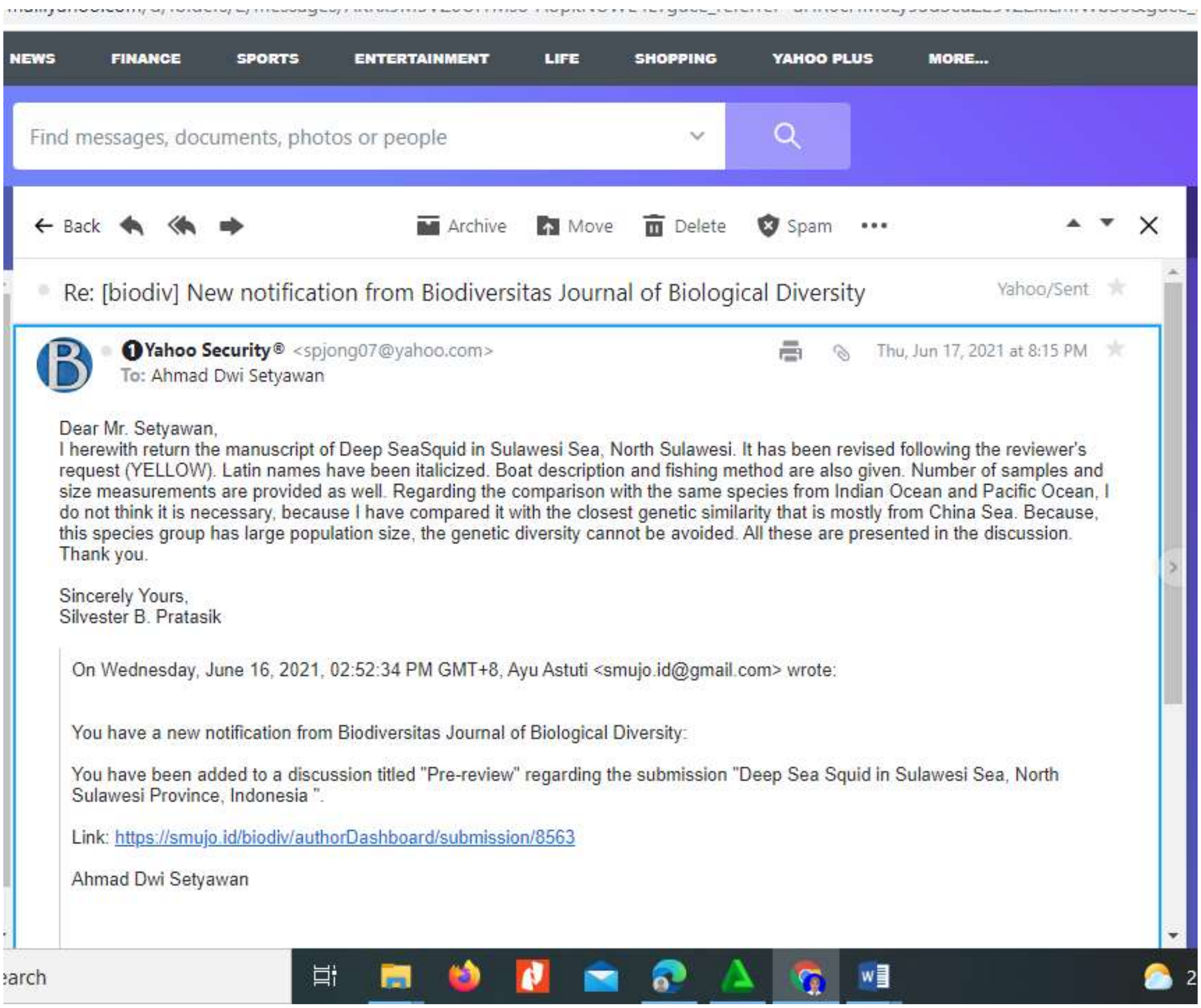

Re: [biodiv] New notification from Biodiversitas Journal of Biological Diversity

Yahoo Security® <spjong07@yahoo.com>
To: Ahmad Dwi Setyawan
Thu, Jun 17, 2021 at 8:15 PM

Dear Mr. Setyawan,
I herewith return the manuscript of Deep SeaSquid in Sulawesi Sea, North Sulawesi. It has been revised following the reviewer's request (YELLOW). Latin names have been italicized. Boat description and fishing method are also given. Number of samples and size measurements are provided as well. Regarding the comparison with the same species from Indian Ocean and Pacific Ocean, I do not think it is necessary, because I have compared it with the closest genetic similarity that is mostly from China Sea. Because, this species group has large population size, the genetic diversity cannot be avoided. All these are presented in the discussion.
Thank you.

