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Incipient speciation in allopatric *Etheostoma rupestre* (Percidae: Etheostomatinae) lineages, with the description of three new subspecies

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Abstract

In recent years, new species descriptions for the North American darters have proliferated. Most species concepts accepted by contemporary ichthyologists require that a valid species be both monophyletic and diagnoseable, yet many lineages exhibit modal or range differences in morphological characteristics without individuals being diagnosable. Such scenarios present difficulties with regards to proper taxonomic recognition of divergent lineages and often prohibit appropriate conservation action. Following the example of recent authors, we provide meristic, geometric morphometric, and pigmentation data to support the recognition of three subspecies of *Etheostoma rupestre*, a species endemic to the Mobile Basin. These morphological data cohere with previous genetic work for *E. rupestre*. The nominate subspecies *Etheostoma rupetsre rupestre* (Tsai's Rock Darter) is endemic to the Tombigbee River and Black Warrior River watersheds in Alabama and Mississippi and is characterized by having lower numbers of lateral blotches, lower range and mean of lateral line scales, lower modal number of scales above the lateral line, and lower degrees of nape squamation than other subspecies. *Etheostoma rupestre piersoni* (Shamrock Darter), **ssp. nov.**, is endemic to the Cahaba and Alabama River Watersheds in Alabama and is characterized by intermediate counts of lateral blotches and higher scale counts and nape squamation than *E. r. rupestre*. *Etheostoma rupestre uphapeense* (Jade Darter), **ssp. nov.**, is restricted to several small, disjunct populations in the Coosa and Tallapoosa watersheds in Alabama, Georgia, and Tennessee. *Etheostoma r. uphapeense* is characterized by having a higher mean number of lateral blotches than both other subspecies and higher scale counts than *E. r. rupestre*. While *E. r. rupestre* and *E. r. piersoni* are widespread and abundant within their respective ranges, *E. r. uphapeense* has a disjunct range and is often uncommon where it occurs. *Etheostoma r. uphapeense* should be monitored where it occurs to discern population trends.

Key words: species concepts, Mobile Basin, endemism, Rock Darter, intraspecific diversity

Introduction

The North American darters (subfamily Etheostomatinae) is a taxa-rich (~240 spp.) group of percid fishes for which new species descriptions have abounded in recent years (Williams *et al.* 2007; Blanton & Jenkins 2008; Near *et al.* 2011; Kozal *et al.* 2017; Near *et al.* 2017; Near *et al.* 2021). Species descriptions for this group of fishes are usually informed by phylogenetic analyses but often still depend on diagnoseable morphological traits (Keck & Near 2013; Near & Thomas 2015; Kozal *et al.* 2017; Piller & Bart 2017; Near *et al.* 2021). While many unique genetic lineages of fishes possess modal or mean differences in pigmentation, meristic, or morphometric characteristics, there is considerable overlap of these traits among lineages that renders any given individual from these populations morphologically undiagnosable (Craig *et al.* 2017; Sterling & Warren 2020; Moyle *et al.* 2023). Controversy often arises when attempting to decide how to appropriately acknowledge divergent lineages of morphologically undiagnosable forms. Recently, some ichthyologists have embraced species concepts that view species as independently evolving lineages that may not necessarily display diagnosable phenotypes (Egge & Simons 2006; Sterling & Warren 2020), while others have considered distinct lineages lacking diagnosability to represent

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subspecies (Craig *et al*. 2017; Moyle *et al.* 2023). While the methods used to identify species and intraspecific units of diversity are the same, the investigator's philosophical view on the nature of a species will ultimately influence the taxonomic rank they assign to a given lineage.

Etheostoma rupestre Gilbert & Swain was described in Gilbert (1887) from specimens collected from the North River, a tributary of the Black Warrior River. *Etheostoma rupestre* is endemic to the Mobile Basin, both above and below the Fall Line, with a disjunct distribution that includes several isolated populations. Tsai (1968) documented meristic differences across the range of *E. rupestre*, including scale counts, vertebrae, dorsal-fin elements, and squamation of the nape and belly. Tsai recognized two races based on differences in meristics and degree of squamation—the Alabama and Tombigbee River races. These populations were not assigned specific or subspecific epithets. Tsai interpreted the North River population as intergrades of the Alabama and Tombigbee races. Near *et al*. (2011) found genetic evidence of two undescribed taxa (three total lineages) in *E. rupestre* (an eastern clade and a central clade). Janosik *et al*. (2023) also found evidence of three lineages in *Etheostoma rupestre*. Two lineages (an Alabama River clade and Coosa/Tallapoosa River clade) exhibited monophyly with the Cytochrome-*b* gene (Fig. 1). One other population was identified from the Tombigbee River and the Sipsey/Black Warrior Rivers but did not exhibit monophyly, possibly due to incomplete lineage sorting, ancestral introgression, or a zone of intergradation between two lineages. *Etheostoma rupestre* appears to exhibit noticeable differences in pigmentation across its disjunct distribution in the Mobile drainage (C. Johnston & A. Janosik, pers. obs.), yet no study has yet examined whether these different color patterns are indicative of distinct lineages.

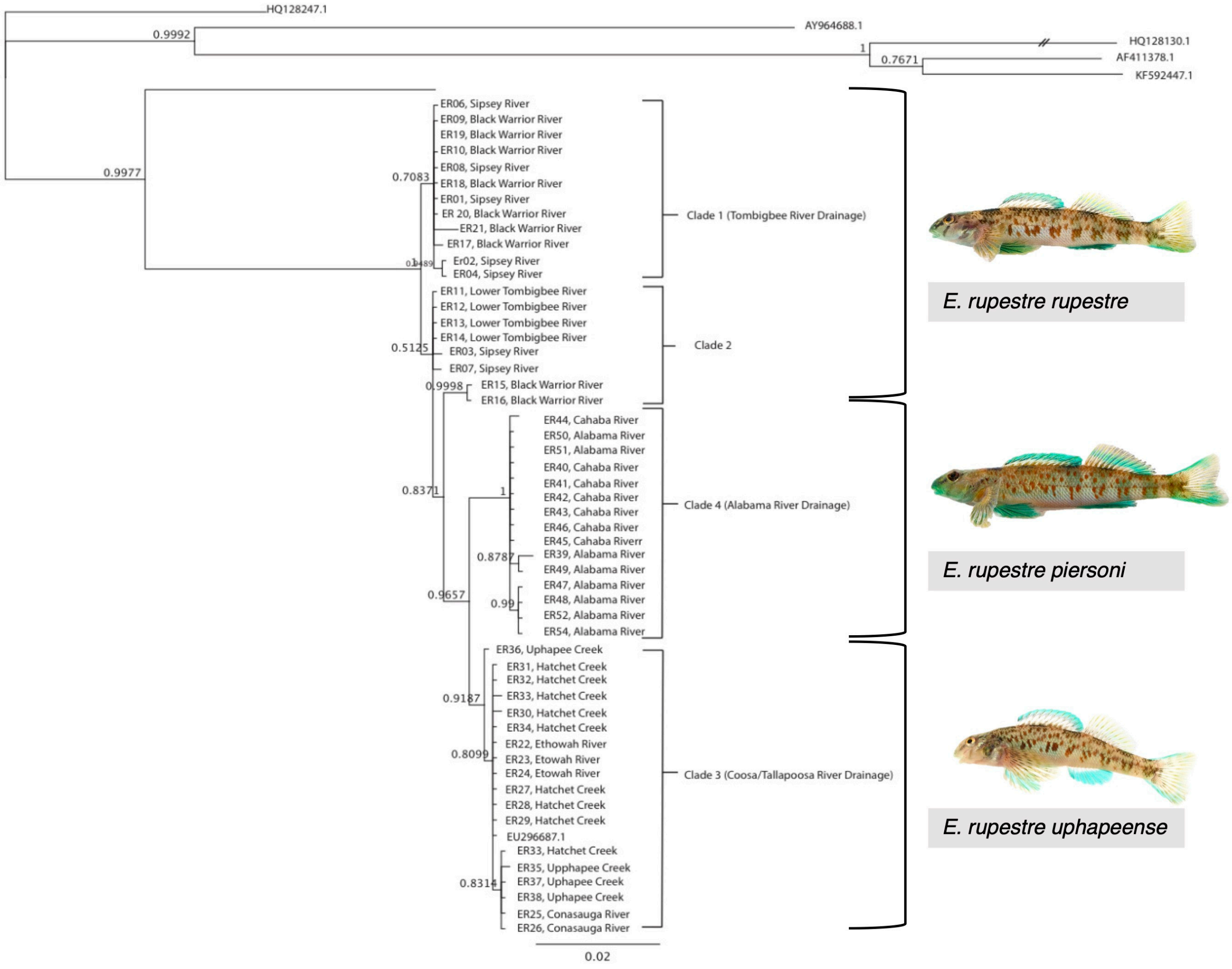

FIGURE 1. Cytochrome-*b* phylogeny (modified from Janosik *et al.* [2023] with permission from Springer Nature Publishing Company; subject to associated copyright policy) displaying the three allopatric lineages of *Etheostoma rupestre*. Bayesian posterior probabilities are displayed on the nodes. EU296687.1 is a GenBank individual from Piller *et al.* (2008). Individuals that did not sort into the three major clades may indicate incomplete lineage sorting or recent gene exchange between lineages.

No further analysis of variation in the *Etheostoma rupestre* complex has been conducted. In this study, we present meristic, pigmentation, and geometric morphometric data to support the recognition of three subspecies of *E. rupestre* and discuss the importance of recognizing and describing intraspecific diversity.

Materials and Methods

Specimens of *Etheostoma rupestre* were obtained from museums and collected with a mesh seine and/or backpack electrofishing unit from across the Mobile Basin (Fig. 2). Institutional abbreviations follow Sabaj (2020).

