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ARTICLE

Genetic data reveals wintering ground affiliation of humpback whales from the Mexican Central Pacific

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ABSTRACT

Two groups of humpback whales inhabit the waters off the Pacific coast of Mexico the coastal wintering aggregation in the north (MX), and the southern Mexico/Central America wintering aggregation (S-MX/CEA) in the south. However, along the coast of the Mexican Central Pacific (MCP), the population affiliation of humpback whales is uncertain. Some studies have concluded that the MCP whales are part of S-MX/CEA, while others have suggested that the MCP may represent an overlap zone between the two wintering aggregations. In this study, data from 354 biopsy samples were collected over a 12-year sampling period, to provide genetic information insight into the affiliation of MCP whales to and the boundaries between the wintering aggregations. Using mitochondrial control region sequences, we find that the majority (73%) of MCP whales are part of MX, but that the boundary between the two wintering aggregations may shift latitudinally depending on environmental conditions. The high haplotypic ($h \pm SD = 0.859 \pm 0.0138$) and nucleotide diversity ($\pi \pm SD = 0.0145 \pm 0.0075$) of the MCP whales are also consistent with our sample, including animals from both wintering aggregations. More research is needed to better describe the ranges of the MX and S-MX/CEA wintering aggregations to ensure their successful conservation and management.

KEYWORDS

Central America, Distinct Population Segment, environmental variability, Mexico, migration, mitochondrial DNA, mixed stock, sex ratio

1 | INTRODUCTION

Three subspecies of humpback whales (*Megaptera novaeangliae*), one in each of the main ocean basins, are recognized based on ecological indicators such as distribution, migratory movements, and genetics, along with their residency in feeding and/or breeding zones (Committee on Taxonomy, 2021; Gambell, 1976; Jackson et al., 2014). Humpback whales in the North Pacific Ocean (*Megaptera novaeangliae kuzira*; Jackson et al., 2014) are distributed between low-latitude wintering grounds and high-latitude feeding grounds (Baker et al., 1994; Dawbin, 1996; Gambell, 1976). Many decades of research (Baker et al., 1993, 1994, 2013; Barlow et al., 2011; Bettridge et al., 2015; Calambokidis et al., 2001, 2008; González-Peral, 2011; Medrano-González et al., 1995; Urbán R. et al., 2000) have led to recognition under the U.S. Endangered Species Act of four Distinct Population Segments (DPSs) in the North Pacific: (1) Central America, (2) Mexico, (3) Hawai‘i, and (4) Western North Pacific, which includes two independent wintering aggregations: (1) the Mariana Archipelago and (2) the Japanese/Philippines waters (Baker et al., 2013; Bettridge et al., 2015; Hill et al., 2020; Oleson et al., 2022).

Although Mexico is considered a single DPS, it includes two geographically distinct wintering aggregations (Figure 1): the

northern coastal Mexico population (MX), which is known primarily from Banderas Bay (BB), and the offshore population, which is found in the Revillagigedo Archipelago (RA) (Urbán R. et al., 2000). The distinctness of the coastal and offshore populations has been established through both genetic studies (Baker et al., 2013) and photo-identification analyses that show a low exchange of whales between both sites and differences in their feeding sites (Barlow et al., 2011; Calambokidis et al., 2001; Medrano-González et al., 1995; Urbán R. et al., 2000). Additionally, Baja California (BC) is also part of the Mexico DPS, but it has been suggested that BC functions as a migratory corridor for various wintering destinations (Baker et al., 2013; González-Peral, 2011; Lagerquist et al., 2008; Martínez-Loustalot et al., 2022; Urbán R. et al., 2000).

The Central America DPS is comprised of a single wintering aggregation. Its range was originally thought to be restricted to the Pacific coast of Central America (Panama, Costa Rica, Nicaragua, Honduras, El Salvador, and Guatemala; Figure 1). However, recent genetic and photo-identification analyses have suggested that its range extends into southern, and possibly central, Mexico (Martien et al., 2021; Martinez-Loustalot et al., 2020, 2022; Taylor et al., 2021). Significant mitochondrial DNA (mtDNA) genetic differentiation was observed when comparing

whales from the southern Mexico states of Guerrero (GRO) and Oaxaca (OAX) to BB and BC (in northern Mexico), but not when comparing to whales sampled off Central America (CEA) (Martínez-Loustalot et al., 2020). Photo-identification analysis showed rates of movement were high between Nicaragua and GRO/OAX, intermediate between BB, the central Mexico state of Colima (COL), and GRO/OAX, but low between RA and all other areas (Martínez-Loustalot et al., 2020). Martínez-Loustalot et al. (2022) noted that some of the apparent interchange between BB, COL, and GRO/OAX could result from animals being photographed off BB and COL during their migration to points further south. They concluded that the CEA wintering aggregation extends into the central and southern Mexico states of COL, and GRO/OAX, though they noted that the small sample size from COL precluded a firm conclusion regarding its affinity (Martínez-Loustalot et al., 2022).

In recent reviews of population structure for the purpose of management under the U.S. Marine Mammal Protection Act, Martien et al. (2021) and Taylor et al. (2021) concluded that the CEA wintering aggregation extends at least as far north as GRO/OAX, but that the wintering ground affiliation of whales off the coasts of Jalisco (JAL), COL, and Michoacan (MIC) is uncertain. Based on the available data, they suggested that the

MCP could represent an area of overlap between the MX and CEA wintering aggregations, perhaps with the proportion of whales from the two aggregations that use the area varying between years. They concluded that additional research efforts are needed to understand the geographic range of the MX and CEA wintering aggregations.

In this study we present mtDNA control region sequences from humpback whales sampled over a 12-year period in the Mexican Central Pacific (MCP; defined as the waters of the states of JAL, COL, and MIC; Figure 1). We compare these genetic data with previously published data generated from humpback whales sampled in BC, RA, BB, GRO/OAX, and CEA to assess the wintering ground affiliation of MCP whales. We use the term S-MX/CEA to refer to the wintering aggregation that occupies southern Mexico and Central America, and the term CEA to refer the geographic stratum off the coast of Central America (Panama through Guatemala; Figure 1). The results of this study provide important information to increase knowledge about the conservation status of humpback whales wintering in the Mexican Pacific, which include the Mexico and Central America DPSs that are listed as threatened and endangered, respectively, under the U.S. Endangered Species Act.

2 | MATERIALS AND METHODS

2.1 | Study area and sample collection

Coastal surveys were conducted during each wintering season (October to April) 2010–2021 along the coast of the MCP, and in 2014, 2017, 2018, and 2020 in Socorro Island, Revillagigedo Archipelago (RA, 18°47.611′N, 110°58.369′W; Figure 1; Table S1). We refer to each season by the calendar year in which it primarily occurred (e.g., the 2010 season was from October 2009 to April 2010). During each humpback whale sighting, photographs of the dorsal and caudal fins (when the latter was shown emerging from the water) were taken using a Canon 50D or 60D camera and a 70–300 mm Sigma lens for individual photo-identification (Katona et al., 1979) to discern between analyzed whales and avoid re-sampling or data duplicates from whales sampled in more than one season. The photo-identification data were analyzed and published by Ortega-Ortiz et al. (2022).

A total of 354 skin and blubber samples were obtained from photo-identified humpback whale, 344 from MCP and 10 from Socorro Island (labeled RA), were collected throughout the study area using special arrows with a biopsy tip (1.5 mm diameter) launched from a Barnett Panzer V crossbow. The tissue samples were stored in sterilized aluminum foil and preserved in liquid nitrogen. The samples from RA were only used for the wintering aggregation comparative analysis. The samples that showed a low

quality of DNA sequences (<80%, through Geneious Prime ver. 2021.1.1; https://www.geneious.com) as well as duplicate samples (i.e., calf samples as maternal replicates) or recaptures identified photographically were eliminated, leaving 257 biopsy samples for genetic analysis. In turn, for the determination of the sex ratio it was possible to analyze a larger number of samples (n = 282), since it was possible to include the sex of some individuals whose sample DNA quality was not limiting for this analysis.

