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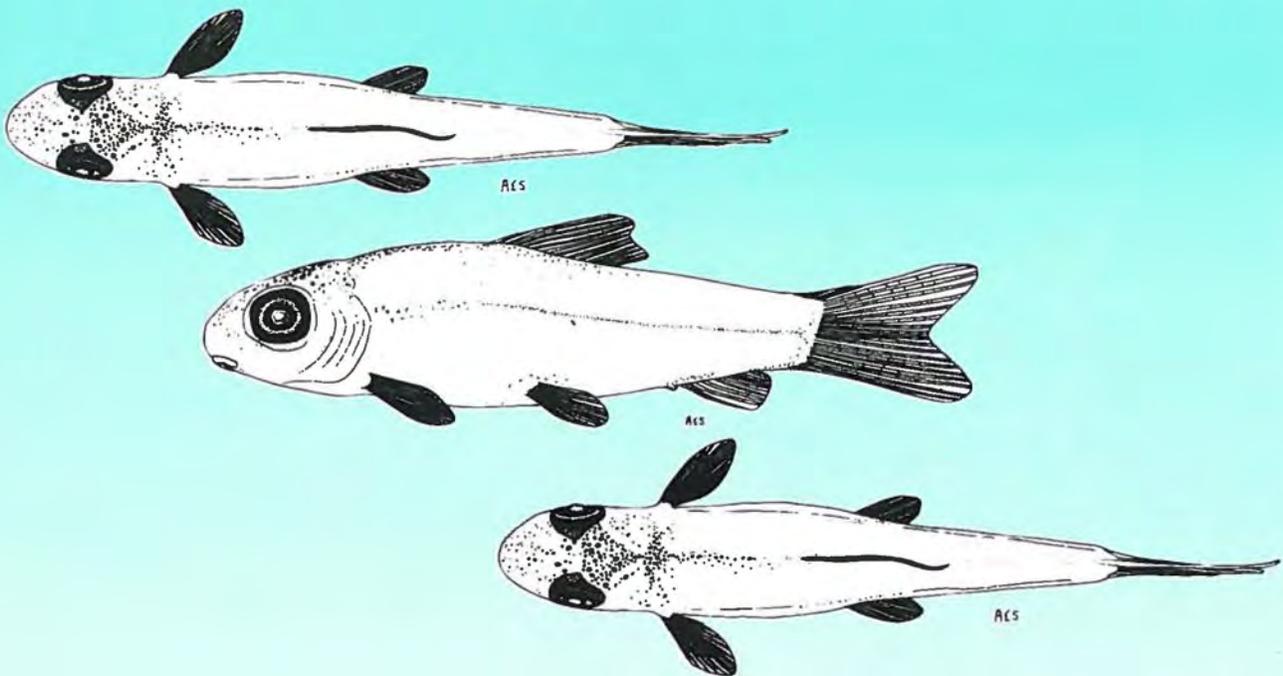
# Bulletin

Bulletin 23

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*Moxostoma robustum*

Systematics, Variation, and Speciation of the  
*Macrhybopsis aestivalis* Complex West of the Mississippi River



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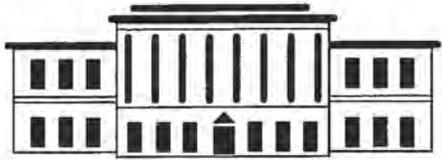
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THE UNIVERSITY OF ALABAMA  
TUSCALOOSA, ALABAMA  
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# Description of Larval and Juvenile Robust Redhorse, *Moxostoma robustum*

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Key words: robust redhorse, larval, *Moxostoma*, description, juvenile, Oconee River, Savannah River

**ABSTRACT:** Looney, Gregory L., Cecil A. Jennings. 2002. Description of Larval and Juvenile Robust Redhorse, *Moxostoma robustum*. Bulletin Alabama Museum of Natural History, Number 23: 1–8. 4 Tables, 5 Figures. Robust redhorse, *Moxostoma robustum*, is a large riverine catostomid that until recently was known only from museum and archeological specimens. A population was discovered in a 60-km reach of the Oconee River, GA during summer 1991. Efforts to locate or to verify continuity of known populations were unsuccessful until individuals were captured at scattered locations in the Savannah River, GA/SC, during 1997–2000; Ocmulgee River, GA, in 1999; and three specimens from the Pee Dee River, NC, during 2000–2001. Little is known about the biology, ecology, and population dynamics of this species, but intensive research in these areas has yielded new information. We describe the morphological development of larval and early juvenile robust redhorse. Newly hatched larvae ranged from 7.2 to 8.1 mm total length (TL). Absorption of yolk is complete at about 14.0 mm TL. Fin development is complete or nearly so by about 22.5 mm TL, at which time scale formation is visible midlaterally on the body from the caudal peduncle to the head. Fin ray development is complete by 23.5 mm TL. Juveniles 72–100 mm TL have acquired most of the morphological characteristics of adults. Of the morphological characters examined, length at hatching was the only morphological characteristic that can be used reliably to distinguish *M. robustum* from *M. collapsum*, a sympatric congener.

## Introduction

Robust redhorse, *Moxostoma robustum*, are large (maximum size approximately 760-mm TL) catostomids that were discovered near the mouth of Commissioner Creek in the Oconee River, Georgia, on 8 August 1991. Edward Cope originally described the species in 1870; however, Cope's original specimens were mislabeled and later lost, thereby erasing any scientific knowledge of the species

(Jenkins and Freeman, unpubl.). A detailed account of this resolution can be found in Jenkins and Burkhead (1993). After discovery of robust redhorse in 1991, a review of archaeological records showed robust redhorse (RRH) remains collected from the Savannah River, Georgia, and Yadkin River, North Carolina. Currently, the Oconee River contains the largest known population. Systematic attempts to locate other populations in the his-

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Fig. 1. Current locations of extant (wild individuals, wild populations, and stocked populations) robust redhorse, *Moxostoma robustum*, in the major drainages of the South Atlantic.

torical range (i.e., Atlantic Slope drainages from the Pee Dee River in North Carolina to the Altamaha River in Georgia, Figure 1) were unsuccessful until recently, when at least 24 individuals were captured at scattered locations in the Savannah River, GA/SC, two adults from the Oconee River, GA, and three specimens from the Pee Dee River, NC.

The estimated 1,000 – 3,000 robust redhorse in the Oconee River (Evans, unpubl.) occur in about a 60 km reach between Milledgeville and Dublin, Georgia. Most individuals are 10 – 26 years (Jenkins et al. 1999, AFS, abstract), and recruitment over the past several years seems to have been negligible. Accordingly, studies have been undertaken to estimate the abundance and distribution of larval robust redhorse in the Oconee River. Because descriptions of larval robust redhorse were unavailable to help identify wild-caught specimens, our objectives were 1) to describe the development of hatchery-reared robust redhorse, and 2) identify morphometric and meristic characters that would aid in distinguishing of wild-caught *M. robustum* and its sympatric congener notchlip redhorse *M. collapsum*.

#### Materials and Methods

The larval and juvenile specimens used were produced from the mating of one male and one female robust redhorse collected from the Oconee River between RM 85.5 and 87.7 on 25 May, 1993, when water temperature was

23° C. The female was stripped and the dry fertilization technique was used to fertilize the eggs. Fertilized eggs and larvae were incubated in a closed, recirculating system at a water temperature of 23° C ± 1.5° C from 25 May until 23 July. Intensively cultured post yolk-sac larvae were fed a combination of *Artemia* *napulii*, chopped blackworms, and commercially prepared diets. Samples were collected periodically (every six to eight hours for yolk sac larvae and every 24 hours for post yolk-sac larvae) from the culture system and preserved in unbuffered 10% formalin solution. Juveniles were pond-reared siblings.

Larval specimens were examined with a stereo-binocular microscope equipped with polarizing filters. Total length (TL) was measured to the nearest 0.1 mm. Measurements of individuals less than 30.0 mm TL were made with an ocular micrometer at magnifications of 6X and 10X. Measurements on juveniles greater than 70 mm TL were made with a ruler graduated in millimeters. Descriptive counts, measurements, and terminology follow Wallus et al. (1990). The described larvae are typical, morphologically, to RRH larvae produced since 1993. The study specimens are currently housed at the Warm Springs Fish Technology Center, Warm Springs, Georgia.

#### Results

The following descriptions are based on preserved specimens and emphasize developmental state, morphology, and pigmentation. Morphometric data are summarized in Table 1. Table 2 contains the numbers of individuals in each size range examined. Myomere count frequencies are in Table 3.

#### Description

**YOLK SAC LARVAE MORPHOLOGY.**— Robust redhorse eggs hatched about 3.5 days after fertilization. Newly-hatched larvae were 7.2–8.1 mm TL and had a large, bright yellow yolk sac. The anterior 40–45% of the yolk-sac was bulbous (appearing almost round); and the remainder was cylindrical or tubular. The head was small and slightly curved around the anterior end of the bulbous portion of the yolk sac. Anterior and posterior myomere development was incomplete, thus accurate myomere counts were not possible for this size range.

At six days post-fertilization, larvae were 9.7–10.5 mm TL. Yolk material was visibly reduced. The yolk sac was no longer bulbous anteriorly; its cylindrical or tubular form along the entire length was retained. The head had lifted and was no longer curved around the yolk sac. The stomodeum was forming, but the mouth was not open.

Larvae at eight days post-fertilization were 11.2–11.7 mm TL (Fig. 2). The yolk-sac was still tubular, but not as thick anteriorly as posteriorly. Depth of the yolk was greater than the depth of the myomeres directly above. The mouth opening was apparent, gill arches had begun to form, and the heart was developing just anterior to the yolk sac.

**Table 1. Morphometry of Robust Redhorse (*Moxostoma robustum*) larvae and juveniles (n=sample size). The range is given in parentheses. (Standard length [SL] - distance from anterior-most part of the head to most posterior point of the notochord or hypural complex; preanal length [PreAL] - distance from anterior-most part of the head to posterior edge of margin of anus; head length [HL] - distance from anterior-most tip of head to the posterior-most part of opercular membrane; eye diameter [ED] - horizontal measurement of the iris of the eye).**

Life stage	n	Total length (mm)	% of total length			
			SL	PreAL	HL	ED
Yolk-sac Larvae	12	7.67 (7.2-8.1)	97.5 (97.2-98)	84.7 (82.9-87.2)	10.8 (9.7-11.5)	4.9 (3.8-5.7)
Yolk-sac Larvae	20	9.99 (9.7-10.45)	96.3 (95.8-97)	79.3 (75.8-81.6)	12.7 (11.2-14.1)	5.3 (4.8-5.8)
Yolk-sac Larvae	10	11.45 (11.2-11.7)	95.6 (94.5-96.5)	76.2 (74.8-77.3)	13.9 (13.0-14.3)	5.5 (5.1-5.8)
Yolk-sac Larvae	10	12.53 (12.1-12.9)	94.8 (94.4-95.9)	74.8 (73.6-76.0)	15.1 (13.8-17.1)	5.9 (5.5-6.3)
Yolk-sac Larvae	17	13.71 (13.0-14.3)	93.9 (89.5-95.7)	70.5 (67.8-73.5)	16.0 (14.8-17.5)	6.4 (5.9-7.0)
Post Yolk-sac Larvae	12	15.26 (14.5-15.9)	87.6 (86.0-89.7)	66.8 (65.6-67.7)	18.2 (17.2-19.1)	6.9 (6.5-7.2)
Post Yolk-sac Larvae	13	16.65 (16.0-17.2)	86.4 (85.2-87.5)	65.7 (65.1-67.5)	19.2 (18.5-20.0)	7.1 (6.8-7.5)
Post Yolk-sac Larvae	10	19.57 (17.7-22.5)	83.3 (82.2-84.7)	62.4 (60.0-63.3)	20.0 (18.7-20.5)	7.2 (6.8-7.6)
Juveniles	6	25.55 (23.5-28.8)	82.3 (81.0-83.0)	60.0 (59.5-60.4)	20.2 (19.8-20.5)	7.0 (6.7-7.2)
Juveniles	5	74.6 (72.0-77.0)	82.3 (81.5-83.1)	61.5 (60.3-62.5)	17.3 (17.1-19.3)	5.6 (5.3-6.0)
Juveniles	5	95.8 (92.0-100.0)	83.5 (82.0-84.9)	62.2 (61.2-63.0)	17.3 (17.0-17.7)	5.2 (5.0-5.4)

Larvae at 10–12 days post-fertilization were 12.2–12.9 mm TL. Yolk was reduced but still tubular and was about equal to the depth of the myomeres directly above. Branchiostegal development was visible, and opercular flaps were forming.

Larvae at 14–18 days post-fertilization were 13.0–14.0 mm TL (Fig. 3). Opercular development continued until the opercular flap covered the gills. Nares were visible, and the optic chamber had formed. The head profile and eyes were slightly flattened. The mouth was subterminal and oblique. The digestive tract was functional in some individuals by 13.6 mm TL. The remaining yolk was still tubular at 13.0 mm TL, its depth about equal to half the depth

of the myomeres directly above. Complete absorption of the yolk, which marked the beginning of the post yolk sac period, occurred at about 14.0 mm TL, although yolk was present on some specimens up to 14.3 mm TL.

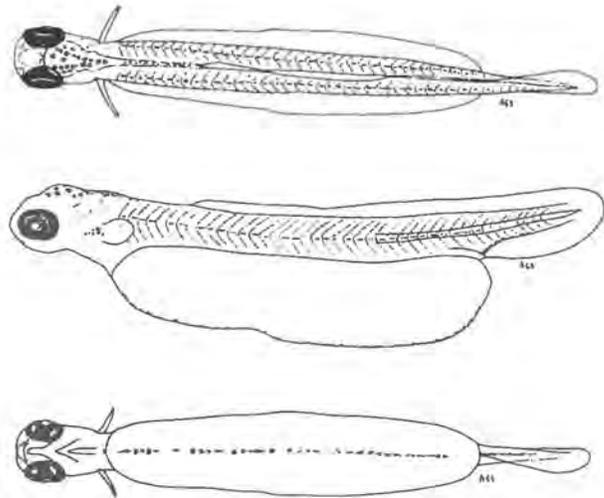
**YOLK SAC LARVAE FIN DEVELOPMENT.**— Newly-hatched yolk sac larvae (7.2–8.1 mm TL) had a median finfold that began dorsally near midbody, extended posteriorly around the notochord, and ended ventrally at the posterior margin of the yolk sac. Other fin development was not apparent.

Larvae of 9.7 mm TL had pectoral flaps present. Median finfold dorsal origin was at an anterior position about 25% the TL. The tip of the notochord was flexed slightly dorsal.

**Table 2. Robust redhorse (n-120) larvae and juveniles, by length increment.**

Life Stage	Size Range	Number (mm TL)
Yolk-sac	7.2-7.9	10
	8.0-8.1	2
	9.7-9.95	10
	10.0-10.45	10
	11.2-11.7	10
	12.2-12.9	10
	13.0-13.8	10
Post Yolk-sac	14.0-14.8	10
	15.2-15.9	9
	16.0-16.9	10
	17.1-17.7	4
	18.3-18.6	2
	19.0-19.2	4
	20.3	1
	21.9	1
	22.5	1
	Juvenile	23.5-23.8
25.3		1
28.2-28		2
72.0-77.0		5
92.0-100.0		5
Total		120

Larvae of 11.2–11.7 mm TL (Fig. 2) showed slight flexing of the notochord, but differentiation of the caudal fin had begun. Rays were not visible in the caudal fin, but basal elements of the hypural complex were forming. Developing pectoral fins were about 0.5 mm long. Dorsal origin of the median finfold was between myomeres 8–10. Ventrally, the finfold was beginning to form on the posterior margin of the yolk sac.

**Fig. 2. Robust redhorse, *Moxostoma robustum*, 11.2–11.7 mm TL.**

Larvae of 12.2–12.9 mm TL showed more obvious flexion of the notochord posteriorly, and basal elements of the caudal fin were well formed. Incipient rays were forming in the caudal fin; 8–12 rays were visible in the caudal fins of 12.9 mm TL fish. The ventral finfold was present anteriorly on the yolk sac to about the position of the pectoral fins. The dorsal profile of the median finfold was beginning to elevate at the future position of the dorsal fin.

Larvae of 13.6–14.3 mm TL (Fig. 3) had pectoral fins about 1.3 mm long. The caudal fin was becoming bilobed. The urostyle extended to the dorsal margin of the caudal fin. The dorsal fin profile was forming in the dorsal finfold, which was much reduced anteriorly. Differentiation in the forming dorsal fin was obvious on some 13.8 mm TL individuals. The anterior and posterior margins of the dorsal fin were nearly defined for fish of 14.0–14.3 mm TL, and incipient rays were forming. The ventral finfold

**Table 3. Myomere count frequencies related to TL (mm) for robust redhorse.**

Life Stage	TL Range	Preanal myomeres						Postanal myomeres						Total myomeres					
		33	34	35	36	37	38	5	6	7	8	9	40	41	42	43	44	45	
Yolk-sac	9.7-9.95				3	7			3	7						6	4		
	10.0-10.45				2	7			6	3					1	7	2		
	11.2-11.7				5	3	1	1	2	8					1	7	1	1	
	12.2-12.9			1	6	3	2		2	8					2	6	2		
	13.0-13.8		1	7	2				1	7	2			1	6	3			
Post Yolk-sac	14.0-14.8		5	4	1				2	6	2			4	6				
	15.2-15.9	2	6	1					8	1		1	7	1					
	16.0-16.9	2	8						2	7	1		3	7					
	17.1-17.7	2	2							4			2	2					
	18.3-18.6	1	1							2			1	1					
	19.0-19.2	1	3							3	1		1	2					

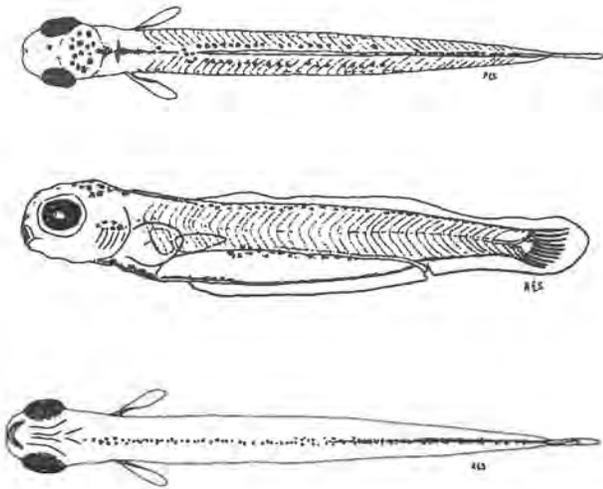


Fig. 3. Robust redhorse, *Moxostoma robustum*, larvae 13.0–14.0 mm TL.

was decreasing in width and extended anteriorly to a position near mid-length of the abdominal cavity. On 14.0–14.3 mm TL fish, pelvic fins appear as narrow flaps positioned ventrolaterally beneath the anterior half of the developing dorsal fin and at the juncture of the gut and the torso.

**YOLK SAC LARVAE PIGMENTATION.**—Newly-hatched larvae lacked pigment in the eyes, head or body. The yolk was yellowish.

Eye pigment first appeared in larvae 9.7–10.5 mm TL. The only body pigmentation consisted of thin dark dashes along the median myosepta, dorsal to the yolk sac, on some specimens.

Larvae 11.2–11.7 mm TL had dark brown eyes. Dorsally, pigmentation was scattered on the head over the brain; this pigmentation narrowed on the occipit to a single middorsal row along the body to about the origin of the dorsal finfold. Scattered melanophores were present dorsally and ventrally at the base of the finfold on the caudal peduncle. Melanophores were present in one mid-ventral row on the yolk sac from the pectoral fin base area to the anus. Lateral pigmentation consisted of a dashed line along the median myosepeta from the head to about mid-length of the caudal peduncle.

Larvae 12.2–12.9 mm TL (Fig. 2) had black eyes. Dorsal pigmentation on the head posterior to the eyes, on the occipit, and in a middorsal row on the body anterior to the finfold, consisted of large, black melanophores. Indistinct rows of small melanophores appeared along each side of the dorsal finfold at about mid-length of the body. The pigmentation outlining the caudal peduncle was darker, and internal pigmentation appeared scattered on the dorsal margin of the yolk sac.

In addition to the previously described pigmentation

patterns, larvae 13.0–14.3 mm TL (Fig. 3) had melanophores present on the head around the tip of the snout at the anterior margins of the nares. A few large melanophores were scattered dorsally on the head between the eyes. Lateral rows of dorsal pigmentation were distinct over the middle of the body and fused posteriorly with the dense, scattered pigmentation on the caudal peduncle. Three to four melanophores in a row were present on the side of the head between the eyes and the pectoral fins, ventral to the optic chamber. This row of pigmentation curved downward anteriorly from about the height of the dorsal margin to the pectoral fin base. Ventral pigmentation on the yolk sac was a wide band of melanophores. Internally, the dorsal margin of the abdominal cavity was covered with melanophores. Scattered pigmentation was present on the caudal fin and by 14.0–14.3 mm TL at the base of the caudal fin. On some individuals, two or three melanophores were present on the chin.

**POST YOLK SAC LARVAE (TL RANGE=14–23 MM) MORPHOLOGY.**—Larvae at 23.5–30.5 days post-hatch were 14.3–16.0 mm TL (Fig. 4) with a ventrally flattened head. The mouth was ventral and had progressed from subterminal-oblique to subterminal-horizontal. Operculum was present to the base of the pectoral fins on larvae 16.0 mm TL. Larvae 18.6–20.0 mm TL had a slightly concave dorsal head profile posterior to the eyes. Larvae at 20.3 mm TL had squamation on the caudal peduncle. Larvae 21.9–22.5 mm TL (Fig. 5) had scales midlaterally from the caudal peduncle to the head.

**POST YOLK SAC LARVAE (TL RANGE=14–23 MM) FIN DEVELOPMENT.**—Larvae 14.3–14.5 mm TL had a distinctly bilobed, well-developed caudal fin with 18 primary rays, some of which were segmented. The urostyle, positioned immediately dorsal to the most anterior primary caudal

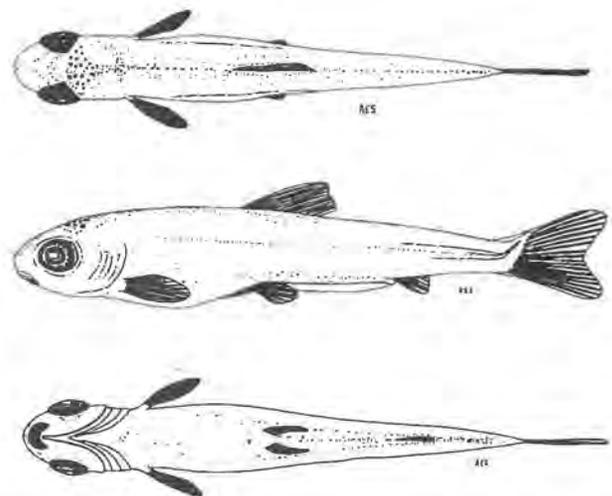


Fig. 4. Robust redhorse, *Moxostoma robustum*, post yolk-sac larvae 14.3–16.0 mm TL.

ray, extended beyond the hypural plate. The anterior and posterior margins of the dorsal fin that originated at myomere 12–13 were defined. Rays were visible in the dorsal fin. The remainder of the dorsal finfold was restricted between the dorsal fin and caudal fin; its depth was less than half the body depth. The ventral finfold also was reduced and extended anterior to the anus to a position near the pectoral fin bases at about the point of the greatest body depth. The anal fin was forming (pterygiophores were present on 14.3 mm TL larvae but rays were not present) posterior to the anus. Rays were visible in the pectoral fins, which were about 1.5 mm long.

Larvae 15.2–15.9 mm TL had rays forming in the anal fin. There were 10–11 rays present in the developing dorsal fin. The pelvic flaps were about half the width of the remaining ventral finfold. The urostyle extended past the hypural plate.

The margin of the anal fin of 16.0–16.9 mm TL larvae (Fig. 4) was rounded and had a defined insertion with five or six rays visible in the fin. Pelvic fins extended to the margin of the remaining ventral finfold. A small amount of dorsal finfold was still present between the dorsal and caudal fins. The ventral finfold was present from the anus anteriorly to about midway between the pectoral and pelvic fins.

Larvae 17.7–19.2 mm TL had lost the dorsal finfold. The ventral finfold was restricted between the pelvic fins and the anus. Fin development was nearing completion in all fins with well developed rays and profiles; however, the urostyle still extended beyond the margin of the hypural plate. The distal margin of the dorsal fin was concave with at least 13 rays visible in the fin. The anal fin had seven or eight rays and; eight or more rays were visible in each pelvic fin. The pectoral fins were well developed and at least 12–14 rays were present.

Larvae 20.0–22.5 mm TL (Fig. 5) had a remnant of finfold immediately anterior to the anus. The finfold was completely gone and fin development was complete on larvae 22.5 mm TL.

**POST YOLK SAC LARVAE (TL RANGE=14–23 MM TL) PIGMENTATION.**—Larvae 14.3–15.9 mm TL were heavily pigmented ventrally. Anterior to the pectoral base, the pigmentation pattern appeared as an arrow with its point near the isthmus. This scattered pigmentation narrowed at the base of the pectoral fins to a double row of melanophores that extended posteriorly to about the anterior margin of the ventral finfold. Dense pigmentation was scattered at the base of the finfold to the anus. Chin pigmentation was present. Pigmentation patterns outlined the gill arches, and pigmentation appeared on the upper lip and snout. Internally, melanophores were scattered dorsally on the gut posterior to the air chambers.

