INTEGR. COMP. BIOL., 44:366–377 (2004)

Phylogeny and Jaw Ontogeny of Beloniform Fishes[1]

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SYNOPSIS. To investigate jaw evolution in beloniform fishes, we reconstructed the phylogeny of 54 species using fragments of two nuclear (RAG2 and Tmo-4C4) and two mitochondrial (cytochrome b and 16S rRNA) genes. Our total molecular evidence topology refutes the monophyly of needlefishes (Belonidae) and halfbeaks (Hemiramphidae), but supports the monophyly of flyingfishes (Exocoetidae) and sauries (Scomberesocidae). Flyingfishes are nested within halfbeaks, and sauries are nested within needlefishes. Optimization of jaw characters on the tree reveals a diverse array of evolutionary changes in ontogeny. During their development, needlefishes pass through a ''halfbeak'' stage that closely resembles the adult condition in the hemiramphid halfbeaks. The reconstruction of jaw transitions falsifies the hypothesis that halfbeaks are paedomorphic derivatives of needlefishes. Instead, halfbeaks make up a basal paraphyletic grade within beloniforms, and the needlefish jaw morphology is relatively derived. The parallel between needlefish ontogeny and beloniform phylogeny is discussed, and clades amenable to future morphological analysis are proposed.

INTRODUCTION

Phylogeny is integral to understanding the evolution of ontogeny. Without a firm understanding of a group's evolutionary relationships, the polarities of ontogenetic transformations are irretrievable. In particular, determining the role of heterochrony (changes in developmental timing during evolution) depends on phylogenetic patterns. Gould (1977) emphasized this point, which was further developed by Alberch *et al.* (1979), and rephrased in a cladistic context by Fink (1982). Two broad heterochronic patterns have been identified, paedomorphosis and peramorphosis. In taxa that exhibit paedomorphosis, descendant adults exhibit morphological features of the juveniles of their putative ancestors. In peramorphosis, descendant taxa extend the ontogeny of ancestors, so that adult features of the ancestor appear in the juveniles of descendants. In both cases, alterations of developmental timing produce parallels between ontogeny and phylogeny. Numerous studies have implicated heterochrony in the evolution of morphology in fishes (*e.g.*, Bemis, 1984; Winterbottom, 1990; Boughton *et al.*, 1991; Johnson and Brothers, 1993; Zelditch *et al.*, 2000). Gould (1977) points out that the endeavour to assess the relative frequencies of peramorphosis *versus* paedomorphosis may be misplaced in a field with nearly limitless empirical potential. However, the examination of specific cases may still yield valuable insight into the relationship between phylogeny, ontogeny and ecology.

The order Beloniformes, a group that currently includes the needlefishes (Belonidae), halfbeaks (Hemiramphidae), flyingfishes (Exocoetidae), sauries (Scomberesocidae) and ricefishes (Adrianichthyidae) (Rosen and Parenti, 1981; Collette *et al.*, 1984) is a good model system for investigating the evolution of ontogeny. Beloniform species exhibit striking variation in jaw structure that varies both ontogenetically and phylogenetically, and appears related to feeding. The most spectacular ontogenetic changes occur in belonid needlefishes. Larval needlefishes have short jaws of equal length. However, in juveniles, the lower jaw elongates first, producing a morphology that is distinctly reminiscent of a related family, the halfbeaks (Hemiramphidae)—indeed, needlefishes in this juvenile ''halfbeak'' form have been mistakenly described as new species of hemiramphids (Collette and Parin, 1970). Later, the upper jaw elongates as well, giving rise to the nearly equal length jaws of most adult needlefishes. These transformations appear linked to ecological shifts: juvenile needlefishes in the ''halfbeak'' morphology feed primarily on plankton, while most adult needlefishes are piscivorous (Boughton *et al.*, 1991).

The similarity in jaw morphology between juvenile needlefishes and the closely related Hemiramphidae has provoked alternative interpretations. Severtsov (1927; summarized in Gould, 1977) thought that the ontogeny of needlefishes paralleled the phylogeny of 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 would thus be an example of the phenomenon of recapitulation or peramorphosis. Nichols and Breder (1928), on the other hand, building on the work of Schlesinger (1909) and Regan (1911) proposed an alternative beloniform phylogeny. In their scheme, needlefishes are the most basal family, and gave rise to hemiramphids, which in turn gave rise to flyingfishes. They hypothesized that hemiramphids are ''fixed larval'' (or paedomorphic) needlefishes (Nichols and Breder, 1928, p. 435). de Beer (1940) reported this finding in his book ''Embryos and Ancestors''

[1] From the Symposium *Patterns and Processes in the Evolution of Fishes* presented at the Annual Meeting of the Society for Integrative and Comparative Biology, 4–8 January 2003, at Toronto, Canada.

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which emphasized the importance of paedomorphosis as an evolutionary pattern.

Clearly, differentiating between the paedomorphosis and recapitulation scenarios for beloniforms is only possible with a robust phylogenetic hypothesis for the group. Lovejoy (2000) presented a phylogenetic analysis based on 2 mitochondrial genes, a nuclear gene, and morphology for representatives of all 5 beloniform families. The result falsified Nichols and Breder's (1928) paedomorphic hemiramphid origin hypothesis, which predicted a basal position for needlefishes. Instead, hemiramphids were found to represent a basal paraphyletic grade, with needlefishes and flyingfishes relatively derived. The phylogenetic position of needlefishes therefore matched the prediction of Severtsov's (1927) recapitulation hypothesis.

Here, we present an extension and test of Lovejoy's (2000) findings. We have expanded the matrix by adding 14 ingroup taxa to the previous 39, which significantly improves taxonomic coverage of hemiramphids and flyingfishes. We have also added a novel source of character data, by sequencing a 1 kilobase fragment of the nuclear gene, Recombination Activating Gene 2, or RAG2. Our analysis is based on the nuclear RAG2 and Tmo-4C4 genes, and the mitochondrial cytochrome-b and 16S rRNA genes. We use the resultant phylogenetic hypothesis to explore the evolution of jaw ontogeny.

METHODS

Fishes were collected in the field by ourselves 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 reliably yields 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 (catalogue numbers for voucher specimens are listed with sequences in GenBank).

Samples represent all beloniform families, and with the addition of new taxa, 30 of 39 beloniform genera are represented in the study: ten of ten needlefish genera, three of four saury genera, nine of thirteen halfbeak genera, seven of seven flyingfish genera, and one of four ricefish genera. Whenever possible, sequences were collected from two individuals of each species, providing a total of 104 terminal taxa for analysis, representing 54 different beloniform species.

Data collection

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 expectation 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. Recombination Activating Gene 2 (RAG2) is a nuclear gene that appears to evolve slightly faster than Tmo-4C4 (Lovejoy and Collette, 2001), and has proven useful for species-level phylogenetic analyses (Sullivan *et al.*, 2000; Lovejoy and Collette, 2001).

For each sample, approximately 25 mg of tissue was rinsed briefly in water, then DNA purified using Qiagen's spin-column tissue kit. Cells were lysed at 55° 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.

