DISTRIBUTION OF TROPICAL EEL GENUS *Anguilla* IN INDONESIA WATER BASED ON SEMI - MULTIPLEX PCR

Melta Rini Fahmi[*)#], Laurent Pouyaud[**)], and Patrick Berrebi[***)]

[*)] *Animal Bioscience Study Program, Graduate School, Bogor Agricultural University, Researcher at Research Institute for Ornamental Fish Culture*
[**)] *Institut de Recherche pour le Développement*
[***)] *Institut des Sciences de l'Evolution (ISEM) UMR 5554 UMII-CNRS-IRD*

(*Received 24 September 2012; Accepted 5 November 2012*)

ABSTRACT

Tropical eels living in Indonesian waters are known to be composed of several species, but their real listing together with their distribution ranges need to be established. The main difficulties are the very high number of islands with perennial rivers where these species are living during the growth phase of their life cycle. It is difficult, sometimes impossible, to determine the species using morphological characters, moreover on glass eels. In order to establish the geographic distribution of tropical eels of the genus *Anguilla* in Indonesian waters, a total 1,115 specimens were collected between 2008 and 2012. Sample collection was done in the growth habitats that are rivers and estuaries by commercial nets of different categories according to the fish size. All samples were identified genetically using the recently developed semi-multiplex PCR method. We recognized four species and subspecies with wide distribution: *Anguilla bicolor bicolor*, *Anguilla bicolor pacifica*, *Anguilla marmorata* and *Anguilla interioris*; two species with limited distribution, close to endemism: *Anguilla celebesensis* and *Anguilla borneensis* and one subspecies *Anguilla nebulosa nebulosa* that is only spread in river flowing into Indian Ocean.

KEYWORDS: ***Anguilla* spp., semi-multiplex PCR, tropical eel, distribution range, Indonesian waters**

INTRODUCTION

The catadromous freshwater eel genus *Anguilla* is distributed nearly world-wide except the South Atlantic and the Eastern Pacific oceans (Ege, 1939). Freshwater eels spawn in the offshore ocean. After hatching, their larvae migrate to coastal areas as pelagic, floating and transparent (Mochioka, 2003). Eel larvae, called leptocephali, are transported passively by warm currents flowing at low latitudes. When they approach the continental shelf, leptocephali metamorphose into glass eels before settling in the continental waters (rivers and lakes) to grow for years until changing into yellow eels or "elver" and then silver eels. The dispersal of leptocephali not only drives the distribution of freshwater eel species on continental areas, but also the phylogeography of the genus and its evolution (Aoyama & Tsukamoto, 1997).

After Johannes Schmidt succeeded collecting anguillid leptocephali in the Sargasso Sea in 1922 (Schmidt, 1922), he and his colleagues, through Carlsberg Foundation's Oceano-

Corresponding author. Research Institute for Ornamental Fish Culture
Jl. Perikanan No. 13, Pancoran Mas, Depok 41152, Indonesia. Telp.: +62 021 7520482
E-mail address: meltarini.fahmi@kkp.go.id

graphic Expedition, continued their efforts by searching for the spawning areas of freshwater eels in the Indo-Pacific region where most of the species of this genus are found. They successfully collected leptocephali in the Indo-Pacific region during their expedition from 1928 to 1930 (Jespersen, 1942). However, most of these leptocephali have overlapping morphological characters, hampering exact identifications. Since then, the spawning areas of the Indo-Pacific anguillid species have remained a mystery. As a result, the studies of Indo-Pacific eels are still poorly understood as well as the exact locations of the spawning areas, and their larval migrations and the recruitment mechanisms.

To solve the problems in identifying anguillid leptocephali, genetic approaches, mtDNA sequences or RFLP, has been successfully used (Aoyama *et al.*, 1999; 2001a; 2001b; Aoyama, 2003; Watanabe *et al.*, 2005). Since species identification of anguillid leptocephali has been developed, projects aimed at learning more about the spawning areas, larvae distribution, and larval ecology of anguillid in the Indo-Pacific region have been organized. The long scientific cruise of the Baruna Jaya, in central Indonesia sea, around Sulawesi Island, from 2001-2002, successfully collected leptocephali of *A. marmorata*, *A. bicolor pacifica*, and *A. interioris*. This survey also collected leptocephali of *A. celebesensis* and *A. borneensis* allowing to deduce the spawning areas of these species (Aoyama *et al.*, 2003, Wouthuyzen *et al.*, 2009). In 2003 this cruise also collected young leptocephali *A. bicolor bicolor* in west Sumatra, positioning a spawning area of *A. b. bicolor* in this zone (Aoyama *et al.*, 2007).

The three Indonesian endemic species spawning areas are also to be discovered: leptocephali of *A. interioris* have been caught in western Sumatra waters (Aoyama *et al.*, 2007) and Sulawesi waters (Aoyama *et al.*, 2003, Wouthuyzen *et al.*, 2009); leptocephali of *A. celebesensis* were recognized in Tomini Bay Sulawesi Island and that of *A. borneensis* were found in Makasar strait (Aoyama *et al.*, 2003; Wouthuyzen *et al.*, 2009).

