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Morphology and Phylogeny of the Studfish Clade, Subgenus *Xenisma* (Teleostei: Cyprinodontiformes)

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Phylogenetic relationships within the studfish clade, subgenus *Xenisma*, were elucidated using parsimony analysis of 21 morphological transformation series, primarily osteology and external morphology. The analysis supports monophyly of subgenus *Xenisma* and the studfishes sensu strictu (*Fundulus bifax*, *Fundulus catenatus*, and *Fundulus stellifer*). *Fundulus julisia* and *Fundulus albolineatus* are recognized as sister taxa and together are recognized as sister to the *F. bifax*, *F. catenatus*, and *F. stellifer* clade. Contrary to a previous allozyme study of the subgenus, *Fundulus rathbuni* is recognized as sister to a monophyletic group composed of all other *Xenisma* species. This relationship is biogeographically consistent with the vicariant pattern previously demonstrated within darters of the subgenus *Percina* and suckers of the genus *Hypentelium*. The biogeography of the rest of subgenus *Xenisma* is complex and the sister-group relationship between *F. catenatus* and *F. bifax* is recognized as anomalous when compared to other Mississippi-Mobile basin biogeographic relationships in North American fishes.

THE approximately 33 species in the genus *Fundulus* are widely distributed in freshwater, brackish, and coastal marine environments of North America, coastal areas of the Yucatan Peninsula, and Bermuda. The studfish clade, subgenus *Xenisma*, includes five extant species, *Fundulus bifax*, *Fundulus catenatus*, *Fundulus julisia*, *Fundulus rathbuni*, and *Fundulus stellifer*, and one recently extinct species, *Fundulus albolineatus*. These fishes are typically found in clear freshwater habitats of the central and eastern United States. Unlike the other subgenera of *Fundulus*, all species in *Xenisma* naturally occur only in low salinity environments. Studfishes are among the largest fundulids and nuptial males of most *Xenisma* species are very brightly colored. The species are allopatric except that *F. julisia* and *F. catenatus* are often syntopic (Etnier and Starnes, 1993), and *F. bifax* and *F. stellifer* both occur in the Lower Coosa drainage (Cashner et al., 1988). Most populations are confined to highland stream systems.

Phylogenetic relationships within *Xenisma* have never been comprehensively addressed using morphological data. Williams and Etnier (1982) described *F. julisia*, diagnosed the subgenus, and suggested that *F. julisia* is more closely related to *F. catenatus* and *F. stellifer* than to *F. rathbuni*. Wiley (1986) in his study of relationships of Fundulus addressed relationships within *Xenisma* but included only characters that were previously mentioned in the literature. Wiley (1986:125) stated that his discussion of character state support for relationships within subgenus *Xenisma* did not constitute a phylogenetic analysis and that ''much work has to be done.'' Wiley's (1986) morphological data supported the monophyly of *Xenisma* and *F. catenatus* plus *F. stellifer* (populations now recognized as *F. bifax* were considered part of *F. stellifer*). Morphological variation within the subgenus *Xenisma* has been described (Brown, 1957; Thomerson, 1969; Williams and Etnier, 1982), but the morphology of *Xenisma* has not yet been subjected to a phylogenetic analysis.

A series of studies using allozyme data (Rogers and Cashner, 1987; Cashner et al., 1988; Grady et al., 1990) first described *F. bifax* from populations previously referred to *F. stellifer* and suggested that *F. rathbuni* was more closely related to the clade containing *F. bifax*, *F. catenatus*, and *F. stellifer*, than to *F. julisia*. Allozyme (Rogers and Cashner 1987, Cashner et al. 1988) and RFLP data (Strange and Burr 1997) support *F. bifax* and *F. catenatus* as sisters, and this clade as sister to *F. stellifer*. There are various DNA sequence studies that included some species in subgenus *Xenisma* (Bernardi and Powers, 1995; Bernardi, 1997). However, none of these DNA sequence studies include all extant *Xenisma* species.

Although Wiley's (1986) study has been the only hypothesis of relationships among all *Xenisma* based on morphological data, this study was not a phylogenetic analysis. We were interested in how a more comprehensive examination of morphological data would contribute to knowledge of relationships within *Xenisma*. In this study, we used osteological and external morphological characteristics, to assess phylogenetic relationships within the subgenus *Xenisma*. In addition, the primarily allopatric and

highland distribution of *Xenisma* species allowed clear evaluation of the biogeographic history of the group.

