Genetic and Morphological Variation of the Redbelly Yellow Tail Fusilier, *Caesio cuning* (Bloch, 1971) from the Nyamuk Waters, Karimunjawa Archipelago

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Abstract

Yellowtail fusilier, Caesio cuning (Bloch, 1971) is the main catch of fishermen on the Nyamuk Island, Karimunjawa. C. cuning also has a unique character in the form of different eye color, white, red, and white-red. These differences were raise question whether the eye colors will be differentiated the morphology, and the genetic diversity of C. cuning. This research aims to study the biodiversity of C. cuning caught by fishermen on Nyamuk Island, Karimunjawa, based on their morphology, and molecular using mtDNA control region gene marker. This study also wanted to test, if different eye colors observed on the sample also reflected at the genetic level. A unique thing was found in morphological observations in the form of eye colors in C. cuning (white, red and white-red). Measurements of 19 morphometric characters were conducted on 44 samples directly in the field. Twenty four (24) samples with three different eye colors were selected for molecular assay of locus control region mtDNA in the laboratory. Morphological analysis showed the total length of 44 samples of C. cuning ranged from 15-29.4 cm. The total length of white eye color ranged from 15-29.4 cm from 20 samples, red-eye color 17-26 cm from 20 samples and white-red eye color 22-24.6 from 4 samples. Molecular test showed that the fish observed was indeed C. cuning with a similarity of 97.07%-99.27% of NCBI data. The genetic distance C. cuning between individuals was 0-3%. Genetic distance in the same eye color and different have the same value of 2%. Twenty haplotypes were obtained from 24 samples with high haplotypes diversity, values ranging from 0.978-1,000. The nucleotide diversity ranges from 0.019-0.021. There is no molecular difference between C. cuning with different eye colors.

Keywords: *Caesio cuning, Genetic, Morphological, Nyamuk Island*

Introduction

Nyamuk Island is part of the Karimunjawa National Park, Jepara Regency, Central Java. It has an area of 486 km^2 with a population of 620 people (Statistik, 2021). The majority people in the Nyamuk Island work as fishermen, who catch several fish species, such as yellow tail fish, red snapper, grouper, tuna, mackerel, squid, and other reef fish (Sumbodo *et al.*, 2017). The *Caesio cuning* is one of the main targets that usually caught with spear gun and traps (Tarigan *et al.*, 2016; Ramadhan and Apriliani, 2017; Darmawan *et al*., 2020). The fishing season in the targeted area occurs twice a year. The first season in the transitional season that span from March to May with the peak in April or May than the second transitional season in September-November which as the fishing peak is in September or October (Kunarso *et al.,* 2016). The fishing activity of *C. cuning* is carried out continuously throughout the year, hence it threaten their sustainability (Darmawan *et al*., 2020) and can lead to extinction (Ambariyanto, 2017). There has been a decline in catches during 2015-2017, starting from 2015 (from 139.3 ton per year),to 2017 (become 70.9 ton per year). The decline in catches, coupled with low migratory activity and relatively narrow migration areas (Irnawati *et al*., 2011), can be a driving factor for population decline (de Mitcheson *et al*., 2020).

Fishes have interesting information and diversity to study (Nelson *et al*., 2016). There are 59 species of reef fish were found in the Karimunjawa national park (Nuranggitasari and Kurniawan, 2021). *C. cuning* caught on Nyamuk Island has eye color variations, white eyes caught at a depth of 7-30 meters, red eyes (2-7 m) and mixed eye colors of white and red (2-30 m). Morphometric data and genetic diversity of *C. cuning* are needed as a basis of fishing fisheries management. This study aims to

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assess the morphological identification, measure the 19 characters of fish morphometric, and conduct phylogenetic analysis based on characters and mitochondrial DNA (mtDNA) control region (CR) sequences. This research is expected to be a recommendation for fisheries management on Nyamuk Island Karimunjawa.

