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Phylogeny, Classification, and Evolution of Salinity Tolerance of the North American Topminnows and Killifishes, Family Fundulidae (Teleostei: Cyprinodontiformes)

Michael J. Ghedotti

Matthew P. Davis

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Cover: *Fundulus heteroclitus* and phylogeny of Family Fundulidae

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Phylogeny, Classification, and Evolution of Salinity Tolerance of the North American Topminnows and Killifishes, Family Fundulidae (Teleostei: Cyprinodontiformes)

Michael J. Ghedotti and Matthew P. Davis

Abstract

The North American topminnows and killifishes in the Family Fundulidae (Cyprinodontiformes) are widely distributed in freshwater, brackish, and coastal marine environments of North America, the Yucatan Peninsula, and Bermuda. Fundulid fishes are often found in habitats that undergo substantial variation in environmental conditions. Salinity tolerance of fundulids varies substantially, and some estuarine species can survive in water that is more than three times marine salinity, whereas other freshwater species can only tolerate up to one-third marine salinity. While the Family Fundulidae has been the focus of numerous phylogenetic investigations, their evolutionary relationships to other cyprinodontiforms and the evolutionary history of species within the family are in need of further investigation with robust taxonomic sampling. In this study we provide the most data-inclusive current hypothesis of evolutionary relationships for the Fundulidae, based on a combination of morphological, karyological, behavioral, and nucleotide (two mitochondrial and two nuclear genes) data. We revise the classification of the family based on this evolutionary framework. Our results indicate that the Family Fundulidae is monophyletic, and the sister group to a clade composed of the Cyprinodontidae, Profundulidae, and Goodeidae within the Cyprinodontiformes. We recognize three genera, *Leptolucania*, *Lucania*, and *Fundulus*, and synonymize *Adinia* with *Fundulus*. We recognize four subgenera within genus *Fundulus*: *Plancterus*, *Zygonectes*, *Fundulus*, and a newly described subgenus containing the two Pacific-coast *Fundulus* species. Ancestral-state reconstructions of salinity tolerance indicate that there may have been as many as five independent shifts from high to low tolerance within the family, and that salinity tolerance evolution likely was an important factor in the biogeographic history of Fundulidae, shaping current fundulid distributions.

Introduction

The fishes in the Family Fundulidae (Cyprinodontiformes), commonly called topminnows or killifishes, are widely distributed in freshwater, brackish, and coastal marine environments of North America, northern coastal areas of the Yucatan Peninsula, and Bermuda (Fig. 1A). Most fundulids inhabit shallow habitats that usually are less than 2 m deep. These habitats also are often marginal with respect to quality of environmental conditions, undergoing substantial variation in temperature, oxygen saturation, and salinity. Many fundulids are called topminnows in reference to their tendency to remain in the upper part of the water column, often immediately under the surface, where they can use the well-oxygenated surface film for respiration. The degree of salinity tolerance of fundulids varies substantially, although in general they are more tolerant of higher salinities than many fishes that inhabit North American freshwaters. Some estuarine fundulids can tolerate more than three times marine salinity (Nordlie, 2000) and other species in freshwater streams in non-arid areas only tolerate one-third marine salinity (Crego & Peterson, 1997).

Although not closely related, the North American killifishes and topminnows (Cyprinodontiformes: Fundulidae) are similar to the true minnows (Cypriniformes: Cyprinidae) in lacking spines in their fins, having a single dorsal fin, and having abdominal pelvic fins. These general similarities resulted in many species' common names including the name topminnow. However, on closer examination fundulids obviously differ from the true minnows in their rounded or truncate caudal fin, upturned mouths, and prominent oral teeth (Fig. 1B, 1C). All fundulids are small in comparison to commercially harvested or recreationally caught fishes, and range in size from the diminutive pygmy killifish, *Leptolucania ommata*, with a maximum standard length of 27 mm, to the giant killifish, *Fundulus grandissimus*, which can reach 180 mm in standard length (Boschung & Mayden, 2004; Miller, 2005). Recently the family has been recognized as including four genera: the monotypic *Adinia*, *Fundulus* with approximately 36 extant and one recently extinct species, the monotypic *Leptolucania*, and *Lucania* with three species (Mayden et al., 1992; Nelson, 2006; Page & Burr, 2011).

Previous phylogenetic analyses based on morphological data (Wiley, 1986; Ghedotti et al., 2004) and nucleotide-sequence data (Bernardi & Powers, 1995; Bernardi, 1997; Whitehead, 2010) conflict in regard to evolutionary relationships within the Fundulidae (Fig. 2). In addition, Wiley's (1986) morphological study was completed before phylogenetic analyses were commonly conducted using computer-based analyses, and

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FIG. 1. (A) Approximate native distribution of the North American killifishes and topminnows, Family Fundulidae. (B) Live adult male *Fundulus grandis*. Photo by C. McMahan. (C) Live adult male *Fundulus chrysotus*. Photo by M. P. Davis.

was based on hand argumentation of character states. The Family Fundulidae is in need of an extensive phylogenetic analysis and revision of its classification. In this study we re-evaluate existing non-nucleotide data and add additional data. Then we use the range of available evidence (skeletal, myological, visceral, external morphological, color pattern, behavioral, karyological characteristics, and nucleotide-sequence data from mitochondrial and nuclear genes) to assess phylogenetic relationships within the family, and the relationships of the family to other cyprinodontiform fishes. In addition, we re-evaluate and test the hypotheses of the evolution of salinity tolerance in the Fundulidae proposed by Griffith (1972) and Whitehead (2010).

Phylogenetic Relationships and Classification

The order Cyprinodontiformes has long been recognized as an acanthomorph group within the series Atherinomorpha together with the orders Atheriniformes (silversides and rainbowfishes) and Beloniformes (halfbeaks, needlefishes, and flying fishes) (Rosen, 1964; Rosen and Parenti, 1981). Parenti (1981) first conducted a hand-argued parsimony-based phylogenetic analysis of the order Cyprinodontiformes in which she reorganized the taxonomy of cyprinodontiform fishes to more accurately reflect phylogenetic relationships.

This landmark study clearly delineated the Fundulidae with its currently recognized composition. Previous authorities recognized a Fundulinae or Fundulini within the Family Cyprinodontidae including the species now in the genera *Adinia*, *Fundulus*, *Leptolucania*, and *Lucania* as well as *Cubanichthys*, *Empetrichthys*, *Oxyzygonectes*, *Profundulus*, and *Valencia* (Hubbs, 1926; Myers, 1931). Parenti's (1981) proposed composition of the Fundulidae, contrary to the assertion of Miller (2005), long has been widely accepted (Wiley, 1986; Page & Burr, 1991, 2011; Mayden et al., 1991; Nelson, 1994; Boschung & Mayden, 2004). However, relationships among the four currently recognized genera included within the Fundulidae and the composition of the largest genus, *Fundulus*, remain controversial (Parenti, 1981; Wiley, 1986; Bernardi, 1997; Whitehead, 2010).

The relationships among cyprinodontiform families also remain uncertain. In the first study to recognize the Suborder Cyprinodontoidei and the modern compositions of most cyprinodontiform families, the Family Fundulidae was recognized as sister to all other cyprinodontoid cyprinodontiforms except members of the genus *Profundulus* (Parenti, 1981). Subsequent phylogenetic studies involving parsimony analysis of *X-src* gene DNA-sequence data (Meyer & Lydeard, 1993), parsimony analysis of combined *X-src* gene and morphological data (Parker & Kornfield, 1995; Parker, 1997), and

FIG. 2. (A) Phylogenetic hypothesis of relationships within the Fundulidae based on hand parsimony argumentation of morphological data presented by Wiley (1986). This figure depicts all fundulid taxa in a single tree based on the discussion in the text and the five trees presented in Wiley (1986). *Fundulus* “*rhizophorae*” as indicated in Wiley (1986) is currently recognized as either *F. relictus* or as a synonym of *F. heteroclitus*. (B) Phylogenetic hypothesis of relationships within the Fundulidae based on partitioned mixed-model maximum-likelihood analysis of nucleotide-sequence data from three genes (cytb, gylt, and RAG1) presented by Whitehead (2010).

parsimony analysis of morphological data (Costa, 1998) found support for a monophyletic group of three families in which the Fundulidae is sister to a Profundulidae–Goodeidae clade.

Although well over 3,000 papers have been published on members of the genus *Fundulus* since the mid-1800s (Huver, 1973; Dimichelle et al., 1986), most of these papers address the physiology, toxicological susceptibilities, and biology of a few species (especially *F. heteroclitus*), the evolutionary relationships within the Fundulidae and among *Fundulus* species are still poorly understood. This group has long been the subject of phylogenetic interest. Farris’s (1968) computer algorithm-based phylogenetic study of the relationships within *Fundulus* was among the first analyses using a clearly phylogenetic methodology. Despite this long history and widespread standard usage of the generic name *Fundulus* for most fundulid fishes, authors have either not found clear evidence supporting its monophyly (Farris, 1968; Wiley, 1986; Grady et al., 2001) or have found evidence suggesting that the genus is not monophyletic (Parenti, 1981; Bernardi, 1997; Whitehead, 2010). Wiley’s (1986) hand-argued parsimony-based phylogenetic study of morphological data remains the most comprehensive phylogenetic and taxonomic treatment of the Fundulidae (Fig. 2A). Most authors have followed the subgeneric classification of Wiley (1986), modified from Brown (1957) and Farris (1968), and subdivide *Fundulus* into five subgenera: *Fontinus*, *Fundulus*, *Plancterus*, *Xenisma*, and *Zygonectes* (Cashner et al., 1992; Wildekamp, 1996)

(Table 1). Wiley (1986) did not include the Pacific coastal species of fundulids, *Fundulus parvipinnis* and *F. lima*, or any fossil *Fundulus* species, thus leaving them unclassified at the subgeneric rank.

Much like the relationships among fundulid genera, the relationships among and within subgenera are controversial with contradictory phylogenetic hypotheses (Fig. 2). Wiley’s (1986) study supported a sister relationship between the subgenera *Fontinus* and *Xenisma* but did not find clear evidence of other groupings of subgenera. Two parsimony-based DNA-sequence studies using a partial sequence of the mitochondrial cytochrome *b* (cytb) gene (Bernardi & Powers, 1995; Bernardi, 1997), a parsimony and maximum-likelihood study using a partial sequence of the mitochondrial cytb gene (Grady et al., 2001), and one study using maximum-likelihood and parsimony analysis of two nuclear genes and cytb (Whitehead, 2010) suggested relationships among subgenera and subgeneric classifications contrary to those presented in Wiley (1986) and to each other but did not recommend formal modification of subgeneric classification or the placement of either genera or subgenera into synonymy.

Among fundulid taxa the relationships of the only species natively distributed west of the continental divide, *Fundulus parvipinnis* and *F. lima*, to other fundulids have been among the most variable. Jordan and Evermann (1896) placed *F. parvipinnis* in a subgenus *Fundulus* that included all of the *Fundulus* species most commonly considered to be brackish or

TABLE 1. Previous classification of the Fundulidae, primarily based on Wiley (1986).

Family Fundulidae, Jordan and Gilbert 1883
Genus <i>Adinia</i> , Girard 1860a
<i>Adinia xenica</i> , (Jordan and Gilbert 1882)
Genus <i>Leptolucania</i> , Myers 1924
<i>Leptolucania ommata</i> (Jordan 1884)
Genus <i>Lucania</i> , Girard 1860b
<i>Lucania goodei</i> , Jordan 1880
<i>Lucania parva</i> , (Baird and Girard in Baird 1855)
<i>Lucania interioris</i> , Hubbs and Miller 1965
Genus <i>Fundulus</i> , Lacépède 1803
Subgenus <i>Plancterus</i> , Garman 1895
<i>Fundulus kansae</i> , Girard 1859b
<i>Fundulus zebrinus</i> , Jordan and Gilbert 1883
Subgenus <i>Zygonectes</i> , Agassiz 1854
<i>Fundulus chrysotus</i> , (Günther 1866)
<i>Fundulus luciae</i> , Baird 1855
<i>Fundulus cingulatus</i> , Valenciennes in Cuvier and Valenciennes 1846
<i>Fundulus rubrifrons</i> , (Jordan 1880)
<i>Fundulus jenkinsi</i> , (Evermann 1892)
<i>Fundulus sciadicus</i> , Cope 1865
The <i>Fundulus notatus</i> species group: <i>Fundulus notatus</i> , (Rafinesque 1820), <i>Fundulus olivaceus</i> , (Storer 1846), <i>Fundulus euryzonus</i> , Suttkus and Cashner 1981
The <i>Fundulus nottii</i> species group: <i>Fundulus dispar</i> , (Agassiz 1854), <i>Fundulus blairae</i> , Wiley and Hall 1975, <i>Fundulus lineolatus</i> , (Agassiz 1854), <i>Fundulus escambiae</i> , (Bollman 1887), <i>Fundulus nottii</i> , (Agassiz 1854)
Subgenus <i>Fundulus</i> , Lacépède 1803
<i>Fundulus confluentus</i> , Goode and Bean in Goode 1879
<i>Fundulus pulvereus</i> , (Evermann 1892)
The <i>Fundulus heteroclitus</i> species group: <i>Fundulus heteroclitus</i> , Linnaeus 1766, <i>Fundulus bermudae</i> , Günther 1874, <i>Fundulus relictus</i> , Able and Felley 1988, <i>Fundulus grandis</i> , Baird and Girard 1853b, <i>Fundulus grandissimus</i> , Hubbs 1936
Subgenus <i>Fontinus</i> , Jordan and Evermann 1896
<i>Fundulus diaphanus</i> , (Lesueur 1817)
<i>Fundulus waccamensis</i> , Hubbs and Raney 1946
<i>Fundulus seminolis</i> , Girard 1859a
The <i>Fundulus majalis</i> species group: <i>Fundulus majalis</i> , (Walbaum 1792), <i>Fundulus persimilis</i> , Miller 1955, <i>Fundulus similis</i> , (Baird and Girard 1854)
Subgenus <i>Xenisma</i> , Jordan 1877
<i>Fundulus rathbuni</i> , Jordan and Meek in Jordan 1896
<i>Fundulus albolineatus</i> , Gilbert 1891—extinct
<i>Fundulus julisia</i> , Williams and Etnier 1982
The <i>Fundulus catenatus</i> species group: <i>Fundulus stellifer</i> , (Jordan 1877), <i>Fundulus catenatus</i> , (Storer 1846), <i>Fundulus bifax</i> , Cashner, Rogers, and Grady 1988
Subgenus <i>Fundulus</i> , Lacépède 1803
<i>Fundulus confluentus</i> , Goode and Bean in Goode 1879
<i>Fundulus pulvereus</i> , (Evermann 1892)
The <i>Fundulus heteroclitus</i> species group: <i>Fundulus heteroclitus</i> , Linnaeus 1766, <i>Fundulus bermudae</i> , Günther 1874, <i>Fundulus relictus</i> , Able and Felley 1988, <i>Fundulus philpisteri</i> , García-Ramírez, Contreras-Balderas, and Lozano-Vilano 2007, <i>Fundulus grandis</i> , Baird and Girard 1853, <i>Fundulus grandissimus</i> , Hubbs 1936
Genus <i>Fundulus</i> incertae cedis, unclassified within a subgenus
<i>Fundulus parvipinnis</i> , Girard 1856
<i>Fundulus lima</i> , Vaillant 1894
† <i>Fundulus detillae</i> , Hibbard and Dunkle 1942—Pliocene, western KS
† <i>Fundulus curryi</i> , Miller 1945—Pliocene–Pleistocene, Death Valley, CA
† <i>Fundulus davidae</i> , Miller 1945—Pliocene–Pleistocene, Death Valley, CA
† <i>Fundulus eulepis</i> , Miller 1945—Pliocene–Pleistocene, Death Valley, CA
† <i>Fundulus lariversi</i> , Lugaski 1977—Miocene, central NV
† <i>Fundulus nevadensis</i> , (Eastman 1917)—Pliocene, northwestern NV

coastal in distribution. Based on a numerical analysis of morphological characteristics, Farris (1968) recognized *F. parvipinnis* and *F. lima* in a monophyletic subgenus *Xenisma* with the *F. catenatus* species group, *F. albolineatus*, *F. rathbuni*, *F. diaphanus*, *F. waccamensis*, *F. seminolis*, and the *F. majalis* species group. Wiley (1986) determined that *Fundulus parvipinnis* and *F. lima* were unable to be placed in any subgenus and recognized them as “other species” in his study of the relationships of the family using morphology.

Based on partial mitochondrial cytb DNA-sequence data, Bernardi (1997) suggested that *F. parvipinnis* and *F. lima* together are sister to all other fundulid species and Grady et al. (2001) depict a *F. parvipinnis* and *F. lima* clade as one of three clades in a trichotomy at the base of the genus *Fundulus*.

Studies focused within subgenera have, likewise, been inconclusive. Allozyme data (Rogers & Cashner, 1987; Cashner et al., 1988; Grady et al., 1990) and morphological data (Ghedotti et al., 2004) were contradictory regarding the

relationships among the *Fundulus catenatus* species group, *Fundulus rathbuni*, and the *F. albolineatus*–*F. julisia* clade. Hand-argued parsimony analysis of morphological data (Wiley, 1986), distance Wagner analysis of allozyme data (Cashner et al., 1992), and parsimony analysis of partial cytb DNA sequence data (Ghedotti & Grose, 1997) also have been contradictory regarding relationships within the subgenus *Zygonectes*.

Prior to this work, Whitehead's (2010) phylogenetic treatment of the Fundulidae (Fig. 2B) was the most taxonomically comprehensive phylogenetic investigation of the family since Wiley's (1986) study. Whitehead (2010) used nucleotide sequence data for three genes—mitochondrial cytb, and nuclear glycosyltransferase (gylt) and recombination activating gene 1 (RAG1)—rooted his topology using three *Profundulus* species, and recognized significantly different relationships within the family than had previously been discussed (Fig. 2). Although most or all taxa previously included in the subgenera *Fundulus*, *Zygonectes*, and *Plancterus* remained in discrete clades together, *Adinia* and *Lucania* were nested within *Fundulus*, and the subgenera *Fontinus* and *Xenisma* were not monophyletic. *Fundulus parvipinnis* and *F. lima* formed a clade sister to a clade of the included *Lucania* species. The genus *Leptolucania* was not included in the taxonomic sampling of Whitehead (2010); thus, the evolutionary relationships among all genera within the Family Fundulidae were not tested.

In this study we re-examine and significantly expand upon existing fundulid morphological data and use these data in conjunction with available behavioral, karyological, and nucleotide-sequence data to further our knowledge of the relationships and evolution of the Fundulidae. In addition we seek to include taxa for which full morphological consideration has not previously been given (e.g., *Fundulus parvipinnis* and *F. lima*) and to include a larger range of outgroup taxa to better investigate the monophyly of the Fundulidae and their relationships among other Cyprinodontiformes.

Evolution of Salinity Tolerance

Members of the Family Fundulidae are notable in the range of salinities commonly inhabited by its members, and all species are rather euryhaline when compared to sympatric ostariophysans (Renfro, 1959; Kendall & Schwartz, 1968). Some fundulid species can acclimate to a very broad range of salinities from freshwater to hypersaline (Griffith, 1972, 1974; Nordlie, 1987, 2000). *Fundulus heteroclitus* is well known to tolerate a wide range of salinity and is a commonly studied model laboratory organism used to understand cellular ionic regulation, especially as this relates to understanding ion-transport disorders in humans (Marshall et al., 2005; Choe et al., 2006; Kidder et al., 2006a, 2006b) and the environmental toxicology of ions (Blanchard & Grosell, 2006). This species also has been the subject of studies of the evolution of genetic differentiation and gene expression (Powers & Schulte, 1998; Oleksiak et al., 2002; Adams et al., 2006; Scott & Schulte, 2005). Because of the medical relevance of ionic regulation as studied in *F. heteroclitus* and the increasingly detailed understanding of the evolution of gene expression within this species, the evolution of salinity tolerance and ionic regulation at a macroevolutionary scale across fundulids will be a fruitful area of future study.

Griffith (1972) hypothesized that except for *F. seminolis* all *Fundulus* species with lower salinity tolerance (the *F. catenatus* species group, *F. julisia*, *F. rathbuni*, and most species of *Zygonectes*) formed a lineage of freshwater species with low salinity tolerance descended from the late Pliocene fossil species †*F. detillae*. He suggested that †*F. detillae* entered freshwater from a brackish environment and lost the ability to tolerate fully marine environments. Whitehead (2010) agreed with Griffith (1972, 1974) that the likely evolutionary transition within fundulids was from broad salinity tolerance to lower salinity tolerance due to ecological specialization for freshwater habitats. However, contrary to Griffith's (1972) suggestion of two shifts to reduced salinity tolerance, Whitehead (2010) concluded that the transition to his lowest category of salinity tolerance was inferred to have occurred at least three times, with a single possible reversal to very high salinity tolerance (“marine physiology”) and at least one reversal to high salinity tolerance (“brackish physiology”).

Although Whitehead's (2010) conclusions are indicated by explicit phylogenetic methods, as opposed to Griffith's (1972) conclusions based on “logical inference,” Whitehead (2010) used only a single cyprinodontoid outgroup (*Profundulus*). Because cyprinodontoid phylogeny is still somewhat tentative and the fact that most recent hypotheses suggest that *Profundulus* is not by itself the sister taxon to the Fundulidae, the use of only *Profundulus* as an outgroup could potentially affect rooting of the Fundulidae which, in turn, substantially affects the inferred directionality of character-state transitions. In addition, the assessment of salinity tolerance from the literature is in need of finer-scale consideration. We seek to test the salinity tolerance hypotheses of both Griffith (1972) and Whitehead (2010) within the context of a more detailed hypothesis of fundulid evolutionary relationships that includes more data, additional cyprinodontiform outgroups, and more refined salinity tolerance information for the family (e.g., Griffith, 1972; Nordlie, 1987, 2000; Dimaggio et al., 2010; Bianco & Nordlie, 2008).

Materials and Methods

Taxonomic Sampling

The taxonomic sampling of this study includes 41 of 43 species of fundulids with representative species from eight families in the Suborder Cyprinodontoidei. We did not include the endemic *Fundulus* of Bermuda (sometimes recognized as the separate species *F. bermudae* and *F. relictus*) as separate operational taxonomic units based on the results of Grady et al. (2001) that indicate these populations may be the result of multiple, possibly recent, colonization events of Bermuda by *F. heteroclitus*. *Fundulus* from Bermuda were generally examined only to verify their substantial morphological similarity to *F. heteroclitus*. In either case, the *Fundulus* on Bermuda are either most closely related to or conspecific with *F. heteroclitus*. Whitehead (2010) did not recover specimens of *F. bermudae* and *F. relictus* as their own clades based on nucleotide data, but rather indicated they were distributed within a *F. heteroclitus* clade, supporting the independent invasions hypothesis previously suggested by Grady et al. (2001).

Like Whitehead (2010), we also did not include *F. philpisteri* and *F. saguanus* Rivas, 1948. *Fundulus philpisteri* was newly

described as a likely inland isolate of *F. grandis* in arid northeastern México (García-Ramírez et al., 2007), and specimens were not available for this study. While not included, previous work suggests *F. plilpisteri* is likely closely related to *F. grandis* (García-Ramírez et al., 2007). *Fundulus saguanus* was described as a subspecies of *F. grandis* distributed in the Florida Keys and along the north coast of Cuba (Rivas, 1948). This taxon was not subsequently treated as a valid species in published literature but frequently was mentioned as possibly subspecifically distinct (Lee et al., 1980; Relyea, 1983; Miller, 2005). Whitehead (2010) treated *F. saguanus* as a valid species but cites Lee et al. (1980), who do not treat it as a valid species. We suspect that this treatment likely was based on its recognition as a species in the online resource FishBase (Froese & Pauly, 2012), in which all the cited publications treat the taxon as a subspecies. Therefore, lacking any published data in support of its recognition as a species, we treat *F. saguanus* as conspecific with *F. grandis*. We did explicitly treat *F. kansae* as a separate species from *F. zebrinus* as recognized by Kreiser et al. (2001), although we acknowledge that these species with adjacent biogeographic ranges are morphologically similar and intraspecifically variable (Poss & Miller, 1983; Kreiser et al., 2001). We recognize *F. cingulatus* and *F. rubrifrons* as separate species and include them separately in this study based on the results of Gilbert et al. (1992). We recognize *F. similis* and *F. majalis* as separate species for the purposes of this analysis, in recognition of their documented morphological and genetic differentiation (Relyea, 1983; Duggins et al., 1995) that demonstrate that they have been and continue to be functioning as separate evolutionary lineages.

The fossil species †*F. detillae* was included because it was available to the authors and its inclusion allows testing of the hypothesis of descent for this species proposed by Griffith (1972). The fossil species †*F. eulepis* was not included in the phylogenetic analyses because it was not available to the authors; however, the description of †*F. eulepis* (Miller, 1945) allowed the unambiguous placement of this taxon in the classification of the family. Other fossil taxa referred to the Fundulidae were not examined, and their descriptions in the literature do not allow their unambiguous phylogenetic placement (Eastman, 1917; Miller, 1945; Lugaski, 1977). We included *Kryptolebias marmoratus* (Poey, 1880) (Family Rivulidae) and *Aplocheilus panchax* (Hamilton, 1822) (Family Aplocheilidae) as outgroups and rooted the analysis on these species because they are members of the cyprinodontiform Suborder Aplocheiloidei (Parenti, 1981; Meyer & Lydeard, 1993; Murphy & Collier, 1997; Costa, 1998) that is sister to the Suborder Cyprinodontoidei, of which Fundulidae is a part.

We obtained non-nucleotide data from *Anableps dowi* Gill, 1861 and nucleotide-sequence data for *A. anableps*. In combined analyses these data were combined and the taxon is referred to as simply *Anableps*. Because this taxon is outside of the Fundulidae and the monophyly of the genus *Anableps*, the four-eyed fishes (Ghedotti, 1998), has never been questioned in the literature, we consider it reasonable to combine data from these two species as a reasonable representative of the genus *Anableps*.

Morphology, Karyology, and Behavioral Data (Non-Nucleotide Data)

Specimens examined morphologically were whole alcohol-preserved specimens or were cleared and stained for bone and

cartilage following Dingerkus and Uhler (1977) and are listed in Appendix I. 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 parasagittal cut through the body wall into the coelom from the anus around the right side of the pelvic girdle and to the pectoral girdle. Dissection for examination of lateral head musculature involved removal of the skin covering the lateral surface of the head, deflection of the lachrymal (if necessary), and the use of a probe to free A1 and A2 muscles from their origins on the dorsal and anterior preopercle and/or hyomandibula.

We surveyed specimens for potentially phylogenetically informative variation using a Leica MZ 12.5 stereomicroscope with an attached Q Imaging MicroPublisher 5.0 RTV photodocumentation system. Specimens of the extinct species *Fundulus albolineatus* were not available for direct examination of internal anatomy. External morphological data were available from Williams and Etnier (1982). Photographs and digital x-rays showing ventral and left lateral views of the lectotype (USNM 125055) and a paralectotype (USNM 225996) were examined. We coded character states that were not identifiable as unknown (?). The character state for the outgroup taxon *Aplocheilus panchax* was designated 0.

We obtained color pattern data, karyology, and reproductive behavior from published literature and unpublished theses. We obtained maximum salinity-tolerance data from descriptions in the literature and used these data to identify categories of salinity tolerance (see Appendix II). Experimental data were treated as more informative than field occurrence data, and experimental data were used wherever possible. The varying nature of how the maximum salinity-tolerance data were gathered in the original literature meant that quantitative assessments of data clustering were not appropriate. Institutional abbreviations are as listed in Leviton et al. (1985). Data were coded using numerical integers.

Nucleotide-Sequence Data, Alignment, Model Choice, and Partitioning

We reanalyzed the DNA-sequence data set from Whitehead (2010) that included one mitochondrial (cytb) and two nuclear genes (gylt and RAG1) with additional cyprinodontiform outgroups (Appendix III) to explore the effect of the more limited outgroup sampling in Whitehead (2010) on the rooting of the Fundulidae. All sequence data from Whitehead (2010) were included in our re-analysis, with the exception of four cytb sequences, two from *F. parvipinnis* and two from *F. lima*. Whitehead (2010) noted that the extremely long branch leading to a *Fundulus parvipinnis* and *F. lima* clade was due to an abnormal amount of cytb mutation, from which he suggested this extreme variation may be due to a non-neutral mitochondrial evolutionary processes. When sequences are compared to fundulids and other Cyprinodontiformes using NCBI (National Center for Biotechnology Information) GenBank BLAST (Basic Logical Alignment Search Tool) searches for nucleotide sequence similarity, the four cytb sequences were indicated as sharing a closer query coverage to other cyprinodontiform taxa (e.g., *Poecilia reticulata* Gill, 1861, *Oryzias latipes* (Temminck & Schlegel, 1846)) than fundulids, which was not the case for any other fundulid

nucleotide sequence from Whitehead (2010). To reduce the potential of inferring false evolutionary relationships because of long-branch attraction (Felsenstein, 2004; Bergsten, 2005), these four sequences were not included in this study. Mitochondrial and nuclear genes were aligned using the program MAFFT v6.0 with default parameters (Kato, 2008). We did not include additional gene-sequence data in this analysis to identify the effects of additional outgroup taxa and elimination of the extremely long branches in *F. parvipinnis* and *F. lima* due to cytb on analysis of Whitehead's (2010) data.

The phylogenetic re-analysis of the nucleotide-sequence data set modified from Whitehead (2010) had a total of 3,402 base pairs (bp), including one mitochondrial gene (cytb; 989 bp), and two protein-coding genes (gylt, 936 bp; RAG1, 1475 bp). The total-evidence data set included the genes from Whitehead (2010) with an additional mitochondrial gene, cytochrome *c* oxidase 1 (CO1; 651 bp) obtained from GenBank, for a combined total of 4054 bp. For each maximum-likelihood analysis, DNA was partitioned by individual gene fragments. A model of molecular evolution was chosen by the program jMODELTEST v.0.1.1 (Posada, 2008) with the best fitting model under the Akaike Information Criterion for each individual gene partition assigned, including RAG1 (TrN+I+ Γ), gylt (TrN+I), cytb (TVM+I+ Γ), and CO1 (TVM+I+ Γ).

Phylogenetic Analyses and Ancestral Character-State Reconstructions

A total evidence data set that included non-nucleotide (morphology, color pattern, karyology, and behavior) and DNA nucleotide characters was created that included 41 Fundulidae species with 20 additional cyprinodontiform species from eight families (Appendix III, Appendix IV). Nucleotide characters in the total evidence analyses included two mitochondrial genes (cytb, CO1) and two nuclear genes (gylt, RAG1); GenBank numbers for all nucleotide data are in Appendix III.

Likelihood analyses for all three data sets (DNA without CO1, morphology + karyology + behavior, and total evidence) were performed in GARLI v2.0 (Zwickl, 2006). For the non-nucleotide data set, a single partition was used under the MK (Markov) model for non-nucleotide data as recommended by Lewis (2001) for morphological data. All morphological characters are treated as unweighted, with each site variable at equal rates. Polymorphisms are treated as missing data in the likelihood analysis. Five partitions were employed in the total evidence analysis, including the morphological partition and four DNA partitions. While certain species in the total-evidence data set are missing DNA information, including fossil and extinct taxa, previous works have indicated that using a combination of morphological and molecular information under a total-evidence approach can provide a robust hypothesis of systematic placement for taxa that have only morphological data (e.g., Egge & Simons, 2009; Davis, 2010). Ten separate likelihood analyses were conducted for each data set, and the tree having the best likelihood score is presented here to evaluate evolutionary relationships. A nonparametric bootstrap analysis (Felsenstein, 1985) was performed for each data set with 100 random pseudoreplicates using the recommended default settings in the GARLI manual.

Parsimony analyses were also performed for the non-nucleotide data and total-evidence data sets in PAUP* (Swofford, 2002) with a heuristic search (10,000 random addition sequence replicates) and tree-bisection-reconnection (TBR) branch swapping. As with the likelihood analysis, all characters were unweighted and unordered. Polymorphisms are treated as multistate. Nonparametric bootstraps (Felsenstein, 1985) were performed with 1000 pseudoreplicates with 30 stepwise TBR replicates.

Ancestral character states were reconstructed using likelihood and parsimony methods in Mesquite 2.75 (Maddison & Maddison, 2012) based on the most likely topology of evolutionary relationships inferred from the maximum-likelihood total-evidence analysis. The Mk1 model (Lewis, 2001) was used to identify the state at each node that maximizes the probability of the states observed in the terminal taxa under the likelihood framework. All character states were unordered for the parsimony analysis. Character states for salinity tolerance are based on previous studies (Appendix I). Taxa for which salinity information is unknown are treated as pruned from the analysis during the calculation of the ancestral state.

Non-Nucleotide Transformation Series

See Appendix I for material examined in identification of morphological transformation series. We coded 154 anatomical, 19 color pattern, 7 behavioral, and 1 karyological transformation series for phylogenetic analysis. Each transformation series description indicates previous phylogenetic studies that have used the same or similar transformation series when relevant and indicates the distribution of the less common states in the taxa in this study. Mention of presence in a particular family, subfamily, genus, subgenus, or species group indicates only its presence in those taxa examined. In the case of non-fundulid taxa this may be a rather limited sampling of taxa. The composition of mentioned species groups follows past usage and is indicated in Tables 1 and 2. Specific figure citations are provided for many past morphological studies to assist the reader in character-state delimitation and also provide visual references for some character states not figured in this study, particularly those that occur outside of the Fundulidae. See Appendix IV for summary distribution of non-nucleotide character states among taxa (data matrix) used in phylogenetic analysis.

Skeletal Morphology

1. Laminar flange on nasals (Fig. 3). Parenti (1981) recognized the presence of medially expanded nasals in *Jenynsia*, *Oxyzygonectes*, the poeciliines, procatopodines, *Aplocheilichthys spilarchus* (Duméril, 1861), cyprinodontids, and goodeids (secondarily reduced in some groups), as synapomorphic for the group containing all cyprinodontoids except for fundulids, profundulids, and *Valencia*. Costa (1998: fig. 10) recognized a similar distribution of this character state but noted its absence in the goodeids. Ghedotti et al. (2004: fig. 1B, 1C) recognized that a studfish clade is diagnosed by more narrow laminar flanges on the nasals compared to the other

TABLE 2. Classification of the Fundulidae.

