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DNA BARCODING APPROACH: ASSESSING DIVERSITY OF ECONOMICALLY IMPORTANT ANCHOVY ON THE NORTHERN COAST OF JAVA, INDONESIA

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ABSTRACT

Anchovy is one of the economically important fish in the western part of the northern coast of Central Java. The production of anchovies extends along the coast from Brebes Regency to Batang Regency. Therefore, this study aims to determine the diversity of anchovies on the northern coast of Central Java (Brebes, Tegal, Pemalang, Batang, and Kendal), using a molecular approach. This study was conducted from April to September 2019 in the Laboratory of Tropical Marine Biotechnology, Faculty of Fisheries and Marine Science, Universitas Diponegoro. The descriptive exploratory method was applied in this study with a random sampling technique, while the cytochrome oxidase I (COI) gene was used for molecular identification. An anchovy sample molecularly identified from each region was found by sampling, including BB 3, PML 2, BTG 1, BTG 2, BTG 4, TGL 2, TGL 4, KDL 1, and KDL 4. The base-pair length of 10 anchovy samples from the COI gene amplification result was 600-700 bp. The sequencing and alignment results of the BLAST analysis showed that the ten anchovy samples were included in the type of *Encrasicholina heteroloba*, *Stolephorus commersoni*, *Stolephorus waitei* and *Atherinomorus* sp., which ranged from 98 to 99% similarity in location. Therefore, it is concluded that there were at least four different species of anchovy on the northern coast of Java. However, further research is suggested to determine the genetic variation of each species for better fisheries management.

How to Cite

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INTRODUCTION

The exploitation rate of fisheries resources on the northern coast (Pantura) of Central Java is subjected to overfishing. This is according to the research results of the National Committee on Fish Stock Assessment (2011). Overfishing causes fishermen to seek another potential fishing ground. Moreover, the behaviour of fishermen who are greedy to catch as much as they can without considering sustainable fisheries by modifying their fishing gear to be larger and with smaller mesh sizes, which worsens the condition.

Marine fisheries production in Central Java Province broadly consists of pelagic fish, demersal fish, and non-fish groups (shrimp, crustacea, and molluscs). Anchovy is one of the abundant catches widely used by the population (Department of Marine and Fisheries of Central Java, 2011).

Anchovy is beneficial to prevent osteoporosis, increase intelligence, improve children's health, and prevent various diseases. To prevent osteoporosis, a person needs 1 (one) gram of calcium per day. According to the research conducted by Haumahu (1995) and Sankar et al. (2013), anchovy contains high calcium and fluorine at around 500 mg per 100 grams of anchovy weight. Thus, calcium requirements can easily be met by anchovies from Indonesian coastal waters. In the eastern region of Indonesia, anchovies are also used extensively as live bait for the skipjack tuna fishery. Therefore, the demand for anchovy consumption is getting higher, while the production is getting lower at 24% from 2000 to 2004. This condition is influenced by the increase in the catch and the fishing gear used (Directorate General of Capture Fisheries - DMF, 2004). The demand for anchovy products is increasing, and both local and national processing companies are expanding (Irnawati et al., 2018).

Anchovy is included as a renewable resource, similar to other fish resources. In other words, when the fish resources are partially caught, the remaining resources are capable of renewing themselves by breeding (Nikijiuluw, 2002; Imran and Yamao, 2014). This trait of anchovy leads to the need for an effort to manage anchovy resources so that they can be sustainably exploited. Essentially, a thorough assessment of the progress made in the fishing attempt in a certain area is required. It includes the biological aspect (fish resources as the fishing target), resources aspect to support the success of the fishing operation, technical aspects such as fishing gear, social aspect related to labour, and economic aspect (Syakila, 2009).

Recently, DNA barcoding has been widely utilized as a global bio-identification system for animals. Taxonomic ambiguity prevails for several fish genera⁄species, and proper identification is crucial for management and trade. The exploitation of DNA sequence diversity among species as DNA-based approaches to taxon diagnosis can be used to identify fishes and resolve taxonomic ambiguities, including the new/cryptic species discovery (Hebert et al., 2003). The barcode system originated from the sequence diversity in a single gene region (a section of the mitochondrial DNA cytochrome c oxidase I gene, COI). New products and specimens can be identified by comparing their DNA barcode sequences with the existing sequence reference library. According to this method, COI sequences produced from the species that were identified morphologically might be used as reference DNA barcodes for the appropriate species. On the basis of similarities to the sequence in question, these reference barcodes would later be used to identify unidentified specimens (Gangan et al., 2019). The lack of information on anchovy DNA barcoding encourages a study on anchovy management on the northern coast of Central Java for the resources to be sustainably utilized.

