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Phylogenetic Relationships of New World Needlefishes (Teleostei: Belonidae) and the Biogeography of Transitions between Marine and Freshwater Habitats

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The New World clade of needlefishes (Belonidae) includes species distributed along the Pacific and Atlantic coasts of the Americas and in freshwater basins of Central and South America. Phylogenetic relationships among 13 species of the group were assessed based on data from two nuclear genes (RAG2 and Tmo-4C4), two mitochondrial genes (cytochrome *b* and 16S rRNA), and a small suite of morphological characters. In general, there was concordance between separate analyses of nuclear and mitochondrial characters, and RAG2 was found to be a particularly useful gene for phylogeny reconstruction. Morphology supported an alternative phylogenetic pattern, but this was probably a result of the small number of characters and the lack of a thorough anatomical survey. The total evidence hypothesis divides the group into two major clades. In one, *Pseudotylosurus* from freshwater in South America is most closely related to a pair of *Strongylura* species from the western and eastern Atlantic; in the other, *Potamorrhaphis* and *Belonion* from South American freshwater are related to a clade of *Strongylura* from marine and freshwater habitats of the eastern Pacific and western Atlantic. Optimization of habitat on the total evidence tree, combined with paleogeographic data, suggests that four independent entries into freshwater have taken place—one in Central America, and three in South America.

THE Belonidae (needlefishes) is an atherinomorph family that includes 32 elongate species distinguished in most cases by lengthened upper and lower jaws studded with numerous sharp teeth (Collette et al., 1984). Many species occur in tropical marine habitats, but a number of taxa are known exclusively from freshwater. Of the 10 needlefish genera, three (*Potamorrhaphis, Pseudotylosurus,* and *Belonion*) are endemics of South American rivers (Collette, 1966, 1974a; Lovejoy and de Araújo, 2000), and one (*Xenentodon*) is an endemic of Southeast Asian rivers (Roberts, 1989). The remaining genera are exclusively marine, with the exception of *Strongylura*, a diverse genus that includes both marine and freshwater species (Collette et al., 1984).

A prerequisite for understanding the evolution and biogeography of transitions between marine and freshwater habitats is a clear understanding of the phylogenetic relationships among taxa. A preliminary phylogenetic analysis of the whole needlefish family (Lovejoy, 1999, 2000) defined a clade (hereafter called the New World clade) that includes all the New World freshwater taxa (*Potamorrhaphis, Pseudotylosurus, Belonion,* and two species of *Strongylura*) and most of the New World inshore marine species, including two *Strongylura* species from the eastern Pacific and three *Strongylura* species from the western Atlantic. *Strongylura senegalensis*, from the eastern Atlantic, was also found to be a member of this group, whereas *Strongylura notata*, from the western Atlantic was found to fall outside the New World clade. A robust phylogeny for the marine and freshwater species of the New World needlefish clade could provide a unique historical perspective on the dynamics of shifts between oceans and Neotropical rivers and lakes.

Previous systematic hypotheses have been proposed for freshwater needlefishes based on morphology. Collette (1966), without providing an explicit character matrix, suggested the following scenario for the evolution of the Neotropical freshwater endemics. First, inshore *Strongylura*-like needlefishes moved from marine waters into freshwater South America. There, one lineage gave rise to *Pseudotylosurus*, while another ''line of development from the same stock led to the ancestors of *Potamorrhaphis* and *Belonion*'' (Collette, 1966:21). Thus, in current terminology, Neotropical freshwater needlefish genera would comprise a monophyletic group, with *Pseudotylosurus* the sister taxon to a clade including *Potamorrhaphis* and *Belonion*. Goulding and Carvalho (1984) presented a similar phylogeny, which they attribute to Collette (1966, 1974a–c, 1982). Most recently Boughton et al. (1991) presented a phylogeny for needlefishes

based on both standard morphological characters and meristic data. Surprisingly, their results group *Potamorrhaphis* and *Belonion* with *Xenentodon*, the Southeast Asian freshwater needlefish, whereas *Pseudotylosurus* was distantly related to this clade.

To better understand relationships within the New World clade of needlefishes, we developed a novel character dataset based on two mitochondrial and two nuclear genes and combined it with currently available morphological information. One of the two nuclear genes has not previously been used for lower level phylogenetic analysis and shows considerable promise for systematic studies. The resultant phylogeny provides a strong framework for examining the evolution of transitions between marine and freshwater habitats.

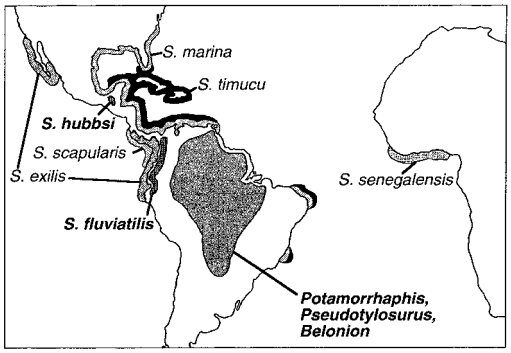

Fig. 1. Estimated geographic distributions for members of the New World needlefish clade. Freshwater species are in bold. Compiled from Cressey and Collette (1970) and Collette (1974a, 1982, unpubl.).

Materials and Methods

We included 13 of the 14 species of the New World needlefish clade defined by Lovejoy (1999, 2000). This clade contains the endemic freshwater needlefishes of South America, *Pseudotylosurus* (2 spp.), *Potamorrhaphis* (3 spp.), and *Belonion* (2 spp.), distributed in the Amazon, Orinoco, Paraná, and Rivers of the Guianas (one of the two species of *Pseudotylosurus* was missing from our analysis). The clade also includes the freshwater species of *Strongylura* from the Atlantic-draining Rio Usumacinta of Mexico and Guatemala (*Strongylura hubbsi*), and the Pacific slope drainages of Ecuador and Colombia (*Strongylura fluviatilis*). The marine species include *Strongylura scapularis* and *Strongylura exilis* from the eastern Pacific coasts, *Strongylura marina* and *Stongylura timucu* from the western Atlantic and Caribbean, and *S. senegalensis* from the Gulf of Guinea in the eastern Atlantic (Fig. 1). The New World clade is one of five distinct lineages that emerge from higher-level phylogenetic analysis of needlefishes. To test its monophyly, we included species from each of the four other lineages: *S. notata, Platybelone argalus, Strongylura incisa,* and *Scomberesox saurus.* The last species is currently classified in a separate family, Scomberesocidae (sauries), but the sauries appear to be nested within Belonidae (Lovejoy, 2000). We also included *Xenentodon cancila*, a Southeast Asian freshwater needlefish, to test Boughton et al.'s (1991) hypothesized relationship between this genus and *Potamorrhaphis* and *Belonion*. *Strongylura* appears to be a polyphyletic genus in need of revision.

Needlefishes were collected in the field by ourselves or colleagues. Specimen and locality information is listed in Material Examined. Gill tissue was preserved in buffer of 20% DMSO, 0.25 M EDTA at pH 8, saturated with NaCl (Seutin et al., 1991) and DNA was extracted using a Quiagen spin-column tissue kit. DNA sequence data were collected from two mitochondrial and two nuclear genes. Whenever possible, two individuals from each species were sequenced. Primers and amplification parameters for the mitochondrial genes (cytochrome *b* and 16S rRNA) and one of the nuclear genes (Tmo-4C4) have been previously described (Lovejoy, 2000). Tmo-4C4 (Tmo) is an anonymous nuclear locus developed by Streelman and Karl (1997). Primers and procedures for the other nuclear gene, recombination activating gene 2 (RAG2), were developed specifically for this study as follows.

