Reconstructing labroid evolution with single-copy nuclear DNA

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SUMMARY

Fifteen per cent of all living fishes are united in a single suborder (Labroidei) and display a dazzling array of behavioural and ecological traits. The labroids are considered monophyletic and members share a pharyngeal jaw apparatus (PJA) modified for crushing and processing prey. Outside of the explicitly functional PJA, there is no corroborative evidence for a monophyletic Labroidei. Here, we report the first molecular phylogenetic analysis of the suborder. Contrary to morphology-based phylogenies, our single-copy nuclear DNA data do not support labroid families as a natural group. Our data indicate that pharyngognathy has evolved independently among labroid families and that characters of the PJA are not reliable markers of perciform evolution. This work 'crushes' conventional views of fish phylogeny and should engender novel concepts of piscine life history evolution.

1. INTRODUCTION

With more than 10 000 species in over 200 families, perciform fishes are the largest and most diverse order of vertebrates. The myriad of taxa, coupled with astonishing variation in size, shape, ecology and behaviour have engendered marked disagreement over the phylogenetic relationships of this assemblage (Greenwood *et al.* 1966; Johnson 1993; Johnson & Patterson 1993; Lauder & Liem 1983; Moyle & Cech 1996; Nelson 1994). Perciform fishes possessing a 'crushing' pharyngeal bite have been accorded natural group status since the work of Müller and Cuvier in the 1880s (Rosen & Patterson 1990). Cuvier's arguments about the pre-eminence of function over form (Rieppel 1990) have sculpted neo-Darwinian concepts of evolution and fostered a functional approach to systematics (Kaufman & Liem 1982; Lauder & Liem 1983; Liem & Greenwood 1981). Recently, molecular and analytical advances have permitted the evaluation of functional morphological evolution using phylogenies independent of focal characters (Albert *et al.* 1992; Cunningham *et al.* 1992). Here, nuclear DNA sequence data offer simultaneous tests of fish phylogeny and the systematic utility of functional characteristics.

The wrasses and parrotfishes (Labridae), damselfishes (Pomacentridae), cichlids (Cichlidae) and surfperch (Embiotocidae) collectively account for 15% of all living fishes (Stiassny 1994). These four families form the suborder Labroidei (Kaufman & Liem 1982; Stiassny & Jensen 1987). The variety of ecological and behavioural attributes of this group offers a panoply of unanswered questions to behavioural ecologists, biogeographers and evolutionary biologists. Cichlids, the only freshwater labroid family, are the paradigmatic example of trophic specialization and adaptive radiation (Fryer & Iles 1972; Meyer *et al.* 1990). They occur throughout the old and new world tropics in large species flocks. Surfperch are viviparous, rock-reef fishes whose temperate-limited range is unique among labroids. Wrasses, parrotfishes and damselfishes are arguably the most abundant organisms found in association with circumtropical coral reefs (Sale 1991). These reef fishes display complex social systems (nest building and protection, territoriality, harem keeping, monogamy), change sex (both protandry and protogyny) and form symbiotic relationships (associations with anemones and fishes). The unparalleled diversity in form, function and behaviour perpetuated in this suborder underscores its evolutionary significance.

The remarkable success and taxonomic proteanism of the Labroidei are believed to reside in the key innovation of a pharyngeal jaw apparatus (PJA) modified to allow efficient crushing and processing of prey. This morphological complex is thought to be responsible for the dramatic radiation of these fishes into nearly all aquatic habitats (Jensen 1990; Kaufman & Liem 1982; Stiassny & Jensen 1987). The elements of the PJA also are the primary taxonomic characters uniting the Labroidei (Kaufman & Liem 1982; Stiassny & Jensen 1987) (figure 1). Unfortunately, these traits cannot reliably be used in the construction of evolutionary hypotheses for three substantial but recondite reasons. First, none of the characters are unique to labroid fishes (e.g. an undivided sphincter oesophagi muscle is also present in *Scorpis*, *Kuhlia*, *Toxotes*, *Kyphosus* and *Pholidichthys*

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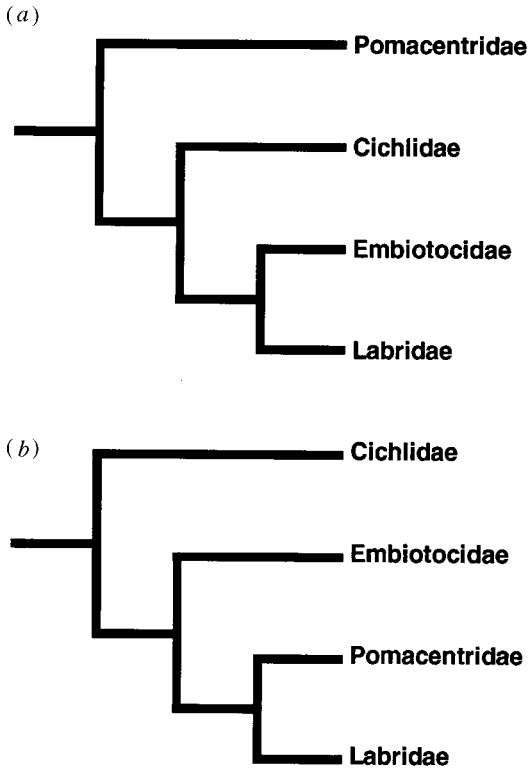

Figure 1. Hypothetical labroid relationships according to (*a*) Kaufman & Liem (1982) and (*b*) Stiassny & Jensen (1987). Characters supporting labroid monophyly are for: (*a*) united or fused fifth ceratobranchials resulting in a single functional unit, true diarthrosis between the upper pharyngeal jaw and the basicranium without an intervening part of the transversus dorsalis anterior muscle, the presence of an undivided sphincter oesophagi muscle; and for (*b*) a neurocranial apophysis of ventrally projecting and rounded form, a muscular sling suspending the lower pharyngeal jaw from the neurocranium, a lower pharyngeal jaw with a well developed ventral keel onto which is inserted a portion of the transversus ventralis VI muscle, presence of a m. transversus pharyngobranchialis II division of the transversus anterior muscle complex, first basibranchial situated partially below the axis of the basihyal and remaining elements of the basibranchial series.

