THE EVOLUTION OF REPRODUCTIVE AND GENOMIC DIVERSITY IN RAY-FINNED FISHES

by

JUDITH ELIZABETH MANK

(Under the Direction of John C. Avise)

ABSTRACT

Using comparative phylogenetic methods, I examined several current hypotheses and patterns in the evolution of reproductive and genomic diversity on a supertree for the ray-finned (Actinopterygii) fishes. The topics examined include the evolution of parental care and the implication of viviparity, the evolution of male mating strategies, the evolution of sex determining mechanisms and the relationship between sex chromosomes and male ornaments, evolutionary genomics and the role of genome dynamics in cladogenesis, and the role of sexual selection in increasing taxonomic diversity.

Regarding reproductive diversity, the Actinopterygii show remarkable convergence in all traits examined (parental care, internal gestation, male alternative reproductive strategies, and sex determining mechanisms). Similar parental care and male reproductive behaviors in diverse species may be caused by convergent selection on the same suite of gonadotropic hormones. Sexual selection is a major force in the evolution of both parental care and male mating strategies, though sexual selection is not correlated with sex determining mechanisms, it generally acts to increase taxonomic diversity. Internal gestation, though a complex adaptions, has originated multiple independent times throughout the Actinopterygii, and there is no evidence that, once evolved, it has ever been lost from a lineage. This may be because the evolution of viviparity is correlated with cladogenetic expansions.

Genomically, actinopterygiian fishes exhibit highly dynamic genomes, possibly due to transposon activity, changes in insertion to deletion ratios, cytogenetic rearrangement, origin and dissolution of sex chromosomes, and gene and genome duplication. This dynamicism may be a factor in the radiation of the teleosts, either by fostering adaptive radiations or via reciprocal silencing. Unexpectedly, the genome dynamics of this clade do not seem to effect higher-level genomic scaffolding, as assessed by somatic chromosome counts. Chromosome complements in the ray-finned fishes are quite stable over vast stretches of evolutionary time.

INDEX WORDS: Parental care, Sex determination, Genomics, Mating systems

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B.A., The University of Florida, 1997

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW: THE EVOLUTION OF DIVERSITY IN RAY-FINNED FISHES

INTRODUCTION

The actinopterygiian (ray-finned) fishes are a large clade (≈ 20,000 species) of vertebrates with an extraordinary diversity in many reproductive characters. Much of this diversity is confined to the teleosts, which are only 235 million years old (Fig. 1.1). Despite the power of this diversity for understanding and testing ideas regarding the evolution of reproductive (Amundsen 2003), genomic (Volff 2005), and other interesting types of characters, the ray-finned fishes have been a largely untapped resource for broad-scale comparative evolutionary analyses. This clade has been largely ignored for broad evolutionary studies primarily due to inconsistencies and polyphylies in the current taxonomy (Johnson 1993). These taxonomic problems have prevented the assembly of a well-resolved phylogeny, hampering efforts to study the evolution of interesting traits across broad groups of the Actinopterygii.

This systematic roadblock has been partially alleviated by the publication of several recent, robust, and large-scale molecular phylogenies (Ishiguro et al. 2003; Miya et al. 2003; Saitoh et al. 2003). While these phylogenies do not themselves resolve all the 435 (Nelson 1994) actinopterygiian families, they do offer, in conjunction with numerous lower-level phylogenies, the possibility for the construction of a supertree (Ragan 1992; Wiens and Reeder 1995), a maximum parsimony amalgam of multiple phylogenies based on otherwise incompatible phylogenetic data matrices. This supertree is the phylogenetic backbone on which the natural history of countless traits can be traced in order to deduce evolutionary patterns, test long-standing hypotheses, and determine correlated traits.

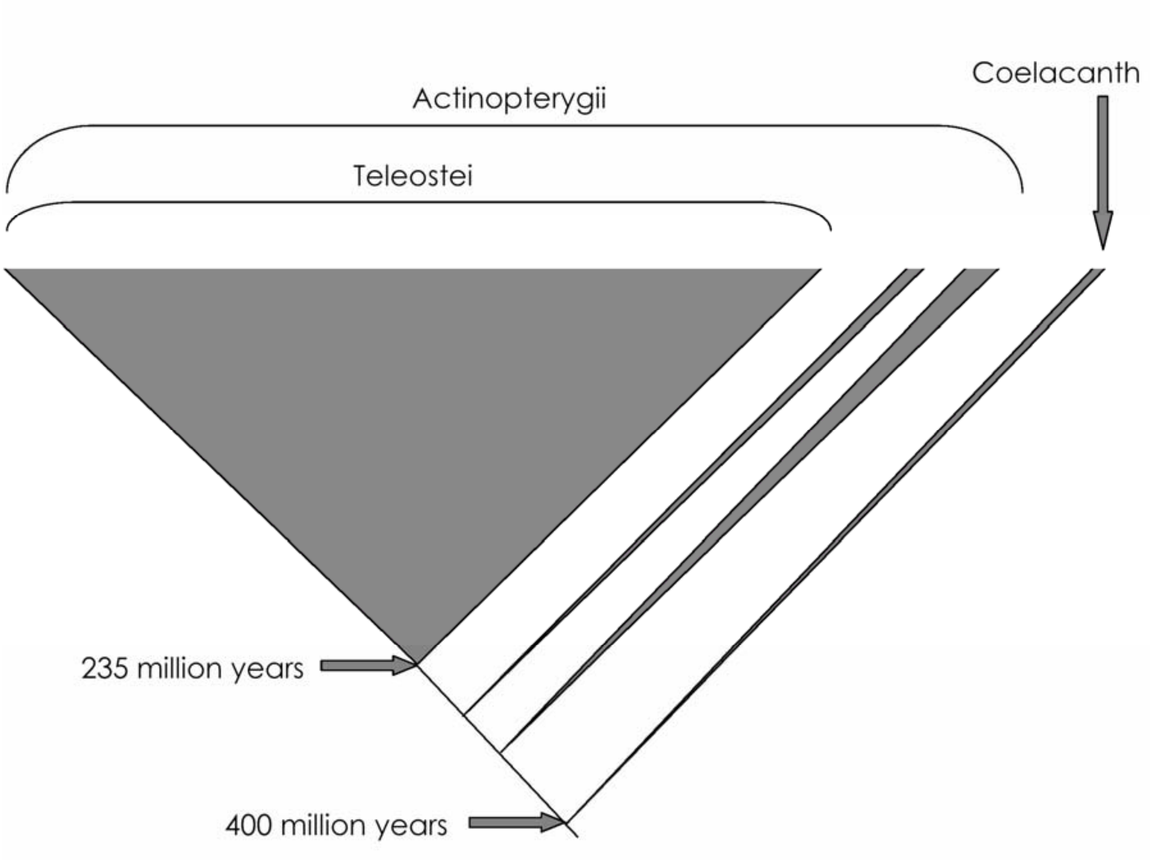

Fig. 1.1 Evolutionary history and relative rate of taxonomic diversification of actinopterygiian fishes.

GOALS OF THIS DISSERTATION

While the supertrees presented here are subject to publication of further phylogenetic data, they offer the first look at the evolution of broad patterns in the natural history of actinopterygiian fishes. The investigations described here are of three primary types. Several chapters in this dissertation are concerned with mapping the pattern of evolution and testing long-standing theories regarding reproductive traits, including sex determination, parental care, reproductive tactics, and sexual selection. Other portions of this dissertation are focused on the genomic peculiarities of fishes. In these analyses, I examine the evolutionary effects of these genomic idiosyncrasies, namely the high rate of gene and genome duplication (Robinson-

Rechavi and Laudet 2001; Robinson-Rechavi et al. 2001) and chromosomal rearrangement (Venkatesh 2003). Finally, the remainder of the chapters in the dissertation relate to the patterns of taxonomic diversity in the ray-finned fishes. These topics are not unrelated. The unusually high rate of genomic change in the ray-finned fishes is likely an ultimate cause of the enormous diversity of this group, both in terms of speciation (Haldane 1933; Holland et al. 1994; Lynch and Conery 2000; Navarro and Barton 2003a; Navarro and Barton 2003b; Ohno 1970; Stephens 1951), as well as underlying behavioral, morphological, and cytogenetic traits (Volff 2005). Similarly, emerging reproductive traits, such as female preference and sex chromosomes, may provide the necessary barriers to gene flow for speciation, and act as proximate mechanisms of diversification.

SIGNIFICANCE OF THE WORK PRESENTED HERE

It has not been previously possible to examine the evolution of actinopterygiian reproductive, genomic, and taxonomic traits in a phylogenetic context. This work represents two primary contributions to the field of evolutionary biology. First, the supertrees of the Actinopterygii and the Atherinomorpha are the first well-resolved phylogenetic frameworks for these clades. In addition to the analyses presented here, these cladograms may prove useful for many other types of comparative analysis. Second, the work presented here is unique in that it synthesizes the sizeable current descriptive literature across the ray-finned fishes in order to search for patterns and processes of evolution that might not be ascertained from work on one or a few species. None of the reproductive and genomic traits presented here have been examined across such a large and diverse group as the Actinopterygii, and therefore this work contributes to evolutionary studies by providing a broad-scale framework for considering evolutionary progressions.

Despite the considerable advantages and potential of phylogenetic comparative analyses, there is a significant caveat that should be remembered. Comparative approaches have the benefit in that they can uncover intriguing broad-scale evolutionary patterns that might not be evident in smaller scale experiments, but they suffer from the fact that the results are purely correlative. Therefore, any hypotheses as to causation that I present are entirely speculative, and are intended only as possible starting points for further experimental analysis.

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CHAPTER 2

EVOLUTION OF ALTERNATIVE MECHANISMS OF SEX DETERMINING IN ACTINOPTERYGIIAN FISHES[1]

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[1] Mank, J.E., D.E.L. Promislow, J.C. Avise. 2006. Biological Journal of the Linnean Society 87: 83-93 Reprinted here with the permission of the publisher.

ABSTRACT

I surveyed the literature for accounts in extant actinopterygiian fishes of male-heterogametic (XY) gonochorism, female-heterogametic (ZW) gonochorism, hermaphroditism, unisexuality, and environmental dependency. Then, using recently published molecular phylogenies based on whole-genomic or partial mitochondrial DNA sequences, I used this information to infer the histories and evolutionary transitions between these reproductive modes with maximum parsimony and maximum likelihood methods. Across a broad actinopterygiian phylogeny involving 25 taxonomic orders, I uncovered a highly patchy distribution of different sex-determination mechanisms, implying numerous transitions between alternative modes. This heterogeneity precluded definitive statements about ancestral states for most clades. Closer inspection of family-level and genus-level phylogenies within each of four orders further bolstered the conclusion that shifts in sex-determining modes are evolutionarily frequent and involve a variety of distinct ancestral-descendant pathways. Possible reasons for the evolutionary lability of sex-determining modes in fishes, in contrast to the conservatism of sex determination within both mammals and birds, are discussed.

INTRODUCTION

Rates and patterns of evolutionary transitions between alternative modes of sex determination are poorly understood. Why, for example, have particular sex-determining mechanisms been retained over vast stretches of evolutionary time in some vertebrate lineages but not others? Extreme conservatism is illustrated by birds and by mammals, all extant species of which share, respectively, a monophyletic ZW system of female heterogamety (Fridolfsson et al. 1998), and an XY system of male heterogamety (Bick and Jackson 1967). In actinopterygiian (ray-finned) fishes, by contrast, a wide variety of sex-determining modes (including the involvement of sex-chromosomes, autosomal genes, and environmental triggers) implies that at least several evolutionary transitions among reproductive systems have occurred, albeit at uncertain rates and in undetermined directions (Maisey 1996; Orzack et al. 1980; Ota et al. 2000).

Recent advances in DNA sequencing technology and phylogenetic analysis afford new opportunities to examine the evolutionary histories of sex-determining mechanisms. Here we employ published estimates of actinopterygiian phylogeny, based on extensive mtDNA sequences from dozens of taxonomically diverse species (Ishiguro et al. 2003; Miya et al. 2003; Saitoh et al. 2003), as phylogenetic backdrop for interpreting current distributions of alternative sex-determination modes (which we compile from a large but scattered literature). Although results of this exercise in phylogenetic character mapping remain provisional due to uncertainties in clade resolution (and other difficulties to be discussed), they do help to address several questions regarding evolutionary trends of sex determination in actinopterygiian fishes, including the following: What are the directions of evolutionary transitions between hermaphroditism and gonochorism (separate sexes), between male heterogamety and female heterogamety, and so on?

Has phylogenetic inertia constrained evolutionary shifts between alternative modes of sex determination? And, to what extent, and why, are bony fishes evolutionarily labile in sex-determining mechanisms vis-à-vis birds and mammals?

MATERIALS AND METHODS

I searched the published literature on teleosts for accounts of the following: female (ZW) or male (XY) heterogametic sex chromosomes; male-first (protandrous), female-first (protogynous), or simultaneous hermaphroditism; unisexuality; and environmental sex determination. A total of 591 species are included in this summary. I used a current classification (Nelson 1994) to reconcile taxonomic discrepancies. A complete list of papers (more than 100 total) that comprise the database for this analysis is available in Appendix A.

I explored two evolutionary levels. First, to examine broad histories of sex determination using the comparative approach (Harvey and Pagel 1991; Martins 1996), I assembled an informal supertree of actinopterygiian families. This composite tree represents an amalgamation of published phylogenies based on whole-genome or partial-genome mtDNA sequences (or in one case on morphological data) for representative species (Table 2.1). When multiple published phylogenies were available, I chose the tree topology based on the largest number of informative characters and that maximized phylogenetic resolution. To increase the sample of taxa included, I grafted monophyletic clades onto sub-tree skeletons from lower level published phylogenies (Donoghue 1989; Janz and Nylin 1998; Sillen-Tullberg 1988; Weiblen et al. 2000). I then mapped sex-determination modes onto the terminal nodes in each phylogeny and, for clarity as well as to minimize bias, pruned clades with unknown mechanisms of sex determination. At this broad level of analysis, I used a maximum parsimony approach of MacClade (Maddison and

Maddison 2000) to estimate minimum numbers of origins and evolutionary transitions between alternative sex-determination modes.

In a second, more detailed level of examination, I focused on four clades that were information-rich and polymorphic regarding sex determination modes, and that were sufficiently characterized to merit comparative phylogenetic analysis. For these lower-level phylogenies, I used both a maximum parsimony approach of MacClade 4 (Maddison and Maddison 2000) and a maximum likelihood approach of DISCRETE (Pagel 1994; Pagel 1997) to reconstruct ancestral states, and to better understand finer-scale evolutionary patterns of sex determination. I used DISCRETE to assess statistical support for these inferred states, and to calculate their relative likelihoods at each ancestral node in a given tree (Schluter et al. 1997). Although Pagel's model can incorporate different branch lengths (ages of clades) into the analysis, the absence of sufficient temporal information in the reconciled molecular trees forced me to consider only the cladogenetic aspect of tree structure. Thus, in DISCRETE, all branch lengths were coded as equal to one (Weiblen et al. 2000).

RESULTS

Broad-scale Phylogenetic Character Mapping

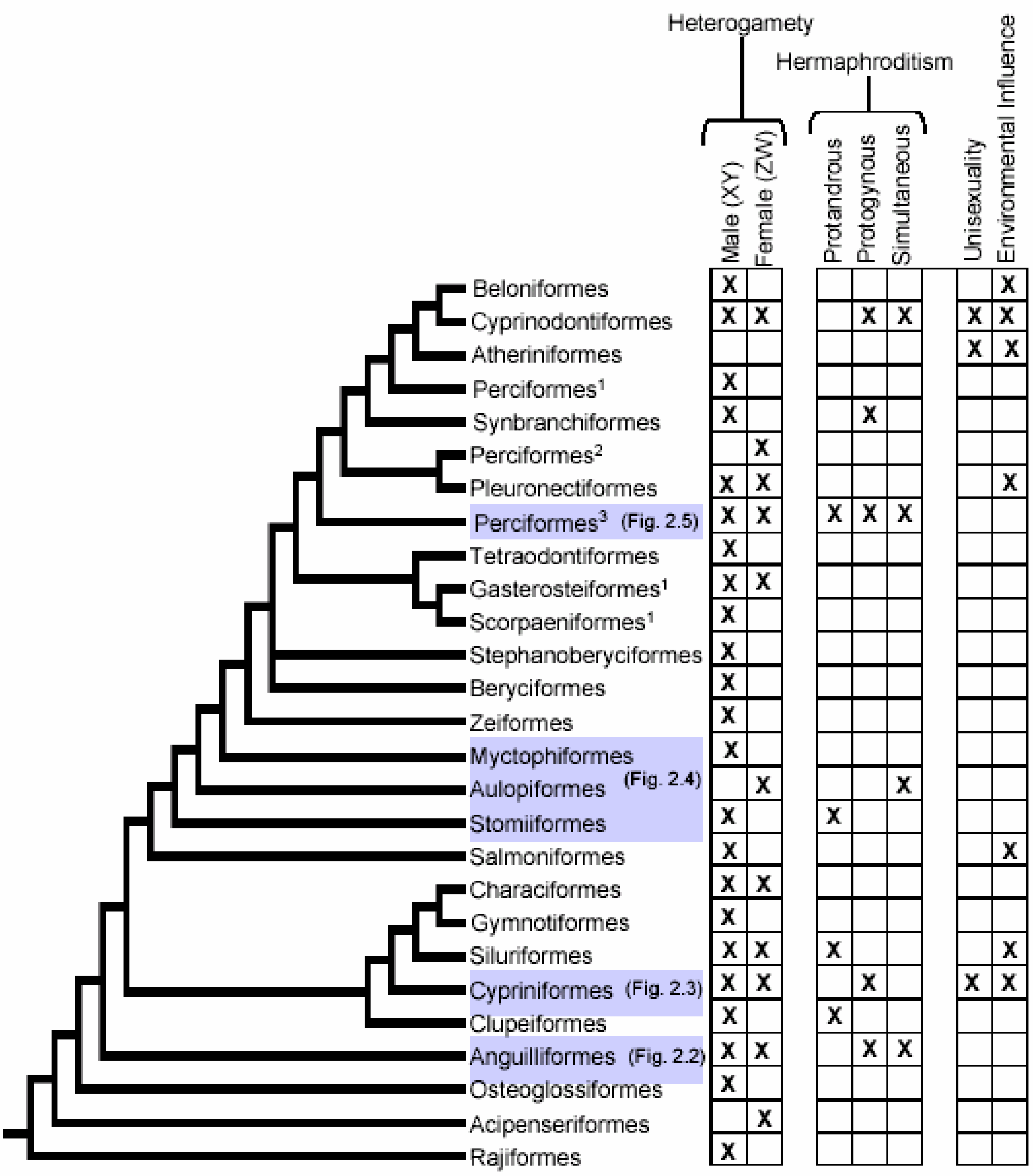
Figure 2.1 shows the condensed ordinal-level composite tree, with sex-determining modes cataloged within each clade. Fifteen actinopterygiian orders contain sex-chromosome gonochorist species with no known hermaphroditic species. The reverse was never true, because all clades with hermaphroditic species also contained members with sex chromosomes. Species representing both sex-chromosome-based and hermaphroditic reproductive modes are known in

Table 2.1 Published phylogenies, and their data matrices, used to construct actinopterygiian composite tree.

Clade	Published phylogenies	Data matrix
1. Actinopterygii	(Ishiguro et al. 2003)	complete mitochondrial genome
	(Miya et al. 2003)	complete mitochondrial genome
	(Saitoh et al. 2003)	complete mitochondrial genome
2. Anguillidae	(Inoue et al. 2001)	complete mitochondrial genome
	(Obermiller and Pfeiler 2003)	mitochondrial 12S and 16S rRNA
3. Cypriniformes	(Liu et al. 2002)	mitochondrial control region
	(Perdices and Doadrio 2001)	mitochondrial ATP syn 6,8, cyt B
4. Basal neoteleosts	(Elmerot et al. 2002)	complete mitochondrial genome
	(Miya et al. 2003)	complete mitochondrial genome
	(Ota et al. 2000)	mitochondrial cyt B
4. Gobioidei	(Akihito et al. 2000)	mitochondrial cyt B
	(Thacker 2003)	mitochondrial ND1, ND2, COI
	(Wang et al. 2001)	mitochondrial 12S rRNA, tRNA
	(Pezold 1993)	morphology

nine orders. With regard to gonochoristic actinopterygiians displaying sex chromosomes, the incidence of male heterogamety (appearing in 22 orders) was approximately double that of female heterogamety (10 orders). In permutation tests (Harvey and Pagel 1991; Manley 1991), isolated monophyletic incidences of XY, ZW, and hermaphroditic reproductive modes showed no significant departures from random-combination expectations (given their frequencies) across the teleost phylogeny (all $p > 0.1$). We found reports of environmental controls on sex differentiation in seven actinopterygiian orders, six of which also included species with chromosomal sex determination. Unisexual taxa were found in three taxonomic orders representing at least two widely separated branches in the teleost tree.

Thus, the phylomap in Fig. 2.1 strongly suggests that particular reproductive modes had polyphyletic origins. For example, maximum-parsimony reconstructions of ancestral states imply that hermaphroditism probably emerged at least nine times during actinopterygiian evolution, and that female heterogamety may have evolved independently 11 times or more. By contrast, the formal analysis indicates just one origin for male heterogamety in actinopterygiian, but this is probably a gross underestimate. Maximum parsimony earmarks XY systems as basal in the tree because they appear at face value to be by far the most common and widespread mode of sex determination in teleosts (Fig. 2.1). However, appearances in this case may be deceiving due to a reporting bias in the literature. Ohno (Ohno 1967) argued that autosomal sex determination is a likely precursor to heterogametic sex chromosomes, but autosomal modes are almost impossible to confirm from cytological evidence (the usual basis for identifying sex chromosomes). Indeed, this lack of certainty forced the omission of “autosomal” sex-determination from this analyses. Therefore, instances of sex-chromosome heterogamety in actinopterygiians might be considerably underestimated from current karyotypic evidence.



	Male (XY)	Female (ZW)	Protandrous	Protogynous	Simultaneous	Unisexuality	Environmental Influence
Beloniformes	X						X
Cyprinodontiformes	X	X		X	X	X	X
Atheriniformes						X	X
Perciformes¹	X						
Synbranchiformes	X			X			
Perciformes²		X					
Pleuronectiformes	X	X					X
Perciformes³ (Fig. 2.5)	X	X	X	X	X		
Tetraodontiformes	X						
Gasterosteiformes¹	X	X					
Scorpaeniformes¹	X						
Stephanoberyciformes	X						
Beryciformes	X						
Zeiformes	X						
Myctophiformes	X						
Aulopiformes		X			X		
Stomiiformes	X		X				
Salmoniformes	X						X
Characiformes	X	X					
Gymnotiformes	X						
Siluriformes	X	X	X				X
Cypriniformes (Fig. 2.3)	X	X		X		X	X
Clupeiformes	X		X				
Anguilliformes (Fig. 2.2)	X	X		X	X		
Osteoglossiformes	X						
Acipenseriformes		X					
Rajiformes	X						

Figure 2.1 Condensed, ordinal-level composite phylogeny for actinopterygiians displaying known sex-determining mechanisms. Polyphyletic clades within the current taxonomy are marked and indicated to the side. Shaded boxes indicate clades that are examined in greater detail in Figs. 2.2-2.5. Polyphyletic orders are indicated on the cladogram, and are as follows: Perciformes^1 = Gobiesocoidei and Blennioidei; Perciformes^2 = Caragnidae; Perciformes^3 = Gobioidei;Gasterosteiformes^1 = Gasterosteioidei; Scorpaeniformes^1 = Cottoidei.

Fine-scale Phylogenetic Character Mapping

Finer-scale character mapping in each of four actinopterygiian clades paints a clearer picture of the polyphyletic origins of all the sex-determining mechanisms studied here. In each case, results from maximum parsimony results were identical to those in maximum likelihood reconstructions. For example, all reconstructed ancestral states that received only poor or marginal support under maximum likelihood were also equivocal under maximum parsimony, and vice versa. Thus for clarity, and also because parsimony can better deal with polytomies in trees, only results from maximum parsimony are presented here.

In various of the four fine-scale analyses, instances of multiple independent origins for hermaphroditism, female and male heterogamety, and/or unisexuality could be inferred. For example, the anguilliform phylogeny (Fig. 2.2) illustrates polyphyletic origins for male heterogamety, with XY lineages apparently having emerged from both hermaphroditic and female-heterogametic lines. All of the hermaphroditic species in this phylogeny were contained in reef-dwelling members of Muraenidae.

The cypriniform phylogeny (Fig. 2.3) likewise implies polyphyletic origins for at least some sex-determining modes, although the great diversity in reproductive systems in this group made it impossible to estimate, with confidence, ancestral states above the genus level. If it is assumed, for example, that hermaphroditism was the ancestral condition, then female heterogamety and unisexuality must each have arisen at least twice; and if it is assumed that female heterogamety was ancestral, then hermaphroditism and unisexuality would each have originated at least twice. Also evidenced in this case is the strong likelihood that male heterogamety (in *Cyprinus*) has indeed arisen from some other ancestral sex-determining mode.

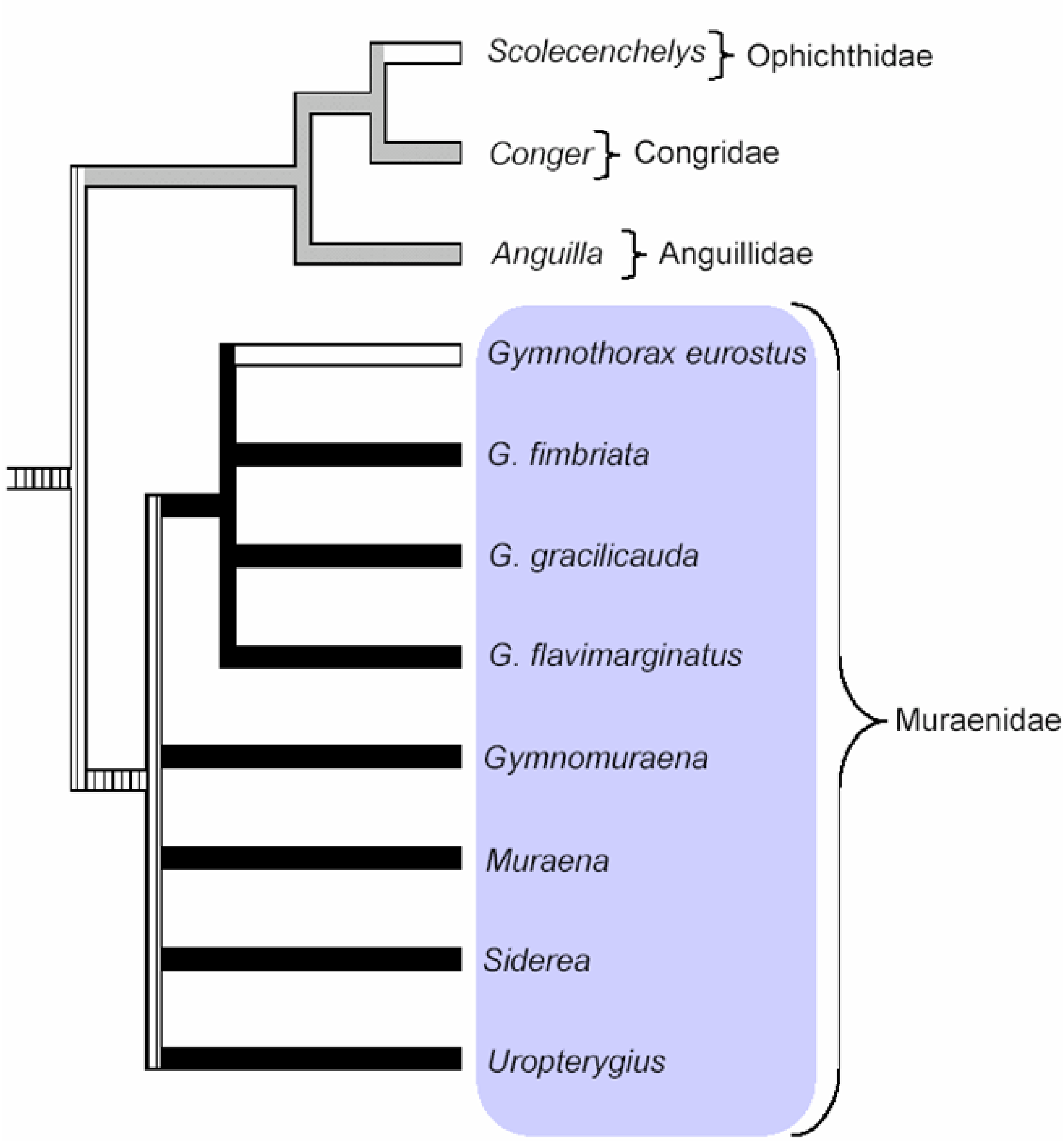


Fig. 2.2 Phylogeny for Anguilliformes. Genera often associated with marine reef habitats are shaded. Phylogenetically reconstructed ancestral modes of sex-determination, shown along branches, are indicated as follows: black lineages, hermaphroditism; white lineages, male heterogamety (XY); gray lineages, female heterogamety (ZW). Hatched branches indicate equivocal ancestral states.

The basal neoteleosts (Fig. 2.4) further illustrate the repeated origin (but often short evolutionary persistence) of hermaphroditic lineages, as well as the emergence of female heterogamety from a probable ancestral mode of male heterogamety in the aulopiforms. Finally, the phylogeny for Gobioidei (Fig. 2.5) again illustrates multiple evolutionary shifts between different reproductive modes, as well as a clustering of hermaphroditic lineages in reef-dwelling taxa.

DISCUSSION

Results from this analysis are provisional for several reasons. First, actinopterygiian phylogeny is far from fully resolved, and the inclusion of many more taxonomic families as well as additional classes of molecular (or other) information should help to further clarify the evolutionary histories of sex-determination modes. For example, the mtDNA-based phylogenies supported long-standing suspicions of polyphyly for several teleost orders, including the Scorpaeniformes, Gasterosteiformes, and Perciformes (Johnson 1993). Also, even whole-genome mtDNA sequences failed to resolve the exact positions of several key groups within the huge order Perciformes, and without this information a complete analysis of actinopterygiian phylogeny retains considerable uncertainty and bias (Sanderson et al. 1998; Weiblen et al. 2000).

Second, current understandings of the genes and mechanistic pathways involved in sex determination are grossly inadequate. Thus, although broad categories of sex determination are clearly polyphyletic in actinoptergyiians, at least some of their component aspects may yet prove to be monophyletic. Finally, karyotyping is another area where better information is needed. Often, fish chromosomes are notoriously small and numerous, so further refinements in

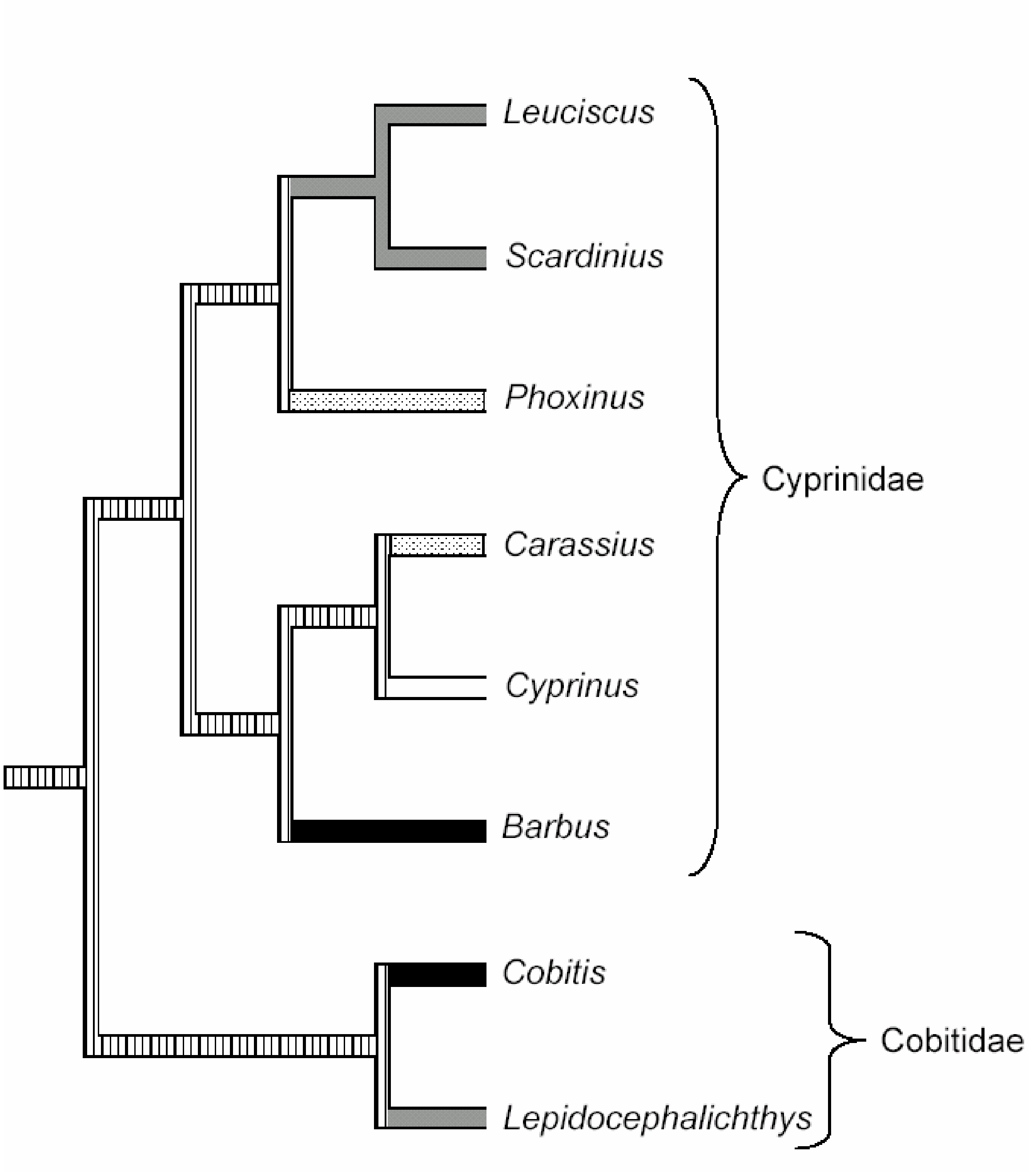


Fig. 2.3 Phylogeny for Cypriniformes. Dotted branches signify unisexuality. See Figure 2.2 for a description of pattern codes for modes of sex determination along other branches in the phylogeny.

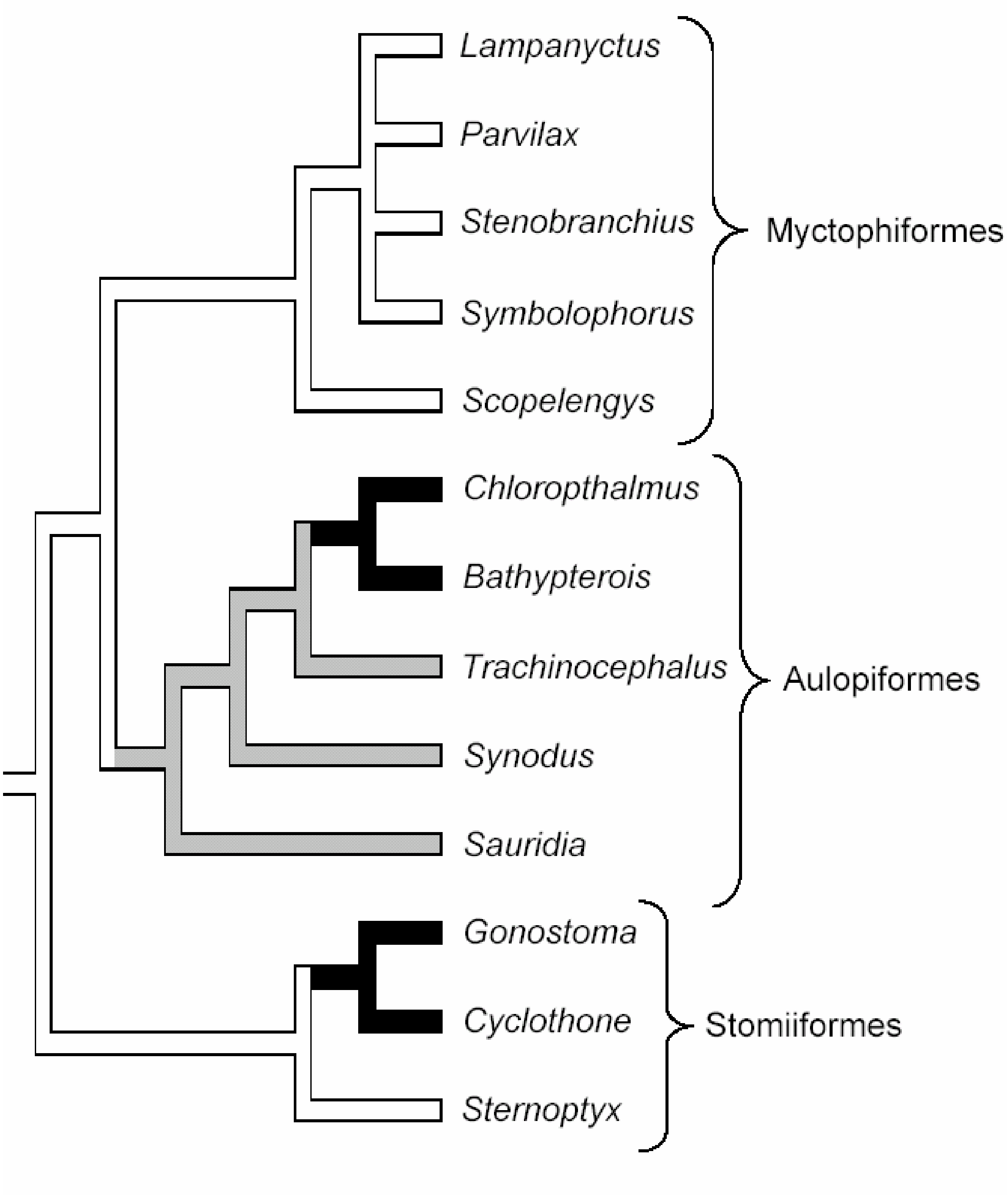


Fig. 2.4 Phylogeny for basal Neoteleosts. See Figure 2.2 for a description of pattern codes for modes of sex determination along branches in the phylogeny.

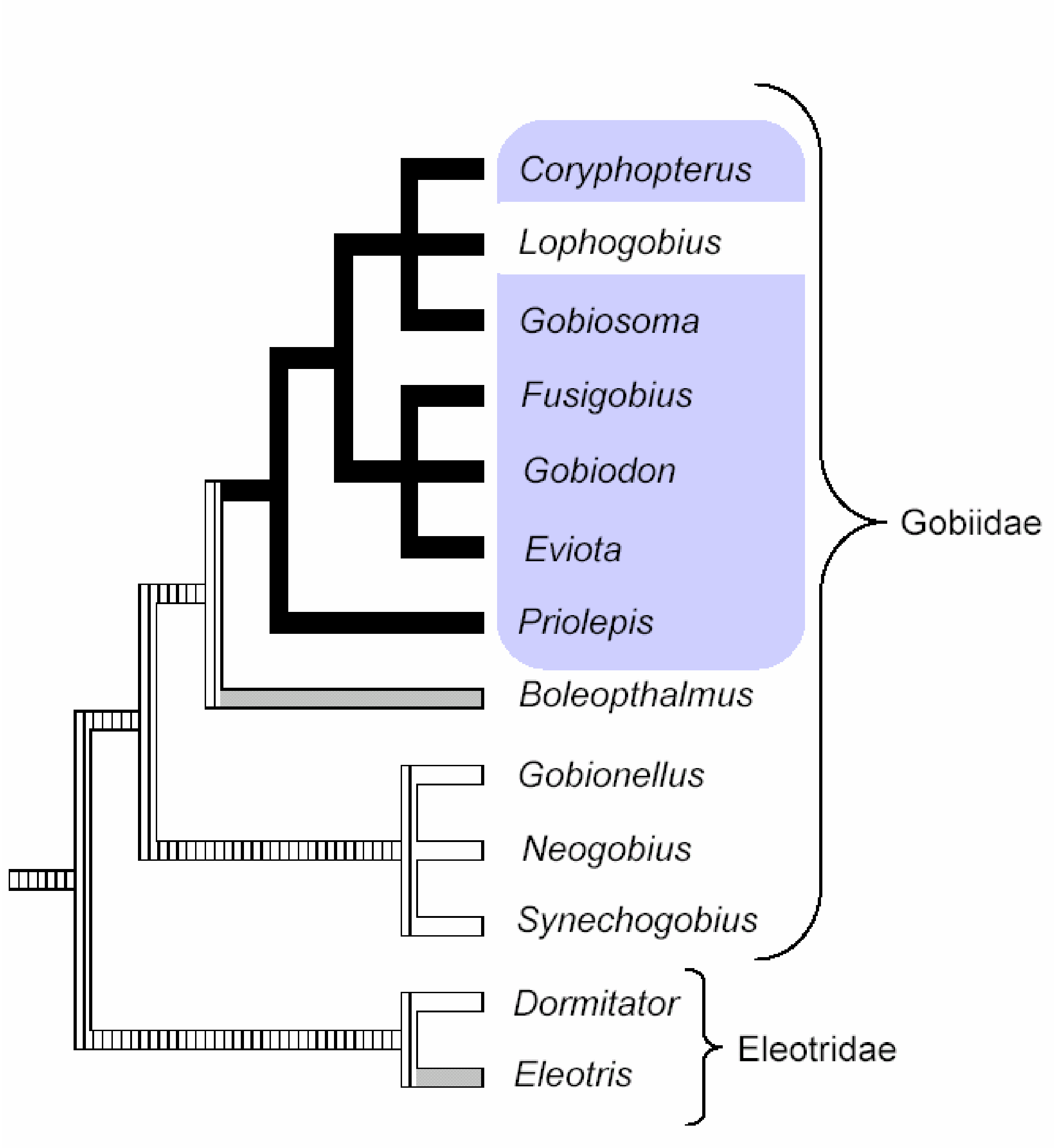


Fig 2.5. Phylogeny for the Gobioidei. Shaded genera are typically associated with reefs. See Figures 2.2 and 2.3 for descriptions of pattern codes for modes of sex determination along branches in the phylogeny.

cytological techniques may help to distinguish, for example, true autosomal modes of sex determination from those that involve now-cryptic sex chromosomes. Despite these limitations, several striking patterns have emerged from these analyses. Most salient is that most if not all of the broad categories of sex-determination in teleosts had multiple evolutionary origins.

Alternative Reproductive Modes

Environmental dependence. Influences of environmental factors, primarily temperature, on sexual differentiation are known in seven actinopterygiian orders, six of which (the exception being Atheriniformes) also contain species with documented chromosomal sex determination. In the atheriniforms, temperature exposures during development are known to interact with genetic factors to determine an individual's sex (Lagomarsino and Conover 1993). The restricted phylogenetic distributions of temperature influences on sex in fishes suggest that this phenomenon may have evolved several times, secondarily, as an auxiliary mechanism that can modify or even over-ride chromosomal sex determination. This could be advantageous, for example, if ecological conditions sometimes favor particular families that can temporarily alter their sex ratios (Conover and Heins 1987; Trivers and Willard 1973). Another possibility, however, is that environmental influences are simply harder to detect (because they normally involve experimental manipulation).

Unisexuality. The current phylogenetic analysis merely adds support to what was already well-known about unisexuality in fish (and other vertebrates), namely that the phenomenon is polyphyletic, evolutionarily ephemeral, and generally confined in extant lineages to the outermost tips of branches in the vertebrate phylogenetic tree (Avise et al. 1992; Dawley and Bogart 1989). Indeed, prior evidence for the polyphyletic origins of unisexuality was even more *prima facie*: across diverse vertebrate taxa, each of the approximately 70 known unisexual biotypes originated via one or more independent hybridization events between closely related sexual species (Avise et al. 1992; Dawley and Bogart 1989).

Sex Chromosomes. Most actinopterygiian lineages for which data are available include representatives with known chromosomal sex determination (Fig. 2.1). Arkhipchuck (Arkhipchuk 1995) estimated that sex-chromosome systems exist in about 10 - 50% of all fish species (although not all of these are evident in the gross karyotypic inspections conducted to date). Whatever the exact tally, due to the many apparent evolutionary transitions among sex-determination modes (as well as missing data for some teleost orders), we cannot definitively conclude that sex chromosomes were the ancestral mode of sex determination in actinopterygiian.

Nor within the XY versus ZW dichotomy can we determine the ancestral condition with certainty. Although the majority of extant lineages include species with male heterogamety (Fig. 2.1), this does not necessarily indicate that the XY mode is plesiomorphic for the group for the following reasons: male and female heterogamety are both dispersed throughout much of the actinopterygiian tree; both XY and ZW systems are evident in some non-teleost fishes, including the chondrichthyan outgroup employed in this study (Fig. 2.1); many other fish species do not have well demarcated sex chromosomes (Harvey et al. 2002; Schwartz and Maddock 2002), and at least some of these probably lack them entirely (Devlin and Nagahama 2002); and in at least one of our finer-focus appraisals (involving Cypriniformes), male heterogamety appears to have secondarily evolved from some other ancestral sex-determination mode. All of these lines of evidence indicate that sex chromosomes of both the XY and ZW type can arise repeatedly and perhaps inter-convert quite readily (but see below) during evolution.

Autosomal sex determination is also thought to be common in actinopterygiians, and has been proposed as the probable ancestral condition prior to the initial evolution of well-differentiated sex chromosomes (Ohno 1967; Traut and Winking 2001). If this is indeed correct (and not merely an artifact of poor karyotypic resolution), then it must be the case that this state commonly gives evolutionary rise to sex-determination systems with a clear XY or ZW basis. This would also provide an alternative model (other than direct XY ⇔ ZW interconversion) for the recurrent origin of the XY or ZW systems along the actinopterygiian evolutionary tree. In summary, the various phylogenies we examined indicate that actinopterygiian lineages may readily switch back and forth between male and female heterogamety over evolutionary time, but we cannot determine from the present analyses whether or not these changes are interspersed with intervals devoid of bona fide sex chromosomes.

The notion that sex chromosomes arise repeatedly from autosomes gains some support from evidence that the X-Y and Z-W chromosome pairs are not homologous (Marshall-Graves and Shetty 2001), and also that actinopterygiian sex chromosomes of the same general heterogametic mode are polyphyletic (Solari 1994; Woram et al. 2003). It is thus improper to assume that recurrent karyotypes displaying the same heterogametic mechanism are necessarily homologous in all actinopterygiians, especially across large evolutionary distances. Further molecular-genetic characterizations (Froschauer et al. 2002) will be required to distinguish genuine homology from analogy.

I prefer not to speculate about the endless variety of eco-biological factors that might influence the evolution of alternative sex-determination modes, except to note one recurring trend (not previously appreciated, to our knowledge) from this literature review: Sex chromosomes were often associated with diadromous species (e.g., catadromous Anguillidae and anadromous Salmonidae). In such species, individuals congregate on spawning grounds, such that a given individual is unlikely to encounter a shortage of potential mates. Thus, I could speculate that by virtue of conferring a relatively equitable sex ratio in such a breeding population, chromosomal sex determination could enhance mean individual fitness under a negative frequency dependent scenario (Fisher 1930). More generally, perhaps the phylogenetic approach that we have taken in this study will be useful in identifying additional such relationships that are worthy of further functional investigation.

Hermaphroditism. This reproductive mode is scattered throughout much of the actinopterygiian phylogeny (Fig. 2.1), but appears to be embedded within clades otherwise characterized by gonochorist species with chromosomal sex determination (Figs. 2.2-2.5). Thus, extant hermaphroditism is most likely polyphyletic and derived, and no single hermaphroditic lineage seems to be evolutionarily ancient.

Hermaphroditism appears to be particularly common in reef-dwelling fishes, as illustrated in this study by the Muraenidae eels (Fig. 2.2) and Gobiinae gobies (Fig. 2.5). Like most reef fish, these eels and gobies have a pelagic larval phase that promotes spatial gene flow (Robertson 2001) but also entails high risks in successful larval settlement and subsequent mate acquisition. Reef fishes tend to have inherently patchy habitat distributions, and many (such as moray eels) have low population densities. Many reef fishes (including gobies) show evidence that fitness advantages to functioning as a male (or as a female) change during life according to an individual's body size or ecological circumstance (St. Mary 1993; St. Mary 1994; St. Mary 1996; St. Mary 1998). All of these factors, which are generally consistent with previous predictive theory (Ghiselin 1969; Smith 1975), might have contributed to selection pressures recurrently promoting evolution of hermaphroditism in various reef fishes. However, this alone would not account for why hermaphroditism typically seems to be evolutionarily short-lived.

Furthermore, the emergence of hermaphroditism in the basal Neoteleosts (Fig. 2.4) seems hard to rationalize by the above logic. Although hermaphroditic Aulopiformes (*Chloropthalmus* and *Bathypterois*) and Stomiiformes (*Cyclothone* and *Gonostoma*) are deep water species in which hermaphroditism might be favored for reasons of low population density (for example), other deep-water representatives of these same orders are sex-chromosome gonochorists (Fig. 2.4). Likewise, it is unclear what if any distinguishing ecologies might apply to hermaphroditic cypriniform genera compared to their gonochoristic relatives (Fig. 2.3). Phylogenetic analyses of the sort conducted here cannot by themselves address the idiosyncrasies of why particular lineages evolved one or another mode of sex-determination; for that, focused ecological and other biological studies are required. Phylogenetic character mapping merely can help to identify particular lineages in which such analyses might meaningfully be focused.

Phylogenetic Constraint Versus Evolutionary Lability

Various hypotheses might be advanced for why sex determination appears evolutionarily labile in fishes but conservative in mammals and birds. Perhaps fish lineages that are polymorphic for sex-determining systems are simply much older and thus have had more evolutionary time to experience shifts between modes. The current analysis demonstrates quite strongly, however, that this is unlikely the case. Although actinopterygiian (ray-finned) fishes originated in the Devonian and thus are collectively older than birds and mammals, which originated about 150 and 200 million years ago, respectively (Feduccia 1996; Vaughan 1986), the earliest known teleosts are from 235 million-year-old fossils (Maisey 1996), and are thus not greatly older than birds or mammals. More telling is the fact that far more recent teleost clades (e.g., within an order, family, or sometimes a genus) often show a greater diversity of sex-determining mechanisms than do the monotypic classes of mammals and birds. The cypriniforms, for example, show enormous diversity in sex-determining mechanisms (Fig. 2.3), but their earliest fossils date only to the Eocene (Carroll 1988). Some shifts between reproductive modes in teleosts are known to have occurred even in contemporary time, as for example in transitions from gonochorism to unisexuality in the genus *Poecilia* (Avise et al. 1992).

Another hypothesis (one that we favor) appeals to developmental and genomic peculiarities of actinopterygiian fishes. Developmentally, testes and ovaries in ray-finned fishes, as well as ovotestes in some species, all derive from the same precursor tissue (Atz 1964), and can rather flexibly differentiate at various life stages within an individual. This differs from the situation in most other vertebrates including birds and mammals, where gonadal differentiation activates early in development and generally is irreversible (Hoar 1969).

