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REINTERPRETING RECAPITULATION: SYSTEMATICS OF NEEDLEFISHES AND THEIR ALLIES (TELEOSTEI: BELONIFORMES)

NATHAN R. LOVEJOY[1]
Section of Ecology and Systematics, Cornell University, Corson Hall, Ithaca, New York 14853-2701

Abstract.—As needlefishes (Belonidae) grow, their jaws pass through a ''halfbeak'' stage that resembles the adult jaw condition of the closely related family of halfbeaks (Hemiramphidae). Based on this pattern, some authors have suggested that halfbeaks are ''developmentally arrested'' or paedomorphic needlefish derivatives, whereas others have supported the notion that needlefishes are descended from halfbeak-like ancestors and that needlefish ontogeny thereby recapitulates phylogeny. To test these ideas and to better understand evolutionary changes in jaw ontogeny, phylogenetic relationships among genera of needlefishes, sauries (Scomberesocidae), halfbeaks, and flyingfishes (Exocoetidae) were assessed using mitochondrial (cytochrome *b* and 16S), nuclear (Tmo-4C4), and morphological characters. The resultant tree provides several novel taxonomic findings: (1) flyingfishes appear to be nested within halfbeaks; (2) sauries appear to be nested within needlefishes; and (3) the Indo-West Pacific freshwater halfbeaks appear to be most closely related to the needlefish/saury clade. The structure of the tree falsifies the idea that halfbeaks are paedomorphic needlefishes. Instead, halfbeaks are basal relative to needlefishes, fitting the pattern predicted by the hypothesis of recapitulation. I discuss limitations to phylogenetic perspectives on recapitulation based on discrete character data by comparing aspects of von Baerian and Haeckelian views of the relation between ontogeny and phylogeny.

Key words.—Haeckel, heterochrony, molecular systematics, ontogeny, paedomorphosis, recapitulation, von Baer.

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The relationship between ontogeny and phylogeny has fascinated both evolutionary biologists and their predecessors (Agassiz 1849; von Baer 1853; Gould 1977; Alberch et al. 1979; McKinney and McNamara 1991; Klingenberg 1998). Heterochrony, defined as evolutionary change in the timing of ontogeny (de Beer 1940), may play an important role in morphological innovation (Bonner 1982); however, the relative importance of two different heterochonic patterns has been debated. Workers of the late 1800s, particularly Haeckel (1866), Cope (1887), and Hyatt (1897) emphasized the importance of recapitulation, defined by Gould (1977) as the repetition of ancestral adult stages in juvenile stages of the descendant (renamed ''peramorphosis'' by Alberch et al. 1979). This idea assumed its most influential form in Haeckel's (1866, p. 300) biogenetic law, which stated ''Ontogeny is the short and rapid recapitulation of phylogeny.'' Under this theory, evolutionary change occurs by the successive addition of stages to the end of an ancestral ontogeny. Later, Garstang (1922) and de Beer (1930, 1940) argued for the primacy of the alternative heterochronic pattern of paedomorphosis, defined as the retention of subadult ancestral stages in the adult stages of the descendant (Gould 1977; McKinney and McNamara 1991).

Gould (1977) pointed out that the relative predominance of recapitulation versus paedomorphosis is an empirical rather than theoretical question. However, some taxa have been interpreted as examples of both patterns. The phylogenetic relationships within Beloniformes, a group that currently includes the needlefishes (Belonidae), halfbeaks (Hemiramphidae), flyingfishes (Exocoetidae), sauries (Scomberesocidae), and ricefishes (Adrianichthyidae; Rosen and Parenti 1981) have long been included in these debates. Morphologists of the early 1900s, engaged in deciphering the relationship between ontogeny and phylogeny, found fuel for their theories in the peculiar development and relationships of belonid needlefishes (Severtzov 1927; de Beer 1930; Gould 1977). Larval needlefishes have short jaws of equal length. However, as they grow, the lower jaw elongates first, producing a morphology that is distinctly reminiscent of a related family, the halfbeaks (Hemiramphidae)—indeed, needlefishes in this ''halfbeak'' form have been mistakenly described as hemiramphids (Collette and Parin 1970). Later, the upper jaw elongates as well, giving rise to the nearly equal length jaws of most adult needlefishes.

Severtzov (1927; summarized in Gould 1977) thought this ontogenetic pattern roughly paralleled phylogeny within beloniforms. He hypothesized that short-jawed ancestral flyingfishes gave rise to descendant halfbeaks, which in turn gave rise to the more advanced needlefishes. Needlefish ontogeny could thus be a prime example of the phenomenon known as recapitulation. de Beer (1930), in contrast cited Schlesinger (1909), Regan (1911), and Nichols and Breder (1928) to suggest instead that halfbeaks are derived from ancestral needlefish stock, perhaps via an arrest in developmental timing. In this case, halfbeaks would be considered an example of paedomorphosis. Clearly, differentiating between these possibilities depends on having an explicit phylogeny that can be used to test hypotheses about the polarity of ontogenetic and morphological transformations (Fink 1982). The two alternative scenarios described above make specific predictions about which taxa (and morphologies) should be relatively basal or derived.

Known variously as ''synentognath'' or ''beloniform'' fishes, a natural grouping of the families Belonidae (needlefishes), Scomberesocidae (sauries), Hemiramphidae (halfbeaks), and Exocoetidae (flyingfishes) has been recognized for more than a century (e.g., Gill 1895; Regan 1911). Recently, Rosen and Parenti (1981) added a fifth family to this clade: the Adrianichthyidae, or Southeast Asian freshwater ricefishes. The most current classification consists of a mono-

[1] Present address: University of California, Museum of Vertebrate Zoology, 3101 Valley Life Science Building, Berkeley, California 94720-3160; E-mail: lovejoy7@uclink4.berkeley.edu.

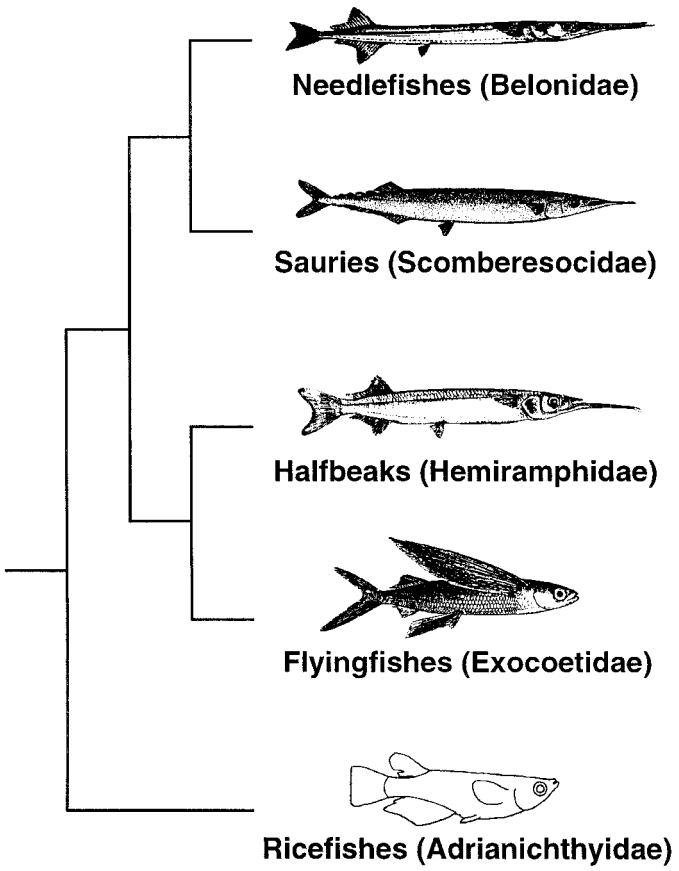

FIG. 1. Hypothesis of beloniform relationships provided by Collette et al. (1984). Fish illustrations after Collette (1977), Parin (1986a,b), Banford and Collette (1993), and Uwa and Parenti (1988).

phyletic order Beloniformes containing all the abovementioned families, with Adrianichthyidae as the basal member (Fig. 1). Beloniformes occupy a diverse range of aquatic habitats including tropical rivers and lakes, coastal mangrove swamps, and epipelagic zones of oceans and seas. They exhibit a variety of reproductive modes: Scomberesocids lay pelagic eggs, whereas some freshwater hemiramphids are viviparous.

In this paper, morphological characters from two previous studies (Collette et al. 1984; Boughton et al. 1991) are combined with a molecular dataset collected from two mitochondrial genes and one nuclear gene for representatives of all five beloniform families. The analysis provides a novel hypothesis of relationships within Beloniformes that can be compared to previous systematic findings. I use this phylogenetic pattern to evaluate the alternative scenarios of jaw evolution advocated by Severtzov (1927) and de Beer (1930).

MATERIALS AND METHODS

Terminal Taxa

Fishes were collected in the field by myself or colleagues. Gill tissue was either frozen immediately in liquid nitrogen or preserved in 95–100% ethanol or buffer of 20% DMSO, 0.25 M EDTA at pH 8, saturated with NaCl (Seutin et al. 1991). Tissue preserved in buffer and stored at room temperature has always yielded amplifiable DNA (after storage for up to four years). Voucher specimens were preserved in 10% buffered formalin, transferred to 70% ethanol or 50–55% isopropanol, and deposited in museum collections. See Appendix 1 for list of vouchers.

