Doctoral Thesis

Molecular genetic variation and fisheries of *Mesopodopsis orientalis* (Crustacea: Mysida) in Indonesian waters, with remarks on fisheries of *Acetes* (Crustacea: Decapoda)

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ABSTRACT

Edible crustaceans belonging to mysids (Crustacea: Mysida) were studied on their molecular analysis and fisheries in Indonesia during 2008 to 2012. The fisheries of *Acetes* (Crustacea: Decapoda) were also involved in this study as remarks because these organisms were found together with mysids.

Samples were obtained by field samplings and buying fresh and dried materials and shrimp paste at local markets across Indonesia for fisheries purposes. In addition to fisheries matter, interview surveys were conducted and fishery data obtained from public offices and processing industries. A variety of sampling gears were employed to collect these crustaceans based on the purpose of this study, such as: plankton-net, push-net, lift-net, scoop-net and boat-seine, and also depended on the type of sampling sites.

The molecular analysis was conducted on *Mesopodopsis orientalis*, as a main fishing target. A total of 458 base pairs (bp) of partial fragment sequences of *COI* region were determined for 136 specimens collected from 10 sites. All sequences were unambiguously aligned, revealing 37 haplotypes. Haplotype diversity (h) and nucleotide diversity (π) were 0.8036 ± 0.0273 and 0.0089 ± 0.0049, respectively. Phylogenetic analyses of the mitochondrial *COI* showed that *M. orientalis* involves several stem lineages, which are genetically divergent at a high level and were subsequently divided into two clades in phylogenetic trees. Haplotypes of *M.*

orientalis population from Indonesia differed from those of Malaysia and Thailand, and formed different clades in the phylogenetic trees. A maximum parsimony tree showed that Indonesian clades are closer to Thailand`s than to Malaysian`s, which may refer to what has happened during the Pleistocene glacial period. The haplotype network also indicated the presence of two genetic clades, Clades 1 and 2 separated from each other by two nucleotide substitutions. Concerning genetic diversity within each local population, the number of haplotypes was polymorphic. Concerning genetic differentiation among populations, average sequences differences (d_A) of 22 of a total 45 pairs were significant after sequential Bonferroni corrections. Haplotype compositions differed among populations, although these populations were divided into two genetic groups. The AMOVA testing significance of Groups 1 and 2 demonstrated that genetic differentiations were significant. Consequently a series of DNA analysis for haplotype sequences of *COI* gene showed the presence of a geographical barrier that separates the two distinct genetic groups across between Java-Madura and Bali-Kalimantan Island systems in Indonesian waters.

There was no exact information regarding the peak seasons of mysids, but they were available in brackish and salt ponds throughout a year. *Acetes* was caught throughout all seasons, but the peak fishing season varied depending on the locations of the fishing grounds and the alternation of monsoons. Fishing time for mysids and *Acetes* was either day or night, but the best time for fishing was during darkness,

either at dusk or dawn. The catches and values of a mixture of mysids and *Acetes* (“rebon”) fluctuated year-by-year and there was much greater annual catch at the sea than in brackish ponds. The catch of “rebon” in Madura was highest of all locations surveyed, which was about 4 tons/day with the value of Indonesian Rupiah (IDR) 15,300,000 (about 1,700 US$) / ton in the peak fishing season from February to March. The economically important species of mysids were *Mesopodopsis orientalis* and *M. tenuipes*. In *Acetes* the following species were fishing targets: *A. indicus*, *A. serrulatus*, *A.erythraeus*, *A. sibogae sibogae*, *A. japonicus*, and *A. vulgaris*. They were all used for making fermented shrimp pastes called “terasi”. There were three types of shrimp pastes in terms of its composition; 1) a mixture of mysids and *Acetes*, 2) *Acetes* only, and 3) mysids only. The price at market for “rebon” and “terasi” varied depending on the quality of the product and the locality. Dried and fresh “rebon” were sold for IDR 5,000 to 15,000/kg (US$ 0.6 - 1.7/ kg), whereas “terasi” for IDR 3,000 - 40,000/kg (US$ 0.3 - 4.4/ kg).

The sustainability of mysid and *Acetes* fisheries depends on exact fisheries statistics and consideration of the genetics of target species.

