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PHYLOGEOGRAPHY OF THE SPOTTED SAND BASS, *PARALABRAX MACULATOFASCIATUS*: DIVERGENCE OF GULF OF CALIFORNIA AND PACIFIC COAST POPULATIONS

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Abstract.—Have the warm tropical waters and currents of the southern Gulf of California, Mexico (also known as the Sea of Cortez), formed a barrier to gene flow, resulting in disjunct populations in the upper gulf that are isolated from the outer Pacific Coast? Phylogeographic and genetic divergences of the spotted sand bass, *Paralabrax maculatofasciatus,* from three Gulf of California and two outer Pacific coastal locations were tested using mitochondrial DNA (mtDNA) control region sequences. Sequence data from two congeners that are sympatrically distributed along the outer Pacific Coast, the barred sand bass, *P. nebulifer,* and the kelp bass, *P. clathratus,* were used to gauge the levels of genetic divergences. Differences among the three species and between the northern gulf and outer Pacific coastal populations of *P. maculatofasciatus* also were analyzed using 40 allozymic presumptive gene loci. Allozyme and mtDNA analyses each revealed many fixed differences among the species. Three significant allozymic frequency differences and two fixed mtDNA substitutions differentiated the gulf and outer Pacific coastal populations of *P. maculatofasciatus.* Three unique mtDNA haplotypes and three unique allozyme alleles were identified from the outer Pacific coastal population. The gulf sites contained four unique mtDNA haplotypes and six unique allozyme alleles. Partitioning of the mtDNA variation revealed that 72% of the variance occurred between the gulf and outer Pacific Coast, 20% between sampling sites in the two regions, and 8% within the sites. There appears to be little gene flow across the waters of the southern Baja Penninsula, producing divergence estimated as 120,000 to 600,000 years between the outer Pacific coastal and the Gulf of California populations. This separation level may date to a hypothesized seaway closure near La Paz, Mexico, during the mid-Pleistocene, and characterizes other fish populations. A second pattern of deeper allopatric species-level divergences in some other fishes may date to a Pliocene closure of a mid–Baja Penninsular seaway. Significant differences also were discerned in *P. maculatofasciatus* between the San Diego and central Baja California coastal sites and between the upper/central and the lower gulf locations. Variation between locations in the two regions may be indicative of larval retention and low adult migration, which needs to be tested further.

Key words.—Genetic divergence, Gulf of California, mitochondrial DNA control region, molecular clock, *Paralabrax maculatofasciatus,* Sea of Cortez, Serranidae.

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Relatively few barriers to gene flow are known for nearshore marine organisms with pelagic early life-history stages (Palumbi 1999). Genetic testing of how current and temperature barriers may affect their distribution patterns, however, has awaited the development of high-resolution genetic markers. As these markers and the methodology for analyzing them have developed, so too has the realization that widespread marine populations formerly believed to be genetically homogeneous often retain genetic mutations that reflect historic and present-day barriers to migration and gene flow (e.g., Bowen and Grant 1997; Palumbi 1999; Stepien 1999; Stepien et al. 2000; Muss et al. 2001).

The warm temperate fauna of the northern Gulf of California, Mexico (also called the Sea of Cortez), appears largely disjunct from the temperate fauna of the outer Pacific Coast, due to their historic separation by the tropical waters in the lower gulf (Briggs 1974). The Gulf of California was formed when the Baja California Peninsula separated from the mainland of Mexico over a period of 3.5 to 12 million years ago along the San Andreas Fault system, opening a new sea (Holt et al. 2000). The upper gulf is older than the lower portion and was formed during the late Miocene (Larson et al. 1968). The present-day southern portion of the Baja California Peninsula formerly was a series of islands, which were uplifted and fused during the separation of the continental plates (Durham and Allison 1960; Holt et al. 2000). Geological evidence indicates that an extensive seaway connected the Gulf of California to the Pacific Ocean dating 3 million to 7 million years ago in the Santa Rosalia region of the present-day central gulf (Fig. 1; Holt et al. 2000). Today, this region along the outer Pacific Coast represents the biogeographic transitional zone between the northerly warm temperate and the southerly tropical provinces (Briggs 1974). Seafloor spreading in the present-day mouth of the gulf occurred about 3.5 million years ago, extending the gulf southward into tropical waters along the East Pacific Rise of the Tamayo Transform Fault (Lonsdale 1989). By this time, the seaway in the Santa Rosalia region had closed. Taxa in the warm temperate waters of the northern gulf presumably became increasingly isolated from those along the outer Pacific Coast.

The present biogeographic conditions characterizing the Gulf of California are believed to have persisted since the

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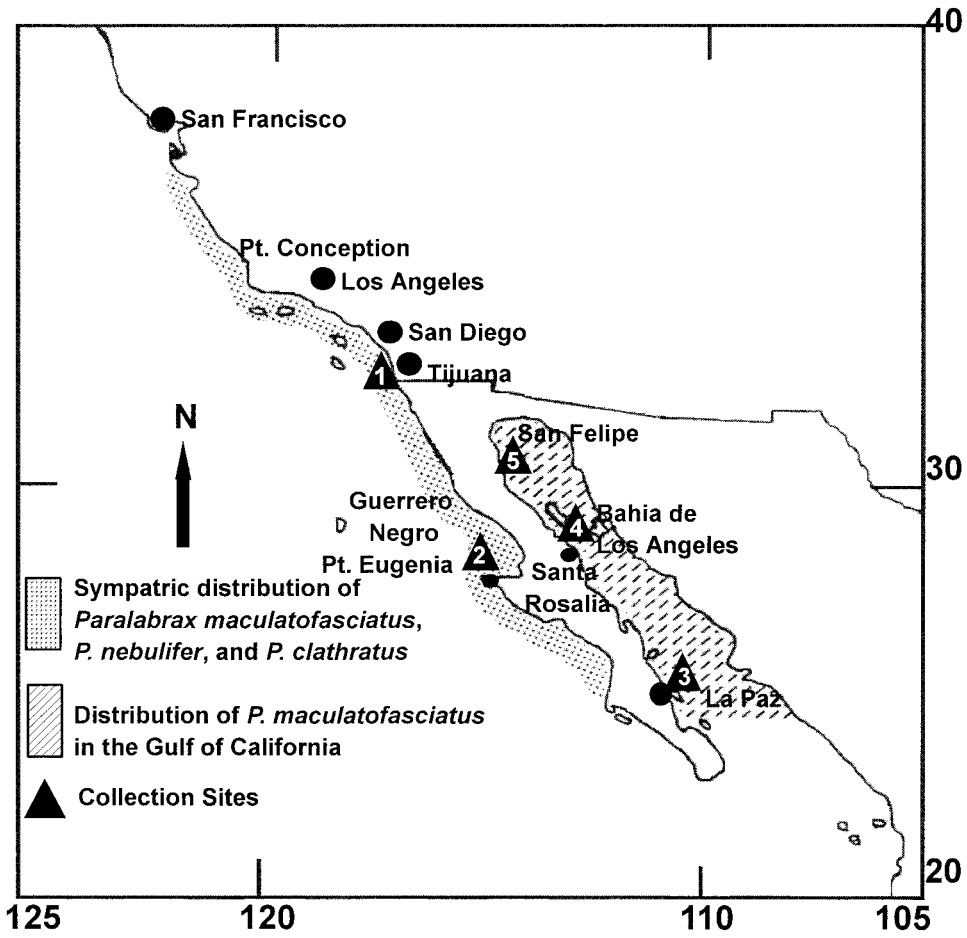

