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Genetic variation of short body mackerel, *Rastrelliger brachysoma* of Jawa Island, Indonesia based on mtDNA control region sequences

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Abstract. *Rastrelliger brachysoma* is the most commercially important small pelagic species of the family Scombridae from five fish landings in Jawa Island (Pelabuhan Ratu, Banten, Lampung, Jakarta and Banyuwangi). Mitochondrial DNA control region sequencing result among 211 individual was approximately 445 base pairs (bp) DNA segment and the nucleotide base composition (%) was 29.1 A; 32.9 T; 22.8 G; and 15.2 C. A total 64 haplotypes were identified. *R. brachysoma* from Pelabuhan Ratu have the highest number of haplotypes (25) and polymorphic site (24). The population of *R. brachysoma* was divided into two clusters. Cluster A (originate population from seas east Jawa) consisted of 53 haplotypes mostly from Banten and cluster B (population from the southern part of the South China Sea) consisted of 13 haplotypes mostly from Lampung. The nucleotide diversity (π) ranged from 0.009 (Banyuwangi) to 0.013 (Lampung) with average nucleotide diversity of 0.010 for all location. This fish population in Indonesia had low levels of nucleotide diversity.
Key Words: haplotypes, phylogenic tree, spanning network, Jawa sea.

Introduction. *Rastrelliger brachysoma* (short body mackerel) is the most commercially important small pelagic species of the family Scombridae because of its low price and providing cheap protein source (Ghazali et al 2012). Therefore, this species have high level of preference among consumers. In Jawa island community, preference for fresh *R. brachysoma* is 7.87% and 5.1% for preserved fish (MMAF 2012a) and 12.7% for fresh fish in Banten province (Indaryanto & Saifullah 2011).

R. brachysoma is distributed abundantly in the Indonesian waters. This genus is caught in the Java Sea and contributes significantly to the national marine fisheries production. The Java Sea only has two species of *Rastrelliger*, there are *R. brachysoma* and *R. kanagurta*, other species are reduced to synonyms (Sudjastani 1976). They have similar morphological characters and can be identified by its body depth ratios and stripes along sides of the body (Burnahuddin et al 1984; Muchlisin et al 2009).

In Indonesian waters, *Rastrelliger* landing make up a large portion of the total catch of small pelagic fishes. *R. brachysoma* catch has declined to 291,863 ton in 2011 or forming 6.2% of the total marine catch, that is highest fisheries production in quantity under Scad in 405.808 ton and Skipjack tuna in 372.211 ton. About 20.4% of *R. brachysoma* catches landed along Jawa Island included Lampung (East Sumatera) and Bali (MMAF 2012b).

The over-exploitation of the pelagic fishery resources, including *R. brachysoma* has been highlighted in Jawa Sea and other Indonesian waters (MMAF 2012a,b). Genetic identification and phylogenetic relationships of species is very important especially in the case of morphological close related taxa for the establishment of adequate fisheries management and conservation for biodiversity studies and not at least for population

dynamics (Jamaluddin et al 2010). Genetic data can provide information about the stock structure and status of the population. Sequence analysis is a widely used technique that is considered good to see a group of organism biodiversity (Wodajo 2015; Wardiatno et al 2015). This technique evolved after creating the DNA sequencer. However, despite its commercial importance, there is only few biological information on *R. brachyosoma*, *i.e.* its helminthic parasites (Indaryanto et al 2014, 2015a), morphometrics (Indaryanto et al 2015b) and RFLP mDNA (Zamroni et al 2007; Indaryanto et al 2015b). On top of that, there is no information about the genetic diversity of the Short body mackerel, and the population structure of this species is mostly unknown, in particular for Jawa Sea.

