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Phylogenetic and Biogeographic Analysis of the Sparidae (Perciformes: Percoidei) from Cytochrome *b* Sequences

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We used complete sequence of the mitochondrial cytochrome *b* gene to test monophyly of the Sparoidea, Sparidae, six subfamilies of Sparidae, and to elucidate the interrelationships of the 33 recognized sparid genera. The analysis included 40 sparid species, 10 closely related species, 10 basal percoids, and two nonperciform outgroup species. The aligned 1140 base pairs of cytochrome *b* yielded 542 parsimony informative characters. Mutational analysis revealed that third codon position transitions were saturated and, therefore, of questionable use in phylogenetic analysis. However, the third codon position transversions and all first and second codon substitutions were not saturated and thus judged more reliable for inferring evolutionary relationship. Parsimony analysis of the equally weighted nucleotide data, weighted nucleotide data set (saturated position transitions given a weight of zero) supported a monophyletic Sparidae with the inclusion of *Spicara*, which is traditionally included in Centracanthidae. The previously proposed composition of genera within the six sparid subfamilies (Boopsinae, Denticinae, Diplodinae, Pagellinae, Pagrinae, and Sparinae) were not monophyletic in all analyses. This suggests the feeding types on which the subfamilies are based were independently derived multiple times within sparid fishes. In all analyses, Lethrinidae were sister to Sparidae. Sparoidea (Sparidae, Centracanthidae, Lethrinidae, and Nemipteridae) were monophyletic only in the weighted nucleotide phylogeny.

SPARIDAE are a diverse group of over 110 mostly neritic species whose putative six subfamilies have been defined primarily on the basis of dentition and feeding type (Smith, 1938; Akazaki, 1962). Monophyly of these subfamilies has not been tested, nor have the phylogenetic relationships of all sparid genera been hypothesized. Smith (1938) and Smith and Smith (1986) initially partitioned sparid genera into four subfamilies based mainly on dentition. Boopsinae have compressed outer incisiform teeth and are typically herbivores or feed on small invertebrates. Denticinae are typical piscivores with enlarged canines in front and smaller conical teeth behind. Pagellinae lack canines, have small conical outer teeth and small inner molars, and are usually carnivorous on small invertebrates. Sparinae have jaws with bluntly rounded molars posteriorly, enlarged front teeth, and are carnivorous on crustaceans, mollusks, and small fishes. Akazaki (1962) erected two new subfamilies, also most easily defined by dentition. He removed the genera *Diplodus*, *Archosargus*, and *Lagodon* from Sparinae and placed them into Diplodinae. He also moved *Pagrus*, *Argyrops*, and *Evynnis* from Sparinae into Pagrinae. Akazaki defined Diplodinae as having six to eight anterior teeth in the jaws and obliquely projecting incisors, and Pagrinae as having four canines on the upper jaw, four to six canines on the lower jaw, scales on the head extending to the interorbital region, molar teeth in two series, and body reddish.

Sparidae have been historically a heterogenous group of fishes, often associated with Lethrinidae, Nemipteridae, Lutjanidae, Caesionidae, and Haemulidae (Jordan and Fesler, 1893; Schultz 1953). Akazaki (1962) used osteology to define ''spariform'' fishes that included the Nemipteridae, Sparidae, and Lethrinidae. Akazaki suggested that spariform fishes had three ''stems'': the primitive Nemipteridae-stem; the intermediate Sparidae-stem; and the highly specialized Lethrinidae-stem. Johnson (1980) proposed the superfamily Sparoidea to include Akazaki's three spariform families and Centracanthidae. He added Centracanthidae based on maxillary-premaxillary distal articulation and other osteological characters. Johnson disagreed with Akazaki's placement of Sparidae between Nemipteridae and Lethrinidae, and he presented tentative anatomical and osteological evidence that Nemipteridae and Lethrinidae were more closely related to each other than they were to either sparids or to centracanthids.

Few molecular studies have examined the evolutionary relationships of Sparidae, and none has employed cladistic analysis to understand the evolutionary history of all sparid genera. Taniguchi et al. (1986) investigated 18 isozyme loci from skeletal muscle, liver, and heart tissues to infer the genetic relationships of 10

species from six genera of Japanese sparids. Their results established a close genetic relationship of Japanese sparids; a genetic distance (Nei, 1978) of less than 0.01 between Japanese members of the genera *Pagrus*, *Evynnis*, *Argyrops*, and *Dentex*. A greater genetic distance (> 0.013) was found between these four genera and *Sparus* and *Acanthopagrus*. Basaglia (1991) analyzed six isozymes from seven different tissues of 15 sparid species to infer phylogenetic relationships based on an ''index of divergence.'' Basaglia and Marchetti (1991) examined white muscle protein using the same 15 species in Basaglia (1991) and presented a more quantitative analysis based on pairwise similarity coefficients that clustered sparids into respective subfamilies—Boopsinae, Diplodinae, and Pagellinae. The pagelline *Lithognathus mormyrus* and the denticine *Dentex dentex* clustered with Sparinae. Garrido-Ramos et al. (1994, 1995, 1999) used centromeric satellite DNA to elucidate the relationships of Mediterranean sparids. The last study sampled 10 taxa from four genera to infer phylogenetic relationships from neighbor-joining and distance analyses. Jean et al. (1995) examined three mitochondrial regions, the displacement loop, $tRNA^{Phe}$, and 12S rRNA gene, of five taxa from two genera of Taiwanese sparids. Their resulting phylogenetic tree was based on Tamura-Nei genetic distances. Hanel and Sturmbauer (2000) used 16S rDNA sequences to examine the evolution of trophic types in Northeastern Atlantic and Mediterranean sparids. Based on their limited subset of sparid genera, they concluded that trophic types evolved more than once in sparid fishes.

In this paper, we present the results of phylogenetic analyses of the complete mitochondrial cytochrome *b* (cyt *b*) gene (1140bp) for 40 sparid species, 10 closely related species, 10 basal percoids, and two nonperciform outgroup species. Cytochrome *b* has proven a valuable evolutionary marker for fishes because it has produced robust phylogenies at various taxonomic levels (Lydeard and Roe, 1997; Schmidt et al., 1998; Song et al., 1998). We used mitochondrial DNA from the complete cyt *b* gene to test monophyly of the Sparoidea, Sparidae, six subfamilies of Sparidae, and to elucidate the interrelationships of the 33 recognized sparid genera. A resulting phylogeny was used to elucidate the biogeographic aspects of sparid evolution.

Materials and Methods

Specimens and tissue samples.—Specimens of species were collected to represent all 33 recognized genera of Sparidae, other members of the superfamily Sparoidea (Centracanthidae, Nemipteridae, and Lethrinidae), and possible close outgroups in Percoidei (Haemulidae, Lutjanidae, and Caesionidae). Basal percoids, Moronidae and Lateolabracidae (Springer and Raasch, 1995), were used to root Sparidae and related families within Perciformes. Sequences of two ostariophysins, *Luxilus* and *Cyprinus*, were used as distant outgroups in this study. GenBank sequences were used for six of the 62 taxa examined. Voucher designations and collection data are provided in Material Examined below. Gill tissue or white muscle tissue was dissected from fresh or frozen samples and placed into a buffer solution of 0.25 M disodium ethylenediaminetetra-acetate (EDTA), 20% dimethyl sulfoxide (DMSO), saturated sodium chloride (NaCl), pH 8.0 (Seutin et al., 1990) and stored at room temperature.

