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# mtDNA variation of humpback whales in their wintering grounds of Guerrero, southern Mexican Pacific

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ABSTRACT: Here, we describe variation in mtDNA haplotypes among 22 individual humpback whales off the Guerrero coast in southern Mexico and compare this to other wintering regions in the eastern North Pacific Ocean using published data. After correcting for different sample sizes, Guerrero humpback whales fit the cline in the eastern North Pacific in which A types are more abundant northwards, while E and F types are more abundant southwards. Whales observed around Guerrero in early winter exhibited a greater frequency of F types that are also observed earlier in winter at Bahía de Banderas in the mainland region, suggesting a seasonal passage of whales from Mexico to Central America. Four diversity indices were higher in different wintering regions between Nayarit and Guerrero. Departures from neutrality were observed in the bordering wintering grounds of the region. Four measures of differentiation by distance indicated boundaries between Nayarit and Southern Jalisco, and between Guerrero and Oaxaca, suggesting a transitional region between wintering regions of Mexico and Central America from 17.5° to 19.5° N.

KEY WORDS: Humpback whale · Eastern North Pacific Ocean · Southern Mexican Pacific · Distinct population segments · mtDNA · Haplotype · Haplogroup

## 1. INTRODUCTION

Humpback whales *Megaptera novaeangliae* exhibit historical gene flow and current migratory connections that complicate the identification of population units for management. Based on the species' wintering areas, Bettridge et al. (2015) identified 14 distinct population segments (DPS) worldwide, of which 4 belong to the North Pacific Ocean, referred to as Western North Pacific, Hawaii, Mexico (Mx), and Central America (CAm) in the US Endangered Species Act. Some DPS are further subdivided into demographically independent populations (DIP; Martien et al. 2019).

During the winter, humpback whales are nearly continuously distributed from southern Baja California to Central America (Section 3 in the Supplement at www.int-res.com/articles/suppl/n054p219_supp.pdf), showing a clinal variation of mtDNA haplotypes (Baker et al. 2013). Two DIP of humpback whales have been recognized in the Mexico DPS, namely the Revillagigedo Archipelago (MxAR), offshore at 18° to 19° N, and the Mexican Mainland between 20° and 23° N (MxML). The coast of southern Baja California (MxBC) has been recognized as an area transited by humpback whales from at least the MxAR and MxML areas (Urbán et al. 2000, González Peral 2011). Recently, others have found evidence for a third population in

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southern Mexico related to the Central America DPS (Martínez-Loustalot et al. 2020, Lizewski et al. 2021, Martien et al. 2021, Taylor et al. 2021, Audley et al. 2022). More recently, Llamas-González et al. (2024) examined mtDNA-haplotype diversity in the Mexican Central Pacific around 19° N (MxCP), and found this region to be genetically intermediate between Bahía de Banderas (BB), the most surveyed zone in the MxML region around 20.5° N, and Oaxaca (MxOax) in southern Mexico around 16° N, with most whales being part of the Mexico DPS, while Central America DPS whales were more frequent in warmer winters.

Here, we provide new data on the variation of mtDNA haplotypes in humpback whales off the Guerrero coast in the southern Mexican Pacific (MxGue; 17.30° to 17.67° N), briefly describing their diversity and differentiation with respect to whales in other areas of the Mexican and Central American Pacific.

## 2. MATERIALS AND METHODS

We collected sloughed skin samples from 40 photo-identified humpback whales and preserved them in 95% molecular-grade ethanol in a refrigerator until sending them to a laboratory for preservation at −86°C. We performed the laboratory protocols described by Baker et al. (2013) for amplifying and sequencing the first 419 bp of the GenBank sequences that correspond to the mtDNA control region of North Pacific humpback whales (accession nos. KF477244 to KF477271) and included all MxGue haplotypes (Section 2.1 in the Supplement).

We determined the sufficiency of the MxGue sample using a resampling procedure to examine the stability of observed haplotype richness ($R_o$), gene diversity ($H$), and nucleotide diversity ($\pi$; Sections 1.2 & 2.2 in the Supplement). We then compared the MxGue sample with the MxAR, MxBC, MxML, MxCP, MxOax, and CAm samples described by Baker et al. (2013) from the SPLASH project (Structure of Populations, Levels of Abundance and Status of Humpbacks), Martínez-Loustalot et al. (2020), and Llamas-González et al. (2024).

