

Species identification of *Ommastrephes bartramii*, *Dosidicus gigas*, *Sthenoteuthis oualaniensis* and *Illex argentinus* (Ommastrephidae) using beak morphological variables

XINJUN CHEN^{1,2,3}, HUAJIE LU¹, BILIN LIU^{1,2,3}, YONG CHEN^{4,3},
SILIANG LI¹ and MA JIN¹

¹ College of Marine Sciences, Shanghai Ocean University, 999 Hucheng Ring Road, Shanghai 201306, China.
E-mail: xjchen@shou.edu.cn

² The Key Laboratory of the Shanghai Education Commission for Oceanic Fisheries Resources Exploitation, 999 Hucheng Ring Road, Shanghai 201306, China.

³ The Key Laboratory of Sustainable Exploitation of Oceanic Fisheries Resources (Shanghai Ocean University), Ministry of Education, 999 Hucheng Ring Road, Shanghai 201306, China.

⁴ School of Marine Sciences, University of Maine, Orono, ME 04469, U.S.A.

SUMMARY: Four oceanic squid species, *Ommastrephes bartramii*, *Dosidicus gigas*, *Sthenoteuthis oualaniensis* and *Illex argentinus*, not only support important commercial fisheries, but also play a vital role in their marine ecosystems. It is therefore important to identify them in the analyses of their predators' stomach contents as this can yield critical information on the trophic dynamics of ecosystems. Hard beaks of the four species frequently found in their predators' stomachs can be used to identify them. In this study, to remove the effect of size differences among individuals, measurements of upper and lower beaks were standardized with an allometric model. A discriminant analysis was carried out to compare morphological differences among the four species and between the sexes for each species. The upper rostral width and upper rostral length showed the greatest interspecific variation in the beak morphological variables of the four Ommastrephidae. The linear discriminant functions of beak morphological variables were developed for the four Ommastrephidae, which resulted in a rate of correct species classification of over 97%. Sexual dimorphism was also found in the beak morphology of *O. bartramii* and *I. argentinus*. This study suggests that morphological variables can be used to reliably classify Ommastrephidae at genus level, which can help identify the specie in the stomachs of cephalopod predators. This helps to improve the understanding of the role cephalopods play in their marine ecosystems.

Keywords: beak morphological variable, species classification, *Ommastrephes bartramii*, *Dosidicus gigas*, *Sthenoteuthis oualaniensis*, *Illex argentinus*.

RESUMEN: IDENTIFICACIÓN DE LAS ESPECIES: *OMMASTREPHEs BARTRAMII*, *DOSIDICUS GIGAS*, *STHENOTEUTHIS OUALANIENSIS* E *ILLEX ARGENTINUS* (OMMASTREPHEIDAE) A TRAVÉS DE MEDIDAS MORFOLÓGICAS DE SUS PICOS. – Las cuatro especies de calamares: *Ommastrephes bartramii*, *Dosidicus gigas*, *Sthenoteuthis oualaniensis* e *Illex argentinus*, sometidas a una importante presión pesquera, juegan un papel significativo dentro de los ecosistemas marinos a los que pertenecen. Al ser los picos de estas especies resistentes, las medidas de diversos aspectos de su morfología pueden servir para identificarlas en análisis de contenidos estomacales de sus depredadores. Ello permite obtener una información crucial sobre la dinámica trófica de los ecosistemas. En el presente estudio, las medidas realizadas en los picos superior e inferior de los Ommastrephidae se han normalizado mediante un modelo de crecimiento alométrico, para evitar la influencia del efecto tamaño de los individuos. A continuación, mediante un análisis discriminante, se han estudiado las diferencias morfológicas entre las cuatro especies, así como entre machos y hembras. Las medidas que presentaban mayores variaciones eran la anchura y longitud del rostro superior. Mediante funciones discriminantes lineales de las medidas morfológicas normalizadas de sus picos, se han conseguido clasificar las cuatro especies de Ommastrephidae, con una fiabilidad superior al 97%. Asimismo, a través de sus medidas morfológicas, se ha encontrado un claro dimorfismo sexual en los picos de *O. bartramii* e *I. argentinus*. El presente estudio sugiere que las medidas morfológicas pueden ser útiles para clasificar correctamente los Ommastrephidae a nivel de

género, y puede permitir identificar la especie en contenidos estomacales de depredadores de cefalópodos, lo cual mejorará el conocimiento del papel de los cefalópodos en los ecosistemas marinos en los que se integran.

Palabras clave: medidas morfológicas de los picos, clasificación de especies, *Ommastrephes bartramii*, *Dosidicus gigas*, *Sthenoteuthis oualaniensis*, *Illex argentinus*.

INTRODUCTION

Cephalopod fisheries, in particular those of Ommastrephidae, have attracted interests worldwide over the last three decades (Rodhouse 2001). Cephalopods play key roles in marine ecosystems both as predators and preys of top predators (Clarke 1996, Boyle and Boletzky 1996). Determining and quantifying their trophic interactions with other species is therefore a key issue in understanding the structure and functioning of marine ecosystems.