(Johnson 1993)). Second, all labroids do not possess all characters (e.g. the muscular sling suspending the lower pharyngeal jaw is lacking in some pomacentrids (Johnson 1993; Stiassny & Jensen 1987)). Third, there is no independent, corroborative evidence for a monophyletic Labroidei outside of the explicitly functional PJA (Gobalet 1989; Johnson 1993; Lauder 1983; Lauder & Liem 1983; Liem 1973; Liem & Greenwood 1981; Liem & Sanderson 1986; Nelson 1967; Richards & Leis 1984; Stiassny & Jensen 1987). In short, the taxonomic support for this suborder is based solely on the PJA, the function of which is to reduce and process prey. The alternative explanation of parallel evolution in a common environmental regime cannot be excluded without an accurate phylogenetic scaffolding on which to trace behavioural and ecological changes. A discussion of the PJA as a key innovation similarly hinges on a phylogenetic hypothesis independent of pharyngeal characters. To this end, we have examined labroid monophyly with single-copy nuclear DNA (scnDNA) sequence data gathered from a variety of percomorph taxa (table 1).

Table 1. *Taxa of fishes included in this study and geographic information for the Cichlidae*

order: Scorpaeniformes	
Scorpaenidae	*Sebastes* sp.
Cottidae	*Scorpaenichthys marmoratus*
order: Perciformes	
Percidae	*Perca fluviatilis*
Embiotocidae	*Amphistichus rhodoterus*
	Micrometrus minimus
	Damalichthys vacca
Pomacentridae	*Abudefduf saxatilis*
	Dascyllus trimaculatus
	Dascyllus aruanus
Pomacanthidae	*Pomacanthus arcuatus*
Acanthuridae	*Acanthurus chirurgus*
Labridae	*Halichoeres maculipinna*
	Sparisoma chrysopterum (Haiti)
	Sparisoma chrysopterum (Florida)
	Sparisoma radians
Cichlidae:	
India	*Etroplus maculatus*
Madagascar	*Paretroplus polyactis*
	Oxylapia polli
Neotropics	*Astronotus ocellatus*
	Crenicichla saxatilis
West Africa	*Pelvicachromis pulcher*
	Hemichromis bimaculatus
Pan-Africa	*Oreochromis leucostictus*
	Tilapia zillii
	Tylochromis polylepis
East Africa	*Boulengerochromis microlepis*
	Julidochromis regani
	Lamprologus brichardi
	Neolamprologus compressiceps
	Astatotilapia calliptera
	Serranochromis robustus
	Tropheus moorii
	Cunningtonia longiventralis
	Enantiopus melanogenys
	Cyprichromis leptosoma
	Labidochromis caereuleus
	Astatoreochromis alluaudi
	Haplochromis sp.
	Pseudotropheus tropheops

2. MATERIALS AND METHODS

(*a*) *Marker isolation*

Our approach generally follows that of Karl & Avise (1993) with some modifications. A genomic DNA library was constructed from a single individual of *Tropheus moorii* (Cichlidae). The genomic copy number of 272 clones from the library was determined. The cloned insert size was estimated by PCR amplification of the recombinant DNA using M13 sequencing primers (Gussow & Clackson 1989). The average insert size was approx-

imately 1000 base pairs (bp) in length. Of the clones assayed for copy number, 175 (65%) appeared to be single-copy, 71 (26%) low-repetitive, 22 (8%) moderate-repetitive and 4 (1%) high-repetitive genomic elements. Independence of the clones was not assessed. Recombinant plasmid DNA was isolated by 'mini-prep' methods from single-copy clones ranging in size from 800–1000 bp in length (Ausubel *et al.* 1993). The sequences of the first 200–600 nucleotide pairs from both ends of a cloned insert were determined by the dideoxy chain termination method (Sanger *et al.* 1977) using the Sequenase T_7 DNA polymerase sequencing kit (US Biochemicals). Flanking and opposing PCR primers from the ends of the inserts were designed with the aid of the computer program OLIGO 4.0 (National Biosciences, Inc.) and primers were synthesized by CyberSyn Inc. (Camden, NJ). One locus-specific primer pair (Tmo-4C4) successfully amplified DNA for nearly all fish species tested and became the focus of this study. Nucleotide and inferred polypeptide sequences from the Tmo-4C4 clone were used as queries in BLAST searches of GENBANK (Altschul *et al.* 1990; Gish & States 1993) using default parameters. Potential open-reading frames were identified in the nucleotide sequence using the program DNA Strider 1.0 (Commissariat à l'Energie Atomique, France).

(*b*) *scnDNA sequencing*

DNA was extracted from individual fish and then amplified with Tmo-4C4 locus-specific primers. Fifty microlitre amplification reactions contained 2 µl of total cell DNA, 1.5 mM of $MgCl_2$, 1X reaction buffer (Promega), 5 µg bovine serum albumin, 12.5 pmols of each primer and 1.25 units of Taq DNA polymerase (Promega). Cycling parameters were 2 min at 95 °C for one cycle, 30 cycles of 30 s at 95 °C, 30 s at 55 °C and 1 min at 72 °C with a final extension of 7 min at 72 °C. After PCR amplification, 5 µl of the reaction mix were assayed for the amount and fidelity of amplification by agarose gel electrophoresis. Free nucleotides and unused primer molecules from successful amplifications were removed by centrifugal filtration with Millipore Ultrafree-MC (30 000 NMWL) filter units. The purified and concentrated DNA was sequenced according to a two phase sequencing strategy. Sequences were obtained either by direct sequencing of PCR products or by cloning amplified DNA (TA cloning kit; Invitrogen). In both cases, sequencing was performed using an ABI automated sequencer. Sequences for all taxa and Tmo-4C4 primers have been deposited in GENBANK under accession numbers U70326–U70363 and U71186.

