

**11<sup>th</sup> MEETING OF THE SCIENTIFIC COMMITTEE**

*11 to 16 September 2023, Panama City, Panama*

**SC11 – SQ02**

**Genetic population structure and genetic diversity of *Dosidicus gigas* along the Pacific Ocean**

*Republic of Chile*

## Genetic population structure and genetic diversity of *Dosidicus gigas* along the Pacific Ocean

Christian M. Ibáñez<sup>1</sup>, Jennifer Catalán<sup>1</sup>, M. Cecilia Pardo-Gandarillas<sup>1</sup>

1.- Department of Ecology and Biodiversity, Faculty of Life Sciences, Universidad Andres Bello, Santiago, Chile

### ABSTRACT

Species with high connectivity usually lack genetic differentiation at regional scale, but isolation by distance may generate genetic differentiation along species distribution. In this study, we tested the genetic population structure of *Dosidicus gigas* along the Pacific Ocean, considering sample sites from Ecuador to southern Chile and sequences from Genbank from Alaska to Chile. To achieve this objective, we sequenced one mitochondrial gene (COI). The analyses of these markers indicated that all the studied localities in Ecuador, Peru and Chile, as well as the region as a whole, are characterised by low genetic diversity. Conversely, the localities in northern hemisphere from Alaska to Panama showed higher genetic diversity. Furthermore, two genetic units were recognized, corresponding to northern and southern hemisphere localities. Therefore, our findings suggest the presence of two genetic units of *D. gigas* along the Pacific Ocean, and this spatial genetic structure is associated with isolation by distance model.

**Keywords:** population genetics, jumbo squid, cephalopods.

### INTRODUCTION

Most coastal and oceanic squids have wide geographical distribution (>1000 km) (Ibáñez et al. 2019, Rosa et al. 2019). These squids undertake large-scale migrations during their lifespan, often moving to feeding grounds during the major period of growth and maturation and migrating to spawning grounds upon maturation (Boyle & Rodhouse 2005). These biological features have implications for the genetic population characteristics of squids. Ommastrephid and loliginid squids, in general, have shown high levels of genetic structure at large geographic scales (ca. 5,000 to 10,000 km; Sandoval-Castellanos et al. 2007, 2010, Staaf et al. 2010), attributed to reduced genetic flow between more isolated squid schools and generating a pattern of isolation by distance. On the other hand, analyses at smaller geographic scales have revealed weak or even absence of genetic structure (ca. 100 to 1,000 km) (Ibáñez et al. 2011, Ibáñez & Poulin 2014), resulting from high gene flow between squid schools.

The jumbo squid *Dosidicus gigas* (d'Orbigny 1835) is a species with a wide latitudinal geographic range, extending from Alaska to southern Chile in the eastern Pacific Ocean (Keyl *et al.* 2008; Ibáñez *et al.* 2015). Previous studies have suggested the existence of two genetic units along the Pacific Ocean: one in the northern hemisphere (Mexico-USA) and another in the southern hemisphere (Peru-Chile), which are explained by a model of isolation by distance (Sandoval-Castellanos *et al.* 2007, 2010; Staaf *et al.* 2010). However, the sample size was not homogeneous for all localities, which could lead to biased interpretations of the results. In addition, geographical explicit models are better for detecting genetic clusters rather than the group comparisons made in the cited works. Therefore, in this study, we aim at testing the presence of genetic structure of *D. gigas* in the Pacific Ocean and characterize its genetic diversity using mitochondrial markers.

## **MATERIALS AND METHODS**

### **Sampling**

Sampling was conducted at 11 localities throughout the South Pacific, from Ecuador to southern Chile. At each locality, we collected 20 to 40 individuals of different body sizes (40 - 85 cm ML), extracting a piece of mantle tissue for fixation in 95% ethanol for genetic analysis. The samples were obtained in previous (2005-2008) and recent (2021) fieldwork. In addition, sequences of COI from Peru, Ecuador, Panama, Mexico, USA, and Canada were obtained from GenBank.