Materials and Methods

Sample collection and morphological identification

A total of 44 samples of individual *C. cuning* were collected for this study. *Twenty* samples with white eyes, 20 samples with red eyes and 4 samples with white-red eyes (Figure 1.). Morphological observations and morphometric measurements were carried out directly in the field for all individual samples. Morphological observations were based on White *et al.* (2013) and Carpenter and Niem (2001). Morphometric measurements followed Fitriadi *et al*. (2012) and Zuhdi and Madduppa (2020). Sample for molecular identification were taken from the caudal fin (fin clip) and preserved in 96% ethanol prior laboratory analysis.

DNA analysis

A total of 24 samples consists of 10 samples with white eyes, 10 samples with red eyes and 4 samples with white-red eyes, were analyzed using molecular approach in the Marine Biodiversity Project Laboratory, Diponegoro University. The molecular test was initiated by extracting the DNA using Chelex 10% (Walsh *et al.,* 1991) with heating at 95 °C for 45 minutes (Akbar and Aris, 2018).

Amplification of mitochondrial control region were utilized forward primer 14F (5'-CTCCCAAAGCTAGGATTCT -3') and reverse primer 14R (5'- CTTAACATCTTCAGTGTTATGC -3') (Oh *et al.,* 2007). The protocol for PCR were as follow: predenaturation at 95 °C for 3 minutes, denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 1 minute post extension 72 °C for 5 minutes (Oh *et al*., 2007). The total cycles for this protocol was 36 cycles. The Sanger Sequencing were conducted at Genetics Science. The *C. cuning* sequences data have been registered at NCBI with accession number OM240836-OM240859.