RAG2, and the closely linked RAG1, encode components of the recombinase involved in recombination of immunoglobin and T-cell receptor genes and appear as conserved single copies in all examined vertebrates (Willett et al., 1997; Hansen and Kaattari, 1996). The RAG1 coding regions in trout and zebrafish are interrupted by one or two introns (Willett et al., 1997); therefore, RAG2 was selected as a better candidate for amplification. The available vertebrate RAG2 sequences were obtained from GenBank and aligned using predicted amino acid sequences; taxa included zebrafish (U71094), trout (U31670), *Xenopus* (L19325), chicken (M58531), mouse (M64796), and human (M94633). Degenerate primers were designed for regions of conserved nucleotide sequence and tested in various combinations yielding a primer pair (RAG2-f1 and RAG2-r4) that successfully amplified a single band for several needlefish species (Table 1). In some cases, primers RAG2-f2 and RAG2-r6 were used in various com-

TABLE 1. PRIMERS USED FOR AMPLIFICATION AND SEQUENCING OF THE RAG2 GENE. Positions are for 3′ end of primer relative to rainbow trout (U31670).

Primer	Sequence	Position
Forward:		
RAG2-f1[a]	5′-TTTGGrCArAAGGGCTGGCC-3′	110
RAG2-f2[a]	5′-ArACGCTCmTGTCCmACTGG-3″	112
RAG2-f6[b]	5′-TACCTGCTGACCACAGACAGC-3′	339
Reverse:		
RAG2-r4[a]	5′-GTrGArTAGTAGGGCTCCCA-3′	1297
RAG2-R6[a]	5′-TGrTCCArGCAGAAGTACTTG-3′	1425
RAG2-r7[b]	5′-AAGTAGAGCTCCTCAGAGTC-3′	1141
RAG2-r8[b]	5′-GCTGCCTTCCAGCTCATGTGGC-3′	1170
RAG2-r9[b]	5′-GTGTGGCCATATCGAGCTCC-3′	403

[a] Primers designed from conserved regions of vertebrate sequences.
[b] Primers designed from needlefish sequences.

binations with f1 and r4 to amplify the initial PCR product. PCR parameters were then optimized by varying $MgCl_2$ concentrations in several different reactions. The optimal reaction mix consisted of 50 μl containing 1 μl of DNA, 1.5 mM $MgCl_2$, 20mM Tris HCl ph 8.4, 50mM KCl, 200μM dNTPs, 0.4μM of each primer, and one unit of Gibco Taq polymerase. PCR cycling was performed using a ''touchdown'' program, with a 30-sec denaturation at 95 C, annealing for 60 sec at 58, 56, 54, and 52 C (two cycles at each temperature), and 90-sec extension at 72 C, for a total of 8 cycles; this regime was followed by 27 cycles of 30 sec at 95 C, 60 sec at 50 C, and 90 sec at 72 C, with a final extension of 72 C for 5 min. Generally, a single bright band approximately 1100 base pairs (bp) long was visualized; 40μl of product was run in a 1% agarose gel, then cut out and cleaned using a Quiagen spin column purification kit. Products were then sequenced directly using the Thermo Sequenase radiolabeled terminator cycle sequencing kit (Amersham Life Science) and the end primers used for PCR. Initial sequence data were used to design additional internal primers for sequencing (Table 1).

We collected a total of 1000 bp of RAG2, 499 bp of Tmo, 641 bp of cytochrome *b* (cyt *b*), and approximately 500 bp of 16S rRNA (16S). Alignment of the three protein coding genes was trivial— no insertions or deletions were observed. For 16S, the Megalign program of the DNAstar package (Lasergene) was used to implement Clustal W alignments. The effects of alignment parameters were explored by varying gap opening and gap length costs and comparing the alignments produced to the secondary structure model for piranha (*Pygocentris nattereri*) proposed by Orti et al. (1996). Gap opening costs between 10 and 20, and gap length costs between five and 20 produced alignments that were considered biologically reasonable because gaps were not inserted in hypothesized stem regions. Regions where alignment varied using combinations of these parameters (three regions and approximately 50 total base pairs) were excluded, producing a final 16S matrix of 468 base pairs. Four unambiguously aligned single base pair indels were found to be phylogenetically informative and coded as presence/absence characters (alignment available from NRL). All sequences have been deposited in GenBank, and accession numbers are listed in Material Examined. The alignments from separate genes were combined in a single matrix. We also added 22 morphological characters (see Appendices 1–2) from Lovejoy (2000) that are derived mainly from Boughton et al. (1991) to produce a final matrix of 2634 characters for 30 individuals representing 18 taxa.

PAUP* (vers. 4.0b3a, D. L. Swofford, Sinauer Assoc., Inc., Sunderland, MA, 1999, unpubl.) was used to find most-parsimonious trees. Data were analyzed together in a total evidence approach and separately (by gene and morphology) to investigate concordance among different data ''partitions.'' Whenever possible, the branch-and-bound algorithm was used, to guarantee discovery of all most-parsimonious trees (this was possible for the nuclear genes). For the full dataset, the mitochondrial genes, and morphology, we used the heuristic search algorithm with random addition of taxa (1000 replications). Bootstrap values (Felsenstein, 1985) were calculated using PAUP* (100 replications using the heuristic search option with 50 replicates of random taxon addition). TreeRot (M. D. Sorenson, Univ. of Michigan, Ann Arbor, 1996, unpubl.) was used to calculate decay indices (heuristic searches with 10 random addi-

tion replications; Bremer, 1988). Consistency indices (CI) and retention indices (RI) were calculated using PAUP* (all CI scores presented exclude uninformative characters). Incongruence length difference (ILD) tests (Farris et al., 1994) were implemented using PAUP* (100 replicates of heuristic searches) to assess data homogeneity. Various character partitions, corresponding to individual genes and gene combinations, were tested, but morphology was not included because the small size of the morphology partition (22 characters) relative to the number of taxa (30) frequently resulted in prohibitively long search times.

To explore patterns of character evolution, the datasets for different genes were separately optimized on the total evidence tree, providing measures of the number of steps, informative characters, and CI. The same procedure was followed with genes partitioned by codon position and by type of mutation (transitions vs transversions). To estimate branch lengths, numbers of changes were optimized (using ACCTRAN) on the total evidence tree using PAUP* and MacClade (vers. 3.0, W. R. Maddison and D. R. Maddison, Sinauer Assoc, Inc., Sunderland, MA, 1992, unpubl.). These branch lengths were used to calculate tree-based or patristic divergence values between species and clades as follows: (1) For each gene, the number of estimated character changes (patristic distance) between each terminal taxon in the tree was calculated; (2) For each comparison between species and/or clades, the mean of all relevant pairwise comparisons was calculated; and (3) This value was divided by the number of characters (base pairs sequenced for a particular gene) to provide a measure of percent divergence. This ''patristic divergence'' should provide a more accurate estimate of the mutational distance between taxa than uncorrected, Kimura 2-parameter, or even more complex measures because the latter do not take into account homoplastic changes that can be reconstructed from the tree.

