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Mitogenomic differentiation in spinner (*Stenella longirostris*) and pantropical spotted dolphins (*S. attenuata*) from the eastern tropical Pacific Ocean

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ABSTRACT

Spinner dolphins (*Stenella longirostris*) and pantropical spotted dolphins (*S. attenuata*) show high intraspecific morphological diversity and endemic subspecies in the eastern tropical Pacific Ocean (ETP). Previous studies of mitochondrial DNA have found low genetic differentiation among most of these groups, possibly due to demographic factors, ongoing gene flow, and/or recent divergence. These species were heavily depleted due to bycatch in the ETP yellowfin tuna fishery. Because understanding population structure is important for accurate management of the recovery of these species, we collected whole mitochondrial genome sequences from 104 spinner and 76 spotted dolphins to test structure hypotheses at multiple hierarchical taxonomic levels. Results show differences between subspecies of spinner and spotted dolphins, but no support for the division of existing offshore stocks of spotted dolphins. We compare these results to previous results of genome-wide nuclear SNP data and suggest high haplotype diversity, female dispersal, and/or relative power of the two data sets explains the differences observed. Interestingly, increasing the amount of mitochondrial

data (base pairs and genes) did not increase ability to delimit population units. This study supports a genetic basis for management units at the subspecies level, and provides critical information for mitigating historical and continued fisheries impacts.

Key words: mitochondrial DNA, conservation genetics, pelagic dolphins.

Intraspecific patterns of diversity in cetaceans often follow a hierarchical pattern: evolutionarily independent lineages (often on the ocean-basin scale) made up of distinct populations at finer scales (*i.e.*, islands or coastlines) (Taylor 2005). Spinner (*Stenella longirostris*) and pantropical spotted dolphins (*S. attenuata*) in the eastern tropical Pacific Ocean (ETP) offer a unique opportunity in cetaceans to test hypotheses of variation at multiple intraspecific levels in two species in the same place. Both species show extensive geographic and morphological variation at multiple hierarchical taxonomic levels. Moreover, thanks to the forethought of biologists during the ETP tuna-dolphin problem of the 1960s and 1970s, genetic samples are available (collected *in situ*) from the remote offshore environments to examine intraspecific variation.

Fisheries bycatch is arguably the largest threat facing cetaceans today (Read *et al.* 2006). One of the largest and best-studied fisheries bycatch events in history occurred in the ETP tuna purse-seine fishery (National Research Council 1992). In this region, two species of pelagic dolphins, spinner (*Stenella*

longirostris) and pantropical spotted (*S. attenuata*), commonly associate with one another as well as with large yellowfin tuna (Scott *et al.* 2012). Both species were historically extremely abundant (numbering in the low millions), but starting in the 1960s, hundreds of thousands were killed annually as bycatch in the fishery (Lo and Smith 1986, National Research Council 1992, Wade 1995, Wade *et al.* 2007). Despite protection under the U.S. Marine Mammal Protection Act of 1972 and multinational protection under the 1999 Agreement on the International Dolphin Conservation Program (Joseph 1994, Gosliner 1999), ETP spinner and spotted dolphin population abundances remain low relative to historical abundance (Wade *et al.* 2007, Gerrodette *et al.* 2008). Wade *et al.* (2007) estimated that bycatch reduced ETP spinner and spotted dolphins to one-third and one-fifth of historical abundance, respectively. Chase and encirclement of dolphins in this fishery are still ongoing and likely create “indirect effects” that impact reproduction (Cramer *et al.* 2008, Kellar *et al.* 2013), disrupt social connections (Wade *et al.* 2012), and/or cause premature separation of mothers and calves (Archer *et al.* 2010, Noren *et al.* 2011, Noren and West 2017). The current

population abundance estimates are based on data from 2006 (Gerrodette *et al.* 2008): eastern spinner (1,062,879, CV = 0.26), whitebelly spinner (734,837, CV = 0.61), northeastern offshore spotted (857,884, CV = 0.23), western/southern offshore spotted (439,208, CV = 0.29), coastal spotted (278,155, CV = 0.59). There is no abundance estimate for Central American spinner dolphins. A critical component of managing their recovery is better understanding how both populations and species are naturally structured within the ETP and how taxa within the ETP are related to nearby populations of conspecifics. Some authors have worried that the population boundaries (designated based on a distributional hiatus) may be leading to the mismanagement of the recovery effort (Gerrodette *et al.* 2008). Genetic studies have presented mixed support for differentiation in these species. Dizon (1991) and Galver (2002) found little evidence for the population boundaries designated by morphology; however, Escorza-Treviño *et al.* (2005), and Andrews *et al*. (2013) found differentiation between subspecies in some loci, and Leslie and Morin (2016) found subspecies and population-level differentiation using a larger nuclear SNP

data. Two issues could arise from a lack of understanding of populations structure. First, populations could be undercharacterized; this would lead to smaller populations not gaining the protection they need. Second, populations could be mischaracterized or not represent biologically meaningful units at all. This would lead to inaccurate and ineffective recovery efforts. We aim to address both of these issues by testing hypotheses of the current management units, and by testing the validity of additional, smaller, hypothesized populations.

Spinner Dolphins (Stenella longirostris)

Globally, there are four subspecies of spinner dolphin (*Stenella longirostris*) defined by morphological differences. The nominate form, the pantropical spinner (*S. l. longirostris*) occurs in all of the tropical waters of the world outside the ETP. In the central and western Pacific, pantropical spinners are usually associated with islands, such as the Hawaiian Islands. In the shallow waters of Southeast Asia, there is a much smaller dwarf spinner subspecies (*S. l. roseiventris*) (Perrin *et al.* 1989, 1999). The Central American subspecies (*S. l. centroamericana*) is found off the Pacific coasts of Southern

Mexico south through Panama, in relatively near-shore waters. The eastern spinner dolphin (*S. l. orientalis*), on the other hand, inhabits offshore waters that extend from Baja California, Mexico, south to Ecuador (Douglas *et al*. 1992, Perrin 1990). Both the Central American and eastern spinner subspecies are endemic to the ETP.

For management purposes within the ETP, there are three stocks of spinner dolphins: the Central American subspecies, regular eastern spinners, and the “whitebelly” spinner, which has been proposed to represent a hybrid swarm between the eastern subspecies and the pantropical subspecies of the central and western Pacific (Perrin *et al.* 1991) (Fig. 1, based on Perrin *et al.* 1985). Taxonomically, whitebellies are classified as part of the nominate (pantropical) spinner subspecies *S. l. longirostris*. Significant geographic overlap exists between the eastern subspecies and the whitebelly form (Perrin *et al.* 1985) (see Fig. 1). Finally, based on external body morphometrics, a distinct morphotype of the eastern spinner dolphin, known as the “Tres Marias” spinner dolphin, has been described from near the islands of the same name off the coast of Mexico (Perryman and

Westlake 1998). The Tres Marias spinner dolphin is not currently recognized as a stock for management purposes.

Some genetic approaches have not found structure among subspecies corresponding to the observed morphological differences (Dizon *et al.* 1994, Galver 2002). Andrews *et al.* (2013) found significant differentiation between the two endemic subspecies using Actin intron data (Φ_{ST} = 0.068, P < 0.001), but not with concatenated mtDNA data (Φ_{ST} = 0.026) or a Y chromosome gene sequence (Φ_{ST} = 0.000). Similarly, this study also found significant differentiation between Central American subspecies and the Tres Marias group using the Actin intron (Φ_{ST} = 0.034, P < 0.05), but not the mtDNA data set (Φ_{ST} = 0.012) or the Y chromosome (Φ_{ST} = 0.000). The only other difference detected was between Central American spinners and whitebelly spinners, using mtDNA (Φ_{ST} = 0.079, P < 0.01). The nonsignificant comparisons had Φ_{ST} values that were small (ranging from −0.013 to 0.026).

Although there was no significant allele frequency difference between groups using the Y chromosome, the authors found a shared Y chromosome haplotype in the eastern and Central American subspecies that was not found in the pantropical or

dwarf subspecies. Interestingly, the Y chromosome locus was found to be polymorphic in whitebellies, supporting the hypothesis of introgression in this form (Andrews *et al.* 2013). The authors proposed that sexual selection was driving the divergence of spinner dolphins in the ETP. Recently, Leslie and Morin (2016) found genetic structure corresponding to the described subspecies and stocks supported by morphological data using genome-wide SNP data. It is worth highlighting, however, that despite these significant differences between subspecies and stocks, the F_{ST} values were quite small (*e.g.*, F_{ST} ranged from 0.0009 to 0.0215) in this study (Leslie and Morin 2016). In addition, multiple independent populations of spinner dolphins were found in the Hawaiian Islands in a study using mtDNA control region and 10 microsatellite loci (Andrews *et al.* 2010).

