduced; dorsal fin positioned directly above pelvic fins (vs. distinctly posterior); a less heavily spotted body; and longer maxillary barbels.

M. tomellerii differs from *M. pallida* (Fig. 1D) in having a single pair of maxillary barbels (vs. two pairs of barbels); anal rays 8 (vs. 7); position of anal opening about two-thirds (70 perecent) of distance between pelvic and anal-fin origins (vs. midway); a slightly more heavily spotted body (vs. body essentially pallid); and a greater average body size, as discussed in the individual species accounts.

Description. Characters listed in the diagnosis are not repeated here, unless additional clarification is required. Variation in meristic characters is presented in Table 1.

Dorsal rays 8; anal rays 8 (rarely 7); pectoral rays usually 15 (occasionally 14 and often 16); pelvic rays usually 8 (rarely 7); lateral-line scales usually 36–37 (range 35 to 38); body circumferential scale rows above and between lateral lines on opposite sides of body usually 11, occasionally 12 (range 10 to 12); scales never present on belly; predorsal scales irregularly distributed and poorly defined, usually numbering 16 to 20 (rarely 15 or 20); total vertebrae usually 36, sometimes 35 or 37 (very rarely 34).

Dorsal fin angular and slightly falcate, the anteriormost rays (when depressed) extending about same distance posteriorly as posteriormost rays; head moderately rounded dorsally and moderately flattened ventrally; mouth inferior and horizontal, its width about 60 percent of head width; lips moderately fleshy, not thickened posteriorly; eyes oval in shape, the diameter about 60 percent of preorbital distance.

None of the 83 specimens of *M. tomellerii* from which meristic data were taken possessed a complete bridge of scales across the belly, as opposed to a small percentage of the superficially similar *M. boschungi* (eleven of 84 specimens [13 percent] having this feature). In addition, *M. tomellerii* appears to exhibit a slight upward shift in predorsal-scale count and a slight downward shift in total vertebrae count (Table 1).

The largest specimen examined (TU 53791) is a 50.7 mm SL female from the Leaf River, Smith County, Mississippi, collected on 5 October 1960. The largest identified male, 47.5 mm, is from the same collection. This information is presented with some qualification, however, in contrast to that for the other four species in this study. First, the above specimens are from a fall collection made well past the late-spring or early-summer breeding season, which made accurate sex determination less certain than for material collected earlier during the year. Second, the total number of specimens of *M. tomellerii* examined, although comparable to the numbers for other species included in this study, represents only a fraction of the total existing in museum collections (primarily Tulane University). Also, the size difference in sexes reported for this species is less than for the other four eastern species. These factors suggest that information reported here on maximum body size may be subject to modification.

Comments. Based on close similarity in overall appearance and geographically contiguous distributions, *M. tomellerii* and *M. boschungi* were initially thought to be conspecific. Genetic analysis has revealed, however, that the relationship of *M. tomellerii* and *M. boschungi* is not intimate. The latter is sister to the morphologically dissimilar *M. pallida*, whereas *M. tomellerii* does not show an intimate relationship to any other eastern species, including evidence of direct decent from *Macrhybopsis hyostoma* (see section on Relationships and Historical Biogeography).

Detailed comparisons of *M. tomellerii* and *M. hyostoma* populations from the lower Mississippi Valley are clearly in order, especially as regards genetic data. Eisenhour's (1997, 2004) morphological analysis included material of *M. hyostoma* from the lower Mississippi, but his findings cannot be readily correlated with those obtained here for *M. tomellerii*. Eisenhour presumed all eastern Gulf slope populations of "*M. hyostoma*" from Lake Pontchartrain to the lower Mobile Bay basin to be specifically identical, and data from those areas were accordingly combined. Based on overall numbers of distribution records of *M. hyostoma* from the mainstem lower Mississippi River and closely adjacent tributaries (Douglas 1974: 101; Pflieger 1975: 138; Burr & Warren 1986: 89; Robison & Buchanan 1988: 182; Etnier & Starnes 1994: 193; Ross 2001: 178), adequate material should be available for analysis.

Distribution. *Macrhybopsis tomellerii* is confined to the Pearl and Pascagoula river drainages of Mississippi and southeastern Louisiana, and presumably also the adjacent Lake Pontchartrain drainage of these two states (Fig. 2).

Western limits of the geographic range of *Macrhybopsis tomellerii* have yet to be precisely defined. The two samples available for this study from the Lake Pontchartrain drainage (total of 21 specimens [INHS 79456, UF 14731]) are identified as this species, and the records are accordingly plotted on the spot-distribution map. Since genetic information is lacking for material from this drainage, it was considered prudent to exclude these specimens from the list of paratypes.

Habitat. *Macrhybopsis tomellerii* inhabits large, moderately clear to turbid rivers and lowermost parts of their major tributaries in the Pearl, Pascagoula, and Lake Pontchartrain drainages of Mississippi and Louisiana. Conditions in these areas are similar to those favored by the superficially similar *M. boschungi* in lower parts of the adjacent Mobile Bay basin.

Conservation status. *Macrhybopsis tomellerii* has, in years past, sometimes been collected in substantial numbers. Although suitable habitats have undoubtedly been subjected to negative modifications, as yet none are believed to have had dramatic impacts on the overall status of the species. The species should be monitored on a regular basis.

Etymology. Named for Joseph R. Tomelleri, biological illustrator living in Leawood, Kansas, whose unsurpassed and meticulously rendered color illustrations of North American freshwater fishes have graced the pages of numerous scientific publications (including the present one), as well as books such as *Fishes of the Central United States* (Tomelleri & Eberle 1990) and *Fishes of Alabama* (Boschung & Mayden 2004).

Key to species of *Macrhybopsis aestivalis* complex east of Mississippi River

1a. Anal-fin rays usually 7; two pairs of maxillary barbels; dorsal-fin origin directly above pelvic-fin origin; pharyngeal teeth always 4-4; anal opening midway between pelvic and anal-fin origins; body essentially pallid, the melanophores tiny and usually not readily visible; belly scaleless anterior to pelvic fins; pectoral-fin rays in males long and pointed, the outer rays notably longer than innermost rays (fins generally shorter in females) and often extending past pelvic-fin origin; average body size smaller, rarely reaching 50 mm SL, with great majority of specimens (all but six of 667 specimens examined) under 46 mm SL . . . . *Macrhybopsis pallida*
Eastern Gulf slope drainages of western Florida and southeastern Alabama, including Escambia, Blackwater Bay, and Choctawhatchee river drainages

1b. Anal-fin rays usually 8; one pair of maxillary barbels; dorsal-fin origin either directly above or distinctly behind pelvic-fin origin; pharyngeal teeth either 4-4, 1,4-4,1, or some combination thereof; anal opening either midway between pelvic and anal-fin origins or distinctly closer to anal-fin origin; body pigmentation variable, the body rarely if ever pallid and melanophores normally prominent and readily visible; belly squamation variable, ranging from scaleless to an obvious bridge of scales across belly immediately anterior to pelvic-fin origin; pectoral fins in males variable in length (fins generally shorter in females), the outermost rays sometimes distinctly longer than inner rays and the fins more pointed and reaching pelvic-fin origin, at other times the outer rays not notably longer and the fin rounded, falling well short of pelvic-fin origin; average body size larger, occasionally reaching 60 mm SL and often exceeding 50 mm SL 2

2a. Anal opening midway between origins of pelvic and anal fins; number of scale rows above and between lateral lines on either side of body usually 9 or 10, rarely 11; pharyngeal teeth usually 1,4-4,1, occasionally with tooth absent from one or (very rarely) both lesser rows; dorsal-fin origin distinctly posterior to hypothetical vertical line extending upward from pelvic-fin origin (Fig. 1C); belly immediately anterior to pelvic fins usually heavily scaled and forming a bridge of two to five scales across belly; body pigmentation comprising a combination of numerous large and small melanophores, these often coalesced

along mid-side of body to form a poorly defined lateral stripe; pectoral fins short and broadly rounded at tip, seldom if ever extending posteriorly to pelvic-fin origin; snout shorter and blunter, the preorbital length either less, or equal to, postorbital length; total vertebrae usually 38 or 39 (counts averaging higher in headwater areas of Georgia and Tennessee). . . . . *Macrhybopsis etnieri*
Mobile Bay basin; restricted to areas above Fall Line in Cahaba, Tallapoosa and Coosa river systems in Alabama, Georgia and Tennessee; occurs sympatrically with Macrhybopsis boschungi (*with no evidence of hybridization*) *over a 40-km section of middle Cahaba River in Fall Line region of Alabama*

2b. Anal opening distinctly closer to anal-fin origin (about 70 percent of distance) than pelvic-fin origin; number of scale rows above and between lateral lines on either side of body usually 11, occasionally 10 or 12; pharyngeal teeth always 4-4; dorsal-fin origin either directly above, or slightly behind, a hypothetical vertical line extending upward from pelvic-fin origin (Figs. 1A–B, D–E); belly immediately anterior to pelvic fins usually scaleless, occasionally with a bridge of scales (usually no more than one or two scales deep) across belly; body pigmentation variable, with prominent melanophores (*M. hyostoma*) or with smaller and less prominent melanophores (*M. boschungi* and *M. tomelleri*); pectoral fins varying from long and pointed to short and rounded (but if latter never as short as in *M. etnieri*); snout longer and more pointed; total vertebrae usually 35 to 38. 3

3a. Melanophores on upper two-thirds of body large, prominent and randomly distributed (Fig. 1A); posterior part of belly usually scaled (ca. 80 percent of specimens examined); dorsal-fin origin slightly behind a hypothetical vertical line extending upward from pelvic-fin origin (Fig. 1A); pectoral fins of males rounded; total vertebrae usually 37 or 38 . . . . *Macrhybopsis hyostoma*
Tennessee River drainage northward to Ohio River drainage in Illinois east to West Virginia; also occurs throughout lower reaches of western tributaries of Mississippi River and most independent river drainages along western Gulf slope west to Rio Grande; however, characters listed above are not uniformly applicable to all those populations

3b. Melanophores on upper two-thirds of body uniformly smaller and less prominent (Figs. 1B, E); posterior part of belly usually scaleless (over 90 percent of specimens examined); dorsal-fin origin directly above pelvic-fin origin (Figs. 1B, D–E); pectoral fins of males usually less distinctly rounded; total vertebrae usually 35 or 36. 4

4a. Snout longer and more acute, the preorbital distance greater than postorbital distance (Fig. 1B); scales sometimes present on posterior part of belly (in ca. 25 percent of specimens examined). . . . . *Macrhybopsis boschungi*
Larger rivers of Mobile Bay basin in Alabama and Mississippi (*Alabama, Cahaba, Coosa and Tallapoosa*)*, where confined below Fall Line; occurs sympatrically with Macrhybopsis etnieri* (*with no evidence of hybridization*) *over a 40-km section of middle Cahaba River in Fall Line area of Alabama*

4b. Snout shorter and less acute, the preorbital distance equal to postorbital distance (Fig. 1E); scales always absent from belly. . . . . *Macrhybopsis tomellerii*
Pearl and Pascagoula river drainages of Mississippi and Louisiana, and likely also geographically contiguous Lake Pontchartrain system in those two states (status of geographically adjacent populations in lower Mississippi Valley uncertain)

Ecology and life histories. Species of the *Macrhybopsis aestivalis* complex are bottom-dwelling fishes found at shallow to moderate depths of medium-sized to large rivers, usually with a moderate to swift current, a sand or gravel/small-rubble substrate, and varying degrees of water clarity. They rarely occur in smaller streams. This is evident from examination of Wallace's (1980: 180) distribution map of "*Hybopsis aestivalis*," as well as maps appearing in various state ichthyologies (Gerking 1945: 51 [Indiana]; Cleary 1956: 293, map 35 [Iowa]; Douglas 1974: 101 [Louisiana]; Pflieger 1975: 138 [Missouri]; Smith 1979: 76 [Illinois]; Trautman 1981: 289 [Ohio]; Becker 1983: 496 [Wisconsin]; Burr & Warren 1986: 89 [Kentucky]; Robison & Buchanan 1988: 182 [Arkansas]; Sublette *et al.* 1990: 120 [New Mexico]; Gilbert & Yerger 1992: 134 [Florida]; Etnier & Starnes 1994: 193 [Tennessee]; Stauffer *et al.* 1995: 103 [West Virginia]; Mettee *et al.* 1996: 218 [Alabama]; Boschung & Mayden 2004: 208 [Alabama]; and Miller *et al.* 2006: 130 [Mexico]). All but the last two publications appeared prior to dismemberment of the complex into the nine species recognized today, and maps for eight states (Indiana, Iowa, Missouri, Illinois, Ohio, Wisconsin, Kentucky, and West Virginia) are applicable only to the species now referable to *Macrhybopsis hyostoma*.

Although the above ecological statement is broadly applicable to the entire complex, individual species occur under conditions that, at their extremes, may be markedly different. For example, the upper Red River of Oklahoma and Texas, to which *Macrhybopsis australis* is endemic, is characterized by an unstable environment, with widely fluctuating water levels and extremes of turbidity and temperature. By contrast, the Guadalupe-San Marcos drainage, in Texas, to which *M. marconis* is largely restricted, is characterized by a more stable environment, typified mostly by relatively clear water emanating from numerous springs located on the Edwards Plateau. Likewise, habitat of one of the new species herein described (*M. etnieri*) is essentially of an upland nature, characterized by generally clear streams with rubble or gravel bottoms. Ecological conditions under which other members of the complex live are variously intermediate to those described above. Contrasting development of morphological characters in the various species, involving aspects of squamation, body pigmentation, eye size, fin length, and sensory structures, are reflective of these diverse ecological conditions (Eisenhour 2004: figs. 4B & F).

No comprehensive life-history study exists for any member of the *Macrhybopsis aestivalis* complex, and available information tends to be anecdotal rather than quantitative. Most information that does exist appeared prior to the 1990's, all under the species name *aestivalis,* although in very few instances was this actually applicable to that species as presently recognized. In most cases, the species in question was *M. hyostoma*, with Bottrell *et al.'s* (1964) study on *M. tetranema*, from western Oklahoma, the lone exception. Eisenhour (1997: 104), in his summary of the situation, stated: "The morphological variation exhibited by the complex and diversity of habitats occupied implies that life histories may be different among the species. Because life-history information from one species may not apply to others, information is needed for all species."

Probably the most complete ecological synthesis is that by Becker (1983: 496–498), who studied *M. hyostoma* in Wisconsin, at the northernmost range limits of the complex. In addition, he included information from studies by Starrett (1950, 1951) in Iowa, and from various state books (involving the same species) by Trautman (1957, 1981) (Ohio), Cross (1967) (Kansas), and Pflieger (1975) (Missouri). He also cited Bottrell *et al's* (1964) work on *M. tetranema* from western Oklahoma. Subsequent books by Etnier & Starnes (1994: 193) (Tennessee), Stauffer *et al.* (1995) (West Virginia), Ross (2001) (Mississippi), and Boschung & Mayden (2004) (Alabama) include summaries from these earlier works. However, no new information was included in these works that is specifically applicable to any of the four new species described in the present study.

Becker (1983) reported that gravid females 55 and 61 mm total length contained 559 and 796 eggs, respectively. Spawning begins in May or June and continues sporadically through late August, at water temperatures of at least 70°F (21.1°C). It occurs during daylight hours in flowing water, with the eggs being broadcast by the female in deeper parts of the water column. The fertilized eggs range from 0.65–1.0 mm in diameter, are slightly heavier than water, and develop as they drift with the current. Hatching is completed within 24 hours, and the larvae begin feeding 2–3 days later, with most food taken from the bottom or as it falls through the water.

The diet of post-larval individuals consists mostly of immature aquatic insects, primarily dipterans, but may also involve other insect groups. Small amounts of detritus and plant matter may also be consumed incidentally. Aquarium observations by Davis & Miller (1967) indicate that food is taken from the bottom, and is located via the numerous taste buds situated on the underside of the head and maxillary barbels, as well as on the pectoral fins.

Individuals tend to be sedentary, resting quietly on the stream bottom when not moving about in search of food (Pflieger 1975). Trautman (1957, 1981) noted that they usually remain in water 4 ft. deep during the day, and venture into the shallows during dark nights.

Individuals in all cases are short-lived, seldom exceeding more than 1.5 years, and reproduction thus is presumably accomplished mostly by year-old fish. Becker (1983) reported that, in Wisconsin, adult specimens of *M. hyostoma* were seldom captured during the end of September, which implies a decrease in population numbers through widespread mortality. Young-of-the-year individuals begin to appear in numbers around the same time. The short life span characteristic of the complex is exemplified by *M. pallida*, one of the new species described in the present paper and evidently the smallest member of the complex. A total of 667 specimens was examined, of which well over 90 percent were less than 38 mm standard length. Only six specimens (all females) exceeded 45 mm standard length, with the largest 51.5 mm SL. These six individuals were included in four separate collections, five specimens in May, June and July, and the other specimen in January. Based on this evidence, it may be concluded that this species normally only lives one year, with a few females surviving into the second year.