(*c*) *Phylogenetic analysis*

Sequences were aligned by eye using the computer program SeqEd (Applied Biosystems). Gaps were included for optimal alignment and to maintain putative open-reading frames. The single region requiring a gap (an amino acid deletion in *Perca*) was treated as missing data in all subsequent analyses. Sequence data were analysed by maximum parsimony using PAUP (heuristic searches with random addition and MULPARS in effect (Swofford 1993)) or the DNAPARS program in PHYLIP (with random addition of sequences (Felsenstein 1989)). Bootstrapping (200 replicates) was performed in PAUP or using the DNAPARS, SEQBOOT, and CONSENSE programs of PHYLIP. Transitions and transversions were assigned either 1:1 or 1:2 weighting. Neighbour-joining analysis using maximum likelihood distances with empirical base frequencies and third codon positions set at a rate category three times that of the first and second positions also was implemented from PHYLIP using the programs DNADIST and NEIGHBOR. The sequences of Tmo-4C4 are biased in favour of adenine (28.44%) and against cytosine (19.68%); maximum likelihood distances are more accurate than the Kimura two-parameter model when nucleotides are not present in equal frequencies. The program MacClade (Maddison & Maddison 1992) was used to estimate tree-based parameters like the number of substitutions at each nucleotide site and the number of changes at first, second and third codon positions. Absolute numbers of transitions (TS) and transversions (TV) for all pairwise comparisons of taxa in table 1 were plotted against corrected genetic distance (maximum likelihood distances as above, DNADIST program of PHYLIP (Felsenstein 1989)).

3. RESULTS

PCR amplifications from individuals in this study produced a single-sized DNA fragment. An open-reading frame extending the entire 511 nucleotides of the Tmo-4C4 locus was identified. The putative reading frame was used to aid in sequence alignment. BLAST searches of the DNA sequence resulted in no probable matches. Searches with the inferred polypeptide, however, revealed several genes of high similarity (e.g. 16 conservative changes and 23 identical amino acids over 78 residues between a region of human titin (Labeit & Kolmerer 1995) and Tmo-4C4). Nearly all significant matches were to muscle-specific proteins (e.g. titin, connectin, a myosin binding protein, twitchin, myosin light-chain kinase, etc.).

Aligned nucleotide sequences for 17 taxa are presented in table 2 with *Tropheus* and *Boulengerochromis* representing the Cichlidae. Other cichlid sequences will be presented in a separate manuscript (J. T. Streelman *et al.*, unpublished data). Weighting of transitions and transversions 1:1 or 1:2 had no effect on the results. Phylogenetic analysis of deduced amino acid sequences produced poor bootstrap support of relationships between families and was not informative at the level of labroid evolution (however, this feature of Tmo-4C4 might be exploited to explore deeper nodes of the fish evolutionary tree). Excluding first and second codon positions from analysis had negligible effects on tree topology but decreased resolution of bootstrapped trees. Third position changes outnumbered first and second position substitutions roughly 5:1:1 when calculated over the most parsimonious trees. The pattern of substitutions in the data did not indicate blocks of sequences differing in their rates of mutation. Numbers of transitions and transversions regressed against corrected genetic distances resulted in linear relationships indicating that substitution saturation has not been reached at Tmo-4C4. Parsimony and distance methods revealed similar tree topologies (figure 2*a*, *b*).

Aspects of the scnDNA phylogenetic tree reproduce previously supported views of perciform evolution. Placement of the Percidae and Pomacanthidae is consistent with convention. The relationships of major groups within the Cichlidae and the grouping

Table 2. *Aligned DNA sequences from select taxa at scnDNA locus Tmo-4C4*

(Sequences from all individuals in table 1 were included in all analyses; here *Tropheus* and *Boulengerochromis* represent the Cichlidae. Dots represent gaps inserted in the sequence to maximize the alignment.)

	22	44	66
Tropheus	ATGAAGAGAAACGTGTTTGAGAACGACACATTAACATTTTATGCTGAGGTGTTTGGCCTACCTTCC		
Boulengerochromis	.....A.....G..T.....		
Sparisoma radians	..T....AG.GA.....A..T..TT.CC.....T.....T		
S. chrysopterum FL	..T....AG.GA.....A..T..AT..C.....T.....T		
S. chrysopterum HT	..T....AG.GA.....A..T..AT..C.....T.....T		
Halichoeres	..A....A..GA.....A..T..GT..C.G.....A..A.....T		
Acanthurus	.....GG.....A..T..TT.GC.C.....A..		
Pomacanthus	..T....A..GA.....A..T...T..C.C.TG.....C.....		
Perca	..T.G..A..GA.....A..T...T.GC....G.....C.....C.....		
Scorpaenichthys	..T.....GA.....T.GC.C.....G.....T		
Amphistichus	..C....A.CGA..C.....A.....T.G..G.TG..C..C..C.....T.....		
Micrometrus	..C....A.CGA..C.....A.....T....G.TG..C..C..C.....T.....		
Damalichthys	..C....A.CGA..C.....A.....T.G..G.TG..C..C..C.....T.....		
Abudefduf	..C....A..GA.....A..T..TT.G..G.....C..C.....G..G...		
Dascyllus trimaculatus	..C....A..GA.....A..T..TT.CC.G.....C.....G.....		
D. aruanus	..C....A..GA.....A..T..TT.TC.G..G.....G.....		
Sebastes	..T....A..GA.....A.C...TT.GC.GG....C..C..C.....C.....		

	88	110	132
Tropheus	CCCGAGGTGAATTGGTTCCGCAACAAAACCCAGCTGTTAGCGGATGACAGAGTTACTATAGAGCGG		
Boulengerochromis	.....G.....		
Sparisoma radians	..T..A..C.GA.....T.....GAG..C.....C.GA.C.GA..G.GAA.A		
S. chrysopterum FL	..T..A..C.GA.....T.....A...GAG..T.....C.GA...GA..G.GAA.A		
S. chrysopterum HT	..T..A..C.GA.....T.....A...GAG..T.....C.GA...GA..G.GAA.A		
Halichoeres	..T.....A..A.....T.T.....T..A...G.G..A.....A.C.TA..G.CAA.A		
Acanthurus	.....C.GA.....T.T.....A...GCG..A..C.....AA..G...A..		
Pomacanthus	..T.....G.....T.....A...G.G..A.....AA..G...A.A		
Perca	..A.....A.....T.....A...G.G..A..C.....AA..G...A..		
Scorpaenichthys	..T.....G.....T.....G..A...G.G..A..C.....AG..G...A.A		
Amphistichus	..G.....G.....T.....G.....G.G..A..C.G...G...GTA.CG...A.A		
Micrometrus	..A.....G.....T.....G.....AG.G..A..C.G...G...GTA.CG...A.A		
Damalichthys	..A.....G.....T.....G.....G.G..A..C.G...G...GTA.CG...A.A		
Abudefduf	..T.....GG.....T.....A...G.G..A..CA.G.....G..G.G.A.A		
Dascyllus trimaculatus	..T.....GG.....T.....A...G.G..T..CAG.....GG.A..G...A.A		
D. aruanus	..T.....GG.....T.....A...G.G..A..CA.....G..A..G...A.A		
Sebastes	..T..A.....G.....T.....A...G.G..A..C.....C...AA..G...A.A		