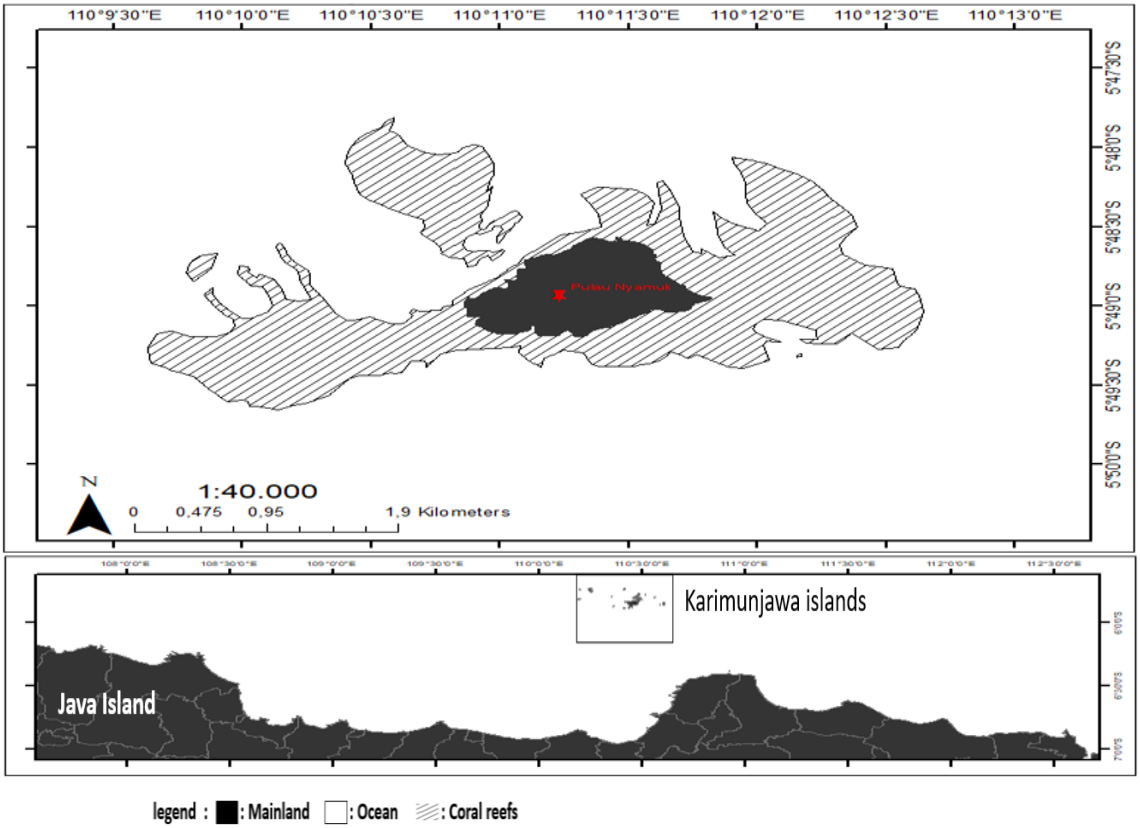

Figure 1. Map of research location (ocean waters Nyamuk Island, Karimunjawa). Source: Indonesian Geospatial Portal - https://tanahair.indonesia.go.id/portal-web and Satellite Google Earth 2021 - https://earth.google.com/web. Map created using Arcgis 10.3.

The forward and reverse sequences were then aligned using MEGA X (Kumar *et al*., 2018). Sequences data then being compared with the basic local alignment search tool (BLAST) into GenBank (NCBI or The National Center for Biotechnology Information; https://www.ncbi.nlm.nih.gov) (Bilderbeek and Etienne, 2018) to get the matching species identification. MEGA X was also used to measure genetic distance (Tamura *et al*., 2011) and phylogenetic trees using Maximum Likelihood and Neighbor-Joining approaches (Kumar *et al*., 2018). Bayesian method is also used in making the phylogenetic tree with software Bayesian Evolutionary Analysis Sampling Trees v2.6.2 (Bilderbeek and Etienne, 2018). The DNAsp v61101 application was used to calculate haplotype diversity (Rozas *et al*., 2017) and the Arlequin 3.1 (Excoffier *et al*., 2005) was used for molecular variance analysis.

Result and Discussion

Morphology

The morphological observations of *C. cuning* showed that the upper bodies (from the snout tip to the dorsal fin) is grayish-yellow (1), the dorsal fin to the tail is yellow (2), the middle of the body is pale-white and the tail is yellow (3) (Figure 2A.). The ventral body from the cheeks to the bottom-base of the tail, pectoral fins, abdomen, and anus are pink (4), a large and slightly elongated body and eyes that are white, red and a mixture white-red (Figure 2.). These results are consistent with White *et al*. (2013) (body deep, greyish blue with yellow caudal fin and upper back and lower sides and head pinkish with yellow markings) and Carpenter and Niem, (2001) (caudal fin, upper caudal peduncle and posterior portion of back yellow; upper body, where not yellow, greyish blue; lower sides and belly white or pinkish; pectoral, pelvic, and anal fins white to pink; axil and upper base of pectoral fins black; dorsal fin yellow posteriorly and greyish blue anteriorly). *C. cuning* with white eye color was found in a depth of 7-30 m with a sand substrate, red-eye color in the depth of 2-7 m in coral reef areas and mixed white and red-eye color was caught in the depth of 2-30 sand and coral reef substrates.

Morphometric of C. cuning

C. cuning were caught by the fishermen using an arrow (spear gun) have size at least 17.9 cm of fork length (mature gonad stage III as recommended at Karimunjawa. 15 individuals out of 20 white eye individuals (75%) were below the recommended size. 10 out of 20 individuals (50%) with red-eye color were also caught outside the recommended size.

