Mitogenomic Exploration of Higher Teleostean Phylogenies: A Case Study for Moderate-Scale Evolutionary Genomics with 38 Newly Determined Complete Mitochondrial DNA Sequences

Masaki Miya, Akira Kawaguchi,† and Mutsumi Nishida†*

*Natural History Museum and Institute, Chiba, Japan; and †Ocean Research Institute, University of Tokyo, Tokyo, Japan

Although adequate resolution of higher-level relationships of organisms apparently requires longer DNA sequences than those currently being analyzed, limitations of time and resources present difficulties in obtaining such sequences from many taxa. For fishes, these difficulties have been overcome by the development of a PCR-based approach for sequencing the complete mitochondrial genome (mitogenome), which employs a long PCR technique and many fish-versatile PCR primers. In addition, recent studies have demonstrated that such mitogenomic data are useful and decisive in resolving persistent controversies over higher-level relationships of teleosts. As a first step toward resolution of higher teleostean relationships, which have been described as the ''(unresolved) bush at the top of the tree,'' we investigated relationships using mitogenomic data from 48 purposefully chosen teleosts, of which those from 38 were newly determined during the present study (a total of 632,315 bp), using the above method. Maximum-parsimony and maximum-likelihood analyses were conducted with the data set that comprised concatenated nucleotide sequences from 12 protein-coding genes (excluding the ND6 gene and third codon positions) and 22 transfer RNA (tRNA) genes (stem regions only) from the 48 species. The resultant two trees from the two methods were well resolved and largely congruent, with many internal branches supported by high statistical values. The tree topologies themselves, however, exhibited considerable variation from the previous morphology-based cladistic hypotheses, with most of the latter being confidently rejected by the mitogenomic data. Such incongruence resulted largely from the phylogenetic positions or limits of long-standing problematic taxa, which were quite unexpected from previous morphological and molecular analyses. We concluded that the present study provided a basis of and guidelines for future investigations of teleostean evolutionary mitogenomics and that purposeful higher-density taxonomic sampling, subsequent sequencing efforts, and phylogenetic analyses of their mitogenomes may be decisive in resolving persistent controversies over higher-level relationships of teleosts, the most diversified group of all vertebrates, comprising over 23,500 extant species.

Introduction

Recently, much attention has been paid to evolutionary genomics as a postgenomics biology (Easteal 2000). In fact, application of genome technologies (e.g., whole-genome shotgun sequencing; Venter, Smith, and Hood 1996) capable of producing sequence data in large quantities is now expected in this field (Pollock et al. 2000). Pollock et al. (2000) designed a method for applying such genome technologies to evolutionary genomics, using vertebrate mitochondrial genomes (mitogenomes) as examples, demonstrating its feasibility on the basis of simulation experiments. In addition, Pollock et al. (2000) convincingly listed many potential benefits as primary products of such large-scale evolutionary genomic sequencing, with one of the most promising being demonstrably more accurate phylogenetic reconstructions with unprecedentedly long DNA sequences from many taxa. For large-scale evolutionary genomics involving multiple laboratories in different countries, these ideas appear to be sound and far-reaching. For moderate-scale evolutionary genomics (e.g., within a specific group of vertebrates), however, a more practical method that is feasible in a single laboratory or a few laboratories would be desirable, owing to various resource limitations and depending on the purposes of the studies.

For fish mitogenomes, one practical method is a PCR-based approach that employs a long PCR technique and many versatile PCR primers and is accurate and faster than sequencing cloned mitochondrial DNA (mtDNA) (Miya and Nishida 1999; for bird mitogenomes, see Sorenson et al. 1999). In fact, complete mtDNA sequences for 12 teleost species have already been determined using this method (Miya and Nishida 1999, 2000*b*; Inoue et al. 2000, 2001*a*, 2001*b*, 2001*c*, 2001*d*; Ishiguro, Miya, and Nishida 2001; Kawaguchi, Miya, and Nishida 2001). Furthermore, including the additional species determined during the present study, the number of complete mtDNA sequences determined using this method (total 50) has become compatible with those determined in other studies for whole vertebrates, including fishes (total 69; Pollock et al. 2000), indicating a certain utility of our approach in moderate-scale evolutionary genomic studies. Thus, technical difficulties in obtaining mitogenomic data from various fishes within a relatively short period of time had been overcome, although there still remained problems as to whether or not they were suitable for inferring phylogenies, thus providing a basis for further comparative evolutionary analyses.

To address this problem, Miya and Nishida (2000*b*) explored the phylogenetic utility and limits of individual and concatenated mitochondrial genes for reconstructing higher-level relationships, using the complete mtDNA sequences of eight teleosts (of noncontroversial relative

Key words: mitogenomics, teleosts, complete mtDNA sequence, long PCR, taxonomic sampling, higher-level relationships.

Address for correspondence and reprints: Masaki Miya, Department of Zoology, Natural History Museum and Institute, Chiba, 955-2 Aoba-cho, Chuo-ku, Chiba 260-8682, Japan. E-mail: miya@chiba-muse.or.jp.

Mol. Biol. Evol. 18(11):1993–2009. 2001

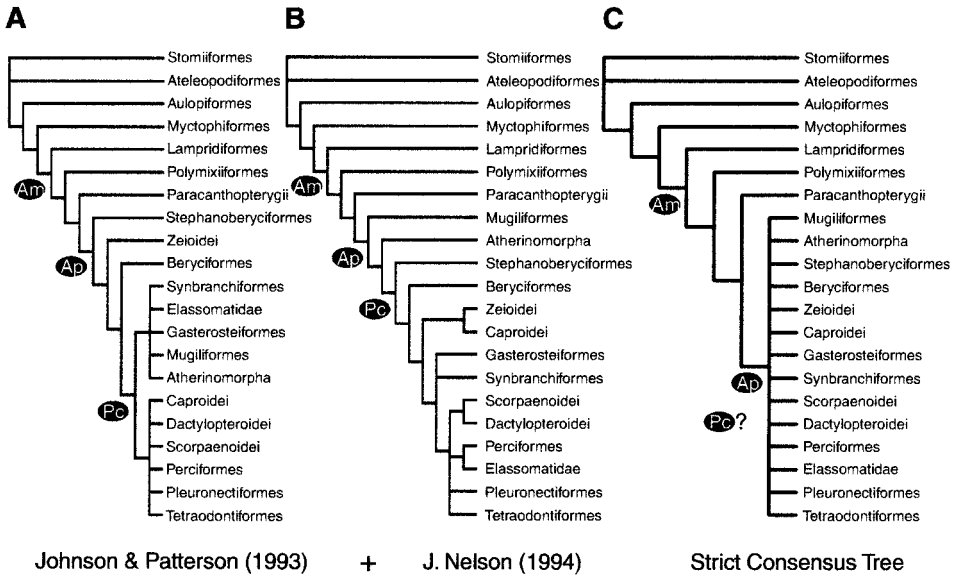

FIG. 1.—Two recently proposed hypotheses on higher-teleostean phylogeny advocated by (*A*) Johnson and Patterson (1993) and (*B*) Nelson (1994). *C*, A strict consensus tree derived from the two former trees. Note that the relationships of the Lampridiformes and below follow those advocated by Johnson (1992) and Olney, Johnson, and Baldwin (1993). Abbreviations: *Am*, Acanthomorpha; *Ap*, Acanthopterygii; *Pc*, Percomorpha.

phylogenetic positions). They demonstrated that nucleotide sequences from concatenated protein-coding (no third codon positions) plus transfer RNA (tRNA; stem regions only) genes (hereinafter called ''mitogenomic data'') were most able to reproduce the expected phylogeny of teleosts with high statistical support, unlike most individual genes. Accordingly, mitogenomic data can be expected to resolve the persistent controversies over the higher-level relationships of teleosts. Subsequently, Inoue et al. (2001*d*) resolved the interrelationships of five major lineages of basal teleosts (Osteoglossomorpha, Elopomorpha, Clupeomorpha, Ostariophysi, and Protacanthopterygii, given various rankings), for which five alternative phylogenetic hypotheses had previously been proposed on the basis of both morphological and molecular data, using mitogenomic data. Thus, the mitogenomic data not only are able to reproduce the expected phylogeny of teleosts (Miya and Nishida 2000*b*), but can also resolve specific phylogenetic questions for higher-level relationships of teleosts (Inoue et al. 2001*d*), the most diversified group of all vertebrates, comprising over 23,500 extant species (J. S. Nelson 1994).

Higher teleosts have been collectively called Acanthomorpha, Acanthopterygii, or Percomorpha, depending on their limits (fig. 1; Rosen 1973; Lauder and Liem 1983; J. S. Nelson 1984, 1994; Stiassny 1986; Stiassny and Moore 1992; Johnson 1993; Johnson and Patterson 1993; Stiassny, Parenti, and Johnson 1996). If higher teleosts are equated with the most comprehensive group, Acanthomorpha (Rosen 1973), they comprise about 14,650 species placed in 20 orders, 296 families, and 2,615 genera (calculated from J. S. Nelson 1994). Their interrelationships have long been controversial and so complex that G. Nelson (1989) described them as the ''(unresolved) bush at the top of the tree,'' which is still evident, as seen in figure 1. A strict consensus tree (fig. 1*C*) generated from two recently proposed hypotheses on higher teleostean relationships (Johnson and Patterson 1993 [fig. 1*A*]; J. S. Nelson 1994 [fig. 1*B*]) clearly included unresolved ''bushes'' (fig. 1*C*). As a first step toward resolution of higher teleostean phylogenies containing such enormous taxonomic diversity, this study attempted (1) to circumscribe a well-supported monophyletic group encompassing such ''bushes'' (=Percomorpha) and (2) to determine the phylogenetic position of such a monophyletic group relative to other major lineages using mitogenomic data. By doing so, we hoped to clarify where the phylogenetic problems lay, thus providing a basis and guidelines for subsequent resolution of the ''bushy top.'' Clearly, this study was not intended to resolve intrarelationships of the bushy top, as such an investigation would require wider and more extensive taxonomic sampling.

Materials and Methods

Taxonomic Sampling

For resolution of a complex phylogeny with enormous taxonomic diversity, such as that seen in higher teleosts (fig. 1), it is essential to conduct purposeful taxonomic sampling that increases phylogenetic accuracy (Hillis 1998). We employed two taxonomic sampling strategies, individually or in combination, according to the following recommendation: (1) ''select taxa within the monophyletic group of interest that will represent the overall diversity of the group'' (strategy 3 in Hillis 1998), and (2) ''select taxa within the monophyletic group of interest that are expected (based on current taxonomy or previous phylogenetic studies) to subdivide long branches in the initial tree'' (strategy 4 in Hillis 1998). Hillis (1998) stated that careful addition of taxa

to ensure coverage of the group of interest and to purposefully break up long branches (a combination of strategies 3 and 4) seemed to be the optimal taxonomic sampling strategy.