This relative developmental plasticity in fishes carries implications with regard to sex determination. First, gonadal development and sexual differentiation are somewhat decoupled. Second, in fish lacking genetically hardwired sex chromosomes, sexual differentiation and even sexual alterations can take place late in development (e.g., even well after sexual maturity in sequential hermaphrodites). This opens wider windows of opportunity for environmental pressures such as social status (Robertson 1972), ecological conditions (Conover 1984; Francis 1992), and population composition (Warner and Hoffman 1980) to play proximate roles in sex determination, and also, via the selection pressures they impose, to influence the genetic evolution of underlying sex-determining mechanisms. Furthermore, negative selection against inter-sex individuals is more likely to be diminished or absent. Unlike most other vertebrates, where mixed signals in sexual differentiation often result in low reproductive fitness or even sterility (Armstrong 1964; Taber 1964), many fish are less prone to suffer reproductive losses when distinctions between the sexes are less than definitive. For example, in some actinopterygiians an ovotestis produces both sperm and ova, and some individuals may have higher fitness than gonochorists (Smith 1975). This may help to explain why hermaphroditic lineages are not uncommon.

In some important regards, fish also display more rapid genomic evolution than most other vertebrates. Notably, a fast pace of genic and genomic duplication (Robinson-Rechavi and Laudet 2001; Robinson-Rechavi et al. 2001), both recent (Allendorf and Thorgaard 1984; Ferris 1984; Van de Poole et al. 2004) and ancient (Amores et al. 1998; Taylor et al. 2003), has been documented in the ray-finned fishes. Such duplications initially provide redundancy and thereby open a potential for replicates of genes and gene pathways to evolve new, often related functions (Ohno 1970). Thus, the increased evolutionary potential afforded by regional and genome-wide duplications probably creates additional evolutionary flexibility in sex-determination pathways.

The retrograde model of pathway evolution (Wilkins 2002) predicts that emergent genes at the apex of a sex-determining hierarchy can requisition downstream components in the pathway. In this way, even if the underlying basis of a sex-determination mode is conserved, that mode nonetheless can become altered by a new set of conditions. For example, any apical sex-determining genes that come under the control of inducible promoters can in theory put sex determination under social and environmental influence. Constitutive promoters, by contrast, presumably inhibit alterations in sex determination, but when recombination is curtailed on chromosomes carrying different male and female alleles at loci encoding such promoters, sex chromosomes may evolve (Charlesworth 1991; Ohno 1967; Rice 1994). Also, female and male heterogamety can interconvert in evolution when emergent upstream sex-determining switches are closely linked to a gene of high adaptive value (Bull and Charnov 1977), or when new upstream switches emerge in systems with large sex ratio biases (Ogata et al. 2003; Wilkins 1995; Wilkins 2002).

Some evidence does exist for this type of evolution in the sex-determining pathways of teleosts. Locus *Dmrt1* is typically a downstream component in sex-determination cascades, and has homologies throughout the animal kingdom from invertebrates (Baker et al. 1987; Shen and Hodgkin 1988) to mammals (Raymond et al. 2000). In fishes, *Dmrt1* has been recognized as a conserved module in the male sex-determination pathway in heterogametic species including *Oncorhynchus mykiss* (Marchand et al. 2000) and *Oryzias latipes* (Brunner et al. 2001), and in hermaphrodites including *Acanthopagrus schlegeli* (He et al. 2003) and *Monopterus albus* (Huang et al. 2002). Duplicate copies of *Dmrt1* may also assume a role of apical initiator in sex determination in some species such as *Oryzias latipes*, where *DmY*, a recent duplicate of the *Dmrt1* gene (Lutfalla et al. 2003), is thought to be the initiating switch in male sex determination (Matsuda et al. 2003; Nanda et al. 2003). In the future, further elucidation of such mechanisms, especially when integrated with phylogenetic considerations, should offer much additional insight into the evolution of sex-determination modes in ray-finned fishes.

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CHAPTER 3

SEX CHROMOSOMES AND MALE ORNAMENTS: A COMPARATIVE EVALUATION IN RAY-FINNED FISHES[1]

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ABSTRACT

Theory predicts that the mechanism of genetic sex determination can substantially influence the evolution of sexually selected traits. For example, female heterogamety (♂ZZ/♀ZW) can favor the evolution of extreme male traits under Fisher's runaway model of sexual selection. I empirically test whether the genetic system of sex determination has played a role in the evolution of exaggerated male ornaments in actinopterygiian fishes, a clade in which both female-heterogametic and male-heterogametic systems of sex determination have evolved multiple times. Using comparative methods both uncorrected and corrected for phylogenetic non-independence, I detected no significant correlation between sex-chromosome systems and sexually selected traits in males. Results suggest that sex-determination mechanism is at best a relatively minor factor affecting the outcomes of sexual selection in ray-finned fishes.

INTRODUCTION

Sexual selection via female preferences has promoted the evolution of elaborate male ornaments in many animal groups. One evolutionary mechanism that can promote the exaggeration of male display traits is indirect selection, in which a female preference evolves because of a genetic correlation that it naturally develops with the male display (Kirkpatrick and Ryan 1991). Under a Fisherian runaway process, if the genetic correlation exceeds a critical value, then the female preference and male display can co-evolve at an explosive rate (Fisher 1952; Lande 1981). Under a good-genes process, if the male display is genetically correlated with traits that are under positive directional selection, then female preference genes that become correlated with male display genes will also become associated with good genes. Both Fisher's runaway and the good-genes processes of sexual selection rely critically on genetic correlations between female preferences and genes either for male displays (in the runaway) or enhanced lifetime fitness (in good genes). Recent theoretical work has considered how sex linkage might influence these correlations and thereby impact the evolution of sexually selected traits (Hastings 1994; Kirkpatrick and Hall 2004).

Some animal clades such as mammals have male heterogamety (that is, males are XY and females XX), while in others such as birds females are heterogametic (males are ZZ and females ZW). The models show that Z-linkage of female preference is especially conducive to a Fisherian runaway, whereas X-linkage of female preference coupled with autosomal inheritance of male displays can favor the operation of a good-genes process. These effects can be substantial (Table 1 of Kirkpatrick & Hall 2004). Furthermore, the sex chromosomes in some organisms may harbor a disproportionately large fraction of the total genetic variation for male traits and perhaps for female mating preferences (Iyengar et al. 2002; Prowell 1998; Reinhold

1998). It thus follows that lineages with different modes of genetic sex determination might show different tendencies to evolve exaggerated male traits. Unfortunately, very few empirical tests of these theoretical predictions are available in the literature. Furthermore, a reporting bias likely exists wherein positive associations between sex chromosome system and sexually selected traits (Iyengar et al. 2002; Reeve and Pfennig 2003) may have appeared in print more often than outcomes in which no such empirical relationship was detected. These factors have complicated efforts to assess any general relationship that might exist between male heterogamety and good-genes processes, or between female heterogamety and Fisherian run-away.

Among major vertebrate clades, ray-finned fishes (Actinopterygii) are unrivaled in the evolutionary lability of their sex-determination mechanisms (Mank et al. 2006; Marshall-Graves and Shetty 2001; Solari 1994; Volff 2005; Woram et al. 2003). ZW and XY (as well as other) modes of sex determination have each arisen multiple times and inter-converted recurrently in actinopterygiian lineages (Mank et al. 2006), thus making these fish ideal for testing hypothesized associations between exaggerated male ornaments and alternative systems of sex determination. Also, the recent construction of a provisional actinopterygiian supertree (Mank et al. 2005) now makes it possible to examine the empirical correlation between male ornaments and sex chromosomes in a comparative phylogenetic context. Here I examine the association between sex determination and the outcome of sexual selection (male ornaments) in ray-finned fishes in order to evaluate the models of indirect selection reviewed above.

MATERIALS AND METHODS

I assembled a bibliographic database on sex-chromosome-based mode of sex determination and male sexually selected characters in 154 species of actinopterygiian fishes (Appendix B.). I focused on male ornament traits that repeatedly have been shown to be the result of female choice in various fish taxa. These include published descriptions of elongate fins (Basolo 1990; Harrington 1997; Kuwamura et al. 2000; Suk and Choe 2002), breeding tubercles (Kortet et al. 2003; Kortet et al. 2004), sexual dichromatism defined as nuptial colorations expressed more noticeably in males than in females of a species (Amundsen and Forgren 2001; Houde and Endler 1990; Reimchen 1989; Stott and Poulin 1996), and electric mating calls (Curtis and Stoddard 2003). I omitted from the database sexually dimorphic traits such as gonopodia and body-size differences that are not unambiguously a consequence of female choice (and for which male-male sexual selection and/or natural selection may largely be responsible). Regarding sexual dichromatism, I did not tally counts of a particular ornament type exhibited by males (specific numbers of stripes, spots, patches, etc.), but simply recorded, from published descriptions in field guides and species accounts, whether males displayed any such traits and if so how many such different trait types. This approach is conservative because it avoids overestimating numbers of “independent” traits (Reeve and Pfennig 2003) that in some studies have proved to be pleiotropically related (Fitzpatrick 2004).

The statistical analyses entailed both qualitative and quantitative appraisals of male ornaments, and were conducted both uncorrected (Harvey and Rambaut 1998; Price 1997; Ricklefs 1996) and corrected (Felsenstein 1985; Harvey and Pagel 1991) for phylogenetic non-independence. In the qualitative assessment, each species was scored for presence versus absence (in published reports) of any sexually selected ornaments; and in the quantitative assessment, each species was scored for total numbers of different male ornament types. For each of these data treatments, phylogeny was either ignored or explicitly accommodated as follows.

First, I treated all 154 surveyed species as independent observations, i.e. without regard to their phylogenetic associations. I calculated the correlation (*r*) between sex-chromosome type and presence and number of male ornaments. I also tested for random association using Fisher's exact test (for presence or absence data) and χ^2 tests (for numbers of male ornament types).

Second, to correct for phylogeny, I used the actinopterygiian supertree topology (Mank et al. 2005), which I augmented with genus- and species-level phylogenies when a given taxonomic family was polymorphic for sex-chromosome system. These lower-level augmented phylogenies, each based on robust analyses that yielded well-resolved tree topologies, were included for Cyprinidae (Briolay et al. 1998; Cunha et al. 2002), Loricariidae (Armbruster 2004), Salmonidae (Phillips et al. 2004), Gobiidae (Penzo et al. 1998; Thacker 2002), Fundulidae (Grady et al. 2001), and Poeciliidae (Breden et al. 1999; Ghedotti 2000; Lydeard et al. 1995; Meyer 1997).

The cladogram was analyzed for possible trait correlations using the maximum likelihood program DISCRETE for presence/absence data, and MULTI-STATE for quantitative data (Pagel 1994; Pagel 1997). In the presence/absence analysis, I compared the model of correlated evolution to a null model of independent evolution between male ornaments and chromosomal sex-determination mode (likelihood ratio test, χ^2 distribution with four degrees of freedom). For the quantitative analysis, I compared the correlated model to a null model in which the evolutionary rate of male ornament acquisition was equal for both of the sex-chromosome types (likelihood ratio test, χ^2 distribution with three d.f.). In the absence of sufficient information to date all internal nodes of the supertree, all branch lengths were coded as equal.

RESULTS AND DISCUSSION

In none of the data analyses could I reject the null hypothesis of no significant relationship between sex-chromosome type and exaggerated male ornamentation in actinopterygiian fishes (Tables 3.1 and 3.2). Indeed, no test result was even marginally significant. Thus, my analysis suggests that female-heterogametic (ZZ-ZW) lineages are not significantly more or less prone to male ornamentation than male-heterogametic (XY-XX) lineages in these fishes. A previous empirical test (Reeve and Pfennig 2003)found a weak positive association between female heterogamety and the number of male ornaments in fish, but that analysis involved many fewer species (29) and the correlation was not statistically significant.

Several reservations about these findings deserve mention. Perhaps this analyses simply lacked the statistical power to detect weak correlations that nonetheless exist. Or, perhaps the (inevitably) provisional and incomplete structure of the supertree employed, or its lack of information on branch lengths, somehow obscured a positive evolutionary association between sex-chromosome systems and sexually selected traits. However, because the evolution of both sex-determination mode and male ornaments under sexual selection are rapid in fishes (see beyond), most of the still-detectable evolutionary effects of sex-chromosome changes should be concentrated near branch tips of the supertree, rather than in deeper portions where phylogenetic uncertainties might often be greatest.

Table 3.1. Statistical relationships between chromosomal mode of sex determination and male ornamentation in 154 surveyed species of actinopterygiian fishes.

Analysis	Correction for Phylogeny?	Test Statistic	Significance
qualitative	no	$r = 0.043$	$p = 0.504$
(presence vs. absence of male ornaments)	yes (by DISCRETE)	likelihood ratio = 3.201	$p = 0.525$
quantitative	no	$r = 0.041$	$p = 0.606$
(number of male ornament types)	yes (by MULTISTATE)	likelihood ratio = 0.329	$p = 0.994$

Table 3.2. Fisher's exact test (presence-absence data) and chi-square test (quantitative data) for possible associations between male ornaments and sex-chromosome system in 154 species of actinopterygiian fishes. The body of each table shows numbers of species observed (and expected under the null hypothesis of random association) to display various combinations of these traits.

Male Ornaments	Sex Chromosome System	
	ZZ-ZW	XX-XY
absent	42 (39.2)	62 (64.8)
present	16 (18.9)	34 (31.2)

(d.f. = 1, $P > 0.2$)

no ornaments	42 (39.2)	62 (64.8)
one ornament type	7 (10.6)	21 (17.4)
two ornament types	9 (8.3)	13 (13.7)

($\chi^2 = 2.38$, d.f. = 2, $p > 0.2$)

Apart from such ‘technical’ concerns, complicating biological and evolutionary factors might also have come into play. First, some male ornaments may be the result of male-male competition more so than female choice (Andersson 1994; Gould and Gould 1997), and this would lower any expected correlation between sex-determination mode and exaggerated male traits. Second, although male display genes are sex-linked in some clades (Lindholm and Breden 2002; Prowell 1998; Reinhold 1998), they are primarily autosomal in others (Fitzpatrick 2004; Ritchie and Phillips 1998), and unfortunately their genetic bases remain completely unknown in most fish taxa (a conspicuous exception being the Poeciliidae; Lindholm and Breden 2002). If preference and display trait genes are often autosomal in the Actinopterygii, any evolutionary effects of sex linkage for the remaining genes might be difficult to detect.

Third, a general pattern may have failed to emerge because the mode of indirect selection (Fisherian, good-genes, or otherwise) has varied across actinopterygiian taxa. To test this possibility, analyses that examined subsets of the full phylogeny could be employed (at least in principle), but the trade-off would be a serious loss of statistical power with the fewer comparisons possible. Fourth, the prediction that sex linkage can have a substantial effect on the evolution of male characteristics assumes that quantities such as genetic variances in male displays and female preferences do not vary in a systematic way with sex linkage (Kirkpatrick and Hall 2004). If this assumption is incorrect, then all predictions would have to be altered accordingly.

A fifth potential concern is that sex-determination systems in fishes might have changed states more rapidly than the male ornaments they theoretically influenced, though this seems unlikely. Although mechanisms of sex determination are indeed highly labile during fish evolution (Mank et al. 2006), evidence for particular taxa (e.g., *Poecilia* and *Xiphophorus*) suggests that rates of male ornament evolution are probably even higher (Basolo 1990; Endler 1980; Houde and Endler 1990; Meyer 1997). Finally, the fast pace of evolution for sex-determining mechanisms and male ornaments might have constrained the extent of influences from sex-chromosome systems on male ornament evolution, and thereby made any association between these variables more difficult to detect (especially in the phylogenetically uncorrected analyses). However, the other side of that coin is that rapid evolution in male ornaments and sex-determination mode should generally have limited unwanted complications otherwise arising from phylogenetic inertia (Blomberg and Garland 2002).

In any event, for all of these biological and technical reasons, these current findings should be viewed as provisional. Further ethological and phylogenetic evaluations will be important, but critical tests of the possible associations between sex chromosomes, male ornaments, and female preferences would profit especially from close genetic dissections of sexually selected traits in many more fish taxa. Only when the exact genetic underpinnings of these phenotypic traits are directly understood in many independent fish lineages will it become more evident as to whether the theoretical association between male ornaments and female heterogamety has been empirically realized. Especially if this association does not exist regularly in nature (as these results suggest), then it will also be important to revisit and perhaps modify the theory itself to take into account additional biological considerations.

In conclusion, my phylogenetic analyses suggest that the particular mode of sex determination has had no consistent and discernible impact on the evolution of sexually selected traits in ray-finned fishes. According to recent theory, an association between male heterogamety and male ornaments should probably have been observed if good-genes processes of sexual selection predominated in fishes, whereas an association between female heterogamety and showy males might have been observed if Fisherian sexual selection was the predominant force. Although various technical and biological complications in my current assessments must be acknowledged (see above), the lack of a clear empirical association between sex-chromosome type and male ornamentation in ray-finned fishes suggests that sex-determination mode has been at best only one of many evolutionary and ecological factors affecting the outcome of sexual selection in this large vertebrate clade.

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CHAPTER 4

PHYLOGENETIC PERSPECTIVES ON THE EVOLUTION OF PARENTAL CARE IN RAY-FINNED FISHES[1]

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[1] Mank, J.E., D.E.L. Promislow, J.C. Avise. 2005. Evolution 59:1570-1578
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ABSTRACT

Among major vertebrate groups, ray-finned fishes (Actinopterygii) collectively display a nearly unrivaled diversity of parental care activities. This fact, coupled with a growing body of phylogenetic data for Actinopterygii, makes these fishes a logical model system for analyzing the evolutionary histories of alternative parental care modes and associated reproductive behaviors. From an extensive literature review, I constructed a supertree for ray-finned fishes and used its phylogenetic topology to investigate the evolution of several key reproductive states including type of parental care (maternal, paternal, or biparental), internal versus external fertilization, internal versus external gestation, nest construction behavior, and presence versus absence of sexual dichromatism (as an indicator of sexual selection). Using a comparative phylogenetic approach, I critically evaluate several hypotheses regarding evolutionary pathways toward parental care. Results from maximum parsimony reconstructions indicate that all forms of parental care, including paternal, biparental, and maternal (both external and internal to the female reproductive tract) have arisen repeatedly and independently during ray-finned fish evolution. The most common evolutionary transitions were from external fertilization directly to paternal care, and from external fertilization to maternal care via the intermediate step of internal fertilization. I also used maximum likelihood phylogenetic methods to test for statistical correlations and contingencies in the evolution of pairs of reproductive traits. Sexual dichromatism and nest construction proved to be positively correlated with the evolution of male parental care in species with external fertilization. Sexual dichromatism was also positively correlated with female-internal fertilization and gestation. No clear indication emerged that female-only care or biparental care were evolutionary outgrowths of male-only care, or that biparental care has been a common evolutionary stepping-stone between paternal and maternal

care. Results are discussed in the context of prior thought about the evolution of alternative parental care modes in vertebrates.

The males of certain fishes do all the work, and afterward take exclusive charge of the young.

Eggs being protected or unprotected by the parents has had little or no influence
on the difference in color between the sexes.

Darwin (1871)

INTRODUCTION

Among vertebrate animals, ray-finned fishes (Actinopterygii) show an unusually high diversity in reproductive features and behaviors. Approximately 20% of the 400+ extant families of actinopterygiians include species that exhibit some form of extended parental care of offspring (Blumer 1979; Blumer 1982). Additionally, some actinopterygiian lineages have evolved highly derived parental care traits such as placental viviparity (Reznick et al. 2002), male pregnancy (Berglund et al. 1986; Jones and Avise 1997), and mouthbrooding (Koblmuller et al. 2004). Such diversity makes the ray-finned fishes a natural model system for studying general patterns and processes of vertebrate parental care evolution (Amundsen 2003).

Studies on a few specific groups of fishes (Gross and Sargent 1985) and anuran frogs (Weygoldt 1987; Zimmermann and Zimmerman 1984; Zimmermann and Zimmerman 1988) have suggested that for species with external fertilization, parental care evolves in stepping-stone fashion, first arising in males, then transitioning to biparental care, and terminating in female-only care upon male desertion. The stepping-stone model is elegantly simple and logically compelling, but it also has been contradicted by recent comparative work on some of the same organisms for which it was developed (Goodwin et al. 1988; Summers et al. 1999).

Additionally, most previous work on the evolution of parental care in fishes has paid scant or no attention to the role of fertilization within a female's reproductive tract. Internal fertilization occurs in at least 21 teleost families, and has led to viviparity (an extreme form of maternal care) in 14 of these (Gross and Sargent 1985; Gross and Shine 1981; Wourms 1981). Because gestation and internal fertilization presumably require elaborate suites of physiological, anatomical, and behavioral adaptations (Amoroso 1968; Schindler and Hamlett 1993), phylogenetic constraints and trade-offs may be particularly important in the evolution of this form of parental care. Such observations suggest that the evolutionary pathways toward parental care might differ between lineages with internal versus external syngamy.

Almost by definition, sexual selection is closely associated with the evolution of reproductive traits (Trivers 1972). It has been found, for example, to be positively associated with male parental care in some vertebrates, primarily birds (Grafen 1990; Heywood 1989; Hoelzer 1989; Price 1984), despite Darwin's pronouncement to the contrary in the opening quotation (Darwin 1871). Many ray-finned fishes also exhibit phenotypic manifestations of sexual selection, most often in the form of sexual dichromatism (Amundsen and Forgren 2001; Houde and Endler 1990; Reimchen 1989; Stott and Poulin 1996). This affords an opportunity to examine the possible role of sexual selection (via its observable surrogate, sexual dichromatism) in the evolution of various forms of parental care in ray-finned fishes.

Despite considerable interest in the diverse modes of parental care in fishes, and the inherent utility of this group as a model for parental care evolution in animals, comparative evolutionary analyses of these behaviors (Balshine-Earn and Earn 1998; Goodwin et al. 1988; Meyer and Lydeard 1993; Wilson et al. 2003) have been severely hampered by uncertainties about teleost phylogeny especially above the level of taxonomic families (Johnson 1993; Nelson 1994). This situation is gradually improving. In particular, recently published estimates of teleost phylogeny based on complete mitochondrial (mt) DNA genomic sequences from dozens of taxonomically diverse species (Inoue et al. 2004; Ishiguro et al. 2003; Miya et al. 2003; Saitoh et al. 2003) have provisionally resolved many problematic fish clades, thereby making it possible to reconsider the evolution of a variety of reproductive traits across Actinopterygii (Mank et al. 2006).

Here I compile information from the literature on parental care and associated traits in more than 200 taxonomic families of fishes. Using a formal supertree (the first attempt to summarize phylogenetic data over such a large group of fishes), I address several long-standing questions including: What were the likely precursor states and evolutionary pathways leading to various forms of parental care? How have alternative fertilization modes (internal versus external) affected the evolution of maternal care (both internal and external) and paternal care in fishes? And, was Darwin correct in his conjecture that sexual dichromatism bears no relationship to parental care of offspring (Darwin 1871)?

MATERIALS AND METHODS

Database Construction

I searched the published literature on actinopterygiian fishes, plus several sarcopterygiian outgroup families, for accounts of the following: presence versus absence of sexual dichromatism and of nest construction, and alternative modes of fertilization, embryo deposition, and post-mating parental care.

Sexual dichromatism, defined as nuptial colorations that differ noticeably between the sexes, is the most common manifestation of sexual selection in fishes (Amundsen and Forgren 2001; Houde and Endler 1990; Reimchen 1989; Stott and Poulin 1996). Sexual dichromatism can vary in degree among species, but due to difficulties of standardizing and quantifying this phenomenon across broad arrays of diverse taxa, I scored sexual dichromatism as being either pronounced (i.e., evident and obvious to the human eye) or absent in a given species. With regard to “nest” building, species that alter their habitat prior to egg deposition were deemed to construct nests (Breder and Rosen 1966).

For current purposes, internal fertilization was defined as syngamy occurring within a female’s body (as opposed to within a male’s body, as occurs in Syngnathidae within Gasterosteiformes). Also for current purposes, modes of embryo deposition were operationally defined as the retention versus lack of retention of fertilized eggs in the ovarian lumen or follicle. Thus, viviparity, which I consider to be a form of maternal care in the current study, was not distinguished here from ovoviviparity as these conditions are not differentiated in much of the available literature (Schindler and Hamlett 1993; Wourms et al. 1988). Similarly, oviparity was operationally defined in the current study to encompass both ovuliparity (where ova are shed prior to external fertilization) and zygoparity (where ova fertilized internally are then deposited outside a female’s body prior to eclosion) (Wourms and Lombardi 1992). Finally, I consider post-mating parental care, which presumably increases mean survival rates of offspring (Gross and Shine 1981), to be any clear protection (internal or external) provided to post-zygotic embryos or juveniles by older females, males, or both. The references used to construct this database are included in Appendix C.

Supertree Construction

I used a formal MRP (Matrix Representation with Parsimony) approach (Ragan 1992) to construct a supertree for actinopterygiian fishes, with representatives of four Sarcopterygiian families included only as outgroups for proper rooting. The underlying or "source" phylogenies for the supertree data matrix were based primarily on molecular data, although several morphological phylogenies were included as well. The supertree data matrix (consisting of cladogenetic information for all species in the source phylogenies) was coded by standard methods (Baum 1992; Ragan 1992). I used the ordinal and super-ordinal classification described in Nelson (1994) as a higher-level organizational framework for the data matrix except when considering the Percomorpha, which has been shown in previous work to be polyphyletic at multiple taxonomic levels (Johnson 1993; Miya et al. 2003; Nelson 1994). Maximum parsimony reconstructions of large supertrees require vast amounts of computational time (Graham and Foulds 1982). To deal with this complexity in identifying most-parsimonious trees using PAUP, I conducted multiple (>100) heuristic searches with random-order additions of taxa (Sanderson et al. 1998). This effort produced a provisional best tree score, and I then searched all computer outputs to identify 25,000 parsimonious trees (the maximum that I could computationally handle) with that identical score. I then constructed a family-level 90% consensus tree from the 25,000 most parsimonious trees identified. The resulting supertree topology for 228 taxonomic families, and a list of the underlying phylogenies that composed the data matrix, are available in Appendix D. I also will make reference to an ordinal-level supertree that represents a "condensed" phylogeny for the 46 taxonomic orders of ray-finned fishes considered.

Comparative Analysis of Parental Care

I first used maximum parsimony as implemented by MacClade v.4 (Maddison and Maddison 2000) to estimate the minimum and maximum numbers of inferred independent evolutionary origins, losses, and transitions between various types of parental care and fertilization systems in the family-level actinopterygiian supertree. I then used maximum likelihood as implemented by DISCRETE (Pagel 1994; Pagel 1997) to test for correlations in the evolution of particular pairs of reproductive traits, each coded as discrete binary characters (such as presence versus absence of nest construction, or maternal versus paternal care of offspring). I evaluated possible correlations by comparing likelihoods in a null model of evolution (i.e., with no correlation between traits) to the alternative correlated model. Each resultant likelihood ratio test has a χ^2 distribution with four degrees of freedom, since there are four fewer parameters in the null model than the correlated model (Pagel 1994).

When a particular correlation was found to be statistically significant, I also used DISCRETE for contingency testing, which indicates if the evolution of one of the traits significantly tended to precede the other (thus suggesting but not proving causal directional evolutionary relationships between the two paired reproductive traits). Contingency testing does not identify where along the phylogeny one trait preceded another, but it does identify broad patterns in correlated trait evolution and provides theoretical frameworks for further analyses. Each contingency test was evaluated using a likelihood ratio statistic with a χ^2 distribution and one degree of freedom (Pagel 1994). Although DISCRETE can incorporate different branch lengths (ages of clades) into the analysis, the amalgamated nature of supertrees (in general, as well as in the current study) typically precludes accurate branch-length estimations. Therefore, as has been conventional in previous such analyses involving other traits and other organisms, I coded all branch lengths in DISCRETE as equal to one.

For the correlation and contingency tests, which involve multiple comparisons among traits, I computed a Bonferroni adjustment. I indicate associations that are significant under

Bonferroni-corrected conditions, and also report associations that are significant under normal statistical rigor (i.e., without invoking this correction).

RESULTS

The supertree resolved 224 of the 400+ families of actinopterygiian fishes, with four sarcopterygiian families included as outgroups. In these cases, all nodes had greater than 90% consensus support, and the phylogenetic topology further substantiates the polyphyly previously reported for several percomorph orders including Perciformes, Gasterosteiformes, and Scorpaeniformes (Johnson 1993; Miya et al. 2003; Nelson 1994). Although I was able to resolve all gasterosteiform and scorpaeniform sub-orders, as well as many perciform suborders including Gobioidei, Blennioidei, Zoarcoidei, and Labroidei, the extreme polyphyly and a lack of higher-level phylogenies for the Percoidei prevented resolution of some percoideian families such as Apongonidae.

I found sufficient information on reproductive characters for 206 of the 228 resolved families. Overall, parental care was present in 31% (62 of 174) of the families included in our analysis. This value is somewhat higher than a previous estimate of 20% (Blumer 1982), but that earlier study included many of the phylogenetically uncharacterized percoideian families.

Among the ovuliparous (externally fertilizing) fishes considered, parental care was present in 25% (49 of 193) of the taxonomic families. For these taxa, male-only parental care (41 families) was far more common in our study than either female-only care (5 incidences) or biparental care (5 incidences) (the Cichlidae showed male-only, female-only, and biparental care). For internal fertilizers, the situation was dramatically different: parental care (either external or internal) was present in 90% (18 of 20) of the families examined.

Maximum Parsimony Inferences

Fig. 4.1 shows the abbreviated (i.e., collapsed), ordinal-level supertree topology. As evidenced by the high number of polymorphic ordinal-level clades in Fig. 4.1, shifts among alternative parental-care modes have occurred frequently in ray-finned fish evolution. Thus, parental care appears to have been evolutionarily quite labile in this group. However, at finer taxonomic scales, taxonomic orders that were polymorphic for parental-care modes usually resolved into families that were monomorphic for one or another mode of care (e.g., Fig. 4.2).

In the 206-family tree (Appendix D), parental care is mostly confined, among modern families, to approximately 35 terminal clades, and many large clades are entirely devoid or nearly so of species in which adults care for their young. For these reasons, and also from basic biological considerations (Baylis 1981), it seems highly likely that external fertilization devoid of parental care is the generalized basal condition for ray-finned fishes, from which more specialized modes of paternal or maternal care arose secondarily and recurrently in various actinopterygiian lineages.

Based on maximum parsimony analyses of the family-level supertree, I estimate that parental care solely by the male emerged independently at least 22 times over the course of ray-finned fish evolution, probably always in lineages in which females release eggs that are fertilized away from their bodies (Fig. 4.3). Biparental care (i.e., by the two sexes either simultaneously or alternately within a species) arose independently on at least four occasions, but again almost certainly only in lineages in which syngamy is external. Parental care solely by the female (other than via internal gestation which I will address later) evolved at least seven independent times in the ray-finned fishes, but in sharp contrast to the male-only care situation,

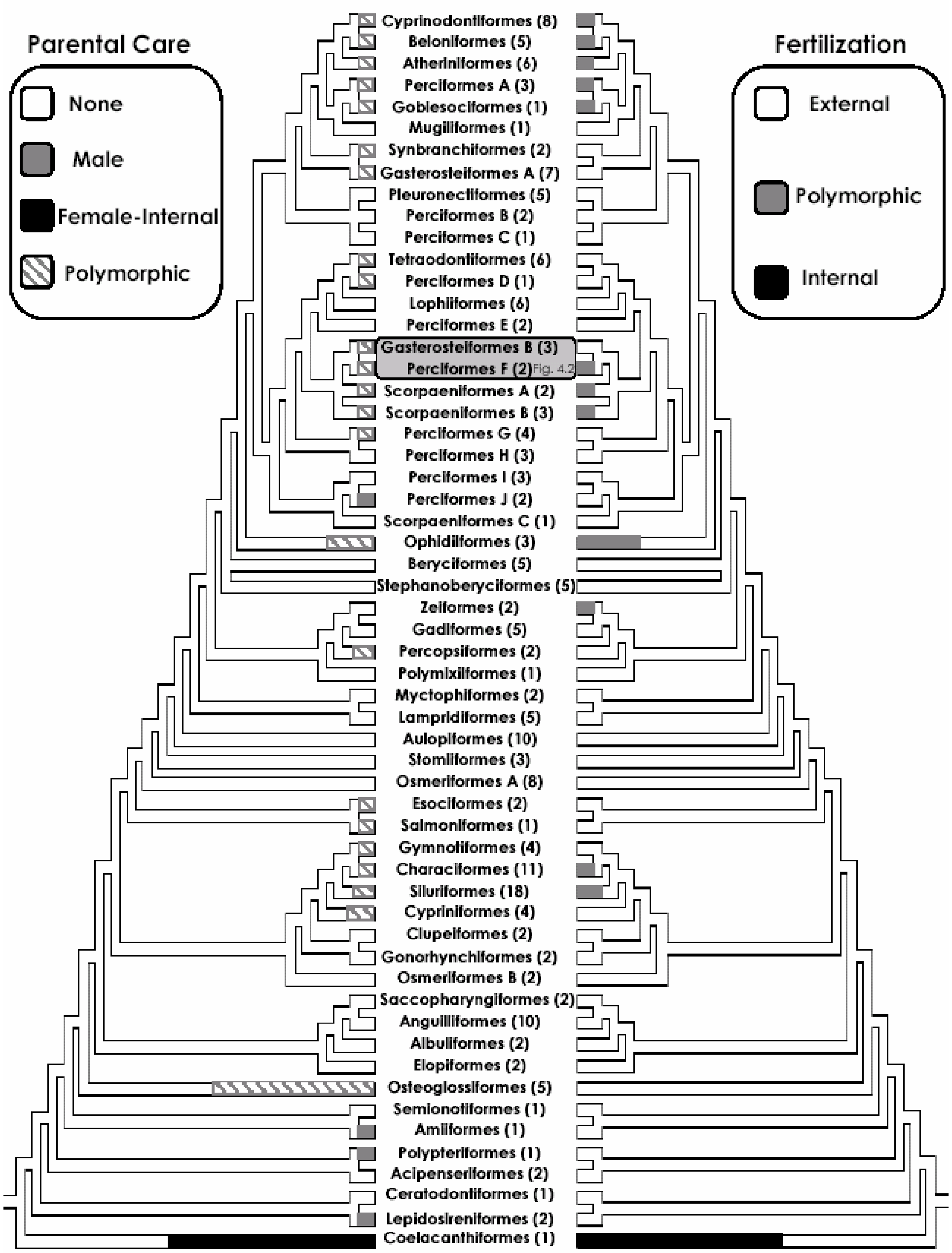

Figure 4.1. Ordinal-level MRP supertree for the actinopterygiian fishes. Plotted on thecladogram are character states for parental care (left) and mode of fertilization (right) as inferred by maximum parsimony reconstructions of finer level (inter familial) molecular phylogenies. In parentheses are the numbers of surveyed families in each collapsed clade. Perciformes A = Blennioidei; Perciformes B = Sphyraenidae, Polynemidae, Menidae; Perciformes C = Carangidae; Perciformes D = Gobioidei; Perciformes E = Emmelichthyidae, Lutjanidae; Perciformes F = Zoarcoidei; Perciformes G = Labroidei, Acanthuroidei; Perciformes H = Notothenoidei, Percidae; Perciformes I = Scombroidei, Stromateidae; Perciformes J = Gobioidei; Gasterosteiformes A = Syngnathoidei; Gasterosteiformes B = Gasterosteioidei; Scorpaeniformes A = Cottoidei; Scorpaeniformes B = Scorpaenoidei; Scorpaeniformes C = Dactylopteroidei; Osmeriformes A = all omseriforms not in Osmeriformes B; Osmeriformes B = Alepocephalidae, Platytrocidae.

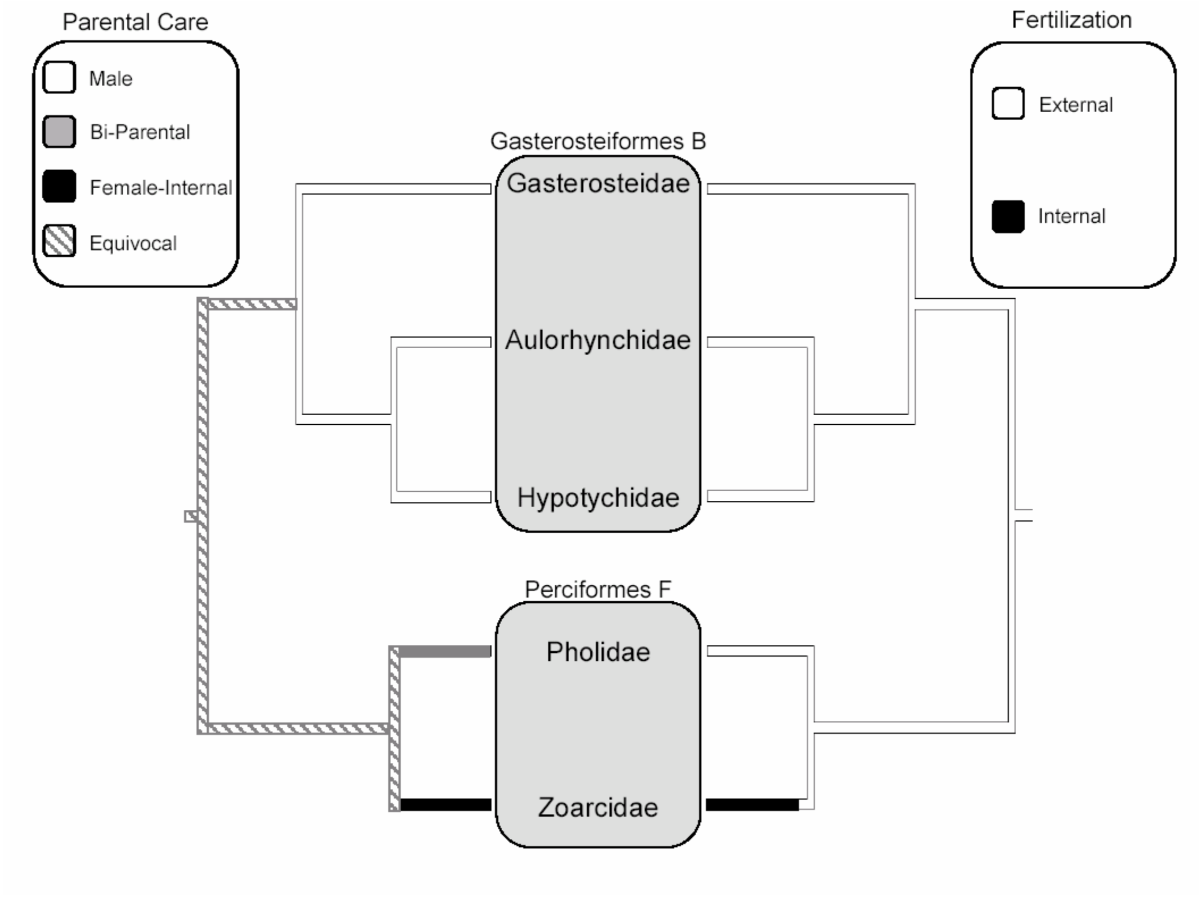

Figure 4.2. Family-level phylogeny for the closely related clades "Gasterosteiformes B" and "Perciformes F" (see Fig. 1). Shown on this portion of the supertree are character states for parental care (left) and mode of syngamy (right), as deduced by maximum parsimony reconstructions.

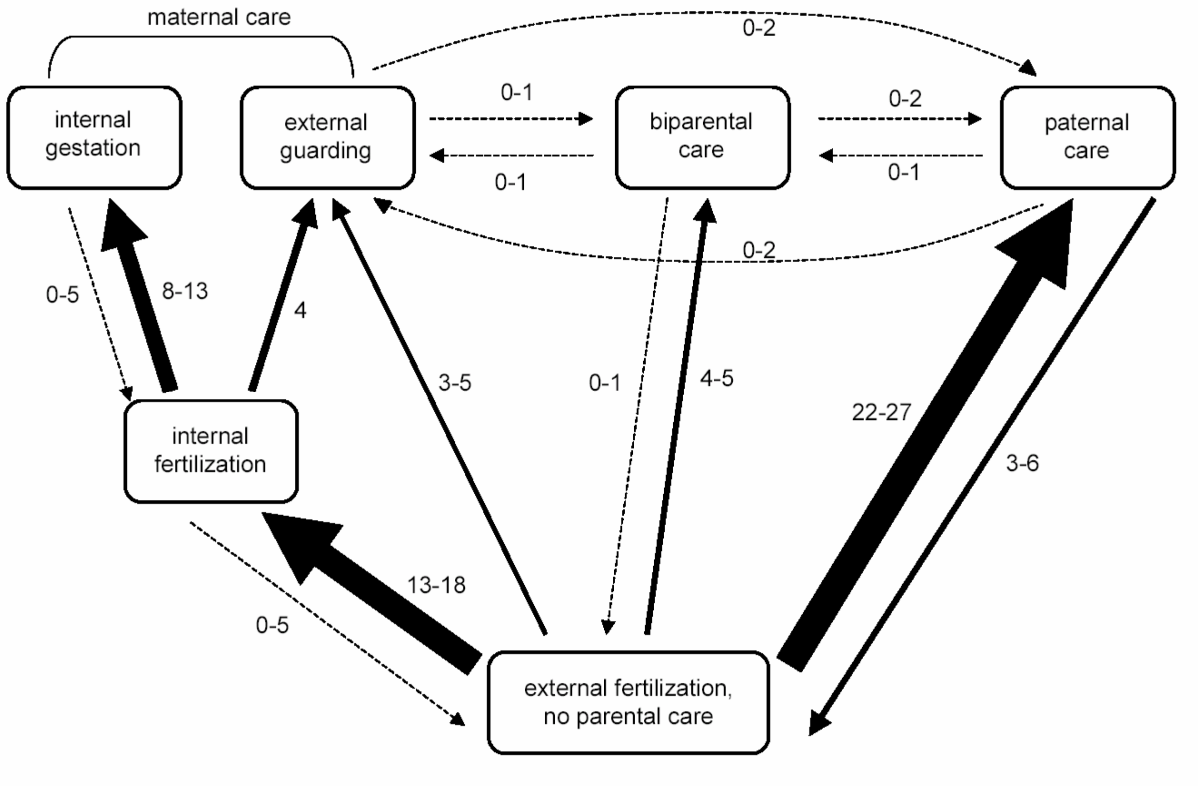

Figure 4.3. Maximum parsimony inferences regarding independent evolutionary transitions among parental care modes in the surveyed Actinopterygiian fishes. Arrow sizes reflect relative numbers of evolutionary transitions; numerals adjacent to the arrows register minimum and maximum estimated numbers of evolutionary transitions. Broken arrows indicate transitions that might or might not have occurred (i.e., those whose estimated minimum number was zero and whose maximum number was ≥ 1).

in only three of these cases (43%) did such female care involve species with external fertilization. The type (male, female, or biparental) of parental differs significantly depending on the type (internal or external) of fertilization ($\chi^2 = 86.3$, $p < 0.0001$).

As judged by the maximum parsimony phylogenetic analyses, fertilization inside the female's body probably arose independently at least 13 times over the course of ray-finned fish evolution (Fig. 4.3). It apparently led to internal gestation (i.e., strict viviparity) in at least eight lineages, and to post-partum external care by females in four other lineages (Fig. 4.3). In no examined lineage did such internal fertilization co-occur with paternal care, a trend also illustrated in Fig. 4.2 (where all cases of internal fertilization occurred in species with either internal or external female care of offspring). [A few reports do exist of the co-occurrence of paternal care and female-internal syngamy, but they concentrate on the Apogonidae (Garnaud 1950; Garnaud 1962), a perciform family omitted from this analysis due to lack of phylogenetic resolution. Although future phylogenetic work should permit inclusion of this family, its peculiar combination of reproductive traits will undoubtedly remain a rare exception due to the strong association of internal fertilization with female care (Gross and Sargent 1985).]

Internal gestation by females involves sheltering and often nourishing embryos within the body. From previous biological considerations (Amoroso 1968; Reznick et al. 2002; Thibault and Schultz 1978; Wourms 1981), this extreme form of maternal care almost certainly is a derived condition in ray-finned fishes, supposedly always preceded by internal fertilization. These parsimony reconstructions are entirely consistent with such notions (Figs. 4.1-4.3).

A summary of these and other historical pathways of evolutionary transition between reproductive states, as inferred from the parsimony analyses of MacClade, is presented in Fig. 4.3.

Maximum Likelihood Inferences

For pairs of reproductive traits, Table 4.1 summarizes the results of the correlation and contingency tests via the maximum likelihood analyses. Several phylogenetic associations were statistically significant, before and sometimes also after Bonferroni corrections for multiple comparisons. The overall pattern is highly significant, as assessed by Fisher’s combined probability test (Sokal and Rohlf 1995) for correlation testing ($p < 0.001$) and even contingency testing ($p < 0.001$). Because it is unlikely that all significant putative relationships between pairs of traits are attributable to type I errors alone, I will briefly describe each phylogenetic association that proved to be significant under standard statistical rigor.

According to the correlation tests, both sexual dichromatism and nest construction were positively correlated with the evolution of male-only parental care. Furthermore, in the case of nest construction (but not of sexual dichromatism), contingency tests revealed a significant evolutionary directionality to the association, with nest construction tending to having preceded (and thus, perhaps, having contributed to the evolution of) male parental care. Unfortunately, similar statistical tests of biparental and female-only external care were not possible due to the rarity of these states in species that shed eggs prior to fertilization.

Sexual dichromatism and internal fertilization were positively correlated ($p = 0.029$), with contingency tests suggesting that the former may precede the latter ($p = 0.006$). Finally, sexual dichromatism was also positively correlated with ($p < 0.007$) and contingently preceded ($p < 0.002$) both internal gestation and viviparity.

DISCUSSION

Inevitably, results from this phylogenetic analysis remain provisional, in part because the actinopterygiian tree is far from fully resolved. As additional phylogenetic information becomes available, especially for families in the Percoidei, the supertree topology should become denser and better resolved at internal nodes.

Table 4.1. Results of tests for correlations and contingencies in the evolution of paired reproductive traits. For correlations that proved to be statistically significant vis à-vis the null model, entries on the first line in each cell are the likelihood ratio and its associated *p*-value (χ^2 distribution, 4 d.f.). For each of these significant correlations, entries on the second line of a cell indicate the deduced evolutionary directionality (trait X in all cases except one tended to precede the evolution of trait Y) and the associated *p*-value (χ^2 distribution, 1 d.f.). Relationships shown in bold are significant even after the Bonferroni correction.

X

		Sexual Dichromatism	Nest Construction
Y	Male Care	10.2 ($p = 0.038$) X↔Y (n.s.)	**82.6 ($p < 0.001$)** **X→Y ($p < 0.001$)**
	Internal Fertilization	10.8 ($p = 0.029$) X→Y ($p = 0.006$)	n.s.
	Viviparity	14.1 ($p = 0.007$) X→Y ($p = 0.002$)	n.s.
	Nest Construction	14.8 ($p = 0.005$) 14.9 **X→Y ($p = 0.001$)**	---

Supertrees (Ragan 1992; Wiens and Reeder 1995), which offer a method for merging phylogenies from diverse and otherwise incompatible data matrices, are becoming increasingly important for phylogenetic analyses across large time spans (tens and hundreds of millions of years), in part because no sequence from a single gene or a set of genes can reasonably be expected to resolve relationships over such a vast span of genetic distances under a single model of molecular evolution (Bininda-Emonds et al. 2002). In a data matrix underlying supertree construction, only tree topologies are employed, with each column in the data matrix corresponding to a monophyletic clade from the various source trees to be amalgamated. Despite the resulting complexity problems, supertree methods extend the upper-limit for the number of taxa that can be surveyed in a single phylogeny. They have made possible the construction of cohesive phylogenies for such groups as Angiosperms (Davies et al. 2004) and Chiroptera (Jones et al. 2002), and even prompted preliminary attempts at the complete tree of life (Maddison and Schultz 2004).

The provisional actinopterygiian supertree presented here is based on numerous source phylogenies, many of which came from molecular data, others were based on relatively robust morphological datasets, and other from multiple kinds of information. Their heterogeneous nature means that these data subsets clearly cannot be treated uniformly or homogenously, so only the amalgamating algorithms of supertree construction can hope to combine these divergent datasets into a cohesive phylogenetic structure.

While the supertree is not a complete survey of all actinopterygiian families, the data from future source phylogenies can be added to the matrix, increasing both taxon sampling and probably the robustness of the cladogram. Despite these current limits to our understanding of actinopterygiian phylogeny, several broad patterns have emerged from our comparative phylogenetic analyses of parental care and associated reproductive traits in ray-finned fishes.

Sexual Dichromatism, Mode of Fertilization, and Parental Care

In this analysis, sexual dichromatism proved to be significantly correlated with the evolution of each of the following: internal fertilization, viviparity (the most common form of maternal care), male parental care, and nest construction. Furthermore, the evolution of internal fertilization, viviparity, and nest construction were significantly contingent upon the presence of sexual dichromatism. This suggests that Darwin's (1871) postulate that dichromatism and parental care are unrelated characters in fishes is incorrect. Sexual dichromatism (especially nuptial coloration) is a manifestation of sexual competition in many fish species (Endler 1980; Okuda et al. 2003; Warner and Schultz 1992; Wedekind et al. 2001). Thus, perhaps the same competition for mates that drives sexual selection also drives the evolution of parental care (both internal and external; see below).

Evolutionary transitions from external fertilization and oviparity to internal fertilization and viviparity are presumably far from trivial mechanistically. Internal fertilization normally requires an intromittant male organ such as a gonopodium, which has a complex physiology and musculature (Rosen and Gordon 1953; Zauner et al. 2003). The female reproductive tract must be physiologically conducive to internal fertilization. And with regard to viviparity, some if not all of the following changes must normally occur along the evolutionary path from egg-laying to live-bearing: the retention of developing embryos in the female reproductive tract to advanced states of development; a reduction in the egg envelop; an increase in egg size with a concomitant decrease in egg number; the emergence of endocrine adaptations; and the development of mechanisms for nutrient and gas exchange between zygote and mother (Amoroso 1968; Guillette 1989; Reznick et al. 2002; Schindler and Hamlett 1993; Wourms 1981; Wourms et al. 1988).

Despite the difficulties presumably associated with the evolution of such suites of complex characters, I estimate that viviparity has evolved on at least eight separate occasions in the Actinopterygii, and this is almost certainly a considerable underestimate due to the necessary omission of several perciform families. Thus, the hurdles have been overcome multiple times, and this suggests that selection pressures for viviparity may sometimes be strong. Viviparity does indeed offer several potential advantages including increased protection of embryos, larger propagule size, and higher trophic level of offspring at the time that progeny become independent (Wourms and Lombardi 1992). Viviparity also appears to be a non-reversible condition in the ray-finned fishes, as no significant (i.e., where the minimum estimate was greater than zero) transitions back toward oviparity were inferred from our phylogenetic analyses. Because of this evolutionary ratchet, and because viviparity places the entire energetic post-mating burden on the female, internal fertilization and viviparity should be considered a distinct pathway from external fertilization in the evolution of parental care in Actinopterygii (see below).

The Evolution of Parental Care

These findings support earlier reports of correlations between external fertilization and male-only care, and between internal fertilization and female-only care (Dawkins and Carlisle 1976; Gross and Sargent 1985; Gross and Shine 1981; Ridley 1978; Trivers 1972). However, evidence also emerged that female-only care occasionally evolves independently of male-only care in the presence of external fertilization. Despite such exceptions, it is clear that male-only care generally evolves in lineages with external syngamy, and female-only care evolves primarily in lineages with internal fertilization (Fig. 4.3). Because of this dichotomy, the evolution of parental care should be thought of as two separate primary pathways with the mode of fertilization being the fundamental point of divergence (Fig. 4.3), and with sexual selection acting on both pathways to foster evolutionary change. Considering these two evolutionary pathways, the one leading toward maternal-only care is the somewhat less traveled route in ray-finned fishes, probably because complex suites of anatomical and physiological adaptations must be required for internal fertilization and viviparity.