Family Fundulidae, Jordan and Gilbert 1883

Genus *Leptolucania*, Myers 1924*Leptolucania ommata*, (Jordan 1884)Genus *Lucania*, Girard 1860b*Lucania goodei*, Jordan 1880*Lucania parva*, (Baird and Girard in Baird 1855)*Lucania interioris*, Hubbs and Miller 1965Genus *Fundulus*, Lacépède 1803Subgenus *Wileyichthys* subgen. nov.†*Fundulus eulepis*, Miller 1945—Pliocene–Pleistocene, Death Valley, CA*Fundulus parvipinnis*, Girard 1856*Fundulus linia*, Vaillant 1894Subgenus *Plancterus*, Garman 1895†*Fundulus detillae*, Hibbard and Dunkle 1942—Pliocene, western KS*Fundulus kansae*, Girard 1859b*Fundulus zebrinus*, Jordan and Gilbert 1883Subgenus *Zygonectes*, Agassiz 1854*Fundulus chrysotus*, (Günther 1866)*Fundulus luciae*, Baird 1855*Fundulus xenicus*, Jordan and Gilbert 1882*Fundulus cingulatus*, Valenciennes in Cuvier and Valenciennes 1846*Fundulus rubrifrons*, (Jordan 1880)*Fundulus sciadicus*, Cope 1865The *Fundulus notatus* species group: *Fundulus notatus*, (Rafinesque 1820), *Fundulus olivaceus*, (Storer 1846), *Fundulus euryzonus*, Suttkus and Cashner 1981The *Fundulus nottii* species group: *Fundulus dispar*, (Agassiz 1854), *Fundulus blairae*, Wiley and Hall 1975, *Fundulus lineolatus*, (Agassiz 1854), *Fundulus escambiae*, (Bollman 1887), *Fundulus nottii*, (Agassiz 1854)Subgenus *Fundulus*, Lacépède 1803The *Fundulus majalis* species group: *Fundulus majalis*, (Walbaum 1792), *Fundulus persimilis*, Miller 1955, *Fundulus similis*, (Baird and Girard 1853b)**Fundulus seminolis*, Girard 1859a*Fundulus rathbuni*, Jordan and Meek in Jordan 1896*Fundulus diaphanus*, (Lesueur 1817)*Fundulus waccamensis*, Hubbs and Raney 1946*Fundulus albolineatus*, Gilbert 1891—extinct*Fundulus julisia*, Williams and Etnier 1982The *Fundulus catenatus* species group: *Fundulus stellifer*, (Jordan 1877), *Fundulus catenatus*, (Storer 1846), *Fundulus bifax*, Cashner, Rogers, and Grady 1988*Fundulus jenkinsi*, (Evermann 1892)*Fundulus confluentus*, Goode and Bean in Goode 1879*Fundulus pulverens*, (Evermann 1892)The *Fundulus heteroclitus* species group: *Fundulus heteroclitus*, Linnaeus 1766, *Fundulus bermudae*, Günther 1874*, *Fundulus relictus*, Able and Felley 1988*, *Fundulus philpisteri*, García-Ramírez, Contreras-Balderas, and Lozano-Vilano 2007, *Fundulus grandis*, Baird and Girard 1853b, *Fundulus grandissimus*, Hubbs 1936Genus *Fundulus* incertae cedis, unclassified within a subgenus**†*Fundulus curryi*, Miller 1945—Pliocene–Pleistocene, Death Valley, CA†*Fundulus davidae*, Miller 1945—Pliocene–Pleistocene, Death Valley, CA†*Fundulus lariversi*, Lugaski 1977—Miocene, central NV†*Fundulus nevadensis*, (Eastman 1917)—Pliocene, northwestern NV

* Some data suggest that these species may be distinct population segments rather than valid species. See Materials and Methods for further discussion.

** Four fossil species currently referred to the genus *Fundulus* remain *incertae cedis*, because we did not have the opportunity to examine specimens and the information available in the literature was not sufficient to reasonably classify them based on likely synapomorphic character states.

fundulids examined. The lateralis canal is bounded by a bony flange or flanges in most taxa and is used as a point of reference in assessing nasal width. In taxa without discrete bony flanges associated with the lateralis canal, the width of the canal is approximated based upon overlying soft tissue and impression of the canal on the underlying nasal bone. A narrow laminar flange on the nasals is present in *Fundulus seminolis*, the *F. majalis* species group, the *F. heteroclitus* species group, *F. confluentus*, *F. pulverens*, and the *F. catenatus* species group, as well as in the *Profundulus* species examined, *Crenichthys baileyi* (Gilbert, 1893), *Ameca splendens* Miller

& Fitzsimmons, 1971, *Anableps dowi*, *Orestias agassizii* Valenciennes, 1846, and *Aphanius dispar* (Rüppell, 1829). A particularly wide laminar flange of the nasals medial to the lateralis canal is present in *Cubanichthys pengelleyi* (Fowler, 1939), *Cyprinodon variegatus* Lacépède, 1803, and *Floridichthys carpio* (Günther, 1866).

(1₀) Laminar flange of nasals mostly lateral to canal and less than twice as long as wide; laterally convex or flat.

(1₁) Laminar flange of nasals mostly lateral to canal and more than twice as long as wide; laterally concave or flat.

FIG. 3. Neurocrania of selected cleared and stained fundulid specimens. Anterior is at top. (A) *Fundulus heteroclitus* KU 15351, dorsal view, (B) ventral view. (C) *F. notatus* KU 18021, dorsal view, (D) ventral view. (E) *F. similis* KU 12827, dorsal view, (F) ventral view. (G) *F. kansae* KU 14726, dorsal view, (H) ventral view. (I) *Lucania goodei* KU 17993, dorsal view, (J) ventral view. Dotted lines show overlying dermal bone margins. Line drawings of *F. heteroclitus* are from different specimens in same lot. Note that in many specimens the left and right posttemporals may be variably attached. Scale bars indicate 1 mm. Abbreviations: boc = basioccipital, dsp = dermosphenotic, epot = epiotic, exoc = exoccipitals, fr = frontal, le = lateral ethmoid, me = mesethmoid, nas = nasal, par = parietal, pro = prootic, psph = parasphenoid, pto = pterotic, ptsph = pterosphenoid, soc = supraoccipital, sp = sphenotic, vo = vomer.

- (1₂) Posterior laminar flange of nasals medial to canal and about twice as long as wide; laterally concave.
2. Bony lateralis canal on nasals (Fig. 3). The absence of the canal is generally correlated with having an open cephalic sensory canal system. However, it is not strictly linked to this because cephalic neuromasts are exposed in *Valencia letourneuxi* (Sauvage, 1880), *Lucania goodei*, *Fundulus xenicus*, and *Orestias agassizi*, all of which have a distinct bony trough around the lateralis canal superficial to the nasal bones. The bony canal is present in all examined fundulids, the examined cyprinodontids, the examined *Profundulus* species, *Crenichthys baileyi*, *Ameca splendens*, and *Valencia letourneuxi*.
 - (2₀) Bony canal on nasals absent or only present as unpaired small flange in middle of nasal bone.
 - (2₁) Bony trough along anterior supraorbital canal present on nasal bone and prominent anteriorly extending distinctly anterior of laminar flange of main body of bone.
3. Mesethmoid ossification (Fig. 3). Parenti (1981: figs. 16, 17) and Costa (1997, 1998: fig. 9) recognized a cartilaginous mesethmoid in the aplocheiloids, procatopodines, and *Aphanius* (including *Kosswigichthys*), as homoplastically synapomorphic for the Aplocheilichthyinae (including the procatopodines and with the mesethmoid condition reversed in *Aplocheilichthys spilauchen*) and the Aplocheiloidei. Tigano and Parenti (1988) noted that the ventral mesethmoid does undergo some ossification and fuses to the dermal vomer. Ghedotti (2000: fig. 3) recognized a cartilaginous mesethmoid as diagnostic of the procatopodines and also present in *Cyprinodon variegatus*. Lucinda and Reis (2005) noted the presence of an ossified mesethmoid in *Aplocheilichthys spilauchen* and all poeciliines except *Priapella*. An ossified and anteriorly convex mesethmoid is present in the examined fundulids, poeciliids, and anablepids, as well as in *Orestias agassizi*, *Cyprinodon variegatus*, *Floridichthys carpio*, and *Valencia letourneuxi*. An ossified and anteriorly concave mesethmoid is found in the examined *Profundulus* species, *Crenichthys baileyi*, and *Ameca splendens*.
 - (3₀) Mesethmoid cartilaginous.
 - (3₁) Mesethmoid ossified; anterior margin concave.
 - (3₂) Mesethmoid ossified, anterior margin convex.
4. Anterior margin of frontals (Fig. 3). Wiley (1986: figs. 6, 7) and Ghedotti et al. (2004: fig. 3B, 3C) recognized the derived state in the then-recognized subgenera *Xenisma* and *Fontinus*. Ghedotti (2000) and Lucinda and Reis (2005) recognized frontals that extend anteriorly between the nasals in most poecilioids. Frontals that extend anteriorly between the nasals are found in the *Fundulus majalis* species group, *F. seminolis*, *F. diaphanus*, *F. waccamensis*, *F. rathbuni*, *F. julisia*, the *F. catenatus* species group, *Oxyzygonectes dovii* (Günther, 1866), *Aplocheilichthys spilauchen*, *Alfaro cultratus* (Regan, 1908), and *Poecilia reticulata*.
 - (4₀) Anterior margin of frontals mostly straight, possibly having low, rounded anterior extension.
 - (4₁) Anterior margin of frontals with distinct anterior extension between the posterior nasals.
5. Size and presence of parietals (Fig. 3). Parenti (1981) recognized the absence of parietals as synapomorphic for a clade composed of all cyprinodontids except for *Cubanichthys* and putatively synapomorphic for all *Fluviophylax*, *Aplocheilichthys spilauchen*, and the procatopodines. Costa (1997) also recognized the absence of parietals as synapomorphic for a clade composed of all cyprinodontids except for *Cubanichthys*. Ghedotti (2000: fig. 3) noted the absence of parietals in *Cyprinodon variegatus*, *Fluviophylax obscurum* Costa, 1996, *Aplocheilichthys spilauchen*, most procatopodines, and some poeciliines and noted small parietals in various poeciliids. Lucinda and Reis (2005) in their extensive study of poeciliines found the condition of the parietals to vary greatly among taxa with multiple inferred events of reduction in size or loss of parietals. We observed small parietals that do not reach the sphenotic anterolaterally in *Fundulus similis* (which just met this length criterion for small size), some individuals of *Profundulus punctatus* (coded as polymorphic [0&1]), the examined anablepids, and the examined poeciliines. Parietals were absent in all cyprinodontids examined (including *Cubanichthys pengelleyi*) and *Aplocheilichthys spilauchen*.
 - (5₀) Parietals present and large, reaching sphenotic anterolaterally.
 - (5₁) Parietals present and small, restricted to epiotic region.
 - (5₂) Parietals absent.
6. Dorsolateral flange on pterotic (Fig. 3). Wiley (1986: fig. 14) recognized the presence of a large dorsolateral flange on the pterotic that contacts a corresponding flange on the sphenotic as a synapomorphy of the *F. nottii* species group. We observed this flange in the *F. nottii* species group and in *Floridichthys carpio*.
 - (6₀) Dorsolateral flange on pterotic small or absent.
 - (6₁) Dorsolateral flange on pterotic present and large, contacting corresponding flange on the sphenotic.
7. Shape of and angle of medial margin of the lateral semicircular canals (Fig. 3). The lateral semicircular canals are visible dorsally through the pterotics in cleared and stained specimens. The medial margin of the lateral semicircular canal is sharply angled, forming a narrow elongate profile in *Lucania*, *Crenichthys baileyi*, *Ameca splendens*, *Cubanichthys pengelleyi*, and *Cyprinodon variegatus*.
 - (7₀) Medial margin of lateral semicircular canal triangular with 20–40° angle laterally.
 - (7₁) Medial margin of lateral semicircular elongate with sharp angle (<20°) laterally.
8. Lateral margin of lateral pterotic flange (Fig. 3). The lateral pterotic flange forms a distinct lateral projection resulting in two apices in the *Fundulus heteroclitus* species group, the *F. majalis* species group, the *F. catenatus* species group, *Crenichthys baileyi*, *Ameca splendens*, and some individuals of *Anableps dowi* (coded as polymorphic [0&1]).
 - (8₀) Lateral margin of pterotic flange continuous.
 - (8₁) Lateral margin of pterotic flange cleft forming two parts.
9. Medial extent of posterior pterotic flange (Fig. 3). Wiley (1986: figs. 11, 14) recognized a prominent posterior

- pterotic flange extending medially, posterior to the lateral portion of the exoccipital, as synapomorphic for the *Fundulus majalis* species group. We observed this degree of medial length of the posterior pterotic in the *F. majalis* species group, *F. parvipinnis*, *F. lima*, *F. kansae*, and *F. zebrinus*. In addition we recognize a less extensive state in this transformation series where the posterior pterotic flange extends posteromedially to the margin of the main body of the pterotic in the *F. heteroclitus* species group, *F. confluentus*, *F. pulvereus*, the *F. catenatus* species group, *F. julisia*, *F. rathbuni*, *F. diaphanus*, *F. waccamensis*, *F. chrysotus*, *Ameca splendens*, and *Floridichthys carpio*.
- (9₀) Posterior pterotic flange does not extend medially to medial margin of main body of pterotic.
- (9₁) Posterior pterotic flange confluent with medial margin of main body of pterotic.
- (9₂) Posterior pterotic flange extends medially beyond margin of main body of bone posterior lateral-most portion of exoccipitals.
10. Length of posterior crest of supraoccipital (Fig. 3). Parenti (1981: figs. 58–61) and Costa (1997, 1998) recognized elongate supraoccipital crests as synapomorphic for anablepids. We observed long supraoccipital crests extending to above the second neural arch or more posterior in *Oxyzygonectes dovii* and *Anableps dowi*. The supraoccipital crest is variably long or moderately long among individuals of *Jenynsia multidentata* (Jenyns, 1842) (coded as polymorphic [0&2]). The supraoccipital crest is short in *Lucania*, *Leptolucania ommata*, and *Orestias agassizi*.
- (10₀) Posterior crest of supraoccipital moderately long, posterior-most extent above first neural arch.
- (10₁) Posterior crest of supraoccipital short, posterior-most extent anterior to first neural arch.
- (10₂) Posterior crest of supraoccipital long, posterior-most extent above second neural arch or more posterior.
11. Presence and length of posterior epiotic crests (Fig. 3). In conjunction with an expanded supraoccipital crest, Parenti (1981: figs. 58–61) recognized expanded exoccipital crests as synapomorphic of anablepids. Ghedotti (1998, 2000: fig. 3) and subsequently Lucinda and Reis (2005) subdivided the transformation series into three states to include absence. These studies recognized elongate epiotic crests as present in *Aplocheilichthys spilauchen*, *Anableps*, *Oxyzygonectes*, and three of nine *Jenynsia* species examined and absence of these crests in all procatopodines, various poeciliines, the included cyprinodontids, *Crenichthys baileyi*, and *Valencia*. We observed long epiotic processes extending above the first neural arch or more posteriorly in all *Fundulus*, *Profundulus*, and anablepids examined, as well as in *Aplocheilichthys spilauchen* and *Alfaro cultratus*. These crests are present and short in *Lucania parva*, *Ameca splendens*, *Poecilia reticulata*, and *Gambusia affinis* (Baird & Girard, 1853a). *Lucania interioris* and *L. goodei* variably lacked or had short supraoccipital crests among individuals and were coded as polymorphic (0&1).
- (11₀) Posterior crests of epiotics absent.
- (11₁) Posterior crests of epiotics short, posterior-most extent anterior to first neural arch.
- (11₂) Posterior crests of epiotics long, posterior-most extent above first neural arch or more posterior.
12. Width of base of epiotic crests (Fig. 3). In the *F. majalis* species group and *Profundulus punctatus* the base of the epiotic processes are notably broad and are associated with a small contiguous flange on the adjacent supraoccipital. This is coded as unknown (?) for species where the observed specimens lacked epiotic processes.
- (12₀) Base of epiotic crests narrow, without adjacent flange of supraoccipital.
- (12₁) Base of epiotic crests broad due to adjacent flange of supraoccipital.
13. Lateral ethmoid position with respect to parasphenoid (Fig. 3). Wiley (1986: fig. 2) recognized lateral ethmoids that overlap the parasphenoid dorsally as synapomorphic of *Fundulus diaphanus*, *F. waccamensis*, *F. seminolis*, and the *F. majalis* species group. We note that more medially positioned lateral ethmoids that overlap the parasphenoid dorsally are present in *F. diaphanus*, *F. waccamensis*, *F. seminolis*, the *F. majalis* species group, *Lucania*, *Aplocheilichthys spilauchen*, *Alfaro cultratus*, and all cyprinodontids examined except *Aphanius dispar*. The lateral ethmoids variably overlap the parasphenoid dorsally in some individuals of *Crenichthys baileyi*, and we coded this taxon as polymorphic (0&1).
- (13₀) Lateral ethmoids positioned more laterally, do not overlap parasphenoid dorsally.
- (13₁) Lateral ethmoids positioned more medially, overlap parasphenoid dorsally.
14. Posterior-facing point on antero-lateral arm of lateral ethmoid (Fig. 3). When present, this process is associated articulation with the head of the autopalatine. Parenti (1981: figs. 16, 17, 57) and Costa (1998: fig. 9) recognized the presence of this process in aplocheiloids and *Profundulus*, although Costa further divided the character state, recognizing a smaller process in *Profundulus* and a larger process in aplocheiloids. Here we treat this as a presence-or-absence transformation series because of the variation in size of this process observed among the *Profundulus* species examined, *Aplocheilus panchax*, and *Kryptolebias marmoratus*.
- (14₀) Posterior-facing point on anterolateral arm of lateral ethmoids present.
- (14₁) Posterior-facing point on anterolateral arm of lateral ethmoids absent.
15. Anterior margin of vomer (Fig. 3). The anterior margin of the vomer can be straight to slightly concave or very distinctly concave forming a distinct U-shape. Costa (1998: fig. 9) recognized the presence of a distinctly concave anterior margin of the vomer (described as a “Y-shaped” vomer) in *Profundulus*. Farris (1968) noted an obviously concave anterior margin of the vomer in *Profundulus*, *Lucania parva*, and a majority of the *Fundulus* taxa he examined. We recognized two discrete concave states, a deeply concave anterior margin of the vomer in the species of *Profundulus* examined and a less pronounced anterior concavity in *F. heteroclitus*, *F. confluentus*, *F. pulvereus*, *F. seminolis*, the *F. majalis* species group, *F. diaphanus*, *F. waccamensis*, *F. rathbuni*, *F. julisia*, the *F. catenatus* species group, *Cyprinodon variegatus*, and *Floridichthys carpio*.

- (15₀) Anterior margin of vomer approximately straight.
- (15₁) Anterior margin of vomer shallowly but distinctly concave.
- (15₂) Anterior margin of vomer deeply concave.
16. Posterolateral processes of head of vomer (Fig. 3). The head of the vomer underlies the ethmoid cartilage and the posterolateral portions of this head may form discrete posterolateral facing processes, which in some cases contact the anterolateral ethmoid ossifications. Wiley (1986: fig. 2) identified long lateral processes of the vomer forming an acute angle with the main body of the vomer as diagnostic of *Fundulus diaphanus*, *F. waccamensis*, *F. seminolis*, and the *F. majalis* species group. We recognized two states with respect to the presence of these processes. Posterolateral processes on the anterior head of the vomer that extend dorsally and form a sharply acute angle with the main shaft of the vomer in ventral view are present in *F. diaphanus*, *F. waccamensis*, *F. seminolis*, the *F. majalis* species group, and *F. confluentus*. Posterolateral processes on the anterior head of the vomer that remain in the same plane as the head of the vomer are present in the *F. heteroclitus* species group, *F. pulvereus*, *F. parvipinnis*, *F. lima*, *F. rathbuni*, *F. julisia*, the *F. catenatus* species group, *F. chrysotus*, *F. luciae*, *F. cingulatus*, *F. rubrifrons*, *F. sciadicus*, the *F. notatus* species group, the *F. nottii* species group, *Lucania*, *Leptolucania ommata*, *Cubanichthys pengelleyi*, *Cyprinodon variegatus*, *Aphanius dispar*, *Anableps dowi*, and *Valencia letourneuxi*.
- (16₀) Posterolateral processes on anterior head of vomer absent.
- (16₁) Posterolateral processes on anterior head of vomer present extending dorsally and form sharply acute angle with main shaft of vomer in ventral view.
- (16₂) Posterolateral processes on anterior head of vomer present in same plane as head of vomer.
17. Width of posterior vomer (Fig. 3). The vomer is about as wide as the overlapping anterior parasphenoid, in *Fundulus lineolatus*, *F. nottii*, and *F. escambiae*, *Cubanichthys pengelleyi*, *Cyprinodon variegatus*, *Aplocheilus panchax*, and *Kryptolebias marmoratus*. It is distinctly narrower with respect to the anterior parasphenoid in other taxa.
- (17₀) Posterior vomer about as wide as overlapping anterior parasphenoid.
- (17₁) Posterior vomer distinctly more narrow than overlapping anterior parasphenoid.
18. Parasphenoid width in region of orbit (Fig. 3). The parasphenoid distinctly narrows in the vicinity of the anterior orbit in *Aplocheilus panchax* and *Kryptolebias marmoratus*. The parasphenoid is very distinctly narrow throughout its length in the orbital region in *Lucania*, *Leptolucania ommata*, *Cubanichthys pengelleyi*, and *Cyprinodon variegatus*.
- (18₀) Parasphenoid distinctly narrows in region of anterior orbit.
- (18₁) Parasphenoid distinctly broadens in region of anterior orbit.
- (18₂) Parasphenoid remains equally narrow throughout orbital region.
19. Ventral extent of pterosphenoid (Fig. 3). The pterosphenoid typically is long, contacting the ascending process of the parasphenoid. In *Fundulus blairae*, *F. nottii*, *F. xenicus*, *Lucania*, *Leptolucania ommata*, *Cubanichthys pengelleyi*, *Cyprinodon variegatus*, *Floridichthys carpio*, and *Orestias agassizi*, the pterosphenoid is short and does not contact the parasphenoid. In *Fundulus parvipinnis*, *F. lima*, *Aplocheilus panchax*, *Aphanius dispar*, the examined anablepids, and the examined poeciliines, the pterosphenoid is short, angled more laterally, and contacts the base of the anterior ascending process of the prootic.
- (19₀) Pterosphenoid short and more laterally angled, contacts base of anterior ascending process of prootic.
- (19₁) Pterosphenoid short, does not contact ascending process of parasphenoid.
- (19₂) Pterosphenoid long, contacts ascending process of parasphenoid.
20. Ventral extent of anterior ascending process of the prootic (Fig. 3). Most commonly in the examined taxa the anterior ascending process of the prootic is long and extends from above the trigeminofacialis opening of the prootic and contacts the ascending process of the parasphenoid and sometimes the pterosphenoid at their tips. Ghedotti et al. (2004: fig. 1D, 1E) recognized a long ascending process of the prootic in the examined species of *Fundulus*, except *F. zebrinus* where it was absent. The process is present but short and does not contact other ossified elements in *F. julisia*, the *F. catenatus* species group, *F. xenicus*, *F. cingulatus*, *F. rubrifrons*, *F. sciadicus*, and *Orestias agassizi*. The ascending process of the prootic is absent in *F. zebrinus*, *F. kansae*, *Lucania*, *Leptolucania ommata*, *Cubanichthys pengelleyi*, *Cyprinodon variegatus*, *Floridichthys carpio*, *Orestias agassizi*, *Valencia*, *Aplocheilus panchax*, and *Kryptolebias marmoratus*.
- (20₀) Anterior ascending process of prootic absent.
- (20₁) Anterior ascending process of prootic short, does not contact ascending process of parasphenoid or pterosphenoid.
- (20₂) Anterior ascending process of prootic long, contacts ascending process of parasphenoid and often pterosphenoid as well.
21. Prootic bridge over trigeminofacialis canal (Fig. 3). Wiley (1986: fig. 10) recognized a distinctly narrow prootic bridge as diagnostic of a clade containing *Fundulus sciadicus*, the *F. notatus* species group, and the *F. nottii* species group, and recognized a wide prootic bridge as synapomorphic of a clade composed of *F. seminolis* and the *F. majalis* species group. Ghedotti (2000: fig. 3) in a broad survey of cyprinodontoids also recognized a narrow prootic bridge in some procatopodines and a broad bridge in most anablepids. A broad prootic bridge is present in *F. seminolis*, the *F. majalis* species group, *F. confluentus*, *F. parvipinnis*, *F. lima*, *F. kansae*, *F. zebrinus*, the examined anablepids, and *Kryptolebias marmoratus*. A distinctly narrow prootic bridge is recognized as present in *F. lineolatus*, *F. nottii*, *F. escambiae*, *F. confluentus*, *Cubanichthys pengelleyi*, *Cyprinodon variegatus*, *Floridichthys carpio*, *Aphanius dispar*, and *Aplocheilus panchax*.

- (21₀) Prootic bridge over trigeminofacialis chamber narrow.
- (21₁) Prootic bridge over trigeminofacialis chamber intermediate in width.
- (21₂) Prootic bridge over trigeminofacialis chamber broad.
22. Lateral prong on ventrolateral exoccipital. A prong extends laterally from the exoccipitals ventral to the medial portion of the pterotic in *Fundulus parvipinnis*, *F. lima*, and *F. chrysotus*. This prong is only present in large individuals of *F. chrysotus*, and it is much more prominent in *F. parvipinnis* and *F. lima*.
- (22₀) Exoccipitals without lateral prong.
- (22₁) Lateral prong extending laterally from exoccipitals ventral to medial portion of pterotic.
23. Size of the intercalar. The intercalar, when present, lies at the point of contact of the lower arm of the posttemporal with the neurocranium, and if the lower arm is unossified at the point of contact of the ligament in which the lower arm ossifies in other taxa. Ghedotti (1998, 2000) recognized an elongate intercalary as present in anablepids, *Aplocheilichthys spilauchen*, and some procatopodines. An elongate intercalar is present in *Profundulus punctatus* (Günther, 1866), *P. guatemalensis* (Günther, 1866), *Aplocheilichthys spilauchen*, and all examined anablepids. Small and absent intercalars are not recognized as separate character states because these two conditions are polymorphic within species and within individuals.
- (23₀) Intercalar small or absent, when present, restricted to site of attachment of lower arm of posttemporal or equivalent ligament.
- (23₁) Intercalar large elongate, extending laterally beyond point of attachment of posttemporal, frequently extending to underlie pterotic.
24. Length of ascending process of premaxilla (Fig. 4). Variation in shape and length of the premaxillary ascending process has been recognized as relevant to cyprinodontoid relations since the first phylogenetic studies of these groups (Farris, 1968; Parenti, 1981: figs. 4, 5, 35, 39, 41). Costa (1998: fig. 1) recognized three ascending process characteristics as variably informative (length, overall shape, and shape of tip), and recognized the presence of long, relatively narrow ascending processes in fundulids and valenciids as likely the ancestral cyprinodontoid condition. Herein we largely follow Costa's use of three transition series, except rather than using overall shape, we instead use the width at the base to better comply with the assumption of independence among transformation series. Most species examined had long premaxillary ascending processes. We observed short processes in the examined poeciliines, and the examined *Profundulus* species, as well as in *Ameca splendens*, *Crenichthys baileyi*, *Cyprinodon variegatus*, *Cubanichthys pengelleyi*, and *Aphanius dispar*. The processes are absent in *Anableps dowi*.
- (24₀) Premaxillary ascending processes long, extending posterior of anterior margin of lateral ethmoids when retracted.
- (24₁) Premaxillary ascending processes short, not extending posterior of anterior margin of lateral ethmoids when retracted.
- (24₂) Premaxillary ascending processes absent.
25. Width of ascending process of the premaxilla (Fig. 4). Most premaxillary ascending processes examined had narrow bases. We observed broad bases of the ascending processes in the examined anablepids, the examined poeciliids, and the examined species of *Profundulus*, as well as in *Aplocheilus panchax*, *Valencia letourneuxi*, *Crenichthys baileyi*, *Ameca splendens*, *Cyprinodon variegatus*, *Cubanichthys pengelleyi*, *Aphanius dispar*, *Fundulus sciadicus*, the *F. notatus* species group, and the *F. nottii* species group. The processes are absent in *Anableps*; thus, we coded *A. dowi* as unknown (?).
- (25₀) Premaxillary ascending processes with broad bases.
- (25₁) Premaxillary ascending processes with narrow bases.
26. Shape of the tips of the ascending process of the premaxilla (Fig. 4). Most species examined had acutely pointed tips of the premaxillary ascending processes. We observed broadly rounded tips of these processes in *Aplocheilus panchax* and *Alfaro cultratus*. Squared tips of the premaxillary ascending processes occur in *Kryptolebias marmoratus*, *Oxyzygonectes dovii*, and the examined species of *Profundulus*. The processes are absent in *Anableps*; thus, we coded *A. dowi* as unknown (?).
- (26₀) Tips of premaxillary ascending processes broadly rounded.
- (26₁) Tips of premaxillary ascending processes squared.
- (26₂) Tips of premaxillary ascending processes acutely angled, forming point.
27. Posterior margin of alveolar process of premaxilla (Fig. 4). Parenti (1981) and Costa (1998) recognized a posteriorly indented alveolar arm producing an "S-shaped" alveolar arm as synapomorphic of the Cyprinodontoidae. We observed the presence of this indentation in all cyprinodontoids examined and its absence in the two aplocheiloids examined.
- (27₀) Posterior margin of alveolar process of premaxilla straight or convex, lacking indentation.
- (27₁) Posterior margin of alveolar process of premaxilla with distinct indentation.
28. Rostral cartilage(s) (Fig. 4). The rostral cartilages, when present, lie in or closely associated with the connective tissue medial to the left and right ventromedial processes of the maxillary heads. Parenti (1981: figs. 4, 5, 35, 39, 41) recognized rostral cartilages as large in aplocheiloids and reduced or absent in cyprinodontoids. Costa (1998: fig. 2) recognized a greater diversity of variation and coded three characteristics related to rostral cartilages (size, shape, and presence of posterior accessory cartilage). Costa (1998) recognized the presence of a second posterior accessory cartilage as apomorphic for the Fundulidae. We had difficulty recognizing discrete states of size variation and were uncertain about the homology of the two chondrifications in fundulids; thus, we recognize only one rostral-cartilage transformation series with multiple states. The most common, but not the only,

FIG. 4. Medial view of right jaws, suspensoria, and opercular series in cleared and stained (A) *Fundulus notatus* KU 18021, (B) *F. kansae* KU 14726, (C) *F. heteroclitus* KU 15351, (D) *F. similis* KU 1282, and (E) *Lucania goodei* KU 17993. The interhyal was left attached to the suspensorium. Dorsal view of snout region in cleared and stained (F) *Fundulus similis* KU 12827 and (G) *Lucania goodei* KU 17993. Rostral cartilages are outlined with white dotted lines. Anterior is at left. Scale bars indicate 1 mm. Abbreviations: ar = articular, de = dentary, enpt = endopterygoid, hy = hyomandibula, iop = interopercle, mc = Meckel's cartilage, mx = maxilla, op = opercle, pal = palatine, pmx = premaxilla, pop = preopercle, qu = quadrate, ra = retroarticular, sop = subopercle, sy = symplectic.

- condition observed in fundulid taxa is the presence of two anterior-to-posterior rostral cartilages, i.e., the presence of a posterior accessory cartilage as described by Costa (1998). *Fundulus jenkinsi* and *F. luciae* have a single anterior-to-posterior elongate rostral cartilage. A single round or squared rostral cartilage is present in *Aplocheilichthys panchax*, *Kryptolebias marmoratus*, *Aplocheilichthys spilauchen*, *Jenynsia multidentata*, *Oxyzygonectes dovii*, *Lucania*, *Leptolucania*, *F. xenicus*, and the *F. nottii* species group. A single laterally elongate rostral cartilage is present in the *Profundulus* species examined, *Cubanichthys pengelleyi*, *Cyprinodon variegatus*, *Floridichthys carpio*, and *Anableps dowi*. *Crenichthys baileyi* has two small rostral cartilages arrayed laterally. A rostral cartilage is absent in the examined poeciliines, *Aphanius dispar*, *Orestias agassizi*, and *Ameca splendens*.
- (28₀) Single round or squared rostral cartilage, approximately as wide as long.
- (28₁) Single anterior-to-posterior elongate rostral cartilage, longer than wide.
- (28₂) Single laterally elongate rostral cartilage, wider than long.
- (28₃) Two rounded rostral cartilages arrayed laterally.
- (28₄) Two rounded rostral cartilages arrayed anterior to posterior, the anterior one usually slightly larger.
- (28₅) Rostral cartilage absent.
29. Direction of main body of ventromedial process of maxillary head (Fig. 4). This process variously is referred to in the literature as the maxillary anterior process, median process of the maxillary head, inner arm of the maxillary, ventral process of the maxilla, and medial arm of the maxilla (Farris, 1968; Parenti, 1981; Wiley, 1986; Costa, 1998; Hernandez et al., 2008). This process extends medially or anteromedially ventral to the ascending process of the premaxilla and is ligamentously attached to the rostral cartilage(s), that is in turn ligamentously attached to the posterior or ventral surface of the ascending processes of the premaxillas (Hernandez et al., 2008). Parenti (1981) recognized an anteriorly directed ventromedial process of the maxillary head as synapomorphic of fundulids. Wiley (1986: 122) stated he had “trouble distinguishing” this character in fundulids. Costa (1998: fig. 1) recognized three informative transformation series associated with this process, identified a slightly or greatly posteriorly curved ventromedial process of the maxillary head as synapomorphic of the aplocheilids and rivulids respectively, and confirmed Parenti’s (1981: figs. 4, 5, 35, 39, 41) observation of an anteriorly directed ventromedial process as synapomorphic of fundulids. Costa (1998) also identified a distinctively widened ventromedial process as synapomorphic of the Poecilioidea, and a ventrally directed tip of the ventromedial process as synapomorphic of a clade composed of the Fundulidae, Profundulidae, and Goodiidae. The variation within these processes is three-dimensionally complex, likely due to their functional significance in jaw opening. We recognize four transformation series that encompass overall direction of the process (29), reduction of the process (30), vertical orientation of the tip of the process (31), and horizontal orientation of the tip of the process (32). We note the presence of a medially directed ventromedial process of the maxillary head as present in *Aplocheilichthys panchax*, *Kryptolebias marmoratus*, *Anableps dowi*, *Ameca splendens*, the *Profundulus* species examined, the *Fundulus notatus* species group, the *F. nottii* species group, and *F. sciadicus*. Various non-fundulids and all fundulids except for a clade within subgenus *Zygonectes* do exhibit an anteriorly directed ventromedial process of the maxillary head.
- (29₀) Main body of ventromedial process of maxillary head angled medially.
- (29₁) Main body of ventromedial process of maxillary head angled distinctly anteromedially.
30. Width of main body of ventromedial process of maxillary head. Although this process is robust in the anablepids and poeciliids examined, we could not distinguish a clearly discernable enlargement of this process as a discrete character state, as identified by Costa (1998). A very narrow and sharply pointed ventromedial process of the anterior maxilla is present in all cyprinodontids examined.
- (30₀) Main body of ventromedial process of maxillary head moderate to robust.
- (30₁) Main body of ventromedial process of maxillary head very narrow, ending in sharp point.
31. Vertical orientation of tip of ventromedial process of maxillary head in lateral or ventrolateral view. The tip of the ventromedial process on anterior head of maxilla is angled ventrally in all fundulids.
- (31₀) Tip of ventromedial process of maxillary head remains in same vertical plane as main arm of ventromedial process, not hooked ventrally.
- (31₁) Tip of ventromedial process of maxillary head hooked ventrally.
32. Horizontal orientation of tip of ventromedial process of maxillary head in dorsal view (Fig. 4). The tip of the ventromedial process on anterior head of maxilla is angled medially or anteromedially in *Valencia letourneuxi*, *Ameca splendens*, the *Fundulus notatus* species group, the *F. nottii* species group, *F. sciadicus*, and the examined species of cyprinodontids, anablepids, and poeciliids.
- (32₀) Tip of ventromedial process of maxillary head angled posteromedially.
- (32₁) Tip of ventromedial process of maxillary head angled medially or anteromedially.
33. The dorsomedial process of maxillary head (Fig. 4). When large, the dorsomedial process of the maxillary head extends anteromedially over the ascending process of the premaxilla. Parenti (1981: figs. 4, 5, 35, 39, 41) recognized reduced dorsomedial process of the maxillary head in *Profundulus* and fundulids as ancestral for cyprinodontoids and, therefore, the presence of well-developed processes as synapomorphic for a clade of all other cyprinodontoids. Costa (1998: fig. 1) recognized three informative transformation series associated with the dorsomedial process: size, shape, and presence or absence of a groove on the dorsal surface. He recognized the presence of a distinct groove on the dorsal surface of the dorsomedial process as synapomorphic of the

