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A New Darter from the Upper Tennessee River Drainage Related to *Percina macrocephala* (Percidae: Etheostomatinae)

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A new species related to *Percina macrocephala* is described from the upper Tennessee River drainage. *Percina williamsi* is distinguished from all species of *Percina* except *P. macrocephala* by the presence of a sickle-shaped suborbital bar and a black bar subtending a medial black spot on the caudal-fin base. It is separated from *P. macrocephala* by having larger scales, including usually 24–26 scales around the caudal peduncle (vs. 27–31 in *P. macrocephala*), 21–23 transverse scales (vs. 23–26), and 70–77 lateral scales (vs. 76–86). Values for the combination of caudal peduncle, transverse, and lateral scales are usually 120–129 in *P. williamsi* and 128–141 in *P. macrocephala*. Phylogenetic analyses of complete mtDNA cytochrome *b* gene sequences resolve *P. macrocephala* and *P. williamsi* as sister species, and intraspecific sampling indicates that mtDNA haplotypes of both species are reciprocally monophyletic. Estimates of divergence times indicate that *P. macrocephala* and *P. williamsi* shared a most recent common ancestor approximately 2.3 million years ago.

EXAMINATION of geographic variation in the morphology of the Longhead Darter, *Percina macrocephala*, resulted in the recognition of three morphologically distinct populations (Page, 1978). Individuals from the upper Tennessee River drainage (above the mouth of Duck River) were found to have the lowest scale counts, shortest snout, and usually a black stripe along the side rather than discrete midlateral blotches. Individuals from the Green, Cumberland, and Duck River systems had the highest scale counts, most cylindrical body, longest snout, and usually a midlateral black stripe. Individuals from the upper Ohio River drainage (above the mouth of Green River) were more deep-bodied than individuals from elsewhere and had better defined (less confluent) midlateral blotches. Scale counts in the upper Ohio were similar to those from Green River and higher than those in Tennessee River individuals.

Information on additional specimens and mitochondrial DNA (mtDNA) sequence data further confirm the distinctiveness of the population occupying the upper Tennessee River, which is diagnosed below as a species and compared to *P. macrocephala*. The Green River population of *P. macrocephala*, although somewhat distinctive from populations in the upper Ohio River drainage, does not appear to be diagnosable morphologically and shares identical mtDNA haplotypes with the upper Ohio River populations.

MATERIALS AND METHODS

Morphometric data.—Morphological counts and measurements were made as described by Hubbs and Lagler (1958) except that the number of transverse scales was counted from the anal-fin insertion anteriodorsally to the base of the first dorsal fin (Page, 1983). Counts of bilateral traits were made on the left side. Color observations were made on live and freshly preserved specimens. Institutional abbreviations are from Leviton et al. (1985) except REJ refers to the collection of R. E. Jenkins and YFTC refers to the Yale Fish Tissue Collection.

Collection and analysis of molecular data.—Collection localities, specimen deposition data, and GenBank accession information for all specimens used in our molecular phylogenetic analyses are given in the Material Examined. We isolated DNA from frozen or ethanol-preserved tissues using standard phenol–chloroform extraction and ethanol precipitation protocols. The complete mtDNA encoded cytochrome *b* (cyt*b*) gene was PCR amplified using primers and cycling conditions given in Near et al. (2000). Products from successful PCR were purified using the Qiagen QIA-quick kit and used as template for Big Dye (Perkin Elmer) terminal cycle sequencing reactions carried out at either the Division of Biological Sciences Automated DNA Sequencing Facility at the University of California, Davis, or the Molecular Systematics and Conservation Genetics Laboratory, Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT.

Complete cyt*b* sequences were aligned by eye to an existing alignment that contains sequences sampled from all recognized species of *Percina* (Near, 2002). Based on previous phylogenetic analyses of cyt*b* (Near, 2002), *Percina aurantiaca*

and the undescribed bridled darter (*Percina* sp.) from the Tallapoosa River (Etnier and Starnes, 1993; Boschung and Mayden, 2004) were used as representative outgroup species within *Percina*. We also included other outgroup percid species, as well as 21 centrarchid species, to provide external fossil calibrations (Near and Keck, 2005).