	154	176	198
Tropheus	GATGGTGACAGCATCTCACTAACAATTCAAAATGTCACTAGAGCTGATCAGGGGGAGTATATTTGT		
Boulengerochromis	.....A.....C		
Sparisoma radians	.....T..C.....T...A....C.AG.....C..C..C		
S. chrysopterum FL	.....T..G.....C...A....C.AG.....A.....C..C		
S. chrysopterum HT	.....T..G.....C...A....C.AG.....A.....C..C		
Halichoeres	.....T.....C.....C.....C.A.....C..A..A.....C...		
Acanthurus	.....G..T.....G.....C.....C.A.....C.....A.....C...		
Pomacanthus	.....T.....G.....G....C.....A.....C.....A.....C		
Perca	.....C..T.....G.....C.....A.A...C..C.....A..A.....C		
Scorpaenichthys	..C..C..T.....G.....G.....C..C....AA.AG..G..C.....A..A.....C...		
Amphistichus	..C..G.....C..C..GG....T.....T...A...C..C.....A.....C..C..C		
Micrometrus	.....G.....T..C..GG....T.....T...A...C.....A.....C..C..C		
Damalichthys	.....G.....C..C..GG....C.....T...A...C..C.....A.....C..C.TC		
Abudefduf	..C.....G.....C..CA....G.AG..C..C.....A..A..C..C...		
Dascyllus trimaculatus	..C.....G..T.....T.C.AG..C..C..A..A..A..C..C...		
D. aruanus	..C.....G..T.....C.....C.AG..C..C..A..A..A..C..C...		
Sebastes	..C..C..T.....G.....C..CA....A.AG..C..C..A..A..A..C..C..C		

of embiotocids with pomacentrids also reflect results of established phylogenies (Stiassny 1991; Stiassny & Jensen 1987; Zardoya *et al.* 1996). The scnDNA data do not, however, corroborate several firmly entrenched perspectives of fish evolution. The sister group relationship of the Acanthuridae with the Labridae is surprising since both families are from distinct suborders (Tyler *et al.* 1989). This relationship is not well supported (43% bootstrap support) and may need to be revised as additional taxa are

Table 2. *(Cont.) Aligned DNA sequences from select taxa at scnDNA locus Tmo-4C4*

	220	242	264
Tropheus	GAAGCTGTGAATTATGTTGGCGAAGCCAGAAGTGTTGCTGTAGTGGTCGTTGTATCACAGGAAGTA		
Boulengerochromis	..........A..		
Sparisoma radians	..G.....C..C.....A....T..G.....T.G.....G..T.....G..C		
S. chrysopterum FL	..G.....C.....A....T..G.....T.G.....T.....G.....C		
S. chrysopterum HT	..G.....C.....A....T..G.....T.G.....T.....G.....C		
Halichoeres	..G..A.....C..C.....A.....G.....C.T.....A.....G.....G		
Acanthurus	..G.....A..C.....C.....C....A..G..C.....G.....G...		
Pomacanthus	..G.....C.....A.....T.....A.....G.....G		
Perca	..G.....C.....C..G.....T.....GA.....G		
Scorpaenichthys	..G.....C..C.....G.....T.....A....A..TG..A.....G		
Amphistichus	.....G.....C..C..C..G..G.....G..C..G..CT.....C..G..T.....G..G		
Micrometrus	..G..G.....C..C..G..G.....G..C..G..CT.....T..C..G..T.....G..G		
Damalichthys	..G..G.....C..C..G.....G..C..G..CT.....C..CC..G..T.....G..G		
Abudefduf	..G.....C.....A.....G..C..C...T.G.....G.....G.....G..G		
Dascyllus trimaculatus	..G.....C.....C..A.....G.....C...T.G.....G.....G.....G..G		
D. aruanus	..G.....C.....C..A.....G.....C...T.G.....T.....G.....G..G		
Sebastes	..G.....C..C..C..G.....C...T.....G.....G		

	286	308	330
Tropheus	AGGTTTACACCGGTCCCACCTGCTGTCTCCCATCAGCACGTCATGAAGTTTGATGTGGAGGAAGAT		
Boulengerochromis	..........C..		
Sparisoma radians	C....C.TG....CT.....G..C...A.....T..G...G.....C		
S. chrysopterum FL	.....C.TG..A.CT.....A.....A.....T..G...G.....C		
S. chrysopterum HT	.....C.TG..A.CT.....A.....A.....T..G...G.....C		
Halichoeres	.....C.TG..A.C.....A.....A.....T..G...G.....		
Acanthurus	.AA.CC.TG..T.C...G.....A.T.....A..T..G...G.....A.....C		
Pomacanthus	..A..C.TG..C.C.....A.....T..G...G.....A.....C		
Perca	.....CCTG..T.C...T.....A.....G...G...G.....---...		
Scorpaenichthys	.....C.TG..T.CT.....A.....T..G...G.....C.....		
Amphistichus	.....C.TG....CT.....C...A.....G...G....C.....G..C		
Micrometrus	.....C.TG....CT.....C...A.....G...G....C.....G..C		
Damalichthys	.....C.TG....CT.....C...A.....G.C.G.A..C.....G..C		
Abudefduf	.....C.TG....CT.....C..TA.....G...G.....G..C		
Dascyllus trimaculatus	.....CCTG..A.CT.....GC...A.....T..G...G....C.....G...		
D. aruanus	.....CCTG..T.CT.....C...A.....T..T...G....C.....G...		
Sebastes	.....C.TG..T.C...G.....C...A.....T..G...G.....G..C		