Sincerely Yours,
Silvester B. Pratasik

On Wednesday, June 16, 2021, 02:52:34 PM GMT+8, Ayu Astuti <smujo.id@gmail.com> wrote:

You have a new notification from Biodiversitas Journal of Biological Diversity:

You have been added to a discussion titled "Pre-review" regarding the submission "Deep Sea Squid in Sulawesi Sea, North Sulawesi Province, Indonesia ".

Link: https://smujo.id/biodiv/authorDashboard/submission/8563

Ahmad Dwi Setyawan

Gambar 3. Korespondensi Revisi 1

Deep sea squid in Sulawesi Sea, North Sulawesi Province, Indonesia

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Abstract. This study aimed to identify one of the deep sea squids caught in artisanal fisheries by traditional fishermen in the Sulawesi Sea, North Sulawesi Province. Samples were collected using vertical hand line of traditional fishermen. The specimen was molecularly identified using Cytochrome c oxidase subunit 1 (CO1). For morphometric characteristics, all body parts were measured and each part was compared with mantle size. Results showed that the specimen was identified as purpleback flying squid *Sthenoteuthis oulaniensis* and had 99.35% similarity to *S. oualaniensis* (CO1) from China (NCBI - MN101944) with sufficient genetic diversity. Based on the body size, the species recorded in the present study belong to the dwarf form, the smallest form of *S. oualaniensis* groups. The species has the following morphometric characteristics: Head length is 0.28 of mantle length, tentacle length is 1.158 mantle length, and fin area is 0.5 mantle length. The fourth arm is the shortest and the second arm is the longest among the squid arms. This finding has contributed to the list of fisheries marine resources, especially squid, in Indonesian waters.

Key Words: species identification, CO1 gene, morphometric, traditional fishermen, hand line.

Running title: Deep Sea Squid

INTRODUCTION

Squids belong to cephalopod, mollusk, living in marine environment. Cephalopod means head feet, since the feet are separated as a number of arms circling the head. This group is an ecological opportunist adapted to exploit favorable environmental conditions (Rodhouse 2013). Squids (*Loligo* sp) are one of the cephalopod members that are important commodities for fisheries worldwide (Prakasa et al. 2014). It is a favorite food type due to its highly nutritional content with high quality protein for human consumption (Roper et al. 1984) and nearly all body parts are edible (Triharyuni and Puspasari 2012). Cephalopod production from fishing has continued to grow (Doubleday et al. 2016), with total commercial annual catches varying between 3.5 and 4.9 million tonnes in 2008–2017 (FAO 2019) and averagely supports about 15 and 20% of marine fishery landings and landed values, respectively (FAO 2019). Demand for this commodity has increased in both fresh and processed forms (Baskoro and Mustaruddin 2019). High market demand for squids as export commodity of Indonesia has made squids become one of major catches besides fish and lobster (Wulandari 2018). This trend will urge fishermen to conduct intensive fishing, while squid production is still dependent upon the stock in the wild (Tresnati et al. 2012). The squids, in general, with the other coleoid cephalopods, are semelparous, have high reproductive rates and generally shortlived approximately one year with a single spawning event, then die (Anusha and Fleming 2014; Rodhouse et al. 2014). They also grow fast with high feeding rates and conversion efficiencies (Arkhipkin et al. 2015).

These biological features make them be ecological opportunists in which they can quickly exploit favorable environmental conditions, but their abundance responds rapidly to poor conditions so that recruitment and abundance may be highly variable on annual time scales (Rodhouse et al. 2014). Therefore, high dependence on the wild stock can lead to stock overfishing.

In Indonesia, squid production is far below the world market demand in spite of its increasing squid production, so that the export development is still wide opened (Triharyuni and Puspasari 2012; Hulalata et al 2013). This condition is caused by uneven distribution of squid fisheries across the country. Only several regions do the squid fisheries, and the other do in very low scale for local consumption.