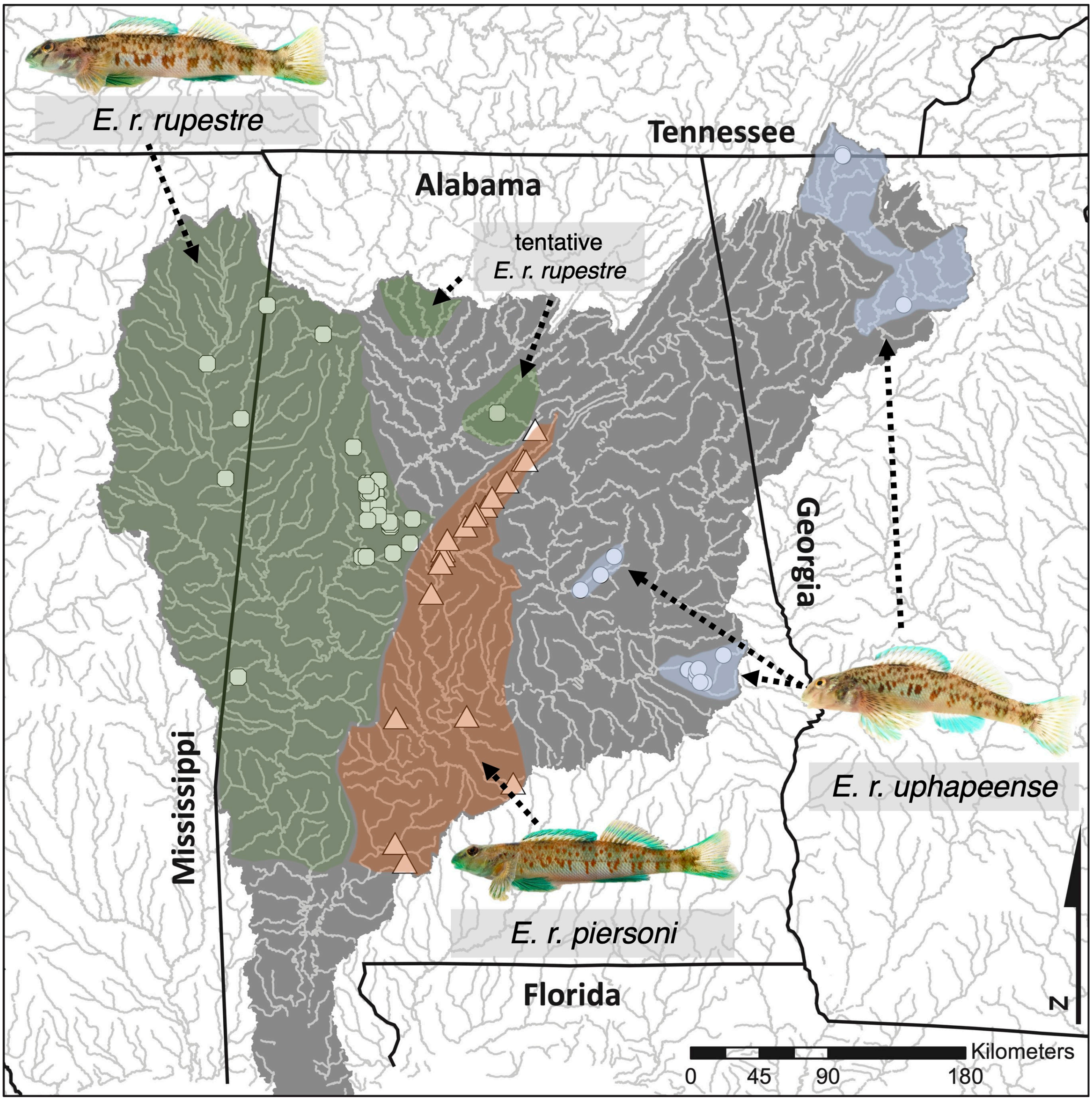

FIGURE 2. Locations of museum material examined that possessed lat/long coordinates. Dark grey background shading indicates the boundaries of the Mobile Basin and colored shading indicates geographic ranges of each subspecies within the Mobile Basin to which *Etheostoma rupestre* is endemic. Populations delimited as *E. r. rupestre* in the current study but for which some taxonomic uncertainty remains are also indicated.

Meristics. Meristic data was collected from 409 specimens of *Etheostoma rupestre* across its range according to the methods of Hubbs & Lagler (1958). Methods for determining nape and belly squamation followed those detailed in Tsai (1968) so our results would be directly comparable. Specifically, we measured 1) Number of lateral line scales, 2) Number of scale rows above the lateral line, 3) Number of scale rows below the lateral line, 4) Number of scales around the caudal peduncle, 5) Number of spines in the first dorsal fin, 6) Number of rays in the second dorsal fin, 7) Percent of nape scaled, and 8) Percent of belly scaled. Means for each meristic trait were calculated and reported for comparison. To further visualize meristic trends within *E. rupestre*, we ran a Principal Component Analysis (PCA) using the aforementioned scale counts and squamation data (percent squamation data was arcsine-square root transformed prior to analysis) and displayed the first two principal components on a biplot.

Geometric Morphometrics. Data on the relative position of 19 morphological landmarks were collected from 45 individuals of *Etheostoma rupestre* (Fig. 3). These analyses were restricted to males >40 mm SL to remove the effects of sex and allometry. We collected data from photos of preserved specimens that appeared straight to the naked eye. Morphological landmarks were digitized in TpsDig2 version 2.31. To remove the effect of specimen size and angle, a Procrustes superimposition was executed in in MorphoJ version 1.07a. Wireframe diagrams displaying the average body shape were then generated using the Procrustes coordinates obtained from the superimposition. We executed a PCA in MorphoJ to discern population-wide trends in body morphology among the three putative lineages.

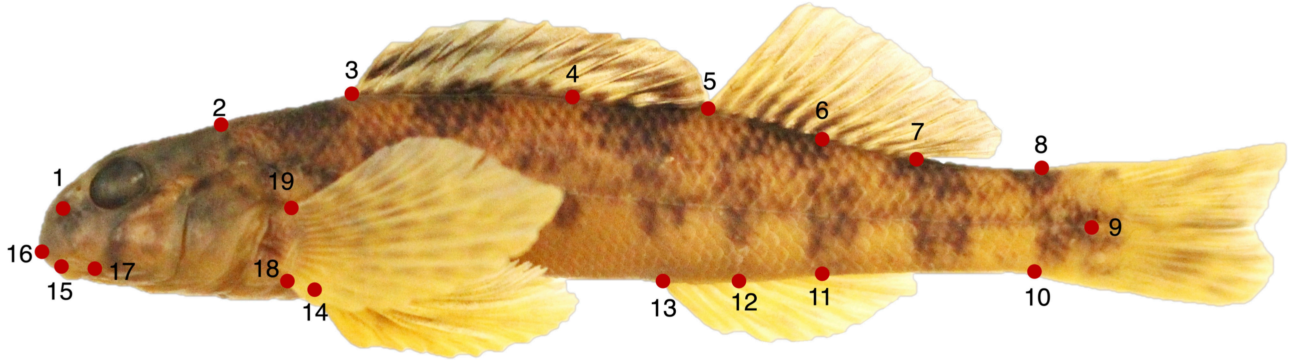

FIGURE 3. Locations of landmarks used in geometric morphometric analysis on specimens of *Etheostoma rupestre* (1: nare, 2: nape, 3: first dorsal-fin origin, 4: 9th dorsal spine insertion, 5: second dorsal-fin origin, 6: 7th dorsal ray insertion, 7: posterior insertion of dorsal fin, 8: caudal fin insertion on dorsum, 9: center edge of hypural plate, 10: caudal fin insertion on ventrum, 11: posterior insertion of anal fin, 12: 5th anal fin element, 13: anal fin origin, 14: pelvic fin origin, 15: tip of lower jaw, 16: tip of upper jaw, 17: posterior edge of maxilla, 18: lower pectoral fin insertion, 19: upper pectoral fin insertion.

Male Pigmentation. Pigmentation was examined from 67 male individuals of *Etheostoma rupestre* with representatives from each lineage. Individuals examined included both museum specimens with well-preserved pigmentation and photographs of live individuals in nuptial or pre-nuptial coloration. All pigmentation measurements were made with digital calipers and reported to the nearest 0.1 mm. We measured 1) Number of lateral blotches, 2) Blotch width, 3) Saddle width, 4) Distance between blotches, 5) Depth of distal band, and 6) Depth of total pigment on the spinous dorsal fins. Individual blotch widths, saddle widths, and distances between blotches were measured and averaged to obtain a single value for each fish. Blotch width, saddle width, and distance between blotches are expressed as percentage of standard length. Depth of distal band and total pigment in the spinous dorsal fin are expressed as percentage of dorsal-fin depth (taken along the 5th dorsal spine, 6th spine in one individual where the 5th was too damaged to measure).

Results and Taxonomy

Meristics. There was considerable differentiation in the number of lateral line scales, number of scales above the lateral line, number of scales around the caudal peduncle, nape squamation, and belly squamation among the three subspecies of *Etheostoma rupestre* (Tables 1–8). The Tombigbee/Black Warrior subspecies (*E. r. rupestre*) showed the greatest level of meristic divergence from the other two subspecies (*E. r. piersoni*, **n. ssp.** and *E. r. uphapeense,* **n. ssp.**). However, no meristic trait measured was able to completely separate subspecies. Additionally, each subspecies was characterized by its own morphological ranges and tendencies in multivariate PC-space, but there was considerable overlap among them (Fig. 4). The differences in these meristic traits among subspecies are discussed in detail in the taxonomic descriptions that follow.

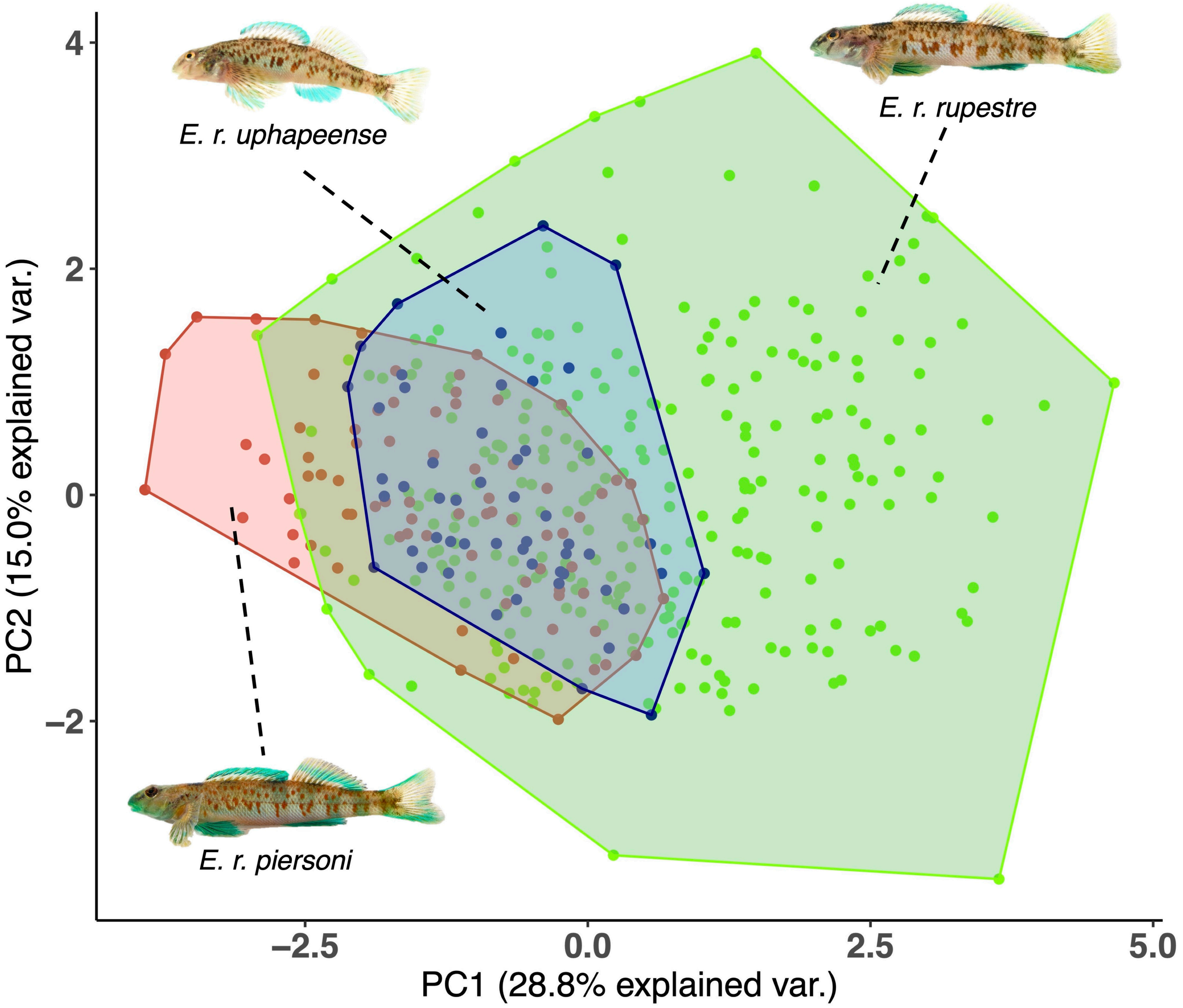

FIGURE 4. First and second principal components for meristic data for the three subspecies of *Etheostoma rupestre*. Variable loadings for each principle component are as follows: PC1—lateral line scales: -0.43, scales above lateral line: -0.47, scales below lateral line: -0.47, caudal peduncle scales: -0.44, dorsal spines: -0.21, dorsal rays: -0.05, nape squamation: 0.21, belly squamation: 0.30; PC2—lateral line scales: 0.12, scales above the lateral line: 0.20, scales below the lateral line: 0.14, caudal peduncle scales: 0.14, dorsal spines: 0.21, dorsal rays: 0.19, nape squamation: 0.71, belly squamation: 0.58.

