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Short communication

Beak length analysis for the identification of two morphologically similar species common names *Uroteuthis edulis* (Hoyle, 1885) and *Uroteuthis duvaucelii* (D'Orbigny, 1835) (Cephalopoda: Loliginidae) in the southern East China Sea

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Introduction

Beaks are a hot spot of cephalopod research as they offer alternate methods for identification (Lu and Ickeringill, 2002). This method becomes useful and available during identification of cephalopods specimen; it allows an objective method to identify cephalopod specimen beyond the family, genus and even specie level (Xavier and Cherel, 2009). By regressing certain beak parameters such as rostral length (RL) against mantle length (ML) or body weight (BW), it also can be possible to estimate the size of individuals and biomass of cephalopods that have been preyed on by many oceanic predators (Jackson, 1995; Gröger *et al*., 2000).

Uroteuthis edulis and *Uroteuthis duvaucelii* are the 2 primary species from the family Loliginidae in the southern East China Sea (ESC) waters (Zhu *et al*., 2014). These 2 species are morphological similar and difficult to distinguish from each other without visual assistance (Jereb *et al*., 2010). Little taxonomic work has been done on either species. This study provided beak length parameters for *U. edulis* and *U. duvaucelii* collected from southern ECS waters and described a biometric method of separating 2 closely related species from beak dimensions. The relationship between both ML and BW and a selected beak dimension were also presented for these 2 species.

Material and methods

U. edulis and *U. duvauceli* were collected in May, September and December 2015 using bottom trawls from the fishery research vessels. Collection locations were in waters of the southern ECS between latitudes 26°30'N and 29'30'N and longitudes 121°00'E and 127°00'E. The basis of distinguishing these 2 species is the teeth of the tentacle central sucker rings.

Measurements used for both species are upper and lower rostral length (URL, LRL), upper and lower hood length (UHL, LHL), upper and lower crest length (UCL, LCL), upper and lower wing length (UWL, LWL) and upper and lower wing width (UWW, LWW) (Clarke, 1977; Ivanovic and Brunetti, 1997) (Fig. 1). A stepwise discriminant analysis was carried out to select the beak morphological variables that identify the 2 species using the beak ratios as a variable (Chen *et al*., 2012). The discriminant analysis was calculated using SPSS 19.0.

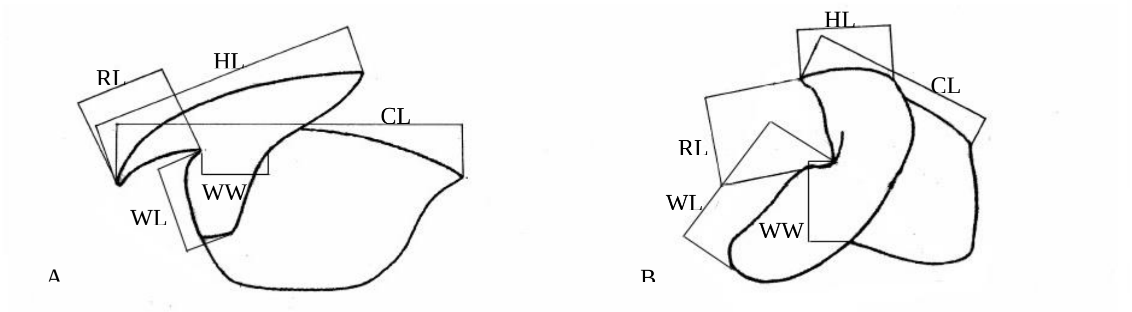

Figure 1: Beak morphology and measurements of upper beak (A) and lower beak (B) (RL: rostrum length, HL: hood length, CL: crest length, WL: wing length, WW: wing width).

Results and discussion

Beak length analysis

A total of 89 specimens of *U. edulis* and 68 specimens of *U. duvaucelii* were analyzed for the beak morphometric description. The beak morphological parameters of *U. edulis* and *U. duvaucelii* were presented in Table 1. All the mean values of measurement parameters of *U. edulis* were about 1.3 times larger than those of *U. duvauceli.*

Table 1: Beak morphological parameters of *Uroteuthis edulis* and *Uroteuthis duvaucelii*.

Parameters	*U. edulis*		*U. duvauceli*	
	Range/mm	Mean(S.D)/mm	Range/mm	Mean(S.D)/mm
URL	1.39-4.31	2.70±0.60	1.47-2.30	1.91±0.23
UHL	5.98-18.40	11.45±2.18	6.05-9.65	8.11±0.83
UCL	8.58-25.79	15.58±3.01	8.72-13.57	11.49±1.16
UWL	2.02-6.80	4.04±0.86	2.08-4.01	2.97±0.40
UWW	1.73-5.45	3.08±0.62	1.54-3.17	2.42±0.33
LRL	1.37-4.61	2.60±0.56	1.54-2.16	1.87±0.17
LHL	2.16-6.21	4.07±0.76	2.43-4.18	3.22±0.40
LCL	5.07-13.86	8.97±1.69	4.94-7.56	6.10±0.65
LWL	3.21-12.65	6.98±1.69	3.78-6.54	5.31±0.63
LWW	1.60-4.96	3.08±0.62	1.72-3.43	2.64±0.35

Relationships between RL and ML

The relationship between URL, LRL and ML for both *U. edulis* and *U. duvaucelii* were linear, when using the nature logarithms of RL and ML for larger r^2 values. The regression equations for *U. edulis* were ln ML= 0.97 ln URL +4.02, r^2=0.79, n=89 and ln ML=1.01 ln LRL + 4.02, r^2=0.80, n=89. The regression equations for *U. duvaucelii* were ln ML=0.58 ln URL+3.99, r^2=0.72, n=68 and ln ML =0.80 ln LRL + 3.87, r^2=0.78, n=68.

