

# On the Status of the Serranid Fish Genus *Epinephelus*: Evidence for Paraphyly Based upon 16S rDNA Sequence

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Received June 14, 2000; revised November 14, 2000; published online March 8, 2001

Historically, attempts to elucidate evolutionary relationships among members of the genus *Epinephelus* (Teleostei: Serranidae), commonly known as groupers, have been hindered by the overwhelming number of species (98, *sensu stricto*), a pan global distribution, and the lack of morphological specializations traditionally used in ichthyological classification. To date, no comprehensive phylogenetic study, morphological or molecular, to evaluate the monophyly of this genus has been presented. In this study, previous hypotheses regarding the relationships among the American grouper species and the allied genera were evaluated by examination of mitochondrial DNA sequences of the 16S ribosomal DNA region. A 590-bp region of the 16S rDNA gene was amplified using a universal primer pair for 42 serranid species, including members of the genera *Epinephelus*, *Mycteroperca*, and *Paranthias* from the New World and selected Indo-Pacific congeners. Maximum-parsimony criteria and neighbor-joining analysis dispute the monophyly of the American *Epinephelus* species as previously hypothesized. The data support the monophyly of *Cephalopholis* only with the inclusion of the morphologically distinct *Paranthias* and the monophyly of *Mycteroperca* with the inclusion of the Indo-Pacific *Anyperodon leucogrammicus*. © 2001 Academic Press

**Key Words:** *Epinephelus*; *Cephalopholis*; *Mycteroperca*; *Paranthias*; *Anyperodon*; groupers; 16S; phylogenetic analysis; perciformes.

## INTRODUCTION

The genus *Epinephelus* (*sensu stricto*) comprises 98 species of perciform fishes commonly known as groupers (Heemstra and Randall, 1993). Extending from tropical to subtropical habitats worldwide, *Epinephelus* species typically inhabit reefs to a depth of 150 m (Heemstra and Randall, 1993). Whereas most groupers are midsized predators, *Epinephelus* species can vary in size from less than 0.5 m to nearly 2.0 m, with some species reaching nearly 455 kg (e.g., *E. itajara*; Robins

*et al.*, 1986). This large size, coupled with their abundance and behavioral adaptation of forming spawning aggregations, has placed the groupers under considerable fishing pressure. The Food and Agriculture Organization (FAO) of the United Nations recognized the substantial contribution of grouper species to the world's fish harvest and estimated the total grouper catch at more than 97,000 metric tons in 1990, highlighting the need for conservation of these species (Heemstra and Randall, 1993).

In general, the many species within *Epinephelus* lack morphological specializations that are typically used to identify individual species in the field. Most often, color pattern and geographic locality are used in the field to identify grouper species. This has led to a great deal of taxonomic confusion within the genus and, coupled with the lack of morphologically distinct attributes, may presumably be a cause for the designation of falsely identified new species (Randall *et al.*, 1993; Heemstra and Randall, 1993). With the exception of the various color schemes among *Epinephelus* species, the gross morphological similarity within *Epinephelus* is a reflection of the generalized body form seen in the entire serranid subfamily Epinephelinae, to which *Epinephelus* has been designated. The homogeneous nature of the morphology has led to problems in reconstructing evolutionary relationships among the grouper species (e.g., Smith, 1971).

Morphological and osteological characters have been the primary data types used to classify grouper species (e.g., Smith, 1971; Randall and Ben-Tuvia, 1983; Randall and Heemstra, 1991; Heemstra and Randall, 1993). The first detailed study utilizing morphological data examined the New World members of the genera *Alphesthes*, *Cephalopholis*, *Dermatolepis*, *Epinephelus*, *Mycteroperca*, and *Paranthias* (Smith, 1971). Focusing primarily on the position and orientation of the three prominent neurocranial crests, Smith (1971) combined the genera *Alphesthes*, *Cephalopholis*, and *Dermatolepis* with *Epinephelus*. The first phylogenetic treatment of such data for the Serranidae (Johnson, 1983) examined the three hypothesized subfamilies Serraninae, Epi-

nephelinae, and Anthiinae (*sensu* Jordan and Eigenmann, 1888; Gosline, 1966). Johnson's (1983) study resulted in the diagnosis of the monophyletic subfamily Epinephelinae, including the hypothesized ancestor *Niphon spinosus*, based upon a single reductive specialization of loss of an autogenous distal radial on the first dorsal pterygiophore, and the division of the subfamily into the five tribes currently recognized (Niphonini, Epinephelini, Diploprionini, Liopropomini, and Grammistini). Subsequently, a morphological phylogenetic hypothesis supporting a monophyletic Epinephelinae and a partially resolved generic phylogeny were provided (Baldwin and Johnson, 1993).

Larval data have also been used with considerable success in the classification of the three subfamilies within the Serranidae (Kendall, 1979; Leis, 1986; Baldwin, 1990; Baldwin *et al.*, 1991; Baldwin and Smith, 1998). From these studies, several ontogenetic characters that may be applicable to phylogenetic hypotheses (e.g., position and movement of melanophores and development of dorsal series) and the identification of characters that aid in assessing the monophyly of the Epinephelinae (e.g., elongation of second dorsal spine in larvae) have been identified. To date, only one biochemical analysis of phylogenetic relationships within the genus *Epinephelus* has been presented (Lopez-Lemus, 1988). Based upon isozyme data, the study examined four species of *Epinephelus* from the eastern Pacific, leaving several questions as to the taxonomic placement of the majority of the remaining species. Thus far, no phylogenetic hypothesis has been provided for either the speciose tribe Epinephelini or the genus *Epinephelus*, leaving much confusion as to the monophyly of *Epinephelus* and its three included subgenera as proposed by Smith (1971).