Both maximum parsimony and Bayesian methods were used to generate phylogenetic trees from the cyt*b* alignment. The computer program PAUP* (4.0b10, D. L. Swofford, PAUP*: Phylogenetic analysis using parsimony [and other methods], Sinauer Associates, Sunderland, MA, 2002) was used to calculate uncorrected pairwise DNA sequence divergences and perform maximum parsimony analysis using a heuristic search with 100 random sequence additions and TBR branch swapping. Bootstrap analysis was used to assess node support through 2,000 pseudoreplications using the branch-and-bound search algorithm in PAUP* 4.0. Relationships of mtDNA haplotypes within species were examined by generating haplotype networks for each species using the computer program TCS (Clement et al., 2000). A partitioned mixed-model Bayesian analysis was also used to estimate phylogenetic relationships (Ronquist and Huelsenbeck, 2003). Each of the three codon positions of cyt*b* were treated as separate data partitions, and the optimal maximum likelihood model of sequence evolution for each partition was determined with likelihood ratio tests using the computer program Modeltest 3.0 (Posada and Crandall, 1998). The different models were assigned to the appropriate data partitions in the computer program MrBayes 3.0 (Ronquist and Huelsenbeck, 2003). MrBayes 3.0 was run for 5×10^6 generations, and the burn-in period was determined by graphically tracking the maximum-likelihood scores to identify the point where it reached a plateau. Trees and parameter values sampled prior to the burn-in were discarded. Bayesian posterior probabilities greater than or equal to 0.95 were considered significant.

We used a likelihood ratio test to determine if there was significant molecular evolutionary rate heterogeneity in the cyt*b* phylogeny (Felsenstein, 1981). We used methods of external fossil calibration that have been applied to other darter clades (Near and Benard, 2004; Near and Keck, 2005). Two centrarchid fossils, ages given in millions of years ago (mya), *Lepomis gulosus* (6.6 mya) and *Archoplites clarkia* (15.5 mya), were used to calibrate the phylogeny. Details regarding these fossils and their performance in calibrating the Centrarchidae phylogeny are detailed in Near et al. (2005). Fossil-dated nodes in the phylogeny were treated as fixed minimal ages in the computer program r8s 1.7 (Sanderson, 2003). Confidence intervals on divergence time estimates were estimated using a bootstrap procedure outlined in Sanderson and Doyle (2001).

Percina williamsi, new species

Sickle Darter

Figure 1

Holotype.—UF 162590, 88.0 mm SL, female, Virginia, Smyth County, 4.0 km E. Broadford, County Route 630, North Fork Holston River, 19 April 1986, R. E. Jenkins et al.

Paratypes.—UAIC 5953.14, 2, 79.8–80.1 mm SL, and UF 162591, 1, 80.2 mm SL, Tennessee, Blount County, 8.6 km ENE Maryville, U.S. Highway 411, Little River, 13 March 1980, D. L. Nieland, B. H. Bauer, and J. L. Harris. UT 91.6349, 2, 88.7–90.5 mm SL, Virginia, Smyth County, above Saltville, river mile 85.1, North Fork Holston River, 25 June 2002. YPM 15471, 2, 75.4–77.8 mm SL, Tennessee, Morgan County, 4.0 km NW Wartburg, State Highway 62, Rock Creek, 27 Feb. 2005, T. J. Near and A. M. Murray. YPM 15472, 1, 83.2 mm SL, Tennessee, Morgan County, 4.0 km NW Wartburg, State Highway 62, Rock Creek, 5 March 2005, T. J. Near, B. P. Keck, and R. C. Harrington.

Diagnosis.—A species that shares a most recent common ancestor (MRCA) with all other species of *Percina* as defined by Bailey et al. (1954) and Page (1974), i.e., darters (Etheostomatinae) with enlarged and strongly toothed scales on the venter. *Percina williamsi* is distinguished from all species of *Percina* except *P. macrocephala* by the presence of a sickle-shaped suborbital bar and a black bar subtending a medial black spot on the caudal-fin base (Fig. 1). *Percina williamsi* is distinguished from *P. macrocephala* by having larger scales (Tables 1–3): usually 24–26 scales around the caudal peduncle (vs. 27–31 in *P. macrocephala*), 21–23 transverse scales (vs. 23–26 in *P. macrocephala*), and 70–77 lateral scales (vs. 76–86 in *P. macrocephala*). Values for the combination of caudal peduncle, transverse, and lateral scales are usually 120–129 in *P. williamsi* and 128–141 in *P. macrocephala* (Table 4). *Percina williamsi* also has a shorter snout on average; snout ranges from 20–28 percent of head length compared to 24–31 percent in *P. macrocephala*.