DNA isolation, amplification, cloning, and sequencing.—DNA was isolated from approximately 0.05–0.1 g of tissue following (Sambrook et al., 1989) or by using the tissue protocol of QIAamp® System DNA extraction kits (QIAGEN, Inc). Primer pairs used for PCR amplification in this study were mapped against the equivalent sequence positions on the mitochondrial genome of *Cyprinus carpio* (Chang et al., 1994; GenBank accession number X61010). The following primer pairs were used: CytbGludgL (TGACTTGAARAACCAYCGTTG, L15249; S. Palumbi, A. Martin, S. Romano, W. O. McMillan, L. Stice, and G. Grabowski, The Simple Fools Guide to PCR, University of Hawaii, Honolulu, HI, 1991, unpubl.), CytbThrdgH (CTCCAGTCTTCGRCTTACAAG, H16565; S. Palumbi, A. Martin, S. Romano, W.O. McMillan, L. Stice, and G. Grabowski, 1991, unpubl.), CytbUnvL (CGAACGTTGATATGAAAAACCATCGTTG, L15242, Kocher and White, 1989), CytbUnvH (ATCTTCGGTTTACAAGACCGGTG, H16458, Cantatore et al., 1994), Cytb4XdgL (TGAYWTGAARAACCAYCGTTG, L15249 modified from S. Palumbi, A. Martin, S. Romano, W. O. McMillan, L. Stice, and G. Grabowski, 1991, unpubl.) and Cytb4xdgH (TGRVNCTGAGCTACTASTGC, H16435, generated during this study). Primers were ordered from Genosys (Genosys Biotechnologies, Inc.). All primers sites were located within the transfer ribonucleic acids (tRNAs) that flank either end of the mtDNA cyt *b* gene ($tRNA^{Glu}$ and $tRNA^{Thr}$).

A 50 μl PCR amplification of cyt *b* was performed with 5–10 ng of each template DNA. The following reagents from the PCR Reagent System (GIBCO BRL Life Technologies) were

used in each reaction: 5 μl 10X PCR Buffer plus Mg (200mM Tris-HCL (pH8.4), 500 mM KCL, 15 mM $MgCl_2$); 1μl 10mM dNTP Mix (10mM each dATP, dCTP, dGTP, dTTP); 50 pmols of each primer, 0.25 μl *Taq* DNA polymerase (5U/μl). Either a Perkin Elmer Cetus or a MJ Research PTC-200 thermocycler was used for PCR amplification with the following cycle parameters: initial denaturation (94 C for 4.0 min); 35 cycles of (denaturation 94 C for 1.0 min, annealing 48–51 C (depending on sample) for 1.0 min; extension 72 C for 3.0 min); final extension (70 C for 5 min); icebox (4 C indefinitely).

Once targets sequences were successfully amplified, they were cloned using the Invitrogen TA Cloning® Kit (Invitrogen Corporation). Ligated PCR product was transformed and cloned into competent *Escherichia coli*. Transformed colonies were grown overnight on LB-Agar plates in the presence of ampicillin and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (Xgal). Colonies with inserts were streaked on new LB-Agar plates in the presence of ampicillin and Xgal. White colonies were screened for appropriate insert using the quick screening methods from Sambrook et al. (1989). Colonies with target insert were grown in 3 mL overnight preparations of terrific broth (Tartof and Hobbs, 1987) and ampicillin and purified plasmid DNA was obtained by either standard plasmid preparation protocols (Sambrook et al., 1989) or by using a PERFECTprep® kit (5Prime→3Prime, Inc). Once pure plasmid DNA was acquired, it was suspended in 65μl diH_20.

Plasmids were sequenced using dideoxynucleotide chain termination Sanger et al. (1977). Plasmid DNA was quantified using a DyNAQuant 200 flourometer (Amersham Pharmacia Biotech) and approximately 300 fmol of plasmid DNA was used in each cycle sequencing reaction. Forward and reverse IRD800 flouresscently labeled M13 primers (Li-Cor, Inc.) were used for sequencing. A heat stable DNA polymerase, Thermo Sequenase™ (Amersham Pharmacia Biotech) was used to incorporate the IRD800 flourescently labeled primer during cycle sequencing. To relax structural stops during electrophoresis, 7-deaza-dGTP was used during chain building. The flourescently labeled termination reactions were electrophoresed through a 66 cm, 0.25 mm thick, 4% LongRanger (FMC BioProducts) acrylamide gel on a Li-Cor 4000-liter automated sequencer. The resulting electronic gel image was analyzed using BaseImage V2.3 software (Li-Cor).

Phylogenetic analysis.—Light and heavy strands were sequenced for all taxa. Light strand sequences were inverted (reversed and complimented) to match the heavy strand sequence. A consensus sequence from combined light and heavy strands was made for all taxa. Cytochrome *b* nucleic acid sequences were aligned by eye and by the Clustal feature of Gene Jockey II (Biosoft). Two cyt *b* sequences from GenBank: accession numbers X81567 (Sparidae: *Boops boops*) and X8156 (Moronidae: *Dicentrarchus labrax*) were used to aid alignment. The resulting alignment introduced no gaps caused by deletions or insertions, and there were no inconsistent alignments between taxa. Once sequences were aligned, they were assigned codon positions and proofed for frame-shifts. Ambiguities were referenced against the sequencing gel image and corrected when possible.

Following Irwin et al., (1991), base compositional bias was calculated across all taxa, ingroup taxa and outgroup taxa, for each codon position, and all codon positions combined. A chi-square test of base heterogeneity was calculated for each codon position and for all codon positions.

Parsimony analysis was performed using PAUP (vers. 4.0b2*, D. L. Swofford, Sinauer Associates, Inc. Sunderland, MA, 1998, unpubl.). The most parsimonious tree or equally parsimonious trees and strict consensus were obtained for each analysis. The number of constant characters, parsimony-uninformative and parsimony informative characters, tree length, consistency index, and the retention index were determined. TreeRot (vers. 2, M. D. Sorensen, Boston University, Boston, MA, 1999, unpubl.) was used to calculate support indices for each node (Bremer, 1988) and to calculate partitioned decay values (Baker and Desalle, 1997; Baker et al., 1998) for each codon position at each node. Wilcoxon sign-ranks, two-tailed probability tests (Templeton, 1983) were used to determine whether unconstrained most-parsimonious trees were significantly different (at $P < 0.05$) from constrained trees.

Biogeographic analysis.—A quantitative biogeographic analysis of sparid evolution was conducted using the method of vicariance biogeography (Brooks, 1985; Wiley 1988; Wiley et al., 1991). Parsimony analysis of a single clade example was used to reconstruct the biogeographic relationships inferred from the cyt *b* phylogeny. A matrix of independent data (areas of occurrence) and dependent data (phylogeny of Sparidae) was constructed. All nodes on the original tree were labeled (terminal taxa, internal nodes = ancestral states). A list of areas of occurrence for each taxon was prepared based

TABLE 1. PAIRWISE VALUES OF MEAN PERCENT SEQUENCE DIVERGENCE DERIVED FROM UNCORRECTED "P" GENETIC DISTANCE.