Results

Figure 2 shows the single most-parsimonious tree based on all available data. The complete tree, generated using all 30 individuals, showed that every species is monophyletic; thus, duplicate individuals have been removed in this and all other trees presented. *Strongylura notata* was used to root the topology because of its basal position in an analysis using a larger number of taxa (Lovejoy, 2000). The New World clade of needlefishes is confirmed to be monophyletic. In accord with the larger study of Lovejoy

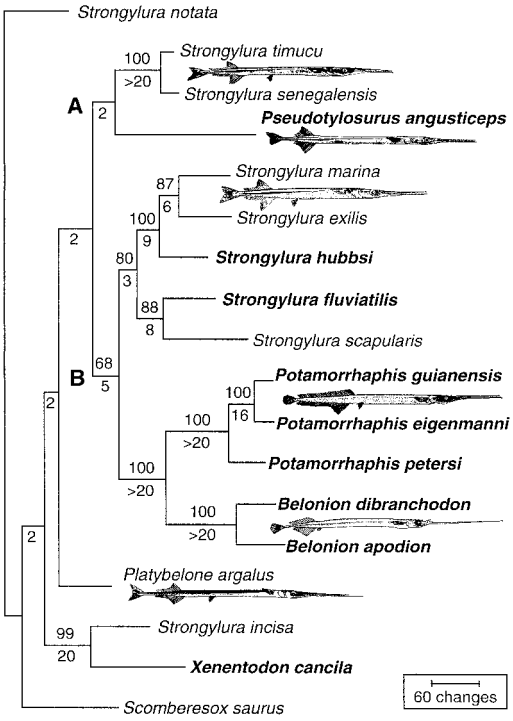

Fig. 2. Total evidence phylogeny for the New World clade of needlefishes and outgroups. Numbers above nodes are bootstrap proportions, and numbers below nodes are decay indices. Freshwater species are in bold. A and B indicate clades A and B of the New World clade (see text). Fish illustrations after Collette (1966, 1974a, 1978, 1982).

(2000), *Strongylura incisa* (marine) and *Xenentodon* (the Southeast Asian freshwater needlefish) make up a monophyletic group; they belong to a clade of Indo-West Pacific needlefishes in the larger study. The sister-group position of *Platybelone*, a marine species, relative to the New World clade also agrees with Lovejoy (2000).

The New World clade is divided into two groups. In one (clade A), the genus *Pseudotylosurus* is most closely related to a pair of marine needlefishes distributed in the Caribbean and western Atlantic (*Strongylura timucu*) and the eastern Atlantic *(S. senegalensis*). In the other group (clade B), the genera *Potamorrhaphis* and *Belonion* make up a monophyletic group related to a clade of *Strongylura* that occupy marine and freshwaters of the western Atlantic and eastern Pacific. Thus, the freshwater needlefishes from the Atlantic basins of South America do not make up a monophyletic group. Also, *Strongylura* is clearly polyphyletic, as predicted by Boughton et al. (1991).

An ILD test using each of the four genes as a separate partition did not indicate significant

heterogeneity ($P = 0.30$), justifying the combined analysis of all molecular data. Although we believe that the total evidence hypothesis offers the best explanation of the available information, we also examined cladograms derived from separately analyzed datasets. The phylogenetic patterns of the total evidence tree are generally congruent with those of individual analyses of nuclear genes. Separate analysis of RAG2 produces 10 equally parsimonious trees with a CI of 0.74 (Fig. 3). The sister relationship of *Pseudotylosurus* with *S. timucu* and *S. senegalensis* is supported, as is the relationship between *Potamorrhaphis* and *Belonion* and their *Strongylura* sister group. Analysis of Tmo produced 64 equally parsimonious trees (CI = 0.77) with less resolution, but all inferred relationships are congruent with the total evidence tree (Fig. 3). Combined analysis of both nuclear genes produces a single most-parsimonious tree (CI = 0.75) that is essentially identical to the total evidence tree, differing only in the positions of some of the taxa outside the New World clade. Bootstrap proportions within this tree are all 75% or higher (not shown).

The two mitochondrial genes are directly linked and should share the same history. Thus, it is most appropriate to analyze them together (data homogeneity between the two genes was confirmed with the ILD test, $P = 0.35$). Combined analysis of 16S and cyt *b* yields two most-parsimonious trees (CI = 0.43) whose consensus (Fig. 3) is identical to the total evidence tree, except with regard to the position of *Pseudotylosurus*. In the total evidence, RAG2, and combined nuclear gene trees, *Pseudotylosurus* is most closely related to *S. timucu* and *S. senegalensis*. In the combined mitochondrial analysis, *Pseudotylosurus* groups with *Strongylura scapularis* instead. To further understand the source of this discrepancy, we conducted separate analyses of the two mitochondrial genes. Analysis of 16S produced 12 most-parsimonious trees whose consensus (not shown) is relatively unresolved but is congruent with the total evidence hypothesis for most species groups. In particular, *Pseudotylosurus* was not grouped with *S. scapularis*, and clade B was supported as a monophyletic group. Analysis of cyt *b* produced two equally parsimonious trees whose consensus (not shown) shows several differences with the total evidence hypothesis. Notably, *Pseudotylosurus* groups with *Strongylura scapularis* in these trees, indicating that cyt *b* is the source of the main topological difference between the nuclear and combined mitochondrial trees. ILD tests confirmed this result: a test of 16S versus the two nuclear genes was not significant ($P = 0.19$), but a test of cyt *b* versus the nuclear genes indicated significant heterogeneity ($P = 0.02$).

Analysis of the morphological data alone produced a single most-parsimonious tree of 32 steps (CI = 0.67; Fig. 3). The topology is less resolved and considerably different from the DNA-based and total evidence trees. Of particular interest is the resolution of a clade composed of the endemic freshwater genera of both South America (*Potamorrhaphis, Belonion*, and *Pseudotylosurus*) and Southeast Asia (*Xenentodon*). The other major clade in the morphology tree includes all ingroup *Strongylura* species, *Platybelone*, and *Scomberesox*.

Table 2 shows characteristics of the data determined from optimizations on the total evidence tree. Most informative changes for the protein-coding genes are at third positions. As expected, the two mtDNA genes show more changes than the nuclear genes. In fact, the two mitochondrial genes combine to provide more than 75% of the total number of informative changes, with third position cyt *b* changes providing 45.9% of the total number of steps. However, these cyt *b* changes are highly homoplastic, as indicated by the low CI value compared to other data partitions. Table 3 shows levels of sequence divergence between various pairs of species and clades—in general there is an inverse relationship between level of divergence and homoplasy. The two nuclear genes, RAG2 and Tmo, show roughly similar levels of divergence that, as expected, are much lower than those of the mitochondrial genes. For most comparisons, cyt b is approximately twice as diverged as 16S.