Description.—Largest specimen 90.5 mm SL (103.3 mm TL) female from Holston River (UT 91.6349). Long, slender body (body depth 17–

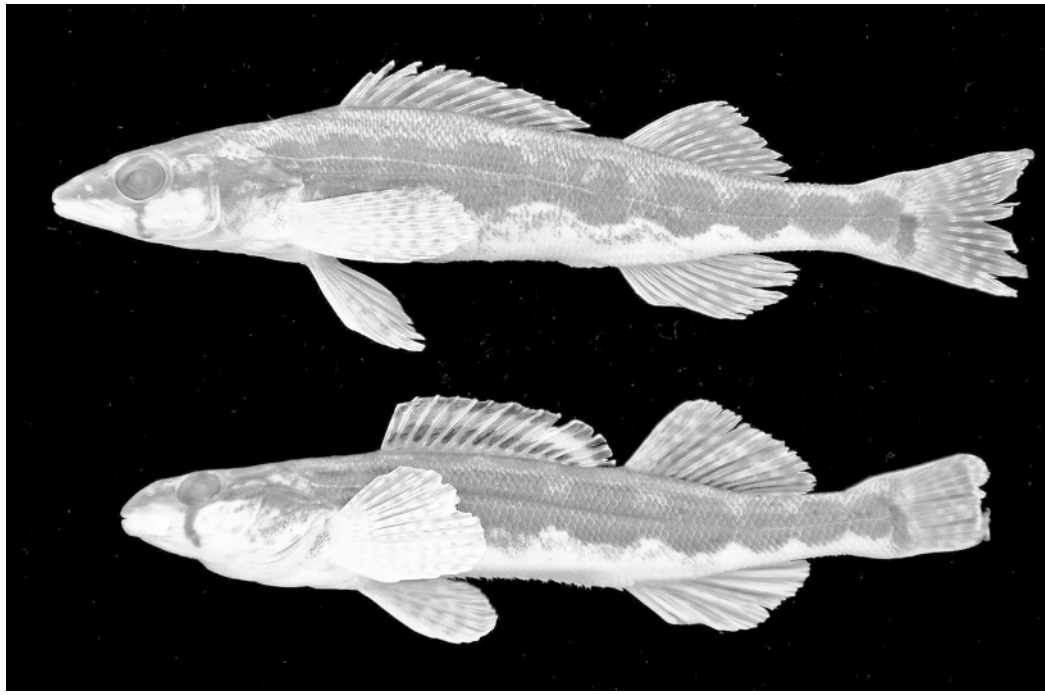

Fig. 1. *Percina williamsi*. Above: UF 162590, holotype, 88.0 mm SL female; below: UF 162591, paratype, 80.2 mm SL male.

TABLE 1. NUMBERS OF SCALES AROUND CAUDAL PEDUNCLE IN *Percina williamsi* AND *P. macrocephala*. Most frequent counts are in bold.

	No. scales													
Species	23	24	25	26	27	28	29	30	31	32	33	*n*	X	SD
P. williamsi	1	**12**	**19**	**12**	2	1						49	25.1	1.00
P. macrocephala			4	6	**26**	**25**	**17**	**18**	**12**	7	1	116	28.6	1.80

TABLE 2. NUMBERS OF TRANSVERSE SCALES IN *Percina williamsi* AND *P. macrocephala*. Most frequent counts are in bold.

	No. scales												
Species	19	20	21	22	23	24	25	26	27	28	*n*	X	SD
P. williamsi	1	3	**11**	**19**	**10**	5					49	22.0	1.14
P. macrocephala		1	3	5	**28**	**29**	**24**	**16**	7	1	114	24.3	1.49

TABLE 3. NUMBERS OF LATERAL SCALES IN *Percina williamsi* AND *P. macrocephala*. Most frequent counts are in bold.