We examined the diversity indices $R$, $H$, $\pi$, and variable sites number, tested neutrality with the Ewens-Watterson and Tajima's analyses, and determined differentiation indices $\Phi_{ST}$ and $F_{ST}$ with the program Arlequin 3.5.2.2 (Excoffier et al. 1992, 2005). Since sample size varied widely among regions, we used program Variatio23 (available upon request) developed by L. Medrano-González to estimate haplotype richness ($R_e$) by the extrapolation method of Foulley & Ollivier

(2006) and to compare its levels with differentiation index $\rho_{ST}$. Variatio23 also compared the variable sites number with differentiation index $v_{ST}$. Variatio23 determined the statistical significance of $\rho_{ST}$ and $v_{ST}$ with 100 permutations of all individual haplotypes between populations to obtain random distributions of both statistics, accepting significance when the actual differentiation was over the upper 5% of the shuffled values. Given the nearly continuous distribution of humpback whales, we normalized all differentiation indices by the straight-line distance between areas (Table S1 in the Supplement), focusing on the cline (MxBC, MxML, MxAR) — MxCP — MxGue — MxOax — CAm.

We compared the seasonal composition of haplogroups A, E, and F (sets of haplotypes in 1 phylogenetic branch; Section 1.1 in the Supplement) in MxGue and BB, using the haplogroup data of Cortés Rodríguez (2013). From the patterns of haplogroup composition and days with data gaps in both regions, we partitioned data into 4 seasonal sets described by the day of the year as (a) −41 (November 20) to 9; (b) 10 to 30; (c) 31 to 55; and (d) 56 to 93 (April 3).

## 3. RESULTS

To obtain a general pattern for describing the mtDNA variation of Guerrero humpback whales, we identified 6 haplogroups defined by their distribution in feeding areas or wintering areas if only found there (Baker et al. 2013). Northern E and Northern F types are absent in the range California to southern British Columbia, while Southern E and F types do occur in these regions. Northern A types, mainly A−, occur in the range California to southern British Columbia with a frequency under 7.4%. Southern A types, A+ only, occur with a frequency of 12.5% in that range.

From 40 samples collected, we obtained only 22 sequences of 419 bp from the mtDNA control region with adequate quality control scores. Our supplementary sufficiency analysis demonstrated that $H$ and $\pi$ attained stability in the small MxGue sample, varying less than 3% between the last 2 data in the real and shuffled series, whereas $R_o$ still varied up to 10%. From the Guerrero and previously published sequences, we identified 26 haplotypes among the 643 individuals from the 7 areas compared. MxGue presented $R_o$ = 10 haplotypes, 4 of which (9 individuals) were Southern E types, 3 were Southern F types (10 individuals), 1 was type A3, 1 was type A−, and 1 was type A+ (Fig. 1, Table S4). The estimated number of haplotypes for MxGue was 23 according to the extrapolation method and 21 to 22 according to the
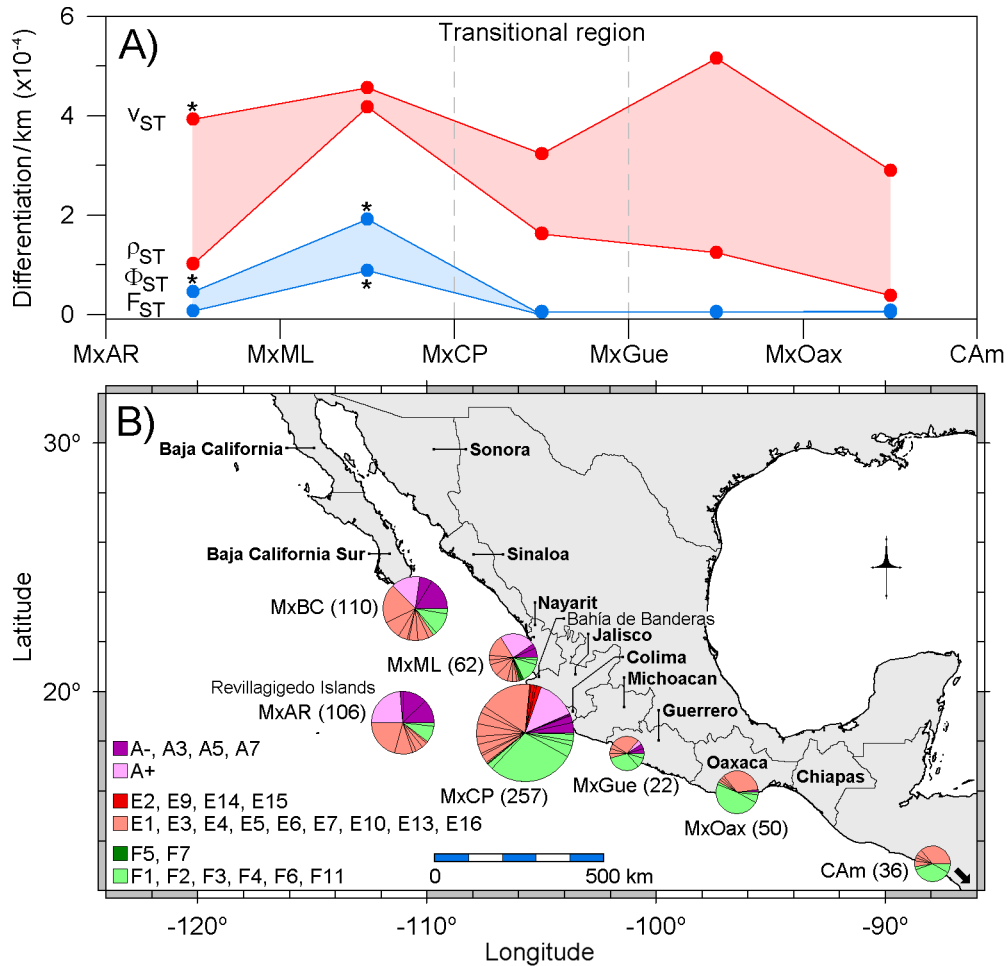
Fig. 1. Diversity and differentiation per distance of mtDNA haplotypes in humpback whales between Baja California and Central America (Baker et al. 2013, Martínez-Loustalot et al. 2020, Llamas-González et al. 2024, present study). (A) Profile of latitudinal differentiation per distance. *p < 0.05 according to the permutational test of Arlequin 3.5.2.2 (Excoffier et al. 1992, 2005). (B) Geographic array of haplotype diversity. Pie size is proportional to sample size, indicated within parentheses. States on the Mexican Pacific coast are shown in **bold**

