STUDY ON THE MORPHOLOGY AND GENETIC CHARACTERS OF TROPICAL FRESHWATER EELS IN THE INDONESIAN WATERS

Hagi Yulia Sugeha*, Rochmadini, Sri Sulandari*****
* Pusat Penelitian Oseanografi-LIPI Jl. Pasir Putih 1, Ancol Timur
** Fakultas MIPA-UI, Kampus UI-Depok, Depok
*** Pusat Penelitian Biologi-LIPI, Jl. Raya Bogor KM 46, Cibinong 16911

ABSTRACT

In order to know morphology and genetic characters of the tropical freshwater eel in Indonesian Waters, a total of 292 specimens collected in 2004 and 2005 from western, center, and eastern parts of Indonesian Waters had been examined based on morphology (pigmentation and body length measurement) and genetic analysis (PCR-RFLP). From pigmentation observation, it was found that all specimens belongs to pigmentation stage VA and VB or in "glass eel" stage and just start to migrate inshore after facing a long distance oceanic migration. From body length measurements, it was found that most specimens belongs to 6 ~ 13 in ADL/%TL (A. borneensis, A. nebulosa, A. celebesensis, A. interioris, A. obscura) while the other species belongs to -3 ~ 3 (A. bicolor) and >13 (A. marmorata) in ADL/%TL. It is suggested that pigmentation and body length measurement could not be used for species identification of tropical freshwater eel during their glass eel stage. Based on PCR-RFLP analysis, it was found that from 7 species identified in the study, each species have to shown one pattern of species specific haplotype as ever reported, except for A. bicolor that have 2 new patterns of species specific haplotypes as well as A. celebesensis. The result on genetic analysis has to shown phenomena of intra-specific variation of tropical freshwater eels in the remote area of Indonesian Waters.

Keywords: glass eel, morphology, genetics, pigmentation, ADL/%TL, PCR-RFLP, haplotype pattern

INTRODUCTION

Anguillid eels traditionally have been an important fish as a food resource in many eastern and western countries. In Japan, the Japanese eel (Anguilla japonica) has long been esteemed as an important food fish that has a unique taste. Presently, as much as 130,000 tons of eels are consumed each year in Japan. Similarly, in the European countries, the Atlantic eels (A. anguilla and A. rostrata) have been an integral part of the cuisine of several countries. However, the decrease of eel resources has been a serious problem in recent years. It is not yet clearly whether this has been caused by global changes in the ocean, atmospheric system, the human impacts of ever-fishing and environmental deterioration, or intra-specific or intra-population biological factors. As an impact, for the last recent years import of tropical anguillid eels from the Southeast Asia countries become a new trend to solve the problem. In order to counteract the decrease in eel resources it is important to understand the underlying causes and mechanisms of these changes, and to develop effective management strategies for maintaining stable eel populations. Therefore, the first goal should be to use a biological approach to gain a complete understanding of the mysterious life cycle of the freshwater eel in Indonesian Waters as the greatest country in the Southeast Asia region.

The catadromous eels of the genus Anguilla appear to have originated in the tropical regions of the Pacific Ocean, where the greatest number of species is found (Aoyama & Tsukamoto 1997). Two thirds of the recognized 18 species and sub species are found in the tropical Pacific, while only 6 are found in temperate regions of both the Pacific and Atlantic Oceans. Seven species and sub species of tropical eels occur in the western Pacific around Indonesia (Ege 1939; Castle & Williamson 1974, Arai et al. 1999a, Sugeha et al. 2001b, c, 2003). Both morphological and genetic studies

indicate that tropical eels are more closely related than temperate eels to the ancestral eel (Ege 1939, Castle & Willimason 1974, Aoyama et al 1998). According to Aoyama et al. (2001), anguillid eels originated near present day Indonesia and dispersed to both the east and west along paleo-circum equatorial current and the authors suggested that A. borneensis from Borneo Island was the most basal species. Thus the long distance catadromous migrations of anguillid eel may have originated in the tropical species, and biological study of the tropical freshwater eels may provide a greater understanding of the origin of the catadromous migration of anguillid eels.

The present study was conducted for biological study of the freshwater eels in the tropic. The objective of the study is to carry out the morphology and genetic characters of the tropical anguillid eels in the Indonesian Waters.

