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Two new species of *Etheostoma* from the Black Warrior River System (Mobile Basin) of Alabama (Teleostei: Percidae)

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Abstract

Etheostoma bellator Suttkus & Bailey, the Warrior Darter, is found in the Black Warrior River system of the eastern Tombigbee River drainage in Alabama. This species inhabits the headwaters of all three major forks of the Black Warrior River, including Sipsey, Mulberry, and Locust forks, as well as a direct tributary to the Black Warrior River (Valley Creek). Previous genetic investigations indicated that *E. bellator* populations from these three forks and *Etheostoma chermocki* were different evolutionary lineages. In the description of *E. bellator* very few specimens were examined from the Sipsey or upper Locust forks of the Black Warrior River. Populations of *E. bellator* from these latter watersheds are morphologically and genetically distinct, diagnosable from other members of the group and are described herein as new species.

Key words: Percidae, Etheostomatini

Introduction

The *Etheostoma chermocki* species group was outlined and discussed by Boschung *et al.* (1992) and examined genetically by Clabaugh *et al.* (1996). The group includes *E. chermocki* Boschung, Mayden & Tomelleri (Fig. 1) and the then undescribed Warrior Darter, *E. bellator* (Fig. 2). *Etheostoma bellator* was described shortly after the description of *E. chermocki* by Suttkus & Bailey (1993) and is a species that has for several years been thought to represent a single species from the Black Warrior River system of the Tombigbee River drainage of the Mobile Basin. Suttkus & Bailey (1993) examined populations of both *E. chermocki* and *E. bellator* and supported the recognition of *E. chermocki*. In the latter study, the authors examined morphological variation in the two species, but for *E. bellator,* their samples were predominately from Valley Creek (a direct tributary to the Black Warrior River) and Mulberry Fork (type locality river system); very few specimens (n=5) were examined from the Sipsey and upper Locust forks of the Black Warrior River.

Clabaugh *et al.* (1996) examined *E. chermocki* and all populations of *E. bellator* in a population genetic and phylogenetic study using allozyme variation. This study not only revealed that *E. chermocki* was diagnosable from *E. bellator*, but also showed two additional genetically divergent, geographically restricted, and diagnosable lineages independent of *E. bellator* and *E. chermocki* masquerading under the name *E. bellator*. These two previously unrecognized species occur allopatrically from *E. chermocki* and populations of *E. bellator.* The two new species are endemic to the Black Warrior System, one from the Sipsey Fork and one from the upper Locust Fork. Boschung & Mayden (2004) and Kuhajda (2004 a,b) provided information on distinguishing characteristics, distribution, habitat, systematics, and conservation, but no formal descriptions were provided. Kim *et al.* (2023) commented on the diversity in this clade in their overview of the area's unique geology. They not only argued for the validity of the two new lineages described herein but presented two dendrograms based on 1) maximum likelihood (IQ-TREE) and 2) PoMo; their findings were presented as evidence for the existence of two additional, genetically independent lineages within what is now recognized *E. bellator* (Gurley Creek and Valley Creek) but provided no data to support their claims.

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Herein, these two genetically and morphologically diagnosable lineages from the Sipsey and Locust forks of the Black Warrior River (Tombigbee River drainage), formerly recognized as distinct, are examined and compared with *E. bellator* for morphological and coloration variation and are described as new species.

Materials and Methods

Pigmentation patterns and coloration are traits that have long been recognized as a critical set of characteristics for diagnosing species of Etheostomatini. Distinct lineages/species diagnosed based on pigmentation and coloration traits have also been further corroborated in the same or subsequent studies by other morphological characters or characters derived from allozymes, isozymes, or molecular studies and analyses. In Percidae, the coloration of live individuals has provided many fundamental diagnostic traits, especially in breeding males, for the delineation of species and subgenera (Page 1983); diagnostic coloration in breeding males can be ephemeral and may only be present for a short period of the breeding season. Excellent examples of ephemeral diagnostic traits include species of the subgenera *Catonotus* (Page & Braasch 1976, 1977; Braasch & Page 1979, Braasch & Mayden 1985, Page *et al.* 2003), *Ulocentra/Nanostoma* (Bailey & Etnier 1988; Suttkus & Etnier 1991; Suttkus & Bailey 1993; Bailey & Bart 1994; Powers & Mayden 2003, 2007), and *Doration* (Layman & Mayden 2012). Herein, the study and verification of diagnostic color patterns involved examining freshly captured live adults between 1992 and 2014, records of coloration, and photographs of freshly caught specimens; following examination, specimens were either fixed/preserved or released.

In diagnoses, the following symbols are used for gene loci and alleles: * = significant allele frequency differences at the locus based on heterogeneity χ^2 analysis; † = locus is a fixed difference between species being compared; 3 = locus is a fixed difference between some but not all populations of *E. bellator* and the other species being compared.

Meristic characters were sampled as described by Mayden (2010). Mensural data follow Hubbs & Lagler (1964) or were sampled between specific homologous landmarks. These included standard length (SL), head length (HL), body depth (BD), body width (BW), head width (HW), orbit width (OW), interorbital width (IOW), snout length (SnL), upper jaw length (UJL), caudal peduncle depth (CPD), caudal peduncle length (CPL), first dorsal-fin base (D1 base), second dorsal-fin base (D2 base), anal fin base (AF base), pectoral-fin base (PC base), tip of snout to lower base of pectoral fin (snout tip to PC base lower), posterior rim of orbit to origin first dorsal fin (posterior orbit to origin D1), origin of first dorsal to origin of second dorsal (origin D1 to origin D2), origin of first dorsal fin to origin of anal fin (origin D1 to origin AF), origin of second dorsal fin to lower base of pectoral fin (origin D2 to PC base lower), origin of second dorsal fin to origin anal fin (origin D2 to origin AF), posterior base of second dorsal fin to posterior base of anal fin (D2 base posterior to AF base posterior), posterior of second dorsal fin base to hypural plate (D2 base posterior to hypural plate), origin of anal fin to lower base of pectoral fin (origin AF to PC base lower), tip of snout to end of maxilla (snout to maxilla end), end of maxilla to lower base of pectoral fin (maxilla end to PC base lower), end of maxilla to upper base of pectoral fin (maxilla end to PC base upper), and posterior rim of orbit to lower base of pectoral fin (posterior orbit to PC base lower).

Statistical analyses were performed using SAS version 9.4. Unless otherwise noted, significance is ≤ 0.05. Ratio data were log-transformed for ANOVA and T-test analyses. Principal Component Analysis was run using SAS v. 9.4; a covariance matrix was used for morphometric variables, while a correlation matrix was used for meristic data. In morphometric analysis Upper jaw length (UJL) and First dorsal fin base (D1 base) were not run in analysis because data were missing for some specimens.

Etheostoma kimberlae Mayden, new species

Locust Fork Darter
Fig. 1

Holotype. UAIC 11031.01, adult male, 39.6 mm standard length. Locality. Alabama, Blount Co., Mill Creek at gravel road 4.3 km SW Oneonta, T13S, R1E, S11, 33.922595 -86.509051, 22 March 1994. Collected by B. R. Kuhajda and R. L. Mayden.

Paratypes. UAIC 11031.03, same data as holotype, n=4; UAIC 10867.06, n=7, Alabama, Blount County, Mill Creek at Old AL Hwy 75, east of AL Hwy 75, T13S, R1E, S1, 33.927813 -86.496049, 2.9 km SSW Oneonta, 16 June 1993, B. R. Kuhajda and R. L. Mayden; UF 188209, n=2, same data as holotype; TU 204140, n=2, same data as UAIC 10867.06. UAIC 14799.01, n=2, same locality data as UAIC 10867.06, 7 October 2005, B.R. Kuhajda; UAIC 16061.01, n=3, Alabama, Blount County, Calvert Prong of the Little Warrior River at AL Hwy 75, 5 km NE of Oneonta, T12S, R2E, S16, 33.985578 -86.442009, 2 April 2016, B.R. Kuhajda and Freshwater Fishes of Alabama class.

Diagnosis. A member of the *Etheostoma chermocki* species group as identified by Boschung *et al*. 1992, Clabaugh *et al.* (1996) and Boschung and Mayden (2004). *Etheostoma chermocki* was diagnosed from the *Etheostoma bellator* complex by Boschung *et al.* (1992) and Suttkus and Bailey (1993). *Etheostoma kimberlae* is distinguished from *E. michellae* new species using the following combination of characters: cream-colored horizontal stripe through lateral band very narrow to absent vs. broad and obvious; snout of breeding males lightly colored with obvious preorbital stripe in breeding males vs. snout dusky and often masking preorbital stripes; suborbital bar diffuse in breeding males, lightly pigmented and short vs. dark, well defined and long.; lateral blotches dark and largely below lateral line vs. diffuse and occurring both above and below lateral line; and crimson line above lateral line formed from spots in continuous to nearly continuous line and not interrupted by lateral blotches vs. crimson line broken up into segments between diffuse upper halves of lateral blotches. *Etheostoma kimberlae* is further distinguished from *E. michellae* in the possession of the following alleles at identified protein loci: *sAat-A* (A vs B)†, *mAcon-A* (B vs B, C)*, *Acp-1* (A vs B)†, *Ada-2* (A vs B)†, *Est-2* (D vs A)†, *Pep-B* (B, C vs B)*, *Pep-F* (D vs B)†, *Pnp-A* (D vs C, D)*. *Etheostoma kimberlae* is distinguished from *E. bellator* using the following characters: cream-colored stripe through lateral band narrow to absent vs broad and obvious; snout lightly colored with obvious preorbital stripes in breeding males vs. snout dusky and often masking preorbital stripes; and suborbital bar diffuse, lightly pigmented and short vs. broad, dark and long. *Etheostoma kimberlae* is further distinguished from *E. bellator* in the possession of the following alleles at identified protein loci: *sAat-A* (A vs B)†, *Acp-1* (A vs B)†, *Ada-2* (A vs B)†, *Pep-B* (B, C vs A, B; allele B fixed in comparisons between Five Mile and Gurley creeks but is polymorphic in Murphy Creek)*, *Pep-F* (D vs B, D)[3], *Pnp-A* (D vs C, D)*. *Etheostoma kimberlae* is distinguished from *E. chermocki* in having a low first dorsal fin with black, red, and blue coloration vs fin tall and mostly red, and ventral orange pigmentation in breeding males in narrow band vs. extending upwards along side to lateral band. *Etheostoma kimberlae* is further diagnosed from *E. chermocki* by the possession of the following alleles at identified protein loci: *sAat-A* (A vs D)†, *Acp-1* (A vs B)†, *Ada-2* (A vs B)†, *Fbp-A* (A vs B)†, *slcdh-A* (B vs A, C)†, *Mpi-A* (B vs B, C)*, *Pep-B* (B, C vs A)†, *Pep-F* (D vs B)†.