In the case of cyt b, 16S, and Tmo, template for sequencing was initially amplified using published PCR primers. For RAG2, primers were developed by comparing available vertebrate sequences. New primers were then designed for sequencing and additional amplifications (for details and primer lists, see Lovejoy, 2000 and Lovejoy and Collette, 2001). 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 second denaturation at 95° to start, followed by 35 cycles of denaturation at 95° for 30 seconds, annealing at 48°–55° for 60 seconds, and 72° extension for 90 seconds, followed by a final extension of 72° for 5 minutes. In cases where faint secondary bands were detected, template was run in 1% agarose gels, then cut out and cleaned using PCR purification spin columns (Qiagen).

PCR products were cleaned using PCR product presequencing purification kits (Qiagen or Amersham Life Science) and then sequenced using a variety of methods. In some cases, we manually sequenced using the Thermo Sequenase radiolabeled terminator cycle sequencing kit (Amersham Life Science). Other samples were cycle sequenced and run on an ABI 377 automated sequencer according to manufacturer specifications (Applied Biosystems, Inc., Foster City, CA). Some samples were sequenced at the University of Calgary Core DNA and Protein Services using an ABI 377 automated sequencer.

The Tmo, cyt b, and RAG2 sequences were aligned unambiguously using Lasergene (DNASTAR, Madison, WI) or Sequencher software (Genecodes, Ann Arbor, MI). 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 imported in ClustalX (Thompson *et al.*, 1997) and an initial multiple alignment was conducted using the parameters: gap cost = 10, gap length = 10, DNA transition weight = 1. This alignment was used to generate a neighbour-joining distance tree (using K2P distances) using PAUP* (version 4.0b10, Swofford, 2002). The distance tree was used as a ''guide tree'' for subsequent multiple alignments in which gap cost and gap length were varied (from 1 to 20). Results were compared to a hypothesized model for secondary structure proposed by Orti *et al.* (1996) for piranhas, and alignments that inserted gaps in stable regions (stems) were excluded from further consideration. Of the remaining alignments (considered more biologically reasonable), sites and regions where alignment was ambiguous were removed. This rather conservative procedure excluded approximately 100 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, since 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. Additional analyses, incorporating the deleted characters, do not alter the findings reported here. New sequences determined for this study have been deposited in Genbank; see Appendix 1 for list of accession numbers.

Phylogenetic analysis

All 2,516 characters (965 bp RAG2, 497 bp Tmo, 636 bp cyt b, and 415 bp 16S) 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. All characters were uniformly weighted. *Oryzias* (the ricefish representative) was used as an outgroup to root all trees. Nuclear, and mitochondrial datasets were also analyzed separately (using the same algorithm and settings) to investigate the contribution provided by each to the total molecular evidence hypothesis. For RAG2, sequence from the outgroup *Oryzias* was unobtainable due to PCR difficulties. To test the rooting of the tree, we conducted a separate RAG2 analysis with additional outgroups from Genbank, including *Danio* (NM_131385), *Takifugu* (AF108420), *Campylomormyrus* (AF201622), *Gnathonemus* (AF201628), *Chitala* (AF201626), and *Gymnarchus* (AF201629). To test the root of the Tmo tree, we included Genbank sequences for labroids deposited by Streelman and Karl (1997). The two mitochondrial genes were analyzed together 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).

The evolution of jaw characters was examined by optimizing juvenile and adult conditions on the total molecular evidence tree using MacClade (Maddison and Maddison, 1992). Jaw states were derived from the literature and from personal observations of specimens by the authors. We examined the effects of slight changes in tree topology by optimizing characters on alternative topologies.

RESULTS

Figure 1 shows the single most parsimonious tree derived from an unweighted parsimony analysis of the total molecular dataset. The tree is 5,670 steps long, with a consistency index (excluding uninformative characters) of 0.27, and a retention index of 0.58. In most respects, the tree is similar to the total evidence topology presented in Lovejoy (2000). Of the five currently recognized beloniform families, only two are monophyletic: the flyingfishes (Exocoetidae) and sauries (Scomberesocidae), whereas the needlefishes (Belonidae) and halfbeaks (Hemiramphidae) are paraphyletic. Only a single ricefish (Adrianichthyidae) was included as an outgroup, prohibiting tests of adrianichthyid monophyly.

The monophyletic sauries, which include the genera *Scomberesox*, *Cololabis*, and *Ellasichthys*, are deeply nested within needlefishes. The clade including sauries and the needlefish genera *Belone* and *Petalichthys* appears well-supported by decay indices and bootstrap scores.

The relationships of halfbeaks are more complex, with the included genera forming three clades. *Zenarchopterus*, *Hemirhamphodon*, *Nomorhamphus*, and *Dermogenys*, which all practice internal fertilization and are distributed in freshwater and estuaries of the Indo-West Pacific, comprise a monophyletic group that is the sister clade to needlefishes/sauries. These halfbeak genera have been recognized as a separate family, Zenarchopteridae, based on evidence from the pharyngeal jaw apparatus (Tibbetts, 1992) and sperm ultrastructure (Jamieson and Grier, 1993). Meisner (2001) provides further anatomical evidence for the monophyly of this clade, with the addition of *Tondanichthys* (not included here). Based on the additional support of molecular data, we hereafter use the name Zenarchopteridae for this monophyletic group of halfbeaks.

The marine halfbeak genera *Hemiramphus*, *Oxyporhamphus*, and *Euleptorhamphus* compose a clade (hereafter referred to as the *Hemiramphus* clade) that is the sister to flyingfishes. Finally, the marine halfbeak genera *Hyporhamphus* and *Arrhamphus* make up a small clade that is the sister to needlefishes/sauries and Zenarchopteridae. The position of the *Hyporhamphus*/*Arrhamphus* clade is not well-supported; in trees that are two steps longer, the clade may be grouped with the flyingfishes and other marine halfbeaks. However, the position of the Zenarchopteridae is well supported,

