Description of a new species of cryptic snubnose darter (Percidae: Etheostomatinae) endemic to north-central Mississippi

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Many subclades within the large North American freshwater fish genus *Etheostoma* (Percidae) show brilliant male nuptial coloration during the spring spawning season. Traditionally, perceived differences in color were often used to diagnose closely related species. More recently, perceived differences in male nuptial color have prompted further investigation of potential biodiversity using genetic tools. However, cryptic diversity among *Etheostoma* darters renders male nuptial color as unreliable for detecting and describing diversity, which is foundational for research and conservation efforts of this group of stream fishes. *Etheostoma raneyi* (Yazoo Darter) is an imperiled, range-limited fish endemic to north-central Mississippi. Existing genetic evidence indicates cryptic diversity between disjunctly distributed *E. raneyi* from the Little Tallahatchie and Yocona river watersheds despite no obvious differences in male color between the two drainages. Analysis of morphological truss and geometric measurements and meristic and male color characters yielded quantitative differences in *E. raneyi* from the two drainages consistent with genetic evidence. Morphological divergence is best explained by differences in stream gradients between the two drainages. *Etheostoma faulkneri*, the Yoknapatawpha Darter, is described as a species under the unified species concept. The discovery of cryptic diversity within *E. raneyi* would likely not have occurred without genetic tools. Cryptic diversity among *Etheostoma* darters and other stream fishes is common, but an overreliance on traditional methods of species delimitation (i.e., identification of a readily observable physical character to diagnose a species) impedes a full accounting of the diversity in freshwater fishes in the southeastern United States.

1 **Description of a New Species of Cryptic Snubnose Darter (Percidae:**

2 **Etheostomatinae) Endemic to North-Central Mississippi**

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20 **Introduction**

21 *Etheostoma raneyi* Suttkus and Bart (Yazoo Darter) is most closely related to other snubnose

22 darters in western Tennessee and Kentucky and Alabama (unranked clade name Adonia, *sensu*

23 Near et al., 2011) (Kozal et al., 2017). The species is distributed across small tributaries of the

24 Little Tallahatchie (L.T.R.) and Yocona rivers (Y.R.) of north-central Mississippi in the upper

25 Yazoo River basin (Figure 1). *Etheostoma raneyi* avoid the bottomland streams of the

26 Mississippi Alluvial Plain and is limited to relatively higher-gradient, perennial streams draining

27 sandy geologic formations of the Upper Gulf Coastal Plain (Stephenson et al., 1928; Randolph &

28 Kennedy, 1974; Thompson & Muncy, 1986; Suttkus et al., 1994; Thompson, 2011; Sterling et

29 al., 2013).

30   A phylogenetic study of Coastal Plain snubnose darters in western Tennessee and Kentucky,

31 and northern Mississippi, including *E. raneyi*, uncovered substantial genetic structure within and

32 among species that was attributed to watershed configurations and the location of stream

33 confluences between large drainages within the unfavorable lowland habitat of the Lower Gulf

34 Coastal Plain and Mississippi Alluvial Plain (Powers & Warren, 2009; Keck & Etnier, 2005)

35 (Figure 1). *Etheostoma raneyi* from the L.T.R. and Y.R. drainages were recovered as

36 reciprocally monophyletic lineages, indicating that individuals from each drainage were

37 evolutionarily divergent and distinct (Powers & Warren, 2009). A more recent phylogenetic

38 analysis using two genes and greater number of samples from across the range of the species also

39 indicated that *E. raneyi* in the two drainages were independently evolving metapopulations

40 (Sterling et al., 2020). Estimated time since divergence (0.4-0.8 my) was similar to estimates for

41 snubnose darters in western Tennessee and Kentucky (Kozal et al., 2017).

42   The original description of *E. raneyi* did not indicate any geographic variation in appearance,

43   male nuptial color, morphology, or meristics except for modal lateral-line scale counts between

44   the L.T.R. and Y.R. (Suttkus et al., 1994). However, an examination of standard lengths (SL)

45   between drainages showed that males and females from the Y.R. were longer compared with

46   males and females from the L.T.R. (Sterling et al., 2013). Because the available evidence

47   suggested possible differences in meristic and morphological characters and because the genetic

48   evidence indicated that *E. raneyi* in the Y.R. were distinct, we investigated possible differences

49   in male nuptial color, meristic characters, and morphology. The description of the new species

50   presented here is based on published data and our new analyses.

52   **Materials and Methods**

53   This study was conducted with the approval of the University of Mississippi IACUC Committee

54   (protocol 09-027), using annual collection permits issued to us from the Mississippi Museum of

55   Natural Science (Mississippi Department of Wildlife, Fisheries, and Parks) for the years 2009-

56   2014: 0604091, 0513101, 0624112, 0622122, 0602132, 0610142.

58   *Male nuptial color*

59   We examined male nuptial color by taking photographs of mature, live fish in the field. Males

60   were captured by seine and deposited into a water-filled, opaque black bucket with a lid and

61   supplemental oxygen (a bubbler) to prevent stress and subsequent loss of color. We used a small,

62   clear Plexiglas photarium with a white foam squeeze plate to obtain images of a lateral view of

63   the entire individual. We made all photographs using an Olympus Stylus TG-3 camera. Though

64   one of us (K.S.) edited images for brightness and contrast, no alterations were made to hue or

65   saturation. We made collections from February to April 2017 and March to April 2018 when

66   males are at the peak of nuptial color (Table 1). We made a total of seven collections from six

67   streams in the L.T.R. drainage (n = 51) and eight collections from five streams in the Y.R.

68   drainage (n = 36). We used resulting images to characterize colors and pigment patterns.

69   To assess possible color and pigment pattern differences between drainages following

70   preliminary comparisons by one of us (K.S.), we asked three colleagues to score images of

71   darters (see Supplement for data) for three characters: 1) the presence and density of black

72   pigment in the pelvic and median fins, breast, branchiostegal membranes, chin, opercle, and

73   cheek, which were scored as 1 = little pigment, 2 = moderate pigment, 3 = heavy pigment; 2) the

74   presence and density of blue pigment in the same areas as for black pigment, which were scored

75   identically; 3) presence of a clear window in the anal fin scored as 1 = window is small to non-

76   existent and covers ≤3 membranes, blue pigment mostly extends to the belly, 2 = window is

77   large and covers ≥3 membranes, blue pigment mostly does not extend to the belly.

78   Images were presented in random order and with no information on the location from which

79   they were sampled. We compiled the scores across all three surveys, and we calculated means

80   and 95% confidence intervals of scores for each drainage. Because this artificially reduces

81   variance, we also present the results from each individual survey in the Supplement. Lastly, we

82   also calculated the proportion of males having orange pigment in the anal fin from each drainage.

83

84   *Meristic, truss morphometric, and geometric morphometric analyses*

85   We counted meristic characters following the methods of Hubbs & Lagler (2004) except we

86   counted transverse scales from the origin of the anal fin diagonally toward the base of the

87   spinous dorsal fin (Page, 1983). For comparison, we compiled meristic data for closely related

88   Adonia snubnose darters (Near et al., 2011) from several sources in the literature (Bailey &

89   Etnier, 1988; Powers & Mayden, 2003; Kozal et al., 2017). Counting methods were clearly

90   different for one source (Kozal et al., 2017) compared with our methods and other data sources

91   as evidenced by differences in modal counts that were consistently either one count higher or

92   lower. In one instance (second dorsal fin rays) this difference in method was cited in the text. We

93   adjusted counts for consistency with other sources of data, except for caudal peduncle scales

94   because of small sample size and no clear modal count.

95   We made morphometric truss measurements following Hubbs & Lagler (2004) and

96   Humphries et al. (1981) (Figure 2). We measured distances between 28 pairs of points (digital

97   calipers, nearest 0.1 mm) and converted them to thousandths of SL to remove the effects of

98   differences in length (Grabowski et al., 2018). We checked distributions of each variable in PC-

99   Ord ver. 6.21 (McCune & Mefford, 2011) and, as expected, variables showed little skew and

100   roughly normal distributions. We then estimated means and 95% confidence intervals for each

101   variable using resampling with replacement and 10,000 samples (Statistics.com LLC, 2009).

102   Specimens were primarily from our own collections now deposited with the National Museum of

103   Natural History (Washington, D.C.) (USNM) or the Mississippi Museum of Natural Sciences

104   (Jackson, MS) (MMNS). We obtained additional specimens from Tulane University Biodiversity

105   Research Institute (Belle Chasse, LA) (TU). *Etheostoma raneyi* mature at about 28-30 mm SL

106   (Suttkus et al., 1994), so we only used specimens >30 mm SL.

107   We used geometric morphometric analyses to test for and describe differences in shape

108   between *E. raneyi* in the L.T.R. and Y.R. We chose to use a morphometric approach rather than

109   more traditional multivariate analysis of linear truss measurements because the morphometric

110   approach describes changes in shape among all landmark points simultaneously and produces

111   clear graphics of shape changes among groups (Bookstein, 1991; Parsons et al., 2003;

112   Klingenberg, 2013).

113   Morphometric analysis requires images of each specimen, and a set of uniform landmark

114   points plotted on each image before analysis. We photographed the lateral view of the left side of

115   the entire fish using an Olympus Stylus TG-3 camera mounted on a vertical camera stand. A

116   glass plate was placed on top of the fish to reduce curvature. Each image included a specimen ID

117   label and a scale (mm). We imported images into tpsUtil (ver. 1.74, Rohlf, 2017a) and tpsDig

118   (ver. 2.30, Rohlf, 2017b) software. We plotted, scaled, and digitized landmarks to produce

119   Cartesian grid coordinates for each individual using 20 homologous landmarks (Figure 3).

120   Because preserved specimens are often vertically curved, we used tpsUtil (ver. 1.74, Rohlf,

121   2017a) to straighten landmark coordinates along the midline of the body.

122   We imported output from the tps software into MorphoJ software for all subsequent analyses

123   (Klingenberg; 2011, 2018). We compared shapes for males and females between drainages and

124   compared shapes between sexes within each drainage (L.T.R. and Y.R.) (Table 2).

125   We used the outlier function in MorphoJ to remove individuals that may have biased the

126   results and performed a least-squares full procrustes superimposition to remove bias resulting

127   from differences in position and orientation among individuals (Mitteroecker & Gunz, 2009;

128   Klingenberg, 2011; Klingenberg & Marugán-Lobón, 2013). Pooled within-group multivariate

129   regression of shape data (Procrustes coordinates) against $\log_{10}$ centroid size removed possible

130   bias resulting from ontogenetic allometry; we used the resulting residual shape variation for all

131   subsequent analyses in MorphoJ (Loy et al., 1998; Klingenberg, 2011). We used discriminant

132   analysis (DA) to test for differences in shape for all comparisons. We estimated mean Procrustes

133   distance (PD) and Mahalanobis distance (M) for each comparison and ran a permutation test

134   (10,000 permutations) to estimate p-values. We used cross-validated classification tables to

135   assess the reliability of the DA results and produced shape change graphics from DA output for

136   all comparisons.

