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UNIVERSITY OF CALIFORNIA, SAN DIEGO

Molecular phylogenetics of the serranid subfamily Epinephelinae: Speciation and Biogeography in a nearshore marine fish clade.

A dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy

in

Marine Biology

by

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2005

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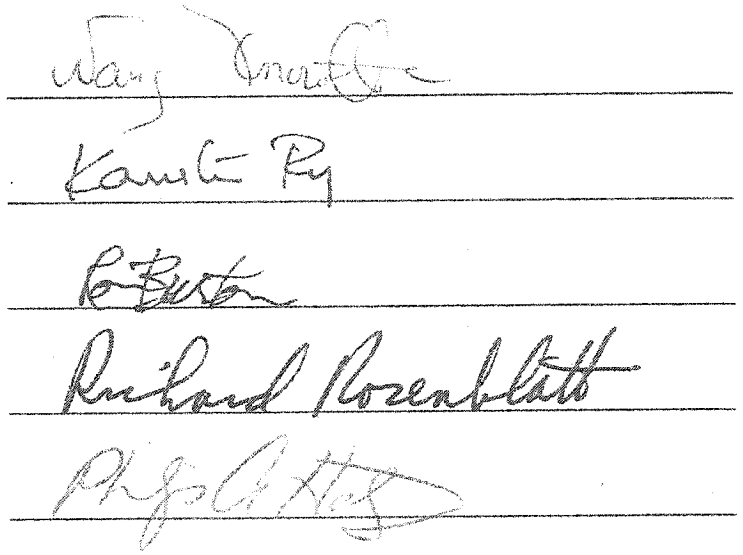

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DEDICATION

This work is dedicated to the memory of several people whose spirit of adventure and passing, however timely or otherwise, has had a profound impact on my life.

Carroll Craig

Robert Leist, Jr.

Captain Ted Thompson

and

Danielle Corey Roth

TABLE OF CONTENTS

LIST OF FIGURES

Chapter IV

Chapter V

LIST OF TABLES

ACKNOWLEDGEMENTS

This dissertation would not have been possible without the help of several people who will be mentioned in this section. First and foremost, I would like to thank my thesis advisor, Philip A. Hastings, who has been an unending source of support and knowledge for the past five years. Many thanks are also due to the remainder of my advisory committee: Ron Burton, Nancy Knowlton, Richard Rosenblatt, and Kaustuv Roy. Nancy Knowlton also provided laboratory space for the duration of this project, without which, this project would not be finished. Ron Burton and his lab (John Flowers, Rosemary Byrne, Chris Willett, and others) provided many insightful discussions that greatly influenced my thinking about evolutionary genetics.

Of course, this project would have never started without the advice and mentorship of Drs. Larry Allen and Dan Pondella. Larry and Dan were the first to take me into the field and show me what marine biology was all about. Dan threw a reference in my lap (he literally threw it) following a collecting trip to Mexico which quickly turned into both a Masters thesis and a Ph. D. Dissertation. Dr. John Hafner also provided valuable mentorship as my thesis advisor at Occidental College. John's insight into evolutionary biology from a terrestrial standpoint allowed me to expand my thinking beyond the marine realm.

Numerous people aided in the collection of samples for this project. Many are named in the acknowledgements that are presented following each chapter. This list is most likely incomplete, and apologies are offered to those whom I have omitted. Thanks also to H. J. Walker, Jr., and Cindy Klepadlo for their curatorial assistance.

Funding for this research was provided by several sources: California Sea Grant, UCSD Academic Senate, UCMEXUS, SIO Graduate Department, ASIH Raney Fund, AMNH Lerner-Grey Fund for Marine Research, Carl and Laura Hubbs Endowment, and the Beyster Family Fund. Many thanks are due to Denise Darling, SIO graduate department, who never let me go broke and somehow managed to find jus the right amount of money to sustain this project. Thanks are also due to Dawn Huffman, Sharon Williams, and Alice Zheng, SIO Graduate Department, who manage to keep track of all of the grad students at SIO, making sure we don't forget to sign critical paperwork and lose our fellowship money.

Lastly I would like to thank all of my family and friends who have encouraged me throughout my journey as a graduate student. Without their constant support this work would not have come together in such a smooth manner.

The text of Chapter II, in full, has been published in Journal of Biogeography, Volume 31, pages 1085-1091. The dissertation author was the primary author and the co-authors listed directed and supervised the research, which forms the basis for this chapter. The text of Chapter III, in full, has been submitted for publication of the material as it appears in Copeia. The dissertation author was the primary author and the co-authors listed directed and supervised the research, which forms the basis for this chapter. The text of Chapter V, in full, has been submitted for publication of the material as it appears in Evolution. The dissertation author was the primary author and the co-authors listed in this publication directed and supervised the research, which forms the basis for this chapter.

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Pondella, Daniel J, II, Robert Snodgrass, Matthew T. Craig, and Hugh S. Kim. 1998. Re-Occurrence of the three-band butterflyfish, *Chaetodon humeralis*, in the Santa Monica Bay, with notes on its distribution and abundance. Bulletin of the Southern California Academy of Sciences Volume 97, No. 3, December.

Craig, Matthew T., Daniel J. Pondella, II, and John C. Hafner, 1999. Analysis of Age and Growth in two Eastern Pacific Groupers (Serranidae: Epinephelinae). Bulletin of Marine Science. Vol. 65(3):807-814.

Craig, Matthew T., Daniel J. Pondella, II, Jens P. C. Franck, and John C. Hafner. 2001. On the status of the serranid fish genus *Epinephelus*: Evidence for paraphyly based on 16s rDNA sequences. Molecular Phylogenetics and Evolution 19(1):121-130.

Pondella, Daniel J. II, and Matthew T. Craig. 2001. First record of the sabertooth blenny, *Plagiotremus azaleus*, in California with notes on its distribution along the Pacific coast of Baja California. Bulletin of the Southern California Academy of Sciences. 100(3):144-148, December.

Pondella, D. J., II, J. S. Stephens, Jr. and M. T. Craig. 2002. Fish productivity of a temperate artificial reef based upon the density of embiotocids (Teleostei: Perciformes). Proceedings of the Seventh International Conference on Artificial Reefs and Related Aquatic Habitats, October. ICES Journal of Marine Science 59:S88-S93.

Craig, Matthew T., Philip A. Hastings, and Paul K. Dayton. 2003. The nearshore fish assemblage of the San Diego/La Jolla Ecological Reserve. (Abstract). Bulletin of the Southern California Academy of Sciences. 2003 supplement.

Craig, Matthew T., F. Joel Fodrie, and P. A. Hastings. 2003. The nearshore fish assemblage of the Scripps Coastal Reserve. Coastal Management 32:341-351.

Pondella, D. J., II, L. G. Allen, J. R. Cobb, M. T. Craig, and B. Gintert. 2003. Evaluation of eelgrass mitigation and fishery enhancement structures in San Diego Bay. (Abstract). Bulletin of the Southern California Academy of Sciences. S2:39.

Pondella, D.J., II, Matthew T. Craig and Jens Franck. 2003. The phylogeny of *Paralabrax* (Perciformes: Serranidae) and allied taxa inferred from 16S and 12S mitochondrial sequence data. Molecular Phylogenetics and Evolution 29(1):176-184.

Craig, Matthew T., Philip A. Hastings, and Daniel J. Pondella, II. 2004. Notes on the systematics of the crestfish genus *Lophotus* (Lampridiformes; Lophotidae), with a new record from California. Bulletin of the Southern California Academy of Sciences. 103(2):57-65.

Craig, Matthew T., Philip A. Hastings, and Daniel J. Pondella, II. 2004. Speciation in the Central American Seaway: The importance of taxon sampling in the identification of geminate species pairs. Journal of Biogeography 31:1085-1091.

Craig, M. T., P. Wirtz, P. Bartsch, and P. Heemstra. 2004. Redescription and validation of *Alphestes afer* (Bloch 1793) as an amphi-Atlantic grouper species. In Review. Copeia.

Rocha, L. A., D. R. Robertson, C. R. Rocha, J.L. Van Tassell, B.W. Bowen and M. T. Craig. 2004. Episodic global warming drives inter-oceanic invasion and transoceanic expansion by a coral-reef fish. In Review. Ecology Letters.

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ABSTRACT OF THE DISSERTATION

Molecular phylogenetics of the serranid subfamily Epinephelinae: Speciation and biogeography in a nearshore marine fish clade.

by

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Doctor of Philosophy in Marine Biology

University of California, San Diego, 2005

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The processes that shape present day distributions of marine organisms have remained a central topic in evolutionary biology, conservation biology, and ecology. In this thesis, genetic data from mitochondrial and nuclear genes were used to create a phylogenetic hypothesis for the groupers of the subfamily Epinephelinae as a means of evaluating the current taxonomy of the group and the geography of speciation in marine organisms.

The molecular phylogenetic hypothesis presented in Chapters I and IV identifies several genera that are paraphyletic. New taxonomic considerations, including the resurrection of the genus *Hyporthodus* Gill, are discussed. We identify four main radiations: *Cephalopholis, Epinephelus, Hyporthodus,* and *Mycteroperca.* These lineages each represent a unique pathway of colonization to the New World and patterns of evolutionary radiation.

In Chapter II, the phylogenetic relationships among the genera *Alphestes* and *Dermatolepis* are discussed based on a molecular analysis of two mitochondrial and

two nuclear genes. Here we show that previously hypothesized trans-isthmian geminate species are not each other's closest living relatives, and that speciation may have been ongoing within the Central American Seway prior to the final closure of the Panamanian Isthmus.

Chapter III discusses the finding of *Alphestes afer* (Bloch 1793), a common western Atlantic species, at São Tomé Island (Gulf of Guinea; West Africa). Mitochondrial and nuclear DNA data indicate that this specimen is conspecific with western Atlantic specimens. Although it was originally described from the coast of Guinea, the presence of this species in the eastern Atlantic has gone unnoticed, and the type locality has been regarded as erroneous. A morphological comparison of the holotype with 44 specimens from western Atlantic and Caribbean localities indicates that the holotype is conspecific with western Atlantic specimens.

Chapter V discusses intra-specific genetic diversity in the flag cabrilla. The mitochondrial Cytochrome B gene was used to examine the phylogeography of two putative eastern Pacific sibling species, the flag cabrilla, *Epinephelus labriformis* (Jenyns 1840) and the Clipperton grouper, *E. clippertonensis* Allen and Robertson 1999. Significant genetic structure corresponding to geographic locality was found These data imply that in some marine fishes, changes in color patterns may evolve more rapidly than genetic markers commonly used in phylogenetic analyses.

CHAPTER I

A molecular phylogeny and revised classification for the groupers of the subfamily Epinephelinae (Serranidae).

Introduction

The family Serranidae is a group of carnivorous marine fishes that inhabit tropical and sub-tropical waters worldwide. While more recent systematic treatments of the family have provided a reasonable classificatory scheme, the family has traditionally been used as a convenient pigeonhole for lower percoid fishes whose affinities are unclear. Jordan and Eigenman (1890) were the first to attempt to resolve the relationships within the assemblage that was the family Serranidae by defining the six subfamilies Serraninae, Epinephelinae, Anthiinae, Grammistinae, Latinae, and Percichthyinae. The first attempt to define a natural classification of the family came with Gosline (1966) who restricted the Serranidae to Jordan and Eigenmann's (1890) Anthiinae, Epinephelinae, and Serraninae. Kendall (1976; 1979) agreed that Jordan and Eigenmann's (1890) Serraninae, Epinephelinae, and Anthiinae were natural groups, however, he expanded the subfamily Grammistinae for Jordan and Eigenmann's (1890) liopropomines and grammistids. Gosline's hypothesis of a restricted Serranidae was corroborated by Johnson (1983) who also placed Kendall's Grammistinae into the Epinephelinae. Johnson (1983) diagnosed the monophyly of the subfamily Epinephelinae based upon the derived feature of loss of an autogenous distal radial on the first dorsal pterygiophore. In this diagnosis, Johnson also included the enigmatic *Niphon spinosus* Cuvier 1828 which he believed represented the ancestral member of the subfamily Epinephelinae. Johnson (1983; 1988) divided the Epinephelinae into the five tribes Niphonini, Epinephelini, Diploprionini,

Liopropromini, and Grammistini, while Baldwin and Johnson (1993) proposed relationships among the tribes and demonstrated their monophyly.

The tribe Epinephelini (sensu Johnson, 1983) is perhaps one of the most speciose percoid assemblages with hypothesized monophyly comprising more than 150 species (Nelson, 1994). Johnson's (1983) Epinephelini includes the genera *Aethaloperca, Alphestes, Anyperodon, Cromileptes, Epinephelus, Cephalopholis, Dermatolepis, Gonioplectrus, Gracilla, Mycteroperca, Paranthias, Plectropomus, Saloptia, Triso*, and *Variola*. Johnson's Grammistini includes the genera *Aporops, Grammistes, Grammistops, Jeboehlkia, Pogonoperca, Pseudogramma, Rypticus* and *Suttonia*. Johnson (1983) also considered *Aulacocephalus, Diploprion,* and *Belonoperca* to be distinctive and allocated them to the tribe Diploprionini. Johnson's (1983) Liopropomini includes the genera *Liopropoma, Bathyanthias* and *Rainfordia*, while his Niphonini is monotypic and restricted to *Niphon spinosus*.

Since Johnson (1983; 1988) and Baldwin and Johnson (1993), few systematic studies have been undertaken to resolve the relationships or confirm the monophyly of the subfamily Epinephelinae or its included genera. Craig et al. (2001) presented the first molecular analysis of the group and provided evidence for a monophyletic Epinephelinae, and a paraphyletic *Cephalopholis* and *Epinephelus*. That study, however, was a preliminary analysis and was based on a limited number of taxa. In the current study, we used DNA sequence data from two mitochondrial and two nuclear genes from 155 taxa as a means of expanding the study of Craig et al. (2001). Herein, we 1) show that the Serranidae as currently defined is polyphyletic; 2) discuss

the interrelationships of the various tribes within the subfamily Epinephelinae; 3) demonstrate the paraphyletic nature of the genera *Cephalopholis, Epinephelus,* and *Mycteroperca* as currently defined, and 4) discuss the nomenclatural implications of these findings.

Materials and Methods

Specimens were collected in the field by various means including spear pole, hook-and-line, or anesthetic, were purchased from fish markets at or near the collecting locality, or purchased from the live aquarium trade. Fin clips, gill clips, and/or muscle tissue were removed from each individual and stored in either 5X net solution (Craig, et al., 2001) or 70-90% Ethanol. When available, voucher specimens were deposited at the Scripps Institution of Oceanography Marine Vertebrates Collection (Apendix I). Other tissues were obtained through various museum collections which maintain frozen or ethanol preserved collections, or from local contacts. When no voucher was available, a photo voucher was retained by MTC. One to 3 individuals per species were sequenced depending on availability. The individual sequences were inspected for significant differences and if none were present, a consensus sequence was used in the final analysis. Overall, tissue samples were obtained for 155 species representing 24 of the 30 epinepheline genera (Appendix I). Within the tribe Epinephelini, these included 68 of 99 species of *Epinephelus,* 14 of 15 species of *Mycteroperca,* 16 of 22 species of *Cephalopholis*, 5 of 7 species of *Plectropomus,* all species of *Alphestes, Dermatolepis, Paranthias,* and *Variola,* and the monotypic genera *Aetheloperca, Anyperodon, Cromileptes, Gracila,*

Saloptia and *Triso*. The Liopropomini was represented by two species of *Liopropoma*, the Diploprionini by *Diploprion bifasciatum* and *Belonoperca chabaudi*, and the Grammistini by species in the genera *Aporops, Grammistes, Pogonoperca, Pseudogramma, Rypticus,* and *Suttonia*. The Niphonini was represented by the monotypic *Niphon spinosus*.

Several outgroups were selected to root both the overall tree and the serranid portion (Appendix I). The beryciform *Hoplostethus mediterraneus* was used to root the Acanthomorpha, while several lower percoids were chosen that have been shown to be closely related to Serranidae based on molecular data (Smith and Craig, in prep.).

Total DNA was isolated from tissues using the DNEasy nucleic acids isolation kit (Qiagen) following manufacturers instructions. The polymerase chain reaction (PCR) was used to amplify portions of two mitochondrial (16S and 12S) and two nuclear (TMO4C4 and Histone III) genes (1,838bp). Primer pairs are listed in Table 1. Twenty-five microliter PCR reactions were prepared following manufacturers instructions included with the RedTaq Readymix (Sigma-Aldrich) with the addition of 10pmol of each primer and 5-50ng of template DNA. Each reaction was subjected to 35 rounds of the following thermal cycling conditions: 94° for 30 sec, 46° for 30 sec, 72° for 1 min. In some instances, PCR failed to amplify one or more genes for a particular taxon (Apendix I). Missing sequences were coded as "?" in the concatenated sequence file.

Sequences in both directions were generated on a MegaBace 500 automated sequencer. Sequence reactions were prepared following manufacturers instructions

for the ET Terminator chemistry with the addition of 5pmol primer (GE Healthcare, formerly Amersham-Biosciences). Sequences were generated for both the forward and reverse directions.

Sequence data were edited for miscalls and/or polymorphism using Sequencher v. 4.2. Edited sequences were aligned using CLUSTAL X with default settings (Thompson, et al., 1997). The alignment was visually optimized using MacClade v. 3.07 (Maddison and Maddison, 1997). A partition homogeneity test was used to determine the suitability of the four genes for use in a combined dataset. Phylogenetic analyses were performed using PAUP* 4.0b10. Due to the large number of taxa leading to computational constraints, the parsimony ratchet of Nixon (1999) was employed using the batch file created by PaupRat v.1b (Sikes and Lewis, 2001). Ten rounds of the ratchet were each performed using default settings (200 ratchets). All trees with the lowest tree score were retained from each ratchet and a consensus tree was created in PAUP*4.0b10. Similarly, the likelihood ratchet was implemented in PAUP* using the batch file created by Vos (2003). Ten rounds of the likelihood ratchet were performed using default settings except that the HKY85+I+G substitution model was used as determined by Modeltest v. 3.6 (Posada, 2005). All trees with the best likelihood score were retained. For the parsimony analysis, gaps were treated as a "fifth base" and for both analyses the tree was rooted with the beryciform *Hoplostethus mediterraneus*. Relative support at nodes was evaluated using the bootstrap as implemented in PAUP*4.0b10 using 1,000 replicates and saving a maximum of 1,000 trees per replicate.

Results

Overall, 1,900 bases were sequenced from the mitochondrial 16S, 12S, and the nuclear TMO4C4 and Histone III genes. Sixty two bases could not be aligned unambiguously and were deleted. Of the final 1,838 bases, 1,011 were constant, 176 were parsimony uninformative, and 651 were parsimony informative. The partition homogeneity test did not support the combination of the four gene datasets (P=0.01), however, this test has been shown to produce inconsistent results when used with molecular data, particularly when variable rates of evolution among genes are apparent (Dolphin, et al., 2000). As both nuclear genes and the mtDNA genes are most certainly evolving at different rates, we chose to combine the datasets for the final analyses. The parsimony ratchet algorithm found 7 trees of length 5,703 (CI=0.2523, HI=0.7477, RI=0.6582). A strict consensus tree is presented in Figures 1 and 3. The maximum likelihood (ML) algorithm found one tree with only minor differences in topology (-LnLikelihood=33996.01835; Fig. 2, 3).

Both tree construction methods found similar topologies with only minor differences at deeper nodes, most notably the placement of *Liopropoma* (see Discussion, below). Some differences were also apparent at tip clades, most of which reflected the increased resolution afforded by ML analyses. Both analyses supported a monophyletic Serranidae with the exclusion of the genera *Acanthistius* and *Niphon*, while the maximum parsimony (MP) analysis supported serranid monophyly with the addition of the genus *Cirrhitus* (Cirrhitidae). Both analyses also supported a monophyletic Epinephelinae with the exclusion of *Niphon*. The genus *Epinephelus*

formed two distinct clades in both ML and MP analyses. Separated by a clade containing the genus *Mycteroperca* and several species currently allocated to *Epinephelus*, the two main *Epinephelus* clades reflect a paraphyletic nature for the genus as currently defined. The genus *Cephalopholis* also formed two distinct, monophyletic clades with the addition of species currently allocated to *Paranthias, Gracila,* and *Aetheloperca.* The genera *Alphestes* and *Dermatolepis* formed two monophyletic clades that were sister to one another. *Saloptia* formed a sister relationship to a monophyletic *Plectropomus*. *Variola* was found to be monophyletic, while the monotypic *Triso* occupied a position that was sister to *Epinephelus* and *Mycteroperca* in the MP analysis, yet embedded within a basal grouper clade in the ML analysis.

Discussion

The genetic data gathered here from both nuclear and mitochondrial genes support the previously hypothesized paraphyly of the genera *Cephalopholis, Epinephelus* and *Mycteroperca* (Craig, et al., 2001). The data also support a monophyletic Serranidae with the exclusion of the genera *Niphon* and *Acanthistius*. There is no evidence to suggest that the previously hypothesized monophyly of the American groupers is valid, nor is there evidence to support most previously assigned subgenera within *Epinephelus* (e.g., Smith, 1971).

Familial Relationships (Serranidae)

The Serranidae as currently defined *sensu* Johnson (1983) and Nelson (1994) is based on the presence of three reductive specializations (absence of a posterior

uroneural, absence of the procurrent spur, and absence of a third preural cartilage) and one uniquely derived feature (presence of three opercular spines). While diagnosing any group based on reductive characters is tenuous, this assemblage has remained intact for some time. However, our genetic data support the monophyly of the Serranidae only with the exclusion of *Niphon* and *Acanthistius*. These differences warrant the discussion provided below.

The first phylogenetic (cladistic) study aiming to determine relationships among serranid fishes and assess their monophyly was by Johnson (1983). He presented morphological data supporting the currently recognized subfamilies Serraninae, Epinephelinae, and Anthiinae. That study also placed the enigmatic *Niphon spinosus* as a basal member of the subfamily Epinephelinae based on a single reductive character, loss of an autogenous distal radial in the dorsal pterygiophores. The presence of three opercular spines in *Niphon* added weight to its placement in the Serranidae. Further corroborative evidence came upon examination of the larvae of *Niphon* which possess a unique modification of the dorsal-fin pterygiophores that presumably aids in the support of an elongate larval dorsal spine typical of other epinephelines (Johnson, 1988). The relationships of *Niphon* have been controversial. Jordan (1923) placed *Niphon* in a monotypic family, yet most subsequent authors treated *Niphon* as a serranid with uncertain affinities (e.g., Berg, 1940, Katayama, 1959; McCully, 1961; Norman, 1966; Greenwood, et al., 1966). Gosline (1966), however, removed *Niphon* from the Serranidae and placed it within the Percichthyidae based in part on the presence of a serrated lacrimal which is not present in the

Serranidae. Greenwood (1977) hypothesized that *Niphon* would eventually be placed into a group with close affinities to Gosline's (1966) Percichthyidae. Although the third opercular spine in *Niphon* and associated larval characters discussed by Johnson (1983; 1988) would seem to indicate a close affinity to the Serranidae (especially the Epinephelinae), we feel that the morphological evidence, combined with the genetic data herein, does not fully support its placement therein. Although relatively uncommon among percoids, three opercular spines are present in some non-serranids, including the epigonid *Sphyraenops*, and two trachinids (*Echiichthys* and *Trachinus*). Additionally, the three-spine condition in *Niphon* only superficially resembles that in more typical serranids (e.g., *Paralabrax*). In *Niphon* the three spines are elongate and thin, forming distinct projections from the posterior margin of the operculum. In *Paralabrax* and other serranids it often difficult to establish the presence of all three spines, especially the most ventral, as they more closely resemble broad flanges rather than distinct projections. Additionally, the typical dorsal-fin in serranids has fewer than 11 spines; among Johnson's (1983) serranids, only *Niphon* and *Acanthistius* possess 13 dorsal-fin spines (*Acanthistius* may have 11-13 dorsal-fin spines; Heemstra and Heemstra, 2004). While number of dorsal-fin spines is clearly a variable character, it would seem to indicate a closer affinity of *Niphon* to the percichthyids which may have up to 12 dorsal-fin spines (Nelson, 1994; See discussion of *Acanthistius* below). Although extremely variable in some percoids, vertebral number in *Niphon* also suggests its removal from the Serranidae. Most serranids have 24 vertebrae (the subfamily Anthiinae and *Acanthistius* have 26; Nelson, 1994; Johnson,

1983), however *Niphon* has 30. Vertebral number in the Percichthyidae is variable (25-36) suggesting a closer relationship among the family with *Niphon*. Lastly, Greenwood (1977) indicated that in *Niphon* the "caudal skeleton is virtually identical with that in the percichthyids." He based this conclusion on the presence of two uroneurals (serranids have only one). Although these uroneurals are fused in *Niphon* (Greenwood, 1977; Johnson 1983), this condition may represent an autapomorphy.

Our genetic data support a close relationship of *Niphon* with the Percidae, a hypothesis that is not entirely likely. As our analysis did not include any members of the Percichthyidae, we hesitate to assign *Niphon* to any family. However we have presented evidence to warrant its removal from the Serranidae.

The affinities of *Acanthistius* are even less clear. Placed in the Epinephelinae by Jordan and Eigenmann (1890), and later into the Serraninae (Johnson, 1983; Kendall, 1984), the genus has most recently been regarded as a member of the Anthiinae (Heemstra and Randall, 1986; Meisler, 1987; Heemstra and Heemstra, 2004). Although Meisler (1987) provided some morphological evidence for his placement of *Acanthistius* within the Anthiinae, however he noted that its placement within the Serranidae remained tenuous. Members of this genus do possess three moderate opercular spines and reductive specializations that characterize the family, however, they also have 11-13 dorsal-fin spines and 26 vertebrae (Johnson, 1983; Nelson, 1994). These morphological characters, coupled with the genetic data here, provide a strong case for its removal from the Serranidae (Figures 1-3).

Further genetic evidence for the placement of *Acanthistius* and *Niphon* is available in a broader study of lower percoid relationships (Smith and Craig, in prep.). In this analysis based on over 60 lower percoid families using both nuclear and mitochondrial genes, the placement of these genera is similarly allied to the Percidae. We therefore suggest that the Serranidae *sensu* Nelson (1994) be redefined to include all genera with the removal of *Acanthistius* and *Niphon*.

Subfamilial relationships (Anthiinae, Epinephelinae, Serraninae)

Three subfamilies of serranid fishes have long been recognized: Anthiinae, Epinephelinae, and Serraninae (Baldwin and Johnson 1993). In their hypothesis based on morphology, Baldwin and Johnson (1993) recognized that these relationships were largely unresolved, yet presented some evidence that the Serraninae are sister to the Anthiinae and Epinephelinae as sister groups (Figure 4). Our molecular analysis confirms their hypothesis that the Serraninae forms a monophyletic group that is sister to the other two subfamilies. Our data also confirm a monophyletic sub-family Epinephelinae (*sensu lato*) with the exclusion of *Niphon spinosus* (see above). This result should permit a robust examination of the intra-relationships of the Epinephelinae as character polarity may now be established in a more confident manner.

Tribal relationships within the Epinephelinae

Baldwin and Johnson (1993) evaluated relationships within the Epinephelinae based on a cladistic analysis of morphological data (Figure 4). In their analysis, the authors confirmed earlier suppositions that *Niphon* represented the basal tribe

(Niphonini; Johnson, 1983; 1988), although that result is refuted by the genetic data herein (see above). Of the remaining four tribes, Baldwin and Johnson (1993) hypothesized that the Grammistini was sister to the Liopropomini, followed by the Diploprionini and Epinephelini (Figure 4). Our genetic data support their hypothesis with the Epinephelini and Diploprionini + Grammistini + Liopropomini forming two distinct, monophyletic lineages. A close relationship between the liopropomins and the grammistin + diploprionin clades has been hypothesized based on morphological data (Kendall, 1979; Johnson, 1983). In our MP analysis, the Liopropomini is sister to the soapfish tribes Diploprionini and Grammistini, while in the ML analysis, the Liopropomini are nested within the soapfish tribes. These alternative hypotheses for the placement of the Liopropomini nevertheless support a close relationship between the liopropomins and grammistin+diplorpionin clades. This placement also poses an interesting question regarding the evolution of the skin toxin grammistin, which is present in both soapfish tribes (Diplopronini and Grammistini). The chemical properties of this toxin have been discussed in detail (Randall, et al., 1971, Oshima, et al., 1974). Baldwin and Johnson (1993) noted that those species traditionally called soapfishes (*Grammistes, Grammistops, Pogonoperca,* and *Rypticus*) not only have grammistin in epidermal cells, but also in specialized dermal glands. They hypothesized that the epidermal toxin was independently derived in the Grammistini and Diploprionini, with a loss of this character in the Liopropomini and those species in the Grammistini which lack the toxin (*Pseudogramma* and *Suttonia*). The presence of dermal toxin cells in the Grammistini thus represented a unique origin of this

feature. Our MP analysis, however, would suggest that the presence of the skin toxin grammistin was uniquely derived within the Grammistini and Diploprinoni, with a subsequent loss in those species in the Gramistini that lack grammistin, as *Liopropoma* occupies a sister relationship to this group. This line of reasoning would seem to favor the MP analysis; however, given the other morphological data presented by Baldwin and Johnson (1993), these relationships remain unresolved. The inclusion of species within the Liopropomini that were not represented in this study (*Jeboehlkia, Pikea, Rainfordia*) may also serve to clarify the placement of this tribe.

Relationships within the Epinephelini

To date, no hypothesis of relationships for the Epinephelini has been presented that adequately represented the large number of taxa therein. Craig et al. (2001) presented a preliminary analysis based on molecular data and hypothesized it's paraphyly. That study, however, severely under sampled the Epinephelinae. While our analysis of molecular data indicate a monophyletic tribe Epinephelini *sensu* Johnson (1983) and Nelson (1994), it also supports recognition of several new monophyletic assemblages (see below).

Leis (1986) discussed the larval development of *Plectropomus* and attempted to assign character polarity based on earlier studies of the ontogenetic development of other epinepheline larvae. In his conclusions, Leis (1986) indicated that the genus *Plectropomus* was most likely the sister group of the remainder of the Epinephelini based in no small part on the development of the spination in the dorsal-fin. Leis (1986) and Johnson (1988) concluded that the eight-or-nine spine condition within the

Epinephelinae was ancestral. Leis (1986) found that in *Cephalopholis* the first eight spines were formed directly, while the ninth was formed indirectly by the transformation of the anteriormost dorsal soft ray. In grouper species with more than nine spinous rays, the anteriormost two soft rays develop into spines (Kendall, 1979). Leis (1986) concluded that the indirect transformation of soft rays into spines led to the increased number of spines seen in the genera *Alphestes, Anyperodon, Cromileptes, Epinephelus,* and *Dermatolepis.* Our molecular analysis supports the hypothesis that the eight dorsal-fin spine configuration is indeed an ancestral character state, as all genera with eight and nine spines (*Aetheloperca, Cephalopholis, Gracila, Paranthias, Plectropomus, Saloptia* and *Variola*) occupy similar positions in both the ML and MP analysis (Figures 1-3). Our data also support the hypothesis that *Plectropomus* is the sister group to the remaining Epinephelini, along with its close ally *Saloptia*.

The placement of *Epinephelus acanthistius* of the eastern Pacific within *Cephalopholis* by earlier authors was based in large part on the presence of nine dorsal-fin spines in this species. Craig, et al. (2001) demonstrated that this species clearly belongs within *Epinephelus* implying a reversal of the fin spine condition. The absence of the transformation of the anteriormost soft rays has apparently been independently lost. The transformation of the dorsal-fin ray series of species of *Epinephelus* with ten spines (*E. analogus, E. exsul, E. nigritus*) is unclear; however, these species clearly belong to *Epinephelus* (Smith, 1971; Heemstra and Randall, 1993; Craig, et al., 2001; Figures 1-3).

The nine-spined groupers in the genera *Aetheloperca, Cephalopholis,* and *Gracila* have long been uncritically assumed to be closely allied (Randall, 1964; Smith-Vaniz, et al., 1988). Randall (1964) erected the genus *Gracila* for the species *Cephalopholis albomarginata* Fowler and Bean 1930. In that study, Randall (1964) indicated that while there was a close relationship between *Gracila* and *Cephalopholis,* the species *albomarginata* did not belong in *Cephalopholis* based on its shorter head and semi-pelagic behavior. Smith (1957) elevated the subgenus *Aetheloperca* Fowler 1904 for the species *Perca rogaa* Forsskål to which he allocated *albomarginata.* Randall (1964) removed *albomarginata* from Smith's *Aetheloperca* based on differences in the dorsal profile of the head (*Aetheloperca* having a much steeper profile) and proportional body depth (*Aetheloperca* being much deeper bodied). Katayama (1974) placed a second species, *G. okinawe* (=*polleni*) into *Gracila* based largely on its truncate caudal fin that is shared with *G. albomarginata.* Smith-Vaniz et al. (1988) provided a re-description of the species *albomarginata* and *polleni* and chose to follow Randall's (1964) allocation of *albomarginata* to *Gracila* while refuting Katayama's placement of *polleni* and placing it back into *Cephalopholis.* Interestingly, our genetic analyses indicate a sister species relationship between *C. polleni* and *G. albomarginata* supporting Katayama's (1974) hypothesis of relationships although his allocation of both species to *Gracila* may have been ill-advised. Additionally, the steeply sloping forehead of *A. rogaa* is shared with *C. igarashiensis* and all species of *Dermatolepis,* indicating that this character is variable within the Epinephelinae and may not be a reliable indicator of relationships.

Heemstra and Randall (1993) also reported that *Aetheloperca, Gracila, Cephalopholis,* and *Paranthias* share tri-segmental pterygiophores, a character absent in many other serranid genera.

Our genetic analysis indicates a monophyletic lineage including *Aetheloperca, Cephalopholis, Gracila,* and *Paranthias* with moderate bootstrap support. The presence of nine spines in all four genera supports this relationship, and the development of these spines in larvae thus far examined indicates their homology (discussed in detail in Leis, 1986 and Craig, et al., 2001). This result is not surprising as these genera share other morphological synapomorphies in addition to nine dorsal-fin spines. McCully (1961) surveyed the scalelets in the posterior field among members of the Epinephelinae. He found that in all genera with fewer than ten dorsal-fin spines (except *Plectropomus*), the scales have the first scalelet fused to the structures anterior to it. In genera with more than ten dorsal-fin spines (except *Alphestes* and *Dermatolepis*), the first scalelet is rarely fused to the main portion of the scale. While *Cephalopholis* and *Aetheloperca* retain the ancestral, fused scalelet, *Gracila* exhibits the derived state of a free first scalelet (Smith-Vaniz, et al, 1988). The remaining morphological characters that have been examined (neurocranial structure, morphometric measurements, robustness of spines, and shape of pectoral fin) appear either uninformative or represent autapomorphic states (e.g., the pectoral-fin in *Aetheloperca* is uniquely asymmetric) and thus not useful for establishing relationships. Based on the evidence above and close genetic relationship, we hereby

reallocate *Aetheloperca rogaa* Forsskål and *Gracila albomarginata* (Fowler and Bean 1930) to *Cephalopholis*.