For species with external syngamy, there is little or no evidence from these analyses that biparental care is an intermediate state between paternal and maternal care. Thus, my analyses do not add support for the conventional stepping-stone model in the evolution of parental care (Gittleman 1962; Gross and Sargent 1985; Weygoldt 1987; Zimmermann and Zimmerman 1984; Zimmermann and Zimmerman 1988). Rather, this work is more consistent with some recent evidence from anuran frogs (Summers et al. 1999) that female-only and biparental care are independent evolutionary transitions from the basal state of no care. An important qualification, however, is that because biparental care is rare and female-only care with external fertilization is even rarer, this analyses simply may lack the requisite sensitivity to detect the intermediacy of the biparental care state (because few evolutionary transitions would be expected to occur from one-parent care to two-parent care).

The relationship between sexual selection and parental care is likely related to competition for limiting resources, which itself might be due in various species to any or a combination of several genetic and ecological factors: e.g., anisogamy (pronounced differences in size between male and female gametes), restrictions on suitable spawning habitats, energetic food constraints, highly unequal sex ratios, or consistent differences between the sexes in mate availabilities. Such limitations foster reproductive competition, usually among males, and this competition can lead to a high variance in male reproductive success that often results in the evolution of sexual dichromatism via sexual selection (Darwin 1871). This process may manifest differently, however, in species with external versus internal syngamy, due to unique phylogenetic constraints and peculiarities of each fertilization system.

For species with external fertilization, this competition is suggested by positive correlations between nest construction and paternal care, as well as between sexual dichromatism and male parental care. The first of these correlations (i.e., between nest construction and male parental care) is a likely outgrowth of territorial defense of spawning sites by males. In other words, nest construction and the protection of progeny therein would merely require a prolongation and elaboration of typical defense behavior, and thus would be especially adaptive when, for example, eggs and fry face heavy predation (Loiselle 1978). Nest construction in this sense may be a “pre-adaptation” for male parental care.

The relationship between sexual dichromatism and male parental care is less straightforward, and it may be that this positive correlation is not causative, but rather that both traits are the result of sexual selection. There are many accounts of the role of sexual selection in the evolution of dichromatism in fish (Amundsen and Forgren 2001; Houde and Endler 1990; Reimchen 1989; Stott and Poulin 1996). My results may suggest that male parental care is also influenced by sexual selection, a relationship that has been proposed both theoretically and empirically for several types of animals (Hoelzer 1989; Iwasa and Pomiankowski 1999; Tallamy 2000) , including fish (Ostlund and Ahnesjo 1998; Pampoulie et al. 2004). In some fishes, males are known to take better care of the offspring when in the presence of breeding females

(Pampoulie et al. 2004) , and females have been shown to choose mates based on the quality of their parental care (Ostlund and Ahnesjo 1998; Ostlund-Nilsson 2000), suggesting that male-care behavior is affected by sexual selection. These effects have also been shown in birds (Moller and Thornhill 1998; Siefferman and Hill 2003; Velando et al. 2001). It is therefore possible that the same forces of intra-sexual (and/or inter-sexual) reproductive competition that foster the evolution of sexual dichromatism also promote the evolution of male parental care.

For species with female-internal fertilization, sexual dichromatism may simply be an evolutionary by-product of enhanced competition among males for mating success. This would likely occur because, in such situations, maternal investment in reproduction becomes higher (almost necessarily), paternal investment in progeny is likely lower, and female fecundity (especially in species with internal gestation) becomes an even more limiting factor in reproduction. Empirically, internal syngamy almost never co-occurs with male parental care, so reproductive involvement by males in these situations must be limited to pre-mating and gametic components, thus creating the classical anisogamy-related limitation on female reproduction that often results in sexual selection (Trivers 1972). But internal fertilization also creates a ready opportunity for male desertion, thus leaving the female with all of the parental care responsibilities. In this way, internal fertilization is not only a pre-adaptation to internal gestation, but also should normally promote competition among males for mates, resulting in sexual dichromatism via sexual selection.

Synopsis

This study represents the first attempt to scrutinize the evolution of parental care in actinopterygiian fishes in a formal phylogenetic context. My comparative analyses indicate the existence of two principal evolutionary routes to extended parental care in fishes: to female-only care (either internal or external) via female-internal fertilization; and to male-only care directly from external fertilization. Other evolutionary pathways exist as well, but appear to be much less frequently traveled. Finally, these phylogenetic results taken at face value (there are caveats) add no support for the conventional notion that biparental care is an evolutionary stepping-stone between male-only care and female-only care. These results should provide a useful backdrop and model for further studies of parental care evolution in other groups of animals.

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CHAPTER 5

COMPARATIVE PHYLOGENETIC ANALYSIS OF MALE ALTERNATIVE REPRODUCTIVE TACTICS IN RAY-FINNED FISHES[1]

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[1] Mank, J.E., J.C. Avise Submitted to Evolution, January 2006

ABSTRACT

Using comparative phylogenetic analysis, I analyzed the evolution of male alternative reproductive tactics (MARTs) in ray-finned fishes (Actinopterygii). Numerous independent origins for each type of MART (involving sneaker males, female mimics, pirates, and satellite males) indicate that these behaviors have been highly labile across actinopterygiian evolution, consistent with a previous notion that convergent selection in fishes can readily mold the underlying suites of reproductive hormones into similar behaviors. The evolutionary appearance of MARTs was significantly correlated with the presence of sexually selected traits in bourgeois males ($p = 0.001$) but not with the presence of male parental care. This suggests that MARTs often arise from selection on some males to circumvent bourgeois male investment in mate monopolization, rather than to avoid male brood care *per se*. I found parsimony evidence for an evolutionary progression of MARTs wherein sneaking is usually the evolutionary precursor to the presumably more complex MARTs of female mimicry and cooperative satellite behavior. Nest piracy appears not to be part of this evolutionary progression, possibly because its late onset in the life cycle of most ray-finned fishes reduces the effects of selection on this reproductive tactic.

INTRODUCTION

Ray-finned fishes (Actinopterygii) display a great diversity of male reproductive tactics, probably more so than any other vertebrate clade. These behaviors range from mass group spawning (where scramble competition and sperm competition are major determinants of male fitness) to distinct pairings between one or a few females and particular territorial or “bourgeois” males (where the effectiveness of mate appropriation has a key impact on male fitness). Under the bourgeois tactic, mate monopolization may occur via monogamous pair bonding or polygamous harems, but in either case the male acts to control the reproductive potential of prospective mate(s) to the relative fitness detriment of other males (Emlen and Oring 1977; Gross 1996; Taborsky 2001). Furthermore, often associated with the bourgeois strategy in various fish species are a variety of parasitic and cooperative male alternative reproductive tactics (henceforth MARTs) by which some males in effect sidestep or even co-opt bourgeois males’ oft-substantial investments in attracting females and defending territories and mates. In other words, effective MARTs can break the monopoly that bourgeois males otherwise hold on fitness-enhancing resources.

These MART behaviors, three of which are parasitic and one cooperative, have been extensively cataloged and reviewed (Gross 1996; Taborsky 1994, 1998, 2001). By definition, males displaying parasitic reproductive behaviors attempt to steal fertilization events from territorial males. Sneaker males do so by means of speed or stealth that gives them access to a spawning opportunity. Female mimics do so by duping territorial males and thereby gaining access to spawning sites in which they deposit sperm. Pirate males steal fertilizations by being massive enough to evict a territorial male from his spawning location. The eviction is often temporary because the pirate may depart after one or a few spawning events, leaving the bourgeois male to guard what may be a mixed-parentage brood. Cooperative reproductive behaviors by males are rarer and less diverse in actinopterygiian fishes, but no less intriguing. Cooperative males, often known as satellites, are tolerated by a bourgeois male and may contribute to female acquisition, territory defense, and parental care in exchange for fertilization opportunities. In general, males displaying most MART adaptations (with the exception of piracy) lack the sexually selected ornaments exhibited by territorial males.

Due to diligent field observations and molecular genetic appraisals of parentage (reviewed in Avise et al. 2002), documentations of species-specific MARTs abound in the literature, and the hormonal and genetic controls of these reproductive tactics are becoming increasingly clarified in laboratory experiments (Bass and Grober 2001; Borg 1994; Fitzpatrick et al. 2005; Foran and Bass 1998; Foran and Bass 1999; Knapp 2004). However, little is understood about phylogenetic patterns of MART evolution in fishes, a shortcoming that we begin to redress here.

The recent publication of a provisional supertree for the Actinopterygii (Mank et al. 2005) makes possible a comparative appraisal of MART evolution across this large and diverse fish clade. Here I use a comparative phylogenetic approach to analyze evolutionary patterns and to assess the evolutionary lability of MARTs. I also test for correlated evolution between MARTs and two other possibly relevant reproductive traits (male parental care, and sexual selection as assessed by the presence of male ornaments).

MATERIALS AND METHODS

I searched the published literature for accounts of the following: mating behavior including patterns of mate pairing and MARTs; paternity identification, primarily from genetic appraisals (Avise et al. 2002), as an indicator of cryptic MARTs; male ornaments or traits presumably indicative of sexual selection; and presence and mode of parental care. MARTs considered here involve sneakers, satellites, female mimics, and pirates (see Introduction). More than 150 references, available in Appendix E, comprise this database.

To reduce problems of ascertainment bias and repeatability, I limited the appraisal of sexually selected traits to published accounts of male ornaments repeatedly shown to be under sexual selection in various fish taxa (Mank et al. 2006). These include elongate fins (Harrington 1997; Kuwamura et al. 2000; Marcus and McCune 1999), breeding tubercles (Kortet et al. 2003, 2004), and sexual dichromatism defined as nuptial colors expressed more noticeably in males than in females (Amundsen and Forgren 2001; Houde and Endler 1990; Reimchen 1989; Stott and Poulin 1996). I omitted from the database sexually dimorphic traits such as gonopodia and body-size differences that likely result at least in part from natural selection and are thus poor proxies for the presence of sexual selection. Finally, taxa in which males tend embryos or larvae subsequent to mating were deemed for current purposes to have male parental care, whether or not female parental care was also involved.

I then mapped this information onto an actinopterygiian supertree phylogeny constructed (via matrix-representation with parsimony; Ragan, 1992) from 58 previously published source cladograms which themselves had been based on molecular data or morphological evidence (Mank et al. 2005). I first estimated numbers of independent origins as well as transitions among MART character states under maximum parsimony criteria using MacClade 4 (Maddison and Maddison 2000). I then tested for patterns of correlated evolution between MARTs and other reproductively associated characters using the maximum likelihood program DISCRETE (Pagel 1994, 1997). Each statistical evaluation of correlated evolution was conducted using a likelihood ratio test of the null model of evolution (i.e., with no correlation between traits) to the alternative correlated model. Each resultant likelihood ratio test has a χ^2 distribution with four degrees of freedom (since there are four fewer parameters in the null model than in the correlated model; Pagel 1994).

Although DISCRETE can incorporate different branch lengths (ages of clades) into the analysis, the amalgamated nature of our supertree (as well as the limited temporal information in the original source cladograms, due in part to the limited actinopterygiian fossil record) precluded estimates of absolute divergence times. Thus, I used the standard convention (Mank et al. 2005; Weiblen et al. 2000) of coding all branch lengths in DISCRETE as equal to one.

RESULTS

I scrutinized published descriptions of mating and reproductive behaviors for 296 species in 86 taxonomic families distributed throughout the Actinopterygii. Fig. 5.1 shows the phylogenetic distribution of MARTs based on this information, as well as the maximum parsimony reconstructions of ancestral states. For clarity of depiction, all of the assessed MARTs have been compressed into a single category in Fig. 5.1. However, the parsimony analysis was performed with each MART behavior coded individually.

Maximum parsimony reconstruction suggests that various MART behaviors arose independently on at least 26 – 43 separate occasions during the course of actinopterygiian evolution (Fig. 5.2). In at least 21 – 27 of these cases, the MART is most-parsimoniously interpreted to have evolved directly from mate monopolization, but the other 5 – 16 evolutionary origins for MARTs seem at face value to trace directly to group spawning (Fig. 5.2). If we assume, however, that distinct pairing or mate monopolization is normally a direct prerequisite for MART evolution (see Taborsky 1994, 2001), then these latter inferred transitions to MARTs might actually have entailed an intermediate but transient stage of female monopolization that was not detected in the parsimony analysis.

Inferred transitions from mate monopolization (which we henceforth assume to be a requisite ancestral state, as described above) to particular types of MARTs are shown in Fig. 5.3. According to maximum parsimony reconstruction**,** by far the most common evolutionary transition has been to sneaking (15 – 20 independent origins). On various occasions, sneaking was also the inferred transitional state between mate monopolization and both female mimicry (3 – 6 evolutionary transitions) and cooperative satellite behavior (3 – 4 evolutionary switches). The parsimony analysis at face value also suggests that mate monopolization can progress directly to satellite and female mimic character states without involving sneaking as an intermediate stage (Fig. 3). However, it is also possible that these latter transitions also progressed through a transitional sneaking state that subsequently was lost.

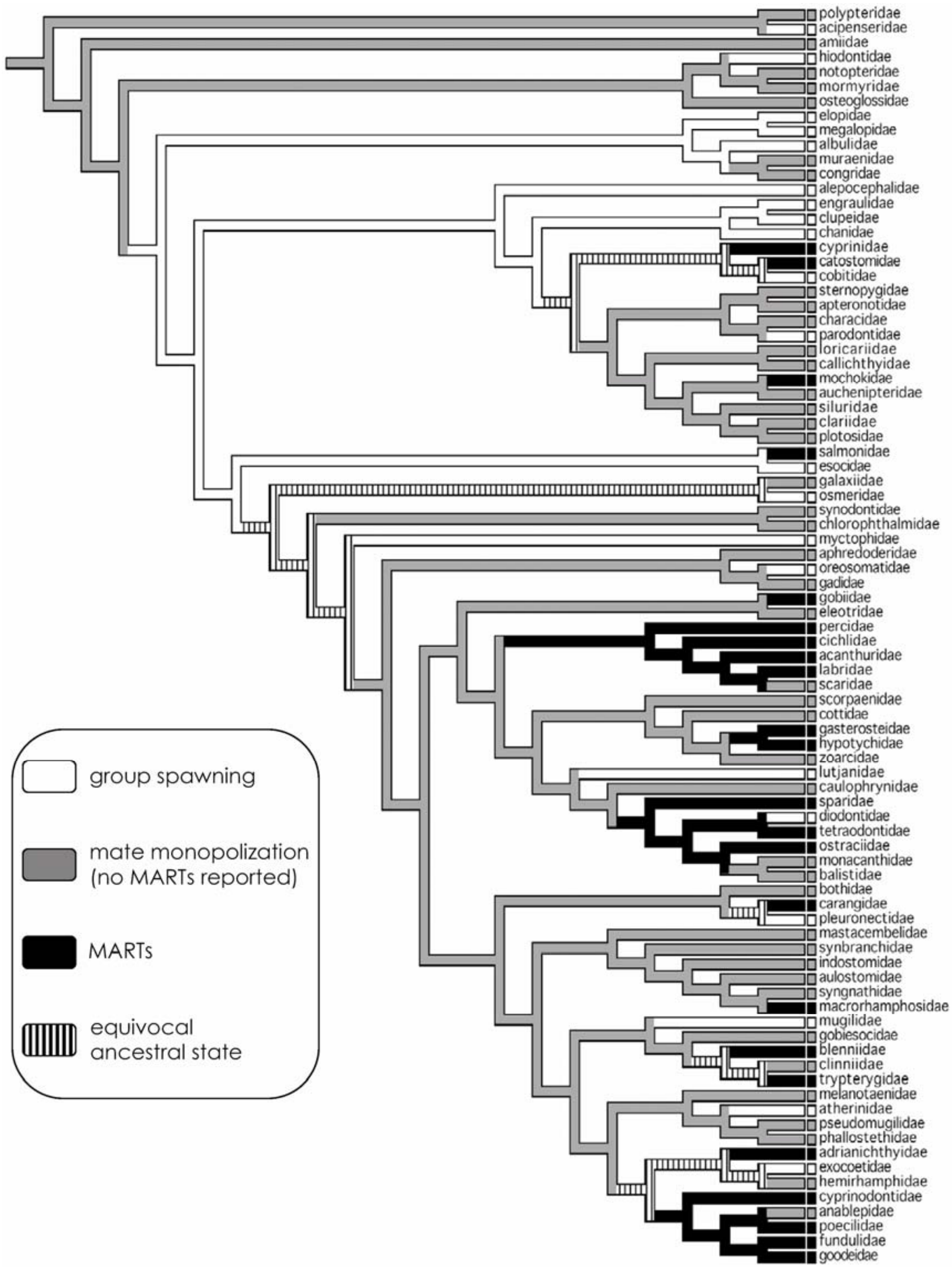

Fig. 5.1 Phylogenetic patterns of MART evolution in ray-finned fishes. Mapped onto the supertree phylogeny are MART observations in extant species and ancestral state reconstructions by maximum parsimony criteria. For simplicity of presentation, the MART condition displayed here (as solid black branches) is any behavior in an aggregate of sneaking, piracy, female mimicry, or satellite activity. Also shown are lineages that exhibit either group spawning or some degree of mate monopolization.

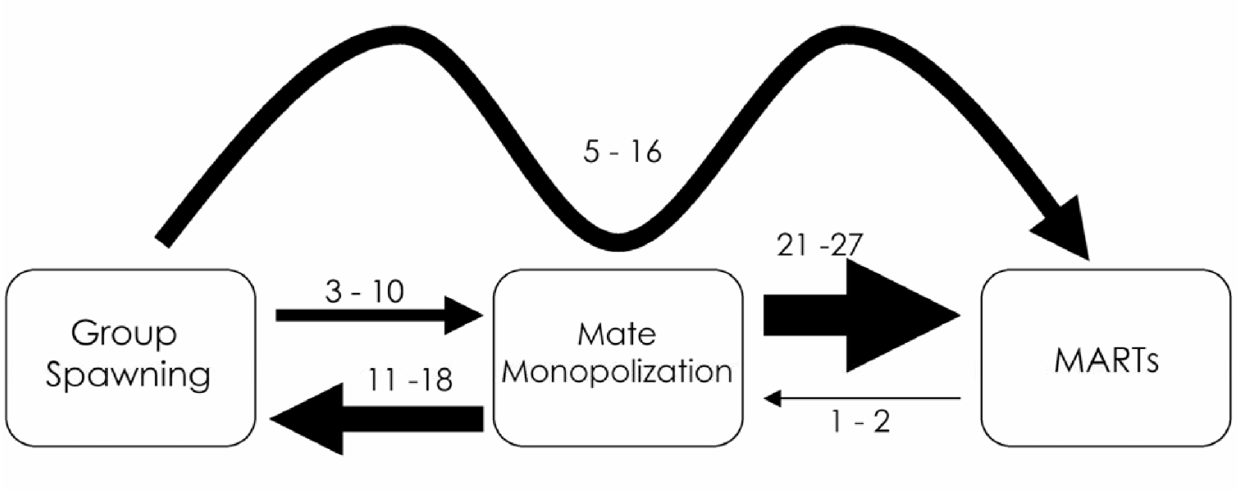

Fig. 5.2. Numbers of independent evolutionary transitions (as inferred from maximum parsimony criteria) from group spawning to alternative MARTs in ray-finned fishes. Arrow sizes correspond to mean numbers of transitions among states; numbers beside arrows indicate minimum and maximum estimates. The transition from group spawning directly to MARTs may represent transitions that went through a mate-monopolization intermediate, but were not presently detected under maximum parsimony (see text).

Finally, I also tested for correlated evolution between MARTs and two other reproductive traits (Table 5.1). The presence of male parental care was not statistically associated with MART evolution, but a significant evolutionary correlation did prove to exist between presence of male sexually selected traits and the evolutionary appearance of MARTs ($p = 0.001$).

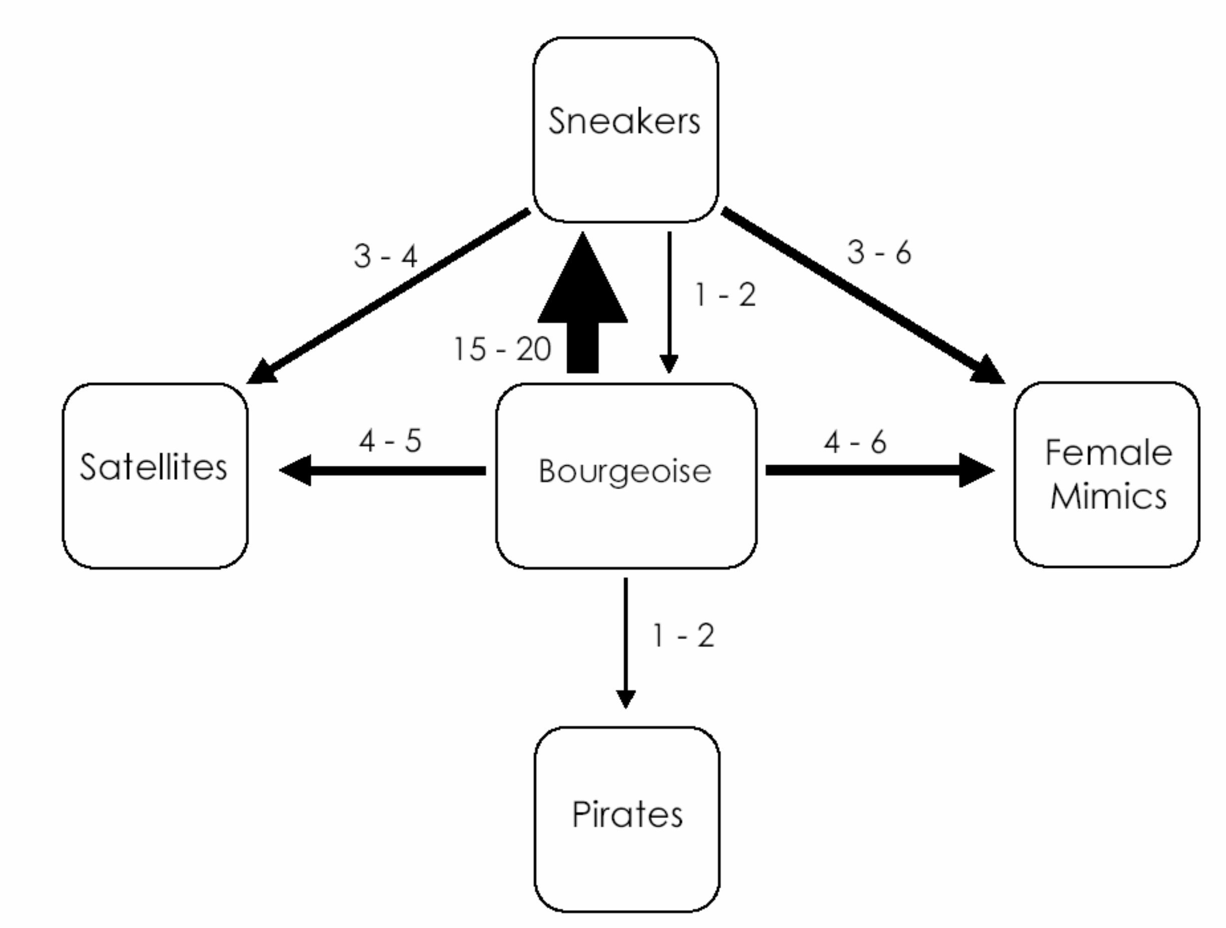

Fig. 5.3. Evolutionary transitions (as inferred from maximum parsimony criteria) between alternative MART character states, assuming that mate monopolization I normally the ancestral state. Arrow sizes correspond to mean numbers of transitions among states; numbers beside arrows indicate minimum and maximum estimates. For clarity, only significant transitions (those where the minimum estimate > 0) are shown.

Table 5.1. Maximum likelihood tests of correlated evolution[1] between MARTs and other reproductive traits.

	likelihood ratio[2] (significance[3])	
	male parental care	male sexually selected traits
MARTs	3.44 (n.s.)	8.91 (*p* = 0.001)

[1] tests conducted with DISCRETE (Pagel 1994, 1997)
[2] of the model of independent to dependent evolution.
[3] χ^2 = 2 * likelihood ratio, 4 degrees of freedom

DISCUSSION

This comparative phylogenetic analyses indicate that similar MARTs have arisen on numerous separate occasions during the evolution of actinopterygiian fishes. Although my current tallies of the number of evolutionary transitions will likely need revision as more published data become available to further resolve the actinopterygiian supertree, the large number of changes already inferred between MARTs clearly paints an overall picture of rapid evolutionary switches among these alternative reproductive behaviors.

How might these presumably complex behavioral pathways, some of which are genetically embedded and therefore heritable (Dominey 1980; Garant et al. 2003; Heath et al. 2002; Ryan and Causey 1989; Ryan et al. 1992; Zimmerer and Kallman 1989), have arisen so many times over the course of actinopterygiian evolution? A proximate or mechanistic answer may lie partly in the hormonal components of reproductive behavior.

In a wide variety of fishes, conditional male reproductive strategies, as well as their associated morphotypes, repeatedly have been shown to be due to differential expression of the same sets of sex hormones. Endocrine analyses have identified argenine vasotocin (Carneiro et al. 2003; Foran and Bass 1998, 1999), gonadotropin-releasing hormone (Bass and Grober 2001; Foran and Bass 1999), and 11-keto-testosterone (Borg 1994; Brantley et al. 1993; Ros et al. 2004) as important hormonal controls affecting MART expression in species throughout the Actinopterygii. Increasing evidence from comparative endocrinology also suggests that the sexual evolutionary plasticity we describe here may be due to convergent selection on these hormones (or their receptor proteins) across the clade (Fitzpatrick et al. 2005; Knapp 2004). Although definitive genetic links between differential hormonal expression and MARTs are not yet firmly established, a working hypothesis is that similar MARTs in different species may share proximate elements of endocrine expression that ultimately have evolved convergently under selection pressures for or against particular reproductive tactics (depending upon ecological circumstances).

This phylogenetic analysis in conjunction with behavioral reasoning also suggests that particular evolutionary pathways for MART progression predominate. As summarized in Figs. 5.2 and 5.3, mate monopolization usually may precede the evolution of sneaking behavior, which in turn may often be a precursor to female mimicry and cooperative male satellite tactics. These progressions make sense, as sneaking is often a conditional strategy based on body size (Aubin-Horth and Dodson 2004; Gross and Charnov 1980; Leiser and Itzkowitz 2004; Mazzoldi and Rasotto 2002) and may require few specialized adaptations. By contrast, female mimicry (which requires that female behaviors and phenotype are decoupled from gonadal development and gamete production) and male satellite behavior (which requires the evolution of cooperation between satellites and bourgeois males) are more complex and may therefore be secondary adaptive add-ons. Because sneaking can be a purely body-size-dependent tactic and thus potentially devoid (at least initially) of a heritable genetic trigger, it may well precede the evolution of heritable mechanisms for sneaking, as well as genetic mechanisms for secondary MARTs involving female mimicry and satellite behaviors.

Interestingly, my phylogenetic analysis suggests that piracy is not a part of this progression. A combination of factors may explain this outcome. Piracy is much less common than sneaking and, being a tactic conditioned primarily on large body-size, is likely to be exhibited only late in a fish's life cycle (most actinopterygiian fish have more-or-less indeterminate growth in which body size continues to increase with age). The combination of rarity and late-onset may reduce the effects of selection on this MART, and prevent it from being readily incorporated into the normal evolutionary pathways of MART progression. However, accounts of piracy are sparse in the scientific literature, so we cannot refute an alternative possibility that our phylogenetic analysis simply lacked the power to accurately place this MART in a clear evolutionary pathway.

Finally, the phylogenetic correlation tests revealed a statistically significant relationship between the presence of sexual selection and the presence of MARTs across evolutionary lineages. This is hardly unexpected, because the same factors that foster the evolution of sexually selected traits (namely mate monopolization through the differential reproductive success of bourgeois males; Emlen and Oring 1977) may also promote the evolution of ARTs by other males to circumvent such monopolization. It is therefore surprising that I did not also observe a phylogenetic correlation between MARTs and male parental care. In a previous analysis of this clade (Mank et al. 2005), we uncovered a significant relationship between male care of offspring and the same sexually selected traits examined here, initially suggesting to us that sexual selection, MARTs, and paternal care are all intertwined forces in the evolution of fish mating systems. Our current analysis may clarify this relationship by suggesting, more basically, that MARTs are evolutionary avoidance responses to the costly investments by bourgeois males in mate monopolization (rather than an avoidance of male brood care *per se*). Such costs of mate monopolization may often include the maintenance of sexually selected traits, defense of territories, and attraction of females.

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CHAPTER 6

CLADOGENETIC CORRELATES OF GENOMIC EXPANSIONS IN THE RECENT EVOLUTION OF ACTINOPTERYGIIAN FISHES[1]

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ABSTRACT

Genomic expansions via regional gene duplications and polyploidization events have been implicated as catalysts for rapid cladogenetic speciation in some fish taxa, but any general relationships between genome sizes and patterns of evolutionary radiation remain poorly characterized. Here I examine empirical correlations between genome size and species richness (number of extant species within a given clade) both across Actinopterygii (ray-finned fishes) and within several large actinopterygiian clades. I conducted the analyses both without and with correction (by independent contrasts) for phylogenetic effects. Across the full suite of 461 surveyed genera, relatively small but significant positive correlations were present between species richness and evolutionary increases in C-value. Although many variables (including ecological and behavioral factors) clearly can influence speciation rates, the current results are consistent with the notion that genomic architecture may play a role in species proliferation as well.

INTRODUCTION

Large-scale genomic expansions or whole-genome duplication events have been documented in early vertebrate evolution (Friedman and Hughes 2001; Ohno 1970; Wang and Gu 2000), near the base of the phylogenetic tree of teleost fishes (Christoffels et al. 2004; Meyer and Schartl 1999; Robinson-Rechavi and Laudet 2001; Wittbrodt et al. 1998), and near the basal roots of several major teleostean clades [such as salmonids (Allendorf and Thorgaard 1984), catastomids (Ferris 1984; Uyeno and Smith 1972), acipenserids (Vasil'ev 1999), and some cyprinids (Larhammar and Risinger 1994)]. Such genomic enlargements have been hypothesized as key factors that enable or perhaps even drive diversification in various vertebrate groups (Holland et al. 1994; Meyer and Malaga-Trillo 1999; Navarro and Barton 2003a; Navarro and Barton 2003b; Ohno 1970; Stephens 1951). Indeed, plausible theories that causally link genomic expansions to evolutionary radiations (Force et al. 1999; Lynch and Conery 2000; Taylor et al. 2001b) have led to a widespread notion that such enlargements routinely accelerate speciation processes (Hoegg et al. 2004; Taylor et al. 2001a). However, little comparative work has explicitly tested for the hypothesized correlations between genome dynamics and cladogenetic patterns.

Genomic architecture in collaboration with ecological or other factors could affect speciation rates via several mechanisms. First, following a genomic expansion event (e.g., by aneuploidy or polyploidization), newly duplicated loci may evolve new functions, as exemplified by the emergence of antifreeze proteins in extreme cold-water fishes (Cheng and Chen 1999). Duplicated loci that evolve new structural, catalytic, or regulatory roles (Dulai et al. 1999; Manzanares et al. 2000; Nanda et al. 2002) may permit a taxonomic group to exploit new habitats and thereby adaptively radiate. Second, most duplicated loci become mutationally silenced over time (Grauer and Li 2000), but these too may promote speciation by fostering chromosomal re-patternings via illicit recombination of non-homologous gene regions (Lynch 2002; Navarro and Barton 2003a; Navarro and Barton 2003b). Third, reciprocal silencing of complimentary duplicate genes (or their regulatory regions) in separate populations is potentially another major source of genomic divergence conducive to the emergence of genetic incompatibilities (Lynch and Conery 2000; Lynch and Force 2000). Finally, some appreciable genomic expansions may be due to repetitive transposable elements, and these too may alter gene expression patterns or otherwise alter genomic profiles in ways that promote speciation events (Brosius 1999; Capy 1997; McDonald 1990; McDonald 1995; McDonald 1998). In theory, any or all of these factors could increase cladogenetic rates in lineages that experience salient genomic expansions. This is the working hypothesis tested here, using comparative phylogenetic methods on fishes.

Among the vertebrates, ray-finned fishes display exceptionally high variation in genome size (Hinegardner 1976; Venkatesh 2003). In contrast to mammals, birds, and reptiles, where in each case genome sizes collectively span only about a two-fold range, fish genomes vary in DNA content (C-values) by more than an order of magnitude: e.g., from the compact genome of the pufferfish (*Fugu rubripes*) with 0.39 picograms (pg) of DNA per cell, to the huge genome of the armored catfish (*Corydoras aeneus*) with 4.4 pg DNA per cell (Hinegardner and Rosen 1972). Such wide variation in genome size in a well-known taxonomic group with more than 20,000 described extant species makes fishes excellent candidates for examining empirical relationships between genome dynamics and evolutionary radiations.

MATERIALS AND METHODS

From recent compendiums (Brainerd et al. 2001; Gregory 2001; Hardie and Herbert 2003; Hinegardner and Rosen 1972), I assembled a database on haploid genome sizes (pg DNA per cell) in 823 surveyed species of actinopterygiian fishes representing 461 genera (appendix F). I then averaged the C-values within each genus, omitting from all calculations the few cases where polyploidy occurred as an intraspecific polymorphism. I also recorded the number of extant species for each genus from the current standard taxonomy (Eschmeyer 1998; Eshmeyer 1990; Froese and Pauly 2004; Nelson 1994).

To examine whether species richness per genus varies with regard to genome size, I employed least squares regression to calculate correlation coefficients (r) and test their significance (p). An ongoing debate about whether phylogeny should be explicitly accommodated (Felsenstein 1985; Harvey and Pagel 1991) or ignored (Harvey and Rambaut 1998; Price 1997; Ricklefs 1996) in comparative evolutionary studies has not yet been resolved, so I present analyses from both types of investigations, as follows.

First, I treated all 461 surveyed genera as independent observations, i.e. without regard to their phylogenetic associations. In these analyses, c-value and species counts were log transformed. Second, to correct for phylogeny, I used a recently constructed supertree for Actinopterygii (Mank et al. 2005), which itself was based primarily on extensive recently published phylogenetic data for various groups of actinopterygiian fishes. This phylogenetic cladogram was analyzed by independent contrasts (Felsenstein 1985; Grafen 1990) as implemented for measures of species richness in the software package macroCAIC (Agapow and Isaac 2002). This method attempts to correct for phylogenetic non-independence among data points by confining attention to trait comparisons across each bifurcating node in an underlying phylogeny, thereby yielding sets of independent data points or “contrasts” (Martins 1996).

In these analyses, soft polytomies were coded as such, and altogether the dataset yielded 189 independent contrasts that I used to test for significant associations, employing linear regression (Harvey and Pagel 1991; Pagel 1993; Purvis and Rambaut 1995). These contrasts proved to be scattered across the supertree (rather than concentrated in particular sets of related genera), as evidenced in part by the fact that 121 of the contrasts (64%) were above the taxonomic level of family. Raw C-value contrasts were square-root-transformed to reduce skew (Quinn and Keough 2002). To prevent a few outlying observations from unduly influencing the regression relationships, I removed two genera (*Haplochromis* and *Barbus*) that were each more than seven standard deviations from the mean species count.

I also conducted comparable analyses on several large actinopterygiian clades for each of which 20 or more data points were available. These involved the superorders Ostariophysi and Atherinomorpha, and the taxonomic orders Tetraodontiformes and Pleuronectiformes. These sub-clade analyses were performed in identical fashion to those described above for the full Actinopterygii.

RESULTS

Haploid genome sizes among the surveyed taxa ranged from 0.39 pg/cell (pufferfish genus *Chelonodon*) to 3.57 pg/cell (sturgeon genus *Acipenser*), with values showing a roughly normal distribution around a mean of 1.19 pg/cell. This distribution is similar to previous reports for fishes (Hardie and Herbert 2003; Hinegardner and Rosen 1972).

Across the full suite of more than 450 actinopterygiian genera surveyed, a statistically significant positive correlation emerged between average genome size and number of species in a genus (Fig. 6.1). This relationship held both for the raw data ($n = 461$, $r = 0.10$, $p = 0.027$; Fig. 6.1A), and for the independent-contrast data corrected for phylogeny ($n = 189$, $r = 0.20$, $p = 0.002$; Fig. 6.1B).

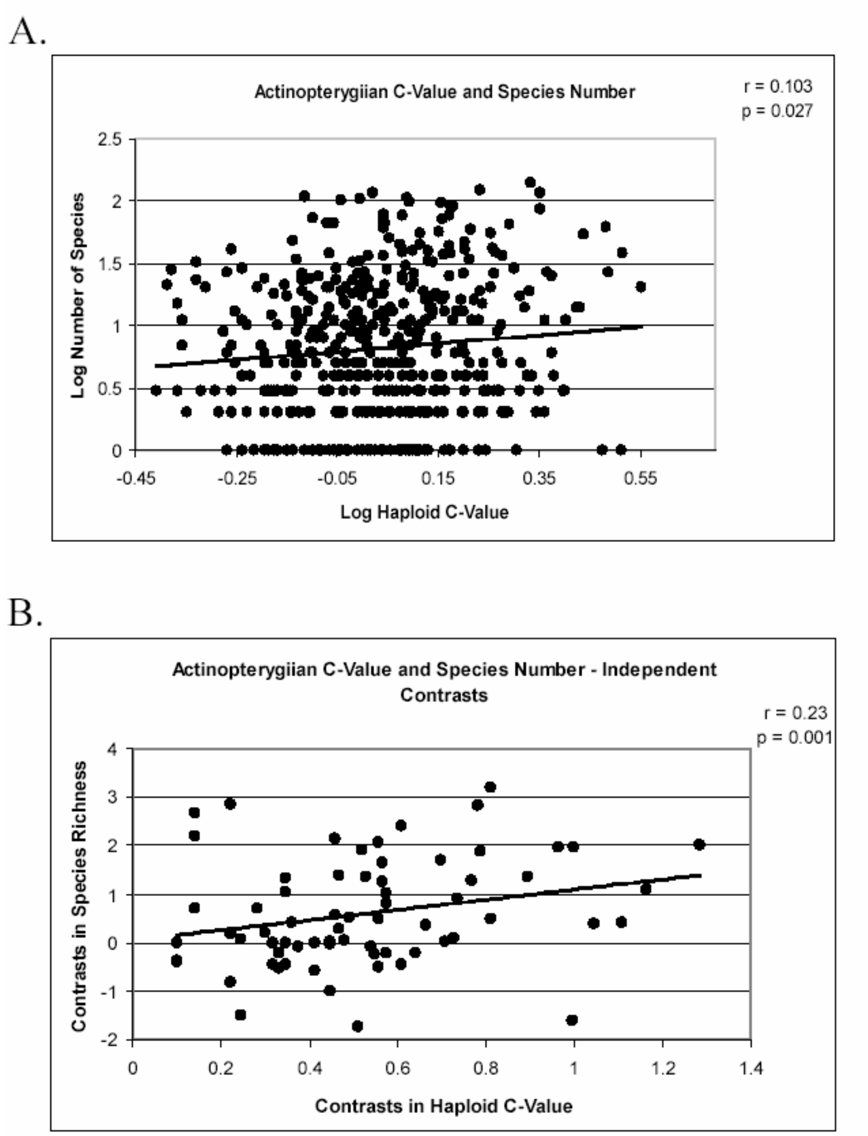

Figure 6.1. Correlation between genome size and species richness in all surveyed actinopterygiian genera. *A*, phylogenetically uncorrected; *B*, phylogenetically corrected by independent contrasts. In both analyses, C-value is measured in picograms DNA per haploid cell. Contrasts in C-value (B) are square root transformed. The trend line in both regressions is shown.

In the finer-scale analysis of taxonomic superorders and orders, several patterns appeared (Table 6.1). Ostariophysi showed a marginally non-significant positive correlation between genome size and generic species richness in the phylogenetically uncorrected analysis ($n = 179$, $r = 0.09$, $p = 0.13$), and the analysis via independent contrasts was significant ($n = 41$, $r = 0.29$, $p = 0.03$). Atherinomorpha showed a stronger positive correlation in both the uncorrected analysis ($n = 24$, $r = 0.28$, $p = 0.08$; Fig.6. 2A) and in the phylogenetically corrected version ($n = 20$, $r = 0.54$, $p = 0.006$; Fig. 6.2B), though the significance of this latter analysis relies on what may be an outlier datapoint. Pleuronectiformes exhibited a positive correlation in the phylogenetically uncorrected analysis ($n = 23$, $r = 0.32$, $p = 0.07$), but independent contrasts failed to recover a significant relationship ($n = 8$, $r = 0.26$, $p = 0.27$). Finally, Tetraodontiformes showed a negative correlation ($n = 26$, $r = 0.50$, $p = 0.005$; Fig. 6.2C) that proved to be statistically significant in the uncorrected analysis but not so when analyzed by independent contrasts ($n = 11$, $r = 0.30$, $p = 0.18$; Fig. 6.2D).

DISCUSSION

The notion that genomic expansions might contribute to speciation was introduced long before the modern molecular era (Haldane 1933; Ohno 1970; Stephens 1951), but interest in the topic has been rekindled with the recent explosion of genome-level data (Christoffels et al. 2004; Meyer and Schartl 1999; Wittbrodt et al. 1998). For example, it now appears likely that the initial evolutionary radiation of teleosts was immediately preceded by large-scale or whole-genome duplication events (Amores et al. 2004; Hoegg et al. 2004; Taylor et al. 2003). Apart from polyploidizations, regionalized duplications of both extensive (Postlethwait et al. 2002;

Table 6.1. Summary of statistical regressions between genome size and species richness for Actinopterygii and various subclades.

clade	# species	# genera	mean C-value[1] (SD)	correction for phylogeny?[2] (# ind. con.)[3]	correlative trend[4]	*r* =	*p* =
Actinopterygii	823	461	1.19	no	+	0.10	0.027
			(0.50)	yes (189)	+	0.20	0.002
Ostariophysi	350	179	1.41	no	n.s.	0.09	0.13
			(0.49)	yes (41)	+	0.29	0.03
Pleuronectiformes	28	23	0.75	no	+	0.32	0.07
			(0.14)	yes (8)	n.s.	0.26	0.27
Tetraodontiformes	41	25	0.62	no	-	0.50	0.005
			(0.18)	yes (11)	n.s.	0.30	0.18
Atherinomorpha	68	26	1.03	no	+	0.28	0.08
			(0.25)	yes (20)	+	0.54	0.006

[1] pg DNA per haploid cell;

[2] correction by independent contrasts;

[3] number of independent contrasts;

[4] positive correlations indicate statistically significant situations in which clades with larger genomes have relatively more extant species; negative correlations are cases in which clades with smaller genomes contain more extant species; n.s. means a non-significant association.

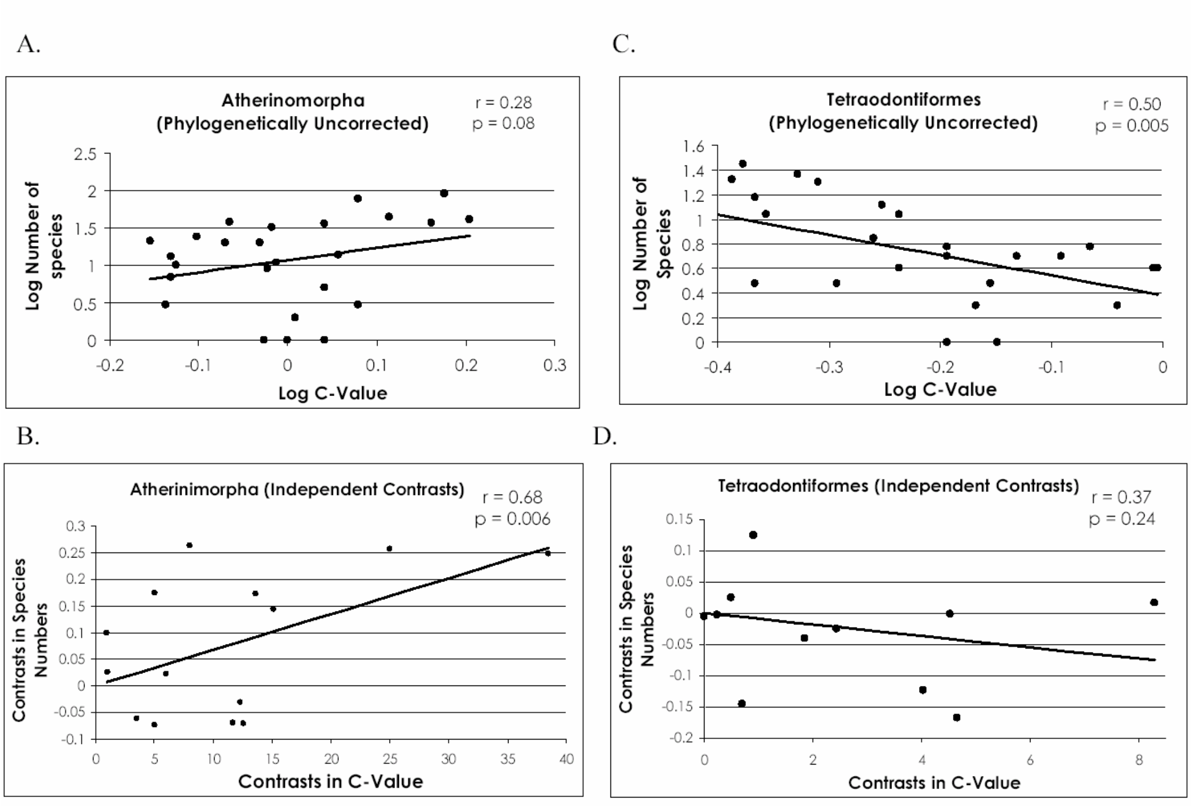

Figure 6.2. Examples of regressions between genome sizes and species numbers in actinopterygiian subclades (see legend to Fig. 6.1 for further explanation). *A and B*, Atherinomorpha (phylogenetically uncorrected and corrected, respectively); *C and D*, Tetraodontiformes (phylogenetically uncorrected and corrected, respectively). C-value is measured in picograms DNA per haploid cell.

Smith et al. 2002) and more limited (Amores et al. 1998; Nanda et al. 2002) genomic sections have been documented in several groups of fishes, as have genomic expansions due to activities of repetitive element families (Nogare et al. 2002; Volff et al. 2001a; Volff et al. 2001b).

Compared to most other vertebrate groups, the genomes of ray-finned fishes are evolutionarily labile in DNA content, apparently expanding and contracting rather quickly via extensive duplications and losses of genetic material (Neafsey and Palumbi 2003; Robinson-Rechavi and Laudet 2001). Despite long-standing suspicions that genomic expansions may often be associated with bursts of cladogenesis, this study is the first to assess this possibility empirically across multiple clades in a large taxonomic group of animals. I addressed net changes in genome content only, because the particular mechanistic reasons for alterations in genome size are not yet well understood in most fish genera.

The current analysis provides some support for the oft-hypothesized link between genome dynamics and cladogenesis. The presence of a statistically significant trend, despite numerous confounding variables (enumerated below), suggests that appreciable genome expansions have indeed been a factor associated with accelerated speciations in ray-finned fishes.

Qualifications

Several sources of biological and statistical noise are nearly inevitable in the type of comparative phylogenetic analyses employed here. First, differential extinction rates across clades could have masked the postulated relationship between genome size and speciation rate in extant clades. Older clades might be most susceptible to this problem because extinctions would tend to accumulate over time following any bursts of cladogenesis. I attempted to minimize such extinction effects by focusing on genera rather than higher taxonomic levels. In other words, because discernable consequences of genomic expansions on cladogenesis might be evolutionarily ephemeral, they might best be examined in recent clades where their historical footprints should remain most evident. Two additional reasons motivated my focus on genus-level species richness: many more comparisons are available at this level than at higher echelons of the taxonomic hierarchy; and the mean half-life of duplicate genes (i.e., before they are silenced by mutations) is about four million years in animals (Lynch 2002; Lynch and Conery 2000; Lynch and Force 2000), so evolutionary radiations promoted by gene duplications might be expected to proceed within the general timeframe associated with congeneric divergences in many vertebrate groups (Avise et al. 1998; Johns and Avise 1998).

Second, taxonomic biases could have introduced noise into this analysis. Suppose, for example, that genomic enlargements tend to spur exceptionally large evolutionary alterations in organismal morphology or behavior. Then, a rapidly speciating clade might have been split by systematists into more genera than a slowly speciating clade, and thereby show fewer (rather than more) extant species per genus on average. I took existing generic assignments at face value, so these or other kinds of taxonomic artifacts would not have been recognized or accommodated in these analyses.

Third, these comparative analyses were based strictly on cladogram structure and did not include information on branch lengths or evolutionary timescales. Unfortunately, neither fossil records nor molecular data for Actinopterygii are as yet adequate to date all relevant nodes in the supertree that provided the phylogenetic framework for this report. This is another reason why indicators of relative speciation rates across genera might be inaccurate.

My fourth reservation is a general caveat that applies to all evolutionary studies of this ilk. The comparative method can only identify trait associations, so mechanisms (e.g., ecological, genetic, or physiological) underlying any correlations remain unspecified. Indeed, the possibility cannot be ruled out that evolutionary variables are correlated merely because they are both influenced by third-party factors (although in the current case it seems difficult to imagine what factor could promote cladogenetic rates and genome size variation jointly but without involving at least some causal links between the two).

Finally, another potential confounding factor is that salient genomic contractions (like salient genomic expansions) might also accelerate cladogenesis, if for example they tend to foster regulatory changes or cytogenetic rearrangements that promote genetic incompatibilities between populations (Lynch and Force 2000; Venkatesh 2003). In the current study, the negative correlation between genome size and species richness in Tetraodontiformes (Fig. 6.2C, D) is consistent with this possibility. This taxonomic order includes species that by virtue of extensive recent deletions of non-functional DNA (Neafsey and Palumbi 2003) display some of the smallest genomes known for any vertebrate taxa (Aparicio et al. 2002).

Genome Dynamics and Cladogenesis

Despite the several reasons (discussed above) for pessimism in detecting any general correlation between changes in genome size and apparent speciation rates, this comparative evolutionary analysis of recently evolved fish taxa nonetheless was able to detect a statistically significant relationship between these two variables. If not spurious, this correlation could be reflective of any of several causal mechanisms by which changes in genome size might translate into increased probabilities of cladogenesis, such as via alterations of gene expression patterns

(Brosius 1999; Capy 1997; McDonald 1998) or via the reciprocal silencing of redundant duplications at different locations in the genome (Lynch and Conery 2000; Lynch and Force 2000). Dissections of such casual processes will require case-by-case functional genomic analyses of particular actinopterygiian taxa.

Speciation is a multifaceted phenomenon (Coyne and Orr 2004), and genomic dynamism is only one plausible category in a complex nexus of causative agents that also includes many ecological and behavioral considerations. Given the diversity of factors impinging on cladogenetic patterns, the current documentation of a significant association between genomic expansion and increased cladogenesis across many piscine genera, as well as within several larger subclades of Actinopterygii, seems quite surprising.