Larvae mm 16.0–16.9 mm TL (Fig. 4) had uniformly scattered pigmentation covering the head, occipit, and optic chamber, dorsally. The large melanophores over the brain and in the single row from the occipit to the

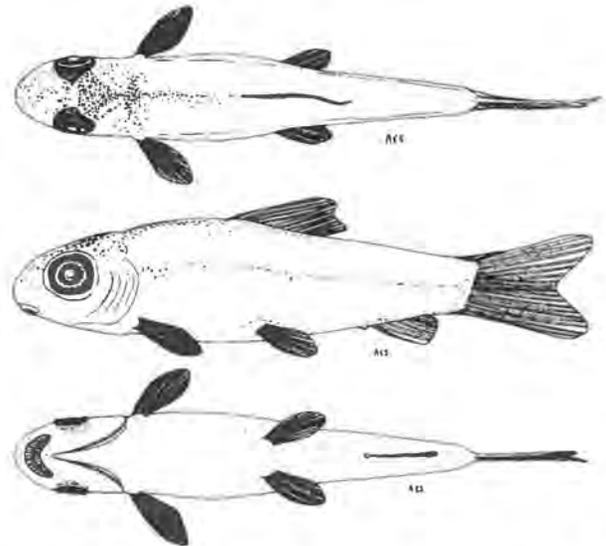


Fig. 5. Robust redhorse, *Moxostoma robustum*, post yolk-sac larvae 21.9–22.5 mm TL.

dorsal fin origin were still present. Scattered small melanophores covered the remainder of the dorsum. This pigmentation consisted of dorsolateral rows of small melanophores with scattered pigmentation between the rows from the dorsal fin origin to the middle of the caudal peduncle. The dark, tightly scattered pigmentation on the middle of the caudal peduncle to the base of the caudal fin remained. Lateral pigmentation was mostly unchanged compared to previous developmental stages. Small melanophores were scattered around the snout with pigmentation on the upper lip and, on some specimens, on the lower lip. Pigmentation was still visible on the chin. There were fewer melanophores ventrally, especially on the gut, anterior to the developing pelvic fins, and the anus was dark. Tightly scattered pigmentation was present on the ventral caudal peduncle between the anal and caudal fins. The arrow pattern anterior to the pectoral fin bases was still present.

Larvae 17.0–19.2 mm TL had small melanophores that increased in number dorso-laterally. In larvae 18.3 mm TL, ventral pigmentation anterior to the pelvic fins was reduced, but there was still dark, tightly scattered pigmentation posterior to the pelvic fins along the gut and posterior to the anal fin. Small melanophores were scattered throughout the caudal fin and on the anterior half of the dorsal fin. Some pigmentation was present in the anal fin on 19.0 mm TL larvae.

Larvae 19.2–22.5 mm TL (Fig. 5) had lateral pigmentation that was continuing to expand. On larvae 19.2 mm TL, pigmentation was scattered laterally to just above the median myosepta; but on larvae 20.3 mm TL, pigment had expanded past the median myosepta on the sides of the body anterior to the anal fin, and small melanophores out-

**Table 4.** Characters for separating, eggs, yolk-sac larvae, post yolk-sac larvae, and juveniles of *Moxostoma robustum*, *M. carinatum*, and *M. collapsum* (Kay et al., 1994).

Characteristic	<i>Moxostoma robustum</i>	<i>M. carinatum</i>	<i>M. collapsum</i>
Eggs	Demersal; nonadhesive	Demersal; nonadhesive	Demersal; adhesive
Length at Hatching	7.2 to 8.1 mm TL	8.7 to 11.7 mm TL	9.0 to 10.2 mm TL
Preanal myomeres	36–37 at 9.7–10.45 mm* 35–38 at 11.2–12.9 mm 33–36 at 13.0–16.9 mm	33–38 at 10.2–14.7 mm 29–35 at 15.0–16.8 mm	34–37 at 9.0–10.2 mm 34–38 at 10.1–13.8 mm 31–35 at 14.0–16.7 mm
Postanal myomeres	6–7 at 9.7–10.45 mm* 5–7 at 11.2–12.9 mm 6–9 at 13.0–16.9 mm	5–9 at 10.2–14.7 mm 5–7 at 15.0–16.8 mm	6–7 at 9.0–10.2 mm 6–8 at 10.1–13.8 mm 6–8 at 14.0–16.7 mm
Total myomeres	42–44 at 9.7–10.45 mm* 42–45 at 11.2–12.9 mm 40–43 at 13.0–16.9 mm	40–45 at 10.2–14.7 mm 35–42 at 15.0–16.8 mm	40–43 at 9.0–10.2 mm 41–45 at 10.1–13.8 mm 38–42 at 14.0–16.7 mm

\* Myomeres unobservable until 9.7 mm TL.

lined scales on the caudal peduncle. Scales were outlined from the caudal fin to the head on larvae 21.9–22.5 mm TL.

**JUVENILE (TL RANGE=23 MM AND GREATER) MORPHOLOGY.**—Juveniles 23.5–28.8 mm TL showed squamation progressing. Body, head, and fin morphology was typical of other redhorses. Mouth was ventral, subterminal, horizontal, and small. The snout was rounded, about as long as the width of the eye. The anterior tip of the snout was at a position about equal to the lower margin of the eye.

Juveniles 72–100 mm TL had a mouth that was still ventral, subterminal, horizontal, and small. The body was elongate. Squamation was complete and a complete lateral line was apparent on 92 mm TL juveniles.

**JUVENILE (TL RANGE=23 MM AND GREATER) FIN DEVELOPMENT.**—Juveniles 23.5–28.8 mm TL had fin ray development apparently complete in all fins by 23.5 mm TL. The caudal fin was deeply forked and typical of redhorses. The anterior five or six rays of the dorsal fin were longer than the rest. On juveniles 28.8 mm TL, the urostyle still extended beyond the hypural plate. Juveniles 72–100 mm TL had 13 dorsal fin rays, 7 anal fin rays, and 18 caudal (primary) rays.

**JUVENILE (TL RANGE=23 MM AND GREATER) PIGMENTATION.**—Juveniles 23.5–28.8 mm TL had scattered pigmentation covering the dorsum of the head and the body. The head had scattered pigmentation laterally to the lower margin of the eye and on the snout to the mouth. The ventrolateral and ventral aspect of the head had little, if any, pigmentation. The body also had very little pigmentation

ventrolaterally and ventrally anterior to the anus. The pigmentation pattern consisted of small melanophores that outlined scales occurring mid-laterally and dorsolaterally, anterior to the anus, and laterally on the caudal peduncle.

Juveniles 30.0 mm TL had scales on the sides of the body that were outlined boldly with pigment. Very little body pigmentation was present ventrally from the ventral margin of the eye posteriorly to the anal fin. Pigment was scarce or lacking on the mouth or ventral surface of the head.

Juveniles 30.0–40.0 mm TL appeared to have about four or five bands of dark pigmentation forming dorsally and extending laterally down the side of the body; one or two anterior to the dorsal fin and two or three posterior to the dorsal fin.

Juveniles 72–100 mm TL were dusky dorsally and laterally, with four or five dark bands apparent laterally. The ventrum lacked pigmentation.

## Discussion

The spawning repertoire and early life history of *M. robustum* are similar to that of other redhorse species (Jennings et al., unpubl.). The ability to reliably distinguish *M. robustum* from sympatric congeners is critical to obtaining meaningful data about the reproductive success of this imperiled fish. Our results provide a description of larval *M. robustum* and identify morphological characters by which it can be distinguished from a sympatric congener. Of the morphological characters examined, length-at-hatching was the only morphological characteristic that can be used reliably to distinguish *M. robustum*, 7.2 to 8.1 mm TL, from *M. collapsum*, 9.0 to 10.2 mm TL

(Table 4). *Moxostoma collapsum* usually spawns much earlier in the year—March and April—and at much cooler temperatures, 11–15° C (Jenkins and Burkhead, 1993) than does *M. robustum*, which spawns from late April to early June at 19–24° C (Jennings et al., unpubl.). This difference and length at hatching are the best methods for differentiating these two species. Difference in length at hatching suggest that there also may be differences in morphological development at a given length (e.g. length at which yolk is absorbed). These differences may help to distinguish between the two species.

*Moxostoma carinatum* and *M. robustum* are the two largest species of *Moxostoma* found in the Southeast and both occur in Georgia, albeit in separate drainages. We include a distinguishing characteristic between these two species to alleviate any potential misidentification. *Moxostoma carinatum* can be distinguished from *M. robustum* primarily by geographical location and length at hatching. *Moxostoma robustum* has been found only in south Atlantic slope drainages whereas *M. carinatum* are restricted to Gulf of Mexico drainages. *M. robustum* are 7.2–8.1 mm TL at hatching, whereas *M. carinatum* are 8.7–11.7 mm TL at hatching (Table 4). Both species spawn at similar water temperatures though *M. carinatum* spawn from mid-April to mid-May (Jenkins and Burkhead, 1993), whereas *M. robustum* spawn from late April to early June (Evans et al., unpubl.).

#### Acknowledgments

J. Evans and W. Clark of the Georgia Department of Natural Resources supplied the broodfish from which the gametes were obtained. R. Wallus supplied the morphological measurements. D. Beyers, G. Pardue, and W. Wayman provided helpful comments on an early draft of the manuscript. A. Schroeer drafted the illustrations. B.

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# Systematics, Variation, and Speciation of the *Macrhybopsis aestivalis* Complex West of the Mississippi River.

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**ABSTRACT:** Eisenhour, D. J. Systematics, Variation, and Speciation of the *Macrhybopsis aestivalis* Complex West of the Mississippi River. *Bulletin of the Alabama Museum of Natural History*, Number 23:9–47, 8 tables, 17 figures. The systematics of the *Macrhybopsis aestivalis* complex (Cypriniformes: Cyprinidae) has been unclear due to confusing morphological variation across the range of the complex. Prior to this study, only one species with six subspecies was recognized. Morphometry, meristics, pigmentation, and tuberculation were examined throughout the western half of the range of the complex. A taxonomic revision is presented with redescriptions of four species, a key to the described species, distributional data, comparisons, and evaluation of contact zones and geographic variation. Analyses of the morphological data support the recognition of five species west of the Mississippi River: *M. aestivalis* in the Rio Grande basin and Río San Fernando drainage; *M. marconis* in the San Antonio, Guadalupe, and Colorado River drainages; *M. australis* in the upper Red River basin; *M. tetranema* in the upper Arkansas River basin; and *M. hyostoma* widespread in the Mississippi River basin and in streams of the West Gulf Slope. *Macrhybopsis hyostoma* is sympatric with *M. marconis* in the middle Colorado River mainstem, *M. australis* in the middle Red River mainstem, and *M. tetranema* in the central Arkansas River basin. Clinal variation along the length of the Rio Grande is exhibited by *M. aestivalis*, and considerable geographic variation is present in *M. hyostoma*.

A phylogeny of the *M. aestivalis* complex was estimated from 17 morphological characters. The monophyly of the *M. aestivalis* complex, a sister relationship of *M. australis* and *M. tetranema*, the monophyly of the remainder of the species of the *M. aestivalis* complex, and a sister relationship of *M. sp.* "Coosa chub" and *M. sp.* "Florida chub" each were supported by consensus and bootstrap trees and at least one character transformation free of homoplasy. Uncertain relationships among the remainder of the species may be attributed to considerable homoplasy among the characters used in the phylogenetic analysis and a pattern of speciation by multiple peripheral isolates. Considerable morphological variation in *M. hyostoma* may be due to periodic isolation of eastern and western populations during Pleistocene glacial advances and adaptation to local environmental conditions.

## Introduction

Members of the *Macrhybopsis aestivalis* (Cypriniformes: Cyprinidae) complex are characterized by black spots scattered over the dorsum of the body and 2–4 prominent maxillary barbels. Most members of this complex are highly adapted for life in turbid streams, possessing well developed barbels, reduced eyes, a well-developed olfactory area of the brain, and large taste buds covering the surface of the body (Hubbs, 1940b; Moore, 1950; Metcalf, 1966; Davis and Miller, 1967; Reno, 1969). The *M. aestivalis* complex occurs in medium to large streams from the

Río San Fernando drainage in Mexico eastward across the Gulf Slope to the Choctawhatchee River in Florida, and northward to Nebraska, Minnesota, and Ohio in the Mississippi River basin. Recent authors have generally recognized the complex as consisting of six subspecies of *M. aestivalis*: *M. a. aestivalis* in clear tributaries of the Rio Grande; *M. a. australis* in the Red River basin; *M. a. hyostomus* east of the Mississippi River; *M. a. marconis* in the San Marcos River; *M. a. sterletus* in the Rio Grande mainstem; and *M. a. tetranemus* in the Arkansas River basin

(Davis and Miller, 1967; Wallace, 1980; Becker, 1983). Prior to my studies, no critical review of the complex has been published although numerous authors have recognized that the *M. aestivalis* complex is polymorphic and badly in need of a detailed systematic analysis (Cross, 1967; Miller and Robison, 1973; Douglas, 1974; Wallace, 1980; Robison and Buchanan, 1988; Gilbert, 1992; Mayden et al., 1992; Etnier and Starnes 1993). Geographic variation in the complex has been documented for number of cutaneous taste buds (Moore, 1950), brain morphology (Davis and Miller, 1967), number of sensory pores (Reno, 1969), nasal rosette morphology (Branson, 1979), maxillary barbel length, eye size, and numbers of lateral-line scales and anal rays (Higgins, 1977).

Recognition of this variation by early workers led to descriptions of six taxa: *Gobio aestivalis* (Girard, 1856); *Ceratichthys sterletus* (Cope, in Cope and Yarrow, 1875); *Nocomis hyostomus* (Gilbert, 1884); *Hybopsis aestivalis marconis* (Jordan and Gilbert, 1886); *Hybopsis tetranemus* (Gilbert, 1886); and *Extrarius australis* (Hubbs and Ortenberger, 1929). Hubbs and Ortenberger (1929) recognized all six nominate taxa as species in the recently erected genus *Extrarius* (Jordan, 1919). Although no publication proposed reduction of the five junior taxa to subspecies, shortly thereafter authors began to refer to the taxa as subspecies (Greene, 1935; Hubbs, 1940a; Gerking, 1945; Eddy and Surber, 1947). Supporting data for taxonomic recognition of the taxa as subspecies were not provided in these publications. Moore (1950:82), listed six subspecies of *Extrarius aestivalis*: *E. a. aestivalis*; *E. a. australis*; *E. a. hyostomus*; *E. a. sterletus*; *E. a. tetranemus*; and an undescribed "plains subspecies" as "recognized by Dr. Hubbs." *Extrarius* and several other genera of barbeled minnows were subsequently consolidated into *Hybopsis* by Bailey (1951). The list of the six subspecies currently recognized first appeared in Davis and Miller (1967), again without comment. Higgin's (1977) unpublished study of geographic variation in the complex was the most comprehensive yet, but was unable to provide taxonomic clarification, in part because of limited geographic coverage. Subsequent phylogenetic analyses led to the breakup of *Hybopsis*, with the complex initially being placed in the monotypic genus *Extrarius* (Mayden, 1989), then in *Macrhybopsis* (Coburn and Cavender, 1992; Dimmick, 1993; Simons and Mayden, 1999). In the early 1990s Carter Gilbert and I agreed to divide a systematic study of the complex. The western populations became the topic of my Ph.D. dissertation (Eisenhour, 1997), while Gilbert, with R. L. Mayden, continued examining the eastern populations. Their studies of eastern populations have revealed the presence of at least three undescribed species from the East Gulf Slope: *M. sp.* "Mobile chub" from the Ponchartrain drainage to the lower Alabama drainage; *M. sp.* "Coosa chub" in the Alabama River drainage above the Fall Line; and *M. sp.* "Florida chub" from the Escambia to Choctawhatchee River drainages.

The portion of my dissertation that has been published (Eisenhour, 1999) redescribed one of the five valid species, *M. tetranema*, of the complex west of the Mississippi River.

A systematic analysis of the complex is critical for comparative studies of the unusual sensory systems exhibited by the *M. aestivalis* complex and other barbeled minnows. Although these barbeled minnows have been the focus of many comparative studies (Branson, 1963, 1979; Moore, 1950; Davis and Miller, 1967; Reno, 1969; Dimmick, 1988), the confusion regarding the relationships among the barbeled minnows has led to inappropriate comparisons resulting from misidentifications of taxa and incorrect assumptions of homology. Additionally, many western populations have been extirpated or undergone severe range reductions (Cross and Moss, 1987; Sublette et al., 1990; Luttrell et al., 1999). Conservation efforts can be assisted by clarification of the taxonomy of the complex.

Recent systematic studies of North American darters have revealed extensive geographic variation in their morphology, resulting in recognition of additional species (Page et al., 1992; Layman, 1994; Ceas and Page, 1997). Because most of these studied complexes of darters inhabiting small, upland streams, populations are largely isolated from one another, and the restricted gene flow tends to facilitate speciation (Page et al., 1992). The *M. aestivalis* complex also exhibits considerable geographic variation in morphology, but inhabits medium to large rivers, with no apparent physical barriers to gene flow among many populations. Examination of geographic variation and biodiversity in the *M. aestivalis* complex is critical in understanding the evolution and zoogeography of large stream fishes.

The primary objective of this study is to clarify the taxonomy of the western populations of the *M. aestivalis* complex. Here, I provide redescriptions of four of the five species occurring west of the Mississippi River. Included in the redescriptions are assessments of the validity of the nominate subspecies, diagnoses and descriptions of geographic variation in morphology, contact zones, and historical distributions. In addition, I provide a taxonomic key for the species west of the Mississippi River and a phylogenetic analysis of all species in the complex. Finally I discuss the speciation history and zoogeography of the complex.

## Methods

**MORPHOLOGICAL METHODS.**—Institutional abbreviations are from Leviton et al. (1985) and Poss and Collette (1995) except MOSU is used as the acronym for the Morehead State University Ichthyology Collection. Twelve meristic variables were collected from 2989 specimens and followed the methods of Hubbs and Lagler (1974) unless defined otherwise. Infraorbital and preoperculo-mandibular pore counts followed the methods of Reno (1969). Soft X-rays (3A, 30 mv, 15 sec) were used to make

vertebral counts, as defined by Jenkins and Lachner (1971). Belly squamation indices were scored following the methods of Eisenhour (1999).

Specimens that lacked obvious distortions or shriveling were chosen for morphometric analyses and encompassed a range of sizes, seasons, and localities within identified drainage units. Measurements were made under a dissecting microscope with a calipers to the nearest 0.1 mm and followed the methods of Hubbs and Lagler (1974). Morphometric data analyzed included 32 variables taken from 1201 specimens selected from the pool of specimens used in the meristic analyses. Measurements follow the methods of Eisenhour (1999) and use, in part, truss-geometric protocol (Bookstein et al., 1985) to archive body shape.

**ANALYTICAL METHODS.**—Univariate and multivariate analyses were performed with programs available in Statistical Analysis Systems 6.11 and as modified by David Swofford. To reduce error associated with small sample sizes, I combined samples from intradrainage or interdrainage localities into 45 drainage units. Populations and proximate drainages were combined when judged to exhibit no meaningful variation based on examination of Student's *t*-tests ( $\alpha = 0.01$ ), frequency tables of meristic variables, and principal component analyses (PCA). In the species descriptions, a frequency distribution expressed as 10–14 (8–17) indicates 90% of the counts are between 10–14, with a range of 8–17.

Twelve meristic variables were subjected to PCA. Principal components were factored from a correlation matrix of the 12 nontransformed variables from 2256 individuals. Individuals with missing variables were not included in the PCA. Multivariate analyses of the morphometric data were accomplished with sheared PCA (Humphries et al., 1981; Bookstein et al., 1985) to eliminate overall size effects. Principal components were factored from the covariance matrix of 32 log-transformed morphometric characters from 1201 specimens following recommendations of Bookstein et al. (1985). Males and females were subjected separately to sheared PCA because of sexual dimorphism. For brevity, loadings of PCA and sheared PCA analyses are summarized in the text and figures. Complete lists of loadings are reported in Eisenhour (1997).

In order to examine the intraspecific and interspecific variation within and among taxa of the *M. aestivalis* complex several sets of data were subjected to PCA or sheared PCA by modifying the geographic area and number of taxa examined. For analyses comparing relatively few individuals (<300) from limited geographic areas, PC scores from individual specimens were examined. To facilitate interpretation of large data sets, means of PC scores or sheared PC scores for drainage units were analyzed. Although using means eliminates variation of individuals within drainage units, it does allow trends and patterns in the data to be summarized (Matthews, 1987). The

approach of Matthews (1987) was used to determine if some individuals from some localities could be distinguished in principal component space, a necessary criterion in using mean scores instead of individual scores.

Spatial autocorrelation analysis (SAA) tests whether values of a variable are spatially (geographically or temporally) independent (Sokal and Oden, 1978). This type of analysis has been useful in detecting clinal and other complicated patterns of geographic variation in *Menidia beryllina* (Chernoff, 1982), *Fundulus zebrinus* (Poss and Miller, 1983), and *Aphredoderus sayanus* (Boltz and Stauffer, 1993). SAA was performed on specimens from 47 localities in the Rio Grande basin and the Río San Fernando drainage using programs available in Legendre and Vaudor (1991). For each locality, latitude and longitude were determined to the nearest degree and sample character means were used as variables. Ten variables were subjected to SAA including meristic PC 1 scores, morphometric sheared PC 2 scores (males and females), and seven characters with high PC or sheared PC loadings, predorsal scales, lateral-line scales, caudal peduncle scales, posterior belly squamation, anterior belly squamation, barbel length, and caudal peduncle depth. The adjacency matrix used in SAA characterizes a fully-connected graph in which each node (locality) is connected to all others. Edge lengths (distances between localities) were weighted by their geographic distance based on arc lengths. Comparisons were made at 100 km intervals. This grouping provided at least 12 pairs in each distance class, and all but the three greatest distance classes had over 50 pairs.

**PHYLOGENETIC ANALYSIS.**—Phylogenetic trees were constructed under the principle of maximum parsimony (Wiley, 1981) using outgroup comparison (Maddison et al., 1984) with PAUP\* 4.0b10 (Swofford, 2002). The branch-and-bound algorithm was used to find all minimum-length and near minimum-length trees. The bootstrap procedure (Felsenstein, 1985) was performed using the branch-and-bound option (1000 replications) to assess the confidence of relationships implied by the most parsimonious trees. Both ACCTRAN and DELTRAN optimization schemes were used for examination of character state reconstructions of minimum-length and near minimum-length trees.

*Macrhybopsis gelida* and *M. meeki* were chosen as outgroup taxa in the phylogenetic analysis based on phylogenies from Mayden (1989), Coburn and Cavender (1992), and Dimmick (1993). Based on Coburn and Cavender's (1992) hypothesis that *M. gelida* is sister to the *M. aestivalis* complex, trees were rooted using only *M. meeki*. This tested the monophyly of the *M. aestivalis* complex by essentially treating *M. gelida* as part of the ingroup taxa.

Character descriptions of 17 discretely coded morphological characters used in the phylogenetic analyses are given in Appendix 1, and the data matrix appears in Appendix 2. Meristic characters were avoided because of

extensive overlap of frequency distributions among species. Characters that showed intraspecific variation were usually defined to encompass the entire range of variation within each species. When this was impossible, species were coded as having multiple states. Although including polymorphic characters may increase homoplasy, it greatly increases the accuracy of phylogenetic analysis (Wiens and Servedio, 1997). Generalized parsimony (Swofford and Olson, 1990) was employed to allow PAUP to assign polymorphic states to internal nodes. Characters were recoded using stepmatrices and methods described in Maddison and Maddison (1992) and Mabee and Humphries (1993). This allowed homology information among character states to be used, while retaining a biologically realistic definition of characters (Mabee and Humphries, 1993). Characters 5, 8, and 17 were treated as ordered because one character state was intermediate between the other two character states (Maddison and Maddison 1992). The sequence of transformation for the remaining characters with more than two states (characters 1 and 4) could not be determined, and these were treated as unordered. To determine how alternate assumptions of character evolution altered implied relationships among species in the complex a second analysis that treated all characters as unordered was performed.

### *Macrhybopsis aestivalis* complex

**DIAGNOSIS.**—Distinguished from all other North American cyprinids except the species of *Oregonichthys* by the combination of random, medium-sized melanophores on the dorsolateral surface of the body, giving a speckled appearance, and one or more pairs of distinct barbels. Differs from species of *Oregonichthys* in having an inferior mouth (terminal or subterminal in species of *Oregonichthys*) and longer barbels (0.02–0.07 standard length [SL] vs. 0–0.01 SL).

**DESCRIPTION.**—Dorsal rays 8; principal caudal rays 19 (15–21); anal rays 7–8 (6–10); pelvic rays 8 (6–9); pectoral rays 13–16 (11–18). Lateral-line scales 32–48; scales above lateral line 4–6 (4–7); scales below lateral line 4–5 (3–7); caudal peduncle scales 12–16 (11–18). Vertebrae 33–39. Lateral line complete; infraorbital canal pores 12–17 (9–20); preoperculomandibular canal pores 10–14 (8–17); supraorbital canal pores 6–11. Breast naked; belly and nape naked to fully scaled.

Individuals small (<75 mm SL); body shape terete to fusiform; mouth inferior and horizontal, with 1–2 pairs of prominent maxillary barbels. Large, prominent compound taste buds present over entire surface of body and fins, enlarged into papillae on interradial membranes of fins and, often, on undersurface of head. Gill rakers absent or rudimentary (4–6 in *M. sp.* “Coosa chub”). Pharyngeal teeth 0,4–4,0 (modally 1,4–4,1 in *M. sp.*

“Coosa chub”), slender, hooked, with little or no grinding surface. Peritoneum silvery; intestine with single S-shaped loop. Nuptial males with tubercles arranged uniserially, with one tubercle per fin ray segment, or biserially, with two tubercles per fin ray segment, on dorsal surface of thickened pectoral rays 2–10 (tubercles minute and rays barely thickened in *M. sp.* “Coosa chub”).

