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SUCCESSFUL GENETIC CHARACTERIZATION OF BULLET TUNA (*Auxis rochei*) USING MICROSATELLITE MARKERS IN KARANGASEM BALI

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ABSTRACT

Bullet Tuna (*Auxis rochei*) is classified under the neritic tuna group, which plays an essential role in small-scale fisheries in Karangasem Bali. The increasing catch of bullet tuna indicated its stock probably under threat. Therefore, genetic characterization is often required as the first step before building a conservation program. This study aims to categorize bullet tuna DNA using microsatellite. Of all five loci used, all were high polymorphism-type, with the number of alleles per locus varied between 18-27. Successful PCR (Polymerase Chain Reaction) created relatively high DNA concentration, ranging from 27.050 to 237.05 ng/ul, with a DNA purity level ranging from 2.073 to 2.239. Overall, the stock condition allegedly still in good condition, marked with high genes diversity (H_o=0.367-0.767 and H_e=0.934-0.966). All loci used can be amplified and well described.

Keywords: Neritic tuna; microsatellite; DNA; genetic diversity

INTRODUCTION

Neritic tuna is dominantly caught by small-scale tuna fisheries and plays a vital role for coastal communities (Naderi, 2016). Auxis, as one under its genus, is a pelagic fish and commonly found in tropical Indo-Pacific waters. It posses a high migration rate and forms large schools that sometimes mix with other Scrombidae species (Agus, 2017). According to Ilhamdi *et al*., (2016), Auxis distribution areas in Indonesian waters include the waters of Sumatra, Java, Bali to Nusa Tenggara.

Bullet tuna (*Auxis roch*ei) is classified under the Auxis genus, however, it oftenly mistaken with frigate tuna (*Auxis thazard*) and sometimes lumped together as one species group. The Indian Ocean Tuna Commission (IOTC, 2019), reported that currently, there is no quantitative stock assessment for bullet tuna in the Indian Ocean, this is due to the lack of bullet tuna data from individual IOTC members. The total number of Auxis catches in Karangasem in 2019 was 8,442.50 tons. In 2018 catches increased from 16,000 tonnes to 32,000 tonnes, largely due to increased catches in Indonesia's small-scale fisheries (IOTC, 2019). Bullet tuna is a species that are widely caught in Indonesia, although it is still in good condition or not in overfishing condition, this condition will be severe if the bullet tuna fisheries resources are not managed properly.

Genetic characterization is the first step in a conservation program. These genetic characters can be used as a guide for genetic diversity which has an important meaning in the stability and resilience of a population (Ferguson et al., 1995). Genetic information can also support sustainable management of fish stocks (Santos et al., 2006). In understanding the genetic character of a population, several methods are used to identify a species. Initial identification was based on morphological similarity and has now been improved on molecular analysis (Puja and Sulabda, 2009). Molecular analysis was carried out with the help of molecular markers. According to Park & Moran (1994), genetic molecular markers can be categorized into 2 types, namely nuclear DNA markers, and mitochondrial DNA (mtDNA).

One of the cell nuclear DNA markers is microsatellite markers. The advantages of microsatellite are high degree of polymorphism, visible band pattern profile that can be easily interpreted as alleles in a locus, is codominant allele and very accurate because the allele size can be distinguished up to a degree of one base pair (1 bp). Catanase et al. (2007) found eight primers that could be used for bullet tuna. The existence of information on the characteristics of bullet tuna is expected to be information to support sustainable and sustainable management of fish stocks.

RESEARCH METHODS

Sampling was carried out in September 2020 at PPI Karangasem Bali (Figure 1), with a total of 30 samples, Mahmud (2011) states that for research using statistical data analysis, the minimum number of samples is 30. It was taken in the form of slices of meat from the pectoral to dorsal part of the fish. This section was chosen because it has thick enough muscle mass to make it easier to take muscle tissue (Ariyanti & Sianturi, (2019). The tissues were obtained using a cutter and put into a labelled bottle filled with 70% alcohol. DNA extraction was carried out using the DNeasy Blood and the Tissue Mini Kit (*Qiagen*) and the concentration measured using a nanophotometer