The large number of species within *Epinephelus*, coupled with their circumglobal distribution, is a key factor in the noted absence of a thorough morphological phylogenetic analysis. Mitochondrial DNA sequence analysis provides an effective means by which we may investigate the phylogenetic relationships of a large group of organisms and has been used recently with considerable success (e.g., Miya and Nishida, 1996; Bernardi and Bucciarelli, 1999; Tringali *et al.*, 1999). In the current study, the mitochondrially encoded 16S rDNA gene was partially sequenced for 42 species from 11 genera of epinepheline serranids. Primarily from North and South America, the species examined were chosen to provide the first systematic attempt to assess the speciose fish genus *Epinephelus* and to address its monophyly and placement within the subfamily Epinephelinae. The evaluation of the phylogenetic relationships within *Epinephelus* on a circumglobal scale including all extant taxa will be key to understanding the true relationships within the genus. Given the logistical constraints presented by this task, however, it seems appropriate to present a preliminary investi-

gation into the monophyly of the genus based upon the species currently available. Here, we present data that provide a partial, well-resolved phylogeny suggesting that three currently recognized genera are paraphyletic: *Cephalopholis*, *Epinephelus*, and *Mycteroperca*. The data also suggest that grouper lineages are not limited to present-day biogeographical provinces.

## MATERIALS AND METHODS

Mitochondrial DNA sequences from a total of 42 species (36 ingroup and 6 outgroup) were used to evaluate phylogenetic relationships within the genus *Epinephelus* (*sensu lato*). Specimens were collected in the field by hook and line and spear pole or obtained from commercial fish markets or fishing vessels. Species were identified in the field by the senior authors (M.C. and D.P.) and confirmed upon being deposited at the Scripps Institution of Oceanography Marine Vertebrates Collection (Appendix). Individuals of unambiguous species and those for which only one individual was available for biochemical analysis were retained in the personal collections of the senior authors at Moore Laboratory of Zoology, Occidental College. Gill filaments were removed from fresh specimens that were sacrificed and retained as vouchers, and pectoral fin clips were taken from frozen and/or live specimens. Two or three individuals from each species were used in sequence analysis. In some instances, however, the exceeding rarity of many grouper species (e.g., *E. itajara*) allowed only one individual for analysis (Appendix).

All tissues were stored at ambient temperature while in the field and at  $-20^{\circ}\text{C}$  under laboratory conditions. Tissues were preserved with 5 $\times$  Net solution (2.5 M NaCl, 0.25 M EDTA, 0.25 M Tris base, pH 8.0). Total genomic and mitochondrial DNA were isolated from approximately 0.5 g of tissue following the protocol included with the Genomic-Prep Cells and Tissues DNA Isolation kit (Amersham Pharmacia Biotech). Homogenized tissues were digested for 45 min at  $65^{\circ}\text{C}$ . Polymerase chain reaction was used to amplify a 590-bp fragment of the mitochondrial 16S RNA gene. One hundred-microliter amplification reactions were prepared with 10–100 ng of DNA, 1.5 mM  $\text{MgCl}_2$ , 2.5 units *Taq* Polymerase, 200  $\mu\text{M}$  dNTP's, and 0.1  $\mu\text{M}$  each primer. Thirty cycles of the following step procedure were performed using an MJ Research PTC-100 Programmable Thermal Controller following a 5-min denaturation at  $94^{\circ}\text{C}$ :  $94^{\circ}\text{C}$  for 1 min 30 s,  $45^{\circ}\text{C}$  for 2 min,  $72^{\circ}\text{C}$  for 1 min 30 s. Primer sequences used were: 16sarL 5'-CGCCTGTTTATCAAAAACAT-3' and 16sbrH 5'-CCGGTCTGAACTCAGATCACGT-3' (Palumbi, 1996). PCR amplification products were purified on a 1% low-melting-point agarose gel stained with ethidium bromide. Desired products were identified by size using  $\phi\text{X174}$  *Hae*III DNA ladder as a reference marker. PCR fragments were separated from

the gel using a Wizard-Prep PCR Purification Kit (Promega Corp., Madison, WI). Automated fluorescent dye-deoxy sequencing of both strands was carried out using an ABI Prism 377 sequencer using the above primers.

### Sequence Analyses

Sequences from both strands of all individuals from each species were assembled into a single consensus sequence using the assembly editor option in the computer program Gene-tool (ver. 1.0, Biotools, Inc). Consensus sequences were aligned using the alignment program Clustal W with default settings (Thompson *et al.*, 1994). Visual optimization using MacClade (V. 3.07, Maddison and Maddison, 1997) was necessary to align regions corresponding to loops in the 16S rRNA secondary structure where hypervariability in nucleotide substitution is known to occur (Meyer, 1993; Ortí and Meyer, 1997). The secondary structure of Ortí *et al.* (1996) developed for piranha 16S rRNA was used as a model as these secondary structures are conserved over large phylogenetic expanses (Meyer, 1993). An 11-bp fragment of the epinepheline 16S sequence (corresponding to characters 240–251 in region “j” of the secondary structure of Ortí and Meyer, 1997) was excluded from the analysis due to the ambiguity of the aligned characters. Indels were coded as a single character so as not to place undue character weight on a single mutational event leading to the insertion or deletion of multiple bases.