Materials and Methods

Specimens examined were whole alcohol preserved or cleared and stained for bone and cartilage following Dingerkus and Uhler (1977). See the Materials Examined section for sample sizes. Counts and measures follow methods outlined in Hubbs and Lagler (1947). Dissection of cleared-and-stained specimens followed Weitzman (1974), except that the branchial basket was removed prior to removal of the suspensorium. Dissection for examination of ventral coelomic viscera involved a right parasaggital cut through the body wall into the coelom from the anus around the right side of the pelvic girdle and to the pectoral girdle.

All specimens were examined using a Leica stereomicroscope for potentially phylogenetically informative variation among included members of the subgenera *Xenisma* and *Fontinus*. Specimens of the extinct species *F. albolineatus* were not available for direct osteological or visceral examination, and some data were unavailable from external examination of specimens or from Williams and Etnier (1982). Digital x-rays showing ventral and left lateral views of the lectotype (USNM 125055) and a paralectotype (USNM 225996) were examined. Because some data were available, we included this species in the phylogenetic analysis. Character states that were not identifiable were coded as unknown (?).

We obtained color pattern data for all species from photographs and descriptions of color pattern in Thomerson (1969), Williams and Etnier (1982), Cashner et al. (1988), Robison and Buchanan (1988), Page and Burr (1991), Etnier and Starnes (1993), Jenkins and Burkhead (1993), Mettee et al. (1996), Wildekamp (1996), Pflieger (1997), and Ross (2001) and from examination of ethanol preserved specimens and field observations of *F. catenatus*. Institutional abbreviations are as listed in Leviton et al. (1985).

Fundulus chrysotus (subgenus *Zygonectes*), *Fundulus heteroclitus* (subgenus *Fundulus*), *Fundulus diaphanus* (subgenus *Fontinus*), *Fundulus seminolis* (subgenus *Fontinus*), and *Fundulus zebrinus* (subgenus *Plancterus*) were included as outgroups based on the family level phylogenies of Parenti (1981) and Wiley (1986). We rooted with *F. zebrinus* because it has been recognized as basal within the Fundulidae (Parenti, 1981), the genus *Fundulus* (Bernardi and Powers, 1995), or within a clade of unresolved relationships composed of *F. zebrinus*, all other *Fundulus*, and *Lucania* (Wiley, 1986).

Transformation series (TS) are grouped into traditional anatomical units and assigned numbers. The character state for *F. zebrinus* was designated 0. Each account includes a description of each character state and a short discussion of the historic usage of each transformation series if applicable. All transformation series were unordered.

Maximum parsimony analyses used the exhaustive search option in PAUP 3.1.1 (D. L. Swofford, Illinois Natural History Survey, 1993, unpubl.). Decay indices (Bremer, 1988) were determined using TreeRot (M. D. Sorenson, University of Michigan, 1996, unpubl.). A Templeton's test (Templeton, 1983) was not run to test statistically the significance of the difference between the phylogeny and the phylogeny obtained from allozyme data (Rogers and Cashner, 1987; Cashner et al., 1988; Grady et al., 1990). The number of character states (4) that varied between the two topologies was low and the power of a Templeton's test to statistically reject a hypothesis was correspondingly low.

Results

No gross characteristics of the coelomic viscera were discretely variable and useful for this analysis. Thomerson (1969) described the molariform pharyngeal teeth and enlarged tooth-bearing bones of *F. stellifer* and noted the absence of these pharyngeal characteristics in *F. catenatus*. This putatively unique trophic morphology suggests that studfishes' diets vary and might be correlated with differing alimentary tract morphology. However, there were no gross morphological differences in digestive tract morphology among *Xenisma* examined. All species had a short digestive tract and a simple intestine with a single U-shaped bend (Fig. 1A).