The aims of the research were morphological and genetic identification of *R. brachysoma*. Genetic identification using mitochondrial DNA sequence analysis (mtDNA) with fish sample came from five fish landing in Jawa Island, Indonesia, *i.e.* Pelabuhan Ratu, Bandar Lampung, Banten, Jakarta and Banyuwangi, which is expected to provide information of *R. brachysoma* biodiversity at the Jawa Sea so it can be used as an input for the proper management of fisheries resources.

Material and Method

Samples of *R. brachysoma* were collected during September to October, 2012 from five fish landing in Indonesia, *i.e*. Pelabuhan Ratu Bay (Pelabuhan Ratu fish landing), Banten Bay (Karangantu fish landing), Lampung Bay (Bandar Lampung fish landing), Jakarta Bay (Muara Angke fish landing) and Banyuwangi Bay (Banyuwangi fish landing) (Figure 1). While samples of *R. Kanagurta* were collected from Pelabuhan Ratu Bay in order to construct the phylogeny tree of the two species. Genetic identification was performed in October to December, 2012 at Biology and Marine Sciences Laboratory, University of the Ryukyus, Japan.

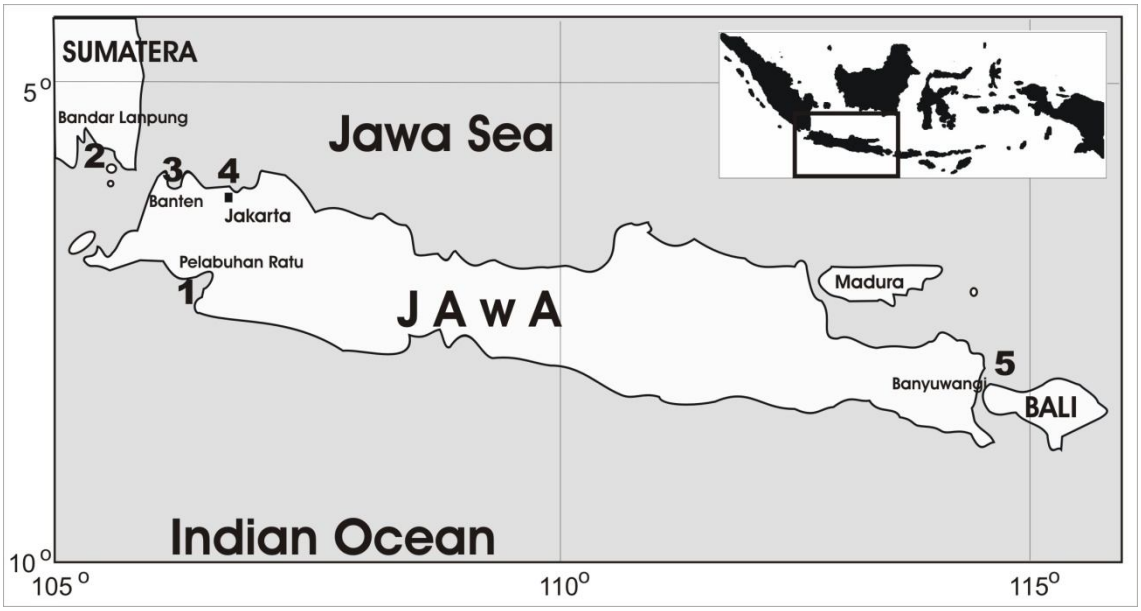

Figure 1. Sampling map. Numbers indicate sites of fish landing where the Indonesian short mackerel (*Rastrelliger brachysoma)* were collected, *i.e.* 1 - Pelabuhan Ratu, 2 - Lampung, 3 - Banten, 4 - Jakarta, 5 - Banyuwangi.