Chapter 1

GENERAL INTRODUCTION

Mysids, the order Mysida, are known as 'opossum shrimps' because they appear similar to some shrimps. The name of opossum shrimp given is because females have brood pouches or marsupiums (located in the thoracic segments between the legs) which carry embryos (Mauchline, 1980; Schram, 1986; Murano, 1999; Heard *et al.*, 2006). This group is planktonic organisms and small in size, about 5 to 20 mm in body length. The size is different among this group, depending on the habitat they live. The deep-sea species tend to be bigger than shallow-water species (Mauchline, 1980).

The Mysida belong to the superorder Peracarida which means "near to shrimps" (Martin and Davis, 2001). The main characteristic of this group is their lack of free-swimming larvae. This characteristic separates them from the superorder Eucarida (Mauchline, 1980). According to Martin and Davis (2001), the order Mysida currently include approximately 160 genera. In 1977, it was reported that this group consisted of 785 species in the world including seawater, freshwater lakes and rivers, and caves and wells (Mauchline and Murano, 1977; Mauchline, 1980; Schram, 1986; Sawamoto and Fukuoka, 2005; Heard *et al.,* 2006), but then now the number of species has increased to more than 1000 (Martin and Davis, 2001).

Mysida in majority are omnivores, feeding on algae, detritus, and zooplankton, but some are scavengers and carnivores (Mauchline, 1980). Mysids such as *Neomysis integer* play important roles as 'bioengineer' or 'ecosystem engineer' for modelling sediment dynamics in the upper estuary (Roast *et al.,* 2004).

The order Mysida are a highly adaptive group (Mauchline, 1980), which occur in vast numbers and usually move in swarms over the substrate or cruise close to the surface of coastal regions. Mysids have a cosmopolitan distribution and are found in both benthic and pelagic areas; they inhabit oceans from the intertidal zone to the depths down to 7210 m, while many are found in brackish waters and a few in freshwaters (Mauchline, 1980; Schram, 1986; Heard *et al.*, 2006). Most mysids are free-living but few species (mostly in the tribe Heteromysini) are associated with invertebrates such as sea anemones, sponges and hermit crabs (Mauchline, 1980; Meland, 2002).

The most important diagnostic characters of this group are the telson and the statocyst in the endopodal uropod, and other features such as stalked compound eyes, and a carapace that covers the head and thoracic segments (Mauchline, 1980; Murano, 1999). Murano (1999) has characterized the general morphology of mysid as follows. (a) The telson is usually furnished with spinules and setae along the margin. It has triangular or trapezoidal shape with broad or narrow apical margin; some have linguiform with broadly or narrowly rounded apical margin, some have

deep or shallow cleft in apical part. (b) The uropods are composed of endopod and exopod, and a statocyst is mostly present basally in the uropodal endopod. (c) The body has external skeleton or integument which is usually thin and poorly calcified. The surface of integument is usually smooth but some species have minute spinules, bristles or scales which partly or totally covering, and in few species with long spines. (d) The cephalon and anterior thoracic somites are mostly covered by the carapace which is a shield-like structure and fused dorsally with the head region and three anterior thoracic somites. A frontal plate or rostrum is formed by the anterior margin of the carapace. (e) A pair of eyes, usually composed of cornea and eyestalk, are uncovered or partly covered with the carapace. (f) The thorax has 8 pairs of pereipods (thoracopods) and the abdomen bears 6 pairs of pleopods.

The classification of mysid below, which is recently improved from Mauchline's (1980) with many additions of new taxa and application of molecular biology, is adopted from Martin and Davis (2001).