Twenty-one transformation series were coded for phylogenetic analysis. See Appendix 1 for descriptions of transformation series. See Table 1 for distribution of character states among taxa. Six equally most parsimonious cladograms were found (length = 40, CI = 0.66, RI = 0.74, RC = 0.48; Fig. 2). Incongruities among the shortest trees involved relationships among *Fundulus bifax*, *F. catenatus*, and *F. stellifer* and among the outgroups. However, all shortest trees include a *Xenisma* + *Fontinus* clade. Monophyly of *F. bifax*, *F. catenatus*, and *F. stellifer*, a sister group relationship between *F. albolineatus* and *F. julisia*, a clade composed of all *Xenisma* except *F. rath-*

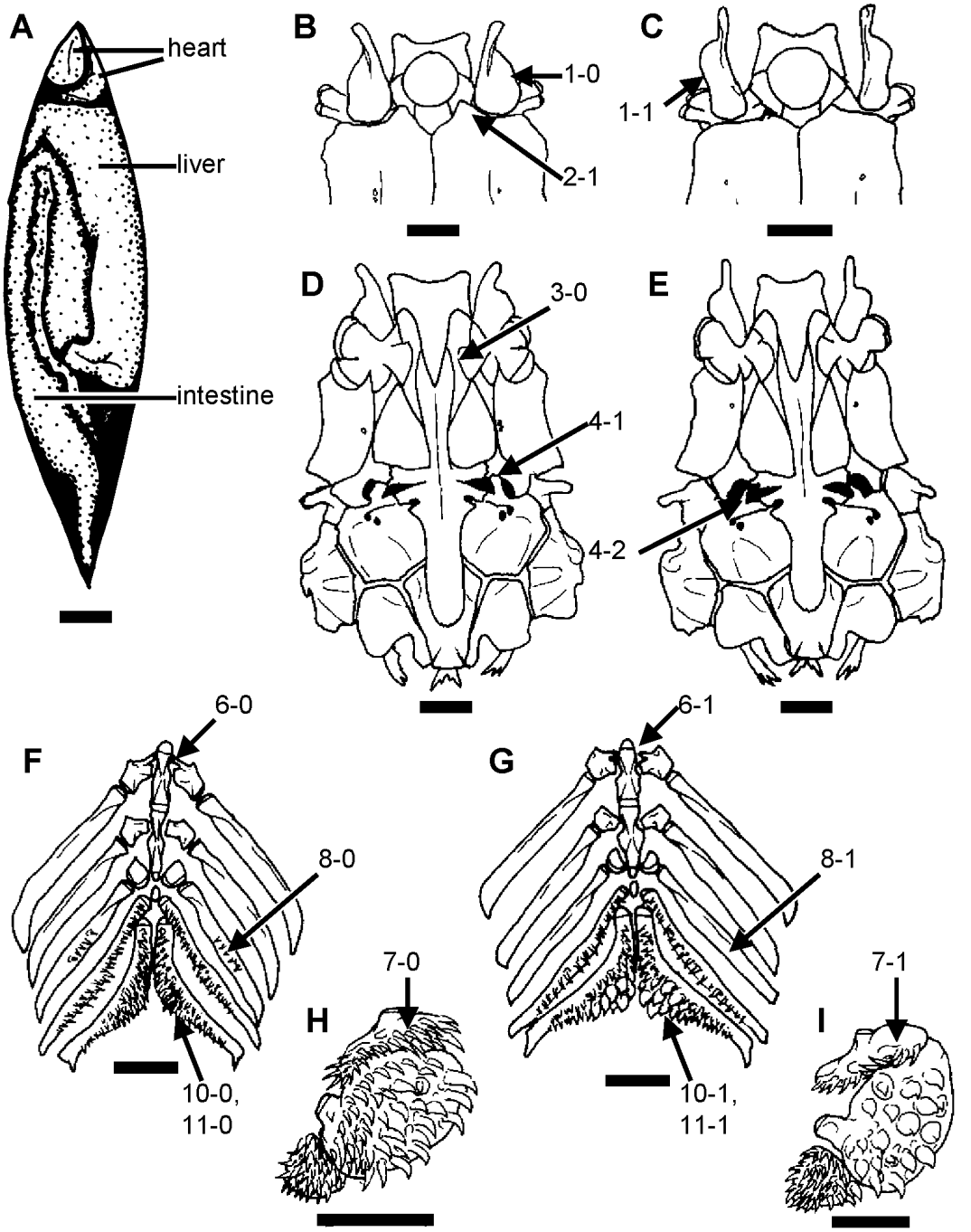

Fig. 1. Semidiagrammatic illustrations. (A) Ventral view of coelomic cavity of *Fundulus stellifer*, KU 20263; (B) dorsal view of anterior neurocranium of *Fundulus rathbuni*, UMMZ 147615; (C) dorsal view of anterior neurocranium of *Fundulus catenatus*, KU 11550; (D) ventral view of neurocranium of *F. rathbuni*, UMMZ 147615; (E) ventral view of neurocranium of *F. catenatus*, KU 11550; (F) dorsal view of ventral branchial skeleton of *F. rathbuni*, UMMZ 147615, (G) dorsal view of ventral branchial skeleton of *F. catenatus*, JFBM 37920; (H) ventral view of dorsal branchial skeleton of *F. rathbuni*, UMMZ 147615; (I) ventral view of dorsal branchial skeleton of *F. catenatus*, JFBM 37920. Anterior is up in all drawings. Scale bars = 1 mm. Arrows indicate transformation series number and character states separated by a hyphen (e.g., 2–1 is transformation series 2, character state 1).

