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Genetic diversity and population structure of *Dosidicus gigas* in the Southeast Pacific Ocean based on genome-wide SNPs by genotyping-by-sequencing

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Abstract

Dosidicus gigas (*D. gigas*) is one of the most abundant squid species supporting a major fishery in the eastern Pacific Ocean, principally in the Gulf of California to northern Chile, and off the coast of Peru. *D. gi*gas display large fluctuations year by year and there are several size-at-maturity groups. Therefore, more reliable information on the population's genetic structure of *D. gigas* in this location is required especially for fishery assessment and management. Based on the simplified genome sequencing technique of Genotyping-by-sequencing (GBS), the genetic structure and diversity of large and small phenotypes of *D. gigas* in the southeast Pacific Ocean were studied in this paper by using Single Nucleotide Polymorphisms (SNPs) markers. The results showed that the observed heterozygosity and expected heterozygosity of large and small phenotypes were 0.23408 and 0.23822, 0.25670 and 0.28057, respectively. The genetic differentiation index (Fst) among two phenotypes was 0.018953, which indicated that the genetic differentiation among two phenotypes was not obvious and there was no obvious geographical isolation. It is suggested that the large and small phenotypes of *D. gigas* should be regarded as a management unit, and this study also enriched the population structure information of *D. gigas*.

1 Introduction

Dosidicus gigas(*D. gigas*), the jumbo flying squid, is a nektonic squid native to the

eastern Pacific. They are widely distributed from from northern California to southern Chile (Markaida et al., 2004) and are typically found in Peruvian and Gulf of California. Three intraspecific groups of *D. gigas* have been distinguished in the light of the size of adult males and females: a large-sized group (mantle length (ML) of adult males>400-500 mm, females 550-650 mm to 1000-1200 mm), a medium-sized group (240-420 mm and 280-600 mm, respectively) and a small-sized group (130-260 mm and 140-340 mm, respectively). Squid of the large-sized group is observed at the northern and southern extremes of the species range, the medium-sized group is found over the whole species range, the small-sized group is discovered predominantly in the equatorial area (Nigmatullin et al., 2001). However, Tafur et al. (2010) showed that the three groups of length-at-maturity may not actually exist, and that may be a continuous alteration as a response to environmental variation and food availability. They also indicated that in the Peruvian Humboldt Current System only 2 groups of size-at-maturity exist.

Due to the important economic role of *D. gigas*， it supports major fisheries in the Gulf of California, Mexico, Central America and off the coasts of Peru, Chile and Ecuador. Over recent years, *D. gigas* has become one of the world's most important cephalopod fisheries, mainly in the Eastern Pacific Ocean such as Japan, Russia, Korea and China (Liu et al., 2015; Morales-Bojórquez and Pacheco-Bedoya, 2016). However, *D. gigas* is short-lived, and production of this species is exhibited large annual fluctuations in response to environmental variability (Waluda et al., 2006). Recently, the abundance and distribution of this species is possibly influenced by the variable El Nino ˜ Southern Oscillation (ENSO) events (such as the 1997/98 ENSO event) (Rodhouse, 2001). Therefore, the management of cephalopod fisheries suffers from difficulties due to lack of basic biological information, such as the density and the population of this species. To aid a proper fisheries management of *D. gigas*, it is essential to define the accurate genetic stocks and its genetic variation in regard to environmental.

