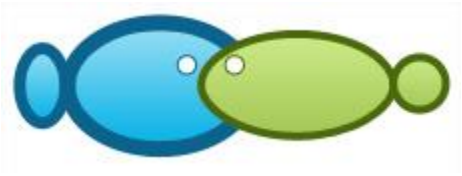

Signature of mitochondrial DNA on the painted spiny lobster (*Panulirus versicolor)* population in Indonesia

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Abstract. This research aimed at describing the identity of the population of *Panulirus versicolor* lobster (Latrelle 1804) genetically based on the COI mitochondrial DNA as a marker. This research investigated 3 populations from Indonesian sea regions, namely Yogyakarta, Malang, and Papua. In this research, COI DNA from 12 individuals of *P. versicolor* s has been extracted using the universal primers LCO-1490 and HCO-2198. A partial sequence of mitochondrial CO1 gene with the length of 687 bp has been analyzed. The results show that the diversity of nucleotides of the Yogyakarta population is included in the medium category (π=0.011), while that of the haplotype is included in the high category (h=1,000). The nucleotide diversity of the Malang population is included in very low category (π=0. 002), whereas that of the haplotype is included in high category (h=0.833); the nucleotide diversity of Papua population is included in very low category (π=0.003), whereas that of the haplotype is included in high category (h=0.900). The genetic diversity of the population indicates that there is a pressure on this population, maybe due to the overfishing.
Key Words: CO1, nucleotides, haplotype, overfishing.

Introduction. Genetic diversity in any population is very important to prevent the environmental stress or environmental changes. The population dynamic in wildlife (wild population) is genetically influenced by various factors. An importand parameter to maintain the genetic diversity levels is the population size. The population size is very important for the preservation of a species or population. Large populations are usually able to maintain the level of genetic diversity because their genetic drift is low and the rate of mutation accumulation is high (Ma et al 2020). The predictions about short-distance distribution and local recruitment of marine organisms can be used as a basis for the spatial management, related to the policy of marine area protectiona (Neo et al 2018). In fact, the distribution of organisms in the pelagic phase, as well as the widespread distribution of larvae, have a key role in both dynamics and genetic structure of marine populations (Levin 2006). The pelagic larvae of marine organisms can indicate the population genetic pattern in microgeographic and macrogeographic scales (Kumar & Kumar 2018; Potkamp & Fransen 2019; Telschow et al 2019).

Genetic studies on marine organisms in large populations have generally emphasized the aspects of diversity, demographic history, or natural selection (Telschow et al 2019). The genetic studies on the populations of marine organisms generally use mtDNA sequences to investigate the natural selection and population history aspects (Studivan & Voss 2018). The mtDNA application is very useful for stock assessment in fisheries and conservation management, since mtDNA is a haploid and maternal DNA, having a rapid evolution rate, being more effective than the nuclear DNA (Brown 1983; Ovenden 1990; Moritz 1994). The use of genes in mitochondrial DNA for a genetic population study refers to the haplotype. The characteristics of the haplotype can be used for the genetic characteriztion of a species or population, because as a maternal DNA, the mtDNA has a rapid mutation rate. The rapid evolution rate of this mtDNA can create

some unique haplotypes in a species or population (Kumar & Kumar 2018). Research on genetic populations using CO1 gene sequences generally uses a variety of haplotypes (h) and nucleotide diversity (π) (Holsinger & Mason-Gamer 1996; Pheng & Chen 2013). Some studies show a positive correlation between h and π (Bird et al 2007). The level of genetic diversity in mtDNA sequencing is seen from the diversity of haplotypes and nucleotides. The diversity of haplotypes and nucleotides can be affected by several factors, such as related to the reproductive system (asexual-sexual), age and population size, and inter-population connectivity (Chiu et al 2013; Silva et al 2019). Thus, mtDNA sequencing can effectively measure the dynamics of the population of organisms, especially marine animals.

Lobster, as arthropod animals living in the ocean, has experienced a very high population pressure. The capture of lobster for export trade is very high. Indonesia is one of the countries that exports lobsters in Asian markets. The volume of lobster export in Indonesia reached the highest level in 2003 (4,892 tonnes), but there was a decline in 2004 (2,803 tonnes) (Holmyard & Franz 2006). Overfishing is included as a threat to the genetic diversity of organism populations in nature or wildlife. Several studies have shown that the pressure from overfishing has suppressed the genetic diversity of a population as in the Neptunae arthritica population (Azuma et al 2014). Overfishing will reduce the effective size of a population, influencing the genetic diversity level of the population (Frankham et al 2002).