	No. scales																							
Species	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	90	*n*	X	SD
P. williamsi	1	1	1	2	**5**	**4**	**3**	**4**	**7**	**3**	**1**	**8**	3			4	1	1						49	74.3	4.07
P. macrocephala							2	1		4	**7**	**8**	**10**	**9**	**15**	**19**	**17**	**8**	**6**	**7**	**5**	3	1	122	80.6	3.31

TABLE 4. NUMBERS OF LATERAL + TRANSVERSE + CAUDAL PEDUNCLE SCALES (TOTAL—100) IN *Percina williamsi* AND *P. macrocephala*. Most frequent counts are in bold.

Species	No. scales																																	*n*	X	SD
	10	12	13	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44			
P. williamsi	1	1	1	1	2	3	3	2	**8**	**6**	**3**	**2**	**4**	**4**		**2**	**1**	**4**		1														49	21.4	4.62
P. macrocephala														3	1	3	**6**	**10**	**5**	**12**	**10**	**6**	**16**	**4**	**8**	**14**	**3**	**2**	**4**	**4**	1	3	1	116	33.6	4.32

18% SL); long snout (snout length 20–28% HL). Separate branchiostegal membranes; complete, straight lateral line; uninterrupted head canals; wide premaxillary frenum. Nape fully scaled to unscaled, usually scaled except anteriorly and medially or with scales embedded anteriorly and medially. Prepectoral area unscaled. Breast unscaled or rarely with embedded scales except for modified scales medially. Belly scaled except anteriorly. Cheeks typically unscaled or with few scales dorsally or anteriorly. Opercles unscaled or with few scales dorsally. Jaw, palatine, and vomerine teeth present.

Usually no (rarely 1) pored scales on caudal fin; 11–16 (usually 13–15) dorsal spines; 11–14 (12–13) dorsal rays; two anal spines; six (rarely five) branchiostegal rays; gill rakers 15–17, those on lower limb vestigial (Etnier and Starnes, 1993); 44–45 vertebrae (Bailey and Gosline, 1955); other counts given in Tables 1–4. Male with incomplete row of 9–22 modified scales on belly midline. Unlike other species of *Percina*, females of *P. williamsi* and *P. macrocephala* typically with midbelly row of modified scales; scales smaller than those of male. Breeding tubercles absent.

Brown to olive above, rarely with poorly developed dusky saddles or wavy lines. Thin black stripe along back from occiput to rear of second dorsal fin; usually a dark spot between dorsal fins (most evident on juveniles and pale specimens). Eight to 14 variably confluent black blotches (with iridescent green cast) along side; blotches wholly fused in some individuals into black stripe with undulating margins; stripe continues onto head past eye and onto upper lip. Narrow yellow stripe along upper side above black blotches or stripe often evident on juveniles and small adults, usually absent on large individuals. Lateral line depigmented at least on front half of body. Sickle-shaped suborbital bar (''teardrop'') extends from eye down and posteriorly onto underside of head (to skin on mandible). Discrete coal-black spot at base of caudal fin; black bar extends from caudal spot to or near ventral edge of caudal fin. White to pale yellow below; often with many black specks on side just below black stripe. First dorsal fin with dusky to black edge, then clear band, then dusky to black band that extends to base of fin or borders narrow clear basal band. Other fins mostly clear (or with yellow cast) with diffuse dark bands.

Distribution.—*Percina williamsi* is restricted to the upper Tennessee River drainage of Tennessee, Virginia, and North Carolina (Fig. 2). Specimens have been collected in the French Broad system