Approximately 50 mg of body tissue was placed in 0.5 mL TNES-8M urea buffer at 1.5 mL sterile tube for pending deoxyribonucleic acid (DNA) extraction. DNA was extracted using the proteinase K, standard phenol-chloroform extraction procedures. DNA template was amplified by polymerase chain reaction (PCR) using KapaTAQ^{TM}DNA polymerase (KapaBiosystems) with the universal primer L15923 5'-TTAAAGCATCGGTCTTGTAA-3 and H16498 5'CCTGAAGTAGGAA CCAGATG-3'. The PCR mixture for KapaTAQ^{TM} consisted of

10-50 ng of template DNA; 1 µL of each primer 12.5 pmole; 10 µL of 5x KAPATaqEXtra Buffer; 1.5 µL of 10 nMdNTPs; 3.5 µL of 25 nM MgCl2; and 0.5 µL of KAPATaq polymerase. Each sample was diluted to 50 µL with sterile distilled water. The process involved initial denaturation (94°C, 2 min); 30 cycles of denaturation (94°C, 30 s); annealing (55°C, 30 s); extension (72°C, 1 min); and a single final extension (72°C, 7 min). PCR product was separated electrophoresed on 1% agaroseTreviGel™ 500 and stained with ethidium bromide (Iwamoto 2008). DNA sequencing was sent to Macrogen-Japan.

Sequence data were initially aligned using ClustalX2ver2.1 (Larkin et al 2007). A neighbor-joining phylogeny of *R. brachysoma* mitochondria was constructed with MEGA5 using Kimura's two-parameter model (Nei & Kumar 2000). Haplotype diversity, nucleotide diversity within populations, minimum spanning network, and population pairwise FSTs were calculated using Arlequin ver 3.5.1.2 (Excoffier & Lischer 2011). The network of haplotypes was drawing manual using CorelDraw software based on minimum spanning tree connection length Arlequin calculating result.

Results and Discussion

The sequencing data were obtained from for 211 individuals of *R. Brachysoma* and 2 individuals of *R. kanagurta. R. Brachysoma* sequencing was approximately 445 base pairs (bp) DNA segment and the mean base composition (%) was 29.1 A; 32.9 T; 22.8 G; and 15.2 C. A total 64 haplotypes were identified (Table 1). *R. Brachysoma* from Pelabuhan Ratu had the highest number of haplotypes (25) and polymorphic site (24). In general, haplotype 01-RB01 (18%) appeared to be the most common to all 5 locations with 38 individuals. There are 4 haplotypes shared with 4 locations, 2 haplotypes shared with 3 locations, 8 haplotypes shared with 2 locations and 48 haplotypes which were singletons and unique. The nucleotide diversity (π) ranged from 0.009 (Banyuwangi) to 0.013 (Lampung) with average nucleotide diversity was 0.010 for all location.

Other study conducted in some area of Java Island was reported that the results of RFLP mDNA analysis on *R. brachysoma* form north Jakarta, Indramayu, Pekalongan, Rembang and Pasuruan (Madura strait) showed low genetic diversity (Zamroni et al 2007).

Table 1

Number of samples, number of haplotypes, nucleotide diversity, and polymorphic sites of each location of *Rastrelliger brachysoma*

Variables	*Pelabuhan Ratu*	*Lampung*	*Banten*	*Jakarta*	*Banyuwangi*
No of samples	50	49	46	48	18
No of haplotypes	25	16	18	21	12
Nucleotide diversity π	0.012	0.013	0.010	0.009	0.009
Tajima's D	0.11	0.91	0.14	-0.32	-0.01
No of polymorphic sites	24	21	19	21	14

Amova population pairwise (FST) indicated the possible existence of two genetic populations of *R. brachysoma* along the west Jawa region (Pelabuhan Ratu, Banten, Lampung and Jakarta) and east Jawa region (Banyuwangi) (Table 2). Zamroni et al (2007) reported that the result of analysis RFLP mDNA in *R. brachysoma* form north Jakarta, Indramayu, Pekalongan, Rembang and Pasuruan (Madura strait) showed no significant differences in fish samples genotip. Both of this study shown that the west Jawa and the central Jawa region are the same population and *R. brachysoma* from east Jawa region is a distinct population. There are two *R. brachysoma* populations in the Java Sea; this supports Hardenberg's hypothesis. Hardenberg (1937) hypothesis is based on *Decapterus* sp. migration patterns which describe the *Rastrelliger* stocks in the area, suggested that at least two populations of mackerel seasonally enter the Java Sea. One population may originate from the seas of eastern Indonesia to the Java Sea and reach the central-western part of the Java Sea during the East monsoon; the other stock may

occupying the southern part of the South China Sea reaches the northwestern part of the Java Sea during the West monsoon.