### **Mitochondrial DNA sequencing**

We sequenced mitochondrial genes cytochrome oxidase I (COI), which have been successfully used in *D. gigas* for phylogeographic studies (Staaf *et al.* 2010; Ibáñez *et al.* 2011). In order to obtain mitochondrial sequences, we followed the salt extraction protocol for total DNA and used universal primers to amplify the mitochondrial gene COI.

## **Analysis of mitochondrial sequences**

### **Genetic diversity and population genealogies**

We estimated standard diversity indices, such as the number of haplotypes (H), the number of polymorphic sites (S), haplotype diversity (Hd), the mean number of pairwise differences ( $\Pi$ ), as well as nucleotide diversity ( $\pi$ ) were estimated for the mitochondrial sequences (COI).

We assessed genealogical relationships between haplotypes from different regions of the Southeast Pacific by constructing median-joining haplotype networks using COI sequence data.

### **Spatial genetic structure**

In order to detect population structure in the jumbo squid fishery, we estimated the number of clusters (k) and the posterior probability density (PPD) of belonging to a given cluster using Bayesian inference in the software Geneland, implemented in R software. We inferred the number of clusters from the modal value of k, running a Markov Chain Monte Carlo (MCMC) with  $5 \times 10^6$  iterations, sampling parameters every 1000 iterations.

## **RESULTS**

### **Analysis of mitochondrial sequences**

#### **Genetic diversity**

The analysis of the COI gene showed generally low genetic diversity at all sampled locations in the Southern hemisphere ( $H_d < 0.55$ ) and high genetic diversity in localities in the Northern hemisphere ( $H_d > 0.75$ ) (Table 1). Additionally, the analyses evidenced less than 1 nucleotide difference on average between haplotypes ( $\Pi < 0.5$ ) from the Southern hemisphere, while in localities from Northern hemisphere average difference was more than 1 ( $\Pi > 1.5$ ) (Table 1).

#### **Genealogical analysis**

The haplotype network constructed using both COI gene showed "star-like" patterns, where one or two highly frequent central haplotypes were surrounded by very rare or unique haplotypes, linked by one to three mutational steps (polymorphic sites) between them (Fig. 1). The most frequent haplotypes were detected at all sampled localities along the Eastern Pacific from Alaska to Chile (Fig. 1).

### **Population genetic structure**

The Bayesian analysis of the georeferenced genetic structure showed a low posterior probability of two groups or populations ( $K = 2$ ) constituting *D. gigas* along its distribution range (Fig. 2). These units are located in the Southern hemisphere (Ecuador, Peru, and Chile) and the Northern hemisphere (Canada, USA, Mexico). This means that the spatial configuration of the posterior probability map consists of individuals assigned to more than one cluster, as jumbo squids have spatial population structure at higher scales  $> 5000$  km. (Fig. 2)

## **DISCUSSION**

This study corroborates that *D. gigas* represents a generically structured unit in the Eastern Pacific using molecular markers (COI). The low probability of belonging to a single genetic cluster found suggests that individuals of *D. gigas* inhabiting the East Pacific experience a great gene flow.

### **Genetic diversity**

The molecular marker used (COI) effectively estimated the genetic diversity of the jumbo squid in the East Pacific consistent with previous studies (Staff et al. 2010, Ibáñez et al. 2011, Ibáñez & Poulin 2014).

### **Population structure**

This study detected two genetic clusters of *D. gigas* in the East Pacific, supporting similar conclusions from previous studies regarding the genetic differences between groups in this region (Sandoval-Castellanos et al. 2007, 2010, Staff et al. 2010). These units are located in the Southern hemisphere (Ecuador, Peru and Chile) and the Northern hemisphere (Canada, USA, Mexico), reinforcing the existence of a single genetic unit of *D. gigas* in the South Pacific.