METHODS

Specimen collection: A total of 292 glass eels collected at the new moon phase in May to October 2004 and 2005 from seven estuaries in the Indonesian waters (Table 1) were used for the present study. They were caught along the beach using 2 triangular scoop nets (mouth 0.3 m^2, 1 mm mesh) following the sampling technique by Sugeha et al (2001b). The glass eels were fixed in ethanol absolute just after capture, labeled, and transported to the laboratory for future analysis. All the specimens were used for morphology analysis while 100 specimens among them were used for genetic analysis.

Morphology and genetic analysis: Prior to genetic analysis, body length measurement including total length (TL) pre-dorsal length (PDL), pre-anal length (PAL), and ano-dorsal length (ADL) of 292 individuals of tropical glass eel were done to the nearest 0.1 mm. Pigmentation observation was determined according to Bertin (1956) in order to adjust the developmental stage of the specimens.

Total genomic DNA (deoxyribonucleic acid) extraction from 100 specimens of glass eel was carried

Table 1. Sample collection

Sampling location	Analyses	Number of sample
Cibaliung River estuary	Morphology	15
	Genetic	14
Batang Antokan River estuary	Morphology	16
	Genetic	13
Mahakam River	Morphology	3
	Genetic	2
Palu River estuary	Morphology	39
	Genetic	17
Dumoga River estuary	Morphology	116
	Genetic	25
Pami River estuary	Morphology	74
	Genetic	15
Akelamo River estuary	Morphology	29
	Genetic	14
Total	Morphology	292
	Genetic	100

out following a standard protocol (Aoyama and Tsukamoto, 1997). DNA was isolated and purified using phenol-chloroform-isoamyl alcohol (25:24:1, v/v) twice with diethyl ether, then concentrated by ethanol precipitation before finally suspended in the TE solution and stored in the freezer. A portion of the mitochondrial 16S ribosomal RNA gene was amplified via polymerase chain reaction (PCR) using the oligo-nucleotide primers that were nested in the 16SrRNA: L1854: 5'-AAA-CCT-CGT-ACC-TTT-TGC-AT- 3' (Aoyama, 1998) and H3058: 5'- TCC-GGT-CTG-AAC-TCA-GAT-CAC-GTA- 3' (Miya and Nishida, 1996). The PCR was carried out with the Gen Amp PCR system 7200 (Applied biosystem), with a 25 ul reaction volume containing 13.8 ul sterile distilled water, 2.5 ul 10XPCR buffer (Perkin Elmer-Cetus), 2.5 ul dNTP (deoxynucleotide triphospate) of 2 mM, 2.5 ul each primer of 5 uM, 0.4 ul of Taq DNA polymerase (AmpliTaq, Perkin Elmer Cetus), and 50 to 1,000 ng of template DNA. Amplification parameters were 30 cycles of denaturation at 94 ^{o}C for 15 sec, annealing at 55 ^{o}C for 15 sec, and extension at 72 ^{o}C for 30 sec.

To develop a new method of identification of anguillid species from the Indonesian Waters, a longer double-stranded mitochondria DNA product from PCR was examined using Restriction Fragment Length Polymorphism (RFLP) analysis with the six type of restriction enzymes Alu I, Hha I, and Bsp 1286I (Promega); EcoT14I and Mva I (Takara Shuzo Co., Ltd); and BbrP I (Toyobo Co., Ltd) which made it possible to identify the species of tropical anguillid eels as described in Aoyama et al (2000b), Watanabe (2000), and Sugeha (2003). Restriction procedures were carried out in a 15ul final volume containing 5 ul PCR product, 1 ul restriction enzyme, 1.6 ul restriction enzyme buffer supplied by manufacturers and 7.5ul sterile distilled water, and incubated at 37 ^{o}C overnight. Restriction fragment length polymorphism (haplotype) was detected by electrophoresis on 1 % agarose gel with ethidium staining.

