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The Phylogenetic Relationships of the Suborder Acanthuroidei (Teleostei: Perciformes) Based on Molecular and Morphological Evidence

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Fragments of 12S and 16S mitochondrial DNA genes were sequenced for 14 acanthuroid taxa (representing all six families) and seven outgroup taxa. The combined data set contained 1399 bp after removal of all ambiguously aligned positions. Examination of site saturation indicated that loop regions of both genes are saturated for transitions, which led to a weighted parsimony analysis of the data set. The resulting tree topology generally agreed with previous morphological hypotheses, most notably placing the Luvaridae within the Acanthuroidei, but it also differed in several areas. The putative sister group of Acanthuroidei, *Drepane*, was recovered within the suborder, and the sister group of the family Acanthuridae, *Zanclus*, was likewise recovered within the family. Morphological characters were included to produce a combined data set of 1585 characters for 14 acanthuroid taxa and a single outgroup taxon. An analysis of the same 15 taxa was performed with only the DNA data for comparison. The total-evidence analysis supports the monophyly of the Acanthuridae. A parametric bootstrap suggests the possibility that the paraphyly of Acanthuridae indicated by the molecular analyses is the result of long-branch attraction. The disagreement between molecular and morphological data on the relationships of the basal acanthuroids and its putative sister taxon is unresolved.

INTRODUCTION

The Acanthuroidei is one of 17 recognized suborders of the teleost order Perciformes (Nelson, 1994). There are about 125 species in the suborder. Most species are marine and many are common reef fishes of the tropical and subtropical seas. In recent years, the concept of the clade has changed from a relatively compact group consisting of rabbitfishes, the Moorish idol, and surgeonfishes to a broader group including spadefishes, scats, and the epipelagic louvar (Tyler *et al.*, 1989; Winterbottom, 1993). Inclusion of the monotypic and unusual louvar based on morphology was suprising because it had been traditionally associated with the suborder Scombroidei (tunas, billfishes, and their allies), a group not thought to be closely related to acanthuroids. The louvar's external appearance gives little indication of its relationship to other acanthuroids. However, larval (Johnson and Washington, 1987) and adult (Tyler *et al.*, 1989; Winterbottom, 1993) morphological evidence strongly indicates that the louvar is in fact the only truly pelagic member of the Acanthuroidei, a group that otherwise comprises strictly shorefishes. The louvar represents a remarkable example of extreme adaptive divergence in morphology, ecology, and behavior in the evolutionary history of Recent fishes. Given the strong morphological support for the relationships among the acanthuroids, the phylogenetic relationships within the suborder and specifically the position of the louvar are particularly interesting hypotheses to test with molecular data. The purposes of this paper are to test the monophyly of the expanded Acanthuroidei using mitochondrial ribosomal DNA sequence data and to test relationships within the suborder using these data and the available morphological data in a total-evidence context.

Taxonomic History

Acanthuroidei traditionally included three families: Siganidae, Zanclidae, and Acanthuridae (e.g., Greenwood *et al.*, 1966; Gosline, 1968; Mok and Shen, 1983). Johnson and Washington (1987) presented evidence from larval morphology that supported the inclusion of the Luvaridae within the Acanthuroidei as the sister group of the Zanclidae and Acanthuridae. The more extensive analysis of Tyler *et al.* (1989) incorporated morphological characters from both adults and larvae.

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Tyler *et al.* (1989) proposed the inclusion of the monotypic Luvaridae in the Acanthuroidei and recognized a monophyletic group comprising the Acanthuroidei, Ephippidae, and Scatophagidae. Tyler *et al.* (1989) based the monophyly of this group of three taxa on six synapomorphies: interarcual cartilage absent, distinctively shaped nonovoid interopercle, articular shorter than dentary, nonprotrusible or nearly nonprotrusible premaxillae, gill membranes broadly united at isthmus, and cancellous frontal and supraoccipital bones. Winterbottom's (1993) phylogenetic analysis combined the Tyler *et al.* (1989) data set with myological characters, producing a tree topologically identical to that of Tyler *et al.* (1989) at the family level and also resolving relationships among the six genera of the Acanthuridae (Fig. 1). Winterbottom (1993) formally expanded the Acanthuroidei to include Ephippidae and Scatophagidae, recognizing one additional synapomorphy: myocommatum in the adductor mandibulae muscle. Currently, the suborder comprises these six families (Scatophagidae, Ephippidae, Siganidae, Luvaridae, Zanclidae, and Acanthuridae), with 18 genera and approximately 125 species, of which more than 70 species are in the family Acanthuridae (Nelson, 1994). The currently accepted hypothesis of phylogenetic relationships (Fig. 1) and classification (Table 1) are based on Winterbottom (1993).

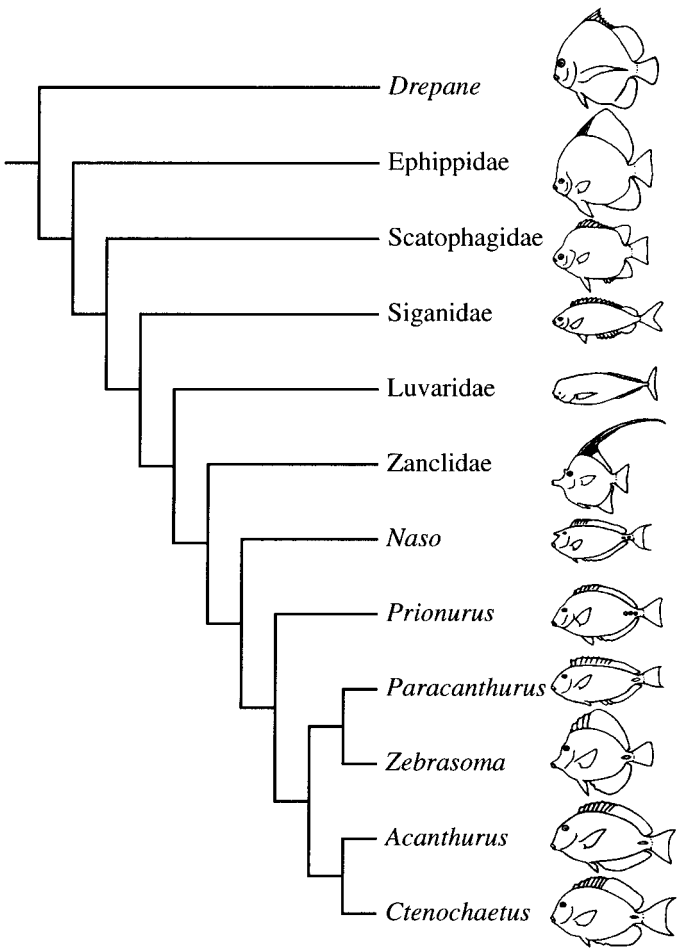

FIG. 1. Previous phylogenetic hypothesis based on morphological evidence (Winterbottom, 1993). Drawings are not to scale. Parts of this figure are from Fig. 1 of Winterbottom and McLennan (1993).

