

Short communication: Conservation genetic of tropical eel in Indonesian waters based on population genetic study

Komunikasi pendek: Konservasi genetik ikan sidat tropis di perairan Indonesia berdasarkan kajian genetika populasi

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Abstrak. Fahmi MR. 2015. Konservasi genetik ikan sidat tropis di perairan Indonesia berdasarkan kajian populasi genetik. *Pros Sem Nas Masy Biodiv Indon 1 (1): 38-43*. Ikan sidat tropis (*Anguilla* spp.) dari Indonesia saat ini menjadi komoditas yang penting di pasar internasional dimikian juga penelitian ikan sidat tropis saat ini menjadi sebuah tantangan baru. Salah satu permasalahan utama pada ikan sidat tropis adalah terjadinya tumpang tindih antara karakter morfologi spesies, sehingga identifikasi spesies dengan menggunakan pendekatan morfologi tidak bisa dilakukan. sehingga identifikasi spesies ikan sidat dilakukan dengan pendekatan molekuler. Metode Semi-multiplek PCR yang dikembangkan pada penelitian ini mampu mengidentifikasi tujuh spesies dan subspecies ikan sidatan yang mendiami perairan Indonesia hanya dengan menggunakan satu step PCR. Berdasarkan identifikasi dengan menggunakan semi-multiplek PRC diketahui ada empat spesies ikan sidat yang memiliki penyebaran luas yaitu *Anguilla bicolor bicolor*, *A. b. pacifica*, *A. marmorata* dan *A. interioris*, dua spesies memiliki penyebaran yang sempit dan mendekati endemis: *A. celebesensis* dan *A. borneensis* dan spesies *A. nebulosa nebulosa* yang hanya ditemukan di perairan barat Indonesia yaitu sungai yang berhubungan dengan Samudera Hindia. Berdasarkan analisis DNA mitokondria gen cytochrome b, tujuh spesies yang mendiami perairan Indonesia memperlihatkan keragaman haplotipe dan nucleotide yang tinggi yaitu masing-masing 0.98 dan 4.57%. Analisa kekerabatan menunjukkan *A. borneensis* merupakan nenek moyang ikan sidat tropis di perairan Indonesia.

Kata kunci: Perairan Indonesia, semi-multiplex PCR, ikan sidat tropis, genetika populasi

Abstract. Fahmi MR. 2015. Conservation genetic of tropical eel (*Anguilla* spp.) in Indonesian waters based on population genetic study. *Pros Sem Nas Masy Biodiv Indon 1 (1): 38-43*. The Indonesian tropical eels become important nowadays in the market, as well as the research on tropical eels also become a new challenge. Taxonomy of tropical eels is still problematic due to overlapping morphological characters, thus species identification based on those characters can be misleading. Molecular approaches have been proposed to resolve the problem. The semi-multiplex method proposed in this study has demonstrated the efficiency for identifying seven species and subspecies of tropical eels with only one step PCR. Based on semi-multiplex PCR, we recognized four species and subspecies with wide distribution: *Anguilla bicolor bicolor*, *A. b. pacifica*, *A. marmorata* and *A. interioris*, two species with limited distribution and close to endemism: *A. celebesensis* and *A. borneensis* and one subspecies *A. nebulosa nebulosa* that can only be found in the river flowing into the Indian Ocean. Based on mitochondrial DNA *cytochrome b* gene, seven species inhabiting in Indonesian waters showed higher haplotype and nucleotide diversity (π) 0.98 and 4.57% respectively. Phylogenetic analysis showed that *A. borneensis* is likely the most basal species in Indonesia waters.

Key words: Indonesian waters, semi-multiplex PCR, tropical eel, population genetic

The freshwater eels (*Anguilla* spp.: Anguillidae) are popular as a commercial important food, because of their good nutritional value. These fish are also well known for their unique catadromous life history. These species breed far from offshore after migrating thousands of kilometers from their growth habitats in freshwater and estuaries to their spawning areas in oceanic waters. Most of the investigations concerning eels are concentrated on temperate species, in the northern hemisphere mainly because of the economic importance of these species. Nowadays, the population of temperate eels, dramatically decrease is caused by habitat damage, illegal fishing and

climatic changes in the ocean. As a consequence, tropical eels become important nowadays in the market, as well as the research on tropical eels become a new challenge. One of primary problems in tropical eels is that they have overlapping range for most morphological characters, so species identification on this genus is no more sufficient. Then molecular approaches have been proposed for eel identification.

