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The phylogeny of *Paralabrax* (Perciformes: Serranidae) and allied taxa inferred from partial 16S and 12S mitochondrial ribosomal DNA sequences

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Abstract

Partial sequences of 16S and 12S mitochondrial ribosomal DNA were used to examine the phylogenetic relationships of the primarily eastern Pacific genus *Paralabrax* (Perciformes: Serranidae) and allied taxa. *Paralabrax* is considered a basal serranine, which is itself considered the basal subfamily in the Serranidae. Multiple serranines reported closely related to *Paralabrax* from the genera *Serranus*, *Hypoplectrus*, *Cratinus*, and *Centropristis* were used as outgroups. Species from the remaining two subfamilies, Epinephilinae and Anthiinae, of the Serranidae were also used in the analyses. The tree of the Serranidae was rooted with the families Polyprionidae and Priacanthidae. *Paralabrax*, the Serranidae, and the Serraninae were monophyletic in this study. *Serranus* was found to be paraphyletic. *Centropristis*, formerly considered the sister taxon to *Paralabrax*, was not closely related in these analyses. *Cratinus agassizii*, a monotypic genus from the eastern Pacific, was found to be the sister taxon to *Paralabrax*. There is greater resolution for intergeneric and subfamily relations than interspecific relationships. A single most parsimonious tree for the interspecific relationships of *Paralabrax* and allied taxa is proposed. This proposed molecular phylogeny is consistent with known biogeographic processes in the eastern Pacific.

1. Introduction

Paralabrax Girard 1856 (Serranidae: Serraninae), the rock basses, is a New World genus of mesocarnivorous fishes that are dominant components of the nearshore rocky-reef marine environment of the eastern Pacific and western Atlantic. Their dominance within this environment has made them extremely important commercially and ecologically. They have been subjected to intensive fishing pressure as all nine species are commercially harvested (e.g., Collyer and Young, 1953 and Allen et al., 1995; Love et al., 1996; Pondella et al., 2001). This accessibility has led to numerous studies of their reproductive strategies (e.g., Oda et al., 1993; Oliva et al., 1992; Smith and Young, 1966). Species of *Paralabrax* have been reported as both gonochores and protogynous hermaphrodites (Hastings, 1989; Oliva et al., 1992; Smith and Young, 1966), with at least one species, *Paralabrax maculatofasciatus*, exhibiting both gonochorism and hermaphroditism in isolated populations (Hovey and Allen, 2000). Their predatory presence in nearshore reef habitats is an important determinant in the structure and evolution of these communities (Hobson, 1994). In addition at least one representative of *Paralabrax* is found in each biogeographic province in the temperate and tropical eastern Pacific making this an appropriate genus for phylogeographic inferences into the processes of speciation among these provinces.

Paralabrax is placed within the subfamily Serraninae, one of three subfamilies in the Serranidae. There are no known synapomorphies for the Serranidae. Instead they are classified based on four symplesiomorphic characters: three opercular spines and the absence of the posterior uroneural, procurrent spur, and third preural

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radial cartilages (Gosline, 1966; Johnson, 1983; Nelson, 1994). Of the three subfamilies (Serraninae, Anthiinae, and Epinephilinae; Jordan and Eigenmann, 1890; Baldwin and Johnson, 1993; Nelson, 1994), the former is considered the basal taxon (Baldwin and Johnson, 1993; Gosline, 1966; Lauder and Liem, 1983) and *Paralabrax* is a considered a basal genus (Meisler, 1987). Thus, it may be one of the ancestral serranids.

Examining the fossil record reveals that the oldest known representative serranid (*sensu lato*) is *Eoserranus hislopi* Woodward (1908) from the Lameta Formation in Dongargaon, Madhya Pradesh, India (Jain, 1986), and dates to the Maastrichtian (66.4–74.5 mya) according to the presence of the Deccan Traps produced by the volcanism of the Cretaceous/Tertiary boundary (Dogra et al., 1994). The fossiliferous site at Dongargaon is characterized by continental facies (Jain, 1986). Strong evidence exists for serranid like fossils in the Danian (66.4–63.6 mya) with diversification by the late Paleocene (Patterson, 1993). In light of the fossil record and the current placement of *Paralabrax* within the Serranidae, this suggests the possibility of describing the phylogenetic relationships of a group that may have started evolving as early as at the beginning of the Cenozoic.