TABLE 1. CHARACTER STATE DATA MATRIX FOR SIX *Xenisma* SPECIES AND FIVE OUTGROUP SPECIES. All transformation series are unordered. See Results for transformation series. Missing data are indicated with a question mark (?). Taxa with polymorphic character states for a given transformation series are indicated with either "A" or "B" (A = 0&1; B = 1&2).

	1 1111111112 2 1234567890 1234567890 1
Fundulus catenatus	110211111B 1111110001 0
Fundulus bifax	1102111111 1111110001 0
Fundulus stellifer	1102111112 1111010011 0
Fundulus julisia	0102101101 0111011110 1
Fundulus albolineatus	????????0? 0?111?1110 1
Fundulus rathbuni	0101100000 0111000100 0
Fundulus diaphanus	0111101011 1100?02000 0
Fundulus seminolis	0111100012 1100?02010 0
Fundulus chrysotus	00010A1001 1001000000 0
Fundulus heteroclitus	0001010001 1000?00000 0
Fundulus zebrinus	0000000000 0000?00000 0

buni, and monophyly of the subgenus *Xenisma* were supported by all most parsimonious trees.

DISCUSSION

Our morphological data support many of the previously proposed relationships among *Xenisma* species. Monophyly of *Xenisma* (Williams and Etnier, 1982; Wiley, 1986) is supported by the unique and unreversed character state of the absence of barring in nuptial males (TS 13–1). Three other homoplastic character states diagnose this node (TS 11–0, 14–1, 18–1). However, this node has a decay index of one because one of the seven trees at 41 steps includes the two *Fontinus* species in this analysis as sister to a clade composed of all *Xenisma* species except *F. rathbuni*, which is sister to the *Fontinus* + other *Xenisma* clade. Previously proposed relationships among species of *Xenisma* corroborated by this analysis are a sister relationship between *F. albolineatus* and *F. julisia* (Williams and Etnier, 1982), and monophyly of the studfishes sensu strictu (*F. bifax, F. catenatus*, and *F. stellifer*; Wiley, 1986, Rogers and Cashner, 1987). Both of these relationships have a decay index of greater than one.

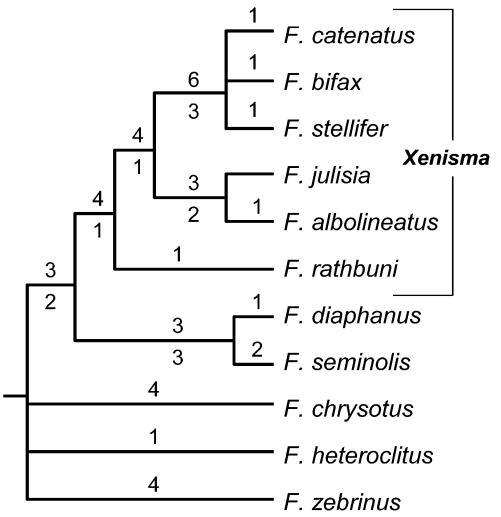

Fig. 2. Strict consensus of three equally most parsimonious phylogenetic trees rooted on *Fundulus zebrinus*. Branch lengths based on ACCTRAN optimization above branches, Bremer (1988) decay indices below branches.