137   Preliminary results indicated shape changes between drainages that suggested possible

138   differences in stream gradients. Therefore, we calculated watershed area (km^2) and stream

139   gradient (relief/length of stream) for all streams with Yazoo Darters in each drainage using

140   DeLorme Topo USA ver. 7.1.0 (2007) and estimated means. We used resampling with

141   replacement to calculate 95% confidence intervals of mean values using Resampling Stats ver.

142   4.0 (Statistics.com LLC, 2009).

143   We evaluated all data and results from analyses under the unified species concept (de

144   Queiroz, 2007). Data used for morphological analyses (MorphoJ) are available from the open

145   access MorphoBank (O'Leary & Kaufman 2012) data repository at:

146   https://morphobank.org/index.php/MyProjects/List/select/project_id/3712. Fishes examined for

147   the truss morphological measurements were borrowed from the Tulane University Biodiversity

148   Research Institute (Belle Chasse, LA) (TU) or were from our own collections now archived at

149   the National Museum of Natural History (Washington, D.C.) (USNM) and the Mississippi

150   Museum of Natural Sciences (Jackson, MS) (MMNS). Images used for male nuptial color

151   comparisons are available in the Supplement.

152   The electronic version of this article in Portable Document Format (PDF) will represent a

153   published work according to the International Commission on Zoological Nomenclature (ICZN),

154   and hence the new name contained in the electronic version are effectively published under that

155   Code from the electronic edition alone. This published work and the nomenclatural acts it

156   contains have been registered in ZooBank, the online registration system for the ICZN. The

157   ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed

158   through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The

159   LSID for this publication is: urn:lsid:zoobank.org:pub:6C5BEC69-22A1-4008-84D3-

160   CA62719AE152. The online version of this work is archived and available from the following

161   digital repositories: PeerJ, PubMed Central and CLOCKSS.

162

163   **Results**

164   *Male nuptial color*

165   Preliminary examination of photographed male darters indicated that possible color or pigment

166   pattern differences were subtle and appeared limited to: 1) generally more extensive and denser

167   black pigment in the pectoral and median fins and the breast, cheek, opercle, and ventral portion

168   of the head region for individuals sampled in the L.T.R.; 2) generally more extensive and denser

169   blue pigment in the same areas for individuals from the Y.R.; 3) complete absence or a small

170   clear window in the anal fin for fish from the Y.R. compared with windows always being present

171   and larger in the L.T.R.; 4) orange pigment occasionally present in the anal fin of fish from the

172   L.T.R. and never present in fish from the Y.R. (Figure 4).

173   Independent scoring of these features revealed that these average differences are consistent

174   between the drainages (Figure 5). Blue pigment was more dense and extensive in the Y.R. males,

175   but black pigment was generally more dense and extensive in the L.T.R. males. Clear windows

176   in the anal fin were generally smaller or absent in the Y.R. individuals (Figure 4). Orange

177   pigment in the anal fin was not found in fish from the Y.R., but was present in 17.7% of males

178   from four of six streams sampled in the L.T.R.

179

180 *Meristics and truss measurements*

181 Count data for lateral-line scales show a bimodal distribution that differ by two scales between

182 individuals from the Y.R. and L.T.R. (Table 3), which is consistent with the counts of Suttkus et

183 al. (1994). There are no other modal differences between the Y.R. and the L.T.R. drainages

184 (Tables 4-9).

185   Mean proportional values for truss measurements indicate that individuals from the Y.R. are

186 more robust with shorter heads and snouts relative to individuals from the L.T.R. Dorsal fins are

187 also longer in the Y.R., but gape width and the position of the mouth relative to the tip of the

188 snout shows no difference. Females in the L.T.R. have a wider inter-orbital width, but females in

189 the Y.R. had a wider body at the pectoral fin insertion. Overall, 13 of 29 characters did not have

190 overlapping 95% CIs for at least one sex between drainages. Mean SL for both males and

191 females in the Y.R. is longer than for males and females in the L.T.R. (Table 10).

192

193 *Geometric morphological analyses*

194 The outlier function in MorphoJ identified two females and two males in the L.T.R. as

195 potentially biasing results and these were removed. No individuals from the Y.R. were identified

196 as possible outliers.

197   There were significant differences in shape between females in the L.T.R. (n = 51) and Y.R.

198 (n = 60) drainages (DA, PD = 0.014, p <0.0001; M = 3.59, p <0.0001). Classification of

199 specimens to groups shows that 12% of individuals from the L.T.R. were incorrectly assigned to

200 the Y.R. drainage and 17% of individuals from the Y.R. were incorrectly assigned to the L.T.R.

201 drainage. Yocona River females had shorter snouts and heads (points 4 and 16), greater body

202 depth, longer second dorsal fin (point 7), longer anal fin associated with an anterior shift in the

203 insertion (point 12), and the posterior edge of the hypural plate shows a shift anteriorly (Figure

204 6).

205   There were significant differences in shape between males in the L.T.R. (n = 50) and Y.R. (n

206 = 49) (DA, PD = 0.009, p <0.0001; M = 2.78, p <0.0001). Classification of specimens to groups

207 shows that 32% of individuals from the L.T.R. were incorrectly assigned to the Y.R. and 29% of

208 individuals from the Y.R. were incorrectly assigned to the L.T.R. Shape changes between

209 drainages show that Y.R. males had shorter snouts and heads (points 4 and 16), greater body

210 depth, and longer second dorsal fins (point 7) (Figure 6).

211   There were significant differences in shape between males (n = 50) and females (n = 51)

212 within the L.T.R. drainage (DA, PD = 0.015, p <0.0001; M = 3.05, p <0.0001. Classification of

213 specimens to groups shows that 20% of females were incorrectly classified as males and 25% of

214 males were incorrectly classified as females. Relative shape changes between males and females

215 show that females are thinner with longer caudal peduncles, the insertion of the pectoral and

216 pelvic fins shifted anteriorly, and the insertion of the anal fin shifted posteriorly relative to males

217 (Figure 7).

218   There were also significant differences in shape between males (n = 49) and females (n = 60)

219 within the Y.R. drainage (DA, PD = 0.013, p <0.0001; M = 4.63, p <0.0001). Cross-validated

220 classification of specimens to groups shows that 3% of females were incorrectly classified as

221 males and 5% of males were incorrectly classified as females. Relative shape changes between

222 males and females show that females are thinner, the snout is shorter, the anal and second dorsal

223 fins are shorter, the insertion of the pectoral and pelvic fins shifts anteriorly, and the insertion of

224 the anal fin shifts posteriorly relative to males (Figure 7).

225 Comparison of watershed area and stream gradients between drainages show that *E. raneyi* in

226 the L.T.R. mostly occur in larger streams with lower gradients compared with the Y.R. (Figure 8,

227 Table S1). Mean watershed area is >twice as large in the L.T.R., and mean gradient is about 45%

228 greater in the Y.R. drainage. Confidence intervals do not overlap for area or gradient.

229

230 *Taxonomy*

231

232 *Etheostoma faulkneri* Sterling and Warren, Yoknapatawpha Darter (Figure 4)

233   urn:lsid:zoobank.org:act:B9FE97A8-A86C-41A9-9CD6-2929362E0C22

234

235 *Etheostoma* (*Ulocentra*) sp. Bouchard, 1974: 41 (distribution).

236

237 *Etheostoma* sp. Clemmer et al., 1975: 8 (listing of an undescribed species of darter in the upper

238   Yazoo River basin, categorized as rare). Jenkins, 1976: 644 (listing of an undescribed

239   species, distribution).

240

241 *Etheostoma* (*Ulocentra*) sp. Kuehne & Barbour, 1983: 99-100 (brief characterization of the

242   "Yazoo Darter" and distribution). Knight & Cooper, 1987: 31-32 (brief description of

243   watershed-scale habitat before additional extensive habitat alteration), 36 (brief description

244   of meso-habitat and distribution within the Otoucalofa Creek drainage), Table 1 (occurrence

245   record).

246

247 *Etheostoma* sp. Page & Burr, 1991: 302, pl. 43, map 345 (brief characterization of the "Yazoo

248   Darter" and distribution.

249

250 *Etheostoma raneyi*, Suttkus et al., 1994: 98-109 (distribution, habitat, description of male and

251   female color, pigment patterns, meristic characters, and morphological measurements

252   subsumed under the description of *Etheostoma raneyi*), fig. 8 (ordination of morphometric

253   data), fig. 9 (distribution). Johnston & Haag, 1996: 47-60 (life history, distribution). Ross &

254   Slack, 2000: 1 (conservation status, distribution), fig. 1 (distribution), fig. 4 (photograph of a

255   male Yazoo Darter from an unknown location). Ross, 2001: 483-484 (distribution and

256   general description and life history account, conservation status). Adams & Warren, 2005:

257   fig. 4 (scatterplot showing post-drought CPUE and immigration probabilities in stream

258   reaches that went dry during an extended drought), Appendix 1 (list of immigration

259   probabilities and standard deviation). Powers & Warren, 2009 (phylogeography and

260   distribution). Near et al., 2011: fig. 3c (phylogenetic relationships among darters), fig. 4

261   (phylogenetic classification among darters). Page & Burr, 2011: 558, pl. 49 (Brief

262   characterization of the "Yazoo Darter" and distribution). Schaefer et al., 2012: Appendix

263   (occurrence and abundance). Sterling et al., 2012: 859-872 (population genetics and effects

264   of habitat alteration on gene flow). Sterling et al., 2013: 816-842 (distribution, abundance,

265   life history, and conservation assessment). Kozal et al., 2017: Table 1 (collection data),

266   Table 4-10 (meristic data), 279, Table 11, and fig. 7 (results from meristic analyses), 279,

267   fig. 5, (phylogenetic results). Sterling & Warren, 2017: 1223-1235 (microhabitat use).

268   Sterling et al., 2020.

269

270 *Holotype*: Adult male, USNM439004 (Figure 4), 43.3 mm SL, Morris Creek, tributary to the

271 Yocona River (Upper Yazoo River system) at County Road 321, 9.28 km south of the Lafayette

272 County courthouse in Oxford (34.283, -89.544), Lafayette County, MS, 1 April 2018, B.D.

273 Sterling, K.A. Sterling, and W.M. Sterling.

274

275 *Allotype*: Adult female, USNM439005 (Figure 4), 41.2 SL, collected with the holotype.

