

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

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6
7 **Abstract.** This study was aimed to identify one of the deep sea squids caught in artisanal fisheries by traditional fishermen in
8 Sulawesi Sea, North Sulawesi Province. Samples were collected using vertical hand line of traditional fishermen. The specimen
9 was ~~molecularly~~ identified using Cytochrome c oxidase subunit I (COI). For morphometric characteristics, all body parts were
10 measured and each part was compared with mantle size. ~~Results showed that~~ The specimen was identified as *Sthenoteuthis*
11 *oulaniensis* and had 99.35% similarity to *S. oulaniensis* (COI) from China (NCBI - MN101944) ~~with sufficient genetic~~
12 ~~variations~~. Based on the body size, species recorded in the present study belongs to the dwarf form, the smallest form of *S.*
13 *oulaniensis* groups. The species has the following morphometric characteristics: Head length is 0.28 of mantle length, tentacle
14 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
15 among the squid arms. *S. oulaniensis* recorded in the present study belonged to dwarf form living in equatorial waters. This
16 finding ~~has may~~ contributed to the list of marine resources of fisheries interest from marine resources, especially squid, in
17 Indonesian waters.

18 **Keywords:** squid species identification, COI gene, morphometric, traditional fishermen, hand line.

19 **Running title:** Deep Sea Squid

20 INTRODUCTION

21 Squids ~~belong to is a~~ cephalopod mollusk, living in the marine environment. ~~Cephalopod means head feet, since~~
22 ~~the feet are separated as a number of arms circling the head.~~ This group is an ecological opportunist adapted to
23 exploit favorable environmental conditions (Rodhouse 2013). ~~Squid (*Loligo* sp) is one of cephalopod members that~~
24 ~~are importantly economic fisheries commodities worldwide (Prakasa et al. 2014).~~ It is a favorite food type due to its
25 highly nutritional content with high-high quality protein for human consumption (Roper et al. 1984) and nearly all
26 body parts are edible (Triharyuni and Puspasari 2012). Cephalopod production from fishing has continued to grow
27 (Doubleday et al. 2016), with total commercial annual catches varying between 3.5 and 4.9 million tonnes in 2008–
28 2017 (FAO 2019) and averagely supports about 15 and 20% of marine fishery landings and landed values,
29 respectively (FAO 2019). Demand for this commodity has increased in both fresh and processed forms (Baskoro and
30 Mustaruddin 2019). High market demand for squids as an export commodity of Indonesia has made squids become
31 one of the major catches besides fish and lobster (Wulandari 2018). ~~This trend will urge fishermen to conduct~~
32 ~~intensive fishing, while the squid production is still dependent upon the stock in the wild (Fresnati et al. 2012).~~ ~~The~~
33 ~~squids, in general, with the other coleoid cephalopods, are semelparous, have high reproductive rates and generally~~
34 ~~shortlived approximately one year with a single spawning event, then die (Anusha and Fleming 2014; Rodhouse et~~
35 ~~al 2014).~~ ~~They~~ Squids also grow fast with high feeding rates and conversion efficiencies (Arkhipkin et al 2015).

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

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

Commented [SM1]: This is a very elaborate introduction. I have tried to trim it. Considerable improvement in the language usage is also necessary.

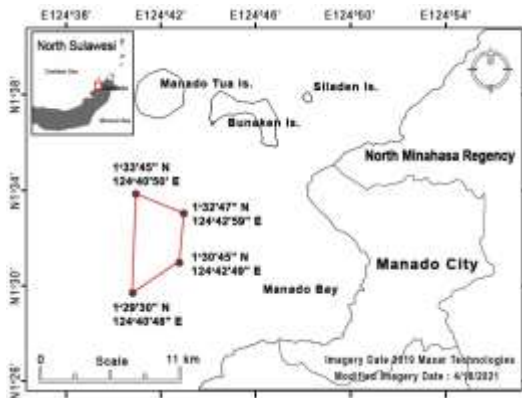
44 Information on this group is, so far, mostly related ~~with to~~ commercial promotion ~~as for~~ fisheries production.
45 ~~This group has been introduced together with other cephalopod groups as export commodity. Very few studies have~~
46 ~~been accomplished, so that people's knowledge on squids is very limited and they know the animal under a common~~
47 ~~name "squid", but there are so many species belonging to this group. At fishermen community's level, squids have~~
48 ~~different names with their morphological characteristics. Nevertheless, several members of this group that usually~~
49 ~~occur in regular period of time have disappeared for longer time.~~ It is evident that there is a major artisanal and
50 small-scale inshore element to the world squid fishing fleet and that large volumes of loliginid squids caught in
51 tropical and subtropical regions have high species diversity. Thus, ~~a species-identification taxonomic~~ study on this
52 group in Indonesian waters is needed. ~~In this area, t~~ The taxonomy of the squid fauna generally is poorly understood.
53 Correct species identification is ~~the~~ basis of ~~the~~ ecological studies (Veijalainen 2011).