Table 2

Population pairwise differences FST of *Rastrelliger brachysoma*

	Jakarta	Lampung	Pelabuhan Ratu	Banten	Banyuwangi
Jakarta	*				
Lampung	-0.00226	*			
Pelabuhan Ratu	0.00060	-0.01617	*		
Banten	-0.00720	0.00499	0.00477	*	
Banyuwangi	0.14047	0.14375	0.17250	0.11562	*

The phylogenetic relationships confirmed the morphological classification of *Rastrelliger* species, the body depth ratio of *R. brachysoma* ranged from 1:2.6 to 1:3.9 and was highest than 1:4.1 for *R. kanagurta*. The dendrograms NJ philogenetic tree collaborated by the minimum spanning network this study was clustered all *R. brachysoma* into two cluster which is a genetically closely related and *R. kanagurta* as out-group.

Figure 2 represents the dendrograms for the neighbor-joining analysis, while the minimum spanning network of mDNA gene of *R. brachysoma* is shown in Figure 3. The phylogenic tree formed can be divided into two cluster that is cluster A and cluster B.

Other study, results virtualization RFLP mDNA using HincII and HindIII enzymes indicates that *R. brachysoma* is divided into two clusters (Indaryanto et al 2015b). Based on this study, is suspected the two groups are *R. brachysoma* and *R. neglectus*. According to Burnahuddin et al (1984), this species is still controversial, some experts say that they are the same species, but other experts say that they are different species. Thailand people use name *R. negletus* (chub mackerel) for this species. Based on this hypothesis, cluster A is the original population from Java seas and cluster B is the population from the southern part of the South China Sea. *R. brachysoma* from cluster B was absent in Banyuwangi population; this indicates that population from South China Sea have never reached Banyuwangi Bay.

Cluster A comprised of 53 haplotypes mostly from Banten and cluster B comprised of 13 haplotypes mostly from Lampung. The overall bootstrap confident shows very low value as the highest bootstrap value 91%. In cluster A, haplotype 01-RB01 appeared to be the most common to all 5 locations with 38 individuals. There are 3 haplotypes shared with 4 locations, 2 haplotypes shared with 3 locations, 7 haplotypes shared with 2 locations and 40 haplotypes which were singletons and unique. In cluster B, haplotype 54-RB54 appeared to be the most common to 4 locations with 19 individuals. There are 1 haplotype shared with 2 locations and 8 haplotypes which were singletons and unique.

Figure 3 shows two major or ancestral haplotype in cluster A which are the haplotype 01-RB01 and haplotype 03-RB03, and one major haplotype in cluster B which are the haplotype 54-RB54. Haplotype 23-RB23 linked Cluster A and Cluster B. The mutation site in this network was low (only 1 to 2 mutation site) that was separated the haplotypes.

The monsoon cycle induces shifts in water circulation and changes in salinity, which coincide with the seasonal migration of adults and influence the dispersion of the larvae of pelagic fishes (Hardenberg 1937; Rohfritsch & Borsa 2005). *R. brachysoma* will overabundance in west of the Java Sea and lack of stock in east during the East monsoon. Other study reported that *R. brachysoma* form north Jakarta, Indramayu, Pekalongan, Rembang and Pasuruan (Madura strait) Indonesia showed the same population (Zamroni et al 2007). The spawning area of short body mackerel was prognostic at Jakarta Bay and Indramayu Bay.