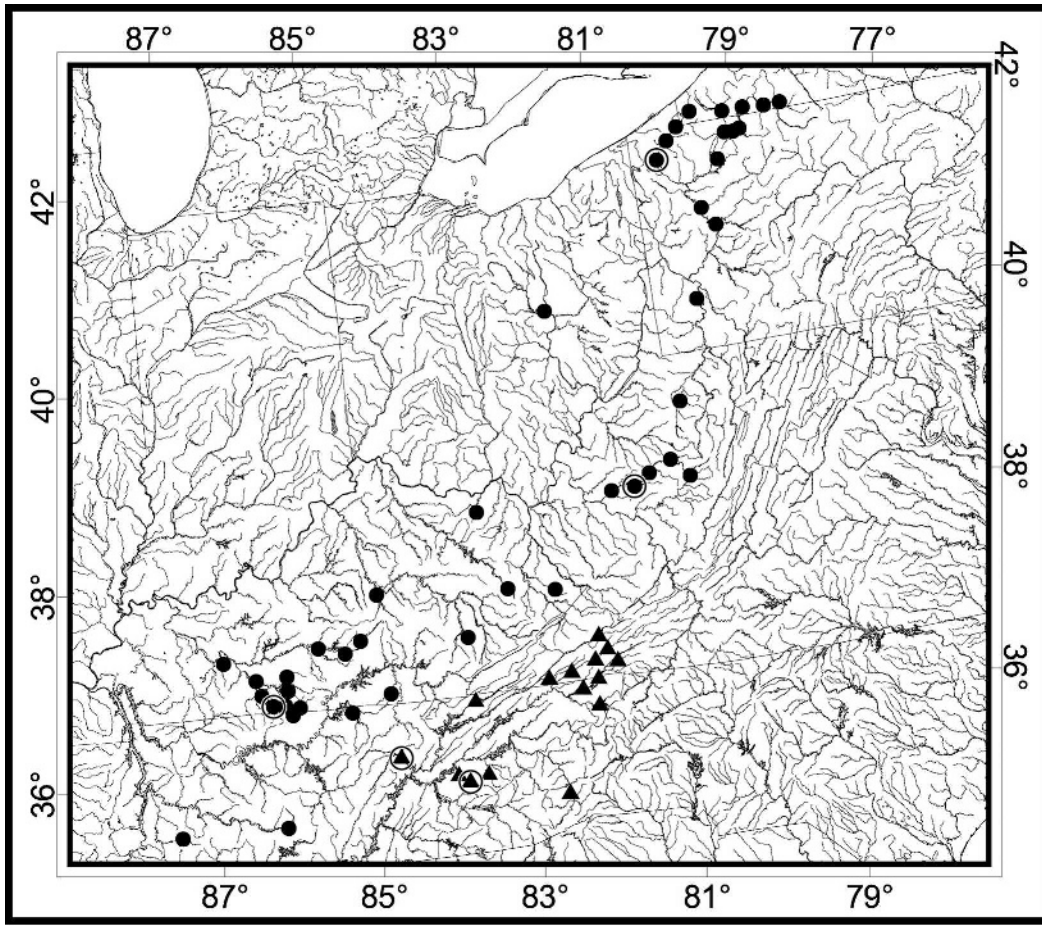

Fig. 2. Localities where *Percina williamsi* (triangles) and *P. macrocephala* (dots) have been collected (Page, 1978; Trautman, 1981; Cooper, 1983; Smith, 1985; Burr and Warren, 1986; Menhinick, 1991; Etnier and Starnes, 1993; Jenkins and Burkhead, 1994; Stauffer et al., 1995). Circled localities indicate samples for mtDNA sequencing.

of Tennessee and North Carolina, the Emory River system in Tennessee, and the Holston and Clinch River systems in Tennessee and Virginia.

Habitat.—Percina williamsi lives in flowing pools over rocky, sandy, or silty substrates in clear creeks or small rivers draining the Appalachian Mountains (Page, 1978; Etnier and Starnes, 1993; Jenkins and Burkhead, 1994). It is found most often near woody debris, vegetation such as water willow, or large boulders (Etnier and Starnes, 1993). As its fusiform shape suggests, it spends most of its time swimming in current in the water column (Greenberg, 1991; Etnier and Starnes, 1993). The prominent black stripe along the side of *P. williamsi* is characteristic of darters living near vegetation in flowing pools (Page, 1983).

In the Little River, spawning *P. williamsi* specimens were collected in March in shallow gravel shoals (Etnier and Starnes, 1993). On 27 February 2005, a male (77.8 mm SL) and female (75.4 mm SL) *P. williamsi* were collected in a tributary of the Emory River in a gravel shoal at a depth of 25 cm and a water temperature of 8 C. Both specimens were in reproductive condition, and the mature ova in the female equaled 27% of her body mass. One hundred of the 355 mature ova had a mean diameter of 1.62 mm. These observations provide evidence that *P. williamsi* spawns in late winter in gravel shoals.