FIG. 1. *Paralabrax* collection sites in the Gulf of California and along the outer Pacific Coast. (1) San Diego, California (outer coast); (2) Guerrero Negro, Baja California, Mexico (outer coast); (3) La Paz, Baja California, Mexico (Gulf of California); (4) Bahia de Los Angeles, Baja California, Mexico (Gulf of California); (5) San Felipe, Baja California, Mexico (Gulf of California).

end of the Pleistocene, about 10,000 years ago (Durham and Allison 1960). During the Pleistocene's duration of about 3 million years, the northerly cold temperate fauna expanded southward and gene flow probably increased across the southern waters. Geological evidence suggests that an oceanic connection in the region of the La Paz Plain (Fig. 1) existed until the mid-Pleistocene, providing another avenue for dispersal (Beal 1948; Lonsdale 1989; Upton and Murphy 1997). The closure of this seaway, along with postglacial warming of the lower gulf (Moore et al. 1980), would have restricted the temperate species to the cooler northerly waters along the outer Pacific Coast and in the gulf (Hubbs 1960; Brusca 1980).

Today, the Gulf of California stretches north through 10° of latitude, deeply invading the continent (Fig. 1). Its salinity (36–37 ppt) is higher than that of the outer Pacific Coast (32–36 ppt) due to evaporation (Brusca 1980). Seasonal surface water temperatures range from 10°C to 32°C and differ markedly between the tropical conditions in the lower gulf and the warm temperate upper gulf. Deep basins characterize the gulf's lower and central regions, extending to more than 3000 m (Walker 1960). The upper Gulf of California is flat and shallow (averaging 200 m) and is regarded as the most isolated large body of water on the northeastern Pacific Coast (Dawson 1960). Extreme seasonal temperature variations, great tidal amplitudes, and a diversity of substrates and habitats characterize the upper gulf. It possesses such a remarkably distinctive biota (Dawson 1960) that it has been classified as a unique biogeographical province (Briggs 1974).

About half of the fish species in the northern warm temperate waters of the Gulf of California are disjunct and are most closely related to sister groups in the San Diego warm temperate province. The generalized distribution of the warm temperate fauna extends along the outer Pacific Coast from Point Conception through central Baja California (Briggs 1974; Fig. 1). Present (1987) listed 42 species or geminate species pairs of fishes that exhibit this disjunct distribution between the upper gulf and outer Pacific Coast (Walker 1960). Some of the disjunct species of the gulf differ morphologically from their counterparts on the outer coast, and several of these are recognized as separate species. Many invertebrates also display this pattern of separation, including arthropods (Garth 1960; Brusca 1980; Correa-Sandoval and Rodriguez-Cortes 1998), bryozoans (Soule 1963), molluscs (Keen 1971; Houston 1980), and polychaetes (Bhaud and Fernandez-Alamo 2000).

Although populations in the upper Gulf of California and the outer Pacific Coast share an apparent common ancestry,

the extent to which the lower gulf acts as a barrier to gene flow—resulting in population genetic divergence—is largely unknown. If the thermal barrier caused a vicariant event due to recent postglacial warming (Moore et al. 1980), then one would expect similar levels of relatively recent genetic divergence to characterize the disjunct pairs of species and populations (if their thermal ranges are the same). If the tropical barrier has been periodically traversed during the last 10,000 years (e.g., by submergence to deeper, cooler waters and/or due to periodic favorable current patterns), then one would expect a variety of degrees of relatively recent genetic separation for different disjunct pairs or no discernable separations due to ongoing gene flow. Alternatively, the divergences may predate the latest ice ages, and at least some of the fauna may have differentiated during the formation of the gulf and due to the closures of proposed seaways.

The present study examined systematic relationships and divergences among three sympatric sea basses (Teleostei: Serranidae) from the nearshore warm temperate eastern Pacific Coast, including: the kelp bass (*Paralabrax clathratus*), the barred sand bass (*P. nebulifer*), and the spotted sand bass (*P. maculatofasciatus*), using mitochondrial DNA (mtDNA) sequences from the highly variable left domain of the control region (see Stepien and Kocher 1997) and 40 presumptive allozyme loci. A study by Graves et al. (1990) examined whole mtDNA restriction fragment length polymorphisms in the three species and resolved interspecific characters, but found little intraspecific variation. The species relationships and divergences were examined to gauge the levels of intraspecific differences characterizing populations of *P. maculatofasciatus.*

This investigation tested phylogeographic and population genetic divergences of *P. maculatofasciatus* that, unlike the other two species, is found in the gulf. Samples were compared from two outer Pacific Coast and three Gulf of California population sites located in the upper, central, and lower temperate regions to evaluate whether, and to what degree, there are barriers to gene flow. Separation of the warm temperate gulf from the outer coastal populations would result in gradual accumulation of selectively neutral mutations, assuming that no appreciable gene flow or migration has occurred between them.