Up to now, population genetics evaluation of *D. gigas* has been limited to allozyme polymorphisms (Yokawa, 1995), random amplified polymorphic DNA PCR (RAPD-PCR) (Sandoval-Castellanos et al., 2009), and sequencing of mtDNA. Only a few

microsatellites were used to study the population structure of *D. gigas* (Sánchez et al., 2016). With the rapid advent of next-generation sequencing (NGS), molecular markers of high resolution like SNPs are applied to estimate the population genetic of species in both freshwater and marine fish recently, such as in white perch (*Morone americana*) (Bian et al., 2016) and yellowfin tuna (*Thunnus albacares*) (Pecoraro et al., 2016). SNPs are characterized by a low diversity and could be detected by short NGS reads nearby or between restriction sites distributed randomly across the entire genome (Peterson et al., 2012). GBS is a technique for genome reduction using restriction enzymes. It has the advantages of rapidity, specificity, and reproducibility, and is suitable for the systems lacking of sequenced reference genomes (Elshire et al., 2011). It has a broad application prospect in population structure and genetic diversity studies (Burrel et al., 2015; Kujur et al., 2015; Gouesnard et al., 2017; Schreiber et al., 2019; Korinsak et al., 2018).

In this report, we applied GBS to develop SNP markers in *D. gigas* and to assess the genetic diversity, population structure of *D. gigas* in the southeast Pacific Ocean

2. Materials and Methods

2.1 Animal materials and DNA extraction

In this study, the materials included 30 samples (small phenotype) from eastern equatorial sea (95°25′W-106°59′W, 1°26′S-3°09′S) in August 2018 and 8 samples (large phenotype) from off Peru water (76°28′W-81°19′W, 17°52′S-20°31′S) in October 2018 (Table 1). In subsequent sequencing and analysis, we have artificially divided the 38 samples into 2 groups according to sampling size (Table 2).

The genomic DNA was extracted from approximately 25 mg of muscle using a modified phenol-chloroform procedure following the described by Gu et al. (2013). In addition, to obtained the high-quality genomic DNA, digested 1 hour with RNase A in 37℃ (Takara, Japan) for removing RNA. The purity and concentration inspected on NanoDrop2000 in terms of ratio of OD260/280 and OD260/230, 1% agarose gel electrophoresis assessed the integrity of the genomic DNA. The final DNA

quantity using create library no less than 800 ng.

Table 1 Sampling area and sample size for the small and large phenotype squid

Phenotypes	Longitude	Latitude	Sample size
Small(S)	95°25′W-106°59′W	1°26′S-3°09′S	30
Large(L)	76°28′W-81°19′W	17°52′S-20°31′S	8

Table 2 Number of the Sample of the small and large phenotype squid

Phenotypes	Sample number
Small(S)	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31
Large(L)	L1, L2, L3, L6, L7, L8, L11, L12

2.2 GBS library preparation and sequencing

The GBS libraries were performed following two restriction enzymes (TaqI and MseI, New England Biolabs, NEB, USA) digesting the double-strand genomic DNA for several fragments which size for a range of 500-600 bp. A sample-specific adapter was ligated to the end of digested fragments and then pooled together. Applying the size selection system filtered 450-700 bp size fragments among the pooled mixture. The target GBS tags were amplified with specific primer to produce the final 400 bp size product for sequencing on Illumina Hiseq^{TM}, PE150. The above steps were conducted at Majorbio Pharm Technology Co., Ltd., Shanghai.

2.3 Bioinformatics and SNP genotyping

The raw GBSseq Illumina sequencing reads were converted to FASTQ format using Base Calling program. The program process_radtags was used check the barcode and demultiplexes the sequencing data from an Illumina platform. We removed the adapters sequence and subsequently discarded the low average quality score bases (quality value < 10) with Stacks 2.3 pipeline.

A set of high quality short-reads were aligned and grouped into loci according to depth parameter: a) minimum depth of coverage (-m) of 5; b) Maximum distance allowed of

2. The cstacks program was used the loci built a catalog in which alleles were merged and consensus, called “stacks”. All of samples were matched against the stacks and determined the allele status using sstacks (Catchen et al., 2013). Based on the putative loci and the maximum likelihood framework identify the SNPs, which further screened by the parameters (minor allele frequency (MAF) ≥ 0.05, missing ratio ≤ 0.2, the lowest depth＝2), obtained high quality SNPs.