Phylogenetic relationships were determined based upon maximum-parsimony criteria with the computer program PAUP\* 4.0b4a (Swofford, 1998) using the heuristic search option with 200 random addition replicates and the tree bisection reconnection (TBR) branch swapping algorithm. The maximum-parsimony method was chosen as it serves to decrease the influence of homoplastic substitutions (Li, 1997) and maximizes character congruence (Forey *et al.*, 1992). A neighbor-joining analysis (Saitou and Nei, 1987) was also performed using default settings in PAUP\* 4.0b4a to evaluate genetic distances between species and aid in evaluating topology achieved by the parsimony tree. This analysis was chosen over other distance-based, tree-building algorithms as it is sensitive to unequal rates of sequence divergence and has been shown to recover the correct tree topology in modeled situations (Saitou and Nei, 1987). Relative support at nodes was evaluated using bootstrap analysis with 1000 replicates (Felsenstein, 1985). Consistency and retention indices (CI and RI, respectively) were generated within the computer program PAUP\* 4.0b4a for the parsimony tree. Transition/transversion ratios among sequences for all pairwise comparisons were calculated using the computer program MEGA (Kumar *et al.*, 1993). In addition to the empirical value, several weighting schemes for transition/transversion ratios (4:1, 3:1, 2:1, 1:1) were used to examine alternative tree

topologies. Skewness and kurtosis estimates ( $g_1$  and  $g_2$ , respectively) were calculated for a distribution of 10,000 random trees generated in PAUP\* 4.0b4a to evaluate the degree of phylogenetic signal contained in the sequence alignment.

### Outgroup Selection

The most recent cladistic refinement of the Epinephelinae (Baldwin and Johnson, 1993) was used as a model for outgroup selection. The serranine *Paralabrax nebulifer* and the Anthiine *Pronotogrammus multifasciatus* were used as outgroups to determine character polarity for the Epinephelinae. In addition, two species of *Plectropomus* (*P. maculatus* and *P. leopardus*), two members of the tribe Grammistini (*Pogonoperca punctata* and *Rypticus saponaceus*), and the monotypic *Anyperodon* from the Indo-Pacific were also examined as outgroups for the genus *Epinephelus*. To evaluate the monophyly of the New World species of *Epinephelus*, three Indo-Pacific species (*E. fasciatus*, *E. undulosus*, and *E. areolatus*) were also examined.

## RESULTS

The mitochondrial 16S ribosomal RNA gene was partially sequenced for all individuals examined. Of the 572 aligned base pairs, 356 were constant, 163 were parsimony informative, and 53 were parsimony uninformative. Eighteen most parsimonious trees (length = 701 steps) were found using the heuristic search option in PAUP\* 4.0b4a. A strict consensus tree (CI = 0.47, RI = 0.61) is depicted in Fig. 1. Transitions were more common than transversions; the average transition/transversion ratio among all pairwise comparisons was 2.28. This suggests that the sequences have not reached the saturation zone (Meyer, 1993). Using various weighting schemes for transition/transversion ratio did not alter the tree topology, and the empirical value of 2.28 was used for the final analysis. The neighbor-joining (NJ) analysis yielded a tree with nearly identical topology as that of the parsimony tree (Fig. 2). The  $g_1$  statistic for 10,000 random trees was highly significant ( $g_1 = -0.824$ ,  $P \ll 0.01$ ), indicating a high level of phylogenetic signal in the sequence data (Hillis and Huelsenbeck, 1992).

The sequence data examined support two distinct clades dividing the genus *Epinephelus*. These clades are recognized both by parsimony criteria and by neighbor-joining analysis (Figs. 1 and 2, respectively). The first clade includes the New World species *E. niveatus*, *E. nigrinus*, *E. niphobles*, *E. acanthistius*, *E. mystacinus*, *E. flavolimbatus*, and *E. cifuentesi*. This clade also includes an internal node uniting the two eastern Pacific species of *Alphestes* (*A. immaculatus* and *A. multiguttatus*), *Dermatolepis dermatolepis*, and *E. drummondhayi*; monophyly of this clade is well supported by bootstrap analysis (87% parsimony, 80% NJ).