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

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

73 MATERIALS AND METHOD

74 Squid samples were obtained from fishermen's catch in ~~the~~ Sulawesi Sea, North Sulawesi, about 5 miles from
75 the shore of Manado Bay. ~~In relation with this study, The~~ fishing operations were carried out at the geographic
76 position covered by the area formed in the east border (1° 30' 45"N and 124° 42' 49"E to 1° 32' 47"N and 124° 42'
77 59"E) and the west one (1° 29' 30"N and 124° 40' 48"E to 1° 33' 45"N and 124° 40' 50"E) (Figure 1).



78
79 **Figure 1.** Sampling location. Red lines point out the sampling area.

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

Commented [SM2]: How many samples? When was this collection made? What was the gear used for catching squids? How did you measure? Did you take the weight of the animal? Did you record the sex and maturity?

85 **Extraction, PCR, and sequencing**

86 Genome DNA extraction of all samples used Innu PREP DNA Micro Kit (Analytic Jena). The CO1 gene was
87 amplified applying universal primer pairs LCO1490: 5'-ggtcaacaatacataaagatattgg-3' and HCO2198:
88 5'taaacttcagggtgaccaaaaaatca-3' (Folmer et al. 1994). Polymerase chain reaction (PCR) was carried out in 35 cycles
89 at 95°C (30 sec.), 50°C (30 sec.), 72°C (50 sec.). The PCR product was visualized in 1% (b/v) agarose gel
90 electrophoresis. Bi-directional sequencing was done by First Base CO (Malaysia) using Big Dye© terminator
91 chemistry (PerkinElmer).

92 **Data analysis**

93 The specimen chromatogram was edited using Mega X v10.1 software (Kumar et al. 2018). The sequences were
94 then compared with Gen Bank data using BLAST (BasicLocal Alignment Search Tools) method (Altschul et al.
95 1997) and BOLD Systems (Ratnasingham and Hebert 2007). In this study, the sample sequence was compared with
96 that of 6 specimens from [the China Sea](#). *Sepia latimanus* was also used as an outgroup ~~in order~~ to strengthen the
97 comparison. This ~~specimen-species~~ is ~~one of a~~ cephalopod ~~members~~ having ~~an~~ internal skeleton called cuttlebone.
98 The phylogenetic tree was built using Neighbor-Joining Method (Saitou and Nei 1987). Similarity index was also
99 calculated.

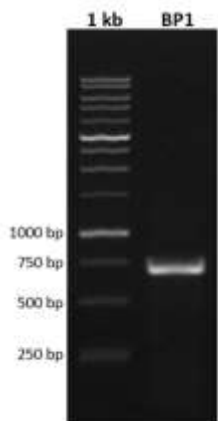
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RESULTS AND DISCUSSION

101 **DNA characteristics**

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

Commented [SM3]: Morphological descriptions first please.



105

106 **Figure 1.** PCR product of the sample specimen

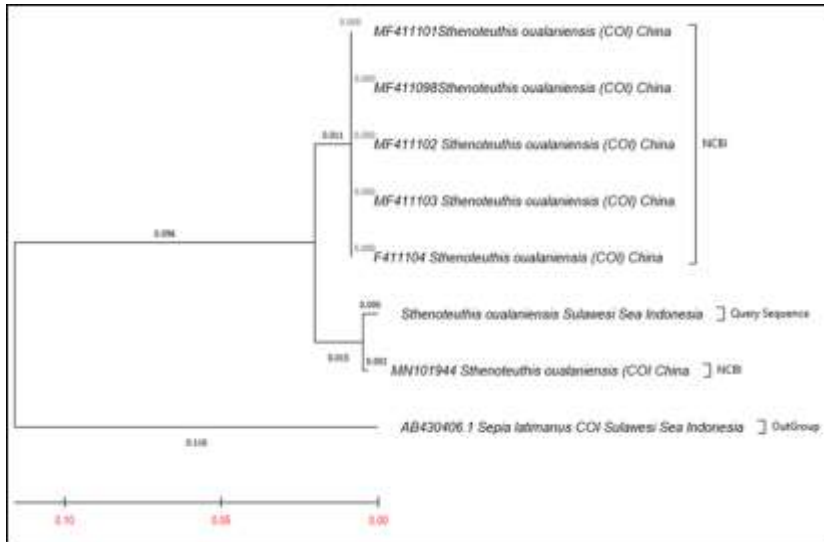
107 The sample was identified as *Sthenoteuthis oualaniensis* as shown in the BLAST results in the Gen Bank of
108 NCBI. This species, according to [the WoRMS details database](#), has synonymized names [such as](#) *Loligo*
109 *brevitentaculata* Quoy & Gaimard, 1832 (synonym), *L. oualaniensis* Lesson, 1830 (original combination), *L.*
110 *vanikoriensis* Quoy & Gaimard, 1832 (synonym), and *Symplectoteuthis oualaniensis* (Lesson, 1830). This species