According to the geographic range of each species, Aoyama *et al.* (2001a/b) and Lin *et al.* (2001) who studied genus *Anguilla* molecular phylogenetics based on partial mtDNA, showed four major subgroups in the world, that are Oceanic, Atlantic, Tropical-Pacific, and Indo-Pacific lineages. However, the complete mtDNA sequence of all species of genus *Anguilla* was determined recently by Minegishi *et al.* (2005) which suggested the geographic structure of eel as follow: *A. mossambica* was the most basal species of anguillid eel, and that the other species (except for *A. borneensis*) formed; two Oceanic species (*A. differenbachii* and *A. australis*), and nine Indo-Pacific species (*A. japonica*, *A. reinhardtii*, *A. marmorata*, *A. nebulosa*, *A. bicolor, A. interioris*, *A. celebesensis*, *A. megastoma*, and *A. obscura*). Seven species/subspecies of the Indo-Pacific Anguilla occur in the western Pacific and eastern Indian Ocean around Indonesian waters (Ege, 1939; Castle & Williamson, 1974). Because of this high diversity, several scientists considered that *"Indonesia is homeland of anguilid*". Besides, Indonesia is also known as "*the origin of anguillid*" because phylogenetic analyses indicated that the endemic tropical species *A. borneensis* from eastern Borneo (Kalimantan) is one of the most basal species of the genus (Aoyama *et al.*, 2001a/b; Minegishi *et al.*, 2005).

Among the 19 existing eel species and subspecies, 7 occupy the Indonesian rivers. Half of them are endemic, limited to Indonesia (*A. borneensis*, *A. cebesensis*, *A. interioris*), the others show larger range distribution (*A. obscura*, *A. nebulosa*), sometimes established at the Indo-Pacific level (*A. bicolor* and *A. marmorata*). However, the species range distribution of the 7 Indonesian species is only an extrapolation of very limited records.

The widespread species *A. bicolor* and *A. marmorata* are of interest because their distribution and abundance place them in central economic position, and because their exceptional geographic distribution (over 18,000 km east-west) and structure made them an important model for eel biology understanding, encompassing probably several spawning areas. Short fin eels *A. bicolor* are considered to be structured into two subspecies *A. b. bicolor* in Indian Ocean, especially at the west of Indonesia and *A. b. pacifica* in Pacific Ocean, at the east of Indonesia (Minegishi *et al.*, 2012). The giant mottled eel *A. marmorata* is not taxonomically divided into subspecies because of its morphological stability, but molecular studies have demonstrated its structure into four differentiated populations: North Pacific, South Pacific, Indian Ocean, and Mariana (Minegishi *et al.*, 2008; Gagnaire *et al.*, 2009; 2011).

One of the most exigent parts of this study is sampling strategy. Indonesia is an archipelago country with counts 17,000 islands, among them several thousand islands possibly hosting eels so a complete sampling is impossible. The selection of representative sampling location is necessary.

Indonesian sea have a complex topography and connectivity between the Pacific and Indian Oceans, so surface heat fluxes, thermo cline and variability in thermocline waters and patterns of Indonesia current was influenced by Pacific and Indian Ocean fluctuations. The eastern part of Indonesia waters influenced by the Indonesian throughflow (ITF) current. ITF are water mass flow passing through Indonesian waters from Pacific to Indian Ocean. This water mass flow occurs as a result of the pressure difference between the two oceans. The water mass drives upper thermocline water from the North Pacific through the western route of the Makassar Strait directly exit through the Lombok Strait or flow eastward into the Banda Sea and further joined with the south equatorial current (SEC) (Wirtky, 1973; Gardon, 2005). The western Indonesian waters affected by South Equatorial Counter Current (SECC), and this current going down the Sumatran coast entry to southern coast of Java by South Java Current (SJC).

Recently, a new molecular identification method has been developed to distinguish seven tropical eel inhabiting Indonesian waters, called semi-multiplex PCR assay. By using multiple species-specific primers in one PCR reaction, the identification of all Indonesian species is now simple, quick, low cost, sensitive, and highly reliable (Fahmi *et al.*, 2012).

In present study we used the semi-multiplex PCR methodology in order to establish a first map distribution of species and subspecies of tropical eel (*Anguilla* spp.) that inhabit in Indonesia water.

MATERIAL AND METHODS

The 1,115 specimens were collected in 28 locations around the Indonesian waters, covering the whole geographic distribution of genus *Anguilla* as known or expected in Indonesian waters (Figure 1, Table 1). Specimens collection was conducted in river estuaries along the coast of Indian Ocean, Pacific Ocean and around Arafuru and Celebes Seas. The specimens were collected from 2008 to 2011 by using traps, nets and pole fishing. Oceanographic data of Indonesian seas (Gardon, 2005) used for looking the movement of water and the possible spread of eels.

All of specimens were identified using semi-multiplex PCR protocol according to Fahmi *et al.* (2012 (*in press*)). Nine species-specific primers were added in one PCR reaction. The PCR was carried out in a total volume of 10 µL containing 2 µL 5x Green GoTaq® reaction buffer, 0.5 µL $MgCl_2$ (25 mM), 1.25 µL dNTP (2 mM), 0.5 µL each primer (10 mM) with 1 µL (10 mM), 0.05 µL GoTaq® DNA polymerase (5 u/µL), 0.2 µL ddH_2O, and 1 µL template DNA (around 20 ng). Semi-multiplex-PCR was carried out in a Bio-Rad Thermal Cycler, programmed to perform a denaturation step at 95ºC for 5 min., followed by 35 cycles with 45 s at 95ºC, 45 s at 50ºC and 1 min. at 72ºC. The final extension step (at 72ºC) lasted 10 min. Five microliters of each PCR product were loaded on a 1.5% agarose electrophoresis gel, stained with Cyber Safe before electrophoresis and migrated at 100 volts for 90 min. The DNA bands were observed under Blue Light and photographed by a Canon camera digital.