The inclusion of both species of the genus *Paranthias* in a monophyletic clade with *Cephalopholis* (*sensu lato*) supports previous hypotheses of close relationship between *Cephalopholis* and *Paranthias* (Smith, 1966; Craig, et al., 2001). The unique, semi-pelagic lifestyle of the two species of *Paranthias* has led to several morphological innovations apparently convergent on those seen in the subfamily Anthiinae (and shared by *Gracila*) and have led to its recognition as a genus independent of *Cephalopholis*. Several morphological and ontogenetic characters (e.g., development of dorsal-fin spines, presence of epineural ribs on vertebrae 1-9) exist, however, that support the inclusion of *Paranthias* within *Cephalopholis* and they have been discussed in detail elsewhere (Heemstra and Randall, 1993; Craig, et al., 2001). Additionally, the ability of *Paranthias furcifer* to hybridize with *Cephalopholis fulva* may indicate a close relationship (Smith, 1966; Craig, et al., 2001; Bostrom, et al., 2002). Sibley (1957) argued that hybridization should indicate evolutionary relatedness; species should lose this ability as they diverge along evolutionary pathways. While this argument may apply in examples where closely allied species are concerned, it is worth considering that the ability to interbreed, if treated as a character in a phylogenetic (cladistic) framework, should represent a primitive, or ancestral state, and hence is phylogenetically uninformative until it is lost and is best treated as autapomorphy (Rosen, 1979). In reality, these arguments reflect differences in species concepts, namely the biological species concept of Mayr (1969)

and other more recent phylogenetic species concepts. The arguments for or against any species concept are beyond the scope of this paper, nevertheless we feel that the ability to interbreed indicates a close relationship. It is therefore necessary to include the species *P. colonus* and *P. furcifer* with the remaining species of *Cephalopholis* in order to maintain the monophyletic definition of the genus.

In summary, we include the species *Aetheloperca rogaa* Forsskål, *Gracila albomarginata* (Fowler and Bean), *Paranthias colonus* (Valenciennes), and *P. furcifer* (Valenciennes) within the genus *Cephalopholis*. As the two species of *Paranthias* form a morphologically distinct monophyletic group in our genetic analysis and *Aetheloperca* and *Gracila* have a unique morphology, we suggest treating *Aetheloperca, Gracila,* and *Paranthias* as subgenera in order to recognize their distinctiveness and retain the nomenclature. A monophyletic nomenclature is retained, however, only if the remaining species of *Cephalopholis* are not allocated to the nominate subgenus.

The only grouper species with a low dorsal-fin spine count (eight) not included in our study is the Spanish Flag, *Gonioplectrus hispanus*. The placement of *Gonioplectrus* within the Epinephelini remains unclear. Johnson (1983) placed *Gonioplectrus* within the Epinephelini with no distinct criteria, and the larvae are unknown, thus a comparison with Kendall's (1979) scheme is impossible. The presence of a low dorsal-fin spine count and epineural ribs on vertebrae 1-9 would seem to indicate a close relationship to more basal genera *Plectropomus* and

Cephalopholis. In the absence of comparative genetic material, we retain *Gonioplectrus* as a distinct genus within the Epinephelini.

This study confirms the monophyly of the genera *Alphestes* and *Dermatolepis* and their sister group relationship (Craig, et al., 2001). Smith-Vaniz, et al. (1988) reported that the smooth scales of *Alphestes* and *Dermatolepis* are unique from all other epinephelines. Additionally, all species in these genera have a high dorsal profile of the head, however, this character occurs in other members of the subfamily. *Alphestes spp.* are unique is possessing a single, antrorse spine at the corner of the pre-operculum (also present in *Gonioplectrus*) and in having larvae with an extremely rugose neurocranium (Johnson and Keener, 1984; Heemstra and Randall, 1993). Although Smith (1971) demoted *Alphestes* and *Dermatolepis* to sub-generic status, subsequent treatments recognized these lineages at the generic level (Heemstra and Randall, 1993; Craig, et al., 2001). Chapter II discussed the interrelationships of the six included species in detail.

A surprising result of both the ML and MP analysis was the clustering of *E. cifuentesi*, *E. drummondhayi,* and *Triso dermopterus* in a clade with *Alphestes* and *Dermatolepis*. However, their affinities appear to lie within *Epinephelus* (*sensu lato*) based on their overall morphology. In the interest of nomenclatural stability and in the absence of any morphological characters that confirm this relationship, we retain the species *drummondhayi, cifuentesi,* and *dermopterus* in their present genera.

In our genetic analysis, all species currently allocated to *Mycteroperca* are closely allied (Figures 1-3). However, the presence of species currently placed in

Epinephelus (*E. marginatus, E. costae, E. caninus, E. goreensis, E. albomarginata, E. morrhua,* and *E. radiatus*) in this same clade renders *Mycteroperca* (*sensu* Heemstra and Randall, 1993) paraphyletic.

Traditionally, *Mycteroperca* and *Epinephelus* are considered as closely related. Species within *Mycteroperca* are traditionally regarded as distinct from *Epinephelus* due to their elongate body form and the presence of 10-12 anal-fin soft rays (*Epinephelus* species typically have 8 or 9; Rosenblatt and Zahuranec, 1963; Smith 1971; Heemstra and Randall; 1993). Most of the species currently allocated to *Epinephelus* that form a clade with the *Mycteroperca* species have 8-9 anal-fin rays, a character heretofore used to justify their placement within *Epinephelus*. Our analysis indicates that the number of anal-fin rays alone is not a reliable indicator of relationships.

No comprehensive systematic treatment exists for the genus *Mycteroperca.* Cervigón and Velasquez (1966) examined the Venezuelan species, Rosenblatt and Zahuranec (1967) discussed the taxonomy of the eastern Pacific members, Smith (1971) treated the American species, and Heemstra (1991) discussed relationships among the *M. rubra* species group. Craig, et al. (2001) discussed genetic relationships among 7 of 15 species within the genus. In order to retain a monophyletic classification, we hereby consider the species *E. marginatus, E. costae, E. caninus, E. goreensis, E. albomarginata, E. morrhua,* and *E. radiatus* to be members of *Mycteroperca.*

The remaining species currently in *Epinephelus* form two distinct clades. The first clade represents species that have previously been allocated to the *E. niveatus* species group (Smith, 1971). Smith (1971) indicated close relationships between the species *E. niveatus, E. flavolimbatus, E. nigritus,* and *E. mystacinus*. Smith (1971) did not examine the eastern Pacific species *E. exsul* and treated the eastern Pacific *E. niphobles* as a synonym of the western Atlantic *E. niveatus*. Heemstra and Randall (1993) recognized the specific status of the latter pair. Our genetic analysis recognizes the monophyly of the *niveatus* species group with the addition of the species *E. exsul, E. ergastularias, E. octofasciatus, E. septemfasciatus, E. quernus,* and *E. acanthistius*.

All species within the *niveatus* species group are characterized by having a much deeper body than the remaining *Epinephelus* species. This character is particularly evident in juveniles which have a disk shaped body (Figure 5). The remaining species of *Epinephelus* and *Mycteroperca* have juveniles and adults with a much more elongate body form (Figure 5). Additionally, all species in the *niveatus* complex share a characteristic drab brown or olive coloration which may or may not have several dark bars along the body. The *niveatus* species group is also characterized by having pelvic fins that insert immediately below or in front of the pectoral insertion, while the remaining *Epinephelus* species have pelvics that insert below or behind the pectoral insertion. In members of the *niveatus* species group, the articulation between the cleithrum and the coracoid forms an elongate, triangular foramen. In the remaining species of *Epinephelus* (*sensu stricto*) and *Mycteroperca* this foramen is distinctly rounded at the same articulation (Figure 6). This character

state is present in both adult and juvenile specimens (Figure 6). In other species of the subfamily (e.g., *Cephalopholis spp.*) this articulation forms the elongate foramen, thus the circular shape serves as a synapomorphy for those species in the *Epinephelus* (*sensu stricto*) and *Mycteroperca* clades.

The members of the *niveatus* species complex clearly represent a monophyletic lineage that is distinct from the remaining species of *Epinephelus*. In this light, it is apparent that the members of this complex should be considered as a unique genus. We hereby propose to allocate the species within the group to the oldest available generic name for a member of this group, *Hyporthodus* Gill.

One troublesome aspect of relationships within the *niveatus* species group is the lack of a sister group relationship between *E. niphobles* of the eastern Pacific and *E. niveatus* of the western Atlantic, two species pairs that are almost surely trans-Isthmian geminates based on morphology (Jordan, 1908; Smith, 1971; Heemstra and Randall, 1993). There does exist a sister group relationship between *E. exsul* of the eastern Pacific and *E. nigritus* of the western Atlantic which have also been defined as geminates. These relationships are most likely due to the relatively small genetic distance between species in this clade which may confound the ability of our analyses to resolve their relationships.

The remaining species of *Epinephelus* form a monophyletic clade designated the "*E. fasciatus* species group" (Craig, et al., 2001) with the inclusion of the monotypic genera *Anyperodon* and *Cromileptes*. This clade is characterized by the

typically slender-bodied species of *Epinephelus* whose pelvic fin insertion is below or behind the pectoral-fin insertion.

Within this clade, there are some monophyletic species groups whose affinities have been discussed based largely on color pattern (Heemstra and Randall, 1993). The reticulated groupers (*E. bilobatus, E. faveatus, E. hexagonatus, E. macrospilos, E. maculatus, E. melanostigma, E. merra, E. spilotoceps*) form a monophyletic clade along with *E. tauvina* and *E. fasciatus*. Heemstra and Randall (1993) state that the juveniles of *E. tauvina* are often confused with members of the reticulated groupers, and its color pattern along with results herein clearly indicate in relationship with the remaining species. *E. fasciatus* is the type species for the genus *Epinephelus*, and while its color pattern does not clearly place it with the reticulated groupers, its inclusion in the clade is supported by high bootstrap support based on our genetic data.

The inclusion of the morphogically distinct *Anyperodon* and *Cromileptes* within the *fasciatus* species group was unexpected, though not surprising. The definitions of these genera reflect uniquely derived features, or autapomorphies, which are phylogenetically uninformative. In this light, it seems most prudent to recognize the monophyletic lineage *Epinephelus* while retaining *Anyperodon* and *Cromileptes* as subgenera to recognize the unique morphology of these two species.

New Generic Classification

One of the central tenets of phylogenetic systematics is the designation of monophyletic groups and a nomenclatural system that reflects groups with shared ancestry (Forey et al., 1992). In light of the genetic and morphological data at hand,

we hereby propose a re-classification of certain genera within the tribe Epinephelini. Many previously described genera are herein found to be monophyletic only with the addition of various morphologically distinct taxa that have been placed in monotypic genera. As a means of preserving a nomenclatural distinction for these morphologically distinct taxa, we propose that they be treated as subgenera. This will also serve to maintain names which are long-standing in the literature. This requires that other members of their respective genera not be placed in subgenera, or the erection of numerous other subgenera. I prefer the former to maintain nomenclatural stability, especially because there are many taxa which were not encountered during the sampling period, particularly within *Epinephelus*. These species are thus referred to the most appropriate genus based on a qualitative assessment of their overall morphology and in accordance with previous classifications, especially Heemstra and Randall (1993). Species whose placement in a distinct clade differed between the ML and MP analyses were placed in the most appropriate genus following the criteria above, but indicated "*incertae sedis*." The genera *Alphestes, Dermatolepis, Gonioplectrus, Plectropomus, Saloptia,* and *Variola* remain as currently defined in Heemstra and Randall (1993). The following is a list of currently recognized species of epinepheline fishes. For a complete list of synonyms, see Heemstra and Randall (1993).

Genus *Epinephelus* Bloch 1793

Type species: *E. marginalis* Bloch 1793 (=*E. fasciatus*) designated under the plenary powers of the IZCN, Opinion 93.

Included Species: *E. adscensionis* (Osbeck 1765), *E. aeneus* (E. Geoffroy Saint-Hiliaire 1817), *E. akaara* (Temminck and Schlegel 1842), *E. amblycephalus* (Bleeker 1857), *E. analogus* Gill 1864, *E. areolatus* (Forsskål 1775), *E. awoarra* (Temminck and Schlegel 1842), *E. bilobatus* Randall and Allen 1987, *E. bleekeri* (Vaillant 1877), *E. bontoides* (Bleekeri, 1855), *E. brunneus* Bloch 1793, *E. caeruleopunctatus* (Bloch 1790), *E. chabaudi* (Castlenau 1861), *E. chlorocephalus* (Valenciennes 1830), *E. chlorostigma* (Valenciennes 1828), *E. clippertonensis* Allen and Robertson 1999, *E. coioides* (Hamilton 1822), *E. corallicola* (Valenciennes 1828), *E. cyanopodus* (Richardson 1846), *E. daemelii* (Günther 1876), *E. diacanthus* (Valenciennes 1828), *E. epistictus* (Temminck and Schlegel 1842), *E. erythrurus* (Valenciennes 1828), *E. fasciatomaculosus* (Peters 1866), *E. fasciatus* (Forsskål 1775), *E. faveatus* (Valenciennes 1828), *E. flavocaeruleus* (Lacepéde 1802), *E. fuscogutattus* (Forsskål 1775), *E. gabriellae* Randall and Heemstra 1991, *E. guttatus* (Linnaeus 1758), *E. heniochus* Fowler 1904, *E. hexagonatus* (Forster 1801), *E. howlandi* (Günther 1873), *E. indistinctus* Randall and Heemstra 1991, *E. irroratus* (Forster 1801), *E. itajara* (Lichtenstein 1822), *E. labriformis* (Jenyns 1843), *E. lanceolatus* (Bloch 1790), *E. latifasciatus* (Temminck and Schlegel 1842), *E. lebretonianus* (Hombron and Jacquinot 1853), *E. longispinis* (Kner 1864), *E. macrospilos* (Bleeker 1855), *E. maculatus* (Bloch 1790), *E. magniscuttis* Postel, Fourmanoir and Guézé 1963, *E. malabaricus* (Bloch and Schneider 1801), *E. melanostigma* Schultz 1953, *E. merra* Bloch 1793, *E. miliaris* (Valenciennes 1830), *E. morio* (1828), *E. multinotattus* (Peters 1876), *E. ongus* (Bloch 1790), *E. poecilonotus* (Temminck and Schlegel 1842), *E.*

polylepis Randall and Heemstra 1991, *E. polyphekadion* (Bleeker 1849), *E. polystigma* (Bleeker 1853), *E. posteli* Formanoir and Crosnier 1964, *E. quoyanus* (Valenciennes 1830), *E. retouti* Bleeker 1868, *E. rivulatus* (Valenciennes 1830), *E. sexfasciatus* (Valenciennes 1828), *E. socialis* (Günther 1873), *E. spilotoceps* Schultz 1953, *E. stictus* Randall and Allen 1987, *E. stolizkae* (Day 1875), *E. striatus* (Bloch 1792), *E. suborbitalis* Amaoka and Randall 1990, *E. summana* (Forsskål 1775), *E. tauvina* (Forsskål 1775), *E. timorensis* Randall and Allen 1987), *E. trimaculatus* (Valenciennes 1828), *E. trophis* Randall and Allen 1987), *E. tuamotoensis* Fourmanoir 1971, *E. tukula* Morgans 1959, *E. undulostriatus* (Peters 1867), *E. undulosus* (Quoy and Gaimard 1824).

Species *incertae sedis*: *E. andersoni* Boulenger 1903.

Genus *Epinephelus*

Subgenus *Anyperodon* Günther 1859

Type species: *Serranus leucogrammicus* (Valenciennes in Cuv. and Val. 1828)

Included Species: *E. (A.) leucogrammicus.*

Genus *Epinephelus*

Subgenus *Cromileptes* Valenciennes 1828

Type species: *Serranus altivelis* (Valenciennes in Cuv. and Val. 1828)

Included Species: *E. (C.) altivelis* (Valenciennes 1828).

Genus *Cephalopholis* Bloch and Schneider 1801

Type species: *C. argus* Bloch and Schneider 1801, type locality East Indies.

Included Species: *C. aitha* Randall and Heemstra 1991, *C. argus* Bloch and Schneider 1801, *C. aurantia* (Valenciennes 1828), *C. boenak* (Bloch 1790), *C. cruentata* (Lacepéde 1802), *C. cyanostigma* (Valenciennes 1828), *C. Formosa* (Shaw and Nodder 1812), *C. fulva* (Linnaeus 1758), *C. hemistiktos* (Rüppell 1830), *C. igarashiensis* Katayama 1957, *C. leopardus* (Lacepéde 1801), *C. microprion* (Bleeker 1852), *C. miniata* (Forsskål 1775), *C. nigri* (Günther 1859), *C. nigripinnis* (Valenciennes IN Cuv. and Val. 1828), *C. oligosticta* Randall and Ben-Tuvia 1983, *C. panamensis* (Steindachner 1876), *C. polleni* (Bleeker 1868), *C. sexmaculata* (Rüppel 1830), *C. sonnerati* (Valenciennes 1828), *C. spiloparaea* (Valenciennes 1828), *C. taeniops* (Valenciennes 1828), *C. urodeta* (Schneider 1801).

Genus *Cephalopholis*

Subgenus *Aetheloperca* Fowler 1904

Type Species: *Perca rogaa* Forsskål 1775

Species: *C. (A.) rogaa* (Forsskål 1775)

Genus *Cephalopholis*

Subgenus *Gracila* Randall 1964

Type Species: *Cephalopholis albomarginata* Fowler and Bean 1930

Included Species: *C. (G). albomarginata* (Fowler and Bean 1930)

Genus *Cephalopholis*

Subgenus *Paranthias* Guichenot 1868

Type Species: *Serranus furcifer* Valenciennes 1828

Species: *C. (P.) colonus* (Valenciennes 1855), *C. (P.) furcifer* (Valenciennes 1828)

Genus *Hyporthodus* Gill 1862

Type species *Hyporthodus flavicauda* [= *Epinephelus niveatus* (Valenciennes in Cuv. and Val., 1828), Type locality Brazil].

Included Species: *H. niveatus* (Valenciennes 1828), *H. niphobles* (Gilbert and Starks 1897), *H. exsul* (Fowler 1944), *H. acanthistius* (Gilbert 1892), *H. flavolimbatus* (Poey 1865), *H. mystacinus* (Poey 1852), *H. septemfasciatus* (Thunberg 1793), *H. octofasciatus* (Griffin 1926), *H. nigritus* (Holbrook 1855), *H. ergastularias* (Whitley 1930), *H. quernus* (Seale 1901), *H. haifensis* (Ben-Tuvia 1953), *H. darwinensis* (Randall and Heemstra 1991).

Species *incertae sedis: H. cifuentesi* (Grove and Lavenberg 1993), *H. drummondhayi* (Goode and Bean 1879), *H. perplexus* (Randall, Hoese, and Last 1991).

Genus *Mycteroperca* Gill 1863

Type species: *Serranus olfax* Jenyns by subsequent designation of Gill, 1866.

Species: *M. acutirostris* (Valenciennes 1828), *M. albomarginata* (Boulenger 1903), *M. bonaci* (Poey 1860), *M. caninus* (Valenciennes 1843), *M. cidi* Cervigón 1966, *M. costae* (Steindachner 1878), *M. fusca* (Lowe 1836), *M. goreensis* (Valenciennes 1830), *M. interstialis* (Poey 1860), *M. jordani* (Jenkins and Evermann 1889), *M. marginatus* (Lowe 1834), *M. microlepis* (Goode and Bean 1880), *M. morrhua* (Valenciennes 1833), *M. olfax* (Jenyns 1843), *M. phenax* Jordan and Swain 1885, *M. prionura* Rosenblatt and Zahuranec 1967, *M. radiatus* (Day 1867), *M. rosacea* (Streets 1877), *M. rubra* (Bloch 1793), *M. tigris* (Valenciennes 1833), *M. venenosa* (Linnaeus 1758), *M. xenarcha* Jordan 1888.

Acknowledgements

Several institutions and individuals provided funds for this research: California Sea Grant, American Museum of Natural History Lerner-Grey Fund for Marine Research, American Society of Ichthyologists and Herpetologists Raney Fund, Beyster Family Fund, UCMexus, Carl and Laura Hubbs Fellowship, Nearshore Marine Fish Research Program (CSUN), Scripps Institution of Oceanography Graduate Department, and the Vantuna Research Group. Numerous individuals also aided in the collection of specimens and/or organization of field trips for this research: E. Ballesteros, R. Chapman, T. Chan , G. Fairclough, S. Fennessy, P. Heemstra, H. Ishimori , P. Lyons, T. Maggio, M. McGrouther, G. Menezes, M. Nishida, M. Rivera, D. R. Robertson, E. Sala , Wm. L. Smith, P. Wirtz, R. Vetter, Y. Yamanoue, T. Yoshino, F. Young, and numerous others whom we most surely have overlooked. We would like to thank Cindy Klepadlo and H. J. Walker, Jr., (SIO) for their curatorial assistance, and the Australian Musuem and University of Kansas Natural History Museum for their maintenance and donation of tissue samples.

References

Baldwin, C. C. and G. D. Johnson. 1993. Phylogeny of the Epinephelinae (Teleostei: Serranidae). Bull. Mar. Sci. 52(1): 240-283.

Berg, L. S. 1940. Classification of fishes, both recent and fossil. Trav. Inst. Zool. Acad. Sci. U.S.S.R. 5:87-517.

Bostrom, M. A., B. B. Collette, B. E. Luckhurst, K. S. Reece, and J. E. Graves. 2002. Hybridization between two serranids, the coney (*Cephalopholis fulva*) and the creole-fish (*Paranthias furcifer*), at Bermuda. Fish. Bull. 100(4):651-661.

Cervigón, M. F. and E. Velasquez. 1966. Las especies del genero *Mycteroperca* de las costas de Venezuela (Pisces-Serranidae). Mem. Soc. Cienc. Nat. LaSalle 26(74): 77-143.

Colgan, D. J., A. McLauchlan, G. D. F. Wilson, S. P. Livingston, G. D. Edgecombe, J. Macaraenas, G. Casis, and M. R. Gray. 1998. Histone III and U2 snRNA DNA sequences and arthropod evolution. Aust. J. Zoology 46:419-437.

Craig, M. T., D. J. Pondella, II, J. C. Hafner, and J. P. C. Franck. 2001. On the status of the serranid fish genus *Epinephelus*: Evidence for paraphyly based on 16S rDNA sequences. Mol. Phyl. Evol., 19(1):121-130.

Dolphin, K., Belshaw, R., Orme, C. D. L., and Quicke, D. L. J. 2000. Noise and incongruence: Interpreting results of the incongruence length difference test. Mol. Phyl. Evol., 17(3):401-406.

Forey, P. L., C. J. Humphries, I. L. Kitching, R. W. Scotland, D. J. Siebert, and D. M. Williams. 1992. Cladistics: A practical course in systematics. The Systematics Association Pub. No. 10. Clarendon Press, Oxford. 191p.

Gosline, W. A. 1966. The limits of the fish family Serranidae, with notes on other lower percoids. Proc. Calif. Acad. Sci. 33: 91-111.

Greenwood, P. H., D. E. Rosen, S. H. Weitzman, and G. S. Myers. 1966. Phyletic studies of teleostean fishes, with a provisional classification of living forms. Bull. Amer. Mus. Nat. Hist. 131:339-455.

Greenwood, P. H. 1977. A review of the family Centropomidae (Pisces, Perciformes): an appendix. Bull. Brit. Mus. (Nat. Hist.) Zool. 31:297-301.

Heemstra, P. C. 1991. A taxonomic revision of the eastern Atlantic groupers (Pisces: Serranidae). Bol. Mus. Mun. Funchal, 43(226):5-71.

Heemstra, P. C. and J. E. Randall. 1986. Serranidae, IN: M. M. Smith and P. C. Heemstra, eds. Smith's Sea Fishes. Macmillan South Africa, Johannesburg.

Heemstra, P. C. and E. Heemstra. 2004. Coastal fishes of southern Africa. South African Institute for Aquatic Biodiversity and National Inquiry Service Centre, Grahamstown, South Africa.

Johnson, G. D. 1983. *Niphon spinosous*: A primitive epinepheline serranid, with comments of the monophyly and intrarelationships of the Serranidae. Copeia 3:777-787.

Johnson, G. D. 1988. *Niphon spinosus*, a primitive epinepheline serranid: corroborative evidence from the larvae. Jap. J. Ichthyol. 35(1):7-18.

Johnson, G. D., and P. Keener. 1984. Aid to identification of American grouper larvae. Bull. Mar. Sci. 34(1):106-34.

Jordan, D. S. 1908. The law of geminate species. *American Naturalist*, 42(494):73-80.

Jordan, D. S. 1923. A classification of fshes including families and genera as far as known. Stanford University Publishers, Univ. Ser. Biol. Sci. 2:77-243.

Jordan, D. S. and C. H. Eigenmann. 1890. A review of the genera and species of serranidae found in the waters of America and Europe. Bull. U.S. Fish Comm. VIII(1888):329-441.

Katayama, M. 1959. Studies on the serranid fishes of Japan (1). Bull. Fac. Ed., Yamaguchi Univ. 8:103-180.

Katayama, M. 1974. Serranid fishes of the Okinawa Islands (II). Bull. Fac. Ed. Yamaguchi Univ. 24(2):99-112.

Kendall, A. W., Jr. 1976. Predorsal and associated bones in serranid and grammistid fishes. Bull. Mar. Sci. 26:585-592.

Kendall, A. W., Jr. 1979. Morphological comparisons of North American seabass larvae (Pisces: Serranidae:). NOAA Tech. Rep. Circular 428:1-50.

Kendall, A. W., Jr. 1984. Serranidae: development and relationships. IN. H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall, Jr., and S. L. Richardson, eds. Ontogeny and systematics of fishes. Amer. Cos. Ichthyol. Herpetol. Spec. Publ. No. 1:499-510.

Thomson, J. D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nuc. Acids Res., 25(24):4876-4882.

Leiss, J. M. 1986. Larval development in four species of Indo-Pacific coral trout *Plectropomus* (Pisces, Serranidae: Epinephelinae) with an analysis of relationships of the genus. Bull. Mar. Sci. 38:525-52.

Maddison, D. R., W. P. Maddison. 1997. MacClade V. 4.06. Sinauer Associates.

Mayr, E. 1969. Principles of Systematic Zoology. McGraw-Hill Book Company. New York.

McCully, H. H. 1961. The comparative anatomy of the scales of serranid fishes. Unpubl. Ph.D. Diss., Stanford University.

Meisler, M. R. 1987. Limits and relationships of serranine seabasses, with revisions of *Serranus* and *Mentiperca* (Pisces: Serranidae). Unpubl. Ph.D. Dissertation, University of Southern California, Los Angeles, Ca.

Nelson, J. S. 1994. Fishes of the world. 3rd edition. John Wiley and Sons, Inc. New York.

Nixon, K. C. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. Cladistics 15:407-414.

Norman, J. R. 1966. A draft synopsis of the orders, families and genera of recent fishes and fish-like vertebrates. British Museum (Nat. Hist.), London.

Oshima, Y., K. Shiomi, and Y. Hashimoto. 1974. Comparison of grammistin from four species of grammistid fishes. Bull. Jap. Soc. Sci. Fish. 40:223-230.

Palumbi, S. . 1996. Nucleic acids II: The polymerase chain reaction. In: D. M. Hillis, C. Moritz, and B. K. Maple, eds. Molecular Systematics. pp. 205-248. Sinauer, Sunderland.

Randall, J. E. 1964. Notes on the groupers of Tahiti, with description of a new serranid fish genus. Pac. Sci. 18(3):281-296.

Randall, J. E., K. Aida, T. Hibiya, N. Mitsuura, H. Kamiya, and Y. Hashimoto. 1971. Grammistin, the skin toxin of soapfishes and its significance in the classification of the Grammistidae. Publ. Seto Mar. Biol. Lab. 19:157-190.

Rosen, D. R. 1979. Fishes from the uplands and intermontane basins of Guatemala: Revisionary studies and comparative geography. Bull. Amer. Mus. Nat. Hist. 162(5):269-375.

Rosenblatt, R.H. and B.J. Zahuranec. 1967. The Eastern Pacific groupers of the genus *Mycteroperca*, including a new species. Cal. Fish and Game 53(4):228-45.

Sibley, C. G. 1957. The evolutionary and taxonomic significance of sexual dimorphism and hybridization of birds. The Condor 59:161-191.

Sikes, D. S. and P. O. Lewis. 2001. beta software, V. 1. PAUPRat: PAUP* implementation of the parsimony ratchet. Distributed by the authors. Dept. of Ecology and Evolutionary Biology, Univ. of Connecticut, Storrs, USA.

Smith, C. L. 1954. Four rare serraniform fishes from east Africa. Ann. Mag. Nat. Hist. Ser. 12. 7:925-933.

Smith, C. L. 1966. *Menephorus* Poey, a serranid genus based on two hybrids of *Cephalopholis fulva* and *Paranthias furcifer*, with comments on the systematic placement of *Paranthias*. Amer. Mus. Nov. No. 2276:1-11.

Smith, C.L. 1971. A revision of the American groupers: *Epinephelus* and allied genera. Bull. Amer. Mus. Nat. Hist. 146(2):1-241p.

Smith-Vaniz, W. F., G. D. Johnson, and J. E. Randall. 1988. Redescription of *Gracila albomarginata* (Fowler and Bean) and *Cephalopholis polleni* (Bleeker) with comments on the generic limits of selected Indo-Pacific groupers (Pisces: Serranidae: Epinephelinae). Proc. Acad. Nat. Sci. Phil. 140(2): 1-23.

Streelman, J. T. and S. A. Karl. 1997. Reconstructing labroid evolution with single-copy nuclear DNA. Proc. Roy. Soc. Lon., 264(1384):1011-1020.

Vos, R. A. 2003. Accelerated likelihood surface exploration: The likelihood ratchet. Syst. Biol. 52(3):368-373.

Table 1. Sequencing and PCR primers.

Primer Name	Gene	Sequence	Reference
16Sar-L	16S	5'-cgcctgtttatcaaaaacat-3'	Palumbi, 1996
16Sbr-H	16S	5'-ccggtctgaactcagatcacgt-3'	Palumbi, 1996
12Sa	12S	5'-aaactgggattatagaccccactat-3'	Palumbi, 1996
12Sb	12S	5'-gagggtgacgggcggtctct-3'	Palumbi, 1996
H3A-L	Histone III	5'- atggctcgtaccaagcagacvgc -3'	Colgan, et al., 1998
H3B	Histone III	5'-atatccttrggcatratrgtgac-3'	Colgan, et al., 1998
TMO-F1-5'	TMO4C4	5'-cctccggccttcctaaaacctctc-3'	Streelman and Karl, 1997
TMO-R1-5'	TMO4C4	5'-catcgtgctcctgggtgacaaagt-3'	Streelman and Karl, 1997

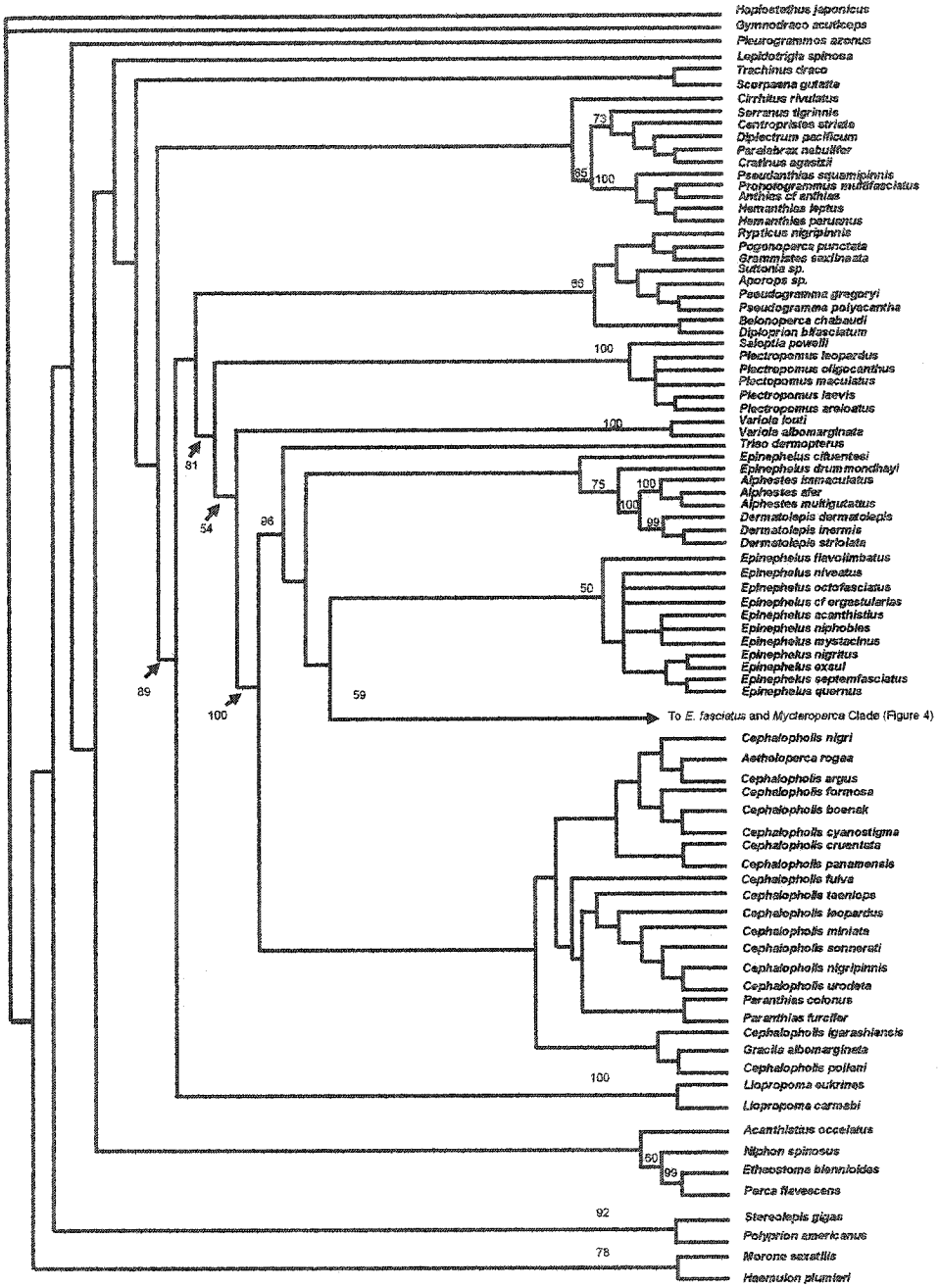

Figure 1. Strict consensus of 7 most parsimonious trees of length 5703 (CI=0.2523, HI=0.7477, RI=0.6582). Numbers above nodes are bootstrap values based on 1000 replicates.

