

**GENETIC DIFFERENTIATION AND INTRASPECIFIC STRUCTURE OF EASTERN  
TROPICAL PACIFIC SPOTTED DOLPHINS, *STENELLA ATTENUATA*, REVEALED  
BY MITOCHONDRIAL AND MICROSATELLITE DNA ANALYSES**

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**ABSTRACT** ADMINISTRATIVE REPORT LJ-02-38

Mitochondrial DNA (mtDNA) control region sequences and microsatellite loci length polymorphisms were used to investigate genetic differentiation in spotted dolphins (*Stenella attenuata*) in the Eastern Tropical Pacific and to examine the intraspecific structure of the coastal subspecies (*S. a. graffmani*). Two hundred and nine animals from several coastal areas and 90 offshore animals were sequenced for 455 bp of the mitochondrial control region, resulting in 121 mtDNA haplotypes. Phylogenetic analyses and the existence of shared haplotypes between the two subspecies suggest recent and/or current gene flow. Analyses using  $\chi^2$ ,  $F_{ST}$  values (based on haplotype frequencies) and  $\Phi_{ST}$  values (based on frequencies and genetic distances between haplotypes) yielded statistically significant separation (bootstrap values  $P < 0.05$ ) among six different coastal and the offshore strata. Ninety-one coastal animals from these six geographic strata and 15 offshore animals were genotyped for three nuclear microsatellite loci. Analysis using  $F_{ST}$  values (based on allelic frequencies) yielded statistically significant separation between each of the coastal strata and offshore animals, but no coastal populations were distinguished. Genotyping of an additional four microsatellite loci in the coastal animals still showed no statistically significant subdivision. This consistent difference between the levels of genetic structure uncovered by mitochondrial and nuclear markers suggests the existence of male-biased dispersal, with females being highly philopatric. These results argue for the existence of at least six distinct coastal populations, which should be treated as separate units for management purposes and suggest the existence of male-biased dispersal among the coastal strata.

## INTRODUCTION

Understanding the processes of genetic subdivision in cetaceans is especially challenging because most species inhabit vast geographic ranges with few geographic boundaries. Yet, populations of these highly mobile animals adapt to local conditions and differentiate and species evolve. Sound marine mammal management argues for the protection of the locally adapted populations (Taylor 1997). Pantropical spotted dolphins (*Stenella attenuata*) represent a good example. They are distributed globally in tropical and warmer temperate waters (Rice 1988). Details on the species distribution are best known for the eastern and central Pacific (Dizon et al. 1994), where the species is killed incidentally to yellow fin tuna purse-seine fishing. In the Eastern Tropical Pacific Ocean (ETP), two subspecies are distinguished: the coastal spotted dolphin (*Stenella attenuata graffmani*) and the offshore spotted dolphin (*S. a. attenuata*). The coastal subspecies can be recognizable by its relatively larger body and heavier spotting (Perrin, 2001), while offshore animals tend to be smaller and more slender, with lighter skulls and smaller teeth (Schnell et al. 1982).

Two stocks of offshore spotted dolphins are recognized in the ETP, based on morphological and tagging data (Perrin et al. 1985, Schnell et al. 1986, Perrin et al. 1994): Northeastern and Western-Southern (Dizon et al. 1994). Only one stock of coastal spotted dolphins is recognized (Dizon et al. 1994), although morphological differences had been described between Gulf of California and Central American coastal animals (Douglas et al. 1984).

Little is known about the migration patterns and separation of coastal and offshore stocks in this species. Our study uses variation in the mitochondrial DNA (mtDNA) and seven nuclear short tandem repeat (microsatellite) loci to investigate genetic differentiation. Specifically, in order to evaluate if the potential for great distance dispersal in these animals translates into a broad gene flow that prevents the development of detectable population subdivision, this paper investigates: (1) the

existence of genetic differentiation between coastal and offshore spotted dolphins and (2) genetic structure within the coastal strata.

