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Evolution of Trophic Types in Emperor Fishes (*Lethrinus,* Lethrinidae, Percoidei) Based on Cytochrome b Gene Sequence Variation

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Abstract. Three trophic categories exist within emperor fishes, genus *Lethrinus,* relating to body form and dentition type. One group contains low-bodied, high speed, stalking predators with conical teeth. Another group comprises high-bodied, slow speed carnivores with molariform teeth capable of crushing hard-shelled benthic prey. A third group is also high-bodied but with conical teeth feeding mostly on small or soft-shelled benthic prey. Inferring the evolution of these trophic types within *Lethrinus* using morphology is problematic since these characters are typically correlated with feeding mode and are potentially homoplasious. We use mitochondrial DNA sequences, to independently determine a phylogenetic hypothesis for *Lethrinus,* which are not dependent on morphological characters relating to trophic categories. We analyzed complete cytochrome b gene sequences (1140 bp) for 20 species of *Lethrinus,* representing the three trophic types, and for 13 outgroup species, including four other representatives of the Lethrinidae. A monophyletic Lethrinidae did not resolve, but the monophyly of *Lethrinus* is well supported. In addition, two major clades within *Lethrinus* are well supported. One of these clades exclusively contains low-bodied species with conical teeth while the other clade only comprises the high-bodied species with molariform teeth. A high-bodied species with conical teeth, *Lethrinus miniatus,* appears most ancestral and sister to all other *Lethrinus* species. We hypothesize that this generalist trophic type was the evolutionary precursor to both of the other primary trophic types.

Key words: Cytochrome b — Molecular phylogeny — Trophic evolution — Lethrinidae — *Lethrinus*

Introduction

The emperor fishes and large-eye breams (Lethrinidae) belong to the suborder Percoidei and have been classified into the superfamily Sparoidea together with the Nemipteridae, Sparidae, and Centracanthidae (Johnson 1981). Akazaki (1962), Johnson (1981), and Carpenter and Johnson (in press) reviewed the evolutionary relationships of these families based on morphological characters and the phyletic sequence Nemipteridae, Lethrinidae, and Sparidae plus Centracanthidae is presently supported. The Lethrinidae is subdivided into the Lethrininae and Monotaxinae based on head scalation patterns and dorsal- and anal-fin ray counts (Carpenter and Allen 1989). *Lethrinus,* with 29 species, comprises the Lethrininae while *Gnathodentex, Gymnocranius, Monotaxis,* and *Wattsia* comprise the Monotaxinae. The monotaxine genera are monotypic with the exception of *Gymnocranius,* which contains eight species.

Lethrinids have strong jaws and their dentition and

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Table 1. Trophic categories, GenBank accession numbers, collection data, and museum catalogue numbers for specimens.

Taxa	Dentition	Body Form	GenBank Accession Number	Collection Locality	Catalogue Number
Lethrininae					
Lethrinus atkinsoni	M	H	AF381255	Philippines	USNM 346713
Lethrinus atlanticus	C	H	AF381264	Ghana	ODU 2990
Lethrinus borbonicus	M	H	AF381268	Kuwait	ODU 2991
Lethrinus erythracanthus	C	H	AF381257	Philippines	USNM 340524
Lethrinus erythropterus	M	H	AF381274	Philippines	USNM 346706
Lethrinus genivittatus	C	L	AF381272	Philippines	AMS uncat.
Lethrinus harak	M	H	AF381258	Philippines	USNM 346850
Lethrinus laticaudis	M	H	AF381251	Australia	AMS I.36447-006
Lethrinus lentjan	M	H	AF381267	Australia	AMS uncat.
Lethrinus microdon	C	L	AF381265	Philippines	USNM 340523
Lethrinus miniatus	C	H	AF381266	Philippines	USNM 345991
Lethrinus nebulosus	M	H	AF381269	Australia	AMS I.36447-010
Lethrinus obsoletus	M	H	AF381271	Philippines	USNM 347032
Lethrinus olivaceus	C	L	AF381252	Philippines	USNM 345988
Lethrinus ornatus	M	H	AF381254	Philippines	USNM 345261
Lethrinus reticulatus	C	L	AF381253	Philippines	USNM 340558
Lethrinus rubrioperculatus	C	L	AF381263	Philippines	USNM 346714
Lethrinus semicinctus	C	L	AF381276	Philippines	USNM 346753
Lethrinus sp. 2	C	L	AF381262	Australia	AMS uncat.
Lethrinus sp. 3	M	H	AF381256	Australia	AMS uncat.
Monotaxinae					
Gymnocranius elongatus	C	L	AF381260	Australia	AMS uncat.
Gymnocranius grandoculis	C	L	AF381275	Australia	AMS uncat.
Gymnocranius griseus	C	L	AF381259	Japan	NSMT uncat.
Wattsia mossambica	C	H	AF381261	Australia	AMS I.36447-005

Museum acronyms are as follows: AMS, Australian Museum of Sydney; NSMT, National Science Museum, Tokyo; ODU, Old Dominion University Ichthyological Laboratory Museum; USNM, United States Natural History Museum.

Dentition abbreviations are as follows: C = conical, M = molariform and submolariform. Body type abbreviations are H = high-bodied and L = low-bodied.

Collection data and museum catalogue numbers for outgroups are presented in Orrell (2000).

body form reflect feeding specificity (Carpenter 1996). Species of *Lethrinus* are demersal feeders with three distinct trophic modes. One group consists of low-bodied species with conical lateral teeth. These are stalking or lie-in-wait predators (mesocarnivore stalkers) that mostly feed on mobile prey such as fishes and certain crustaceans. The other group contains high-bodied species with molariform (or submolariform, the "intermediate" of Carpenter, 1996) teeth. These "mesocarnivore specialists" typically consume slow moving invertebrates. Molariform teeth allow these species to process a wide range of benthic invertebrates including both hard and soft-shelled prey such as mollusks, sea urchins, and some crustaceans. The remaining trophic type exhibits high-bodied species with conical teeth. These "mesocarnivore generalists" typically consume slow moving invertebrates and since they lack molars, are limited to soft-shelled or small hard-shelled invertebrates. The evolution of trophic types has never been examined within the family Lethrinidae.

