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Morphological variation and stock classification of *Phidippoteuthis* flying squid based on the statolith shape using wavelet analysis

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Morphological variation and stock classification of jumbo flying squid,  
*Dosidicus gigas*, based on the statolith shape using wavelet analysis

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## Introduction

Oceanic squid, especially ommastrephid species, plays an important role in marine ecosystems, both as prey and predators (Navarro *et al.*, 2013). They prefer to gather in areas with high primary productivity (Ichii *et al.*, 2002; Waluda and Rodhouse, 2006). The dramatic fluctuations of annual landings for these species indicate that they are heavily susceptible to the surrounding environmental changes (Anderson and Rodhouse, 2001; Rodhouse 2001; Zeidberg and Robison, 2007; Keyl *et al.*, 2011; Robinson *et al.*, 2013; Yu *et al.*, 2016). The local population, or stock, also possess distinct biological characteristics, reflecting genetic structures and experienced ambient environment (Ichii *et al.*, 2002; Rocha and Vega, 2003; Sandoval-Castellanos *et al.*, 2007, 2010; Arkhipkin *et al.*, 2014; Liu *et al.*, 2015a).

Jumbo flying squid, *Dosidicus gigas*, mainly located in regions with comparatively high productivity that is induced by two main upwelling zones (Ichii *et al.*, 2002; Lavín *et al.*, 2006; Radenac *et al.*, 2012), are the target of commercial fisheries for Asian and Latin American countries in east Pacific Ocean (Chen *et al.*, 2008; Ibáñez and Poulin, 2014; Ibáñez *et al.*, 2015; Guevara-Carrasco *et al.*, in press).

This largest ommastrephid species is highly mobile and often migrate on a large scale (Keyl *et al.*, 2008; Rodhouse, 2008). Historically, the general habitat for *D. gigas* ranged from 30°N to 25°S, with occasional occupation at 40°N in the eastern tropical Pacific (ETP) (Nigmatullin *et al.*, 2001). However, this species has undergone an unusual change in distribution since the severe El Niño and La Niña event during 1997-1999 (Waluda *et al.*, 2006; Arkhipkin *et al.*, 2014), including an expansion of habitat stretched to Alaska in the north and Antarctic in the south (Keyl *et al.*, 2008; Hoving *et al.*, 2013; Stewart *et al.*, 2014). The behavior of these extreme generalists seeking for suitable habitat strengthens the connectivity among different populations, potentially influencing the intrinsic population structure of *D. gigas* (Staaf *et al.*, 2010; Morales-Bojórquez and Pacheco-Bedoya, 2016; Sanchez *et al.*, 2016).

It is critically important to fully understand the population structure of commercial marine organisms for investigating the population dynamics and developing management strategies (Thorrold *et al.*, 2001; Gillanders, 2002). The population structure of *D. gigas* is complicated and usually defined using mantle length size (Nigmatullin *et al.*, 2001) where the small-sized group is thought to be restricted to equatorial waters; the medium-sized group has been found in the entire range of the species; and large-sized group only occurs in the northern and southern peripheries of its ranges. These size based sub-populations are regarded as distinct stocks (Nigmatullin *et al.*, 2001). The seasonal schooling of *D. gigas* in different fishing grounds can be regarded as separated geographical stocks (Liu *et al.*, 2015). However, some researchers have indicated that *D. gigas* should be regarded as two

separate stocks, “northern” and “southern”, based on supposed migration patterns that only occur northern hemisphere or south hemisphere, respectively (Nesis, 1983; Wormuth, 1998; Clarke and Paliza, 2000; Staaf *et al.*, 2010). This perspective is supported by recent RAPD-genetic and mitochondrial DNA analysis, which concluded that samples from the two hemispheres are genetically distinct (Sandoval-Castellanos *et al.*, 2007, 2010). The results of elemental signatures in the statolith nucleus (embryonic stage) and postnucleus (paralarval stage) also showed significant differences among different geographic locations, representing the northern and southern stocks (Liu *et al.*, 2015a).

Cephalopod statolith has been considered as an archival tissue that records valuable ecological information during the life history within a stable configuration (Lipiński, 2001; Arkhipkin, 2005). Like fish otolith, cephalopod statolith has been widely used for age estimation via the daily deposited increments as observed under microscope (Lipiński, 1981, 1986, 1993; Arkhipkin and Shcherbich, 2012). Statoliths embedded in the statocyst also have unique characteristics of shape (Clarke, 1978). Droplet-shaped statoliths are usually anomalous, compared to the oval-shaped otolith in most fish species (Clarke, 2003), although they have similar function to keep balance (Budelmann, 1975; Lecomtefiniger, 1999). Arkhipkin (2005) reported that the features of statoliths in different sections and modified the terminology based on previous studies (Clarke, 1978; Lipiński, 1993). Genetic structure and environmental factors (e.g. temperature, salinity) during ontogenetic processes are the two main determinants that influence the statolith shape variation in species and ontogenetic

levels (Thomas and Moltschaniwskyj, 1998; Lipiński, 2001; Arkhipkin, 2005).