Geometric Morphometrics. The geometric morphometric analysis generated similar wire-frame plots for each putative subspecies and showed a great degree of overlap among each of the three subspecies in PC-space, but nevertheless showed a break between the Western subspecies (*Etheostoma rupestre rupestre*) and the Central + Eastern subspecies (*Etheostoma rupestre piersoni*, **n. ssp.** + *Etheostoma rupestre uphapeense,* **n. ssp.**) (Fig. 5, Fig. 6). The first principal component explained 24.5% of the variation in body shape and the second principal component explained 18.7% of the variation. *Etheostoma rupestre* from the Western subspecies were the most divergent in PC space and appeared to be characterized by having more ventrally flattened/decurved body profiles.

Pigmentation. Blotch shape, size, and spacing often differed even within the same population (Fig. 7). There were considerable differences in pigmentation among subspecies, particularly with regards to the number of lateral blotches. While each subspecies had modally seven blotches, the means and ranges differed trenchantly, with fish from the Eastern subspecies (*E. r. uphapeense*) having the greatest mean number of lateral blotches (7.7), the Central subspecies (*E. r. piersoni*) having an intermediate number of lateral blotches (mean = 7.0), and the Western subspecies (*E. r. rupestre*) having the fewest (mean = 6.4). Pigmentation differences are summarized in Tables 9–11 and are detailed in the following descriptions.

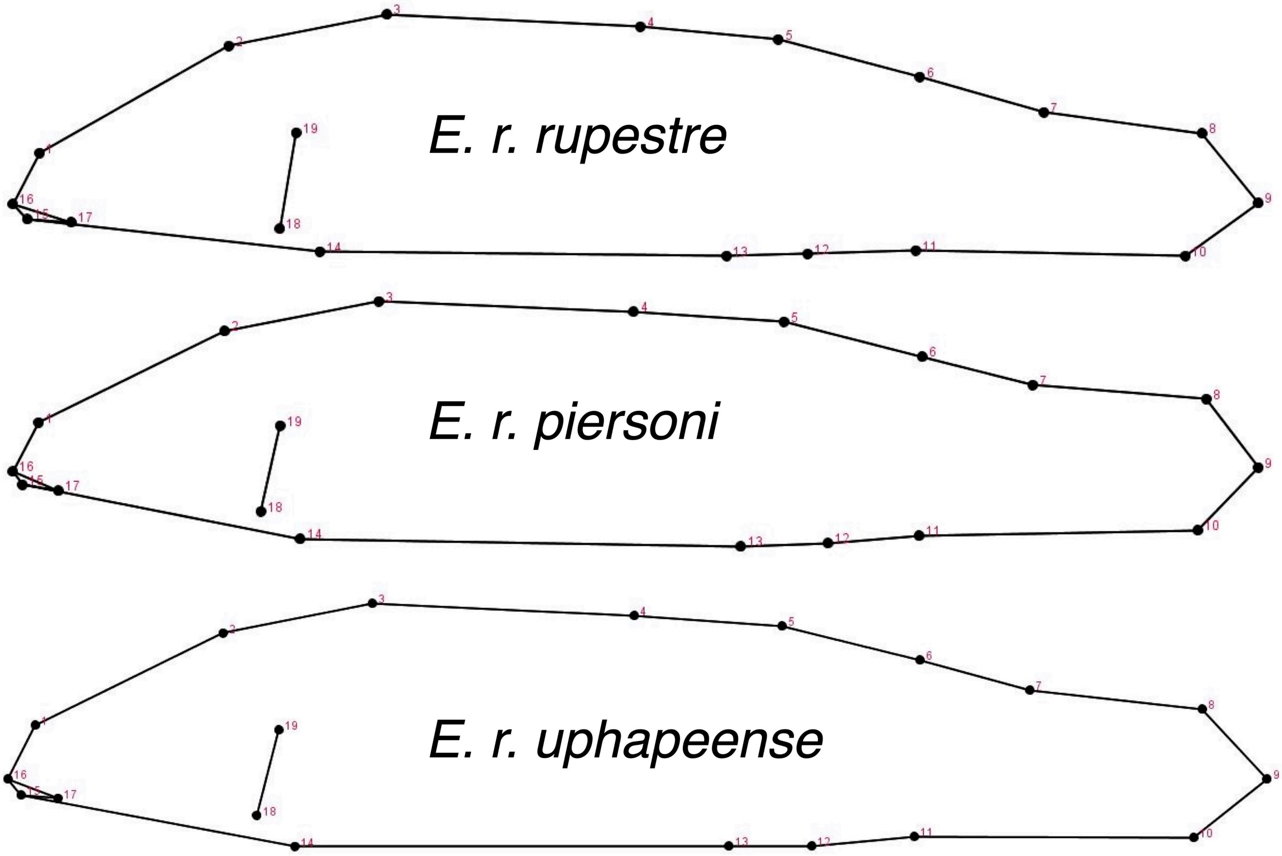

FIGURE 5. Wireframe diagrams for *E. r. rupestre*, *E. r. piersoni*, and *E. r. uphapeense*.

Etheostoma rupestre **Gilbert and Swain 1887**
Rock Darter
Figure 8

Etheostoma **(*Rhothoeca*)** ***rupestre*** **Gilbert and Swain *in* Gilbert 1887:57.** Type locality: North River, tributary of Black Warrior River, near Tuscaloosa, Alabama.

Lectotype. North River. Alabama. USNM 36695, 32 mm SL, North River, Tuscaloosa, Alabama.

Paralectotypes. USNM 188971, (17, 22–33).

Etheostoma rupestre was described from the North River by Gilbert and Swain (Gilbert 1887). Additional diagnostic and descriptive characteristics are provided in Tsai (1968) and Boschung & Mayden (2004). The following diagnosis and description are based on characteristics provided by these sources as well as our own measurements.

Diagnosis. A species of clade *Neoetheostoma* (*sensu* Near *et al*. 2011) That differs from all other *Etheostoma* by the following combination of characteristics: 3–9 distinct lateral blotches (blotch shape includes diffuse irregular lines, clusters of dots, square markings, grouped sets of 1–3 vertical lines, and/or *W*-shaped tessellations), six dorsal saddles, and offset rows of stippling on the pectoral fins. Male nuptial coloration green (tends towards blue in pre-spawn individuals), diffuse black medial band in first dorsal fin with a green distal band, thick green medial band in second dorsal fin, diffuse green bars on side that usually encompass lateral blotches, anal fin infused with green, pelvic fins green usually with a transparent margin, caudal fin with dorsal and ventral edges colored in green occasionally forming thick green margin on posterior of caudal fin, nuptial color often present on anterior portion of head on mouth bleeding into branchiostegal membranes. Closely related congeners include the *Etheostoma* *blennioides* (Rafinesque) species complex + *E. gutselli* (Hildebrand), *E. zonale* (Cope)*, E. lynceum* (Hay), and *E. histrio* Jordan and Gilbert. *Etheostoma rupestre* differs from species in the *E. blennioides* complex by lacking a sheathed premaxilla, red nuptial coloration, and often-interconnected rows of *U*-shaped markings on the side. It differs from *E. zonale* and *E. lynceum* by possessing large lateral blotches, lacking red nuptial coloration, and by possessing fewer and less distinct vertical nuptial bars. It differs from *E. histrio* (the only one of these species with which it occurs sympatrically) by possessing a series a black or burgundy irregular lateral blotches and by lacking mottling on the underside of the head and lacking red in fin nuptial coloration.

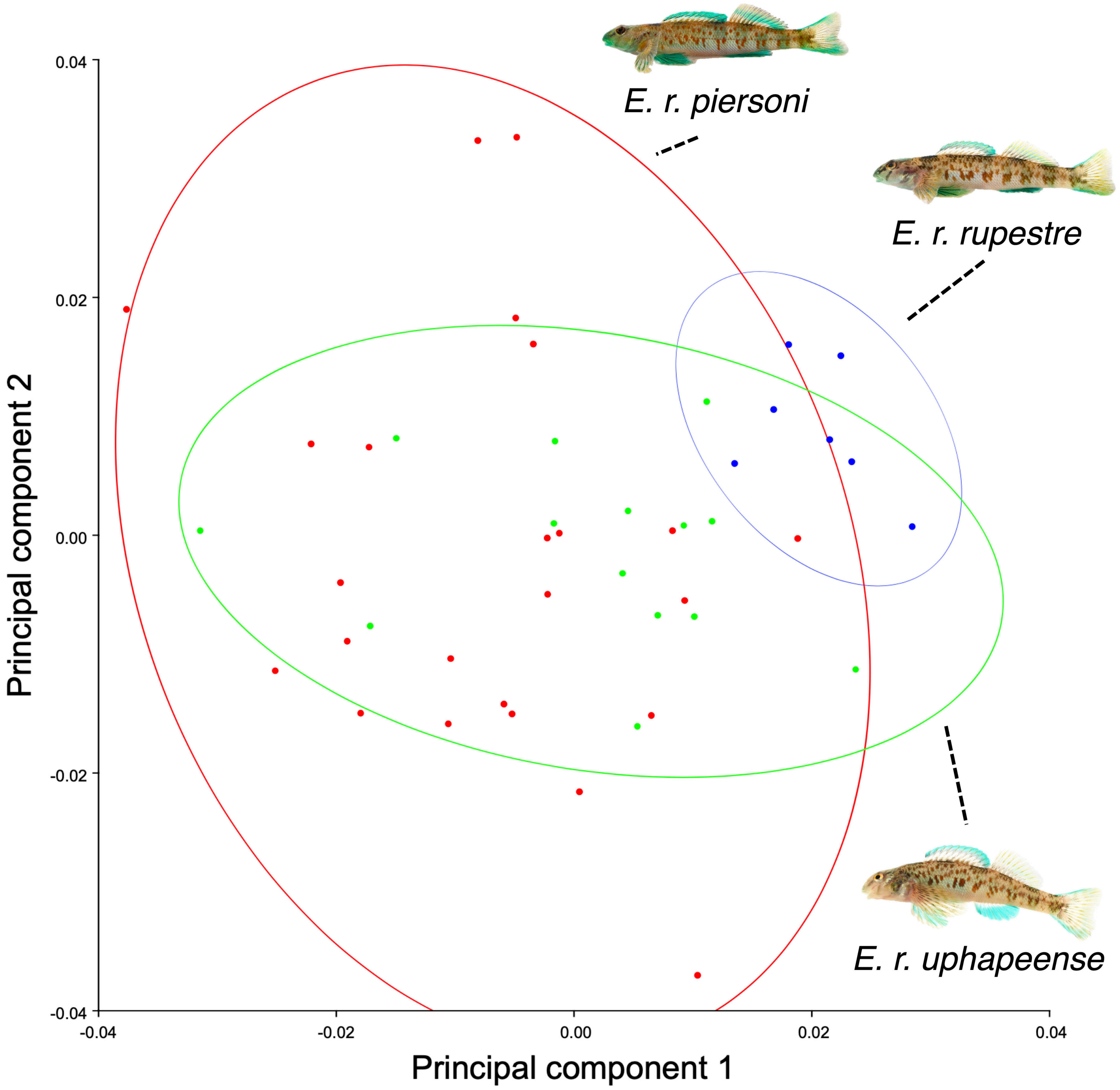

FIGURE 6. Biplot of first and second principal components from Procrustes coordinates generated from geometric morphometric analysis of *Etheostoma rupestre* subspecies (n = 45; PC1 = 24.5% variance explained, PC2 = 18.7% variance explained).