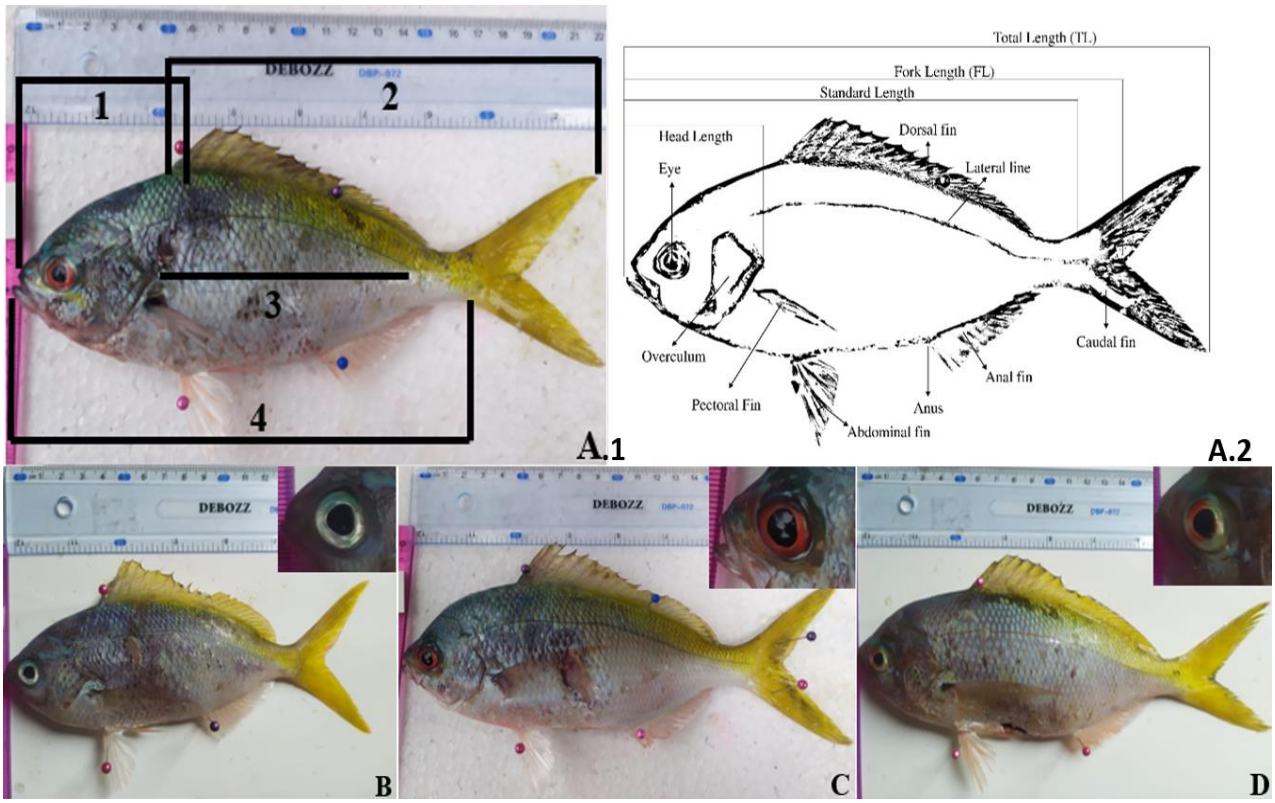

Figure 2. Morphology of *C. cuning* (A.1 and A.2), *C. cuning* with white eyes (B), *C. cuning* with red eyes (C) and *C. cuning* with white-red eyes (D).

This is presumably because the study was carried out during the full moon phase (Suhendar *et al*., 2016), where the catch was low even though it was in the second transition season (Kunarso *et al*., 2016). The number of fish caught below the minimum size can lead to a decrease in intrapopulation diversity.

Morphometric measurements were carried out on one side of the *C. cuning*'s body. The total length of all the 44 samples of *C. cuning* ranged from 15 to 29.4 cm. The total length of the white eyes samples ranged from 15-29.4 cm, the red-eye samples ranged from 17-26 cm and the white-red eye samples ranged from 22-24.6 cm (Table 1.). These results shows that the total length of *C. cuning* in this study is higher compared with the previous research in Muara Baru Market, North Jakarta were 9-15.5 cm (Zuhdi and Madduppa, 2020) and at the Nusantara Sungai Liat fishing port, Bangka Regency it is 12-18.3 cm (Gustomi *et al*., 2019). Morphometric of *C. cuning* varied in both present and previous studies. Morphometric variations are a response to the physical environment in which they live, such as adaptation to their habitat (Alamsyah *et al*., 2020). It also caused by sex and environmental conditions (Setiawan and Maduppa, 2020).