hyperbolic model of saturation (Fig. S2). Haplotypes from MxGue exhibited 20 of the 27 variable sites found among the 7 areas (Table 1).

MxGue whales corroborated the pattern of larger mtDNA diversity on the Mexican Pacific coast, with highest $H$ in MxML (0.894), highest $R_o$, $R_e$, and vari-

Table 1. mtDNA diversity and neutrality of humpback whales at their wintering grounds in the eastern North Pacific Ocean (Baker et al. 2013, Martínez-Loustalot et al. 2020, Llamas-González et al. 2024, this work). Estimated haplotypes were determined by the extrapolation method. $R_o$: observed haplotype richness; $R_e$: haplotype richness estimated by the extrapolation method. For an explanation of Ewens-Watterson's p and Tajima's $D$ see Section 1.1 in the Supplement. **Bold**: largest sample size, highest diversity index value, or significant at p < 0.05

|  | Sample size | $R_o$ | $R_e$ | $H$ | $\pi$ | Variable sites | Ewens-Watterson's p ($F_{rand} \geqslant F_{obs}$) | Tajima's $D$ |
|---|---|---|---|---|---|---|---|---|
| MxBC | 110 | 13 | 21 | 0.887 | 0.011 | 20 | **0.022** | 0.621 |
| MxAR | 106 | 12 | 20 | 0.860 | 0.008 | 19 | 0.085 | −0.074 |
| MxML | 62 | 16 | 23 | **0.894** | 0.013 | 22 | 0.377 | 0.439 |
| MxCP | **257** | **25** | **26** | 0.857 | 0.016 | **27** | 0.930 | 0.705 |
| MxGue | 22 | 10 | 23 | 0.840 | **0.017** | 20 | 0.877 | 1.055 |
| MxOax | 50 | 9 | 19 | 0.661 | 0.015 | 18 | **0.989** | **1.791** |
| CAm | 36 | 8 | 20 | 0.744 | 0.016 | 18 | 0.701 | **1.940** |

