



# Morphological, molecular, and ecological evidence in population determination and fishery management of purpleback flying squid *Sthenoteuthis oualaniensis* in the South China Sea

Chunxu ZHAO<sup>1,2</sup>, Bin KANG<sup>3,\*</sup>, Xiongbo HE<sup>2</sup>, Yunrong YAN<sup>2,4</sup>

1. Fishery College, Jimei University, Xiamen 361021, China.

2. Marine Resources Big Data Center of South China Sea, Southern Marine Science and Engineering Guangdong Laboratory, Zhanjiang 524013, China.

3 Fisheries College, Ocean University of China, Qingdao 266003, China.

4 Fisheries College, Guangdong Ocean University, Zhanjiang 524088, China.

\*Corresponding author's Tel: +86-13012425395; E-mail: bkangfish@163.com

(Manuscript received 13 May 2020; Accepted 8 May 2021; Online published 13 May 2021)

**ABSTRACT:** Purpleback flying squid *Sthenoteuthis oualaniensis* has great potential to be an important fishery in the South China Sea, while its complex population structure causes difficulties for fishery management. In this study, specimens were sampled monthly throughout 2018 and identified at the population scale from the aspects of morphological, molecular, and ecological traits. Corresponding to the appearance of the photophore, the purpleback flying squid could be divided into two populations as the medium population and the dwarf population, also indicated by significant differences in mantle length. In the phylogenetic tree, all individuals fell into two lineages, and in each lineage, there was no sub-lineage corresponding to geological isolation or seasonal change. Fishes were the main food for purpleback flying squid, occupying approximately half of the total, followed by squid and crustaceans. Stable isotope analysis suggested that neither body size nor gender affected trophic niches. Spring and autumn were the main feeding periods of both the medium and the dwarf populations, followed by the spawning peak in winter and the second peak in summer. Despite the morphological differences at the population or even species scale, two populations of purpleback flying squid mixed in terms of habitat space, food selectivity, and developmental process, with no need for differentiated management.

**KEY WORDS:** Developmental stage, mantle length, photophore, phylogenetic, trophic niche.

## INTRODUCTION

Continuous increase in resource consumption has led to the loss of biodiversity and ecosystem services (Bullock *et al.*, 2011). Globally, fishermen catch more than 90 million tons of food fishes each year, accounting for more than 8% of the animal protein (FAO, 2014), and this value does not include unsold commodities (e.g., recreational fishing products). Under the threats of overfishing and climate change, managing fisheries utilization in a sustainable way is a large challenge. Species is an integral part of the ecosystem and bring unexpected benefits to humans in many ways (Gascon *et al.*, 2015). Because different species play different roles on the planet, it is important to clarify the species or population in an area to improve the use and management of resources (Martin *et al.*, 2016).

Traditional species classification methods are based on the morphological characteristics of the individual (Cronquist, 1978). However, these methods cannot distinguish species that have evolved from convergence but are not truly closely related and individuals who differ slightly in morphology (such as color or size) but belong to the same species (Scoville, 2020). Therefore, it is more accurate to use behavioral and molecular evidence to determine species, e.g., biological species are populations of actual or potential hybrid natural

populations reproductively separated from other populations, except for asexually reproduced species (Mayr, 1942; Hey *et al.*, 2005; Mallet, 2020). These differences are reflected in the phylogenetic tree, forming new lineages from a common ancestor species and discovering the secret of species life history and evolutionary time on Earth (Scoville, 2020). Considering ecosystem operation, more attention is given to the functional role of species in ecosystems using species biological and behavioral characteristics as important evidence for species identification, leading to the discovery of many cryptic species (Bickford *et al.*, 2007). For example, the degree of ecological interchangeability of certain taxa that lack obvious morphology and extensive gene transfer may be a decisive taxonomic feature that distinguishes closely related species (Leaché *et al.*, 2009), such as bacteria (Cohan, 2006; Fraser *et al.*, 2009). Criteria for selecting traits were that they could be applied to show the differentiation in habitat heterogeneity, food utilization, and population expansion, such as diet, body size, and reproduction (Costello *et al.* 2015).

Purpleback flying squid *Sthenoteuthis oualaniensis* (Lesson, 1830) belongs to the family Ommastrephidae, Teuthoidea, Coleoidea, Mollusca; the species enjoys tropical and temperate waters and is distributed in the Indian Ocean and the Western Pacific (Dunning, 1998).



Purpleback flying squid was classified into four different populations (Nesis, 1993): large-sized population that occurs only in the Red Sea, the Gulf of Aden, and the Arabian Sea in the northern Indian Ocean (mantle length is generally 400–500 mm with a maximum of 650 mm); medium-sized population that is recognized as the "typical" type and widely distributed throughout the species range (mantle lengths of the mature male and female are 190–250 mm and 120–150 mm, respectively); small-sized population that is similar to the medium population, but the body length of the sexually mature individual is smaller than that of the medium population (the range of mantle length in mature female is mostly 120–140 mm); and dwarf population that appears in the waters near the equator (mantle lengths of the mature male and female are 90–120 mm and 90–100 mm, respectively), with the appearance of photophore. In the South China Sea, purpleback flying squid could be divided into two populations based on the differences in the length of mantle and arms (Zhu *et al.*, 2019), while three geological populations were identified according to the morphological characters of otolith (Li *et al.*, 2019a). Furthermore, samplings from the same area in the Bashi Channel and the South China Sea showed no significant intraspecific differences, suggesting the effects from environments rather than phylogenetic (Wang *et al.*, 2019). However, a genetic analysis based on mitochondrial ND2, COI, and 16S rDNA showed the middle-sized population and the dwarf population could be distinguished as two species, supported by significant differences beyond the request for interspecies differentiation (Li *et al.*, 2019b).

In the South China Sea (SCS), there are two types of phenotypic populations (medium population and dwarf population) in the purpleback flying squid. However, the photophore is not obvious in the early developmental stage of the individual; thus, the two stemmed wrists, gonads, and inner shell were suggested to distinguish the differences of species at the initial developmental stage (Liu, 2006). Besides, morphological analysis showed significant differences between the two populations (Zhu *et al.*, 2019) and even among several populations from different geographical locations (Yan *et al.*, 2015). Molecular evidence showed that the difference between the two populations was significant at the species level (Li *et al.*, 2019b), while not enough genetic data was adopted to explain the population structure. Besides, the spawning time in different sea areas was used to divide purpleback flying squid into corresponding seasonal populations (Okutani and Tung, 1978).