Genetic population studies can become a reference to determine a strategy and conservation management. The study generally emphasizes the local area aspect and connectivity between populations (locality), as well as the genetic diversity in the population. Genetic diversity is the main objective in the management of wild populations and cultivation of high economic value species. The genetic diversity is closely related to genetic erosion and reproduction potential (Crnokrak & Roff 2001; Keller & Waller 2002). Information on genetic diversity and connectivity in the population can be used to assess the dynamics of the ecosystem and to predict the level of the resilience of exploited populations. Nowadays, population genetics is becoming a main study topic in molecular ecology and biology conservation, in particular the genetic diversity between populations, and is associated with demographic history in populations, based on molecular data (Pool et al 2010). A population will develop according to its evolution history, geographical conditions, and environmental factors (Kumar & Kumar 2018).This research aimed at determining the status and identity of the population based on genetic diversity within the population and between the populations related to the environmental situation.

Material and Method

The samples of this research were 12 individuals of *P. versicolor* from Malang (4 individuals), Yogyakarta (3 individuals), and Papua (5 individuals). Sampling locations are shown in Figure 1. The lobster DNA was extracted using an extraction kit (Qiagen). The DNA was extracted with a High Pure PCR Template Preparation Kit (Qiagen) from approximately 10 mg of the tissue sample and heated with a heating block at 90°C for 25 minutes. After that, it was treated in the vortex and centrifuged (13,000 rpm speed), each time for 30 seconds. The DNA of the lobster genome obtained at this stage was in the form of a supernatant. The supernatant was further amplified by using the Jg-LCO1490 primer pair 5'-TNTCNACNAAYCAYAARGAYATTGG-3' and Jg-HCO2198 5'-TANACYTCNGGRTGNCCRAARAAYCA-3' (Geller et al 2013) for the amplification of COI gene fragments. Composition of PCR process (25 µL) was 2.5 µl dNTPs (8 µM), 2.5 µL PCR Buffer (10x), 1.25 µL primer forward (10 mM), 1.25 µL primer reverse (10 mM), 2 $MgCl_2$ (25 mM), 0.125 µL Amplitag (5 unit L^{-1}), 2 µLDNA genome, and 14.5 µL ddH_2O. The amplification was done in the Thermal cycler Biorad 48-Well engine. The PCR reaction was performed for 30 cycles with pre-denaturation at 94^{o}C (5 min), denaturation at 94^{o}C (30 seconds), primary agitation at 50^{o}C (30 sec), elongation at 72^{o}C (45 sec), and final elongation at 72^{o}C (10 min). The PCR results were sequenced using commercial laboratory services in Malaysia to produce sequences from lobster samples.

The genetic analysis of the population characteristics in this research was based on the measurement of DNA polymorphism, nucleotide (p), and haplotype (Hd) diversity in the population sample using DnaSP version 5.10. The analysis of genetic diversity among and within the population used method analysis of Wright's fixation index of FST using Arlequin version 3.1. The phylogeography was analyzed using the Network program. The demographic analysis used the mismatch distributions analysis.

