Trends in Genetics and Evolution (2023) Volume 6 Issue 1
doi: 10.24294/tge.v6i1.2350

ORIGINAL RESEARCH ARTICLE

Phylogenetic and structure genetic of *Rastrelliger sp* in north Maluku Sea, Indonesia

Nebuchadnezzar Akbar[1,*], Eko S. Wibowo[1], Abdurrachman Baksir[1], M. Janib Achmad[1], Rustam E. Paembonan[1], Ikbal Marus[1], Irmalita Tahir[1], Najamuddin[1], Riyadi Subur[1], Firdaut Ismail[1], Abdul Ajiz Siolimbona[1], Aradea Bujana Kusuma[2], Iswandi Wahab[3], Edwin Jefri[4], Beginer Subhan[5], Nyoman M. N. Natih[5], Dondy Arafat[5], Dandi Saleky[6], Waluyo[7]

[1] *Marine Science Department, Faculty of Fisheries and Marine Science, Khairun University, Ternate 97719, North Maluku Province, Indonesia*

[2] *Department of Fisheries, Faculty of Fisheries and Marine Science, University of Papua, Manokwari 98314, West Papua, Indonesia*

[3] *Marine Science Department, Fisheries and Marine Faculty, Pasifik University, South Morotai 97771, North Maluku Province, Indonesia*

[4] *Fisheries and Marine Faculty, Mataram University, Lombok 83125, Nusa Tenggara Barat Province, Indonesia*

[5] *Department of Marine Science and Technology, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, Bogor 16680, West Java Province, Indonesia*

[6] *Fisheries and Marine Faculty, Musamus University, Marauke 99611, Papua Province, Indonesia*

[7] *Aquaculture Department, Tidar University, Magelang City 56116, Province of Central Java, Indonesia*

*** Corresponding author:** Nebuchadnezzar Akbar, nezzarnebuchad@yahoo.co.id

ABSTRACT

Mackerel is a small pelagic fish that has potential value and can be found throughout Indonesian waters. It is feared that high exploration activities will have an impact on the population. Sampling was carried out at the fish landing port and fish auction place (Bacan, Morotai and Ternate). The sample was photographed and the 3 cm swimming fin was taken. The sample is then stored in a tube that has been filled with 96% ethanol solution. The next stage is the process of extraction, amplification, electrophoresis and DNA sequencing. Phylogenetic reconstruction of comparison of primary data (Bacan, Morotai and Ternate) and secondary data (Indian) clarified to form two different populations (clades). Amova population pairwaise (Fst) showed the genetic flow of *R. kanagurta* population in the waters of the north Maluku Sea (Bacan, Morotai and Ternate). On the other hand, genetic distance shows that populations (Bacan, Morotai and Ternate) were closely related and have strong genetic connectivity.

Keywords: evolution; molecular; tropical fish; ancestry; ocean dynamics

ARTICLE INFO

Received: 3 July 2023
Accepted: 8 August 2023
Available online: 15 November 2023

1. Introduction

Mackerel is a small pelagic fish that has potential value and can be found throughout Indonesian waters. Mackerel is a fish catch that has important economic value in Indonesia[1]. This is because many of these species are caught for consumption because they have a dense meat texture and contain high nutritional value and have a profitable selling value compared to other pelagic fish[2]. In addition, the high level of utilization and the possibility of a high level of exploitation of this fish resource, demands good management efforts so that the use of fish is sustainable[3]. Climate change will greatly affect the physiology and behavior of individuals, populations and fish communities. Extreme conditions with rising sea surface temperatures, low dissolved

oxygen concentrations and water pH can result in the death of fish. An environment with extreme conditions will hamper the growth and reproduction patterns of fish. All of these changes directly affect the population and community structure of mackerel. Population structure analysis for estimating fish populations can be determined using several methods where genetic analysis is the most accurate method for determining population structure, because genetic characters are passed from one generation to the next with very little chance of changed[4]. This genetic method to find out information on the genetic diversity of a population is the DNA sequencing technique. This method is used to obtain the sequence of nucleotide bases in a DNA molecule with a fast and efficient technique[5]. In addition, knowledge of the kinship of a species is needed to study the evolution of several related taxa by comparing their DNA sequences[6]. Molecular phylogenetics serves to determine kinship and genetic differences within a population or subspecies that are geographically different[7]. The stated that phylogenetics can show the evolutionary relationship of an organism which is inferred from morphological and molecular data[8]. The purpose of this study was to determine the phylogenetic and genetic population structure of mackerel based on DNA information.

