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Geographic variations of jumbo squid (*Dosidicus gigas*) based on gladius morphology

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Abstract—The jumbo flying squid (*Dosidicus gigas*) is widely distributed in the eastern Pacific Ocean and its population structure is complex. Morphometric analysis of hard structures of cephalopods is frequently employed to explore the geographic variation within discreet population units. In this study, 8 morphometric characteristics of the gladius, or pen, were measured and compared from 562 jumbo squid specimens collected off Peru, the Costa Rica Dome, and from the equatorial central eastern Pacific. The growth rate of the gladius was evaluated by comparing it with the ages determined from statoliths. Results showed significant differences in gladii morphology between sexes and geographic populations. Stepwise discriminant analysis indicated that gladius morphometric characteristics were suitable discriminatory variables with an overall correct classification rate of 78.6% for distinguishing different populations. Higher daily growth rates were found in the proostracum than in the conus, possibly because of the different biological functions of these anatomical parts. These results support our general hypothesis that gladius morphology provides an alternative way to distinguish potential geographic populations of *D. gigas* and it adds new information regarding the gladius of the Ommastrephidae.

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Morphometric analysis has been an accepted method for studying the geographic variation of population units within species of cephalopods (Nesis, 1993; Crespi-Abril et al., 2010; Liu et al., 2015a; van der Vyver et al., 2016). Cephalopods show high phenotypic plasticity in both soft and hard structures owing to their biological characteristics, such as rapid growth, short life span, and highly migratory activity (Crespi-Abril et al., 2010; Arkhipkin et al., 2015; van der Vyver et al., 2016). Therefore, morphometric analysis may be a useful approach for studying geographic variations in pelagic cephalopods that have an extensive distribution range.

The jumbo flying squid (*Dosidicus gigas*) is widely distributed in the eastern Pacific Ocean, extending from Alaska (60°N) to Chile (46°S) and stretching to the west (125°–140°W) at the Equator (Nigmatullin et al., 2001; Ibáñez and Cubillos, 2007). As a pelagic cephalopod, *D. gigas* has an important ecological function in marine ecosystems, not only as a voracious predator but also as a valuable source of prey (Field et al., 2007; Alegre et al., 2014). It also supports an important commercial fishery, and in 2014 the annual catch exceeded 1,000,000 metric tons (FAO, 2016). The main fishing regions are located off the coasts of Chile and Peru, off the Costa Rica Dome and within the Gulf of California.

Throughout its geographic range, the population structure of *D. gigas* is complicated and often debated. In the past, three distinguishable groups with a different size-at-ma-

