

Demographic inference and genetic diversity of *Octopus mimus* (Cephalopoda: Octopodidae) throughout the Humboldt Current System

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Abstract Climatic and oceanographic events occurring during the last glacial cycle in the Humboldt Current System (HCS) have left genetic footprints in marine invertebrate populations. The objective of this study was to evaluate the effect of the glacial period on *Octopus mimus* populations found throughout the HCS. This species lays a large number of small eggs which hatch into planktonic paralarvae with the potential to undergo wide dispersal. We sequenced the COIII gene to perform phylogeographic analyses

of 197 octopuses sampled from seven localities. The genetic diversity of *Octopus mimus* was low and decreased towards the southern end of the distribution range, which comprises a single population. The haplotype genealogy and Bayesian Skyride plot suggest that *O. mimus* underwent a demographic expansion after the last glacial maximum (LGM). This would imply a contraction of the range of this organism toward northern latitudes during the LGM followed by southward expansion and recolonization once the contemporary interglacial period began.

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Introduction

Most of the Northern Hemisphere has been affected during glaciations, and many species have survived in glacial refugia or have emigrated southwards and recolonized higher latitudes during post-glacial expansions (Hewitt & Ibrahim, 2001; Hewitt, 2004; Provan & Bennett, 2008; Marko et al., 2010; Kelly & Palumbi, 2010). In biogeographic studies, the Contraction–Expansion (C–E) model has often been invoked to describe the genetic and demographic response of species to the glacial cycles of the Pleistocene (Gates, 1993; Provan & Bennett, 2008; Marko et al., 2010; Kelly & Palumbi, 2010). Recently,

there has been an increase in the number of phylogeographic studies conducted in the Southern Hemisphere, and these have made it possible to compare the variable effects of glacial cover on coastal species from both hemispheres. Despite this, there is still a lack of knowledge regarding the effects of glacial refugia on species inhabiting the Southern Hemisphere (Ruzzante et al., 2006; Beheregaray, 2008). There is, however, genetic and demographic evidence suggesting that marine species in southern South America were affected by the glacial period during the Pleistocene (Cárdenas et al., 2009a; Macaya & Zuccarello, 2010; Fraser et al., 2010; Sánchez et al., 2011; González-Wevar et al., 2012). Some marine species found throughout the Humboldt Current System (HCS) also appear to have experienced a demographic history of range contraction and subsequent expansion caused by the glacial period (Cárdenas et al., 2009a, b; Haye et al., 2010; Ibáñez et al., 2011; Ibáñez et al., 2012; Haye et al., 2014).

The distribution and composition of marine populations in the HCS are the result of multiple climatic and oceanographic events (Camus, 2001; Thiel et al., 2007; Echevin et al., 2008; Cárdenas et al., 2009a). The progressive glaciation of Antarctica due to the formation of the Circumpolar Current at the Eocene–Oligocene boundary (Kennett, 1977) generated the HCS, which cooled the region and contributed to conditions for upwelling along the southeastern Pacific coast (Camus, 2001; Tsuchi, 2002). This was marked by radical changes in the coastal fauna during the Tertiary specifically, subtropical species found in warmer environments were replaced by cold-water fauna similar to those presently inhabiting the southeastern Pacific coast (Camus, 2001). During the Pliocene and Pleistocene, glacial cycles caused displacement of the Antarctic Circumpolar Current to lower latitudes. Subsequently, the strength and location of the South Pacific Anticyclone, the belt of southwest winds, and the West Wind Drift Current were altered (Romero et al., 2006; Kaplan et al., 2008). Additionally, increasing advection of Sub-Antarctic water masses and reduction of the sea surface temperature (SST, Hebbeln et al., 2002) brought about the formation of an ice sheet throughout southern South America (Hulton et al., 2002; Lamy et al., 2004), thus changing the oceanographic conditions of the HCS.