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CHAPTER 7

PHYLOGENETIC CONSERVATION OF CHROMOSOME NUMBERS IN ACTINOPTERYGIIAN FISHES[1]

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ABSTRACT

The genomes of ray-finned fishes (Actinopterygii) are well known for their evolutionary dynamism as reflected by drastic alterations in DNA content often via regional and whole-genome duplications, differential patterns of gene silencing or loss, shifts in the insertion-to-deletion ratios of genomic segments, and major re-patternings of chromosomes via non-homologous recombination. In sharp contrast, chromosome numbers in somatic karyotypes have been highly conserved over vast evolutionary timescales-- a histogram of available counts is strongly leptokurtic with more than 50% of surveyed species displaying either 48 or 50 chromosomes. Here I employ comparative phylogenetic analyses to examine the evolutionary history of alterations in fish chromosome numbers. The most parsimonious ancestral state for major actinopterygiian clades is 48 chromosomes. When interpreted in a phylogenetic context, chromosome numbers evidence many recent instances of polyploidization in various lineages but there is no clear indication of a singular polyploidization event that has been hypothesized to have immediately preceded the teleost radiation. After factoring out evident polyploidizations, a correlation between chromosome numbers and genome sizes across the Actinopterygii is marginally statistically significant ($p = 0.012$) but exceedingly weak ($R^2 = 0.0096$). Overall, this phylogenetic analysis indicates a mosaic evolutionary pattern in which the forces that govern labile features of fish genomes must operate largely independently of those that operate to conserve chromosome numbers.

INTRODUCTION

Recent comparative analyses have shown that ray-finned fishes (Actinopterygii) exhibit rapid genomic changes compared to other vertebrate clades (Robinson-Rechavi et al. 2001; Venkatesh 2003; Volff 2005). The sources of this evolutionary dynamism have been hypothesized to include any combination of the following: elevated rates of gene and genome duplication (Christoffels et al. 2004; Meyer and Schartl 1999; Robinson-Rechavi and Laudet 2001; Wittbrodt et al. 1998) functional sub-partitioning of duplicate genes (Amores et al. 2004; Force et al. 1999); elevated transposon activity (Ozouf-Costaz et al. 2004) changes in the insertion-to-deletion ratio of genomic segments (Neafsey and Palumbi 2003); cytogenetic rearrangements (Arkhipchuk 1995; de Almeida-Toledo et al. 2002; Postlethwait et al. 2002; Smith et al. 2002; Thomas et al. 2003); and rapid origins and dissolutions of sex chromosomes (Devlin and Nagahama 2002; Mank et al. 2006). Causal links have also been suggested between this unusually high genomic variability and the exuberant species diversity of actinopterygiian fishes (Holland 1994; Mank and Avise 2006; Meyer and Schartl 1999; Navarro and Barton 2003a; Navarro and Barton 2003b; Ohno 1970; Stephens 1951).

This rapid pace of genomic change in ray-finned fishes might suggest that the overarching chromosomal scaffolding should be highly variable as well, an impression further reinforced by assessments of genome size. In actinopterygiian fishes, haploid C-values span roughly an order of magnitude-- from 0.39 picograms (pg) of DNA per cell in the pufferfish (*Fugu rubripes*) to 5.85 pg DNA per cell in the bichir (*Polypterus palmas*) (Hinegardner and Rosen 1972). This huge span of genomes sizes in actinopterygiian fishes is several-fold greater than those in most other major vertebrate groups (Gregory 2005; Hinegardner 1976; Venkatesh 2003).

Here I examine another feature of actinopterygiian genomes: genetic scaffolding as reflected in chromosome numbers. By interpreting somatic chromosome counts (and genome sizes) in a phylogenetic context provided by a recently published supertree for the Actinopterygii (Mank et al. 2005), I further document a surprising ultraconservatism in chromosome numbers that contrasts dramatically with the evolutionary dynamisms displayed by numerous other features of actinopterygiian genomes.

MATERIALS AND METHODS

For 1546 vertebrate species, chromosome numbers (per somatic cell) and haploid genome sizes (C values) were retrieved from the Animal Genome Size Database (Gregory 2005, online at www.genomesize.com). From histograms of these data for each of several major vertebrate taxa, I computed standard summary statistics, including kurtosis or the sharpness of the distribution peak (Sokal and Rohlf 1995).

For the actinopterygiian species, I then mapped chromosome numbers onto the supertree topology of Mank et al. (2005), using MacClade 4 (Maddison and Maddison 2000), and reconstructed putative ancestral states under maximum parsimony criteria (a full phylogeny showing all the species analyzed and their somatic chromosome counts are available in the supplemental materials). I inferred putative polyploidization events where terminal or internal nodes showed roughly a two-fold or higher chromosome count than the nearest relative or sister clade.

I also analyzed the relationship between chromosome number and genome size through linear regression, both for all surveyed actinopterygiian species (n = 615) and for diploids only (i.e., after removing 78 species identified as evident polyploids). For both analyses, standard correlation coefficients (R^2) and their probabilities (p) were computed.

RESULTS

Chromosome numbers in the 615 species of ray-finned fishes ranged from 22 to 250, but the frequency distribution was strongly leptokurtic (peaked) with a mode at 48 (Fig. 1; Table 1). Most actinopterygiians displayed either 48 chromosomes (29.3% of the species surveyed) or 50 chromosomes (25.4%). With the blatant exception of mammals, other vertebrate taxonomic classes (amphibians, reptiles, and birds) exhibited similarly leptokurtic distributions of chromosome counts, albeit with each group having a different mode (Figure 1).

Across all surveyed actinopterygiian species, I uncovered a highly significant association between genome size and chromosome number ($R^2 = 0.26$, $p < 0.001$). However, this relationship was greatly diminished (but remained marginally significant; $R^2 = 0.01$, $p = 0.012$) when evident polyploids were removed from consideration (Fig.7.2). Approximately 78 such polyploid species were discernible in our survey, and we estimate from the phylogeny that they stem from 7 - 20 separate polyploidization events within the Actinopterygii. Understandably, most of these still-recognizable polyploidization events were concentrated near tips of the supertree, occurring at the genus or species level in all groups except Chondrostei (where all extant acipenseriform taxa appear to be of deeper polyploidy ancestry). Polyploidization events have also been common in Cypriniformes and Salmoniformes, where polyploid lineages clearly are phylogenetically interspersed with diploid lineages.

Table 7. 1. Summary statistics for chromosome numbers in major vertebrate groups.

Clade (n)	2*N* Range	Mean	Mode	Variance	Kurtosis
Actinopterygii (615)	22-250	55	48	22	30.27
diploid species (537)	22-78	48	48	6	6.69
Amphibia (326)	20-108	28	26	11	22.91
Reptilia (170)	22-66	38	36	8	1.39
Aves (104)	50-138	76	80	10	14.99
Mammalia (327)	6-102	46	48	18	-0.32

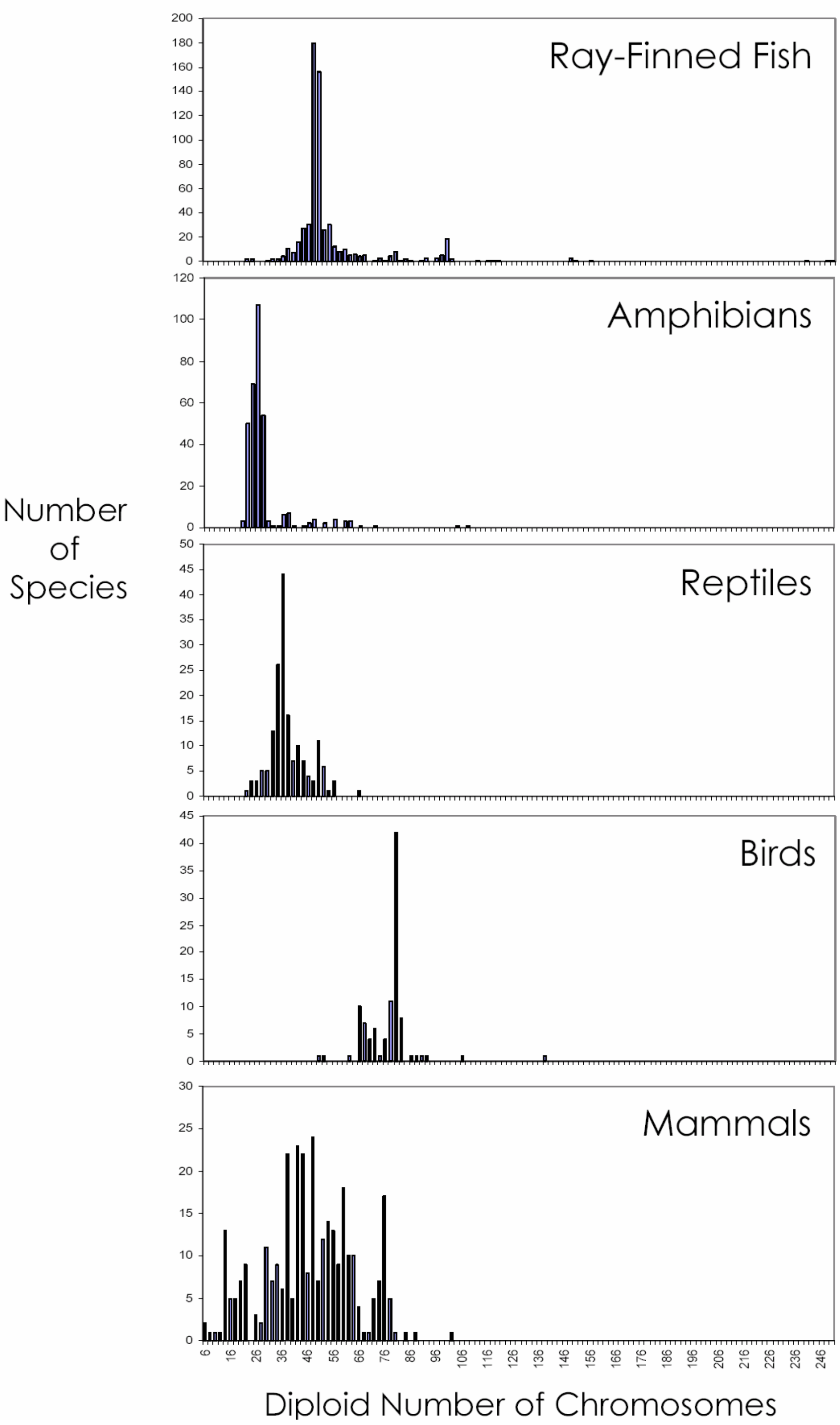

Figure 7.1. Histogram of diploid chromosome numbers for several taxonomic groups of vertebrates (data taken from Gregory, 2005). See also Table 7.1.

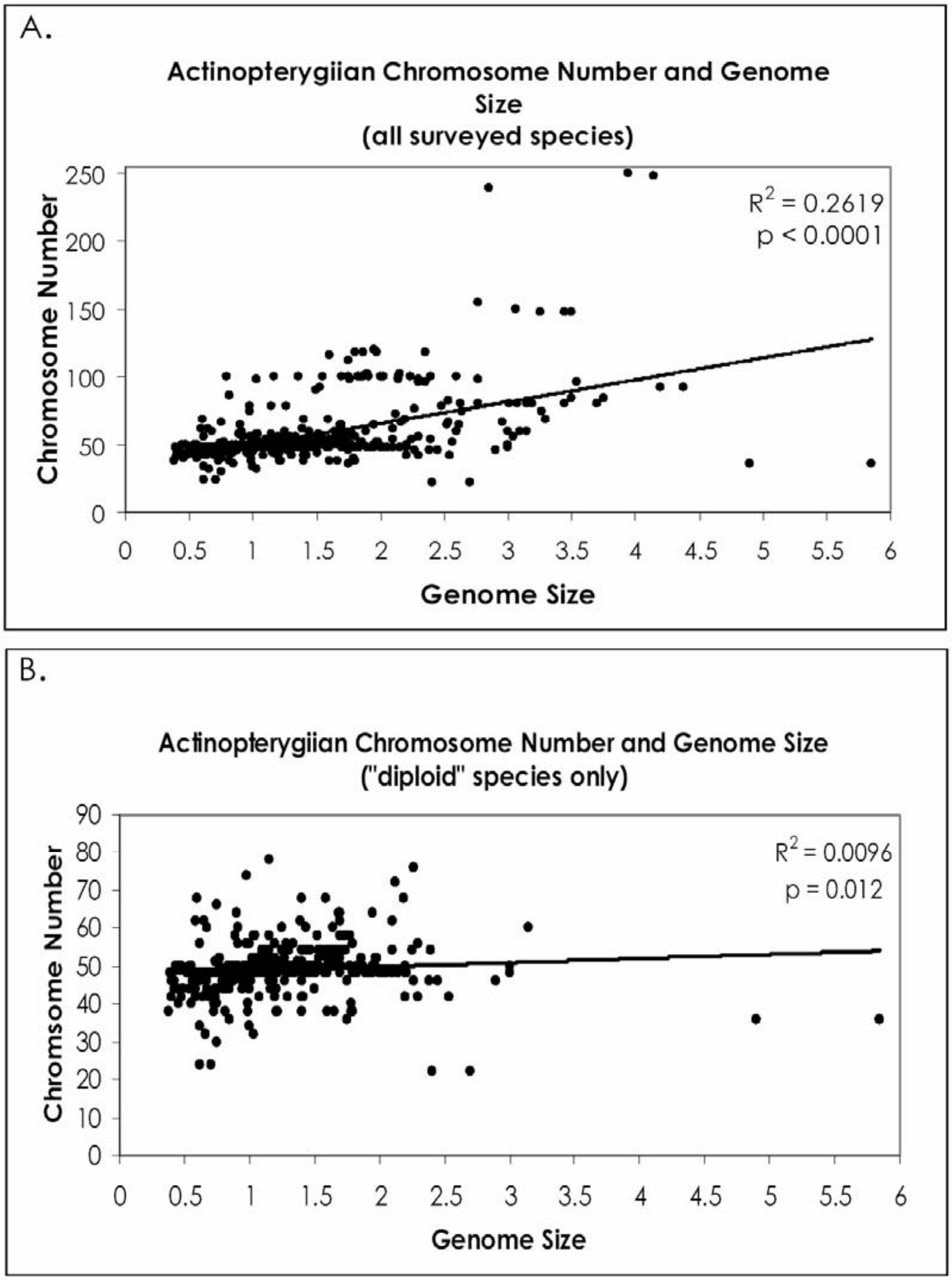

Figure 7.2. Scatterplot of the relationship between genome size (as measured by haploid C-value) and somatic chromosome number. Panel A shows the relationship for all 615 surveyed species of Actinopterygii; panel B shows the relationship for 537 "diploid" species of Actinopterygii, after removal of putatively polyploid taxa.

Figure 7.3 provides a condensed summary of the maximum parsimony reconstruction of chromosomal evolution on the phylogenetic supertree for ray-finned fishes. Despite numerous small departures from the modal number, 48 chromosomes per somatic cell is the most common extant condition as well as the most parsimonious ancestral state for Teleostei and several major subclades therein (Fig. 7.3). Interestingly, most acanthopterygiian lineages display 48 chromosomes, whereas most ostariophysiian lineages exhibit 50 chromosomes (although 48 remains the most parsimonious ancestral count for the basal Ostariophysi). Overall, the Ostariophysi also exhibit far more variation in chromosome numbers than do the Acanthopterygii, with several lineages exhibiting small or modest reductions, expansions, and also polyploid deviations from the probable ancestral state.

The precise ancestral chromosome number at the base of the full actinopterygiian clade could not be reconstructed with confidence, due primarily to variability in this trait among ancient Chrondrostei. However, that original ancestral condition was probably less than 48 chromosomes, according to the parsimony analysis.

DISCUSSION

This analysis has added a phylogenetic perspective to several interesting patterns previously reported in fish chromosomal evolution. First, somatic chromosome numbers are indeed remarkably stable in acanthopterygians, and this evolutionary conservatism contrasts with the striking diversity of actinopterygiian lineages in terms of genome size, composition, and synteny. Thus, dramatic evolutionary changes in these latter genomic features have been accomplished within a relatively steadfast framework of genomic scaffolding as reflected in chromosome numbers. Second, this evolutionary conservatism in chromosome numbers holds

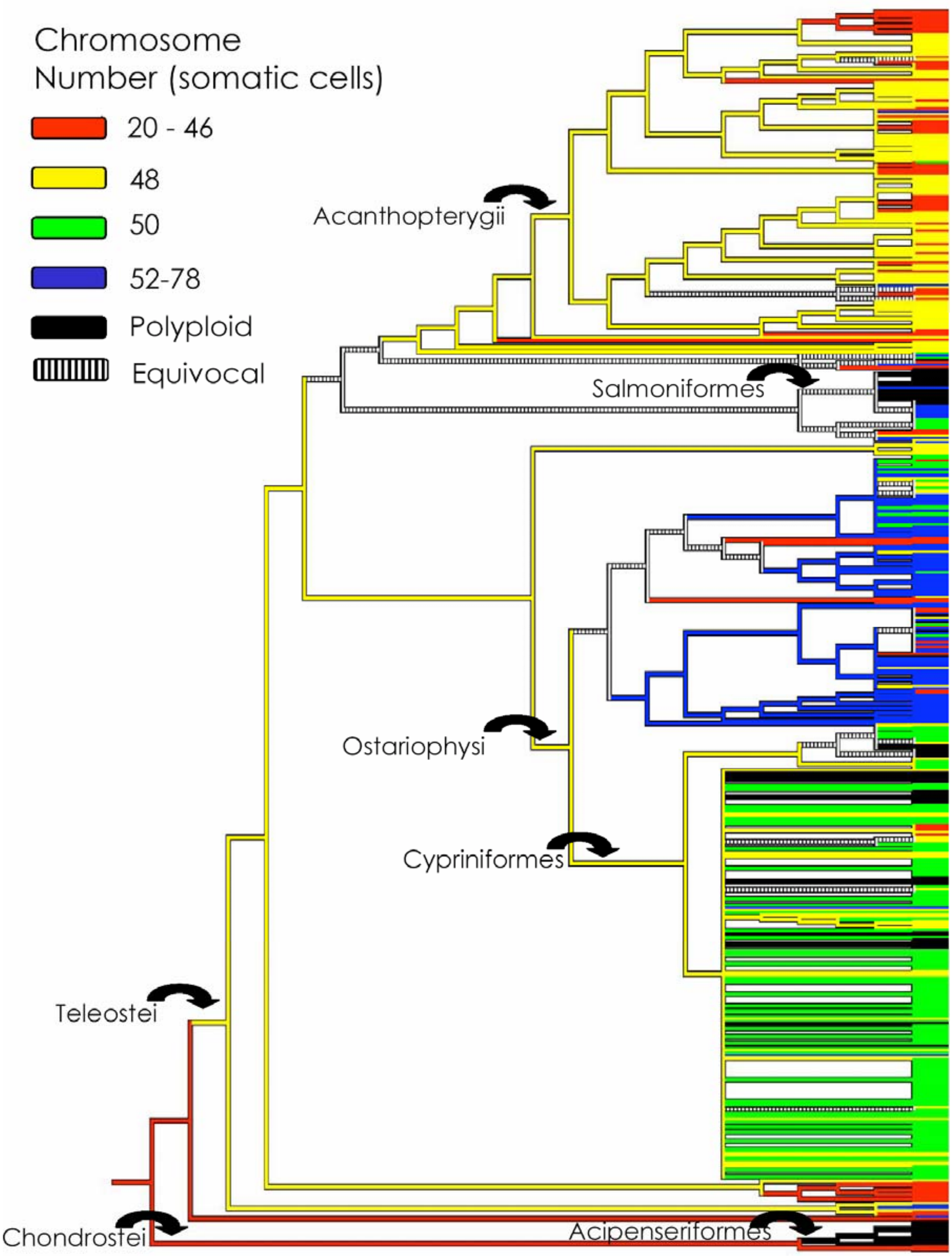

Figure 7.3. Actinopterygiian phylogeny showing chromosome numbers in extant taxa as well as results of a maximum parsimony reconstruction of ancestral character states. Clades mentioned in the text are indicated.

despite the evident capacity of actinopterygiian lineages to accommodate large karyotypic alterations via occasional polyploidization events. Third, chromosome numbers in actinopterygiian species are centered at 48 and 50 per somatic cell, with the frequency distribution being strongly leptokurtic. Maximum parsimony reconstruction suggests that the somatic cell count in ancestral teleosts was probably 48, but also that numerous small permutations from this or a similar number have occurred throughout the teleost clade.

These reconstructions also agree with previous assertions that ray-finned fishes exhibit a high tolerance for polyploidy (Allendorf 1984; Christoffels et al. 2004; Ferris 1984; Larhammar and Risinger 1994; Uyeno and Smith 1972; Vasil'ev 1999). Based on current C-value comparisons, about 7 - 20 polyploidization events were inferred in the present phylogenetic analysis. These were usually most evident on recent twigs of the phylogenetic tree, where the evolutionary footprints of sudden large shifts in chromosomal numbers are expected to be best preserved. I found no discernable phylogenetic evidence for a previously proposed whole-genome duplication at the root of the teleosts (Christoffels et al. 2004; Meyer and Malaga-Trillo 1999; Meyer and Schartl 1999; Wittbrodt et al. 1998). However, this observation carries a significant caveat: over time, genome dynamics including large-scale deletions and chromosome re-patternings could likely have erased most direct karyotypic evidence for ancient genomic doublings.

My analysis lacks the karyotypic resolution to determine how polyploidization, changes in genome size, and other forms of genomic dynamism manifest cytologically. Although the current literature lacks sufficient data to permit a comparative analysis of detailed cytogenetics across the Actinoptergyii, preliminary analyses based on available genome sequence data

(Thomas et al. 2003) suggest that synteny is not well conserved in this group. This suggests that there may be a great deal of cryptic cytological diversity at finer karyotypic levels.

I have no compelling explanation for the general conservation of chromosome numbers in actinopterygian fishes. An ad hoc (but unenlightening) possibility is that phylogenetic inertia generally has inhibited changes in chromosomal numbers (Blomberg and Garland 2002), especially since considerable modifications in genome size in fishes can evidently occur largely independent of changes in chromosome counts (Fig. 7.2). But this merely begs the question of why such phylogenetic inertia might exist for this but not many other genomic features. Perhaps there are cytokinetic constraints of some sort on shifts in chromosome numbers in fishes. But then why would such constraints appear to apply with much less force to some other, younger vertebrate clades (e.g., mammals; Fig. 7.1)?

Even more perplexing is why actinopterygiian fishes display a highly leptokurtic distribution of chromosome counts centered at 48 - 50 chromosomes per somatic cell. If one speculates that this outcome reflects something inherently important about the absolute number and distribution of chromosomes (or perhaps associated genetic factors such as chromosomal break-points or total recombination potential), then one must also be prepared to explain why various other vertebrate groups show leptokurtic distributions centered on very different chromosome numbers (26, 30, and 80 in amphibia, reptiles, and birds, respectively; Fig. 7.1; Table 7.1).

If definitive answers to these and related conundrums are eventually to emerge, comparative genome analyses on larger comparative scales may be required, perhaps coupled with novel lines of thought about the possible evolutionary forces that shape chromosomal dynamics. Novel insights about other aspects of vertebrate genomes (e.g., regarding molecular mechanisms of rampant DNA loss in pufferfish; Neafsey and Palumbi 2003) have already emerged from massive sequencing efforts (Aparicio et al. 2002; Jaillon et al. 2004), so perhaps the evolutionary patterns that I have summarized here will someday be understood also in terms of evolutionary mechanism and process.

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CHAPTER 8

SUPERTREE ANALYSIS OF THE ROLES OF VIVIPARITY AND HABITAT IN THE EVOLUTION OF ATHERINOMORPH FISHES[1]

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[1] Mank JE, JC Avise. In press. Journal of Evolutionary Biology
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ABSTRACT

Using supertree phylogenetic reconstructions, I investigate how livebearing and freshwater adaptations may have shaped evolutionary patterns in the Atherinomorpha, a large clade (≈1500 extant species) of ray-finned fishes. Based on maximum parsimony reconstructions, livebearing appears to have evolved at least four times independently in this group, and no reversions to the ancestral state of external brooding were evident. With respect to habitat, at least five evolutionary transitions apparently occurred from freshwater to marine environments, at least two transitions in the opposite direction, and no clear ancestral state was identifiable. All viviparous clades exhibited more extant species than their oviparous sister taxa, suggesting that transitions to viviparity may be associated with cladogenetic diversification. Transitions to freshwater were usually but not invariably associated with increased species richness, but the trend overall was not significant across pairs of sister clades. Additionally, I investigated whether livebearing and freshwater adaptations are currently associated with elevated risks of extinction as implied by species' presence on the 2004 IUCN Red List. Despite being correlated with decreased brood size, livebearing has not significantly increased extinction risk in the Atherinomorpha. However, freshwater species were significantly more likely than marine species to be listed as endangered.

INTRODUCTION

Atherinomorphs are unusual among the ray-finned (actinopterygiian) fishes in that they have repeatedly evolved both livebearing (Lydeard 1993; Mank et al. 2005; Reznick et al. 2002) and freshwater adaptations (Helfman et al. 1997). Both of these traits have profound effects on dispersal, reproductive rate, and life history, characters that have been implicated in diversification and extinction in a variety of organisms (Crooks and Soule 1999; Diamond 1984; Lydeard 1993; MacArthur and Wilson 1967; Purvis et al. 2000; Trewick 1997; Vrba 1983; Waters and Wallis 2001; Winker 2000).

Two forms of livebearing-- lecithotrophy (wherein the embryo is nourished by egg yolk that was provisioned prior to fertilization) and matrotrophy (where nutrient transfer occurs directly from mother to embryo)-- have each evolved numerous times in fishes (Gross and Sargent 1985; Gross and Shine 1981; Mank et al. 2005; Meyer and Lydeard 1993; Reznick et al. 2002; Wourms 1981). Livebearing (as contrasted with external embryonic development) profoundly affects both maternal-fetal relationships and the mating behaviors by both sexes. In particular, each evolutionary transition to viviparity from oviparity requires the establishment of complex suites of physiological, anatomical, and behavioral adaptations for internal brooding (Amoroso 1968; Guillette 1989; Schindler and Hamlett 1993; Wourms et al. 1988) as well as the evolution of intromittant organs and behavioral modifications for internal fertilization (Rosen and Gordon 1953; Zauner et al. 2003).

Being presumably intricate, these physiological and anatomical changes might also act as an evolutionary ratchet for livebearing, perhaps inhibiting the loss of viviparity even in lineages that may no longer benefit from it. Indeed, the precocity of live-born progeny presents a reproductive tradeoff: viviparous females normally produce fewer progeny but these offspring may experience a higher survival rate than oviparous progeny (Wourms 1981; Wourms et al. 1988; Wourms and Lombardi 1992). In other animals, higher extinction risks have sometimes been associated with smaller litter sizes (Bennett and Owens 1997; Jones et al. 2003; Saether et al. 2005) and higher trophic levels (Crooks and Soule 1999; Diamond 1984).

Many atherinomorph fishes also spend all or most of their lives in freshwater (Helfman et al. 1997). Due to the discontinuous physical nature of such habitats, stream and lake-dwelling fishes often experience lower dispersal and inter-population gene flow than comparable marine species (DeWoody and Avise 2000), and these factors can promote vicariant speciations (Vrba 1983; Waters and Wallis 2001; Winker 2000). But disjunct freshwater habitats can also reduce local population sizes and perhaps thereby increase inbreeding, two classic correlates of increased extinction risk (Lande 1999).

My aims in this study are threefold: to test whether important evolutionary adaptations have cladogenetic effects; to ascertain whether an elevated extinction risk is associated with these adaptations; and to test the feasibility of supertree construction (given current computational capabilities) for an exceptionally large clade with more than 1,500 terminal taxa. The Atherinomorpha, comprised of Cyprinodontiformes (ca. 1,000 species of guppies, platyfish, and allies), Beloniformes (nearly 250 species of needlefish, ricefish, and allies), and Atheriniformes (about 300 species of silversides, rainbowfish, and allies), is phylogenetically one of the best-characterized clades of ray-finned fishes, making it an ideal group for these goals. To that end, I have constructed a species-level supertree for Atherinomorpha and used its topology to identify sister clades with alternate character states for both livebearing and freshwater adaptations. By definition, sister taxa are equally old (Cracraft 1981), therefore independent contrasts between many such pairs permit tests of possible lifestyle associations with other evolutionary features such as speciation rate and extinction risk.

MATERIALS AND METHODS

Supertree Construction

I constructed a formal MRP (matrix representation with parsimony) supertree (Ragan 1992) from available phylogenetic literature for all currently recognized species (Eshmeyer 1990) of Atherinomorpha, being careful to omit phylogenetic inferences based on livebearing or its associated anatomies *per se* (to avoid circular reasoning). The data matrix underlying this supertree reconstruction consisted of cladogenetic information from 58 published source phylogenies (listed in Appendix G), each generated from molecular or morphological data for overlapping subsets of atherinomorph species. I reconciled all nomenclature in these source phylogenies according to recent taxonomic work (Eshmeyer 1990; Nelson 1994). In the data matrix, which was coded in standard binary format (Baum 1992; Ragan 1992), each column represents a provisional monophyletic clade as identified in a given source phylogeny, with the information from all 58 source phylogenies concatenated into a supermatrix that consisted of 1355 columns (putative clades) and 1544 rows (species).

Because recent phylogenetic appraisals have shown good support for the monophyly of each atherinomorph order (Miya et al. 2003; Parenti 1981), and because datasets with > 200 taxa create massive computational complexity, I performed the following two parsimony-based analyses on each taxonomic order independently before reassembling the ordinal-level topologies into an atherinomorph supertree. First, using PAUP* 4.0b (Swofford 2003), I conducted 100 heuristic searches of the data matrix using random-order addition. Each search starts in a different area of tree space, so multiple searches help ensure that a local optimum in not mistaken for the global optimum. I computed a 90% consensus tree from 100,000 trees with the best tree score, and used its topology for subsequent comparative phylogenetic analyses. Second, to confirm the best tree score, I performed 1000 iterations (five sets of 200 iterations each) of the parsimony ratchet (Nixon 1999) as implemented by PAUPrat (Sikes and Lewis 2001). Agreement between the heuristic searches and the parsimony ratchet does not guarantee the best possible tree, but it does suggest a good provisional tree. For Atheriniformes and Cyprinodontiformes, the parsimony ratchet did not result in a lower tree score than did the heuristic PAUP* searches with random order addition. In Beloniformes, however, the parsimony ratchet returned a lower tree score than the standard PAUP* searches, so in this case I used the ratchet to generate 10,000 trees with this lower score and then used these equally parsimonious trees to generate a 90% consensus phylogeny. Current molecular (Miya et al. 2003), morphological (Parenti 1981), and supertree meta-analysis (Mank et al. 2005) concordantly indicate that Atheriniformes is the outgroup to Beloniformes and Cyprinodontiformes, so I assembled the ordinal phylogenies accordingly.

Species Diversity and Extinction Risk

I assembled a database on fertilization mode and embryonic development from several sources for all recognized species of Atherinomorpha (Breder 1922; Breder and Rosen 1966; Constanz 1989; Froese and Pauly 2004; Ghedotti 2000). Then, using the supertree topology and maximum parsimony reconstruction as implemented in MacClade 4 (Maddison and Maddison 2000), I examined the evolutionary histories of livebearing. I assumed that the ancestral state for Atherinomorpha was oviparity with external fertilization, as viviparity has previously been shown to be a derived trait in ray-finned fishes (Lydeard 1993; Mank et al. 2005). I identified sister clades with alternate reproductive modes, and then evaluated whether viviparous clades are more diverse than oviparous clades in terms of numbers of extant species using 1000 iterations of a randomization test (Nee et al. 1996; Vamosi and Vamosi 2005). Such randomization tests evaluate in this case whether a particular adaptation is correlated with increased diversity across (not within) all analyzed sister-clades. Only non-nested sister clades that were exclusively viviparous or oviparous were considered in these comparisons.

I also examined whether transitions to freshwater habitat may have promoted species diversity. I assembled a database on numbers of extant freshwater and marine species for all the atherinomorphs (Froese and Pauly 2004), and then performed the same types of comparative phylogenetic analyses as described above for livebearing. As with the above-described analysis, all comparisons were non-nested.

Finally, I mapped extinction risk as determined by the IUCN 2004 Red List (Baillie et al. 2004) onto the supertree phylogeny. Species with the following IUCN designations were treated as those "at elevated risk" for extinction: extinct, extinct in the wild, critically endangered, endangered, vulnerable, near threatened, and conservation dependent. Many sister clades contained few taxa and thus provided very low statistical power, so I examined possible associations of elevated extinction risk with livebearing and freshwater adaptations across the entire Atherinomorpha. According to the IUCN Red List, 9.85% of atherinomorph species are now in jeopardy. Accordingly, I used this figure to calculate expected numbers of at-risk species given the observed numbers of taxa with viviparous versus oviparous and with freshwater versus marine lifestyles, assuming random associations between the variables. For each pair of reproductive or habitat states, I compared the random distribution to the observed distribution using one-tailed Fisher's exact test.

RESULTS

The full supertree for Atherinomorpha is presented in condensed form in Figure 8.1. Although the published data were insufficient to resolve the phylogenetic placement of all 1500+ species, nearly all relationships among genera were resolved in the 90% consensus tree.

Based on this phylogenetic analyses, viviparity in Atherinomorpha apparently evolved on at least four separate occasions: once in Beloniformes and thrice in Cyprinodontiformes (Fig. 8.1). I found no evidence that livebearing, once gained, was ever subsequently lost from any atherinomorph lineage. In sister-clade analyses, each of the viviparous clades identified proved to be significantly more species-rich than their oviparous sister-lineages (Table 8.1), a trend that overall was marginally non-significant (randomization test, $p = 0.0625$). Furthermore, viviparity proved not to be significantly associated with elevated extinction risk at the present time (Fisher's exact test, $p = 0.09$; Table 8.2).

Cyprinodontiform fishes inhabit freshwaters primarily, but inter-conversions between marine and freshwater habitats clearly have occurred on multiple occasions in Beloniformes and Atheriniformes. These inter-conversions (at least five transitions from freshwater to marine, two from marine to freshwater) were primarily at the congeneric or confamilial level and therefore are not shown in Fig. 8.1. Compared to marine lineages, freshwater lineages do not appear to have experienced a higher rate of diversification (randomization test, $p = 0.125$), but they do appear to suffer a higher current risk of species extinction (Fisher's exact test , $p < 0.0001$; Tables 8.3 and 8.4). Because lifestyle transitions between freshwater and marine environments have been rather frequent and scattered across Atherinomorpha, no ancestral state for this trait could be identified unequivocally.

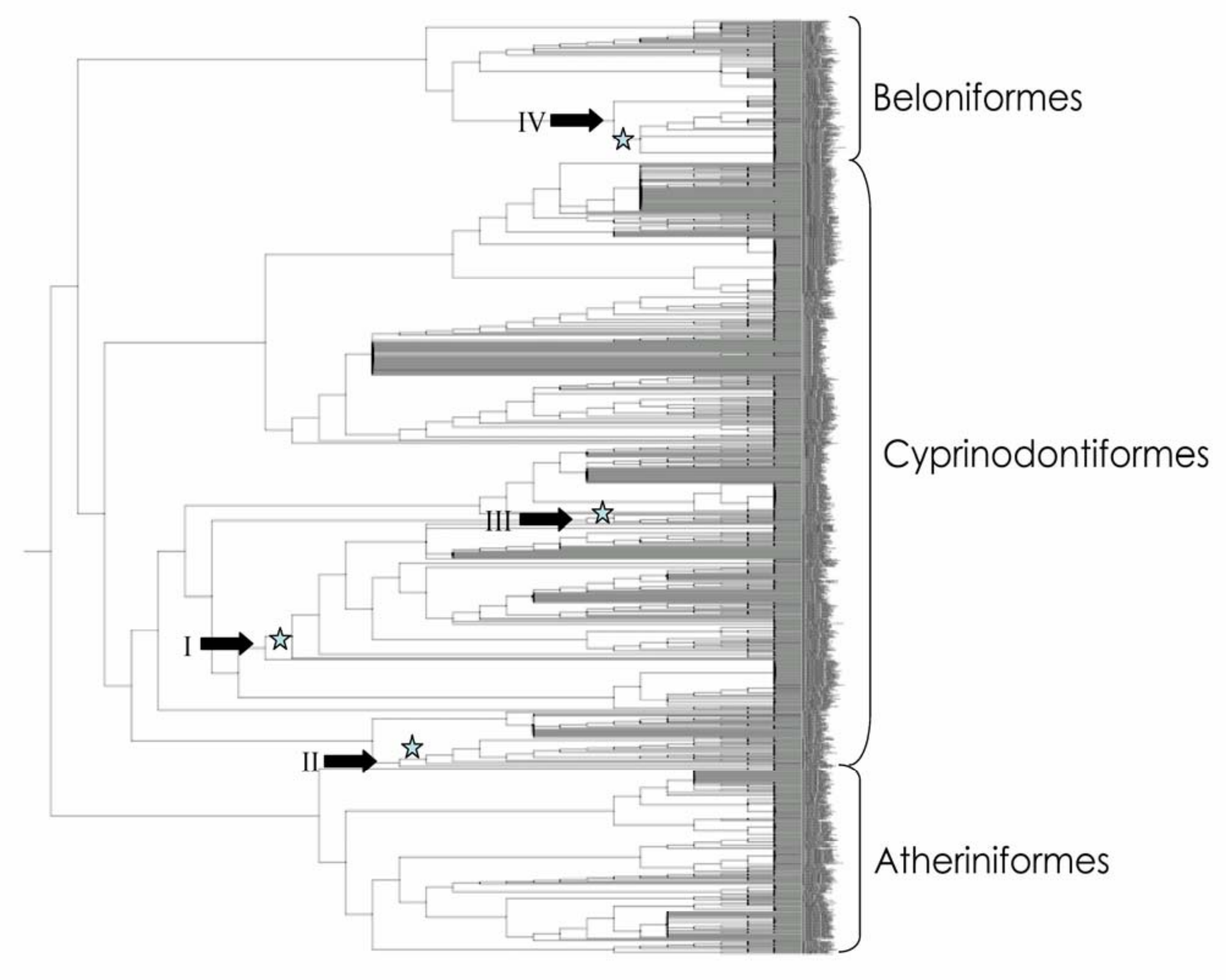

Figure 8.1. Condensed supertree topology for Atherinomorpha. Shown is the condensed, 90% consensus of 100,000 equally parsimonious trees. Taxonomic orders within the Atherinomorpha are indicated to the right of the topology. Stars indicate independent origins of livebearing in this group, and arrows show the root of sister-clade comparisons. Roman numerals adjacent to sister clades correspond to the statistical comparisons in Table 8.1.

Table 8.1. Sister-clade comparisons of species richness in relation to livebearing.

Comparison	Clade	Number viviparous species	Number oviparous species
I	Poeciliinae	230	1
II	Goodeidae/Profundulidae	46	5
III	Anablepinae	14	1
IV	Hemiramphidae	95	21

Randomization matched pairs test: p = 0.0625

Table 8.2. Extinction risk in relation to reproductive mode. Livebearing and external brooding were evaluated, using Fisher's exact test, for a possible significant difference in the current number of threatened species.

Reproductive Mode[a]	Observed (and expected[c]) number of threatened taxa	Significantly higher risk of extinction than expected?
Livebearing (4)	34 (37)	no
External Brooding (1)[b]	135 (114)	no[d]

[a] estimated number of unambiguous origins under maximum parsimony
[b] assumed ancestral state, and no reversions from livebearing
[c] expected under random association with lifestyle or habitat
[d] $p = 0.09$, Fisher's exact test

Table 8.3. Sister-clade comparisons of extant species richness in relation to freshwater or marine habitats.

Taxonomic order (Family)	Freshwater clade (number of extant taxa)	Marine clade (number of extant taxa)
Beloniformes (Hemiramphidae)	*Nomorhamphus* and *Dermogenys* (31)	*Hemirhamphodon* (6)
Beloniformes (Belonidae)	*Xenentodon* (2)	Stronglyura, Tylosurus, *and* Ablennes (21)
Atheriniformes (Atherinidae)	*Craterocephalus* (24)	*Atherinion* (3)
Atheriniformes (Atherinopsidae)	*Basilichthys* and *Odontesthes* (24)	*Atherinops, Atherinopsis, Colpichthys*, and *Leuresthes* (5)
Atheriniformes (Telmatherinidae)	all other telmatherinids (16)	*Kalyptatherina* (1)

Randomization matched pairs test: $p = 0.156$

Table 8.4. Extinction risk in relation to aquatic habitat. Marine and freshwater adaptations were evaluated, using Fisher's exact test, for a possible significant difference in the current number of threatened species.

Habitat (number of independent origins)[a]	Observed (and expected[b]) number of threatened taxa	Significantly higher risk of extinction than expected?
Freshwater (2)	151 (132)	yes[c]
Marine (5)	1 (20)	no

[a] estimated number of unambiguous origins under maximum parsimony

[b] expected under random association with lifestyle or habitat

[c] $p < 0.00001$, Fisher's exact test

DISCUSSION

Supertree reconstruction is the only approach currently available for combining phylogenies from diverse and otherwise incompatible data matrices (Ragan 1992). Supertree methods have made possible the construction of cohesive phylogenies from disparate data sets for several major taxonomic groups, such as angiosperms (Davies et al. 2004) and bats (Jones et al. 2002). The phylogeny for Atherinomorpha presented here, with 1,544 included species, is to my knowledge the largest supertree yet reported for any animal assemblage (Bininda-Emonds 2004). Most of the source phylogenies for this supertree were based on molecular data (notably mtDNA sequences), but a sizable portion also utilized morphological evidence.

Supertrees with large taxon samples present enormous challenges for maximum parsimony searches. I tried to minimize this complexity in two ways. First, I divided the sample according to monophyletic taxonomic order. Restricting the supertree searches to monophyletic clades reduced complexity of the supertree reconstruction. Second, I also employed the parsimony ratchet, which has been shown to outperform standard heuristic parsimony searches in some cases (Nixon 1999), including supertrees (Price et al. 2005). Despite these shortcuts, the analyses presented here represent the equivalent of two+ years of computational time for one computer (to greatly reduce the search time, I actually used 14 dual-processor G4 MacIntosh machines). Without far more powerful search methods, it seems doubtful that supertrees with much larger numbers of taxa could be recovered. For example, a species-level supertree for all Actinopterygii, with >20,000 extant species, would require either enormous computational capacity or much cleverer search methods than those available at present.

The supertree presented here is of course preliminary and potentially subject to topological revision at internal nodes (especially at genus and species levels) as additional phylogenetic information becomes available. The same applies to supertrees constructed for other taxa. Although biological conclusions from supertree approaches are inherently provisional, I expect that similar analysis methods will soon become increasingly feasible and popular for comparative phylogenetic applications in many taxonomic groups and biological settings.

Implications of Viviparity

In agreement with previous appraisals (Lydeard 1993; Mank et al. 2005), I estimate at least four separate origins of livebearing in Atherinomorpha. My results show that each of these four viviparous clades was much more species-rich than its respective oviparous sister-lineage (Table 8.1), but also that the overall trend *across* these clades was only marginally significant (p = 0.0625) in randomization tests (due to the inevitably poor power of this test statistic when only a few sister-clades are available for analysis). Whether or not viviparity increases diversification, it does not appear to be associated with an increased risk of extinction at the present time (Table 2). Whether or not viviparity increases diversification, it does not appear to be associated with an increased risk of extinction at the present time (Table 8.2). Both of these findings suggest that any benefits of livebearing, including larger offspring born to a higher trophic level, may generally outweigh potential disadvantages of smaller clutch sizes (Wourms and Lombardi 1992).

These findings raise questions as to why viviparity is relatively uncommon in Atherinomorpha (only about 25% of species are livebearers) and even rarer across the entire Actinopterygii. This probably reflects, at least in part, the difficulty of evolving sophisticated suites of physiological, anatomical, and behavioral features associated with livebearing. Several key biological modifications are entailed in any evolutionary transition from oviparity to viviparity, and these evolutionary hurdles may be difficult to overcome. On the other hand, as judged by recurrent evolutionary origins of viviparity in Atherinomorpha, at least some lineages in this clade appear pre-disposed for transitions to livebearing, possibly due to the high incidence of internal fertilization in this group (Breder and Rosen 1966).

Implications of Freshwater Habitat

Any evolutionary transition from marine to freshwater lifestyles could have both positive and negative impacts on a lineage's prospects for cladogenesis. Freshwater habitats to most fishes are like land islands to many terrestrial organisms (MacArthur and Wilson 1967), physically subdividing a species and limiting gene flow in ways that can promote local diversification. On the other hand, these same fragmenting processes can produce small and specialized populations that individually may be subject to higher risks of extinction (Lande 1999). Although four of the five analyzed sister-clades showed higher species richness in freshwater lineages, the low power of the randomization test prevented demonstration of a significant trend overall (Table 8.3). Despite the lack of a consistent correlation between freshwater habitat and higher species diversity, freshwater occupancy does appear to significantly increase current risks of species' extinction (Table 8.4).

There are several inter-related characteristics of freshwater fishes that may increase extinction risk. Freshwater fishes typically experience larger barriers to dispersal than marine fishes, this can decrease effective population size, lead to problems with inbreeding and mutational load, and accelerate the evolution of specialized adaptations to limited local and ecologies that are poorly suited to other habitats. These barriers to dispersal also often result in endemic freshwater species that are limited to small geographic areas, making them more vulnerable to localized ecological and environmental fluctuations. All these factors may contribute to the elevated extinction risk for freshwater species, though more detailed analyses will be needed to parse out the separate effects of these different potential factors.

Regardless of the cladogenetic implications, the biological alterations required for each successful evolutionary transition between marine and freshwater environments would seem to be fewer and less complex than those involved in the evolutionary transitions between oviparity and viviparity. Freshwater adaptations primarily involve osmo-regulatory adjustments (Helfman et al. 1997), and many freshwater (and diadromous) lineages clearly retain a tolerance for high salinity regimes (Froese and Pauly 2004). The suspected relative ease with which many fish lineages can make evolutionary transitions between freshwater and marine habitats is consistent with the rapid and recent inter-conversions (many at the level of taxonomic genus) between these lifestyles that are evident in our current phylogenetic appraisals.

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CHAPTER 9

SEXUAL SELECTION AND TAXONOMIC DIVERSITY IN RAY-FINNED FISHES [1]

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[1] Mank, J.E. In review. American Naturalist, submitted Febraury 2006.

ABSTRACT

Evolutionary theory predicts that sexual selection may increase taxonomic diversity when emergent mating preferences result in reproductive isolation and therefore speciation. This theory has been invoked to explain patterns of diversity in ray-finned fishes (most notably in the cichlids), but the theory has not been tested comparatively in fish. Additionally, several other unrelated factors have been identified as promoters of cladogenesis, so it is unclear how important sexual selection might be in diversification of this group. Using sister-clade analysis, I tested the relationship between the presence of sexually selected traits and taxonomic diversification in actinopterygiian fishes, a large clade that shows substantial diversity in mating preferences and related sexually selected traits. In all identified sister-families that differed with regard to the proportion of species manifesting sexually selected traits, sexual selection was correlated with increased diversification, and this association was significant across all sister clades ($p = 0.008$). This suggests that sexual selection, when present, is a substantial driver of diversification in the ray-finned fishes, and lends further empirical support to the theoretical link between mating preferences and accelerated cladogenesis.

INTRODUCTION

Changes in mating preferences can theoretically lead to speciation events if the emergent mating preferences result in assortative mating and reproductive (prezygotic) isolation. Increased prezygotic isolation would be expected to accelerate taxonomic diversification compared to postzygotic barriers to gene flow, which can require long periods of time to accumulate. This suggests that sexual selection, a common response to the pressures of mating preferences, may be associated with taxonomic diversification (Darwin 1871; Lande 1981; Lande 1982; West-Eberhard 1983). This correlation has been supported by some large-scale comparative analyses, primarily in birds and invertebrates (Barraclough et al. 1995; Gleason and Ritchie 1998; Masta and Maddison 2002; Polak et al. 2004).

The theory linking sexual selection and cladogenesis has been anecdotally invoked to explain observed patterns of diversity in some clades of ray-finned (actinopterygiian) fishes (McMillan et al. 1999; Mendelson 2003), most often for the cichlids (Dominey 1984; Knight et al. 1998; Maan et al. 2004). Sexual selection via mating preference has been documented in shaping a variety of traits in the ray-finned fishes, including dichromatism (Endler 1980; Houde and Endler 1990), breeding tubercles (Kortet et al. 2003; Kortet et al. 2004) and elongated fins (Basolo 1990; Meyer 1997). Despite these clear documentations linking mating preferences and sexually selected traits (usually, though not always displayed in males) for species or small clades of Actinopterygii, some comparative studies in the ray-finned fishes have failed to uncover the expected manifestations of female preference (Mank et al. 2006; Ritchie et al. 2005), casting doubt as to the pervasiveness and magnitude of female preference through the clade.

Additionally, the relative importance of sexual selection in explaining patterns of actinopterygiian diversity is unclear as several other unrelated factors have recently been identified as promoting diversification in this clade. Changes in genomic architecture (Hoegg 2004; Mank and Avise 2006), key innovations such as internal gestation (Lydeard 1993; Mank and Avise in press) and antifreeze genes (Bargelloni et al. 1994; Eastman and McCune 2000; Near et al. 2004), as well as vicariance (Hurwood and Hughes 1998; Murphy and Collier 1996; Murphy and Collier 1997; Near et al. 2003; Planes and Fauvelot 2002) have all been demonstrably linked to increased cladogenesis in the ray-finned fishes. It is therefore likely that sexual selection, if a major factor at all, acts in combination with other unrelated causes to create the current patterns in actinopterygiian diversity.

Despite the evidence for and against sexual selection as a driver of diversification in ray-finned fishes, the theory has not been tested across the clade, probably due to a combination of problems with the underlying phylogeny and the difficulty in gathering sufficient data on sexually selected traits. The recent construction of a well-resolved provisional supertree (Mank et al. 2005) for the Actinopterygii has partially resolved this problem and provides the necessary phylogenetic framework for a broad-scale comparative analysis. This supertree framework, in conjunction with numerous species accounts and field guides, offers the first opportunity to test the role of mating preference in promoting taxonomic diversification across the Actinopterygii. Using sister clade comparisons identified from the supertree, I test the relationship between the presence of sexually selected traits, a proxy for mating preference, and increased diversification in the ray-finned fishes.

MATERIALS AND METHODS

From the actinopterygiian supertree (Mank et al. 2005), I identified all potential sister families. Sister families are pairs of families that are taxonomically more related to one another than they are to any other family, and are equally old by definition (Cracraft 1981). This type of comparative analysis therefore automatically corrects for shared ancestry in the assessment of any correlative relationship.

For each of these sister taxa, I first assessed whether there were any manifest sexually selected traits, using a family level compendium (Breder and Rosen. 1966). I focused on sexually dimorphic traits that have been shown unambiguously to be the result of sexual selection in fishes, and have been used in other comparative appraisals of sexual selection in the Actinopterygii (Mank and Avise in review; Mank et al. 2006; Mank et al. 2005), rather than sexually dimorphic traits that may be the result (at least partially) of natural selection. Sister families that completely lacked evidence of sexual selection in both clades were removed from any further analysis. These sister clades are uninformative regarding the relationship between manifest sexual selection and diversification because any quantitative cladogenetic differences must be due to other factors.

For the sister families with some degree of manifest sexual selection, I searched numerous field guides, species accounts, and aquarium references for descriptions of sexually selected traits (see appendix H) in all currently taxonomically recognized species (Eschmeyer 1998; Eshmeyer 1990; Froese and Pauly 2004). Sexually selected traits including in this analysis have been shown in fish to be the result of mating preferences, and they include extended or elongate fins or rays (Harrington 1997; Kuwamura et al. 2000; Marcus and McCune 1999), breeding tubercles (Kortet et al. 2003; Kortet et al. 2004), and sexual dichromatism (Amundsen and Forgren 2001; Houde and Endler 1990; Reimchen 1989; Stott and Poulin 1996).