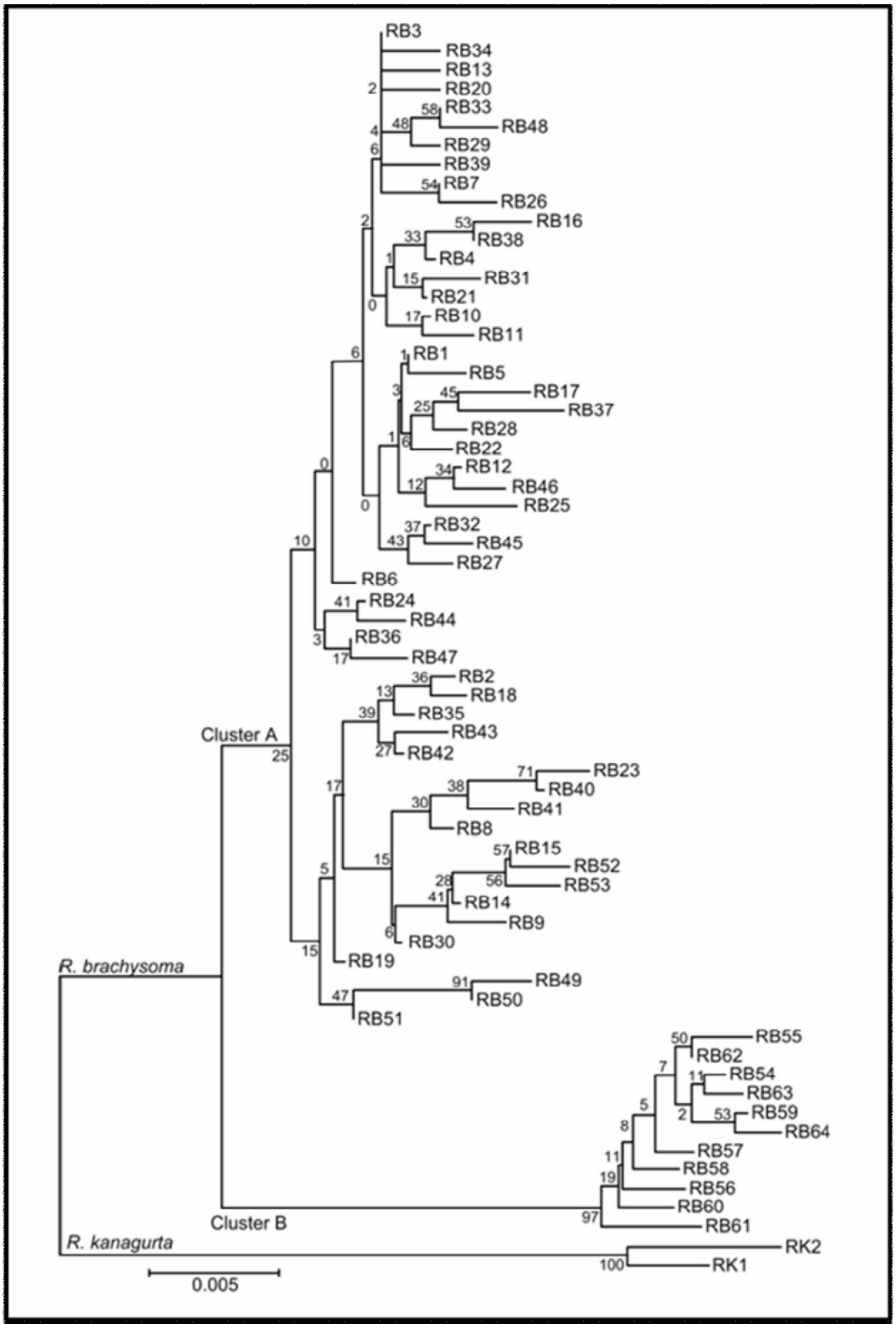

Figure 2. Dendrograms for the neighbor joining phylogenic tree of *Rastrelliger brachysoma* constructed with MEGA5 using Kimura's two-parameter.