- Cyprinodontidae, a reduced dorsomedial process of the maxillary head as synapomorphic for a clade composed of fundulids, *Profundulus*, and goodeids, and a broad dorsomedial process as synapomorphic of a clade composed of anablepids, poeciliids, and cyprinodontids. Because we found additional recognizable shape variation and because the group of focus in this study exhibits a reduced dorsomedial process of the maxillary head, we chose to treat this as a single transformation series. We recognize a reduced dorsomedial process of the maxillary head in all examined fundulids, the species of *Profundulus* examined, *Crenichthys baileyi*, and *Ameca splendens*. The process is tapered and comes to a blunt point in *Kryptolebias marmoratus*, *Valencia letourneuxi*, *Poecilia reticulata*, and *Gambusia affinis*. All cyprinodontids examined have an especially large and medially expanded dorsomedial process of the maxillary head that also bears a distinct dorsal groove. The co-occurrence of the medial expansion of the dorsomedial process and a dorsal groove on this process in all taxa with either made us question their independence; thus, they are treated as a single character state.
- (33₀) Dorsomedial process of maxillary head broadly squared or rounded anteromedially.
- (33₁) Dorsomedial process of maxillary head large and squared medially with distinct medial-lateral groove on dorsal surface.
- (33₂) Dorsomedial process of maxillary head tapering to blunt point anteromedially.
- (33₃) Dorsomedial process of maxillary absent or present as low indistinct ridge.
34. The maxilla in region of association with head of the autopalatine (Fig. 4). The head of the autopalatine is in close association with the posterior maxilla in the snout region, likely bracing the anterior maxilla during jaw opening. *Fundulus parvipinnis*, *F. lima*, *F. diaphanus*, *F. waccamensis*, the *F. majalis* species group, *F. rathbuni*, *F. julisia*, and the *F. catenatus* species group exhibit a small ridge ventral to the head of the autopalatine that is absent in other examined taxa.
- (34₀) Maxilla without distinct ridge in the region of head of autopalatine.
- (34₁) Maxilla with distinct ridge immediately ventral to head of autopalatine.
35. Width of distal maxilla in lateral view. Parenti (1981: figs. 34, 36, 37) and Costa (1998: fig. 1) recognized a distinctively widened distal maxilla as shared, at least ancestrally, by poeciliids and anablepids. The distal maxilla is broad compared to the subdistal maxilla in the examined anablepids, *Aplocheilichthys spilauchen*, and *Cubanichthys pengelleyi*.
- (35₀) Distal maxilla narrow, approximately as wide as subdistal maxilla.
- (35₁) Distal maxilla broad, obviously wider than subdistal maxilla.
36. Tooth arrangement on premaxilla and dentary (Fig. 4). The teeth are uniserial, forming a single row at the margin of the oral jaws in *Lucania parva*, *L. interioris*, *Cyprinodon variegatus*, *Floridichthys carpio*, and *Aphanius dispar*.
- (36₀) Teeth on premaxilla and dentary multiserial, in more than one row or clustered.
- (36₁) Teeth on premaxilla and dentary uniserial, in a single row.
37. Tooth shape on premaxilla and dentary (Fig. 4). Parenti (1981) recognized bicuspid teeth as diagnostic of the Goodeidae with some reversals in included taxa and recognized tricuspid teeth as diagnostic of anablepids, although reversed within *Anableps*, and in some cyprinodontids. The teeth are bicuspid in *Crenichthys baileyi* and *Ameca splendens*, and are triuspid in the anablepids examined (in *Anableps dowi* only in neonates), *Cyprinodon variegatus*, *Floridichthys carpio*, and *Aphanius dispar*. All other examined taxa have unicuspid conical teeth.
- (37₀) Teeth on premaxilla and dentary unicuspid.
- (37₁) Teeth on premaxilla and dentary tricuspid.
- (37₂) Teeth on premaxilla and dentary bicuspid.
38. Shape of dentary (Fig. 4). This and all subsequent transformation series referring to the lower jaw use directional terminology with the mouth fully open, rendering the long axis of the lower jaw horizontal. Parenti (1981: figs. 31, 33) recognized a shorter, more robust dentary as synapomorphic for cyprinodontoids. Wiley (1986: fig. 4) recognized a more elongate and rectangular jaw as synapomorphic of the *Fundulus* subgenus *Zygonectes*. We recognize the variation identified by both authors and combine them into a single transformation series. The dentary is extremely elongate in the aplocheiloids *Kryptolebias marmoratus* and *Aplocheilus panchax*. The dentary is somewhat elongate and rectangular in *F. jenkinsi*, *F. sciadicus*, the *F. notatus* species group, and the *F. nottii* species group. All other examined taxa have roughly triangular dentaries that obviously narrow anteriorly.
- (38₀) Dentary very long and narrow, distinctly longer than anguloarticular, distinctly narrowing anteriorly.
- (38₁) Dentary roughly rectangular and somewhat elongate appearing, similar in length or only slightly longer than anguloarticular, distinct anterior narrowing not obvious.
- (38₂) Dentary roughly triangular, similar in length or only slightly longer than anguloarticular, distinct anterior narrowing obvious.
39. Ventromedial margin of dentary. In some taxa the anterior part of the dentary has a concavity along the medioventral margin of the bone that usually is accentuated by a small medioventral process. The dentary has a distinct concavity on the anterior half of the ventromedial margin in *Lucania*, *Leptolucania ommata*, *Aphanius dispar*, *Orestias agassizi*, *Poecilia reticulata*, and *Aplocheilus panchax*. All other examined taxa have roughly continuous ventromedial dentary margin that lacks an obvious cleft.
- (39₀) Anterior part of ventromedial margin of dentary with distinct concave cleft.
- (39₁) Ventromedial margin of dentary continuous, lacking distinct cleft.
40. Posteroventral extent of dentary (Fig. 4). The posteroventral dentary may extend approximately the same

- distance posteriorly as the posterodorsal dentary, extend distinctly farther posteriorly than the posterodorsal dentary, or stop distinctly anterior of the posterodorsal dentary. Costa (1998: fig. 3) recognized a reduced posteroventral process of the dentary as shared by anablepids and poeciliids. The posteroventral dentary is distinctly long in *Fundulus kansae*, *F. zebrinus*, *Lucania*, *Leptolucania ommata*, *Aphanius dispar*, *Orestias agassizi*, *Poecilia reticulata*, and *Aplocheilichthys panchax* and notably short in *F. jenkinsi*, *F. luciae*, *Aplocheilichthys panchax*, *Kryptolebias marmoratus*, all poeciliids examined, *Oxyzygonectes dovii*, and *Anableps dowi*. All other examined taxa have rightly equal posterodorsal and posteroventral dentaries.
- (40₀) Posteroventral dentary distinctly shorter than posterodorsal dentary.
- (40₁) Posteroventral dentary distinctly longer than posterodorsal dentary.
- (40₂) Posteroventral dentary about as long as posterodorsal dentary.
41. Position of coronoid process of anguloarticular (Fig. 4). Typically the posterior margin of the coronoid process of the anguloarticular is at or slightly anterior to a vertical dorsal to the jaw articulation. In some taxa the coronoid process is displaced posteriorly such that the posterior margin of the coronoid process of the anguloarticular is at a vertical obviously posterior to the jaw articulation, resulting in the main body of the coronoid process being dorsal to the jaw articulation. The coronoid process of the anguloarticular is displaced posteriorly in *Fundulus jenkinsi*, *F. luciae*, *F. sciadicus*, *F. xenicus*, the *F. nottii* species group, *Crenichthys baileyi*, *Ameca splendens*, and all cyprinodontids examined except *Cubanichthys pengelleyi*.
- (41₀) Coronoid process of anguloarticular largely anterior to vertical at point of lower jaw articulation with quadrate.
- (41₁) Coronoid process of anguloarticular clearly on vertical at point of lower jaw articulation with quadrate.
42. Dorsal margin of coronoid process of anguloarticular (Fig. 4). Typically in cyprinodontiforms the dorsal margin of the coronoid process is rounded or approximately squared. The *F. majalis* species group and *Ameca splendens* have a dorsal margin of the coronoid process that forms a blunt point.
- (42₀) Dorsal margin of coronoid process of anguloarticular rounded or squared.
- (42₁) Dorsal margin of coronoid process of anguloarticular forming a blunt point.
43. Length of ventral process of anguloarticular (Fig. 4). Costa (1998: fig. 3) recognized a long retroarticular process as co-occurring with a long retroarticular, both together as a single character state supporting a monophyletic Poecilioidea. Ghedotti (2000: fig. 5) treated these two separately because in the taxa he examined they varied independently. All examined fundulids, profundulids, goodeids, and cyprinodontids, and *Valencia letourneuxi* have a short retroarticular process of the anguloarticular. *Orestias agassizi* lacks the ventral process of the anguloarticular and was coded as unknown (?).
- (43₀) Ventral process of anguloarticular long, extends anterior to vertical at point of overlap between anterior process of anguloarticular and dentary.
- (43₁) Ventral process of anguloarticular short.
44. Shape of anterior margin of ventral process of anguloarticular (Fig. 4). Costa (1998: fig. 3) recognized an expanded ventral process of the anguloarticular in aplocheilids, *Profundulus guatemalensis*, and two species of *Rivulus*. We had difficulty developing explicit criteria for expansion but were able to clearly recognize differences in the shape of the anterior margin. The anterior margin of the ventral process of the anguloarticular is pointed in *Fundulus heteroclitus*, *F. confluentus*, *F. pulvereus*, *F. parvipinnis*, *F. lima*, *F. seminolis*, the *F. majalis* species group, *F. rathbuni*, the *F. nottii* species group, *F. xenicus*, *Lucania goodei*, *L. interioris*, *Leptolucania ommata*, *Ameca splendens*, all cyprinodontids examined with a ventral process, *Anableps dowi*, and *Kryptolebias marmoratus*. The species in the *F. catenatus* species group show polymorphism for this characteristic, with some individuals showing each character state. These taxa were coded as polymorphic (0&1). *Orestias agassizi* lacks the ventral process of the anguloarticular and was coded as unknown (?).
- (44₀) Anterior margin of ventral process of anguloarticular squared, with anterior point and second more obtuse point more posteroventrally.
- (44₁) Anterior margin of ventral process of anguloarticular pointed, with single anterior point.
45. Ventral margin of articular facet with quadrate. Typically the articular component of the anguloarticular forms the entire articulatory facet with the head of the quadrate. However, in *Crenichthys baileyi* and *Ameca splendens* the posteroventral portion of the facet is formed by the head of the retroarticular.
- (45₀) Head of quadrate articulates with articular component of anguloarticular.
- (45₁) Head of quadrate articulates with articular component of anguloarticular and head of retroarticular.
46. Angle between head and anterior process of retroarticular (Fig. 4). The angle between the two portions of the retroarticular when viewed medially can vary from approximately 90° to distinctly obtuse. *Fundulus xenicus*, *Anableps dowi*, *Kryptolebias marmoratus*, the examined *Profundulus* species, the examined cyprinodontids, *Crenichthys baileyi*, and *Ameca splendens* exhibit obtusely angled retroarticular.
- (46₀) Angle between head and anterior process of retroarticular approximately 90°.
- (46₁) Angle between head and anterior process of retroarticular clearly obtuse (greater than 90°).
47. Comparative lengths of head of retroarticular and anterior process of retroarticular (Fig. 4). Parenti (1981: figs. 31, 33, 34) and Costa (1998: fig. 3) recognized an elongate retroarticular as diagnostic of the Poecilioidea. Ghedotti (2000: fig. 5) recognized the degree of elongation as varying in the Poecilioidea and also differentiated between more robust and more gracile long anguloarticulars. In the taxa examined in this study, some species

- clearly exhibit long retroarticular heads and long anterior retroarticular processes (corresponding to the long retroarticular of previous authors), that could be delineated by comparing these two components of the retroarticular. In most examined taxa, the head and the anterior process of the retroarticular are similar in size. The members of the *Fundulus majalis* species group exhibit a distinctly elongate head of the retroarticular as compared to the anterior process. *Oxyzygonectes dovii*, *Anableps dowi*, *Jenynsia multidentata*, *Alfaro cultratus*, *Gambusia affinis*, and *Poecilia reticulata* exhibit a distinctly elongate anterior process of the retroarticular as compared to the head.
- (47₀) Head and anterior process of retroarticular similar in size.
- (47₁) Head of retroarticular obviously longer than anterior process of retroarticular.
- (47₁) Anterior process of retroarticular obviously longer than head of retroarticular.
48. Dorsomedial flange on head of retroarticular. In the examined *Profundulus* species, *Crenichthys baileyi*, *Ameca splendens*, and all cyprinodontids examined except *Cubanichthys pengelleyi*, there is a dorsomedial flange on the head of the retroarticular that medially overlaps the edge of the articular facet of the anguloarticular.
- (48₀) Head of retroarticular lacks dorsomedial flange.
- (48₁) Head of retroarticular with dorsomedial flange.
49. Shape of coronomeckelian (Fig. 4). The coronomeckelian is an ossification just dorsal to the junction of Meckel's cartilage with the articular portion of the anguloarticular. It also is the site of insertion of the more medial insertion of the A2/3 portion of the adductor mandibulae on the lower jaw. It is notably elongate in *Aplocheilichthys panchax*, *Floridichthys carpio*, *Lucania*, and all *Fundulus* except *F. xenicus*, *F. sciadicus*, the *F. notatus* species group, and the *F. nottii* species group. *Orestias agassizi* and *Leptolucania ommata* have coronomeckelians that are very small and ovoid, appearing much reduced compared to the condition in the other examined taxa.
- (49₀) Coronomeckelian large, narrow, and elongate.
- (49₁) Coronomeckelian large and roughly triangular to ovoid.
- (49₂) Coronomeckelian small and present only as approximately ovoid remnant.
50. Length of medial process of lachrymal. Ghedotti (2000: fig. 4) recognized a short medial process of the lachrymal as present in *Cyprinodon* and most poeciliids (but not *Aplocheilichthys spilarchus*). The process is notably short in the examined *Profundulus* species, *Ameca splendens*, *Cyprinodon variegatus*, *Floridichthys carpio*, and the poeciliines examined.
- (50₀) Medial process of lachrymal long, length longer than width of base.
- (50₁) Medial process of lachrymal short, length shorter than width of base.
51. Shape of medial process of lachrymal. Wiley (1986: fig. 3) called this process the dorsal process and recognized a convex or sigmoid posterior margin as synapomorphic for his subgenera *Fontinus* and *Xenisma*. We could not consistently discern this shape; however, the medial process does exhibit significant discernable shape variation. The medial process of the lachrymal tapers only slightly and has a broadly rounded tip in *Aplocheilichthys panchax* and *Kryptolebias marmoratus*. The process is very narrow, tapering to a sharp point in *Fundulus luciae*, *Leptolucania ommata*, and *Valencia letourneuxi*. The process is very narrow but becomes wider distally in the *F. heteroclitus* species group, *F. confluentus*, and *F. pulvereus*. In the remaining taxa the medial process of the lachrymal is more broadly triangular.
- (51₀) Medial process of lachrymal broad and approximately rectangular, ending in bluntly rounded tip.
- (51₁) Medial process of lachrymal broad and approximately triangular, tapering to point.
- (51₂) Medial process of lachrymal very narrow, width of base at least four times into length, and approximately triangular, tapering to sharply pointed tip.
- (51₃) Medial process of lachrymal very narrow, width of base at least four times into length, and widening distally.
52. Width of posterior laminar shelf of lachrymal. Wiley (1986: fig. 3) noted that the posterior notch on the lachrymal, equivalent to a wide posterior shelf, is wide in his subgenera *Fontinus* and *Xenisma*. Ghedotti et al. (2004) confirmed this distribution within *Fundulus*. In this study we note the presence of a wide posterior shelf of the lachrymal in the same *Fundulus* taxa identified by Wiley (1986) and *Oxyzygonectes dovii*.
- (52₀) Posterior shelf of lachrymal narrow, narrower than the adjacent lachrymal lateralis canal.
- (52₁) Posterior shelf of lachrymal wide, wider than the adjacent lachrymal lateralis canal.
53. Angle of head of autopalatine with respect to body of palatine (Fig. 4). The head of the autopalatine is in close association with the posterior maxilla in the snout region, likely bracing the anterior maxilla during jaw opening. Parenti (1981: figs. 29, 30) recognized a sharply angled head of the autopalatine as apomorphic for cyprinodontoids that was confirmed by Costa (1998: fig. 4). This study used a more geometric criterion of an angle of approximately 90° or less for identifying a sharply angled autopalatine head, which resulted in some cyprinodontoids being recognized as not having this condition (the examined *Profundulus* species, the examined anablepids, *Aplocheilichthys spilarchus*, and *Alfaro cultratus*). We recognize an autopalatine head angled approximately 90° or less in all fundulids, goodeids, and cyprinodontids examined, as well as in *Gambusia affinis*, *Poecilia reticulata*, and *Valencia letourneuxi*.
- (53₀) Head of autopalatine angled greater than 90° with respect to body of palatine.
- (53₁) Head of autopalatine angled approximately 90° or less with respect to body of palatine.
54. Direction of head of autopalatine in dorsal view (Fig. 4). Costa (1998) noted that in all cyprinodontoids, the autopalatine was angled laterally as visible in dorsal view. We also observed a laterally angled autopalatine head (in dorsal view) in all cyprinodontoids.
- (54₀) Head of autopalatine not obviously angled laterally or medially in dorsal view.

- (54₁) Head of autopalatine angled laterally in dorsal view.
55. Size of head of autopalatine (Fig. 4). Parenti (1981: fig. 39) and Costa (1998: fig. 4) noted that the condition of the head of the autopalatine in goodeids was clearly sharply angled but not obviously so because of reduction of the autopalatine head. We recognize a reduced head of the autopalatine head in *Crenichthys baileyi* and *Ameca splendens*.
- (55₀) Head of autopalatine distinct as cylindrical shaft.
- (55₁) Head of autopalatine reduced to small cartilaginous head on short raised prominence.
56. Position of head of autopalatine with respect to lateral ethmoids (Fig. 4). Parenti (1981) and Wiley (1986) recognized anteriorly projecting autopalatines that do not articulate with the lateral ethmoids as diagnostic of the Fundulidae. In prepared specimens the possible articulation of the autopalatine head with the lateral ethmoids is not obvious and difficult to ascertain if it occurs during jaw opening. We used the anterior margin of the lateral ethmoids as a reference point to identify relative position of the palatine heads. Based on this criterion, we observed anteriorly positioned autopalatine heads in *Orestias agassizi*, all anablepids examined, and all *Fundulus* examined except *F. chrysotus*, *F. luciae*, *F. rubrifrons*, *F. cingulatus*, *F. sciadicus*, and *F. jenkinsi*.
- (56₀) Head of autopalatine approximately at anterior margin of lateral ethmoids in dorsal view.
- (56₁) Head of autopalatine distinctly anterior to anterior margin of lateral ethmoids in dorsal view.
57. Dorsoposterior process on head of autopalatine. The dorsoposterior process of the head of the autopalatine is located at the point of bend between the main body and the head of the autopalatine and because of the lateral displacement of the head of the autopalatine in cyprinodontoids is best observed in dorsal view. Ghedotti (2000: fig. 6) recognized the degree of development and angle of this process as variable among the poecilioid taxa examined. Many taxa exhibit a low indistinct dorsoposterior process. A pronounced dorsoposterior process that narrows toward the tip is present in *Crenichthys baileyi*, *Ameca splendens*, the examined cyprinodontids, and most fundulids except *Fundulus rathbuni*, *F. kansae*, *F. zebrinus*, *F. parvipinnis*, *F. lima*, the *F. heteroclitus* species group, *F. confluentus*, and *F. pulvereus*. The *F. heteroclitus* species group, *F. confluentus*, and *F. pulvereus* have a pronounced dorsoposterior process with a squared tip. The examined anablepids exhibit a distinctive dorsoposterior process that is expanded distally with respect to a proximal "neck" of the process.
- (57₀) Dorsoposterior process of head of autopalatine low and indistinct.
- (57₁) Dorsoposterior process of head of autopalatine pronounced and narrowing toward tip.
- (57₂) Dorsoposterior process of head of autopalatine pronounced and forming approximately squared tip.
- (57₃) Dorsoposterior process of head of autopalatine pronounced and broadening toward tip.
58. Anterior flange of symplectic (Fig. 4). Most commonly the anterior flange of the symplectic originates from the dorsal and anterior surfaces of the horizontal and vertical arms of the symplectic, respectively. The flange is restricted to the vertical arm of the symplectic in *Aplocheilichthys spilauchen*, *Gambusia affinis*, *Poecilia reticulata*, and *Valencia letourneuxi*. It is absent in *Profundulus*, the goodeids examined, and all cyprinodontids examined except *Aphanius dispar*.
- (58₀) Anterior flange of symplectic originates from both vertical and horizontal arms of symplectic.
- (58₁) Anterior flange of symplectic originates entirely or almost entirely from vertical arm of symplectic.
- (58₂) Anterior flange of symplectic absent.
59. Metapterygoid. Parenti (1986: figs. 29, 30) recognized the absence of a metapterygoid as synapomorphic for the Cyprinodontidae. We observed a metapterygoid only in the two aplocheiloids we examined, *Aplocheilus panchax* and *Kryptolebias marmoratus*.
- (59₀) Metapterygoid present.
- (59₁) Metapterygoid absent.
60. Anterior flange of preopercle ventral to posterior hyomandibula (Fig. 4). Ghedotti (1998: fig. 8, 2000: fig. 6) recognized a robust posteroventral process of the hyomandibula by a cleft or concave anterior shelf of the preopercle as diagnostic of the Anablepidae within the Poecilioidea but variably present among other non-poecilioid cyprinodontiforms. We observed a continuous anterior shelf of the preopercle in *Leptolucania onimata*, *Orestias agassizi*, and all poeciliids examined.
- (60₀) Anterior shelf of hyomandibula concave or cleft ventral to posteroventral process of hyomandibula.
- (60₁) Anterior shelf of hyomandibula continuous laterally overlying posteroventral process of hyomandibula.
61. Shape of dorsal process of subopercle (Fig. 4). Ghedotti (2000: fig. 6) recognized length of the dorsal process of the subopercle as variable within poecilioids. We observed a long, acutely pointed dorsal process in *Valencia letourneuxi*, all anablepids examined, *Aplocheilichthys spilauchen*, all cyprinodontids examined, *Ameca splendens*, *Leptolucania onimata*, *Lucania*, *Fundulus chrysotus*, *F. rubrifrons*, *F. cingulatus*, the *F. catenatus* species group, *F. julisia*, *F. rathbuni*, the *F. majalis* species group, *F. diaphanus*, *F. waccaniensis*, *F. zebrinus*, *F. kansae*, †*F. detillae*, *F. lima*, *F. pulvereus*, *F. confluentus*, *F. grandis*, and *F. grandissimus*. *Profundulus labialis* (Günther, 1866) and *P. punctatus* exhibit a long, squared dorsal process.
- (61₀) Dorsal process of subopercle short, forming dorsal point.
- (61₁) Dorsal process of subopercle long, forming distinct acute dorsal point.
- (61₂) Dorsal process of subopercle long, squared with two points, anterior one more obtuse.
62. Proportion of length of basihyal cartilaginous (Fig. 5). Parenti (1981: fig. 11) recognized a large basihyal cartilage as synapomorphic for the Aplocheiloidei and figured large and small basihyal cartilages. Costa (1998: fig. 6) noted that non-cyprinodontiform atherinomorphs had large basihyal cartilages and recognized a smaller basihyal cartilage as synapomorphic for the Cyprino-

FIG. 5. Dorsal views of branchial skeletons in cleared and stained (A) *Fundulus heteroclitus* KU 15351, (B) *F. similis* KU 1282, and (C) *Lucania goodei* KU 17993. Anterior is at top. Scale bars indicate 1 mm. Dotted lines show margins of the left dorsal branchial elements. Abbreviations: ach = anterior ceratohyal, bb = basibranchials, bh = basihyal, cb = ceratobranchial, eb = epibranchial, hb = hypobranchial, iac = interarcual cartilage, pb = pharynogobranchial toothplate, pch = posterior ceratohyal.

dontoidei and figured a notably large and a notably small basihyal. In our examination of specimens, we also identified very small basihyal cartilages and used a percent-of-length criterion for assigning large, moderate, and small character states. The basihyal cartilage is notably large, greater than 50% by length of the basihyal, in *Aplocheilichthys panchax*, *Kryptolebias marmoratus*, *Valencia letourneuxi*, *Alfaro cultratus*, *Aplocheilichthys spilauchen*, *Anableps dowi*, *Oxyzygonectes dovii*, and *Aphanius dispar*. The basihyal cartilage is notably small, less than 25% by length of the basihyal, in *Cyprinodon variegatus*, *Floridichthys carpio*, *Cubanichthys pengelleyi*, *Leptolucania ommata*, *Lucania*, *Fundulus xenicus*, *F. luciae*, *F. chrysotus*, *F. cingulatus*, *F. rubrifrons*, *F. jenkinsi*, *F. pulvereus*, *F. confluentus*, the *F. heteroclitus* species group, *F. diaphanus*, *F. waccamensis*, *F. seminolis*, the *F. majalis* species group, *F. rathbuni*, *F. julisia*, and the *F. catenatus* species group.

- (62₀) Basihyal cartilage large, 50% or more by length cartilaginous.
- (62₁) Basihyal cartilage large, between 25% and 50% by length cartilaginous.
- (62₂) Basihyal cartilage small, less than 25% by length cartilaginous.

- 63. Shape of dorsal process of urohyal. Costa (1998: fig. 5) recognized an absent or vestigial dorsal process of the urohyal as synapomorphic for Fundulidae and poeciliines as variable in the orientation of the dorsal process. A long, straight, dorsal process is present in *Fundulus xenicus*, *Profundulus*, *Crenichthys baileyi*, *Cyprinodon variegatus*, *Floridichthys carpio*, *Jenynsia multidentata*, *Valencia letourneuxi*, and *Kryptolebias marmoratus*. We recognize a vestigial or absent dorsal process of the urohyal in all fundulids examined except *Fundulus xenicus*.

- (63₀) Dorsal process of urohyal long and angled posterodorsally.
- (63₁) Dorsal process of urohyal long, approximately straight, and projecting dorsally.
- (63₂) Dorsal process of urohyal present as very small indistinct prominence or absent.

- 64. Shape of main body of urohyal in ventral view. Farris (1968) and Wiley (1986: fig. 12) recognized a laterally expanded ventral urohyal producing an ovate shape in ventral view as diagnosing the *F. majalis* species group. We observed the same distribution of this character state.
 - (64₀) Ventral urohyal narrow and elongate in ventral view, greatest width three or more times into length.
 - (64₁) Ventral urohyal wide and ovate in ventral view, greatest width less than three times into length.
- 65. Dorsal hypohyal. Parenti (1981: fig. 28) recognized the absence of a dorsal hypohyal as synapomorphic for cyprinodontoids. We did not observe a dorsal hypohyal in the examined cyprinodontoids.
 - (65₀) Dorsal hypohyal present.
 - (65₁) Dorsal hypohyal absent.
- 66. Relationship of anterior ceratohyal to hypohyal. Parenti (1981: fig. 28) recognized the absence of an anterior process of the anterior ceratohyal extending ventral to the ventral hypohyal in cyprinodontoids and ventral to the dorsal hypohyal and the posterior-most ventral hypohyal in aplocheiloids as synapomorphic for the Poeciliidae. We observed the same distribution of this character state.
 - (66₀) Anterior ceratohyal with anterior process ventrally underlying hypohyal elements.
 - (66₁) Anterior ceratohyal lacking anterior process, abutting ventral hypohyal.

67. Number of branchiostegal rays. Most commonly in cyprinodontiforms, there are six branchiostegal rays associated with each ceratohyal, the first two associated with the narrow anterior portion of the anterior ceratohyal. In cases with fewer than six branchiostegals, it is the more anterior branchiostegal rays that are absent. Ghedotti (2000: fig. 7) recognized substantial variation in the number of branchiostegal rays from six to four within the Poecilioidea. Five branchiostegal rays are present in *Aplocheilichthys spilauchen*, *Jenynsia multidentata*, *Orestias agassizi*, *Aphanius dispar*, *Crenichthys baileyi*, *Ameca splendens*, *Lucania*, *Fundulus xenicus*, *F. chrysotus*, *F. sciadicus*, the *F. notatus* species group, the *F. nottii* species group, *F. kansae*, *F. zebrinus*, †*F. detillae*, *F. parvipinnis*, *F. lima*, *F. pulvereus*, *F. confluentus*, and the *F. heteroclitus* species group. *Leptolucania ommata* has only three branchiostegal rays.
- (67₀) Six branchiostegal rays.
 (67₁) Five branchiostegal rays.
 (67₂) Three branchiostegal rays.
68. Number of basibranchials. Parenti (1981: fig. 11) recognized and Costa (1998: fig. 6) confirmed the presence of two rather than three basibranchials due to either loss of the first basibranchials or fusion of the first and second basibranchials as synapomorphic for cyprinodontoids. All examined cyprinodontoids had two basibranchials.
- (68₀) Three basibranchials.
 (68₁) Two basibranchials.
69. Anteromedial and posteromedial heads of first hypobranchial (Fig. 5). The medial heads of the first hypobranchial abut the lateral surface of the first basibranchial in cyprinodontoids and the lateral surface of the first and second basibranchials in aplocheiloids. Only a single medial head with a single continuous terminal cartilage, often narrowed or with a depression in the middle indicating that this is a fusion rather than a loss of one head and an enlargement of the remaining head, is present in *Aplocheilus panchax*, *Aplocheilichthys spilauchen*, *Alfaro cultratus*, *Anableps dowi*, *Floridichthys carpio*, *Ameca splendens*, and *Crenichthys baileyi*. Two medial heads of the first hypobranchial separated by a wide angle with a separate terminal cartilages are present in *Kryptolebias marmoratus*, *Valencia letourneuxi*, *Jenynsia multidentata*, *Oxyzygonectes dovii*, *Aphanius dispar*, *Leptolucania ommata*, *Fundulus xenicus*, *F. luciae*, *F. chrysotus*, *F. sciadicus*, the *F. notatus* species group, *F. rathbuni*, *F. julisia*, *F. stellifer*, *F. diaphanus*, *F. zebrinus*, *F. kansae*, *F. parvipinnis*, and *F. lima*. The remaining taxa examined have two medial heads of the first hypobranchial separated by a narrow angle.
- (69₀) Single medial head of first hypobranchial, single continuous terminal cartilage.
 (69₁) Two medial heads of first hypobranchial with separate terminal cartilages, and narrow angle between two heads.
 (69₂) Two medial heads of first hypobranchial with separate terminal cartilages, and wide angle between two heads.
70. Relative length of anteromedial head of first hypobranchial (Fig. 5). The anteriomedial head is longer than the posteromedial head in *Fundulus kansae*, *F. zebrinus*, *F. diaphanus*, *F. rathbuni*, *F. julisia*, and *F. xenicus*. Ghedotti et al. (2004: fig. 1F, 1G) recognized the same distribution of this character state, but did not examine *F. xenicus* or *F. zebrinus*.
- (70₀) Medial heads of first hypobranchial approximately same length.
 (70₁) Anteriomedial head of first hypobranchial longer than posteromedial head.
71. Anterolateral flange on posterolateral head of first hypobranchial (Fig. 5). In *Kryptolebias marmoratus* and many fundulid taxa, the anteromedial flange on the posterolateral head of the first hypobranchial extends posterolaterally beyond the cartilaginous tip. In *Lucania goodei*, *Fundulus luciae*, *F. chrysotus*, *F. julisia*, the *F. majalis* species group, *F. seminolis*, *F. diaphanus*, *F. parvipinnis*, *F. lima*, and the *F. heteroclitus* species group, this process was present in the observed adult specimens. The flange variably extends beyond the cartilaginous tip, with some adult individuals exhibiting the posterolateral extension and some not, in *Lucania parva*, *L. interioris*, the *F. notatus* species group, the *F. nottii* species group, and the *F. catenatus* species group (coded as polymorphic [0&1]).
- (71₀) Anterolateral flange on posterolateral head of first hypobranchial does not extend beyond cartilaginous tip of posterolateral head.
 (71₁) Anterolateral flange on posterolateral head of first hypobranchial extends beyond cartilaginous tip of posterolateral head.
72. Teeth on posterior third ceratobranchial. Costa (1998) recognized an edentulous third ceratobranchial as synapomorphic of the Fundulidae. Ghedotti et al. (2004: fig. 1F, 1G) noted variation in the presence or absence of teeth within fundulids. The third ceratobranchial is edentulous in many fundulid taxa, but is not universal, and also occurs in *Anableps dowi*. Within the Fundulidae, *Leptolucania ommata*, *Fundulus grandissimus*, *F. julisia*, the *F. catenatus* species group, *F. rubrifrons*, *F. cingulatus*, *F. sciadicus*, the *F. notatus* species group, the *F. nottii* species group exhibit teeth on the posterior third ceratobranchial. *Fundulus parvipinnis* has adult individuals that have an edentulous third ceratobranchial and some individuals with a few teeth on the posterior third ceratobranchial, and was coded as polymorphic (0&1).
- (72₀) Teeth present on posterior third ceratobranchial.
 (72₁) Third ceratobranchial edentulous.
73. Anterior extent of ventrolateral flange on head of fourth ceratobranchial. There is a small anterolateral flange that extends along the ventrolateral surface of the anterior head of the fourth ceratobranchial. In many taxa this flange extends beyond the ossified margin of the anterior head of this bone, forming a short process ventrolateral to the anterior cartilaginous tip of the fourth ceratobranchial. Wiley (1986: fig. 9) recognized the presence of this process as synapomorphic for a clade containing *F. seminolis* and the *F. majalis* species group. This process is obvious, visible dorsally, and well developed in these taxa, but it also is present across a wide range of cyprinodontiform taxa. The ventrolateral flange does not extend beyond the ossified margin of the anterior head of the fourth ceratobranchial in the examined poeciliines, *Anableps*

- dowi, *Jenynsia multidentata*, *Orestias agassizi*, *Leptolucania ommata*, *Lucania*, *Fundulus xenicus*, *F. luciae*, *F. chrysotus*, *F. rubrifrons*, *F. cingulatus*, *F. sciadicus*, the *F. nottii* species group, *F. julisia*, *F. diaphanus*, *F. waccamensis*, *F. kansae*, *F. zebrinus*, *F. jenkinsi*, *F. pulvereus*, and *F. confluentus*.
- (73₀) Lateral flange on head of fourth ceratobranchial extends beyond ossified margin of anterior head, forming short process lateral to cartilaginous tip of fourth ceratobranchial.
- (73₁) Lateral flange on head of fourth ceratobranchial does not extend beyond ossified margin of anterior head.
74. Posteromedial shelf on medial surface of anterior fourth ceratobranchial visible dorsally (Fig. 5). The anterior head of the fifth ceratobranchial dorsally overlaps the posteromedial shelf when present. Wiley (1986: fig. 9) recognized the presence of this shelf combined with lateral expansion of the anterior fifth ceratobranchial as synapomorphic for a clade containing *F. seminolis* and the *F. majalis* species group. Costa (1998: fig. 7) recognized the presence of this shelf as synapomorphic for cyprinodontoids but reversed in some cyprinodontids and goodeines. The shelf is not present and visible dorsally in *Aplocheilichthys panchax*, *Kryptolebias marmoratus*, *Valencia letourneuxi*, *Aphanius dispar*, *Cyprinodon variegatus*, *Floridichthys carpio*, *Fundulus chrysotus*, *F. julisia*, the *F. catenatus* species group, *F. kansae*, and *F. zebrinus*.
- (74₀) Posteromedial surface of anterior fourth ceratobranchial simple, lacking dorsally visible shelf.
- (74₁) Posteromedial shelf visible dorsally on anterior fourth ceratobranchial.
75. Teeth on fourth ceratobranchial. Ghedotti (2000: fig. 9) noted variation in presence and absence of fourth ceratobranchial teeth among cyprinodontoids. However, in this study we recognize both presence-absence and extent of teeth together defining four clearly identifiable character states. Most commonly among the examined taxa, the fourth ceratobranchial bears teeth in multiple rows that extend at least half the length of the bone. Teeth are present in a single row along more than half the length of the fourth ceratobranchial in *Floridichthys carpio*, *Cubanichthys pengelleyi*, *Crenichthys baileyi*, *Profundulus punctatus*, *P. labialis*, *Leptolucania ommata*, *Lucania*, *Fundulus rubrifrons*, *F. cingulatus*, *F. julisia*, and the *F. catenatus* species group. Teeth are present only on the anterior quarter of the fourth ceratobranchial in *Ameca splendens*. The fourth ceratobranchial is edentulous in *Cyprinodon variegatus* and *Orestias agassizi*.
- (75₀) Teeth present on fourth ceratobranchial, in multiple rows and extending more than half length of bone.
- (75₁) Teeth present on fourth ceratobranchial, in single row and extending more than half length of bone.
- (75₂) Teeth present on fourth ceratobranchial, restricted to anterior quarter of bone in either one short row or as a few scattered teeth.
- (75₃) Fourth ceratobranchial edentulous.
76. Lateral flange on anterior fifth ceratobranchial (Fig. 5). Wiley (1986: fig. 9) recognized the presence of a lateral expansion of the anterior fifth ceratobranchial overlapping the posterior medial shelf of the anterior fourth ceratobranchial as synapomorphic for a clade containing *Fundulus seminolis* and the *F. majalis* species group. This lateral expansion is present in *F. seminolis*, the *F. majalis* species group, and *Ameca splendens*.
- (76₀) Lateral surface of anterior fifth ceratobranchial continuous.
- (76₁) Lateral surface of anterior fifth ceratobranchial with distinct flange.
77. Shape of fifth ceratobranchial (Fig. 5). The fifth ceratobranchials may be slender such that the left and right fifth ceratobranchials diverge away from each other posterolaterally, robust such that they maintain their proximity along the first half of their length then diverge posterolaterally, or very robust such that they articulate medially and generally exhibit a strongly triangular shape. Wiley (1986) recognized a firm articulation between the fifth ceratobranchials as synapomorphic for a clade containing *F. seminolis* and the *F. majalis* species group. Ghedotti et al. (2004: fig. 1F, 1G) recognized variation in the robustness of the posterior fifth ceratobranchials within *Fundulus*. Robust but unfused fifth ceratobranchials are present in *Kryptolebias marmoratus*, *Orestias agassizi*, *Floridichthys carpio*, *Ameca splendens*, *Lucania*, *Fundulus stellifer*, *F. bifax*, *F. diaphanus*, *F. waccamensis*, *F. seminolis*, *F. parvipinnis*, and *F. lima*. *Fundulus catenatus* varies between having slender and robust among individuals and was coded as polymorphic (0&1). The *F. majalis* species group and *Crenichthys baileyi* exhibit very robust and fused fifth ceratobranchials.
- (77₀) Posterior fifth ceratobranchial slender, left and right posteromedial ceratobranchials not closely approaching each other.
- (77₁) Posterior fifth ceratobranchial robust, forming angle medially, left and right posteromedial ceratobranchials closely approaching each other.
- (77₂) Posterior fifth ceratobranchial very robust, forming distinct angle medially, left and right posteromedial ceratobranchials in contact and fused in moderate-sized to large adults.
78. Shape of medial fifth ceratobranchial teeth. The medial fifth ceratobranchial teeth typically share a common morphology with the teeth on the opposing pharyngo-branchial tooth plate (not coded as a separate transformation series). Wiley (1986) recognized molariform teeth as synapomorphic for a clade composed of *F. seminolis* and the *F. majalis* species group. Ghedotti et al. (2004: fig. 1F, 1G) recognized differing robustness of fifth ceratobranchial teeth within *Fundulus*, with *F. seminolis* and *F. stellifer* recognized as having rounded molariform teeth. Robust medial fifth ceratobranchial teeth that retain a distinct point are present in all cyprinodontids examined, *Leptolucania ommata*, *Lucania*, *F. xenicus*, *F. luciae*, *F. chrysotus*, *F. cingulatus*, *F. rubrifrons*, the *F. nottii* species group, *F. catenatus*, *F. bifax*, *F. diaphanus*, *F. waccamensis*, *F. pulvereus*, and *F. confluentus*. Very robust rounded molariform teeth are present in *Crenichthys baileyi*, *F. stellifer*, *F. seminolis*, and the *F. majalis* species group.
- (78₀) Medial fifth ceratobranchial teeth slender and conical.