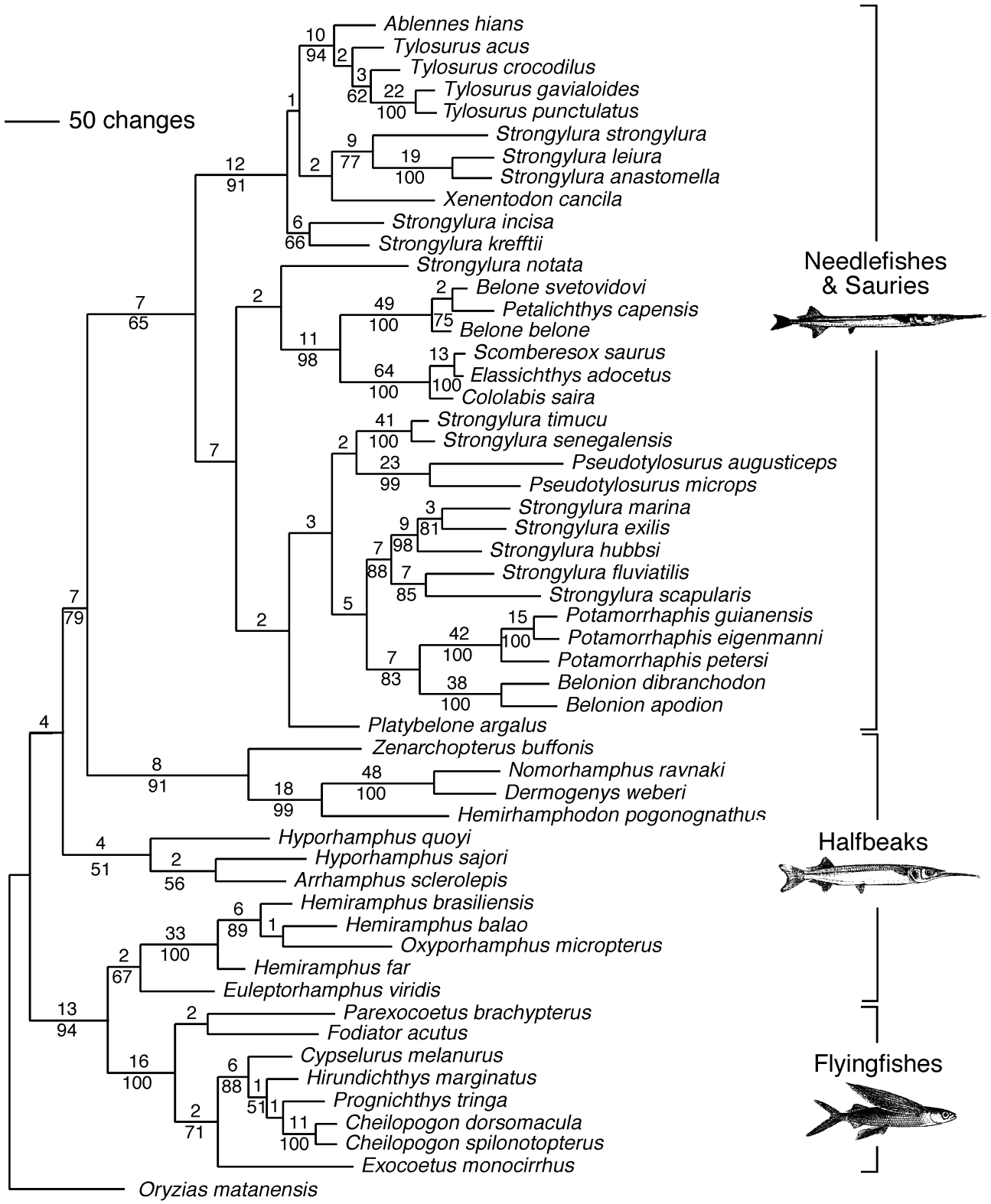

FIG. 1. Total molecular evidence (Rag2, Tmo, 16S, cyt b) phylogenetic hypothesis for beloniform fishes. Branch lengths correspond to amounts of character change. Numbers above branches are decay indices; number below branches are bootstrap proportions.

as is the position of the marine *Hemiramphus* clade as the sister group to flyingfishes.

Separate analyses of the nuclear genes are largely congruent with the total molecular evidence hypothesis. Two analyses were conducted with RAG2. In the first, only the taxa from the total molecular evidence dataset were included (Fig. 2A). In the second, additional outgroups were used to root the tree. In this analysis (Fig. 2B), the Zenarchopteridae are grouped with the needlefish/saury clade; the *Hemiramphus* clade is grouped with the flyingfishes; and *Hyporhamphus* is the sister to the *Hemiramphus* clade and flyingfishes.

The two analyses of Tmo were stopped before completion, owing to the large number of equally parsimonious trees (>60,000). This is most likely a result of the small size of the Tmo dataset relative to the number of OTUs analyzed (>100). Strict consensus trees of the results (Fig. 2C,D) showed congruence with RAG2 and with the total molecular evidence hypothesis. These trees were similar to those with smaller numbers of taxa that were presented in Lovejoy (2000).

Separate analysis of the mtDNA genes resulted in four equally parsimonious trees. The consensus (Fig. 2E) disagrees in many ways with the total molecular evidence and nuclear gene trees. Although some of the more recent relationships seen in the total molecular evidence tree are reiterated (such as the close relationship between the saury genera and *Petalichthys*/*Belone*), many of the deeper parts of the tree are fundamentally different. For example, in the mtDNA trees, the freshwater South American *Potamorrhaphis*/*Belonion* clade (not shown) is the basal beloniform lineage, while in the total molecular evidence and nuclear trees, it is deeply nested in the tree. Lovejoy (2000) suggested that these differences may be the result of the high levels of homoplasy in cyt b 3rd codon positions. To examine this possibility, cyt b 3rd codon positions were downweighted 1/5, and 1/10th relative to other changes in additional mtDNA analyses. These alternative weighting schemes resulted in topologies that were more similar to the total molecular evidence and nuclear trees (Fig. 2F).

The general pattern of relationships indicated by the total molecular evidence analysis places halfbeaks as a series of paraphyletic lineages originating near the base of the tree. Needlefishes and sauries are nested within these halfbeak clades, as are flyingfishes. The taxonomic distribution and optimization of jaw characters on this topology is shown in Figure 3. The basal condition within beloniforms to the exclusion of ricefishes is the presence of an extended lower jaw in juveniles and in adults. The extended upper jaw appears in the needlefish/saury clade, and in some taxa (*Xenentodon cancila*, and *Tylosurus crocodilus*) the extended upper jaw is also present in juveniles. In flyingfishes, and in some halfbeaks such as *Arrhamphus* (and other genera not included in this study, *e.g.*, *Chriodorus* and *Melapedalion*), the extended lower jaw is lost in adults. In most flyingfish genera, the extended lower jaw in juveniles is also lost.

DISCUSSION

Phylogeny

The expansion of the Lovejoy (2000) matrix by 1 kilobase of nuclear gene sequence and 14 new taxa confirms the main conclusions of that study regarding beloniform relationships. The family Belonidae is not monophyletic without the inclusion of sauries, traditionally regarded as a distinct family (Scomberesocidae). Halfbeaks are also non-monophyletic; however, salvaging the family Hemiramphidae is more problematic. Halfbeaks are divided into three main clades: Zenarchopteridae (the Indo-West Pacific freshwater halfbeaks), the *Hemiramphus* clade (which includes *Euleptorhamphus* and *Oxyporhamphus*), and the *Hyporhamphus*/*Arrhamphus* clade. Perhaps the most surprising result is the sister group relationship between Zenarchopteridae and needlefishes/sauries. This relationship is supported by high decay indices and bootstrap proportions, and by its presence in separate analyses of the nuclear genes. Similarly, the sister group relationship between the *Hemiramphus* clade and flyingfishes is quite strong. The position of the *Hyporhamphus* clade is less clear. In the most parsimonious tree, it is placed as the sister group to the zenarchopterids and needlefishes/sauries; however, in trees two steps longer (and in separate analyses of the nuclear genes), it is grouped with the *Hemiramphus* clade and flyingfishes. Increased taxonomic sampling of halfbeaks may resolve this issue; *Hyporhamphus*, the most taxonomically diverse halfbeak genus, with two distinct subgenera, is represented by only two species in this study.