Information on this group is, so far, mostly related with commercial promotion as fisheries production. This group has been introduced together with other cephalopod groups as export commodity. Very few studies have been accomplished, so people’s knowledge on squids is very limited and they know the animal under a common name ”squid”, but there are many species belonging to this group. At fishermen community’s level, squids have different names with their morphological characteristics. Nevertheless, several members of this group that usually occur in regular period of time have disappeared for longer time. It is evident that there is a major artisanal and small-scale inshore element to the world squid fishing fleet and that large volumes of loliginid squids caught in tropical and subtropical regions have high species diversity. Thus, species identification study on this group in Indonesian waters is needed. In this area, the taxonomy of the squid fauna generally is poorly understood. Correct species identification is basis of the ecological studies (Veijalainen 2011).

There are 290 species of squids and about 30–40 species have substantial commercial importance (Arkhipkin et al 2015) belonging to family Ommastrephidae, Loliginidae, Onychoteuthidae, and Gonatidae. Triharyuni and Puspasari (2012) have grouped family Lolinginidae from Indonesian waters into several genera, Afrololigo, Allotheuthis, Dorytheuthis, Heterololigo, Loliolus, Lollinguncula, Pickfordiateuthis, Sepioteuthis, and Urotheuthis (Wulandari 2018), but there are much more species described (Jerep and Roper 2010). In Rembang waters, Central Java, four species were found, *Loligo chinensis*, *L. singhalensis*, *L. edulis,* and *L. duvaucelli,* in which *L. duvaucelli* is the most often caught species.

In North Sulawesi, Indonesia, there is no squid fisheries and the squid catches are bycatch of other fisheries, but squid production of different species is available every month. Squid catches are obtained at certain moon period, especially in early new moon, when the large squid schools come near the surface. The local fishermen catch them using small mesh-sized seine, lift net or using scoop net for those who have simple fishing facility. A small group of fishermen along Manado Bay, North Sulawesi, Indonesia, have benefitted deep sea squid as income source and food ingredients by relying on hand line fishing or jig fishing as practiced by foreign squid fishermen (Sundaram and Sawant 2014). Nevertheless, very few fishermen do squid fishing, the amount of catches is very low. This animal has become very important resource, so that its sustainability needs to be maintained, and thus, basic information on this resource needs to be provided. This study is intended to identify the deep sea squid caught by local fishermen of Manado Bay in Sulawesi waters. The findings may enrich the inventory of importantly economic squid species and develop squid fisheries in this area for future regional economic growth.

MATERIALS AND METHOD

Squid samples were obtained from fishermen's catch in Sulawesi Sea, North Sulawesi, about 5 miles from the shore of Manado Bay. In relation with this study, fishing operations were carried out at the geographic position covered by the area formed in the east border (1º 30' 45"N and 124º 42' 49"E to 1º 32' 47"N and 124º 42' 59"E) and the west one (1º 29' 30"N and 124º 40' 48"E to 1º 33' 45"N and 124º 40' 50'E) (Figure 1). Fishing operations used a 76 traditional outboard-motored outrigger boat and fishing activities relied on hand-line facilitated with flashlight 77 artificial bait that was lowered down to 20-25 m depth to get the squid to bite.