2.4 Data analysis

2.4.1 Population structure analysis

In the population structure investigation, we used a free available software program ADMIXTURE 1.3.0 (http://www.genetics.ucla.edu/software/admixture/) which depends on the model-based Bayesian analyzed all of the samples based on the filtered SNPs locus. Briefly, the number of subpopulation (k) of samples were estimated ranged from 2 to 19, the optimal K determined by the cross validation (CV) error (Cross validation error), where the lowest position of CV error corresponds to K.

2.4.2 Phylogenetic tree analysis

To assess the phylogenetic relationship for samples from 3 different waters, using RAxML version8 a tool for phylogenetic analysis calculated the genetic relationship between species and represented it by a tree-like diagram. The analysis based on the SNPs and maximum likelihood algorithm.

2.4.3 PCA analysis

We assessed the proportion of phenotypic variance by using principal component analysis (PCA) with GCTA 1.26.0 (Price et al., 2006). Based on the SNPs and introduced a mathematical method, 29 samples were clustered in terms of complex traits.

2.4.5 Genetic diversity

The population genetic diversity statistics, including the private SNPs number (Private), observed heterozygosity (Obs-He), expected heterozygosity (Exp-He), observed

homozygosity (Obs-Ho) expected homozygosity (Obs-Ho), the Nucleotide diversity (Pi), population coefficient of inbreeding (Fis) (Catchen et al., 2011) and and the genetic differentiation index (Fst). The Fis was used to assessed whether exist inbreeding cause generate a hidden population, lead to reduction in heterozygosity (Catchen et al., 2013). The Fst is an important parameter to measure the extent among populations.

3 Results and Discussions

3.1 GBS development and analysis

Molecular markers, including isozymes, microsatellites, mitochondrial DNA, SNPs and other molecular markers, have high value in genetics because of their genotyping ability (Grover et al., 2014). In recent years, the simplified genome sequencing technology based on the NGS technology has reduced the complexity and cost of genome sequencing by sequencing only a part of the genome, and has a high throughput, which is helpful to find a large number of SNPs and improved the comprehensiveness and accuracy of genetic diversity assessment and genome-wide association analysis. At present, SNPs development technologies based on sequencing mainly include specific-locus amplified fragmentation sequencing (SLAF-seq), restriction-site-associated DNA sequencing (RAD-seq), and genotyping by sequencing (GBS), and so on (Appleby et al., 2009; Martin et al., 2013). RAD-seq can develop high-density genetic markers suitable for the study of low-quality reference genomic species, but sequencing requires a lot of DNA, library construction is cumbersome (Baird et al., 2008). GBS has become the main technique to simplify genome sequencing because there is little demand for DNA based on high-throughput parallel sequencing and no need to select the length of fragments in the process of constructing the library (Elshire et al., 2011). In addition, the restriction enzyme used to digest genome belongs to type II enzyme, which can avoid methylation region, improve sequencing efficiency and save time. In this study, the genetic diversity and population structure of 38 *D. gigas* individuals were analyzed for the first time by using GBS sequencing technique in two areas of the southeast Pacific Ocean. A total of 182.72 G primitive data were obtained by Illumina double-

ended sequencing, with an average of 4.81 G per sample and a base mass Q30 ratio of 93.93%. A total of 8088906 SNP markers were obtained by mutation detection, and the initial SNP was screened. According to the screening parameters (MAF not less than 0.05, deletion rate not more than 0.2, sample depth up to 2), 35510 high-quality SNP markers were finally obtained for further population evolution analysis.