276

277 *Paratopotypes*: USNM439006 (1 male), collected with the holotype; USNM439007 (2 males, 1

278 female), 10 April 2014, Morris Creek at the holotype location; MMNS79801 1 (4 males), 22

279 April 2014, Morris Creek at the holotype location.

280

281 *Paratypes*: MMNS79802 (2 males, 4 females), 11 June 1999, Pumpkin Creek at County Road

282 266, Lafayette County, Mississippi, 12.5 km E of Oxford ; MMNS79803 (2 males, 5 females), 9

283 June 1999, Yellow Leaf Creek on private property, Lafayette County Mississippi, 6.5 km E of

284 Oxford; MMNS79804 (1 male, 3 females), 10 April 2014, Gordon Branch at CR 121, Yalobusha

285 County, Mississippi, 7.6 km ESE of Water Valley; MMNS79805 (1 female), 10 April 2014,

286 Johnson Creek at County Road 436, Yalobusha County, Mississippi, 3.9 km S of Water Valley;

287 MMNS79806 (4 males), 21 April 2014, Gordon Branch at CR 121, Yalobusha County,

288 Mississippi, 7.6 km ESE of Water Valley; MMNS79807 (5 males, 1 female), 22 April 2014, Mill

289 Creek at State Highway 315, Yalobusha County, Mississippi, 5.7 km WSW of Paris;

290 MMNS79808 (3 males, 2 females), 12 October 2017, Splinter Creek at State Highway 328,

291 Lafayette County, Mississippi, 8.9 km N of Water Valley; USNM439008 (2 males, 4 females),

292 12 October 2017, Splinter Creek at County Road 348, Lafayette County, Mississippi, 10 km N of

293 Water Valley; MMNS79809 (4 females, 1 juvenile), 9 June 1999, Yellow Leaf Creek on private

294 property downstream of a confluence with an unnamed tributary, Lafayette County, Mississippi,

295 8.2 km E of Oxford and 16.3 km SSE of Abbeville; TU152109 (4 males, 6 females), 11 May

296 1988, Un-named tributary to Taylor Creek at Old Taylor Road, Lafayette County, Mississippi,

297 2.7 km N of Taylor; TU3116 (5 males, 6 females), 24 May 1952, Pumpkin Creek at State

298 Highway 6, Lafayette County, Mississippi, 12 km ESE of Oxford; TU155210 (6 males, 15

299 females), 14 June 1989, Un-named tributary to Otoucalofa Creek at State Highway 32,

300 Yalobusha County, Mississippi, 4.2 km SSE of Water Valley; TU155225 (1 males, 2 females),

301 15 June 1989, Gordon Branch at County Road 121, Yalobusha County, Mississippi, 7.8 km ESE

302 of Water Valley.

303

304 *Diagnosis and description*

305 *Etheostoma faulkneri* is one of at least 27 species, undescribed forms, or Evolutionary

306 Significant Units of snubnose darters in the unranked clade Adonia (s*ensu* Near et al., 2011; see

307 Blanco, 2001; Brogdon et al., 2003; Boschung & Mayden, 2004; Gabel, 2007; Kozal et al., 2017)

308 (Table S2) and is indicated as the sister species of *E. raneyi* (Sterling et al., 2020). Consistent

309 with other Adonia snubnose darters, *E. faulkneri* lack a frenum and usually have several long,

310 thin teeth on the vomer (Suttkus et al., 1994; Kozal et al., 2017). Modal lateral line scale counts

311 differ between *E. faulkneri* (45) and *E. raneyi* (47). *Etheostoma faulkneri* males and females

312 have shorter snouts and heads and more robust bodies relative to *E. raneyi* and are longer.

313 Compared with *E. raneyi*, male *E. faulkneri* usually have a smaller or no clear window in the

314 blue anal fin and more extensive blue pigment on the anal fin, second dorsal fin, procurrent rays

315 of the caudal fin, the cheek, opercle, and tip of the snout during the spawning season.

316 *Etheostoma faulkneri* also lack orange pigment in the anal fin which is present in a small

317 proportion (17.7%) of male *E. raneyi*, and the black pigment on the opercle, cheek, chin,

318 branchiostegal rays, pelvic fins, second dorsal fin, and caudal fin is generally less dense and less

319 extensive for *E. faulkneri* than for *E. raneyi*. This results in spawning male *E. faulkneri* usually

320 appearing brighter blue overall than *E. raneyi*, which appear duskier overall (Figure 4).

321   *Etheostoma faulkneri* can be distinguished from *E. raneyi* by fixed allele differences at 12

322 loci on the mitochondrial *cytb* gene and one difference and two deletions on the nuclear *S7* gene

323 (Sterling et al., 2020). Maximum length for *E. faulkneri* is a 64 mm SL male sampled from

324 Morris Creek. For females, three 49 mm SL individuals were each sampled from Yellow Leaf,

325 Johnson, and Morris creeks. Maximum SL for *E. raneyi* is 57 mm for males and 50 mm for

326 females (unpubl. data).

327   The lateral line is complete with 41-51 scales (mode = 45) (Table 3). The last scale is

328 sometimes unpored. Transverse scales range from 11-15 (Table 4), and scales around the caudal

329 peduncle range from 16-19 (Table 5). Dorsal fin spines range from 9-11 (mode = 10), second

330 dorsal fin rays range from 9-11 (mode = 11) (Tables 6-7), anal fin rays range from 6-8 (mode =

331 7), and pectoral fin rays range from 13-15 (mode = 14) (Tables 8-9). The belly, nape, cheek, and

332 opercle are scaled, and the breast is usually naked but frequently has several scales, especially

333 for larger individuals. The prepectoral area is often scaled, but for some individuals scales are

334 limited in extent.

335   The spinous dorsal fin of male *E. faulkneri* have a bright turquoise margin, an incomplete

336 medial band of bright red in the posterior 3-5 interradial membranes that fades anteriorly into a

337 metallic gold color best seen against a black background but appears as beige patches of pigment

338 against light backgrounds (described as pale cream in Suttkus et al., 1994). A band of black that

339 is usually incomplete is basal to the red and gold band and is present in the anterior 1-6 fin

340 membranes. During the spawning season, this black band is often suffused or overlain with

341   turquoise pigment. The soft dorsal fin has a diffuse posterior patch of turquoise on the fin

342   margin; the turquoise pigment becomes limited to the fin rays anteriorly and does not compose a

343   band of color (there is no marginal band of turquoise in the soft dorsal fin as described in Suttkus

344   et al., 1994 for either *E. raneyi* or *E. faulkneri*). A bright orange medial band of color in the

345   interradial membranes extends from the posterior edge of the soft dorsal fin and fades and

346   becomes thinner anteriorly through 5-8 membranes. A basal patch of black pigment often occurs

347   where the fin meets a dorsal saddle. Black pigment is frequently present in the interradial

348   membranes of the fin but is usually less dense and less extensive in area compared with *E.*

349   *raneyi*, though in some individuals the black pigment is well developed.

350   The anal and pelvic fins are bright turquoise in nuptial males. A small, thin, clear to creamy

351   medial window in the anal fin is often present in the posterior 2-3 interradial membranes.

352   Turquoise pigment is well developed from the margin of the anal fin to the belly. In contrast, *E.*

353   *raneyi* usually have larger windows that cover 3-4 membranes and are wider. In some

354   individuals, black pigment is present in the membranes of the pelvic fins, which is usually denser

355   and more extensive in *E. raneyi*. The pectoral fins are mostly devoid of pigment, but the rays

356   have intense orange pigment proximal to the fin insertion. The caudal fin is mostly clear, but the

357   procurrent rays of nuptial males are bright turquoise blue, and the primary rays may have some

358   green, blue, or black pigment.

359   The background color of adult males is a warm cream color dorsally that becomes paler

360   ventrally. Large males have intense dark orange pigment from the mid-ventral area up to the

361   mid-lateral stripe that fades to a lighter, bright orange anteriorly. In some males, the orange is

362   also present in the prepectoral area. In smaller males, the orange is reduced to a band of pigment

363   along the side of the belly that does not extend to the mid-lateral stripe or to the anal fin. Two

364 basicaudal spots of orange are almost always present. The mid-lateral stripe consists of a series

365 of blotches bisected by a depigmented lateral line that becomes indistinct posteriorly. Blotches

366 alternate between a dark brown to black color and a dark red color. The dark red blotches are

367 usually elongate rectangles. The dark brown or black blotches are often the ventral portion of a

368 y-shaped pattern that extends to the dorsal fins and is connected by the dorsal saddles, of which

369 there are eight (see Figure 4). However, these blotches are irregularly shaped and patterns are

370 often irregular, especially anteriorly. The dark brown and black pigment becomes an irregular

371 series of spots and blotches on the head. A dark to dusky suborbital bar extending below the eye

372 is usually present as is a dark pre-orbital stripe. The top of the head is the same dark brown as the

373 dorsal saddles. In nuptial males, there may be green to turquoise tint to the top of the head and

374 anterior saddles as well as the tip of the snout. A clear, bright turquoise color is present on the

375 pre-opercle, opercle, cheek, chin, branchiostegal rays, and breast. In some individuals, black

376 spots of pigment are present in these areas which cause the bright turquoise to appear dusky, an

377 effect more often seen in *E. raneyi*.

378   Females are more cryptically colored although the pigment patterns of the mid-lateral stripe

379 and dorsal surface of the body are highly similar to the male. The background color is a neutral

380 tan dorsally and becomes an unsullied bright white ventrally. The mid-lateral stripe is an

381 alternating series of dark maroon to warm brown and dark brown to black blotches bisected by

382 an unpigmented lateral line that becomes more indistinct posteriorly. The maroon or warm

383 brown blotches are elongate rectangles and the dark brown to black blotches are usually irregular

384 in size and shape, sometimes connected to the dorsal saddles. Blotches become more irregular

385 anteriorly and are small on the head and opercle. A dark to dusky pre-orbital stripe and

386 suborbital bar usually are present. The belly is usually devoid of pigment, though larger females

387   may have some small spots of orange pigment. Two orange basicaudal spots are almost always

388   present. In nuptial females, a light wash of turquoise may occur on the breast, tip of the snout,

389   and top of the head. Fins are all mostly devoid of bright pigment with clear membranes and a

390   series of elongate dark brown to black areas of pigment on the straw-colored dorsal and caudal

391   fin rays. Occasionally, the red median band in the spinous dorsal fin of males is present in

392   females though the color is much less intense and often appears as an indistinct series of spots in

393   the most posterior 1-3 membranes. The pelvic, pectoral, and anal fins are usually clear though an

394   indistinct wash of turquoise may be present in the anal fin.

395

396   *Distribution: Etheostoma faulkneri* is endemic to perennial headwater tributaries of the Y.R.