Analisis hubungan panjang-bobot ikan dit Amplification in the nucleus of DNA cells was carried out using 5 microsatellite DNA primers, namely: Aro1–10 (Forward: HEX 5'- CCC-ACC-CAC-CCA-GCC-CTT-CC -3'Reverse: 5'- TCA-TCC-CTT-GGT- ACC-TGC-GTT-TCT-ATT-TTC-3'); Aro 1-59 (Forward: FAM 5'- CTA CGT GCA TGT CAG GTT GGA TTC A -3'Reverse: 5'-TTG TCT AAG TTT TTC TCC TGT GCT TTT ATT GGT C -3'); Aro 2-15 (Forward: HEX 5'- CCA-TTT-TTT-CCT-CAA-ACC-AAA-CTG-CCA-TT-3'Reverse: 5'- GTG-GGT-GTG-TTG-TAA-ACT- CTG-AGC-AGG-TGT-3'); Aro 3-37 (Forward: 5'- CTT-

TAT-ATT-GGC-AAG-AGT-ATT-GTT-CAC-TCA-TTT-3'Reverse: HEX 5'-TTG-AGC-CCA-CAT-GGT- TGA-TAG-CAG-GAT-3'); Aro 4-13 (Forward: HEX 5'- AAT-CCA-TCC-ATC-ACA-CAC-AGC-CAG-A-3'Reverse: 5'-TTA-AGT-GTA-TGT-GTT-GTA-GAG- ACA-GAG-CGA-GA -3') (Catanese et al., 2007).

Polymerase Chain Reaction (PCR) amplification process was conducted inside the PCR tube contained 12.5 µL MyTaq HS Red Mix , and then added with NFW (Nuclease-Free Water), primer F, primer R and DNA template (total concentration 25 µL) (Zedta & Setyadji, 2019). The PCR tube was inserted into a DNA cycler machine (Labcycler-Sensoquest). The amplification temperature configuration used is as follows: Pre Denaturation at 95˚C for 2 minutes for 1 cycle, followed by 34 cycles of denaturation at 95˚C for 30 seconds, Anneling with temperature and time based on the journal, extension at 72˚C for 45 seconds and one final extension cycle at 72˚C for 5 minutes.

Microsatellite loci polymorphisms were screened using the QIAxcel fragment analysis tool and using a high resolution DNA Screening gel cartridge with a size marker of 25 bp - 500 bp with an alignment marker measuring 15 bp/600 bp (Qiagen, 2017). The number of alleles, allele frequency, heterozygosity and variability (Ho/He) were calculated using the Arlequin version 3.5 program (Excoffier et al., 2005) and the polymorphism values for each locus were obtained with the help of the GeneAlex 6.5 software application.