2.2 | DNA extraction, mtDNA control region sequencing, and sex ratio determination

Genomic DNA was isolated from skin using a modified phenol-chloroform with CTAB protocol (Baker et al., 1994; Murray & Thompson, 1980). A partial fragment of approximately 600 base pairs (bp) of the mtDNA control region was amplified using the primers M13Dlp1.5 and Dlp8G (Garrigue et al., 2004). Each of the reactions was performed in a final volume of 12.5 µl containing the following components: 7.23 µl of nuclease-free water, 0.75 µl of 25 mM MgCl2, 0.66 µl of 10 mM of each dNTP, 2.5 µl of 5× Buffer Green, 0.26 µl of 100 µM of both primers, 0.1 µl of 5µ/µl Taq Polymerase and 1 µl of extracted DNA (approximately 3–480 ng/µl; through the Qubit 4 fluorometer). Conditions for the fragment amplification are presented in Table S2.

The PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega) following the manufacturer's guidelines. All purified amplicons were sent for sequencing in both directions using the Sanger sequencing method to Macrogen, Seoul, Korea. All sequences were manually edited and aligned using Geneious Prime ver. 2021.1.1. In each case, the sequences were checked for good quality, that considered greater than 80% provided by Geneious prime software ver. 2021.1.1 and an alignment of the forward and reverse sequences was carried out to obtain a consensus sequence and eliminate the extremes if they showed low quality. The consensus sequences were aligned by ClustalW through the parameters Gap Opening Penalty of 15 and Gap Widening Penalty of 6.66 for Pairwise Alignment and Multiple Alignment, and a transition weight of 0.5 with the software MEGA ver. 10.2.2 (Kumar et al., 2018). Sequences were trimmed to a size of 500 bp after aligning them to haplotypes registered in the North Pacific as reference (Baker et al., 2013).

The methodology of Palsbøll et al. (1992), which targets the SRY, ZFY, and ZFX genes, was used to determine the genetic sex of sampled whales. Each of the reactions was performed in a final volume of 12.5 µl in the same way as for the haplotype methodology, and the amplification parameters are shown in Table S2. For the ZFY and ZFX genes, a digestion with a TaqI

restriction endonuclease was performed to separate the restriction fragments of the two loci. Samples collected from whales identified as mothers with calves were used as a control for the identification of females.

2.3 | Statistical analyses

2.3.1 | Genetic diversity of MCP

The number of haplotypes was determined with the software DnaSP ver. 6.12.03 (Rozas et al., 2017). A Templeton-Crandall-Sing (TCS) haplotype network was generated using the software PopART ver. 4.8.4 (Clement et al., 2022; https://popart.maths.otago.ac.nz/) using a statistical parsimony approach to group the haplotypes into the "AE" and "CD" clades (monophyletic groups) defined by Baker et al. (2013). Additionally, to determine whether the haplotypes found in the MCP are unique, they were compared with the haplotypes previously recorded in the North and South Pacific (Baker et al., 2013; Martien et al., 2020; Olavarría et al., 2007) under a Bayesian inference analysis (Baker et al., 2013; Olavarría et al., 2007) using MrBayes ver. 3.2.1 (Ronquist et al., 2012) under the HKY + G evolutionary substitution model (computed with Mega ver. 10.2.2 (Kumar et al., 2018)) with two Markov Chain Monte Carlo (MCMC) simulations of over 25,000,000 generations carried out with sampling every 1,000 generations. The

appropriate burning value was determined by examining the standard deviation of split frequencies. A 50% majority rule consensus tree was constructed from all generations sampled after the burning and discarding 25% of the original samples. Sequences of *Balaenoptera physalus* (GenBank: AY582748) and *Balaenoptera musculus* (GenBank: NC_001601) were used as outgroup to root the tree (Figure S1). Haplotypic diversity ($h \pm SD$), nucleotide diversity ($\pi \pm SD$), and the frequency of the haplotypes were estimated with the software Arlequin ver. 3.5.2. (Excoffier & Lischer, 2010).

2.3.2 | Population genetic structure

Genetic differentiation among the sampling seasons in the MCP and among the geographically distinct areas was quantified using the software Arlequin ver. 3.5.2.2 (Excoffier & Lischer, 2010). We calculated both F_{ST}, which only accounts for haplotype frequencies, and Φ_{ST}, which also accounts for the genetic distance between haplotypes. We used Mega ver. 10.2.2 (Kumar et al., 2018) to determine the best substitution model for our data. We assessed statistical significance using a permutation test with 10,000 permutations. The data from 2016 were excluded from the sampling season comparisons due to low sample size (n = 3) as during that season, although sampling effort was similar to previous years, whale density was lower (Ortega-Ortiz et al.,

2022). For the comparison among geographically distinct areas, our data were combined with published sequences from Baker et al. (2013; *n* = 314; GenBank access KF477244-KF477271), Martien et al. (2020; *n* = 96), and Martínez-Loustalot et al. (2020; *n* = 174) (both sets of sequences were provided from the author/publication) to evaluate potential differences between the MCP in relation to the MX and S-MX/CEA wintering aggregations (Tables S1, S3).

We estimated the proportion of animals sampled in MCP that belonged to the MX and S-MX/CEA wintering aggregations using the MCMC mixed stock analysis described in Bolker et al. (2003). We combined the data from CEA and GRO/OAX to represent the S-MX/CEA source population (*n* = 130 number of samples) and used the data from BB (*n* = 109 number of samples) to represent the MX source population. We first ran the analysis using the entire MCP sample as the unknown population, then reran the analysis separately for each sampling year to examine temporal variation in the composition of the MCP animals. The analysis was not run for the 2016 samples due to small sample size (*n* = 3). In all runs, we used a chain length of 40,000 and default values for all other parameters. We tested convergence of the chains using the Gelman-Rubin criterion (Gelman et al., 1995). The mixed stock analysis was conducted in R software (R Core Team, 2022)

using the *mixstock* package version 0.9.5.1 (https://github.com/bbolker/mixstock). All scripts and data used in the analyses are available at https://github.com/kmartien/MCP_humpback_analyses.

We visually compared changes in the composition of MCP samples between years to changes in environmental conditions as reflected by the Oceanic Niño Index (ONI), which is the primary index used for tracking El Niño and La Niña events (ocean-atmosphere climate system). ONI values were retrieved using the R software with package *rsoi* (Albers, 2020).

To determine whether the whales sampled in the MCP in a given year could all belong to the same wintering aggregation, we used the group exclusion-assignment test (GELATo) developed by O'Corry-Crowe et al. (2015). GELATo assumes all of the individuals in an unknown sample (in our case the MCP whales sampled in a given season) came from the same source population and determines which known population is the most likely source. It does this by randomly partitioning the samples from a known population, K, into the groups K_u, whose sample size is equal to that of the unknown sample, and $K_{k'}$, which contains the remaining samples. F_{ST}-null is calculated between $K_{k'}$ and K_u, and F_{ST}-obs is calculated between $K_{k'}$ and the unknown sample. This process is repeated 1,000 times to generate distributions for F_{ST}-null and

F_{ST}-obs, and a Normal distribution is fitted to F_{ST}-null. The likelihood of the unknown sample having originated from *K* is calculated as the product of the likelihoods of all values in F_{ST}-obs given the fitted null distribution. The relative odds of known populations A or B being the source of the unknown sample is equal to the likelihood of A divided by the likelihood of B.