At present, the HCS interacts with the oceanographic dynamics of the equatorial zone causing

periodic advection of warm water masses towards the south (Montecino & Lange, 2009), which may be either multi-decadal (Pacific Decadal Oscillation; Chavez et al., 2003; Montecino et al., 2005; Chavez et al., 2008; Ramos et al., 2008) or inter-annual (El Niño Southern Oscillation). Current oceanic and atmospheric variability has ecological effects, which may be episodic and local, or may persist over long periods of time and occur over large spatial scales (Ahumada et al., 2000; Ichii et al., 2002; Chavez et al., 2003, 2008). Specifically in marine ecosystems of the HCS, communities can become reorganized and trophic relations can be altered as a result of this variability (Steele, 2004; Collie et al., 2004; Cahuín et al., 2009). As such, the HCS is an interesting study system to evaluate the relative effects of glacial history on benthic organisms with larval development and high dispersal potential. The species *Octopus mimus* Gould, 1852 is a suitable model to evaluate these effects, since it is widely distributed throughout the HCS from 26°S in northern Chile to 4°S in northern Peru near Tumbes (Defeo & Castilla, 1998; Guerra et al., 1999; Cardoso et al., 2004). In addition, adult octopuses produce numerous small eggs which hatch into planktonic paralarvae with a high dispersal potential (Boletzky, 1992, 1997; Cortez et al., 1995; Villanueva & Norman, 2008). Therefore, the aims of this study were (1) to assess the influence of historic and contemporary climatic events on the genetic diversity, population structure, and demographic history of *O. mimus* inhabiting the HCS; and (2) to examine whether the patterns observed in this species support a Pleistocene contraction–expansion (C–E) biogeography model.

Materials and methods

Sampling

Samples of *O. mimus* were obtained from seven localities covering approximately 2000 km of the Southeastern Pacific coast, from 4° to 26°S latitude (Fig. 1). A total of 197 octopuses were sampled between August and October 2009. The samples from off Chile were obtained by artisanal fishing and scuba diving, while those off Peru were collected by IMARPE (Instituto del Mar del Perú). The necessary permits for the capture of octopuses for scientific

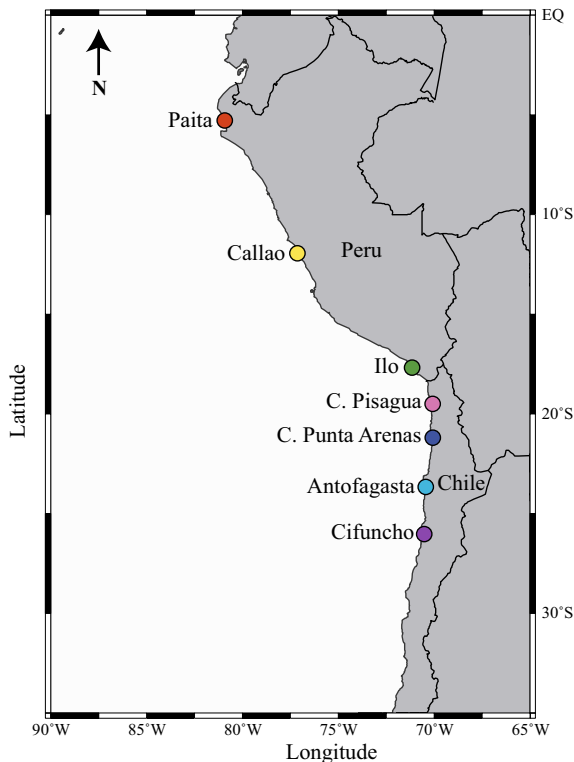


Fig. 1 Map showing the localities throughout the Humboldt Current System where samples of *Octopus mimus* were collected

purposes were obtained from local government institutions. All octopus samples were transported to the laboratory, where morphological measurements were made for a different investigation. A small piece of muscle tissue (2 cm²) was cut from the mantle of each octopus and stored in 96% ethanol to await molecular analysis. Subsequently, all octopus samples were frozen for other biological studies by IMARPE and Project-FIP 2008-39.