## METHODS

### *Samples*

Two hundred and nine coastal animals from several geographic strata and 90 offshore animals were used in this study. The geographic location and number of samples are summarized in Figure 1. Skin from coastal spotted dolphins was obtained from biopsies of free-ranging animals, while samples of offshore animals were obtained from fisheries bycatch. Samples were stored in an aqueous solution of 20% (v/v) dimethyl sulfoxide (DMSO) saturated with sodium chloride (NaCl) (Amos & Hoelzel 1991) or kept frozen until DNA extraction. Coastal samples span waters of the Eastern Tropical Pacific Ocean, from the southern Gulf of California to Ecuador. Coastal and offshore individuals were identified based on both morphology and distance from the coast (Dizon et al. 1994). Each coastal individual was assigned, *a priori*, to one of eleven provisionally defined sampling strata, (#1 through #11 in Figure 1), based on sample discontinuities, depth profiles between sampling locations, and number of samples per sample site.

### *DNA extraction*

Tissue (100 - 300 mg) was digested in cetyltrimethylammonium bromide (CTAB; Winnepennickx et al. 1993) extraction buffer, and DNA was purified by standard phenol/chloroform/isoamyl alcohol (25:24:1) extractions (modified from Sambrook et al. 1989). The precipitate was resuspended in TE buffer to an average concentration of 1.5 µg/µl. The quality of the DNA was examined via electrophoresis on 1% or 2% agarose gels using approximately 1.5 µg of DNA. Those samples that exhibited some visible product in the size range above 12,000 bp usually could be successfully amplified.

### *Mitochondrial DNA*

A DNA fragment of about 650 base pairs (bp) comprising the proline transfer RNA gene and the hypervariable region I of the control region was amplified from 180 individuals using the Polymerase Chain Reaction (PCR). Reactions were performed in 25 µl volumes, containing 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 150 µM each dNTP, 0.3 µM each primer, 1.25 units of *Taq* DNA polymerase (Perkin Elmer Cetus, Promega, or Gibco BRL), and approximately 50 ng of genomic DNA. The thermal cycling profile included an initial hot start of 2 minutes and 30 seconds at 90°C, followed by 35 amplification cycles. Each of these cycles consisted of denaturation for 45 seconds at 94°C, annealing for 1 minute at 48°C, and extension for 1 minute and 30 seconds at 72°C. An additional 5-minute interval at 72°C was added at the end of the cycle series to ensure complete extension of the PCR products. The following primers, which anneal between the tRNA threonine gene and the tRNA proline gene (L-strand) and the B region (H-strand), were used (numbers refer to the 3' base of the primer with reference to the human mtDNA sequence of Anderson *et al.* [1981]): L15965 5'-CCTCCCTAAGACTCAAGG-3' (developed at our laboratory) and H00034 5'-TACCAAATCTATGAAACCTCAG-3' (Rosel *et al.* 1994).

Successful amplification products were then cleaned by filtration through purification columns (QIAquick® 250, QIAGEN) according to the manufacturer's specifications. Both heavy and light strands were cycle-sequenced using the PRISM® DyeDeoxy Terminator Cycle Sequencing

Ready Reaction kit (Applied Biosystems Inc.). Primers used for sequencing were L15965 (described above) and H16498 5'-CCTGAAGTAAGAACCAGATG-3' (Rosel *et al.* 1994). Sequencing chemistry was optimized for 20 µl reactions containing 60-200 ng double stranded PCR product, 0.2 µM primer, and 6 µl terminator ready reaction mix. The cycling profile was 10 seconds denaturation at 96°C, 5 seconds annealing at 50°C, and 4 minutes extension at 60°C, for 25 cycles. Sequenced products were purified by ethanol precipitation and then run on an ABI 377 DNA automated sequencer. Editing of opposing strands was done simultaneously using SeqEd v. 1.0.3 software, designed to deal with the output files of the automated sequencer.

#### *Microsatellite Genotyping*

Seven microsatellite loci (dinucleotide repeats) shown to be polymorphic in several cetacean species were used in this study: EV14, EV37, EV94, and EV104 (Valsecchi & Amos 1996); and SI849, SI969, and SI1026 (Galver 2002). None of these loci had been originally screened on spotted dolphin.