RESULT

A total of 1,115 freshwater eels were collected and genetically identified as *A. marmorata* (487), *A. b. bicolor* (510), *A. b. pacifica* (34), *A. interioris* (16), *A. n. nebulosa* (15), *A. celebesensis* (47), and *A. borneensis* (3). Only 3 specimens were not identifiable because of the DNA was damage. This is a first investigation on freshwater eel distribution in Indonesian waters, using species, and subspecies specific primers. The first four species and subspecies are wide distribution anguillids spread from western to eastern Indonesia waters (Figure 2a,b,c) and far outside this zone.

A. marmorata was found in almost all sampling locations except Station 4, 8, 13, and 15 (Figure 2b). However, by enhancing the sampling frequency, it is likely that this species would be seen everywhere. The highest abundances of *A. marmorata* are in Sulawesi and Ambon waters, whereas around Sumatra Island this species is commonly found in Bengkulu and Mentawai waters.

Another species has a wide distribution. The shortfin *A. bicolor*, according to its geographic distribution, is divided into two sub-

Table 1. List of sampling locations of tropical eels in Indonesia

No	Sampling location	Code	Sampling date	No. of individuals	Stade
1	Estuary of Tadu, Aceh	Ace	Jan-10	40	*Silver eel*
2	Sea Waters of Smeulue	Ace	Mar-12	8	*Silver eel*
3	Sea Waters of Mentawai	Men	Jun-11	42	*Silver eel*
4	Estuary of Bungus River, Padang (1-45)	Pad 1	Aug-08	50	*Silver eel*
5	Tarusan River, Painan, Padang (46-97)	Pad 2	Feb-08	37	*Silver eel*
6	-Estuary of Ketaun River, Bengkulu, Sumatra	Ben	Dec-09	23	*Silver eel*
	-Estuary of Ketaun River, Bengkulu, Sumatra	Ben	Mar-09	8	*Silver eel*
	-Estuary of Ketaun River, Bengkulu, Sumatra	Ben	Jun-08	51	*Glass eel*
7	-Estuary of Cimadiri, Pelabuhan Ratu, Java	Pel 8	Aug-09	30	*Glass eel*
	-Estuary of Cimadiri, Pelabuhan Ratu, Java	Pel 9	Sep-09	30	*Glass eel*
	-Estuary of Cimadiri, Pelabuhan Ratu, Java	Pel 10	Oct-09	43	*Glass eel*
	-Estuary of Cimadiri, Pelabuhan Ratu, Java	Pel 11	Nov-09	23	*Glass eel*
	-Estuary of Cimadiri, Pelabuhan Ratu, Java	Pel R	Jun-08	163	*Glass eel*
8	Estuary of Pangandaran, Java	Pang	Jun-09	20	*Silver eel*
9	Estuary of Cilacap, Java	Cil	Jun-09	18	*Silver eel*
10	Estuary of Mengereng River, Bali (1-30)	Bal 1	Jun-09	30	*Silver eel*
11	Unde River, Bali (31-62)	Bal 2	Dec-09	31	*Silver eel*
12	Estuary of Labuan Haji River, Lombok	Lom	Sep-09	14	*Silver eel*
13	Estuary of Bengalon River, Borneo	Beng	Oct-10	11	*Silver eel*
14	Estuary of Sangata River, Borneo	Sang	Oct-10	14	*Silver eel*
15	Estuary of Mahakam River, Borneo	Mah	Oct-10	3	*Silver eel*
16	-Estuary of Lasusua River, Celebes	Las 1	Oct-08	64	*Silver eel*
	-Estuary of Lasusua River, Celebes	Las 2	Sep-09	40	*Silver eel*
17	Estuary of Salusuna River	Sal	Oct-10	1	*Silver eel*
18	Estuary of Donggala River, Celebes	Dong	Oct-10	18	*Silver eel*
19	-Estuary of Poso River, Celebes	Pos	Apr-10	47	*Glass eel*
	-Estuary of Poso River, Celebes	Pos	Dec-11	30	*Glass eel*
	-Estuary of Poso River, Celebes	Pos-S	Jun-08	37	*Glass eel*
	-Estuary of Poso River, Celebes	Pos-J	Jun-08	24	*Glass eel*
20	Estuary of Tantena River,Clebes	Pal	Aug-10	7	*Silver eel*
21	Estuary of Tanah merah, Seram, Ambon	Amb	Nov-10	26	*Silver eel*
22	Obi Island, Maluku	Obi	Nov-11	12	*Silver eel*
23	Ware mare River, Papua (1-5, 9-10)	Pap	Nov-10	7	*Silver eel*
24	Kali Besar River, Papua (11-16)	Pap	Nov-10	6	*Glass eel*
25	Tanjung Boy River, Papua (6-8)	Pap	Nov-10	3	*Silver eel*
26	Estuary of Amurang, North Celebes	Amu	Nov-11	51	*Silver eel, glass eel*
27	Estuary of Poigar, North Celebes	Poig	Jan-12	30	*Glass eel*
28	Estuary of Inobonto, North Celebes	Ino	Nov-11	23	*Silver eel*
	Total specimens			**1,115**	