RESULTS

External morphology analysis

Prior to species adjustment using genetic analysis, there were a total of 292 glass eels that were morphologically analyzed in the study based on measurement of body length. Two third (243 specimens) of these glass eel were longfin eel species and the rest (59 specimens) were shortfin eel, based on their percentage of ano-dorsal length to total length (ADL/%TL) as reported by Sugeha et al. (2001). Based on the geographic distribution and the range in ADL/%TL of the tropical anguillid eel species that ever reported (Ege, 1939; Jespersen, 1942; Watanabe, 2000; Arai, 1999; Sugeha, 2001, 2003), it was predict that the shortfin eels belongs to A. bicolor including its two sub species of A. bicolor bicolor and A. bicolor pacifica and to A. obscura while the other longfin eels (4~13 in range of ADL/%TL) belongs to Anguilla spp (overlap area of ADL/%TL), except for the longfin eel A. marmorata that reported to have more than 14 in range of ADL/%TL (Sugeha, 2001). Therefore, based on the external morphology analysis it was found that all specimen could be separated only in 3 species: A. bicolor, Anguilla spp, and A. marmorata (Figure 1).

Further observation on the pigmentation development of the glass eel specimens shown that all specimens belongs to stage VA (75 %) and VB (25%) and suggested that there are finished the metamorphosis stage and just enter the freshwater area or in the glass eel stage when caught.

PCR-RFLP analysis

The PCR analysis of the 16S ribosomal RNA gene from DNA mitochondria of the tropical anguillid eels (Figure 2a) showed several different restricted fragment patterns or different haplotype after RFLP-analysis (Figure 2b). The restriction enzymes Alu I, BbrP I, and Mva I showed two different haplotypes. The enzymes EcoT14 I and Bsp1286 I exhibited five different haplotypes. The restriction enzymes Hha I only showed one haplotypes. Thus the 16SrRNA processed by the six

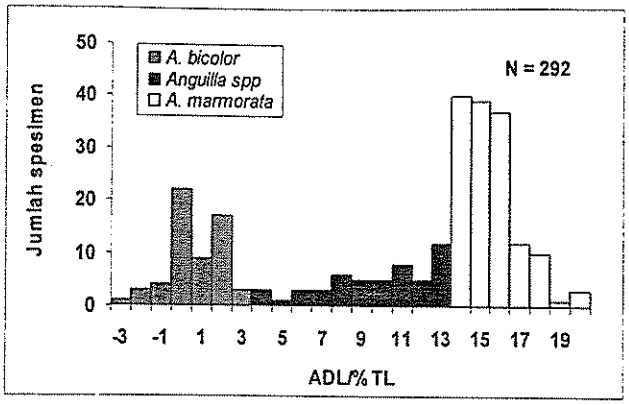


Figure 1. External morphology character of all specimens of tropical anguillid eels collected in the present study before species adjustment by genetic analysis

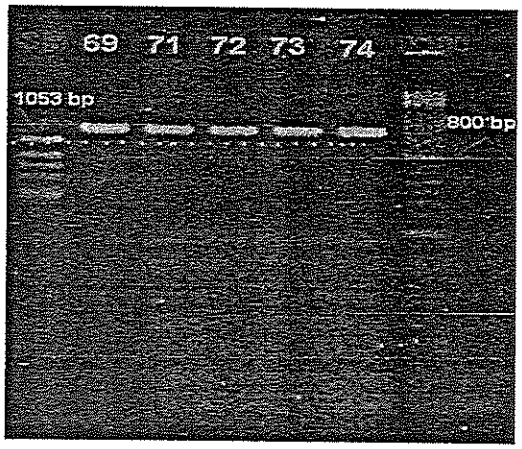


Figure 2a. Photograph PCR product of the 16S ribosomal RNA gene from DNA mitochondria of the tropical anguillid eels

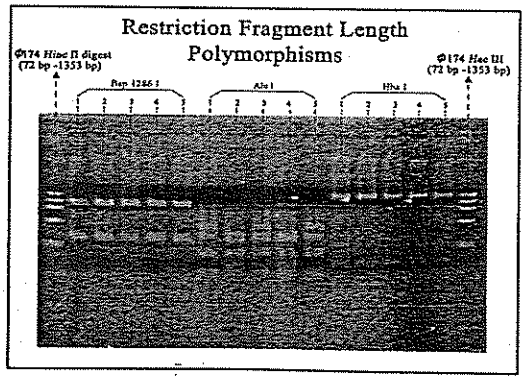


Figure 2b. Photograph RFLP analysis of the PCR product

restriction enymes of Alu I, BbrP I, Mva I, EcoT14 I, Bsp1286 I and BbrP I, clearly exhibit unambiguous fragment pattern whose haplotypes were designated alphabetically (Table 2).