Few studies have examined the evolution of trophic types in closely related fishes since the morphological characters required to infer phylogeny often rely on features such as dentition and body shape, which are correlated with feeding type. Molecular data allows for phylogenetic inference independent of morphology. Based on mitochondrial 16S rDNA from a subset of representatives of the Sparidae, Hanel and Sturmbauer (2000) hypothesized that the same trophic types evolved multiple times within this family. Orrell et al. (in press) generated a comprehensive phylogeny of Sparidae genera using complete cytochrome b sequences. They demonstrated that the subfamilies of Sparidae, which were previously described based on trophic type, are not monophyletic. Carpenter (1996) studied the relationship of dentition, body shape, and feeding mode of species of *Lethrinus* and suggested that examining an independent data set such as an ontogenetic series may provide clues to the evolution of these feeding types. For *Lethrinus* in particular, an independent data set is necessary to construct a phylogeny since the morphological characters are very conservative and simple identification of species is often problematic (Carpenter and Allen 1989). The purpose of this paper is to examine the evolution of the three primary feeding types in 20 species of *Lethrinus* using complete cytochrome b gene sequences. The cytochrome b gene has proved useful for phylogenetic inference in Actinopterygian fishes in general (Lydeard

Table 2. Percent mean uncorrected pairwise genetic distances among all species. Column numbers correspond to species rows.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 *Lethrinus harak*																
2 *Lethrinus laticaudis*	10															
3 *Lethrinus species 3*	10	9														
4 *Lethrinus nebulosus*	9	10	9													
5 *Lethrinus lentjan*	10	11	9	8												
6 *Lethrinus ornatus*	10	12	10	8	7											
7 *Lethrinus obsoletus*	11	11	10	10	10	10										
8 *Lethrinus borbonicus*	14	14	13	12	11	13	13									
9 *Lethrinus atkinsoni*	12	12	11	11	10	12	12	10								
10 *Lethrinus genivittatus*	16	16	17	16	16	17	16	17	16							
11 *Lethrinus atlanticus*	16	15	15	14	15	15	15	14	14	17						
12 *Lethrinus reticulatus*	16	16	16	16	15	16	16	15	16	18	16					
13 *Lethrinus rubrioperculatus*	16	15	16	15	16	16	15	15	16	18	16	9				
14 *Lethrinus microdon*	16	16	15	14	15	15	16	15	15	17	16	11	11			
15 *Lethrinus olivaceus*	17	16	16	16	16	16	16	16	17	18	16	14	13	12		
16 *Lethrinus semicinctus*	15	15	15	15	15	15	15	15	14	17	15	13	13	13	13	
17 *Lethrinus species 2*	15	16	15	15	15	15	16	16	14	15	16	15	15	13	14	10
18 *Lethrinus erythropterus*	16	17	15	16	14	15	16	17	16	17	16	16	16	15	16	16
19 *Lethrinus erythracanthus*	16	15	14	14	14	14	14	15	14	16	16	14	13	13	15	13
20 *Lethrinus miniatus*	17	17	16	15	16	17	17	18	17	19	18	17	17	17	17	16
21 *Gymnocranius grandoculis*	22	22	21	21	21	21	20	21	21	21	21	21	21	21	21	21
22 *Gymnocranius griseus*	21	21	21	20	20	21	21	21	21	21	20	21	21	21	21	21
23 *Gymnocranius elongatus*	23	22	21	22	21	21	21	21	21	22	21	24	23	22	23	22
24 *Wattsia mossambica*	20	21	21	21	20	20	21	22	21	22	22	22	22	22	21	21
25 *Scolopsis ciliatus*	24	24	23	22	23	23	23	23	23	23	24	22	22	22	22	21
26 *Nemipterus marginatus*	23	24	23	23	23	23	24	24	24	24	24	25	24	24	24	24
27 *Dentex tumifrons*	22	22	21	22	23	22	21	21	23	22	22	22	22	21	22	22
28 *Sparus auratus*	23	23	22	22	22	23	23	22	22	23	24	23	23	23	22	22
29 *Lutjanus decussatus*	19	20	18	18	18	19	19	20	19	20	19	19	19	19	21	18
30 *Dipterygonotus balteatus*	21	21	20	20	21	21	21	21	20	20	21	20	20	21	21	20
31 *Caesio caerulaurea*	20	20	20	20	19	20	20	21	20	21	21	19	20	20	21	20
32 *Morone americana*	23	24	22	23	23	23	24	23	25	24	24	24	23	24	24	23
33 *Morone saxatilis*	22	24	22	23	23	22	22	24	22	22	24	25	23	24	24	23

and Roe 1997) and within families of percoid fishes (Allegrucci et al. 1999; Orrell et al. in press).