MATERIALS AND METHODS

Specimen collection

A survey method was used in this study with simple random sampling as the sampling technique, allowing the sampling to be done randomly so that each member of the population has an equal opportunity to be in the sample (Notoarmodjo, 2002). The sample was collected once from the ten sampling points along the coast in the north of Java, as shown in Fig. 1. The ten coasts are located in several cities, including Brebes, Tegal, Pemalang, Pekalongan, Batang, and Kendal. Each sample collected at each sampling point was stored in a cool box and frozen to prevent tissue damage before it was analysed further in the Integrated Laboratory, Universitas Diponegoro. Then, all samples were morphologically identified, resulting in 15 different species. Subsequently, the fifteen anchovy species were verified using a molecular approach, called DNA barcoding.

DNA barcoding approach

The anchovy samples stored in a frozen condition were processed using an appropriate method for DNA extraction. A piece of white meat of anchovies was taken for DNA extraction (Huang et al., 2021). The Chelex method was applied for the DNA extraction of the anchovies with the following steps: (1) around 25 mg of the muscle meat of the anchovies was inserted into a 1.5 ml microtube pre-filled with 20% Chelex 100; (2) the mixture of meat and Chelex was heated at 95 °C for 45 minutes and homogenized with a vortex for 20 minutes (to ensure that the meat and Chelex solution were perfectly mixed); (3) the mixture of the second-step product was centrifuged at 2000 rpm for 10 minutes; (4) supernatant was extracted and stored at -4 °C (Susilowati et al., 2015; Pringgenis & Susilowati, 2016). The concentration and purity of the DNA extraction results were evaluated using nanodrop by observing the A260/280 value at around 1.8-2.00.

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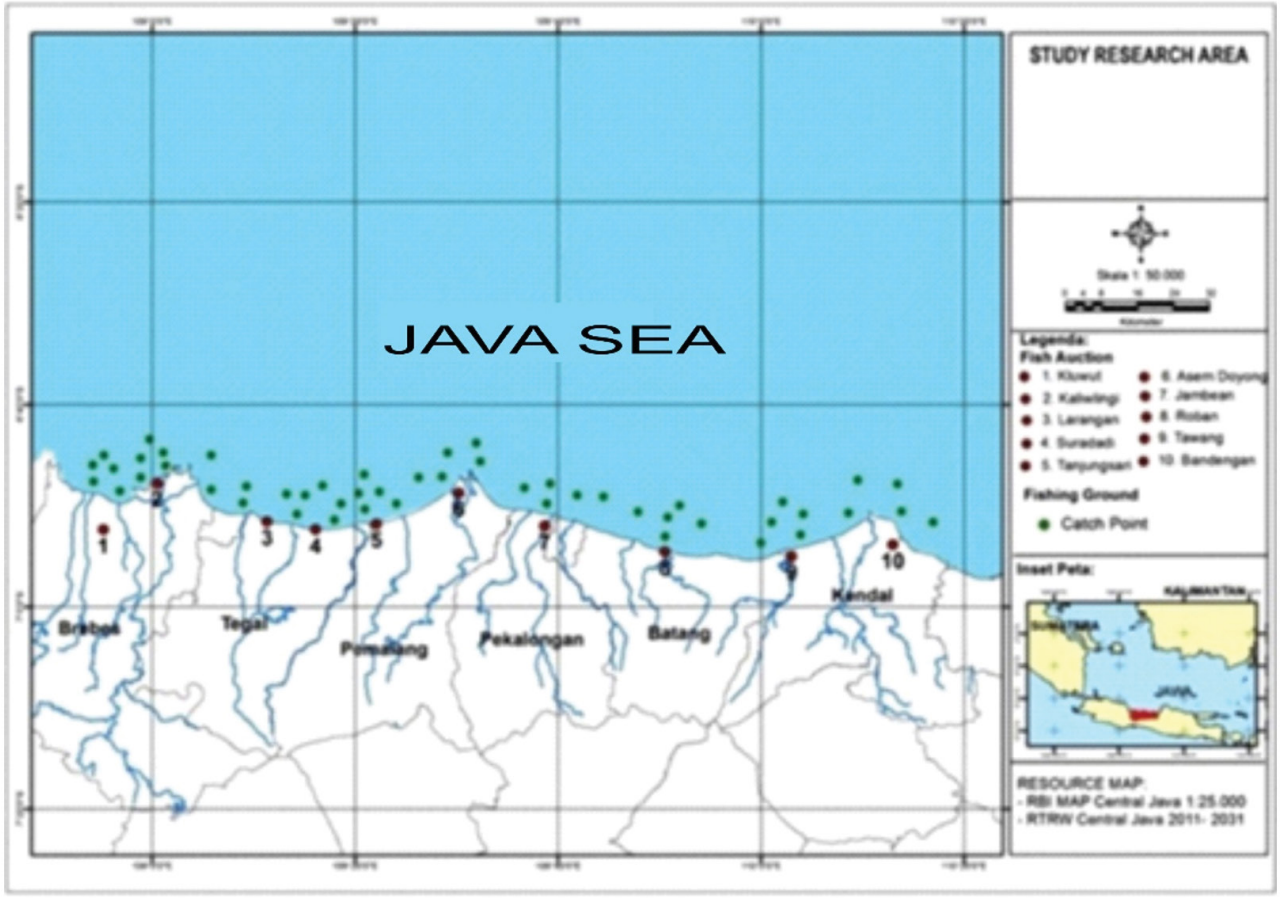