Samples represent all beloniform families. However, greater numbers of needlefishes were examined because this study represents a component of research specifically targeting needlefish relationships. Nine of 10 needlefish genera, two of four saury genera, seven of 13 halfbeak genera, two of seven flyingfish genera, and one of four ricefish genera were included in the study. The limited representation of ricefishes, flyingfishes, and sauries was not considered problematic because each is strongly supported as monophyletic by morphology (Collette et al. 1984). The same is not true for needlefishes and halfbeaks. Whenever possible, sequences were collected from two individuals of each species, providing 76 terminal taxa for analysis (see Appendix 1 for a full list of specimens included).

DNA Sequencing

Both mitochondrial and nuclear genes were used for analysis. However, rather than sequencing a single complete mitochondrial gene, smaller segments of two separate genes, cytochrome *b* (cyt *b*) and 16S rRNA (16S), were examined. This decision was based on the hope that sampling a range of genes, with different rates and patterns of molecular evolution, would provide phylogenetic information that spanned a broader range of taxonomic divergence. The nuclear gene, Tmo-4C4 (Tmo), is an anonymous, putative protein-coding locus identified and used for phylogeny by Streelman and Karl (1997). It provided resolution of families and genera within labroids and was thus expected to provide useful information for deeper parts of the beloniform tree.

For each sample, approximately 25 mg of tissue was rinsed briefly in water, then DNA purified using Qiagen's (Valencia, CA) spin-column tissue kit. Briefly, cells were lysed at 55°C in 20 μl of Proteinase K (20 mg/ml) for three to six hours. Lysate was bound to the spin-column membrane and washed twice by centrifugation. DNA was then eluted by centrifugation twice with 200 μl of low-salt buffer.

Template for sequencing was initially amplified using published polymerase chain reaction (PCR) primers. New primers were then designed for sequencing and additional amplifications (see Table 1). Generally, DNA was amplified in 50 μl reactions containing 1 μl of DNA, 3 mM $MgCl_2$, 20 mM Tris HCl ph 8.4, 50 mM KCl, 200 μM dNTPs, 0.4 μM of each primer, and one unit of Gibco Taq polymerase. PCR amplifications were performed using the following conditions: 30-sec denaturation at 95°C to start, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 48–55°C for 60 sec, and 72°C extension for 90 sec, followed by a final extension of 72°C for 5 min.

PCR products were cleaned using a PCR product presequencing kit (Amersham Life Science, Piscataway, NJ) and then directly sequenced using the Thermo Sequenase radiolabeled terminator cycle sequencing kit (Amersham Life Science). Template that proved difficult to sequence was run in

TABLE 1. Primers used for polymerase chain reaction and sequencing.

Gene	Name	Position[1]	Sequence	Reference
Cyt b	GLUDG-5′	15269	5′-CGAAGCTTGACTTGAArAACCAyCGTTG-3′	Palumbi 1996
	Cyt-b1-5′	15387	5′-AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA-3′	Kocher et al. 1989
	Cyt-b2-3′	15695	5′-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3′	Kocher et al. 1989
	Cyt-b3-3′	16103	5′-GGCAAATAGGAArTATCATTC-3′	Palumbi 1996
	Cyt-bg-3′	16043	5′-GAGTAAAGTTGTCTGGGTCdCC-3′	this study
16S	16Sar-5′	2938	5′-CGCCTGTTTATCAAAAACAT-3′	Palumbi 1996
	16Sho-5′	3171	5′-CATAAGACGAGAAGACCCTGTGGAGC-3′	this study
	16SBr-3′	3520	5′-CCGGTCTGAACTCAGATCACGT-3′	Palumbi 1996
Tmo-4C4	Tmo-fl-5′	24	5′-CCTCCGGCCTTCCTAAAACCTCTC-3′	Streelman and Karl 1997
	Tmo-f2-5′	236	5′-ATCTGTGAGGCTGTGAACTA-3′	this study
	Tmo-f3-5′	391	5′-ATCCCCTCAGGAGATTCTGC-3′	this study
	Tmo-r1-3′	536	5′-CATCGTGCTCCTGGGTGACAAAGT-3′	Streelman and Karl 1997
	Tmo-r2-3′	328	5′-TCCACGTCAAACTCCATCAC-3′	this study

[1] Positions for cyt b and 16S correspond to the position of the 3′ end of the primer in the carp mitochondrial genome (Chang et al. 1994). Positions of the Tmo primers are based on the *Strongylura notata* sequence.

1% agaraose gels, then cut out and cleaned using PCR purification spin columns (Quiagen).

The Tmo and cyt *b* sequences were aligned unambiguously using Lasergene (Madison, WI) software (DNASTAR). As in Streelman and Karl's (1997) study, an open reading frame for Tmo was determined that produced amino acid translations with no stop codons in any sequences. Tmo is therefore considered a protein-coding gene, with positions determined by the hypothesized open reading frame. The 16S sequences were aligned using a variety of parameters (gap cost and gap length cost) and compared to a hypothesized model for secondary structure proposed by Orti et al. (1996) for piranhas. Alignments that inserted gaps in stable regions (stems) were excluded from further consideration. Of the remaining alignments (which were considered more biologically reasonable), sites and regions where alignment was ambiguous were removed. This rather conservative procedure excluded approximately 130 positions, leaving only regions of 16S that were conserved over the full range of taxa. The sacrifice of potential characters for clear topographical identity of sites (as per Brower and Schawaroch 1996) was considered acceptable, because preliminary analysis of the mitochondrial protein-coding genes indicated that many characters for resolving recent nodes were available, while conservative characters for deeper parts of the tree would be at a premium. All sequences have been deposited in Genbank under accession numbers AF243857–AF244082.

Morphology

Morphological characters were culled from the literature (see Appendixes 2, 3). When possible, codings were confirmed through observations of cleared and stained material from the collections of the U.S. National Museum (USNM) and the American Museum of Natural History (AMNH). Two additional morphological characters were discovered during the course of this study. Characters supporting the monophyly of Exocoetoidei (Beloniformes to the exclusion of Adrianichthyidae, the outgroup) were not included because the use of a single adrianichthyid outgroup automatically makes the ingroup, Exocoetoidei, monophyletic. Autapomorphies for terminal taxa were also excluded from the analysis.

Phylogenetic Analysis

All 1532 characters (484 bp Tmo, 641 bp cyt *b*, 371 bp 16S, and 36 morphological) were combined in a single matrix and the heuristic search algorithm of PAUP* (100 replicates of random taxon additions, TBR branch swapping) was used to search for most parsimonious trees (Swofford 1999). All characters were unweighted. *Oryzias* (the ricefish representative) was used as an outgroup to root all trees. Nuclear, mitochondrial, and morphological datasets were also analyzed separately (using the same algorithm and settings) to investigate the contribution provided by each to the total evidence hypothesis. Individual mitochondrial genes were not each analyzed separately because it was assumed that the small size of each fragment would prevent effective phylogeny reconstruction. Decay indices for nodes were calculated using TreeRot (Sorenson 1996), and bootstrap proportions were calculated using PAUP* (100 replicates with 50 random taxon additions per replicate). Investigation of the sensitivity of results to various weighting schemes was also carried out using PAUP*.

Mapping Jaw Ontogeny

The evolution of jaw characters was examined by optimization on the total evidence tree using MacClade (Maddison and Madisson 1992). Characters 34 to 36 were optimized using both ACCTRAN and DELTRAN. The effects of slight changes in tree topology were also considered by optimizing characters on these alternative topologies. Some authors have cautioned against the inclusion of ''characters of interest'' in phylogenetic analyses that are then used to evaluate those same characters (Brooks and McLennan 1991). However, following the argument that hypotheses of homology can only be tested by congruence of characters in a phylogenetic context (Patterson 1982; de Pinna 1991; Deleporte 1993; Kluge and Wolf 1993), jaw characters were included in the total evidence analysis.

RESULTS

Figure 2 shows a strict consensus of the two most parsimonious trees based on all available data (3622 steps). The