The phylogenetic challenges continue because the Serraninae is a complex assemblage of at least 13 genera (Nelson, 1994) that lacks a resolved phylogeny. As such, a phylogenetic treatment of *Paralabrax* poses many difficulties. *Paralabrax* appears to be monophyletic, however that has yet to be demonstrated. The sister taxon is believed to be the genus *Centropristis* based upon four characters: the width of parietal crests, intermediate body size, temperate affinities, and reproductive status (Smith and Young, 1966). Unfortunately only four species were examined in that phenetic study. Gonochorism, the character used to differentiate *Paralabrax* from *Centropristis* is now known to be variable. Originally *Paralabrax* was considered to be solely gonochoristic and subsequently they have been referred to as 'secondary gonochores' based upon the belief that they evolved from a protogynous ancestor basal to the Serraninae. *Centropristis*, another presumed basal serranine, is protogynous and reproduction in a gonochoristic *Paralabrax* would then be a reversal from the gonochoristic Percichthyidae that may be ancestral to the Serranidae (Gosline, 1966; Smith and Young, 1966; Smith, 1967). Gosline (1966) referred to the Serranidae as the 'wastebasket of lower percoids' due to their dubious monophyly and problematic taxonomy as he removed a polyphyletic Percichthyidae from them (Johnson, 1983). He used three species of *Paralabrax* in this survey, thus placing them at the heart of a larger problem, the monophyly of the serranids. Unfortunately various revisions of other lower percoid families including the Percichthyidae have not resolved the interfamilial relationships (Johnson, 1984, 1993).

The goal of this study is to utilize partial mitochondrial DNA sequences of the 16S and 12S ribosomal genes (994 bp) to reconstruct the phylogeny of *Paralabrax*. These genes are useful for phylogenetic questions since their secondary structures of loop and stem regions allows both conservative and relatively variable regions of the mitochondrial genome to be analyzed with a potential to resolve both deep and shallow clades. These genes have also been successfully used in reconstructing phylogenies for related marine fishes, the Centropomidae (snooks) and Epinephilnae (groupers) (Craig et al., 2001; Tringali et al., 1999).

2. Materials and methods

2.1. Material examined

Specimens were collected in the field by hook and line, spear, quinaldine or purchased from commercial fish markets. Gill filaments or pectoral fin clips were used. Two individuals from each species were sequenced with the exception of *Pristigenys serrula* and *Serranus tabacarius*, for which only one specimen was available. Fishes were placed on ice in the field and tissues were preserved in 5× Net solution (Craig et al., 2001). Representative species of the Serranininae, Epinephilinae and Anthiinae were analyzed as outgroups including two epinephelines, *Epinephelus adscenscionis* and*Rypticus saponaceus* and the anthiine *Pronotogrammus multifasciatus*. To root the basal Serraninae, other percomorphs that are potentially close to the Serranidae were used in the analysis. These included a polyprionid, *Stereolepis gigas*, and a priacanthid, *Pristigenys serrula*.

Smith and Young (1966) felt that the two most closely related genera to *Paralabrax* are *Serranus* and *Centropristis*, citing Robins and Starck's (1961) redefinition of the tropical genus *Serranus* in the Atlantic. Their analysis is based upon 17 morphological characters (many of which are shared with *Paralabrax*), along with the temperate affinities and skull similarities between *Paralabrax* and *Centropristis*. Thus, three species of *Serranus* (*S. baldwini*, *S. tabacarius*, and *S. tigrinus*) from the Atlantic and *Centropristis striata* are used in the analysis. All nine species of *Paralabrax*, and four other Serraninae are used: *Cratinus agassizii*, *Hypoplectrus nigricans*, *H. gemma*, and *H. unicolor*. *Hypoplectrus* is thought to be relatively close to *Paralabrax* (Meisler, 1987) and *C. agassizii*, a monotypic genus, has been included because it morphologically appears to be very similar to *Paralabrax nebulifer*, and it occurs in the eastern Pacific. The specimens used and their taxonomy are listed in Table 1.