Body translucent in life, olive or gray dorsally, silvery-white ventrally with silver lateral stripe. Black spots, from scale-size to slightly larger than background melanophores, present on dorsolateral surface of body. Lateral stripe absent to well-developed, centered on lateral line to just above lateral line. Ventral surface of body usually lacking pigment except for few characteristic scattered medium-sized melanophores. Dorsal-fin origin over pelvic-fin origin. Caudal fin with white ventral margin and light to moderate pigment on caudal rays. No chromatic colors present except in nuptial male *M. marconis*, which have lemon-yellow pectoral fins.

**SEXUAL DIMORPHISM.**—In addition to lacking tubercles, females typically attain a larger maximum size. Analysis of sexual dimorphism in 14 males and 15 females of *M. aestivalis* from the Rio San Fernando drainage is presented as representative of the complex. Sheared PCA separated males and females into nearly nonoverlapping clusters, with most separation occurring on the sheared PC 2 axis (Fig. 1). Loading values indicated males were less robust and had longer fins. Other populations and taxa of the complex have similar patterns of sexual dimorphism (Eisenhour, 1997).

**HABITAT.**—All species occupy flowing water over coarse sand and fine gravel substrates in medium to large streams. Although most species are typically found in race-

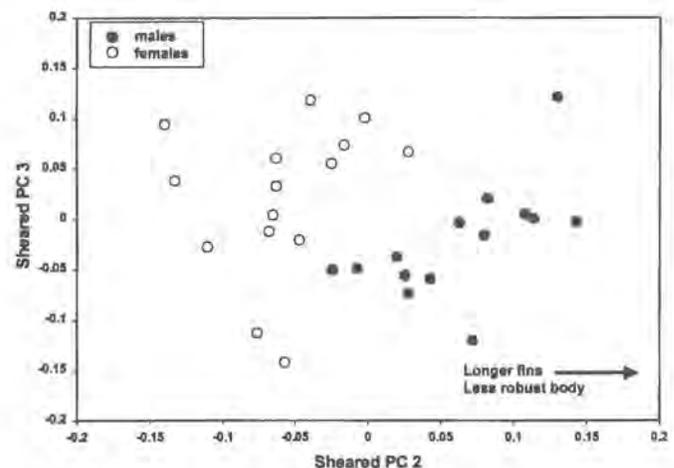


Figure 1. Morphometric scores on sheared PC axes 2 and 3 for 14 male and 15 female *Macrhybopsis aestivalis* from the Rio San Fernando drainage.

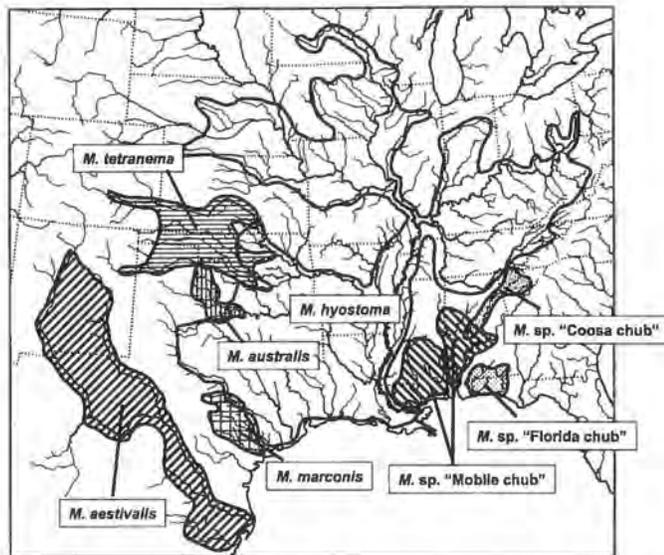


Figure 2. Distribution of the *Macrhybopsis aestivalis* complex.

ways and runs, I found *M. marconis* most abundant in riffles over large gravel and cobble. Two species, *M. australis* and *M. tetranema*, occupy intermittent streams that may dry to isolated, salt-encrusted pools. Echelle et al. (1972) recorded *M. australis* from waters with salinities up to 19.6 parts per thousand.

**KEY TO THE SPECIES OF THE *MACRHYBOPSIS AESTIVALIS* COMPLEX.**—On the basis of data collected during this study, I recognize five species in the *M. aestivalis* complex west of the Mississippi River (Fig. 2). The following key can be used to identify these species. Following the key are redescriptions of four of these five species [*M. tetranema* was redescribed by Eisenhour (1999)]. Synonymies presented list only original descriptions. Complete synonymies for all species can be found in Eisenhour (1997).

- 1a. One or two pairs of barbels present, posterior barbels usually < orbit length, anterior barbels absent or < 50% of orbit length; pectoral-fin ray tuberculation of nuptial males uniserial, with 1–2 rows of tubercles at midsection of rays (Fig. 3A); lips not fleshy and greatly expanded posteriorly. . . . . 2
- 1b. Two prominent pairs of barbels present, posterior barbels usually > orbit length, anterior barbels usually > 50% of orbit length; pectoral fin-ray tuberculation of nuptial males usually biserial on primary branches, with 3–4 rows of tubercles at midsection of rays (Fig. 3B); lips fleshy and greatly expanded posteriorly. . . . . 4
- 2a. Nuptial males without tubercles on head; lateral stripe absent or darkest on caudal peduncle and fading anteriorly (Fig. 4A–B, D–E) . . . . . 3
- 2b. Nuptial males with tubercles on head; prominent lateral stripe present from base of caudal fin to operculum (Fig. 4F) . . . . . *M. marconis*

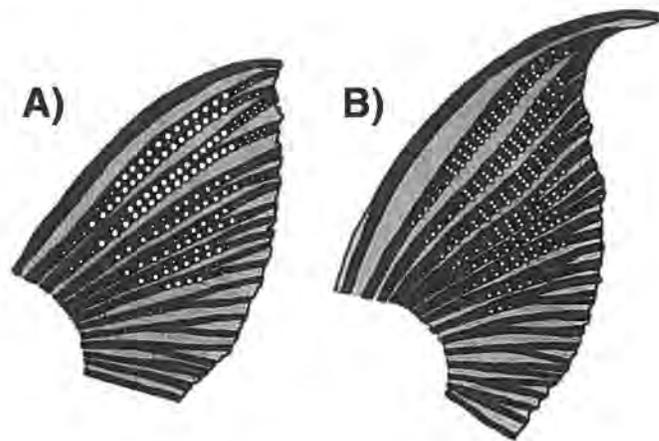


Figure 3. Nuptial male right pectoral-fin tuberculation. A) uniserial, *Macrhybopsis hyostoma* (41 mm SL, SIUC 26485 Sabine River, Panola County, Texas, 26 June 1996). B) biserial, *M. australis* (50 mm SL, SIUC 24730 Salt Fork Red River, Greer County, Oklahoma, 7 August 1995).

- 3a. Eyes round or nearly so (orbit depth > 80% of orbit length); usually with 20–50 dorsolateral scales bearing clusters of melanophores; small melanophores never concentrated on dorsolateral scale margins or submargins; lateral stripe absent (Fig. 4A–B) . . . . . *M. aestivalis*
- 3b. Eyes oval (orbit depth < 80% of orbit length); few (1–10) or no dorsolateral scales with clusters of melanophores; small melanophores often concentrated on dorsolateral scale margins or submargins; lateral stripe usually present (Fig. 4D–E) . . . . . *M. hyostoma*
- 4a. Anal rays modally 7; pectoral fins of adult males extending beyond pelvic bases; vertebrae 34–36. . . . . *M. australis*
- 4b. Anal rays modally 8; pectoral fins of adult males just reaching pelvic bases; vertebrae 36–39. . . . . *M. tetranema*

**Species descriptions**

*Macrhybopsis aestivalis* (Girard)

Speckled Chub  
FIG. 4A–B

*Gobio aestivalis* Girard 1856:189 (Río San Juan at Cadereita, Nuevo León, Mexico).

*Ceraticthys sterletus* Cope in Cope and Yarrow 1875:652, Pl. XXXVII, Figs. 3, 3a (Río Grande at San Ildefonso, New Mexico).

Following the original descriptions of *Gobio aestivalis* (Girard, 1856) and *Ceraticthys sterletus* (Cope, in Cope

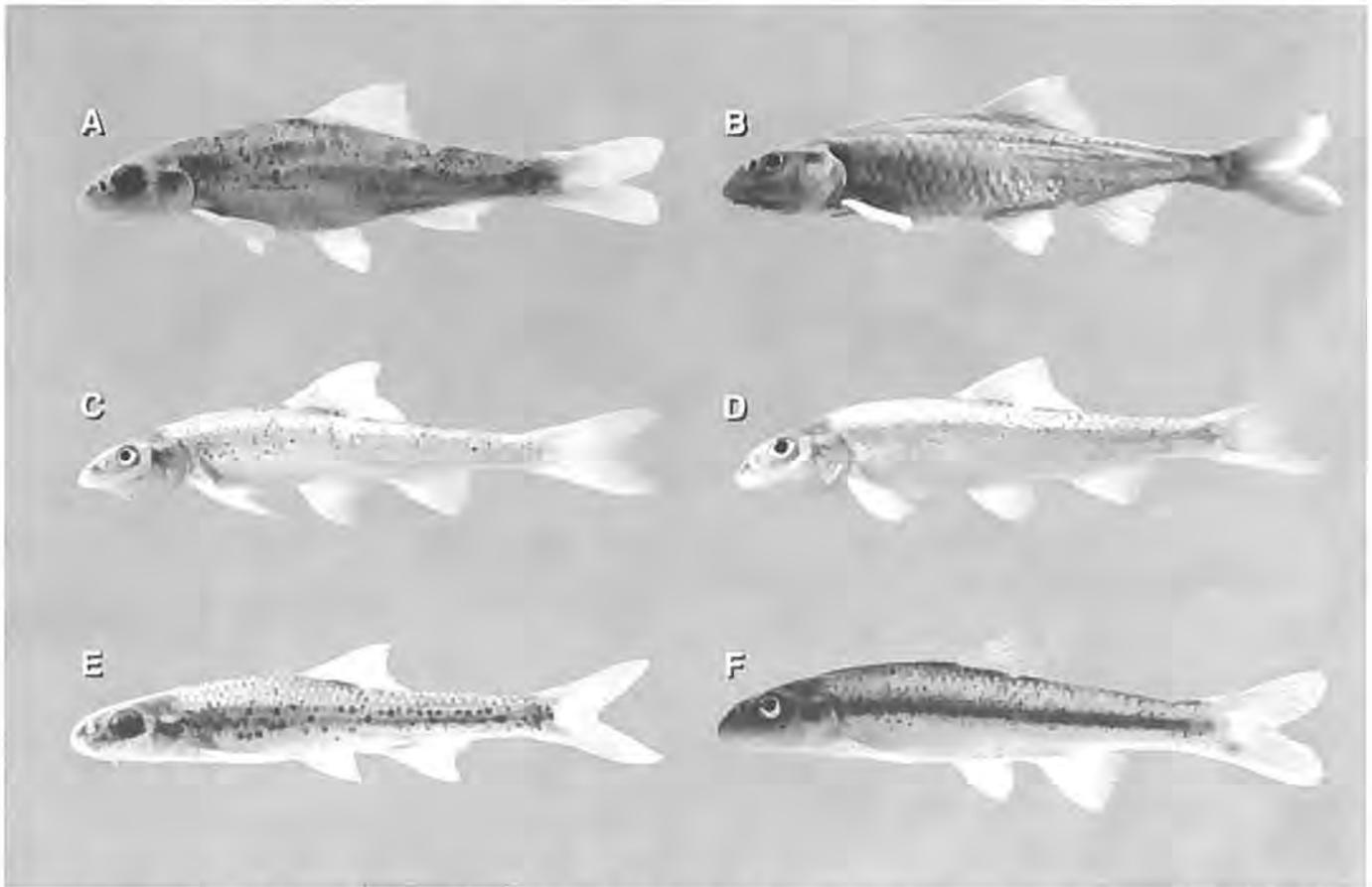


Figure 4. A) *Macrhybopsis aestivalis* (female, 50 mm SL, UMMZ 97389 Río San Juan, Nuevo León, Mexico, 16 April 1930). B) *M. aestivalis* (female, 75 mm SL, MSB 1875 Rio Grande, Bernalillo County, New Mexico, 14 September 1941). C) *M. australis* (male, 51 mm SL, SIUC 24730 Salt Fork of Red River, Greer County, Oklahoma, 7 August 1995). D) *M. hyostoma* (female, 48 mm SL, SIUC 26042 Red River, Jefferson County, Oklahoma, 29 June 1996). E) *M. hyostoma* (male, 43 mm SL, UT 44.5712 French Broad River, Knox County, Tennessee, 28 July 1992). F) *M. marconis* (male, 50 mm SL, SIUC 26492 San Marcos River, Caldwell County, Texas, 27 June 1996).

and Yarrow, 1875), Jordan (1885a, b) synonymized *sterletus* and placed *aestivalis* in the genus *Hybopsis*, subgenus *Erinemus*. Jordan and Evermann (1896) later transferred *H. aestivalis* to the subgenus *Erinystax* and expanded the range of *E. aestivalis* to include populations from the Rio Grande to the Arkansas River. Subsequently, both *aestivalis* and *sterletus* were elevated to species and placed in either the genus *Extrarius* (Hubbs and Ortenberger, 1929) or *Macrhybopsis* (Jordan, 1930). A manuscript concerning the validity of *sterletus* was drafted by C. L. Hubbs and M. Gordon (Hubbs, 1940a:5) but never published. Moore (1950:82) treated both nominal species as subspecies in the genus *Extrarius*, apparently following unpublished studies by "Dr. Hubbs." Recent authors have generally followed Moore in recognizing two subspecies in the Rio Grande: *Hybopsis aestivalis aestivalis* in clear Rio Grande tributaries and *M. a. sterletus* in the Rio Grande mainstem (Davis and Miller 1967, Wallace 1980, Becker, 1983). Higgins (1977), in a study of geographic variation in the morphology of the *M. aestivalis* complex, concluded *M. a.*

*aestivalis* and *M. a. sterletus* were identical and only recognized *M. a. aestivalis* as a valid subspecies. However, the only specimens examined were from geographically proximate areas in the middle Rio Grande basin, not near either of the type localities of *Ceratichthys sterletus* Cope or *Gobio aestivalis* Girard.

Although populations from the upper Rio Grande basin are well differentiated in several morphological characters from populations in the lower Rio Grande basin and Río San Fernando drainage, populations in the central portion of the range of *M. aestivalis* are intermediate in these characters. Numerous characters show concordant geographic patterns of clinal variation along the length of the Rio Grande. Herein, populations in the Rio Grande basin and Rio San Fernando drainage are treated as a single species, *M. aestivalis*. *Ceratichthys sterletus* (Cope) is considered here a junior synonym of *M. aestivalis*.

**TYPES.**—The holotype of *Gobio aestivalis* Girard (USNM 79 [39.5 mm SL], Río San Juan, near Cadereita, Nuevo

León, Mexico, winter 1852–1853, by D. N. Couch) is apparently no longer extant. Jordan (1885a) reported examining the type of *Gobio aestivalis* at USNM, but later Jordan and Evermann (1896) did not list an extant type, suggesting it may have been lost by that time.

The description, though brief, seems to be based on what is referred to currently as *M. aestivalis*, as it is the only barbeled minnow in the Río San Juan. However, the illustration of the holotype (Fig. 17, Plate LVII in Girard, 1858), clearly is not *M. aestivalis*. The illustration departs from typical *M. aestivalis* from the Río San Juan in several characters: 1) lack of spots; 2) lack of barbels; 3) terminal mouth; 4) tiny eyes; and 5) pointed snout. Despite this dubious illustration of the holotype, a second illustration of the holotype (Fig. 18 in Girard, 1858), depicting the underside of the head with an inferior mouth and barbels is clearly *M. aestivalis*. In addition, Jordan (1885a) reported comparing the holotype with USNM 16978 (which does contain *M. aestivalis*) and decided they were the same species. Although one illustration of the holotype appears to be erroneous, I believe the original description to be based on what is currently referred to as *M. aestivalis*. Extant topotypes: FMNH 4406 (14); UMMZ 97389 (10).

Nine specimens were originally in the syntype series of *Ceratichthys sterletus* Cope (USNM 16978, Rio Grande at San Ildefonso, New Mexico, August 1874, E. D. Cope and H. C. Yarrow, collectors). Two of these specimens are *Notropis jemezianus*, one specimen is *Cyprinella lutrensis* (Gilbert, 1998), and two specimens have been lost, leaving four extant specimens (40.2–59.1 mm SL) that conform to the description of *C. sterletus*. An additional specimen (MCZ 35925) labeled as a syntype of *C. sterletus* is *N. jemezianus* (Gilbert, 1998). The original description was based on a single large specimen “.075 m” [=75 mm total length (TL)] that is extant. The substantial morphological variation within *M. aestivalis* and the presence of several species in the syntype series of *C. sterletus* warrants designation of a lectotype. I designate the original 75 mm TL specimen (USNM 16978, female, 59.1 mm SL, Rio Grande at San Ildefonso, New Mexico, August 1874, E. D. Cope and H. C. Yarrow, collectors) as lectotype. The remaining three specimens of *C. sterletus* (now USNM 345460; 40.2–49.2 mm SL) become paralectotypes.

**DIAGNOSIS.**—Distinguished from all other members of the *M. aestivalis* complex by the combination of a single pair of barbels, round eyes (orbit depth > 80% of orbit length), absence of a lateral stripe, melanophores not concentrated on scale margins or submargins of dorsolateral scales, and usually bearing many dorsolateral scales (>20) with clusters of small melanophores. Additional distinguishing characteristics include: anal rays modally 8; tubercles lacking on head of nuptial males; and pectoral ray tubercles uniserial.

**DESCRIPTION.**—Selected meristic counts for 745 total

specimens appear in Tables 1–8. Largest female 73.1 mm SL (90 mm TL, UMMZ 133234); largest male 60.6 mm SL (77 mm TL, UMMZ 178711). Principal caudal rays 19 (16–21); anal rays 8 (7–9); pelvic rays 8 (6–9); pectoral rays 14–16 (11–18). Lateral-line scales 34–38 (31–42); predorsal scales 0–19; scales above lateral line 4–5 (4–6); scales below lateral line 4–5 (3–6); caudal peduncle scales 12–14 (12–18). Nape and belly fully scaled to naked. Infraorbital pores 13–17 (10–19); preoperculo-mandibular pores 10–13 (9–17). Total vertebrae 35–37 (34–38); precaudal vertebrae 17–19 (16–19); caudal vertebrae 17–19 (16–19).

Body terete and often robust anteriorly; snout rounded and blunt. Mouth horizontal and inferior; gape width not as wide as head when viewed ventrally. Lips only moderately fleshy. One pair of maxillary barbels present, variable in length. Eye round or nearly so, variable in size. Taste buds enlarged into barbel-like papillae on the gular region. Genital papillae absent or poorly developed as small, conical flap. Anus near base of anal fin. Gill rakers absent or present as 1–4 dorsal rudiments. Pharyngeal teeth 0,4–4,0.

Life colors not observed, but published descriptions (Sublette et al., 1990) are similar to those of other species of the complex. Most specimens with clusters of small melanophores concentrated on single scales. Other small melanophores randomly scattered over dorsolateral surface of body, not concentrated on margins or submargins of scales. Lateral stripe absent. Dorsal rays weakly outlined with pigment; pigment darkest basally on first three rays. Pectoral fin with some pigment on rays 1–7 or absent; pigment absent in pelvic and anal fins.

Pectoral rays 2–8 to 2–10 greatly thickened in nuptial males, with conical, slightly antrorse curved uniserial tubercles (Fig. 3A). Minute tubercles present on dorsal, anal, and pelvic rays of large (>50 mm SL) nuptial males in peak breeding condition. Cephalic and predorsal sensory papillae more pronounced on nuptial males, but not cornified into tubercles. Females lack tubercles, although a few large specimens (>55 mm SL) examined had slightly thickened pectoral rays.

**GEOGRAPHIC VARIATION.**—Specimens of *M. aestivalis* from the southeast part of its range (Río San Fernando, San Juan, and Salado drainages) differed from specimens from the upper Rio Grande mainstem in several meristic and morphometric characters. Upper Rio Grande specimens had a smaller orbit length (0.050–0.065 SL vs. 0.065–0.080 SL) and caudal peduncle depth (0.070–0.090 SL vs. 0.100–0.115 SL), longer barbels (0.045–0.065 SL vs. 0.025–0.050 SL), more lateral-line scales (35–38 vs. 33–36), caudal peduncle scales (12–16 vs. 12) and vertebrae (36–37 vs. 34–36), and less belly squamation (naked vs. some scales present) and predorsal squamation (scales absent or embedded vs. fully exposed). Multivariate analyses of the meristic and morphometric data sets grouped

Table 1. Frequency distribution of anal rays and pectoral rays in four species of the *Macrhybopsis aestivalis* complex.

Species/Drainage unit	anal rays									pectoral rays									
	6	7	8	9	10	n	mean	SD	11	12	13	14	15	16	17	18	n	mean	SD
<i>Macrhybopsis aestivalis</i>																			
San Fernando		4	97	3		104	7.99	0.26		1	2	17	40	36	7	1	104	15.28	0.98
San Juan		2	48	1		51	7.98	0.24			1	12	22	13	2		50	15.06	0.87
Salado		1	69			70	7.99	0.12			2	10	28	25	5		70	15.30	0.91
Lower R. Grande		5	91	3		99	7.98	0.28		1	2	12	49	29	6		99	15.22	0.89
Lower middle R. Grande		4	111	3		118	7.99	0.24			5	32	55	22	3		117	14.88	0.85
Pecos		3	70	2		75	7.99	0.26		1	3	21	31	13	1		75	14.87	0.92
Upper middle R. Grande		5	104			109	7.95	0.23		3	4	36	48	15	2		108	14.69	0.92
Upper R. Grande		2	113	3		118	8.01	0.21	1	-	6	23	62	26			118	14.89	0.87
<i>Macrhybopsis australis</i>																			
Upper Red	1	83	7			91	7.07	0.29		2	9	43	31	5	1		92	14.34	0.86
Middle Red	1	54	1			56	7.00	0.19			7	31	15	1			54	14.19	0.68
Wichita		58	6			64	7.09	0.29		1	11	25	25	2			64	14.25	0.84
<i>Macrhybopsis hyostama</i>																			
Colorado		5	84			89	7.94	0.23		4	6	37	28	12	1		88	14.47	1.01
Brazos		10	115			125	7.92	0.27			15	48	48	11	-	1	123	14.48	0.88
Middle Red		21	73			94	7.78	0.42			10	38	35	11			94	14.50	0.84
Washita		11	21	1		33	7.70	0.53		1	6	16	7	3			33	14.15	0.94
Lower Red		11	66			77	7.86	0.35		2	7	30	31	7			77	14.44	0.88
Canadian		3	21	1		25	7.92	0.40			3	12	8	1	1		25	14.40	0.91
Cimarron		6	33			39	7.85	0.37		1	5	13	13	1	1		34	14.32	0.98
Salt Fork Arkansas		13	58	1		72	7.83	0.41			4	27	30	11			72	14.67	0.80
Arkansas		10	69			79	7.87	0.33	1	-	3	26	34	13	2		79	14.76	0.95
Platte-Elkhorn		24	46			70	7.66	0.48		2	13	31	21	1	1		68	14.01	0.82
Blue-Republican		6	43	1		50	7.90	0.36		3	13	30	4				50	13.70	0.71
Kansas		3	72	1	1	77	8.00	0.32	1	4	18	38	13	2			76	13.84	0.91
Grand-Chariton-Osage		13	59	1		73	7.84	0.41		1	14	27	24	7			73	14.30	0.94
Upper Missouri		5	28	1		34	7.88	0.41		2	5	18	5	3			30	14.06	0.80
Lower Missouri		6	39			45	7.87	0.34			6	16	15	7	1		45	14.58	0.99
Iowa-Des Moines		9	10			19	7.53	0.51				11	5	3			19	14.58	0.77
Lower Mississippi		14	116	2		132	7.91	0.34			7	35	55	33	1		132	14.92	0.91
Upper Mississippi		15	44			59	7.75	0.44			6	29	17	5	1		58	14.41	0.86
Middle Mississippi		2	41			43	7.95	0.21		1	5	16	16	5			43	14.44	0.93
Illinois		1	18			19	7.95	0.23			2	9	7	1			19	14.37	0.76
Wabash		7	89			96	7.93	0.26			6	18	48	20	3		95	14.96	0.89
Sabine-Calcasieu		9	106	2		117	7.94	0.30		1	6	52	39	18	1		117	14.60	0.87
Ouachita		3	36			39	7.92	0.27			4	16	14	5			39	14.51	0.85
White-St. Francis		12	80	1	1	94	7.90	0.42	1	3	21	49	17	4			95	13.95	0.89
Upper Ohio		4	51			55	7.93	0.26			1	18	29	5			53	14.72	0.66
Licking		3	39			42	7.93	0.26			2	11	23	6			42	14.79	0.75
Kentucky		1	16			17	7.94	0.24				8	8	1			17	14.59	0.62
Green		2	42	1		45	7.98	0.26			1	11	27	5	1		45	14.87	0.73
Tennessee			24	1		25	8.04	0.20			1	6	14	3			24	14.79	0.72
<i>Macrhybopsis marconis</i>																			
Guadalupe-San Antonio		6	136	1		143	7.97	0.22		3	34	75	28	3			143	13.96	0.78
Colorado			27			27	8.00	0.00			8	19					27	13.70	0.47

Table 2. Frequency distribution of lateral-line scales in four species of the *Macrhybopsis aestivalis* complex.