The jumbo squid, *D. gigas*, undertakes extensive seasonal migrations in the South Pacific, covering distances of up to 1,000 km. These migrations have been associated to active feeding, migration, and maturation (Nesis 1970, Nigmatullin et al. 2001, Keyl et al. 2008). During these migrations, these squids can move at speeds ranging from 8 to 30 km per day, covering distances of 200 to 1000 km in a span of just a few weeks (Markaida et al. 2005, Gilly et al. 2006, Stewart et al. 2012). Additionally, passive migrations of pelagic egg masses and paralarvae might contribute to high gene flow and long-distance dispersal throughout their range. Furthermore, the remarkable fecundity of females, capable of producing 15 to 18

million eggs (Ibáñez et al. 2015), substantially increases the probability of dispersal throughout the expanse of the Pacific Ocean.

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Table 1. Genetic diversity of *D. gigas* along East Pacific using COI.

Localities	Gene : COI					
	N	K	S	Hd	p	P
Tofino, Canada	16	8	9	0.850	0.0033	2.142
Gulf of California	9	4	4	0.778	0.0019	1.222
Baja California	3	3	5	1.000	0.0051	3.333
Nicaragua	1	1	1			
Panama	1	1	1			
Puerto López, Ecuador	15	5	4	0.476	0.0008	0.533
Huarmey, Peru	27	6	7	0.342	0.0008	0.519
Callao	33	8	9	0.544	0.0013	0.848
Coquimbo	29	8	7	0.431	0.0009	0.613
COQUIMBO 2022	30	6	5	0.455	0.0009	0.554
Valparaíso	28	4	4	0.206	0.0004	0.286
QUINTAY	16	3	2	0.242	0.0004	0.250
SAN_ANTONIO	16	3	2	0.342	0.0006	0.358
Isla Santa María	29	6	5	0.320	0.0006	0.409
LEBU	32	7	7	0.393	0.0008	0.496
Isla Mocha	23	5	5	0.324	0.0007	0.435
TOTAL	308	45	45	0.445	0.0011	0.713