Description. A large species of *Etheostoma* that attains a maximum size of 71 mm SL. Body elongate and cylindrical, slightly compressed, anterior portion of head highly convex, distinct frenum present, eyes large, oval-shaped, and high-set. Paired fins large. Dorsum coloration brassy or olive, ventral surface light olive, 3–9 lateral blotches along the midline (variable in shape and degree of separation) that range 3.8–8.2% of SL in average width and 2.8–7.5% of SL in average spacing, six dorsal saddles (highly variable in width, 5.7–9.1% of SL), numerous small dorsolateral dots or tessellations, suborbital and lacrimal bars present. Male nuptial coloration dark green to turquoise, distal band in the first dorsal fin 21.4–51.1% of dorsal-fin depth, total pigment (distal + medial band) depth 49.2–78.0% of dorsal-fin depth. Female genital papilla long and tubular, flattened distally, male genital papilla short and pointed. Lateral scale rows 39–65 (45–64 >99% of the time), scales above the lateral line 4–7, scales below the lateral line 6–12, scales around the caudal peduncle 16–23, dorsal-fin spines IX–XII (>99% X–XII) rays 9–13, anal-fin spines 2, anal-fin rays 5–9, nape squamation naked to fully scaled (0–100%), belly squamation 10–90%, vertebrae 36–40.

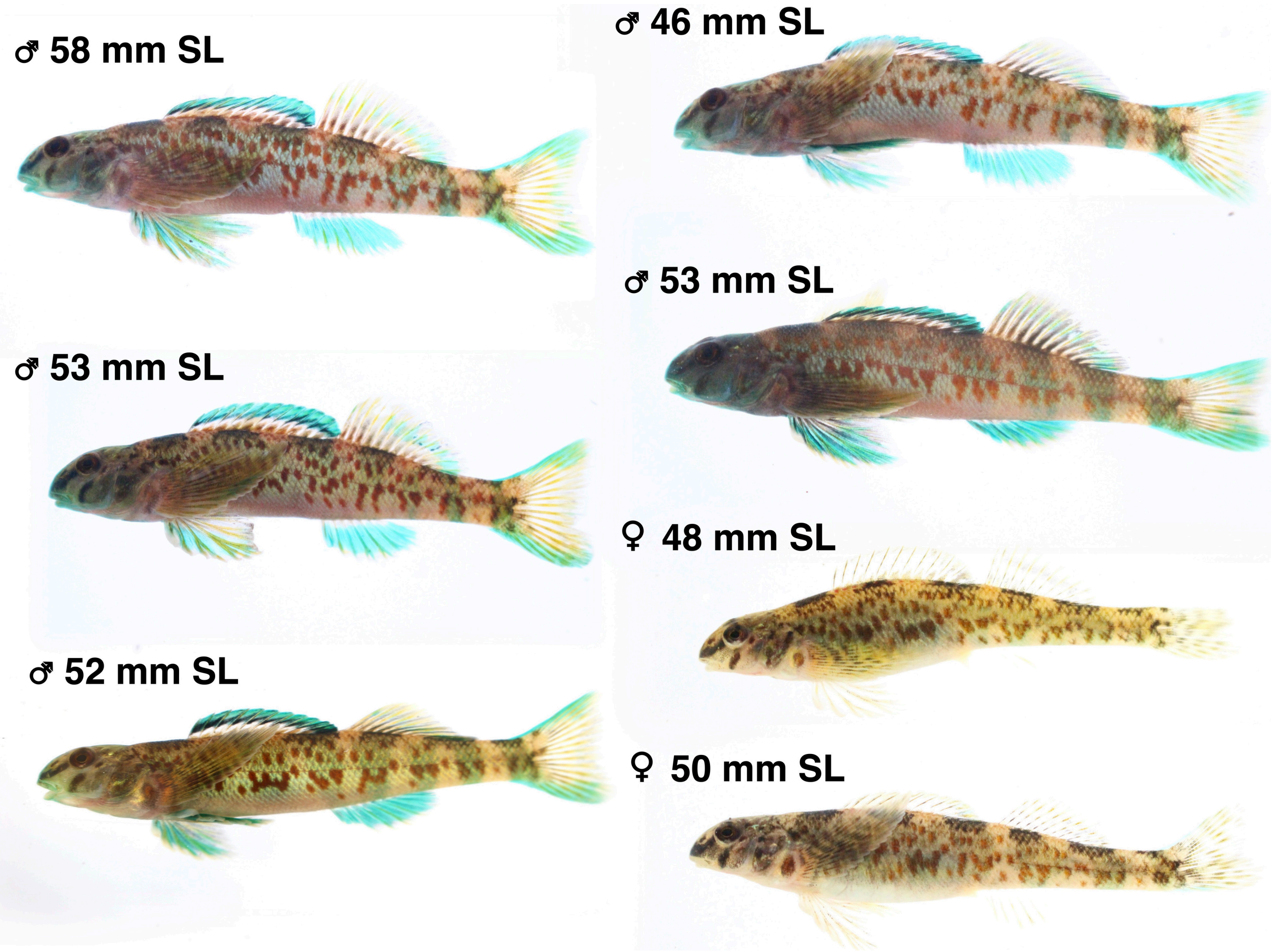

FIGURE 7. Example of variable lateral blotch pigmentation within a single population of *Etheostoma rupestre* (Caffee Creek, Cahaba River watershed).

Distribution. *Etheostoma rupestre* is endemic to the Mobile Basin in Mississippi, Alabama, Georgia, and Tennessee. In the Tombigbee River drainage, it occurs from ~40 air-km north of the Tombigbee-Alabama River confluence and extends north to the Black Warrior drainage and northeast Mississippi. In the Alabama and Cahaba River Basins it occurs from ~40 air-km north of the Tombigbee-Alabama River confluence north to the headwaters of the Cahaba River in Central Alabama. *E. rupestre* also occurs sporadically in several disjunct populations in the Coosa and Tallapoosa watersheds—namely in the Uphapee Creek and Hatchet Creek systems in east Alabama, and the Etowah, Talking Rock Creek (Coosawattee), and Conasauga systems in northwest Georgia and extreme southeastern Tennessee. The disjunct range and geographic variation in *E. rupestre* are discussed in the subspecies descriptions that follow.

Ecology. *Etheostoma rupestre* typically inhabits fast riffles and runs of large creeks and rivers above gravel and small cobble (Kuehne & Barbour 1983). Previous authors have speculated that the spawning season of *E. rupestre* lasts from mid-March to April or May (Joachim *et al.* 2003; Boschung & Mayden 2004). However, in lower Hatchet Creek male *E. rupestre* have been observed in peak nuptial coloration in mid-February (J. Dattilo, Alabama Division of Wildlife and Freshwater Fisheries, pers. comm.). Males in peak nuptial coloration and gravid females that appeared ready to spawn were collected from Caffee Creek (Cahaba drainage) on 22 March 2022.

Male *E. rupestre* collected from Opintlocco Creek (Uphapee system) on 17 May 2022 had nuptial coloration that had nearly completely receded. We assume that the spawning season of *E. rupestre* ranges from mid-February to early April based on our anecdotal observations. Fecundity ranges from 18–94 eggs/female (Joachim *et al.* 2003). The morphology of the female genital papilla suggests that *E. rupestre* is an egg-attacher—a strategy common to other *Neoetheostoma* darters (Page 1983). A life history study that incorporates populations from all subspecies is needed.

Etymology. From the Latin *rupestris* meaning living on rocks.

Etheostoma rupestre rupestre Gilbert and Swain 1887

Tsai's Rock Darter

Figure 8A, Tables 1–11

Holotype. USNM 36695, same as species lectotype.

Paratypes. USNM 188971 (17, 22–33), same as species paralectotypes.

Materials examined (nontypes).

Tombigbee River drainage: Alabama. Fayette Co., USNM 398674, (1, 56), Harris Creek, co. rd. 53, 33.7451, -87.7593, 8 March 2010, A. M. Janosik, C. E. Johnston and T. H. Haley; USNM 398675, (5, 42–52) mm SL, same locality, 8 March 2010, A. M. Janosik, C. E. Johnston and T. H. Haley. **Marion Co.**, AUM 49010, Buttahatchee River, downstream site of Buttahatchee Dam, .75 miles NNE of Pearces Mill (1, 45), 18 July 2007, 34.1261º, -87.8369º; AUM 49032, same locality, (7, 46–49), 14 July 2007, 34.1261º, -87.8369º. AUM 23708, McConegal Creek, tributary to Bull Mountain Creek, 8.9 km NNW of Bexar Co. Road 56 (3, 32–38), 12 July 1982, 34.2663°, -88.1667°. **Sumter Co.**, AUM 68120, Alamuchee Creek, at Hwy. 80, 3.8 km ENE of Cuba (13, 40–55), 11 November 2016, 32.4387º, -88.3376º. **Mississippi. Lowndes Co.**, AUM 20642, Tombigbee River, Buzzard Island 8.4 km S of Columbus (35, 30–40), 11 September 1979, 33.4200º, -88.4110º. AUM 3351, unnamed creek, small tributary to Buttahatchee River 8.0 km N of Caledonia on gravel road (2, 36–43), 02 October 1968, 33.7126º, -88.3200º. **Monroe Co.**, AUM 20489, Tombigbee River, 6.6 km W of Amory on Highway 278 (13, 29–41), 12 September 1980, 33.9820º, -88.5190º.