The hypothesized division of halfbeaks into three groups has implications for patterns of morphological evolution. The fusion of the third pair of upper pharyngeal bones has been considered a synapomorphy of Hemiramphidae (Collette *et al.*, 1984). In light of the topology presented here, these bones either fuse independently in two or more halfbeak lineages, or become unfused in needlefishes and flyingfishes Similarly, homoplasy is required in the evolution of the fourth upper pharyngeal toothplates. The plates are absent in halfbeaks and flyingfishes (Rosen and Parenti, 1981; Collette *et al.*, 1984), thus the present topology requires their independent loss in the three halfbeak/flyingfish lineages, or their reappearance in the needlefish/saury lineage. Before more definitive hypotheses concerning the evolution of the characters can be made, a complete anatomical survey needs to be undertaken for beloniforms, and incorporated into the matrix.

The inclusion of additional halfbeak and flyingfish taxa provides some interesting results concerning the evolution of gliding. Parin (1961), Collette *et al.*, (1984), Dasilao *et al.* (1997), and Dasilao and Sasaki (1998) have presented phylogenetic hypotheses for

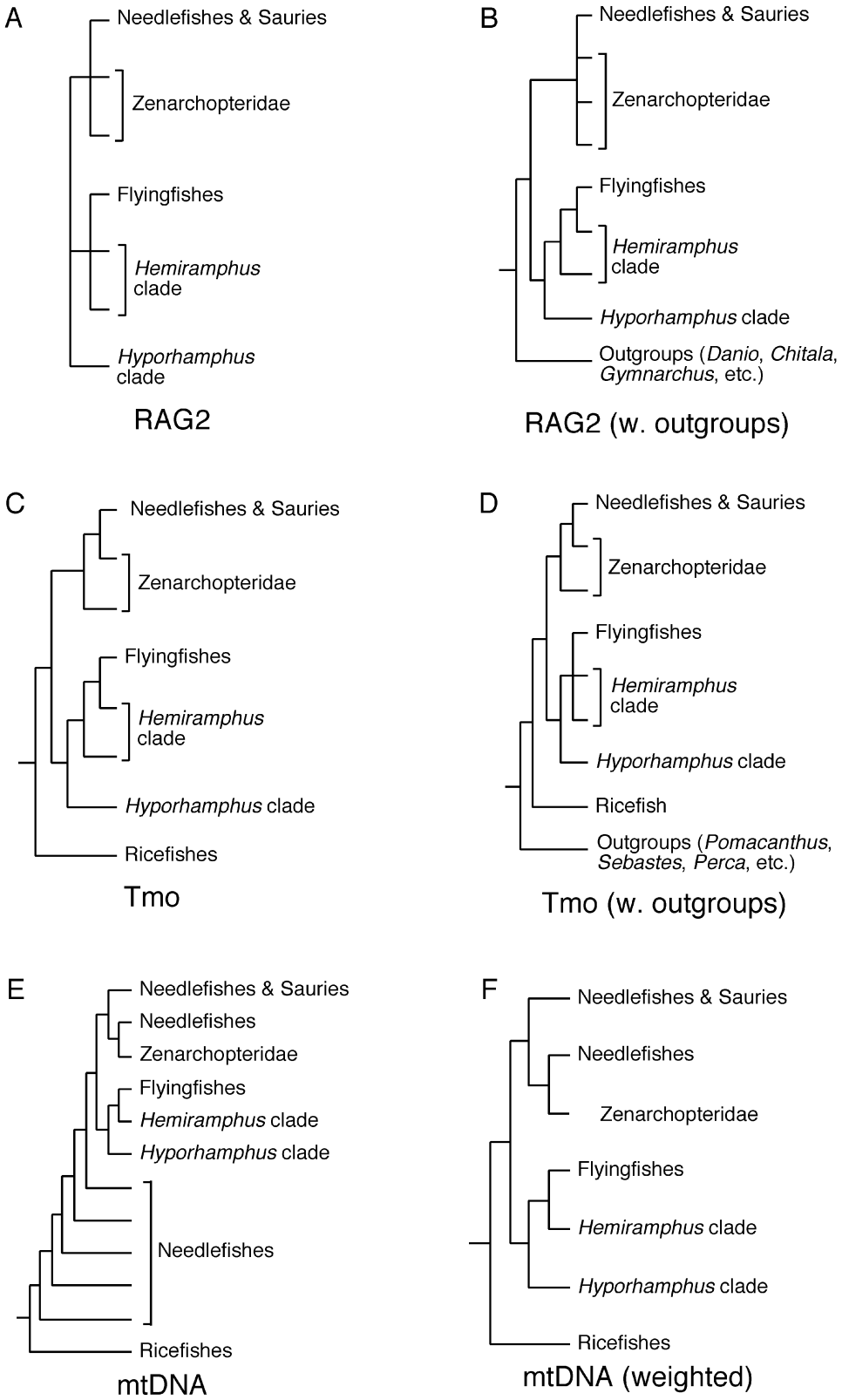

FIG. 2. Simplified separate analyses of nuclear and mitochondrial genes. Separate analyses of Rag2 and Tmo include additional outgroup taxa (B and D). In weighted analysis of mtDNA (E), third codon positions of cyt b were weighted 1/10th other changes in cyt b and 16S.