- (78₁) Medial fifth ceratobranchial teeth robust, somewhat molariform but with distinct point.
- (78₂) Medial fifth ceratobranchial teeth robust, molariform, and rounded.
79. Shape of anterior arm of first epibranchial (Fig. 5). A short anterior arm of first epibranchial narrowing anteromedially to the tip is present in *Aplocheilichthys panchax*, *Kryptolebias marmoratus*, *Valencia letourneuxi*, *Jenynsia multidentata*, *Anableps dowi*, *Orestias agassizi*, *Cyprinodon variegatus*, *Cubanichthys pengelleyi*, *Ameca splendens*, *Crenichthys baileyi*, *Profundulus guatemalensis*, and *P. labialis*. A long anterior arm of first epibranchial narrowing anteromedially to the tip is present in most fundulids and various non-fundulid cyprinodontoids examined. A distinctive long anterior arm of first epibranchial that broadens anteromedially to a large cartilage cap is present in *Fundulus luciae*, *F. parvipinnis*, and *F. lima*.
- (79₀) Anterior arm of first epibranchial short and slender, narrowing anteromedially to tip.
- (79₁) Anterior arm of first epibranchial long and slender, narrowing anteromedially to tip.
- (79₂) Anterior arm of first epibranchial long and broad, widening to broad cartilaginous cap.
80. Vertical orientation of anterior arm of first epibranchial. Typically the anterior arm of the first epibranchial extends anteromedially roughly in the same plane as the posterior portion of the first epibranchial. In *Fundulus sciadicus*, the *F. notatus* species group, and the *F. nottii* species group, the anterior arm of the first epibranchial extends anteroventrally.
- (80₀) Anterior arm of first epibranchial extends anteromedially.
- (80₁) Anterior arm of first epibranchial extends anteroventrally.
81. Width of ventrolateral head of first epibranchial in dorsal view. Costa (1998: fig. 8) recognized an expanded ventral head of the first epibranchial as diagnosing the Goodeidae. He also noted that the expansion resulted in two separate medial and lateral cartilaginous caps. We observed this character state in the two goodeids examined, *Crenichthys baileyi* and *Ameca splendens*. However, we also observed a wide ventrolateral head of the first epibranchial in *Aplocheilichthys panchax*, *Orestias agassizi*, and *Aphanius dispar*.
- (81₀) Ventrolateral head of first epibranchial wide, often forming two cartilaginous caps.
- (81₁) Ventrolateral head of first epibranchial moderate with single cartilaginous caps.
82. Orientation of lateral head of second epibranchial. The lateral head is directed laterally, resulting in the second epibranchial appearing straight in anterior view in *Kryptolebias marmoratus*, *Floridichthys carpio*, *Cyprinodon variegatus*, and all fundulids examined. In the other taxa the lateral head is directed ventrolaterally, resulting in the second epibranchial appearing bent in anterior view.
- (82₀) Lateral head of second epibranchial angled ventrolaterally, bone appearing bent in anterior view.
- (82₁) Lateral head of second epibranchial angled laterally, bone appearing straight in anterior view.
83. Dorsolateral process on second epibranchial. In *Aplocheilichthys panchax*, *Ameca splendens*, *Crenichthys baileyi*, and the examined *Profundulus* species, there is a distinct dorsolateral prominence or process where the medial and lateral heads of the second epibranchial meet.
- (83₀) Dorsolateral process present on second epibranchial where medial and lateral heads meet.
- (83₁) Dorsolateral process present on second epibranchial absent.
84. Arrangement of teeth on second pharyngobranchial toothplate. Ghedotti et al. (2004: fig. 1H, 1I) recognized the presence of only one or two tooth rows on the second pharyngobranchial toothplate as supporting a sister relationship between the *Fundulus catenatus* species group and a clade composed of *F. julisia* and *F. albolineatus*. The teeth on the second pharyngobranchial toothplate are in more than two rows in *Valencia letourneuxi*, *Aplocheilichthys spilauchen*, the examined anablepids, *Orestias agassizi*, *Floridichthys carpio*, *Cyprinodon variegatus*, *Lucania*, *Fundulus xenicus*, *F. luciae*, *F. rathbuni*, *F. diaphanus*, *F. waccamensis*, *F. seminolis*, the *F. majalis* species group, *F. kansae*, *F. zebrinus*, *F. parvipinnis*, *F. lima*, *F. pulvereus*, *F. confluentus*, and the *F. heteroclitus* species group.
- (84₀) Teeth on second pharyngobranchial toothplate in narrow band in one or two rows.
- (84₁) Teeth on second pharyngobranchial toothplate in broad band in more than two rows.
85. Dorsal flange on fourth epibranchial. Ghedotti et al. (2004) recognized a robust fourth epibranchial with a dorsal flange as present in the *Fundulus catenatus* species group, *F. diaphanus*, and *F. seminolis* among the taxa examined. This condition is best observed in posterior view. There is a robust fourth epibranchial with a dorsal flange in *Aplocheilichthys panchax*, *Ameca splendens*, the *F. catenatus* species group, *F. diaphanus*, *F. waccamensis*, *F. seminolis*, the *F. majalis* species group, *F. parvipinnis*, and *F. lima*.
- (85₀) Fourth epibranchial robust with dorsal flange.
- (85₁) Fourth epibranchial slender, lacks dorsal flange.
86. Articulation of first vertebra with exoccipitals. Parenti (1981: figs. 59–62) recognized the direct contact of the neural arch with the exoccipitals and the absence of condyles on the exoccipitals and the centrum of the first vertebra as independently derived in the ancestor of the poeciliines and the New World cyprinodontines. Concordant with this, we observed that condyles are absent on the exoccipitals and the first vertebra, and the neural arch directly contacts the exoccipitals in the examined poeciliines, *Floridichthys carpio*, and *Cyprinodon variegatus*.
- (86₀) First vertebra articulates via distinct vertebral and exoccipitals condyles.
- (86₁) First vertebra articulates via direct contact of neural arch with exoccipitals, condyles absent.
87. Condition of neural arch and spine on first vertebra (Fig. 6). Parenti (1981: figs. 58–62) recognized an open neural arch as synapomorphic of the Poeciliinae as

FIG. 6. Diagrammatic illustrations of character states of the first neural arch and spine in anterior view. Character-state numbers are indicated with each illustration.

compared to *Fluviophylax* and the Procatopodines. Costa (1998: fig. 18) recognized an open neural arch with neurapophyses that do not unite near their tips as synapomorphic of all cyprinodontoids and the presence of a median neural spine as variably present within aplocheiloids. Ghedotti (2000: fig. 11) recognized a neural arch closed at the neuropophyseal tips in *Aplocheilichthys spilauchen* and procatopodines and a neural arch with free neuropophyseal tips but closed by a more ventral ossified bridge in most poeciliines. We were able to discern five discrete states of the neural arch and spine on the first vertebra and use the term neural spine to indicate a median ossification extending dorsally from an ossification closing the neural canal. We do not necessarily assert *a priori* homology of these structures in using this terminology. *Aplocheilus panchax* and *Kryptolebias marmoratus* have neurapophyses with separate tips that are joined more ventrally by a horizontal bridge of bone that closes the neural canal dorsally and supports a small median neural spine between the free tips of the neurapophyses. *Crenichthys baileyi*, *Ameca splendens*, the examined *Profundulus* species, *Aphanius dispar*, the examined anablepids, all poeciliines examined, and *Valencia letourneuxi* exhibit a similar connection between the neurapophyses, except that the ossified connecting bridge is further from the neuropophyseal tips in a more ventral position, and lack the neural spine extending dorsally from the bridge between the neurapophyses. Smaller individuals often exhibited only partial fusion or near fusion of left and right bony shelves along the midline. All fundulids examined exhibit neurapophyses that are separate and not joined either at the tips or by a more ventral bridge of bone, although some individuals do exhibit partially developed bony processes approximately where such a bridge develops in other taxa, but even large individuals did not have these meet along the midline. *Cubanichthys pengelleyi*, *Orestias agassizi*, and *Aplocheilichthys spilauchen* exhibit shortened parapophyses that extend medially and meet, closing the neural canal and forming a small neural spine. *Cyprinodon variegatus* and *Floridichthys carpio* exhibit neurapophyses

that touch at the tips but are anteriorly inclined and contact the neurocranium.

- (87₀) Tips of neurapophyses of first vertebra separate, but neural arch closed dorsally by horizontal bridge of bone immediately below tips of neurapophyses, bearing median neural spine.
 - (87₁) Tips of neurapophyses of first vertebra separate, but neural arch closed dorsally by horizontal bridge of bone below tips of neurapophyses.
 - (87₂) Tips of neurapophyses of first vertebra separate, and neural arch open.
 - (87₃) Tips of neurapophyses of first vertebra join and form a ridge or small median neural spine, neural arch low and closed.
 - (87₄) Tips of neurapophyses of first vertebra meet but do not necessarily fuse along midline and are anteriorly inclined and contact neurocranium, neural arch effectively closed.
88. Number of anteroposteriorly enlarged neural spines on second through sixth vertebrae. *Cubanichthys pengelleyi*, *Cyprinodon variegatus*, *Floridichthys carpio*, *Orestias agassizi*, *Leptolucania ommata*, and all three *Lucania* species have only one or two expanded anterior neural spines. Whereas, all other examined taxa had three to five expanded anterior neural spines. The two–three point is used as the division between the two character states because individuals within the same species frequently vary within these ranges but do not vary between the two ranges.
- (88₀) Three to five expanded neural spines on second through sixth vertebrae.
 - (88₁) One or two expanded neural spines on second through sixth vertebrae.
89. Number of caudal vertebrae. *Aplocheilus panchax*, *Valencia letourneuxi*, *Aplocheilichthys spilauchen*, *Cubanichthys pengelleyi*, *Aphanius dispar*, *Cyprinodon variegatus*, *Floridichthys carpio*, *Leptolucania ommata*, all three *Lucania* species, and *Fundulus xenicus* have 16 or fewer caudal vertebrae. The 16–17 point is used as the division between the two character states because individuals within the same species frequently vary within these ranges but do not vary between the two ranges. The one exception was *F. chrysotus*, which was found to vary across this range, occasionally having 16 caudal vertebrae. Thus, *F. chrysotus* was coded as polymorphic (0&1).
- (89₀) Sixteen or fewer caudal vertebrae.
 - (89₁) Seventeen or more caudal vertebrae.
90. Hypural plate in adults. The symmetrical caudal fin and hypural plate of cyprinodontiforms was recognized as diagnostic for the order by Parenti (1981: fig. 2) and Costa (1998: fig. 16). Ghedotti (2000: fig. 17) noted that the presence of a single hypural element or a split hypural plate varied among poecilioid fishes. *Aplocheilus panchax*, *Kryptolebias marmoratus*, *Valencia letourneuxi*, all examined poeciliids, *Oxyzygonectes dovii*, *Anableps dowi*, and the examined *Profundulus* species have a hypural plate with dorsal and ventral component separated horizontally along the vertebral axis (corresponding to a dorsal plate composed of hypurals three, four, and five and a ventral plate composed of hypurals one and two). Other taxa exhibit a single solid hypural plate, sometimes with a

- proximal small oval opening near the terminal centrum. Adult *Crenichthys baileyi* were variable with respect to this transformation series and were coded as polymorphic (0&1).
- (90₀) Hypural plate in adults divided by cleft at vertebral axis into dorsal and ventral parts.
- (90₁) Hypural plate in adults a single undivided plate.
91. Contact between dorsal intermuscular bones and proximal pleural ribs. The terminology of the dorsal intermuscular bones of percomorph teleosts remains in dispute due to questions about their homology with the intermuscular bones of non-percomorphs. Patterson and Johnson (1995) recognize the single series of intermuscular bones dorsal to the ribs as epineurals and Gemballa and Britz (1998) recognize them as epicentrals. Because the elements present in cyprinodontiform fishes are either epineurals or epicentrals, the use of the term epipleurals that has been used extensively for these elements in percomorphs is incorrect. Because the previous term is no longer valid and there is disagreement regarding the appropriate homologous term, we will refer to them using the non-specific terminology “dorsal intermuscular bones.” Wiley (1986) recognized dorsal intermuscular bones contacting pleural ribs laterally as synapomorphic of the Fundulidae. This condition is present in all fundulids examined as well as in *Aplocheilichthys panchax*, *Kryptolebias marmoratus*, *Aplocheilichthys spilarchus*, *Anableps dowi*, *Cubanichthys pengelleyi*, *Crenichthys baileyi*, and *Ameioba splendens*.
- (91₀) Proximal dorsal intermuscular bones overlap pleural ribs laterally.
- (91₁) Proximal dorsal intermuscular bones abut pleural ribs posterolaterally.
92. Contact between dorsal intermuscular bones and parapophyses of first five vertebrae. Wiley (1986) recognized contact between the parapophyses of the five vertebrae with the proximal heads of the associated dorsal intermuscular bones as synapomorphic for a clade composed of *Leptolucania ommata* and *Fundulus xenicus*. In these cases the dorsal intermuscular bone continues to contact the pleural rib as well. The proximal tips of the dorsal intermuscular bones contact the vertebral parapophyses in *L. ommata*, *F. xenicus*, and *Anableps dowi*.
- (92₀) Proximal heads of dorsal intermuscular bones contact only the pleural ribs.
- (92₁) Proximal heads of first five dorsal intermuscular bones contact parapophyses on first five vertebrae.
93. Ossification of dorsal intermuscular bones associated with posterior-most two abdominal vertebrae. The dorsal intermuscular bones associated with the last two abdominal vertebrae are well enough ossified proximally to contact the proximal ribs and/or the vertebrae in *Kryptolebias marmoratus*, *Valencia letourneuxi*, *Orestias agassizi*, *Aphanius dispar*, *Floridichthys carpio*, *Cyprinodon variegatus*, *Leptolucania ommata*, *Fundulus xenicus*, and *F. luciae*.
- (93₀) Dorsal intermuscular bones associated with last two abdominal vertebrae unossified or only ossified distally, do not contact pleural ribs.
- (93₁) Dorsal intermuscular bones associated with last two abdominal vertebrae ossified and contact pleural ribs or parapophyses of vertebrae.
94. Ossification of dorsal intermuscular bones associated with first caudal vertebra. The intermuscular ligaments associated with the first caudal vertebra are ossified in *Kryptolebias marmoratus*, *Valencia letourneuxi*, all poeciliines examined, *Aphanius dispar*, *Floridichthys carpio*, *Cyprinodon variegatus*, and *Fundulus xenicus*. In all three species of *Lucania*, the presence or absence of a dorsal intermuscular ossification associated with this vertebra was variable among individuals; thus, all three species were coded as polymorphic (0&1). In most cases the intermuscular ossification does not contact the pleural rib or vertebra and only is ossified distally.
- (94₀) Dorsal intermuscular bones associated with first caudal vertebra absent, dorsal intermuscular ligaments unossified.
- (94₁) Dorsal intermuscular bones associated with first caudal vertebra present.
95. Proximal anal-fin radials in adult males. In cyprinodontiforms with elongate anal-fin-derived intromittent organs, some of the anal-fin radials are fused to form a specialized skeletal suspensorium. Parenti (1981: figs. 65–68), Costa (1998), Ghedotti (2000: fig. 14), and Lucinda and Reis (2005: fig. 16) recognized distinctive patterns of fusion in the intromittent organ suspensorium as synapomorphic for the Anablepinae and the Poeciliinae. Our observations support this distribution, with the Poeciliinae and the Anablepinae (*Anableps* and *Jenynsia*) having different radial fusion patterns in each subfamily.
- (95₀) Proximal anal-fin radials in adult males separate and similar to female condition.
- (95₁) Second, third, and fourth proximal anal-fin radials in adult males fused together, leaving first proximal radial free.
- (95₂) First through fourth proximal anal-fin radials in adult males fused together, in some cases including additional further posterior proximal anal-fin radials.
96. Lateral processes on dorsal portion of fifth proximal anal-fin radial in adult males. Ghedotti (2000: fig. 16) recognized lateral processes on the fifth proximal anal-fin radial as synapomorphic for all poeciliines except *Alfaro*. Our observations support this distribution, with these processes observed in *Gambusia affinis* and *Poecilia reticulata*.
- (96₀) Dorsal portion of fifth proximal anal-fin radials in adult males simple, lacking lateral processes.
- (96₁) Dorsal portion of fifth proximal anal-fin radials in adult males with distinct lateral processes.
97. Ossification of ventral arm of posttemporal (Fig. 3). The posttemporal in cyprinodontiforms connects the dorsal pectoral girdle via the supracleithrum with the dorsal neurocranium via the epiotics. A ligament runs from the anteroventral surface of the posttemporal to the intercalar on the ventral neurocranium. This ligament may be variously ossified. Parenti (1981: fig. 7) and Wiley (1986) recognized that in all fundulids except *Fundulus zebrinus* and *F. kansae* (subgenus *Plancterus*) this ligament is

unossified and that this provided evidence supporting *Planterus* as either sister to the remaining Fundulidae or of uncertain position within the Fundulidae, respectively. Ghedotti (2000: fig. 12) and Lucinda and Reis (2005) recognized wide variation in the presence of an ossified lower posttemporal arm within the Cyprinodontoidae. We observed an ossified lower arm of the posttemporal in *Kryptolebias marmoratus*, *Fundulus kansae*, *F. zebrinus*, and †*F. detillae* as well as the examined poeciliines, anablepids, cyprinodontids, and *Profundulus*.

(97₀) Ligamentous ventral arm of posttemporal unossified.

(97₁) Ligamentous ventral arm of posttemporal ossified.

98. Length of supracleithrum (Fig. 7). The shape of the reduced supracleithrum as found in atherinomorphs and mugilomorphs (Stiassny, 1993) varies within cyprinodontiforms in its general shape. Ghedotti (2000: fig. 12) recognized a reduced supracleithrum as synapomorphic for the Poeciliidae but homoplastically reversed in some procatopodines. We observed a short supracleithrum in the poeciliids examined and in *Oxyzygonectes dovii*.

(98₀) Supracleithrum elongate, length at least 1.5 times width or more.

(98₁) Supracleithrum short, length less than 1.5 times width.

99. Width of posterior supracleithrum (Fig. 7). Costa (1998: fig. 14) recognized a posteriorly expanded supracleithrum as synapomorphic for goodeids and profundulids, and recognized that some poeciliines also exhibit a supracleithrum that appears expanded. We observed a posteriorly expanded supracleithrum in the poeciliines examined, *Ameca splendens*, *Crenichthys baileyi*, and the *Profundulus* species examined.

(99₀) Posterior supracleithrum narrow, no more than two times width of more anterior supracleithrum.

(99₁) Posterior supracleithrum wide, two times or more width of more anterior supracleithrum.

100. Shape of posterior process of dorsal enclosure of cleithrum (Fig. 7). Most examined taxa had a rounded posterior process. We observed a squared posterior process in *Aplocheilichthys panchax* and *Alfaro cultratus*. *Profundulus punctatus*, *Fundulus catenatus*, some individuals of *F. bifax* (coded as polymorphic [1&2]), *F. julisia*, *F. albolineatus*, *F. rathbuni*, *F. seminolis*, *F. diaphanus*, *F. waccamensis*, *F. kansae*, *F. zebrinus*, and †*F. detillae* have a posterior process of the dorsal enclosure of the cleithrum that comes to a distinct dorsoposterior point.

(100₀) Posterior process of dorsal enclosure of cleithrum squared posteriorly.

(100₁) Posterior process of dorsal enclosure of cleithrum with distinct posterior point.

(100₂) Posterior process of dorsal enclosure of cleithrum rounded posteriorly.

101. Ventral notch in posterior process of dorsal enclosure of cleithrum. Wiley (1986) recognized a dorsal process of the cleithrum that hooks downward as synapomorphic for *Fundulus diaphanus* and *F. waccamensis*. Ghedotti (2000: fig. 12) recognized the presence or absence of a ventral notch on the dorsal process of the dorsal enclosure of the cleithrum as variable among the cyprinodontoids. We did

not observe an obvious notch in the posterior process in *F. diaphanus* and *F. waccamensis*. The examined *Profundulus* species, *Crenichthys baileyi*, *Ameca splendens*, *Cubanichthys pengelleyi*, and *Jenynsia multidentata* have a posterior process of the dorsal enclosure of the cleithrum with a distinct ventral notch.

(101₀) Posteroventral surface of posterior process of dorsal enclosure of cleithrum continuous.

(101₁) Posteroventral surface of posterior process of dorsal enclosure of cleithrum with distinct notch or cleft.

102. Shape of scapular process of cleithrum (Fig. 7). The scapular process of the cleithrum laterally overlaps the scapula dorsal to the scapular foramen. Wiley (1986: fig. 5) recognized a scapular process of the cleithrum that was well below the junction of the scapula and the cleithrum as synapomorphic for a clade composed of the subgenera *Xenisma* and *Fontinus*. We found this character state to be associated with a prolonged scapular process and to be broadly present within cyprinodontoids. The scapular process of the cleithrum extends posteroventrally to at least the posterior margin of the scapular foramen, forming a blunt but acute tip in *Kryptolebias marmoratus*, *Anableps dowi*, all cyprinodontids examined except *Cubanichthys pengelleyi*, *Ameca splendens*, *Fundulus xenicus*, *F. sciadicus*, the *F. nottii* species group, *F. jenkinsi*, *F. pulvereus*, *F. confluentus*, the *F. heteroclitus* species group, *F. kansae*, *F. zebrinus*, *F. diaphanus*, *F. waccamensis*, *F. seminolis*, the *F. majalis* species group, *F. rathbuni*, *F. julisia*, and the *F. catenatus* species group.

(102₀) Scapular process of cleithrum rounded and indistinct, does not extend posterior of posterior margin of scapular foramen.

(102₁) Scapular process of cleithrum prominent and coming to acute but blunt point, extends posterior of posterior margin of scapular foramen.

103. Angle of posterior coracoid process with dorsoposterior margin of coracoid (Fig. 7). The dorsal margin of the posterior coracoid process most commonly forms an approximately 90° angle with the dorsoposterior margin of the coracoid in most taxa examined. The posterior coracoid process forms an acute angle with the dorsoposterior coracoid in the *Profundulus* species examined, *Ameca splendens*, *Lucania parva*, *L. interioris*, *Fundulus chrysotus*, *F. kansae*, *F. zebrinus*, †*F. detillae*, *F. parvipinnis*, and *F. lima*. The posterior coracoid process forms an obtuse angle with the dorsoposterior coracoid in *Poecilia reticulata* and *Gambusia affinis*.

(103₀) Dorsal margin of posterior coracoid process at approximately 90° angle with dorsoposterior margin of coracoid.

(103₁) Dorsal margin of posterior coracoid process at distinctly acute angle with dorsoposterior margin of coracoid.

(103₂) Dorsal margin of posterior coracoid process at distinctly obtuse angle with dorsoposterior margin of coracoid.

104. Posterior coracoid process shape. Ghedotti (2000: fig. 12) recognized a rounded posterior process of the coracoid associated with a broadly rounded posteroventral coracoid as synapomorphic for Poeciliidae. We observed this condition in all examined poeciliids. *Aplocheilichthys panchax*

FIG. 7. Left lateral views of pectoral skeletons (anterior at left) in cleared and stained (A) *Fundulus heteroclitus* KU 15351, (B) *F. kansae* KU 14726, (C) *F. notatus* KU 18021, and (D) *Lucania goodei* KU 17993. The supracleithrum and posttemporal remain *in situ* in *Lu. goodei*; they have been removed from the other specimens shown. Pectoral images photo reversed. Ventral views of pelvic skeletons (anterior at top) in cleared and stained (E) *F. heteroclitus* KU 15351, (F) *F. notatus* KU 18021, and (G) *Lu. goodei* KU 17993. Left pelvic-fin rays removed on *F. heteroclitus* and *F. notatus*. Caudal skeleton (anterior at left) of (H) *Lu. parva* KU 17042. Scale bars indicate 1 mm. Abbreviations: ce = vertebral centrum, cl = cleithrum, co = coracoid, dar = distal anal-fin radial, ddr = distal dorsal-fin radial, decl = dorsal enclosure of the cleithrum, eu = epural, hs = hemal spine, hyp = hypural plate, mar = middle anal-fin radial, mdr = middle dorsal-fin radial, mpg = medial process of pelvic girdle, nar = neural arch, ns = neural spine, par = proximal anal-fin radial, pdr = proximal dorsal-fin radial, pg = main body of pelvic girdle, ph = parhypural, ppg = posterior process of pelvic girdle, ptm = posttemporal, pu = preural centrum, rad = radial, sc = scapula, scl = supracleithrum.

- does exhibit some ventral expansion of the coracoid, but it does not exhibit the broadly rounded profile of poeciliids.
- (104₀) Posterior coracoid process forms distinct point or acute rounded process.
- (104₁) Posterior coracoid process broadly rounded associated with a broadly rounded postero-ventral coracoid.
105. Shape of ventral-most proximal pectoral radial. A compressed ventral-most proximal pectoral radial is present in *Aphanius dispar*, *Orestias agassizi*, *Floridichthys carpio*, and *Cyprinodon variegatus*.
- (105₀) Ventral-most proximal pectoral radial approximately square, trapezoidal, or slightly antero-posteriorly elongate.
- (105₁) Ventral-most proximal pectoral radial compressed, dorsoventrally elongate.
106. Dorsal postcleithrum. Parenti (1981: figs. 7, 8) and Costa (1998) recognized the absence of a dorsal postcleithrum to be a synapomorphy of Rivulidae and independently lost in *Orestias*, *Anableps*, *Leptolucania*, and various poeciliids. Ghedotti (2000) noted that all poeciliids he examined, except for *Aplocheilichthys spilauchen*, lacked a dorsal postcleithrum. In addition to the presence and absence of the dorsal postcleithrum, the shape of the postcleithrum also varies. The dorsal postcleithrum is absent in *Rivulus marmoratus*, the poeciliines examined, *Anableps dowi*, *Orestias agassizi*, *Leptolucania ommata*, and the *Fundulus nottii* species group. *Aplocheilus panchax*, *Valencia letourneuxi*, *Aplocheilichthys spilauchen*, *Jenynsia multidentata*, *Oxyzygonectes dovii*, *Floridichthys carpio*, *Cyprinodon variegatus*, *Cubanichthys pengelleyi*, *Fundulus chrysotus*, *F. luciae*, and *F. parvipinnis* have stoutly oval dorsal postcleithra. The other examined taxa have elongately oval dorsal postcleithra.
- (106₀) Dorsal postcleithrum slightly oval, less than 1.5 times longer dorsoventrally than wide.
- (106₁) Dorsal postcleithrum elongately oval, about 1.5 times longer dorsoventrally than wide.
- (106₂) Dorsal postcleithrum absent.
107. Overlap of dorsal and ventral postcleithra. The dorsal and ventral postcleithra overlap in *Aphanius dispar*, *Crenichthys baileyi*, *Ameca splendens*, the examined *Profundulus* species, and *Lucania*. Taxa lacking a dorsal postcleithrum were coded as unknown (?).
- (107₀) Dorsal postcleithrum does not obviously overlap the ventral postcleithrum.
- (107₁) Dorsal postcleithrum distinctly overlaps the ventral postcleithrum.
108. Presence of posterior flange on ventral postcleithrum. Ghedotti (1998: fig. 13, 2000) recognized an expanded ventral postcleithrum with a posterior flange as synapomorphic for the Anablepidae and also present in *Crenichthys* and *Valencia*. In this study we observed differing levels of development of the flange on the ventral postcleithrum. We observed a ventral postcleithrum with a narrow flange in *Fundulus chrysotus*, *F. sciadicus*, the *F. notatus* species complex, *F. escambiae*, *F. nottii*, *F. kansae*, *F. zebrinus*, *F. parvipinnis*, *F. lima*, and *F. jenkinsi*. An enlarged posterior flange on the ventral postcleithrum is present in *Valencia letourneuxi*, all examined anablepids, *Crenichthys baileyi*, *F. rathbuni*, *F. julisia*, the *F. catenatus* species group, *F. diaphanus*, *F. waccamensis*, *F. seminolis*, the *F. majalis* species group, *F. pulvereus*, *F. confluentus*, *F. grandis*, and *F. heteroclitus*. The ventral postcleithrum lacks a posterior flange in the other examined taxa.
- (108₀) Ventral postcleithrum slender, lacking posterior flange.
- (108₁) Ventral postcleithrum with narrow posterior flange, as wide as main ventral postcleithral shaft or less.
- (108₂) Ventral postcleithrum with broad posterior flange, 1.5 to 2.5 times as wide as main ventral postcleithral shaft.
109. Orientation of posterior flange on ventral postcleithrum. *Fundulus escambiae* and *F. nottii* have a unique orientation of the posterior postcleithral flange, in that wraps medially around the associated pleural rib. Taxa lacking a ventral postcleithral flange were coded as unknown (?).
- (109₀) Postcleithral flange oriented posteriorly.
- (109₁) Postcleithral flange oriented posteromedially, wrapping around adjacent pleural rib.
110. Absence of pelvic fins. Parenti (1981) recognized the absence of pelvic fins as separate synapomorphic losses supporting the Crenichthyinae and the genus *Orestias*. Pelvic fins and their skeletal supports are absent in *Crenichthys baileyi* and *Orestias agassizi*.
- (110₀) Pelvic fins and skeletal supports present.
- (110₁) Pelvic fins and skeletal supports absent.
111. Length of posterior process of pelvic girdle (Fig. 7). Ghedotti (2000: fig. 13) recognized the absence of posterior processes in *Anableps* and the presence of long posterior processes in some procatopodines and *Cyprinodon variegatus*. Posterior processes of the pelvic girdle are absent in *Kryptolebias marmoratus*, the examined poeciliines, *Anableps dowi*, *Leptolucania ommata*, *Lucania interioris*, *Fundulus sciadicus*, *F. cingulatus*, and *F. rubrifrons*. A long posterior process of the pelvic girdle is present in *Aplocheilichthys spilauchen*, *Oxyzygonectes dovii*, *Aphanius dispar*, *Floridichthys carpio*, *Cyprinodon variegatus*, *Ameca splendens*, the *Profundulus* species examined, *Fundulus xenicus*, *F. luciae*, *F. chrysotus*, the *F. notatus* species group, the *F. nottii* species group, *F. seminolis*, *F. waccamensis*, *F. jenkinsi*, *F. confluentus*, and the *F. heteroclitus* species group. Taxa lacking a pelvic girdle were coded as unknown (?).
- (111₀) Posterior process of pelvic girdle present and short, about as long as width of process at base.
- (111₁) Posterior process of pelvic girdle present and long, obviously longer than width of process at base.
- (111₂) Posterior process of pelvic girdle absent or present as only small indistinct protrusion.
112. Shape of tip of posterior process of pelvic girdle (Fig. 7). *Jenynsia multidentata* and all fundulids examined that had posterior processes except *Fundulus xenicus* had rounded or squared tips of their posterior processes. All other examined taxa with pelvic girdles that have posterior processes had pointed tips. Taxa lacking a pelvic girdle or posterior processes of the pelvic girdle were coded as unknown (?).

