

# The Phylogeny of Atherinomorphs: Evolution of a Novel Fish Reproductive System

Lynne R. Parenti



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Division of Fishes, MRC NHB 159, Department of  
Vertebrate Zoology, National Museum of Natural History,  
Smithsonian Institution,  
Washington, DC, USA.

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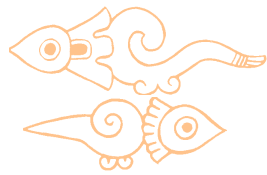
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## Abstract

The fishes commonly known in English as silversides, rainbowfishes, phallostethids, killifishes, ricefishes, halfbeaks, needlefishes, flying fishes, and sauries were combined by Rosen (1964), into one taxon, now known as the Atherinomorpha, largely using osteological and reproductive characters. Subsequent reviews have supported atherinomorph monophyly, adding characters to the diagnosis. Today atherinomorphs are diagnosed as monophyletic by derived characters of the testis, egg, reproductive mode, circulatory system, jaw musculature, olfactory organ, and various parts of the skeleton including the ethmoid region of the skull, gill arches, pelvic girdle, among others. Support for monophyly of each of the three included taxa, now classified as the orders Cyprinodontiformes, Beloniformes, and Atheriniformes, and the relationships among them, varies in quantity and quality. During the past twenty years, reproductive and molecular data used to infer atherinomorph relationships have grown significantly. In general, molecular data support hypotheses based on morphology, and, in some cases, provide novel hypotheses and unique challenges to morphological data. A growing body of data indicates that all atherinomorphs share a unique testis-type that is correlated with an array of reproductive modifications such as coupling during mating, relatively long developmental period, sperm-bundle formation, internal fertilization, superfetation, embryo retention, diapause, delayed hatching, hermaphroditism, and live-bearing. Corroboration of an atherinomorph sister group may include identification of some unique aspects of this reproductive system in other taxa.

## Resumen

Los peces comúnmente conocidos en inglés como: *silversides*, *rainbowfishes*, *phallostethids*, *killifishes*, *ricefishes*, *halfbeaks*, *needlefishes*, *flying fishes* y *sauries* fueron integrados por Rosen (1964) en un *taxon* ahora conocido como Atherinomorpha, utilizando esencialmente caracteres osteológicos y reproductivos. Revisiones posteriores han sostenido que Atherinomorpha es un grupo monofilético y han agregado otros caracteres a la diagnosis. Hoy, los atherinomorfos están definidos como monofiléticos por características derivadas del testículo, huevos, formas reproductoras, sistema circulatorio, musculatura de la mandíbula, órgano olfatorio, y varias partes del esqueleto, incluyendo la región etmoidea del cráneo, arcos branquiales, y cintura pélvica, entre otros. El carácter monofilético de cada uno de los tres *taxa* incluidos actualmente, clasificados en los órdenes Cyprinodontiformes, Beloniformes, y Atheriniformes, se define en sus relaciones entre ellos, con variaciones de cantidad y calidad. Durante los últimos veinte años, ha aumentado significativamente el uso de datos sobre reproducción y moleculares para inferir relaciones en los atherinomorfos. En general, datos moleculares apoyan hipótesis basadas en la morfología y, en algunos casos, sustentan hipótesis nuevas y únicas referentes a los datos morfológicos. Un número creciente de datos indica que todos los atherinomorfos tienen un tipo testicular único, correlacionado con una serie de modificaciones reproductivas, tales como el apareamiento, un periodo relativamente largo de desarrollo, formación de paquetes de espermatozoides, fertilización interna, superfetación, retención de los embriones, diapausa, eclosión demorada, hermafroditismo y viviparidad. Corroborar que algún grupo de otro *taxa*, esté emparentado con los atherinomorfos, puede incluir la identificación de alguno de estos aspectos del sistema reproductor.



## Introduction

In 1964, Donn E. Rosen, then curator in the Department of Ichthyology, American Museum of Natural History, New York, published a monograph as a *Bulletin of the American Museum of Natural History*, in which he brought together three disparate groups of teleost fishes in one order, the Atheriniformes (Rosen, 1964). The three suborders in Rosen's Atheriniformes were the Atherinoidei (the silversides, rainbowfishes, and phallostethids), the Cyprinodontoidei (the killifishes and ricefishes), and the Exocoetoidei (the sauries, needlefishes, halfbeaks, and flying fishes). Support of this taxon included evidence largely from two systems, osteology and reproductive biology, which Rosen described in an essay, as was common then, rather than enumerating putative synapomorphies. Skeletal characters that Rosen considered diagnostic of his Atheriniformes included a disc-shaped dorsal and ventral ossified mesethmoid and decoupling of the rostral cartilage from the ascending processes of the premaxillae. Reproductive characters included a large, demersal egg with long, adhesive chorionic filaments and large oil globules (Rosen, 1964:253-255).

The new taxon, classified as the series Atherinomorpha by Greenwood *et al.* (1966), was not accepted readily by all systematic ichthyologists. In particular, Gosline (1971, Fig. 28B) argued that Rosen had brought together fishes from different evolutionary grades that did not share an evolutionary history. Atherinoids (now classified as the order Atheriniformes following Dyer and Chernoff, 1996; Table 1) were

considered by Gosline to be "higher teleosts" because they have characters such as two dorsal fins, both with anterior spines or thickened rays, and an I,5 pelvic-fin ray formula. In contrast, the Cyprinodontoidei and Exocoetoidei (Cyprinodontiformes and Beloniformes, following Greenwood *et al.*, 1966) were considered by Gosline to be "intermediate teleosts." Both have a single, soft-rayed dorsal fin and may have more than six pelvic-fin rays, among other characters, that they share with taxa that Gosline thought to be less advanced (see review by Parenti, 1993). Cyprinodontiformes and Beloniformes were not considered closely related by Gosline (1971), who postulated that they were derived from, or most closely related to the Beryciformes and Myctophiformes, respectively.

Monophyly of atherinomorphs and monophyly and relationships of the three included taxa was reviewed by Rosen and Parenti (1981) in conjunction with a phylogenetic analysis of Cyprinodontiformes by Parenti (1981). The first explicitly cladistic analyses of atherinomorph phylogeny were presented in these two papers. Atherinomorph monophyly was supported by ten characters, again largely those of the skeleton, but also including two reproductive characters (Rosen and Parenti, 1981:20), one of the egg ("a large demersal egg with long adhesive and short filaments and many lipid globules that coalesce at the vegetal pole"), and one of the testis ("the spermatogonia forming only at the blind end of the tubule near the tunica albuginea").

Table 1.

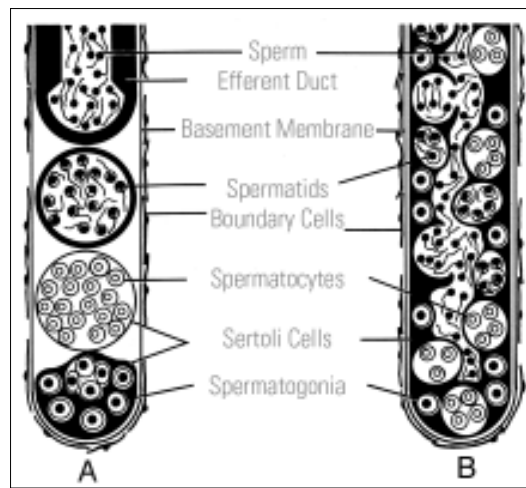
Annotated classification of Atherinomorph fishes (following Rosen and Parenti, 1981; Collette *et al.*, 1984; Parenti, 1993; Costa, 1998a; Dyer and Chernoff, 1996).