The monophyly of *F. bifax*, *F. catenatus*, and *F. stellifer* was well supported by the morphological data with a decay index of three. Although well diagnosed as a group, morphological variation among these three species was limited to three transformation series, which did not suggest a single most parsimonious resolution. The low level of morphological variation is not surprising since *F. bifax* was not recognized as distinct from *F. stellifer* until relatively recently (Cashner et al., 1988) when allozyme data highlighted the distinctness of these populations. Three of the six trees *F. bifax* and *F. catenatus* as sister taxa, which is supported by the arrangement of lateral spots into horizontal lines (TS 15–1). This also occurs homoplastically in *F. albolineatus*. The other three shortest trees support a sister relationship between *F. bifax* and *F. stellifer*, which is supported by a loss of a yellow band in the caudal fin of nuptial males (TS 18–0). The former relationship is supported by both allozyme and restriction analysis (Rogers and Cashner, 1987; Cashner et al., 1992; Strange and

Burr, 1997). Therefore, the hypothesis of relationships among these species supported by consideration of most data when considering all published data form a sister-group relationship between *F. bifax* and *F. catenatus*.

The morphological data suggest a novel relationship of *F. rathbuni* as sister to all other *Xenisma*. This relationship is supported by the shared possession by all members of *Xenisma* except *F. rathbuni* of a long ascending process of the prootic that does not fuse to the alisphenoid (TS 4–2), teeth on the second pharyngobranchial toothplate present in a narrow band of only one or two rows (TS 7–1), the absence of teeth on the third ceratobranchial (TS 8–1), and distinct iridescent blue coloration in nuptial males (TS 16–1). This relationship differs from Rogers and Cashner's (1987) hypothesis based on allozyme data, which recognized *F. julisia* as sister to all other *Xenisma* and *F. rathbuni* as sister to *F. bifax*, *F. catenatus*, and *F. stellifer*. No morphological character states support this relationship. Although the clade including all *Xenisma* species except *F. rathbuni* is supported by a decay index of one, none of the seven trees one step longer (41 steps) than the most parsimonious tree include *F. rathbuni* as sister to the *F. bifax*, *F. catenatus*, and *F. stellifer* clade. The difference between our best supported hypothesis and trees derived from the allozyme data (Rogers and Cashner, 1987) also could be the result of different rooting of subgenus *Xenisma* because of outgroup choice. In both studies species in subgenus *Fontinus*, hypothesized to be sister to *Xenisma* by Wiley (1986), and subgenus *Fundulus* were included as outgroups. However, we also included *F. chrysotus* and *F. zebrinus*, in subgenera *Zygonectes* and *Plancterus*, respectively. Although the outgroups used in both studies were similar, outgroup choice could account for the differences between the morphological and molecular (allozyme and RFLP) trees.

Relationships within the subgenus *Xenisma* suggest that both dispersal and vicariance have shaped the geographic distributions of *Xenisma* species. *Fundulus rathbuni*, the sister to the rest of the subgenus, occurs in four Atlantic slope drainages, the Roanoke, Neuse, Peedee, and Santee River drainages, within the Piedmont physiographic province of Virginia and North Carolina. Meristic variation among populations from these drainages (Brown, 1955) suggests limited gene flow. The remaining species of *Xenisma* occur in the Mississippi and Mobile basin drainages. A pattern in which a species distributed in Atlantic slope drainages is sister to a more widespread taxon distributed west of the Appalachians is repeated in several distantly related fish groups. For example, *Percina rex* is restricted to the Roanoake and Chowan River drainages of Virginia (Jenkins and Burkhead, 1993) and is sister to the rest of the subgenus (Near, 2002), which is widely distributed in the Mississippi and Gulf coast drainages. Also, *Hypentelium roanokense* occurs in the Roanoke River drainage of Virginia and is sister to the rest of the genus (Berenzen et al. 2003); sympatry with *H. nigricans* in the Roanoke is likely the result of recent dispersal (Berendzen et al. 2003). Phylogenetic and geographic concordance among the unrelated groups suggest a historic event dividing the ranges of the common ancestors of subgenera *Xenisma* and *Percina* and the genus *Hypentelium* between the Atlantic and Gulf drainages.

Fundulus albolineatus and *F. julisia* had or have very restricted ranges in the Tennessee and Cumberland River drainages. The extinct *F. albolineatus* was known from a single spring in what is now downtown Huntsville, Alabama (Mettee et al., 1996). The ancestor of *F. albolineatus* and *F. julisia* separated from the ancestor of *F. bifax, F. catenatus*, and *F. stellifer* after the isolation of *F. rathbuni* on the Atlantic slope. The syntopic occurrence of the narrowly distributed *F. julisia* and the widely distributed *F. catenatus* suggests dispersal. This was likely dispersal by *F. catenatus* rather than *F. julisia* because molecular data suggest that much of the disjunct distribution of *F. catenatus* can be explained by more recent dispersal than by vicariance (Strange and Burr, 1997).