Purpleback flying squid is characterized by a short life cycle, high fertility and natural mortality, high growth rate and yield (Zuyev *et al.*, 2002), forming a complex intraspecies structure. Generally, the biomass of purpleback flying squid is determined by acoustic survey and was estimated to be 8–11.2 million tons in

the SCS (Jereb and Roper, 2010), including approximately 2.44 million tons of available catch (Yang *et al.*, 2013). With the continuous technical development of acoustic evaluation, the result is expected to be more and more accurate. Based on 1998–2001 survey data, the biomass in the SCS was considered to be 1.13 million tons (Siriraksophon, 2001), and this data was increased to 2.05 million tons by Kringing method in 2001–2003 survey data (Feng *et al.*, 2014). According to the results of a recent survey, the amount of available catch was expected to be more than 5 million tons (Liao, 2019), indicating that purple squid in the SCS has great potential to be an important fishery. Purpleback flying squid shows obvious diel vertical movement, perching in water layer deeper than 300 m in the daytime and floating on the surface to feed at night. Due to the high metabolic and growth rate, purpleback flying squid feeds on a large amount of food, including most fish, shellfish, and other mollusks; meanwhile, purpleback flying squid is the main food source for many large predators (Olson and Young, 2007). As a vital link in the food web, purpleback flying squid plays an important role in the ecosystem (Fang *et al.*, 2015). Though studies of purpleback flying squid covered morphology, biology, genetics, and resources, the results remained controversial, in species identification (Li *et al.*, 2019b), population structure (Li *et al.*, 2019a; Zhu *et al.*, 2019), and trophic niche (Huang *et al.*, 2019). In this study, we collected samples monthly for a year and measured all the specimens from the aspects of morphological traits, phylogenetic relationships, and trophic function. We attempted to identify morphological differentiation, molecular linkage, and ecological traits cluster of purpleback flying squid in the SCS. On top of that, the population structure of purpleback flying squid in the SCS was discriminated against, supporting effective management and sustainable fisheries.

## MATERIALS AND METHODS

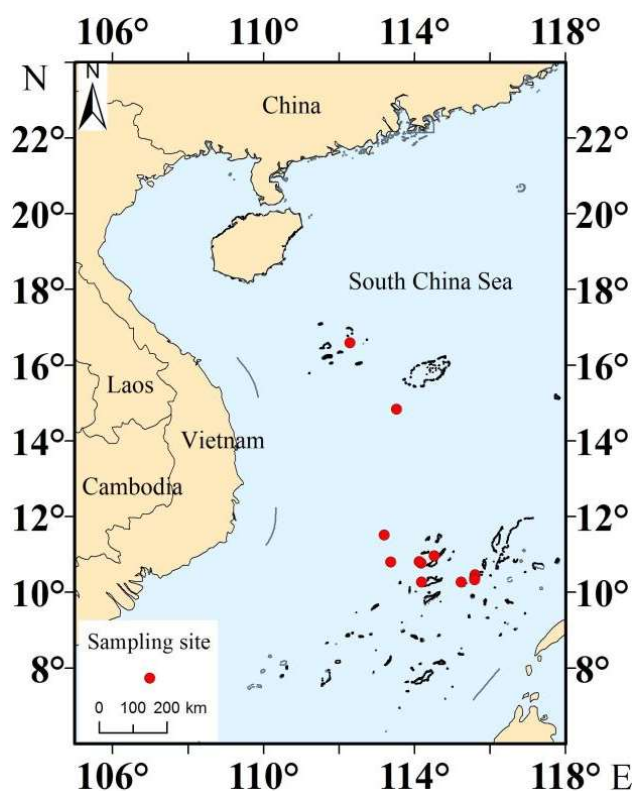
### *Sampling collection*

A total of 12 locations were taken at random each month in 2018 in the area between latitude 10.27°N to 16.59°N and longitude 112.28°E to 115.59°E in purpleback flying squid (Fig. 1). The net fishing boat was 55 m long and 622 kw host power, equipped with a net with 90 m height and 300 m outlet circumference and a total of 700 fish-collection lights (each at 1 kw). In total 300–400 lights were turned on for attracting squid from 7 pm to 10 pm, and an hour later at 11 pm, the nets were cast and then hauled out (Table 1).

In total, 2143 individuals were collected and divided into four populations: females with photophores (FPs), females without photophores (FnPs), males with photophores (MPs), and males without photophores (MnPs). Sex determination was based on the appearance

**Table 1.** Detailed information of sampling sites.

Sampling time	Longitude (°E)	Latitude (°N)	Depth (m)
Jan. 22, 2018	113.46	14.95	2779.66
Feb. 23, 2019	114.52	10.97	946.18
Mar. 14, 2018	113.19	11.51	4298.99
Apr. 04, 2018	115.23	10.27	1021.70
May 04, 2018	115.60	10.46	1392.78
June 3, 2018	114.17	10.77	1490.70
July 5, 2018	115.59	10.38	1392.78
Aug. 16, 2018	114.18	10.27	1502.88
Sep. 06, 2018	114.12	10.81	1490.70
Oct. 04, 2018	112.28	16.59	633.69
Nov. 02, 2018	115.59	10.33	1392.78
Dec. 06, 2018	113.37	10.80	4145.76

**Fig.1.** Sampling sites of purpleback flying squid *Sthenoteuthis oualaniensis* in the South China Sea in 2018.

of suckers in the hectocotylized arm for mature individuals, or the reproductive structures in the mantle cavity for immature individuals (Carpenter and Niem, 1998). Gonad maturity was adopted to be the criteria for dividing the developmental stage into I-V, as I, *immature* (the female nidamental gland and the male spermatophoric organ are semi-transparent); II, *maturing* (the female nidamental gland is white with a granular surface, and the male spermatophoric organ has a few granules and testis is milk-white); III, *mature*, the female ovary is full of eggs, and the volume of male testis has increased; IV, *late mature*, the surfaces of the female oviduct and the male white testis turn red, and the female

eggs become larger; V, *spawning*, genitals are undergoing atrophy (State Oceanic Administration, 2006).

### Morphological analysis

To test the morphological evidence in identifying the samplings, 14 morphological traits, namely, mantle length (ML), mantle width (MW), head width (HW), right arm I length (RAL1), right arm II length (RAL2), right arm III length (RAL3), right arm IV length (RAL4), left arm I length (LAL1), left arm II length (LAL2), left arm III length (LAL3), left arm IV length (LAL4), right tentacle length (RTL), left tentacle length (LTL), and fin length (FL) were measured. Except for mantle length, the remaining 13 traits were transformed into percentages by dividing by mantle length for effective comparison. First, we used the 13 traits to test the different populations in the SCS corresponding to body shape or geological distribution using cluster analysis. Second, the growth equations of the four populations were determined as:

$$W = aL^b$$

where  $W$  is the body weight,  $L$  is the mantle length (Froese, 2006). To test whether two populations were corresponding to the appearance of the photophore, the mantle length and power coefficient  $b$  were used to test the differences among the four populations by student t-test. To further describe the differences in the developmental process, mantle length (mean value and standard deviation, maximum and minimum values) of samplings at different stages was measured. A statistical t-test was used to test the difference between paired populations from different sexuality or developmental stages.