Numerous fish species are described on the basis of a single preserved type specimen, often collected long before the taxonomy is evaluated and described. Since color patterns often rapidly fade in preservation jars, it is not possible to ascertain from preserved type specimens whether sexual dichromatism, the most common manifestation of sexual selection in fish, exists. In order to avoid underestimating the incidence and importance of sexual dichromatism in the dataset, I did not use species accounts based solely on preserved specimens. This strategy presented an alternative problem, as some families are described almost entirely based on pickled individuals. I therefore removed all sister families that were insufficiently characterized (<10% of recognized species described in detail) from further analysis.

I analyzed the remaining sister families according to the recommendations of Barraclough et al. (1995), Nee et al. (1996), and Vamosi and Vamosi (2005). For each sister family, I calculated the proportion of species that exhibited sexually selected traits, as well as determined the current number of recognized species (Eschmeyer 1998; Eshmeyer 1990). Under the null expectation that female preference does not influence patterns of diversity, we would expect families with a higher proportion of species with manifestations of sexual selection to be no more or less taxonomically diverse than their sister families. I evaluated the data against this null expectation with a randomization test for matched pairs according to Nee et al. (1996) and Barraclough et al. (1995), which I solved probabilistically rather than with repetitions. The randomization test is similar to the Wilcoxon sign test, which is not applicable to small numbers of comparisons. The randomization test computes the probability that the observed patterns of diversity, the correlation between greater taxonomic diversity and a higher proportion of manifest sexual selection, is due to chance alone across all the analyzed clades.

Two sister-families showed no significant difference from one another in the proportion of species with sexually selected traits (less than 10% difference between sister families). Because I was unable to gather information on all the recognized species in these sister-clades, sampling error obscures whether these small differences are significant. I therefore treated these comparisons in the same manner as the comparisons that lacked manifest sexual selection entirely. Comparisons with the same degree of sexual selection in both sister families are uninformative, as any differences in diversification must be due to factors other than mating preferences. These clades were therefore not included in the randomization analysis (Barraclough et al. 1995).

RESULTS

Of the 66 potential sister families, 42 (64%) lacked sexually selected traits entirely. I was unable to find sufficient data for another 15 identified sister clades.

The nine informative sister clades are shown in Table 1, and are distributed across seven taxonomic orders. Of the 816 species characterized in these 18 taxonomic families, 36% exhibited sexually selected traits. This is most definitely an overestimate

Table 9.1.Sister-families analyzed in this study.

	Sister Families (Order)	Number of Species[A]	Number of Characterized Species (%)	Proportion Manifesting Sexual Selection	Direction of Correlation[B]
I.	Poeciliidae	309	55 (18)	0.42	
	Anablepidae	15	11 (73)	0.00	+
	(Cyprinodontiformes)				
II.	Goodeidae	47	9 (19)	0.89	
	Profundulidae	5	5 (100)	0.20	+
	(Cyprinodontiformes)				
III.	Melanotaenidae	67	16 (24)	0.69	
	Bedotiidae	11	11 (100)	0.27	+
	(Atheriniformes)				
IV.	Belonidae	34	8 (24)	0.13	
	Scomberesocidae	4	4 (100)	0.00	+
	(Beloniformes)				
V.	Monacanthidae	107	28 (26)	0.39	
	Balistidae	42	18 (43)	0.00	+
	(Tetraodontiformes)				
VI.	Bothidae	157	32 (20)	0.41	
	Cynoglossidae	136	36 (26)	0.00	+
	(Pleuronectiformes)				
VII.	Labridae	481	133 (28)	0.52	
	Scaridae	95	36 (78)	0.58	n.s.
	(Perciformes)				
VIII.	Gobiidae	1426	227 (16)	0.22	
	Eleotridae	161	45 (28)	0.28	n.s.
	(Perciformes)				
IX.	Characidae	1113	116 (10)	0.58	
	Alestiidae	111	26 (23)	0.31	+
	(Characiformes)				

[A] Number of recognized species according to Eschmeyer (1994)

[B] Direction of correlation between sexual selection and species diversity. Where the proportion of species exhibiting sexually selected traits in sister clades differed by less than 0.1, the clade was not included in the randomization test.

of the incidence of sexually selected traits for the entire Actinopterygii, as all sister clades that lacked manifestations of mating preferences are not included. The percentage of species in a given family exhibiting manifestations of sexual selection ranged from 0 to 89, as shown in Table 9.1. Sexual dichromatism was the most common trait, present in 75% of species that manifested sexual selection, followed by elongate rays or fins (29%). Breeding tubercles were not documented in any of the species surveyed for this analysis.

For the seven comparisons that differed with respect to the proportion of species exhibiting sexual selected traits, all showed a positive association between the presence of sexual selection and taxonomic diversity. This was significant across all seven comparisons ($p = 0.008$, randomization test).

Neither of the perciform comparisons (Gobiidae-Eleotridae and Labridae-Scaridae) differ significantly between sister families with regard to the proportion of recognized species manifesting sexually selected traits (the differences between the sister families in both comparisons was less than 10% in my sample). As with previous estimates of sexually selected traits and taxonomic diversity (Barraclough et al. 1995), these comparisons were not assessed in the randomization test, as they are uninformative regarding the relationship between sexual selection and diversification. Despite the fact that these families do not differ significantly with regard to percent exhibiting sexually selected traits, if they are included in the analysis, the relationship between sexual selection and taxonomic diversity remains marginally significant ($p = 0.022$, randomization test).

DISCUSSION

This analysis supports the theoretical link between sexual selection and taxonomic diversification, through the presumed intermediate of shifting mating preferences. Additionally, these findings are concordant with previous comparative work in birds (Barraclough et al. 1995), suggesting that sexual selection acts in a similar manner throughout the vertebrates to accelerate cladogenesis. Because the observed pattern was significant across several taxonomically diverse actinopterygiian orders, this work implies that mating preference is another mechanism to explain the heterogeneity in taxonomic diversity, as well as some instances of rapid cladogenesis exhibited by many clades of ray-finned fishes (Clements et al. 2003; Johns and Avise 1998; Ruber et al. 2003; Ruber and Zardoya 2005).

It is somewhat surprising that all the informative comparisons showed a concordant pattern of positive association between sexual selection and taxonomic diversity. Previous comparative work has identified other important factors that drive diversification in the ray-finned fishes. These other factors, such as genome duplication (Hoegg 2004; Mank and Avise 2006), and key innovations like internal gestation and anti-freeze proteins (Bargelloni et al. 1994; Lydeard 1993; Mank and Avise in press), would be expected to obscure the association between sexual selection and cladogenesis in some taxa. Both genomic changes and transitions to viviparity have been observed in some of the sister-clades analyzed here, and are therefore likely responsible for at least some of the observed pattern of taxonomic diversity. Because each comparison in this analysis recovered the same positive relationship despite these possibly confounding factors, it is likely that sexual selection via mating preference is a common force shaping the diversity of the ray-finned fishes.

There are several caveats to this analysis that are worth careful consideration. First, the evidence described here is indirect. Not only are comparative analyses strictly correlative in nature, my analysis relies on a proxy for mating preferences, i.e. manifestations of sexual selection. Sexual dichromatism and elongate fins, the sexually selected traits in this study, have been shown to be the result of mating preference in the ray-finned fishes (Basolo 1990; Houde and Endler 1990; Maan et al. 2004). However, the sexually selected traits used here are only indirect indicators of mating preferences, and since mating preferences are not the only way in which sexual selection can influence cladogenesis (Arnqvist et al. 2000), it is difficult to parse out the specific effects of mating preference from other sexual selection factors, such as male-male competition in this analysis.

Other caveats are more related to the current state of available data. The actinopterygiian supertree used to identify possible sister clades is by its very nature provisional, as it is an amalgam of all the applicable and robust phylogenetic information available in the current literature. As more relevant phylogenetic information is published, it may be prudent to revisit this topic with a new and improved version of the supertree. Also, my analysis relies upon extant taxa, and does not account for differential extinction rates, which could be a potential source of noise in the data. However, since sexually selected lineages may experience an elevated extinction risk compared to sexually monomorphic lineages (Kokko and Brooks 2003; McLain et al. 1995; McLain et al. 1999; Morrow and Pitcher 2003), it is logical to conclude that extinction rates would obfuscate the relationship between sexual selection and cladogenesis rather than spuriously suggest it. Finally, roughly a quarter of identified sister clades were insufficiently characterized and were excluded from this study, and these omissions only hint at the lack of information available for the Actinopterygii. It is conceivable that emerging systematic databases, such as FishBASE (Froese and Pauly 2004) will eventually solve this problem, and make a more complete analysis possible in the future.

Despite these caveats, this analysis is useful in that it suggests a relationship between manifestations of sexual selection and taxonomic diversification, and lends supports to theories linking mating preferences to cladogenesis (Darwin 1871; Lande 1981; Lande 1982; West-Eberhard 1983) in another large vertebrate clade. Shifting mating preferences have been suggested to explain several actinopterygiian radiations (Danley and Kocher 2001; Dominey 1984; Jones et al. 2003; McMillan et al. 1999; Mendelson 2003), though this analysis is the first to comparatively test the role of sexual selection in taxonomic diversification across the ray-finned fishes.

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CHAPTER 10

CONCLUSIONS: PHYLOGENETIC INTERPRETATIONS OF REPRODUCTIVE AND GENOMIC DIVERSITY IN RAY-FINNED FISHES[1]

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[1] Mank J.E., J.C. Avise. In review. Journal of Fish Biology, submitted January 2006

ABSTRACT

Collectively, ray-finned fishes (Actinopterygii) display far more diversity in many reproductive and genomic features than any other major vertebrate group. Recent large-scale comparative phylogenetic analyses have begun to reveal the evolutionary patterns and putative causes for much of this diversity. Several such recent studies have offered clues to how different reproductive syndromes evolved in these fishes, as well as possible physiological and genomic triggers. In many cases, repeated independent origins of complex reproductive strategies have been uncovered, probably reflecting convergent selection operating on common suites of underlying genes and hormonal controls. For example, phylogenetic analyses have uncovered multiple origins and predominant transitional pathways in the evolution of alternative male reproductive tactics, modes of parental care, and mechanisms of sex determination. They have also shown that sexual selection in these fishes is repeatedly associated with particular reproductive strategies. Collectively, studies on reproductive and genomic diversity across the Actinopterygii illustrate both the strengths and the limitations of comparative phylogenetic approaches on large taxonomic scales.

INTRODUCTION

With more than 20,000 living species representing more than 400 taxonomic families, ray-finned fishes (Actinopterygii) are ideal subjects for comparative analyses of reproductive evolution because they display a remarkable diversity of morphologies and behaviors related to procreation (Amundsen 2003). This variety manifests at multiple levels of reproductive biology: e.g., sex-determining mechanisms including male-heterogamety (XY), female-heterogamety (ZW), and autosomal genetic triggers (Devlin and Nagahama 2002), pre-mating reproductive behaviors of many kinds (Gross 1996; Henson and Warner 1997), and post-mating parental tactics ranging from no offspring care to extended internal gestation of embryos by females and sometimes even by males (Blumer 1979; Blumer 1982). Accompanying this reproductive diversity are striking morphological and behavioral manifestations of sexual selection, as well as interesting heterogeneity in genomic features such as DNA content (Gregory 2005). Furthermore, various reproductive traits have probably evolved independently on many occasions across the Actinopterygii, thus creating a wealth of opportunities for comparative phylogenetic approaches in which cladogenetic structure provides historical backdrop for the study of evolutionary patterns and processes (Felsenstein 1985; Harvey and Pagel 1991; Martins 1996).

Most exercises of comparative phylogenetics in fishes have been conducted on single traits in relatively small clades, often with great success. Platyfishes and swordtails (*Xiphophorus*) provide an example germane to the evolution of decorative features under sexual selection. Swordtails are named for the long and pointed extension of the male's caudal fin. This sword is present in some *Xiphophorus* lineages, but absent in others. Comparative analyses (see Fig. 10.1) helped to reveal that swords evolved several times independently (Meyer 1997;

Meyer et al. 1994), and that female mating biases for males displaying this feature predated evolution of the sword itself (Basolo 1990; Basolo 1995; Basolo 1996). Apparently, sexual selection based on female choice promoted repeated evolution of this flamboyant male feature despite its hindrance to an individual's mobility (a problem for males especially in predator-rich environments). Recent molecular analyses have further suggested that convergent sexual selection for male swords has acted on the same underlying genetic mechanisms (Zauner et al. 2003).

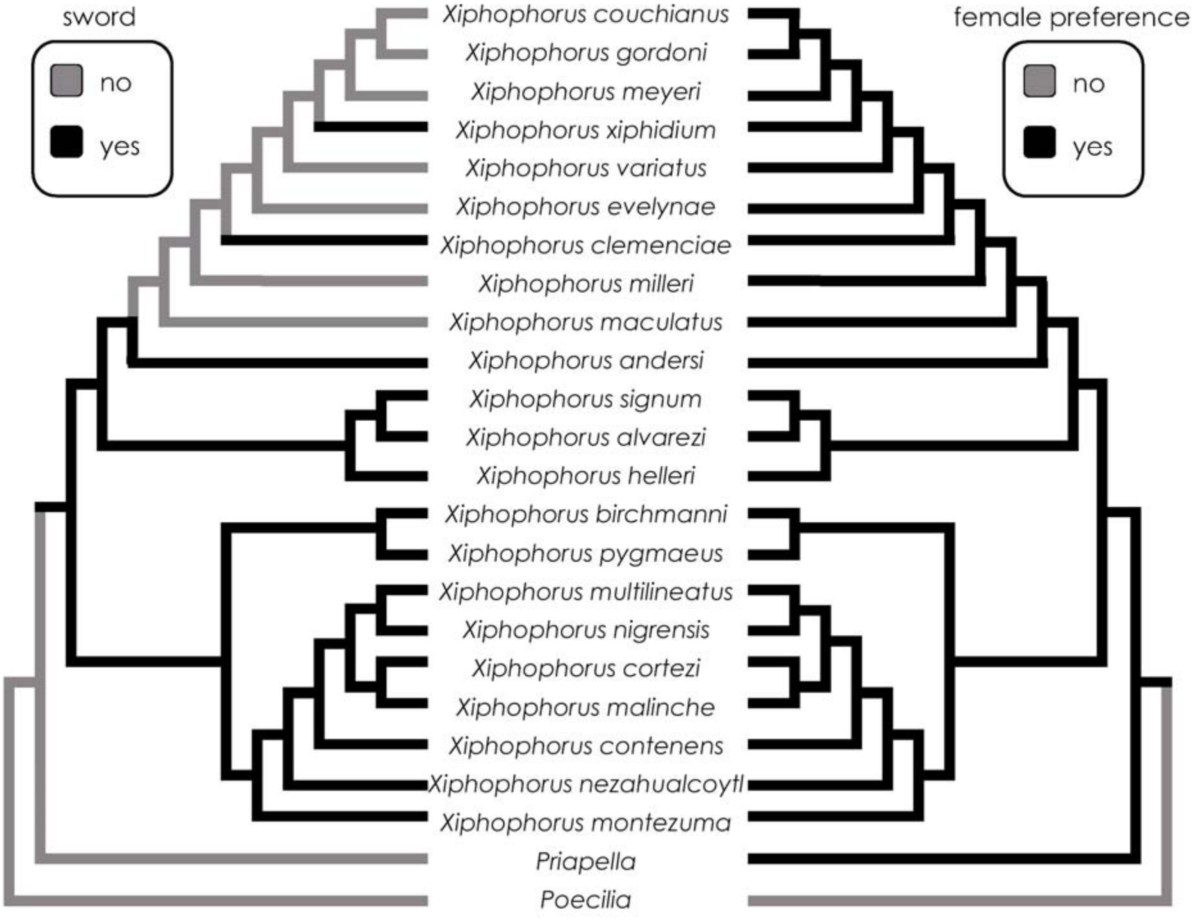


Fig. 10.1. Comparative phylogenetic (maximum parsimony) analysis of sexual selection in *Xiphophorus* and related genera. The left panel plots presence versus absence of a sworded tail, which evolved independently at least three times in this clade (Meyer 1997). The right panel likewise plots the females' preference for sworded males, which predates the actual evolutionary appearance of swords (Basolo 1995).

Similar analyses have been conducted on various other reproductive traits in fishes, such as male brood-pouch designs in syngnathids (Wilson et al. 2003; Wilson et al. 2001), and livebearing and placental structures in poeciliids (Meyer and Lydeard 1993; Reznick et al. 2002). Although such studies on specific features in particular small clades can offer many evolutionary insights, the study of multiple categories of traits in much larger clades offer novel challenges as well as opportunities. The general goals in such analyses are to uncover pervasive evolutionary patterns and processes that occur over long timescales.

The stage for extending the scope of comparative phylogenetics to the full actinopterygiian clade was set by the availability of extensive catalogs of reproductive behaviors in thousands of these species (Blumer 1979; Blumer 1982; Breder and Rosen. 1966; Devlin and Nagahama 2002; Taborsky 1994) coupled with recent advances in knowledge about actinopterygiian phylogeny (Elmerot et al. 2002; Inoue et al. 2004; Ishiguro et al. 2003; Miya et al. 2003; Saitoh et al. 2003). In addition, a recent expansion of genomic information for these fishes (Volff 2005) has allowed researchers to investigate possible mechanistic underpinnings of reproductive diversity. Here I will illustrate the comparative phylogenetic approach as applied to large taxonomic assemblages by reviewing recent work on ray-finned fishes and placing results in the context of current thought about the evolutionary sources of reproductive and genomic diversity in this huge vertebrate clade.

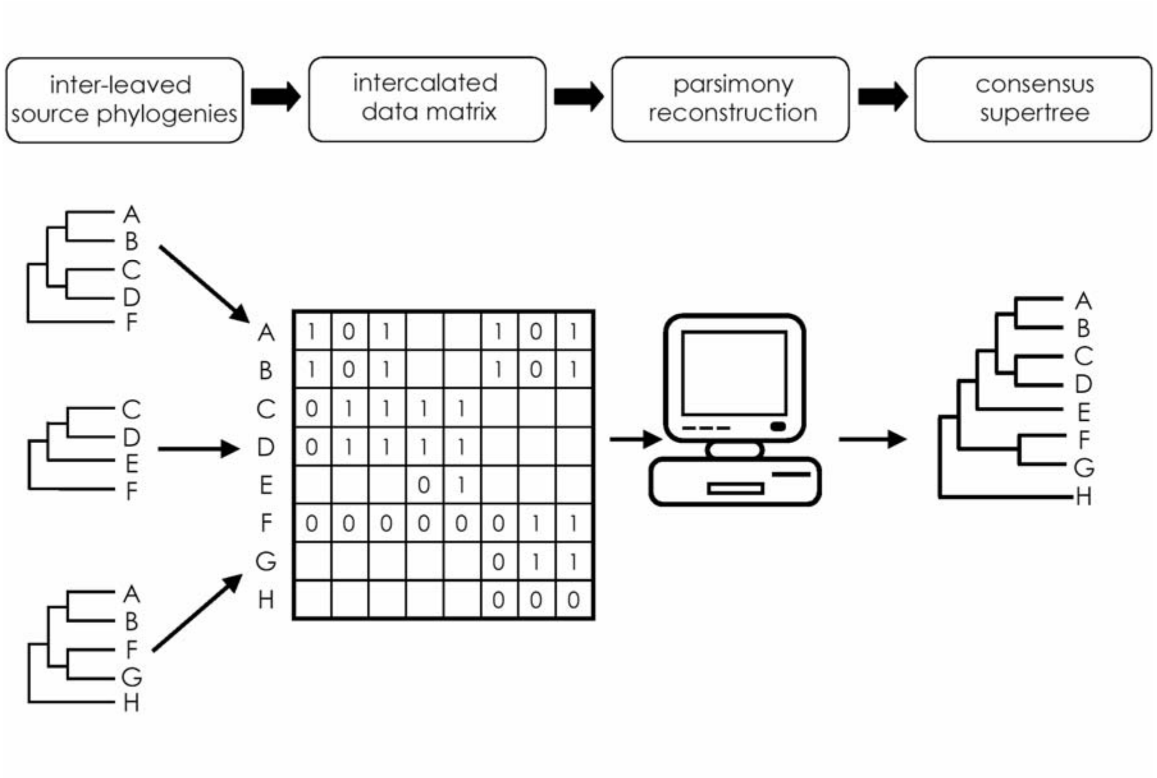
BACKGROUND

Comparative Methodologies

The basic notion of comparative phylogenetics has been a part of evolutionary biology for more than a century, but only recently (Felsenstein 1985) have quantitative and statistical frameworks for comparative methods been formally developed. Several methods now exist to "correct" for phylogenetic non-independence of lineages, i.e., to accommodate the possibility that a given trait is possessed by two or more lineages by virtue of shared ancestry rather than separate origins. Cladograms are the usual starting points for such analyses (Felsenstein 1985; Harvey and Pagel 1991). Although debate continues on the appropriateness and need for phylogenetic corrections (Harvey and Rambaut 1998; Price 1997; Ricklefs 1996), comparative phylogenetic approaches have gained popularity for at least two basic reasons: ancestral states themselves are often of inherent interest; and spurious correlations (or lack thereof) can be a problem in comparative data sets when phylogeny is neglected.

For comparative analyses at lower-taxonomic levels (as exemplified in Fig. 10.1), it is customary to estimate molecular phylogenies from DNA sequences at one or a few loci. This expedience disappears as the number of taxa in the data set increases. For analyses of very large clades or higher taxonomic levels, building a custom molecular phylogeny may require incorporating information from multiple loci in many hundreds of taxa, a task that may be prohibitive in cost and time and also computationally intractable. On the other hand, large phylogenies are attractive for comparative analyses because they permit views of broad patterns in the evolutionary forest that would not necessarily be discernable from close inspections of individual trees.

This conundrum has been partially alleviated by supertree construction (Baum 1992), the basic methodology of which is outlined in Fig. 10.2. Supertrees are amalgamated representations of smaller inter-leaved cladograms, including those based on entirely different data sets (molecular or otherwise). Supertrees have become such an important tool for evolutionary analyses (Cardillo et al. 2004; Davies et al. 2004; Fernandez and Vrba 2005; Grotkopp et al. 2004; Jones et al. 2002; Pisani et al. 2002; Ruta et al. 2003), that they themselves have merited thorough reviews (Bininda-Emonds 2004; Bininda-Emonds et al. 2002).



A	1	0	1			1	0	1
B	1	0	1			1	0	1
C	0	1	1	1	1			
D	0	1	1	1	1			
E				0	1			
F	0	0	0	0	0	0	1	1
G						0	1	1
H						0	0	0

Fig. 10.2. Flow chart outlining the basic steps of supertree construction (see text). Each column in the matrix represents a monophyletic clade from a source phylogeny, dichotomously coded for presence (1) or absence (0) of a taxon in a given clade.

After a cladogram or a supertree has been developed, there are several potential types of comparative analysis depending on the questions to be investigated. All of these methods can be performed by hand, but as the analysis of large clades can become quite tedious, various computer programs allow for a certain degree of automation. The primary methods and examples of commonly used computer programs are summarized in Table 10.1.

The most basic type of comparative phylogenetic analysis examines how a single trait has evolved in terms of number of independent origins, direction of evolutionary transitions, and likelihood of reversion to the ancestral state. MacClade (Maddison and Maddison 2000) and MULTISTATE (Pagel 1994; Pagel 1997), are popular software programs for these purposes. MacClade attempts to optimize ancestral states (at internal nodes) to accommodate the fewest required evolutionary transitions (maximum parsimony), whereas MULTISTATE (maximum likelihood) calculates the probability of observing the data under a set of evolutionary models specified by the investigator. Different models can be compared with a likelihood ratio test. MacClade and MULTISTATE usually are implemented on discrete data, although they can also handle continuous data if categories are assigned.

More complex analyses can test for correlated evolution, or the statistical propensity for associations between two or more traits across lineages. Suites of correlated characters could have resulted from the same evolutionary forces, or they could be present if one of the correlated traits (an independent variable) has causally influenced the evolution of another (a dependent variable). Independent contrast (IC) methods (Felsenstein 1985) search for possible associations by computing the numerical relationship between pairs of traits at each node in a phylogeny, after which the data points can be regressed and analyzed for overarching patterns. The most commonly used IC software is CAIC (Comparative Analysis by Independent Contrasts), which also permits corrections for varying branch lengths. CAIC is intended for continuously distributed traits and operates essentially by computing, at each internal node, an ancestral state that is a trait average of the respective daughter lineages (Purvis and Rambaut 1995). Maximum likelihood (ML) methods also exist to examine correlated evolution via independent contrasts. The program DISCRETE is applicable to pairs of binary traits, whereas CONTINUOUS can be used on pairs of continuously distributed characters (Pagel 1994; Pagel 1997).

To examine the possible roles of particular traits in cladogenesis, MacroCAIC (Agapow and Isaac 2002) uses independent contrasts (in a fashion similar to CAIC) to test whether or not lineages with a given character state are more prone to diversification.

Of course, it is also possible to assess trait correlations without phylogenetic corrections, using standard statistical association tests such as correlation analyses or chi-square tests. However, because standard statistical approaches entail an assumption that extant taxa provide independent data points, they lack the historical perspective that is the hallmark of comparative phylogenetics.

Sister clade comparisons can be implemented when some (but not all) of a clade's phylogeny is known, or when the nature of the dataset violates underlying assumptions of other IC methods. These are similar to IC except that sister clades diverged from a single node (by definition) and thus can be assumed to be of the same age (Cracraft 1981). Thus, there is no need to correct for time (branch length). In favorable cases (when knowledge about a cladogram's structure is strong and a trait in question originated multiple times independently), it is possible to conduct many sister clade comparisons and search for overall statistical signal using a sign test, a randomization test for matched pairs, or some other appropriate statistical method. Sister clade comparisons are advantageous in that the researcher need not know the

Table 10.1. Common comparative approaches and associated computer programs that correct for shared ancestry among traits.

Questions	Program	Algorithm	Applications
Single trait evolution	MacClade (Maddison and Maddison 2000)	Maximum parsimony	Transitions among states
	MULTISTATE (Pagel 1994; Pagel 1997)	Maximum likelihood	Directional evolution of discrete traits
Correlated evolution	CAIC (Purvis and Rambaut 1995)	Independent contrasts	Correlation testing between continuous traits
	CONTINUOUS (Pagel 1994; Pagel 1997)	Maximum likelihood	Correlation testing between continuous traits
	DISCRETE (Pagel 1994; Pagel 1997)	Maximum likelihood	Correlated evolution between binary traits
Species richness	MacroCAIC (Agapow and Isaac 2002)	Independent contrasts	Testing continuously distributed traits as possible causes of diversification

structure of the entire cladogram, but they can suffer from serious reductions in statistical power because attention is confined to less than all internal nodes.

Actinopterygiian Phylogeny

Large-scale comparative phylogenetics requires cohesive cladograms involving perhaps hundreds or thousands of taxa. Although such phylogenies exist (at least in provisional form) for several major eukaryotic groups (Davies et al. 2004; Jones et al. 2002; Sibley and Ahlquist 1990), until recently the paucity of useful phylogenetic data and confusion surrounding several key taxonomic assemblages (Johnson 1993) had hindered even preliminary attempts to phylogenetically resolve the entire Actinopterygii. This situation is changing, thanks in large part to the publication of full mitochondrial genomic sequences from numerous fish species (Inoue et al. 2004; Ishiguro et al. 2003; Miya et al. 2003; Saitoh et al. 2003). These recent molecular studies sampled broadly across the entire Actinopterygii, and when combined with numerous lower-level phylogenies (both morphological and molecular) they have created the first opportunities to address higher-level supertrees for the entire actinopterygiian clade.

In this review, reference will often be made to a family-level actinopterygiian supertree (Fig. 3A) that was generated by Mank et al. (2005) based on the published cladogenetic structures in 38 source phylogenies for various partially overlapping actinopterygiian groups. The source phylogenies themselves had been based on diverse types of molecular and phenotypic data. More than 500 binary-coded bits of intercalated cladogenetic data (as illustrated by the matrix in Fig. 10.2) went into construction of the supertree, which actually is a 90% consensus phylogeny of 25,000 equally parsimonious trees based on extensive heuristic computer searches. That consensus supertree resolved 228 of the 421 recognized actinopterygiian families representing all of the 42 recognized orders and their approximately 10,000 extant species. Most of the unresolved families were in the traditional order Perciformes, which actually has proved to be highly polyphyletic (Johnson 1993; Miya et al. 2003). Other findings summarized in this review came from smaller supertrees (as for the Atheriniformes in Fig. 10.3B), or from more specific lower-taxa phylogenies.

New phylogenetic information can be incorporated into existing supertree data matrices with relative ease (although subsequent maximum parsimony searches of these large matrices can be computationally intensive and time consuming). This relative ease of revision is advantageous because all supertrees are inherently provisional, always pending improvement as additional phylogenetic information becomes available.

MALE REPRODUCTIVE TACTICS

Ray-finned fishes probably display more diversity in male reproductive tactics than any other vertebrate clade. Many fishes spawn in mass conspecific aggregations where scramble competition and sperm competition are likely to be primary reproductive determinants of male fitness. At the other end of the spectrum, in many fish species a territorial male (who often exhibits costly sexually selected traits) pairs with just one or a few females. In effect, these "bourgeois" males attempt to monopolize the reproductive output of associated females to the fitness detriment of other males (Emlen and Oring 1977; Gross 1996; Taborsky 2001). In response to this reproductive challenge, various alternative reproductive tactics have evolved by which other males seek to break the monopoly that bourgeois males otherwise hold on fitness enhancing resources.

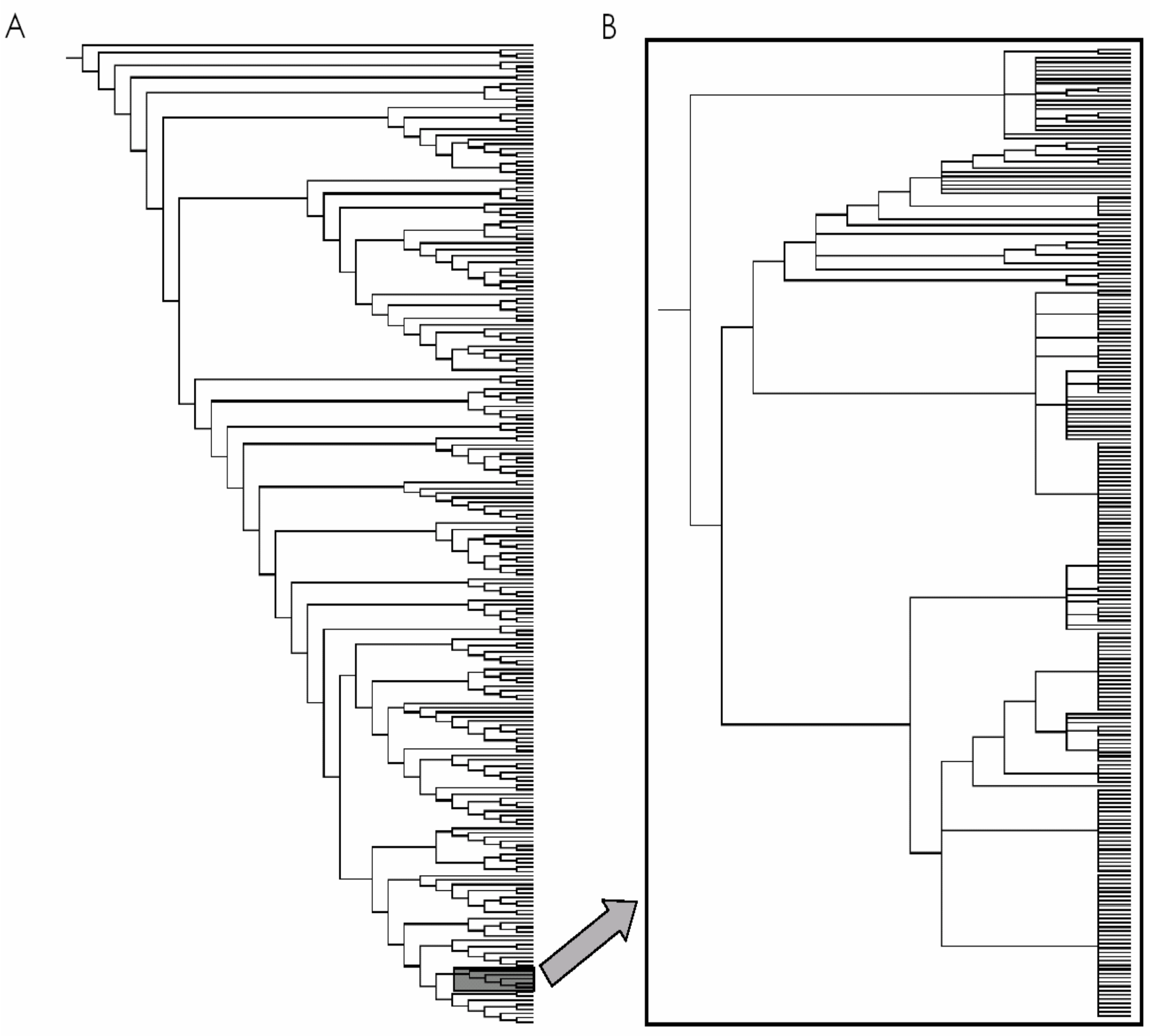


Fig. 10.3. Supertree topologies for ray-finned fishes. A: Provisional family-level supertree for Actinopterygii, where each terminal node is a taxonomic family with many species (Mank et al. 2005). B: Species-level supertree for the taxonomic order Atheriniformes, where each terminal node is a surveyed species (Mank and Avise in press-b). Taxon names for the 228 families in 3A and the 309 species in 3B can be obtained from the above-mentioned references.

These male alternative reproductive tactics (MARTs) have been cataloged (Gross 1996; Taborsky 1994; Taborsky 1998; Taborsky 2001) and can generally be divided into parasitic and cooperative behaviors. Sneaking, the most common parasitic tactic, involves sneaker males that lack sexually selected body ornaments and by virtue of speed or stealth attempt to steal fertilizations by releasing sperm onto the nests of bourgeois males during spawning episodes. Female mimicry, another form of parasitic behavior, involves males who look or behave like females and thereby dupe territorial males to gain access to spawning sites where they deposit sperm. Piracy is the least common parasitic MART, usually employed only by large males that display sexually selected traits. A pirate can fertilize eggs after evicting a territorial male from a spawning site, but he may also depart after one or a few spawns, leaving the bourgeois male to guard what could be a mixed-parentage brood. Finally, cooperative MARTs may be employed by satellite males (who often lack sexually selected phenotypic traits). Satellite males are tolerated by bourgeois males and may help to attract females, defend territories, or care for offspring in exchange for fertilization opportunities.

The Origin and Evolution of MARTs

Various MARTs described above have proved to be scattered throughout the actinopterygiian supertree (Mank and Avise in review). However, comparative endocrinological analyses suggest that similar parental behaviors observed in distantly related species are proximally mediated by similar hormone profiles (Fig. 10.4). Thus, selection appears likely to have shaped the expression of reproductive hormones (or their receptor proteins) to produce convergent MARTs repeatedly across the ray-finned fishes (Fitzpatrick et al. 2005; Knapp 2004). Hormones under potential convergent selection include arginine vasotocin which results in secondary sexual characteristics and spawning behavior both in females and female mimics (Carneiro et al. 2003; Foran and Bass 1998; Foran and Bass 1999), and 11-keto-testosterone which controls sexually selected traits and spawning behavior both in bourgeois and pirate males (Borg 1994; Brantley et al. 1993; Ros et al. 2004).

In addition to implicating evolutionary convergence for the hormonal controls of MARTs, comparative phylogenetic analyses have identified evolutionary pathways of increasing MART complexity (Fig. 10.5). Namely, the first and numerically predominant evolutionary response to attempted mate monopolization often appears to involve sneaker tactics. This makes biological sense because, being the simplest of the MARTs, sneaking is often a conditional strategy based on body size, with small males acting as sneakers early in life and later transitioning to bourgeois tactics (Aubin-Horth and Dodson 2004; Gross and Charnov 1980; Leiser and Itzkowitz 2004; Mazzoldi and Rasotto 2002). On some occasions, this adaptation may subsequently become genetically embedded in lineages when a heritable mechanism evolves such that sneaker fathers tend to sire sneaker sons (Heath et al. 2002).

Furthermore, according to the phylogenetic analysis, in some lineages sneaking has transitioned to one or the other of two presumably more complex MARTs: female mimicry and satellite behavior (Fig. 10.5). This too seems biologically plausible. Female mimicry requires the addition of “female” reproductive behavior and morphology to sperm production, so it would seem to represent a level of complexity beyond simple sneaking; and satellite tactics would seem to require the addition of elaborate cooperative behaviors to mere sneaking per se (Stiver et al. 2005). However, the phylogenetic reconstructions have also implied more direct routes to female mimicry and satellite behavior from bourgeois tactics, suggesting that sneaking may not always be a necessary intermediate step.

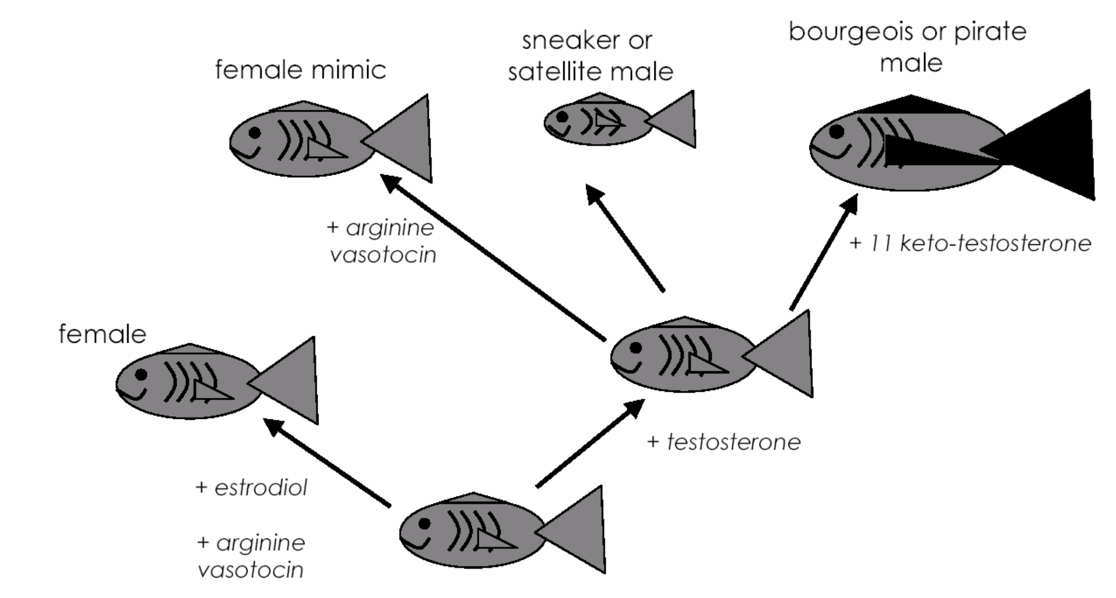


Fig. 10.4. A generalized model for endocrine hormone profiles underlying various MARTs in actinopterygiian fishes (see text).

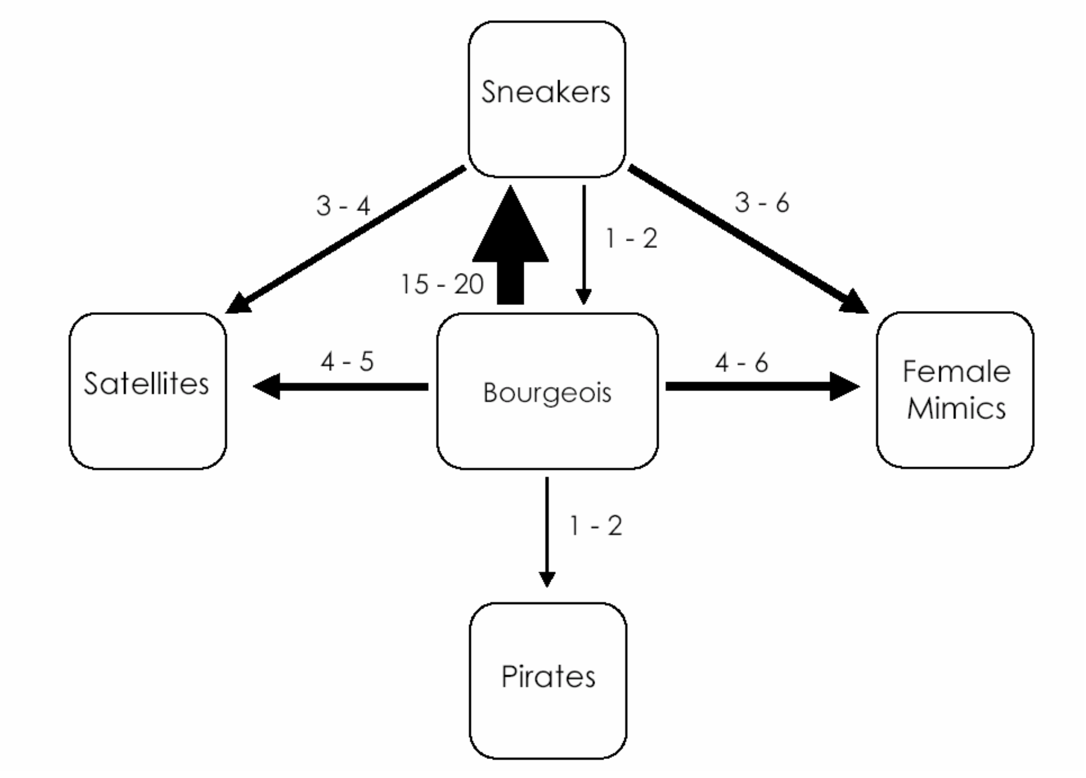


Fig. 10.5. Results from comparative phylogenetic analyses of MARTs in actinopterygiian fishes (Mank and Avise in review). Arrow sizes correspond to mean estimated numbers of parsimony-inferred evolutionary transitions between different MARTs, and numbers beside arrows indicate minimum and maximum estimates.

Finally, phylogenetic analyses gave no indication that piracy is consistently integrated as a component of these evolutionary pathways (Mank and Avise in review). Perhaps the phenomenon is under-reported in the literature, or perhaps selection forces acting upon this late-in-life type of adaptation are rather weak, reducing the likelihood that this tactic could be incorporated into a generalized evolutionary pathway.

PARENTAL CARE

Parental strategies influence fecundity and other life history traits, and have also been shown to effect cladogenetic patterns in fishes (Lydeard 1993). Extensive catalogs of parental behaviors in ray-finned fishes (Blumer 1979; Blumer 1982; Breder and Rosen. 1966) indicate that approximately 20% of actinopterygiian taxonomic families contain at least some species in which adults provide post-zygotic care of one sort or another, ranging from internal gestation by females (or by males in the case of syngnathid pipefishes and seahorses), to external brooding by either or both parents. The evolution of parental care can be conceptually divided into two pathways: one for species with external fertilization, and the other for species in which syngamy occurs inside the female reproductive tract.

Species With External Fertilization

Although maternal care or bi-parental care of offspring is displayed by a few actinopterygiian fishes with external fertilization, parental care (when present) is normally provided primarily or exclusively by males (Blumer 1979; Blumer 1982). Typically, a male maintains and defends a spawning territory (often including a nest) where he may mate with several females successively during a spawning cycle. Thus, males tend to be in close physical proximity to their progeny from earlier spawns, probably accounting in part for a common evolutionary progression from defense of a mating territory to paternal care for developing embryos and fry. Indeed, a phylogenetic reconstruction of care-giving behaviors on an actinopterygiian supertree has indicated that paternal care tends to evolve repeatedly in lineages in which males build and defend spawning sites (Mank et al. 2005).

Results from parsimony reconstructions have further indicated that maternal care and bi-parental care have also arisen on multiple occasions in the Actinopterygii, thus making it possible to evaluate competing models of parental care evolution (Fig. 6). Under the stepping-stone hypothesis originally developed for labroid fishes (Barlow 1974; Gittleman 1962; Gross and Sargent 1985), bi-parental care is an intermediate transitional state between paternal and maternal care. Under a competing independent-origins model developed for anuran frogs (Summers et al. 1999), the three categories of parental care (maternal, paternal, and bi-parental) arise separately from care absence and are not linked in any evolutionary progression. For actinopterygiian fishes, comparative phylogenetic analyses indicate that the evolution of parental care conforms better to the independent origins model (Mank et al. 2005).

Species With Internal Fertilization

Internal fertilization obviously affects proximity relationships between parents and embryos. For species with female-internal syngamy (both in zygoparity where fertilized eggs are laid shortly after mating and in viviparity where embryos are delivered live-born after a lengthy internal gestation), the mother is closely associated with her developing offspring whereas males typically have long departed. Given this spatio-temporal situation, males tend to be freed from obligate offspring care, undoubtedly explaining why female-internal fertilization and maternal-

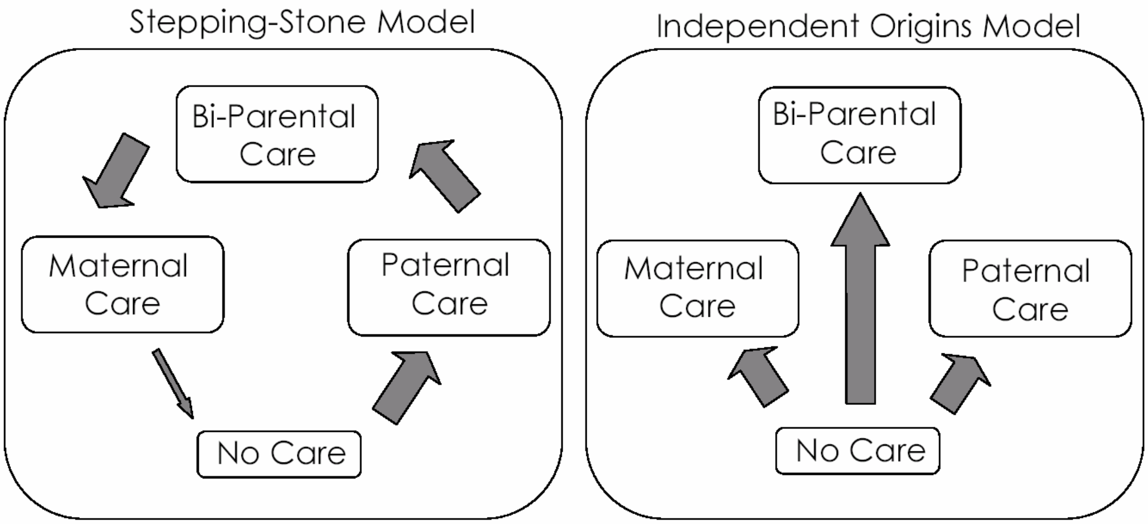


Fig.10. 6. Competing hypotheses regarding the evolution of parental care evolution in species with external fertilization (see text). Recent comparative phylogenetic analyses tend to favor the independent origins model for actinopterygiian fishes.

only care (via pregnancy and/or external brooding) are closely linked phylogenetically (Gross and Shine 1981; Mank et al. 2005).

Internal gestation requires complex suites of behavioral, morphological, and physiological adaptations (Amoroso 1968; Schindler and Hamlett 1993) related to the requirements for copulations (as opposed to gamete release) as well as subsequent offspring nurturing. Despite these seemingly major biological hurdles, live-bearing has evolved multiple times in the ray-finned fish clade (Breder and Rosen. 1966; Lydeard 1993; Mank and Avise in press-b). Preliminary evidence further suggests that livebearing may sometimes have involved evolutionary convergence at particular loci such as the gene encoding an insulin-like factor that controls fetal growth and development (Lawton et al. 2005).

Phylogenetic analyses also add strength to the notion that live-bearing is highly adaptive, because most lineages with internal fertilization have proceeded to internal gestation whereas relatively few lineages exhibit external brooding (Mank et al. 2005). Furthermore, livebearing lineages in the Actinopterygii appear to exhibit significantly higher rates of cladogenesis than externally brooding sister clades (Lydeard 1993; Mank and Avise in press-b).

GENOMICS

A growing understanding of extant actinopterygiian genomes, although not yet as sophisticated as genetic knowledge for mammals and birds, is also providing new opportunities for comparative phylogenetic evaluations of evolutionary patterns and processes.

Modes of Sex Determination

The mechanism by which sex is determined can greatly influence the sex ratio in a population. Under sex-chromosome systems, for example, Mendelian segregation and syngamy tend to produce 1:1 sex ratios in the absence of confounding factors, whereas environment-conditioned sex determination can in some cases produce highly unequal numbers of males and females. Sex ratio in turn is an important component in such evolutionary considerations as effective population size and mating systems (Hartl and Clark 1997), and it can play an enormous role in the evolution of sexually selected traits (Kvarnemo and Ahnesjo 1996; Wade and Shuster 2004).

In some large vertebrate clades, particular modes of sex determination have been conserved over vast stretches of evolutionary time. For example, birds have maintained a single sex-chromosome system (female ZW heterogamety) across their 150-million-year existence

(Fridolfsson et al. 1998; Handley et al. 2004), and mammals have retained another sole mechanism of sex determination (male XY heterogamety) since their origin approximately 250 million years ago (Foster and Marshall-Graves 1994; Lahn and Page 1999). Such evolutionary conservatism can aid in analyses of how a single category of sex determination may change through time (Ellegren and Carmichael 2001; Iwase et al. 2003), but this lack of diversity also precludes studies of how categorically different sex-determination systems originate and interconvert.

The situation in actinopterygiian fishes is remarkably different, with many distinct types of sex-determining systems having evolved over the last 230 million years. These include both XY and ZW systems, constitutive autosomal sex determination, hermaphroditism, unisexuality, and various environmentally mediated mechanisms of sex determination (Devlin and Nagahama 2002). Indeed, sex-determining mechanisms in actinopterygiian fishes are so variable that the primordial ancestral state remains unresolved (Mank et al. 2006a), and even the basic molecular mechanisms of sex determination are poorly characterized for the vast majority of this clade (Kondo et al. 2003; Nanda et al. 2002; Volff et al. 2003).

Phylogenetic investigations have made clear that the major categories of sex determination have each arisen on multiple independent occasions throughout the evolutionary history of actinopterygiians. Few if any other vertebrate clades match this level of diversity, nor do they generally show such high rates of evolutionary change in this seemingly complex trait.

Closer phylogenetic examination has revealed more. First, despite its fairly common occurrence and multiple origins, unisexuality (gynogenesis and hybridogenesis) clearly is a derived and evolutionarily short-lived condition in fish lineages (Avise et al. 1992). Second, much the same can be said for hermaphroditism (Mank et al. 2006a; Smith 1975), an adaptation often associated with reef species, possibly due to their pelagic dispersal syndrome (Ghiselin 1969). Third, although it was previously understood that convergent evolution must have resulted in multiple independent origins of both male-heterogametic and female-heterogametic sex chromosomes (Ota et al. 2000; Solari 1994; Woram et al. 2003), supertree analyses have further revealed just how remarkably labile heteromorphic sex-chromosomes systems in fishes can be (Mank et al.2006a). In some cases, single genera and even individual species simultaneously display both XY and ZW modes of sex determination (Devlin and Nagahama 2002).

Much remains to be learned at the DNA sequence level about sex chromosome evolution in fishes. The genomes of only two species (both pufferfishes) have been fully sequenced to date (Aparicio et al. 2002; Jaillon et al. 2004), but they both lack discernible sex chromosomes; and only limited regions of sex chromosomes have been sequenced in a handful of other fish species (Felip et al. 2004; Harvey et al. 2003; Peichel et al. 2004).