Spotted Dolphins (Stenella attenuata)

The pantropical spotted dolphins (*Stenella attenuata*) in the ETP are split into two subspecies based on morphometric analyses: a coastal endemic subspecies (*S. a. graffmani*; Perrin 1975, Perrin *et al.* 1987) and an offshore pantropical subspecies (*S. a. attenuata*). Offshore pantropical spotted dolphins in the

ETP are divided into two management stocks: (1) the northeastern (NE) stock is defined geographically as north of 5ºN, east of 120ºW, and (2) the western-southern (WS) stock is defined as south and west of this northeastern area (Fig. 2) (Perrin *et al.* 1994). A distributional hiatus along 5ºN is the basis for the north-south boundary between NE and WS stocks (Perrin *et al.* 1994), and this has recently been supported by SNP analyses (Leslie and Morin 2016). Although Leslie and Morin (2016) found a significant difference in the F_{ST} test using nuclear SNP data, the F_{ST} value was small (F_{ST} = 0.0019). Currently managed as one stock, some researchers have speculated that the WS stock may be two different stocks based on difference found *via* habitat modeling.[3]

Genetic analyses of mitochondrial control region and microsatellites show high genetic diversity in ETP spotted dolphins and support some differentiation between subspecies (Escorza-Treviño *et al.* 2005). This study identified at least four demographically independent populations within the coastal subspecies (*S. a. graffmani*) and differences between southern populations of the coastal subspecies and the offshore

subspecies (microsatellite DNA F_{ST} values were as low as 0.0202, with $P < 0.001$, for comparisons between offshore and coastal subpopulations). However, this study found no differences between the northern populations of the coastal subspecies and the offshore subspecies in either microsatellite or mtDNA data sets (F_{ST} = −0.0032 and −0.0126, respectively). Escorza-Treviño *et al.* (2005) posited that interchange continues between the northern *S. a. graffmani* populations and the offshore pantropical subspecies. Leslie and Morin (2016) found population structure between coastal spotted dolphins and both putative offshore stocks (NE and WS) of pantropical spotted dolphins using a large nuclear SNP data set (F_{ST} = 0.0416 and 0.0734, respectively, with $P < 0.001$ in both). A recent study of pantropical spotted dolphins in the Hawaiian Islands used mtDNA and microsatellite loci to test for population genetic structure (Courbis *et al*. 2014). The authors found at least three differentiated populations along the archipelago.

To help place these ETP populations within a global context, Leslie and Morin (2018) conducted a broad phylogeographic study. Using a SNP data set similar to Leslie

and Morin (2016) the authors found the ETP spinner dolphin populations occupy a distant branch removed from the pantropical and dwarf subspecies. For spotted dolphins, their analyses revealed two main lineages, corresponding to the pantropical and the coastal spotted dolphin subspecies.

Objectives

Given the morphological differentiation between subspecies and recent evidence of nuclear DNA genetic differentiation, we hypothesize the previous results using one or two mtDNA loci (Dizon *et al.* 1994, Galver 2002, Escorza-Treveño *et al.* 2005, Andrews *et al.* 2013) lacked power to resolve these close intraspecific relationships. In addition, studies of other cetacean species have shown whole mitochondrial genomes to be a useful tool for resolving intraspecific relationships when single mtDNA genes cannot (Morin *et al.* 2010, 2018; Archer *et al.* 2013). We expanded upon previous mtDNA data sets and included the whole mitochondrial genome to test for population structure in two species of dolphins.

Because mitochondrial DNA (mtDNA) is more abundant in cells and has a higher rate of mutation—thus accruing variability on a

time-scale typical of population divergence—it has been the preferred marker for population genetic studies of wildlife (Moritz 1994, Allendorf 2017). Moreover, mtDNA has a much lower effective population size (four-fold lower, because it is haploid and maternally inherited), thus it experiences more drift and may display higher F_{ST} values when compared to nuclear DNA (nuDNA). Because of the strictly maternal inheritance of mtDNA, comparing the strength of genetic structure between mtDNA and nuDNA can provide insights into maternal genetic structure and sex-biased dispersal in wildlife populations (Moritz 1994); however, the difference in effective population size potentially confounds these comparisons, which may not necessarily reflect sex-biased dispersal. Mitochondrial DNA data are particularly useful for species with matrilineal social structure—such as several toothed whale species (*i.e.*, killer whales (*Orcinus* spp.), sperm whales (*Physeter macrocephalus*), and pilot whales (*Globicephala macrorhynchus*) Hoelzel *et al.* 2007, Van Cise *et al.* 2017, Morin *et al.* 2018).

We used DNA capture array library enrichment and highly parallelized DNA sequencing to collect whole mitochondrial

genome sequence data from 104 spinner and 76 spotted dolphins to test hypotheses of population genetic structure at multiple hierarchical taxonomic levels in the eastern tropical Pacific Ocean. We performed analyses of whole mtDNA genomes (mitogenomes) and individual mtDNA genes to test observed levels of differentiation between recognized and proposed management stocks. We also tested for structure supporting the Tres Marias spinner dolphin and alternative stock boundaries in the offshore spotted dolphins. Although still only representing one locus, mitogenomes allow us to examine matrilineal population structure and contrast our findings with those found in previous studies using nuclear DNA (Escorza-Treviño *et al.* 2005, Andrews *et al.* 2013, Leslie and Morin 2016).

METHODS

Sample Collection and DNA Extraction

Skin samples used in this study were collected from free-ranging animals *via* biopsy dart (Lambertsen 1987) on research cruises or from dead specimens killed as bycatch in the tuna purse-seine fishery between 1982 and 2010 (104 spinner dolphins and 76 spotted dolphins; Fig. 1, 2; Tables S1, S2). Spinner

dolphin samples collected from research cruises were assigned to a stock based on the external morphology of the majority of animals in the school, rather than the morphology of the individual sampled or the geographic location of the school. This method was preferable because (1) only after observing the group (which could contain >1,000 individuals) for some time could observers classify it to stock; (2) the external characters distinguishing subspecies are subtle, therefore researchers collecting biopsies from the bow of the research vessel could not confidently classify fast-swimming individuals in real time; and (3) the ranges of ETP spinner dolphin subspecies overlap making geography an unreliable predictor of stock identity. Because of school mixing, it is common to observe a small number of spinner dolphins of alternate morphology (*i.e.*, possibly different subspecies) within a school of dolphins comprised mostly of another morphotype/subspecies. Therefore, there is a chance for some samples to be misassigned, which would potentially bias our population differentiation results toward homogeneity. In addition, some samples were used from areas where the eastern and whitebelly spinners are known

to overlap geographically (see Fig. 1). Spinner dolphin samples from Hawaii spanned the breadth of the main islands and also Midway Atoll.

Because there is little overlap of subspecies distribution in ETP pantropical spotted dolphins, geographic location of the sampling site was used to assign samples to subspecies and stocks. To avoid misassigned individuals near the borders of the NE and WS offshore stocks, we did not use samples collected between 4°N and 6°N east of 125°W. Hawaiian spotted dolphin samples were collected from the Kona Coast of Hawaii and Oahu.

Biopsy samples were stored in salt-saturated 20% DMSO, 70% ethanol, or frozen with no preservative. We extracted DNA using silica-based filter membranes (Qiagen, Valencia, CA) on an automated workstation (Perkin Elmer, Waltham, MA). DNA was quantified using Pico-Green fluorescence assays (Quant-it Kit, Invitrogen, Carlsbad, CA) and a Tecan Genios microplate reader (Tecan Group Ltd, Switzerland).

Library Preparation and Sequencing

Next-generation sequencing libraries were generated as described by Hancock-Hanser *et al.* (2013), using unique 6bp and

7bp index sequences for each individual to allow up to 100 samples to be multiplexed. Multiplexed libraries were enriched for whole mitogenomes and 85 nuclear DNA loci using Sure Select DNA Capture Arrays (Agilent Technologies, Inc., Santa Clara, CA, USA) as described by Hancock-Hanser *et al.* (2013). Sequence data from the 85 nuclear loci were not used in this study. Target sequences for capture enrichment included the reference pantropical spotted dolphin mitochondrial genome (Genbank No. EU557096; Xiong *et al.* 2009) and a suite of 85 nuclear loci (not included in this study). Three identical arrays—designed with the eArray software package (Agilent Technologies, Inc., Santa Clara, CA)—were used to capture a multiplexed mix of both species. Each array contained one replicate of the mitogenome probes at a probe interval of 15 bp as well as 13 replicates of probes for the nuclear loci at a probe interval of 3 bp. Each enriched library was then sequenced using 1 × 100 bp Illumina HiSeq technology (two using Illumina HiSeq2000 and one using HiSeq2500).