Research on fish genetics in Indonesia has been carried out including genetic studies of populations of *bigeye tuna* (*Thunnus obesus*) in Benoa Bali[9], *kandra fish* (*Torsoro*) originating from North Sumatra and west Java Provinces[10], *red snapper* (*Lutjanus malabaricus*) originating from several fishing areas on the North Coast of Java and the eastern part of the Java Sea[11], *yellowfin tuna* (*Thunnus albacores*) from the areas of Bali, North Maluku and North Sulawesi[12], The same research has also been carried out, where fish samples were taken from Spain and the Philippines[13], *Betutu fish* from the Brebes Penjalin reservoir[14], study of the population structure of the *bigeye tuna* in the Indian Ocean of west Sumatra, South Java and Nusa Tenggara [15], on the genetic diversity of *yellowfin tuna* from two populations in the Maluku Sea, Indonesia[5], the preliminary study of yellowfin tuna populations from two populations in the Maluku Sea, Indonesia[16], molecular phylogenetics of grouper genus *Epinephelus* from collections from traditional markets[17], genetic variants of *Sardinella lemuru* in the Bali Strait sea[18], genetic and phylogenetic variations of yellowfin tuna as the basis for sustainable fisheries management in north Maluku[19], genetic diversity of mackerel scads, decapterus macarellus[20] in the Indian Ocean, about morphometric and genetic identification of Mackerel (*Rastrelliger sp*)[21] collected from Muara Baru fish market, Jakarta and about DNA barcoding and morphometric of *Rastrelliger sp*[22] in north Maluku Sea, Indonesia.

2. Material and method

Mackerel tissue DNA were collected in Ternate (3 samples), Morotai (3 samples) and Bacan (3 samples) at the fish landing port and fish auction place (**Figure 1**). Tissue collection was carried out on the pectoral fin 3 cm and then put into a tube containing 96% ethanol. Extraction, amplification, electrophoresis and sequencing were carried out in the Bionesia laboratory, Bali. DNA sequencing method with primer CRK-CRE.

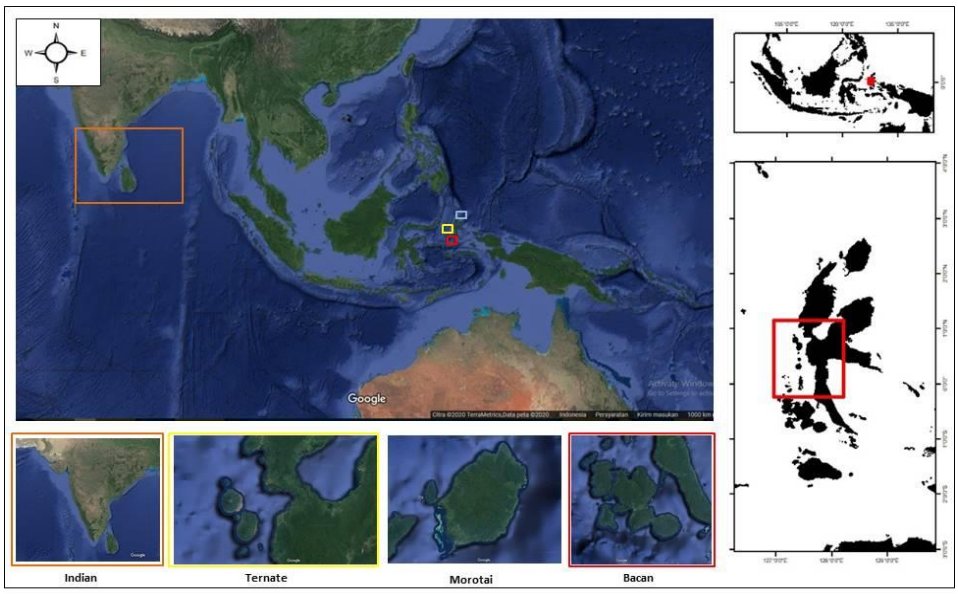

Figure 1. Research location *R. kanagurta*, primer data (Ternate, Morotai and Bacan) and secondary data (Indian)[22].

3. Extraction, PCR and electrophoresis DNA

Sample extraction was carried out using a 10% Chelex solution[23]. +2 mm sample tissue was taken using tweezers and put into a tube containing the sample tissue Chelex solution, vortexed and centrifuged +20 s, then heated in a heating block with a temperature of 95 ℃ + 45 min. After heating, the tube was vortexed again and centrifuged for +20 s. The extraction solution is ready to be used for amplification. Amplified at the CR (control region) locus with the Hotstart method. denaturation at 94 ℃ for 30 s, annealing at 50 ℃ for 30 s, and extension at 72 ℃ for 45 s, and the PCR process was repeated for 38 cycles, and finally the final extension at 72 ℃ for 5 min[24]. Two primers were used, namely the forward primer L15923 with the following nucleotide sequence 5′-TTAAAGCATCGGTCTTGTAA-3′ and the reverse primer H16498 with the following nucleotide sequence 5′-CCTGAAGTAGGAACCAGATG-3′. Electrophoresis is a standard method used for the identification, separation and purification of DNA. Electrophoresis is useful to be able to see the success of PCR through 1% agarose gel by adding 1 gram of agarose to Erlenmeyer then adding 100 mL of TAE 1X. After that it was heated in the microwave, when it was evenly dissolved, 4 µL EtBr was added. Agarose gel was poured into a mold that had been installed with a well-making comb and allowed to stand for 30 min. The electrophoresis results were then visualized using an ultraviolet machine with a voltage of 200 V and a current of 400 mA for 15 min.