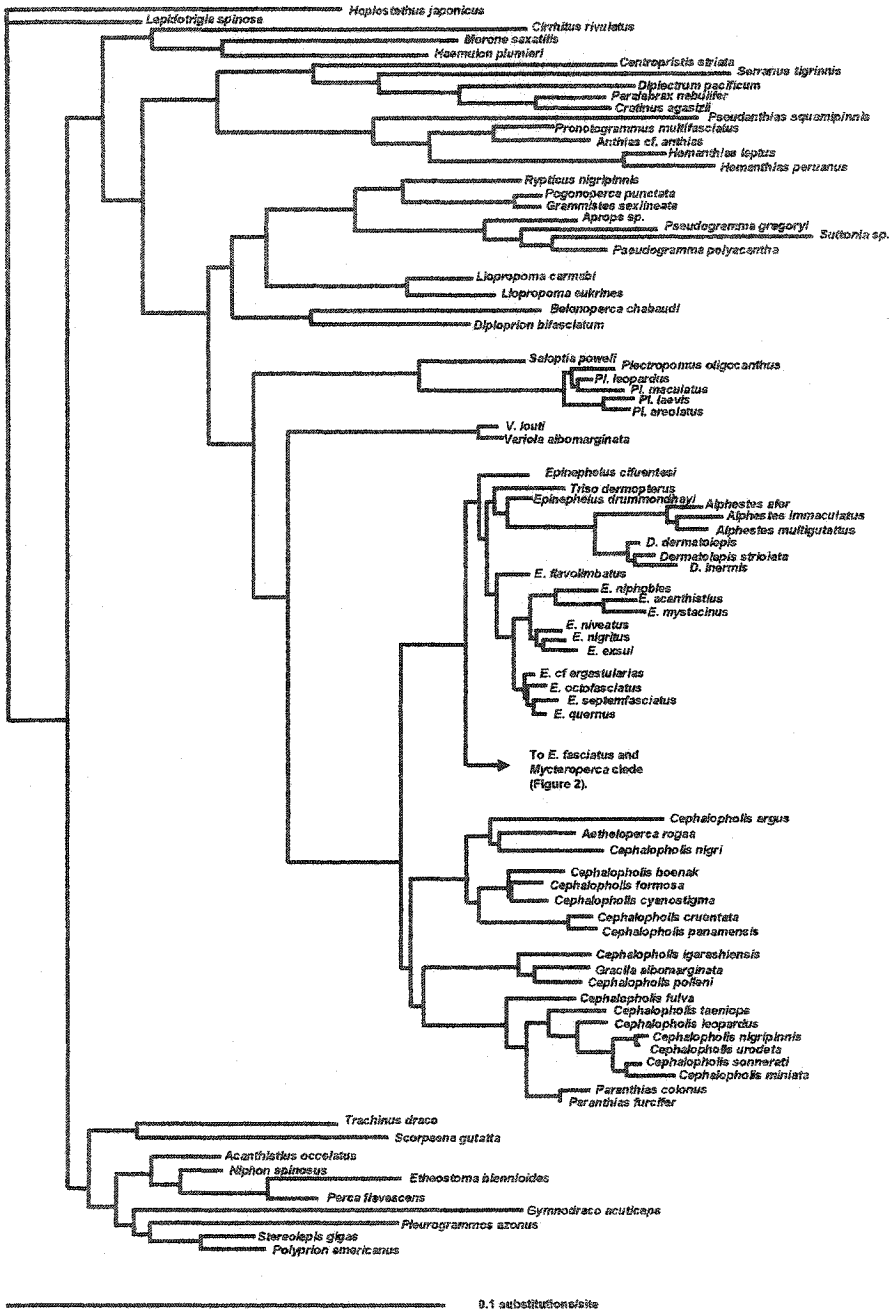

Figure 2. Maximum likelihood tree (-lnLikelihood=33996.01835) for 155 species of perciforme fishes.

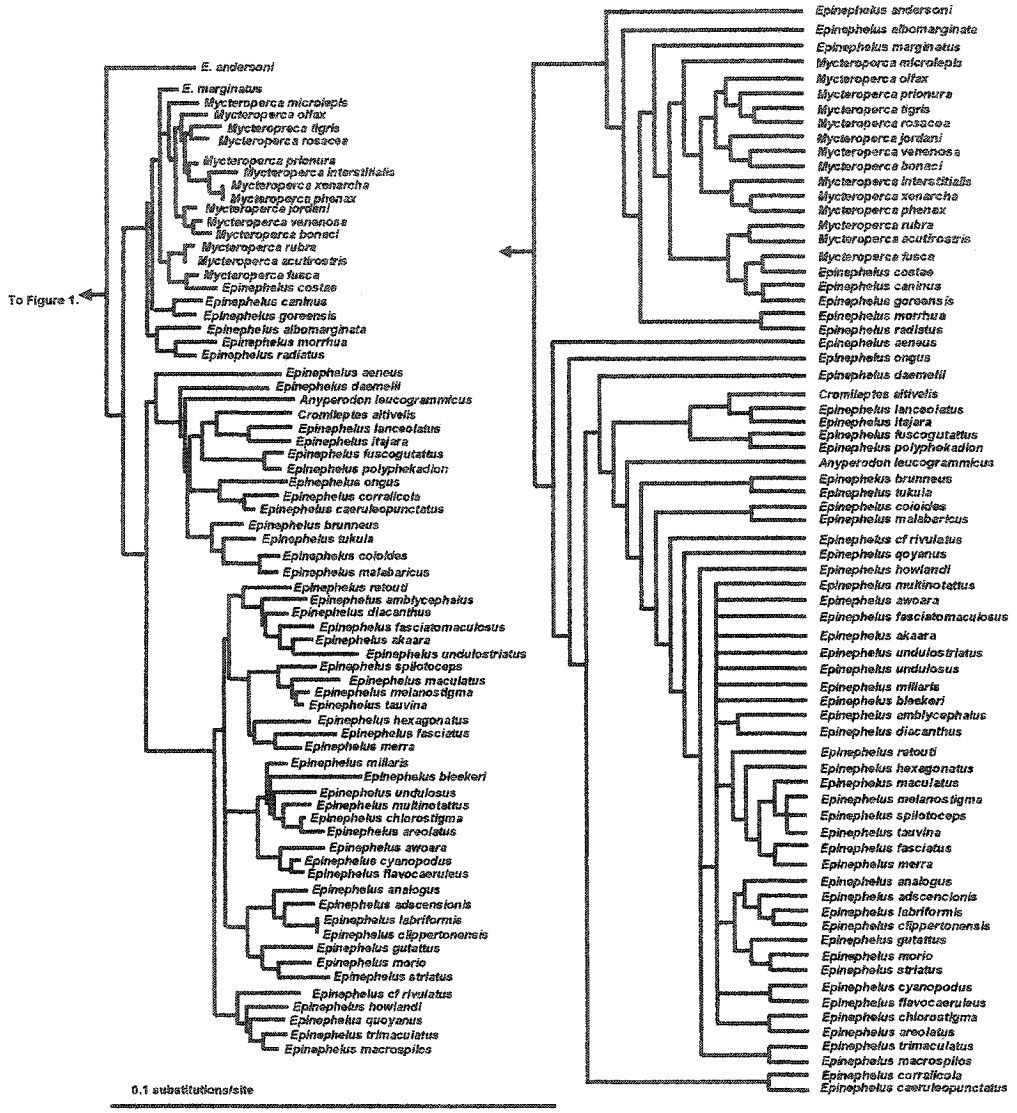

Figure 3. Relationships among the *Epinephelus fasciatus* and *Mycteroperca* clades as determined by maximum likelihood (A) and maximum parsimony (B) criteria. Numbers above nodes are bootstrap values based on 1000 replicates. Scale bar pertains to likelihood tree only (A).

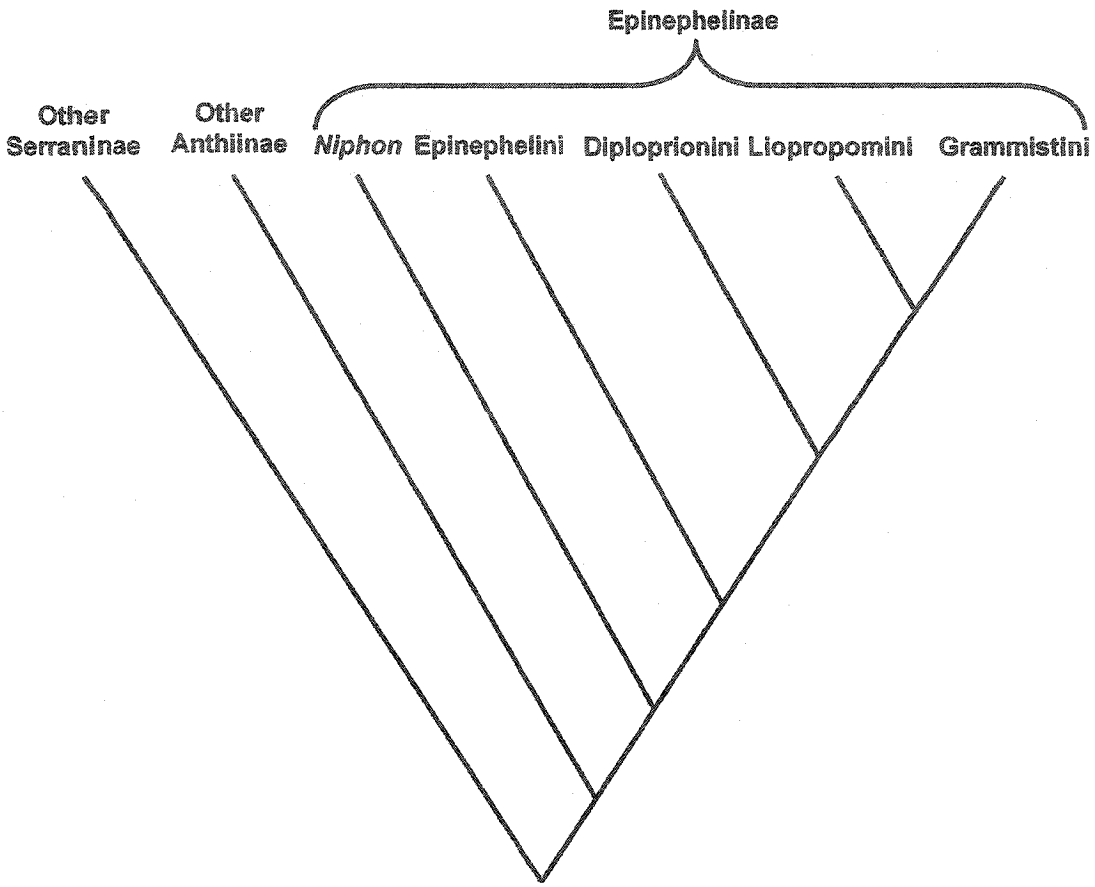

Figure 4. Phylogenetic hypothesis for tribes in the subfamily Epinephelinae based on Baldwin and Johnson (1993).

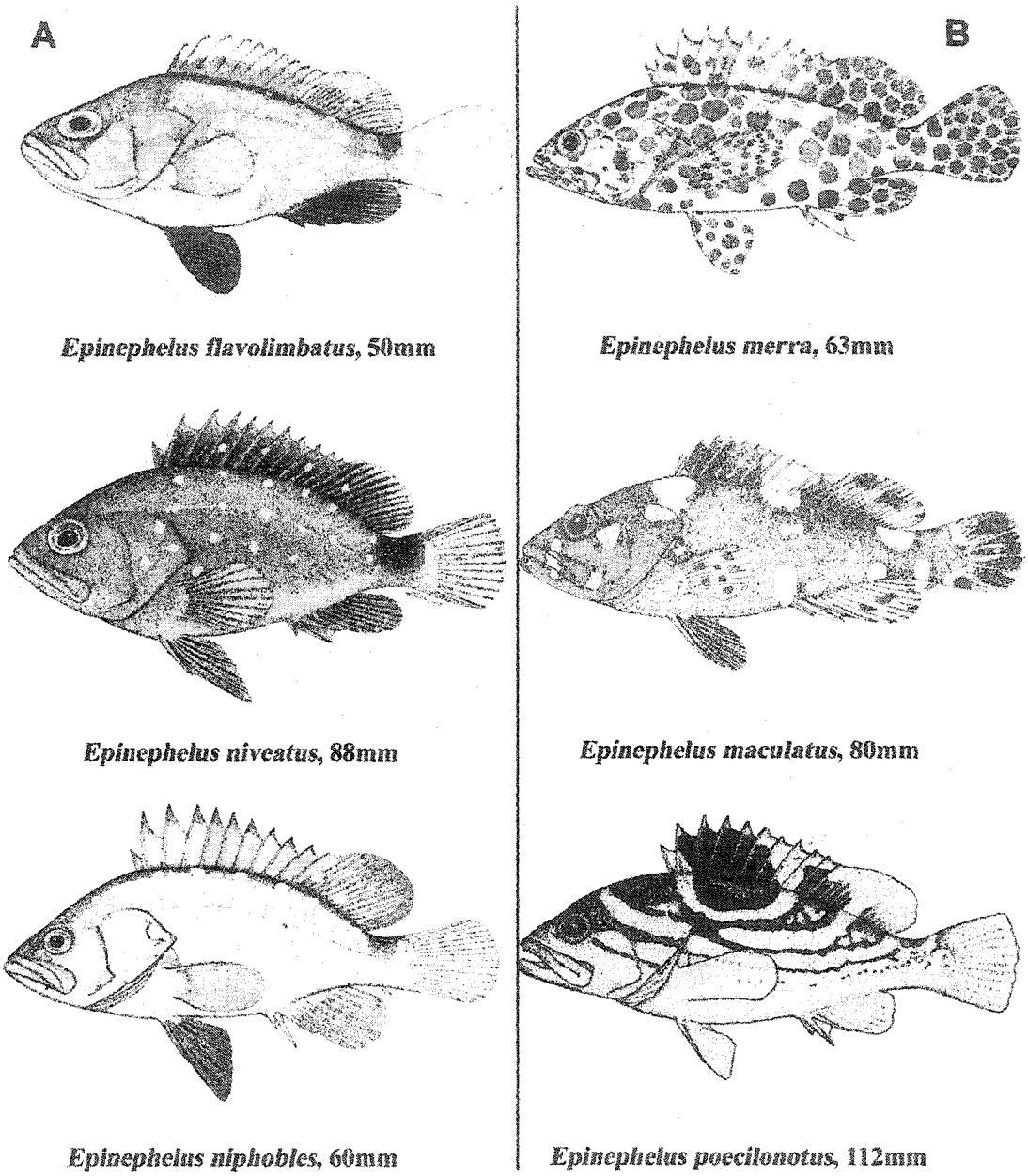

Figure 5. Juvenile specimens of the *E. niveatus* species complex (A) and the *E. fasciatus* complex (B). Pictures are reproduced by permission of the author (PCH) from Heemstra and Randall (1993).

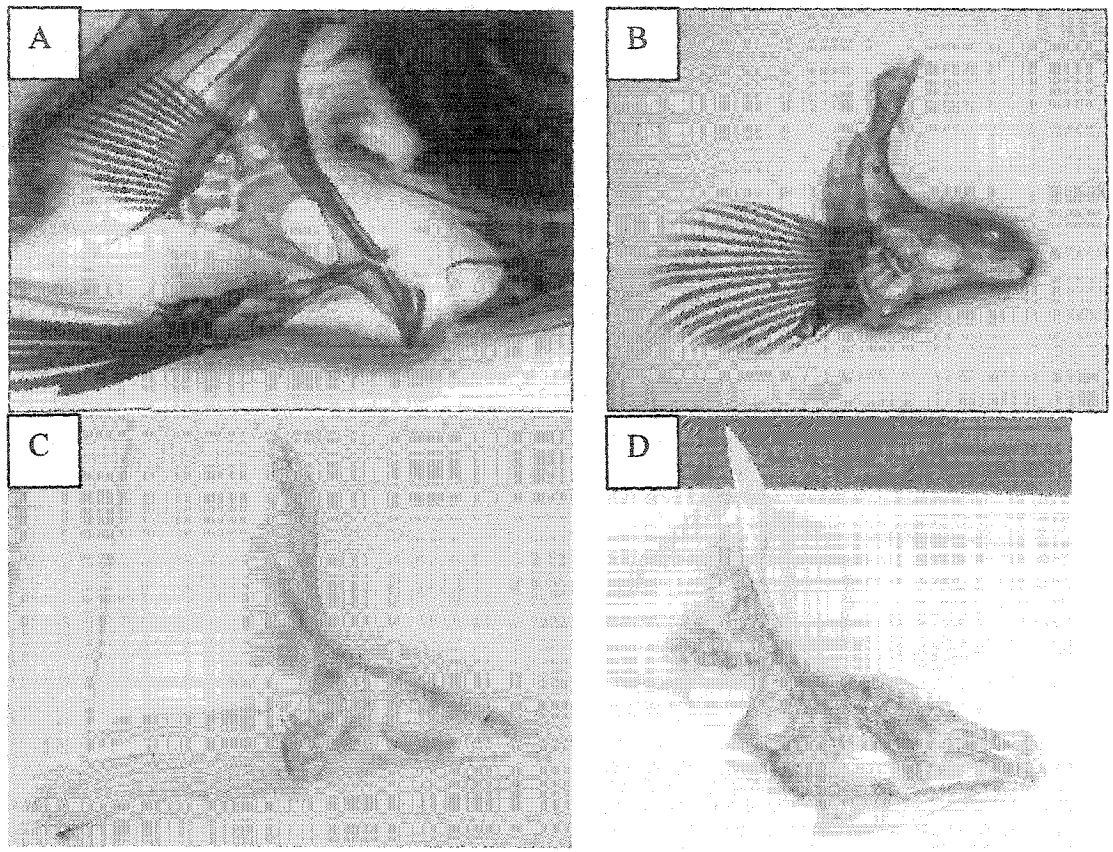

Figure 6. Pectoral girdle for four species of *Epinephelus* and *Hyporthodus*: A: *H. septemfasciatus*, B: *E. adscensionis*, C: *H. acanthistius*, D: *E. labriformis*. "A" and "C" represent the character state for the deep bodied members of the genus *Hyporthodus*, while "B" and "D" represent the character state for the slender bodied members of the genera *Epinephelus* and *Mycteroperca*.

CHAPTER II

Speciation in the Central American Seaway: the importance of taxon sampling in the identification of transisthmian geminate pairs.

Journal of Biogeography (J. Biogeogr.) (2004) 31, 1085–1091

ORIGINAL ARTICLE

Speciation in the Central American Seaway: the importance of taxon sampling in the identification of trans-isthmian geminate pairs

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ABSTRACT

Aim To create a molecular phylogenetic hypothesis for the closely related serranid genera *Alphestes* Bloch and Schneider and *Dermatolepis* Gill and assess the role of the Panamanian Isthmus in speciation within these reef fishes.

Location Tropical eastern Pacific, Caribbean, and Indian Oceans.

Methods Sequence data from one nuclear (TMO-4C4) and three mitochondrial genes (16S, 12S, and cytochrome *b*) were used in maximum parsimony and maximum likelihood analyses.

Results Here we show that previously hypothesized trans-isthmian geminate species are not each other's closest living relatives. Species of *Alphestes* Bloch and Schneider in the eastern Pacific are sister taxa indicating post-closure speciation. Within *Dermatolepis* Gill, we identify a sister group relationship between the Caribbean and western Indian Ocean species, a rarely reported biogeographic pattern. Based on sequence divergence, speciation among the three species of *Dermatolepis* was, however, nearly simultaneous around the time of the isthmian closure event.

Main conclusions Our molecular phylogenetic analysis of two closely related genera of reef fishes, each with presumed trans-isthmian geminates, cautions against the uncritical use of morphological similarity in identification of geminates, as well as the assumption that trans-isthmian sister groups date to the isthmian closure event. These findings suggest that in some instances incomplete sampling of species within a clade including putative geminates may lead to improper conclusions regarding the pattern and timing of speciation, as well as incorrect estimation of the rate at which evolution has proceeded.

Keywords

Alphestes, *Dermatolepis*, Serranidae, Isthmus of Panama, speciation, biogeography.

INTRODUCTION

The well-dated final closure of the Central American seaway provides a key focal point for testing the tempo of allopatric speciation in the marine realm (Coates & Obando, 1996; Haug & Tiedeman, 1998) and justifiably has received an extraordinary amount of attention (e.g. Knowlton *et al.*, 1993; Collins, 1996; Marko & Jackson, 2001; Marko, 2002). The so-called 'geminate' species pairs, defined by David Starr Jordan as '... twin species – each one representing the other on opposite sides of some form of barrier' (Jordan, 1908, p. 75), have provided model study systems for testing hypotheses regarding the effects of the disruption of gene flow in an allopatric setting. This is particularly true for species pairs on either side of the Panamanian Isthmus that are assumed to be descendant from a common population at the time of partial or complete closure of the Central American Seaway not less than 3.5 million years ago (Ma) (Coates & Obando, 1996). The availability of an independent estimate of the minimum time since last genetic contact (Coates & Obando, 1996; Haug & Tiedeman, 1998) allows for critical analysis of sequence divergence of trans-isthmian geminates. This process has become a prominent tool used to study the rate at which evolution proceeds, using the closure of the seaway as a reference point for calibrating a

'molecular clock' in many marine organisms (e.g. Bermingham *et al.*, 1997; Arbogast *et al.*, 2002; Marko, 2002). Knowlton and Weight (1998) cautioned that the generally accepted time of 3.5 Ma for the final closure of the seaway should be viewed as a minimum time since divergence because the dispersal of some species was apparently disrupted much earlier by the rising, but still incomplete, isthmus of Panama. Results from our study add other cautions: that apparent trans-isthmian geminates may not, in all instances, be each other's closest living relatives and that their divergence may be unassociated with the rise of the isthmus.

Geminates are typically identified based on morphological similarity, but rarely is a phylogeny available for larger clades including the hypothesized geminate pair. We present data for all heretofore described species of two closely related genera of reef fishes, both containing morphologically similar members in the eastern Pacific and western Atlantic, which show very different biogeographic relationships. This suggests that the assumption that morphologically similar species occurring in these oceans are geminates can be flawed and that the paradigm of trans-isthmian speciation may not hold true in all otherwise apparent cases.

BACKGROUND

The epinepheline serranids, commonly known as groupers, are nearshore reef fishes found worldwide in tropical and subtropical reef habitats (Heemstra & Randall, 1993). They include some of the most economically valuable species of reef fishes, contributing a substantial percentage of the world's fish harvest, particularly within developing nations. Groupers are thought to be effective dispersers both in early life as broadcast spawners with pelagic eggs and larvae that may remain in the plankton for as long as 60 days, and in adult life as predators with large home ranges, sometimes undertaking long spawning migrations, and reaching ages in excess of 25 years (Johnson & Keener, 1984; Leis, 1986; Craig *et al.*, 1999; Liao *et al.*, 2001; Sala *et al.*, 2001; White *et al.*, 2002).

The grouper genera *Alphestes* Bloch and Schneider and *Dermatolepis* Gill each contain three species and have representatives on both sides of the Panamanian Isthmus. *Alphestes* comprises two species, *Alphestes multiguttatus* Günther and *Alphestes immaculatus* Breder, in the tropical eastern Pacific (TEP) and one species, *Alphestes afer* Bloch, in the tropical western Atlantic (WA) and Caribbean (C). *Dermatolepis* includes one TEP species, *Dermatolepis dermatolepis* Boulenger, one WA/C species, *Dermatolepis inermis* Valenciennes, and a third Indian Ocean (IO) endemic, *Dermatolepis striolata* Playfair. A recent study based on molecular sequence data provided evidence that *Alphestes* and *Dermatolepis* together form a monophyletic clade nested within the polyphyletic genus *Epinephelus* Bloch (Craig *et al.*, 2001). In his 1908 paper, Jordan provided several examples of species pairs of fishes separated by the Panamanian Isthmus that he thought were geminates. His list included members of both *Alphestes* and *Dermatolepis*: *A. afer* and *A. multiguttatus* (Jordan's paper was published prior to the description of *A. immaculatus*) and *D. inermis* and *D. dermatolepis* (the latter under the nominal species *Dermatolepis punctata*). Since this initial assumption and in the absence of further study on their intra-relationships testing his hypothesis, these species continue to be regarded as geminate pairs (e.g. Thomson *et al.*, 2001).

MATERIALS AND METHODS

Fishes were collected in the field by hook and line or spear pole, or were purchased from commercial fish markets. Tissue samples (gill filaments, fin clips, or muscle tissue) were removed and preserved in either 5× Net solution (Craig *et al.*, 2001) or 95% ethanol and stored at ambient temperature in the field prior to freezing at −80 °C in the laboratory. When available, voucher specimens were deposited in the Scripps Institution of Oceanography Marine Vertebrates Collection. Museum numbers for specimens used in this study and GenBank accession numbers for sequence data are listed in Table 1. One to three individuals per species were used for both ingroup and outgroup taxa depending on availability. The speckled hind, *Epinephelus drummondhayi* Goode and Bean was included because it is the putative sister species to the *Alphestes/Dermatolepis* complex (Craig *et al.*, 2001). The flag cabrilla, *Epinephelus labriformis* Jenyns, and the white-blotched grouper, *Epinephelus multinotatus* (Peters), were also included to increase taxon sampling within this lineage of nearly 100 species. The barred sand bass, *Paralabrax nebulifer* (Girard) was used as an outgroup taxon to root the serranid tree as this genus has been hypothesized to be a basal member of the family Serranidae (Pondella *et al.*, 2003).

Total genomic DNA was isolated using the DNeasy kit (Qiagen, Inc, Valencia, CA, USA) and protocols followed manufacturer's recommendations. Polymerase chain reaction (PCR) was used to amplify portions of three mitochondrial (mt) genes (16S, 12S, and cytochrome *b*) as well as one putative protein coding nuclear gene (TMO-4C4). Fifty microlitre PCR reactions were prepared using Red-Taq Ready Mix (Sigma–Aldrich, St. Louis, MO, USA) following the manufacturer's instructions with the addition of approximately 10–100 ng of template DNA and 10 pmol of both forward and reverse primers. Qiaquick spin columns (Qiagen, Inc., Valencia, CA, USA) were used to remove primers and unincorporated dyes from PCR reactions. PCR reactions were carried out on an MJ Research PTC-200 thermal cycler. PCR reactions were subjected to 35 cycles of the following cycling protocol following a 1-min denaturation at 94 °C for 1 min: 94 °C for 30 s, 50 °C for 45 s, 72 °C for 30 s. Sequencing was performed on a Megabace 1000 capillary sequencer using ET-Chemistry (Amersham Biosciences, Piscataway, NJ, USA) following manufacturer's protocols. Sequencing and PCR primers used are listed in Table 2.

Sequences were aligned using CLUSTAL-X (Thomson *et al.*, 1997) with default settings and alignments were visually optimized using MACCLADE 4.0 (Sinauer Associates, Sunderland, MA, USA). A 19 bp segment of the 16S gene

Table 1 Museum numbers for specimens deposited at Scripps Institution of Oceanography Marine Vertebrates Collection (SIO) and GenBank accession numbers for sequence data in this study. Photo vouchers are retained in the personal collection of MFC.

		GenBank accession number			
Species	Museum number	Cyt-B	16S	12S	TMO4C4
Alphestes multigutattus	SIO 00-95	AY313995	AF297305	AY313981	AY313991
Alphestes immaculatus	SIO 00-92	AY314002	AF297290	AY313980	AY313994
Alphestes afer	SIO 03-49	AY313996	AY314003	AY313982	AY313992
Dermatolepis dermatolepis	Photo voucher	AY314000	AF297317	AY313984	AY313988
Dermatolepis inermis	Photo voucher	AY314001	AY314005	AY313979	AY313987
Dermatolepis striolata	Photo voucher	AY313999	AY314004	AY313989	AY313989
Epinephelus multinotatus	Photo voucher	AY426254	AY428594	AY426252	AY425675
Epinephelus labriformis	SIO 00-137	AY426255	AF297296	AY426252	AY425676
Epinephelus drummondhayi	SIO 00-50	AY313997	AF297317	AY313985	AY313993
Paralabrax nebulifer	SIO 00-97	AY313998	AF297328	AY072662	AY313990

Table 2 Sequencing and PCR primers.

Primer name	Gene	Sequence	Reference
16Sar-L	16S	5′-cgcctgtttatcaaaaacat-3′	Palumbi (1996)
16Sbr-H	16S	5′-ccggtctgaactcagatcacgt-3′	Palumbi (1996)
12Sa	12S	5′-aaactgggattatagaccccactat-3′	Palumbi (1996)
12Sb	12S	5′-gagggtgacgggcggtctct-3′	Palumbi (1996)
28For	Cyt-B	5′-cgaacgttgatatgaaaaaccatcgttg-3′	Gilles *et al.* (2000)
34Rev	Cyt-B	5′-aaactgcagccccctcagaatgatatttctccttca-3′	Gilles *et al.* (2000)
TMO-F1-5′	TMO4C4	5′-cctccggccttcctaaaacctctc-3′	Streelman and Karl (1997)
TMO-R1-5′	TMO4C4	5′-catcgtgctccctgggtgacaaagt-3′	Streelman and Karl (1997)

was removed due to ambiguity of the aligned base pairs. Indels in protein coding genes (cyctochrome *b* and TMO4C4) were treated as single events in all analyses. Exhaustive search criteria for maximum parsimony analysis and heuristic search criteria for likelihood analysis were employed in PAUP*4b10 (Sinauer Associates, Sunderland, MA, USA). For the maximum likelihood analysis, the default settings in PAUP*4b10 were used except that a gamma distribution of rates at variable sites was employed. For the maximum parsimony analysis, default settings in PAUP*4b10 were used with the exception that the Delayed Transformation Series (DELTRAN) was used, and gaps in the data were treated as a fifth base. A partition homogeneity test was performed in PAUP*4b10 to assess the validity of combining the four molecular datasets. Bootstrap support values were calculated using default settings in PAUP*4b10 with 1000 replicates, and Bremer support indices were calculated using NONA (V. 2.0; Goloboff, unpubl. program) in conjunction with the program WINCLADA (1.00.08; Nixon, unpubl. program). Bremer supports were calculated using a heuristic search with a maximum of 100,000 trees, holding 10 trees per replicate, with 100 random addition sequences each with Tree Bisection Reconnection (TBR) branch swapping.

RESULTS

Using partial DNA sequences for one nuclear (TMO4C4; 535 bp) and three mitochondrial genes (16S, 12S, and cytochrome *b*; 1447 bp), we explored the phylogenetic relationships of species within *Alphestes* and *Dermatolepis*. The partition homogeneity test supported the combination of all genetic data sets ($P = 0.44$), and the combined data were used in all further analyses. Maximum parsimony and maximum-likelihood analyses of the combined data set recovered similar trees with only minor differences in topology (Figs 1 & 2). In both topologies, relationships within *Dermatolepis* are identical: *D. inermis* of the WA is sister to *D. striolata* of the IO, while *Dermatolepis dermatolepis* of the TEP is sister to these two. Within *Alphestes*, the parsimony analysis includes an unresolved trichotomy, while the likelihood analysis supports the sister group relationship of *A. immaculatus* and *A. multigutattus*, both of the TEP, with *A. afer* of the WA sister to this pair. This suggests that the ancestor of the two eastern Pacific species and *A. afer* evolved via a trans-isthmian speciation event.

Genetic distances were calculated for all species pairs and ranged from 2.2 to 5.7% among ingroup taxa (Table 3). When calibrated using the average genetic distance and likelihood branch lengths between the species pairs *A. immaculatus* + *A. afer*, and *A. multigutattus* + *A. afer* (thus accounting for the amount of sequence divergence between the ancestor of *A. immaculatus* + *A. multigutattus* and *A. afer*, the hypothesized trans-ithmian speciation event) and a disruption of gene flow at 3.5–4.5 Ma (Coates & Obando, 1996; Knowlton & Weight, 1998), speciation within

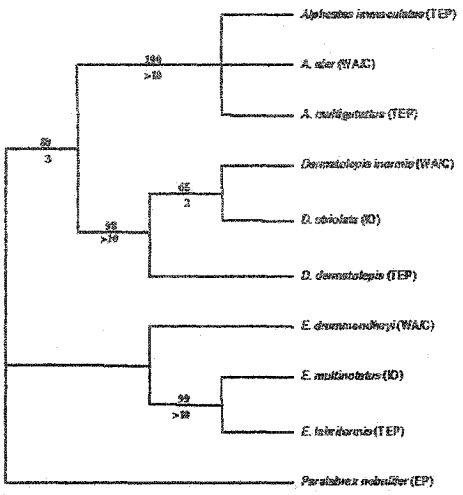

Figure 1 Consensus of two most parsimonious trees (669 steps) for the serranid genera *Alphestes* Bloch and Schneider and *Dermatolepis* Gill. Numbers above and below nodes are Bootstrap (1000 replicates) and Bremer support values, respectively. Geographic range is indicated after species names as follows: WA, Western Atlantic; C, Caribbean; TEP, Tropical Eastern Pacific; EP, Eastern Pacific; and IO, Indian Ocean. Consistency index (CI) and retention index (RI) were identical for both trees (CI = 0.728, RI = 0.546).

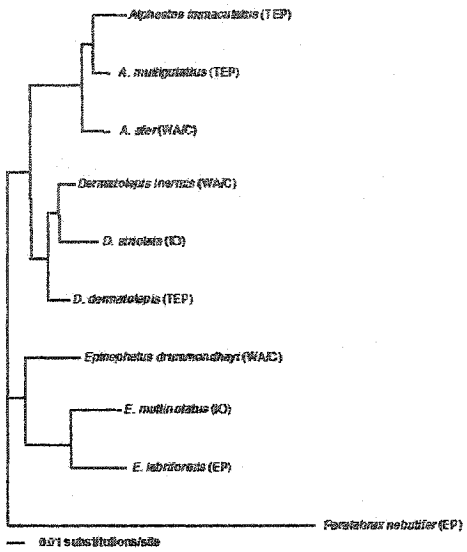

Figure 2 Maximum likelihood tree (–ln likelihood = 6246.8547) obtained for the serranid genera *Alphestes* Bloch and Schneider and *Dermatolepis* Gill. Abbreviations of geographic ranges are as in Fig. 1.

this group was most likely initiated during the middle-late Miocene, approximately 7–9 Ma.

DISCUSSION

The analyses presented here confirm the previously hypothesized monophyly of *Alphestes* and *Dermatolepis* and confirm the validity of generic status for these clades (Craig *et al.*, 2001). Our molecular phylogenetic hypotheses for these two closely related genera of reef fishes reveal some quite unexpected biogeographic patterns. In both analyses, there is no evidence for an extant trans-isthmian species pair in either genus. The timing of the speciation events in each genus implies that partial or complete closure of the Panamanian isthmus did not play as crucial a role in precipitating the most recent speciation events in these reef fishes as was previously hypothesized (Jordan, 1908).

The topologies presented for each genus in both analyses are not biogeographically incongruent. The closure of the Central American Seaway apparently precipitated allopatric speciation

Table 3 Percent sequence divergence between species of *Alphestes* Bloch and Schneider and *Dermatolepis* Gill and selected outgroups. Abbreviations of geographic ranges are as in Fig. 1

Species	1	2	3	4	5	6	7	8	9
1. *Alphestes immaculatus* (TEP)	*	*	*	*	*	*	*	*	*
2. *Alphestes afer* (WA/C)	0.033	*	*	*	*	*	*	*	*
3. *Alphestes multiguttatus* (TEP)	0.022	0.023	*	*	*	*	*	*	*
4. *Dermatolepis inermis* (WA/C)	0.054	0.057	0.048	*	*	*	*	*	*
5. *Dermatolepis dermatolepis* (TEP)	0.055	0.054	0.053	0.022	*	*	*	*	*
6. *Dermatolepis striolata* (IO)	0.054	0.057	0.055	0.026	0.029	*	*	*	*
7. *Epinephelus drummondhayi* (WA/C)	0.063	0.067	0.064	0.055	0.054	0.062	*	*	*
8. *Epinephelus multinotatus* (IO)	0.074	0.079	0.076	0.063	0.064	0.068	0.062	*	*
9. *Epinephelus labriformis* (TEP)	0.077	0.081	0.075	0.068	0.068	0.071	0.06	0.047	*
10. *Paralabrax nebulifer* (EP)	0.132	0.142	0.138	0.132	0.131	0.133	0.13	0.138	0.137

in *Alphestes*, with subsequent speciation occurring within the TEP. Within ocean speciation in Alphestes is plausible because, although these species overlap in distribution, *A. multiguttatus* generally occurs in deeper water where it co-occurs with *A. immsculatus* (M.T.C., pers. obser.). Post-isthmian speciation within the TEP has been hypothesized for other fishes (e.g. Rosenblatt, 1963).