	352	374	396
Tropheus	GACTCTTCTCGTTCACCATCTCCTCAAGAGATTCTGCTTGAAGTAGAGCTGGATGAAAATGAAGTC		
Boulengerochromis	.....T.....		
Sparisoma radians	.....G.....T..T..A..G..G..A..C.....G..G...T.A.....G..G		
S. chrysopterum FL	.....G.....T..T..A..G..G..A.....G.....AT.A.....G..G		
S. chrysopterum HT	.....G.....T..T..A..G..G..A.....G.....AT.A.....G..T		
Halichoeres	.....T..T..C..A.....A.....T..A.....C..G..G		
Acanthurus	.....A.....T.....C.....G..A.....T.A.....C..G..G		
Pomacanthus	.....T..T..C.....G.....A.....C.....A.....G..G		
Perca	.....T..C..A.....G.....C.....AT.....C..G..G		
Scorpaenichthys	.....G..T..T..C.....G..A.....C.....A.....T.C..G..G		
Amphistichus	.....C.....C..T.....G.....C.....G.....G..A.....C..G..G		
Micrometrus	.....C.....C..T.....C.....G.....C.....G.....G..A.....C..G..G		
Damalichthys	.....C.....C..T.....C.....G.....C.....G.....G..A.....C.....G		
Abudefduf	.....C.....T.....C.....G.....C.....G..A.....G..G		
Dascyllus trimaculatus	..T..C.....T..G..A.....G.C.....C.....G..A.....G..C..G..G		
D. aruanus	..T..C.....T.....C.....G.....G.....G..A.....G..C..G..G		
Sebastes	.....T..G.....G.....G.....G..A.....C..C..G		

added to the tree. Embiotocids and pomacentrids do not group with labrids and cichlids as previously believed (Stiassny & Jensen 1987). These families not only fail to cluster with other members of the suborder Labroidei (rejecting monophyly), but appear as basal members of the tree along with fishes of an entirely different order (cottids and scorpaenids). This relationship is upheld in both parsimony and distance analyses. Ironically, we included the cottids and scorpaenids as outgroup taxa. The association of these fishes with the embiotocids and pomacentrids markedly contradicts traditional taxonomic understanding (Lauder & Liem 1983; Moyle & Cech 1996) and suggests that both Scorpaeniformes and

Table 2. *(Cont.) Aligned DNA sequences from select taxa at scnDNA locus Tmo-4C4*

	418	440	462
Tropheus	AAAGAATTTGAGAAACAGGTGAAGATCATCACCATACCTGAATACACAGCTGACAATAAGAGTATG		
Boulengerochromis	.....		
Sparisoma radians	.....G.....T.....T.....C.....C.....C...		
S. chrysopterum FL	.....T.....T.....C.....C.G.....		
S. chrysopterum HT	.....G.....T.....T.....C.....C.....		
Halichoeres	.....C..G..G.....G..A.....C...		
Acanthurus	.....A..C.....T.....C.....G.....C.....C...		
Pomacanthus	.....G.....T.....T..T.....T.....G.....C.....C.....C...		
Perca	.....G...A..T.....T..T.....G.....C.....C..A..C...		
Scorpaenichthys	..G.....T..T.....T..A..G.....C..C.....C.....C...		
Amphistichus	.....G.....G.....T..T.....G.....G..C.....C.....C...		
Micrometrus	.....G.....G.....T..T.....G.....G..C.....C.....C...		
Damalichthys	.....G.....G.....T..T.....G.....G..C.....C.....C...		
Abudefduf	.....C.....G.....T.....T.....G.....G..C.....C.....C...		
Dascyllus trimaculatus	.....G.....C.....T.....G.....G..C.....C.....C...		
D. aruanus	...A.G...A....G..A..C.....AT.....G.....G..C.....C.....C...		
Sebastes	.....G.....T.....T..C..G.....G.....C.....C...		

	484	506
Tropheus	ATCATATCTCTGAATGTGTTACCAAGTATTTATGAGGAGGGTGCTGTGG	
Boulengerochromis	.....G.....	
Sparisoma radians	.....T..A...G....T.....G.....C.....G..C....	
S. chrysopterum FL	.....T..A...G....T.....G.....C.....G..C....	
S. chrysopterum HT	.....T..A...G....T.....G.....C.....G..C....	
Halichoeres	.....T..G....A.....A.....A.....A..A.	
Acanthurus	..T..T...T..G.....CC.G.....A.	
Pomacanthus	.....T..G.....C.....C.....A.C.....T.	
Perca	.....T..G....T.....G.....C..C....	
Scorpaenichthys	.....T.AG.....G.....A.....C..C....	
Amphistichus	.....G.....G...C.C..C.....C....	
Micrometrus	.....G.....G...C.C..C.....C..C....	
Damalichthys	.....G.....G...C.C..C.....C.T.....	
Abudefduf	.....C.....G.C.....G..G.....C.	
Dascyllus trimaculatus	G....C.....G.....G..G.....C.....	
D. aruanus	.....C.....G.....C.G..G.....C.....	
Sebastes	.....T..G.....C.G..G.....C.....C..C.G..	

Perciformes may be paraphyletic (Johnson & Patterson 1993). Nonetheless, additional outgroup taxa are necessary before a firm conclusion can be drawn concerning the monophyly of these orders.

4. DISCUSSION

Phylogenetic results from scnDNA locus Tmo-4C4 challenge central tenets of labroid evolution and the traditional methods used to reconstruct it. Our data indicate that a modified PJA has developed independently among families of the taxon Labroidei. The multiple evolution of this character suite weakens the hypothesis that the PJA is a key innovation explaining diversity in the suborder Labroidei. Characters of the PJA do not appear to be accurate markers of labroid evolution, although convergence in this functional complex is no less exciting. Because other perciform fishes share pharyngeal characters with the labroids, it has been suggested (Liem & Greenwood 1981; Nelson 1967; Stiassny & Jensen 1987) that labroid families are part of a larger pharyngognathous assemblage. It could be argued that a proper test of labroid monophyly should include fishes from other families thought to be pharyngognathous teleosts (e.g. Gerreidae, Sparidae, Kyphosidae). We counter with the notion that the present study is not only a test of labroid monophyly, but also an evaluation of functionally important morphological characters (i.e. the PJA) in determining perciform phylogeny. By sampling non-pharyngognathous taxa we present a more extreme examination of both conventions. Due to the apparent flexibility of the PJA in ontogeny (Smits *et al.* 1996) and now evolution, a re-evaluation of other fish clades defined by similar functional characters (e.g. the neoteleosts (Lauder & Liem 1983) and sunfishes of the genus Lepomis (Mabee 1993)) may be appropriate. Furthermore, the nuclear DNA-based phylogeny suggests that several ecological characteristics such as nest building, sex reversal and territoriality appear to have evolved convergently among labroid groups.