397   drainage. To the east, distribution appears limited by the increased presence of intermittent

398   streams in terrain with less relief and more extensive agricultural land (Figure 1). Otoucalofa

399   Creek, a large tributary to the Y.R., may have the largest connected population of *E. faulkneri*

400   (Sterling et al.; 2012, 2013). The species is known from 33 sites in 20 streams; 11 streams are in

401   the Otoucalofa Creek system (Table S3), and future sampling will likely yield *E. faulkneri* in

402   several additional streams that appear to have suitable habitat. However, repeated sampling

403   indicates that *E. faulkneri* are uncommon in most streams of occurrence and may even be

404   extirpated from Smith Creek in Calhoun County (unpubl. data). The earliest known collection

405   was by R.D. Suttkus on May 24, 1952 when 11 individuals were taken in Pumpkin Creek at State

406   Highway 6, Lafayette Co., Mississippi (Tulane University: TU3116).

407

408   *Habitat and biology*

409   The biology and habitat use of *E. faulkneri* is apparently similar to that of *E. raneyi* (Knight &

410   Cooper, 1987; Suttkus et al., 1994; Sterling & Warren, 2017). A life history study of *E. faulkneri*

411   was conducted at Morris Creek (Johnston & Haag, 1996), results of which are consistent with the

412   literature for other clade Adonia snubnose darters (Carney & Burr, 1989; Khudamrongsawat &

413   Kuhajda, 2007; Barton & Powers, 2010). In our experience in the field, *E. faulkneri* are most

414   commonly sampled from less-disturbed small streams with strong perennial flow and plentiful

415   instream cover (wood or hard clay riffles). In streams lacking cover because of channelization,

416   incisement, and sedimentation, the species is often collected from the rubble, wood pilings, and

417   human refuse associated with bridge crossings and is sparse elsewhere. The first known

418   description of habitat use by *E. faulkneri* (specific to the Otoucalofa Creek watershed) indicates

419   that the species are usually found in densely canopied first-order streams that have not been

420   clear-cut, and are associated with hard-clay riffles (Knight & Cooper, 1987).

421

422   *Conservation management*

423   *Etheostoma faulkneri* is restricted to one headwater drainage in the upper Yazoo River basin with

424   a total area of only about 1500 km^2 (relative to about 3200 km^2 for *E. raneyi*) and is distributed

425   almost entirely on private lands (Sterling et al., 2013). As described earlier, the species has low

426   genetic diversity and is nowhere abundant outside of small stream reaches associated with some

427   road crossings. The upper Yazoo River basin is highly modified, in common with neighboring

428   drainages to the north in western Tennessee with snubnose darters, and stream alterations have

429   obliterated most suitable habitat (Shields et al., 1995; Warren et al., 2002; Keck & Etnier, 2005;

430   Fore et al., 2019). Urban development in the Y.R. drainage is increasing rapidly in association

431   with the explosive growth of the city of Oxford and, to a lesser extent, Water Valley, MS. As a

432 result, *E. faulkneri* faces increasing habitat degradation through time across most of its range.

433 However, the Otoucalofa Creek watershed (Figure 1) is not yet under the direct threat of

434 increased rates of development and appears to hold the largest connected population of *E.*

435 *faulkneri* (Sterling et al.; 2012, 2013). Land use is still mostly dominated by timber plantations

436 and row crop agriculture upstream of Water Valley. The USFWS (Jackson, MS) and USDA

437 NRCS (Jackson, MS) have also taken steps to improve aquatic organism passage and riparian

438 habitat in two streams in the Otoucalofa Creek watershed (NRCS, 2020; USFWS, 2020). Time

439 possibly remains for the public to become aware that a unique part of their natural heritage

440 swims in the streams in their backyards, on their farms, and the lands they hunt. Increased

441 awareness may help to protect enough habitat for *E. faulkneri* to persist in the Otoucalofa Creek

442 watershed over the coming century.

443

444 *Etymology*

445 We have named the species *Etheostoma faulkneri* to honor the great writer and Nobel Laureate

446 William C. Faulkner (1897-1962), a native of the Oxford, Mississippi area who was also an avid

447 hunter and fisher. The landscape was an important theme in many of his works, and the actions

448 of his characters were often influenced by the lands and streams surrounding his fictional

449 Jefferson, Mississippi, including the Yocona River, which he renamed the Yoknapatawpha.

450

451 **Discussion**

452 *Etheostoma faulkneri* masqueraded as *E. raneyi* for decades mostly because of the lack of

453 distinctive color differences in nuptial males during spawning. Even so, patterns of distribution

454 and quantitative differences in lateral line scale counts (Table 3), male nuptial color (Figures 4

455   and 5), length (Table 10), and morphology (Table 10; Figure 6) between individuals sampled

456   from the Y.R. and L.T.R., as well as population genetic and phylogenetic data (Powers &

457   Warren, 2009; Sterling et al.; 2012, 2020), support the recognition of *Etheostoma faulkneri* as a

458   separately evolving metapopulation and valid species under the unified species concept (de

459   Queiroz, 2007).

460   Species descriptions of other snubnose darters have often relied on colors of breeding males

461   and examination of meristic and morphological characters for diagnosis. Though distinct

462   differences in male color among closely related taxa are sometimes described (Boschung et al.,

463   1992), subjective descriptions of color differences are likely to vary according to the source

464   (Suttkus & Bailey, 1993). In some cases, described differences in male color are less than

465   distinct (Suttkus et al., 1994) and are not reliable across the range of individual variation within

466   species (see *Ulocentra* key in Boschung & Mayden, 2004). Qualitative differences in meristic

467   and morphological characters are rare among Adonia snubnose darters and are limited to counts

468   of branchiostegal rays which separate *E. coosae* from the rest of the clade (except for *E. scotti*,

469   Bauer et al., 1995). Our results are consistent with these observations and the quantitative

470   differences that we detected are similar to those found for other species of snubnose darters

471   (Suttkus et al., 1994; Bauer et al., 1995; Powers & Mayden, 2003).

472   Because pigment patterns, meristics, and body shape cannot reliably distinguish individuals

473   from the L.T.R. and Y.R., and the only diagnosable characters that qualitatively distinguish

474   between *E. faulkneri* and *E. raneyi* are genetic (Powers & Warren, 2009; Sterling et al., 2020), it

475   is reasonable to categorize *E. faulkneri* as a cryptic species. Cryptic species can occur through

476   several mechanisms that are not mutually exclusive: recent divergence, phylogenetic niche

477   conservatism, and evolutionary convergence (Fišer et al., 2017). Evolutionary convergence is

478   clearly not a factor. However, the estimated time of separation between *E. faulkneri* and *E.*

479   *raneyi* (<1 my) is recent (Sterling et al., 2020) and niche conservatism is apparent between the

480   two species (Johnston & Haag, 1996; Sterling et al., 2017; Ruble et al., 2019) and other snubnose

481   darters as well (O'Neil, 1981; Carney & Burr, 1989; Hicks, 1990; Khudamrongsawat &

482   Kuhajda, 2007; Barton & Powers, 2010; Hubbell & Banford, 2019). *Etheostoma faulkneri* and *E.*

483   *raneyi* exist in adjacent drainages with little latitudinal gradient, no elevational gradient, and no

484   clear differences in surface geology, water chemistry, or aquatic communities. Their Eltonian

485   and Grinnellian niches appear to be identical in all respects but one: there is a small difference in

486   stream size and gradient between streams that the two species inhabit. Though this difference has

487   likely influenced the subtle morphological divergence that we detected, it is clear that in the

488   absence of other biotic or abiotic differences between the L.T.R. and Y.R. drainages, stabilizing

489   selection for their ancestral niche across a short time span since separation has prevented the

490   evolution of readily observable divergent morphological, meristic, or color characters.

491   Cryptic diversity is common among diverse taxa (Pfenninger & Schwenk, 2007; Adams et al.,

492   2014; Fennessy et al., 2016), including freshwater fishes in North America (Egge & Simons,

493   2006; April et al., 2011). Among darters (Etheostomatinae), cryptic diversity is especially

494   common (April et al., 2011) and is linked to niche conservatism, vicariant events and allopatric

495   distributions, and relatively stable habitat conditions over geologic time-scales (Bauer et al.,

496   1995; Page et al., 2003; Near & Benard, 2004; Hollingsworth & Near, 2009; Kozal et al., 2017).

497   These conditions are consistent with the literature on *E. faulkneri* and *E. raneyi* and our results

498   (Johnston & Haag, 1996; Powers & Warren, 2009; Sterling & Warren, 2017; Sterling et al.,

499   2020), as they are for other Adonia snubnose darters (Carney & Burr, 1989; Bauer et al., 1995;

500   Powers & Mayden, 2003; Khudamrongsawat & Kuhajda, 2007; Kozal et al., 2017).

501

Conclusions

503 The results we present here, existing genetic evidence (Powers & Warren, 2009; Sterling et al.;

504 2012, 2020), and a growing acceptance among biologists that the presence of readily observable

505 qualitative diagnostic characters are not necessary to describe biodiversity (Egge & Simons,

506 2006; de Queiroz, 2007; Fišer et al., 2017), supports the recognition and description of

507 *Etheostoma faulkneri*, the Yoknapatawpha Darter, as an independently evolving metapopulation

508 lineage and valid species under the unified species concept (de Queiroz, 2007). The description

509 of *E. faulkneri* represents an increase in the accuracy of our understanding of freshwater fish

510 evolution and diversity, which is the foundation for research and conservation efforts for the

511 many imperiled freshwater fishes of the southeastern United States.

512

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Figure 1

Map of northern Mississippi showing the distribution of *Etheostoma raneyi* (Yazoo Darter) and *E. faulkneri* (Yoknapatawpha Darter).

Distribution is indicated by green circles in the Little Tallahatchie River drainage (Yazoo Darter) and green triangles in the Yocona River drainage (Yoknapatawpha Darter). The location of the old confluence (blue square) of the two drainages is shown as well as the new confluence (blue diamond) after stream alterations; the approximate boundaries of the Mississippi Alluvial Plain, Lower Gulf Coastal Plain, and Upper Gulf Coastal Plain are indicated from west to east, respectively.