Groups	% Divergence	Number	SD
All	20.22	1891	0.0419
Ingroup Taxa	16.27	861	0.0209
Outgroup Taxa	22.73	190	0.0187
Sparidae vs All	23.06	880	0.0274
Lutjanidae vs All	21.20	121	0.0169
Haemulidae vs All	22.49	121	0.0153
Nemipteridae vs All	23.66	121	0.0117
Lethrinidae vs All	22.52	121	0.0118
Centracanthidae vs Lutjanidae	19.93	4	0.0120
Centracanthidae vs Haemulidae	22.13	4	0.0099
Centracanthidae vs Nemipteridae	22.87	4	0.0144
Centracanthidae vs Lutjanidae	22.39	4	0.0072
Haemulidae vs Lutjanidae	20.02	4	0.0141
Haemulidae vs Nemipteridae	23.77	4	0.0082
Haemulidae vs Lethrinidae	22.48	4	0.0161
Lethrinidae vs Lutjanidae	19.82	4	0.0063
Lethrinidae vs Nemipteridae	23.27	4	0.0113
Lutjanidae vs Nemipteridae	22.96	4	0.0061
Sparidae vs Centracanthidae	16.46	84	0.0158
Sparidae vs Haemulidae	22.38	84	0.0122
Sparidae vs Lethrinidae	22.44	84	0.0076
Sparidae vs Lutjanidae	20.98	84	0.0111
Sparidae vs Nemipteridae	23.38	84	0.0097
Lutjanidae	12.28	1	N/A
Haemulidae	18.33	1	N/A
Lethrinidae	17.02	1	N/A
Nemipteridae	22.81	1	N/A
Sparidae	16.27	861	0.0209

on distributional data. Areas were defined as objectively as possible by using the Food and Agriculture Organization of the United Nations (FAO) fishing areas as established by FAO (1995). Each fishing area provides a defined distributional zone typically used for fisheries statistical purposes. Each node was assessed for each of the areas. A binary data-matrix was developed based on the presence (1) or absence (0) of each dependent variable (node) for each independent variable (area). The data matrix was converted to a NEXUS file and analyzed with PAUP*. The resulting tree was overlaid onto a map of the world.

RESULTS

Sequence divergence and mutation analysis.—Mean uncorrected pairwise genetic distance was calculated between all taxa (Table 1). The mean uncorrected pairwise sequence divergence between all taxa was 20.22%, between outgroup taxa was 22.73%, and between ingroup was 16.27%. The smallest mean pairwise sequence divergence between families was that of the Sparidae and the Centracanthidae (16.76%). This value was similar to the mean pairwise divergence between members of the family Sparidae (16.27%). The largest mean pairwise sequence divergence between families was that of the Haemulidae and the Nemipteridae (23.77%).

Because of the substantial difference in sequence divergence between ingroup and outgroup taxa, mutation analysis was restricted to ingroup taxa only. The total number of substitutions from the third codon position and the total number of substitutions from pooled first and second codon positions were plotted as a function of sequence divergence (Fig. 1A–B). Most substitutions were found in the third codon position. Third positional transitions appeared to asymptote at about 15% sequence divergence (Fig. 1B). Because third positional changes accounted for most of all substitutions, the contribution of transitions and transversions to the total number of third position substitutions was plotted as a function of sequence divergence (Fig. 1D–E). The contribution of tran-

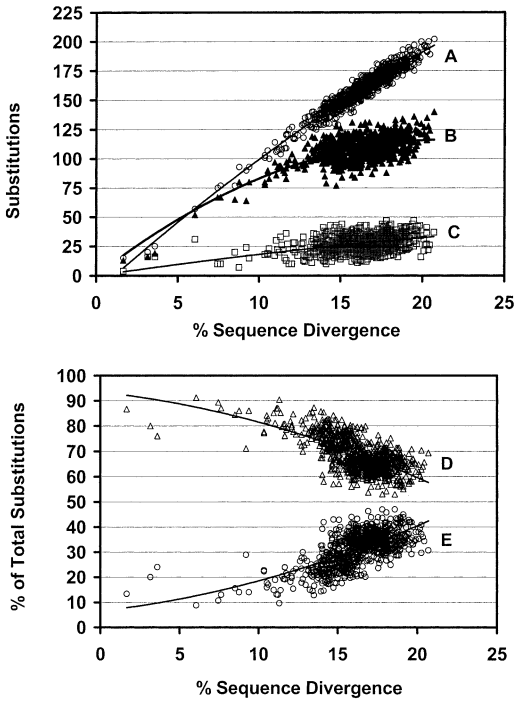

Fig. 1. Ingroup substitutions as a function of sequence divergence for (A) all substitutions third codon position; (B) transitions only third codon position; (C) all substitutions pooled first and second codons; the contribution of third position transitions (D) and transversions (E) to the total number of third position substitutions.

sitions as a percentage of total substitutions decreased with increasing sequence divergence, and the converse relationship was true for transversions (although the relative number of total substitutions was contributed from transitions). As sequence divergence increased, the contribution of transversions to the total number of substitutions increased. Because transitions are saturated, they are less informative at uncorrected sequence divergence greater than 15%. Past 15% uncorrected sequence divergence, transversions provide a more reliable phylogenetic signal. Therefore, transversions should become increasingly more important in deriving phylogenetic relationships from third codon position characters as sequence divergence increases. Because of saturation, third positional transitions were weighted to a value of zero in a subsequent weighted phylogenetic analysis. The sequence divergence and third positional substitution saturation reported here were equivalent to other cyt *b* studies that include percoid fishes (e.g., Lydeard and Roe, 1997; Song et al., 1999).

Base compositional bias.—The overall base compositional bias was 0.133 for all taxa. There was no significant difference in base compositional bias between ingroup and outgroup species. The highest bias was found in the third codon position (0.274) followed by the second codon position (0.215), and the smallest observed bias was in the first codon position (0.042). There was a strong antiguanine bias in the third codon position with a subsequent shift to procytosine. The first codon position ($\chi^2 = 34.22$, df = 183, $P > 0.995$) and second codon position ($\chi^2 = 9.68$, df = 183, $P > 0.995$) did not demonstrate significant heterogeneity among taxa in base composition. The frequencies of the four bases in the third codon position was strongly unequal (antiguanine, procytosine) and the chi-square test demonstrated significant heterogeneity among taxa in third codon position base frequency ($\chi^2 = 477.6$, df = 183, $P < 0.001$). This bias was comparable to other cyt *b* studies (Lydeard and Roe, 1997).