Several studies of statolith morphometrics have been proposed mostly based on measurements of linear distances (Clarke, 1978; Arkhipkin and Murzov, 1986; Flores and Garland, 2002; Barcellos and Gasalla, 2015). This method, however, has a degree of uncertainty and may increase the risk of potential bias of measurements (Francis and Mattlin, 1986; Bookstein, 1998; Adams *et al.*, 2004). Geometric morphometrics (GM), which can effectively represent and reconstruct the shape of object, has been proposed and developed for more than two decades (Adams *et al.*, 2013). This powerful method, including the landmark (Cadrin, 2013) and outline analyses (Rohlf and Marcus, 1993; Marcus *et al.*, 1996), has been widely used in stock and population identification based on fish otoliths (Stransky *et al.*, 2008; Farias *et al.*, 2009; Crespo *et al.*, 2012). There are limited reports for cephalopod statolith shapes, except for genera and species discrimination for some local species in Europe (Lombarte *et al.*, 1997; 2006; Dommergues *et al.*, 2000).

For outline analysis, Fourier transform (FT) analysis (including Elliptical Fourier Analysis, EFA and Fast Fourier Analysis, FFA) is more commonly used due to its well-known mathematic background that serves to characterize statolith shape with a series of harmonic expansions (Lombarte *et al.*, 1997; Green *et al.*, 2015). It also provides a representation of decomposing the curve in the form of sines and cosines, which are problematic to capture the localized sharp irregularities and different singularities (Libungan *et al.*, 2015). This can therefore weaken the description of morphology for sharp margins (Graps, 1995; Parisi-Baradad *et al.*, 2005). The

Wavelet transform (WT), with the ability to measure sharp transitions and establish locations that effectively and adequately represent morphological characteristics, providing a more powerful alternative to solve the problems inherent with FT analysis (Parisi-Baradad *et al.*, 2005). Previously, WT was found to be an effective tool for species discrimination and stock identification (Lombarte *et al.*, 2006; Libungan *et al.*, 2015, 2016).

In this study, the squid samples commercial caught in four main fishing grounds of *D. gigas* was collected, the effect of wavelet analysis is evaluated to identify different stocks of *D. gigas* using statolith shapes. A large number of wavelet variables were automatically extracted with statistical software and then described positional differences and inter-population variations were derived from the shape of statolith edge. Different partitioning approaches were also compared to improve classification rate and select an appropriate method for stock discrimination. This study aims to enhance the understanding of *D. gigas* statolith morphological features in different stocks and give us an alternative method for stock classification. The statolith shape description and follow-up discriminating method in this study are also applicable to the investigation of other cephalopod species.

## **Materials and methods**

### **Sampling**

A total of 350 *D. gigas* squid samples were randomly obtained on the deck from the Chinese commercial jigging vessel *F/V Zheyuanyu 807*, which operated off the

Chilean Exclusive Economic Zone (75°-80°W and 20°-24°S) in 2008; the *F/V New Century 52*, which operated off the Peruvian Exclusive Economic Zone (82°-86°W and 10°-13°S) in 2009; the *F/V Puyuan 802*, which operated off the equatorial area (114°-119°W and 2°N-3°S) in 2013; and the *F/V Fenghui 16*, which operated off the Costa Rica Dome (90°-98°W and 6°-10°N) in 2009 (Table.1, Fig. 1). All samples were immediately frozen at -18°C on deck.

Table 1 Sample information of *D. gigas* in this study

Location	Code	Time	Fishing vessel	Coordinates	N	ML (mm) [Mean (range)]
Chile	CH	May-Jul. 2008	<i>Zheyuanyu 807</i>	75°-80°W 20°-24°S	103	395.6(312.4-517.1)
Costa Rica	CR	Jun.-Aug. 2009	<i>Fenghui 16</i>	91°-97°W 7°-10°N	55	307.4(256.1-356.8)
Equator	EQ	Apr.-Jun.2013	<i>Puyuan 802</i>	115°-119°W 2°N-1°S	103	296.0(226.7-397.5)
Peru	PE	Aug.-Sep. 2009	<i>New Century 52</i>	82°-85°W 10°-13°S	102	342.4(220.7-494.5)