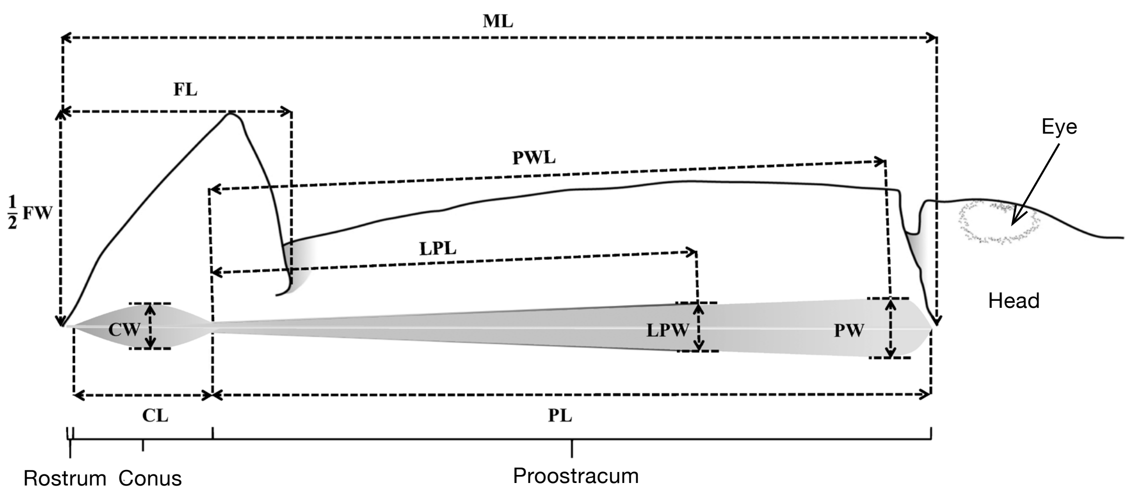

Figure 1

Morphometric measurements of the gladius and mantle recorded for jumbo squid (*Dosidicus gigas*) sampled off the Costa Rica Dome (CRD), off the Peruvian exclusive economic zone (PE), and in offshore waters of the central eastern Pacific (CEP) in 2009, 2013, and 2014 and examined in this study (measurements were taken on the basis of work by Arkhipkin et al., 2012). The measurements are mantle length (ML), conus length (CL), maximum width of conus (CW), proostracum length (PL), maximum width of prostracum (PW), length from anterior tip of conus to the widest point of proostracum (PWL), length from anterior tip of conus to proximal end of lateral plates (LPL), width between two anterior apices of lateral plates (LPW), fin length (FL), and fin width (FW). Adapted from Lorrain et al. (2011).

turity have been identified (Nigmatullin et al. 2001). It has been assumed that there is a small group that occurs in the near-equatorial waters, a medium-size group that is distributed within the entire distribution range, and a large-size group that occurs at the northern and southern peripheries of its distribution range. However, a high degree of variability in the size of the groupings was observed along the eastern Pacific Ocean (Keyl et al., 2011; Morales-Bojórquez and Pacheco-Bedoya, 2016); for example, the mantle length at first maturity was found to vary between 31.0 to 77.0 cm off Mexico (Markaida, 2006). Moreover, little genetic diversity has been found among these three groups on the basis of microsatellite loci (Sanchez et al., 2016). In contrast to this hypothetical spatial distribution, other researchers have divided *D. gigas* into northern and southern populations on the basis of genetic structure (Sandoval-Castellanos et al., 2007) or elemental signatures in the statolith (Liu et al., 2015b). In this study, not only *D. gigas* from northern and southern populations were examined, but also individuals from equatorial central eastern Pacific. For squid whose population structure is uncertain, a holistic management approach should always be considered on the assumption that it is a single population. However, if it can be unequivocally shown that separate stocks are present in the *D. gigas* population, stock-specific harvest strategies may be more effective in providing a sustainable biomass.

In *D. gigas*, the gladius, or pen, is a flexible internal structure that grows from the tail fin toward the head (Fig. 1). Morphologically, the gladius consists of a feather-shaped plate, the proostracum that tapers toward the posterior end of the gladius, becoming a funnel-shaped conus with a smallish rostrum (Arkhipkin et al, 2012). The gladius lies within the shell sac, which attaches to the fin cartilage in the vicinity of the conus and rostrum, whereas, the cartilage attaches to the fin muscles (Young and Vecchione, 1996; Arkhipkin et al, 2012). The gladius is a metabolically inert tissue that grows continuously throughout the lifetime of the species. Previous studies have shown that it is useful in age studies and can reveal ontogenetic patterns associated with changes in diet and habitat (Perez et al., 1996; Ruiz-Cooley et al., 2010; Li et al., 2017). Nonetheless, little attention has been paid to variations in gladius growth although the differences in age, growth, and population structure have often been observed (Sandoval-Castellanos et al., 2007; Chen et al., 2013; Ibáñez et al., 2016).

In this study, the morphometric characteristics of the gladius were analyzed. Our goal was to identify potential sexual dimorphism and spatial variations of gladius shape among population units within *D. gigas*. This study provides an alternative way to identify the potential geographic populations of *D. gigas* and contributes new information on the gladius of Ommastrephidae.