- (112₀) Posterior process of pelvic girdle with acute pointed tip.
- (112₁) Posterior process of pelvic girdle with squared or rounded blunt tip.

Myology

The differentiation of the adductor mandibulae in teleosts provides a system with significant interspecific variation (Hertwig, 2008). The divisions of the adductor mandibulae examined in this study follow a terminology similar to that employed by Winterbottom (1974) and Hernandez et al. (2008). We chose to use this terminology to be concise and clear. We do not imply any homologies of muscles outside of the Cyprinodontiformes in using this terminology, nor do we question Hertwig's (2008) less specific terminology based on his recognition of uncertain homology broadly across teleosts.

We recognize three major sections of the adductor mandibulae identified as A1, A2, and A3 based on their origins from superficial to deep. More dorsally positioned sections of a muscle are designated as α and more ventral components are designated as β . The A1 α section (A1 larger head in Hertwig, 2008) originates on the preopercle, and in some cases on some more medial suspensorial elements, and inserts posteriorly on the ventral arm of the maxilla. The A1 β section (A1 smaller head, A1 ventral head, and A1 medial head in Hertwig, 2008) originates on the ventral quadrate and the anterior symplectic and variably on the anterior arm of the preopercle inserting posteriorly on the ventral arm of the maxilla near or at the insertion of A1 α . In some cases the origin of A1 β may be medial rather than ventral to A1 α .

A2 and A3 originate on the preopercle and other posterior suspensorial elements and insert on the medial lower jaw. Anteriorly there is a single laterally thickened A2/3 muscle portion (anterior A2/3 in Hertwig, 2008) that originates from posterior and ventral tendons or aponeuroses with other adductor mandibulae portions and inserts via two separate tendons on the medial lower jaw. Most commonly the more lateral insertion is on the posterior dentary and/or anguloarticular (attributed to A2 by Hernandez et al., 2008), and the more medial insertion is on the coronomeckelian (attributed to A3 by Hernandez et al., 2008). In some cases the A2/3 portion has a third tendon that inserts on the medial ventral alveolar arm of the premaxilla.

The A2 α portion (A2/3 dorsolateral head in Hertwig, 2008) originates medial to the A1 α on the preopercle and in some cases on some more medial elements of the posterior suspensorium and inserts via a tendon or aponeurosis on the posterolateral surface of the A2/3 portion. The A2 β portion (A2/3 intermediate head in Hertwig, 2008) originates on the dorsal surface of the anterior arm of the preopercle and/or quadrate, medial to the A1 sections, and inserts via an aponeurosis on the ventral surface of the A2/3 portion. The A2 α and the A2 β sections are separated by the ramus mandibularis of the facial nerve.

The A3 portion (A2/3 medial head in Hertwig, 2008) originates on elements of the posterior suspensorium, medial to the A2 α portion and the insertion of the levator palatoquadrati, and inserts via an aponeurosis on the posteromedial surface of the A2/3 portion. The A ω (A ω and A ω Q in Hertwig, 2008) portion of the adductor mandibulae

originates on the anteromedial quadrate and/or anguloarticular and inserts on the medial dentary and/or anguloarticular. The A ω section was not examined in this study.

113. Relationship between dorsal origins of A1 α and A2 α portions of adductor mandibulae (Fig. 8). In *Aplocheilichthys panchax* and *Kryptolebias marmoratus*, the origin of A1 α extends only 1/3 to 1/2 the way up the dorsal arm of the preopercle, revealing a large triangular portion of the origin of A2 α laterally. A vertically much larger area of origination for A1 α extends greater than 1/2 the way up the vertical area of the preopercle but still does not cover the dorsal-most part of A2 α in *Fundulus parvipinnis*, *F. lima*, *F. kansae*, *F. zebrinus*, the *F. majalis* species group, *F. seminolis*, *F. diaphanus*, *F. waccamensis*, *F. rathbuni*, *F. julisia*, the *F. catenatus* species group, *Profundulus*, *Crenichthys baileyi*, *Ameca splendens*, *Cubanichthys pengelleyi*, *Orestias agassizi*, *Oxyzygonectes dovii*, *Jenynsia multidentata*, *Aplocheilichthys spilarchus*, *Alfaro cultratus*, and *Valencia*. In the other examined taxa the area of origination for A1 α covers all of the area of origination of A2 α laterally. In *Lucania* and *Gambusia affinis* A1 α varies among individuals if it covers A2 α laterally, and is coded as polymorphic (1&2) in these taxa.

(113₀) Origin of A1 α extends only 1/3 to 1/2 up dorsal arm of preopercle, revealing large portion of origin of A2 α laterally.

(113₁) Origin for A1 α extends greater than 1/2 up vertical area of preopercle but does not cover dorsal-most part of origin of A2 α .

(113₂) Origin for A1 α covers all of origin of A2 α laterally.

114. Position of A1 β with respect to A1 α portion of adductor mandibulae (Fig. 8). Hertwig (2008: fig. 1) recognized a ventral position of the A1 β with respect to A1 in the examined fundulids, cyprinodontids, poeciliids, *Crenichthys baileyi*, and *Valencia*. Our observations on more fundulid taxa (and fewer other taxa) are consistent with his.

(114₀) A1 β mostly medial to A1 α .

(114₁) A1 β mostly ventral to A1 α .

115. Extent of origin of A1 β portion of adductor mandibulae (Fig. 8). Hertwig (2008: fig. 1) recognized a long origin of A1 β portion of the adductor mandibulae arising substantially from the preopercle in all fundulids he examined but not in *Fundulus xenicus*. We identified a long origin of A1 β (also obviously including the preopercle) in the fundulids we examined (including *F. xenicus*) and all cyprinodontids examined except *Cubanichthys pengelleyi*.

(115₀) A1 β origin short, originating almost entirely on posterior process of quadrate and anterior symplectic.

(115₁) A1 β origin long, obviously originating on preopercle as well as posterior process of quadrate and symplectic.

116. Insertion of A1 portion of adductor mandibulae on maxilla. Hertwig (2008: fig. 3) recognized a broad insertion of the A1 portion of the adductor mandibulae in a wide range of cyprinodontiforms. We only recognize those taxa with very wide insertions as having that condition because we could not easily differentiate the

FIG. 8. Right lateral views of suborbital adductor-mandibulae myology with visible portions of the adductor mandibulae labeled using the terminology discussed in the text in (A) *Fundulus diaphanus* JFBM 39057, right superficial view with skin removed, (B) deep view showing A2 with A1 reflected (reflected A1 components also labeled), and (C) dorsolateral view with A1 and A2 freed from their origins and partially ventrolaterally deflected showing major components of the adductor mandibulae; (D) *F. notatus* JFBM 38463, superficial view with skin removed and (E) deep view with A1 reflected. Scale bars indicate 1 mm. Dotted lines show margins of muscle portions. Muscle portions labeled as described in text. The asterisk (*) is on the levator arcus palatini that runs between the posterior-most portions of A2 and A3. Abbreviation: rm = ramus mandibularis of the trigeminal nerve.

somewhat widened insertions from the narrow insertions as identified by Hertwig (2008). The insertion is short and very broad in all cyprinodontids examined except *Cubanichthys pengelleyi*.

(116₀) Insertion of A1 on maxilla via narrow ligament.

(116₁) Insertion of A1 on maxilla short and broad, ligament inconspicuous.

117. Insertion of A1β portion of adductor mandibulae. Hertwig (2008) noted a separate inserting ligament from A1β from that of the A1α portion of the adductor mandibulae in the fundulids and the poeciliines, with the exception of *Fundulus zebrinus* and *Alfaro cultratus* in each respective group. We confirmed this and recognize a separate A1β ligament in all fundulids except *F. kansae* and *F. zebrinus*, as well as in *Gambusia affinis* and *Poecilia reticulata*.

(117₀) A1β inserts via same ligament as A1α.

(117₁) A1β inserts via separate ligament from A1α.

118. Posterior aponeurosis of A2/3 portion where A2α and/or A2β portions of adductor mandibulae insert (Fig. 8). The posterior A2/3 aponeurosis of the adductor mandibulae is at a right angle with the dorsal margin of the A2/3 portion in *Cyprinodon variegatus* and *Floridichthys carpio*.

(118₀) Posterior A2/3 aponeurosis angles to dorsoposterior point.

(118₁) Posterior A2/3 aponeurosis is at right angle with dorsal margin of A2/3 portion.

119. A2α insertion on posterior A2/3 portion of adductor mandibulae (Fig. 8). Hertwig (2008) recognized the insertion of A2α on A2/3 as via a tendon rather than a broad aponeurosis in *Aplocheilichthys* and *Alfaro cultratus*. A2α inserts on the posterior A2/3 portion via a discrete tendon in the *Fundulus notatus* species group, *Cubanichthys pengelleyi*, *Alfaro cultratus*, and *Aplocheilichthys panchax*.

(119₀) A2α inserts on posterior A2/3 portion via broad aponeurosis.

(119₁) A2α inserts on posterior A2/3 portion via tendon.

120. Extent of origin of A2α portion of adductor mandibulae. Hertwig (2008: fig. 1) recognized a reduced A2α compared to A2β as present in cyprinodontids and the two *Anableps* species he examined. Herein we restrict this character state to a small rectangular-shaped A2α that is not substantially wider at its origin than at its insertion. A small A2α is present in all cyprinodontids examined except *Cubanichthys pengelleyi*.

(120₀) A2α large, flared posteriorly.

(120₁) A2α small, roughly rectangular.

FIG. 9. Diagrammatic illustrations of character states concerning the looping of the intestine in ventral view. Character states are indicated with each illustration.

121. Extent of origin of A2 β portion of adductor mandibulae. Hertwig (2008) recognized a short origin of A2 β as present in almost all poeciliids he examined. Among the taxa examined in this study, a short origin of A2 β is present in *Oxyzygonectes dovii*, *Aplocheilichthys spilauchen*, *Alfaro cultratus*, *Poecilia reticulata*, and *Gambusia affinis*.

- (121₀) Origin of A2 β long, extending from quadrate and symplectic to preopercle.
(121₁) Origin of A2 β short, almost entirely restricted to quadrate and symplectic.

122. Condition of the A3 portion of adductor mandibulae (Fig. 8). Most commonly the A3 portion of the adductor mandibulae is a single muscle element. In *Fundulus jenkinsi*, *F. seminolis*, *F. chrysotus*, *F. xenicus*, the *F. notatus* species group, the *F. nottii* species group, *Lucania parva*, *L. interioris*, *Oxyzygonectes dovii*, and *Jenynsia multi-dentata*, the A3 portion is comprised of two distinct lateral and medial components, with the lateral component inserting more posteriorly on A2/3 than the medial component. The A3 portion of the adductor mandibulae is absent in *Leptolucania ommata*, *Aphanius dispar*, and *Orestias agassizi*.

- (122₀) A3 portion of adductor mandibulae single.
(122₁) A3 with separate lateral and medial portions.
(122₂) A3 portion absent.

Visceral Morphology

123. Condition of middle intestine (Fig. 9). Cyprinodontiform fishes lack a discrete stomach. The intestine extends from

the esophagus posteriorly along the left surface of the liver and up around the posterior and posterior-most right side of the liver. In the simplest condition exhibited by most fundulids, there is a single anterior bend before the intestine continues mostly straight posterior to the anus. In *Fundulus parvipinnis*, *F. lima*, and *Alfaro cultratus*, the intestine does not appear coiled on quick examination but additionally proceeds laterally, forming a right lateral bend. *Fundulus kansae*, *F. zebrinus*, the *Profundulus* species examined, and the examined anablepids have a longer and somewhat more complicated intestine that loops laterally as in *F. parvipinnis* and *F. lima* but also loops posteriorly, producing a distinct posterior bend. *Crenichthys baileyi*, *Floridichthys carpio*, and *Orestias agassizi* have an even longer, more complicated intestine that loops laterally and posteriorly as in *Fundulus kansae* and *F. zebrinus* but also loops anteriorly again, producing a distinct secondary anterior bend. *Ameca splendens*, *Aphanius dispar*, and *Poecilia reticulata* have a complexly spirally coiled intestine that spirals inwards counterclockwise superficially and then spirals outwards clockwise more deeply.

- (123₀) Middle intestine simple with single anterior bend.
(123₁) Middle intestine simple with right lateral bend.
(123₂) Middle intestine appears simply coiled, looping laterally and posteriorly with distinct posterior bend.
(123₃) Middle intestine appears coiled, looping laterally and posteriorly and then anteriorly with distinct secondary anterior bend.
(123₄) Middle intestine complexly coiled, spiraling inwards counterclockwise superficially and spiraling outwards clockwise more deeply.

124. Condition of posterior intestine. The posterior intestine loops right in *Cubanichthys pengelleyi*, *Cyprinodon variegatus*, and *Floridichthys carpio*.

- (124₀) Posterior intestine largely straight.
(124₁) Posterior intestine loops right.

125. Gestation and ovarian structure in mature females. Fertilization occurs in the follicles and embryos remain within the follicles until birth in *Anableps* and poeciliines. Fertilization occurs in the follicles, and embryos develop in the central ovarian cavity until birth in *Jenynsia* and goodeines such as *Ameca splendens*.

- (125₀) Ovary not site of extensive embryonic development.
(125₁) Ovarian follicles site of fertilization and embryonic development until parturition.
(125₂) Ovarian follicles site of fertilization, embryonic development occurs in central ovarian lumen.

External Morphology

Terminology of the dorsal head squamation and cephalic lateralis system follow Hoedeman (1958) and Gosline (1949), respectively.

126. Anterior naris. The anterior naris extends away from the head via a fleshy extension (tubular anterior naris). Parenti (1981) recognized a tubular anterior naris in aplocheiloids, *Anableps*, and *Cubanichthys*. Costa (1998)

also noted a similar condition in *Profundulus* and considered the aplocheiloids and *Anableps* to have a truly tubular anterior naris and *Cubanichthys* and *Profundulus* to have a cylindrical anterior naris. We could not differentiate these two conditions and simply recognize a tubular anterior naris in *Fundulus confluentus*, *F. pulvereus*, the examined *Profundulus* species, *Cubanichthys pengelleyi*, *Oxyzygonectes dovii*, *Anableps dowi*, *Aplocheilus panchax*, and *Kryptolebias marmoratus*.

(126₀) Anterior naris tubular.

(126₁) Anterior naris non-tubular.

127. Preorbital squamation. Scales are present in front of the eye in the lachrymal region in the *Fundulus majalis* species group, the *F. notatus* species group, the examined *Profundulus* species, *Aphanius dispar*, *Orestias agassizi*, the examined anablepids, *Aplocheilichthys spilauchen*, and *Alfaro cultratus*.

(127₀) Scales absent in lachrymal region anterior to orbit.

(127₁) Scales present in lachrymal region anterior to orbit.

128. Dorsal cephalic squamation pattern. Squamation pattern based on the scales that are the most superficial and not overlapped by other cephalic scales. A-type squamation is present in the *F. heteroclitus* species group, *F. confluentus*, *F. pulvereus*, *F. jenkinsi*, *F. diaphanus*, *F. waccamensis*, *F. seminolis*, the *F. majalis* species group, *F. rathbuni*, *F. julisia*, *F. albolineatus*, the *F. catenatus* species group, *F. parvipinnis*, *F. lima*, *F. kansae*, *F. zebrinus*, *F. chrysotus*, *F. luciae*, *F. sciadicus*, and *Orestias agassizi*. G-type squamation is present in *F. dispar*, *F. blairae*, and *Aplocheilus panchax*. Wiley (1986) recognized G-type squamation as synapomorphic for *F. dispar* and *F. blairae*. The remaining taxa exhibit E-type squamation.

(128₀) G-type cephalic squamation.

(128₁) A-type cephalic squamation.

(128₂) E-type cephalic squamation.

129. Number of E-scales on top of head. Most cyprinodontiforms have two E-scales. However, *Lucania*, *Cubanichthys pengelleyi*, *Cyprinodon variegatus*, and *Floridichthys carpio* have one E-scale.

(129₀) Two E-scales.

(129₁) One E-scale.

130. Condition of lachrymal canal. The lachrymal canal is open in *Orestias agassizi*, *Gambusia affinis*, and *Poecilia reticulata*.

(130₀) Lachrymal canal closed with four pores.

(130₁) Lachrymal canal open.

131. Condition of snout pit neuromasts. The snout pit neuromasts are enclosed in canals in the members of the *Fundulus heteroclitus* species group.

(131₀) Snout pit neuromasts exposed on dorsal surface of snout.

(131₁) Snout pit neuromasts enclosed in canals.

132. Anterior supraorbital lateralis system and relationship to middle supraorbital canal. The most common condition in the examined fundulid taxa is a closed anterior supraorbital canal with pores 1 and 2a, distinctly separated from the middle portion of the canal (usually

starting with pore 2b). In this condition pores 2a and 2b are far from each other, greater than a single pore width. A similar condition with a separate anterior portion of the canal but with pore 4a close to pore 4b is present in *F. sciadicus*, the *F. notatus* species group, *Ameca splendens*, and *Oxyzygonectes dovii*. The middle anterior part of the supraorbital canal is continuous with the middle portion of the canal, thus having only a single pore 2 over the canal in *Lucania parva*, *L. goodei*, *Aphanius dispar*, and *Floridichthys carpio*. This portion of the supraorbital canal is open with an exposed neuromast in *Fundulus xenicus*, *Leptolucania ommata*, *Orestias agassizi*, all examined poeciliids, *Valencia letourneuxi*, *Aplocheilus panchax*, and *Kryptolebias marmoratus*. Wiley (1986) recognized this condition as synapomorphic of a *F. xenicus* (as *Adinia xenica*) and *Leptolucania ommata* clade. The posterior-most anterior supraorbital lateralis canal pore is adjacent to an open portion with an exposed neuromast in *F. luciae* and *Lucania interioris*.

(132₀) Anterior supraorbital lateralis canal open.

(132₁) Anterior supraorbital lateralis canal closed adjacent to open part of middle portion of canal with one exposed neuromast.

(132₂) Anterior supraorbital lateralis canal closed and continuous with middle supraorbital canal with single pore 2.

(132₃) Anterior supraorbital lateralis canal closed and separate from middle supraorbital canal with pore 2a close to pore 2b.

(132₄) Anterior supraorbital lateralis canal closed and separate from middle supraorbital canal and with pore 2a far from pore 2b.

133. Middle supraorbital lateralis system and relationship to posterolateral supraorbital canal. The most common condition in the examined fundulid taxa is a closed middle supraorbital canal with pores 3 and 4a, distinctly separated from the posterolateral portion of the canal (usually starting with pore 4b). This portion of the supraorbital canal is open with exposed neuromasts in *Fundulus luciae*, *F. xenicus*, *Lucania interioris*, *Leptolucania ommata*, *Orestias agassizi*, all examined poeciliids, *Valencia*, *Aplocheilus panchax*, and *Kryptolebias marmoratus*. The posterior-most middle supraorbital lateralis canal pore is adjacent to an open portion with exposed neuromasts in *F. sciadicus*, *F. jenkinsi*, and *Aphanius dispar*. A closed middle supraorbital canal with pores 3 and 4, continuous with the posterolateral portion of the canal is present in *F. escambiae*, *F. nottii*, *Lucania parva*, *L. goodei*, *Ameca splendens*, *Crenichthys baileyi*, *Cubanichthys pengelleyi*, *Cyprinodon variegatus*, and *Floridichthys carpio*. Wiley (1986) recognized this condition as diagnostic of a sister group relationship between *Fundulus nottii* and *F. escambiae*.

(133₀) Middle supraorbital lateralis canal open.

(133₁) Middle supraorbital lateralis canal ending at pore 4a/4 and followed by open portion with exposed neuromasts.

(133₂) Middle supraorbital lateralis canal continuous with posterolateral canal with pore 4.

(133₃) Middle supraorbital lateralis canal closed and discontinuous with respect to posterolateral supraorbital canal ending with pore 4a.

134. Posterolateral supraorbital lateralis system. The most common condition in the examined taxa is a closed posterolateral supraorbital canal with pore 5 positioned anterolaterally with respect to pores 4b (or 4) and 6. The posterolateral supraorbital canal is closed, with pore 5 positioned posteromedially with respect to pores 4b (or 4) and 6 in *Fundulus cingulatus* and *F. rubrifrons*. The posterolateral supraorbital canal is closed anteriorly and open between what would be pores 5 and 6 in *F. notatus* and *F. sciadicus*. This portion of the supraorbital canal is open with exposed neuromasts in *F. kansae*, *F. zebrinus*, *F. jenkinsi*, *F. xenicus*, *F. luciae*, *Lucania interioris*, *Aphanius dispar*, *Orestias agassizi*, *Oxyzygonectes dovii*, *Jenynsia multidentata*, all examined poeciliids, *Valencia letourneuxi*, *Aplocheilus panchax*, and *Kryptolebias marmoratus*.
- (134₀) Posterolateral supraorbital lateralis canal open.
- (134₁) Posterolateral supraorbital lateralis canal closed, with pore 5 positioned anterolaterally with respect to pores 4b (or 4) and 6.
- (134₂) Posterolateral supraorbital lateralis canal closed, with pore 5 positioned posteromedially with respect to pores 4b (or 4) and 6.
- (134₃) Posterolateral supraorbital lateralis canal closed anteriorly and open between what would be pores 5 and 6.
135. Condition of pore 6 in posterolateral supraorbital lateralis system. The most common condition in the examined taxa is a closed, continuous posterior supraorbital canal between pores 5, 6, and 7. The posterior supraorbital canal is discontinuous with a more anterior canal terminating in pore 6a and a more posterior canal between pore 6b and 7 in *Fundulus pulvereus*, *F. lima*, *F. rathbuni*, *F. julisia*, *F. bifax*, *F. stellifer*, *F. jenkinsi*, and *F. euryzonus*. This condition varies among individuals in *F. parvipinnis* and *F. catenatus*, and these taxa are coded as polymorphic (0&1). Species with open posterior supraorbital canals are coded as unknown (?).
- (135₀) Single supraorbital pore 6 over continuous canal.
- (135₁) Separate supraorbital pores 6a and 6b marking point of discontinuity between canals.
136. Preopercular lateralis canal. The most common condition in the examined taxa is a closed, continuous preopercular canal with seven pores, three on the dorsal arm, three on the anterior arm, and one at the angle of the canal. The preopercular canal is completely open with exposed neuromasts in *Orestias agassizi*, *Gambusia affinis*, *Poecilia reticulata*, and *Kryptolebias marmoratus*. The preopercular canal is closed and continuous with six or fewer pores in *Fundulus cingulatus*, *F. rubrifrons*, and *Leptolucania onnata*. The preopercular canal is closed and discontinuous with eight pores, four on the dorsal arm and four on the anterior arm separated at the angle of the preopercle, in *F. sciadicus*, *Valencia letourneuxi*, and some individuals of *F. kansae* and *F. zebrinus* (both coded as polymorphic [1&3]). *Fundulus lima* is unique in having a partially closed preopercular canal with three pores.
- (136₀) Preopercular lateralis canal closed and continuous with seven pores.
- (136₁) Preopercular lateralis canal open.
- (136₂) Preopercular lateralis canal closed and continuous with six or fewer pores.
- (136₃) Preopercular lateralis canal closed and discontinuous with eight pores.
- (136₄) Preopercular lateralis canal partially closed and continuous with three pores.
137. Distance between anterior-most preopercular-canal pore and posterior-most mandibular-canal pore. These pores are close, closer than the width of the distal (ventral) maxilla in the *Fundulus majalis* species group, *Profundulus*, *Jenynsia multidentata*, *Aplocheilichthys spilauchen*, and *Alfaro cultratus*. Species with open preopercular and/or mandibular canals are coded as unknown (?).
- (137₀) Distance between anterior-most preopercular-canal pore and posterior-most mandibular-canal pore large.
- (137₁) Distance between anterior-most preopercular-canal pore and posterior-most mandibular-canal pore small, distance less than width of distal maxilla.
138. Mandibular lateralis canal. Most commonly in fundulids the mandibular lateralis canal is closed and continuous with four pores. Wiley (1986) recognized an increased number of mandibular pores as diagnostic of *F. grandis* and *F. grandissimus*. *Fundulus grandis* and *F. grandissimus* have a closed continuous mandibular canal with five or six pores. *Fundulus kansae*, *F. zebrinus*, *F. luciae*, *F. sciadicus*, *Profundulus labialis*, *Anableps dowi*, *Jenynsia multidentata*, and *Alfaro cultratus* have a closed discontinuous mandibular canal with five or six pores, where the canal between the posterior two pores is disjunct from the more anterior canal. *Fundulus cingulatus* and *F. rubrifrons* have a shortened closed continuous mandibular canal with three pores. The mandibular lateralis canal is represented by exposed neuromasts in an open groove in its anterior portion in *F. julisia*, *F. albolineatus*, *F. xenicus*, *Lucania*, *Leptolucania onnata*, *Aphanius dispar*, *Orestias agassizi*, *Gambusia affinis*, *Poecilia reticulata*, *Valencia*, and *Kryptolebias marmoratus*. The mandibular canal is open posteriorly with two pores associated with the anterior portion only in *Aplocheilus panchax*.
- (138₀) Mandibular lateralis canal open posteriorly and closed and continuous with two pores anteriorly.
- (138₁) Mandibular lateralis canal closed and continuous with four pores.
- (138₂) Mandibular lateralis canal closed and continuous with five to six pores.
- (138₃) Mandibular lateralis canal closed and discontinuous with five to six pores.
- (138₄) Mandibular lateralis canal closed and continuous with three pores.
- (138₅) Mandibular lateralis canal open.
139. Fleishy flap extends posteriorly beyond bony margin of operculum. A fleshy opercular flap is visible laterally in *Fundulus lima*, *F. parvipinnis*, the *F. majalis* species group, *F. xenicus*, *Leptolucania onnata*, *Aneca splendens*, *Cubanichthys pengelleyi*, *Cyprinodon variegatus*, *Floridichthys carpio*, *Aphanius dispar*, and *Oxyzygonectes dovii*.
- (139₀) Posterior margin of operculum without fleshy flap.

- (139₁) Fleshy flap extends posteriorly beyond bony margin of operculum.
140. Branchiostegal membrane crosses isthmus. The branchiostegal membrane joining the opercular membranes is visible across the isthmus in *Fundulus heteroclitus*, *F. lima*, *F. parvipinnis*, *F. luciae*, *F. xenicus*, *Leptolucania onnmata*, *Ameca splendens*, *Cubanichthys pengelleyi*, *Cyprinodon variegatus*, *Floridichthys carpio*, and *Aphanius dispar*.
- (140₀) Branchiostegal membrane not visibly crossing isthmus.
- (140₁) Branchiostegal membrane visibly crossing isthmus.
141. Position of dorsal-fin origin with respect to anal-fin origin in adult females. The dorsal-fin origin is distinctly posterior to the anal-fin origin in *Fundulus chrysotus*, *F. luciae*, *F. cingulatus*, *F. rubrifrons*, *F. sciadicus*, the *F. notatus* species group, the *F. nottii* species group, *Leptolucania onnmata*, *Oxyzygonectes dovii*, *Anableps dowi*, all examined poeciliids, *Aplocheilus panchax*, and *Kryptolebias marmoratus*. The dorsal-fin origin is distinctly anterior to the anal-fin origin in *F. parvipinnis*, *F. lima*, *F. kansae*, *F. zebrinus*, †*F. detillae*, *F. diaphanus*, *F. waccamensis*, *F. seminolis*, *F. xenicus*, *Lucania*, *Ameca splendens*, and all examined cyprinodontids.
- (141₀) Dorsal-fin origin distinctly posterior to a vertical at anal-fin origin.
- (141₁) Dorsal-fin origin distinctly anterior to a vertical at anal-fin origin.
- (141₂) Dorsal-fin origin above anal-fin origin.
142. Vertical position of pectoral fins. Parenti (1981) recognized high-set pectoral fins as diagnostic of the Poeciliidae (including the poeciliines, procatopodines, and *Aplocheilichthys spilarchen*). Costa (1998) confirmed this and noted that *Aplocheilus* also exhibits high-set pectoral fins. Using the criterion of position of dorsal pectoral origin in relation to a horizontal from the ventral margin of the orbit, the pectoral fins are positioned more dorsally in the poeciliids included in this study as well as in *Oxyzygonectes dovii* and *Aplocheilus panchax*.
- (142₀) Dorsal origin of pectoral fins positioned high, above horizontal line from ventral margin of orbit.
- (142₁) Dorsal origin of pectoral fins positioned low, near or ventral to horizontal line from ventral margin of orbit.
143. Urogenital sheath in mature females (Fig. 10). A urogenital sheath as found in fundulids is a fleshy structure that extends between the anus and the urogenital opening posteroventrally, contacting the anal fin in some species. Wiley (1986) recognized a urogenital sheath (also called an anal sheath) in mature females as diagnostic for a clade composed of *Fundulus* and *Lucania*. Adult female fundulids in all species except *F. lima* have an unscaled urogenital sheath. In some cases it is small (e.g., *F. xenicus* and *Leptolucania onnmata*). The character varies in *F. xenicus* populations, with some females possessing an unscaled fleshy urogenital sheath and others lacking it. This species was coded as polymorphic (0&1). Adult female *Oxyzygonectes* have a densely scaled urogenital sheath. Adult female *Anableps* have a scaled asymmetrical flap.
- (143₀) Urogenital sheath absent.
- (143₁) Urogenital sheath present as symmetrical fleshy structure with few or no scales.
- (143₂) Urogenital sheath present as densely scaled symmetrical structure.
- (143₃) Urogenital sheath present as scaled asymmetrical lateral flap.
144. Size of urogenital sheath in mature females (Fig. 10). *Fundulus grandis*, *F. grandissimus*, *F. parvipinnis*, *F. kansae*, *F. zebrinus*, and the *F. majalis* species group have moderate-sized urogenital sheaths. *Fundulus heteroclitus* has a notably long urogenital sheath. Species lacking an unscaled fleshy urogenital sheath are coded as unknown (?).
- (144₀) Urogenital sheath small, extends less than 1/10 length of first anal-fin ray.
- (144₁) Urogenital sheath of moderate size, extends between 1/10 and 1/4 length of first anal-fin ray.
- (144₂) Urogenital sheath long, extends more than 1/4 length of first anal-fin ray.
145. Position of urogenital sheath in mature females (Fig. 10). *Lucania* species have an anteriorly positioned urogenital sheath that results in the urogenital opening being visible in ventral view. In other fundulids the urogenital sheath is positioned and angled posteriorly such that the urogenital opening is not visible ventrally. In a few cases *Lucania* may have a more posteriorly positioned urogenital sheath and some *Zygonectes* species may have an anteriorly positioned urogenital sheath. However, these are uncommon. Species lacking an unscaled fleshy urogenital sheath are coded as unknown (?).
- (145₀) Urogenital positioned posteriorly, covering urogenital opening.
- (145₁) Urogenital positioned anteriorly, such that urogenital opening is visible in ventral view.
146. Male genital laterality. Parenti (1981) recognized genital laterality as diagnostic of anablepids, with variable dextral or sinistral gonopodia in *Anableps* and *Jenynsia* and variably dextral and sinistral urogenital papillae in *Oxyzygonectes*.
- (146₀) Adult males lacking morphological genital laterality.
- (146₁) Adult males exhibiting morphological genital laterality.
147. Branching of anal-fin ray 3 in adult females. Parenti (1981) recognized an unbranched third anal-fin ray in female poeciliines and anablepids as a homoplastic phylogenetic character independently derived in the two groups. Observations confirm this distribution.
- (147₀) Third anal-fin ray in adult females branched.
- (147₁) Third anal-fin ray in adult females unbranched.
148. Male intromittent organs. Classification in cyprinodontiforms was long focused on the intromittent organs, recognizing three or four viviparous families diagnosed by their differing intromittent organ structures, with the remaining taxa recognized in a single oviparous family (Greenwood et al., 1966; Nelson, 1976). Parenti (1981) radically changed this taxonomy and also demonstrated that the differing intromittent organ structures were, in fact, independent evolutionary innovations. Poeciliines have a rod-like intromittent organ composed of anal-fin

rays 3–5. Anablepines (*Anableps* and *Jenynsia*) have a tubular intromittent organ supported by more than three anal-fin rays. Goodeines have an intromittent organ composed of partially separated and shortened anterior anal-fin rays.

(148₀) Males lack intromittent organ.

(148₁) Adult males with rod-like intromittent organ composed of anal-fin rays 3–5.

(148₂) Adult males with tubular intromittent organ composed of more than three anal-fin rays.

(148₃) Adult males with intromittent organ composed of partially separated and shortened anterior anal-fin rays.

149. Condition of posterior surface of posterior branch of anal-fin ray 4. The fourth anal-fin ray in adult males bears serrae in *Gambusia affinis* and *Poecilia reticulata*.

(149₀) Fourth anal-fin ray in adult males lacking serrae.

(149₁) Fourth anal-fin ray in adult males bearing serrae.

150. Branching of anal-fin ray 6 in adult males. Ghedotti (2000) recognized an unbranched sixth anal-fin ray in males as diagnostic of a clade containing all poeciliines except *Alfaro*. The sixth anal-fin ray in adult males is unbranched in *Gambusia affinis* and *Poecilia reticulata*.

(150₀) Sixth anal-fin ray in adult males branched.

(150₁) Sixth anal-fin ray in adult males unbranched.

151. Caudal-fin shape. Cyprinodontiforms do not have the falcate caudal fin common in most teleosts and have rounded, oval, or truncate caudal fins in lateral profile. The most common condition in cyprinodontoids is a truncate or subtruncate caudal fin. The outgroup taxa (from the Suborder Aplocheiloidei), *Aplocheilichthys panchax* and *Kryptolebias marmoratus*, have caudal fins that appear short and rounded in lateral profile. Members of the *Fundulus notatus* species group, the *F. nottii* species group, and *Leptolucania ommata* have elongate oval caudal fins.

(151₀) Caudal fin round.

(151₁) Caudal fin truncate or subtruncate.

(151₂) Caudal fin elongate and oval.

Color Pattern

We obtained color pattern data for all species from photographs and/or descriptions of color pattern in Thomerson (1969), Williams and Etnier (1982), Cashner et al. (1988), Robison and Buchanan (1988), Etnier and Starnes (1993), Jenkins and Burkhead (1993), Mettee et al. (1996), Wildekamp (1996), Pflieger (1997), Ross (2001), Boschung and Mayden (2004), Miller (2005), McGinnis (2006), Minckley and Marsh (2009), and Page and Burr (2011) as well as from examining of ethanol-preserved specimens and author field observations. All color pattern data are necessarily lacking for the fossil taxon †*Fundulus detillae*. Color pattern data for the recently extinct taxon *F. albolineatus* are derived from the original description (Gilbert, 1891), the comparative examination of *F. albolineatus* in the description of *F. julisia* (Williams and Etnier, 1982), and examination of a cotype specimen of *F. albolineatus*. For *Kryptolebias marmoratus*, the condition in hermaphrodites was coded as for adult females because females are not known in

this species and hermaphrodites have a color pattern very similar to females of other *Kryptolebias* species. *Kryptolebias marmoratus* does produce males in some populations.

152. Vertical barring on lateral surface of live nuptial males. Black to brown barring is very common on the lateral surface of juvenile cyprinodontiforms of both sexes. However, the presence or absence of these bars does vary among adults. Wiley (1986) recognized the absence of bars in males as diagnostic for *F. rathbuni*, *F. julisia*, *F. albolineatus*, and the *F. catenatus* species group. These bars in both males and females frequently can fade in alcohol-preserved specimens. Therefore, their presence primarily was determined via photographs of live specimens. We observed an absence of lateral barring in *F. rathbuni*, *F. julisia*, *F. albolineatus*, the *F. catenatus* species group, *F. linna*, *F. sciadicus*, *Lucania*, the examined *Profundulus* species, *Crenichthys baileyi*, *Orestias agassizi*, *Anableps dowi*, *Jenynsia multidentata*, *Poecilia reticulata*, and *Gambusia affinis*. The presence or absence of bars in nuptial males varies among populations of *Aplocheilichthys panchax* and was coded as polymorphic (0&1).