Species identification is a basic problem in determining the feeding ecology of cephalopods and their predators. Morphological characteristics of body and hard structures have often been used to identify cephalopod species with close affinities (Roper *et al.* 1984, Jackson 1995, Doubleday *et al.* 2006). Since chitinous beaks have a relatively consistent shape (Smale 1996, Clarke 1996, 1998, Neige and Boletzky 1997) and are more resistant to fragmentation than other hard structures, such as the statolith and inner shell, they have been proven to be valuable for studying cephalopod predators (Lu and Ickeringill 2002, Cherel and Hobson 2005).

Many studies have been carried out on cephalopod species identification using beaks. Clarke (1962) used beaks to distinguish families and found that the lower beaks were more useful for species identification. Clarke and Macleod (1974) were able to distinguish cephalopod species with various beak characteristics. Clarke (1986) and Xavier and Cherel (2009) identified cephalopod beaks based on the beak structural features. Lu and Ickeringill (2002) produced a diagnostic illustrating key for identifying 75 cephalopod beaks in the diets of marine vertebrates from southern Australian waters, and analyzed the relationships between beak morphometrics and animal body attributes. An international workshop and training course on cephalopod beaks was held in Faial Island of the Azores during April 2007 to review the current status of using beaks to identify cephalopods (Xavier *et al.* 2007). The beaks were proven to be more accurate than soft body parts for separating populations of *Loligo gahi* from Peruvian waters, southern Chilean waters and waters around the Falkland Islands (Vega *et al.* 2002).

Previous studies have shown that beak morphometric characteristics can provide good materials for identifying species and populations of cephalopods (Clarke 1986). Traditional morphometrics is commonly applied in the study of cephalopod beaks due to its simple and convenient measurements (Jackson and McKinnon 1996, Ogden *et al.* 1998, Gröger *et*

al. 2000). Ogden *et al.* (1998) suggested that seven size-standardized ratios for nine species of Southern Ocean octopods could be used as taxonomic characters for distinguishing between genera, but not between species. Stepwise discriminant function analysis also indicated that all seven ratios were required to maximize the discrimination between beaks. Multivariate discriminant analysis of three *Illex* species resulted in a high rate of correct classification (83%) based on beak characters (Martínez *et al.* 2002). Other geomorphometric methods for identifying cephalopods that have been applied in recent years include coordinate (landmarks) morphometrics and boundary (outline) morphometrics (Hsu 2003, Neige 2006). Hsu (2003) successfully applied coordinate morphometrics to examine the differences between sexes, local populations and among 11 different octopus species.

Four economically and ecologically important species of Ommastrephidae, *Ommastrephes bartramii*, *Dosidicus gigas*, *Sthenoteuthis oualaniensis* and *Illex argentinus* are widely distributed in the three oceans. *I. argentinus* is distributed along the shelf and slope in the western South Atlantic from 22° to 54°S (Hatfield *et al.* 1990), which are subject to subtropical convergence formed by the Falklands current and Brazilian current (Fedulov *et al.* 1990). *D. gigas* is commonly found in the southeastern Pacific Ocean, which is closely associated with the Humboldt Current (Chen *et al.* 2008). *O. bartramii* is widely distributed in subtropical and temperate oceanic waters, and is commercially exploited in the northwestern Pacific Ocean, which is strongly affected by the Kuroshio and Oyashio currents (Chen and Chiu 1999, Chen *et al.* 2008). *S. oualaniensis* is found in the northwestern Indian Ocean, which is closely related to the Somalia upwelling (Chen *et al.* 2008). Of the four species, the distribution of *O. bartramii* overlaps with that of the other species. The other three species have a much more limited geographical distribution and do not overlap with each other. These four important squid support a world fishery that had a annual catch ranging from 1.1 to 1.65 million tonnes in 2005 to 2007 (FIGIS 2009). In addition, they play a vital role in their marine ecosystems, in particular as important prey for predators such as tuna, swordfish, sharks and whales (Desportes and Mouritsen 1988, Clarke 1996, Benjamins 2000). Identification and differentiation of these squid species in the stomachs of predators is important for the study of the marine ecosystem.

The objectives of this study are to quantify beak characteristics, develop an approach for identifying species of *O. bartramii*, *D. gigas*, *S. oualaniensis* and

TABLE 1. – The fishing area, fishing date, sample number and mantle length of the different species included in this study.

Species	Fishing area	Fishing date	Sample size	Mantle length(mm)
<i>O. bartramii</i>	151°7'-158°36'E, 40°6'-44°38'N	Jul-Nov, 2007	754	201-426
	82°5'-85°30'W, 10°32'-13°32'S	Sep, 2008-Feb, 2009	277	209-1060
<i>D. gigas</i>	75°-79°30'W, 20°-23°30'S	May, 2008	353	269-517
<i>S. oualaniensis</i>	59°40'-64°8'E, 13°10'-20°10'N	Apr, 2005 and Oct-Dec, 2005	103	142-575
<i>I. argentinus</i>	57°55'-60°43'W, 40°2'-46°53'S	Feb-May, 2007	408	174-346

I. argentinus using beak morphometric variables, and evaluate possible differences between male and female beak morphology. This study provides an approach that can be used to distinguish species of *O. bartramii*, *D. gigas*, *S. oualaniensis* and *I. argentinus* based on their beaks, which is essential information for improving the understanding of the role cephalopods play in their marine ecosystems.