able sites in MxCP (25, 26, and 27 respectively), and highest π in MxGue (0.017; Table 1). Our new data also confirmed the north-to-south cline in the Northeast Pacific wintering grounds, in which A-types were more frequent northwards, whilst E and F types were more frequent southwards (Medrano-González et al. 1995, Baker et al. 2013). Moreover, A, Northern E, and Northern F types were also more frequent in the northern wintering areas, whilst Southern E and Southern F types increased in frequency southwards; Southern E types reached their maximum at CAm and Southern F types at MxOax. Note the abrupt increase in F types between MxML and the adjacent MxCP area, and the similarity between MxCP and MxGue (Fig. 1, Table S2). Significant departures from neutrality occurred in the bordering wintering grounds, with a low fixation index in the north and a high fixation index and Tajima's $D$ values in the south (Table 1). The 4 differentiation indices were higher between MxML and MxCP, with only $\Phi_{ST}$ and $F_{ST}$ being statistically significant. MxAR and MxML showed lower differentiation, with only $\Phi_{ST}$ and $v_{ST}$ being significant. Indices $\Phi_{ST}$ and $F_{ST}$ were

zero south of MxCP, but $\rho_{ST}$ and $v_{ST}$ showed a degree of differentiation between MxGue and MxOax, and even between MxOax and CAm (Fig. 1, Table S2).

A higher proportion of F types was found during early and late winter in BB, in the MxML region, and in MxGue (Fig. 2). The higher occurrence of F types from November to early January was followed by a brief increase in A types during January and thereafter of E types during February in both areas. $\Phi_{ST}$ and $F_{ST}$ were largest between seasonal sets BB-a and BB-b, and both were statistically significant. This differentiation signal was apparent in Guerrero between seasonal sets MxGue-b and MxGue-c but was not significant. No differentiation was observed between seasonal sets c and d in either area (Fig. 2).

## 4. DISCUSSION

Episodic gene flow between humpback whales from the Southeast and Northeast Pacific since the Illinoian glaciation and their northward expansion after
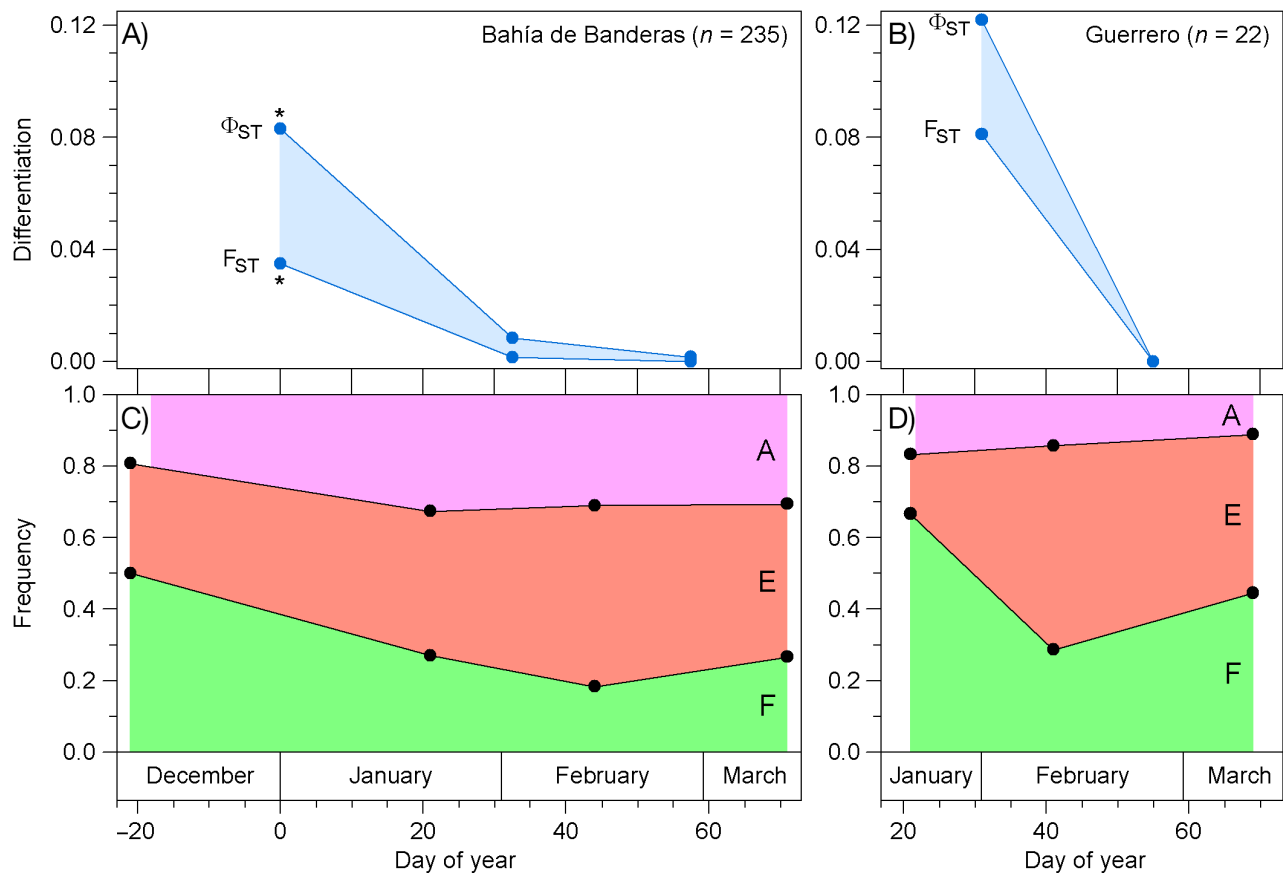