Mitogenome Assembly

Raw read data were filtered for quality (minimum quality

score of 15) and demultiplexed by unique barcode. Consensus sequences for each sample were generated from mitogenome sequence reads using a custom pipeline (Dryad data repository doi:10.5061/dryad.cv35b) in R v2.15.0 (R Core Team 2014). Reads were first mapped to the reference spotted dolphin sequence with the short-read alignment tool BWA (Li and Durbin 2009). The mpileup module in SAMTOOLS (Li *et al.* 2009) was then used to convert the resulting BAM-format alignment file into a "pileup" text format, which was then parsed by custom R code to create the consensus sequence for each individual. The following rules were used in this process: A "N" was inserted at a position if the assembly had <3 reads, <5 reads, where not all contained the same nucleotide, or >5 reads, where no one nucleotide (*i.e.*, A, C, G, T) was present in >70% of the reads. All mitogenome sequences were initially aligned with MAFFT using the automatic selection of an appropriate handling strategy ("auto") and default parameters (Katoh *et al.* 2009) followed by a refinement of alignments by eye.

Diversity Estimates and Population Structure Analyses

Two mitogenome data sets were created for each species to

examine gene-level and whole mitogenome differentiation, and to determine if increasing the amount of mitochondrial data (bps and genes) helped delineate populations. First, we partitioned each species' mitogenome into 14 loci (12 coding sequences and two rRNA genes). The control region was removed in all analyses due to large sections of missing data (most like due to poor capture across the hypervariable region), making alignment and haploytping difficult. ND6 and tRNA loci were removed prior to analyses because they conform to different evolutionary models and ND6 falls on the opposite strand from the remaining genes (Duchene *et al.* 2011). Sequences were aligned to the pantropical spotted dolphin reference and locus start/stop positions were annotated in GENEIOUS v5.4 (Biomatters Limited) using the GENEIOUS alignment tool and the amino acid translation tool, respectively.

Second, we removed the control region because of high variation in this region and concatenated the remaining 14 regions to make the concatenated mitogenome sequences. The final sequence lengths for the concatenated data were 13,426 bp and 13,425 bp for spinner and spotted dolphins, respectively. An

individual was removed entirely from analyses if it contained >10% missing data across the entire concatenated sequence.

For both data sets, we estimated haplotypic diversity (h, Nei 1987) and nucleotide diversity (π, Tajima 1983), and assigned individual genes and whole mitochondrial genome sequences to unique haplotypes using tools from the *strataG* package in R (v. 2.3.1; Archer *et al.* 2017). Two pairwise estimates of population genetic structure, F_{ST} (Wright 1949) and Φ_{ST} (Excoffier *et al.* 1992), were also performed using the *strataG* package. The significance of each estimate was tested using 5,000 nonparametric random permutations of the data matrix variables. For Φ_{ST}, pairwise distances were calculated using the best substitution model as identified by Akaike's information criterion in JModelTest version 2.1.4 (Posada 2008). Models were determined for individual gene regions and the entire concatenated data set.

Given the large population sizes, expected low divergence, and relatively low power due to high mitogenome diversity and low sample size relative to population size, we present results for population structure tests using a relaxed threshold for

significance ($P < 0.1$) to highlight possible differentiation accepting a wider acceptance of potential false positives.

We performed a substitution rate test on each species' mitogenome data set to determine if mutations had reached a point of saturation. For this test, we generated pairwise percent differentiation and plotted this against a Jukes and Cantor (1969) correction factor generated using MEGA 5.2.2 (Tamura *et al.* 2011). We chose this model because of its simplicity; if deviations were seen here then general saturation could be assumed.

Although mitochondrial loci are assumed to be under purifying selection (Stewart *et al.* 2008) we, nonetheless, tested spinner dolphin mitochondrial genes for evidence of positive selection using both Tajima's *D* and Codon-based *Z*-test as implemented in MEGA 5.2.2 (Tamura *et al.* 2011). We did not test for positive selection in spotted dolphins because there were no individual mtDNA genes that supported differentiation between the two ETP subspecies.

RESULTS

Hancock-Hanser *et al.* (2013) present information on the

success rate of the DNA capture method including summary statistics of the data analyzed in this paper. As it relates to our analyses, questions might arise about how using arrays designed from closely related species affected our results. As presented in tables 4 and 5 of Hancock-Hanser *et al.* (2013), spinner dolphin samples had a slightly higher number of mtDNA reads per individual than spotted dolphin samples, despite use of the spotted dolphin mitogenome as the capture bait. The same pattern was found for the nuDNA capture; spinner dolphins had more mapped reads per individual than spotted dolphins, despite all the baits being common bottlenose dolphin (*Tursiops truncatus*) DNA sequence (table 4 in Hancock-Hanser *et al.* 2013). We interpreted this consistency as an indication that interspecific capture worked well and that any decrease in capture success (as evidenced in reads per individual for a given species) was more likely due to a combination of other factors (sample quality, multiplexing rate, sequencing technology, and/or variation in library preparation) rather than reduced capture due to interspecific baits. The one area that might have been an issue for interspecific capture was the

hypervariable section of the control region. The hypervariable section of the control region had consistently lower coverage in many individuals and was removed from the concatenated and partitioned data sets.

Spinner Dolphins

We assembled 104 complete or nearly complete (<10% missing data) concatenated spinner dolphin mtDNA data sets (Genbank accession numbers in Table S1). Sample sizes, summary statistics, and genetic diversity measures for each subspecies and stock are listed in Table 1A. At the subspecies level, haplotypic diversities were high and nucleotide diversity was low (>0.9722, <0.0073, respectively). The substitution rate test did not show any signs of saturation. The best nucleotide substitution model estimated by JModelTest (Posada 2008) was JC69 (Jukes and Cantor 1969) for each individual gene region and the entire concatenated data set. The results of F_{ST} and Φ_{ST} analyses of the mtDNA concatenated genes and Φ_{ST} of the individual gene regions for spinner dolphins are shown in Table 2 (F_{ST} for individual gene regions shown in Table S3 and S4).

At the subspecies level, between Central American and

eastern spinner dolphins, the Φ_{ST} test of the concatenated data was not significant, and only one gene showed differentiation (ATP8). F_{ST} was significant in the concatenated data set (0.0133, P = 0.034) and three individual genes (Table S3). Φ_{ST} comparisons of the pelagic whitebelly form and the coastal Central American subspecies indicated differentiation in the concatenated data set (Φ_{ST} = 0.0490, P = 0.0542). Eight individual gene regions were significant (Table 2) in the tests between Central American and whitebelly. We also found significant differences between the whitebelly and the eastern subspecies with Φ_{ST}, but not F_{ST}, using the concatenated mitogenome data (Φ_{ST} = 0.0181, P = 0.0741). Eight individual mitochondrial genes showed significant differentiation between this pair. Φ_{ST} tests showed no differentiation between Tres Marias spinners and either ETP spinner dolphin subspecies in either the concatenated or partitioned data sets, but F_{ST} was significant between Tres Marias and the Central American strata for the concatenated data. Significant differentiation was found between Tres Marias and whitebelly spinners (Φ_{ST} = 0.0263; P = 0.0807), with eight individual gene regions showing significant differentiation in

this comparison.

All tests involving comparisons with Hawaiian spinner dolphins (*S. l. longirostris*) using the concatenated data set were significant. Differentiation between Hawaiian and other Pacific Ocean populations supports previous studies that demonstrated this (Galver 2002, Andrews *et al.* 2013). Between ten and thirteen of the mitogenome genes also showed significant Φ_{ST} and F_{ST} differences between Hawaii and the ETP populations (Tables 2, S3).

All individual gene partitions in spinner dolphins were found to be under purifying selection using Tajima's *D* tests for selection (Table S3) and *Z*-test for positive selection using the Nei-Gojobori method (Nei and Gojobori 1986) (Table S6).

Spotted Dolphins

We assembled 76 complete or nearly complete (<10% missing data) spotted dolphin mitogenomes (Genbank accession numbers in Table S2). Sample sizes, summary statistics and genetic diversity measures are listed in Table 1B. At the level of subspecies, nucleotide diversity was higher in spotted dolphins (>0.0162) than spinner dolphins. Haplotypic diversity (*h*) was

high in both species (>0.9529), but ETP spotted dolphins subspecies had slightly lower levels (0.9529 and 0.9804 for the coastal and offshore groups, respectively) than spinner dolphin subspecies (0.9722 and 0.9985) in this region. The coastal ETP subspecies for both spinner (*S. l. centroamericana*) and pantropical spotted dolphins (*S. a. graffmani*) in the ETP showed reduced *h* compared to their offshore ETP counterparts (Table 1). Similar to the spinner dolphin mitogenome data, the substitution rate test did not detect any signs of saturation, and JC69 was the best substitution model for all individual gene regions and the entire concatenated data set.