Relationships between RL and BW

The linear model also provides the best adjustment for the relationships between URL, LRL and BW, when using nature logarithms of RL and BW. Other analysis using raw data of RL and BW, resulted in curvilinear relationships with lower r^2 values. The regression equations for *U. edulis* were ln BW=2.22 ln URL+2.65, r^2=0.82, n=89 and ln BW=2.29 ln LRL+2.67, r^2 =0.82, n=89. The regression equations for *U. duvauceli* were ln BW=1.54 ln URL+2.39, r^2=0.66, n=68 and ln BW=2.23 ln LRL+2.04, r^2=0.78, n=68.

U. edulis and *U. duvauceli* play an important role in the marine ecosystem, both as predators and prey (Zhu *et al*. 2014). There is a need for a greater understanding and quantifying their tropic relationships in ECS waters. As the regressions between RL versus ML and BW were linear and had high correlation coefficients; thus this data provided here describes a method for determining the size and weight of a cephalopod and estimating cephalopod biomass from beaks found in the stomach contents of their predators (Clarke *et al*., 2002).

Beak identification

Stability of beak ratios

The data presented here indicated a trend that the beak length indices increased with the ML. However, the beaks ratios seemed to be very stable, and were not observably different despite the changes of the ML. Using t-test, beak ratio pairs were compared between the species. Significant differences (p<0.05) were found between 5 upper beak ratios and 6 lower beak ratios (Table 2).

Table 2: Beak ratios and interspecific difference between *Uroteuthis edulis* and *Uroteuthis duvauceli*.

Ratios	Upper beak			Lower beak		
	U. edulis	***U. duvauceli***	***p* value**	***U.edulis***	***U. duvauceli***	***p* value**
RL/HL	0.24±0.02	0.24±0.02	0.74	0.64±0.07	0.58±0.005	0.13
RL/CL	0.17±0.01	0.17±0.02	0.90	0.29±0.02	0.31±0.02	0.48
RL/WL	0.67±0.07	0.65±0.07	0.72	0.38±0.05	0.35±0.03	0.08
RL/WW	0.88±0.15	0.80±0.10	0.00	0.85±0.13	0.71±0.07	0.00
HL/CL	0.73±0.02	0.71±0.01	0.02	0.45±0.03	0.53±0.03	0.79
HL/WL	2.86±0.20	2.74±0.18	0.42	0.59±0.07	0.61±0.04	0.02
HL/WW	3.72±0.36	3.37±0.19	0.00	1.33±0.13	1.22±0.08	0.00
CL/WL	3.89±0.25	3.88±0.25	0.73	1.30±0.13	1.15±0.06	0.01
CL/WW	5.07±0.55	4.77±0.30	0.00	2.94±0.30	2.32±0.15	0.00
WL/WW	1.31±0.15	1.23±0.09	0.00	2.27±0.29	2.02±0.11	0.00

Stepwise discriminant analysis

Based on the results from the stability analysis and the t-test procedure, a total of 5 upper beak ratios and 6 lower beak ratios were used as variables to identify the 2 loliginid family squids in a stepwise discriminant analysis. Wilks' Lambda was estimated from the stepwise discriminant analysis to have a value of 0.478 (p<0.0001); suggesting a high rate of correct identification (Table 3).

The linear discriminant functions of the upper beak length ratios for *U. edulis* and *U. duvaucelii* were Y=3825.86 UHL/UCL+198.81 UCL/UWW–460.01 URL/UWW–1708.70 and Y=3678.24 UHL/UCL+ 191.92 UCL/UWW–447.46 URL/UWW–1578.92, respectively. The linear discriminant functions of the lower beak length ratios for *U. edulis* and *U. duvauceli* were Y= 642.75LHL/LWW+4471.89 LCL/LWL +2144.64 LWL/LWW-64.20 LRL/LWW-1573.11 LRL/LWL-1949.77 LCL/LWW– 2420.11 and Y = 518.21 LHL/LWW+4261.07 LCL/LWL +2111.69 LWL/LWW–47.30 LRL/LWW–1256.15 LRL/LWL–1878.26 LCL/LWW–2323.00, respectively.

Table 3: The percentage of correct species classification of the two loliginids using the stepwise discriminant analysis.

Beaks	Error estimation method	Species	Species		Total	Accuracy
			U.edulis	*U. duvauceli*		
Upper beak	Resubstitution	*U. edulis*	81	8	89	91.00%
		U. duvaucelii	4	64	68	94.10%
	Cross-validation	*U. edulis*	79	10	89	88.80%
		U. duvaucelii	6	62	68	91.20%
Lower beak	Resubstitution	*U. edulis*	86	3	89	96.5 %
		U. duvaucelii	2	66	68	97.0 %
	Cross-validation	*U. edulis*	85	4	89	95.5 %
		U. duvaucelii	4	64	68	94.1 %

Stepwise discriminant analysis provides a method for identifying these 2 morphologically similar species. The high correct classification rates for the 2 Loliginid squids estimated demonstrated the success of this approach. Based on its stability, beak ratios for species determination appear to be the frequently-used and accurate method for future development of beak identification.

Many existing studies had demonstrated that there were geographical variations in morphometric characteristics of cephalopod species with either disjunction or widespread distributions (Wolff, 1982; Liu *et al*., 2015 a or b). *U. edulis* and *U. duvauceli* are widely distributed, including but not limited to the ECS. Compared with the specimens collected from the fish market (Liu *et al*., 2015 a or b), the survey data used in this paper is more practical and restricted to the southern ECS. The beak lengths described for the species identification could be assumed to be regional. Despite some limitations, the regression equations and linear discriminant functions that we give here will be essential and helpful in further ecological studies.

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