Meristic and morphometric comparisons. Morphological studies of the *Macrhybopsis* species complex by Eisenhour (1997, 1999, 2004) revealed "cryptic" diversity across drainages and species relationships, based on 17 morphological characters derived from body and fin coloration, body shape, tooth count, brain morphology, tuberculation, and other external features. These studies clearly demonstrated the inconsistency between considering this group a single species and the observed morphological divergences. Herein, morphological analyses of populations of the *M. aestivalis* complex from rivers of the Gulf Coastal Plain of the southeastern United States have also revealed previously unknown diversity that heretofore had not been taxonomically described. While divergence of these taxa is present with respect to morphological characters, variation in biochemical traits is also characteristic of these new species (see below).

Eisenhour (1997, 2004: 36, fig. 16) employed principal component analysis of 17 discrete morphological characters in his study of species relationships of *Macrhybopsis*, in which western populations were strongly emphasized. This method of analysis is very useful for examining variation of morphological characters, especially those involving mensural traits. Eisenhour found cases of considerable overlap in ranges of species, along with very clear differences diagnostic to species.

For the *Macrhybopsis aestivalis* complex east of the Mississippi River divergence was observed in both morphometric and meristic characters. Analysis of mensural characters revealed considerable overlap in variation of the species but in some cases species were notably different. Examination confined to female mensural characters displayed little separation between species on sPC2; however, *M. boschungi* and *M. pallida* showed no overlap on sPC3 (Fig. 3). There was a small degree of overlap between *M. pallida* and *M. tomellerii* on sPC3, and even less overlap between *M. pallida* and *M. hyostoma*. *Macrhyposis etnieri* also showed a low to moderate level of overlap with *M. boschungi* and *M. hyostoma*. The sPCA of females from all species loaded heaviest on sPC2 for maxillary barbel length (-0.9561). Anal-fin insertion to lower caudal peduncle (0.36825), anal-fin insertion to upper caudal peduncle (0.32418), and upper jaw length (-0.3841) (Table 2) loaded heaviest on sPC3.

TABLE 2. Loadings of 33 standard and truss measurements taken from female (n = 153) *Macrhybopsis boschungi*, *M. etnieri*, *M. tomellerii, M. hyostoma* and *M. pallida* specimens in sheared principal component analysis.

Measurement	sPC2	sPC3
Standard length	0.04315	0.09438
Snout length	0.04714	-0.3579
Orbital diameter	0.05853	-0.2216
Bony interorbital width	0.0516	-0.0296
Caudal peduncle depth	0.01548	0.14323
Mouth gape	0.03101	-0.2317
Upper jaw length	0.02097	-0.3841
Maxillary barbel length	-0.9561	-0.0507
Dorsal-fin base	0.08961	-0.0543
Anal-fin base	0.05854	-0.1314
Predorsal Length	0.06001	0.01173
Prepelvic length	0.07375	-0.0222
Dorsal origin to anal origin	0.07104	0.012
Dorsal origin to anal insertion	0.0633	-0.0043
Dorsal origin to pelvic origin	0.08887	-0.1473
Dorsal insertion to pelvic origin	0.00495	0.18658
Dorsal insertion to anal origin	0.05399	0.12179
Dorsal insertion to anal insertion	0.0614	0.00913
Dorsal insertion to upper caudal peduncle	0.02572	0.19457
Dorsal insertion to lower caudal peduncle	0.02867	0.17071
Pectoral origin to dorsal origin	0.01649	0.1691
Pectoral origin to pelvic origin	0.07072	0.11661
Pectoral origin to branchiostegal junction	0.02981	-0.0246
Pelvic origin to anal origin	0.05069	0.24739
Pelvic origin to branchiostegal junction	0.06201	0.07203
Anal insertion to upper caudal peduncle	0.03106	0.32418
Anal insertion to lower caudal peduncle	-0.0348	0.36825
Pectoral-fin length	-0.0332	0.07757
Pelvic-fin length	0.00257	-0.0015
Depressed dorsal-fin length	0.04788	-0.0623
Depressed anal-fin length	0.04398	-0.0354
Tip of snout to top of gill slit	0.05686	-0.1583
Tip of snout to branchiostegal junction	0.05291	-0.2119

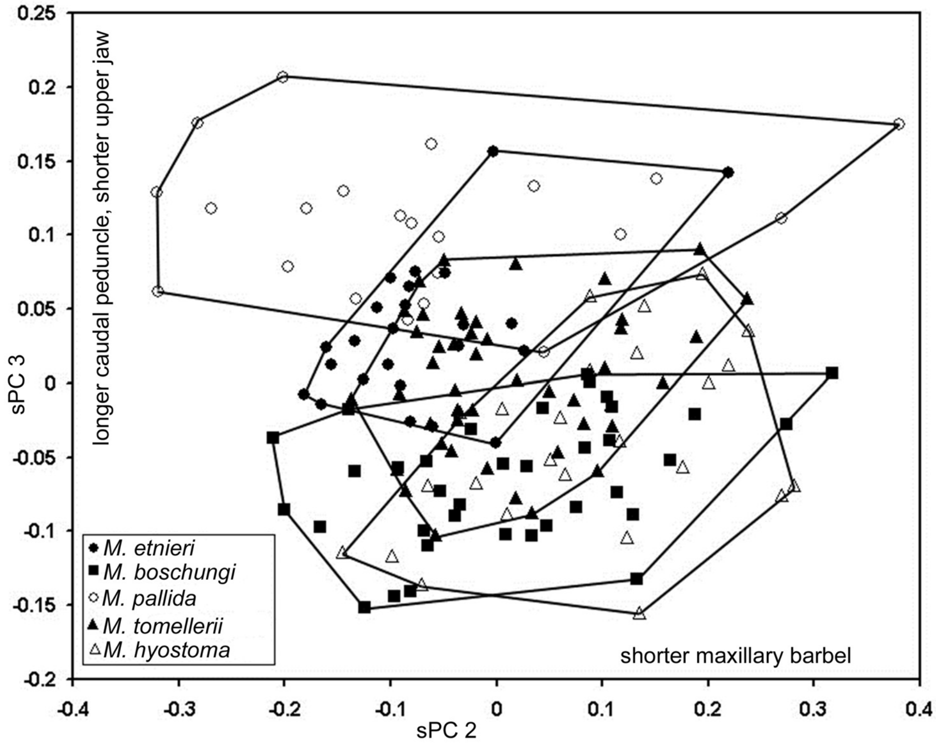


FIGURE 3. Scatterplot of sPC2 and sPC3 from sheared principal components analysis of 33 standard and truss measurements taken from female (n = 153) *Macrhybopsis boschungi*, *M. etnieri*, *M. pallida*, *M. tomellerii* and *M. hyostoma* from Mississippi River drainage.

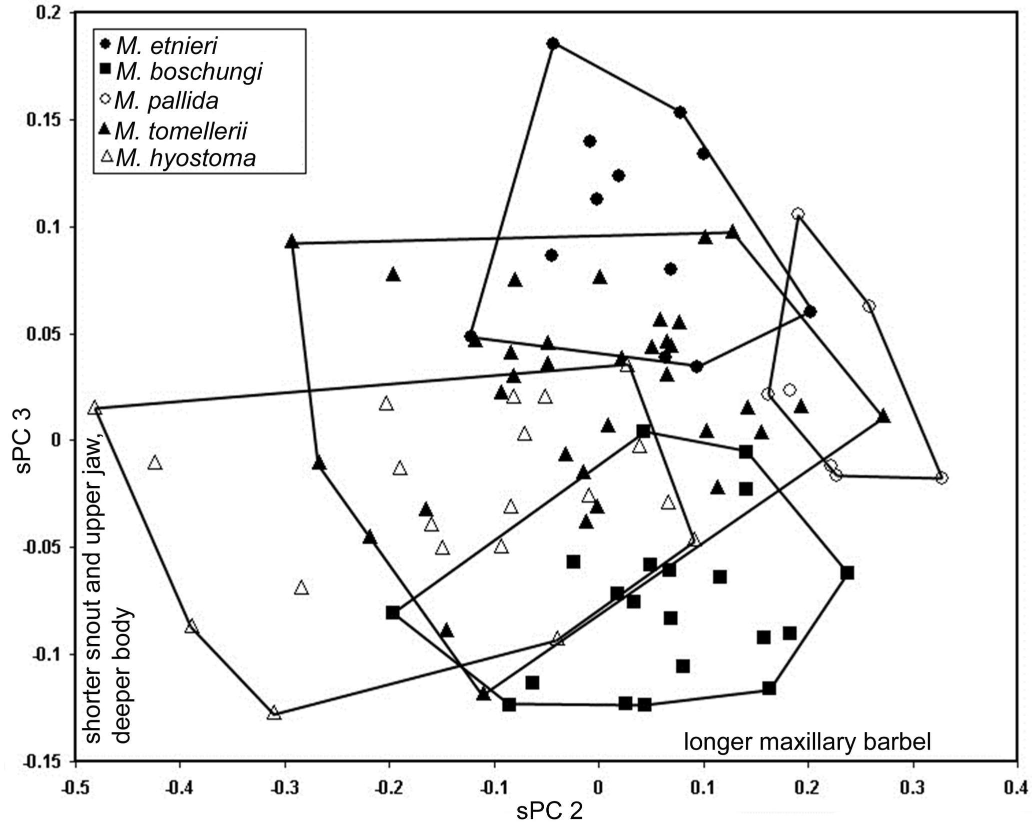


FIGURE 4. Scatterplot of sPC2 and sPC3 from sheared principal components analysis of 33 standard and truss measurements taken from male (n = 93) *Macrhybopsis boschungi*, *M. etnieri*, *M. pallida*, *M. tomellerii*, and *M. hyostoma* from Mississippi River drainage.

Examining only males for all species, maxillary barbel length (0.90754) had the highest loading of all characters in the sPC2 (Table 3). *Macryhybopsis pallida* showed complete separation from *M. hyostoma* and *M. boschungi* on this axis, and there was only slight overlap with *M. etnieri* and *M. tomellerii,* with *M. pallida* having longer maxillary barbels (Fig. 4). In this analysis, sPC3 loaded heaviest for upper jaw length (-0.35313), snout length (-0.42624), and dorsal fin insertion to pelvic fin origin (0.3622) (Table 3). Complete separation of *M. etnieri* from *M. boschungi* and *M. hyostoma* occurred along sPC3, with *M. etnieri* having a generally deeper body and shorter snout and upper jaw (Fig. 4).

TABLE 3. Loadings of 33 standard and truss measurements taken from male (n = 93) *Macrhybopsis boschungi*, *M. etnieri*, *M. tomellerii, M. hyostoma* and *M. pallida* specimens in sheared principal component analysis.

Measurement	sPC2	sPC3
Standard length	-0.06247	0.04751
Snout length	-0.00859	-0.42624
Orbital diameter	-0.09105	-0.13401
Bony interorbital width	-0.12454	0.06137
Caudal peduncle depth	-0.07683	0.10158
Mouth gape	-0.02884	-0.00513
Upper jaw length	0.04114	-0.35313
Maxillary barbel length	0.90754	0.13061
Dorsal-fin base	-0.01631	-0.16812
Anal-fin base	-0.12102	0.03644
Predorsal length	-0.04335	0.04833
Prepelvic length	-0.04128	-0.06855
Dorsal origin to anal origin	-0.04489	-0.07396
Dorsal origin to anal insertion	-0.06291	-0.05894
Dorsal origin to pelvic origin	-0.09589	0.15092
Dorsal insertion to pelvic origin	-0.07506	0.3622
Dorsal insertion to anal origin	-0.07006	0.05082
Dorsal insertion to anal insertion	-0.07404	0.03477
Dorsal insertion to upper caudal peduncle	-0.09017	0.05767
Dorsal insertion to lower caudal peduncle	-0.06459	0.09157
Anal insertion to upper caudal peduncle	-0.09238	0.2507
Anal insertion to lower caudal peduncle	-0.06916	0.21283
Pectoral origin to dorsal origin	-0.0742	0.28849
Pectoral origin to pelvic origin	-0.03846	0.13688
Pectoral origin to branchiostegal junction	-0.05329	-0.12764
Pelvic origin to anal origin	-0.07293	0.16184
Pelvic origin to branchiostegal junction	-0.04334	0.08079
Pectoral-fin length	-0.03138	-0.13231
Pelvic-fin length	-0.01494	-0.08447
Depressed dorsal-fin length	-0.0479	-0.10812
Depressed anal-fin length	-0.07031	0.00281
Tip of snout to top of gill slit	-0.04682	-0.26562
Tip of snout to branchiostegal junction	0.05291	-0.2119

The sPCA analysis of only *Macryhybopsis boschungi* and *M. tomellerii* females also revealed maxillary barbel length loading heavily on sPC2 (-0.9442), but little separation occurred between these species on this axis (Fig. 5). However, sPC3 loaded heavily for pectoral-fin origin to branchiostegal junction (0.50187), snout length (0.3887), and mouth gape (-0.3483) (Table 4) and resulted in moderate separation between the species, suggesting that *M. boschungi* has a generally longer anterior body and snout and smaller mouth than *M. tomellerii.*

The sPCA analysis of only male *Macryhybopsis boschungi* and *M. tomellerii* also loaded heavily on sPC2 for maxillary barbel length (-0.8946), whereas sPC3 loaded heaviest for snout length (-0.4968) and upper jaw length (-0.24776) (Table 5). Moderate separation occurred between these species in the scatterplot of sPC2 and sPC3, with *M. tomellerii* generally having a shorter snout (Fig. 6).

TABLE 4. Loadings of 33 standard and truss measurements taken from female (n = 84) *Macrhybopsis boschungi* and *M. tomellerii* from Gulf Coast drainages in sheared principal component analysis.

Measurement	sPC2	sPC3
Standard length	0.04807	-0.0092
Snout length	0.02668	0.3887
Orbital diameter	0.0128	0.19556
Bony interorbital width	0.08087	-0.0253
Caudal peduncle depth	0.08586	-0.3071
Mouth gape	0.02377	-0.3483
Upper jaw length	0.04353	0.19262
Maxillary barbel length	-0.9442	-0.0505
Dorsal-fin base	0.01874	0.03489
Anal-fin base	-0.0334	0.12592
Predorsal Length	0.06269	0.0172
Prepelvic length	0.06616	0.08942
Dorsal origin to anal origin	0.04632	-0.0367
Dorsal origin to anal insertion	0.03762	0.02433
Dorsal origin to pelvic origin	0.02349	0.00323
Dorsal insertion to pelvic origin	0.08163	-0.1835
Dorsal insertion to anal origin	0.08283	-0.1757
Dorsal insertion to anal insertion	0.02044	-0.1098
Dorsal insertion to upper caudal peduncle	0.05786	-0.0368
Dorsal insertion to lower caudal peduncle	0.06313	-0.1106
Anal insertion to upper caudal peduncle	0.08651	-0.2246
Anal insertion to lower caudal peduncle	0.07639	-0.1181
Pectoral origin to dorsa origin	0.06601	-0.1682
Pectoral origin to pelvic origin	0.08374	-0.0567
Pectoral origin to branchiostegal junction	0.09025	0.50187
Pelvic origin to anal origin	0.05596	-0.0675
Pelvic origin to branchiostegal junction	0.06629	0.04194
Pectoral-fin length	-0.0987	-0.0668
Pelvic-fin length	-0.0458	0.16687
Depressed dorsal-fin length	0.0077	0.10783
Depressed anal-fin length	0.00526	0.04577
Tip of snout to top of gill slit	0.03649	0.16975
Tip of snout to branchiostegal junction	0.00391	0.09074