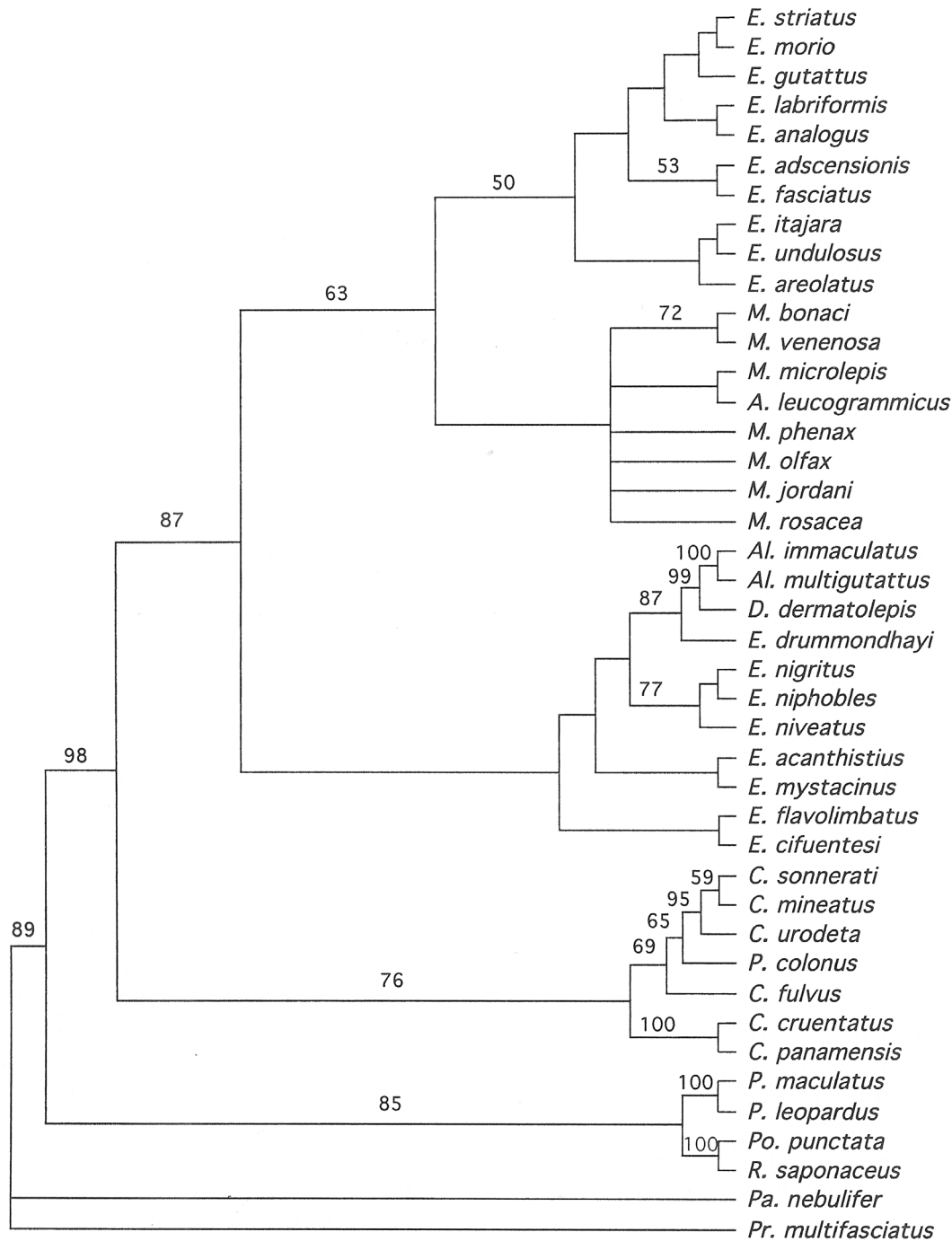


FIG. 1. Strict consensus of 18 most parsimonious trees depicting phylogenetic relationships among 42 species of epinepheline Serranids based upon 16S rDNA sequences. Numbers above nodes represent bootstrap values (%) with 1000 replicates. CI = 0.47, RI = 0.61.

The two species of *Alphestes* are grouped by a common 5-bp indel in the 16S rDNA gene. The second clade comprises the New World species *E. itajara*, *E. labriiformis*, *E. analogous*, *E. gutattus*, *E. striatus*, *E. morio*, and *E. adscensionis*, and the Indo-Pacific species *E. areolatus*, *E. fasciatus*, and *E. undulosus*. Strong bootstrap support exists for the splitting of these two clades (87% parsimony, 100% NJ); yet, relationships within the clades are not well resolved.

Both parsimony criteria and neighbor-joining analysis show that there is support for a paraphyletic grouping of the six species of *Cephalopholis* examined, with the inclusion of the morphologically

divergent *Paranthias colonus*. The Indo-Pacific species (*C. sonnerati*, *C. urodeta*, and *C. mineatus*) form a clade with the New World species (*C. fulvus*, *C. cruentatus*, and *C. panamensis*). The three Indo-Pacific species (*C. sonnerati*, *C. urodeta*, and *C. mineatus*) are united by a 3-bp indel in the 16S rDNA sequence and are supported by a high bootstrap value (95% parsimony, 97% NJ). Both parsimony criteria and neighbor-joining analysis support a *Cephalopholis* + *Paranthias* clade with a high bootstrap value (76% parsimony, 81% NJ).

The seven species of the genus *Mycteroperca* are united in a paraphyletic group with the inclusion of the