Similarly, the closure of the Central American Seaway could have precipitated allopatric speciation in *Dermatolepis* with subsequent dispersal to and speciation in the Indian Ocean by the western Atlantic member via a stepping-stone model, first across the Atlantic, and subsequently around the southern tip of Africa into the Indian Ocean. Trans-Atlantic dispersal has been noted in several shore fish species (Joyeux *et al.*, 2001) and genetic data show minimal distance between western Atlantic and mid-Atlantic populations (Rocha *et al.*, 2002). Additionally, sister species status has been hypothesized for eastern and western Atlantic species in the seahorse genus *Hippocampus* Rafinesque (Jones *et al.*, 2003) and several species of fishes occur in both western and eastern Atlantic localities [e.g. the serranids *Paranthias furcifer* Valenciennes and *Epinephelus marginatus* Lowe, the sperid *Sparisoma rubripinne* (Valenciennes), the labrid *Bodianus pulcheilus* (Poey), the chaetodontid *Chaetodon sedentarius* (Poey), and the pomacanthid *Pomacanthus paru* (Bloch)] (Afonso *et al.*, 1999; Bernardi *et al.*, 2000; Joyeux *et al.*, 2001).

Species with long-lived larval stages such as the serranids (Liao *et al.*, 2001) would be particularly suited to these trans-Atlantic crossings, and while there are several island groups that may provide stepping stones (e.g. Ascension Island), they may not be required as the south equatorial counter current may be sufficient to disperse larvae across the Atlantic (Joyeux *et al.*, 2001). Three other related grouper species, *Mycteroperca rubra* (Bloch), *Mycteroperca fusca* (Lowe), and *Mycteroperca acutirostris* (Valenciennes) are known from both sides of the Atlantic basin, but are absent from the mid-Atlantic island groups (Heemstra & Randall, 1993) supporting the hypothesis that trans-Atlantic crossings are possible in these fishes. Additionally, a 1.8 m specimen of the warsaw grouper, *Epinephelus nigritus* (Holbrook), was reported from the coast of Brittany (46°47′ N, 3°50′ W) in summer of 1997, and a specimen identified as the scamp, *Mycteroperca phenax* (Jordan and Swain), was recently reported from the Azores (P.C. Heemstra, pers. comm.).

While the crossing of the Atlantic basin has been demonstrated by several species (Joyeux *et al.*, 2001), dispersal around the southern tip of Africa is poorly documented. However, both the dusky grouper, *Epinephelus marginatus* (Lowe), and the comber, *Serranus cabrilla* (Linnaeus), range from the Mediterranean Sea, along the entire western coast of the African continent, and into a portion of the western Indian Ocean, the area from which *D. striolata* has been recorded (Heemstra & Randall, 1993). *Epinephelus marginatus* also is known from the western Atlantic (Heemstra & Randall, 1993).

The hypothesis of a sister group relationship between western Atlantic and Indian Ocean shore fish species, to our knowledge, has never been reported. While corals of the family Meandrinidae show a similar distribution (Veron, 2000), the timing of their diversification is more consistent with dispersal through the now closed Tethys Sea. This explanation does not apply to the species of *Dermatolepis* as the closure of the Tethys Sea 18–20 Ma (Hallum, 1994) well predated the hypothesized speciation events within this genus. While this area relationship is rare, it is consistent with dispersal into the eastern Atlantic and around the tip of Africa by *Dermatolepis*, with subsequent extinction along the western coast of Africa or persistence of an unnoticed population in this remote and relatively poorly known area. The latter possibility is supported by the known distribution of the dusky grouper, *E. marginatus* (see above).

While the biogeographic pattern hypothesized for the species within *Dermatolepis* is unique, the timing of this process offers another interesting finding for speciation hypotheses associated with the Central American Seaway. Examination of the rates of sequence divergence between these species and corresponding branch lengths in our phylogenetic analysis supports a pre-closure dispersal scenario. Percent sequence divergences between sister species are comparable for *Dermatolepis* and *Alphestes* (Table 3). These data imply that the first speciation within *Dermatolepis* predated the first (trans-isthmian) speciation event in *Alphestes*, while subsequent speciation resulting in *D. striolata* and *D. inermis* was more or less concomitant with the closure of the isthmus (Fig. 2 and Table 3). Additionally, the uncorrected genetic distance between the eastern Pacific *D. dermatolepis* and the Caribbean *D. inermis* is less than that between *D. inermis* and *D. striolata*, despite the sister species status afforded to the latter pair in our phylogenetic analyses. This is consistent with a nearly simultaneous divergence of all three species within *Dermatolepis* (given the short branch lengths between the ancestor of the *D. striolata* and *D. inermis* clade and *D. dermatolepis*).

These findings provide a strong argument for complete taxon sampling of clades including putative trans-isthmian geminates prior to evaluating hypotheses regarding their evolution. Had all species within *Alphestes* and *Dermatolepis* not been sampled, particularly *D. striolata* from the Indian Ocean, several alternative and incorrect hypotheses may have been deduced for the patterns of speciation in these fishes. For example, without this species being included, *D. dermatolepis* and *D. inermis* would necessarily have been sister taxa, leading to incorrect hypothesized rates of molecular evolution within this group. Alternatively, we might have hypothesized that the rising isthmus interrupted gene flow at different times in the evolutionary history of *Dermatolepis* and *Alphestes* as suggested by the model presented by Knowlton & Weight (1998). These and similar findings indicate that the evolutionary history of trans-isthmian geminate species is often far more complex than previously thought.

ACKNOWLEDGMENTS

The authors wish to thank H.J. Walker, Jr., and C. Klepadlo, Scripps Institution of Oceanography Marine Vertebrates Collection, for their curatorial assistance. We would also like to thank D.R. Robertson, R. Beaver, and F. Young for collection of specimens used in this research. We would like to thank W.A. Newman and P.C. Heemstra for additional distribution information and recent collection records. We are grateful to N. Knowlton for use of laboratory facilities, and to N. Knowlton and R. Burton for helpful comments on earlier drafts of this manuscript. Funding was provided by the SIO Graduate Department, SIO Marine Biology Research Division, UCSD Academic Senate, the American Museum of Natural History Lerner-Grey Fund for Marine Research, and the Vantuna Research Group, Occidental College.

REFERENCES

Afonso, P., Porteiro, P.M., Santos, R.S., Barreiros, J.P., Worms, J. & Wirtz, P. (1999) Coastal marine fishes of São Tomé Island (Gulf of Guinea). *Arquipélago*, **17A**, 65–92.

Arbogast, B.S., Edwards, S.V., Wakeley, J., Beerli, P. & Slowinski, J.B. (2002) Estimating divergence times from molecular data on phylogenetic and population genetic timescales. *Annual Review of Ecology and Systematics*, **33**, 707–741.

Bermingham, E., McCafferty, S.S. & Martin, A.P. (1997) Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus. *Molecular systematics of fishes* (ed. by C. Stepien and T. Kocher), pp. 113–128. Academic Press, London.

Bernardi, G., Robertson, D.R., Clifton, K.E. & Azzurro, E. (2000) Molecular systematics of and evolutionary ecology of the Atlantic parrotfish genus *Sparisoma*. *Molecular Phylogenetics and Evolution*, **15**, 292–300.

Coates, A.G. & Obando, J.A. (1996) The geologic evolution of the Central American Isthmus. *Evolution and environment in tropical America* (ed. by J. Jackson, A.F. Budd and A.G. Coates), pp. 21–56. The University of Chicago Press, Chicago, IL.

Collins, T. (1996) Molecular comparisons of transisthmian species pairs: rates and patterns of evolution. *Evolution and environment in tropical America* (ed. by J. Jackson, A.F. Budd and A.G. Coates), pp. 303–334. The University of Chicago Press, Chicago, IL.

Craig, M.T., Pondella, D.J., II. & Hafner, J.C. (1999) Analysis of age and growth in two eastern Pacific groupers (Serranidae:Epinephelinae). *Bulletin of Marine Science*, **65**, 807–814.

Craig, M.T., Pondella, D.J., II, Hafner, J.C. & Franck, J.P.C. (2001) On the status of the serranid fish genus *Epinephelus*: evidence for paraphyly based on 16S rDNA sequences. *Molecular Phylogenetics and Evolution*, **19**, 121–130.

Gilles, A., Miquelis, A., Quignard, J-P. & Faure, E. (2000) Molecular phylogeography of Western Mediterranean dusky grouper, *Epinephelus marginatus*. *Comptes rendus de l'Académie des sciences. Série III, Sciences de la vie*, **323**, 195–205.

Hallum, A. (1994) *An outline of Phanerozoic biogeography*. Oxford University Press, Oxford.

Haug, G.H. & Tiedeman, R. (1998) Effect of the formation of the Isthmus of Panama on Atlantic Ocean thermohaline circulation. *Nature*, **393**, 673–676.

Heemstra, P.C. & Randall, J.E. (1993) *Groupers of the world*. FAO Species Catalogue, 16, Rome.

Johnson, G.D. & Keener, P. (1984) Aid to identification of American grouper larvae. *Bulletin of Marine Science*, **34**, 106–134.

Jones, A.G., Moore, G.I., Kvarnemo, C., Walker, De E. & Avise, J.C. (2003) Sympatric speciation as a consequence of male pregnancy in seahorses. *Proceedings of the National Academy of Sciences*, **100**, 6598–6603.

Jordan, D.S. (1908) The law of geminate species. *American Naturalist*, **42**, 73–80.

Joyeux, J.C., Floeter, S.R., Ferreira, C.E.L. & Gasparini, J.L. (2001) Biogeography of tropical reef fishes: the south Atlantic puzzle. *Journal of Biogeography*, **28**, 831–841.

Knowlton, N.K. & Weight, L.A. (1998) New dates and new rates for divergence across the Isthmus of Panama. *Proceedings of the Royal Society of London, Series B: Biology*, **265**, 2257–2263.

Knowlton, N., Weight, L.A., Solorzano, L.A., Mills, D.K. & Bermingham, E. (1993) Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. *Science*, **260**, 1629–1632.

Leis, J.M. (1986) Larval development in four species of Indo-Pacific coral trout *Plectropomus* (Pisces, Serranidae: Epinephelinae) with an analysis of relationships of the genus. *Bulletin of Marine Science*, **38**, 525–552.

Liao, I.C., Su, H.M. & Chang, E.Y. (2001) Techniques in finfish larvaculture in Taiwan. *Aquaculture*, **200**, 1–31.

Marko, P.B. (2002) Fossil calibration of molecular clocks and the divergence times of geminate species pairs separated by the Isthmus of Panama. *Molecular Biology and Evolution*, **19**, 2005–2021.

Marko, P.B. & Jackson, J.B.C. (2001) Patterns of morphological diversity among and within arcid bivalve species pairs separated by the Isthmus of Panama. *Journal of Paleontology*, **75**, 590–606.

Palumbi, S. (1996) Nucleic acids II: the polymerase chain reaction. *Molecular systematics* (ed. by D.M. Hillis, C. Moritz and B.K. Mable), pp. 205–248. Sinauer, Sunderland.

Pondella, D.J. II., Craig, M.T. & Franck, J.P.C. (2003) The phylogeny of *Paralabrax* (Perciformes; Serranidae) and allied taxa inferred from partial 16S and 12S mitochondrial ribosomal DNA sequences. *Molecular Phylogenetics and Evolution*, **29**, 176–184.

Rocha, L.A., Bass, A.L., Robertson, D.R. & Bowen, B. (2002) Adult habitat preferences, larval dispersal, and phylogeography of three Atlantic surgeonfishes (Teleostei: Acanthuridae). *Molecular Ecology*, **11**, 243–252.

Rosenblatt, R.H. (1963) Some aspects of speciation in marine shore fishes. Systematics Association Publication, No. 5, pp. 171–180.

Sala, E., Ballesteros, E. & Starr, R.M. (2001) Rapid decline of Nassau grouper spawning aggregation in Belize: Fishery management and conservation needs. *Fisheries*, **26**, 23–30.

Streelman, J.T. & Karl, S.A. (1997) Reconstructing labroid evolution with single-copy nuclear DNA. *Proceedings of the Royal Society of London*, **264**, 1011–1020.

Thomson, D.A., Findley, L.T. and Kerstitch, A.N. (2001) *Reef fishes of the Sea of Cortez*. The University of Texas Press, Austin, TX.

Thomson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **25**, 4876–4882.

Veron, J.E.N. (2000) *Corals of the world* (scientific ed. and producer M.S. Smith). Australian Institute of Marine Science, Townsville.

White, D.B., Wyanski, D.M., Eleby, B.M. & Lilyestrom, C.G. (2002) Tiger grouper (*Mycteroperca tigris*): profile of a spawning aggregation. *Bulletin of Marine Science*, **70**, 230–240.

ADDENDUM ADDED IN PRESS

Recently, a specimen of *Alphestes afer* (Bloch 1873) was reported from a fish market at Sao Tome Island (eastern Atlantic) by Dr Peter Wirtz. Dr Wirtz was able to photograph the specimen and obtain a tissue sample for analysis. Examination of DNA sequence data from the mitochondrially encoded Cytochrome B, 16S, and 12S genes, the nuclear TMO-4C4 locus, and characters in the photograph indicate that the specimen from Sao Tome is conspecific with specimens from the Florida Keyes (uncorrected p distance = 0.004). Originally described from the coast of Guinea, the type locality of *A. afer* has long been regarded as a locality error (report forthcoming by M. T. Craig and P. C. Heemstra). The incorporation of this information provides further support for our biogeographic hypothesis of trans-Atlantic dispersal in *Dermatolepis*. The data suggest that the eastern Atlantic served as a stepping stone for *Dermatolepis* prior to its dispersal around the Cape of Good Hope and subsequent speciation within the western Indian Ocean.

BIOSKETCHES

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The text of Chapter II, in full, has been submitted for publication of the material as it appears in Journal of Biogeography, Volume 31, pages 1085-1091. The dissertation author was the primary author and the co-author listed in this publication directed and supervised the research, which forms the basis for this chapter.

CHAPTER III

Redescription and validation of *Alphestes afer* (Bloch 1793) as an amphi-Atlantic grouper species (Perciformes: Serranidae).

Redescription and validation of *Alphestes afer* (Bloch 1793) as an amphi-Atlantic grouper species (Perciformes: Serranidae).

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Abstract of Chapter III

On 4 March 2004, a specimen of *Alphestes afer* (Bloch 1793) a common western Atlantic species of grouper, was photographed and a tissue sample was taken at São Tomé city fish market on São Tomé Island (Gulf of Guinea; West Africa). Mitochondrial and nuclear DNA evidence indicates that this specimen is conspecific with western Atlantic specimens. Although it was originally described from the coast of Guinea (western Africa), the presence of this species in the eastern Atlantic has gone unnoticed in that region, and the type locality has long been regarded as erroneous. A morphological comparison of the holotype with 44 specimens from various western Atlantic and Caribbean localities indicates that the holotype is indeed conspecific with western Atlantic specimens. The species is re-described. The holotype, the São Tomé specimen, and a re-consideration of the species synonymy validate the amphi-Atlantic distribution of this species.

INTRODUCTION

The genus *Alphestes* comprises three species of serranid fishes commonly known as Mutton Hamlets: These small grouper species rarely reach sizes in excess of 30 cm total length. The Rivulated Mutton Hamlet, *Alphestes afer* (Bloch 1793), has been recorded from Bermuda south to Brazil. Originally described from the coast of Guinea by Bloch (1793) as *Epinephelus afer*, this species has, however, not been reported from the eastern Atlantic since the original description, and the type locality has long been regarded as erroneous (Smith, 1971, Heemstra and Randall, 1993).

On 4 March 2004, P. Wirtz observed a grouper in a fish market on São Tomé Island (Gulf of Guinea, western Africa) that was unfamiliar to him. He photographed the fish and obtained a tissue sample for genetic analysis. The fish was identified from the photograph as *Alphestes afer* by P.C. Heemstra and an examination of DNA sequences by M.T. Craig indicated that the specimen was conspecific with western Atlantic specimens of *Alphestes afer* Bloch 1793. Herein, we re-describe Bloch's *Alphestes afer* and confirm its amphi-Atlantic distribution.

MATERIALS AND METHODS

Counts and measurements were performed following Hubbs and Lagler (1958) and Heemstra and Randall (1993). Institutional abbreviations follow Leviton, et al. (1985). For the Sãão Tomé specimen, DNA extraction, PCR, and sequencing protocols followed Craig, et al. (2004). Sequence data was obtained for the mitochondrial Cytochrome B, 16S rDNA, and 12s rDNA genes, as well as the nuclear TMO 4C4 genes (2001 total base pairs). Sequence data was added to an existing dataset designed to determine relationships among *Alphestes* and the closely related grouper genus *Dermatolepis* (Craig, et al., 2004). Sequences were aligned using CLUSTAL X with default settings, and visually optimized in MacClade (V 4.0). A maximum parsimony and maximum likelihood analysis were performed in PAUP*4b10 using default settings except that the Delayed Transformation Series (DELTRAN) was used. Bootstrap supports were calculated in PAUP*4b10 using 1000 replicates.

RESULTS

Both maximum parsimony and maximum likelihood analysis of the DNA sequence data recovered identical tree topology. The maximum parsimony tree is depicted in Figure 1. The three species of *Alphestes* formed a monophyletic clade with the specimen from São Tomé occupying a sister-taxon relationship with *A. afer* from Florida. The three species of *Dermatolepis* also formed a monophyletic clade. The interrelationships among *Alphestes* and *Dermatolepis* were discussed in detail in Craig, et al. (2004). Total sequence divergence (p) between the São Tomé specimen and *A. afer* from Florida was 0.00454, while that between *A. afer* and *A. immaculatus* was 0.04282, and between *A. afer* and *A. multigttatus*, 0.02519.

Alphestes afer (Bloch)

Figures 2, 3.

Epinephelus afer Bloch, 1793: 12, Plate 327 [Original description; holotype ZMB 143, 224 mm SL; type locality Guinea (western Africa)]. Boulenger, 1895: 254 (description based on Western Atlantic specimens; *Serranus armatus* Osorio, 1894 listed in synonymy with a question mark; distribution given as "Atlantic coasts of America from West Indies to Falkland Islands; coast of Guinea?"). Smith, 1971 (in part, not eastern Pacific specimens, which are *Alphestes immaculatus* Breder, 1936). Paepke, 1999: 135 (Guinea type locality noted as "wrong")

Alphestes afer: Bloch and Schneider, 1801: 236; Peters, 1865: 105 (examined Bloch's holotype and synonymized it with the Western Atlantic species *Plectropoma chloropterum* and *Plectropoma monacanthus*); Jordan and Swain, 1884: 396 (distribution given as "West Indies"; queried Bloch's type locality, Guinea); Jordan and Eigenmann, 1890: 350 (description based on Western Atlantic fish; synonymy); Jordan and Evermann, 1896: 1164 (description, synonymy; noted that "only the original type of Bloch recorded from Africa"); Heemstra and Randall, 1993: 20, Pl. I, Fig. B, (diagnosis, synonymy, distribution; Bloch's type locality, Guinea said to be

erroneous; distinguished from *Alphestes immaculatus* Breder, 1936 and *Alphestes multiguttatus* (Günther, 1867)).

Plectropoma chloropterum Valenciennes in Cuv. and Val., 1828: 398 [type locality Dominican Republic and Martinique].

Plectropoma monocanthus Müller and Troschel, 1848: 665 [type locality Barbados].

Plectropoma afrum (non Bloch): Günther, 1868: 411 [mis-identification].

Serranus armatus Osorio, 1894: 174 (type locality São Tomé).

Epinephelus lightfooti Fowler, 1907: 258, Fig. 3 [type locality Santo Domingo, Dominican Republic]

Diagnosis.- (Based on 44 western Atlantic specimens (64 – 253 mm SL; data from Bloch's holotype given in parentheses.)

Body depth slightly less than head length, depth contained 2.4-3.1 (2.9) in standard length; caudal peduncle depth contained 7-9 (8.5) times in standard length; eye diameter greater than or equal to snout length and contained 8.1-15 (13) times in standard length; snout length 10-16 (16) times in standard length; preopercle rounded, the posterior edge serrate, with a large antrorse spine at the angle directed downward and forward and usually covered with skin. Gill rakers 5-8 on upper limb, 14-17 on lower limb, 19-25 total, including rudiments. Dorsal fin with 11 spines and 17 to 20 (18) rays, anal fin with 3 spines and 9, rarely 10 rays; pectoral fins with 16 - 18 (17) rays; caudal fin rounded, with 15 branched rays. Scales smooth; lateral-line scales 53-66; lateral scale series 68 - 78. Color in life: Head, body, and median fins olivaceous or light brown, irregularly blotched and barred with dark brown. Some individuals densely spotted with orange; head, body, and all fins often with scattered white spots; body sometimes covered with scattered small black dots; pectoral fins may be orange or yellow with faint dark reticulations.

Description.-A small grouper species, rarely reaching greater than 33 cm total length. Meristic counts and morphometric data are presented in Table 1. Spinous dorsal fin with membranes slightly incised, the third or fourth spine longest, and

soft rays distinctly longer than posteriormost spines. Spinous and soft portion of dorsal fin covered with scales approximately half way up the fin. Pectoral fins reaching beyond pelvics, the origin ahead of pelvic fin origin. Second and third anal-fin spines about equal. Interorbital space with minute, embedded scales extending forwards to rear nostrils. Middle opercular spine strongest, often exposed; upper and lower opercular spines covered with skin. Dorsal head profile high, slightly concave in some individuals. Nostrils close together, the anterior nostril in a short tube with rear margin expanded as a short skin flap. Branchiostegal membranes separate, attached to anterior end of isthmus. Gill rakers slender, shorter than gill filaments. Maxilla naked, reaching slightly beyond orbit Teeth on lower jaw with three to 5 anterior rows and two rows along the lateral edges; a pair of small canines (hidden by lips) on outer edge of tooth bands at front of both jaws; inner teeth near symphysis largest; palatine and vomerine teeth well developed. Belly scaly and lightly pigmented. Anus situated about two thirds of distance from origin of pelvic fins to origin of anal fin. Lateral line parallel to curvature of dorsal body profile, with two or three pored scales extending onto caudal fin.

Distribution.-Western Atlantic: Bermuda, south Florida, Gulf of Mexico Bahamas, Cuba, West Indies, Panama, Venezuela, and southward to the São Paulo, Brazil. Boulenger's (1895) record of *E. afer* from the Falkland Islands is based on the stuffed specimen reported by Günther (1859). This specimen probably came from Brazil, and was mislabeled or mixed up with specimens from the Falklands. Western Africa: Two specimens, one from the coast of Guinea (holotype) and the specimen reported herein from São Tomé Island (Gulf of Guinea).

DISCUSSION

While the existence of *A. afer* in the eastern Atlantic has gone unreported for more than two centuries, its presence there is now confirmed. The morphological characteristics of the holotype indicate that Bloch's (1793) description indeed represents *A. afer* of both eastern and western Atlantic populations. The sequence data presented herein confirm that the specimen

photographed on São Tomé is conspecific with *A. afer* (Bloch 1793) based on both the low level of sequence divergence (0.4%) and its sister group relationship with *A. afer* from Florida.

Osório (1893) described *Serranus armatus* as a new species from São Tomé. His description (in French) reads as follows: "Species near *Serranus undulosus* Cuvier, [= *Mycteroperca acutirostris*] but it differs by the following characters. The formula of the fins is D 11/18; A 3/10. The caudal is rounded also the soft part of the dorsal and not truncate as in *S. undulosus*. The serrae at the angle of the preopercle are larger than those on the vertical edge but particularly the hindmost, which is 2 mm long and curved downward and directed anteriorly. The irregular spots on the dorsal surface of the body and flanks have a black margin. The soft dorsal-fin is one and half times higher than the spinous part and the anal fin rays almost two times longer than the anal-fin spines. Color generally pale reddish brown. A black line extends from suborbital along the upper edge of the maxilla, another less dark line runs from the edge of the suborbital to the angle of the preopercle. Length of the largest of our specimens 12 cm. Habitat: São Tomé."

Alphestes afer is the only fish known from the eastern Atlantic that matches Osorio's description of *Serranus armatus*. The syntypes of this species were housed at the Museu Bocage in Lisbon but were destroyed by fire in 1978. The species name, preceded by a question mark, was placed in the synonymy of *Epinephelus afer* by Boulenger (1895). Although Fowler (1936: 763) was aware of Boulenger's identification of *Serranus armatus*, he considered the species to be a synonym of *Mycteroperca rubra* as "it seems more nearly to approach the present species. " Fowler's re-assignment of *Serranus armatus* was perhaps influenced by the apparent absence or doubtful occurrence of *Alphestes afer* in the eastern Atlantic. Fowler's misidentification of *Serranus armatus* was uncritically accepted by Smith (1990: 702) and doubtfully followed by Heemstra (1991: 53) and Heemstra and Randall (1993: 275).

Alphestes afer is apparently rare in the eastern Atlantic; no specimens were reported by Poll (1954) in his extensive trawling survey off the Congo River and

northern Angola. And, except for the listing (as *Serranus armatus*) in the synonymy of *Mycteroperca rubra*, it is not reported in the literature on eastern Atlantic groupers (Fowler, 1936; Smith, 1990; Heemstra, 1991).

Several amphi-Atlantic grouper species are known to exist on both sides of this ocean basin (e.g., *Epinephelus adscensionis, Epinephelus marginatus, Epinephelus itajara, Paranthias furcifer*) and there are putative sister species on either side of this basin (e.g., *Mycteroperca rubra and M. acutirostris*; cf. Heemstra and Randall, 1993; Luiz-Júnior, et al., 2004))

The Gulf of Guinea appears to be particularly rich in amphi-atlantic fishes and invertebrates (see Wirtz, 2004 and references therein). Even though planktonic larvae could cross the Atlantic in as little as 35 to 105 days in the Equatorial Undercurrent (Scheltema, 1971), it remains unclear in most cases if the western and eastern Atlantic populations of amphi-Atlantic species are still linked genetically today. A genetic study of the fish *Ophioblennius atlanticus* (Valenciennes, in Cuvier & Valenciennes, 1836 ; Muss, et al., 2001) suggested that eastern and western Atlantic populations of this species have been genetically distinct for about 5.5 million years and should probably be considered sister species. In contrast, there appears to be ongoing gene flow between American and African populations of the seaurchin *Eucidaris tribuloides* (Lamarck, 1816; Lessios et al. 1999).

Materials Examined.-Forty-five specimens: Holotype, 212 mm SL, Guinea, ZMB 143; ANSP 83729, Bahamas, 201 mm; ANSP 121416; ANSP 103289; ANSP 87101; ANSP 105415; ANSP 113327, St Lucia, 5 (64-180 mm); FMNH 4838, Bermuda, 253 mm; FMNH 49047, Bermuda,134 mm; LACM 7733, Puerto Rico, 132 mm; MCZ 9785, Cuba, 6 (136- 180 mm; MCZ 9797 Cuba, 191 mm; MCZ 43873, Panama, 130 mm; MZUSP uncataloged, Brasil: Ubatuba, 150 mm; UPR 819, Puerto Rico, 220 mm; UPR 2569, Puerto Rico, 148 mm; USNM 247245; USNM 33239; USNM 43348; USNM 235696.

ACKNOWLEDGEMENTS

The authors wish to thank ZMB for permission to reproduce Figure 2. P. Wirtz would like to thank CCMAR for partial funding of the collecting trip to São Tomé. We would like to thank M. Sabaj (ANSP), J. Seigel and R. Feeney (LACM), and J. Williams (USNM) for their curatorial assistance.

LITERATURE CITED

BLOCH, M.E. 1793. Naturgeschichte der auslädischen Fische. Berlin, Vol. 7: 1-144.

BLOCH, M.E. AND J. SCHNEIDER. 1801. Systema Ichthyologiae iconibus cx illustratum., 1-584.

BOULENGER, G.A. 1895. Catalogue of the Perciform Fishes in the British Museum. Vol. 1. Second Edition. Taylor and Francis, London. 394 p.

CRAIG, M. T., P. A. HASTINGS, AND D. J. PONDELLA, II. 2004. Speciation in the Central-American Seaway: the importance of taxon sampling in the identification of trans-isthmian geminate pairs. J. Biogeography 31:1085-1091.

FOWLER, H.W. 1907. Notes on Serranidae. Proc. Acad. Nat. Sci. Phila. 59: 249-269.

FOWLER, H.W. 1936. The marine fishes of West Africa based on the collection of the American Museum Congo Expedition, 1909-1915. Part II. Bull. Am. Mus. Nat. Hist., 70 (2): 607-1493.

HEEMSTRA, P.C. 1991. A taxonomic revision of the eastern Atlantic groupers (Pisces: Serranidae). Bol. Museu Municipal do Funchal, 43 (226): 5-71.

HEEMSTRA, P. C., AND J.E. RANDALL. 1993 . FAO Species Catalogue, Vol. 16. Groupers of the world (Family Serranidae, Subfamily Epinephelinae). An annotated and illustrated catalogue of the grouper, rockcod, hind, coral grouper and lyretail species known to date. FAO Fisheries Synopsis No. 125, Vol. 16. Rome, FAO. 382 p.

HUBBS, C. L., AND K. F. LAGLER. 1958. Fishes of the great lakes region. Cranbrook Inst.Sci. Bull. No. 26, Bloomfield Hills, Michigan.

JORDAN, D.S. AND C.H. EIGENMANN. 1890. A review of the genera and species of Serranidae found in the waters of America and Europe. Bull. U.S. Fish Comm., 8 (1888): 329-441,

JORDAN, D.S. AND B.W. EVERMANN. 1896. The fishes of North and Middle America. Bull. U.S. Nat. Mus., 47(1): 1240 p.

JORDAN, D.S. AND J. SWAIN. 1885. A review of the American species of *Epinephelus* and related genera. Proc. U.S. Nat. Mus., 7(1884): 358-410.

LESSIOS, H. A., B. D. KESSING, D. R. ROBERTSON, AND G. PAULAY. 1999. Phylogeography of the pantropical sea urchin genus *Eucidaris* in relation to land barriers and ocean currents. Evolution 53:806-817.

LEVITON, A. E., R. H. GIBBS, JR., E. HEAL, AND C. E. DAWSON. 1985. Standards in herpetology and ichthyology. Part I. Standard symbolic codes for institutional resources collections in herpetology and ichthyology. Copeia 1985(3): 802-832.

LUIZ-JÚNIOR, O. J., S. R. FLOETER, J. L. GASPARINI, C. E. L. FERREIRA, AND P. WIRTZ. 2004. The occurrence of *Acanthurus monroviae* (Perciformes; Acanthuridae) in the south-western Atlantic, with comments on other eastern Atlantic reef fishes occurring in Brazil. J. Fish Biol. 65:1173-1179.

MÜLLER, J. AND F.H. TROSCHEL. 1848. The vertebrate animals. In: Schomburgk. R.H., *The History of Barbados; Comprising a GeographicalDescription of the Island and an account of its Geology and Natural Productions.* Longman, Brown, Green and Longmans, London. 772p.

MUSS, A., D. R. ROBERTSON, C. STEPIEN, P. WIRTZ, AND B. BOWEN. 2001. Phylogeography of *Ophioblennius*: The role of ocean currents and geography in reef fish evolution. Evolution 55(3):561-572.

OSORIO, B. 1894. Estudo ichthyológicos àcerca da fauna dos domínios Portuguezes da Africa. 3. nota – peixes marítimos das ilhas de S. Thomé, do Princip e ilheo das Rolas. Jorn. Sci. Math. Phys. Nat., ser. 2, 5: 173-182.

PAEPKE, H.J. 1999. Bloch's Fish Collection in the Museum für Naturkunde der Humboldt Universität zu Berlin, an illustrated catalog and historical account. Theses Zoologicae, Vol. 32, Gantner Verlag KG, 216 pp, 32 pls.

PETERS, W.C.H. 1865. Ûber einige Bloch'sche Arten der Fischgattung *Serranus*. Monatsber. Dtsch. Akad. Wiss. Berlin (1865): 97-111.

POLL, M. 1954. Poissons IV. Téléostéens Acanthoptérygiens (Première Partie). Rés. Sci. Expéd Océanogr. Belg. Eaux Côt. Afr. Atlan. Sud. (1948-49). Mém. Inst. R. Sci. Nat. Belg., 4(3A): 1-390.

SCHELTEMA, R. S. 1971. The dispersal of the larvae of shoal-water benthic invertebrate species over long distances by ocean currents. In: Crisp, P. J. (ed), Fourth European Marine Biology Symposium. Cambridge University Press, Cambridge, Mass.

SMITH, C.L. 1971. A revision of the American groupers: *Epinephelus* and allied genera. Bull. Aml. Mus. Nat. History, 146 (2): 67- 241.

SMITH, C.L. 1990. Serranidae, (pp 695-706) *In* Clofeta II, Check-list of the fishes of the eastern tropical Atlantic, Vol. II. Junta Nacional de Investigacão Cientifica e Tecnologica, Lisbon. 519-1079.

WIRTZ, P. 2004. Four amphi-Atlantic shrimps new for São Tomé and Príncipe (eastern central Atlantic). Arquipélago Life and Marine Sciences 21 A, 83-85.

Table 1: Meristic counts and measures for 24 specimens of *Alphestes afer*. "*" indicates holotype.