Lateral line scale rows of *Etheostoma kimberlae* usually 47 or 48 (vs. *E. michellae* with 45–48, usually 45, and 47–51 in *E. bellator).* Scale rows below lateral line usually 7 or 8 (vs. usually 8 in *E. michellae* and 7 or 8 *E. bellator).* Transverse scale rows usually 15 (vs. usually 14 and 15 in *E. michellae).* Transverse scale rows plus scale rows below lateral line usually 15 (vs. usually 14 or 15 in *E. michellae* and *E. bellator).* Dorsal fin rays usually 11 or 12 (vs. usually 11 or 12 in *E. michellae* and 11 in *E. bellator).* Pectoral fin rays usually 13 (vs. usually 14 in *E. michellae* and *E. bellator).* Caudal fin rays usually 16 or 17 (vs. usually 16 or 17 in *E. michellae* and 14 in *E. bellator*).

Etheostoma kimberlae differs from *E. michellae* in having usually 7 infraorbital pores (mean = 7.1) (vs. usually 8, mean = 8.1). *Etheostoma kimberlae* is a small member of the *E. chermocki* species group, reaching recorded 45.0 mm SL (male) and 41.4 mm SL (female) vs. *E. bellator* (male = 58 mm SL, Suttkus & Bailey 1993; female = 48.2 herein) and *E. chermocki* (male = 55 mm SL; female = 51 mm SL; Boschung *et al*. 1992).

Description. General head and body shape and coloration illustrated in Figure 3. Morphometric variables for males and females (sexually dimorphic) provided in Table 1. Distribution of lateral line scales, caudal fin rays, and pectoral fin rays provided in Table 2.

Lateral line complete and virtually straight, from upper margin of gill opening to base of caudal fin. Scale rows above lateral line 4 (3 spms), 5 (34) or 6 (3) (Mean = 5.0, SD = 0.37). Scale rows below lateral line 6 (3), 7 (7), 8 (26), or 9 (4) (Mean = 7.8, SD = 0.84). Transverse scale rows 14 (6), 15 (27), or 16 (7) (Mean = 14.8, SD = 0.88). Transverse scale rows plus scale rows below lateral line 13 (5), 14 (6), 15 (22), 16 (6) or 17 (1) (Mean = 14.8, SD = 0.98). Caudal peduncle scale rows 10 (3), 11 (20), 12 (15) or 13 (2) (Mean = 11.3, SD = 0.82). Dorsal saddles 8 (40). Dorsal fin spines 6 (3), 7 (20), 8 (13), or 9 (1) (Mean = 7.3, SD = 0.67). Dorsal fin rays 9 (1), 10 (3), 11 (20), 12 (14), or 13 (2) (Mean = 11.3, SD = 0.82). Anal spines = 2 (40). Pelvic spines = 1 (40). Pelvic rays = 5 (40).

FIGURE 1. Male (Top) and Female (Midde) Vermilion Darter, *Etheostoma chermocki* Boschung, Mayden, & Tomelleri. Images from Boschung, Mayden & Tomelleri (1992) and Boschung & Mayden (2004; plate 80B and A) and copyrighted by Joseph R. Tomelleri (used with permission). (Bottom) General habitat of *E. chermocki*, Turkey Creek, Jefferson Co., Alabama, 12 July 2014. Photography copyrighted by B. R. Kuhajda.

FIGURE 2. Male (Top) and Female (Middle) Warrior Darter, *Etheostoma bellator* Suttkus & Bailey. Images from Boschung, Mayden & Tomelleri (1992) and Boschung & Mayden (2004; plate 79B and A) and copyrighted by Joseph R. Tomelleri (used with permission). (Bottom) General habitat of *E. bellator*, Murphy Creek, Blount Co., Alabama 13 June 2006. Photography copyrighted by B. R. Kuhajda.

FIGURE 3. (Top) Male, Locust Fork Darter, *Etheostoma kimberlae* new species Mayden. Images from Boschung & Mayden (2004; plate 79D). Image copyrighted by Joseph R. Tomelleri (used with permission). (Bottom) General habitat of *E. kimberlae*, Calvert Prong Creek, Jefferson Co., Alabama, 18 February 2017. Photography copyrighted by B. R. Kuhajda.

Infraorbital pores 6 (2 spms), 7 (5) or 8 (3) (Mean = 7.1, SD = 0.74). Preopercularmandibular pores 7 (1), 8 (5) or 9 (2) (Mean = 8.3, SD = 0.67). Lateral canal pores 2 (10). Supratemporal canal complete, pores 2 (10). Supraorbital pores 3 (10). Coronal pore single (10).

Nape scaled. Belly fully scaled. Breast naked.

Coloration of breeding males. Dorsum of head and body cream colored except for series of black saddles on body, normally numbering 8. Dorsolateral scales lightly pigmented along edges. Lateral blotches black and continuous or nearly so across very narrow light cream line along lateral line. Scale row directly above lateral line deep crimson and continuous or nearly continuous; lateral blotches not oval; above lateral line lateral blotch small, circular, short and not distinct; most of lateral blotch below lateral line and obvious; above lateral line, blotches not clearly separated. Below lateral line single crimson-colored scale row separating dark blotches. Crimson line usually one scale row and dissipating posteriorly directly vertical origin to middle of second dorsal fin. Dark caudal

TABLE 1. Body and head measurements as proportions of standard or head length for *E. bellator*, *E. michellae*, and *Etheostoma kimberlae*. D1 = First dorsal Fin, D2 = second dorsal fin, PC = pectoral fin, AF = anal fin.

Males	***Etheostoma bellator* (N = 19)**				***Etheostoma michellae* (N = 20)**				***Etheostoma kimberlae* (N = 20)**			
	Min	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**
SL	43.75	54.08	48.81	3.09	29.87	44.94	38.40	4.46	36.05	47.17	41.35	2.87
Percent standard length												
HL	0.21	0.27	0.24	0.01	0.23	0.25	0.24	0.01	0.23	0.26	0.25	0.01
BW	0.13	0.15	0.14	0.01	0.10	0.14	0.12	0.01	0.11	0.14	0.12	0.01
HW	0.13	0.15	0.14	0.01	0.12	0.15	0.14	0.01	0.12	0.15	0.14	0.01
Tip of snout to PC base lower	0.23	0.26	0.24	0.01	0.22	0.25	0.24	0.01	0.23	0.25	0.24	0.00
Posterior orbit to origin of D1	0.20	0.22	0.21	0.01	0.20	0.23	0.21	0.01	0.19	0.22	0.21	0.01
Origin D1 to origin to D2	0.27	0.31	0.30	0.01	0.28	0.32	0.30	0.01	0.27	0.31	0.28	0.01
Origin D1 to origin AF	0.31	0.36	0.34	0.01	0.30	0.35	0.33	0.01	0.31	0.34	0.33	0.01
Origin of D2 to lower PC base	0.37	0.41	0.39	0.01	0.37	0.39	0.38	0.01	0.35	0.38	0.36	0.01
D2 base length	0.15	0.21	0.18	0.01	0.14	0.19	0.17	0.01	0.16	0.21	0.19	0.01
Origin D2 to origin AF	0.16	0.20	0.18	0.01	0.15	0.18	0.17	0.01	0.15	0.18	0.17	0.01
Posterior of D2 base to posterior of AF base	0.13	0.16	0.14	0.01	0.11	0.16	0.13	0.01	0.11	0.15	0.13	0.01
Caudal peduncle depth	0.09	0.11	0.10	0.01	0.10	0.11	0.10	0.00	0.09	0.11	0.10	0.01
Posterior of D2 base to hypural plate	0.21	0.25	0.22	0.01	0.21	0.27	0.24	0.01	0.21	0.26	0.23	0.01
Caudal peduncle length	0.28	0.32	0.30	0.01	0.29	0.32	0.30	0.01	0.28	0.32	0.30	0.01
Base of AF	0.11	0.14	0.13	0.01	0.09	0.14	0.12	0.01	0.10	0.15	0.13	0.01
Origin AF to PC base lower	0.31	0.36	0.34	0.01	0.32	0.36	0.34	0.01	0.31	0.36	0.34	0.01
PC base	0.05	0.07	0.06	0.00	0.05	0.06	0.06	0.00	0.05	0.07	0.06	0.00
Percent head length												
Interorbital width	0.16	0.22	0.18	0.02	0.14	0.21	0.18	0.02	0.16	0.21	0.19	0.01
Preorbital	0.24	0.33	0.27	0.02	0.21	0.29	0.24	0.02	0.22	0.28	0.26	0.02
Snout to end of maxilla	0.21	0.35	0.25	0.03	0.22	0.28	0.25	0.02	0.18	0.29	0.24	0.03
End of maxilla to PC base lower	0.65	0.86	0.72	0.05	0.68	0.82	0.73	0.03	0.66	0.76	0.71	0.02
End of maxilla to PC base upper	0.70	0.90	0.76	0.05	0.72	0.93	0.78	0.05	0.72	0.85	0.77	0.04
Orbit width	0.23	0.31	0.26	0.02	0.24	0.30	0.27	0.02	0.22	0.28	0.26	0.01
Posterior orbit to PC base lower	0.51	0.66	0.55	0.03	0.51	0.61	0.56	0.03	0.49	0.62	0.54	0.03

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TABLE 1. (Continued)