- Series Atherinomorpha Greenwood *et al.*, 1966 [= Atheriniformes of Rosen, 1964]
  - Order Atheriniformes *sensu* Dyer and Chernoff, 1996 [= Atherinoidei of Rosen, 1964; Division I of Rosen and Parenti, 1981] Classification is sequenced.
    - Family Atherinopsidae
    - Suborder Atherinoidei
      - Family Notocheiridae (including Isonidae)
    - Infraorder Atherines
      - Family Melanotaeniidae (including Bedotiidae, Pseudomugilidae)
      - Family Atherionidae
    - Superfamily Atherinoidea
      - Family Phallostethidae (including Dentatherinidae)
      - Family Atherinidae
  - Superorder Cyprinodonteia of Dyer and Chernoff, 1996 [Division II of Rosen and Parenti, 1981, order Cyprinodontiformes of Nelson, 1984, not recognized by Rosen, 1964]
    - Order Cyprinodontiformes [= Cyprinodontoidea of Rosen, 1964]
      - Suborder Aplocheiloidei
        - Family Aplocheilidae
        - Family Rivulidae
      - Suborder Cyprinodontoidei
        - Superfamily Funduloidea
          - Family Profundulidae
          - Family Fundulidae
          - Family Goodeidae
        - Superfamily Valencioidea of Costa, 1998a [= Sept 1 of Parenti, 1981]
          - Family Valenciidae
  - Unranked category including superfamilies Cyprinodontoidea and Poecilioidea
    - Superfamily Cyprinodontoidea
      - Family Cyprinodontidae
    - Superfamily Poecilioidea of Parenti, 1981 [= unnamed clade of Costa, 1998a]
      - Family Anablepidae
      - Family Poeciliidae
  - Order Beloniformes [not recognized by Rosen, 1964]
    - Suborder Adrianichthyoidei [= Adrianichthyoidea of Rosen, 1964]
      - Family Adrianichthyidae (including Horaichthyidae and Oryziidae)
    - Suborder Exocoetoidei
      - Superfamily Exocoetoidea
        - Family Exocoetidae
        - Family Hemiramphidae
      - Superfamily Scomberesocoidea
        - Family Belonidae
        - Family Scomberesocidae

The many, unique characteristics of the atherinomorph egg are correlated with reproductive and developmental modifications. Filaments are derived from the secondary or outer layer of the *zona pellucida* which is secreted by follicle cells (Wourms, 1976; Wourms and Sheldon, 1976; Loureiro and deSá, 1996). Filaments vary in number, shape, and relative length (*e.g.*, Able, 1984; Collette *et al.*, 1984; White *et al.*, 1984; Loureiro and deSá, 1996). Oil globule number ranges from one to over 100 (White *et al.*, 1984: table 93). The relatively long developmental period in both oviparous and viviparous taxa is correlated with direct development, that is, loss or reduction of a distinct larval stage, most notably in cyprinodontiforms and beloniforms (Rosen, 1964:253).

The long developmental period and relatively large, desiccation-resistant egg is correlated with the evolution of delayed hatching (Martin, 1999) or diapause (Wourms, 1972). Delay of hatching of fertilized eggs has been reported in the atheriniform grunions of the genus *Leuresthes*, and the cyprinodontiforms *Fundulus heteroclitus*, *F. confluentus*, and *Adinia xenica* (Martin, 1999). Delay of hatching in these taxa is facultative; fertilized, fully-developed eggs can hatch, but are stranded in relatively dry habitats and must await waves, tides, or rains to stimulate hatching (Martin, 1999; Griem and Martin, 2000). This is in contrast to fertilized eggs of annual killifishes, which undergo diapause and for which delay of hatching is obligatory (Wourms, 1972). Stranding of fertilized eggs out of water has been reported also in the Baja California endemic, *Fundulus lima*, by Brill (1982). Facultative delayed hatching of teleost eggs has been reported outside atherinomorphs only in the lower teleost osmeroid *Galaxias maculatus* (Martin, 1999).

The evolutionary relationship between facultative and obligatory delay of hatching in atherinomorphs is unknown. Phylogenetic analyses based on molecules (*e.g.*, Murphy and Collier, 1997) or morphology (*e.g.*, Costa, 1990, 1998a) have led to various, sometimes conflicting, conclusions concerning single or multiple origins of developmental diapause. A molecular phylogenetic analysis of the family Rivulidae by Hrbek and Larson (1999: Fig. 4) supported the hypothesis that diapause was present in two distantly related groups of South American killifishes. Rather than interpret the cladogram literally, they considered it unlikely that diapause had originated twice, but that presence or ab-



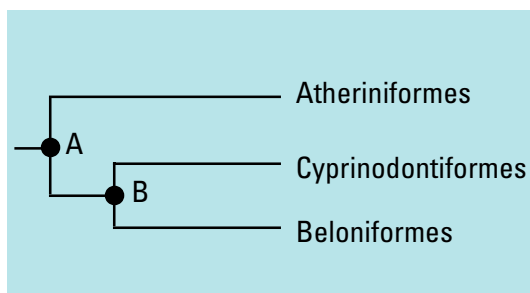
**Figure 1.** Diagrammatic representation of two different testis-types in higher teleosts: A: testis lobule in representative atherinomorph, with spermatogonia restricted to the distal end of the lobule; B: testis lobule in representative non-atherinomorph higher teleost, with spermatogonia distributed throughout the length of the testis lobule (from Grier *et al.*, 1980: Fig. 1)

sence of diapause results from "...developmental switches between alternative stabilized pathways" (Hrbek and Larson, 1999:1200). This was said another way by Parenti (1981:364): "...the annual habit is no more than an exaggeration, due to extreme environmental fluctuations, of a capability of all cyprinodontiforms to survive stress that involves desiccation". Now, with our increased knowledge of delayed hatching patterns, I would rewrite that sentence by substituting "atherinomorphs" for "cyprinodontiforms" (see also Parenti, 1993). Delayed hatching patterns of atherinomorphs, whether facultative or obligatory, may be homologous and represent another atherinomorph synapomorphy.

The testis character proposed as an atherinomorph synapomorphy by Rosen and Parenti (1981) had been described just the year before by Harry Grier and colleagues (Grier *et al.*, 1980; Fig. 1) who reported this distinctive testis-type in 31 atherinomorph species representing each of the three orders. Since Grier *et al.* (1980), the atherinomorph testis has been reported or confirmed in a total of 79 atherinomorph species (Parenti and Grier, 2004), including the beloniform adrianichthyid *Horaichthys setnai* (Grier, 1984), seven species of atheriniform phallostethids (Grier and Parenti, 1994), an anablepid, *Jenynsia multidentata* (Martínez and Monasterio de Gonzo, 2002), the internally-fertilizing halfbeak genus, *Zenarchopterus* (Grier and Collette, 1987), and the viviparous halfbeaks, *Dermogenys*, *Hemirhamphodon* and *Nomorhamphus* (Downing and Burns, 1995; Meisner and Burns, 1997a). A possibly similar testis-type in viviparous surperches, family Embiotocidae, was noted by Grier *et al.* (1980) but dismissed a year later by Grier (1981).