Catalog #	SL	TL	Body Depth	Dept of CP	Snout	Orbit	D	A	Pc.	GR Upper	GR Lower	GR Total	LLS	LSS
ANSP 105415 6	28	37	10.5	4	2.5	3	XI, 18	III, 9	16	7	17	24	61	78
ANSP 121416 2	33	42	14	4	2.5	4	XI, 19	III, 9	16	7	16	23	-	-
UNSM 247245	36	44	13.5	5	3.5	4	XI, 18	III, 9	16	7	16	23	63	81
ANSP 121416 1	57	71	20.5	6.5	4	7	XI, 18	III, 9	16	7	17	24	66	88
ANSP 103289 3	62	79	23	8	6	6.5	XI, 18	III, 9	16	7	16	23	55	73
ANSP 105415 4	63	78	24	8	4.5	6.5	XI, 18	III, 9	16	7	16	23	62	78
ANSP 105415 5	68	82	25	8	5.5	7.5	XI, 19	III, 9	16	7	17	24	60	81
ANSP 105415 2	72	83	25.5	8.5	5.5	7	XI, 18	III, 9	16	7	15	22	60	75
ANSP 113327 4	72	89	27.5	8.5	5	6	XI, 18	III, 9	17	8	17	25	63	83
ANSP 105415 3	72	89	25.5	8.5	5	7	XI, 18	III, 10	15	7	16	23	63	86
ANSP 113327 3	72	91	26	8.5	5.5	7	XI, 18	III, 9	16	8	17	25	65	88
ANSP 105415 1	76	92	28.5	9	5.5	7.5	XI, 19	III, 10	16	7	16	23	66	78
ANSP 103289 2	77	96	31	10	6.5	6.5	XI, 18	III, 9	17	7	16	23	66	80
USNM 235696 8	84	110	30.5	9.5	8	8	XI, 18	III, 10	16	6	16	22	65	86
USNM 235696 1	96	113	35.5	12	8.5	9	XI, 18	III, 9	16	5	17	22	63	81
ANSP 103289 1	120	150	48.5	16	9.5	10	XI, 19	III, 9	17	7	17	24	53	70
LACM 7733	135	163	48.5	17	11	10	XI, 18	III, 9	16	5	14	19	49	80
ANSP 87101 1	152	192	55	19.5	10	13.5	XI, 18	III, 9	16	7	17	24	59	72
USNM 43348	153	186	63	20	10.5	12	XI, 19	III, 9	16	6	17	23	58	79
ANSP 87101 2	155	192	60	18	11	13	XI, 18	III, 9	16	7	16	23	61	78
ANSP 113327 2	168	24	62	22.5	14	14.5	XI, 18	III, 9	16	6	16	22	60	82
ANSP 113327 1	184	230	67	24	11.5	15	XI, 18	III, 9	16	6	16	22	53	70
ZMB 143*	224	-	75	24.8	14.7	15.1	XI, 19	III, 10	17	7	16	23	-	-
USNM 33239	230	286	78	30	16	16	XI, 19	III, 9	16	5	14	19	60	80

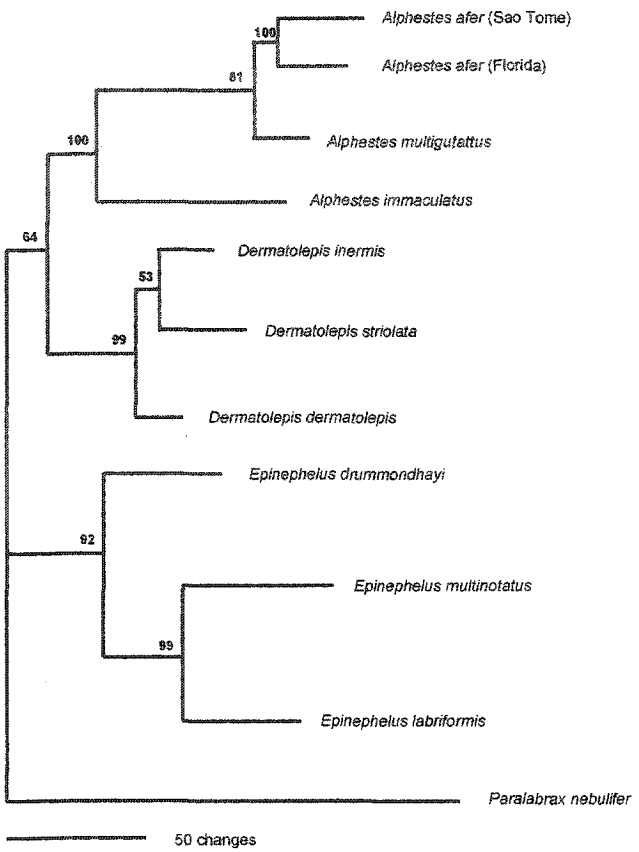

Figure 1. Maximum parsimony tree depicting relationships among species of *Alphestes* and *Dermatolepis* based on gene sequence data from the mitochondrial Cytochrome B, 16S, 12S, and nuclear TMO4C4 genes. Numbers above nodes are bootstrap percentages based on 1000 replicates. Branch lengths are proportional to sequence divergence. Tree length=738, CI=0.7304, HI=0.2696, and RI=0.6052.

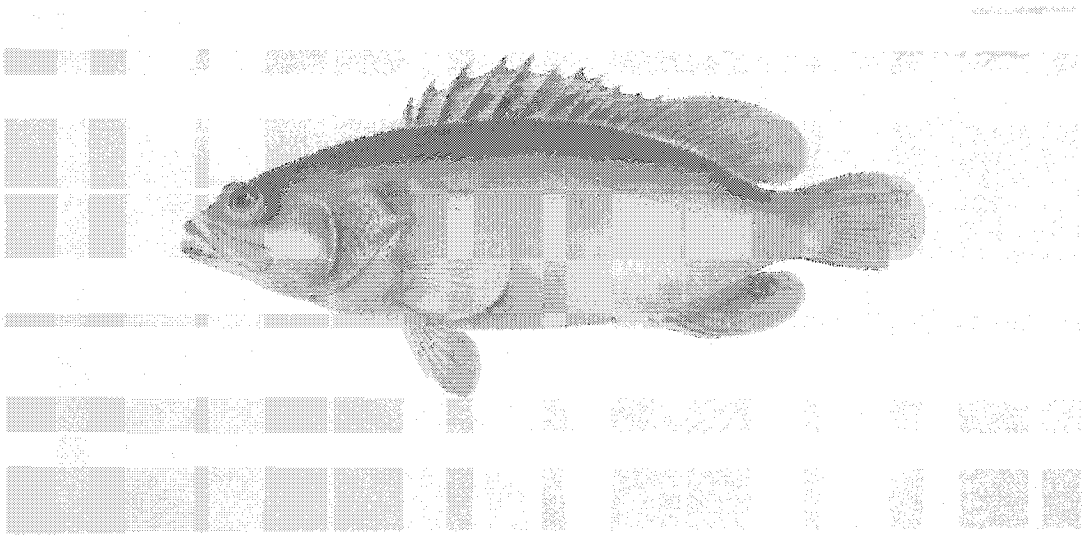

Figure 2. Original, unpublished drawing of Bloch's (1793) *Epinephelus afer*. Plate is provided courtesy of the archives of the Museum für Naturkunde der Humboldt-Universität zu Berlin (ZMB).

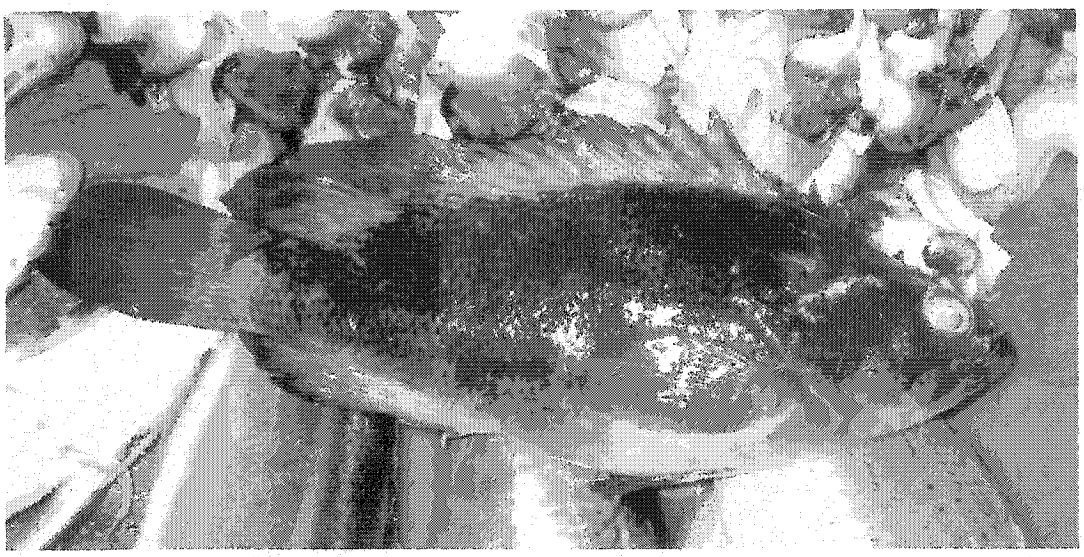

Figure 3. Photograph of *Alphestes afer* (Bloch 1793) from São Tomé City fish market (Gulf of Guinea, eastern Atlantic). Photograph was taken by P. Wirtz.

The text of Chapter III, in full, has been submitted for publication of the material as it appears in Copeia. The dissertation author was the primary author and the co-author listed in this publication directed and supervised the research, which forms the basis for this chapter.

CHAPTER IV

Global biogeography in nearshore marine fishes: A case study using the groupers of the tribe Epinephelini (Serranidae).

Introduction

Detecting historical patterns of diversification in the marine environment has remained a challenging topic for evolutionary biologists, particularly due to the scale upon which evolutionary forces operate in the world's oceans. Processes that have traditionally been thought to cause populations to become genetically distinct and ultimately drive speciation such as local adaptation and genetic drift are potentially overwhelmed by the extreme degree to which gene flow operates in the sea. The unifying effects of gene flow in the ocean environment are exaggerated in many marine species due to the presence of a pelagic larval stage (reviewed in Palumbi, 1996 and Avise, 2000). The pelagic larval stage, which may last days, weeks, or months, gives organisms that possess it expanded dispersal ability, as well as the capability to traverse the most significant physical barriers that are present in the ocean. This in turn leads to species ranges that may be greater than 10,000 km (Jablonski and Lutz, 1983). The effects of a pelagic larval stage make understanding and characterizing divergence in the ocean a daunting task.

Several factors have been hypothesized to facilitate genetic differentiation in the marine environment, although it is rarely the case that any single, direct cause can be unequivocally demonstrated in the field (Rosen 1985, 1988; Palumbi, 1997; Paulay, 1997; Bellwood and Wainwright, 2002; Connolly, et al., 2003). It is more likely that several factors in combination play a role in the genetic divergence of populations such as limited dispersal ability (Waples, 1987; Doherty et al., 1995), local adaptation (Powers, et al., 1991; Schmidt and Rand, 1999), oceanographic currents (Palumbi et

al, 1997; Rocha-Olivares and Vetter, 1999), habitat discontinuities (Hastings, 2000; Burton and Feldman, 1981; Stepien and Rosenblatt, 1991), isolation by distance (Palumbi, et al, 1997), and historical vicariance (Avise, 1992).

Biogeography of coastal organisms has been studied in fairly good detail on local and regional scales (e.g., Hastings, 2000; Joyeux, et al, 2001) however, global biogeography of near-shore species has only recently been examined from a phylogenetic perspective. The lack of global biogeographic studies is most likely due to the paucity of large data sets that are conducive to examining biogeographic patterns; few species groups provide the appropriate combination of diversity, distributional range, and well supported phylogenetic hypothesis of relationships that are essential in examining historical patterns of genetic divergence that may have led to speciation events and clade diversification. Global biogeography has been examined with molecular data for organisms such as sea urchins of the genus *Diadema* (Lessios, et al., 2001), and gastropods of the genus *Echinolittorina* (Williams and Reid, 2004), while large-scale oceanic biogeography has been investigated in such taxa as the urchin genus *Echinometra* (Palumbi, 1996; Palumbi, et al., 1997), some near shore fishes (e.g., Planes and Galzin, 1997), and various other taxa (e.g., Sturmbauer, et al., 1996; Lydeard, et al., 2002).

Classic studies in population genetics have focused their efforts on observing genetic differentiation across hypothesized boundaries and barriers to dispersal (e.g., Burton, 1998). Often, these boundaries are described by the overlapping end points of distributional ranges of many species (Valentine, 1966; Roy, et al., 1994; 1998). Such

biogeographic provinces have long been hypothesized to be a reflection of some physical barrier to dispersal. Thus over evolutionary time, these barriers should be effective at reducing gene-flow, setting the stage for speciation events. This idea holds for some important geographic regions such as Cape Canaveral on the Florida peninsula separating the Atlantic and Gulf of Mexico faunas (e.g., Avise, et al., 1987; Reeb and Avise, 1990), and the Isthmus of Panama (reviewed in Knowlton, 1993). Other hypothesized boundaries have not been shown to be effective in preventing gene flow in extant populations (e.g., Pt. Conception separating the Californian and Oregonian fauna; Burton, 1998).

The tropical portions of the world's coastal oceans have been broken into four large biogeographic regions that contain unique assemblages of marine species: the Indo-west Pacific, the eastern Pacific, the western Atlantic, and the eastern Atlantic (Briggs, 1974). The present patterns of biodiversity in these regions are the result of the interactions of several processes such as origination, extinction, and migration (Williams and Reid, 2004). The major biogeographic regions are separated by four barriers: The east Pacific barrier, the New World land barrier, the mid-Atlantic barrier, and the Old World land barrier (Briggs, 1974). These barriers vary in their absolute effectiveness in preventing species movement or gene flow, yet all have been hypothesized to be of considerable importance in affecting species distributions (e.g., Briggs, 1974). These barriers also vary in their duration over evolutionary time. The New World barrier gradually altered the connectivity of Caribbean and Pacific faunas until the final closure of the Panamanian Isthmus some 3-4 million years ago (Coates

and Obando, 1996), and the Old world land barrier was established in the mid-lower Miocene (approx. 14 mya), closing what remained of the Tethys Sea (Ruggieri, 1967; Hallam, 1994). As a result of these tectonic processes, a former hotspot of diversity in the Tethyan Sea shifted to the East Indies Triangle, encompassing the Philippines, Indonesia, and New Guinea (Wilson and Rosen, 1998; Vermeij, 2001; Briggs, 2003).

Understanding speciation in the marine environment requires careful evaluation of a complex body of knowledge. In addition to knowing a complete taxonomy, distribution, and phylogenetic hypothesis for a given clade, it is important to consider three other factors. First, the prevailing mode of speciation in the marine realm must be established. Geographical isolation that involves large-scale barriers has been hypothesized to drive most speciation events (e.g., Colborn, et al., 2001; Lessios, et al., 2001; Meyer, 2003), however, sympatric and parapatric speciation has also been highlighted as a distinct possibility (Knowlton, 1993; Palumbi, 1998; Vacquier, 1998). Second, we must establish the major geographical patterns of marine speciation. Vicariant splitting of historical populations or founder events may drive allopatric speciation (Knowlton et al., 1993; Lessions, et al., 2001) to achieve the current patterns in biodiversity. Speciation events might also be concentrated in certain areas (i.e., a center of origin) or may occur on the periphery of a region (i.e., a center of overlap). Lastly we must ask how old speciation events are in marine systems. Extant species may have been formed as a by product of tectonic events, such as the rising of the Isthmus of Panama, or may be more recent products of sea

level changes during the glacio-eustatic cycles over the past million years (McMillan and Palumbi, 1995; Palumbi, 1997; Benzie, 1999; Bellwood and Wainwright, 2002).

Many studies in biogeography have been based solely upon the distribution of single organisms (species), or a combination of the overlapping distributions of several species across a broad geographic range. Molecular genetics can contribute to biogeography in several ways that allow us to ask previously unanswerable questions about patterns of speciation. Palumbi (1996) outlined three keys areas in which molecular genetics may contribute to marine biogeography. First, genetic data allow for the detection of cryptic species (Knowlton, 1993). Despite the large degree to which morpho-species are recognized and studied, many populations are known to be genetically distinct, therefore representing independent gene pools. Second, molecular data may help to infer the timing of speciation. With a suitable calibration event and the assumption of a molecular clock (Zuckerkanndl and Pauling, 1965; Kimura, 1983), it is possible to differentiate between biogeographic hypotheses based upon vicariance associated with movement of plates (Springer, 1982), or alternatively, to Plio-Pleistocene sea level changes (e.g., Palumbi, et al., 1997). A third way in which molecular data may contribute to studies of biogeography is by providing a robust phylogeny for a particular study group. These data allow us to examine the geography of speciation, thus addressing mechanistic explanations for observed distributions of extant organisms. This may be particularly useful in addressing the observation that species diversity on coral reefs in the Indo-Pacific decreases with increasing distance

from the Indo-west Pacific (i.e., elucidating "center of overlap" versus "center of origin" hypotheses).

The epinepheline serranids comprise a large assemblage of generalized perciform fishes commonly known as groupers (Nelson, 1994). Extending from tropical to sub-tropical habitats worldwide, the diversity within this group of fishes is dominated by members of the genus *Epinephelus* comprising 99 currently recognized species (Heemstra and Randall, 1993; Allen and Robertson, 1999). Groupers are often top predators on a given reef system where they feed primarily on fishes and macro-crustacea (Randall, 1967; Hobson, 1968; Randall and Allen, 1987; Beets and Hixon, 1994). While most groupers are less than 1 m in length, *Epinephelus* species may reach a length of nearly 2.0 m (e.g., *E. itajara*, Robins, et al., 1986). Groupers are one of the most highly prized food fishes worldwide. The United Nations Food and Agriculture Association (FAO) estimated the total grouper catch at 97,000 metric tons in the year 1990 (Heemstra and Randall, 1993). This value is undoubtedly an underestimate given that a large proportion of grouper is taken in artisanal fisheries worldwide and thus not recorded in most fisheries statistics.

All groupers are broadcast spawners and possess a pelagic larval stage that may last as long as 60 days (Liao, et al., 2001; B. Victor, pers. comm.). Many groupers are known to be protogynous hermaphrodites, switching from female to male (Smith, 1959; Smith, 1965; Moe, 1969; Shapiro, 1987; Sadovy, et al., 1992; Bullock, et al., 1996; Brule, et al., 2000) and several species are known to reach ages in excess of 25 years (e.g. Bullock, et al, 1992; Craig, et al., 1999). These life history

characteristics, coupled with the behavioral adaptation seen in many species of forming immense spawning aggregations (Domeir and Colin, 1997), make the groupers particularly susceptible to over fishing. Human impacts on local grouper populations have been documented in many species (Sadovy, et al., 1992; Beets and Hixon, 1994; Schirripa and Burns, 1997; Coleman, et al., 2000; Sala, et al., 2001).

The Serranidae has long been regarded, systematically, as a problematic family. Several taxonomic revisions, based largely upon morphological similarity, have suggested the dissolution of the wastebasket assemblage of genera historically placed within the family and have provided working hypotheses for the monophyly of portions of the group. Recently, molecular analysis established the monophyly of the Epinephelinae and provided a hypothesis of relationships (Chapter I). This resulted in a re-classification of several members of the subfamily and the identification of several deep lineages within this group. This permits us to evaluate repeated biogeographic patterns within a monophyletic group of closely related species.

The subfamily Epinephelinae (*Epinephelus* and its allies) is particularly suited to answering questions regarding global biogeography. The numerous species within the subfamily are distributed circumglobally in tropical and sub-tropical habitats. Representative of other lineages within the Epinephelinae, diversity patterns within *Epinephelus* are similar to other shore fishes, showing numerical dominance of species in the Indo-Pacific (76), followed by the western Atlantic (11), eastern Pacific (9), and eastern Atlantic (9). While most species are restricted to one biogeographic province, the western Atlantic and eastern Pacific share two species, *E. itajara* and *E.*

mystacinus, and the western Atlantic and eastern Atlantic share three (*A. afer, E. itajara* and *E. marginatus*; Heemstra and Randall, 1993).

The goal of this study is to examine the pattern and mode of speciation with the groupers of the genus *Epinephelus* and its allies, as well as to determine the geography and timing of these events. This analysis is based on the phylogenetic hypothesis of Chapter I which represents the most thorough and recent sampling of this diverse group.

Materials and Methods

Sampling

Specimens were collected in the field by various means including spear pole, hook-and-line, or anesthetic, were purchased from fish markets at or near the collecting locality, or purchased from the live aquarium trade. Fin clips, gill clips, and/or muscle tissue were removed from each individual and stored in either 5X net solution (Craig, et al., 2001) or 70-90% Ethanol. When available, voucher specimens were deposited at the Scripps Institution of Oceanography Marine Vertebrates Collection (Apendix I). Other tissues were obtained through various museum collections which maintain frozen or ethanol preserved collections, or from local contacts. When no voucher was available, a photo voucher was retained by MTC or the individual who collected the specimen. One to three individuals per species were sequenced depending on availability. The individual sequences were inspected for significant differences and if none were present, a consensus sequence was used in the final analysis. Overall, tissue samples were obtained for 155 species representing 24 of the 30 epinepheline

genera (Appendix I). Within the tribe Epinpehelini, these included 68 of 99 species of *Epinephelus,* 14 of 15 species of *Mycteroperca,* 16 of 22 species of *Cephalopholis*, 5 of 7 species of *Plectropomus,* all species of *Alphestes, Dermatolepis, Paranthias,* and *Variola,* and the monotypic genera *Aetheloperca, Anyperodon, Cromileptes, Gracila, Saloptia* and *Triso.* The Liopropomini was represented by two species of *Liopropoma*, the Diploprionini by *Diploprion bifasciatum* and *Belonoperca chabanaudi*, and the Grammistini by species in the genera *Aporops, Grammistes, Pogonoperca, Pseudogramma, Rypticus,* and *Suttonia.* The Niphonini was represented by the monotypic *Niphon spinosus*.

Several outgroups were selected to root both the overall tree and the serranid portion (Appendix I). The beryciform *Hoplostethus mediterraneus* was used to root the Acanthomorpha, while several lower percoids were chosen that have been shown to be closely related to Serranidae based on molecular data (Smith and Craig, in prep.).

DNA Isolation and Sequencing

Total DNA was isolated from tissues using the DNEasy nucleic acids isolation kit (Qiagen) following manufacturer's instructions. The polymerase chain reaction (PCR) was used to amplify portions of two mitochondrial (16S and 12S) and two nuclear (TMO4C4 and Histone III) genes (1838bp). Primer pairs are listed in Chapter I, Table 1. Twenty-five microliter PCR reactions were prepared following manufacturers instructions included with the RedTaq Readymix (Sigma-Aldrich) with the addition of 10pmol of each primer and 5-50ng of template DNA. Each reaction was subjected to 35 rounds of the following thermal cycling conditions: 94° for 30

sec, 46° for 30 sec, 72° for 1 min. In some instances, PCR failed to amplify one or more genes for a particular taxon (Apendix I). Missing sequences were coded as "?" in the concatenated sequence file.

Sequences in both directions were generated on a MegaBace 500 automated sequencer. Sequence reactions were prepared following manufacturer's instructions for the ET Terminator chemistry with the addition of 5pmol primer (GE Healthcare, formerly Amersham-Biosciences). Sequences were generated for both the forward and reverse directions.

Sequence data were edited for miscalls and/or polymorphism using Sequencher v. 4.2. Edited sequences were aligned using CLUSTAL X with default settings (Thompson, et al., 1997). The alignment was visually optimized using MacClade v. 3.07 (Maddison and Maddison, 1997). A partition homogeneity test was used to determine the suitability of the four genes for use in a combined dataset. Phylogenetic analyses were performed using PAUP* 4.0b10. Due to the large number of taxa leading to computational constraints, the likelihood ratchet was implemented in PAUP* using the batch file created by Vos (2003). Ten rounds of the likelihood ratchet were performed using default settings except that the HKY85+I+G substitution model was used as determined by Modeltest v. 3.6 (Posada and Crandall, 1998). All trees with the best likelihood score were retained. For all analyses the tree was rooted with the beryciform *Hoplostethus mediterraneus*. Computational limitations prevented the production of bootstrap values for the ML analysis. Therefore, relative support at nodes representing major lineages was evaluated using the bootstrap under

parsimony criteria as implemented in PAUP*4.0b10 using 1000 replicates and saving a maximum of 1000 trees per replicate.

Biogeographic Analysis

In serranids as in many other percoid fish lineages, there is a nearly complete absence of a fossil record (Bellwood and Wainwright, 2002). Hence we must rely on the availability of an independent estimate of the minimum time since last genetic contact to allow for critical analysis of rates of sequence divergence. The well-dated final closure of the Central American seaway provides a key focal point for testing the tempo of allopatric speciation in the marine realm (Coates and Obando, 1996; Haug and Tiedeman, 1998) and justifiably has received an extraordinary amount of attention (e.g., Knowlton, et al., 1993; Collins, 1996; Marko and Jackson, 2001; Marko, 2002;). This event has become a prominent tool used to study the rate at which evolution proceeds, using the final closure of the seaway 3.5 million years ago (MYA) as a reference point for calibrating a "molecular clock" in many marine organisms (e.g., Bermingham, et al., 1997; Arbogast, et al., 2002; Marko, 2002). We used the average pairwise genetic distance between sister taxa identified as trans-isthmian geminate pairs, as well as the average distance between any three taxon clade whose most common ancestor was a transithmian geminate to calibrate a local mitochondrial DNA "clock" for the groupers. This rate was then fitted to the branch lengths of the ML analysis after the branch lengths were transformed using nonparametric rate smoothing (NPRS; Sanderson, 1997) as implemented in TreeEdit (V. 1.0a10, A. Rambaut and M. Charleston, http://evolove.zoo.ox.ac.uk). Knowlton and Weight

(1998) cautioned that the generally accepted time of 3.5 MYA for the final closure of the seaway should be viewed as a minimum time since divergence because the dispersal of some species was apparently disrupted much earlier by the rising, but still incomplete, Isthmus of Panama. We therefore calculated two rates; a "fast" rate was based on the final closure of the isthmus 3.5 MYA, and a "slow" rate was based on a disruption of gene flow at 4.5 MYA. Although there are two transisthmian species (*E. itajara/H. mystacinus*) we chose not to use these species when calibrating the molecular clock as there are no studies to date that demonstrate complete lack of mtDNA differentiation.

Mode of Speciation

Modes of speciation were inferred using unreduced area cladograms following Rosen (1975, 1985). Areas of occurrence for each species were determined by examination of range maps included in the excellent taxonomic revision of the groupers by Heemstra and Randall (1993). Two levels of resolution were examined. First, species were assigned to the major biogeography areas of Briggs (1974). These regions are the Indo-west Pacific, the eastern Pacific, the western Atlantic, and the eastern Atlantic. Second, species restricted to areas within those regions were analyzed to detect more fine-scale phenomena. This analysis, however, may be compromised by the widely accepted notion that secondary overlap following range expansion of newly formed species overshadows much of the phylogeographic signal in the grouper lineages.

Results

The partition homogeneity test did not support the combination of the four genes (p=0.01), however, this test has been noted to underestimate the suitability of genetic partitions for combination, especially when the partitions may evolve at varying rates (Dolphin, et al., 2000). We therefore chose to utilize the combined dataset in all analyses. The ML hypothesis of Craig and Hastings (2005) is presented as Figures 1 and 2. This method found one best tree at –lnLikelihood=33996.01835. This tree indicates the paraphyly of many previously recognized genera.

Chapter I discussed the relationships among species and the taxonomic implications of the molecular phylogenetic hypothesis presented here. That chapter suggested treating members of the *E. niveatus* clade (*sensu* Smith, 1971; Craig, et al., 2001; Chapter II) as members of the genus *Hyporthodus* Gill 1862. Therefore, all members of this species group will be referred to as *Hyporthodus* spp. throughout the remainder of this paper. In Chapter I of this thesis, several other taxonomic considerations were suggested and they are followed in this paper as well.

Our molecular data support the presence of six previously hypothesized transithmian species pairs, and three clades of three species whose common ancestor was presumably a transisthmian species whose distribution was interrupted by the rise of the Isthmus (Table 1). Our data do not support the sister taxa relationship between *Hyporthodus niphobles* of the eastern Pacific and *H. niveatus* of the western Atlantic/Caribbean (Smith, 1971). The average pairwise genetic distance between all five species pairs and all seven transithmian trichotomies was p=1.91 (Table 1).

Using the final closure of the Panamanian Isthmus at 3.5 MYA as a "fast" calibration point, we found a mtDNA evolutionary rate of 0.54% per MY, while a "slow" clock based on an interruption of geneflow 4.5 MYA yields a rate of 0.42% per MY. This rate is considerably slower than the generally accepted mtDNA evolutionary rate of 2% per MY, highlighting the importance of developing a local molecular clock for a particular study group. Using the local rate determined by the genetic data herein, the radiation of the main grouper lineage (genera) was most likely initiated in the early Miocene, some 10-14 MYA (Figure 3). Terminal speciation events within genera were most likely initiated around the time of the closure of the Panamanian Isthmus some 3.5-4.5 MYA (Coates and Obando, 1996) with a few instances of post-closure speciation. The timing of diversification within the grouper lineage suggests that the only major tectonic event shaping the distribution of species was the closure of the Panamanian Isthmus as the final closure of what remained of the Tethys Sea was some 14-16 MYA (Hallam, 1994).

Discussion

Origins of diversification

Our molecular phylogenetic hypothesis supports an origin for the grouper clade in the western Pacific region. Nearly all basal genera have a majority of their members in this area, while all New World members occupy more derived positions on the tree (Figure 1). From this area, three main lineages are readily distinguishable as either radiating outwards or speciating within this area: *Cephalopholis, Epinephelus,* and *Mycteroperca.* Surprisingly, one lineage, that leading to the genus

Hyporthodus, apparently originated in the New World, with radiation within the Central American Seaway, and dispersal across the east Pacific barrier into the western Pacific (see discussion below).

New World origins

Smith (1971) performed the first study evaluating the relationships among the many grouper species. In his 1971 paper, Smith commented that "...a classification can never be complete until the family is studied on a world-wide basis." (Smith, 1971, p. 71). With this caveat, he presented a dendrogram depicting relationships of all New World species and proposed a new classification. Smith (1971) failed to demonstrate the monophyly of the New World species, thus his taxonomic recommendations were not put into widespread usage. Craig, et al. (2001) presented a preliminary analysis of genetic data for the groupers, and commented that the New World may have been colonized multiple times as their hypothesis of relationships did not show a monophyletic grouping of New and Old World species. Our analysis here yields considerable insight into the colonization of the New World by grouper lineages. We have identified four major grouper lineages with members in the New World: *Cephalopholis, Epinephelus, Hyporthodus,* and *Mycteroperca*. The pathways and timing leading to the New World by each lineage are unique, and each will be discussed below.

In *Cephalopholis* the pattern of geographic diversification in the New World is consistent with multiple and independent dispersal events into this region (Figure 1, node D). Five species are present in New World waters representing three lineages:

C. cruentata, C. (P.) colonus, C. fulva, C. (P.) furcifer, and *C. panamensis*. Each of these lineages occupies a unique position in the tree indicating non-monophyly of a New World group (Figure 1; Node D). Two trans-Isthmian geminate pairs are confirmed (*C. cruentata/C. panamensis, C. (P.) colonus/C. (P.) furcifer*; see discussion of trans-Isthmian evolution below), while the fifth New World species (*C. fulva*) is sister to a predominantly Indo-Pacific clade. Although the New World has been colonized three times independently by members of the *Cephalopholis* clade, the pathway of this transgression (trans-Atlantic or trans-Pacific) is unclear.

In the *Epinephelus* (*sensu stricto*) lineage, a majority of the species inhabit the western Pacific and Indian Oceans (Figure 2, Node H). However, there is one, monophyletic lineage that apparently represents a single colonization to and diversification within the New World. The seven species *E. analogus, E. adscensionis, E. clippertonensis, E. gutattus, E. labriformis, E. morio,* and *E. striatus* all form a monophyletic clade, and all reside in the eastern Pacific, and/or western Atlantic (Figures 2, 3; Node I). Within this monophyletic lineage, the *E. labriformis* clade (Figure 3) appears to have been influenced by the rising of the Panamanian Isthmus, as well as a dispersal event to the offshore islands producing both peripatric and allopatric speciation events. In the *E. morio* clade (Figure 3) we see a pattern consistent with sympatric speciation or allopatric speciation with post-speciational dispersal in the Caribbean/western Atlantic, as all three members of this clade are found within the same biogeographic area. Based on the molecular clock estimates of 0.54% per MY (fast) and 0.42% per MY (slow) the dispersal event leading to this New

World clade occurred between 5.5-7.1 MYA. Determining the pathway of the dispersal event leading to this New World clade is equivocal as the sister clades to this New World clade are Indo-Pacific in origin. The occurrence of *E. adscencionis* at Ascension Island (Mid-Atlantic) may represent a recent dispersal from the western Atlantic to this offshore Island. This hypothesis is confirmed by intra-specific genetic data that show little structure between populations at Ascension and Caribbean localities (Carlin, et al., 2003). This pattern of an eastward migration across the Atlantic has been hypothesized for other shore fish species including the epinepheline serranid *C. (P.) furcifer* (Joyeux, et al., 2001).

Within the *Epinephelus* (*sensu stricto*) lineage, there is only one other New World species, *E. itajara*. This species is known to occur in the eastern Pacific, the western Atlantic, and the eastern Atlantic. Noted for its large size (individuals may reach up to 250 cm total length and a weight of up to 320 kg), *E. itajara* is certainly capable of dispersing long-distances both as an adult and larva (Heemstra and Randall, 1993). Its sister species, *E. lanceolatus*, is also known for its large size, and may be found throughout the western Pacific and Indian Oceans. Two scenarios could account for the biogeography of these large, widely distributed species. First, a tropical ancestor may have dispersed from the Indian Ocean to the eastern Atlantic via a westward movement around the Cape of Good Hope, or may have crossed the East Pacific Barrier into the New World. The cold water Benguela Current and associated upwelling, however, usually prevents movement of tropical species northward along the coast of western Africa (Bowen, et al., 2005), while the east Pacific Barrier

reduces movement from the western Pacific to the eastern pacific (Briggs, 1974; Leis, 1984). Given that *E. lanceolatus* occurs in the mid-Pacific Pitcairn Islands (Heemstra and Randall, 1993), it would seem plausible that an ancestral species could have dispersed across the East Pacific Barrier to colonize the New World. However neither extant species occurs at the Galápagos Islands, which might be expected in any island hopping model of dispersal. The phylogeography of this species is certainly worthy of a more fine-scale examination of genetic data to infer directionality of colonization to the New World. Trans-Pacific dispersal would be supported if the eastern Pacific population was nearest the outgroup, while trans-Atlantic dispersal would be supported if the eastern Atlantic population was nearest the outgroup.

Within *Hyporthodus*, a contrasting pattern of relationships and diversification is revealed. This lineage is represented by roughly equal numbers of species in the New World and the western Pacific/Indian Ocean (Figures 1, 4, Node E). Based on the ML analysis, the origins of the group are in the New World, as the sister species of the remaining species, *H. flavolimbatus*, resides in the western Atlantic and its sister clades are predominantly New World species. The remaining New World species appear to have radiated in response to the allopatric splitting of populations influenced by the rising of the Isthmus of Panama (see discussion of trans-isthmian speciation below). Within this clade, there is one species that inhabits the offshore islands and seamounts of the eastern Pacific and Caribbean (*H. mystacinus*), one species endemic to the mid-Pacific Hawaiian Islands and Johnston Atoll (*H. quernus*), and three species restricted to the western Pacific (*H. ergastularias/H. septemfasciatus*), one of

which reaches the Indian Ocean (*H. octofasciatus*). This pattern implies that trans-Pacific dispersal has played a large role in shaping the distribution of species within this clade as no species are present in the eastern Atlantic and only one (*H. octofasciatus*) is present in the Indian Ocean. While a New World origin is surprising, it is supported by the relative sequence divergence between species. The trans-isthmian species pair *H. nigritus/H. exsul* has genetic distance p=0.022. The distance between the Hawaiian endemic *H. quernus* and its western Pacific sister species *H. septemfasciatus* is p=0.013, consistent with speciation after the closure of the Panamanian Isthmus.

The genus *Mycteroperca* appears to have radiated in the eastern and western Atlantic, with an independent radiation in the Indian Ocean. A majority of the species, however, are found in the New World (Figure 2, Node G). This lineage includes one species that is present on both sides of the Atlantic (*M. marginatus*), one sister pair separated by the Atlantic Ocean (*M. rubra/M. acutirostris*), and one trichotomy of Indian Ocean species (*M. albomarginata/M. morrhua/M. radiatus*). The origins of the *Mycteroperca* lineage (Indian Ocean or eastern Atlantic) cannot be unequivocally determined. However, the sister group to the remainder of this lineage, as well as several outgroup members are found in the Indian Ocean and western Pacific. If this represents the point of origin for diversification of the *Mycteroperca* lineage, a subsequent dispersal event into the eastern Atlantic is required to explain subsequent diversification and geographical patterns. While dispersal around the Cape of Good Hope is unlikely for tropical species, two lines of evidence support this pattern. First,

one of the Indian Ocean species (*M. albomarginata*) is well suited to a more temperate environment, as it is known to occur as far south as East London, South Africa (33° South latitude). This would enable it to cope with the cooler water temperatures of the Benguela Current system. Second, large eddy currents comprised of warm water from the Agulhas current system are known to "leak" around the tip of South Africa (Gordon, 2003). This retroflexion of warm water provides a mechanical process that could move larvae into the eastern Atlantic (Gordon, 2003). The pattern of relationships of the remaining, non-Indian Ocean species indicates a westward dispersal across the Atlantic into the New World with subsequent radiation there (see discussion of trans-isthmian speciation below). This pattern of a westward dispersal across the Atlantic has been documented in the shore fish genus *Centropyge* (Bowen, et al., 2005, in review), while an eastward migration has been hypothesized for the epinepheline serranids of the genus *Dermatolepis* (Craig, et al., 2004; Chapter II).