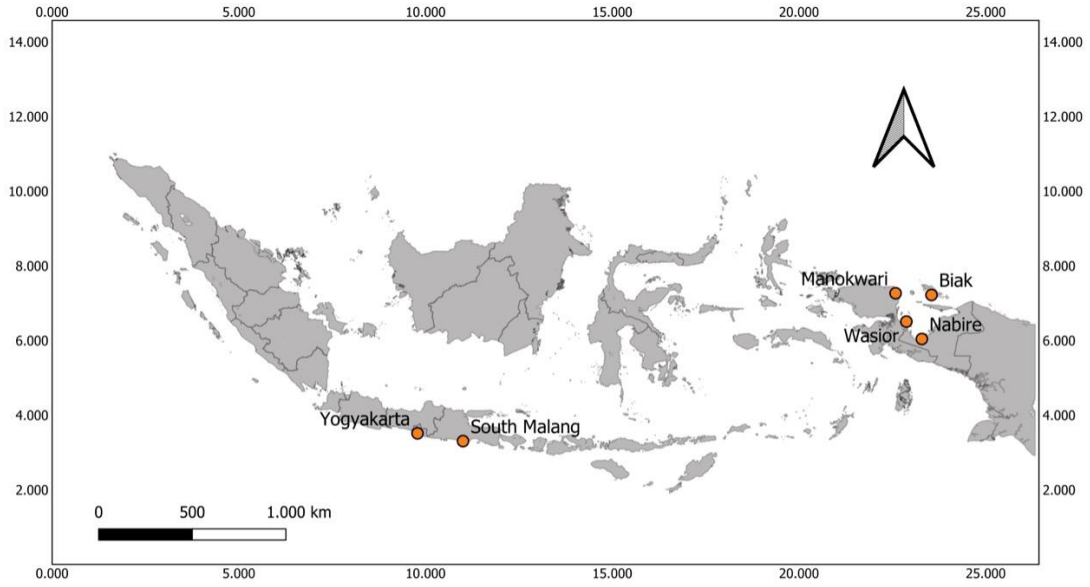

Figure 1. Map of Indonesia sample location: 1. Yogyakarta (3 individuals), 2. South Malang (4 individuals), 3-6 Papua (3. Manokwari, 4. Biak, 5. Wasior and 6. Nabire).

Results. In this research, the diversity of haplotype (h) in all three populations ranges between 0.83-1.0 and the diversity of nucleotides (π) ranges between 0.0021-0.11. The diversity of haplotypes of the samples in Malang was 0.83, in Yogyakarta 1.00, and în Papua 0.9 (Table 1). The value of the haplotype diversity in all three locations is categorized as high, and conversely, the value of the nucleotide diversity in all three populations is low.

Table 1

Genetic diversity of CO1 genes in Java and Papua *Panulirus versicolor* populations

Populations	*Genetic diversity*				
	N	*H*	*S*	*h*	*π*
Malang	4	3	2	0.83	0.002
Yogya	3	3	8	1.00	0.011
Papua	5	4	4	0.90	0.003
Total	12	9	13	0.93	0.005

In this research, 9 haplotypes are obtained from 12 sequence samples. H1 (Malang1, Malang2), H2 (Manokwari, Wasior1), H3 (Malang4), H4 (Yogyakarta1), H5 (Yogyakarta2), H6 (Yogyakarta3), H7 (Biak), H8 (Nabire), H9 (Wasior2). The results of the network analysis of the haplotype distribution from 12 sample sequences by using the median-joining network method indicate that there is a starburst pattern (Figure 2). Figure 3 shows that 'H2' is the haplotype center. Haplotype 'H2' is common to the populations of only three sampling locations. H2 is a haplotype that shares the character of the sample population from Malang2, Manokwari, Wasior1.

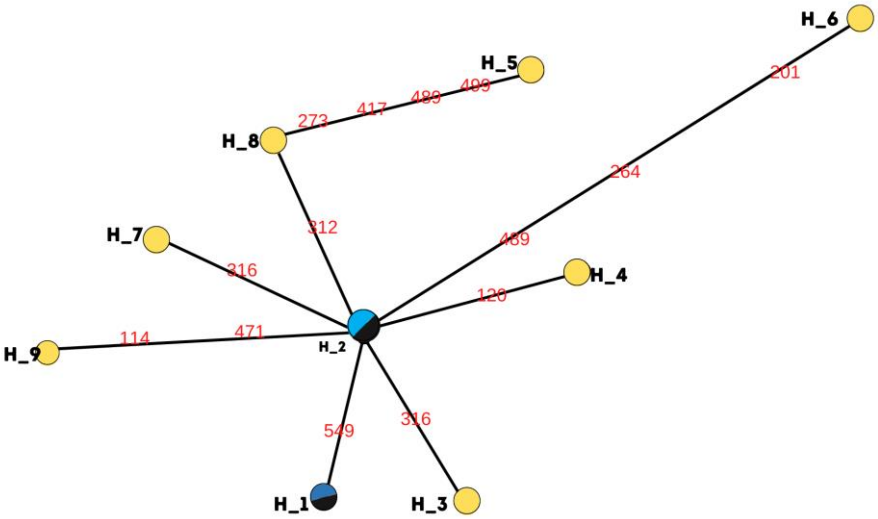

Figure 2. Haplotype network using the Median-joining network method of the samples of *Panulirus versicolor*. The circle shows the haplotype of each individual (H1-H9).