Materials and Methods

The left domain (5′ end) from tRNA proline to the central conserved section of the mtDNA control region was amplified and sequenced using the polymerase chain reaction (PCR) and Sanger dideoxy sequencing (following Stepien 1995) with conserved primers (given in Kocher et al. 1989; Meyer et al. 1990). Specimens of *P. maculatofasciatus* were analyzed from two sites along the outer Pacific Coast at San Diego, California (site 1, Fig. 1; $N = 17$), and Laguna Guerrero Negro, central Baja California, Mexico (site 2; $N = 10$). Three sites were sampled in the Gulf of California, including La Paz, Mexico, in the southern reaches of the warm temperate waters (site 3; $N = 10$); Bahia de Los Angeles, Mexico, in the central gulf (site 4; $N = 10$); and San Felipe, Baja California, Mexico, in the north (site 5; $N = 16$). The mtDNA control regions of two closely related species, the kelp bass, *P. clathratus* (site 1, San Diego; $N = 2$), and the barred sand bass, *P. nebulifer* (site 1; $N = 4$), were sequenced for comparison. In addition, the same individuals of *P. maculatofasciatus* from the furthest separated sites, at San Diego (plus three additional specimens, totaling $N = 20$) and San Felipe ($N = 16$), and the samples of *P. nebulifer,* were analyzed for allozyme electrophoretic variability at 40 presumptive gene loci. Specimens from the other sites were sequenced only, because a small amount of gill filament tissue was available (precluding allozyme analysis).

Procedures followed for allozyme electrophoresis are described in Stepien and Rosenblatt (1996). The 40 presumptive gene loci scored were *sAat-A2, sAat-B1, Acp-A2, Acp-B1, Adh-A1, Adh-B2, sAh-1, Ak, Ck-A3, Ck-B1, Ck-C2, Est-1, Est-2, Fh-A1, Gapdh-1, Gapdh-2, Gludh, G3pdh-A2, G3pdh-B1, G6pdh-1, G6pdh-2, Gpi-A1, Gpi-B2, Iddh-A, sIdhp, mIdhp, Ldh-A3, Ldh-B3, Ldh-C1, sMdh-A2, sMdh-B1, sMdh-C3, Mpi-1, Pep-A1, Pep-B2, Pep-3, Pgdh-1, Pgm-1, sSod-A,* and *Xdh-A.* Allozyme data were analyzed using BIOSYS-2 (Swofford 1990; Swofford and Selander 1990).

Sequence differences among individual haplotypes and their geographic areas were analyzed in four ways: (1) clustering of pairwise (p-) and Tajima-Nei (1984) genetic distances (the latter were used to correct for differences in nucleotide composition; see Kumar et al. (1993) with the neighbor-joining algorithm (Saitou and Nei 1987) in MEGA (Kumar et al. 1993); (2) comparing nucleotide diversity and divergence levels within and among populations using REAP 4.0 (McElroy et al. 1992) and AMOVA (ver. 1.55, Excoffier et al. 1992; Excoffier 1995); (3) testing for selective neutrality using Tajima's (1989) and Chakraborty's (1990) tests in Arlequin (ver. 2.0, Schneider et al. 2000); and (4) calculating parsimonious relationships among haplotypes with PAUP (Swofford 2001). Sequences were compared with those for the related species *P. nebulifer* and *P. clathratus,* and the latter was used as the outgroup to root the trees. Bootstrap tests with 1000 replications calculated support for individual nodes of the neighbor-joining (MEGA, Kumar et al. 1993) and maximum-parsimony trees (PAUP, Swofford 2001).

A phylogenetic network was constructed to summarize the relationships among the mtDNA haplotypes and their geographic concordance (see Avise 2000). The distribution of haplotypes also was tested between pairs of sampling sites using modified chi-square contingency tests and Bonferroni corrections for multiple post hoc tests (Sokal and Rohlf 1995). The relationship of ϕ_{ST}-values (the F_{ST} analog from the AMOVA analyses; Excoffier 1995) and the geographical distances between pairs of sampling sites was tested with correlation and least-squares regression analyses (Sokal and Rohlf 1995) using Excel. This analysis also was done for pairwise genetic distances among pairs of haplotypes versus the geographic distances between sampling sites (with the closest locations used for cases of haplotypes that were shared among sampling locations).

Results

Sequence data were aligned manually among the three species and deposited in GenBank (accession nos. AF314926–AF314928) for 384 bp of the left domain of the mtDNA

TABLE 1. Mean (±SE) nucleotide diversity (above diagonal) and divergence (below diagonal) between pairs of sampling sites, calculated using REAP 2.0 (McElroy et al. 1992).

Sites	San Diego	Guerrero Negro	La Paz	Bahia de Los Angeles	San Felipe
San Diego	—	0.0040 ± 0.0030	0.0086 ± 0.0040	0.0119 ± 0.0040	0.0123 ± 0.0040
Guerrero Negro	0.0036 ± 0.0030	—	0.0123 ± 0.0040	0.0179 ± 0.0050	0.0183 ± 0.0050
La Paz	0.0069 ± 0.0040	0.0110 ± 0.0040	—	0.0058 ± 0.0030	0.0064 ± 0.0030
Bahia de Los Angeles	0.0115 ± 0.0040	0.0179 ± 0.0050	0.0045 ± 0.0030	—	0.0011 ± 0.0030
San Felipe	0.0108 ± 0.0040	0.0172 ± 0.0050	0.0040 ± 0.0030	0.0036 ± 0.0030	—

control region. *Paralabrax nebulifer* differed from *P. maculatofasciatus* by 32 fixed base substitutions and a single nucleotide insertion. *Paralabrax clathratus* differed from *P. maculatofasciatus* by 60 fixed nucleotides, including an insertion of a single nucleotide and two deletions of single bases. *Paralabrax clathratus* diverged from *P. nebulifer* by 50 fixed differences and two single nucleotide deletions. The two *P. clathratus* individuals also differed from each other by four transitional substitutions.

Nucleotide diversity levels for *P. maculatofasciatus* generally were lower within (0.000 to 0.003) than among (0.001 to 0.018) the population sites (Tables 1, 3). Haplotypic diversity within the sites was low, with sampling locations having one to three haplotypes (Tables 2, 3). Only one haplotype (G) was shared among sites (in all three Gulf of California samples). Differences in nucleotide diversity and divergence levels were greatest between the outer coast and the gulf populations (Table 1). Tajima's (1989) and Chakraborty's (1990) tests of selective neutrality revealed no significant deviations from random predictions ($P = 0.21$ and $P = 0.27$, respectively).