Although surprising, these results are not explained by substitution saturation, since both transitions and transversions accrue linearly with increasing corrected genetic distance. Furthermore, experimental weighting of transversions up to five times

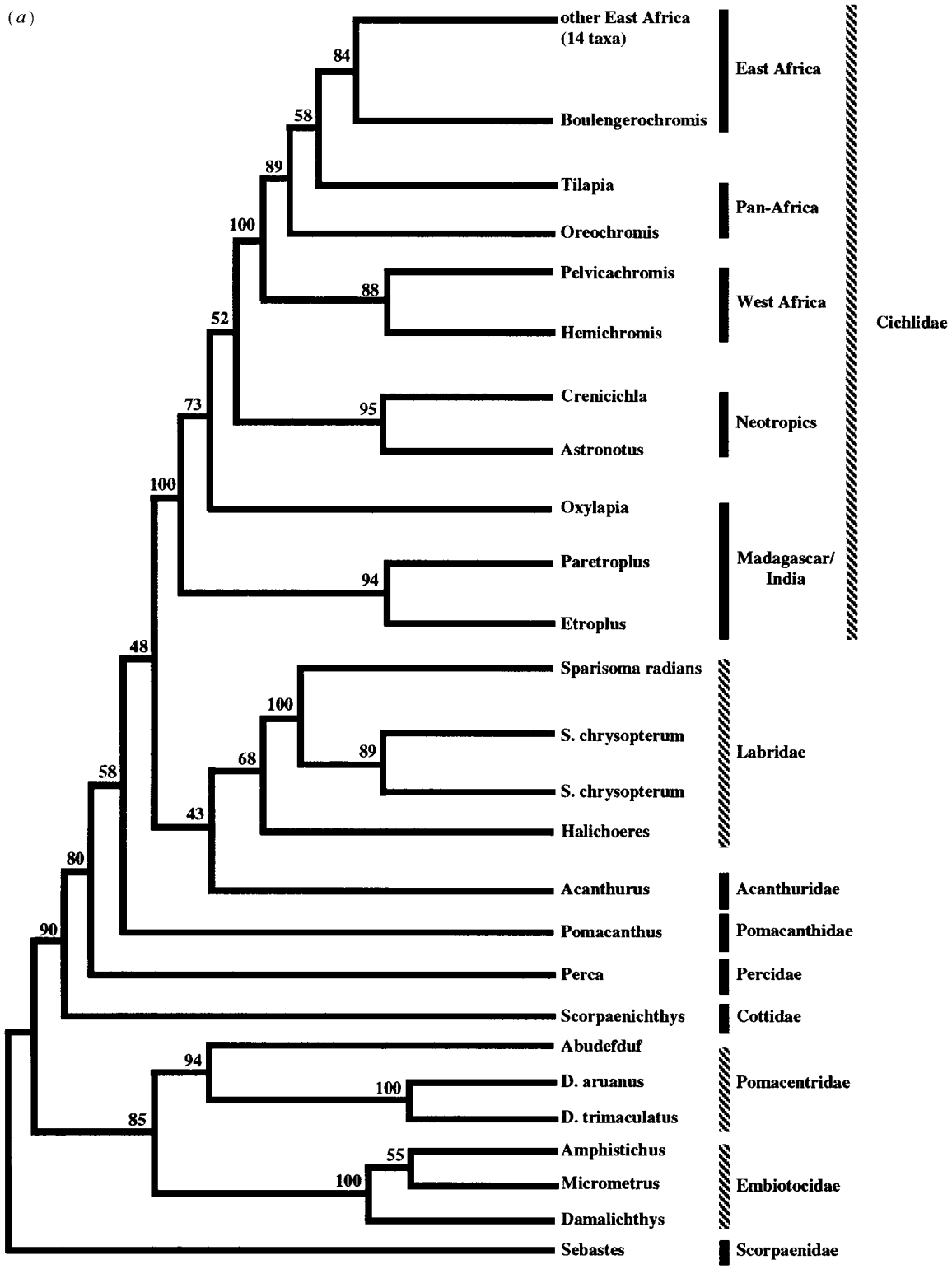

Figure 2. (*a*) One of two most parsimonious trees showing familial and cichlid major group relationships. *Sebastes* (Scorpaenidae) was used as outgroup. The two most parsimonious trees differed only in the relationships among the three embiotocid taxa. Numbers at nodes represent bootstrap values from the parsimony analysis (200 replicates; PAUP (Swofford 1993)). Labroid families are indicated by cross-hatched vertical bars.

that of transitions has no effect on labroid familial relationships. We can discount selection on amino acid sequence as an explanation for the phylogenetic pattern because using substitutions at third codon positions only (i.e. synonymous substitutions) does not alter tree topology among families. DNA sequence data from the mitochondrial COI gene from representatives of each family corroborate our intrafamilial groupings, but provide no support for nodes relevant to labroid evolution (J. T. Streelman, unpublished data). The lack of resolution at 50–150 million years may be a general feature of mitochondrial cod-