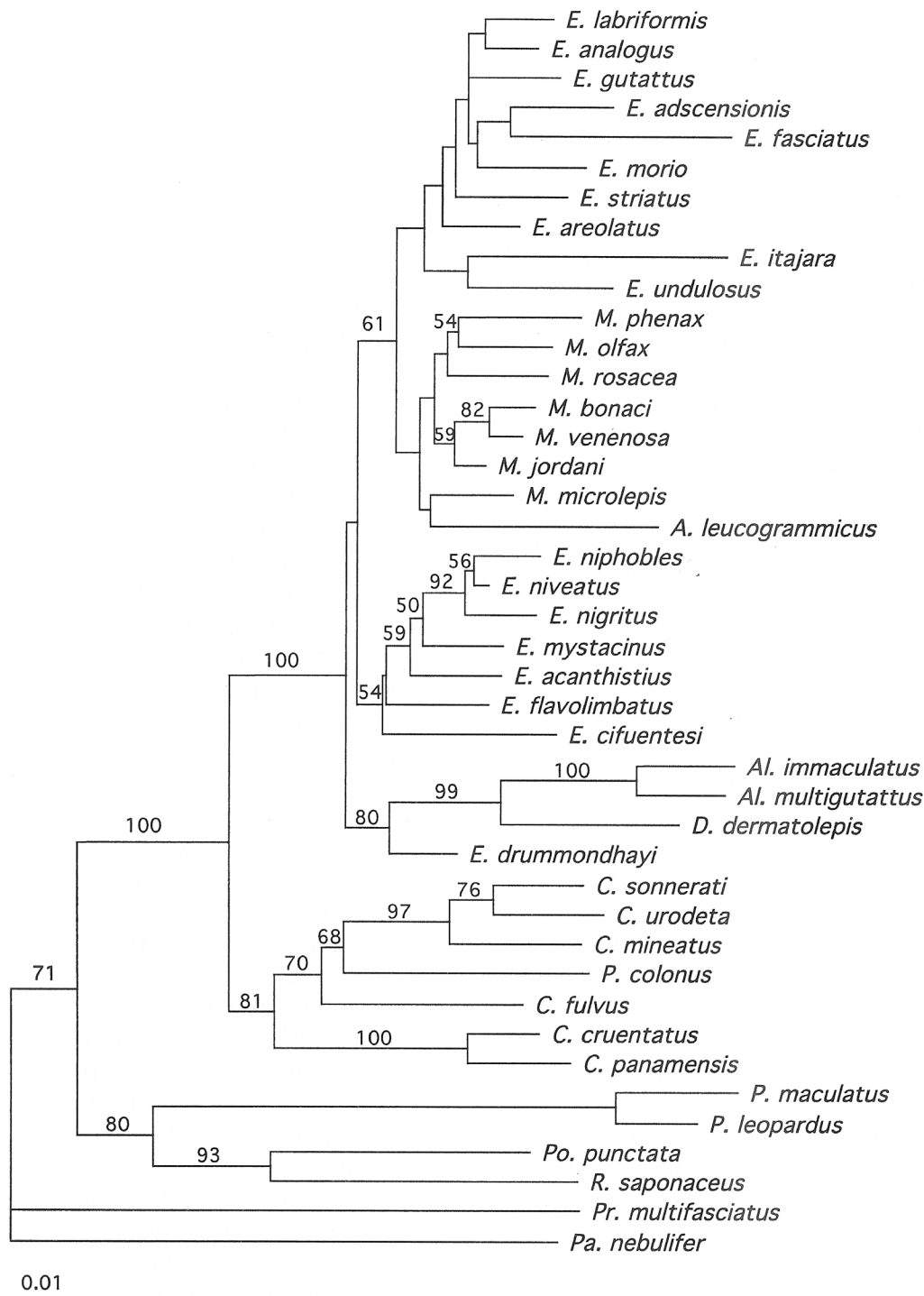


FIG. 2. Neighbor-joining tree depicting relationships for 42 species of epinepheline Serranids based upon genetic similarity of 16S rDNA sequences. Scale is uncorrected "p" distance. Numbers at nodes are bootstrap values (%) based on 1000 replicates.

monotypic *Anyperodon*. Although this clade lacks strong bootstrap support, support does exist for a *Mycteroperca*-*Epinephelus* clade (63% parsimony, 61% NJ). The two members of the soapfish tribe Grammistini (*Pogonoperca punctata* and *Rypticus saponaceus*) are united with strong support (100% parsimony, 93% NJ). Strong support exists also for the formation of a clade including the members of the soapfish tribe Grammistini and the two species of *Plectropomus* examined (85% parsimony, 80% NJ).

## DISCUSSION

The data collected in this study do not corroborate previous hypotheses of the interrelationships among the New World grouper genera (Smith, 1971). There is no genetic evidence to support the monophyly of the American groupers, nor is there evidence to support the proposed subgenera of Smith (1971). The data suggest that, as currently defined, the genera *Epinephelus*, *Cephalopholis*, and *Mycteroperca* are paraphyletic.

### *Dermatolepis* and *Alphestes*

Strong genetic affinities exist among *Dermatolepis*, *Alphestes*, and *E. drummondhayi*. Within *Alphestes*, Smith (1971) synonymized *A. immaculatus* and *A. afer*, but retained *A. multiguttatus*. However, Johnson and Keener (1984) noted differences in the larval morphology of Atlantic *A. afer* and Pacific *A. immaculatus*, and Heemstra and Randall (1993) documented the validity of all three species based on differences in color pattern and modal gill raker counts. *Alphestes* differs from all other epinepheline genera (except *Gonioplectrus* and *Niphon*) in having a single, antrorse spine at the angle of the preopercle. Further, the dorsal and lateral surfaces of the neurocranium in larval *Alphestes* are uniquely rugose (Johnson and Keener, 1984). Although Smith united *Alphestes* with *Epinephelus* based upon the similarity of the postocular skull process, the generic status of *Alphestes*, *Dermatolepis*, and *E. drummondhayi* may need reevaluation and is certainly worthy of further study.

### *Mycteroperca*

The genus *Mycteroperca* seems to be a well-defined assemblage based upon 7 of 15 species examined herein. *Mycteroperca* is distinct from *Epinephelus* in having a greater number of anal fin rays (10–13 in *Mycteroperca*, 7–10 in *Epinephelus*) and in differing shapes of the caudal fin. Several species of *Mycteroperca* (*M. acutirostris*, *M. interstitialis*, *M. cidi*, *M. rubra*, *M. fusca*, *M. prionura*, *M. xenarcha*, and *M. tigris*) were not encountered during the sampling period and were not included in this analysis. Thus, it is premature to assess intrageneric relationships. It is worth noting, however, that two previous studies identified similar species groups within *Mycteroperca* from the western Atlantic that are reflected in this study (Cervigón and Velasquez, 1966; Smith, 1971). The first group contains the species *M. venenosa*, *M. bonaci*, *M. jordani*, and *M. tigris*; the second group includes the species *M. interstitialis*, *M. microlepis*, *M. phenax*, *M. cidi*, *M. xenarcha*, *M. prionura*, *M. rosacea*, *M. olfax*, and *M. rubra* (Smith, 1971, included the eastern Pacific species as members of this group). Although not recognized under parsimony criteria, the neighbor-joining analysis of the 16S sequence data recaptures the same species groups with the exception of the placement of *M. microlepis*, which together with *Anyperodon* is the sister group of the remaining *Mycteroperca* species.