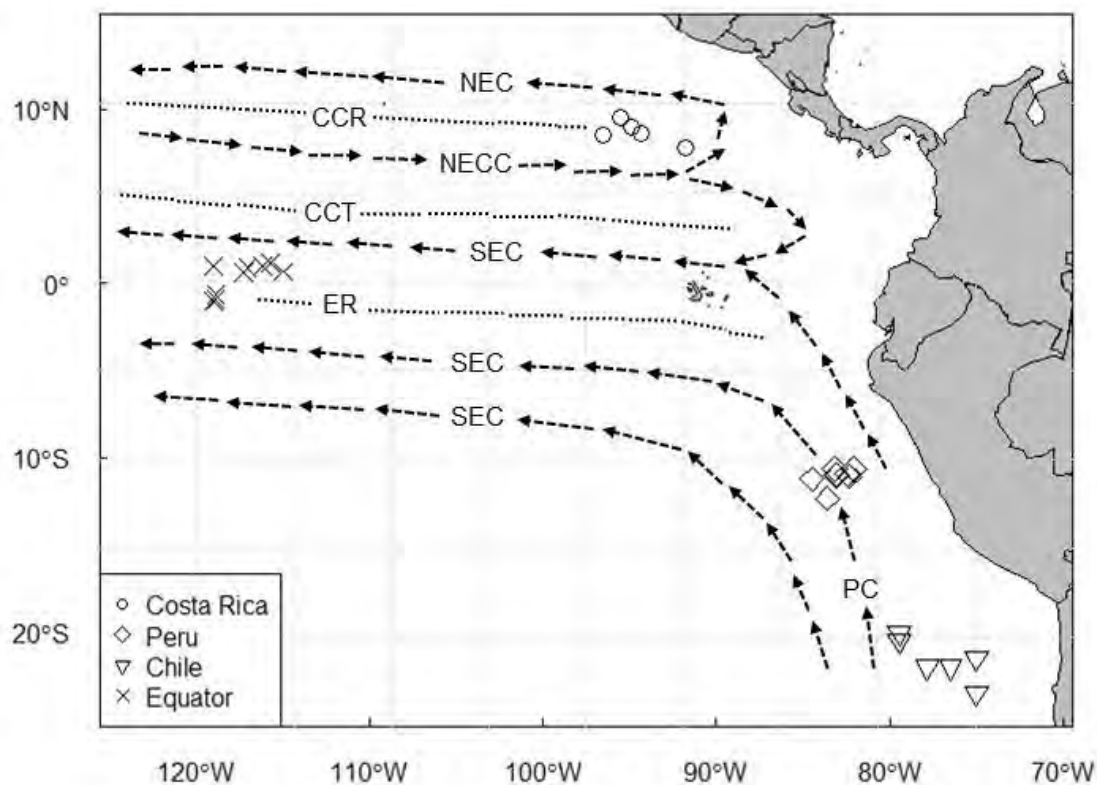


Fig 1 Sampling locations for *Dosidicus gigas* and related surface currents in eastern Pacific Ocean. NEC: North Equatorial Current; NECC: North Equatorial Countercurrent; SEC: South Equatorial Current; PC: Peru Current; CCR: Countercurrent Ridge; CCT: Countercurrent Trough; ER: Equatorial Ridge. Revised from Ichii et al (2002)

Dorsal mantle length (ML) was measured to the nearest 0.1 cm in the laboratory after samples were thawed. Sex maturity stage was evaluated by naked eyes following Lipinski and Underhill (1995). Maturing and matured squid (stage III to stage V) was selected for the future analysis to avoid the effect of allometric growth. Finally, 260 complete statoliths were extracted from the statocyst and preserved in a 0.5 ml centrifuge tube. As the left and right statoliths were tested with no significant difference in morphometrics (t-test,  $P > 0.05$ ), only the right statolith was used in the



latter process.

### **Image capture and shape analysis**

Images of the 260 statoliths were taken with Charge-coupled Device (CCD) with stereomicroscope (Olympus Corp. Tokyo). The contour of statolith in every picture should be clear to ensure the margin of the statolith visibly. The wavelet coefficients were obtained using the wavethresh package (Nason, 2012) and scaled by adjusting for allometric relationships with ML as in Lleonart *et al.* (2000) to avoid the influence of squid size. The image analysis was conducted with R 3.1.3 (R Core Team, 2015) using the package shapeR (Libungan and Pálsson, 2015) to reconstruct the outline of statolith with wavelet analysis.

A series of size variables concerning the shape of the statolith were automatically calculated by shapeR (Libungan and Pálsson, 2015) based on the digitized images and MLs and used to calculate the shape indices according to Tuset *et al.* (2003) (Table 2). The relationship between ML and statolith length were assess the effect of body size on statolith shape using linear regression. And the stock differences of statolith growth were evaluated using ANCOVA. Statolith shape indices were analyzed using an ANOVA test to assess the stock variation of *D. gigas*. In order to detect the section variation in statolith shape, the wavelet coefficients were plotted against the angle according to its mean and standard deviation. The proportion of variation within stocks along the outline was concluded with intraclass correlation (ICC).

Table 2 Statolith shape indices for different stocks of *D. gigas*

Size variables	Shape indices			
Area (A)	Formfactor = $(4\pi A)P^{-2}$			
Perimeter (P)	Roundness = $(4A)(\pi SL^2)^{-1}$			
Statolith Length (SL)	Circularity = $P^2A^{-1}$			
Statolith Width (SW)	Rectangularity = $A(SL \times SW)^{-1}$			
Shape indices	Mean±standard deviation			
	CH	CR	EQ	PE
Formfactor	3.83±0.24	2.94±0.14	3.19±0.18	3.43±0.27
Roundness	11.05±2.07	5.27±0.96	6.21±1.38	8.06±2.50
Circularity	19.67±0.94	21.37±1.00	20.24±0.98	20.24±0.90
Rectangularity	0.65±0.05	0.56±0.03	0.58±0.04	0.63±0.05

Note: CH = Chile; CR = Costa Rica; EQ = Equator; PE = Peru

Intra-species statolith shape variation was also analyzed by applying a canonical analysis of principal coordinates (CAP) (Anderson and Willis, 2003) with the “capscale” function in package vegan (Oksanen *et al.*, 2013). Ordination of the population averages was also examined graphically with the shape descriptors (wavelet coefficients).

### Statistical analyses with different classification methods

There were 52 wavelet coefficients retained for latter data processing after the wavelet data standardization. Combined with the four shape indices, a total of 56 shape variables were selected for stock discriminant analysis. The assumption of normality and homogeneity of variance for each variable was examined using Kolmogorov–Smirnov (KS) and Levene's tests (Zar, 2007). The shape index “Roundness” and 14 wavelet coefficients were excluded due to their failure to pass these normality and homogeneity tests. In order to unify the data analysis, the

remained 41 variables were prepared for future analysis.