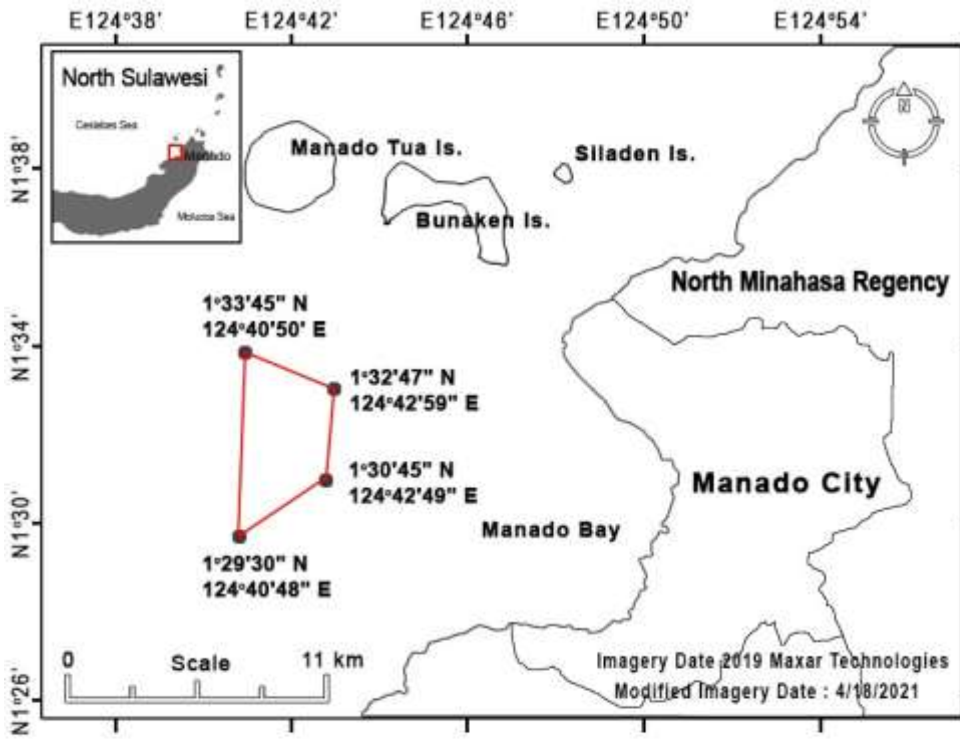

Figure 1. Sampling location. Red lines point out the sampling area.

A total of 600 squid samples were measured in order to describe the species physical characteristics through comparison of mantle size and other morphometric characteristics, such as head length, tentacle length, arm length, fin length, and fin width based on fresh samples. For DNA identification, a piece of the squid arm was taken and preserved in 95% ethanol solution before extraction to wash the sample from salt water and draw water from the cell. All samples were preserved in 95% ethanol and stored at room temperature before DNA extraction.

Extraction, PCR, and Sequencing.

Genome DNA extraction of all samples used Innu PREP DNA Micro Kit (Analytic Jena). The CO1 gene was amplified applying universal primer pairs LCO1490: 5'-ggtcaacaaatcataaagatattgg-3' and HCO2198: 5'taaacttcagggtgaccaaaaaatca-3' (Folmer et al. 1994). Polymerase chain reaction (PCR)was carried out in 35 cycles at 95ºC (30 sec.), 50ºC (30 sec.), 72ºC (50 sec.). The PCR product was visualized in 1% (b/v) agarose gel electrophoresis. Bi-directional sequencing was done by First Base CO (Malaysia) using Big Dye© terminator chemistry (PerkinElmer).

Data analysis.

The specimen chromatogram was edited using Mega X v10.1 software (Kumar et al. 2018). The sequences were then compared with Gen Bank data using BLAST (BasicLocal Alignment Search Tools) method (Altschul et al. 1997) and BOLD Systems (Ratnasingham and Hebert 2007). In this study, the sample sequence was compared with that of 6 specimens from China Sea. *Sepia latimanus* was also used as an outgroup in order to strengthen the comparison. This specimen is one of cephalopod members having internal skeleton called cuttlebone. The phylogenetic tree was built using Neighbor-Joining Method (Saitou and Nei 1987). Similarity index was also calculated.

RESULTS AND DISCUSSION

DNA characteristics.

Based on molecular identification using the Internal Transcribed Spacer (ITS) region, the DNA bands obtained were around 500-750bp, the success of PCR was detected by the presence of a single DNA band around 680 bp, the PCR results can be seen in Figure 2.

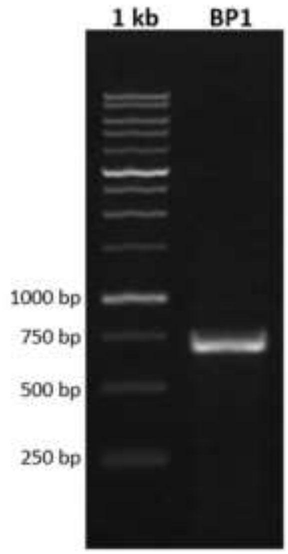

Figure 1. PCR product of the sample specimen

The sample was identified as purpleback flying squid *Sthenoteuthis oualaniensis* as shown in the BLAST results in the Gen Bank of NCBI. This species, according to WoRMs details, has synonymized names as *Loligo brevitentaculata* Quoy & Gaimard, 1832 (synonym), *L. oualaniensis* Lesson, 1830 (original combination), *L. vanikoriensis* Quoy & Gaimard, 1832 (synonym), and *Symplectoteuthis oualaniensis* (Lesson, 1830). This species belongs to class Cephalopod, subclass Coleoides, suborder Decapodiformes, order Oegopsida, superfamily Ommastrephidea, family Ommastrephidae, subfamily Ommastrephinae, and genus *Sthenoteuthis* (Jerep and Roper 2010). *Sthenoteuthis oualaniensis* from Sulawesi Sea has similarity rate of 99.35% to NCBI record (acc. no. MN101944.1) and 96.8% to five other NCBI records from China Sea (Table 1). *Sepia latimanus* used as outgroup clearly indicates great difference from the specimen and supports the kinship status of the specimen on study.