In this research, the differentiation level between samples was analyzed using the fixation index value (FST). This fixation index is useful to know the genetic differentiation among samples in this research. The test results show that the FST value in this research is 0.48 (Table 3), showing that there is no differentiation among the three populations genetic structure(the populations are not isolated from each other).

Table 2

Analysis of molecular variance (ANOVA) in two populations of *Panulirus versicolor* (Java and Papua). Fixation index F ST=0.48 (p<0.05)

Source of variation	*D f*	*Sum of squares*	*Variance components*	*Percentage of variation*
Among regions	1	220.083	36.50838	57.44
Within regions	1	12.883	-5.85494	-9.21
Total	9	296.167	32.9071	51.77

The history of *P. versicolor* population in this research was analyzed using the mismatch distribution analysis. The graph of mismatch distribution shows a multimodal graph pattern on the observation line (Figure 3). The graph pattern shows a stable population history.

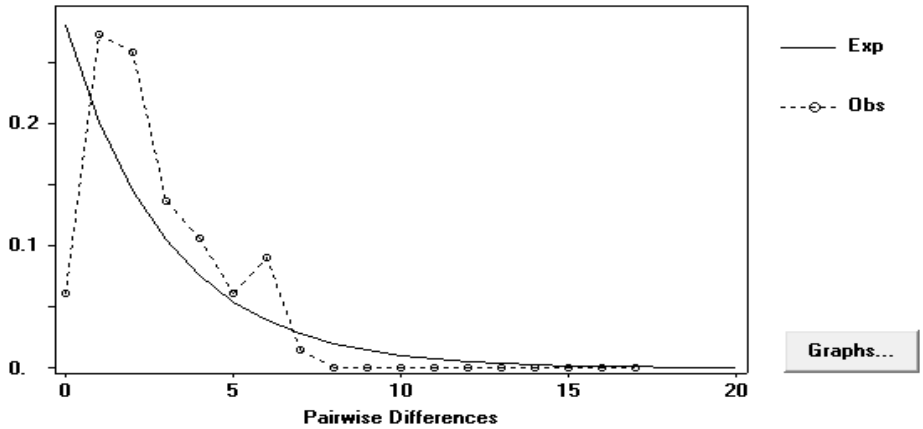

Figure. 3. Distributions of pairwise differences (mismatch distribution) indicating bimodal distribution pattern based on COI sequence of the of *Panulirus versicolor* population.

The results of this research indicate that the diversity of haplotypes within the population is high. The mean value of the haplotype diversity in the population is 0.95, and the nucleotide diversity is 0.005. There are several explanations for this condition of large populations such as *P. versicolor*. The diversity of nucleotide and haplotype can provide demographic information relating to the history of the population (Goodall-Copestake et al 2012). These widely distributed populations allow connectivity between them and gene flow occurring.

Discussion. The populations which are linked generally show a high level of genetic diversity. In this research, the haplotype diversity is high, and the nucleotide diversity is very low. The high haplotype diversity and the low nucleotide diversity are influenced by environmental factors and population history (White et al 2010; Young et al 2015). The environmental stress on the population of *P. versicolor* lobster is allegedly due to habitat destruction and over-harvesting pressures. The pressure factor caused by human activity can also become the cause of such a condition (Kochzius & Haryanto 2008). The environmental stress in the *P. versicolor* lobster population is presumably influenced by various environmental factors. The diversity of haplotype and nucleotide in the population is also related to environmental factors (White et al 2008) and over-exploitation occurrences (Kochzius & Nuryanto 2008). The environmental aspects include habitat destruction and overfishing or overharvesting. As already known, *P. versicolor* is one of the lobsters that have high economic value, and it experiences overharvesting in the southern sea of Java (Khadafi 2006; Saputra 2009). Overharvesting on high-value species commonly happens. These conditions also resulted in a population decline that affects the decrease in genetic diversity in the population (Pinsky & Palumbi 2014).