Several topics on phylogeography of tropical eel in Indonesia has been investigated in the present study, including the establishment of accurate taxonomy and identification of tropical eels by using molecular approach,

understanding exact distribution and dispersal pattern, uncovering phylogenetic relationship among species that inhabit in Indonesian water and population genetic structure of species that have widespread distribution. All of the information obtained in this study is needed for conservation management of anguillids in Indonesian water.

Conservation of eel nowadays has become a big issue in the world. Historically it seems that little attention was given by fisheries managers to eel and their fisheries in most part of the world, and only recently, as recruitment has drastically declined in some regions, awareness raised about eel conservation issue. After these species continued to show drastic recruitment decline in some areas and was listed in the Appendix II of the Convention on International Trade on Endangered species of Wild Fauna and Flora (CITES Red List), there has been increasing concern among researchers (Watanabe 2004). Accordance to the decrease of eel population in the Northern Hemisphere temperate, so researchers suggested the management and conservation of tropical eel should be more cautious.

The classical conservation theory focused on population abundance and diversity of species, but since molecular genetics develop recently the classical conservation also switching towards to conservation genetic. Conservation genetics is conservation and management of species by using molecular genetics analyses to understand aspects of species biology. Conservation genetic was involved interdisciplinary science such as; ecology, molecular biology, population genetics, mathematical modeling and evolutionary systematic. The first aim of conservation genetics is to reduce extinction risk by minimizing inbreeding depression, loss genetic diversity and ability to evolve in response to environmental change, resolving taxonomic uncertainties and defining management units within species (Frankham et al. 2007).

A critical first step in species conservation is to gain a clear understanding of its taxonomy. The taxonomy status must be accurately established for several reasons: such as legal protection of endangered species and populations, identification of species dispersal, determination of species that have high economic value, stock assessment, determination of endemic or invasive status of species, help to decisions for development and sustainable management of eel farming and to determination of the fitness level of population from variation of species or composition species (Frankham et al. 2007).

A comprehensive taxonomy study of the genus *Anguilla* has been conducted by Ege (1939) based on morphological characters. The systematic classification proposed by Ege remains widely accepted by many biologists for long period. However, Watanabe et al. (2004) showed that the morphological characters used by Ege were insufficient to distinguish all the *Anguilla* species without considering their geographic distribution. It can be a problem when sampling location is unknown, especially in the tropical area, where there are some cases of sympatric distributions between two species with all morphological characters overlapping. In these cases, two species cannot be strictly identified. In addition, potential

problems of the geographic distribution of species will be increased dramatically considering passive transport of leptocephali and international trading of young eel. Passive transport of leptocephali for long periods can easily change the range of glass eels recruitment, thus the geographic distribution of the species can change year after year. Furthermore the international trade of glass eels and young eels for aquaculture purpose lead to introduction of non-native species eels in several areas of the world (Watanabe et al. 2004, Aoyama 2009).

After establishment that morphological characters were not sufficient to identify all species, molecular genetics have been recommended (Aoyama et al. 2005). Since then various molecular identification methods of eels began to be developed; some of them using non specific primer such as RAPD (Lehmann et al. 2000; Kim et al. 2009) and RFLP (Aoyama et al. 2000), whereas the specific primers were used to distinguish two different species such as *A. japonica* and *A. anguilla* (Sezaki et al. 2005), *A. interioris* and *A. celebesensis* (Aoyama et al. 2000) or *A. anguilla* and *A. rostrata* (Trautner 2006). Most of them use simple PCR followed by sequencing, while sequencing is not convenient for large samples because of its cost, time and money. In addition most of these researches are concerned in temperate area only.

Considering Indonesian waters that are inhabited by several sympatric species of tropical eels, almost all morphological character in this genus are overlapping. So a rapid and efficient identification technique is needed. Semi-multiplex PCR developed in the present study has successfully distinguished seven species that inhabit the waters of Indonesia through one-step PCR (Fahmi et al. 2012). The semi-multiplex method proposed in this study has demonstrated the efficiency for identifying seven species and sub-species of tropical eels with only one step PCR. By using this method, one could reduce the number of necessary sequences while the results are sure for each species determination; it is easily to identify 1,112 specimens). Figure 1 shows seven different sizes of amplification fragments. All species obtained in this study showed overlapping morphology and distribution, especially for small specimens (mainly glass eels).