Females	***Etheostoma bellator* (N = 20)**				***Etheostoma michellae* (N = 19)**				***Etheostoma kimberlae* (N = 17)**			
	Min	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**
SL	37.25	48.15	41.91	2.86	34.40	41.44	38.08	1.99	32.32	43.30	37.51	3.51
Percent standard length												
HL	0.21	0.26	0.24	0.01	0.23	0.25	0.24	0.01	0.23	0.28	0.25	0.01
BW	0.13	0.18	0.16	0.02	0.12	0.16	0.14	0.01	0.12	0.18	0.15	0.02
HW	0.13	0.15	0.14	0.01	0.13	0.14	0.14	0.00	0.13	0.14	0.14	0.00
Tip of snout to PC base lower	0.22	0.25	0.23	0.01	0.23	0.25	0.24	0.01	0.22	0.26	0.24	0.01
Posterior orbit to origin of D1	0.20	0.24	0.22	0.01	0.20	0.23	0.22	0.01	0.20	0.22	0.21	0.01
Origin D1 to origin to D2	0.29	0.32	0.30	0.01	0.29	0.32	0.30	0.01	0.27	0.31	0.29	0.01
Origin D1 to origin AF	0.33	0.36	0.34	0.01	0.31	0.36	0.34	0.01	0.32	0.36	0.33	0.01
Origin of D2 to lower PC base	0.38	0.43	0.40	0.02	0.36	0.41	0.39	0.01	0.35	0.40	0.38	0.01
D2 base length	0.14	0.18	0.17	0.01	0.12	0.19	0.17	0.02	0.16	0.21	0.18	0.01
Origin D2 to origin AF	0.16	0.19	0.18	0.01	0.13	0.18	0.16	0.01	0.16	0.18	0.17	0.01
Posterior of D2 base to posterior of AF base	0.11	0.15	0.13	0.01	0.11	0.13	0.12	0.01	0.11	0.14	0.13	0.01
Caudal peduncle depth	0.09	0.11	0.10	0.01	0.09	0.11	0.10	0.00	0.08	0.10	0.09	0.01
Posterior of D2 base to hypural plate	0.21	0.26	0.23	0.01	0.20	0.26	0.23	0.02	0.21	0.25	0.23	0.01
Caudal peduncle length	0.28	0.33	0.30	0.01	0.27	0.34	0.29	0.02	0.28	0.31	0.29	0.01
Base of AF	0.10	0.13	0.11	0.01	0.10	0.13	0.11	0.01	0.09	0.14	0.12	0.01
Origin AF to PC base lower	0.34	0.40	0.36	0.01	0.33	0.37	0.35	0.01	0.32	0.42	0.36	0.02
PC base	0.05	0.07	0.06	0.00	0.05	0.06	0.06	0.00	0.05	0.07	0.06	0.01
Percent head length												
Interorbital width	0.16	0.22	0.19	0.02	0.17	0.23	0.19	0.02	0.17	0.20	0.18	0.01
Preorbital	0.23	0.29	0.26	0.02	0.22	0.29	0.25	0.02	0.24	0.30	0.27	0.02
Snout to end of maxilla	0.22	0.28	0.25	0.02	0.20	0.28	0.24	0.02	0.20	0.27	0.24	0.02
End of maxilla to PC base lower	0.64	0.79	0.71	0.04	0.67	0.77	0.72	0.03	0.60	0.78	0.70	0.05
End of maxilla to PC base upper	0.66	0.86	0.75	0.05	0.73	0.85	0.79	0.03	0.71	0.83	0.75	0.04
Orbit width	0.24	0.32	0.27	0.02	0.25	0.31	0.28	0.02	0.25	0.31	0.27	0.02
Posterior orbit to PC base lower	0.47	0.62	0.54	0.04	0.51	0.58	0.55	0.02	0.45	0.59	0.53	0.04

TABLE 2. Distributions of Scale and Fin- Ray Counts in *Etheostoma bellator*, *E. kimberlae* and *E. michellae*. Underlined numbers are modes.

	Lateral Line Scale Rows												
	40	43	44	45	46	47	48	49	50	51	N	Mean	SD
Etheostoma bellator		1	2	2	4	10	11	7	8	8	56	48.4	2.21
Etheostoma michellae	1	1	2	10	6	6	6	3	2		37	46.3	2.03
Etheostoma kimberlae		2	2	4	8	14	11	6	6	3	63	48.0	2.54

	Caudal Fin Rays										
	12	13	14	15	16	17	18	19	N	Mean	SD
Etheostoma bellator	3	8	17	7	9	8	3	1	56	14.9	1.70
Etheostoma michellae		2	6	5	8	10	6		37	17.0	1.50
Etheostoma kimberlae		1	1	6	21	24	10		63	16.5	1.03

	Pectoral Fin Rays						
	12	13	14	15	N	Mean	SD
Etheostoma bellator	3	16	34	3	56	14.2	0.64
Etheostoma michellae		13	23	1	37	13.7	0.53
Etheostoma kimberlae	2	46	15		63	13.2	0.48

spot present; above and posterior to caudal spot short crimson stripe; stripes overlapping light cream-colored spots dorsally and ventrally at base of fin. Some ventrolateral scales lightly pigmented with melanophores, especially along distal portion of scales. Broad reddish-orange ventrolateral stripe present and involving multiple scale rows, extending length of body and 3–4 scale rows from above base of anal fin and pelvic fin insertion. Ventrolateral color stripe narrow and not connected with crimson scales around complex lateral stripe.

Opercle darkly pigmented dorsally becoming lighter ventrally; dark oblique line between cheek and preopercle; remainder of opercle lightly pigmented to immaculate; subopercle, branchiostegals, under side of head, preorbital area, breast and tip of snout turquoise. Distinct narrow suborbital bar, dark preorbital bar, middle of upper lip pigmented; remainder of lips yellowish to turquoise. Snout lightly pigmented and in distinct contrast to preorbital bars.

Pectoral-fin rays pigmented and with two stripes, one a basal band covering 1/3 of rays and boarded distally by turquoise colored stripe; distal to this basal band light area, bordered distally by darker stripe, and distal-most 1/3 of fin with lightly pigmented rays; membranes without pigmentation. Base of fin and area of insertion reddish-orange. Spine of pelvic fin immaculate to turquoise; remaining rays turquoise to darkly pigmented. Base of fin and first three branched rays turquoise. Posterior rays immaculate. Spine and first three rays also pigmented with melanophores medially, creating a dark stripe; all membranes turquoise. Spinous dorsal fin with four stripes. Base of fin with narrow dark green stripe, area between this stripe and adjoining more distal stripe narrow and light cream. Distal dark stripe with pigment on rays and membranes. Subdistal stripe beginning with dark red irregular shaped spot between first and second spines and stripe that is less intense in color, continuing posteriorly from being narrow in anterior membranes and significantly increasing in width towards last membranes. Narrow blue distal band beginning at sixth spine and extending to posterior of fin. Distal margin of anterior five rays clear. Distal-most black stripe separated from brick red stripe by narrow clear stripe. Soft (second) dorsal fin with three distinct bands. Distinct broad median brick red stripe below and above darkly pigmented median stripe formed from pigmented rays and membranes. Basal half of first three rays may have alternating black and yellow/gold narrow stripes. Procurrent, principle and two adjacent branched rays of caudal fin turquoise. Two-three dark vertical bands located medially on caudal fin separated by yellow- to cream-colored narrow bars; distal margin lightly pigmented, appearing almost transparent.

Coloration of Preserved breeding males. Adult males with dark brown dorsal saddles extending from occiput to posterior extent of caudal peduncle; occasionally blotches may be connected; blotches formed from dense

concentrations of melanophores, and separated by pale areas having only light concentrations of melanophores. Dorsolateral area above lateral line with stripe, usually 1.5 to 2.5 scales high, extending posteriorly to vertical of middle of second dorsal fin to near hypural plate; stripe formed largely from dorsal halves of slightly lighter scale areas relative to dark lateral blotches (hereafter referred to as lighter) that, in live specimens, were dark red; dark lateral blotches weakly developed above lateral line and most strongly developed below lateral line where appearing somewhat oval in shape; blotches separated by lighter scale areas. Posterior to middle of second dorsal fin base dark lateral blotches connecting across and centered on lateral line; lighter intervening scale areas only present above lateral line. Lighter blotches below lateral line forming continuous stripe to hypural plate, becoming darker and centered on lateral line posterior to middle of second dorsal fin. Belly and breast lightly pigmented.

Dorsum of head tan to brown. Two horizontally rectangular dark blotches immediately posterior to head along lateral line poorly developed, usually small, if present, and consisting of broken lines. Snout with melanophores uniformly distributed except for distinct dark line connected to preorbital strip; line directly above upper lip and continuous across snout in adult males; smaller males with interruption in snout band; upper lip pigmented medially. Suborbital bar present; bar most distinct in females. Postorbital stripe usually not continuous but broken into two distinct, dark spots; first spot immediately posterior to orbit and second at junction of dorsal arm of preopercle and anterodorsal area of opercle. Remainder of cheek, opercle, preopercle, and subopercle only lightly pigmented except for distinct irregularly shaped concentrations of melanophores formed on the cheek and upper operculum. Cheek spot located slightly ventral and posterior to postorbital spot immediately behind orbit. Opercular spot located near center or posterodorsal area of opercle below dorsal margin of opercle. Lower cheek, branchiostegals, and gular areas with light scattering of melanophores to immaculate; lower lip immaculate.

Color pattern of dorsal fins as described for live specimens except colors muted. Caudal fin with light pigmentation on rays only, forming two to four bands. Anal and pelvic fins with few melanophores on membranes and little to no pigment on rays. Pectoral fin with bars formed from melanophores on rays separated by depigmented portions of rays; membranes clear. Spot at base of caudal fin only darkly pigmented like lateral blotches and surrounded by pale oval areas.

Coloration of live breeding females. Without bright coloration. Dorsum of body with distinct dark dorsal saddles separated by lighter scales cream in color. Dorsolateral scales with cream bases and darker posterior edges; some scales darker than others and in short continuous lines, creating mottled appearance; scattered scales partially brick red to orange. Scale row directly above lateral line brick red to orange and continuous or nearly continuous. Lateral blotches mostly below lateral line where dark pigment covers 1–2 rows of scales; blotches, if present, above lateral line may be half scale row. Anterior five blotches with oblique line of pigmented scales (best developed anteriorly) directed anteroventrally; posterior blotches with short (1–2 scales) pigmented along ventral margins. Scattered scales one scale row below lateral-line and between lateral blotches may be orange. Some ventrolateral scales may have some orange in center of scale.

Dorsum of head dark brown; preorbital and postorbital stripes and suborbital bar brown and well developed. Preorbital stripes connecting along pigmented upper lip. Cheek with distinct dark blotch posteriorly; opercle mottled. Venter of head and body cream. Pectoral, second dorsal, and caudal with pigment on rays only, creating lines on fins; membranes transparent. First dorsal fin with dark pigment on rays and membranes, small red to orange blotch in first membrane. Anal fin immaculate except for pigment on membranes between spines and posterior spine and first ray. Spot at base of caudal fin darkly pigmented and surrounded by pale cream oval areas.

Coloration of preserved females and juveniles. Dorsum of body with dark saddles; areas between saddles and ventrally to lateral line somewhat lighter but less obvious than in males; most scales with darkened centers, creating a mosaic pattern of dark and light irregularly shaped spotting pattern. Below lateral line distinct dark and squarish blotches, 3–4 scales deep, separated by areas of near equivalent width with light or depigmented scales; most larger females with distinct narrow anteroventral extension of dark lateral blotch, usually connected to blotch and sometimes extending anteriorly to connect with or nearly connect with immediate blotch anteriorly; anteroventral extensions of dark blotches creating pattern of pale circles or ovals between dark blotches below lateral line. Lighter pigmented line, as described in males, not as well developed and occurring below lateral line posteriorly to usually origin of second dorsal fin. Narrow light line below lateral line only anteriorly, extending posteriorly to near terminus of first dorsal fin. Ventrolateral areas, belly, breast and lower caudal peduncle immaculate.