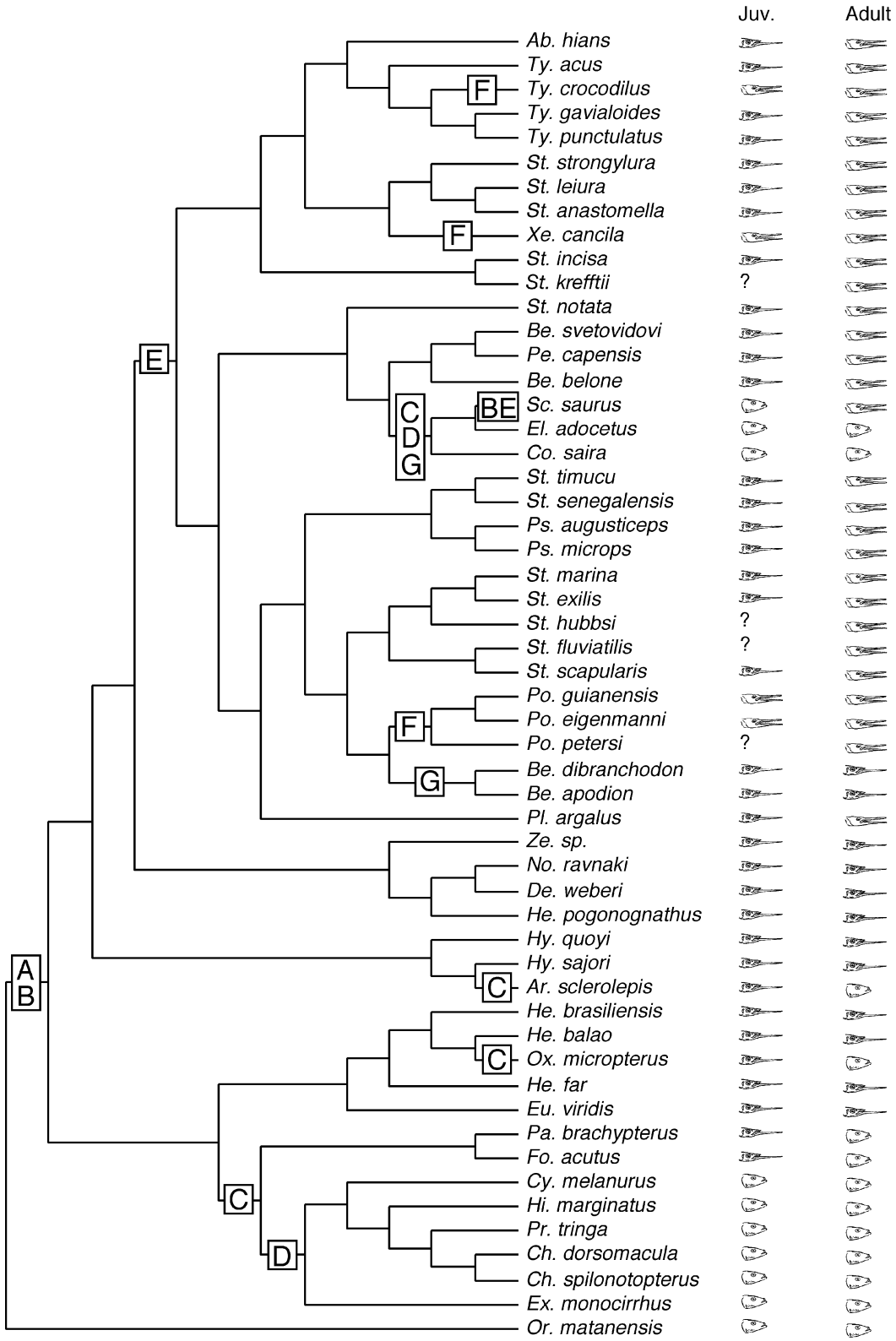

FIG. 3. Total molecular evidence (Rag2, Tmo, 16S, cyt b) phylogenetic hypothesis for beloniform fishes, with juvenile and adult jaw characters to the right of each taxon. ACCTRAN optimized jaw ontogenetic changes are:A, lower jaw elongate in juveniles; B, lower jaw elongate in adults; C, lower jaw short in adults; D, lower jaw short in juveniles; E, upper jaw elongate in adults; F, upper jaw elongate in juveniles; G, upper jaw short in adults.

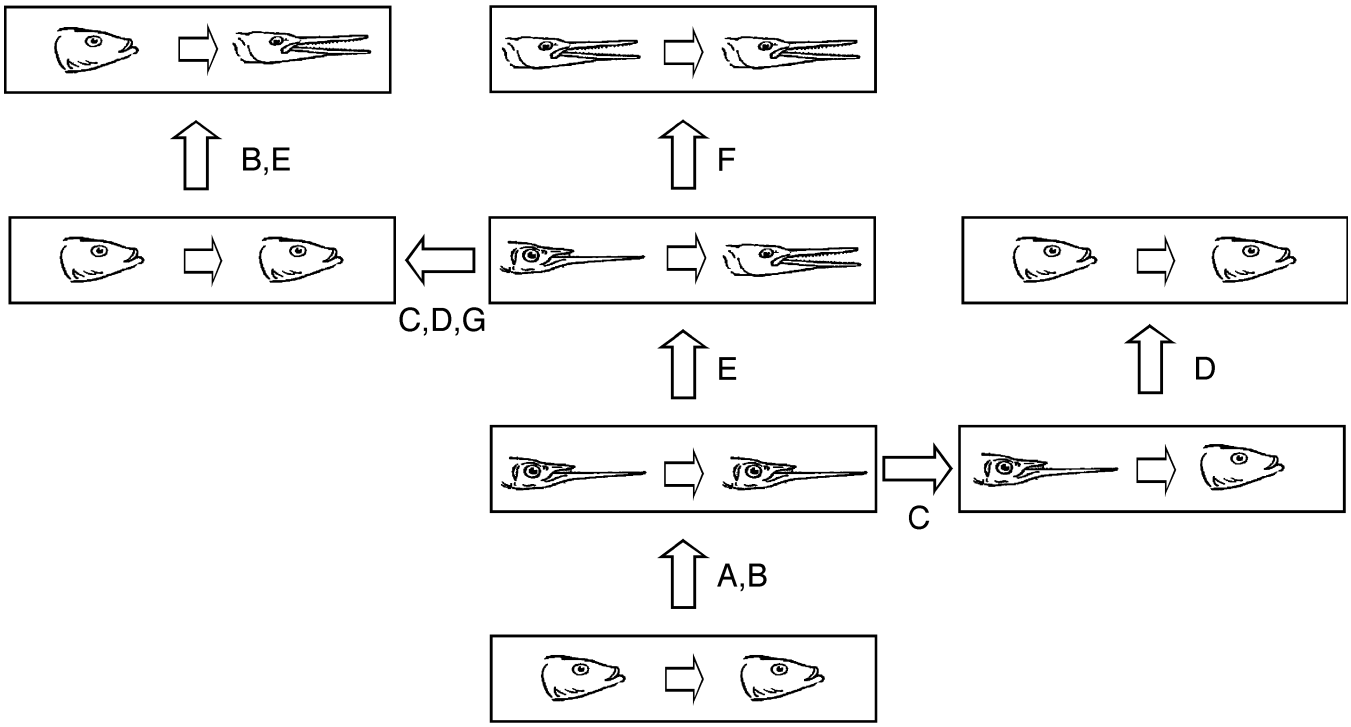

FIG. 4. Hypothesized summary of evolutionary transitions between jaw ontogenies in beloniform fishes. Transition codes are listed in Figure 3.

flyingfishes. The results presented here are largely in agreement with these previous studies. *Fodiator* is generally considered the least sophisticated glider, along with *Parexocoetus*, and *Exocoetus*. These three genera are ''monoplane'' gliders, having greatly expanded pectoral fins but not pelvics. *Cypselurus*, *Prognichthys*, and *Hirundichthys*, and *Cheilopogon* (the Cypselurinae) are ''biplane'' gliders, and have expanded pelvic as well as pectoral fins, which help to control gliding stability (Davenport, 1992). Morphological studies place the monoplane gliders at the base of the exocoetid tree, suggesting a progressive refinement of gliding ability. The molecular data support this idea. Dasilao *et al.* (1997) also showed that, based on morphology, the halfbeak *Oxyporhamphus*, should be considered the basal flyingfish taxon. The molecular data presented here strongly disagree with this hypothesis, placing *Oxyporhamphus* deeply within the *Hemiramphus* clade. Several of the characters used by Dasilao *et al.* (1997) are related to gliding (in particular, a strengthened caudal complex for take-offs), and might be convergent features of gliding behavior.