For circumscription of the upper bushes, we chose at least one species each to represent all of the major lineages above Paracanthopterygii (=Acanthopterygii; fig. 2); of those major lineages that occupied the three most basal positions in either Johnson and Patterson's (1993) or J. S. Nelson's (1994) hypotheses (see fig. 1*A* and *B*; Mugiliformes, Atherinomorpha, Stephanoberyciformes, Beryciformes, and Zeioidei), we added one to three species so as to locate their phylogenetic positions more correctly, assuming possible cases in which they would be placed outside the upper bushes. For more accurate determination of the relative phylogenetic positions of the upper bushes, we chose two to three species from all major lineages up to the Paracanthopterygii in order to break up possible long branches (fig. 2). Final rooting was done using a clupeid, *Sardinops melanostictus*, with reference to the recent mitogenomic analysis of basal teleostean phylogeny by Inoue et al. (2001*d*). All species used in this study are listed in table 1, along with references and DDBJ/EMBL/GenBank accession numbers.

DNA Extraction

A portion of the epaxial musculature (ca. 0.25 g) was excised from fresh specimens of each species and immediately preserved in 99.5% ethanol. Total genomic DNA was extracted using the Qiagen QIAamp tissue kit following the manufacturer's protocol.

Mitochondrial DNA Purification by Long PCR

The mitogenomes of the 38 species were amplified in their entirety using a long PCR technique (Cheng et al. 1994; Miya and Nishida 1999). Seven fish-versatile long PCR primers (S-LA-16S-L, L2508-16S, L12321-Leu, H12293-Leu, H15149-CYB, H1065-12S, and S-LA-16S-H; for locations and sequences of these primers, see Miya and Nishida 2000*b*; Inoue et al. 2000, 2001*d*; Ishiguro, Miya, and Nishida 2001; Kawaguchi, Miya, and Nishida 2001) were used in various combinations so as to amplify the entire mitochondrial genome in a single reaction or two reactions. When long PCR using the above primers proved to be unsuccessful for a certain segment, species-specific primers were alternatively designed with reference to the partial nucleotide sequences from either 16S rRNA, ND5, or cytochrome *b* (cyt *b*) genes, determined from total DNA with the fish-versatile primers listed below.

Long PCR was done in a Perkin-Elmer Model 9700 thermal cycler, with reactions being carried out with 30 cycles of a 25-μl reaction volume containing 13.25 μl sterile distilled H_2O, 2.5 μl 10× LA PCR buffer (Takara), 4.0 μl dNTP (4 mM), 2.5 μl each primer (5 μM), 0.25 μl of 2.5 U LA *Taq* (Takara), and 1 μl template. The thermal cycle profile was that of ''shuttle PCR'': denaturation at 98°C for 10 s, with annealing and extension combined at the same temperature (68°C) for 16–20 min. Long PCR products were electrophoresed on a 1.0% L 03 agarose gel and later stained with ethidium bromide for band characterization via ultraviolet transillumination. The long PCR products were diluted with sterile TE buffer (1:10–100) for subsequent use as PCR templates.

PCR and Sequencing

A total of 182 fish-versatile PCR primers were used in various combinations to amplify contiguous, overlapping segments of the entire mitochondrial genome for each of the 38 species (for primer locations and sequences of these primers, see Miya and Nishida 1999, 2000*b*; Inoue et al. 2000, 2001*a*, 2001*b*, 2001*c*, 2001*d*; Ishiguro, Miya, and Nishida 2001; Kawaguchi, Miya, and Nishida 2001). Species-specific primers were designed in cases in which no appropriate fish-versatile primers were available. A list of PCR primers used for a specific species is available from M.M. on request.

PCR was done in a Perkin-Elmer Model 9700 thermal cycler, and reactions were carried out with 30–32 cycles of a 15-μl reaction volume containing 8.3 μl sterile, distilled H_2O, 1.5 μl 10× PCR buffer (Takara), 1.2 μl dNTP (4 mM), 1.5 μl of each primer (5 μM), 0.07 μl *Taq* DNA polymerase (Z *Taq*, Takara), and 1 μl template (diluted long PCR products). All PCR products overlapped by approximately 50–300 bp.

The thermal cycle profile was as follows: denaturation at 98°C for 1 s; annealing at 50–53°C, depending on primer specificity, for 5 s; and extension at 72°C for 10–20 s, depending on the expected size of the PCR products. The PCR products were electrophoresed on a 1.0% L 03 agarose gel (Takara) and stained with ethidium bromide for band characterization via ultraviolet transillumination.

Double-stranded DNA products, purified using a Pre-Sequencing Kit (USB), were subsequently used for direct cycle sequencing with dye-labeled terminators (Applied Biosystems Inc.). Primers used were the same as those for PCR. All sequencing reactions were performed according to the manufacturer's instructions. Labeled fragments were analyzed on a Model 373S/377 DNA sequencer (Applied Biosystems Inc.). All DNA sequence electropherograms were carefully checked to see whether or not they included the mitochondrial pseudogenes in the nuclear genome (for checkpoints, see Mindell et al. 1999). As pointed out by Mindell et al. (1999), features that were consistent with mitochondrial origin were (1) the presence of a conserved reading frame in protein-coding genes among all taxa, with decreasing rates of variability at third, first, and second codon positions, respectively; (2) the absence of extra stop codons, frameshifts, or unusual amino acid substitutions; and (3) no sequence changes resulting in losses of known secondary structure in tRNA and rRNA genes.

Phylogenetic Analysis

The DNA sequences were edited with EditView, version 1.0.1; AutoAssembler, version 2.1 (Applied Biosystems); and DNASIS, version 3.2 (Hitachi Soft-

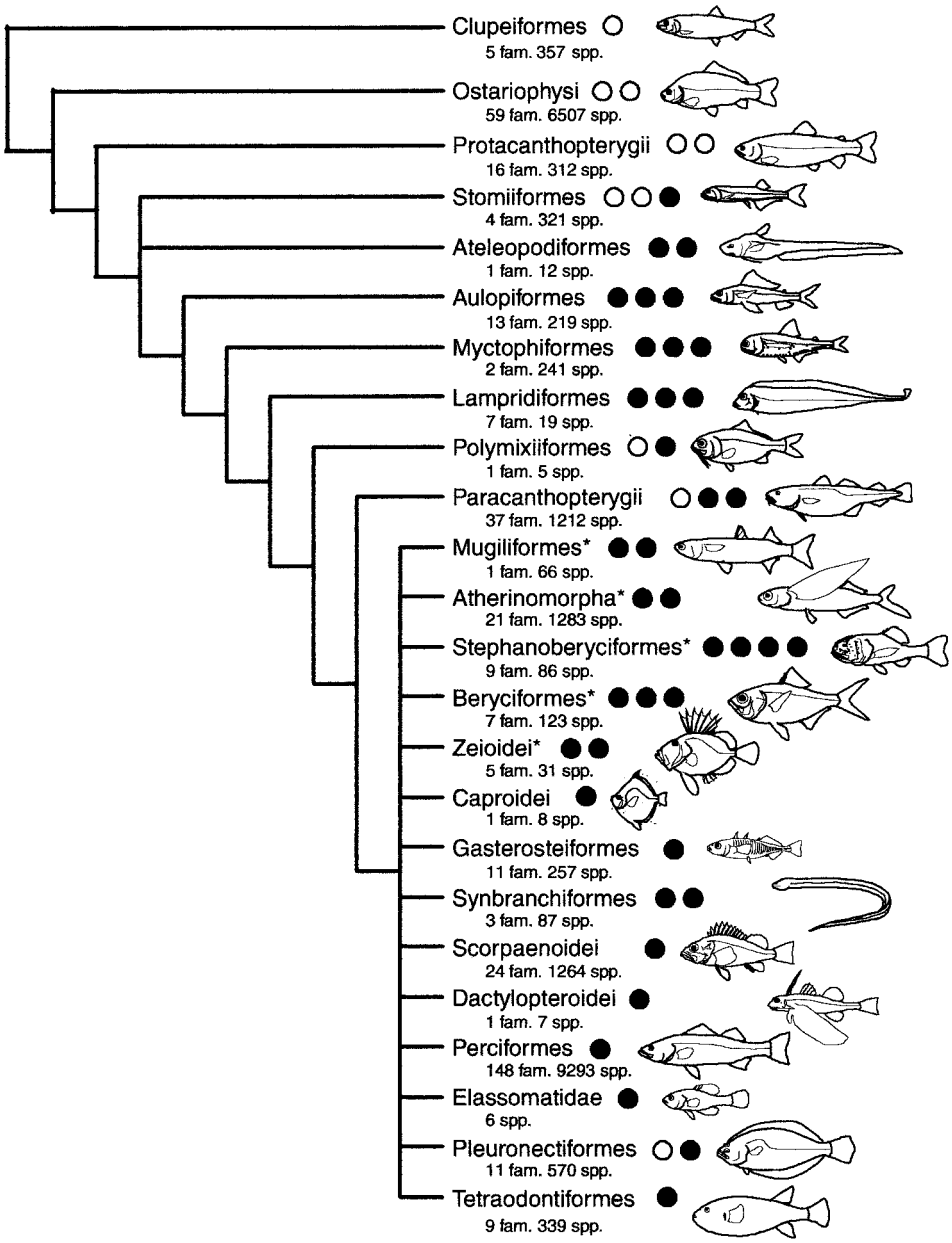

FIG. 2.—Schematic representation of the taxonomic sampling strategy plotted on the strict consensus tree (fig. 1*C*). Open and filled circles represent those species for which complete mtDNA sequences have been determined in previous studies and the present study, respectively. Asterisks denote the major lineages that occupied the three most basal positions in Acanthopterygii in either Johnson and Patterson's (1993) or J. S. Nelson's (1994) hypotheses (for detailed explanation, see text). Numbers of included taxa follow J. S. Nelson (1994).

ware Engineering). Individual gene sequence alignments for the 48 teleosts were initiated with Clustal X (Thompson et al. 1997) with default gap penalties and adjusted manually using DNASIS. Amino acids were used for alignments of the protein-coding genes and secondary-structure models (Kumazawa and Nishida 1993) for alignment of tRNA genes. Since unambiguous alignments of the two rRNA genes (12S and 16S) on the basis of secondary-structure models (e.g., Miya and Nishida 1998, 2000*a*; Yamaguchi et al. 2000) were not feasible, they were not used in the analyses. The ND6 gene was not used in the phylogenetic analyses because of its heterogeneous base composition and consistently poor phylogenetic performance (Zardoya and Meyer 1996; Miya and Nishida 2000*b*). Also, tRNA loops and other ambiguous alignment regions, such as the 5′ and 3′ ends of several protein-coding genes, were excluded from the analyses. In addition, third codon positions in the protein-coding genes that would positively mislead an analysis of higher-level relationships of teleosts (Miya and Nishida 2000*b*) were excluded from the analyses, leaving 7,002 and 908 available nucleotide positions from the 12 protein-coding and 22 tRNA genes, respectively. Amino acid sequences were not used in phylogenetic analysis, as the resulting trees were not well resolved, with many internal branches being sup-