Head coloration as in males except for the following; preorbital stripe present, suborbital bar usually well developed, and concentrations of melanophores on check and posterodorsal area of opercle darker and may include more than two spots.

Membranes of dorsal fins clear; rays with concentrations of melanophores separated by areas of same size with no melanophores, creating a pattern of bands in the fin; no indication of a red blotch on first membrane of first dorsal fin. Like dorsal fins, caudal and pectoral fins with melanophores on rays and melanophore distributions form two or more bands. Anal and pelvic immaculate. Spot at base of caudal fin distinct, similar in intensity as dark lateral blotches, and surrounded by pale ovals.

Distribution and Habitat. This species is only known from the upper Locust Fork of the Black Warrior River drainage (Figure 5). It is presently known from Calvert Prong at AL Hwy 75 just north of Oneonta and a tributary to Calvert Prong, Mill Creek, in and around Oneonta, Blount County, Alabama. It is known historically from another Calvert Prong tributary, Sand Valley Creek, Blount County, Alabama (UMMZ 158288, 1939) and Little Cove Creek, Etowah County, Alabama (UAIC 3307.11, 1969) (Suttkus & Bailey 1993, Kuhajda 2004a). No systematic status survey has been done for this species, and it is predicted to occur at other localities in the Calvert Prong system and different streams of geologies dominated by the Chapultepec and Copper Ridge Dolomites undifferentiated formation in Blount and Etowah counties. The species is found in small to moderate-sized upland creeks associated with moderate to little current over a sand/gravel to cobble substrate, typically in the glide above riffles and in the transition to pool habitat below riffles, but can occur in riffles proper (Kuhajda 2004a).

Relationships. Clabaugh *et al.* (1996) discussed possible relationships of this species relative to others of the *E. chermocki* group based on allozyme variation. Near *et al.* (2011), using sequence variation, identified *E. kimberlae* (therein referred to as *E.* cf. *bellator* (Locust Fork Darter) as the sister species of a clade composed of *E. bellator* and *E. chermocki*. Interestingly, in all of the supplemental dendrograms by Near *et al.* (2011) (cytb, S7 intron 1, RAG1) presented for the group, there are no data for the Locust Fork Darter. Yet the species appears in the concatenated dendrogram in the body of the paper (two specimens each of Locust Fork and Sipsey Fork darters). In the definition of the clade *Adonia*, Near *et al.* (2011) mentions the Locust Fork Darter but not the Sipsey Darter. Kim *et al.* (2023) provide a short dialog about the genetic variation in this group relative to the geological formations in the area. Like Clabaugh *et al.* (1996), using only a relatively small number of allozyme loci, Kim *et al.* (2023), using 25,393 ddRAD loci revealed the exact similarities between *E. bellator* (*sensu lato*), *E. michellae*, and *E. kimberlae* in both the maximum likelihood concatenated dendrogram and the unrooted PoMo dendrogram. *Etheostoma kimberlae* forms the basal sister group to all other members of the *E. chermocki* clade. In their analysis, the authors confidently discuss the existence of not only *E. bellator* in the Mulberry Fork of the Black Warrior River but also two undescribed forms in Valley and Gurley creeks (no data were provided to substantiate any of the forms). Hence, this is why we refer to *E. bellator* as *sensu lato*. Neither lineage was identified in our analyses (genetic or morphological), the coloration of breeding and nonbreeding males and females based on descriptions, personal observations, and photographs of breeding and nonbreeding coloration, meristic, morphometrics, and morphological observations analyses.

Etymology. This species is named after my daughter Kimberly Linnae Mayden. The common name Locust Fork Darter refers to the river system where *Etheostoma kimberlae* is endemic.

Conservation Status.

Species is currently only recorded from Calvert Prong north of Oneonta and approximately 5 km of Mill Creek in and surrounding Oneonta, Blount County, Alabama (Kuhajda 2004a). Given the very small distribution of *E. kimberlae* and localized industrial activities, urbanization, and agriculture, this species is endangered (Jelks *et al.* 2008) and needs to be considered for State and Federal protection. It is currently listed as a species of High Conservation Concern (P2) by the State of Alabama (Kuhajda 2004a, ADCNR 2015) and S1 by the Alabama Natural Heritage Program but is not on the State list of protected species (ALNHP 2015). A complete systematic status survey is needed (Kuhajda 2004a), followed by long-term monitoring of extant populations.

Etheostoma michellae Mayden, new species

Sipsey Fork Darter

Fig. 1

Holotype—UAIC 11065.05. Male. Alabama, Lawrence Co., Borden Creek at Forest Service Road 224, William Bankhead National Forest. T8S, R8W, S21/28, 34.329885 -87.377174. 18 April 1994. B. R. Kuhajda and R. L. Mayden.

Paratypes—UAIC 11065.07, n=14, from UAIC 11065.05; UF 188210 (n=2); TU 204141 (n=2). All with the same data as the holotype. UAIC 11290.06 (n=5). UAIC 11040.01, n=3, Alabama, Lawrence Co., Flannigan Creek at Forest Service Road 208, 8.8 km S Youngtown, T8S, R8W, S 21, 34.338848 -87.388241. 10 May 1994. AUM 54703, n=6 same locality as UAIC 11040.01. 5 August 2011. Alabama, Lawrence County: UAIC 1696.14 (5), Borden Creek at Bunyan Hill Road (Co. Rd. 5, Forest Service Road 224), 34.309532 -87.394673, T8S, R8W, S32, 12 July 1978; UAIC 3868.08 (11), 22 August 1970; UAIC 6264.09 (19), 11 October 1980; UAIC 11065.05 (19), 18 April 1994; BRK16-17, 5 May 2016. UAIC 4963.12 (4), Borden Creek at Forest Service Road 229 (208) (Co. Rd. 9), 34.329867 -87.377157, T8S, R8W, S21/28, 19 August 1974; UAIC 13309.12 (2). 24 June 2001. UAIC 11040.01 (3), Flannigan Creek at Forest Service Road 229 (208) (Co. Rd 9), 34.338849 -87.388339, T8S, R8W, S21, 10 May 1994. Winston County: UAIC 4329.18 (7), Sipsey Fork at Sipsey Recreation Area on Co. Rd. 60, 34.285416 -87.399144, T9S, R8W, S8, 2 November 1978. UAIC 3852.12 (7), Sipsey Fork at mouth of Hurricane Creek, 34.252967 -87.367050, T9S, 8W, S22, 3 November 1971.

Diagnosis. Member of *Etheostoma chermocki* species group as identified by Boschung *et al.* 1992, Clabaugh *et al.* (1996) and Boschung & Mayden (2004). *Etheostoma chermocki* was diagnosed from the *Etheostoma bellator* complex by Boschung *et al.* (1992) and Suttkus & Bailey (1993). *Etheostoma michellae* is distinguished from *E. kimberlae* using the following combination of characters; cream-colored horizontal stripe through lateral band broad obvious vs. very narrow to absent; snout of breeding males dusky and often masking preorbital stripes vs. snout lightly colored with obvious preorbital stripes; suborbital bar long, dark and well defined vs. usually shorter, diffuse, and lightly pigmented; lateral blotches diffuse and occurring both above and below lateral line vs. lateral blotches dark and largely below lateral line; crimson line above lateral line broken up into segments between diffuse upper halves of lateral blotches vs. crimson lines formed by spots continuous to nearly continuous and not interrupted by lateral blotches; distinct coloration pattern in spinous and soft dorsal fins of breeding males as described below in color description. *Etheostoma michellae* is further distinguished from *E. kimberlae* in the possession of the following alleles at identified protein loci: *sAat-A* (B vs. A)†, *mAcon-A* (B, C vs. B)*, *Acp-1* (B vs. A)†, *Ada-2* (B vs. A)†, *Est-2* (A vs. D)†, *Pep-B* (B vs. B, C)*, *Pep-F* (B vs. D)†, *Pnp-A* (C, D vs. D)*. *Etheostoma michellae* is diagnosed from *E. bellator* based on the following characters: crimson line above lateral line broken up into segments between upper halves of lateral blotch vs. crimson line irregular in shape and passing over lateral blotches. *Etheostoma michellae* is further distinguished from *E. bellator* in the possession of the following alleles at identified protein loci: *mAcon-A* (B, C vs. B)*, and *Est-2* (A vs. D)†.

Etheostoma michellae usually has 45–48 lateral line scale rows (46–50 in *E. kimberlae,* usually 45, and 47–51 in *E. bellator).* Scale rows below lateral line usually 8 (usually 7 or 8 in *E. kimberlae* and *E. bellator).* Transverse scale rows usually 14 or 15 (usually 15 in *E. kimberlae).* Transverse scale rows plus scale rows below lateral line usually 14 or 15 (usually 15 in *E. kimberlae,* 14 or 15 in *E. bellator).* Dorsal fin rays usually 11 or 12 (usually 11 in *E. bellator,* 11 or 12 in *E. kimberlae).* Pectoral fin rays usually 14 (usually 13 in *E. kimberlae* and 14 in *E. bellator).* Caudal fin rays 15 or 16 (usually 14 in *E. bellator* and *E. kimberlae*)

Etheostoma michellae differs *E. kimberlae* in usually having 8 infraorbital pores (mean = 8.1) (vs. usually 7, mean 7.1). Nape, cheek, opercle scaled; breast naked. *Etheostoma michellae* is a small member of the *E. chermocki* species group, reaching a recorded 45.0 mm SL (male) or 41.4 mm SL (female) vs. *E. bellator* (male = 58 mm SL, Suttkus & Bailey 1993; female = 48.2 herein) and *E. chermocki* (male = 55 mm SL; female = 51 mm SL; Boschung *et al.* 1992) and slightly larger than *E. kimberlae* (male = 45.0 mm SL; female = 41.4 mm SL).

Description. General head and body shape and coloration illustrated in Figure 4. Morphometric variables for males and females (sexually dimorphic) provided in Table 1. Distribution of lateral line scales, caudal fin rays, and pectoral fin rays provided in Table 2.

Small snubnose darter, reaching a recorded 47.7 mm SL (male) or 42.6 mm SL (female).