Materials and Methods

Table 1 shows the trophic information, GenBank Accession Numbers, and collection data for the sequenced lethrinids. *Lethrinus sp. 2* and *Lethrinus sp. 3* are undescribed species (Carpenter 2001). Collection data and cytochrome b sequences for the outgroups *Lutjanus decussatus* (Lutjanidae), *Morone americana,* and *Morone saxatilis* (Moronidae), *Scolopsis ciliatus* and *Nemipterus marginatus* (Nemipteridae), and *Dentex tumifrons* and *Sparus auratus* (Sparidae) were provided by Orrell (2000). The GenBank accession numbers for these respective outgroup species are as follows: AF240750, AF240744, AF240746, AF240753, AF240754, AF240708, and AF240735. Two caesionid species, collected in the Philippines, were sequenced and also served as outgroup taxa. Accession numbers and museum catalogue numbers for *Caesio caerulaurea* are AF381273 and USNM 344393, respectively; and for *Dipterygonotus balteatus,* AF381270 and USNM uncatalogued, respectively. Muscle or gill tissue was collected for lethrinids and caesionids between 1995 and 2000 and preserved in a dimethyl sulfoxide (DMSO)-salt solution as described by Seutin et al. (1991). Tissues were digested in 500 μl of Isolation Buffer (50 mM EDTA, 50 mM Tris, 150 mM NaCl), 60 μl SDS (10%), 10 μl of RNAse (10 mg/ml), and 10 μl of proteinase K (25 mg/ml) for 24 h at 37°C. SDS was precipitated with 8M KoAc. DNA was extracted by a phenol chloroform method as described in Sambrook et al. (1989). DNA was precipitated with ethanol (100%), washed with ethanol (70%), and re-suspended in sterile TE buffer.

The Gibco BRL® PCR Reagent System (Life Technologies) was utilized for amplification reactions. PCR master mix protocol and PCR primer sequences L14249 and H16435 were provided by Orrell (2000). PCR was performed on a MJ MiniCycler™ with the following parameters; initial denaturation (95°C for 5 min, 94°C for 1 min); 35 cycles of (denaturation 94°C for 1 min, annealing 45°C for 1.0 min, extension 65°C for 3.0 min); and final extension (65°C for 7 min). Amplification products were purified with the QIAquick™ PCR Purification kit (Qiagen). PCR amplifications from *Lethrinus nebulosus, Gymnocranius grandoculis,* and *Lethrinus semicinctus* were cloned using Invitrogen's Topo-TA® cloning kit for sequencing to generate primers for cycle sequencing. From the remaining PCR amplifications, approximately 200 ng of double-stranded PCR product was used in cycle sequencing reactions as outlined in the ABI Prism BigDye® sequencing kit (PE Applied Biosystems). The following primers were used in cycle sequencing reactions: L14724 5′TGA CTT GAA RAA CCA YCG TTG 3′ (Palumbi et al. 1991), L15171 5′GAG GAC AAA TRT CYT TCT GAG G 3′ (Reed et al. 2001), H15403 5′GAG AAG TAR GGR TGG AAG G 3′ (Reed et al. 2001), H15889 5′TGG RAC TGA GCT ACT AGT GC 3′ (Reed et al. 2001), L15171b 5′GAG GAC AAA TGT CTT TCT GAG G 3′, L15171c 5′GAG GAC AGA TAT CTT TCT GAG G 3′, H15314a 5′GTT GTC CGG GTC TCC GAG AAG 3′, H15314b 5′ATT GTC CGG GTC CC GAG GAG 3′. Amplified fragments of cytochrome b were cloned and sequenced in order to design the sequencing primers specific to lethrinid species (L15171b, L15171c,

Table 2. Extended

17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
14															
15	14														
16	16	16													
20	20	21	21												
20	22	22	21	9											
22	22	22	23	16	14										
20	20	22	22	16	15	16									
22	23	22	23	22	22	23	22								
23	24	23	23	23	23	24	22	23							
21	24	21	23	22	22	22	22	24	24						
21	23	21	23	24	23	24	24	23	22	18					
19	20	19	20	20	21	21	21	22	23	20	22				
19	21	20	21	21	21	22	21	24	24	21	22	15			
19	20	20	20	22	22	23	22	23	22	21	22	13	13		
24	23	24	24	22	23	24	22	25	24	24	24	23	24	23	
24	23	23	23	21	23	22	22	24	26	25	24	23	22	22	13

H15314a, and H15314b). Primer names indicate the DNA strand (H = heavy strand and L = light strand) and the position of the 3′ end of the oligonucleotide primer relative to the human mitochondrial DNA sequence (Anderson et al. 1981). Unincorporated dyes were removed from sequencing reaction products by ethanol and sodium acetate precipitation following the protocol outlined in the ABI Prism BigDye manual. Reaction products were electrophoresed on an ABI 377 automated DNA sequencer for approximately 7 h. Sequencing reactions were performed for both light and heavy DNA strands. Contiguous DNA fragments were reconciled using Sequencher v 3.1 (GeneCodes) and submitted to GenBank. DNA sequences contained no gaps and were unambiguously aligned by eye. The entire cytochrome b gene (1140 bp) was sequenced for each specimen.

Phylogenetic Analysis

Maximum parsimony (MP) analysis was performed with PAUP* (Swofford 2001). The most parsimonious tree, or equally parsimonious trees were obtained using the heuristic search algorithm with sequences added randomly (n = 1, 100 replicates with 100 trees held per replicate) and tree-bisection-reconnection (TBR) branch swapping. This analysis was repeated using all substitutions and with third codon substitutions weighted to zero. The analyses provide the number of constant characters, parsimony informative characters, parsimony uninformative characters, tree length, consistency index, and the retention index. Genetic distances were generated using PAUP* (Swofford 2001). Sepal (Salisbury 2000) was used to calculate Bremer decay values (Bremer 1988) and jackknife (36% deletion) support (Farris et al. 1996) for each node. Bootstrap values were obtained from MEGA2 (Kumar et al. 2000) at 100 replicates.