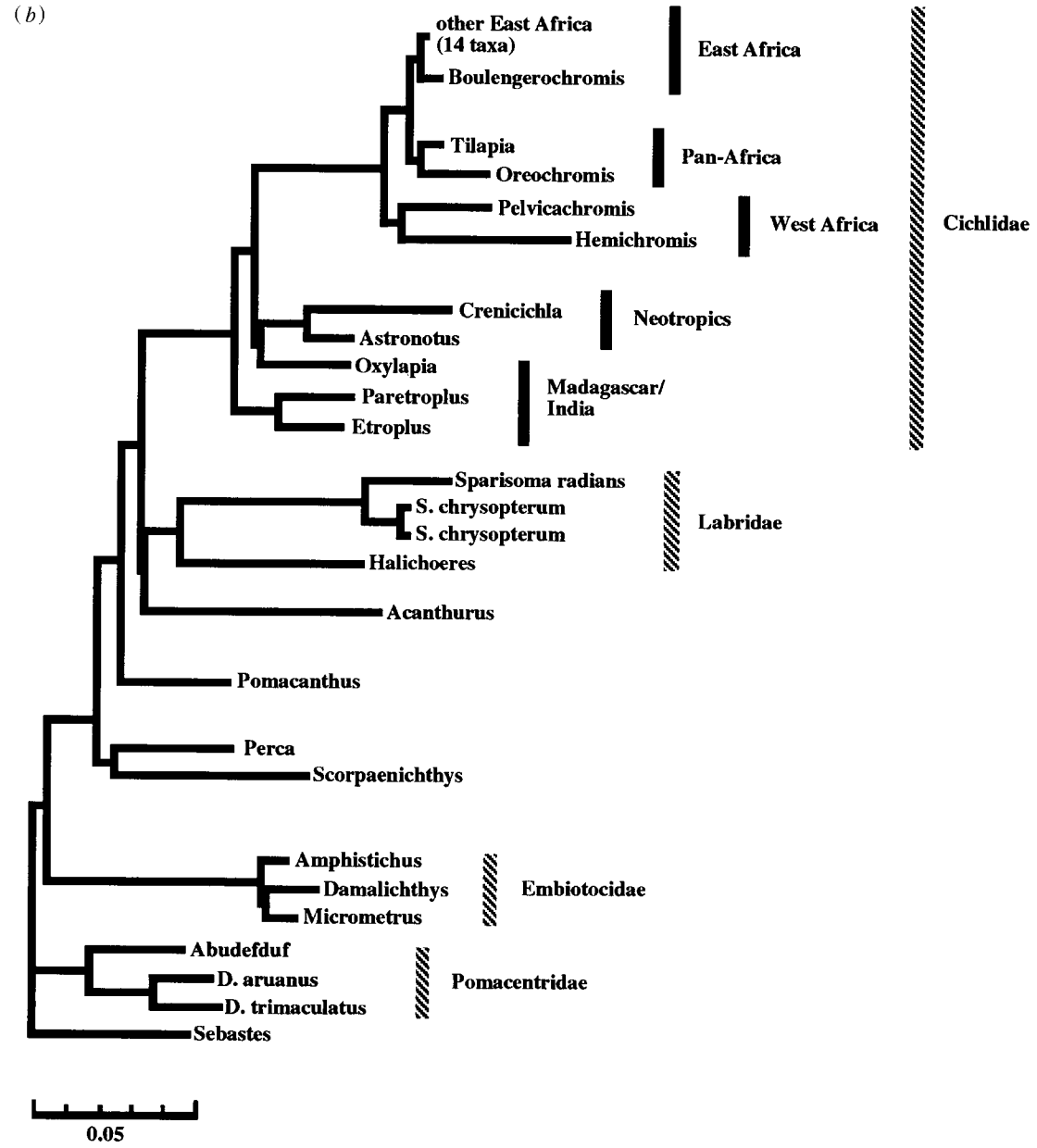

Figure 2. (*b*) Neighbour joining tree constructed from maximum likelihood distances using the DNADIST and NEIGHBOR programs of PHYLIP (Felsenstein 1989). *Sebastes* was used as outgroup. Empirical base frequencies were used in this analysis and the rate of third position substitutions was set to three times first and second position changes. Scale indicates maximum-likelihood distance. Note the position of the Embiotocidae and *Oxylapia*. Labroid families are indicated as in figure 2*a*.

ing regions where third position mutation saturation has been reached in a background of amino acid conservation (Cantatore *et al.* 1994; Meyer 1994). That the scnDNA data support previous hypothetical relationships of cichlid major groups thought to have diverged at least 75 million years ago (Lundberg 1993; Stiassny 1991; Zardoya *et al.* 1996) further substantiates the use of this nuclear locus to investigate deep divergence among perciform taxa. Unfortunately, the lack of independent corroborating data from COI (or any other locus) forces us to draw cautious conclusions from this single-gene genealogy.

These results have manifold implications for perciform phylogeny. The fossil record has been a difficult cipher of perciform relationships, revealing an uninterpretable explosion of taxa between 50 and 75 million years ago (Bellwood 1996; Carroll 1988; Patterson 1993). Molecular hypotheses of teleost phylogeny are few (Bernardi *et al.* 1993; Cantatore *et al.* 1994) and have not addressed perciform interfamilial relationships. It is possible that much of perciform evolution has gone unrecorded by the fossil record, a prospect consistent with recent molecular analyses of other taxonomic radiations (Hedges *et al.* 1996; Wray *et al.* 1996). The ultimate resolution of perciform phylogeny will require continuing effort by systematists (molecular as well as morphological), paleontologists and ecologists, with perhaps greater attention given to the biogeography and natural history of this diverse array of fishes.

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REFERENCES

Albert, V. A., Williams, S. E. & Chase, M. W. 1992 Carnivorous plants: phylogeny and structural evolution. *Science* **257**, 1491–1495.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. 1990 Basic local alignment search tool. *J. Molec. Biol.* **215**, 403–410.

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., Struhl, K., Wang-Iverson, P. & Bonitz, S. G. 1993 *Current protocols in molecular biology*. New York: Greene Publishing Associates and Wiley-Interscience.

Bellwood, D. R. 1996 The Eocene fishes of Monte-Bolca the earliest coral-reef fish assemblage. *Coral Reefs* **15**, 11–19.

Bernardi, G., D'Onofrio, G., Caccio, S. & Bernardi, G. 1993 Molecular phylogeny of bony fishes, based on the amino acid sequence of the growth hormone. *J. Molec. Evol.* **37**, 644–649.

Cantatore, P., Roberti, M., Pesole, G., Ludovico, A., Milella, F., Gadaleta, M. N. & Saccone, C. 1994 Evolutionary analysis of cytochrome *b* sequences in some Perciformes: evidence for a slower rate of evolution than in mammals. *J. Molec. Evol.* **39**, 589–597.

Carroll, R. L. 1988 *Vertebrate paleontology and evolution*. New York: Freeman.

Cunningham, C. W., Blackstone, N. W. & Buss, L. W. 1992 Evolution of king crabs from hermit crab ancestors. *Nature* **355**, 539–542.

Felsenstein, J. 1989 PHYLIP—phylogeny inference package (version 3.2). *Cladistics* **5**, 164–166.

Fryer, G. & Iles, T. D. 1972 *The cichlid fishes of the great lakes of Africa: their biology and evolution*. Edinburgh: Oliver and Boyd.

Gish, W. & States, D. J. 1993 Identification of protein coding regions by database similarity search. *Nat. Genet.* **3**, 266–272.

Gobalet, K. W. 1989 Morphology of the parrotfish pharyngeal jaw apparatus. *Am. Zool.* **29**, 319–331.