DNA fragments encompassing the target microsatellite regions were amplified from 91 coastal individuals using PCR. Fifteen offshore individuals were also genotyped for three loci: EV37, EV94 and SI969. Reactions were performed in 25 µl volumes, containing 10-100 ng of genomic DNA, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 150 µM each dNTP, 0.3 µM each primer (one fluorescently labeled), and 1.25 units of *Taq* DNA polymerase (Perkin Elmer Cetus, Promega, or Gibco BRL). The thermal cycling profile included an initial hot start of 3 minutes at 97°C, followed by 35 amplification cycles. Each of these cycles consisted of denaturation for 1 minute at 90°C, annealing for 1 minute at a locus-specific temperature, and extension for 1:30 minutes at 72°C. Annealing temperatures were for EV14, 64°C; EV37, 50°C; EV94, 55°C; EV104, 43°C for 10 cycles and 46°C for 25 cycles; SI849, 49°C for 10 cycles and 52°C for 25 cycles; SI969, 54°C; and SI1026, 55°C. For every locus, an additional 5-minute interval at 72°C was added at the end of the cycle series to ensure complete extension of the PCR products.

The fragment sizes of the successful amplification products were measured with an ABI ABI 377 DNA automated sequencer running in the “genotyping” mode.

No statistically significant linkage disequilibrium was observed between any pair of loci, so it was assumed all seven loci were unlinked (Raymond & Rousset 1995).

#### *Data Analysis*

Phylogeny. Both parsimony and genetic distance-based methods were used to reconstruct the phylogenetic relationships among the haplotypes:

The heuristic search algorithm of PAUP (Phylogenetic analysis Using Parsimony, Version 3.1.1, Swofford 1993) was used and 1000 minimum trees were saved. This algorithm uses the criterion of maximum parsimony to find the tree(s) that require the least number of evolutionary changes.

The genetic distance between haplotypes, measured as the proportion of differences, was used to construct a neighbor-joining tree (Saitou & Nei 1987) with the aid of the computer program MEGA version 1.0 (Kumar *et al.* 1993). Neighbor-Joining operates on the principle of finding pairs of OTUs in consecutive stages of clustering that result in the minimum total branch length (Saitou & Nei 1987).

Population Differentiation and Structure. The extent of population subdivision was examined using an

analysis of molecular variance (AMOVA, Excoffier et al. 1992) on both the mitochondrial and the microsatellite data, and  $\chi^2$  tests (Roff and Bentzen 1989) on mitochondrial data. The AMOVA analyses were performed using the program Arlequin v. 2.000 (Schneider et al. 2000), which calculates F-statistics (Wright's fixation index, Wright 1965; Cockerham & Weir 1993), for both mitochondrial and microsatellite data, and their analogs ( $\Phi$ -statistics) in the case of mitochondrial data. F-statistics and  $\Phi$ -statistics indicate which proportion of the genetic variance is due to subdivision into a priori determined populations. The genetic distance between a pair of haplotypes was estimated as the proportion of the nucleotide differences between them, and the null distribution of pairwise  $F_{ST}$  and  $\Phi_{ST}$  values under the hypothesis of panmixia was obtained by 10,000 permutations of the original data set.

Samples were tested for the existence of closely related animals in the same sampling location by examining the sharing of mitochondrial haplotypes and common microsatellite alleles. No cases of related individuals were found.

## RESULTS

### *Mitochondrial Genetic Diversity*

Two hundred and ninety nine specimens were sequenced and 121 different haplotypes identified, 77 of which were unique. Of the 44 haplotypes common to more than one individual, 14 were common to offshore and coastal animals. No heteroplasmy was detected, either in the length or in the nucleotide sequence of the amplified fragment. Only one single bp insertion-deletion (indel) was necessary to align the 121 haplotypes. The indel was located at site #157 of haplotype 114 (two animals). In total, 108 sites were variable and 44 were phylogenetically informative.