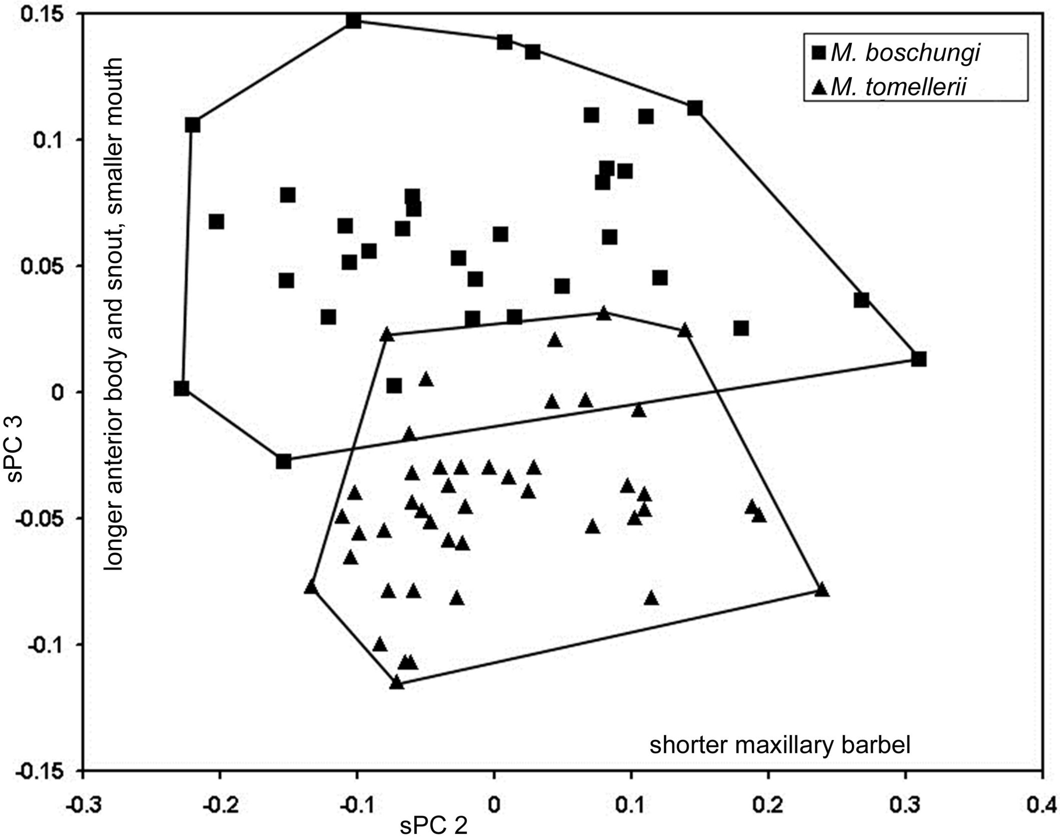


FIGURE 5. Scatterplot of sPC2 and sPC3 from sheared principal components analysis of 33 standard and truss measurements taken from female (n = 84) *Macrhybopsis boschungi* and *M. tomellerii.*

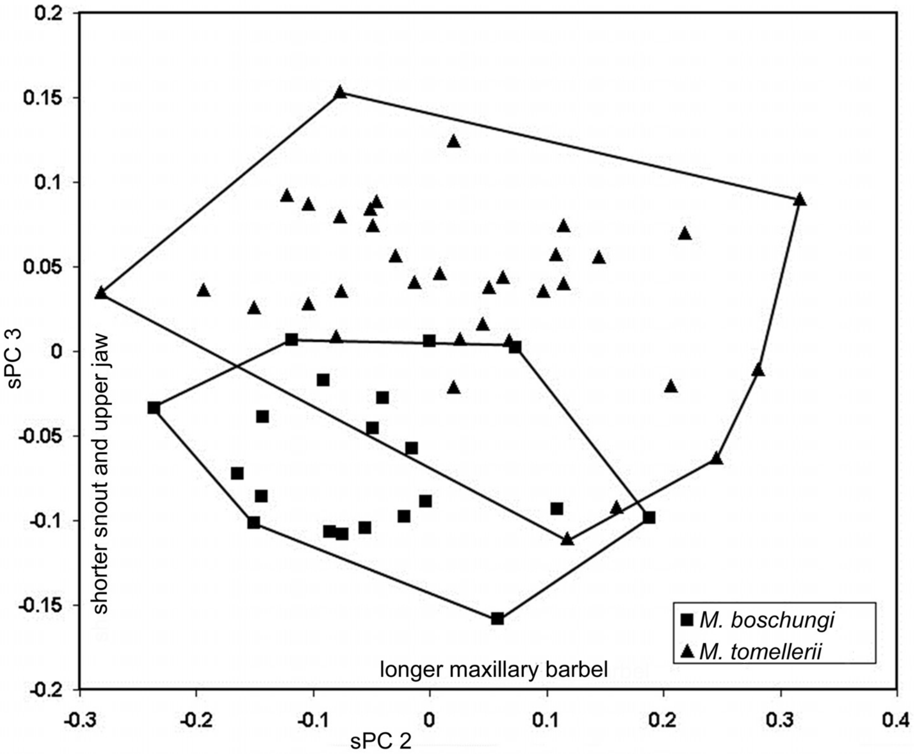


FIGURE 6. Scatterplot of sPC2 and sPC3 from sheared principal components analysis of 33 standard and truss measurements taken from male (n = 55) *Macrhybopsis boschungi* and *M. tomellerii*.

TABLE 5. Loadings of 33 standard and truss measurements taken from male (n = 55) *Macrhybopsis boschungi* and *M. tomellerii* from Gulf Coast drainages in sheared principal component analysis.

Measurement	sPC2	sPC3
Standard length	0.07149	0.05183
Snout length	-0.00432	-0.4968
Orbital diameter	0.07517	-0.11564
Bony interorbital width	0.09734	-0.19283
Caudal peduncle depth	0.13081	0.06845
Mouth gape	0.01313	-0.03243
Upper jaw length	0.04021	-0.24776
Maxillary barbel length	-0.8946	0.20247
Dorsal-fin base	-0.00865	-0.15105
Anal-fin base	0.08285	0.05289
Predorsal length	0.03785	0.01848
Prepelvic length	0.03317	-0.04757
Dorsal origin to anal origin	0.0587	0.0255
Dorsal origin to anal insertion	0.04071	0.00375
Dorsal origin to pelvic origin	0.03299	0.00238
Dorsal insertion to pelvic origin	0.06626	0.25902
Dorsal insertion to anal origin	0.12501	0.18196
Dorsal insertion to anal insertion	0.09739	0.23838
Dorsal insertion to upper caudal peduncle	0.12016	0.1676
Dorsal insertion to lower caudal peduncle	0.10649	0.15686
Anal insertion to upper caudal peduncle	0.15502	0.16704
Anal insertion to lower caudal peduncle	0.15697	0.12255
Pectoral origin to dorsal origin	0.09815	0.17008
Pectoral origin to pelvic origin	0.06816	0.1298
Pectoral origin to branchiostegal junction	0.00353	-0.33915
Pelvic origin to anal origin	0.11052	0.21203
Pelvic origin to branchiostegal junction	0.05928	0.03325
Pectoral-fin length	0.07064	0.12085
Pelvic-fin length	0.04055	-0.05296
Depressed dorsal-fin length	0.01959	-0.07913
Depressed anal-fin length	0.06121	-0.00701
Tip of snout to top of gill slit	0.00156	-0.21316
Tip of snout to branchiostegal junction	0.0084	-0.1771

Meristic characters exhibited disparate levels of variation among species (Table 1). Pelvic-fin rays and dorsal-fin rays showed no modal differences among species, with nearly all specimens having 8 of each. Anal-fin rays showed little variation among *Macryhybopsis hyostoma, M. boschungi, M. tomellerii,* and *M. etnieri*, with all species having modally 8 anal-fin rays. By contrast, *M. pallida* showed a strong modal divergence, with 7 anal-fin rays. Similarly, all species except *M. etnieri* have a 4-4 pharyngeal teeth pattern with no variation. Only two of the 71 *M. etnieri* examined had a 4-4 pattern, with the overwhelming majority displaying a 1,4-4,1 pattern. Predorsal scales varied little among *M. hyostoma, M. boschungi, M. etnieri,* and *M. pallida,* with all having a mode of 16. *Macrhybopsis tomellerii* had 17 predorsal scales, with nearly one-third of the specimens examined having 18 predorsal scales. Despite a limited number of specimens examined for body circumferential scales, *M. etnieri* showed a modal divergence of 23 total body-circumferential scales, with *M. hyostoma* and *M. boschungi* having a mode of 25, while the few *M. pallida* had equally 24 and 25. Total pectoral-fin rays for *M. hyostoma, M. boschungi,* and *M. tomellerii* were modally 15, whereas *M. etnieri* and *M. pallida* had a mode of 14. *Macrhybopsis etnieri* had modally 38 lateral-line scales, whereas *M. hyostoma* and *M. pallida* had modally 37. Equal numbers of *M. tomellerii* had 36 and 37 lateral-line scales, and *M. boschungi* had a mode of 36. *Macrhybopsis boschungi, M. tomellerii,* and *M. pallida* had 36 vertebrae modally, *M. hyostoma* had a mode of 37, and *M. etnieri* a mode of 38. Combined, these characters indicate that *M. etnieri* is the most meristically divergent of these species, with all other species having small modal differences useful for diagnosis.

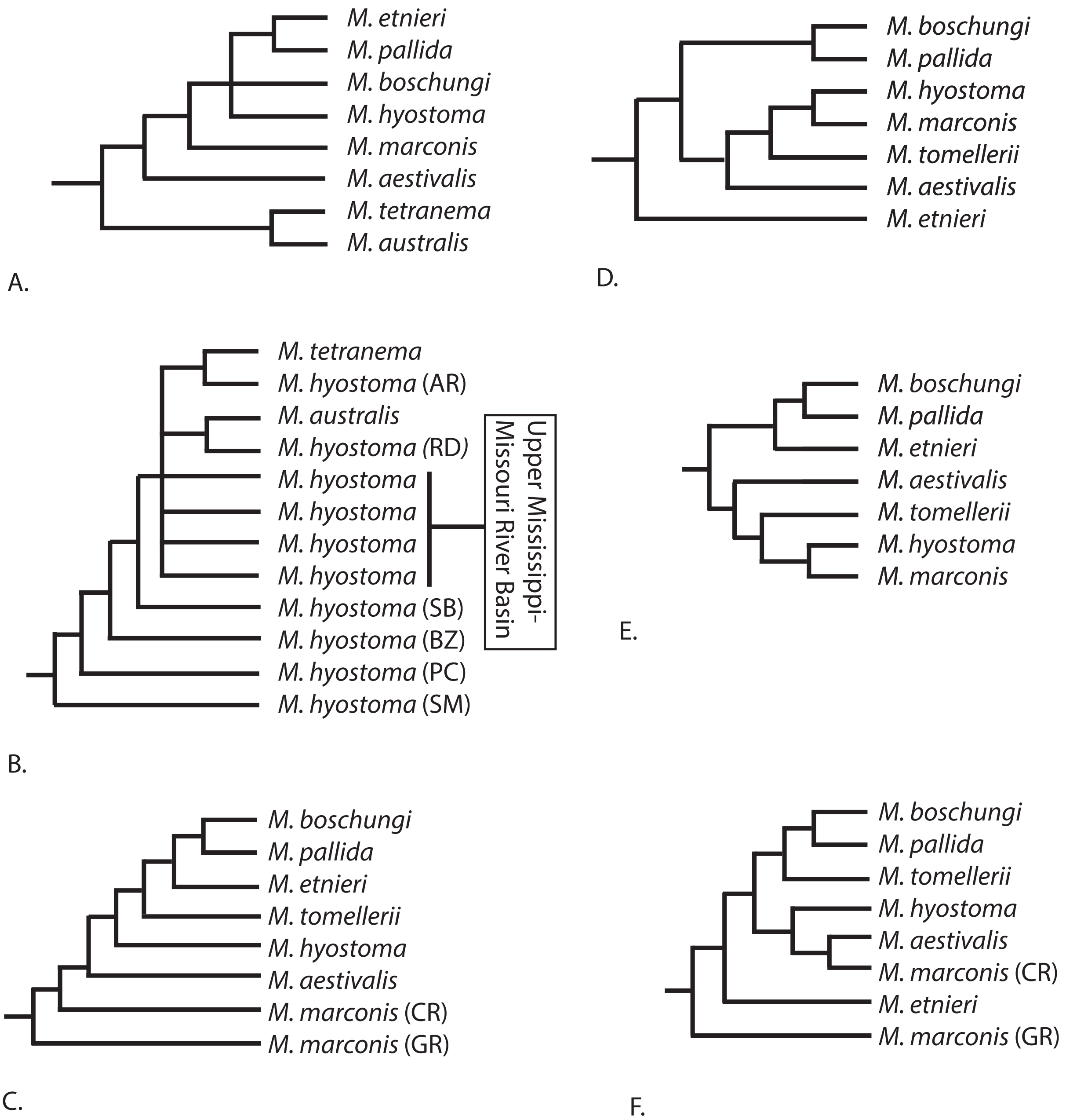


FIGURE 7. Proposed phylogenetic relationships among various species/lineages recognized in the *Macrhybopsis aestivalis* complex of eastern North America. Not all species in the complex were evaluated. One study is based on morphological characters (Eisenhour 2004 [A]), whereas the other two studies are based on allozyme variation. Underwood *et al.* (2003 [B]) focused on western diversity (SB = Sabine, BZ = Brazos, PC = Pecos River, SM = San Marcos River); and Mayden & Powers (2004 [C-G]) mostly involved eastern lineages, excluding Colorado River (CR) and Guadalupe River (GR). In Mayden & Powers (2004) different distances were examined, as well as parsimony: C = Edwards and Cavalli-Sforza Edwards Chord distances; D = Prevosti and Rogers distances; E = Cavalli-Sforza Edwards Arc and Modified Rogers distances; F = Fitch Generalized Parsimony with coded characters.

Genetic analyses. Genetic diversity and relationships in *Macrhybopsis* have been examined in two studies (Underwood *et al.* 2003, Mayden & Powers 2004), focusing on western and eastern diversity, respectively. In addition to examining relationships, the study by Mayden & Powers (2004: tables 1 and 2) (Tables 6 and 7, respectively, in present paper) validated five eastern species as distinct and diagnosable lineages based on allozyme data. Four of these species were recognized as distinct evolutionary lineages, although no formal descriptions were included. Considering that the latter paper appeared in a source unavailable to many, the results of that study are summarized below and in Tables 6–9.

Species diversity in the *Macrhybopsis aestivalis* complex east of the Mississippi River has been viewed as equally complex to that observed west of the Mississippi River (Boschung & Mayden 2004). Although some eastern populations of the *Macrhybopsis aestivalis* complex were examined by Eisenhour (1997, 2004) for comparative purposes, this diversity was not the focus of the latter's investigations, and his analysis did not accurately portray species diversity of *Macrhybopsis* within this area. Rather, in referring to populations of *Macrhybopsis* east of the Mississippi River, Eisenhour (1997, 2004) underrepresented this diversity and referred to the species in this region as *M.* sp. "Coosa Chub," *M.* sp. "Florida Chub," and *M. hyostoma* (Gilbert) or "Shoal Chub." However, Boschung & Mayden (2004: plates 20D, 21A–D), in reference to the current study, more accurately identified diversity within the group from Gulf Coastal rivers east of the Mississippi River. They identified these as *Macrhybopsis aestivalis hyostoma* (Shoal Chub) (now *M. hyostoma*), occurring from the Tennessee drainage northward to the Ohio drainage; *M.* sp. A (Gulf Chub [herein called Mobile Chub] (now *M. boschungi*), endemic to the Mobile Basin below the Fall Line; *M.* sp. B (Coosa Chub) (now *M. etnieri*), endemic to the Mobile Basin above the Fall Line; *M.* sp. C (Pallid Chub) (now *M. pallida*), endemic to Gulf Coastal Plain rivers east of the Mobile Basin from the Escambia to Choctawhatchee River systems in Alabama and Florida; and *M.* sp. D (Coastal Chub) (now *M. tomellerii*), occurring in rivers west of the Mobile Basin to Lake Pontchartrain. In the accompanying text, however, Boschung & Mayden (2004: 209–210) lumped the Gulf and Coastal chubs together under the former name. This contradiction resulted from then ongoing uncertainty regarding specific distinctness of the Gulf (=Mobile) and Coastal chubs.

Although only three of the above eastern Gulf slope species were initially recognized on the basis of morphological divergence (*M.* sp. A–C), investigations into patterns of variation in biochemical traits offered additional evidence for lineage independence and divergence, as well as for reconstruction of phylogenetic relationships. In this section we examine levels of divergence within *Macrhybopsis*, using allozyme electrophoresis among populations of members of the *Macrhybopsis aestivalis* complex (or *M. hyostoma sensu* Eisenhour 2004) across the Gulf Coastal river drainages. Variation in 39 gene loci are used herein to reveal diversity and test for evidence of lineage independence for the morphologically divergent Gulf Coastal species of *Macrhybopsis*, and to examine species relationships among some of the species within the traditionally recognized *M. aestivalis* complex.

The products of 39 allozyme loci were resolved for samples from 11 populations of the *Macrhybopsis aestivalis* complex; six samples were derived from the southeastern members of the *Macrhybopsis aestivalis* complex (*M. boschungi*, *M. etnieri*, *M. pallida*, and *M. tomellerii*), one sample of *M. aestivalis,* two samples of *M. marconis*, two samples of *M. hyostoma*, and the outgroup species *M. storeriana* (Table 6). Fourteen loci (sAcon, Ada-A, sAp-A, Cbp-2, G3pdh-A, mIcdh, sIcdh-A, sIcdh-B, Ldh-C, Pgdh-A, Pep-A, Pep-D, Pep-F, and Sod) were monoallelic for all ingroup and outgroup samples. For the remaining 25 loci, geographic and phylogenetic variation in the ingroup is provided in Tables 6 and 7. Within the ingroup samples the mean number of alleles per locus, proportion of polymorphic loci, and observed and expected mean heterozygosity are provided in Table 7.

