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Contrasting phylogeographic patterns among Northern and Southern Hemisphere fin whale populations revealed by new data from the Southeastern Pacific and Gulf of California

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ABSTRACT

Three sub-species of fin whales are currently considered valid: *Balaenoptera physalus physalus* in the Northern Hemisphere (NH), and *B. p. quoyi* and *B. p. patachonica* in the Southern Hemisphere (SH). The latter was described as a pygmy-type sub-species located in low to mid latitudes in the SH. In the NH, a strong genetic differentiation was previously detected between North Pacific (NP) and North Atlantic (NA) fin whales, which lead to a current debate of a taxonomic division between these two groups. Additionally, a highly isolated population has been detected in the Gulf of California (GoC), Mexico. Little is known, however, for the SH, impeding a global biogeographic and taxonomic revision of the taxon. This study includes sequences previously reported for NA, NP, and South Atlantic (SA), the first samples of the Southeast Pacific (SEP) (n=37 as well as new mtDNA sequences from GoC (n=107) improving the worldwide phylogeographic and demographic picture of the species. SEP sequences recovered 25 haplotypes with nine shared by two or more individuals, a haplotype diversity (h) of 0.979 and nucleotide diversity (π) of 0.8%. In contrast GoC showed only five haplotypes (h = 0.3 and π = 0.06%). A strong genetic structure was observed as previously between (1) NP and the GoC populations, where a low and unidirectional and rare dispersal event flow from SP to NP was further confirmed. Contrary to the NH, (3) no significant phylogeographic structure was detected within the SH (SP and Atlantic Southern Occan; Φ_{ST} = 0.00582, p = 0.235), which suggests the existence of a single evolutionary unit and challenge the validity of the proposed pygmy fin whale sub-species. Finally, (4) *B. physalus* would include four major population units (three for the NH and one for the SH).

KEYWORDS: FIN WHALE, GENETICS, CHILE, FEEDING GROUNDS, PYGMY FIN WHALE

INTRODUCTION

Fin whale populations background

The fin whale (*Balaenoptera physalus*) occurs in all major oceans (Mizroch et al. 1984), particularly in middle and high latitudes (Mackintosh 1966, Miyashita et al. 1995, Branch & Butterworth 2001, Reilly et al. 2013). This species has been proposed to be separated into three sub-species, *B. physalus physalus* in the Northern Hemisphere (NH), *B. physalus quoyi* and *B. physalus patachonica* in the Southern Hemisphere (SH, Committee on Taxonomy 2016). The latter, although not supported by genetic data, is described as a pygmy-type sub-species and is proposed to be located mainly in low to mid latitudes in the SH (Clarke 2004). In the NH a strong genetic differentiation was detected between North Pacific (NP) and North Atlantic (NA) (Berubé 1998, Hoelzel 1994, Archer 2013, Cabrera et al 2019) which lead to a current debate of a taxonomic division between these two groups (Cabrera et al 2019). Additionally, a highly isolated and thus evolutionary unique population is proposed in the Gulf of California (GoC,

Berubé et al 2002). These authors found that all mtDNA haplotypes identified within the GoC are unique to the NP and are not shared with mtDNA data from NA and Mediterranean (Berubé et al 2002), while recently, Jimenez-Lopez et al (2019) evidence that fin whales from GoC conform a resident population, that remains year-round there (Urban-Ramirez et al. 2005; Jimenez-Lopez et al. 2019). Phylogenetics analysis between NP and SH shows that NP and SH clades are not reciprocally monophyletic (Archer et al 2013, Pérez-Alvarez et al 2018). However, sample size for the SP was reduced, impeding a global taxonomic revision of this taxon. The reduced representation of different stocks/populations in the SH have precluded more detailed analyses, including investigating the validity of the putative *B. p. patachonica* sub-species (Jackson et al 2018).