Results of F_{ST} and Φ_{ST} analyses of the mtDNA concatenated genes and Φ_{ST} of the individual gene regions for spotted dolphins are presented in Table 3 (individual F_{ST} measures are shown in Table S4). Our analyses at the subspecies level for spotted dolphins (coastal *vs*. offshore) show no significant differentiation using Φ_{ST} for the concatenated or partitioned data sets. F_{ST} was significant in the concatenated data set, however (0.0125, P = 0.0402), and in four of the individual genes (Table S4).

Estimates of differentiation between the current management stocks were not statistically significantly different within the offshore subspecies (NE and WS stocks) using the whole mitogenome data, but were significantly different with F_{ST} for four of the individual genes. Using Φ_{ST}, no significant differences were observed between the coastal subspecies and either the NE or WS offshore stocks, however, F_{ST} was significant for both (concatenated and 4–5 individual genes).

Within the WS offshore stock, Φ_{ST} (0.17) between the southern and western offshore regions show significant differentiation (P = 0.067) for the concatenated mitogenome and ten individual mtDNA genes. The western portion of the WS stock was also differentiated from the NE stock). The only significant differences between the NE stock and the southern portion of the WS stock were in F_{ST} of three individual genes (Table S4).

There was very limited signal of differentiation between the coastal subspecies and either of the two portions of the western-southern offshore stock. The southern portion was significantly differentiated only by F_{ST} (0.026) of the concatenated data, and the western portion differed only in Φ_{ST}

and F_{ST} of four and five individual genes, respectively. Ideally, we would have partitioned the coastal subspecies south of central Mexico into the population units described by Escorza-Triveño *et al.* (2005), but our smaller sample size precluded this.

Significant Φ_{ST} differentiation was detected between Hawaii and all other stocks using the concatenated data set (see Table 3). ATP was the only gene in these comparisons that did not show significant differences in at least one pairwise test. In some individual gene comparisons, some strata had fewer than five individuals, leading to both low power and high variability in estimates of the test statistics.

Finally, we tested hypotheses of differences between Hawaii and divided western and southern portions of the WS stock. Hawaii and the western portion were differentiated using the concatenated data set (Φ_{ST} = 0.4932, P = 0.0179). Eleven individual genes showed differentiation between these two strata (see Table 3). Hawaii and the southern portion of the WS stock were not differentiated based on our concatenated data sets, but did show significant differentiation in seven individual genes

($P < 0.1$). Again, some of the tests for genetic structure with individual genes were conducted with strata composed of fewer than five individuals.

Discussion

Spinner and spotted dolphins in the eastern tropical Pacific offer a unique opportunity to study genetic differentiation at multiple scales in species with intraspecific morphological differences. Recent divergence, high genetic diversity, large population sizes, and ongoing gene flow likely contribute to low power to detect genetic differentiation (Taylor and Dizon 1996, Waples 1998, Galver 2002, Escorza-Treviño *et al.* 2005, Andrews *et al.* 2013). Using complete mitogenomes, we find some genetic support for endemic subspecies of spinner and spotted dolphins, although the strength of this support varied (Table 4). We did not find support for the division of offshore stocks of spotted dolphins, though there was significant differentiation when only the western portion of the WS stock was compared to the NE stock. We also did not find separation between the Tres Marias spinner dolphins and the eastern spinner dolphin subspecies. In contrast, nuclear SNP

analysis recovered these stock-level differences (Leslie and Morin 2016). The difference in our findings compared to those of Leslie and Morin (2016) could reflect the limitations of our mtDNA data or something biologically meaningful about the populations.

Spinner Dolphins

Traditional F_{ST} was very low as expected (Dizon *et al.* 1994, Galver 2002, Andrews *et al.* 2013, Leslie and Morin 2016), but supported endemic subspecies distinction (Central American and eastern). We found nonsignificant results from $\Phi_{ST,}$ a metric that weights frequency differences by the genetic distances among sequences. Thus, we conclude that haplotypes within these two subspecies are very similar, but that haplotype frequencies are significantly different. Nevertheless, our results provide evidence of genetic differentiation between the accepted ETP endemic subspecies concordant with morphology (Perrin *et al.* 1991), and nuclear DNA (Andrews *et al.* 2013, Leslie and Morin 2016).

F_{ST} and Φ_{ST} tests for differentiation between whitebellies and Central American spinners, using the mitogenome data set,

indicated differentiation concordant with the nuclear SNP differentiation (Leslie and Morin 2016). In our mitogenome data set, every whitebelly sample had a unique haplotype, meaning F_{ST} was likely underestimated, as variance of the haplotype frequencies is underestimated due to inadequate sampling (Meirmans and Hedrick 2011; see further discussion below). Using slightly different samples, Andrews *et al.* (2013) also found differentiation between Central American and whitebelly spinners using control region and cytochrome *b* sequences. However, Andrews *et al.* (2013) included 10 samples of Central American spinners that had questionable subspecific assignment in the field (based on further investigation of the sample collection records at SWFSC by MSL). Removal of these samples reduced our representation of Central American spinners (n = 9), but intraspecific structure was still detected.

We detected significant differentiation between whitebelly and eastern spinner using Φ_{ST} but not F_{ST}. These differences are supported by those found in the analysis of SNP loci between the same groups (Leslie and Morin 2016). Andrews *et al.* (2013) did not find structure between these two groups using any of the

markers they examined, and inferred low levels of gene flow between whitebelly and eastern spinner dolphins (30.1 migrants per generation from whitebelly to eastern and 57.9 migrants from eastern to whitebelly) using mtDNA and nuclear intron sequences. These results are not in disagreement with the structure we detected, as it is possible that there is both ongoing geneflow and population structure between these groups.

Although not a major focus of our study, the differences we detected between the Hawaiian population and the ETP pelagic populations were higher (in terms of F_{ST} and Φ_{ST}) than any comparisons within the ETP, supporting the hypothesis that this is an insular population or possibly subspecies. Andrews *et al.* (2013) found significant differentiation between all comparisons with Hawaiian spinner dolphins. In addition, these authors found lower, but significantly different from zero, migration rates between populations of pantropical (Hawaiian and other Pacific Island groups) and whitebelly spinners (3.22 migrants per generation into pantropical and 1.6 into whitebelly spinners). The rate of migration into pantropical spinner populations from the eastern population was estimated to be less than one (0.82),

but significantly different from zero. Because the statistical power to estimate levels of migration between very large populations with low relative sample sizes is weak (Waples 1998, Taylor *et al.* 2000), we did not attempt to estimate migration rates with these data.

Our mtDNA results do not show the overall differences detected using genome-scale nuclear DNA sequencing (Leslie and Morin 2016). A number of factors could contribute to this pattern. Breeding biology and movement patterns could affect the degree of differentiation observed in different markers between the Central American, whitebelly, and eastern spinner dolphins. In particular, assortative mating can decrease N_e, which could serve to amplify signal of structure in the nuDNA genome. The eastern spinner dolphin is thought to have a polygynous mating system, wherein relatively few males are involved with mating, which would serve to reduce N_e and potentially increase genetic structure (Perrin and Mesnick 2003). Conversely, a skewed breeding system might also increase dispersal, as adult male dominance might promote movements of juvenile males, which then become established breeders outside their natal range.

Unfortunately, little is known about the movement patterns of individual dolphins in the ETP (Scott and Chivers 2009), and less is known about differences in movement based on sex. High site fidelity in males could also restrict male-mediated gene flow between populations and increase nuDNA differentiation. Different degrees of male site fidelity have been found in delphinids (*e.g.,* Möller and Beheregaray 2004, Sprogis *et al.* 2016), but this has not been studied spinner or spotted dolphins.

Support for a unique Tres Marias population (*e.g.*, Perryman and Westlake 1998) differing from the Central American subspecies was found in the concatenated mitochondrial gene data sets using the F_{ST} ; Φ_{ST} tests did not support differentiation. None of our tests showed significant differences between the eastern subspecies and the Tres Marias group. Given the small genetic differences we found between the accepted endemic subspecies with much more marked morphological differences, this result may not be surprising. Although the Tres Marias spinner dolphins are morphologically distinct, it is unclear from our genetic data whether their difference rises to the level of

subspecies (Taylor *et al.* 2017). Future studies should approach this question using larger sample sets and additional data.

Spotted Dolphins

Spotted dolphin mitogenomes have lower haplotypic diversity and higher nucleotide diversity than spinner dolphins, despite double the historical population sizes in the former (Wade *et al.* 2007). We did not test for significance of these two measures between the two species, and thus cannot conjecture on the potential reasons for this pattern; however, this might be an interesting avenue for possible future research comparing past demographic histories (*e.g.*, Vijay *et al*. 2018).