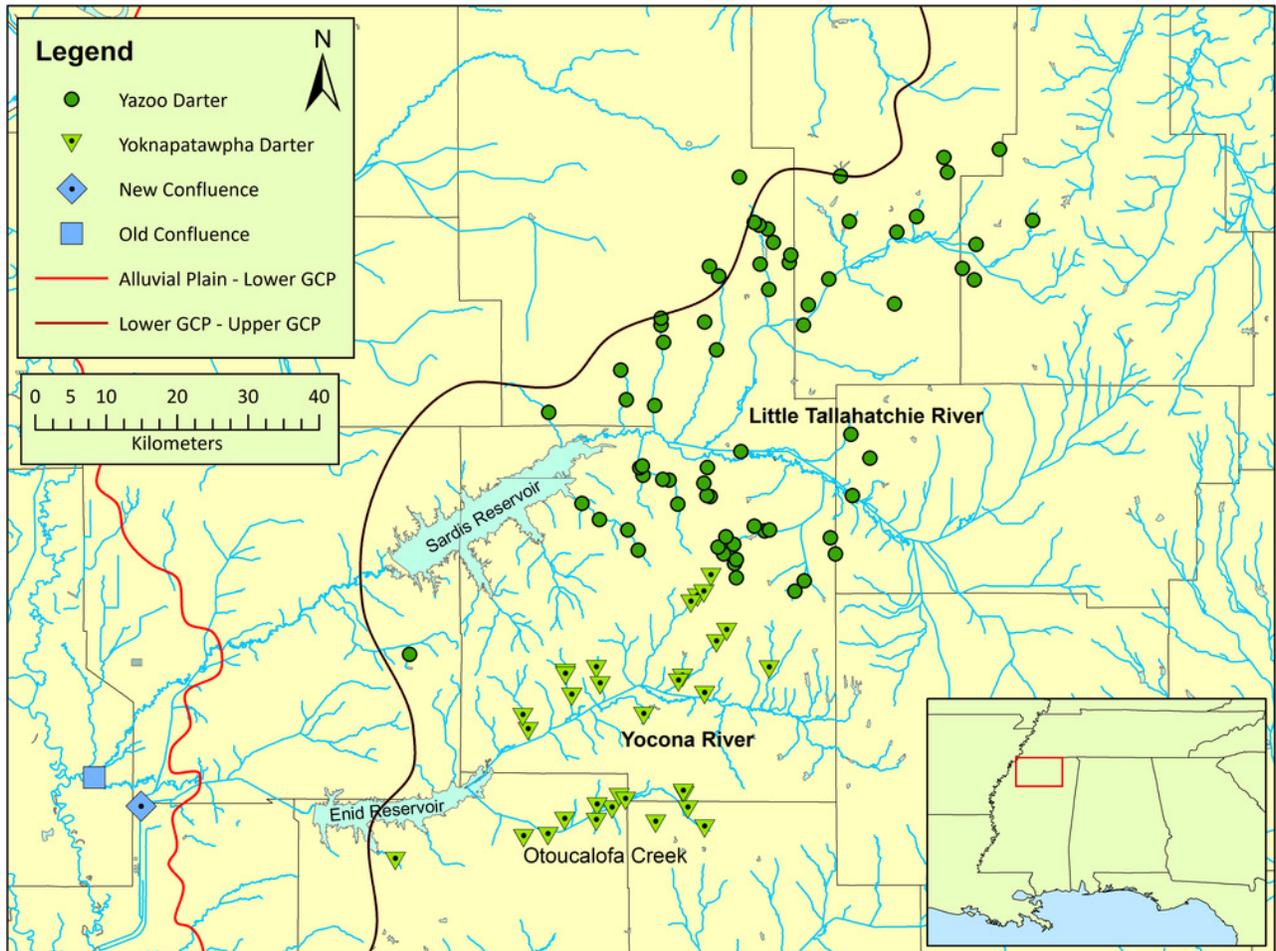



Figure 2

Location of landmarks used for truss measurements is shown (after Powers & Mayden, 2003).

Numbers correspond to Table 10.

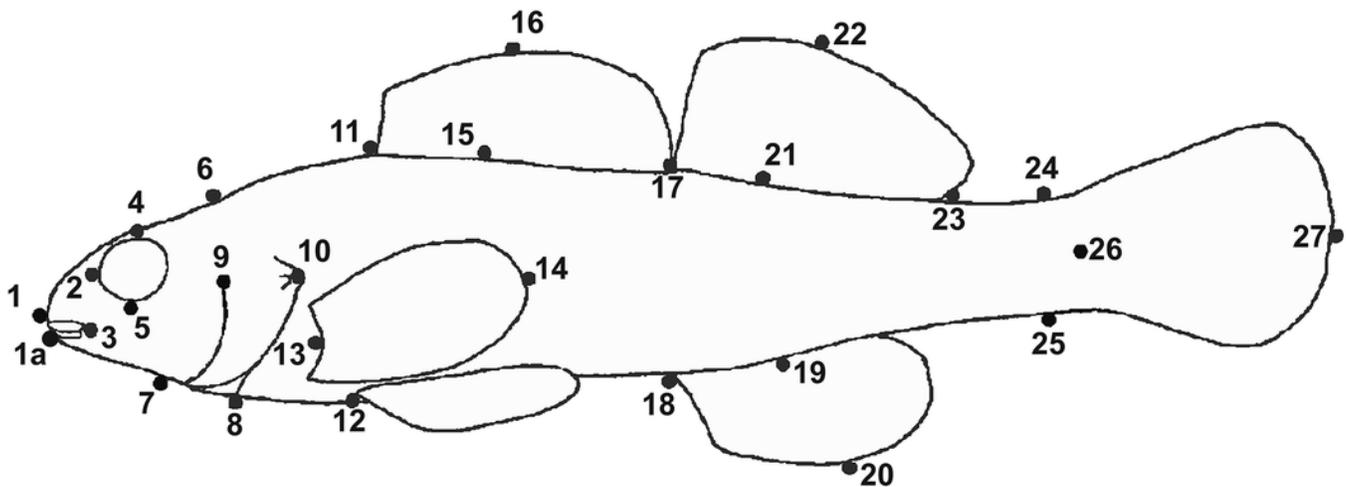



Figure 3

Landmark locations used for geometric morphological analyses are shown.

The label Y1 in the photograph indicates individual number one from the Yocona River drainage.

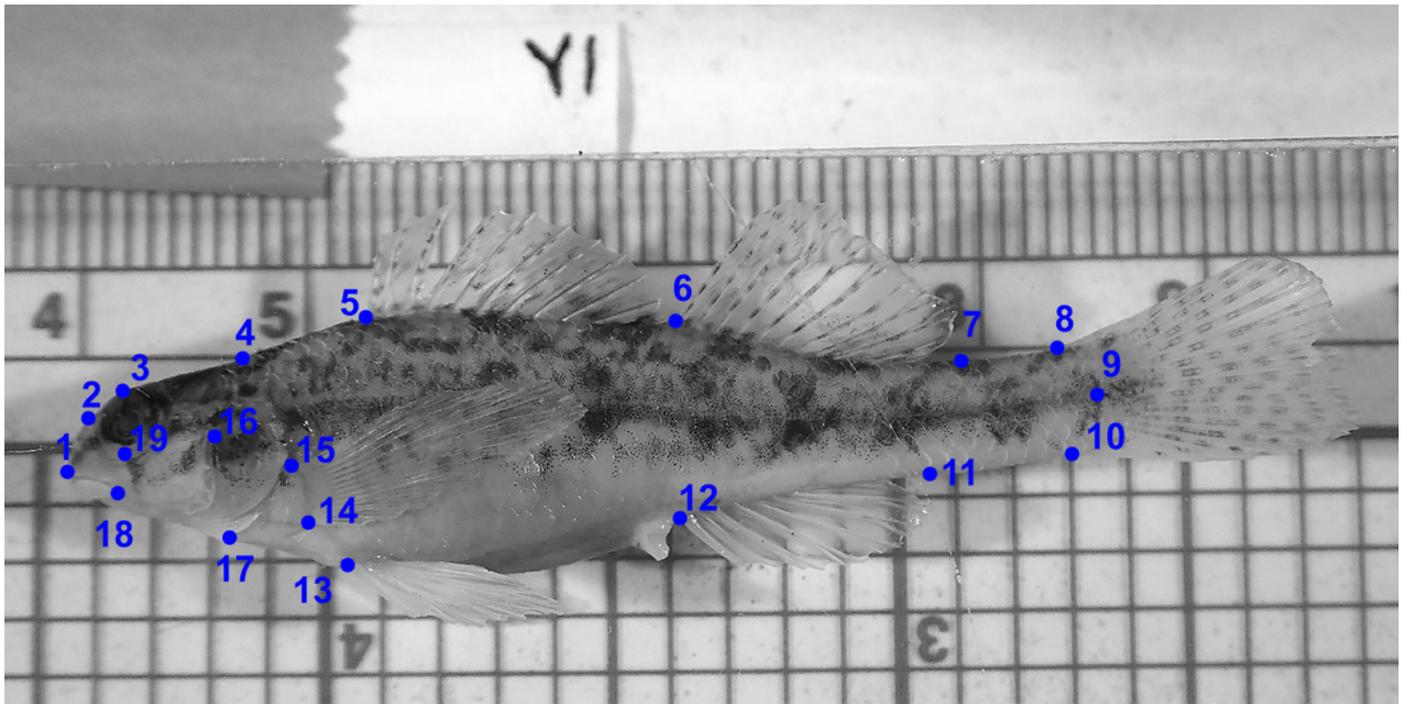



Figure 4

Photographs of *Etheostoma faulkneri* (Yoknapatawpha Darter) and *E. raneyi* (Yazoo Darter).

Shown are *E. faulkneri* male holotype (c) and female allotype (a) (see text for specimen data), and male (d) and female (b) *E. raneyi* for comparison; scale bar = 1 cm.

Figure 5

Bar graph showing the results of male nuptial color surveys.

Each graph (a, b, c) represents mean values (± 95% C.I.) scored by one of three colleagues for each character (Black, Blue, Window, see text for definitions of characters); L.T.R. = Little Tallahatchie River, n = 153; Y.R. = Yocona River, n = 108.

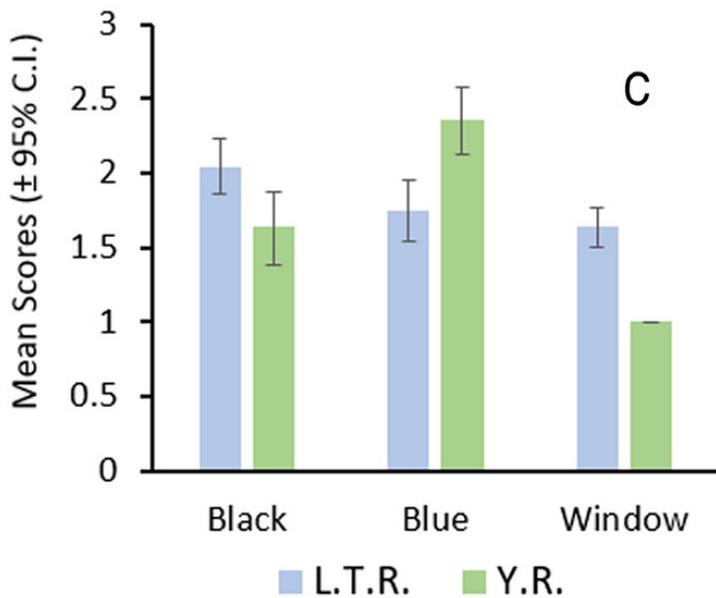
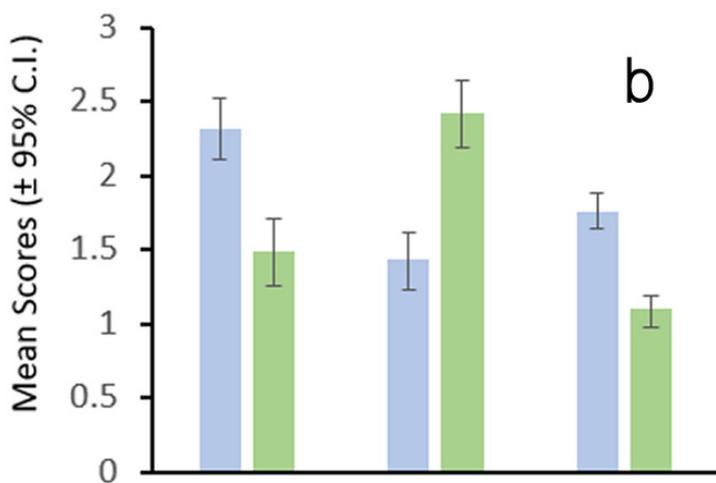
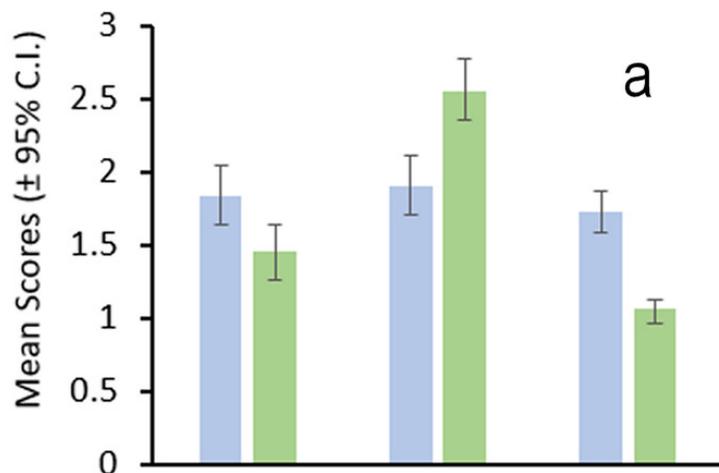