Materials and methods

Sampling and preparation

D. gigas samples were collected during various commercial jigging vessels operating in 2009, 2013, and 2014 in the waters of northern (off the Costa Rica Dome, [CRD]) and southern hemisphere (off the Peru Exclusive Economic Zone, [PE]) and equatorial Pacific Ocean (offshore waters of the central eastern Pacific, [CEP]) (Fig. 2). All squid were frozen on board and then transported to the Key Laboratory of Sustainable Exploitation of Oceanic Fisheries Resources at Shanghai Ocean University where they were defrosted under normal room temperature. Dorsal mantle length (ML), fin length (FL), and fin width (FW) were recorded to the nearest 1 mm, and body weight (BW) was measured to the nearest 1 g. Sex and maturity stage were determined on the basis of visual evaluation of the gonad morphometric characteristics defined by Lipiński and Underhill (1995). The gladius was extracted from its dorsomedial site in the mantle cavity and cleaned in an ultrasonic cleaner with distilled water. Gladii which appeared to be damaged were eliminated. Statoliths were extracted for age determination and the number of growth increments were counted under the assumption that the increment is deposited daily (Jackson and Forsythe, 2002).

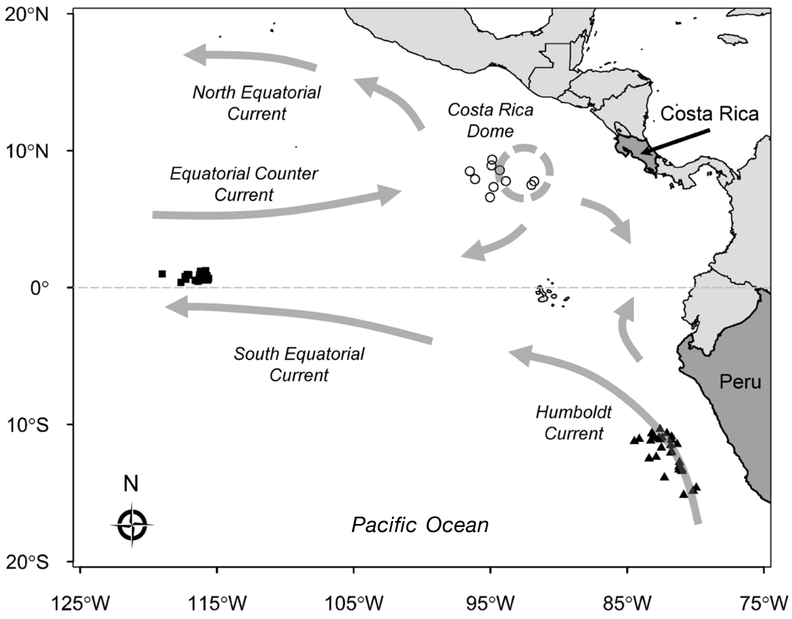

Figure 2

Map of the major surface currents (adapted from Anderson and Rodhouse, 2001) and the locations where jumbo squid (*Dosidicus gigas*) were sampled in 2009, 2013, and 2014 off the Costa Rica Dome (CRD). Sampling locations off the CRD are indicated with open circles, sampling locations off the Peruvian exclusive economic zone (PE), are indicated with black triangles, and those in offshore waters of the central eastern Pacific (CEP) are indicated with black squares. Morphological features were used to distinguish between populations in different geographic zones

Morphometric measurements

A total of 562 gladii from the three regions were used in the subsequent analyses, and detailed information on the samples can be seen in Table 1. Eight morphometric characteristics of the gladius (gladius length [GL], conus length [CL], maximum width of conus [CW], proostracum length [PL], maximum width of prostracum [PW], length from anterior tip of conus to the widest point of proostracum [PWL], length from anterior tip of conus to proximal end of lateral plates [LPL], the width between two anterior apices of lateral plates [LPW]) were measured from each sample to the nearest 1 mm (Fig. 1). All morphometric measurements were made by a single researcher throughout our study.

Statistical analyses

All morphometric data used for the analyses of sexual dimorphism and geographic variation were standardized to remove the allometric effects of body size and meet the assumptions of normality. To accomplish this, raw data were transformed by using the following formula (see Lleonart et al., 2000 for details):

$$Y_i^* = Y_i\left(\frac{ML_0}{ML_i}\right)^b, \qquad (1)$$

where Y = one of the morphometric variables of the gladius;

Y_i^* = the standardized value for the individual I;

Y_i and ML_i = the observed values of Y and ML for the individual I;

ML_0 = the arithmetic mean for the study population; and

b can be predicted from the following formula:

$$\ln(Y) = \ln(a) + b\ln(ML) + \varepsilon \;\; \varepsilon \sim N(0,\sigma^2), \qquad (2)$$

where a = a parameter to be estimated; and

σ^2 = the variance for the normally distributed unexplained errors ε.