Comparative Genomics

Several rounds of large-scale or even genome-wide gene duplications have been provisionally documented within the Actinopterygii, both near the evolutionary root of teleostean fishes (Christoffels et al. 2004; Meyer and Schartl 1999; Robinson-Rechavi et al. 2001; Wittbrodt et al. 1998) and near the base of several major clades nested therein (Allendorf 1984; Ferris 1984; Larhammar and Risinger 1994; Uyeno and Smith 1972; Vasil'ev 1999). These duplications, together with the proliferation of several families of repetitive elements (Nogare et al. 2002; Volff et al. 2001a; Volff et al. 2001b), rapid changes in insertion/deletion ratios (Neafsey and Palumbi 2003), repeated origin and dissolution of heteromorphic sex chromosomes

(Mank et al. 2006b), and smaller scale chromosomal re-patternings (e.g., via illicit recombination of non-homologous chromosome segments), indicate that genomic architecture in the Actinopterygii is evolutionarily dynamic. These genomic alterations have also resulted in reduced levels of genetic synteny compared to some otherwise comparable vertebrate clades (Thomas et al. 2003). It thus seems utterly remarkable that chromosome counts show a strongly leptokurtic or peaked frequency distribution (centered at 2N = 48 and 2N = 50) across teleostean species and higher taxa (Mank and Avise in press-a). Thus, for reasons that remain completely unknown, the extreme evolutionary dynamism displayed by many internal structural features of fish genomes have all occurred within the context of an extreme evolutionary conservatism in chromosome numbers.

Genomic Catalysts of Diversity

The genomic enlargements and rearrangements mentioned above have been suggested as engines that may have helped to drive the diversification of the teleost fishes (Hoegg 2004; Meyer and Malaga-Trillo 1999; Meyer and Schartl 1999; Taylor et al. 2003). These ideas are based on empirical findings coupled with conceptual models of how evolutionary radiations might relate to these kinds of genomic alterations (Lynch 2002; Lynch and Force 2000; Navarro and Barton 2003a; Navarro and Barton 2003b; Ohno 1970; Stephens 1951). Comparative phylogenetic analyses of extant actinopterygiian genera are consistent with these notions: substantial increases in genome size appear to be significantly correlated with increased bursts of cladogenesis (Mank and Avise 2006). Whether these changes in genome size arose through proliferations of transposable elements, regional gene duplications, or whole-genome polyploidizations generally remains unknown, however.

Unusually high rates of genomic change may also partially explain the diversity of sex-determining mechanisms in actinopterygiian fishes (Devlin and Nagahama 2002; Mank et al. 2006a). Illicit recombination, in particular, is perhaps an important factor behind the repeated origin of particular sex-chromosome modes and the frequent and rapid evolutionary inter-conversions among them. This process could, for example, move sex-determining genes from constitutive to inducible promoters (and vice versa), fostering changes to and from ecological (or other condition-dependent) controls of sex.

Non-homologuous recombination of chromosomes or chromosomal segments is often promoted when repetitive elements proliferate in a genome (Brosius 1999; Capy 1997; McDonald 1990; McDonald 1995; McDonald 1998), a phenomenon that has been documented in several fish lineages (Kawakami et al. 2000; Nogare et al. 2002). Cytogenetic rearrangements can also be fostered via recombination among translocated gene duplicates, perhaps further contributing to the rapid evolution of sex-determining mechanisms in fishes. Particular gene duplications might also play a role. Gene duplicates often assume new but related functions (Dulai et al. 1999; Manzanares et al. 2000) that can alter the flow of existing molecular pathways. This phenomenon has been linked to changes in the sex-determination pathway for at least one fish species, *Oryzias latipes* (Nanda et al. 2002), and future comparative genomic analyses may well reveal examples in other species as well. All of this genomic action would be evolutionarily ineffective if it were not also the case the fishes in general show enormous developmental flexibility (compared to mammals and birds, for example) with regard to gonadal and sexual differentiation.

THE MULTIFARIOUS ROLE OF SEXUAL SELECTION

Sexual selection derives from inequities in reproductive potentials between males and females, an imbalance that results in mate-choice and mate-access competition among individuals of either sex (Gould and Gould 1997). In fishes, sexual selection has been shown to be a cause of the following: sexual dichromatism wherein one sex is brighter or more ornately patterned than the other (Amundsen and Forgren 2001; Endler 1980; Houde and Endler 1990), elongated fins (Harrington 1997; Kuwamura et al. 2000; Marcus and McCune 1999), presence of breeding tubercles (Kortet et al. 2003; Kortet et al. 2004), and presence of electrical mating calls (Curtis and Stoddard 2003).

Many of the reproductive syndromes described in previous sections of this paper alter the reproductive efforts required of males or females. Theoretically, any exacerbation or alleviation of sexual selection could result in a gain or loss, respectively, of sexually selected traits. Recent comparative work has uncovered many of the ways in which sexual selection is linked to the evolution of phenotypic diversity in the ray-finned fishes. Additionally, the widespread occurrence of sexually selected traits in conjunction with other forms of phenotypic diversity makes Actinopterygii an ideal clade for testing numerous theoretical predictions about the causes and consequences of sexual selection.

MARTs and Sexual Selection

MARTs can be interpreted as a response to mate monopolization (Taborsky 1994; Taborsky 1998; Taborsky 2001), and some theoretical relationships between mate acquisition tactics and sexual selection seem relatively clear. Mate monopolization often promotes differential reproductive output among males, a classic contributor to sexual selection (Emlen and Oring 1977). From this perspective, MARTs and sexually selected traits might be phylogenetically associated with one another by virtue of having arisen from the same sets of evolutionary pressures. In support of this hypothesis, phylogenetic correlation analysis across the ray-finned fishes (Mank and Avise in review) has shown that lineages exhibiting MARTs also tend to be those in which sexually selected ornaments are present in bourgeois males. Furthermore, MARTs were not phylogenetically associated with male brood care, thus suggesting that they often have arisen from selection on males to circumvent mate monopolization rather than brood care avoidance.

Sexual Selection and Parental Care

Investments in parental care can alter the skew between the sexes in terms of reproductive energy burdens, theoretically resulting in an evolutionary link between sexual selection and parental behaviors. Indeed, like other physical manifestations of sexual selection in male fishes such as bright colors, elongate fins, and other bodily features, sexual selection has been implicated as a contributing factor in the evolution of paternal care. For example, nest-tending male sticklebacks and gobies have been shown to act as better parents when in the presence of potential mates (Ostlund and Ahnesjo 1998; Pampoulie et al. 2004). Comparative phylogenetic analysis of the actinopterygiian supertree is consistent with the notion that sexual selection and paternal care are intertwined, because lineages with male parental care also significantly tend to exhibit sexually selected traits (Mank et al. 2005). Further analyses will be needed to clarify whether sexual selection causally influences or simply is correlated with male parental investment.

In the ray-finned fishes, sexual selection on males is also significantly associated with live-bearing, according to comparative phylogenetic analyses (Mank et al. 2005). Internal gestation, which entails protection and nourishment of embryos, in effect merely amplifies an inherent asymmetry already present between males and females with respect to energetic investment in individual gametes (by virtue of anisogamy— the pronounced difference in size between egg cells and sperm cells). By increasing the differential reproductive potential between the sexes, internal gestation can theoretically exacerbate the forces of sexual selection on males.

Sex Chromosomes and Sexual Selection

The evolutionary lability of sex chromosomes in fishes makes these animals uniquely well suited for phylogenetic tests of several models predicting that sexually selected male ornaments (such as showy fins) should arise more often under female heterogamety than under male heterogamety (Albert and Otto 2005; Fisher 1952; Kirkpatrick and Hall 2004; Kirkpatrick and Ryan 1991; Lande 1981; Reeve and Pfennig 2003). This theoretical prediction is based on the special inheritance pattern of heterogametic sex chromosomes, combined with the assumption that genes controlling expression of a sexually selected male ornament and genes that control female preference for that trait are tightly linked on a sex chromosome. More specifically, only under female heterogamety would a showy male bequeath his Z chromosome (containing this linkage group) to his sons (who would be showy) as well as to his daughters (who would prefer showy males). No analogous outcome arises under male heterogamety because a showy male would either bequeath his Y chromosome to his sons only, or his X chromosome to his daughters only. Thus, the combined sex-linkage of genes for both male ornament and female preference should increase the likelihood that a male ornament would sweep through a female-heterogametic population and thereby facilitate the spread of sexually selected traits in species with ZW sex chromosome systems.

Empirical support for this theory has come from studies on a few isolated species (Iyengar et al. 2002; Prowell 1998), and the association between female heterogamety and male ornaments has been anecdotally invoked to explain the profusion of male ornaments in birds (which are all ZW) compared to mammals (which are XY). However, a critical comparative phylogenetic evaluation of this theory across the Actinopterygii found no such expected association (Mank et al. 2006a). This indicates either that the theoretical models do not apply to fishes (for any of several possible reasons, such as autosomal control of sexually selected traits), or that the relationship between the chromosome mode of sex determination and male adornments is too small to be detected.

This “negative” result does not mean that sex-determination mode plays no role in the evolution of sexual selected characters. For example, any autosomal (Chourrout 1986; Sola et al. 1981; Solari 1994) or environmental (Devlin and Nagahama 2002) factors that might act to bias sex ratios could thereby also effect both the form and intensity of sexual selection.

Sexual Selection and Taxonomic Diversification

Sexual selection can lead to increased taxonomic diversification when mating preferences result in assortative mating and reproductive (prezygotic) isolation (Darwin 1871; Lande 1981; Lande 1982; West-Eberhard 1983). Increased prezygotic isolation would be expected to accelerate taxonomic diversification compared to post-zygotic barriers to gene flow, which can require long periods of time to accumulate. This theory linking sexual selection and cladogenesis has been anecdotally invoked to explain observed patterns of diversity in some clades of ray-finned (actinopterygiian) fishes (McMillan et al. 1999; Mendelson 2003), especially for the cichlids (Dominey 1980; Knight et al. 1998; Maan et al. 2004). These theoretical predictions and anecdotal invocations were recently substantiated by a comparative appraisal throughout the Actinoptergyii that found a link between manifestations of sexual selection and increased rates of taxonomic diversification (Mank in review).

LIMITATIONS OF THE COMPARATIVE METHOD

Although comparative phylogenetic analyses can add insights into evolutionary processes, they also suffer from several inherent limitations that stem either from the underlying biological phenomena addressed or from technical aspects of the analyses themselves. First, the comparative method is necessarily limited by data available from extant lineages (or, in the case of paleontological approaches, secure fossil evidence). This means that brief transitional states that leave little or no trace in extant species, or traits that increase extinction risks and thereby remove lineages from available modern samples, are difficult to identify and study with the comparative method. An example germane to the current review involves MARTs. Phylogenetic analyses suggest that these alternative reproductive tactics by males often evolve along pathways of increasing complexity, with sneaking arising first and subsequently transitioning in some cases to female mimicry or satellite behaviors (Mank and Avise in review). At face value, the phylogenetic analyses also indicate that these latter behaviors sometimes arose directly from the bourgeois tactic (Fig. 10.5). However, the available analyses cannot eliminate the possibility that sneaking typically existed as a transient intermediate that simply has gone undetected in the phylogenetic reconstructions.

More generally, the incidence of short-lived adaptations can be underestimated by comparative phylogenetic methods. The phenomenon of unisexuality provides another example. Supertree reconstructions identified three separate origins for unisexuality in the actinopterygiian clade (Mank et al. 2006b), but finer-scale examinations and more direct evidence (Avise et al. 1992) prove that this is a gross underestimate. Unisexual lineages suffer from reduced adaptive ability, which greatly increases their extinction risk (Stanley 1975; Vrijenhoek et al. 1977; Vrijenhoek et al. 1985). Without extant modern descendents, even common ancient states can be overlooked in comparative phylogenetic appraisals alone.

A second category of difficulties arises when evolutionary transitions among character states (including those that survive to the present) have been frequent in the clade under consideration. In such cases, maximum parsimony and other reconstruction methods usually cannot specify with any precision the actual numbers of exact cladogenetic placements of particular transitions. In such cases, researchers may have to be content in concluding merely that the trait in question is evolutionarily labile.

Another limitation is that a truly thorough comparative phylogenetic analysis would often require corrections for tree branch lengths (or times since shared ancestry). In the usual absence of a complete and accurate fossil record, researchers are forced to rely on molecular clocks to date internal nodes in a phylogeny, but these can be rather erratic and inaccurate (Kolaczkowski and Thornton 2004; Langley and Fitch 1974; Rodriguez-Trelles et al. 2001). Divergence dates can be especially difficult to determine for many supertrees due to the amalgamated and often heterogeneous nature of their underlying data matrices. Because time-dated phylogenies (i.e, phylograms) are normally far more difficult to estimate reliably than are cladograms, this general problem of temporal estimation afflicts nearly all current phylogenetic research (comparative or otherwise), at least to some extent.

Finally, it is wise to remember that any findings from comparative phylogenetics are inherently descriptive (rather than experimental) and correlational (rather than causal). Although comparative phylogenetics offers some powerful and relatively novel tools for biological inquiry, its findings should normally be interpreted mostly as helpful starting points for further evolutionary investigations.

FUTURE DIRECTIONS

Further comparative work on the actinopterygiians should proceed on several fronts. With respect to phylogeny estimation per se, much work remains. Although great strides have recently been made in clarifying historical relationships among many of the 400+ taxonomic families of ray-finned fishes, the phylogenetic information available to date (as published in the first actinopterygiian supertrees) is sufficient to resolve only about 50% of extant families. Most lower-level phylogenies are no less incomplete.

With respect to genomic features, the molecular era has barely dawned on the Actinopterygii, and researchers studying this clade lack the extensive comparative genomic tools available for higher vertebrate clades. Furthermore, the only two genera (*Fugu* and *Tetraodon*) with completed draft sequences (Aparicio et al. 2002; Jaillon et al. 2004) were chosen precisely because they contain unusually compact genomes that were relatively straightforward to sequence. But this fact could also make these species poor or misrepresentative models for evolutionary genomics in fishes. Additional genomic resources are in development for many fish species (Volff 2005), but the great diversity of actinopterygiian fishes means that researchers will need many reference points for meaningful comparative analyses.

Another promising front involves new developments linking reproductive evolution to its underlying molecular mechanisms. For a handful of well studied species, researchers have worked out key molecular details of sex determination (Nanda et al. 2002; Volff et al. 2003), viviparity (Lawton et al. 2005), internal fertilization (Zauner et al. 2003), and spawning strategies (Ros et al. 2004), but much work remains to be accomplished before we can know whether particular molecular mechanisms are shared across the Actinopterygii.

CONCLUSIONS

Despite their spectacular diversity of morphologies, life histories, behaviors, and genomic features related to reproduction, ray-finned fishes historically have been underutilized in comparative evolutionary studies. This situation is gradually changing with recent developments in actinopterygiian genomics and the elaboration of analytical methods for generating and analyzing large cladograms in a comparative phylogenetic context. As we have tried to illustrate here, these scientific advances are creating many exciting opportunities for capitalizing upon the exuberant biological diversity of the world's largest vertebrate clade.

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Appendix A

SUPPLEMENTAL REFERENCES FOR ACTINOPTERYGIIAN SEX DETERMINATION

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Appendix B

DATA AND SUPPLEMENTAL REFERENCES FOR ACTINOPTERYGIIAN SEX CHROMOSOMES AND MALE ORNAMENTS.

Genus	**Species**	**Male Ornaments**	**Sex Chromosomes**
Acipenser	*transmontanus*	Brighter (Breder and Rosen 1966)	ZZ-ZW (Van Eenennaam et al. 1999)
Anguilla	*anguilla*	No (Breder and Rosen 1966)	ZZ-ZW (Chourrout 1986; Devlin and Nagahama 2002; Sola et al. 1981)
Anguilla	*japonica*	No (Breder and Rosen 1966)	ZZ-ZW (Chourrout 1986; Devlin and Nagahama 2002; Sola et al. 1981)
Anguilla	*rostrata*	No (Breder and Rosen 1966)	ZZ-ZW (Chourrout 1986; Devlin and Nagahama 2002)
Conger	*myriaster*	No (Breder and Rosen 1966)	ZZ-ZW (Chourrout 1986; Sola et al. 1981)

Gymnothorax	*eurostus*	No (Breder and Rosen 1966)	XX-XY (Devlin and Nagahama 2002)
Scolecenchelys	*gymnotus*	No (Breder and Rosen 1966)	XX-XY (Devlin and Nagahama 2002)
Aulopus	*japonicus*	Brighter (Froese and Pauly 2004)	ZZ-ZW (Devlin and Nagahama 2002)
Saurida	*elongata*	No (Breder and Rosen 1966)	ZZ-ZW (Chourrout 1986; Devlin and Nagahama 2002; Sola et al. 1981)
Saurida	*undosquamis*	No (Breder and Rosen 1966)	ZZ-ZW (Chourrout 1986; Devlin and Nagahama 2002; Sola et al. 1981)
Synodus	*hoshinonus*	No (Breder and Rosen 1966)	ZZ-ZW (Devlin and Nagahama 2002)
Synodus	*ulae*	No (Breder and Rosen 1966)	ZZ-ZW (Devlin and Nagahama 2002)
Trachinocephalus	*myops*	No (Breder and Rosen 1966)	ZZ-ZW (Devlin and Nagahama 2002)
Oryzias	*curvinotus*	No (Breder and Rosen 1966)	XX-XY (Matsuda et al. 2003)

Oryzias	*latipes*	No (Breder and Rosen 1966)	XX-XY (Chourrout 1986; Francis 1992)
Beryx	*splendens*	No (McEachran and Fechhelm 1998)	XX-XY (Devlin and Nagahama 2002)
Leporinus	*conirostris*	Brighter, longer fins (Breder and Rosen 1966)	ZZ-ZW (Moreira-Filho et al. 1993)
Leporinus	*elongatus*	Brighter, longer fins (Breder and Rosen 1966)	ZZ-ZW (Devlin and Nagahama 2002; Moreira-Filho et al. 1993)
Leporinus	*lacustris*	Brighter, longer fins (Breder and Rosen 1966)	XX-XY (Chourrout 1986; Devlin and Nagahama 2002; Moreira-Filho et al. 1993)
Leporinus	*macrocephalus*	Brighter, longer fins (Breder and Rosen 1966)	ZZ-ZW (Moreira-Filho et al. 1993)
Leporinus	*obtusidens*	Brighter, longer fins (Breder and Rosen 1966)	ZZ-ZW (Chourrout 1986; Moreira-Filho et al. 1993)
Leporinus	*reinhardti*	Brighter, longer fins (Breder and Rosen 1966)	ZZ-ZW (Moreira-Filho et al. 1993)

Leporinus	*silvestrii*	Brighter, longer fins (Breder and Rosen 1966)	ZZ-ZW (Chourrout 1986; Devlin and Nagahama 2002)
Leporinus	*trifasciatus*	Brighter, longer fins (Breder and Rosen 1966)	ZZ-ZW (Moreira-Filho et al. 1993)
Characidium	*fasciatum*	Spotted dorsal (Sanford 1995)	ZZ-ZW (Devlin and Nagahama 2002; Maistro et al. 1998)
Triportheus	*albus*	No (Axelrod and Schultz 1983)	ZZ-ZW (Moreira-Filho et al. 1993)
Triportheus	*angulatus*	No (Paysan 1975)	ZZ-ZW (Moreira-Filho et al. 1993)
Thoracocharax	*stellatus*	No (Breder and Rosen 1966)	ZZ-ZW (Caravalho et al. 2002)
Semaprochilodus	*taeniurus*	No (Sanford 1995)	ZZ-ZW (Devlin and Nagahama 2002; Moreira-Filho et al. 1993)
Brevoortia	*aurea*	No (Breder and Rosen 1966)	XX-XY (Devlin and Nagahama 2002)
Lepidocephalichthys	*guntea*	Brighter (Breder and Rosen 1966)	ZZ-ZW (Devlin and Nagahama 2002)

Barbonymus	*gonionotus*	No (Froese and Pauly 2004)	XX-XY (Pongthana et al. 1995)
Barilius	*shacra*	No (Jayaram 1981)	XX-XY (Devlin and Nagahama 2002)
Carassius	*auratus*	Tubercles (Froese and Pauly 2004)	XX-XY (Chourrout 1986; Li and Gui 2003; Yamamoto and Kajishima 1969)
Carassius	*carassius*	No (Jayaram 1981)	XX-XY (Devlin and Nagahama 2002; Fujioka 2002)
Ctenopharyngodon	*idella*	No (Sanford 1995)	XX-XY (Stanley 1976)
Cyprinus	*carpio*	No (Gilbert and Williams 2002)	XX-XY (Chourrout 1986; Golovinskaya 1972)
Leuciscus	*carolitertii*	No (Miller and Loates 1997)	ZZ-ZW (Devlin and Nagahama 2002)
Leuciscus	*pyrenaicus*	No (Miller and Loates 1997)	ZZ-ZW (Devlin and Nagahama 2002)
Scardinius	*erythrophthalmus*	No (Reeve and Pfennig 2003)	ZZ-ZW (Devlin and Nagahama 2002; Koehler et al. 1995)

Vimba	*vimba*	Brighter (Miller and Loates 1997)	XX-XY (Devlin and Nagahama 2002)
Aplocheilus	*panchax*	No (Breder and Rosen 1966)	ZZ-ZW (Chourrout 1986; Devlin and Nagahama 2002)
Nothobranchius	*guentheri*	No (Breder and Rosen 1966)	XX-XY (Devlin and Nagahama 2002)
Pterolebias	*hoignei*	No (Breder and Rosen 1966)	XX-XY (Devlin and Nagahama 2002)
Garmanella	*pulchra*	No (Goldstein et al. 2000)	XX-XY (Devlin and Nagahama 2002)
Megupsilon	*aporus*	Brighter (Goldstein et al. 2000)	XX-XY (Devlin and Nagahama 2002)
Fundulus	*diaphanus*	No (Goldstein et al. 2000)	XX-XY (Chourrout 1986; Devlin and Nagahama 2002; Kornfield 1981)
Fundulus	*heteroclitus*	Brighter stripes (Robins and Ray 1986)	XX-XY (Kornfield 1981)
Fundulus	*parvipinnis*	Male darker (Goldstein et al. 2000)	XX-XY (Chourrout 1986; Kornfield 1981; Sola et al. 1981)

Gambusia	*affinis*	No (Breder and Rosen 1966; Froese and Pauly 2004)	ZZ-ZW (Chourrout 1986; Devlin and Nagahama 2002; Sola et al. 1981)
Gambusia	*hurtadoi*	No (Breder and Rosen 1966; Froese and Pauly 2004)	ZZ-ZW (Chourrout 1986; Devlin and Nagahama 2002; Sola et al. 1981)
Gambusia	*puncticulata*	No (Breder and Rosen 1966; Froese and Pauly 2004)	ZZ-ZW (Devlin and Nagahama 2002; Moreira-Filho et al. 1993)
Mollienesia	*sphenops*	No (Breder and Rosen 1966; Froese and Pauly 2004)	ZZ-ZW (Chourrout 1986; Sola et al. 1981)
Poecilia	*latipinna*	Brighter, large dorsal (Breder and Rosen 1966; Reeve and Pfennig 2003)	ZZ-ZW (Devlin and Nagahama 2002)
Poecilia	*reticulata*	Brighter, longer fins (Breder and Rosen 1966; Reeve and Pfennig 2003)	XX-XY (Bacci 1965; Devlin and Nagahama 2002; Francis 1992)
Poecilia	*sphenops*	Larger dorsal (Breder and Rosen 1966; Reeve and Pfennig 2003)	multiple sex chroms = ZZ-ZW, XX-XY (Devlin and Nagahama 2002)

Poecilia	*velifera*	Larger dorsal (Breder and Rosen 1966; Froese and Pauly 2004)	ZZ-ZW (Devlin and Nagahama 2002)
Xiphophorus	*alvarezi*	Sword, dark caudal (Breder and Rosen 1966; Meyer 1997)	ZZ-ZW (Volff and Schartl 2001)
Xiphophorus	*cortezi*	Sword, dark caudal (Breder and Rosen 1966; Meyer 1997)	XX-XY (Volff and Schartl 2001)
Xiphophorus	*maculatus*	Dark dorsal (Breder and Rosen 1966; Meyer 1997)	XX'-XY (Bellamy 1928)
Xiphophorus	*milleri*	No (Breder and Rosen 1966; Meyer 1997)	XX-XY (Kallman and Atz 1966)
Xiphophorus	*nezahualcoyotl*	Sword, dark caudal (Breder and Rosen 1966; Meyer 1997)	XX-XY (Volff and Schartl 2001)
Xiphophorus	*nigrensis*	Sword (Breder and Rosen 1966; Meyer 1997)	XX-XY (Volff and Schartl 2001)
Xiphophorus	*pygmaeus*	Sword, dark caudal (Breder and Rosen 1966; Meyer 1997)	XX-XY (Kallman and Atz 1966)
Xiphophorus	*variatus*	No (Breder and Rosen 1966; Meyer 1997)	XX-XY (Kallman and Atz 1966)

Xiphophorus	*xiphidium*	Sword (Breder and Rosen 1966; Meyer 1997)	XX-XY (Volff and Schartl 2001)
Apeltes	*quadracus*	Brighter (Breder and Rosen 1966)	ZZ-ZW (Chourrout 1986; Devlin and Nagahama 2002; Sola et al. 1981)
Gasterosteus	*wheatlandi*	Brighter (Breder and Rosen 1966)	XX-XY (Chourrout 1986; Devlin and Nagahama 2002)
Diademichthys	*lineatus*	Brighter (Breder and Rosen 1966)	XX-XO (Devlin and Nagahama 2002)
Lepidogaster	*candollei*	Brighter (Breder and Rosen 1966)	XX-XY (Devlin and Nagahama 2002)
Eigenmannia	*virescens*	Electric song (Froese and Pauly 2004)	multiple sex chroms =XX-XY, ZZ-ZW (de Almeida-Toledo et al. 2002; Moreira-Filho et al. 1993)
Lampanyctus	*ritteri*	No (Breder and Rosen 1966)	XX-XO (Chourrout 1986; Devlin and Nagahama 2002; Sola et al. 1981)
Parvilux	*ingens*	No (Breder and Rosen 1966)	XX-XO (Devlin and Nagahama 2002)

Stenobrachius	*leucopsarus*	No (Breder and Rosen 1966)	XX-XY (Chourrout 1986)
Symbolophorus	*californiensis*	No (Breder and Rosen 1966)	XX-XY (Chourrout 1986; Devlin and Nagahama 2002)
Scopelengys	*tristis*	No (Breder and Rosen 1966)	XX-XY (Chourrout 1986; Devlin and Nagahama 2002)
Argentina	*silus*	No (Breder and Rosen 1966)	XX-XY (Chourrout 1986; Devlin and Nagahama 2002)
Galaxias	*platei*	No (Breder and Rosen 1966)	XX-XO (Devlin and Nagahama 2002)
Brienomyrus	*brachistius*	Electric song (Breder and Rosen 1966; Froese and Pauly 2004)	XX-XY (Devlin and Nagahama 2002)
Osteoglussum	*bicirrhosum*	No (Breder and Rosen 1966)	XX-XY (Devlin and Nagahama 2002)
Parablennius	*tentacularis*	Brighter (Breder and Rosen 1966)	XX-XY (Devlin and Nagahama 2002)

Alepes	*djedaba*	No (Kyushin et al. 1982)	ZZ-ZW (Devlin and Nagahama 2002)
Trachinotus	*ovatus*	No (Muus et al. 1999)	ZZ-ZW (Devlin and Nagahama 2002)
Chaenodraco	*wilsoni*	No (Froese and Pauly 2004)	XX-XY (Devlin and Nagahama 2002)
Cichlasoma	*nigofasciata*	Long rays, dark bars (Reeve and Pfennig 2003)	XX-XY (Reeve and Pfennig 2003)
Geophagus	*brasiliensis*	No (Sanford 1995)	XX-XY (Chourrout 1986; Devlin and Nagahama 2002; Sola et al. 1981)
Oreochromis	*aureus*	Brighter (Froese and Pauly 2004)	ZZ-ZW (Chourrout 1986; Pandian and Koteeswaran 1999)
Oreochromis	*mossambicus*	Brighter (Froese and Pauly 2004)	XX-XY (Chourrout 1986; Pandian and Koteeswaran 1999)
Pseudocrenilabrus	*multicolor*	Brighter (Froese and Pauly 2004)	XX-XY (Chourrout 1986)
Dormitator	*maculatus*	Brighter (Froese and Pauly 2004)	XX-XY (Moreira-Filho et al. 1993)

Eleotris	*pisonis*	No (Breder and Rosen 1966)	ZZ-ZW (Devlin and Nagahama 2002)
Boleophthalmus	*boddaerti*	No (Jayaram 1981)	ZZ-ZW (Devlin and Nagahama 2002)
Gobiodon	*citrinus*	No (Breder and Rosen 1966; Reeve and Pfennig 2003)	XX-XO (Chourrout 1986; Devlin and Nagahama 2002; Sola et al. 1981)
Gobionellus	*shufeldti*	No (Robins and Ray 1986)	XX-XY (Devlin and Nagahama 2002)
Gobius	*bucchichi*	No (Miller and Loates 1997)	XX-XY (Devlin and Nagahama 2002)
Gobius	*cobitis*	Darker (Miller and Loates 1997)	XX-XY (Devlin and Nagahama 2002)
Gobius	*niger*	No (Muus et al. 1999)	XX-XY (Chourrout 1986)
Gobius	*pagenellis*	No (Muus et al. 1999)	XX-XY (Chourrout 1986)
Proterorhinus	*marmoratus*	No (Middaugh et al. 1981)	XX-XY (Devlin and Nagahama 2002)
Acerina	*cernua*	No (Muus et al. 1999)	XX-XY (Devlin and Nagahama 2002)

Parapercis	*sexfasciata*	No (Andryashev 1971)	XX-XY (Devlin and Nagahama 2002)
Bothus	*podas*	Longer fins (Breder and Rosen 1966)	XX-XY (Devlin and Nagahama 2002)
Cynogloassus	*puncticeps*	No (Breder and Rosen 1966)	ZZ-ZO (Devlin and Nagahama 2002)
Symphurus	*plagiusa*	No (Breder and Rosen 1966)	XX-XO (Devlin and Nagahama 2002; Sola et al. 1981)
Microchirus	*ocellatus*	Longer fins (Breder and Rosen 1966)	XX-XY (Devlin and Nagahama 2002)
Coregonus	*sardinella*	No (Breder and Rosen 1966; Reeve and Pfennig 2003)	XX-XY (Devlin and Nagahama 2002)
Hucho	*hucho*	Red color (Miller and Loates 1997)	XX-XY (Devlin and Nagahama 2002)
Oncorhynchus	*gorbuscha*	Hooked jaw, brighter (Breder and Rosen 1966)	XX-XY (Chourrout 1986)
Oncorhynchus	*kisutch*	Hooked jaw, brighter (Breder and Rosen 1966)	XX-XY (Chourrout 1986)

Oncorhynchus	*lagocephalus*	Hooked jaw, brighter (Breder and Rosen 1966)	XX-XY (Chourrout 1986)
Oncorhynchus	*mykiss*	Hooked jaw, brighter (Breder and Rosen 1966)	XX-XY (Chourrout 1986)
Oncorhynchus	*tshawytscha*	Hooked jaw, brighter (Breder and Rosen 1966)	XX-XY (Chourrout 1986; Devlin and Nagahama 2002)
Salmo	*salar*	Hooked jaw, brighter (Froese and Pauly 2004)	XX-XY (Woram et al. 2003)
Salmo	*trutta*	Hooked jaw, brighter (Froese and Pauly 2004)	XX-XY (Chourrout 1986)
Salvelinus	*alpinus*	Brighter (Froese and Pauly 2004)	XX-XY (Woram et al. 2003)
Salvelinus	*namaycush*	Brighter (Froese and Pauly 2004)	XX-XY (Devlin and Nagahama 2002)
Netuma	*barba*	No (Breder and Rosen 1966)	XX-XY (Devlin and Nagahama 2002)
Clarias	*batrachus*	No (Breder and Rosen 1966)	ZZ-ZW (Devlin and Nagahama 2002; Pandey and Lakra 1997)

Clarias	*fuscus*	No (Breder and Rosen 1966)	XX-XY (Devlin and Nagahama 2002)
Clarias	*gariepinus*	No (Breder and Rosen 1966)	XX-XY (Galbusera et al. 2000)
Ictalurus	*punctatus*	No (Breder and Rosen 1966)	ZZ-ZW (Nakamura et al. 1998; Wolters et al. 1982)
Notorus	*taylori*	No (Breder and Rosen 1966)	XX-XY (Chourrout 1986; Devlin and Nagahama 2002)
Hypostomus	*ancistroides*	No (Breder and Rosen 1966)	XX-XY (Moreira-Filho et al. 1993)
Hypostomus	*macrops*	No (Breder and Rosen 1966)	XX-XY (Moreira-Filho et al. 1993)
Hypostomus	*sp.*	No (Breder and Rosen 1966)	ZZ-ZW (Artoni et al. 1998)
Loricariichthys	*platymetopon*	No (Breder and Rosen 1966)	ZZ-ZW (Devlin and Nagahama 2002)
Microlepidogaster	*leucofrenatus*	No (Breder and Rosen 1966)	ZZ-ZW (Devlin and Nagahama 2002; Moreira-Filho et al. 1993)

Plecostomus	*ancistroides*	No (Breder and Rosen 1966)	XX-XY (Chourrout 1986; Devlin and Nagahama 2002; Sola et al. 1981)
Plecostomus	*macrops*	No (Breder and Rosen 1966)	XX-XY (Devlin and Nagahama 2002)
Pseudotocinclus	*tietensis*	No (Breder and Rosen 1966)	XX-XY (Devlin and Nagahama 2002; Moreira-Filho et al. 1993)
Hemisynodontis	*membranaceous*	No (Breder and Rosen 1966)	ZZ-ZW (Devlin and Nagahama 2002)
Synodontis	*bastiani*	No (Breder and Rosen 1966)	ZZ-ZW (Devlin and Nagahama 2002)
Synodontis	*budgetti*	No (Breder and Rosen 1966)	ZZ-ZW (Devlin and Nagahama 2002)
Synodontis	*courteti*	No (Breder and Rosen 1966)	ZZ-ZW (Devlin and Nagahama 2002)
Synodontis	*filamentosus*	No (Breder and Rosen 1966)	ZZ-ZW (Devlin and Nagahama 2002)
Synodontis	*ocellifer*	No (Breder and Rosen 1966)	ZZ-ZW (Devlin and Nagahama 2002)

Synodontis	*schall*	No (Breder and Rosen 1966)	ZZ-ZW (Devlin and Nagahama 2002)
Synodontis	*sorex*	No (Breder and Rosen 1966)	ZZ-ZW (Devlin and Nagahama 2002)
Synodontis	*violaceus*	No (Breder and Rosen 1966)	ZZ-ZW (Devlin and Nagahama 2002)
Imparfinis	*mirini*	No (Breder and Rosen 1966)	ZZ-ZW (Devlin and Nagahama 2002)
Pimelodella	*sp.*	No (Breder and Rosen 1966)	XX-XY (Moreira-Filho et al. 1993)
Ompok	*bimaculatus*	No (Breder and Rosen 1966)	XX-XY (Sola et al. 1981)
Melamphaes	*parvus*	No (Breder and Rosen 1966)	XX-XY (Chourrout 1986; Devlin and Nagahama 2002)
Scopeloberyx	*robustus*	No (Breder and Rosen 1966)	XX-XY (Chourrout 1986; Devlin and Nagahama 2002)
Scopelogadus	*mizolepis*	No (Breder and Rosen 1966)	XX-XY (Devlin and Nagahama 2002)

Sternoptyx	*diaphana*	No (Paxton and Echenmeyer 1998)	XX-XO (Devlin and Nagahama 2002)
Mastacembelus	*aculeatus*	No (Breder and Rosen 1966)	XX-XY (Liu et al. 2002)
Odonus	*niger*	No (Sanford 1995)	XX-XY (Devlin and Nagahama 2002)
Rhinecanthus	*aculeatus*	No (Smith and Heemstra 1986)	XX-XY (Devlin and Nagahama 2002)
Rhinecanthus	*reticulangus*	No (Smith and Heemstra 1986)	XX-XY (Devlin and Nagahama 2002)
Stephenolepis	*cirrhifer*	No (Smith 1997)	XX-XY (Devlin and Nagahama 2002)
Stephenolepis	*hispidus*	No (Gilbert and Williams 2002)	XX-XY (Devlin and Nagahama 2002)
Arothron	*nigropunctatus*	No (Breder and Rosen 1966)	XX-XY (Devlin and Nagahama 2002)
Zeus	*faber*	No (Breder and Rosen 1966)	XX-XY (Devlin and Nagahama 2002)

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Appendix D

SOURCE PHYLOGENIES AND ACTINPTERYGIIAN SUPERTREE TOPOLOGY

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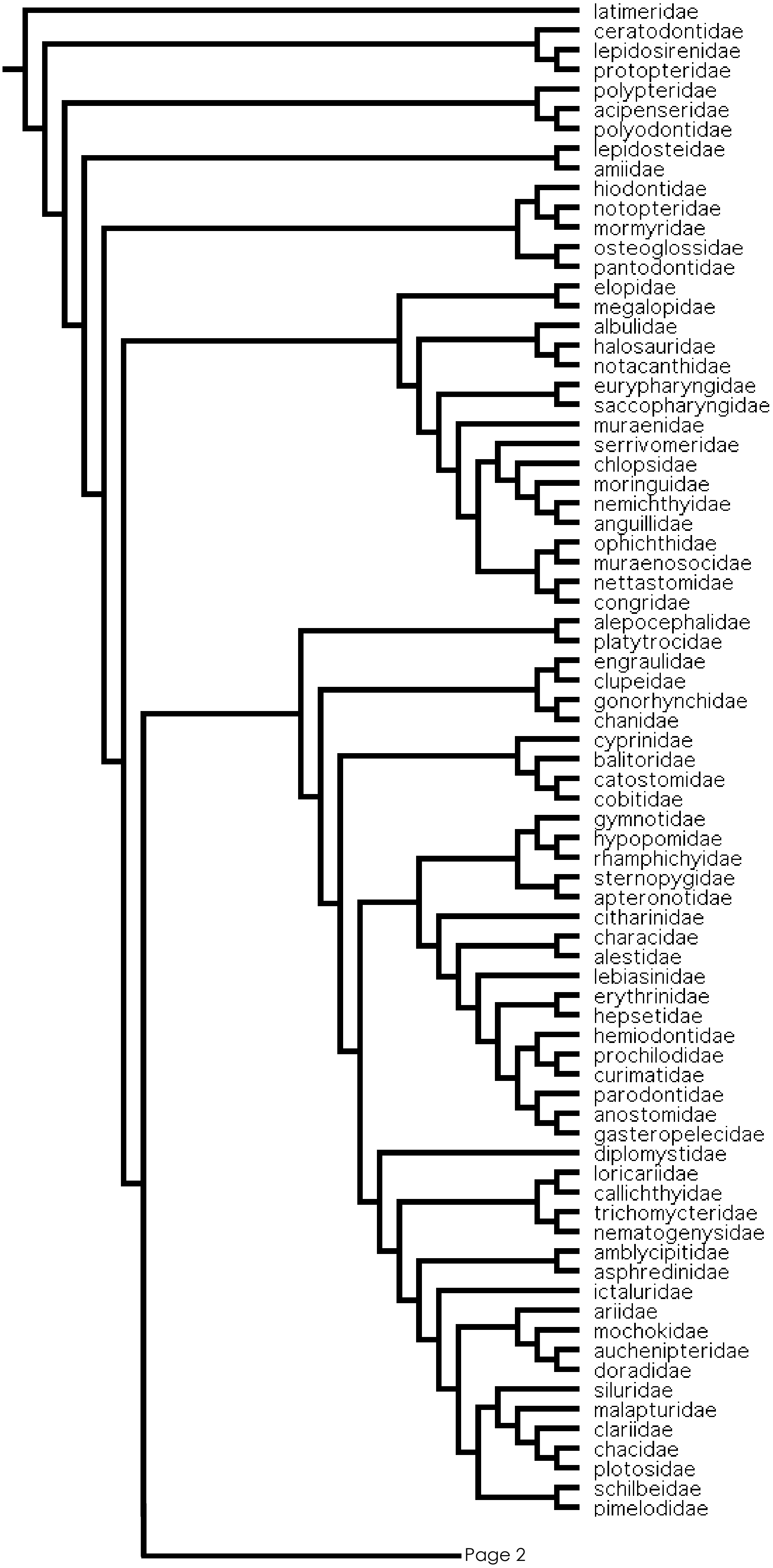
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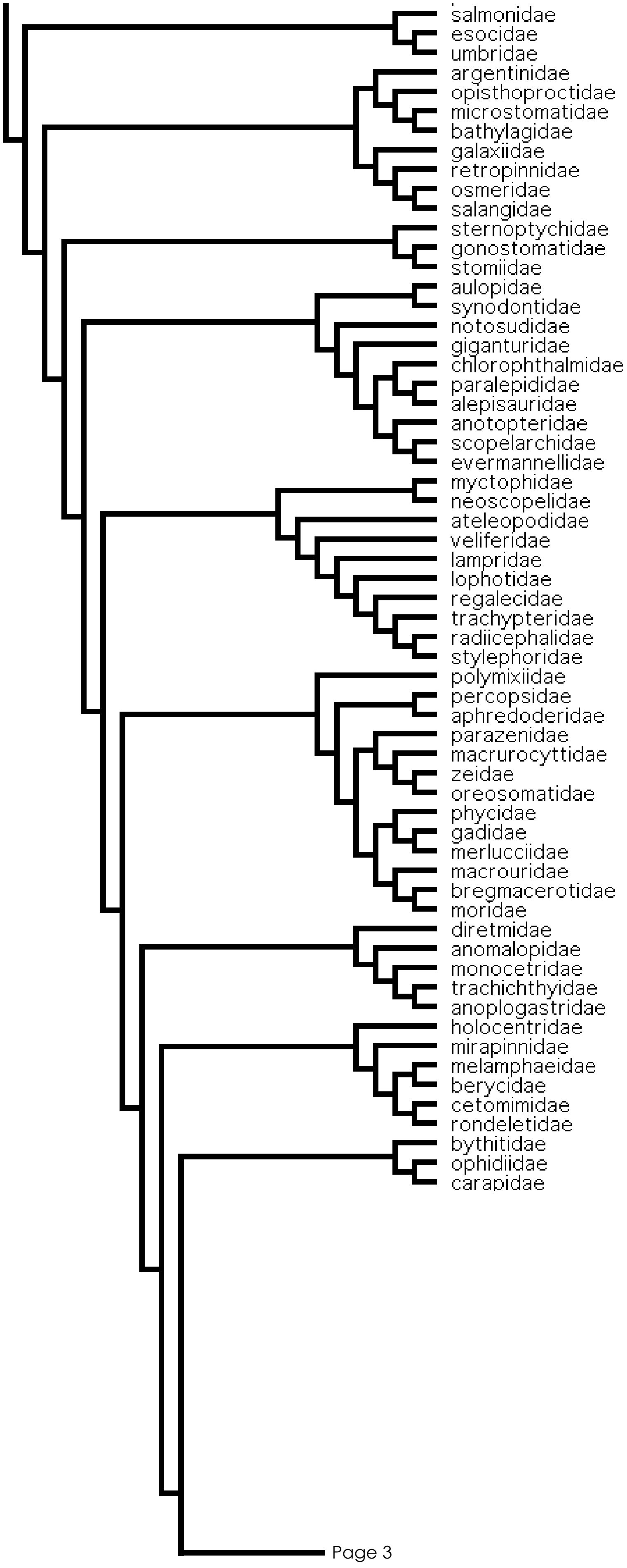
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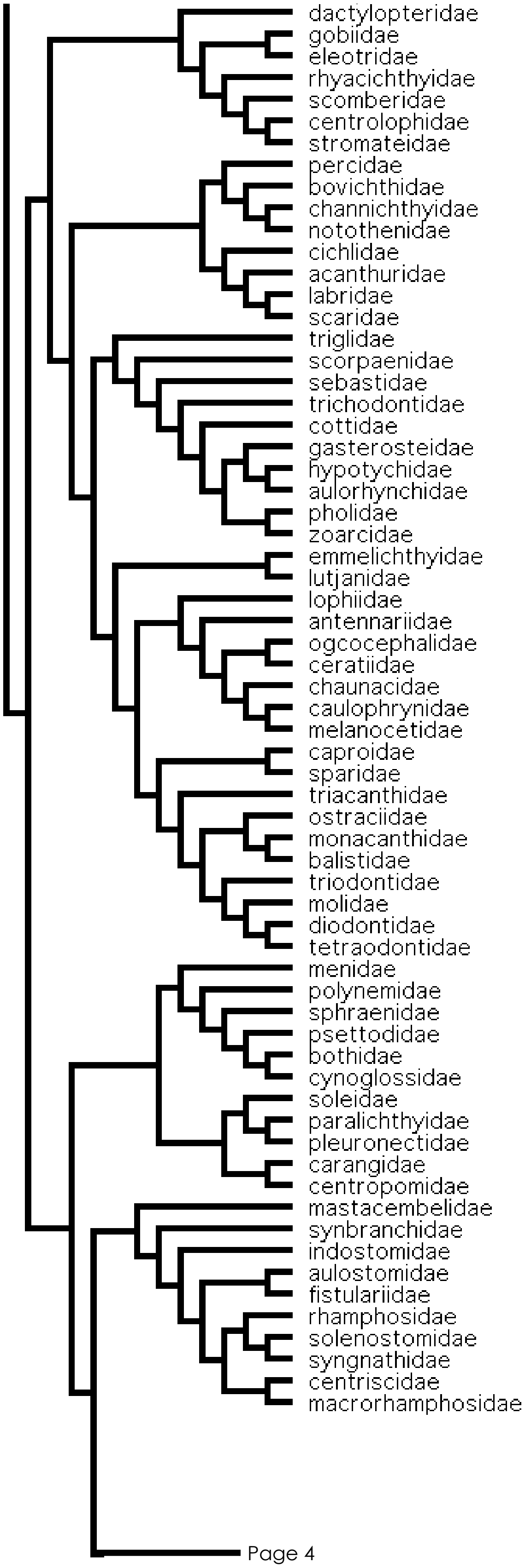
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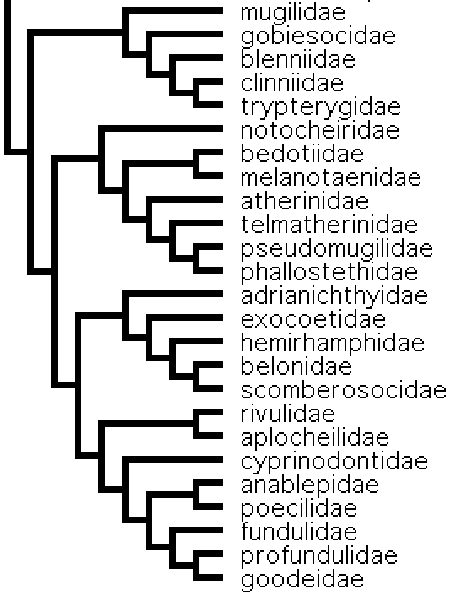
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profundulidae
goodeidae

Appendix E

SUPPLEMENTAL REFERENCES FOR ACTIOPTERYGIIAN MALE ALTERNATIVE REPRODUCTIVE TACTICS

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Appendix F

C-VALUES AND SPECIES COUNTS FOR ALL SURVEYED ACTINOPTERYGIIAN GENERA.