With this identification technique have several advantage as follows: (i) more accurate and sensitive, because we used species and subspecies specific primers, (ii) quick, because only by one-step PCR was enough to distinguish seven species, (iii) lower cost detection by using simple agarose gel electrophoresis and (iv) also applicable to leptocephali and glass eels missing adult morphological characters. Specimens used in this study were collected all around Indonesian waters, covering the geographic distribution of the *Anguilla* species that were expected to occur in Indonesia water.

After finding out and solve the taxonomic uncertainties, next step in conservation genetics is producing a map of distribution and species composing tropical eels in Indonesia. The 16 species of freshwater eels are widely distributed in the world, there are 5 temperate and 11 tropical anguillids species, and all of them have unique catadromous life histories. According to Ege (1939) and

Watanabe et al. (2004), Indonesian waters are inhabited by seven tropical eel species and subspecies including *A. celebesensis*, *A. borneensis*, *A. marmorata*, *A. interioris*, *A. bicolor bicolor*, *A. b. pacifica*, and *A. nebulosa nebulosa*. That means that two-thirds of tropical eel species inhabit Indonesian waters. Because all unexpected semi-multiplex result led to sequencing, it is possible to affirm that no eel other species was present in the sampled localities. We presented the distribution and species composition of tropical eels in Indonesia in Figure 2. This map is the first detailed distribution of Indonesian eels published.

Based on the distribution map, several species showed a wide distribution such as *A. marmorata*, *A. b. bicolor* and *A. interioris*, and some have a limited distribution, known as endemic species such as *A. celebesensis* and *A. borneensis*. The distribution pattern is not permanent for each species in geologic time. Moreover, due to their particular life cycle, distribution patterns can change seasonally, linked with the dispersion of their leptocephali driven by the current system in their habitat (Miller 2003).

So the current system has an important role on anguillids distribution. There are four species sympatric in the western of Sumatra and south of Java, they are *A. marmorata*, *A. interioris*, *A. n. nebulosa* and *A. b. bicolor*, the three first species having most similar morphological characters. The same case is also found in Sulawesi waters where there are four sympatric species: *A. marmorata*, *A. interioris*, *A. celebesensi* and *A. b. pacifica*, the first three species also have overlapping morphological characters. In these two cases of overlapping nearly sibling species, the molecular determination is now indispensable.

The accurate taxonomy status of eel in this study has been successfully determined dispersal and endemic status of species, that useful to decisions for development and sustainable management of eel conservation. This map is more useful to inform the fishing companies the location where is their fishing zone which is not present an endangered species. This map also is more important to policy or government decision that the rare or endemic species are no fishing activity.