Fig. 2. Composition and differentiation of mtDNA haplogroups A, E, and F during the winter in (A,C) Bahía de Banderas in the Mexican Mainland and (B,D) the Guerrero coast; $n$: sample size. *p < 0.05 according to the permutational test of Arlequin 3.5.2.2 (Excoffier et al. 1992, 2005)

the Little Ice Age, have shaped the current cline of mtDNA in the eastern North Pacific. This is evident in A types being more abundant northwards, whilst E and F types are more frequent southwards (Medrano-González et al. 1995, Baker et al. 2013; our Fig. 1), and in F types migrating further south, appearing more during the early and late winter, at least in BB (Medrano González et al. 2009, Cortés Rodríguez 2013) and MxGue (Fig. 2). Evidence for the seasonal procession of humpback whales between the Mexican and Central American Pacific coasts is provided by the graded exchange and positive-movement indices between Bahía de Banderas and Nicaragua obtained by photo-identification (Martínez-Loustalot et al. 2023).

We observed 2 apparent mtDNA-haplotype boundaries among humpback whales along their wintering grounds in the eastern North Pacific: one between MxML and MxCP for $\Phi_{ST}$, $\rho_{ST}$, and $F_{ST}$ and another between MxGue and MxOax for $v_{ST}$. MxCP and MxGue did not show differentiation in the 4 indices (Section 1.4 in the Supplement). These results suggest that the recently described sample MxCP and the MxGue sample described here constitute a transitional unit between the Mexico and CAm DPS that deserves further attention for research and management. The region between MxCP and MxGue also shows an apparent distribution hiatus for the humpback whale (Section 3 in the Supplement) and other cetaceans such as *Grampus griseus* (Leatherwood et al. 1980) and *Delphinus delphis* (Perrin et al. 1983), suggesting that this region contains an ecological frontier for different cetacean species that needs to be investigated. Such a gap could also correspond to the negative movement index between MxCP and MxGue, which is the only negative one between Nicaragua and Bahía de Banderas (Martínez-Loustalot et al. 2023). A relevant issue to solve is the dynamics of stay, dispersion, and migration of humpback whales that keeps the Mexico and CAm DPS separated, or not, over their spatial and seasonal overlaps along the Mexican coast of the Pacific Ocean.

Humpback whale populations have differentially recovered from near extinction in the 1960s through changing marine ecosystems and emerging anthropic threats. The fact that whales from the Mexico and CAm DPS breed in waters under Mexican jurisdiction with their respective threatened and endangered designations, and that there is a transitional population unit between these DPS with still-unknown relationships, raises the need for reviewing national policies on fisheries, urban development, tourism, and vessel traffic, as the protection of humpback whales and other migratory species in Mexico is of international relevance.

## LITERATURE CITED

Audley K, Garcia Chavez A, Ramirez R, Dobson E, Flynn K, Cheeseman T, Calambokidis J (2022) Humpback whale photo-identifications from Guerrero indicate southern Mexico is similar to Central America as a wintering area of whales that feed primarily on the US West Coast. Book of Abstracts, 24th Biennial Conference on the Biology of Marine Mammals, 1—5 August 2022, Palm Beach, FL. Society for Marine Mammalogy, p 41—42. www.smmconference.org/wp-content/uploads/2022/08/SMM2022-Abstract-Book-August_11.pdf

Baker CS, Steel D, Calambokidis J, Falcone E and others (2013) Strong maternal fidelity and natal philopatry shape genetic structure in North Pacific humpback whales. Mar Ecol Prog Ser 494:291—306