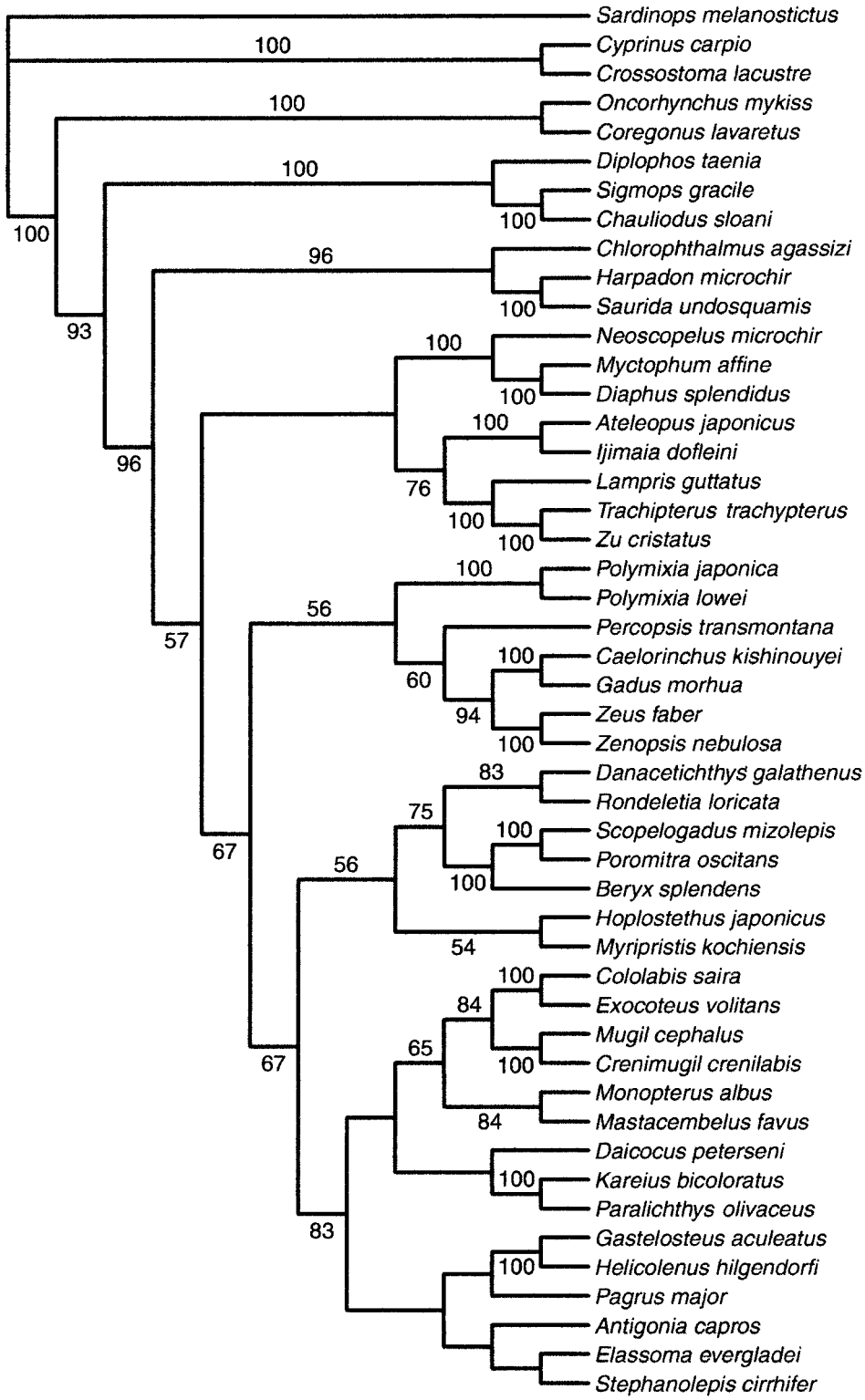

FIG. 3.—The single most-parsimonious (MP) tree derived from unweighted analysis of mitogenomic data comprising concatenated nucleotide sequences from 12 protein-coding (excluding the ND6 gene and third codon positions) and 22 transfer RNA (tRNA) genes (stem regions only) from all 48 species examined. Tree length = 22,932 steps; consistency index = 0.245; retention index = 0.345; rescaled consistency index = 0.087. Numbers next to internal branches indicate bootstrap values (only those >50%) obtained for 500 replicates using the heuristic search option in PAUP 4.0b4a (Swofford 1998) with 20 random-addition sequences being performed in each replication.

Table 1
List of Species Used in this Study, Along with DDBJ/EMBL/GenBank Accession Numbers and References

Species	Accession No.	Reference
Clupeiformes		
Sardinops melanostictus	AB032554	Inoue et al. (2000)
Ostariophysi		
Cyprinus carpio	X61010	Chang, Huang, and Lo (1994)
Crossostoma lacustre	M91245	Tzeng et al. (1992)
Protacanthopterygii		
Oncorhynchus mykiss	L29771	Zardoya, Garrido-Pertierra, and Bautista (1995)
Coregonus lavaretus	AB034824	Miya and Nishida (2000*b*)
Stomiiformes		
Diplophos taenia	AB034825	Miya and Nishida (2000*b*)
Sigmops gracile[a,b]	AB016274	Miya and Nishida (1999)
Chauliodus sloani[b]	AP002915	This study
Ateleopodiformes		
Ateleopus japonicus	AP002916	This study
Ijimaia dofleini	AP002917	This study
Aulopiformes		
Chlorophthalmus agassizi[b]	AP002918	This study
Harpadon microchir[b]	AP002919	This study
Saurida undosquamis[b,c]	AP002920	This study
Myctophiformes		
Neoscopelus microchir	AP002921	This study
Myctophum affine[b]	AP002922	This study
Diaphus splendidus[b]	AP002923	This study
Lampridiformes		
Lampris guttatus[b,d]	AP002924	This study
Trachipterus trachypterus[b]	AP002925	This study
Zu cristatus[b]	AP002926	This study
Polymixiiformes		
Polymixia japonica	AB034826	Miya and Nishida (2000*b*)
Polymixia lowei	AP002927	This study
Paracanthopterygii		
Percopsis transmontana	AP002928	This study
Caelorinchus kishinouyei	AP002929	This study
Gadus morhua	X99772	Johansen and Bakke (1996)
Atherinomorpha		
Mugil cephalus	AP002930	This study
Crenimugil crenilabis[b]	AP002931	This study
Cololabis saira	AP002932	This study
Exocoteus volitans	AP002933	This study
Stephanoberyciformes		
Scopelogadus mizolepis[b]	AP002934	This study
Poromitra oscitans[b]	AP002935	This study
Danacetichthys galathenus	AP002936	This study
Rondeletia loricata	AP002937	This study
Beryciformes		
Hoplostethus japonicus	AP002938	This study
Beryx splendens	AP002939	This study
Myripristis kochiensis	AP002940	This study
Zeioidei		
Zeus faber	AP002941	This study
Zenopsis nebulosus	AP002942	This study
Caproidei		
Antigonia capros	AP002943	This study
Gasterosteiformes		
Gasterosteus aculeatus[b]	AP002944	This study
Syngnathiformes		
Monopterus albus	AP002945	This study
Mastacembelus favus	AP002946	This study

Table 1
Continued

Species	Accession No.	Reference
Scorpaenoidei		
Helicolenus hilgendorfi	AP002947	This study
Dactylopteroidei		
Daicocus peterseni	AP002948	This study
Perciformes		
Pagrus major	AP002949	This study
Elassomatidae		
Elassoma evergladei[b]	AP002950	This study
Pleuronectiformes		
Kareius bicoloratus[b]	AP002951	This study
Paralichthys olivaceus	AB028664	Saitoh et al. (2000)
Tetraodontiformes		
Stephanolepis cirrhifer[b]	AP002952	This study

[a] *Sigmops gracile* = *Gonostoma gracile* (see Miya and Nishida 2000*a*).
[b] Complete mtDNA sequences except for a portion of the control region.
[c] *Saurida undosquamis* = *Saurida* sp. 2 in Nakabo (2000).
[d] $tRNA^{Thr}$ and $tRNA^{Pro}$ gene sequences could not be determined owing to technical difficulties.

ported by relatively low bootstrap values compared with those obtained by nucleotide sequences. Also, there was no noticeable improvement in tree statistics or bootstrap values when greater weight was given to amino acid replacements, which required more nucleotide changes, by using the PROTPARS weight matrix for amino acids provided in MacClade (Maddison and Maddison 1992). Similarly, although Naylor and Brown (1997, 1998) suggested that isoleucine (I), leucine (L), and valine (V) were the amino acids responsible for phylogenetic inconsistency, exclusion of these three amino acids through conversion of L and V into I from the data matrix (see Cao et al. 1998; Mindell et al. 1999) did not improve the tree statistics or the bootstrap values.

MacClade, version 3.08 (Maddison and Maddison 1992) was used in various phases of the phylogenetic analyses, such as preparing data matrices in NEXUS format, exporting tree files, and exploring alternative tree topologies. Aligned sequence data in NEXUS format are available from M.M. on request.

All phylogenetic analyses were performed using PAUP 4.0b4a (Swofford 1998). Heuristic maximum-parsimony (MP) analyses were conducted with tree bisection-reconnection branch swapping and 100 random-addition sequences. Support for internal branches was assessed using 500 bootstrap replications, with 20 random-addition sequences performed in each replication. All phylogenetically uninformative sites were ignored. Gaps were considered as missing data rather than as fifth characters, to prevent those longer than one or two bases from being taken as representing multiple events (Swofford 1993).

Heuristic maximum-likelihood (ML) analyses were conducted to determine the statistically most likely phylogeny with the following parameters: substitution model set at transition/transversion ratio = 2; the HKY85 (Hasegawa, Kishino, and Yano 1985) two-parameter model variant for unequal base frequencies; empirical base frequencies; starting branch lengths obtained using the Rogers-Swofford approximation method; and molecular clock not enforced. Nucleotide positions, including gaps, were all excluded.

Tests of alternative phylogenetic hypotheses were accomplished using the constraint tree option in PAUP 4.0b4a (Swofford 1998). Differences in tree topologies were compared between the unconstrained and the constrained MP trees, with tree length differences being statistically evaluated using the Templeton (1983) test implemented in PAUP 4.0b4a (Swofford 1998).

Results

Genome Organization

The complete L-strand nucleotide sequences from the mitogenomes of the 38 species (except for a portion of the putative control region and a few tRNA genes for some species; see table 1) have been registered in DDBJ/EMBL/GenBank under accession numbers AP02915–AP02952. The genome content of the 38 species included 2 rRNA, 22 tRNA, and 13 protein-coding genes, plus the putative control region, as found in other vertebrates (*Lampris guttatus* $tRNA^{Thr}$ and $tRNA^{Pro}$ genes located downstream of the cyt *b* gene could not be sequenced owing to technical difficulties). As in other vertebrates, most genes were encoded on the H-strand, except for the ND6 and eight tRNA genes, which were encoded on the L-strand.