Linear Discriminant Analysis (LDA), Classification Tree (CT), Naive Bayes (NB), Random Forest (RF) and Support Vector Machine (SVM) were employed to evaluate the classification effect. The first method is a traditional classification method commonly used for stock identification based on linear regression analysis (Martínez *et al.*, 2002; Chen *et al.*, 2012; Fang *et al.*, 2014; Liu *et al.*, 2015b). The latter four methods, which belong to supervised learning method, were used in varied fields due to their powerful classification ability (Lantz, 2013) for fish stock discrimination (Mercier *et al.*, 2011).

The NB is a family of simple probabilistic classifiers based on applying Bayesian rules with strong independence assumptions between the features. This method requires a number of linear parameters in the number of predictors in a learning problem, and has also used in fish recruitment forecasting (Fernandes *et al.*, 2014).

The response variable of decision trees (DTs), which have a branching structure that distinguishes values in a hierarchical form (Breiman *et al.*, 1984), is either categorical (classification trees, CT) or numeric (regression trees, RT), and has also been used in the establishment of cephalopod maturity stages (Crespi-Abril *et al.*, 2015).

The RF (Breiman, 2001) utilizes an ensemble of classification or regression trees to predict the dependent variable as a result of majority vote or average assignment across trees. The advantage of RF is that it allows correlated predictors without any data transformation or exclusion to estimate the importance of variables (Cutler *et al.*,

2007).

The SVM, also referred to Maximum Margin Classifiers (MMC), is a method that minimizes empirical errors and maximizes geometric margins. It constructs an N-dimensional hyper-plane based on an algorithm that finds the maximum-margin hyper plane with the greatest division between classes (Hu *et al.*, 2012). This method has been considered as one of the most influential data mining algorithms (Wu *et al.*, 2008).

All the above analyses were carried out with R 3.1.3 (R Core Team, 2015) using the packages “MASS” for LDA, “C50” for CT, “e1071” for NB and SVM, “randomForest” for RF. The classification results from different methods were compared with the K-fold cross validation, which is a popular method to compare different models (Arlot and Celisse, 2010). The 10-fold cross-validation procedure has shown good performance in model selection (Kohavi, 1995) and was used to evaluate the performance of each model. Considering the sample size in this study, a stratified sampling was performed in the cross-validation (Zhao *et al.*, 2014), where 70% of the total samples were chosen as training data and the remaining 30% as validation data to test the model performance. Some relevant measurements (e.g. sensitivity, specificity, Kappa) were listed to assess the effect among different methods (Kuhn, 2008).

## **Results**

### ***Statolith shape indices variation among stocks***

Statolith length was not changed by the squid body growth in each stock (Table.3). Statolith shape also differed among four geographic stocks. The result showed that the Chilean (CH) stock had the highest value of the shape indices, except for circularity, among the four geographic stocks (Fig. 2). Chilean, Peruvian (PE) and equatorial (EQ) stocks had the similar circularity, except for Costa Rica (CR), which had the highest value (Fig. 2). The shape indices had significant difference between stocks (ANOVA,  $F=90.66$ ,  $P<0.01$ ). Pairwise analysis (Post-hoc, Tukey HSD) also showed significant differences between stocks (Fig.3,  $P<0.01$ ). Individuals in PE and CH had the most similar shape indices, while CR and CH had the most different shape indices among stocks (Fig.3). The statolith in different stocks also presented distinguish growth pattern with their body size (ANCOVA,  $F=54.171$ ,  $P<0.01$ ).

Table 3 Regression analysis of mantle length and statolith length of *D. gigas*

Location	Code		df	SS	MS	F	R <sup>2</sup>	Probability
Chile	CH	Regression	1	0.1497	0.1497	20.9732	0.172	<0.01
		Residual	102	0.7209	0.0071			
Costa Rica	CR	Regression	1	0.0591	0.0591	9.4013	0.150	<0.01
		Residual	53	0.3337	0.0063			
Equator	EQ	Regression	1	0.1940	0.1940	32.1856	0.241	<0.01
		Residual	102	0.6089	0.0060			
Peru	PE	Regression	1	0.3643	0.3643	47.8026	0.323	<0.01
		Residual	100	0.7620	0.0076			