Mean allozymic heterozygosities were comparable for *P. maculatofasciatus* from the two populations: 0.085 ± 0.018 from the San Diego site and 0.090 ± 0.011 for San Felipe in the upper gulf. Total polymorphism was 42.50% for each site. There were 17 polymorphic allozyme loci, for which six alleles appeared restricted to the Gulf of California sampling site and three to the outer Pacific Coast. Unique allozyme alleles were identified in the Gulf of California population at the following loci: *sAat*-B1 (which migrated 190%, compared to the migration of the most common allele at 100%), *Acp-B1* (105%), *Fh-A1* (110%), *Gludh* (80%), and *Iddh-A* (–200%). Alleles exclusively found in the outer Pacific coastal population included *sAat-A2* (130%), *Adh-A2* (50%), and *Acp-B1* (110%).

Three additional allozyme loci had significantly different allelic frequencies for the Gulf of California versus the outer Pacific Coast samples, including *Acp-A2* ($\chi^2 = 4.28$, $P < 0.04$), *Adh-B2* ($\chi^2 = 4.29$, $P < 0.04$), and *Iddh-A* ($\chi^2 = 8.34$, $P < 0.02$). Both populations corresponded to Hardy-Weinberg equilibrium expectations for all loci sampled. The mean Wright's (1978) F_{ST}-value averaged across all polymorphic allozyme loci was 0.021, supporting genetic divergence between the two regions (Hartl 1988). The Nei's (1972) genetic distance *D* separating the gulf and coastal populations was 0.0040 ± 0.0001. The Nei's *D* separating the congeners *P. maculatofasciatus* and *P. nebulifer* was 0.186 ± 0.005.

The outer coastal haplotypes diverged from those in the Gulf of California by two fixed nucleotide substitutions (Table 2), forming a dumbbell pattern in the phylogenetic network shown in Figure 2. Greater genetic divergence between the coastal and gulf sites was revealed with left domain mtDNA control region sequences than with the allozyme data, as shown in the neighbor joining tree (Fig. 3). An exhaustive maximum-parsimony search using PAUP (Swofford 2001) yielded a g-1 skewness of −2.57, indicating significant skew (Swofford et al. 1996). Five maximum-parsimony trees were obtained with lengths of 88 steps and consistency indices excluding uninformative characters of 0.97. A 50% majority rule consensus of the most parsimonious trees also revealed separation of the Gulf of California from the outer Pacific coastal haplotypes (Fig. 4). Haplotypes F and G from the upper and central Gulf of California were sister groups, forming the sister taxon to the remaining gulf haplotypes (Figs. 3, 4).

Seven haplotypes (A–G; Table 2) were obtained for *P. maculatofasciatus*, which differed at nine nucleotide positions. Haplotypes A–C characterized individuals from the outer coast (Table 3), which shared guanine nucleotides at mtDNA control region positions 139 and 189 (Table 2). No

TABLE 2. Sequences of spotted sand bass, *Paralabrax maculatofasciatus*, mitochondrial DNA haplotypes. Nucleotide positions refer to GenBank accession nos. AF314926–AF314928.

Haplotype	Nucleotide position									
	139	189	226	250	259	267	289	308	325	*N*
A	G	G	T	T	C	T	A	T	A	16
B	G	G	T	T	T	T	A	T	A	1
C	G	G	T	C	C	T	A	T	A	10
D	A	A	C	T	C	T	A	A	A	1
E	A	A	T	T	C	T	A	T	A	8
F	A	A	T	T	C	C	G	T	T	1
G	A	A	T	T	C	C	G	T	A	26
Hypothesized ancestral state[1]	C/T	A	T	T	T	T	A	T	A	—

[1] Ancestral state hypothesized due to characters shared by congeners *P. nebulifer* and *P. clathratus.*

TABLE 3. Distribution of spotted sand bass, *Paralabrax maculatofasciatus,* mitochondrial DNA haplotypes among collection sites and haplotype and nucleotide diversities in collection locations.

Haplotype	San Diego	Guerrero Negro	La Paz	Bahia de Los Angeles	San Felipe
A	16	0	0	0	0
B	1	0	0	0	0
C	0	10	0	0	0
D	0	0	0	0	1
E	0	0	8	0	0
F	0	0	0	0	1
G	0	0	2	10	14
Total *N*	17	10	10	10	16
Haplotype diversity ± SE	0.24 ± 0.14	0.00	0.36 ± 0.16	0.00	0.12 ± 0.10
Nucleotide diversity ± SE	0.0022 ± 0.0032	0.0000	0.0026 ± 0.0032	0.0000	0.0007 ± 0.0013

haplotypes were shared between the two outer coastal sites (Table 3). Four haplotypes (D–G) were found in the Gulf of California, which differed from the outer coast samples by adenine substitutions at positions 139 and 189.

The sample from San Diego contained two haplotypes (A and B), and the Guerrero Negro location had only a single haplotype (C). Haplotype G was found in all three sites in the Gulf of California, comprising 88% in the upper, 100% in the central, and 20% in the lower region (Table 3). No other haplotypes were shared between sites. Haplotypes F and G were most closely related to each other, clustering together in both the neighbor-joining and maximum-parsimony trees (Figs. 3 and 4). All pairs of sampling locations had significantly different haplotype compositions, except San Felipe (upper Gulf) and Bahia de Los Angeles (central gulf; Table 4).

Analysis of molecular variance (AMOVA; Excoffier 1995) revealed significant partitioning of variance ($P < 0.001$) between the outer Pacific Coast versus the Gulf of California (72%), between the two outer coastal sites, and between the upper/central versus the lower sites in the gulf (20%; Table 5). The mean genetic distance (both pairwise and Tajima-Nei [1984]) between the outer coastal and the Gulf populations was 0.012 ± 0.005, which is equivalent to an estimated 600,000 years of separation using a 2% per million year calibration (slow clock calibration) and 120,000 years using a 10% per million year calibration (fast clock calibration; see Grant and Bowen 1998; Avise 2000). AMOVA tests also showed significant divergences between all pairs of sites, except between the central (Bahia de Los Angeles) and upper Gulf (San Felipe) locations (Table 4, above diagonal). The mean genetic distances among the Gulf of California haploytpes was 0.0074 ± 0.004 (both pairwise and Tajima-Nei distances), corresponding to an estimated 74,000 (fast clock) to 370,000 years (slow clock). Genetic distances between the outer Pacific coastal haplotypes averaged 0.0035 ± 0 0.003 (both pairwise and Tajima-Nei distances), or about 35,000 (fast clock) to 170,000 years (slow clock).