DNA extraction, PCR amplification, and sequencing

DNA was extracted from mantle muscle tissue using the salting-out method (Aljanabi & Martinez, 1997). Amplification was performed using the primers Co3a2: F-5'TTATTTATTGCATCAGAAGT3' and Co3b2: R-5'TCAACAAAGTGTCAGTATCA3' (Allcock et al., 2008) to amplify 597 base pairs (bp) of the mitochondrial cytochrome oxidase subunit III gene (COIII). Amplifications were done in a 25 µl reaction volume consisting of 0.25 µl *Taq* DNA polymerase

(1.0 units), 2.5 µl buffer 10X (50 mM KCl, 10 mM Tris-HCl pH 8.0), 2 µl dNTPs (10 µM), 1.0 µl of 50 mM MgCl₂, 0.5 µl (10 pg/µl) of primer Co3a2 and 0.5 µl of primer Co3b2, 17.25 µl of double-distilled water, and 1.0 µl (50 ng/µl) DNA. The optimum amplification conditions were: initial denaturation at 94°C for 120 s, 40 cycles of denaturation at 94°C for 40 s, annealing at 48°C for 40 s, and extension at 72°C for 90 s, with a final extension at 72°C for 10 min. Double stranded PCR products were purified and sequenced in both directions using an Automatic Sequencer ABI3730 × 1 at Macrogen, Inc. (Seoul, Korea). Alignment and confirmation of polymorphic sites were conducted using ProSeq version 2.9 (Filatov, 2002). These COIII sequences were deposited in the NCBI GenBank database (Accession Numbers: KC886287–KC886305).

Data analysis

Genetic diversity

Genetic diversity was characterized using the number of polymorphic sites (S), number of haplotypes (K), haplotype diversity (H_d), nucleotide diversity (π), average number of nucleotide differences between pairs of sequences (Π), theta K (Θ_K), theta S (Θ_S), theta H_d (Θ_{H_d}), and theta Π (Θ_Π). All diversity indices were calculated using Arlequin software version 3.5.1.3 (Excoffier & Lischer, 2010). The effective population size (N_e) and mutation rate (μ) were estimated using the estimated theta values. The genealogical relations among haplotypes were estimated using the Median-Joining algorithm for sequence data with the Network software version 5.0 (Bandelt et al., 1999).

Population structure and gene flow

The Φ_{ST} (the number of differences between pairs of sequences) and F_{ST} (the haplotype frequencies) indices between pairs of localities were estimated in Arlequin, and the statistical significance of differences was estimated based on 10,000 permutations. Phylogeographic structuring was evaluated by comparing the observed genetic differentiation coefficients G_{ST} and N_{ST} proposed by Pons & Petit (1995, 1996). This analysis used the methodology proposed by Burban

et al. (1999) in the PERMUT software version 1.2.1 (Pons & Petit, 1995, 1996); 10,000 permutations were performed using a genetic distance matrix to evaluate whether N_{ST} was greater than G_{ST} , which would indicate the existence of phylogeographic structure.

Demographic history

Demographic history of previously identified populations of *O. mimus* was assessed. Firstly, Tajima's D (Tajima, 1989) and Fu's F_s (Fu, 1997) indices were calculated in Arlequin. These indices detect deviations from Wright-Fisher mutation-drift equilibrium based on the excess or deficit of low frequency haplotypes generated by population expansion, bottlenecks, or selection events which have occurred in the past. Secondly, a Bayesian Skyride plot (BSR; Minin et al., 2008) was generated using a coalescence framework chosen by means of the Bayes Factor (BF = 3.541) described by Newton & Raftery (1994) and modified by Suchard et al. (2001); this was done in the software Tracer 1.5 (Rambaut & Drummond 2009). This analysis was conducted to estimate the effective population size of *O. mimus*, and the time to the Most Recent Common Ancestor (MRCA) based on the coalescence of maximum credibility trees, performed in BEAST version 1.8.4 (Drummond et al., 2012). For COIII mitochondrial sequences, HKY 85 nucleotide substitution models were chosen using Bayesian Information Criterion (BIC) in the jModelTest software ($-\ln L = 757.76$, $BIC = 4002.56$) (Posada, 2008). The mutation rate usually used for COI in cephalopods is 2% substitutions per million years (Pérez-Losada et al., 2007; Ibáñez et al., 2011, 2012; Sales et al., 2017). We chose to use the same mutation rate used for COI for our COIII analyses, since in octopods mitochondrial coding genes have showed similar nucleotide variability: COI (0.345), COII (0.339), COIII (0.356), and Cytb (0.336) (Cheng et al., 2013). Additionally, a relaxed uncorrelated log-normal molecular clock model was chosen by means of the Bayes Factor (BF = 3.186) to perform the BSR. The Markov-Chain Monte Carlo method was carried out using a single chain of 250 million iterations. The first iterations that did not reach convergence were discarded from the analysis. Subsequently, parameters such as effective population size and the time to the MRCA were assessed in the coalescence credibility trees, sampling every 1000 generations. The posterior

parameter distribution (highest posterior density, 95% HPD interval) represented the uncertainty of the effective population size (N_e) and time to the MRCA (Drummond et al., 2005). Finally, the results were visualized in Tracer.