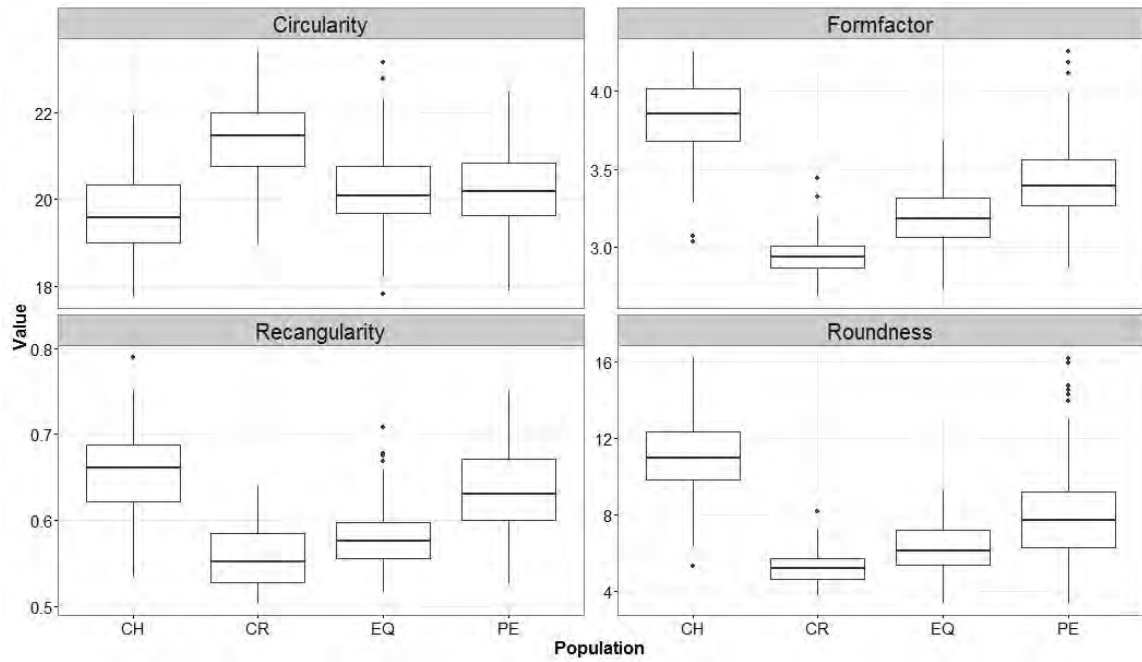


Fig 2 Shape indices of statoliths for the four stocks of *Dosidicus gigas*. CH: Chile; CR: Costa Rica; EQ: equatorial; PE: Peru.

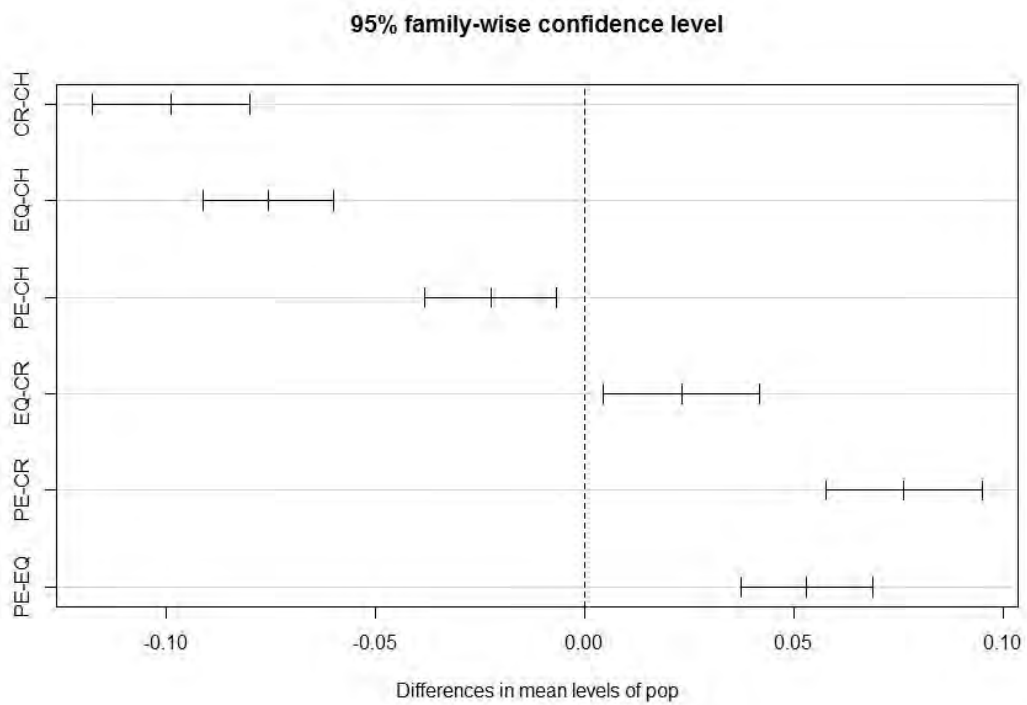


Fig 3 Pair-wise comparisons of shape indices among the four geographic stocks of *Dosidicus gigas*

### *Shape variation of different geographic stocks*

The reconstructed mean statolith shapes were shown in Fig. 4 and differed among four stocks. The most obvious modification sections of statolith shape were located in rostrum (0 to 30°), rostral angle (30 to 60°) and wing (300 to 360°) (Figs. 4 and 5). The rostrum section of CR was the most outward from the centroid, CH was moved inward toward the centroid, PE and CR were between the above two stocks. On the contrary, CR presented a sharp rostrum with an innermost rostral angle, CH presented a blunt rostrum with an outermost rostral angle (Fig. 4). For the wing, CH had a plump wing than any other stocks, PE and EQ were similar and more outward than CR (Fig. 4). Dorsal section (150 to 210°) showed little variation among stocks, which was confirmed by examining the proportion of variation within groups summarized with the ICC, although the mean and standard deviation of wavelet coefficients were relatively higher than other sections (Fig. 5).