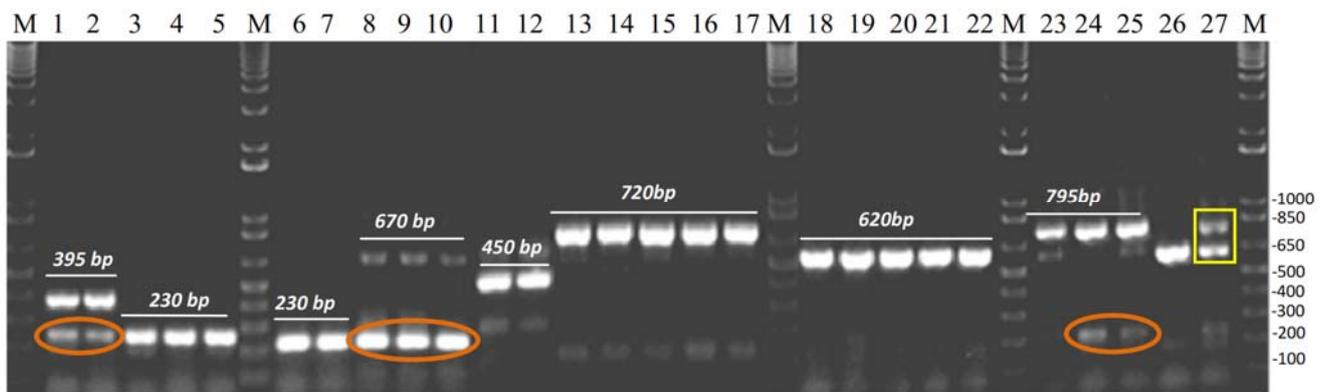


Figure 1. Identification of species and subspecies of *Anguilla* by semi-multiplex PCR samples. The order of the sample is the following : 1-2 are *A. n. nebulosa*, 3-7 are *A. b. bicolor*, 8-10 are *A. b. pacifica*, 11-12 are *A. borneensis*, 13-17 are *A. celebesensis*, 18-22 and 26 are *A. marmorata*, 23-25 and 27 are *A. interioris*, M= 100 bp ladder. ○ = unspecific bands, □ = unexpected band.

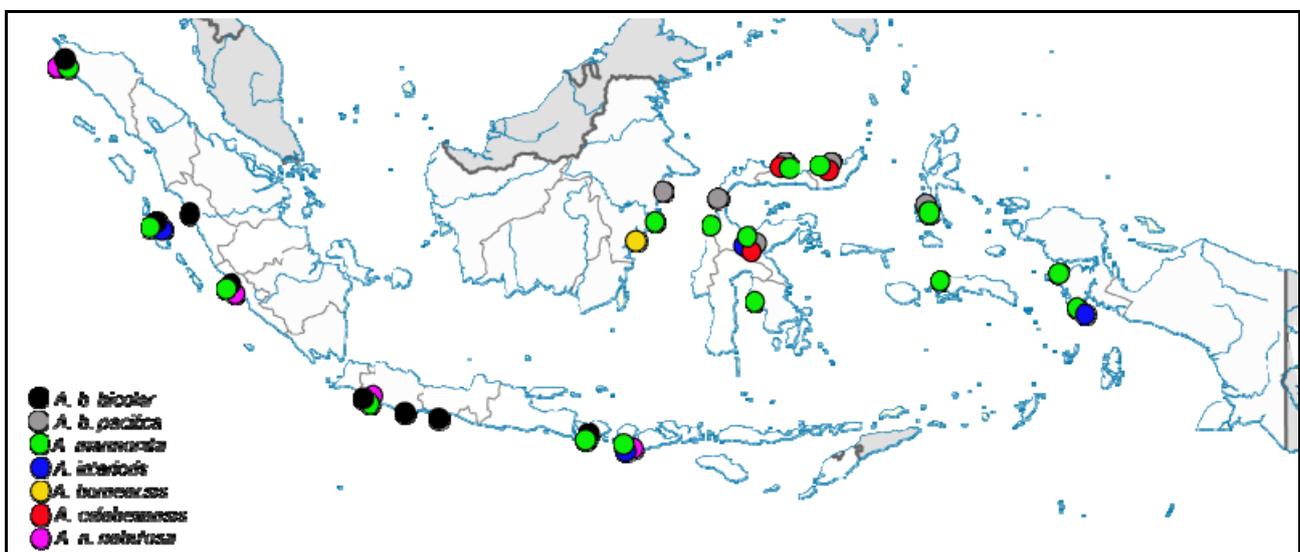


Figure 2. Distribution and species composition of tropical eel in Indonesia water.

The endemic eel species with typically small population are more prone to extinction, because as the genetic consequences of small population are more inbreeding depression and less of population size. Loss of genetic diversity in endemic species was reduced the ability of species to respond the environmental change. Here, we proposed the management and conservation of eel endemic species. The necessary precursor for genetic management of endemic species is increased their population size. One efforts could be done is restricting the capture and trading the species through in situ conservation. Based on the mapping of eel distribution we suggested to conserve three locations that could be an in situ conservation of eel; Mentawai, Poso and Mahakam. These locations were suspected as the spawning area and grow up of eel both endemic and non-endemic species.