Trans-Isthmian Speciation

Jordan (1908) provided a list of fish species pairs that he believed were sister taxa separated by the rising of the Isthmus of Panama. His suppositions were based on the best available data at the time and were solely based on overall morphological similarity. Within the grouper lineage, Jordan (1908) explicitly identified three trans-isthmian species pairs: *Alphestes afer/A. multiguttatus, Dermatolepis inermis/D. punctatus* (=*dermatolepis*), and *Epinephelus analogus/E. adscencionis*. This list of grouper geminates has been further expanded by subsequent authors (e.g., Smith, 1971, Heemstra and Randall, 1993) to include four other pairs: *Cephalopholis*

panamensis/C. cruentata, Hyporthodus exsul/H. nigritus, H. niphobles/H. niveatus, and *C. (P.) colonus/C. (P.) furcifer.* A close relationship has also been identified between species of *Mycteroperca* that have representatives on either side of the Isthmus (Smith 1971). As these groups contain more than two species, no single pair has been designated as a geminate. These groups are the "*venenosa*" group comprising *M. venenosa/M. jordani/M. bonaci,* and the "*interstialis*" group comprising *M. microlepis, M. interstitialis, M. phenax, M. cidi, M. xenarcha, M. prionura, M. rosacea, M. olfax,* and *M. rubra* (Smith, 1971; Craig, et al., 2001). Craig, et al. (2004; Chapter II) cautioned that the uncritical use of morphology in the designation of trans-isthmian species pairs may lead to incorrect hypotheses of relationships. They found that species previously described as geminates were in fact not each others closest living relatives. The presence and proper identification of trans-isthmian geminate pairs is of the utmost importance here as in the absence of a fossil record for the group, they provide the only means for calibrating the tempo of speciation in the groupers.

The rising isthmus eliminated gene flow between populations that were separated by it, leading to allopatric sister species on either side of the isthmus. This vicariant event is one of the most well document examples of allopatric speciation in the marine realm, and our results support this event as one of the major tectonic phenomena that shape the distribution and formation of shore fish species. No fewer than twelve possible transisthmian events are implied by the phylogeny of the Epinephelinae (Figure 1).

In some cases, however, the pattern is not clear. For example, the relationships of *Alphestes* and *Dermatolepis* imply nearly simultaneous speciation within the Central American Seaway (Craig, et al., 2004; Chapter II). Another similar species group (*Mycteroperca jordani/M. bonaci/M. venenosa*) provides an interesting perspective of trans-isthmian relationships. In this clade, *M. jordani* of the eastern Pacific is the basal species leading to a sister clade containing two species found in the western Atlantic/Caribbean (*M. bonaci* and *M. venenosa;* Figure 2). This hypothesis of relationships assumes that the rising of the isthmus accounted for the allopatric divergence of *M. jordani* and the common ancestor of *M. bonaci* and *M. venenosa.* The sister group relationship between *M. bonaci* and *M. venenosa* implies post-closure, sympatric speciation or allopatric speciation and subsequent dispersal in the Caribbean/western Atlantic. Morphologically, *M. bonaci* and *M. venenosa* are quite similar, with only minor color pattern differences. *Mycteroperca bonaci* is usually found in depths of less than 30m, while *M. venenosa* may be found to depths of 137m (Heemstra and Randall, 1993). This difference in depth preference may have facilitated a micro-allopatric separation leading to a restriction in gene flow and subsequent speciation of these two populations.

Within the New World *Epinephelus* lineage, the rising of the Isthmus of Panama also played a role in the formation and distribution of species. The eastern Pacific species *E. analogus* is sister to a clade including a trans-isthmian trichotomy including the western Atlantic *E. adscencionis* and the eastern Pacific *E. labriformis* and *E. clippertonensis* (Figure 3, Node I). The phylogeography and specific status of

the *E. labriformis* and *E. clippertonensis* clade is discussed in detail in Chapter V of this thesis. The ancestor of these two species, however, appears to have been a geminate of *E. adscencionis* (Figure 2). The sister-group relationship of this clade and *E. analogus* suggests a pre-closure speciation event within the Central American Seaway.

Two other geminate pairs were revealed within the *Cephalopholis* lineage: *C. cruentata/C. panamensis* and *C. (P.) colonus/C. (P.) furcifer*. These two clades clearly represent the allopatric separation of ancient populations within the Central American Seaway, although they have little influence on the biogeography of the remaining *Cephalopholis* species whose evolution is largely confined to the Indo-Pacific. The patterns of diversification within this lineage are not discussed further as many species of *Cephalopholis* were not included in this study.

Other geographical patterns

Wallace's line has long been regarded as a major biogeographic boundary, separating Oriental and Occidental terrestrial faunas (Wallace, 1859). Huxley (1868) modified Wallace's line in a northward direction to include the Phillipines. Barber, et al. (2000) discussed the presence of a marine Wallace's line as applied to the mantis shrimp *Haptosquilla pulchella*. DeBruyn, et al. (2004) confirmed this result in a freshwater prawn (*Macrobrachium*). These and other authors hypothesize that lowered sea-levels during the glacio-eustatic cycles of the Pleistocene resulted in a land barrier preventing exchange between western Pacific and Indian Ocean faunas (Springer and Williams, 1990; Randall, 1998). Our phylogenetic hypothesis reveals

some patterns that are consistent with these findings. Despite the presence of the few grouper species that are restricted to the Indian Ocean, and the more numerous species that are restricted to the western Pacific, there is little phylogeographic signal indicating an ancient or recent separation of these faunas. However, there are two examples of sister taxa whose distribution suggests that this process may have been a factor in shaping their evolution. *Cephalopholis nigripinnis* is a small grouper species of the Indian Ocean (Heemstra and Randall, 1993). Its sister species, *C. urodeta* seems to only be found in the western Pacific region. Similarly, *Epinephelus cyanopodus* and its sister species *E. flavocaeruleus* are distributed in the same fashion (*E. cyanopodus* in the western Pacific, *E. flavocaeruleus* in the Indian Ocean). The percent sequence divergence between these two sister pairs is low (0.4% and 0.5%, respectively). Based on the local molecular clock estimates, a divergence time between these species pairs was between 750,000 and 950,000 years and between 925,000 and 1.2 MYA respectively. These data suggest that divergence along Wallace's Line with a subsequent expansion in the species ranges may have resulted in present day distributional patterns.

Other distributional patterns are noted within the grouper lineages that would seem to indicate allopatric divergence based on geography. Five species of *Epinephelus* (*E. akaara, E. awoara, E. brunneus, E. fasciatomaculosus,* and *E. trimaculatus*) are restricted in the western Pacific to more northward, tropical localities, such as the southern waters of Japan. These species, however, show no phylogeographic structure, i.e., they are not a monophyletic unit ruling out a single

dispersal event to the more northern west Pacific (Figure 2). In the case of *E. akaara* and its sister *E. undulostriatus* it appears as though an ancient divergence some 3.8-5 MYA (p=0.021) led to the anti-tropical distribution of these species. With the exception of *E. trimaculatus* which diverged from its sister species *E. macrospilos* some 1.8-2.3 MYA (p=0.01), the remaining species diverged from their sister species or clades between 3.5 and 6.1 MYA, again ruling out a single vicariant event leading to divergent lineages transgressing into the northwest Pacific.

Conclusions

We have thus identified four main radiations of the grouper lineages: *Cephalopholis, Epinephelus, Hyporthodus,* and *Mycteroperca.* Two of these lineages (*Cephalopholis* and *Epinephelus*) appear to have originated in the western Pacific, while *Hyporthodus* originated in the New World, and *Mycteroperca* in the western Indian Ocean. We have outlined two pathways of dispersal into the New World, one via a trans-Atlantic crossing, the other via a trans-Pacific island hopping model. The relative timing estimates place diversification among these lineage in the early Miocene (approx. 10-14 MYA; Figure 5). The only major tectonic event shaping the distribution of grouper species since that time was the rising of the Isthmus of Panama (Hallam, 1994). This event has clearly played a large role in the formation of many New World species. The remaining lineages appear to have undergone speciation and subsequent radiation from a biodiversity hotspot in the western Pacific. This is a well documented pattern for nearshore marine fishes and is not surprising (Briggs, 1974). Some peripatric speciation events are noted resulting in species with relatively

restricted ranges. This process of peripatric speciation may have played a much larger role, however, than can be inferred from the data herein. Recent range expansions in many grouper taxa imply that the geographical distributions of these species are constantly changing, thus any historical signal of divergence in populations may be obscured (Gill and Kemp, 2002; Craig, et al, 2005). The complex patterns of geographical distributions of shorefish species provide a valuable tool for examining the patterns and processes that yield current patterns in biodiversity. It is hoped that this example will serve to clarify some of these patterns and elucidate the processes that shape the distributions of other shorefish clades.

References

Allen, G.R., and D. R. Roberston. 1999. *Epinephelus clippertonensis*, a new species of serranid fish from the tropical eastern Pacific. Revue Fr. Aquariol. 26:11-15.

Arbogast, B. S., S.V. Edwards, J. Wakeley, P. Beerli, and J. B. Slowinski. 2002. Estimating divergence times from molecular data on phylogenetic and population genetic timescales. Ann. Rev. Ecol. Syst., 33:707-741.

Avise, J. C. 1992. Molucular population structure and biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos 63:62-76.

Avise, J. C. 2000. Phylogeography: The history and formation of species. Harvard University Press, Cambridge.

Avise, J. C., C. A. Reeb, and N. C. Saunders. 1987. Geographic population structure and species differences in mitochondrial DNA of mouthbrooding marine catfishes (Arridae) and demersal spawning toadfishes (Batrachoididae). Evolution 41:991-1002.

Barber, P. H., S. R. Palumbi, M. V. Erdmann, and M. K. Moosa. 2000. A marine Wallace's line? Nature 406:692-693.

Beets, J. and M. A. Hixon. 1994. Distribution, persistence, and growth of groupers (Pisces: Serranidae) on artifical and natural patch reefs in the Virgin Islands. Bull. Mar. Sci. 55(2-3):470-483.

Bellwood, D. R., and P. C. Wainwright. 2002. The history and biogeography of fishes on coral reefs. IN. Coral reef fishes: dynamics and diversity in a complex ecosystem. P. F. Sale, Ed. Academic Press, Inc. San Diego, California.

Benzie, J. A. H. 1999. Genetic structure of coral reef organisms: ghost of dispersal past. Am. Zool. 39:131-145.

Bermingham, E., S. S. McCafferty, and A. P. Martin. 1997. Fish biogeography and molecular clocks: Perspectives from the Panamanian Isthmus. Molecular Systematics of Fishes. C. Stepien and T. Kocher, Eds.) pp. 113-128. Academic Press.

Bowen, B. W., A. Muss, L. A. Rocha, and W. S. Grant. 2005. Shallow mtDNA coalescence in Atlantic pygmy angelfishes (genus *Centropyge*) indicates a recent invasion from the Indian Ocean. Unpubl. Manuscript.

Briggs, J. C. 1974. Marine Zoogeography. McGraw-Hill Book Co.

Briggs, J. C. 2003. Marine centres of origin as evolutionary engines. J. Biogeog. 27:1183-1188.

Brule, T., T. Colas-Marrufo, A. Tuz-Sulub, and C. Deniel. 2000. Evidence for protogynous hermaphroditism in the serranid fish *Epinephelus drummondhayi* (Perciformes: Serranidae) from the Campeche Bank in the southern Gulf of Mexico. Bull. Mar. Sci. 66 (2): 513-521.

Bullock, L. H., M. D. Murphy, F. Godcharles, and M. E. Mitchell. 1992. Age, growth, and reproduction of jewfish, *Epinephelus itajara*, in the eastern Gulf of Mexico. Fish. Bull. 90:243-49.

Bullock, L. H., M. F. Godcharles, and R. E. Crabtree. 1996. Reproduction of yellowedge grouper, *Epinephelus flavolimbatus*, from the eastern Gulf of Mexico. Bull. Mar. Sci. 59:216-224.

Burton, R. S. 1998. Intraspecific phylogeny across the Point Conception biogeographic boundary. Evolution 35:1192-1205.

Carlin, J. L., D. R. Robertson, and B. Bowen. 2003. Ancient divergences and recent connections in two tropical Atlantic reef fishes *Epinephelus adscensionis* and *Rypticus saponaceous* (Percoidei: Serranidae). Mar. Biol. 143:1057-1069.

Coates, A. G., and J. A. Obando. 1996. The geologic evolution of the Central America isthmus. In: Jackson, J. B. C., A. F. Budd, and A. G. Coates, Eds. Evolution and Environment in Tropical America. University of Chicago Press.

Colborn, J., R. E. Crabtree, J. B. Shaklee, E. Pfeiler, and B. W. Bowen. 2001. The evolutionary enigma of bonefishes (*Albula* spp.): cryptic species and ancient separations in a globally distributed shorefish. Evolution 55:807-820.

Coleman, F. C., C. C. Koenig, G. R. Huntsman, J. A. Musick, A. M. Eklund, J. C. McGovern, R. W. Chapman, G. R. Sedberry, and C. B. Grimmes. 2000. Long-lived reef fishes: The grouper-snapper complex. Fisheries 25(3):14-21.

Collins, T. 1996. Molecular comparisons of transithsmian species pairs: Rates and patterns of evolution. Evolution and Environment in Tropical America. (ed. By J. Jackson, A. F. Budd, and A. G. Coates) pp. 303-334 The University of Chicago Press. Chicago.

Connolly, S. R., D. R. Bellwood, and T. P. Hughes. 2003. Indo-Pacific biodiversity of coral reefs: deviations from a mid-domain model. Ecology 84:2178-2190.

Craig, M. T., D. J. Pondella, and J. C. Hafner. 1999. Analysis of age and growth in two eastern Pacific groupers (Serranidae: Epinephelinae). Bull. Mar. Sci. 65(3):807-814.

Craig, M. T., D. J. Pondella, J. P. C. Franck, and J. C. Hafner. 2001. On the status of the serranid fish genus *Epinephelus*: Evidence for paraphyly based on 16S rDNA sequence. Mol. Phy. Evol. 19(1):121-130.

Craig, M. T., P. A. Hastings, and D. J. Pondella, II. 2004. Speciation in the Central American Seaway: the importance of taxon sampling in the identification of trans-isthmian species pairs. J. Biogeo. 31:1085-1091.

Craig, M. T., D. J. Pondella, II, and R. N. Lea. 2005. New records of the flag cabrilla, *Epinephelus labriformis* (Serranidae; Epinephelinae) from the Pacific coast of Baja California with notes on the distribution of other rare groupers in California. Unpubl. Manuscript.

de Bruyn, M., J. C. Wilson, and P. B. Mather. 2004. Huxley's line demarcates extensive genetic divergence between eastern and western forms of the giant freshwater prawn *Macrobrachium rosenbergii*. Mol. Phy. Evol. 30:251-257.

Doherty, P. J., S. Planes, and P. Mather. 1995. Gene flow and larval duration in seven species of fish from the Great Barrier Reef. Ecology 76:2373-2391.

Dolphin, K., R. Belshaw, C. D. L. Orme, and D. L. J. Quicke. 2000. Noise and incongruence: Interpreting results of the incongruence length difference test. Mol. Phy. Evol. 17(3):401-406.

Gill, A. C., and J. M. Kemp. 2003. Wide-spread Indo-Pacific shorefish species: a challenge for taxonomists, ecologists, and fishery and conservation managers. Env. Biol. Fishes 65:165-174.

Gordan A. L. 2003. The brawniest retroflexion. Nature 421:904-905.

Hallam, A. 1994. An outline of Phanerozoic Biogeography. Oxford Biogeography Series No. 10. Oxford University Press, Oxford.

Hastings, P. A. 2000. Biogeography of the tropical eastern Pacific: distribution and phylogeny of chaenopsid fishes. Zoo. J. Linn. Soc. 128:319-335.

Haug, G. H., and R. Tiedeman. 1998. Effect of the formation of the Isthmus of Panama on Atlantic Ocean thermohaline circulation. *Nature*, 393:673-676.

Heemstra, P. C. and J. E. Randall. 1993. Groupers of the World. FAO Species Catalogue 16(1):1-382. Rome.

Hobson, E. S. 1968. Predatory behavior of some shore fishes in the Gulf of California. U.S. Fish and Wildlife Service Res. Rep., 73:1-92.

Huxley, T. H. 1868. On the classification and distribution of the Alectoromorphae and Heteromorphae. Proc. Zool. Soc. Lond. 294-319

Jablonski, D., and R. A. Lutz. 1983. Larval ecology of marine benthic invertebrates: paleobiological implications. Bio. Rev. 58:21-89

Jordan, D. S. 1908. The law of geminate species. Am. Nat., 42(494):73-80.

Joyeux, J. C., S. R. Floeter, C. E. L. Ferreira, and L. J. Gasparini. 2001. Biogeography of tropical reef fishes: the south Atlantic puzzle. J. Biog. 28:831-841.

Kimura, M. 1983. The neutral theory of evolution. Cambridge University Press, Cambridge.

Knowlton, N. 1993. Sibling species in the sea. Ann. Rev. Ecol. Syst. 24:189-216.

Knowlton, N., L. A. Weigt, L. Aníbal Solórzano, D. K. Mills, and E. Bermingham. 1993. Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. Science 260:1629-1632.

Knowlton, N. K. and L. A. Weight. 1998. New dates and new rates for divergence across the Isthmus of Panama. Proc. R. Soc. Lond. (Series B: Biology), 265(1412):2257-2263.

Leis, J. M. 1984. Larval fish dispersal and the East Pacific Barrier. Oceanogr. Trop. 19(2):181-192.

Lessios, H. A., B. D. Kessing, and J. S. Pearse. 2001. Population structure and speciation in tropical seas: Global phylogeography of the sea urchin *Diadema*. Evolution 55(5):955-975.

Liao, I. C., H. M. Su, and E. Y. Chang. 2001. Techniques in finfish larvaculture in Taiwan. Aquaculture 200:1-31.

Lydeard, C., W. E. Holznagel, M. Glaubrecht, and W. R. Ponder. 2002. Molecular phylogeny of a circum-global, diverse Gastropod superfamily (Cerithioidea: Mollusca: Caenogastropoda): Pushing the deepest phylogenetic limits of mitochondrial LSU rDNA sequences. Mol. Phy. Evol. 22(3):399-406.

McMillan, W. O., and S. R. Palumbi. 1995. Concordant evolutionary patterns among Indo-West Pacific butterflyfishes. Proc. R. Soc. Lond. B 260:229-236.

Maddison, D. R., and W. P. Maddison. 1997. MacClade V. 4.03. Sinauer Associates, Sunderland, Mass.

Marko, P. B. 2002. Fossil calibration of molecular clocks and the divergence times of geminate species pairs separated by the Isthmus of Panama. *Molecular Biology and Evolution,* 19(11):2005-2021.

Marko, P. B., J. B. C. Jackson. 2001. Patterns of morphological diversity among and within arcid bivalve species pairs separated by the Isthmus of Panama. *Journal of Paleontology,* 75(3):590-606.

Meyer, C. P. 2003. Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. Bio. J. Linn. Soc. 79:401-459.

Moe, M. A. Jr. 1969. Biology of the red grouper (*Epinephelus morio* Valenciennes) from the eastern gulf of Mexico. Prof. Pap. Ser. Mar. Lab. Fla. No. 10. 1-95.

Nelson, J. 1994. Fishes of the World. 3rd edition. John Wiley and Sons, Inc. New York.

Palumbi, S. R. 1996. What can molecular genetics contribute to marine biogeography? An urchin's tale. J. Exp. Mar. Biol. 203:75-92.

Palumbi, S. R. 1997. Molecular biogeography of the Pacific. Coral Reefs 16:S47-S52.

Palumbi, S. R. 1998. Species formation and the evolution of gamete recognition loci. IN. D. J. Howard and S. H. Berlocher, eds. Endless forms: species and speciation. Oxford University Press, Oxford.

Palumbi, S. R., G. Grabowski, T. Duda, L. Geyer, and N. Tachino. 1997. Speciation and population genetic structure in tropical Pacific sea urchins. Evolution 51:1506-1517.

Paulay, G. 1997. Diversity and distribution of reef organisms. IN: C. Birkeland, ED. Life and Death of Coral Reefs. Chapman and Hall, New York.

Planes, S., and R. Galzin. 1997. New perspectives in biogeography of coral reef fish in the Pacific using phylogeography and population genetic approaches. Vie et Milieu, 47(4):375-380.

Posada D. and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14 (9): 817-818.

Powers, D. A., T. Lauerman, D. Crawford, and L. DiMichele. 1991. Genetic mechanisms for adapting to a changing environment. Ann. Rev. Gen. 25:629-659.

Randall, J. E. 1967. Food habits of reef fishes of the West Indies. Stud. Trop. Oceanogr. (Miami) 5:665-847.

Randall, J. E. 1998. Zoogeography of shore fishes of the Indo-Pacific region. Zool. Studies 37(4):227-268.

Randall, J. E., and G. R. Allen. 1987. Four new serranid fishes of the genus *Epinephelus* (Perciformes: Epinephelinae) from Western Australia. Rec. West. Aust. Mus. 13(3):387-411

Reeb, C. A., and J. C. Avise. 1990. A genetic discontinuity in a continuously distributed species: Mitochondrial DNA in the American oyster, *Crassostrea viginica.* Genetics 124:397-406.

Robins, C. R., G. C. Ray, and J. Douglas. 1986. A Field Guide to Atlantic Coast Fishes. Houghton Mifflin Co., New York.

Rocha-Olivares, A., and R. D. Vetter. 1999. Effects of oceanographic circulation on the gene flow, genetic structure, and phylogeography of the rosethorn rockfish (*Sebastes helvomaculatus*). Can. J. Fish. Aq. Sci. 56:803-813

Rosen, D. E. 1975. A vicariance model for Caribbean biogeography. Syst. Zool. 24:431-464.

Rosen, D. E. 1985. Geological hierarchies and biogeographic congruence in the Caribbean. Ann. Missouri Botanical Gardens 72:636-659.

Rosen, D. E. 1988. Progress, problems and patterns in the biogeography of reef corals and other tropical marine organisms. Helgol. Meeresunters. 42:269-301.

Roy, K., D. Jablonski, and J. W. Valentine. 1994. Eastern Pacific molluscan provinces and latitudinal diversity gradient: No evidence for "Rapoport's rule." Proc. Natl. Acad. Sci. 91:8871-8874.

Roy, K., D. Jablonski, J. W. Valentine, and G. Rosenberg. 1998. Marine latitudinal diversity gradients: Tests of causal hypotheses. Proc. Natl. Acad. Sci. 95:3699-3702.

Ruggieri, G. 1967. The Miocene and later evolution of the Mediterranean sea. Syst. Ass. Pub. 7:283-290.

Sadovy, Y., M. Figuerola, and A. Roman. 1992. Age and growth of red hind *Epinephelus guttatus* in Puerto Rico and St. Thomas. Fish. Bull. 90:516-528.

Sala, E., E. Ballesteros, and R. M. Starr. 2001. Rapid decline of Nassau grouper spawning aggregations in Belize: Fishery management and conservation needs. Fisheries 26(10):23-30.

Sanderson, M. J. 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. Mol. Biol. Evol. 11:458:463.

Shapiro, D. Y. 1987. Reproduction in groupers. IN: Tropical snappers and groupers: biology and fisheries mangagment, J. J. Polovina and S. Ralston, eds. Westview Press, Boulder, CO.

Schirripa, M. J., and K. M. Burns. 1997. Growth estimates for three species of reef fish in the Eastern Gulf of Mexico. Bull. Mar. Sci. 61(3):581-91.

Schmidt, P. S. and D. M. Rand. 1999. Intertidal microhabitat and selection at MPI: interlocus contrasts in the northern acorn barnacle, *Semibalanus balanoides*. Evolution 53:135-146.

Smith, C. L. 1959. Hermaphroditism in some serranid fishes from Bermuda. Papers Michigan Acad. Sci., Arts Letts. 44:111-118.

Smith, C. L. 1965. The patterns of sexuality and the classification of serranid fishes. Amer. Mus. Novitates 2297, 20pp.

Smith, C.L. 1971. A revision of the American Groupers: *Epinephelus* and allied genera. Bull. Amer. Mus. Nat. Hist. 146(2):241p.

Stepien, C. A., and R. H. Rosenblatt. 1991. Patterns of gene flow and genetic divergence in the northeastern Pacific Clinidae (Teleostei: Blennioidei), based on allozyme and morphological data. Copeia 4:873-896.

Sturmbauer, C., J. S. Levinton, and J. Christy. 1996. Molecular phylogeny analysis of fiddler crabs: Test of the hypothesis of increasing behavioral complexity in evolution. Proc. Nat. Acad. Sci. 93:10855-10857.

Springer, V. G. 1982. Pacific plate biogeography, with special reference to shorefishes. Smithsonian Contributions to Zoology, Washington, D.C.

Springer, V. G., and J. T. Williams. 1990. Widely distributed pacific plate endemics and lowered sea-level. Bull. Mar. Sci. 47(3):631-640.

Thomson, J. D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nuc. Acids Res., 25(24):4876- 4882.

Valentine, J. W. 1966. Numerical analysis of marine molluscan ranges on the extratropical northeastern Pacific shelf. Limnol. Oceanogr. 11(2):198-211.

Vacquier, V. D. 1998. Evolution of gamete recognition proteins. Science 281:1995-1998.

Vermeij, G. J. 2001. Community assembly in the sea: geological history of the living shore biota. IN. M. D. Bertness, S. D. Gaines, and M. E. hay, eds. Marine Community Ecology. Sinauer Associates, Sunderland.

Wallace, A. R. 1859. On the Zoological Geography of the Malay Archipelago [communicated by Charles Darwin to the LSL meeting of 3 Nov. 1859]. J. Proc. Linn. Soc. Zoology. 4:172-184 (1860).

Waples, R. S. 1987. A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution 41:385-400.

Williams, S. T. and D. G. Reid. 2004. Speciation and diversity on tropical rocky shores: A global phylogeny of snails of the genus *Echinolittorina*. Evolution 58(10):2227-2251.

Wilson, M. E. J. and B. R. Rosen. 1998. Implications of paucity of corals in the Paleogene of SE Asia: plate tectonics or centre of origin? IN: R. Hall and J. D. Holloway, eds. Biogeography and Geological Evolution of SE Asia. Backhuys Publishers, Leiden, The Netherlands.

Zuckerkandl, E., and L. Pauling. 1965. Evolutionary divergence and convergence in proteins. IN: V. Bryson and H. J. Vogel, eds. Evolving Genes and Proteins. Academic Press, New York.

Table 1. Geminate species pairs and uncorrected "p" distance between Atlantic and Pacific members for 16S and 12S rDNA sequences. "Slow" rate was calculated using an interruption of geneflow at 4.5 MYA, and the "fast" rate was calculated based on an interruption of geneflow at 3.5 MYA.

Geminate Pairs		
Pacific Species	Caribbean Species	% mtDivergence
Cephalopholis panamensis	*Cephalopholis cruentata*	1.6
Mycteroperca rosacea	*Mycteroperca tigris*	1.3
Mycteroperca xenarcha	*Mycteroperca phenax*	0.2
Paranthias colonus	*Paranthias furcifer*	0.9
Epinephelus exsul	*Epinephelus nigritus*	2.2
Geminate Trichotomies		
Alphestes immaculatus	*Alphestes afer*	3.1
Alphestes multiguttatus	*Alphestes afer*	2.6
Dermatolepis dermatolepis	*Dermatolepis inermis*	1.3
Dermatolepis dermatolepis	*Dermatolepis striolata* (IO)	2.3
Epinephelus labriformis	*Epinephelus adscencionis*	1.7
Epinephelus labriformis	*Epinephelus clippertonensis*	1.7
Mycteroperca jordani	*Mycteroperca bonaci*	1.3
Mycteroperca jordani	*Mycteroperca venenosa*	0.9
	Average % mt Divergence	1.916666667
	mt DNA evolutionary rate (fast)	0.54% per MY
	mt DNA evolutionary rate (slow)	0.42% per MY

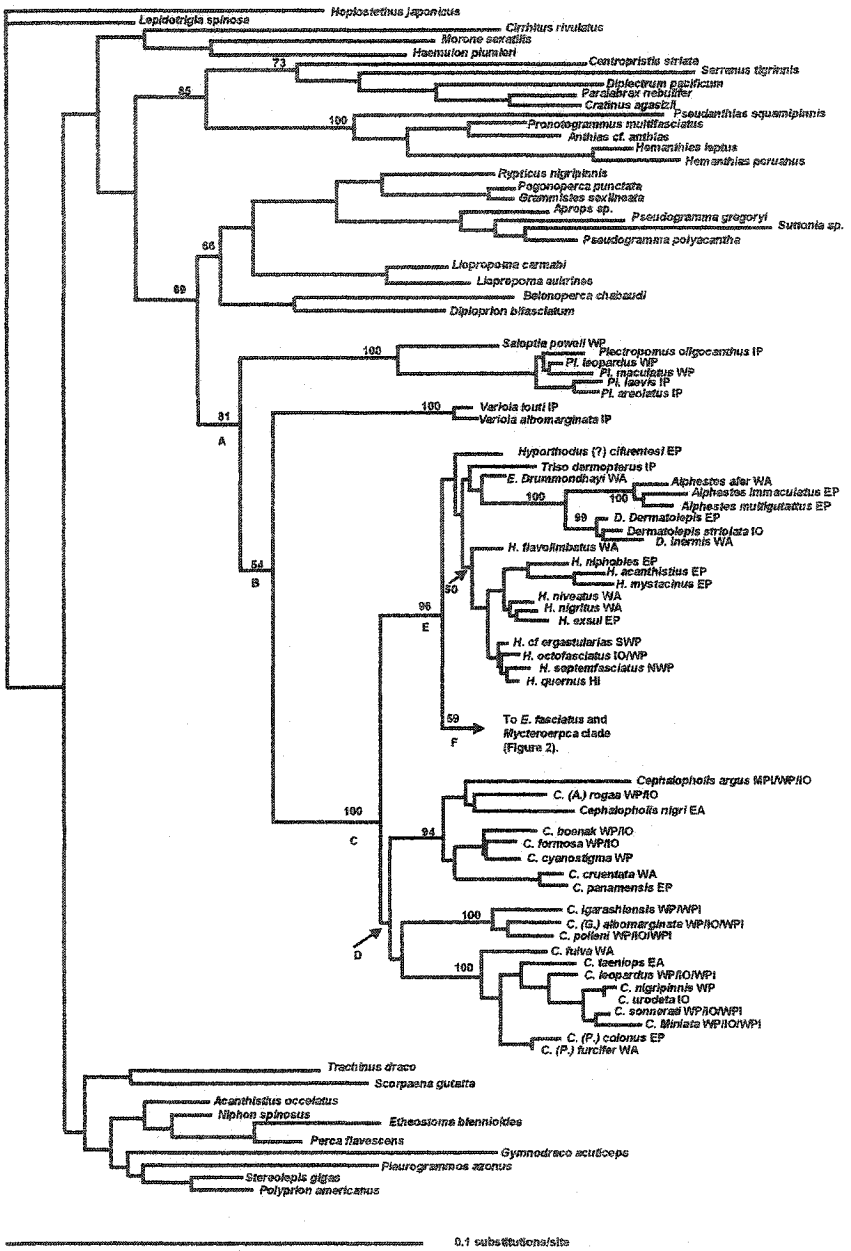

Figure 1. Maximum likelihood tree (-lnLikelihood=33996.01835) depicting relationships among the epinepheline serranids. Numbers above nodes are bootstrap values for a maximum parsimony analysis. Letters below nodes are for reference throughout the text. EP=Eastern Pacific, WP=Western Pacific, IO=Indian Ocean, WPI=Western Pacific Islands, MPI=mid-pacific Islands, NWP=North-Western Pacific, SWP=South-Western Pacific, IP=Indo-Pacific.

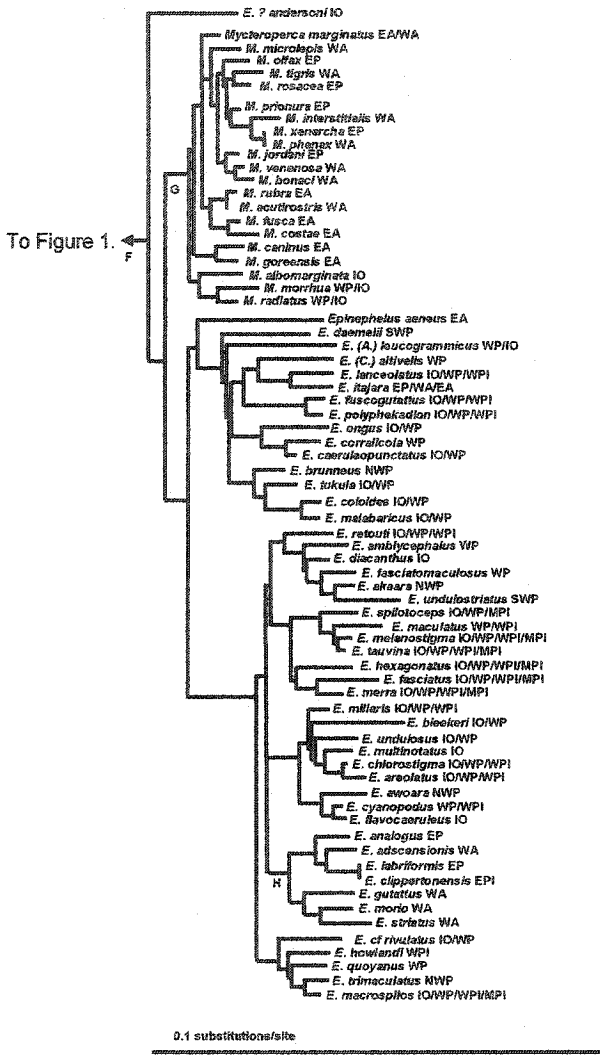

Figure 2. Continuation of the Maximum Likelihood tree in Figure 1. Abbreviations follow those in Figure 1.

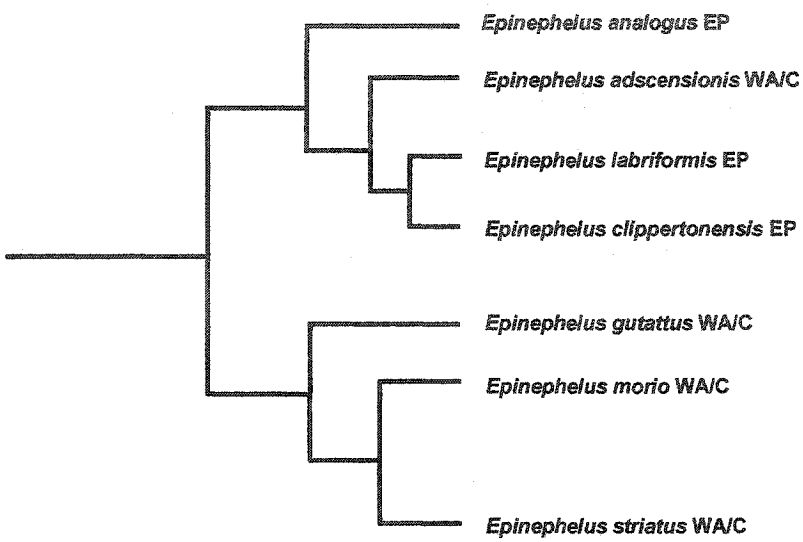

Figure 3. New World clade of *Epinephelus* spp. Branch lengths have been removed and abbreviations are as in Figure 1.