3.2 Estimation of genetic diversity

Heterozygosity can reflect the genetic variation of a population at multiple loci and is an important parameter to measure the genetic diversity of population. Liu et al. (2014) analyzed the genetic variation of small and large phenotypes of *D. gigas* in the Peruvian open sea using microsatellite DNA (SSR) markers. The observed heterozygosity (Obs-He) and expected heterozygosity (Exp-He) of large phenotype were 0.716 and 0.868 respectively. The Obs-He of the small phenotype was 0.721 and the Obs-He was 0.883. Both of them have high genetic diversity. Sánchez et al. (2016) measured the average Obs-He and Exp-He of two phenotypes of *D. gigas* in northern and south-central Peruvian waters using SSR markers, which were 0.712 and 0.658, 0.828 and 0.825, respectively. The heterozygosity, homozygosity, nucleotide polymorphism (Pi) and population inbreeding coefficient (Fis) of the two phenotypes were determined based on the screening data of 35510 SNPs. The Obs-He of the large phenotype was 0.23408 and the Exp-He was 0.25670. The Obs-He and Exp-He of the small phenotype were 0.23822 and 0.28057, respectively (Table 3). These results showed that the genetic diversity of the two phenotypes was lower than that of the other populations. Although the heterozygosity of the small phenotype was slightly higher than that of the large phenotype, the observed heterozygosity of the two phenotypes was lower and the observed homozygosity was higher, indicating a decrease in genetic diversity. The average Obs-He of the two phenotypes was lower than expected, and the Fis value was positive (0.11376 and 0.1804, respectively), indicating that the population was seriously inbred (Table 3). Liu et al. (2014) also detected the loss of heterozygosity in the *D. gigas* populations of the coast of Peru, which is consistent with the results of our experiment. Sánchez et al. (2016) showed that there are also heterozygote deletions in

the northern Humboldt Current system and the observed heterozygosity is significantly lower than that of other commercially important oegopsid squid, such as *Illex argentinus* (Obs-He=0.84) and *Todaropsis eblanae* (Obs-He=0.82-0.91) (Adcock et al., 1999; Dillaneet al., 2005). Loss of heterozygosity leads to an increase in homozygosity, which resulting an increase in inbreeding withdrawal rates and a decrease in genetic diversity, as well as a decrease in the ability of species to cope with adverse environmental conditions (Yang et al., 2000). According to the results of genetic diversity parameters, effective management measures should be taken to reduce the loss of heterozygosity and inbreeding as much as possible in order to protect the genetic diversity of *D. gigas*.

Table 3 Genetic diversity of the small and large type squid

Phenotypes	Private	Num indv	Obs-He	Obs-Ho	Exp-He	Exp-Ho	Pi	Fis
Small(S)	61	6.81323	0.23408	0.76592	0.2567	0.7433	0.2775	0.11376
Large(L)	6471	26.14712	0.23822	0.76178	0.28057	0.71943	0.28606	0.1804

Private, number of group-specific SNP; Num indv, the average number of individuals per locus in a population; Obs-He, observed heterozygosity; Obs-Ho, observed homozygosity; Exp-He, expected heterozygosity; Exp-Ho, expected homozygosity; Pi, nucleotide polymorphism; Fis, population inbreeding coefficient.

3.3 Population structure

Based on mitochondrial DNA (mtDNA) markers, Liu et al. (2014) studied the genetic differentiation of large and small phenotypes of *D. gigas* off the coast of Peru. The results showed that the genetic differentiation index (Fst) between the two phenotypes was less than 0.05. Sánchez et al. (2016) use microsatellite DNA markers to analyze that genetic differentiation between two phenotypes of *D. gigas* in the northern Humboldt Current system. These results showed that the Fst value was small and the P value was not significant. In order to analyze the degree of genetic differentiation between the two phenotypes, Fst were calculated by using high-quality SNPs, and the Fst was 0.18953, which indicated that the genetic differentiation among the phenotypes was weak. The result was consistent with those of Liu et al. (2014) and Sánchez et al. (2016), which indicated that there was no obvious genetic differentiation between large

and small phenotypes of *D. gigas*.