Similar to our findings for spinner dolphins, traditional F_{ST} analyses support differentiation of the offshore *S. a. attenuata* and the endemic coastal *S. a. graffmani* subspecies, whereas Φ_{ST} failed to identify differences, either for the entire mitogenome or within any single gene. The finding of haplotype frequency differences between the NE offshore stock and coastal subspecies is counter to the results found by Escorza-Treviño *et al.* (2005), which suggested a connection between the NE stock and the coastal subspecies based on seven microsatellite loci.

In that study, the authors detected population structure between offshore pantropical spotted dolphins and coastal spotted dolphins except in comparisons between the offshore group and the coastal subspecies off northern Mexico. The study also showed gene flow between the coastal and offshore subspecies in northern Mexico. Escorza-Treviño *et al.* (2005) used different sampling (including more samples from the northern portion of the coastal spotted dolphin range) and markers (*i.e.,* biparentally inherited microsatellites *vs*. the maternally inherited mitogenomes), but it is entirely possible that population structure and gene flow cooccur between these groups. Certainly, significant F_{ST} values cannot be interpreted as an indication of no gene flow between groups.

A main objective of this work was to test for difference between existing (NE, WS, and coastal) and proposed (independent W and S) management stocks. Using the whole mitogenome data set, we found no evidence for differentiation between the two current offshore stocks (NE and WS), which could be due to current genetic connectivity or because our data lack power to detect differentiation at this fine scale (due to low sample size

relative to abundance and high mtDNA haplotypic diversity). There was differentiation between the western portion of the WS stock and the southern portion, and between the western portion of the WS stock and the NE stock. The NE stock and the offshore southern group were not significantly different in any test, suggesting that the distributional hiatus at 5º north is not a barrier to gene flow. However, we note that our sample size for the southern portion was small (n = 9), which likely means we had low power to detect differences if they truly exist.

Leslie and Morin (2016) found significant nuclear divergence between the offshore and coastal spotted dolphin subspecies, but did not include data from individuals from the NE offshore stock of spotted dolphins. Therefore, this comparison includes animals from the most geographically separate portions of the offshore (WS) and coastal subspecies range. Additional nuclear data from the NE stock are needed to determine whether proximate populations of these two subspecies are also as genetically divergent.

Overall Patterns

Our mitogenome results show subtle patterns of population

genetic structure in these two species in this region despite strong morphological differences. The levels of divergence we measured with these new data were smaller than what we expected. Increased sequence length (*via* the whole mitogenome data) may still not provide enough statistical power to detect differences arising from recent divergence where there is continued low-level gene flow and/or high diversity due to historically abundant populations. Our initial hypothesis was that sequencing more of the mitogenome would split haplotypes shared between populations, which would then be reflected in frequency-based statistics. F_{ST} indicates genetic structure when haplotype frequencies are similar within populations and different between populations (such as those that would result *via* drift in small populations; Excoffier *et al.* 1992). However, when overall haplotype diversity is high, very large sample sizes are needed to accurately characterize haplotype frequencies (Excoffier *et al.* 1992). In this situation, point values of F_{ST} will be underestimated (Meirmans and Hedrick 2011). In other words, one drawback of using longer sequences (*e.g.*, more base pairs) in studies of populations with high genetic diversity is that the

discovery of new haplotypes may not reach a plateau without substantial increases of sample size. In addition, adding base pairs should have increased the genetic distance between haplotypes (as measured by Φ_{ST}). However, this may have had little impact in our study because increased genetic distance between haplotypes (found in different populations) was likely small relative to the genetic distances found between haplotypes within populations (the latter increasing faster than the former). Moreover, sampling effects can become important drivers of F_{ST} beyond the base frequency of alleles present and result in false positive results (Excoffier *et al.* 1992).

Although not the magnitude we expected, we found significant differences between subspecies of both spinner and spotted dolphins using F_{ST}, but not Φ_{ST}. F_{ST} and Φ_{ST} provide slightly different perspectives on population differentiation and we believe it is important to present both measures. Our results show inconsistencies between these two metrics, which does not necessarily mean analytical problems or inaccuracies, but reflects something interesting about our data. F_{ST} tests for population differentiation are based on haplotype frequencies

and do not provide direct insights into levels of molecular divergence (Weir and Cockerham 1984, Excoffier *et al.* 1992, Meirmans and Hedrick 2011). Φ_{ST} estimates capture more information regarding differentiation due to sequence divergence in addition to differences in haplotype frequencies. The high diversity issues mentioned above can still affect Φ_{ST}. We have provided results and discussion on Φ_{ST} and F_{ST} to compare the two metrics.

In addition, our analyses resulted in several negative Φ_{ST} values. Pairwise values that are below zero result from a high frequency of individuals in one population that are also closely related to individuals in another population, without being fully outbreeding. When this occurs, the null distribution shifts more negative than it would be with actual population structure because random permutations will shift individuals away from other closely related individuals. Despite being negative, these distributions can still have low *P*-values in significance tests.

Given the subtle patterns distinguishing these groups noted in previous research, we accepted *P*-values greater than the

customary 0.05 as significant ($P < 0.1$). In doing so, we accepted a higher degree of possible error. In a study such as this, the use of hypothesis tests and focus on *P*-values is necessary, but it is worth reiterating that nearly all of the F_{ST} values were low, indicating that these populations are closely related. The tests for significance are an important way to delimit populations, but they should not be interpreted in a vacuum. In other words, despite being significantly different, the ETP populations are likely interbreeding (or have interbred in the recent past). Moreover, researchers should use additional lines of evidence to define management units in cases such as this where the metrics of population genetic structure have lower power. In addition, because of the difficulty accessing these remote areas, and importance of accurate sample assignment, our sample sizes were low in some partitions. This could result in the allele frequencies of populations being poorly characterized, which could skew results in uncertain ways. Small sample sizes tend to lead to a lack of power, which is seen in nonsignificant results between strata with low levels of gene flow that are truly demographically independent

populations (Avise 1995, Taylor and Dizon 1996). Efforts should be made to collect more samples for future studies.

The discordance in magnitude of signal we observed between the mitogenome results and those using nuDNA data (Leslie and Morin 2016) could also reflect biological factors. One possibility is female-mediated exchange diluting the signal of structure in mtDNA or male site-fidelity increasing structure in the mtDNA. Although there is some evidence from radio tagging studies that spinner and spotted dolphins can move relatively large distances (Perrin *et al.* 1979), a thorough investigation into the differences between sexes is lacking. At least for spinner dolphins it is likely that the polygynous breeding system described by Perrin and Mesnick (2003) would contribute to increased signal of structure in the nuclear genome.

One clear pattern of differentiation in the mitogenomes is between ETP stocks and Hawaii. Interestingly, there were genes within the mitogenome that showed a lesser degree of structure, or none at all between these groups. This might prove to be a useful model for examining the mechanisms driving patterns of structure between large pelagic populations in different highly

linked genes. In particular, future studies might want to ask why, in both species, ATP8 consistently had less evidence of population structure between these groups compared to the rest of the mitogenome.

Finally, all of the mtDNA regions with significant Φ_{ST} were found to be under purifying selection (negative Tajima's *D*, Table S5; and nonsignificant *Z*-tests, Table S6) indicating that the within-mitogenome differences are accumulating by neutral drift rather than *via* positive selection in ETP spinner dolphins. Positive selection in spotted dolphins was not examined because there was no evidence for structure between the two ETP subspecies in individual mtDNA genes.

Conclusions

Defining population genetic structure is challenging for pelagic species with large historical population sizes and high mobility. These populations may retain high genetic variation even as abundance becomes relatively low, which could obscure signals of genetic structure used to designate stock boundaries for estimating population abundance and setting stock-specific mortality limits. Ultimately, without information on structure,

populations could be underclassified and unique evolutionary units and populations could suffer losses of genetic diversity that make them less able to adapt to changing conditions. Alternatively, there is a cost to managing populations as separate when there is no biological basis to do so. Such errors can have economic, social, and political consequences resulting from unnecessary restrictions on human activity. Furthermore, a consistent pattern of these errors will "stiffen the resolve of skeptics and make it difficult to accomplish sound resource management in the future" (Waples 1998).

This unique system of two historically abundant, pelagic delphinids, with available samples collected *in situ* from remote offshore environments encompassing extensive geographic and morphological variation, was used to test for population genetic structure at multiple hierarchical taxonomic levels in species with high intraspecific morphological variation. Our results show a complex pattern of genetic structure within each species. Although complex, the subtle signatures of structure are important findings. The mitogenome data show support for the endemic ETP spinner and spotted dolphin subspecies (in F_{ST} but

not Φ_{ST}). However, increasing the amount of mitochondrial data did not overwhelmingly increase our ability to delimit population units. For mtDNA specifically, researchers working in similar systems (large N_e, high gene flow, pelagic organisms) should consider sequencing more individuals as well as adding longer sequence.