The standardized variables are represented by subscript "S", i.e. CL_S, CW_S, PL_S, PW_S, PWL_S, LPL_S, LPW_S, FL_S and FW_S.

One-way analysis of variance (ANOVA) and stepwise discriminant analysis (SDA) were used to analyze sexual dimorphism. The SDA is a method of linear modeling to estimate the proportion of correct classification with a leave-one out stepwise jack-knife procedure. Owing to the small sample size of males, samples from off the CRD was excluded from this analysis.

Table 1

Summary information, including mean dorsal mantle length (ML) and body weight (BW) with standard deviations (SDs), for jumbo squid (*Dosidicus gigas*) sampled in 2009, 2013, and 2014 off the Costa Rica Dome (CRD), off the Peruvian exclusive economic zone (PE), and in offshore waters of the central eastern Pacific (CEP) to examine the use of morphological features to distinguish between populations in different geographic zones. *n*=number of squid in a sample

					ML (cm)				BW (g)			
Geographic population	*n* (F, M)	Sampling date	Coordinates	Maturity stage	Maximum	Minimum	Mean	SD	Maximum	Minimum	Mean	SD
CRD	105 (89, 16)	Jul. to Aug. 2009	91°48′– 99°30′W 6°36′– 9°30′N	I–IV	40.9	21.8	29.5	3.1	1907.5	252.6	737.2	256.9
PE	32 (27, 5)	Sep. to Oct. 2009	82°06′– 84°29′W 10°21– 11°17′S	I–III	44.7	26.4	32.7	4.6	2928.7	490.0	1088.2	555.4
	179 (116, 63)	Jul. to Oct. 2013	79°57′– 83°24′W 10°54′– 15°09′S	I–IV	39.6	21.8	26.8	4.3	1647.0	278.0	548.8	379.8
	71 (38, 33)	Feb. to Sep. 2014	74°57′– 83°13′W 10°26′–22°38′S	I–IV	48.5	19.1	28.1	7.3	3361.0	177.0	767.9	721.4
CEP	121 (57, 64)	Apr. to Jun. 2013	114°59′–119°00′W 0°28′N–1°00′S	I–IV	37.2	22.2	29.2	3.7	1523.0	291.0	746.7	300.7
	54 (45, 9)	Apr. to May. 2014	116°00′– 117°19′W 0°42′– 0°58′N	I–III	41.2	27.5	33.0	3.0	1849.0	608.0	1059.8	300.9

Principal component analysis (PCA) was used to determine the main morphometric variables among three population units by reducing the multidimensional data. SDA was performed on the results of PCA to identify the variables that would distinguish the geographic populations and calculate the classification rates.

Moreover, to remove the potential impacts of the disproportionate number of female *D. gigas* and different sex ratios for the three sites, PCA and SDA were repeated with female data only as an independent data set to evaluate the geographic divergence of females.

The analyses of growth rates were performed on untransformed morphometric and age data. The absolute daily growth rate (DGR, mm/day) was calculated for each 25-day interval with the following equation (Forsythe and Van Heukelem, 1987):

$$DGR = \frac{L_2 - L_1}{t_2 - t_1}, \tag{3}$$

where L = one of the morphometric variables of the gladius;

L_1 and L_2 = the observed values (mm) at the beginning (t_1) and the end (t_2) of each interval of time (day).

Multiple comparison was examined by using ANOVA with post-hoc Tukey's honestly significant difference (HSD) test. For nonnormal and ordinal data, a matched comparison with the Friedman test was applied. A P value of ≤0.05 was considered significant for all statistical tests, and statistical analyses were carried out by SPSS Statistics,[1] vers. 19.0 (IBM Corp., Armonk, NY).