We ran GELATo separately for each sampling season for the MCP, including 2016 because GELATo accounts for sample size in the unknown sample. We again used the data from CEA and GRO/OAX to represent the S-MX/CEA source population and used the data from BB to represent the MX source population. We ran the analyses in R (R Core Team, 2022) using the *strataG* package version 2.5.01 (Archer, 2016).

3 | RESULTS

3.1 | Genetic diversity within MCP

From the 257 samples, a total of different 25 haplotypes were obtained, defined by 29 polymorphic sites (Table 1); these sequences have been submitted to GenBank (OQ383642-OQ383666). Of these 25 haplotypes, 23 were previously reported by Baker et al. (2013) and González-Peral (2011), while two were newly discovered in this study. The new haplotypes, MCP-E16 and MCP-F11, each differed from a previously published haplotype (E5 and F1, respectively) by a single transition. In both new

haplotypes, the forward and reverse sequences agreed. The position that distinguishes MCP-E16 from E5 had a Phred score of 20 in the forward sequence and 58 in the reverse, while the position that distinguishes MCP-F11 from F1 had Phred scores over 50 in both directions.

The number of total and shared haplotypes was higher in MCP (25 and 20, respectively), and lower in CEA (13 and 10, respectively). Haplotype diversity ($h \pm SD$) within the MCP was 0.859 ± 0.0138, while nucleotide diversity ($\pi \pm SD$) was 0.0145 ± 0.0075 (Table 2). The substitution model that best fit our data was the Tamura 3-parameter model (Tamura, 1992), which we used for all F_{ST} calculations.

3.2 | Clade distribution

The TCS network shows the haplotypes from the MCP grouped into the "AE" and "CD" clades defined by Baker et al. (2013; Figure 2). The larger clade is AE, which includes 18 haplotypes and 61.4% of the total samples. The new haplotype MCP_E16 falls within the AE clade. Clade CD is composed of seven haplotypes and 38.5% of the samples. It includes the new haplotype MCP_F11 (Figure 2).

3.3 | Temporal variation within MCP

During the sampling period, a shift in the haplotype frequencies was found. Between the years 2010 and 2015, the "A" haplotypes

(A−, A+, A3, A5, and A7) were observed in 27% of sampled animals, while from 2016 to 2020 that percentage dropped to only 5% before returning to 22% in 2021 (Figure 3, Table S1). The most notable difference was in the frequency of the A+ haplotype, which is common in BB (16%) but absent in CEA (Figure 4; Baker et al., 2013). This haplotype was also common in MCP in the years 2010–2015 (18%) and 2021 (17%), but rare or absent in the intervening years (2016–2020; Figure 3). The "F" haplotypes showed the opposite trend, comprising about one-third of the samples in 2010–2015 and 2021, but over half of the samples in 2016–2020 (Figure 3).

We found no significant genetic differentiation ($p > .05$) using F_{ST} or Φ_{ST} among the 2010–2015 seasons, or between any of those seasons and 2021. For the 2017–2020 seasons, the only significant differentiation was between 2017 and 2018, and only for F_{ST}. Approximately half of comparisons between the 2017–2020 seasons and other seasons did show significant differentiation using F_{ST}, Φ_{ST}, or both (Table 3). However, most of the significant comparisons involved either the 2010 or 2017 seasons, both of which had very low sample size.

3.4 | Comparison to surrounding regions

The expanded data set (n = 851), which included samples from this study (n = 257) and other published sequences (n = 584),

contained 29 haplotypes with 35 polymorphic sites. The overall haplotypic diversity ($h \pm SD$) was 0.8711 ± 0.0059, with a range between 0.6928 and 0.8948 (Table 2). The overall average nucleotide diversity ($\pi \pm SD$) was 0.0136 ± 0.0070, with a range between 0.0083 and 0.0149 (Table 2). Haplotypic diversity for MCP was comparable to that from BC, RA, and BB, and higher than from GRO/OAX and CEA. Nucleotide diversity, in contrast, was highest in CEA and generally declined towards the north (Table 2). Of the 29 haplotypes in the expanded data set, five were present in all regions, while nine were exclusive to a single region (Table 2). The MCP was the area with the highest number of haplotypes (25), while RA had the lowest number of haplotypes (12) (Figure 4). Additionally, CEA had the lowest number of shared haplotypes, while the RA, BC, and GRO/OAX did not possess exclusive haplotypes (Table 2).

Significant genetic differentiation ($p < .05$) was observed using both F_{ST} and Φ_{ST} in most pairwise comparisons of wintering aggregations. MCP and BB differed significantly when compared with all areas except each other (Table 4). Similarly, RA differed significantly from all areas except BC, and CEA differed significantly from all areas except for GRO/OAX (Table 4).

The mixed stock analysis indicated that, overall, the

median proportion of BB humpback whales in our MCP samples is 0.72, 95% CI [0.57, 0.95], with the remaining 0.28, 95% CI [0.05, 0.43] coming from S-MX/CEA. There was considerable variation between years in the composition of MCP, though 95% confidence intervals on most of the yearly estimates are quite large (Figure 5). The Gelman-Rubin criterion was close to one for all parameters in all analyses, indicating that the chains had converged. Figure 5 shows the yearly mixed stock analysis results overlayed on the ONI, which is the primary index used for tracking El Niño and La Niña events.

The log-likelihood estimates from the GELATo analysis were higher for the MX than for the S-MX/CEA wintering aggregation in 2010–2013, 2015, and 2021 (Table 5). For each of these years, the odds of the sample belong to MX were high (range 78.3–$9.8e^{22}$). Conversely, the S-MX/CEA wintering aggregation had high log-likelihoods in 2016, 2017, and 2020, with the odds of the MCP animals having come from MX in those years much less than 1 (range 0.002–0.031). The odds of the two wintering aggregations being the source of the MCP samples were approximately equal in the years 2014, 2018, and 2019 (range 0.237–1.878).

3.5 | Sex ratio composition

The sex ratio in general was close to one, with a slight bias towards males (1:1.2; 127 females and 155 males), which did not

differ significantly from the expected 1:1 (χ^2 = 2.78, p > .05). In the case of the sex ratio by season, a higher ratio of males was observed in the years 2013, 2015, 2018, 2019, and 2020 (leaving aside that the years 2016 and 2017 did not meet the number of samples necessary to perform a statistical analysis). However, they do not differ significantly from the expected 1:1 (Table S4).

4 | DISCUSSION

The present research is the first to describe the genetic diversity of humpback whales from MCP and to assess whether these whales are genetically more similar to the S-MX/CEA wintering aggregation or the MX wintering aggregation. The results of this 12-year consecutive time series provided insight into the geographic range of the wintering aggregations currently recognized for Mexican and Central America waters.

4.1 | Distributions of the Mexican and Central American Wintering Aggregations

Numerous studies have aimed to improve our understanding of the population structure of humpback whales in their wintering areas in the North Pacific (Baker et al., 1993, 1994, 2013; Barlow et al., 2011; Bettridge et al., 2015; Calambokidis et al., 2001; González-Peral, 2011; Martien et al., 2020; Martínez-Loustalot et al., 2020, 2022; Medrano-González et al., 1995; Urbán R. et

al., 2000, 2017). However, there are still considerable gaps in our knowledge (Hill et al., 2020; Lammers et al., 2023). Recently, the central and southern region of Mexico is considered an important region due to a lack of information regarding humpback whales. It has been demonstrated through genetic analysis that whales from GRO/OAX and CEA belong to the same wintering aggregation (Martínez-Loustalot et al., 2020). This conclusion is also supported by photo-identification analysis, in which there is a considerable amount of whale movement between GRO/OAX and CEA (Martínez-Loustalot et al., 2022). Thus, it has been determined that the CEA wintering aggregation extends north into Mexico at least as far as GRO/OAX.

