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**Genetic Diversity and Population Structure of *Dosidicus gigas***

*Chile*

## Genetic diversity and population structure of *Dosidicus gigas* (Cephalopoda: Ommastrephidae) in the Pacific Ocean

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### ABSTRACT

Jumbo squid samplings were carried out in northern (Coquimbo), central (San Antonio and Quintay) and southern Chile (Lebu). The squid collected were large sized (> 60 dorsal mantle length) and most males were maturing and mature (stages II and III), while all females were immature (stage II). DNA extractions were conducted for the samples collected during this project. Preliminary results of mitochondrial cytochrome oxidase I (COI) gene sequences generated here are presented. The genetic diversity inferred from COI gene sequences was low, which is similar to previous studies. The genealogical network and pairwise comparisons suggested an absence of population structure comparing samples from the four localities along the northern, central and southern Chilean coast.

### INTRODUCTION

The jumbo squid *Dosidicus gigas* (d'Orbigny 1835) is the most abundant and largest squid in the Pacific Ocean (Nigmatullin et al. 2001, Ibáñez et al. 2015). *Dosidicus gigas* exhibits sporadic and short-term pulses in abundance related to environmental changes and recruitment patterns (e.g., ENSO) (Keyl et al. 2008, Zúñiga et al. 2008). Recently, after the 1997-98 ENSO event, *D. gigas* experienced a range expansion in both hemispheres linked to an increasing abundance related to the expansion of the oxygen minimum zone (Zeidberg & Robinson 2007, Keyl et al. 2008).

This squid species undergoes multiple spawning, producing over 32 million eggs, which entails the highest fecundity among cephalopods (Nigmatullin & Markaida 2009). Females spawn a large egg mass (3-4 m diameter) near the surface, embedded in a gelatinous matrix dispersing thousands of eggs (Staaf et al. 2008) that possibly drift several kilometers (Ibáñez et al. 2011). Additionally, the squids hatch into small paralarvae (Yatsu et al. 1999, Staaf et al. 2008) with a high dispersal potential.

Genetic studies evaluating the jumbo squid revealed an absence of structuring at relatively small distances (< 5000 km) and isolation at larger distances (> 5000 km) along the Pacific Ocean (see review in Ibáñez et al. 2015). This pattern has been associated with the biological characteristics of *D. gigas* (e.g., horizontal migrations, dispersive egg masses and paralarvae) (Ibáñez et al. 2011).

This report is based on preliminary results on the development of new genetic data of jumbo squid from Chile.

### METHODS

Sampling was carried out in the following three areas of Chile: northern zone (E3), central zone (E4) and southern zone (E5). For each squid (total = 130), a piece of mantle tissue was extracted

for fixation in specific solutions (RNAlater) for genetic analyses. All squids correspond to large specimens (> 60 cm dorsal mantle length) and most of the males were maturing and mature (stages II and III), while all females were immature (stage II).

To obtain mitochondrial sequences, total DNA was extracted following the saline extraction protocol (Alhjanabi & Martinez 1997). DNA extractions have been performed for 96 squids. Mitochondrial sequences of cytochrome oxidase I (COI) gene have been obtained via PCR for around 15 individuals per sampling site. The collected jumbo squid samples were in good condition, presenting high DNA concentrations (>500 ng/ $\mu$ l) and an adequate 260/280 absorbance ratio (~2.0) (Table 3). DNA integrity was also verified by agarose gel (2%) electrophoresis.

For this project, the DArTseq genotyping technology will be applied. This reduced-representation sequencing technique includes a library preparation by digesting DNA with two restriction enzymes. The procedure will be performed by the Diversity Arrays Technology company once the optimal restriction enzymes combination and the fragment size to be sequenced (usually between 75 – 150 pb) are determined in an Illumina HiSeq platform. Samples have been shipped for DArTseq genotyping.