The population history is one aspect of the current population assessment of an area. This is in line with what is presented by Grant & Bowen (1998) that the population history can be observed based on the diversity of the haplotypes and nucleotides. The analysis of population history in this research is performed using the median-joining network method. The results of the analysis indicate that the haplotype pattern in this lobster population forms a star-like or starburst pattern. This star pattern indicates a population expansion history from one source. In some populations, the pattern indicates the presence of haplotypes originating from their ancestors. Meanwhile, unique haplotypes can develop from several mutation events in the population (Clark et al 1998; Teixeira et al 2011).

The first haplotype pattern is related to the demographic history factor. The demographic history of the *P. versicolor* lobster population experienced population expansion in the Pleistocene era (Pulgarin & Burg 2012). Rapid population expansion as depicted in the haplotype network (Figure 2) is also supported by the analysis of the missmatch distribution graph (Figure 3). The second haplotype pattern suggests that a genetic link of selective sweep events at a locus of the mitochondrial gene is thought to cause a decrease in the genetic diversity (mtDNA) at the 'neutral' loci (Baeza 2018; Gershoni et al 2009). Thus there is an advantageous haplotype that spreads in the population, due to the selective sweep event at one of the loci in mtDNA. Related to the research on some animal populations using genetic data from mtDNA, some experts suspect the presence of populations of several species during the glacial period in all continental regions or trans-continental glaciers (about 18,000 years ago). The emergence of several new species occurred in those days, and then some of these species allegedly began to spread to form populations during the Pleistocene time (Kumar & Kumar 2018). Several independent lines indicate the spread of populations in the post-ice melting period over the past millions of years. The star pattern on this haplotype network indicates a predominant haplotype and a mutation phase of the main haplotype. Thus, the current widespread *P. versicolor* lobster population is thought to have originated from a population that emerged in the Pleistocene era.

The preservation of genetic diversity in the population enhances adaptability and sustains the population from environmental stresses. The populations with high genetic diversity can deal with environmental stresses (Studivan & Voss 2018). Genetic diversity between and within populations is also very important for the continuity of evolution of

the population in the future. The loss of genetic diversity in the population forms a threat to the sustainability, in conjunction with the over-exploitation and natural factors (Frankham et al 2002; Aguirre & Marshall 2012).

In this research, the FST value of the population is 0.48, indicating the absence of differentiation between populations, caused by many factors. Each population is still connected to the nearest population (neighbor population) so that no subpopulation is formed. In some cases, dynamics of lobster populationsare related to inter-population connectivity. The larvae of lobster are generally active so that they actively spread (migration) and choose the habitat they want. This characteristics of the larvae can help to restore the stock of the population (adult animals) in the area, as in the case of the extensive larval distribution of *P. gilchristi* lobster on the southern coast of South Africa. The study on *P. gilchristi* lobsters using mitochondrial DNA indicates the presence of panmyxic reproduction in the lobster population (Silva et al 2019).

Conclusions. The study using the diversity of haplotype as well as nucleotide and the netwok pattern on the haplotype shows the possibility of *P. versicolor* experiencing population expansion (recently). The high level of haplotype diversity and the low level of nucleotide diversity observed in the population research of *P. versicolor* lobster indicates a pressure (or stress) on this population. High-value lobster species, such as *P. versicolor*, generally experience a population decline due to overharvesting. This population decline can be indicated by the low level of nucleotide diversity. The decline in *P. versicolor* population may threaten genetic diversity and population sustainability in Indonesia's marine areas. Natural resource conservation stakeholders immediately formulate policies targeting the general marine lobster population and *P. versicolor* populations in particular, so that recovery and recruitment of the population in the future can occur.

Conflict of interest. The authors declare no conflict of interest.

References

Aguirre J. D., Marshall D. J., 2012 Genetic diversity increases population productivity in a sessile marine invertebrate. Ecology 93(5):1134-1142.

Baeza J. A., 2018 The complete mitochondrial genome of the Caribbean spiny lobster Panulirus argus. Scientific Reports 8(1):1–10.

Chiu Y., Bor H., Tan M., Lin H., Jean C., 2013 Phylogeography and genetic differentiation among populations of the moon turban snail *Lunella granulata* Gmelin, 1791 (Gastropoda: Turbinidae). International Journal of Molecular Sciences 14:9062-9079.