MATERIALS AND METHODS

Four species of Ommastrephid squid, *O. bartramii*, from the northwest Pacific Ocean, *D. gigas*, from the southeast Pacific Ocean, *S. oualaniensis*, from the southwest Indian Ocean, and *I. argentinus*, from the southwest Atlantic Ocean, were randomly sampled in the surveys conducted by the Chinese squid jigging vessels from 2005 to 2007 (Table 1). Sizes of the sampled individuals varied from 201 to 426 mm dorsal mantle length (ML) for *O. bartramii*, from 209 to 1060 mm ML for *D. gigas*, from 142 to 575 mm ML for *S. oualaniensis*, and from 174 to 346 mm ML for *I. argentinus* (Table 1).

All samples were immediately frozen and preserved at -18°C . The beaks were thawed at room temperature in the lab, then extracted according to the technique described in Bizikov (1991). They were preserved in 75% ethyl alcohol. The photographs of the beaks are shown in Figure 1.

A total of 13 morphometric characteristics of the body and beaks were measured. ML was measured to the nearest 1 mm, whereas the rest of the variables were measured to the nearest 0.01mm using digital calipers. We followed Clarke (1986) for measuring the beak morphological variables (Fig. 2): upper hood length (UHL), lower hood length (LHL), upper crest length (UCL), lower crest length (LCL), upper rostral length (URL), lower rostral length (LRL), upper rostral width (URW), lower rostral width (LRW), upper wing length (UWL), lower wing length (LWL), upper lateral wall length (ULWL), and lower baseline length (LBL). The morphological variables of each beak were measured independently by two different people.

We evaluated the differences in these beak morphological variables among the four Ommastrephid species and between the sexes for each species. The measured beak morphological variables were standardized to remove possible allometric effects of body size in the morphological analyses (Leonart *et al.* 2000, Pineda *et al.* 2002, Vega *et al.* 2002, Lefkaditou and Bekas 2004). In the standardization, the UHL was chosen as the fixed independent variable, and the other variables were measured as the dependent variables. The following allometric model was used to fit the data:

$$\ln(y) = \ln(a) + b \ln(\text{UHL}) + \varepsilon \quad \varepsilon \sim N(0, \sigma^2) \quad (1)$$

where y is the value of one of the other beak morphological variables aside from UHL; a and b are the pa-

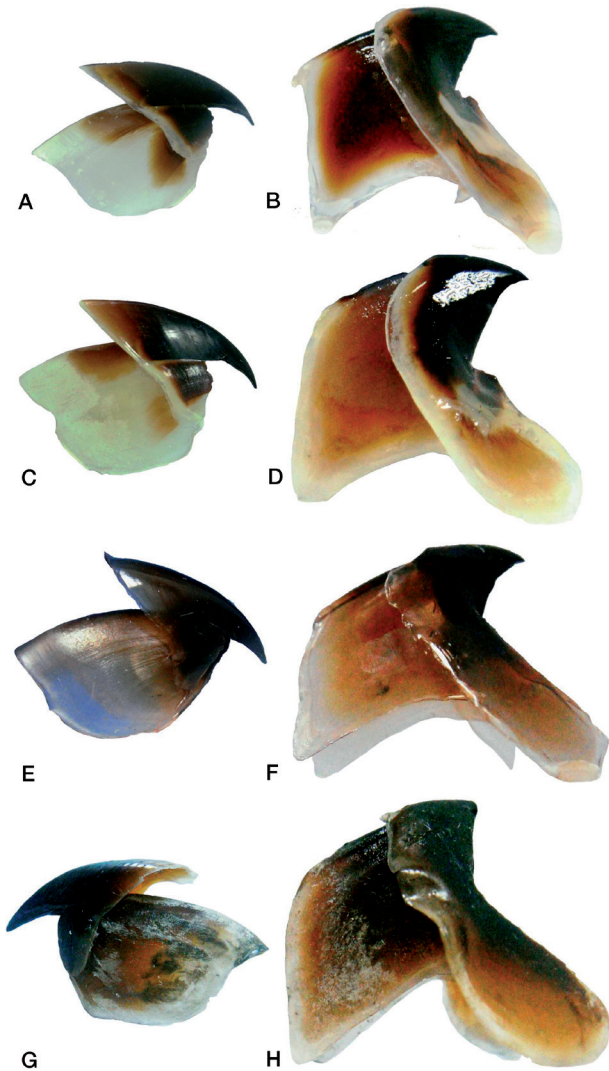


FIG.1. – The photographs of the beaks of the four Ommastrephid species: A, upper beak and B, lower beak of *O. bartramii*; C, upper beak and D, lower beak of *D. gigas*; E, upper beak and F, lower beak of *S. oualaniensis*; and G, upper beak and H, lower beak of *I. argentinus*.