A plot of ϕ_{ST}-values among sampling locations from the AMOVA analyses versus geographic distances was not significant in a regression analysis ($F = 3.82$, $P = 0.086$; Fig. 5A). That analysis considered the overall genetic divergences among sites, and included the haplotype shared among locations in the gulf. Separate analyses that excluded single sites decreased the overall linear correspondence, as did a log-log transformation. A plot of p-distances between pairs of haplotypes versus the geographic distances separating them (Fig. 5B), which included only the nearest location (La Paz) to the outer coast for the shared haplotype, revealed a linear relationship that was significant in a regression analysis ($F = 11.92$, $P = 0.003$). The latter analysis also included pairs of haplotypes located in the same sampling area, which were thus separated by distances of 0 km. Overall, the genetic versus geographic distance analyses indicate significant correspondence to an isolation-by-distance pattern for the evolutionary origin of the haplotypes (Fig. 5B), but their distribution among the sampling sites differed significantly from an isolation-by-distance pattern (Fig. 5A).

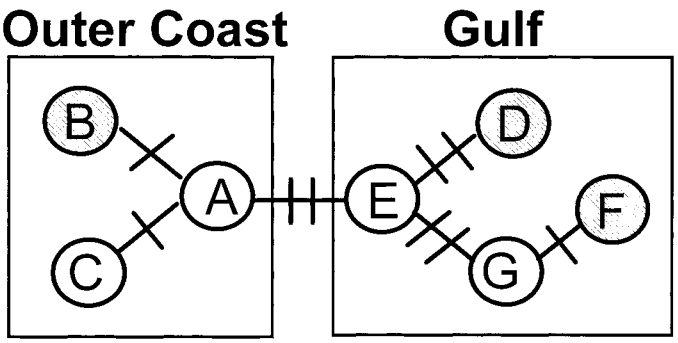

FIG. 2. Phylogenetic network of mitochondrial DNA control region haplotypes (circles A–G) of *Paralabrax maculatofasciatus,* following Avise (2000). Slashes across lines linking the haplotypes denote nucleotide substitutions. Shaded circles designate rare haplotypes.

DISCUSSION

Species Divergences and Molecular Clock Calibrations

Genetic distance molecular clock calibration estimates for mtDNA control region pairwise sequence divergences in fishes commonly have ranged from 2% (a slow clock; see Bernatchez et al. 1992; Bernatchez and Danzmann 1993; Stepien and Faber 1998) to 10% per million years (a fast clock estimate; summarized by Avise 2000), which suggests that the species *P. maculatofasciatus* and *P. nebulifer* have been differentiated for 1.05 million to 5.25 million years (corresponding to a mtDNA control region pairwise sequence divergence [p] of 0.105). This level of sequence divergence is

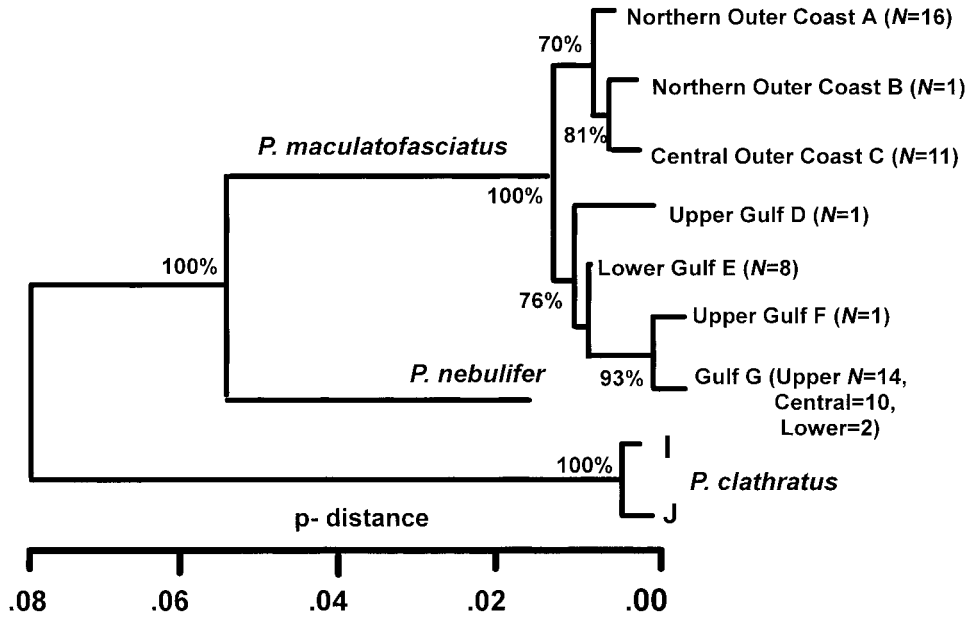

FIG. 3. Neighbor-joining tree of *Paralabrax maculatofasciatus* mitochondrial DNA control region haplotypes from pairwise (p) and Tajima-Nei (1984) genetic distances (which were the same), using MEGA (Kumar et al. 1993). Bootstrap percentage support for nodes calculated using 1000 replications are indicated.