The average pairwise distance (nucleotide diversity,  $d$ , Nei 1987), i.e., the probability that two randomly chosen homologous nucleotides are different among all individuals in the sample, was 1.48 % (S.D. = 0.77 %), while the overall haplotype diversity ( $h$ , Nei 1987), i.e., the probability of two sampled animals having different haplotypes, was 97.24 % (S.D. = 0.46 %).

### *Microsatellite Genetic Variation*

Varying levels of polymorphism were observed in all microsatellite loci used. The number of alleles per locus ranged from four for locus EV104 to 30 for locus EV37 (data available from authors upon request).

Observed heterozygosity (Table 1) for each locus differed among populations but was never below 0.65, consistent with the high levels of polymorphism found at all loci.

### *Phylogeny*

Because of the very low number of informative characters ( $n = 44$ ) relative to the number of unique haplotypes ( $n = 121$ ), maximum-parsimony analysis resulted in numerous polytomies and a large number of equally parsimonious trees, lending little phylogenetic resolution. A neighbor-joining tree (Saitou & Nei 1987) showed no strict concordance between clades and geographic origins or morphotype of the samples, with haplotypes found in offshore and in coastal animals present throughout the tree (Figure 2).

### *Population Structure*

Mitochondrial Results. Statistically significant genetic differentiation was detected when a comparison was made between coastal and offshore samples ( $F_{ST} = 0.0239$ ,  $P < 0.001$ ;  $\Phi_{ST} = 0.0236$ ,  $P < 0.001$ ;  $\chi^2 = 217.662$ , d.f. = 120,  $P < 0.001$ ).

Within the coastal animals, pairwise comparisons were made among the eleven provisionally defined sampling strata. Adjacent strata for which statistically significant subdivision was not

detected by the AMOVA were pooled (results not shown) and subsequently reanalyzed until maximum population structure was attained. When coastal samples were divided into six groups (Northern Mexico: #1; Southern Mexico: #2, #3, and #4; Central America: #5 and #6; Costa Rica: #7; Panama: #8; and Ecuador: #9, #10, and #11), statistically significant levels of genetic subdivision for  $\chi^2$  resulted for all pairwise comparisons among coastal strata, as well as for all but two pairwise comparisons between coastal and offshore strata (Table 2). Statistically significant levels of genetic subdivision for  $F_{ST}$  resulted for all but one of the comparisons between pairs of adjacent coastal strata, and for four of the six comparisons between coastal and offshore strata (Table 3, upper matrix). In the case of  $\Phi_{ST}$ , three of the five comparisons between coastal strata and four of the six comparisons between coastal and offshore strata were statistically significant (Table 3, lower matrix). Overall, between 6 and 7% of the total molecular variance was accounted for by stratifying the sample into the six coastal and the one offshore populations, although it was highly significant ( $\chi^2 = 1106.882$ , d.f. = 720,  $P < 0.001$ ;  $F_{ST} = 0.0603$ ,  $P < 0.001$ ;  $\Phi_{ST} = 0.0720$ ,  $P < 0.001$ ).

**Microsatellite Results.** Genetic differentiation between coastal and offshore samples was highly statistically significant ( $F_{ST} = 0.0911$ ,  $P < 0.001$ ).

For coastal animals, pairwise comparisons using AMOVA were made among the eleven provisionally defined sampling strata. Adjacent strata for which statistically significant subdivision was not detected were pooled (results not shown) and subsequently reanalyzed. Strata distinguished by the prior mtDNA analysis were not pooled, although statistically non-significant results (not shown) were obtained. Thus, the final pairwise comparisons were performed using the above mentioned six coastal strata and the offshore stratum.

Almost 3.3% of the total molecular variance overall was accounted for by the stratification ( $F_{ST} = 0.0322$ ,  $P < 0.0001$ ). Results from pairwise comparisons between pairs of adjacent strata are shown in Table 4. Statistically significant results for  $F_{ST}$  were found for all the pairwise comparisons between coastal and offshore strata, while no comparison between coastal strata, either when using three or seven loci, resulted in a statistically significant  $F_{ST}$  value (Table 4).