Lateral line complete and virtually straight from upper margin of gill opening to base of caudal fin. Scale rows above lateral line 4(2 spms), 5 (56) or 6 (5) (Mean = 5.0, SD = 0.33). Scale rows below lateral line 6 (1), 7 (27), 8 (27), or 9 (8) (Mean = 7.7, SD = 0.72). Transverse scale rows 14 (27), 15 (27), 16 (7), or 17 (Mean = 14.7, SD = 0.76). Transverse scale rows plus scale rows below lateral line 13 (1), 14 (26), 15 (29), or 16 (8) (Mean = 14.7, SD = 0.71). Caudal peduncle scale rows 10 (3), 11 (38), or 12 (22) (Mean = 11.3, SD = 0.55). Dorsal saddles 6 (1), 7 (2), or 8 (60) (mean = 7.9; SD = 0.30). Dorsal fin spines 6 (3), 7 (51), 8 (8), or 9 (1) (Mean = 7.4, SD = 0.42). Dorsal fin rays 9 (1), 10 (3), 11 (38), or 12 (22) (Mean = 11.2, SD = 0.63). Anal spines 2 (67). Pelvic spines 1 (67 specimens). Pelvic rays 5 (65).

FIGURE 4. (Top) Male, Sipsey Fork Darter, *Etheostoma michellae* new species Mayden. Images from Boschung & Mayden (2004; plate 79C). Images copyrighted by Joseph R. Tomelleri (used with permission). (Bottom) General habitat of E. *kimberlae*, Bordon Creek, Lawrence Co., Alabama 5 May 2016. Photography copyrighted by B. R. Kuhajda.

Infraorbital pores 8 (9 spms) and 9 (1) (Mean = 8.1, SD = 0.32). Preopercularmandibular pores 8 (7) and 9 (3) (Mean = 8.3, SD = 0.48). Lateral canal pores 2 (9) and 3 (1) (mean = 1.75, SD = 0.45). Supratemporal canal complete, pores 2 (10). Supraorbital pores 3 (10). Coronal pore single (10).

Coloration of live breeding males. Dorsum of head and body cream colored except for series of black saddles on body, normally numbering 8. Dorsolateral scales lightly pigmented along edges. Deep crimson-colored scale row(s) directly above lateral line broken/interrupted by diffuse, dorsally elongate lateral blotches; diffuse lateral blotches tall and cross moderately broad light stripe along lateral light. Crimson colored scales above lateral line broken into distinct segments, not continuous single line; segments of crimson-colored scales between distinct lateral blotches above lateral line beginning anteriorly as one scale row and sometimes terminate posteriorly near next blotch as two scale rows deep. Dark caudal spot present and posterior to hypural plate, sometimes narrowly connected to last lateral blotch; short crimson stripe above and posterior to caudal spot usually absent or poorly

developed; caudal spot bordered dorsally and ventrally by cream-colored circular to oval spots. Some ventrolateral scales lightly pigmented with melanophores, especially along distal portion of scales. Above and below caudal spot and yellowish areas small but distinct black spots medial to procurrent rays. Narrow reddish-orange ventrolateral stripe present and involving usually 1–2 scale rows (sometimes 3); narrow reddish-orange stripe above base of anal fin and pelvic fin insertion, and extending anteriorly to under pectoral fin to insertion. Ventrolateral color stripe not connected with crimson scales around complex lateral stripe.

Opercle dusky and darkest at dorsal third; dark oblique bar between cheek and preopercle absent; remainder opercle lightly pigmented to immaculate, especially progressing ventrally; opercle with greenish hue. Underside of head, gular region, branchiostegals, and tip of snout turquoise. Suborbital bar dark and long; preorbital stripe light and extending to tip of snout; stripes often masked by darkly pigmented snout.

Pectoral fin without prominent stripes. Pectoral fin spine and distal tips of branched rays 1–2 immaculate; remaining rays with greenish-yellow hue; membranes pigmented. Base of pectoral fin and surrounding areas dusky to immaculate with reddish hue visible from blood vessels below surface. Pelvic fin spines and distal tips of branched rays 1–2 immaculate; remaining rays turquoise; membranes pigmented; fins without dark stripes or darkly pigmented rays; base light turquoise to immaculate. Pelvic fin spines turquoise; remaining rays turquoise to darkly pigmented. Narrow median clear stripe present, especially posteriorly. Base and distal portions of fin membranes and rays turquoise; membranes, except in clear area, with melanophores. Spinous dorsal fin with five bands. Basal band narrow and composed of black and golden pigment on bases of spines; band separated by narrow clear area from a distinct black stripe with pigment on membranes and spines; black pigment horizontal across spines and membranes but extending dorsally on membranes as narrow lines, creating generally concave black line; cream-colored area filling in membranes and rays anterior to dorsal extension of black; broad black band separated from sub-distal red band formed from distinct, oblique to horizontal stripes of crimson red on membranes (rays white to clear); red band begins with distinct red spot on first membrane, is narrow anteriorly but expands to broad band on membranes at the posterior-most portion of fin. Distal-most band clear or white anteriorly and becoming black posteriorly; rays white to clear and contrasting with distal-most stripe. Second dorsal fin with five distinct bands: basally, fin with yellow band; distal to this is a narrow black band; distal to this band is a broad median crimson red band where pigment limited to membranes and rays golden to white; sub-distal band cream-yellow; distal-most band black, basal portion of anterior rays with alternating black and golden stripes, as wide as median red band. Caudal fin generally dusky but with three distinct black bands, one at base of fin posterior to caudal spot and adjacent yellow-cream spots, all posterior to hypural plate; second and third black bands separated from each other and basal-most band by narrow cream to golden bands; distal one third of fin clear; procurrent rays and base of principle caudal ray turquoise.

Coloration of preserved breeding males. Adult males with dark brown dorsal saddles extending from occiput to posterior extent of caudal peduncle; blotches formed from dense concentrations of melanophores and separated by pale areas having only light concentrations of melanophores. Dorsolateral area above lateral line with disjunct, dark, and relatively small and irregularly shaped upper halves of more or less vertical (not oval) blotches; dorsolateral halves of dark blotches separated by lightly pigmented areas occurring in same scale regions where dark red coloration occurred in live specimens. Both types of blotches generally forming an irregularly shaped stripe; stripe above lateral line notably paler than stripe along lateral line and below lateral line; paler stripe both above and below lateral line and extending posteriorly to near middle of base of second dorsal fin; dark blotches may be weakly contiguous across lateral line posteriorly, but usually by only a few melanophores; lighter blotches not contiguous across lateral line. Dark blotches best developed below lateral line and 1–2 scale rows high; lighter blotches below lateral line forming continuous stripe to hypural plate, becoming darker and centered on lateral line posterior to middle of second dorsal fin. Posterior to middle of second dorsal fin base both types of blotches smaller than those anteriorly and frequently connected across lateral line; dark blotches may be oval posteriorly but light blotches retain rectangular shape. Breast pigmented as belly.

Dorsum of head tan to brown. Area immediately posterior to head along lateral line with two distinct horizontally rectangular dark blotches separated by lighter area along lateral line; blotches different in shape from lateral blotches. Snout with melanophores uniformly distributed and dusky, often dark enough to mask preorbital stripe. Suborbital bar diffuse, appearing narrow and lightly pigmented relative to other melanistic bars, spots, or lines on head; suborbital bar more distinct in females. Postorbital stripe usually not continuous but usually broken into two distinct, dark spots; first spot immediately posterior to orbit and second at junction of dorsal arm of preopercle and anterodorsal area of opercle. Remainder of cheek, opercle, preopercle, and subopercle only lightly pigmented

except for distinct concentrations of melanophores formed the cheek and upper operculum. Cheek spot located slightly ventral and posterior to postorbital spot immediately behind orbit. Opercular spot located near center or posterodorsal area of opercle below dorsal margin of opercle. Lower cheek, branchiostegals, and gular areas with light scattering of melanophores; lower lip immaculate.

Coloration of dorsal fins as described for live specimens except colors muted. Caudal fin with light pigmentation on rays only, forming two to three vertical bands. Anal and pelvic fins with dense concentrations of melanophores on membranes and little to no pigment on rays. Pectoral fin with bars formed from melanophores on rays separated by depigmented portions of rays; membranes clear. Spot at base of caudal fin only lightly pigmented and surrounded by pale oval areas.

Coloration of live breeding females. Without bright coloration. Dorsum of body with distinct dark dorsal saddles separated by lighter scales cream in color. Dorsolateral scales with cream bases and darker posterior edges, some scales darker; most scales in row above lateral line partially brick red to orange, as well as scattered partially orange scales elsewhere dorsolaterally. Lateral stripe below lateral line similar to males except that blotches are more intense and contrast strongly with cream background coloration. A few small, dark olive clusters of melanophores may interdigitate between blotches. Some ventrolateral scales may have orange in scale center.

Dorsum of head dark olive; preorbital stripe and suborbital bar dark brown, dorsal half of opercle mottled. Anal, pelvic and pectoral fins immaculate; no melanophores on spines, rays or membranes. Dorsal fins with two or three dusky bands formed from dark melanophores along rays and membranes, small subdistal red to orange blotch in first membrane, subdistal colored blotches may be present in other membranes. Flanks below lateral blotches and venter, from gular area to caudal fin, immaculate. Medial spot at base of caudal fin small but darkly pigmented and surrounded by pale cream to yellow oval areas. Smaller young-of-the-year without any red, orange, yellow, or any other colors other than cream, olive, and black on body, head and fins.

Coloration of preserved females and juveniles. Dorsum of body with dark saddles separated from one another by lighter scales extending ventrally to lateral line; lighter scales, unlike males, with darkened centers. Sides with dark taller than wide vertical blotches often separated by narrow light line centered on lateral line; light line may extend to posterior terminus of second dorsal fin or hypural plate. Some blotches above lateral line sometimes connecting with dorsal saddles by thin and irregularly shaped vertical line. Below lateral line dark blotches well developed and 2–3 scales high; blotches lacking anteroventral lines connected to blotch but may have small pigmented spot between ventral portions of lateral blotches; scale around blotches and those extending ventrally to and on belly and caudal peduncle immaculate.

Head coloration as in males except for the following; dark lines on snout usually weakly developed, suborbital bar usually well developed, and concentrations of melanophores on check and posterodorsal area of opercle darker and may include more than two spots.

Membranes of dorsal fins clear; rays with concentrations of melanophores separated by areas of same size with no melanophores, creating pattern of bands in fin; no indication of red blotches on membranes (first membrane especially) of first dorsal fin. Like dorsal fins, caudal fin with melanophores on rays in distributions forming 2–3 bands. Anal, pelvic, and pectoral fins immaculate. Distinct dark spot present at base of caudal fin surrounded by pale ovals.