We found no support for the division of offshore stocks of spotted dolphins and only weak support for the unique form of Tres Marias spinner dolphins as compared to the eastern or Central American subspecies. This is not to say that these biological entities do not exist, just that our mtDNA data do not support them or may not have sufficient power to detect the subtle genetic differences between them. Because our sample size for the Central American spinner dolphin subspecies is low, we recommend the collection and analysis of additional samples to compare to existing offshore subspecies samples collected from fisheries bycatch and research cruises.

Management Implications

What does this mean for managing the recovery of ETP dolphin stocks? This work, combined with recent work by Andrews

et al. (2013) and Leslie and Morin (2016), has improved our understanding of ETP spinner and spotted dolphin population genetic structure. The management units already in place are supported by a large nuclear SNP data set that also supports the addition of a new Tres Marias spinner dolphin stock. Results from our mitogenome analyses were not as clear-cut. However, the results do support the current subspecies and stocks of spinner dolphins and the subspecies of spotted dolphins, albeit with the caveats discussed. Additional studies on movements and habitat modeling may provide additional clarity into stock-level boundaries, as they might help determine the degree of ongoing gene flow and the movement of stock boundaries over time. Further studies of population structure should try to incorporate environmental variables to better describe potential population boundaries in this area. Such an approach might be more accurate than using fixed latitude and longitude and could be incorporated into adaptive management strategies. Finally, researchers and managers should focus on other possible causes for the lack of recovery in ETP pelagic dolphins, including the possibility that ongoing fishing activities may be having a

negative impact on reproduction (Archer et *al.* 2010, Noren 2013, Noren and West 2017).

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Supporting Information

The following supporting information is available for this article online at http://

Table S1. Sample information and mitochondrial genome haplotype assignment for *S. longirostris*. The Genbank accession numbers ("Genbank #") are for mitochondrial genomes.

Table S2. Sample information and mitochondrial genome haplotype assignment for *S. attenuata*. The Genbank accession numbers ("Genbank #") are for mitochondrial genomes.

Table S3. Pairwise divergence estimates for subspecies and

stocks of spinner dolphins based on partitioned mitogenomic data (F_{ST} only).

Table S4. Pairwise divergence estimates for subspecies and stocks of spotted dolphins using partitioned mitogenomic data (F_{ST} only).

Table S5. Results from Tajima's neutrality test for spinner dolphin mtDNA loci. Samples were not partitioned into populations for this test. Conducted in Mega 5 (https://www.megasoftware.net/).

Table S6. Results from Codon-based test of positive selection for analysis averaging over all mitochondrial DNA sequence pairs within spinner dolphin groups. Test statistic is shown in the *Z* column. Probability of rejecting the null hypothesis (dN = dS) in favor of the alternative hypothesis (dN > dS) is shown as the *P*-value.

Figure 1. Sampling localities and range map for spinner dolphins within the ETP. Subspecies and stock boundaries based on Perrin *et al*. 1985. Red dots indicate Central American spinners. Blue symbols indicate eastern spinners; boxes are the proposed Tres Marias form. Note that for some analyses these two strata are combined (n = 53) as they are both classified as eastern spinners. Green dots indicate whitebelly spinners, a proposed intergrade between the pantropical (orange diamonds) and the eastern subspecies. mtDNA sample sizes are in the legend.

Figure 2. Sampling localities for spotted dolphins with ETP subspecies and stock boundaries based on Perrin *et al.* 1985. Coastal spotted dolphins (*S. a. graffmani*) are in red and offshore (*S. a. attenuata*) are in blue. Blue circles indicate sampling locations for the northeastern stock of offshore spotted dolphins. Blue triangles indicate samples from Hawaii. Inverted triangles indicate southern offshore samples that were removed from analyses of offshore stocks because they were collected between 4ºN and 6ºN; these samples were included in subspecies-level analyses. Animals that represent the western

substock were the group of blue squares west of 120°W and animals representing the southern sub-stock were the group of blue squares taken from south of the 5°N stock boundary. Samples sizes for mtDNA analyses are presented in the legend.

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Table 1. Summary statistics for ETP spinner (A) and spotted (B) dolphin mitogenome data. n_H: number of haplotypes; PS: polymorphic sites; *h*: haplotype diversity; π: nucleotide diversity; %: percent of unique haplotypes.

A. Spinner dolphins *Stenella longirostris* (*n* = 104).

Subspecies/stock	*n*: female/male/unknown	n_H	PS	*h*	π	%
Central American *S. l. centroamericana*	9:4/4/1	8	648	0.9722	0.0057	0.7778
Eastern[a] *S. l. orientalis*	53: 28/19/6	51	648	0.9985	0.0073	0.9245
Putative stocks						
Whitebelly *S. l. longirostris*	27: 16/11/0	27	457	1	0.0043	1
Tres Marias[a,b] *S. l. orientalis*	21: 8/10/3	20	373	0.9952	0.0078	0.9048
Hawaii *S. l. longirostris*	15: 1/4/10	9	104	0.9921	0.0068	0.8260

B. Spotted dolphins *Stenella attenuata* (*n* = 76).

Subspecies	*n*: female/male/unknown	n_H	PS	*h*	π	%

Coastal *S. a. graffmani*	24: 11/13/0	16	234	0.9529	0.0162	0.5000
ETP offshore[c] *S. a. attenuata*	47: 20/19/8	43	519	0.9804	0.0198	0.7222
Offshore stocks (*S. a. attenuata*): current and putative.[b]						
Northeastern	25: 10/8/7	22	400	0.9867	0.0238	0.8000
Western-southern	17: 9/7/1	17	298	1	0.0096	1
Offshore western[b]	8: 7/1/0	8	191	1	0.0087	1
Offshore southern[b]	9: 2/6/1	9	253	1	0.0092	1
Hawaii	5: 1/3/1	3	36	0.7000	0.0244	0.4000

[a]The Tres Marias spinner samples are part of the eastern stratum.

[b]Stocks that are not recognized for management purposes.

[c]Includes data for five samples that were omitted from stock comparisons because they were sampled too close to geographic stock boundaries.

Table 2. Pairwise divergence estimates for subspecies and stocks of spinner dolphins based on concatenated mitogenome data (F_{ST} and Φ_{ST}) and partitioned mitogenomic data (Φ_{ST} only).