Martínez-Loustalot et al. (2022) found that COL had relatively high rates of movement with both BB, to the north, and GRO/OAX and CEA to the south. Due to these mixed results and a low sample size from COL, they were unable to draw a conclusion regarding the wintering aggregation affiliation of whales off COL. The results from the present study demonstrated that humpback whales from MCP, including COL, are more genetically similar to whales from BB, which is part of the MX wintering aggregation, than they are to the whales from the S-MX/CEA wintering aggregation. We found significant genetic

differentiation between MCP and every other geographic location except BB (Table 4). However, the mixed stock analysis also showed that whales from both wintering aggregations use the waters off the MCP, with the majority of the animals we sampled (73%) belonging to the MX aggregation.

The Mexican coast functions as a migratory corridor for whales that have southern Mexico and Central America as a destination. Thus, it is possible that the S-MX/CEA individuals that we sampled offshore of MCP were all in transit and that only MX whales use the MCP as a wintering destination. However, our temporal analyses suggest this is not the case. We observed a marked shift in the haplotype frequencies observed over the course of our study. Between 2010 and 2015, frequencies were similar to those observed in BB (Figures 3 and 4), but in 2016 the sample changed to be more similar to that from GRO/OAX and CEA, before changing back in 2021. The fluctuation in haplotype frequencies apparent in Figure 3 is also supported by both the mixed stock analysis and the GELATo analysis, which both show that the whales sampled offshore of MCP were more likely to be part of the same population as BB in some years, and more likely to be part of the same population as GRO/OAX and CEA in others (Figure 5, Table 5). If MCP were part of the wintering ground of the MX aggregation and CEA whales only used it as a migratory

corridor, we would expect the relative frequencies of MX and CEA whales in our sample to be consistent through time. Rather, our results suggest the wintering ranges of the MX and CEA whales shift latitudinally from year to year, with the MCP occupied primarily by MX whales in most years and primarily by CEA whales in other years.

The latitudinal shift in the distributions of the MX and CEA wintering aggregations is likely related to environmental variability. Humpback whales have been shown to alter their behavior and distribution in response to environmental change (Fleming et al., 2016; Szesciorka et al., 2022). The change in haplotype frequencies we observed in 2016 coincided with a strong El Niño, as well as a marine heatwave event in the North Pacific (Di Lorenzo & Mantua, 2016; Holbrook et al., 2019). These climate anomalies resulted in shifts in the distribution of humpbacks on both feeding and wintering grounds around the world (Askin et al., 2017; Félix et al., 2020; Gabriele et al., 2022; Schall et al., 2021), including for the MX and CEA whales (Ortega-Ortiz et al., 2022; Pelayo-González et al., 2022). Sighting rates dropped precipitously in the 2016 season compared to other years in both Costa Rica, which is part of the CEA wintering area (Pelayo-González et al., 2022), and in COL (Ortega-Ortiz et al., 2022). In both cases, the authors

hypothesized that whales migrate further south in colder years, and end their migrations further north in warm years, like 2016.

Though our data suggest that a shift in the distributions of the MX and S-MX/CEA wintering aggregations was likely associated with the 2016 climate anomalies, the distributions do not appear to have fully shifted back to their pre-2016 configuration until 2021 (Figure 5, Table 5). Future research should focus on investigating the environmental drivers of the distributions of the MX and CEA wintering aggregations.

4.2 | Genetic Diversity in the MCP

The haplotypic diversity observed in humpback whales from MCP was similar to that diversity observed in RA, BC, and BB humpback whales. This result is consistent with our finding that MCP shows a greater genetic affinity for BB than for GRO/OAX and CEA, which have lower haplotypic diversity. However, nucleotide diversity for the MCP whales was also high, similar to the values for GRO/OAX and CEA, not BB. The high nucleotide diversity in MCP, GRO/OAX, and CEA results from the relatively high frequencies of the F haplotypes, which are highly divergent from the A and E haplotypes (Baker et al., 2013; Olavarría et al., 2007). The frequencies of F haplotypes are substantially higher in MCP than in BB, presumably due to a quarter (~27%) of MCP whales that belong to the S-MX/CEA wintering aggregation.

We recorded two haplotypes (MCP_E16, and MCP_F11) not previously identified in other studies. Each of the new haplotypes differed from a previously published haplotype by a single mutation (Table 1, Figure 2). The detection of two new haplotypes suggests that genetic variation in humpback whale populations is not yet fully characterized. Such is the case of haplotypes H35 and H36, which are identical to the haplotypes SP98 and SP62 identified in the southern hemisphere (Olavarría et al., 2007) and were recently recorded for the first time in southern Mexico (Martien et al., 2020).

We also detected two haplotypes, E9 and E15, that have not been seen previously in the MX or CEA wintering aggregations (Baker et al., 2013; González-Peral, 2011; Martien et al., 2020). Haplotype E15 has only been identified in individuals sampled in the Western Gulf of Alaska (Baker et al., 2013) and California/Oregon (Martien et al., 2020) feeding areas. The wintering destinations of these individuals are unknown, but based on our results, they might potentially winter in MCP or adjacent regions. Haplotype E9 is relatively common in the western North Pacific, but otherwise has only been detected once in the northern Gulf of Alaska and once in Hawai‘i (Baker et al., 2013).

Our detections of haplotypes F3 and A7 are also noteworthy.

Haplotype F3, which is primarily associated with CEA (Baker et al., 2013; Martien et al., 2020; Martínez-Loustalot et al., 2020; but see also González-Peral, 2011) was detected in a whale that was photo-identified off the MCP coast and recaptured there seven years later. Given our finding that both MX and CEA whales use MCP waters, it is unclear which wintering aggregation this whale belongs to. Haplotype A7 has previously only been found in one individual from BC during data collection for the SPLASH project (Calambokidis et al., 2008; González-Peral, 2011). We detected this haplotype A7 in a mature female (observed with a calf) in the MCP.

4.3 | Sex ratio

Regarding the sex ratio of PCM humpback whale individuals, our finding that the sex ratio in the MCP did not differ significantly from 1:1 is unusual; in all other wintering areas, approximately twice as many males as females have been reported. The 1:1 sex ratio could indicate both sexes are philopatric to the area. Among the recaptures found in this study, three whales were females and five were males, which suggests that the return to reproductive areas is undifferentiated between males and females. However, this result must be handled with caution since the number of resightings was low. Druskat et al. (2019) recently reported that variation in the sex ratio of migrating

southern hemisphere humpback whales closely tracks body condition, suggesting that skewed sex ratios may result from females opting not to migrate in years with poor feeding conditions. However, in all feeding grounds except for the east of the Aleutians, the sex ratio is similar to the MCP, with no apparent significant differences in the quantity of males and females (Baker et al., 2013). Nevertheless, these sites are the destination of whales from different wintering areas, so these values cannot be considered to belong to a single wintering grouping. We observed the strongest male-bias in animals sampled in the MCP during the 2016 El Niño and the following year. However, our sample size from these years were also very low. Further research is needed to determine the influence that environmental variability may play in humpback sex ratios in the MCP.

4.4 | Conclusions

Although the MCP region is relatively small geographically, it is important to understanding the distribution of the Mexico and Central America DPSs. Our data set, generated from whales sampled over a 12-year period in the MCP, revealed high haplotypic and nucleotide diversity, varying haplotype frequencies between years, and significant genetic differentiation from all other sampling areas except BB. Taken

together, these findings suggest that most whales using the MCP are part of the MX wintering aggregation, rather than the S-MX/CEA. However, temporal shifts in the frequencies of mtDNA haplotypes identified in MCP whales also suggest that the proportion of S-MX/CEA whales in the area may be higher in years when warm El Niño events are occurring. Therefore, more scientific research is needed (e.g., movement data from photo-id or potentially nuclear data) to elucidate ecological parameters of the MX and S-MX/CEA wintering aggregations to ensure the conservation and successful management of these humpback whales.