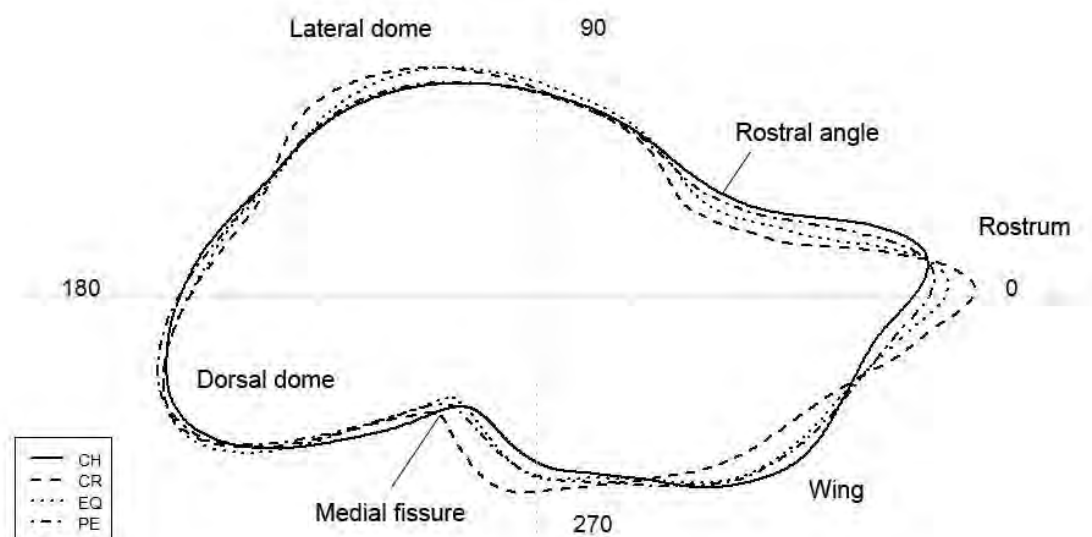


Fig 4 Mean shape of statoliths for the four stocks of *Dosidicus gigas*. CH: Chile; CR: Costa Rica; EQ: equatorial; PE: Peru. The four numbers (0, 90, 180 and 270) represent angle in degrees (°) along the outline

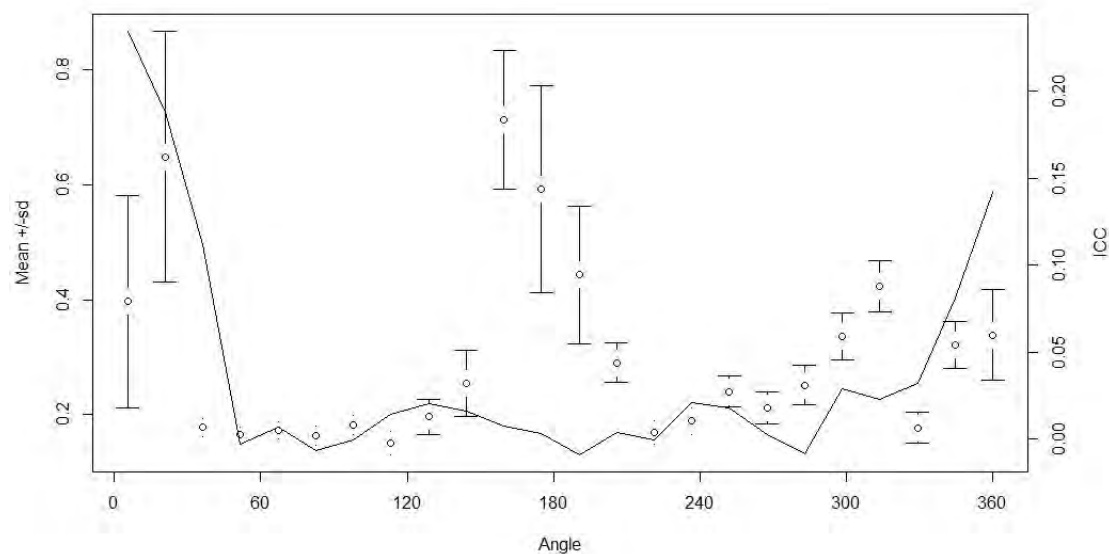


Fig 5 Mean and standard deviation (SD) of the wavelet coefficients (dot with error bar) for all combined statoliths and the proportion of variance within stocks, or the intraclass correlation (ICC, black solid line)

A total of 52 wavelet coefficients were selected to analyze the stock variation by canonical analysis of principal coordinates (CAP). The canonical scores revealed large differences between stocks (Fig. 6). The first two canonical axes explained a large proportion (96.0%) of the variation between stocks (CAP 1: 87.1%; CAP 2: 8.9%). The first scores (CAP 1) showed that southern hemisphere stocks (CH and PE) had negative values while northern hemisphere stock (CR) had positive values (Fig. 6). CAP 2 also showed difference between neritic stocks (CH, PE and CR) with negative values and oceanic stocks (EQ) with positive value (Fig. 6).