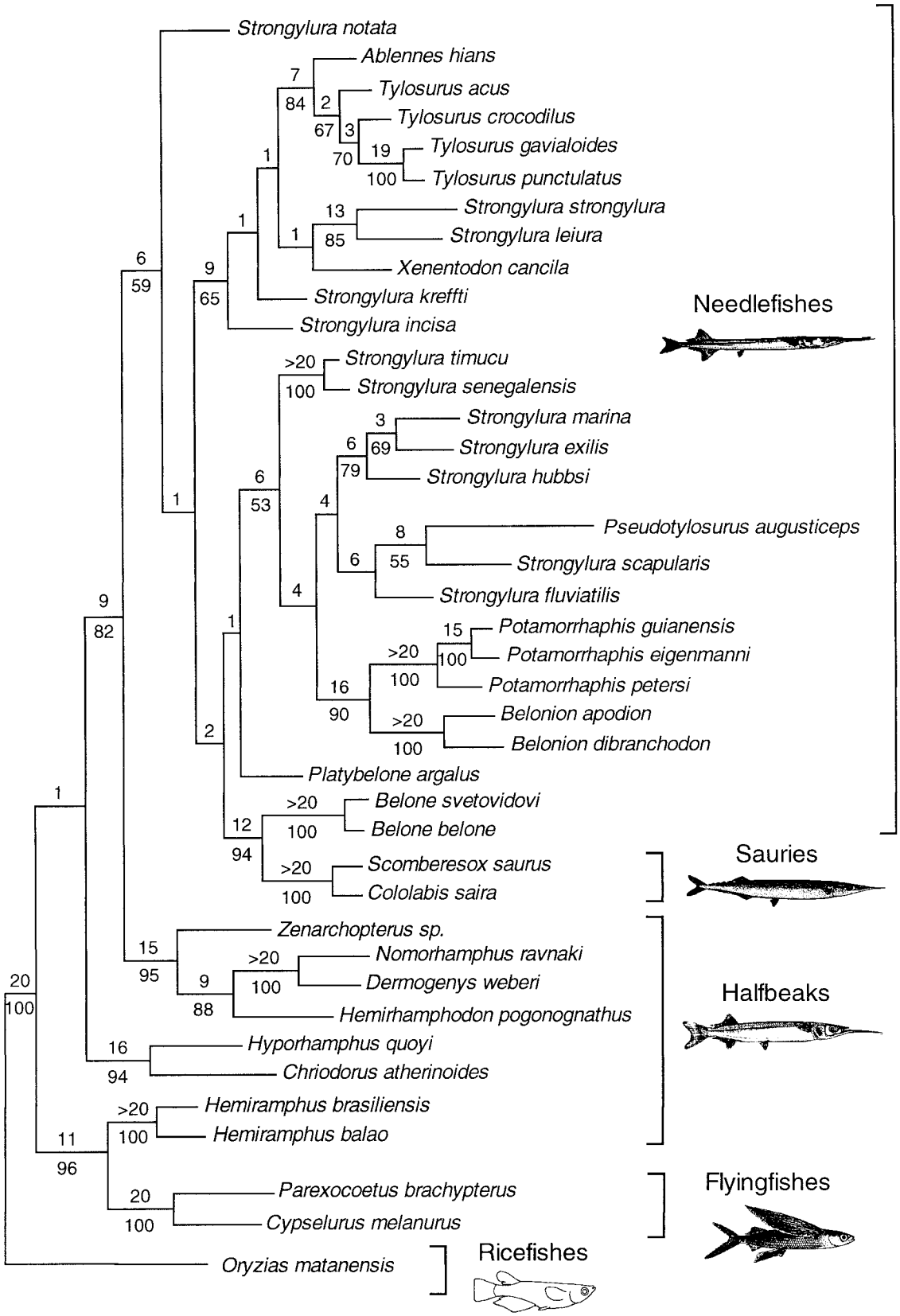

FIG. 2. Consensus of two most parsimonious trees produced by unweighted analysis of all data (total evidence hypothesis). Conspecific individuals were found to be monophyletic, thus only a single represenative of each species is shown. Numbers above nodes are decay indices. Numbers below nodes are bootstrap proportions. Branch lengths are proportional to numbers of changes, CI 0.27, RI 0.73.

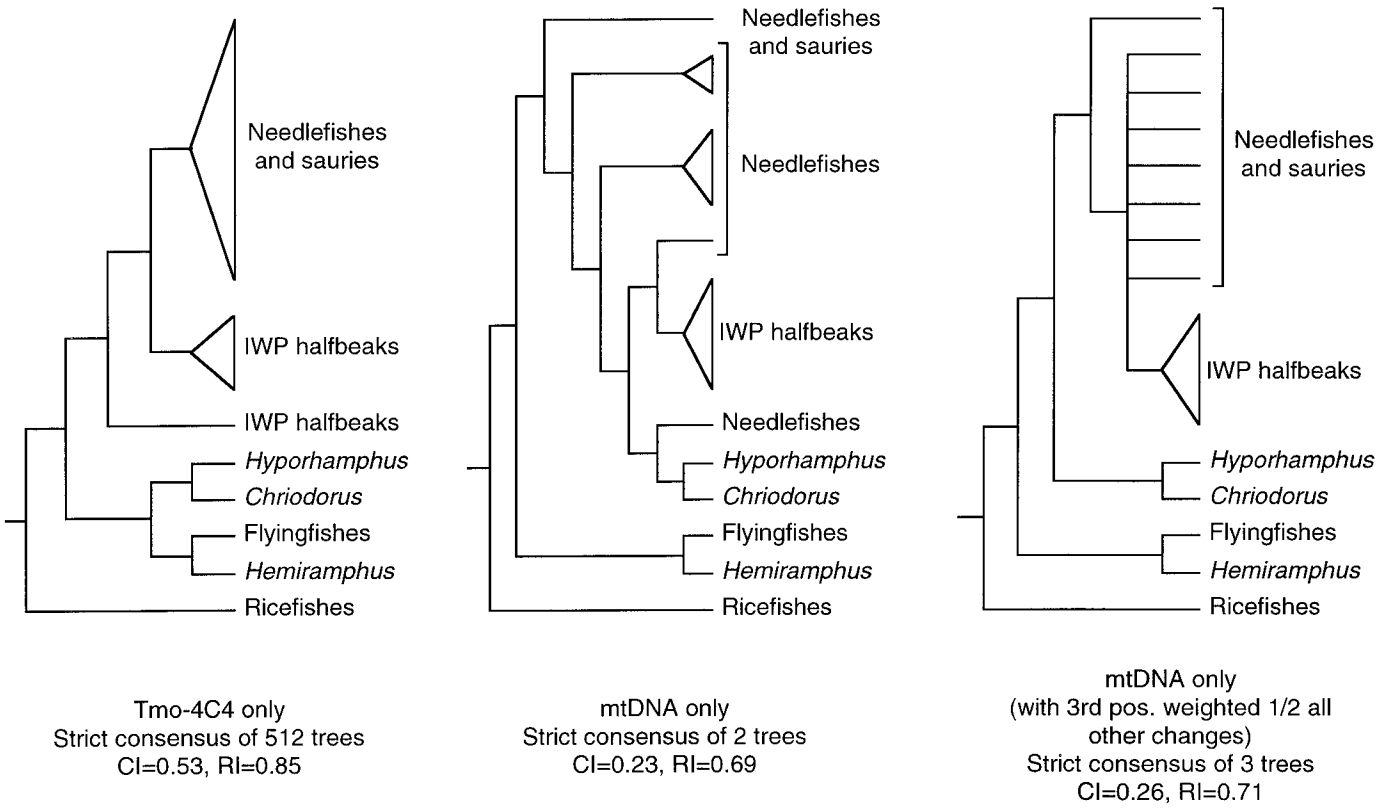

FIG. 3. Simplified strict consensus trees for nuclear and mitochondrial data analyzed separately. Triangles indicate monophyletic clades.

only difference between the trees results from minor variation in the relationships among the three *Pseudotylosurus* individuals; the consensus is hereafter referred to as the total evidence hypothesis. Of the five currently recognized beloniform families, two are monophyletic, the sauries (Scomberesocidae) and flyingfishes (Exocoetidae); whereas two others, the needlefishes (Belonidae) and halfbeaks (Hemiramphidae), are paraphyletic. In the case of needlefishes, the sauries were found to be the sister group of the needlefish genus *Belone*. Thus, Belonidae can be preserved as a monophyletic family only by the inclusion of the sauries. This is a strong result because the *Belone*/saury clade is well supported by decay indices and bootstrap proportions.

The nonmonophyly of Hemiramphidae is more complicated. Halfbeaks of the marine genus *Hemiramphus* appear most closely related to flyingfishes, a result that is supported by high decay indices and is found in all analyses. The internally fertilized halfbeaks (*Zenarchopterus, Nomorhamphus, Dermogenys*, and *Hemirhamphodon*) form a clade (hereafter referred to as the IWP freshwater halfbeak clade, because all species are Indo-West Pacific estuarine or freshwater forms) and are more closely related to needlefishes and sauries than to the marine halfbeaks. Finally, a clade of the marine halfbeak genera, *Hyporhamphus* and *Chriodorus*, emerges as a separate lineage from the main stem of the cladogram. The node that defines all taxa excluding *Hemiramphus* and flyingfishes has a decay index of only one. In trees one step longer, the *Chriodorus*/*Hyporhamphus* lineage clusters with the *Hemiramphus* and flyingfish clade. This alternative, less parsimonious topology does not affect the interpretation of jaw evolution (see below).

The general pattern of the tree is that halfbeaks form a paraphyletic assemblage that is basal relative to needlefishes and sauries. Support for nodes that define this pattern are strong (as assessed by decay indices and bootstrap proportions). Also, separate analyses of nuclear and mitochondrial datasets repeat this overall topology (see Fig. 3). In the separate Tmo analysis, marine halfbeaks and flyingfishes form a clade that is the first to diverge from the main stem of the tree, IWP halfbeaks form a paraphyletic assemblage that is next to branch off, and finally needlefishes and sauries are more deeply nested. In the separate mitochondrial analysis (cyt *b* and 16S combined), the *Hemiramphus* and flyingfish clade shows the same basal position as in the total evidence tree, but IWP halfbeaks and the *Chriodorus*/*Hyporhamphus* clade are nested within the needlefish/saury clade. Because the morphological dataset was relatively small, the resultant tree (not shown) is largely unresolved.

To further test the position of the root and the general structure of the beloniform tree, a larger-scale analysis of Tmo was conducted that included both the beloniform taxa and the 21 sequences for labroids provided by Streelman and Karl (1997). The resultant topology (with labroids used as outgroups) confirms the position of ricefishes at the base of the beloniform tree and the relatively basal position of halfbeaks and flyingfishes relative to needlefishes and sauries (the topology is essentially identical to the one shown in Fig. 3).

The sensitivity of the phylogenetic analyses to weighting

TABLE 2. Summary statistics for data partitions as summarized on the total evidence tree.