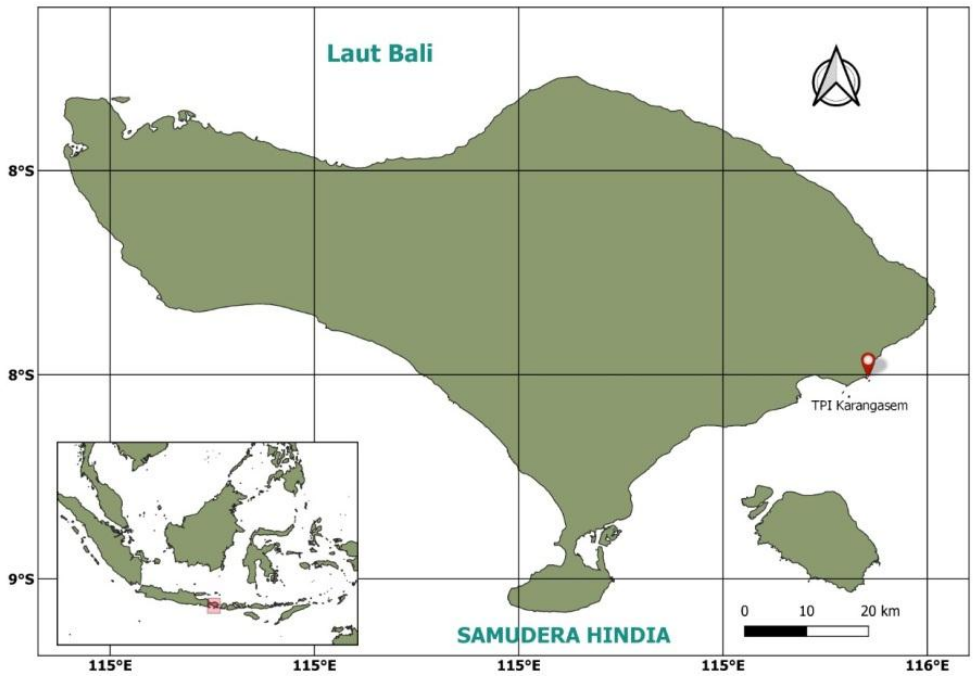

Figure 1. Map of Research Site

RESULTS AND DISCUSSION

A total of 30 samples of bullet tuna tissue was successfully extracted using the DNeasy Blood and the Tissue Mini Kit (extraction kit), according to Ariyanti and Sianturi (2019), this method have several advantages, namely a shorter processing time, simpler, non-toxic and high-purity DNA harvest. In addition, according to Hajibabaei et al., (2006) extraction using a kit is safer and minimizes contamination. This extraction method is also recommended for specimens undergoing a storage (preservation) process. The value of DNA concentration obtained was relatively high, ranging from 27.05 to 237.05 ng/ul. Which is higher compared to Ariyanti & Sianturi (2019) study, in which grouper DNA isolated using an extraction kit ranged from 6.25 to 16.25 ng/ul. The right concentration of template DNA will produce a good amplification product (Mulyani et al., 2011). Since, the DNA concentration values have relatively high variation, so it is necessary to dilute the DNA first by adding NFW (Nuclease Free Water) to the desired concentration (Mulyani et al., 2011).

The level of DNA purity is correlated with the quality of the DNA. Measurement of DNA concentration with ananophotometer was carried out at a wavelength of 260 nm, while protein was measured at a wavelength of A280. The purity of the DNA solution can be calculated by comparing A260 nm with A280 nm (Sentani et al., 2017). DNA is declared to have a purity level of 85% if it has a ratio value of A260/A280 (optical density) ranging from 1.8–2.0 (Sambrook and Russell, 2001). The purity level of bullet tuna DNA in this study, namely for A260/A280, ranged from 2.07 to 2.24, meaning that the DNA obtained could be categorized as having a high level of purity.

The bullet tuna DNA samples were then amplified using five specific loci, namely Aro1–10, Aro1–59, Aro2–15, Aro3–37 and Aro4–13. Each locus had different annealing temperature and annealing time (Table 1). Based on Table 1, it can be seen that the five loci used had the same annealing time of 30 seconds but the annealing temperature used was different at each locus. The PCR mix used by all loci had the same amount of 25 ul with a PCR product of 3 ul. Then repeated 34

cycles, with a total PCR time of one hour and fifteen minutes. In contrast to Zedta & Setyadji (2019), which used 4 ul for DNA samples of frigate tuna and bullet tuna in PCR mix to produce clear DNA bands.

PCR products that have been electrophoresed using agarose and give positive results, are then screened using the QIAxcel Advanced fragment analyzer. The electrophoresis process using QIAxcel will produce sample electrogram information with base pair lengths displayed in the form of images and numbers (Figure 2).

Table 1. PCR Optimization Results from Five Microsatellite Locus

Locus	Time (seconds)	Temperature (°C)	PCR *Mix* (ul)
Aro1–10	30	55	25
Aro1–59	30	55	25
Aro2–15	30	54	25
Aro3–37	30	50	25
Aro4–13	30	54	25