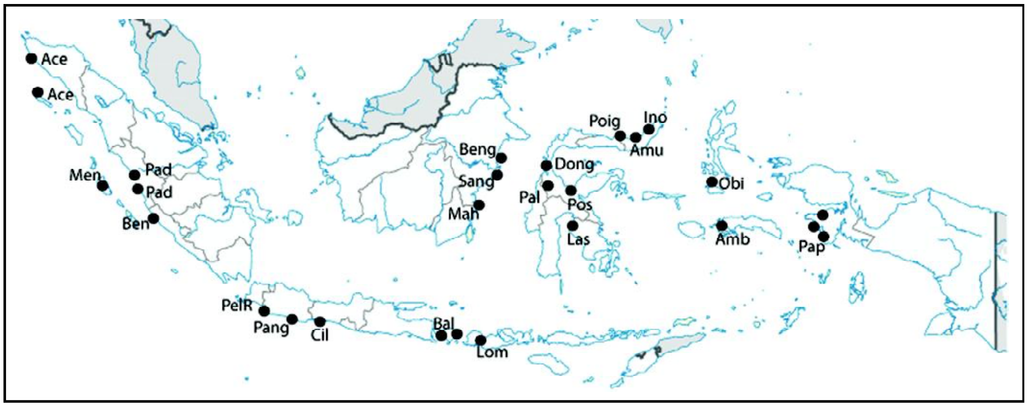

Figure 1. Map of the stations around Indonesia Sea where samples have been collected for this study

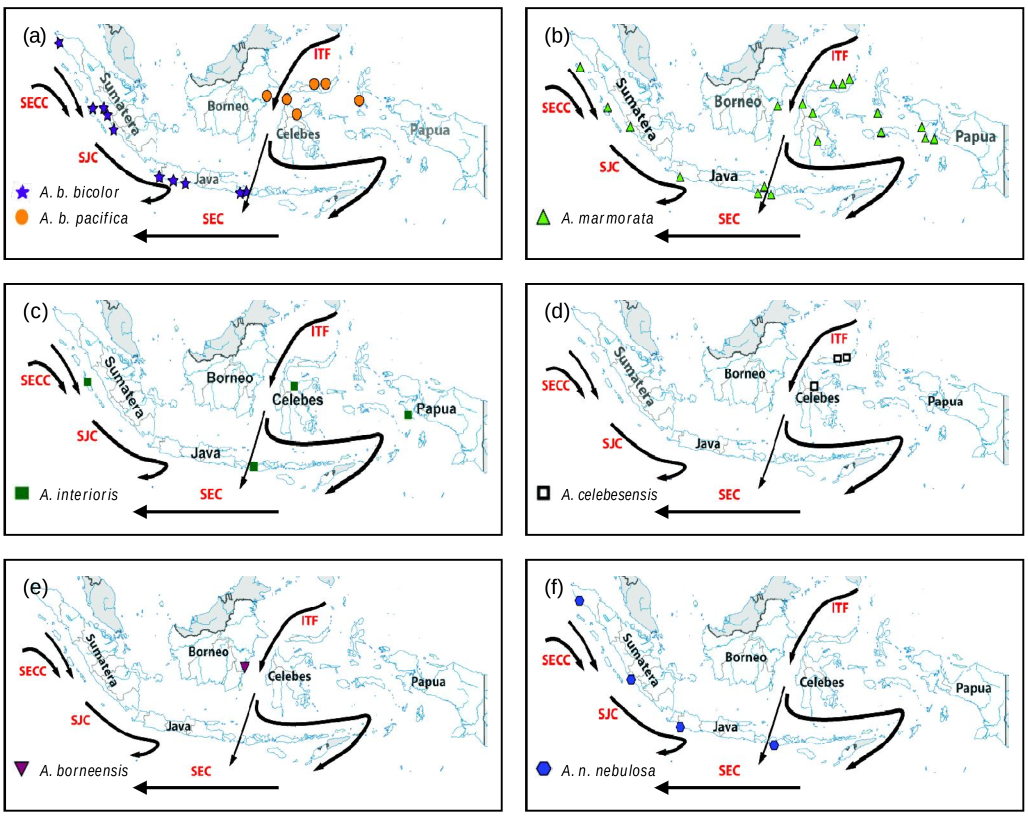

Figure 2. Distribution of the seven species and subspecies of freshwater eels which were found around Indonesia during this study: a) *A. b. bicolor* and *A. b. pacifica*, b) *A. marmorata,* c) *A. interioris*, d) *A. celebesensis*, e) *A. borneensis,* and f) *A. n. nebulosa*. The current flow pattern in Indonesia waters, probably influencing the spread of eel larvae, is given through simplified arrows, adapted from Wirtky (1973, 2005). ITF (Indonesian Troughflow), SEC (South Equatorial Current), SECC (South Equatorial Countercurrent), SJC (South Java Current)

species, *A. b. bicolor* in western Indonesia, in waters connected with Indian Ocean and *A. b. pacifica* spread in the east of Indonesia receiving Pacific Ocean waters (Figure 2a). *A. b. pacifica* has been recorded in the north of Sulawesi, eastern of Borneo, and Maluku waters (Figure 2a). This paper was constituted the first report of the presence of *A. b. pacifica* in Borneo and Maluku. The abundance of *A. b. pacifica* in eastern Borneo is so high, the harvest of this species has been done intensively by fishermen for economic purposes.