Although the gene arrangements of most species were identical to those in typical vertebrates, those of five species (*Sigmops gracile, Chauliodus sloani, Myctophum affine, Diaphus splendidus,* and *Caelorinchus kishinouyei*) were unique among vertebrate mitogenomes. To date, only two patterns of gene rearrangements have been found in teleosts (*S. gracile* [=*Gonostoma gracile*] [Miya and Nishida 1999]; *Conger myriaster* and their allies [Inoue et al. 2001*c*]). Notwithstanding,

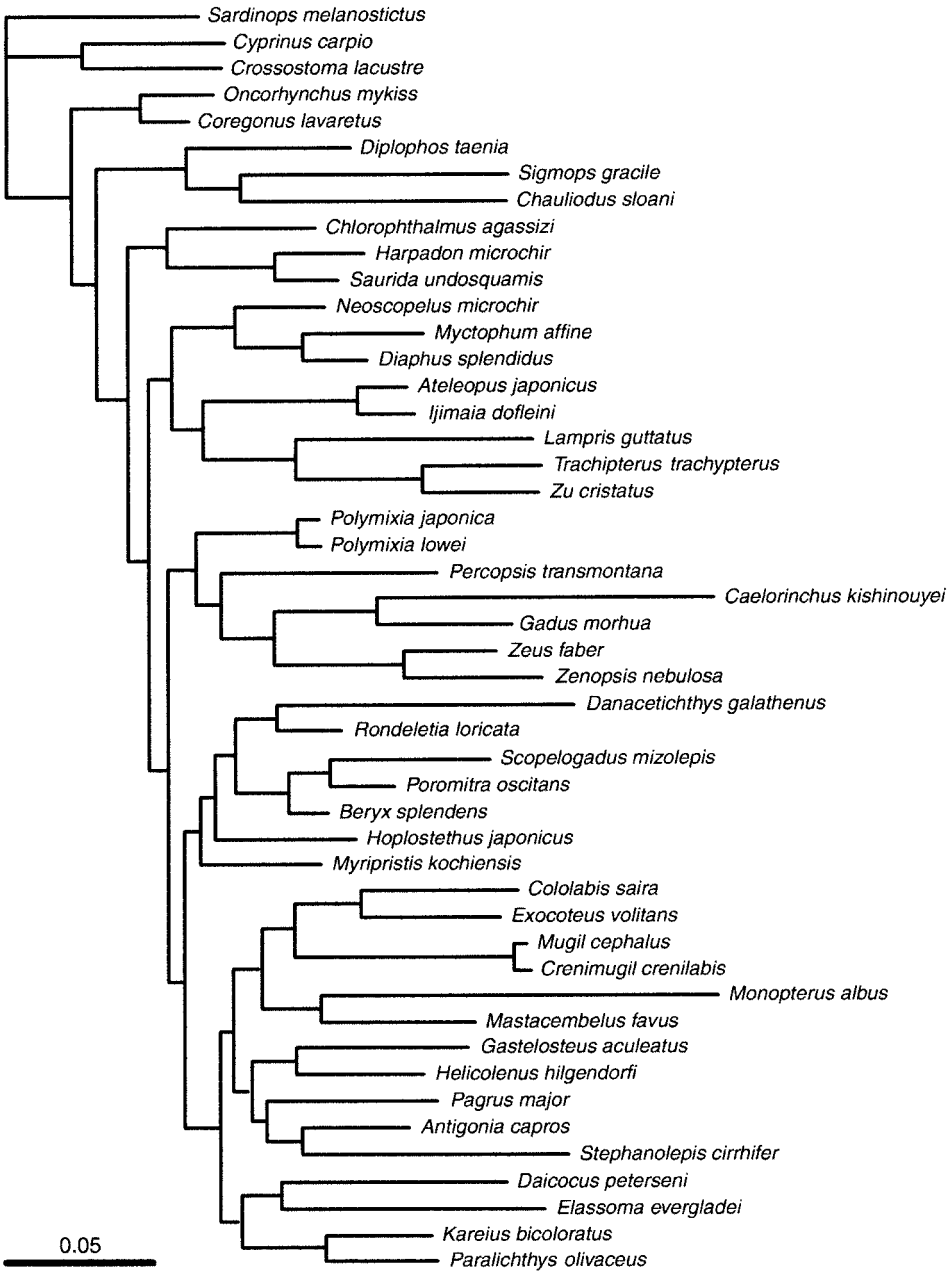

FIG. 4.—The maximum-likelihood (ML) tree derived from mitogenomic data comprising concatenated nucleotide sequences from 12 protein-coding (excluding the ND6 gene and third codon positions) and 22 transfer RNA (tRNA) genes (stem regions only) from all 48 species examined. The HKY85 model of sequence evolution (Hasegawa, Kishino, and Yano 1985) was used. The scale indicates expected nucleotide substitutions per site.

the five species listed above exhibited gene arrangements that differed completely from the two examples. These unique mitogenomes will be described elsewhere, with discussions of their phylogenetic utility and the possible mechanisms generating such gene arrangements.

Phylogenetic Analysis

Heuristic MP analysis of the nucleotide sequences from the concatenated 12 protein-coding (no third codon positions) and 22 tRNA (stem regions only) genes yielded a single most-parsimonious tree (fig. 3) with a length of 22,932 steps (consistency index = 0.245; retention index = 0.345; rescaled consistency index = 0.087). Many internal branches were supported by moderate (60%–79%) to high (80%–100%) bootstrap values, except for a within-monophyletic group that was taken to represent upper bushes (fig. 3), with the observation of many unbisected long branches (fig. 4) probably being due to the lower-density taxonomic sampling compared with their taxonomic diversity (fig. 2).

ML analysis of the same data set using the HKY85 model of sequence evolution produced a tree similar to that found in the MP analysis (fig. 4), with $-\ln L = 126{,}245.01$. Topological differences were minor, being

observed where bootstrap values in the MP tree were around or lower than 50% (fig. 3). No statistically significant differences were found between the two topologies ($z = -0.756$, $P = 0.449$).

Discussion

Molecular studies have been expected to prove decisive in resolving persistent controversies over higher-level relationships of teleosts (J. S. Nelson 1984; G. Nelson 1989), although they have not yet fulfilled their promise (Stepien and Kocher 1997; Miya and Nishida 2000*b*). Indeed, an earlier study using partial amino acid sequences from the three mitochondrial protein-coding genes (Normark, McCune, and Harrison 1991) suggested an unorthodox tree (e.g., nonmonophyletic teleosts) that Patterson, Williams, and Humphries (1993) criticized as ''goofy'' from the morphologist's point of view. Bernardi et al. (1993) and Rubin and Dores (1995) analyzed amino acid sequences of growth hormones on the basis of different data sets, including 25 and 24 teleosts, respectively, comparing them with those from two outgroups, and reported that the resulting MP trees agreed well with the morphology-based trees. Lê, Lecointre, and Perasso (1993) analyzed nuclear 28S rRNA sequences from 31 gnathostomes (including 18 teleosts) and found a highly supported node within the teleosts (Clupeomorpha + Ostariophysi) that was incongruent with the morphology-based tree. Using entire mitochondrial cyt *b* gene sequences from 31 fishes (including 30 teleosts), Lydeard and Roe (1997) reported that the resulting MP trees were largely congruent with the morphology-based tree, although some incongruities (e.g., paraphyletic Neoteleostei) were observed. With the exception of the sister relationship of Clupeomorpha + Ostariophysi (Lê, Lecointre, and Perasso 1993), however, no novel molecular phylogenetic hypotheses have been considered significant in recent studies dealing with higher-level relationships among major teleostean lineages (Lecointre and Nelson 1996; Johnson and Patterson 1996). Also, it should be noted that Meyer (1994) and Ortí and Meyer (1997) explicitly demonstrated limits of partial mitochondrial gene sequences for resolving higher-level relationships of teleosts. Recently, Wiley, Johnson, and Dimmick (2000) employed a total-evidence approach in analyzing acanthomorph relationships, combining partial nucleotide sequences of the mitochondrial 12S rRNA (572 bp) and nuclear 28S rRNA (1,112 bp) genes and morphological data (38 characters) from 27 species, thereafter subjecting the combined data to MP analyses. Although they had expected that a combination of mitochondrial and nuclear ribosomal genes would provide a strong database from which they could evaluate acanthomorph relationships, their expectation was only partly met. Their molecular data seemed to be unsuitable for such an analysis of higher-level relationships owing to the high level of saturation observed in both the highly variable mitochondrial 12S rRNA and the extremely conservative nuclear 28S rRNA genes (Wiley, Johnson, and Dimmick 2000). Consequently, their molecular data were of little help in evaluating alternative hypotheses unless used in a total-evidence context, as the authors frankly acknowledged.

It appears that adequate resolution of higher-level relationships of any organism will require longer DNA sequences (Miya and Nishida 2000*b*). If so, the question remains as to which types of genes or genomes are useful in reconstructing the higher-level relationships of teleosts. It remains unclear whether or not mitochondrial or nuclear genes are generally more efficacious for such a purpose, although the transition from an apparently unsolvable problem to a solvable problem has come about mainly from the availability of complete mtDNA sequences in mammals (Penny et al. 1999; Springer et al. 1999). Additional complete mtDNA sequences have also been expected to be useful in answering some questions about fish relationships (Stepien and Kocher 1997). Inoue et al. (2001*d*), in fact, resolved the interrelationships of five major lineages of basal teleosts, for which five alternative phylogenetic hypotheses had been proposed on the basis of both morphological and molecular data, using mitogenomic data. Accordingly, mitogenomic data can be expected to resolve the persistent controversies over higher teleostean relationships, which are apparently more complex and difficult than those for basal teleosts (G. Nelson 1989). Below is a brief discussion of phylogenetic issues for the higher teleosts from the standpoint of both global and local taxonomic congruence between the MP tree obtained in this study (fig. 3; statistically indistinguishable from the ML tree) and previously proposed hypotheses, most of them based on morphological analyses. Our discussions of phylogenetic issues are restricted to recent major contributions and are not intended to be exhaustive.

Global Congruence

Recently, two major hypotheses on higher teleostean relationships have been proposed (Johnson and Patterson 1993 [fig. 1*A*]; J. S. Nelson 1994 [fig. 1*B*]). Johnson and Patterson (1993) presented a cladogram (their fig. 24) summarizing their ''views'' on acanthomorph interrelationships on the basis of their own extensive, comparative anatomical survey. Although they provided many putative synapomorphies (a total of 34 characters) substantiating their hypothesis, along with detailed discussions on their validity, they did not construct a character matrix including all of the species that they examined. Alternatively, their ''views'' were corroborated by MP analysis of an abbreviated character matrix comprising 39 anatomical features taken from 16 acanthomorph species (their fig. 25). Johnson and Patterson (1993, p. 621) stated that the number of taxa examined by them and ideally included in their sample far exceeded the limits of available parsimony programs at that time. Major topological differences between the present MP tree (fig. 3) and that of Johnson and Patterson (1993) were in the placements of zeiforms, stephanoberyciforms, and beryciforms (for details, see below). When topological constraints of Johnson and Patterson's (1993) tree (those of Johnson [1992] and Olney, Johnson, and Baldwin [1993] being followed below Lam-