The closely related *P. macrocephala* also occupies flowing pools. The Green River population of *P. macrocephala* was found to live three or four years and to feed on crayfishes and mayflies (Page, 1978). The long snout and large mouth of *P. macrocephala* facilitates the ingestion of large food items such as crayfishes and mayflies; *P. williamsi* shares the long snout and large mouth

and probably has a similar diet and habits. This hypothesis is supported by the presence of the heptageniid mayfly, *Stenonema vicarium,* in the stomachs of three *P. williamsi* collected in the Emory River system (YPM 15471 and YPM 15472).

Conservation status.—*Percina williamsi* can be observed with regularity in a few streams, but populations are widely scattered and the species has been extirpated from several streams where it was collected in the late 1800s and early to mid-1900s. It is considered extirpated in North Carolina (Menhinick, 1991), rare in Virginia (Jenkins and Burkhead, 1994), and threatened in Tennessee (Etnier and Starnes, 1993). Similarly, its close relative, *P. macrocephala*, has been extirpated from much of its range (Page, 1978; Trautman, 1981; Burr and Warren, 1986), and the species appears to be common only in the Allegheny River, Pennsylvania (Cooper, 1983). Proximate principal threats to both *P. macrocephala* and *P. williamsi* are most likely increased turbidity and siltation resulting from agricultural, industrial, and municipal development, the ultimate result of population growth in *Homo sapiens*.

Molecular phylogeny and divergence time estimates.—Complete cyt*b* sequences were collected for seven specimens of *P. macrocephala* and five of *P. williamsi*. Uncorrected pairwise sequence divergences of cyt*b* ranged from 2.8 to 2.9% between *P. macrocephala* and *P. williamsi*, and were much less than 1.0% within either of the two species. Maximum parsimony resulted in two equally parsimonious trees that showed reciprocal monophyly of mtDNA haplotypes in both species. Bayesian analysis resulted in an identical tree topology with regard to relationships of *P. macrocephala* and *P. williamsi* (Fig. 3). The haplotype network for *P. williamsi* revealed that all five sampled specimens contained a unique haplotype, haplotypes within a sampling area were more similar than between the two sampling areas, and four of the nine reconstructed haplotypes were missing (Fig. 3). Although with limited sampling of individuals, the haplotype network recovered for *P. macrocephala* revealed very little haplotype divergence among a very wide geographic sampling area, haplotype sharing between sampling localities in the Allegheny River system and the Barren River system, and no missing haplotypes in the reconstructed network (Fig. 3).

Using the external fossil calibrations in r8s 1.7 with a smoothing parameter value of 31.26 resulted in a divergence time of 2.23 ± 0.90 mya for the MRCA of *P. macrocephala* and *P. williamsi* (Fig. 3). Estimated intraspecific coalescent times were 0.62 ± 0.43 mya for *P. williamsi* and 0.15 ± 0.06 mya for *P. macrocephala*.

Etymology.—Named for James D. Williams, a contemporary ichthyologist, malacologist, and natural historian *extraordinaire*. The common name, Sickle Darter, refers to the distinctive sickle-shaped suborbital bar, a synapomorphy for *P. williamsi* and *P. macrocephala*.

Discussion

Percina williamsi and *P. macrocephala* uniquely share two morphological synapomorphies: a sickle-shaped suborbital bar that extends down and posteriorly onto the underside of the head and a black bar subtending a medial black spot on the caudal-fin base. These two species share another uncommon trait, the presence of a midbelly row of modified scales on females and males (although scales are larger and have larger teeth on males). The usual presence of a midbelly row of modified scales on females may also be a synapomorphy; however, a row of modified scales is present, albeit rarely, on females in at least five other species of Percina in which a midbelly row is well developed on males (Page, 1976). Given that these species appear to be disparately related to one another in the context of a molecular phylogeny (Near, 2002), it is difficult to interpret the evolution of modified belly scales in female *Percina*.

Bailey and Gosline (1955) placed *Percina macrocephala* (then including *P. williamsi*) with *P. maculata, P. peltata,* and *P. notogramma* in the subgenus *Alvordius*. Page (1974) enlarged and diagnosed *Alvordius* as a subgenus including *P. maculata, P. peltata, P. notogramma, P. crassa, P. roanoka, P. pantherina*, and the undescribed Bridled Darter from the Coosa River (Etnier and Starnes, 1993). *Percina gymnocephala* was described as an additional member of *Alvordius* by Beckham (1980). In an analysis of mitochondrially encoded cyt*b* sequence data, Near (2002) found no support for the monophyly of *Alvordius* and found no described species to be particularly closely related to *P. macrocephala*. These results suggest that the morphological traits used to diagnose *Alvordius* are plesiomorphic or independently derived among species assigned to the subgenus. Identification of the species of *Percina* that are the closest relatives of the *P. macrocephala–P. williamsi* clade will require the analysis of additional data.