The last widespread species found in Indonesian waters was *A. interioris*. This species is not found in many Indonesian sampling stations as the other two widespread species. *A. interioris* was found in the waters of Mentawai, Lombok, Poso, and the western part of Papua. The abundance of this species was relatively high in the waters of Mentawai and the harvest of adult eels is done intensively for its high economic benefice (Figure 2c).

Hereinafter are two species with limited distribution: *A. borneensis* and *A. celebesensis* (Figure 2d, e). Leptocephali of both species have been reported by Wouthuyzen *et al.* (2009) during their expedition around Sulawesi Island. *A. borneensis* was only found in Mahakam Rivers, Borneo, during our investigations. Another Indonesian endemic eel, *A. celebesensis*, showed a limited range since only found from the north to the mid part Sulawesi. This is supported by the discovery of a *A. celebesensis* leptocephalus in the Sulawesi Sea and the Tomini Bay by Aoyama *et al.* (2003).

A. n. nebulosa was only found in estuaries open to the Indian Ocean, from northern Sumatra until Lombok Island (Figure 2f).

DISCUSSION

Producing new data on Indonesian eel species is an important challenge for several reasons. Both of biodiversity knowledge for conservation and economical exploitation need species determination and distribution ranges.

For most eel species, the timing of the vital cycle of Indonesian populations is not known. Efficient sampling periods are not well known, even by Indonesian people, because eels are not commonly consumed. Moreover, these fishes are often considered as sacred or mystic symbols by many people, because of some mystery in their life cycle.

Currently, freshwater eel has been harvested intensively in some regions of Indonesia so that, to overcome the excessive exploitation, the Indonesian government established a regulation for exportation, banning juvenile of eels. *A. marmorata* and *A. bicolor* are the two most important commercial species because they are widespread and abundant in Indonesian waters so catching of both species has been done intensively. However, five other species, less abundant, can make confusion at the very young stages (glass eel and elver).

Specimen Collection Design

The most important phase in this study is the samples collection, because Indonesia is an archipelago with more than 17,000 islands and the choice of sampling locations is determinant for the survey design. In our study, sampling strategy was influenced by the surface current pattern in Indonesia Sea, known to induce 'migration loops', differentiation and speciation (Tsukamoto *et al.*, 2002).

The larval migration of the Atlantic eels *A. anguilla* and *A. rostrata* and Japanese eels *A. japonica* have been fairly well studied and these species were found to have spawning sites associated with oceanographic frontal feature such as temperature (McCleave, 1993) or salinity (Tsukamoto, 1992; Kimura *et al.*, 1994). On the contrary, tropical species, and especially Indonesian ones, are waiting for basic researches on the spawning areas and on larvae migration.

The western part of Indonesian Sea is influenced by current SECC that developed during the whole year and lies just crossing the equator (Wyrtki, 1973). Anguillid leptocephali could have been transported into the sampling area from further offshore-around Mentawai- by the flow of the SECC to south and north Sumatra (Aoyama *et al.*, 2007). This current flow generally towards south-east along the coast of Sumatra continued along the coast of Java (SJV). In this study we collected eels entering to the rivers of this coast, forming populations which can be found here all around the year.

While in the eastern part of Indonesian Sea is influenced majority by current ITF. The first flow through of ITF was via the Makassar Strait with one branch entering the Indian Ocean through the Lombok Strait, while the bulk of the Makassar transport turns eastward in the

Flores Sea, into the Banda Sea, eventually passing into the Indian Ocean on either side of Timor. The second flow through of ITF was passages east of Sulawesi and connections of the Banda Sea to the Indian Ocean also on either side of Timor (Gardon, 2005). Thus the present study was conducted sampling of specimen along the flow through by ITF.

Anguilla bicolor Distribution

The semi-multiplex PCR assay proposed by Fahmi *et al*. (2012) has been successfully used here to distinguish between *A. b. bicolor* and *A. b. pacifica*. Previously publications only distinguished both species through their geographic distribution, even when they used molecular approaches (Aoyama, 2007). Using Fahmi *et al.* (2012) semi-multiplex method, the present study clearly showed that *A. b. bicolor* is only found in waters connected to the Indian Ocean and *A. b. pacifica* to the Pacific ocean. The present results did not found any overlapping area between both subspecies (Figure 5a). This sub-species distributions contrast with that found by Sugeha *et al.* (2008) who recorded *A. b. pacifica* in the western of Sumatra and south of Java waters by using RFLP-PCR for taxa determination. In accordance with the discovery of leptochepali of *A. b. bicolor* by Aoyama *et al.* (2007), in the waters at the west of Sumatra constitute spawning area of this subspecies.

The discovery of young leptocephali (TL 44-55 mm) of *A. b. bicolor* by Aoyama *et al.* (2007) during their expedition in the west of Sumatra Island, indicated that the spawning area of this subspecies was somewhere in front of the island. The leptochepali of *A. b. bicolor* are then passively transported by the flow of SECC and SJV into the southern region of Java (Herunadi, 2003; Aoyama *et al.*, 2007). The age of glass eel of *A. b. bicolor* entering Cimandiri river estuary, Pelabuhan Ratu has been determined (at least 118-262 days) by using otolith microstructure (Setiawan *et al.*, 2001). Calculating back the passive migration of leptocephali, taking into account the larvae phase duration and the speed of the flow of SJC, it could be estimated that spawning area of *A. b. bicolor* was around Mentawai Island (Haerunadi, 2003). This author found glass eel of *A. b. bicolor* in the south west coast of Sumatra, in Bengkulu and Lampung Rivers, in the south western coast of Java, in Malimping and Cimandiri Rivers, and abundant *A. b. bicolor* glass eels on the south eastern coast of Java, in Cilacap River and in the northern part of the Sumatra, in Aceh River.