The phylogenetic relationship and genetic diversity was constructed and calculated based on mitochondrial DNA *cytochrome b* gene sequence of *Anguilla* that covered all of the geographical distribution in Indonesian waters. The genetic structure of populations responsible for a given genetic architecture and evolutionary factors is an important objective of population genetics. In this study we present genetic diversity and phylogenetic relationship among and within species in the genus *Anguilla* to understand the evolutionary process of Anguillids that inhabit in Indonesian waters. Seven species of Indonesian tropical eels showed a higher haplotype and nucleotide diversity at the *cyt b* locus with haplotype and nucleotide diversity (π) 0.98 and 4.57% respectively. Phylogenetic tree showed *A. borneensis* is most likely as basal species in Indonesia water (Figure 3).

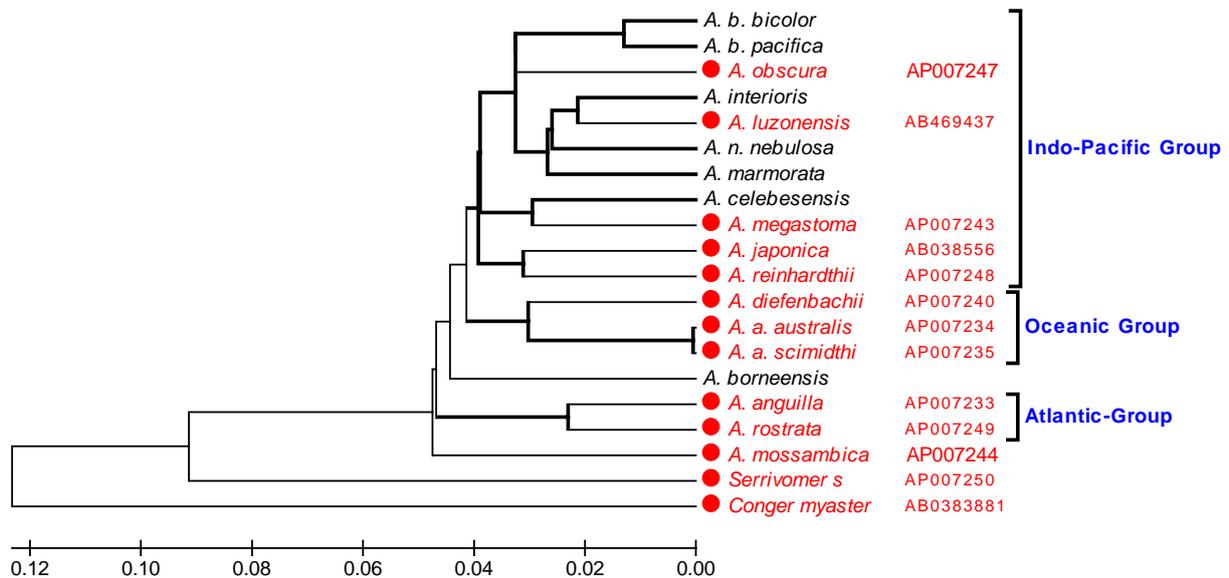


Figure 3. Dendrogram of genetic distance of Indonesian tropical eel based on cytochrome b gene sequence, without dot (●): *cyt b* sequence from this study and with dot (●): based on sequence from GenBank.

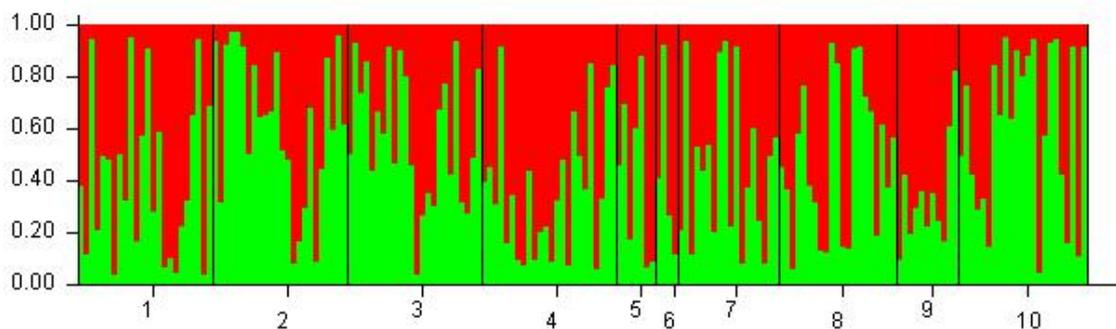


Figure 4. Clustering analyses (K=2) for the *Anguilla bicolor* data set (180 individual, 10 population and 7 loci) performed using STRUCTURE program (Pritchard et al. 2000).