Gene	Position	Number of characters	Number informative[1]		Steps		CI (excluding uninformative characters)	
Tmo	1st	162	36	(13)	65	(19)	0.63	(0.77)
	2nd	161	12	(5)	29	(12)	0.72	(0.62)
	3rd	161	116	(40)	378	(91)	0.48	(0.54)
Cyt b	1st	214	74	(33)	396	(81)	0.28	(0.44)
	2nd	214	30	(10)	69	(24)	0.53	(0.58)
	3rd	213	212	(130)	2208	(705)	0.19	(0.18)
16S		371	98	(33)	398	(110)	0.36	(0.33)
Morphology		36	36		79		0.47	
Total		1532	614	(264)	3622	(1042)	0.27	(0.27)

[1] Transversions shown in parentheses.

schemes was also tested. Relative weights were derived from estimates of the consistency and homoplasy of different data partitions. Table 2 shows confidence indes (CI) values estimated from optimizations of various groups of characters on the total evidence tree. Third positions of cyt *b*, although contributing most of the steps, are also the most homoplastic (both transitions and transversions). Therefore, these changes were downweighted by 1/2, 1/5, 1/10, and zero relative to other characters. The total evidence topology, particularly the relationship between the major clades and the position of halfbeaks relative to needlefishes, was robust to these manipulations. In the separate analysis of mitochondrial data, downweighting cyt *b* third positions brought the resultant trees more in line with the total evidence pattern, that is, IWP halfbeaks and the *Chriodorus*/*Hyporhamphus* fell outside the needlefish/saury clade.

The evolution of jaw characters was examined by optimization. Figure 4 shows optimized changes on a simplified version of the total evidence phylogeny (the same optimizations are observed on the full tree). The elongation of the upper jaw in adult fishes (character 36) unambiguously defines the clade of needlefishes and sauries, whereas the elongation of the lower jaw in juveniles (34) unambiguously defines Exocoetoidei (Beloniformes to the exclusion of ricefishes). Elongation of the lower jaw in adults (35) has multiple, equally parsimonious optimizations, but always occurs more basally in the tree than the elongation of the upper jaw in adults (36). Because the *Chriodorus*/*Hyporhamphus* lineage can move to a sister taxon position with the *Hemiramphus* and flyingfish clade in trees that are one step longer, jaw optimizations were also examined on this topology (not shown). Elongation of the upper jaw in adults (36) was again found to be relatively derived within the tree, compared to elongation of the lower jaw in juveniles (34) and adults (35). These patterns suggest the a hypothetical ancestor to needlefishes would have been characterized by a ''halfbeak'' juvenile and adult morphology.

DISCUSSION

Phylogeny and Taxonomy

In the following, results from the total evidence analysis are emphasized; the separate analysis of Tmo, and the weighted analysis of the mitochondrial genes are generally in agreement. Historically, Beloniformes to the exclusion of Adrianicthyidae is well supported by a number of morphological characters (summarized in Collette et al. 1984) and has been divided into two groups, with needlefishes and sauries united in one, and halfbeaks and flyingfishes in the other (Fig. 1; Schlesinger 1909; Regan 1911; Nichols and Breder 1928; Collette et al. 1984). This division was initially based on scale size, but later received support from other character systems, including lateral lines (Parin and Astakhov 1982), and branchial arches and pharyngeal teeth (Rosen 1964; Collette 1966; Rosen and Parenti 1981; Collette et al. 1984). The results presented here suggest that beloniform taxonomy will require an overhaul to reflect phylogenetic relationships; in particular, the families Belonidae and Hemiramphidae will likely require revision.

Belonidae (needlefishes) is not currently monophyletic unless sauries (Scomberesocidae) are also included in the family. The relationship between the sauries and *Belone* is well supported; however, the position of this clade within the rest of the needlefishes is not, as indicated by low decay indices in the basal parts of the needlefish portion of the tree. Addition of further data may show that the *Belone* and saury clade is basal within needlefishes. If this is the case, *Belone* could be added to Scomberesocidae, and the rest of the needlefishes would then represent a monophyletic Belonidae.

''Hemiramphidae,'' as currently defined, appears to be a paraphyletic assemblage from which other beloniform taxa have diverged. The flyingfishes are strongly supported as the sister group of *Hemiramphus*. Previously, flyingfishes have been considered the sister group to all halfbeaks rather than a subset thereof. However, in light of comments by Nichols and Breder (1928, p. 437), ''it is from the *Hemiramphus* type of halfbeak that flyingfishes would have developed,'' these novel results may not be unexpected. These authors based their suggestion on similarities of pelvic fins and juvenile coloration between *Hemiramphus* and two-winged flyingfishes. Also, Parin and Astakhov (1982) pointed out that no clear lateral line characters could be used to separate flyingfishes and halfbeaks, as evidenced in the classification scheme of Greenwood et al. (1966) uniting these groups in a single family. By clarifying the relationship of flyingfishes to the rest of the beloniforms, this study sets the stage for subsequent analyses of this group and the elucidation of the evolution of gliding in fishes.

The position of the *Hyporhamphus* and *Chriodorus* lineage (both are marine halfbeaks) is currently uncertain. In the total

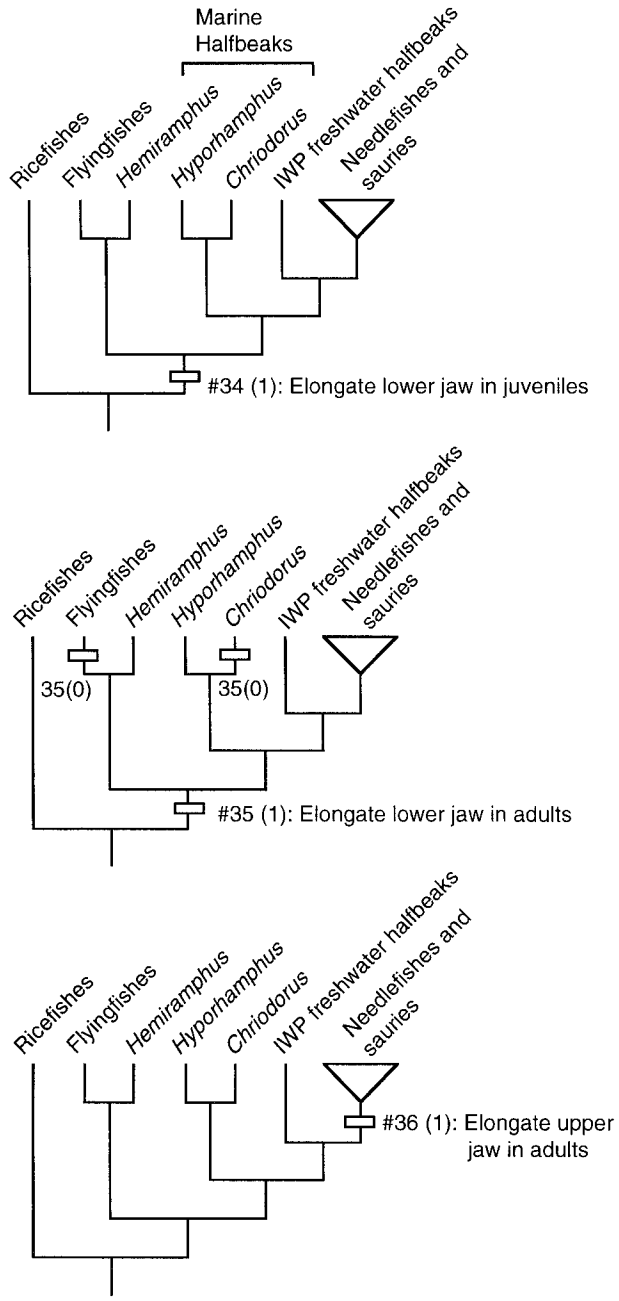

FIG. 4. Reconstructions of jaw characters on simplified version of beloniform tree. Character 35 has three equally parsimonious optimizations, of which only one is shown; in all cases, 35(1) occurs before 36(1). 35(0) occurs where adults lose the extended lower jaw: In flyingfishes and in one marine halfbeak group (for *Chriodorus*, which has a short lower jaw as an adult).

evidence tree, these taxa form a separate clade that branches from the main stem of the tree, however, in trees one step longer, the group clusters with the *Hemiramphus* and flyingfish clade. If further evidence supports the latter topology, marine halfbeaks and flyingfishes could be united in a monophyletic family. IWP halfbeaks (*Zenarchopterus, Nomorhamphus, Dermogenys,* and *Hemirhamphodon*), surprisingly, appear to be the sister group to needlefishes and sauries, indicating that they may merit separate familial status.

Ontogeny and Phylogeny: Reassessing Recapitulation

The total evidence hypothesis suggests that de Beer (1930) was wrong about the evolution of jaw development in Beloniformes. Nichols and Breder (1928, fig. 171) had earlier presented a tree that showed needlefishes as the basal taxon within beloniforms (ricefishes had not yet been added to the clade), with halfbeaks evolved from a ''primitive'' needlejawed form. Because needlefishes pass through a ''halfbeak'' stage during growth (and because they were considered primitive), Nichols and Breder (followed by de Beer) concluded that halfbeaks were essentially derived ''fixed larval'' needlefishes, or paedomorphs.

The tree presented here shows the opposite. Hemiramphids are relatively basal, in agreement with the hypotheses of Parin (1961) and Rosen (1964). It is therefore most parsimonious to assume that the ''halfbeak'' form is plesiomorphic and the ''needlefish'' morphology is derived. Collette et al. (1984), from a tree based on a small suite of morphological characters (see Fig. 1), drew approximately the same conclusion. Here, relationships are more complicated, but allow less equivocal reconstructions of changes in ontogeny. The principal evolutionary changes in jaw characters, as inferred from the new topology, show that the elongation of the upper jaw in adult fishes (36) is derived relative to the elongation of the lower jaw in juveniles (34) and in adults (35; Fig. 4). This suggests that the ancestral condition within Exocoetoidei was a ''halfbeak'' juvenile and adult.