Results

Gladius structure

Morphometric variables of the three geographic populations are presented in the Supplementary Table.

Regression analysis performed on the GL and ML showed strong correlations within the three geographic populations (CRD: r=0.95, ML=1.005GL+1.267; PE: r=0.98, ML=0.94GL+0.99; CEP: r=0.99, ML=1.03GL–0.63). As main morphological parts of the gladius, the ratio of CL to PL (CL/PL) showed significant differences (ANOVA, $P<0.05$, $F_{2,\,558}=87.30$) among regions. The CL/PL values for squid off the CRD (0.22 ±0.02) were lower than those off the PE (0.24 ±0.02) and CEP squid (0.26 ±0.02). In addition, the CL showed significant positive relationships with FW (CR: r=0.77; PE: r=0.90; CEP: r=0.70) and FL (CR: r=0.82; PE: r=0.89; CEP: r=0.72).

Sexual dimorphism

When comparing morphometric characteristics between females and males, we found that only CL_S, CW_S and

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[1] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 2

Results of analysis of variance between sexes for standardized ($_{\mathrm{S}}$) gladius morphometric variables of jumbo squid (*Dosidicus gigas*) sampled in 2009, 2013, and 2014 off the Peruvian exclusive economic zone (PE) and in offshore waters of the central eastern Pacific (CEP). The morphometric variables are conus length (CL), maximum width of conus (CW), proostracum length (PL), maximum width of prostracum (PW), length from anterior tip of conus to the widest point of proostracum (PWL), length from anterior tip of conus to proximal end of lateral plates (LPL), and width between 2 anterior apices of lateral plates (LPW). n=number of samples; F=variance between the means of female and male values; ns=nonsignificant, probability (P)>0.05; *=P<0.05; **=P<0.01

	PE		CEP	
Variable	F	P	F	P
n	282		175	
$\mathrm{CL_S}$	3.72	ns	38.67	**
$\mathrm{CW_S}$	2.40	ns	27.06	**
$\mathrm{PL_S}$	8.21	**	281.11	**
$\mathrm{PW_S}$	5.93	*	89.90	**
$\mathrm{PWL_S}$	26.29	**	57.87	**
$\mathrm{LPL_S}$	3.07	ns	78.39	**
$\mathrm{LPW_S}$	4.34	*	60.13	**

Table 3

The percentages of cross-validated classification from stepwise discriminant analysis for each sex of 2 geographic populations of jumbo squid (*Dosidicus gigas*) sampled in 2013 and 2014 off the Peruvian exclusive economic zone (PE) and in offshore waters of the central eastern Pacific (CEP).

	PE		CEP	
	Female	Male	Female	Male
Female	62.0%	38.0%	96.1%	3.9%
Male	36.7%	63.3%	8.2%	91.8%
Total	62.4%		94.3%	
P	<0.001		<0.001	

$\mathrm{LPL_S}$ of PE specimens were not different for the sexes (Table 2). Meanwhile, the SDA results showed that the correct cross-validated classification rates reached from 62.4 % to 94.3 % for the region off PE and within the CEP, respectively (Table 3). The results may indicate a significant difference between the sexes within each population.

Geographic variations

For all individuals, the PCA of the morphometric features of squid gladii retained two principal components (PC1 and PC2), having eigenvalues greater than 1 and accounted for 62.40 % and 14.81 % of the total variance, respectively. The main variables counting for variation in PC1 were $\mathrm{CL_S}$, $\mathrm{PL_S}$, $\mathrm{PW_S}$, $\mathrm{PWL_S}$, $\mathrm{LPL_S}$, and $\mathrm{LPW_S}$. Variation of PC2 was mainly influenced by $\mathrm{CW_S}$. The SDA performed on all samples identified four valid gladius morphometric features ($\mathrm{CL_S}$, $\mathrm{CW_S}$, $\mathrm{PL_S}$ and $\mathrm{PW_S}$) that served as discriminatory variables for the three geographic populations (Table 4). The first canonical function explained 83.9% of the total variance. Although there was considerable overlap, the final correct cross-validated classification rates among the geographic populations reached 80.0% (off the CRD), 74.8% (off the PE) and 84.0% (within the CEP), respectively (Table 5A).