Species/Drainage unit	31	32	33	34	35	36	37	38	39	40	41	42	43	44	n	mean	SD
<i>Macrhybopsis aestivalis</i>																	
San Fernando	1	4	18	43	27	4									97	34.06	0.94
San Juan			2	11	21	11	1	1							47	35.02	0.97
Salado			1	14	28	16	3								62	35.10	0.86
Lower R. Grande			2	15	49	21	7								94	35.17	0.86
Lower middle R. Grande				12	50	40	12	1	1						116	35.50	0.91
Pecos				2	10	27	23	9	3						74	36.49	1.09
Upper middle R. Grande				6	20	36	11	12	1	1					87	36.11	1.21
Upper R. Grande				1	9	42	32	22	7	3	-	1			117	36.89	1.27
<i>Macrhybopsis australis</i>																	
Upper Red					6	5	10	16	14	10	5	2	1		69	38.35	1.85
Middle Red				1	1	3	10	10	9	4	3	4			45	38.42	1.88
Wichita				1	2	3	14	10	15	12	3	2	1	1	64	38.58	1.88
<i>Macrhybopsis hyostoma</i>																	
Colorado			3	9	17	20	15	3	2	1					70	35.81	1.42
Brazos				14	27	48	12	14	2	1					118	35.96	1.26
Middle Red				4	16	25	25	5	6	3					84	36.49	1.38
Washita				1	3	3	6	3	3	2	-	1			21	37.29	1.95
Lower Red			1	2	12	26	15	11	2	4					73	36.55	1.43
Canadian			1	-	3	7	5	3	1						28	37.61	1.79
Cimarron				1	8	5	4	5	-	1	-		1		25	37.56	1.83
Salt Fork Arkansas				1	4	17	20	9	7	4	1	1			64	38.25	1.55
Arkansas				1	16	18	14	11	3	1					64	37.48	1.30
Platte-Elkhorn			1	-	8	15	22	11	5	4	1				67	37.03	1.49
Blue-Republican				5	11	13	8	5	1	1					44	37.09	1.35
Kansas				1	4	20	23	16	4	2					70	36.99	1.17
Grand-Chariton-Osage			1	1	7	19	12	5	6	1					52	36.62	1.42
Upper Missouri				1	3	8	5	2							19	36.21	1.03
Lower Missouri				1	3	9	15	2	2						32	37.47	1.39
Iowa-Des Moines				1	1	5	8	2	1						18	36.67	1.14
Lower Mississippi			1	4	19	27	37	20	6	2	1				117	36.67	1.38
Upper Mississippi				4	12	9	3	2							30	35.57	1.07
Middle Mississippi			1	-	7	11	5								24	35.79	0.93
Illinois				1	4	4	-	1	1						11	36.91	1.45
Wabash				1	9	22	30	9	7						78	36.74	1.13
Sabine-Calcasieu		1	3	25	42	24	10	1	1						102	35.16	1.12
Ouachita				5	6	5	3	1							20	35.45	1.19
White-St. Francis				5	23	22	8	6	3	-	-	1			68	36.03	1.43
Upper Ohio				1	2	8	8	6	2						27	36.81	1.21
Licking				1	7	11	5	5	1						30	37.30	1.21
Kentucky					3	2	3	4	1						13	37.95	1.34
Green					12	14	9	2							37	37.03	0.90
Tennessee				5	6	7	1	2							21	36.48	1.21
<i>Macrhybopsis marconis</i>																	
Guadalupe-San Antonio				12	48	44	25	3							132	36.69	0.96
Colorado				10	8	5	1								24	35.88	0.90

Table 3. Frequency distribution of predorsal scales in four species of the *Macrhybopsis aestivalis* complex.

Species/Drainage unit	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	n	mean	SD	
<i>Macrhybopsis aestivalis</i>																										
San Fernando										1	1	8	15	21	24	14	10	2	3					99	13.68	1.76
San Juan							1	—	—	—	—	5	9	16	8	8	1	1						49	13.14	1.81
Salado											2	2	4	17	16	7	9	4	1					62	14.03	1.71
Lower R. Grande									1	—	2	6	22	17	26	11	7	4	—	1				97	13.47	1.77
Lower middle R. Grande	1	—	1	2	3	2	4	6	4	3	10	12	23	16	10	5	7	3						112	11.15	3.48
Pecos	1	—	—	—	—	2	—	4	2	1	2	6	8	12	12	10	12	1						73	12.71	3.18
Upper middle R. Grande						1	4	—	2	4	5	9	17	19	20	12	5	1	—	1				100	12.57	2.50
Upper R. Grande	21	7	14	12	7	3	4	6	3	5	5	5	4	2	6	5	3	1						113	5.77	5.18
<i>Macrhybopsis australis</i>																										
Upper Red	1	1	—	1	3	4	1	2	8	7	8	4	5	5	4	9	12	9	—	1				85	11.61	4.37
Middle Red	2	—	6	—	1	4	2	—	6	3	1	1	4	5	4	5	2	—	1	2				49	9.71	5.25
Wichita	1	1	3	1	5	3	2	1	5	5	9	4	5	4	8	7								64	9.56	4.17
<i>Macrhybopsis hyostoma</i>																										
Colorado										2	—	4	4	17	16	17	12	9	2	5				88	14.61	2.11
Brazos			1	—	—	—	—	1	2	1	9	6	14	21	27	21	11	4	3	1				122	13.45	2.41
Middle Red											2	3	11	20	21	19	11	2	1	2				92	14.02	1.72
Washita											1	—	—	3	9	11	2	2	1	1				30	14.77	1.65
Lower Red												3	6	19	19	6	5	2						76	14.14	1.57
Canadian											1	1	—	4	9	4	1	—	1					21	13.94	1.63
Cimarron										1	—	2	—	6	11	8	5	1	1					35	14.23	1.72
Salt Fork Arkansas													4	10	12	22	8	5	4	1			66	14.85	1.60	
Arkansas												1	2	11	14	25	15	3	2				73	14.74	1.35	
Platte—Elkhorn								2	3	2	1	5	6	13	11	14	7	3	3				70	13.47	2.56	
Blue—Republican								3	1	2	2	2	1	10	13	6	3	1					44	12.91	2.51	
Kansas			2	3	—	2	2	4	1	9	5	6	6	10	15	7	—	3	2				77	11.32	3.77	
Grand—Chariton—Osage								2	1	—	2	2	3	19	15	11	8	4	4				77	13.94	2.26	
Upper Missouri						1	—	2	1	1	1	—	2	4	7	5	4	2					30	13.10	3.12	
Lower Missouri											1	1	5	7	11	6	3	2	1				37	13.95	1.70	
Iowa—Des Moines							1	1	1	1	—	1	3	5	1	2	1						17	11.88	2.85	
Lower Mississippi											1	1	7	15	36	30	16	12	5	1	1		125	14.74	1.66	
Upper Mississippi													2	2	8	10	7	7	1	—	1		38	15.29	1.64	
Middle Mississippi													5	9	8	8	1	3	2				36	14.22	1.68	
Illinois														3	4	3	—	1	1				12	14.58	1.56	
Wabash											2	—	10	18	23	18	10	6	—	—	1	1	89	14.26	1.79	
Sabine—Calcasieu											1	4	7	20	28	36	12	6	1	1			116	14.33	1.55	
Ouachita													1	4	9	3	4	5	1	1	1	1	30	15.40	2.19	
White—St. Francis											1	1	3	13	17	23	13	8	7	3			89	15.01	1.82	
Upper Ohio												1	5	17	10	11	2	2					48	13.81	1.32	
Licking													4	4	13	9	4	2					36	15.31	1.31	
Kentucky													1	2	6	4	3	1					17	14.53	1.28	
Green													1	7	17	9	8	1					43	14.44	1.12	
Tennessee													1	7	9	5	1	1	1				25	14.20	1.35	
<i>Macrhybopsis marconis</i>																										
Guadalupe—San Antonio			1	1	2	1	—	1	1	2	5	1	4	15	22	31	15	17	10	4	3			136	14.51	3.19
Colorado	4	1	1	1	1	1	1	—	—	1	—	—	4	2	6	2	1							26	9.00	5.88

**Table 4. Frequency distribution of caudal peduncle scales in four species of the *Macrhybopsis aestivalis* complex.**

Species/Drainage unit	11	12	13	14	15	16	17	18	n	mean	SD
<i>Macrhybopsis aestivalis</i>											
San Fernando		98	2						100	12.02	0.14
San Juan		44	3	1					48	12.10	0.37
Salado		61	4	1					66	12.09	0.34
Lower R. Grande		56	20	14	4	3			97	12.74	1.05
Lower middle R. Grande		78	21	11	4	3			117	12.57	0.98
Pecos		36	13	14	4	5			72	13.01	1.25
Upper middle R. Grande		38	18	13	16	5			90	13.24	1.32
Upper R. Grande		35	32	21	12	15	—	1	116	13.52	1.42
<i>Macrhybopsis australis</i>											
Upper Red		34	9	8	4	2			57	12.79	1.15
Middle Red		23	10	6	6	1			46	12.96	1.17
Wichita		33	6	4	8	12	1		64	13.42	1.70
<i>Macrhybopsis hyostoma</i>											
Colorado		71	6	3	1				81	12.19	0.55
Brazos		117	2						119	12.02	0.13
Middle Red		70	7	7	2				86	12.31	0.72
Washita		22	4	1					27	12.22	0.51
Lower Red		43	15	9	1				68	12.53	0.78
Canadian		17	2	1					21	12.20	0.52
Cimarron		24	3	2	—	2			31	12.48	1.09
Salt Fork Arkansas		33	14	10	3	4			64	12.92	1.20
Arkansas		38	13	5	4	2			62	12.69	1.08
Platte-Elkhorn		67							67	12.00	0.00
Blue-Republican	1	42	2	1					46	12.07	0.39
Kansas		61	11	1	1				74	12.22	0.53
Grand-Chariton-Osage	1	52	4	1					58	12.09	0.39
Upper Missouri	1	24							25	11.96	0.20
Lower Missouri		30	1	1					32	12.09	0.39
Iowa-Des Moines	1	17							18	11.94	0.24
Lower Mississippi		104	8	4	3				119	12.21	0.62
Upper Mississippi		29							29	12.00	0.00
Middle Mississippi		28							28	12.00	0.00
Illinois		12							12	12.00	0.00
Wabash		77	1	1					79	12.04	0.25
Sabine-Calcasieu	1	106	4	3					114	12.08	0.38
Ouachita		23	2						25	12.08	0.28
White-St. Francis		67	—	1	1				69	12.07	0.43
Upper Ohio		38							38	12.00	0.00
Licking	1	32	1						34	12.00	0.25
Kentucky		16							16	12.00	0.00
Green		45							45	12.00	0.00
Tennessee		22							22	12.00	0.00
<i>Macrhybopsis marconis</i>											
Guadalupe-San Antonio	1	134	6	1	1				143	12.08	0.43
Colorado		26							26	12.00	0.00

**Table 5. Frequency distribution of anterior belly squamation index in four species of the *Macrhybopsis aestivalis* complex.**

Species/Drainage unit	Index/Percent squamation						n	mean index	SD
	0 0%	1 20%	2 40%	3 60%	4 80%	5 100%			
<i>Macrhybopsis aestivalis</i>									
San Fernando		3	1	8	60	30	103	4.10	0.82
San Juan	2	10	10	14	9	5	50	2.66	1.36
Salado	4	12	17	28	7		68	3.32	1.07
Lower R. Grande	1	31	16	15	16		99	1.74	1.38
Lower middle R. Grande	40	40	18	7	11	2	118	1.28	1.33
Pecos	58	15	2				75	0.25	0.50
Upper middle R. Grande	83	22	3	1	1		110	0.32	0.67
Upper R. Grande	119						119	0.00	0.00
<i>Macrhybopsis australis</i>									
Upper Red	76	14	1				91	0.18	0.41
Middle Red	48	6	2				56	0.18	0.47
Wichita	59	5					64	0.08	0.27
<i>Macrhybopsis hyostoma</i>									
Colorado	29	40	13	3	3	1	89	1.03	1.05
Brazos	96	22	5	1	1		125	0.31	0.66
Middle Red	38	37	10	5	3	1	94	0.95	1.09
Washita	14	12	2	2	3		33	1.03	1.26
Lower Red	23	38	8	7	5	1	77	1.23	1.23
Canadian	12	6	3	3	1		25	1.00	1.22
Cimarron	11	21	5	—	2		39	1.00	0.95
Salt Fork Arkansas	27	37	5	1	2		72	0.81	0.85
Arkansas	14	52	9	1	1	2	79	1.10	0.93
Platte—Elkhorn	54	13	1	1	1		70	0.31	0.71
Blue—Republican	28	19	2	2			51	0.57	0.76
Kansas	52	23	1	—	—	1	77	0.39	0.73
Grand-Chariton-Osage	27	32	5	8	—	1	73	0.97	1.05
Upper Missouri	18	9	4	2	1		34	0.79	1.07
Lower Missouri	11	23	7	—	3		44	1.11	1.02
Iowa—Des Moines	10	9					19	0.47	0.51
Lower Mississippi	19	70	20	11	8	4	132	1.48	1.20
Upper Mississippi	10	35	10	1	2		58	1.38	0.85
Middle Mississippi	7	23	6	3	3	1	43	1.42	1.20
Illinois	3	10	2	2	1		18	1.33	1.08
Wabash	14	51	12	11	5	2	95	1.45	0.16
Sabine—Calcasieu		10	12	16	50	9	117	3.65	1.21
Ouachita	2	16	9	6	2	3	38	1.97	1.32
White—St. Francis	13	43	16	8	11	4	95	1.72	1.37
Upper Ohio	6	16	11	13	7	1	54	2.05	1.30
Licking	3	20	8	7	3	1	42	1.76	1.19
Kentucky			2	4	4	7	17	3.94	1.09
Green	1	5	5	5	9	20	45	3.69	1.52
Tennessee			1	2	2	18	23	4.61	0.84
<i>Macrhybopsis marconis</i>									
Guadalupe-San Antonio	1	12	10	25	49	46	143	3.73	1.26
Colorado		4	4	5	7	7	27	3.33	1.41

**Table 6. Frequency distribution of posterior belly squamation index in four species of the *Macrhybopsis aestivalis* complex.**

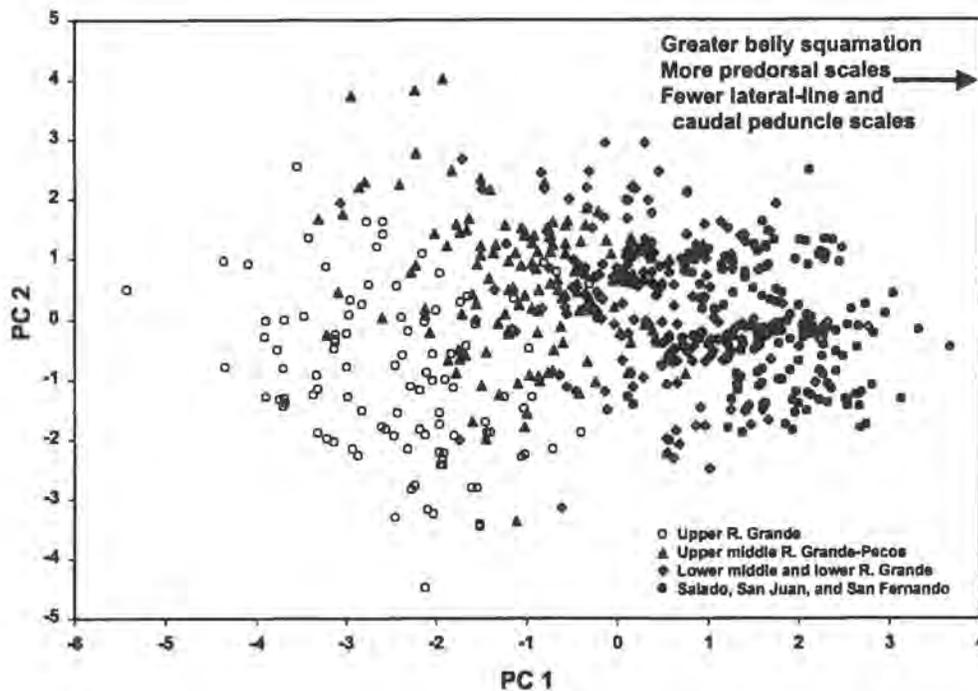
Species/Drainage unit	Index/Percent squamation			n	mean index	SD
	0 0-33%	1 34-67%	2 67-100%			
<i>Macrhybopsis aestivalis</i>						
San Fernando			104	104	2.00	0.00
San Juan			51	51	2.00	0.00
Salado		3	67	70	1.96	0.20
Lower R. Grande		2	97	99	1.98	0.14
Lower middle R. Grande	3	11	103	117	1.85	0.42
Pecos	16	36	23	75	1.09	0.72
Upper middle R. Grande	10	48	51	109	1.38	0.65
Upper R. Grande	104	15		119	0.13	0.33
<i>Macrhybopsis australis</i>						
Upper Red	53	24	14	91	0.57	0.75
Middle Red	27	15	14	56	0.77	0.83
Wichita	28	18	18	64	0.84	0.84
<i>Macrhybopsis hyostoma</i>						
Colorado		7	82	89	1.92	0.27
Brazos	32	23	70	125	1.30	0.85
Middle Red	5	15	74	94	1.73	0.55
Washita	3	4	26	33	1.70	0.64
Lower Red		9	68	77	1.88	0.32
Canadian	2	6	17	25	1.60	0.65
Cimarron	3	3	32	38	1.76	0.59
Salt Fork Arkansas	8	11	53	72	1.63	0.68
Arkansas	4	15	59	78	1.71	0.56
Platte-Elkhorn	10	28	32	70	1.31	0.71
Blue-Republican	3	12	36	51	1.65	0.59
Kansas	22	23	32	77	1.13	0.83
Grand-Chariton-Osage	5	19	49	73	1.60	0.62
Upper Missouri	10	9	15	34	1.15	0.86
Lower Missouri	2	10	32	44	1.68	0.56
Iowa-Des Moines	5	8	6	19	1.05	0.78
Lower Mississippi	10	24	98	132	1.67	0.61
Upper Mississippi	1	17	38	56	1.66	0.51
Middle Mississippi	7	9	27	43	1.47	0.77
Illinois	4	6	8	18	1.22	0.81
Wabash	13	13	69	95	1.59	0.72
Sabine-Calcasieu			117	117	2.00	0.00
Ouachita		1	37	38	1.97	0.16
White-St. Francis	1	5	89	95	1.93	0.30
Upper Ohio	1	1	52	54	1.94	0.31
Licking			42	42	2.00	0.00
Kentucky			17	17	2.00	0.00
Green			45	45	2.00	0.00
Tennessee			24	24	2.00	0.00
<i>Macrhybopsis marconis</i>						
Guadalupe-San Antonio			143	143	2.00	0.00
Colorado			27	27	2.00	0.00

Table 7. Frequency distribution of infraorbital pores in four species of the *Macrhybopsis aestivalis* complex.

Species/Drainage unit	10	11	12	13	14	15	16	17	18	19	20	n	mean	SD
<i>Macrhybopsis aestivalis</i>														
San Fernando			4	19	31	25	12	1	—	1		93	14.32	1.20
San Juan			1	4	10	19	8	5	1	1		49	15.08	1.35
Salado		4	3	10	18	12	13	—	3			63	14.35	1.62
Lower R. Grande		4	6	25	33	19	5	3	1			96	13.98	1.32
Lower middle R. Grande				5	20	32	31	16	6			110	14.46	1.23
Pecos		1	3	8	22	25	9	3	2			73	14.59	1.31
Upper middle R. Grande	1	1	4	14	27	19	18	13				97	14.67	1.51
Upper R. Grande		1	2	9	29	30	21	9	4	2		107	15.02	1.45
<i>Macrhybopsis australis</i>														
Upper Red		1	6	25	24	19	13					88	14.06	1.22
Middle Red	1	5	9	15	15	9	1					55	13.25	1.32
Wichita	1	4	11	22	18	6	2					64	13.22	1.21
<i>Macrhybopsis hyostoma</i>														
Colorado		1	9	29	25	17	5	1				87	13.77	1.16
Brazos	1	6	24	40	32	14	4	3				124	13.37	1.32
Middle Red	3	6	10	27	26	15	5					92	13.43	1.39
Washita		3	6	10	9	2	1					31	13.13	1.20
Lower Red		1	3	28	27	11	1					71	13.66	0.89
Canadian		1	3	10	5	1	1	1				22	13.41	0.91
Cimarron	1	2	5	15	7	2	1					33	13.06	1.20
Salt Fork Arkansas		1	5	17	22	16	10	1				72	14.13	1.24
Arkansas		3	8	28	19	13	5	1				77	13.65	1.25
Platte-Elkhorn		3	10	17	11	11	8	—				61	13.75	1.52
Blue-Republican		2	4	8	19	12	2	1				48	13.94	1.23
Kansas		4	10	21	26	12	4					77	13.57	1.21
Grand-Chariton-Osage		3	12	20	20	14	3	1				73	13.59	1.28
Upper Missouri	1	—	3	7	10	6	3					30	13.83	1.34
Lower Missouri			4	13	14	7	3	3				44	14.02	1.32
Iowa-Des Moines		1	1	6	2	6	2					18	13.94	1.39
Lower Mississippi	1	5	7	21	41	29	16	2				122	14.11	1.35
Upper Mississippi		1	2	8	23	12	10	—	1			57	14.37	1.23
Middle Mississippi		1	5	13	12	8	4					43	13.77	1.23
Illinois		1	2	7	4	2	1	1				18	13.61	1.46
Wabash		2	—	7	17	27	28	9	5			95	15.23	1.39
Sabine-Calcasieu		2	10	14	30	32	23	3	1			115	14.44	1.38
Ouachita			3	7	11	7	7	3				38	14.45	1.41
White-St. Francis		1	6	12	17	27	14	5				82	14.52	1.36
Upper Ohio			1	—	6	7	22	7	1			44	15.68	1.12
Licking			1	1	10	8	12	4	3	2		41	15.54	1.57
Kentucky				1	1	1	2	1				5	15.60	1.14
Green				1	4	7	9	4	11	—	1	37	16.32	1.60
Tennessee		1	—	1	5	4	6	3	2			22	15.32	1.67
<i>Macrhybopsis marionis</i>														
Guadalupe-San Antonio		1	7	25	40	45	17	6				141	14.39	1.22
Colorado		1	—	6	11	8	1					27	14.04	1.02

**Table 8.** Frequency distribution of vertebrae in four species of the *Macrhybopsis aestivalis* complex.

Species/Drainage unit	34	35	36	37	38	39	n	mean	SD
<i>Macrhybopsis aestivalis</i>									
San Fernando	7	17					24	34.71	0.46
San Juan	1	8	5	1			15	35.40	0.73
Salado		3	9	1			13	35.85	0.55
Lower R. Grande		9	8	1			18	35.56	0.62
Lower middle Grande		7	21	1	1		30	35.87	0.63
Pecos	1	4	6	3			14	35.79	0.89
Upper middle R. Grande			13	8			21	36.38	0.50
Upper R. Grande			8	13			21	36.62	0.50
<i>Macrhybopsis australis</i>									
Upper Red	1	9	3				13	35.15	0.56
Wichita		14	5				19	35.26	0.45
<i>Macrhybopsis hyostoma</i>									
Colorado	1	6	7				14	35.43	0.65
Brazos		8	5				13	35.38	0.51
Middle Red	1	3	7	2	1		14	35.93	1.00
Arkansas		2	5	7	2		16	36.65	0.89
Kansas			6	8			14	36.57	0.51
Grand-Chariton-Osage		1	7	4			12	36.25	0.62
Lower Mississippi			1	15	1		17	37.00	0.34
Upper Mississippi		2	10	3	1		16	36.19	0.75
Wabash			6	22	10	3	41	37.24	0.80
Sabine-Calcasieu	4	16	12	1			33	35.30	0.73
White-St. Francis		4	9				13	35.69	0.48
Upper Ohio				6	1		7	37.14	0.38
Licking				7	3	1	11	37.45	0.69
Tennessee			1	4	5		10	37.40	0.70
<i>Macrhybopsis marconis</i>									
Guadalupe-San Antonio			4	30	10		44	37.14	0.55
Colorado			9	5			14	36.36	0.50

**Figure 5.** Meristic scores on PC axes 1 and 2 for 603 *Macrhybopsis aestivalis*.

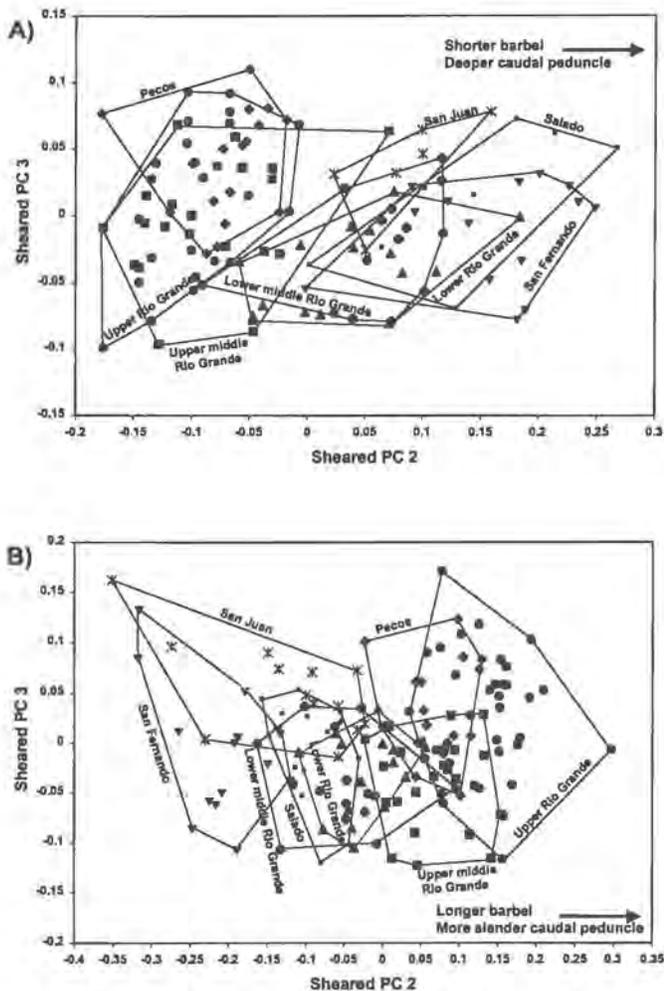


Figure 6. Morphometric scores on sheared PC axes 2 and 3 for *Macrhybopsis aestivalis*. A) 122 males. B) 149 females. Polygons bound individuals of each drainage unit.

specimens from these populations into nonoverlapping clusters (Figs. 5–6).