## DISCUSSION

### *Phylogeny*

High levels of genetic variation were observed in the haplotype distribution used in the present study, with values well within the range expected for cetaceans. The high haplotypic diversity found in ETP spotted dolphins, though, stems from the existence of a very high proportion of unique haplotypes that differ by only a small number of bases. This low nucleotide diversity (1.48 %) was smaller than values reported between closely related species in cetaceans within the same ocean basin, such as 2.1% for common dolphins, *Delphinus delphis* and *D. capensis* (Rosel et al. 1994) or 2.7% for bottlenose dolphins, *Tursiops truncatus* and *T. aduncus* (Curry 1997). This, together with the phylogenetic signal derived from the presence of mitochondrial haplotypes from both coastal and offshore animals in both clusters shown in the neighbor-joining tree (Fig. 2), and, above all, the existence of shared haplotypes between both morphotypes, suggests a long history of gene flow between coastal and offshore populations that might be still taking place.

### *Population Structure*

Analysis of variance in the mitochondrial control region within and among population strata

uncovered substantial levels of genetic partitioning. When coastal samples were divided into six strata, significant differences were found for all pairwise comparisons among those strata (Table 2). For comparisons between all pairs of adjacent divisions, pairwise differences were larger between strata in the southern part of the range: the largest differences were between Panama and Ecuador ( $\Phi_{ST} = 0.1473$ ;  $F_{ST} = 0.0834$ ) and between Costa Rica and Central America ( $\Phi_{ST} = 0.0998$ ;  $F_{ST} = 0.1020$ ). These would indicate that the southern strata were the first to diverge, while northern strata (Northern Mexico and Southern Mexico) are the most recently diverged. The different nature of  $F_{ST}$  and  $\Phi_{ST}$  values would also support this conclusion.  $F_{ST}$  is based solely on haplotype frequencies, while  $\Phi_{ST}$  takes into consideration both haplotype frequencies and genetic distances among those haplotypes. Thus, in cases where the evolutionary time for these genetic differences to evolve is large enough,  $\Phi_{ST}$  would enhance the ability to detect population structure. This would be the case for the southern populations, with the highest  $\Phi_{ST}$  value between Panama and Ecuador followed by the value between Costa Rica and Central America. In contrast, when there has not been enough time for haplotypes to diverge, interhaplotypic distances will be small, even if the frequencies of these haplotypes differ, and only  $F_{ST}$  would detect population differentiation. This is indeed the case for the northern strata, where only  $F_{ST}$  but not  $\Phi_{ST}$  values are statistically significant.

Mitochondrial results also revealed genetic differentiation between the offshore population and the coastal strata. Statistically significant differentiation was detected when comparing overall coastal and offshore samples ( $F_{ST} = 0.0239$ ,  $P < 0.001$ ;  $\Phi_{ST} = 0.0236$ ,  $P < 0.001$ ;  $\chi^2 = 217.662$ , d.f. = 120,  $P < 0.001$ ). Additionally, all the pairwise comparisons between offshore and coastal strata were statistically significant, except for northern Mexico and Central America (Tables 2 and 3), supporting a strong subdivision throughout the study area.

Contrasting with the mitochondrial results, analysis of variance of microsatellite loci uncovered very low levels of genetic partitioning among the coastal strata, but showed differentiation when overall offshore and coastal samples were compared ( $F_{ST} = 0.0911$ ,  $P < 0.001$ ), as well as between each of these and the offshore population (Table 4). Again,  $F_{ST}$  values were higher for pairwise comparisons between the offshore population and southern coastal strata than for northern coastal strata. Still, although genetic differentiation between offshore and coastal animals was highly significant, no structure was detected within the coastal region, even when the number examined of loci was increased to seven (Table 4, lower matrix).

A possible explanation for this lack of structure would be low sample sizes, which would cause population differences to be statistically non-significant (Waples 1998), resulting in an underestimate of subdivision. Nevertheless, this bias should be lower for microsatellites than for mtDNA, since the ability to reliably detect genetic differentiation is enhanced by considering multiple and independent loci (Waples 1998). A more plausible explanation would be the existence of differential dispersal rates between sexes, since the population structure shown by mtDNA, which is maternally inherited (Hutchison *et al.* 1974), is much higher than that shown by microsatellite loci (bi-parentally transmitted). It seems possible, then, that females exhibit a higher degree of philopatry in this region and, while males disperse and homogenize the populations, different maternal lineages separate and define the different coastal strata. A separate analysis of genetic structure for each gender is underway, as sample numbers increase, in order to further test this hypothesis.