It is most probable, based on the whole data obtained on *A. b. bicolor*, that this species is locally constituted by a single population using a single spawning area. This is supported by the researchers conducted by Minegishi *et al.* (2012) who described the genetic population structure of *A. bicolor*, showing that *A. b. bicolor* is genetically homogeneous in the whole Indian Ocean.

In the eastern part of Indonesia, *A. b. pacifica* is widely distributed but less abundant. Allopatric isolation between two oceans have probably split *A. bicolor* into two subspecies. According to Wouthuyzen *et al.* (2009) spawning area of *A. b. pacifica* occurs at the north of Sulawesi Sea.

Similarly with *A. b. bicolor*, the population structure analysis of *A. b. pacifica* showed no significant genetic divergence within its south Pacific Ocean distribution (Minegishi *et al.*, 2012). The widely distribution of *A. b. pacifica* at the east of Indonesia Sea may be due to dispersive transportation of leptocephali in the whole eastern Indonesia. The dynamics of water currents in eastern Indonesia is strongly influenced by the ITF current. This current flow is important to local climate, it is also essential to the planktonic eggs and larvae of eels transportation.

Anguilla marmorata Distribution

A. marmorata Quoy & Gaimard (1842) was considered by Ege's as a species which has the widest geographic distribution among the 15 species of genus *Anguilla.* This species is abundant in Indo-Pacific zone and spread from east Africa to the central and south Pacific, passing Indonesia water where apparently it has multiple spawning areas. By using morphological characters Ege (1939) also divide *A. marmorata* into three races, but morphological studies showed not sufficient differences between them, the term of race in *A. marmorata* is no longer used (Watanabe *et al.*, 2004a). Recently by molecular approaches, some studies established the genetic population structure of this species (Isikawa *et al.*, 2004; Minegishi *et al.*, 2008; Gagnaire *et al.*, 2009).

The broad distribution of *A. marmorata* was confirmed by our sampling, this species has been found in almost all the sampling locations. The spawning areas of this species have been evaluated according to several findings. In the western part of Indonesia, Aoyama *et al.* (2007) captured a young leptocephalus (around 46.8 mm TL) of *A. marmorata*. The *A. marmorata* glass eel entering Cimandiri River, Pelabuhan Ratu are about 121-233 days old (Setiawan *et al.*, 2001). Harvest glass eel of *A. marmorata* also have been done by fishermen in southern Sumatra Island. The spawning area of *A. marmorata* is considered to be around Mentawai waters. Transported by current flow, the larvae swim passively toward their growth habitat in the west of Sumatra and south of Java.

A spawning area of *A. marmorata* has also been found in the northern Sulawesi waters (Aoyama *et al.*, 2003; Wouthuyzen *et al.*, 2009) after the capture of small leptocephali between mid-August and end October. The juvenile of *A. marmorata* can be found throughout almost the whole year at the mouth of Poigar River, Sulawesi (Arai *et al*., 1999).

The Three Endemic Species

All of the morphological characters of *A. interioris* and *A. celebesensis* described by Ege (1939) are overlapping as well as their distribution in the east of Indonesia (Watanabe, 2003). Aoyama *et al.* (1999) suggested molecular genetics approach for identification of both species. The development of semi-multiplex PCR by Fahmi *et al.* (2012) permitted to avoid any confusion in the present study.

Surprisingly, *A. interioris* has been captured on a broad distribution (Mentawai, Lombok, Poso, and Papua) but their abundance is generally limited in each sampling location. The glass eel stage was commonly found in Poso and Papua, while the large one are found in considerable abundance in the waters of Mentawai. *A. interioris* leptocephali discovered by a scientific expedition; Aoyama *et al.* (2007) captured small leptocephali (44.1 to 55.5 mm TL) in the western part of Sumatra waters and Wouthuyzen *et al.* (2009) and Aoyama *et al.* (2003) collected small leptocephali (33.1-49.5 mm TL) in the northern of Sulawesi waters. From these discovery, they concluded that the western part of Sumatra and northern part of Sulawesi constitute the spawning area of *A. interioris.*

However, Setiawan *et al.* (2001) did not found *A. interioris* in the mouth of Cimandiri River, nor Arai *et al.* (1999) at the mouth of the Poigar River, at any season. Two spawning areas of *A. interioris* have been found but their spread is very limited, so the migration patterns of this eel need further studies.

The other endemic tropical species is *A. celebesensis*. Young leptocephali of this species have been collected in two different seasons and in two different areas (Sulawesi Sea and Tomini Bay); the leptocephali in Tomini Bay found in May, while lepthocephali in Celebes Sea found in February (Aoyama *et al.*, 2003). In this study we collected specimens from both areas. The spawning and growth area of *A. celebesensis* in considered be in Tomini Bay (Wouthuyzen *et al.*, 2009). This suggests that migration distance of *A. celebesensis* is the shortest in the genus Anguilla since its approximate migration loop is of 80 km large. In contrast, the European eel distance from growth to spawning areas in the Sargasso Sea ranges from 4,000 to 8,000 km.