Discussion

Congruence between mtDNA, morphology, and nuclear genes.—In terms of relationships among members of the New World clade, the cladograms based on RAG2 and Tmo, although not fully resolved, are congruent with the total evidence hypothesis. The combined mitochondrial tree suggests a somewhat different pattern, with disagreement over the position of *Pseudotylosurus*. It is difficult to know whether this difference results from variation in the histories of the genes involved (for example, because of differential lineage sorting), or whether it is a result of erroneous phylogenetic reconstruction. Separate analysis of the two mitochondrial genes indicate that 16S is congruent with the nuclear and total evidence trees and that cyt *b* data group *Pseudotylosurus* with *S. scapularis*. The fact that many cyt *b* characters are highly homoplastic, particularly at third codon positions,

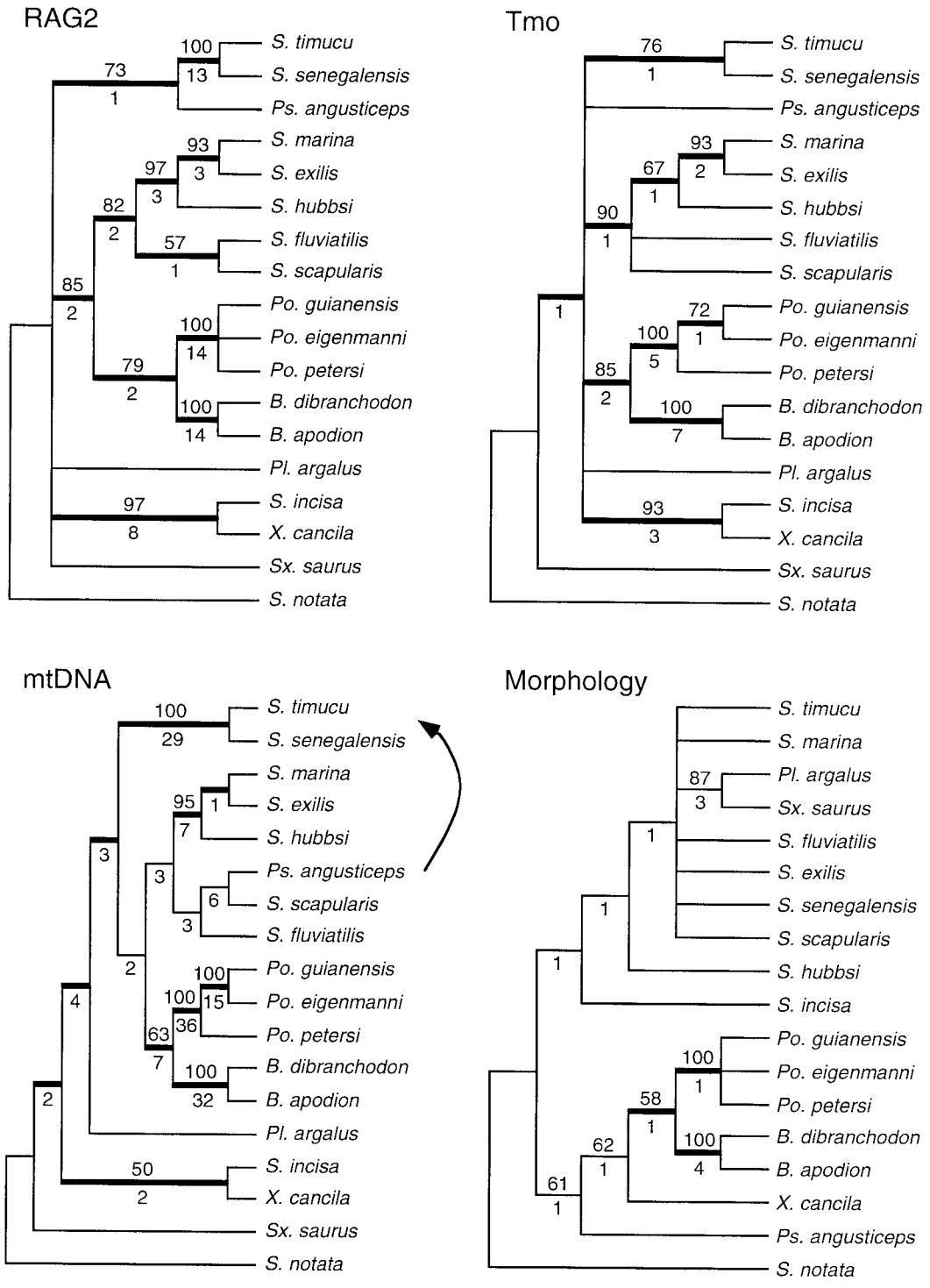

Fig. 3. Separate analyses of genes and morphology. Dark lines indicate nodes that are present in the total evidence hypothesis (Fig. 2). Numbers above nodes are bootstrap proportions, and numbers below nodes are decay indices. The arrow in the mtDNA tree indicates the change in position of *Pseudotylosurus* required to make the mtDNA topology fully congruent with the total evidence hypothesis.

TABLE 2. CHARACTERISTICS OF DIFFERENT DATA PARTITIONS DETERMINED FROM OPTIMIZATIONS ON THE TOTAL EVIDENCE HYPOTHESIS. # Char = number of characters per partition. % Informative = % characters that are informative within each partition. % Steps = % steps contributed by each partition to the total number of steps for the tree. Numbers in parentheses are transversions only.

	# Char	% Informative	# Steps	% Steps	CI
RAG2					
1st positions	334	8.7	67 (23)	3.4 (1.1)	0.68 (0.85)
2nd positions	333	6.6	48 (17)	2.4 (0.8)	0.61 (0.85)
3rd positions	333	28.5	191 (60)	9.6 (3.0)	0.79 (0.69)
Tmo					
1st positions	167	8.9	22 (4)	1.1 (0.2)	0.94 (1.0)
2nd positions	166	3.0	8 (2)	0.4 (0.1)	0.71 (0.5)
3rd positions	166	25.9	96 (16)	4.8 (0.8)	0.74 (0.85)
Cyt *b*					
1st positions	214	28.5	193 (38)	9.7 (1.9)	0.43 (0.68)
2nd positions	214	9.9	43 (16)	2.2 (0.8)	0.69 (0.67)
3rd positions	213	91.5	913 (275)	45.9 (13.8)	0.38 (0.39)
16S	468	27.1	359 (103)	18 (5.2)	0.53 (0.53)
16S gaps	4	100	8	0.4	0.50
Morphology	22	81.1	42	2.1	0.47
Total	2634	24.1	1990 (566)	100 (27.7)	0.49 (0.51)

combined with the relatively small number of sites sequenced (641 base pairs), suggests that the differences in topology between cyt *b* and the total evidence hypothesis may be an artifact of inaccurate phylogeny estimation by cyt *b*. To test this hypothesis, third positions of cyt *b* were downweighted by one-fifth to one-tenth in combined mitochondrial analyses. The results showed consistency with the nuclear and total evidence hypotheses—*Pseudotylosurus* grouped with *S. timucu* and *S. senegalensis* rather than *S. scapularis*. More data are required to fully test the congruence of mtDNA and the nuclear genes, but we expect that cyt *b* supports a different topology because of homoplasy and random error rather than real differences in gene history. Interestingly, although cyt *b* third positions contribute 46% of the steps on the total evidence tree, compared to 21.7% (Table 2) for the two nuclear genes combined, it is the pattern supported by the nuclear genes that emerges when the data are combined. This sug-

TABLE 3. AVERAGE PATRISTIC SEQUENCE DIVERGENCE (%) BETWEEN SELECTED SPECIES AND CLADES.