Distribution and Habitat. *Etheostoma michellae* is found only in the upper Sipsey Fork system of the Black Warrior River drainage (Figure 5). It is currently found in the Sipsey Fork mainstem from its origin to just upstream of the Lewis Smith Reservoir embayment, and in three tributaries; Thompson, Borden (including Flannigan and Braziel creeks), and Caney creeks, Lawrence and Winston counties, Alabama. It does not occur in Hubbard Creek. Although there are no records, the species likely occurred historically in the impounded section of the Sipsey Fork (Suttkus & Bailey 1993, Kuhajda 2004b). The species is found in small to large upland creeks associated with moderate to little current over a sand/gravel to cobble substrate, typically in the glide above riffles and in the transition to pool habitat below riffles (Powers *et al.* 2003, Kuhajda 2004b).

Relationships. Clabaugh *et al.* (1996) discussed possible relationships of *Etheostoma michellae* relative to other species of the *E. chermocki* group based on allozyme variation. Near *et al.* (2011), using sequence variation, identified *E. michellae* (therein referred to as *E.* cf. *bellator* (Sipsey Darter)) as the sister species to the entire *E. chermocki* clade. Kim *et al.* (2023) discuss relationships in this group about geological formations in the area.

Kim *et al.* (2023) using 25,393 loci and ddRAD in the maximum likelihood concatenated analysis revealed *E. michellae* as the unlikely sister lineage to a purportedly undescribed lineage restricted to Gurley Creek. However, in the unrooted PoMo species tree *E. michellae* was resolved in an unresolved polytomy with *E. bellator* (Mulberry

Fork Black Warrior) and purportedly undescribed (no data provided to substantiate any of the forms) lineages in Gurley and Valley creeks.

Etymology. The species is named in honor of my wife Michelle Joy Mayden. The common name Sipsey Fork Darter refers to the upper Sipsey Fork system where *Etheostoma michellae* is endemic.

Conservation status. Species is currently only known from the Sipsey Fork mainstem above Lewis Smith Reservoir embayment and in three tributaries in Lawrence and Winston counties, Alabama (Kuhajda 2004b). Compared to historical collections, numbers of individuals in recent collections are few. Although most of this species' range is within the William B. Bankhead National Forest and in part the Sipsey Wilderness area, there are sedimentation issues associated with poor forestry practices, especially in tributaries (Kuhajda 2004b), and recent extreme droughts drying headwater streams. This species is threatened (Jelks *et al*. 2008) and needs to be considered for State and Federal protection. It is currently listed as a species of High Conservation Concern (P2) by the State of Alabama (Kuhajda 2004b, ADCNR 2015) and S2 by the Alabama Natural Heritage Program, but is not on the State list of protected species (ALNHP 2015). An updated status survey is needed, followed by long term monitoring of extant populations.

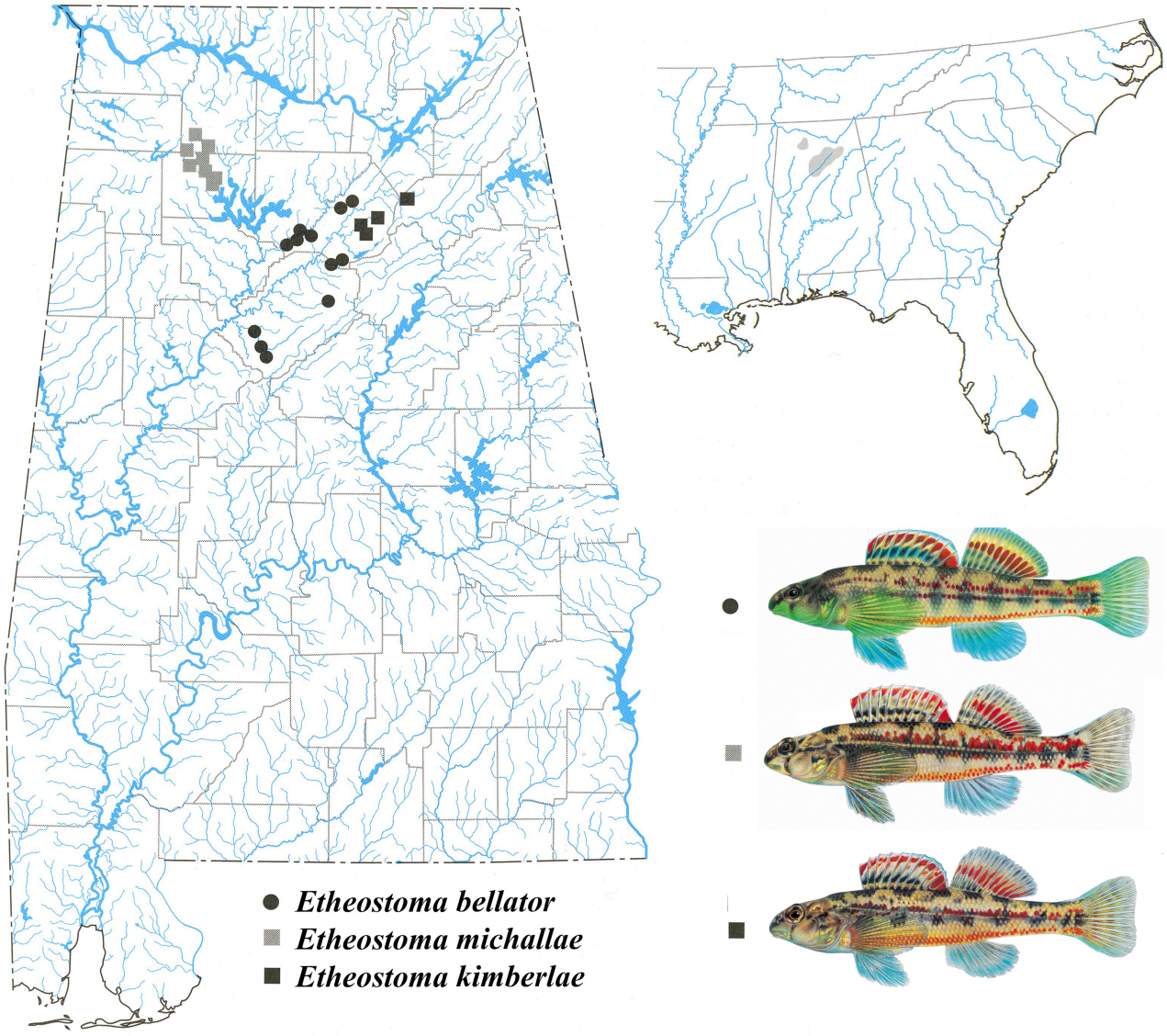

FIGURE 5. Distributions of *Etheostoma bellator* (circles), *E. kimberlae* (black squares), and *E. michellae* (gray squares) in the river systems of Alabama. The inset to the right represents the distributions of these species relative to the major river systems of the Southeastern United States. Images of each species are provided for reference. Maps modified from Boschung & Mayden (2004) and used with permission. Images of darters copyrighted by Joseph R. Tomelleri (used with permission.)

Results

Principal component analysis. Meristic and morphometric variables were examined separately for *E. bellator, E. michellae,* and *E. kimberlae*. No distinct patterns of interspecies relationships were revealed using meristic data or morphometric data for females. Among morphometric data for males *E. michellae* was almost 100% separable from *E. kimberlae* along PC2 (Fig. 7). *Etheostoma bellator* had substantial overlap with *E. michellae* but not with *E. kimberlae*. All species overlapped along PC3. Factors with high negative loading along PC2 included origin D1 to origin D2, origin D2 to PC base lower, and origin AF to PC base lower (Table 3). The single positive factor loading heavily along PC2 was D2 base.

TABLE 3. Variable loadings for Principal Component Analysis for mensural data from male specimens of *Etheostoma bellator, E. kimberlae,* and *E. michellae.* See Methods for measurements and abbreviations.

Measurement	PC 1	PC 2	PC 3
SL	0.676425	0.193859	-0.128355
HL	0.171364	0.121019	0.240434
BW	0.119662	-0.091633	0.239782
BD	0.111654	-0.009834	0.326788
HW	0.110258	0.013836	0.193814
IOW	0.030582	0.032806	0.056682
SnL	0.053507	0.06764	0.122893
Snout to maxilla end	0.040753	-0.013901	0.077844
Maxilla end to PC base lower	0.117397	0.009551	0.114341
Maxilla end to PC base upper	0.118792	0.12033	0.112409
Snout tip to PC base lower	0.159697	0.057731	0.138821
Orbital width	0.039926	-0.030741	0.052538
Posterior orbit to PC base lower	0.096788	0.03612	0.024823
Posterior orbit to origin D1	0.142203	-0.128488	0.140577
Origin D1 to origin D2	0.211947	-0.439466	-0.097578
Origin D1 to origin AF	0.259551	-0.12817	-0.227697
Origin D2 to PC base lower	0.290064	-0.425706	0.132392
D2 base	0.146116	0.571519	0.146686
Origin D2 to origin AF	0.156234	0.008978	0.074991
D2 base posterior AF base posterior	0.116787	0.125588	0.192302
CPD	0.07228	-0.052306	0.022005
D2 base posterior to hypural plate	0.119149	0.173627	-0.484501
CPL	0.18857	0.179994	-0.459559
AF base	0.09814	0.165747	0.284614
Origin AF to PC base lower	0.244186	-0.249518	-0.082526
PC base	0.046776	-0.000538	0.05845

Biogeography of the *Etheostoma chermocki* group. Most of the of Black Warrior River drainage above the Fall Line lies upon the two main geologic formations of the southern Cumberland Plateau physiographic province, the Pottsville Formation, upper (A) and lower (B) parts (Osborne *et al.* 1988, Szabo *et al.* 1988) (Figure 2). The distribution of the four species of the *Etheostoma chermocki* species group are mostly or completely restricted to other geologic formation that border or extend onto these main Cumberland Plateau geologies (Figure 6). *Etheostoma chermocki* and populations of *E. bellator* in Valley and Fivemile creeks occur on the divide between the Cumberland Plateau (Warrior Basin physiographic district) and the Alabama Valley and Ridge (Birmingham-Big Canoe Valley physiographic district) in geological formations dominated by Chepultepec and Copper Ridge

Dolomites undifferentiated as well as Ketona Dolomite, Bangor Limestone, and Chickamauga Limestone formations (C) (Osborne *et al.* 1988, Shepard *et al.* 2004). These same geologic formations extend as a narrow band in the Murphrees Valley physiographic district to the northeast onto the Cumberland Plateau, bisecting eastern tributaries