Clark A. G., Weiss K. M., Nickerson D. A., Taylor S. L., Buchanan J., Stengard V., Salomaa E., Vartiainen M., Perola E., Boerwinkle, Sing C. F., 1998 Haplotype structure and population genetic inferences from nucleotide sequence variation in human lipoprotein lipase. The American Journal of Human Genetics 63:595–612.

Frankham R., Ballou J. D., Briscoe D. A., 2002 Introduction of conservation genetics, Cambridge University Press, Cambridge, UK, 617 p.

Geller J., Meyer C., Parker M., Hawk H., 2013 Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. Molecular Ecology Resources 13:851-861.

Gershoni M., Templeton A. R., Mishmar D., 2009 Mitochondrial bioenergetics as a major motive force of speciation. Bioessays 31:642–650.

Grant W. A. S., Bowen B. W., 1998 Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. Journal of Heredity 89(5):415-426.

Holsinger K. E., Mason-Gamer R. J., 1996 Hierarchical analysis of nucleotide diversity in geographically structured population. Genetics 142:629-639.

Kochzius M., Nuryanto A., 2008 Strong genetic population structure in the boring giant clam, *Tridacna crocea*, across the Indo-Malay Archipelago: Implications related to evolutionary processes and connectivity. Marine Ecology 17:3775-3787.
Kumar R., Kumar V., 2018 A review of phylogeography: biotic and abiotic factors. Geology, Ecology, and Landscapes 2(4):268–274.
Levin L. A., 2006 Recent progress in understanding larval dispersal: new directions and digressions. Integrative and comparative biology 46:282–297.
Ma Z. S., Li L., Zhang Y. P., 2020 Defining individual-level genetic diversity and similarity profiles. Scientific Reports 10(1):1–12.
Neo M. L., Liu L. L., Huang D., Soong K., 2018 Thriving populations with low genetic diversity in giant clam species, *Tridacna maxima* and *Tridacna noae*, at Dongsha Atoll, South China Sea. Regional Studies in Marine Science 24:278–287.
Pinsky M. L., Palumbi S., 2014 From the cover, meta-analysis reveals lower genetic diversity in overfished populations. Molecular Ecology 23:29–39.
Potkamp G., Fransen C. H. J. M., 2019 Speciation with gene flow in marine systems. Contributions to Zoology 88(2):133–172.
Pulgarin R. P. C., Burg T. M., 2012 Genetic signals of demographic expansion in downy woodpecker (*Picoides pubescens*) after the last north american glacial maximum. PLoS ONE 7(7):e40412.
Silva C. N. S., Villacorta-Rath C., Woodings L. N., Murphy N. P., Green B. S., Hartman K., Gardner C., Bell J. J., Strugnell J. M., 2019 Advancing our understanding of the connectivity, evolution and management of marine lobsters through genetics. Reviews in Fish Biology and Fisheries 29(3):669–687.
Studivan M. S., Voss J. D., 2018 Assessment of mesophotic coral ecosystem connectivity for proposed expansion of a marine sanctuary in the Northwest gulf of Mexico: Population genetics. Frontiers in Marine Science 5:1–11.
Teixeira S., Cambon-Bonita E. A., Serrao D., Arnaud-haond S., 2011 Recent population expansion and connectivity in the hydrothermal shrimp Rimicaris exoculata along the Mid-atlantic Ridge. Journal Biology 38(3):5674-574.
Telschow A., Gadau J., Werren J. H., Kobayashi Y., 2019 Genetic incompatibilities between mitochondria and nuclear genes: Effect on gene flow and speciation. Frontiers in Genetics 10:1–12.
White C., Selkoe K. A., Watson J., Siegel D. A., Zaherl D. C., Toonen R. J., 2008 Ocean currents help explain population genetic structure. Proceedings of the Royal Society B: Biological Sciences 277(1688):1685-1694.
Young E. F., Belchier M., Hauser L., Horsburgh G. J., Meredith M. P., Murphy E. J., Pascoal S., Rock J., Tysklind N., Carvalh G. R., 2015 Oceanography and life history predict contrasting genetic population structure in two Antarctic fish species. Evolution Application 8:486-505.

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