### Molecular analysis

A total of 114 specimens of *Sthenoteuthis oualaniensis* were randomly selected for molecular analysis. The sampling locations were examined in detail, with latitude and longitude, sample sizes for analysis, haplotypes identified. Genomic DNA was extracted from the muscle tissue in the dorsal mantle using the Animal Genomic DNA Extraction Kit (Sangon Biotech, Shanghai, China) according to the manufacturer's recommended protocol. The partial COI gene was amplified by polymerase chain reaction (PCR) using the primers SthenoF (5'-CCATAAAGACATTGGTACTC-3') and SthenoR (5'-ATAAACTTCTGGGTGACC-3') (Xu *et al.*, 2017). Each 25  $\mu$ l PCR mixture contained 5 ng of template DNA, 5  $\mu$ l of 10x reaction buffer, 4  $\mu$ l of dNTP mix (10 mM), 5 pmol of each primer, and 2 U of Taq polymerase (TaKaRa, Taq polymerase). The PCR conditions for amplification were as follows: one cycle of denaturation at 94°C for 5 min, 40 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s and extension at 72°C for 1 min 30 s, followed by a 72°C extension for 10 min and 4°C for storage. The purified PCR products were sequenced using an ABI 377



automated sequencer (Applied Biosystems, Foster City, CA). All nucleotide sequences were deposited in GenBank (accession numbers: MW183552-MW183666). The partial COI genes of the mtDNA sequences were aligned in the program CLUSTALX v1.81 (Thompson *et al.*, 1997) and then optimized manually. The selection of the best-fit nucleotide substitution models was performed using the Bayesian information criterion (BIC) by the Smart Model Selection (SMS) procedure in PhyML (Lefort *et al.*, 2017), and the most appropriate nucleotide substitution model was HKY85. The phylogenetic relationships among all individuals were inferred using neighbor-joining (NJ) and maximum likelihood (ML) in MEGA 7.0 (Kumar *et al.*, 2016) and PhyML 3.0 (Guindon *et al.*, 2010). The ML analysis was used with the GTR+I model of substitution for the nucleotide dataset. Bootstrap resampling was carried out using the rapid option with 1,000 iterations and branch support was assessed with 100 pseudo replicates. For NJ analysis, we chose Kimura 2-parameter model, and bootstrapping was performed within 1000 replications. The values for the time to the most recent common ancestor (TMRCA) were determined using the software BEAST v1.8.2 (Drummond *et al.*, 2012). In this study, a mutation rate of 0.7% to 2.4% per million years has been calibrated for the mtDNA genes in *S. oualaniensis* for molecular dating (Staaaf *et al.*, 2010).

### Ecological analysis

To effectively represent the food utilization and population reproduction, traits as trophic niche and gonad repletion index were adopted. The trophic niche of purpleback flying squid was calculated by combining stomach content analysis and stable isotope analysis. As an annual species, the gonadal stage and repletion index RI (representing the percentage of food weight in eviscerated body weight (Yin, 1995) at each month were determined. In total, 664 purpleback flying squid individuals were analyzed, including 279 from dwarf group and 385 from medium group. Gastric dissection was performed on all the 2143 individuals, and the residual foods were identified to taxonomic category. For stable isotope analysis, three individuals were randomly selected from each ML group with 10mm interval at each station, and a total of 442 individuals were collected. The abdominal muscle of each purpleback flying squid was sampled and stored in a freeze dryer for 48 h, and then ground into powder for determination of carbon and nitrogen stable isotope composition using an Elemental Analyzer (FLASH2000, Thermo Scientific, Germany) and isotope mass spectrometer (Delta V, Thermo Scientific, Germany), as:

$$\delta^{15}\text{N}(\text{or } \delta^{13}\text{C}) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000\text{‰}$$

where  $R_{\text{sample}}$  is the measured isotope value, and

$R_{\text{standard}}$  is the isotopic value of the standard material (Vander Zanden and Fetzer, 2007). The standard material PDB (Blendrite) was used for the determination of carbon stable isotopes, and standard nitrogen was used for the determination of nitrogen stable isotopes. Seven indicators concerning the ecological niche were determined, including  $\delta^{13}\text{C}$  range (CR, distance between the two species with the most enriched and most depleted  $\delta^{13}\text{C}$  values, i.e., maximum  $\delta^{13}\text{C}$  - minimum  $\delta^{13}\text{C}$ ) and  $\delta^{15}\text{N}$  Range (NR, distance between the two species with the most enriched and most depleted  $\delta^{15}\text{N}$  values, i.e., maximum  $\delta^{15}\text{N}$  - minimum  $\delta^{15}\text{N}$ ), both providing niche diversification at the base of a food web; mean distance to centroid (CD, average Euclidean distance of each species to the  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  centroid), providing a measure of the average degree of trophic diversity within a food web; mean nearest neighbor distance (MNND, mean of the Euclidean distances to each species' nearest neighbor in bi-plot space) and standard deviation of nearest neighbor distance (SDNND, a measure of the evenness of species packing in bi-plot space), measuring the overall density of species packing and divergence of trophic niche; total area of trophic niche (TA, convex hull area encompassed by all species in  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  bi-plot space) and standard ellipse area (SEA, the standard ellipse describing  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ), representing the niche space occupied by target species (Layman *et al.*, 2007; Jackson *et al.*, 2011). A student t-test was used to test the difference between paired populations from different sexuality or developmental stages.

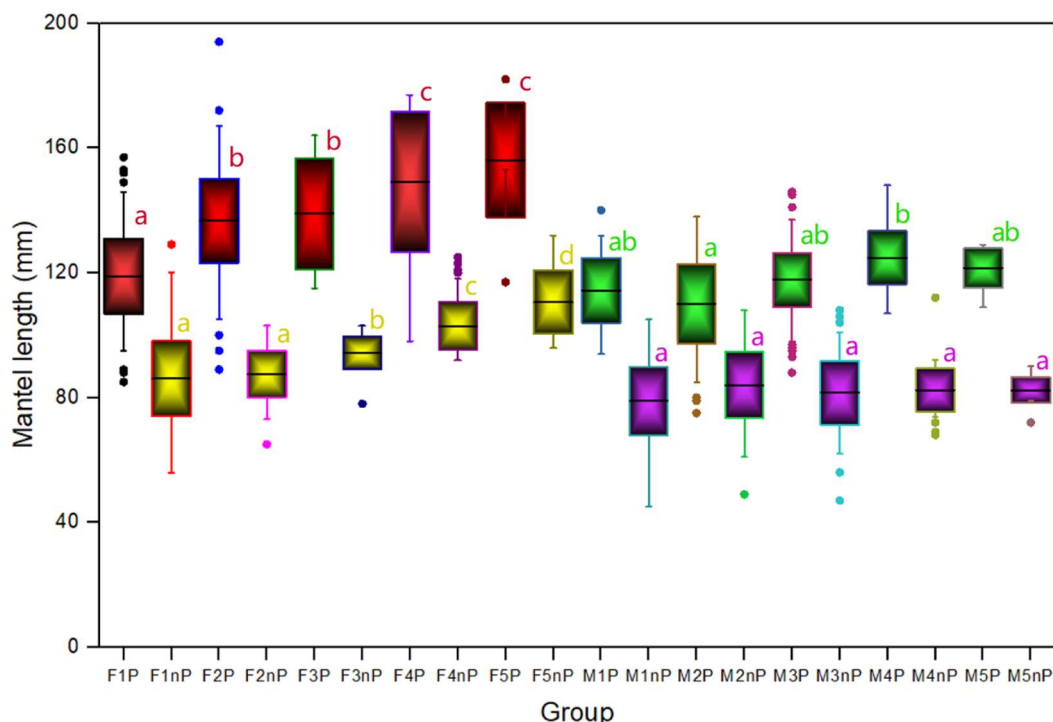
## RESULTS

### Morphological population

Based on the dissimilarity of ratios of 13 morphological traits to the mantle length, samplings characterized by the presence - absence of photophores could be divided into two populations by cluster analysis. However, the sexual difference did not significantly influence morphological traits ( $p > 0.05$ ), except both the right and left tentacle length in percentage to the mantle length (Table 2).

Considering the mantle length, significant differences existed not only between female and male populations but also between photophore and non-photophore populations. The growth efficiency  $b$  showed significant differences existed between different sexual populations, but not in populations with or without photophores (Table 3). In females,  $b$  was greater than 3, showing positive allometric growth, while in males  $b$  was close to 3 at a constant growth rate.