Phylum : Arthropoda

Subphylum : Crustacea Pennant, 1777

Class : Malacostraca Latreille, 1806

Superorder : Peracarida Caiman, 1904

Order : Lophogastrida Boas, 1883

Family : Lophogastridae Sars, 1870

Family : Gnathophausiidae Bacescu. 1984

Family : Eucopiidae Sars, 1870

Order : Mysida Boas. 1883

Family : Petalophthalmidae Czerniavsky, 1882

Family : Mysidae Dana, 1850

Subfamily : Boreomysinae Holt and Tattersall, 1905

Subfamily : Siriellinae Norman, 1892

Tribe : Metasiriellini Murano, 1986

Tribe : Siriellini Murano, 1986

Subfamily : Rhopalophthalminae Hansen, 1910

Subfamily : Gastrosaccinae Norman. 1892

Subfamily : Mysinae Hansen, 1910

Tribe : Erythropini Hansen. 1910

Tribe : Leptomysini Hansen. 1910

Tribe : Mancomysini Bacescu and Iliffe, 1986

Tribe : Mysini Hansen, 1910

Tribe : Heteromysini Hansen, 1910

Subfamily : Mysidellinae Hansen, 1910

Mysids are also important organisms for human beings (Table 1-1). In many Asian countries, they are a common part of local cuisine (Omori, 1975, 1978; Mauchline, 1980; Jadhav and Josekutty, 2003; Paul and Josekutty, 2005; Mantiri *et al*., 2012) and mainly used to make shrimp paste and sauce (Omori, 1978; Mantiri *et al*., 2012). In Japan mysids become an excellent supplementary food, which is boiled, dried in the sun and frequently marketed as a preserved cooked food called 'tsukudani' (Mauchline, 1980). In India, at the markets of Calcutta, *Mesopodopsis orientalis* mixed with smaller numbers of *Gangemysis assimilis* are sold as food called 'Kada chingri', while elsewhere *M. orientalis* was eaten as 'Sridhar' (Mauchline, 1980). *M. orientalis* and *M. zeylanica* are caught regularly in India for human consumption (Aravindakshan *et al*., 1988; Jadhav and Josekutty, 2003; Paul and Josekutty, 2005). In Cirebon and Sungai Raya, Indonesia, fresh *M. orientalis* is found to be sold being mixed with fresh pelagic shrimp such as *Acetes vulgaris, A. japonicus, A. serrulatus, and A. erythraeus,* whereas *Mesopodopsis* sp. mixed with *Acetes* sp. and also *M. orientalis* alone are found in the composition of shrimp paste 'terasi' from Madura, Indonesia (Mantiri *et al*., 2012).

Table 1-1. Target species of mysids for fisheries in the world.

Localities	Species	References
Japan	***Neomysis intermedia***	**Murano (1963); Mauchline (1980)**
	N. japonica	**Mauchline (1980); Hanamura (2001)**
	N. awatschensis	**Hanamura (2001)**
	***Orientomysis mitsukurii* (*Acanthomysis mitsukurii*)**	**Mauchline (1980)**
Indonesia (Madura and Bali Islands)	***Mesopodopsis orientalis***	**Mantiri *et al.* (2012)**
	M. tenuipes	**Mantiri *et al.* (2012)**
India	***M. orientalis***	**Mauchline (1980); Aravindakshan *et al.* (1988); Paul and Josekutty (2005)**
	M. zeylanica	**Aravindakshan *et al.* (1988); Jadhav and Josekutty (2003)**
	Gangemysis assimilis	**Mauchline (1980)**
Thailand	***M. orientalis***	**Ohtsuka *et al.* (unpublished)**
France (Channel Islands)	***Praunus flexuosus***	**Mauchline (1980)**
Korea	**mysids**	**Omori (1975)**
South Vietnam	**mysids**	**Omori (1975)**

There are various species of mysids fished locally in Japan, India, China, Korea, and some southeast Asian countries (Omori, 1978; Mauchline, 1980; Aravindakshan *et al*., 1988; Jadhav and Josekutty, 2003; Paul and Josekutty, 2005; Mantiri *et al*., 2012). In Japan, *Neomysis intermedia*, *N. japonica* and *Orientomysis mitsukurii* (*Acanthomysis mitsukurii*) are harvested in thousands of tons each year (Mauchline, 1980). According to Murano (1963), *N. intermedia* has played an important role for lake fishery in Japan. In Indonesia, so far there is no detailed and complete statistics available at the national level, nevertheless *M. orientalis* and *M. tenuipes* become the fishery target, because they were found in fresh and dried materials sold, and in the composition of shrimp paste 'terasi' (Mantiri *et al*., 2012).

Mysids have been known as one of the fisheries in Indonesia since 1930s (Djajadiredja and Sachlan, 1956; Nouvel, 1957). They are usually mixed with the pelagic shrimp *Acetes* and other different transparent shrimps, and are called as 'udang terasi' (terasi shrimps), a technical term used in Indonesian government services (Djajadiredja and Sachlan, 1956), and by common people as 'Rebon' or 'Jembret' (Djajadiredja and Sachlan, 1956; Nouvel, 1957; Mantiri *et al.*, 2012). 'Rebon' has bigger size varying between 3 and 20 mm, whereas 'Jembret' the size varying between 5 and 15 mm (Djajadiredja and Sachlan, 1956). 'Rebon' consist mainly of *Acetes,* whereas 'Jembret' consist mainly of mysids (Mantiri *et al.*, 2012). Both 'Rebon' and 'Jembret' are usually used for making 'terasi', a shrimp paste

which constitutes an important smyce of protein for human beings (Djajadiredja and Sachlan, 1956; Nouvel, 1957; Mantiri *et al.*, 2012). Since the contribution of Omori (1975) is not so detail, and the current status of these fisheries has never been addressed clearly, I would like to get detailed information about fisheries of the mysids and the pelagic shrimps in the present study.