The third endemic tropical species is A. borneensis. The expedition undertaken by Aoyama *et al.* (2003) succesfully collected a small leptocephalus (8.5 and 13.0 mm) of *A. borneensis* in Sulawesi Sea, around 16 and 26 days after hatching, whereas the larger specimen (35.4 mm) was collected in the south in Makassar Strait, probably passively transported from the Sulawesi Sea (Aoyama *et al.*, 2003). As a summary, the growth habitat of *A. borneensis* is limited to the east-central part of Borneo, and this species spawns in the Sulawesi Sea. In this study we only found specimens of *A. borneensis* in one of eight locations that have contact with the Sulawesi Sea and the Makasar Strait. *A. borneensis* were only found in Mahakam River. The migration distance of *A. borneensis* leptocephali is at least of 450-650 km and its freshwater distribution is narrow, from Equator to 7 N of latitude (Aoyama *et al*., 2003).

The distribution range of freshwater eel is linked with the distribution of their leptocephali and with the current system in their habitat (Miller, 2003). Temperate eels appear to make longer spawning migrations to spawn in low-latitude westward-flowing current such as the North and South Equatorial Currents, and their leptocephali have been collected far from their freshwater habitat. Whereas the tropical eel have the unique pattern distribution and mi-

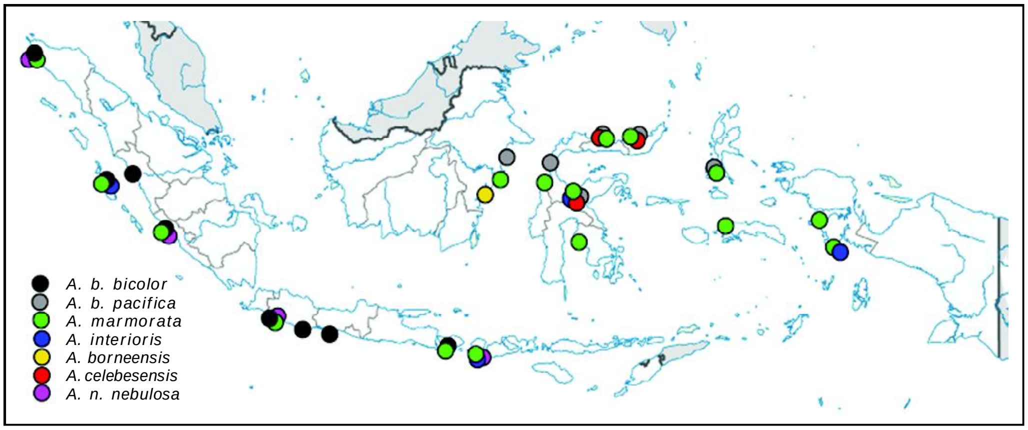

Figure 3. Distribution and species composition of tropical eel in Indonesia water

gration such as: they have short migration distance, short metamorphosis duration, they spawning whole the years and some of them widely distribution and the other narrow. So, in several things the tropical eel different from temperate eel, the other hand, until now the theory of migration, distribution patterns, and evolution of eel has been widely adopted from the temperate Anguillid. Future the study of distribution pattern, migration theory, and evolution of tropical eel needed and it is most important to complete information of biological eel.

Sympatric Dispersal of Tropical Eel in Indonesia Water

The compilation of the distribution map of all species eel that inhabit the waters of Indonesia eel comprehensively presented in Figure 3. 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. celebesensis*, 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.

REFERENCES

Aoyama, J. & Tsukamoto, K. 1997. Evolution of the freshwater eels. *Naturwissenschaften*, 84: 17-21.

Aoyama, J., Watanabe, S., Ishikawa, S., Nishida, M., & Tsukamoto, K. 1999. Are morphological characters effective enough to discriminate two species of freshwater eels, *Anguilla celebesensis* and *A. interioris*? *Ichthyol. Res.*, 47: 157-161.

Aoyama, J., Nishida, M., & Tsukamoto, K. 2001a. Molecular Phylogeny and evolution of freshwater eel, genus *Anguilla*. *Mol. Phylogenet Evol.*, 20: 450-459.

Aoyama, J., Ishikawa, S., Otake, T., Mochioka, N., Suzuki, Y., Watanabe, S., Shinoda, A., Inoue, J., Lokman, P.M., Inagaki, T., Oya, M., Hasumoto, H., Kubokawa, K., Lee, T.W., Fricke, H., & Tsukamoto, K. 2001b. Molecular approach to species identification of eggs with respect to determination of the spawning site of the Japanese eel *Anguilla japonica*. *Fisheries Science*, 67: 761-763.

Aoyama, J. 2003. Origin and evolution of the freshwater eels genus *Anguilla. In*: Aida K, Tsukamoto K., Yamauchi K. (eds). Eel Biology. Springer, Tokyo. p. 19-29.

Aoyama, J., Wouthyuzen, S., Miller, M.J., Inagaki, T., & Tsukamoto, K. 2003. Short-distance spawning migration of tropical freshwater eels. *Biol. Bull.*, 204: 104-108.

Aoyama, J., Wouthuyzen, S., Miller, M.J., Minegishi, Y., Minagawa, G., Kuroki, M., Suharti, S.R., Kawakami, T., Sumardihargo, K.O., & Tsukamoto, K. 2007. Distribution of the lepthocephali of the freshwater eels, genus *Anguilla* in the waters off west Sumatra in the Indian Ocean. *Environ. Biol. Fish*, 80: 445-452.