Species or clades	RAG2	Tmo	cyt *b*	16S
Strongylura timucu/S. senegalensis	>0.00	0.01	0.04	0.02
Pseudotylosurus angusticeps/P. microps	>0.00	>0.00	>0.00	>0.00
Clade A *Strongylura/Pseudotylosurus*	0.04	0.03	0.25	0.12
S. marina/S. exilis	0.02	>0.00	0.14	0.06
S. marina, S. exilis/S. hubbsi	0.02	0.01	0.15	0.07
S. fluviatilis/S. scapularis	0.01	0.01	0.19	0.08
S. marina, S. exilis, S. hubbsi/S. fluviatilis, S. scapularis	0.05	0.04	0.30	0.15
Potamorrhaphis guianensis/P. eigenmanni	0.01	0.01	0.05	0.01
P. guianensis, P. eigenmanni/P. petersi	0.01	>0.00	0.12	0.04
Belonion dibranchodon/B. apodion	>0.00	0.01	0.11	0.07
Potamorrhaphis/Belonion	0.05	0.04	0.12	0.14
Clade B *Strongylura/Potamorrhaphis, Belonion*	0.05	0.05	0.25	0.12
Clade A/clade B	0.06	0.04	0.32	0.17
*Platybelone/*clades A & B	0.07	0.08	0.26	0.12
Strongylura incisa/Xenentodon	0.05	0.04	0.16	0.06
*Strongylura notata/*ingroup	0.05	0.08	0.23	0.07

gests that the strong hierarchical information in the nuclear genes is not ''swamped'' by the more numerous and homoplastic cyt *b* changes.

The morphology-only topology also differs from the total evidence hypothesis. It should be borne in mind that the morphological data included in this study were not explicitly collected for phylogenetic analysis of needlefishes (particularly the New World clade) but are primarily derived from taxonomic revisions (e.g., Collette and Berry, 1965), new species descriptions (e.g., Collette, 1966), and higher-level phylogenetic investigations (e.g., Parin and Astakhov, 1982) as compiled by Boughton et al. (1991) and Lovejoy (2000). The small number of characters (18 informative) relative to the number of taxa (18 species) results in the mostly unresolved tree. The small dataset also makes it difficult to assess confidence in particular nodes. For example, a single character (18, secondary tubes of the body lateral line extend dorsally and ventrally) supports the clade that groups *Potamorrhaphis* and *Pseudotylosurus* (the endemic South American genera) with *Xenentodon* (the Southeast Asian freshwater endemic). Until a more detailed anatomical survey of belonid fishes is completed, we place limited reliance on the morphology-only tree and would hesitate to suggest that morphology conflicts with genes.

Although an ILD test using each of the four genes as a separate partition suggests no significant incongruence, independent phylogenetic analyses and ILD tests using alternative partitions (cyt *b* vs nuclear data) suggest that some of the data partitions used here may be ''incongruent'' (Bull et al., 1993). However, this incongruence may result from the small size of some partitions, the effects of homoplasy, and even the partitioning strategy (DeSalle and Brower, 1997; Siddall, 1997) rather than differences in the history of the partitions. We assume here that maximal hierarchical and phylogenetic information emerges from the combination of all available data (Barrett et al., 1991; Kluge and Wolf, 1993; Nixon and Carpenter, 1996) and use the total evidence tree (Fig. 2) as the basis for the following discussions.

Phylogeny.—The phylogeny presented here provides both new hypotheses and confirmation of previous proposals for the relationships of Neotropical freshwater needlefishes. Collette (1966) in his description of *Belonion* as a new genus of miniaturized needlefishes from the Orinoco and Amazon basins, hypothesized that its closest relative was *Potamorrhaphis* based on several characters, including reduction of the pharyngeal dentition (morphological characters 6 and 14 in this study), and the position of the first neural spine relative to the supraoccipital crests (5). Superficially, *Belonion* resembles a closely related family, the halfbeaks (Hemiramphidae), because it matures with an elongate lower jaw and a short upper jaw (most needlefishes pass through a similar ''halfbeak'' stage during development). The total evidence and molecular datasets strongly support a *Potamorrhaphis/Belonion* clade; the node is one of the best supported in the tree and appears in all the different analyses. This supports Collette's (1966) hypothesis that *Belonion* is ''developmentally arrested'' or paedomorphic relative to a *Potamorrhaphis*-like ancestor.

A relationship between the *Potamorrhaphis/Belonion* clade and *Pseudotylosurus* was implied by Collette (1966) in his hypothesized scenario for the evolution of freshwater Neotropical needlefishes and explicitly presented by Goulding and Carvalho (1984) in a tree that groups these genera without supporting characters. In both cases, the hypothesis appears to have been based more on biogeographic rather than morphological evidence. Because these three genera are endemic to freshwater South America, it was perhaps assumed that they would comprise a monophyletic group. The results presented here instead indicate that *Pseudotylosurus* and the *Potamorrhaphis/Belonion* clade are each related to different groups of *Strongylura* species, suggesting multiple transitions between marine and freshwater habitats. Neotropical freshwater needlefishes, therefore, show a different pattern than the three endemic genera of Neotropical freshwater stingrays, which do form a monophyletic group, indicating only a single transition to freshwater (Lovejoy, 1996; Lovejoy et al., 1998).

In Boughton et al.'s (1991) phylogeny, and in the morphology-only analysis of data presented here, the *Potamorrhaphis/Belonion* clade shows a sister-group relationship with *Xenentodon*, the Southeast Asian freshwater needlefish. The characters that unambiguously support this clade include elongate nasal papillae (character 4 in this study), and reduced or absent second upper pharygeal toothplates (14). However, in the total evidence tree, and the molecule-only analyses, *Xenentodon* is only distantly related to *Potamorrhaphis* and *Belonion*, and characters 4 and 14 are homoplastic. Collette (1966) had earlier suggested that the apparent similarities in pharygeal dentition between *Xenentodon* and the South American needlefishes were independently derived. Boughton et al. (1991) noted that the elongate nasal papilla may be a convergent adaptation to freshwater, because the char-

acter also appears in the four Southeast Asian freshwater halfbeak genera. The preponderance of data placing *Xenentodon* outside the New World needlefish clade supports the contention that characters 4 and 14 evolved convergently in different freshwater lineages. Whether these characters represent adaptations to freshwater is a question that awaits functional analyses. The reduction of pharyngeal dentition is a noticeable trend in several independent freshwater lineages (*Pseudotylosurus, Potamorrhaphis/Belonion*, and *Xenentodon* and deserves a more detailed anatomical investigation. An additional character that might represent a freshwater adaptation, as identified by its optimization on the tree, is 18 (secondary tubes of the body lateral line that extend both dorsally and ventrally), which occurs independently in *Potamorrhaphis, Pseudotylosurus,* and *Xenentodon* and groups these taxa together in the morphology-only analysis.

The total evidence phylogeny also clearly resolves relationships among various New World *Strongylura* species. As previously predicted (Boughton et al., 1991), *Strongylura* is not monophyletic. Instead, the genus consists of several small monophyletic species groups, such as the *S. timucu* and *S. senegalensis* clade and the *S. marina, S. exilis, S. hubbsi, S. scapularis,* and *S. fluviatilis* clade. The composition of these two clades, and the relationships among species in the latter are fully congruent with a study conducted by H. Banford, E. Bermingham, and B. B. Collette (unpubl.) based on a different set of mitochondrial and nuclear genes.

Estimating the number of marine to freshwater transitions. —Freshwater needlefish taxa of the New World clade are interleaved among marine sister groups, complicating inference of transitions between habitat types. To trace the evolution of these transitions, we coded habitat as a binary character (with states: freshwater or marine) and optimized it on the total evidence tree. The sister group and several sequential outgroups to the New World clade are marine; thus, this condition was considered the plesiomorphic state. Figure 4 shows the three equally parsimonious optimizations. They range from four independent invasions of freshwater (Fig. 4A) to a single invasion followed by three independent returns to marine waters (Fig. 4C). However, not all these scenarios are equally likely if geological, biogeographic, and chronological information is considered.