111 belongs to class Cephalopod, subclass Coleoides, suborder Decapodiformes, order Oegopsida, superfamily
 112 Ommastrephidea, family Ommastrephidae, subfamily Ommastrephinae, and genus *Sthenoteuthis* (Jerep-Jereb and
 113 Roper, 2010). *Sthenoteuthis oualiansensis* from the Sulawesi Sea has a similarity rate of 99.35% to NCBI record
 114 (acc. no. MN101944.1) and 96.8% to five other NCBI records from the China Sea (Table 1). *Sepia latimanus* used
 115 as an outgroup clearly indicates the great difference from the specimen and supports the kinship status of the
 116 specimen on-studied.

117 **Table 1.** Similarity rate of *S. oualiansensis* from Sulawesi Sea and those from the Gen Bank DNA sequence database.

	1	2	3	4	5	6	7
<i>Sthenoteuthisoualiansensis</i> , Sulawesi Sea, Indonesia (Query Sequence)							
MN101944, <i>Sthenoteuthis oualiansensis</i> (COI), China (NCBI)	0.007						
F411104, <i>Sthenoteuthis oualiansensis</i> (COI), China (NCBI)	0.032	0.028					
MF411103, <i>Sthenoteuthis oualiansensis</i> (COI), China (NCBI)	0.032	0.028	0.000				
MF411102, <i>Sthenoteuthis oualiansensis</i> (COI), China (NCBI)	0.032	0.028	0.000	0.000			
MF411101, <i>Sthenoteuthis oualiansensis</i> (COI), China (NCBI)	0.032	0.028	0.000	0.000	0.000		
MF411098, <i>Sthenoteuthis oualiansensis</i> (COI), China (NCBI)	0.032	0.028	0.000	0.000	0.000	0.000	
AB430406.1 <i>Sepia latimanus</i> (COI), Sulawesi Sea, Indonesia (OutGroup)	0.220	0.224	0.217	0.217	0.217	0.217	0.217

118 The difference is demonstrated in specimen grouping (Figure 3) as well in which there are 3 different groups
 119 formed, and only one compared *S. oualiansensis* of six NCBI records is in the same group as *S. oualiansensis*
 120 specimen on-used in the study. It reflects that this species has sufficient genetic variations. Group 1 consists of 5
 121 NCBI specimens from China waters, group 2 comprises specimen from Sulawesi Sea and 1 NCBI China specimen,
 122 and- group 3 is an outgroup.



123
 124 **Figure 3.** Phylogenetic tree of *S. oualiansensis*

125 **Morphological description**

126 *Sthenoteuthis oualiansensis* has the following morphometric characteristics: Head length is 0.28 of mantle length
 127 (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
 128 the second arm is the longest among the squid arms (Figure 4).

Commented [SM4]: Do the morphometric characters match with previous descriptions? You mention a lot of morphometric measurements in Methods, but none of these are described here.



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

129
130

131 *S. oulaniensis* is known as purpleback flying squid, a tropical Indo-Pacific species that occurs in the Pacific
 132 from southern Japan to southern Queensland and from just south of Baja California to northern Chile. *S.*
 133 *oulaniensis* belongs to family Ommastrephidae widely distributed in the tropical and subtropical areas of the
 134 Pacific Ocean and Indian Ocean, and this species is important for resource exploitation in South China Sea as one of
 135 the major target species of large-scale light falling-net fishing (Yu et al 2019; Zhao et al 2021). There is relationship
 136 between purpleback flying squid abundance and environmental variables (Alabia et al 2016; Mohamed et al 2018).
 137 Cephalopods are sensitive to water temperature (Li et al 2020) that becomes one of the major environmental factors
 138 affecting squid activities, including aggregation, breeding, and emigration (Klemas 2013). Deep sea squids
 139 *Sthenoteuthis oulaniensis* are distributed from 0-4,500 M depth with a peak at 0-500 M depth, water temperature of
 140 25 – 32°C, salinity of 34-35 PSU (Jerep and Roper 2010). These seawater temperature ranges covers that of
 141 Sulawesi Sea that enables to support the occurrence of this squid species based on 10 years of Sulawesi Sea water
 142 temperature data (Sea water temperature Sulawesi today | Indonesia (seatemperature.info). Besides sea surface
 143 temperature (SST), sea surface height anomaly (SSHA) at -0.05-0.05 m and chlorophyll-a concentration higher
 144 than 0.18 µg/L are required to gain higher catch (Zhao et al 2021).