Severtzov (1927), as discussed in de Beer (1930) and Gould (1977), interpreted needlefish evolution from the perspective of recapitulation. Needlefish ontogeny paralleled (and recapitulated) beloniform phylogeny. Thus, larval needlefishes with short jaws represented a flyingfish ancestor and juvenile needlefishes with an elongate lower jaw represented a halfbeak ancestor. Each ontogenetic stage in needlefishes thus represented the adult morphology of an ancestor. Severtzov was off the mark with his assessment of needlefish ancestry—flyingfishes are clearly nested within halfbeaks, and thus do not represent an ancestral form (the impossibility of establishing ''ancestors'' aside). However, in the context of character polarity, his hypothesis of jaw evolution in Beloniformes is more correct than that of de Beer (1930).

Is jaw evolution in needlefishes recapitulatory? Juvenile needlefishes certainly resemble adult halfbeaks, and the ontogeny of needlefishes does appear to parallel phylogenetic changes in jaw characters (Fig. 5). Below, some difficult issues that still surround the idea of recapitulation are highlighted using a discrete character-based context (e.g., see Mabee 1993), rather than a growth trajectory approach (Alberch et al. 1979). The three jaw characters are considered a functional complex (related to feeding). Thus far, I have conflated the morphological expressions of heterochronic change (paedomorphosis and peramorphosis) with the phylogenetic phenomena they produce (respectively, reverse recapitulation and recapitulation), according to Alberch et al. (1979). Below, recapitulation is considered a phylogenetic phenomenon resulting from several possible peramorphic processes (acceleration, hypermorphosis, or predisplacement) that occur at the level of individual organisms. The

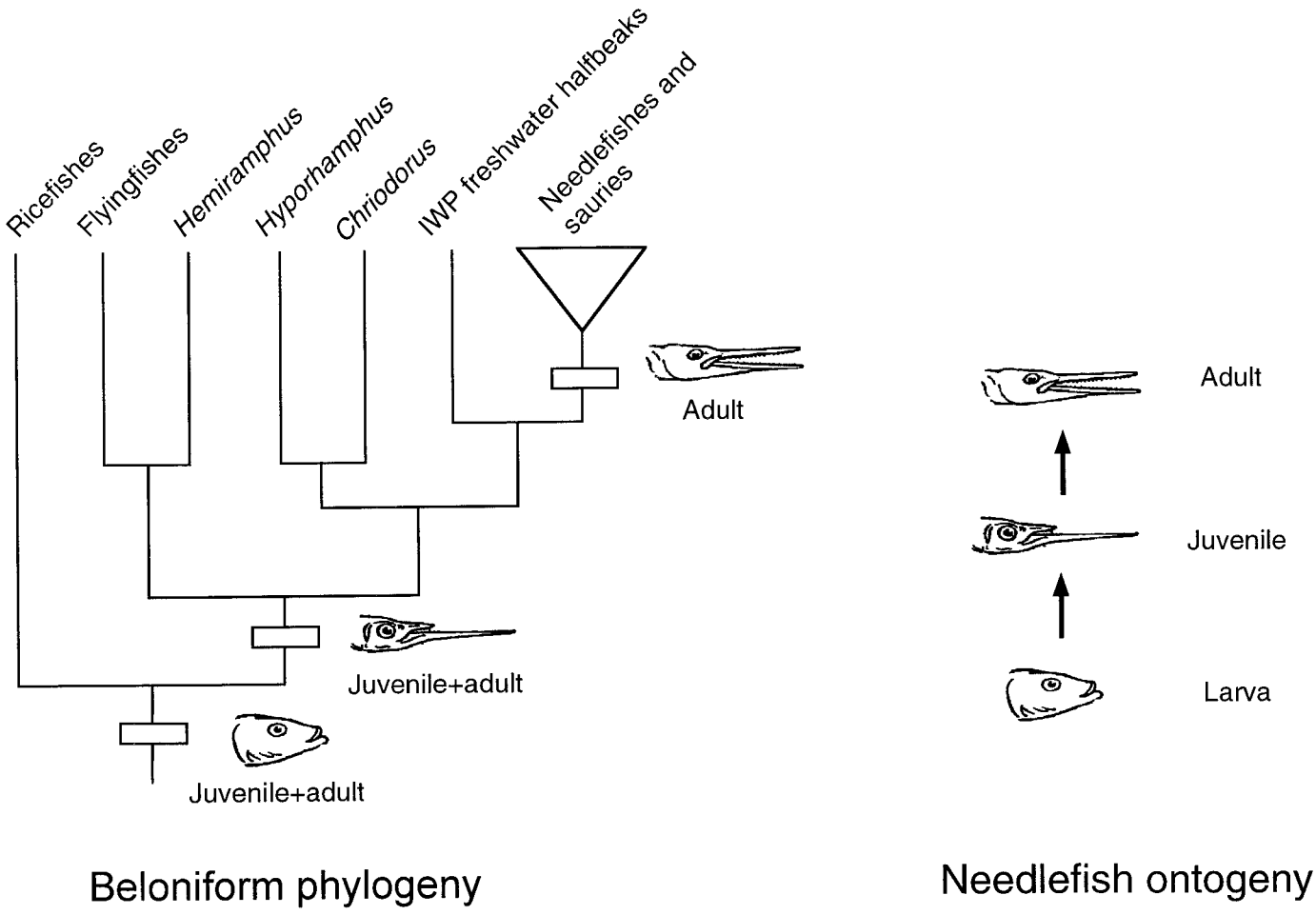

FIG. 5. Simplified beloniform phylogeny with jaw change reconstructions and generalized needlefish ontogeny.

main point is that an adequate phylogenetic approach for clearly identifying or refuting ''true'' or Haeckelian recapitulation has not been proposed.

In Haeckel's original formulation, recapitulation represented the repetition of adult stages of the ancestor during the ontogeny of the descendant (Gould 1977). However, a Haeckelian recapitulation scenario for needlefishes is subject to the criticisms of Garstang (1922), who would argue that the ''halfbeak'' form of juvenile needlefishes represents (or is homologous with) the ''halfbeak'' form of juvenile halfbeaks, rather than the morphology of any adult halfbeak. Halfbeak adults have simply diverged less from their juvenile morphology, thus they appear similar to needlefish juveniles. This argument derives from von Baer's laws (1853, p. 214): ''It is only because the least developed forms of animals are but little removed from the embryonic condition, that they retain a certain similarity to the embryos of higher animals.'' This criticism was broadly recognized, and further developed in the early 20th century, particularly by German biologists (see de Beer 1930). Gould (1977, p. 234) summarizes the situation by stating his belief that there are many ''cases that have nothing to do with recapitulation, but only mimic it in the workings of von Baer's laws.'' In a discrete, character-based, phylogenetic approach to the evolution of development, there is no strategy for separating such ''von Baerian'' recapitulation (sensu Lovtrup 1978; also called ''Meckelian'' recapitulation by Garstang 1922) from the authentic phenomenon of ''true'' or Haeckelian recapitulation.

Haeckelian recapitulation must result strictly from terminal additions to the ontogeny of a taxon, relative to its sister group (Gould 1977; Lovtrup 1978). In contrast, a pattern of von Baerian recapitulation results from the differential divergence of adults in different groups from shared juvenile forms (Garstang 1922). Figure 6 shows a hypothetical example of the difference in phylogenetic patterns predicted by ''true'' versus von Baerian recapitulation. A represents a character that, in taxon X and Y, is similar between juveniles and adults (e.g., the ''halfbeak'' morphology of juvenile and adult halfbeaks). In Figure 6a, B is a terminal addition to the ancestral ontogeny of taxon Z, and a pattern of Haeckelian recapitulation is the result. In Figure 6b, B is a modification or substitution of A_{adult} and produces a pattern of von Baerian recapitulation.

The critical test between types of recapitulation is therefore the identity of character A between taxa X, Y, and Z. If taxon Z has character A_{adult}, then Haeckelian recapitulation is the pattern, but if Z has character $A_{juvenile}$, then only von Baerian recapitulation is observed. The problem, of course, is that

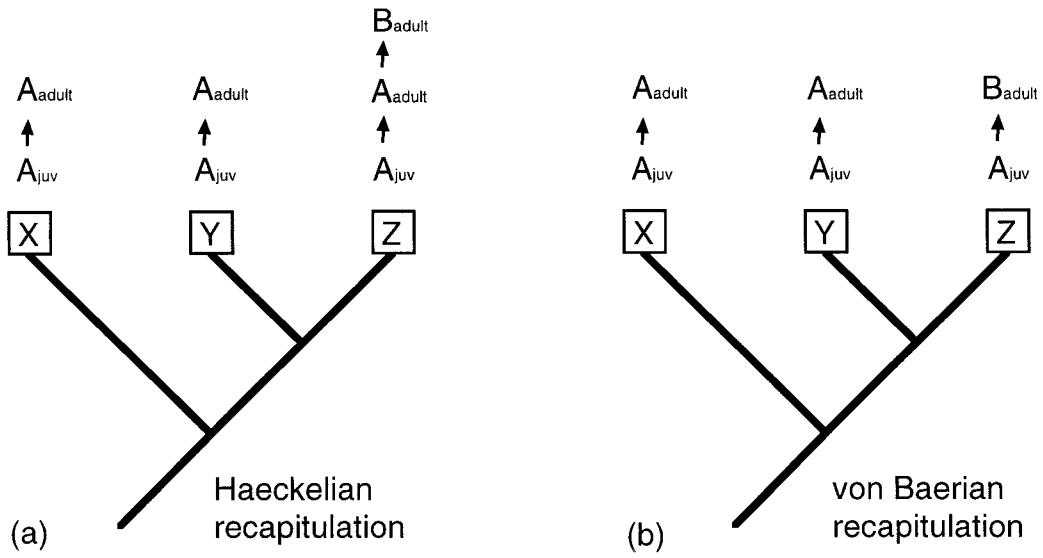

FIG. 6. Alternative evolutionary patterns of character ontogeny. (a) Terminal addition via peramorphosis that produces a pattern of Haeckelian recapitulation. (b) Terminal modification produces a pattern of von Baerian recapitulation. X, Y, and Z are taxa. A_{juv} is the juvenile character state, A_{adult} and B_{adult} are alternative adult character states. Arrows represent ontogenetic change.