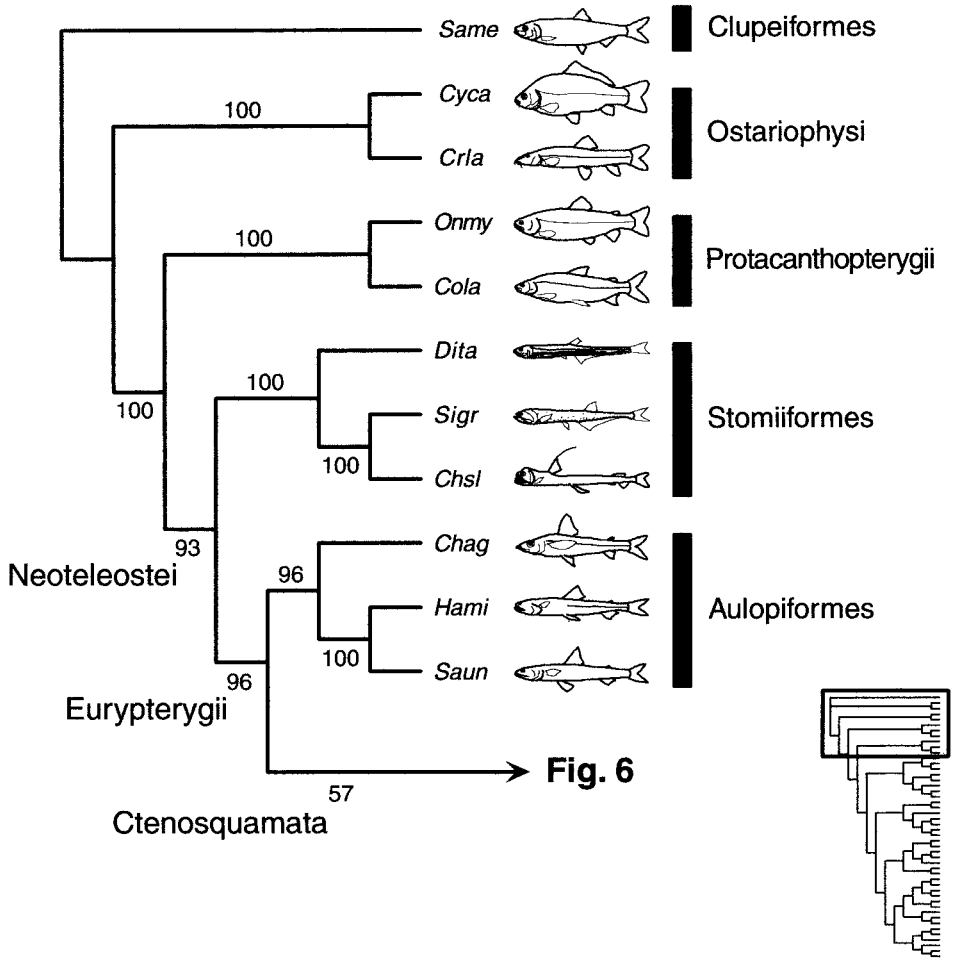

FIG. 5.—Basal relationships of the higher teleosts. For details, see text and figure 3 legend. Abbreviations: Same, *Sardinops melanostictus*; Cyca, *Cyprinus carpio*; Crla, *Crossostoma lacustre*; Onmy, *Oncorhynchus mykiss*; Cola, *Coregonus lavaretus*; Dita, *Diplophos taenia*; Sigr, *Sigmops gracile* (=*Gonostoma gracile*; see Miya and Nishida 2000*a*); Chsl, *Chauliodus sloani*; Chag, *Chlorophthalmus agassizi*; Hami, *Harpadon microchir*; Saun, *Saurida undosquamis.*

pridiformes) were enforced, two minimum-length trees that required an additional 413 steps were found, with the differences being highly significant ($z = -11.847$, $P < 0.0001$; $z = -10.428$, $P < 0.0001$).

J. S. Nelson (1994) subsequently presented different views on higher teleostean relationships in his comprehensive guide to fish classification (now in its third edition), which has been used as a standard reference and has been highly influential in systematic ichthyology. Although his hypothesis was presented in the form of two separate, sequential cladograms (J. S. Nelson 1994, pp. 194, 215), he failed to provide any relevant evidence or corroborative statements for such relationships. His hypothesis seemed to have been based on the conventionally accepted views on acanthomorph relationships advocated by Rosen (1973) and subsequently reviewed by Lauder and Liem (1983), with partial modifications being made where he agreed with Johnson and Patterson's (1993) radically different views. When topological constraints of J. S. Nelson's (1994) tree were enforced, one minimum-length tree that required an additional 588 steps was found, with the difference being highly significant ($z = -13.977$, $P < 0.0001$).

Basal Relationships

Basal interrelationships of the four major lineages (fig. 5) generally followed previously proposed hypotheses (Rosen 1973; Lauder and Liem 1983; Fink 1984; Johnson 1992), with Ostariophysi, Protacanthopterygii, Stomiiformes, and Aulopiformes sequentially diverging in this sequence. Notwithstanding, the monophyly of each taxon, particularly Ostariophysi and Protacanthopterygii, should be substantiated on the basis of more extensive taxonomic sampling, considering their heterogeneous compositions. It should be noted that two comprehensive major clades within the teleosts, Neoteleostei and Eurypterygii, were strongly supported, with bootstrap values of 93% and 96%, respectively.

Although Olney, Johnson, and Baldwin (1993) thought that Ateleopodiformes formed an unresolved trichotomy with Stomiiformes and more advanced neoteleosts (fig. 1), the former did not occupy a position close to the stomiiforms in the present tree (fig. 5), instead forming a sister group relationship with Lampridiformes (fig. 6), with a moderate bootstrap value (76%). This sister group relationship should not be entirely unex-

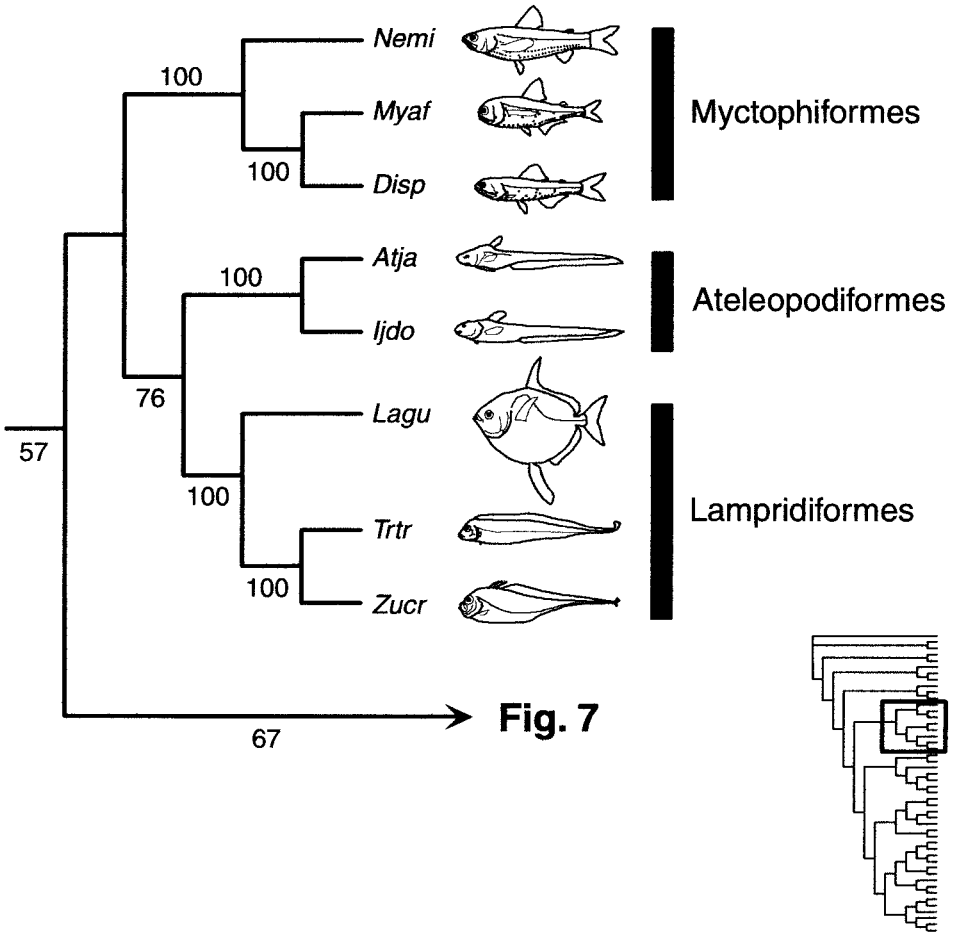

FIG. 6.—Relationships of the Myctophiformes, Ateleopodiformes, and Lampridiformes. For details, see text and figure 3 legend. Abbreviations: Nemi, *Neoscopelus microchir*; Myaf, *Myctophum affine*; Disp, *Diaphus splendidus*; Atja, *Ateleopus japonicus*; Ijdo, *Ijimaia dofleini*; Lagu, *Lampris guttatus*; Trtr, *Trachipterus trachypterus*; Zucr, *Zu cristatus.*

pected by systematic ichthyologists, because present ateleopodiforms (=ateleopodids) had previously been included in lampridiforms (J. S. Nelson 1976, 1984). Although Olney, Johnson, and Baldwin's (1993) hypothesis could not be rejected statistically ($z = -1.683$, $P = 0.092$), it appears that Ateleopodiformes had diverged after Stomiiformes, because the bootstrap value for Aulopiformes and above (=Eurypterygii) was as high as 96% (fig. 5).

Rosen (1973) considered Myctophiformes a sister group of the Acanthomorpha (Lampridiformes and above; fig. 1), although he subsequently rejected that suggestion (Rosen 1985). Later, however, Johnson (1992) confirmed the initial hypothesis (Rosen 1973) on the basis of an extensive comparative anatomical survey. In the present tree, myctophiforms formed a sister group relationship with ateleopodiforms + lampridiforms, although such a relationship was supported only weakly (bootstrap value < 50%; fig. 6). Furthermore, Rosen's (1973) hypothesis could not be rejected statistically ($z = -1.342$, $P = 0.179$). More extensive taxonomic sampling from these groups should indicate clearly which relationship patterns are the most likely. It should also be noted that the three lampridiforms formed a strongly supported monophyletic group, with a bootstrap value of 100% (fig. 6), despite their disparate external morphologies (see also Olney, Johnson, and Baldwin 1993; Wiley, Johnson, and Dimmick 1998).

Monophyly and Interrelationships of Zeiformes and Paracanthopterygii

The monophyly and interrelationships of Zeiformes have long been one of the most problematic issues in systematic ichthyology (for details, see Johnson and Patterson 1993, p. 592–599). Zeiformes currently comprise two suborders, Zeioidei and Caproidei (J. S. Nelson 1994), although recent authors have questioned the reality of a relationship between those two groups (e.g., Johnson and Patterson 1993). In the present tree, monophyly of the two zeioid species was strongly supported by a bootstrap value of 100%. However, a caproid, *Antigonia capros*, was convincingly placed within a well-supported monophyletic group (bootstrap value = 83%) at the top of the tree (fig. 3). When topological constraints were enforced on the monophyly of zeiforms (including caproids), one minimum-length tree that required an additional 111 steps was found, with the difference being highly significant ($z = -4.267$, $P = 0.0008$). The superficial similarity between the two