**Figure 2.**

Phylogenetic relationships among the three orders of atherinomorph fishes, following Rosen and Parenti (1981), Collette *et al.* (1984), Parenti (1993), Dyer and Chernoff (1996), and this paper. Synapomorphies are: A) testis a restricted spermatogonial type; egg demersal, with several to many chorionic filaments, and several oil globules that coalesce at the vegetal pole; coupling during mating; prolonged developmental period; separation of embryonic afferent and efferent circulation by development of heart in front of head; ossified portion of ethmoid region of skull highly reduced; infraorbital series represented by the lacrimal, dermosphenotic, and two, one or no anterior infraorbital bones; lateral process of pelvic bone and distal end of pleural rib in close association, and, in some taxa, connected via a ligament; supraclithrum reduced or absent; dorsal portion of gill arches with a large fourth epibranchial the prominent supporting bone and no fourth pharyngobranchial element; medial hooklike projection and ventral flange on the fifth ceratobranchial bone; supraneural bones absent; superficial division of adductor mandibulae with two tendons, one inserting on the maxilla, a second inserting on the lacrimal bone; olfactory sensory epithelium arranged in sensory islets; absence of the saccus vasculosus; B) second infraorbital bone absent; first epibranchial bone with an expanded base and no separate uncinata process; first pharyngobranchial element absent; second and third epibranchials smaller than the first and fourth; stomach, pyloric caecae and pneumatic duct absent



Evolution of viviparity is considered independent in atherinomorphs and embiotocids (see Lydeard, 1993). The phylogenetic significance of reproductive characters in embiotocids, including both egg and testis, are evaluated relative to those of atherinomorph fishes elsewhere in this volume (Grier *et al.*, this volume). The derived testis and egg are correlated in atherinomorphs with a vast array of reproductive modifications including coupling during mating, relatively long developmental period, sperm-bundle formation, internal fertilization, superfetation, embryo retention, diapause, delayed hatching, hermaphroditism, and live-bearing. Understanding the phylogeny of atherinomorph fishes will be enhanced by an understanding of their derived reproductive modifications and evolution of this novel reproductive system.

The purpose of this paper is to review the current state of our knowledge of atherinomorph phylogeny, with a focus on live-bearing taxa, summarizing molecular and morphological cladistic analyses published during the past two decades. Some phylogenetic analyses of solely oviparous taxa are cited but not discussed in detail. It is not my goal to summarize all cladistic analyses of atherinomorph taxa, which is well beyond the scope of this review.

### Atherinomorph Monophyly

Atherinomorph monophyly was reviewed by Parenti (1993) who listed 14 diagnostic characters, five of which were of reproduction and development (Fig. 2, node A). The atherinomorph testis-type and associated reproductive modifications remain among the strongest evidence for monophyly, as argued above.

Here, I add a fifteenth character to the atherinomorph diagnosis: absence of the saccus vasculosus, a hypothalamic circumventricular organ of unspecified function (Tsuneki, 1992). The saccus vasculosus has been reported to produce a parathyroid hormone-related protein in the perciform sea bream, *Sparus aurata* (Devlin

*et al.*, 1996). Approximately 200 teleost species, both freshwater and marine, representing all major teleost lineages, were surveyed by Tsuneki (1992) for presence or absence of the saccus vasculosus and extent of its development when present. A well-developed saccus vasculosus was considered to be a generalized condition, present in some Osteoglossiformes, Anguilliformes, and ostariophysans among lower teleosts, and Mugiliformes, Gasterosteiformes, Scorpaeniformes, Perciformes, Pleuronectiformes, and Tetraodontiformes among higher teleosts. The saccus vasculosus is reduced or absent in a variety of taxa, including atherinomorphs, African cichlids, some anabantoids, and a synbranchid eel among higher teleosts.

The most “clear-cut” result of this survey, according to Tsuneki (1992:74), is that absence of the saccus vasculosus is characteristic of atherinomorphs, and I agree. Fifteen atherinomorphs surveyed, representing all three orders, from both freshwater and marine habitats unambiguously lack the saccus vasculosus. Its absence is proposed here as an atherinomorph synapomorphy.

A sixteenth atherinomorph synapomorphy, of the oocyte, was proposed and illustrated by Parenti and Grier (2004: Figs. 3,4). Atherinomorph yolk is fluid, rather than granular, throughout vitellogenesis.

### Atherinomorph Sister Group

The Series Atherinomorpha was classified as sister to the Series Percomorpha by Rosen and Parenti (1981) without an explicit proposal of relationship to any particular percomorph taxon. In diagnosing atherinomorphs, Rosen (1964) made deliberate comparisons with taxa such as mullets (Mugilidae) or percopsiform fishes that had at one time been proposed as closely related to one or another atherinomorph order (see Parenti, 1993). In discussing atherinomorph sister-group relationships, Parenti (1993: Fig. 1c) could come to no firm conclusion, arguing that evidence linked atherinomorphs to paracanthopterygians on one hand and to percomorphs on the other.

The close relationship of mullets to atherinomorphs was proposed by Stiassny (1990) and explored further by Stiassny (1993) who, although she concluded that there was good evidence for a sister group relationship, discussed numerous characters that contradicted that proposal. Of seven characters in support of a mul-



let-atherinomorph sister-group relationship (Stiassny, 1993: Fig. 1), four were of the pectoral girdle, two of the branchial muscles, and one of the vertebral column. Three pelvic fin characters were considered to have reversed in atherinomorphs (*viz.* Stiassny and Moore, 1992). The study was not intended as an exhaustive review of acanthomorph relationships, however, because, for example, another reversal in atherinomorphs would be loss of transforming ctenoid scales (Roberts, 1993).

The hypothesis of a close mullet-atherinomorph relationship was taken a step further by Johnson and Patterson (1993) who proposed a new taxon, the Smegmamorpha, to include synbranchoid eels, mastacembeloid eels, the centrarchid *Elassoma*, gasterosteiforms, mullets, and atherinomorphs. A single character was proposed for smegmamorph monophyly (Johnson and Patterson, 1993:572): the first two epineural bones originate at the tip of transverse processes or fused parapophyses on the first two vertebral centra. In addition, Johnson and Patterson (1993:table 2) tabulated selected, shared derived characters found in some, but not all smegmamorphs, such as, for example, infraorbital series with three or fewer bones between the lacrimal and the dermosphenotic, present in atherinomorphs, *Elassoma*, gasterosteiforms, and synbranchoids, but absent in mullets and mastacembeloids.

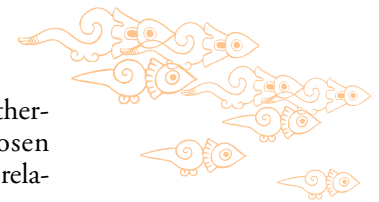
Smegmamorph monophyly was one hypothesis tested by Wiley *et al.* (2000) in a total evidence analysis of acanthomorph phylogeny combining molecular and morphological data. The strict consensus of 137 equally parsimonious trees based on morphological data recovered a monophyletic Atherinomorpha with unresolved relationships to an array of higher taxa (Wiley *et al.*, 2000: Fig. 8c). A strict consensus of four equally parsimonious trees based on combined molecular and morphological data recovered a group that included the mullet *Mugil* and the four atherinomorph taxa analyzed (the atheriniforms *Melanotaenia* and *Atherinomor*, the beloniform *Strongylura*, and the cyprinodontiform *Gambusia*), but did not recover a monophyletic Smegmamorpha (Wiley *et al.*, 2000: Fig. 6). *Mugil* was considered to be more closely related to the two atheriniforms than either is to the cyprinodontiform or the beloniform; that is, atherinomorph monophyly was refuted as well. Morphological characters surveyed by Wiley *et al.* (2000), however, did not include characters such as the atherino-

morph testis-type (not found in mullets; Grier *et al.*, 1980), absence of the saccus vasculosus (well-developed in mullets; Tsuneki, 1992), or innervation of the pectoral and pelvic fin muscles by branches of spinal nerve 2 (present and derived in mullets, absent in atherinomorphs; Parenti and Song, 1996). Detailed molecular analyses of acanthomorph phylogeny based on molecular data alone (*e.g.*, Miya *et al.*, 2003) confirm atherinomorph monophyly, but include other taxa, such as blennioids and gobioids, along with mullets, as putative atherinomorph sister taxa. Broader surveys of morphology, as well as molecules, are needed to further test monophyly of smegmamorphs and evaluate the hypothesis of a sister group relationship of mullets and atherinomorphs.