A_{adult} and $A_{juvenile}$ are similar. Nevertheless, it will be important to try to distinguish the two if we want to understand the prevalence of heterochrony. The effects of not differentiating between ''true'' and von Baerian recapitulation are evident from Mabee's (1993) study on ontogeny and phylogeny in centrarchid fishes. She considers eight separate types of ontogenetic modification, of which one is identical to Figure 6 (with A-to-A changes in the outgroup, and an A-to-B change in the ingroup), and is labeled ''terminal addition.'' By calling this type of change an ''addition,'' it is linked to peramorphosis, the heterochronic pattern associated with recapitulation by Alberch et al. (1979). Mabee finds that approximately 30% of morphological evolution in centrarchids occurs via terminal addition, thus one might conclude that peramorphosis (and recapitulation) are relatively common in sunfishes. However, by not considering the possibility of recapitulation operating under von Baer's laws rather than Haeckel's, this estimate may be exaggerated.

Alberch (1985) noted the problem of inferring homology between different developmental stages of different taxa. Because the most powerful test of homology, character congruence (Patterson 1982; de Pinna 1991), is usually carried out on individuals of the same development stage (semaphoronts), the problem of assessing homology between different developmental stages is an open one. Clearly, what we really would like to know is the mechanism by which one state is transformed into another over evolutionary time. The growth trajectory approach of Alberch et al. (1979; for phylogenetic modifications, see Fink 1982) describes the processes that would produce peramorphosis (and thus the recapitulatory pattern of Fig. 6). For example, if needlefish jaws are truly peramorphic and therefore recapitulate halfbeak jaws, one might expect their growth (particularly the growth of their upper jaw) to have earlier onset time, delayed offset time, or a higher rate. In contrast, if the evolution of the upper jaw is more complex and cannot be described by simple changes in parameters of timing and rate, peramorphosis and recapitulation are not the answer.

Without age and growth trajectory data for these fishes, I hesitate to speculate on patterns and processes of heterochrony. However, the phylogeny presented here provides a guide for future consideration of jaw evolution. A first step would be the collection of age/growth series data for exemplars of each clade that bracket the transition between jaw morphology character states. Other interesting taxa to consider include *Belonion*, a South American freshwater needlefish, which matures at a very small size (2 in) without elongating its upper jaw (thus it looks superficially like a halfbeak); Collette (1966) called this taxon paedomorphic. Four halfbeak genera (like some flyingfishes) develop an elongate lower jaw as juveniles, but subsequently lose it as adults; the developmental mechanisms of this change are unexplored.

It should be noted that, whereas some authors have proposed coding ontogenetic transformations themselves as characters (Lundberg 1973; de Queiroz 1985), I follow most recent authors (e.g., Wheeler 1990; Mabee 1993) by using morphological attributes as characters. Mabee (1993) has provided an excellent discussion of the effects of alternative character coding on discernment of evolutionary changes in ontogeny. The coding used here embodies the caution of Alberch (1985) that similar morphological features from different ontogenetic stages may not be ''homologous'' with one another—adult and juvenile stages are considered separately. For example, the presence of an extended lower jaw is a distinct character for juveniles (34) and adults (35). This coding allows seemingly counterintuitive reconstructions, such as the gain of characters by adults that are already present in juveniles. Nevertheless, the (hypothetical) evolution of a new flyingfish lineage whose adults had extended lower jaws, would embody just this situation. By treating ontogenetic stages separately and testing homology only through

character congruence (Patterson 1982), the coding used here is compatible with de Beer's (1971) warning that ontogeny cannot be used to establish homology, a perspective strengthened by findings in developmental genetics (Abouheif 1997).

Conclusion

The total evidence tree provides a number of taxonomic surprises. Both halfbeaks and needlefishes are nonmonophyletic groups, despite their distinctive morphologies. Sauries are nested within needlefishes; sauries, needlefishes, and flyingfishes are all nested within halfbeaks. It is clear that considerable taxonomic revision will be required, but I do not suggest any changes here—increased sampling of halfbeaks and flyingfishes combined with morphological work are prior requirements. An exciting future goal will be clarification of the phylogenetic position of IWP freshwater halfbeaks relative to needlefishes.

The combined morphological and DNA phylogeny resolves the dispute between Severtzov (1927) and de Beer (1930): Needlefishes are derived relative to halfbeaks, rather than the other way around. Whether needlefish jaws ''recapitulate'' the halfbeak morphology, and what role heterochrony has played in the group remains to be elucidated. Aside from jaw morphology beloniforms express a rich variety of ontogenetic changes in morphology (e.g., the melanistic lobe of the dorsal fin in *Tylosurus*, body bars in *Hemiramphus*) that could also be profitably examined with genetic and growth data.

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Literature Cited

Abouheif, E. 1997. Developmental genetics and homology: a hierarchical approach. Trends Ecol. Evol. 12:405–408.

Agassiz, L. 1849. Twelve lectures on comparative embryology. Henry Flanders, Boston, MA.

Alberch, P. 1985. Problems with the interpretation of devlopmental sequences. Syst. Zool. 34:46–58.

Alberch, P., S. J. Gould, G. F. Oster, and D. B. Wake. 1979. Size and shape in ontogeny and phylogeny. Paleobiology 5:296–317.

Anderson, W. D., III, and B. B. Collette. 1991. Revision of the freshwater viviparous halfbacks of the genus Hemirhamphodon (Teleostei: Hemiramphidae). Ichthyol. Explor. Freshwaters 2: 151–176.

Banford, H. M., and B. B. Collette. 1993. *Hyporhamphus meeki*, a new species of halfbeak (Teleostei: Hemiramphidae) from the Atlantic and Gulf Coasts of the United States. Proc. Biol. Soc. Wash. 106:369–384.

Berry, F. H., and L. R. Rivas. 1962. Data on six species of needlefishes from the western Atlantic. Copeia 1962:152–160.

Bonner, J. T., ed. 1982. Evolution and development. Springer-Verlag, New York.

Boughton, D. A., B. B. Collette, and A. R. McCune. 1991. Heterochrony in jaw morphology of needlefishes (Teleostei: Belonidae). Syst. Zool. 40:329–354.

Brooks, D. R., and D. A. McLennan. 1991. Phylogeny, ecology, and behavior. Univ. of Chicago Press, Chicago.

Brower, A. V. Z., and V. Schawaroch. 1996. Three steps of homology assesment. Cladistics 12:265–272.

Chang, Y.-S., F.-L. Huang, and T.-B. Lo. 1994. The complete nucleotide sequence and gene organization of carp (*Cyprinus carpio*) mitochondrial genome. J. Mol. Evol. 38:138–155.

Collette, B. B. 1966. *Belonion*, a new genus of fresh-water needlefishes from South America. Am. Mus. Novit. No. 2274:1–22.

———. 1974. South American freshwater needlefishes (Belonidae) of the genus *Pseudotylosurus*. Zool. Meded. (Leiden) 48: 169–186.

———. 1977. Belonidae: FAO species indentification sheets, west central Atlantic. FAO, Rome.

Collette, B. B., and N. V. Parin. 1970. Needlefishes (Belonidae) of the Eastern Atlantic Ocean. Atl. Rep. 11:1–60.

Collette, B. B., G. E. McGowen, N. V. Parin, and S. Mito. 1984. Beloniformes: development and relationships. Pp. 335–354 *in* H. G. Moser, ed. Ontogeny and systematics of fishes. American Society of Ichthyologists and Herpetologists. Spec. Publ. no. 1.

Cope, E. D. 1887. The origin of the fittest. Macmillan, New York.

de Beer, G. R. 1930. Embryology and evolution. Clarendon Press, Oxford, U.K.

———. 1940. Embryos and ancestors. Clarendon Press, Oxford, U.K.

———. 1971. Homology: an unsolved problem. Oxford Biology Readers no. 11. Oxford Univ. Press, London.

Deleporte, P. 1993. Characters, attributes, and tests of evolutionary scenarios. Cladistics 9:427–432.

de Pinna, M. C. C. 1991. Concepts and tests of homology in the cladistic paradigm. Cladistics 7:367–394.

de Queiroz, K. 1985. The ontogenetic method for determining character polarity and its relevance to phylogenetic systematics. Syst. Zool. 34:280–299.

Fink, W. L. 1982. The conceptual relationship between ontogeny and phylogeny. Paleobiology. 8:254–264.

Garstang, W. 1922. The theory of recapitulation: A critical restatement of the biogenetic law. J. Linn. Soc. Zool. 35:81–101.

Gill, T. 1895. The families of synentognathous fishes and their nomenclature. Proc. U. S. Nat. Mus. 18:167–178.

Gould, S. J. 1977. Ontogeny and phylogeny. Harvard Univ. Press, Cambridge, MA.