Fundulus bifax, *F. catenatus*, and *F. stellifer* exhibit a much wider distribution than their putative sister clade. *Fundulus stellifer* is known from the Coosa River system and the Alabama River system of the Mobile basin (Mettee et al., 1996). *Fundulus bifax* also is distributed in the Mobile basin in the Tallapoosa river system with a single population in a tributary to the lower Coosa (Cashner et al., 1988; Mettee et al., 1996). *Fundulus catenatus* has a widespread distribution with disjunct populations in the Ozark, Ouachita, and Eastern Highlands and small populations in the White River of Indiana and the Homochitto drainage of Mississippi. We accept the relationships among these species supported by the allozyme (Rogers and Cashner, 1987; Cashner et al., 1988; Grady et al., 1990) and restriction fragment (Strange and Burr, 1997) analysis data since the morphological data are equivocal. *Fundulus bifax* and *F. catenatus* are recognized as sister taxa, yet the current distribution of *F. stellifer* lies between them. The existence of a connection between the Tennessee River system, which is part of the Missis-

sippi basin and the Mobile basin has been proposed by Mayden (1988) and Etnier and Starnes (1993). Some examples of clades where there is support for a sister group relationship between taxa in the Mobile and Mississippi basins include *Hypentelium etowanum* and *Hypentelium nigricans* (Berendzen et al., 2003); *Percina antesella*, *Percina tanasi*, and *Percina uranidea* (Near, 2002); *Percina evides*, *Percina aurantiaca*, *Percina palmaris*, and *Percina* (*Alvordius*) sp.; and *Phenacobius catostomus*, *Phenacobius uranops*, and *Phenacobius crassilabrum* (Mayden, 1989; Dimmick and Burr, 1999). However, in these cases, there is no evidence of paraphyly of taxa in the Mobile basin with respect to taxa in the Mississippi basin as is the case with *F. stellifer* and *F. bifax* (Rogers and Cashner, 1987; Cashner et al., 1988). A better understanding of this unique distributional pattern requires further phylogenetic and geologic study.

Material Examined

The number of alcohol preserved specimens examined is indicated after the catalog number followed by cleared and stained specimens examined in parentheses. Ingroup taxa are listed first followed by outgroup taxa. *Fundulus albolineatus*: UMMZ 157692, cotype, Alabama, Madison Co., Spring Cr. *Fundulus bifax*: JFBM 35197, 5(3), Alabama: Tallapoosa Co., Josie Leg Cr; UMMZ 213930, holotype, Alabama, Tallapoosa Co., Tallapoosa R; UMMZ 213931, 4 paratypes, Alabama, Tallapoosa Co., Tallapoosa R. *Fundulus catenatus*: JFBM 37334, 3 (2), Mississippi: Lincoln Co., Homochitto R.; JFBM 37590, 5(1); Tennessee; Blount Co.; Little R.; JFBM 37773, 4(1); Indiana, Johnson Co., Leatherwood Cr.; JFBM 37821, 4(1), Tennessee, Lewis Co., Buffalo R.; JFBM 37920, 4(2), Arkansas, Pike Co., Caddo R.; KU 11550, 4(1), Tennessee, Jackson Co., Roaring R.; KU 17616, (7), Missouri, Jefferson Co., Big R. *Fundulus julisia*: KU 20999, 2(1), Tennessee, Cannon Co., tributary to McMahan Cr.; UMMZ 120861, 3 paratypes, Tennessee, Coffeee Co., Spring Branch.; UMMZ 120914, 8 paratypes, Tennessee, Coffee Co., Little Duck R.; UMMZ 121013 and 21014, (1)7 paratypes, Tennessee, Coffee Co., Hunt Cr.; UMMZ 207690, 2, Tennessee, Coffee Co., W Fork Hickory Cr. *Fundulus rathbuni*: JFBM 38544, 5, North Carolina, Caswell Co., County Line Cr.; JFBM 38634, 4(1), North Carolina, Randolph Co., Uwharrie R.; JFBM 38675, 3(2), North Carolina, Randolph Co., Deer R.; UMMZ 147615, (5), North Carolina, Sugartree Cr. *Fundulus stellifer*: JFBM 19834, 2(1), Tennessee, Bradley Co., Conasauga R.; JFBM 35224, 4(2), Alabama, Coosa Co., Hachemendega Cr.; KU 18168, (3), Alabama, Calhoun Co., Little Hillabee Cr.; KU 20263, 9, Alabama, Calhoun Co., Little Hillabee Cr. *Fundulus chrysotus*: KU 18165, 5(6), Florida, Lake Co., Hogeye Sink. *Fundulus diaphanus*: JFBM 12707, 20, Polk Co., Minnewaska L.; KU 18192, 5(3), Pennsylvania, Franklin Co., Conococheaque Cr. *Fundulus heteroclitus*: KU 15351, 5(5), Massachusetts, Norfolk Co., Quincy. *Fundulus seminolis*: KU 18195, 3(11), Florida, Putnam Co., St. John's R. *Fundulus zebrinus*: KU 5362, 16, Kansas, Barton Co., Arkansas R.; KU 14726, (5), Kansas, Edwards Co., Arkansas R.