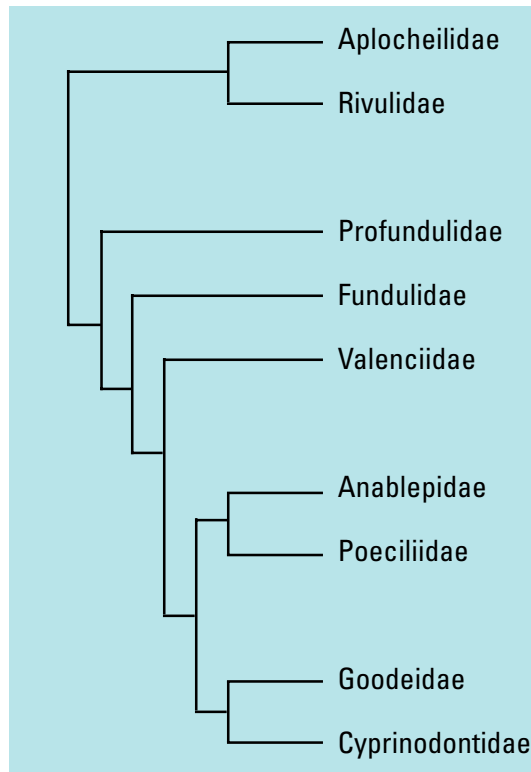
### Cyprinodontea

Relationships among the three groups of atherinomorph fishes were unspecified by Rosen (1964). Monophyly of each group and the relationships among them were considered by Rosen and Parenti (1981:21-23) who proposed a sister group relationship between Cyprinodontiformes and Beloniformes, together called Division II atherinomorphs. Four characters were proposed to support monophyly of Division II (Rosen and Parenti, 1981:21; Fig. 2, node B). A fifth character may be added from Li (2001:585): absence of a stomach, including absence of pyloric caecae and a pneumatic duct.

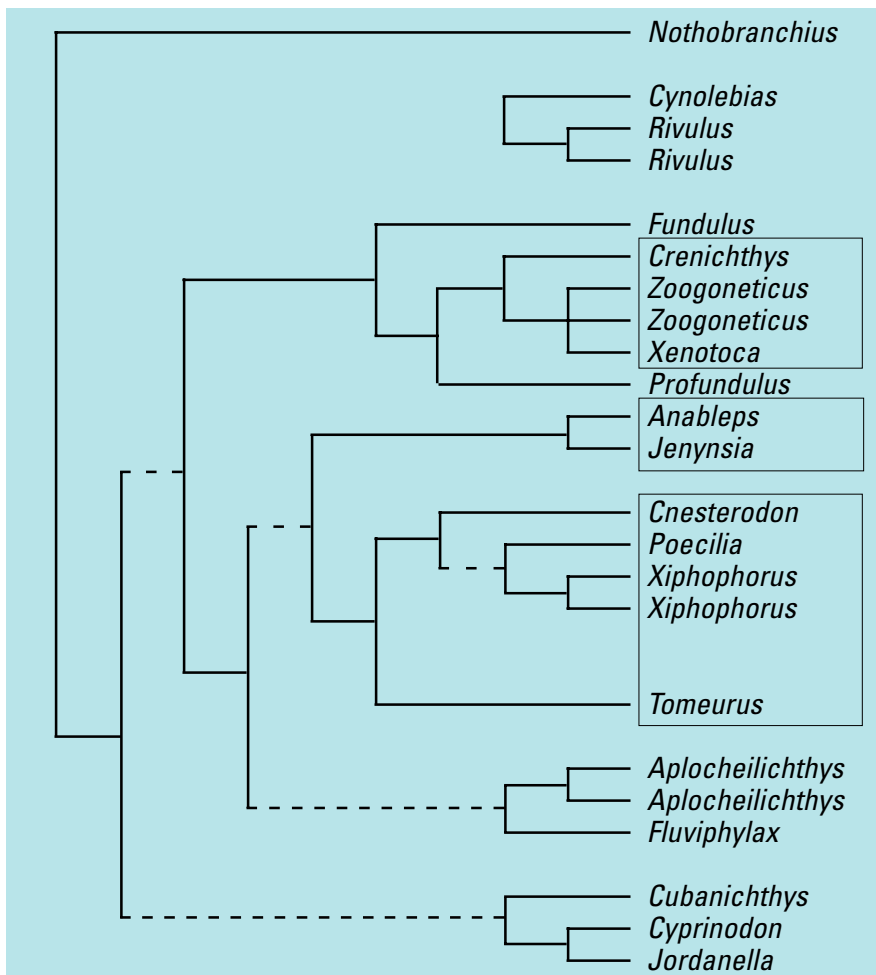
The sister group relationship between Cyprinodontiformes and Beloniformes has been corroborated (Stiassny, 1990; Saeed *et al.*, 1994; Dyer and Chernoff, 1996; Wiley *et al.*, 2000; Li, 2001). The relationship was recognized by Nelson (1984:214) who in his second edition of *Fishes of the World* synonymized the two orders in an expanded Cyprinodontiformes. This decision was reversed in the third edition (Nelson, 1994:264, with the three orders Atheriniformes, Beloniformes, and Cyprinodontiformes recognized without inter-ordinal relationships expressed). The current edition of *Fishes of the World* is used worldwide as a standard reference for teleost classification, however, and an enlarged order Cyprinodontiformes, including beloniforms, was incorporated into numerous publications, particularly during the decade between 1984 and 1994 (*e.g.*, Tsuneki, 1992; Kottelat *et al.*, 1993). The superorder Cyprinodontea was proposed by Dyer and Chernoff (1996) as a formal name for Rosen



**Figure 3.** Phylogenetic relationships among families of Cyprinodontiformes as proposed by Parenti (1981)



**Figure 4.** Phylogenetic relationships among select genera of Cyprinodontiformes as proposed by Meyer and Lydeard (1993) based on partial DNA sequences of the tyrosine kinase gene *X-src*. Genera in boxes represent families or subfamily groupings of live-bearing taxa and their close relatives, as also proposed by Parenti (1981), from top to bottom, family Goodeidae, family Anablepidae, and subfamily Poeciliinae



and Parenti's (1981) Division II, the Cyprinodontiformes and Beloniformes, and is adopted here (Table 1).