Table 1
GenBank accession numbers (12S and 16S) and museum voucher numbers are listed for all species used in this study organized by current classification

Taxon	Museum voucher	Accession Nos.	
		12S	16S
Family Serranidae			
Subfamily Serraninae			
Centropristis striata	UCLA W97-22	AY072656	AY072667
Cratinus agassizii	LACM 47328-1	AY072647	AY072668
Hypoplectrus gemma	SIO 01-126	AY072648	AY072678
H. nigricans	SIO 01-128	AY072657	AY072686
H. unicolor	SIO 01-126	AY072649	AY072680
Paralabrax albomaculatus	UCLA W98-18	AY072651	AY072670
P. auroguttatus	UCLA W97-19	AY072653	AY072671
P. callaensis	UCLA W98-19	AY072665	AY072682
P. clathratus	UCLA W96-7	AY072660	AY072672
P. dewegeri	LACM 47550-1	AY072655	AY072677
P. humeralis	UCLA W98-19	AY072661	AY072673
P. loro	UCLA W99-2	AY072652	AY072674
P. maculatofasciatus	UCLA W97-21	AY072654	AY072675
P. nebulifer	UCLA W96-8	AY072662	AF297328
Serranus baldwini	SIO 01-126	AY072658	AY072681
S. tabacarius	SIO 01-128	AY072650	AY072687
S. tigrinus	SIO 01-127	AY072659	AY072688
Subfamily Anthiinae			
Pronotogrammus multifasciatus	SIO 00-139	AF472574	AF297330
Subfamily Epinephelinae			
Epinephelus adscensionis	SIO 00-145	AF472575	AF297314
Rypticus saponaceus	SIO 70-179	AF472573	AF297327
Family Polyprionidae			
Stereolepis gigas	SIO 94-28	AY072666	AY072683
Family Priacanthidae			
Pristigenys serrula	SIO 94-142	AY072664	AY072685

All taxa are in the suborder Percoidei (Order Perciformes). Institutional abbreviations follow Leviton et al. (1985).

2.2. DNA amplification and sequencing

Extraction, amplification, and sequencing followed the protocols in Craig et al. (2001) with the addition of the 12s rRNA gene. Mitochondrial DNA was isolated from approximately 0.5 g of tissue (gill filaments or fin clips) using the Genomic-Prep Cells and Tissues DNA Isolation kit (Amersham Pharmacia Biotech). These tissues were homogenized and then digested for 45 min at 65 °C. Polymerase chain reaction (PCR) was used to amplify fragments of the mitochondrial 16s rRNA and 12s rRNA genes. One hundred microliter amplification reactions were prepared with 10–100 ng of DNA, 1.5 mM $MgCl_2$, 2.5 U *Taq* Polymerase, 200 μM of dNTP's, and 0.1 μM for the following primers shown in Table 2 (Palumbi, 1996). Thirty cycles of the following step procedure were performed using an MJ Research PTC-100 Programmable Thermal Controller following a 2-minute denaturation at 94 °C (94 °C for 1 min 30 s, 45 °C for 2 min, and 72 °C for 1 min 30 s). PCR amplification products were purified on a 1% low melting point agarose gel stained with ethidium bromide. Desired products were identified by size using ϕX174 *Hae*III DNA ladder as a reference marker. PCR fragments were separated from the gel using a Wizard-Prep PCR Purification Kit (Promega, Madison, WI). Automated fluorescent dye-deoxy sequencing of both strands was carried out using an ABI Prism 377 sequencer using the above primers.