However, specimens from the middle Rio Grande basin were intermediate for most of these characters. Eight characters exhibited some degree of univariate clinal variation in *M. aestivalis*: lateral-line scales, predorsal scales, caudal peduncle scales, posterior and anterior belly squamation, vertebrae, barbel length, and caudal peduncle depth (Fig. 7, Tables 2–6, 8). Examination of a PCA of the meristic data set revealed multivariate clinal variation present among the drainage units (Figs. 5, 7). Separation was primarily along the PC 1 axis; loading values indicated specimens from more upstream areas had more lateral-line scales and caudal peduncles scales, fewer predorsal scales, and lower indices of posterior and anterior belly squamation. Similar patterns of variation were present in a sheared PCA of the morphometric variables (Figs. 6–7). Specimens from the middle Rio Grande basin were intermediate in multivariate space, with separation primarily

along the sheared PC 2 axis; loadings were highest for barbel length and caudal peduncle depth.

Correlograms from a spatial autocorrelation analysis of seven characters and three mean PC and sheared PC scores are shown in Fig. 8. The autocorrelation coefficient (Moran's I) decreased clinally for mean meristic PC 1 scores and mean morphometric sheared PC 2 scores. Variables with the highest PC or sheared PC loadings (e.g., belly squamation indices; Fig. 8C) had correlograms similar to those produced by the PC scores, while variables with only moderate PC loadings (e.g., predorsal scales; Fig. 8A) showed lower coefficients of correlation and shallower, more irregular clinal patterns. In general, significant positive spatial autocorrelation occurred for localities within 500 km and significant negative spatial autocorrelation for localities 900–1400 km apart. Thus, values for geographically proximate populations are similar (correlated), while values for geographically distant populations are different (negatively correlated); that is, the values are clinal (Sokal and Oden, 1978).

In summary, populations of *M. aestivalis* at opposite ends of the Rio Grande basin are morphologically distinct. Univariate and multivariate analyses reveal a concordant pattern of clinal variation for numerous morphological characters along the length of the Rio Grande basin, without meaningful discontinuities.

**COMPARISONS.**—*Macrhybopsis aestivalis* is the largest member of the complex and the only member with numerous scales bearing clusters of small melanophores. It is most similar to *M. marconis* in overall morphology but lacks a strong lateral stripe and head tubercles on nuptial males. *Macrhybopsis australis* and *M. tetranema* differ in having two pairs of barbels.

Although *M. aestivalis* is easily separable from eastern, well-pigmented populations of *M. hyostoma*, it is morphologically similar to some western, pallid populations. In addition to lacking clusters of melanophores, many western specimens of *M. hyostoma* have a second pair of barbels (never found in *M. aestivalis*). Sheared PCA of all members of the *M. aestivalis* complex showed no overlap of mean PC scores of drainage units of male *M. aestivalis* and *M. hyostoma*, although some overlap occurred for mean PC scores of females (Fig. 9). Plots of individuals from the same PCA showed considerably more overlap among species and are not presented. Loading values indicated *M. aestivalis* has larger fins, a more posterior pelvic fin base, a shorter caudal peduncle, and a more robust body than *M. hyostoma*.

**DISTRIBUTION AND STATUS.**—Endemic to the Rio San Fernando drainage and Rio Grande basin (Fig. 2). This species remains common in most of the Pecos River and the Rio Grande mainstem between the mouths of the Rio Conchos and Pecos River (Hubbs, 1940a; Hubbs et al., 1977; Sublette et al., 1990). Recent surveys indicate that

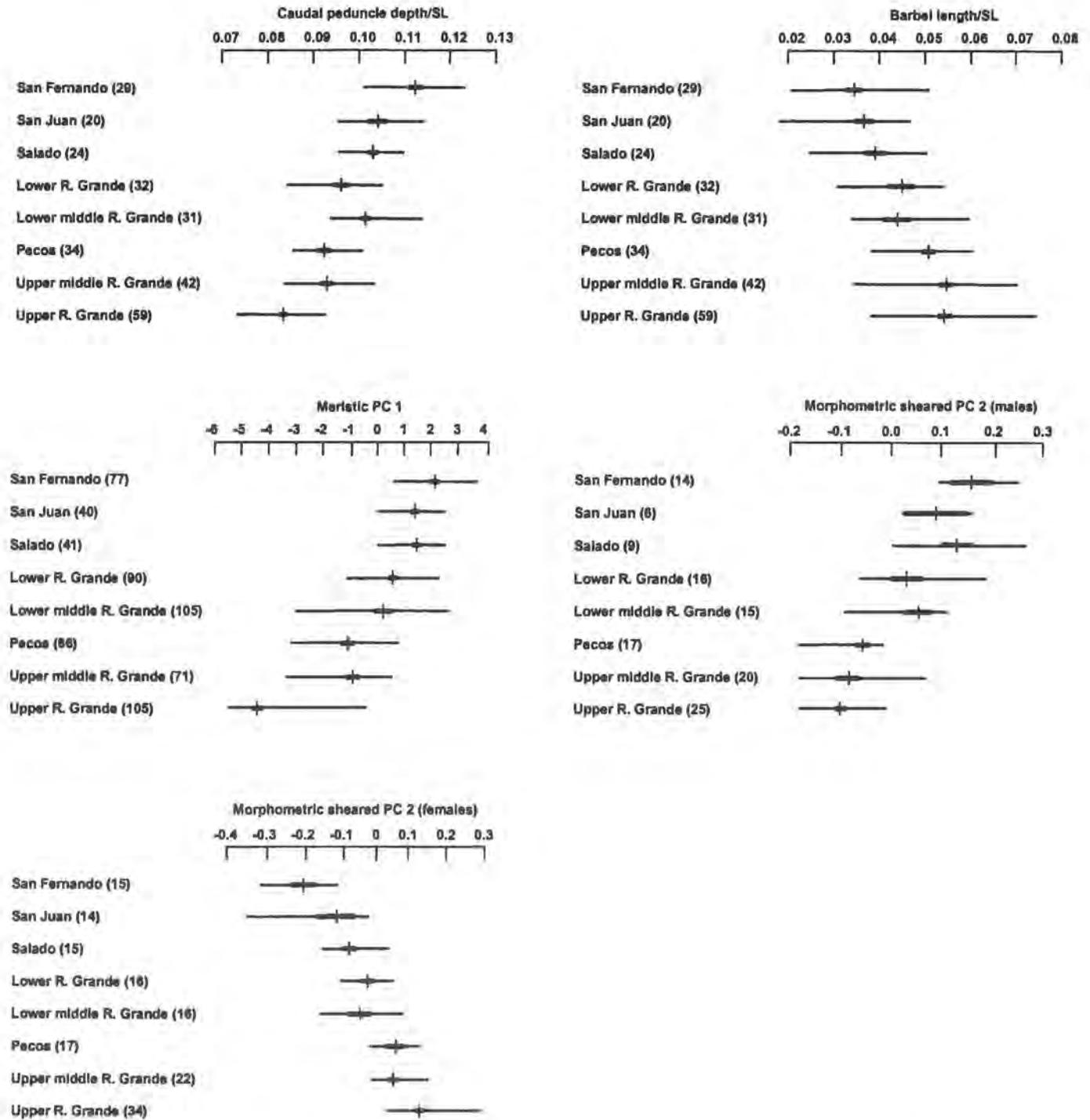


Figure 7. Means, ranges, and two standard errors for two characters and three mean multivariate scores showing clinal variation in *Macrhybopsis aestivalis*. Numbers in parentheses are sample sizes.

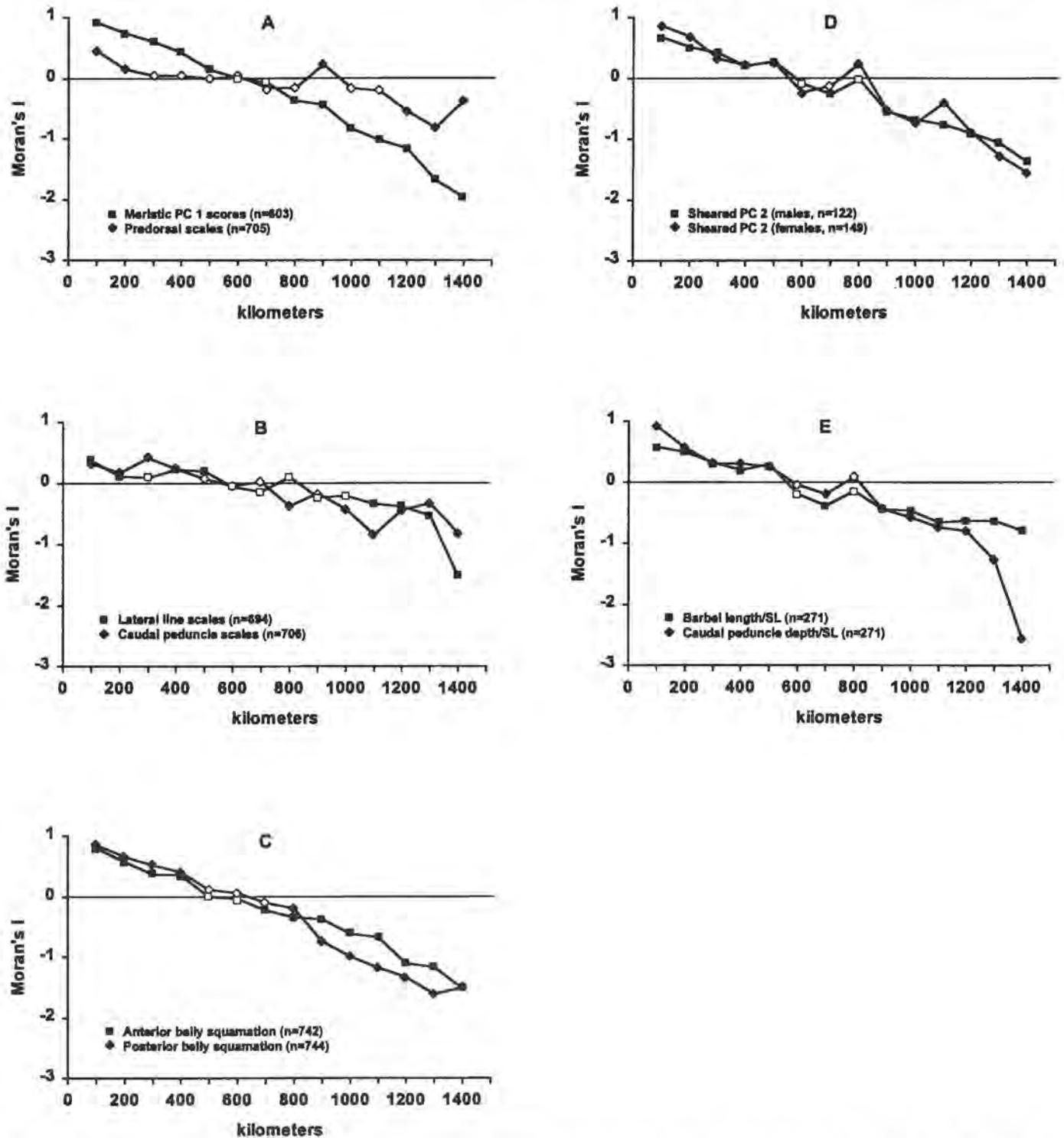


Figure 8. Correlograms of seven characters and three mean multivariate scores of *Macrhybopsis aestivalis*. Significant autocorrelation ( $P < 0.05$ ) is indicated by solid symbols. Numbers in parentheses are sample sizes.

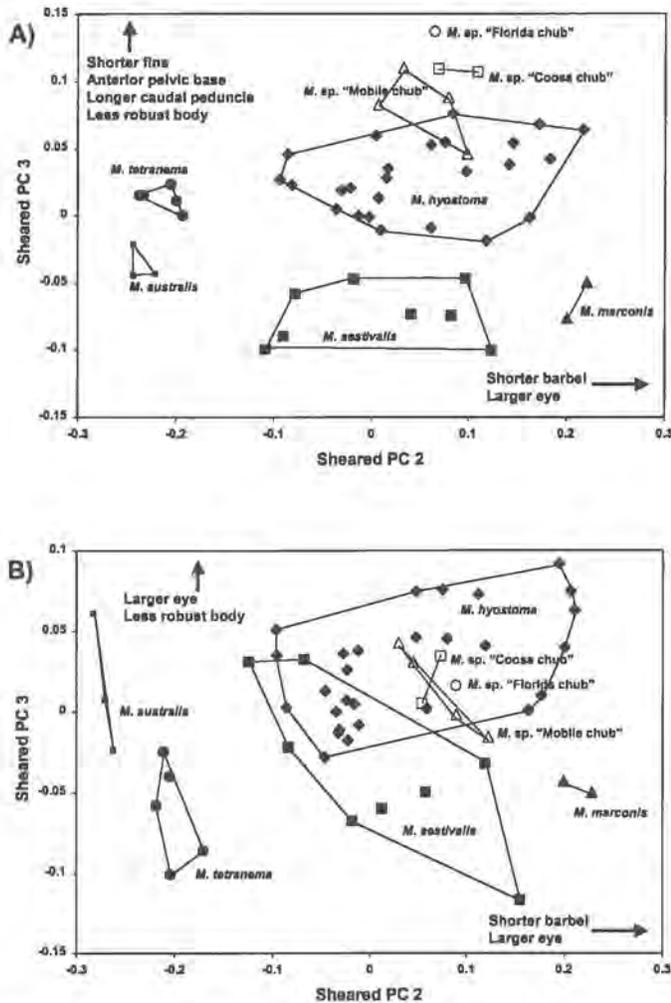


Figure 9. Mean morphometric scores on sheared PC axes 2 and 3 for drainage unit groups of members of the *Macrhybopsis aestivalis* complex. A) 619 males. B) 763 females. Polygons bound mean scores of drainage units of each species cluster.

*M. aestivalis* has greatly declined in the lower Rio Grande mainstem, likely resulting from reservoirs, channelization, and reduced stream flows due to irrigation withdrawal (Edwards and Contreras-Balderas, 1991).

Numerous, large ( $n=147$ ) MSB collections from 1930–1950 from the upper Rio Grande suggest the species was common there before recent, extensive changes to the Rio Grande. The last collections from the upper Rio Grande were in 1964 at Las Cruces, Dona Ana County, New Mexico ( $n=5$ ) and Peña Blanca, Sandoval County, New Mexico ( $n=7$ ). *Macrhybopsis aestivalis* is considered extirpated in the upper Rio Grande mainstem (Sublette et al., 1990).

Of the five characteristic cyprinids originally inhabiting the upper Rio Grande mainstem, four (*M. aestivalis*, *N. jemezianus*, *Notropis orca*, and *Notropis simus simus*) are now extinct or extirpated, and the remaining species,

*Hybognathus amarus*, is confined to less than 10% of its former range (Bestgen and Propst, 1996). The disappearance of these cyprinids is likely due to numerous anthropogenic disturbances, including deforestation, overgrazing, intensive irrigation, mining of groundwater, reservoir construction, and channelization, which have dewatered the main channel, altered salinity and water temperature, blocked migration of fishes, and created heavy sediment loads (Jordan, 1891; Bestgen and Platania, 1990; Sublette et al., 1990). *Macrhybopsis aestivalis* has been listed as “amenazados” (=threatened) in Mexico (Pérez et al., 1993) and “rare” in Nuevo León (Contreras-Balderas et al., 1995).

**TAXONOMIC CONSIDERATIONS.**—Populations on opposite ends of a cline generally have not been given formal taxonomic recognition among North American freshwater fishes (Page and Smith, 1976; Chernoff et al., 1981; Poss and Miller, 1983). Proponents of the biological species concept (BSC) (Mayr, 1963, 1969) and the evolutionary species concept (ESC) (Simpson, 1961; Wiley, 1981) recommended recognition of only one species for cases involving broad clinal variation/contact zones (Mayr, 1969:46; Wiley, 1981:63).

Under the phylogenetic species concept (PSC), species are both diagnosable (Cracraft, 1983) and monophyletic (Cracraft, 1989). Because no reproductive isolation criterion is required, even sister taxa with broad areas of hybridization can be recognized as species as long as they can be diagnosed (Cracraft, 1983). Populations from the upper Rio Grande basin and the Río San Fernando-San Juan-Salado drainages certainly are diagnosable and each conceivably could be designated as species, (i.e., *M. sterletus* and *M. aestivalis*, respectively) under the PSC. Individuals from the middle Rio Grande basin would then be considered hybrids. However, diagnosability is technique dependent and thus arbitrary; even small family groups or individuals may be diagnosed with recent molecular technology (Avise, 1994). Even though the upper Rio Grande was, for a short time, an endorheic drainage (Belcher, 1975), based on data from this study, it appears unlikely that populations in the Rio Grande basin presently exhibit any meaningful phylogenetic partitions. The lack of identifiable breaks in morphological characters examined during this study suggests gene flow historically has occurred throughout the range of *M. aestivalis*.

It has been noted that for allopatric groups exhibiting apparent clinal variation, intermediacy may be attributed to retained ancestral polymorphism instead of historical gene flow (Mayden and Wood, 1995). However, the clinal variation in *M. aestivalis* does not appear to fit a model of retained ancestral polymorphism for a couple of reasons. First, *M. aestivalis* does not consist of a series of allopatric populations (except for the Río San Fernando population), but instead is composed of continuous populations that cannot be broken up into discrete geographic units. Because continuous populations along a cline do not

exhibit hierarchical relationships (de Querioz and Good, 1997), imposition of a phylogeny building technique that constructs hierarchical relationships is likely to produce a phylogeny that suggests an incorrect evolutionary history (Avisé, 1994; de Querioz and Good, 1997). Second, populations in the middle of the range of *M. aestivalis* are not polymorphic; that is, they do not encompass the range of morphological variation exhibited by populations at either end of the Rio Grande.

In summary, populations at either end of the Rio Grande basin do not appear to have their own "evolutionary tendencies and fates," a requirement of the ESC (Wiley, 1981:25). Because I judge only one independently evolving lineage identifiable in the Rio Grande basin and Rio San Fernando drainage, recognition of only one evolutionary species, *M. aestivalis*, is warranted.

**ETYMOLOGY.**—The specific epithet, *aestivalis*, is derived from the Latin *aestivus* (=pertaining to summer). The common name, speckled chub, is in reference to the prominent black body spots.

### *Macrhybopsis australis*

(Hubbs and Ortenberger)

Prairie Chub

FIGS. 3B, 4C

*Extrarius australis* Hubbs and Ortenberger 1929:26–28 (Red River 6–9 miles southwest of Hollis, Harmon County, Oklahoma).

Moore (1950:82) treated the nominal *Extrarius australis* as a subspecies of *Extrarius aestivalis* occupying the Red River basin, as "recognized by Dr. Hubbs." The distribution of this taxon was unclear because the number of barbels, supposedly diagnostic for the subspecies, was variable in populations from the lower and middle Red River basin (Davis and Miller 1967, Miller and Robison 1973, Douglas 1974, Robison and Buchanan 1988). Miller and Robison (1973) recognized that populations in the Red River basin were polymorphic and suggested two species or subspecies might be present.

I recognize two species, *M. australis* and *M. hyostoma*, in the *M. aestivalis* complex in the Red River basin. These species are syntopic in the middle section of the Red River mainstem. Analysis of morphometric, meristic, and other morphological data did not reveal evidence of meaningful introgression. In addition, phylogenetic analysis of the morphological data do not support a sister relationship between *M. australis* and *M. hyostoma*. In addition, recent analysis of allozymic variation supported monophyly in *M. australis* and demonstrated differences in allele frequencies between *M. australis* and *M. hyostoma* in a syntopic collection (Underwood et al., 2003). Herein, *M. australis* is

treated as a distinct species restricted to the upper Red River basin (including the Wichita River) from Jefferson County, Oklahoma, to its headwaters and in the upper Washita River.

**TYPES.**—The holotype (UMMZ 80347) is a male 43.5 mm SL (54 mm TL), Red River 6–9 miles southwest of Hollis, Harmon County, Oklahoma, 16 June 1926, C. L. Hubbs and A. I. Ortenberger, collectors. Paratopotypes: AMNH 10105 (2), BMNH 1933–124:6–8 (3), MCZ 32694 (1), TCWC 1110.1 (1), OKMNH 6337 (585), UMMZ 80348 (3), UMMZ 80349 (407), USNM 93433 (1), USNM 117528 (10). Paratypes: UMMZ 80383 (43), UMMZ 80384 (1), OKMNH 6181 (31), UMMZ 80410 (1), OKMNH 6202 (1), UMMZ 80376 (37), OKMNH 6294 (46), UMMZ 80367 (7), OKMNH 6369 (11).

**DIAGNOSIS.**—Distinguished from all other members of the *M. aestivalis* complex by the combination of two pairs of well-developed barbels, the posterior pair > orbit length and the anterior pair > 50% of orbit length, and modally seven anal rays. Additional distinguishing characters include head conical with a relatively pointed snout; pectoral fins very long and falcate in males (Fig. 3B), reaching past pelvic bases; poorly pigmented body with small melanophores randomly scattered over dorsolateral surface; belly without bridge of scales just anterior to pelvic bases; vertebrae 34–36; nuptial males with biserial pectoral fin tuberculation (Fig. 3B); lateral stripe weakly expressed and centered one scale row above lateral line or absent; and lips greatly thickened posteriorly.

**DESCRIPTION.**—Selected meristic counts from 212 specimens are given in Tables 1–8. Largest female 56.3 mm SL (70 mm TL, OSUS 26888); largest male 51.2 mm SL (65 mm TL, OSUS 26888). Principal caudal rays 19 (16–20); anal rays 7 (6–8); pelvic rays 7–8 (6–9); pectoral rays 13–15 (12–17). Lateral-line scales 36–42 (34–44); predorsal scales 2–16 (0–19); scales above lateral line 5–6 (4–7); scales below lateral line 4–5 (4–6); caudal peduncle scales 12–16 (12–17). Nape fully scaled in about 50% of individuals, otherwise with scattered embedded scales, rarely naked. Belly posterior to pelvic fin bases naked to fully scaled; 79% of specimens with 66% or less area covered with exposed scales. Belly just anterior to pelvic fin bases naked in about 85% of individuals, otherwise with few scales not forming bridge across belly. Infraorbital pores 12–16 (10–16); preoperculo-mandibular pores 10–12 (9–14). Total vertebrae 35–36 (34–36); precaudal vertebrae 16–18 (16–19); caudal vertebrae 17–19.

Body fusiform with moderately slender caudal peduncle. Anal and dorsal fins slightly falcate; pelvic fins pointed; pectoral fins long and falcate, reaching past bases of pelvic fins in adult males, just reaching bases of pelvic fins in adult females. Head conical and flattened ventrally with long and relatively pointed snout. Mouth inferior and horizontal; width equal to head width when viewed ven-

trally. Lips very fleshy and thickened posteriorly. Eyes tiny and round or nearly so. Two prominent pairs of maxillary barbels present, more posterior pair > orbit length and anterior pair > 50% of orbit length. Cutaneous taste buds expanded into large papillae on gular area. Genital papillae poorly developed as small conical or cylindrical extensions in both sexes. Gill rakers on first arch absent or present as 1–3 dorsal rudiments. Pharyngeal teeth 0,4–4,0.

In life pallid and translucent, often with a pink wash throughout, pale yellow or gray dorsally, silvery white ventrally, with broad silver lateral stripe. Small melanophores randomly scattered over dorsolateral surface of body, not concentrated on margin or submargin of scales. Poorly defined lateral stripe present to nearly absent, composed of small, often X-shaped melanophores, and centered one scale row above lateral line. Dorsal fin with fairly dark pigment on basal third of first 3–5 rays; pigment on distal portion of rays lacking or reduced. Pectoral, pelvic, and anal fins generally lacking pigment; rarely some pigment on pectoral rays.

