

Gambar 10. Korespondensi

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

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Abstract. This study was aimed to identify one of the deep sea squids caught in artisanal fisheries by traditional fishermen in 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 I (COI). For morphometric characteristics, all body parts were measured and each part was compared with mantle size. Results showed that the specimen was identified as *Sthenoteuthis oulaniensis* and had 99.35% similarity to *S. oulaniensis* (COI) from China (NCBI - MN101944) with sufficient genetic variations. Based on the body size, species recorded in the present study belongs to the dwarf form, the smallest form of *S. oulaniensis* 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. *S. oulaniensis* recorded in the present study belonged to dwarf form living in equatorial waters. This finding has contributed to the list of fisheries marine resources, especially squid, in Indonesian waters.

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Commented [JB3]: Species – or one individual squid?

Commented [JB4]: fishermen in the Sulawesi Sea

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Commented [JB6]: genetic diversity

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

Running title: Deep Sea Squid

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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 ecological opportunist adapted to exploit favorable environmental conditions (Rodhouse 2013). Squid (*Loligo* sp) is one of cephalopod members that are importantly economic fisheries commodities 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 the 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 (Arkipkin et al 2015).

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Commented [JB11]: Important commodities for fisheries worldwide

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.

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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.

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

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

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

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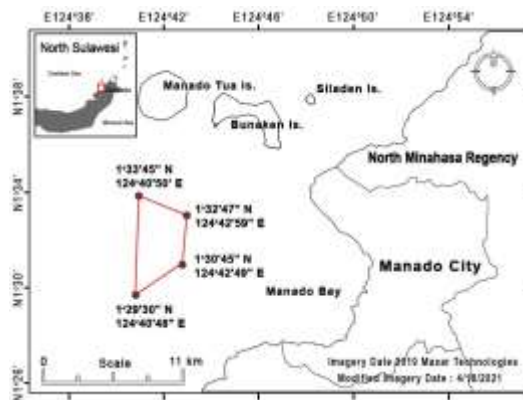
Commented [JB17]: economically valuable squid species

71 **MATERIALS AND METHOD**

72 Squid samples were obtained from fishermen's catch in Sulawesi Sea, North Sulawesi, about 5 miles from the
 73 shore of Manado Bay. In relation with this study, fishing operations were carried out at the geographic position
 74 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' 59"E) and
 75 the west one (1° 29' 30"N and 124° 40' 48"E to 1° 33' 45"N and 124° 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 bite.

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Commented [JB19]: When did the sample collection take place. Please give dates and times



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

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

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'-ggtcaacaatacataagatattgg-3' and HCO2198:
88 5'taaacttcaggtgaccaaaaaatca-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 China Sea. *Sepia latimanus* was also used as an outgroup in order to strengthen the
97 comparison. This specimen is one of cephalopod members having internal skeleton called cuttlebone. The
98 phylogenetic tree was built using Neighbor-Joining Method (Saitou and Nei 1987). Similarity index was also
99 calculated.

Commented [JB20]: As in from 600 individual squids?
Were they all from the same species? Did you use any keys
to identify species?

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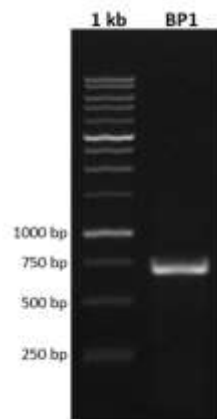
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100 **RESULTS AND DISCUSSION**

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.



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 NCBI.
108 This species, according to WoRMs details, has synonymized names as *Loligo brevitentaculata* Quoy & Gaimard,
109 1832 (synonym), *L. oualaniensis* Lesson, 1830 (original combination), *L. vanikoriensis* Quoy & Gaimard,

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mention

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110 1832 (synonym), and *Symplectoteuthis oualaniensis* (Lesson, 1830). This species belongs to class Cephalopod,
 111 subclass Coleoides, suborder Decapodiformes, order Oegopsida, superfamily Ommastrephidea, family
 112 Ommastrephidae, subfamily Ommastrephinae, and genus *Sthenoteuthis* (Jerep and Roper, 2010). *Sthenoteuthis*
 113 *oualaniensis* from Sulawesi Sea has similarity rate of 99.35% to NCBI record (acc. no. MN101944.1) and 96.8% to
 114 five other NCBI records from China Sea (Table 1). *Sepia latimanus* used as outgroup clearly indicates great
 115 difference from the specimen and supports the kinship status of the specimen on study.