Table 2
The following amplification primers and sequencing were used

Primer	Sequence (5′–3′)
16sarL	CGCCTGTTTATCA AAAACAT
16sbrH	CCGGTCTGAACTCAGATCACGT
12SA	AAACTGGGATTAGATACCCCACTAT
12SB	GAGGGTGACGGGCGGTGTGT

2.3. DNA alignment

Sequences from both strands of each species were assembled into a single consensus sequence using the assembly editor option in the computer program GeneTool 1.0 (Biotools). An initial sequence alignment of the consensus sequences was completed using the program Clustal W for both genes with default settings (Thompson et al., 1994). Visual optimization using MacClade 3.07 (Maddison and Maddison, 1992) was

used to align regions corresponding to loops in the 16S rRNA secondary structure due to hyper-variability in nucleotide substitution (Meyer, 1993; Ortí and Meyer, 1997). There were large indels in the loop regions of the 16S sequence of *Centropristis striata*. These were coded as a single character change prior to analyses to reduce the weighting of these events (Craig et al., 2001). The 12S rRNA alignment was comparably straightforward necessitating only minor visual optimization.

2.4. Phylogenetic analyses

Heuristic searches using equally weighted parsimony (ACCTRAN) as the optimality criterion were performed on both the single-gene (16S and 12S) datasets as well as the combined (16S + 12S) dataset. A partition homogeneity test (ILD; Farris et al., 1995) was performed within PAUP* 4.0b10 (Swofford, unpublished) to assess statistical confidence for combining the 16S and 12S data sets. Using the neighbor-joining algorithm branch lengths were assessed in the combined analysis (Saitou and Nei, 1987). Branch support was assessed for all topologies using 1000 bootstrap replications for both the parsimony and neighbor joining trees (Felsenstein, 1985). In addition, Bremer decay indices (Bremer, 1988) were calculated using the program NONA 2.0 (Goloboff, unpublished) in conjunction with the software package WincladA 1.00.08 (Nixon, unpublished). Consistency and retention indices transition/transversion ratios as well as percent sequence divergences were calculated in PAUP. Also in PAUP random trees ($n = 1000$) were generated to examine phylogenetic signal (Hillis and Huelsenbeck, 1992).

3. Results

The partition homogeneity test supported the combination of the 16S and 12S data sets ($p = 0.073$). Although this value approaches statistical significance at the 95% confidence interval, we chose to combine the data sets as the intermediate level of random noise