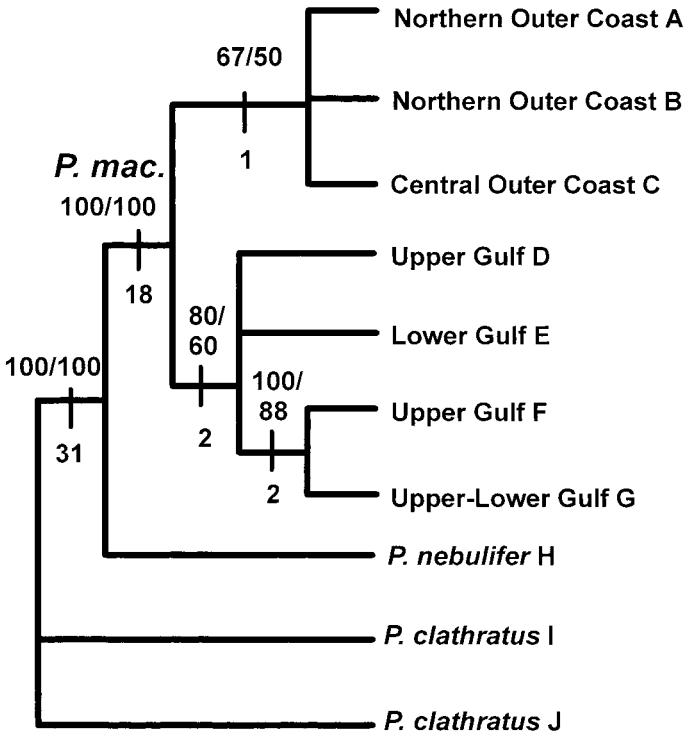

FIG. 4. Maximum-parsimony 50%-majority-rule consensus tree of *Paralabrax maculatofasciatus* mitochondrial DNA control region haplotypes, using PAUP 4.0b5 (Swofford 2001). Numbers above nodes indicate consensus percentage/bootstrap percentage support from 1000 replications. Numbers below slashes on the nodes denote the number of synapomorphies supporting that relationship.

relatively high among those separating sister species for fishes (Johns and Avise 1998; Avise 2000), indicating a Pliocene separation. *Paralabrax clathratus* may have diverged from an ancestor shared with the *P. nebulifer/ P. maculatofasciatus* clade about 1.72 million to 8.6 million years ago (based on a mean p-distance of 0.172). Whole mtDNA RFLP digestions by Graves et al. (1990) for the three species revealed a sequence divergence of 0.069 ± 0.019 between *P. maculatofasciatus* and *P. nebulifer,* and 0.142 ± 0.027 between *P. nebulifer* and *P. clathratus,* which is consistent with a more rapid evolutionary rate for the control region versus the entire mtDNA molecule in fishes (see Stepien and Kocher 1997). Very low intraspecific variation was discerned in the Graves et al. (1990) study from 11 restriction enzyme endonucleases, and from the allozyme data in this investigation, in contrast to these results from the mtDNA control region sequences.

Phylogeographic Divergence between the Pacific Coast and the Gulf of California

Results of the present investigation support historic separation of the Gulf of California and outer Pacific coastal populations of *P. maculatofasciatus.* The separation pattern among them (depicted in Fig. 2) was noted as a Type I phylogeographic pattern in a recent classification by Avise (2000), in which the lineages are defined as deeply allopatric. That example was based on a preliminary analysis of the data by Stepien (1995) using the two furthest separated sampling locations (San Diego and San Felipe). Addition of the three intermediate sites in the present study shows that the divergence pattern between the Gulf of California versus the outer Pacific Coast is moderately divergent (distinguished by two single nucleotide substitutions; see Figs. 2, 3), and thus appears intermediate between Types I and III, the latter designating a more recent allopatric separation. This pattern in-

TABLE 4. Genetic differences between pairs of collection sites. Below diagonal: χ^2 and probability values from modified χ^2 contingency test comparisons (Sokal and Rohlf 1995) of the numbers of shared versus different haplotypes among pairs of sampling sites (from Table 3), with Bonferroni corrections (Sokal and Rohlf 1995) for multiple tests (df = 2). Overall χ^2 among all sites = 176.16, df = 24, $P < 0.001$ calculated using REAP 4.0 (McElroy et al. 1992). Above diagonal: ϕ_{ST}-values among pairs of sampling sites from AMOVA (ver. 1.55; Excoffier 1995) and probability calculated from 1000 iterations, with Bonferroni corrections (Sokal and Rohlf 1995) for multiple tests.

Sites	San Diego	Guerrero Negro	La Paz	Bahia de Los Angeles	San Felipe
San Diego	—	0.93	0.86	0.98	0.91
		<0.001	<0.001	<0.001	<0.001
Guerrero Negro	27.43	—	0.90	1.00	0.92
	<0.001		<0.001	<0.001	<0.001
La Paz	27.43	20.98	—	0.78	0.61
	<0.001	<0.001		<0.001	<0.001
Bahia de Los Angeles	27.43	20.98	13.33	—	−0.32
	<0.001	<0.001	<0.01		n.s.
San Felipe	74.53	26.99	9.93	1.01	—
	<0.0001	<0.001	<0.01	n.s.	

dicates low gene flow due to lineage sorting and random drift and is characteristic of a separation dating to the Pleistocene (Avise 2000). Levels of genetic divergences of the mtDNA control region data suggest that the gulf and coastal populations may have been isolated for about 120,000 (fast clock calibration) to 600,000 years (slow clock). This divergence may date to the closure of a seaway at La Paz during the mid-Pleistocene (see Walker 1960).

The California Current and Gulf of California surface waters converge to form a persistent oceanic front in the cape region (Roden and Groves 1959; Griffiths 1968; Castro et al. 2000), which may effectively block dispersal and augment a tropical temperature barrier. Occasionally, adult *P. maculatofasiatus* have been taken as far south as Mazatlan (Miller and Lea 1972; Present 1987), but such records are relatively rare and anomalous (R. N. Lea, Dept. of California Fish and Game, pers. comm.). It would be very interesting to genetically analyze these fish. Tranah and Allen (1999) recently found that *P. maculatofasciatus* samples from the outer Pacific Coast had significantly smaller bodies and fins than those in the Gulf of California, revealing morphological divergence that parallels the genetic differentiation discerned in the present study. Ecological and tagging studies have found that *P. maculatofasciatus* are relatively sedentary, structure-oriented, and do not migrate long distances (Allen et al. 1995). These life-history factors may account for the paucity of shared haplotypes among locations in the present investigation, except between the upper and central gulf sites.

Korsmeyer (1991) proposed that *P. maculatofasciatus* in the upper and central Gulf of California have regionally evolved to withstand high temperature fluctuations, resulting in physiological differences from the outer Pacific coastal population. An investigation conducted at the central gulf sampling site examined in the present study (Bahia de Los Angeles) found that populations of *P. maculatofasciatus* remained large and constant despite radical temperature changes of 8°C (Ferry et al. 1997). Results from the present study suggest that site fidelity and larval retention (Sinclair 1988; Swearer 1999; Cowen et al. 2000) may occur, because there is considerable genetic divergence among most locations. Additional sampling of larvae and adults is necessary to address these hypotheses. Although results show that different sites are geographically isolated, the pattern of genetic divergence does not directly correspond to an isolation-by-distance relationship among locations (Fig. 5A). It is likely that current and gyre patterns, rather than geographic distance, are the primary determinants of passive dispersal at pelagic early life-history stages in nearshore marine organisms (see Palumbi 1999; Stepien 1999).