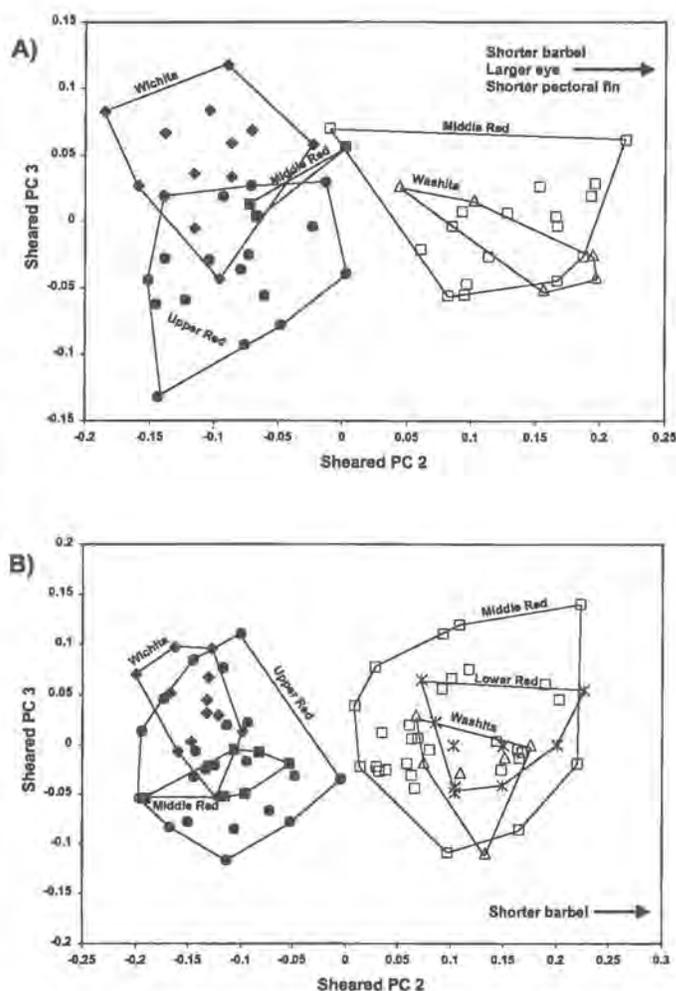
Pectoral rays 2–10 greatly thickened in large nuptial males, with rows of small, conical, antrorse, recurved biserial tubercles. Basal part of rays and the primary branches each bear 1–2 rows of tubercles. Two tubercles per fin ray segment on posterior primary branch, 1–2 tubercles per segment on the anterior primary branch (Fig. 3B). Tubercles arranged uniserially on secondary branches.

**GEOGRAPHIC VARIATION.**—Relatively little geographic variation was evident in *M. australis*. Specimens from the upper Red River basin had more mean predorsal scales and infraorbital pores (Tables 3, 7) than specimens from the remainder of the range.

**COMPARISONS.**—Most similar to the upper Arkansas River basin endemic, *M. tetranema*. *Macrhybopsis australis* differs from *M. tetranema* in the following (those of *M. tetranema* in parentheses): anal rays modally 7 (modally 8); vertebrae 34–36 (36–39); pectoral fins usually extending past the bases of the pelvic fins in adult males (just reaching pectoral fin bases); and fewer mean lateral-line and caudal peduncle scales. In addition, *M. australis* has a shorter caudal peduncle, longer pectoral fin, and larger eye than *M. tetranema* for males and a narrower body and larger eye for females (Eisenhour, 1999).

Specimens of *M. sp.* “Florida Chub”, an undescribed member of the *M. aestivalis* complex inhabiting the Escambia, Blackwater Bay, and Choctawhatchee river drainages (Gilbert, 1992), are similar to *M. australis* in having two pairs of barbels and modally seven anal rays. They differ from specimens of *M. australis* in having larger eyes, shorter barbels, uniserial fin tuberculation, well-developed genital papillae, and an anus midway between the pelvic bases and anal fin origin.

Where sympatric with *M. hyostoma*, *M. australis* is distinguished by the following (those of *M. hyostoma* in paren-



**Figure 10.** Morphometric scores on sheared PC axes 2 and 3 for *Macrhybopsis australis* (solid symbols) and *M. hyostoma* (open symbols) from the Red River basin. A) 32 male *M. australis* and 20 male *M. hyostoma*. B) 40 female *M. australis* and 27 female *M. hyostoma*. Polygons bound individuals of each drainage cluster.

theses): anal rays modally 7 (modally 8); posterior barbels > orbit length (< orbit length); anterior barbels > 50% orbit length (< 50% orbit length, or absent); belly just anterior to pelvic bases naked in 85% of specimens (naked in 35% of specimens); belly just posterior to pelvic bases fully scaled or nearly so in 20% of specimens (fully scaled or nearly so in 80% of specimens); head conical (head more rounded, with a “hump” above nares); pectoral fins strongly falcate in males, reaching past pelvic bases (pectoral fins pointed or weakly falcate, not reaching past pelvic bases); nuptial males with biserial pectoral fin tuberculation (usually with uniserial pectoral fin tuberculation) (Fig. 3); mouth as wide as head when viewed ventrally (not as wide as head); and lips greatly thickened posteriorly (not thickened posteriorly). In addition, *M. australis* has more mean lateral-line scales (38.42 vs. 36.49), fewer mean predorsal scales (9.71 vs. 14.02), and

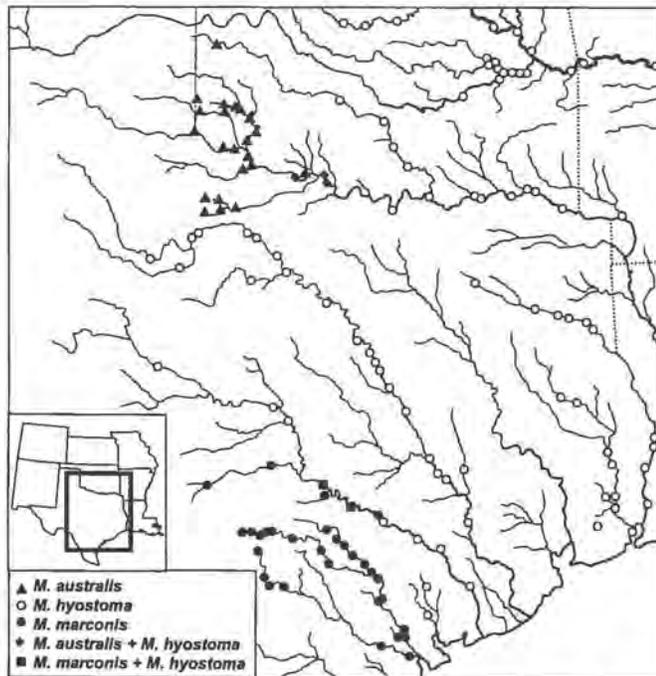


Figure 11. Distribution of *Macrhybopsis australis*, *M. hyostoma*, and *M. marconis* in the Red River basin and West Gulf Slope. Only localities from which specimens were examined are plotted.

is more pallid, with small dorsolateral melanophores randomly distributed (usually concentrated on margins or submargins of scales).

**DISTRIBUTION AND STATUS.**—Endemic to the upper Red River basin from Jefferson County, Oklahoma, to its upper reaches (Fig. 11), although it has not been reported from the Prairie Dog Town Fork of Red River in Texas. It remains a common and characteristic minnow over most of its former range. *Macrhybopsis australis* is presumed extirpated in the Washita River where it was known from only two specimens (UMMZ 80410, OKMNH 6202) collected in 1926 in Roger Mills County, Oklahoma. Winston et al. (1991) considered *M. australis* extirpated in the upper North Fork of Red River as a result of reservoir construction, although preimpoundment collections from there did not contain *M. australis* (Hubbs and Ortenberger, 1929).

If this species is a flood-pulse spawner like its putative sister species, *M. tetranema* (Bottrell et al., 1964), alteration of present stream flows (e.g., reservoir construction, channelization, and excessive removal of groundwater) likely would disrupt reproduction or recruitment. Many of the streams inhabited by *M. australis* dry to isolated pools in late summer (Winston et al., 1991). Downstream refugia in the form of large, permanent flowing streams may be necessary for *M. australis* to recolonize tributaries that suffer periodic local extirpations. Stream modifications that disrupted recolonization have already resulted in extirpation of most populations of *M. tetranema* (Luttrell et al., 1999). An additional potential threat is a large scale chloride removal project planned for the upper Red River basin (A. A. Echelle, pers. comm.). Distributions of several fish species in the upper Red River basin, including *M. australis*, are correlated with high lev-

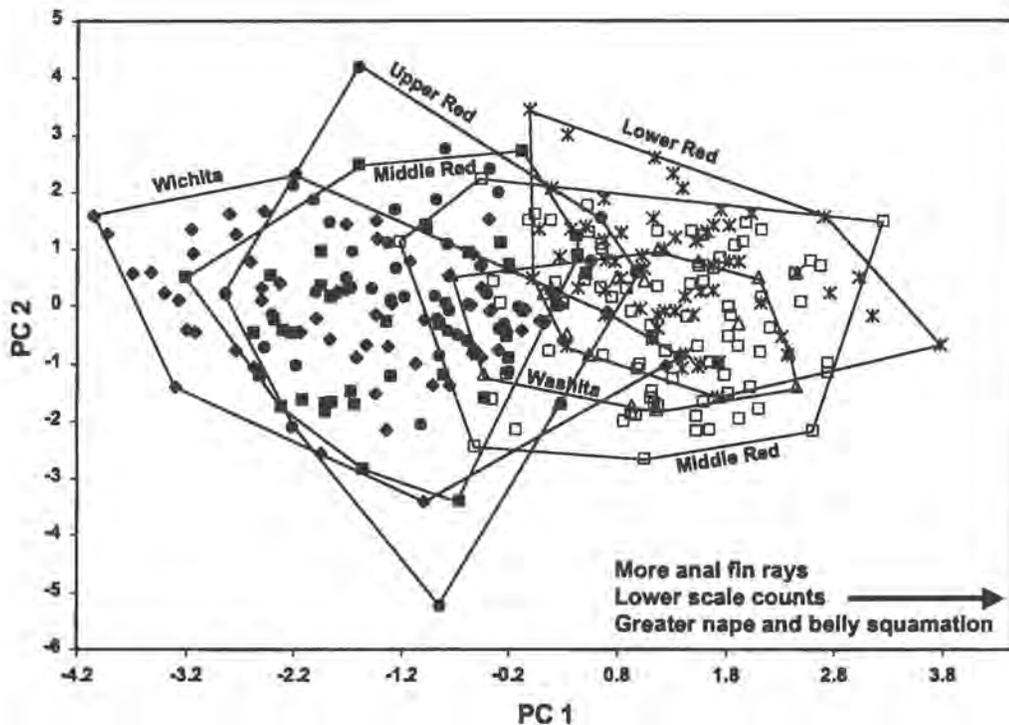


Figure 12. Meristic scores on PC axes 1 and 2 for 151 *Macrhybopsis australis* (solid symbols) and 162 *M. hyostoma* (open symbols) from the Red River basin. Polygons bound individuals of each drainage cluster.

els of dissolved salts (Echelle et al., 1972; Taylor et al., 1993). The effects of chloride removal are uncertain but could be detrimental to *M. australis* and other associated cyprinids (e.g., *Hybognathus placitus*, *Notropis bairdi*).

**CONTACT ZONE.**—*Macrhybopsis australis* and *M. hyostoma* are syntopic in the Red River mainstem in Cotton and Jefferson counties, Oklahoma (Fig. 11). Analysis of morphological data collected during this study did not reveal evidence of meaningful gene flow in the contact zone. Sheared PCA of morphometric variables separated these species into two non-overlapping clusters for both males and females (Fig. 10). Discrimination was primarily along the PC 2 axis, with *M. australis* having longer barbels, smaller eyes, and longer pectoral fins, particularly in males. Individuals of *M. australis* from the contact zone ("Middle Red") were not intermediate in morphometry, as might be expected if substantial hybridization has occurred but, instead, clustered with *M. australis* individuals from allopatric populations (Fig. 10). In addition, individuals of *M. hyostoma* from the Red River basin were more similar in morphometry to *M. hyostoma* from the Arkansas River basin than to *M. australis* (Eisenhour, 1999). A PCA of the meristic variables indicated *M. australis* had more lateral-line and caudal peduncle scales, fewer anal rays and predorsal scales, and lower indices of belly squamation (Fig. 12). Specimens from the Washita River suggest some gene flow has occurred, a hypothesis with some support from recent allozyme studies (Underwood et al., 2003). A series (EKU 529) of *M. hyostoma* from the Washita River in Garvin County, Oklahoma contained three males typical in most respects, but with biserial pectoral fin tuberculation. Specimens of *M. hyostoma* from the Washita River have a higher frequency (33%) of seven anal rays than other Red River *M. hyostoma* (19% with seven anal rays). It is uncertain whether this is evidence of limited introgression with *M. australis* or simply geographic variation.

**ETYMOLOGY.**—The specific epithet, *australis*, is Latin for "southern" and may refer to the more southerly range of *M. australis* compared to its putative sister species, *M. tetranema*. The proposed common name, prairie chub, refers to the prairies drained by streams in the range of *M. australis*.

### *Macrhybopsis hyostoma* (Gilbert)

Shoal Chub  
FIGS. 3A, 4D–E

*Nocomis hyostomus* Gilbert 1884:203 (East Fork of White River at Bedford, Lawrence County, Indiana).

Jordan and Evermann (1896) treated this taxon as a species in the genus *Hybopsis* and expanded its known range from the type locality to include "Indiana to Iowa, and south

to the Alabama River." Apparently following an unpublished classification by C. L. Hubbs, the taxon was reduced to a subspecies of *Extrarius aestivalis* (Greene, 1935; Gerking, 1945) and its range restricted to the Ohio River basin (Moore, 1950). Trautman (1957) later mapped the distribution of this subspecies to also include the upper Mississippi River basin, but noted that the western limits of the subspecies were unknown. Populations from Nebraska to Illinois and south in the Mississippi River and Texas were observed to differ in morphology from Ohio River basin populations, and were considered to represent an undescribed "plains subspecies" (Moore, 1950). The manuscript name "*sesqualis*" that C. L. Hubbs used for this form (F. Cross, pers. comm.) appears on handwritten notes in numerous KU lots from the Midwest and is a *nomen nudum* in Breukelman (1940). Most recent authors follow Davis and Miller's (1967) restriction of the range of *Hybopsis aestivalis hyostomus* to areas east of the Mississippi River, leaving populations in much of the upper Midwest and Texas not assigned to a subspecies. Populations considered to represent *M. hyostoma* in this study from downstream parts of the Red and Arkansas rivers populations have been identified as *australis* and *tetranemus*, respectively (Davis and Miller, 1967; Miller and Robison, 1973; Douglas, 1974; Higgins, 1977; Wallace, 1980; Becker, 1983; Robison and Buchanan, 1988). *Macrhybopsis hyostoma* is treated herein as a species occurring in streams of the West Gulf Slope and Mississippi River basin.

**TYPES.**—Syntypes include USNM 34980 (6), CAS-SU 888 (30), and CAS 58626 (4) East Fork of White River near Bedford, Lawrence County, Indiana, date unknown, C. H. Gilbert et al., collectors. Böhlke (1953) listed USNM 34980 as "holotype" based on Jordan and Evermann's (1896) reference to USNM 34980 as "type," but USNM 34980 contains six specimens and cannot be considered the holotype.

Meek (1884) described *Hybopsis montanus* as having barbels and 0,4–4,0 pharyngeal teeth, consistent with specimens of the *M. aestivalis* complex from the Missouri River. Metcalf (1966), after examining the three type specimens (USNM 36882), suggested *Hybopsis montanus* Meek could be an additional available name for the *M. aestivalis* complex. Hubbs and Ortenberger (1929) previously had identified the types as *Notropis dorsalis*. The specimens presently in the type series have 1,4–4,1 teeth and lack barbels, and are referable to *N. dorsalis* (Gilbert, 1978; personal observation). The labels with the types state locations of "E. Slope of Rocky Mts." and "upper Missouri Region," well outside the range of the *M. aestivalis* complex (Fig. 2) and *N. dorsalis* or any other species matching the original description. The conflict in Meek's (1884) description, the stated type locality, and the specimens presently in the type jar, indicates substitution of the type specimens likely occurred prior to Hubbs and Ortenberger's examination (Gilbert, 1998). Because of this confusion, *Hybopsis montanus* Meek is not presently identifiable.



but fades anteriorly, centered on lateral line to one scale row above lateral line. Pigmentation on fin rays highly variable. Pectoral fin usually well-pigmented along rays; anal, pelvic, and dorsal fins with only scattered melanophores along rays or unpigmented.

Pectoral rays 2–10 thickened in large nuptial males and bear rows of conical, antrorse uniserial tubercles (Fig. 3A). Tiny tubercles rarely present on rays of dorsal and pelvic fins in large (>50 mm SL) nuptial males in peak condition.

**GEOGRAPHIC VARIATION.**—Univariate and multivariate analyses of morphological characters conducted during this study revealed several geographic trends in *M. hyostoma*. Sheared PCA and PCA of morphometric and meristic variables, respectively, of five randomly selected drainages discriminated at least some drainage units (Eisenhour, 1997), indicating use of group means of sheared PC and PC scores is appropriate (Matthews, 1987). Considerable geographic variation in morphology was evident in sheared PCA of 314 males and 382 females of *M. hyostoma* (Fig. 13). For both sexes, mean PC scores of drainage units primarily were separated along an east-west direction. Populations from the Plains, especially the Red and Arkansas River basins, had longer barbels and smaller eyes, while populations from the East, especially the Ohio River basin, had larger eyes and smaller barbels (Fig. 13). Populations from the central portion of the Mississippi River basin tended to be intermediate in PC space. An east-west trend also was apparent along the PC 2 axis of the meristic data set (Fig. 14). Examination of loading values indicated eastern populations had higher indices of belly squamation, more infraorbital pores, and fewer caudal peduncle scales. Separation of mean scores along the PC 1 axis was greatest between populations from the East Gulf slope and those from the Red and Arkansas River basins. Drainage units with high mean PC 1 scores (i.e., Red and Arkansas River basins) had relatively high scale counts and pectoral rays.

To simplify descriptions of geographic variation, the 33 drainage units of *M. hyostoma* were combined into six groups identified from examination of univariate and multivariate analyses and qualitative characters. These groupings are based on phenotypic similarity and may not necessarily represent evolutionary units. Boundaries between most groups are not discreet, but, rather, populations at the geographic margin of a particular group's range tend to be intermediate in morphology.

A "Southern Plains" group, consisting of populations from the Red and Arkansas River basins, are characterized by morphological adaptations to turbid river conditions. Specimens are pallid overall (Fig. 4D), with poorly defined or absent lateral stripes and submarginal dorsolateral scale pigmentation. They have high mean scale counts (Tables 2–4), few mean infraorbital pores (Table 7), long barbels, small eyes (Fig. 13), and, usually, a sec-

ond pair of barbels. Specimens from the lower Red and Arkansas rivers have shorter barbels, larger eyes, and darker pigmentation than specimens from the middle section of these river basins (Fig. 13).

A "West Gulf Slope" group consists of populations from the Colorado and Brazos River drainages. This group is similar in morphology to the Southern Plains group but always lacks a second pair of barbels and has fewer lateral-line scales and vertebrae (Tables 2, 8). In addition, lateral stripe and submarginal dorsolateral scale pigmentation is most reduced in this group. Specimens from the turbid Brazos River are more pallid, and have lower mean belly squamation indices, smaller eyes, and longer barbels than specimens from the clearer Colorado River (Tables 5–6; Figs. 13–14).

A "Northern Plains" group consists of populations from the Missouri River basin, the Des Moines and Iowa River drainages, and the Mississippi River mainstem below the mouth of the Missouri River. The extension of the basically Plains distribution of this group into the lower Mississippi River is similar to the range of several other Plains fishes including *Scaphirhynchus albus*, *Hybognathus argyritus*, *H. placitus*, *M. gelida*, *M. meeki*, and *P. gracilis*. This group is similar to the Southern Plains group, except that morphological adaptations to turbid waters are not as pronounced. Specimens from the Northern Plains have shorter and fewer barbels, fewer scales, and darker pigmentation than specimens from the Southern Plains (Tables 2–4; Figs. 13–14). The lateral stripe on the caudal peduncle and dorsolateral pigment pattern nearly always are present but poorly expressed on specimens from the Northern Plains. In general, specimens collected from clearer streams (Platte River tributaries, upper Des Moines River, and the Thompson River) have larger eyes and darker pigment than specimens taken from more turbid streams (Missouri River).

The shortest barbels and darkest pigment of any member of the *M. aestivalis* complex are present in the "Ohio" group of *M. hyostoma*. This group consists of populations from the Ohio River basin, exclusive of the Wabash River drainage. Also, specimens are characterized by large eyes, high infraorbital pore and vertebral counts, high belly squamation indices, and the complete absence of a second pair of barbels (Tables 5–8; Figs. 4E, 13–14).

The "Central Coastal Plain" group consists of populations from the Sabine, Calcasieu, Ouachita, White, and St. Francis River drainages. These populations are similar to the Ohio group in having large eyes and dark pigmentation but tend to be intermediate between the Ohio group and specimens from the Southern and Northern Plains in barbel length and number, and infraorbital pore counts (Table 7; Fig. 13). Also, this group is characterized by relatively low lateral-line scale and vertebral counts (Tables 2, 8). Considerable geographic variation is present within this group, with specimens from the Sabine and Calcasieu River drainages having higher mean belly squamation indices and shorter mean

barbel lengths than specimens from the Ouachita and White-St. Francis drainages (Tables 5–6; Figs. 13–14).

The "Upper Midwest" group consists of populations from the Mississippi River basin above the mouth of the Missouri River (exclusive of the Des Moines and Iowa River drainages) and the Wabash River drainage. Several characters in this group are intermediate between the Ohio group and the Northern and Southern Plains groups, including orbit length, infraorbital pore counts, belly squamation indices, pigmentation, and number of barbels (Tables 5–7; Figs. 13–14). Populations from the more turbid middle Mississippi drainage unit are more similar to the Northern Plains group, whereas populations from relatively clear tributaries (Wisconsin River, Vermilion River, Tippecanoe River) are more similar to the Ohio group.

**COMPARISONS.**—Populations sympatric with *M. australis* and *M. marconis* are compared in their respective species accounts. Sympatry with *M. tetranema* is discussed in Eisenhour (1999).

**DISTRIBUTION AND STATUS.**—Widely distributed in the Mississippi River basin and the West Gulf Slope west to the Lavaca River drainage, Texas (Fig. 2). Although generally common over much of its range, habitat fragmentation resulting from reservoir construction, channelization, and other anthropogenic disturbances to the hydrology of the streams has isolated many populations. *Macrhybopsis hyostoma* has been extirpated from the Cumberland River (Etnier and Starnes, 1993), the upper Kansas River drainage (Sanders et al., 1993), the Arkansas River mainstem in Arkansas (Luttrell et al., 1999), and the Osage River, Missouri (last collected in 1950). This species has declined in abundance and distribution in the Mississippi mainstem above the mouth of the Missouri (J. Lyons, pers. comm.) and in the Nebraska portion of the Missouri River (Hesse et al., 1993).

**TAXONOMIC CONSIDERATIONS.**—Although considerable geographic variation in morphology is present, no populations are diagnosable based on data from this study. Some characters do show east-west trends but exhibit too much discordant variation to permit identification of discrete geographic groups. Two processes are hypothesized to have created this pattern of variation in *M. hyostoma*.

The first pattern is one of local differentiation resulting from diverse selective pressures. Populations inhabiting turbid streams have longer and more numerous barbels, smaller eyes, and reduced pigmentation and belly squamation. Researchers have commented on the incredible plasticity of this species (Davis and Miller, 1967; Reno, 1969) and the amount of homoplasy and polymorphisms present in the characters examined in the phylogenetic analyses below tends to support their observations. For example, the Brazos and Colorado rivers were connected

as recent as 8,000 ya (Conner and Suttkus, 1986), yet there are substantial differences in meristics, morphometrics, and pigmentation between populations from these drainages. Specimens from the more turbid Brazos River exhibit a suite of characters adapted for reduced visual but increased chemosensory orientation.

A second process overlaying the adaptive morphology pattern involves vicariance of eastern and western groups prior to and during glacial advances. Prior to the mid-Pleistocene, most of the Plains was drained by the south-flowing "Plains Stream" (Frye and Leonard, 1952; Metcalf, 1966; Cross et al., 1986). At the height of "Kansan" glaciation, the upper Missouri drainage was diverted into the Plains Stream (Metcalf, 1966; Pflieger, 1971; Cross et al., 1986), completely isolating the Plains from the Mississippi-Teays basin. After glacial retreat, the Missouri, Arkansas, and Red rivers established their present courses (Quinn, 1958; Metcalf, 1966; Cross et al., 1986), permitting enough gene flow between western and eastern populations of *M. hyostoma* to eliminate discrete breaks in morphological variation (Metcalf, 1966).

A recent phylogenetic analysis of selected western populations using allozyme data did not demonstrate a monophyletic *M. hyostoma* (Underwood et al., 2003). However, these authors suggested the apparent paraphyly of *M. hyostoma* could be attributed to secondary contact and introgressive hybridization of *M. hyostoma* with *M. australis* and *M. tetranema*.

Given the uncertain and possibly reticulate evolutionary history among populations considered to represent *M. hyostoma* in this study, taxonomic subdivision of *M. hyostoma* is premature. Additional geographic studies of variation incorporating genetic markers are needed to determine whether taxonomically meaningful phylogenetic partitions exist in *M. hyostoma*.

**ETYMOLOGY.**—The specific epithet, *hyostoma*, is from the Greek *hypo* (hog) and *stoma* (mouth). The specific epithet was emended from *hyostomus* to *hyostoma* to match the gender of the feminine *Macrhybopsis* (Bailey, pers. comm.). The proposed common name, shoal chub, refers to the typical habitat of this species, coarse sand and gravel raceways with strong current.