Phylogenetic Relationships

Equally weighted.—A heuristic search of 1000 random addition replicates of the cyt *b* nucleotide dataset resulted in four equally parsimonious trees (tree length = 6416, CI = 0.1900; RI = 0.4258, starting seed 796159554; all characters unordered and equal weight of 1; outgroup taxa *Cyprinus carpio* and *Luxilus zonatus*; branch-swapping = stepwise addition; swapping algorithm = tree bisection-reconnection; no topological restraints; character-state optimization = accelerated transformations). Complete (BS) and partitioned decay (PBS) values (20, unrestricted random addition sequences per node) and jackknife support (JS) of 1000 replicates (37% character deletions, five random addition replicates) are shown on a strict consensus of four equally parsimonious trees (Fig. 2). Of the 1140 characters sampled across all taxa, 483 (42%) were constant, 115 (10%) variable characters were parsimony uninformative and 542 (48%) variable characters were parsimony informative. Of all informative characters, 69% came from the third codon position. Third codon position bases were more variable (two constant, two uninformative, 376 informative) than first codon position bases (203 constant, 49 uninformative, 128 informative) and second codon position bases (276 constant, 66 uninformative, 38 informative). For each codon position, most of the parsimony informative characters were from transitions (third TS = 68%, first TS = 66%, and second TS = 59%).

The family Sparidae formed a fully resolved

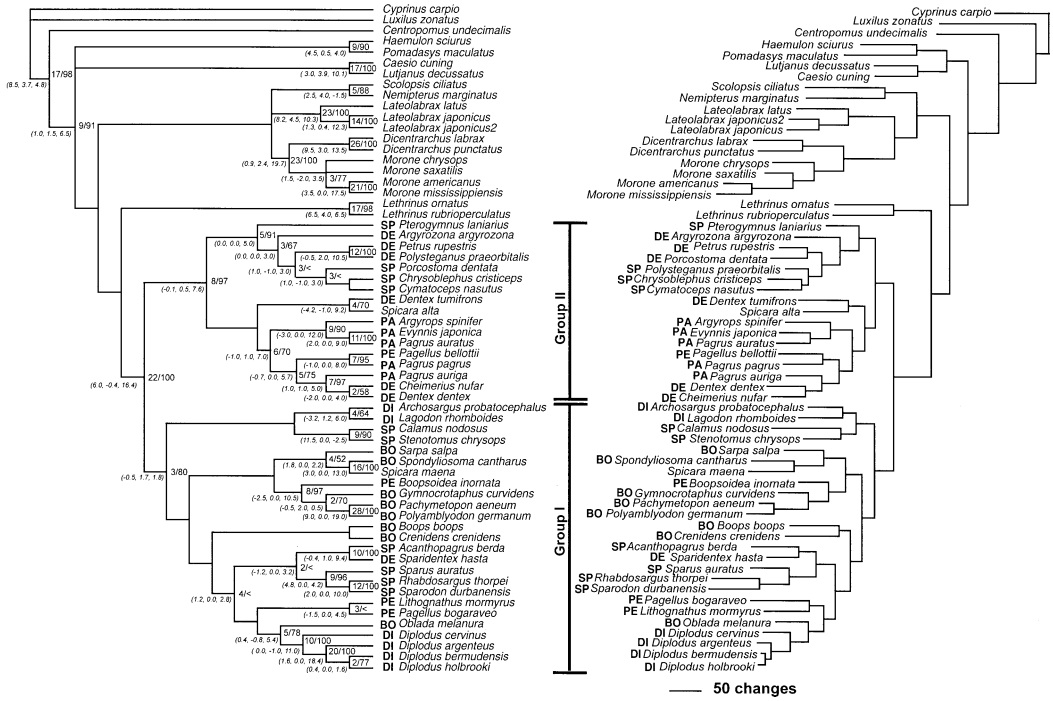

Fig. 2. Left tree is a strict consensus of four equally parsimonious trees from the equally weighted cytochrome *b* nucleotide data. The numbers within nodes are total decay values followed by jackknife values and the numbers within the parentheses below nodes are partitioned decay values for codons one, two, and three. Subfamilies are labeled as follows (BO = Boopsinae, DE = Denticinae, DI = Diplodinae, PA = Pagrinae, PE = Pagellinae, and SP = Sparinae). Right tree is a phylogram of one of four equally parsimonious trees.

clade with strong support (BS = 22, JS = 100) for sparid monophyly. Most of the partitioned decay support for sparid monophyly was based on third codon position (PBS = 16.4) substitutions and the remainder was from first codon substitutions (PBS = 6.0). Two species of the centracanthid genus *Spicara* were included in the monophyletic Sparidae clade. Pending further evidence (see Discussion) *Spicara* was considered a member of Sparidae in this paper. Within the monophyletic Sparidae + *Spicara* clade, two distinct groups were found. There was minimal support (BS = 3, JS = 80) for sparid taxa in ''Group I'' (Fig. 2), whereas sparid taxa in ''Group II'' (Fig. 2) were much more robust (BS = 8, JS = 97). There was no support for any of the previously defined subfamilies. Templeton tests (Table 2) of constrained subfamilies showed the Boopsinae ($P = < 0.001$), Denticinae ($P = 0.016$), Diplodinae ($P = 0.004$), Pagellinae ($P = < 0.001$), Pagrinae ($P = 0.011$), and Sparinae ($P = < 0.001$) were significantly different than the unconstrained equally parsimonious trees. Within Group I taxa, there were solid sister relationships between *Archosargus probatocephalus* + *Lagodon rhomboides* and between *Calamus nodosus* + *Stenotomus chrysops*, but there was no jackknife or decay support for the combined clade. There was strong support (BS = 16, JS = 100) for *Spondyliosoma cantharus* sister to the centracanthid *Spicara maena* and for *Pachymetopon aeneum* + *Polyamblyodon germanum* (BS = 28, JS = 100). A robust clade of *Rhabdosargus thorpei* + *Sparodon durbanensis* was sister to *Sparus auratus* and the Mediterranean *Oblada melanura* was basal to members of *Diplodus*. Within Group II taxa, *Argyrops spinifer* was basal to a strong clade of *Evynnis japonica* + *Pagrus auratus*. The centracanthid *Spicara alta* was sister to *Dentex tumifrons*, and *Petrus rupestris* was strongly sister to *Polysteganus praeorbitalis* (BS = 12, JS =100). *Diplodus* was monophyletic within Group I, but *Pagellus* and the centracanthid *Spicara* were paraphyletic occurring in both Group I and II and when constrained were found to be significantly different than in the equally parsimonious trees ($P = 0.001$ and $P \leq 0.001$, respectively). *Dentex* and *Pagrus* were polyphyletic within Group II, but only *Pagrus* had a significant Templeton value ($P \leq 0.001$). The sister group to Sparidae were Lethrinidae (*Lethrinus ornatus*, *Lethrinus rubriop-*

TABLE 2. REULTS OF WILCOXON SIGN-RANKS, TWO-TAILED PROBABILITY TESTS (TEMPLETON, 1983).