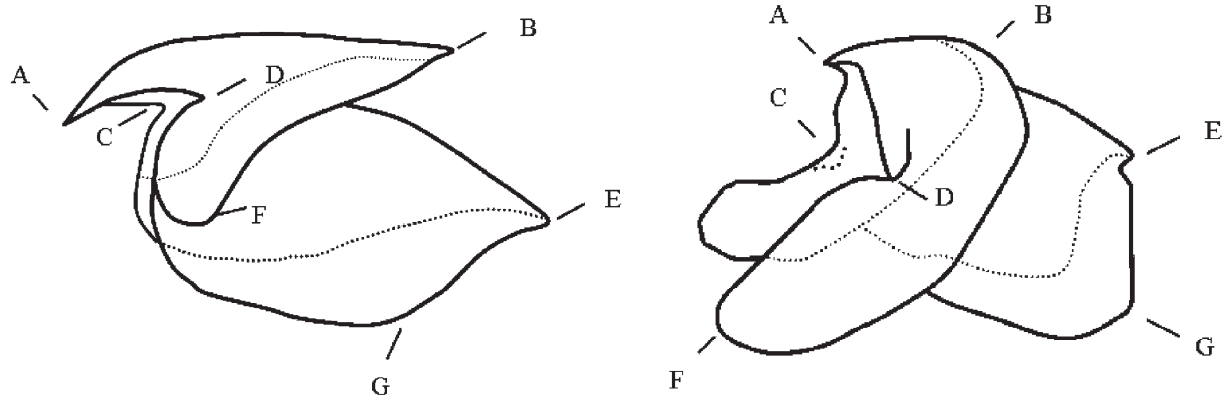


FIG. 2. – Beak morphological variables of the Ommastrephidae: (a) upper beak: hood length (A-B, UHL), crest length (A-E, UCL), rostral length (A-C, URL), rostral width (C-D, URW), wing length (D-F, UWL), lateral wall length (A-G, ULWL); (b) lower beak: hood length (A-B, LHL), crest length (A-E, LCL), rostral length (A-C, LRL), rostral width (C-D, LRW), wing length (D-F, LWL), and baseline length (F-G, LBL).

rameters to be estimated and σ^2 is the variance for the normally distributed random errors ϵ .

The beak morphological variables were then standardized using the following formula, derived from (1):

$$\ln(y/aUHL^b) \quad (2)$$

For the approach described above, theoretically the variance of standardized beak morphological variables within the group was not larger than the variance of the original morphological variables (Pineda *et al.* 2002, Vega *et al.* 2002). The standardized morphological variables were represented by adding a lower case letter “s” after each variable, i.e., LHLs, UCLs, LCLs, URLs, LRLs, URWs, LRWs, UWLs, LWLs, ULWLs and LBLs.

Finally, a stepwise discriminant analysis was performed to select the beak morphological variables that were significant ($P < 0.05$; Rencher 2002). In order to test potential differences among these four species and between males and females for *O. bartramii*, *D. gigas* and *I. argentinus* (excluding *S. oualaniensis* because of the small number of females), a linear discriminant analysis was carried out using the selected beak morphological variables (Rencher 2002). Errors in group classification were estimated using the resubstituti-

tion method and cross validation (Jackknife) method (Lachenbruch and Mickey 1968). An unweighted pair group mean analysis (UPGMA) phenogram was derived from the Mahalanobis distance matrix (Mahalanobis 1936) of beak morphological variables among the four Ommastrephidae (Sneath and Sokal 1973). All statistical analyses were conducted with the SAS (Version 9.1.3)

RESULTS

Data standardization

The 11 beak morphological variables for four species were fitted with UHL using the allometric model separately. The parameters *a* and *b* were estimated (Table 2). The beak morphological variables for interspecific and intraspecific (sexual dimorphism) identifications were standardized with the allometric model.

Identification of Ommastrephid squid

A total of 11 variables were selected using the stepwise discriminant analysis to identify the four Ommastrephid squid (Table 3). Wilks’ λ was estimat-

TABLE 2. – The coefficients of the allometric growth models for the four Ommastrephid squid.

Variables	<i>O. bartramii</i>		<i>D. gigas</i>		<i>S. oualaniensis</i>		<i>I. argentinus</i>	
	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>
UCL	1.450	0.953	1.444	0.955	1.226	1.010	1.481	0.945
URL	0.465	0.890	0.311	1.041	0.298	1.030	0.216	1.108
URW	0.274	0.982	0.327	0.989	0.221	1.066	0.253	0.947
ULWL	1.220	0.943	1.131	0.974	0.883	1.060	1.048	0.999
UWL	0.332	0.959	0.250	1.021	0.280	1.024	0.343	0.952
LHL	0.492	0.858	0.294	0.974	0.670	0.717	0.418	0.898
LCL	0.807	0.915	0.705	0.945	0.797	0.896	0.729	0.943
LRL	0.355	0.926	0.380	0.958	0.249	1.078	0.330	0.930
LRW	0.402	0.878	0.366	0.963	0.306	1.007	0.323	0.914
LWL	0.599	0.957	0.595	0.933	0.485	1.015	0.545	1.023
LBL	0.854	0.962	0.893	0.950	0.764	0.968	0.510	1.125

TABLE 3. – Stepwise discriminant analysis of beak morphological variables for the four Ommastraphidae.