Figure 4B–C show reconstructions in which the hypothetical ancestor of clade B was originally present in fresh water, and transitions to

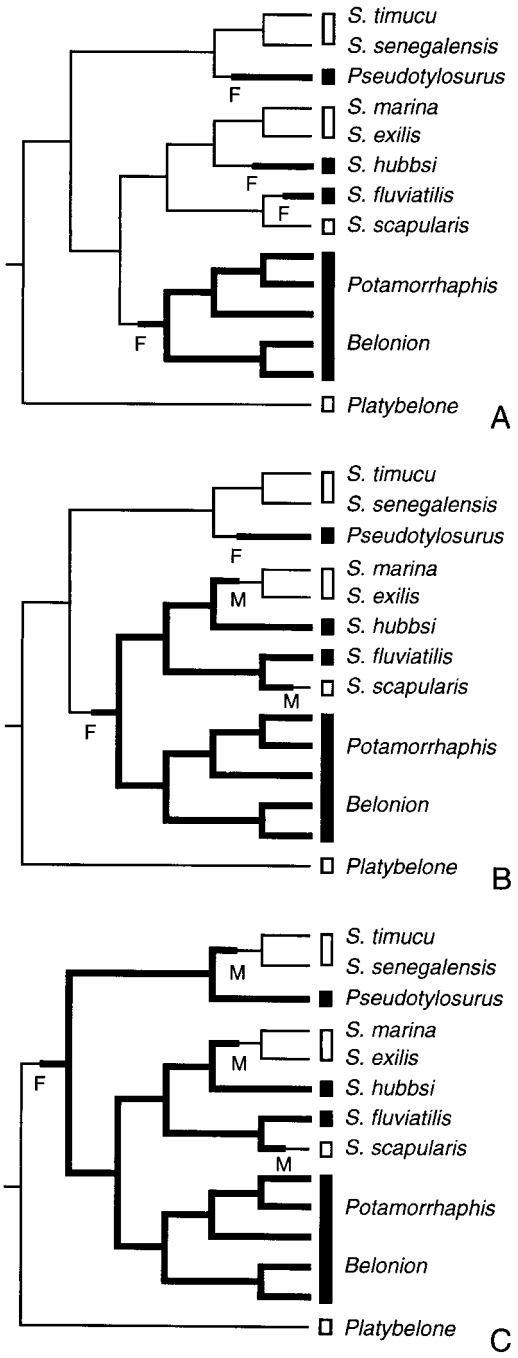

Fig. 4. Alternative optimizations of freshwater/marine transitions in the New World needlefish clade. Dark boxes indicate freshwater taxa, and F indicates a transition to freshwater. Open boxes indicate marine taxa, and M indicates a transition to a marine habitat.

marine habitats independently occurred on the branches leading to *S. scapularis* and *S. marina/S. exilis*. This reconstruction implies that the freshwater ancestor of clade B was originally distributed from the Atlantic drainage of the Rio Usumacinta of Mexico and Guatemala (*S. hubbsi*) to the Pacific slopes of South America (*S. fluviatilis*) and the Atlantic drainages of South America (*Potamorrhaphis* and *Belonion*). If we entertain a dispersal scenario, it is necessary to hypothesize movement of needlefishes across the isthmus of Panama in a northern or southern direction to give rise to these geographically separated species. The important point is that needlefishes must have been distributed across the isthmus of Panama or to have traversed it to explain the patterns seen in Figure 4B or C.

This seems unlikely. If needlefishes crossed the isthmus of Panama during its Pliocene emergence, we would expect to observe remnant populations in the rivers of Panama, Costa Rica, and intervening regions. Because these have not been recorded, we would have to invoke extinction throughout this area. Numerous South American fish taxa are hypothesized to have dispersed northward from South America across the isthmus (Myers, 1966; Bussing, 1985; Bermingham and Martin, 1998), but they show evidence of this movement in the form of remnant populations and species along their route. The phylogenetic and geographic distributions of the sister species *S. marina* and *S. exilis* are also difficult to reconcile with an isthmian freshwater distribution. These species are distributed in the Atlantic/Caribbean (*S. marina*) and Pacific (*S. exilis*) and, thus, would appear to be geminate taxa (Jordan, 1908) divided by the emergence of the isthmus. However, if the isthmus was already present (to allow a geographic connection between freshwater *Strongylura*), we must instead posit that *S. marina* and *S. exilis* represent separate reinvasions from freshwater into each ocean. These additional hypotheses of extinction and dispersal required to explain the hypothesized patterns of Figure 4B–C make it more parsimonious to assume that the transitions between marine and freshwater habitats occurred according to the pattern shown in Figure 4A and that multiple invasions of fresh water took place rather than the converse.

The analysis above makes the assumption that transitions between habitat types (and therefore dispersals) have occurred. From a strict vicariance perspective, we would instead assume that the distribution of an ancestral taxon represents the sum of the distributions of its descendants (Humphries and Parenti, 1999). Thus, a clade with freshwater and marine taxa would be assumed to be descended from an ancestral needlefish species whose range included both habitat types. This assumption seems unrealistic in light of the biology of belonid fishes: no species currently have large ranges encompassing both freshwater and marine areas (although some species, such as *S. marina* and the Indo-West Pacific *S. strongylura* have large marine ranges, and minor freshwater distributions). We believe that our optimization of habitat on the needlefish tree, combined with the distributional data outlined above, provides a reasonable hypothesis of multiple freshwater origins. More complete understanding of the biogeographic history of New World needlefishes will derive from phylogenetic analyses of other taxa with similar distributions, allowing detailed comparisons of pattern.

Freshwater Strongylura.—Based on the discussion above, we hypothesize that needlefishes have made four independent transitions into Neotropical freshwater habitats. Two of these produced the geographically restricted *S. hubbsi*, centered in the upper Rio Usumacinta drainage of Guatemala and Mexico, and *S. fluviatilis*, distributed in rivers of northwestern South America (Ecuador and Colombia) that drain to the Pacific and Caribbean (Rio Atrato). *Strongylura hubbsi* and *S. fluviatilis* are nested within clade B marine *Strongylura*, and the ecology of the latter provides a good explanation for the propensity for freshwater invasion within this group. *Strongylura marina* is a frequent invader of rivers—the species has been collected 622 km up the Black Warrior River at Tuscaloosa, Alabama (Boschung and Hemphill, 1960), and maintains freshwater populations in the St. John's river system of Florida (Collette, 1974b). *Strongylura exilis* has been collected in estuaries, rivers, and sloughs (Miller, 1966; Fitch and Lavenberg, 1975). These taxa are physiologically well adapted to salinity fluctuations and provide a model for how freshwater *Strongylura* may have originated.

The distribution of *S. hubbsi* is paralleled by several other predominantly marine groups, including halfbeaks of the genus *Hyporhamphus* (Collette, 1974b) and toadfishes of the genus *Batrachoides* (Collette and Russo, 1981), both of which are primarily marine but have endemic representatives in the Usumacinta. Because shared biogeographic patterns may be indicative of shared responses to historical (geological) events (Nelson and Platnick, 1981; Humphries and Parenti, 1999), these distributions may be indicative of an ancient vicariance event

that isolated marine or estuarine taxa in the Usumacinta region. Alternatively, the pattern may signal particular ecological conditions of the Usumacinta that facilitate invasions from the sea. Movements between freshwater and marine habitats are inhibited by gradients in osmotic pressure and ionic concentrations (Lee and Bell, 1999). The Usumacinta drains an eroded carbonate and limestone karst formation—a habitat of potentially high ionic concentration that may have facilitated movement from marine to freshwater habitats (H. Banford, E. Bermingham, and B. B. Collette, unpubl.).