### Cyprinodontiformes

The first explicitly cladistic analysis of cyprinodontiform fishes was based largely on osteological characters (Parenti, 1981; Fig. 3). Cyprinodontiform monophyly was corroborated by six complex characters (Parenti, 1981: Fig. 9, node a), and it has been challenged only by Li (2001; see comments below under Beloniformes). One cyprinodontiform character of Parenti (1981), prolonged embryonic development, is discussed above as an atherinomorph synapomorphy (see also Parenti, 1993). A major conclusion of Parenti's (1981) study that refuted earlier notions of cyprinodontiform relationships was that viviparity had evolved at least three times within the order, not once. That is, oviparous sister groups of each group of viviparous taxa were hypothesized. Also, cyprinodontiforms were divided into two monophyletic suborders, the Aplocheiloidei and Cyprinodontoidei. This proposal of relationships was tested by Meyer and Lydeard (1993; Fig. 4) using partial DNA sequences of the tyrosine kinase gene *X-src*, an oncogene chosen in part because cyprinodontiforms of the genus *Xiphophorus* have long been known to inherit melanomas (see Schartl, 1995). No non-cyprinodontiform taxon was used as an outgroup by Meyer and Lydeard, so their analysis was not a test of cyprinodontiform monophyly. Also, at least one pivotal taxon, the oviparous *Oxyzygonectes*, sister to the viviparous *Anableps* and *Jenynsia* in the Anablepidae, according to Parenti (1981), was not included. Nonetheless, the maximum parsimony phylogenetic hypothesis of Meyer and Lydeard (1993) corroborated Parenti's (1981) conclusions that viviparity had evolved at least three times within cyprinodontiforms, in anablepids, goodeids, and poeciliids. Further, the analysis recovered the controversial sister group relationship of the viviparous goodeids and their oviparous relatives, *Empetrichthys* and *Crenichthys* (see also Grant and Riddle, 1995).

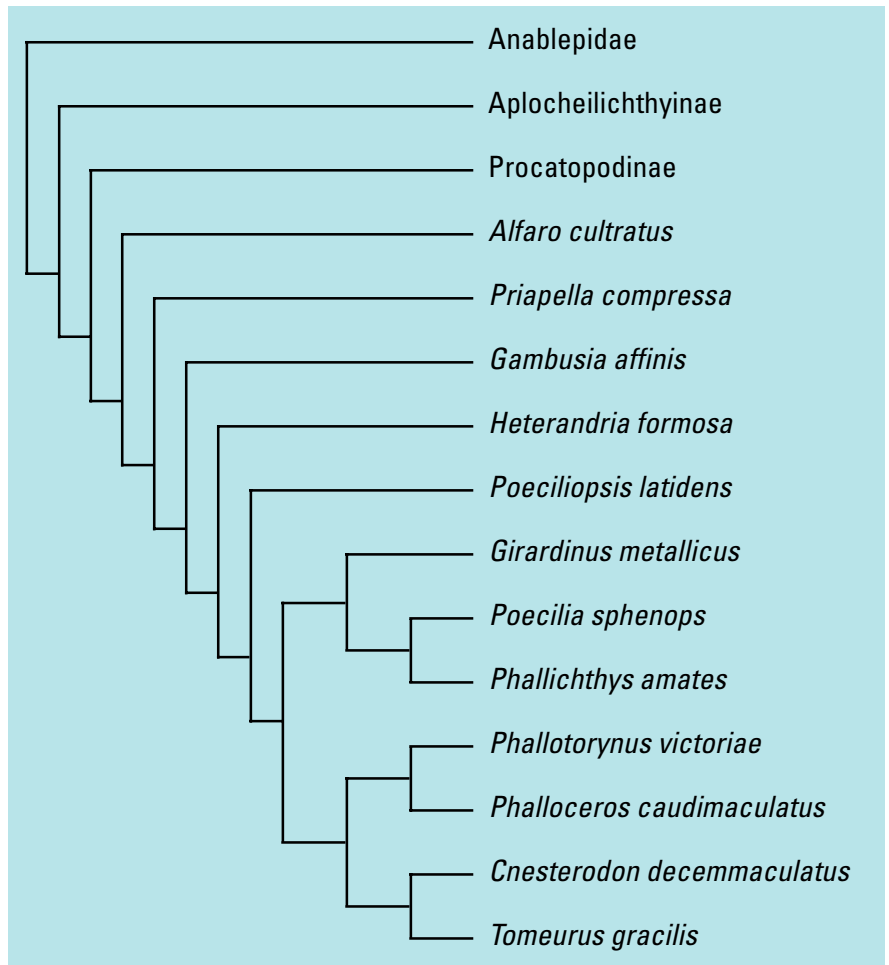
Cyprinodontiforms were used as a test taxon by Parker (1997) to evaluate the consequences of combining morphological and molecular data in a phylogenetic analysis. Morphological characters described by Parenti (1981, 1984a) are listed and character states coded in a detailed appendix (Parker, 1997:184-185). Although this study was not intended as a thorough re-analy-



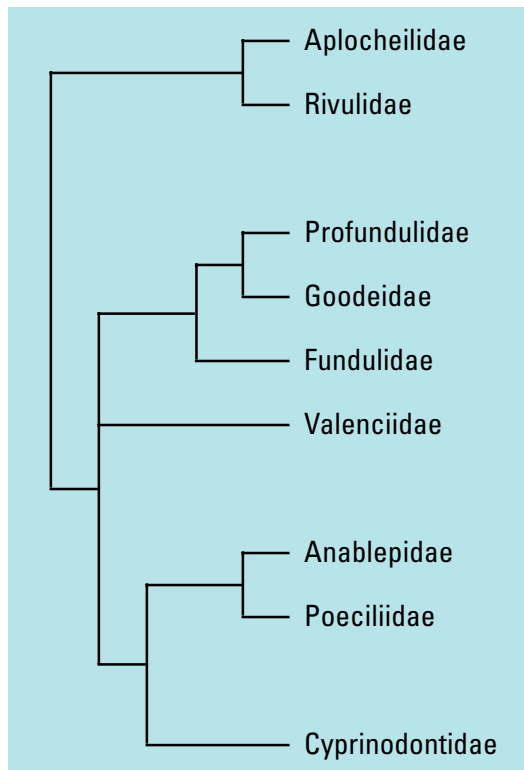
sis of Parenti's (1981) hypothesis, some of Parker's results anticipated those of other studies. For example, *Tomeurus*, the ovoviparous poeciliine, had been considered primitive to viviparous poeciliines since Rosen and Bailey's (1963) classic review, and this relationship was corroborated by Meyer and Lydeard (1993; Fig. 4). In contrast, a partitioned *X-src* dataset of first and second codon positions only, analyzed under maximum parsimony (Parker, 1997: Fig. 2), supported the sister group relationship of *Tomeurus* and *Cnesterodon*, a viviparous poeciliine, together considered derived, not basal, poeciliines. This relationship was corroborated by an array of synapomorphies in a parsimony analysis of morphological characters by Ghedotti (2000; Fig. 5), including neural arch of first vertebra open dorsally, lateral processes on ventral portion of seventh, eighth, and ninth proximal anal-fin radials in adult males present and in contact, and distal tip of the gonopodium with elongate, bony processes. These morphological characters were of course known to Rosen and Bailey (1963) who likely accepted the constraint that an ovoviparous taxon such as *Tomeurus* was basal to a group of viviparous taxa. This constraint was relaxed by Parker (1997) and Ghedotti (2000) who independently recovered a novel hypothesis of relationships of *Tomeurus*.

Division of Cyprinodontiformes into two suborders, Aplocheiloidei and Cyprinodontoidei, was corroborated by Costa (1998a; Fig. 6) who re-analyzed killifish phylogenetic relationships using morphology. The hypothesis of Costa (1998a; Fig. 6) differs from that of Parenti (1981; Fig. 3) in the placement of Goodeidae as sister to Profundulidae, not Cyprinodontidae, and the resolution of the relationships of Valenciidae. Both studies used osteology as a principal source of data, but differed in interpretation of significance of character states of the premaxilla, among others. The sister group relationship of Goodeidae and Profundulidae was recovered also in the molecular hypotheses of Meyer and Lydeard (1993) and Parker (1997). The close relationships of fundulids, goodeids, and profundulids is reflected in the written classification of Cyprinodontiformes (Table 1).