	Equally weighted tress				Weighted trees			
Constraint	TL	*n* trees	Average *P*-value	SD	TL	*n* trees	Average *P*-value	SD
Boopsinae	6475	3	<0.001*	0	2583	12	<0.001*	0
Denticinae	6452	5	0.016*	0.009	2555	92	0.023*	0.010
Diplodinae	6450	6	0.004*	0.001	2558	16	0.002*	0.001
Pagellinae	6480	1	<0.001*	n/a	2586	56	<0.001*	0
Pagrinae	6440	11	0.011*	0.007	2544	26	0.307*	0.091
Sparinae	6481	5	<0.001*	0	2604	8	<0.001*	0
Centracanthidae	6473	4	<0.001*	0	2572	8	<0.001*	0
*Diplodus***	6416	4	1.0	0	2536	2	1.0	0
Pagrus	6459	6	<0.001*	0	2554	2	0.003*	0.003
Dentex	6444	3	0.109	0.009	2548	90	0.148	0.027
Pagellus	6466	10	0.001*	0.001	2574	14	0.001*	0

Note. All trees generated by a heuristic search of 100 random addition sequences with five trees held at each step. Unconstrained search generated four equally parsimonious trees L = 6416. *P*-values are averaged across all comparisons of unconstrained and constrained trees. * indicates significant difference at $P < 0.05$. ** indicates that trees generated with this constraint are the same length as those generated during an unconstrained search.

erculatus). However, decay values did not support lethrinids as separate from other percoids. Nemipteridae were included with an unresolved clade of moronids + *lateolabracids* and haemulids and lutjanids + caesionids formed an unresolved trichotomy with other percoids. Strong decay and jackknife support (BS = 9, JS = 91) separated *Centropomus undecimalis* from other perciform fishes.

Weighted.—Saturation in third codon position transitions occurred at the approximate mean pairwise sequence divergence within the Sparidae. Because transitions at a saturated site might not reflect homologous changes between taxa, evolutionary relationships inferred from saturated sites are less likely to be reflective of phylogenetic history. Down-weighting or eliminating the contribution of saturated transitions within the analysis might decrease systematic error because of homoplasy (Swofford et al., 1996). Third codon position transitions appeared saturated based on mutation analysis. However, it was not possible to separate those positions that were saturated from those that were unsaturated. Therefore, all third position transitions were eliminated in this analysis (although some informative data were potentially sacrificed). A step matrix was developed that weighted all third position transitions 0 and transversions 1 and was applied to third positions under the ''Set Character Types'' of the Data option of PAUP4.0b2*. A heuristic search of 1000 random addition replicates yielded two equally parsimonious trees (tree length = 2536, CI = 0.2504, RI = 0.5816 starting seed 303516572, cyt *b* nucleotide data set using all changes at first and second position and only transversions in third position, all other parameters of this analysis were similar to the previous analysis). The strict consensus of these trees (Fig. 3) revealed a monophyletic Sparidae and a monophyletic Sparoidea (Sparidae + *Spicara* + Lethrinidae + Nemipteridae) although there was only minimal support (BS = 2) from the third (PBS = 3.4) and first (PBS = 1.1) codons for the Sparoidea node. The Sparoidea were sister to Lutjanidae + Caesionidae followed by Haemulidae and a clade containing Moronidae + Lateolabracidae. The relationship of Lateolabracidae sister to Moronidae is novel, although unsupported. To date no cladistic study has placed the Moronidae sister to *Lateolabrax*, although the relationship was hypothesized by McCully (1962) and Waldman (1986). Within the Sparidae, *Calamus*, *Stenotomus*, and *Archosargus* + *Lagodon* were removed from their previous placement and were basal to all other Sparidae. Minus these taxa, the two clades (Group I and Group II) rendered in the previous analysis remained, but the relative location of taxa was not stable. Notably, *Boops* shifted in placement from *Boops* + *Crenidens* in the previous tree to (*Boops* + *Sarpa*) + (*Spondyliosoma* + *Spicara*) and *Crenidens* shifted to a clade with *Lithognathus*. As in the equally weighted analysis, all subfamilies as previously defined were not monophyletic

Biogeography.—The monophyletic Sparidae clade within the weighted cyt *b* phylogeny (Fig. 3) was used as the source of dependent data for biogeographic analysis. The Sparidae clade was pruned from the original tree and all ancestral states were numbered (Fig. 4). The geographic distribution was defined for terminal taxa using FAO areas (see Material Examined) and these

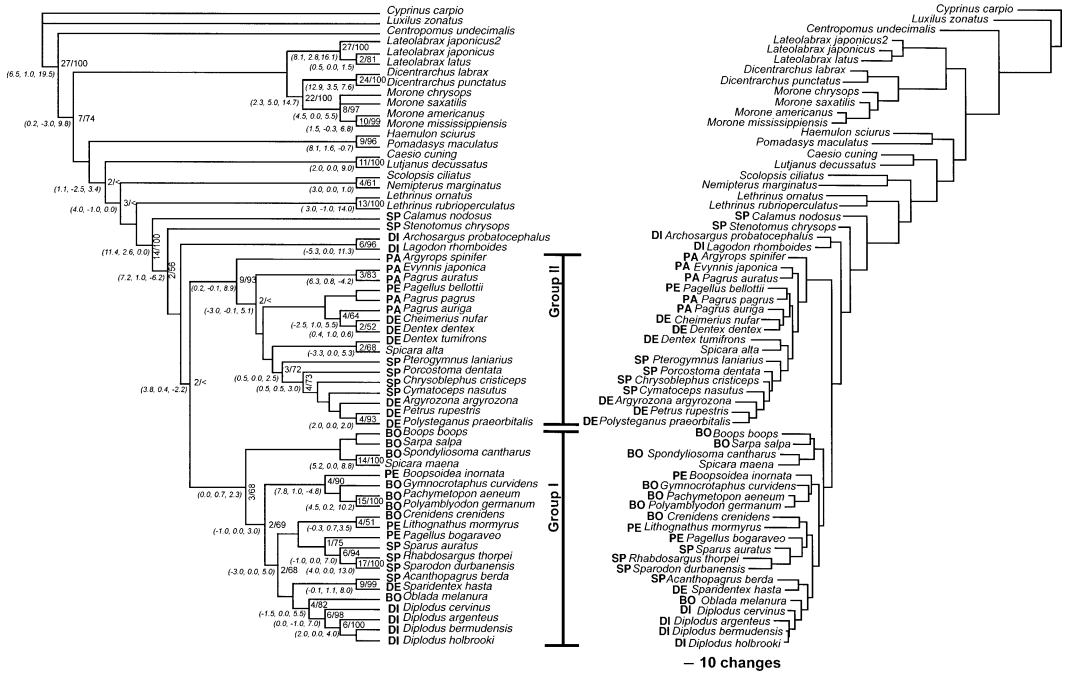

Fig. 3. Left tree is a strict consensus of two equally parsimonious trees from the weighted cytochrome *b* nucleotide data. The numbers within nodes are total decay values followed by jackknife values and the numbers within the parentheses are partitioned decay values for codons one, two, and three. Subfamilies as defined in Figure 2. Right tree is a phylogram of one of two equally parsimonious trees.