Results

Genetic diversity

The COIII mitochondrial gene sequences from 197 *O. mimus* specimens clustered into a total of eight haplotypes. The seven localities had low haplotype and nucleotide diversities, few polymorphic sites, and a low mean number of nucleotide differences between pairs of sequences; interestingly, even less diversity was found southward (Table 1). These low diversity indices could be a consequence of demographic changes resulting from changes in oceanographic conditions throughout the HCS. In addition, low diversity (0.27, 0.46, and 0.85) and departure from Hardy–Weinberg equilibrium have been found for this species, which is relatively low even for highly variable markers such as microsatellites (Galleguillos et al., 2011). Therefore, our results and those of Galleguillos et al. (2011) suggest that demographic effects could be influencing genetic diversity rather than selective pressure on genes. Estimates based on the distribution range of *O. mimus* and according to

Table 1 Genetic diversity of *Octopus mimus* based on a 597 bp region of the mitochondrial COIII gene

Localities	N	K	S	Hd	π	Π
Peru						
Paíta	30	5	7	0.457	0.003	1.338
Callao	24	5	7	0.370	0.003	1.370
Ilo	33	2	1	0.117	0.000	0.117
Chile						
Caleta Pisagua	23	5	9	0.324	0.002	0.783
Caleta Punta Arenas	36	1	0	0.056	0.000	0.522
Antofagasta	25	1	0	0.000	0.000	0.000
Cifuncho	26	2	5	0.077	0.001	0.385
Total	197	13	14	0.609	0.002	1.110

N number of sampled specimens, K number of haplotypes, S polymorphic sites, Hd haplotype diversity, π nucleotide diversity, Π average number of nucleotide differences

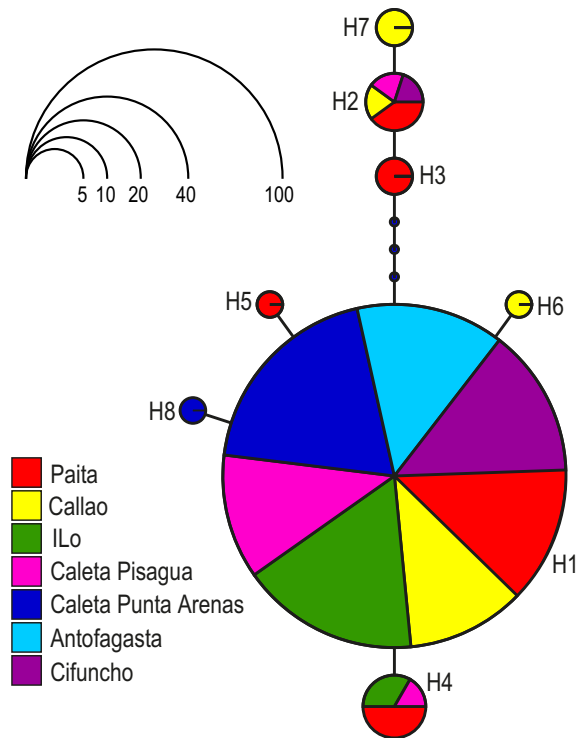


Fig. 2 Haplotype network of 197 *Octopus mimus* mtDNA sequences. Each haplotype is represented by a colored circle whose size is proportional to its frequency in the whole dataset. Black points correspond to a specific mutation