Evolution of jaw ontogeny

The optimization of jaw characters on the total molecular evidence beloniform tree clearly falsifies the scenario proposed by Nichols and Breder (1928) and supported by de Beer (1940). These authors hypothesized that needlefishes are the basal members of Beloniformes, and that halfbeaks represent a more derived paedomorphic lineage. Instead, our hypothesis suggests that halfbeaks are relatively basal members of Beloniformes, and the needlefish morphology is relatively derived. The presence of elongate lower jaws in both juveniles and adults is relatively plesiomorphic, whereas the evolution of an elongate upper jaw in adults is a relatively derived condition. Collette *et al.* (1984) also supported this scenario, based on a phylogeny in which each of the traditional beloniform families was monophyletic. In the topology presented here, the basal paraphyletic position of the halfbeaks provides additional confidence in the optimization of the elongate lower jaw in juvenile and adult as the relatively plesiomorphic states.

The increased number of taxa and the more detailed presentation of jaw condition (Fig. 2) compared to Lovejoy (2000), makes possible a more complete compilation of evolutionary changes in beloniform jaw ontogeny. Figure 4 is a schematic summary of these transitions. The story of beloniform jaws is clearly not limited to the simple transition between the ''halfbeaked'' and ''needle-jawed.'' The hypothesized patterns of transformation are complex. The generalized halfbeak ontogeny leads independently to ontogenies in which adults lose the elongate lower jaw (*e.g.*, *Oxyporhamphus*, *Arrhamphus*). In most flyingfishes, the elongate lower jaw is absent in juveniles. The generalized needlefish ontogeny leads to ontogenies in which juveniles have elongate upper and lower jaws (*e.g.*, *Tylosurus crocodilus*), and others in which the elongate upper jaw is absent in adults (*Belonion*). In sauries the transitions are particularly striking. *Cololabis* and *Elassichthys* have short upper and lower jaws as adults and juveniles, while *Scomberesox* has elon-

gate upper and lower jaws in adults (Collette *et al.*, 1984). Thus, the placement and relationships of sauries requires the loss of elongate upper and lower jaws, followed by their reappearance in *Scomberesox*.

What drives the evolution of jaw ontogeny? Boughton *et al.* (1991) summarized evidence for the importance of jaw shape for the feeding ecology of needlefishes. Needlefishes in the ''halfbeak'' stage often consume prey such as shrimp, mysids, amphipods, and phytoplankton, but after developing the elongate upper jaw, shift to a fish diet. The forceps-like adult needlefish morphology is well-suited to piscivory. Taxa such as *Tylosurus crocodilus* and *Xenentodon cancila*, which have no ''halfbeak'' juvenile stage, feed almost exclusively on fishes (Breder, 1932; Foster, 1974). Thus, it seems likely that changes in jaw ontogeny may be at least partially related to diet and food availability. Our phylogenetic approach suggests an additional consideration. While most needlefishes are marine, there are a number of freshwater taxa, including several freshwater endemic genera: *Xenentodon* from Southeast Asia, and *Pseudotylosurus, Potamorrhaphis*, and *Belonion* from South America. Most of these taxa display notable changes in ontogenetic patterns; however, not all are in the same direction. *Xenentodon*, and *Potamorrhaphis* have lost the ''halfbeak'' stage as juveniles, while *Belonion* maintains the halfbeak stage as an adult. The concentration of evolutionary change in ontogeny along freshwater lineages may be related to high diversity of food types in tropical freshwater habitats. For example, in South America, *Belonion* feeds mainly on zooplankton, insect larvae, and bryozoans, *Potamorrhaphis* on terrestrial insects (particularly ants), and *Pseudotylosurus* on fishes (Goulding and Carvalho, 1984). Specialization on alternative food resources might drive evolutionary shifts in jaw ontogeny and morphology. Several species of *Strongylura* are also freshwater inhabitants (*S. krefftii*, *S. hubbsi*, and *S. fluviatilis*), but data for the juvenile condition are not available for these taxa. The relationship between ecology and jaw ontogeny will remain speculative until more detailed studies of diet, broader surveys of ontogeny, and functional investigations of morphology are completed.

What role has heterochrony played in the evolution of beloniform jaw ontogeny? Severtsov (1927; summarized in Gould, 1977) proposed that needlefish phylogeny recapitulated beloniform ontogeny. The phylogenetic position of needlefishes relative to halfbeaks fits his prediction. The juvenile jaw morphology of the relatively derived needlefish lineage is similar to the adult morphology of the more basal halfbeak lineages. Thus, the ontogenetic sequence in needlefish of short-jawed larva to ''halfbeaked'' juvenile to ''needle-jaw'' adult parallels the phylogenetic transition of short-jawed outgroup to hemiramphid halfbeak to belonid needlefish. However, Lovejoy (2000) pointed out that von Baer's (1853) critique of recapitulation may apply—the similarities between needlefish juveniles and halfbeak adults may simply be a byproduct of limited divergence of the halfbeak adult from the halfbeak juvenile. In other words: (1) needlefish juveniles look like halfbeak juveniles due to shared ancestry, (2) halfbeak jaw structure does not change dramatically during the transition from juvenile to adult, thus (3) needlefish juveniles look like halfbeak adults. This ''von Baerian'' explanation does not require heterochrony (Gould, 1977).

Unfortunately, the discrete character-based approach used here does not allow differentiation between ''von Baerian'' and true recapitulation caused by changes in developmental timing. One alternative would be to compare ontogenetic trajectories, as did Boughton *et al.* (1991), who optimized upper and lower jaw length trajectories on a needlefish phylogeny. However, Zelditch *et al.* (2000, p. 1363) pointed out that one-dimensional features such as jaw length ''. . . necessarily both develop and evolve along the same dimension so they always suggest parallelism between ontogeny and phylogeny.'' These authors advocate a morphometric shape-based approach, which permits a comparative assessment of the roles of heterochrony and heterotopy (evolutionary change in spatial patterning of development) (Wray and McClay, 1989; Zelditch *et al.*, 2000).

Beloniform fishes offer rich possibilities for such investigations. Our phylogeny highlights some limitations and opportunities. The transition to ''needle-jaw'' adults (transition E in Figs. 3 and 4) is deep within beloniform phylogeny. Reconstruction of hypothetical ancestral ontogenetic trajectories at this node will require extrapolation from large numbers of needlefish and zenarchopterid halfbeak species, and would be relatively speculative. However, a number of ontogenetic transitions are shallow in the tree. In particular, *Belonion* has lost the elongate upper jaw in adults, matures at a very small size, and is hypothesized to be paedomorphic (Collette, 1966). Detailed developmental analysis of this taxon and its sister clade *Potamorrhaphis* could be extremely informative. Similarly, analyses of *Tylosurus* and *Xenentodon* will provide data on the elimination of the juvenile ''halfbeak'' stage. We view our beloniform phylogeny as a valuable roadmap for further forays into the evolution of ontogeny in this model group of fishes.

ACKNOWLEDGMENTS

We thank the collectors of beloniforms and field assistants who made this study possible, many of whom are listed in Lovejoy (2000). In addition, we gratefully acknowledge the assistance of P. Heemstra, J. Fu, A. Summers, M. Littman, M. Sabaj, J. Albert, L. Ballance, and W. Crampton. R. Pitman generously provided nearly all of the flyingfish samples. L. Parenti, G.D. Johnson, and an anonymous reviewer provided valuable comments on the manuscript. We thank F. Santini and G. Ybazeta for organizing an excellent symposium. Support from NSERC (National Science and Engineering Research Council) and CFI (Canada Foundation for Innovation) made the study possible.