Fig 1. Map of sampling locations as indicated by the point and number. Sampling points 1 and 2 were located in Brebes Regency, 3 and 4 in Tegal, 5 and 6 in Pemalang Regency, 7 in Pekalongan, 8 in Batang Regency, and 9, 10 in Kendal Regency.

The gene marker used for molecular identification was *cytochrome oxidase* I gene, which had a fragment length between 600 and 700 bp (Fahmi et al., 2020). Fish F1 and Fish R1 were used as the set primer, with the following base sequences for each primer: Fish F1: 5'- TCA ACC AAC CAC AAA GAC ATT GGC AC-3'; Fish R1: 5'- TAG ACT TCT GGG TGG CCA AAG AAT CA-3' (Ward et al., 2005).

During gene amplification, the following materials were used in the composition of the PCR mixture (PCR *cocktail*): GoTaq®Green Master Mix Promega (12.5 µL), Fish F1 primer (1 µL), Fish R1 primer (1 µL), DNA template (1 µL), and Nuclease-Free Water (9.5 µL). Therefore, a total of 25 µL of the PCR *cocktail* volume was used during amplification. To amplify the PCR *cocktail*, several stages of the program were performed. The PCR tubes were positioned in a thermal cycler programmed with the following PCR protocols: initial step at 94 °C for one min, 35 cycles at 94 °C for 15 s, 54 °C for 15 s and 72 °C for 30 s., and 8 min of final extension at 72 °C was preserved (Ude et al., 2020). The PCR products were evaluated with 1% agarose to observe the existence of the target gene ribbons with a base length of 600-700 bp through electrophoresis at 100 volts for 30 minutes, and visualized using UVIDoc.

The visualization results of the PCR products, which showed that the base length was on target at 600-700 bp, were sent to the 1st Base, Malaysia via Genetika Science for sequencing. *Single pass sanger sequencing* was used as the sequencing method.

BLAST homology and phylogenetic tree

The sequencing results were analysed using MEGA X program (Kumar et al., 2018) by combining two sequences of the amplification results from both forward and reverse primers to form a consensus sequence. Then, the consensus sequence was matched with other sequences listed in the gene bank (https://www.ncbi.nlm.nih.gov/) to determine the similarity with other species using BLAST homology (https://blast.ncbi.nlm.nih.gov/). Species with the highest similarity index were taken as the reference species to construct a phylogenetic tree. The phylogenetic tree construction used neighbour-joining mode with 1000 times bootstrap evaluation (Ayuningrum et al., 2019).