Greenwood, P. H., D. E. Rosen, S. H. Weitzman, and G. S. Myers. 1966. Phyletic studies of teleostean fishes, with a provisional

classification of living forms. Bull. Am. Mus. Nat. Hist. 131: 339–456.
Haeckel, E. 1866. Generelle morphologie der organismen. 2 vols. Georg Reimer, Berlin, Germany.
Hyatt, A. 1897. Cycle in the life of the individual (ontogeny) and in the evolution of its own group (phylogeny). Proc. Am. Acad. Arts. Sci. 32:209–224.
Klingenberg, C. P. 1998. Heterochrony and allometry: the analysis of evolutionary change in ontogeny. Biol. Rev. Camb. Philos. Soc. 73:79–123.
Kluge, A. G., and A. J. Wolf. 1993. Cladistics: what's in a word? Cladistics 9:183–199.
Kocher, T. D., W. K. Thomas, S. A. Meyer, S. V. Edwards, S. Paabo, F. X. Villablanca, and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86:6196–6200.
Leviton, A. E., R. J. Gibbs Jr., E. Heal, and C. E. Dawson. 1985. Standards in herpetology and ichthyology. Part 1. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. Copeia 1985:802–832.
Lovtrup, S. 1978. On Von Baerian and Haeckelian recapitulation. Syst. Zool. 27:348–352.
Lundberg, J. G. 1973. More on primitiveness, higher level phylogenies and ontogenetic transformations. Syst. Zool. 22:327–329.
Mabee, P. M. 1993. Phylogenetic interpretation of ontogenetic change: sorting out the actual and artefactual in an empirical case study of centrarchid fishes. Zool. J. Linn. Soc. 1993: 172–291.
Maddison, W. P., and D. R. Maddison. 1992. MacClade. Cers. 3.0. Sinauer, Sunderland, MA.
McKinney, M. L., and K. J. McNamara. 1991. Heterochrony: the evolution of ontogeny. Plenum Press, New York.
Nichols, J. T., and C. M. J. Breder. 1928. An annotated list of the Synentognathi with remarks on their development and relationships. Zoologica (N.Y.) 8:423–448.
Orti, G., P. Petry, I. R. Porto Jorge, M. Jegu, and A. Meyer. 1996. Patterns of nucleotide change in mitochondrial ribosomal RNA genes and the phylogeny of piranhas. J. Mol. Evol. 42:169–182.
Palumbi, S. R. 1996. Nucleic acids. II. The polymerase chain reaction. Pp. 205–247 *in* D. M. Hillis, C. Moritz, and B. K. Mable, eds. Molecular systematics. Sinauer, Sunderland, MA.
Parin, N. V. 1961. Principles of the classification of flyingfishes (families Oxyporhamphidae and Exocoetidae). Tr. Inst. Okeanol. Akad. Nauk. SSSR 43:92–183. (In Russian.)
———. 1967. Review of marine needlefishes of the West Pacific and Indian Oceans. Tr. Okeanol. Inst. 84:3–83. (In Russian; English translation in Natl. Mar. Fish. Serv. Transi. 681.)
———. 1986a. Scomberesocidae. Pp. 610–611 *in* P. L. P. Whitehead, M.-L. Bauchot, J.-C. Hureae, J. Neilsen, and E. Tortonese, eds. Fishes of the North-eastern Atlantic and the Mediterranean. Vol. 2. United Nations Educational, Scientific and Cultural Organization, Paris.
———. 1986b. Exocetidae. Pp. 612–619 *in* P. L. P. Whitehead, M.-L. Bauchot, J.-C. Hureae, J. Neilsen, and E. Tortonese, eds. Fishes of the North-eastern Atlantic and the Mediterranean. Vol. 2. United Nations Educational, Scientific and Cultural Organization, Paris.
Parin, N. V., and D. A. Astakhov. 1982. Studies on the acoustico lateralis system of beloniform fishes in connection with their systematics. Copeia 1982:276–291.
Patterson, C. 1982. Morphological characters and homology. Pp. 21–74 in K. A. Joysey and A. E. Friday, eds. Problems in phylogenetic reconstruction. Academic Press, London.
Regan, C. T. 1911. The classification of the teleostean fishes of the order Synentognathi. Ann. Mag. N. Hist. 8(7):327–335.
Rosen, D. E. 1964. The relationships and taxonomic position of the halfbeaks, killifishes, silversides, and their relatives. Bull. Am. Mus. Nat. Hist. 127:217–268.
Rosen, D. E., and L. R. Parenti. 1981. Relationships of *Oryzias* and the groups of atherinomorph fishes. Am. Mus. Novit. 2719:1–25.
Schlesinger, Z. 1909. Zur phylogenie und ethologie der Scombresciden. Verh. Zool.-Bot. Ges. Wien. 59:302–339.
Seutin, G., B. N. White, and P. T. Boag. 1991. Preservation of avian blood and tissue samples for DNA analyses. Can. J. Zool. 69: 82–90.
Severtsov, A. N. 1927. Uber die Beziehungen zwischen der Ontogenese und der Phylogenese der Tiere. Jena. Z. Naturwiss. 56: 51–180.
Sorenson, M. D. 1996. TreeRot. Univ. of Michigan, Ann Arbor.
Streelman, J. T., and S. A. Karl. 1997. Reconstructing labroid evolution with single-copy nuclear DNA. Proc. R. Soc. Lond. B Biol. Sci. 264:1011–1020.
Swofford, D. L. 1999. PAUP*: phylogenetic analysis using parsimony (* and other methods). Vers. 4. Sinauer, Sunderland, MA.
Uwa, H., and L. R. Parenti. 1988. Morphometric and meristic variation in ricefishes, genus *Oryzias*: a comparison with cytogenetic data. Jpn. J. Ichthyol. 35:159–166.
von Baer, K. E. 1853. Fragments relating to philosophical zoology. Pp. 176–238 *in* A. Henfrey and T. H. Huxley, eds. Scientific memoirs, selected from the transactions of foreign academies of science and from foreign journals. Taylor and Francis, London.
Wheeler, Q. D. 1990. Ontogeny and character phylogeny. Cladistics 6:225–268.

Corresponding Editor: L. Bernatchez

APPENDIX 1

List of specimens used in analysis. Institutional abbreviations follow Leviton et al. (1985).

Species	Preparation	Catalog number	Locality
Ablennes hians	N-2a	CU 75115	Plantation Fisheries, Florida
A. hians	N-2b	USNM 347820	Philippine Islands
Belone belone	N-35a	USNM 352451	Courtsmacsherry Bay, Ireland
B. belone	N-35b	USNM 352453	Courtsmacsherry Bay, Ireland
Belone svetovidovi	N-15	CU 78066	Courtsmacsherry Bay, Ireland
B. svetovidovi	N-16	USNM 352454	Courtsmacsherry Bay, Ireland
Belonion dibranchodon	N-14a	CU 78499	Rio Atabapo, Venezuela
B. dibranchodon	N-14b	CU 78499	Rio Atabapo, Venezuela
Belonion apodion	N-55	INPA 14339	Rio Negro, Brazil
Platybelone argalus argalus	N-12a	UF 99881	Tortugas Bank, Florida
Platybelone argalus platyura	N-12b	USNM 348277	Bolinaro Mkt., Philippines
Potamorrhaphis eigenmanni	N-17	CU 77949	Rio Matos, Bolivia
P. eigenmanni	N-18	CU 77950	Arroyo Mururita, Bolivia
Potamorrhaphis guianensis	N-13a	CU 76874	Rio Caicara, Venezuela
P. guianensis	N-13b	CU 76873	Rio Caicara, Venezuela
Potamorrhaphis petersi	N-27a	CU 78500	Rio Atabapo, Venezuela
Pseudotylosurus augusticeps	N-28a	CU 78505	Rio Napo, Ecuador
P. augusticeps	N-28b	CU 78505	Rio Napo, Ecuador
P. augusticeps	N-41	INPA 13132	Rio Tapajos, Brazil
Strongylura exilis	N-38a	STRI 00192	E Pacific, Panama
S. exilis	N-38b	STRI 00192	E Pacific, Panama
Strongylura hubbsi	N-30a	CU 77876	Rio Usumacinta, Guatemala
S. hubbsi	N-30b	CU 77876	Rio Usumacinta, Guatemala
Strongylura fluviatilis	N-29a	CU 78507	Rio Cayapas, Ecuador
S. fluviatilis	N-29b	CU 78507	Rio Cayapas, Ecuador
Strongylura incisa	N-19	CU 78063	Cubao Mkt., Philippines
S. incisa	N-20	CU 78064	Malaban Mkt., Philippines
Strongylura krefftii	N-31a	NTM S. 14320-001	Shady Camp, Australia
S. krefftii	N-31b	NTM S. 14320-001	Shady Camp, Australia
Strongylura leiura	N-32a	AMS I.37226001	Wallis Lake, Australia
S. leiura	N-32b	AMS I.37226002	Wallis Lake, Australia
Strongylura marina	N-7a	USNM uncat.	Virginia Institute of Marine Science, Atlantic
S. marina	N-7b	ID by H. M. Banford	Virginia Institute of Marine Science, Atlantic
Strongylura notata forsythia	N-1a	CU 75110	Long Key, Florida
Strongylura notata notata	N-1b	CU 77875	Caye Caulker, Belize
Strongylura scapularis	N-48	STRI 00194	E Pacific, Panama
Strongylura senegalensis	N-39a	USNM 348315	Volta estuary, Ghana
S. senegalensis	N-39b	CU 78068	Volta estuary, Ghana
Strongylura strongylura	N-21	CU 78065	Bolinaro, Philippines
S. strongylura	N-22	USNM 347834	Bolinaro, Philippines
Strongylura timucu	N-4b	CU 75117	N Hobe Beach, Florida
S. timucu	N-4a	CU 75113	N Hobe Beach, Florida
Tylosurus acus acus	N-3a	CU 75116	Plantation Fisheries, Florida
Tylosurus acus melanotus	N-3b	CU 77948	Makassar Strait, Sulawesi
Tylosurus crocodilus	N-23	USNM 347836	Manila Mkts., Philippines
T. crocodilus	N-24	USNM 348293	Bolinaro, Philippines
Tylosurus gavialoides	N-33a	no specimen	Wallis Lake, Australia
T. gavialoides	N-33b	ID by J. Paxton	Wallis Lake, Australia
Tylosurus punctulatus	N-34	NTM S. 14343-001	Fannie Bay, Darwin, Australia
Xenentodon cancila	N-25	CU 77144	India–West Bengal (aquarium)
X. cancila	N-26	no specimen	Cambodia–Tonle Sap
Scomberesox saurus	N-36b	no specimen	Majorca
S. saurus	N-36c	ID by E. Massuti	Majorca
Cololabis saira	N-43a	NMST-P 54046	Japan
Hemiramphus balao	N-11a	UF 99879	Tortugas Bank
H. balao	N-11b	UF 99879	Tortugas Bank
Hemiramphus brasiliensis	N-05a	CU 75111	Long Key, Florida
H. brasiliensis	N-05b	UF 99880	Tortugas Bank
Hyporhamphus quoyi	N-49a	ZRC 40626	Singapore
H. quoyi	N-49b	ZRC 40626	Singapore
Chriodorus atherinoides	N-51a	no specimen	Florida Bay–Bamboo Bank
C. atherinoides	N-51b	ID by R. McBride	Florida Bay–Bamboo Bank
Zenarchopterus buffonis	N-50a	CU 77844	Manado, Sulawesi
Z. buffonis	N-50b	CU 77844	Manado, Sulawesi