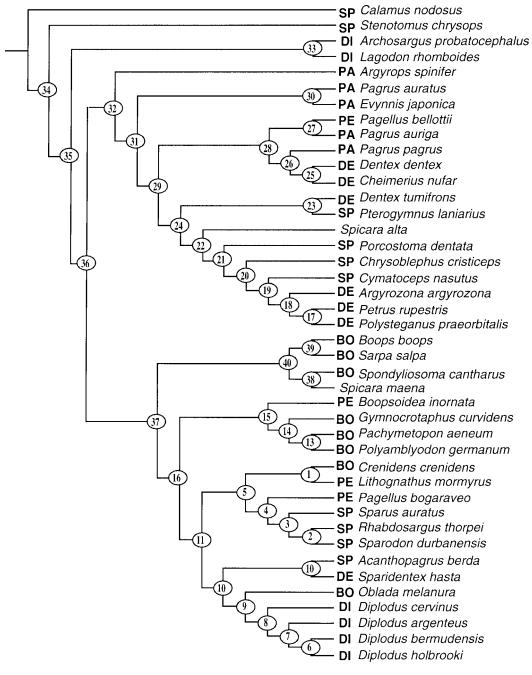

Fig. 4. Numbered ancestral nodes of the Sparidae clade, pruned from the equally weighted cytochrome b (Fig. 3). Ancestral nodes were used for biogeographical analysis. Subfamilies as defined in Figure 2.

areas were treated as independent data. Areas of occurrence were determined relative to each terminal taxa or ancestral node and coded into a binary matrix; presence = 1, absence = 0 (Table 3).

Parsimony analysis of the data matrix resulted in a single most parsimonious tree, that is overlaid on a worldwide map in Figure 5 (tree length = 102; CI = 0.7549; RI = 0.8377; of 82 total characters; all characters unordered and equal weight, 5 characters were constant, 7 variable characters were parsimony uninformative, 70 variable characters were parsimony informative; 1000 replicates of random stepwise addition; 100 trees held at each step; starting seed = 1674512988; character-state optimization: Delayed transformation). All taxa were found in more than one FAO area, except the Bermuda endemic *Diplodus bermudensis* and the east Asian endemic *Evynnis japonica*. Many of the taxa were assigned to multiple areas (for example, *Acanthopagrus berda*). The tree was rooted to the node of southwestern Pacific Ocean and eastern Indian Ocean + western central Pacific Ocean + northwestern Pacific Ocean. The western Indian Ocean/southeastern Atlantic clade was sister to eastern Atlantic/Mediterranean-Black Sea species. These were in turn sister to western At-

TABLE 3. BIOGEOGRAPHIC MATRIX. FAO areas were treated as independent data. Taxa and ancestral nodes were treated as dependent data during parsimony analysis.

Areas	Characters .....1.....2.....3.....4. 12345678901234567890123456789012345678901
Atlantic, Eastern Central	00001000000100010000110110110000001001111
Atlantic, Northeast	00001000000100010000110100110000001001101
Atlantic, Northwest	01000010000000000100100000001000000000000000
Atlantic, Southeast	00111101111000010010111010101111111100011
Atlantic, Southwest	01000000000001000000000000001000000000000000
Atlantic, Western Central	01000010000001101001000000010000000000000000
Indian Ocean, Eastern	10100000000010000000000000100000000000010000
Indian Ocean, Western	10110101111000000010101000001111111110000
Pacific, Northwest	10100000000010000100000001000000000000000000
Pacific, Southwest	00000000000000000000000000100000000000000000
Pacific, Western Central	10100000000001000000000000010000000000000000

Areas	Characters 4.....5.....6.....7.....8.. 23456789012345678901234567890123456789012
Atlantic, Eastern Central	01011100110110001000000111111101101111111
Atlantic, Northeast	01011100110110001000000001111101101111111
Atlantic, Northwest	10000011110110001000000000011101111111000
Atlantic, Southeast	01111100110111111111111111111101101111111
Atlantic, Southwest	00000001110110001000000000011101111111000
Atlantic, Western Central	10000011110110001000000000011101111111000
Indian Ocean, Eastern	00000000001110001000000110000111101111011
Indian Ocean, Western	01111100001111111111111011101101101111011
Pacific, Northwest	00000000001110001000000110000111101111000
Pacific, Southwest	00000000000000000000000000000011101110000
Pacific, Western Central	00000000001110001000000110000111101111000

Note: AN = ancestral node. Characters are: 1 *Acanthoparus berda*; 2 *Achosargus probatocephalus*; 3 *Argyrops spinifer; 4 Argyrozona argyrozona*; 5 *Boops boops*; 6 *Boopsoidea inornata*; 7 *Calamus nodosus*; 8 *Cheimerius nufar*; 9 *Chrysoblephus criticeps*; 10 *Crenidens crenidens*; 11 *Cymatoceps nasutus*; 12 *Dentex dentex*; 13 *Dentex tumifrons*; 14 *Diplodus argenteus*; 15 *Diplodus bermudensis*; 16 *Diplodus cervinus*; 17 *Diplodus holbrooki*; 18 *Evynnis japonia*; 19 *Gymnocrotaphus curvidens*; 20 *Lagodon rhomboides*; 21 *Lithognathus mormyrus*; 22 *Oblada melanura*; 23 *Pachymetopon aeneum*; 24 *Pagellus bogaraveo*; 25 *Pagellus bellotti*; 26 *Pagrus auratus*; 27 *Pagrus auriga*; 28 *Pagrus pagrus*; 29 *Petrus rupestris*; 30 *Polyamblyodon germanum*; 31 *Polysteganus praeorbitalis*; 32 *Porcostoma dentata*; 33 *Pterogymnus laniarius*; 34 *Rhabdosargus thorpei*; 35 *Sarpa salpa*; 36 *Sparodon durbanensis*; 37 *Sparidentex hasta*; 38 *Sparus awratus*; 39 *Spicura maena*; 40 *Spicara alta*; 41 *Spondyliosoma cantharus*; 42 *Stenotomus chrysops*; 43 AN1; 44 AN2; 45 AN3; 46 AN4; 47 AN5; 48 AN6; 49 AN7; 50 AN8; 51 AN9; 52 AN10; 53 AN11; 54 AN12; 55 AN13; 56 AN14; 57 AN15; 58 AN16; 59 AN17; 60 AN18; 61 AN19; 62 AN20; 63 AN21; 64 AN22; 65 AN23; 66 AN24; 67 AN25; 68 AN26; 69 AN27; 70 AN28; 71 AN29; 72 AN30; 73 AN31; 74 AN32; 75 AN33; 76 AN34; 77 AN35; 78 AN36; 79 AN37; 80 AN38; 81 AN39; 82 AN4.

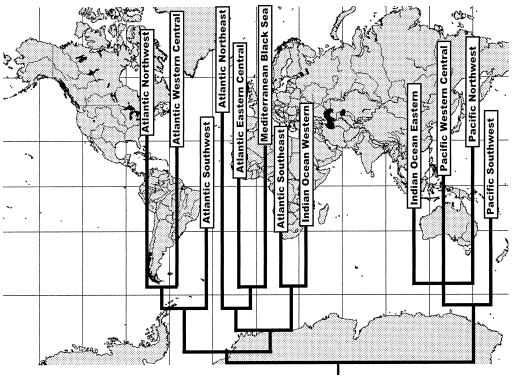

Fig. 5. Vicariance biogeography clade of the Sparidae. No distances are implied by branch lengths or internodal spaces.

lantic species. All Atlantic Sparidae + the western Indian Ocean/Atlantic species were sister to Pacific Ocean/eastern Indian Ocean clade. The eastern Indian Ocean-western central and northwestern Pacific taxa formed an unresolved polytomy.