Table 1.

Similarity rate of *S. oulaniensis* from Sulawesi Sea and those from the Gen Bank DNA sequence database.

No.		1	2	3	4	5	6	7
1	*Sthenoteuthis oualaniensis,* Sulawesi Sea, Indonesia (Query Sequence)							
2	MN101944, *Sthenoteuthis oualaniensis*(COI), China (NCBI)	0.007						
3	F411104, *Sthenoteuthis oualaniensis*(COI), China (NCBI)	0.032	0.028					
4	MF411103, *Sthenoteuthis oualaniensis*(COI), China (NCBI)	0.032	0.028	0.000				
5	MF411102, *Sthenoteuthis oualaniensis*(COI), China (NCBI)	0.032	0.028	0.000	0.000			
6	MF411101, *Sthenoteuthis oualaniensis*(COI), China (NCBI)	0.032	0.028	0.000	0.000	0.000		
7	MF411098, *Sthenoteuthis oualaniensis*(COI), China (NCBI)	0.032	0.028	0.000	0.000	0.000	0.000	
8	AB430406.1_*Sepia_latimanus* (COI), Sulawesi Sea, Indonesia (OutGroup)	0.220	0.224	0.217	0.217	0.217	0.217	0.217

The difference is demonstrated in specimen grouping (Figure 3) as well in which there are 3 different groups formed, and only one compared *S. oulaniensis* of six NCBI records is in the same group as *S. oulaniensis* specimen on study. It reflects that this species has sufficient genetic diversity. Group 1 consists of 5 NCBI specimens from

China waters, group 2 comprises specimens from the Sulawesi Sea and 1 NCBI China specimen, and group 3 is an outgroup.

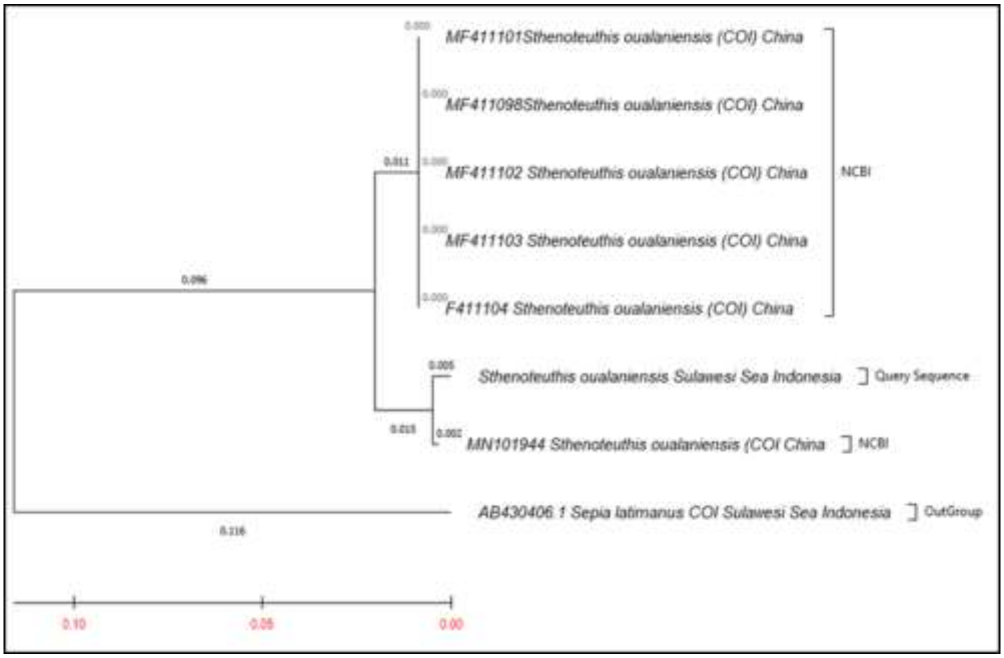

Figure 3. Phylogenetic tree of *S. oulaniensis*

Morphological description

Sthenoteuthis oulaniensis has the following morphometric characteristics based on mean size: Head length is 0.28 of mantle length (ML), tentacle length is 1.158 ML, fin width is 0.3 ML, and fin length is 0.4 ML. The fourth arm is the shortest and the second arm is the longest among the squid arms (Figure 4).