Genetic diversity of C. cuning

A total of 24 individuals *C. cuning* was successfully sequenced for the mtDNA control region markers. The results showed that *C. cuning* species have similarity between 97.07-99.27% with the sequences from the NCBI database. The genetic distance (the level of gene difference in a population or species) (Nei, 1987) between individual rage from 1- 3%. It shows that all samples are the same species where the limit for a genetic distance of the

Tabel 1. List of morphometric characters of *C. cuning* samples obtained from this study.

Morphometric characters (cm)	Mix data white, red and white-red eye color (cm)	White eye color		Red eye color		White-red eye color	
		Min-Max (cm)	Mean±SD	Min-Max (cm)	Mean±SD	Min-Max (cm)	Mean±SD
TL	15-29.4	15-29.4	19.7±3.6	17-26	21.3±2.8	22-24.6	23.5±1.2
FL	12.3-26.4	12.3- 26.4	16.8±3.12	13.9-21.8	17.8±2.4	18.8-20.5	19.7±0.9
SL	11-22.7	11-22.7	15.0±2.9	12-19.2	16.0±2.2	16.9-19.1	18.2±1.0
HL	3.1-6.3	1-2.6	1.7±0.42	1.2-2	1.7±0.3	2-2.1	2.0±0.1
BH	4.6-9.5	3.1-6.3	4.2±0.91	3.5-5.5	4.5±0.5	5-5.2	5.1±0.1
TBH	0.9-2.8	4.6-9.5	6.3±1.2	5.1-8.5	6.6±0.9	7-7.5	7.3±0.3
EDR	0.8-1.1	1.2-2	1.7±0.3	0.9-2.5	1.8±0.4	1.2-2.8	2.0±0.7
EDE	0.8-1.2	0.8-1.1	1.0±0.1	0.9-1.1	1.1±0.0	1.1-1.1	1.1±0.0
BW	1-2.6	0.8-1.1	1.0±0.1	0.8-1.2	1.1±0.1	1.1-1.1	1.1±0.0
LBDF	3.6-7.3	3.6-7.3	4.8±0.9	4-7	5.1±0.8	5.6-6	5.8±0.2
LBVF	3.3-7.9	3.8-7.9	5.1±1.0	3.3-6.5	5.3±0.8	5.2-6.1	5.7±0.5
LBAF	7.3-21.5	7.3-21.5	12.4±4.1	8.8-13	10.7±1.2	16.5-17.3	16.9±0.3
DFBL	7-13.5	7-13.5	9.2±1.6	8.5-12.3	10.4±1.2	11-13	12.0±1.1
VFBL	1.5-4.6	2-4	2.7±0.6	1.5-4.6	3.0±0.7	3-4.5	3.8±0.9
FBLAFL	3-7	3-7	4.3±0.9	3.2-5.6	4.6±0.6	4.8-5	4.9±0.1
PFBL	3-9.8	3.1-9.8	4.6±1.4	4.1-6.5	5.0±0.6	5-5.2	5.1±0.1
UCFL	4-6.8	4-6.7	4.7±0.8	4.3-6.8	5.3±0.7	5.1-5.5	5.3±0.2
MCFL	1.9-4	1.9-4	2.3±0.5	1.5-3	2.0±0.4	1.6-1.9	1.8±0.1
LCFL	4-6.8	4-6.7	4.7±0.8	4.3-6.8	5.3±0.7	5.1-5.5	5.3±0.2

Description : Total length (TL), Fork length (FL), Standard length (SD), Head length (HL), Body height (BH), Tail base height (TBH), Eye diameter (EDR), Eye distance (EDE), Body width (BW), Length before dorsal fin (LBDF), Length before ventral fin (LBVF), Length before anal fin (LBAF), Dorsal fin base length (DFBL), Ventral fin base length (VFBL), Fin base length anal fin length (FBLAFL), Pectoral fin base length (PFBL), Upper caudal fin length (UCFL), Middle caudal fin length (MCFL) and Lower caudal fin length (LCFL).

same species is 3% (Bucklin *et al*., 2011;(Leray *et al*., 2013). Genetic distance within the same eye color was 2%, meanwhile, the genetic distance between the eye color difference was 2%. These results indicate that different eye colors do not differentiate the genetic diversity in *C. cuning* species in Karimunjawa.