3.1. DNA Analysis

Control region mtDNA sequences were analyzed using the MEGA5 (Molecular Evolutionary Genetic Analysis)[25] and Arlequin 3.5 application[26] to obtain phylogenetic and genetic distances. Secondary data were downloaded from GenBank (3 samples) with accession numbers.

3.2. Result and Discussion

3.2.1. Phylogenetic

Phylogenetic relationships of mackerel (*R. kanagurta*) in north Maluku Sea waters (primary data) did not find differentiation and clade formation between locations (**Figure 2**). The entire individuals (Bacan, Morotai and Ternate) form clades showed random mixing of individuals. phylogenetic reconstruction provided an explanation of the evolutionary processes that occur. Phylogenetic relationships confirmed the morphological classification of *Rastrelliger* species[27] Phylogenetic reconstruction using primary data (Bacan, Morotai and Ternate) and secondary data (Indian) clarified to form two different populations (clades) (**Figure 2**). The

results of this reconstruction provided an explanation that, there were sub populations (*R. kanagurta*) which distributed globally in tropical waters. The results of phylogenetic reconstruction were supported by genetic distance analysis among locations (**Table 1**). Natural factors such as oceanography and circulation patterns of location currents are the barriers which resulting in genetic differentiation. Regional lineage segregation of marine organisms is associated with spreading barriers such as ocean circulation, upwelling, geological history, heterogeneity of ocean views and self-recruitment behavior strategies which play the important roles in fish philogeography[28].

Phylogenetic studies of *mackerel* (*Rastrelliger sp*) were also reported by Indaryanto et al.[27] on *mackerel* (*R. brachysoma*) Java, Indonesia, where the results of the study showed no population grouping. The *mackerel* (*R. kanagurta*) at Raja Empat, Kendari, Gorontalo, Ambon and Dobo locations, two main population groups were found[20]. The identification of four lineages in mackerel (*R. kanagurta*) in Banda Aceh and Trang (Andaman Sea) and Can Tho (South China Sea)[29]. Previous studies discussing phylogenetic pelagic fish have been reported such as *yellowfin tuna*[5,19], *tuna* and *mackarel* in the Indonesian Archipelago[30] in *julung-julung* fish (*Hemirampus sp*)[31].

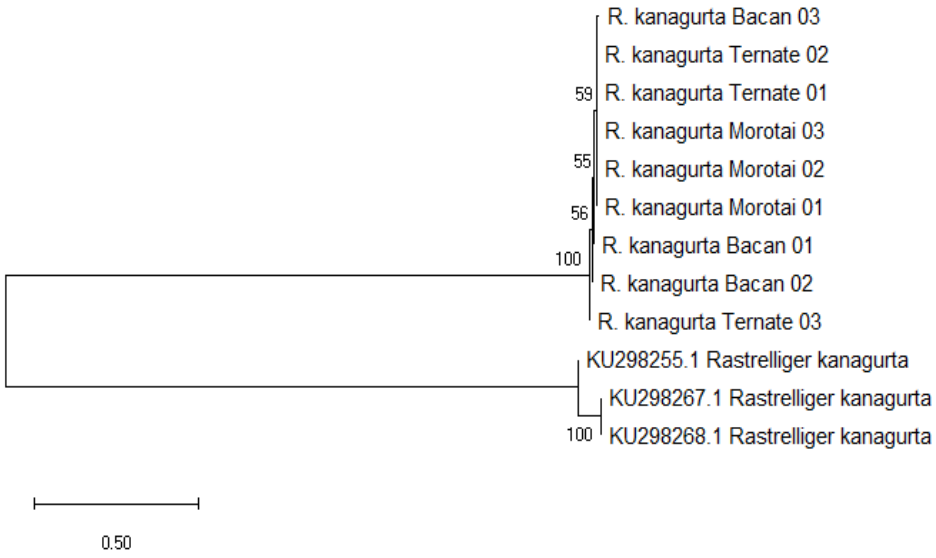

Figure 2. Phylogenetic tree *R. kanagurta*, primer data (Ternate, Morotai and Bacan) and secondary data (Indian).

Substitution probability values were found to have similarities in all nucleotide elements (**Table 1**). The results of the nucleotide frequency presentation calculation were divided into four segments namely *A* = 25%, *T/U* = 25%, *C* = 25% and *G* = 25%. The higher the nucleotide frequency value, the greater the chance of substitution.

Table 1. Maximum likelihood Estimate of substitution Matrix.