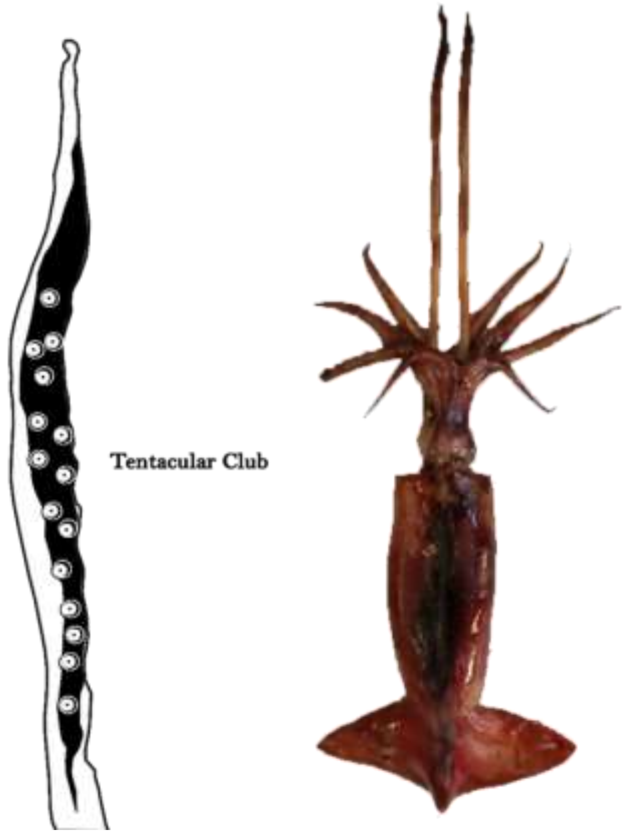

Figure 4. Tentacular club (left) and Squid *Sthenoteuthis oulaniensis*.

S. oualaniensis is known as purpleback flying squid, a tropical Indo-Pacific species that occurs in the Pacific from southern Japan to southern Queensland and from just south of Baja California to northern Chile. *S. oualaniensis* belongs to family Ommastrephidae widely distributed in the tropical and subtropical areas of the Pacific Ocean and Indian Ocean, and this species is important for resource exploitation in South China Sea as one of the major target species of large-scale light falling-net fishing (Yu et al 2019; Zhao et al 2021). This species is known to do diurnal vertical migration between the 134 surface at night to the deeper layer during the day, and thus, the species is caught by local fishermen of Manado Bay using 135 the flashlight artificial bait near the surface water There is a relationship between purpleback flying squid abundance and environmental variables (Alabia et al 2016; Mohamed et al 2018). Cephalopods are sensitive to water temperature (Li et al 2020) that becomes one of the major environmental factors affecting squid activities, including aggregation, breeding, and emigration (Klemas 2013). Deep sea squids *Sthenoteuthis oulaniensis* are distributed from 0-4,500 M depth with a peak at 0-500 M depth, water temperature of 25 – 32ºC, salinity of 34-35 PSU (Jerep and Roper 2010). These seawater temperature ranges covers that of Sulawesi Sea that enables to support the occurrence of this squid species based on 10 years of Sulawesi Sea water temperature data (Sea water temperature Sulawesi today | Indonesia (seatemperature.info). Besides sea surface temperature (SST), sea surface height anomaly (SSHA) at −0.05–0.05 m and chlorophyll-a concentration higher than 0.18 µg/L are required to gain higher catch (Zhao et al 2021).