APPENDIX 1
Continued.

Species	Preparation	Catalog number	Locality
Nomorhamphus ravnaki	N-52a	USNM 338497	Bontonomai, Sulawesi
N. ravnaki	N-52b	USNM 338351	Malawa, Sulawesi
Dermogenys weberi	N-53a	CU 78509	Lake Matano, Sulawesi
D. weberi	N-53b	CU 78509	Lake Matano, Sulawesi
Hemirhamphodon pogonognathus	N-54a	uncataloged	Singapore
H. pogonognathus	N-54b	uncataloged	Singapore
Cypselurus melanurus	N-10b	UF 99882	Tortugas Bank, Florida
C. melanurus	N-10a	UF 99877	Tortugas Bank, Florida
Parexocoetus brachypterus	N-09b	UF 99883	Tortugas Bank, Florida
P. brachypterus	N-09a	UF 99876	Tortugas Bank, Florida
Oryzias matanensis	N-44a	CU 78508	Lake Matano, Sulawesi
O. matanensis	N-44b	CU 78508	Lake Matano, Sulawesi

APPENDIX 2
Morphological characters used for phylogenetic analysis.

1. Extension of the lateral line onto caudal peduncle: 1, present; 0, absent (Parin and Astakhov 1982).
2. Number of pores on the vertical limb of the canalis preopercularis: 1, two pores; 0, many pores (Parin and Astakhov 1982).
3. Gill rakers: 1, present; 0, absent (Berry and Rivas 1962).
4. Posterior lobe on dorsal fin in juveniles: 1, present; 0, absent (Berry and Rivas 1962).
5. Shape of nasal papillae: 1, elongate; 0, spatulate (Collette 1974).
6. Shape of first neural spine: 1, elongate; 0, not elongate (Collette 1966).
7. Density of pharyngeal teeth: 1, sparse; 0 dense (Collette 1966).
8. Canalis sphenoticalis/pteroticalis: 1, present; 0, absent (Parin and Astakhov 1982; Boughton et al. 1991).
9. Canalis postemporalis: 1, present; 0, absent (Parin and Astakhov 1982; Boughton et al. 1990).
10. Canalis epioticalis: 1, present; 0, absent (Parin and Astakhov 1982; Boughton et al. 1990).
11. Curvature of canalis frontalis: 1, acutely recurved; 0, shallowly curved (Parin and Astakhov 1982; Boughton et al. 1990).
12. Gap in canalis frontalis: 1, gap; 0, no gap (Parin and Astakhov 1982).
13. Medial branch of canalis frontalis: 1, present; 0, absent (Parin and Astakhov 1982).
14. Postorbital segment of the canalis frontalis: 1, present; 0, absent (Parin and Astakhov 1982; Boughton et al. 1990).
15. Anteromedial branch of the canalis nasalis: 1, present; 0, absent (Parin and Astakhov 1982; Boughton et al. 1990).
16. Shape of caudal fin: 1, strongly forked; 0, shallowly forked or truncate (Boughton et al. 1990).
17. Length of ventral lob of caudal fin: 1, longer than dorsal lobe; 0, same length or shorter than dorsal lobe (Boughton et al. 1990).
18. Posterior lobe of dorsal fin in adults: 1, present; 0, absent (Collette and Parin 1970).
19. Second upper pharyngeal toothplate: 1, reduced or absent; 0, present (Collette 1966).
20. Paired frontal bones: 1, fused; 0, separated (Collette 1966).
21. Pectoral branch of the lateral line: 1, absent; 0, present (Parin and Astakhov 1982).
22. Premaxillary canal: 1, present; 0, absent (Parin and Astakhov 1982).
23. Length of premaxillary canals: 1, short, extends only along proximal third of upper jaw; 0, long (Parin and Astakhov 1982).
24. Superficial secondary canals in head scales: 1, present; 0, absent (Parin and Astakhov 1982).
25. Secondary tubes of the body lateral line: 1, extend both dorsally and ventrally; 0, extend ventrally only or are absent (Collette 1974).
26. Third upper pharyngeal toothplate: 2, fused; 1, joined, but unfused; 0, separate (Rosen 1964; Collette et al. 1984).
27. Fourth upper pharyngeal toothplate: 1, absent; 0, present (Collette et al. 1984).
28. Shape of lower pharyngeal plate: 1, long and narrow; 0, expanded posteriorly (this study).
29. Relation between preorbital and maxillary: 1, preorbital covers lower margin of maxillary; 0, lower margin of maxillary uncovered (Parin 1967; this study).
30. Anal fin rays in males: 1, modified; 0, unmodified (Anderson and Collette 1991).
31. Internal fertilization: 1, present; 0, absent (Anderson and Collette 1991).
32. First anal pterygiphore: 1, enlarged; 0, unmodified (Anderson and Collette 1991).
33. Anterior rays of male anal fin: 1, modified; 0, unmodified (Anderson and Collette 1991).
34. Lower jaw in juveniles: 0, short; 1, elongate (Collette et al. 1984).
35. Lower jaw in adults: 0, short; 1, elongate (Collette et al. 1984).
36. Upper jaw in adults: 0, short; 1, elongate (Collette et al. 1984).

APPENDIX 3.
Morphological character matrix.

Species	1	11	21	31
Strongylura notata	1100000010	1100000000	0100000000	000111
Ablennes hians	1101000000	1111010100	0100000100	000111
Tylosurus acus acus	1101000000	1111011000	0100000100	000111
Tylosurus acus melanotus	1101000000	1111011000	0100000100	000111
Strongylura timucu	1100000010	1101100000	0100000?10	000111
Hemiramphus brasiliensis	0111000000	0?00011000	00?10210?0	000110
Strongylura marina	1100000010	1101100000	0100000?10	000111
Parexocoetus brachypterus	0110000000	0?00011000	00?10110?0	000000
Cypselurus melanurus	0110000000	0?00011000	00?10110?0	000000
Hemiramphus balao	0111000000	0?00011000	00?10210?0	000110
Platybelone argalus	1110000010	0001110000	0100000000	000111
Potamorrhaphis guianensis	1100111000	1101100010	0100100010	000111
Belonion apodion	1100111000	0100000011	00?0101010	000110
Belone svetovidovi	1110000111	0001110000	0110000000	000111
Potamorrhaphis eigenmanni	1100111000	1101100010	0100100010	000111
Strongylura incisa	1100000000	1001000000	0100000?10	000111
Strongylura strongylura	1100000000	1001000000	0100000?10	000111
Tylosurus crocodilus	1101000000	1111011000	0100000100	000111
Xenentodon cancila	1100100000	1100000010	0100101110	000111
Potamorrhaphis petersi	1100111000	1101100010	0100100010	000111
Pseudotylosurus augusticeps	1100000000	1100100000	0100100110	000111
Strongylura fluviatilis	1100000010	1101100000	0100000?10	000111
Strongylura hubbsi	1100000000	1101100000	0100000?10	000111
Strongylura kreffti	1100000000	1101000000	0100000110	000111
Strongylura leiura	1100000000	1101000000	0100000?00	000111
Tylosurus gavialoides	1101000000	1111011000	0100000100	000111
Tylosurus punctulatus	1101000000	1111011000	0100000100	000111
Belone belone	1110000111	0001110000	0110000000	000111
Scomberesox saurus	0010000011	0001100000	1110000010	000111
Strongylura exilis	1100000010	1101100000	0100000?10	000111
Strongylura senegalensis	1100000010	1101100000	0100000010	000111
Cololabis saira	0010000011	0001100000	1110000010	000111
Oryzias matanensis	0010000000	??00000000	?0??0010?0	000000
Strongylura scapularis	1100000010	1101100000	0100000?10	000111
Hyporhamphus quoyi	0110000000	0?00010000	00?10210?0	000110
Zenarchopterus sp.	0110100000	0?00000000	00?00210?1	100110
Chriodorus atherinoides	0110000000	0?00010000	00?10210?0	000100
Nomorhamphus ravnaki	0110100000	0?00000000	00?00210?1	111110
Dermogenys weberi	0110100000	0?00000000	00?00210?1	111110
Hemirhamphodon pogonognathus	0110100000	0?00000000	00?00210?1	100110
Belonion dibranchodon	1100111000	0100000011	00?0101010	000110