Review of Families

The suborder Acanthuroidei is composed of six families. The family Ephippidae (spadefishes) are deep-bodied marine fishes from the Atlantic, Indian, and Pacific Oceans, with some species that enter brackish water. This family comprises seven genera with approximately 20 species (Nelson, 1994). The Scatophagidae (scats) are a family of laterally compressed fishes comprising two genera and four species distributed in the Indo–West Pacific. It includes marine and brackish-water species that inhabit estuaries, as well as species that enter freshwater.

The remaining four families (Acanthuroidei *sensu stricto:* Tyler *et al.*, 1989) share a specialized, planktonic larval stage: the acronurus (Lauder and Liem, 1983; Tyler *et al.*, 1989), a term that was originally restricted to the Acanthuridae (Leis and Richards, 1984). Tyler (1970) suggested that the pelagic larvae of the Chaetodontidae (butterflyfishes) might support a relationship with the Acanthuridae, but the larvae of the two groups share only general similarities associated with an extended pelagic existence (Leis and Rennis, 1983).

The family Siganidae (rabbitfishes) comprise 30 species placed in a single genus, *Siganus*, and are found in the Indo–West Pacific and, vicariously, the Mediterranean Sea. Siganids have 13 venomous dorsal-fin spines and are unique in having a spine at each end of the pelvic fin flanking three soft rays.

A notable exception to the inshore lifestyle of other acanthuroids is the epipelagic louvar, *Luvarus imperialis*. It is the only extant member of the family Luvaridae and is, by far, the largest species in this suborder (more than 1.8 m SL and 140 kg). This species was widely considered to be a member of the largely pelagic suborder Scombroidei (e.g., Nelson, 1984). That hypothesis, originating with Regan (1903), was based on putative adaptations for a pelagic lifestyle found in both louvars and scombroids. In fact, Regan (1902) originally proposed that the louvar is a highly modified acanthuroid, but, after noting some striking similarities between certain aspects of the caudal skeleton of the louvar and the scombroids (particularly the billfishes), Regan (1903) rejected his previous hypothesis and proposed that the louvar is related to the Scombroidei. Because of its morphological specializations, other ideas about the relationships of the louvar have been proposed; e.g., Gregory and Conrad (1943) suggested that the louvar may be related to the Carangoidei, another predominately pelagic suborder. However, this bizarre and enigmatic fish was generally placed in or

near the Scombroidei in subsequent classifications (see Tyler *et al.*, 1989, for a detailed history of its classification).

The family Zanclidae is also monotypic, its only member being the striking Moorish idol, *Zanclus cornutus*. Like many acanthuroids, this species occurs in association with coral reefs in the tropical Indo–Pacific. Zanclids are considered the closest relatives of the Acanthuridae and at times had been included in that family (e.g., Nelson, 1984). However, numerous morphological characters distinguish this species from the members of the Acanthuridae.

The largest and best known family in the Acanthuroidei is the Acanthuridae (surgeonfishes, tangs, and unicornfishes). The common name surgeonfish is derived from the modified scales found on the caudal peduncle of all species in the family. The members of the Acanthuridae are widely distributed in most tropical and subtropical seas of the world. The approximately 70 species in the Acanthuridae are placed in the subfamilies Nasinae, which includes *Naso* (unicornfishes), and Acanthurinae, which comprises three tribes: Prionurini, Zebrasomini, and Acanthurini (Winterbottom, 1993). The status of the two genera in the tribe Acanthurini, *Acanthurus* and *Ctenochaetus*, has been somewhat controversial. It has been suggested that species of the genus *Ctenochaetus* are derivatives of the largest genus, *Acanthurus* (Aoyagi, 1943; Randall, 1955). Species in *Acanthurus* have two types of stomachs: thin-walled and thick-walled (Randall, 1956; Hiatt and Strasburg, 1960; Jones, 1968). The thick-walled stomach is found in 14 species of *Acanthurus* as well as in all species of *Ctenochaetus* (Winterbottom, 1993). Species with thick-walled stomachs are all benthic grazers that ingest large amounts of substrate during feeding and use their gut like a gizzard (Jones, 1968), suggesting that thick-walled *Acanthurus* species and *Ctenochaetus* might form a clade.

Goals of the Study

The major goal of this study is to use DNA sequence data from the 12S and 16S mitochondrial genes and existing morphological data to infer the phylogenetic relationships among the Acanthuroidei and their putative relatives. The strength of the DNA data alone as well as in a total-evidence context will also be addressed. The analysis of the DNA data will be compared to two aspects of the existing hypothesis of acanthuroid relationships: that of Johnson and Washington (1987) and Tyler *et al.* (1989) that Luvaridae is included in the Acanthuroidei and that of Winterbottom (1993) regarding the interrelationships among the Acanthuroidei *sensu lato*.

MATERIALS AND METHODS

Material Examined

At least one species representing every acanthuroid genus was included except for ephippids, for which representatives of two of the seven genera were examined. Two species of *Acanthurus*, one with a thin-walled gut and one with a thick-walled gut, were included to minimally test the monophyly of the genus *Acanthurus*. The composition and relationships among putative members of the Perciformes are much debated (Johnson, 1993). As a result, outgroup taxa were drawn from a wide range of perciform families: Moronidae, Pomatomidae, Scombridae, Carangidae, Chaetodontidae, Pomacanthidae, and Drepanidae. *Morone chrysops* was chosen to represent a "typical" basal perciform. Two members of the Scombroidei, *Scomber scombrus* and *Pomatomus saltatrix*, and a carangoid, *Caranx latus*, were included because of the previously hypothesized relationship of the Luvaridae to these groups. The percoids *Chaetodon striatus* and *Holacanthus ciliaris* were included because of previous suggestions that Chaetodontidae and Pomacanthidae are related to acanthuroids (Tyler, 1970; Tyler *et al.*, 1989). *Drepane punctata* was chosen because it has been proposed as the closest outgroup to the Acanthuroidei (Tyler *et al.*, 1989; Winterbottom, 1993). See Table 1 for material examined.