Substantial allelic divergence occurred at a number of loci for representative populations or groups of populations across the range of the ingroup (Tables 6–8); some loci and alleles were diagnostic for species from the eastern Gulf Coastal Plain (Table 9). *Macrhybopsis boschungi* and *M. pallida* are differentiated from other members of the *M. aestivalis* complex by fixed allelic variation at Cbp-1; *M. tomellerii* is diagnosed from other taxa by fixed mAat-A allele A. *Macrhybopsis hyostoma* populations share the derived allele B for mAcon-A; *M. aestivalis* shares the derived and unique fixed allele D at locus Pgm-A. *Macrhybopsis marconis* populations from the Guadalupe and Colorado rivers can be diagnosed from one another and other members of the group at locus Gpi-A. *M. marconis* from the Guadalupe River is fixed for allele A, whereas the population from the Colorado River is fixed for allele B. No significant variation was noted within the multiple populations sampled of *M. hyostoma* or *M. etnieri*.

TABLE 6. Allozyme variation in the southeastern *Macrhybopsis aestivalis* species complex.[1] From Mayden & Powers (2004); reproduced with permission.

	Macrhybopsis etnieri			*Macrhybopsis boschungi*	*Macrhybopsis pallida*	*Macrhybopsis tomellerii*
Locus	Cahaba River I	Cahaba River II	Etowah River	Cahaba River	Conecuh River	Leaf River
mAat-A	BB:26	BB:02	BB:08	BB:32 BC:01	AB:01 BB:21	AA:25
sAat-A	DD:12 DE:05 EE:03	DD:06 DE:05 EE:02	DD:08	DD:33 DE:01	DD:21 DE:01	DD:24 DE:01
mAcon-A	CC:28	CC:02	CC:08	CC:11 CD:14 DD:09	CC:22	CC:25
Acp-1	AA:28	AA:22	AA:08	AA:31 AB:03	AA:22	AA:09 AB:15 BB:01
Ak-A	AA:28	AA:22	AA:08	AA:34	AA:22	AA:25
Ald-A	BB:28	BB:22	BB:08	BB:34	BB:22	BB:25
Cbp-1	BB:28	BB:22	BB:08	AA:34	AA:22	BB:25
Ck-A	BB:28	BB:05	BB:08	BB:34	BB:22	BB:16 BC:05 CC:04
Est-1	DD:27 DE:01	DD:22	DD:08	CE:02 CC:02 CD:05 DD:12 DE:06 EE:07	DD:22	BD:01 DD:24
Fum-A	AA:28	AA:22	AA:08	AA:34	AA:22	AA:25
G6pdh-A	AA:03 AB:03 BB:22	BB:22	BB:08	AB:01 BB:31 BC:01 BD:01	AB:01 BB:19 BC:01	BB:25
Gpi-A	AA:20 AB:07 BB:01	BB:22	AA:04 AB:03 BB:01	AB:01 BB:33	BB:22	AA:01 BB:24
Gpi-B	BB:28	BB:22	BB:08	BB:26 AB:05 AA:02 BC:01	AB:02 BB:20	BB:25

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TABLE 6. (Continued)

	Macrhybopsis etnieri			*Macrhybopsis boschungi*	*Macrhybopsis pallida*	*Macrhybopsis tomellerii*
Locus	Cahaba River I	Cahaba River II	Etowah River	Cahaba River	Conecuh River	Leaf River
Ldh-A	CC:28	CC:22	CC:08	CC:34	CC:22	CC:25
Ldh-B	CC:27 BC:01	CC:22	CC:08	BB:14 CC:02 BC:18	CC:22	CC:25
sMdh-A	CC:28	CC:22	CC:08	CC:33 AC:01	CC:22	AC:07 CC:18
mMdh-B	BB:28	BB:22	BB:08	BB:34	BB:22	BB:25
mMdh-A	CC:28	CC:22	CC:08	CC:34	BC:01 CC:21	AC:01 CC:24
sMdhp-A	CC:01 CD:12 DD:13	CC:01 CD:08 DD:08	CC:08	CC:34	BB:01 CC:21	CC:25
mMdhp-A	AB:01 BB:27	BB:15	BB:04 BD:04	CC:30 CB:02 BB:02	BB:22	BB:25
Mpi-A	CC:17 CD:11	CC:04 CD:03 DD:01	BC:02 CC:06	CC:34	BC:02 CC:20	CC:24 CD:01
Pep-B	BB:25 CC:01 BC:01	BB:22	BB:08	BB:34	BB:22	BB:25
Pgm-A	CC:22	CC:10	CC:08	AB:01 BB:26 BC:02 AC:01 AA:02 CC:01	BC:10 CC:07 BB:04	BB:04 BC:03 CC:18
Tpi-A	BB:20 AB:04 AA:04	AA:05 AB:05 BB:12	BB:03 AB:03 AA:02	BB:34	BB:18 AB:03 AA:01	BB:25
Tpi-B	CC:28	CC:22	CC:08	CC:33 BC:01	BB:01 BC:01 CC:20	BB:01

……*continued on the next page*

TABLE 6. (Continued)

	Macrhybopsis hyostoma		*Macrhybopsis aestivalis*	*Macrhybopsis marconis*		*Macrhybopsis storeriana*
Locus	Mississippi River	Kansas River	Rio Grande	Colorado River	Guadalupe River	
mAat-A	AA:12	AA:24	AA:08	AA:12	AA:24 DD:01	AA:20
sAat-A	BD:02 DD:10	CD:01 DD:22	DD:08	DD:12	AA:02 AD:03 DD:18 BD:01	DD:19 DF:01
mAcon	BB:12	BB:24	CC:08	CC:12	AA:01 CC:24	AA:20
Acp-1	AA:04 AB:04 BB:04	AA:17 AB:05	AA:03 AB:03 BB:02	BB:12	AA:01 AB:02 BB:22	AA:20
Ak-A	AA:11 AB:01	AA:24	AA:08	AA:12	AA:25	AA:20
Ald-A	BB:12	BB:24	BB:08	BB:12	BB:25	AA:20
Cbp-1	BB:12	BB:24	BB:08	BB:12	AA:01 BB:24	AA:20
Ck-A	CC:12	CC:24	BB:08	BB:12	AA:01 BB:24	BB:20
Est-1	EE:12	EE:24	DD:08	BB:11 AB:01	DD:25	DD:20
Fum-A	AA:12	AA:24	AA:08	AA:12	AA:25	BB:20
G6pdh-A	BB:12	BB:24	BB:03 BD:03 DD:02	BB:12	AA:01 BB:24	AA:01 BB:19
Gpi-A	BB:12	BB:24	BB:08	BB:12	AA:25	AA:20
Gpi-B	BB:12	BC:07 BB:14 CC:03	BB:07 BC:01	BB:06 BC:05 CC:01	CC:22 BB:02 BC:01	BB:17 CC:02 BC:01
Ldh-A	AA:01 AC:03 CC:08	AA:02 AC:10 CC:12	CC:08	CC:12	BB:01 CC:24	CC:20

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TABLE 6. (Continued)

Locus	*Macrhybopsis hyostoma* Mississippi River	*Macrhybopsis hyostoma* Kansas River	*Macrhybopsis aestivalis* Rio Grande	*Macrhybopsis marconis* Colorado River	*Macrhybopsis marconis* Guadalupe River	*Macrhybopsis storeriana*
Ldh-B	CC:12	CC:24	CC:08	CC:12	AA:01 CC:24	CC:20
sMdh-A	CC:12	CC:24	CC:08	CC:12	AA:01 CC:24	BB:20
mMdh-A	BC:02 CC:10	AC:03 BC:02 CC:19	CC:08	CC:12	CC:24 DD:01	CC:20
mMdh-B	BB:12	BB:24	BB:08	BB:12	AA:01 BB:24	AA:20
sMdhp-A	CC:12	CC:24	CC:08	AC:04 CC:07 AA:01	CC:24 DD:01	DD:20
mMdhp-A	BB:02 BD:04 DD:06	DD:10 CC:13	BB:08	BB:12	BB:01 BE:01 EE:23	AA:02 BB:18
Mpi-A	CC:12	BC:01 CC:23	CC:08	AA:12	AA:23 CC:02	BB:16 BC:04
Pep-B	BB:12	BB:23 AD:01	BB:08	BB:12	BB:24 BE:01	BB:20
Pgm-A	CC:07	CC:24	DD:08	CC:12	CC:22 BB:01 BC:01	CC:20
Tpi-A	BB:12	BB:22 BC:01	BB:05 BC:03	BB:12	BB:24 AA:01	AA:20
Tpi-B	CC:11 CD:01	BC:01 CC:21 CD:02	CC:08	CC:12	AA:01 CC:24	AA:20

[1] Classification of newly-described species follows morphological studies in present paper; classification of other species follows revisionary studies by Eisenhour (1997, 1999, 2004).

Significant genetic heterogeneity occurred at 22 of 25 polyallelic loci for the combined 33 samples of the ingroup ($X2 = 12219.390$, 744 df, $P < 0.001$). All loci contributed significantly to this measure of differentiation except Ak-A, mMdh-A, and Pep-B. Most genetic variation occurred as a result of within and among species subdivision, as evidenced by significant genetic heterogeneity measures and the high F_{IT} and F_{ST} values. All two or more composite species comparisons (Table 8) suggested very high subdivision among samples and genetic divergence (F_{IT} = 0.630–0.803; F_{ST} = 0.557–731); heterogeneity due to geographic isolation among samples was also extremely high (Table 8). For all ingroup populations the high F_{ST} was indicative of high levels of divergence within this complex (comparison 1; F_{ST} = 0.731). F_{ST} values were also very high for comparisons limited to the undescribed species from eastern Gulf Coastal Plain rivers, further supporting the existence of diversity based on genetic heterogeneity across independent lineages (comparisons 2, 3, 4).

TABLE 7. Genetic variability at 25 loci in all populations of the *Macrhybopsis aestivalis* complex examined in this study (standard errors in parentheses). From Mayden & Powers (2004); reproduced with permission.

Population	Mean sample size per Locus	Mean no. of alleles per locus	Percentage of loci polymorphic[1]	Mean heterozygosity	
				Directcount	HdyWbg expected[2]
Macrhybopsis etnieri					
Cahaba River (I)	27.2	1.4	28.0	0.070	0.090
	(0.4)	(0.1)		(0.026)	(0.029)
Cahaba River (II)	17.8	1.2	16.0	0.058	0.073
	(1.4)	(0.1)		(0.028)	(0.034)
Etowah River	8.0	1.2	16.0	0.060	0.065
	(0.0)	(0.1)		(0.029)	(0.032)
Macrhybopsis boschungi	33.9	1.7	24.0	0.080	0.104
	(0.1)	(0.2)		(0.029)	(0.036)
Macrhybopsis pallida	21.9	1.4	12.0	0.043	0.053
	(0.1)	(0.1)		(0.020)	(0.022)
Macrhybopsis tomellerii	24.0	1.4	16.0	0.054	0.067
	(1.0)	(0.1)		(0.027)	(0.027)
Macrhybopsis hyostoma					
Mississippi River	11.8	1.3	20.0	0.062	0.073
	(0.2)	(0.1)		(0.023)	(0.029)
Kansas River	23.8	1.5	24.0	0.058	0.082
	(0.1)	(0.1)		(0.022)	(0.030)
Macrhybopsis aestivalis	8.0	1.2	16.0	0.050	0.060
Rio Grande	(0.0)	(0.1)		(0.025)	(0.031)
Macrhybopsis marconis					
Colorado River	12.0	1.1	8.0	0.033	0.036
	(0.0)	(0.1)		(0.021)	(0.023)
Guadalupe River	24.9	1.8	24.0	0.016	0.083
	(0.1)	(0.1)		(0.008)	(0.013)

[1] A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95

[2] Unbiased estimate (see Nei, 1978)

TABLE 8. Summary of F-statistics for 25 polyallelic loci within the *Macrhybopsis aestivalis* species group and, in one instance an outgroup taxon, *Macrhybopsis storeriana*. From Mayden & Powers (2004); reproduced with permission.

Species/Populations	FIS	FIT	FST
1. *M. aestivalis* species group	0.232	0.794	0.731
2. *M. aestivalis, M. boschungi, M. etnieri, M. pallida*	0.165	0.637	0.566
3. *M. aestivalis*, *M. boschungi, M. pallida*	0.163	0.630	0.557
4. All but *M. aestivalis, M. boschungi, M. etnieri, M. pallida* & *M. storeriana*	0.297	0.803	0.720

Population genetics of samples examined herein from some members of the *Macrhybopsis aestivalis* species group have revealed patterns of allozyme variation confirming a history of isolation and differentiation for these species. The *M. aestivalis* complex east of the Mississippi River is hypothesized to consist of five species, including *M. boschungi*, *M. etnieri*, *M. pallida*, *M. tomellerii*, and *M. hyostoma*. It is predicted that additional sampling and analyses of morphological and/or biochemical/molecular characters for more populations of *M. hyostoma* will reveal additional species diversity. The highly significant levels of heterogeneity at 22 of 25 polymorphic loci and extremely high F_{ST} for the collection of populations traditionally recognized as *M. aestivalis,* and even among populations across the southeastern Gulf Coastal rivers, clearly supports the recognition of multiple independent lineages (Tables 6–9). Evidence is also presented for *M. marconis* possibly representing a complex of more than one species. These data corroborate the hypothesized morphological divergence within the group. These data, in concert with phylogenetic hypotheses, argue that *M. aestivalis* east of the Mississippi River is not a single evolutionary lineage or species *sensu* the Evolutionary Species Concept (Wiley & Mayden 2000a, b, c; Mayden 1997, 1999) and diagnosable species *sensu* the Phylogenetic Species Concept (Mayden & Wood 1995; Mayden 1997, 1999). Thus, recognizing *M. aestivalis* as a single species is a poor reflection of existing evolutionary diversity within this complex.

TABLE 9. Diagnostic loci and alleles for southeastern Gulf Coast species of the *Macrhybopsis aestivalis* species complex. For each locus in a cell, the allele(s) presented before the "vs" is characteristic of the taxon in the row and the allele(s) after the "vs" is characteristic of the taxon of the column. From Mayden & Powers (2004);_reproduced with permission.

Species	*M. boschungi* (A)	*M. etnieri* (B)	*M. pallida* (C)
M. etnieri (B)	Cbp-1 (B vs. A) Pgm-A (C vs. A, B, C) Tpi-A (AB vs. B)		
M. pallida (C)	Pgm-A (BC vs. C)	Cbp-1 (A vs. B)	
M. tomellerii (D).	mAat-A (A vs. B) Ck-A (BC vs. B) Cbp-1 (A vs. B) Pgm-A (BC vs. C)	mAat-A (A vs. B) Ck-A (BC vs. B) Tpi-A (B vs. AB) Tpi-B (B vs. C)	mAat-A (A vs. B) Ck-A (BC vs. B) Cbp-1 (B vs. A)

Relationships and historical biogeography. In comparisons of all hypotheses of relationships of the *Macrhybopsis* species complex not all taxa have ever been examined in one study, and although there are several consistent patterns of descent there are also incongruencies. Thus, the historical biogeography of co-distributed species and geological hypotheses of ancestral rivers and sea level shifts provide important information in formulating biogeographic hypotheses below. Although the relationships of species in the *Macrhybopsis aestivalis* complex remain partially unresolved, the evolution of the group is thought to have occurred over three time sequences (i.e., epochs), each of which is associated with pronounced changes in sea levels. These include the mid to late Miocene (>5 million years BP), characterized by consistently low sea levels; Pliocene (5 to 2 million years BP), with consistently high levels; and Pleistocene (<2 million years BP), involving a combination of widely fluctuating sea levels (Vail & Hardenbol 1979; Gilbert 1987: 37, fig. 5). The Miocene is viewed here as a time of faunal dispersal, the Pliocene as a time of differentiation and consolidation, and the Pleistocene as including a combination of both.