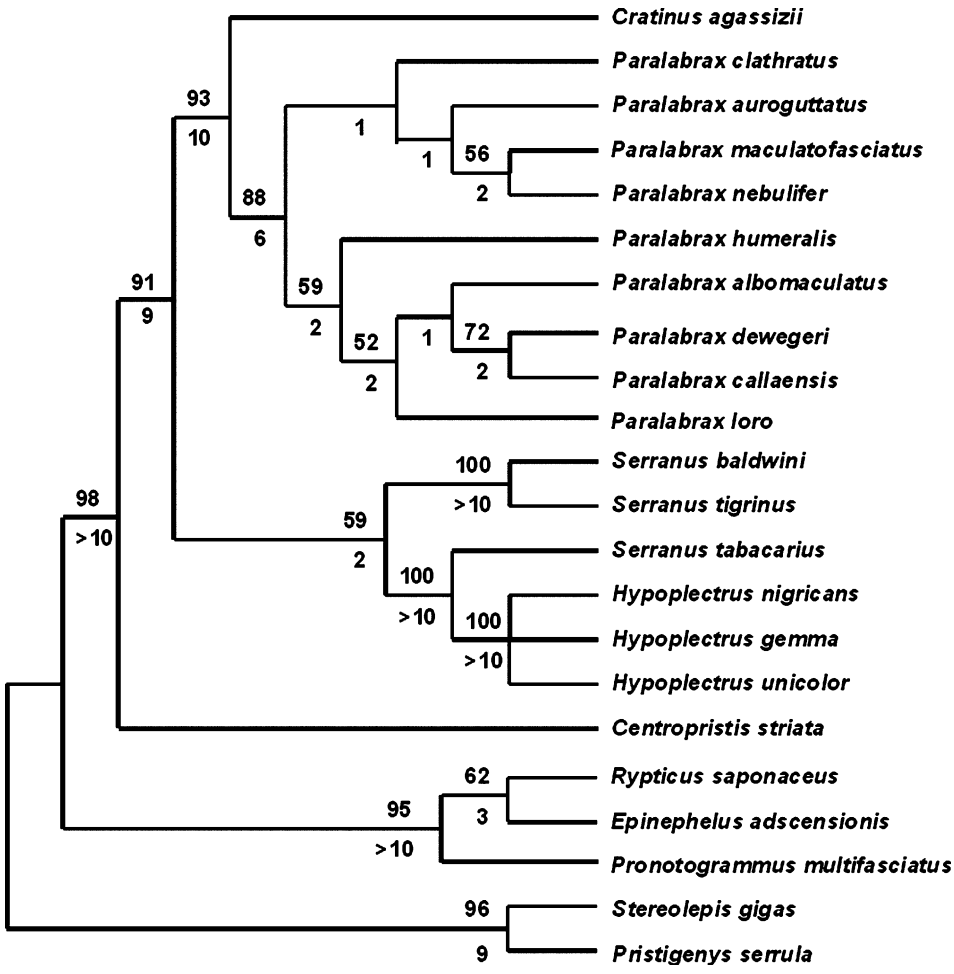

Fig. 1. A strict consensus of three most parsimonious trees having 1052 steps from the partial 12S + 6S combined mitochondrial genes and having 292 informative characters (CI = 0.54, RI = 0.62). Bootstrap support (50% majority consensus from 1000 reps) are given above each node and Bremer decay indices are given below each node.

inherent in molecular data has been demonstrated to cause artifactual significance when the ILD test is employed (Dolphin et al., 2000). Of 994 characters, 292 were parsimony informative and the distribution of random trees is skewed indicating phylogenetic signal ($g_1 = -1.65$, $p < 0.01$; Hillis and Huelsenbeck, 1992). The heuristic search produced three most parsimonious trees at 1052 steps (CI = 0.54, RI = 0.62) from which a strict consensus tree is presented (Fig. 1). The difference between the trees is found for the three species of *Hypoplectrus*. For the partial 16S sequences, the transition versus transversion ratio within *Paralabrax* is 1.4 with an average of 3.2% sequence divergence. There is an average of 6.5% sequence divergence to *Cratinus* and 14.6% to *Centropristis*. For the partial 12S sequences, there is an average of 2.15% sequence divergence within *Paralabrax* and a ratio of 2.38 transitions to transversions. There is an average of 5.98% sequence divergence to *Cratinus agassizii*. There is strong support from both the bootstrap and decay indices for the monophyly of *Cratinus* + *Paralabrax* as well as the monophyly of *Paralabrax*.

The parsimony and distance analyses reveal two major clades in *Paralabrax*. *Paralabrax humeralis* is the basal member of a group of South and Central American taxa. This clade includes *Paralabrax loro* as the sister taxon to a clade containing *Paralabrax dewegeri*, *P. callaensis*, and *P. albomaculatus*. While there is support for *P. dewegeri* and *P. callaensis* being sister taxa, there is weak branch support for the arrangement of the rest of this clade. *Paralabrax auroguttatus* is the sister group to the geminate species *P. maculatofasciatus* and *P. nebulifer*, with *P. clathratus* as the basal member of a North American clade in the parsimony analysis. In the distance analysis *P. auroguttatus* and *P. clathratus* are sister taxa (Fig. 2).