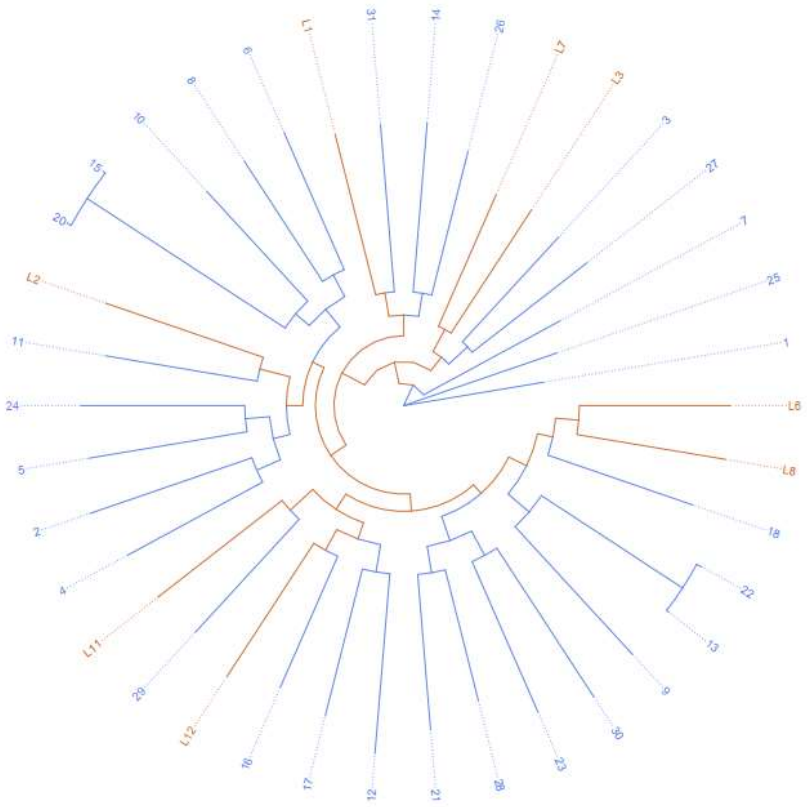

Fig.1 A maximum likelihood tree for *Dosidicus gigas* based on the high-quality SNPs.

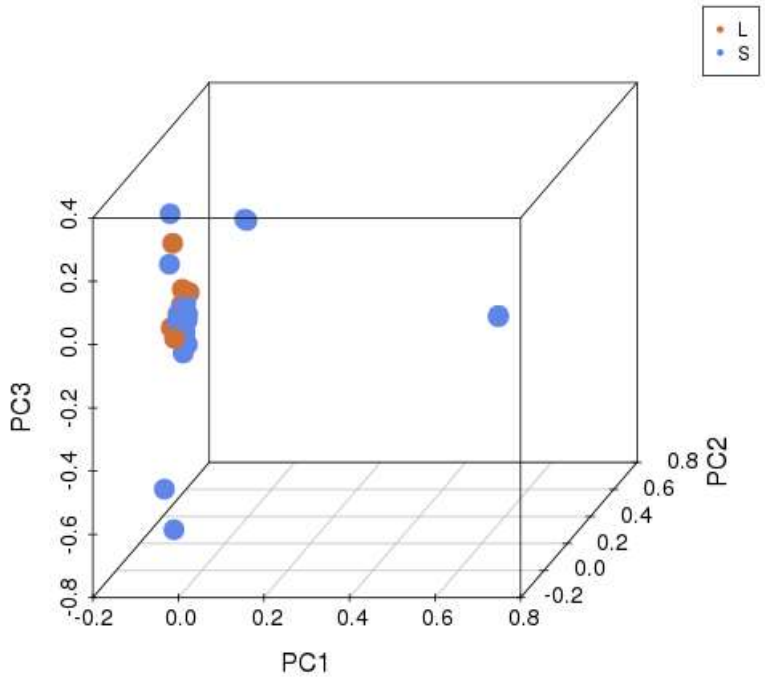

Fig.2 Principal component analysis (PCA) of *Dosidicus gigas* was implemented in GCTA.