The PCA performed on data sets for females detected only one principal component with eigenvalues greater than 1 and explaining 65.30% of the total variance. All morphometric features showed high contributions to the principal component analysis. The SDA selected $\mathrm{CL_S}$, $\mathrm{CW_S}$, $\mathrm{PL_S}$, and $\mathrm{PW_S}$ as suitable variables to distinguish female *D. gigas* from the three regions, with a misclassification rate of 16.8% (Tables 4 and 5B).

A total of 340 statoliths from the three sites were satisfactorily prepared for age determination. In all three population units, values of DGR for PL were larger than CL during all studied periods for each sex (Friedman test, P<0.01, Table 6), although small sample sizes may have influenced the reliability of calculated growth rates in some age groups.

For each geographic population, the maximum DGR in CL was found from 201 to 225 days of age for female *D. gigas* (Table 6). Although the maximum DGR in PL occurred at different periods, the value for off the CRD (1.44 mm/d, 201–225 days) and off the PE (1.12 mm/d, 201–225 days) were larger but occurred later than the CEP value (1.11 mm/d, 176–200 days). For males, the maximum DGR in CL (0.41 mm/d) was reached from 201to 225 days of age for PE, wheras in the CEP the maximum is smaller (0.34 mm/d) and it is reached before, at 176–200 days. In contrast, the maximum DGR in PL was observed at 201–225 and 176–200 days for males off the PE and within the CEP (Table 6).

Discussion

Gladius structure

In *D. gigas*, a strong correlation was found between GL and ML in each geographic population. This finding indicates that the gladius can be considered a hard tissue that supports the mantle and internal organs. Similar results were reported for other ommastrephids, *Illex illecebrosus* (Perez et al., 1996) and *Ommastrephes bartramii* (Kato et al., 2016). Also, GL may be a more reliable measurement than ML when individuals are

Table 4

Function coefficients for 3 geographic populations of jumbo squid (*Dosidicus gigas*) sampled off the Costa Rica Dome (CRD), off the Peruvian exclusive economic zone (PE), and offshore waters of the central eastern Pacific (CEP) in 2009, 2013, and 2014. The morphometric features used for the examination are proostracum length (PL), conus length (CL), the maximum width of conus (CW), and the maximum width of prostracum (PW).

		CR	PE	CEP
All individuals	PL_S	19.56	20.17	23.78
	CL_S	12.02	18.08	25.72
	CW_S	31.57	29.65	32.15
	PW_S	–37.22	–31.43	–37.54
	(constant)	–392.63	–365.74	–441.60
Females	PL_S	25.07	25.63	30.48
	CL_S	42.88	47.98	62.08
	CW_S	40.57	38.26	42.58
	PW_S	–26.65	–18.64	–25.87
	(constant)	–532.33	–498.69	–629.86

stored frozen because soft tissues can be deformed during the thawing process. In this study, positive correlations were found between the CL with FL and FW in all three population units. The conus, which is located in the middle of the tail fin, is the main morphological section of the gladius (Arkhipkin et al., 2012). With their anatomical analysis, Young and Vecchione (1996) found that fin cartilage connected the fin muscles and shell sac, which secretes the conus and cone flags. Therefore, the conus could be considered a supporting structure that helps the fin resist the dynamic pressures generated during swimming (Bizikov and Arkhipkin, 1997; Arkhipkin et al., 2012). A longer conus might also provide appropriate support for the fin when the animal is experiencing high speed currents.