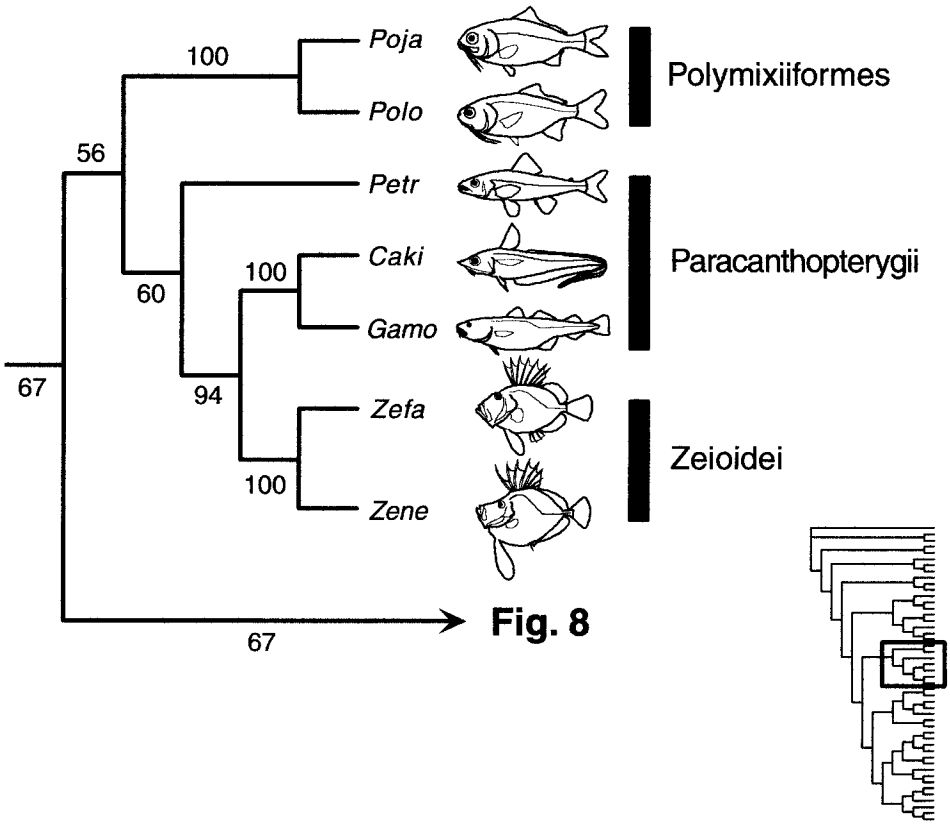

FIG. 7.—Relationships of the Polymixiiformes, Paracanthopterygii, and Zeioidei. Note that Paracanthopterygii are paraphyletic. For details, see text and figure 3 legend. Abbreviations: Poja, *Polymixia japonica*; Polo, *Polymixia lowei*; Petr, *Percopsis transmontana*; Caki, *Caelorinchus kishinouyei*; Gamo, *Gadus morhua*; Zefa, *Zeus faber*; Zene, *Zenopsis nebulosus.*

groups, such as the laterally flattened and extremely deep body (see fig. 2), would surely have misled phylogenetic inferences based on morphology.

At no time has convincing evidence been demonstrated regarding zeiform affinities with other higher teleosts. Rosen (1984) suggested a close affinity to Tetraodontiformes, although this idea has not been generally accepted (Johnson and Patterson 1993). In fact, when topological constraints on the monophyly of tetraodontiforms + zeiforms (including caproids) were enforced, one minimum-length tree that required an additional 84 steps was found, with the difference being highly significant ($z = -2.793$, $P = 0.0052$). In a total-evidence analysis of the combined molecular and morphological data, Wiley, Johnson, and Dimmick (2000) found that two zeids and two gadiforms formed a well-supported monophyletic group (bootstrap value = 94%), which has never before been proposed. However, this sister group relationship has been reproduced in the present analysis with a high bootstrap value of 94%. Evidently, the extremely disparate morphologies of the two groups (see fig. 7) has deterred most systematic ichthyologists from exploring such a possibility.

Paracanthopterygii has also been a controversial group since Greenwood et al. (1966) established this comprehensive higher taxon on the basis of their own extensive comparative anatomical observations. Since the original proposal (Greenwood et al. 1966) and a subsequent review (Rosen and Patterson 1969) of this group, taxonomic modifications (addition or deletion of various higher teleosts) have been so numerous that Patterson and Rosen (1989) described the situation as ''disorder'' in their second review of Paracanthopterygii. Although paracanthopterygians were represented by only two gadiforms (*Gadus morhua* and *Caelorinchus kishinouyei*) and a percopsiform (*Percopsis transmontana*) in the present analysis, they were reciprocally paraphyletic, because the two zeioids formed a sister group relationship with the two gadiforms (fig. 7). When topological constraints were enforced on the monophyly of the Paracanthopterygii, one minimum-length tree that required an additional 39 steps was found, with the difference being highly significant ($z = -3.433$, $P = 0.0006$). Further taxonomic sampling from other paracanthopterygians, such as species representing the Batrachoidiformes, the Lophiiformes, and the Ophidiiformes, would be necessary to clarify the limits of this problematic group.

Monophyly of Beryciformes and Stephanoberyciformes

The limits of Beryciformes have undergone considerable reduction, with the currently recognized Polymixiiformes, Stephanoberyciformes, and Trachichthyiformes (*sensu* Moore 1993) having been removed from

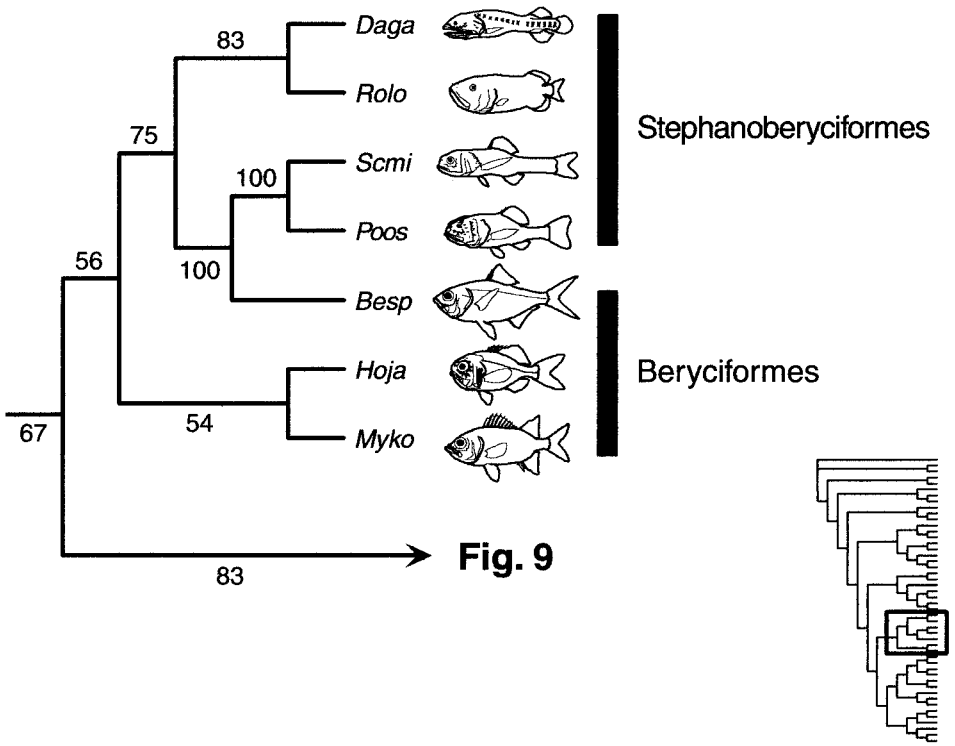

FIG. 8.—Relationships of the Stephanoberyciformes and the Beryciformes. Note that both groups are paraphyletic. For details, see text and figure 3 legend. Abbreviations: Daga, *Danacetichthys galathenus*; Rolo, *Rondeletia loricata*; Scmi, *Scopelogadus mizolepis*; Poos, *Poromitra oscitans*; Besp, *Beryx splendens*; Hoja, *Hoplostethus japonicus*; Myko, *Myripristis kochiensis.*

the group during the past 30 years (for details, see Moore 1993; Johnson and Patterson 1993). All of these groups, however, with the exception of polymixiiforms (fig. 7), formed a weakly supported monophyletic group (fig. 8), although the two major components (Stephanoberyciformes and Beryciformes; *sensu* J. S. Nelson 1994; Johnson and Patterson 1993) within the lineage were not monophyletic because a berycid (*Beryx splendens*) was confidently placed as a sister species of the two melamphaids represented (*Scopelogadus mizolepis* + *Poromitra oscitans*) with a bootstrap value of 100%. Such a close affinity has not previously been asserted with confidence (but see Colgan, Zhang, and Paxton 2000), apparently owing to the disparate morphologies and ecology of the component taxa. When topological constraints on the monophyly of stephanoberyciforms and beryciforms were enforced, one minimum-length tree, requiring an additional 67 and 86 steps, respectively, was found for each, with the differences being highly significant ($z = -7.183$, $P < 0.0001$; $z = -7.786$, $P < 0.0001$, respectively).

''Upper Bushes'' (=Percomorpha)

Many authors have investigated limits, interrelationships, and intrarelationships of the crown group of teleosts, Percomorpha (for details, see Johnson 1993; Johnson and Patterson 1993), although no consensus has been reached regarding these. One of the purposes of the present study was to circumscribe a well-supported, comprehensive monophyletic group encompassing such ''bushes'' that presumably correspond to the Percomorpha. By doing so, we had hoped to clarify wherein phylogenetic problems lay, enabling the formation of a basis in addition to providing guidelines for the next step in resolving the ''bushy top.'' In this investigation, a comprehensive monophyletic group was found at the top of the tree, supported by a high bootstrap value of 83% (fig. 9). The composition of this monophyletic group exhibited complete congruence to the Percomorpha that has been circumscribed by Johnson and Patterson (1993).

We did not expect to resolve intrarelationships of this group because of the sparse taxonomic sampling compared with the tremendous taxonomic diversity of the group (>13,000 species placed in 230 families; calculated from J. S. Nelson 1994). In fact, while many internal branches were only weakly supported, with bootstrap values of <50% (mostly <20%; fig. 8), an unexpected relationship was found between *Gasterosteus aculeatus* (Gasterosteiformes) and *Helicolenus hilgendorfi* (Scorpaeniformes), which formed a strongly supported monophyletic group with a bootstrap value of 100%. Such a close affinity between the two groups has not previously been suggested, and recent preliminary phylogenetic analyses using mitogenomic data from an additional gasterosteiform have confirmed such a relationship (unpublished data). Again, further taxonomic sampling and subsequent sequencing efforts should clarify these previously unexplored relationships.