DISCUSSION

Phylogenetic relationships of sparoid families.—Sparidae were monophyletic with the inclusion of the centracanthid genus *Spicara* in all phylogenies derived from the cyt *b* sequence data. Centracanthidae were considered members of Sparidae by Jordan and Fesler (1893) and very closely related to Sparidae based on jaw morphology by Regan (1913) and Smith (1938). Johnson (1980) noted the close relationship of Centracanthidae to Sparidae, but he retained the fam-

ily status of Centracanthidae, ''pending a more complete understanding of sparoid interrelationships.'' The molecular evidence reported in this study support the provisional insertion of *Spicara* within sparids, and for the remainder of the discussion when we refer to Sparidae, it refers to Sparidae + *Spicara*. However, these same data question the monophyly of *Spicara*; it was polyphyletic in the equally weighted and weighted nucleotide analyses. This study included two representatives of *Spicara* but not the other centracanthid genus, *Centracanthus*. The phylogenetic relationship of *Centracanthus* to all species of *Spicara*, and the ultimate status of Centracanthidae remains pending a more thorough analysis of all species of this putative polyphyletic family.

Lethrinidae were sister to Sparidae in both analyses. The superfamily Sparoidea (Sparidae, Centracanthidae, Lethrinidae, and Nemipteridae) was supported by the weighted nucleotide analysis. Sparoidea were not monophyletic in the equally weighted nucleotide analysis possibly because of homoplasy caused by saturated third positional substitutions.

The Sparidae appear derived within Sparoidea. Lethrinidae were sister to Sparidae and Nemipteridae were basal to Lethrinidae + Sparidae. These results agreed with a morphological analysis (Carpenter and Johnson, 2002). The cyt *b* nucleotide data did not support a strong relationship between nemipterids and lethrinids as proposed by Johnson (1980). The sequence data did support an overall sister relationship between Sparidae and Lethrinidae, but there was little clade support for placement of Nemipteridae sister to Lethrinidae in the weighted analysis, and nemipterids were sister to moronids + lateolabracids in the equally weighted tree. A weak decay value supported Sparoidea in the weighed analysis, but there was no support for the monophyly of Sparoidea in the equally weighted tree.

In the weighted analysis, the relationship of Sparoidea to other percoids was better defined than in the equally weighted analysis. Lutjanoids (*Lutjanus* + *Caesio*) were sister to Sparoidea, and Haemulidae were sister to lutjanoids + Sparoidea. Moronidae + Lateolabracidae were basal to Haemulidae + lutjanoids + Sparoidea. *Centropomus undecimalis* was sister to all other percoids.

Phylogenetic relationships of sparid subfamilies.—Of the four subfamilies of Sparidae (Boopsinae, Denticinae, Pagellinae, and Sparinae) proposed by Smith (1938) and Smith and Smith (1986) and the two (Pagrinae and Diplodinae) proposed by Akazaki (1962), none was found to be monophyletic based on cyt *b* nucleotide data. The Boopsinae as defined by Smith and Smith (1986) were contained within Group I of the family Sparidae but were rendered paraphyletic within the group. Most genera of Denticinae were found within sparid Group II except for *Sparidentex hasta*, which was found in Group I. *Sparidentex hasta* has conical teeth typical of denticines but an upper jaw morphology more typical for *Acanthopagrus* (K. Carpenter, unpubl. data). The Diplodinae (*Archosargus*, *Diplodus*, and *Lagodon*) were restricted to sparid Group I in the equally weighted nucleotide clade but were polyphyletic in the weighted nucleotide tree. *Diplodus* formed a well-supported, monophyletic clade in the equally weighted and weighted analyses. *Diplodus holbrooki* was sister to the Bermuda endemic species, *Diplodus bermudensis* and each were sister to the southwestern Atlantic *D. argenteus*. The South African *D. cervinus* was basal to all other *Diplodus*. *Oblada melanura*, a Mediterranean/Eastern Atlantic monotypic sparid, allied with *Diplodus* in the equally weighted and weighted analyses. The placement of *Oblada* outside of Boopsinae is novel. The subfamily Pagellinae was polyphyletic within Sparidae. The genus *Pagellus* was polyphyletic in the equally weighted and the weighted data analyses. *Pagellus* was the only genus that was found in both sparid Group I and Group II. The Pagrinae (*Argyrops*, *Evynnis*, *Pagrus*) were paraphyletic within sparid Group II. Within Pagrinae the genus *Pagrus* was problematic. *Pagrus* was paraphyletic within Group II of Sparidae. The genetic relationship of *Pagrus auratus* to its geographic siblings rather than to other members of *Pagrus* is enigmatic and is supported by the close genetic distance found among Japanese sparids of Taniguchi et al. (1986). Either the taxonomic placement of *Evynnis* and *Argyrops* outside of *Pagrus* is in error, or *Pagrus* is not a monophyletic group. In the equally weighted and weighted analyses, the subfamily Sparinae was distributed across sparid Group I and Group II.

Because these subfamilies are defined mostly by trophic type, the molecular results suggests that different feeding modes have evolved multiple times within Sparidae. This is similar to the conclusions of Hanel and Sturmbauer (2000).

Biogeography.—Results of biogeographic analysis suggested a strong vicariant explanation to structuring of genera within Sparidae. There were two areas of sparid evolution, eastern Indian Ocean-western Pacific and western Indian Ocean-Mediterranean/Atlantic. These species

probably had a Tethyan Sea common ancestor. Sparids are known from Tertiary fossil records of Europe, north Africa, Australia, New Zealand and North America (Frickhinger, 1995). Fossil sparids from Paleocene formations of Europe and north Africa lived at least 65 million years ago during the time of a continuous Tethyan Sea. The Tethys was partitioned into distinct provinces when Africa and Eurasia collided in the Miocene and with formation of the Panama land bridge in the Pliocene (Hocutt, 1987). This partitioning of a continuous habitat might explain the two areas of sparid evolution found during biogeographic analysis. However, little more can be drawn from this analysis, because the vicariance biogeography clade was based on a single tree example and this is considered equivalent to a single transformation series explaining a clade (Wiley et al., 1991). It would be preferable to have multiple dependent data to test the biogeographic relationships of a group. Therefore, the power of this analysis is little more than can be derived from purely descriptive biogeography.

Material Examined

Museum abbreviations are following (Leviton et al., 1985 and Leviton and Gibbs, 1988). GenBank accession numbers are in parentheses. FAO fishing area assignments (FAO, 1995) are in brackets and are given for ingroup species only. We have assigned the following numbers to corresponding FAO areas: 1-Atlantic, Eastern Central; 2-Atlantic, Northeast; 3-Atlantic, Northwest; 4-Atlantic, Southeast; 5-Atlantic, Southwest; 6-Atlantic, Western Central; 7-Indian Ocean, Eastern; 8-Indian Ocean, Western; 9-Mediterranean and Black Sea; 10-Pacific, Northwest; 11-Pacific, Southwest; 12-Pacific, Western Central