### *Analysis Limitations*

It should be noted that, although efforts have been made to avoid sampling error, the low sample sizes might affect interpretation of patterns of population structure. Small sample sizes severely limit the power of the analysis to detect differentiation (Peterman 1990; Dizon et al. 1995), generally causing population differences to be statistically non-significant (Waples 1998). This bias, however, would tend to underestimate structure. The fact that population differentiation is detected despite the relatively small sample sizes indicates that at least six different strata exist in ETP coastal spotted dolphins. The number of strata could increase, and the boundaries reassessed, upon examination of a larger sample. For instance, in the present study, only seven samples were available from Ecuador, and these were pooled with samples from Panama to attain a higher sample size in the southern-most strata. It is likely, though, that some structure exists within this area, and that this structure could be unveiled as more samples are added and the sampling gaps are filled.

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## TABLE AND FIGURE LEGENDS

Table 1.

Table 2. Pairwise comparisons among the five coastal strata and the offshore stratum of spotted dolphins based on mtDNA.  $F_{ST}$  values are shown in the upper matrix and  $\Phi_{ST}$  values in the lower matrix. Statistically significant results (calculated from 10000 random permutation tests) are shown in bold type.

Table 3.  $F_{ST}$  values for pairwise comparisons among the five coastal strata and the offshore stratum of spotted dolphins based on three microsatellite loci are shown in the upper matrix.  $F_{ST}$  values for pairwise comparisons only among the five coastal strata based on seven microsatellite loci are shown in the lower matrix. Statistically significant results (calculated from 10000 random permutation tests) are shown in bold type

Figure 1. Geographic location of spotted dolphin samples collected for this study (numbers indicate each of the 11 provisional initial strata).

Figure 2. Unrooted Neighbor–Joining tree of the 121 haplotypes of spotted dolphins found in this study. Genetic distances were calculated as proportion of differences among haplotypes. Haplotypes found in both coastal and offshore animals are shown with a black circle. Haplotypes unique to offshore animals are shown with a blank circle.



Table 1. Measures of genetic diversity ( $\pm$  SD) in strata of spotted dolphins for mtDNA and microsatellites.

Division	Number of mtDNA sampled	Number of mtDNA Haplotypes	Haplotype Diversity (%) $h$	Number of Chromosomes sampled	Mean Number of Alleles per Locus	Mean Observed Heterozygosity	Mean Expected Heterozygosity
Northern Mexico	28	21	96.83	56 (7 loci)	13.57	0.768	0.784
Southern Mexico	31	17	92.47	38 (7 loci)	9.00	0.682	0.725
Central America	11	11	1.00	22 (7 loci)	6.71	0.725	0.708
Costa Rica	14	6	80.22	16 (7 loci)	7.29	0.748	0.731
Panama	92	22	85.21	8 (7 loci)	5.17	0.875	0.798
Ecuador	33	20	96.59	42 (7 loci)	11.57	0.694	0.778
Offshores	90	60	98.35	30 (3 loci)	10.67	0.929	0.868
Total	299	121	97.24				

Table 2.  $\chi^2$  pairwise comparisons among the six coastal strata and the offshore stratum of spotted dolphins based on mtDNA. Statistically significant results (calculated from 10,000 random permutation tests) are shown in dark.