Our analyses show that *P. macrocephala* and *P. williamsi* share a MRCA that dates to 2.23 ± 0.90 mya at the later part of the Pliocene

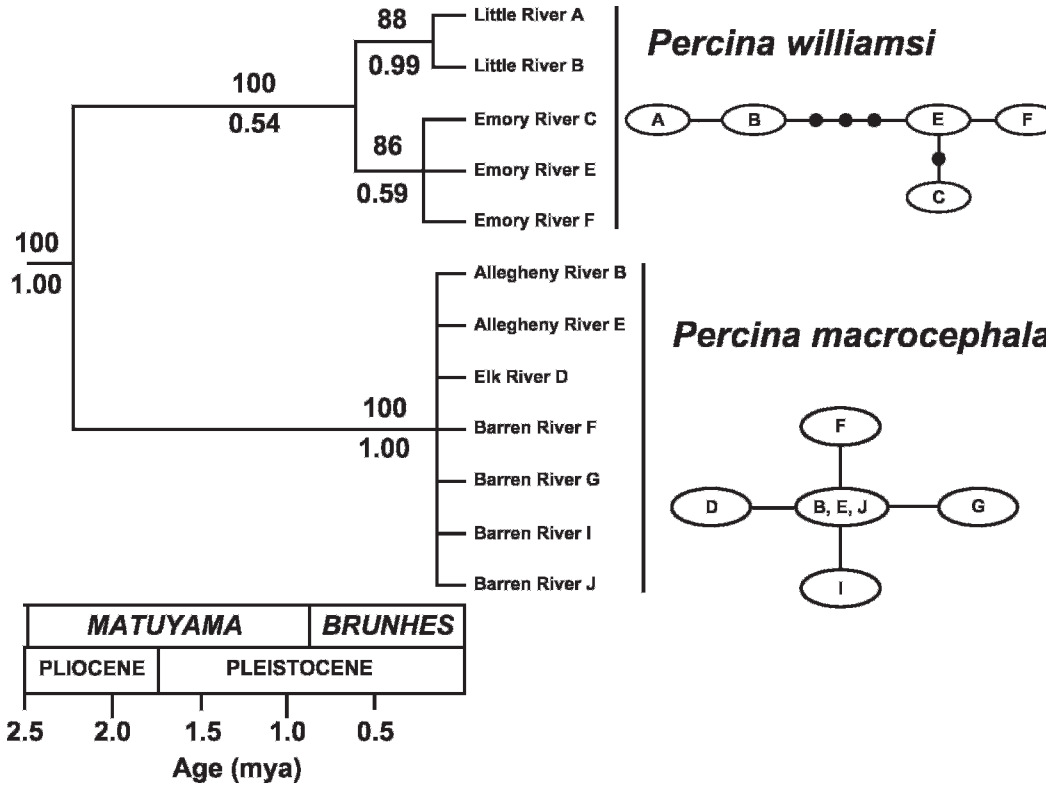

Fig. 3. Time-calibrated phylogeny (chronogram) of mtDNA sequences from *Percina williamsi* and *P. macrocephala* (outgroups not shown). Maximum parsimony bootstrap pseudoreplicate scores above nodes and Bayesian posterior probabilities below nodes. Branch lengths scaled to estimated evolutionary age from penalized likelihood analysis. Chronogram is plotted against geological time scale of paleomagnetic chrons and epochs. Networks of mtDNA haplotypes of *P. williamsi* and *P. macrocephala* shown on right. Observed haplotypes indicated with ovals; letters in ovals correspond to individuals in phylogeny. Lines connect haplotypes separated by one observed nucleotide change. Black dots represent unobserved haplotypes.