Phylogenetic relationships. Relationships of species of *Macrhybopsis* were examined by Eisenhour (2004: 36,: fig. 16), based on 17 discrete morphological traits. Genetic diversity and relationships in *Macrhybopsis* were examined in two studies (Underwood *et al.* 2003, Mayden & Powers 2004), focusing on western and eastern diversity, respectively. Minimal overlap in taxa exists between these latter studies. The phylogenetic relationships generated by Underwood *et al.* (2003: 496, fig. 3) provide some evidence of taxonomic complexities within the *Macrhybopsis aestivalis* species complex, specifically with regard to populations of *M. hyostoma* west of the Mississippi River in the Red, Arkansas, and Brazos rivers and the upper Mississippi and Missouri rivers. Populations of *M. hyostoma* inhabiting the Red and Arkansas rivers were genetically more similar to the respective endemics, *M. australis* and *M. tetranema,* than to other populations of *M. hyostoma* from the upper Mississippi and Missouri rivers. Those authors considered the three species as distinct, however, and discussed their independence and likely explanations for shared alleles. Populations from the Missouri, upper and middle Mississippi, and Brazos rivers were resolved in the gene tree as multiple independent lineages. Paraphyletic gene trees of these populations of *M. hyostoma* may be indicative of an unnatural grouping recognized under one taxonomic name. Further research is required to resolve this pattern of lineage diversification. With respect to the potential gene flow between *M. tetranema* and *M. australis* with *M. hyostoma*, an alternative hypothesis not considered by those authors is that the genetic variation maintained in some populations represents an earlier history of "ancestral gene flow," perhaps associated with divergence events of the two endemics from a "*hyostoma*"-like ancestor, with the pattern of genetic variation being maintained by local stabilizing selection. *M. hyostoma* from the Brazos and Sabine river drainages were basal in this analysis, as was *M. aestivalis* from the Pecos and *M. marconis* from the San Marcos-Guadalupe rivers.

Mayden & Powers (2004) used four distance measures in distance Wagner analyses of biochemical variation that resulted in three different topologies (Fig. 7). Resolved relationships were identical between Edwards and CS&E chord distances (Fig. 7C), Prevosti and Rogers distances (Fig. 7D), and CS&E arc and Modified Rogers (Fig. 7E). However, all three topologies shared certain relationships. In all three sets of relationships *Macrhybopsis boschungi* and *Macrhybopsis pallida* were resolved as sister taxa; in three of the four trees *Macrhybopsis etnieri* formed the sister group to this clade. In the fourth topology *Macrhybopsis etnieri* formed the sister group to all other members of the *Macrhybopsis* species complex. In all topologies, *Macrhybopsis hyostoma* from the Mississippi and Kansas rivers formed a monophyletic group exclusive of any populations of *Macrhybopsis* from rivers of the Gulf Coastal Plain. *Macrhybopsis tomellerii* did not share any evidence of descent with *Macrhybopsis hyostoma,* but was either sister to other southeastern species plus *M. aestivalis,* or was sister to *M. marconis* plus *M. hyostoma*. *Macrhybopsis marconis* formed a monophyletic group in topologies derived from Prevosti and Rogers (Fig. 7D) and CS&E arc (Fig. 7C), but was represented as a basal paraphyletic assemblage in both Edwards and CS&E chord topologies (Fig. 7C). Finally, *Macrhybopsis aestivalis* either formed the sister group to a clade inclusive of *Macrhybopsis boschungi, M. etnieri*, and *M. pallida,* or was the sister group to *Macrhybopsis tomellerii* plus *M. hyostoma* and *M. marconis* (Figs. 7C, E). From these analyses, Mayden & Powers (2004) gave preference to Prevosti and Rogers distances (Fig. 7D).

Phylogenetic analysis of allozyme variation coded as discrete characters yielded topologies with some similarities and differences with distance Wagner trees. Fitch parsimony yielded a single topology with poor resolution of species relationships (Fig. 7F). In this tree most of the taxa of the *Macrhybopsis* species complex formed an unresolved basal polytomy; *M. hyostoma* formed a monophyletic group, *Macrhybopsis boschungi* and *Macrhybopsis pallida* formed a clade, and samples of *Macrhybopsis etnieri* from the Cahaba River formed a clade. The use of stepmatrix or generalized parsimony resulted in a single, more fully resolved phylogeny (Fig. 7F). In this topology, *M. marconis* was not monophyletic, with the population from the Colorado River forming the sister group to *M. aestivalis* and the population from the Guadalupe River forming the basal sister group to all members of the *M. aestivalis* species group. *Macrhybopsis etnieri* formed a monophyletic group. *Macrhybopsis tomellerii* formed the sister group to a clade inclusive to *Macrhybopsis boschungi* plus *Macrhybopsis pallida*. This clade was sister to a clade inclusive of a monophyletic *M. hyostoma* (separate from *Macrhybopsis tomellerii*), *M. aestivalis*, and *M. marconis* from the Colorado River.

Eisenhour (2004) discussed speciation in the complex and favored a peripheral isolation model, proposing that *Macrhybopsis hyostoma* was the common ancestor to all other species of the complex. This pattern of speciation would be supported only if there were a polychotomous resolution of relationships and if *M. hyostoma* possessed no apomorphies. Relationships of this nature were not found in any analysis, except in a Fitch Parsimony analysis of discrete coding of allozyme variation (Mayden & Powers 2004), but in this analysis the two samples of *M. hyostoma* possessed autapomorphic alleles. All other trees from multiple analyses present dichotomous relationships, thus precluding the hypothesis that *M. hyostoma,* as currently understood, was the common ancestor to all other taxa (expected polychotomous tree). However, Eisenhour's hypothesis of peripheral isolation or vicariance, and the hypothesis that *M. tetranema, M. australis, M. marconis*, and *M. aestivalis* diverged in their present geographical locations, following some period of previous ancestral dispersal, is very plausible and can be considered consistent with all trees resolved for the group. Divergence of *M. marconis* and *M. aestivalis* would have necessarily predated the divergence of *M. tetranema* and *M. australis* (Underwood *et al.* 2003), also falsifying the hypothesis of a "*hyostoma*"-like widespread ancestor. The possibility that *M. australis* and *M. tetranema* diverged from an ancestral form similar to *M. hyostoma* and the evidence of genetic mixing still remains a viable hypothesis. Other populations of *M. hyostoma* from the upper Mississippi and Missouri rivers are unresolved. Further detailed analysis of populations of the complex referred to as *M. hyostoma* is warranted.

Biogeography. The late Miocene was characterized by a prolonged period of low sea levels (Vail *et al.* 1977; Vail & Hardenbol 1979: 77, fig. 8; also see Gilbert 1987: 37, fig. 5) (Fig. 8). Continental margins extended outward toward the edges of the continental shelf, which resulted in the interconnection of many previously independent river drainages in their lower reaches, including those entering the Gulf of Mexico. Given the associated habitat of members of the *M. aestivalis* complex and the anastomosed connections in the lower reaches of these rivers, this is hypothesized to have provided opportunities for multiple lateral transfers of various aquatic taxa. It would have allowed fishes and other aquatic organisms, including an early ancestral form of the *M. aestivalis* complex (thought to resemble *M. hyostoma*), to disperse both east and west along the Gulf Slope to the Mobile Bay basin and Rio Grande, respectively, from a presumed ancestral range in the centrally-located Mississippi River basin, where *M. hyostoma* currently resides.

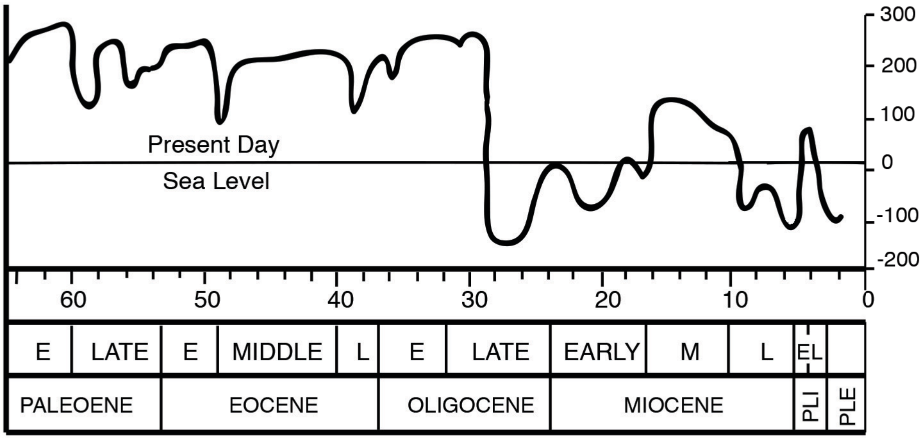

FIGURE 8. Tertiary eustatic changes in sea level. Meters above or below present sea level are tentative. From Vail & Hardenbol (1979); reproduced in Gilbert (1987: 37, fig. 5).

An example of this late-Miocene period of low sea levels was the extended isolation and eventual desiccation of the Mediterranean Sea and the geographically proximate Black Sea (Hsű 1972, 1978). The subsequent Pliocene epoch, which persisted around 3 million years, was characterized by consistently elevated sea levels at least 30 m higher than today (Vail & Hardenbol 1979: 77, fig. 8; also see Gilbert 1987: 35, fig. 4). During the Miocene-Pliocene transition, the rising Atlantic Ocean began to spill into the empty Mediterranean basin at its western end, thus creating what must have been a spectacular marine waterfall of extended duration.

The Pliocene rise in sea level (Fig. 8) would have had a significant effect on drainage connections along the coasts of North America and led to a long period of isolation, also promoting speciation (see Nagel & Simons 2012). This rise in sea level resulted in the disarticulation of many previously interconnected river drainages entering the northern Gulf of Mexico and elsewhere, and is hypothesized to have led to the isolation and eventual differentiation of taxa in the aquatic faunas. This isolation of drainages during this period is believed to have been the fundamental event, or series of events, that resulted in the divergence of at least three or four eastern Gulf Slope species of *Macrhybopsis*, as well as members from coastal drainages west of the Mississippi River.

The Pleistocene epoch was characterized by periodic changes in sea levels (Fig. 8). This was precipitated by glaciation in the northern hemisphere, and consisted of at least four major glacial advances and retreats. Each glacial advance was accompanied by a concordant drop in world sea levels at least 100 meters lower than present (Anderson *et al.* 2004; Greene *et al.* 2007; Anderson 2011; Williams 2011). Each of the glacial advances and periods of lowered sea level is thought to have reestablished lowland riverine conditions and the possibility for renewed exchanges in the lowermost portions of basins.

Biogeographers have long been aware of the relationships of sea-level changes to fish distributions, but generally speaking such studies have focused mostly on late Pleistocene (i.e., post Wisconsin) events (Swift *et al.* 1986: 220–221). Recent unpublished information (Anderson 2011; Williams 2011), which dealt mostly with Pleistocene events in the northeastern Gulf of Mexico, has served to reawaken interest in the subject. The hypothesis is that sea-level changes dating well back in geological time have been of utmost significance in the evolution and distributions of Gulf Coast freshwater fish faunas, including the *Macrhybopsis aestivalis* complex. Although sea-level changes are hypothesized as being the major factor associated with distribution and evolution of this species complex, other geological processes have also been involved, including headwater stream captures and lateral stream transfers, specifically as regards eastern species such as *Macrhybopsis etnieri* and the western species *M. tetranema* and *M. australis*.

Our preferred evolutionary hypotheses for the evolution and biogeography of this group are ones that are repeated by multiple other co-distributed species across a diverse range of taxa. These hypotheses are derived from the allozyme study by Mayden & Powers (2004) and include the tree based on Prevosti distance (Fig. 7D). However, other trees provide equally important information that may vary in a biogeographic history only in the timings of divergences of isolated populations. Despite its apparent sister relationship to other species of the *Macrhybopsis aestivalis* complex, *M. etnieri* does not appear to have played a major role in evolution of the remainder of the group. Rather, divergence in the remaining species seems to be centered around a fish likely similar to *M. hyostoma*. Based partly on its broad and centralized geographic distribution, as well as commonality of morphological characters relative to other members of the complex (see section involving diagnosis and description of *M. hyostoma*), Eisenhour (1997: 38) presumed *M. hyostoma* to be the group's most basal and widespread species, which could have served as an ancestral stock to other species through some peripheral isolation events (allopatric model H [(Wiley 1981; Wiley & Mayden 1985]).

The deep divergence and old basal sister group relationship between *M. etnieri* and remaining species of the complex is a pattern that is repeated in many fish groups and is discussed below under "Upper Coosa-Tennessee connection." In all but one of the phylogenies presented by Mayden & Powers (2004), the relationship between *M. boschungi* and *M. pallida* is a consistently repeated pattern. The one notable exception is the phylogeny based on morphology put forth by Eisenhour (2004: 36–37, fig. 16; Fig. 7A in present paper), in which *M. pallida* and *M. etnieri* were proposed as sister species. (This supposition is believed to have been based largely, if not entirely, on the shared presence of an intermediate position of the genital opening and well developed genital papillae). *Macrhybopsis boschungi* and *M. tomellerii* were considered by Eisenhour (2004) to be eastern populations of *M. hyostoma,* which in turn was regarded by him as most closely related to the western species *M. marconis* and *M. aestivalis.* Underwood *et al.* (2003) identified similar genotypes involving many western Gulf Slope populations of *M. hyostoma*, plus *M. aestivalis* and *M. marconis*. Support for the relationships of these western lineages is poor and they could be considered a polytomy and thus consistent with other hypotheses outlined by Eisenhour (2004) or Mayden & Powers (2004). An eastern and western Gulf slope pattern of relationships is also observed in a number of fish clades that are co-distributed with the four species east of the Mississippi River and at least two species west of the Mississippi River, plus *M. hyostoma* in the Mississippi and Missouri rivers. This pattern of relationships is discussed below under "Eastern and Western Gulf Slope".

Upper Coosa-Tennessee connection. Generalized parsimony analysis of allozyme data by Mayden & Powers (2004), together with morphological evidence presented elsewhere in this paper, provide support for a sister relationship between *Macrhybopsis etnieri* and remaining members of the *aestivalis* complex. *Macrhybopsis etnieri* is restricted to areas above the Fall Line in eastern tributaries of Mobile Bay (Cahaba, Coosa, and Tallapoosa river systems), where its distribution is similar to other Mobile basin endemic fishes, including *Hybopsis lineapunctata, Phenacobius catostomus, Lythrurus lirus, Luxilus c. chrysocephalus, Etheostoma rupestre, E. jordani* species group*, Cyprinella gibbsi* and *C. trichroistia,* and *Fundulus bifax* and *F. stellifer*. Each of these species, species pairs, or subspecies is closely related to populations/taxa in the geographically contiguous Tennessee River drainage and, in some cases, other areas of the Mississippi River basin. These include *Hybopsis amblops*, *Phenacobius uranops*, *Lythrurus lirus, Luxilus c. chrysocephalus***,** *Ethestoma blennioides, E. acuticeps,* and *Fundulus catenatus.* The *Cyprinella gibbsi and C. trichroistia* clade is a basal sister group to all other species in the genus, supporting the great age-early divergence of the *M. etnieri* lineage (Broughton & Gold 2000, Schönhuth & Mayden 2010).

These examples suggest a former well-established distributional pathway between the two drainages in times past, likely involving a large river as opposed to a short-term stream capture event. The connection involved the "Appalachian River" that existed well into the Miocene, would have included the present-day upper Tennessee and Coosa rivers, and is hypothesized to have extended southward to the Gulf of Mexico (Mayden 1988: 341, fig. 4). The lower section of the present-day Tennessee River was at that time greatly reduced in size, and extended upstream only as far as northern Alabama and south-central Tennessee. Subsequent headwater cutting of the Tennessee River resulted in capture of the upper portion of the Appalachian River (including the Coosa River) in the vicinity of Waldens Ridge, near present-day Chattanooga, Tennessee (Starnes & Etnier 1986: 332, fig. 10.2; Boschung & Mayden 2004: 35). Later, following geological uplifting in this Tennessee-Coosa contact area, the Coosa was disconnected and experienced a reversal of flow, to become reconnected with what today is the eastern part of the upper Mobile Bay basin, the Alabama River drainage.

Eastern and Western Gulf Slope. During the late Miocene, which was characterized by significantly low sea levels, an ancestral form, likely similar to the wide-ranging "*M. hyostoma,*" could have extended its range west and east of the Mississippi River from the central Mississippi River valley (separate from events associated with the Upper Coosa-Tennessee connection). Westward movement, through anastomosing lower ends of coastal rivers, extended to the Rio Grande (including major upstream drainages such as the Pecos and Conchos rivers) and perhaps also the independent Rio San Fernando, immediately to the south.

Divergence in the combined rios San Fernando and Grande likely occurred later, during the Pliocene-Pleistocene, when river drainages became isolated with elevated sea levels. Another segment of the complex became isolated in the geographically intermediate Colorado and/or Guadalupe-San Antonio river systems, where it evolved into *M. marconis*. In some trees appearing in Mayden & Powers (2004) it is clear that the Guadalupe and Colorado rivers are genetically distinct; however, the distant relationship depicted in some trees is not considered a viable hypotheses here. Divergences of lineages along the Gulf Coast of Texas and Louisiana warrants additional attention.