OUTGROUP TAXA—Centropomidae: *Centropomus undecimalis*, (AF240739), no voucher, Gulf of Mexico, Florida. Cyprinidae: *Cyprinus carpio*, (X61010), sequence from GenBank, Chang et al. (1994); *Luxilus zonatus*, (U66600), sequence from GenBank, Dowling and Naylor (1997). Haemulidae: *Haemulon sciurus*, (AF240747), no voucher, Big Pine Key, w. of bridge, Florida; *Pomadasys maculatus*, (AF240748), no voucher, Manila Fish Market, Luzon, Manila, Philippines. Lateolabracidae: *Lateolabrax japonicus*, (AF250741), VIMS 10381, picture voucher, fish market, Japan; *Lateolabrax japonicus* 2, (AF240742), VIMS 10381, picture voucher, fish market, Japan; *Lateolabrax latus*, (AF240743), MTUF 27451, Sasebo, Nagasaki Prefecture, Japan. Lethrinidae: *Lethrinus ornatus*, (AF240751), USNM 345259, Bolinao, Luzon, Philippines; *Lethrinus rubrioperculatus*, (AF240752), no voucher, W. Australia CSIRO SS 8/95/45, Australia. Lutjanidae: *Caesio cuning*, (AF240749), USNM 345193, fish market, Iloilo Panay, Philippines; *Lutjanus decussatus*, (AF240750), USNM 346695, fish market, Northern Negros, Philippines. Moronidae: *Dicentrarchus labrax*, (X81566), sequence from GenBank, Cantatore et al. (1994); *Dicentrarchus punctatus*, (AF240740), no voucher, fish market, Spain; *Morone americanus*, (AF240744), no voucher, VIMS Trawl Survey, Chesapeake Bay; *Morone chrysops*, (AF240745), UT 85.91, Cherokee Reservoir, Grainger Co, TN; *Morone mississippiensis*, (AF045362), sequence from GenBank, Song et al. (1998); *Morone saxatilis* (AF240746), no voucher, VIMS Trawl Survey, Chesapeake Bay. Nemipteridae: *Nemipterus marginatus* (AF240754), USNM 345202, Manila Market, Manila, Luzon, Philippines; *Scolopsis ciliatus* (AF240753), USNM 346853, Guimaras Island, JTW 95–1, Philippines.

INGROUP TAXA (Sparidae + Centracanthidae)—Sparidae: Boopsinae: *Boops boops* (X81567), sequence from GenBank, Cantatore et al. (1994) [1, 2, 4, 9]; *Crenidens crenidens* (AF240699), no voucher, Qatif Market, eastern Saudi Arabia [4, 8]; *Gymnocrotaphus curvidens* (AF240700), RUSI 49447, Kenton-on-Sea, South Africa [4, 8]; *Oblada melanura* (AF240701), no voucher, Azohia, Bay of Cartagena, Spain [1, 2, 4, 9]; *Pachymetopon aeneum* (AF240702), RUSI 49672, Kenton-on-Sea, South Africa [4, 8]; *Polyamblyodon germanum* (AF240703), RUSI 49690, Kenton-on-Sea, South Africa [4, 8]; *Sarpa salpa* (AF240704), RUSI 49456, Kenton-on-Sea, South Africa [1, 2, 4, 8, 9]; *Spondyliosoma cantharus* (AF240705), ODU 2782, Fiumicino Fish Market, Fiumicino, Italy [1, 2, 4, 9]; Denticinae: *Argyrozona argyrozona* (AF240706), RUSI 58449, fish market, Durban, South Africa [4, 8]; *Cheimerius nufar* (AF240707), RUSI 49443, Kenton-on-Sea, South Africa [4, 8]; *Dentex dentex* (AF143197), sequence from GenBank, Allegrucci et al. (1999) [1, 2, 8]; *Dentex tumifrons* (AF240708), AMS I.36450–002, Nelson Bay, Australia [7, 10, 12]; *Petrus rupestris* (AF240709), RUSI 49684 Kenton-on-Sea, South Africa [4, 8]; *Polysteganus praeorbitalis* (AF240710), RUSI 49686 Kenton-on-Sea, South Africa [4, 8]; *Sparidentex hasta* (AF240734), ODU 2783 Shuwaik Market, Kuwait City, Kuwait [7, 8]; Diplodinae: *Archosargus probatocephalus* (AF240716), VIMS 010192, Chesapeake Bay [3, 5, 6]; *Diplodus argenteus* (AF24072), NSMT-P 48013, Sea Life Park Tokyo, origin: Argentina [5, 6]; *Diplodus bermudensis* (AF240722), no voucher, Bermuda [6]; *Diplodus*

cervinus (AF240723), RUSI 49680, Kenton-on-Sea, South Africa [1, 2, 4, 9]; *Diplodus holbrooki* (AF240724), ODU 2789, Atlantic, South Carolina [3, 6]; *Lagodon rhomboides* (AF240726), no voucher, Bahia Honda-ocean side, Florida Keys, Florida [3, 6]; Pagellinae: *Boopsoidea inornata* (AF240711), ODU 2791, St. Sebastian Bay, South Africa [4, 8]; *Lithognathus mormyrus* (AF240712), ODU 2784, Fiumicino Fish Market, Fiumicino, Italy [1, 2, 4, 8, 9]; *Pagellus bogaraveo* (AF240713), ODU 2785, Fiumicino Fish Market, Fiumicino, Italy [1, 2, 9]; *Pagellus bellottii* (AF240714), ODU 2792, R/V African, Station 17491, South Africa [1, 4, 9]; Pagrinae: *Argyrops spinifer* (AF240717), AMS I.36447–001, N. Territory, Australia [4, 8, 7, 10, 12]; *Evynnis japonica* (AF240725) , NSMT-P 47497, Miyazaki, Kyushu Prefecture, Japan [10]; *Pagrus auratus* (AF240727), no voucher, Sydney Fish Market, New Zealand [7, 10, 11, 12]; Table 1. Cont.; *Pagrus auriga* (AF240728), ODU 2786, V. Emmanul Fish Market, Rome, Italy [1, 2, 4, 9]; *Pagrus pagrus* (AF240729), ODU 2790, Atlantic, North Carolina [1, 2, 3, 5, 6, 9]; Sparinae: *Acanthopagrus berda* (AF240715), USNM 345989, Manila Market, Manila, Luzon, Philippines [8, 7, 10, 12]; *Calamus nodosus* (AF240718), no voucher, Atlantic, Charleston, SC [3, 6]; *Chrysoblephus cristiceps* (AF240719), RUSI 49441, Kenton-on-Sea, South Africa [4, 8]; *Cymatoceps nasutus* (AF240720), RUSI 49445, Kenton-on-Sea, South Africa [4, 8]; *Porcostoma dentata* (AF240730), RUSI 58450, Durban, South Africa [4, 8]; *Pterogymnus laniarius* (AF240731), no voucher, Plettenberg Bay, South Africa [4, 8]; *Rhabdosargus thorpei* (AF240732), RUSI 49683, Ponta do Ouro, Mozambique [4, 8]; *Sparodon durbanensis* (AF240733), RUSI 49673, Kenton-on-Sea, South Africa [4, 8]; *Sparus auratus* (AF240735), ODU 2787, Fiumicino Fish Market, Fiumicino, Italy [1, 2, 9]; *Stenotomus chrysops* (AF240736), no voucher, Chesapeake Bay [3, 6]. Centracanthidae: *Spicara alta* (AF240737), ODU 2793, Angola [1, 4]; *Spicara maena* (AF240738), ODU 2788, Fiumicino Fish Market Fiumicino, Italy [1, 2, 9].

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