Bootstrap support for *Serranus tabacarius* as the sister taxon to *Hypoplectrus* is 100% in both analyses. The

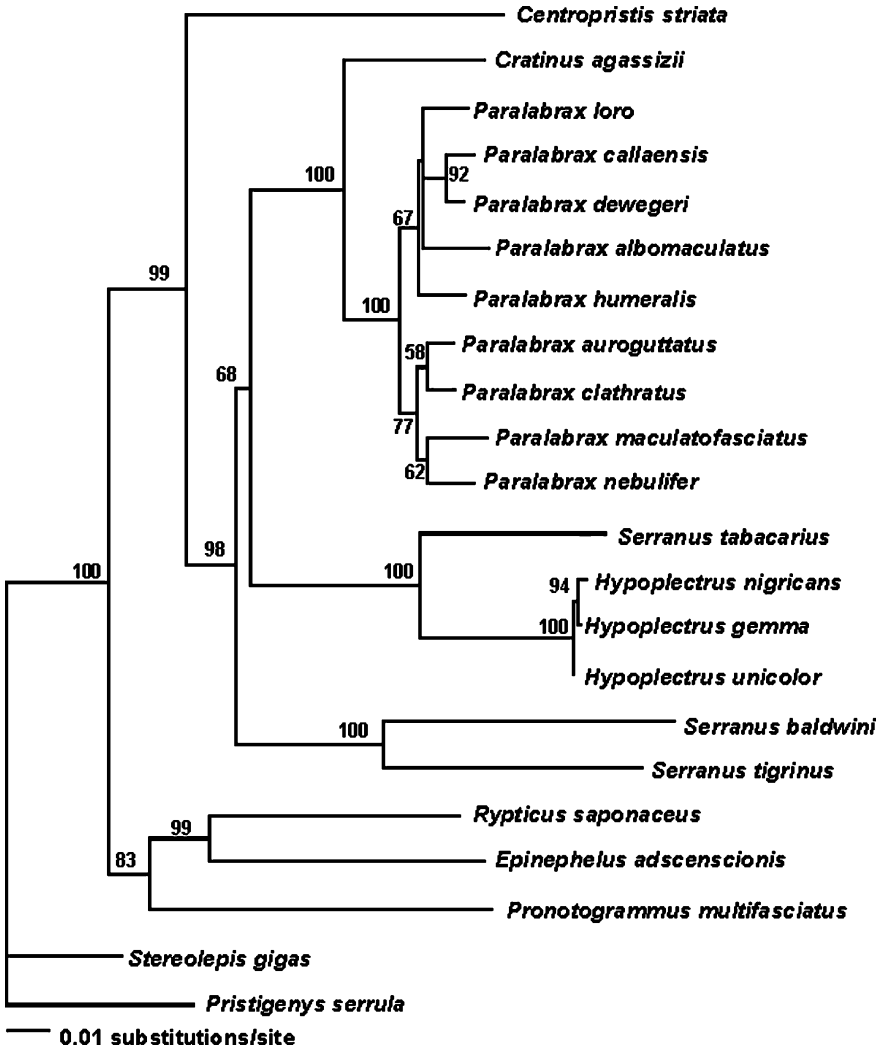

Fig. 2. A neighbor-joining reconstruction of the combined data sets. Bootstrap support (50% majority consensus from 1000 reps) are given next to each node.

clade of *S. tigrinus* and *S. baldwini* also has 100% support, and appears as the sister clade to the *S. tabacarius*/*Hypoplectrus*. *Serranus* is paraphyletic in these analyses. *Centropristis striata* is the basal serranine. The epinephelines and anthiines formed a well supported clade with the anthiines located basal to the epinephelines. The Serranidae appear monophyletic in both analyses with their root between the serranines and anthiines.

4. Discussion

The combination of the 16S and 12S sequence databases allowed the phylogenetic challenges associated with the genus *Paralabrax* to be addressed. *Centropristis*, originally placed as the sister group to *Paralabrax*, is basal within the Serraninae. The placement of *Cratinus agassizii*, a monotypic genus, is highly supported as the sister taxon of *Paralabrax*. There is appreciable evidence that the genus *Serranus* is paraphyletic, consistent with a previous morphological treatment (Meisler, 1987). The paraphyly of this genus is not a surprise; nearly every serranid was originally assigned to *Serranus* and the genus now contains an artificial assemblage of taxa. This molecular hypothesis is consistent with a previous morphological analysis of the relationship among the three subfamilies of the Serranidae (Baldwin and Johnson, 1993). In this morphological treatment, the serranines were hypothesized to be basal to a clade containing the anthiines and the epinephelines. The epinephelines were considered most derived. While there was strong support for the root of this serranid tree between the Anthiinae and the Serraninae, this hypothesis may or may not overtake the original hypothesis that serranines are the basal forms of the Serranidae. With nearly 450 serranid species (Nelson, 1994) a greater sampling effort within the family is necessary to address this hypothesis. The priacanthids and polyprionids are only two of many families that could be used to root this tree (Gosline, 1966). Considering that the origin of these families dates to at least the Cretacious/Tertiary boundary, it is reasonable to assume that there may be long branch attraction limitations at this level of the analysis (Felsenstein, 1978; Marshall, 1997). Thus, these families may not be the sister group to the Serranidae and the rooting of the tree may change with further analyses. Nonetheless, the four-symplesiomorphic characters traditionally used to unite the Serranidae as monophyletic are supported in this analysis.