Collection and Preservation of Material

Fishes were collected and preserved with a variety of methods. Tissue was either preserved in 95% ethanol and stored at −20°C or stored without preservation at −70°C in an ultracold freezer. One tissue sample (*Luvarus imperialis*) was preserved in 20% dimethyl sulfoxide (DMSO) solution in 0.25 M EDTA buffer and stored at −20°C.

DNA Protocols

All genomic extractions were done from muscle tissue. Approximately 25 mg of tissue were dissected and DNA was extracted using QIAamp tissue extraction kits (Qiagen). Target regions of the mtDNA were amplified using the polymerase chain reaction (Saiki, 1990). Amplitaq DNA polymerase (Perkin–Elmer Cetus Corp.) and primers listed in Table 2 were used to amplify approximately 900 bp of the 12S mtDNA gene and approximately 650 bp of the 16S mtDNA gene using the following thermal cycling profile: 94°C denaturing for 30 s, 55°C annealing for 30 s, and 70°C extension for 2 min and 30 s for 35 cycles. Amplified products were loaded onto NuSieveGTG (FMC) agarose gels and electrophoresed at 85–90 V for approximately 1 h. The target band was excised from the gel and purified using QIAquick gel extraction kits (Qiagen). The purified PCR product was amplified (profile: 96°C denaturing for 30 s, 50°C annealing for 15 s, and 60°C extention for 4 min for 26 cycles) and sequenced with an Applied Biosystems 310 automated sequencer using ABI Prism dye terminator sequencing kits and the primers indicated in Table 2. Both heavy and light strands were sequenced for most of both gene regions. All sequences were deposited in GenBank (Table 1).

TABLE 1

Species Used with Higher Level Classification (following Winterbottom, 1993; Nelson, 1994) and GenBank Accession Nos.

Taxon	Catalog number (KU tissue number)	Accession no. (12S)	Accession no. (16S)
Order Perciformes			
Suborder Acanthuroidei			
Family Ephippidae			
Chaetodipterus faber[a,b]	KU photo voucher slide 1040 (T131)	AF055596	AF055617
Platax orbicularis[a]	ROM 68386 (T1828)	AF055597	AF055618
Family Scatophagidae			
Scatophagus argus[a,b]	ROM 69920 (T1829)	AF055598	AF055619
Selenotoca multifasciata[a]	ROM 68452 (T1831)	AF055599	AF055620
Family Siganidae			
Siganus canaliculatus[a,b]	ROM 69808 (T1832)	AF055600	AF055621
Family Luvaridae			
Luvarus imperialis[a,b]	USNM 345269 (T1849)	AF055601	AF055622
Family Zanclidae			
Zanclus cornutus[a,b]	USNM 334326 (T786)	AF055602	AF055623
Family Acanthuridae			
Subfamily Nasinae			
Naso lituratus[a,b]	KU 26887 (T1836)	AF055603	AF055624
Subfamily Acanthurinae			
Tribe Prionurini			
Prionurus maculatus	ROM 68379 (T1835)	AF055604	AF055625
Tribe Zebrasomini			
Paracanthurus hepatus[a,b]	KU 22978 (T46)	AF055605	AF055626
Zebrasoma scopas[a,b]	USNM 334159 (T711)	AF055606	AF055627
Tribe Acanthurini			
Acanthurus guttatus	USNM 331074 (T797)	AF055609	AF055630
A. xanthopterus[a,b]	USNM 334425 (T757)	AF055607	AF055628
Ctenochaetus binotatus	USNM 334119 (T664)	AF055608	AF055629
Suborder Carangoidei			
Family Carangidae			
Caranx latus	KU photo voucher slide 1024 (T63)	AF055590	AF055611
Suborder Percoidei			
Family Chaetodontidae			
Chaetodon striatus[a]	USNM 327589 (T196)	AF055592	AF055613
Family Drepanidae			
Drepane punctata[a,b]	ROM 69918 (T1837)	AF055595	AF055616
Family Moronidae			
Morone chrysops	KU 22901 (T823)	AF055589	AF055610
Family Pomacanthidae			
Holacanthus ciliaris[a]	USNM 327591 (T175)	AF055593	AF055614
Suborder Scombroidei			
Family Pomatomidae			
Pomatomus saltatrix	KU 22947 (T6)	AF055591	AF055612
Family Scombridae			
Scomber scombrus	KU 26888 (T1483)	AF055594	AF055615

Note. KU, University of Kansas; ROM, Royal Ontario Museum; USNM, Smithsonian Institution, United States National Museum.
[a] Examined by Tyler *et al.* (1989).
[b] Examined by Winterbottom (1993).

DNA Data and Alignment Protocols

DNA sequences were inspected individually for quality and then spliced together using the computer program Sequence Navigator 1.01 (Applied Biosystems). A consensus sequence (light strand) was produced by comparing the heavy and the light strand sequences generated for each taxon against the raw data. An initial alignment was made using the CLUSTAL algorithm in Sequence Navigator. Sequence variation between species was compared against the original electropherograms as a further check on sequence quality. The aligned data were then exported as a NEXUS file and organized into stem and loop regions using models presented by Van de Peer *et al.* (1994) and de Rijk *et al.* (1994) for 12S and 16S mitochondrial DNA, respectively. Each stem was examined visually for base-pair complementarity and alignments in loops were adjusted where needed. Stem and loop regions