### *Macrhybopsis marconis* (Jordan and Gilbert)

Burrhead Chub  
FIG. 4F

*Hybopsis aestivalis marconis* Jordan and Gilbert 1886:315–316 (Río San Marcos at San Marcos).

This taxon was elevated to species by Hubbs and Ortenberger (1929), although they did not provide supporting data. Moore (1950) did not include this taxon in

his list of subspecies of *M. aestivalis*. Because Moore did not provide a list of specimens examined, it is unclear whether he chose not to recognize *marconis* as a distinct taxon, or simply did not examine specimens from its range. Hubbs et al. (1953), after examining numerous specimens from the Guadalupe River, concluded "no specimens . . . indicated any tendency toward *Extrarius aestivalis marconis*," and identified them as *H. a. aestivalis*. The conclusion of Hubbs et al. (1953) is surprising, considering the unique patterns of tuberculation and pigmentation of populations in the Guadalupe, San Antonio, and Colorado River drainages. The taxon again was recognized as a subspecies as *H. a. marconis* by Davis and Miller (1967) and others (Higgins, 1977; Wallace, 1980; Becker, 1983), referencing Hubbs and Ortenberger's 1929 paper. The distinctive morphology of *M. marconis* and the presence of an area of sympatry with *M. hyostoma* in the Colorado River indicates that *M. marconis* warrants species recognition and is herein treated as such.

**TYPES.**—Syntypes include: USNM 36524, USNM 345458, and USNM 345459 (16, 27.5–53.6 mm SL, plus one specimen of *Notropis volucellus*; all originally USNM 36524) Río San Marcos at San Marcos, Texas, July–September 1884, D. S. Jordan and C. H. Gilbert, collectors; UMMZ 61573 (1, 30.9 mm SL; originally IU 5289), Río San Marcos just below mouth of Río Blanco, Texas, summer 1884, D. S. Jordan and C. H. Gilbert, collectors; USNM 125136 (2, 31.9–39.3 mm SL; originally USBF 140), Río San Marcos Spring, Texas, date and collectors unknown.

I designate USNM 36524 (female, 53.6 mm SL, collected July–September 1884, Río San Marcos at San Marcos, Texas, D. S. Jordan and C. H. Gilbert, collectors) as lectotype. The remaining syntypes (USNM 345458, n=15; USNM 125136, n=2; and UMMZ 61573, n=1) become paralectotypes.

**DIAGNOSIS.**—Distinguished from all other members of the *M. aestivalis* complex by the presence of tubercles on the head of nuptial males and a well-defined lateral stripe continuous from the operculum to the base of the caudal fin, approximately one scale wide (Fig. 4F).

Additional distinguishing characters include: eye large and round; with a single pair of short barbels, usually < 45% of orbit length; snout rounded and blunt; caudal peduncle deep, > 50% of maximum body depth; pectoral fins short and rounded or bluntly pointed, lemon-yellow in nuptial males; small melanophores tend to be concentrated on submargin of scales, creating diamond pattern on dorsolateral surface of body; few or no scales with clusters of melanophores; bridge of scales usually present just anterior of pelvic bases; anal rays modally eight.

**DESCRIPTION.**—Selected meristic counts of 170 total specimens appear in Tables 1–8. Largest female 56.4 mm SL (70 mm TL, UF 56781); largest male 59.8 mm SL (73 mm

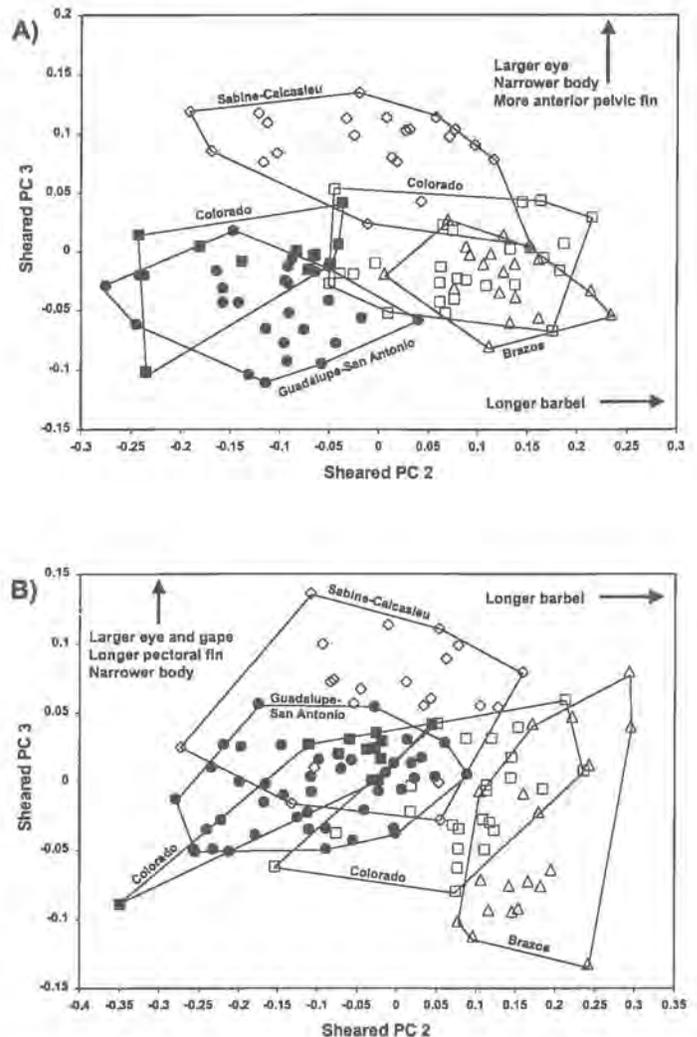
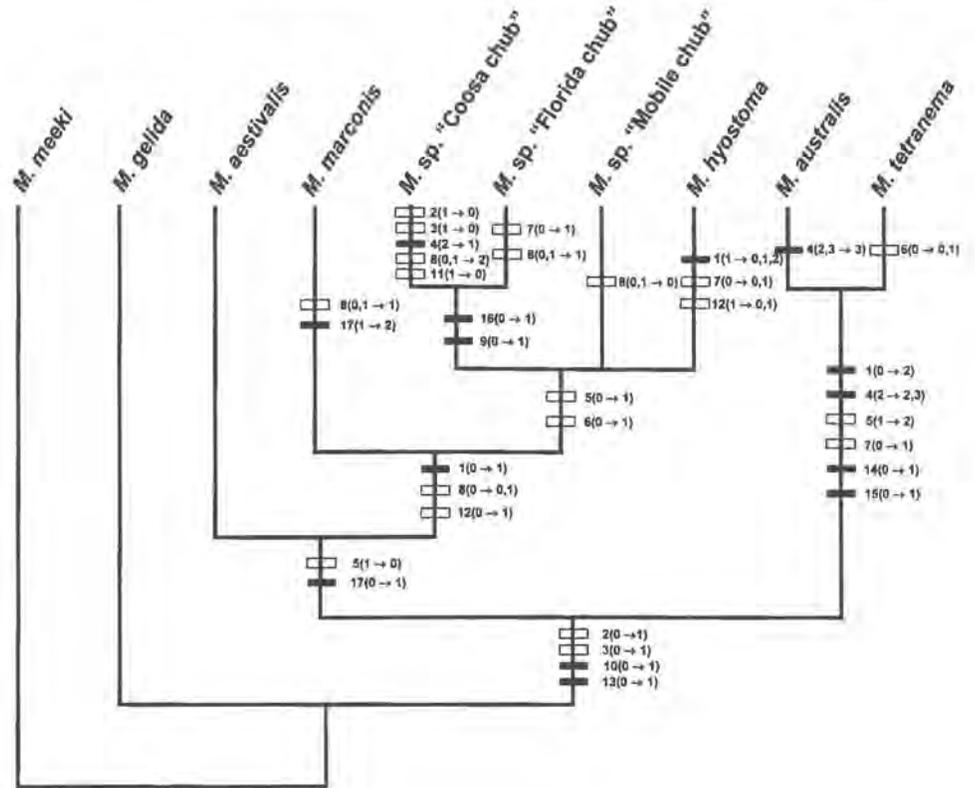


Figure 15. Morphometric scores on sheared PC axes 2 and 3 for *Macrhybopsis hyostoma* (open symbols) and *M. marconis* (solid symbols) from the West Gulf Slope. A) 67 male *M. hyostoma* and 37 male *M. marconis*. B) 65 female *M. hyostoma* and 56 female *M. marconis*. Polygons bound individuals of each drainage unit cluster.

TL, USNM 46220). Caudal rays 19 (17–20); anal rays 8 (7–9); pelvic rays 8 (8–9); pectoral rays 13–15 (12–16). Lateral-line scales 35–38 (35–39); predorsal scales 0–20; scales above lateral line 5 (4–6); scales below lateral line 4–5 (4–6); caudal peduncle scales 12 (11–15). Nape fully scaled in 88% of specimens examined. Belly posterior to pelvic bases fully scaled. Usually with bridge of scales on belly just anterior to pelvic bases, occasionally with only few scales or naked. Infraorbital pores 13–16 (11–17); preoperculo-mandibular pores 11–13 (9–14). Total vertebrae 36–38; precaudal vertebrae 17–18 (17–19); caudal vertebrae 18–19 (17–20).

Body terete with deep and long caudal peduncle. Dorsal and anal fins bluntly pointed; pelvic fins rounded; pectoral fins rounded or bluntly pointed and short, failing

Figure 16. The most conservative tree and strict consensus tree of three shortest trees generated from parsimony analysis of 17 morphological characters for eight species of the *Macrhybopsis aestivalis* complex and two outgroups. This tree has a length of 67, a consistency index of 0.727, and a retention index of 0.705. Character numbers and derived character states are shown for ACCTRAN optimization; see Appendix 1 for character descriptions and Appendix 2 for data matrix. Homoplasious transformations are indicated by open rather than solid bars.



to reach origin of pelvic fins in adults. Snout rounded, more so than in any other member of *M. aestivalis* complex. Mouth horizontal and inferior; gape width not as wide as head when viewed ventrally; lips only slightly fleshy. One pair of short maxillary barbels present, usually < 45% of orbit length. Eye large, round or nearly so (orbit depth > 80% of orbit length). Small taste buds not as prominent as in other members of the *M. aestivalis* complex; present as small papillae on gular region. Genital papilla absent or poorly developed as small conical flap in both sexes. Gill rakers absent or present as 1–4 dorsal rudiments. Pharyngeal teeth 0,4–4,0.

Somewhat translucent in life, pale olive dorsally and silvery-white ventrally with broad silver lateral stripe. Small melanophores on posterior dorsolateral scales concentrated to form submarginal band on scales giving appearance of vague diamond pattern. In preserved specimens, dark and continuous lateral stripe present from operculum to caudal peduncle, centered on lateral line and one scale wide (obscured by silvery coloration in live specimens). All dorsal rays outlined with pigment, first 6–8 pectoral rays pigmented; pelvic and anal fins with little or no pigment. Nuptial males with lemon-yellow pectoral fins.

Pectoral rays 2–8 to 2–10 thickened in nuptial males and bear rows of conical, slightly antrorse uniserial tubercles (Fig. 3A) on the dorsal surface. Tubercles also present on head of nuptial males, most concentrated anterior and ventral to eye and on dorsum of head. Cornified portion of these tubercles a narrow cone, resting upon a round, fleshy base. Smaller tubercles present on predorsal sur-

face of body in large males in peak nuptial condition. Females lack tubercles although large females (>50 mm SL) have slightly thickened pectoral rays. Cephalic taste buds enlarged on females during summer, although not cornified.

**GEOGRAPHIC VARIATION.**—Little variation was apparent, with the exception of a series from the Blanco River (INHS 8378) with longer barbels (48–63% of orbit length) than specimens from the remainder of the range (25–50% of orbit length). Specimens from the Colorado drainage had fewer mean lateral-line scales and predorsal scales (Tables 2–3).

**COMPARISONS.**—Sheared PCA moderately separated *M. marconis* from sympatric and geographically proximate populations of *M. hyostoma* (Fig. 15). Separation occurred primarily on the sheared PC 2 axis, with loading values indicating *M. marconis* had shorter barbels. Specimens of *M. hyostoma* from the Brazos and Colorado River drainages had lower mean indices of belly squamation (Tables 5–6) and fewer mean preoperculomandibular pores than specimens of *M. marconis* (10.59–11.37 vs. 12.17).

**DISTRIBUTION AND STATUS.**—Distributed throughout the larger streams of the Guadalupe and San Antonio River drainages, and the Colorado River drainage, where it is nearly confined to the Edwards Plateau (Fig. 11). Although common to abundant in the Guadalupe and

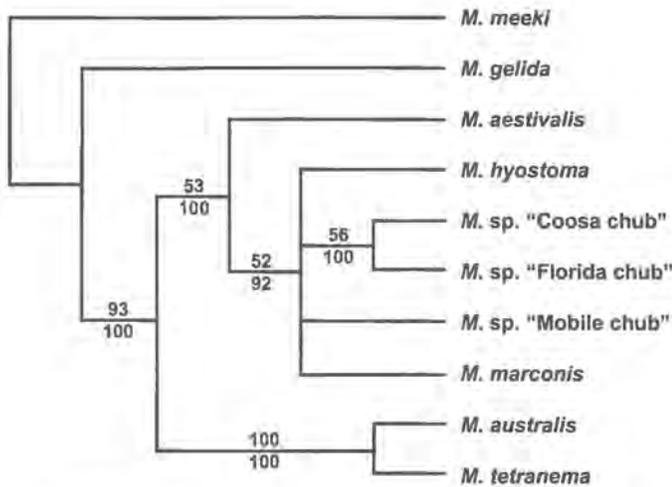


Figure 17. Bootstrap majority-rule consensus tree. This is also the majority-rule consensus tree for all minimum-length trees and trees one step longer (12 total trees). Numbers above branches indicate the percentage of bootstrap replications supporting a clade. Numbers below branches indicate percentage of trees from consensus procedure supporting the clade.

San Antonio drainages, it has declined in the Colorado River drainage. All Colorado River mainstem historical localities now are impounded or affected by reservoir discharge, and the lower Pedernales River is impounded. *Macrhybopsis marconis* was last collected from the Pedernales River in 1952 and the Llano River in 1976. My recent (1995–1996) attempts to collect *M. marconis* in the Pedernales and Llano rivers were unsuccessful. The species was collected in 2002 in the Colorado River Bastrop by A. A. Echelle, indicating it persists in the lower part of the drainage.

**CONTACT ZONE.**—*Macrhybopsis marconis* was sympatric with *M. hyostoma* in the Colorado River in Burnet, Travis, and Bastrop counties. Collections from Burnet County (TNHC 1362) and Bastrop County (OSUS 27540–27541) contained both species. These species are easily distinguished where they occur together and no morphologically intermediate specimens were found in this study.

**ETYMOLOGY.**—The specific epithet, *marconis*, refers to San Marcos, type locality for the species (Jordan and Evermann, 1896). The proposed common name, burrhead chub, refers to the diagnostic head tubercles of nuptial males.

#### Relationships within the *Macrhybopsis aestivalis* complex

Phylogenetic analysis of the morphological data with mixed ordered and unordered characters produced three minimum-length trees with lengths of 67, consistency

indices (CI, Kluge and Farris, 1969) of 0.727, and retention indices (RI, Farris, 1989) of 0.705. The most conservative of these trees (Fig. 16), which is also the strict consensus tree, has an unresolved trichotomy consisting of *M. hyostoma*, *M. sp. "Mobile chub,"* and a *M. sp. "Coosa chub"*-*M. sp. "Florida chub"* clade. One of the minimum length tree resolves this trichotomy by placing *M. sp. "Mobile chub,"* as sister to the other three species. The other minimum length tree places *M. hyostoma* sister to the other three species. These clades in the two fully resolved trees are not supported by character transformations free of homoplasy. A second phylogenetic analysis treating all characters as unordered resulted in three minimum-length trees identical in topology to the three minimum-length trees produced by using the mixed ordered and unordered characters.

Three clades in the most conservative of the shortest trees are strongly supported upon examination of character transformations, the consensus tree of the minimum and near minimum-length trees, and the bootstrap tree. The *M. aestivalis* complex and *M. australis*-*M. tetranema* clades are each supported by at least two characters free of homoplasy, all of the 12 shortest trees, and 93–100% of the replicates in the bootstrap procedure (Figs. 16–17). Two character transformations free of homoplasy, presence of body spots and loss of strongly bicolored ventral lobe of caudal fin, support the *M. aestivalis* complex. The *M. australis*-*M. tetranema* clade is supported by four character transformations free of homoplasy: lateral stripe above lateral line, some individuals with biserial pectoral fin tuberculation, fleshy lips, and relatively long barbels. Although the bootstrap value (56%) is lower in a clade containing *M. sp. "Coosa chub"* and *M. sp. "Florida chub"* (Fig. 17) all 12 shortest trees and two character transformations free of homoplasy, genital papilla prominent and anterior position of the anus, support this clade (Fig. 16–17). The surprisingly low bootstrap values may be due to the low character to taxon ratio (1.7), numerous reversals in *M. sp. "Coosa chub"*, and uneven branch lengths within this clade (Fig. 16) (Sanderson, 1989; Hillis and Bull, 1993). In addition, bootstrapping has been shown to underestimate values above 50%, as actual probabilities representing the "true" phylogeny are somewhat higher (Hillis and Bull, 1993).

Two additional clades have moderate support. The clade containing all of the *M. aestivalis* complex except *M. australis* and *M. tetranema* is supported by all of the 12 shortest trees, 53% of the bootstrap replications, and one character transformation free of homoplasy, moderately enlarged optic lobes (Figs. 16–17). A clade containing *M. hyostoma*, *M. marconis*, *M. sp. "Mobile chub,"* *M. sp. "Coosa chub,"* and *M. sp. "Florida chub"* is supported by 11 of the 12 shortest trees, 52% of the bootstrap replications and one character transformation free of homoplasy, presence of a lateral stripe centered on the lateral line.

Remaining clades have weak support. These clades are

supported by less than 50% of the bootstrap replications, less than 50% of the 12 shortest trees, and no character transformations free of homoplasy. The relatively high amount of homoplasy, as indicated by the low CI values, RI values, and uneven branch lengths, likely contributed to the low support for these nodes (Felsenstein, 1978; Hendy and Penny, 1989; Swofford and Olson, 1990).

A possible sister relationship of *M. marconis* and *M. aestivalis*, suggested by overall phenotypic similarity and geographic proximity, was tested by constraining the analysis to contain a clade of *M. aestivalis* and *M. marconis*. The resulting tree is one step longer (length = 68) and supports monophyly of these species with one synapomorphy, blunt snout.

Many of the characters used in this study, especially pigmentation and sensory structures, exhibit reversals and parallel evolution, apparently as a result of similar environmental selective pressures (Hubbs, 1940b; Metcalf, 1966). In general, populations associated with relatively clear streams (*M. sp.* "Coosa chub," *M. marconis*, eastern populations of *M. hyostoma*, and southeastern populations of *M. aestivalis*) are more darkly pigmented, and have larger eyes and smaller and fewer taste buds and barbels than populations from relatively turbid streams (*M. australis*, *M. tetranema*, western populations of *M. hyostoma*, and northwestern populations of *M. aestivalis*). Because of the high degree of homoplasy in the data and general lack of support for certain nodes, the most conservative of the three trees is presented as the preferred tree (Fig. 16).

## Discussion

**SPECIATION HYPOTHESIS.**—Part of the difficulty of uncovering the evolutionary history of the *M. aestivalis* complex may be traced to the mechanism of speciation within the complex. *Macrhybopsis hyostoma* is widespread in North America, whereas the remaining species occupy limited geographic areas at the periphery of the range of *M. hyostoma* (Fig 2). This geographic pattern suggests the possibility of speciation through peripheral isolation with multiple speciation events (allopatric model II; Wiley, 1981; Wiley and Mayden, 1985). Climatic changes resulting in Great Plains streams changing from depositional to downcutting at the start of the Pleistocene (discussed below) may have been the event that isolated many populations in a relatively short period of time. Although some species have distributions rather large to be considered peripheral isolates (*M. aestivalis*), they are still much smaller than the presumed central and ancestral species (*M. hyostoma*). Because this difference in size of distribution is likely sufficient to result in the central species surviving the speciation event, phylogenetic and zoogeographic predictions of speciation through peripheral isolation or "microvicariance" apply.

One of the predictions of speciation of several peripheral isolates is that branching patterns as evidenced by synapomorphies are polychotomous rather than dichoto-

mous (Wiley, 1981; Frey, 1993). Even if speciation of peripheral isolates was not simultaneous, speciation events may have been too close together to recover the phylogeny. Derived character states are unlikely to evolve during short periods of time between successive speciation events (Wiley, 1981; Lanyon, 1985). In addition, due to segregation of ancestral polymorphisms, there is a high probability of obtaining an incorrect phylogeny unless speciation events are far apart in time, especially when relatively few characters or genes are examined (Wu, 1991; Avise, 1994). Thus, polychotomous branching patterns in the *M. aestivalis* complex phylogeny, whether present in minimum-length trees or suggested by lack of support or agreement for clades, may reflect an evolutionary history of speciation of multiple peripheral isolates.

Speciation by the peripheral isolate formation model in the *M. aestivalis* complex is generally consistent with other predictions of phylogenetic and biogeographic patterns (Wiley and Mayden, 1985; Frey, 1993). Peripheral isolates should be similar or have phylogenetic affinities to proximate populations of the parental species (Wiley and Mayden, 1985). *Macrhybopsis tetranema* and *M. australis* are most similar in morphology and allozymes (Underwood et al., 2003) to proximate populations of *M. hyostoma*. In addition, phylogenetic analysis of the allozyme data placed *M. hyostoma* from the Red and Arkansas river basins with the associated endemic, rather than with other *M. hyostoma* populations (Underwood et al., 2003). Additional phylogenetic studies incorporating genetic characters, are needed to better address this prediction. The prediction that peripheral isolates should be relatively apomorphic compared to the central population (Frey, 1993) is rather equivocal. Although this study found no apomorphies for the central population (i.e., *M. hyostoma*), most of the other taxa lack multiple apomorphies (*M. marconis* is a notable exception). An additional result of peripheral isolation is that fixation of characters may proceed more rapidly in peripheral isolates than in the parental species (Wiley, 1981). This seems to be true in the *M. aestivalis* complex, as the widespread *M. hyostoma* is polymorphic for four characters, whereas the remaining peripheral species are polymorphic for 0–2 characters each (Appendix 2).

Although peripheral isolation was important during the evolution of the *M. aestivalis* complex, some speciation events were characterized by vicariance (Allopatric model I; Wiley, 1981), perhaps the most common mode of speciation for North American freshwater fishes (Wiley and Mayden, 1985). Vicariant speciation of *M. australis* and *M. tetranema* likely occurred following the breakup of the ancestral Plains Stream and the subsequent establishment of the modern Arkansas and Red rivers.

**ZOOGEOGRAPHY.**—It is likely the *M. aestivalis* complex had a Mississippi-Missouri basin origin, probably in the northern plains. The sensory systems of *Macrhybopsis* sug-

gest evolution in a place of high turbidity, as were the rivers of the glacial and preglacial Plains (Frye and Leonard, 1952). A northern Plains origin has been suggested for putative sister taxa *M. gelida* and *M. meeki*, which nearly are confined to the Missouri River basin (Metcalf, 1966; Cross et al., 1986).

The paleohydrography of the Plains supports a view of dispersal in the Miocene-Pliocene followed by a period of vicariance during the Pliocene-Pleistocene. The ancestor of the complex apparently became widespread throughout much of its present range prior to the Pleistocene. The south-flowing preglacial Plains Stream (Metcalf, 1966) and extensive crossgrading of Ogallala Formation streams during the Miocene-Pliocene (Frye and Leonard, 1959; Gustavson and Finley, 1985) allowed for extensive north-south ichthyofaunal transfer (Conner and Suttkus, 1986) in the Plains. Access to the Rio Grande basin could have been gained during the early to middle Pliocene, when the upper Rio Grande drained eastward across the Ogallala Formation, into the ancestral Brazos or Colorado rivers (Thomas, 1972; Belcher, 1975).

The second phase in the zoogeography of the complex may have begun at the middle of the Pliocene when climatic and tectonic changes altered the drainage patterns of the Plains, generally impeding ichthyofaunal movements. Tectonic uplifts in New Mexico diverted the ancestral upper Rio Grande south (Belcher, 1975), isolating *M. aestivalis* from the rest of the complex. Entrenchment of Ogallala formation streams during the increasingly mesic conditions of the late Pliocene, as streams changed from depositional to erosional (Walker, 1978; Dolliver, 1984; Gustavson and Finley, 1985), further contributed to isolation of populations of the *M. aestivalis* complex. Thus, *M. aestivalis*, *M. marconis*, *M. hyostoma*, and the *M. australis-M. tetranema* ancestor may have become isolated in a relatively short period of time. This hypothesis of concurrent speciation by multiple peripheral isolation is consistent with the phylogenetic hypothesis presented in this study.

The zoogeographic origin of *M. marconis* is uncertain. It may have gained access to its present range via an eastward-flowing Ogallala stream and subsequently became isolated at the end of the Pliocene when streams began to entrench on the Edwards Plateau (Thornbury, 1965). Alternatively, if Rio Grande-San Antonio Bay connections during low oceanic levels of the Pleistocene (Morton and Price, 1987) allowed gene flow with Rio Grande populations, isolation would have occurred more recently, resulting in a sister relationship with *M. aestivalis*. However, this relationship was not supported in minimum-length trees of the phylogenetic analysis. An additional route of dispersal from the east is less likely, as there is no geologic evidence of a connection with the Colorado River (Conner and Suttkus, 1986).