REFERENCES

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

Bemis, W. 1984. Paedomorphosis and the evolution of the Dipnoi. Paleobiology 10:293–307.

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

Breder, C. M., Jr. 1932. On the habits and development of certain Atlantic Synentognathi. Carnegie Inst. Wash. Publ. 435, Pap. Tortugas Lab 28:1–35.

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

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

Collette, B. B. and N. V. Parin. 1970. Needlefishes (Belonidae) of the eastern Atlantic Ocean. Atl. Rep. No. 11:1–60.

Collette, B. B., G. E. McGowen, N. V. Parin, and S. Mito. 1984. Beloniformes: Development and relationships. *In* H. G. Moser *et al.* (eds.), *Ontogeny and systematics of fishes*, pp. 335–354. Am. Soc. Ichthyol. Herpetol. Spec. Publ. 1.

Dasilao, J. C. Jr. and K. Sasaki. 1998. Phylogeny of the flyingfish family Exocoetidae (Teleostei, Beloniformes). Ichthyol. Res. 45: 347–353.

Dasilao, J. C. Jr., K. Sasaki, and O. Okamura. 1997. The hemiramphid, *Oxyporhamphus*, is a flyingfish (Exocoetidae). Ichthyol. Res. 44:101–107.

Davenport, J. 1992. Wing-loading, stability, and morphometric relationships in flying fish (Exocoetidae) from the north-eastern Atlantic. J. Mar. Biol. Assoc. UK 72:25–39.

de Beer, G. R. 1940. *Embryos and ancestors.* Clarendon Press, Oxford, U.K.

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

Foster, N. R. 1974. Behavior, development, and early life history of the Asian needlefish, *Xenentodon cancila*. Proc. Acad. Nat. Sci. Phila. 125:77–88.

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

Goulding, M. and M. L. Carvalho. 1984. Ecology of Amazonian needlefishes (Belonidae). Rev. Bras. Zool. 2:99–111.

Jamieson, B. G. M. and H. J. Grier. 1993. Influences of phylogenetic position and fertilization biology on spermatozoal ultrastructure exemplified by exocoetoid and poeciliid fish. Hydrobiologia 271:11–25.

Johnson, G. D. and E. B. Brothers. 1993. *Schindleria*: A paedomorphic goby. Bull. Mar. Sci. 52:441–471.

Lovejoy, N. R. 2000. Reinterpreting recapitulation: Systematics of needlefishes and their allies (Teleostei:Beloniformes). Evolution 54:1349–1362.

Lovejoy, N. R. and B. B. Collette. 2001. Phylogenetic relationships of New World needlefishes (Teleostei:Belonidae) and the biogeography of transitions between marine and freshwater habitats. Copeia 2001:324–338.

Maddison, W. P., and D. R. Maddison. 1992. MacClade ver. 3.0. Sinauer Assoc. Inc., Sunderland.

Meisner, A. D. 2001. Phylogenetic systematics of the viviparous halfbeak genera *Dermogenys* and *Nomorhamphus* (Teleostei: Hemiramphidae:Zenarchopterinae). Zool. J. Linn. Soc. 133: 199–283.

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.

Parin, N. V. 1961. Osnovy sistemy letuchikh ryb (semeistva Oxyporhamphidae y Exocoetidae) [in Russian; Principles of the classification of flyingfishes (families Oxyporhamphidae and Exocoetidae)]. Tr. Inst. Okeanol. Akad. Nauk. SSSR 43:92–183.

Regan, C. T. 1911. The classification of the teleostean fishes of the order Synentognathi. Ann. & Mag. Nat. Hist. Ser. 8 7:327–335.

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. London B 264:1011–1020.

Swofford, D. L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods). version 4. Sinauer Associates, Sunderland, Massachusetts.

Sullivan, J. P., S. Lavoue, and C. D. Hopkins. 2000. Molecular systematics of the African electric fishes (Mormyroidea:Teleostei) and a model for the evolution of their electric organs. J. Exp. Biol. 203:665–683.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24:4876–4882.

Tibbetts, I. R. 1992. The trophic ecology, functional morphology and phylogeny of the Hemiramphidae (Beloniformes). Ph.D. Diss. University of Queensland, Brisbane.

von Baer, K. E. 1853. Fragments relating to philosophical zoology. *In* A. Henfrey and T. H. Huxley (eds.), *Scientific memoirs, delected from the transactions of foreign academies of science and from foreign journals*, pp. 176–238. Taylor and Francis, London.

Winterbottom, R. 1990. The *Trimmatom nanus* species complex (Actinopterygii, Gobiidae): Phylogeny and progenetic heterochrony. Syst. Zool. 39:253–265.

Wray, G. A. and D. R. McClay. 1989. Molecular heterochronies and heterotopias in early echinoid development. Evolution 43:803–813.

Zelditch, M. L., H. D. Sheets, and W. L. Fink. 2000. Spatiotemporal reorganization of growth rates in the evolution of ontogeny. Evolution 54:1363–1371.