RESULTS AND DISCUSSION

Sample morphology identification

Anchovy can commonly be found in coastal and estuary regions in varying sizes. Anchovy is commonly small,

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its size varies between 6-9 cm. An overview of anchovy morphology is the forking caudal fin which does not merge with the anal fin, and the abdominal spines which only consist of pectoral and ventral fins, colourless or slightly reddish. Anchovy possesses an elongated and oval body shape (fusiform) or laterally compressed, with a silvery-white line on the side of the body extending from the head to the tail. It has easy-to-lose small and thin scales, and the upper jaw bones extend to the gill slits. The teeth are located on the jaw, palate, pterygoid, and tongue (Saanin, 1984). The morphology of anchovies collected successfully from the northern coast of Java was not completely different from the common features mentioned above. The identification results of the anchovy samples from the northern coast of Java are presented in Fig. 2.

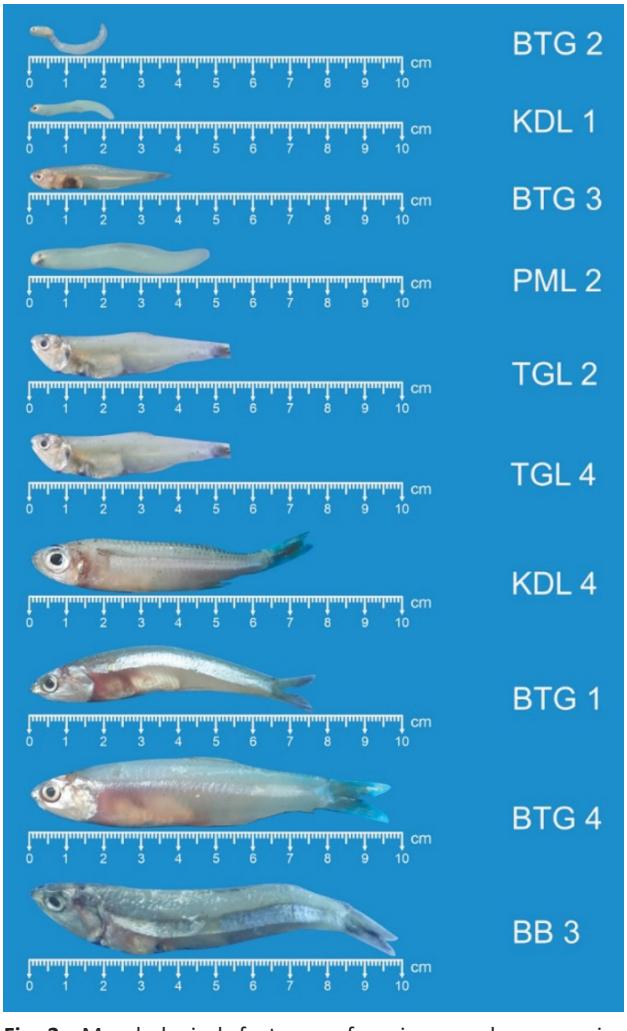

Fig 2. Morphological features of various anchovy species collected along the northern coast of Java. The sample codes showed different regions, including BTG (Batang), KDL (Kendal), PML (Pemalang), TGL (Tegal), BB (Brebes).

DNA barcoding approach of anchovy

Mitochondrial DNA or mtDNA is DNA derived from the mitochondrion, a DNA structure in the cell cytoplasm that cannot be found in the nucleus. Mitochondrial DNA is maternally inherited, i.e. entirely inherited from the "mother" line; therefore, the descendant of a population will possess a similar mtDNA to her/his mother. In addition, it shows that mtDNA changes from generation to generation will be smaller than the nuclear DNA, by approximately 50% for each generation (Aliah, 2009). Due to the ease of measurement for the mutation rate, mtDNA is considered a proper instrument to trace the family lineage. Furthermore, mitochondrial DNA holds the potential to be used as a system to observe the interspecies or intraspecies (intraspecies with a close relationship) genetic relationship. The high polymorphism degree exhibited by mitochondrial DNA makes it suitable to be used in the study of the genetic diversity of the organism. MtDNA is used to study DNA barcoding by targeting its gene, cytochrome oxidase I (COI) gene.

COI gene was selected due to the short fragment of COI and can be used as a marker of a variation that can accurately identify a variety of animals to the species level (Hebert, 2003). The common primers used to amplify this gene include Fish F1: 5'- TCA ACC AAC CAC AAA GAC ATT GGC AC-3'; Fish R1: 5'- TAG ACT TCT GGG TGG CCA AAG AAT CA-3'. In addition, the COI gene is considered to be appropriate for determining the genetic diversity in a species or between populations. The COI gene length that had been successfully amplified was around ± 650 bp (base pair), as evident in the quantitative test using agarose gel electrophoresis (Fig 3). The electrophoresis result showed that only the DNA ribbons of the 10 anchovy samples were observed clearly.