Arai, T., Limbong, D., Otake, T., & Tsukamoto, K. 1999. Metamorphosis and inshore migration of tropical eels, *Anguilla* sp. in the Indo-Pacific. *Marine Ecology Progress Series*, 182: 283-293.

Castle, P.H.J. & Williamson, G.R. 1974. On the validity of the freshwater eel species *Anguilla ancestralis* Ege, from Celebes. *Copeia*, 2: 569-570.

Ege, V. 1939. A revisi of The Genus *Anguilla* Shaow, A systemic, phylogenetic and geographical study. London: Oxford University Press, 260 pp.

Gagnaire, P.A., Minegishi, Y., Aoyama, J., Réveillac, E., Robinet, T., Bosc, P., Tsukamoto, K., Feunteun, E., & Berrebi, P. 2009. Ocean currents drive secondary contact between *Anguilla marmorata* populations in the Indian Ocean. *Mar. Ecol. Prog. Ser*.; 379: 267-278. doi: 10.3354/meps07895.

Gardon, A.L. 2005. Oceanography of the Indonesia and their troughflow. *Oceanography*, 18: 4.

Ishikawa, S., Tsukamoto, K., & Nishida, M. 2004. Genetics evidence for multiple geographic population of the giant mottle eel *Anguilla marmorata* in the Pacific and Indian Oceans. *Ichthyol. Res.*, 51: 343-353.

Jespersen, P. 1942. Indopacific Leptocephali of the genus *Anguilla*: systematic and biology studies. *Dana Rep.*, 22: 1-128.

Kimura, S., Tsukamoto, K., & Sugumoto, T. 1994. Model of the larval migration of the Japanese eel: role of the trade winds and salinity front. *Mar. Biol.*, 119: 185-190.

Lin, Y.S., Poh, Y.P., & Tzeng, C.S. 2001. A phylogeny of freshwater eels inferred from mitochondrial genomes. *Mol. Phylogenet. Evol.*, 20: 252-261.

McCleave, J.D. 1993. Physical and behavioral controls on the oceanic distribution and migration of leptocephali. *J. Fish Biol*., 43 (suppl A): 243-273.

Michioka, N. 2003. Leptocephali. *In*: Aida K., Tsukamoto, K., Yamauchi, K. (eds). Eel Biology. Springer, Tokyo, p. 19-29.

Miller, M.J. 2003. The worldwide distribution of Anguillid leptocephali. *In*: Aida K., Tsukamoto K., Yamauchi K. (eds). Eel Biology. Springer, Tokyo, p. 157-168.

Minegishi, Y., Aoyama, J., Inoue, J.G., Miya, M., Nishida, M., Tsukamoto, K. 2005. Molecular phylogeny and evolution of the freshwater eel genus *Anguilla* based on the whole mitochondrial genome sequence. *Mol. Phylogen. Evol.*, 17: 3,109-3,122.

Minegishi, Y., Aoyama, J., & Tsukamoto, K. 2008. Multiple population structure of the giant mottle eel *Anguilla marmorata. Mol. Ecol.*, 17: 3,109-3,122.

Minegishi, Y., Gagnaire, P.A., Aoyama, J., Bosc, P., Feunteun, E., Tsukamoto, K., & Berrebi, P. 2012. Present and past genetic connectivity of the Indo-Pacific tropical eel *Anguilla bicolor*. *J. Biogeogr.*, 39: 408-420.

Schmidt, J. 1922. The breeding places of the eel. *Phil. Trans. R. Soc. Lond*, 211: 179-208.

Setiawan, I.E., Michioka, N., Amarullah, H., & Nakazono, A. 2001. Inshore migration and spawning season of the tropical eel *Anguilla bicolor bicolor* recruiting to the Cimandiri River estuary, Java Island. Indian Ocean. *In*: Aida K, Tsukamoto K, Yamauchi K (eds*). Proceedings of the International Symposium Advances in Eel Biology.* The University of Tokyo. Tokyo, p. 125-127.

Sugeha, H.Y., Suharti, S.R., Wouthuyzeu, S., & Sumadhiharga, K. 2008. Biodiversity, distribution and abundance of the tropical Anguillid eel in the Indonesia waters. *Marine Research in Indonesia*, 33: 129-13.

Tsukamoto, K., Aoyama, J., & Miller, M.J. 2002. Migration, speciation and the evolution of diadromy in anguillid eels. *Canadian Journal of Fisheries and Aquatic Science*, 59: 1989-1998.

Tsukamoto, K. Discovery of spawning area of the Japanese eel. *Nature*, 356: 789-791.

Watanabe, S., Aoyama, J., & Tsukamoto, K. 2005. A molecular genetic evaluation of the taxonomy of the genus *Anguilla* (Pisces: Anguilliformes). *Bulletin of Marine Science*, 76(3): 675-690.

Wouthuyzen, S., Aoyama, J., Sugeha, H.Y., Miller, M.J., Kuroki, M., Minegishi Y., Suharti, S.R., & Tsokamoto, K. 2009. Seasonality of spawning by tropical anguillid eels around Sulawesi Island, Indonesia. *Naturwissenschaften*, 96: 153-158.

Wyrtki, K. 1961. Physical oceanography of the Southeast Asian waters. Naga Report 2. Scripps Institution of Oceanography. La Jolla. CA, 195 pp.