Mysids are very important creatures for aquatic organisms as well. In aquarium, most fish seem to feed on mysids rather than other foods once accustomed to capturing them (Hemdal, 2002). For examples, *Mysidopsis bahia* is favourite food for Red backed butterflyfish, *Chaetodon paucofasciatus* (Hemdal, 2002); new born *Mysis* sp. is excellent for growing juvenile seahorses (Weiss, 2002, 2006); mysids *M. orientalis* are good for grouper *Epinephelus fuscoguttatus* (Eusebio *et al.*, 2010). In Indonesia, *M. orientalis* and *M. tenuipes* are fed by seahorses cultured in cement tank (Mantiri *et al*., 2012). In Thailand, mysids are as live food for cultured organisms (Mauchline, 1980; Ohtsuka *et al.*, unpublished). In Japan, *Neomysis awatschensis* and *N. japonica* are said to be as food for cultured organisms (Hanamura, 2001).

The taxonomic and ecological information of mysids has been scare in Indonesia. Taxonomical study of mysids in Indonesian waters started in the late 19th century by Sars through the 'Challenger Expedition' of 1872-1876 in Arafura and celebes seas (Sars, 1883) and by Hansen through the 'Siboga Expedition' of 1899-

1900 (Hansen, 1910). Sars' works were the preliminary notices on the Schizopoda (Sars, 1883). The first species of mysids recognized in Indonesian waters are *Siriella gracilis* (in Arafura and celebes seas) and *Promysis pussila* (in Celebes sea), found through the 'Challenger Expedition' of 1872-1876 by Sars (1883). Subsequently, Hansen (1910) recognized the following species of mysids in Indonesian waters through the 'Siboga Expedition' of 1899-1900 : *Haplostylus indicus* and *Gymnerythrops anomala*. So far not so many species of Mysida have been described from these two expeditions. As for the coastal mysids of Indonesian waters people started to work in 1950s when Djajadiredja and Sachlan (1956) and Nouvel (1957) examined the composition of shrimp paste called 'terasi'. They found that the shrimp paste in Indonesia was composed not only by *Acetes* but also by mysids (Djajadiredja and Sachlan, 1956; Nouvel, 1957).

There was no more expedition or research after these two expeditions until 1950s by Djajadiredja and Sachlan (1956) and Nouvel (1957), and then there was no more study on mysids. Considering such a scientific situation, I have been studying mysids in coastal waters of Indonesia. *Mesopodopsis orientalis* and *M. tenuipes* were one of the most dominant species, and ordinarily utilized as "rebon" and "terasi" (Mantiri *et al.*, 2012). However several genetic types of these species exist in Malaysia and Thailand (Hanamura *et al*., 2008). Since the genetics of the Indonesian populations of *M. orientalis* remains unknown, I have tried to disclose the genetic

types of these populations using *COI* of mtDNA (Chapter 2). In addition, fisheries of mysids have never been addressed in Indonesia. Since I have found out in the present study that mysids *Mesopodopsis* and the pelagic shrimp *Acetes* are captured together by Indonesian fishermen, I investigated fisheries of these two pelagic crustacean groups and then tried to collect information of fishing grounds, gears, seasonality and captures of edible mysids from several islands of Indonesia (Chapter 3).

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Chapter 2

GENETIC DIVERSITY AND PHYLOGEOGRAPHIC STRUCTURE OF THE POPULATIONS OF *MESOPODOPSIS ORIENTALIS* (Tattersall, 1908) IN INDONESIAN WATERS

2.1 Introduction

Mesopodopsis orientalis, the shallow-water Indo-Australasian mysid has been recorded from various localities throughout the southwest coast of India and the Philippines, including Malaysia, Singapore, Indonesia and Thailand (Mauchline, 1980; Mŭller 1993; Pinkaew *et al*., 2001; Hanamura *et al*., 2008a,b, 2009). This species is considered to be one of the most important mysids in the shallow-water crustacean community in tropical Asian waters, and is often found in vast numbers in inshore waters and estuaries of the region. It has been reported that swarming *M. orientalis* are a food source of the Irrawaddy dolphin *Orcealla brevirostris* in Chilka Lake (Tattersall, 1915), for human consumption in India and Thailand (Mauchline, 1980; Chaitiamwongse and Yoodee, 1982), and also for making shrimp paste called 'terasi' in Madura, Indonesia (Mantiri *et al.*, 2012).