TABLE 2

Sequencing (S) and Amplification (A) Primers Used

Name	Sequence	Strand	Use
Mitochondrial 12S Gene			
Phe2-L[a]	5′ AAAGCATAACACTGAAGATGTTAAGATG 3′	Light	A,S
12Sb-H[b]	5′ AGGAGGGTGACGGGCGGTGTGT 3′	Heavy	A,S
12Sd-L[c]	5′ GGGTTGGTAAATCTCGTGC 3′	Light	S
12Sd-R	5′ GCTGGCACGAGTTTTACCGGCC 3′	Heavy	S
Mitochondrial 16S Gene			
16Sa-L[d]	5′ CGCCTGTTTACCAAAAACATCGCCT 3′	Light	A,S
16Sb-H[d]	5′ CCGGTCTGAACTCAGATCACGT 3′	Heavy	A,S

[a] *Oncorhynchus mykiss* position 946–965.
[b] Modified from Kocher *et al.* (1989).
[c] Modified 503 primer of John Patton, Washington University. *Oncorhynchus mykiss* position 1216–1233.
[d] See Palumbi (1996).

were examined for possible site saturation (multiple mutations at a site) by plotting the number of mutations between pairs of taxa against the Tamura–Nei genetic distances generated from MEGA 1.01 (Kumar *et al.*, 1993). A NEXUS format file with the complete alignment is available from the senior author.

Morphological Data

The morphological data (186 transformation series) used in this study are taken from Winterbottom (1993). This matrix includes original data as well as character data taken from the literature (Mok, 1977; Johnson and Washington, 1987; Tyler *et al.*, 1989; Guiasu and Winterbottom, 1993). See Winterbottom (1993) for character descriptions.

Phylogenetic Analyses

Phylogenetic analyses were carried out using the heuristic search option, 10 random addition sequence replicates, and tree-bisection-reconnection of PAUP* 4.0d61a (Swofford, unpublished). In all analyses, loop regions were weighted using a step matrix that accorded transitions a weight of zero, so that loops were analyzed for transversions only. This weighting was done to account for the saturation of transitions in the more variable loop regions, as indicated by the analyses for site saturation (see Results). Gaps were common in loop regions and these were included in the analyses as characters. All morphological data were analyzed as equally weighted and unordered characters.

Analyses were performed on three different data sets. There was one large data set analysis consisting of 21 taxa using only DNA data, with *Morone* designated as the outgroup. This is referred to as the "21-taxon data set." This analysis was done to ascertain the utility of molecular data in reconstructing the phylogeny of the suborder. A total-evidence analysis using both DNA and morphological characters of these 21 taxa would have been ideal. However, the morphological data for six of the seven outgroups were not available. To incorporate the available morphological evidence and conduct a total-evidence analysis, those six outgroups were removed from the analysis, leaving the 15 taxa for which there was a complete morphological data set. A second small data set analysis examined these 15 taxa utilizing only DNA data to provide a basis of comparison for the total-evidence analysis. Both small data set analyses employed two different outgroup topologies (see Results).

Phylogenetic trees were evaluated using summary values reported by PAUP (e.g., tree length, ensemble consistency index). Support for internodes (monophyletic groups) was evaluated by calculating branch support values (Bremer, 1988, 1994) using TreeRot (Sorenson, 1996). Bootstrap values (Felsenstein, 1985) were calculated using a heuristic search and 1000 bootstrap replications. A Templeton's test (Templeton, 1983), based on a Wilcoxon's signed-ranks test, was used to determine whether the tree topology derived from the 15-taxon molecular data differed significantly from the tree topology derived from morphological data (Winterbottom, 1993). The problem of possible long branch attraction (Swofford *et al.*, 1996) was addressed only in the analyses of the small data set (*Drepane* and the acanthuroids). Parametric bootstrap analyses were performed via 100 replicate data sets. Data replication consisted of inputting likelihood parameters derived from a null-hypothesis phylogenetic tree using Seq-Gen 1.04 (Rambaut and Grassly, 1997), as outlined in Results. The parametric test follows Huelsenbeck and Rannala (1997).

RESULTS

A DNA matrix of 1463 aligned base positions (columns of data) was obtained. Two regions each of the 12S and 16S mtDNA data, comprising a total of 64 data columns, were excluded from all analyses due to inability to align these regions with confidence. All deleted columns were from loop regions. Of the remaining 1399 characters, 828 positions were from the 12S mtDNA gene and 571 from the 16S mtDNA gene. After plotting the number of mutations between pairs of taxa against their Tamura–Nei genetic distances, loop regions of both genes appear to have been saturated for transitions as indicated by deviation from a linear relation between the number of mutations and the genetic distance (Figs. 2 and 3). The more conservative stem regions do not display this site saturation (Figs. 2 and 3).

Analysis of the 21-taxon data set included a total of 640 base positions from stem regions and 759 base positions from loop regions. Of the 1399 data columns, 816 were invariant, 526 were parsimony informative, and 57 were variable but uninformative. A single most-parsimonious tree of 1054 steps (CI = 0.514,