The economic important short-finned eel, *Anguilla bicolor*, has a relatively wide geographic distribution compared to the 19 species and subspecies of the genus *Anguilla*, it is distributed longitudinally from the eastern coasts of Africa through the seas around Indonesia to New Guinea in the Pacific Ocean. The genotypes of seven microsatellite DNA were analyzed for 180 specimens collected from 10 representative locations where two subspecies have been found. Analyses with seven microsatellite loci showed expected (H_e) and observed (H_o) heterozygosity of each locus ranging from 0.594 to 0.921 and from 0.250 to 1.000 respectively. All of locus showed high polymorphic. Based on F_{ST} value and clustering test, there is no structure and fragmentation of *A. bicolor* in Indonesian water (Figure 4).

While the economic important and widespread of eel, *A. marmorata* and *A. bicolor*, can be utilized under controlled or quota fisheries. The most important consideration on the utilization of economically eel is maintenance of genetic diversity and maintenance the population from endangered species. In temperate areas where eel is economically important fish, conservation and management glass eel has been managed. Management on utilization of the glass eel in temperate areas included natural recruitment was low, restriction on fishing, fishing gear control, imposing size limits or requiring fishing licenses and facilitating the passage of glass eel over dams (Ringuelet 2002).

Next discussion we would like to emphasize that Indonesian water is a "center of the origin of Anguillia" of eels because there are a lot of species. But to propose these hypotheses, we must find the more basal lineage of eels in the phylogeny of the genus and distribution of this basal species. Accordance to Minegishi et al. (2005) was constructed the phylogenetic tree based on complete mtDNA that suggested *A. mossambica* as basal species, followed by the six species of *A. borneensis* and the Atlantic (two species) and Oceanian (three species) species with vary distribution. Another clade is 11 Indo-Pacific species were the geographic distributions adjacent to each other. So there are two large phylogenetic groups of anguillid eels which have different evolutionary histories. Therefore, the evolutionary process of the genus *Anguilla* might not be simple, and the present geographic distribution could be attributed to, for example, multiple dispersal events, multidirectional dispersion, or past extinctions. As the result, the question the origin of freshwater anguillid eels genus *Anguilla* not really clear and that is always intriguing for scientists (Minegishi et al. 2005).

The international trade in glass eel has become increasingly global in its influences in recent decades due in part to more extensive eel aquaculture effort in China and Korean, so a lot of glass eel from tropical area especially from Indonesia has been sent to support aquaculture there. To overcome the over exploitation of the glass eel for export purpose so Indonesian government through the Ministry of Marine Affairs and Fisheries have made a regulation that banning the export of small eel less

than 150 g (PERMEN No.19/MEN/2012). The objective of this regulation is to increase the aquaculture production of eel. However there is still need a new regulation to reduce anthropogenic influences, to manage and conservation of eels and to restrict all collections of glass eel.

The last question only tested in this survey is the research of structure inside the large distribution species. In Indonesia, *A. interioris* is scarcely observed and no intraspecific structure could be investigated. But *A. marmoratus* and *A. b. bicolor* have been sampled in most parts of the country and deserve more detailed investigations. These species have been recently analyzed in their whole distribution from western Indian Ocean to eastern Pacific Ocean (Gagnaire et al. 2009, 2011; Minegishi et al. 2012) but a detailed phylogeography using both mtDNA and nuclear markers in Indonesian, crossroad country between several oceans, is still missing. A preliminary study of *A. b. bicolor* is proposed in this thesis.

A critical first step in species conservation is to gain a clear understanding of its taxonomy. Considering Indonesian water is inhabited by several sympatric species of tropical eels, almost all morphological characters in this genus are overlapping. So a rapid and efficient identification technique is needed. The semi-multiplex PCR that has been developed in the present study has successfully distinguished seven species that inhabit the waters of Indonesia through one-step PCR. After establishing the identification species and subspecies methods then we constructed the distribution and dispersal pattern, phylogenetic relationship among species that inhabit Indonesian waters and the population genetic structure of species that have widespread distribution. All the information obtained in this study was needed for conservation management of anguillids in Indonesian waters.

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