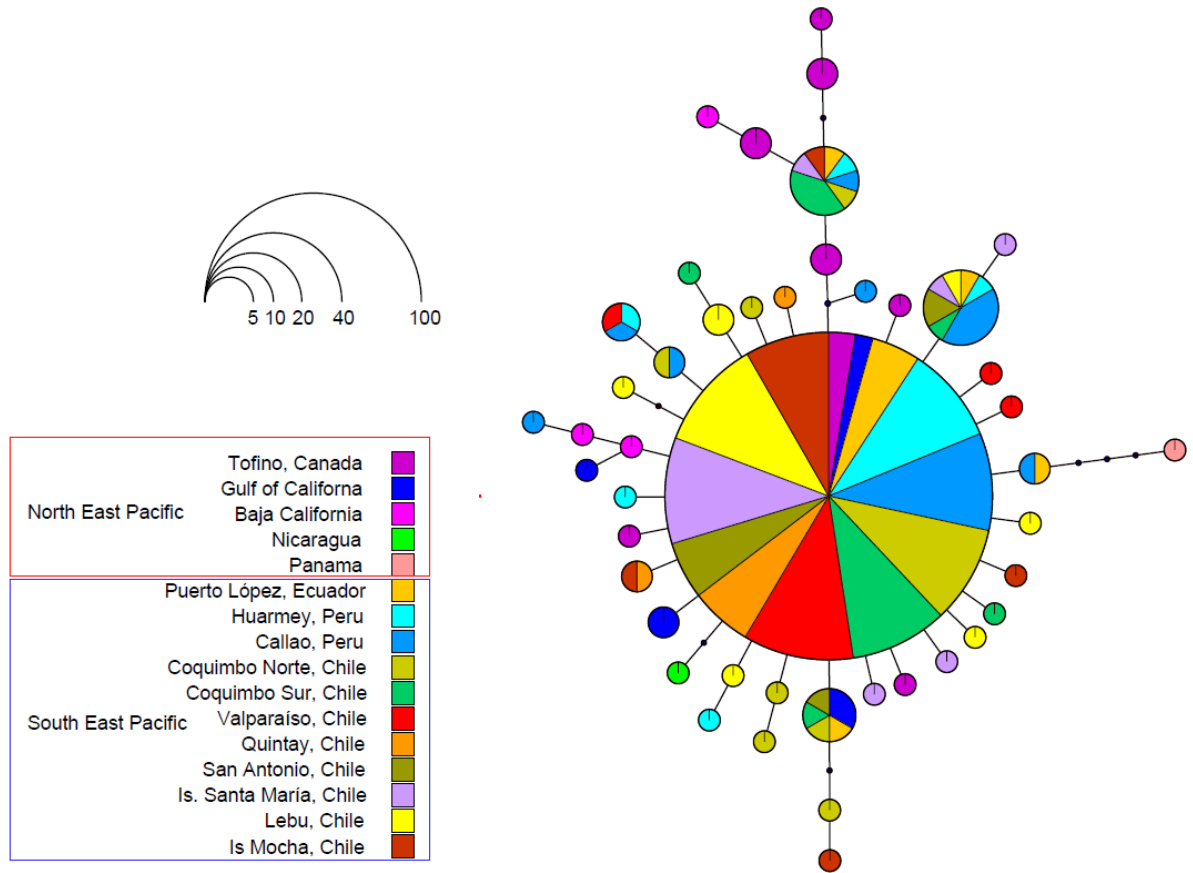


Figure 1. Haplotype network of *D. gigas* along East Pacific.



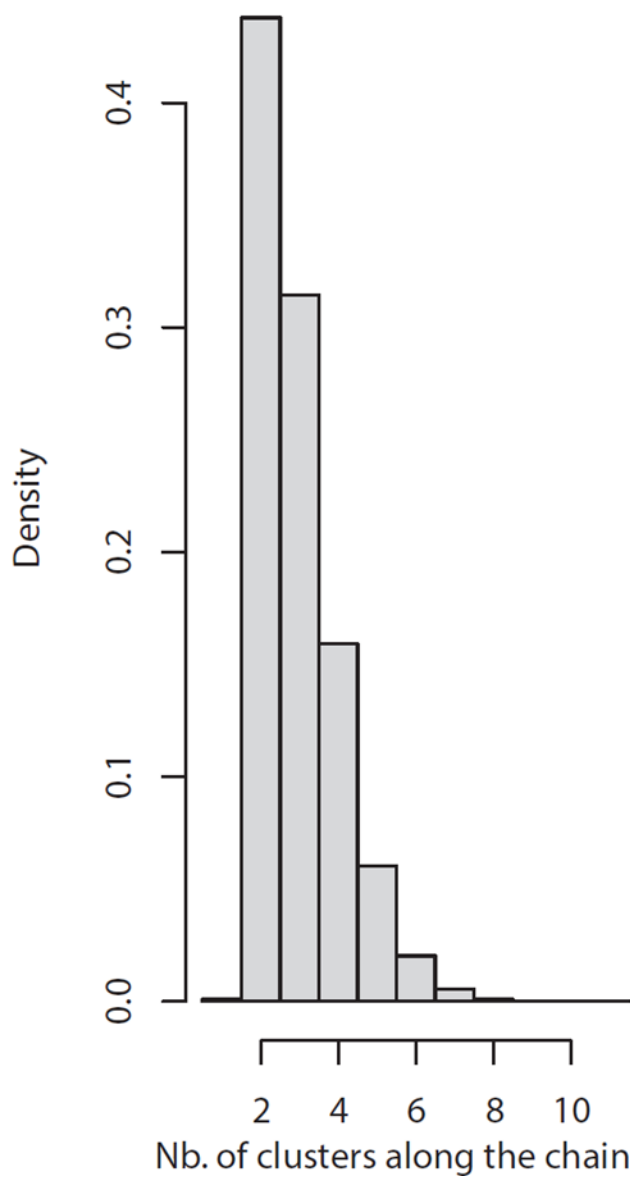


Figure 2. Posterior probability density histogram of *D. gigas* genetic groups along East Pacific.