Sexual size dimorphism

Sexual size dimorphism is a common phenomenon that occurs in the hard structures of cephalopods (Fang et al., 2014; Liu et al., 2015a). Our results also showed that the shapes of gladii were different for the sexes within two of the populations (i.e., off the PE and within the CEP). These results are in agreement with those from Liu et al. (2015a) who observed sexual dimorphism in another hard structure, the beak of *D. gigas*, from these same two populations which are found in the waters off Peru and Ecuador, termed PE and CEP, respectively in our study. This difference in shapes was despite the fact that both populations experienced similar environmental conditions and food availability. However, sex-specific different nutritional requirements have recently been described for female and male squid and may explain the sexual dimorphism we observed (e.g. *Nototodarus gouldi*, (Steer and Jackson, 2004), *Sepioteuthis australis* (Pecl and Moltschaniwskyj, 2006) and *Illex argentines* (Lin et al., 2015)). In these cases, females and males within the population show divergent energetic needs for growth or reproduction, and one might expect this to result in squid with different growth-related parameters, which could have a potential impact on the gladius morphometric characteristics (van der Vyver et al., 2016). Additionally, there may be sexually related behavioral differences where females and males might forage on different prey items at different depths in a given habitat (Arkhipkin and Middleton, 2002).

Geographic variations

Dosidicus gigas is a species endemic to the eastern Pacific and has a complex population structure. Geographic variations in age, growth, and reproduction of this species have often been observed (Sandoval-Castellanos et al., 2007; Chen et al., 2013; Ibáñez et al., 2016). As reported by Liu et al. (2015a), spatial variations in the beak structure of *D. gigas* could be used to distinguish geographic populations. This difference was attributed to a phenotypic response resulting from distinct environmental conditions that a population experiences at a given geographic location (Liu et al., 2015a; van der Vyver et al., 2016), but further research is needed to confirm the exact relationship between a species' physiological response to variations in the environment.

In this study, the SDA revealed a high rate of correct classification of gladii shapes among geographic populations, even with the female data as an independent data set (Table 5B). When looking at the environmental conditions these populations experience,

Table 5

Results of the stepwise procedure of discriminant analysis showing the order of standardized ($_S$) morphometric variables to entry for (**A**) 3 geographic populations and (**B**) the female jumbo squid (*Dosidicus gigas*) sampled in 2009, 2013, and 2014 off the Costa Rica Dome (CRD), off the Peruvian exclusive economic zone (PE), and in offshore waters of the central eastern Pacific (CEP). Correct cross-validated classification rates are shown in bold type. The morphometric variables are proostracum length (PL), conus length (CL), the maximum width of conus (CW), and the maximum width of prostracum (PW). At each step, the variable with the largest *F* value that exceeds the entry criteria is added.

A

Step no.	Variable	*F*	df 1	df 2	*P*
1	PL_S	264.60	2	559.00	<0.01
2	CL_S	193.95	4	1116.00	<0.01
3	CW_S	151.85	6	1114.00	<0.01
4	PW_S	119.01	8	1112.00	<0.01

	Number and percentage of samples classified to a population			
Population	CR	PE	CEP	Total
CR	84	18	3	105
%	**80.0**	17.1	2.9	100
PE	62	211	9	282
%	22.0	**74.8**	3.2	100
CEP	14	14	147	175
%	8.0	8.0	**84.0**	100
			Total correct (%)	**78.6**

B

Step no.	Variable	*F*	df 1	df 2	*P*
1	PLs	331.32	2	372.00	<0.01
2	CLs	204.84	4	742.00	<0.01
3	CWs	165.34	6	740.00	<0.01
4	PWs	129.74	8	738.00	<0.01

	Number and percentage of samples classified to a population			
Population	CR	PE	CEP	Total
CR	72	14	3	89
%	**80.9**	15.7	3.4	100
PE	39	141	4	184
%	21.2	**76.6**	2.2	100
CEP	3	0	99	102
%	2.9	0	**97.1**	100
			Total correct (%)	**83.2**

there is reason to believe that our SDA results can be explained by these environmental differences. For example, the Costa Rica Dome (Fig. 2) is an oceanic upwelling center where the thermocline is shallow (Sasai et al., 2007), whereas the waters off Peru (PE) are primarily affected by the cold northward flowing Humboldt Current and are associated with strong upwelling of cool subsurface waters (Chavez et al., 2008). Individuals in the CEP were from an area primarily influenced by a warm Equatorial Counter Current and the cooler South Equatorial Current (Fig. 2). The differences in oceanographic conditions in each region may consequently result in morphometric variations in the gladius. For example, several studies have shown that ambient temperature has a significant influence on the growth and development of *D. gigas* (Keyl et al.,

Table 6

Absolute daily growth rates (DGR) in mm/day for conus length (CL) and proostracum lengh (PL) for female and male jumbo squid (*Dosidicus gigas*) sampled off the Costa Rica Dome (CRD), off the Peruvian exclusive economic zone (PE), and in offshore waters of the central eastern Pacific (CEP) in 2009, 2013, and 2014. *n*=number of squid in a sample. na=data not available.