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TABLE 1 Polymorphic sites that define the haplotypes determined in the present research obtained from samples of humpback whales from Mexican Central Pacific during the 2010–2021 seasons. The clade (as defined by Baker et al., 2013) to which each haplotype belongs and the number of individuals with each haplotype are denoted in the rightmost columns.

Haplotypes	Polymorphic sites																																	Clade	Quantity
	23	82	83	87	98	115	123	131	143	144	158	159	160	164	236	237	243	244	245	261	262	264	266	270	313	314	377	379	444	489		
A+	G	T	T	G	C	G	C	G	T	G	T	T	T	C	G	T	T	C	C	T	C	A	T	A	T	C	C	A	T	A	AE	34
A-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	AE	9
A3	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	AE	6
A5	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	AE	1
A7	A	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	AE	1
E1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	AE	45
E2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	C	-	-	-	-	-	-	-	AE	4
E3	-	-	C	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	AE	6
E4	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	C	-	-	-	-	-	-	-	AE	10
E5	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	AE	11
E6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	C	-	-	-	-	-	-	-	AE	7
E7	-	C	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	AE	8
E9	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	AE	2
E10	-	-	C	-	-	-	-	-	C	-	-	-	-	-	-	-	C	-	-	-	T	-	C	-	-	-	T	-	-	G	AE	2
E13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	G	C	-	A	-	-	-	C	-	AE	7
E14	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	C	-	-	-	-	-	-	-	AE	3
E15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	G	-	-	AE	1
MCP_E16[a]	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	AE	1
F1	-	-	C	-	A	-	T	A	C	-	-	-	-	-	-	-	C	-	-	-	T	-	-	G	C	T	-	-	-	G	CD	5
F2	-	-	C	-	A	-	T	A	C	-	-	C	-	-	-	-	C	-	-	-	T	-	-	G	C	T	-	-	-	G	CD	75
F3	-	-	C	-	A	-	T	A	C	-	-	C	-	-	-	C	-	-	-	-	T	-	-	G	C	T	-	-	-	G	CD	10
F4	-	-	-	-	A	-	T	A	C	-	-	C	-	-	-	-	C	-	-	-	T	-	-	G	C	T	-	-	-	G	CD	4
F6	-	-	C	-	A	-	T	A	C	-	-	C	-	-	-	-	C	-	-	-	T	G	-	G	C	T	-	-	-	G	CD	3
F7	-	-	C	-	A	-	T	A	C	-	-	-	-	-	-	-	C	T	-	-	T	-	-	G	C	T	-	-	-	G	CD	1

MCP_F 11[a]	-	-	C	-	A	-	T	A	-	-	-	-	-	-	-	-	C	-	-	-	T	-	-	G	C	T	-	-	-	G	CD	1

Note: The hyphens indicate that the base is conserved in reference to the first sequence (A+).

[a] The haplotypes are new records for the Mexican Central Pacific.

TABLE 2 Number of humpback whale samples per geographically distinct area, number of control region haplotypes (500 bp), polymorphic sites, exclusive and shared haplotypes, and nucleotide diversity ($\pi \pm SD$) and haplotypic ($h \pm SD$) in each region, using the expanded data set which included sequences from this study (n = 257), Baker et al. (2013) (n = 314), Martien et al. (2020) (n = 96), and Martínez-Loustalot et al. (2020) (n = 174). (BC = Baja California; RA= Revillagigedo Archipelago; BB = Banderas Bay; MCP = Mexican Central Pacific; GRO/OAX = Guerrero/Oaxaca; CEA = Central American).

Area	*n*	Haplotypes	Polymorphic sites	Exclusive haplotypes	Shared haplotypes	Nucleotide diversity	Haplotype diversity
BC	239	17	23	0	17	0.010741 ± 0.005751	0.8818 ± 0.0082
RA	116	12	20	0	12	0.008387 ± 0.004647	0.8592 ± 0.0141
BB	109	18	24	1	17	0.013809 ± 0.007249	0.8948 ± 0.0143
MCP	257	25	29	5	20	0.014562 ± 0.007568	0.8590 ± 0.0138
GRO/OAX	63	13	23	0	13	0.014416 ± 0.007591	0.6928 ± 0.0435
CEA	67	13	27	3	10	0.014916 ± 0.007824	0.7580 ± 0.0424
Overall	851	29	34	—	—	0.013612 ± 0.007097	0.8711 ± 0.0059

TABLE 3 Pairwise estimates of genetic differentiation among sampling year for the control region of humpback whales sampled in the Mexican Central Pacific. The first column in parentheses shows the sample size by year. Differentiation based on haplotype frequency (F_{ST}) above the diagonal, values based on nucleotide differentiation (Φ_{ST}) are below the diagonal. Permutation *p*-values are in parentheses. The values in bold represent significant differences ($p < .05$) without correction for multiple comparisons.

	2010	2011	2012	2013	2014	2015	2017	2018	2019	2020	2021
2010 (5)		−0.012 (0.553)	0.061 (0.146)	0.034 (0.250)	0.208 (0.347)	−0.017 (0.627)	**0.308 (0.014)**	0.089 (0.109)	**0.111 (0.035)**	**0.127 (0.036)**	0.049 (0.150)
2011 (46)	0.047 (0.133)		0.007 (0.267)	−0.007 (0.627)	0.002 (0.354)	−0.027 (0.985)	**0.157 (0.000)**	**−0.035 (0.049)**	**0.049 (0.002)**	**0.067 (0.000)**	0.006 (0.270)
2012 (19)	0.115 (0.129)	−0.013 (0.503)		−0.013 (0.637)	−0.017 (0.714)	−0.002 (0.444)	0.076 (0.082)	−0.007 (0.496)	−0.010 (0.612)	0.008 (0.276)	−0.016 (0.787)
2013 (24)	0.120 (0.151)	−0.005 (0.400)	−0.039 (0.863)		−0.018 (0.741)	−0.031 (0.915)	**0.145 (0.011)**	0.011 (0.290)	0.036 (0.054)	0.040 (0.055)	−0.005 (0.548)
2014 (22)	0.180 (0.069)	0.033 (0.141)	−0.030 (0.682)	−0.029 (0.697)		−0.008 (0.523)	**0.107 (0.047)**	−0.029 (0.836)	0.008 (0.251)	0.004 (0.328)	−0.058 (0.535)
2015 (15)	0.062 (0.150)	−0.030 (0.818)	−0.038 (0.655)	−0.043 (0.851)	−0.015 (0.437)		**0.176 (0.007)**	0.039 (0.125)	**0.056 (0.027)**	**0.071 (0.017)**	−0.010 (0.616)
2017 (7)	**0.732 (0.015)**	**0.439 (0.000)**	**0.317 (0.023)**	**0.317 (0.011)**	**0.240 (0.041)**	**0.374 (0.007)**		0.127 (0.058)	0.028 (0.177)	0.011 (0.314)	0.064 (0.076)
2018 (16)	0.176 (0.114)	0.006 (0.310)	−0.050 (0.900)	−0.038 (0.651)	−0.044 (0.769)	−0.029 (0.550)	**0.280 (0.044)**		0.004 (0.346)	0.006 (0.325)	0.023 (0.151)
2019 (32)	**0.269 (0.030)**	**0.102 (0.015)**	0.012 (0.250)	0.021 (0.195)	−0.015 (0.444)	0.052 (0.150)	0.137 (0.070)	−0.013 (0.433)		−0.010 (0.731)	0.006 (0.260)

2020 (32)	**0.363 (0.009)**	**0.191 (0.000)**	0.082 (0.066)	**0.083 (0.045)**	0.029 (0.176)	**0.127 (0.037)**	0.047 (0.225)	0.048 (0.150)	−0.010 (0.438)		0.014 (0.152)
2021 (36)	0.134 (0.055)	0.008 (0.231)	−0.033 (0.899)	−0.028 (0.893)	−0.026 (0.775)	−0.028 (0.677)	**0.275 (0.025)**	−0.038 (0.912)	0.010 (0.241)	**0.068 (0.047)**	

TABLE 4 Pairwise estimates of genetic differentiation among sampling locations for the control region of humpback whales sampled in Mexico and Central America wintering aggregations. Differentiation based on haplotype frequency (F_{ST}) above the diagonal, values based on nucleotide differentiation (Φ_{ST}) are below the diagonal. Permutation *p*-values are in parentheses. The values in bold represent significant differences ($p < .05$) without correction for multiple tests. (BC = Baja California; RA = Revillagigedo Archipelago; BB = Banderas Bay; MCP = Mexican Central Pacific; GRO/OAX = Guerrero/Oaxaca; CEA = Central American).