Fin whale in Chile

In Chile, this species represented the main target of whaling operations from 1929 to 1983, between 18°S to 22°S and 29°S to 40°S (Aguayo 1974, Clarke et al. 1978, IWC catch records). Post-whaling information on the presence of fin whales in Chile has come from sighting cruises between Antofagasta (23°29' S) and Cape Horn (56°48' S) (Clarke 1962, Clarke et al. 1978, Aguayo et al. 1998, Acevedo et al. 2012, Pérez et al. 2006, Pacheco et al. 2015, Sepúlveda et al. 2016, Toro et al. 2016, Sepúlveda et al 2018), including the Archipelago of Juan Fernández (33°77' S; 80°78' W), located 670 km off the Chilean coast (Aguayo et al. 1998). Most sightings were reported further than 100 km from the coast, leading to the belief that in Chilean waters fin whales occur more in oceanic habitats (Clarke 1962). However, this notion is now changing, due to the regular presence of fin whales in coastal waters at latitudes between 23°S and 29°S during spring and summer seasons (Pérez et al. 2006, Pacheco et al. 2015, Sepúlveda et al. 2016, Toro et al. 2016, Sepúlveda et al 2018), where summer-spring foraging areas have been identified for the species (Pérez et al 2006, Toro et al 2016).

This report

Here we present a global phylogeographic and demographic study of the species including Southeast Pacific fin whales from the feeding area off coastal north-central Chile (ca. $29^{\circ}02$ 'S, $71^{\circ}36$ 'W), as well as new samples from the GoC. In particular, we (1) evaluate the existence genetic structure between NP and SP populations, (2) improve the comparison between Northeast Pacific fin whales and GoC resident population, (3) test for genetic structure between fin whale populations within SH (Southeast Pacific versus South Atlantic Southern Ocean), and finally, (4) discuss the taxonomic status of the putative pygmy fin whale sub-species *B. physalus patachonica*.

MATERIALS AND METHODS

From 2003 to 2017 boat-based surveys were conducted off the north-central Chilean coast, as part of a wider program of ecological studies on fin whales and other marine mammal species. Surveys were undertaken around the Marine Reserves Isla Chañaral (29°02'S, 71°36'W), Isla Choros (29° 14' S, 71° 32' W) and Isla Damas (29° 13' S, 71° 32' W) (Figure 1). Skin samples from 37 free-swimming fin whales were obtained using a PAXARM. All samples were stored in 90% ethanol.



Figure 1. Study area off coastal north-central Chile, Southeast Pacific, where fin whales were sampled. It is included the location of fin whale samples from Archer et al (2013), with a black dot in that figure to indicate our study area general location (n=37).

Laboratory procedures

In the laboratory sex of each individual was identified by simultaneously amplifying a fragment of the ZFX/ZFY genes and a fragment of the SRY gene (Aasen and Medrano, 1990; Gilson et al., 1998). A 750 base pair (bp) fragment of the mitochondrial DNA control region (Dloop) was amplified, for both forward and reverse strands. Our haplotype sequences were then aligned and compared with the mitogenomes published by Archer et al. (2013), and trimmed to our sequence length (624pb). This way, 153 additional samples from the NP (n=92), GoC (n=5), Southeast Pacific (SEP, n=1), SA (n=41), NA (n=13) and western Australia (n=1) were retrieved from (Archer et al., 2013). Additionally, 107 sequences from the GoC were added, resulting in a global total of 296 sequences. Samples were then grouped according to their geographic origin. Five groups were build: NP (n=92), SEP (n=37), GoC (n=112), SA (n=41) and NA (n=13). The sample from western Australia (Archer et al., 2013) was included only in the haplotype network and left out of further analyses.

Genetic analyses

The possible presence of duplicate individuals among the GoC samples was ruled out by microsatellite typing. Sampling in the SEP was guided by the photo-identification of individuals. Genetic diversity indices segregative sites (k), number of haplotypes (h), haplotypic diversity (Hd), mean number of differences between two random sequences (Π) and nucleotide diversity (π), together with analyses of genetic structure (F_{ST}) and phylogeographic structure (Φ_{ST}) were conducted in Arlequin v3.5.2 (Excoffier and Lischer, 2010) with 1000 permutations and a significance level of 0.05. Additionally, Snn tests of genetic differentiation (Hudson, 2000) with 1000 permutations were conducted in Dnasp (5). The haplotype network was constructed in Hapview, a software that uses phylogenetic trees to construct haplotype genealogies. Previously, a Neighbour-Joining tree was constructed in MEGA v7 (Kumar et al., 2016) to serve as input file for the reconstruction of the haplotype network