Step	Entered characters	Partial F Value	P value of F	Overall Wilks' λ	P value of Wilks' λ
1	URW _S	1746.05	<0.0001	0.264	<0.0001
2	URL _S	767.01	<0.0001	0.121	<0.0001
3	LWL _S	594.32	<0.0001	0.063	<0.0001
4	LHL _S	385.12	<0.0001	0.037	<0.0001
5	LRL _S	158.15	<0.0001	0.031	<0.0001
6	LBL _S	99.26	<0.0001	0.026	<0.0001
7	ULWL _S	115.83	<0.0001	0.023	<0.0001
8	UCL _S	90.73	<0.0001	0.021	<0.0001
9	LRW _S	49.63	<0.0001	0.018	<0.0001
10	LCL _S	35.61	<0.0001	0.017	<0.0001
11	UWL _S	4.75	<0.0001	0.016	<0.0001

ed from the stepwise discriminant analysis to have a value of 0.016 ($p < 0.0001$). The canonical correlation analysis was used to derive the first three canonical variables (CV1, CV2 and CV3) with correlation coefficients of 0.935, 0.810 and 0.751 respectively. These canonical variables could explain 69.40%, 17.81% and 12.77% of the variations in the original data respectively (Fig. 3).

Based on the results from the linear discriminant functions (Table 4), all correct identification percentages of the four Ommastraphidae were above 97% using both the resubstitution and cross validation methods (Table 5), and their average error rates were 1.25% and 1.45% respectively. Misclassification mainly occurred between *O. bartramii* and *I. argentinus* as *O. bartramii* was occasionally misclassified as *I. argentinus* and vice versa (Table 5).

The Mahalanobis distance matrix of beak morphological variables estimated for the four squid indicated that there were significant differences among the four squid ($P < 0.0001$; Table 6). The nearest distance of 19.46 was found to be between *O. bartramii* and *I. argentinus* and the largest distance of 56.58 was between *S. oualaniensis* and *I. argentinus* (Table 6).

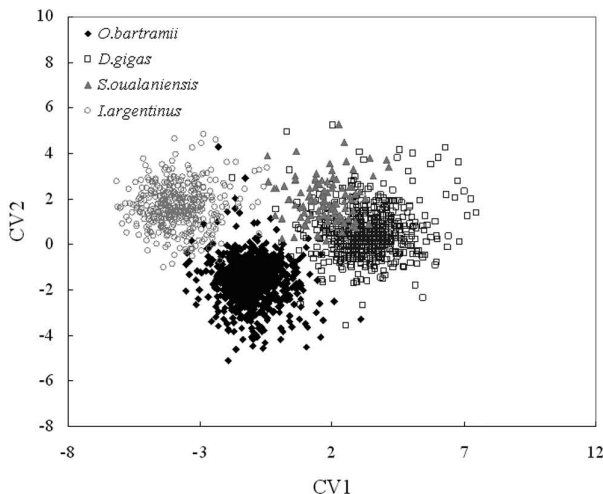


TABLE 4. – Coefficients of linear discriminant functions of beak morphological variables for the four Ommastraphidae.

Variables	<i>O. bartramii</i>	<i>D. gigas</i>	<i>S. oualaniensis</i>	<i>I. argentinus</i>
UCL _S	-15.12	32.46	-33.97	-192.40
URL _S	123.12	227.30	132.45	196.61
URW _S	141.65	94.85	147.80	214.69
ULWL _S	211.02	195.12	80.60	214.71
UWL _S	-14.93	40.61	109.10	-13.47
LHL _S	45.27	40.18	-6.35	63.10
LCL _S	46.62	63.11	106.06	73.89
LRL _S	197.61	167.81	195.40	177.80
LRW _S	57.03	134.80	105.94	47.98
LWL _S	67.26	43.38	21.92	67.70
LBL _S	15.40	2.34	22.39	11.70
Constant	-266.01	-286.04	-287.05	-303.85

Intraspecific identification

O. bartramii

URL_S, ULWL_S, LHL_S, LRL_S, UWL_S, LBL_S, URW_S and LWL_S were used in turn in the stepwise discriminant analysis (Table 7). The total Wilks' λ was 0.368 ($p < 0.0001$), suggesting a high rate of correct identification. CV1 explained almost 100% of the variation in the data and had a correlation coefficient of 0.790 (Fig. 4).

When linear discriminant functions were used, the rates for misidentifying males and females were 11.1% and 7.9% respectively, and the average misclassification rate was 9.5% for the resubstitution method. The misclassification rates were 11.3% and 8.4% respectively, for males and females when the cross validation method was used (Table 8).