We used the computer program ModelTest (Posada and Crandall 1998) as a guide to determine a best-fit maximum likelihood (ML) model as described by Cunningham et al. (1998). ModelTest examines ML models ranging from simple to complex. This method incrementally increases the number of parameters in the ML model until the addition of a new parameter no longer significantly increases the fit between the model and the data. ModelTest calculated likelihood scores for 56 nested ML models and used hierarchical likelihood ratio tests (LRTs) to determine the best-fit model. We performed post-hoc LRTs to examine several ML models that were not evaluated by the program ModelTest. The best-fit ML model contained the nucleotide substitution rate matrix; A–C = 0.19, A–G = 5.27, A–T = 0.38, C–G = 0.46, C–T = 4.42, and G–T = 1.00. The estimated nucleotide frequencies were A = 0.287, C = 0.385, G = 0.097, and T = 0.231. The proportion of invariable sites was estimated to be 0.50 and the shape of the gamma parameter was estimated to be 0.77. We incorporated this model of nucleotide evolution in PAUP* (Swofford 2001) using the ML optimality criterion.

We used PAUP* to generate an initial neighbor joining tree (using the default settings) and estimated the ML parameters from that topology. Using the parameter estimates, we performed a heuristic search with random sequence addition (n = 1) and TBR branch swapping. We then re-estimated the ML parameters from the new tree topology. These

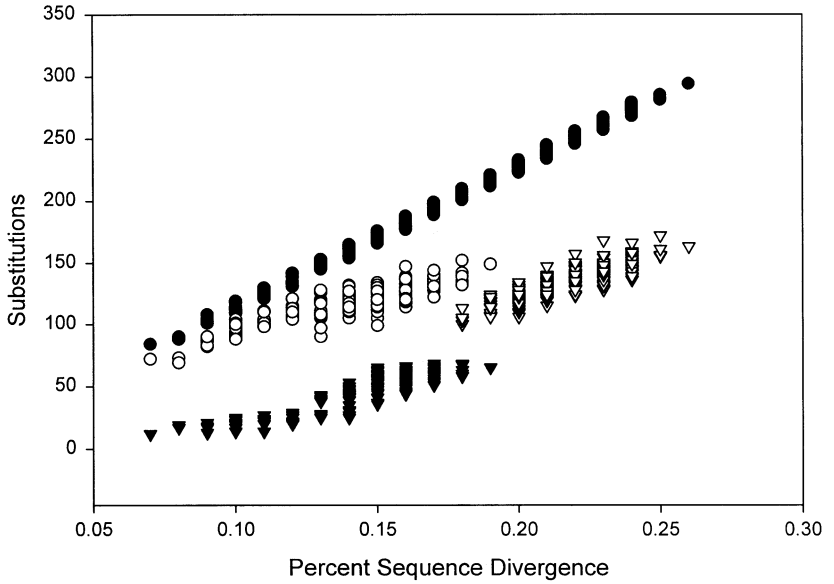

Fig. 1. Substitutions as a function of percent sequence divergence. Shaded circles represent transitions and transversions combined including all ingroup and outgroup comparisons. Non-shaded circles represent all transitions from comparisons within the ingroup *Lethrinus.* Non-shaded triangles represent transitions from comparisons between *Lethrinus* and representatives of the Monotaxinae. Shaded triangles represent transversions from comparisons within *Lethrinus.*

values were used in another heuristic search with random sequence addition (n = 1) and TBR branch swapping. Again we reestimated the ML parameters from the new topology. This iterative method of refining the parameter estimates was repeated until the estimates remained unchanged in three successive iterations. A final heuristic search was performed using random sequence addition (n = 10) and TBR branch swapping. We performed 100 bootstrap replicates (TBR branch swapping) to test relative support for nodes in the topology (Felsenstein 1985).

Results

Sequence Analysis

Table 2 shows the percent uncorrected pairwise genetic distances among *Lethrinus* species and outgroup taxa. Mean pairwise percent sequence differences were 19.09% between all taxa (ranging from 7–26%), 14.47% between *Lethrinus* species (ranging from 7–19%), 21.20% between *Lethrinus* species and monotaxine species (ranging from 20–24%), and 21.91% between *Lethrinus* species and all outgroup taxa (ranging from 18–25%). Plots of sequence divergence as a function of substitutions are given in Fig. 1. Pooled transition and transversion substitutions increase linearly with sequence divergence. Transition substitutions increase without reaching an asymptote in comparisons between species of *Lethrinus* but do reach an asymptote when all taxa are included. Transversions appear to increase linearly in comparisons between species of *Lethrinus* and for comparisons between *Lethrinus* and all outgroups (not shown in Fig. 1, the scatter of points overlap the outgroup transition scatter).

The aligned sequences from all species exhibited 481 parsimony informative variable sites and 60 parsimony uninformative variable sites. The maximum parsimony analysis excluding third codon positions exhibited 460 phylogenetically informative characters.

Phylogenetic Analyses

Figure 2 illustrates the consensus tree from two equally parsimonious trees from the unweighted MP analysis. Phylogenetic signal was detected in the data set determined by the skewed distribution of 1000 random trees ($g_1 = -0.521$). The consistency index from this analysis was 0.284 and the retention index was 0.412.

Monophyly of the Lethrinidae was not supported in both the unweighted MP tree (Fig. 2) and the ML tree (Fig. 3). However, both analyses did support the monophyly of *Lethrinus* with high Bremer decay, jackknife, and bootstrap support. *Lethrinus miniatus* resolves as the most ancestral species and positions sister to all remaining *Lethrinus.* Two major clades resolve within *Lethrinus* with high node support. One of these clades consists of *Lethrinus sp. 2, Lethrinus semicinctus, Lethrinus olivaceus, Lethrinus microdon, Lethrinus rubrioperculatus,* and *Lethrinus reticulatus.* This clade is comprised exclusively of mesocarnivore stalkers. The other well-supported clade includes all mesocarnivore specialists and includes *Lethrinus atkinsoni, Lethrinus borbonicus, Lethrinus obsoletus, Lethrinus ornatus, Lethrinus lentjan, Lethrinus nebulosus, Lethrinus sp. 3, Lethrinus laticaudis,* and *Lethrinus harak.* These two clades within *Lethrinus* correlate with both dentition and body form (Figs. 2 and 3). The placement of *Lethrinus obsoletus, Lethrinus nebulosus, Lethrinus harak, Lethrinus erythropterus, Lethrinus erythracanthus, Lethrinus atlanticus,* and *Lethrinus genivittatus* differs slightly between the tree generated from the MP analysis and the tree created from the ML analysis. However, support for the placement of these species is minimal in both analyses.