The addition of *Anyperodon leucogrammicus* to the *Mycteroperca* clade was unexpected, although few studies to date have examined the phylogenetic position of *A. leucogrammicus*. Heemstra and Randall (1993) hypothesized that the affinities of the monotypic *Anyperodon* lie within *Epinephelus*. They based this on the observation that both genera share 11 dorsal spines and lack trisegmental pterygiophores, characters that would also place *Anyperodon* with *Myctero-*

*perca*. Furthermore, Heemstra and Randall (1993) commented that *Anyperodon* is unique among the groupers in that it lacks palatine teeth and has a markedly elongate body form, yet in their key to grouper genera they diagnosed *Mycteroperca* as having an elongate body form relative to members of *Epinephelus*.

### *Cephalopholis*

Although Smith demoted *Cephalopholis* to subgeneric status in 1971, he subsequently referred to the group as a valid genus (1978, 1981). The monophyly of *Cephalopholis* has yet to be addressed. The genetic data recognize close phylogenetic affinities among the New World species of *Cephalopholis*; as demonstrated by the high bootstrap value, strong support is present between *Cephalopholis cruentatus* and *C. panamensis*. It appears that these two species are a geminate group, separated by the rising of the isthmus of Panama, which is consistent with previous hypotheses (Smith, 1971; Graves *et al.*, 1983).

The support for the reevaluation of the generic status of *Cephalopholis* is supported both by the genetic data in the current study and by that of previous morphological studies. All members of *Cephalopholis* are united in having nine dorsal spines, a character that is shared with only 1 species of *Epinephelus* (*E. acanthistius* of the eastern Pacific) and four other epinephelin genera, two of which are monotypic (*Aethaloperca*, *Gracila*, *Paranthias*, and *Variola*; Heemstra and Randall, 1993). Data compiled on larval development also characterize members of *Cephalopholis* as distinct from *Epinephelus* based upon number and movement of ventral melanophores in pre- and postflexion larvae (Leis, 1986). Heemstra and Randall (1993) commented that 21 species of *Cephalopholis* surveyed by autoradiograph displayed trisegmental pterygiophores in the dorsal fin, whereas all species of *Epinephelus* examined by X ray (41 species) have bisegmental pterygiophores supporting the dorsal elements. The presence of trisegmental pterygiophores may be a primitive serranid character lost independently in some members of the subfamilies Serraninae and Epinephelinae (Baldwin and Johnson, 1993). Thus, the presence of trisegmental pterygiophores in *Cephalopholis* may indicate an ancestral relationship to the remaining Epinephelini (as suggested by this study), and the loss of trisegmental pterygiophores may be a synapomorphy of the remaining epinephelins. The presence of trisegmental pterygiophores, however, is noted in other serranid lineages; hence, the homoplasticity of this character is evident and its phylogenetic implications should be addressed with caution.

The inclusion of *Paranthias* as a derived member of *Cephalopholis* in the current study is the first indication of the paraphyly of *Cephalopholis*. The existence of a clade including *Paranthias* and *Cephalopholis* is not

surprising, however, as both genera share 9 dorsal spines and typically do not reach large size as adults. *Paranthias* also shares ctenoid midlateral scales, epineural ribs on vertebrae 1–9 (*Epinephelus* and *Mycteroperca* typically have epineurals on vertebrae 1–10), and trisegmental pterygiophores with *Cephalopholis* (Baldwin and Johnson, 1993; Heemstra and Randall, 1993). *Cephalopholis* and *Paranthias* also show similar ontogenetic development of the spinous dorsal fin. In larval *Paranthias*, the 8 anterior dorsal spines form directly, followed by the transformation of the anterior-most dorsal soft ray into the posterior-most dorsal spine (Kendall, 1979). In *Cephalopholis*, a similar transformation of the 9th dorsal spine takes place in ontogenetic development (Leis, 1986). This differs from larval Indo-Pacific *Epinephelus* (all with 11 dorsal spines) and American species of *Epinephelus* with 11 dorsal spines, in which 9 spines form directly and the posterior-most 2 form by transformation of soft rays (Kendall, 1979; Leis, 1986). In larval *Mycteroperca*, the first 10 spines form directly, and the 11th forms by transformation of the soft ray (Kendall, 1979). This suggests that the 9-dorsal-spine condition of adult *Cephalopholis* and *Paranthias* is homologous. Additional evidence supporting a close relationship between *Paranthias* and *Cephalopholis* is Smith's (1966) report that *P. furcifer* and *C. fulvus* hybridize. The hybrid, previously reported as *Menephorus dubius* (Poey, 1860), has been collected at Morant Bank, Jamaica (Thompson and Munro, 1978).

The semipelagic behavior of *Paranthias* is unique among epinephelins as are the concomitant morphologically distinct features accompanying the shift in niche occupancy (deeply forked tail, high gill raker counts [37–44 in *Paranthias*], and small teeth; Randall, 1967). This behavior and morphology is apparently convergent on that seen in most species of the subfamily Anthiinae (e.g., *Anthias* spp.; Kendall, 1979).