(Gibbard and Kolfschoten, 2004). This divergence time is similar to the age estimate of the MRCA of *Etheostoma chlorobranchium*, an upper Tennessee River endemic, and its sister clade. However, the divergence time estimated for another upper Tennessee endemic darter, *E. vulneratum*, is much younger than the MRCA age estimates for *P. williamsi* and *E. chlorobranchium* (Near and Keck, 2005). *Percina macrocephala* occupies areas that were directly impacted by glaciation in the Pleistocene, as well as highland areas south of the Ohio River that escaped glaciation (Flint, 1957; Thornbury, 1965; Teller and Goldthwait, 1991). The discontinuous distribution of *P. macrocephala* throughout the Ohio River drainage appears to have resulted from post-Pleistocene dispersal and extinction. This inference is based on very little mtDNA divergence and the presence of a shared mtDNA haplotype in the Allegheny River in Pennsylvania and Barren River in Kentucky (Figs. 2, 3). Eastern populations of *Percina evides* exhibit a similar phylogeographic pattern of mtDNA haplotype diversity (Near et al., 2001). On the other hand, given a more limited sampling, *P. williamsi* exhibits more structure between populations that are in closer geographic proximity (Fig. 2). We expect that as more populations of *P. williamsi* are sampled for mtDNA sequences, there will be few, if any, haplotypes shared between major river systems of the upper Tennessee River drainage. More morphological variation among populations of *P. williamsi*, e.g., in snout shape (Fig. 1), than is presently appreciated may also become evident, and possibly taxonomically significant, when more specimens are available for study.

Material Examined

Percina williamsi. Holston R. Dr.: Watauga R.: UMMZ 157401 (1); North Fork: UF 162590 (1),

UMMZ 96879 (3), 187253 (1), SU 813 (1), UT 91.6349 (2); South Fork: UMMZ 157577 (1), 157610 (1). French Broad R. Dr.: West Prong Little Pigeon R.: UMMZ 129326(1); Little R.: TU 65971 (1), 78365 (3); UAIC 4924.09 (2), 5953.14 (2); UF 162591 (1); UT 91.375 (4), 91.480 (1), 91.545 (7), 91.595 (10). Clinch R. Dr.: Emory R.: UT 91.579 (1), 91.1462 (1), 91.6496 (2); YPM 15741 (2), 15742 (1); Copper Cr.: Roanoke College (REJ) 348 (1), 349 (1), 365 (1); Little R.: Roanoke College (REJ) uncat. (1). Specimens for mtDNA sequencing. Little R: PwilA YFTC 184, UT 91.6796 PwilB YFTC 3316; Emory R.: UT 91.6870 PwilC YFTC 4884, UT 91.6874 PwilE YFTC 4911, UT 91.6949 PwilF YFTC 5206.

Percina macrocephala. Upper Ohio R. Dr: Allegheny R.: CU 6207 (1), 9429 (1), 62509 (1), 62753 (2); MCZ 24500 (5); PSU 24 (6), 330 (4), 1585 (1), 1592 (8); UL 9381 (1); UMMZ 102362 (1), 180964 (1), 86386 (1); USNM 1164 (2), 1194 (4). Monongahela R.: ANSP 22626 (lectotype); UL 9332 (1). Walhonding R.: OSUMZ 2-14 (2), 977 (5). Kanawha R.: KFW uncat. (3); UMMZ 119637 (1), 119661 (1). Big Sandy R.: KFW 1013 (1); UMMZ 154793 (1). Kinniconick Cr.: UL 12003 (1). Kentucky R.: USNM 144267 (2). Green R. Dr.: CAS-SU 3263 (1); INHS 76641 (9); KFW 1397 (12), 1670 (3), 1780 (10), 1737 (6); TU 19401 (1); UL 11525 (1), 12004 (3), 12006 (2), 12008 (2); UMMZ 154701 (1), 177436 (7); USNM 63773 (4). Cumberland R. Dr.: Little South Fork: USNM 46215 (2); Obey R.: USNM 126474 (2). Duck R.: UT 91.737 (1), 91.544 (3), 91.1544 (3). Specimens for DNA sequencing. Allegheny R.: INHS 39196 PmceB YFTC 318, PmceE YFTC 616; Elk R.: INHS 37876 PmceD YFTC 217; Green R.: no voucher PmceF R58, UT 91.7318 PmceG YFTC 6364, JBFM 43299 PmceH YFTC 6890, PmceI YFTC 6891, and PmceJ YFTC 6892.

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