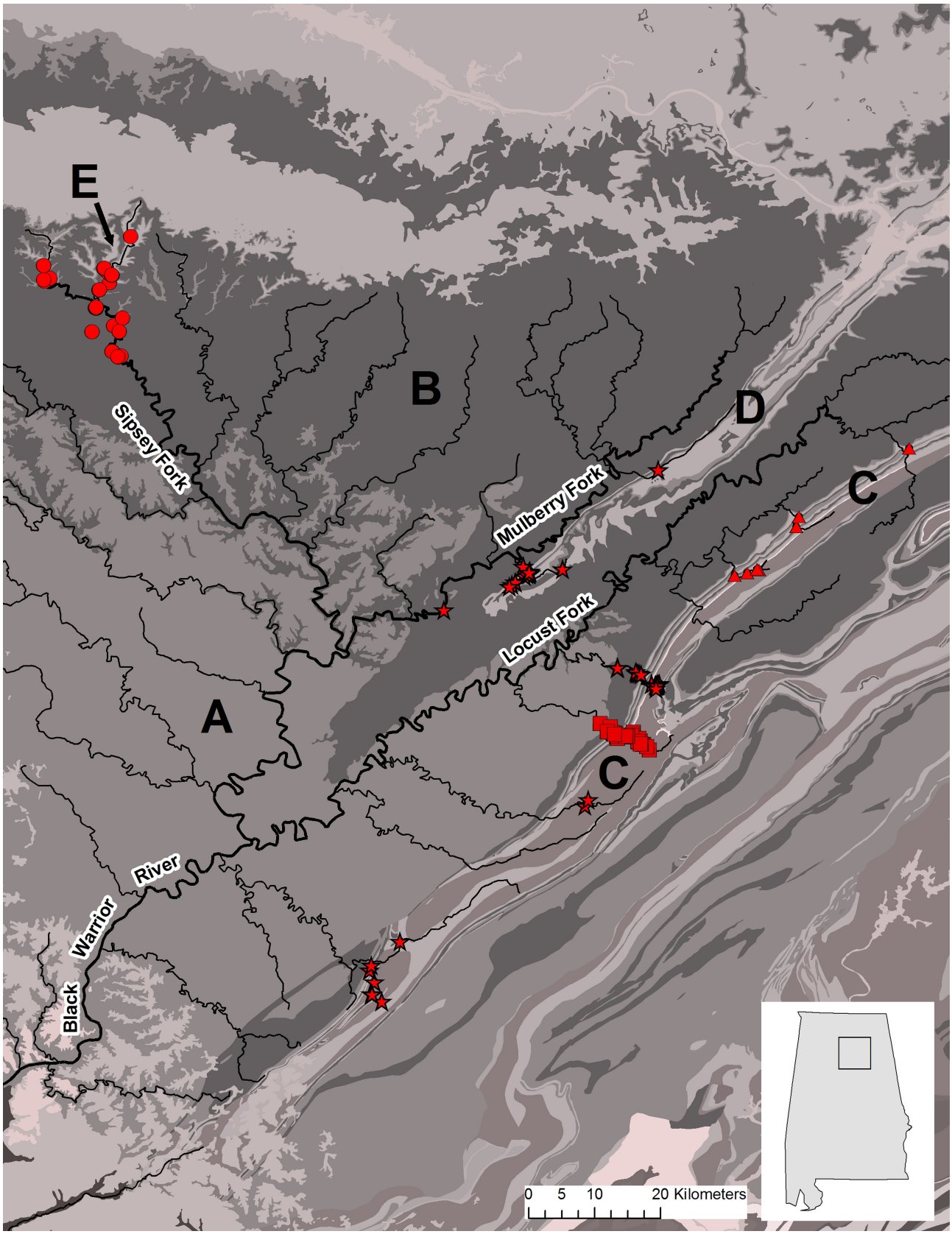

FIGURE 6. Distributions of *Etheostoma chermocki* (squares), *E. bellator* (stars), *E. kimberlae* (triangles), and *E. michellae* (circles). Letters denote geologic formations referenced in the text.

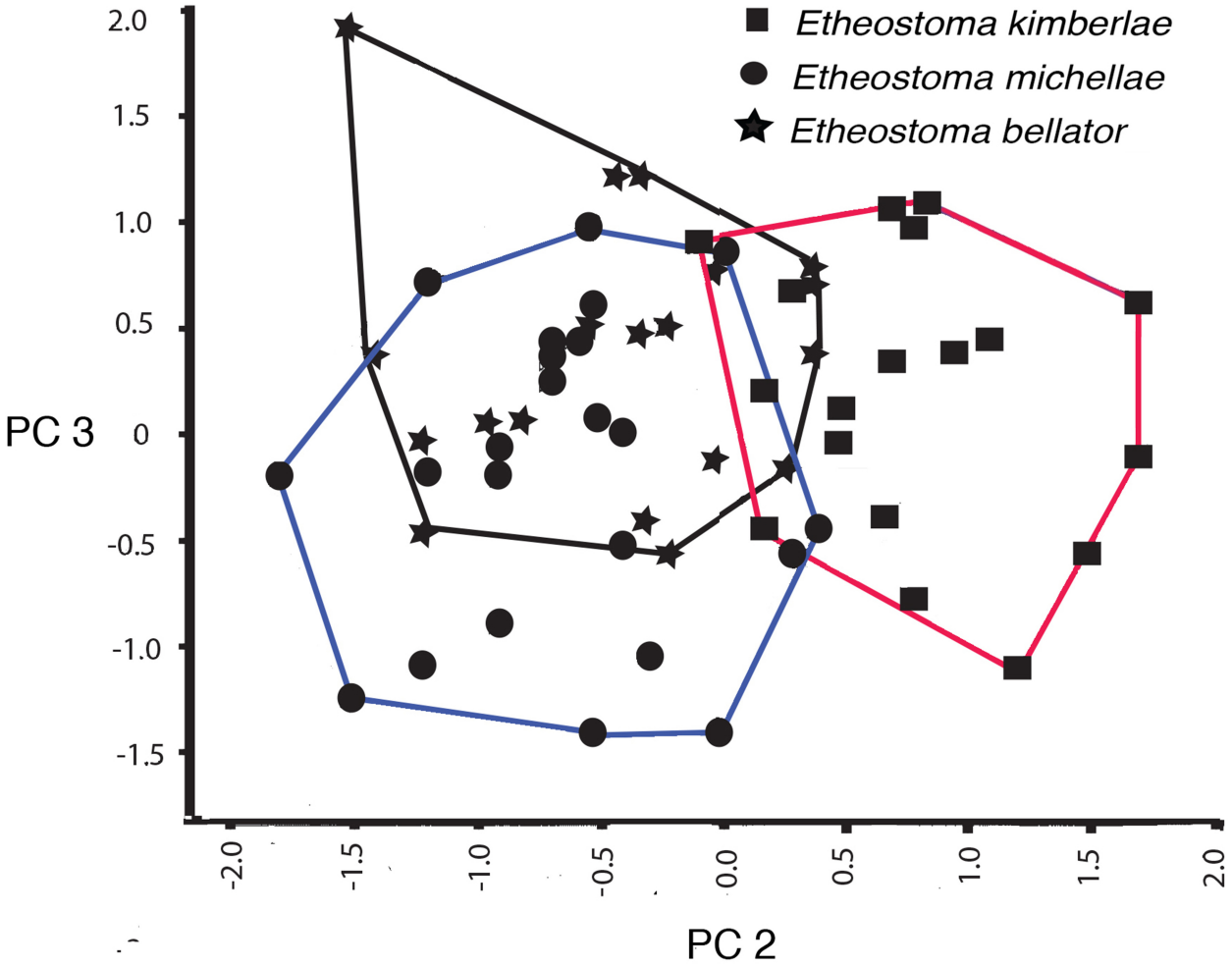

FIGURE 7. Principle Component Analysis of male specimens and their morphometrics for *Etheostoma bellator*, *E. kimberlae*, and *E. michellae*.

to the upper Locust Fork and extreme upper Locust Fork proper (C) (Osborne *et al.* 1988, Shepard *et al.* 2004); the Gurley Creek population of *E. bellator* is found on the divide between the Murphree Valley and Warrior Basin districts whereas *E. kimberlae* is restricted to the geologic formations associated with Murphrees Valley district (Figure 6). The Sequatchie Valley physiographic district is a narrow band of geology onto the Cumberland Plateau that bisects eastern tributaries to Mulberry Fork (D). Geologies associated with this district are typical of the Highland Rim physiographic province, including Bangor Limestone and the Tuscumbia Limestone and Fort Payne Chert undifferentiated formation (Osborne *et al.* 1988, Szabo *et al.* 1988). Almost all of the occurrences of *E. bellator* in the Mulberry Fork system occur within the Sequatchie Valley district (Figure 2). Upper Sipsey Fork and its tributaries (exclusive of Cane Creek and Hubbard Creek) flow over unique geologic formations for the Warrior Basin physiographic district, Bangor Limestone and the Parkwood Formation (E) (Szabo *et al.* 1988), and most occurrences of *E. michellae* are on these geologies (Figure 2).

Other narrow endemic upland darters in the Black Warrior River drainage follow a similar pattern. Locust Fork populations of *E. phytophilum* (Rush Darter) and all four native populations of *E. nuchale* (Watercress Darter) are restricted to formations dominated by Chepultepec and Copper Ridge Dolomites undifferentiated, either just east of the boundary of the Cumberland Plateau and Valley and Ridge physiographic provinces (*E. nuchale* and Turkey Creek populations of *E. phytophilum*) or in the Murphrees Valley district bisecting eastern tributaries to the upper Locust Fork (*E. phytophilum* Little Cove and Bristow creeks) (Figure 6, C) (Bart & Taylor 1999, Fluker *et al.* 2010, Fluker 2011, Cook *et al.* 2014). A population of *E. phytophilum* is also present in a tributary to the Sipsey Fork (Clear Creek), but this is within the Pottsville Formation lower part (B), downstream of *E. michellae* and the unique geologic formations for the Warrior Basin physiographic district (Bangor Limestone and the Parkwood Formation)

(Bart & Taylor 1999, Fluker 2011). The colonization of these areas by ancestors of *E. nuchale* and *E. phytophilum* are hypothesized to have occurred as multiple complex upland dispersal from specific populations of more widespread Coastal Plain species (*E. swaini* and *E. parvipinne*, respectively) (Fluker *et al.* 2010, Fluker 2011). Another narrow endemic upland darter in the Black Warrior River drainage, *E. cyanoprosopum*, is in Hubbard Creek of the Sipsey Fork and in Bear Creek of the Tennessee River drainage (Fluker *et al.* 2019). Both populations are restricted to the Pottsville Formation lower part (B), and Hubbard Creek individuals have never been found sympatric with *E. michellae* in Thompson Creek and upper Sipsey Fork (Bangor Limestone and the Parkwood Formation (E)) even though there are no known barriers to dispersal; both species are restricted to these unique geologies.