### *Epinephelus*

Included in Smith's (1971) expansion of *Epinephelus* was the synonymy of the genus *Promicrops* (which included the exceptionally large species *itajara* and *lanceolatus*) and the synonymy of *E. niphobles* with *E. niveatus*. The sequence data indicate that the affinities of the highly derived *E. itajara* lie with the Indo-Pacific members of the genus. Smith's synonymy of *E. niphobles* and *E. niveatus* requires further investigation, as the parsimony criteria and neighbor-joining analysis results are not congruent with respect to these species. Nonetheless, the genetic similarities among *E. niveatus*, *E. niphobles*, and *E. nigritus* suggest that they form a monophyletic group.

One of Smith's (1971) most significant findings was the inclusion of *Bodianus acanthistius* Gilbert in *Epinephelus*, which had previously been reassigned to the genus *Cephalopholis* (Meek and Hildebrand, 1925).

The placement of this species within *Cephalopholis* was due to the presence of nine dorsal spines, a character typically associated with the genus. This confusion resulted in the erection of the subgenus *Enneistus* (Jordan and Evermann, 1896) with subsequent elevation to generic status (Jordan *et al.*, 1930). Previous hypotheses suggested that *E. acanthistius* was most likely part of an "*E. niveatus* species group" encompassing *E. niveatus*, *E. flavolimbatus*, *E. nigritus*, and *E. mystacinus* (Smith, 1971). With the addition of the newly described *E. cifuentesi* (Grove and Lavenberg, 1997), the *niveatus* species group is recognized as a monophyletic group in the current study.

The genetic affinities of *E. acanthistius*, *C. panamensis*, *E. analogus*, and *E. labriformis* were evaluated based on isozyme data (Lopez-Lemus, 1988). These data suggest that *E. acanthistius* is genetically dissimilar to *E. analogus* and *E. labriformis*, supporting the presence of the *niveatus* species group. The phylogenetic analyses in this study support the inclusion of *E. acanthistius* within *Epinephelus* and indeed within the proposed *E. niveatus* species group.

### Outgroup Relationships

The molecular phylogenetic analysis in this study provides considerable support for a monophyletic Epinephelinae (Figs. 1 and 2). The placement of the representative species within the Serraninae and the Anthiinae as sister taxa to the Epinephelinae agrees with previous hypotheses based on morphological data (Johnson, 1983; Baldwin and Johnson, 1993). The sequence data do not support a monophyletic tribe Epinephelini *sensu* Johnson (1983); rather, they suggest that the tribe is paraphyletic with the inclusion of *Plectropomus*. However, considering the implication that members of the soapfish tribe Grammistini are sister to the genus *Plectropomus*, this conclusion is highly unlikely based upon previous morphological data and may be a result of long-branch attraction. Potential rooting problems are also evident and may be resolved upon the inclusion of additional lower percoid representatives to the dataset.

Under the assumptions of maximum-parsimony, several clades that lack strong bootstrap support, most notably within the *Mycteroperca* and *Epinephelus* clades, are recognized. The neighbor-joining analysis of the data demonstrates that between many of these species there is little genetic distance, indicating a very close relationship among species. Perhaps many of these species diverged too recently for the 16S gene to reveal species-level differences.

Few differences exist between the topology of the parsimony tree and that of the tree derived from the neighbor-joining analysis. One difference is the position of *E. striatus*. The parsimony tree suggests that *E. striatus* is a derived member of the *fasciatus* species group, whereas the neighbor-joining analysis suggests

that it is genetically similar to the Indo-Pacific members occupying a primitive position on the tree. It is most likely that sequence comparisons of a faster-evolving gene would elucidate the interrelationships of this species group and that lack of genetic differentiation is the cause for the discrepancy observed in the two analyses.

The second, and perhaps more interesting, difference is the placement of the *Alphestes* + *Dermatolepis* clade. Invariably, *Alphestes* and *Dermatolepis* not only form a clade with each other, but also with *E. drummondhayi*. This relationship is supported by high bootstrap support (87% parsimony, 80% NJ). Under parsimony criteria, the clade appears to be nested within the *niveatus* species complex. The neighbor-joining analysis, however, places this same clade as sister to the *niveatus* species group. The neighbor-joining analysis also reveals that the genetic distance between the *Alphestes* + *Dermatolepis* clade and the *niveatus* species group is quite small. This, coupled with the lack of support for the topology described by the parsimony analysis, suggests that further investigation of this clade is required before interrelationships at the generic level can be discussed. The inclusion of the remaining species of *Alphestes* and *Dermatolepis* may also elucidate genus-level relationships.

## CONCLUSIONS

Based upon the data presented in the current study, the serranid fish genus *Epinephelus* as currently described is paraphyletic, forming two distinct clades under both parsimony criteria and neighbor-joining analysis. The examination of genetic data and a thorough morphological analysis of the many *Epinephelus* species not examined in the current study may further elucidate the interrelationships within the genus. There is strong evidence, both in the current study and in established literature, for the treatment of *Cephalopholis* as a valid genus. However, from a strict cladistic interpretation of this molecular data, *Paranthias* should be included within this genus, despite its ecological and morphological distinctiveness. The alteration of long-standing nomenclature, however, seems premature, given the number of species within *Cephalopholis* yet to be examined. Several researchers have addressed the importance of larval characters with respect to serranid phylogeny (e.g., Johnson, 1988; Baldwin, 1990); thus, the larval data supporting a *Cephalopholis* + *Paranthias* clade is particularly convincing when coupled with the genetic data and the potential for hybridization. The paraphyletic nature of the genus *Mycteroperca* is also apparent from the data presented here. The monotypic *Anyperodon* should be included in *Mycteroperca* to adhere to a strict cladistic definition of the genus, however, the formal designa-

tion of this taxonomic change is withheld pending further clarification of interspecific relationships.