Order	**Family**	**Genus**	**Average C-Value**	**Number of Species**
Acipenseriformes	Acipenseridae	*Acipenser*	3.57	20
Acipenseriformes	Acipenseridae	*Huso*	1.95	2
Acipenseriformes	Acipenseridae	*Pseudoscaphirhychus*	1.74	3
Acipenseriformes	Acipenseridae	*Scaphirhynchus*	2.06	3
Acipenseriformes	Polyodontidae	*Polyodon*	3	1
Amiiformes	Amiidae	*Amia*	1.22	1
Anguilliformes	Anguillidae	*Anguilla*	1.405	18
Anguilliformes	Chlopsidae	*Ophichthus*	1.96	65
Anguilliformes	Congridae	*Ariosoma*	1.8	20
Anguilliformes	Congridae	*Conger*	1.36	14
Anguilliformes	Muraenidae	*Enchelycore*	2.3	11
Anguilliformes	Muraenidae	*Gymnothorax*	2.25	115
Anguilliformes	Muraenidae	*Muraena*	2.54	11

Anguilliformes	Muraenosocidae	*Muraenesox*	1.13	2
Atheriniformes	Atherinidae	*Atherinops*	1.1	1
Atheriniformes	Melanotaenidae	*Melanotaenia*	1.3	45
Aulopiformes	Synodontidae	*Saurida*	1.57	16
Aulopiformes	Synodontidae	*Synodus*	1.35	33
Aulopiformes	Synodontidae	*Trachinocephalus*	0.8	1
Beloniformes	Adrianichthyidae	*Oryzias*	0.85	20
Beloniformes	Belonidae	*Platybelone*	1	1
Beloniformes	Belonidae	*Potamorrhaphis*	1.2	3
Beloniformes	Belonidae	*Strongylura*	1.14	14
Beloniformes	Belonidae	*Tylosurus*	1.1	5
Beloniformes	Exocoetidae	*Cypselurus*	0.97	11
Beloniformes	Hemirhamphidae	*Dermogenys*	0.74	13
Beloniformes	Hemirhamphidae	*Euleptorhamphus*	1.02	2
Beloniformes	Hemirhamphidae	*Hemiramphus*	0.75	10
Beloniformes	Hemirhamphidae	*Hyporhamphus*	1.1	36
Beryciformes	Berycidae	*Beryx*	0.85	3
Beryciformes	Diretmidae	*Diretmichthys*	1.46	1
Beryciformes	Holocentridae	*Holocentrus*	0.74	4
Beryciformes	Holocentridae	*Myripristis*	1.01	27
Characiformes	Alestiidae	*Arnoldichthys*	1.2	1
Characiformes	Anostomidae	*Anostomus*	1.4	8
Characiformes	Anostomidae	*Chilodus*	1.6	4

Characiformes	Anostomidae	*Leporinus*	1.49	76
Characiformes	Anostomidae	*Schizodon*	1.46	13
Characiformes	Characidae	*Aphyocharax*	1.38	13
Characiformes	Characidae	*Astyanax*	1.51	90
Characiformes	Characidae	*Brachycalcinus*	1.74	5
Characiformes	Characidae	*Brycon*	1.2	41
Characiformes	Characidae	*Bryconamericus*	1.64	59
Characiformes	Characidae	*Bryconops*	1.1	13
Characiformes	Characidae	*Chalceus*	1.07	5
Characiformes	Characidae	*Characidium*	1.19	45
Characiformes	Characidae	*Charax*	1.44	16
Characiformes	Characidae	*Colossoma*	1.5	1
Characiformes	Characidae	*Exodon*	1.7	1
Characiformes	Characidae	*Galeocharax*	1.6	3
Characiformes	Characidae	*Gymnocorymbus*	1.88	3
Characiformes	Characidae	*Hemigrammus*	1.82	42
Characiformes	Characidae	*Hyphessobrycon*	1.43	96
Characiformes	Characidae	*Markiana*	1.08	2
Characiformes	Characidae	*Metynnis*	1.7	11
Characiformes	Characidae	*Mimagoniates*	1.53	6
Characiformes	Characidae	*Moenkhausia*	1.3	55
Characiformes	Characidae	*Mylossoma*	1.46	3
Characiformes	Characidae	*Odontostilbe*	1.86	9

Characiformes	Characidae	*Oligosarcus*	1.67	16
Characiformes	Characidae	*Piabina*	1.18	1
Characiformes	Characidae	*Piabucus*	1.2	3
Characiformes	Characidae	*Poptella*	1.74	4
Characiformes	Characidae	*Pseudocorynopoma*	1.26	2
Characiformes	Characidae	*Roeboides*	1.32	19
Characiformes	Characidae	*Salminus*	1.31	3
Characiformes	Characidae	*Serrasalmus*	1.61	4
Characiformes	Characidae	*Tetragonopterus*	1.74	2
Characiformes	Characidae	*Triportheus*	1.54	13
Characiformes	Curimatidae	*Curimata*	1.46	13
Characiformes	Curimatidae	*Cyphocharax*	1.63	34
Characiformes	Curimatidae	*Potamorhina*	1.9	5
Characiformes	Curimatidae	*Prochilodus*	1.59	13
Characiformes	Curimatidae	*Semaprochilodus*	1.86	6
Characiformes	Curimatidae	*Steindachnerina*	1.59	1
Characiformes	Erythrinidae	*Hoplias*	1.28	9
Characiformes	Gasteropelecidae	*Carnegiella*	1.4	4
Characiformes	Gasteropelecidae	*Gasteropelecus*	1.4	3
Characiformes	Gasteropelecidae	*Thoracocharax*	1.19	2
Characiformes	Hemiodontidae	*Apareiodon*	1.15	13
Characiformes	Leniasinidae	*Pyrrhulina*	1.1	18
Clupeiformes	Clupeidae	*Alosa*	1.27	22

Clupeiformes	Clupeidae	*Clupea*	0.9	2
Clupeiformes	Clupeidae	*Dorosoma*	0.99	5
Clupeiformes	Clupeidae	*Harengula*	1.02	4
Clupeiformes	Clupeidae	*Herklotsichthys*	0.76	11
Clupeiformes	Clupeidae	*Nematalosa*	0.88	11
Clupeiformes	Clupeidae	*Sardinella*	1.11	21
Clupeiformes	Clupeidae	*Sardinops*	1.35	1
Clupeiformes	Engraulidae	*Anchoa*	1.9	36
Clupeiformes	Engraulidae	*Engraulis*	1.58	7
Cypriniformes	Balitoridae	*Barbatula*	0.54	6
Cypriniformes	Balitoridae	*Homaloptera*	0.47	32
Cypriniformes	Balitoridae	*Lefua*	0.48	3
Cypriniformes	Balitoridae	*Pseudogastromyzon*	0.44	7
Cypriniformes	Balitoridae	*Triplophysa*	1.1	79
Cypriniformes	Catostomidae	*Carpiodes*	2.24	3
Cypriniformes	Catostomidae	*Catostomus*	2.37	25
Cypriniformes	Catostomidae	*Erimyzon*	1.86	3
Cypriniformes	Catostomidae	*Moxostoma*	2.14	19
Cypriniformes	Catostomidae	*Myxocyprinus*	2.02	1
Cypriniformes	Cobitidae	*Botia*	0.89	29
Cypriniformes	Cobitidae	*Cobitis*	1.84	39
Cypriniformes	Cobitidae	*Leptobotia*	0.56	5
Cypriniformes	Cobitidae	*Misgurnus*	2.12	4

Cypriniformes	Cobitidae	*Pangio*	1.01	24
Cypriniformes	Cobitidae	*Paramisgurnus*	1.09	1
Cypriniformes	Cyprinidae	*Abbottina*	1.54	4
Cypriniformes	Cyprinidae	*Abramis*	1.2	3
Cypriniformes	Cyprinidae	*Acheilognathus*	0.99	33
Cypriniformes	Cyprinidae	*Acrossocheilus*	1.25	20
Cypriniformes	Cyprinidae	*Alburnus*	1.39	17
Cypriniformes	Cyprinidae	*Anaecypris*	1.24	1
Cypriniformes	Cyprinidae	*Aphyocypris*	1.15	3
Cypriniformes	Cyprinidae	*Aristichthys*	1.03	1
Cypriniformes	Cyprinidae	*Aspius*	1.24	2
Cypriniformes	Cyprinidae	*Balantiocheilus*	1.03	1
Cypriniformes	Cyprinidae	*Barbodes*	1.12	28
Cypriniformes	Cyprinidae	*Barbus*	1.77	349
Cypriniformes	Cyprinidae	*Blicca*	1.26	1
Cypriniformes	Cyprinidae	*Campostoma*	1.14	5
Cypriniformes	Cyprinidae	*Carassius*	2.17	4
Cypriniformes	Cyprinidae	*Catlocarpio*	1.76	1
Cypriniformes	Cyprinidae	*Chela*	1.6	6
Cypriniformes	Cyprinidae	*Chondrostoma*	1.59	26
Cypriniformes	Cyprinidae	*Ctenopharyngodon*	1.03	1
Cypriniformes	Cyprinidae	*Culter*	1.15	7
Cypriniformes	Cyprinidae	*Cyprinella*	1.22	30

Cypriniformes	Cyprinidae	*Cyprinus*	1.73	15
Cypriniformes	Cyprinidae	*Danio*	1.83	26
Cypriniformes	Cyprinidae	*Dionda*	1.02	8
Cypriniformes	Cyprinidae	*Epalzeorhynchos*	1.25	5
Cypriniformes	Cyprinidae	*Erythroculter*	0.91	1
Cypriniformes	Cyprinidae	*Garra*	1.1	61
Cypriniformes	Cyprinidae	*Gnathopogon*	1.11	11
Cypriniformes	Cyprinidae	*Gobio*	1.71	19
Cypriniformes	Cyprinidae	*Hemibarbus*	1.11	9
Cypriniformes	Cyprinidae	*Hemiculter*	1.22	5
Cypriniformes	Cyprinidae	*Hemigrammocypris*	0.9	1
Cypriniformes	Cyprinidae	*Hemitremia*	1.19	1
Cypriniformes	Cyprinidae	*Hybognathus*	1.41	7
Cypriniformes	Cyprinidae	*Hypophthalmichthys*	1	2
Cypriniformes	Cyprinidae	*Ischikauia*	1.29	1
Cypriniformes	Cyprinidae	*Labeo*	1.23	105
Cypriniformes	Cyprinidae	*Lavinia*	1.25	1
Cypriniformes	Cyprinidae	*Leuciscus*	1.26	40
Cypriniformes	Cyprinidae	*Luxilus*	1.24	9
Cypriniformes	Cyprinidae	*Lythrurus*	1.3	9
Cypriniformes	Cyprinidae	*Macrhybopsis*	1.25	4
Cypriniformes	Cyprinidae	*Megalobrama*	1.17	5
Cypriniformes	Cyprinidae	*Moroco*	1.22	2

Cypriniformes	Cyprinidae	*Mylopharodon*	1.33	1
Cypriniformes	Cyprinidae	*Mylopharyngodon*	1.06	1
Cypriniformes	Cyprinidae	*Nocomis*	1.25	7
Cypriniformes	Cyprinidae	*Notemigonus*	1.14	1
Cypriniformes	Cyprinidae	*Notropis*	1.24	98
Cypriniformes	Cyprinidae	*Ochetobius*	1.06	1
Cypriniformes	Cyprinidae	*Opsariichthys*	1.41	2
Cypriniformes	Cyprinidae	*Opsopoeodus*	1.04	1
Cypriniformes	Cyprinidae	*Parabramis*	0.98	1
Cypriniformes	Cyprinidae	*Paracanthobrama*	1.28	1
Cypriniformes	Cyprinidae	*Percocypris*	2.3	2
Cypriniformes	Cyprinidae	*Phenacobius*	1.63	5
Cypriniformes	Cyprinidae	*Phoxinus*	1.37	14
Cypriniformes	Cyprinidae	*Pimephales*	1.13	4
Cypriniformes	Cyprinidae	*Platypharodon*	1.49	1
Cypriniformes	Cyprinidae	*Pseudogobio*	1.59	2
Cypriniformes	Cyprinidae	*Pseudolaubuca*	1.39	3
Cypriniformes	Cyprinidae	*Ptychocheilus*	1.32	4
Cypriniformes	Cyprinidae	*Pungtungia*	1.49	1
Cypriniformes	Cyprinidae	*Puntius*	0.91	100
Cypriniformes	Cyprinidae	*Rasbora*	1.44	71
Cypriniformes	Cyprinidae	*Rhinichthys*	1.29	8
Cypriniformes	Cyprinidae	*Rhodeus*	1.05	15

Cypriniformes	Cyprinidae	*Richardsonius*	1.31	2
Cypriniformes	Cyprinidae	*Rutilus*	1.34	16
Cypriniformes	Cyprinidae	*Sarcocheilichthys*	1.3	10
Cypriniformes	Cyprinidae	*Scardinius*	1.26	4
Cypriniformes	Cyprinidae	*Schizothorax*	2.74	54
Cypriniformes	Cyprinidae	*Semotilus*	1.25	4
Cypriniformes	Cyprinidae	*Sinocyclocheilus*	2.33	27
Cypriniformes	Cyprinidae	*Squalidus*	1.25	11
Cypriniformes	Cyprinidae	*Tanakia*	1.03	4
Cypriniformes	Cyprinidae	*Tinca*	0.98	1
Cypriniformes	Cyprinidae	*Tribolodon*	0.9	4
Cypriniformes	Cyprinidae	*Xenocyris*	1.23	5
Cypriniformes	Cyprinidae	*Zacco*	1.2	5
Cyprinodontiformes	Aplocheilidae	*Aphyosemion*	1.2	77
Cyprinodontiformes	Aplocheilidae	*Aplocheilus*	0.74	7
Cyprinodontiformes	Aplocheilidae	*Pachypanchax*	0.73	3
Cyprinodontiformes	Aplocheilidae	*Rivulus*	1.5	91
Cyprinodontiformes	Cyprinodontidae	*Cyprinodon*	1.6	41
Cyprinodontiformes	Fundulidae	*Fundulus*	1.45	37
Cyprinodontiformes	Poeciidae	*Belonesox*	0.94	1
Cyprinodontiformes	Poeciidae	*Gambusia*	0.86	38
Cyprinodontiformes	Poeciidae	*Heterandria*	0.95	9
Cyprinodontiformes	Poeciidae	*Limia*	0.93	20

Cyprinodontiformes	Poeciidae	*Poecilia*	0.96	32
Cyprinodontiformes	Poeciidae	*Poeciliopsis*	0.7	21
Cyprinodontiformes	Poeciidae	*Xiphophorus*	0.79	24
Elopiformes	Elopidae	*Elops*	1.2	6
Esociformes	Esocidae	*Esox*	1.17	5
Esociformes	Umbridae	*Dallia*	1.26	1
Esociformes	Umbridae	*Novumbra*	1.04	1
Esociformes	Umbridae	*Umbra*	2.51	3
Gadiformes	Gadidae	*Boreogadus*	0.88	1
Gadiformes	Gadidae	*Gadus*	0.67	3
Gadiformes	Gadidae	*Microgadus*	0.9	2
Gadiformes	Macrouridae	*Caelorinchus*	0.77	108
Gadiformes	Macrouridae	*Coryphaenoides*	0.85	66
Gadiformes	Macrouridae	*Macrourus*	0.94	4
Gadiformes	Macrouridae	*Mesobius*	0.79	2
Gadiformes	Merluccidae	*Merluccius*	0.96	12
Gadiformes	Moridae	*Halargyreus*	0.94	1
Gadiformes	Phycidae	*Urophycis*	0.89	7
Gasterosteiformes	Aulostomidae	*Aulostomus*	0.7	3
Gasterosteiformes	Centriscidae	*Aeoliscus*	0.45	2
Gasterosteiformes	Centriscidae	*Centriscops*	0.55	2
Gasterosteiformes	Fistulariidae	*Fistularia*	0.76	5
Gasterosteiformes	Gasterosteidae	*Apeltes*	0.58	1

Gasterosteiformes	Gasterosteidae	*Culaea*	0.67	1
Gasterosteiformes	Gasterosteidae	*Gasterosteus*	0.64	3
Gasterosteiformes	Syngnathidae	*Corythoichthys*	1.34	11
Gasterosteiformes	Syngnathidae	*Hippocampus*	0.55	41
Gasterosteiformes	Syngnathidae	*Nerophis*	1.78	3
Gasterosteiformes	Syngnathidae	*Syngnathus*	0.58	29
Gymnotiformes	Apteronotidae	*Apteronotus*	0.71	17
Gymnotiformes	Rhamphichthyidae	*Rhamphichthys*	0.93	10
Gymnotiformes	Sternopygidae	*Eigenmannia*	1	8
Gymnotiformes	Sternopygidae	*Gymnotus*	0.99	18
Gymnotiformes	Sternopygidae	*Sternopygus*	0.99	5
Lepidosteiformes	Lepidosteidae	*Atractosteus*	1.2	3
Lepidosteiformes	Lepidosteidae	*Lepisosteus*	1.42	4
Lophiiformes	Antennariidae	*Antennarius*	0.78	17
Lophiiformes	Lophiidae	*Lophius*	1.02	7
Lophiiformes	Ogcocephalidae	*Ogcocephalus*	0.74	13
Mugiliformes	Mugilidae	*Liza*	0.77	23
Mugiliformes	Mugilidae	*Mugil*	0.8	16
Mugiliformes	Mugilidae	*Myxus*	0.84	4
Myctophiformes	Myctophidae	*Lampanyctus*	2	29
Myctophiformes	Myctophidae	*Parvilux*	1.95	2
Myctophiformes	Myctophidae	*Triphoturus*	1.9	2
Myctophiformes	Neoscopelidae	*Neoscopelus*	2.52	3

Myctophiformes	Neoscopelidae	*Scopelengys*	1.3	2
Ophidiiformes	Ophidiidae	*Ophidion*	0.76	24
Osmeriformes	Argentinidae	*Argentina*	0.85	12
Osmeriformes	Bathylagidae	*Bathylagus*	2.7	14
Osmeriformes	Bathylagidae	*Leuroglossus*	1.7	2
Osmeriformes	Osmeridae	*Hypomesus*	0.74	6
Osmeriformes	Osmeridae	*Osmerus*	0.66	3
Osmeriformes	Osmeridae	*Spirinchus*	0.84	3
Osteoglossiformes	Mormyridae	*Brienomyrus*	1.2	10
Osteoglossiformes	Mormyridae	*Gnathonemus*	1.2	4
Osteoglossiformes	Mormyridae	*Mormyrus*	1	21
Osteoglossiformes	Mormyridae	*Pollimyrus*	1	17
Osteoglossiformes	Notopteridae	*Chitala*	1.08	4
Osteoglossiformes	Notopteridae	*Xenomystus*	1.3	1
Osteoglossiformes	Osteoglossidae	*Arapaima*	0.98	1
Osteoglossiformes	Osteoglossidae	*Osteoglossum*	1	2
Osteoglossiformes	Pantodontidae	*Pantodon*	0.77	1
Perciformes	Acanthuridae	*Acanthurus*	0.74	34
Perciformes	Acanthuridae	*Ctenochaetus*	0.84	8
Perciformes	Acanthuridae	*Zebrasoma*	0.72	7
Perciformes	Blenniidae	*Aidablennius*	0.61	1
Perciformes	Blenniidae	*Blennius*	0.86	3
Perciformes	Blenniidae	*Crossosalarias*	0.83	1

Perciformes	Blenniidae	*Ecsenius*	0.73	48
Perciformes	Blenniidae	*Hypsoblennius*	0.86	14
Perciformes	Blenniidae	*Lipophrys*	0.79	8
Perciformes	Blenniidae	*Parablennius*	0.76	26
Perciformes	Blenniidae	*Paralipophrys*	1.19	1
Perciformes	Blenniidae	*Petroscirtes*	0.68	10
Perciformes	Blenniidae	*Plagiotremus*	0.53	9
Perciformes	Blenniidae	*Salaria*	1.07	3
Perciformes	Carangidae	*Carangoides*	0.62	20
Perciformes	Carangidae	*Caranx*	0.67	18
Perciformes	Carangidae	*Chloroscombrus*	0.78	2
Perciformes	Carangidae	*Decapterus*	0.66	12
Perciformes	Carangidae	*Seriola*	0.8	9
Perciformes	Carangidae	*Seriolina*	0.68	1
Perciformes	Carangidae	*Trachinotus*	0.86	20
Perciformes	Carangidae	*Ulua*	0.64	2
Perciformes	Channichthyidae	*Chionodraco*	1.83	3
Perciformes	Channichthyidae	*Cryodraco*	1.93	2
Perciformes	Channichthyidae	*Pagetopsis*	2.21	2
Perciformes	Cichlidae	*Aequidens*	1.2	23
Perciformes	Cichlidae	*Astronotus*	1.09	2
Perciformes	Cichlidae	*Cichlasoma*	1.35	40
Perciformes	Cichlidae	*Crenicichla*	1.1	74

Perciformes	Cichlidae	*Haplochromis*	1.12	209
Perciformes	Cichlidae	*Oreochromis*	1.03	36
Perciformes	Cichlidae	*Pelvicachromis*	1	5
Perciformes	Cichlidae	*Pterophyllum*	1.11	3
Perciformes	Cichlidae	*Sarotherodon*	0.84	10
Perciformes	Cichlidae	*Satanoperca*	1.2	7
Perciformes	Cichlidae	*Symphysodon*	1.22	2
Perciformes	Cichlidae	*Tilapia*	1.2	39
Perciformes	Emmelichthyidae	*Erythrocles*	1.04	6
Perciformes	Gobiidae	*Chaenogobius*	1.18	8
Perciformes	Gobiidae	*Cryptocentrus*	1.38	32
Perciformes	Gobiidae	*Gobiodon*	1.07	18
Perciformes	Gobiidae	*Gobius*	0.64	24
Perciformes	Gobiidae	*Istigobius*	1.65	11
Perciformes	Gobiidae	*Neogobius*	1.34	17
Perciformes	Gobiidae	*Oplopomus*	1.33	2
Perciformes	Gobiidae	*Paragobiodon*	1.24	5
Perciformes	Gobiidae	*Rhinogobius*	1.5	38
Perciformes	Gobiidae	*Signigobius*	0.86	1
Perciformes	Gobiidae	*Stigmatogobius*	1.4	6
Perciformes	Gobiidae	*Typhlogobius*	1.2	1
Perciformes	Gobiidae	*Valenciennea*	0.93	15
Perciformes	Labridae	*Cheilio*	0.88	1

Perciformes	Labridae	*Choerodon*	1.03	23
Perciformes	Labridae	*Coris*	1.01	25
Perciformes	Labridae	*Gomphosus*	1.05	2
Perciformes	Labridae	*Halichoeres*	0.8	72
Perciformes	Labridae	*Labroides*	0.89	5
Perciformes	Labridae	*Labrus*	1.44	4
Perciformes	Labridae	*Novaculichthys*	0.72	2
Perciformes	Labridae	*Pseudolabrus*	1.07	12
Perciformes	Labridae	*Symphodus*	1.05	10
Perciformes	Labridae	*Tautoga*	0.93	1
Perciformes	Labridae	*Tautogolabrus*	0.91	2
Perciformes	Labridae	*Thalassoma*	0.98	26
Perciformes	Lutjanidae	*Aprion*	0.68	1
Perciformes	Lutjanidae	*Etelis*	0.95	4
Perciformes	Lutjanidae	*Lutjanus*	1.11	67
Perciformes	Lutjanidae	*Ocyurus*	1.2	1
Perciformes	Nototheniidae	*Dissostichus*	1.02	2
Perciformes	Nototheniidae	*Trematomus*	1.51	11
Perciformes	Percidae	*Perca*	1.13	3
Perciformes	Percidae	*Sander*	1.14	2
Perciformes	Scaridae	*Chlorurus*	2.06	17
Perciformes	Scaridae	*Scarus*	1.49	44
Perciformes	Scaridae	*Sparisoma*	1.89	10

Perciformes	Scombridae	*Katsuwonus*	1	1
Perciformes	Scombridae	*Sarda*	0.88	4
Perciformes	Scombridae	*Scomber*	0.97	3
Perciformes	Scombridae	*Scomberomorus*	0.96	18
Perciformes	Scombridae	*Thunnus*	0.91	8
Perciformes	Sparidae	*Boops*	0.52	2
Perciformes	Sparidae	*Calamus*	0.98	13
Perciformes	Sparidae	*Diplodus*	0.74	12
Perciformes	Sparidae	*Lithognathus*	0.6	4
Perciformes	Sparidae	*Pagellus*	0.65	5
Perciformes	Sparidae	*Pagrus*	0.93	6
Perciformes	Sparidae	*Sarpa*	0.54	1
Perciformes	Sparidae	*Stenotomus*	0.98	2
Perciformes	Sphyraenidae	*Sphyraena*	0.82	25
Perciformes	Stromateidae	*Peprilus*	0.81	8
Perciformes	Trypterigidae	*Ucla*	0.82	1
Perciformes	Zoarcidae	*Bothrocara*	0.8	6
Perciformes	Zoarcidae	*Gymnelus*	0.92	16
Perciformes	Zoarcidae	*Lycodichthys*	1.35	2
Perciformes	Zoarcidae	*Pachycara*	1.46	17
Perciformes	Zoarcidae	*Zoarces*	0.91	4
Pleuronectiformes	Bothidae	*Psettina*	0.59	10
Pleuronectiformes	Cynoglossidae	*Paraplagusia*	0.73	6

Pleuronectiformes	Cynoglossidae	*Symphurus*	1.1	66
Pleuronectiformes	Paralichthyidae	*Citharichthys*	0.96	23
Pleuronectiformes	Paralichthyidae	*Etropus*	1	9
Pleuronectiformes	Paralichthyidae	*Paralichthys*	0.76	23
Pleuronectiformes	Paralichthyidae	*Pseudorhombus*	0.54	27
Pleuronectiformes	Paralichthyidae	*Xystreurys*	0.78	2
Pleuronectiformes	Pleuronectidae	*Eopsetta*	0.75	2
Pleuronectiformes	Pleuronectidae	*Glyptocephalus*	0.84	4
Pleuronectiformes	Pleuronectidae	*Hippoglossus*	0.73	2
Pleuronectiformes	Pleuronectidae	*Lepidopsetta*	0.72	3
Pleuronectiformes	Pleuronectidae	*Limanda*	0.79	6
Pleuronectiformes	Pleuronectidae	*Lyopsetta*	0.73	1
Pleuronectiformes	Pleuronectidae	*Microstomus*	0.97	5
Pleuronectiformes	Pleuronectidae	*Parophrys*	0.65	1
Pleuronectiformes	Pleuronectidae	*Platichthys*	0.65	3
Pleuronectiformes	Pleuronectidae	*Pleuronectes*	0.55	3
Pleuronectiformes	Pleuronectidae	*Pleuronichthys*	0.63	7
Pleuronectiformes	Pleuronectidae	*Pseudopleuronectes*	0.7	5
Pleuronectiformes	Pleuronectidae	*Reinhardtius*	0.71	3
Pleuronectiformes	Psettodidae	*Psettodes*	0.71	3
Pleuronectiformes	Soleidae	*Soleichthys*	0.73	3
Salmoniformes	Salmonidae	*Coregonus*	3.04	62
Salmoniformes	Salmonidae	*Oncorhynchus*	2.66	14

Salmoniformes	Salmonidae	*Prosopium*	2.38	6
Salmoniformes	Salmonidae	*Salmo*	3.07	27
Salmoniformes	Salmonidae	*Salvelinus*	3.28	38
Salmoniformes	Salmonidae	*Stenodus*	3.27	1
Salmoniformes	Salmonidae	*Thymallus*	2.07	5
Scorpaeniformes	Cottidae	*Alcichthys*	0.73	2
Scorpaeniformes	Cottidae	*Clinocottus*	0.93	5
Scorpaeniformes	Cottidae	*Gymnocanthus*	0.74	6
Scorpaeniformes	Cottidae	*Icelinus*	0.82	9
Scorpaeniformes	Cottidae	*Myoxocephalus*	1.37	12
Scorpaeniformes	Cottidae	*Triglopsis*	0.92	2
Scorpaeniformes	Scorpaenidae	*Pterois*	0.99	8
Scorpaeniformes	Scorpaenidae	*Scorpaena*	1.42	57
Scorpaeniformes	Scorpaenidae	*Scorpaenopsis*	1.2	25
Scorpaeniformes	Sebastidae	*Helicolenus*	0.96	9
Scorpaeniformes	Sebastidae	*Sebastes*	0.99	104
Scorpaeniformes	Sebastidae	*Sebastiscus*	0.92	3
Scorpaeniformes	Trigldiae	*Prionotus*	0.89	23
Siluriformes	Ariidae	*Arius*	2.25	85
Siluriformes	Ariidae	*Bagre*	2.4	4
Siluriformes	Ariidae	*Galeichthys*	2.5	3
Siluriformes	Callichthyidae	*Aspidoras*	0.76	19
Siluriformes	Callichthyidae	*Brochis*	1.17	3

Siluriformes	Callichthyidae	*Callichthys*	1.45	2
Siluriformes	Callichthyidae	*Corydoras*	2.16	141
Siluriformes	Callichthyidae	*Dianema*	0.59	2
Siluriformes	Callichthyidae	*Hoplosternum*	0.68	3
Siluriformes	Callichthyidae	*Megalechis*	1.58	2
Siluriformes	Clariidae	*Clarias*	1.13	50
Siluriformes	Diplomystidae	*Diplomystes*	1.29	3
Siluriformes	Doradidae	*Acanthodoras*	1.6	3
Siluriformes	Ictaluridae	*Ameiurus*	1.08	2
Siluriformes	Ictaluridae	*Ictalurus*	1.02	9
Siluriformes	Loricariidae	*Ancistrus*	1.8	55
Siluriformes	Loricariidae	*Farlowella*	1.49	25
Siluriformes	Loricariidae	*Hypostomus*	1.71	123
Siluriformes	Loricariidae	*Liposarcus*	1.69	4
Siluriformes	Loricariidae	*Microlepidogaster*	0.89	2
Siluriformes	Loricariidae	*Otocinclus*	2.1	14
Siluriformes	Loricariidae	*Rineloricaria*	1.6	47
Siluriformes	Malapturidae	*Malapterurus*	1	11
Siluriformes	Mochokidae	*Synodontis*	1.05	117
Siluriformes	Pimelodidae	*Imparfinis*	1.03	20
Siluriformes	Pimelodidae	*Pimelodella*	0.88	66
Siluriformes	Pimelodidae	*Pimelodus*	1.27	26
Siluriformes	Plotosidae	*Euristhmus*	1.75	3

Siluriformes	Schilbeidae	*Schilbe*	0.98	19
Siluriformes	Siluridae	*Kryptopterus*	0.91	19
Siluriformes	Siluridae	*Silurus*	1.21	15
Stomiiformes	Stomiidae	*Idiacanthus*	1.3	3
Symbranchiformes	Mastacembelidae	*Macrognathus*	0.78	13
Symbranchiformes	Synbranchidae	*Monopterus*	0.74	9
Tetraodontiformes	Balistidae	*Abalistes*	0.64	1
Tetraodontiformes	Balistidae	*Balistapus*	0.71	1
Tetraodontiformes	Balistidae	*Balistes*	0.55	7
Tetraodontiformes	Balistidae	*Balistoides*	0.68	2
Tetraodontiformes	Balistidae	*Melichthys*	0.7	3
Tetraodontiformes	Balistidae	*Sufflamen*	0.64	5
Tetraodontiformes	Balistidae	*Xanthichthys*	0.74	5
Tetraodontiformes	Diodontidae	*Cyclichthys*	0.86	6
Tetraodontiformes	Diodontidae	*Diodon*	0.81	5
Tetraodontiformes	Molidae	*Mola*	0.91	2
Tetraodontiformes	Monacanthidae	*Aluterus*	0.64	6
Tetraodontiformes	Monacanthidae	*Cantherines*	0.58	11
Tetraodontiformes	Monacanthidae	*Monacanthus*	0.58	4
Tetraodontiformes	Monacanthidae	*Pseudomonacanthus*	0.43	3
Tetraodontiformes	Monacanthidae	*Stephanolepis*	0.64	5
Tetraodontiformes	Monacanthidae	*Thamnaconus*	0.56	13
Tetraodontiformes	Ostraciidae	*Acanthostracion*	0.99	4

Tetraodontiformes	Ostraciidae	*Lactophrys*	0.98	4
Tetraodontiformes	Tetraodontidae	*Arothron*	0.43	15
Tetraodontiformes	Tetraodontidae	*Canthigaster*	0.42	28
Tetraodontiformes	Tetraodontidae	*Chelonodon*	0.39	3
Tetraodontiformes	Tetraodontidae	*Lagocephalus*	0.44	11
Tetraodontiformes	Tetraodontidae	*Sphoeroides*	0.49	20
Tetraodontiformes	Tetraodontidae	*Takifugu*	0.41	21
Tetraodontiformes	Tetraodontidae	*Tetraodon*	0.47	23
Tetraodontiformes	Triacanthidae	*Tripodichthys*	0.51	3
Zeiformes	Oreosomatidae	*Neocyttus*	1.26	4
Zeiformes	Zeidae	*Zenopsis*	1.23	3

C-value are averaged from Gregory (2005), and species counts are from Froese and Pauly (2004)

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Appendix G

SOURCE PHYLOGENIES FOR ATHERINOMORPH SUPERTREE

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Appendix H

ACCOUNTS OF SEXUALLY SELECTED TRAITS IN SURVEYED SISTER TAXA.

Sister Taxa	Family	Genus	Species	Sexually Selected Traits	Reference
I	Anablepidae	*Anableps*	*microlepis*	none	Froese and Pauly 2004
I	Anablepidae	*Anableps*	*anableps*	none	Sanford 1995
I	Anablepidae	*Jenysia*	*alternimaculata*	none	Froese and Pauly 2004
I	Anablepidae	*Jenysia*	*eirmostigma*	none	Froese and Pauly 2004
I	Anablepidae	*Jenysia*	*lineata*	none	Froese and Pauly 2004
I	Anablepidae	*Jenysia*	*multidentata*	none	Froese and Pauly 2004

I	Anablepidae	*Jenysia*	*onca*	none	Froese and Pauly 2004
I	Anablepidae	*Jenysia*	*sanctaecatarinae*	none	Froese and Pauly 2004
I	Anablepidae	*Jenysia*	*unitaenia*	none	Froese and Pauly 2004
I	Anablepidae	*Jenysia*	*weitzmanni*	none	Froese and Pauly 2004
I	Anablepidae	*Oxyzygonextes*	*dovii*	none	Froese and Pauly 2004
I	Poeciliidae	*Alfaro*	*cultitrans*	none	Sanford 1995
I	Poeciliidae	*Aplocheilichthys*	*katangae*	males brighter	Sanford 1995
I	Poeciliidae	*Belonesox*	*belizanus*	none	Smith 1997
I	Poeciliidae	*Brachyrhaphis*	*episcopi*	males brighter	Sanford 1995
I	Poeciliidae	*Cnesterodon*	*carnegiei*	none	Sanford 1995
I	Poeciliidae	*Gambusia*	*manni*	brighter	Bohlke et al. 1993

I	Poeciliidae	*Gambusia*	*hurtadoi*	none	Breder and Rosen1966
I	Poeciliidae	*Gambusia*	*puncticulata*	none	Breder and Rosen1966
I	Poeciliidae	*Gambusia*	*affinis*	none	Goldstein et al. 2000
I	Poeciliidae	*Gambusia*	*amistadensis*	none	Goldstein et al. 2000
I	Poeciliidae	*Gambusia*	*gagei*	none	Goldstein et al. 2000
I	Poeciliidae	*Gambusia*	*geiseri*	none	Goldstein et al. 2000
I	Poeciliidae	*Gambusia*	*georgei*	none	Goldstein et al. 2000
I	Poeciliidae	*Gambusia*	*heterochir*	none	Goldstein et al. 2000
I	Poeciliidae	*Gambusia*	*holbrooki*	none	Goldstein et al. 2000
I	Poeciliidae	*Gambusia*	*nobilis*	none	Goldstein et al. 2000
I	Poeciliidae	*Gambusia*	*rhizophorae*	none	Goldstein et al. 2000

I	Poeciliidae	*Gambusia*	*senilis*	none	Goldstein et al. 2000
I	Poeciliidae	*Gambusia*	*regani*	none	Sanford 1995
I	Poeciliidae	*Gambusia*	*vittata*	males brighter	Sanford 1995
I	Poeciliidae	*Girardinius*	*falcatus*	none	Sanford 1995
I	Poeciliidae	*Girardinius*	*metallicus*	none	Sanford 1995
I	Poeciliidae	*Heterandria*	*formosa*	brighter	Goldstein et al. 2000
I	Poeciliidae	*Heterandria*	*bimaculata*	none	Sanford 1995
I	Poeciliidae	*Limia*	*melanogaster*	males brighter	Sanford 1995
I	Poeciliidae	*Limia*	*vittata*	males brighter	Sanford 1995
I	Poeciliidae	*Limia*	*zonata*	none	Sanford 1995
I	Poeciliidae	*Mollienesia*	*sphenops*	none	Blumer 1982

I	Poeciliidae	*Phallichthys*	*amates*	bars darker	Froese and Pauly 2004
I	Poeciliidae	*Phalloceros*	*caudacancaudi-maculatusa*	none	Sandford 1995
I	Poeciliidae	*Poecilia*	*reticulata*	brighter, longer fins	Blumer 1982
I	Poeciliidae	*Poecilia*	*sphenops*	larger dorsal	Blumer 1982
I	Poeciliidae	*Poecilia*	*latipinna*	sailfin	Goldstein et al. 2000
I	Poeciliidae	*Poecilia*	*mexicana*	none	Goldstein et al. 2000
I	Poeciliidae	*Poecilia*	*caucana*	none	Sanford 1995
I	Poeciliidae	*Poecilia*	*sphenops*	elongate fins	Sanford 1995
I	Poeciliidae	*Poecilia*	*velifera*	males have sail	Sanford 1995
I	Poeciliidae	*Poecilia*	*vivipara*	none	Sanford 1995
I	Poeciliidae	*Poeciliopsis*	*occidentalis*	brighter	Goldstein et al. 2000

I	Poeciliidae	*Poeciliopsis*	*gracilis*	none	Sanford 1995
I	Poeciliidae	*Priapella*	*compressa*	none	Sanford 1995
I	Poeciliidae	*Priapella*	*intermedia*	none	Sanford 1995
I	Poeciliidae	*Procatopus*	*aberrans*	males brighter	Sanford 1995
I	Poeciliidae	*Procatopus*	*similis*	males brighter	Sanford 1995
I	Poeciliidae	*Quintana*	*atrizona*	none	Sanford 1995
I	Poeciliidae	*Xiphophorus*	*alvarezi*	sword, dark caudal	Breder and Rosen 1966, Meyer 1997
I	Poeciliidae	*Xiphophorus*	*cortezi*	sword, dark caudal	Breder and Rosen 1966, Meyer 1997
I	Poeciliidae	*Xiphophorus*	*nezahualcoyotl*	sword, dark caudal	Breder and Rosen 1966, Meyer 1997
I	Poeciliidae	*Xiphophorus*	*nigrensis*	sword	Breder and Rosen 1966,

					Meyer 1997
I	Poeciliidae	*Xiphophorus*	*helleri*	sword	Sanford 1995
I	Poeciliidae	*Xiphophorus*	*maculatus*	none	Sanford 1995
I	Poeciliidae	*Xiphophorus*	*milleri*	none	Sanford 1995
I	Poeciliidae	*Xiphophorus*	*pygmaeus*	sword	Sanford 1995
I	Poeciliidae	*Xiphophorus*	*variatus*	none	Sanford 1995
I	Poeciliidae	*Xiphophorus*	*xiphidium*	sword	Sanford 1995
II	Profundulidae	*Profundulus*	*candalarius*	none mentioned	Froese and Pauly 2004
II	Profundulidae	*Profundulus*	*hildebranchi*	none mentioned	Froese and Pauly 2004
II	Profundulidae	*Profundulus*	*labialis*	none mentioned	Froese and Pauly 2004
II	Profundulidae	*Profundulus*	*punctatus*	males brighter	Froese and Pauly 2004
II	Profundulidae	*Profundulus*	*quatemalensis*	none	Froese and

				mentioned	Pauly 2004
II	Goodeidae	*Ameca*	*splendens*	males brighter	Sanford 1995
II	Goodeidae	*Characodon*	*lateralis*	males brighter	Sanford 1995
II	Goodeidae	*Crenichthys*	*baileyi*	none	Goldstein et al. 2000
II	Goodeidae	*Crenichthys*	*nevadae*	males brighter	Goldstein et al. 2000
II	Goodeidae	*Empetrichthys*	*latos*	males brighter	Goldstein et al. 2000
II	Goodeidae	*Ilyodon*	*whitei*	males brighter	Sandford 1995
II	Goodeidae	*Xenotoca*	*eiseni*	males brighter	Sandford 1995
II	Goodeidae	*Xenotoca*	*variata*	males brighter	Sandford 1995
II	Goodeidae	*Zoogoneticus*	*tequila*	darker	Webb and Miller 1998
III	Bedotiidae	*Bedotia*	*madagascariensis*	none	Froese and Pauly 2004
III	Bedotiidae	*Bedotia*	*marojejy*	none	Froese and

					Pauly 2004
III	Bedotiidae	*Bedotia*	*masoala*	males mottled	Froese and Pauly 2004
III	Bedotiidae	*Bedotia*	*geayi*	males brighter	Sanford 1995
III	Bedotiidae	*Rheocles*	*alaotrensis*	none	Froese and Pauly 2004
III	Bedotiidae	*Rheocles*	*derhami*	males brighter	Froese and Pauly 2004
III	Bedotiidae	*Rheocles*	*lateralis*	none	Froese and Pauly 2004
III	Bedotiidae	*Rheocles*	*pellegrini*	none	Froese and Pauly 2004
III	Bedotiidae	*Rheocles*	*sikorae*	none	Froese and Pauly 2004
III	Bedotiidae	*Rheocles*	*vatosoa*	none	Froese and Pauly 2004
III	Bedotiidae	*Rheocles*	*wrightae*	none	Froese and Pauly 2004
III	Melanotaeniidae	*Chilatherina*	*axelrodi*	males brighter	Sanford 1995
III	Melanotaeniidae	*Chilatherina*	*bleheri*	males	

				brighter	Sanford 1995
III	Melanotaeniidae	*Chilatherina*	*fasciata*	males brighter	Sanford 1995
III	Melanotaeniidae	*Chilatherina*	*sentaniensis*	males brighter	Sanford 1995
III	Melanotaeniidae	*Glossolepis*	*incisus*	males brighter	Sanford 1995
III	Melanotaeniidae	*Glossolepis*	*maculosus*	none	Sanford 1995
III	Melanotaeniidae	*Glossolepis*	*multissquammatus*	males brighter	Sanford 1995
III	Melanotaeniidae	*Glossolepis*	*wanamensis*	males brighter, longer fins	Sanford 1995
III	Melanotaeniidae	*Melanotaenia*	*affinis*	males brighter	Sanford 1995
III	Melanotaeniidae	*Melanotaenia*	*boesemani*	males brighter	Sanford 1995
III	Melanotaeniidae	*Melanotaenia*	*lacustris*	males brighter	Sanford 1995
III	Melanotaeniidae	*Melanotaenia*	*maccullochi*	none	Sanford 1995

III	Melanotaeniidae	*Melanotaenia*	*nigrans*	none	Sanford 1995
III	Melanotaeniidae	*Melanotaenia*	*parkinsoni*	none	Sanford 1995
III	Melanotaeniidae	*Melanotaenia*	*splendida*	none	Sanford 1995
III	Melanotaeniidae	*Melanotaenia*	*trifasciata*	males brighter	Sanford 1995
IV	Beloniidae	*Ablennes*	*hians*	none	Bohlke et al. 1993
IV	Beloniidae	*Platybelone*	*argulus*	none	Bohlke et al. 1993
IV	Beloniidae	*Strongylura*	*notata*	none	Bohlke et al. 1993
IV	Beloniidae	*Strongylura*	*timicu*	none	Bohlke et al. 1993
IV	Beloniidae	*Strongylura*	*marina*	none	Boschung 2004
IV	Beloniidae	*Tylosurus*	*acus*	none	Bohlke et al. 1993
IV	Beloniidae	*Tylosurus*	*crocodilus*	none	Bohlke et al. 1993

IV	Beloniidae	*Xenentodon*	*cancila*	black-edged dorsal	Sanford 1995
IV	Scomberesocidae	*Cololabis*	*adocetus*	none	Froese and Pauly 2004
IV	Scomberesocidae	*Cololabis*	*saira*	none	Froese and Pauly 2004
IV	Scomberesocidae	*Scomberesox*	*saurus*	none	Robins and Ray 1986
IV	Scomberesocidae	*Scomberesox*	*simulans*	none	Froese and Pauly 2004
IX	Alestiidae	*Micralestes*	*occidentalis*	brighter	Paysan 1975
IX	Alestiidae	*Lepidarchus*	*adonis*	darker	Sanford 1995
IX	Alestiidae	*Ladigesia*	*roloffi*	longer fins	Sanford 1995
IX	Alestiidae	*Alestes*	*baremoze*	longer fins	Froese and Pauly 2004
IX	Alestiidae	*Brycinus*	*longipinnis*	longer fins	Paysan 1975
IX	Alestiidae	*Micralestes*	*interruptus*	longer fins	Sanford 1995

IX	Alestiidae	*Micralestes*	*eburneensis*	longer fins	Froese and Pauly 2004
IX	Alestiidae	*Phenacogrammus*	*interruptus*	longer fins, brighter	Riehl and Baensch 1989
IX	Alestiidae	*Alestes*	*emberri*	none	Paysan 1975
IX	Alestiidae	*Alestes*	*dentex*	none	Froese and Pauly 2004
IX	Alestiidae	*Alestes*	*grandisquammis*	none	Froese and Pauly 2004
IX	Alestiidae	*Alestopetersius*	*caudalis*	none	Froese and Pauly 2004
IX	Alestiidae	*Arnoldichthys*	*spiliopterus*	none	Axelrod and Schultz 1983
IX	Alestiidae	*Bathyaeiops*	*caudomaculatus*	none	Froese and Pauly 2004
IX	Alestiidae	*Brycinus*	*imberbi*	none	Riehl and Baensch 1989
IX	Alestiidae	*Brycinus*	*affinis*	none	Froese and Pauly 2004
IX	Alestiidae	*Brycinus*	*ferox*	none	Froese and Pauly 2004

IX	Alestiidae	*Brycinus*	*jacksonii*	none	Froese and Pauly 2004
IX	Alestiidae	*Brycinus*	*lateralis*	none	Froese and Pauly 2004
IX	Alestiidae	*Brycinus*	*macrolepidotus*	none	Froese and Pauly 2004
IX	Alestiidae	*Brycinus*	*sadleri*	none	Froese and Pauly 2004
IX	Alestiidae	*Hydrocynus*	*brevis*	none	Froese and Pauly 2004
IX	Alestiidae	*Hydrocynus*	*vittatus*	none	Froese and Pauly 2004
IX	Alestiidae	*Micralestes*	*acutidens*	none	Riehl and Baensch 1989
IX	Alestiidae	*Micralestes*	*elongatus*	none	Froese and Pauly 2004
IX	Alestiidae	*Rhabdalestes*	*maunensis*	none	Froese and Pauly 2004
IX	Characidae	*Aphyocharax*	*alburnus*	none	Riehl and Baensch 1989
IX	Characidae	*Aphyocharax*	*anisitsi*	longer fins	Sanford 1995

IX	Characidae	*Astyanax*	*bimaculatus*	brighter	Riehl and Baensch 1989
IX	Characidae	*Astyanax*	*fasciatus*	none	Sanford 1995
IX	Characidae	*Astyanax*	*mutator*	brighter	Axelrod and Schultz 1983
IX	Characidae	*Axelrodia*	*riesei*	none	Riehl and Baensch 1989
IX	Characidae	*Axelrodia*	*stigmatias*	brighter	Sanford 1995
IX	Characidae	*Boehlkea*	*fredcochii*	none	Sanford 1995
IX	Characidae	*Brachychalcinus*	*orbicularus*	none	Paysan 1975
IX	Characidae	*Brycon*	*falcatus*	none	Riehl and Baensch 1989
IX	Characidae	*Bryconops*	*affinis*	brighter	Axelrod and Schultz 1983
IX	Characidae	*Bryconops*	*melanurus*	none	Sanford 1995
IX	Characidae	*Carlastyanax*	*aurocaudatus*	none	Riehl and Baensch 1989

IX	Characidae	*Catoprion*	*mento*	none	Paysan 1975
IX	Characidae	*Chalceus*	*erythrurus*	brighter	Axelrod and Schultz 1983
IX	Characidae	*Characidium*	*fasciatum*	brighter	Sanford 1995
IX	Characidae	*Characidium*	*rachovii*	brighter	Sanford 1995
IX	Characidae	*Charax*	*gibbosus*	none	Axelrod and Schultz 1983
IX	Characidae	*Charax*	*stenopterys*	none	Axelrod and Schultz 1983
IX	Characidae	*Cheirodon*	*kriegi*	none	Sanford 1995
IX	Characidae	*Cheirodon*	*parahybae*	longer fins	Riehl and Baensch 1989
IX	Characidae	*Colossoma*	*macropomum*	none	Axelrod and Schultz 1983
IX	Characidae	*Corynopoma*	*riisei*	longer fins	Sanford 1995
IX	Characidae	*Creagrutus*	*beni*	brighter	Sanford 1995

IX	Characidae	*Crenuchus*	*spilururs*	longer fins	Paysan 1975
IX	Characidae	*Ctenobrycon*	*spilurus*	brighter	Paysan 1975
IX	Characidae	*Elachocharax*	*pulcher*	none	Sanford 1995
IX	Characidae	*Exodon*	*paradoxus*	longer fins	Axelrod and Schultz 1983
IX	Characidae	*Gephyrocharax*	*atrocaudatus*	brighter	Axelrod and Schultz 1983
IX	Characidae	*Glandulocauda*	*inequalis*	none	Axelrod and Schultz 1983
IX	Characidae	*Gymnocorymbus*	*bondi*	none	Sanford 1995
IX	Characidae	*Gymnocorymbus*	*ternetzi*	longer fins, brighter	Riehl and Baensch 1989
IX	Characidae	*Gymnocorymbus*	*thayeri*	longer fins	Riehl and Baensch 1989
IX	Characidae	*Hasemannia*	*nana*	brighter	Sanford 1995
IX	Characidae	*Hemibrycon*	*guppyi*	none	Axelrod and Schultz 1983

IX	Characidae	*Hemigrammus*	*armstrongi*	none	Paysan 1975
IX	Characidae	*Hemigrammus*	*bleheri*	brighter	Sanford 1995
IX	Characidae	*Hemigrammus*	*erythrozonus*	none	Sanford 1995
IX	Characidae	*Hemigrammus*	*gracilis*	brighter	Axelrod and Schultz 1983
IX	Characidae	*Hemigrammus*	*hyanuary*	longer fins	Paysan 1975
IX	Characidae	*Hemigrammus*	*ocellifer*	brighter	Mills 1988
IX	Characidae	*Hemigrammus*	*pulcher*	brighter	Paysan 1975
IX	Characidae	*Hemigrammus*	*rhondostomus*	longer fins	Paysan 1975
IX	Characidae	*Hemigrammus*	*rodwayi*	brighter	Paysan 1975
IX	Characidae	*Hemigrammus*	*ulreyi*	brighter	Sanford 1995
IX	Characidae	*Hemigrammus*	*unilineatus*	none	Paysan 1975

IX	Characidae	*Hyphessobrycon*	*anisitsi*	brighter	Paysan 1975
IX	Characidae	*Hyphessobrycon*	*bentosi*	longer fins	Paysan 1975
IX	Characidae	*Hyphessobrycon*	*bifasciatus*	longer fin	Paysan 1975
IX	Characidae	*Hyphessobrycon*	*callistus*	longer fins	Mills and Lambert 2004
IX	Characidae	*Hyphessobrycon*	*eos*	brighter	Axelrod and Schultz 1983
IX	Characidae	*Hyphessobrycon*	*erythrostoma*	longer fins, brighter	Mills 1988
IX	Characidae	*Hyphessobrycon*	*flammeus*	brighter	Sanford 1995
IX	Characidae	*Hyphessobrycon*	*griemi*	none	Paysan 1975
IX	Characidae	*Hyphessobrycon*	*herbertaxelrodi*	none	Paysan 1975
IX	Characidae	*Hyphessobrycon*	*heterhabdus*	brighter	Mills 1988
IX	Characidae	*Hyphessobrycon*	*loretoensis*	none	Sanford 1995

IX	Characidae	*Hyphessobrycon*	*macrolepidotus*	none	Paysan 1975
IX	Characidae	*Hyphessobrycon*	*megalopterus*	longer fins, brighter	Mills 1988
IX	Characidae	*Hyphessobrycon*	*pulchripinnis*	darker	Paysan 1975
IX	Characidae	*Hyphessobrycon*	*roseus*	longer fins	Paysan 1975
IX	Characidae	*Hyphessobrycon*	*scholzei*	none	Paysan 1975
IX	Characidae	*Hyphessobrycon*	*serpae*	brighter	Mills and Lambert 2004
IX	Characidae	*Hyphessobrycon*	*socolfi*	darker	Sanford 1995
IX	Characidae	*Hyphessobrycon*	*sweglesi*	brighter	Sanford 1995
IX	Characidae	*Hyphessobrycon*	*vilmae*	none	Paysan 1975
IX	Characidae	*Iguanodectes*	*spilarius*	longer fins	Sanford 1995
IX	Characidae	*Inpaichthys*	*kerri*	brighter	Sanford 1995

IX	Characidae	*Metynnis*	*hypsaucher*	none	Paysan 1975
IX	Characidae	*Metynnis*	*lippinocottianus*	longer fins	Paysan 1975
IX	Characidae	*Metynnis*	*maculata*	none	Axelrod and Schultz 1983
IX	Characidae	*Mimagoniates*	*barberi*	longer fins	Paysan 1975
IX	Characidae	*Mimagoniates*	*microlepis*	longer fins	Axelrod and Schultz 1983
IX	Characidae	*Moenkhausia*	*oligolepis*	none	Axelrod and Schultz 1983
IX	Characidae	*Moenkhausia*	*pitteri*	longer fins, brighter	Sanford 1995
IX	Characidae	*Moenkhausia*	*sanctaefilomenae*	none	Sanford 1995
IX	Characidae	*Myleus*	*arnoldi*	none	Paysan 1975
IX	Characidae	*Myleus*	*schultzei*	longer fins	Paysan 1975
IX	Characidae	*Mylosoma*	*aureum*	none	Axelrod and Schultz 1983

IX	Characidae	*Mylosoma*	*duriventris*	none	Paysan 1975
IX	Characidae	*Nematobrycon*	*palmeri*	longer fins, brighter	Sanford 1995
IX	Characidae	*Paracheirodon*	*axelrodi*	none	Sanford 1995
IX	Characidae	*Paracheirodon*	*innesi*	brighter	Sanford 1995
IX	Characidae	*Paracheirodon*	*simulans*	none	Sanford 1995
IX	Characidae	*Paragoniates*	*alburnus*	none	Riehl and Baensch 1989
IX	Characidae	*Petitella*	*georgiae*	brighter	Sanford 1995
IX	Characidae	*Phenagoniates*	*macrolepis*	brighter	Riehl and Baensch 1989
IX	Characidae	*Phoxinopsis*	*typicus*	brighter	Axelrod and Schultz 1983
IX	Characidae	*Prionobrama*	*filigera*	longer fins, brighter	Riehl and Baensch 1989
IX	Characidae	*Pristella*	*maxellaris*	brighter	Mills and Lambert 2004