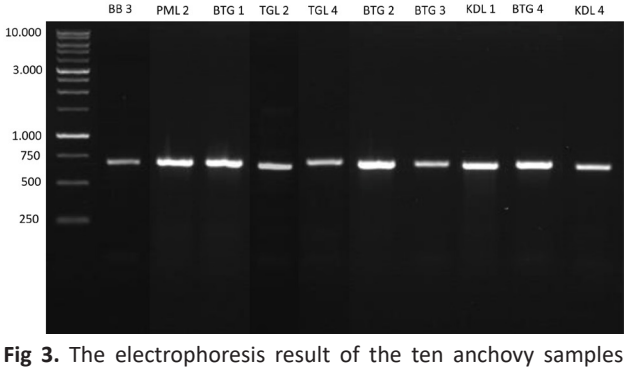

Fig 3. The electrophoresis result of the ten anchovy samples from various locations on the northern coast of Java

The thickness of the DNA ribbon from each sample showed different amplicon concentrations. The thicker the DNA ribbon, the higher the concentration, resulting in the sequencing process running effectively. Several factors affected the success of a target gene amplification process, including the concentration of the components in the PCR mix, primer of the target gene, amount and purity of the DNA template, and technical and non-technical issues such as contamination (Sjafaraenan et al., 2018).

The sequencing result showed that amplification using either Fish F1 or Fish R1 primer resulted in a properly-read chromatogram. The sequence combination or

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consensus sequence from both primers resulted in an intact sequence with a length of 600-700 bp in 10 anchovy samples. The analysis of the alignment of the sequence with the sequence data of the whole anchovy species in the world using the BLAST feature revealed that several fish from different locations were included in the same species, including those from Brebes, Pemalang, Batang, and Tegal. Also, more than one species of anchovy can be found in one location, such as 3 species in Batang, including *Encrasicholina heteroloba*, *Stolephorus waitei*, and *Stolephorus commersonnii*. The detailed BLAST analysis results are presented in Table 1.

The similarity value ranged from 98 to 99% and showed a high similarity level between the species of anchovy samples and those listed in the gene bank. Sofro (1994) states that a population with a high level of closeness of kinship relationships has many morphological and genetic similarities, and is influenced by environmental circumstances. The gene mutation is one of the most contributing factors in creating new species. The gene mutation is a factor leading to the genetic diversity that resulted in life diversity.

The kinship level of the anchovy species was analysed using a phylogenetic tree, a method for determining the evolution of a species and the level of kinship. Anchovy species with similar DNA chains will form a proximity branch and form a large group (clade). Phylogenetic analysis of a species can be done using mitochondrial DNA sequence on the morphological characters and genes inside and outside of the body. The application of mitochondrial DNA sequences clarifies the indistinct evolution relationship of species due to the morphological variation (Avise, 1994). Mitochondrial DNA sequence showed the DNA variation of a population, the breeding changes of an individual, and the isolation of the population (Liu et al., 2000; Tjong et al., 2007). The comparison between 10 COI gene sequences of anchovies on the northern coast of Java and 12 sequences of anchovies in the Gene Bank resulted in the phylogenetic tree displayed in Figure 4, which was made with the MEGA X program based on the algorithm of neighbour-joining trees using Kimura2parameter (K2P) evolution model. The construction results of the phylogenetic tree showed that 10 COI gene sequences of anchovies from the northern coast of Java were not categorized in accordance with the landing areas respectively, but were combined into two large sub-clades.

There are two large groups (clades) of anchovy, the genus *Stolephorus* and genus *Encrasicholina*. The genus *Stolephorus* clade was divided into 2 sub-clades, species *S. waitei* and *S. commersonii*; while the *Stolephorus* clade formed several branching categories, indicating that the *Stolephorus* clade consisted of different populations. Although BTG 3, KDL 1, BTG 2 sequences were located in different branching, the *root* remained the same, which was *Stolephorus waitei*. In addition, an anchovy species from the genus *Stolephorus*, namely *Stolephorus commersonii*, with a code of BTG 4 was identified in Batang coastal waters. The following large clade consisted of two different genera, *Encrasicholina* and *Atherinomorus*. The genus *Encrasicholina* was found in the coastal waters of Tegal, Brebes, Pemalang, and Tegal with the species *E.heteroloba*. Meanwhile, *Atherinomorus* sp. was found in Kendal coastal waters.