Black Warrior River and North River drainage: Alabama. Jefferson Co., UAIC 3324, Cunningham Creek, 1.6 km S of Morris (Locust Fork) U.S. Route 31 (3, 40–45), 17 August 1969, 33.7403º, -86.8119º. **Tuscaloosa Co.**, UAIC 676, Big Sandy Creek (1, 41), 07 February 1959, 33.0360º, -87.5870 º; UAIC 834, (10, 38–48), 16 February 1962, 33.0337º, -87.5821º; UAIC 409, Gulf States Paper Company Picnic Grounds (5, 36–47), 10 August 1954, 33.0333º, -87.6083º. UAIC 804, Big Sandy Creek and Big Sandy Spring (3, 34–42), 12 May 1961, 33.0337º, -87.5821º. UAIC 1218, Big Sandy Spring, 8.0 km SE of Coaling (1, 33), 20 February 1964, 33.1011º, -87.3261º; UAIC 1225, same locality (3, 34–44), 08 March 1964, 33.0337º, -87.5821º; UAIC 1445, same locality (3, 35–41), 12 August 1964, 33.0337º, -87.5821º. UAIC 1243, Carroll Creek, Highway 69, 16.1–24.1 km N of Tuscaloosa (North River) (4, 32–39), 16 April 1964, 33.29417º, -87.5697º; UAIC 3029, Highway 69, 6.4 km N of junction of Highway 69 and U.S. Highway 82 (North River) (1, 53), 11 August 1968, 33.2942º, -87.5697º. UAIC 929, Cottondale Creek, Keene's Mill Road (4, 31–46), 05 November 1962, 33.1828º, -87.4425º; UAIC 1792, 1.6 km N of Cottondale (3, 31–33), 04 December 1965, 33.2006º, -87.4462º. UAIC 1158.03, Hammer Creek, 6.4 km off Watermelon Road, 13.8 km N of Tuscaloosa (9, 31–48), 30 January 1963, 33.3389º, -87.5258º. UAIC 59, Hurricane Creek, 183 m downstream of confluence of North Fork (1, 45), 26 September 1950, 33.2203º, -87.3108º. UAIC 61, Lower Cottondale Creek (1, 36), 09 October 1956, 33.1947º, -87.4397º; UAIC 58, 0.8 km upstream of confluence of Hurricane Creek (1, 31), 05 July 1950, 33.2057º, -87.4467º. USNM 36695, North River, Tuscaloosa (1, 31), 1884, no lat/long. available; UAIC 1181, crosses Highway 69 17.7 km N of Tuscaloosa (30, 34–48), 23 September 1963, 33.3539º, -87.5572º; UAIC 1182, Watermelon Road, 11.3 km NE Tuscaloosa (10, 30–44), 01 October 1963, 33.2914º, -87.5128º; UAIC 1194.03, 3.2 km upstream of Highway 69, 12.9 km N of Northport (43, 28–47), 18 November 1964, 33.3461º, -87.5786º; UAIC 2696, 18.0 air km N of Northport (8, 33–44), 19 September 1967, 33.3964º, -87.5797º; UAIC 3026, unnamed dirt road 1.6 km W of Highway 69 at Macedonia Church (1, 28), 31 July 1968, 33.3528º, -87.5725º; UAIC 3522, 12.9 air km N of junction of U.S. Highway 82 and Highway 69 (2, 40–42), 25 April 1969, 33.3528º, -87.5725º. UAIC 2576, Rice Mine Creek, 3.2 km E of U.S. Highway 82 (3, 36–45), 21 January 1967, 33.2369º, -87.5125º. UAIC 1303, Swampy area, Springbranch on N side of U.S. Highway 82 and Big Sandy Creek at Duncanville (2, 35–35), 22 June 1964, 33.053º, -87.4267º. UAIC 1169, Turkey Creek, Highway 69,

TABLE 1. Number of lateral line scales for each of the three *E. rupestre* subspecies. Modes are listed in bold.

Species/Drainage	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	mean	*n*
E. r. rupestre																												
Black Warrior River	1	-	-	-	-	-	-	-	3	9	9	19	9	14	**17**	12	6	4	-	-	1	-	-	-	-	1	51.6	102
North River								1	2	3	10	10	16	14	**20**	18	14	7	5	2	1						52.6	123
Tombigbee River							1	4	5	6	**12**	6	11	4	1	1	2										49.5	53
E. r. piersoni																												
Cahaba River																3	2	5	7	2	3	**6**	-	1			57.5	29
Alabama River											1	9	5	7	2	6	**6**	2	5	1	1	2					53.4	47
E. r. uphapeense																												
Uphapee Creek											1	-	-	3	3	4	**6**	5	4	3	-	-	2	-	1		55.5	32
Hatchet Creek													1	1	1	2	**2**	**2**	-	-	1	1					55.0	11
Etowah River															**1**	-	-	-	**1**								55.0	2
Conasauga River																	**2**	-	-	-	1	-	**2**				58.2	5
																											Total	404

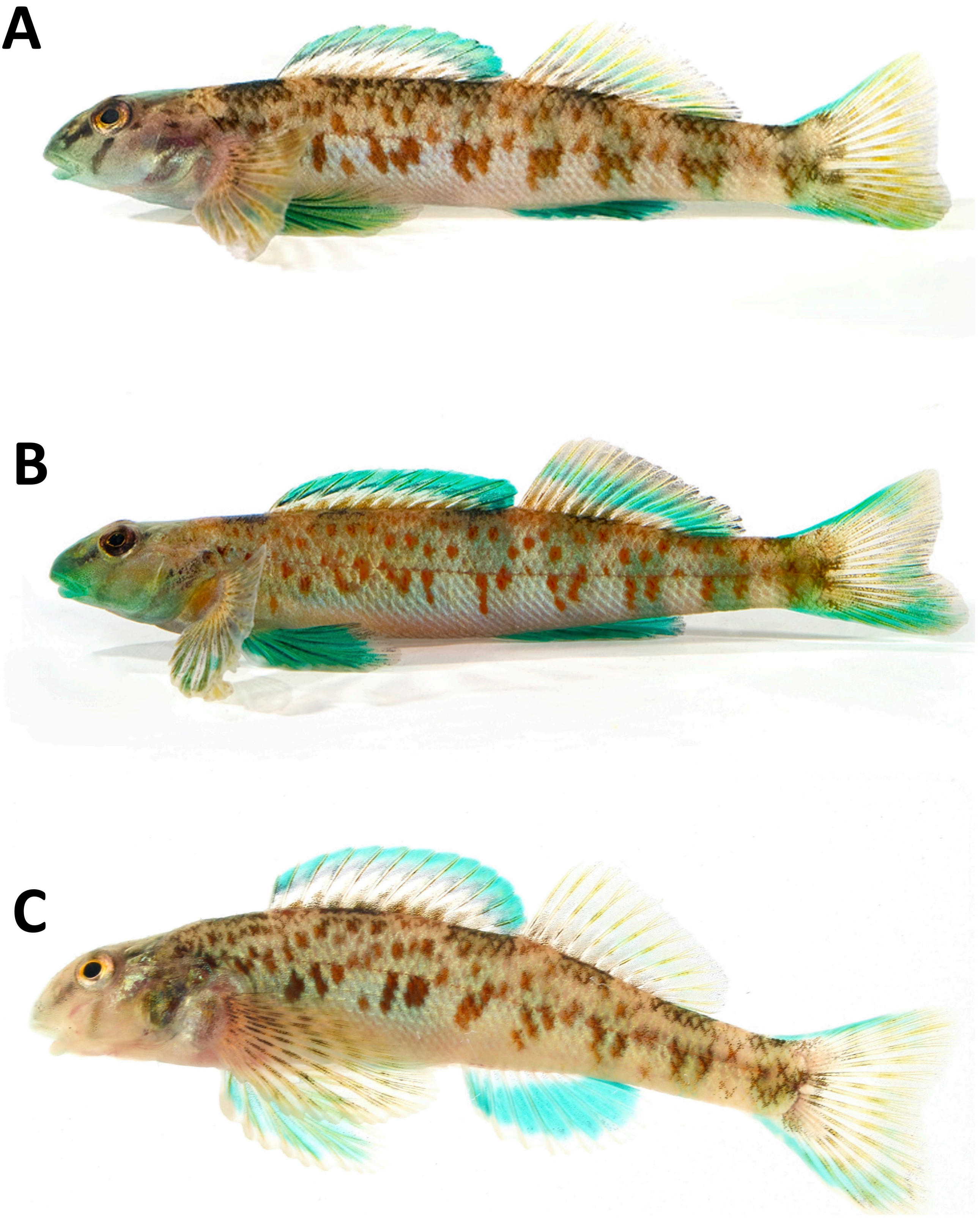

FIGURE 8. Male pigmentation of A) *Etheostoma rupestre rupestre*—47 mm SL, Cottondale Creek, County Road 32, Tuscaloosa, Alabama (photo by K. Boone), B) *E. r. piersoni*—61 mm SL, Chilatchee Creek, Highway 5, 1 mile north of Alberta, Alabama (photo by K. Boone), and C) *E. r. uphapeense*—45 mm SL, Opintlocco Creek, County Highway 20, ~3 miles East of Tuskegee, Alabama (Photo by B. Hilburn).

22.5 km N of Tuscaloosa (2, 32–32), 13 March 1963, 33.4097º, -87.5111º; UAIC 1172, 4.0 km W of Highway 69, 19.3 km N of Tuscaloosa (6, 30–37), 15 March 1963, 33.3969º, -87.5533º; UAIC 1170, N of Tuscaloosa on Highway 69 (1, 36), 16 June 1963, 33.4097º, -87.5111º; UAIC 3182, Highway 69 (1, 31), 16 January 1973, 33.4097º, -87.5111º; UAIC 5847.09, Highway 69 (3, 30–41), 19 November 1979, 33.4097º, -87.5111º; UAIC 5835.13, Highway 69 (2, 29–30), 24 October 1979, 33.4097º, -87.5111º; UAIC 5879.04, Highway 69 (1, 51), 18 December 1979, 33.4097º, -87.5111º; UAIC 5888.10, Highway 69 (3, 28–39), 29 January 1980, 33.3523 º, -87.5537 º; UAIC 5897.06, Highway 69 (5, 28–33), 21 February 1980, 33.4097, -87.5111; UAIC 7876.04, 91 m downstream of Highway 69 (bridge crossing) (1, 47), 11 July 1987, 33.41º, -87.5092º; USNM 188971, Tuscaloosa (10, 28–31), 1884, no lat/long. available. UAIC 1198, unnamed creek, at confluence of North River on Highway 69, 19.3 km N of Tuscaloosa (16, 31–46), 14 October 1965, 33.3556º, -87.5583º. UAIC 1150, unnamed creek, Highway 69 at bridge crossing North River 15.3 km N of Tuscaloosa (6, 37–50), 10 January 1963, 33.3539º, -87.5572º; UAIC 1151, bridge crossing North River off Highway 69, 15.3 km N of Tuscaloosa (5, 35–46), 08 March 1963, 33.3539º, -87.5572º. AUM 862, unnamed creek, tributary to North River 14.8 km N of Junction 69 (U.S. Highway 82 and Highway 69) (1, 44), 14 April 1968, 33.3840º, -87.5840º. USNM 166029, (12, 33–46), unnamed creek, tributary to North River, 11.3 km N of New Lexington on U.S. Highway 43, 07 April 1952, 33.5740º, -87.6581º. UAIC 1647, Black Warrior River, below dam at Tuscaloosa (1, 41), 29 June 1965, 33.2141º, -87.5756º.