Monophyly of each of the nine families recognized by Parenti (1981) was corroborated by Costa (1998a) and the terminal taxa of Meyer and Lydeard's (1993; Fig. 4) hypothesis are also consistent with Parenti's hypothesis. Monophyly of the Anablepidae and Poeciliidae (*sensu* Parenti, 1981) was corroborated in Ghedotti's

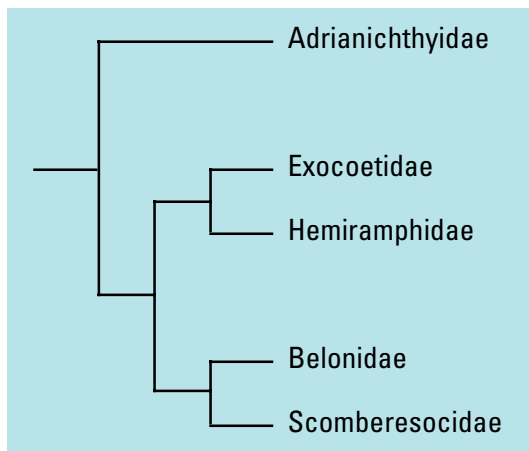


**Figure 5.** Phylogenetic relationships among the poecilioid fishes, simplified from Ghedotti (2000: Fig. 20). Species listed are members of the subfamily Poeciliinae



**Figure 6.** Phylogenetic relationships among families of Cyprinodontiformes as proposed by Costa (1998a)

**Figure 7.** Phylogenetic relationships among the families of beloniform fishes, following Rosen and Parenti (1981) and Collette *et al.* (1984)



(2000; Fig. 5) morphological analysis, whereas Meyer and Lydeards' (1993; Fig. 4) analysis proposed a paraphyletic Poeciliidae. The dotted lines in Meyer and Lydeard's (1993; Fig. 4) cladogram indicate relationships for which they found weak support, however. Morphology and molecules do equally well, *i.e.* agree, in resolving relationships at the tips of the tree, but vary in their ability to recover higher taxa. A molecular phylogenetic analysis of aplocheiloids by Murphy and Collier (1997) conflicts, in part, with the proposals of Parenti (1981) and Costa (1998a) based on morphology. A stable classification of cyprinodontiforms, at the family level and above, is within reach, however, and is expected to include components common to the above morphological and molecular analyses.

Additional molecular phylogenies of families or other subgroups of aplocheiloids include those of Murphy and Collier (1999, *Aphyosemion* and *Fundulopanchax*), Murphy *et al.* (1999a, West African aplocheiloids), and Murphy *et al.* (1999b, Rivulidae). Morphological phylogenies or surveys include Costa (1990, 1998b, Rivulidae; 1995a, *Cynopoecilus*; 1995b, Cynolebiatinae; 1996a, *Simpsonichthys*), Loureiro and deSá (1998, *Cynolebias*), and Aarn and Shepherd (2001, epiplatines). Taxonomy, biology, and conservation status of Brazilian annual killifishes was reviewed by Costa (2002, and references therein).

Molecular phylogenies of families or other subgroups of cyprinodontoids include Bernardi (1997, Fundulidae), Breden *et al.* (1999, *Poecilia*), Grady *et al.* (2001, *Fundulus*), Hamilton (2001, *Limia*), Hrbek and Meyer, 2003 (*Aphanius*) Lüßen *et al.* (2003, *Orestias*), Lydeard *et al.* (1995, *Gambusia*), Mojica *et al.* (1997, *Brachyrhaphis*), Parker and Kornfield (1995,

cyprinodontids), and Webb *et al.* (2004, livebearing Goodeidae). Morphological phylogenies or surveys include Parenti (1984a, *Orestias*), Chambers (1987, cyprinodontiform gonopodia; 1990, cnesterodontin gonopodia), Ghedotti (1998, Anablepidae), Rauchenberger (1989, *Gambusia*), Costa (1996b, *Fluviphylax*; 1997, cyprinodontids), and Rodríguez (1997, Poeciliini). Conclusions of these studies that bear on higher order relationships of cyprinodontiforms were summarized by Lazara (2001:ix-xiv).

The genus *Xiphophorus*, the swordtails and platyfishes, serves as a model taxon in analyses of congruence of phylogenetic pattern with behavior, development, morphology, and molecules (see, for example, Basolo, 1991; Haas, 1993; Meyer *et al.*, 1994, Marcus and McCune, 1999, and Morris *et al.*, 2001).

### Beloniformes

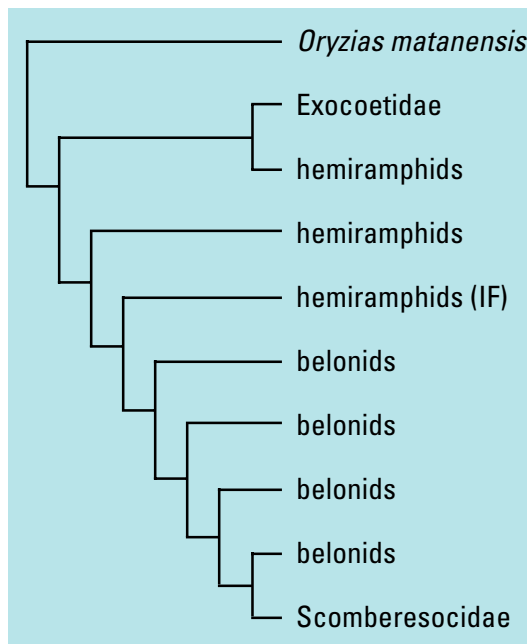
Beloniform monophyly was supported by seven synapomorphies (Rosen and Parenti, 1981:17), including absence of the interhyal bone, reduction or loss of the interarcual cartilage, presence of only a single, ventral hypohyal bone, modifications of the gill arch skeleton, as well as a distinctive caudal skeleton characterized by the lower caudal lobe with more principal rays than in the upper caudal lobe.

Beloniform relationships were reviewed by Collette *et al.* (1984; Fig. 7) who accepted a monophyletic Beloniformes including Rosen and Parenti's (1981) proposal that ricefishes (family Adrianichthyidae) are more closely related to exocoetoids (families Exocoetidae, Hemiramphidae, Belonidae, and Scomberesocidae) than to cyprinodontiforms. This proposal has been criticized recently by Li (2001) who argued that adrianichthyoids are more closely related to Cyprinodontiformes than to exocoetoids. In particular, Li (2001) claimed that some characters proposed as diagnostic of Beloniformes *sensu lato*, although absent in the primitive cyprinodontiform suborder Aplocheiloidei, are present in the derived suborder Cyprinodontoidei. For example, Li (2001:584) rejected Rosen and Parenti's (1981) beloniform synapomorphy of a single, ventral hypohyal because cyprinodontoids have a single hypohyal. If we accept cyprinodontiform monophyly, however, which is supported by a symmetrical caudal fin skeleton, absent in adrianichthyoids, and first pleural rib on the second, rather than

the third vertebra, among other characters, then similarities between cyprinodontoids and adrianichthyoids must be interpreted as convergent rather than indicative of close relationship. Cyprinodontiforms and beloniforms, *sensu* Rosen and Parenti (1981), are readily distinguished by their distinct caudal fin skeletons. Finally, both cyprinodontiforms and adrianichthyoids were characterized by Li (2001) as lacking elongate jaws, whereas Parenti (1987, 1989a) argued that the elongate jaws of the large-bodied adrianichthyoids of Sulawesi, some of which have been called 'duck-billed' represent additional support for their close relationship to exocoetoids. Despite rejection of Li's (2001) hypothesis, I appreciate his comments as they call for continued critique of the monophyly of clades within the Atherinomorpha as recognized herein.