A RFLP study of the nuclear internal transcribed spacer (ITS) regions of rDNA by Tranah and Allen (1999) of *P. maculatofasciatus* also revealed significant divergence between samples from the outer Pacific Coast and Gulf of California (due to frequency differences, not fixed differences as discerned here), but no differentiation among sites in the gulf. Because the mtDNA control region is believed to evolve more quickly than the ITS regions in fishes (see Stepien et al. 1993, 1997), the present investigation yielded more characters useful for discerning population genetic and phylogeographic differences than were found by Tranah and Allen (1999).

Present (1987) found six significantly different allelic frequency distributions in allozyme loci between disjunct populations of the blenny *Hypsoblennius jenkinsi* in the upper Gulf of California versus the outer Pacific Coast. A Nei's (1972) genetic distance (*D*) of 0.037 was determined, sug-

TABLE 5. Results of AMOVA (ver. 1.55; Excoffier 1995) tests for partitioning of variation within and among populations (outer Pacific Coast and the Gulf of California) among sampling sites within groups and within the sampling locations.

Hierarchical level	Variance	% Variance	ϕ_{ST}	*P*
Between outer coast and gulf	1.44	72.2	0.92	<0.001
Among sampling sites within groups (overall)	0.40	20.1	0.73	<0.001
Within sampling sites (overall)	0.17	7.7	0.72	<0.001
Between outer coast sites	0.49	92.9	0.93	<0.001
Among gulf sites	0.35	59.1	0.59	<0.001

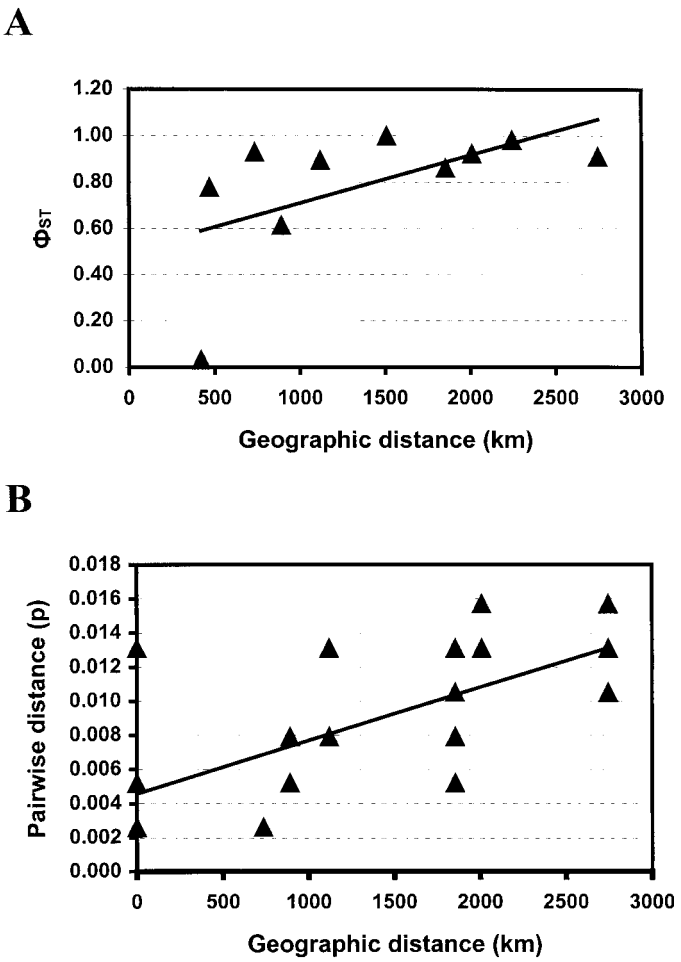

FIG. 5. Pairwise comparisons of genetic divergences versus geographic distance. (A) Plot of genetic distance values between collection sites, measured as ϕ_{ST} from AMOVA (Excoffier 1995) versus their geographical distances (km); $R = 0.569$, $R^2 = 0.324$; multiple regression analysis of y on x: $F = 3.82$, $P = 0.086$). A log-log transformation was markedly less significant, as were separate analyses omitting single sites. (*B*) Plot of pairwise genetic distances between haplotypes versus geographical distances (km) between sampling sites (closest locations were used for haplotypes occurring in more than one site; $R = 0.631$, $R^2 = 0.398$; multiple regression analysis of y on x: $F = 11.91$, $P < 0.003$).

gesting a possible genetic divergence of 700,000 years (using the calibration of Grant 1987). The divergence level for *H. jenkinsi* appears somewhat higher than the differentiation for *P. maculatofasciatus* determined in the present study, but similarly is intermediate between a Type I and III divergence pattern, having no fixed differences and dating to the Pleistocene epoch (Avise 2000).

Genetic distances separating some of the disjunct fish taxa suggest a second, and apparently much older, divergence pattern. These differences are species-level separations and fit a Type I deep divergence pattern (Avise 2000). For example, Crabtree (1983) described fixed allozymic differences between the atherinid disjunct species pair *Leuresthes tenuis* and *L. sardina* at six presumptive allozyme loci and a Nei's *D* of 0.283 ± 0.100 (equivalent to an estimated divergence of 5.3 million years). Rocha-Oliveres et al. (1999) discerned species-level divergences between outer Pacific coastal and Gulf of California taxa in the rockfish genus *Sebastomus*, including mean pairwise distances of 5% for the left domain of the mtDNA control region and 1.6% for the mtDNA cytochrome *b* gene. This level of divergence is significantly greater than that discerned for *P. maculatofasciatus* in our study, corresponding to an estimated 500,000 (fast clock calibration) to 2.5 million years (slow clock). A study of mtDNA control region sequences by Terry et al. (2000) similarly found a large difference between the sister species *Girella nigricans* from the outer Pacific Coast and *G. simplicidens* in the Gulf of California ($F_{St} = 0.84$, Nm = 0.10), accounting for 31.9% of the total genetic variance (somewhat less than the 71.8% shown in Table 5 for *P. maculatofasciatus*). However, the average sequence divergence in the Terry et al. (2000) study between the gulf and outer coastal populations was very high (4.4%), suggesting a possible separation of 440,000 (fast clock calibration) to 2.2 million years (slow clock). This mtDNA divergence was much greater than an allozyme divergence estimate by Orton and Buth (1984), who found no fixed differences and no significant frequency differences. A discrepancy between the allozyme and mtDNA divergence levels also occurred in the present study, with the former being considerably smaller than the latter (Results). This discrepancy may be due to differences in organellar versus nuclear DNA inheritance and differences in their relative effective population sizes (see Avise 1994, 2000), which outlines the need to critically examine different molecular datasets. The level of mtDNA divergence in *Girella* indicates a species-level separation (Terry et al. 2000), and predates the separation for the *P. maculatofasciatus* populations in the present study. It is possible that these older divergences followed the closure of an earlier seaway near Santa Rosalia, leading to speciation in *Leuresthes, Girella,* and *Sebastomus.*