Areas	**MCP**	**RA**	**BC**	**BB**	**CEA**	**GRO-OAX**
MCP		**0.035 (0.000)**	**0.020 (0.000)**	0.002 (0.209)	**0.019 (0.005)**	**0.029 (0.002)**
RA	**0.110 (0.000)**		0.002 (0.202)	**0.015 (0.009)**	**0.108 (0.000)**	**0.114 (0.000)**
BC	**0.072 (0.000)**	0.005 (0.139)		**0.007 (0.038)**	**0.077 (0.000)**	**0.085 (0.000)**
BB	0.005 (0.148)	**0.067 (0.002)**	**0.030 (0.006)**		**0.047 (0.000)**	**0.059 (0.000)**
CEA	**0.078 (0.006)**	**0.350 (0.000)**	**0.285 (0.000)**	**0.136 (0.000)**		−0.005 (0.585)
GRO/OAX	**0.031 (0.025)**	**0.277 (0.000)**	**0.211 (0.000)**	**0.077 (0.002)**	−0.003 (0.384)	

TABLE 5 Results of the GELATo (group exclusion-assignment test) analyses. The columns "lnL MX" and "lnL S-MX/CEA" show the log-likelihood of the MCP samples in each year belong exclusively to the MX or S-MX/CEA wintering aggregations, respectively. "Odds MX" shows the odds of the sampled animals all belonging to the MX wintering aggregation rather than the S-MX/CEA aggregation.

Year	lnL MX	lnL S-MX/CEA	Odds MX
2010	2.25	−2.11	78.257
2011	3.89	−49.05	9.807E+22
2012	3.21	−2.52	307.969
2013	3.55	−4.18	2,275.602
2014	3.58	2.95	1.878
2015	2.88	−5.38	3,866.094
2016	−2.66	0.80	0.031
2017	−3.75	1.35	0.006
2018	2.66	2.76	0.905
2019	1.39	2.83	0.237
2020	−3.13	3.27	0.002
2021	3.73	−3.31	1,141.388

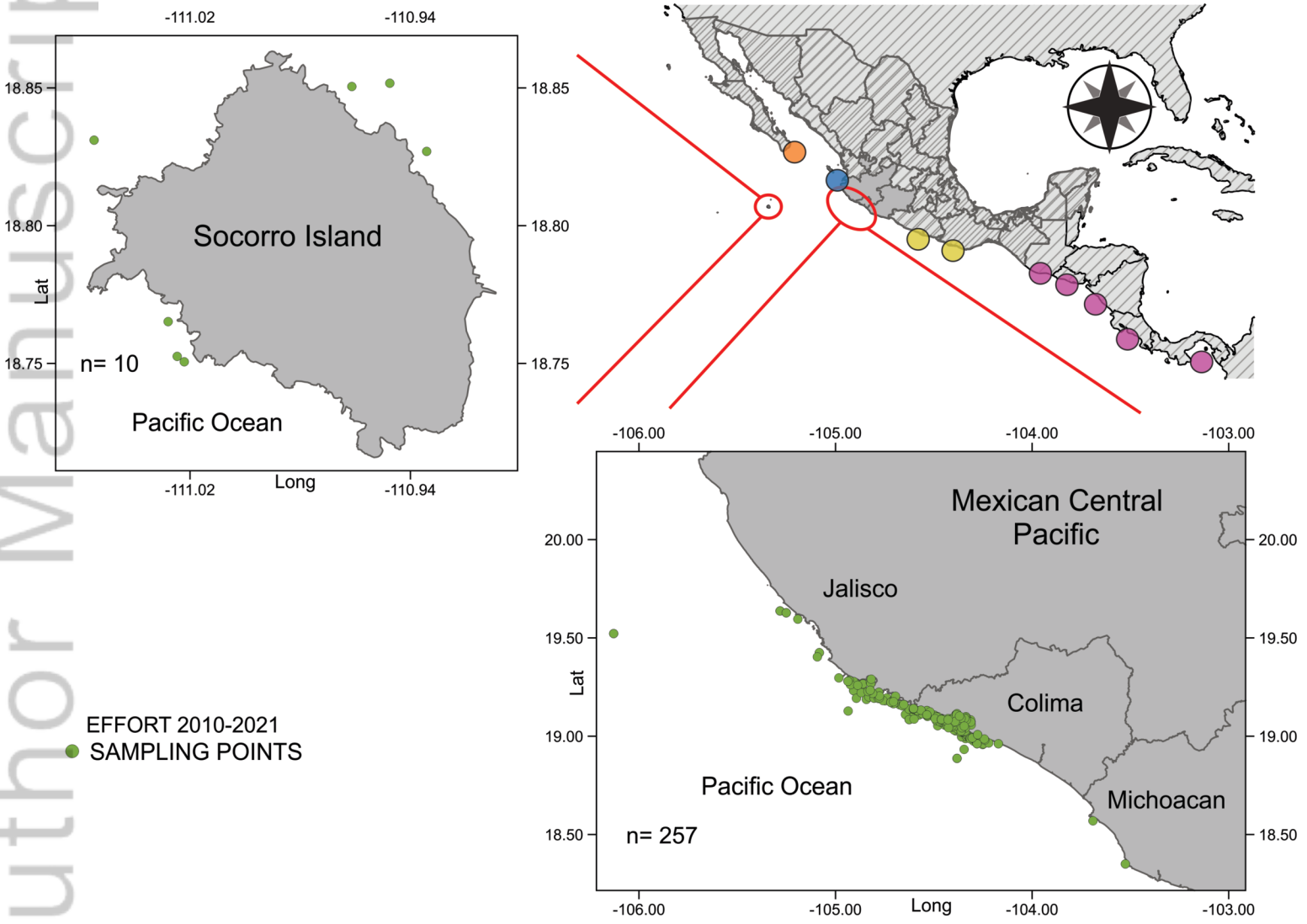
FIGURE 1 Geographic location of the study area, along the coast of the Mexican Central Pacific (MCP), and Socorro Island in the Revillagigedo Archipelago (RA). Green dots show sampling locations of humpback whales during the 2010–2021 winter seasons. Larger colored circles show sampling locations for data from previously published studies: Baja California (BC, orange circle), Banderas Bay (BB, blue circle), Guerrero-Oaxaca (GRO/OAX, yellow circles), and Central America (CEA, pink circles).

FIGURE 2 Haplotype network of the 25 mtDNA control region haplotypes of humpback whales from the Mexican Central Pacific during the 2010–2021 seasons. The size of the circle indicates the frequency of the haplotypes, blue letters represent the new haplotypes, and clades were previously determined by Baker et al. (2013).

FIGURE 3 Proportion of the haplotypes of humpback whales determined during the sampling seasons in the Mexican Central Pacific. Sample size (*n*) in the parenthesis.