Higher-order beloniform *sensu lato* relationships were reviewed by Lovejoy (2000; Fig. 8) who combined data from nuclear and mitochondrial gene sequences with morphology in a total evidence analysis. The families Exocoetidae (flyingfishes) and Scomberesocidae (sauries) were considered monophyletic by both Collette *et al.* (1984; Fig. 7) and Lovejoy (2000; Fig. 8). The families Hemiramphidae (halfbeaks) and Belonidae (needlefishes), considered monophyletic by Collette *et al.* (1984), were hypothesized to be paraphyletic by Lovejoy (2000).

No non-beloniform taxon was included in Lovejoy's analysis and a single adrianichthyid species (*Oryzias matanensis*) was an outgroup to the ingroup exocoetoids. Therefore, the analysis was not a test of beloniform or of exocoetoid monophyly. Further, some intriguing taxa were not included in the study, such as the southern African needlefish, *Petalichthys*, which remains in a "halfbeak stage" of development for a relatively long time before both upper and lower jaws become elongate (Collette *et al.*, 1984:342; Boughton *et al.*, 1991). Also missing was the hemiramphid *Oxyporhamphus* recently reclassified as a flyingfish, family Exocoetidae, based on a re-analysis of morphology (Dasilao *et al.*, 1997). Nonetheless, Lovejoy's (2000; Fig. 8) hypothesis offers a novel reinterpretation of traditional beloniform morphology and invites further study of the relationship between morphological and molecular data as used in phylogenetic analyses. Molecular sequences and morphology were combined in a total evidence analysis of phylogenetic relationships of New World needlefishes by Lovejoy and Collette (2001).



**Figure 8.** Phylogenetic relationships among beloniform fishes, simplified from Lovejoy (2000: Fig. 2). The families Exocoetidae and Scomberesocidae were each considered monophyletic; the families Hemiramphidae and Belonidae paraphyletic. "Hemiramphids (IF)" refers to the four internally fertilizing genera, *Zenarchopterus*, *Nomorhamphus*, *Dermogenys* and *Hemirhamphodon*

Monophyly of the internally-fertilizing halfbeaks, genera *Zenarchopterus*, *Hemirhamphodon*, *Dermogenys*, and *Nomorhamphus*, was supported by the morphological studies of Anderson and Collette (1991), Downing and Burns (1995), Meisner and Burns (1997b), Meisner and Collette (1999), and Meisner (2001). *Petalichthys* and *Oxyporhamphus* were included in a reanalysis of beloniform phylogeny by Lovejoy *et al.* (2004) and paraphyly of hemiramphids and belonids corroborated. The last three halfbeak genera are viviparous and together form a monophyletic group as corroborated by these analyses. A fifth genus, the monotypic *Tondanichthys*, described from the type series of ten specimens that does not include a mature male, is inferred to be internally fertilizing (Collette, 1995; Meisner and Collette, 1999).

Reproductive biology has been used to infer phylogenetic relationships among live-bearing halfbeaks. *Dermogenys* is diagnosed by large sperm bundles and intrafollicular development; whereas, *Nomorhamphus* is diagnosed by small sperm bundles and a long intraluminal developmental period (Downing and Burns, 1995; Meisner and Burns, 1997b; Meisner and Collette, 1999; Meisner, 2001). These generic limits are in contrast to those of Brembach (1991).

Ricefish females are known to carry bundles of fertilized eggs until hatching (Fig. 9), rather than depositing them on over-hanging vegetation or the substrate. Aquarium-maintained *O.*



**Figure 9.**  
*Oryzias dancena*, USNM 313908,  
female, 23.8 mm SL, with cluster of  
fertilized eggs. Photo by H. H. Tan



**Fig. 9**

**Figure 10.**  
*Xenopoeilus sarasinorum*, CMK  
6557, female, 53.4 mm SL. Above,  
embryo cluster held to body  
posterior to pelvic fins. Below, same  
specimen, chorion measures  
approximately 2 mm in diameter



**Fig. 10**

*nigrimas* females reportedly carry large bundles of eggs, but deposit them among plants or on the substrate soon after spawning (Kottelat, 1990a:54). In spawning in open water, ricefishes are similar to the above mentioned *Fundulus lima* (Brill, 1982). Embryos in the clusters are relatively well developed, with large, well-formed eyes and pigmented bodies, and appear near hatching (Fig. 10). Because these fertilized eggs may be carried until hatching, Kottelat (1990a:62) proposed that ricefishes be considered a distinct reproductive guild for which he coined the term “pelvic brooders.” This represents one of the few cases of parental care in oviparous atherinomorphs. Females carrying clusters of fertilized eggs, long known in the medaka, *Oryzias latipes* (Yamamoto, 1975:7), has been reported in at least eight other ricefish species, *O. dancena* (Fig. 9), *O. nigrimas* (Kottelat, 1990a:54) *X. oophorus* (Kottelat, 1990a: Fig. 6), *X. sarasinorum* (Fig. 10), *O. marmoratus* (Kottelat, 1990b: Fig. 5), *O. matanensis* (Kottelat, 1990b:161), *O. javanicus* (BMNH 1970. 7.22:38-39), *O. luzonensis* (Blanco, 1947) and is likely to occur in others.

The Indian ricefish, *Horaichthys setnai*, is internally fertilizing and lays fertilized eggs (Kulkarni, 1940). Internal fertilization and embryo retention is facultative in some ricefishes. Facultative embryo retention was reported in the medaka, *Oryzias latipes*, by Amemiya and Murayama (1931). One specimen of *Adrianichthys kruyti*, a large, pelagic ricefish from Lake Poso, Sulawesi, was reported to be hermaphroditic, having both testis and ovary, by Klie (1988, in Kottelat, 1990a:57). Much information available on the reproduction of the large

ricefishes is anecdotal (see Weber and de Beaufort, 1922; Kottelat, 1990a; Rosen, 1964). More detailed study of the reproductive morphology of the large Sulawesi ricefishes is needed to determine the extent of internal fertilization, embryo retention, and hermaphroditism in the Adrianichthyidae.

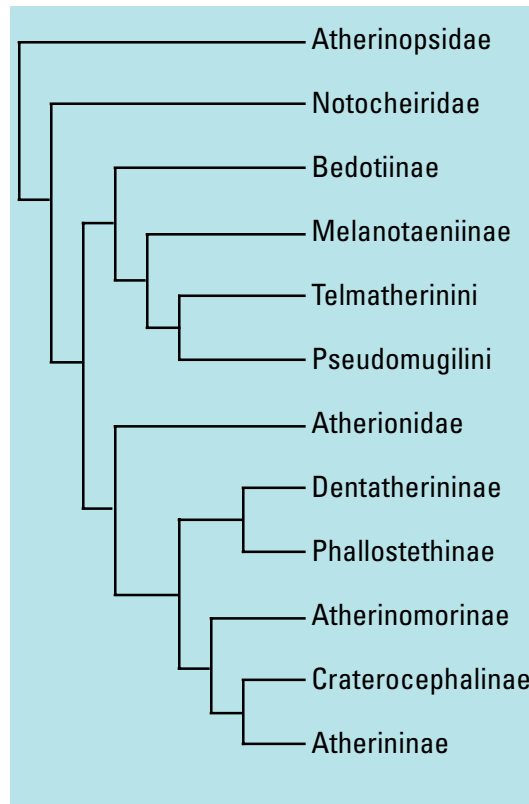
### Atheriniformes

Since 1981, there have been several, solely morphological, phylogenetic analyses of atheriniform fishes (White *et al.*, 1984; Stiassny, 1990; Saeed *et al.*, 1994; and Dyer and Chernoff, 1996; Fig. 11). Monophyly of atheriniforms was not supported by Rosen and Parenti (1981) or Parenti (1984b), but it has been argued for strongly in these other studies.