These genes are well suited for addressing the relationships between genera and subfamilies of serranids and may also be useful for interfamilial relationships. However, they do not strongly describe all of the interspecific relationships of *Paralabrax*. The distance analysis illustrates why there is not strong phylogenetic support for the basal interrelationships for the two major clades of *Paralabrax*. There are very short branch lengths on these parts of the trees bracketed by relatively longer branches. This indicates rapid divergence of these genes and speciation events that are temporally close. This pattern is consistent with phylogenetic and phenetic analyses of the primarily eastern Pacific *Sebastes*, the rockfishes, which has been described as a species flock (Johns and Avise, 1998).

Hastings (2000) proposed an allopatric speciation model for rocky-reef shore fishes in the eastern Pacific based upon the Chaenopsidae (Perciformes: Blennioidei). These are primarily New World rocky-reef fishes found in the Tropical Eastern Pacific Biogeographic Region extending from California to northern Peru (Stephens, 1963; Stephens et al., 1989). In the chaenopsid model, speciation events occur allopatrically across habitat gaps. These habitat gaps are due to long stretches of coastline without rocky-reefs, notably the Sinaloan Gap from Topolobampo on the eastern shore of the Gulf of California to Mazatlan and the 'Pacific Central American Faunal Gap' from the Golfo de Tehuantepec in Southern Mexico to the Golfo de Fonseca, Nicaragua. These two habitat gaps plus the Isthmus of Panama and the pelagic gap to the oceanic eastern Pacific islands, including the Galápagos, Malpelo, and Cocos islands delineate four provinces in the tropical eastern Pacific. The tropical and subtropical eastern Pacific is delineated by thermal transitions to the temperate provinces to the north (San Diegan province) and south (Chilean province).

To ascertain whether or not the molecular phylogeny is a reasonable hypothesis, it has been superimposed upon the current range of *Paralabrax* (Fig. 3). Starting with the protogynous North American *P. maculatofasciatus* and *P. nebulifer*, the most derived members of this clade, these two species would have speciated between the Cortez and San Diegan province perhaps due to limited gene flow around the tip of Baja California. *P. maculatofasciatus* is an abundant nearshore rocky-reef fish in the Gulf of California and *P. nebulifer* is found on nearshore reefs of the Pacific coast of California and Baja California. *P. maculatofasciatus* is also found in southern California. The rocky-reef *P. maculatofasciatus* from the Gulf of California assumes a different ecological niche in southern California and the Pacific coast of Baja California, living primarily in bays, estuaries and harbors around eelgrass and riprap reefs (Fitch and Lavenberg, 1975; Allen et al., 1995). Two studies have demonstrated genetic structure among populations of *P. maculatofasciatus* around the Baja California Peninsula (Tranah and Allen, 1999; Stepien et al., 2001) further supporting this allopatric hypothesis. The next two species in this clade, *P. clathratus* and *P. auroguttatus* are also from these two separate provinces. Although their placement as sister taxa or *P. clathratus*