D. gigas

The stepwise discriminant analysis indicated that seven morphological variables, LHL_S, UWL_S, LCL_S, LWL_S, LRL_S, ULWL_S and LBL_S could describe

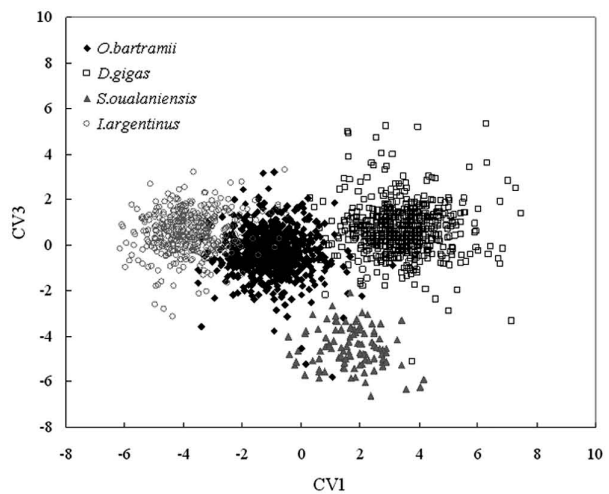


FIG. 3. – Plot of canonical variables (CV1, CV2 and CV3) of beak morphological variables of *O. bartramii*, *D. gigas*, *S. oualaniensis* and *I. argentinus*.

TABLE 5. – The percentage of correct species classification of the four Ommastrephidae using the discriminant analysis method.

Error estimation Method	Species	Classified species			
		<i>O. bartramii</i>	<i>D. gigas</i>	<i>S. oualaniensis</i>	<i>I. argentinus</i>
Resubstitution	<i>O. bartramii</i>	97.8%	0.4%	0.4%	1.4%
	<i>D. gigas</i>	0.8%	98.2%	0.7%	0.3%
	<i>S. oualaniensis</i>	0%	0%	100%	0%
	<i>I. argentinus</i>	1.0%	0%	0%	99.0%
Cross validation	<i>O. bartramii</i>	97.6%	0.4%	0.5%	1.5%
	<i>D. gigas</i>	0.8%	97.8%	1.1%	0.3%
	<i>S. oualaniensis</i>	0%	0%	100%	0%
	<i>I. argentinus</i>	1.2%	0%	0%	98.8%

TABLE 6. – Mahalanobis distances of beak morphological variables between the four Ommastrephidae and their significance levels.

Comparisons	Mahalanobis distance	F value	P value
<i>O. bartramii</i> - <i>D. gigas</i>	24.49	763.06	<0.0001
<i>O. bartramii</i> - <i>S. oualaniensis</i>	38.65	316.68	<0.0001
<i>O. bartramii</i> - <i>I. argentinus</i>	19.46	465.53	<0.0001
<i>D. gigas</i> - <i>S. oualaniensis</i>	32.29	254.58	<0.0001
<i>D. gigas</i> - <i>I. argentinus</i>	53.20	1182.02	<0.0001
<i>S. oualaniensis</i> - <i>I. argentinus</i>	56.58	423.51	<0.0001

the beak features of *D. gigas* (Table 7), and the total Wilks' λ for these seven variables was 0.768 ($P < 0.01$). The canonical correlation analysis indicated that CV1 could explain almost 100% of the data variations and had the correlation coefficient of 0.478 ($P < 0.0001$). The distribution of male and female squid on CV1 was partially overlapped, but could still be identified approximately (Fig. 5).

When linear discriminant functions were used, the misclassification rate for male squid (52.6%) was higher than that for female squid (7.9%), and the average misclassification rate was 30.25% for the resubstitution method (Table 8). The misclassification rate for male squid (52.6%) was still higher than that for female squid (8.8%), and the average misclassification was 30.7% for the cross validation method (Table 8).

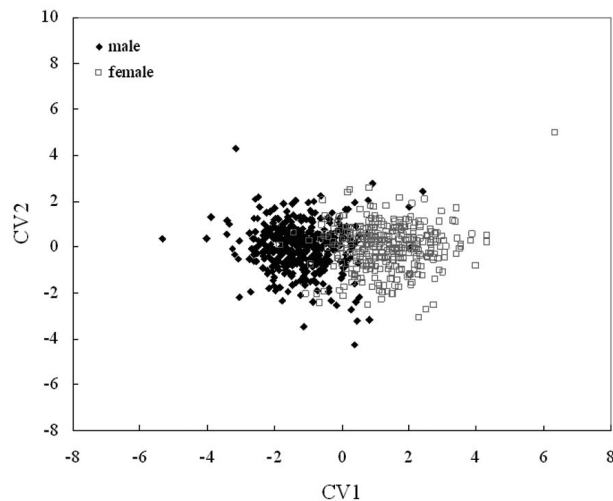


FIG. 4. – Plot of canonical variables (CV1 vs. CV2) for male and female beak morphological variables of *O. bartramii*.

TABLE 7. – Coefficients of linear discriminant functions of beak morphological variables for the intraspecific identification.