	A	***T/U***	***C***	***G***
A	-	*4.04*	*4.04*	**16.92**
T/U	*4.04*	-	**16.92**	*4.04*
C	*4.04*	**16.92**	-	*4.04*
G	**16.92**	*4.04*	*4.04*	-

Note—Each entry is the probability of substitution (*r*) from one base (row) to another base (column). Substitution pattern and rates were estimated under the Kimura (1980) 2-parameter model (+G) . A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories, [+*G*], parameter = 1.8612). Rates of different transitional substitutions are shown in bold and those of transversionsal substitutions are shown in italics. Relative values of instantaneous *r* should be considered when evaluating them. For simplicity, sum of *r* values is made equal to 100, The nucleotide frequencies are *A* = 25.00%, *T/U* = 25.00%, *C* = 25.00%, and *G* = 25.00%. For estimating *ML* values, a tree topology was automatically computed. The maximum Log likelihood for this computation was 017.484. This analysis involved 12 nucleotide sequences. There was a total of 374 positions in the final dataset. Evolutionary analyses were conducted in MEGA X[2].

Several works such as reported, where in mackerel (*R. kanagurta*) at Raja Empat, Kendari, Gorontalo, Ambon and Dobo locations, two main population groups were found[20]. The identification of four lineages in *mackerel* (*R. kanagurta*) in Banda Aceh and Trang (Andaman Sea) and Can Tho (South China Sea)[29]. Previous studies discussing phylogenetic pelagic fish have been reported such as yellowfin tuna[5,19], tuna and mackarel in the Indonesian archipelago[30] in julung-julung fish (*Hemirampus sp*)[31].

Substitution probability values were found to have similarities in all nucleotide elements (**Table 1**). Probability values are closely related to nucleotide frequencies[13]. The results of the nucleotide frequency presentation calculation were divided into four segments namely *A* = 25%, *T/U* = 25%, *C* = 25% and *G* = 25%. The higher the nucleotide frequency value, the greater the chance of substitution. The probability of transition substitution was greater than that of transversion substitution, because transition substitution that occurs between purine nucleotides with purines and between pyrimidines and pyrimidines will be easier than transversion substitution that occurs between purines and pyrimidines[13]. This change was likely due to the similarity of the molecules that exist, besides that there were structural differences between purines and pyrimidines. The same research results were also found on samples of *yellowfin tuna* from the Philippines and Spain[13].

3.2.2. Structure genetic

Amova population pairwaise (Fst) shows the genetic flow of *R. kanagurta* population in the north Maluku Sea waters (Bacan, Morotai and Ternate) (**Table 2**). Population comparison analysis showed the possibility of a high gene flow between *R. kanagurta* in the of north Maluku waters (Bacan, Morotai and Ternate) and Indians (**Table 2**). Comparison of Fst *R. kanagurta* values (primary and secondary data) found Bacan-Indian (0.993), Morotai-Indian (0.998), Ternate-Indian (0.993) (**Table 2**). Values testing of Fst in *R. kanagurta* (Bacan, Morotai and Ternate) found a strong genetic link between the population of Morotai-Bacan (0.625), Ternate-Bacan (0.052) and Morotai-Ternate (0.665). Nevertheless, genetic distance provided the different results in the two populations. Population grouping was possible due to differences in genetic structure, although Fst analysis showed a strong genetic flow.

Overall genetic distance results showed that populations (Bacan, Morotai and Ternate) are closely related and have strong genetic connectivity (**Table 3**). The results of genetic distance analysis between populations found genetic similarities between Morotai-Ternate (0.003) and Bacan-Ternate (0.006). Genetic differentiation has showed between Bacan-Indian (3625), Morotai-indian (3524) and Ternate-Indian (3524). Meanwhile, genetic distance provided an explanation that there was no significant genetic difference of *R. kanagurta* in the Bacan Sea, Morotai and Ternate. High genetic distance among populations (Bacan, Morotai and Ternate) and Indians, indicated that the two populations were separated due to the global geographical system, several barriers and oceanography factors.

Table 2. Amova population pairwise (Fst) *R. kanagurta*.

Locations	Indian	Bacan	Morotai	Ternate
Indian	-	-	-	-
Bacan	0.993	-	-	-
Morotai	0.998	0.625	-	-
Ternate	0.993	0.052	0.665	-

Table 3. Genetic distance *R. kanagurta.*

Locations	Indian	Bacan	Morotai	Ternate
Indian	-	-	-	-
Bacan	3.625	-	-	-
Morotai	3.524	0.008	-	-
Ternate	3.524	0.006	0.003	-

Probability values are closely related to nucleotide frequencies[13]. The probability of transition substitution was greater than that of transversion substitution, because transition substitution that occurs between purine nucleotides with purines and between pyrimidines and pyrimidines will be easier than transversion substitution that occurs between purines and pyrimidines[13]. This change was likely due to the similarity of the molecules that exist, besides that there were structural differences between purines and pyrimidines. The same research results were also found byon samples of *yellowfin tuna* from the Philippines and Spain[13].