The genetic data examined in this study suggest that the current practice of evaluating evolutionary relationships among grouper genera that are subdivided by geographic locality may not be effective in discerning the true relationships among the *Epinephelini*. Although the number of species that must be considered is great (more than 100 to evaluate *Epinephelus* alone), our data suggest that this technique is a practical approach for reconstructing the phylogenetic relationships of groupers.

## APPENDIX

### Specimens Examined

*Alphestes immaculatus*: SIO 00-92, AF297290, (*N* = 1). *Alphestes multiguttatus*: SIO 00-95, AF297305, (*N* = 2). *Anyperodon leucogrammicus*: SIO 64-235, AF297306, (*N* = 2). *Cephalopholis cruentatus*: AF297323, (*N* = 2). *Cephalopholis fulvus*: SIO 00-146, AF297292, (*N* = 2). *Cephalopholis mineatus*: SIO 64-235, AF297321, (*N* = 1). *Cephalopholis panamensis*: AF297313, (*N* = 3). *Cephalopholis sonnerati*: SIO 64-235, AF297307, (*N* = 2). *Cephalopholis urodeta*: AF297325, (*N* = 1). *Dermatolepis dermatolepis*: SIO 64-235, AF297317, (*N* = 2). *Epinephelus acanthistius*: SIO 00-142, AF297318, (*N* = 1). *Epinephelus adscensionis*: SIO 00-145, AF297314, (*N* = 2). *Epinephelus analogus*: AF297302, (*N* = 1). *Epinephelus areolatus*: SIO 00-235, AF297316, (*N* = 1). *Epinephelus cifuentesi*: SIO 00-138, AF297295, (*N* = 2). *Epinephelus drummondhayi*: SIO 00-152, AF297308, (*N* = 2). *Epinephelus fasciatus*: SIO 64-235, AF297319, (*N* = 2). *Epinephelus flavolimbatus*: SIO 00-150, AF297293, (*N* = 1). *Epinephelus guttatus*: SIO 00-143, AF297299, (*N* = 2). *Epinephelus itajara*: AF297294, (*N* = 1). *Epinephelus labriiformis*: SIO 00-137, AF297296, (*N* = 3). *Epinephelus morio*: SIO 00-145, AF297324, (*N* = 2). *Epinephelus mystacinus*: SIO 00-138, AF297304, (*N* = 2). *Epinephelus nigrurus*: SIO 00-149, AF297297, (*N* = 1). *Epinephelus niphobles*: SIO 64-235, AF297309, (*N* = 1). *Epinephelus niveatus*: SIO 00-151, AF297310, (*N* = 2). *Epinephelus striatus*: SIO 00-146, AF297311, (*N* = 2). *Epinephelus undulosus*: SIO 64-235, AF297326, (*N* = 1). *Mycteroperca bonaci*: SIO 00-145, AF297315, (*N* = 1). *Mycteroperca jordani*: SIO 00-144, AF297329, (*N* = 2). *Mycteroperca microlepis*: SIO 00-148, AF297312, (*N* = 2). *Mycteroperca olfax*: AF317512, SIO 00-89, (*N* = 2). *Mycteroperca phenax*: SIO 00-145, AF297303, (*N* = 2). *Mycteroperca rosacea*: SIO 00-92, AF297300, (*N* = 2). *Mycteroperca venenosa*: SIO 00-147, AF297291, (*N* = 2). *Paranthias colonus*: SIO 00-89, AF297301, (*N* = 1). *Plectropomus leopardus*: SIO 64-235, AF297298, (*N* = 2). *Plectropomus maculatus*: SIO 64-235, AF297320, (*N* = 1). *Pogonoperca punctata*: SIO 64-235, AF297322, (*N* = 1). *Paralabrax*



*nebulifer*: SIO 00-97, AF297328, ( $N = 2$ ). *Pronotogrammus multifasciatus*: SIO 00-139, AF297330, ( $N = 2$ ). *Rypticus saponaceus*: AF297327, ( $N = 2$ ).

*Note.* Museum numbers are listed for the Marine Vertebrates Collections at Scripps Institution of Oceanography. GenBank accession numbers are provided for nucleotide data. Sample size ( $N$ ) is number of individuals examined in the current study.

## ACKNOWLEDGMENTS

Several institutions graciously provided funds in support of this project: The Vantuna Research Group, Occidental College; Larry G. Allen, California State University Northridge (Nearshore Marine Fish Research Program); Wayne Ishimoto, Chevron Products Company; The American Museum of Natural History Lerner Grey Fund for Marine Research; the Paul K. and Evelyn E. Cook Richter Trust Fund Committee; and David Axeen, Dean of Faculty, Occidental College. We thank the Charles Darwin Research Station and Galápagos National Park System for coordinating fieldwork in Ecuador. The authors acknowledge the following people for their assistance in collecting specimens used in this project: Gregory R. Fairclough, National Marine Fisheries Service, Southeast Fisheries Science Center; Milton Love and Donna Schroeder, Marine Science Institute, University of California, Santa Barbara; Dan Richards, Channel Islands National Park Service; Jorge Rosales Casian and Oscar Sosa Nishizaki, Centro Investigación Científica y de Educación Superior de Ensenada, B.C.; and Bill Neblett, Occidental College, Department of Philosophy. We thank Jan McMurdie-Wengert, California State University, Northridge, Automated Sequencing Facility for generation of DNA sequences. We thank G. David Johnson and Phillip A. Hastings for critical reviews of the manuscript. We are indebted to H. J. Walker and C. Klepladlo, Scripps Institution of Oceanography Marine Vertebrates Collection for their curatorial assistance. The authors also thank A. A. Lures for donation of collection supplies.

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