theta values ($\Theta_k = 1.53$, $\Theta_s = 1.71$, $\Theta_{Hd} = 0.16$, $\Theta_{\Pi} = 0.53$), the effective population size (N_e) was estimated to be between 7350 and 142,000 octopuses. The genealogical relationships among haplotypes graphically demonstrated the low genetic diversity of *O. mimus* (Fig. 2). This genealogical network was characterized by one dominant haplotype (H1) surrounded by one lower frequency haplotype (H4) and three unique haplotypes (H5, H6, and H8) that were linked to three other low frequency haplotypes (H7, H2, and H3) by five, four, and three mutational steps, respectively (Fig. 2). The H1 haplotype was found in 179 octopuses (90.9%), dominating in all sampled localities throughout the distribution range. Haplotypes H3, H5, H6, H7 were only found off the coastal of Peru, and haplotype H8 was only found off the coastal of Chile, whereas H2 and H4 were found off the both Peru and Chile. The low overall diversity continued to decrease in the southern part of the range, where all octopuses except four specimens were characterized as having the H1 haplotype.

Population structure and gene flow

The pairwise Φ_{ST} and F_{ST} values were all low and not significant after Bonferroni correction, with the exception of the comparisons between Paita with Caleta Punta Arenas and Antofagasta, which were significant but equally low (Table 2). The within-locality genetic diversity levels were very low ($h_s = 0.169$) and similar to the total diversity ($h_T = 0.176$), which means that a relatively low proportion of the total diversity was due to differences among localities ($G_{ST} = 0.040$). The intra-population haplotype number ($vs = 0.142$) was also similar to the overall estimation ($v_T = 0.146$). Finally, permutations showed that 69.3% of the permuted values of N_{ST} were greater than the observed N_{ST} , suggesting the absence of phylogeographic structure.

Demographic inference

The Tajima's D and Fu's F_s indices estimated for *O. mimus* with COIII gene were negative and statistically significant ($D = -1.612$, $p = 0.0209$; $F_s = -4.024$, $p = 0.0479$); thus, the neutral hypothesis of mutation-drift equilibrium was rejected. The deviations from neutrality likely are due to deficits of low frequency haplotypes generated by population expansion, bottlenecks, or selection events in the past.

The Bayesian Skyride plot revealed one pulse of demographic expansion approximately 40,000 years ago with the effective population size expanding by

Table 2 Phylogeographical and genetic structuring of *Octopus mimus*

	1	2	3	4	5	6	7
1		0.007	0.061	0.023	0.118*	0.127*	0.079
2	0.021		0.028	0.007	0.059	0.079	0.026
3	0.074	0.094		0.027	0.003	0.019	0.006
4	0.001	0.003	0.002		0.005	0.027	0.024
5	0.096*	0.104	0.023	0.016		0.011	0.015
6	0.076	0.080	0.019	0.004	0.011		0.002
7	0.017	0.013	0.013	0.038	0.009	0.002	

Φ_{ST} (below the diagonal) and F_{ST} (above the diagonal) pairwise comparisons among localities in the Humboldt Current System. Bonferroni correction level, $\alpha = 0.0071$