Macrhybopsis populations were simultaneously undergoing eastward dispersal from the Mississippi Valley. Evidence suggests that the Mobile Bay basin likely was the eastern terminus of this early dispersal, based on the sizeable number of coastal endemics with widespread northern congeners throughout extensive areas of the Mississippi River basin. Included among these are (northern relatives appear in parentheses) *Scaphirhynchus suttkusi* (*S. platorynchus*) (Willliams & Clemmer 1991), *Noturus munitus* (*N. stigmosus*) (Taylor 1969), *Notropis amplamala* (*N. buccatus*) (Pera & Armbruster 2006), *Cycleptus meridionalis* (*C. elongatus*) (Burr & Mayden 1999), and *Ammocrypta meridiana* (*A. pellucida*) (Williams 1975). The ancestral species to *M. boschungi, M. pallida*, and *M. tomellerii* is hypothesized to have reached the lowland habitats below the Fall Line at this time. For reasons discussed below, the highly endemic fish fauna occurring in smaller coastal drainages east of Mobile Bay is believed to be of subsequent (i.e., Pleistocene) origin.

The presence of separate species of *Macrhybopsis* in the Mobile basin (*M. boschungi*) and the adjacent combined Pearl and Pascagoula river drainages (*M. tomellerii*) invites speculation. These species are sufficiently similar in outward appearance that they might logically be considered sister species, with *M. tomellerii* the end result of a segment of the long established *M. boschungi* that likely had reached its present home and evolved during an early Pleistocene low-water stage. Surprisingly, however, the genetic data do not indicate a sister relationship, which suggests that the biogeographic history of *M. tomellerii*, at least, is more complicated than first appears. It should be noted that these comparative distributions are mirrored by the percid species *Percina brevicauda* (Mobile Bay basin) and *P. aurora* (Pearl-Pascagoula drainage) (Suttkus *et al.* 1994), with the ancestral *Percina copelandi* being widespread in rivers of the mid to upper Mississippi Valley and in several eastern Great Lakes drainages (Gilbert & Burgess 1980). Although the similar geographic distributions of these two groups of fishes would seem to suggest similar biogeographic histories, confirmation based on genetic analysis of the *Percina* species has yet to be attempted.

The remaining eastern Gulf slope species, *Macrhybopsis pallida*, is endemic to the Escambia, Blackwater, and Choctawhatchee river drainages of western panhandle Florida and southeastern Alabama. It is one of the most morphologically distinct members of the genus, and, despite marked differences in outward appearance, genetic analysis identifies it as sister to *M. boschungi.* This Mobile Bay-eastern Gulf coast faunal relationship is repeated many times, as demonstrated by the following examples (Mobile Bay form listed first): *Hybopsis winchelli-H.* cf *winchelli*, *Notropis longirostris-N.* cf *longirostris*, *Lythrurus roseipinnis-L. atrapiculus*, *Ammocrypta beanii–A. bifascia, Etheostoma chlorosoma-E. davisoni*, *Etheostoma ramseyi-E. colorosum, Percina suttkusi-P. austroperca,* and *Micropterus punctulatus-M.* cf *punctulatus*. Other than the two species of *Macrhybopsis,* all the above species pairs are very similar morphologically, and the *Notropis longirostris* pair can be distinguished only on the basis of genetics. At least ten other widespread species with distributions comparable to the above appear not to have undergone species differentiation. This group includes *Moxostoma carinatum, Moxostoma poecilurum,* *Hybognathus hayi, Noturus funebris, Fundulus blairae, Crystallaria asprella, Etheostoma histrio, Etheostoma proeliare, Etheostoma stigmaeum* and *Percina vigil*. It is hypothesized that the above eastern Gulf coast species have common biogeographic histories, all having arrived from the Mobile basin during one or more Pleistocene low-sea stands.

During glacial maxima, rivers arising from glacial runoff were characterized by substantially reduced size and flow. The Mississippi River itself was much smaller and presumably much clearer than today, which in turn permitted temporary habitation by species not normally found there. This is evidenced by the presence today of notably isolated populations of various northern species in the generally smaller, clearer west-flowing tributaries of the Mississippi River in southwestern Mississippi, well south of their normal ranges. These include *Chrosomus erythrogaster* (Cashner *et al.* 1979; Starnes & Starnes 1980; Cross *et al.* 1986: 384, fig. 11.5; Ross 2001: 211, map 80)*, Moxostoma erythrurum* (Jenkins 1980c; Ross 2001: 288, map 106), *Fundulus catenatus* (Bart & Cashner 1980; Shute, 1980; Ross, 2001: 354, map 137)*,* and *Etheostoma caeruleum* (Stauffer & Hocutt 1980; Ross 2001: 461, map 191). Assuming these reflect natural occurrences, these species could only have reached here by downstream movement through the Mississippi River during times when ecological conditions were dramatically different from those existing today.

Glacial retreat was not a continuous process, but instead was punctuated by intermittent periods of advances and retreats. This led to periodic changes in drainage patterns and stream flow (Mayden 1985: fig. 1; Wiley & Mayden 1985: fig. 22; Cross *et al.* 1986: fig. 11.3), which resulted in temporary and unpredictable periods of isolation for resident populations of species such as *M. hyostoma*. These would have been of sufficient duration to permit some degree of genetic change, but apparently not long enough to allow taxonomic differentiation as independent lineages; however, much remains to be studied in *M. hyostoma* in terms of genetic and morphological diversity and potential independent lineages.

Western diversification. The upper Red and Arkansas rivers are considered to have once formed the headwaters of the preglacial "Plains Stream," which flowed southward to the Gulf of Mexico in eastern Texas through the valley occupied today by the adjacent Neches and Trinity rivers (Mayden 1988: 341, fig. 4) (i.e., the Galveston Bay drainage). The upper portions of this prehistoric river are hypothesized to have been beheaded and captured by the present-day Red and Arkansas rivers (Quinn 1958), along with the respective resident populations of *Macrhybopsis*, which upon further geographic separation and isolation evolved into *M. australis* and *M. tetranema*, respectively. This above scenario seems plausible, except that no member of the *M. aestivalis* complex is known today from the Galveston Bay drainage (Conner & Suttkus 1986: 419; Eisenhour 2004: 30, fig. 11). Adding further confusion are the findings of Underwood *et al.* (2003: 496, fig. 3) suggesting that *M. australis* and *M. tetranema* both show strong genetic ties to downstream populations in the Red and Arkansas rivers, respectively, which are currently assigned the species name *hyostoma*. The situation described above leaves major unanswered questions, for which there are at the moment no suitable answers.

Macrhybopsis hyostoma. The concluding part of this evolutionary chronology centers around *Macrhybopsis hyostoma*, which, as presently conceived, is one of the most morphologically variable of any eastern North American freshwater fish species. Populations east of the Mississippi River appear to show little variation, in marked contrast to those in western drainages.

The unusual morphological complexity characteristic of trans-Mississippi populations of *Macrhybopsis hyostoma* is here hypothesized to be a combination of pre-Pleistocene evolutionary processes, together with subsequent long-term instability and changes in stream-drainage patterns and flow regimes related to periodic advances and retreats of the Pleistocene ice sheets (Mayden 1985: figs. 1, 5B–C; Wiley & Mayden 1985: fig. 22; Cross *et al.* 1986: fig. 11.3).

Eisenhour (1997) presented a detailed analysis of meristic and morphometric characters for western populations of *M. hyostoma,* which later appeared in published form (Eisenhour 2004). Among his most notable findings was that populations from the lower Red and Arkansas river drainages are unusual in having a very high percentage of individuals with two pairs of maxillary barbels, which may range from 76–90 percent of individuals examined from the former drainage and 90–100 percent from the latter (Eisenhour 1997: table 44). This condition is also found in varying degrees in some other western populations to the north, most notably the Platte and Illinois rivers, where up to 27 percent of specimens examined exhibited this feature. An added pair of barbels is unknown for any eastern populations of *M. hyostoma*, and they were also absent from all specimens examined by Eisenhour (1997) from the two most southern populations of the species (Colorado and Brazos river drainages, in Texas).

The unusual morphological variation cited above for western populations of *Macrhybopsis hyostoma* has been a major contributing factor to the taxonomic uncertainty long associated with the entire *M. aestivalis* complex. The problem was further enhanced by the presence of two morphologically distinct taxa, *M. australis* and *M. tetranema*, from the upper Red and Arkansas river drainages, respectively, which differ in a seemingly confusing and enigmatic manner from *Macrhybopsis* populations downstream. Eisenhour's studies have gone far toward resolving these issues. Sheared PCA analyses revealed near-complete morphometric separation of upstream and downstream populations in both these drainages (Eisenhour 1999: fig. 5; 2004: fig. 10), as well as high degrees of meristic separation (*op. cit.* 1999: fig. 4; 2004: fig. 12). Equally important are examples of sympatry and syntopy in both drainages (*op. cit*., 1999: fig. 3; 2004: fig. 11), with only occasional introgression (*op. cit.* 1999: 973; 2004: 31). As discussed elsewhere, the genetic findings of Underwood *et al.* (2003) have confirmed that *M. australis* and *M. tetranema* are sister species, and that together they also have a close relationship to the respective downstream populations of *M. hyostoma*. In addition, Eisenhour (2004: 37, fig. 11) reported examples of sympatry, with no evidence of gene interchange, involving *M. hyostoma* and another member of the complex (*M. marconis*) in the lower Colorado River drainage of Texas.

During glacial maxima, the southern limits of the ice sheets were in close proximity to the northern boundary of the Mississippi embayment, which resulted in a narrow bottleneck that served to isolate highland faunas to the east and west (Mayden 1985: figs. 5B–C; Burr & Page 1986: 291, fig. 9.2; Mayden 1987: fig. 3). As glaciers subsequently retreated, repopulation of previously glaciated areas was possible. However, the newly exposed areas were often unsuitable for these upland forms, which resulted in varying degrees of evolution (sometimes to species) of these now isolated eastern and western populations (Wiley & Mayden 1985; Mayden 1987: figs. 7, 9; *et al*). Examples include cyprinid fishes such as *Notropis percobromus* (Wood *et al.* 2002; Berendzen *et al.* 2008; Berendzen *et al.* 2009), *Notropis nubilus* (Berendzen *et al.* 2010), and *Hybopsis amblops* (Berendzen *et al.* 2008); we well as the percid species *Percina evides* (Near *et al.* 2001) and *Etheostoma caeruleum* (Ray *et al.* 2006). Although northern populations of *M. hyostoma* were likewise temporarily extirpated during these glacial advances, the species is not basically an upland inhabitant, and western and eastern populations thus did not become similarly isolated.

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We also acknowledge and thank Joseph R. Tomelleri for permission to reproduce his superlative illustrations of the five species treated in this paper, which originally appeared in the book *Fishes of Alabama* (Boschung & Mayden 2004). In recognition of this, one of the new species herein described (*Macrhybopsis tomellerii*) is named in his honor.

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(along with Dr. Mayden) of the landmark book *Fishes of Alabama* (Boschung & Mayden 2004). This book is notable, in addition to its sheer size, for the superlative color illustrations (all by Tomelleri), carefully constructed distribution maps, and detailed taxonomic accounts. It was the culmination of Bo's long ichthyological career, and sets the standard for all past and future state and regional ichthyological works. One of the new species described in the present paper (*Macrhybopsis boschungi*) is named in his honor, and it is to be regretted that he did not live long enough to witness its formal description.

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APPENDIX 1. Material examined.

Ranges of length refer to standard length. Sex of specimens not indicated.

Macrhybopsis hyostoma (non types unless indicated otherwise) (43 lots; 409 specimens)

Area of coverage includes only that part of overall range (as delineated by Eisenhour, 2004) east of Mississippi River, from Tennessee River drainage, in northern Alabama, northward to Ohio River drainage in West Virginia and Ohio, and west to the Wabash River, bordering Indiana and Illinois.

The following collections are from within this area of coverage, as follows: Tennessee River drainage, Alabama (Limestone Co.) and Tennessee (Anderson, Cocke, Claiborne, Decatur, Giles, Humphreys, Knox, Loudon, Unicoi, Washington, and Wayne cos.); Cumberland River drainage, Tennessee (Davidson, Jackson, and Pickett cos.). Following are river systems within Ohio River drainage: Green River, Kentucky (Warren Co.); Kentucky River, Kentucky (Owsley Co.); Licking River, Kentucky (Bath, Nicholas, and Rowan cos.); Muskingum River, Ohio (Washington Co.); Kanawha River, West Virginia (Kanawha Co.); Wabash River, Indiana (Carroll, Sullivan, and Vigo cos.); White River, Indiana (Lawrence and Owen cos.).

Additional localities under names *Hybopsis aestivalis, Macrhybopsis aestivalis,* or *Macrhybopsis aestivalis* complex for Alabama are plotted in Mettee et al. (1996: 218) and Boschung and Mayden, 2004: 208), for Tennessee in Etnier and Starnes (1994: 193), for Kentucky in Burr and Warren (1986: 89), for West Virginia in Stauffer et al. (1995: 103),), for Ohio in Trautman (1981: 289), and, under name *Extrarius aestivalis hyostomus,* for Indiana in Gerking (1945: 51).

Alabama:

Limestone Co.: UAIC 6220.01 (26, 24.3–54.5), Elk R., at Mason Id. (river mi. 31.3), 2 June 1980.

Indiana:

Wabash River collections sometimes extend across river into Illinois, but only Indiana locality data are listed.

Carroll Co.: USNM 39616 (1, 43.9), Wabash R., at Delphi, summer 1884. **Lawrence Co**.: USNM 34980 (6, 44.0–47.4), White R., near Bedford, 16 June 1884 **(syntypes)**. **Owen Co.:** USNM 36495 (1, 44.6), White R., at Gosport, summer, 1884. **Sullivan Co.:** UT 44.2000 (5, 42.3–48.2), Wabash R., at Riverview riffle, 1.7 mi. below Breed power plant, 22 June 1978. UF 78630 (1, 30.4), UF 78676 (4, 37.4–43.1), Wabash R., near York (river mi. 178), 1 September 1988. UF 78655 (15, 32.7–43.3), Wabash R., at York (river mi. 176). near York, 22 September 1988. UF 78588 (7, 37.2–46.8), Wabash R., near Riverview (river mile 182), 22 September 1988 **Sullivan Co**.: UF 78374 (7, 21.8–47.0), Wabash R., vicinity of Breed power plant, ca. 30 mi. downstream from Terre Haute (borders Vigo Co.), 1988. **Vigo Co.:** UF 78419 (13, 32.5–55.1), Wabash R., vicinity of Darwin, Illinois (river miles 190–192), 21 September 1988. UF 78715 (6, 19.9–53.5), UF 78761 (1, 36.5), Wabash R., 3 mi. upstream from Terre Haute, near Wabash River power plant (river mile 218), 22 September 1988. UF 78847 (1, 25.2), Wabash R., 4 mi. upstream from Terre Haute, below mouth of Otter Creek (river mile 221), 1988.

Kentucky:

Bath Co.: INHS 88045 (14, 39.4–54.0), Licking R., at Moore's Ferry, 30 September 1982. UF 43877 (1, 40.8), Licking R., ¼ mi. S of jct. with U.S. hwy. 60, beside st rt. 801 (borders Rowan Co.), 7 September 1969. USNM 230467 (25, 38.0–52.), same locality as preceding, 17 June 1970. **Nicholas Co**.: UMMZ 171441 (7 originally [now 6], 21.8–36.8), UF 7388 (*ex* UMMZ 171441) (1), Licking R., on st. hwy. 32, at jct. of Cassady Cr., 12 September 1955. **Owsley Co.:** UMMZ 168876 (3, 41.0–42.5), Redbird Cr., at mouth of Sexton Cr., 9 mi. N of Oneida, 14 September 1954. UF 44016 (1, 38.8), S. Fk. of Kentucky R., at Boonesville, 13 August 1972. **Warren Co**.: UT 44.1511 (36, 41.0–61.3), Barren R., at Claypool, 5 August 1977.

Ohio:

Washington Co.: UMMZ 87766 (6, 25.2–45.5), Muskingum R., Dam 3 at Lowell, 28 September 1929. UMMZ 107288 (4, 33.7–41.0), Muskingum R., Dam 2, west-central Muskingum twp., 28–29 June 1930.