Panmiksia populations were shown based on the phylogenetic reconstruction of mackerel samples (Bacan, Morotai and Ternate) **(Figure 3**). Population mixing according to the phylogenetic reconstruction was likely due to the large genetic flow and the low genetic distance between populations. The fish population originated from the same offspring and has a pattern of residence in the same location, resulting in these two populations being genetically similar[16]. Primary data clades (Bacan, Morotai and Ternate) and secondary data clades (Indian) form separate clades (**Figure 3**). The reconstruction explained that the two locations (primary and secondary data) had genetic differences in mackerel (*Rastrelliger sp*). Phylogenetic trees provided an explanation of the evolutionary processes that occur within or between populations. The history of species populations can be interpreted into four categories based on their combination of genetic diversity and nucleotide values from small to large[29]. Genetic data provides information on barriers to the geographical distribution[32].

Research on *Rastrelliger sp* found two genetic populations of *R. brachysoma* along west Java (Pelabuhan Ratu, Banten, Lampung and Jakarta) and east Java (Banyuwangi)[27]. Zamroni et al.[33] found that the results of RFLP mDNA analysis in *R. brachysoma* formed the population at north of Jakarta, Indramayu, Pekalongan, Rembang and Pasuruan (Madura Strait) showed no significant differences in fish genotype samples. Both of these studies showed that the western part of Java and the central Java region were the same population and *R. brachysoma* from the east of the Java Region was the population[29] revealed that the two separate populations of *R. kanagurta* namely the populations of southeast Asia (South China Sea, Malacca Strait, Sulu Sea, Sulawesi Sea, Andaman Sea) and Iranian population (West Indian Ocean). The results of the study indicated that the Andaman Sea stock of Indian mackerel is a population with a moderate level of genetic variation[34].

Specifically, genetic closeness can be explained, suggesting that the entire population of mackerel (*R. kanagurta*) were a descendant. The inferred demographic history also showed that the population of *R. kanagurta* has the potential to be expanded in the late Pleistocene and gene flow appears to be ongoing among the population that still exists to date[29]. Gene connection between populations may be caused by between populations having the same parent origin and genetic proximity[35]. Reports on the genetic proximity between other marine species[35] on Sarcophyton trocheliophorum soft corals in Indonesia, and species of Epinephelus spp groupers in Indonesia[17]. Genetic closeness was strongly influenced by current flow patterns, high larval spread, appropriate habitat conditions and migration capability[5,17,35–37].