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

	1	2	3	4	5	6	7
<i>Sthenoteuthisoualaniensis</i> , Sulawesi Sea, Indonesia (Query Sequence)							
MN101944, <i>Sthenoteuthis oualaniensis</i> (COI), China (NCBI)	0.007						
F411104, <i>Sthenoteuthis oualaniensis</i> (COI), China (NCBI)	0.032	0.028					
MF411103, <i>Sthenoteuthis oualaniensis</i> (COI), China (NCBI)	0.032	0.028	0.000				
MF411102, <i>Sthenoteuthis oualaniensis</i> (COI), China (NCBI)	0.032	0.028	0.000	0.000			
MF411101, <i>Sthenoteuthis oualaniensis</i> (COI), China (NCBI)	0.032	0.028	0.000	0.000	0.000		
MF411098, <i>Sthenoteuthis oualaniensis</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

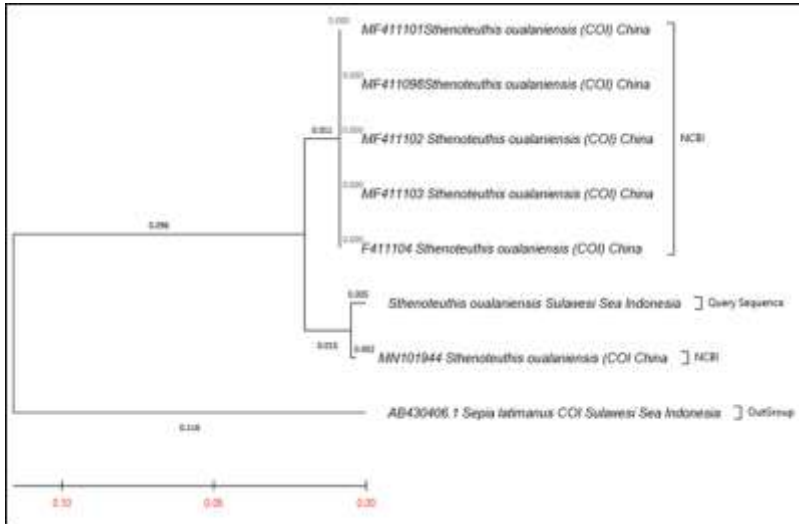
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117 The difference is demonstrated in specimen grouping (Figure 3) as well in which there are 3 different groups formed, and
 118 only one compared *S. oulaniensis* of six NCBI records is in the same group as *S. oulaniensis* specimen on study. It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



Commented [JB30]: genetic diversity

Commented [JB31]: Chinese

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121
 122 **Figure 3.** Phylogenetic tree of *S. oulaniensis*

123 **Morphological description**

124 *Sthenoteuthis oulaniensis* has the following morphometric characteristics: Head length is 0.28 of mantle length (ML),
 125 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
 126 arm is the longest among the squid arms (Figure 4).

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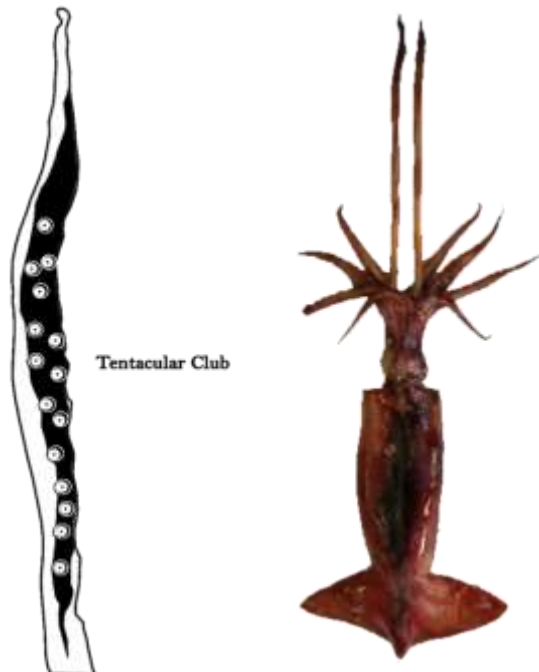


Figure 4. Tentacular club (left) and Squid *Sthenoteuthis oulaniensis*.