Conclusions

Following the publication of the seminal work by Greenwood et al. (1966) and the advent of cladistic the-

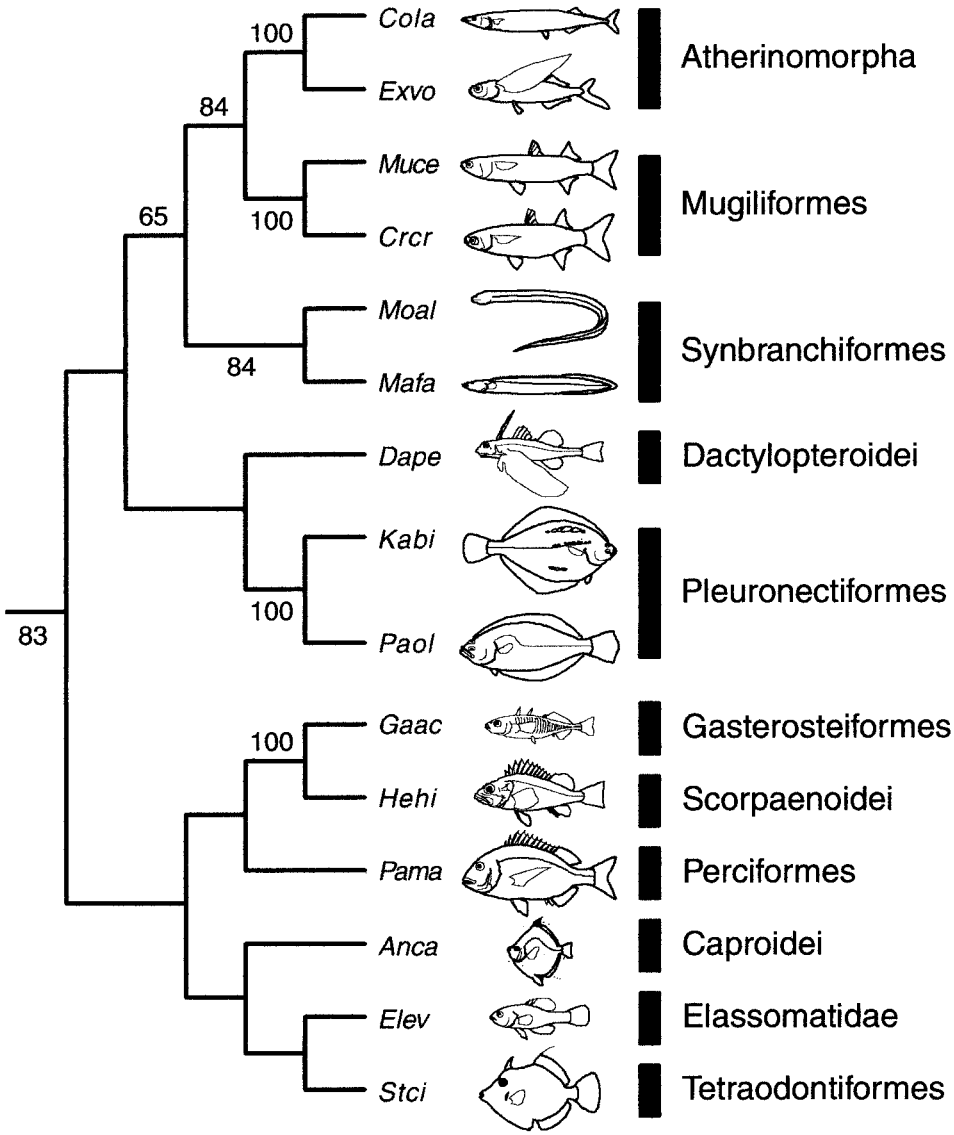

FIG. 9.—A comprehensive monophyletic group encompassing ''upper bushes'' (=Percomorpha *sensu* Johnson and Patterson [1993], with the exception of zeioids; for details, see text). Abbreviations: Cosa, *Cololabis saira*; Exvo, *Exocoteus volitans*; Muce, *Mugil cephalus*; Crcr, *Crenimugil crenilabis*; Moal, *Monopterus albus*; Mafa, *Mastacembelus favus*; Dape, *Daicocus peterseni*; Kabi, *Kareius bicoloratus*; Paol, *Paralicthys olivaceus*; Gaac, *Gasterosteus aculeatus*; Hehi, *Helicolenus hilgendorfi*; Pama, *Pagrus major*; Anca, *Antigonia capros*; Elev, *Elassoma evergladei*; Stci, *Stephanolepis cirrhifer.*

ory (Hennig 1966; Wiley 1981), numerous comparative anatomical studies have been conducted in attempts to resolve the interrelationships of teleosts at various taxonomic levels (for recent reviews, see G. Nelson 1989; Johnson and Patterson 1993; J. S. Nelson 1994; Helfman, Collette, and Facey 1997). Despite these efforts, there remains much controversy over higher-level relationships among the major teleostean lineages, especially evident in the transitions of the classification systems adopted in the first to third editions of J. S. Nelson's *Fishes of the World* (J. S. Nelson 1976, 1984, 1994). Considering the enormous species diversity involved (>23,500 species) and the wide-ranging variations not only in morphology (see figs. 2 and 5–9) but also in behavior and ecology (see Helfman, Collette, and Facey 1997), it is no wonder that comparative anatomical approaches have faced a number of difficulties (e.g., homology assessment among characters) in unraveling the higher-level relationships of teleosts. The same is true of molecular phylogenetic studies which have employed shorter sequences (mostly <1,000 positions) based on limited taxonomic representation (Stepien and Kocher 1997; Miya and Nishida 2000*b*).

The present study was based on unprecedentedly long DNA sequences (>15 kb) from many purposefully chosen species that represented the overall diversity of the higher teleosts. The resultant trees were well resolved and provided a number of new insights into higher teleostean phylogeny, many of them quite unexpected from previous analyses, including both morphology-based cladistic analyses and molecular phylogenetic studies. Also, the present study clearly identified major

phylogenetic problems. In particular, the limits and interrelationships of the Paracanthopterygii, the Zeiformes, the Beryciformes, and the Stephanoberyciformes should be reconsidered, this being the most obvious ''next investigative step.'' By doing so, the Percomorpha could be circumscribed more accurately, leading to the resolution of that group.

It should also be noted that this study not only embodies the recent expectation of Pollock et al. (2000) that increasing the mitogenomic data set would be likely to have a large impact on confidence in the resolution of tree structure, but also indicates the practicality of our PCR-based approach for sequencing fish mitogenomes during moderate-scale evolutionary genomic research under limited resources. Although our long PCR primers were originally designed for fish mitogenomes, they should be effective for other vertebrates, including humans, with little or no modification (Miya and Nishida 2000*b*). By using long PCR products (9–17 kb) as purified templates of mtDNA for subsequent full-nested PCR (500–1,500 bp), a number of published primers (e.g., Kocher et al. 1989; Palumbi 1996; Miya and Nishida 1999, 2000*b*; Sorenson et al. 1999) may work well for various vertebrate mitogenomes. Direct sequencings of these contiguous, overlapping PCR products that cover the entire mitogenomes have proven to be successful, providing consistent results (Miya and Nishida 1999, 2000*b*; Sorenson et al. 1999; Mindell et al. 1999). Also, with the aid of commercially available sequence editors, the complete mtDNA sequence and associated information (e.g., location of features) from a single species are obtainable within a few weeks or so from DNA extraction to submission of the data to DNA databases, such as DDBJ/EMBL/GenBank. Thus, our PCR-based approach is feasible in a single laboratory equipped with standard experimental facilities for molecular biology with a moderate level of manpower and funds. We believe that similar kinds of moderate-scale evolutionary genomics should be conducted in parallel with larger-scale evolutionary genomics employing genome technologies capable of producing sequence data in large quantities (Pollock et al. 2000), which would consequently cover the entire vertebrate biodiversity in the near future.

Acknowledgments

This study would not have been possible without the donation of the study materials, for which we sincerely thank H. Arai, H. Endo, T. Komai, S. Matsumoto, S. Mori, M. Motegi, T. Mukai, S. Okada, S. Ono, H. Shikatani, S. M. Shirai, T. T. Sutton, M. J. Yamaguchi, and particularly E. O. Wiley. We also thank J. G. Inoue, R. J. Machida, K. Nanba, and N. B. Ishiguro, and M. Iwashita for their assistance in the sequencing experiments, and M. J. Yamaguchi and J. R. Paxton for some species identifications. Thanks are also due to Y. Fukuyo, K. Furuya, and K. Nanba and graduate students at Fisheries Oceanography Laboratory, University of Tokyo, for generously allowing us to use their experimental facilities. A portion of this study was supported by grants-in-aid from the Showa-Seitoku Foundation and the Ministry of Education, Science, Sports and Culture, Japan (numbers 09740644, 11640705, 10660189, and 12NP0201).

LITERATURE CITED

BERNARDI, G., G. D'ONOFRIO, S. CACCIÒ, and G. BERNARDI. 1993. Molecular phylogeny of bony fishes, based on the amino acid sequence of the growth hormone. J. Mol. Evol. **37**:644–649.

CAO, Y., P. J. WADDELL, N. OKADA, and M. HASEGAWA. 1998. The complete mitochondrial DNA sequence of the shark *Mustelus manazo*: evaluating rooting contradiction to living bony vertebrates. Mol. Biol. Evol. **15**:1637–1646.

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

CHENG, S., S.-Y. CHANG, P. GRAVITT, and R. RESPESS. 1994. Long PCR. Nature **369**:684–685.

COLGAN, D. J., C.-G. ZHANG, and J. R. PAXTON. 2000. Phylogenetic investigations of the Stephanoberyciformes and Beryciformes, particularly whalefishes (Euteleostei: Cetomimidae), based on partial 12S rDNA and 16S rDNA sequences. Mol. Phylogenet. Evol. **17**:15–25.

EASTEAL, S. 2000. Evolutionary annotation of the genome. Mol. Biol. Evol. **17**:1775.

FINK, W. 1984. Basal euteleosts: relationships. Pp. 202–206 *in* H. G. MOSER, W. J. RICHARDS, D. M. COHEN, M. P. FAHAY, A. W. KENDALL JR., and S. L. RICHARDSON, eds. Ontogeny and systematics of fishes. Special Publication No. 1. American Society of Ichthyologists and Herpetologists, Lawrence, Kans.

GREENWOOD, P. H., D. E. ROSEN, S. H. WEITZMAN, and G. S. MYERS. 1966. Phyletic studies of teleostean fishes, with a provisional classification of living forms. Bull. Am. Mus. Nat. Hist. **131**:339–456.

HASEGAWA, M., H. KISHINO, and T. YANO. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. **22**:160–174.

HELFMAN, G. S., B. B. COLLETTE, and D. E. FACEY. 1997. The diversity of fishes. Blackwell Science, Boston, Mass.

HENNIG, W. 1966. Phylogenetic systematics. University of Illinois Press, Urbana, Ill.

HILLIS, D. M. 1998. Taxonomic sampling, phylogenetic accuracy, and investigator bias. Syst. Biol. **47**:3–8.

INOUE, J. G., M. MIYA, J. AOYAMA, S. ISHIKAWA, K. TSUKAMOTO, and M. NISHIDA. 2001*a*. Complete mitochondrial DNA sequence of the Japanese eel, *Anguilla japonica*. Fish. Sci. **67**:118–125.

INOUE, J. G., M. MIYA, K. TSUKAMOTO, and M. NISHIDA. 2000. Complete mitochondrial DNA sequence of the Japanese sardine, *Sardinops melanostictus.* Fish. Sci. **66**:924–932.

———. 2001*b*. Complete mitochondrial DNA sequence of the Japanese anchovy, *Engraulis japonicus.* Fish. Sci. (in press).

———. 2001*c*. Complete mitochondrial DNA sequence of *Conger myriaster* (Teleostei: Anguilliformes): novel gene order for vertebrate mitochondrial genomes and the phylogenetic implications for anguilliform families. J. Mol. Evol. **52**:311–320.

———. 2001*d*. A mitogenomic perspective on the basal teleostean phylogeny: resolving higher-level relationships with longer DNA sequences. Mol. Phylogenet. Evol. (in press).

ISHIGURO, N., M. MIYA, and M. NISHIDA. 2001. Complete mitochondrial DNA sequence of ayu, *Plecoglossus altivelis.* Fish. Sci. **67**:474–481.

JOHANSEN, S., and I. BAKKE. 1996. The complete mitochondrial DNA sequence of Atlantic cod (*Gadus morhua*): relevance to taxonomic studies among codfishes. Mol. Mar. Biol. Biotechnol. **5**:203–214.

JOHNSON, G. D. 1992. Monophyly of the euteleostean clades—Neoteleostei, Eurypterygii, and Ctenosquamata. Copeia **1992**:8–25.

———. 1993. Percomorph phylogeny: progress and problems. Bull. Mar. Sci. **52**:3–28.