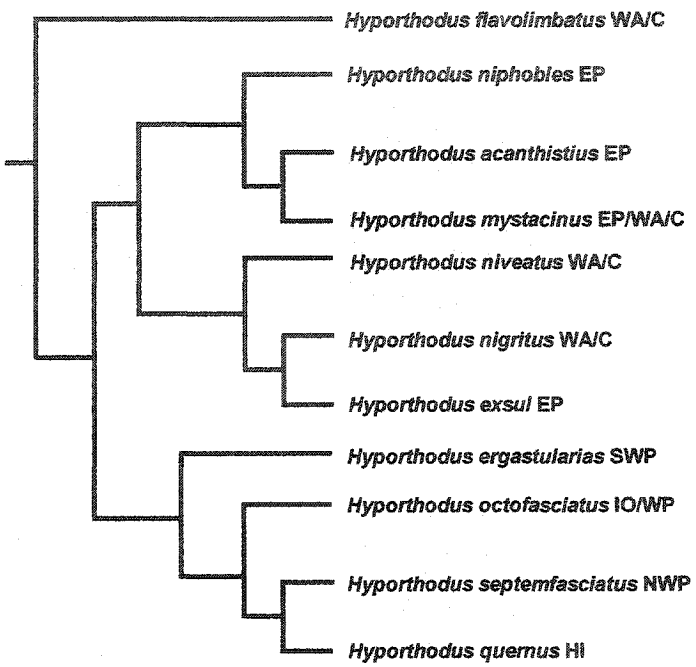

Figure 4. *Hyporthodus* clade. Branch lengths have been removed and abbreviations follow Figure 1.

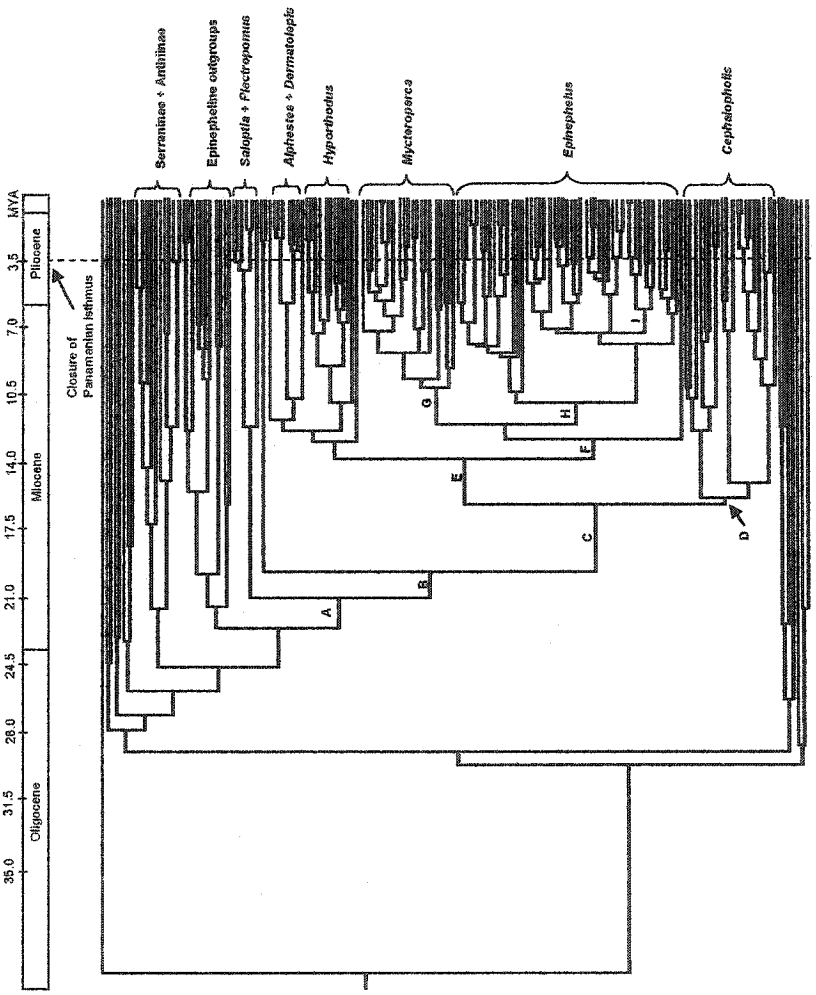

Figure 5. Chronogram of relationships for the tribe Epinephelini (Serranidae). Letters above nodes correspond to Figure 1 and text.

CHAPTER V

Phylogeography of the flag cabrilla, *Epinephelus labriformis* (Serranidae): implications for the biogeography of the Tropical Eastern Pacific and the early stages of speciation in a marine shore fish.

Phylogeography of the flag cabrilla, *Epinephelus labriformis* (Serranidae): implications for the biogeography of the Tropical Eastern Pacific and the early stages of speciation in a marine shore fish.

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Abstract of Chapter V

The mitochondrial Cytochrome B gene was used to examine the phylogeographic relationships within two putative eastern Pacific sibling species, the flag cabrilla, *Epinephelus labriformis* (Jenyns 1840) and the Clipperton grouper, *E. clippertonensis* Allen and Robertson 1999 (Serranidae). Overall, 49 haplotypes were found within 304 total samples, and there was significant structure corresponding to geographic locality (AMOVA Φ_{ct}=0.0830.19814; p=0.00010, Φ_{st}=0.20671, p=0.00; F_{st}=0.1689, p=0.00, F_{ct}=0.15072, p=0.036). Our results suggest that while some previously described barriers to dispersal in the Tropical Eastern Pacific may impinge upon the dispersal ability of marine species that have long-lived pelagic larva, including these groupers, others may not. Our data support a restriction in gene flow between mainland and island populations of *Epinephelus labriformis* and the putative morphospecies *E. clippertonensis*. These data also imply that, in some marine fishes, changes in external color patterns may

evolve more rapidly than do genetic markers commonly used to delimit species boundaries, and that these characters coupled with a lack of reciprocal monophyly may be a good indication of incipient speciation in the marine environment.

Introduction

Understanding the process and underlying mechanisms of speciation remains a challenging subject in evolutionary biology. Identifying genes that are essential to confer reproductive isolation is difficult and time consuming, and those that have been described as speciation genes vary in terms of a demonstrable effect (Ritchie and Noor, 2004; Swanson and Vaquier, 2002; Noor, 2003). We are therefore faced with the challenge of using putatively neutral markers as a means of developing hypotheses regarding the underlying mechanisms of speciation based on genetic data.

It has become commonplace in evolutionary biology to evaluate the degree of connectivity among populations of organisms. Such studies are useful in terms of assessing conservation strategies, evaluating ecological processes, and determining the evolutionary history of a group of organisms. Populations may diverge due to several mechanisms such as drift, selection, or mutation (Avise, 2000). Usually, these processes require the additional feature of restricted gene flow in order for populations to diverge sufficiently for speciation to occur (cf. Avise 2002 and references therein). Such restrictions in gene flow have long been thought to be most influenced by complete geographic isolation or disjunction (Endler, 1977; Terry, et al., 2000; Bernardi, et al., 2003). Our evaluation of

population divergence is of considerable interest as it is often thought to reflect the early stages of speciation. In the marine environment, the processes governing population divergence are further altered by the homogenizing effects of pelagic larval transport. Nonetheless, it is expected that at some early stage of speciation, populations will show certain characteristics, such as subtle differentiation with a lack of reciprocal monophyly, that indicate so called incipient speciation.

It has long been a contention in marine zoogeography that the presence and duration of the pelagic larval stage plays a critical role in shaping the distributions of species and the degree of connectivity among populations (Ekman, 1953; Hedgecock, 1986; Bonhomme and Planes, 2000). Dispersal theory suggests that organisms possessing long-lived pelagic larvae should, on average, display relatively extensive ranges in comparison to related species with shorter larval periods (Scheltema, 1968). Using the same reasoning, population genetic theory predicts that such organisms should experience high gene flow among populations, and species with large ranges should show little genetic structuring relative to geographic locality of populations sampled (Avise, 2000 and references therein). It is therefore surprising that recent studies have shown that not only do some species that have restricted ranges also have long pelagic larval stages relative to congeners with larger geographic ranges, but also that despite time spent in the plankton, local retention of larvae may play a more important role in settlement processes and population structuring than previously hypothesized (Swearer, et al., 1999; Leis and Carson-Ewart, 2000; Taylor and Hellberg, 2003). As restrictions in gene flow are

often assumed to be a requisite of speciation, these studies provide mechanisms by which speciation may occur in the absence of physical barriers to dispersal.

The Tropical Eastern Pacific Ocean (TEP) has been considered a model system for examining how the distributions of marine shorefishes are affected by habitat discontinuities (e.g. Springer, 1959; Rosenblatt, 1967; Allen and Robertson, 1994; Hastings, 2000; Mora and Robertson, 2004). In this area, long stretches of rocky coastline are interrupted by two large expanses of sandy shore: the 370 km wide Sinaloan Gap extending south from Topolobampo, Sinaloa, MX, to Mazatlán, MX, and the 1000km wide Central American Gap extending south from the Isthmus of Tehuantepec to the Gulf of Fonseca (Springer, 1959; Walker, 1960; Rosenblatt, 1967; Dawson, 1975; Hastings, 2000; Fig 1). These barriers may either represent an environment that prohibits adult movement in species that have strong affinity for reef systems, and/or may limit larval movement due to the distance that they must travel before encountering suitable habitat for settlement. These gaps have been hypothesized to play a large role in driving speciation within this region (e.g. Hastings, 2000; Pondella, et al., 2003). Based on shared overlapping distributions of marine shorefish species, the TEP has been divided into three mainland biogeographic provinces which are separated by the gaps above: the Cortez Province, the Mexican Province, the Panamic Province (Fig. 1). The offshore oceanic islands in this region harbor a unique ichthyofauna, but with the exception of the Galápagos Islands (Brusca and Wallerstein, 1979), none have been otherwise designated as a distinct province. The unique setting of this coastline

with its hypothesized barriers to dispersal makes this area a prime setting to test the ability of marine species with pelagic larval stages to traverse these barriers, as well as to assess the extent to which habitat gaps play a key role in driving speciation.

The flag cabrilla, *Epinephelus labriformis* Jenyns 1840, is a member of an exclusively marine family commonly known as groupers (Serranidae). A dominant predator on many reef systems throughout the TEP, the flag cabrilla is commonly taken in artisinal and commercial fisheries throughout its range (Ramírez and Rodríguez, 1990; MTC pers. obs.). The flag cabrilla lives over rocky substrate where it feeds on small fishes and crustaceans (Hobson, 1967; pers. obs.). Typical of other species in the family, the flag cabrilla exhibits a rapid first year of growth, followed by an asymptotic slowing in upper age classes, and may reach ages in excess of 25 years (Craig, et al., 1999). The flag cabrilla possesses a pelagic larval stage that may last as long as 60 days (B. Victor, pers. comm.) and recent evidence suggests that it is a protogynous hermaphrodite (Erisman and Craig, unpublished data).

Widespread throughout the TEP, the flag cabrilla has been recorded from San Diego, California, to Peru, including the offshore islands of the Revillagigedos, Cocos, and Galápagos (Heemstra and Randall, 1993; Craig, et al., in prep.). Despite previous reports of *E. labriformis* at another east Pacific island, Clipperton Atoll, this species appears to be replaced there by a newly described species, *E. clippertonensis* Allen and Robertson 1999. That species is presumed to be the sister species of *E. labriformis* based on morphology (Robertson and Allen, 1999).

A newly discovered population of groupers that bares strong resemblance to *E. clippertonensis* occurs at the Alijos Rocks (Baja California, Mexico). Lacking the characteristic white spots and olive green background of the flag cabrilla, the Clipperton grouper has a color pattern that is readily identifiable in the field (Figure 2). However, similarities in their overall morphology make it very difficult to distinguish preserved specimens of *E. labriformis* and *E. clippertonensis*, although they have differences in relative eye size and scalation (Allen and Robertson, 1999).

Population genetic data for many grouper species in the TEP is scarce, yet may contribute not only towards an understanding of the evolutionary implications of dispersal ability and the strength of hypothesized dispersal barriers, but also to the growing body of knowledge surrounding mechanisms of speciation.

In an effort to understand the phylogeographic relationships within an economically and ecologically important shorefish species, address the implications of these relationships for the biogeography of the TEP, and to investigate population divergence and subsequent indicators of early speciation, we sampled genetic data from numerous individuals throughout the TEP. While these data are a prime example of those which may provide insight into biogeographic proceses, this system also provides an opportunity to explore incipient speciation in a marine organism. We considered three hypothesis to explain our data: 1. Complete speciation despite ongoing gene flow, 2. Complete speciation with secondary contact (hybridization), and 3. Incomplete or recent speciation with incomplete

lineage sorting. Our findings are most consistent with a recent or incomplete speciation event.

Materials and Methods

Overall, sequence data was collected for 304 individuals (268 *E. labriformis*, and 36 *E. clippertonensis*) at 11 sample sites throughout the TEP (Figure 1). Tissue samples (muscle, fin clips, or gill clips) were taken from individuals collected by spear pole or hook and line, or purchased from artisinal fish markets from 1997-2004. Voucher specimens were deposited at the Scripps Institution of Oceanography Marine Vertebrates Collection (SIOMVC). Tissues were stored in either 95% ethanol or 5X Net solution (Craig, et al., 2001) and maintained at ambient temperature while in the field, and at -20°C in the laboratory. Total genomic DNA was isolated using the DNEasy DNA isolation kit (Qiagen) following manufacturers instructions. A 468 bp portion of the mitochondrial CYT B gene was amplified using the polymerase chain reaction (PCR), and unique haplotypes were deposited in Genbank (AY…). Fifty microliter PCR reactions were prepared following manufacturer's instructions included with the Sigma RedTaq Ready Mix, with the addition of 10-100 ngm of template DNA and 10 pmol of forward and reverse primer. The CYT B gene has proven useful in evaluating population level differentiation in other grouper species (Gilles, et al., 2000). PCR and sequencing primers were taken from Gilles, et al. (2000): 28-for 5'-cgaacgttgatatgaaaaaccatcgttg-3', 34-rev 5'-aaactgcagcccctcagaatgatatttgtcctca-3'. PCR reactions consisted of thirty-five cycles of the following step procedure

following a 30 second denaturation step at 94°C: 94°C for 30 sec, 50°C for 30 sec, 72°C for 45 sec. Unincorporated dNTPs and primers were removed using the Millipore Amicon filter plate. Direct sequencing of PCR products was accomplished using a Megabace 1000 automated DNA sequencer. Ten microliter sequencing reactions were prepared following manufacturer's instructions included with the ET Dye Terminator chemistry (Amersham Biosciences). Sequences for both the forward and reverse directions were used to create a consensus sequence for the final analysis.

Gene diversity (H) and nucleotide diversity (π) were calculated for each site and for the overall sample group using the computer program package Arlequin, V. 2.00 (Schneider, et al., 2000). Neutrality (equilibrium) was assessed by calculating Tajima's D for each population (Tajima, 1989). Significance was tested using 1000 permutations in Arlequin, V. 2.00. A statistical parsimony network was constructed using the computer program TCS (Clement, Posada, and Crandall, 2000) using default settings. Φ_{ct} and Φ_{st} values were calculated using Arlequin for samples grouped by the TEP provinces, Clipperton, and Alijos Rocks, and significance was tested using 1000 permutations of the dataset. Conventional "F" statistics based only on haplotype frequency were also calculated according to the same procedure. An exact test of haplotype frequencies among populations was performed using 20,000 replicates of a Markov chain as implemented in Arlequin.

Results

We analyzed 468 base pairs of the mt CYT B gene in 304 flag cabrilla (*E. labriformis*) and Clipperton grouper (*E. clippertonensis*) from throughout the TEP. We found 49 unique haplotypes among all individuals. Nucleotide diversity (π) within sampled groups ranged from 0.0012 to 0.004856, and gene diversity (H) from 0.7 to 0.87 (Table 1). Analysis of molecular variance for all sampled groups (AMOVA) indicated significant population structuring (Φ_{ct}=0.19814; p=0.00010, Φ_{st}=0.20671, p=0.00; F_{st}=0.1689, p=0.00, F_{ct}=0.15072, p=0.036). A pairwise comparison of population Φ_{st} values indicated that a majority of the significance was found in comparisons involving the Alijos Rocks and Clipperton Atoll populations (Table 2). Tajima's test of neutrality yielded negative values for all groups (Table 1). Negative values indicate a predominance of low frequency haplotypes.

A statistical parsimony network is presented in Figure 3. Four of 24 (16.6%) individuals collected at Clipperton Atoll had unique alleles, 16 of 24 (66.6%) had alleles that were present only in very low frequencies on the mainland (4/222; 1.8%), and three of 18 (16.6%) had the most common mainland allele (Figure 3). For the Alijos Rocks 11 of 12 individuals (91.6%) had alleles that were present only at that locality, while one individual had the most common allele found at the Clipperton Atoll. Despite sharing some mitochondrial CYT B alleles with the mainland groups, all individuals collected at Clipperton Atoll displayed the *E. clippertonensis* color pattern, while those on the mainland were exclusively

of the *E. labriformis* color pattern (Figure 2). At the Alijos Rocks, all individuals showed a color pattern similar to individuals at Clipperton Atoll, except for one individual which showed the typical *E. labriformis* color pattern. While some mainland groups possessed private alleles, they did not cluster together in any discernable units. In contrast in the Clipperton group the private alleles clustered with the most dominant Clipperton haplotypes.

A tree-based phylogenetic analyses based on the CYTB data including only *E. clippertonensis*, *E. labriformis* and the putative sister taxon *E. analogus* Gill 1863 produced a large "comb," indicating a lack of strong phylogeographic signal (Craig, et al., 2001; tree not shown).

Discussion

While the conservative morphology of the family Serranidae has confounded the ability to diagnose many species, genetic data have provided keen insight into delimiting species boundaries of closely related species (Craig, et al., 2001, 2004; Pondella, et al., 2003). In many instances, the life color pattern is the most useful characteristic for distinguishing species in the field (Heemstra and Randall, 1993). Unfortunately colors quickly fade in preserved fish, and are thus not generally useful for identification of many museum specimens. The description of the Clipperton grouper, *E. clippertonensis* Allen and Robertson, illustrates this situation. While the color pattern of this fish clearly separates it from it closest relative (*E. labriformis*), only slight differences in traditional morphological measurements and meristic counts are able to distinguish these species in preserved

specimens. Our analysis of genetic data from the mitochondrial CYT B gene adds a useful perspective in the present case. The clustering of the private and shared alleles of the Clipperton and Alijos populations and lack of such grouping of private alleles at other island sites suggests a restriction in gene flow between Clipperton Atoll, Alijos Rocks, and the mainland (Figure 3).

The AMOVA analysis provides a unique perspective on the evolutionary distinctiveness of the two populations (species) in this study. A comparison of Φ_{st} and F_{st} values indicates a deeper level of divergence when sequence similarity is incorporated (Φ_{st}=0.20671, p=0.00; F_{st}=0.1689, p=0.00). Quattro et al. (2002) suggested that a greater depth in Φ_{st} relative to F_{st} indicates a phylogenetic component in regards to the separation of populations (species). Our data reflect a pattern consistent with this notion, and indicate that the divergence in the populations discussed here reflect a phylogenetic distinctiveness of each population (species).

Several hypotheses may explain the marked differences in life colors, the more subtle differentiation of other morphological features, and the concurrent lack of complete genetic differentiation between *E. clippertonensis* and *E. labriformis*. One hypothesis is that speciation has occurred despite gene flow in these two sister taxa. While an unlikely scenario, computer simulations indicate that this can occur when additional developmental mechanisms develop (Porter and Johnson, 2002). In an influential paper, Felsenstein (1981) described the unlikely possibility of reproductive isolation when gene flow is prominent. In that paper, Felsenstein

(1981) concluded that recombination between fitness loci and assortative mating loci causes a breakdown in the possible allelic combinations that are favorable and thus produces less fit individuals. Divergence at these island localities with subsequent secondary contact with mainland individuals may also explain these data. However, the *E. labriformis* color morph has not been observed at Clipperton, nor has the Clipperton color pattern appeared on the mainland. The mainland color morph is, however, present at the Alijos Rocks. The occurrence of individuals at Clipperton Atoll and Alijos Rocks with certain color features that resemble those of mainland *E. labriformis* (e.g., prominent red tip of spinous dorsal fin) support this hypothesis, however, in the absence of further data, it impossible to makes this ascertation. Furthermore, it seems unlikely that individuals with such radically different coloration would be successful in breeding endeavors.

Another more likely hypothesis is that the evolution of external colors precedes or occurs more rapidly than changes in molecular markers traditionally used to delimit species boundaries in fishes (i.e., neutral markers), and that we are capturing speciation at a relatively early stage. For this hypothesis to remain tenable, one would expect a pattern of genetic diversification that reflects recent speciation with incomplete lineage sorting. Our data is consistent with this notion in that the populations at Clipperton Atoll and Alijos Rocks have not reached reciprocal monophyly at the locus examined, yet show statistically significant differences in gene flow estimates. Our observations further support this idea as the Clipperton Atoll and Alijos Rocks populations harbor several unique

haplotypes that consistently cluster together in a statistical parsimony network, yet share a few "mainland" haplotypes in CYT B. The pairwise comparisons of F_{st} also support this hypothesis as both the Clipperton Atoll and Alijos Rocks populations show significantly different values in all comparisons (Table 2). This pattern is consistent with the expectations of incipient species, namely that a current or historical restriction in gene flow has led to incomplete lineage sorting. Coupled with the morphological data presented here, there is a strong case for the hypothesis that we are capturing speciation as it is occurring in nature.

While this phenomenon is relatively uncommon, distinct color morphs that are thought to represent different species occur in other fish taxa that lack genetic distinctiveness (e.g., rockfish of the genus *Sebastes* and hamlets of the genus *Hypoplectrus*; Domeir, 1994; Aguilar-Perrera, 2003; McCartney, et al., 2003; Garcia-Machado, et al., 2004). Given the importance of coloration and subtle morphological variation in closely related fish species, it is imperative that those performing molecular analyses deposit voucher material, including both whole specimens and photographs taken at time of capture, such that future investigators may confirm identifications.

Our genetic data thus support the recognition of the Clipperton Atoll and Alijos Rocks populations as genetically distinct. Despite the lack of reciprocal monophyly at the mitochondrial DNA locus examined and the incomplete lineage sorting, these populations are most likely traveling on unique evolutionary trajectories. The CYT B data from this analysis, the morphological differences

previously discussed (Allen and Robertson, 1999), and the distinctive color pattern support this hypothesis. Given that there exists a specific name in the literature for the Clipperton Atoll population, we therefore suggest that the Alijios Rocks population be treated as *E. clippertonensis* Allen and Robertson given that they share the morphological attributes which distinguish it from *E. labriformis* (i.e. scale counts, morphology, and color pattern).

The CYT B gene has been used with considerable success in elucidating population genetic structure in several fishes (e.g., Gilles, et al. 2000; Muss, et al., 2001; Yamamoto, et al., 2004). While other loci may indeed be more useful as they have been shown to evolve at more rapid speeds (e.g. mitochondrial control region), our CYT B data indicate that this molecule is appropriate. Indeed, when the hypervariable control region of the mitochondria was sequenced for a subset of samples from the most distant localities (Loreto and the Galápagos Islands) similar levels of variation were found as for CYT B (data not shown). The maternal inheritance of mitochondria may confound the ability to detect subtle population differentiation or recent effects of bottlenecks (Avise, 2000). Future studies on this species should aim to assess variation in nuclear markers, including microsatellites which may provide greater phylogeographic signal.

The TEP has been divided into biogeographic regions based on the distributions of marine shore fishes and habitat breaks (see discussion above; Hastings, 2000). These habitat discontinuities have been hypothesized to play a major role in shaping the distribution and evolution of some fishes within this

oceanic region. It would be expected that reef species with long-lived larval stages would be most likely to traverse these boundaries, and should be present in more than one faunal province. Given the distance that larvae must travel before settlement, some genetic discontinuities would be expected that correlate with the geography of the habitat breaks. While the long lived pelagic larvae of *Epinephelus* species is of key importance and is suspect as a mechanism for gene flow, earlier studies have hypothesized that this attribute may not indeed be a causal factor in predicting species geographic ranges or genetic connectivity. Victor and Wellington (2000) recorded the larval duration of 49 species of TEP shore fishes in the families Pomacentridae (damselfishes) and Labridae (wrasses) from daily otolith increments, and searched for a correlation between geographic range and length of time spent as a pelagic larva. Surprisingly, these authors found that many species endemic to small areas possessed some of the longest pelagic larval stages, while species whose ranges were known to extend over large distances had some of the shortest larval durations. Their data suggest a somewhat counter-intuitive hypothesis, that beyond a threshold length of time, the planktonic duration of pelagic larvae may not drive speciation.

While damselfishes and wrasses are an important ecological component of marine fish communities, they are often not of critical importance to fisheries. Species that do contribute substantially to the total fisheries catch of the TEP are often long-lived and slowly maturing species that are particularly vulnerable to exploitation such as groupers (family Serranidae) and snappers (family

Lutjanidae)(Manooch, 1987; Ramírez and Rodríguez, 1990; Heemstra and Randall, 1993).

Our data support a panmictic population for mainland *E. labriformis*, as there is little to no genetic divergence correlated with distance along the quasi-linear coastline of the TEP. Despite the long-standing hypothesis that such biogeographic breaks drive population differentiation, it has been shown that some of these breaks do not (e.g. Pt. Conception, Burton, 1998), while others seem to play a more profound role in shaping population differentiation (e.g., the Florida Peninsula, Avise, et al., 1987; Bowen and Avise, 1990).

The Clipperton Atoll, which lies some 1100km from the mainland and 950km from the nearest offshore islands of the Revillagegedos (Robertson and Allen, 1996), includes an endemic species of grouper. The genetic differentiation of this species from its sibling species on the mainland and other eastern Pacific islands is most likely facilitated by the geographic isolation of the island. The fish fauna of the Clipperton Atoll has been described in detail (Robertson and Allen, 1996) and is a combination of TEP and western Pacific species. The atoll's isolation and reduced habitat diversity (Robertson and Allen, 1996) most likely contribute to its relative paucity of species in comparison to other oceanic islands, both in the TEP and western Pacific. While these and other islands of the TEP (e.g., Cocos, Galápagos) harbor several unique species, it appears as though their degree of isolation is insufficient to drive speciation in the flag cabrilla. In fact, even though the Galápagos Islands lie approximately the same distance from the

mainland as the Clipperton Atoll, the former is more "connected" to the mainland by the dominant North Equatorial Current. In contrast, Clipperton Atoll is under the influence of the much weaker North Equatorial Counter Current during the period of time when propagules would likely be dispersed in the plankton (June-September; B. Erisman, pers. comm..), and would thus have little input of recruits from mainland populations (Figure 5). This situation is uniquely paralleled by that seen in the genus *Ophioblennius* for mid-Atlantic crossings (Muss, et al., 2001). The lack of gene flow to and from the Clipperton Atoll suggests that there exists a mechanism for larval retention. The small current vectors surrounding the island during the likely spawning period for the Clipperton grouper (Figure 5) indicate that perhaps larvae are not dispersed long distances from the island. Alternatively, eddy currents emanating from the islands landmass may return larvae to their natal site.

While a similar evolutionary pattern has occurred at the Alijos Rocks resulting in the differentiation of the population there, a subtly different mechanism may be responsible. Lying only 300km from the outer coast of Baja California, the Alijos Rocks appear to be close enough to allow a large degree of larval transport between the island and the mainland. However, this small group of elevated rocks lies at the northernmost range of *E. labriformis* (Craig, et al., 2004), thus there are very few individuals on the mainland which may supply the island with propagules throughout most of the year. Instead, it would seem probable that during warm water periods facilitated by the El Nino Southern Oscilation larvae that would

normally be transported in a southerly direction are redirected towards the Alijos Rocks. These individuals could have then formed a founder population that is not under considerable influence by mainland populations but may, however, be interconnected with other island groups given the sharing of haplotypes between Clipperton Atoll and the Alijos Rocks (Fig. 3). This hypothesis is supported by the occurrence of an adult *E. labriformis* taken at the Alijos Rocks. The individual was 260mm standard length and was taken in 2004. Based on previous age and growth analysis for this species (Craig et al., 1999), the individual was most likely 6-7 years old. This would indicate that the individual settled during one of the strongest ENSO events on record during 1997-98. Alternatively, if there is no or little gene flow among island populations, the population at the Alijos Rocks may be a striking example of convergent evolution in that the individuals there share many of the same morphological attributes which distinguish them from mainland *E. labriformis*.

Fisheries management strategies are transitioning from a species by species strategy to a more ecosystem based model (Sala, et al., 2002; Pikitch, et al., 2004). However, in order to effectively maintain biological diversity of any ecosystem, it is of the utmost importance to assess how populations of organisms are interconnected. With a growing desire to implement networks of marine reserves, such data have become critical in understanding population dynamics, identifying source and sink populations, and targeting new areas for new reserves (Sala, et al., 2002). Additionally, all management decisions should take into account the recent

evolutionary history of organisms and an accurate assessment of their taxonomic status (Templeton, 2004). Human induced changes in both population genetic structure and life history parameters of marine organisms have been documented, suggesting that anthropogenic impacts over relatively short time scales may have evolutionary time scale effects. Our data provide one of the few examples which may be applicable for management decisions within the TEP by confirming the specific status of a highly endemic island species and the lack of genetic structure in a broadly distributed species. If indeed the observed lack of genetic diversity is a result of a high degree of migratory individuals, the flag cabrilla may be resilient to local extirpation and able to recolonize an overfished area relatively quickly. While our data suggest panmixia for the broadly distributed flag cabrilla, it is important to remember that a high degree of genetic connectivity does not necessarily indicate demographic connectedness given that the number of migrants to maintain genetic diversity is low (Avise, 2000). The Clipperton grouper, however, should be treated as an endemic island species that is highly susceptible to extinction and should be managed accordingly.

Acknowledgments

We would like to thank N. Knowlton for use of laboratory facilities. Thanks to J. M. Flowers and R. Burton for thoughtful discussions throughout the duration of this project and comments on earlier versions of the manuscript. We would like to thank H. J. Walker, Jr., and C. Klepadlo, SIO Marine Vertebrates Collection for

their curatorial assistance. L. G. Allen (CSUN) created the map figure. Funding was provided by the UC Mexus dissertation research grant program, the Carl L. and Laura C. Hubbs Marine Biology Endowment, the UCSD Academic Senate, and the SIO Graduate Department. DRR collected samples at Clipperton Atoll under sponsorship from a National Geographic Society Grant (NGS 5831-96).

References

Allen, G. R., and D. R. Robertson. 1994. *Fishes of the Tropical Eastern Pacific.* University of Hawaii Press, Honolulu.

Allen, G. R., and D. R. Robertson. 1999. *Epinephelus clippertonensis*, a new species of serranid fish from the tropical eastern Pacific. *Revue Francaise d'Aquariologie Herpetologie*, **26,** 11-15.

Aguilar-Perrera, A. 2003. Abundance and distribution of hamlets (Teleostei: *Hypoplectrus*) in coral reefs off south western Puerto Rico: support for the multiple species hypothesis. *Caribbean Journal of Science*, **39**,151-154.

Avise, J. C. 2000. *Phylogeography: The history and formation of species.* Harvard University Press. Cambridge, Mass.

Avise, J. C. H, C. A. Reeb, and N. C. Saunders. 1987. Geographic population structure and species differences in mitochondrial DNA of mouthbrooding marine catfishes (Ariidae) and demersal spawning toadfishes (Batrachoididae). *Evolution*, **41**, 991-1002.

Bernardi, G., L. Findley, and A. Rocha-Olivares. 2003. Vicariance and dispersal across Baja California in disjunct marine populations. Evolution 57(7):1599-1609.

Bonhomme, F., and S. Planes. 2000. Some evolutionary arguments about what maintains the pelagic interval in reef fishes. *Environmental Biology of Fishes*, **5,** 365-383.

Bonjean F. and G.S.E. Lagerloef. 2002: Diagnostic Model and Analysis of the Surface Currents in the Tropical Pacific Ocean, *Journal of Physical Oceanography*,**32**, 2938-2954.

Bowen, B., and J. C. Avise. 1990. Genetic structure of Atlantic and Gulf of Mexico populations of sea bass, menhaden, and sturgeon: Influence of

zoogeographic factors and life-history patterns. *Marine Biology,* **107**, 371-381.

Brusca, R. C., and B. R. Wallerstein. 1979. Zoogeographic pattern of idoteid isopods in the northeast Pacific, with a review of shallow water zoogeography of the area. Bulletin of the Biological Society of Washington, **3**, 67-105.

Burton, R. S. 1998. Intraspecific phylogeography across the Point Conception biogeographic boundary. *Evolution*, **52**, 734-745.

Craig, M. T., D. J. Pondella, and J. C. Hafner. 1999. Analysis of age and growth in two eastern Pacific groupers (Serranidae; Epinephelinae). *Bulletin of Marine Science*, **65**, 807-814.

Craig, M. T., D. J. Pondella, J. C. Hafner, and J. P. C. Franck. 2001. On the status of the serranids fish genus *Epinephelus*: Evidence for paraphyly based upon 16S rDNA sequence. *Molecular Phylogenetics and Evolution*, **19**, 121-130.

Craig, M. T., P. A. Hastings, and D. J. Pondella. 2004. Speciation in the Central American seaway: the importance of taxon sampling in the identification of trans-isthmian geminate pairs. *Journal of Biogeography*, **31**, 1085-1091.

Dawson, C. E. 1975. Studies on the eastern Pacific sand stargazers (Pisces: Dactyloscopidae) 2. Genus *Dactyloscopus*, with descriptions of new species and subspecies. *Natural History Museum of Los Angeles County Scientific Bulletin* **22,** 1-61.

Domeir, M. L. 1994. Speciation in the serranid fish *Hypoplectrus. Bulletin of Marine Science*, **54**, 103-141.

Ekman, S. 1953. *Zoogeography of the sea.* Sidgwick and Jackson Limited, London.

Endler, J. 1977. Geographic variation, speciation, and clines. Princeton University Press, Princeton, New Jersey.

Felsenstein, J. 1981. Skepticism towards Santa Rosalia, or why are there so few kinds of animals? Evolution 35:124-138.

Garcia-Machado, E., P. Chevalier Monteagudo, and M. Solignac. 2004. Lack of mt differentiation among hamlets (*Hypoplectrus*, Serranidae). *Marine Biology*, **144**, 147-152.

Gilles, A., A. Miquelis, J. P. Quignard, and E. Faure. 2000. Molecular phylogeography of western Mediterranean dusky grouper *Epinephelus marginatus*. *Comptes rendus de l'Académie des sciences. Série III, Sciences de la vie,* **323,** 195-205.