Phylogenetic trees are used to describe the evolutionary relationships among species.

According to the relationship between individuals, the evolution of species can be expressed intuitively. The phylogenetic tree constructed by maximum likelihood method (Fig. 1) showed that there was no significant clustering between individuals of large phenotype and small phenotype, indicating that there was no significant genetic differentiation between the two phenotypes. The result of principal component analysis (PCA) showed (Fig. 2) that the two phenotypes were not clustered and could not be well distinguished, which was consistent with the results of genetic differentiation index analysis.

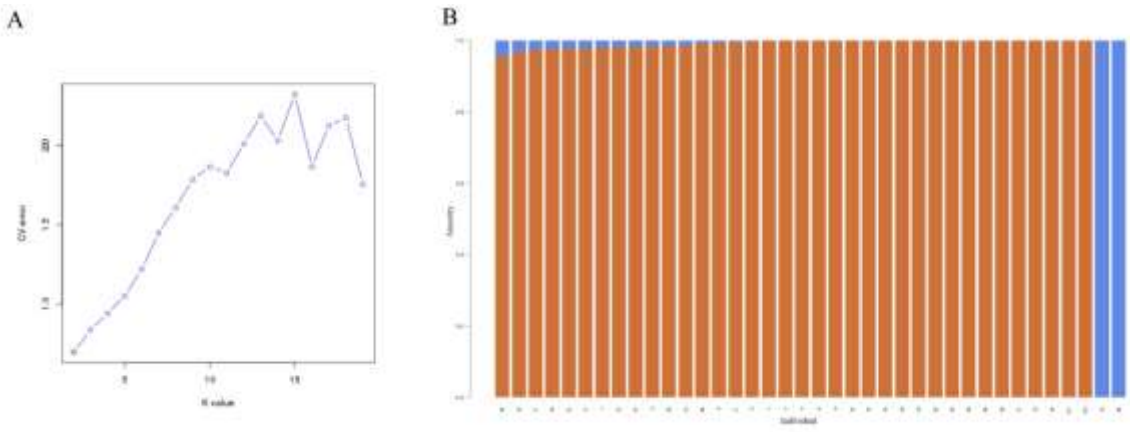

Fig.3 Population genetic structure. (A) CV error value for each K value; (B) Population structure of all samples when divided into two genetic components and each color represents one putative ancestry background. CV, cross validation.

Population structure analysis can provide information on the origin and composition of species. In this study, we analyzed the population structure of all the samples, and assumed that the number of groups K (K value) of all the samples was 2-19, respectively, for clustering. According to the K value corresponding to the lowest point of CV error, the optimal number of clusters is determined as 2 (Fig. 3A), as shown in Fig. 3B. K=2 suggests that all samples of the *D. gigas* in this study may have come from two primitive ancestors. Geographical isolation and environmental differences are one of the important factors leading to genetic differentiation. Although all of the *D. gigas* in our study may have come from two primitive ancestors, as an oceanic cephalopod, it has a strong ability to swim. With the reproductive migration of equatorial current system

and Peruvian cold current, it greatly promotes gene exchange among populations and reduces the genetic difference between populations, which results in no significant genetic differentiation between the two phenotypes.

4. Conclusion

In this study, the genetic diversity and structure of large and small types of *D. gigas* in the southeast Pacific Ocean were analyzed by using GBS technique. These results showed that the genotyping technique was suitable for evaluating the genetic diversity of oceanic fishes with high diffusion potential. In terms of genetic diversity, the two phenotypes have shown a decline in genetic diversity and loss of heterozygosity. According to population differentiation, the two phenotypes did not differentiate significantly and did not form geographical isolation, so they could be managed as a whole. In order to obtain a more accurate population genetic structure of *D. gigas*, we need to expand the sampling area and increase the number of samples in the next step.

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