	Northern Mexico	Southern Mexico	Central America	Costa Rica	Panama	Ecuador	Offshores
Northern Mexico							
Southern Mexico	d.f. = 35 $\chi^2 = 50.98$ P < 0.001						
Central America	d.f. = 28 $\chi^2 = 52.12$ P < 0.001	d.f. = 26 $\chi^2 = 38.56$ P = 0.006					
Costa Rica	d.f. = 26 $\chi^2 = 42.12$ P < 0.001	d.f. = 21 $\chi^2 = 42.67$ P < 0.001	d.f. = 16 $\chi^2 = 25.01$ P = 0.001				
Panama	d.f. = 40 $\chi^2 = 112.31$ P < 0.001	d.f. = 36 $\chi^2 = 115.93$ P < 0.001	d.f. = 29 $\chi^2 = 82.38$ P < 0.001	d.f. = 23 $\chi^2 = 37.64$ P = 0.036			
Ecuador	d.f. = 39 $\chi^2 = 57.78$ P < 0.001	d.f. = 34 $\chi^2 = 58.33$ P < 0.001	d.f. = 28 $\chi^2 = 38.67$ P = 0.010	d.f. = 21 $\chi^2 = 29.87$ P = 0.033	d.f. = 34 $\chi^2 = 90.60$ P < 0.001		
Offshores	d.f. = 74 $\chi^2 = 78.29$ P = 0.141	d.f. = 73 $\chi^2 = 102.62$ P < 0.001	d.f. = 67 $\chi^2 = 81.42$ P = 0.090	d.f. = 62 $\chi^2 = 86.83$ P = 0.002	d.f. = 78 $\chi^2 = 167.60$ P < 0.001	d.f. = 74 $\chi^2 = 100.76$ P < 0.001	

Table 3. Comparisons between pairs of adjacent coastal strata and between these and the offshore stratum of spotted dolphins based on mtDNA.  $F_{ST}$  values are shown in the upper matrix and  $\Phi_{ST}$  values in the lower matrix. Statistically significant results (calculated from 10,000 random permutation tests) are shown in dark.

	Northern Mexico	Southern Mexico	Central America	Costa Rica	Panama	Ecuador	Offshores
Northern Mexico		0.0426 P < 0.001					0.0017 P = 0.301
Southern Mexico	0.0122 P = 0.188		0.0348 P = 0.036				P < 0.001
Central America		0.0140 P = 0.244		0.1020 P = 0.007			0.0012 P = 0.531
Costa Rica			0.0998 P = 0.037		0.0088 P = 0.200		0.0916 P < 0.001
Panama				0.0528 P = 0.036		0.0834 P < 0.001	0.0973 P < 0.001
Ecuador					0.1473 P < 0.001		0.0168 P < 0.001
Offshores	-0.0141 P = 0.676	0.0185 P = 0.043	-0.0141 P = 0.378	0.0320 P = 0.014	0.0464 P < 0.001	0.0538 P = 0.001	

Table 4.  $F_{ST}$  values for pairwise comparisons between adjacent coastal strata and between these and the offshore stratum of spotted dolphins based on three microsatellite loci are shown in the upper matrix.  $F_{ST}$  values for pairwise comparisons only between adjacent coastal strata based on seven microsatellite loci are shown in the lower matrix. Statistically significant results (calculated from 10,000 random permutation tests) are shown in dark.

	Northern Mexico	Southern Mexico	Central America	Costa Rica	Panama	Ecuador	Offshores
Northern Mexico		0.0091 P = 0.170					0.0400 P = 0.005
Southern Mexico	0.0013 P = 0.328		-0.0013 P = 0.763				0.0855 P < 0.001
Central America		-0.0490 P = 0.997		-0.0063 P = 0.475			0.1243 P < 0.001
Costa Rica			-0.0062 P = 0.432		-0.0303 P = 0.818		0.1398 P < 0.001
Panama				-0.0083 P = 0.497		-0.0390 P = 0.956	0.1099 P = 0.001
Ecuador					-0.0296 P = 0.875		0.1043 P < 0.001

Figure 1. Geographic location of spotted dolphin samples collected for this study (numbers indicate each of the 11 provisional initial strata).





Figure 2. Unrooted Neighbor-Joining tree of the 121 haplotypes of spotted dolphins found in this study. Genetic distances were calculated as proportion of differences among haplotypes. Haplotypes found in both coastal and offshore animals are shown with a black circle. Haplotypes unique to offshore animals are shown with a blank circle.