(152₀) Lateral barring present in nuptial males.

(152₁) Lateral barring absent in nuptial males.

153. Vertical barring on lateral surface of live nuptial females. Lateral barring is less common in nuptial females than in nuptial males. It was found to be absent in *F. rathbuni*, *F. julisia*, *F. albolineatus*, the *F. catenatus* species group, *F. linna*, *F. chrysotus*, *F. sciadicus*, the *F. notatus* species group, *F. blairae*, *F. dispar*, *F. nottii*, *Lucania*, *Leptolucania ommata*, the examined *Profundulus* species, *Crenichthys baileyi*, *Aneca splendens*, *Cubanichthys pengelleyi*, *Oxyzygonectes dovii*, *Anableps dowi*, *Jenynsia multidentata*, *Poecilia reticulata*, *Gambusia affinis*, and *Aplocheilichthys panchax*.

(153₀) Lateral barring absent in nuptial females.

(153₁) Lateral barring present in nuptial females.

154. Multiple horizontal stripes on lateral surface of mature females. Wiley (1986) recognized horizontal black or dark brown lines in females as diagnostic of the *Fundulus nottii* species group. Lateral stripes in females are found in two forms, as five or more narrow stripes in the *F. nottii* species group and *Jenynsia multidentata* and as four or fewer thick stripes in *F. majalis*.

(154₀) Multiple horizontal midlateral stripes absent.

(154₁) Five or more narrow midlateral stripes present.

(154₂) Four or fewer midlateral stripes present.

155. Midlateral stripe (Fig. 11). Wiley (1986) recognized a broad lateral stripe as diagnostic of the *F. notatus* species group. A black or brown midlateral stripe is present in a range of fundulids but varies in its extent and width. A prominent midlateral stripe extends from anterior to the eye to the caudal-fin base in the *F. notatus* species group, *Lucania goodei*, and *Cubanichthys pengelleyi*. A prominent midlateral stripe that extends from the posterior margin of the operculum to the base of the caudal fin and is often discontinuous, forming a broken line, is present in *F. parvipinnis*, *F. linna*, the examined *Profundulus* species, *Crenichthys baileyi*, *Aneca splendens*, *Aphanius dispar*, *Orestias agassizi*, and *Anableps dowi*. A faint narrow continuous midlateral stripe extends from the

FIG. 10. Left lateral views of urogenital sheath in adult female (A) *Fundulus heteroclitus* FMNH 60131, dotted line indicates margin of urogenital sheath, (B) *F. majalis* FMNH 109220, (C) *F. notatus* FMNH 51470, (D) *F. olivaceus* FMNH 60553, and (E) *Lucania parva* FMNH 113089. Scale bars indicate 1 mm.

posterior margin of opercle to the caudal-fin base in *Lucania parva* and *L. interioris*. Female *Leptolucania ommata* exhibit a somewhat faint brown continuous midlateral stripe extending from anterior to the eye and posterior to the caudal-fin base.

(155₀) Midlateral stripe absent.

(155₁) Midlateral stripe present and prominent from anterior of eye to caudal-fin base, continuous.

(155₂) Midlateral stripe present and prominent from posterior margin operculum to caudal-fin base, continuous or discontinuous as large blotches.

(155₃) Midlateral stripe present and faint from posterior margin of opercle to caudal-fin base, continuous but usually very faint.

(155₄) Midlateral stripe present and faint from anterior of eye to caudal-fin base, continuous, in females only.

156. Reddish-brown or brown lateral spots in nuptial males. Brown and red or reddish-brown spots are coded in the same transformation series because in either type of spot melanophores (brown or black), erythrophores (red), or some combination likely are present. Silver spots do not have this composition and likely are structural. Williams

- and Etnier (1982) and Wiley (1986) recognized reddish-brown spots as diagnostic of a clade containing *F. rathbuni*, *F. julisia*, and the *F. catenatus* species group. Ghedotti et al. (2004) recognized their presence in these taxa as well as in *F. chrysotus*. For this transformation series we did not consider the red blotches often present in *Poecilia reticulata* to represent the same color pattern. We observed red to reddish-brown scattered or regularly arranged spots on the flank in *Aplocheilichthys panchax*, *Floridichthys carpio*, *Fundulus sciadicus*, *F. chrysotus*, *F. rubrifrons*, *F. cingulatus*, *F. julisia*, *F. albolineatus*, and some nuptial male *F. heteroclitus* (coded as polymorphic [0&2]).
- (156₀) Multiple scattered or regular red or reddish-brown spots on lateral surface of nuptial males.
- (156₁) Multiple scattered or regular dark brown lateral spots of nuptial males.
- (156₂) No brown, reddish-brown, or red spots scattered or in regular rows in nuptial males.
157. Arrangement of reddish-brown or brown lateral spots in nuptial males. Cashner et al. (1988) and Ghedotti et al. (2004) recognized the arrangement of reddish-brown lateral spots into lines as supporting a sister relationship between *Fundulus bifax* and *F. catenatus*. We identified dark brown, reddish-brown, or red spots arranged into discrete lines in *F. bifax*, *F. catenatus*, *F. albolineatus*, *F. seminolis*, the *F. nottii* species group, *Floridichthys carpio*, and *Jenynsia multidentata*. The many species lacking brown or reddish-brown spots on the lateral surface are coded as unknown (?).
- (157₀) Red, reddish-brown, or brown spots on lateral surface in nuptial males irregularly scattered.
- (157₁) Red, reddish-brown, or brown spots on lateral surface in nuptial males in discrete horizontal lines.
158. Reddish-brown or brown spots on cheek and ventral to eye. Reddish-brown or brown spots are present ventral to the eye in nuptial males of *Fundulus rathbuni*, *F. julisia*, *F. albolineatus*, and the *F. catenatus* species group. Reddish spots may be present under the eye in some males of *F. cingulatus* and *F. rubrifrons*, and these species were coded as polymorphic (0&1).
- (158₀) No reddish-brown to brown spots under eye in nuptial males.
- (158₁) Reddish-brown to brown spots on lateral surface of cheek under eye in nuptial males.
159. Distinct scattered iridescent silvery spots on lateral surface in nuptial males. Scattered iridescent silvery spots are present in nuptial males of the *F. heteroclitus* species group, *F. confluentus*, *F. pulvereus*, *Aplanius dispar*, and *Valencia letourneuxi*.
- (159₀) No iridescent silvery spots on lateral surface in nuptial males.
- (159₁) Iridescent silvery spots on lateral surface in nuptial males.
160. Iridescent blue or blue-green background color on lateral surface in nuptial males. Williams and Etnier (1982) suggested that a blue-green lateral background coloration in males suggested a relationship between *F. julisia* and the *F. catenatus* species group. Ghedotti et al. (2004) included this character in their phylogenetic analysis which recovered a relationship between *F. julisia* and the *F. catenatus* species group. We recognized iridescent blue to blue-green coloration in nuptial males in *F. julisia*, the *F. catenatus* species group, *F. jenkinsi*, *F. rubrifrons*, *F. cingulatus*, *F. chrysotus*, *F. luciae*, *F. sciadicus*, the *F. nottii* species group, *Cubanichthys pengelleyi*, *Cyprinodon variegatus*, *Floridichthys carpio*, *Aplocheilichthys spilauchen*, *Alfaro cultratus*, *Poecilia reticulata*, and *Aplocheilichthys panchax*.
- (160₀) Iridescent blue or blue-green on lateral surface in nuptial males.
- (160₁) No iridescent blue or blue-green on lateral surface in nuptial males.
161. Red chromatophores anterior to the eye and on operculum in nuptial males. Red pigment is present anterior to the eye and on the operculum in the *Fundulus nottii* species group, *F. cingulatus*, and *F. rubrifrons*.
- (161₀) No red pigment anterior to eye and on operculum in nuptial males.
- (161₁) Red pigment anterior to eye and on operculum in nuptial males.
162. Dark subocular teardrop. Wiley (1986) recognized a subocular teardrop marking as diagnostic of the *F. nottii* species group. A vertical dark chromatophore teardrop mark under eye is present in the *F. nottii* species group and *Gambusia affinis*.
- (162₀) Teardrop absent.
- (162₁) Teardrop marking through eye present.
163. Iridescent spot on top of head. A distinct spot on the top of the head, which is frequently visible from above the surface, is present in the *F. notatus* species group, the *F. nottii* species group, and *Aplocheilichthys panchax*.
- (163₀) Iridescent spot on top of head.
- (163₁) No iridescent spot on top of head.
164. Predorsal pigment line. A median dark chromatophore line extends from dorsal-fin origin to mid-nape or head in alcohol-preserved specimens. Ghedotti et al. (2004) recognized variability of this line among *Fundulus*. A prominent line from the dorsal-fin origin to the back of the head characterizes *F. confluentus*, *F. pulvereus*, *F. diaphanus*, *F. similis*, *F. rathbuni*, *F. julisia*, *F. albolineatus*, *F. lima*, *F. parvipinnis*, *F. chrysotus*, *F. luciae*, *F. xenicus*, *F. rubrifrons*, *F. cingulatus*, *F. sciadicus*, the examined *Profundulus* species, *Crenichthys baileyi*, the examined poeciliids, and *Aplocheilichthys panchax*. A prominent line from the dorsal-fin origin to approximately halfway between head and dorsal-fin origin is seen in *F. majalis* and *Aplanius dispar*. A faint, in some cases to the point of being discontinuous, line from the dorsal-fin origin to the back of the head is in *F. waccamensis*, *F. seminolis*, *F. jenkinsi*, *F. zebrinus*, *F. kansae*, the *F. nottii* species group, *Lucania parva*, *L. interioris*, *Aneca splendens*, *Oxyzygonectes dovii*, *Jenynsia multidentata*, and *Valencia letourneuxi*.
- (164₀) Prominent continuous predorsal line from dorsal-fin origin to head.
- (164₁) Prominent continuous predorsal line from dorsal-fin origin to mid-nape.
- (164₂) Faint predorsal line from dorsal-fin origin to head.
- (164₃) No predorsal line.

165. Predorsal pigment spot. A median dark chromatophore mark immediately anterior to the dorsal-fin origin in alcohol-preserved specimens is present as either a small or larger elongate mark. A small predorsal mark is present in *Fundulus confluentus*, *F. pulvereus*, *F. diaphanus*, *F. waccamensis*, *F. rathbuni*, the *F. nottii* species group, and *Aplocheilichthys panchax*. An elongate predorsal mark is present in the *F. heteroclitus* species group, the *F. catenatus* species group, *F. julisia*, the *F. majalis* species group, *F. lima*, *F. parvipinnis*, *F. kansae*, *F. zebrinus*, *F. xenicus*, *Cyprinodon variegatus*, *Jenynsia multidentata*, and *Alfaro cultratus*. *Fundulus seminolis* variably lacks a predorsal spot or has a small spot and was coded as polymorphic (0&1).
- (165₀) Small predorsal spot, about as long as wide.
 (165₁) No predorsal spot.
 (165₂) Anteroposteriorly elongate spot, distinctly longer than wide.
166. Cleithral bar. Wiley (1986) recognized the presence of a cleithral bar in *F. nottii* and *F. escambiae*. A dark chromatophore cleithral bar extends from the angle of operculum to the dorsal base of pectoral fin in *F. lineolatus*, *F. escambiae*, *F. nottii*, *F. euryzonus*, and *Aphanius dispar*.
- (166₀) Cleithral bar absent.
 (166₁) Dark chromatophore cleithral bar from angle of operculum to dorsal base of pectoral fin.
167. Red, reddish-orange, or pink pelvic and anal fins in nuptial males. Red chromatophores are present on the pelvic and anal fins in nuptial males, resulting in red, reddish-orange, or pink background color in *Fundulus zebrinus*, *F. kansae*, *F. rathbuni*, *F. julisia*, *F. jenkinsi*, *F. luciae*, *F. chrysotus*, *F. rubrifrons*, *F. cingulatus*, *F. sciadicus*, *Lucania parva*, *Leptolucania*, *Profundulus*, *Cubanichthys pengelleyi*, *Cyprinodon variegatus*, *Aphanius dispar*, *Oxyzygonectes dovii*, and *Valencia letourneuxi*. This varies between populations in *Aplocheilichthys panchax* and *Lucania goodei*, and these taxa were coded as polymorphic (0&1).
- (167₀) Pelvic and anal-fin background color not red, reddish orange, or pink.
 (167₁) Red, reddish-orange, or pink background color in nuptial male pelvic and anal fins.
168. A black spot in the anterior dorsal fin in nuptial males is present in *Lucania*.
- (168₀) Dorsal fin of nuptial males lacks anterior black spot.
 (168₁) Dorsal fin of nuptial males has anterior black spot.
169. Ocellus in posterior dorsal fin in nuptial males. Wiley (1986) recognized the presence of an ocellus as diagnostic of the subgenus *Fundulus* composed of the *F. heteroclitus* species group, *F. confluentus*, and *F. pulvereus*, and also present in *F. luciae*. A discrete spot of dark chromatophores surrounded by light chromatophores (i.e., an ocellus) is present in the posterior dorsal fin of nuptial males in the *F. heteroclitus* species group, *F. confluentus*, *F. pulvereus*, and *F. luciae*. Some nuptial male *F. xenicus* exhibit a particularly darkly pigmented area of the posterior dorsal fin surrounded by unpigmented windows.
- Because of the only occasional occurrence combined with the less discrete nature of these ocellus-like areas, we coded *F. xenicus* as polymorphic (0&1).
- (169₀) Dorsal fin of nuptial males lacks ocellus.
 (169₁) Dorsal fin of nuptial males with ocellus.
170. "Windowed" dorsal and caudal fins in nuptial males. Nuptial males of the *F. heteroclitus* species group, *F. confluentus*, *F. pulvereus*, *F. xenicus*, and *Oxyzygonectes dovii* have dorsal and caudal fins that are darkly pigmented and interrupted by spots lacking dark chromatophores, thus forming small transparent "windows" in nuptial males.
- (170₀) Dorsal and caudal fins without small transparent "windows" in nuptial males.
 (170₁) Darkly pigmented dorsal caudal fins with small transparent "windows" in nuptial males.
171. A black spot is present on the caudal-fin base of *Lucania goodei* and *Leptolucania ommata*.
- (171₀) Black spot on caudal-fin base absent.
 (171₁) Black spot on caudal-fin base present.
172. Yellow submarginal band on posterior sixth of caudal fin in nuptial males. Wiley (1986) followed Williams and Etnier (1982) in recognizing a pigmented caudal-fin band as diagnostic of a subgenus *Xenisma* composed of the *Fundulus catenatus* species group, *F. albolineatus*, *F. julisia*, and *F. rathbuni*. Ghedotti et al. (2004) broke this characteristic into a submarginal yellow band and or gray marginal band due to consistent positional and color differences (next transformation series). Nuptial males of *F. catenatus*, *F. julisia*, and *F. rathbuni*, *Ameca splendens*, and *Aphanius dispar* have a distinct yellow submarginal caudal band.
- (172₀) Submarginal region of posterior sixth of caudal fin in nuptial males without yellow band.
 (172₁) Yellow submarginal band on posterior sixth of caudal fin in nuptial males.
173. Black or dark gray marginal band on caudal fin of nuptial males. *Fundulus julisia* and *F. albolineatus* have a narrow dark marginal caudal band. *Fundulus similis*, some populations of *F. stellifer* (coded as polymorphic [0&2]), *Lucania parva*, *Floridichthys carpio*, *Cyprinodon variegatus*, *Valencia letourneuxi*, and some populations of *Aplocheilichthys panchax* (coded as polymorphic [0&2]) have a broad dark marginal caudal band.
- (173₀) Margin of caudal fin of nuptial males lacks black or dark gray marginal band.
 (173₁) Narrow black or dark gray marginal band on caudal fin of nuptial males.
 (173₂) Broad black or dark gray marginal band on caudal fin of nuptial males.

Karyology

174. Diploid chromosome number (2n). We obtained karyological data from descriptions in Chen and Ruddle (1970), Chen (1971), and Gold et al. (1980). In cases where the 2n number is less than 48, there are large metacentric chromosomes approximately twice the length of the other chromosomes that likely are

FIG. 11. *Fundulus parvipinnis*, cleared and stained specimen from KU 19046, whole ethanol-preserved specimen from FMNH 61255. (A) Dorsal view and (B) ventral view of neurocranium with left nasal removed, dotted lines show overlying dermal bone margins, anterior at top. (C) Left lateral view of pectoral girdle with supracleithrum and posttemporal removed, anterior at left, image photo reversed. (D) Left lateral view of adult male. (E) Medial view of right jaws, suspensorium, and opercular series, anterior at left. (F) Dorsal and (G) ventral views of branchial skeleton, dotted lines show element margins of the left dorsal branchial elements, anterior is at top. (H) Ventral view of pelvic girdle, fin rays of left fin removed, anterior at top. (I) Dorsal view of snout region, anterior is at left. Please see previous figure captions (Figs. 3–7) for morphological structure abbreviations.

equivalent to two small fused acrocentric chromosomes (Chen, 1971). Diploid numbers were used as character states because no studies have provided information that would allow identification of individual chromosomes across fundulids and thus identify specific likely fusions or

divisions of chromosomes. Karyotypes are not known for many species. The most common karyotype in the taxa studied was $2n = 48$. *Fundulus cingulatus*, the *F. nottii* species group, *Lucania parva*, and *Poecilia reticulata* have a karyotype of $2n = 46$. *Fundulus sciadicus* has a

karyotype of $2n = 44$. *Fundulus notatus* exhibits population-dependent, diploid chromosome numbers of either 40 or 44 and was coded as polymorphic (3&4). *Aplocheilichthys panchax* has a karyotype of $2n = 36$. *Fundulus chrysotus* has a karyotype of $2n = 34$. *Fundulus luciae* and *F. xenicus* have a karyotype of $2n = 32$. *Ameioba splendens* has a karyotype of $2n = 26$.

- (174₀) $2n = 36$.
- (174₁) $2n = 48$.
- (174₂) $2n = 46$.
- (174₃) $2n = 44$.
- (174₄) $2n = 40$.
- (174₅) $2n = 34$.
- (174₆) $2n = 32$.
- (174₇) $2n = 26$.

Reproductive Behavior

We obtained reproductive behavior data from two unpublished theses (Foster, 1967a; Arndt, 1971), books primarily published for aquarists (Wildekamp, 1993, 1995, 1996, 2004), and other published accounts (Newman, 1907; Leitholf, 1917; Mayer, 1932; Koster, 1948; Kopec, 1949; Breder & Rosen, 1966; Foster, 1967b; Baugh, 1981; Baugh et al., 1986; Taylor & Pedretti, 1993; Taylor & Burr, 1997). Pertinent data are relatively limited and could only be coded for a subset of taxa with well-documented courtship; the remainder was coded as unknown (?). All behavioral data are necessarily lacking for the fossil taxon †*Fundulus detillae*. Because authors vary in the descriptions of behavior, we included only the most visually distinctive aspects of behavior that would be unlikely to be missed by observers.

- 175. Male looping and circling in nuptial display. Male looping and circling in front of female during early courtship is common in many cyprinodontiforms. In *F. rubrifrons*, *F. notatus*, *F. olivaceus*, *F. dispar*, *F. lineolatus*, *F. nottii*, *F. xenicus*, and the poeciliines, the male does not exhibit this behavior.
 - (175₀) Male loops and circles in front of female during early courtship.
 - (175₁) Male does not loop and circle in front of female during early courtship, approaches directly.
- 176. Male head bobbing in nuptial display. In *F. chrysotus*, *F. notatus*, *F. olivaceus*, and *F. bifax* the male bobs his head vertically during courtship.
 - (176₀) Male does not head bob during courtship.
 - (176₁) Male bobs head vertically during courtship.
- 177. Male head flicking in nuptial display. In *F. rubrifrons*, *F. chrysotus*, *Lucania goodei*, *Leptolucania*, and *Valencia*, the male flicks his head laterally during courtship.
 - (177₀) Male flicks head laterally during courtship.
 - (177₁) Male does not head flick during courtship.
- 178. Male rubbing of female isthmus in nuptial display. During later courtship in *Lucania* and *Leptolucania*, the male swims under the female and rubs her isthmus with his dorsal snout.
 - (178₀) Male does not isthmus rub during courtship.
 - (178₁) Male rubs the isthmus of the female with his snout during courtship.
- 179. Male jaw nudging in nuptial display. In all fundulids with well-described courtship, except *Fundulus xenicus* and

Cyprinodon variegatus, at some point during courtship the male will usually swim with his head above the head or back of female during which he opens his mouth downward, often contacting the female.

- (179₀) Male swims with head above head or back of female and opens mouth downward.
- (179₁) Male does not jaw nudge during courtship.
- 180. Female substrate biting before spawning. The female bites or nudges the spawning location with her mouth immediately prior to spawning in *F. majalis*, *F. notatus*, *F. olivaceus*, *F. lineolatus*, *F. escambiae*, *F. nottii*, *Lucania parva*, *L. goodei*, and *Leptolucania ommata*.
 - (180₀) Female nips or nudges spawning location with snout immediately prior to spawning.
 - (180₁) Female does not nip or nudge spawning location immediately prior to spawning.
- 181. Spawning substrate. Although cyprinodontiform fishes exhibit some plasticity in spawning substrate, most oviparous cyprinodontiforms spawn on a fibrous aquatic structure, as is recognized by aquarists who frequently use yarn “mops” for aquarium spawning (Wildekamp, 1993, 1995, 1996, 2004). *Fundulus parvipinnis*, *F. kansae*, *F. zebrinus*, *F. seminolis*, the *F. majalis* species group, the *F. catenatus* species group, and *Cyprinodon variegatus* usually spawn on or into loose substrates such as mud, sand, or gravel. *Fundulus lima* usually adheres eggs to solid structure above the substrate, often even above the surface of the water, during spawning (Wildekamp, 1996). *Fundulus xenicus* variously has been reported to adhere eggs to solid structure above the substrate or to spawn on loose substrates or on aquatic plants (Arndt, 1971; Wildekamp, 1996) and was coded as polymorphic (0&1&2). The poeciliines, *Anableps*, *Jenynsia*, and the goodeines (including *Ameioba splendens*) are viviparous.
 - (181₀) Usually spawn on macrophytes, algae, or other branching or fibrous aquatic structure.
 - (181₁) Usually spawn on loose substrate such as mud, sand, and/or gravel.
 - (181₂) Usually adhere eggs to solid structure such as rock or wood above substrate during spawning.
 - (181₃) Viviparous eggs retained and fertilized in ovary.

Phylogenetic Relationships and Classification

All 10 separate likelihood runs recovered identical topologies within their respective data sets, with differences solely in branch lengths. The maximum-likelihood re-analysis of mitochondrial and nuclear data from Whitehead (2010), which included cyprinodontiform outgroups in addition to *Profundulus*, resulted in a different hypothesis of relationships within the Fundulidae (Fig. 12, likelihood $-28,947.05$) from those presented in Whitehead (2010). Notably, the genus *Fundulus* was paraphyletic in Whitehead (2010), with the genus *Lucania* sister to a clade comprised of *F. parvipinnis* and *F. lima*. Our re-analysis of data from Whitehead (2010) indicates that the genus *Lucania* is sister to a monophyletic *Fundulus*, with the *F. parvipinnis* and *F. lima* clade sister to the remaining *Fundulus* taxa. As noted previously, cytb sequence data for *F. parvipinnis* and *F. lima* were excluded from our re-analysis because of their

FIG. 12. Phylogenetic hypothesis of relationships within the Fundulidae based on partitioned mixed-model maximum-likelihood analysis of Whitehead's (2010) cytb, gylt, and RAG1 data after inclusion of additional outgroup taxa and removal of cytb data for *Fundulus parvipinnis* and *F. lima*.

abnormally high sequence divergence that resulted in exceedingly long branches, which may have resulted in the inference of a false evolutionary relationship (e.g., sister relationship to *Lucania*) because of long-branch attraction (e.g., Felsenstein, 2004; Bergsten, 2005). The remaining relationships within *Fundulus* were fairly consistent with those proposed by Whitehead (2010), and we recognize *Adinia xenica* in this study as belonging to the genus *Fundulus* as *F. xenicus*.

A fifty-percent majority-rule tree of the 728 equally parsimonious trees (tree length of 896, consistency index of 0.31, retention index of 0.69, 181 parsimony informative characters) for the morphological, karyological, and behavioral (non-nucleotide) data set is presented in Figure 13. The maximum-likelihood topology for the non-nucleotide data set was largely congruent with these results (not shown). With the non-nucleotide data, the Family Fundulidae was recovered as monophyletic with strong bootstrap support. While a clade including *Leptolucania* and *Lucania* had strong bootstrap support in both analyses (parsimony, likelihood), support for a monophyletic genus *Fundulus* (excluding *F. xenicus*) was weakly supported (Fig. 13). Within *Fundulus*, of note is that a strong relationship was recovered with non-nucleotide data that indicated the North American west-coast clade *F. parvipinnis* and *F. lima* was sister to a clade including the westerly distributed taxa *F. zebrinus*, *F. kansae*, and the extinct †*F. detillae*.

The total-evidence analysis was reconstructed from a concatenated matrix of DNA characters from four genes (Appendix III) with the non-nucleotide characters (Appendix IV) presented in this study. The recovered topology with the best likelihood score (−38,068.79) of the 10 independent maximum-likelihood analyses is presented in Figure 14.

Parsimony analyses resulted in 36 equally parsimonious trees (tree length of 8150, consistency index of 0.31, retention index of 0.52, 1265 parsimony informative characters). The fifty-percent majority-rule tree (not shown) is largely consistent in relationships with the most likely topology (Figs. 14, 15), with the exception of reduced resolution at deeper nodes among the relationships in the genus *Fundulus*, as shown in Figure 15, where clades not recovered in all equally parsimonious trees are indicated by dashed lines. Based on the results of our total-evidence data set, we revised the classification of the topminnows, as this is the most taxonomically robust and data-inclusive hypothesis of evolutionary relationships for the Family Fundulidae to date (Figs. 14, 15). Throughout the remainder of this monograph we will discuss the phylogenetic relationships within Cyprinodontiformes, relationships within the Family Fundulidae, the evolution of salinity tolerance, and biogeography in the context of the total-evidence hypothesis of evolutionary relationships (Figs. 14, 15).

Our non-nucleotide and total-evidence analyses support the Fundulidae as the sister taxon to a clade composed of the Cyprinodontidae, Profundulidae, and Goodeidae (Figs. 13, 14). Although previous studies have supported a Fundulidae, Profundulidae, and Goodeidae clade (Meyer & Lydeard, 1993; Parker, 1997; Costa, 1998), the inclusion of the Cyprinodontidae has not previously been proposed. There are no unique and unreversed morphological character states supporting this relationship, yet all analyses using the non-nucleotide data support this relationship, which additionally has a robust bootstrap value of 90. All analyses also support Valenciidae as sister to the combined Fundulidae, Cyprinodontidae, Profundulidae, and Goodeidae clade, as well as a sister relationship

FIG. 13. Phylogenetic hypothesis of relationships based on maximum-parsimony and partitioned maximum-likelihood analysis of non-nucleotide data (morphology, karyology, and behavior). Numbers above branches indicate percent maximum-parsimony bootstrap support over 50, and numbers below branches indicate percent maximum-likelihood bootstrap support over 50. Tree shown is fifty-percent majority-rule tree of 728 equally most parsimonious trees (length = 896; CI = 0.31) in the maximum-parsimony analysis. Dashed lines indicate clades that are not present in all 728 equally parsimonious trees. Non-fundulid families indicated in parentheses.

between Anablepidae and Poeciliidae (Figs. 13, 14). Bootstrap support for these clades is low, less than 50 in both cases. However, the examined members of the Valenciidae, Fundulidae, Cyprinodontidae, Profundulidae, and Goodeidae all uniquely share the character state of a nasal bone with a prominent bony trough (2_1). Based on these relationships we recognize three monophyletic superfamily-rank taxa within

the Suborder Cyprinodontoidei. The Poecilioidea includes the Anablepidae and the Poeciliidae. The Valencioidae includes the two species in the European genus *Valencia*. The Cyprinodontoidea includes the Fundulidae, Cyprinodontidae, Profundulidae, and Goodeidae.

All conducted phylogenetic analyses support the monophyly of the Family Fundulidae with robust bootstrap values

FIG. 14. Phylogenetic hypothesis of relationships among all included species with branch lengths based on partitioned mixed-model maximum-likelihood analysis of all compiled available data. Numbers indicate percent maximum-likelihood bootstrap support for clades over 50. Families of non-fundulid taxa indicated in parentheses.

(Figs. 12–15). The Fundulidae is diagnosed by three unique and unreversed morphological synapomorphies. The tip of the ventromedial process of the maxillary head is angled ventrally (31₁, Fig. 4) in all fundulids, and is associated with the states

of this process recognized as synapomorphic of the Fundulidae by Parenti (1981) and Costa (1998). The neural arch on the first vertebra is open, lacking a more ventral horizontal bridge of bone (87₂, Fig. 6) in all fundulids. Female fundulids

sequences for *F. parvipinnis* and *F. linia* (see Materials and Methods) obtained the same result (Fig. 12). Our non-nucleotide (Fig. 13) and total-evidence analyses (Figs. 14, 15) found *Lucania* and *Leptolucania* forming a clade sister to a monophyletic genus *Fundulus*. Therefore, we continue to recognize *Lucania* and *Leptolucania* as valid genera within the Fundulidae.

Unlike *Lucania* and *Leptolucania*, the monotypic genus *Adinia* was not consistently recovered outside of *Fundulus*. Most of the 728 equally most parsimonious trees in the non-nucleotide data analyses recover *A. xenica* outside the genus *Fundulus* (Fig. 13) as either sister to all fundulids or sister to a *Lucania* and *Leptolucania* clade. But many of the equally most parsimonious trees did have *A. xenica* within *Fundulus* as sister to either *F. luciae* or a large clade of *Fundulus*. Nucleotide-sequence analyses and total-evidence analyses strongly support a sister relationship between *F. luciae* and *A. xenica* (Figs. 12, 14, 15). This suggests that the distinctive deep body in this species is not a body form evolved very early in the history of the family. Rather, it evolved after divergence from *F. luciae*. Because of the strong support for *A. xenica* in a clade with *F. luciae* nested well within *Fundulus*, we synonymize the monotypic genus *Adinia* with the genus *Fundulus* as well as with the subgenus *Zygonectes*. Thus, we have referred to this species as *F. xenicus* rather than *A. xenica* throughout this study. We recognize a Fundulidae with three genera: *Fundulus*, *Lucania*, and *Leptolucania*.

The analyses of non-nucleotide data did not provide strong support for a monophyletic genus *Fundulus* including *F. xenicus*, with most but not all equally parsimonious trees including a monophyletic *Fundulus* (Fig. 13). The total-evidence analyses (Figs. 14, 15) do provide strong support for a monophyletic genus *Fundulus* including *F. xenicus*. Within *Fundulus*, the taxa recognized by Wiley (1986) as comprising the subgenus *Zygonectes* largely formed a monophyletic group in the total-evidence analyses (Figs. 14, 15). The removal of *F. jenkinsi* and the inclusion of *F. xenicus* in subgenus *Zygonectes* restore the monophyly of this subgenus. The non-nucleotide analyses only provide consistent support for a clade composed of *F. sciadicus*, the *F. notatus* species group, and the *F. nottii* species group, with the nucleotide data in the total-evidence analyses supporting a clade composed of these taxa as well as *F. cingulatus*, *F. rubrifrons*, *F. chrysotus*, *F. luciae*, and *F. xenicus*. Whitehead (2010) and our total-evidence analyses recovered a clade composed of *F. chrysotus*, *F. luciae*, and *F. xenicus* as sister to a clade composed of the other *Zygonectes* (Figs. 12, 14, 15). Before Whitehead (2010) this clade had not previously been proposed. Interestingly, *F. luciae* and *F. xenica* both uniquely share the lowest karyotype yet observed among fundulids, $2n = 32$ (174₆), and *F. chrysotus* uniquely exhibits the next lowest karyotype among fundulids, $2n = 34$ (174₅). Homoplastic morphological characteristics of the coronoid process of the anguloarticular (41₁), the relationship of the dorsal intermuscular bones to the last two abdominal vertebrae (93₁), and the supraorbital canal system (133₀) also provide clear support for a sister relationship between *F. luciae* and *F. xenicus* that, at least in part, account for this sister relationship appearing in some of the equally most parsimonious trees in the non-nucleotide data analysis.

A large clade composed of *F. jenkinsi* and the members of Wiley's (1986) subgenera *Fundulus*, *Fontinus*, and *Xenisma* was present in the total-evidence analysis (Figs. 14, 15) and our re-analysis of Whitehead's (2010) data (Fig. 12). This same clade

without *Fundulus jenkinsi* also was present in most of the equally parsimonious trees resulting from analyses of the non-nucleotide data (Fig. 13). These non-nucleotide analyses also recover *Fundulus*, *Xenisma*, and *Fontinus* as monophyletic and relationships within them largely similar to those presented in Wiley (1986) and Ghedotti et al. (2004). Due to the effect of the nucleotide-sequence data, the total-evidence analyses do not recover any of these subgenera as monophyletic and suggest some novel relationships (Figs. 14, 15). Most notably, *Fundulus jenkinsi* is sister to the *F. heteroclitus* species group. This relationship is surprising given the appearance of the relatively gracile *F. jenkinsi* compared to the robust species of the *F. heteroclitus* group and most of the other members of the larger clade containing *Fundulus*, *Xenisma*, and *Fontinus*. However, an allozyme study (Cashner et al., 1992) recovered *F. jenkinsi* as sister to *F. pulvereus* in a clade with the *F. heteroclitus* species group. The relationships in this part of the phylogeny require revision of the subgeneric classification. To ensure that the taxonomy reflects phylogeny, we expand subgenus *Fundulus* to include the previous members of subgenera *Fundulus*, *Fontinus*, and *Xenisma*, as well as *F. jenkinsi* (removed from *Zygonectes*). The specific relationships within this newly recognized and more inclusive subgenus *Fundulus* and the low bootstrap support for some of the larger clades within it make the placement of subgenera *Fontinus* and *Xenisma* into synonymy with subgenus *Fundulus* likely a more stable classification change and a simpler taxonomic solution than re-arranging the membership in all three subgenera.

The relationships of the western Plains species *F. kansae* and *F. zebrinus* to other fundulids has long been unclear, with some authors recognizing them in a separate fundulid genus, *Plancterus* (Parenti, 1981; Bernardi, 1997), rather than as members of a subgenus *Plancterus* within *Fundulus*. Our total-evidence results clearly recover these taxa as a clade within *Fundulus* that also includes the Pliocene–Pleistocene fossil species from western Kansas, †*F. detillae* (Figs. 14, 15). Smith (1962) suggested that †*F. detillae* is closely related to *F. zebrinus* and *F. kansae*, although he noted that they differ slightly in meristic characteristics. †*Fundulus detillae*, *F. kansae*, and *F. zebrinus*, uniquely among fundulids, share an ossified lower arm of the posttemporal (97₁). We recognize subgenus *Plancterus* with its current extant species composition of *F. kansae* and *F. zebrinus* and further include the fossil species †*F. detillae* from the Pliocene of western Kansas (Figs. 14, 15).