Variables	<i>I. argentinus</i>		<i>D. gigas</i>		<i>O. bartramii</i>	
	Males	Females	Males	Females	Males	Females
URL _s	36.8	45.2	-	-	272.4	342.2
ULWL _s	128.2	142.1	179.3	169.0	119.1	94.9
LHL _s	-	-	99.6	118.1	361.3	314.8
LRL _s	41.9	14.1	28.3	47.4	119.2	164.5
UWL _s	-	-	41.9	14.1	173.7	141.6
LBL _s	28.3	47.4	69.4	74.4	60.6	77.6
URW _s	-	-	-	-	117.6	100.5
LWL _s	-	-	-	-	169.1	160.5
LCL _s	99.6	118.1	36.8	45.2	-	-
LWL _s	-	-	128.2	142.1	-	-
UCL _s	179.3	169.0	-	-	-	-
Constant	-168.9	-177.3	168.9	177.3	272.7	-264.1

I. argentinus

Stepwise discriminant analysis indicated that six morphometric variables, LCL_s, LRW_s, URL_s, ULWL_s, LBL_s and UCL_s, could effectively identify differences between the sexes for *I. argentinus* (Table 7), and the total Wilks' λ was 0.392 ($P < 0.0001$). Canonical correlation analysis showed that CV1 could explain almost 100% of the variation in data with a correlation coefficient of 0.775 ($P < 0.001$).

The misclassification rates for males and females with the resubstitution method were 10.5% and 12.2% respectively, with an average misclassification rate of 11.35% (Table 8). The misclassification rates estimated using the cross validation method were the same as those derived using the resubstitution method (Table 8).

DISCUSSION

It is considered difficult to identify cephalopods based on their beaks (Xavier *et al.* 2007). However, this study obtained a low misclassification rate for the four Ommastrephid squid, as it was only 1.25% with the resubstitution method and 1.45% with the cross validation method. The correct classification rate estimated using the resubstitution and cross validation methods reached more than 97%, suggesting that there was a great difference in beak morphology among the four squid, and thus beak morphology could be used to identify them. Therefore, we recommend that beak

TABLE 8. – The percentage of correct intraspecific classification for *O. bartramii* using the linear discriminant function.

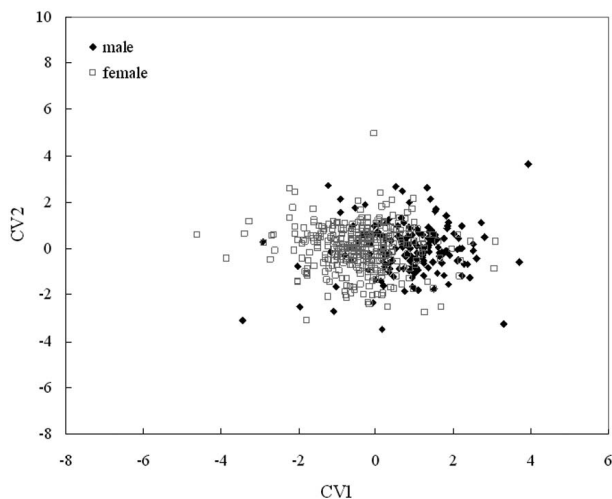
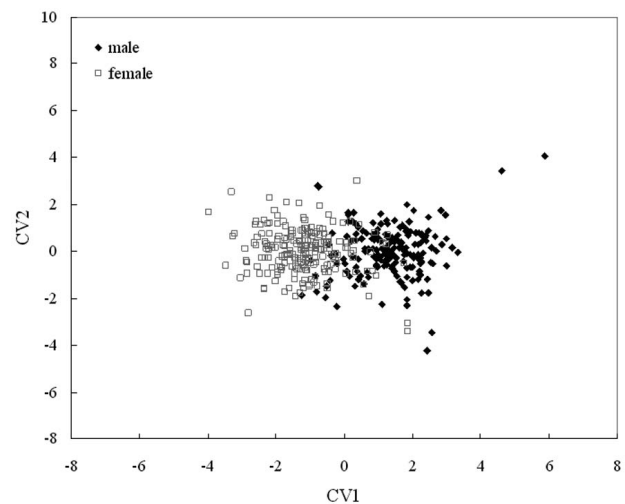
Method	Sex	<i>O. bartramii</i> Classified sex		<i>D. gigas</i> Classified sex		<i>I. argentinus</i> Classified sex	
		Female	Male	Female	Male	Female	Male
Resubstitution	Female	88.9%	11.1%	92.1%	7.9%	89.5%	10.5%
	Male	7.9%	92.1%	52.6%	47.4%	12.2%	87.8%
Cross validation	Female	8.4%	91.6%	52.6%	47.4%	12.2%	87.8%
	Male	88.9%	11.1%	92.1%	7.9%	89.5%	10.5%

morphological variables should be standardized using the approach we used in this study prior to being used in species classification.