Tennessee:

Anderson Co.: USNM 36623 (1, 46.0), Clinch R., at Clinton, summer 1884. **Cocke Co.:** UF 24249 (1, 49.4), Nolichucky R., 3 mi. S of Beulah, at Steele Id. (river mile 19.7; on Greene Co. boundary), 9 July 1977. **Claiborne Co.:** UMMZ 158355 (4, 29.7–43.9), Powell R., 3 mi. SE of Harrogate, at U.S. hwy. 25E bridge, 8 November 1939. **Davidson Co.:** USNM 36840 (3, all 44.6), Stones R., E of Nashville, summer 1884. **Decatur Co.:** UT 44.690 (146, 23.0–39.0), Tennessee R., at head of Eagle Nest Island, 5 August 1977. **Giles Co.:** UT 44.2162 (5, 44.3–53.2), Elk R., at Whitfield Id., ¾ mi. above co. rd. 6234 bridge, 2 June 1980. **Humphreys Co**.: UF 24114 (1, 38.2), Duck R., at and above mouth of Hurricane Cr., ca. 3 air mi. NW of Hurricane Mills, 11 July 1977. UT 44.957 (3, 22.0–34.2), same locality as preceding, 1974. UT 44.1635 (3, 44.2–48.5), Duck R., at end of unnumbered co. rd., ca. 3.4 air mi. N of Buffalo, 1978. **Jackson Co**.: UMMZ 168201 (3, 41.4–51.2), Cumberland R., 6½ mi. NE of Gainesboro, 23 August 1954. **Knox Co.:** UT 44.180 (6, 42.0–??), Holston R., 1.5 mi. below U.S. hwy. 11E bridge, 1 August 1967. UT 44.501 (30, 24.8–44.9), Holston R., on east side of island, ca. 1 mi. below U.S. hwy. 70–11 bridge, 4 June 1970. USNM 230767 (1, 42.0), Holston R., on Gov. John Sevier hwy., S of U.S. hwys. 11-70-25W bridge, 9 April 1968. **Loudon Co.:** UT 44.954 (1, 42.3), Little Tennessee R., main channel side of Davis Id., ca. 3 river mi. below U.S. hwy. 411 bridge, 4 October 1974. **Pickett Co.:** UMMZ 125648 (1, 41.4), Wolf R., below ford and dam at Miller's Chapel, NW of Byrdstown, 24 July 1939. **Unicoi Co.:** UT 44.727 (5, 42.4–46.9), Nolichucky R., beside st. hwy. 81, at co. line (on Washington Co. boundary), 11 November 1972. **Washington Co**.: UT 44.673 (8, 36.0–54.3), Nolichucky R., at Embreeville, 0.25 mi. above st. hwy. 81 bridge, 18 August 1972. **Wayne Co.:** UT 44.631 (3, 42.8–44.2), Tennessee R., at head of island above Clifton Ferry, 6–7 June 1972.

West Virginia:

Kanawha Co.: UMMZ 119350 (2, 16.6–18.7), Kanawha R., at mouth of Paint Cr., 1 August 1935.

Macrhybopsis boschungi (all paratypes unless otherwise noted) (26 lots; 617 specimens)

Collections listed below, all from Mobile Bay basin, are from Alabama, Cahaba, Tombigbee, Coosa and Tallapoosa river drainages (or systems) in Alabama, and from Tombigbee River drainage in Mississippi, as follows: Alabama drainage (Mobile Co., Alabama); Cahaba system (Bibb, Dallas and Perry cos., Alabama); Coosa system (Elmore Co.); Tallapoosa system (Macon and Montgomery cos., Alabama); Tombigbee drainage (Pickens and Tuscaloosa cos., Alabama); Clay and Lowndes cos., Mississippi).

Additional localities are plotted for Alabama in Boschung and Mayden (2004: 208) (as *Macrhybopsis aestivalis* complex), and for Alabama and Mississippi in Mettee et al. (1996: 218), and Mississippi in Ross (2001: 178) (as *Macrhybopsis aestivalis*).

Alabama:

Bibb Co.: UAIC 1443.06 (1, 49.2), Cahaba R., 2 mi. N of U.S. hwy. 82, at Centreville, 29 August 1964. (Following three Bibb Co. collections include mixed series of *M. boschungi* and *M. etnieri*): UF 116293 (1, 45.8) (+4 *M. etnieri* [UF 15434]) (*ex* lot that includes 24 additional specimens of *M. etnieri* [UAIC 1611.03]), Cahaba R., ca. 7½ mi. SSE of Blocton, at Pratt Ferry bridge, 5 June 1965. UAIC uncat. (2, 29.3–31.4) (+7 *M. etnieri* [UAIC 7198.02]), Cahaba R., ca. 0.7 mi. downstream from U.S. hwy. 82 bridge at Centreville, 8 June 1984. UMMZ 250267 (2, 39.8–50.9) (+ 28 *M. etnieri* [UMMZ 250266]) (*ex* mixed series overall totaling 273 specimens), Cahaba R., ca. 6 mi. E of Centreville, at co. rd. 27 bridge, 23 May 1956. **Dallas Co.:** UAIC 7188.01 (20, 25.2–47.1). UAIC 7189.04 (62, 23.0–39.8), Cahaba R., ca. 1.5 mi. upstream from U. S. hwy. 80, 7 September 1984. UF 175766 (57.0) (*ex* UT 44.2312) **(holotype)**, UT 44.2312 (76 originally [now 40], 34.9–58.2), UF 175767 (5), USNM 437193 (5), MCZ 171826 (5), ANSP 200789 (5), TU 204138 (5), KU 41378 (5) (preceding eight lots, all *ex* UT 44.2312, are paratopotypes), Cahaba R., at U.S. hwy. 80 bridge, 17 May 1981. **Elmore Co.:** UF 116296 (5, 26.6–36.4), Coosa R., ca. 3 mi. downstream from Jordan Dam, 1.8 mi. W of Wallsboro, T19N, R18E, Sec. 35, 18 February 1983. **Macon Co.:** UAIC 1516.04 (2, 38.5–42.2), Uphapee Cr.,, 0.2 mi. N of Franklin, on unmarked road, NE of Tuskegee, R22E, T17N, Sec. 1, 8 November 1964. UT 44.1949 (2, 37.4–50.5), Uphapee Cr., ca. 3.0 air mi. NNE of Tuskegee, at I-85 hwy. crossing, 16 November 1979. UF 116295 (13, 29.4–41.1), Tallapoosa R., 5.0 mi. W of Franklin, 1.3 mi. upstream of st. hwy. 229, T17JN, R22E, Sec. 17, 27 March 1985. **Mobile Co.:** UF 20859 (16, 27.6–34.8), Middle R. (fork of Tensaw R.), at mouth of Lizard Cr., 11 May 1975. UF 20862 (5, 19.0–27.6), Middle R., (fork of Tensaw R.,), ca. ½ mi. up from mouth of Tensaw R., 11 May 1975. **Montgomery Co.:** UF 116297 (3, 30.6–37.5), Tallapoosa R., 4.3 mi. NNW of Mt. Meigs, T17N, R19E, Sec. 26, 18 April 1985. **Perry Co.:** UAIC 962.06 (5, 37.0–50.5), Cahaba R., ca. 5.7 mi. E of Marion and 1.9 mi. W of Sprott, on st. hwy. 14, 6 October 1962. UAIC 1437.19 (2, 45.4–45.9), Cahaba R., 1 mi. W of Sprott, 16 September 1964. UAIC 5819.01 (1, 35.0), Cahaba R., 1.1 mi. WSW of Sprott, 16 September 1964. UAIC 6430.04 (1, 43.0), Cahaba R., at st. hwy. 181, T20N, R8E, Sec. 35, 11 July 1981. (Following four Perry Co. collections involve mixed series of *M. boschungi* and *M. etnieri*): UAIC uncat. (5, 42.3–50.2) (+18 *M. etnieri* [UAIC 6791.03]), Cahaba R., ca. 6.5 mi. NE of Marion, 7 October 1983. UAIC uncat. (2, 30.4–31.2) (+7 *M. etnieri* [UAIC 6799.02]), Cahaba R., ca. 1.2 mi. SW of Sprott, 20 August 1983. UAIC uncat. (20, 40.6–56.2) (+ 10 *M. etnieri* [UAIC 7194.03]), same locality as preceding, 19 June 1984. UAIC uncat. (1, 41.8) (+ 4 *M. etnieri* [UAIC 7199.03]), same locality as preceding, 2 September 1983. **Pickens Co.:** UAIC 4330.03 (192, 27.0–44.5), Tombigbee R., ca. 600 yds. below boat landing at Vienna, 18 August 1971. **Tuscaloosa Co.:** USNM 36715 (3, 29.0–40.0), Black Warrior R., at Tuscaloosa, summer 1884. USNM 43531 (18, 31.6–42.4), same locality as above, 1889.

Mississippi:

Clay Co.: UF 28985 (7, 24.9–34.0), Tombigbee R., just upstream from st. hwy. 50 bridge, 5 September 1978. **Lowndes Co.:** UT 44.1204 (150 originally, now 145, 27.1–48.2), Tombigbee R., at U.S. hwy, 82 bridge, 24 May 1976; UF 175768 (5) (*ex* UT 44.1204).

Macrhybopsis etnieri (all paratypes unless otherwise noted) (69 lots; 266 specimens)

Although listed here, the 15 collections (totaling 260 specimens) in AUM collection, were not examined by the authors and are not designated paratypes; however, they are listed here since collection localities were included for mapping purposes. Paratypes selected for distribution to other museums will come from UF 86244.

Collections listed below, all from the Mobile Bay basin, are from Cahaba River system in Alabama; Coosa River system in Alabama, Georgia and Tennessee; and Tallapoosa River system in Alabama and Georgia. These are as follows: Cahaba system (Bibb and Perry cos., Alabama); Coosa system (Clay, Cleburne, Coosa and Talladega cos., Alabama; Cherokee, Forsyth and Lumpkin cos., Georgia; and Bradley and Polk cos., Tennessee); Tallapoosa system (Chambers, Lee, Randolph and Tallapoosa cos., Alabama; Haralson Co., Georgia). Collections are arranged, in alphabetical and chronological order, by state and county. All the above are regarded as paratypes, except AUM lots which were not personally examined by the authors but are listed for purposes of map plotting. Also, size ranges of individual AUM specimen lots are not indicated, as this information was unavailable.

Additional localities are plotted (under the name *Macrhybopsis aestivalis*) for Tennessee in Etnier and Starnes (1994: 193); for Tennessee, Georgia and Alabama in Mettee *et al.* (1996: 218); and for Alabama (under name *Macrhybopsis aestivalis* complex) in Boschung and Mayden (2004: 208).

In addition, a locality is noted here from the Coosa River proper, which is based on several large rotenone collections (during July–August, 1949), from two closely adjacent localities near Childersburg, Talladega County, Alabama. A fish identified by Scott (1951: 37) as *Hybopsis hyostomus* was reported to be the most abundant cyprinid present. Although no voucher specimens were saved, the record is considered by us, based on locality, to refer to *Macrhybopsis etnieri*, and the localities are combined as a single plot, with notation, on the distribution map in the present paper.

Alabama:

Bibb Co.: UF 116298 (12, 32.1–45.4), Cahaba R., 3.6 mi. downstream from U.S. hwy. 82, 8 June 1984. UAIC 2029.04 (2, 35.4–40.5), Cahaba R., at Pratts Ferry, NE of Centreville, at co. rd. 27 bridge, 7 June 1966. UAIC 4679.04 (1, 30.0), Cahaba R., T22N, R9E, Sec. 29, 13 March 1974. UAIC 5584.01 (2, 26.0–27.2), Cahaba R., at co. rd. 27 bridge, 18 February 1978. UAIC 6452.01 (12, 26.9–31.6), same locality as above, 14 November 1981. UAIC 6788.03 (3, 27.2–31.1), UAIC 6789.03 (36, 26.7–41.8), Cahaba R., ca. 6 mi. NE of Centreville, 16 September 1983. UAIC 7702.03 (4, 29.7–34.8), same locality as above, 12 October 1984. UAIC 6797.03 (2, 42.6–45.4), Cahaba R., ca. 7.5 mi. SSW of Centreville, 22 July 1983. UAIC 7186.03 (9, 24.0–46.4), Cahaba R., ca. 8.3 air mi. SW of Centreville, T22N, R9E, Sec. 31, 22 September 1984. UAIC 7187.03 (25, 26.8–44.3), Cahaba R., 7.9 air mi. SW of Centreville, T22N, R9E, Sec. 31, 22 September 1984. UAIC 11053.01 (female, 44.0 mm specimen used for illustration). INHS 76341 (5, 28.9–36.3), Cahaba R., 9 mi. N of Centreville, 14 October 1971. INHS 76335 (3, 30.8–34.0), Cahaba R., 2 mi. N of Centrevillle, 14 October 1971. (Following three Bibb Co. collections include mixed series of *M. etnieri* and *M. boschungi*): UAIC 1611.03 (24, 33.2–43), UF 15434 (4, 33.2–44.3) (+1 *M. boschungi* [now UF 116293]), Cahaba R., ca. 7½ mi. SSE of Blocton, at Pratt Ferry bridge, 5 June 1965. UAIC 7198.02 (7, 20.3–42.7) (+2 *M. boschungi* [UAIC uncat.]), Cahaba R., ca. 0.7 mi. downstream from U.S. hwy. 82 bridge at Centreville, 8 June 1984. UMMZ 250266 (28, 39.6–43.1) (+ 2 *M. boschungi* [UMMZ 250267]) (*ex* mixed series totaling 273 specimens; remainder are UMMZ 171750), Cahaba R., ca. 6 mi. NE of Centreville, at co. rd. 27 bridge, 23 May 1956. **Chambers Co.:** UF 116299 (6, 29.1–37.7), Tallapoosa R., 1.6 mi. W of Abanda, 1.5 mi. SSE of Wadley, T24N, R25E, Sec. 4, 24 June 1982. **Clay Co.:** AUM 1093 (2), Hatchet Cr., 5.2 mi. N of Goodwater, on hwy. 7, 6 April 1968. **Cleburne Co.:** AUM 1585 (8), Shoal Cr., 5.7 mi. NW of Edwardsville, 13 August 1968. **Coosa Co.:** AUM 16763 (7), Hatchet Cr., 3.8 air miles N of Rockford, at US hwy. 231 bridge, 20 May 1978; AUM 18177 (3), same locality as preceding, 8 Sept. 1978; AUM 18710 (18), same locality as preceding, 29 March 1979; AUM 20908 (14), same locality as preceding, 6 May 1981; AUM 21474 (1), same locality as preceding, 25 May 1981; AUM 23267 (1), same locality as preceding, 4 April 1982; AUM 35067 (125), same locality as preceding, 21 June 1980; AUM 41781 (43), same locality as preceding, 20 June 1980; AUM 56630 (2), same locality as preceding, 31 August 1979; AUM 58687 (27), same locality as preceding, 14 May 1985 (**preceding seven AUM lots from Coosa County are not paratypes**). **Lee Co.:** UMMZ 111192 (1, 38.3), UMMZ 111193 (5, 29.7–40.7), creek at Loachapoka, W of Auburn, 3 June 1931. UMMZ 111194 (12, 22.2–34.7), Solugahatchee Cr., near Auburn, 24 October 1930. UMMZ 142909 (1, 38.4), same locality as above, 27 October 1940. **Perry Co.:** UF 116294 (6, 24.2–35.1), Cahaba R., 1.2 mi. SW of Sprott, 2 September 1983. UAIC 6798.03 (6, 30.5–44.1), same locality as above, 20 August 1983. UAIC 7197.02 (6, 28.9–34.2), same locality as above, 11 March 1984. UAIC 7191.03 (3, 33.0–46.4), Cahaba R., 3 mi. NE of Heiberger, 2.0 mi. upstream from Jericho Bridge, T21N, R8E, Sec. 15, 23 September 1984. (Following four Perry Co. collections involve mixed series of *M. etnieri* and *M. boschungi*): UAIC 6791.03 (18, 28.5–40.4) (+5 *M. boschungi* [UAIC uncat.]), Cahaba R., ca. 6.5 mi. NE of Marion, 7 October 1983. UAIC 6799.02 (7, 21.1–41.9) (+2 *M. boschungi* [UAIC uncat.]), Cahaba R., ca. 1.2 mi. SW of Sprott, 20 August 1983. UAIC 7194.03 (10, 30.8–43.0) (+ 20 *M. boschungi* [UAIC uncat.]), same locality as preceding, 19 June 1984. UAIC 7199.03 (4, 25,1–33.2) (+

1 *M. boschungi* [UAIC uncat.]), same locality as preceding, 2 September 1983. **Randolph Co.**: UT 44.2293 (3, 30.4–37.0), Little Tallapoosa R., 4.0 air mi. N of Wedowee, at U.S. hwy. 431 crossing, 16 May 1981. **Talladega Co.:** AUM 409 (6), tributary to Choccolocco Cr., 13.1 mi. NNE of Talladega, on st. rd. 21, 31 August 1967 (non-paratypes). **Tallapoosa Co**.: UF 91617 (1, 33.7), Hillabee Cr., 5.9 air mi. ENE of Alexander City, at st. rt. 22 bridge, 6 April 1992. UT 44.2300 (1, 44.5), same locality as above, 16 May 1981. UAIC 1040.03 (13, 26.8–39.5), Hillabee Cr., 6.5 mi. E of Alexander City, on st. hwy. 22, 12 September 1963.