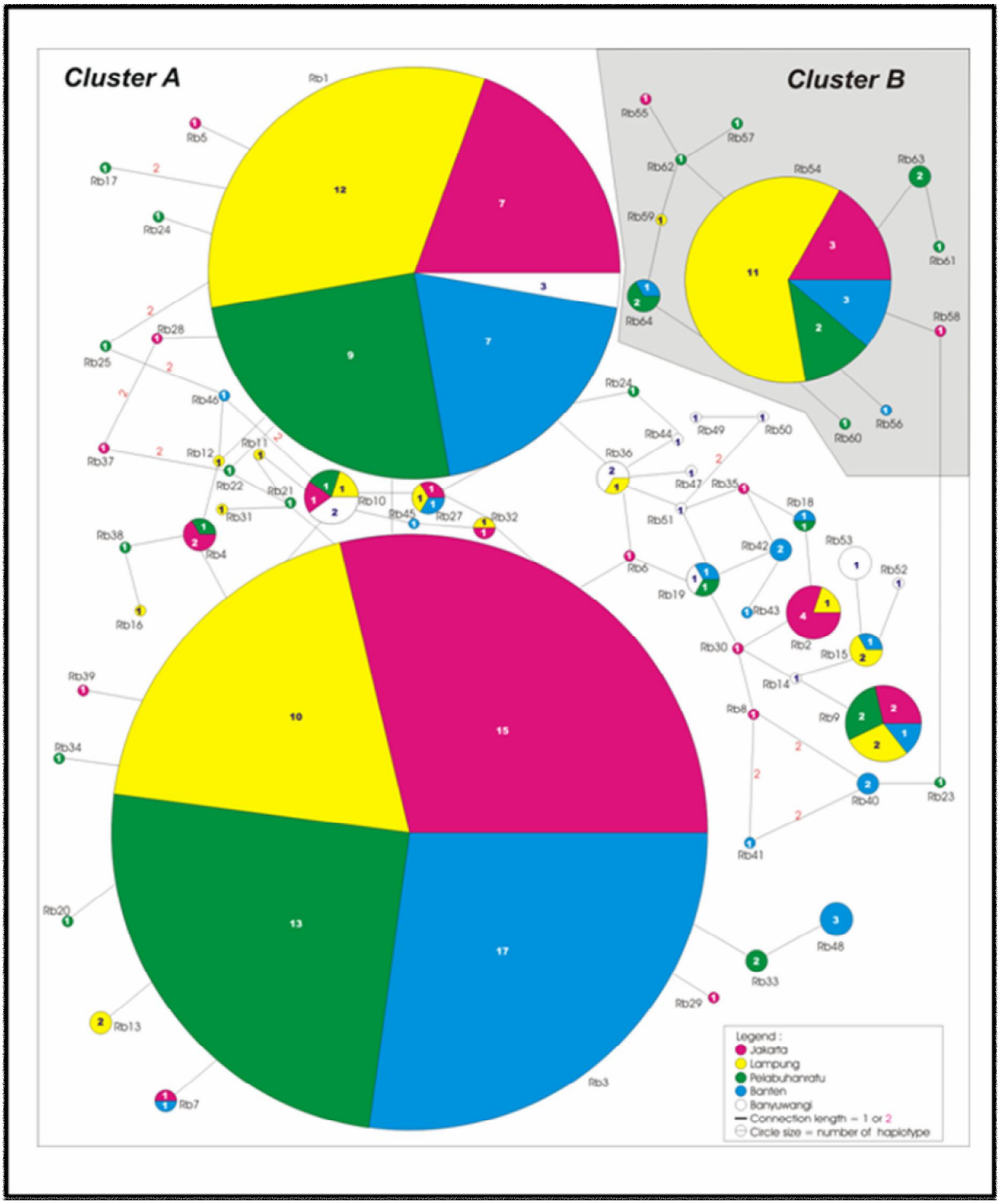

Figure 3. The minimum spanning network of mtDNA gene of *Rastrelliger brachysoma.*