The MP analysis performed with the exclusion of third codon positions (not shown) supported the monophyly of the *Lethrinus* with *Lethrinus miniatus* as the sister to all remaining *Lethrinus* species. This analysis

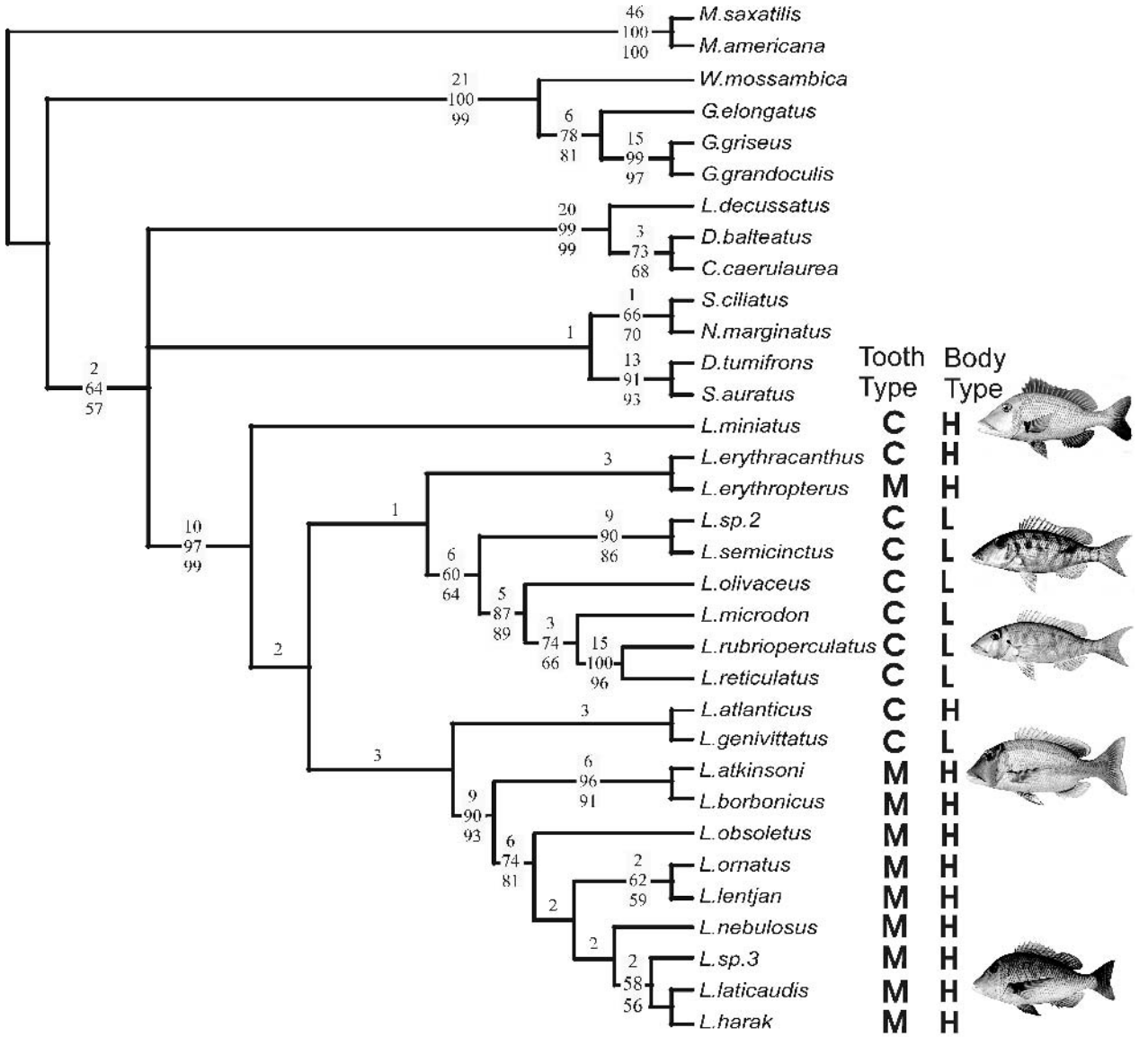

Fig. 2. A strict consensus phylogeny of two equally parsimonious trees from the maximum parsimony analysis of the unweighted cytochrome b data. Numbers at the base of the nodes are from measures of support and include Bremer decay values on top, jackknife support in the middle, and bootstrap support below. Jackknife and bootstrap support lower than 50 are not shown. Dentition abbreviations are C = conical, M = molariform and submolariform. Body type abbreviations are H = high-bodied and L = low-bodied. Fish figures represent the typical body shape for the different trophic types and include, from top to bottom: *Lethrinus miniatus, Lethrinus semicinctus, Lethrinus rubrioperculatus, Lethrinus atkinsoni,* and *Lethrinus laticaudis.*

supported the monophyly of the same two well-supported clades within *Lethrinus* as in the unweighted analyses, however, the remaining species of *Lethrinus* formed a polytomy. This analysis also did not result in a monophyletic Lethrinidae.