IX	Characidae	*Pseudocorynopoma*	*doriae*	longer fins	Riehl and Baensch 1989
IX	Characidae	*Pygocentrus*	*nallereri*	none	Paysan 1975
IX	Characidae	*Pygocentrus*	*piraya*	none	Paysan 1975
IX	Characidae	*Roeboides*	*dayi*	longer fins	Riehl and Baensch 1989
IX	Characidae	*Roeboides*	*guatemalensis*	longer fins	Paysan 1975
IX	Characidae	*Serrasalmus*	*rhmbeus*	none	Paysan 1975
IX	Characidae	*Tetragonopterus*	*chalceus*	longer fins	Paysan 1975
IX	Characidae	*Thayeria*	*boehlkei*	none	Sanford 1995
IX	Characidae	*Thayeria*	*obliqua*	none	Sanford 1995
IX	Characidae	*Thayeria*	*sanctae-mariae*	none	Paysan 1975
IX	Characidae	*Triportheus*	*angulatus*	none	Sanford 1995

IX	Characidae	*Triportheus*	*elongatus*	none	Paysan 1975
IX	Characidae	*Tyttocharax*	*cochui*	none	Paysan 1975
IX	Characidae	*Xenagoniates*	*bondi*	none	Riehl and Baensch 1989
IX	Characidae	*Bryconamericus*	*mennii*	brighter	Miquelarena et al 2002
IX	Characidae	*Bryconamericus*	*rubropictus*	brighter	Brage 2000
IX	Characidae	*Gephyrocharax*	*venezuelae*	brighter	Bonilla and Lopez 1997
IX	Characidae	*Astyanax*	*ojiara*	longer fins	Azpeliculeta and Garcia 2000
IX	Characidae	*Bryconamericus*	*turiuba*	none	Langeani et al. 2005
IX	Characidae	*Gymnocharacinus*	*bergi*	none	Cussac and Ortubay 2002
IX	Characidae	*Characidium*	*vestigipinne*	brighter	Buckup and Hahn 2000
IX	Characidae	*Bryconamericus*	*eigenmanni*	longer fins	Miquelarena and Aquino 1999

IX	Characidae	*Kolpotocheirodon*	*figueiredoi*	longer fins	Malabarba et al. 2004
IX	Characidae	*Bryconamericus*	*iheringii*	longer fins	Lampert et al. 2004
IX	Characidae	*Moenkhausia*	*intermedia*	none	Hojo et al. 2004
IX	Characidae	*Bryconamericus*	*pectinatus*	brighter	Vari and Siebert 1990
V	Balistidae	*Abalistes*	*stellaris*	none	Froese and Pauly 2004
V	Balistidae	*Abalistes*	*stellatus*	none	Smith and Heemstra 1986
V	Balistidae	*Balistapus*	*vetula*	none	Smith and Heemstra 1986
V	Balistidae	*Balistoides*	*virescens*	none	Smith and Heemstra 1986
V	Balistidae	*Canthidermis*	*maculatus*	none	Smith and Heemstra 1986
V	Balistidae	*Melichthys*	*indicus*	none	Smith and Heemstra 1986
V	Balistidae	*Melichthys*	*vidua*	none	Smith and Heemstra 1986

V	Balistidae	*Melichthys*	*niger*	none	Smith and Heemstra 1986
V	Balistidae	*Odonus*	*niger*	none	Smith and Heemstra 1986
V	Balistidae	*Pseudobalistes*	*flavimarginatus*	none	Smith and Heemstra 1986
V	Balistidae	*Pseudobalistes*	*fuscus*	none	Smith and Heemstra 1986
V	Balistidae	*Rhinecanthus*	*aculeatus*	none	Smith and Heemstra 1986
V	Balistidae	*Rhinecanthus*	*rectacngulus*	none	Smith and Heemstra 1986
V	Balistidae	*Sufflamen*	*bursa*	none	Smith and Heemstra 1986
V	Balistidae	*Sufflamen*	*chrysopterus*	none	Smith and Heemstra 1986
V	Balistidae	*Sufflamen*	*fraenatus*	none	Smith and Heemstra 1986
V	Balistidae	*Xanichthys*	*lineopunctatus*	none	Smith and Heemstra 1986
V	Balistidae	*Xenobalistes*	*punctatus*	none	Smith and Heemstra 1986

V	Monacanthidae	*Aluterus*	*monoceros*	none	Smith and Heemstra 1986
V	Monacanthidae	*Aluterus*	*scriptus*	none	Smith and Heemstra 1986
V	Monacanthidae	*Amanses*	*scopas*	none	Smith and Heemstra 1986
V	Monacanthidae	*Cantherhines*	*dumerilii*	bands in fins	Smith and Heemstra 1986
V	Monacanthidae	*Cantherhines*	*pardalis*	none	Smith and Heemstra 1986
V	Monacanthidae	*Monacanthus*	*tuckeri*	none	Froese and Pauly 2004
V	Monacanthidae	*Monacanthus*	*ciliatus*	spines	Froese and Pauly 2004
V	Monacanthidae	*Oxymonacanthus*	*longirostris*	longer fins	Froese and Pauly 2004
V	Monacanthidae	*Paraluteres*	*prionurus*	none	Smith and Heemstra 1986
V	Monacanthidae	*Paramonacanthus*	*japonicus*	longer fins	Froese and Pauly 2004
V	Monacanthidae	*Paramonacanthus*	*congalensis*	none	Smith and Heemstra 1986

V	Monacanthidae	*Paramonacanthus*	*otisensis*	none	Froese and Pauly 2004
V	Monacanthidae	*Paramonacanthus*	*choirocephalus*	none	Froese and Pauly 2004
V	Monacanthidae	*Paramonacanthus*	*barnardi*	stripes	Smith and Heemstra 1986
V	Monacanthidae	*Pervagor*	*spilosoma*	none	Froese and Pauly 2004
V	Monacanthidae	*Pervagor*	*nigrolineatus*	none	Froese and Pauly 2004
V	Monacanthidae	*Pervagor*	*melanocephalus*	spikes	Froese and Pauly 2004
V	Monacanthidae	*Pervagor*	*janthinosoma*	spikes	Froese and Pauly 2004
V	Monacanthidae	*Pervagor*	*aspricaudus*	spikes	Froese and Pauly 2004
V	Monacanthidae	*Pseudalutarius*	*nasicornis*	none	Smith and Heemstra 1986
V	Monacanthidae	*Rudarius*	*excelsus*	none	Froese and Pauly 2004
V	Monacanthidae	*Rudarius*	*minutus*	ocellus	Froese and Pauly 2004

V	Monacanthidae	*Stephanolepis*	*auratus*	none	Smith and Heemstra 1986
V	Monacanthidae	*Stephanolepis*	*auratus*	none	Froese and Pauly 2004
V	Monacanthidae	*Stephanolepis*	*setifer*	spikes	Froese and Pauly 2004
V	Monacanthidae	*Thamnaconus*	*modestoides*	brighter	Smith and Heemstra 1986
V	Monacanthidae	*Thamnaconus*	*arenaceus*	none	Smith and Heemstra 1986
V	Monacanthidae	*Thamnaconus*	*fajardoi*	none	Smith and Heemstra 1986
VI	Bothidae	*Arnaglossus capensis*	*brunneus*	eye stalks, longer fins	Froese and Pauly 2004
VI	Bothidae	*Arnaglossus capensis*	*oxyrhynchus*	eye stalks, longer fins	Froese and Pauly 2004
VI	Bothidae	*Arnaglossus capensis*	*tenuis*	none	Froese and Pauly 2004
VI	Bothidae	*Arnaglossus capensis*	*dalgleeshi*	none	Smith and Heemstra 1986
VI	Bothidae	*Bothus*	*ocellatus*	none	Froese and Pauly 2004

VI	Bothidae	*Bothus*	*mancus*	eye stalks, longer fins	Smith and Heemstra 1986
VI	Bothidae	*Bothus*	*myriaster*	eye stalks, longer fins	Smith and Heemstra 1986
VI	Bothidae	*Bothus*	*pantherinus*	eye stalks, longer fins	Smith and Heemstra 1986
VI	Bothidae	*Bothus*	*podas*	none	Smith and Heemstra 1986
VI	Bothidae	*Chascanopsetta*	*lugubris*	none	Smith and Heemstra 1986
VI	Bothidae	*Crossorhombus*	*howensis*	eye stalks, longer fins	Froese and Pauly 2004
VI	Bothidae	*Crossorhombus*	*valderostratus*	eye stalks, longer fins	Smith and Heemstra 1986
VI	Bothidae	*Engyprosopon*	*grandisquama*	eye stalks, longer fins	Smith and Heemstra 1986
VI	Bothidae	*Engyprosopon*	*smithi*	none	Smith and Heemstra 1986
VI	Bothidae	*Engyprosopon*	*macrolepis*	eye stalks, longer fins	Smith and Heemstra 1986
VI	Bothidae	*Engyprosopon*	*natalensis*	eye stalks, longer fins	Smith and Heemstra 1986

VI	Bothidae	*Grammatobothus*	*polyophthalmus*	darker	Froese and Pauly 2004
VI	Bothidae	*Laeops*	*parviceps*	none	Froese and Pauly 2004
VI	Bothidae	*Laeops*	*pectoralis*	none	Smith and Heemstra 1986
VI	Bothidae	*Laeops*	*nigromaculatus*	none	Smith and Heemstra 1986
VI	Bothidae	*Laeops*	*natalensis*	none	Smith and Heemstra 1986
VI	Bothidae	*Lophonectes*	*gallus*	longer fins	Froese and Pauly 2004
VI	Bothidae	*Mancopsetta*	*milfordi*	none	Smith and Heemstra 1986
VI	Bothidae	*Neolaeops*	*microphthalmus*	none	Smith and Heemstra 1986
VI	Bothidae	*Psettina*	*gigantea*	none	Froese and Pauly 2004
VI	Bothidae	*Psettina*	*brevitictis*	none	Smith and Heemstra 1986
VI	Bothidae	*Pseudorhombus*	*elevatus*	none	Smith and Heemstra 1986

VI	Bothidae	*Pseudorhombus*	*natalensis*	none	Smith and Heemstra 1986
VI	Bothidae	*Pseudorhombus*	*arsius*	none	Smith and Heemstra 1986
VI	Bothidae	*Syacium*	*micurum*	eye stalks, longer fins	Smith and Heemstra 1986
VI	Bothidae	*Taenopsetta*	*ocellata*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Cynoglossus*	*acutirostris*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Cynoglossus*	*blilineatus*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Cynoglossus*	*cynoglossus*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Cynoglossus*	*heterolepis*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Cynoglossus*	*lachneri*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Cynoglossus*	*lingua*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Cynoglossus*	*acaudatus*	none	Smith and Heemstra 1986

VI	Cynoglossidae	*Cynoglossus*	*attebuatus*	none	Smith and Heemstra 1986
VI	Cynoglossidae	*Cynoglossus*	*capensis*	none	Smith and Heemstra 1986
VI	Cynoglossidae	*Cynoglossus*	*durbanensis*	none	Smith and Heemstra 1986
VI	Cynoglossidae	*Cynoglossus*	*gilchristi*	none	Smith and Heemstra 1986
VI	Cynoglossidae	*Cynoglossus*	*lachneri*	none	Smith and Heemstra 1986
VI	Cynoglossidae	*Cynoglossus*	*lida*	none	Smith and Heemstra 1986
VI	Cynoglossidae	*Cynoglossus*	*marleyi*	none	Smith and Heemstra 1986
VI	Cynoglossidae	*Cynoglossus*	*zanzibarensis*	none	Smith and Heemstra 1986
VI	Cynoglossidae	*Paraplagusia*	*blineata*	none	Smith and Heemstra 1986
VI	Cynoglossidae	*Symphurus*	*microlepis*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Symphurus*	*oligomerus*	none	Froese and Pauly 2004

VI	Cynoglossidae	*Symphurus*	*ommaspilus*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Symphurus*	*plagusia*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Symphurus*	*prolatinaris*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Symphurus*	*strictus*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Symphurus*	*trewavasae*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Symphurus*	*undecimplerus*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Symphurus*	*varius*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Symphurus*	*williamsi*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Symphurus*	*melasmatotheca*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Symphurus*	*leei*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Symphurus*	*jenynsi*	none	Froese and Pauly 2004

VI	Cynoglossidae	*Symphurus*	*gorgonae*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Symphurus*	*ginsburgi*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Symphurus*	*fasciolaris*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Symphurus*	*elongatus*	none	Froese and Pauly 2004
VI	Cynoglossidae	*Symphurus*	*ocellatus*	none	Smith and Heemstra 1986
VI	Cynoglossidae	*Symphurus*	*strictus*	none	Smith and Heemstra 1986
VI	Cynoglossidae	*Symphurus*	*variegatus*	none	Smith and Heemstra 1986
VII	Labridae	*Acantholabrus*	*palloni*	none	Muus et al. 1999
VII	Labridae	*Anamphes*	*elegans*	none	Ayling and Cox 1982
VII	Labridae	*Anampses*	*cuvieri*	none	Axelros and Burgess 1987
VII	Labridae	*Anampses*	*meleagris*	bands on fins	Oliver 1977

VII	Labridae	*Anampses*	*caeruleopunctatus*	brighter	Smith and Heemstra 1986
VII	Labridae	*Anampses*	*lineatus*	none	Smith and Heemstra 1986
VII	Labridae	*Anampses*	*meleagris*	brighter	Smith and Heemstra 1986
VII	Labridae	*Anchichoerops*	*nataliensis*	none	Smith and Heemstra 1986
VII	Labridae	*Bodianus*	*bilunulatus*	none	Axelros and Burgess 1987
VII	Labridae	*Bodianus*	*vulpinus*	brighter	Ayling and Cox 1982
VII	Labridae	*Bodianus*	*diptotaenia*	brighter	Grove and Lavenberg 1997
VII	Labridae	*Bodianus*	*eclancheri*	brighter	Grove and Lavenberg 1997
VII	Labridae	*Bodianus*	*pulchellus*	none	Sanford 1995
VII	Labridae	*Bodianus*	*rufus*	none	Sanford 1995
VII	Labridae	*Bodianus*	*anthoides*	none	Smith and Heemstra 1986

VII	Labridae	*Bodianus*	*axillaris*	darker	Smith and Heemstra 1986
VII	Labridae	*Bodianus*	*diana*	none	Smith and Heemstra 1986
VII	Labridae	*Bodianus*	*leucostictus*	none	Smith and Heemstra 1986
VII	Labridae	*Bodianus*	*perdito*	none	Smith and Heemstra 1986
VII	Labridae	*Cheilinus*	*bimaculatus*	none	Axelros and Burgess 1987
VII	Labridae	*Cheilinus*	*chlorourus*	none	Smith and Heemstra 1986
VII	Labridae	*Cheilinus*	*diagrammus*	none	Smith and Heemstra 1986
VII	Labridae	*Cheilinus*	*oxycephalus*	none	Smith and Heemstra 1986
VII	Labridae	*Cheilinus*	*trilobatus*	none	Smith and Heemstra 1986
VII	Labridae	*Cheilinus*	*undulatus*	none	Smith and Heemstra 1986
VII	Labridae	*Cheilio*	*inermis*	brighter	Axelros and Burgess 1987

VII	Labridae	*Cheilio*	*inermis*	brighter	Smith and Heemstra 1986
VII	Labridae	*Choerodon*	*gymnoginys*	brighter	Smith and Heemstra 1986
VII	Labridae	*Cirrhilabrus*	*rubriventialis*	brighter	Sanford 1995
VII	Labridae	*Cirrhilabrus*	*exquisitus*	none	Smith and Heemstra 1986
VII	Labridae	*Clepticus*	*parrai*	none	Robins and Ray 1986
VII	Labridae	*Coris*	*flavovittata*	none	Axelros and Burgess 1987
VII	Labridae	*Coris*	*formosa*	none	Axelros and Burgess 1987
VII	Labridae	*Coris*	*sandageri*	brighter	Ayling and Cox 1982
VII	Labridae	*Coris*	*angulata*	none	Mills 1980
VII	Labridae	*Coris*	*gaimard*	none	Sanford 1995
VII	Labridae	*Coris*	*julis*	longer fins	Oliver 1977

VII	Labridae	*Coris*	*aygula*	brighter	Smith and Heemstra 1986
VII	Labridae	*Coris*	*caudimacula*	none	Smith and Heemstra 1986
VII	Labridae	*Ctenolabrus*	*exoletus*	none	Muus et al. 1999
VII	Labridae	*Ctenolabrus*	*rubestris*	none	Muus et al. 1999
VII	Labridae	*Cymolutes*	*praetextatus*	none	Smith and Heemstra 1986
VII	Labridae	*Decodon*	*puellaris*	none	Robins and Ray 1986
VII	Labridae	*Decodon*	*grandisquamis*	none	Smith and Heemstra 1986
VII	Labridae	*Doratonotus*	*megalepis*	longer fins	Robins and Ray 1986
VII	Labridae	*Epibulus*	*insidiator*	brighter	Smith and Heemstra 1986
VII	Labridae	*Gomphosus*	*coerulens*	brighter	Mills 1980
VII	Labridae	*Gomphosus*	*varius*	brighter	Sanford 1995

VII	Labridae	*Gomphosus*	*caeruleus*	brighter	Smith and Heemstra 1986
VII	Labridae	*Halichoeres*	*dispilius*	brighter	Grove and Lavenberg 1997
VII	Labridae	*Halichoeres*	*nicholsi*	brighter	Grove and Lavenberg 1997
VII	Labridae	*Halichoeres*	*notospilus*	none	Grove and Lavenberg 1997
VII	Labridae	*Halichoeres*	*bathyphilus*	none	Robins and Ray 1986
VII	Labridae	*Halichoeres*	*bivittatis*	brighter	Robins and Ray 1986
VII	Labridae	*Halichoeres*	*caudalis*	brighter	Robins and Ray 1986
VII	Labridae	*Halichoeres*	*cyanocephalus*	none	Robins and Ray 1986
VII	Labridae	*Halichoeres*	*garnoti*	darker	Robins and Ray 1986
VII	Labridae	*Halichoeres*	*maculipinna*	brighter	Robins and Ray 1986
VII	Labridae	*Halichoeres*	*pictus*	brighter	Robins and Ray 1986

VII	Labridae	*Halichoeres*	*poeyi*	darker	Robins and Ray 1986
VII	Labridae	*Halichoeres*	*radiatus*	none	Robins and Ray 1986
VII	Labridae	*Halichoeres*	*cosmetus*	none	Smith and Heemstra 1986
VII	Labridae	*Halichoeres*	*dussumieri*	none	Smith and Heemstra 1986
VII	Labridae	*Halichoeres*	*hortulanus*	brighter	Smith and Heemstra 1986
VII	Labridae	*Halichoeres*	*iridus*	none	Smith and Heemstra 1986
VII	Labridae	*Halichoeres*	*lapillus*	red	Smith and Heemstra 1986
VII	Labridae	*Halichoeres*	*marginatus*	brighter	Smith and Heemstra 1986
VII	Labridae	*Halichoeres*	*nebulosus*	brighter	Smith and Heemstra 1986
VII	Labridae	*Halichoeres*	*scapularis*	brighter	Smith and Heemstra 1986
VII	Labridae	*Hemigymnus*	*fasciatus*	none	Axelros and Burgess 1987

VII	Labridae	*Hemigymnus*	*melapterus*	none	Smith and Heemstra 1986
VII	Labridae	*Hemipteronotus*	*pentadactylus*	none	Axelros and Burgess 1987
VII	Labridae	*Hemipteronotus*	*taeniurus*	none	Axelros and Burgess 1987
VII	Labridae	*Hologynosus*	*doliatus*	brighter	Smith and Heemstra 1986
VII	Labridae	*Iniistius*	*niger*	none	Axelros and Burgess 1987
VII	Labridae	*Labridichthys*	*unileatus*	brighter	Smith and Heemstra 1986
VII	Labridae	*Labroides*	*phthirophagus*	none	Axelros and Burgess 1987
VII	Labridae	*Labroides*	*bicolor*	brighter	Smith and Heemstra 1986
VII	Labridae	*Labroides*	*dimidiatus*	none	Smith and Heemstra 1986
VII	Labridae	*Labrus*	*bergylta*	none	Muus et al. 1999
VII	Labridae	*Labrus*	*bimaculatus*	brighter	Muus et al. 1999

VII	Labridae	*Labrus*	*viridis*	brighter	Oliver 1977
VII	Labridae	*Lachnolaimus*	*maximus*	darker	Robins and Ray 1986
VII	Labridae	*Macropharyngodon*	*meleagris*	none	Axelros and Burgess 1987
VII	Labridae	*Macropharyngodon*	*bipartitus*	brighter	Smith and Heemstra 1986
VII	Labridae	*Macropharyngodon*	*cyanoguttatus*	none	Smith and Heemstra 1986
VII	Labridae	*Macropharyngodon*	*vivienae*	brighter	Smith and Heemstra 1986
VII	Labridae	*Novaculichthys*	*taeniorus*	none	Sanford 1995
VII	Labridae	*Novaculichthys*	*macrolepidatus*	none	Smith and Heemstra 1986
VII	Labridae	*Oxyjulis*	*californica*	none	Gilbert and Williams 2002
VII	Labridae	*Pseudocheilinus*	*evanidus*	none	Smith and Heemstra 1986
VII	Labridae	*Pseudocheilinus*	*octotaenia*	none	Smith and Heemstra 1986
VII	Labridae	*Pseudodax*	*moluccanus*	none	Smith and

					Heemstra 1986
VII	Labridae	*Pseudojuloides*	*elongatus*	blue lines	Ayling and Cox 1982
VII	Labridae	*Pseudojuloides*	*cerasinus*	brighter	Smith and Heemstra 1986
VII	Labridae	*Pseudolabrus*	*celidotus*	bands on fins	Ayling and Cox 1982
VII	Labridae	*Pseudolabrus*	*cinctus*	brighter	Ayling and Cox 1982
VII	Labridae	*Pseudolabrus*	*incriptus*	brighter	Ayling and Cox 1982
VII	Labridae	*Pseudolabrus*	*luculentus*	brighter	Ayling and Cox 1982
VII	Labridae	*Pseudolabrus*	*miles*	brighter	Ayling and Cox 1982
VII	Labridae	*Pseudolabrus*	*pucicola*	brighter	Ayling and Cox 1982
VII	Labridae	*Pterogogus*	*flagellifer*	darker	Smith and Heemstra 1986
VII	Labridae	*Pterogogus*	*pelycus*	none	Smith and Heemstra 1986
VII	Labridae	*Pterogogus*	*taeniops*	none	Smith and

					Heemstra 1986
VII	Labridae	*Semicossyphus*	*pulcher*	darker	Gilbert and Williams 2002
VII	Labridae	*Semicossyphus*	*darwini*	brighter	Grove and Lavenberg 1997
VII	Labridae	*Stethojulis*	*bandanensis*	brighter	Axelros and Burgess 1987
VII	Labridae	*Stethojulis*	*albovittata*	none	Smith and Heemstra 1986
VII	Labridae	*Stethojulis*	*interupta*	brighter	Smith and Heemstra 1986
VII	Labridae	*Stethojulis*	*strigivenila*	brighter	Smith and Heemstra 1986
VII	Labridae	*Symphodus*	*melops*	brighter	Muus et al. 1999
VII	Labridae	*Tautoga*	*onitis*	darker	Robins and Ray 1986
VII	Labridae	*Tautogolabrus*	*adspersus*	none	Robins and Ray 1986
VII	Labridae	*Thalossoma*	*ballieui*	none	Axelros and Burgess 1987
VII	Labridae	*Thalossoma*	*duperreyi*	brighter	Axelros and

					Burgess 1987
VII	Labridae	*Thalossoma*	*lucasanum*	brighter	Axelros and Burgess 1987
VII	Labridae	*Thalossoma*	*grammaticum*	none	Grove and Lavenberg 1997
VII	Labridae	*Thalossoma*	*bifasciatum*	brighter	Robins and Ray 1986
VII	Labridae	*Thalossoma*	*pavo*	none	Oliver 1977
VII	Labridae	*Thalossoma*	*amblycephalum*	brighter	Smith and Heemstra 1986
VII	Labridae	*Thalossoma*	*genivitattum*	brighter	Smith and Heemstra 1986
VII	Labridae	*Thalossoma*	*hardwicke*	none	Smith and Heemstra 1986
VII	Labridae	*Thalossoma*	*hebraicum*	brighter	Smith and Heemstra 1986
VII	Labridae	*Thalossoma*	*lunare*	brighter	Smith and Heemstra 1986
VII	Labridae	*Thalossoma*	*purpureum*	brighter	Smith and Heemstra 1986
VII	Labridae	*Thalossoma*	*quinquevittatum*	none	Smith and

					Heemstra 1986
VII	Labridae	*Thalossoma*	*trilobatum*	brighter	Smith and Heemstra 1986
VII	Labridae	*Xyrichthys*	*victori*	brighter	Grove and Lavenberg 1997
VII	Labridae	*Xyrichthys*	*martinicensis*	brighter	Robins and Ray 1986
VII	Labridae	*Xyrichthys*	*novacula*	none	Robins and Ray 1986
VII	Labridae	*Xyrichthys*	*splendens*	brighter	Robins and Ray 1986
VII	Labridae	*Xyrichthys*	*pavo*	none	Smith and Heemstra 1986
VII	Labridae	*Xyrichthys*	*pentadactylus*	red spots	Smith and Heemstra 1986
VII	Scaridae	*Bolbometopon*	*bicolor*	none	Oliver 1977
VII	Scaridae	*Bolbometopon*	*muricatum*	none	Smith and Heemstra 1986
VII	Scaridae	*Calotomus*	*carolinus*	brighter	Smith and Heemstra 1986
VII	Scaridae	*Calotomus*	*spinidens*	brighter	Smith and

					Heemstra 1986
VII	Scaridae	*Cryptotomus*	*roseus*	none	Robins and Ray 1986
VII	Scaridae	*Hipposcarus*	*harid*	brighter	Smith and Heemstra 1986
VII	Scaridae	*Leptoscarus*	*vaigiensis*	none	Smith and Heemstra 1986
VII	Scaridae	*Nicholsina*	*denticulata*	none	Grove and Lavenberg 1997
VII	Scaridae	*Nicholsina*	*usta*	none	Robins and Ray 1986
VII	Scaridae	*Scarus*	*taeniopterus*	brighter	Gilbert and Williams 2002
VII	Scaridae	*Scarus*	*compressus*	brighter	Grove and Lavenberg 1997
VII	Scaridae	*Scarus*	*perrico*	none	Grove and Lavenberg 1997
VII	Scaridae	*Scarus*	*coelestinus*	none	Robins and Ray 1986
VII	Scaridae	*Scarus*	*coeruleus*	none	Robins and Ray 1986
VII	Scaridae	*Scarus*	*guacamaia*	none	Robins and Ray

					1986
VII	Scaridae	*Scarus*	*vetula*	brighter	Robins and Ray 1986
VII	Scaridae	*Scarus*	*atrilunila*	brighter	Smith and Heemstra 1986
VII	Scaridae	*Scarus*	*caudofasciatus*	brighter	Smith and Heemstra 1986
VII	Scaridae	*Scarus*	*cyanascens*	none	Smith and Heemstra 1986
VII	Scaridae	*Scarus*	*enneacanthus*	none	Smith and Heemstra 1986
VII	Scaridae	*Scarus*	*festicus*	none	Smith and Heemstra 1986
VII	Scaridae	*Scarus*	*ghobban*	brighter	Smith and Heemstra 1986
VII	Scaridae	*Scarus*	*globiceps*	brighter	Smith and Heemstra 1986
VII	Scaridae	*Scarus*	*niger*	longer fins	Smith and Heemstra 1986
VII	Scaridae	*Scarus*	*psittacus*	brighter	Smith and Heemstra 1986
VII	Scaridae	*Scarus*	*rubroviolasceus*	brighter	Smith and

					Heemstra 1986
VII	Scaridae	*Scarus*	*russelli*	brighter	Smith and Heemstra 1986
VII	Scaridae	*Scarus*	*scaber*	brighter	Smith and Heemstra 1986
VII	Scaridae	*Scarus*	*sordicus*	brighter	Smith and Heemstra 1986
VII	Scaridae	*Scarus*	*tricolor*	brighter	Smith and Heemstra 1986
VII	Scaridae	*Sparisoma*	*viride*	none	Bohkle and Chaplin 1993
VII	Scaridae	*Sparisoma*	*aurofrenatum*	none	Hoese and Moore 1998
VII	Scaridae	*Sparisoma*	*atomarium*	brighter	Robins and Ray 1986
VII	Scaridae	*Sparisoma*	*chrysopterum*	brighter	Robins and Ray 1986
VII	Scaridae	*Sparisoma*	*radians*	brighter	Robins and Ray 1986
VII	Scaridae	*Sparisoma*	*rubripinne*	brighter	Robins and Ray 1986
VIII	Eleotridae	*Butis*	*butis*	none	

					Sanford 1995
VIII	Eleotridae	*Butis*	*melanostigma*	none	Smith and Heemstra 1986
VIII	Eleotridae	*Calumia*	*godeffroyi*	none	Smith and Heemstra 1986
VIII	Eleotridae	*Dormitator*	*latifrons*	none	Goldstein et al 2000
VIII	Eleotridae	*Dormitator*	*maculatus*	none	Goldstein et al 2000
VIII	Eleotridae	*Eleotrica*	*cableae*	none	Grove and Lavenberg 1997
VIII	Eleotridae	*Eleotris*	*ambilyopsis*	none	Bohkle and Chaplin 1993
VIII	Eleotridae	*Eleotris*	*picta*	none	Goldstein et al 2000
VIII	Eleotridae	*Eleotris*	*pisonis*	none	Goldstein et al 2000
VIII	Eleotridae	*Eleotris*	*africana*	none	Paysan 1975
VIII	Eleotridae	*Eleotris*	*marmorata*	longer fins	Riehl and Baensch 1989
VIII	Eleotridae	*Eleotris*	*fusca*	none	Smith and Heemstra 1986

VIII	Eleotridae	*Eleotris*	*mauritianus*	none	Smith and Heemstra 1986
VIII	Eleotridae	*Eleotris*	*melanosoma*	none	Smith and Heemstra 1986
VIII	Eleotridae	*Erotelis*	*smargdus*	none	Robins and Ray 1986
VIII	Eleotridae	*Gobiomorus*	*australis*	brighter	Allen 1989
VIII	Eleotridae	*Gobiomorus*	*dormitator*	none	Goldstein et al 2000
VIII	Eleotridae	*Gobiomorus*	*maculatus*	none	Grove and Lavenberg 1997
VIII	Eleotridae	*Gobiomorus*	*coxii*	brighter	Merrick and Schmida 1984
VIII	Eleotridae	*Grahamichthys*	*radiata*	none	Ayling and Cox 1982
VIII	Eleotridae	*Hypseleotris*	*aurea*	none	Allen 1989
VIII	Eleotridae	*Hypseleotris*	*galii*	darker	Allen 1989
VIII	Eleotridae	*Hypseleotris*	*ejuncida*	brighter	Merrick and Schmida 1984

VIII	Eleotridae	*Hypseleotris*	*kimberleyensis*	dark head	Merrick and Schmida 1984
VIII	Eleotridae	*Hypseleotris*	*klunzingeri*	red fins	Merrick and Schmida 1984
VIII	Eleotridae	*Hypseleotris*	*regalis*	longer fins	Merrick and Schmida 1984
VIII	Eleotridae	*Hypseleotris*	*compressa*	brighter	Sanford 1995
VIII	Eleotridae	*Hypseleotris*	*dayi*	none	Smith and Heemstra 1986
VIII	Eleotridae	*Kimberleyeleotris*	*notata*	none	Allen 1989
VIII	Eleotridae	*Mileringa*	*veritas*	none	Merrick and Schmida 1984
VIII	Eleotridae	*Mogurnda*	*adspersa*	bright spots	Allen 1989
VIII	Eleotridae	*Mogurnda*	*mogurnda*	none	Sanford 1995
VIII	Eleotridae	*Ophieleotris*	*aporos*	none	Merrick and Schmida 1984
VIII	Eleotridae	*Ophiocara*	*porocephala*	none	Merrick and Schmida 1984
VIII	Eleotridae	*Oxyeleotris*	*aruensis*	none	Allen 1989
VIII	Eleotridae	*Oxyeleotris*	*finbriatus*	none	

					Allen 1989
VIII	Eleotridae	*Oxyeleotris*	*nullipora*	none	Allen 1989
VIII	Eleotridae	*Oxyeleotris*	*herwerdeni*	none	Merrick and Schmida 1984
VIII	Eleotridae	*Oxyeleotris*	*lineolatus*	none	Merrick and Schmida 1984
VIII	Eleotridae	*Oxyeleotris*	*marmoratus*	brighter	Sanford 1995
VIII	Eleotridae	*Philypnodon*	*grandiceps*	darker	Merrick and Schmida 1984
VIII	Eleotridae	*Prionobutis*	*microps*	none	Merrick and Schmida 1984
VIII	Eleotridae	*Prionobutis*	*koilomatodon*	none	Smith and Heemstra 1986
VIII	Eleotridae	*Tateurndina*	*ocellatus*	brighter	Sanford 1995
VIII	Eleotridae	*Xenisthmus*	*africanus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Acanthogobius*	*flavimanus*	none	Lee et al. 1980
VIII	Gobiidae	*Acentrogobius*	*audax*	none	Smith and Heemstra 1986

VIII	Gobiidae	*Amblyeleotris*	*aurora*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Amblyeleotris*	*wheeleri*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Amblygobius*	*albimaculatus*	dark spots	Smith and Heemstra 1986
VIII	Gobiidae	*Amblygobius*	*sphynx*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Amoya*	*signatus*	dark spots	Smith and Heemstra 1986
VIII	Gobiidae	*Aphania*	*minuta*	longer fins	Muus et al. 1999
VIII	Gobiidae	*Asterropteryx*	*semipunctatus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Austrolethops*	*wardi*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Awaous*	*crassilabrus*	none	Merrick and Schmida 1984
VIII	Gobiidae	*Awaous*	*tajasica*	none	Robins and Ray 1986
VIII	Gobiidae	*Awaous*	*aeneofuscus*	none	Smith and Heemstra 1986

VIII	Gobiidae	*Barbulifer*	*antennatus*	none	Bohkle and Chaplin 1993
VIII	Gobiidae	*Barbulifer*	*ceuthoecus*	none	Robins and Ray 1986
VIII	Gobiidae	*Barbuligobius*	*boehlkei*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Bathygobius*	*fuscus*	none	Axelros and Burgess 1987
VIII	Gobiidae	*Bathygobius*	*soporator*	brighter	Goldstein et al 2000
VIII	Gobiidae	*Bathygobius*	*lineatus*	none	Grove and Lavenberg 1997
VIII	Gobiidae	*Bathygobius*	*curacao*	bands on fins	Robins and Ray 1986
VIII	Gobiidae	*Bathygobius*	*mystacium*	none	Robins and Ray 1986
VIII	Gobiidae	*Bathygobius*	*albopunctatus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Bathygobius*	*cocosensis*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Bathygobius*	*cotticeps*	none	Smith and Heemstra 1986

VIII	Gobiidae	*Bathygobius*	*cyclopterus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Bathygobius*	*laddi*	darker	Smith and Heemstra 1986
VIII	Gobiidae	*Bathygobius*	*niger*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Bollmannia*	*bogueronensis*	none	Robins and Ray 1986
VIII	Gobiidae	*Bollmannia*	*communis*	bands on fins	Robins and Ray 1986
VIII	Gobiidae	*Brachygobius*	*aggregatus*	brighter	Axelrod and Schultz 1990
VIII	Gobiidae	*Brachygobius*	*doriae*	brighter	Axelrod and Schultz 1990
VIII	Gobiidae	*Brachygobius*	*nunos*	brighter	Riehl and Baensch 1989
VIII	Gobiidae	*Brachygobius*	*xanthozona*	none	Sanford 1995
VIII	Gobiidae	*Buenia*	*jeffreysi*	none	Muus et al. 1999
VIII	Gobiidae	*Caffrogobius*	*caffer*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Caffrogobius*	*multifasciatus*	none	Smith and

					Heemstra 1986
VIII	Gobiidae	*Caffrogobius*	*natalensis*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Caffrogobius*	*nudiceps*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Caffrogobius*	*saldanha*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Callogobius*	*flavobrunneus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Callogobius*	*maculipinnis*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Callogobius*	*plumatus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Callogobius*	*sclateri*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Chalmydogobius*	*eremius*	bands on fins	Merrick and Schmida 1984
VIII	Gobiidae	*Chirolepis*	*torgus*	none	Grove and Lavenberg 1997
VIII	Gobiidae	*Chlamydogobius*	*eremius*	bands on fins	Allen 1989
VIII	Gobiidae	*Chriolepis*	*fisheri*	none	Bohkle and

					Chaplin 1993
VIII	Gobiidae	*Clevelandia*	*ios*	darker	Goldstein et al 2000
VIII	Gobiidae	*Coryphopterus*	*nicholsi*	brighter	Goldstein et al 2000
VIII	Gobiidae	*Coryphopterus*	*urospilus*	brighter	Grove and Lavenberg 1997
VIII	Gobiidae	*Coryphopterus*	*alloides*	bands on fins	Robins and Ray 1986
VIII	Gobiidae	*Coryphopterus*	*dicrus*	none	Robins and Ray 1986
VIII	Gobiidae	*Coryphopterus*	*eidolon*	none	Robins and Ray 1986
VIII	Gobiidae	*Coryphopterus*	*glaucofrenum*	none	Robins and Ray 1986
VIII	Gobiidae	*Coryphopterus*	*hyalinus*	none	Robins and Ray 1986
VIII	Gobiidae	*Coryphopterus*	*lipernes*	none	Robins and Ray 1986
VIII	Gobiidae	*Coryphopterus*	*personatus*	none	Robins and Ray 1986
VIII	Gobiidae	*Coryphopterus*	*punctipectophorus*	none	Robins and Ray

					1986
VIII	Gobiidae	*Coryphopterus*	*thrux*	none	Robins and Ray 1986
VIII	Gobiidae	*Croilia*	*mossambica*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Cryptocentrus*	*cryptocentrus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Cryptocentrus*	*pretoriusi*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Cryptocentrus*	*strigilliceps*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Cryptocentrus*	*pavoninoides*	brighter	Statopoomin and Winterbottom 2002
VIII	Gobiidae	*Crystallogobius*	*lineaaris*	none	Muus et al. 1999
VIII	Gobiidae	*Ctenogobius*	*boleossoma*	brighter	Goldstein et al 2000
VIII	Gobiidae	*Ctenogobius*	*shufeldti*	none	Goldstein et al 2000
VIII	Gobiidae	*Drombus*	*key*	none	Smith and Heemstra 1986

VIII	Gobiidae	*Drombus*	*simulus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Elacatinus*	*nestoides*	none	Grove and Lavenberg 1997
VIII	Gobiidae	*Eucyclogobius*	*newberryi*	none	Lee et al. 1980
VIII	Gobiidae	*Evermannichthys*	*metzelaari*	none	Bohkle and Chaplin 1993
VIII	Gobiidae	*Evermannichthys*	*spongicola*	none	Robins and Ray 1986
VIII	Gobiidae	*Eviota*	*albolineata*	longer fins	Smith and Heemstra 1986
VIII	Gobiidae	*Eviota*	*prasina*	longer fins	Smith and Heemstra 1986
VIII	Gobiidae	*Evorthodus*	*lyricus*	longer fins	Goldstein et al 2000
VIII	Gobiidae	*Favonigobius*	*tamarensis*	bands on fins	Merrick and Schmida 1984
VIII	Gobiidae	*Favonigobius*	*melanobranchus*	bands on belly	Smith and Heemstra 1986
VIII	Gobiidae	*Favonigobius*	*reichei*	darker	Smith and Heemstra 1986
VIII	Gobiidae	*Fusigobius*	*duospilus*	none	Smith and

					Heemstra 1986
VIII	Gobiidae	*Fusigobius*	*longispinus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Fusigobius*	*neophytus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Gillichthys*	*mirabilis*	none	Goldstein et al 2000
VIII	Gobiidae	*Ginsburgellus*	*novemlineatus*	none	Robins and Ray 1986
VIII	Gobiidae	*Glassogobius*	*biocellatus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Glassogobius*	*callidus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Glassogobius*	*giurus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Glossogobius*	*aureus*	none	Allen 1989
VIII	Gobiidae	*Glossogobius*	*celebius*	none	Allen 1989
VIII	Gobiidae	*Glossogobius*	*concavifrons*	none	Allen 1989
VIII	Gobiidae	*Glossogobius*	*giurus*	bands on	

				fins	Allen 1989
VIII	Gobiidae	*Glossogobius*	*giurus*	brighter	Merrick and Schmida 1984
VIII	Gobiidae	*Gnatholepis*	*thompsoni*	none	Robins and Ray 1986
VIII	Gobiidae	*Gobiodes*	*broussoneti*	none	Goldstein et al 2000
VIII	Gobiidae	*Gobiodon*	*citrinus*	none	Axelros and Burgess 1987
VIII	Gobiidae	*Gobioides*	*broussoneti*	none	Robins and Ray 1986
VIII	Gobiidae	*Gobionellus*	*atripinnis*	none	Lee et al. 1980
VIII	Gobiidae	*Gobionellus*	*oceanicus*	none	Goldstein et al 2000
VIII	Gobiidae	*Gobionellus*	*claytoni*	none	Hoese and Moore 1998
VIII	Gobiidae	*Gobionellus*	*boleosoma*	brighter	Robins and Ray 1986
VIII	Gobiidae	*Gobionellus*	*hastatus*	none	Robins and Ray 1986
VIII	Gobiidae	*Gobionellus*	*saepepallens*	none	Robins and Ray

					1986
VIII	Gobiidae	*Gobionellus*	*stigmalophis*	none	Robins and Ray 1986
VIII	Gobiidae	*Gobionellus*	*stigmaticus*	none	Robins and Ray 1986
VIII	Gobiidae	*Gobionellus*	*stigmaturus*	none	Robins and Ray 1986
VIII	Gobiidae	*Gobiopsis*	*atrata*	none	Ayling and Cox 1982
VIII	Gobiidae	*Gobiopsis*	*pinto*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Gobiossoma*	*dilepis*	longer fins	Bohkle and Chaplin 1993
VIII	Gobiidae	*Gobiossoma*	*evelynae*	none	Bohkle and Chaplin 1993
VIII	Gobiidae	*Gobiossoma*	*gemmatum*	longer fins	Bohkle and Chaplin 1993
VIII	Gobiidae	*Gobiossoma*	*louisae*	none	Bohkle and Chaplin 1993
VIII	Gobiidae	*Gobiossoma*	*palliens*	none	Bohkle and Chaplin 1993
VIII	Gobiidae	*Gobiossoma*	*bosc*	none	Goldstein et al

					2000
VIII	Gobiidae	*Gobiossoma*	*ginsburgi*	none	Goldstein et al 2000
VIII	Gobiidae	*Gobiossoma*	*longipala*	none	Goldstein et al 2000
VIII	Gobiidae	*Gobiossoma*	*robustium*	none	Goldstein et al 2000
VIII	Gobiidae	*Gobiossoma*	*genie*	none	Robins and Ray 1986
VIII	Gobiidae	*Gobiossoma*	*grosvenori*	none	Robins and Ray 1986
VIII	Gobiidae	*Gobiossoma*	*horsti*	none	Robins and Ray 1986
VIII	Gobiidae	*Gobiossoma*	*macrodon*	none	Robins and Ray 1986
VIII	Gobiidae	*Gobiossoma*	*multifasciatum*	none	Robins and Ray 1986
VIII	Gobiidae	*Gobiossoma*	*oceanops*	none	Robins and Ray 1986
VIII	Gobiidae	*Gobiossoma*	*xanthiprora*	none	Robins and Ray 1986
VIII	Gobiidae	*Gobius*	*niger*	none	Muus et al.

					1999
VIII	Gobiidae	*Gobius*	*paganellus*	none	Muus et al. 1999
VIII	Gobiidae	*Gobius*	*xanthozona*	brighter	Riehl and Baensch 1989
VIII	Gobiidae	*Gobiusculus*	*flavescens*	spotted pectorals	Muus et al. 1999
VIII	Gobiidae	*Gobullus*	*myersi*	none	Robins and Ray 1986
VIII	Gobiidae	*Hetereleotris*	*apora*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Hetereleotris*	*caminata*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Hetereleotris*	*margaretae*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Hetereleotris*	*tentacula*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Hetereleotris*	*zanzibarensis*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Hetereleotris*	*zonata*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Ioglossus*	*calliurus*	none	Robins and Ray

					1986
VIII	Gobiidae	*Istigobius*	*decoratus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Istigobius*	*ornatus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Istigobius*	*spence*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Lebetus*	*guilleti*	none	Muus et al. 1999
VIII	Gobiidae	*Lebetus*	*scorpoides*	none	Muus et al. 1999
VIII	Gobiidae	*Lepidogobius*	*lepidus*	none	Goldstein et al 2000
VIII	Gobiidae	*Lesuerigobius*	*gunnellus*	none	Muus et al. 1999
VIII	Gobiidae	*Lophogobius*	*cyprinoides*	darker	Goldstein et al 2000
VIII	Gobiidae	*Lythrypnus*	*elasson*	none	Bohkle and Chaplin 1993
VIII	Gobiidae	*Lythrypnus*	*heterochroma*	longer fins	Bohkle and Chaplin 1993
VIII	Gobiidae	*Lythrypnus*	*okapia*	none	Bohkle and

					Chaplin 1993
VIII	Gobiidae	*Lythrypnus*	*gilberti*	none	Grove and Lavenberg 1997
VIII	Gobiidae	*Lythrypnus*	*rhizophora*	none	Grove and Lavenberg 1997
VIII	Gobiidae	*Lythrypnus*	*nestoides*	none	Robins and Ray 1986
VIII	Gobiidae	*Lythrypnus*	*spilus*	none	Robins and Ray 1986
VIII	Gobiidae	*Lythrypnus*	*dalli*	none	Sanford 1995
VIII	Gobiidae	*Mahidolia*	*mystacina*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Microgobius*	*gulosus*	bands on fins	Goldstein et al 2000
VIII	Gobiidae	*Microgobius*	*thalassinus*	brighter	Goldstein et al 2000
VIII	Gobiidae	*Microgobius*	*carri*	none	Robins and Ray 1986
VIII	Gobiidae	*Microgobius*	*microlepis*	brighter	Robins and Ray 1986
VIII	Gobiidae	*Monishia*	*sordida*	none	Smith and Heemstra 1986

VIII	Gobiidae	*Monishia*	*william*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Mugilogobius*	*durbanensis*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Mugilogobius*	*inhacae*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Nemareleotris*	*magnifica*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Neogobius*	*melanostomus*	none	Werner 2004
VIII	Gobiidae	*Nes*	*longus*	none	Robins and Ray 1986
VIII	Gobiidae	*Oligolepis*	*acutipennis*	longer fins	Smith and Heemstra 1986
VIII	Gobiidae	*Oligolepis*	*keiensis*	longer fins	Smith and Heemstra 1986
VIII	Gobiidae	*Oplopomus*	*oplopomus*	longer fins	Smith and Heemstra 1986
VIII	Gobiidae	*Oxyurichthys*	*lemayi*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Oxyurichthys*	*microlepis*	none	Smith and Heemstra 1986

VIII	Gobiidae	*Oxyurichthys*	*ophthalmonema*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Oxyurichthys*	*papuensis*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Palatogobius*	*paradoxus*	none	Robins and Ray 1986
VIII	Gobiidae	*Pandaka*	*silvana*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Parachaeturichthys*	*polynema*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Paragobiodon*	*echinocephalus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Paragobiodon*	*lacunicolus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Paragobiodon*	*modestus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Paragobiodon*	*xanthosomus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Periophthalmus*	*barbarus*	none	Sanford 1995
VIII	Gobiidae	*Periophthalmus*	*papilio*	none	Sanford 1995

VIII	Gobiidae	*Periophthalmus*	*koelreuteri*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Periophthalmus*	*sobrinus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Pleurosicya*	*annandalei*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Pleurosicya*	*mossambica*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Pleurosicya*	*muscarum*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Pomatoscistus*	*lazonoi*	bands on fins	Muus et al. 1999
VIII	Gobiidae	*Pomatoscistus*	*microps*	none	Muus et al. 1999
VIII	Gobiidae	*Pomatoscistus*	*minutus*	none	Muus et al. 1999
VIII	Gobiidae	*Pomatoscistus*	*norvegicus*	none	Muus et al. 1999
VIII	Gobiidae	*Pomatoscistus*	*pictus*	none	Muus et al. 1999
VIII	Gobiidae	*Priolepis*	*inhaaca*	none	Smith and Heemstra 1986

VIII	Gobiidae	*Proterorhinus*	*marmoratus*	none	Werner 2004
VIII	Gobiidae	*Psammogobius*	*knysnaensis*	darker	Smith and Heemstra 1986
VIII	Gobiidae	*Pseudogobius*	*olorum*	brighter	Allen 1989
VIII	Gobiidae	*Psilotris*	*alepis*	none	Bohkle and Chaplin 1993
VIII	Gobiidae	*Psilotris*	*batrachodes*	none	Bohkle and Chaplin 1993
VIII	Gobiidae	*Psilotris*	*celsus*	none	Bohkle and Chaplin 1993
VIII	Gobiidae	*Pteroleotris*	*evides*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Pteroleotris*	*heteroptera*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Quientula*	*y-cauda*	bands on fins	Goldstein et al 2000
VIII	Gobiidae	*Quisquilius*	*hipoliti*	none	Robins and Ray 1986
VIII	Gobiidae	*Redigobius*	*balteatops*	none	Smith and Heemstra 1986

VIII	Gobiidae	*Redigobius*	*bikolanus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Redigobius*	*dewaali*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Rhinogobius*	*nicholsi*	black disk	Gilbert and Williams 2002
VIII	Gobiidae	*Rhinogobius*	*brunneus*	longer fins	Suk and Choe 2002
VIII	Gobiidae	*Risor*	*ruber*	none	Robins and Ray 1986
VIII	Gobiidae	*Sicyopus*	*chloe*	brighter	Watson et al. 2001
VIII	Gobiidae	*Silhouettea*	*insinuans*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Silhouettea*	*sibayi*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Stenogobius*	*kenyae*	brighter	Smith and Heemstra 1986
VIII	Gobiidae	*Stigmatogobius*	*sadanandio*	longer fins	Sanford 1995
VIII	Gobiidae	*Stiphodon*	*elegans*	none	Sanford 1995

VIII	Gobiidae	*Sufflogobius*	*bibartatus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Taenoides*	*esquicei*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Taenoides*	*jacksoni*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Tasmanogobius*	*lordi*	none	Merrick and Schmida 1984
VIII	Gobiidae	*Thorogobius*	*ephippiatus*	none	Muus et al. 1999
VIII	Gobiidae	*Tridentiger*	*trigonocephalus*	none	Goldstein et al 2000
VIII	Gobiidae	*Trimma*	*corallina*	longer fins	Smith and Heemstra 1986
VIII	Gobiidae	*Trimma*	*macrophthalmus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Trypauchen*	*microcephalus*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Typhlogobius*	*californiensis*	none	Goldstein et al 2000
VIII	Gobiidae	*Valenciennea*	*helsdingenii*	none	Smith and Heemstra 1986

VIII	Gobiidae	*Valenciennea*	*sexguttata*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Valenciennea*	*strigata*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Vanderhorstia*	*deladoae*	none	Smith and Heemstra 1986
VIII	Gobiidae	*Yongeichthys*	*nebulosus*	none	Smith and Heemstra 1986

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