To further test the influence at the developmental stage, we divided all the samples into 20 populations (Fig. 2). Among all the samplings, male samplings with photophores at stage 3 accounted for 17.62% of the total samplings, followed by female samplings with photophores



**Fig. 2.** Mantle length of *Sthenoteuthis oualaniensis* of four groups at different developmental stages, including mean value, standard deviation, minimum and maximum values, and outliers. The digit in group name refers to developmental stage, e.g., F1P as FP in gonad stage 1. Red square: female with photophore; Green square: male with photophore; Yellow square: female without photophore; Purple square: male without photophore. Different letters meant significant differences among samplings at different developmental stages in each group.

**Table 2.** Measurements of 14 morphological traits of purpleback flying squid from females with photophores (FP), females without photophores (FnP), males with photophores (MP), males without photophores (MnP). Different letters meant significant differences among groups.

Morphological traits	FP	MP	FnP	MnP
Mantle length (ML, mm)	128.59 <sup>a</sup>	91.6 <sup>b</sup>	119.02 <sup>c</sup>	81.52 <sup>d</sup>
Mantle width/ML	0.32 <sup>a</sup>	0.31 <sup>a</sup>	0.31 <sup>a</sup>	0.31 <sup>a</sup>
Head width/ML	0.24 <sup>a</sup>	0.22 <sup>ab</sup>	0.19 <sup>b</sup>	0.18 <sup>b</sup>
Right arm I length/ML	0.42 <sup>a</sup>	0.39 <sup>b</sup>	0.37 <sup>b</sup>	0.36 <sup>b</sup>
Right arm II length/ML	0.49 <sup>a</sup>	0.47 <sup>ab</sup>	0.46 <sup>ab</sup>	0.44 <sup>b</sup>
Right arm III length/ML	0.54 <sup>a</sup>	0.52 <sup>ab</sup>	0.49 <sup>bc</sup>	0.48 <sup>c</sup>
Right arm IV length/ML	0.48 <sup>a</sup>	0.50 <sup>a</sup>	0.41 <sup>b</sup>	0.38 <sup>b</sup>
Left arm I length/ML	0.41 <sup>a</sup>	0.39 <sup>a</sup>	0.37 <sup>ab</sup>	0.35 <sup>b</sup>
Left arm II length/ML	0.50 <sup>a</sup>	0.48 <sup>ab</sup>	0.45 <sup>b</sup>	0.44 <sup>b</sup>
Left arm III length/ML	0.55 <sup>a</sup>	0.53 <sup>ab</sup>	0.50 <sup>bc</sup>	0.48 <sup>c</sup>
Left arm IV length/ML	0.48 <sup>a</sup>	0.46 <sup>a</sup>	0.42 <sup>b</sup>	0.40 <sup>b</sup>
Right tentacle length/ML	1.33 <sup>a</sup>	1.24 <sup>b</sup>	1.16 <sup>c</sup>	1.09 <sup>d</sup>
Left tentacle length/ML	1.37 <sup>a</sup>	1.27 <sup>b</sup>	1.18 <sup>c</sup>	1.11 <sup>d</sup>
Fin length/ML	0.87 <sup>a</sup>	0.84 <sup>a</sup>	0.73 <sup>b</sup>	0.69 <sup>b</sup>

**Table 3.** Descriptive statistical information of mantle length in four groups of *Sthenoteuthis oualaniensis*, as well as the growth efficiency. Different letters meant significant differences among groups.

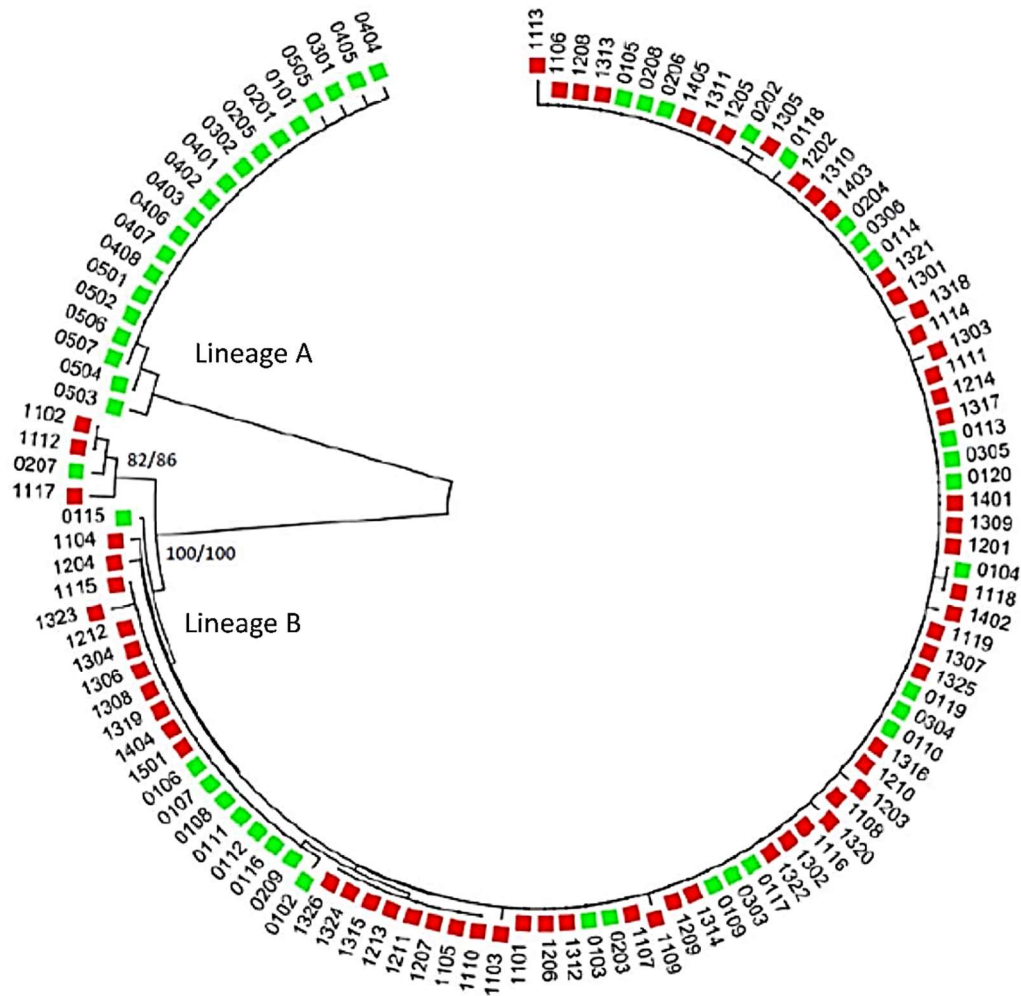
Group	Number	Mean±SD (mm)	Max (mm)	Min (mm)	<i>b</i>
FP	659	128.6±16.62 <sup>a</sup>	194 <sup>a</sup>	85 <sup>a</sup>	3.504 <sup>a</sup>
MP	728	119±10.23 <sup>b</sup>	148 <sup>b</sup>	75 <sup>b</sup>	3.009 <sup>b</sup>
FnP	374	91.61±13.04 <sup>c</sup>	132 <sup>c</sup>	56 <sup>c</sup>	3.479 <sup>a</sup>
MnP	382	81.52±10.18 <sup>d</sup>	112 <sup>d</sup>	45 <sup>d</sup>	2.857 <sup>c</sup>

at stage 2 at 13.85% of the total. The mantle length of the population with photophores was significantly higher than that of the populations without photophores at all developmental stages ( $p < 0.05$ ). In FP, there were three gradients in increasing mantle length, like stage 1, stages 2 and 3, and stages 4 and 5; in FnP, mantle length in stages 1 and 2 were similar and then increased at each stage. The mantle length in FP was significantly higher than that in FnP ( $p < 0.01$ ), even the mantle length at stage 1 in FP was higher than that at stage 5 in FnP. In contrast, no significant differences in mantle length were found among different stages in MP, as was that in MnP ( $p > 0.05$ ) (Fig. 2). Except for the regular trends in mantle length at different stages, there were some outlier values in each stage, and the overlapping of mantle length at different stages showed a greater variation in growth of purpleback flying squid.