Figure 6

Output from lateral view geometric morphological analyses (MorphoJ) showing shape changes for male and female *Etheostoma raneyi* and *E. faulkneri* between drainages.

Numbers represent fixed points in the analysis (see Figure 3).

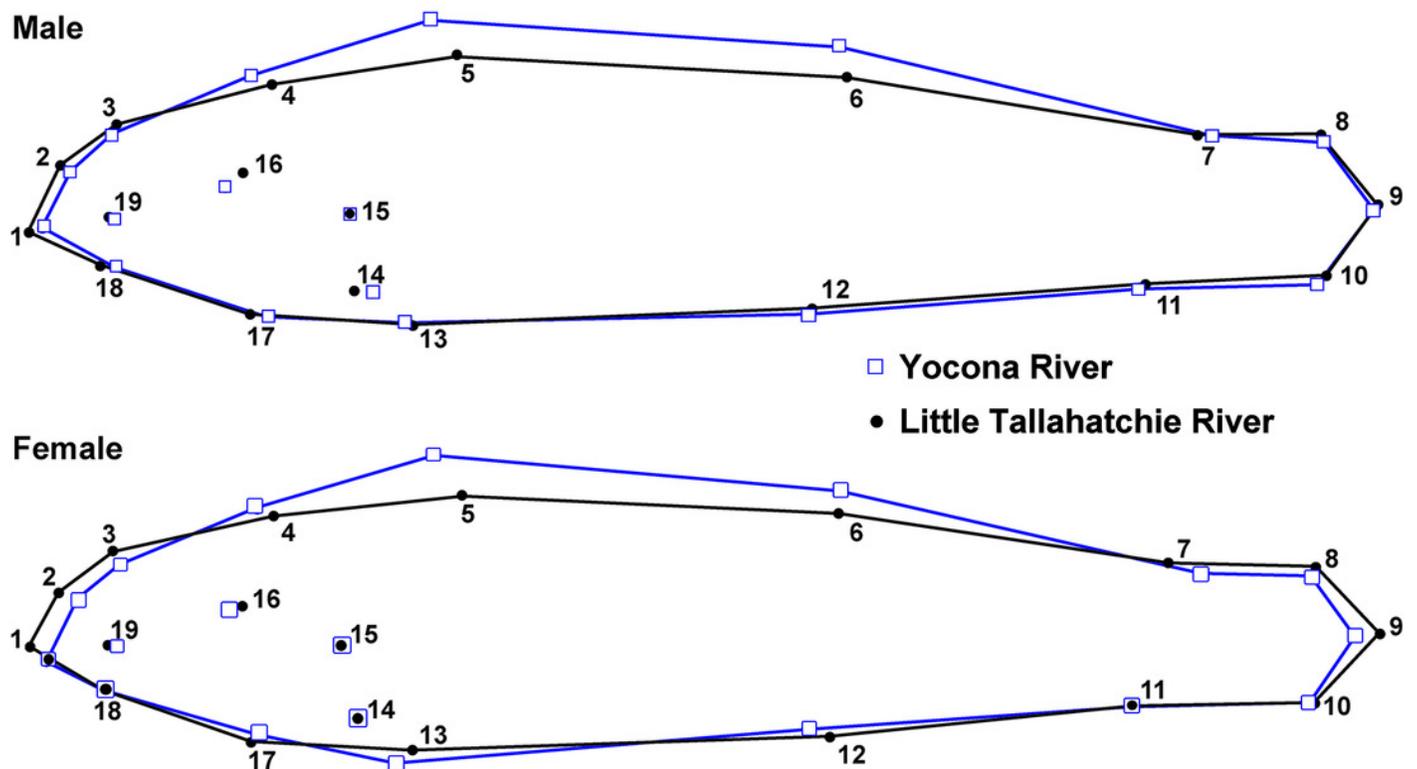



Figure 7

Output from lateral view geometric morphological analyses showing shape changes for male and female *Etheostoma raneyi* (Little Tallahatchie River) and *E. faulkneri* (Yocona River) between sexes within drainages.

Numbers represent fixed points in the analysis (see Figure 3).

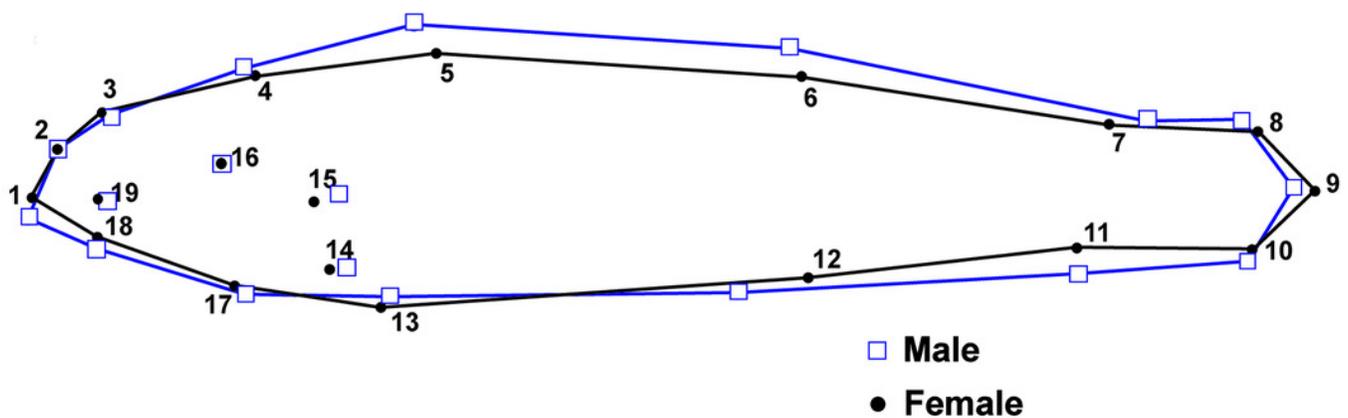
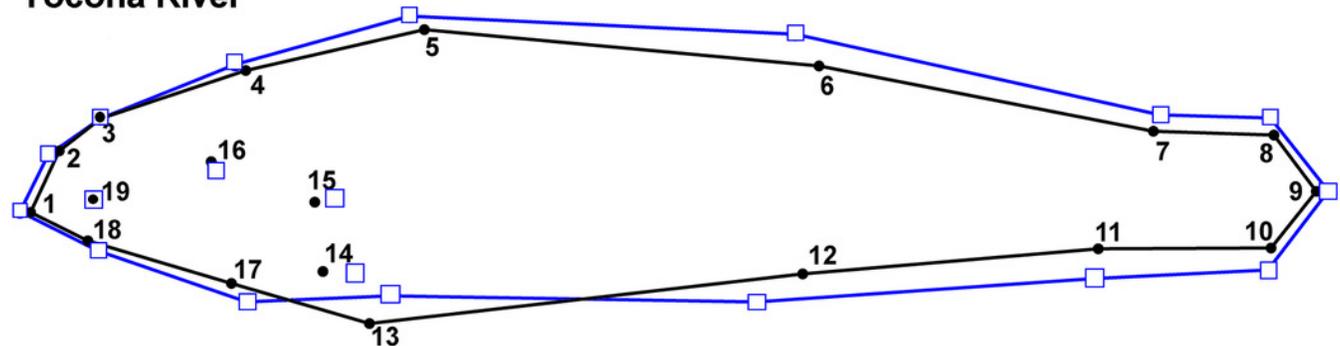
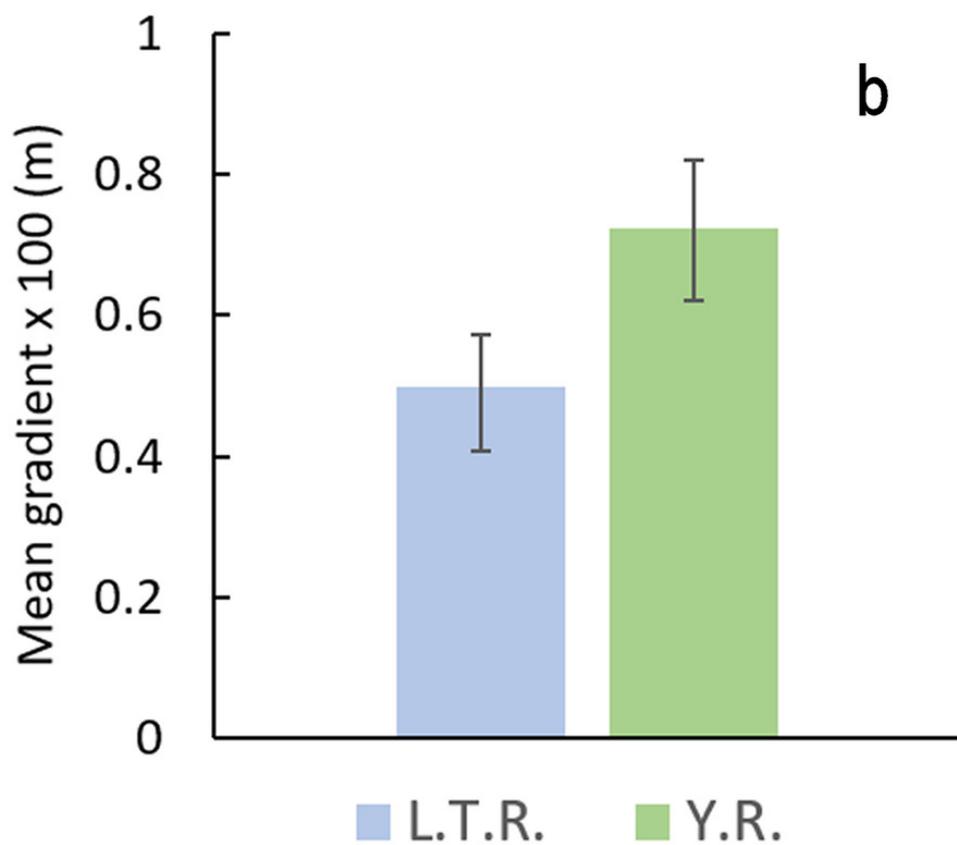
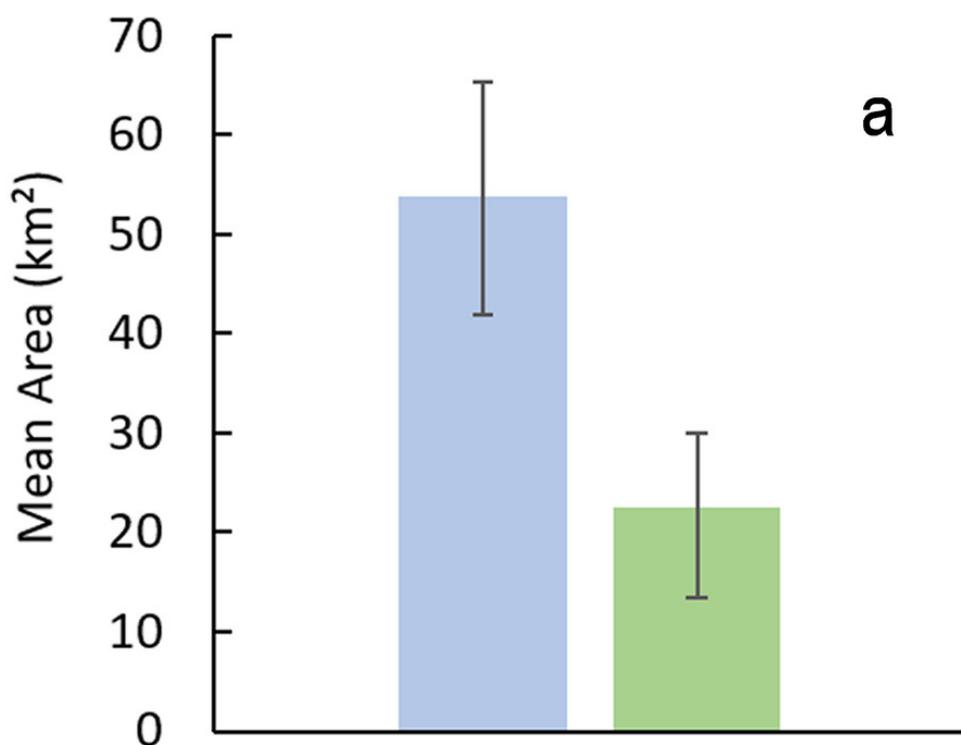