The sister taxon of *S. hubbsi* consists of an Atlantic and Pacific species pair; thus, *S. hubbsi* diverged prior to the last connection between these oceans, dated at 3 MYBP (Duque-Caro, 1990; Coates and Obando, 1996; Nesbitt and Young, 1997). Less can be said of the origin of *S. fluviatilis*, but it appears that this freshwater species originated from the Pacific, because of the Pacific distribution of its sister taxon, *S. scapularis*. A similar origin has been proposed for the freshwater Atrato toadfish *Daector quadrizonatus*, whose relatives also occur in the Pacific (Collette, 1973). *Strongylura fluviatilis* is distributed in both Pacific slope drainages of the Andes and the Caribbean draining Rio Atrato, and thus its origin must date at least to the last connection between these systems.

Amazon invasions.—The endemic genera of needlefishes from the large Atlantic drainages of South America, *Pseudotylosurus, Potamorrhaphis,* and *Belonion*, are in the company of other Neotropical freshwater representatives of predominantly marine groups, such as stingrays, anchovies, flatfishes, toadfishes, and drums (Roberts, 1972; Goulding, 1980). Phylogenetic and biogeographic analyses have been presented for freshwater anchovies (Nelson, 1984), stingrays (Lovejoy, 1996; Lovejoy et al., 1998), and stingray parasites (Brooks et al., 1981; Lovejoy, 1997), offering hypotheses for the origination of these taxa from their marine relatives. There are several similarities in ecological, phylogenetic, and biogeographical patterns between freshwater Neotropical needlefishes and stingrays (Potamotrygonidae). In both groups, freshwater endemic genera are most closely related to near-shore marine relatives that are physiologically tolerant of freshwater habitats. The potamotrygonid stingrays are most closely related to euryhaline rays that enter estuaries and rivers (Lovejoy, 1996). *Potamorrhaphis* and *Belonion* are related to a clade whose constituents are either exclusively freshwater (*Strongylura hubbsi* and *S. fluviatilis*), or known to ascend rivers (*Strongylura marina, S. exilis*; Myers, 1966; Collette, 1974b). Similarly, the sister species of *Pseudotylosurus* (*Strongylura timucu* and *S. senegalensis*) have been reported from lakes and brackish bays (Collette and Parin, 1970; Collette, 1974b). These patterns suggest that ancestors of the ''freshwater invaders'' were preadaptated for inland survival.

Needlefishes (like stingrays) occupy a broad range in South America that includes the Amazon, Orinoco, Guyana, and Parana basins. This suggests a relatively ancient association with fresh water, and the phylogenetic position of the freshwater needlefishes supports the idea—the divergence of the *Pseudotylosurus* and *Potamorrhaphis/Belonion* lineages are among the deepest in the New World clade. Hypotheses for the origination of ancient marine-derived taxa in the Neotropics have been proposed that invoke the role of Andean orogeny (Brooks et al., 1981; Brooks, 1995) and marine incursions (Lovejoy et al., 1998) as vicariance events that isolated marine taxa in newly forming freshwater habitats. Unlike freshwater stingrays, which comprise a monophyletic group, the *Pseudotylosurus* and *Potamorrhaphis/Belonion* lineages had independent origins from marine relatives. In one case (clade B), biogeographic patterns are similar to those of stingrays, stingray parasites, and anchovies—freshwater taxa are the sister group of species distributed in both the Atlantic and Pacific. Was the *Potamorrhaphis/Belonion* clade affected by the same geological events that produced freshwater stingrays? We defer further examination of the needlefish biogeographic patterns to a later publication (N. R. Lovejoy, unpubl.). A critical criterion for differentiating between the Andean orogeny and marine incursion scenarios is some estimate of the age of freshwater lineages; comparisons of sequence evolution hold promise for this purpose, but interpretation of these data is complex (Hillis et al., 1996; Arbogast and Slowinsky, 1998; Slowinsky and Arbogast, 1999).

RAG2 for phylogenetic analysis.—The realization that mitochondrial genes may have particular limitations for phylogeny reconstruction (e.g., Meyer, 1994; however see Lydeard and Roe, 1997) has led to widespread attempts to diagnose nuclear markers that will offer new sources of systematic data (e.g., Orti, 1997; Quattro and Jones, 1999). Based on our study, RAG2 appears to be an excellent choice for molecular systematic studies of fishes. The amplified fragment of the gene provided a sufficient number of informative characters to almost fully resolve a species-level tree (see also Sullivan et al., 2000). Se-

quence divergence between species and clades is lower for RAG2 (and Tmo) than for the mitochondrial genes (Table 3), and correspondingly, RAG2 shows less homoplasy (Table 2). The phylogeny based on RAG2 is largely concordant with both the total evidence tree and with other individual genes analyzed separately. We detected no evidence of multiple copies or pseudogenes during PCR and sequencing. Finally, the primers described here have been successfully tested on a range of taxa, including Clupeiformes (N. R. Lovejoy, unpubl.), Osteoglossiformes (J. Sullivan, unpubl.), and mormyrids (Sullivan et al., 2000). We predict that RAG2 will be a useful source of characters for phylogenetic studies over a broad taxonomic range. The low levels of sequence divergence between deep clades in our needlefish tree suggests that RAG2 may be particularly useful for resolving more ancient divergences.

Material Examined

Institutional abbreviations follow Leviton et al. (1985). GenBank accession numbers for RAG2, Tmo, cyt *b*, and 16S are in brackets.