The phylogenetic relationships within genus *Mesopodopsis* are still poorly understood. Only few have conducted the molecular studies on this genus; Hanamura *et al.* (2008b) have conducted on *M. orientalis* and *M. tenuipes* from

Thailand and Malaysia, and Biju *et al*.(2008) have conducted on *M. orientalis* from India; Remerie *et al.* (2006) have conducted on *M. wooldridgei* from south Africa; Biju and Francis (2011) have conducted on *M. zeylanica* from India; Cristescu and Hebert (2005) have conducted on *M. slabberi* from Black Sea, Europe.

Since no one has ever done the molecular work on *Mesopodopsis* in Indonesia before, in this chapter, genetic diversity and phylogeographic structure of the populations of *M. orientalis* in Indonesian waters were investigated by analyzing DNA sequences for mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene. The evolutionary and zoogeographical patterns of the Indonesian populations are also considered.

2.2 Materials and methods

2.2.1 Collection and Identification of specimens

Specimens of *Mesopodopsis orientalis* used in this study were collected from 10 Indonesian water sites (Table 2-1, Fig. 2-1): Jakarta, Cirebon, Tegal and Tuban in Java Island; Karanganyar and Madura strait in Madura Island; Guris, Perancak and Serangan in Bali Island; Sungai Raya in Kalimantan Island. These collections were derived from three types of habitats that were classified into three types of habitats; brackish and salt ponds (site nos 1, 5, 7, 8 and 9 in Table 2-1; B and C in Fig. 2-2), intertidal zone and shallow waters (site nos 2, 3, 4 and 6 in Table 2-1; A in Fig. 2- 2) and mouth of rivers (site no 10 in Table 2-1; D in Fig. 2-2).

The collections of specimens in these sampling sites were performed both at day and night from June to July in 2009 and 2010 (Table 2-1); using a plankton-net and scoop-nets with a short or long handle. All specimens were preserved in 99.5% ethanol immediately upon sampling, and then stored at 23 ˚C until subsequent analysis.

Before to complete DNA analysis, mysid specimens were identified using a dissecting microscope (Olympus SZ60), following the identification keys of Hanamura *et al*. (2008a). Male specimens attributable to *M. orientalis* were sorted. Male was chosen because its long 4th pleopod character sets it apart from the

female. A total of one hundred and thirty six (136) male specimens (three to thirty three specimens per collection site) were used in subsequent DNA analysis (Table 2-1). Specimens from Jakarta and Sungai Raya (collection site nos 1 and 10) were given by Dr. Mulyadi (a staff of LIPI, Jakarta) and Mr. K. Handoko (a staff of Regional Office for Marine and Coastal Resource Management, Pontianak, Kalimantan), respectively.

Table 2-1. List of localities, number of specimens (*n*), type of habitat and date and time in collection sites for genetic analysis of *Mesopodopsis orientalis*.

No	Locality	*n*	Type of habitat	Date/Time
1	Jakarta, Java Is. 6°09.000' S, 106°49.000' E	3	Coastal area (brackish pond)	June 2009/Day time
2	Cirebon, Java Is. 6°42.561' S, 108°34.310' E	22	Seashore (sandy and muddy bottom)	5th July 2009/14:00 25th June 2010/5:00
3	Tegal, Java Is. 6°50.681' S, 109°08.506' E	16	Shallow seawater (sandy and muddy bottom)	3rd July 2009/23:00
4	Tuban, Java Is. 6°51.435' S, 112°01.665' E	8	Seashore (sandy and muddy bottom)	1st July 2009/13:00
5	Karanganyar, Madura Is. 7°38.000' S, 113°37.000' E	33	Salt pond (1 ha)	12th July 2010/7:00
6	Madura strait, Madura Is. 7°38.533' S, 113.33.417'E	3	Shallow seawater	11thJuly2010/10:00
7	Guris, Bali Is. 8°25.556' S 114°34.420' E	11	Brackish culture pond	4thJune 2010/18:00
8	Perancak, Bali Is. 8°24.361' S 109°08.506' E	8	Brackish culture pond	3rdJune 2010/19:00
9	Serangan, Bali Is. 8°50.681' S, 115°14.575' E	29	Shrimp brackish culture pond (2 500 m^2)	1st June 2010/5:00
10	Sungai Raya, Kalimantan Is. 0°42.448' N, 108°52.260' E	3	Mouth of river (sandy and muddy bottom)	8th July 2010/4:00