Based on the phylogenetic tree result, *C. cuning* was divided into 2 clades. 13 samples in clade 1 and 11 samples in clade 2. The bootstrap value were below 80% in both clades. It shows that the high similarity between the individual sequences. 20 haplotypes were observed from 24 samples. Eight haplotypes associated with white eyes (Haplotype 1-3, 5, 7-10), seven haplotypes with red eyes (Haplotype 11-17), three haplotypes with mixed eyes (haplotype 18-20), and two haplotypes shared between different eye color (haplotype 4 dan 6) (Table 3.). High haplotype variation in a population can indicate that the population has a high genetic diversity (supported by the value of h= < 0.9) due to high mobility and migration in larval and adult stages of the marine animals (Ackiss *et al*., 2013).

The results showed high haplotype diversity between different eye color (white eye color: h=1,000, red: h=0.978 and white-red: h=1,000 (Table 3). High haplotype diversity can be caused by large populations (protecting populations from genetic drift caused by overfishing) and random mating between individuals (interbreeding) (ensuring the stability of allele frequencies from one generation to the next) (Ardiana *et al*., 2021). The result also supported by Ackiss *et al*. (2013). The categories of genetic diversity values ranged from 0.8 to 1 high, 0.5 to 0.7 and low 0.1 to 0.4 (Nei, 1987). However, since this study only utilized a small number of samples, conclusion cannot be made easily. Further study using more sample number were recommended.