All the six restriction enzymes used in the study (Table 2) were showed a similar genetic character as ever reported except for the restriction enzymes Bsp1286 I that exhibited different genetic character of fragment pattern which were never described by Aoyama et al. (2000), Watanabe (2001), and Sugeha (2003). Based on the similarity of the restriction fragment pattern between the previous and the present study than the species-specific haplotype of the tropical anguillid eels in the present study could be precise as follows as A. marmorata, A. borneensis, A. interioris, A. celebesensis, A. bicolor and A. megastoma. Furthermore, there were five new haplotypes found in the present study and still categories as "Anguilla sp 1" to "Anguilla sp 5".

DISCUSSION

External morphology characters of the tropical glass eel

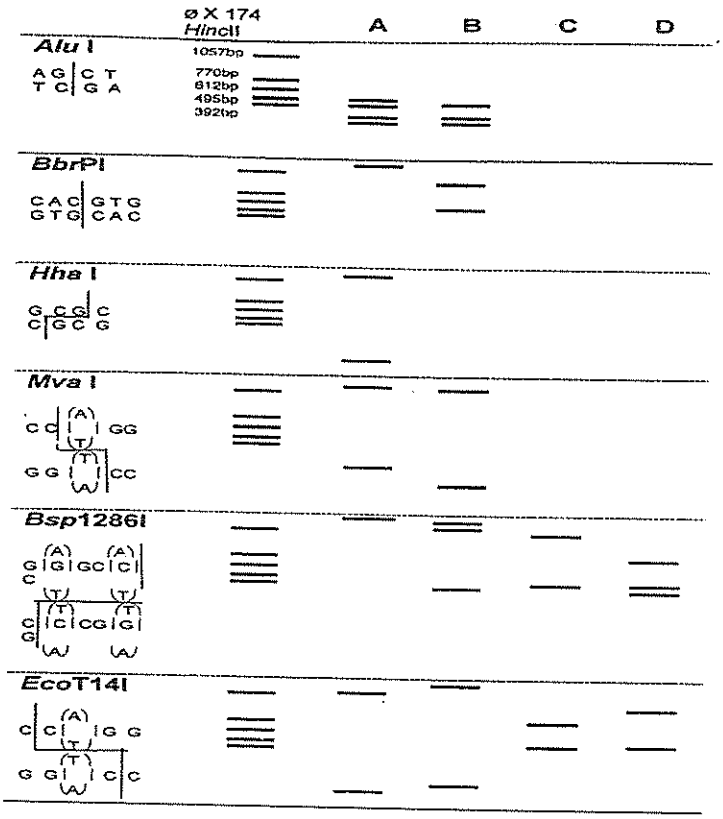
Using external morphological analysis of the quantitative catch sample of glass eels at some estuaries around Indonesian Waters, the same several species of Anguilla were identify by the morphology and genetic analysis of Aoyama et al (2000), Arai et al (1999), Watanabe (2001) and Sugeha (2001). These were shortfin eel A. bicolor with a range of ADL/%TL about -4 to 6 and the long fin eels with the range of ADL/%TL 7 to 20. The sub-species of shortfin eel A. bicolor bicolor and A. bicolor pacifica could be distinguished genetically by different haplotype but their range of ADL/%TL were overlapped from -4 to 4 and from 2 to 9 respectively, and could not be used for sub-species identification. The similar condition was observed in the longfin eels. Genetically the longfin eel species of A. nebulosa, A. borneensis, A. interioris, A. celebesensis, A.marmorata and A. megastoma could be identify based on their range of ADL/%TL were shown a great overlapped from 7 to 20. It was found also that all characters of body measurement outside ADL/%TL showed overlapping in ranges including total length (TL), pre-dorsal length (PDL), and pre-anal length (PAL). These finding reconfirmed that the external morphology (body measurement) alone could not be used for species identification of tropical anguillid eels around Indonesian waters (Figure 3). This study also has to complete similar study on the genetic diversity that ever reported.