Bettridge S, Baker CS, Barlow J, Clapham PJ and others (2015) Status review of the humpback whale (*Megaptera novaeangliae*) under the Endangered Species Act. NOAA Tech Memo NOAA-TM-NMFS-SWFSC-540. https://repository.library.noaa.gov/view/noaa/4883

Cortés Rodríguez X (2013) Variación regional y estacional de los linajes mitocondriales de las ballenas jorobadas del Pacífico mexicano. MSc thesis, Universidad Nacional Autónoma de México, Mexico City

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479—491

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evol Bioinform Online 1: 47—50

Foulley JL, Ollivier L (2006) Estimating allelic richness and its diversity. Livest Sci 101:150—158

González Peral UA (2011) Definición y características de las unidades poblacionales de las ballenas jorobadas que se congregan en el Pacífico mexicano. PhD thesis, Universidad Autónoma de Baja California Sur, La Paz

Leatherwood S, Perrin WF, Kirby VL, Hubbs CL, Dahlheim M (1980) Distribution and movements of Risso's dolphin, *Grampus griseus*, in the eastern North Pacific. Fish Bull 77:951—962

Lizewski K, Steel D, Lohman K, Albertson GR and others (2021) Mixed-stock apportionment of humpback whales from feeding grounds to breeding grounds in the North Pacific based on mtDNA. Scientific Committee of the International Whaling Commission, SC/68C/IA/01Rev 01

Llamas-González M, Ortega-Ortiz CD, Enríquez-Paredes LM, González-Peral UA, Bautista-Guerrero E, Martien KK (2024) Genetic data reveals wintering ground affiliation of humpback whales from the Mexican Central Pacific. Mar Mamm Sci 40:54—72

Martien KK, Lang AR, Taylor BL, Simmons SE, Oleson EM, Boveng PL, Hanson MB (2019) The DIP delineation handbook: a guide to using multiple lines of evidence to delineate demographically independent populations of marine mammals. NOAA Tech Memo NOAA-TM-NMFS-SWFSC-622. https://repository.library.noaa.gov/view/noaa/22660

Martien KK, Taylor BL, Archer FI, Audley K and others (2021) Evaluation of Mexico distinct population segment of humpback whales as units under the Marine Mammal Protection Act. NOAA Tech Memo NOAA-TM-NMFS-SWFSC-658. https://repository.library.noaa.gov/view/noaa/33497

Martínez-Loustalot P, Guzón O, Audley K, Villegas F and others (2020) Population assignment of humpback whales from the southern Mexican Pacific. Scientific Committee of the International Whaling Commission, SC/68B/CMP/26Rev01

Martínez-Loustalot P, Audley K, Cheeseman T, De Weerdt J and others (2023) Towards the definition of the humpback whale population units along the Mexican and Central American coasts in the Pacific Ocean. Mar Mamm Sci 39:422—437

Medrano-González L, Aguayo-Lobo A, Urbán-Ramírez J, Baker CS (1995) Diversity and distribution of mitochondrial DNA lineages among humpback whales, *Megaptera novaeangliae*, in the Mexican Pacific Ocean. Can J Zool 73:1735—1743

Medrano González L, Vázquez Cuevas MJ, Robles Saavedra MR, Rodríguez Cortés X, Núñez López MT, Peters Recagno E (2009) Identidad y heterogeneidad poblacional de la ballena jorobada en la Bahía de Banderas y aguas circundantes. II. Identidad y heterogeneidad genética. Report to Instituto Nacional de Ecología, Mexico City

Perrin WF, Scott MD, Walker GJ, Ralston FM, Au DWK (1983) Distribution of four dolphins (*Stenella* spp. and *Delphinus delphis*) in the eastern tropical Pacific, with an annotated catalog of data sources. NOAA Tech Memo NOAA-TM-NMFS-SWFSC-38. https://swfsc-publications.fisheries.noaa.gov/publications/TM/SWFSC/NOAA-TM-NMFS-SWFC-38.PDF

Taylor BL, Martien KK, Archer FI, Audley K and others (2021) Evaluation of humpback whales wintering in Central America and southern Mexico as a demographically independent population. NOAA Tech Memo NOAA-TM-NMFS-SWFSC-655

Urbán RJ, Jaramillo LA, Aguayo LA, Ladrón de Guevara PP and others (2000) Migratory destinations of humpback whales wintering in the Mexican Pacific. J Cetacean Res Manag 2:101—110