Although the Pacific Slope species *F. parvipinnis* and *F. linia* have long been recognized as sister taxa, their relationship to other fundulids has long remained unclear. Brown (1957) classified them in subgenus *Fundulus*. Wiley (1986) simply classified them as “other species” that had relationships that could not at that time be determined. Other authors proposed conflicting relationships (Farris, 1968; Wiley, 1986; Bernardi, 1997; Grady et al., 2001). Our non-nucleotide analyses and our total-evidence analyses recover these taxa as a clade sister to the next westernmost extant fundulids, *F. kansae* and *F. zebrinus* (Figs. 13–15). However, this clade has low bootstrap support (under 50) in the total-evidence analysis. The re-analysis of Whitehead's (2010) corrected nucleotide data with additional outgroup taxa (Fig. 12) finds the Pacific *Fundulus* to be sister to all other members of the genus (including *F. xenicus*). Although our total-evidence hypothesis indicates

that the Pacific *Fundulus* are sister to subgenus *Plancterus*, this relationship is not necessarily robustly supported.

Based on our total-evidence hypothesis and the geographic, anatomical, and genetic distinctiveness of the Pacific *Fundulus*, we describe a new subgenus below to include *F. parvipinnis*, *F. lima*, and the fossil species †*F. eulepis* from the Pliocene–Pleistocene of Death Valley, California. This subgenus includes the two extant fundulids native west of the North American continental divide. †*Fundulus eulepis* is referred to this subgenus based on the described meristic characters and the description of the expanded posterior anal fin in presumed males (Miller, 1945). Miller (1945) noted that the latter is uniquely shared with *F. parvipinnis*.

Genus *Fundulus* Lacépède 1803

Wileyichthys subgen. nov.

TYPE—*Fundulus parvipinnis* Girard, 1856.

CURRENT COMPOSITION—*Fundulus parvipinnis* (Fig. 11); *Fundulus lima* Vaillant, 1894; †*Fundulus eulepis* Miller, 1945.

DIAGNOSIS—Diagnostic but homoplastic morphological characters include: (19₀) Alisphenoid short and more laterally angled, contacts base of anterior ascending process of prootic (also in *Aphanius dispar*, poeciliines, and *Aplocheilichthys panchax*). (22₁) Lateral prong on exoccipital present, extending ventral to medial pterotics (also present but smaller in large individuals of *Fundulus chrysotus*). (79₂) Anterior arm of first epibranchial long and broad, widens to broad cartilaginous cap (also in *F. luciae*). (123₁) Middle intestine simple with right lateral bend (also in *Alfaro cultratus*). (155₂) Midlateral stripe present and prominent from posterior margin operculum to caudal-fin base, continuous or discontinuous as large blotches (also in *Profundulus*, *Crenichthys baileyi*, *Ameca splendens*, *Aphanius dispar*, *Orestias agassizi*, and *Anableps dowi*).

HABITAT AND DISTRIBUTION—West of the North American continental divide. *Fundulus parvipinnis*: fresh to hypersaline waters, relatively shallow coastal waters, and coastal streams from Moro Bay California, U.S.A., to southern Baja California Sur, México. *Fundulus lima*: freshwater springs and streams in Baja California Sur, México. †*Fundulus eulepis*: known only from Pliocene–Pleistocene deposits in Death Valley, California.

ETYMOLOGY—This subgenus honors E. O. Wiley for his many contributions to our understanding of fundulid fishes, North American biogeography, and to phylogenetic systematics.

Evolution of Salinity Tolerance

The wide range of habitats exhibited by the Fundulidae provides a special opportunity to study the evolution of physiological salinity tolerances. Whitehead's (2010) study of salinity-tolerance evolution used parsimony and likelihood optimization of three salinity-tolerance states identified as "freshwater" (with maximum salinity tolerances between 20‰ and 25‰), "brackish" (with maximum salinity tolerances between 50‰ and 75‰), and "marine" (with maximum salinity tolerances between 80‰ and 120‰) on a phylogeny derived from sequences of three genes (Fig. 16A). He concluded that there were multiple contractions of physiological plasticity in the Fundulidae as broadly salinity-tolerant

FIG. 16. (A) Maximum-likelihood phylogeny depicting the evolution of salinity tolerance using parsimony ancestral-state reconstruction as depicted in Whitehead (2010). Uses three salinity-tolerance categories: marine tolerance ($>75\%$), brackish tolerance ($50\text{--}75\%$), and freshwater tolerance ($<30\%$). (B) Maximum-likelihood phylogeny based on re-analysis of Whitehead (2010) with additional non-fundulid taxa and the removal of cytb nucleotide-sequence data for *Fundulus parvipinnis* and *F. lima*. Parsimony ancestral-state reconstruction uses Whitehead's (2010) salinity-tolerance categories and assignment of taxa to these categories corrected based on re-assessment of Griffith (1972, 1974). Clades of individuals of the same species in Figure 12 collapsed to a single branch when possible. Pie charts indicate reconstructed ancestral-species character-state optimizations.

ancestors gave rise to species or clades with more restricted tolerances (Fig. 16A). In reviewing the literature that was the source of the salinity data, we recognized that there were a few small errors of attribution of data to the species as recognized today. *Fundulus* sp. and *F. swampinus* in Griffith (1974) were *F. julisia* and *F. lineolatus*, respectively, based on distribution and past taxonomic usage. In addition, salinity-tolerance data that were attributed to *F. notti* in Griffith (1974) were from populations today recognized as *F. escambiae*. Addition and modification to reflect these changes and use of the phylogeny produced by re-analysis of the data from Whitehead (2010) (Fig. 12), with more extensive outgroups and removal of cytb sequences for *F. parvipinnis* and *F. lima*, do not substantially alter Whitehead's (2010) conclusion that there have been at least three transitions from broad to narrow salinity tolerances (Fig. 16B).

FIG. 17. Phylogenetic hypothesis of relationships among all included species based on maximum-parsimony and partitioned mixed-model maximum-likelihood analysis of all compiled available data. Ancestral-state reconstruction of two salinity-tolerance categories, marine tolerance ($>36\text{‰}$) and freshwater tolerance ($<36\text{‰}$), to which taxa were assigned by consideration of salinity tolerances reported in the literature (see Appendix II). (A) Maximum-likelihood state reconstruction. (B) Parsimony character-state reconstruction. Pie charts indicate reconstructed ancestral-species character-state optimizations.

After examining the available salinity data in the literature, we prefer to use two rather than three categories of salinity tolerance as Whitehead (2010) used. Compiled experimental salinity-tolerance data from studies where daily acclimation typically was 1.0–3.5‰ (see Appendix II) demonstrate a gap in maximum salinity tolerance around 36‰. A lower experimental salinity-tolerance group of 14 species had maximum experimental salinity tolerances between 19.1‰ (*F. escambiae*) and 35‰ (*F. rathbuni*), the calculated mean salinity tolerances ranged from 18.7‰ (*F. escambiae*) to 24.7‰ (*F. catenatus*) for the 12 species with calculated mean tolerances available. The lower salinity-tolerance group, all of which could tolerate moderately brackish salinities up to 16.0‰, largely corresponds to Whitehead's (2010) "freshwater physiology" group. A higher experimental salinity-tolerance group of 14 species had maximum experimental salinity tolerances between 60‰ (*F. chrysotus* Biloxi, MS, population) and 120.3‰ (*F. heteroclitus*), and the calculated mean salinity tolerances ranged from 55.4‰ (*F. waccamensis*) to 113.9‰ (*F. heteroclitus*) for the nine species with calculated mean tolerances available. The minimum

salinity tolerance experimentally observed in *F. waccamensis* was 37.2‰. The higher salinity-tolerance group roughly corresponds to a combination of Whitehead's (2010) "brackish physiology" and "marine physiology" groups. Two sets of data for *F. chrysotus* differ substantially in the maximum salinity tolerances ascribed to this species, with one population falling in the "high tolerance" category and the other in the "low tolerance" category. Crego and Peterson (1997) examined *F. chrysotus* collected around Biloxi, Mississippi, and noted a maximum salinity tolerance of about 60‰. This differs substantially from Griffith's (1972, 1974) determination of a maximum tolerance of 20.5‰ for a population from the Florida Panhandle (Wakulla Co.). Whitehead (2010) used Crego and Peterson's (1997) data in preference to Griffith's (1972, 1974) and recognized *F. chrysotus* as having "brackish" physiology. We follow Whitehead (2010) in treating *F. chrysotus* as having high salinity tolerance, but we also examined the effects of treating the Florida population of *F. chrysotus* as the source of the maximum tolerance for the species. Differences in methods among the experimental salinity-tolerance studies, and the

FIG. 18. Native geographic distributions of *Lucania*, *Leptolucania*, and *Fundulus* species in the subgenera *Wileyichthys* subgen. nov. and *Plancterus* and fossil localities for taxa currently recognized in these subgenera.

contradiction of field data with the compiled experimental data for *F. parvipinnis* (which would move it from “brackish” to “marine” categories) made the use of more fine-scale division of tolerance categories difficult to justify.

The experimentally identified maximum salinity tolerance separating the two groups, 36‰, also is within the range of “typical” open-ocean surface-water salinity and is approximately the salinity of offshore areas in the Gulf of Mexico and North Atlantic (Antonov et al., 2006). As a result, we also attributed species reliably reported from marine waters or other waters of higher than marine salinity to the higher salinity-tolerance group. Although salinity nearshore often is lower than offshore, the still, shallow-water habitats of most cyprinodontiforms also frequently exhibit increased salinity due to localized evaporation. The attribution of naturally entering marine or highly saline habitat as indicative of high salinity tolerance also is supported by the experimental data where all species reported as primarily brackish or as entering marine waters also experimentally have maximum salinity tolerances well above 36‰ (see Appendix II). The reverse is not always the case. *Fundulus waccamensis* is found exclusively

in low-salinity waters, and *F. chrysotus* is only very rarely reported from brackish waters. Yet *F. waccamensis* and *F. chrysotus* from a Mississippi population (but not a Florida population) exhibit salinity tolerance in which all tested individuals could survive up to 36‰. Thus, strict freshwater habitat use could not categorize species’ salinity tolerance.

Our two-state salinity-tolerance categories optimized using likelihood and parsimony methods on the total-evidence phylogeny result in an ancestral-state reconstruction of high salinity tolerance for the most recent common ancestor of the Fundulidae (Fig. 17), and is generally consistent with Griffith (1972, 1974) and Whitehead’s (2010) assertion that the trend is from broad to more narrow salinity tolerance. The common ancestor of each subgenus was indicated as most likely possessing high salinity tolerances, with potentially five independent transitions from a higher salinity tolerance to a low tolerance within these clades (Fig. 17). Three independent transitions from high to low salinity tolerances potentially occurred in the species *Leptolucania ommata*, *Lucania goodei*, and *Fundulus seminolis* (Fig. 17). A transition from high to low salinity tolerance also likely occurred in the common ancestor

FIG. 19. Native geographic distributions of species in the subgenus *Zygonectes*: *Fundulus xenicus*, *F. luciae*, *F. chrysotus*, *F. sciadicus*, *F. cingulatus*, *F. rubrifrons*, and the *F. notatus* species group.

of the *F. stellifer*, *F. bifax*, *F. catenatus*, *F. albolineatus*, *F. julisia*, *F. rathbuni*, *F. diaphanus*, and *F. waccamensis* clade within subgenus *Fundulus*, with a transition back from low to high salinity tolerance occurring in the common ancestor of *F. diaphanus* and *F. waccamensis* (Fig. 17). A fifth potential transition from high to low salinity tolerance most likely occurred in the common ancestor of the *F. cingulatus*, *F. rubrifrons*, *F. sciadicus*, *F. notatus*, *F. euryzonus*, *F. olivaceus*, *F. blairae*, *F. dispar*, *F. lineolatus*, *F. nottii*, and *F. escambiae* clade within the subgenus *Zygonectes*. Within this clade there are no transitions back to high salinity tolerance (Fig. 17).

The fossil taxon †*F. detillae* was hypothesized by Griffith (1972) to be the ancestor of a large freshwater radiation of *Fundulus*. In our study †*F. detillae* is within subgenus *Plancterus* and is from a locality within the current distribution of *F. kansae* (Fig. 18). Therefore, it is most parsimonious to assume that †*F. detillae* likely shared a high salinity tolerance with contemporary members of subgenus *Plancterus* and necessarily was not the ancestor of most freshwater *Fundulus* as hypothesized by Griffith (1972).

An evolutionary interpretation that favors transitions from high to low salinity tolerance in Fundulidae is contingent upon coding *F. chrysotus* as having high tolerance based on Crego and Peterson's (1997) study of populations from Mississippi. If

F. chrysotus is treated as having low tolerance based on Griffith's (1972, 1974) study of specimens from a Florida population, the parsimony optimization of tolerance for the ancestor of Fundulidae is equivocal for low and high salinity tolerance, and the percent likelihood optimization of the common ancestor and the deeper nodes within the Fundulidae favor a low salinity tolerance (52–80%) over high tolerance (20–48%). This would tentatively support the opposite hypothesis to that proposed in both Griffith (1972) and Whitehead (2010), with five inferred salinity tolerance transitions from low to high salinity tolerance, representing repeated expansions rather than contractions of physiological plasticity.

Our data provide further support for the hypothesis proposed by Whitehead (2010), that salinity tolerance most likely evolved via contraction of physiological plasticity multiple times (at least three times in Whitehead and potentially as many as five times in our study). However, it must be recognized that this support is tentative as a low salinity tolerance coding for *F. chrysotus* based on a different population (Griffith, 1972, 1974) alters the ancestral reconstruction to be equivocal at deeper nodes in the Fundulidae phylogeny. We can more definitively conclude that evolution of salinity tolerance in the Fundulidae has been complicated with multiple transitions and possibly variation in tolerance among populations that is in need of further investigation. More

FIG. 20. Native geographic distributions of species in the subgenus *Zygonectes*: the *Fundulus notti* species group.

extensive salinity-tolerance data from fundulids and cyprinodontiforms are needed to further study the evolution of physiological change in this highly plastic and fascinating system.

Evolution of the Urogenital Sheath

The urogenital sheath (143₁) is diagnostic for the Fundulidae and shows some variation that co-occurs with a high salinity tolerance. Mature females of the *Fundulus majalis* species group, *F. parvipinnis*, *F. kansae*, *F. zebrinus*, and the *F. heteroclitus* species group all have moderate to long urogenital sheaths (144₁ and 144₂, Fig. 10). Although many species with high salinity tolerance do not have moderate to large urogenital sheaths (e.g., *F. confluentus*, *F. luciae*, *F. xenicus*), all of the taxa with moderate to large urogenital sheaths also exhibit high salinity tolerances. This may bear some relationship to spawning substrate because the *F. majalis* species group, *F. parvipinnis*, *F. kansae*, and *F. zebrinus* frequently spawn on or in a gravel or sand substrate (Koster, 1948; Breder & Rosen, 1966; Foster, 1967b; Baugh, 1981). However, females of the *F. heteroclitus* species group most commonly spawn, as is more typical of oviparous cyprinodontiforms, on fibrous materials such as submerged vegetation (Newman, 1908; Breder & Rosen, 1966). The extremely long urogenital sheath

in *F. heteroclitus* may be associated with spawning site selection because the southern populations of this species very commonly spawn inside of mussel shells, an activity that a long urogenital sheath would help facilitate. However, *F. heteroclitus* from northern populations have not been observed to do this, even when given the opportunity to do so in aquaria (Able & Hata, 1984). Additional behavioral work would help clarify if urogenital sheath extent and use is directly associated with habitat, spawning site availability, and/or preference of spawning substrate.

Biogeography

The biogeography of the Fundulidae is intriguing given the presence of both primarily inland, usually freshwater, and coastal brackish to marine distributions. A particularly interesting biogeographic clade includes the subgenera *Plancterus* and *Wileyichthys* subgen. nov. that comprise the extant *Fundulus* from the western Great Plains and the west coast (Fig. 18). Fossils placed in both subgenera demonstrate that these clades have been distributed in this area at least since the Pleistocene. The large geographic gap in native distribution for *Fundulus* between *Plancterus* and *Wileyichthys* subgen. nov. is bridged by multiple *Fundulus* fossil taxa distributed in eastern California

FIG. 21. Native geographic distributions of species in the subgenus *Fundulus*: the *F. majalis* species group, *F. seminolis*, the *F. heteroclitus* species group, *F. jenkinsi*, *F. pulvereus*, and *F. confluentus*.

and Nevada that are currently *incertae sedis* within the family. These fossils are of particular interest in understanding the past distribution of the family, which may have had a more continuous distribution across the southwestern United States during the Pleistocene before the Holocene aridification of the North American Southwest.

There are two different biogeographic patterns observed among species groups of freshwater *Zygonectes*, broad sympatry in the *F. notatus* species group (Fig. 19) and substantial allopatry in the *F. cingulatus*–*F. rubrifrons* species group (Fig. 19) and the *F. nottii* species group (Fig. 20). The broadly sympatric species of the *F. notatus* species group exhibit some ecological partitioning (Braasch & Smith, 1965; Howell & Black, 1981; Suttkus & Cashner, 1981), although all three species may be taken syntopically. While the chromosomal difference between *F. notatus* and the other two species does not prevent the production of viable hybrids, the three species do exhibit partially effective prezygotic and postzygotic isolation mechanisms (Vigueira et al., 2008). The largely allopatric distribution of members of the *F. nottii* species group has been explained by vicariance with small-scale

subsequent dispersal corresponding to well-documented biogeographic patterns (Wiley & Hall, 1975; Wiley, 1986; Soltis et al., 2006).

In three cases within the Fundulidae a freshwater species with part or all of its distribution within peninsular Florida is sister to a clade distributed coastally from the western Gulf of Mexico to the mid-Atlantic coast: *Lucania goodei* sister to the *L. parva*–*L. interioris* clade (Fig. 18), *F. chrysotus* sister to the *F. xenicus*–*F. luciae* clade (Fig. 19), and *Fundulus seminolis* sister to the *F. majalis* species group (Fig. 21). During the Pleistocene glaciations the Florida peninsula was broadly expanded with more extensive freshwater areas and a warmer climate than the rest of eastern North America. Whereas, during the highest sea levels of the interglacials, the Ocala highlands of peninsular Florida were partially or completely separated from the North American landmass, producing more extensive coastal habitat in the area (Riggs, 1984). Optimization of salinity tolerance (Fig. 17) suggests three separate transitions of fundulids from coastal brackish environments to freshwater environments in peninsular Florida, and in two of these cases a subsequent contraction

FIG. 22. Native geographic distributions of species in the subgenus *Fundulus*: the *F. catenatus* species group, *F. julisia*, *F. albolineatus* (extinct), *F. rathbuni*, *F. waccamensis*, and *F. diaphanus*. Fossil localities for *F. diaphanus* are indicated. On the large map at bottom, the approximate greatest extent of the last Pleistocene glaciation (Wisconsinian) is indicated by a dashed line.

of salinity-tolerance plasticity. However, the opposite pattern, movement from freshwater to coastal high-salinity environments, is inferred as more likely to have occurred if when optimizing salinity tolerance, *F. chrysotus* is treated as having low tolerance based on populations from Florida. This alternative optimization suggests three establishments of high-salinity-tolerance fundulids coastally from low-salinity-tolerance populations in peninsular Florida, all three of which underwent substantial expansions of physiological plasticity. More definitive determination of the origin of this pattern will require additional study of salinity tolerance for fundulid species from multiple populations, the incorporation of additional fossils into the fundulid phylogeny to calibrate molecular clocks, and possibly molecular phylogeographic studies of these populations.

In another three cases within the Fundulidae, a coastally distributed species exhibits a pattern whereby an Atlantic coastal species or clade is sister to a species or clade distributed coastally in the Gulf of Mexico, including *Fundulus luciae* sister to *F. xenicus* (Fig. 19), *F. majalis* sister to the *F. similis*–

F. persimilis clade (Fig. 21), and *F. heteroclitus* sister to the *F. grandis*–*F. grandissimus* clade (Fig. 21). In all three cases, the point of disjunction is in northeastern Florida. This pattern is repeated both with respect to sister species and haplotype variation among a range of nearshore marine and coastal species, including near-coastal terrestrial species (Avice & Nelson, 1989; Gold & Richardson, 1998; Soltis et al., 2006). However, this coincident disjunction in so many organisms is likely due to multiple separate biogeographic events that led to this same pattern recurring and cannot, for example, be traced to a single glacial maximum or interglacial period. Within the Fundulidae, the branch lengths (Figs. 12, 14) and degree of morphological differentiation suggest that the divergence of *F. luciae* and *F. xenicus* occurred well before the divergence in the other two species pairs separated by the northeastern Florida disjunction. The fact that this area is approximately the northeastern limit of the mangrove community, an area of subtropical to temperate climate transition, and that the northeastern flow of the Gulf Stream restricts movement from the Atlantic to the Gulf of Mexico likely play some role in

either the formation and/or the maintenance of this boundary in northeastern Florida (Wise et al., 2004).

The recovery of a well-supported clade composed of *F. rathbuni*, *F. diaphanus*, and *F. waccamensis* (Fig. 22) unites all the frequently freshwater inhabiting species in subgenus *Fundulus* with distributions entirely east of the Appalachians. This clade is nested within a clade with low salinity tolerance and includes an unambiguous transition from low tolerance to high tolerance in the ancestor of *F. diaphanus* and *F. waccamensis* (Fig. 17). Interestingly, *F. rathbuni* also has the highest salinity tolerance of the low-tolerance species, with one individual tolerating up to 35‰ (Appendix II) despite living in an upland freshwater habitat. The large range of *F. diaphanus* is noteworthy, extending north into Canada and as far west as the Dakotas, well into areas covered during the most recent Pleistocene glaciation (Fig. 22). The expansion of *F. diaphanus* into the Dakotas must have begun before 10,000 years ago. Fossil *F. diaphanus* in North Dakota dated to this time colonized the area coincident with warming associated with a change from a spruce-forest community to deciduous forest (Newbrey & Ashworth, 2004). Of particular interest is the source of *F. diaphanus* populations that expanded after the glacial retreat. The Northern Leopard Frog, *Rana pipiens* Schreber, 1782, which has a similar, though more extensive, distribution, likely colonized its current range from separate eastern and western refugia (Hoffman & Blouin, 2004). Because two recognized eastern and western forms of *F. diaphanus* were previously recognized as subspecies, *F. d. diaphanus* and *F. d. menona* Jordan & Copeland, 1877 with intergrades in the region of Lake Erie, a similar pattern of separate eastern and western refugia and reinvasion may explain the present distribution of *F. diaphanus*. However, high salinity tolerance may have offered routes of colonization unavailable to freshwater-restricted species, like the amphibian *Rana pipiens*. The salinity tolerances of western populations of *F. diaphanus* (i.e., the populations previously considered to be *F. d. menona*) have not been determined. The presence of fossils of *F. diaphanus* documenting entry into previously unavailable glaciated habitat, the availability of a phylogeny for the family, and documented variation in salinity tolerance make this clade a particularly attractive subject for future phylogeographic study of the interplay between the evolution of physiological plasticity and postglacial colonization.

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Appendix I: Morphological Material Examined

The number of alcohol-preserved specimens examined is indicated after the catalog number, followed by number of cleared and stained specimens examined in parentheses.

Family Fundulidae. Genus Fundulus (Subgenus Fundulus). *Fundulus albolineatus*: UMMZ 157692, cotype, Alabama, Madison Co., Spring Cr. *F. 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. *F. 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, Tennessee, Lewis Co., Buffalo R.; JFBM 37920, 4(2), Arkansas, Pike Co., Caddo R.; KU 11550, 4(5), Tennessee, Jackson Co., Roaring R.; KU 17616, (7), Missouri, Jefferson Co., Big R.; KU 6730, 11, Missouri, Shannon, Mahan Cr.; JFBM 38436, 10, Kentucky, Boyle Co., Salt R.; JFBM 38200, 5, Tennessee, Clay Co., Breen R., Hurricane Cr.; JFBM 39057, 30, Arkansas, Montgomery Co., Quachita R.; JFBM 39352, 30, Arkansas, Carroll Co., White R., Kings R.; JFBM 38181, 6, Kentucky, Allen Co., Breen R.; JFBM 38401, 8, Kentucky, Lincoln Co., Kentucky R., White Oak Cr. *F. confluentus*: KU 17415, 3(2), Florida, Levy Co., marsh 1.7 mi. north of Cedar Key; UMMZ 183957, 18, Florida, Palm Beach Co., culvert on Indiantown Rd. 11 mi. west of Jupiter; UMMZ 139228, 17(5), Florida, Hillsborough ditch beside Palm R.; UMMZ 111786, 16(5), Florida, Manatee Co., Mill Cr. *F. diaphanus*: JFBM 12707, 20, Polk Co., Minnewaska Lake; JFBM 24362, 10, MN, Otter Tail Co., Red R. of the North; JFBM 30380, 10, Massachusetts, Hampshire Co., Connecticut R.; KU 18192, 5(3), Pennsylvania, Franklin Co., Conococheague Cr. *F. grandis*: KU 17051, 23(3), Texas, Nueces Co., Stedman Island on causeway off Arkansas Pass; KU 17416, 12, Florida, Levy Co., 1.7 mi. north of Cedar Key; KU 17062, 10, Texas, Nueces Co., canal along Mustang Island; UMMZ 153584, 21(5), Florida, Pinellas Co., Boca Ciega Bay; UMMZ 209509, 7, Mexico, Veracruz, unnamed arroyo trib. to south end of Laguna de Tamiahua. *F. grandissimus*: UMMZ 196532, 5, Mexico, Yucatan, marshy lagoon on east side of Rio Lagartos-Valladolid Hwy.; UMMZ 143095, 10(4), Mexico Yucatan, El Rio o La Cienega, 200 ft. *F. heteroclitus*: FMNH 60131, 64, New York, Westchester Co., Playland Lake; KU 15351, 5(5), Massachusetts, Norfolk Co., Quincy; KU 5363, 16, North Carolina, Carteret Co., North River Estuary; JFBM 18999, 9, Massachusetts, Dukes Co., Atlantic Ocean at Martha's Vineyard on Chappaquiddick Island; UMMZ 138841, 25(4), Connecticut, New London Co., Mystic R. *F. jenkinsi*: KU 17310, 2(3), Mississippi, Harrison Co., Biloxi Bay. *F. julisia*: KU 20999, 2(1), Tennessee, Cannon Co., trib. to McMahan Cr.; UMMZ 120861, 3 paratypes, Tennessee, Coffee Co., Spring Branch; UMMZ 120914, 3 paratypes, Tennessee, Coffee Co., Little Duck R.; UMMZ 121013 and 21014, (1) and 7 paratypes, Tennessee, Coffee Co., Hunt Cr.; UMMZ 207690, 2, Tennessee, Coffee Co., W Fork Hickory Cr. *F. majalis*: FMNH 109220, 13, North Carolina, Beaufort Co.; KU 18180, (3), Maryland, Crisfield Co., Sand Dunes; KU 17061, 4(2), Texas, Nueces Co., canal along Mustang Island; KU 12827, (5), Florida, Pinellas Co., W end Howard Franklin Causeway; KU 15387, 10, North Carolina, Carteret Co., Pilot Island; JFBM 19000, 14, Massachusetts, Dukes Co., Atlantic Ocean at Martha's Vineyard on Chappaquiddick Island; JFBM 17000, 17, Louisiana, Gulf of Mexico, Grande Isle. *F. persimilis*: UMMZ 177529, 10(5), Mexico, Yucatan, Rio Lagartos across

from San Felipe. *F. pulvereus*: KU 19453, 32(4), Louisiana, Orleans Parish, W Coast Lake Pontchartrain. *F. 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, 25(5), North Carolina, Sugartree Cr. *F. seminolis*: KU 18195, 3(11), Florida, Putnam Co., St. John's R. *F. stellifer*: JFBM 19834, 2, Tennessee, Bradley Co., Conasauga R.; JFBM 35229, 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.; UMMZ 177784, (5), Georgia, Cobb Co., Allatoona Cr.; UMMZ 35229, 4, Alabama, Coosa Co., Hachemendega Cr. *F. waccamensis*: UMMZ 218715, 14, North Carolina, Columbus Co., Lake Waccamaw; UMMZ 138474, 25(5), North Carolina, Columbus Co., Lake Waccamaw. **Genus Fundulus (Subgenus Plancterus).** †*F. detillae*: KUVF 848, 852, 856, 861, 865, 872, 878, 880, 881, 1137, 1143, Kansas, Logan Co., Ogallala formation. *F. kansae*: KU 5362, 16, Kansas, Barton Co., Arkansas R.; KU 14726, (5), Kansas, Edwards Co., Arkansas R.; UMMZ 145022, 25, Nebraska, Dundee Co., Arikaree R.; KU 22588, 16, Kansas, Barton Co., Arkansas R. *F. zebrinus*: UMMZ 196785, 25(10), Texas, Brewster Co., Terlingua Cr. **Genus Fundulus (Subgenus Wileyichthys subgen. nov.).** *F. lima*: KU 18197, (2), Baja California Sur, Darr Ignacio; UMMZ 197525, 21(4), Mexico, Baja California Sur, Arroyo San Ignacio; UMMZ 197527, 25(5), Mexico, Baja California, stream 5 mi. east of La Purisima. *F. parvipinnis*: FMNH 61255, 26, California, San Diego, Slough south of Imperial Beach; KU 19046, 10(5), California, San Diego Co., San Diego Bay; KU 19744, (9), California, Santa Barbara Co., Goleta Slough; UMMZ 168966, 25(5), Mexico, Baja California Sur, Bahia Magdalena. **Genus Fundulus (Subgenus Zygonectes).** *F. blairae*: KU 19675, 2(1), Louisiana, Vernon Parish, Anacoco Cr. *F. chrysotus*: KU 18165, (6), Florida, Lake Co., Hogeys Sink; UMMZ 158585, 51, Florida, Brevard Co., ditch pond; UMMZ 155297, 56(5), Louisiana, Orleans Parish, road side pools on south side of US 11 & 90; KU 16924, 5, Louisiana, Orleans Co., marsh 1 mi. E jct. US Rt. I-70; KU 17990, 6, Florida, Dixie Co., trib. to Stein Hatches R. *F. cingulatus*: UMMZ 163524, 25(5), Florida, Santa Rosa Co., borrow pit pond and small tribs. to Sweetwater Cr. *F. dispar*: KU 16518, 7(1), Arkansas, Calhoun Co., west Locust Bayou. *F. escambiae*: KU 17890, 7(5), Florida, Santa Rosa Co., Coldwater Cr. *F. euryzonus*: JFBM 22774, 8(2), Mississippi, Amite Co., W Amite R. *F. lineolatus*: KU 23987, (4), no data; KU 16925, 5, Georgia, Brantley Co., Satilla R.; KU 18193, 14, Florida, Marion Co., Mill Dam L.; KU 33898, 1, Georgia, Charleton Co., Mims Cr. *F. luciae*: KU 18175, 10(6), North Carolina, Dare Co., Hatteras; UMMZ 157690, 32(5), Delaware, Sussex Co., White Oak Cr. *F. notatus*: FMNH 51470, 32, Indiana, Kankakee R.; JFBM 38463, 4, Tennessee, Sevier Co., Middle Cr.; KU 18021, (4), Texas, Dewitt Co., creek 1.6 mi. south jct. US Rt. 183 & Farm Rd. 237; KU 26305, 20, Kansas, Neosho Co., Spring Cr. *F. nottii*: JFBM 16998, 5, Louisiana, St. Tammany Parish, Talisheek Cr.; KU 15471, 2(1), Texas, Tyler Co., Steinhagen L.; KU 17371, 8(4), Alabama, Washington Co., Bassetts Cr. *F. olivaceus*: FMNH 60553, 24, Missouri, Butler Co., Keener Spring; KU 17617, (4), Missouri, Jefferson Co., Big R.; KU 17619, (4), Missouri, Jefferson Co., Big River; KU 20765, 19, Tennessee, Benton Co., creek at east edge of Camden city limits; JFBM 17219, 11, Mississippi, Yalobusha Co., small creek near Scobby; JFBM 37991, 7, Arkansas, Lawrence Co., White R., Chaplin Cr. *F. rubrifrons*: KU 18170, 7(5), Georgia, Clinch Co., roadside ditch about 12 mi. north of I-10 on US 441; UMMZ 181442, 25, Florida, Volusia Co., roadside ditch west of Samoula on old FL 40. *F. sciadicus*: KU 11047, (5), Missouri, Texas and Pulaski Cos., Big Piney R.; UMMZ 127456, 25(5), South Dakota, Todd Co., Sand Cr.; KU 10579, 12, Nebraska, Niobrara Co., Niobrara R.; JFBM 22712, 26, Nebraska, Keya Paha Co., Holt Cr.; JFBM 38117, 30, Minnesota, Nobles Co., Champepadan Cr. *F. xenicus*: KU 17048, 10(4), Texas, Nueces Co., Stedman Island on causeway off Arkansas Pass; UMMZ 158860, 35(5), Florida, Levy Co., Cedar Key. **Genus Lucania.** *Lu. goodei*: KU 17993, 10(11), Florida, Dixie Co., trib. to Steinhatchee R., 0.5 mi. SE main channel; JFBM 16944, 28, Florida, Wakulla Co., Gulf of Mexico, Wakulla R.; UMMZ 178963, 16, Florida, Dade Co., canal beside US 27, 7 mi. south of jct. with US 41. *Lu. interioris*: KU 7433, 10(5), Mexico, Coahuila, La Angostura Canal, near Garbota R.; JFBM 19403, 37, Mexico, Coahuila, Cuatro Ciénegas; UMMZ 179853, 25, Mexico, Coahuila, La Angostura Canal. *Lu. parva*: FMNH 113089, 18, Louisiana, Price Lake at Rockefeller State Wildlife Refuge; KU 17042, (5), Texas, Refugio Co., Copano Bay; UMMZ 145104, 25(5), Louisiana, Plaquemine Parish, 8 mi. W of Pilot Town, near end of Rapheal Pass; KU 17340, 20, Mississippi, Hinds Co., Jackson Marsh. **Genus Leptolucania.** *Le. ommata*: KU 16835, 1(2), Florida, Taylor Co., Econfinia R.; UMMZ 135866, (5), Georgia, Billy Lake, Okefenokee; JFBM

19718, 10, Florida, Alachua Co., 12 mi. northeast of Gainesville. **Family Anablepidae.** *Anableps dowi*: KU 18689, 14(3), Nicaragua, Chinandega, Rio Estero Real. *Jenynsia multidentata*: KU 21307, (3), Argentina, Buenos Aires, Buenos Aires Zoo; KU 19190, 27(4), Argentina, Mendoza, Dique Benegas; KU 23873, 16(3), Argentina, Buenos Aires, Punta Lake; KU 22416, 21(4), Uruguay, Montevideo, Santiago Vasquez. *Oxyzygonectes dovii*: KU 18174, 2(5), Panama, Veraguas, Rio San Pablo. **Family Aplocheilidae.** *Aplocheilus panchax*: KU 28518, 4(3), Nepal, Saptari, Koshi R.; KU 29193, 4(2), Nepal, Morang, Bilbari. **Family Cyprinodontidae.** *Aphanius dispar*: UMMZ 209943, 7(3), Oman, Wadi Sumail, 100 km west southwest of Muscat. *Cubanichthys pengelleyi*: UMMZ 213381, 5, Jamaica, Springfed roadside pool in St. Elizabeth Park; UMMZ 166660, (1), Jamaica, spring on road from Black R. to Parrotte, 6 mi. southeast of Black R. *Cyprinodon variegatus*: KU 17117, 25(4), Georgia, Liberty Co., St. Catherines Island at Yankee Bridge Cr.; KU 17040, (3), Texas, Refugio Co., Copano Bay; KU 19634, (4), Florida, Lake Co., Lake Eustis. *Floridichthys carpio*: UMMZ 189707, 18(2), Florida, Dade Co., Biscayne Bay. *Orestias agassizi*: KU 19186, 5(3), Bolivia, La Paz, Lake Titicaca. **Family Goodeidae.** *Ameca splendens*: UMMZ 233594, 30, Mexico, Jalisco, Rio Teuchitlan;

UMMZ 172229, (18), Mexico, Jalisco, Rio Teuchitlan. *Crenichthys baileyi*: KU 14031, 15(4), Nevada, White Pine Co., White Pine irrigation ditch in Preston; KU 11862, (3), Nevada, White Pine Co., Preston Springs. **Family Poeciliidae.** *Alfaro cultratus*: KU 11122, 13(3), Costa Rica, Heredia, Rio Santa Clara and trib. east of Guapiles. *Aplocheilichthys spilauchen*: CAS 163440, 3(2), West Africa, Ghana, Eastern Region, Volta R. mouth; CAS 163435, 5, Africa, Ghana, Avedzake Cr. *Gambusia affinis*: KU 22033, 26(4), Nebraska, Colfax Co., Schulyer City Park, Lost Cr.; KU 22589, (4), Kansas, Barton Co., Arkansas R. *Poecilia reticulata*: UMMZ 172618, 26(4), Puerto Rico, Rio Grande de Marati. **Family Profundulidae.** *Profundulus guatemalensis*: UMMZ 190542, 24(4), Guatemala, Escuinta, Rio Marina; UMMZ 197100, 10, Guatemala, Baja Verapaz, Rio Chicruz. *Profundulus labialis*: KU 18163, 20(7), Guatemala, Rio Carchda; UMMZ 197100, 10, Guatemala, Baja Verapaz, Rio Chicruz. *Profundulus punctatus*: KU 18177, (4), Guatemala, Rio Camaya; UMMZ 184831, 30(5), Mexico, Guerrero, trib. of Rio Papagayo. **Family Rivulidae.** *Kryptolebias marmoratus*: UMMZ 213942, 3(2), Florida, No Name Key. **Family Valenciidae.** *Valencia hispanica*: AMNH 38401, 1(2), Spain, A. Casinos. *Valencia letourneuxi*: UMMZ 213901, 8, Greece, Epirus, Louros R.; UMMZ 213902, 10(3), Greece, Epirus, Acheron Basin.