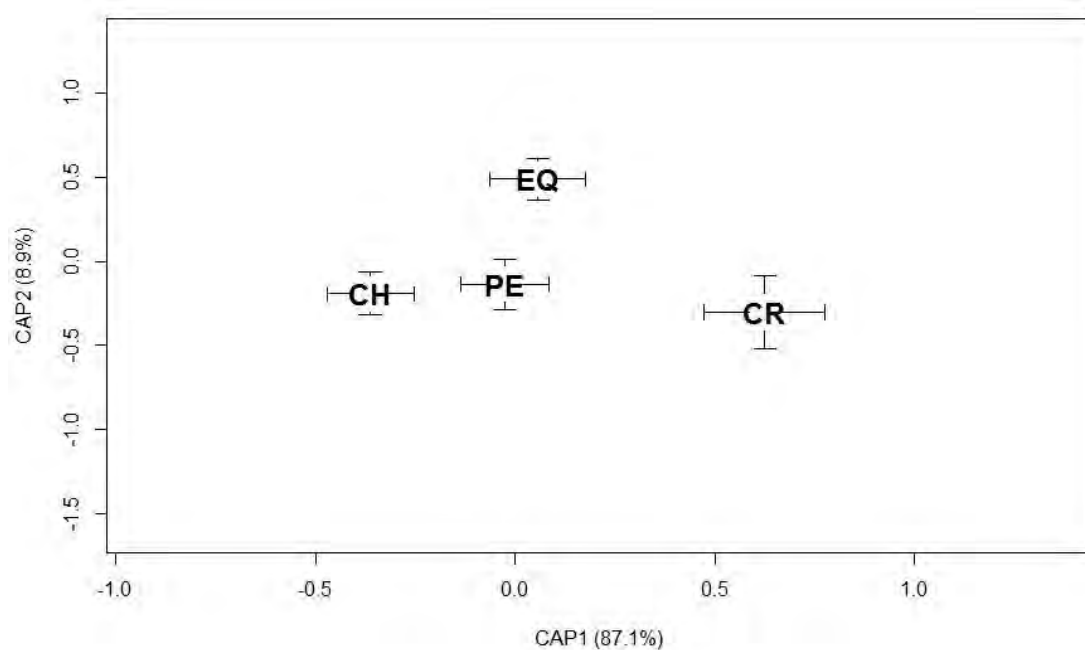


Fig 6 Canonical scores on discriminating axes 1 and 2 for each squid geographic stock.

#### ***Discrimination rate comparison among difference methods***

Among these four stocks, PE had a relatively higher misclassification rate than the other two stocks (Table 4). The classification rate in CH changed little among these five methods (Table 4). CR had a higher classification rate using CT and RF. EQ had the highest rate using SVM (Table 4).

Comparing traditional method (LDA) with machine learning methods (NB, DT, SVM and RF), the discrimination rates of machine learning methods were higher (Table 4). All the results showed that these methods could effectively partition different stocks of *D. gigas* with high classification rates (more than 60%, Table 4). For the result of classification in every stock, the performance of RF was better than SVM due to the precisely partitioning CR stock (92.86%) rather than the result in SVM (73.68%, Table 4).

Table 4 Discriminant analysis results and accuracy measures of different methods considered in this study

Method	Original	Predict (%)				Total (%)	Sensitivity (%)	Specificity (%)	Kappa
		CH	CR	EQ	PE				
LDA	CH	68.93	1.94	2.91	26.21	64.46	54.7	85.6	0.396
	CR	0.00	63.64	23.64	12.73		58.3	93.4	
	EQ	4.85	9.71	78.64	6.80		63.9	88.9	
	PE	27.45	7.84	18.63	46.08		36.2	73.4	
NB	CH	67.57	0.00	8.11	24.32	65.09	76.7	81.6	0.440
	CR	0.00	58.33	37.50	4.17		68.7	90.0	
	EQ	5.88	0.00	76.47	17.65		53.3	84.2	
	PE	14.29	7.14	17.86	60.71		40.0	88.2	
CT	CH	69.70	0.00	3.03	27.27	68.87	66.7	86.8	0.333
	CR	0.00	92.86	7.14	0.00		56.2	93.3	
	EQ	3.33	10.00	70.00	16.67		56.7	75.0	
	PE	20.69	0.00	24.14	55.17		26.7	77.6	
SVM	CH	74.07	0.00	3.70	22.22	70.75	80.0	89.5	0.487
	CR	0.00	73.68	21.05	5.26		62.5	94.4	
	EQ	4.17	4.17	83.33	8.33		56.7	78.9	
	PE	25.00	2.78	13.89	58.33		50.0	85.5	
RF	CH	71.88	0.00	3.12	25.00	70.74	80.0	89.5	0.472
	CR	0.00	92.86	7.14	0.00		62.5	96.7	
	EQ	6.90	6.90	72.41	13.79		63.3	76.3	
	PE	16.13	3.23	22.58	58.06		40.0	84.2	

Note: LDA = Linear Discriminant Analysis; NB = Naive Bayes; CT = Classification Tree; SVM = Support Vector Machine; RF = Random Forest

## Discussion

Since the statolith was discovered and studied in the 1960s (Clarke, 1962), its morphology has attracted numerous researchers' interests and is considered as an appropriate material to explore cephalopod biology and evolution (Clarke, 2003). The statolith analyses of linear measurements based on the terminology of previous studies revealed that some statolith morphometrics (e.g. statolith length, SL) had a strong relationship with ML (Arkhipkin and Murzov, 1986; Flores and Garland 2002;

Barcellos and Gasalla, 2015) and that some applications were also utilized to identify the stock variation (Fang *et al.*, 2012; 2014; Vyver *et al.*, 2016). However, it is a problematic method to describe the whole statolith shape with linear measurements. In this study, the otolith-derived shape indices (Tuset *et al.*, 2003) and wavelet coefficients quantitatively described the irregular-shaped statolith via images and computer software, producing credible results, which were statistically in accordance with the statolith shape reconstruction (Fig. 4). These shape indices are supplemental variables for describing statolith morphometrics which help distinguish different stocks, as the significant variation presented in the result of this study was able to demonstrate.