The migratory behavior of Indian mackerel may lead to a congregation of fish from different geographical origins. For marine species, migration can have a substantial impact on the extent of population substructure (Hellberg 2009). The Jawa Sea population of short body mackerel exhibited low level of the nucleotide diversity, this is in accordance with the studies of Zamroni et al (2007), and Darlina et al (2011). For marine fishes, nucleotide diversities are generally considered to be low where they are less than 0.5 (Hobbs et al 2013). Overfishing also was reported to be a major factor causing a significant reduced genetic diversity in an exploited stock, because of overlapping

generations, skewed sex ratios and the variation in the reproductive success of breeders such as genetic drift and inbreeding (Munpholsri et al 2013).

Conclusions. Based on the mtDNA gene, the population of *R. brachysoma* in Jawa Island was divided into two populations with low levels of nucleotide diversity. This genetic data can be integrated with population dynamic data to establish the stock extent and composition for the effective management of the fisheries. These findings also support a management strategy for Indonesian Ministry of Marine Affair and Fisheries. Further analysis with more sampling effort, detailed morphmetrics and more efficient molecular marker are needed to provide a clear understanding of *R. brachysoma* in Indonesia.

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References

Burnahuddin, Martosewojo S., Adrim, M Hutomo M., 1984 [Indian mackerel resources]. Oceanologi Institute – The Indonesian Institute of Sciences, Jakarta, 25 pp. [In Indonesian].

Darlina M. S., Masazurah A. R, Jayasankar P., Jamsari A. F. J., Siti A. M. N., 2011 Morphometric and molecular analysis of mackerel (*Rastrelliger* spp.) from the west coast of Peninsular Malaysia. Genetics and Molecular Research 10(3):2078–2092.

Excoffier L., Lischer H., 2011 Arlequin ver 3.5.1.3 User Manual. Swiss Institute of Bioinformatics, 174 pp.

Ghazali A. F., Abidin D. H. Z., Nor S. A. M., Nalm D. M., 2012 Genetic variation of Indian mackerel (*Rastrelliger kanagurta*) (Cuvier, 1816) of Sabah water based on mitochondrial D-loop region: a preliminary study. Asian Journal of Biology and Biotechnology 1(1):1–10.

Hardenberg J. D. F., 1937 Preliminary report on a migration of fish in the Java sea. Treubia 16:295–300.

Hellberg M. E., 2009 Gene flow and isolation among populations of marine animals. Annual Review Ecological System 40:291–310.

Hobbs J. P. A., van Herwerden L., Jerry D. R., Jones G. P., Munday P. L., 2013 High genetic diversity in geographically remote populations of endemic and widespread coral reef Angelfishes (genus: *Centropyge*). Diversity 5(1):39–50.

Indaryanto F. R., Abdullah M. F., Wardiatno Y., Tiuria R., Imai H., 2015a A description of *Lecithocladium angustiovum* (Digenea: Hemiuridae) in short mackerel, *Rastrelliger brachysoma* (Scombridae), of Indonesia. Tropical Life Sciences Research 26(1):31-40.

Indaryanto F. R., Wardiatno Y., Imai H., 2015b Genetics and morphometrics of short body mackerel (*Rastrelliger brachysoma*). In: Proceeding of National Seminar on Marine Affairs and Fisheries, 5 November 2014, Serang, Banten, Indonesia. [In Indonesian].