Population	Sex	Age class (d)	*n*	Average CL (mm)	DGR (mm/d)	Average PL (mm)	DGR (mm/d)
CR	F	126-150	2	37.5	na	167.5	na
		151-175	15	43.1	0.22	198.0	1.22
		176-200	36	49.2	0.24	222.9	1.00
		201-225	29	57.9	0.35	259.0	1.44
		226-250	3	63.0	0.20	288.0	1.16
PE	F	126-150	8	46.0	na	188.0	na
		151-175	31	49.2	0.13	200.6	0.50
		176-200	41	51.9	0.11	211.0	0.42
		201-225	7	62.1	0.41	239.0	1.12
		226-250	2	68.5	0.26	255.5	0.66
		251-275	2	78.0	0.38	278.5	0.92
	M	126-150	6	42.5	na	186.3	na
		151-175	17	47.3	0.19	194.8	0.34
		176-200	21	49.8	0.10	204.2	0.38
		201-225	8	60.1	0.41	233.4	1.17
CEP	F	126-150	5	50.6	na	191.6	na
		151-175	6	54.7	0.16	208.3	0.67
		176-200	26	59.7	0.20	236.0	1.11
		201-225	12	65.8	0.24	250.7	0.59
		226-250	4	69.5	0.15	276.0	1.01
	M	126-150	5	45.8	na	185.0	na
		151-175	13	51.6	0.23	200.5	0.62
		176-200	27	60.1	0.34	234.7	1.37
		201-225	13	64.5	0.18	252.50	0.71
		226-250	1	67.0	0.10	268.00	0.62

2011; Staaf et al., 2011). The proostracum contributes to a large proportion of GL (>80 %) and has a higher longitudinal growth rate than conus (Table 6). With the assumption that the conus provides a supporting structure for the fin during swimming, the higher growth rate of the proostracum can be considered a structure that meets the requirements for rapid growth of squids because the gladius is a tissue that supports the growth of the mantle and internal organs (Bizikov and Arkhipkin, 1997; Arkhipkin et al., 2012). Comparisons in CL/PL values and growth rates between geographic populations show spatial variations in these two structures. The squid from CEP had higher CL/PL values than the squid from the area of the CRD and PE. We suggest that this result is possibly due to the environmental conditions experienced by the squid off the CRD and PE that are reflected in the longitudinal growth of proostracum. The Humboldt Current or upwelling (or both) supply nutrients to the waters off Peru and the CRD, while the waters of the central equatorial Pacific have lower primary productivity (Anderson and Rodhouse, 2001; Fiedler, 2002). Because food availability is considered as one of the central factors that influences *D. gigas* growth (Chen et al., 2013; Liu et al., 2013), regional differences in primary productivity may be strongly correlated with the observed differences in the growth of gladius.

Although the squid populations can be distinguished on the basis of morphological evidence, there is still considerable overlap, as shown in Table 5, A and B, which suggests that stable population substructuring is not the norm. Similar overlap has also been observed in elemental signatures in statoliths o *D. gigas* from waters off the Costa Rica Dome and Peru (Liu et al., 2015b). Indeed it was reported that a weak northern and southern hemisphere divergence was found for *D. gigas* with the use of molecular methods. Sandoval-Castellanos et al. (2010) and Staaf et al. (2010), and some of our results from CEP individuals have also indicated a close similarity to the other two jumbo squid populations. This finding would suggest that although the morphological studies reported here suggest clear spatial subpopulations, further research will reveal the stability of these populations units over time.

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