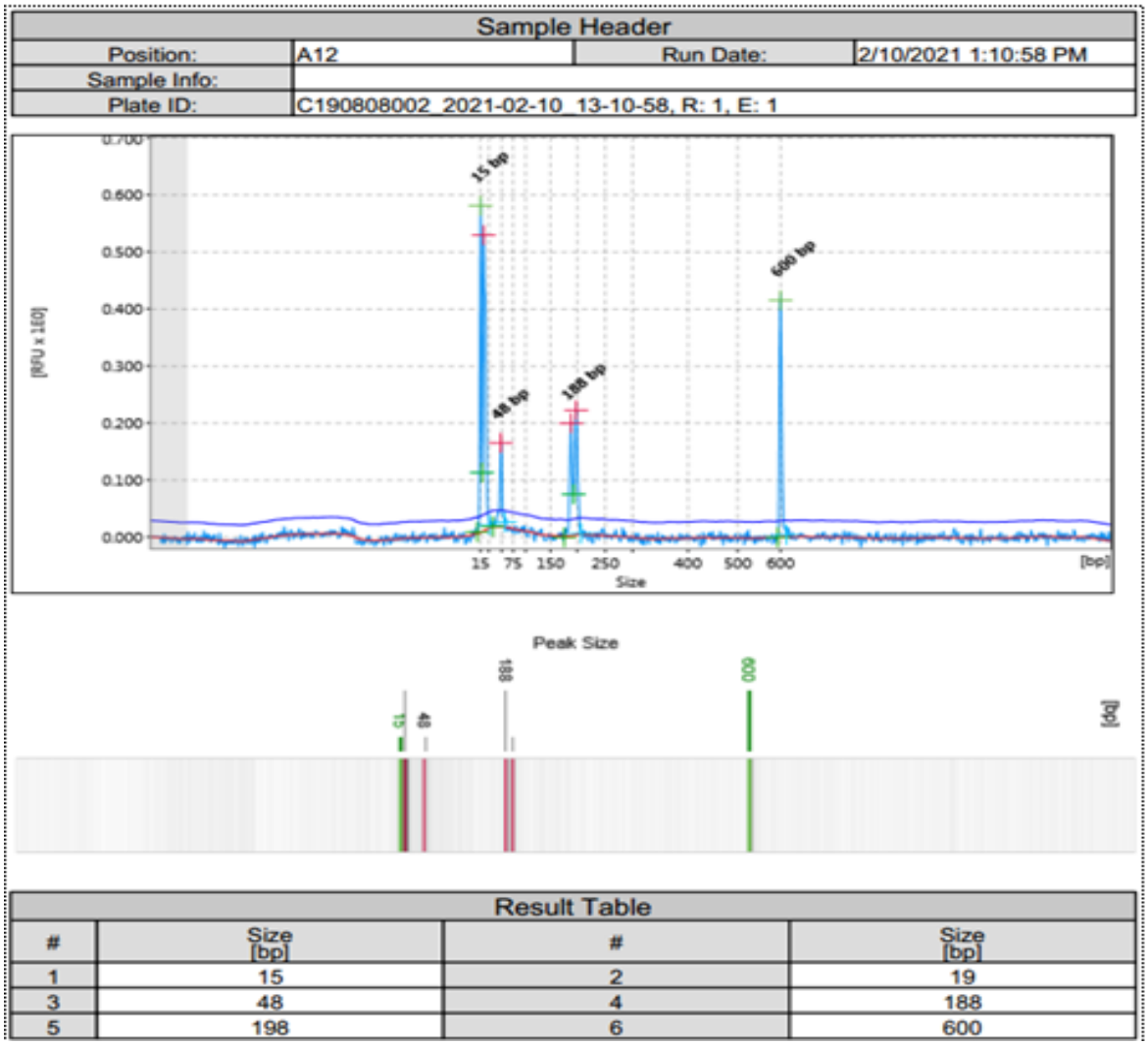

Figure 2. QIAxel Advanced Screening Results

The observation of genetic characteristics of bullet tuna was carried out on 150 PCR products resulting from the extraction of 30 samples of bullet tuna obtained from the PPI. Karangasem. PCR products that have been electrophoresed been giving rise to polymorphism properties in bullet tuna. Based on data on the number of alleles obtained at each microsatellite locus, more than one allele was found. This shows that the loci used are polymorphic (Ulfa & Retnoningsih, 2010). The five microsatellite loci successfully amplified DNA fragments with high polymorphism. The value of polymorphism can be determined by the frequency of occurrence of alleles at each locus (Lestami A., 2019).

Polymorphism can give an idea of whether the life of a population is safe or threatened. A population with low polymorphism tends to have long-term life threatened, so it is necessary to select appropriate population-saving measures in the future (Wandia et al., 2009). Information on the resulting allele length (bp) is presented in Table 2.

The number of bullet tuna DNA alleles obtained from Karangasem varied at each locus. The Aro1-10 locus had a total of 21 alleles with an allele length of 184-244 bp. At this locus there are 10 heterozygous alleles and 20 homozygous alleles. The observed heterozygosity (Ho) was 0.367 and the expected heterozygosity (He) was 0.942. The Aro1-59 locus had 18 alleles with an allele length of 120-170 bp. At this locus there are 21 heterozygous alleles and 9 homozygous alleles. For the observed heterozygosity (Ho) of 0.700 and the expected heterozygosity (He) of 0.940. The Aro2-15 locus had 18 alleles with an allele length of 158-206 bp. At this locus there are 24 heterozygous alleles and 6 homozygous alleles.