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APPENDIX 1

Transformation series used in this analysis. The consistency index (ci) for each transformation series follows the account in parentheses.

1. Laminar flange on nasals: (0) less than twice as long as wide, laterally convex (Fig. 1B); (1) more than twice as long as wide, laterally concave or flat (Fig. 1C). (1.00).

2. Anterior margin of frontals: (0) mostly straight, possibly having a low, rounded anterior extension; (1) with a distinct, pointed anterior process (Fig. 1B). Wiley (1986) described the derived character state as including elongate frontals and contact between the an-

terior process and the lateral ethmoids. It was difficult to differentiate elongate from non-elongate and contact between the anterior frontals and the lateral ethmoids was observed in outgroup taxa. (1.00).

3. Position of lateral ethmoids: (0) lateral ethmoids do not overlap parasphenoid (Fig. 1D); (1) lateral ethmoids overlap parasphenoid dorsally. Wiley (1986: fig. 2) described two derived states at the node supporting the monophyly of *Fontinus*, the dorsal overlap of the parasphenoid by the lateral ethmoids and the lateral processes of the vomer forming an acute angle with the main body of the vomer. The condition of the vomerine processes are not recognized as separate transformation series because the lateral processes of the vomer contact the lateral ethmoids and the angle of the lateral processes of the vomer depends upon the position of the lateral ethmoids. (1.00).

4. Ascending process of prootic: (0) absent; (1) long, contacts and fuses to alisphenoid and ascending process of parasphenoid (Fig. 1D); (2) long, may contact alisphenoid and/or ascending process of parasphenoid but does not fuse to them (Fig. 1E). (1.00).

5. Posterior laminar shelf of the lacrymal: (0) narrow, not wider than the width of the lacrymal canal; (1) wide, wider than the width of the lacrymal canal. Wiley (1986:fig. 3) described and figured two derived states supporting the monophyly of *Fontinus* plus *Xenisma*, the posterior margin of the lacrymal convex or sigmoid and a wide posterior notch of the lacrymal. Wiley (1986) acknowledged the possibility of nonindependence of these character states and we agree with this assessment and consider this transformation series synonymous with the two identified by Wiley (1986). (1.00).

6. Anterior medial process of the first hypobranchial: (0) long extending further medially than more posterior medial process (Fig. 1F); (1) short extending less further medially than more posterior medial process (Fig. 1G). (0.67).

7. Teeth on second pharyngobranchial toothplate: (0) in more than two rows (Fig. 1H); (1) in slender band of only one or two rows (Fig. 1I). (0.33).

8. Teeth on third ceratobranchials: (0) present (Fig. 1F); (1) absent (Fig. 1G). (1.00).

9. Fourth epibranchial: (0) slender, lacking dorsal flange; (1) broad, with distinct dorsal flange. (0.50).

10. Medial teeth on the posterior fifth ceratobranchial in adults: (0) slender and conical (Fig. 1F); (1) robust, somewhat molariform, possessing a distinct point (Fig. 1G); (2) robust, molariform, rounded lacking point. Thomerson (1969) described the possession of character state 2 in *Fundulus stellifer* and state 1 in *Fundulus catenatus*. However, we observed an adult specimen from the Wabash drainage (JFBM 37773) that exhibited rounded, molariform teeth. Therefore, *F. catenatus* was coded as polymorphic (1 and 2). (0.75).