1 Paita, 2 Callao, 3 Ilo, 4 Caleta Pisagua, 5 Caleta Punta Arenas, 6 Antofagasta, 7 Cifuncho

* Significant difference among comparisons

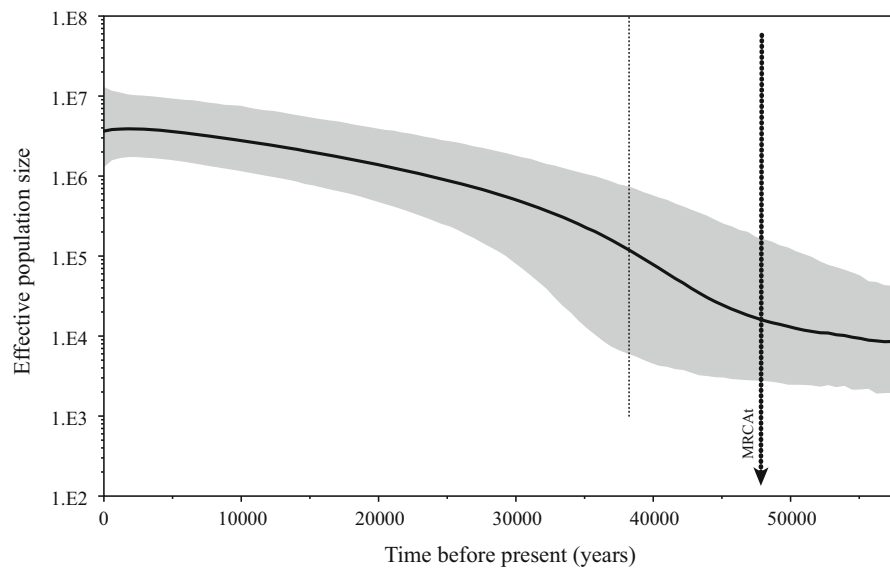


Fig. 3 Bayesian Skyride plot showing changes in effective population size over time in *Octopus mimus*. Time estimates along the x-axis were obtained by assuming an average pairwise sequence divergence rate of 2% per million years. Population size on the y-axis is given on a logarithmic scale. The *thick solid*

line represents the mean estimate of population size; the *thin solid lines* show the 95% HPD (highest posterior density) intervals. The *solid vertical dotted line* represents the mean timing of the Most Recent Common Ancestor and thin dotted lines the 95% HPD

approximately two orders of magnitude (Fig. 3). Moreover, the estimated time to the *O. mimus* MRCA was approximately 47,837 years ago (95% HPD: 38,239–58,015, Fig. 3).

Discussion

Genetic diversity and structure of *Octopus mimus* in the HCS

The genetic diversity of *Octopus mimus*, as measured in this study using the COIII mitochondrial gene, is reduced throughout its distribution range in the HCS. Interestingly, genetic diversity continued to decrease southwards. Similar patterns in COIII diversity have also been found for *Octopus vulgaris* Cuvier, 1797 off the coast of South Africa (Oosthuizen et al., 2004). Contrastingly, high genetic diversity (COIII Hd = 0.91) has been found for *Octopus variabilis* (Sasaki, 1929) off the coast of China (Xu et al., 2011). These contrasting results suggest that this mitochondrial gene is not as conserved as has been suggested for cephalopods (Cheng et al., 2012, 2013). Furthermore, the low diversity found here could be a consequence of demographic fluctuations or selective pressure in the

HCS. Results employing the mitochondrial COI gene for the same specimens showed even lower variability than the COIII gene (Hd = 0.01, $n = 20$) in this study. Galleguillos et al. (2011) also reports low observed heterozygosities for *O. mimus* microsatellites. Therefore, these data together support the hypothesis that low genetic diversity observed in this species is primarily a consequence of demographic fluctuations that have occurred in this population. Low genetic diversity of COI mtDNA has also been found for other marine invertebrates in the HCS (Haye et al., 2010; Haye et al., 2014) including squids (Ibáñez et al., 2011, 2012). This suggests that, as for *O. mimus*, low diversity is a consequence of the drastic changes in oceanographic conditions in this ecosystem during the last glacial maximum (LGM; Montecino & Lange 2009).

The haplotype network illustrates the low genetic diversity of *O. mimus*. It shows that most of the octopuses sampled in all localities are characterized by the dominant haplotype, and few of the other haplotypes are represented (Fig. 2). Additionally, no significant genetic or phylogeographic breaks were detected with the exception of localities at the edges of the distribution of this species. However, overall genetic differences were quite low (Table 1). *Octopus*

mimus has planktonic paralarvae with high dispersal potential; hence dispersal could maintain high levels of connectivity. Previously, Pardo-Gandarillas et al. (2016) have suggested that *Robsonella fontaniana* D'Orbigny, 1835, another octopus inhabiting the HCS, has a high dispersal potential based on the genetic similarity of paralarvae and adults over 2000 km of distance. Vega et al. (1999) collected *O. mimus* paralarvae at depths between 20 and 100 m off the Mejillones Peninsula in northern Chile, and have indicated that migration of this species might occur in currents and undercurrents at no more than 150–200 m depth; that is, in the Humboldt Coastal Current (50–80 m) and by the surface flow of Chilean Coastal Current (0–30 m) (Ahumada et al., 2000; Montecino et al., 2005).