Morphological differentiation among populations of *M. aestivalis* was likely facilitated by periodic isolation of the upper Rio Grande during periods of endorheism in

the late Pliocene to middle Pleistocene (Thomas, 1972; Belcher, 1975; Smith and Miller, 1986). Access to the Rio San Fernando drainage from the Rio Grande basin could have occurred by stream capture in the headwaters of the Rio San Juan (Conant, 1969) or confluence of the lower channels during lowered sea levels.

The ancestor of the *M. australis-M. tetranema* clade likely inhabited a south-flowing stream in western Kansas, Oklahoma, and Texas. The *Notropis girardi* complex has a distribution similar to *M. australis-M. tetranema*, with endemics in the upper Brazos, upper Red, and upper Arkansas rivers (Cross, 1953). This shared zoogeographic tract supports the presence of a Pliocene-early Pleistocene drainage (ancestral upper Red River) to the west and largely independent of the Plains Stream. Geologic evidence for this stream is limited to southwestern Kansas (Frye and Leonard, 1952), although faunal evidence presented here and in Metcalf (1966) and Connor and Suttkus (1986) indicate this stream likely extended south to the present Brazos River. Establishment of the modern Arkansas River basin in the middle Pleistocene by capture of part of the Plains Stream (Quinn, 1958; Cross et al., 1986) apparently isolated *M. australis* and *M. tetranema*.

Drainage evolution of the Mississippi basin contributed to the geographic pattern of morphological variation of *M. hyostoma*. The present Red, Arkansas, Missouri, and Des Moines rivers (and possibly the Brazos and Colorado rivers) were all connected by the Plains Stream but were isolated from the Mississippi River basin during early glacial advances (Metcalf, 1966; Mayden, 1985; Cross et al., 1986). Access to the Mississippi River basin was accomplished during the mid-Pleistocene when the headward eroding Arkansas and Red rivers captured sections of the Plains Stream (Quinn, 1958; Mayden, 1988) and glacial retreat reopened the outlet of northern Plains streams through the present Missouri River (Frye and Leonard, 1952; Cross et al., 1986). This would have allowed western *M. hyostoma* to invade the lower Red, Arkansas, Missouri, and Mississippi rivers and provided opportunity for genetic exchange with eastern populations of *M. hyostoma*.

The origin of Brazos-Colorado *M. hyostoma* is enigmatic. A dispersal origin along the West Gulf Coast is possible during various interdrainage connections created by fluctuating sea levels (Conner and Suttkus, 1986), but the absence of *M. hyostoma* in the Trinity River drainage, and the phenotypic dissimilarity of Brazos-Colorado and Sabine-Calcasieu populations makes this scenario dubious. Affinities of Brazos-Colorado *M. hyostoma* with northern *M. hyostoma* are more tenable. Brazos-Colorado connections with the glacial Plains Stream or Brazos-Red stream capture events may have occurred as late as the middle Pleistocene (Stricklin, 1961; Conner and Suttkus, 1986). Connections this late would require that the stream inhabited by the *M. australis-M. tetranema* clade would have been integrated with the Plains Stream at this point, necessitating divergence of the *M. australis-M.*

*tetranema* clade prior to the formation of the glacial Plains Stream. Earlier vicariance of Brazos-Colorado populations, during dissection of the Ogallala formation in the Pliocene, permits a later date of divergence of the *M. australis*-*M. tetranema* clade. Although this hypothesis would indicate *M. hyostoma* (as construed herein) is not monophyletic, *M. hyostoma* may not be expected to be monophyletic as evidenced by phylogenetic analysis if speciation within the complex was a pattern of multiple speciation events spaced too close to permit lineage sorting.

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#### Materials Examined

Parenthetical numbers after catalog numbers refer to number of specimens used in meristic and morphometric data, respectively.

*Macrhybopsis aestivalis*.—San Fernando drainage: Nuevo León (Mexico): USNM 55727 (4,0), UMMZ 162123 (10,1), UMMZ 97390 (10,0), OSUS 12525 (1,1), FMNH 4438 (35,8), FMNH 4429 (10,3). Tamaulipas (Mexico): TCWC 6402.2 (9,4), TCWC 6403.2 (20,8), UF 43048 (5,0), CU 24390 (8,1), UMMZ 192468 (1,0), TU 43577 (10,3). San Juan drainage: Nuevo León (Mexico): UMMZ 169592 (23,6), UMMZ 164720 (4,0), FMNH 4406 (14,8), UMMZ 97389 (10,5). Salado drainage: Coahuila (Mexico): UMMZ 130345 (25,1), TU 87156 (2,2). Nuevo León (Mexico): FMNH 5571 (43,21). Lower R. Grande basin: Texas: Hidalgo County, ANSP 161581 (1,1), THNC 11705 (1,1), TU 11330 (2,0); Starr County, TNHC 4659 (95,30). Lower Middle R. Grande basin: Texas: Maverick County, MSB 6982 (5,1), UMMZ 170128 (13,3), TU 11342 (32,4); Webb County, OSUS 12523 (2,1) TNHC 4453 (21,12), TU 11323 (20,1), TNHC 4769 (19,5); Zapata County, UMMZ 170192 (1,1). Coahuila (Mexico): UMMZ 196746 (3,3), KU 3019 (2,0). Pecos drainage: New Mexico: Chavez County, INHS 81963 (1,1); De Baca County, OSUS 14325 (20,19), MSB 13542 (20,4); Eddy County, INHS 88342 (7,1); Guadalupe County, UMMZ 133134 (1,1). Texas: Reeves County, UMMZ 170092 (18,0); Val Verde County, INHS 67913 (8,7); Ward County, UMMZ 137106 (1,1). Upper Middle R. Grande drainage: Chihuahua (Mexico): KU 4995 (2,0), TNHC 4061 (30,10). Coahuila (Mexico): UMMZ 20153 (4,3). Texas: Brewster County, INHS 83247 (5,0), UMMZ 201486 (5,1), OKMNH 31273 (6,4), OKMNH 31301 (4,0), INHS 83257 (1,1), FMNH 35265 (3,0), MSB 10450 (9,1), UMMZ 201504 (2,1); Presidio County, UMMZ 20476 (10,0), MSB 25152 (7,3), MSB 25191 (8,4), MSB 25169 (22,11), MSB 4915 (1,0); Terrill County, UMMZ 201532 (5,3). Upper R. Grande drainage: New Mexico: Bernalillo County, UMMZ 13324 (1,1), KU 4250 (1,0), MSB 1851 (3,3), MSB 1869 (4,4), MSB 1863 (1,0), MSB 1864 (10,0), MSB 1875 (25,3), MSB 1848 (10,3); Dona Ana County, UMMZ 124735 (2,2); Sandoval County, UMMZ 133241 (3,2), MSB 1865 (14,11), MSB 1855 (7,6); Santa Fe County, UMMZ 178711 (8,6), USNM 16978 (4,0), MSB 3412 (1,1); Socorro County, MSB 1859 (7,5). Texas: El Paso County, UMMZ 207685 (1,0), TNHC 4239 (3,3), TNHC 4619 (2,2).

*Macrhybopsis australis*.—Upper Red basin: Oklahoma: Beckham County, OKMNH 33446 (1,1); Greer County, UMMZ 80383 (9,5), OKMNH 6181 (5,0), SIUC 24730 (8,6); Harmon County, UMMZ 80349 (10,5), OKMNH 6337 (1,0), UMMZ 80347 (1,1), OKMNH 6369 (5,0), UMMZ 80367 (5,1), OKMNH 6294 (2,0); Jackson County, OKMNH 33379 (4,0), OKMNH 33389 (6,2), OKMNH 33379 (4,0); Kiowa County, OKMNH 33260 (3,3), OKMNH 33276 (3,3), OKMNH 33283 (19,9); Roger Mills County, OKMNH 6202 (1,0), UMMZ 80410 (1,0); Tillman County, OKMNH 33241 (2,2). Middle Red basin: Oklahoma: Cotton County, KU 2439 (8,0), OKMNH

33147 (5,1), OKMNH 33137 (5,0); Jefferson County, OKMNH 33099 (5,1), OKMNH 33389 (6,2); Tillman County, OKMNH 33185 (3,2), OKMNH 33192 (4,1), OKMNH 33207 (1,1). Texas: Wichita County, INHS 67830 (10,0), TCWC 3910.02 (5,0); Wilbarger County, TU 149600 (10,1). Wichita drainage: Texas: Baylor County, OSUS 26962 (20,8); Foard County, OSUS 26888 (11,10), OSUS 26894 (23,6); Knox County, OSUS 26902 (10,0).

*Macrhybopsis hyostoma*.—Colorado drainage: Texas: Burnet County, TNHC 1362 (17,12); Coke County, TU 39884 (10,0); Colorado County, TU 73405 (9,4); Fayette County, MCZ 56154 (40,30); Jackson County, TNHC 1253 (1,0), TNHC 1255 (2,1); San Saba County, TNHC 2486 (9,3), TNHC 2497 (1,0). Brazos drainage: Texas: Bosque County, TU 4949 (10,3); Brazos County, UF 29435 (2,0), KU 14275 (1,1), UMMZ 110598 (3,0), TCWC 7551.09 (14,5); McLennon County, UMMZ 167773 (5,0), TU 35963 (3,2); Palo Pinto County, KU 2311 (18,15), OSUS 12121 (6,5); Robertson County, INHS 67877 (3,3), UF 29626 (1,0); Stonewall Co., TNHC 12246 (14,0); Young County, OKMNH 31310 (20,2), OSUS 11845 (24,0), SIUC 26469 (2,2). Sabine-Calcasieu drainages: Louisiana: Allen Parish, TU 64268 (9,2); Vernon Parish, UAIC 2678.14 (2,0). Texas: Gregg County, ANSP (40,9); Jasper County, TU 120745 (20,8); Nacogdoches County, SIUC 26035 (5,2); Newton County, UF 50891 (19,0); Panola County, INHS 67847 (27,21), UT 44.1611 (3,0), SIUC 26485 (10,2). Middle Red basin: Oklahoma: Bryan County, OKMNH 36968 (1,1), OKMNH 29889 (4,4), OKMNH 33762 (3,3); Choctaw County, KU 14217 (42,10); Cotton County, OKMNH 33137 (1,1), KU 2439 (7,0); Jefferson County, OKMNH 33099 (1,0), SIUC 26042 (10,7); McCurtain County, UT 44.1382 (7,3). Texas: Wichita County, INHS 67830 (14,14), TCWC 3910.02 (4,4). Washita drainage: Oklahoma: Garvin County, UF 25504 (5,0), EUK 529 (12,3); Grady County, OKMNH 36252 (11,7); Murray County, OKMNH 31575 (5,1). Lower Red basin: Arkansas: Hempstead County, USNM 36342 (3,0). Louisiana: Bossier Parish, USNM 173317 (3,0); Natchitoches Parish, UAIC 2675.08 (2,0); Rapides Parish, TU 42265 (64,10); parish unknown, USNM 242264 (5,0). Ouachita drainage: Louisiana: Ouachita Parish, UMMZ 169788 (4,0), UMMZ 184123 (4,0), UMMZ 211888 (2,0), UMMZ 184244 (4,0), KU 22144 (24,18); Union Parish, UMMZ 184089 (1,0). Canadian drainage: Oklahoma: Haskell County, OKMNH 36236 (2,0); McIntosh County, OKMNH 36211 (1,0), OKMNH 35103 (1,0), OKMNH 36223 (12,0), OKMNH 34533 (5,0), OKMNH 36301 (1,0); Pittsburg County, KU 5952 (3,0). Cimarron drainage: Oklahoma: Kingfisher County, OSUS 26854 (3,3); Logan County, OKMNH 33985 (1,1), OSUS 26839 (26,10); Pawnee County, OKMNH 39025 (5,0); Payne County, USNM 161636 (4,1). Salt Fork Arkansas drainage: Oklahoma: Alfalfa County, OKMNH 29157 (5,3), OSUS 1869 (2,2), TU 2265 (4,0); Grant County, OSUS 18061

(18,10), OSUS 19586 (20,11), OSUS 19152 (3,3); Noble County, UMMZ 127283 (5,0), TU 13840 (15,0). Arkansas basin: Arkansas: Pope County, OSUS 7224 (10,0). Kansas: Sumner County, UMMZ 67816 (1,0), KU 8311 (12,7), KU 8285 (1,1). Oklahoma: Osage County, OSUS 26784 (19,10); Tulsa County, OKMNH 39017 (30,4), OSUS 26682 (5,0), OSUS 26609 (1,1). White-St. Francis drainages: Arkansas: Prairie County, INHS 33605 (1,0); Randolph County, TU 54593 (2,0), TU 57250 (2,0), TU 65584 (2,0), TU 59683 (2,0), TU 57153 (2,0), TU 57228 (17,2); St. Francis County, UF 64345 (1,0). Missouri: New Madrid County, SIUC 3096 (5,0), INHS 81120 (2,0), UMMZ 153120 (3,0), SIUC 24448 (7,6); Pemiscot County, UMMZ 139708 (2,0); Scott County, UMMZ 188560 (40,22); Stoddard County, INHS 81729 (8,2). Platte-Elkhorn drainage: Nebraska: Blaine and Buffalo counties, USNM 125132 (3,0); Buffalo County, UMMZ 135694 (8,0), USNM 76047 (3,0), KU 17819 (6,6), KU 17817 (10,7); Madison County, EUK 316 (20,9); Platte County, EUK 584 (20,2). Blue-Republican drainages: Kansas: Jewell County, KU 4870 (4,0); Marshall County, KU 4175 (20,11); Washington County, KU 4384 (4,4). Nebraska: Dundy County, UMMZ 134343 (6,0); Red Willow County, FMNH 78288 (10,9); Webster County, UMMZ 135057 (7,1). Kansas: Douglas County, USNM 242263 (10,0), KU 24655 (1,1), KU 13984 (49,5); Shawnee County, KU 8130 (4,0); Wyandotte County, KU 21821 (14,10). Grand-Chariton-Osage drainages: Missouri: Chariton County, INHS 80241 (7,7), INHS 68091 (1,1), UMMZ 148979 (6,6), SIUC 24863 (2,0); Clair County, UMMZ 150253 (1,0); Gentry County, UMMZ 148095 (4,3); Grundy County, UMMZ 148187 (5,5), SIUC 24924 (39,5); Harrison County, UMMZ 148068 (5,2); Livingstone County, UMMZ 148928 (2,2); Vernon County, INHS 81845 (1,1). Upper Missouri basin: Missouri: Cooper County, UT 44.1866 (5,1), UT 44.5129 (10,0); Jackson County, UMMZ 152578 (5,0); Lafayette County, KU 9782 (13,2). Nebraska: Washington County, UT 44.3981 (1,0). Lower Missouri basin: Illinois: Madison County, INHS 19848 (11,0). Missouri: St. Charles County, SIUC 24558 (1,1), SIUC 28896 (6,1); St. Louis County, KU 9674 (22,1), UT 44.7181 (4,0), UT 44.7182 (1,1). Upper Mississippi basin: Illinois: Henderson County, INHS 20613 (5,0), INHS 20709 (10,0); Jo Daviess County, INHS 30908 (1,0); Mercer County, INHS 64272 (5,0); Rock Island County, INHS 24337 (10,1). Minnesota: Houston County, USNM 118190 (1,0); Wabasha County, INHS 79712 (1,0). Wisconsin: Iowa County, UMMZ 76580 (1,0), USNM 242267 (25,20). Iowa-Des Moines: Iowa: Boone County, SIUC 26568 (3,3); Muscatine County, UMMZ 146921 (15,1), SIUC 26581 (1,0). Middle Mississippi basin: Illinois: Adams County, INHS 19168 (2,0); Calhoun County, INHS 14042 (10,0). Missouri: Lewis County, KU 23598 (23,4); Marion County, UMMZ 150036 (8,0). Illinois drainage: Illinois: Calhoun County, INHS 25150 (4,1); Grundy County, INHS 29754 (1,0); Mason County,

INHS 25735 (3,0), INHS 25703 (1,0); Morgan County, INHS 25797 (1,0); Pike County, INHS 26210 (1,0); Schuyler County, INHS 26423 (1,0); Tazewell County, UT 38.3813 (5,0), INHS 14845 (2,2). Lower Mississippi basin: Arkansas: Crittendon County, UAIC 6400.02 (3,0), UT 44.2274 (5,0). Illinois: Alexander County, SIUC 23911 (5,5), INHS 5758 (1,0); Jackson County, SIUC 7657 (5,2), SIUC 23100 (5,1), INHS 67681 (4,2), SIUC 8378 (5,3), SIUC 7668 (4,6); Madison County, SIUC 22802 (4,0); Monroe County, INHS 20244 (1,0). Louisiana: Pointe Coupee Parish, UT 44.4366 (13,8); West Feliciana Parish, TU 110894 (5,0). Mississippi: Hines County, UT 44.4354 (6,6). Missouri: Cape Girardeau County, KU 23550 (4,2); Perry County, KU 10046 (7,0); Mississippi County, INHS 80839 (5,3). Tennessee: Lake County, UT 44.5971 (5,0); Tipton County, UT 44.2545 (7,4), UT 44.3040 (2,2). Upper Ohio basin: Ohio: Washington County, UMMZ 87766 (7,0). Kentucky: Carter County, SIUC 5644 (3,0); Floyd County, SIUC 11401 (9,0); Greenup County, SIUC 10900 (1,0), SIUC 16011 (1,1); Johnson County, SIUC 8005 (1,0). West Virginia: Logan County, CU 70199 (6,6), CU 75241 (3,2); Mingo County, CU 69303 (32,7). Licking drainage: Kentucky: Bath County, SIUC 9960 (32,32), SIUC 12488 (1,0), SIUC 12484 (1,1), SIUC 10414 (3,3); Pendleton County, SIUC 18092 (1,1), SIUC 9978 (2,2); Robertson County, SIUC 15003 (2,1). Kentucky drainage: Kentucky: Clay County, SIUC 21093 (2,2); Owsley County, SIUC 21003 (1,1) MOSU 1278 (1,0), SIUC 21832 (12,12); Perry County, SIUC 21003 (1,1). Green drainage: Kentucky: Butler County, SIUC 10252 (1,0), SIUC 16144 (3,3), SIUC 16812 (8,5); Edmonson County, SIUC 19228 (6,6), SIUC 18952 (4,0); Green County, SIUC 10286 (1,0), SIUC 10545 (2,2), SIUC 15513 (8,6); Hart County, SIUC 10072 (6,6), SIUC 7317 (6,2). Wabash drainage: Illinois: Clark County, INHS 2449 (5,5), UF 78419 (10,0); Crawford County, UF 78655 (10,0); Lawrence County, INHS 9141 (10,5), INHS 9104 (1,1); Vermilion County, SIUC 14267 (1,1); Wabash County, INHS 7785 (10,3); White County, SIUC 25110 (28,0). Indiana: Carroll County, INHS 68829 (9,3); Gibson County, INHS 7700 (5,4); Knox County, UT 44.3849 (10,5); Lawrence County, USNM 34980 (6,0); Martin County, INHS 69002 (3,3); Posey County, INHS 73686 (4,1), INHS 68022 (3,0), UT 44.1664 (8,3); Sullivan County, UT 44.3767 (8,8); Vermilion County, INHS 73759 (1,1), UT 44.3943 (7,7), INHS 73790 (5,0). Tennessee drainage: Tennessee: Claiborne County, USNM 70583 (1,0); Cocke County, UT 44.5778 (1,1); Giles County, UT 44.6143 (4,4); Humphreys County, SIUC 20569 (2,2), UT 44.6217 (5,5); Knox County, UT 44.6022 (3,0), UT 44.5712 (9,9).

*Macrhybopsis marconis*.—Guadalupe-San Antonio drainages: Texas: Bandera County, TCWC 157.02 (20,8); Bexar County, TCWC 156.1 (2,0), TCWC 163.1 (1,0); Caldwell County, UF 65768 (1,1), UF 50616 (1,0), SIUC 24487 (30,8), SIUC 26492 (7,7); Comal County, USNM 46220

(7,0); Goliad County, UMMZ 170235 (2,0); Guadalupe County, UF 14989 (6,6), UF 65781 (21,16); Hays County, UF 29646 (1,1), UF 9290 (3,3), UF 51686 (3,0), UF 26592 (5,3), USNM 166097 (2,1), INHS 83238 (8,7), USNM 36524 (2,0); Kendall County, MCZ 56153 (3,2), UMMZ 211201 (5,1); Medina County, USNM 242265 (1,1), TNHC 1858 (12,8). Colorado drainage: Texas: Bastrop County, TNHC 3273 (1,0); Burnet County, TNHC 1362 (2,2); Kimble County, TU 97387 (2,2); Llano County, TCWC 925.02 (3,2); Travis County, UMMZ 166050 (1,0), TNHC 1421 (7,7), TNHC 5383 (4,4), TU 22366 (7,4).

*Macrhybopsis* sp. "Mobile chub".—Ponchartrain drainage: Louisiana: East Feliciana Parish, TU 83566 (0,7). Pearl drainage: Mississippi: Leake County, UT 44.4385 (0,13). Tombigbee drainage: Mississippi: Lawrence County, UT 44.624 (0,8). Alabama basin: Alabama: Lowndes County, UT 44.4606 (0,1); Bibb County, SIUC 18114 (0,1); Dallas County, UT 44.4323 (0,14).

*Macrhybopsis* sp. "Coosa chub".—Cahaba drainage: Alabama: Bibb County, SIUC 18114 (0,13), UT 44.4312 (0,3). Coosa drainage: Georgia: Cherokee County, UT 44.1600 (0,1), UT 44.5877 (0,12). Tennessee: Bradley County, UT 44.484 (0,2), UT 44.303 (0,1).

*Macrhybopsis* sp. "Florida chub".—Choctawhatchee drainage: Alabama: Dale County, OSUS 6498 (0,15).

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## APPENDICES

**APPENDIX 1.** Character descriptions of 17 morphological characters used in phylogenetic analyses of the *Macrhybopsis aestivalis* complex.

1. Lateral stripe of surface melanophores: 0=absent; 1=present and centered on lateral line; 2=present and centered above lateral line. Unordered.
2. Pharyngeal tooth formula: 0=modally 1,4-4,1; 1=0,4-4,0.
3. Gill rakers on first gill arch: 0=present as 2-6 short, but distinct rakers; 1=absent or occasionally with 1-4 dorsal rudiments.
4. Nuptial male pectoral ray tuberculation: 0=absent; 1=a few tiny tubercles on rays, but no regular pattern; 2=well developed, arranged uniserially on rays; 3=well developed, arranged biserially on some primary branches. Unordered.
5. Head/snout shape (females): 0=snout very blunt and rounded, dorsal slope of head convex; 1=snout moderately blunt, dorsal slope of head slightly convex, often with a pronounced "hump" over nares; 2=head conical, with a fairly pointed snout, dorsal slope of head flat. Ordered.
6. Orbit shape: 0=round; 1=oval.
7. Anterior pair of maxillary barbels: 0=absent; 1=present.
8. Gular area: 0=barbel-like papillae present; 1=bumps present, but not enlarged into barbel-like structures; 2=raised structures absent. Ordered.
9. Genital papillae: 0=absent or extremely reduced; 1=prominent.
10. Body spots: 0=absent; 1=present.
11. Nuptial male pectoral-fin ray width: 0=not thickened; 1=thickened.
12. Small melanophore arrangement on dorsolateral scales: 0=evenly scattered; 1=concentrated on scale margins or submargins.
13. Caudal fin coloration: 0=bicolored, with a darker lower lobe; 1=not bicolored, both lobes pigmented equally.
14. Lip shape: 0=not expanded posteriorly, only moderately fleshy; 1=expanded posteriorly, greatly fleshy.
15. Posterior barbel length: 0=short, 2.5-5.3% of SL; 1=long, 6.3-7.8 % of SL.
16. Anus position: 0=posterior, nearer anal fin; 1=anterior, midway between anal and pelvic fins.
17. Brain morphology: 0=optic lobes small, facial lobe width > optic lobe width; 1=optic lobes moderate, facial lobe width 0.65-1.0 optic lobe width; 2=optic lobes large, facial lobe < 0.65 optic lobe width. Ordered.

APPENDIX 2. Data matrix used in phylogenetic analysis of the *Macrhybopsis aestivalis* complex for 17 characters (Appendix 1). \*=outgroups.

Character Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
<i>M. meeki*</i>	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>M. gelida*</i>	0	0	0	2	1	1	0	0	0	0	1	0	0	0	0	0	0
<i>M. aestivalis</i>	0	1	1	2	0	0	0	0	0	1	1	0	1	0	0	0	1
<i>M. australis</i>	2	1	1	3	2	0	1	0	0	1	1	0	1	1	1	0	0
<i>M. hyostoma</i>	0,1,2	1	1	2	1	1	0,1	0,1	0	1	1	0,1	1	0	0	0	1
<i>M. marconis</i>	1	1	1	2	0	0	0	1	0	1	1	1	1	0	0	0	2
<i>M. tetranema</i>	2	1	1	2,3	2	0,1	1	0	0	1	1	0	1	1	1	0	0
<i>M. sp. "Coosa chub"</i>	1	0	0	1	1	1	0	2	1	1	0	1	1	0	0	1	1
<i>M. sp. "Florida chub"</i>	1	1	1	2	1	1	1	0	1	1	1	1	1	0	0	1	1
<i>M. sp. "Mobile chub"</i>	1	1	1	2	1	1	0	0	1	1	1	1	1	0	0	0	1

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