Statoliths and statocysts were described as receptors that control and regulate squid position and movement in the water (Budelmann, 1977). Different feeding and swimming behaviors are the main reason for morphological variations in statoliths (Arkhipkin and Bizikov, 2000). The rostrum and wing of statoliths have the most significant difference between the reconstruction image and statistical result (Figs 3 and 4). These two sections (rostrum and wing) are the main parts that regulate accelerations during linear movement for pelagic squids (Arkhipkin and Bizikov, 2000). It has been reported that the statolith is only attached with macula statica princeps (MSP, located in the anterior chamber of statocysts) in the region of the wing (Budelmann, 1990). The main role of rostrum is inducing the endolymph flows across the crista transversalis anterior (CTA) in the inner posterior corner of statocysts and serves mainly to support the lower part of the wing (Budelmann, 1990; Arkhipkin and

Bizikov, 2000). As the above structures described, the gravity of center of statolith lies outside the wing. When the squid move or change direction, the statolith body deviates by a small acute angle to maintain a certain endolymph stream flows from under the statolith body along the anterior statocyst wall outwards (Arkhipkin and Bizikov, 2000). The size of wing and the volume of endolymph stream are closely related to the size of statolith and statocyst, which indicate the intensity of movement, including swimming speed. The CR squid have a narrower and longer rostrum and wing than those of CH (Fig. 4). Considering the explanation of the statolith morphological difference between demersal and pelagic decapods (Arkhipkin and Bizikov, 2000), an appropriate hypothesis of stock statolith variation in this study is that the northern stock (CR) has a greater sensitivity to the low angular accelerations compared with those of other geographic stocks in this study.

The movement of squid is highly related to its ambient oceanographic environment and the mobility of its prey and predators. *D. gigas* are mainly distributed in a high productivity region in the eastern tropical Pacific Ocean (Ichii *et al.*, 2002). The mechanism of squid aggregation in each geographic area depends on the local oceanographic characteristics. The Peruvian and Chilean waters are dominated by Peru Current (PC) in the Humboldt Current System (HCS), characterized by a low surface temperature and higher primary production levels (Pennington *et al.*, 2006). The North Equatorial Countercurrent (NECC) veers to the north in a counter-clockwise direction to merge with the North Equatorial Current (NEC) in the area of the countercurrent ridge (Costa Rica Dome) concentrating

surface nutrients to attract the aggregation of squid (Wyrski, 1966; Lavín *et al.*, 2006). A divergence of westward component of South Equatorial Current (SEC), from 3°N to 3°S and across the eastern tropical Pacific, is called “Equatorial cold tongue” and also known for macronutrients in euphotic zone (Chavez and Barber, 1987; Pennington *et al.*, 2006). These features are the important factors that attract squid in the equatorial area in eastern tropical Pacific (Sanchez *et al.*, 2016). Characterized by a higher surface temperature and a more complicated, weaker current system in the Costa Rica Dome, the squids living in this area tend to move more to prey and swim in such a wind-driven eddy area (Kessler, 2006) in comparison to their counterparts in south hemispheres of eastern Pacific with low temperature and strong current (Kessler *et al.*, 2006). Thus, the statolith shapes of CH and PE individuals become “rounded”, the result of living in relatively cold temperatures and stronger currents, while the northern stock (CR) statolith shape (Fig. 3) is “sharp”. *D. gigas* prey on zooplankton over all life stages (Liu, 2012). Some studies reported that the zooplankton feature in Costa Rica Dome, including the species and biomass, was distinctively different from other area in the ETP (Fernández-Álamo and Färber-Lorda, 2006). Food composition and availability also lead to the variability of squid prey behavior, which indirectly influences the statolith shape diversity (Nigmatullin *et al.*, 2001; Ulloa *et al.*, 2012).

Stock identification is a critically important priority for stock assessment, as biased results can lead to mismanagement (Ying *et al.*, 2011). Some materials and methods have been proposed and used for stock discrimination with successful results (Chen *et al.*, 2012; Fang *et al.*, 2012, 2014; Liu *et al.*, 2015b). The results of this study

have also successfully classified the different stocks from north and south hemispheres, supporting the results of previous studies (Sandoval-Castellanos *et al.*, 2007, 2010; Staaf *et al.*, 2010; Liu *et al.*, 2015b). Conventional statistical methods should rigorously keep the normality and homogeneity for all the data (Zuur *et al.*, 2010). This rule limited the data availability in some extents. The machine learning approaches, which create of algorithm from data and build models for decisions, can process all kinds of data with related statistical analysis, regardless of the data format (Lantz, 2013). Supervised learning methods have been widely used in fisheries science because of the merits of automatically building a model to make prediction from data without any human assistance, (Dub *et al.*, 2013; Fernandes *et al.*, 2014; Crespi-Abril *et al.*, 2015) and these methods have also been tested as an effective way to solve classification issues (Vyver *et al.*, 2016). This is also verified within this study, with a higher classification rate than traditional methods (Table 4). Comprehensively considering the sensitivity, specificity and Kappa values, SVM and RF were good models for the *D. gigas* statolith shape classification in this study (Table 4). The SVM and RF techniques produced a better result than other methods, which has been observed in other studies (Li *et al.*, 2015). RF tended to be more successful than SVM in our study because of the diminution associated the averaging results in SVM (Table 4).

The stock classification of *D. gigas* was evaluated based on the statolith shape in this study. The statolith shape of CH stock had the largest size and CR stock had the smallest size within the shape indices and all the shape indices had significant

differences among stocks. Statolith shapes from different geographic stocks were reconstructed by wavelet coefficients derived from the statolith images via computer software, and stock classification was performed. The results showed that reconstructed statolith shapes could realistically reflect the stock morphological difference, which was mainly observed in the rostrum and wing sections of statoliths. Machine learning methods were also used for stock classification in this study and produced a superior classification to traditional methods. This study supports the conclusions of previous studies that two separate stocks of *D. gigas* exist in the ETP. Physical oceanography and feeding behaviour are the two main causes of statolith shape variation among stocks. This study further reinforces the idea that analysis of statolith shape, using techniques such as RF and SVM, is a useful tool for stock recognition.

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