145 *S. oulaniensis* are sexually dimorphic and the females tend to grow larger than the males in most cases
 146 (Chembian and Mathew 2014). Purpleback squid *S. oulaniensis* has a short lifecycle, a rapid growth rate, and high
 147 fecundity (Zhang et al 2013). According to Roper et al (2010), this species is highly active predator with major prey
 148 groups of fish, cephalopods, crustaceans, and others. This species is known to be one of the fastest-growing squid
 149 species with daily length increase of about 1.0 mm in the dwarf and middle-sized forms and 3.8 mm in the giant
 150 form is about 3.8 mm. For this, *S. oulaniensis* can move at high speeds through the water, easily manoeuver and
 151 quickly respond to their environment changes. Under distress of external factors, such as predation, this species can
 152 reach a high speed and glide above the surface over ten meters. They also found that the cruising speed of an adult
 153 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

154 important for sudden changes in movement and escape behavior. They can occur into shoals from 2 individuals to
155 800 individuals, and when geographical distributions overlap they can exist with other members of similar-sized
156 family Ommastrephidae schools, *Dosidicus gigas* and *Ommastrephes bartramii* that are distributed from the Indo-
157 Pacific to Indian Ocean as well (Liu et al 2016). Ommastrephidae squids are known as voracious and adaptable
158 predators of a broad range of prey including small crustaceans and fishes at early life stages and shift to
159 micronekton, larger fishes, and cephalopods (including cannibalism) (Gong et al. 2020). These species are highly
160 migratory, undertake diel vertical migrations of several hundred meters and seasonal migrations between the shelf
161 and open ocean (Stewart et al 2013). Thus, they can work as important linkages between both neritic and oceanic
162 food webs (Arkhipkin 2013; Alegre et al 2014).

163 In North Sulawesi, this squid species fishing is done by artisanal fishermen using jigging with artificial flashing
164 bait, and the fishing operation has been done in Sulawesi Sea. In the present study, the squids *S. oulaniensis* caught
165 in 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
166 ML for mature males and 90-120 mm ML in mature females (Nesis 1993). The occurrence of *S. oulaniensis* in
167 Sulawesi waters could result from the influence of environmental factors. Similar finding is also reported that
168 changes in marine environments affect cephalopod fishery resources at different spatial and temporal scales (Zhang
169 et al 2012). Furthermore, the distribution of purpleback flying squid *S. oulaniensis* in this area could not be
170 separated from the impact of the Kuroshio from the Pacific Northern Equatorial current toward the east coast of
171 Luzon, Philippines that forms the southward-flowing Mindanao Current (Qiu and Lukas 1996) and transfers the
172 upper ocean waters from the Pacific to the Indian Ocean through the Indonesian Seas (Taufiqurrahman et al 2020).
173 As the water mass enters the Indonesian Seas, the warm and relatively salty characteristics of the Pacific water tend
174 to disappear due to strong vertical tidal mixing. The mixing is believed to affect the carbon, oxygen, and nutrient
175 (nitrate, phosphates) concentrations in the eastern Indonesian Seas and subsequently in the Indian Ocean (Ayers et
176 al. 2014). This event makes Indonesian marine waters be fertile to support the high occurrence of marine animals in
177 the area. The Indonesian Throughflow is the only ocean connector pathway in the equator (Sprintall et al 2014), and
178 it has an important purpose in the transport of mass and heat from the Pacific into the Indian Ocean (Feng et al
179 2018). The throughflow brings the eggs and larvae, along with the rich detritus of the sea that is swept up from the
180 offshore deep basins. It is in agreement with Dell et al (2011) that the biophysical environment plays an essential
181 role in controlling the distribution and abundance of pelagic predators in the ocean. Cheng et al (2018) who studied
182 the swordtip squid *Uroteuthis edulis* found that complex oceanographic conditions might affect their population in
183 the Southern East China Sea due to seasonal changes in the Kuroshio Current and Mainland China Coastal Current
184 during the northeasterly monsoon and southwesterly monsoon seasons.

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

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198 but passed away in the middle of study.

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Commented [SM5]: These descriptions are not relevant to the main objective of your study which was to identify the deep-sea squids in the area. The text provided here are available from literature and have little relevance to this study.

I suggest that this MS is rewritten keeping in mind the data collected and inferences from the data.

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