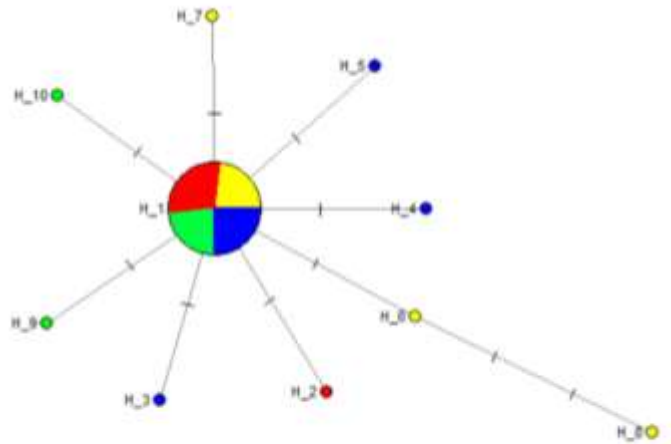
## RESULTS

As DArTseq genotyping is on process, only the results of partial COI gene sequences are presented here. These COI gene sequences revealed a total of 10 haplotypes from the 657 bp fragments obtained in the 57 individuals examined, respectively. Haplotype and nucleotide diversity were low in all locations (for pooled samples  $H_d = 0.29$ , Table 1).

**Table 1.** Genetic diversity of *Dosidicus gigas* from four localities along the Chilean coast.

Locality	Sample size	Haplotypes	Polymorphic sites	Haplotype diversity ( $H_d$ )	Nucleotide diversity
Coquimbo	14	4	4	0.3956	0.0011
San Antonio	15	2	1	0.1333	0.0002
Quintay	13	3	2	0.2949	0.0005
Lebu	15	4	3	0.3714	0.0006
Total	57	10	10	0.2932	0.0006

The median-joining network revealed a clear starlike pattern with a dominant central haplotype (H1) and a very short genealogy (Fig. 1). The central haplotype (H1) was the most common and the most widely distributed geographically. The rest of the haplotypes were separated from H1 by one or 2 mutations (Fig. 1).



**Figure 1.** Haplotype network of jumbo squid from Chile. Small black lines in the network represent mutation steps and circle size represents the number of individuals in each haplotype. Yellow = Coquimbo, red = San Antonio, green = Quintay, blue = Lebu.

The low  $F_{st}$  values suggested that the jumbo squid exhibits a large and genetically uniform population across all the sampled Chilean localities (Table 2).

**Table 2.** Fixation index ( $F_{st}$ ) values of *Dosidicus gigas* between localities from Chile.

Localities	Localities	$F_{st}$
San_Antonio	Lebu	0.0000
San_Antonio	Coquimbo	0.0026
San_Antonio	Quintay	0.0000
Lebu	Coquimbo	0.0020
Lebu	Quintay	0.0000
Coquimbo	Quintay	0.0022

## DISCUSSION

### Genetic diversity

Theoretically, a very large population size should maintain high levels of genetic variability as genetic drift is low and the rate of mutation accumulation is high. Even though *D. gigas* exhibits a very large population size, this species shows low mtDNA diversity (Sandoval-Castellanos et al. 2010, Ibáñez et al. 2011). In addition, only to decrease haplotype diversity, *D. gigas* shows a star-like network characterized by very short branches as well as a marked L-shaped distribution of pairwise differences (Sandoval-Castellanos et al. 2010, Ibáñez et al. 2011).

### Population structure

Studies at the intra-specific level suggest that there are two genetic units, one in the Northern Hemisphere (Mexico-USA) and the other in the Southern Hemisphere (Peru-Chile), which are

explained by the isolation by distance model (Sandoval-Castellanos et al., 2007, 2010; Staaf et al., 2010). This absence of genetic structure within each hemisphere is consistent with the life history of species with high dispersal or large migration capacities (long-lived planktotrophic larvae or migratory behavior). In the case of *D. gigas*, extensive seasonal active migrations of up to 1,000 km have been related to active feeding, migration and maturation (Nesis 1970, Nigmatullin et al. 2001, Ibáñez & Cubillos 2007, Keyl et al. 2008, Ibáñez et al. 2015). Jumbo squids can move at a speed of eight to 30 kilometers per day during their migrations, thus, being able to migrate up to 200 km in seven days (Markaida et al. 2005, Gilly et al. 2006b). Furthermore, the passive migrations of pelagic egg masses and paralarvae of this species could contribute to the high gene flow and long-distance dispersal along its distribution range.

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