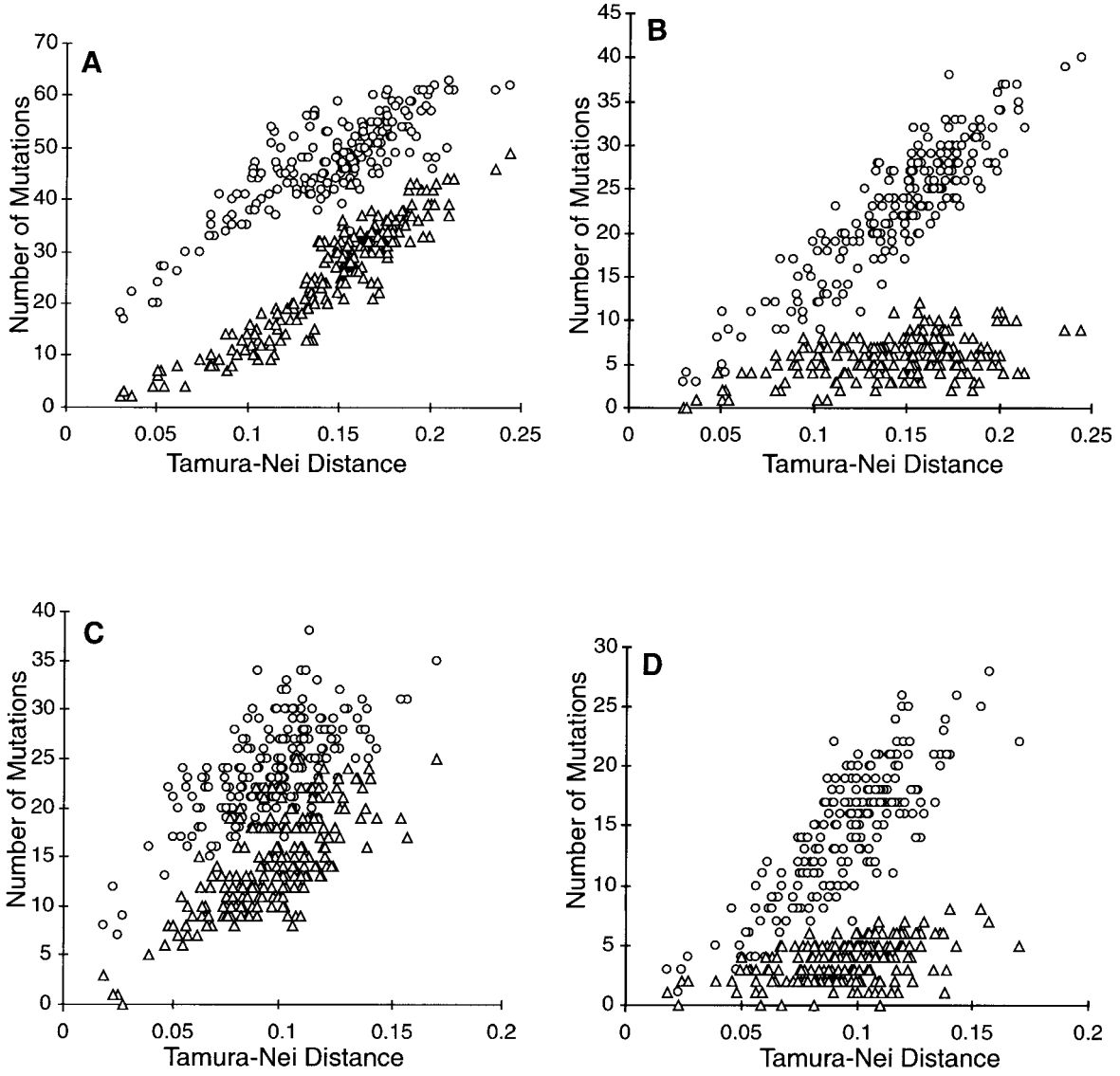

FIG. 2. Scatter plots of the number of mutations vs Tamura–Nei genetic distance in pairwise comparisons for the 21-taxon data set. (A) Rate of site saturation in 12S loop regions. (B) Rate of site saturation in 12S stem regions. (C) Rate of site saturation in 16S loop regions. (D) Rate of site saturation in 16S stem regions. Transitions are indicated by circles (○), and transversions are indicated by triangles (△).

HI = 0.486, RI = 0.480, RC = 0.247; these indices do not include stepmatrix characters) was obtained (Fig. 4). Bootstrap and Bremer decay index values were calculated and are shown in Fig. 4. Additional analyses were performed to examine longer trees in order to further assess stability. These results are presented under Discussion and tree topologies are available from the senior author.

Analysis of the 15-taxon total-evidence matrix consisted of a combined data set of 1585 data columns. Of these 1585 data columns, 952 were invariant, 421 were parsimony informative, and 212 were variable but uninformative. Analysis of this total-evidence matrix employed two different outgroup topologies. The first outgroup option used *Drepane*, Scatophagidae, and Ephippidae as outgroups for acanthuroids *sensu stricto* (i.e., Acanthuroidei *sensu* Tyler *et al.*, 1989). This outgroup topology is derived from the topology of relationships determined from the 21-taxon data set analysis (Fig. 4) and differs from the morphological hypotheses. The results of this analysis nearly matched the results of the 21-taxon analysis, except that the two *Acanthurus* species and *Ctenochaetus* collapse into a trichotomy (Fig. 5a). Two most-parsimonious trees with a length of 754 steps (CI = 0.690, HI = 0.310, RI = 0.766, RC = 0.528; these indices do not include stepmatrix characters) resulted from this 15-taxon analysis (Fig. 5a). An alternate outgroup option used *Drepane* as the only outgroup for Acanthuroidei *sensu lato.* This choice of outgroup, while different from the relation-