Hastings, P. A. 2000. Biogeography of the Tropical Eastern Pacific: distribution and phylogeny of chaenopsid fishes. *Zoological Journal of the Linnaean Society*, **128,** 319-335.

Hedgecock, D. 1986. Is gene flow from larval dispersal important in the adaptation and evolution of marine invertebrates? *Bulletin of Marine Science*, **39**, 550-564.

Heemstra, P. C., and J. Randall. 1993. *Groupers of the World.* FAO Species Catalog **16**. Rome.

Hobson, E. S. 1968. Predatory behavior of some shore fishes in the Gulf of California. *Bureau of Sports Fisheries and Wildlife Research Reports*, **73**, 1-92.

Leis, J. M., and B. M. Carson-Ewart. 2000. *In situ* swimming speeds of the late pelagic larvae of some Indo-Pacific coral-reef fishes. *Marine Ecology Progress Series*, **159**, 165-174.

Manooch, C. S. 1987. Age and growth of snappers and groupers. IN: *Tropical Snappers and Groupers*: *Biology and Fisheries Management* (ed. J. J Polovina and S. Ralston). Westview Press, London.

McCartney, M. A., J. Acevedo, C. Heredia, et al. 2003. Genetic mosaic in a marine species flock. *Molecular Ecology*, **12,** 2963-2973.

Mora, C., and D. R. Robertson. 2004. Factors shaping the range-size frequency distribution of the endemic fish fauna of the Tropical Eastern Pacific. In Press. *Journal of Biogeography*.

Muss, A., D. R. Robertson, C. A. Stepien, P. Wirtz, and B. W. Bowen. 2001. Phylogeography of *Ohpioblennius*: The role of ocean currents and geography in reef fish evolution. *Evolution*, **55**, 561-572.

Noor, M. A. F. 2003. Evolutionary biology: Genes to make new species. Nature 423:699-700.

Pikitch, E. K., C. Santora, E. A. Babcock, et al. 2004. Ecosystem-based fishery management. *Science,* **305**, 346-347.

Pondella, D.J., II, M. T. Craig, and J. P. C. Franck. 2003. The phylogeny of *Paralabrax* (Perciformes: Serranidae) and allied taxa inferred from 16S and 12S mitochondrial sequence data. *Molecular Phylogenetics and Evolution*, **29**, 176-184.

Porter, A. H., and N. A. Johnson. 2002. Speciation despite gene flow when developmental pathways evolve. *Evolution,* **56**, 2103-2111.

Quattro, J. M., T. W. Greig, D. K. Coykendall, B. W. Bowen, and J. D. Baldwin. 2002. Genetic issues in aquatic species management: the shortnose sturgeon (*Acipenser brevirostrum*) in the southeastern United States. Conservation Genetics 3:155-166.

Ramírez, R. M., and C. M. Rodríguez. 1990. Specific composition of the small scale fishery capture at Isla Cerralvo, B.C.S., México. *Investigaciones marinas Cicimar,* **5**, 137-141.

Ritchie, M. G., and M. A. F. Noor. 2004. Gene replacement and the genetics of speciation. Heredity 94:1-2.

Robertson, D. R., and G. R. Allen. 1996. Zoogeography of the shorefish fauna of Clipperton Atoll. *Coral Reefs,* **15**, 121-131.

Rosenblatt, R. H. 1967. The zoogeographic relationships of the marine shore fishes of tropical America. *Studies in Tropical Oceanography*, **5**, 579-592.

Sala, E. O. Aburto-Oropeza, G. Paredes, I. Parra, et al. 2002. A general model for designing networks of marine reserves. *Science*, **298**, 1991-1993.

Scheltema, R. S. 1968. Dispersal of larvae by equatorial ocean currents and its importance to the zoogeography of shoal-water tropical species. *Nature*, **217**, 1159-1162.

Springer, V. G. 1959. Systematics and zoogeography of the clinid fishes of the subtribe Labrisomini Hubbs. *Institute of Marine Science Publications*, **5**, 417-492.

Swanson, W. J., and V. D. Vaquier. 2002. The rapid evolution of reproductive proteins. Nat. Rev. Genetics 3:137-144.

Swearer, S. E., J. E. Caselle, D. W. Lea, and R. R. Warner. 1999. Larval retention and recruitment in an island population of a coral reef fish. *Nature*, **40**, 799-802.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, **123**, 585-596.

Taylor, M. S., and M. E. Hellberg. 2003. Genetic evidence for local retention of pelagic larvae in a Caribbean marine fish. *Science*, **299**, 107-109.

Templeton, A. R. 2004. Using haplotypes trees for phylogeographic and species inference in fish. *Environmental Biology of Fishes*, **69**, 7-20.

Terry, A., G. Bucciarelli, and G. Bernardi. 2000. Restricted gene flow and incipient speciation in disjunct Pacific Ocean and Sea of Cortez populations of a reef fish species, *Girella nigricans*. Evolution, 54(2):652-659.

Victor, B. C., and G. M. Wellington. 1998. Endemism and the pelagic larval duration of reef fishes in the eastern Pacific Ocean. *Marine Ecology Progress Series*, **205**, 241-248.

Walker, B. W. 1960. The distribution and affinities of the marine fish fauna of the Gulf of California. *Systematic Zoology*, **9**, 123-133.

Yamamoto, S., K. Morita, S. Kitano, K. Watanabe, I. Koizumi, K. Maekawa, and K. Takamura. 2004. Phylogeography of white-spotted char (*Salvelinus leucomaeneus*) inferred from mitochondrial DNA sequences. *Zoological Science* (Tokyo), **21**, 229-240.

Table 1. Sample size and descriptive statistics for Cytochrome B data for 304 *E. labriformis* and *E. clippertonensis*.

Site	N	# Haplotypes	# Unique Haplotypes	Gene Diversity	Nucleotide Diversity	Tajima's D
Cortez Province						
Loreto	38	10	5	0.4609	0.002343	-1.78099*
La Paz	5	3	2	0.7	0.001709	-0.97256
Cabo san Lucas	7	3	0	0.5238	0.001832	-1.35841
Mexican Province						
Mazatlan	30	7	3	0.4644	0.002659	-1.82477*
Puerto Vallarta	49	8	4	0.4464	0.001741	-1.67569*
Huatulco	26	9	3	0.76	0.002774	-1.19676
Panamic Province						
El Salvador	21	5	3	0.5381	0.003663	-1.72296*
Panama	46	9	4	0.5295	0.002147	-1.62131*
East Pacific Islands						
Alijos rocks	12	7	5	0.7727	0.004856	-0.56737
Cocos Island	11	7	3	0.8727	0.004274	-0.66206
Clipperton Atoll	24	6	5	0.4964	0.0012	-1.68244
Galapagos Islands	35	6	2	0.6034	0.002492	-0.54846

Table 2. Pairwise population Φst values and corresponding significance values (p values) for 11 populations of *Epinephelus clippertonensis* and *E. labriformis*. Fst values are below the diagonal, and p values are above the diagonal.

	Clipperton	Mazatlan	Pto. Vallarta	Panama	Oaxaca	Cocos	Galapagos	Loreto	El Salvador	East Cape	Alijos Rocks
Clipperton	-	0*	0*	0*	0*	0*	0*	0*	0*	0*	0*
Mazatlan	0.46239	-	0.3566	0.2573	0.17068	0.09187	0.03693*	0.4155	0.19305	0.72904	0*
Pto. Vallarta	0.52191	0.00021	-	0.74894	0.23186	0.02663*	0.07504	0.50292	0.34571	0.50124	0*
Panama	0.47746	0.00578	-0.011	-	0.5045	0.04227*	0.20859	0.49698	0.45778	0.35987	0*
Oaxaca	0.44075	0.01509	0.00842	-0.00789	-	0.13613	0.58707	0.15612	0.69805	0.24552	0*
Cocos	0.49537	0.04992	0.09755	0.07337	0.03972	-	0.12573	0.02693*	0.15335	0.0597	0.0003*
Galapagos	0.4925	0.05157	0.03558	0.01203	-0.01358	0.04518	-	0.03693*	0.42521	0.10148	0*
Loreto	0.46893	-0.00078	-0.0057	-0.00516	0.01523	0.07429	0.04761	-	0.20879	0.67904	0*
El Salvador	0.43917	0.014	0.00149	-0.0071	-0.01685	0.0357	-0.00775	0.01015	-	0.35699	0*
East Cape	0.54137	-0.02012	-0.01295	-0.00431	0.01661	0.06436	0.06397	-0.01842	-0.00026	-	0.0003*
Alijos Rocks	0.66178	0.43473	0.55838	0.51581	0.41816	0.35231	0.46131	0.50135	0.4007	0.47503	-

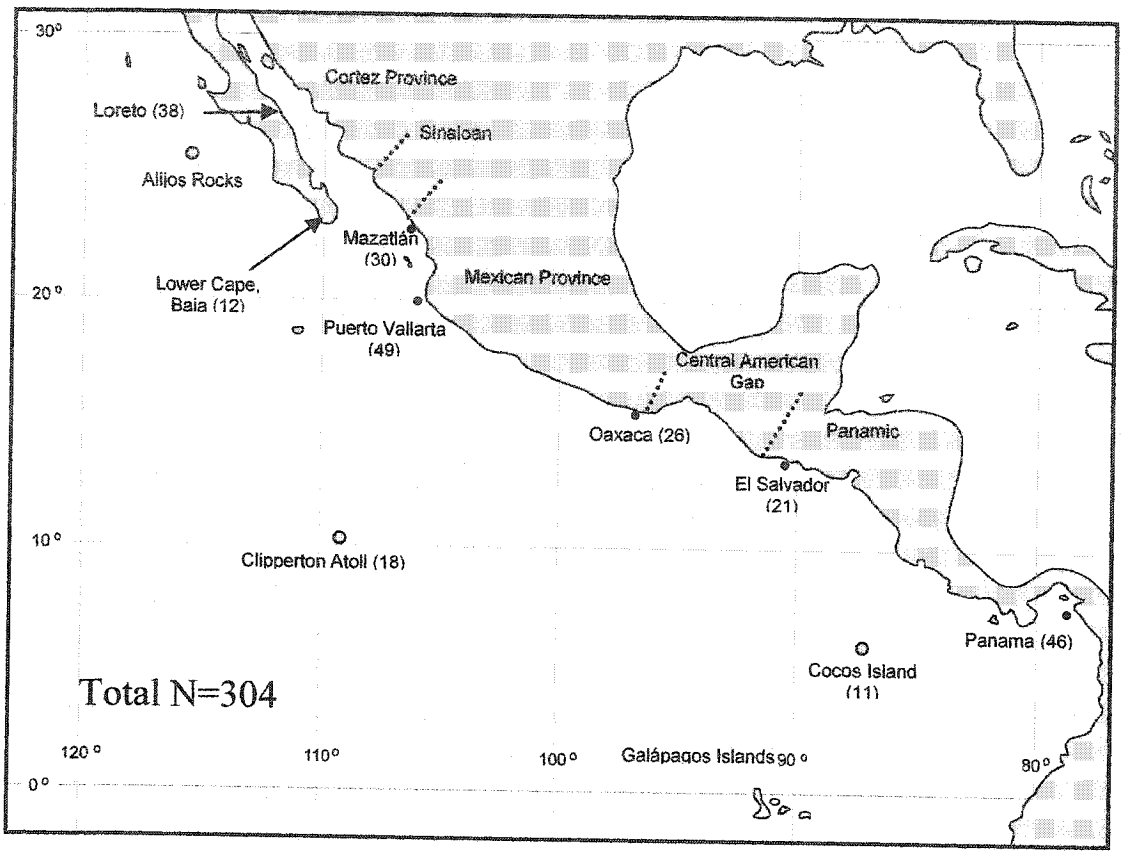

Figure 1. Map of the Tropical Eastern Pacific (TEP) showing biogeographic provinces and collecting localities for 288 specimens of *Epinephelus labriformis* and *E. clippertonensis*. Numbers in parentheses are samples sizes for each collecting site.

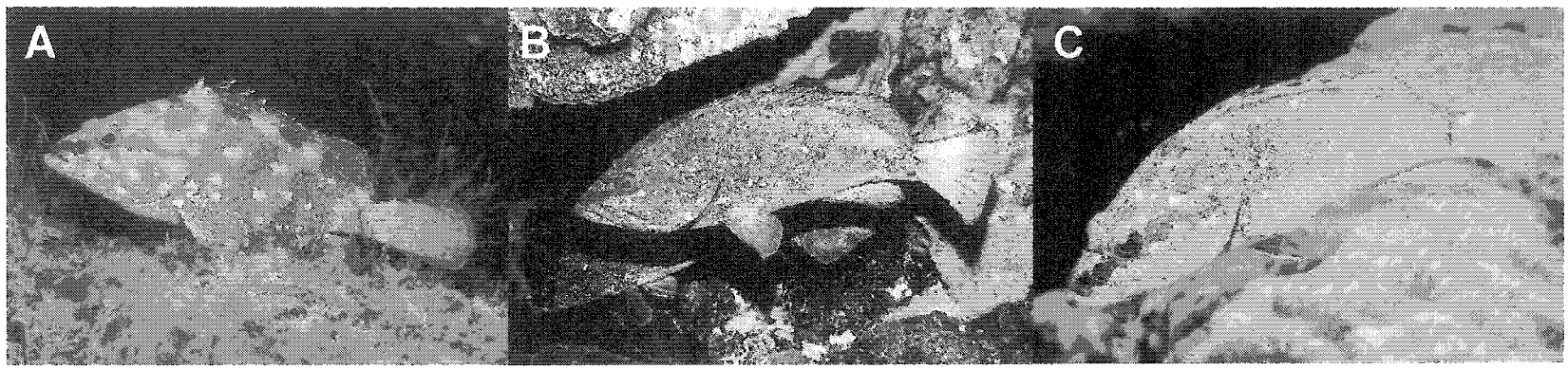

Figure 2. Photographs of the flag cabrilla, *Epinephelus labriformis* (A), *E. clippertonensis* intermediate color morph (B), and *E. clippertonensis* (C).

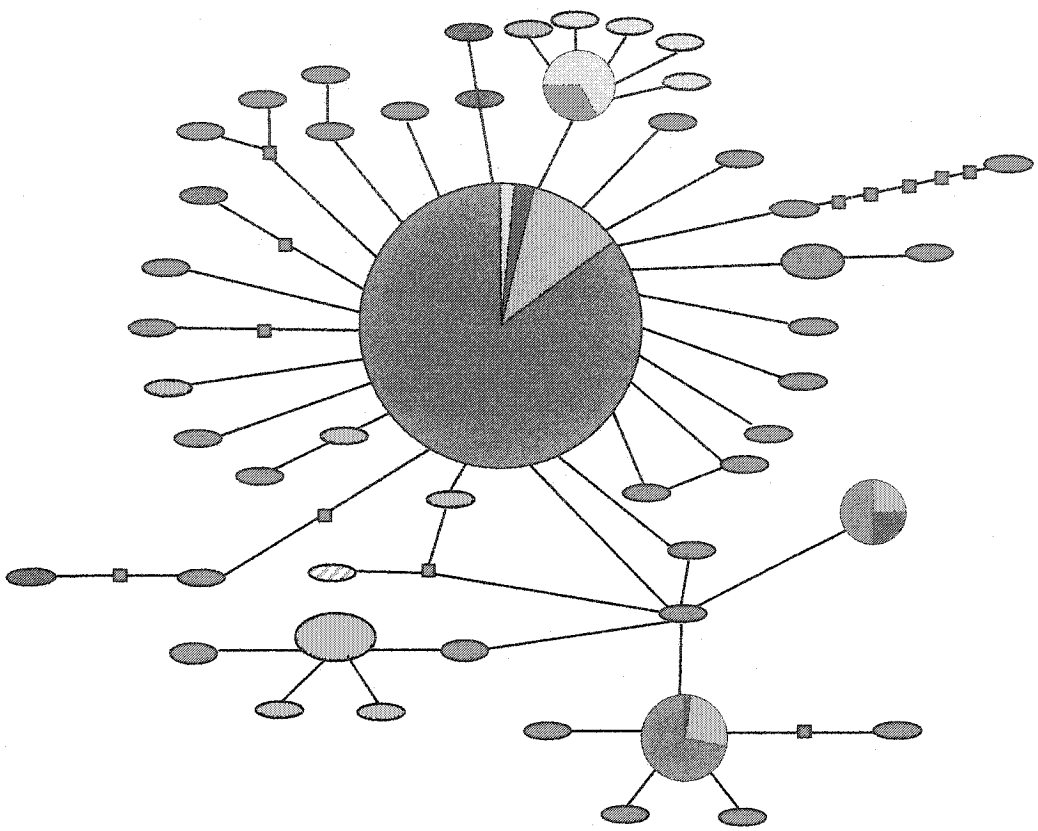

Figure 3. Statistical parsimony network for 304 individuals of *Epinephelus spp.* based on 41 haplotypes of the mitochondrial CYT B gene. Yellow indicates that the haplotype was found at Clipperton Atoll, dark blue at the mainland, green at the Galápagos Islands, light blue at the Alijos Rocks, and red indicates haplotype was present at Cocos Island. Hashed light blue is the mainland color morph found at the Alijos Rocks. Circles are proportional to the number of individuals displaying the haplotypes, ovals are singleton haplotypes, and small squares are single nucleotide changes (i.e. missing haplotypes).

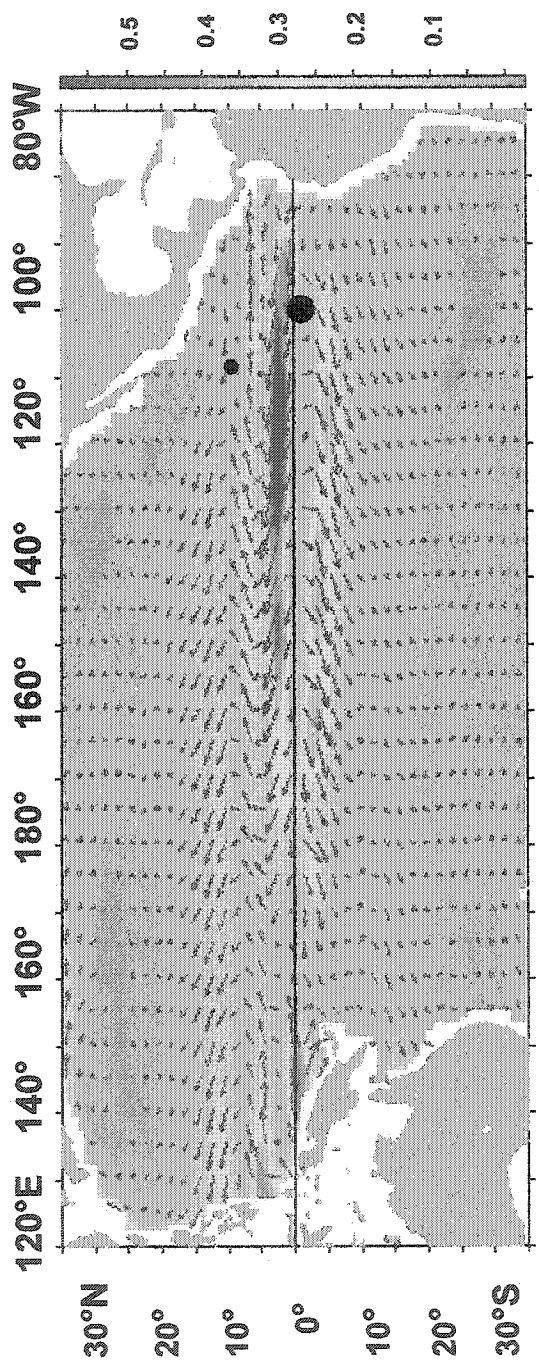

Figure 4. Mean surface current vectors overlying long-term mean June-September, 1994-2004. Colored scale bar is long term mean in meter/second; large scale vector is 1meter/second. Data taken from http://www.oscar.noaa.gov (Bonjean and Lagerloef,,2002). Small dot is the Clipperton Atoll, large dot is the Galápgos Islands.

The text of Chapter V, in full, has been submitted for publication of the material as it appears in Evolution. The dissertation author was the primary author and the co-author listed in this publication directed and supervised the research, which forms the basis for this chapter.

Appendix I. Specimens collected, voucher specimen numbers, and GenBank accession numbers for data gathered in the current study. "X" indicates sequence available but not yet deposited in GenBank. Blanks indicate no sequence available. PV=Photo Voucher, SIO=Scripps Institution of Oceanography Marine Vertebrates Collection, LACM=Los Angeles County Museum, AMNH=American Museum of Natural History, KUNHM-BRC=University of Kansas Natural History Museum and Biological Research Collection, AM=Australian Museum.

Species	N	Voucher Number	16S	12S	TMO4C4	H III
Epinephelini						
Aethaloperca rogaa	2	SIO 02-138	AY947565	AY949367	AY949225	AY949552
Alphestes afer	2	SIO 03-49	AY314003	AY313982	AY313992	AY949455
A. immaculatus	1	SIO 00-92	AF297290	AY313980	AY313994	AY949456
A. multiguttatus	2	SIO 00-95	AF297305	AY313981	AY313991	
Anyperodon leucogrammicus	3	SIO 64-235	AF297306	AY949379		AY949577
C. argus	2	PV, D. R. Robertson	AY947555	AY949357	AY949223	AY949472
C. boenak	2	SIO 02-138	AY947598	AY949325	AY949293	AY949520
C. cruentata	2	SIO 04-192	AF297323	AY949385	AY949266	AY949533
C. cyanostigma	1	SIO 04-191	AY947594	AY949389	AY949290	AY949517
C. formosa	1	SIO 04-191	AY947603	AY949370	AY949291	AY949588
C. fulva	2	SIO 00-146	AF297292	AY949395	AY949282	AY949589
C. igarashiensis	2	SIO 02-138	AY947599	AY949326	AY949292	AY949457
C. leopardus	1	PV, D. R. Robertson	AY947560	AY949327	AY949323	AY949473
C. miniata	1	SIO 64-235	AF297321	AY949400	AY949318	AY949523
C. nigri	1	SIO 04-39	AY947604	AY949451	AY949279	AY949581
C. nigripinnis	1	SIO 04-67	AY947605	AY949382	AY949280	AY949504
C. panamensis	3	SIO 00-92	AF297313	AY949396	AY949272	AY949531
C. polleni	1	SIO 04-191	AY947627	AY949371	AY949278	AY949553
C. sonnerati	2	SIO 64-235	AF297307	AY949404	AY949297	AY949534
C. taeniops	2	SIO 04-39	AY947589	AY949387		AY949498
C. urodeta	1	SIO 02-139	AF297325	AY949408	AY949277	AY949538
Cromileptes altivelis	3	SIO 02-141	AY947628	AY949328	AY949286	AY949500
Dermatolepis dermatolepis	2	SIO 64-235	AF297317	AY313984	AY313988	AY949536

Appendix I (cont.)

D. inermis	1	PV, MTC	AY314005	AY313979	AY313987	ay949573
D. striolata	1	PV, D. R. Robertson	AY314004	AY313989	AY313989	AY949474
Epinephelus acanthistius	1	SIO 00-142	AF297318	AY949376		AY949590
E. adscencionis	2	SIO 00-145	AF297314	AY949381	AY949284	AY949487
E. aeneus	1	PV, P. Wirtz	AY947593	AY949441	AY949226	AY949476
E. akaara	1	R. Chapman	AY947600	AY949442		AY949569
E. albomarginata	1	PV, S. Fennesey	AY947590	AY949378	AY949298	AY949477
E. amblycephalus	1	SIO 64-228	AY731070	AY949434	AY949312	AY949513
E. analogus	1	SIO 00-185	AF297302	AY949330	AY949220	AY949499
E. andersoni	2	SIO 04-60	AY947592	AY949383	AY949315	AY949478
E. areolatus	1	SIO 00-235	AY731076	AY949391		AY949479
E. awoara	1	SIO 02-137	AY947558	AY949331	AY949227	AY949576
E. bleekeri	1	PV, MTC	AY947626	AY949366		AY949554
E. bruneus	1	PV, MTC	AY947562	AY949399	AY949228	AY949555
E. caeruleopunctatus	3	SIO 02-139	AY947563	AY949374	AY949229	AY949580
E. caninus	1	PV, E. Sala	AY947585	AY949428	AY949294	
E. chlorostigma	2	PV, D. R. Robertson	AY731075	AY949407	AY949231	AY949508
E. cifuentesi	2	SIO 00-138	AF297295	AY949397	AY949209	AY949480
E. clippertonensis	2	SIO 00-186	AY731077	AY949332	AY949304	AY949521
E. coioides	2	SIO 64-235	AY947608	AY949333	AY949295	AY949518
E. corrallicola	2	PV, MTC	AY947568	AY949334	AY949232	AY949459
E. costae	1	PV, E. Sala	AY947596	AY949368	AY949296	AY949506
E. cyanopodus	2	SIO 02-138, AM I.39542007	AY731074	AY949335	AY949233	AY949460
E. daemelii	1	PV	AY947635	AY949453		AY949587
E. diacanthus	1	PV, MTC	AY947619	AY949406	AY949274	AY949549
E. drummondhayi	2	SIO 00-152	AF297317	AY313985	AY313993	AY949541
E. ergastularius	2	AM I.39542007	AY947606	AY949432	AY949230	AY949575
E. exsul	2	SIO 02-21	AY947556	AY949358	AY949222	AY949461
E. fasciatomaculosus	2	PV, MTC	AY947622	AY949398	AY949324	AY949579

Appendix I (cont.)

E. fasciatus	1	SIO 64-235	AF297319	AY949401		AY949524
E. flavocaeruleus	1	SIO 04-67	AY947607	AY949384	AY949316	AY949585
E. flavolimbatus	1	SIO 00-150	AF297293	AY949336	AY949269	AY949528
E. fuscogutattus	1	AM I.42844005	AY947561	AY949415	AY949234	AY949510
E. guttatus	2	SIO 00-140	AF297299	AY949437	AY949281	AY949545
E. goreensis	1	PV, G. Menenzes	AY947621	AY949438	AY949305	AY949551
E. hexagonatus	2	AMNH 120080	AY947623	AY949380	AY949319	AY949462
E. howlandi	3	SIO 02-139	AY947620	AY949414	AY949317	AY949583
E. itajara	1	SIO 00-185	AF297294	AY949337	AY949235	AY949592
E. labriformis	3	SIO 00-137	AF297296	AY426252	AY949236	AY949566
E. lanceolatus	2	SIO 04-191	AY947588	AY949377	AY949237	AY949463
E. macrospilos	1	SIO 02-141	AY731072	AY949416	AY949238	AY949481
E. maculatus	1	SIO 02-138, AM I.42844011	AY731068	AY949338	AY949313	AY949482
E. malabaricus	2	SIO 02-140	AY947609	AY949390	AY949275	AY949544
E. marginatus	2	SIO 04-62	AY947595	AY949369	AY949239	AY949483
E. melanostigma	2	SIO 02-138	AY947633	AY949339	AY949240	AY949591
E. merra	2	SIO 02-141	AY947629	AY949427	AY949288	AY949515
E. miliaris	1	PV, D. R. Robertson	AY947634	AY949418	AY949299	AY949516
E. morio	2	SIO 00-145	AF297324	AY949425	AY949322	AY949484
E. morrhua	2	SIO 02-137	AY947630	AY949340	AY949287	AY949464
E. multinotatus	2	PV, D. R. Robertson	AY428594	AY426252	AY425675	AY949567
E. mystacinus	2	SIO 00-138	AF297304	AY949341	AY949307	AY949485
E. nigritus	1	SIO	AF297297	AY949405	AY949309	AY949532
E. niphobles	1	SIO 64-235	AF297309	AY949342	AY949241	AY949584
E. niveatus	2	SIO 00-151	AF297310	AY949343	AY949262	AY949535
E. octofasciatus	2	SIO 02-138	AY947564	AY949388	AY949242	AY949501
E. ongus	3	SIO 02-138	AY947566	AY949344	AY949243	AY949496
E. polyphekadion	2	SIO 02-141	AY947569	AY949431	AY949244	AY949509
E. quernus	2	PV, M. Rivera	AY947570	AY949429	AY949245	AY949465

Appendix I (cont.)

E. quoyanus	1	R. Chapman	AY731073	AY949394	AY949285	AY949502
E. radiatus	2	SIO 02-141	AY947602	AY949430	AY949301	AY949519
E. retouti	2	SIO 02-139	AY947625	AY949345	AY949246	AY949466
E. rivulatus	1	SIO 02-141	AY947586	AY949410	AY949224	AY949458
E. septemfasciatus	2	SIO 02-137	AY947559	AY949346	AY949247	AY949568
E. spilotoceps	1	PV, D. R. Robertson	AY731069	AY949440	AY949321	AY949564
E. striatus	2	SIO 00-146	AF297311	AY949433	AY949283	AY949539
E. tauvina	1	SIO 02-138	AY731067	AY949347	AY949248	AY949467
E. trimaculatus	2	R. Chapman	AY731071	AY949403	AY949264	AY949486
E. tukula	2	R. Chapman	AY947557	AY949443	AY949249	AY949507
E. undulosus	1	SIO 64-235	AF297326	AY949409	AY949302	AY949505
E. undulostriatus	1	PV, D. R. Robertson	AY947636	AY949454		AY949586
Gracila albomarginata	1	PV, MTC	AY947582	AY949348	AY949250	AY949574
Mycteroperca acutirostris	1	PV, R. Chapman	AY947591	AY949411	AY949251	AY949514
M. bonaci	1	SIO 00-145	AF297315	AY949449	AY949270	AY949546
M. fusca	2	PV, P. Wirtz	AY947597	AY949448	AY949252	AY949489
M. interstitialis	2	SIO	AY947632	AY949359	AY949221	ay949556
M. jordani	2	SIO 00-144	AF297329	AY949435	AY949303	AY949522
M. microlepis	2	SIO 00-148	AF297312	AY949373	AY949253	AY949490
M. olfax	2	SIO 00-89	AF317512	AY949360	AY949276	AY949537
M. phenax	2	SIO 00-145	AF297303	AY949450	AY949265	AY949548
M. prionura	1	PV, D. J. Pondella, II	AY947583	AY949361	AY949254	AY949557
M. rosacea	2	SIO 00-92	AF297300	AY949350	AY949268	AY949540
M. rubra	3	PV, T. Maggio	AY947587	AY949364	AY949255	AY949468
M. tigris	2	UKNHM-BRC T104	AY947574	AY949452	AY949217	AY949560
M. venenosa	2	SIO 00-147	AF297291	AY949419	AY949273	AY949527
M. xenarcha	1	SIO UN-CAT	AY947637	AY949445		AY949571
Paranthias colonus	1	SIO 00-89	AF297301	AY949351		AY949491
P. furcifur	2	SIO 00-125	AY947584	AY949372	AY949263	AY949595
Plectropomus areolatus	1	PV, MTC	AY947613	AY949447	AY949267	AY949565

Appendix I (cont.)

P. laevis	1	SIO 64-236	AY947614	AY949444	AY949320	AY949542
P. leopardus	1	AM I.42844017	AF297298	AY949352	AY949211	AY949525
P. maculatus	1	SIO 64-235	AF297320	AY949423		AY949570
P. oligocanthus	1	PV, MTC	AY947615	AY949386	AY949300	AY949547
Saloptia powelli	2	SIO 02-139	AY947631	AY949375		AY949578
Triso dermopterus	1	AM I.41217002	AY947601	AY949365	AY949260	AY949469
Variola albomarginata	2	SIO 02-138	AY947567	AY949412	AY949261	AY949495
V. louti	2	SIO 04-191	AY947577	AY949363	AY949219	AY949494
Niphonini						
Niphon spinosus	2	SIO 00-174	AY947575	AY949420	AY949210	AY949596
Diploprionini						
Diploprion bifascaitum	2	SIO 04-191	AY947576	AY949329	AY949214	AY949475
Belonoperca chabanaudi	1	SIO 04-191	AY947580	AY949422		AY949561
Lioprompimini						
Liopropoma eukrines	1	SIO 01-11	AY947581	AY949426	AY949208	AY949488
Liopropoma carmabi	1	PV, MTC	AY947579	AY949349	AY949310	AY949558
Gramistini						
Aporops sp.	1	UKNHM-BRC T804	AY947573	AY949356	AY949271	AY949471
Pseudogramma polyacantha	2	UKNHM-BRC T695, T696	AY947512	AY949362	AY949212	AY949493
Psuedogramma gregoryi	2	UKNHM-BRC T100, T155	AY947571	AY949417	AY949213	AY949492
Pogonoperca punctata	1	SIO 64-235	AF297322	AY949353	AY949218	AY949582
Rypticus nigripinnis	1	SIO 00-182	AY947578	AY949402	AY949258	AY949593
Sutonia sp.	1	UKNHM-BRC T805	AY947618	AY949355	AY949311	
Grammistes sexlineata	1	PV, MTC	AY539050	AY949413	AY539458.1	AY949572
Anthiine outgroups						

Appendix I (cont.)

Pronotogrammus multifasciatus	2	SIO 00-139	AF297330	AY949354	AY949257	AY949511
Hemanthias peruanus	1	SIO 00-185	AY947610	AY949393	AY949306	AY949594
Pseudanthias squamipinnis	2	SIO 04-51	AY947624	AY949436	AY949308	AY949543
Hemanthias leptus	1	MTC	AY947611	AY949392	AY539459.1	AY949512
Anthias cf anthias	1	PV, G. Menezes	AY947617	AY949446		AY949550
Serranine outgroups						
Paralabrax nebulifer	2	SIO 00-97	AF297328	AY072662	AY313990	AY949497
Diplectrum pacificum	1	PV, D. J. Pondella, II	AY072669	AY072663	AY949215	AY949529
Centropristes striata	1	UCLA W97-22	AY072667	AY072656.1	AY949216	AY949530
Serranus tigrinnis	1	SIO 01-127	AY072688	AY072659.1	AY949259	AY949503
Cratinus agasizi	1	LACM 47328-1	AY072668	AY072647.1	AY949289	AY949526
Other outgroups						
Polyprion americanus	1	AM I.42844002	AY947616	AY949424	AY949256	AY949562
Morone saxatatilis	1	Wm. L. Smith	AY539046.2	x	AY539454.1	AY539255.1
Hoplostethus medditeraneus	1	Wm. L. Smith	AY538968.2	AY141335	AY539384	AY539177
Haemulon plumieri	1	Wm. L. Smith	AY539057.2	x	AY539465.1	AY539266.
Cirrhitus rivulatus	1	Wm. L. Smith	AY539059.2	x	AY539467.1	AY539268.1
Lepidotrigla spinosus	1	Wm. L. Smith	AY539001.2	x		AY539210.1
Pleurogrammos azonus	1	SIO 01-34	AY539012	AY949439	AY539424.1	AY949563
Scorpaena gutatta	1	Wm. L. Smith	AY538984.2	x	AY539400.1	AY539193.1
Perca flavescens	1	Wm. L. Smith	AY539055.2	x	AY539463.1	AY539264.1
Etheostoma blenniooides	1	Wm. L. Smith	AY539054.2	AY372771.1	AY539462.1	AY539263.1
Trachinus draco	1	Wm. L. Smith	AY539068.2	AY141378.1	AY539476.1	AY539277.1
Gymnodraco acuticeps	1	Wm. L. Smith	AY539064.2	U90413	AY539472.1	AY539273.1
Acanthistius occelatus	1	AM I.42844022	AY947612	AY949421	AY949314	AY949470
Stereolepis gigas	2	SIO 03-74	AY072683.1	AY072666		AY949559