Demographic history of *Octopus mimus*

Hypothetically, large population sizes should maintain high levels of genetic diversity, since the effects of genetic drift are insignificant compared to the rate of accumulation of mutations (Freeland, 2005). In this context, the theta values estimated from genetic diversity suggest small effective *O. mimus* population sizes ($N_e = 7350\text{--}142,000$). Indeed, the effective population size found here for *O. mimus* is smaller than that expected for an economically important species in both Chile and Peru (Defeo & Castilla 1998). Chilean as well as Peruvian octopus landings could be used as indirect demographic measures. Approximately 500 to 3000 tons of octopus are caught annually (1998–2014; Sernapesca, 2015; Produce, 2014), which is equivalent to approximately 300,000 to 2 million octopuses (N_t) with a mean wet weight of 1.5 kg per adult (Cortez et al., 1995). These numbers suggest that this species should have a considerable population size, and therefore high genetic diversity but the low genetic diversity found here indicates otherwise, a small population size. In an ideal population, $N_e = N_t$, but normally this ratio is near 0.1 due to the loss of genetic diversity by fluctuating population sizes (Freeland, 2005). The low N_e/N_t ratios estimated (0.02–0.07) for *Octopus mimus* suggest a faster loss of genetic diversity than that expected from genetic drift alone. Thus, demographic fluctuations could also be influential in the genetic diversity.

The negative and significant Tajima's D and Fu's Fs indices indicate that *O. mimus* is not in Wright-

Fisher mutation-drift equilibrium (Fu, 1997). Hence, the demographic inferences support a historical demographic population expansion. However, the value obtained for the expansion time estimated in this study (approximately 40,000 years ago; Fig. 3) could vary depending on whether or not rates of molecular evolution are considered (Ho et al., 2005; Ho & Shapiro, 2011). Evolutionary studies on birds, mammals, and invertebrates have shown the importance of considering rates of evolution depending on the time frame over which they are measured, in other words rates estimated at species levels should be used carefully at population level, since substitution rates inferred at population levels are much higher than at species level (Ho et al., 2005; Ho & Shapiro, 2011; Subramanian & Lambert, 2011). Mutation rates of mitochondrial DNA should be two to fifteen times higher (including fish, butterfly, birds, and primates) than long-term substitution rates (Ho et al., 2005; Burridge et al., 2008; Gratton et al., 2008; Subramanian et al., 2009). We used a substitution rate of 2% (substitutions/site/my), which has been used at the species level, although it has also been used for several studies at population level (Strugnell & Lindgren, 2007; Pérez-Losada et al., 2007; Ibáñez et al., 2011, 2012; Sales et al., 2017). Thus, when using this substitution rate in our analysis, the estimated time of demographic expansion for *O. mimus* was of 40,000 years, which may be an overestimation. Therefore, it incorporated a simple rate correction of two to ten-fold, following Gonzalez-Wevar et al. (2011), resulting in a corrected expansion time of 20,000–4000 years ago. This would align with the post-glacial period after the LGM (which occurred 25,000–23,000 years ago; Rabassa, 2008; Rabassa et al., 2011). Accordingly, the results of demographic expansion estimates, small effective population size, and low genetic diversity support a Pleistocene biogeographic Contraction–Expansion (C–E) model. Under this scenario, *O. mimus* populations should have undergone: (1) A contraction of the distribution range to the north, probably caused by the strong reduction of sea surface temperatures in the HCS during the LGM (25,000 years ago, Kim et al., 2002; Feldberg & Mix, 2003; Romero et al., 2006); followed by (2) a demographic expansion southwards during the beginning of the post-glacial period (Rabassa et al., 2005, Rabassa, 2008), coinciding with the

entry of subtropical waters into the HCS (Keefer et al., 1998; Rodbell et al., 1999; Ortlieb et al., 2011).

Finally, this study suggests that the *O. mimus* samples gathered here derive from one homogenous population with low genetic diversity. The low genetic diversity and simple haplotype network indicate a reduced effective population size, which is a consequence of demographic changes in the past. *Octopus mimus* may represent an example of a Pleistocene biogeographic C–E model (Provan & Bennett, 2008; Marko et al., 2010; Kelly & Palumbi, 2010) which was not affected directly by the glacial ice sheet, but rather by other climatic and oceanographic changes which historically cooled the oceans towards low latitudes. The effects of glaciations were so widespread that a Pleistocene biogeographic pattern as mentioned above can be recognized in species that currently inhabit warmer latitudes, such as in the Humboldt Current System.

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