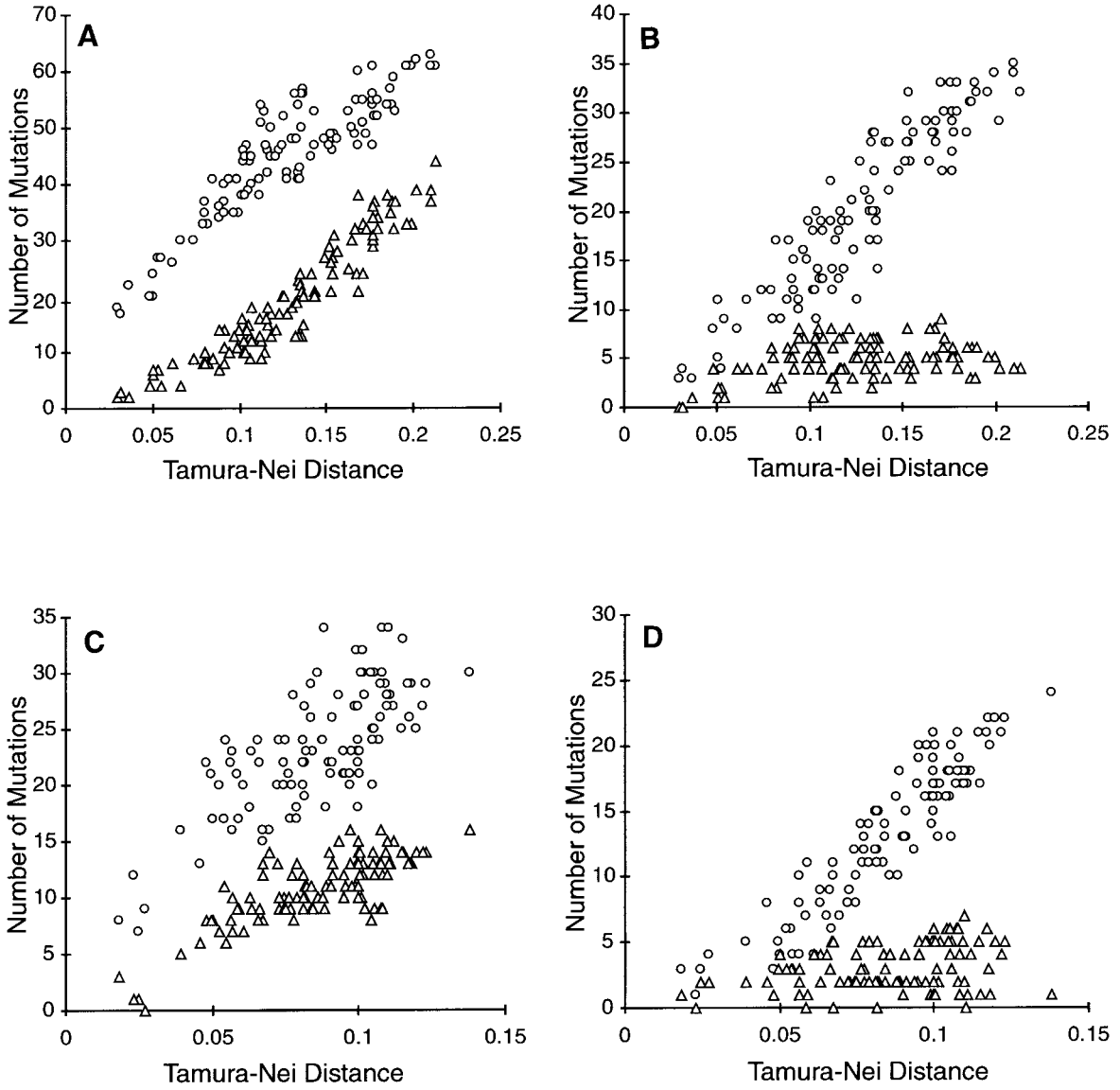

FIG. 3. Scatter plots of the number of mutations vs Tamura–Nei genetic distance in pairwise comparisons for the 15-taxon data set. (A) Rate of site saturation in 12S loop regions. (B) Rate of site saturation in 12S stem regions. (C) Rate of site saturation in 16S loop regions. (D) Rate of site saturation in 16S stem regions. Transitions are indicated by circles (○), and transversions are indicated by triangles (△).

ships recovered from the 21-taxon analysis, is congruent with previous morphological hypotheses (Tyler *et al.*, 1989; Winterbottom, 1993). The topology that resulted from this alternative rooting is shown in Fig. 5b.

Analysis of the 15-taxon matrix using only the DNA data was performed to provide a basis of comparison with the 15-taxon total-evidence analysis. A single most-parsimonious tree with a length of TL = 562 steps (CI = 0.623, HI = 0.377, RI = 0.601, RC = 0.374; these indices do not include stepmatrix characters) resulted from this analysis (Fig. 6a). As with the total-evidence analysis, the rooting decision significantly affects the topology of relationships among the basal acanthuroids. Use of multiple outgroups (*Drepane,* Scatophagidae, and Ephippidae) results in a tree identical to the 21-taxon analysis (Fig. 6a), whereas use of only one outgroup (*Drepane*) produces a tree similar to previous morphological hypotheses (Fig. 6b). The relationships among the higher acanthuroids in either topology are nearly identical to the corresponding total-evidence tree, except that in the DNA-only analysis *Zanclus* and *Naso* form a clade and the *Acanthurus* + *Ctenochaetus* clade is resolved, both of which are consistent with the topology generated from the 21-taxon analysis. Comparison of this most-parsimonious DNA-only tree (TL = 562) with the morphological tree (TL = 576) using the Templeton's test indicates that these two trees do not differ significantly ($P = 0.142$).

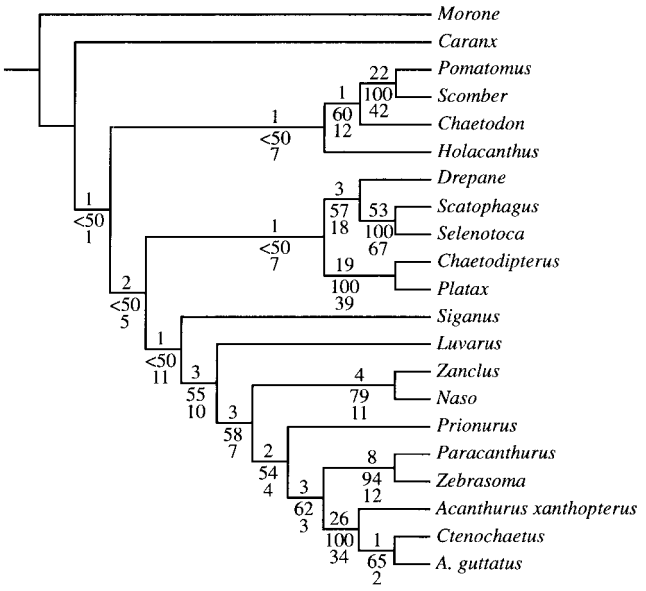

FIG. 4. Single most-parsimonious tree resulting from a weighted parsimony analysis of 12S and 16S ribosomal mtDNA genes in 21 taxa. Bremer decay index support (top), bootstrap values for 1000 replicates (middle), and branch lengths (bottom) are reported for each node. TL = 1054, CI = 0.514, HI = 0.486, RI = 0.480, and RC = 0.247 (these indices do not include stepmatrix characters).

The total-evidence analysis rejects a sister-group relationship between *Zanclus* and *Naso*, while corroborating the hypothesis that *Zanclus* is the sister group of a monophyletic Acanthuridae (Fig. 5). This contradicts both the 21-taxon and the 15-taxon DNA-only analyses (Figs. 4 and 6), raising the suspicion that other factors may be confounding the DNA analyses. A parametric bootstrap simulation was performed to explore the possibility that some aspect of the nature of DNA evolution was causing this discrepency. A parametric bootstrap simulation was conducted to test the hypothesis that rates of evolution along branches did not interfere with reconstructing the phylogeny of the group. The null hypothesis was the tree topology from Winterbottom (1993) for the 15-taxon data set, and the alternate hypothesis was the DNA-only tree topology for the 15-taxon data set with *Drepane*, scatophagids, and ephippids as outgroups. The simulation used maximum likelihood values from optimization of the Winterbottom (1993) tree using values derived from the original data matrix. Sequence length was 1463 bases with base frequencies of: A = 0.300, C = 0.261, G = 0.220, and T = 0.219. A total of 100 replicated data sets was generated using an estimated γ rate heterogeneity of $\alpha = 0.5141$ (four categories of data) and a transition/transversion ratio of $t = 3.983$ under the HKY model of DNA substitution (Hasegawa *et al.*, 1985). The 100 simulated data sets yielded 137 equally most-parsimonious trees. The majority consensus tree was topographically identical to the null tree except for one grouping: 62% of the trees contained the *Zanclus* + *Naso* clade that was present in the most-parsimonious DNA-only tree topology.