Meanwhile, the observed heterozygosity (Ho) was 0.767 and the expected heterozygosity (He) was 0.934. The Aro3-37 locus had 27 alleles with an allele length of 106-190 bp. At this locus there are 23 heterozygous alleles and 7 homozygous alleles. Meanwhile, the observed heterozygosity value (Ho) is 0.700 and the expected heterozygosity (He) is 0.966. The Aro4-13 locus had 18 alleles with an allele length of 120-158 bp. At this locus there are 19 heterozygous alleles and 11 homozygous alleles. Meanwhile, the observed heterozygosity (Ho) was 0.600 and the expected heterozygosity (He) was 0.945.

Table 2. Allele Length (bp) and Average Number of Alleles per Microsatellite Locus

Locus	n	Allele length (bp)	k	Ho	He
Aro1–10	30	184-244	21	0,367	0,942
Aro1–59	30	120-170	18	0,700	0,940
Aro2–15	30	158-206	18	0,767	0,934
Aro3–37	30	106-190	27	0,700	0,966
Aro4–13	30	120-158	18	0,600	0,945
Rerata	150		20	0,627	0,945

Information: n = Number of PCR Products, k = Number of Alleles, Ho = Observation Heterozygosity, He = Expected Heterozygosity

The average number of alleles obtained is almost the same when compared to the average number of alleles identified in bullet tuna populations in the Mediterranean, Atlantic and Pacific, which ranges from 13-27 alleles per locus. Similar to the number of alleles, the Expected Heterozygosity (He) value obtained in this study is also not much different from the He obtained by Catanese et al., (2007) while the Ho obtained is smaller than the previous study. The mean value of observed heterozygosity (Ho) obtained in this study is smaller than the expected heterozygosity (He). The observed heterozygosity value and the expected heterozygosity value can be used to estimate the inbreeding value in a population (Ulupi et al., 2014). Nei (1987) explained that the range of expected heterozygosity values (He) for the high genetic diversity category was 0.8-1.0 so that the genetic diversity of the bullet tuna landed in Karangasem Bali was included in the category of high genetic diversity.

The allele frequencies produced by the five microsatellite loci used can be seen in Figure 1. From Figure 1, it can be seen that the aro2-15 locus has the highest allele frequency compared to the other four loci. However, all the loci used can be amplified and well described

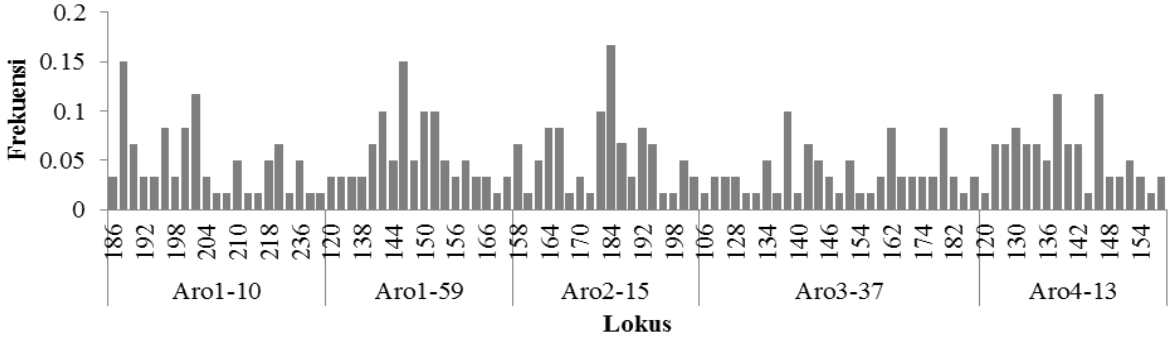

Figure 3. Allele Frequency of Bullet Tuna Genetic Sample

CONCLUSION

The conclusion of this study is that the number of alleles per locus varies between 18-27 alleles. The DNA concentration obtained was relatively high, ranging from 27.050 to 237.05 ng/ul with a DNA purity level ranging from 2.073 to 2.239. The locus used has a high polymorphism with Expected Heterozygosity (He) values ranging from 0.934 to 0.966 and Ho values ranging from 0.367 to 0.767. All loci used can be amplified and well described.

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