Figure 8

Mean stream gradient (b) and watershed area (a) (± 95% C.I.) are shown for all tributary streams with *Etheostoma raneyi* and *E. faulkneri*.

Abbreviations indicate Y.R. = Yocona River and L.T.R. = Little Tallahatchie River drainages.

Specimen data used for male nuptial color comparisons is shown.

Drainage basins are indicated by L.T.R. = Little Tallahatchie River (*Etheostoma raneyi*) and Y.R. = Yocona River (*E. faulkneri*); stream name, date of sample, water temperature, and the number of males photographed are also shown.

Drainage	Stream	Date	Temp. (°C)	n	Coordinates
L.T.R.	Bay Springs Branch	2/23/2017	15	12	34.428, -89.395
L.T.R.	Hurricane Creek	3/16/2017	9.5	7	34.425, -89.496
L.T.R.	Yellow Rabbit Creek	4/7/2017	14	7	34.819, -89.106
L.T.R.	Big Spring Creek	4/17/2017	16	9	34.664, -89.413
L.T.R.	Chewalla Creek	3/22/2018	8.5	7	34.725, -89.305
L.T.R.	Chewalla Creek	3/22/2018	13	5	34.76, -89.333
L.T.R.	Graham Mill Creek	3/22/2018	13	4	34.503, -89.491
	Total			**51**	
Y.R.	Morris Creek	2/24/2017	15	12	34.282, -89.544
Y.R.	Morris Creek	3/8/2018	11.5	5	34.282, -89.544
Y.R.	Morris Creek	4/1/2018	15.5	3	34.282, -89.544
Y.R.	Johnson Creek	4/6/2017	14	5	34.124, -89.641
Y.R.	Gordon Branch	4/6/2017	13	1	34.14, -89.549
Y.R.	Mill Creek	1/1/2017	-	3	34.167, -89.52
Y.R.	Mill Creek	3/8/2018	9.5	5	34.167, -89.52
Y.R.	U.T. Otoucalofa Creek	3/23/2018	11	2	34.126, -89.611
	Total			**36**	

Specimen data used for geometric morphological analyses (MorphoJ) is shown.

Locations and sample sizes for collections by drainage: L.T.R. = Little Tallahatchie River (*Etheostoma raneyi*); Y.R. = Yocona River (*E. faulkneri*); U.T. = unnamed tributary) are also given.

Drainage	Stream	Coordinates	Male n	Female n
L.T.R.	Yellow Rabbit Creek	34.819, -89.106	6	5
L.T.R.	Chewalla Creek	34.726, -89.305	13	7
L.T.R.	Bay Springs Branch	34.428, -89.395	8	0
L.T.R.	Big Spring Creek	34.664, -89.413	12	9
L.T.R.	Shelby Creek	34.844, -89.039	1	2
L.T.R.	Fice Creek	34.421, -89.247	1	1
L.T.R.	U.T. Tippah River	34.682, -89.281	1	1
L.T.R.	U.T. Tippah River	34.712, -89.254	0	4
L.T.R.	Oak Chewalla	34.583, -89.511	0	2
L.T.R.	Chilli Creek	34.682, -89.173	0	1
L.T.R.	Mitchell Creek	34.521, -89.203	0	1
L.T.R.	Puskus Creek	34.447, -89.345	1	5
L.T.R.	Cypress Creek	34.383, -89.299	5	6
L.T.R.	Lee Creek	34.498, -89.457	0	1
L.T.R.	Wagner Creek	34.768, -89.229	2	2
L.T.R.	Hurricane Creek	34.425, -89.496	2	6
	Total		**52**	**53**
Y.R.	Johnson Creek	34.124, -89.641	0	1
Y.R.	Morris Creek	34.282, -89.544	4	9
Y.R.	Mill Creek	34.167, -89.52	5	1
Y.R.	Gordon Branch	34.14, -89.549	9	4
Y.R.	Yellow Leaf Creek	34.376, -89.421	2	5
Y.R.	Pumpkin Creek	34.285, -89.445	14	9
Y.R.	U.T. Otoucalofa Creek	34.126, -89.611	6	19
Y.R.	Taylor Creek	34.297, -89.589	3	6
Y.R.	Smith Creek	34.168, -89.439	1	0
Y.R.	Splinter Creek	34.236, -89.635	5	6
	Total		**49**	**60**

Compilation of frequency distributions of lateral line scale counts for six species of snubnose darters and one undescribed form.

Modal counts shown in bold; sources of data are as follows: *Etheostoma cervus* (Bailey & Etnier, 1988; Powers & Mayden, 2003; Kozal et al., 2017); *E. pyrrhogaster*, *E. zonistium*, *E.* cf. *zonistium*, and *E. cyanoprosopum* (Bailey & Etnier, 1988; Kozal et al., 2017); S.D. = standard deviation.

	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	n	x̄	S.D.
E. cervus	2	11	30	**49**	40	29	8	3		2									174	39.5	1.51
E. pyrrhogaster			8	10	40	44	**47**	26	13	10	3								201	42.5	1.73
E. zonistium (Tennessee R.)				5	7	18	38	45	61	**85**	65	50	50						424	45.8	2.13
E. cf. zonistium (Hatchie R.)						5	5	6	12	**33**	22	15	15	5	2	1	1		122	45.7	2.11
E. cyanoprosopum									1	2	11	18	**31**	23	15	11	3	2	117	48.5	1.73
E. raneyi (Suttkus et al., 1994)						1	7	15	24	**34**	25	32	11	11	1	2	1	1	165	45.7	2.09
E. faulkneri (Kozal et al., 2017)									5	**10**	7	5	4	4	1				36	46.3	1.70
E. raneyi (new data)							3	3	9	19	21	**34**	21	19	15	7	1		152	47.2	2.10
E. faulkneri (new data)						1	5	6	16	**29**	22	19	13	7	1	1			120	45.8	1.88

Compilation of frequency distributions of transverse scale counts for six species of snubnose darters and one undescribed form.

Modal counts are in bold; sources of data not cited in the table are as follows: *Etheostoma cervus* (Powers & Mayden, 2003; Kozal et al., 2017); *E. pyrrhogaster*, *E. zonistium*, *E.* cf. *zonistium*, and *E. cyanoprosopum* (Kozal et al., 2017); S.D. = standard deviation.

	10	11	12	13	14	15	16	17	18	n	□	S.D.
E. cervus	3	20	**32**	11	3					69	11.9	0.89
E. pyrrhogaster	1	**16**	8	1	1					27	11.4	0.80
E. zonistium (Tennessee R.)			27	**82**	63	9				181	13.3	0.78
E. cf. *zonistium* (Hatchie R.)	4	**47**	7	1						59	11.1	0.50
E. cyanoprosopum			3	13	21	**35**	6	1	3	82	14.5	1.21
E. raneyi (Suttkus et al., 1994)		2	36	**57**	51	16	3			165	13.3	1.02
E. faulkneri (Kozal et al., 2017)		9	10	**17**						36	13.7	5.34
E. raneyi (new data)		17	42	**75**	19					153	12.6	0.84
E. faulkneri (new data)		2	38	**61**	19	1				121	12.8	0.74

Compilation of frequency distributions of caudal peduncle scale counts for six species of snubnose darters and one undescribed form.

Modal counts are in bold; sources of data not cited in the table are as follows: *Etheostoma cervus* (Bailey & Etnier, 1988; Powers & Mayden, 2003; Kozal et al., 2017); *E. pyrrhogaster*, *E. zonistium*, *E.* cf. *zonistium*, and *E. cyanoprosopum* (Bailey & Etnier, 1988; Kozal et al., 2017); S.D. = standard deviation.