RESULTS

Diversity in the Southeastern Pacific and Gulf of California

Analysis of the 37 samples from the SEP revealed a total of 26 different haplotypes together with high haplotype (Hd=0.980) and nucleotide diversities (π =0.81%). Nine haplotypes were shared by more than one individual, lowering the possibility of resampling in this area. Such a high diversity was found in four of the five localities (NP, NA, SEP and SA), despite differences in sample size (Hd range=0.962-0.996, sample size range=13-97) (Table 1). The GoC contrasted sharply with the other four localities, presenting a diversity approximately three times lower, despite the higher sampling size (n=112). A single haplotype was present in 89 of the 112 samples (83%). A sixth shared haplotype was found between SEP and the western Australia sample.

Table 1. Sample sizes (N) by geographic origin: North Pacific (NP), Gulf of California (GoC), Southeast Pacific (SEP), South Atlantic (SA) and North Atlantic (NA). Genetic diversity indices: number of haplotypes (h), number of polymorphic sites, haplotypic diversity (Hd), mean number of differences between two random sequences (\prod) and nucleotide diversity (π).

	NP	GOC	SEP	SA	NA
Ν	92	112	37	41	13
h	48	5	26	39	11
S	36	6	25	36	15
Hd	0.979	0.313	0.98	0.996	0.962
П	5.23	0.367	5.08	5.58	5.53
П (%)	0.84	0.06	0.81	0.89	0.89

Phylogeographic structure

In a geographical coherence, four haplogroups were recognizable in the resulting haplotype network (Figure 2). The GoC haplogroup are nearly isolated but connected to the NP haplogroup by four haplotypes shared (three of them are the most frequent in GoC). The thirteen NA samples (from Archer et al 2013) formed a separate group as well. The SEP (new sequences) and SA (sequences from Archer et al 2013) shared six haplotypes with a homogeneous distribution within the SH topology. Two haplotypes sampled in the North Pacific nested genetically into the South hemisphere haplogroup.

High values of phylogeographic structure indices were obtain between localities from NH (NP, NA Φ_{ST} range from 0,72 to 0,9, P<0.001 Table 2) except for the comparison between GoC and NP (Φ_{ST} = 0,28 P<0.001). On contrary, a lack of statistical significance of the Snn test of genetic differentiation (Snn=0.591, P=0.06) and low values of phylogeographic structure were obtained between SH (SEP vs SA, Φ_{ST} =0,26). An intermediate value of Φ_{ST} =0,43, P<0.001) was obtained between NP and SP populations.



Figure 2: Haplotype network of the 296 samples from the five localities, coded by color: North Pacific (green), Gulf of California (yellow), Southeastern Pacific (white), South Atlantic (blue) and North Atlantic (red). Circle size is proportional to sample size, detailed within each haplotype



Figure 3: Pairwise comparison between five general localities, incluiding the analysis of genetic structure (F_{ST}) and phylogeographic structure (Φ_{ST})

CONCLUDING REMARKS

- A strong genetic structure, with some few shared haplotypes, was observed as previously between NP and the GoC populations, the latter being a differentiated unit under a recent population expansion process.
- A genetic structure analysis between North Pacific and South Pacific was observed (considering for first time fin whale genetic information from Chile) obtaining an intermediate phylogeographic index. Two haplotypes sampled in the North Pacific nested genetically into the South hemisphere haplogroup, thus a low and unidirectional and rare dispersal event from SP to NP was further confirmed.

- No phylogeographic structure was detected between Southern Hemisphere localities (SP and SA) suggesting a single genetic unit.
- These results challenge the validity of the putative pygmy fin whale sub-species. However, there is still a possibility that the potential pigmy fin whales have not been sampled and consequently not included in the analyses. However, we strongly believe that our sampling effort should have included potential pigmy fin whales during the long study period. Indeed, six individuals included in this study satellite tagged by Sepúlveda et al (2018), remained in lower latitudes during summer months within the distribution range proposed to pygmy fin whales by Clarke 2004.
- Finally, our analyses points to that four major population units (three for the NH and one for the SH) should be considered for *B. physalus*

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