Diagnosis. While individuals of *Etheostoma. r. rupestre* cannot be morphologically distinguished from other subspecies due to overlap in all traits measured, entire populations can be diagnosed by the following tendencies: populations of *E. r. rupestre* have modally five scales above the lateral line (as opposed to six in both *E. r. piersoni* and *E. r. uphapeense*) (Table 2), tend to have low numbers of lateral line scales (usually 45–59 vs. 49–63 in other subspecies) (Table 1), tend to have wide ranges of nape squamation (naked to fully scaled in *E. r. rupestre* vs. ≥40% in other subspecies) (Table 7), and by having few lateral blotches along the midline that may often be diffuse (3–8 distinct blotches in *E. r. rupestre* vs. 5–9 in *E. r. piersoni* and 7–9 in *E. r. uphapeense*) (Table 9).

TABLE 2. Frequency distribution for number of scales above the lateral line for each of the three *Etheostoma rupestre* subspecies. Modes are listed in bold.

Taxon/Drainage	4	5	6	7	mean	*n*
E. r. rupestre						
Black Warrior River	9	**54**	38	4	5.4	105
North River	1	**65**	54	3	5.5	123
Tombigbee River	6	**44**	3	-	4.9	53
E. r. piersoni						
Cahaba River	-	7	**17**	5	5.9	29
Alabama River	-	3	**39**	5	6.0	47
E. r. uphapeense						
Uphapee Creek	-	13	**21**	-	5.6	34
Hatchet Creek	-	4	**5**	2	5.8	11
Etowah River	-	**1**	**1**	-	5.5	2
Conasauga River	-	2	**3**	-	5.6	5
					Total	409

Description. Populations of *Etheostoma r. rupestre* possess the following traits: body ventrally flattened, lateral line scales 45–59 in >99% of specimens (modally 49–53) (Table 1), scale rows above the lateral line 4–7 (modally 5) (Table 2), scales below lateral line 6–12 (modally 8–9) (Table 3), scales around the caudal peduncle 16–23 (modally 18–20) (Table 4), dorsal-fin spines IX–XII (modally XI) (Table 5), dorsal-fin rays 9–13 (modally 11–12) (Table 6), nape squamation 0–100% (naked to fully scaled) (mean 75–80% squamation) (Table 7), 10–90% belly squamation (mean 44–58%) (Table 8), lateral blotches tend towards being few and diffuse (3–8 distinct blotches, mean 6.4) (Table 9), distance between blotches 3.5–7.5% of SL (mean 4.8%), lateral blotch width 4.2–6.6% of SL (mean 5.5%), dorsal saddle width 5.8–9.1% of SL (mean 8.0) (Table 10), distal band in first dorsal fin 21.4–48.2% of fin depth (mean 38.3%), total first dorsal pigment 59.0–74.6% of fin depth (mean 66.9) (Table 11), nuptial coloration ranging from light blue to dark green.

TABLE 3. Frequency distribution for number of scales below the lateral line for each of the three *Etheostoma rupestre* subspecies. Modes are listed in bold.

Taxon/Drainage	6	7	8	9	10	11	12	mean	*n*
E. r. rupestre									
Black Warrior River	12	18	**34**	26	13	2	-	8.2	105
North River	-	11	41	**46**	21	3	1	8.7	123
Tombigbee River	3	24	**21**	5	-	-	-	7.5	53
E. r. piersoni									
Cahaba River	-	-	5	**12**	11	1	-	9.3	29
Alabama River	1	2	**18**	14	10	2	-	8.8	47
E. r. uphapeense									
Uphapee Creek	-	6	11	**14**	2	1	-	8.4	34
Hatchet Creek	-	-	3	**5**	3	-	-	9.0	11
Etowah River	**1**	-	**1**	-	-	-	-	7.0	2
Conasauga River	-	1	**2**	-	**2**	-	-	8.6	5
								Total	409

TABLE 4. Frequency distribution for number of caudal peduncle scales for each of the three *Etheostoma rupestre* subspecies. Modes are listed in bold.

Taxon/Drainage	16	17	18	19	20	21	22	23	mean	*n*
E. r. rupestre										
Black Warrior River	4	13	17	**28**	14	15	13	1	19.3	105
North River	1	-	18	26	**33**	23	19	3	20.0	123
Tombigbee River	2	14	**18**	16	3	-	-	-	18.1	53
E. r. piersoni										
Cahaba River	-	-	-	2	6	**8**	9	4	21.2	29
Alabama River	-	-	9	**10**	9	8	9	2	20.1	47
E. r. uphapeense										
Uphapee Creek	-	-	2	5	10	**13**	4	-	20.4	34
Hatchet Creek	-	1	1	**3**	2	**3**	1	-	19.7	11
Etowah River	-	-	-	**2**	-	-	-	-	19.0	2
Conasauga River	-	-	1	-	**2**	1	1	-	20.2	5
									Total	409

Distribution. The range of *Etheostoma. r. rupestre* extends from the lower Tombigbee watershed in Alabama to the upper Tombigbee watershed of Mississippi and the Black Warrior watershed (Fig. 2). *Etheostoma r. rupestre* is disjunct from other *E. rupestre* subspecies.

TABLE 5. Frequency distribution for number of dorsal-fin spines for each of the three *Etheostoma rupestre* subspecies. Modes are listed in bold.

Taxon/Drainage	IX	X	XI	XII	XIII	mean	*n*
E. r. rupestre							
Black Warrior River	-	10	**63**	32	-	11.2	105
North River	1	11	**73**	38	-	11.2	123
Tombigbee River	-	3	**46**	4	-	11.0	53
E. r. piersoni							
Cahaba River	-	3	9	**17**	-	11.5	29
Alabama River	-	1	**29**	16	1	11.4	47
E. r. uphapeense							
Uphapee Creek	-	-	**23**	11	-	11.3	34
Hatchet Creek	-	3	**7**	1	-	10.8	11
Etowah River	-	-	-	**2**	-	12.0	2
Conasauga River	-	-	**3**	2	-	11.4	5
						Total	409

TABLE 6. Frequency distribution for number of dorsal-fin rays for each of the three *Etheostoma rupestre* subspecies. Modes are listed in bold.

Taxon/Drainage	9	10	11	12	13	mean	*n*
E. r. rupestre							
Black Warrior River	-	3	**54**	45	3	11.5	105
North River	1	2	**60**	57	3	11.5	123
Tombigbee River	-	1	15	**36**	1	11.7	53
E. r. piersoni							
Cahaba River	-	2	9	**16**	2	11.6	29
Alabama River	-	-	**24**	19	4	11.6	47
E. r. uphapeense							
Uphapee Creek	-	2	12	**16**	4	11.6	34
Hatchet Creek	-	1	1	**9**	-	11.7	11
Etowah River	-	-	-	**2**	-	12.0	2
Conasauga River	-	-	2	**3**	-	11.6	5
						Total	409

Etymology. As species. The common name honors Chu-fa Tsai, who has contributed to the understanding of morphological divergence in *E. rupestre*.

***Etheostoma rupestre piersoni*, Hilburn, Janosik, and Johnston, New Subspecies**
Shamrock Darter

Figure 8B, Tables 1–11

***Etheostoma* cf. *rupestre*—**Near *et al.* 2011: 568, 578, Tbl 1., Fig. 3 (recognition as a distinct lineage, termed Central Rock Darter).

TABLE 7. Nape squamation for each of the three *Etheostoma rupestre* subspecies expressed as percentage of nape scaled. Modes are listed in bold.

Taxon/Drainage	0	20	40	60	80	100	mean	*n*
E. r. rupestre								
Black Warrior River	2	12	8	12	27	44	74.7	105
North River	1	5	2	26	35	54	80.8	123
Tombigbee River	-	6	2	13	11	21	74.7	53
E. r. piersoni								
Cahaba River	-	-	-	-	6	23	95.9	29
Alabama River	-	-	-	-	19	28	91.9	47
E. r. uphapeense								
Uphapee Creek	-	-	-	1	4	29	96.5	34
Hatchet Creek	-	-	-	-	2	9	96.4	11
Etowah River	-	-	1	-	-	1	70.0	2
Conasauga River	-	-	-	1	1	3	88.0	5
							Total	409

TABLE 8. Belly squamation for each of the three *Etheostoma rupestre* subspecies expressed as percentage of belly scaled. Modes are listed in bold.

Taxon/Drainage	10	20	30	40	50	60	70	80	90	mean	*n*
E. r. rupestre											
Black Warrior River	7	9	11	13	9	**28**	15	9	4	51.0	105
North River	2	3	3	5	34	**42**	21	11	2	57.9	123
Tombigbee River	-	3	8	**17**	**17**	6	2	-	-	44.0	53
E. r. piersoni											
Cahaba River	-	-	-	1	7	**13**	6	2	-	60.3	29
Alabama River	-	-	-	5	**15**	14	10	2	1	58.3	47
E. r. uphapeense											
Uphapee Creek	-	-	1	11	**13**	5	4	-	-	50.0	34
Hatchet Creek	-	-	2	-	**4**	**4**	1	-	-	51.8	11
Etowah River	-	-	-	1	1	-	-	-	-	45.0	2
Conasauga River	-	1	-	-	1	1	**2**	-	-	54.0	5
										Total	409

TABLE 9. Frequency Distribution of lateral blotch counts for *Etheostoma rupestre* subspecies. Blotch counts of 5 or below indicate the presence of blotches that were too diffuse too count. Blotch counts of 6 include both fish that had 6 distinct blotches and those that had some blotches too diffuse to count.

	Number of Blotches								
Taxon	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Mean**	***n***
E. r. rupestre	1	2		5	13	2		6.4	23
E. r. piersoni			1	6	10	4	2	7.0	23
E. r. uphapeense					11	6	4	7.7	21
								Total	67

TABLE 10. Means and ranges (parentheses) of widths of lateral blotches and dorsal saddles as well as distances between blotches of the *Etheostoma rupestre* subspecies. Distances are expressed as percent of standard length.

	BLOTCHES			SADDLES	
Taxon	**Mean Dist. Btwn. Blotches (%)**	**Blotch Width (%)**	***n***	**Mean Saddle Width (%)**	***n***
E. r. rupestre	4.8 (3.5–7.5)	5.5 (4.2–6.6)	19	8.0 (5.8–9.1)	19
E. r. piersoni	4.6 (2.8–6.6)	6.2 (4.6–8.2)	16	7.9 (6.6–8.8)	17
E. r. uphapeense	5.0 (4.0–6.1)	4.6 (3.8–5.3)	17	6.9 (5.7–8.0)	17
		Total	52	*Total*	53

TABLE 11. Means and ranges (parentheses) for distal and total pigment (medial band + distal band) band depths of the spinous dorsal fin of the *Etheostoma rupestre* subspecies. Band depths are expressed as percentage of total fin depth.