S. oualaniensis are sexually dimorphic and the females tend to grow larger than the males in most cases (Chembian and Mathew 2014). Purpleback squid *S. oualaniensis* has a short lifecycle, a rapid growth rate, and high fecundity (Zhang et al 2013). According to Roper et al (2010), this species is highly active predator with major prey groups of fish, cephalopods, crustaceans, and others. This species is known to be one of the fastest-growing squid species with daily length increase of about 1.0 mm in the dwarf and middle-sized forms and 3.8 mm in the giant form is about 3.8 mm. For this, *S. oualaniensis* can move at high speeds through the water, easily manoeuver and quickly respond to their environment changes. Under distress of external factors, such as predation, this species can reach a high speed and glide above the surface over ten meters. They also found that the cruising speed of an adult squid is between 3 to 10 km per hour, their burst speed achieves greater speeds of up 35 km per hour, and it can be important for sudden changes in movement and escape behavior. They can occur into shoals from 2 individuals to

800 individuals, and when geographical distributions overlap they can exist with other members of similar-sized family Ommastrephidae schools, *Dosidicus gigas* and *Ommastrephes bartramii* that are distributed from the Indo-Pacific to Indian Ocean as well (Liu et al 2016). Ommastrephidae squids are known as voracious and adaptable predators of a broad range of prey including small crustaceans and fishes at early life stages and shift to micronekton, larger fishes, and cephalopods (including cannibalism) (Gong et al. 2020). These species are highly migratory, undertake diel vertical migrations of several hundred meters and seasonal migrations between the shelf and open ocean (Stewart et al 2013). Thus, they can work as important linkages between both neritic and oceanic food webs (Arkhipkin 2013; Alegre et al 2014).

In North Sulawesi, this squid species fishing is done by artisanal fishermen using jigging with artificial flashing bait, and the fishing operation has been done in the Sulawesi Sea. In the present study, the squids *S. oualaniensis* caught in the Sulawesi Sea had a size range of 60 mm to 11.0 cm ML. They belong to dwarf form with body size of 90-100 mm ML for mature males and 90-120 mm ML in mature females (Nesis 1993), occurs in the equatorial waters, and lacks of dorsal mantle photophore patch. The occurrence of *S. oulaniensis* in Sulawesi waters could result from the influence of environmental factors. Similar finding is also reported that changes in marine environments affect cephalopod fishery resources at different spatial and temporal scales (Zhang et al 2012). Furthermore, the distribution of purpleback flying squid *S. oulaniensis* in this area could not be separated from the impact of the Kuroshio from the Pacific Northern Equatorial current toward the east coast of Luzon, Philippines that forms the southward-flowing Mindanao Current (Qiu and Lukas 1996) and transfers the upper ocean waters from the Pacific to the Indian Ocean through the Indonesian Seas (Taufiqurrahman et al 2020). As the water mass enters the Indonesian Seas, the warm and relatively salty characteristics of the Pacific water tend to disappear due to strong vertical tidal mixing. The mixing is believed to affect the carbon, oxygen, and nutrient (nitrate, phosphates) concentrations in the eastern Indonesian Seas and subsequently in the Indian Ocean (Ayers et al. 2014). This event makes Indonesian marine waters be fertile enough to support the high occurrence of marine animals in the area. The Indonesian Throughflow is the only ocean connector pathway in the equator (Sprintall et al 2014), and it has an important purpose in the transport of mass and heat from the Pacific into the Indian Ocean (Feng et al 2018). The throughflow brings the eggs and larvae, along with the rich detritus of the sea that is swept up from the offshore deep basins. It is in agreement with Dell et al (2011) that the biophysical environment plays an essential role in controlling the distribution and abundance of pelagic predators in the ocean. Cheng et al (2018) who studied the swordtip squid *Uroteuthis edulis* found that complex oceanographic conditions might affect their population in the Southern East China Sea due to seasonal changes in the Kuroshio Current and Mainland China Coastal Current during the northeasterly monsoon and southwesterly monsoon seasons.

This evidence is supported by the closest kinship to the deep sea squid *S. oulaniensis* specimens from China waters of NCBI record and they could originate from the same ancestor despite its genetic diversity. Genetic diversity could result from population size, in which the larger population size within species is, the higher genetic diversity will be. This finding has reconfirmed the distribution of *S. oulaniensis* reported in previous studies. The present study has provided information on the occurrence of one of the importantly economic squid resources in Indonesian waters, especially the Sulawesi Sea, that can be used for national economic development. As a center of biodiversity, we strongly believe that there are more squid species living in these waters that need to be described, so that there are more taxonomic works need to be done on species richness of squids in Sulawesi Sea waters as well for future development. Besides, other biological studies of the species need to be done as well in order to have better understanding on this species that future management and conservation efforts could be well prepared.

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