DISCUSSION

The 21-taxon data set produced a tree topology that has many areas of agreement with previous morphological hypotheses (Johnson and Washington, 1987; Tyler *et al.*, 1989; Guiasu and Winterbottom, 1993; Winterbot-

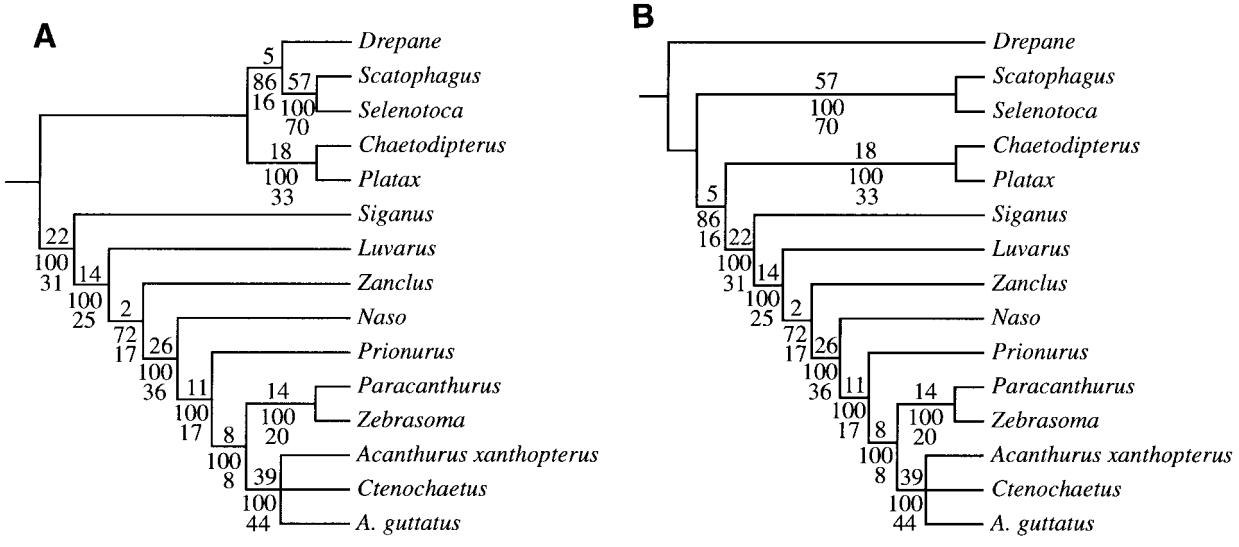

FIG. 5. Weighted parsimony analysis of 15-taxon total-evidence data set with (A) *Drepane*, Ephippidae, and Scatophagidae designated as outgroups; and (B) *Drepane* designated as the outgroup. Bremer decay index support (top), bootstrap values for 1000 replicates (middle), and branch lengths (bottom) are reported for each node. TL = 754, CI = 0.690, HI = 0.310, RI = 0.766, and RC = 0.528 (these indices do not include stepmatrix characters).

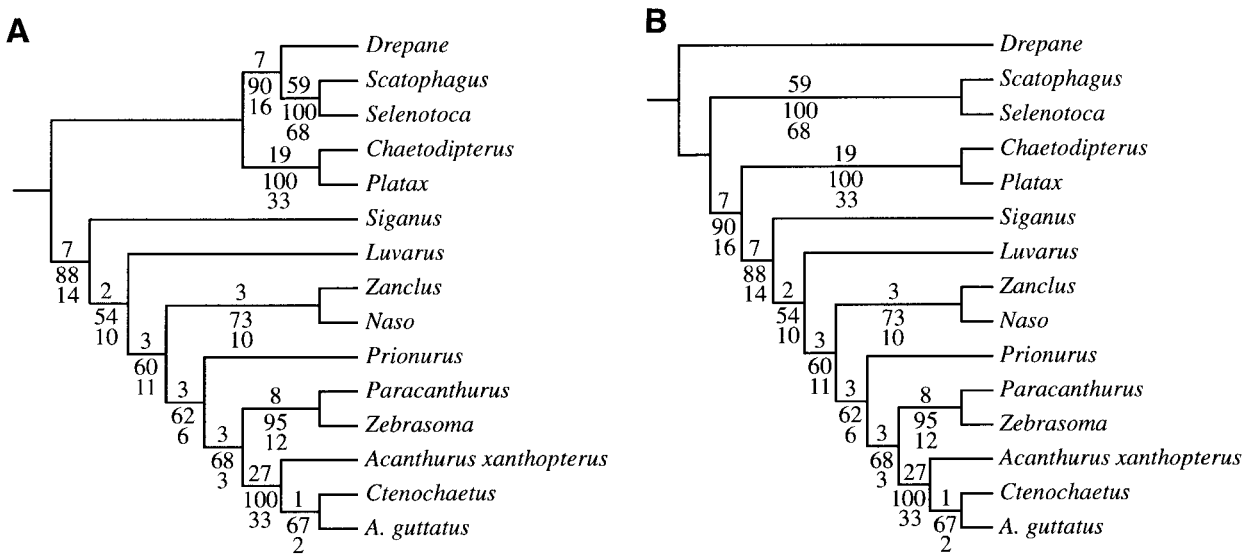

FIG. 6. Weighted parsimony analysis of 15-taxon DNA data set with (A) *Drepane*, Ephippidae, and Scatophagidae designated as outgroups; and (B) *Drepane* designated as the outgroup. Bremer decay index support (top), bootstrap values for 1000 replicates (middle), and branch lengths (bottom) are reported for each node. TL = 562, CI = 0.623, HI = 0.377, RI = 0.601, and RC = 0.374 (these indices do not include stepmatrix characters).