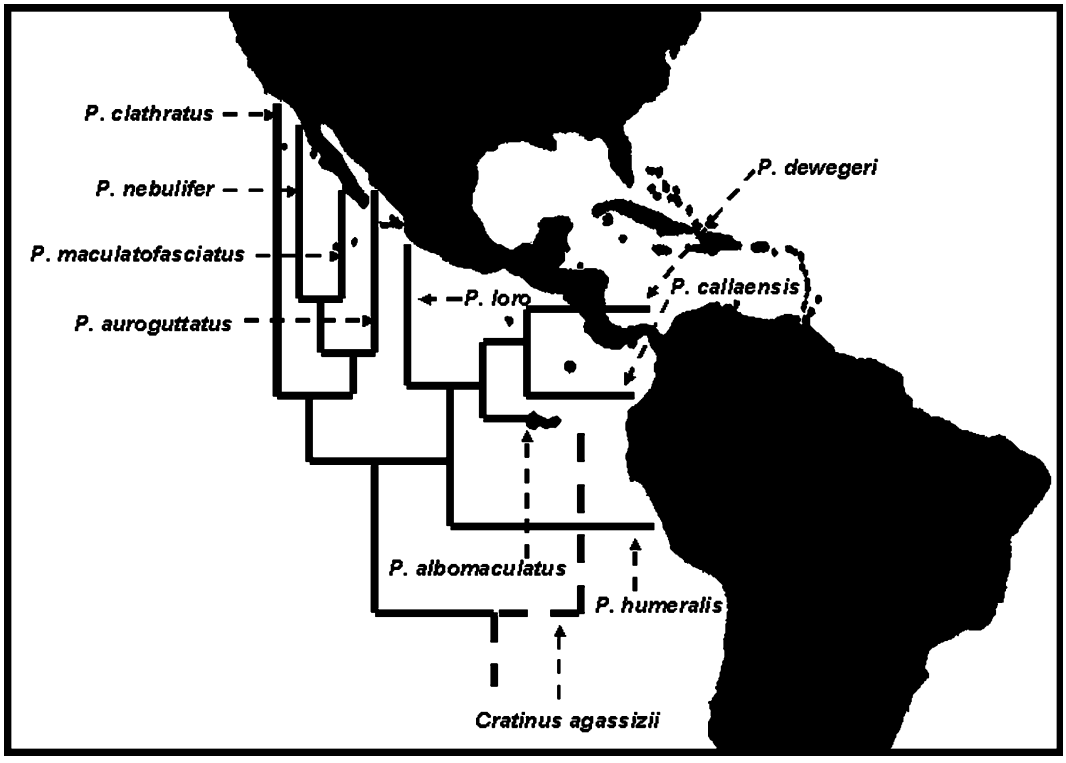

Fig. 3. *Paralabrax* phylogeny with the outgroup *Cratinus agassizii* (dashed line) superimposed on current range in the Eastern Pacific. The tips of the branches are drawn to the center of the current range of each species.

as ancestor (Figs. 1 and 2) is unclear, either scenario fits the allopatric model.

The allopatric model also fits the processes observed in the Central and South American clade. Speciation between *P. callaensis* and *P. dewegeri* is explained readily by the rise in the Isthmus of Panama with their common ancestor rising from a speciation event with its sister that became the Galápagos endemic *P. albomaculatus*. This event would have had to happen prior to the rise of the Isthmus of Panama, not an unrealistic assumption since the rise of the volcanic Galápagos Islands dates to the Pliocene (Chrisite et al., 1992; Sinton et al., 1996). Sister to this clade is a speciation event across the Central American Gap to form *P. loro*. Finally the basal member *P. humeralis* is sister to this clade across the temperate subtropical boundary in the Southern Hemisphere. The fact that all of these events have taken place in the eastern Pacific is confirmed in a cladistic fashion by the presence of *Cratinus agassizii*, the sister taxon off the coast of the Galápagos, Peru and Northern Chile. The basal members of *Paralabrax*, *P. clathratus*, and *P. humeralis*, are the most temperate representatives of this group. This temperate preference is similar to the affinities of *C. agassizii*. The presented phylogeny of *Paralabrax* is consistent with known biogeographical processes in the eastern Pacific and these analyses provide an appropriate template for future research.

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