Atheriniform monophyly was supported by two developmental characters by White *et al.* (1984:357): short preanal length of flexion larvae and a single row of melanophores on the dorsal margin of larvae. Eight adult characters were added to the diagnosis by Dyer and Chernoff (1996:1): vomerine ventral face concave, long A1 muscle tendon to lacrimal, two anterior infraorbital bones, pelvic-rib ligament, pelvic medial plate not extended to anterior end, and second dorsal-fin spine flexible.

This atheriniform diagnosis requires homoplasy within several characters. For example, newly hatched adrianichthyids also have a single row of dorsal melanophores (White *et al.*, 1984:359). Number of anterior infraorbital bones ranges from one to three in atheriniforms (Dyer and Chernoff, 1996). The three anterior infraorbital bones of the rainbowfishes *Melanotaenia* and *Chilatherina* were considered evidence of their sister-group relationship by Dyer and Chernoff (1996:67), whereas Rosen and Parenti (1981) considered the character to be primitive for atherinomorphs. Outgroups of atheriniforms in Dyer and Chernoff's (1996: Table 2) data matrix include representative cyprinodontiform and beloniform taxa and the mullet, *Mugil*. Given the strong support for atherinomorph monophyly and the tentative support for a mullet-atherinomorph sister group relationship, as argued above, additional acanthomorph outgroups may yield alternate interpretations of phylogeny when included in a parsimony analysis of either morphological or molecular data.

Despite the ambiguity in distribution of characters discussed above, I use the formal term order Atheriniformes for the included taxa be-



**Figure 11.** Phylogenetic relationships among the subfamilies, families or tribes of atheriniform fishes as proposed by Dyer and Chernoff (1996), following Dyer (1998: Fig. 1d)

cause it is more popular than the vernacular "atherinoids."

Phylogenetic analyses of atheriniform subgroups include an analysis of the internally fertilizing freshwater and coastal family Phallostethidae, proposed as sister taxon of the marine *Dentatherina* (Parenti, 1984b, 1989b). This sister group relationship was corroborated by Dyer and Chernoff (1996) who classified *Dentatherina* in an expanded Phallostethidae. Atheriniformes are oviparous, although report of facultative embryo retention would not be surprising. Phallostethids (*sensu* Parenti, 1989b) are internally fertilizing and lay fertilized eggs. Internal fertilization was reported in the brook silverside, *Labidesthes sicculus*, by Grier *et al.* (1990).

Other morphological phylogenies of subgroups of Atheriniformes include Chernoff (1986, menidiines), Saeed *et al.* (1989, Pseudomugilidae), and Dyer (1998, Atherinopsidae).

### Conclusions

The series Atherinomorpha is a well-corroborated, monophyletic taxon. As for other such well-corroborated taxa, the list of characters diagnostic of the Atherinomorpha continues to grow (Rosen and Parenti, 1981; Parenti, 1993;



herein). Each of its included orders, the Cyprinodontiformes, Beloniformes, and Atheriniformes is monophyletic, although the quality and quantity of support for each is variable.

Molecular tests of morphological hypotheses of atherinomorph relationships agree, in large part. Where they differ, molecules present novel hypotheses of relationship, most notably in beloniforms (Lovejoy, 2000), and invite reinterpretations of our traditional understanding of morphological characters. Not all molecular analyses agree, however. Two phylogenetic hypotheses based on partial sequences of the oncogene, *X-src*, yielded different interpretations of relationships of the poeciliid *Tomeurus*. Notably, Parker's (1997: Fig. 2) hypothesis of the sister group relationship of *Tomeurus* and *Cnesterodon* was corroborated in a reinterpretation of morphological data by Ghedotti (2000). Molecular and morphological hypotheses may inform each other and point to weak areas in the other analysis. The Cyprinodontiformes has been studied most intensively during the past twenty years; morphological and molecular analyses share repeated statements of relationship to such a degree that a stable classification of the order is within reach.

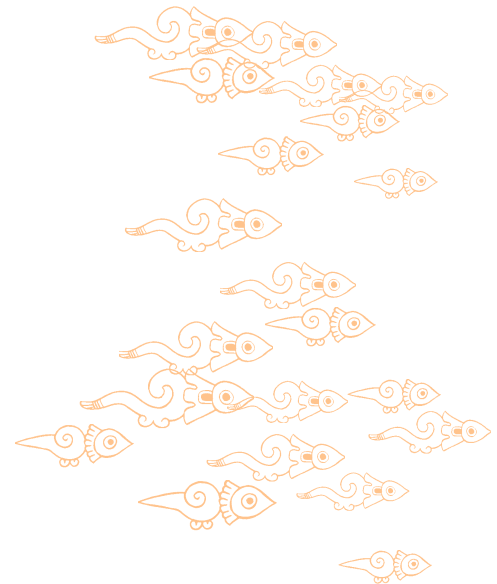
Monophyly of the order Atheriniformes has been supported by numerous morphological studies, most recently that of Dyer and Chernoff (1996). Interpretation of polarity of the characters used by Dyer and Chernoff (1996) to diagnose atheriniforms hinges on the choice of an atherinomorph outgroup, however. Their choice of mullets may be appropriate, but a broader range of outgroup taxa may yield other interpretations of atheriniform relationships. A molecular test of the Dyer and Chernoff (1996) hypothesis may reveal novel relationships as well. As for cyprinodontiforms and beloniforms, however, a molecular hypothesis of atheriniforms will not substitute for that based on morphology.

Atherinomorph fishes likely will continue to be studied broadly, especially as they include numerous model organisms, such as the poeciliids *Xiphophorus*, *Poecilia*, and *Gambusia*, the ricefishes *Oryzias*, as well as a wide range of live-bearing teleost fish taxa in the families Goodeidae and Anablepidae and other poeciliids. Phylogenetic hypotheses of atherinomorphs and their subgroups are among the most examined and re-examined within bony fishes, which makes their continued use as model organisms and their unique role in understanding evolution of fish reproduction even more justifiable.

## Acknowledgments and abbreviations

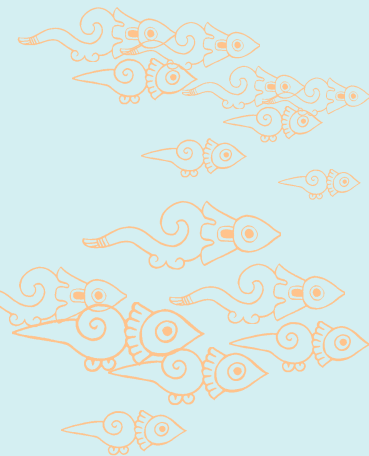
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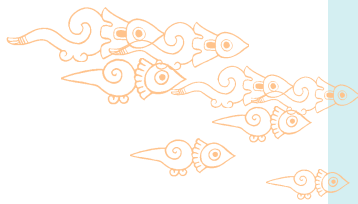
Institutional abbreviations: BMNH, The Natural History Museum, London; USNM, United States National Museum of Natural History, Washington, DC; CMK, Collection of Maurice Kottelat, Cornol, Switzerland.



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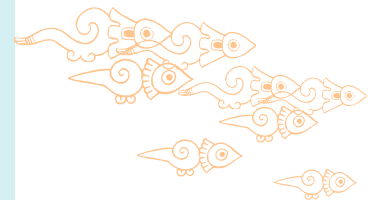
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