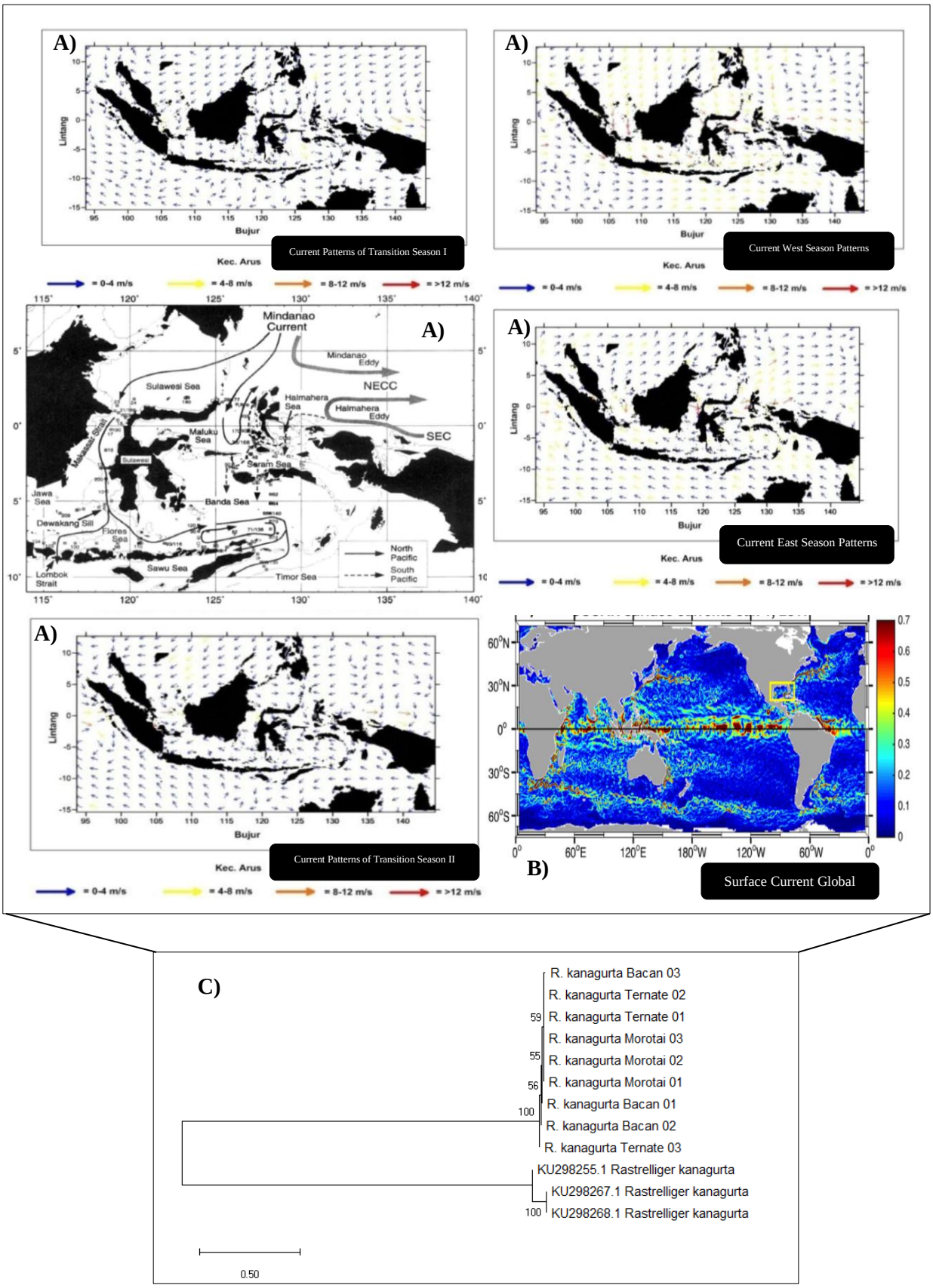

Figure 3. (a) Indonesia throughflow and surface ocean current in Indonesia; **(b)** global current surface and Dendogram *R.Kanagurta*.

The wide distribution in the ocean indicated that the large population sizes in mackerel, this certainly has the effect of genetic flow between populations. However, fish size has an influence on the ability of migration, where there are variations in the environmental conditions of each waters area. Factors causing limited genetic

flow are current patterns, changes in temperature and salinity or physical barriers between continents or oceanic sediments[38–40]. Global current circulation is a barrier to the distribution of small pelagic fish, due to their low migration ability. The results of research indicated that currents not only limit gene flow between two regional biogeographies, but also the formation of each lineage in another habitat may be limited by physiological adaptation to regional environmental conditions[41]. The currents influence the distribution of larval populations and make sub-populations in each region. However, in extreme conditions, sea level currents are a barrier to population migration. The process of population genetic distribution that is different from the population (primary and secondary), indicated that sub-populations were formed among the world's ocean waters in a dispersal manner. The distribution of genetic diversity is influenced by the strength of the ocean circulation in recruitment larvae[28]. In the world's oceans, fish are abundant and distributed, but observed levels of genetic differentiation among populations are often low[42]. The significant differences in the genetic structure of three populations due to physical oceanographic patterns[32].

4. Conclusion

Phylogenetic reconstruction using primary data (Bacan, Morotai and Ternate) and secondary data (Indian) clarified to form two different populations (clades). Panmixia population was shown based on phylogenetic reconstruction in mackerel samples (Bacan, Morotai and Ternate). Comparative analysis of population showed the possibility of high gene flow between *R. kanagurta* in north Maluku Sea waters (Bacan, Morotai and Ternate) and Indians. High genetic distance between populations (Bacan, Morotai and Ternate) and Indians, indicated that the two populations were separated due to global geographical and oceanography factors.

Author contributions

Conceptualization, NA and ESW; methodology, NA, ESW and ABK; software, IW; validation, EJ and ABK; formal analysis, NA and IW; investigation, IT, N, RS, AB and MJA; resources, NA, BS, DA, IM, REP; writing—original draft preparation, NA, NMNN, FI, AAS, DS and W; writing—review and editing, DS, NA and W; visualization, IW and W; supervision, NA, NMNN, BS and EJ; project administration, ESW; funding acquisition, NA and ESW. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

References

1. Sarasati W. *Population dynamics and multispecies reproductive biology of mackerel (Rastrelliger faughni, R. kanagurta R. brachysoma) in the waters of the Sunda Strait* [Master's thesis]. Bogor Agricultural Institute; 2017.
2. Permatachani, A, Boer M,Kamal MM. Fish stock assesment of fish resources common Ponyfish (Leiognathus equulus) caught rampus net in Sunda Strait. *Jurnal Teknologi Perikanan dan Kelautan* 2016;7(2): 107-116.
3. Ibrahim PS, Setyobudiandi I, Sulistiono. Relationship between long weight and condition factors for Selaroides leptolepis yellow trevally in the Sunda Strait waters. *Jurnal Ilmu Dan Teknologi Kelautan Tropis* 2018; 9(2): 577–584. doi: 10.29244/jitkt.v9i2.1929
4. Syahailatua A. Fish Stock Identification, The Princip and Its Significance. *Oseana* 1993; 18(2): 55–63.
5. Akbar N, Zamani NP, Madduppa HH. Genetic diversity of yellowfin tuna (Thunnus albacares) from two populations in the Moluccas Sea, Indonesia. *Depik* 2014; 3(1): 65–73.
6. Ubadillah R, Sutrisno H. *Pengantar biosistematik : teori dan praktek. museum zoologicum bogoriense, pusat penelitian biologi. Lembaga Ilmu Pengetahuan Indonesia. Bogor.* Lipi press; 2009.
7. Pertiwi ND. *Identification of Coral Fish of the Pseudochromidae (Dottyback) Family in the Coral Triangle Region* [Master's Thesis]. Denpasar Udayana University; 2015.
8. Safitri A, Simarmata L, Hardani HW (editors). Lestari ABR (translator). *Biologi. Jilid 1*. Jakarta : Erlangga; 2002.
9. Nugraha B. *Studi Tentang Genetika Populasi Ikan Tuna Mata Besar (Thunnus Obesus) Hasil Tangkapan Tuna Longline yang Didaratkan di Benoa* [Master's Thesis]. Pascasarjana Institut Pertanian Bogor; 2009.