Discussion

The phylogenies inferred from cytochrome b sequences using both MP and ML methods clearly support the monophyly of groups of taxa, but fail to support the monophyly of the family Lethrinidae or the superfamily Sparoidea. The lack of resolution at the base of the tree may be the result of taxon sampling issues. Also, the rate of evolution within our group may not reflect the phylogenetic resolution provided by the cytochrome b gene. Perhaps analysis of another gene that more closely mirrors the evolutionary rate of this group would provide additional phylogenetic resolution. Strong node support from both MP (bootstrap, jackknife, and Bremer decay) and ML (bootstrap) is evident for monophyly of the monotaxine genera *Wattsia* + *Gymnocranius,* and the genus *Lethrinus.* In addition, monophyly for the outgroup families, with the exception of the Nemipteridae is also strongly supported. However, there is only weak support for nodes uniting these families. The MP and ML analyses differ in phylogenetic placement of taxa where

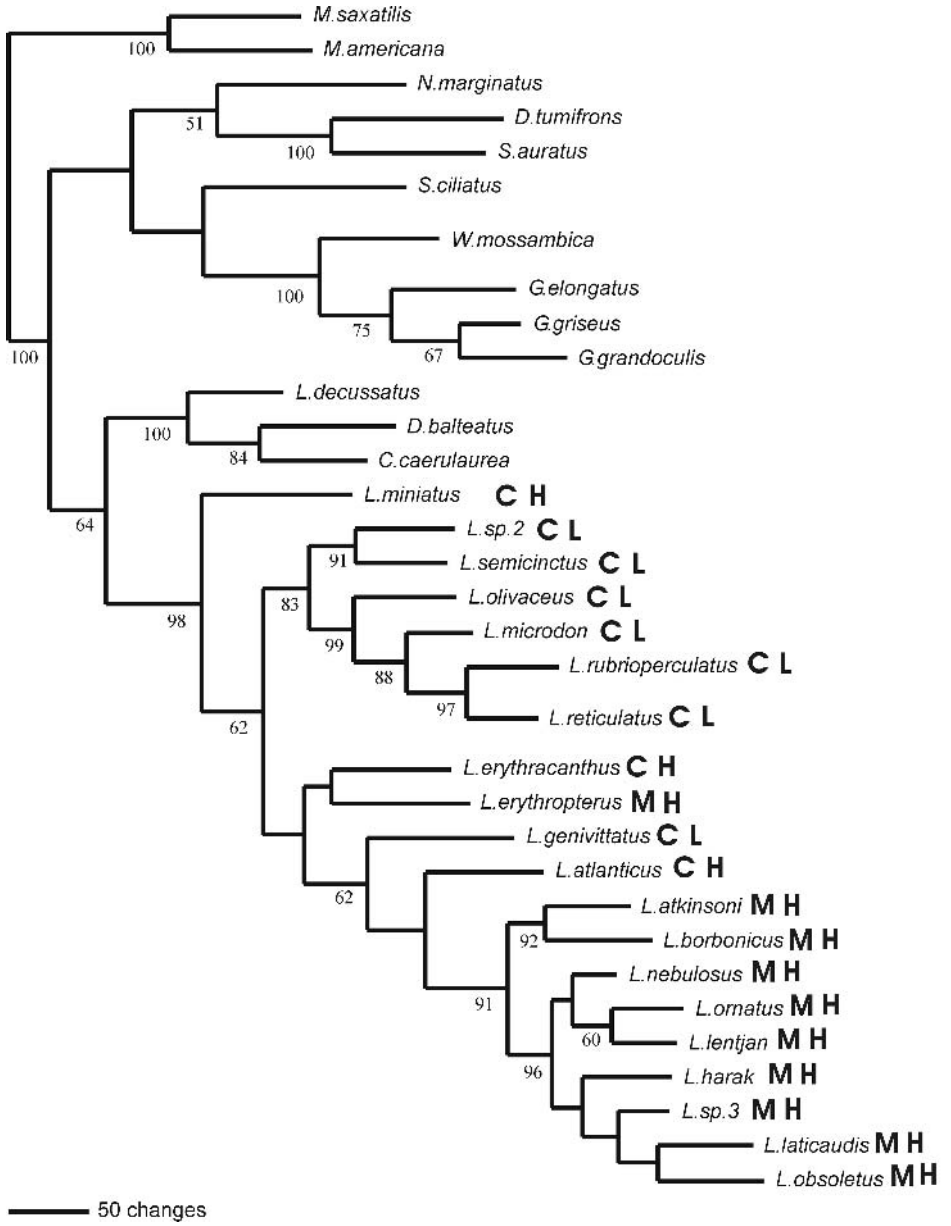

Fig. 3. Phylogenetic tree from the maximum likelihood analysis. Numbers below branches indicate bootstrap support. Bootstrap support lower than 50 is not shown. Branch lengths are proportional to number of changes. Dentition abbreviations are C = conical, M = molariform and submolariform. Body type abbreviations are H = high-bodied and L = low-bodied.

node support is weakest. The monophyly of the Lethrinidae is not supported potentially because sequences for the other genera within this family, *Gnathodentex* and *Monotaxis,* could not be obtained. These two taxa may represent intermediate forms. Transition substitutions are also clearly saturated for comparisons between the Lethrininae and the available monotaxine genera. However, excluding saturated data from the analysis still failed to produce a monophyletic Lethrinidae. It is possible that use of a more conservative gene may be necessary to adequately test the monophyly of this family. Orrell et al. (in press) inferred a weakly supported monophyletic Sparoidea from complete cytochrome b sequences when third position transitions were excluded from a MP analysis. We did not obtain similar results in our study. Although both studies include representatives of the three main sparoid lineages, the taxonomic coverage within families differs. More complete coverage of each of the families or a more conservative gene may also be necessary to test the monophyly of the Sparoidea.