FIGURE 4 Percentage of the haplotypes of humpback whales in Mexican and Central American. S-MX represents the combined samples from GRO and OAX. Sample size (*n*) in the parenthesis. See Figure 1 for location of sampling sites.

FIGURE 5 (a) Results of mixed stock analysis indicating the proportion of MCP samples belonging to the Banderas Bay (BB) stratum, which represents the MX wintering aggregation. For each year, the horizontal line represents the median value, the box represents the central 50% of the distribution (also called the interquartile range, IQR), vertical lines span 1.5× IQR beyond the box, and dots represent outliers. (b) ONI values shown in red indicate El Niño conditions (≥0.5), while those in blue indicate La Niña conditions (≤−0.5). Sample size for 2016 was too small to include in the analysis.



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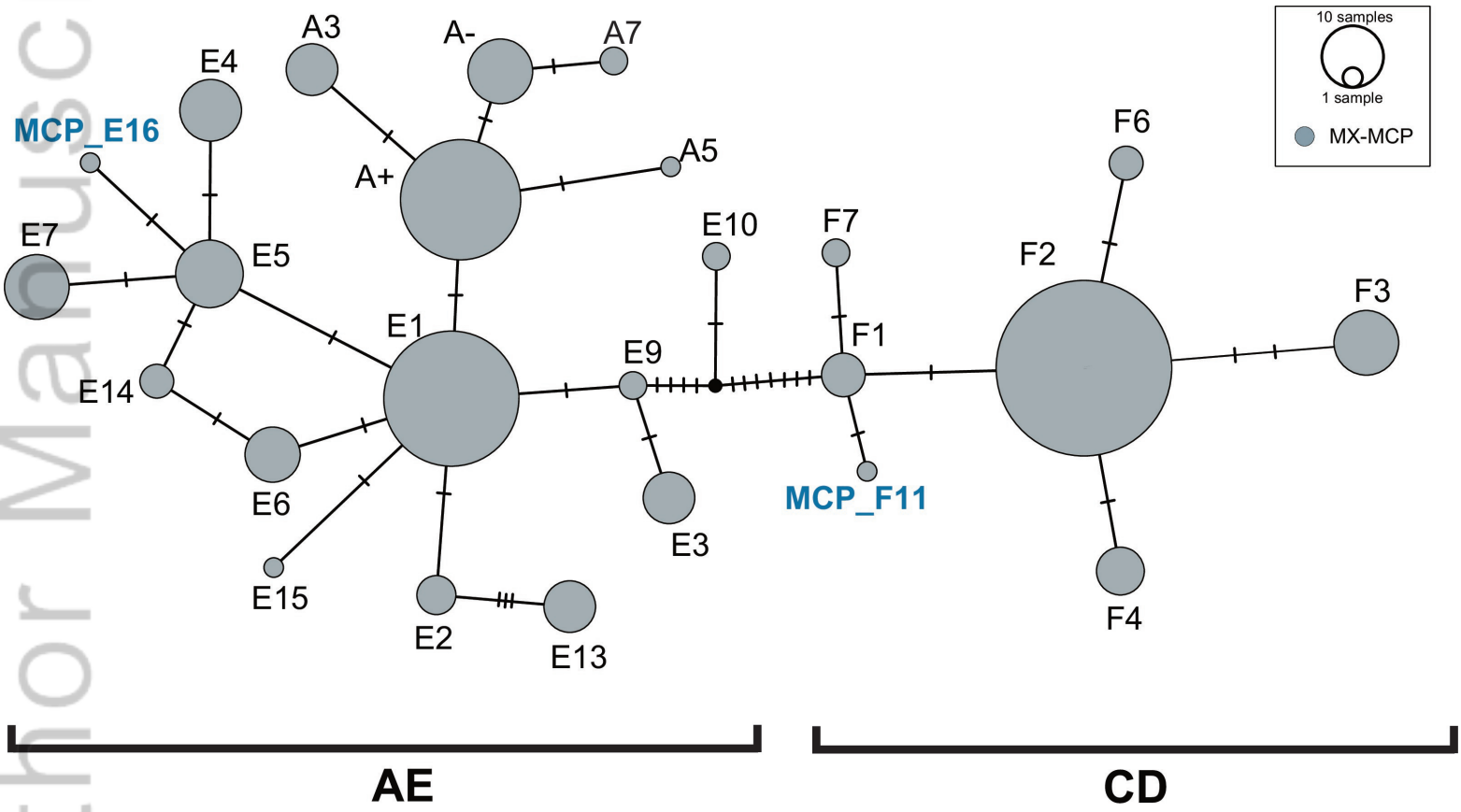

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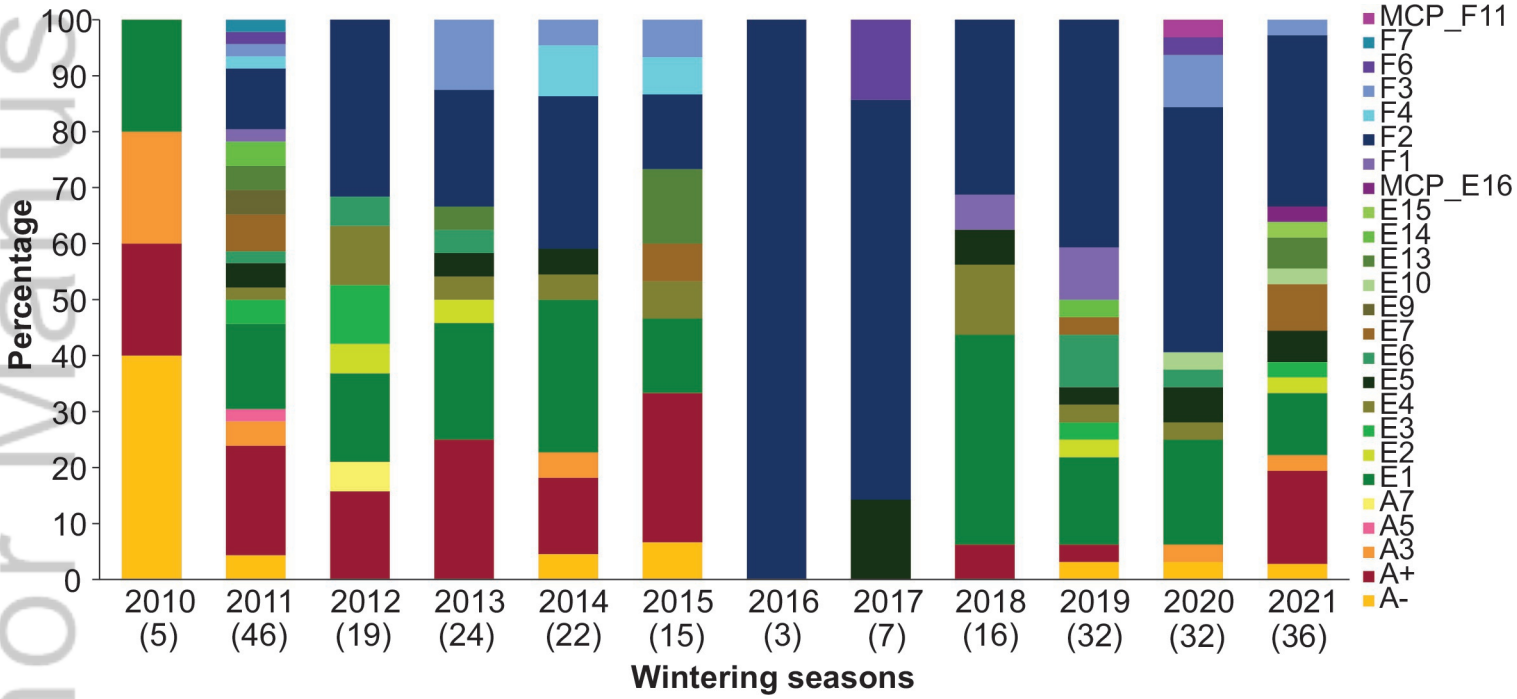