Taxon 1 (*n*) *vs.* Taxon 2 (*n*)	Concatenated mitogenome		Partitioned mitogenome Φ_{ST}													
	F_{ST}	Φ_{ST}	12S n_H = 24	16S n_H = 24	ATP6 n_H = 53	ATP8 n_H = 11	COI n_H = 65	COII n_H = 39	COIII n_H = 47	CYTB n_H = 61	ND1 n_H = 59	ND2 n_H = 53	ND3 n_H = 21	ND4 n_H = 56	ND4L n_H = 22	ND5 n_H = 70
Central American (9) *vs.* eastern (53)	0.0133 (0.034)[b]	−0.0127 (0.5235)	−0.0120 (0.5265)	0.0061 (0.4977)	−0.0076 (0.4001)	0.0590 (0.0801)[a]	−0.0276 (0.8640)	−0.0199 (0.6988)	−0.0158 (0.5766)	−0.0094 (0.4711)	0.0148 (0.2501)	−0.0260 (0.7376)	0.0338 (0.1325)	−0.0287 (0.6950)	−0.0268 (0.7444)	−0.0139 (0.5368)
Central American (9) *vs.* whitebelly (27)	0.0128 (0.056)[a]	0.0490 (0.0542)[a]	−0.0165 (0.5882)	0.0217 (0.1947)	0.0311 (0.1277)	0.1279 (0.0189)[b]	0.0351 (0.0903)[a]	0.0936 (0.0144)[b]	0.0086 (0.2995)	0.0601 (0.0456)[b]	0.0844 (0.0362)[b]	0.0113 (0.2833)	0.1505 (0.0054)[c]	0.0870 (0.0464)[b]	0.0478 (0.0931)[a]	0.0273 (0.1203)
Eastern (53) *vs.* whitebelly (27)	0.0007 (0.2867)	0.0181 (0.0741)[a]	0.0307 (0.0414)[b]	0.0159 (0.0835)[a]	0.0051 (0.2421)	−0.0065 (0.5546)	0.0264 (0.0288)[b]	0.0342 (0.0152)[b]	−0.0020 (0.4501)	0.0154 (0.1165)	0.0260 (0.0468)[b]	0.0104 (0.1687)	0.0638 (0.0018)[c]	0.0464 (0.0422)[b]	0.0343 (0.0214)[b]	0.0026 (0.2859)
Tres Marias (21) *vs.* Central American (9)	0.0155 (0.0914)[a]	−0.0345 (0.7576)	0.0113 (0.2921)	−0.0283 (0.7240)	−0.0436 (0.7284)	−0.0082 (0.2863)	−0.0393 (0.8636)	−0.0318 (0.7150)	−0.0301 (0.6752)	−0.0238 (0.5872)	−0.0158 (0.5219)	−0.0451 (0.8698)	0.0022 (0.4025)	−0.0558 (0.8900)	−0.0638 (0.9470)	−0.0383 (0.7888)
Tres Marias (21) *vs.* eastern (32)	0.0009 (0.4107)	−0.0116 (0.7084)	−0.0109 (0.6474)	−0.0217 (0.9462)	−0.0088 (0.5169)	0.0019 (0.3119)	−0.0124 (0.7654)	−0.0182 (0.8772)	−0.0150 (0.8116)	−0.0062 (0.5291)	−0.0117 (0.6898)	−0.0031 (0.4447)	−0.0206 (0.8894)	−0.0185 (0.7898)	−0.0049 (0.4887)	−0.0105 (0.6442)
Tres Marias (21) *vs.* whitebelly (27)	0.0024 (0.1934)	0.0263 (0.0807)[a]	0.0421 (0.0643)[a]	0.0111 (0.1979)	0.0086 (0.2423)	0.0175 (0.2421)	0.0323 (0.0519)[a]	0.0406 (0.0362)[b]	-0.0005 (0.3907)	0.0311 (0.0765)[a]	0.0359 (0.0636)[a]	0.0243 (0.1087)	0.0676 (0.0052)[c]	0.0859 (0.0789)[a]	0.0485 (0.0448)[b]	0.0124 (0.1807)
Hawaii (15) *vs.* whitebelly (27)	0.0456 (0.0001)[c]	0.1964 (0.0002)[c]	0.0236 (0.1667)	0.3590 (0.0002)[c]	0.2560 (0.0002)[c]	−0.0127 (0.6582)	0.1964 (0.0002)[c]	0.1885 (0.0006)[c]	0.0154 (0.1363)	0.1031 (0.0004)[c]	0.0818 (0.0026)[c]	0.3302 (0.0002)[c]	0.4467 (0.0002)[c]	0.1324 (0.0002)[c]	−0.0137 (0.2197)	01858 (0.0002)[c]
Hawaii (15) *vs.* eastern (53)	0.0449 (0.0001)[c]	0.1849 (0.0002)[c]	0.0428 (0.0605)[a]	0.3293 (0.0002)[c]	0.2268 (0.0002)[c]	−0.0002 (0.4031)	0.2061 (0.0002)[c]	0.2104 (0.0002)[c]	0.0338 (0.0625)[a]	0.1182 (0.0026)[c]	0.1406 (0.0012)[c]	0.3090 (0.0002)[c]	0.3283 (0.0002)[c]	0.1339 (0.0025)[c]	0.0170 (0.1643)	0.1494 (0.0007)[c]
Hawaii (15) *vs.* Central Amer. (9)	0.0636 (0.0219)[b]	0.3284 (0.0002)[c]	−0.0083 (0.4045)	0.5265 (0.0002)[c]	0.3328 (0.0004)[c]	0.1600 (0.0631)[a]	0.3983 (0.0002)[c]	0.4280 (0.0002)[c]	0.1415 (0.0034)[c]	0.2474 (0.0002)[c]	0.3091 (0.0002)[c]	0.4352 (0.0004)[c]	0.3863 (0.0002)[c]	0.2728 (0.0002)[c]	0.1597 (0.0701)[a]	0.2854 (0.0004)[c]
Hawaii (15) *vs.* Tres Marias (21)	0.0487 (0.0004)[c]	0.2260 (0.0002)[c]	0.0796 (0.0478)[a]	0.3900 (0.0002)[c]	0.2552 (0.0002)[c]	0.0339 (0.2507)	0.2576 (0.0002)[c]	0.2351 (0.0002)[c]	0.0703 (0.0272)[b]	0.1398 (0.0004)[c]	0.1958 (0.0002)[c]	0.3454 (0.0002)[c]	0.3608 (0.0002)[c]	0.1667 (0.0004)[c]	0.0630 (0.0669)[a]	0.1828 (0.0002)[c]

Note: *P*-values in parentheses.

[a]*P* < 0.1.

[b]*P* < 0.05.

[c]*P* < 0.01.

Table 3. Pairwise divergence estimates for subspecies and stocks of spotted dolphins using concatenated mitogenome data (F_{ST} and Φ_{ST}) and partitioned mitogenomic data (Φ_{ST} only). n_H listed below each gene name is the number of haplotypes for that gene.

Taxon 1 (*n*) *vs.* Taxon 2 (*n*)	Concatenated mitogenome		Partitioned mitogenome Φ_{ST}													
	F_{ST}	Φ_{ST}	12S $n_H = 6$	16S $n_H = 7$	ATP6 $n_H = 20$	ATP8 $n_H = 5$	COI $n_H = 21$	COII $n_H = 13$	COIII $n_H = 11$	CYTB $n_H = 20$	ND1 $n_H = 15$	ND2 $n_H = 17$	ND3 $n_H = 10$	ND4 $n_H = 23$	ND4L $n_H = 2$	ND5 $n_H = 29$
Coastal (24) vs. offshore (47)[a]	0.0125 (0.0402)[c]	−0.0091 (0.4961)	0.0089 (0.2553)	−0.0316 (0.9932)	−0.0149 (0.6788)	−0.0169 (0.7536)	0.0018 (0.3265)	−0.0133 (0.6182)	−0.0243 (0.8890)	−0.0143 (0.6067)	−0.0099 (0.5357)	−0.0198 (0.7610)	−0.0222 (0.9006)	−0.0042 (0.4085)	0.0041 (0.3023)	−0.0056 (0.4217)
Northeastern (25) *vs.* western-southern (17)	0.0045 (0.2099)	−0.0076 (0.4111)	−0.0014 (0.3779)	0.0079 (0.2841)	−0.0156 (0.5332)	0.0057 (0.2691)	−0.0194 (0.6164)	0.0003 (0.3637)	−0.0193 (0.5067)	−0.0211 (0.5423)	0.0086 (0.2585)	−0.0021 (0.3473)	−0.0068 (0.4139)	0.0039 (0.3077)	−0.0038 (0.3771)	−0.0187 (0.5574)
Coastal (24) *vs.* northeastern (25)	0.0302 (0.0002)[d]	−0.0082 (0.4405)	0.0032 (0.3375)	−0.0326 (0.8096)	−0.0204 (0.7070)	−0.0061 (0.4689)	−0.0007 (0.3651)	−0.0060 (0.4325)	−0.0271 (0.7540)	−0.0201 (0.6148)	0.0031 (0.3041)	−0.0119 (0.4797)	−0.0105 (0.4947)	0.0055 (0.2923)	0.0016 (0.3249)	−0.0125 (0.5309)
Coastal (24) *vs.* western-southern (17)	0.0144 (0.0884)[b]	−0.0342 (0.8102)	−0.0186 (0.5621)	−0.0356 (0.6598)	−0.0297 (0.7402)	0.0081 (0.3153)	−0.0313 (0.8118)	−0.0355 (0.8624)	−0.0449 (0.8950)	−0.0385 (0.8666)	−0.0392 (0.9142)	−0.0335 (0.7224)	−0.0393 (0.9112)	−0.0311 (0.7582)	−0.0360 (0.7624)	−0.0285 (0.6812)
Offshore southern (9) *vs.* offshore western (8)	0.0771 (0.2249)	0.1666 (0.0668)[b]	−0.1717 (0.0781)[b]	0.2129 (0.0618)[b]	0.1167 (0.1039)	0.1117 (0.0801)[b]	0.1229 (0.0743)[b]	0.1361 (0.0939)[b]	0.1816 (0.0767)[b]	−0.0471 (0.4611)	0.1767 (0.0575)[b]	0.1771 (0.0743)[b]	0.1155 (0.1183)	0.2148 (0.0394)[d]	0.1382 (0.1231)	0.1895 (0.0529)[b]
Northeastern (25) *vs.* offshore western (8)	0.0027 (0.4291)	0.1135 (0.0517)[b]	0.0853 (0.0945)[b]	0.1848 (0.0352)[c]	0.0728 (0.1223)	0.1128 (0.0252)[c]	0.0575 (0.1397)	0.1164 (0.0504)	0.1117 (0.0749)[b]	0.0064 (0.3259)	0.1525 (0.0372)[c]	0.1179 (0.0775)[b]	0.1142 (0.0697)[b]	0.1497 (0.0394)[d]	0.1309 (0.0689)[b]	0.0894 (0.0957)[b]
Northeastern (25) *vs.* offshore southern (9)	0.0073 (0.3755)	−0.0400 (0.7446)	−0.0242 (0.5728)	−0.0537 (0.8008)	−0.0468 (0.8162)	−0.0691 (0.9552)	−0.0291 (0.6287)	−0.0387 (0.7512)	−0.0491 (0.7828)	−0.0509 (0.8168)	−0.0379 (0.7394)	−0.0392 (0.7150)	−0.0551 (0.9540)	−0.0238 (0.5626)	−0.0694 (0.9756)	−0.0327 (0.6092)
Coastal (24) *vs.* offshore southern (9)	0.0255 (0.0762)[b]	−0.0130 (0.4065)	−0.0323 (0.5874)	−0.0277 (0.4713)	−0.0279 (0.5721)	−0.0147 (0.4071)	−0.0122 (0.4423)	−0.0079 (0.3971)	−0.0227 (0.4611)	−0.0579 (0.8690)	−0.0012 (0.3477)	−0.0419 (0.6714)	−0.0309 (0.5854)	0.0051 (0.3209)	−0.0160 (0.4301)	−0.0030 (0.3453)
Coastal (24) *vs.* offshore western (8)	0.0049 (0.4321)	0.0749 (0.1331)	0.1368 (0.0559)[b]	0.1366 (0.0855)[b]	0.0594 (0.1583)	0.1363 (0.0167)[c]	0.0751 (0.1281)	0.0406 (0.1953)	0.0769 (0.1535)	−0.0089 (0.3361)	0.0609 (0.1541)	0.0901 (0.1101)	−0.0372 (0.2059)	0.1067 (0.0881)[b]	0.0239 (0.2425)	0.0841 (0.1269)
Hawaii (5) *vs.* coastal (24)	0.1430 (0.0026)[d]	0.2773 (0.0208)[c]	0.4166 (0.0019)[d]	0.2176 (0.0572)[c]	0.2767 (0.0174)[c]	−0.0502 (0.5687)	0.4032 (0.0028)[c]	0.1687 (0.0762)[b]	0.2175 (0.0585)[b]	−0.2859 (0.0254)	0.3643 (0.0049)[d]	0.1575[e] (0.0947)	0.3085 (0.0042)[d]	0.2660 (0.0252)[c]	0.2811 (0.0202)[c]	0.2541 (0.0244)[c]
Hawaii (5) *vs.* offshore (47)	0.1181 (0.0006)[d]	0.1582 (0.0389)[c]	0.1806 (0.0422)[c]	−0.1282 (0.1107)	0.1882 (0.0352)[c]	−0.0459 (0.6156)	−0.2138 (0.0124)[c]	−0.0818 (0.1323)	0.1361 (0.0632)b	−0.0849 (0.1481)	0.2598 (0.0082)[d]	0.1146[e] (0.1449)	0.2609 (0.0054)d	0.1239 (0.0593)[b]	0.1689 (0.0545)[b]	0.1303 (0.0517)[b]
Hawaii (5) *vs.* northeastern (25)	0.0576 (0.2709)	0.1308 (0.0645)	0.1153 (0.0962)[b]	0.0809 (0.1793)	(0.1584 (0.0353)[c]	−0.0198 (0.4695)	−0.1981 (0.0206)[c]	0.0638 (0.1739)	0.1099 (0.1123)	0.1478 (0.0714)	0.2499 (0.0102)[c]	0.0869[e] (0.1279)	0.2751 (0.0051)[d]	0.0984 (0.1029)	0.1446 (0.0843)[b]	0.0951 (0.1355)