Belonion apodion, INPA 14339, Rio Negro, Brazil, (AF306488, AF244082, AF243931, AF244007). *Belonion dibranchodon*, CU 78499, Rio Atabapo, Venezuela, (AF306468, AF244030, AF243878, AF243954). *Belonion dibranchodon*, CU 78499, Rio Atabapo, Venezuela, (AF306469, AF244031, AF243879, AF243955). *Platybelone argalus argalus*, UF 99881, Tortugas Bank, Florida, (AF306464, AF244026, AF243874, AF243950). *Platybelone argalus platyura*, USNM 348277, Bolinaro Mkt., Philippines, (AF306465, AF244027, AF243875, AF243951). *Potamorrhaphis eigenmanni*, CU 77949, Rio Matos, Bolivia, (AF306470, AF244034, AF243882, AF243958). *Potamorrhaphis eigenmanni*, CU 77950, Arroyo Mururita, Bolivia, (AF306471, AF244035, AF243883, AF243959). *Potamorrhaphis guianensis*, CU 76874, Rio Caicara, Venezuela, (AF306466, AF244028, AF243876, AF243952). *Potamorrhaphis guianensis*, CU 76873, Rio Caicara, Venezuela, (AF306467, AF244029, AF243877, AF243953). *Potamorrhaphis petersi*, CU 78500, Rio Atabapo, Venezuela, (AF306474, AF244044, AF243892, AF243968). *Pseudotylosurus angusticeps*, CU 78505, Rio Napo, Ecuador, (AF306475, AF244045, AF243893, AF243969). *Pseudotylosurus angusticeps*, CU 78505, Rio Napo, Ecuador, (AF306476, AF244046, AF243894, AF243970). *Pseudotylosurus angusticeps*, INPA 13132, Rio Tapajos, Brazil, (AF306486, AF244066, AF243914, AF243990). *Scomberesox saurus*, no specimen, Mediterranean Sea, Majorca, (AF306481, AF244060, AF243908, AF243984). *Strongylura exilis*, STRI 00192, Eastern Pacific, Panama, (AF306482, (AF244062, AF243910, AF243986). *Strongylura exilis*, STRI 00192, Eastern Pacific, Panama, (AF306483, AF244063, AF243911, AF243987). *Strongylura hubbsi*, CU 77876, Rio Usumacinta, Guatemala, (AF306479, AF244049, AF243897, AF243973). *Strongylura hubbsi*, CU 77876, Rio Usumacinta, Guatemala, (AF306480, AF244050, AF243898, AF243974). *Strongylura fluviatilis*, CU 78507, Rio Cayapas, Ecuador, (AF306477, AF244047, AF243895, AF243971). *Strongylura fluviatilis*, CU 78507, Rio Cayapas, Ecuador, (AF306478, AF244048, AF243896, AF243972). *Strongylura incisa*, CU 78063, Cubao Mkt., Philippines, (AF306472, AF244036, AF243884, AF243960). *Strongylura marina*, USNM uncat., ID by H. Banford, Western Atlantic, Virginia, (AF306462, AF244018, AF243866, AF243942). *Strongylura marina*, USNM uncat., ID by H. Banford, Western Atlantic, Virginia, (AF306463, AF244019, AF243867, AF243943). *Strongylura notata forsythia*, CU 75110, Long Key, Florida, (AF306489, AF244008, AF243856, AF243932). *Strongylura scapularis*, STRI 00194, Eastern Pacific, Panama, (AF306487, AF244069, AF243918, AF243994). *Strongylura senegalensis*, USNM 348315, Volta estuary, Ghana, (AF306484, AF244064, AF243912, AF243988). *Strongylura senegalensis*, CU 78068, Volta estuary, Ghana, (AF306485, AF244065, AF243913, AF243989). *Strongylura timucu*, CU 75117, North Hobe Beach, Florida, (AF306460, AF244014, AF243862, AF243938). *Strongylura timucu*, CU 75113, North Hobe Beach, Florida, (AF306461, AF244015, AF243863, AF243939). *Xenentodon cancila*, CU 77144, West Bengal, India (aquarium), (AF306473, AF244042, AF243890, AF243966).

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APPENDIX 1. MORPHOLOGICAL CHARACTERS FOR PHYLOGENETIC ANALYSIS. Numbers in brackets refer to character numbers in Lovejoy (1999).

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. Gillrakers: 1 = present; 0 = absent (Collette and Berry, 1965).
4. (5). Shape of nasal papillae: 1 = elongate; 0 = spatulate (Collette, 1974a).
5. (6) Shape of first neural spine: 1 = elongate; 0 = not elongate (Collette, 1966).
6. (7) Density of pharyngeal teeth: 1 = sparse; 0 = dense (Collette, 1966).
7. (9) Canalis postemporalis: 1 = present; 0 = absent (Parin and Astakhov, 1982; Boughton et al., 1991).
8. (10) Canalis epioticalis: 1 = present; 0 = absent (Parin and Astakhov, 1982; Boughton et al., 1991).
9. (11) Curvature of canalis frontalis: 1 = acutely recurved; 0 = shallowly curved (Parin and Astakhov, 1982; Boughton et al., 1991).
10. (12) Gap in canalis frontalis: 1 = gap; 0 = no gap (Parin and Astakhov, 1982).
11. (14) Postorbital segment of the canalis frontalis: 1 = present; 0 = absent (Parin and Astakhov, 1982; Boughton et al., 1991).
12. (15) Anteromedial branch of the canalis nasalis: 1 = present; 0 = absent (Parin and Astakhov, 1982; Boughton et al., 1991).
13. (16) Shape of caudal fin: 1 = strongly forked; 0 = shallowly forked or truncate (Boughton et al., 1991).
14. (19) Second upper pharyngeal toothplate: 1 = reduced or absent; 0 = present (Collette, 1966).
15. (20) Paired frontal bones: 1 = fused; 0 = separated (Collette, 1966).
16. (21) Pectoral branch of the lateral line: 1 = absent; 0 = present (Parin and Astakhov, 1982).
17. (22) Premaxillary canal: 1 = present; 0 = absent (Parin and Astakhov, 1982).
18. (25) Secondary tubes of the body lateral line: 1 = extend both dorsally and ventrally; 0 = extend ventrally only, or are absent (Collette, 1974a).
19. (27) Fourth upper pharyngeal toothplate: 1 = absent; 0 = present (Collette et al., 1984).
20. (28) Shape of lower pharyngeal plate: 1 = long and narrow; 0 = expanded posteriorly (Collette, 1974a).
21. (29) Relation between preorbital and maxillary: 1 = preorbital covers lower margin of maxillary; 0 = lower margin of maxillary uncovered (Collette, 1978; this study).
22. (36) Upper jaw in adults: 0 = short; 1 = elongate (Collette et al., 1984).

APPENDIX 2. MORPHOLOGICAL CHARACTER MATRIX.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Belonion apodion	1	1	0	1	1	1	0	0	0	1	0	0	0	1	1	0	0	0	1	0	1	0
Belonion dibranchodon	1	1	0	1	1	1	0	0	0	1	0	0	0	1	1	0	0	0	1	0	1	0
Platybelone argalus	1	1	1	0	0	0	1	0	0	0	1	1	1	0	0	0	1	0	0	0	0	1
Potomorrhaphis eigenmanni	1	1	0	1	1	1	0	0	1	1	1	1	0	1	0	0	1	1	0	0	1	1
Potamorrhaphis guianensis	1	1	0	1	1	1	0	0	1	1	1	1	0	1	0	0	1	1	0	0	1	1
Potamorrhaphis petersi	1	1	0	1	1	1	0	0	1	1	1	1	0	1	0	0	1	1	0	0	1	1
Pseudotylosurus augusticeps	1	1	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	1	0	1	1	1
Scomberesox saurus	0	0	1	0	0	0	1	1	0	0	1	1	0	0	0	1	1	0	0	0	1	1
Strongylura exilis	1	1	0	0	0	0	1	0	1	1	1	1	0	0	0	0	1	0	0	0	1	1
Strongylura hubbsi	1	1	0	0	0	0	0	0	1	1	1	1	0	0	0	0	1	0	0	0	1	1
Strongylura fluviatilis	1	1	0	0	0	0	1	0	1	1	1	1	0	0	0	0	1	0	0	0	1	1
Strongylura incisa	1	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	1	1
Strongylura marina	1	1	0	0	0	0	1	0	1	1	1	1	0	0	0	0	1	0	0	0	1	1
Strongylura notata	1	1	0	0	0	0	1	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1
Strongylura scapularis	1	1	0	0	0	0	1	0	1	1	1	1	0	0	0	0	1	0	0	0	1	1
Strongylura senegalensis	1	1	0	0	0	0	1	0	1	1	1	1	0	0	0	0	1	0	0	0	1	1
Strongylura timucu	1	1	0	0	0	0	1	0	1	1	1	1	0	0	0	0	1	0	0	0	1	1
Xenentodon cancila	1	1	0	1	0	0	0	0	1	1	0	0	0	1	0	0	1	1	1	1	1	1