#### Molecular population

In the phylogenetic analyses, the trees reconstructed with different methods (NJ and ML) were identical. In the phylogenetic tree, all individuals fell into two lineages, A and B (Fig. 3). Lineage A included 7 haplotypes in 20 individuals, all without photophores belonging to dwarf populations. The mantle length of individuals in this lineage was at an average of 101.3 mm between 65 and 132 mm. In lineage B there were 22 haplotypes in 95 individuals, 65 individuals belonged to





**Fig. 3.** Neighbor-joining (NJ) tree of genetic relationships among 114 individuals in *Sthenoteuthis oualaniensis* in the South China Sea using the mitochondrial COI region. The values above the branches are the posterior probabilities for bootstrap values for the NJ and ML analysis. The species code was composed of 4 digits: the first was 1 with photophore or 0 without photophore; the second showed the developmental stage; the last two digits refer to the species number. The red represented individuals with photophore; the green represented individuals without photophore.

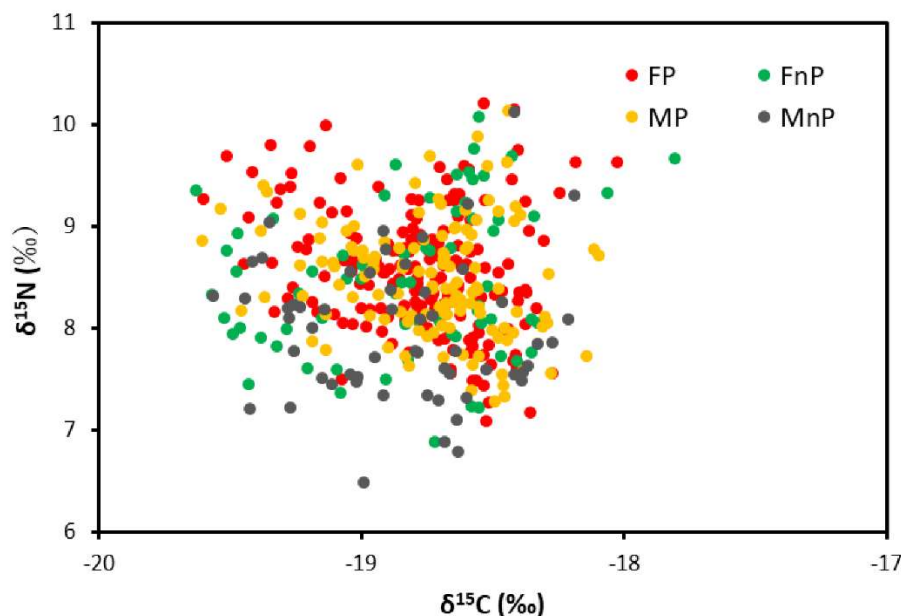
the medium-sized populations with photophores, with an average mantle length of 118.5 mm between 95 and 170 mm. The remaining 30 individuals in lineage B without photophores were the smallest with an average mantle length of 92.1 mm, all in immature at developmental stages 1 and 2 in which the photophores were not yet developed. In the BEAST analyses, the time to coalescence was estimated to be 1.92 – 6.62 myr in *S. oualaniensis*.

### Ecological population

In the stomach of purpleback flying squid, the remaining foods were identified including fishes, mollusks, and crustaceans, and then counted. Fishes were the main food, occupying 55.5% in total weight. In terms of food composition, fishes were detected in 969 of 1397 individuals with photophores and 221 of 756 individuals without photophores, followed by squid in 727 individuals with photophores and 121 individuals without photophore

The medium group mainly fed on fishes (especially species from Myctophidae), followed by cephalopods (cephalopods were detected in stomachs of 100 percent of samples with ML greater than 160mm, and this value decreased to 60 percent in the samples with ML less than 160mm). Besides, some small individuals from medium group also consumed crustaceans. The dwarf population mainly feeds on fish, followed by crustaceans appearing in 20% of the samples. Cephalopods were rarely detected in the samples.

The female population without photophore occupied the largest area of the trophic area in all the samplings. The standard niche area of the population without photophore was higher than those in the population with photophore, and sexuality did no effects (Table 4). Gender showed significant effects of the  $\delta^{15}\text{N}$  range, and body size was responsible for the variation of  $\delta^{13}\text{C}$ . Regardless of the remarkable variations of the niche



**Fig. 4.** Biplots of stable isotopes  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in four groups of *Sthenoteuthis oualaniensis* in the South China Sea in 2018. All the values mixed without significance between all paired groups. FP, female with photophore; FnP, female without photophore; MP, male with photophore; MnP, male without photophore. The  $\delta^{13}\text{C}$  values (mean $\pm$ standard deviation) were  $-18.79\pm 0.33$ ,  $-18.83\pm 0.40$ ,  $-18.75\pm 0.29$  and  $-18.86\pm 0.35$  for FP, FnP, MP and MnP respectively. The  $\delta^{15}\text{N}$  values (mean $\pm$ standard deviation) were  $8.55\pm 0.61$ ,  $8.48\pm 0.73$ ,  $8.49\pm 0.56$  and  $7.98\pm 0.68$  for FP, FnP, MP and MnP respectively.

**Table 4.** Trophic niche characters of purpleback flying squid in different groups under stable isotope analysis. Different letters meant significant differences among groups. CR, distance between the two species with the most enriched and most depleted  $\delta^{13}\text{C}$  values, i.e., maximum  $\delta^{13}\text{C}$  - minimum  $\delta^{13}\text{C}$ ; NR, distance between the two species with the most enriched and most depleted  $\delta^{15}\text{N}$  values, i.e., maximum  $\delta^{15}\text{N}$  - minimum  $\delta^{15}\text{N}$ ; CD, average Euclidean distance of each species to the  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  centroid; MNND, mean of the Euclidean distances to each species' nearest neighbor in bi-plot space; SDNND, a measure of the evenness of species packing in bi-plot space; TA, convex hull area encompassed by all species in  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  bi-plot space; SEA, the standard ellipse describing  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

Indicator	FP	MP	FnP	MnP
CR	1.58 <sup>a</sup>	1.51 <sup>a</sup>	1.82 <sup>b</sup>	1.38 <sup>c</sup>
NR	3.12 <sup>a</sup>	2.85 <sup>b</sup>	3.19 <sup>a</sup>	3.64 <sup>c</sup>
CD	0.565 <sup>a</sup>	0.545 <sup>a</sup>	0.734 <sup>b</sup>	0.664 <sup>c</sup>
MNND	0.065 <sup>a</sup>	0.075 <sup>a</sup>	0.129 <sup>b</sup>	0.137 <sup>b</sup>
SDNND	0.054 <sup>a</sup>	0.058 <sup>b</sup>	0.094 <sup>b</sup>	0.138 <sup>c</sup>
SEA	0.53 <sup>a</sup>	0.511 <sup>a</sup>	0.896 <sup>b</sup>	0.758 <sup>b</sup>
TA	3.38 <sup>a</sup>	2.82 <sup>b</sup>	3.67 <sup>c</sup>	3.27 <sup>a</sup>

indicators, the mean  $\delta^{13}\text{C}$  of the four populations varied from  $-19.03$  to  $-18.76$ ; the mean  $\delta^{15}\text{N}$  of the four populations varied from  $7.98$  to  $8.62$ ; both showed no significant differences between all paired populations ( $p > 0.05$ ), suggesting that neither body size nor gender affected the results (Fig. 4).