Appendix II: Compiled Salinity Tolerances of Taxa in This Study

Experimental Salinity-Tolerance Data

Included experimental limits of salinity tolerance were determined by death of fish, loss of balance, or loss of responsiveness to touch after gradually increasing salinity (typically by 1.0–3.5‰ per day). Min. = minimum tolerance observed in an experimental trial. Max. = maximum tolerance observed in an experimental trial. Taxa ordered by decreasing maximum salinity tolerance. The midline indicates division between identified categories of relatively higher and lower salinity tolerance.

Species	Mean tolerance, ‰ (mean)	Min. (‰)	Max. (‰)	Citation
Fundulidae				
<i>Fundulus heteroclitus</i>	113.9 (6)	106.0	120.3	Griffith, 1972, 1974
<i>Fundulus grandis</i>			110.0	Nordlie, 2000
<i>Fundulus pulvereus</i>	100.8 (4)	95.6	106.0	Griffith, 1972, 1974
<i>Fundulus majalis</i>	98.8 (6)	73.4	106.0	Griffith, 1972, 1974
<i>Fundulus luciae</i>	101.3 (7)	73.4	106.0	Griffith, 1972, 1974
<i>Fundulus confluentus</i>	99.0 (5)		99.0	Griffith, 1972, 1974
			100	Nordlie, 2000
<i>Fundulus similis</i>			99	Stanley and Fleming, 1977
			100	Nordlie, 2000
<i>Fundulus kansae</i>	88.9 (5)	87.2	95.6	Griffith, 1972, 1974
<i>Fundulus xenicus</i>			95.0	Nordlie, 1987
<i>Lucania parva</i>			80+	Dunson and Travis, 1991
			~90.0	Whitehead, 2010
<i>Fundulus jenkinsi</i>	73.6 (3)	67.2	80.2	Griffith, 1972, 1974
<i>Fundulus diaphanus</i>	69.6 (6)	56.5	73.4	Griffith, 1972, 1974
<i>Fundulus parvipinnis</i>			70	Valentine and Miller, 1969
				See field data below as well
<i>Fundulus waccamensis</i>	55.4 (4)	37.2	67.2	Griffith, 1972, 1974
<i>Fundulus chrysotus</i> , MS		40	60.0	Crego and Peterson, 1997
<i>Fundulus rathbuni</i>	24.4 (15)	18.8	35.0	Griffith, 1972, 1974
<i>Fundulus seminolis</i>	23.3 (4)	19.4	33.4	Griffith, 1972, 1974
			32.0+	Dimaggio et al., 2010
<i>Lucania goodei</i>			~30	Dunson and Travis, 1991
<i>Leptolucania ommata</i>			<30	Dunson and Travis, 1991
<i>Fundulus lineolatus</i>	27.3 (5)	26.3	27.8	Griffith, 1972, 1974 (as <i>F. swampinus</i> (Lacepède, 1803))
<i>Fundulus catenatus</i>	24.7 (10)	24.0	26.1	Griffith, 1972, 1974
<i>Fundulus olivaceus</i>	23.7 (7)	21.6	24.8	Griffith, 1972, 1974
<i>Fundulus sciadicus</i>	24.3 (6)	24.3	24.3	Griffith, 1972, 1974
<i>Fundulus stellifer</i>	20.5 (9)	18.5	23.6	Griffith, 1972, 1974
<i>Fundulus julisia</i>	23.3 (2)	23.1	23.5	Griffith, 1972 (as <i>F. albolineatus</i>), 1974 (as <i>Fundulus</i> sp.)
<i>Fundulus cingulatus</i>	22.7 (9)	20.6	23.2	Griffith, 1972, 1974
<i>Fundulus notatus</i>	19.9 (14)	16.1	23.2	Griffith, 1972, 1974
<i>Fundulus chrysotus</i> , FL	20.1 (8)	19.5	20.5	Griffith, 1972, 1974
<i>Fundulus escambiae</i>	18.7 (7)	18.5	19.1	Griffith, 1972, 1974 (as <i>F. nottii</i>)
<i>Fundulus nottii</i>			15.0	Crego and Peterson, 1997
Non-fundulids				
<i>Aphanius dispar</i>			145.0	Lotan, 1971
<i>Cyprinodon variegatus</i>			125.0	Nordlie 1985
<i>Kryptolebias marmoratus</i>			114	King et al., 1989
<i>Floridichthys carpio</i>			90.0	Nordlie and Walsh, 1989
<i>Poecilia reticulata</i>		39.0	58.5+	Chervinski, 1984
<i>Gambusia affinis</i>		39.0	58.8+	Chervinski, 1983
<i>Valencia letourneuxi</i>			46	Bianco and Nordlie, 2008

Appendix II: Continued.

Field Salinity-Tolerance Data

These data are provided when available only for taxa without experimental data or where experimental data were in question. Max. = maximum tolerance observed in an experimental trial.

Experimental data or field indication of the ability to tolerate marine salinity are not available for *Profundulus*, *Crenichthys baileyi*, *Ameca splendens*, *Cubanichthys pengelleyi*, *Orestias agassizi*, *Alfaro cultratus*, *Fundulus bifax*, *F. lima*, *F. rubrifrons*, *F. euryzonus*, *F. blairae*, and *F. dispar*. All of these taxa primarily inhabit freshwater habitats and have not undergone laboratory study of salinity tolerance; thus, maximum tolerance for these taxa is treated as unknown. Fundulid taxa for which these data are lacking; all are closely related to species for which these data are available.

Species	Max. collected salinity (‰)	Citation and notes
Fundulidae		
<i>Fundulus zebrinus</i>	140–150	Echelle et al., 1972—Collected where many individuals had already died; thus, this likely is close to or at an upper field salinity tolerance
<i>Fundulus parvipinnis</i>	128	Feldmeth and Waggoner, 1972—Griffith (1974) questioned the experimental data of Valentine and Miller (1969) above based on this reported field collection
<i>Fundulus persimillis</i>	51.7	Miller, 2005
<i>Fundulus grandissimus</i>	saltwater	Miller, 2005
<i>Lucania interioris</i>	saline and very saline waters	Miller, 2005
<i>Fundulus chrysotus</i>	24.7	Kilby, 1955
Non-fundulids		
<i>Oxyzygonectes dovii</i>	estuarine, occasionally marine	Bussing, 1987
<i>Anableps dowi</i>	enters full seawater	Miller, 2005
<i>Jenynsia multidentata</i>	can live in seawater	Ringuelet et al., 1967
<i>Aplocheilichthys spilauchen</i>	enters saltwater	Wildekamp, 1995
<i>Aplocheilus panchax</i>	thrive in fresh- and saltwater	Lim & Ng, 1990

Appendix III: GenBank and Non-Nucleotide Data for Total-Evidence Analysis

Accession numbers for each individual used in the combined analysis are provided. See Whitehead (2010) for GenBank information for the additional individuals in the genetic data re-analysis (Fig. 14). Gene abbreviations: cytb = cytochrome *b*; CO1 = cytochrome *c* oxidase 1; RAG1 = recombination activating gene 1; gylt = glycosyltransferase. An X in the N category corresponds to taxa for which non-nucleotide data were available and included. Individual/Isolate tag refers to the specific individual from Whitehead (2010) used in the total-evidence analysis.

Taxon	Individual/Isolate	RAG1	gylt	cytb	CO1	N
<i>Fundulus heteroclitus</i>	MD AWMDCIII02	GQ119889	GQ119807	GQ119718	EU524630	X
<i>Fundulus grandis</i>	LA AW4	GQ119886	GQ119804	GQ119715	HQ557162	X
<i>Fundulus grandissimus</i>		NA	NA	NA	NA	X
<i>Fundulus confluentus</i>	FL F162711	GQ119873	GQ119789	GQ119700	NA	X
<i>Fundulus pulvereus</i>	LA K1737	GQ119916	GQ119836	GQ119748	NA	X
<i>Fundulus rathbuni</i>	NC M224 01	GQ119918	GQ119838	GQ119750	NA	X
<i>Fundulus julisia</i>	TN NC30733	GQ119891	GQ119811	GQ119722	NA	X
<i>Fundulus stellifer</i>	AL M362 01	GQ119928	GQ119850	GQ119763	JN026687	X
<i>Fundulus bifax</i>		NA	NA	NA	NA	X
<i>Fundulus catenatus</i>	MO DD	GQ119865	GQ119780	GQ119691	JN026621	X
<i>Fundulus diaphanus</i>	MD AWMDCIII 01	GQ119875	GQ119791	GQ119702	EU524058	X
<i>Fundulus waccamensis</i>		NA	NA	NA	JN026688	X
<i>Fundulus seminolis</i>	FL JF1	GQ119926	GQ119848	GQ119761	NA	X
<i>Fundulus persimilis</i>		NA	NA	NA	NA	X
<i>Fundulus similis</i>	FL K2458	GQ119923	GQ119845	GQ119757	HQ557158	X
<i>Fundulus majalis</i>	VA AWCt2 8	GQ119903	GQ119823	GQ119735	NA	X
<i>Fundulus lima</i>	CA K2452	GQ119898	GQ119818	NA	HQ579118	X
<i>Fundulus parvipinnis</i>	CA K2462	GQ119915	GQ119835	NA	GU440324	X
<i>Fundulus kansae</i>	KS K2746	GQ119893	GQ119813	GQ119724	JN026653	X
<i>Fundulus zebrinus</i>	NM BK Pec12	GQ119930	GQ119852	GQ119765	JN026695	X
<i>Fundulus jenkinsi</i>	MS MP1	NA	GQ119809	GQ119720	HQ557160	X
<i>Fundulus xenicus</i>	TX K1638	GQ119858	GQ119771	GQ119680	JN024716	X
<i>Fundulus chrysotus</i>	FL K538	GQ119867	GQ119782	GQ119693	HQ579039	X
<i>Fundulus luciae</i>	MA K3288	GQ119900	GQ119820	GQ119732	NA	X
<i>Fundulus rubrifrons</i>	FL AT	GQ119920	GQ119842	GQ119754	HQ937017	X
<i>Fundulus cingulatus</i>	FL AT	NA	GQ119788	GQ119699	HQ557444	X
<i>Fundulus sciadicus</i>	MO K526	GQ119922	GQ119844	GQ119756	JN026679	X
<i>Fundulus notatus</i>	AL M4440 01	GQ119906	GQ119826	GQ119738	EU524064	X
<i>Fundulus olivaceus</i>	LA K522	GQ119911	GQ119831	GQ119743	JN026673	X
<i>Fundulus blairae</i>	LA K527	GQ119862	GQ119776	GQ119687	JN026619	X
<i>Fundulus dispar</i>	LA K7500	GQ119881	GQ119797	GQ119708	HQ557217	X
<i>Fundulus lineolatus</i>	GA F160834	GQ119896	GQ119816	GQ119728	JN026657	X
<i>Fundulus escambiae</i>	FL K6587	GQ119883	GQ119799	GQ119710	HQ557447	X
<i>Fundulus nottii</i>	LA K524	GQ119905	GQ119825	GQ119737	NA	X
† <i>Fundulus detillae</i>		NA	NA	NA	NA	X
<i>Fundulus albolineatus</i> Ex.		NA	NA	NA	NA	X
<i>Lucania parva</i>		GQ119934	GQ119856	GQ119769	HQ579046	X
<i>Lucania interioris</i>		NA	NA	NA	NA	X
<i>Lucania goodei</i>		GQ119933	GQ119855	GQ119768	HQ557449	X
<i>Leptolucania ommata</i>		NA	NA	NA	HQ557457	X
<i>Profundulus labialis</i>		NA	NA	AY155567	HQ682638	X
<i>Profundulus punctatus</i>		NA	NA	AY155566	HQ682639	X
<i>Profundulus guatemalensis</i>		GQ119857	GQ119770	AY155568	JN028283	X
<i>Crenichthys baileyi</i>		FJ185089	NA	AF510819	AY356571	X
<i>Ameca splendens</i>		NA	NA	NA	AY356564	X
<i>Cubanichthys pengelleyi</i>		NA	NA	NA	AY356593	X
<i>Cyprinodon variegatus</i>		NA	NA	AY902067	JN025276	X
<i>Floridichthys carpio</i>		NA	NA	NA	JN026617	X
<i>Aphanius dispar</i>		NA	NA	NA	NA	X
<i>Orestias agassizii</i>		NA	NA	NA	NA	X
<i>Oxyzygonectes dovii</i>		EF017407	NA	EF017510	AY356581	X
<i>Anableps anableps</i>		EF017405	NA	EF017508	NA	
<i>Anableps dowi</i>		NA	NA	NA	NA	X
<i>Jenynsia multidentata</i>		EF017406	NA	EF017509	NA	X
<i>Aplocheilichthys spilauchen</i>		NA	NA	NA	NA	X
<i>Alfaro cultratus</i>		EF017429	NA	EF017531	NA	X
<i>Poecilia reticulata</i>		EF017434	NA	EF017536	NA	X
<i>Gambusia affinis</i>		EF017411	NA	NC004388	NC004388	X
<i>Valencia letourneuxi</i>		NA	NA	NA	NA	X
<i>Kryptolebias marmoratus</i>		EF455707	NA	NC003290	NC003290	X
<i>Aplocheilus panchax</i>		JQ073285	NA	NC011176	NC011176	X

Appendix IV: Anatomical, Color Pattern, Karyological, and Behavioral Data Matrix

Data matrix of character states of 181 non-nucleotide transformation series for 61 species, including 154 anatomical (1–154), 19 color pattern (155–173), 1 karyological (174), and 7 behavioral (175–181) transformation series. All transformation series are treated as unordered. Unknown = ?. Polymorphic symbols: A = (0&1); B = (0&2); C = (1&2); D = (0&3); E = (3&4); F = (0&1&2).

	1	111111112	222222223	333333334	444444445	555555556	666666667	777777778	888888889
	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890
<i>Fundulus heteroclitus</i>	1120000110	2001121122	1000121410	1030000212	0011000000	3011012010	0220101110	1101000010	1111102011
<i>Fundulus grandis</i>	1120000110	2001021122	1000121410	1030000212	0010000000	3011012010	1220101110	1101000010	1111102011
<i>Fundulus grandissinus</i>	1120000110	2001021122	1000121410	1030000212	0010000000	3011012010	1220101110	1001000010	1111102011
<i>Fundulus confluentus</i>	1120000010	2001111122	1000121410	1030000212	0011000000	3011012010	1220101110	0111000110	1111102011
<i>Fundulus pulvereus</i>	1120000010	2001121122	1000121410	1030000212	0011000000	3011012010	1220101110	0111000110	1111102011
<i>Fundulus rathbuni</i>	0121000010	2001121122	1000121410	1031000212	0011000000	1111010010	1220100121	0101000010	1111102011
<i>Fundulus albolineatus</i>	?????????	??????11??	???0121?10	10???00212	001?300??0	11110?01?	??????0???	??????0???	??????011
<i>Fundulus julisia</i>	0121000010	2001121121	1000121410	1031000212	0010000000	1111011010	1220100121	1010100010	1110102011
<i>Fundulus stelleri</i>	1121000110	2001121121	1000121410	1031000212	001A000000	1111011010	1220100120	A000101210	1110002011
<i>Fundulus bifax</i>	1121000110	2001121121	1000121410	1031000212	001A000000	1111011010	1220100110	A000101110	1110002011
<i>Fundulus catenatus</i>	1121000110	2001121121	1000121410	1031000212	001A000000	1111011010	1220100110	A00010A110	1110002011
<i>Fundulus diaphanous</i>	0121000010	2011111122	1000121410	1031000212	0010000000	1111011010	1220100121	1111001110	1111102011
<i>Fundulus waccamensis</i>	0121000010	2011111122	1000121410	1031000212	0010000000	1111011010	1220100110	0111001110	1111102011
<i>Fundulus seminolis</i>	1121000010	2011111122	2000121410	1030000212	0011000000	1111011010	1220100110	1101011210	1111002011
<i>Fundulus similis</i>	1121000120	2111111122	2000121410	1031000211	0111001000	1111011010	1221100110	1101012210	1111002011
<i>Fundulus majalis</i>	1121000120	2111111122	2000121410	1031000211	0111001000	1111011010	1221100110	1101012210	1111002011
<i>Fundulus lima</i>	1121000120	2111111122	2000121410	1031000211	0111001000	1111011010	1221100110	1101012210	1111002011
<i>Fundulus parvipinnis</i>	0120000020	2001021102	2100121410	1031000212	0011000000	1011010010	1120101120	1101001020	1111002011
<i>Fundulus kansae</i>	0120000020	2001001120	2000121410	1030000211	0010000000	1011010010	1120101121	0110000010	1111102011
<i>Fundulus zebrinus</i>	0120000020	2001001120	2000121410	1030000211	0010000000	1011010010	1120101121	0110000010	1111102011
† <i>Fundulus detillae</i>	?????????	???100?1??	???0121?10	103?00021?	0010000??0	10110?????	1?20??1????	??????0???	?11??02?11
<i>Fundulus jenkinsi</i>	0120000000	2001021122	1000121110	1030000110	1010000000	1011001010	0220100110	0111000010	1111102011
<i>Fundulus xenticus</i>	0120000000	0?01001111	1000121010	1030000212	1011010010	1011011010	0210101121	0111000110	1111102001
<i>Fundulus chrysotus</i>	0120000010	2001021122	1100121410	1030000212	0010000000	1011001010	1220101120	1110000110	11101020A1
<i>Fundulus luciae</i>	0120000000	2001021122	1000121110	1030000210	1010000000	2011001010	0220100120	1111000120	1111102011
<i>Fundulus rubrifons</i>	0120000000	2001021121	1000121410	1030000212	0010000000	1011001010	1220100110	0011100110	1110102011
<i>Fundulus cingulatus</i>	0120000000	2001021121	1000121410	103?000212	0010000000	1011001010	1220100110	0011100110	1110102011
<i>Fundulus sciadicus</i>	0120000000	2001021121	1000021400	1130000112	1010000010	1011001010	0120101120	0011000011	1110102011
<i>Fundulus notatus</i>	0120000000	2001021122	1000021400	1130000112	0010000010	1011011010	0120101120	A001000011	1110102011
<i>Fundulus olivaceus</i>	0120000000	2001021122	1000021400	1130000112	0010000010	1011011010	0120101120	A001000011	1110102011
<i>Fundulus euryzonus</i>	0120000000	2001021122	1000021400	1130000112	0010000010	1011011010	0120101120	A001000011	1110102011
<i>Fundulus blairae</i>	0120010000	2001021112	1000021000	1130000112	1011000010	1011011010	0120101110	A011000111	1110102011
<i>Fundulus dispar</i>	0120010000	2001021122	1000021000	1130000112	1011000010	1011011010	0120101110	A011000111	1110102011
<i>Fundulus lineolatus</i>	0120010000	2001020122	0000021000	1130000112	1011000010	1011011010	0120101120	A011000111	1110102011
<i>Fundulus escambiae</i>	0120010000	2001020122	0000021000	1130000112	1011000010	1011011010	0120101120	A011000111	1110102011
<i>Fundulus notti</i>	0120010000	2001020112	0000021000	1130000112	1011000010	1011011010	0120101120	A011000111	1110102011
<i>Lucania parva</i>	0120001001	A011021210	1000121010	1030010202	0010000000	1011001010	1220101110	A111101110	1111102101
<i>Lucania interioris</i>	0120001001	A011021210	1000121010	1030010202	0010000000	1011001010	1220101110	A111101110	1111102101
<i>Lucania goodie</i>	0120001001	1011021210	1000121010	1030000202	0011000000	1011001010	1220101110	1111101110	1111102101
<i>Leptolucania omnata</i>	0120000001	0?01021210	1000121010	1030000202	0011000020	2011001011	1220102120	0011100110	1110102101
<i>Profundulus labialis</i>	1110000000	2000201122	1001011200	0030000212	0010010111	1001000210	2110100110	0001100000	1000101010
<i>Profundulus punctatus</i>	1110A00000	2100201122	1011011200	0030000212	0010010111	1001000210	2110100110	0001100010	1000101010
<i>Profund. guatemalensis</i>	1110000000	2000201122	1011011200	0030000212	0010010111	1001000210	0110100110	0001000000	1000101010
<i>Crenichthys baileyi</i>	1110001100	0?A1001120	1001021310	0030002212	1010110110	1011101210	0110101100	0001102200	000010100A
<i>Ameca splendens</i>	1110001110	1011001120	1001021500	0130002212	1111110111	1011101210	1100101100	0001211000	0000001011

Appendix IV: Continued.

	1	1111111112	2222222223	3333333334	4444444445	5555555556	6666666667	7777777778	8888888889
	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890
<i>Cubanich. pengelleyi</i>	2100201000	0?11021110	0001021211	0110100212	0011010010	1011001210	1200100110	0001100100	1010103101
<i>Cyprinodon variegates</i>	2100201000	0011120210	0001021211	0110011212	1011010111	1011001210	1210100110	0000300100	1111114101
<i>Floridichthys carpio</i>	2100210010	0?11100210	0000121211	0110011212	1011010101	1011001210	1210100100	0000101110	1111114101
<i>Aplanius dispar</i>	1100200000	0?01021101	0001021511	0110011202	1011010110	1011001010	1000101120	0000000110	0010101001
<i>Orestias agaszii</i>	1120200001	0?11??1110	1000121511	0110000202	10?0?01021	1011011211	1100101110	0011301100	0011103111
<i>Oxyzygonectes dovii</i>	0021100002	2001001122	2010011010	0100101210	0000002010	1101013010	1000100120	0001000010	1011101000
<i>Anableps dowi</i>	1020100A02	2001021122	2012??1200	0100101210	0001012010	1001013010	1000100100	0111000000	1011101010
<i>Jenynsia multidentata</i>	002010000B	2001001122	2010021010	0100101212	0000002010	1001013010	1110101120	0011000000	1011101011
<i>Aplocheilich. spilauchen</i>	0021200000	2011001121	1010021010	0100100210	0000000010	1001000111	1000111100	0001000010	1011103000
<i>Alfaro cultratus</i>	0021100000	2011001102	1001001510	0100000210	0000002011	1001000011	0000110100	0011000010	1010111010
<i>Poecilia reticulata</i>	0020100000	1001001102	1001021510	0120000200	0000002011	1011000111	0100110110	0011000010	1010111010
<i>Gambusia affinis</i>	0020100000	1001001102	1001021510	0120000210	0000002011	1011000111	0100110110	0011000010	1010111010
<i>Valencia letourneuxi</i>	0120000000	0?01021120	1000021010	0120000212	0010000010	2011000110	1010100120	0001000000	1011101000
<i>Kryptoleb. marmoratus</i>	0000000000	0?00000020	2000110000	0020000010	0001010010	0000000000	0010000020	1000001000	1110100010
<i>Aplocheilus panchax</i>	0000000000	0?00000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000
	1	1111111111	1111111111	1111111111	1111111111	1111111111	1111111111	1111111111	1111111111
	9999999990	0000000001	1111111112	2222222223	3333333334	4444444445	5555555556	6666666667	7777777778
	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890
<i>Fundulus heteroclitus</i>	0000000002	0100010200	1121101010	0000010100	1431000101	2112000000	20100B0011	0013200011	00010010?0
<i>Fundulus grandis</i>	0000000012	0100010200	1121101010	0000010100	1431000200	2111000000	201002?011	0013200011	0001?0?0??
<i>Fundulus grandissimus</i>	0000000012	0100010100	1121101010	0000010100	1431000200	2111000000	201002??11	001320?011	000?0?0?0??
<i>Fundulus confluentus</i>	0000000002	0100010200	1121101010	0000000100	0431001100	2110000000	2010010011	0010000011	0001?0?0??
<i>Fundulus pulverous</i>	0000000002	0100010200	0121101010	0000000100	0431100100	2110000000	2010010011	0010000011	00010?0?0??
<i>Fundulus rathbuni</i>	0000000001	0100010200	0111101010	0000010100	0431100100	2110000000	2100010101	0010001000	0101?0?0??
<i>Fundulus albolineatus</i>	00?00?0??1	0?000?0??0	??????????	?????0?010?	043?0?0?5??	21100?0000	210000110?	??0?0?0000	0?1?0?0?0??
<i>Fundulus julista</i>	0000000001	0100010200	0111101010	0000?10100	043A100500	2110000000	2100000100	0010201000	01110?0?10
<i>Fundulus stellifer</i>	0000000002	0100010200	0111101010	0000010100	0431100100	2110000000	2100000100	0013200000	00B1?0?0??
<i>Fundulus bifax</i>	000000000C	0100010200	0111101010	0000010100	0431100100	2110000000	2100001100	0013200000	000?011010
<i>Fundulus catenatus</i>	0000000001	0100010200	0111101010	0000010100	0431A00100	2110000000	2100001100	0013200000	010?0?0?0??
<i>Fundulus diaphanous</i>	0000000001	0100010200	0111101010	0000010100	0431000100	1110000000	201002?001	0010000000	00010?0?0?
<i>Fundulus waccanensis</i>	0000000001	0100010200	1111101010	0000010100	0E31000100	1110000000	201002?001	0012000000	0001?0?0??
<i>Fundulus seminolis</i>	0000000001	0100010200	1111101010	0100010100	0431000100	1110000000	2010011001	0012A00000	0001000010
<i>Fundulus persimilis</i>	0000000002	0100010200	0111101010	0000011?00	0431001110	2111000000	201002?001	0012200000	000?0?0?0??
<i>Fundulus similis</i>	0000000002	0100010200	0111101010	0000011100	04310011A0	2111000000	201002?001	0010200000	0021000010
<i>Fundulus majalis</i>	0000000002	0100010200	0111101010	0000011100	0431001110	2111000000	201202?001	0011200000	0001000011
<i>Fundulus lima</i>	0000000002	0010010100	0111101010	00100101?0	0431140111	110?000000	210022?001	0010200000	000?0?0?0??
<i>Fundulus parvipinnis</i>	0000000002	0010000100	0111101010	0010010100	0431A00111	1111000000	201022?001	0010200000	0001?0?0??
<i>Fundulus kansae</i>	0000001001	0110010100	0111100010	0020010100	0430?D0300	1111000000	201002?001	0012201000	0001001000
<i>Fundulus zebrinus</i>	0000001001	0110010100	0111100010	0020010100	0430?D0300	1111000000	201002?001	0012201000	0001001000
† <i>Fundulus detillae</i>	???0?1001	0?1001?0?0	?1?0?0?0??	??????????	??????????	11?0?00000	2?0?0?0?0??	??????????	??????????
<i>Fundulus jenkinsi</i>	0000000002	0100010100	1121100010	0100010100	0410100300	0110000000	2010010000	0012101000	000?0?0?0??
<i>Fundulus xenicus</i>	0111000002	01000100?0	1021100010	0100010200	0000?00511	11A0000000	201002?001	0010200001	0006101000
<i>Fundulus chrysotus</i>	0000000002	0010000100	11C1101010	0100010100	0431000100	0110000000	2000000000	0010101000	0005010010
<i>Fundulus luciae</i>	0010000002	00000000?0	1121100010	0000010100	0100?00301	0110000000	201002?000	0010101010	0006?0?0??
<i>Fundulus rubrifrons</i>	0000000002	00000100?0	2?21101010	0000010200	0432020400	0110000000	2010001A00	1010101000	000?100010
<i>Fundulus cingulatus</i>	0000000002	00000100?0	2?21101010	0000010200	0432020400	0110000000	2010001A00	1010101000	0002?0?0??
<i>Fundulus sciadicus</i>	0000000002	0100010100	2?21101010	0000010100	0313?30300	0110000000	2100000000	0010101000	0003?0?0??
<i>Fundulus notatus</i>	0000000002	0000010100	1121101000	0100011200	0333?00100	0110000000	1000110001	0003100000	000E111011

Appendix IV: Continued.

	1	111111111	111111111	111111111	111111111	111111111	111111111	111111111	111111111	111111111	111111111	1
	999999999	000000001	111111112	222222223	333333334	444444445	555555556	666666667	777777778			8
	123456789	123456789	123456789	123456789	123456789	123456789	123456789	123456789	123456789	123456789	123456789	1
<i>Fundulus olivaceus</i>	000000002	0000010100	1121101000	0100011200	03310000100	0110000000	1000110001	0003100000	0001111011	0003100000	0001111011	0
<i>Fundulus euryzonus</i>	000000002	0000010100	1121101000	0100011200	0331100100	0110000000	1000110001	0003110000	0001?????	0003110000	0001?????	?
<i>Fundulus blairae</i>	000000002	010002?0?0	1121101010	0100010000	04310000100	0110000000	1A01011000	1102000000	0002?????	1102000000	0002?????	?
<i>Fundulus dispar</i>	000000002	010002?0?0	1121101010	0100010000	04310000100	0110000000	1001011000	1102000000	0002101011	1102000000	0002101011	0
<i>Fundulus lineolatus</i>	000000002	010002?0?0	1121101010	0100010200	04310000100	0110000000	1011011000	1102010000	0002101011	1102010000	0002101011	0
<i>Fundulus escambiae</i>	000000002	010002?110	1121101010	0100010200	04210000100	0110000000	1011011000	1102010000	0002?????	1102010000	0002?????	?
<i>Fundulus nottii</i>	000000002	010002?110	1121101010	0100010200	04210000100	0110000000	1001011000	1102010000	0002101011	1102010000	0002101011	0
<i>Lucania parva</i>	000A000002	00100110?0	11C1101010	0100010210	02210000500	1110100000	210032?001	0012101100	0022001111	0012101100	0022001111	0
<i>Lucania interioris</i>	000A000002	00100110?0	2?C1101010	0100010210	0100?000500	1110100000	210032?001	001210?100	000???????	001210?100	000???????	0
<i>Lucania goodie</i>	000A000002	00000110?0	11C1101010	0000010210	02210000500	1110100000	210012?001	001310A100	100?000111	001310A100	100?000111	0
<i>Leptolucania omnata</i>	0110000002	000002?0?0	2?21101010	0200010??0	0001020501	0110000000	100042?001	0013101000	100?000111	0013101000	100?000111	0
<i>Profundulus labialis</i>	1000001012	10100110?0	1010000010	0020001200	0431001300	210?000000	2100210001	001010?0A0	000???????	001010?0A0	000???????	?
<i>Profundulus punctatus</i>	1000001011	10100110?0	1010000010	0020001200	0431001100	210?000000	2100210001	0010101000	000???????	0010101000	000???????	0
<i>Pro. guatemalensis</i>	1000001012	10100110?0	1010000010	0020001200	0431001100	210?000000	2100210001	0010101000	000???????	0010101000	000???????	?
<i>Crenichthys baileyi</i>	0000001012	1000011201	??11000A10	00300010200	0421000100	210?000000	2100210001	0010100000	000???????	0010100000	000???????	0
<i>Ameca splendens</i>	0000001012	11100110?0	1011000010	0050210200	0321010111	110?000300	2000210001	0012100000	0107?????	0012100000	0107?????	3
<i>Cubanichthys pengelleyi</i>	0000001002	10000000?0	0011000000	0001000210	0421000111	110?000000	200012?000	0013101000	0001?????	0013101000	0001?????	?
<i>Cyprinodon variegates</i>	1011001002	01001000?0	1021110111	0001010210	0421000111	110?000000	201002?000	0013201000	0021001010	0013201000	0021001010	1
<i>Floridichthys carpio</i>	1011001002	01001000?0	1021110111	0031010210	0221000111	110?000000	2010001000	0013100000	0021?????	0013100000	0021?????	0
<i>Aphanius dispar</i>	1011001002	01001110?0	1021110011	02500011200	0210?0?511	110?000000	201022?011	0011111000	0101?????	0011111000	0101?????	0
<i>Orestias agassizii</i>	1010000002	010012?0?1	??11110011	02300011101	0000?1?500	110?000000	2110210001	0013100000	000???????	0013100000	000???????	?
<i>Oxyzygonectes dovii</i>	1000001102	0000000200	1010000010	1120001200	0330?00110	002??10000	200002?001	0012101001	000???????	0012101001	000???????	0
<i>Anableps dowi</i>	0100201002	010002?200	2?20000010	0020101200	0431000300	013??11200	21002?001	0013100000	000???????	0013100000	000???????	3
<i>Jenynsia multidentata</i>	1000201002	1000000200	0110000010	0120211200	0430?01300	210??10200	2101011001	0012200000	0001?????	0012200000	0001?????	3
<i>Aplocheilichthys spilarchen</i>	0000000102	00010000?0	1011000010	1000001200	0000?01100	000??00000	201002?000	0010100000	0001?????	0010100000	0001?????	0
<i>Alfaro cultratus</i>	1001101110	000102?0?0	2?11000000	1010111200	0000?01300	000??01100	201002?000	0010200000	000???????	0010200000	000???????	3
<i>Poecilia reticulata</i>	1001111112	002102?0?0	2?21001010	1050110201	0000?1?500	000??01111	210002?000	0010100000	000210000?	0010100000	000210000?	3
<i>Gambusia affinis</i>	1001111112	002102?0?0	2?C1001010	A000110201	0000?1?500	000??01111	210002?000	0110100000	000110000?	0110100000	000110000?	3
<i>Valencia letourneuxi</i>	0011000002	0000000200	0011000010	0100010200	0000?30500	210??00000	201002?010	0012100000	002??0?0?	0012100000	002??0?0?	0
<i>Krypto. marmoratus</i>	0011001002	01000200?0	2?00000010	0000000200	0000?1?500	010??00000	0010010001	0013100000	0001?????	0013100000	0001?????	?
<i>Aplocheilichthys panchax</i>	0000000000	00000000?0	0000000000	0000000000	0000?0?000	000??00000	0A00000000	000000A000	00B0?????	000000A000	00B0?????	0

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