Greenwood, H. P., Rosen, D. E., Weitzman, S. H. & Myers, G. S. 1966 Phyletic studies of teleostean fishes, with a provisional classification of living forms. *Bull. Am. Mus. Nat. Hist.* **131**, 339–456.

Gussow, D. & Clackson, T. 1989 Direct clone characterization from plaques and colonies by the polymerase chain reaction. *Nucl. Acids Res.* **17**, 4000–4001.

Hedges, S. B., Parker, P. H., Sibley, C. G. & Kumar, S. 1996 Continental breakup and the ordinal diversification of birds and mammals. *Nature* **381**, 226–229.

Jensen, J. S. 1990 Plausibility and testability: assessing the consequences of evolutionary innovation. In *Evolutionary innovation* (ed. M. H. Nitecki), pp. 171–190. Chicago, IL: University of Chicago Press.

Johnson, G. D. 1993 Percomorph phylogeny: progress and problems. *Bull. Mar. Sci.* **52**, 3–28.

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

Karl, S. A. & Avise, J. C. 1993 PCR-based assays of Mendelian polymorphisms from anonymous single-copy nuclear DNA: techniques and applications for population genetics. *Molec. Biol. Evol.* **10**, 342–361.

Kaufman, L. S. & Liem, K. F. 1982 Fishes of the suborder Labroidei (Pisces: Perciformes): phylogeny, ecology and evolutionary significance. *Brevortia* **472**, 1–19.

Labeit, S. & Kolmerer, B. 1995 Titins: giant proteins in charge of muscle ultrastructure and elasticity. *Science* **270**, 293–296.

Lauder, G. V. 1983 Functional design and evolution of the pharyngeal jaw apparatus in euteleostean fishes. *J. Zool. Lin. Soc.* **77**, 1–38.

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

Liem, K. F. 1973 Evolutionary strategies and morphological innovations: cichlid pharyngeal jaws. *Syst. Zool.* **22**, 425–441.

Liem, K. F. & Greenwood, P. H. 1981 A functional approach to the phylogeny of the pharyngognath teleosts. *Am. Zool.* **21**, 83–101.

Liem, K. F. & Sanderson, S. L. 1986 The pharyngeal jaw apparatus of labrid fishes: a functional morphological perspective. *J. Morph.* **187**, 143–158.

Lundberg, J. G. 1993 African–South America freshwater fish clades and continental drift: problems with a paradigm. In *Biological relationships between Africa and South America* (ed. P. Goldblatt), pp. 156–199. New Haven, CT: Yale University Press.

Maddison, W. P. & Maddison, D. R. 1992 *MacClade: analysis of phylogeny and character evolution*, v. 3.0. Sunderland, MA: Sinauer Associates.

Mabee, P. M. 1993 Phylogenetic interpretation of ontogenetic change: sorting out the actual and artifactual in an empirical case study of centrarchid fishes. *J. Zool. Lin. Soc.* **107**, 175–291.

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

Meyer, A., Kocher, T. D., Basasibwaki, P. & Wilson, A. C. 1990 Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature* **347**, 550–553.

Moyle, P. B. & Cech, J. J. 1996 *Fishes: an introduction to ichthyology*. Englewood Cliffs, NJ: Prentice Hall.

Nelson, G. 1967 Gill arches of some teleostean fishes of the families Girellidae, Pomacentridae, Embiotocidae, Labridae, and Scaridae. *J. Nat. Hist.* **1**, 289–293.

Nelson, J. S. 1994 *Fishes of the world*. New York: Wiley.

Patterson, C. 1993 An overview of the early fossil record of acanthomorphs. *Bull. Mar. Sci.* **52**, 29–59.

Richards, W. J. & Leis, J. M. 1984 Development and relationships. In *Ontogeny and systematics of fishes* (ed. H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendals & S. L. Richardson), pp. 542–547. American Society of Ichthyologists and Herpetologists.

Rieppel, O. 1990 Structuralism, functionalism, and the four Aristotelian causes. *J. Hist. Biol.* **23**, 291–320.

Rosen, D. E. & Patterson, C. 1990 On Muller's and Cuvier's concepts of the pharyngognath and labyrinth fishes and the classification of perciform fishes, with an atlas of percomorph dorsal gill arches. *Am. Mus. Nov.* **2983**, 1–57.

Sale, P. F. 1991 *The ecology of fishes on coral reefs*. New York: Academic.

Sanger, F., Miklen, S. & Coulson, A. R. 1977 DNA sequencing with chain terminating inhibitors. *Proc. Natn. Acad. Sci. USA* **74**, 5463.

Smits, J. D., Witte, F. & Povel, G. D. E. 1996 Differ-

ences between interspecific and intraspecific architectonic adaptations to pharyngeal mollusk crushing in cichlid fishes. *Biol. J. Linn. Soc.* **59**, 367–387.

Stiassny, M. L. J. 1991 Phylogenetic intrarelationships of the family Cichlidae: an overview. In *Cichlid fishes—behavior, ecology and evolution* (ed. M. H. A. Keenleyside), pp. 1–31. London: Croom Helm.

Stiassny, M. L. J. 1994 A tangled web: cichlids and their relatives. *The Cichlids Yearbook* **4**, 91–94.

Stiassny, M. L. J. & Jensen, J. S. 1987 Labroid interrelationships revisited: morphological complexity, key innovations, and the study of comparative diversity. *Bull. Mus. Comp. Zool.* **151**, 269–319.

Swofford, D. L. 1993 *PAUP: phylogenetic analysis using parsimony*, version 3.1.1. Champagne, IL: Illinois Natural History Survey.

Tyler, J. C., Johnson, G. D., Nakamura, I. & Collette, B. B. 1989 Morphology of *Luvarus imperialis* (Luvaridae), with a phylogenetic analysis of the Acanthuroidei (Pisces). *Smith. Cont. Zool.* **485**, 1–78.

Wray, G. A., Levinton, J. S. & Shapiro, L. H. 1996 Molecular evidence for deep precambrian divergences among metazoan phyla. *Science* **274**, 568–573.

Zardoya, R., Vollmer, D. M., Craddock, C., Streelman, J. T., Karl, S. A. & Meyer, A. 1996 Evolutionary conservation of microsatellite flanking regions and their utility in resolving the phylogeny of cichlid fishes (Pisces: Perciformes). *Proc. R. Soc. Lond.* B **263**, 1589–1598.

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