Totally 866 individuals were females at stages 1–2 and 753 individuals were males at stages 3–4. In the monthly gonadal stage, there were two peaks in stage 5 and stage 3–4 respectively at March–May and October–

November except for only one peak at stage 5 at March–May in FP. Considering the request for enough food preparation for spawning, the RI peak was suggested to be a month before the spawning peak in September–October, and the second peak was in March–May (Fig. 5).

## DISCUSSION

Since the suggestion of dividing purpleback flying squid into large-, medium-, small- and dwarf-sized populations were proposed, discussions on the population structure of purpleback flying squid have not stopped. For example, the large individual was recognized as a morphological plastic phenotype of medium-sized species (Snyder, 1998); additionally, dwarf populations without photophores were speculated to be independent species different from the medium-sized population (Clarke, 1965; Young, 1975). In the SCS, purpleback flying squid was traditionally considered to contain medium and dwarf populations (Liu, 2006; Huang *et al.*, 2019; Zhu *et al.*, 2019). In this study, despite the overlapping mantle lengths, the external variables, including head, carcass, and arms, supported the separation of purpleback flying squid into two populations as medium and dwarf populations, corresponding to the appearance of photophores on the back. This result suggested that the existence of photophores could be effectively adopted as a criterion for the identification of different populations in purpleback flying squid. Further analysis showed the

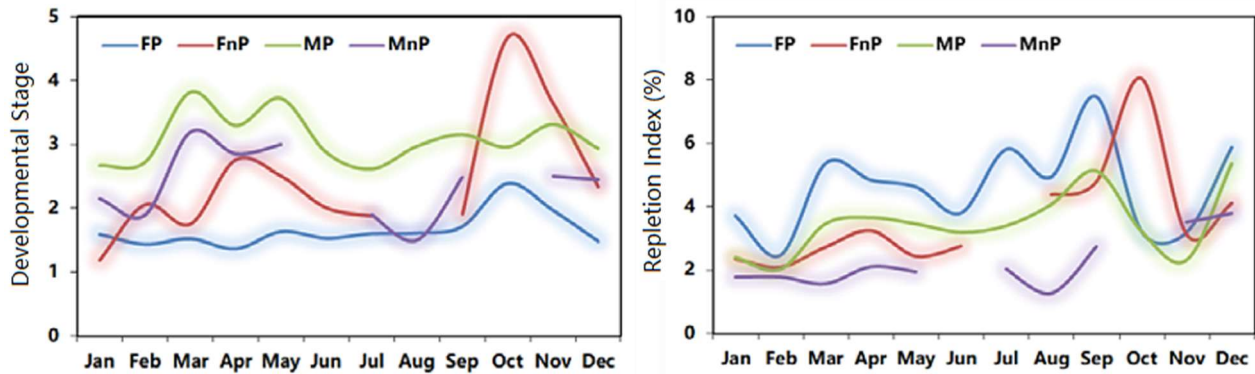


Fig. 5. Monthly variations in developmental stage (left) and repletion index RI (right) in four groups of *Sthenoteuthis oualaniensis* in the South China Sea in 2018. FP, female with photophore; FnP, female without photophore; MP, male with photophore; MnP, male without photophore.

sexual difference in each population. Actually, from the morphological aspect, the presence/absence of an organ can even be considered credible evidence to distinguish individuals at the species level (Aldhebiani, 2018). The shortcoming is that if the organ is developed with growth, it will cause mistakes in identifying species at the initial stage (Kishimoto and Kohno, 1992; Bower *et al.*, 1999).

As a widely distributed oceanic species, purpleback flying squid has a very complex population structure (Nesis, 1993). In this study, genomic DNA was used to map the population genetic structure: one lineage was composed of dwarf individuals; the other lineage was mixed by individuals with or without photophores. To be mentioned, in the latter lineage the individuals without photophores were all immature, and it was most likely that photophores had not yet developed (Jereb and Roper, 2010). Therefore, it can be inferred that in the SCS, *S. oualaniensis* could be divided into two populations as the medium population and the dwarf population, corresponding to the presence of photophores. Additionally, in each lineage, there was no sub-lineages corresponding to geological or seasonal differences. Xu *et al.* (2020) suggested that *S. oualaniensis* with different morphs varying in size at maturity contains two species, a dwarf species and a medium-sized species, separated by molecular markers. These results are consistent with our study, *S. oualaniensis* at maturity can distinguish into two populations as medium and dwarf populations, corresponding to the appearance of photophores on the back, but immature individuals cannot. This may be due to the fact that the photophores is not obvious or the photophores is not generated in the medium group before the stage II (Gonad maturity is immature).

Purpleback flying squids play an important role in ecosystems as a key species by connecting predators and prey. Purpleback flying squids consume large amounts of food to support their high growth rate, e.g., consuming 8–10% of body weight in food composition every day (Shulman *et al.*, 1992). Along with growth, purpleback flying squid showed a diet shift from crustaceans and

squid to fishes, e.g., purpleback flying squid with mantle length 40–100 mm mainly fed on crustaceans and substituted by myctophids and other squids in individuals with mantle length larger than 150 mm (Shchetinnikov, 1992). Meanwhile, the large quantities of purpleback flying squid make it a main component of food for high trophic level predators such as mammals, seabirds, and large fishes, e.g., purpleback flying squid accounted for 26% of the food of sperm whale in the Taiwan strait (Laurs and Lynn, 1991); purpleback flying squid was found in the stomachs of 34–97% of the total seabirds in the mid-Pacific equator (Ashmole and Ashmole, 1967).

There were significant differences among all the four groups at some indicators reflecting trophic niche, however, no congruent tendency of the seven indicators could be concluded. Though these indicators showed significant changes, the mean values of between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  showed no significant differences among the four populations, suggesting that from the ecological aspect, the medium and dwarf populations were indistinguishable with the same diet selectivity. In contrast, the feeding intensity showed monthly variations in the four populations. Spring and autumn were the main feeding periods, indicating preparation for the spawning peak in winter and the second peak in summer, which was different from other studies that suggested only one peak in July–August (Jiang *et al.*, 2019). Interestingly, in this study, mature individuals were sampled every month, suggesting no time break in spawning for purpleback flying squid, which was verified in most species of cephalopods (Rocha *et al.*, 2001).