10. Nugroho E, Subagja J, Asih S, et al. Evaluasi keragaman genetik ikan Kancra dengan menggunakan marker mt DNA D-loop dan Random Amplified Polymorphism DNA (RAPD). *Jurnal Riset Akuakultur* 2016; 1(2): 211–217. doi: 10.15578/jra.1.2.2006.211-217
11. Suwarso. *Variasi Geografik dalam Struktur Genetik Populasi Ikan Kakap Merah, Lutjanus malabaricus, (Lutjanidae) dan Interaksi Lingkungan di Laut Jawa* [Master's Thesis]. Program Pascasarjana, Institut Pertanian Bogor; 2020.
12. Permana GN, Hutapea JH, Haryanti, Sembiring SBM. Variasi genetik ikan tuna sirip kuning (*Thunnus albaceras*) dengan analisis elektroforesis allozyme dan mtDNA. *Jurnal Riset Akuakultur* 2007; 2(1): 41–50. doi: 10.15578/jra.2.1.2007.41-50
13. Wijana IMS, Mahardika IGN. Struktur Genetik dan Filogeni Yellowfin Tuna (Thunnus albacares) berdasarkan Sekuen DNA Mitkondria control region sitokrom oksidase I pada diversitas zone biogeografi. *Jurnal Bumi Lestari* 2010; 10(2): 270–274.
14. Susanto AH, Suryaningsih S. Variasi biokimia genetik ikan betutu (Oxyeleotris marmorata. BLKR) di waduk penjalin Brebes. *Jurnal Biota* 2006; 11(3): 136–141.
15. Suman A, Irianto HE, Amri K. Population structure and reproduction of bigeye tuna (Thunnus obesus) in Indian Ocean at western part of Sumatera and southern part of Java and Nusa Tenggara. *Indian Ocean Tuna Commission* 2013; 8: 1–14.
16. Akbar N, Pertiwi D, Neviaty PZNP, et al. A pilot study on the population genetics of yellowfin tuna (Thunnus albacares) from two populations in the Moluccas Islands, Indonesia. *Depik* 2020; 9(1): 69–106. doi: 10.13170/depik.9.1.10585
17. Jefri E, Zamani N P, Subhan B, Madduppa HH. Molecular phylogeny inferred from mitochondrial DNA of the grouper Epinephelus spp. in Indonesia collected from local fish market. *Biodiversitas Journal of Biological Diversity* 2015; 16(2). doi: 10.13057/biodiv/d160221
18. Kartika GRA, Sartimbul A, Widodo W. Genetic Variance OF Sardinela lemuru in the Bali Strait Waters. *Jurnal Kelautan* 2017; 10(1). doi: 10.21107/jk.v10i1.1615
19. Aris M, Akbar N, Labenua R. Genetic and phylogenetic variations of yellowfin tuna (thunnus albacares) as a basis for sustainable fishery resources management in north moluccas. *International Journal of Pharma and Bio Sciences* 2017; 8(4): 419-426. http://dx.doi.org/10.22376/ijpbs.2017.8.4.b419-426
20. Zamroni A, Suwarso S, Kuswoyo A. Genetic Variation of Indian Mackerel, Rastrelliger kanagurta (Cuvier, 1817) in the Waters of Eastern Indonesia. *Bawal* 2017; 9(2): 123–131.
21. Salma U, Madduppa H. Morphometric and genetic identification of Mackerel (*Rastrelliger sp.*) collected from muara baru fish market, jakarta. *Jurnal Ilmu Kelautan Kepulauan* 2021; 4(2). doi: 10.33387/jikk.v4i2.3880
22. Akbar N, Wibowo ES, Irfan M, et al. DNA barcoding and morphometric of Rastrelliger spp. in North Maluku Sea, Indonesia. *Aceh Journal of Animal Science* 2022; 7(3): 73–81. doi: 10.13170/ajas.7.3.26530
23. Walsh PS, Metzger DA, Higuchi R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 1991; 10(4): 506–513.
24. Barber PH, Erdmann MV, Palumbi SR. Comparative phylogeography of three codistributed stomatopods: origins and timing of regional lineage diversification in the coral triangle. *Evolution* 2006; 60(9): 1825–1839. doi: 10.1111/j.0014-3820.2006.tb00526.x
25. Tamura K, Peterson D, Peterson N, et al. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 2011; 28(10): 2731–2739. doi: 10.1093/molbev/msr121
26. Excoffier L, Lischer H. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 2010; 10(3):564–567. doi: https://doi.org/10.1 111/j.1755-0998.2010.02847.x
27. Indaryanto FR, Imai H, Yusli Wardiatno Y. Genetic variation of short body mackerel, Rastrelliger brachysoma of Jawa Island, Indonesia based on mtDNA control region sequences. *Bioflux* 2015; 8(5): 648-655
28. Torquato F, Range P, Ben-Hamadou R, et al. Consequences of marine barriers for genetic diversity of the coral-specialist yellowbar angelfish from the Northwestern Indian Ocean. *Ecology and Evolution* 2019; 9(19): 11215–11226. doi: 10.1002/ece3.5622
29. Akib NAM, Tam BM, Phumee P, Abidin MZ, Tamadoni S, Mather PB, Nor SAM. High Connectivity in Rastrelliger kanagurta: Influence of Historical Signatures and Migratory Behaviour Inferred from mtDNA Cytochrome b. *PLoS ONE* 2015; 10(3): e0119749. https://doi.org/10.1371/journal.pone.0119749
30. Jackson AM, Ambariyanto, Erdmann MV, Toha AHA, Steven LA, Barber. Phylogeography of commercial tuna and mackerel in the Indonesian Archipelago. Bulletin of Marine Science 2014; 90(1):471–492. http://dx.doi.org/10.5343/bms.2012.1097
31. Achmad MJ, Djamhur M, Fabanyo M, Akbar N. DNA barcoding application of garfish (Hemirhampus sp.) in North Maluku Sea. *Jurnal Iktiologi Indonesia* 2019; 19(3): 463-473 DOI: ttps://doi.org/10.32491/jii.v19i3.506
32. Lee PLM, Dawson MN, Neill SP, et al. Identification of genetically and oceanographically distinct blooms of jellyfish. *Journal of the Royal Society Interface* 2013, 10(80). doi: 10.1098/rsif.2012.0920