Allozyme variation indicated the *Etheostoma chermocki* species group was not monophyletic, with other snubnose darters *E. ramseyi* and *E. simoterum* (now *E. occidentale*) more closely related to other members of the *E. chermocki* species group than *E. michellae* (Clabaugh *et al.* 1996). Based on mitochondrial and nuclear gene sequences, the *E. chermocki* species group is monophyletic and is in a clade with two Coastal Plain species of snubnose darters, *E. lachneri* restricted to the Tombigbee River drainage and *E. colorosum* found in Gulf of Mexico drainages east of the Mobile Basin (Near *et al.* 2011). Neither of these studies are appropriate for examining the origin of the *E. chermocki* species group because they either lack the necessary intraspecific population sampling or species sampling of other snubnose darters (Clabaugh *et al.* 1996, Near *et al.* 2011).

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Literature Cited

ADCNR [Alabama Department of Conservation and Natural Resources] (2015) *Alabama Wildlife action plan, draft.* Alabama Department of Conservation and Natural Resources, Montgomery, Alabama, 503 pp.

ALNHP [Alabama Natural Heritage Program] (2015) *Alabama inventory list: rare, threatened and endangered plants & animals of Alabama*. Alabama Natural Heritage Program, Auburn, Alabama, 100 pp.

Bailey, R.M. & Etnier, D.A. (1988) Comments on the subgenera of darters (Percidae) with descriptions of two new species of *Etheostoma* (*Ulocentra*) from Southeastern United States. *Miscellaneous Publications Museum Zoology, University of Michigan*, 175, 1–48.

Bailey, R.M. & Bart, H.L. (1994) Three new species of *Etheostoma*, subgenus *Ulocentra*, from the gulf coastal plain of southeastern United States. *Tulane Studies in Zoology and Botany*, 29, 97–126.

Bart, H.L. Jr. & Taylor, M.S. (1999) Systematic review of subgenus *Fuscatelum* of *Etheostoma* with description of a new species from the upper Black Warrior River system, Alabama. *Tulane Studies in Zoology and Botany*, 31, 23–50.

Boschung, H.T. Jr. & Mayden, R.L. (2004) *Fishes of Alabama*. Smithsonian Press, Washington, D.C., 736 pp., 112 color pls.

Boschung, H.T., Mayden, R.L. & Tomelleri, J.R. (1992) *Etheostoma chermocki*, a new species of darter (Teleostei: Percidae) from the Black Warrior River drainage of Alabama. *Bulletin Alabama Museum of Natural History*, 13, 11–20.

Braasch, M.E. & Page, L.M. (1979) Systematic studies of darters of the subgenus *Catonotus* (Percidae), with the description of a new species from Caney Fork. *Occasional Papers of the Museum of Natural History of the University of Kansas*, 78, 1–10.

Braasch, M.E. & Mayden, R.L. (1985) Review of the subgenus *Catonotus* (Percidae), with descriptions of two new species of the *Etheostoma squamiceps* species group. *Occasional Papers of the Museum of Natural History of the University of Kansas*, No. 119, 1–83.

Clabaugh, J., Knott, E.K., Wood, R.M. & Mayden, R.L. (1996) Systematics and biogeography of snubnose darters, genus *Etheostoma* (Teleostei: Percidae) from the Black Warrior River system, Alabama. *Biochemical Systematics and Ecology*, 24 (2), 119–134.
https://doi.org/10.1016/0305-1978(95)00110-7

Cook, M.R., Murgulet, D. & Rogers, A.L. (2014) Hydrogeologic characterization of Thomas Spring, Jefferson County, Alabama. *Geological Survey of Alabama open file report*, 1403, 1–21.

Fluker, B.L. (2011) *Spring-adapted darters (Percidae: Etheostoma) as a model to understand factors that influence diversification, gene flow, and genetic variation in freshwater fishes*. *Dissertation*. University of Alabama, Alabama, 204 pp. [unpublished]

Fluker, B.L., Jones, K.D. & Kuhajda, B.R. (2019) Genetic structure and diversity of the blueface darter *Etheostoma cyanoprosopum* a microendemic freshwater fish in the southeastern USA. *Endangered Species Research*, 40, 133–147. https://doi.org/10.3354/esr00986

Fluker, B.L., Kuhajda, B.R., Lang, N.J. & Harris, P.M. (2010) Low genetic diversity and small long-term population sizes in the spring endemic Watercress Darter, *Etheostoma nuchale*. *Conservation Genetics*, 11, 2267–2279. https://doi.org/10.1007/s10592-010-0111-y

Hubbs, C.L. & Lagler, K.F. (1964) *Fishes of the Great Lakes Region.* The University of Michigan, Ann Arbor, Michigan, 213 pp.

Jelks, H.L., Walsh, S.J., Burkhead, N.M., Contreras-Balderas, S., Diaz-Pardo, E., Hendrickson, D.A., Lyons, J., Mandrak, N.E., McCormick, F., Nelson, J.S., Platania, S.P., Porter, B.A., Renaud, C.B., Smitter-Soto, J.J., Taylor, E.B. & Warren, M.J. (2008) Conservation status of imperiled North American freshwater and diadromous fishes. *Fisheries*, 33 (8), 372–407. https://doi.org/10.1577/1548-8446-33.8.372

Kim, D., Stokes, M.F., Ebersole, S. & Near, T.J. (2023) Erosional exhumation of carbonate rock facilitates dispersal-mediated allopatric speciation in freshwater fishes. *Evolution*, 77 (11), 2442–2455. https://doi.org/10.1093/evolut/qpad156

Kuhajda, B.R. (2004a) Locust Fork Darter *Etheostoma* sp. cf. *bellator*. *In*: Mirarchi, R.E., Garner, J.T., Mettee, M.F. & O'Neil, P.E. (Eds.), *Alabama Wildlife. Volume 2. Imperiled Aquatic Mollusks and Fishes*. The University of Alabama Press, Tuscaloosa, Alabama, pp. 225.

Kuhajda, B.R. (2004b) Sipsey Darter *Etheostoma* sp. cf. *bellator*. *In*: Mirarchi, R.E., Garner, J.T., Mettee, M.F. & O'Neil, P.E. (Eds.), *Alabama Wildlife. Vol. 2. Imperiled Aquatic Mollusks and Fishes*. The University of Alabama Press, Tuscaloosa, Alabama, pp. 226.

Layman, S.R. & Mayden, R.L. (2012) Morphological diversity and phylogenetics of the Subgenus *Doration* (Percidae: *Etheostoma*), with descriptions of five new species. *Bulletin Alabama Museum of Natural History*, 30, 1–75.

Mayden, R.L. (1985) Nuptial structures in the subgenus *Catonotus*, genus *Etheostoma* (Percidae). *Copeia*, 1985 (3), 580–583. https://doi.org/10.2307/1444747

Mayden, R.L. (2010) Morphological and molecular systematics of the *Etheostoma punctulatum* species group (Teleostei: Percidae), with descriptions of new species. *Copeia*, 2010 (4), 716–734. https://doi.org/10.1643/CG-10-056

Near, T.J., Bossu, C.M., Bradburd, G.S., Carlson, R.L., Harrington, R.C., Hollingsworth, P.R., Keck, B.K. & Etnier, D.A. (2011) Phylogeny and Temporal Diversification of Darters (Percidae: Etheostomatinae). *Systematic Biology*, 60 (5), 565–595. https://doi.org/10.1093/sysbio/syr052

Osborne, W.E., Szabo, M.W., Neathery, T.L. & Copeland, Jr. C.W. (1988) Geologic map of Alabama, northeast sheet. *In*: *Geological Survey of Alabama Special Map 220.* Geological Survey of Alabama, Tuscaloosa, Alabama, sheet 4. [map]

Page, L.M. (1983) *Handbook of darters*. Tropical Fish Hobbiest Publications, Inc., Harrisburg, Pennsylvania, 272 pp.

Page, L.M. & Braasch, M.E. (1976) Systematic studies of darters of the subgenus *Catonotus* (Percidae), with the description of a new species from the lower Cumberland and Tennessee River systems. *Occasional Papers of the Museum of Natural History of the University of Kansas*, 60, 1–18.

Page, L.M. & Braasch, M.E. (1977) Systematic studies of darters of the subgenus *Catonotus* (Percidae), with the description of a new species from the Duck River system. *Occasional Papers of the Museum of Natural History of the University of Kansas*, 63, 1–18.

Page, L.M., Hardman, M. & Near, T.J. (2003) Phylogenetic Relationships of Barcheek Darters (Percidae: *Etheostoma*, Subgenus *Catonotus*) with Descriptions of Two New Species. *Copeia*, 2003, 512–530. https://doi.org/10.1643/CI-02-259R

Powers, S.L., Jones, G.L., Redinger, P. & Mayden, R.L. (2003) Habitat associations with upland stream fish assemblages in Bankhead National Forest, Alabama. *Southeastern Naturalist*, 2, 85–92. https://doi.org/10.1656/1528-7092(2003)002[0085:HAWUSF]2.0.CO;2

Powers, S.L. & Mayden, R.L. (2003) *Etheostoma cervus*, a new species from the Forked Deer River System in Western Tennessee with comparison to *Etheostoma pyrrhogaster* (Percidae: subgenus *Ulocentra*). *Copeia*, 2003 (3), 576–582. https://doi.org/10.1643/CI-02-121R2

Powers, S.L. & Mayden, R.L. (2007) Evolution, systematics and biogeography of the *Etheostoma simoterum* species complex (Percidae: subgenus *Ulocentra*). *Bulletin of the Alabama Museum of Natural History*, 25, 1–23.

Shepard, T.E., O'Neil, P.E., McGregor, S.W. & Mettee, M.F. (2004) *Biomonitoring in the Locust Fork watershed, Alabama, 1997–98. Geological Survey of Alabama Bulletin. Vol. 175.* Geological Survey of Alabama, Tuscaloosa, Alabama, 61 pp.

Suttkus R.D. & Bailey, RM. (1993) *Etheostoma colorosum* and *E. bellator*, two new darters, subgenus *Ulocentra*, from southeastern United States. *Tulane Studies in Zoology and Botany*, 29, 1–28.

Suttkus, R.D. & Etnier, D.A. (1991) *Etheostoma tallapoosae* and *E. brevirostrum*, two new darters, subgenus *Ulocentra*, from the Alabama River Drainage. *Tulane Studies in Zoology and Botany*, 28, 97–126.

Szabo, M.W., Osborne, W.E. & Copeland, C.W. Jr. (1988) Geologic map of Alabama, northwest sheet. *In*: *Geological Survey of Alabama Special Map 220.* Geological Survey of Alabama, Tuscaloosa, Alabama, sheet 5. [map]