Overall, on the northern coast of Java, particularly in the Kendal, Batang, Pekalongan, Pemalang, Tegal, and Brebes regions, there were four different species of anchovies, as presented in Table 1. The most dominating anchovy species distributed in each region was *E.heteroloba* species, which was found in almost every location except Kendal and Pekalongan.

Table 1. The BLAST homology of anchovy fish samples from different locations on the northern coast of Java

No.	Isolate code	BLAST results	Similarity	BP length
1	BB 3	*Encrasicholina heteroloba* (Rüppell, 1837)	99.15%	713
2	PML 2	*Encrasicholina heteroloba*	99.57%	713
3	BTG 1	*Encrasicholina heteroloba*	99.43%	711
4	TGL 2	*Encrasicholina heteroloba*	99.7%	680
5	TGL 4	*Encrasicholina heteroloba*	99.85%	682
6	BTG 2	*Stolephorus waitei*	99%	711
7	BTG 3	*Stolephorus waitei*	98.98%	708
8	KDL 1	*Stolephorus waitei*	99.43%	706
9	BTG 4	*Stolephorus commersonnii*	99.71%	679
10	KDL 4	*Atherinomorus* sp.	98.86%	680

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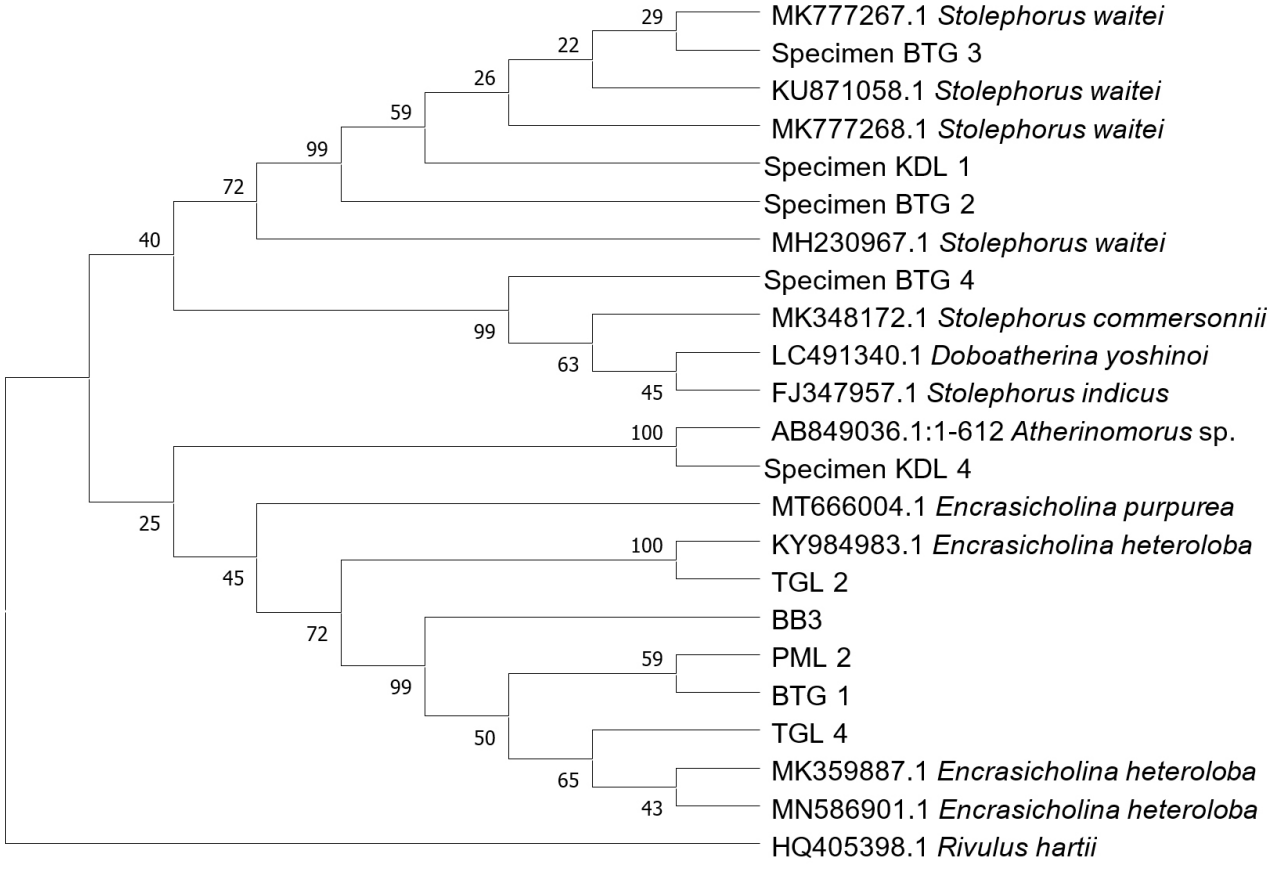