A similar condition of overlapping in external morphology key character of ADL/%TL also has been found in specimens that recognized as "Anguilla sp 1" to "Anguilla sp 5". Genetically they could be separate in five different haplotypes but morphologically they was overlap in range of ADL %TL from 1 to 16 and suggested that the five new characters could belongs to long and shortfin eel based on Ege's description (1939). The present study has been rising up a new perspective on the critical condition of species identification of anguillid eels in the tropic. It is become important to understand that the morphology criteria of shortfin and longfin anguillid eel could not be applied for the tropical species and the new criteria of taxonomy works in the tropical angullid eels are strongly required based on confirmation and crossing check between morphology and genetic study.

Genetic characters of the tropical anguillid eels

The PCR-RFLP analyses clearly identified the species of the 100 glass eels from some estuaries around Indonesian Waters based on thespecies specific haplotype found in the study (Table 3). There are six species that genetically identify in the study as following as: A. borneensis, A. celebesensis, A. marmorata, A. interioris, A. megastoma and A. bicolor. The first five species completely supported previous study on the diversity of anguillid eel species in the Indonesian Waters (Aoyama, 1998) who reported about 7 species and sub species are inhabits on the region. However, the other two species A. nebulosa and A. obscura has not yet been found in the present study even the range of distribution of these species

Table 2. Genetic character of tropical angullid eels after PCR-RFLP analyses that symbolized with different alphabet, showing several restriction fragment pattern after digested with by six restriction enzymes



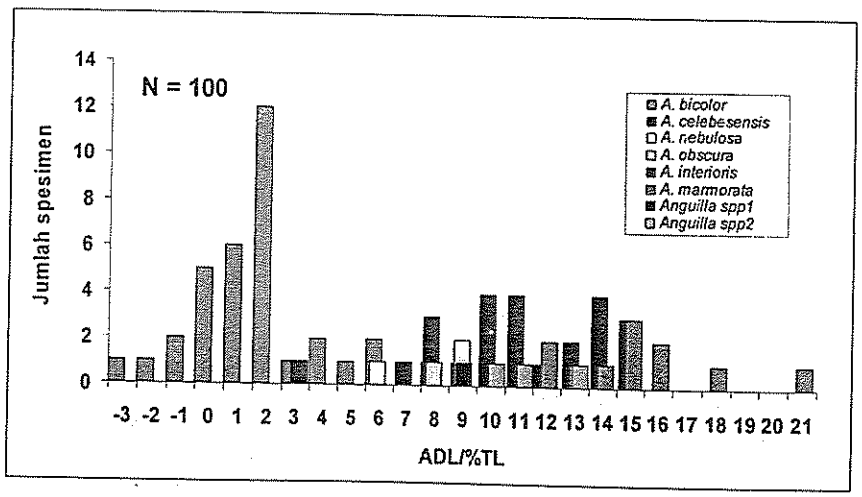


Figure 3. Overlapping external morphology character of ADL/%TL of tropical glass eel after genetic PCR-RFLP analyses suggested that external morphology alone could not be used for species identification of tropical anguillid eel in glass eel stage

Tabel 3. Species-specific haplotypes obtained from RFLP analyis with six restriction enzymes for the tropical anguillid eel

No.	Restriction Enzymes	Species specific haplotype											
		A. bic bic	A. bic pac	A. cel	A. int	A. bor	A. meg	A. mar	Ang sp 1	Ang sp 2	Ang sp 3	Ang sp 4	Ang sp 5
1	Alu I	A	A	B	A	A	B	A	B	B	A	A	A
2	BbrP I	A	A		B		B	A			A	A	B
3	Bsp 1286	C	B	B	B	D	A	B	C	B	B	C	C
4	EcoT14 I	A	A	A	A	C	A	A	A	B	B	B	A
5	Hha I	A	A	A	A	A	A	A	A	A	A	A	A
6	Mva I	D	B	B	B	B	B	B	D	B	B	B	B

Remarks:

A. bic bic	: A. bicolor bicolor	Ang sp 1	: Anguilla sp 1
A. bic pac	: A. bicolor pacifica	Ang sp 2	: Anguilla sp 2
A. cel	: A. celebesensis	Ang sp 3	: Anguilla sp 3
A. int	: A. interioris	Ang sp 4	: Anguilla sp 4
A. bor	: A. borneensis	Ang sp 5	: Anguilla sp 5
A. meg	: A. megastoma	Alpabeth with blue color = new types	
A. mar	: A. marmorata		

were located in the western and eastern part of Indonesian Waters, respectively. In contrast, the longfin eel species of A. megastoma that usually reported to be found and distributed from the eastern part of Papua New Guinea to the southern part of Pacific Ocean (Ege, 1939; Watanabe, 2001) were found in the Northern Sulawesi and Western Papua. The finding on the changes in the geographic distribution of the tropical eels around Indonesian waters might be cause of change in distribution pattern of the organism regulated by the change of global environmental condition. The other possibility that the sampling area conducted in the previous studies still not yet covered the range of distribution of A. megastoma. Therefore, a new range of distribution of A. megastoma carried out from the present study would be expanded the range of distribution of the species in the world.

Especially for A. bicolor, both genetic sequence and PCR-RFLP analysis had been applied in previous study by Aoyama (2000) and Sugeha (2003). From the study, the authors found two different haplotypes derivate from A. bicolor that could be used for recognized and distinguished genetic characters in form of sub species restriction fragment pattern for A. bicolor bicolor and A. bicolor pacifica. In the present study, both genetic characters also found. The finding were supported the previous studies, and proved that the two sub species of the tropical shortfin eels A. bicolor were inhabit in the Indonesian Waters.

Five new genetic characters were found in the present study. All the genetic characters were shown five different haplotypes that never reported in previous studies. The result suggested a possibility of new finding on the new species in the genus. The possibility of new finding on the intra-specific variation of tropical anguillid eels also promised. Study on the genetic diversity of tropical anguillid eels in Indonesian Waters using an advance method of DNA sequencing analyses will be an interesting research works in the future.

Acknowledgements

This study was supported by research grant from Program Competitive, Sub Program Census of Marine Life - LIPI from 2004 to 2006o of fiscal years. We wish to send our grateful to the students of Andalas Univ., Sam Ratulangi Univ., Tadulako Univ., Negeri Papua Univ. as well as the local government of Banten, East Kalimantan, and North Maluku who helped us during the field research.

LITERATURE CITED

Aoyama, J. and K. Tsukamoto. 1997. Evolution of the freshwater eels. Naturwissenscaften 84 : 17-21

Aoyama, J. 1998. Molecular phylogeny and evolution of the freshwater eels genus Anguilla. Ph.D thesis. The University of Tokyo

Aoyama J, Watanabe S, Nishida N, Tsukamoto K. 2000. Discrimination of catadromous eels of genus Anguilla using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism Analysis of the Mitochondrial 16S Ribosomal RNA Domain. Trans Am Fish Soc 129: 873-878.

Arai T., J. Aoyama, D. Limbong. K. Tsukamoto. 1999b. Species composition and inshore migration of tropical eels, Anguilla spp., recruiting to the estuary of the Poigar River, Sulawesi Island. Mar. Ecol. Prog. Ser. 188:299-303

Castle PHJ, GR Williamson. 1974. On the validity of the freshwater eel species Anguilla ancestralis Ege from Celebes. Copeia 2:569-570.

Ege, V. 1939. A revision of the genus Anguilla Shaw. A systematic, phylogenetic and geographical study. Dana Rep 16:1-256

Jespersen P. 1942. Indo-Pasific leptocephali of the genus Anguilla. Dana Rep 22:1-128

Miya, M and Nishida M. 1996. Molecular phylogeny perspective on the

evolution of the deep-sea fish genus Cyclothone (Stomiiformes: Gonostomatidae). Icht Res 43:375-398

Sugeha HY, Arai T, Miller MJ, Limbong D, Tsukamoto K. 2001b. Inshore migration of the tropical eels Anguilla spp recruiting to the Poigar River estuary on north Sulawesi Island. Mar Ecol Prog Ser 182:283-293

Sugeha HY. 2003. Life History of Tropical eel Anguilla marmorata in the Indonesian Waters. Ph.D Disertasi. The University of Tokyo, Jepang.

Watanabe, S. 2001. Taxonomic study on the freshwater eels, genus Anguilla Schrank. Ph.D thesis. The University of Tokyo