JOHNSON, G. D., and C. PATTERSON. 1993. Percomorph phylogeny: a survey of acanthomorphs and a new proposal. Bull. Mar. Sci. **52**:554–626.

———. 1996. Relationships of lower euteleostean fishes. Pp. 251–332 *in* M. L. J. STIASSNY, L. R. PARENTI, and G. D. JOHNSON, eds. Academic Press, San Diego, Calif.

KAWAGUCHI, A., M. MIYA, and M. NISHIDA. 2001. Complete mitochondrial DNA sequence of *Aulopus japonicus* (Teleostei: Aulopiformes), a basal Eurypterygii: longer DNA sequences and higher-level relationships. Ichthyol. Res. (in press).

KOCHER, T. D., W. K. THOMAS, A. MEYER, S. V. EDWARDS, S. F. PÄÄBO, F. S. VILLABLANCA, and A. C. WILSON. 1989. Dynamics of mtDNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA **86**:6196–6200.

KUMAZAWA, Y., and M. NISHIDA. 1993. Sequence evolution of mitochondrial tRNA genes and deep-branch animal phylogenetics. J. Mol. Evol. **37**:380–398.

LAUDER, G. V., and K. F. LIEM. 1983. The evolution and interrelationships of the actinopterygian fishes. Bull. Mus. Comp. Zool. **150**:95–197.

LÊ, H. L. V., G. LECOINTRE, and R. PERASSO. 1993. A 28S rRNA-based phylogeny of the gnathostomes: first steps in the analysis of conflict and congruence with morphologically based cladograms. Mol. Phylogenet. Evol. **2**:31–51.

LECOINTRE, G., and G. NELSON. 1996. Clupeomorpha, sister group of Ostariophysi. Pp. 193–207 *in* M. L. J. STIASSNY, L. R. PARENTI, and G. D. JOHNSON, eds. Interrelationships of fishes. Academic Press, San Diego, Calif.

LYDEARD, C., and K. J. ROE. 1997. The phylogenetic utility of the mitochondrial cytochrome *b* gene for inferring relationships among actinopterygian fishes. Pp. 285–303 *in* T. D. KOCHER and C. A. STEPIEN, eds. Molecular systematics of fishes. Academic Press, San Diego, Calif.

MADDISON, W. P., and D. R. MADDISON. 1992. MacClade. Version 3. Sinauer, Sunderland, Mass.

MEYER, A. 1994. Shortcomings of the cytochrome *b* gene as a molecular phylogenetic marker. Trends. Ecol. Evol. **9**: 278–280.

MINDELL, D. P., M. D. SORENSON, D. E. M. DIMCHEFF, M. HASEGAWA, J. C. AST, and T. YURI. 1999. Interordinal relationships of birds and other reptiles based on whole mitochondrial genomes. Syst. Biol. **48**:138–152.

MIYA, M., and M. NISHIDA. 1998. Molecular phylogeny and evolution of the deep-sea fish genus *Sternoptyx.* Mol. Phylogenet. Evol. **10**:11–12.

———. 1999. Organization of the mitochondrial genome of a deep-sea fish *Gonostoma gracile* (Teleostei: Stomiiformes): the first example of transfer RNA gene rearrangements in bony fishes. Mar. Biotechnol. **1**:416–426.

———. 2000*a.* Molecular phylogeny of the deep-sea fish genus *Gonostoma* (Teleostei: Stomiiformes): two paraphyletic clades and resurrection of *Sigmops.* Copeia **2000**:378–389.

———. 2000*b.* Use of mitogenomic information in teleostean molecular phylogenetics: a tree-based exploration under the maximum-parsimony optimality criterion. Mol. Phylogenet. Evol. **17**:437–455.

MOORE, J. A. 1993. Phylogeny of the Trachichthyiformes (Teleostei: Percomorpha). Bull. Mar. Sci. **52**:114–136.

NAKABO, T., ed. 2000. Fishes of Japan with pictorial keys to the species. 2nd edition. Tokai University Press, Tokyo.

NAYLOR, G. J. P., and W. M. BROWN. 1997. Structural biology and phylogenetic estimation. Nature **388**:527–528.

———. 1998. Amphioxus mitochondrial DNA, chordate phylogeny, and the limits of inference based on comparisons of sequences. Syst. Biol. **47**:61–76.

NELSON, G. 1989. Phylogeny of major fish groups. Pp. 325–336 *in* B. FERNHOLM, K. BREMER, and H. JÖRNVALL, eds. The hierarchy of life. Excerpta Medica, Amsterdam.

NELSON, J. S. 1976. Fishes of the world. John Wiley & Sons, New York.

———. 1984. Fishes of the world. 2nd edition. John Wiley & Sons, New York.

———. 1994. Fishes of the world. 3rd edition. John Wiley & Sons, New York.

NORMARK, B. B., A. R. MCCUNE, and R. G. HARRISON. 1991. Phylogenetic relationships of neopterygian fishes, inferred from mitochondrial DNA sequences. Mol. Biol. Evol. **8**: 819–834.

OLNEY, J. E., G. D. JOHNSON, and C. C. BALDWIN. 1993. Phylogeny of lampridiform fishes. Bull. Mar. Sci. **52**:137–169.

ORTÍ, G., and A. MEYER. 1997. The radiation of characiform fishes and the limits of mitochondrial ribosomal DNA sequences. Syst. Biol. **46**:75–100.

PALUMBI, S. R. 1996. Nucleic acids II. The polymerase chain reaction. Pp. 205–221 *in* D. M. HILLIS, C. MORITZ, and B. K. MABLE, eds. Molecular systematics. 2nd edition. Sinauer, Sunderland, Mass.

PATTERSON, C., and D. E. ROSEN. 1989. The Paracanthopterygii revisited: order and disorder. Pp. 5–36 *in* D. M. COHEN, ed. Papers on the systematics of gadiform fishes. Natural History Museum of Los Angeles County, Los Angeles, Calif.

PATTERSON, C., D. M. WILLIAMS, and C. J. HUMPHRIES. 1993. Congruence between molecular and morphological phylogenies. Annu. Rev. Ecol. Syst. **24**:153–188.

PENNY, D., M. HASEGAWA, P. J. WADDELL, and M. D. HENDY. 1999. Mammalian evolution: timing and implications from using the LogDeterminant transform for proteins of differing amino acid composition. Syst. Biol. **48**:76–93.

POLLOCK, D. D., J. A. EISEN, N. A. DOGGETT, and M. P. CUMMINGS. 2000. A case for evolutionary genomics and the comprehensive examination of sequence biodiversity. Mol. Biol. Evol. **17**:1776–1788.

ROSEN, D. E. 1973. Interrelationships of higher teleostean fishes. Pp. 397–513 *in* P. H. GREENWOOD, R. S. MILES, and C. PATTERSON, eds. Interrelationships of fishes. Academic Press, London.

———. 1984. Zeiforms as primitive plectognath fishes. Am. Mus. Novit. **2782**:1–45.

———. 1985. An essay on euteleostean classification. Am. Mus. Novit. **2827**:1–57.

ROSEN, D. E., and C. PATTERSON. 1969. The structure and relationships of the paracanthopterygian fishes. Bull. Am. Mus. Nat. Hist. **141**:357–474.

RUBIN, D. A., and R. M. DORES. 1995. Obtaining a more resolute teleost growth hormone phylogeny by the introduction of gaps in sequence alignment. Mol. Phylogenet. Evol. **4**: 129–138.

SAITOH, K., K. HAYASHIZAKI, Y. YOKOYAMA, T. ASAHIDA, H. TOYOHARA, and Y. YAMASHITA. 2000. Complete nucleotide sequence of Japanese flounder (*Paralichthys olivaceus*) mitochondrial genome: structural properties and cue for resolving teleostean relationships. J. Hered. **91**:271–278.

SORENSON, M. D., J. C. AST, D. E. DIMCHEFF, T. YURI, and D. P. MINDELL. 1999. Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. Mol. Phylogenet. Evol. **12**:105–114.

SPRINGER, M. S., H. M. AMRINE, A. BURK, and M. J. STANHOPE. 1999. Additional support for Afrotheria and Paenungulata, the performance of mitochondrial versus nuclear genes, and the impact of data partitions with heterogeneous base composition. Syst. Biol. **48**:65–75.

STEPIEN, C. A., and T. D. KOCHER. 1997. Molecules and morphology in studies of fish evolution. Pp. 1–11 *in* T. D. KOCHER and C. A. STEPIEN, eds. Molecular systematics of fishes. Academic Press, San Diego, Calif.

STIASSNY, M. L. J. 1986. The limits and relationships of the acanthomorph teleosts. J. Zool. Lond. B **1986**:411–460.

STIASSNY, M. L. J., and J. A. MOORE. 1992. A review of the pelvic girdle of acanthomorph fishes, with comments on hypotheses of acanthomorph interrelationships. Zool. J. Linn. Soc. **104**:209–242.

STIASSNY, M. L. J., L. R. PARENTI, and G. D. JOHNSON, eds. 1996. Interrelationships of fishes. Academic Press, San Diego, Calif.

SWOFFORD, D. L. 1993. PAUP: phylogenetic analysis using parsimony. Version 3.1.1. Smithsonian Institution, Washington, D.C.

———. 1998. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.0. Sinauer, Sunderland, Mass.

TEMPLETON, A. R. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of human and the apes. Evolution **37**: 221–244.

THOMPSON, J. D., T. J. GIBSON, F. PLEWNIAK, F. JEANMOUGIN, and D. G. HIGGINS. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **25**:4876–4882.

TZENG, C. S., C. F. HUI, S. C. SHEN, and P. C. HUANG. 1992. The complete nucleotide sequence of the *Crossostoma lacustre* mitochondrial genome: conservation and variations among vertebrates. Nucleic Acids Res. **20**:4853–4858.

VENTER, J. C., H. O. SMITH, and L. HOOD. 1996. A new strategy for genome sequencing. Nature **381**:364–366.

WILEY, E. O. 1981. Phylogenetics: the theory and practice of phylogenetic systematics. John Wiley & Sons, New York.

WILEY, E. O., G. D. JOHNSON, and W. W. DIMMICK. 1998. The phylogenetic relationships of lampridiform fishes (Lampridiformes, Acanthomorpha), based on a total evidence analysis of morphological and molecular data. Mol. Phylogenet. Evol. **10**:417–425.

———. 2000. The interrelationships of Acanthomorph fishes: a total evidence approach using molecular and morphological data. Biochem. Syst. Ecol. **28**:319–350.

YAMAGUCHI, M., M. MIYA, M. OKIYAMA, and M. NISHIDA. 2000. Molecular phylogeny and larval morphological diversity of the lanternfish genus *Hygophum* (Teleostei: Myctophidae). Mol. Phylogenet. Evol. **15**:103–114.

ZARDOYA, R., A. GARRIDO-PERTIERRA, and J. M. BAUTISTA. 1995. The complete nucleotide sequence of the mitochondrial DNA genome of the rainbow trout, *Oncorhynchus mykiss*. J. Mol. Evol. **41**:942–951.

ZARDOYA, R., and A. MEYER. 1996. Phylogenetic performance of mitochondrial protein-coding genes in resolving relationships among vertebrates. Mol. Biol. Evol. **13**:933–942.

NARUYA SAITOU, reviewing editor

Accepted June 27, 2001