Taxon	Distal Band Depth (%)	Medial + Distal Depth (%)	*n*
E. r. rupestre	38.3 (21.4–48.2)	66.85 (59.0–74.6)	19
E. r. piersoni	39.6 (24.9–51.1)	64.0 (49.2–78.0)	17
E. r. uphapeense	43.4 (24.5–50.7)	64.7 (53.41–76.9)	15
		Total	51

Holotype. Alabama. Dallas/Wilcox Co., USNM 398677, 61 mm SL, Chilatchee Creek, Hwy 5, 32.2359°, -87.4091°, 26 March 2010, C. E. Johnston, A. M. Janosik, D. E. Holt and T. H. Haley.

Paratopotypes. USNM 398678, (2, 43–46) mm SL, same locality, 32.2359°, -87.4091°, 26 March 2010, C. E. Johnston, A. M. Janosik, D. E. Holt and T. H. Haley.

Paratypes. Alabama and Cahaba River drainages: Alabama. Bibb Co., AUM 39178, Cahaba River, 6.2 airmi NE of Centreville, Hwy 27 (5, 47–55), 23–24 October 1978, no lat/long available; AUM 38753, 0.6 miles downstream of the Harrisburg Bridge, at the first gravel island (14, 43–55), 6 November 1983 32.8545°,-87.2001°; AUM 2516, 13.4 km N of Centerville on Highway 27 (2, 46–51), 13 May 1969, 33.0636°, -87.1308°; AUM 5880, 10.0 air km NE of Centerville on Highway 27 (21, 40–51), 21 November 1971, 33.0394°, -87.1308°. AUM 18172, Schultz Creek, 6.3 air km NNW of Centerville on Highway 219 (2, 30–48), 08 September 1978, 33.0021°, -87.1483°. **Butler Co.**, AUM 68107, Cedar Creek, at Cedar Creek Plantation, 13 km NW of Greenville (1, 64), 13 October 2016, 31.90968º, -86.71909º. AUM 74489, Caffee Creek, riffle near Griffin Town Road (5, 46–58), 22 March 2022, 33.12001º, -87.10619º. **Dallas Co.**, AUM 7481, Mush Creek, 4.8 air km S of Sardis on Highway 41 (2, 46–46), 03 April 1972, 32.2444°, -86.9928°; AUM 7653, 4.8 air km S of Sardis on Highway 41 (4, 34–51), 11 July 1972, 32.2444°, -86.9928°; AUM 7813, 4.8 air km S of Sardis on Highway 41 (14, 35–47), 17 October 1972, 32.2444°, -86.9928°. **Jefferson Co.**, AUM 47076, Cahaba River, Grant's Mill, downstream of Grant's Mill Road bridge (1, 50), 5 October 2006, 33.5110º, -86.6584º; AUM 49331, Mill Dam site of Grant's Mill, 1 mi SE of Moore Corner, AL (2, 47–51), 6 June 2007, 33.5089º, -86.6436º; AUM 18694, 11.1 air km WSW of Leeds on Highway 143 (5, 35–46), 28 March 1979, 33.6604°, -86.5894°. **Monroe Co.**, AUM 15507, Big Flat Creek, 12.9 air km NW of Monroeville on Highway 14 (24, 34–54), 16 October 1977, 31.6078°, -87.415°. USNM 199547, Unnamed creek, tributary of Limestone Creek between Monroeville and Mexia (1, 47), 27 November 1962, 31.5148°, -87.3559°. **Monroe Co.**, AUM 48923, Big Flat Creek, downstream site of Rikard's Mill, 1 mile NNW of Fountain (2, 49–51), 22 July 2007, 31.6084º, -87.4143º. **Shelby Co.**, AUM 5699, Cahaba River, 4.2 air km N of Marvel (1, 49), 16 May 1971, 33.1867°, -86.9997°; AUM 56142, 2.5 airmi WSW of Helena, Hwy 52 (1, 57), 5 December 1978, 33.2846º, -86.8827º; AUM 6660, at river mile 3.4 on J Foshee's Cahaba Section 8 map (1, 53), 26 September 1981, 33.2495º, -86.9370º; AUM 65819, at river mile 1.5 on J. Foshee's Cahaba Section 5 map (2, 44–57), 07 November 1981 33.4037º, -86.7572º; AUM 41174, at Booth Ford, 4.5 airmi N of Marvel, Hwy 251 (2, 57–61), 6 October 1978, no lat/long available; AUM 66613, at river mile 5.0 on J. Foshee's Cahaba Section 8 map (1, 48), 26 September 1981, 33.2360º, -86.9430º; AUM 66657, at river mile 4.0 on J. Foshee's Cahaba Section 6 map (1, 46), 24 October 1981, 33.3289º, -86.8441º.

Diagnosis. While there is considerable overlap among all traits measured rendering individuals undiagnosable, populations can be diagnosed. Populations of *E. r. piersoni* differ from *E. r. rupestre* by possessing modally six scales above the lateral line (as opposed to five in *E. r. rupestre*) (Table 2), by possessing a higher range of lateral line scales than populations of *E. r. rupestre* (49–63 in *E. r. piersoni* vs. usually 45–59 in *E. r. rupestre*) (Table 1), by having a much narrower range of nape squamation (≥80% scaled in *E. r. piersoni* vs. 0–100% scaled in *E. r. rupestre*) (Table 7), and by having a higher number of lateral blotches (5–9 in *E. r. piersoni* vs. 3–8 in *E. r. rupestre*) (Table 9). Populations of *E. r. piersoni* can be distinguished from populations of *E. r. uphapeense* by possessing a lower range and mean of blotches that tend to be slightly more diffuse (5–9 distinct blotches in *E. r. piersoni* vs. 7–9 in *E. r. uphapeense*) (Table 9). *E. r. piersoni* and *E. r. uphapeense* can be further distinguished with genetics (see Janosik *et al.* 2023).

Description. Populations of *E. r. piersoni* possess the following traits: lateral line scales 49–62 (modally 54–60) (Table 1), scales above the lateral line 5–7 (modally 6) (Table 2), scales below the lateral line 6–11 (modally 8–9) (Table 3), scales around the caudal peduncle 18–23 (modally 19–21) (Table 4), dorsal spines X–XIII (modally XI–XII) (Table 5), dorsal-fin rays 10–13 (modally 11–12) (Table 6), Nape squamation 80–100% (mean 92–96%) (Table 7), belly squamation 40–90% (mean 58–60%) (Table 8), distinct lateral blotches 5–9 (mean 7.0) (Table 9), distance between lateral blotches 2.8–6.6% of SL (mean 4.6%), average lateral blotch width 4.6–8.2% of SL (mean 6.2%), average dorsal saddle width 6.6–8.8% of SL (mean 7.9%) (Table 10), male distal band in first dorsal fin 24.9–51.1% of fin depth (mean 39.6%), total first dorsal pigment 49.2–78.0% of fin depth (mean 64.0%) (Table 11), nuptial coloration ranging from light blue to dark green.

Distribution. *Etheostoma rupestre piersoni* is endemic to the Alabama and Cahaba watersheds of central Alabama (Fig. 2). It is allopatric to *E. r. rupestre* and *E. r. uphapeense*.

Etymology. Named in honor of J. Malcom Pierson, an ichthyologist who made outstanding contributions to the study of Alabama's ichthyofauna. Shamrock Darter refers to the nuptial color and pattern as it resembles that of Shamrock leaves. The common name serves as a double entendre for a derivation of the original Rock Darter ("*sham-"* (false derivative) "*-rock"* (Rock Darter)).

Etheostoma rupestre uphapeense, Hilburn, Janosik, and Johnston, New Subspecies
Jade Darter

Figure 7C, Tables 1–11

***Etheostoma* cf. *rupestre*—**Near *et al.* 2011: 568, 578, Tbl 1., Fig. 3 (recognition as a distinct lineage, termed Eastern Rock Darter).

Holotype. Alabama. Macon Co., USNM 398679, 53 mm SL, Uphapee Creek, I85, 32.4745º -85.6881º, 19 April 2010, K. M. Dowling, T. H. Haley and R. K. Bolton.

Paratopotypes. Alabama. Macon Co., USNM 398680, same locality, (14, 35–45), 3/8 November 2008; USNM 398681, same locality, (1, 50), 9 January 2008; USNM 398682, same locality, (1, 40), 9 January 2008; USNM 398683, (1, 38), same locality, 9 January 2008; AUM 5147, Uphapee Creek, 5.6 air km N of Tuskegee on Interstate-85 (4, 29–34), 16 August 1969, 32.4747º, -85.6864º; AUM 6564, 5.6 air km N of Tuskegee on Interstate-85 (24, 38–62), 29 October 1971, 32.475º, -85.6869º; AUM 47401, at SR 81, 3.65 miles N of Tuskegee (3, 47–51), 9 January 2008, 32.4747º, -85.6864º.

Paratypes. Coosa River drainage: Alabama. Coosa Co., AUM 18036, Hatchet Creek, 6.1 air km N of Rockford on Highway 231 (1, 39), 14 July 1978, 32.9441º, -86.2042º; AUM 18179, 6.1 air km N of Rockford on Highway 231 (1, 31), 08 September 1978, 32.9442º, -86.2042º; UAIC 10590.15, 9.7 km W of Rockford, 3.7 km N of Kelly's Crossroads on gravel logging road (2, 35–42), 26 October 1990, 32.8697º, -86.3211º; UAIC 10591.20, 6.4 km NNE of Rockford, 0.3 km N of U.S. Highway 231 (4, 36–40), 27 October 1990, 32.9442º, -86.2036º; UAIC 11534.10, U.S. Highway 280, 7.2 km WSW of Goodwater (3, 33–37), 16 September 1996, 33.03611º, -86.1233º. **Georgia. Cherokee Co.**, UAIC 12413, Etowah River, Coker's Church Road 1.6 km SE of Gober (2, 42–47), 26 February 2000, 34.2692º, -84.4186º. **Whitfield Co.**, AUM 9111, Conasauga River, Beaverdale on Highway 2 at Murray Co. line (2, 50–56), 01 August 1974, 34.9901º, -84.7744º. **Tennessee. Bradley Co.**, USNM 231113, Conasauga River, Highway 74 (2, 38–39), 16 May 1970, 35.0016º, -84.7785º; UAIC 12835.10, Highway 74, downstream to mouth of Mill Creek (1, 51), 15 May 1977, 33.2942º, -87.5697º.

Tallapoosa River drainage: Alabama. Macon Co., UAIC 9696.21, Chewacla Creek, Co. Road 22, 9.7 km E of Tuskegee (6, 33–42), 20 October 1987, 32.4097º, -85.5936º; AUM 69405, at gauging station at old CR 33 bridge crossing, 4.3 miles S of Auburn (1, 57), 28 July 2017, 32.5477º, -85.4810º. AUM 47177, Uphapee Creek, at swamp trib. inlet, unmarked dirt road immediately before Hwy 29 crossing, 4 miles NW of Tuskegee (1, 51), 22 July 2002, 32.4394º, -85.6403º. AUM 42074, Choctafaula Creek, at junction with Uphapee Creek (1, 51), 30 October 1991, no lat/long available; AUM 66500, at FSR 900, 4 miles NE of Tuskegee, Tuskegee National Forest (5, 46–59), 16 August 2001, 32.4676º, -85.6374º. AUM 34523, Choctafaula Creek, ST 186, 6 miles NE of Tuskegee (1, 44), 20 June 2001, 32.4792º, -85.6258º. AUM 74479, Opintlocco Creek, at Old Columbus Road (CR 26), first run upstream (6, 48–60), 17 May 2022, 32.4125º, 85.6166º.