Fig 4. Phylogenetic tree construction of the ten anchovy samples collected on the northern coast of Java showing a kinship with 4 different species in the gene bank

Meanwhile, Batang was the region with the greatest diversity of anchovy species since three anchovy species were identified, including *S. commersonii*, *S. waitei*, and *E. heteroloba*. The distance between each sampling location is relatively small, which eases the genetic movement and combination of each species. The Indonesian Through Flow (ARLINDO) was considered one of the factors affecting anchovy distribution. ITF (ARLINDO) is the flow of the water mass forming ocean currents in the waters of the Indonesian territory from the north, originating from the Pacific Ocean to the south in the Indian Ocean (Rizal, 2015). These factors led to the genetic combination between anchovies in Bali strait coastal waters and the anchovies in other regions, since anchovies followed the ITF flow in search for food. It is in line with the statement of Tomascik et al. (1997) that the ITF water mass will be combined with other waters when it flows through Indonesian waters, and this leads to the combination of the water mass from two different oceans. Included as one of the ITF flows, these coastal waters are rich in important nutrients for phytoplankton. In the food chain, phytoplankton is eaten by small fish, and then by big fish.

CONCLUSION

Based on the results of the present study, four different species of anchovy were successfully identified from the northern coast of Java by applying the barcoding approach. Those four species include *Encrasicholina heteroloba*, *Stolephorus commersoni*, *Stolephorus waitei* and *Atherinomorus* sp. with the similarity level ranging from 98 to 99%.

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DNA BARKODIRANJE: PROCJENA RAZNOLIKOSTI EKONOMSKI VAŽNIH INĆUNA NA SJEVERNOJ OBALI JAVE, INDONEZIJA

SAŽETAK

Inćun ekonomski važna vrsta u zapadnom dijelu sjeverne obale središnje Jave. Proizvodnja inćuna proteže se duž obale od Brebes Regency do Batang Regency. Stoga je cilj ove studije odrediti raznolikost inćuna na sjevernoj obali središnje Jave (Brebes, Tegal, Pemalang, Batang i Kendal), koristeći molekularni pristup. Ovo je istraživanje provedeno od travnja do rujna 2019. u Laboratoriju za biotehnologiju tropskog mora, Fakulteta za ribarstvo i znanost o moru, Sveučlišta Diponegoro. U ovom istraživanju primijenjena je deskriptivna eksplorativna metoda metodom slučajnog uzorka, dok je za molekularnu identifikaciju korišten gen citokrom oksidaze I (COI). Uzorkovanjem je pronađen uzorak inćuna koji je molekularno identificiran iz svake regije, uključujući BB 3, PML 2, BTG 1, BTG 2, BTG 4, TGL 2, TGL 4, KDL 1 i KDL 4. Duljina para baza od 10 uzoraka inćuna iz rezultata amplifikacije gena COI bila je 600-700 bp. Rezultati sekvenciranja i poravnanja BLAST analize pokazali su da je deset uzoraka inćuna uključeno u vrstu *Encrasicholina heteroloba*, *Stolephorus commersoni*, *Stolephorus waitei* i *Atherinomorus* sp., koja se kretala od 98 do 99% sličnosti u lokaciji. Stoga se zaključuje da su na sjevernoj obali Jave postojale najmanje četiri različite vrste inćuna. Međutim, predlažu se daljnja istraživanja kako bi se utvrdila genetska varijacija svake vrste radi boljeg upravljanja.

Ključne riječi: inćun, molekularna analiza, identifikacija, COI, sjeverna obala Jave

REFERENCES

Aliah, R. S. (2009): Keragaman Genetik Induk Udang Windu di Perairan Selatan Pangandaran dan Binuangeun, Jawa Barat. 5 (3): 209-217.