	14	15	16	17	18	19	20	n	☐	S.D.
E. cervus	2	9	35	**69**	17	1	1	134	16.7	0.90
E. pyrrhogaster	4	18	**19**	14	1			56	15.8	0.96
E. zonistium (Tennessee R.)		6	38	**148**	68	32	2	294	17.3	0.92
E. cf. *zonistium* (Hatchie R.)	2	18	**41**	36				97	16.1	0.79
E. cyanoprosopum			5	**45**	28	26	8	112	17.9	1.05
E. raneyi (Suttkus et al., 1994)		16	57	**75**	13	4		165	16.6	0.86
E. faulkneri (Kozal et al., 2017)		9	**15**	10	1			35	16.1	0.82
E. raneyi (new data)	1	11	37	**66**	30	4		149	16.8	0.94
E. faulkneri (new data)			9	**59**	29	11		108	17.4	0.78

Compilation of frequency distributions of first dorsal fin spine counts for six species of snubnose darters and one undescribed form.

Modal counts are in bold; sources of data not cited in the table are as follows: *Etheostoma cervus* (Bailey & Etnier, 1988; Powers & Mayden, 2003; Kozal et al., 2017); *E. pyrrhogaster*, *E. zonistium*, *E*. cf. *zonistium*, and *E. cyanoprosopum* (Bailey & Etnier, 1988; Kozal et al., 2017); S.D. = standard deviation.

	9	10	11	12	n	☐	S.D.
E. cervus		59	**76**	1	136	10.6	0.51
E. pyrrhogaster	8	**42**	27	1	78	10.3	0.66
E. zonistium (Tennessee R.)	20	**252**	96	2	370	10.2	0.54
E. cf. *zonistium* (Hatchie R.)	8	**99**	15		122	10.1	0.43
E. cyanoprosopum	4	**60**	48	2	114	10.4	0.59
E. raneyi (Suttkus et al., 1994)	14	**114**	35	2	165	10.2	0.57
E. faulkneri (Kozal et al., 2017)	2	**31**	3		36	10.0	0.38
E. raneyi (new data)	18	**111**	24		153	10.0	0.52
E. faulkneri (new data)	8	**86**	21		115	10.1	0.49

Compilation of frequency distributions of second dorsal fin ray counts for six species of snubnose darters and one undescribed form.

Modal counts are in bold; sources of data not cited in the table are as follows: *Etheostoma cervus* (Bailey & Etnier, 1988; Powers & Mayden, 2003; Kozal et al., 2017); *E. pyrrhogaster*, *E. zonistium*, *E.* cf. *zonistium*, and *E. cyanoprosopum* (Bailey & Etnier, 1988; Kozal et al., 2017); S.D. = standard deviation.

	9	10	11	12	13	n	⬜	S.D.
E. cervus			**53**	40	4	97	11.5	0.58
E. pyrrhogaster			**41**	34	3	78	11.5	0.58
E. zonistium (Tennessee R.)	3	58	**249**	59		369	11.0	0.59
E. cf. *zonistium* (Hatchie R.)	1	21	**68**	2		92	10.8	0.49
E. cyanoprosopum	2	26	**81**	3		112	10.8	0.52
E. raneyi (Suttkus et al., 1994)	1	50	**105**	9		165	10.7	0.56
E. faulkneri (Kozal et al., 2017)		5	**28**	3		36	10.9	0.47
E. raneyi (new data)	3	57	**86**	6		152	10.6	0.60
E. faulkneri (new data)	1	53	**61**			115	10.5	0.52

Compilation of frequency distributions of anal fin ray counts for six speciesof snubnose darters and one undescribed form.

Modal counts are in bold; sources of data not cited in the table are as follows: *Etheostoma cervus* (Bailey & Etnier, 1988; Powers & Mayden, 2003; and Kozal et al., 2017); *E. pyrrhogaster*, *E. zonistium*, *E*. cf. *zonistium*, and *E. cyanoprosopum* (Bailey & Etnier, 1988; Kozal et al., 2017); S.D. = standard deviation.

	5	6	7	8	9	n	□	S.D.
E. cervus		2	**74**	58	2	136	7.4	0.55
E. pyrrhogaster			18	**52**	8	78	7.9	0.57
E. zonistium (Tennessee R.)	2	30	**260**	54	1	347	7.1	0.52
E. cf. *zonistium* (Hatchie R.)		23	**64**	5		92	6.8	0.52
E. cyanoprosopum		10	**70**	34		114	7.2	0.59
E. raneyi (Suttkus et al., 1994)		11	**133**	21		165	7.1	0.44
E. faulkneri (Kozal et al., 2017)		2	**26**	8		36	7.2	0.51
E. raneyi (new data)		11	**99**	9		119	7.0	0.41
E. faulkneri (new data)		7	**86**	9		102	7.0	0.40

Compilation of frequency distributions of pectoral fin ray counts for six species of snubnose darters and one undescribed form.

Modal counts are in bold; sources of data not cited in the table are as follows: *Etheostoma cervus* (Bailey & Etnier, 1988; Powers & Mayden, 2003; Kozal et al., 2017); *E. pyrrhogaster*, *E. zonistium*, *E.* cf. *zonistium*, and *E. cyanoprosopum* (Bailey & Etnier, 1988; Kozal et al., 2017); S.D. = standard deviation.

	12	13	14	15	16	n	⬚	S.D.
E. cervus		20	**72**	5		97	13.8	0.49
E. pyrrhogaster		27	**41**			68	13.6	0.49
E. zonistium (Tennessee R.)	1	98	**328**	63		490	13.9	0.58
E. cf. *zonistium* (Hatchie R.)		38	**83**	1		122	13.7	0.48
E. cyanoprosopum	1	8	**87**	18		114	14.1	0.50
E. raneyi (Suttkus et al., 1994)		11	**126**	28		165	14.1	0.48
E. faulkneri (Kozal et al., 2017)		3	**28**	4	1	36	14.1	0.55
E. raneyi (new data)		11	**86**	15		112	14.0	0.48
E. faulkneri (new data)		10	**80**	10		100	14.0	0.45

Means and 95% confidence intervals (C.I.) for measurements of morphometric truss variables in thousandths of SL (except for SL) for *Etheostoma faulkneri* and *E. raneyi* males and females.

Variable labels correspond to landmarks in Figure 2; measurements in bold font with asterisks indicate that 95% confidence intervals did not overlap within sexes between drainages.

Variable	*E. faulkneri* males (n = 43)	*E. raneyi* males (n = 68)	*E. faulkneri* females (n = 51)	*E. raneyi* females (n = 77)
	mean (95% C.I.)	mean (95% C.I.)	mean (95% C.I.)	mean (95% C.I.)
SL	***42.9 (41.3-44.6)**	***39.7 (38.4-41.1)**	***39.3 (38.4-40.1)**	***37.3 (36.3-38.3)**
1-3	***63 (62.6-64.9)**	***67.6 (66.5-68.5)**	***61.2 (60.3-62)**	***66 (65.2-66.8)**
1a-3	***56.5 (55.1-57.8)**	***59.6 (58.6-60.6)**	***54.2 (53.4-55.1)**	***58.4 (57.5-59.4)**
1-1a	7.3 (6.3-8.4)	7.9 (7.3-8.6)	6.9 (6.4-7.5)	7.6 (7.1-8.1)
3-3	73.1 (71.4-74.9)	70.7 (69.3-72)	66.8 (65.5-68)	68.8 (67.8-69.8)
1-2	***47.4 (45.9-48.9)**	***51.8 (50.3-53.2)**	***45.6 (44-46.9)**	***48.6 (47.4-49.7)**
3-5	36 (34.4-37.7)	36 (34.5-37.7)	30.6 (29.3-31.9)	32.2 (30.7-33.8)
1-9	***159.2 (157-161.3)**	***164.8 (162.8-166.9)**	***156.9 (155.2-158.5)**	***162.8 (160.8-164.7)**
9-10	88.2 (86.3-90.2)	89.3 (87.8-90.7)	84.4 (83-85.9)	84.3 (83-85.6)
4-4	44.1 (43.1-45.1)	45.8 (44.9-46.8)	***42.1 (40.9-43.2)**	***45.6 (44.9-46.4)**
13-13	141.9 (138.2-145.7)	139.7 (137.5-141.8)	***145.4 (142.6-148.3)**	***137.6 (135.3-139.8)**
13-14	272 (266.3-277)	272.7 (269.5-275.9)	271.7 (268-275.2)	271.7 (269-274.5)
15-16	137 (134-139.8)	140.3 (138.1-142.6)	126.7 (124.2-129.1)	129.9 (120.5-133.5)
19-20	118.7 (115-122.2)	121.4 (118.3-124.7)	118.5 (115.7-121.4)	117.1 (114.5-119.7)
21-22	155.1 (151.7-158.5)	154.1 (151.6-156.5)	152.4 (149.3-155.3)	151.7 (149.6-153.7)
23-24	217.1 (214-220.1)	216.2 (211.7-220.8)	215.5 (211.3-219.5)	218.1 (214.9-220.9)
24-25	104.2 (102.4-106)	101.5 (100.3-102.7)	94.7 (93.5-96)	95.8 (94.9-96.7)
26-27	222.4 (218.9-225.7)	227.9 (224.2-231.9)	227.6 (224.6-230.5)	226.6 (224.3-229)
1-6	***196.3 (194.6-197.9)**	***204.2 (202.4-206)**	***195.7 (194.3-197.1)**	***202.2 (200.6-203.8)**
1-7	***106.6 (102.9-110.1)**	***122.8 (120.4-125.2)**	***110.4 (107.4-113.4)**	***117.3 (115.2-119.3)**
6-11	143.7 (141.5-145.9)	142.4 (140.6-144.3)	145.3 (143-147.8)	142.9 (141.3-144.5)
7-12	***183.4 (179.9-187)**	***175.8 (172.4-179.2)**	176.5 (172.9-179.9)	179 (175.7-182.4)
11-12	***212.7 (208.5-216.9)**	***204.3 (201.5-207.1)**	***214.8 (210.7-219)**	***196.3 (193.7-198.8)**
11-17	***298.7 (295-302.3)**	***287.1 (283.1-291.2)**	***286.4 (283.2-289.5)**	***270.6 (266.9-274.4)**
11-18	***344.4 (341.4-347.6)**	***335.2 (332-338.4)**	***343.5 (340.3-346.7)**	***333.5 (330.6-336.5)**
12-17	371.5 (366.2-376.7)	370.9 (367.3-374.5)	377.1 (373-381.3)	368.3 (364.5-372)
17-18	***187.2 (183.4-191.2)**	***176.9 (174.6-179.3)**	171.3 (169-173.6)	167.6 (165.8-169.4)
17-25	396.3 (392.8-399.8)	393.7 (390.4-397)	389.7 (386.9-392.6)	388.7 (386.4-391.1)
18-24	409.6 (405.9-413.3)	413.5 (410.5-416.5)	401.6 (398-405.2)	402.4 (399.6-405.2)