APPENDIX 1. *Genbank accession numbers for sequences used in analysis.*

Species	Isolate	RAG2	Tmo	cyt b	16S
Ablennes hians	N02a	no sequence	AF244010	AF243858	AF243934
Ablennes hians	N02b	AY693520	AF244011	AF243859	AF243935
Arrhamphus sclerolepis	N72a	no sequence	AY693450	AY693510	AY693480
Arrhamphus sclerolepis	N72b	no sequence	AY693451	AY693511	AY693481
Belone belone	N35a	AY693546	AF244058	AF243906	AF243982
Belone belone	N35b	AY693547	AF244059	AF243907	AF243983
Belone svetovidovi	N15	AY693531	AF244032	AF243880	AF243956
Belone svetovidovi	N16	AY693532	AF244033	AF243881	AF243957
Belonion apodion	N55	AF306488	AF244082	AF243931	AF244007
Belonion dibranchodon	N14a	AF306468	AF244030	AF243878	AF243954
Belonion dibranchodon	N14b	AF306469	AF244031	AF243879	AF243955
Cheilopogon dorsomacula	N60a	AY693568	AY693437	AY693497	AY693467
Cheilopogon dorsomacula	N60b	AY693569	AY693438	AY693498	AY693468
Cheilopogon spilonotopterus	N63a	AY693574	AY693443	AY693503	AY693473
Cheilopogon spilonotopterus	N63b	AY693575	AY693444	AY693504	AY693474
Cololabis saira	N43a	AY693549	AF244067	AF243915	AF243991
Cololabis saira	N43b	AY693550	AY693428	AY754822	no sequence
Cypselurus melanurus	N10a	AY693527	AF244022	AF243870	AF243946
Cypselurus melanurus	N10b	AY693528	AF244023	AF243871	AF243947
Dermogenys weberi	N53a	AY693557	AF244078	AF243927	AF244003
Dermogenys weberi	N53b	AY693558	AF244079	AF243928	AF244004
Elassichthys adocetus	N74a	AY693580	AY693452	AY693512	AY693482
Elassichthys adocetus	N74b	AY693581	AY693453	AY693513	AY693483
Euleptorhamphus viridis	N62a	AY693572	AY693441	AY693501	AY693471
Euleptorhamphus viridis	N62b	AY693573	AY693442	AY693502	AY693472
Exocoetus monocirrhus	N59a	AY693566	AY693435	AY693495	AY693465
Exocoetus monocirrhus	N59b	AY693567	AY693436	AY693496	AY693466
Fodiator acutus	N61a	AY693570	AY693439	AY693499	AY693469
Fodiator acutus	N61b	AY693571	AY693440	AY693500	AY693470
Hemirhamphodon pogonognathus	N54a	AY693559	AF244080	AF243929	AF244005
Hemirhamphodon pogonognathus	N54b	no sequence	AF244081	AF243930	AF244006
Hemiramphus balao	N11a	AY693529	AF244024	AF243872	AF243948
Hemiramphus balao	N11b	AY693530	AF244025	AF243873	AF243949
Hemiramphus brasiliensis	N05a	AY693523	AF244016	AF243864	AF243940
Hemiramphus brasiliensis	N05b	AY693524	AF244017	AF243865	AF243941
Hemiramphus far	N76a	AY693582	AY693456	AY693516	AY693486
Hemiramphus far	N76b	AY693583	AY693457	AY693517	AY693487
Hirundichthys marginatus	N57a	AY693562	AY693431	AY693491	AY693461
Hirundichthys marginatus	N57b	AY693563	AY693432	AY693492	AY693462
Hyporhamphus quoyi	N49a	AY693551	AF244070	AF243919	AF243995
Hyporhamphus quoyi	N49b	AY693552	AF244071	AF243920	AF243996
Hyporhamphus sajori	N71c	AY693579	AY693448	AY693508	AY693478
Hyporhamphus sajori	N71d	no sequence	AY693449	AY693509	AY693479
Nomorhamphus ravnaki	N52a	AY693555	AF244076	AF243925	AF244001
Nomorhamphus ravnaki	N52b	AY693556	AF244077	AF243926	AF244002
Oryzias matanensis	N44a	no sequence	no sequence	AF243916	AF243992
Oryzias matanensis	N44b	no sequence	AF244068	AF243917	AF243993
Oxyporhamphus micropterus	N56a	AY693560	AY693429	AY693489	AY693459
Oxyporhamphus micropterus	N56b	AY693561	AY693430	AY693490	AY693460
Parexocoetus brachypterus	N09a	AY693525	AF244020	AF243868	AF243944
Parexocoetus brachypterus	N09b	AY693526	AF244021	AF243869	AF243945
Petalichthys capensis	N68c	AY693577	AY693446	AY693506	AY693476
Petalichthys capensis	N68d	AY693578	AY693447	AY693507	AY693477
Platybelone argalus argalus	N12a	AF306464	AF244026	AF243874	AF243950
Platybelone argalus platyura	N12b	AF306465	AF244027	AF243875	AF243951
Potamorrhaphis eigenmanni	N17	AF306470	AF244034	AF243882	AF243958
Potamorrhaphis eigenmanni	N18	AF306471	AF244035	AF243883	AF243959
Potamorrhaphis guianensis	N13a	AF306466	AF244028	AF243876	AF243952
Potamorrhaphis guianensis	N13b	AF306467	AF244029	AF243877	AF243953
Potamorrhaphis petersi	N27a	AF306474	AF244044	AF243892	AF243968
Prognichthys tringa	N58a	AY693564	AY693433	AY693493	AY693463
Prognichthys tringa	N58b	AY693565	AY693434	AY693494	AY693464
Pseudotylosurus augusticeps	N28a	AF306475	AF244045	AF243893	AF243969
Pseudotylosurus augusticeps	N28b	AF306476	AF244046	AF243894	AF243970
Pseudotylosurus microps	N640	AY693576	AY693445	AY693505	AY693475
Pseudotylosurus microps	N810	AY693584	AY693458	AY693518	AY693488
Scomberesox saurus	N36b	AF306481	AF244060	AF243908	AF243984
Scomberesox saurus	N36c	AY693548	AF244061	AF243909	AF243985
Strongylura anastomella	N75a	no sequence	AY693454	AY693514	AY693484

APPENDIX 1. *Continued.*

Species	Isolate	RAG2	Tmo	cyt b	16S
Strongylura anastomella	N75b	no sequence	AY693455	AY693515	AY693485
Strongylura exilis	N38a	AF306482	AF244062	AF243910	AF243986
Strongylura exilis	N38b	AF306483	AF244063	AF243911	AF243987
Strongylura fluviatilis	N29a	AF306477	AF244047	AF243895	AF243971
Strongylura fluviatilis	N29b	AF306478	AF244048	AF243896	AF243972
Strongylura hubbsi	N30a	AF306479	AF244049	AF243897	AF243973
Strongylura hubbsi	N30b	AF306480	AF244050	AF243898	AF243974
Strongylura incisa	N19	AF306472	AF244036	AF243884	AF243960
Strongylura incisa	N20	AY693533	AF244037	AF243885	AF243961
Strongylura krefftii	N31a	AY693539	AF244051	AF243899	AF243975
Strongylura krefftii	N31b	AY693540	AF244052	AF243900	AF243976
Strongylura leiura	N32a	AY693541	AF244053	AF243901	AF243977
Strongylura leiura	N32b	AY693542	AF244054	AF243902	AF243978
Strongylura marina	N07a	AF306462	AF244018	AF243866	AF243942
Strongylura marina	N07b	AF306463	AF244019	AF243867	AF243943
Strongylura notata forsythia	N01a	AF306489	AF244008	AF243856	AF243932
Strongylura notata notata	N01b	AY693519	AF244009	AF243857	AF243933
Strongylura scapularis	N48	AF306487	AF244069	AF243918	AF243994
Strongylura senegalensis	N39a	AF306484	AF244064	AF243912	AF243988
Strongylura senegalensis	N39b	AF306485	AF244065	AF243913	AF243989
Strongylura strongylura	N21	AY693534	AF244038	AF243886	AF243962
Strongylura strongylura	N22	AY693535	AF244039	AF243887	AF243963
Strongylura timucu	N04b	AF306461	AF244015	AF243863	AF243939
Strongylura timucu	N04a	AF306460	AF244014	AF243862	AF243938
Tylosurus acus acus	N03a	AY693521	AF244012	AF243860	AF243936
Tylosurus acus melanotus	N03b	AY693522	AF244013	AF243861	AF243937
Tylosurus crocodilus	N23	AY693536	AF244040	AF243888	AF243964
Tylosurus crocodilus	N24	AY693537	AF244041	AF243889	AF243965
Tylosurus gavialoides	N33a	AY693543	AF244055	AF243903	AF243979
Tylosurus gavialoides	N33b	AY693544	AF244056	AF243904	AF243980
Tylosurus punctulatus	N34	AY693545	AF244057	AF243905	AF243981
Xenentodon cancila	N25	AF306473	AF244042	AF243890	AF243966
Xenentodon cancila	N26	AY693538	AF244043	AF243891	AF243967
Zenarchopterus buffonis	N50a	AY693553	AF244072	AF243921	AF243997
Zenarchopterus buffonis	N50b	AY693554	AF244073	AF243922	AF243998