Unlike the lack of resolution at the family and superfamily level, cytochrome b produces strongly supported clades within genera and between genera within subfamilies of percoid fishes. Orrell et al. (in press) obtained well-supported cladistic relationships among sparid genera using cytochrome b. Our results clearly infer a monophyletic *Lethrinus* and two well-defined lineages within *Lethrinus.*

The two strongly supported clades within *Lethrinus* exhibit two distinct trophic types. These types relate to body shape and dentition as delineated for *Lethrinus* by Carpenter (1996). One distinct clade includes *Lethrinus sp. 2, Lethrinus semicinctus, Lethrinus olivaceus, Lethrinus microdon, Lethrinus rubrioperculatus,* and *Lethrinus reticulatus.* These species are low-bodied forms with conical teeth. They are the mesocarnivore stalkers that feed on relatively high-speed prey such as fishes and crustaceans (Carpenter 1996). The other well-resolved clade contains *Lethrinus atkinsoni, Lethrinus borbonicus, Lethrinus obsoletus, Lethrinus ornatus, Lethrinus lentjan, Lethrinus nebulosus, Lethrinus sp. 3, Lethrinus laticaudis,* and *Lethrinus harak.* These species are high-bodied forms with molariform or submolariform teeth. These mesocarnivore specialists consume hard or soft-shelled invertebrates such as mollusks, sea urchins, and crustaceans (Carpenter 1996). The distinctness of each of the main *Lethrinus* clades indicates that the primary radiation within *Lethrinus* occurred separately within these two primary trophic clades. This differs from the Sparidae wherein the same trophic type evolved separately several times (Hanel and Sturmbauer 2000; Orrell et al. in press). However, the sparids are much more speciose with 33 genera and approximately 110 species.

The only other well-supported *Lethrinus* clade, aside from the two primary trophic groups, is the clade that supports monophyly of *Lethrinus* with *Lethrinus miniatus* as the basal species, sister to all other species of *Lethrinus.* This high-bodied species is a mesocarnivore generalist and has conical teeth. It feeds predominantly on benthic invertebrates (Carpenter and Allen 1989) but lacks molariform teeth. Based on the ancestral placement of this species, we hypothesize that the two primary trophic types within *Lethrinus* both evolved from a high-bodied, conical-toothed ancestor. Thus, a *Lethrinus miniatus*-like ancestor gave rise to both a low-bodied conical-toothed form and a high-bodied molariform-toothed form. Some of the high-bodied forms with molariform teeth retain nearly conical teeth as juveniles and develop submolariform teeth and finally more molariform teeth as they age (the "intermediate" tooth type of Carpenter 1996). This ontogenetic sequence supports the notion of a conical-toothed ancestor for this clade.

Node support is low for the remaining species of *Lethrinus.* The placement of *Lethrinus erythracanthus, Lethrinus erythropterus, Lethrinus atlanticus,* and *Lethrinus genivittatus* differs between the MP to the ML analyses. Two of these species, *Lethrinus erythracanthus* and *Lethrinus atlanticus* have conical teeth and high bodies and therefore are mesocarnivore generalists, similar to *Lethrinus miniatus.* In the MP analysis, one of each of these was placed in a clade sister to each of the two main trophic types. This further supports the hypothesis that the mesocarnivore stalker and mesocarnivore specialist trophic types in *Lethrinus* derived from a mesocarnivore generalist. In both the MP and ML analyses, *Lethrinus erythracanthus* is placed sister to *Lethrinus erythropterus.* The latter has molars and is high-bodied. Superficially, these two species appear closely related since they share anal fin characteristics that are unique among species of *Lethrinus* (Carpenter and Allen 1989). This suggests that the high-bodied form with molars may have evolved more than once within *Lethrinus.* However, support for their sister relationship and phylogenetic placement within *Lethrinus* is weak. The addition of closely related species to the analyses may change the phylogeny with respect to these two taxa. *Lethrinus atlanticus* is another high-bodied species with conical teeth and is the only species of *Lethrinus* that occurs in the Atlantic. Assuming this represents the ancestral body form, we hypothesize that *Lethrinus atlanticus* diverged fairly early from the *Lethrinus* lineage as it became isolated from the remaining lethrinids in the Indo-Pacific. *Lethrinus genivittatus* is the only low-bodied form with conical teeth that does not appear closely related to all the other similar forms in *Lethrinus.* This species is unique in many respects with a number of autapomorphies that distinguish it from all other species of *Lethrinus* (Carpenter and Allen 1989). With a maximum size of 25 cm total length, it is smaller than the other low-bodied forms with conical teeth in this study, which range between 35 and 100 cm total length. It is possible that this trophic type evolved more than once within *Lethrinus* or that its correct phylogenetic placement is with members of the same trophic type.

Given the strong support for two trophic clades within *Lethrinus,* we can predict the placement of those species of *Lethrinus* for which tissues specimens were not available. *Lethrinus amboinensis, Lethrinus sp. 1* (Carpenter and Allen 1989), and *Lethrinus xanthochilus* are low-bodied forms with conical teeth, are active piscivores, and would predictably cluster with the mesocarnivore stalkers. *Lethrinus variegatus* has conical teeth and the most fusiform body of all lethrinids but differs from other low-bodied lethrinids with conical teeth by its small maximum size of approximately 20 cm in total length and its habit of feeding on small benthic invertebrates. This species may be closely related to *Lethrinus genivittatus,* which has similar feeding habits and is also relatively small. Alternatively, *Lethrinus variegatus* may be a highly derived low-bodied form with conical teeth. *Lethrinus crocineus, Lethrinus enigmaticus,* and *Lethrnius mahsena* show characteristics that would indicate placement in the clade containing high-bodied forms with molariform teeth. *Lethrinus haematopterus* is a high-bodied species with conical teeth and may be closely related to *Lethrinus miniatus.* If this can be tested and found to be valid, we can further formulate a hypothesis about the origin and radiation of *Lethrinus. Lethrinus haematopterus,* found in East Asia, is the only lethrinid restricted to temperate waters. *Lethrinus minia-*