Indaryanto F. R., Wardiatno Y., Tiuria R., 2014 [Community structure of helminth parasites of mackerel (Rastrelliger *spp.*) from Banten Bay and Pelabuhan Ratu Bay]. Jurnal Ilmu Pertanian Indonesia 19(1):1-8. [In Indonesian].

Indaryanto F. R., Saifullah, 2011 [Fish consumer preference analysis at Rau fish market and Lama fish market in Serang City – Province Banten]. Jurnal Penelitian Ilmu-Ilmu Sosial dan Eksakta 22(3):56–60. [In Indonesian].

Iwamoto K., 2008 Molecular ecological study of *Siganus spinus* and *S. guttatus* from Okinawa waters based on mitochondrial DNA control region sequences. Journal of Oceanography 65(1):103–112.

Jamaluddin J. A. F., Ahmad A. T., Basir S., Rahim M. A., Nor S. A. M., 2010 *Rastrelliger* systematics inferred from mitochondrial cytochrome b sequences. African Journal of Biotechnology 9(21):3063–3067.
Larkin M. A., Blackshields G., Brown N. P., Chenna R., McGettigan P. A., McWilliam H., Valentin F., Wallace I. M., Wilm A., Lopez R., Thompson J. D., Gibson T. J., Higgins D. G., 2007 Clustal W and Clustal X version 2.0. Bioinformatics 23(21):2947–2948.
Muchlisin Z. A., Masazurah A. R., Abu T. A., Siti A. M. N., Samsudin B., Jamsari A. F. J., 2009 Genetic identification of four Malaysian mackerel species off coast of Peninsular Malaysia based on molecular marker. In: 8th Malaysia Genetic Congress: Role of Genetics in Wealth Creation and Quality of Life , 4–6 August 2009, Awana Genting, Pahang, Malaysia, pp. 74–77.
Munpholsri N., Poompuang S., Senanan W., Kamonrat W., 2013 Microsatellite markers suggested moderate genetic variation in Indian mackerel (*Rastrelliger kanagurta*) populations from the Andaman Sea, Thailand. Kasetsart Journal (Natural Science) 47:853–863.
MMAF (Ministry of Marine Affair and Fisheries), 2012a Jawa Island, the potential market for fisheries products. Ministry of Marine Affairs and Fisheries. Available at: http://www.seafoodservicecentre.com/index.php?option=com_content&view=article&id=70:pulau-jawa-pasar-potensial-untuk-hasil-perikanan&catid=34. Accessed: 3 May 2012.
MMAF (Ministry of Marine Affair and Fisheries), 2012b Capture fisheries statistics of Indonesia, 2011 Ministry of Marine Affairs and Fisheries, Jakarta, 182 pp.
Nei M., Kumar S., 2000 Molecular evolution and phylogenetics. Oxford University, New York, p. 333.
Rohfritsch A., Borsa P., 2005 Genetic structure of Indian scad mackerel *Decapterus russelli*: pleistocene vicariance and secondary contact in the Central Indo-West Pacific Seas. Heredity 95(4):315–326.
Sudjastani T., 1976 The species of *Rastrelliger* in the Jawa Sea: their taxonomy and morphometry (Perciformes, Scombridae). Marine Research in Indonesia 16:1–29.
Zamroni A., Suwarso, Mukhlis N. A., 2007 Reproductive biology and genetic population of short mackerel (*Rastrelliger brachysoma*, Scombridae) in the coastal waters of northern Jawa. Indonesian Fisheries Research Journal 14(2):215–226.
Wardiatno Y., Ardika P. U., Farajallah A., Butet N. A., Mashar A., Kamal M. M., Renjaan E. A., Sarong M. A., 2015 Biodiversity of Indonesian sand crabs (Crustacea, Anomura, Hippidae) and assessment of their phylogenetic relationships. AACL Bioflux 8(2):224-235.
Wodajo B., 2015 DNA-barcoding for taxonomical classification: it's implication species identification. International Journal of Multidisciplinary Research and Information 1(1):63-74.

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