Georgia:

Cherokee Co.: UF 86141 (3, 37.9–44.8), Etowah R., at co. rd. 782 bridge, 2.9 air mi. SSW of Ball Ground, 17 September 1990. UF 86274 (1, 29.0), Etowah R., 1.4 air mi. E of mouth of Long Swamp Cr., at Big Shoal, 20 September 1990. UF 86289 (12, 30.3–45.8), Etowah R., 1.2 air mi. E of mouth of Long Swamp Cr., 20 September 1990. UF 91296 (17, 31.7–40.0), Long Swamp Cr., at co. rd. 445 bridge (Conn Creek Road), 2.0 air mi. SE of center of Ball Ground, 10 July 1991. UF 91414 (1, 37.8), Settingdown Cr., ca. 0.2 mi. from stream mouth, 6.2 mi. SW from the center of Ball Ground, on st. rt. 369, 21 January 1992. UT 44.1891 (9, 37.5–39.5), Etowah R., on co. rd. 90861, N of st. rt. 369, 12 June 1979. UT 44.1926 (6, 27.3–39.7), Etowah R., E of Canton, at L & N railroad bridge, along co. rd. 100, 2 mi. S of st. hwy. 5, summer 1979. UT 44.1937 (13, 22.8–50.1), UT 44.1942 (3, 38.8–50.4), Etowah R., at co. rd. 90861 crossing, N of st. hwy. 369, 23 August 1979. UT 44.1937 (13, 22.8–50.1), UT 44.1942 (3, 38.8–50.4), Etowah R., at co. rd. 9086 crossing, N of st. hwy. 369, 23 August 1979. UT 44.2230 (6, 27.6–43.8), Etowah R., at co. rd. S-1018, 23 September 1980. UT 44.2240 (2, 46.0–47.3), above mouth of Conn Creek, 22 Sept. 1980. **Dawson Co.:** UF 15785 (2, 45.0–46.0), Etowah R., 0.7 mi. NW of Landrum, on st. hwy. 136, 21 April 1968. UF 86162 (9, 26.2–40.4), Etowah R., 2.35 air mi. ESE of mouth of Shoal Cr., 18 September 1990. UF 86182 (1, 28.9), Etowah R., 0.65 air mi. SE of mouth of Shoal Cr., 18 September 1990. UF 86203 (3, 26.8–29.1), Etowah R., ca. 40 yds. downstream from mouth of Shoal Cr., 18 September 1990. UF 86227 (9, 30.0–51.1), Etowah R., 0.95 air mi. NE of mouth of Yellow Cr., 19 September 1991. UF 90100 (52.2) (**holotype**). UF 237855 (14 paratopotypes [ex UF 90100], 34.8–?), Etowah R., just off co. rd. 76, 5.4 air mi. SE of center of Dawsonville, 19 November 1990. UF 97259 (3, 36.4–44.4), Amicalola Cr., just W of Dawsonville, ca. 4.7 river km below st. hwy. 53 bridge, coordinates 34°24’11”N, 84°12’64”W, 15 June 1994. UF 97284 (30, 31.2–47.8), Amicalola Cr., SW of Dawsonville, ca. 9 river km below st. hwy. 53 bridge, coordinates 34°22’41”N, 84°11’16”W, 15 June 1994. UT 44.1934 (6, 37.3–52.5), Etowah R., ca. 4.0 mi. SE of Dawsonville, at st. rt. 53 crossing, 24 August 1979. UMMZ 175589 (1, 36.6), Etowah R., on US hwy. 19, ca. 5 mi. S of Dawsonville, 9 September 1958. **Forsyth Co.:** UF 86244 (59 originally [now 34], 27.6–44.8), USNM 437193. (5), MCZ 171825 (5), ANSP 200788 (5), TU 204137 (5), KU 41377 (5), Etowah R., 0.5 air mi. SW of mouth of Yellow Cr., 19 September 1990. **Haralson Co.:** UT 44.2794 (1, 38.5), Tallapoosa R., 3 mi. NNE of Tallapoosa, on co. rd. 52340, 2 May 1982. **Lumpkin Co.:** UF 90116 (3, 39.2–42.5), Etowah R., at co. rd. 2 bridge (Castleberry Bridge), 5.2 air mi. SSW from center of Dahlonega, 20 November 1991.

Tennessee:

Bradley Co.: UF 42694 (1, 30.2), Conasauga R., at st. rd. 74 bridge, ca. ½ mi. N of Georgia st. line, 14 June 1985. UF 42743 (1, 45.0), same locality as above, 9 June 1985. UT 44.303 (1, 42.9), same locality as above, 22 October 1969. UT 44.1692 (2, 32.0–33.3), same locality as above, 25 August 1978. UT 44.484 (2, 28.1–29.5), Conasauga R., around lower end of “The Island,” just above Georgia st. line, 9–10 April 1970. INHS 74581 (2, 27.4–28.6), Conasauga R., 3 mi. W of Conasauga, 13 October 1971. **Polk Co.:** UT 44.400 (1, 42.1), Conasauga R., between mouth of Minnewauga Cr. and Boanerges Church bridge, 2 July 1969. UT 44.413 (11, 27.1–48.0), Conasauga R., ford above US hwy. 411 bridge, 11 October 1969. USNM 230851 (1, 45.3), Conasauga R., at Easley Ford bridge, off US hwy. 411, 20 October 1968.

Macrhybopsis pallida (all paratypes unless otherwise noted) (44 collections; 666 specimens)

Collections listed below are from the Escambia, Blackwater Bay (Yellow River system), and Choctawhatchee river drainages in Alabama and Florida, as follows: Escambia drainage (Escambia Co., Alabama; Escambia and Santa Rosa cos., Florida); Blackwater Bay (Covington Co., Alabama; Okaloosa Co., Florida); Choctawhatchee drainage (Dale and Houston cos., Alabama; Holmes and Washington cos., Florida).

Localities for Florida (as *Extrarius* n. sp. cf *aestivalis*) are plotted in Gilbert and Yerger (1992: 134), and additional localities for Alabama (as *Macrhybopsis aestivalis*) are plotted in Mettee et al. (1996: 218) and (as *Macrhybopsis aestivalis* complex) in Boschung and Mayden (2004: 208).

Alabama:

Covington Co.: UAIC 4188.03 (1, 44.0), Five Runs Cr., 12 air mi. S of Andalusia, T2N, R15E, Sec. 26, 17 September 1972. TU 73150 (1, 42.6), Yellow R., 10 mi. SW of Opp, 21 November 1971. TU 72958 (1, 41.1), Yellow R., 9.4 mi. NNW of Florala, on hwy. 55, 6 October 1971. **Dale Co.:** UF 73336 (29, 23.0–46.0), Choctawhatchee R., at st. rt, 92, SE of Ft. Rucker, 23 February 1975. UF 73488 (2, 22.8–25.2), same locality as preceding, 25 March 1975. **Dale/Houston cos.:** UF 73313 (1, 50.3), Choctawhatchee R., at U.S. hwy. 84 bridge, E of Clayhatchee, 10 January 1975 (Holotype). UF 73468 (1, 30.5), same locality as preceding, 25 March 1975. **Escambia Co.:** UF 44666 (68, 26.0–35.8), Conecuh R., on co. rd. 4, 6 mi. E of Brewton, T2N, R11E, Sec. 35, 1 November 1986. UF 73320 (10, 20.7–28.5), Conecuh R., ca. 10 mi. E of Brewton, near co. rd. 4, 10 January 1975. UAIC 1823.03 (2, 16.3–18.5), Conecuh R., at U.S. hwy. 29 bridge, T2N, R13 W, 26 December 1965; UAIC 10855.04 (1, 36.0 [*illustrated specimen*, from presumably larger series]), Conecuh R., at Pollards Landing, at end of Old River Road, T1N, R9E, Sec. 33, 15 July 1993.. TU 15948 (6, 29.9–45.4), Conecuh R., 3 mi. SE of Flomaton, 18 July 1957. TU 81354 (9, 26.1–29.3), Conecuh R., 9.4 mi. E of East Brewton, 23 April 1972. **Houston Co.:** UAIC 3508.11 (14, 27.3–34.9), Choctawhatchee Cr., 2 mi. E of Clayhatchee, on U.S. hwy. 84, 4 April 1969.

Florida:

Escambia Co.: UF 9333 (11, 18.8–26.4), Escambia R., at point due E of Pine Barren, 14 October 1954. UF 53534 (2, 33.0–35.5), Escambia R., 5 river mi. N of bridge E of Century, on st. hwy. 4, 26 August 1958. UF 54366 (4, 31.5–33.8), Escambia R., 6 river mi. N of bridge E of Century, on st. hwy. 4, 26 August 1958. UF 75361 (2, 15.2–16.8), Escambia R., 1.7 mi. E of Century, ca. 1 mi. S of st. rt. 4 bridge, 11 September 1976. UF 75441 (2, 19.5–24.9), same locality as preceding, 13 November 1976. UF 130433 (8, 22.2–29.3), Escambia River, sand bar on west side of river, ca. 1.5 mi. below Bluff Springs boat ramp, 15 May 2002. UF 130470 (1, 31.7), Escambia River, gravel and sand bar on right bank of river, upstream of Bluff Springs boat ramp, 14 May 2002. UF 138618 (2, 25.5–27.0), Escambia River, bar N of Exxon Landing and south of Alabama state line, 19 February 2003. UF 143879 (4, 18.9–21.8), Escambia River, west bank of river, between Exxon Landing and st. hwy. 4, 20 August 2003. UF 150156 (1, not measured), Escambia River, vicinity of Jay, 4 March 1972. UF 171841 (3, 22.7–29.8), Escambia River, at st. hwy. 4 bridge, at Jay, 12 November 1986. UF 171848 (9, 18.8–28.8), same locality as preceding, 25 November 1985. UF 172372 (8, 35.1–51.5), same locality as preceding, 14 May 1986. **Holmes Co.:** UF 55457 (21, 18.8–34.9), Choctawhatchee R., 6 air mi. S of Geneva, Alabama, on st. rt. 2, 11 September 1959. UF 75477 (1, 14.3), Choctawhatchee R., ca. 3 mi. upstream from U.S. hwy. 90 bridge at Caryville, 17 November 1976. UAIC 3195.06 (12, 24.4–33.6), Choctawhatchee R., on st. hwy. 2, 1½ mi. W of Pittman, 22 November 1968. UAIC 3126.13 (196 originally [now 166], 21.3–36.7), USNM 437191 (5), UMMZ 250265 (5), MCZ 171824. (5), ANSP 200787 (5), UT 44.13095 (5), KU 41376 (5), same locality as preceding, 28 September 1968. UAIC 4449.08 (4, 26.0–31.0), mouth of a branch of Choctawhatchee R., 2.25 air mi. N of Caryville, 21 October 1972. TU 20811 (28, 25.5–31.9), Choctawhatchee R., 3 mi. S of Browns, on st. hwy. 2, 24 July 1959. TU 102794 (32, 25.0–37.5), Choctawhatchee R., at st. hwy. 2, 24 June 1977. **Okaloosa Co.:** UF 57640 (5, 30.6–50.5), mouth of Shoal R., 7 air mi. SW of Crestview, 6 June 1961. TU 82620 (5, 24.5–32.0), Yellow R., 7 mi. SW of Laurel Hill, on st. hwy. 2, 23 April 1972. TU 79682 (2, 31.1–35.5), same locality as preceding, 24 June 1972. TU 83078 (11, 27.3–37.5), Yellow R., 3.5 mi. SW of Crestview, at U.S. hwy. 90 bridge, 22 April 1972. TU 102299 (1, 37.5), same locality as preceding, 9 June 1977. TU 101906 (4, 28.6–37.6), Yellow R., 4 mi. WSW of Crestview, at U.S. hwy. 90 bridge, 29 April 1977. **Santa Rosa Co.:** UF 73352 (1, 22.8), Escambia R., 1 mi. below st. rt. 4 bridge, 28 November 1974. UF 73438 (15, 22.0–29.7), Escambia R., above entrance to Look and Tremble oxbow, below st. hwy. 4, 8 March 1975. UF 145534 (6, 21.0–24.2), Escambia River, bar on east side of river, south of Exxon Landing, 17 September 2003. UF 145898 (36, 17.9–28.2), Escambia River, bar on east side of river, south of Exxon Landing, 30 September 2003. **Washington Co.:** UAIC 3191.02 (84, 22.6–32.8), Choctawhatchee R., beside st. hwy. 20 bridge, 1½ mi. W of Ebro, 9 November 1968.

Macrhybopsis tomellerii (all paratypes unless otherwise noted) (19 lots; 591 specimens)

Collections listed below are from the Pascagoula River drainage in Mississippi; the Pearl River drainage in Louisiana and Mississippi; and from the Lake Pontchartrain drainage in Louisiana, as follows: Pascagoula drainage, Mississippi (Forrest, Jones, Lauderdale, Smith, Stone, and Wayne cos.); Pearl drainage, Louisiana (Washington Psh.) and Mississippi (Leake and Simpson cos.); Lake Pontchartrain drainage, Louisiana (East Baton Rouge and Tangipahoa parishes). Lots from the Lake Pontchartrain drainage are not regarded as paratypes for reasons discussed in text.

Additional localities (as *Hybopsis aestivalis* are plotted for Louisiana in Douglas, 1974: 101) and (as *Macrhybopsis aestivalis*) for Mississippi in Ross (2001: 178).

Louisiana:

East Baton Rouge Psh.: INHS 79456 (10, 29.8–40.6), Sandy Cr., ca. 1½ mi. N of Greenwell Springs, 7 April 1974 (**non-paratypes**). **Tangipahoa Psh.:** UF 14731 (11, 33.5–42.2), Tangipahoa R., ca. 6 air mi. E of Hammond, on U.S. hwy. 190, 13 July 1966 (**non-paratypes**). **Washington Psh.:** TU 45061 (30, 32.7–45.4) (*ex* lot of 192 specimens [remaining 162 specimens are not paratypes]), Bogue Chitto R., just below sill, 1 mi. below lock 3, 1 April 1967. TU 62182 (30, 35.4–47.2) (*ex* lot of 2058 specimens [remaining **2028 specimens are not paratypes**]), Pearl R., just below sill at Pools Bluff, 4 mi. S of Bogalusa, 21 April 1970.

Mississippi:

Forrest Co.: UAIC 6391.02 (9, 29.9–36.5), Bowie R., ca. 1.0 river mi. downstream from hwy. I-59 bridge, NW of Hattiesburg, 6–7 May 1981. UAIC 6392.02 (2, 33.2–39.1), Leaf R., E of Hattiesburg, ca. 0.25 river mi. of U.S. hwy. 11 bridge, 7–8 May 1981. UAIC 6393.03 (5, 31.0–41.8), same locality as preceding, 7–8 May 1981. UAIC 6412.02 (12, 40.9–51.1), Bowie R., T5N, R33W, Sec. 31 (NW¼), 26–27 August 1981. UAIC 6413.06 (6, 38.9–49.2), Leaf R.,T5N, R13W, sec. 34 (SE¼), 26–27 August 1981. **Jones Co.:** INHS 79942 (6, 37.3–43.7), Leaf R., 2 mi. W of Moselle, below I-59 hwy. bridge, 16 October 1971. INHS 76268 (81, 28.2–49.4), same locality as preceding, 17 April 1977. TU 57445 (63, 25.7–44.5), same locality as preceding, 26 April 1969. UT 44.1206 (159, 34.5–53.0), same locality as preceding, 25 May 1976. **Lauderdale Co.:** UT 44.2317 (20, 33.0–49.0), Chunky R., ca. 0.1 mi. N of Clarke Co. line, on hwy. I-59 hwy., 18 May 1981. **Leake Co.:** UF 28146 (1, 48.8), Pearl R., ca. ½ mi. S of Sunrise, on unmarked rd., T11N, R9E, Secs. 25 & 30 (**holotype**). UF 237859 (9, 31.5–47.1), same data as preceding (paratopotypes). **Simpson Co.:** TU 57271 (30, 33.5–48.8 (*ex* lot of 141 specimens [remaining 111 specimens are not paratypes), Strong R., 2 mi. W of Pinola, at rapids upstream from st. rd. 28 bridge, 23 April 1969. **Smith Co.:** TU 53791 (87 originally, now 57, 37.2–51.3), UF 237864 (5), USNM 437190 (5), UMMZ 250264 (5), ANSP 200786 (5), MCZ 171824 (5), KU 41375 (5). Leaf R., 1.4 mi. E of Taylorsville, on st. rd. 28, 5 October 1968. **Stone Co.:** UAIC 893.02 (3, 24.1–29.2), Black Cr., 13 mi. E of Wiggins, at st. hwy 26, 27 October 1962. **Wayne Co.:** TU 15395 (17, 34.2–45.0), Chickawawhay R., near Waynbesboro, November 1956.