Purpleback flying squid is recommended as a potential species for resource exploitation in the SCS and can sustain intensive fishing activities (Liao, 2019). Correspondingly, effective management is needed based on the understanding of population structure to support sustainable exploitation. In this study, the purpleback flying squid could be divided into two populations as medium and dwarf populations, and this result could be





supported by both morphologic and molecular evidence. In contrast, the difference between the two populations was not supported by ecological evidence, as the feeding habits and developmental process of both populations combined. Despite the differences in the population or even species scale, two populations of purpleback flying squid were mixed in habitat space, food selectivity and developmental process, with no need to treat resource management in different ways. Considering the two reproductive peaks and differentiated food composition between the two populations, the time and quantity of catch require careful plans.

## ACKNOWLEDGMENTS

This study was supported by National Programme on Global Change and Air-Sea Interaction (GASI-02-SCS-YDsum), Southern Marine Science and Engineering Guangdong Laboratory (Zhanjiang) [Zhanjiang Bay Laboratory ZJW-2019-08] and National Key R&D Program of China [Grant/Award Number: 2018YFD0900905].

## LITERATURE CITED

- Aldhebiani, A.Y.** 2018. Species concept and speciation. *Saudi J. Biol. Sci.* **25**(3): 437–440.
- Ashmole, N.P. and M.J. Ashmole.** 1967. Comparative feeding ecology of tropical seabirds. *Bull. Am. Mus. Nat. Hist.* **24**: 191–198.
- Bickford, D., D.J. Lohman, N.S. Sodhi, P.K. Ng, R. Meier, K. Winker, K.K. Ingram and I. Das.** 2007. Cryptic species as a window on diversity and conservation. *Trends Ecol. Evol.* **22**(3): 148–155.
- Bower, J.R., M.P. Seki, R.E. Young, K.A. Bigelow, J. Hirota and P. Flament.** 1999. Cephalopod paralarvae assemblages in Hawaiian Islands waters. *Mar. Ecol. Prog. Ser.* **185**: 203–212.
- Bullock, J.M., A. Aronson, A.C. Newton, R.F. Pywell and J.M. Rey-Benayas.** 2011. Restoration of ecosystem services and biodiversity: conflicts and opportunities. *Trends Ecol. Evol.* **26**(10): 541–549.
- Carpenter, K.E. and V.H. Niem.** 1998. FAO species identification guide for fisheries purposes. The living marine resources of the Western Central Pacific. Volume 2. Cephalopods, crustaceans, holothurians and sharks. Rome, FAO. pp. 687–1396.
- Clarke, M.R.** 1965. Large light organs on the dorsal surface of the squids *Ommastrephes pteropus*, '*Symplectoteuthis oualaniensis*' and '*Dosidicus gigas*'. *J. Molluscan Stud.* **36**(5): 319–321.
- Cohan, F.M.** 2006. Towards a conceptual and operational union of bacterial systematics, ecology, and evolution. *Phil. Trans. R. Soc. B.* **361**(1475): 1985–1996.
- Cronquist, A.** 1978. Once again, what is a species? In: L.V. Knutson (Ed.), *Biosystematics in Agriculture*, Allenheld Osmun, Montclair, New Jersey, U.S.A. pp. 3–20.
- Dunning, M.C.** 1998. A review of the systematics, distribution, and biology of the arrow squid genera *Ommastrephes* Orbnigny, 1835, *Sthenoteuthis* Verrill, 1880, and *Ornithoteuthis* Okada, 1927 (Cephalopoda: Ommastrephidae). *Smithson Contrib. Zool.* **586**: 425–434.
- Fang, Z., L. Xu, X. Chen, B. Liu, J. Li and Y. Chen.** 2015. Beak growth pattern of purpleback flying squid *Sthenoteuthis oualaniensis* in the eastern tropical Pacific equatorial waters. *Fish Sci.* **81**(3): 443–452.
- FAO.** 2014. The State of World Fisheries and Aquaculture: Opportunities and Challenges. (Rome: FAO).
- Feng, B., Yan, Y.R., Zhang, Y.M., Yi, M.R. and H.S. Lu.** 2014. A new method to access the population of *Sthenoteuthis oualaniensis* in South China Sea. *Progress in Fishery Sciences* **35**(4): 1–6.
- Fraser, C., E.J. Alm, M.F. Polz, B.G. Spratt and W.P. Hanage.** 2009. The bacterial species challenge: making sense of genetic and ecological diversity. *Science* **323**(5915): 741–746.
- Froese, R.** 2006. Cube law, condition factor and weight–length relationships: history, meta-analysis and recommendations. *J. Appl. Ichthyol.* **22**(4): 241–253.
- Gascon, C., T.M. Brooks, T. Contreras-MacBeath, N. Heard, W. Konstant, J. Lamoreux, F. Launay, M. Maunder, R.A. Mittermeier, S. Molur, R.K.A. Mubarak, M.J. Parr, A.G.J. Rhodin, A.B. Rylands, P. Soorae, J.G. Sanderson and J.C. Vié.** 2015. The importance and benefits of species. *Curr. Biol.* **25**(10): R431–R438.
- Guindon, S., J.F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk and O. Gascuel.** 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst. Biol.* **59**(3): 307–321.
- Hey, J., W.M. Fitch and F.J. Ayala.** 2005. Systematics and the origin of species: an introduction. *Proc. Natl. Acad. Sci. USA* **102**(Suppl 1): 6515–6519.
- Huang, J.X., Y.Y. Gong, S.N. Xu, Z.Z. Chen, J. Zhang and W.M. Yu.** 2019. Trophic niche of medium-form and dwarf-form of purple flying squid *Sthenoteuthis oualaniensis* in the central and western South China Sea. *Chinese J. Appl. Ecol.* **30**(8): 2822–2828.
- Jackson, A.L., R. Inger, A.C. Parnell and S. Bearhop.** 2011. Comparing isotopic niche widths among and within communities: SIBER—stable isotope Bayesian ellipses in R. *J. Anim. Ecol.* **80**(3): 595–602.
- Jereb, P. and C.F. Roper.** 2010. Cephalopods of the world. An annotated and illustrated catalogue of cephalopod species known to date. Volume 2. Myopsid and Oegopsid Squids. FAO.
- Jiang, Y.E., Z.Z. Chen, Z.J. Lin, Y.S. Qiu, P. Zhang and Z.Q. Fang.** 2019. Comparison of fishery biology between medium-form and dwarf-form of *Sthenoteuthis oualaniensis* in South China Sea. *J. Fish. China* **43**: 454–466.
- Kishimoto, H. and H. Kohno.** 1992. Development of the luminous organ in the purpleback flying squid, *Sthenoteuthis oualaniensis*, as shown by alcian blue stain techniques. *Bull. Inst. Oceanic Res. & Develop.* **13**: 71–83.
- Kumar, S., G. Stecher and K. Tamura.** 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **33**(7): 1870–1874.
- Laurs, R.M. and R.J. Lynn.** 1991. Biology, oceanography, and fisheries of the North Pacific Transition Zone and subarctic frontal zone National Marine Fisheries Service, NOAA Technical Report NMF. North Pacific albacore ecology and oceanography 105, 69–87.
- Layman, C.A., D.A. Arrington, C.G. Montana and D.M. Post.** 2007. Can stable isotope ratios provide community-wide measures of trophic structure? *Ecology* **88**(1): 42–48.