Based on the results from the stepwise discriminant analysis, URW_S and URL_S showed the greatest interspecific variation in the beak morphological variables of the four Ommastrephidae, suggesting that there are significant differences in beak width and length. In previous studies, URL and lower rostrum length (LRL) were mainly used in beak length analyses (Jackson and McKinnon 1996, Jackson *et al.* 1997, Gröger *et al.* 2000, Santos and Haimovici 2000, Lu and Ickerlingill 2002, Cherel *et al.* 2004). Therefore, URL is an important length measurement in beak morphology. The four squid all belong to the family Ommastrephidae, but *I. argentinus* belongs to the genus *Illex* of sub-family *Illicinae*. The other squid belong to the sub-family Ommastrephinae and come from different genera: *O. bartramii* from the genus *Ommastrephes*, *D. gigas* from the genus *Dosidicus*, and *S. oualaniensis* from the genus *Sthenoteuthis*. In the paralarval phase of the cephalopod, the protrusion of rostral tips suggests changes in prey type, feeding mode and behavior (O'Dor *et al.* 1985, Vidal and Haimovici 1998, Uchikawa *et al.* 2009). Such differences in feeding ecology can result in different beak morphologies for different species since the cephalopod beak is primarily a feeding tool. Future studies need to evaluate the relationship between beak variation and feeding modes in different life history stages, including juveniles

Clarke and Maddock (1988) suggested that the beak shape might be related to phylogenetic affinities. A detailed comparison between phylogenetic analysis and morphometric analysis is, however, out of the scope of this study. Our results obtained from the distance matrix of beak morphological variables are not consistent with the conclusions made by Yokawa (1994). This indicates that the beak morphometric information may not yield consistent results with allozyme analyses of cephalopods. However, more studies with more samples are needed to further evaluate the consistency of studies with different methods, including morphometrics, life history, and genetic analysis.

Several studies have revealed that there is sexual dimorphism in cephalopods (Pineda *et al.* 2002, Vega *et al.* 2002, Bolstad 2006). Using intraspecific discrimination, our study also found sexual dimorphism in the beaks of three Ommastrephid squid (*O. bartramii*, *D. gigas* and *I. argentinus*). However, Martínez *et al.* (2002) suggested that *I. argentinus* did not have sexual dimorphism in either body or beak morphology. The evaluation of sexual dimorphism may also be influenced by the choice of beak morphological variables and the data analysis methods used. The standardization of beak morphological variables used in the present study reduced the impacts of size effectively. If the 11 variables had not been standardized, LHL would have been the only variable selected for discrimination for *I. argentinus* (total Wilks' λ 0.935). Thus the data standardization used in this study could be one of the

FIG. 5. – Plot of canonical variables (CV1 vs. CV2) for male and female beak morphological variables of *D. gigas*.FIG. 6. – Plot of canonical variables (CV1 vs. CV2) for male and female beak morphological variables of *I. argentinus*.

reasons behind the difference in the results obtained in this study and those of previous studies.

Simultaneous sampling was recommended for reducing the size effect in morphometric studies (Pierce *et al.* 1994, Vega *et al.* 2002). Biases in sampling might result in a lower intraspecific morphometric variation than the true variation existing in nature, and thus the error rate in the discriminating process would be underestimated (Yatsu *et al.* 1997), in particular for widely distributed and rapidly growing species like cephalopods. Previous studies indicated that the ratio between morphological variables could reduce the size effect and yield shape information (Martínez *et al.* 2002, Vega *et al.* 2002). This study sampled 1895 individuals in total, and the sampling period of *O. bartramii* and *D. gigas* lasted for four months. More studies with large samples with wide size ranges are needed to identify factors leading to this discrepancy with different studies.

Since Ommastrephid squid play a key role in their marine environments, both as predators and preys for top predators, determining and quantifying their trophic relationships are key issues for understanding the structure and functioning of marine ecosystems. The allometric regression models between beak size versus mantle length and body weight of cephalopods can yield estimates of cephalopod biomass (Lu and Ickerignill 2002). Other biological attributes of beaks, such as pigment deposits (Ivanovic and Brunetti 1997, Hernandez-García *et al.* 1998), rings (Hernández-López *et al.* 2001) and stable isotopes (Cherel and Hobson 2005), can also help improve our understanding of cephalopod life history and ecology. For example, the stable isotopic signatures of beaks found in predators' stomachs can be used to determine trophic relationships and migration patterns, and thus are a powerful tool for investigating the role played by poorly known cephalopods in the marine environment (Hobson and Cherel 2006, Xavier *et al.* 2007). The results derived from this study can be used to identify the four Ommastrephidae species and estimate the biomass of the species identified to be consumed by a given predator. A similar approach can also be applied to distinguish other cephalopod species and estimate their biomass based on beak morphological variables (Clarke 1986, Gröger *et al.* 2000).

In conclusion, beak morphological variables are a convenient tool for providing reliable information for identifying Ommastrephidae to genus level. The standardization method for beak morphological variables used in this study extracted shape information effectively. However, more studies are needed to compare the results for identifying species using beak morphological variables and those obtained using other methods, including genetic analysis.

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