11. Medial extent of fifth ceratobranchial: (0) slender, left and right ceratobranchials not closely apposed anteriorly (Fig. 1F); (1) robust, left and right ceratobranchials closely apposed anteriorly approaching each other more posteriorly, forming distinct angle medially (Fig. 1G). Thomerson (1969) described the possession of character state 1 in *Fundulus stellifer* and this species does exhibit the most extreme development of this character state. All *F. catenatus* show robust fifth ceratobranchials. However, the extent of development varies within populations of this species. The fifth ceratobranchials of some *F. catenatus* specimens (e.g., JFBM 37773) approach the extremely robust condition seen in *F. stellifer*. (0.33).

12. Baudelot's ligament: (0) connects posttemporal to basioccipital and exoccipital; (1) connects cleithrum to basioccipital and exoccipital. The derived state was recognized as diagnostic for *Xenisma* by Wiley (1986), but is present in all *Xenisma* and *Fontinus* species examined. (1)

13. Vertical barring in live nuptial males: (0) present, (1) absent. Wiley (1986) recognized the absence of lateral barring in males as diagnostic for *Xenisma*. However, barring is present in alcohol-preserved small individuals (< 25 mm SL) of all species and may be found on alcohol-preserved specimens of *Fundulus catenatus* up to approximately 85 mm SL. This barring is visible in a female 69.4 mm SL in Thomerson (1969:fig. 1). (1.00).

14. Reddish-brown lateral spots in nuptial males: (0) absent; (1) present. Present in all *Xenisma* species and first recognized as diagnostic for *Xenisma* by Williams and Etnier (1982). Also present in *F. chrysotus*. (0.50).

15. Arrangement of reddish-brown lateral spots in nuptial males: (0) scattered; (1) arranged into straight horizontal lines. Cashner et al. (1988) recognized the arrangement of the reddish-brown spots into lines as synapomorphic of a *Fundulus bifax* and *F. catenatus* clade. Taxa without reddish-brown spots are coded with a question mark (?). (0.50).

16. Iridescent blue or blue-green background in nuptial males: (0) absent; (1) present. Williams and Etnier (1982) suggested that the presence of iridescent blue coloration in nuptial males provided evidence of a relationship between *F. julisia* and the studfishes, *F. catenatus*, and *F. stellifer*. (1.00)

17. Median dark chromatophore line in front of dorsal fin origin in alcohol preserved specimens: (0) forms elongate mark, longer than wide; (1) forms short mark, approximately as long as wide; (2) forms line extending from dorsal-fin origin to occiput. (0.67).

18. Yellow band on posterior sixth of caudal fin in nuptial males: (0) absent; (1) present. Williams and Etnier (1982) and Wiley (1986) recognized the presence of a chromatic band on the distal margin of the caudal fin as diagnostic for *Xenisma*. In this study, this has been broken into two transformation series (TS

18 and TS 19). Thomerson (1969) indicated that this band was absent from the Homochitto population of *F. catenatus* in Mississippi. However, Ross (2001) contradicts this and identifies a yellow band as present in nuptial males from the Homochitto drainage. *Fundulus catenatus* was coded with the derived condition (1) in this analysis. (0.67).

19. Narrow marginal black band on posterior caudal fin in nuptial males: (0) absent; (1) present. This marginal black band is present in *Fundulus albolineatus*, *F. julisia*, and 58% of the individuals of *F. stellifer* examined by Thomerson (1969). According to Thomerson (1969) 12–44% of individuals in all populations of *F. catenatus* except the Homochitto population have a black band proximal to the marginal yellow band found in this species. This subdistal band is not treated as homologous to the band described in this transformation series due to positional dissimilarity. (0.67).

20. Number of anal-fin rays: (0) 12 or fewer; (1) 13 or greater. Wiley (1986) recognized the possession of a higher modal number of anal-fin rays as diagnostic of a studfish clade. The difference between 12 and 13 was chosen as a cut-off point because 12 was the upper limit of anal-fin ray number in *Fundulus albolineatus*, *F. julisia*, and *F. rathbuni* and 13 was the lower limit for *F. bifax*, *F. catenatus*, and *F. stellifer*. The mean and modal number of individuals with each count for each species was likewise distinctly above or below the 12–13 boundary. *Fundulus zebrinus* broadly overlaps this range as it is a meristically very variable species (Poss and Miller, 1983) and is coded as polymorphic. (1.00).

21. Contact organs on pelvic fins of nuptial males: (0) present; (1) absent. Williams and Etnier (1982) recognized the absence of contact organs on the pelvic fins of nuptial males as shared by *F. julisia* and *F. albolineatus*. (1.00)