- Leaché, A.D., M.S. Koo, C.L. Spencer, T.J. Papenfuss, R.N. Fisher and J.A. McGuire. 2009. Quantifying ecological, morphological, and genetic variation to delimit species in the coast horned lizard species complex (Phrynosoma). *Proc. Natl. Acad. Sci. USA* **106**(30): 12418–12423.
- Lefort, V., J.E. Longueville and O. Gascuel. 2017. SMS: smart model selection in PhyML. *Mol. Biol. Evol.* **34**(9): 2422–2424.
- Li, B., X.F. Yang, X.Y. Qiu, J.X. Wang, B.H. Zhou, J.Y. Xie and Y.R. Yan. 2019a. Discriminant Analysis of Purpleback Flying Squid (*Sthenoteuthis oualaniensis*) Groups Based on Statolith Morphology in the South China Sea. *J. Guangdong Ocean University* **39**(2): 58–66.
- Li, M., P. Zhang, J. Zhang, K. Zhang and Z.Z. Chen. 2019b. Genetic differentiation of the purpleback flying squid *Sthenoteuthis oualaniensis* in the South China Sea: population or species divergence. *J. Fish. Sci. China* **26**(1): 133–140.
- Liao, J. 2019. Resources survey helps orderly development of distant fishery science; purpleback squid catch up to 5 million tons in South China Sea. *Ocean and Fishery* **20**(4): 78–79.
- Liu, J.L. 2006. Identifying population structure in the northwestern Indian Ocean using morphological and RAPD analysis of the purpleback flying squid *Symplectoteuthis oualaniensis* (Master's Thesis). Shanghai: Shanghai Ocean University.
- Mallet, J. 2020. Alternative views of biological species: reproductively isolated units or genotypic clusters? *National Science Review* **7**(8): 1401–1407.
- Martin, J., Maris, V. and D.S. Simberloff. 2016. The need to respect nature and its limits challenges society and conservation science. *PNAS* **113**(22): 6105–6112.
- Mayr, E. 1942. Systematics and the origin of species. Columbia University Press. New York, 334.
- Nesis, K.N. 1993. Population structure of oceanic ommastrephids, with particular reference to *Sthenoteuthis oualaniensis*: a review. In: Okutani T, O'Dor RK, KUBODERA T (eds.) Recent advances in cephalopod fishery biology. Tokyo: Tokai University Press, 375–383.
- Okutani, T. and I.H. Tung. 1978. Reviews of biology of commercially important squids in Japanese and adjacent waters. I. *Symplectoteuthis oualaniensis* (Lesson). *The Veliger* **21**(1): 87–94.
- Olson, R.J. and J.W. Young. 2007. Role of squid in open ocean ecosystems: report of a GLOBEC-CLIoTOP/PFRP workshop, 16–17 November 2006, Honolulu, Hawaii, USA.
- Rocha, F., A. Guerra and Á.F. González. 2001. A review of reproductive strategies in cephalopods. *Biol. Rev.* **76**(3): 291–304.
- Scoville, H. 2020. "The Species Concept." <http://www.thoughtco.com/the-species-concept-1224709>.
- Shchetinnikov, A.S. 1992. Feeding spectrum of squid *Sthenoteuthis oualaniensis* (Oegopsida) in the eastern Pacific. *J. Mar. Biol. Ass. U.K.* **72**(4): 849–860.
- Shulman, G. E., G.I. Abolmasova and A.Y. Stolbov. 1992. On the utilization of proteins in energy metabolism of epipelagic squids of genus *Sthenoteuthis*. *Doklady Akademii Nauk* **325**: 630–632.
- Siriraksophon, S, Y. Nakamura and N. Sukramonkol. 2001. Exploration for the purpleback flying squid, *Sthenoteuthis oualaniensis* resources in the South China Sea. Southeast Asian Fish Dev Cent: Training Department **2001**: 1–81.
- Snyder, R. 1998. Aspects of the biology of the giant form of *Sthenoteuthis oualaniensis* Cephalopoda: Ommastrephidae) from the Arabian Sea. *J. Molluscan Stud.* **64**(1): 21–34.
- Staaf, D.J., R.I. Ruiz-Cooley, C. Elliger, Z. Lebaric, B. Campos, U. Markaida and W. F. Gilly. 2010. Ommastrephid squids *Sthenoteuthis oualaniensis* and *Dosidicus gigas* in the eastern Pacific show convergent biogeographic breaks but contrasting population structures. *Mar Ecol Prog Ser* **418**: 165–178.
- State Oceanic Administration. 2006. Specifications for Oceanographic Survey-Part 6: Marine Biological Survey (GB/T 12763.6-2007). Standards Press of China. Beijing, China, 85.
- Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin and D.G. Higgins. 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25**(24): 4876–4882.
- Wang, X.H., B.L. Liu, Y.S. Qiu, P. Zhang, F.Y. Du, X.J. Chen and H. Su. 2019. Regional and intraspecific variation in *Sthenoteuthis oualaniensis* (Cephalopoda: Ommastrephidae) morphology from the western central Pacific Ocean. *Bull. Mar. Sci.* **95**(1): 1–12.
- Xu, L., P. Liu, X. Wang, K. Van Damme and F. Du. 2020. Phylogenetic relationships and cryptic species in the genus *Sthenoteuthis* (Cephalopoda: Ommastrephidae) in the South China Sea. *Mol. Phylogenetics Evol.* **149**: 106846.
- Xu, L., Q. Huang, S. Xu, X. Wang, P. Zhang, L. Xu and F. Du. 2017. A new set of primers for COI amplification from purpleback flying squid (*Sthenoteuthis oualaniensis*). *Mitochondrial DNA Part B* **2**(2): 439–443.
- Yan, Y.R., M.R. Yi, B. Feng, C.X. Zhao, Z.H. Li and H.S. Lu. 2015. Morphological variations and discriminant analysis of three different geographic cohorts of *Sthenoteuthis oualaniensis* in the South China Sea. *J. Guangdong Ocean University* **35**(3): 43–50.
- Yang, Q., Y. Li, P. Zhang, Y. Tang, G. Chen and J. Zhang. 2013. Acoustic survey of the *Symplectoteuthis oualaniensis* resources in the South China Sea based on light falling-net. *J. Fish. China* **37**(7): 1032–1039.
- Yin, M.C. 1995. Fish ecology. *Beijing: Agricultural Press of China*, **134**(272): 134–272.
- Young, R.E. 1975. A brief review of the biology of the oceanic squid, *Symplectoteuthis oualaniensis* (Lesson). *Comp. Biochem. Physiol. B: Comp. Biochem.* **52**(1): 141–143.
- Zanden, M.J.V. and W.W. Fetzer. 2007. Global patterns of aquatic food chain length. *Oikos* **116**(8): 1378–1388.
- Zhu, K., X.H. Wang, F.Y. Du, P. Zhang and Y.S. Qiu. 2019. A study on morphological indicators variations and discrimination of medium and dwarf forms of purpleback squid *Sthenoteuthis oualaniensis* in the Central and Southern South China Sea. *Periodical Ocean Univ. China* **49**: 43–54.
- Zuyev, G., C. Nigmatullin, M. Chesalin and K. Nesis. 2002. Main results of long-term worldwide studies on tropical nektonic oceanic squid genus *Sthenoteuthis*: an overview of the Soviet investigations. *Bull. Mar. Sci.* **71**(2): 1019–1060.