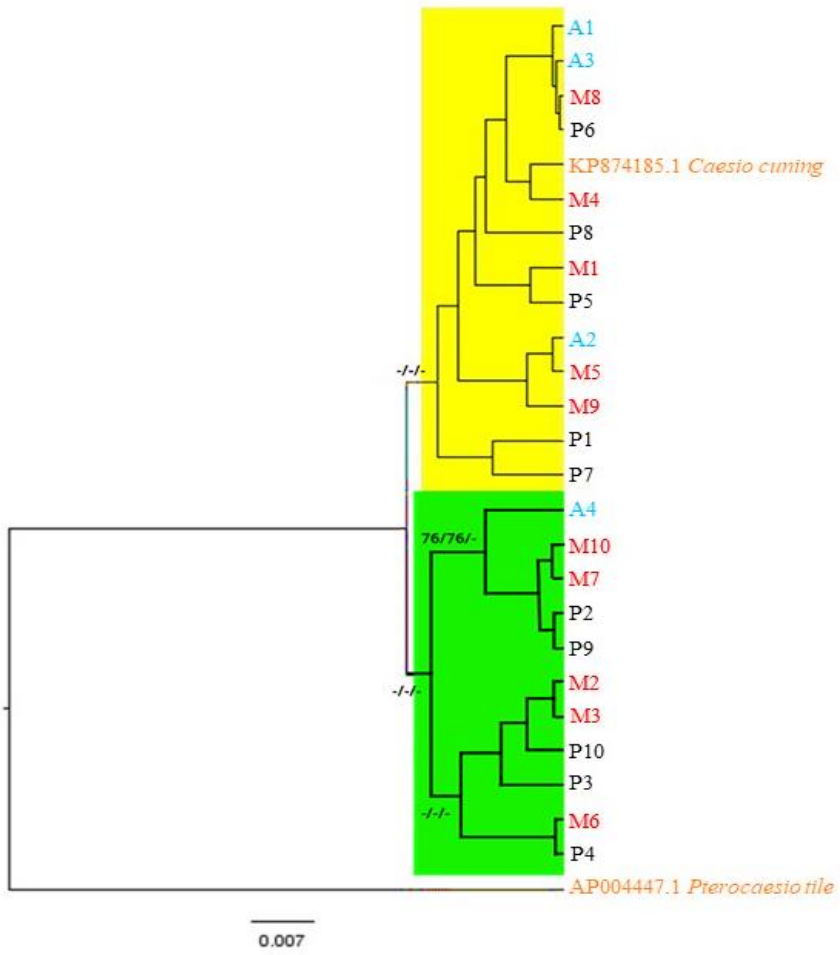

Figure 3. Phylogenetic tree using the neighbor joining method (first number), Maximum Likelihood (second number) and Bayesian posterior probability (third number) with ingroup (*Caesio cuning*) and outgroup (*Pterocaesio tile*) taken from GeneBank (www.ncbi.nlm.nih.gov). The writing color is black (white eyes), red writing color (red eyes), blue writing color (white-red eyes) and brown writing color (ingroup and outgroup).

Table 3. Number of haplotypes (N*h*), Haplotype Diversity (*h*) and Nucleotide Diversity (π) of *Caesio cuning* from Nyamuk Island, Karimunjawa. Haplotypese were named using "Hap_" and the code inside the bracket shows the sample number.

eye color	*h*	N*h*	π
white eyes (10 samples)	1,000	10 *haplotype*s of white eye color (Hap_1 (P1), Hap_2 (P2), Hap_3 (P3), Hap_4 (P4), Hap_5 (P5), Hap_6 (P6), Hap_7 (P7), Hap_8 (P8), Hap_9 (P9) and Hap_10 (P10))	0,021
Red eye (10 samples)	0,978	9 *haplotype*s of red eye color (Hap_4 (M6), Hap_6 (M8), Hap_11 (M1), Hap_12 (M2 dan M3), Hap_13 (M4), Hap_14 (M5), Hap_15 (M7), Hap_16 (M9) and Hap_17 (M10))	0,019
Eyes between white and red (4 samples)	1,000	4 *haplotype*s of White-red eye color (Hap_6 (A3), Hap_18 (A1), Hap_19 (A2) and Hap_20 (A4))	0,021

The results showed that the nucleotide diversity in white and white-red eyes was 0.021. The value of red-eye nucleotide diversity was lower at 0.019. The value of nucleotide diversity in all eye colors is lower when compared to previous research conducted in Karimunjawa (π =0.036) (Ackiss *et al.,* 2013). The value of nucleotide diversity as a result of the research is still in the range of *C. cuning* diversity values that have been carried out in various regions in Indonesia ranging from 0.017-0.036 (Ackiss *et al.,* 2013). The low number of nucleotide diversity shows the high similarity between the individuals DNA, meaning the population tend to be homogen in term of nucleotide diversity. The results of the analysis of Molecular Variance (AMOVA) showed that the genetic structure did not differ significantly between eye color in *C. cuning* with a *P*-value < 0.05.

Conclusion

The morphological observations of *C. cuning* showed that the upper bodies (from the snout tip to the dorsal fin) is grayish-yellow, the dorsal fin to the tail is yellow, the middle of the body is pale-white and the tail is yellow. The ventral body from the cheeks to the bottom-base of the tail, pectoral fins, abdomen, and anus are pink. The *C. cuning* samples from the Nyamuk Island, Karimunjawa had a size of 15-29.4 cm, with 75% of white eye individual and 25% red eye individual caught below the standard size. High diversity of *C. cuning* was seen in the genetic distance between each individual (0%-3%) and between eye colors 2%. The high haplotype diversity (0.978-1,000) were reported with 20 haplotype identify from the 24 individuals samples. The nucleotide diversity was low (0.01902-0.02180), indicate the high similarity in the DNA sequences between individual within population. The results of the AMOVA analysis showed that the genetic structure was not significantly different between the eye color of *C. cuning*. In the future, it is hoped that there will be strict regulations and controls regarding the size of *C. cuning* that can be caught, this study is expected to provide a basic data to protect the abundance and genetic diversity of *C. cuning* in Karimunjawa.

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