33. Zamroni A, Suwarso, Mukhlis NA, Reproductive biology and genetic population of short mackerel (Rastrelliger brachysoma, Scombridae) in the coastal waters of northern Jawa. *Indonesian Fisheries Research Journal* 2007; 14(2):215–226
34. Munpholsri N, Poompuang S, Senanan W, Kamonrat W.Microsatellite markers suggested moderate genetic variation in Indian mackerel (Rastrelliger kanagurta) populations from the Andaman Sea, Thailand. *Kasetsart Journal (Natural Science)* 2013; 47:853–863
35. Kusuma BA, Bengen DG, Madduppa H, Subhan B, Arafat D, Negara BFSP. Close genetic connectivity of soft coral Sarcophyton trocheliophorum in Indonesia and its implication for marine protected area. *Aceh Journal of Animal Science* 2016; 1(2): 50-57. https://doi.org/10.13170/ajas.1.2.4867
36. Grant WS. Biochemical genetic stock structure of the southern African anchovy, Engraulis capensis Gilchrist. *Journal of Fish Biology* 1985; 27(1): 23–29. doi: 10.1111/j.1095-8649.1985.tb04006.x
37. White C, Selkoe KA, Watson J, et al. Ocean currents help explain population genetic structure. *Proceedings of the Royal Society B: Biological Sciences* 2010; 277(1688): 1685–1694. doi: 10.1098/rspb.2009.2214
38. Finnerty JR, Block BA. Direct sequencing of mitochondrial DNA detects highly divergent haplotypes in blue marlin (Makaira nigricans). *Molecular Marine Biology and Biotechnology* 1992; 1(3): 206–214.
39. Chow S, Ushiama H. Global population structure of albacore (Thunnus alalunga) inferred by RFLP analysis of the mitochondrial ATPase gene. *Marine Biology* 1995; 123: 39–45. doi: 10.1007/BF00350321
40. Menezes MR, Kumar G, Kunal SP. Population genetic structure of skipjack tuna Katsuwonus pelamis from the Indian coast using sequence analysis of the mitochondrial DNA D-loop region. *Journal of Fish Biology* 2012; 80(6): 2198–2212. doi: 10.1111/j.1095-8649.2012.03270.x
41. Teske PR, Papadopoulos I, Newman BK, et al. Oceanic dispersal barriers, adaptation and larval retention: an interdisciplinary assessment of potential factors maintaining a phylogeographic break between sister lineages of an African prawn. *BMC Evolutionary Biology* 2008; 8(1): 1–14. doi: 10.1186/1471-2148-8-341
42. O'Reilly PT, Canino MF, Bailey KM, Bentzen P. Inverse relationship between FST and microsatellite polymorphism in the marine fish, walleye pollock (Theragra chalcogramma): implications for resolving weak population structure. *Molecular Ecology* 2004; 13(7): 1799–1814. doi: 10.1111/j.1365-294X.2004.02214.x