Hawaii (5) *vs.* western-southern (17)	0.2474 (0.0702)[b]	0.2273 (0.0238)[d]	0.2942 (0.0284)[b]	0.2139 (0.0774)[b]	0.2670 (0.0297)[c]	−0.0542 (0.8308)	0.2583 (0.0244)[c]	0.1353 (0.1133)	−0.1992 (0.0547)[b]	0.2259 (0.0286)[c]	0.3089 (0.0196)[c]	0.1793[e] (0.1473)	0.2751 (0.0234)[c]	0.1925 (0.0356)[c]	0.2673 (0.0342)[c]	0.2062 (0.0366)[c]
Hawaii (5) *vs.* offshore western (8)	0.4958 (0.0732)[b]	0.4932 (0.0179)[c]	0.4558 (0.0318)[c]	0.5298 (0.0168)[c]	0.4984 (0.0148)[c]	0.0285 (0.3925)	0.4640 (0.0119)[c]	0.4572 (0.0364)[c]	0.5036 (0.0352)[c]	0.0013 (0.4061)	0.5523 (0.0039)[d]	0. 4484[e] (0.0328)[c]	0.4915 (0.0033)[d]	0.5093 (0.0114)[c]	0.1309 (0.0689)[c]	0.0924 (0.1393)
Hawaii (5) *vs.* offshore southern (9)	0.1509 (0.2207)	0.1274 (0.1167)	0.3012 (0.0268)[c]	0.0306 (0.2757)	0.1750 (0.0718)[b]	0.0443 (0.1961)	0.2126 (0.0202)[c]	0.0036 (0.4437)	0.0161 (0.3045)	0.1384 (0.0872)[b]	0.2138 (0.0178)[b]	0.0039[e] (0.2641)	−0.0551 (0.0206)[c]	0.0808 (0.1389)	0.1382 (0.1231)	0.5038 (0.0198)[c]

Note: *P*-values in parentheses. “NA” indicates comparisons where Φ_{ST} could not be estimated because all individuals in both strata share the same haplotype.

[a] Includes data for five samples that were omitted from stock comparisons because they were sampled too close to geographic stock boundaries.

[b] $P < 0.05$.

[c] $P < 0.01$.

[d] $P < 0.001$.

[e] Where one stratum was $n < 5$.

Table 4. Summary table of pairwise comparisons using mtDNA and nuDNA data sets (sample sizes in parentheses). In the mtDNA column, a "✓" deno one measure (see Tables 2–4), "ns" = nonsignificant, "# Genes" is the number of significant mtDNA genes. For the nuDNA column, a "✓" denote Morin 2016).

	Taxon 1 (n_{mt}/n_{nuc})	Taxon 2 (n_{mt}/n_{nuc})	mtDNA		nuDNA
			Whole	# Genes	
Spinner dolphins					
Test of endemic subspecies	Central American (9/9)	Eastern (53/36)	✓	1	✓
Testing whitebelly intergrade	Central American (9/7)	Whitebelly (27/15)	✓	8	✓
Testing whitebelly intergrade	Eastern (53[a]/36)	Whitebelly (27/15)	✓	8	✓
Alternative stock hypotheses	Tres Marias (21/12)	Central American (9/9)	✓	0	✓
	Tres Marias (21/12)	Eastern (32/36)	ns	0	✓
	Tres Marias (21/12)	Whitebelly (27/12)	✓	8	✓

	Hawaii (15/0)	Whitebelly (27/0)	✓	10	NA
	Hawaii (15/0)	Eastern (32/0)	✓	12	NA
	Hawaii (15/0)	Central American (9/0)	✓	13	NA
	Hawaii (15/0)	Tres Marias (21/0)	✓	13	NA
Spotted dolphins					
Testing subspecies	Offshore all (47/13)	Coastal (24/27)	✓	0	✓[b]
Testing existing stocks	Offshore northeastern (25/15)	Offshore western-southern (17/16)	ns	0	✓
Testing existing stocks	Offshore northeastern (25/15)	Coastal (24/27)	✓	0	✓
Testing existing stocks	Offshore western-southern (17/16)	Coastal (24/27)	✓	0	✓
Alternative stock hypotheses	Offshore southern (9/0)	Offshore western (8/0)	✓	10	NA
	Offshore northeastern (25/0)	Offshore western (8/0)	✓	11	NA
	Offshore northeastern (25/0)	Offshore southern (9/0)	ns	0	NA
	Offshore southern (9/0)	Coastal (24/0)	✓	0	NA
	Offshore western (8/0)	Coastal (24/0)	ns	4	NA
	Hawaii (5/0)	Coastal (24/0)	✓	13	NA
	Hawaii (5/0)	Offshore (47/0)	✓	9	NA

	Hawaii (5/0)	Offshore northeastern (25/0)	✓	7	NA
	Hawaii (5/0)	Offshore western-southern (17/0)	✓	11	NA
	Hawaii (5/0)	Offshore western (8/0)	✓	11	NA
	Hawaii (5/0)	Offshore southern (9/0)	ns	7	NA

[a] Includes data from both the offshore eastern and the Tres Marias that are currently classified as part of the eastern subspecies.

[b] Escorza-Treviño *et al*. 2005.