tom, 1993); perhaps the most significant is placing *Luvarus* as the sister group of *Zanclus* + Acanthuridae (Fig. 4). This result is in complete agreement with the morphological hypotheses and reinforces the contention that the louvar is in fact a highly derived, pelagic acanthuroid. These results differ from the previous morphological hypotheses on three major points. First, the analysis suggests that *Drepane* is the sister taxon of Scatophagidae rather than the sister group of Acanthuroidei, as proposed in earlier hypotheses. Second, it suggests that a clade composed of Ephippidae plus *Drepane* + Scatophagidae is sister to the remaining acanthuroid fishes (Acanthuroidei *sensu stricto*). Third, it suggests a sister-group relationship between *Zanclus* and *Naso*. None of these hypotheses are well supported. The best-supported node, the *Zanclus* + *Naso* clade, has a bootstrap value of 75% and a Bremer decay index value of 4. The *Drepane* + Scatophagidae hypothesis also has weak support (bootstrap = 66%, Bremer decay index = 3), but the basal clade of *Drepane*, Scatophagidae, and Ephippidae is very weakly supported (bootstrap $<$ 50%, Bremer decay index = 1) due to instability of the *Drepane* + Scatophagidae clade. In some trees that are two steps longer than the most-parsimonious one, the *Drepane* + Scatophagidae clade falls outside of the Acanthuroidei entirely. Similarly, the support for Acanthuroidei *sensu stricto* is weak (bootstrap $<$ 50%, Bremer decay index = 1) due to instability of the position of Siganidae, which falls outside of the acanthuroids in some trees that are one step longer than the most-parsimonious one. In general, support values (bootstrap and Bremer) at various nodes in the 21-taxon tree are lower than the support values at those same nodes in the 15-taxon trees. Removal of the various outgroup taxa probably decreases the noise in the data. The outgroup taxa in some cases are distant relatives to the acanthuroids, and their inclusion in the data matrix likely reduced the phylogenetic signal, resulting in the lower support. In the total-evidence tree, the morphological data provided additional support, thereby further improving the support values at all the nodes (Fig. 5).

Restricted (15-taxon) analyses were performed to integrate the available morphological data, providing a better estimate of phylogeny. Although the total-evidence tree using three functional outgroups (Fig. 5a) appears different from the one using only *Drepane* (Fig. 5b) as the outgroup, this is an artifact of rooting choices, as the unrooted trees are identical. The corresponding DNA-only analyses of the 15-taxon data set are similar, wherein the apparent differences in tree topology are entirely due to rooting and outgroup choice (Fig. 6). This conflict makes the relationships of the basal acanthuroids and *Drepane* difficult to resolve. The best solution would be to include additional outgroups other than *Drepane* so that rooting choice is not an issue. Although the solution is clear, the necessary morphological data are not available to execute a total-evidence analysis. However, the DNA evidence (the 21-taxon data set), which is the only available data with additional outgroups, supports the topology derived from rooting with three outgroups, favoring a basal clade of *Drepane*, Scatophagidae, and Ephippidae within the Acanthuroidei (Fig. 4). A more conclusive resolution might result from a better sampling of outgroup taxa that are more closely related to the

Acanthuroidei. Unfortunately, the still limited understanding of perciform relationships does not allow us to identify the most appropriate outgroup taxa for such an analysis.

The *Zanclus* + *Naso* clade appeared in 62% of the trees generated from the simulated data sets based on values obtained from the null hypothesis: Winterbottom's (1993) tree. That this clade appears despite modeling based on the null hypothesis leads to the conclusion that some intrinsic properties of the DNA data are causing this group to appear. Assuming that the model of DNA evolution is minimally realistic, the *Zanclus* + *Naso* grouping is likely an artifact of long-branch attraction. That this grouping may be artificial is unsurprising, because morphological hypotheses do not place *Zanclus* within the Acanthuridae, as *Zanclus* is distinguished from the acanthurids by a number of characters, most notably its lack of any kind of caudal spine or plate from which the surgeonfishes derive their common name. However, no such long-branch attraction can be invoked to explain the unexpected basal position of the Scatophagidae relative to the other acanthuroids.

The genus *Acanthurus* appears to be paraphyletic relative to *Ctenochaetus*. A prevalent hypothesis is that species with a thick-walled stomach, such as *Acanthurus xanthopterus*, are more closely related to *Ctenochaetus*, species of which all have a thick-walled gut, than they are to thin-walled species such as *Acanthurus guttatus* (Aoyagi, 1943; Randall, 1955). Although the results of the DNA-only analyses (Figs. 4 and 6) suggest that *Acanthurus* is not monophyletic, they do not support the preceding hypothesis, as the thin-walled *A. guttatus* forms a clade with the representative *Ctenochaetus*, with *A. xanthopterus* as the sister to that group (Figs. 4 and 6). Support for the latter relationship is weak (bootstrap $< 50\%$, Bremer decay index $= 1$), collapsing entirely in the total-evidence analyses (Fig. 5). Nonetheless, this result again raises the issue of *Acanthurus* monophyly. This is a question that merits further investigation but is beyond the scope of this study.

The 12S and 16S mtDNA data in the 21-taxon analysis convincingly place *Luvarus* within the Acanthuroidei. This analysis provides an independent test of its relationship to the acanthuroids, a hypothesis for which compelling morphological evidence has been presented (Johnson and Washington, 1987; Tyler *et al.*, 1989; Winterbottom, 1993). Therefore this result may really be a test of the veracity of the DNA data at this level of analysis. However, no strong conclusions about the relationships among the nonacanthuroid outgroups can be drawn from this analysis. Taxon sampling is too sparse and support is too weak for the results to be meaningful. The relationships within the Acanthuroidei *sensu stricto* derived from the DNA data are nearly congruent with Tyler *et al.* (1989) and Winterbottom (1993). One exception (*Zanclus* + *Naso*) can be reasonably explained as a result of long-branch attraction, but the position of scatophagids, ephippids, and *Drepane* is not satisfactorily resolved and will require a more extensive DNA and morphological sampling of perciform outgroups.

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