Avise, J. C. (1994): Molecular Markers, Natural History and Evolution. Chapman and Hall. USA.

Ayuningrum, D., Liu, Y., Riyanti, Sibero, M. T., Kristiana, R., Asagabaldan, M. A., Wuisan, Z. G., Trianto, A., Radjasa, O. K., Sabdono, A., Schaeberle, T. F. (2019): Tunicate-Associated Bacteria Show Great Potential for the Discovery of Antimicrobial Compounds. PLoS ONE 14(3):e0213797, 14 p.

Fahmi, M. R., Kusrini, E., Hayuningtyas, E. P., Sinansari, S., Gustiano, R. (2020): DNA Barcoding Using COI Gene Sequences of Wild Betta Fighting Fish from Indonesia: Phylogeny, Status and Diversity. Indonesian fisheries research journal 26 (2): 83-96.

Gangan, S. S., Pavan-Kumar, A. (2019): Multigene barcoding and phylogeny of selected Engraulidae species. Mitochondrial DNA Part A, 30(3), 548-555.

Hebert, P. D., Cywinska, A., Ball, S. L., DeWaard, J. R. (2003): Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences, 270(1512), 313-321.

Imran, Z., Yamao, M. (2014): Factors Contributing to the Decline of the Anchovy Fisheries in Krueng Raya Bay, Aceh, Indonesia. Annals of Tropical Research. 36 (2):23-44.

Irnawati, R., Surilayani, D., Susanto, A., Munandar, A., Rahmawati, A. (2018): Potential Yield and Fishing Season of Anchovy (*Stolephorus* sp.) in Banten, Indonesia. AACL Bioflux. 111 (3): 804 – 809.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. (2018): MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549.

Liu, W., Lathrop, A., Fu, J., A. D., Murphy, R. W. (2000): Phylogeny of the East Asian Bufonids Inferred from Mitochondrial DNA Sequences (Anura: Amphibia): Molecular Phylogenetics and Evolution, 14(3): 423-435.

Liyan, Qu, Chunyan, Ma, Hongyu, Ma, Yao Dong, Wei Wang, Guijing Ren, Lingbo Ma. (2017): The complete mitochondrial genome of *Stolephorus commersonii*. Mitochondrial DNA Part B, 2 (2), 573-574.

Rizal, F., Hassan, W., Pratomo, Y., Andrew, E. (2015): Identifikasi Komponen Harmonik di Selat Lombok Berdasarkan Data Arus Time Series. Depik, 4 (1), 24-32.

Saanin, H. (1984): Taxonomy and identification key of the Fish. Jakarta: Bina Cipta.

Sankar, T.V., Anandan, R., Mathew, S., Asha, K. K., Lakshmanan, P. T., Varkey, J., Mohanty, B. P. (2013): Chemical Composition and Nutritional Value of anchovy (*Stolephorus commersonii*) Caught from Kerala Coast, India. European Journal of Experimental Biology, 3 (1), 85 – 89.

Sjafaraenan, Lolodatu, H., Johannes, E., Agus, R., Sabran, A. (2018): The DNA Profiles of Genes Follicle Stimulating Hormone Receptor (FSHR) in Women Acne with the Techniques of PCR and DNA Sequencing. Biomes: Journal of Biological Makassar 3 (1): 1 – 11.

Sofro, A. S. M. (1994): Keanekaragaman Genetik. Yogyakarta: Andi Offset. 127 hal.

Tjong, D. H., Matseu, M., Kuramoto, M., Belabut, D. M. Sen, Y. H., Nishioka, M., Sumida, M. (2007): Morphological Divergence, Reproductive Isolating Mechanism and Molecular Phylogenetic Relationship, Among Indonesia, Malaysia, and Japan Populations of the Fejervaria limnocharis Complex (Anura, Ranidae): Zoological Science, 24, 1197 - 1212.

Tomascik, T., Mah, A. J., Nontji, A., Moosa, M. K. (1997): The Ecology of the Indonesian Seas. Part I, Periplus Editions Ltd., Singapore.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., Hebert, P.D.N. (2005): DNAbarcoding Australia's fish species. Philos. Trans. R. Soc. Lond. B. Biol. Sci.,(1462), 1847-1857.

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