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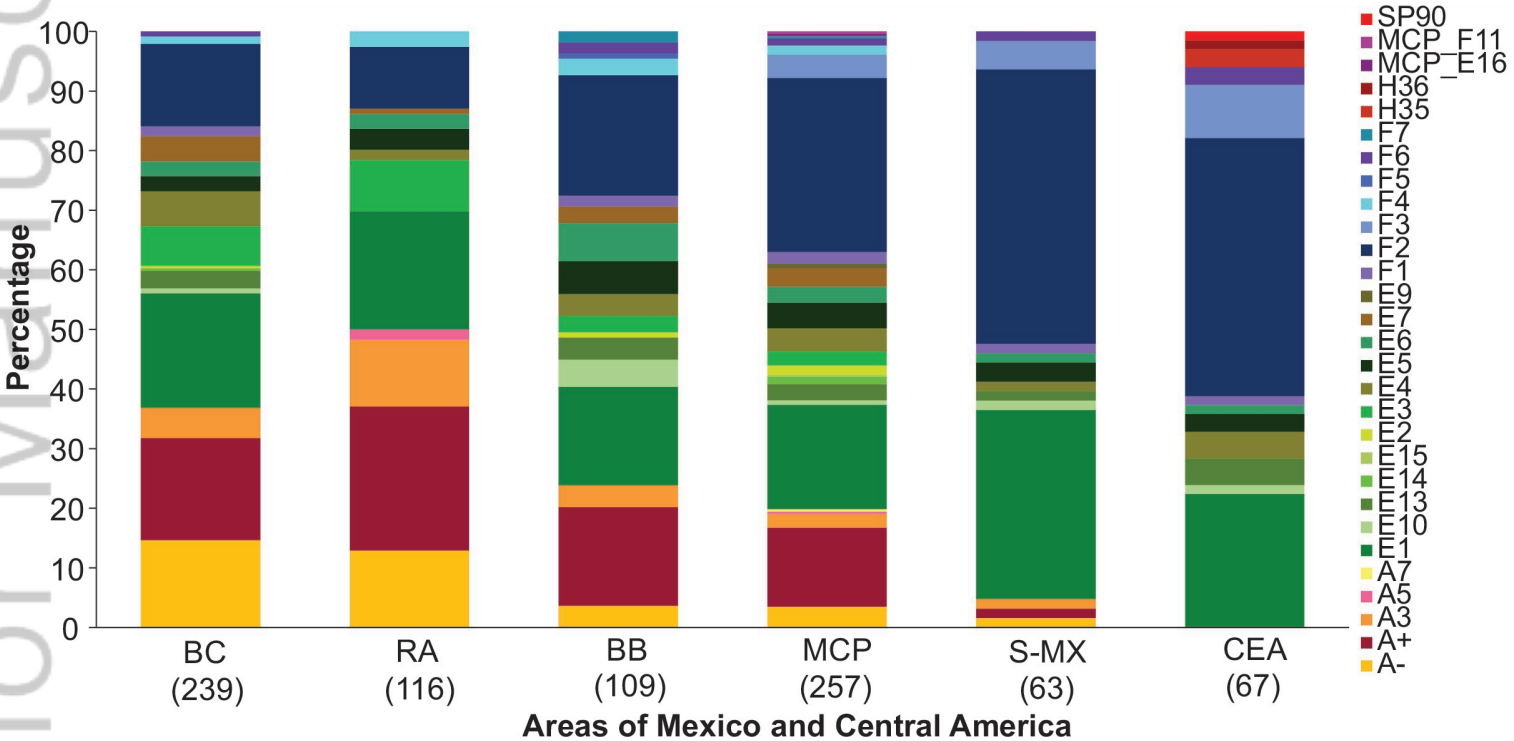

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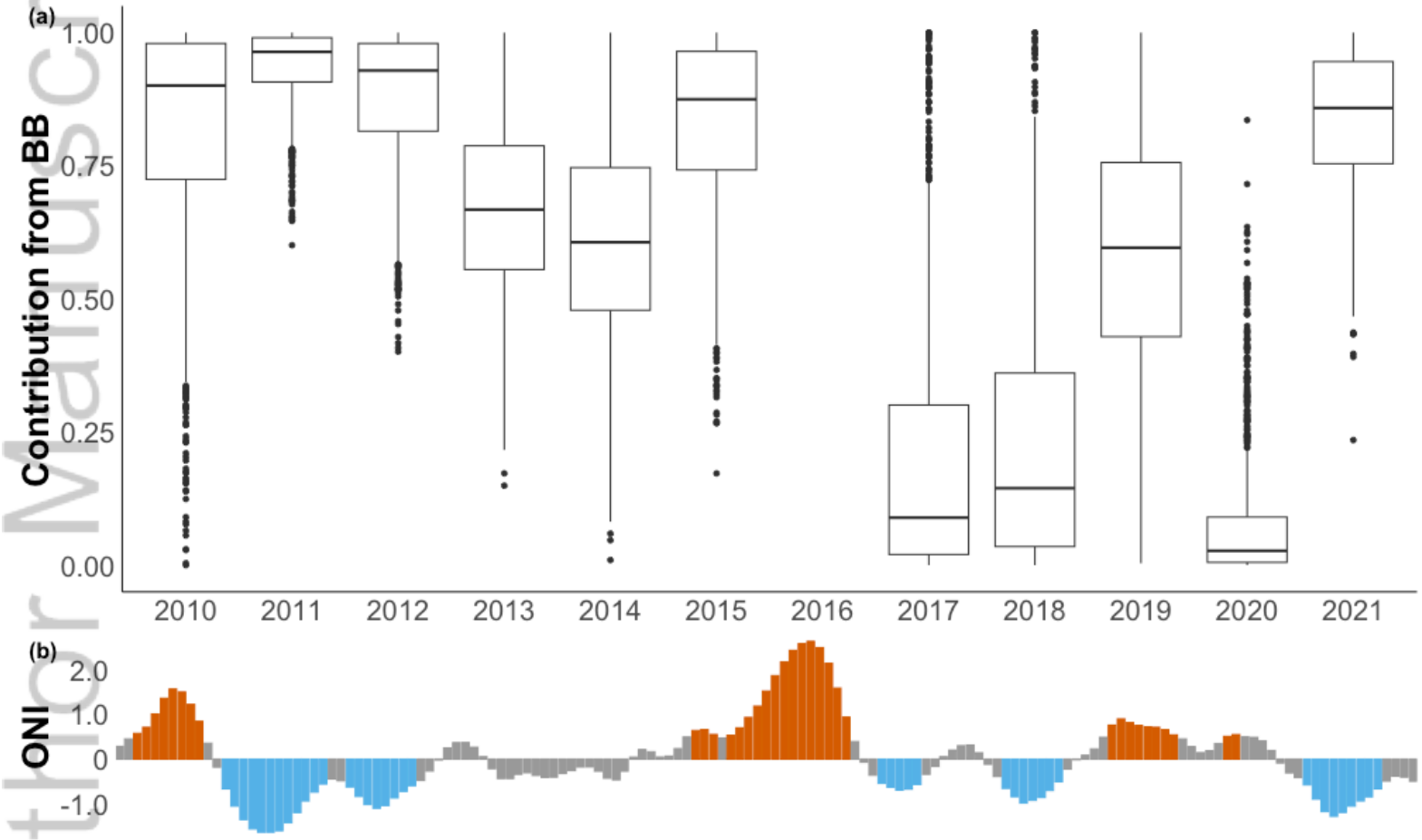

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