Diagnosis. While *Etheostoma r. uphapeense* has large degrees of overlap with other subspecies in every trait measured, populations can be diagnosed and distinguished from other subspecies based on the following morphological tendencies: populations of *E. r. uphapeense* differ from *E. r. rupestre* by possessing modally six scales above the lateral line (as opposed to five in *E. r. rupestre*) (Table 2), by possessing a higher range of lateral line scales than populations of *E. r. rupestre* (49–63 in *E. r. uphapeense* vs. usually 45–59 in *E. r. rupestre*) (Table 1), by having a much narrower range of nape squamation (≥40% scaled in *E. r. uphapeense* vs. 0–100% scaled in *E. r. rupestre*) (Table 7), and by possessing a higher number of lateral blotches (7–9 in *E. r. uphapeense* vs. 3–8 in *E. r. rupestre*). Populations of *E. r. uphapeense* possess 7–9 distinct lateral blotches (mean 7.7) as opposed to 5–9 (mean 7.0) in *E. r. piersoni* (Table 9). *Etheostoma r. uphapeense* and *E. r. piersoni* are genetically distinct and monophyletic on cytochrome *b* (Janosik *et al*., 2023).

Description. Populations of *Etheostoma r. uphapeense* possess the following traits: lateral line scales 49–63 (modally 54–60) (Table 1), scale rows above the lateral line 5–7 (modally 6) (Table 2), scale rows below the lateral line 6–11 (modally 8–10) (Table 3), scales around the caudal peduncle 17–22 (modally 19–21) (Table 4), dorsal spines X–XII (modally XI–XII) (Table 5), dorsal-fin rays 10–13 (modally 12) (Table 6), nape squamation 40–100% (mean 70–97%) (Table 7), belly squamation 20–70% (mean 45–54%) (Table 8), Lateral blotches tend towards being well-defined and typically range 7–9 (mean 7.7) (Table 9), distance between lateral blotches 4.0–6.1% of SL (mean 5.0%), average lateral blotch width 3.8–5.3% of SL (mean 4.6%), dorsal saddle width 5.7–8.0% of SL (mean 6.9%) (Table 10), male distal band in first dorsal fin 24.5–50.7% of fin depth (mean 43.4%), total first dorsal pigment 53.4–76.9% of fin depth (mean 64.7%) (Table 11), nuptial coloration ranging from light blue to dark green.

Distribution. *Etheostoma rupestre uphapeense* is restricted to several small, disjunct populations in the Coosa and Tallapoosa basins of eastern Alabama, Northwest Georgia, and extreme southeastern Tennessee (Fig. 2). The following are the systems known to possess populations of *E. r. uphapeense*: In the Tallapoosa system: Uphapee Creek and its tributaries; In the Coosa system: Hatchet Creek, The Conasauga River, Talking Rock Creek (Coosawattee system), and the Etowah River. Very few individuals of *E. r. uphapeense* have been reported historically from certain other streams in the Coosa (e.g., Choccolocco Creek), and additional isolated populations may eventually be discovered in the Coosa watershed.

Comments. *Etheostoma rupestre uphapeense* has a spotty distribution and is often uncommon where it occurs. We recommend routine monitoring to ensure population numbers are stable.

Etymology. The subspecific epithet *uphapeense* is derived from Uphapee Creek from which the holotype was collected. The word Uphapee's origin (alternative spellings *Euphapee* or *Euphaube*) is traditionally attributed to the Muskogean language family and may derive from the word *nofapi* (Hitchiti dialect) which means beech tree (Bright 2004). We assign the common name Jade Darter to this subspecies, as jade is a green-colored rock and its appearance can be likened to that of *E. r. uphapeense*.

Discussion

Our findings are similar to those of Tsai (1968) who recognized two races of *E. rupestre* confined to the Tombigbee River and the Alabama River watersheds. However, we found evidence of two lineages within the Alabama River race that had differences in pigmentation and are genetically monophyletic on cytochrome *b* (Janosik *et al*. 2023). Previous genetic data as well as the morphological data presented in this paper suggest that *E. r. rupestre* is the most divergent lineage and is sister to *E. r. piersoni* + *E. r. uphapeense*. Like Tsai (1968), we interpret differences among lineages as incipient speciation but assign subspecific epithets and common names (see discussion below on application of common names) to each lineage.

Though *Etheostoma r. rupeste* did not resolve as monophyletic on cytochrome-*b* in previous genetic work (Janosik *et al.* 2023), we nevertheless consider it a valid subspecies that may bear the hallmarks of past reticulate evolution via introgressive hybridization with other lineages. *Etheostoma r. rupestre* (which corresponds to the Tombigbee River race identified by Tsai [1968]), is the most morphologically divergent lineage, and is the only subspecies to possess modally five scales above the lateral line in all populations (as opposed to six in the other two subspecies) and additionally has markedly lower lateral line scale count ranges than other subspecies. Certain authors consider modal differences in meristic characters to support species-level recognition if lineages resolve as monophyletic (e.g., Near *et al.* 2023). Our decision to classify *E. r. rupestre* as a subspecies reflects its apparently recent gene exchange with other lineages but its separate phenotypic trajectory. Alternatively, the non-monophyly of *E. r. rupestre* observed by Janosik *et al.* (2023) might be explained by a retained genetic ancestral polymorphism within this morphologically variable basal subspecies. The distribution of *E. r. rupestre* is replicated in the putative sister pair *Etheostoma lachneri* Suttkus and Bailey + the *Etheostoma bellator* Suttkus and Bailey species complex (Near *et al.* 2011) which evidently have had no secondary contact with other closely related species. However, the latter taxa are smaller-bodied and headwater-restricted which would allow for less gene exchange among populations and therefore higher rates of speciation, whereas the larger-bodied *E. rupestre* tends to occupy large streams and rivers which would inhibit allopatric isolating mechanisms. Whether the non-monophyly of the nominate subspecies is due to historic gene exchange or incomplete lineage sorting, with the current drainage configuration in the Mobile Basin *E. r. rupestre* represents an incipient lineage and distinct metapopulation with its own evolutionary trajectory, morphological tendencies, and modal differences. Nevertheless, we recognize that future authors may be warranted in following the interpretation of Tsai (1968) in recognizing narrow areas of intergrade between *E. r. rupestre* and *E. r. piersoni* and we note that the few specimens of *E. r. rupestre* examined from the Sipsey Fork and Locust Fork appear to be somewhat meristically divergent (Tsai 1968, B. Hilburn, pers. obs.).

The most recent subspecies descriptions for darters were published over 40 years ago (*Etheostoma blennius blennius* Burr and *E. b. sequatchiense* Burr) (Burr 1979), and there have been many recent studies elevating former subspecies of darters to specific status (Powers & Mayden 2007; Blanton & Schuster 2008; Piller & Bart 2017; Matthews & Turner 2019; MacGuigan *et al*. 2023). Under the diagnoseable version of the phylogenetic species concept, such redescriptions are often warranted as lineages are morphologically diagnoseable, monophyletic, and lack zones of intergradation. In fact, many current subspecies of darters likely warrant elevation to species status because they exhibit diagnoseable morphological traits (Bailey & Richards 1963; Kuehne & Barbour 1983). However, in many cases lineages display morphological tendencies towards certain extremes but overlap in all characters measured, indicating that these lineages are on the evolutionary trajectory towards becoming species under the diagnoseable version of the phylogenetic species concept but have not yet attained this status (incipient speciation). In cases where populations display only modal or statistically significant mean differences in measurable morphological traits, subspecies status is likely appropriate (Craig *et al*. 2017).

Subspecies designations are often viewed as archaic and are controversial in current taxonomy, causing many authors to dispense with subspecies concepts altogether (see Patten 2015 for a discussion on this). Much of the modern skepticism of subspecies concepts in ichthyology is warranted as early subspecies descriptions were often made arbitrarily with no objective criterion provided to determine whether a particular taxon should be recognized as a species or subspecies. Most authors that are proponents of the use of subspecies designations consider subspecies to represent populations that are on the evolutionary trajectory towards becoming species but have not yet arrived (Burr 1979; Patten 2015; Craig *et al*. 2017; Moyle & Campbell 2022; Moyle *et al.* 2023). Under the diagnoseable version of the phylogenetic species concept, a valid subspecies by extension would be a metapopulation that possesses its own morphological and genetic trajectories (i.e., modal or range differences in measurable morphological traits and that tend towards monophyly).

Often in taxonomy there is an impetus to describe slightly divergent populations as species or to elevate subspecies to garner more intensive conservation efforts (Karl & Bowen 1999). Excessive elevation of subspecies to species status can lead to taxonomic inflation which can confound ecological studies and confuse conservation efforts (Isaac *et al*. 2004). A researcher's view of species or subspecies will inevitably be informed by an ontological perspective on the degree of distinctiveness a certain lineage must possess to qualify for a particular taxonomic rank (Ghiselin 2002; Patten 2015), but regardless of philosophical perspective taxon descriptions should be informed by data rather than social pressure. Nevertheless, we recognize the importance of proper taxonomic recognition to conservation efforts. This creates a dilemma regarding how to appropriately recognize and protect incipient lineages under the diagnoseable version of the phylogenetic species concept. Subspecific designations for morphologically undiagnosable taxa with separate phenotypic and genetic trajectories could help to ameliorate this issue. Not only would such subspecies designations be supported by objective criteria, they would also provide taxonomic rank to distinct, partially-diagnoseable lineages which would facilitate their conservation. These criteria may also be useful for determining which previously described darter subspecies should be elevated to species status.

We assign common names to each subspecies of *E. rupestre* to emphasize the importance of recognizing subspecies as unique evolutionary entities that warrant unique management and conservation efforts. In the taxonomy of North American nongame fishes, common names are rarely applied to subspecies (Bailey & Richards 1963; Burr 1979), while for game and commercial fishes it seems to be the convention to associate common names with subspecies (examples include Gulf Sturgeon *Acipenser oxyrhinchus desotoi* Vladykov, Grass Pickerel *Esox americanus vermiculatus* Lesueur, Neosho Bass *Micropterus dolomieu velox* Hubbs & Bailey, and Rio Grande Cutthroat Trout *Oncorhynchus clarkii virginalis* (Girard)). In future subspecies descriptions for nongame fishes (if any should follow), we recommend assigning common names to taxa.

Regardless of philosophical perspective on lower taxonomic ranks, most ichthyologists and conservation biologists acknowledge the importance of maintaining the genetic integrity of locally adapted lineages (Blanton & Jenkins 2008; Baker *et al.*, 2013; Mussmann *et al*. 2020; Moyle & Campbell 2022, Moyle *et al.* 2023), an idea with which we concur. In the Mobile Basin, numerous geographically isolated lineages likely warrant species or subspecies status (Boschung & Mayden 2004; Near *et al*. 2011). This is particularly evident in the *Adonia* darters, and several isolated populations in the Warrior, Cahaba, and upper Tombigbee watershed likely warrant species recognition. We recommend further investigations into geographically isolated populations of darters and suggest that researchers be open to the use of subspecies as an extension of the diagnoseable version of the phylogenetic species concept.

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