tus was originally thought to have an antitropical distribution (Carpenter and Allen 1989) ranging from temperate southern Japan to temperate Australia, but has now been reported also in the Philippines (Carpenter 2001). Most other *Lethrinus* are primarily tropical but some also range into warm temperate waters. Since the two putative ancestral forms, *Lethrinus miniatus* and *Lethrinus haematopterus* are the most cold-water adapted *Lethrinus,* the ancestor for this genus may also have been a temperate water form. This is interesting when we note that the sister group of the Lethrinidae are the Sparidae (Akazaki 1962; Orrell et al. in press). Sparids are typically more cold-water adapted with diversity centers around South Africa, the Mediterranean, and the western Atlantic. Sparids also tend to be more speciose in southern Australia and in Japan than lethrinids. Lethrinids are more warm-water adapted with their current center of diversity in the Indo-West Pacific. Based on the relationships of these ecological and biogeographical equivalents, we can hypothesize that ancestors of both families probably arose from a cold water ancestor.

The cytochrome b gene provided a well-corroborated phylogenetic data set within *Lethrinus,* independent of morphological characters. It strongly supports the inference that the primary radiation of *Lethrinus* from a *Lethrinus miniatus*-like ancestor, occurred within two main trophic types. However, lack of resolution at the level of intergeneric and interfamilial relationships implies that additional taxon sampling or analyzing a gene with an appropriate evolutionary rate may be necessary to test monophyly above the genus level for lethrinids.

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References

Akazaki M (1962) Studies on the spariform fishes. Anatomy, phylogeny, ecology, and taxonomy. Misaki Mar Biol Inst, Kyoto Univ., Spec. Rep. 1

Allegrucci G, Caccone A, Sbordoni V (1999) Cytochrome b sequence divergence in the European sea bass (*Dicentrarchus labrax*) and phylogenetic relationships among some Perciformes species. J Zool Syst Evol Research 37:149–156

Anderson S, Bankier AT, Barrell BG, de Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Straden R, Young IG (1981) Sequence and organization of the human mitochondrial genome. Nature 290:457–465

Bremer K (1988) The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42:795–803

Carpenter KE (2001) Lethrinidae. In: Carpenter KE, Niem V (eds.) FAO species identification guide for fishery purposes. The living marine resources of the western central Pacific. Volume 5. Bony fishes part 3. FAO, Rome, pp 3297–3298

Carpenter KE (1996) Morphometric pattern and feeding mode in emperor fishes (Lethrinidae, Perciformes). In: Marcus LF et al. (eds.) Advances in morphometrics. Plenum Press, New York, pp 479–487

Carpenter KE, Allen GR (1989) FAO species catalogue. Vol. 9. Emperor fishes and large-eye breams of the world (family Lethrinidae). An annoted and illustrated catalogue of lethrinid species known to date. FAO Fisheries Synopsis. No. 125, Volume 9. FAO, Rome

Carpenter KE, Johnson GD (in press). A phylogeny of sparoid fishes (Perciformes, Percoidei) based on morphology. Ichthyological Research

Cunningham CW, Zhu H, Hillis DM (1998) Best-fit maximum likelihood models for phylogenetic inference: Empirical tests with known phylogenies. Evolution 52:978–987

Farris JS, Albert VA, Källersjö M, Lipscomb D, Kluge AG (1996) Parsimony jackknifing outperforms neighbor-joining. Cladistics 12:99–124

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783–791

Hanel R, Sturmbauer C (2000) Multiple recurrent evolution of trophic types in northeastern Atlantic and Mediterranean seabreams (Sparidae, Percoidei). J Mol Evol 50:276–283

Johnson GD (1981) The limits and relationships of the Lutjanidae and associated families. Bull Scripps Inst Ocean 24:1–114

Kumar S, Tamura K, Jakobsen I, Masatoshi N (2000) MEGA. Molecular evolutionary genetics analysis, version 2.0b. Arizona State University, Phoenix

Lydeard C, Roe KJ (1997) The phylogenetic utility of the mitochondrial cytochrome b gene for inferring relationships among Actinopterygian fishes. In: Kocher TD, Stepien CA (eds.) Molecular systematics of fishes. Academic Press, San Diego, pp 285–302

Orrell TM (2000) A molecular phylogeny of the Sparidae (Perciformes: Percoidei). College of William and Mary, Williamsburg, VA

Orrell TM, Carpenter KE, Musick JA, Graves JE (in press) A phylogenetic and biogeographic analysis of the Sparidae (Perciformes: Percoidei) based on cytochrome b sequences. Copeia

Palumbi S, Martin A, Romano S, McMillan WO, Stice L, Grabowski G (1991) The simple fool's guide to PCR. University of Hawaii, Honolulu

Posada D, Crandall KA (1998) ModelTest: testing the model of DNA substitution. Bioinformatics 14:817–818

Reed DL, de Gravelle MJ, Carpenter KE (2001) Molecular systematics of *Selene* (Perciformes: Carangidae) based on cytochrome b sequences. Molecular Phylogenetics and Evolution 21:468–475

Salisbury BA (2001) SEPAL. Strongest evidence and parsimony analyzer, version 1.2. Yale University, New Haven

Sambrook J, Fritsch EF, Maniatis T (1989) Extraction with phenol: chloroform. In: Molecular cloning: A laboratory manual. Volume 3. Cold Spring Harbor Laboratory Press, Plainview, pp E.3–E.4

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. Can J Zool 69:82–90

Swofford DL (2001) PAUP*: Phylogenetic analysis using parsimony (and other methods), version 4.0b8. Sinauer Associates, Sunderland, MA