S. oulaniensis 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. oulaniensis* 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 surface at night to the deeper layer during the day, and thus, the species is caught by local fishermen of Manado Bay using the flashlight artificial bait near the surface water column. There is 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 – 32°C, 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. oulaniensis are sexually dimorphic and the females tend to grow larger than the males in most cases (Chembian and Mathew 2014). Purpleback squid *S. oulaniensis* 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. oulaniensis* 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

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157 shift to micronekton, larger fishes, and cephalopods (including cannibalism) (Gong et al. 2020). These species are highly
158 migratory, undertake diel vertical migrations of several hundred meters and seasonal migrations between the shelf and
159 open ocean (Stewart et al 2013). Thus, they can work as important linkages between both neritic and oceanic food webs
160 (Arkhipkin 2013; Alegre et al 2014).

161 In North Sulawesi, this squid species fishing is done by artisanal fishermen using jigging with artificial flashing bait,
162 and the fishing operation has been done in Sulawesi Sea. In the present study, the squids *S. oulaniensis* caught in
163 Sulawesi Sea had a size range of 68 mm to 119 mm ML, with mean mantle length of 89.833±0.9000 mm, reflecting that
164 the squid catches are dominated by large individuals. According to the classification of Nesis (1993), this size range
165 belong to dwarf form with body size of 90-100 mm ML for mature males and 90-120 mm ML in mature females, and
166 occurs in the equatorial waters, and lacks of dorsal mantle photophore patch. The occurrence of *S. oulaniensis* in Sulawesi
167 waters could result from the influence of environmental factors. Similar finding is also reported that changes in marine
168 environments affect cephalopod fishery resources at different spatial and temporal scales (Zhang et al 2012). Furthermore,
169 the distribution of purpleback flying squid *S. oulaniensis* in this area could not be separated from the impact of the
170 Kuroshio from the Pacific Northern Equatorial current toward the east coast of Luzon, Philippines that forms the
171 southward-flowing Mindanao Current (Qiu and Lukas 1996) and transfers the upper ocean waters from the Pacific to the
172 Indian Ocean through the Indonesian Seas (Taufiqurrahman et al 2020). As the water mass enters the Indonesian Seas, the
173 warm and relatively salty characteristics of the Pacific water tend to disappear due to strong vertical tidal mixing. The
174 mixing is believed to affect the carbon, oxygen, and nutrient (nitrate, phosphates) concentrations in the eastern Indonesian
175 Seas and subsequently in the Indian Ocean (Ayers et al. 2014). This event makes Indonesian marine waters be fertile to
176 support the high occurrence of marine animals in the area. The Indonesian Throughflow is the only ocean connector
177 pathway in the equator (Sprintall et al 2014), and it has an important purpose in the transport of mass and heat from the
178 Pacific into the Indian Ocean (Feng et al 2018). The throughflow brings the eggs and larvae, along with the rich detritus of
179 the sea that is swept up from the offshore deep basins. It is in agreement with Dell et al (2011) that the biophysical
180 environment plays an essential role in controlling the distribution and abundance of pelagic predators in the ocean. Cheng
181 et al (2018) who studied the swordtip squid *Uroteuthis edulis* found that complex oceanographic conditions might affect
182 their population in the Southern East China Sea due to seasonal changes in the Kuroshio Current and Mainland China
183 Coastal Current during the northeasterly monsoon and southwesterly monsoon seasons.

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

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194 ACKNOWLEDGEMENTS

195 We would greatly appreciate Sam Ratulangi University Manado for providing small research grant and laboratory
196 facilities. Our high appreciation is also addressed to Ms. Isroja Paransa who has involved in this study, but passed away in
197 the middle of study.

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