The estimated times separating disjunct pairs of taxa in the Gulf of California versus the outer Pacific Coast, reflect the considerable caveats in calibrating molecular clocks and comparing different types of molecular datasets (see Kocher and Carleton 1997; Avise 2000). Nonetheless, the two disparate levels of differences among them suggest that the disjunct populations follow two different patterns of separation times. These separations may follow the history of seaway closures for the Gulf of California fauna. The patterns suggest exchange of individuals and gene flow several million years ago via a Santa Rosalia seaway in the central gulf and throughout the mid-Pleistocene via a more southerly La Paz seaway. This hypothesis merits testing a variety of fish taxa displaying this disjunct distribution using mtDNA and nuclear DNA sequence data from the same gene regions in a phylogenetic and phylogeographic framework for each taxon. Differential temperature tolerances and dispersal abilities at various life-history stages presumably also account for species-specific divergence differences among the various disjunct populations. Testing of larval versus adult distribution patterns and temperature tolerances would further resolve these questions.

Phylogeography of the Outer Pacific Coast along the Baja California Peninsula

Samples of *P. maculatofasciatus* from the two outer coastal sites, representing southern California and the central Baja

California Peninsula, lack shared haplotypes and show a significant genetic divergence estimated as 35,000 (fast clock calibration) to 174,000 years (slow clock). Tranah and Allen (1999) discerned congruent and statistically significant morphological differences between samples from southern California and the central coast of the Baja peninsula (including the same individuals sampled from the Guerrero Negro site in the present study), with the latter having larger heads and more robust facial characters. Similarly significant genetic divergences characterized populations of warm temperate nearshore fishes from southern California versus the outer coast of central Baja in an allozyme analysis by Waples and Rosenblatt (1986). A recent mtDNA control region sequence study of the kyphosid *G. nigricans* (Terry et al. 2000) likewise found considerable population divergence between populations from San Diego/northern Baja California versus central/southern coastal Baja California. This pattern of genetic divergence within the warm temperate biogeographic province (Briggs 1974) thus appears common to a variety of nearshore taxa along the outer Pacific Coast of Baja California.

Genetic divergences in the present study indicate restricted genetic exchange despite the southerly transport of the California Current, which may result from retention of larvae (Sinclair 1988; Stepien 1999; Cowen et al. 2000) in gyres and/or the cold-water upwelling patterns off the outer coast of Baja California (Moser et al. 1993). The upwellings lower surface temperatures by 3°C to 9°C year-round and support an admixture of fishes characteristic of both the warm and cold temperate biogeographic provinces; the latter largely skip southern California and then reappear in the upwelling regions (Hubbs 1948; Stepien et al. 1991). Gyre patterns are especially common off the coastline of north and central Baja California and may retain larvae near adult populations (see Waples 1987). These hydrological factors may reduce gene flow between populations along southern California and the central Baja California Peninsula, accounting for the appreciable genetic divergences.

The genetic differentiation and lack of shared haplotypes between the two outer Pacific coastal sites in the present study denote considerable historic separation and gene flow restrictions between southern California and the Pacific Coast of central Baja California. This finding merits additional sampling of intermediate locations and further investigation of this pattern at different life-history stages for *P. maculatofasciatus* and other taxa.

Genetic and Phylogeographic Relationships within the Gulf of California

Samples of *P. maculatofasciatus* from the lower reaches of the warm temperate zone in the Gulf of California have smaller pelvic fins and longer dorsal fin spines than those from the upper and central areas (Tranah and Allen 1999). The present study similarly found significant genetic differentiation between sampling locations in the gulf. These differences may be due to site fidelity and/or larval retention, which need to be tested. Additional investigations also should examine whether similar patterns of divergence occur in other Gulf of California fishes.

Summary and Conclusions

Phylogeographic studies to date indicate significant genetic divergence between the disjunct taxa in the northern temperate waters of the Gulf of California versus the outer Pacific Coast of Baja California, but varying degrees of temporal separations. An examination of the literature, and results from the present study, suggest two general patterns of historic divergence among pairs of taxa. The earlier pattern corresponds to long-term allopatric divergences (Avise 2000), which are supported by many fixed differences in molecular markers and species-level separations in *Leuresthes, Girella*, and *Sebastomus.* These divergences may have resulted from a seaway closure in the Santa Rosalia region several million years ago. The more recent divergence pattern includes *P. maculatofasciatus* and *H. jenkinski*, which are separated at the allopatric population level by significant allozyme frequency differences and two fixed mtDNA nucleotide substitutions (in the former). This moderate allopatric divergence pattern (Avise 2000) dates to the mid-Pleistocene ice ages and may follow the closure of a seaway in the La Paz region along a transform fault. The tropical waters and current patterns of the lower gulf presented a further barrier to gene flow and migration since the end of the Pleistocene. Divergence level variations within the two groupings of disjunct taxa probably reflect their differential abilities to occasionally traverse the tropics. This presumably is due to variation in a species' dispersal ability and temperature tolerance at various life-history stages, which needs to be tested. For example, retention of larvae near adult spawning sites may limit long-distance dispersal at early life-history stages, and spawning site philopatry may decrease adult dispersal. The present investigation, as well as other recent studies, indicates that molecular genetic analyses of nearshore marine populations may reveal considerable phylogeographic structure.

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