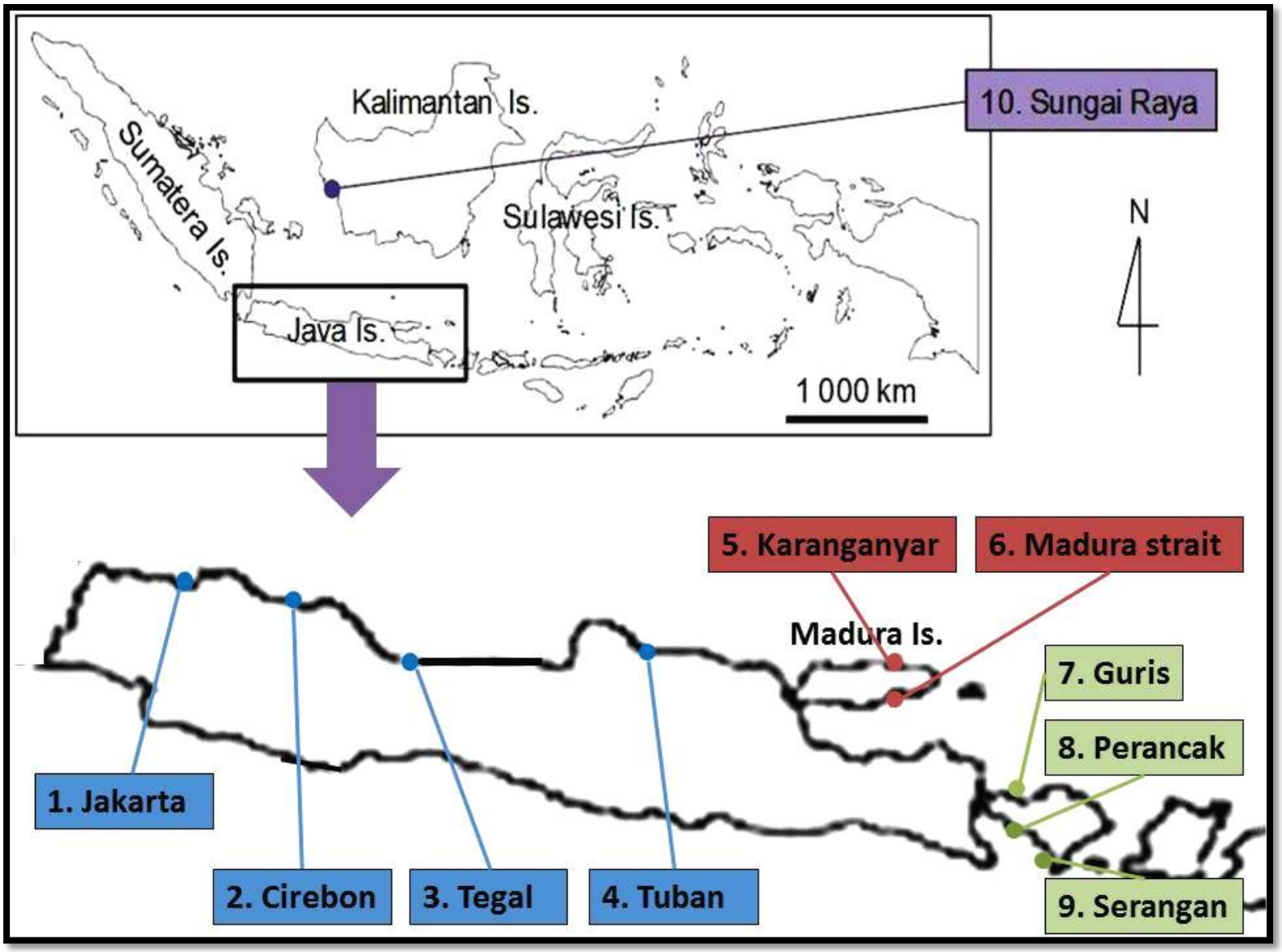

Fig. 2-1. Map of 10 collection sites of specimens of *Mesopodopsis orientalis* in Indonesian waters.

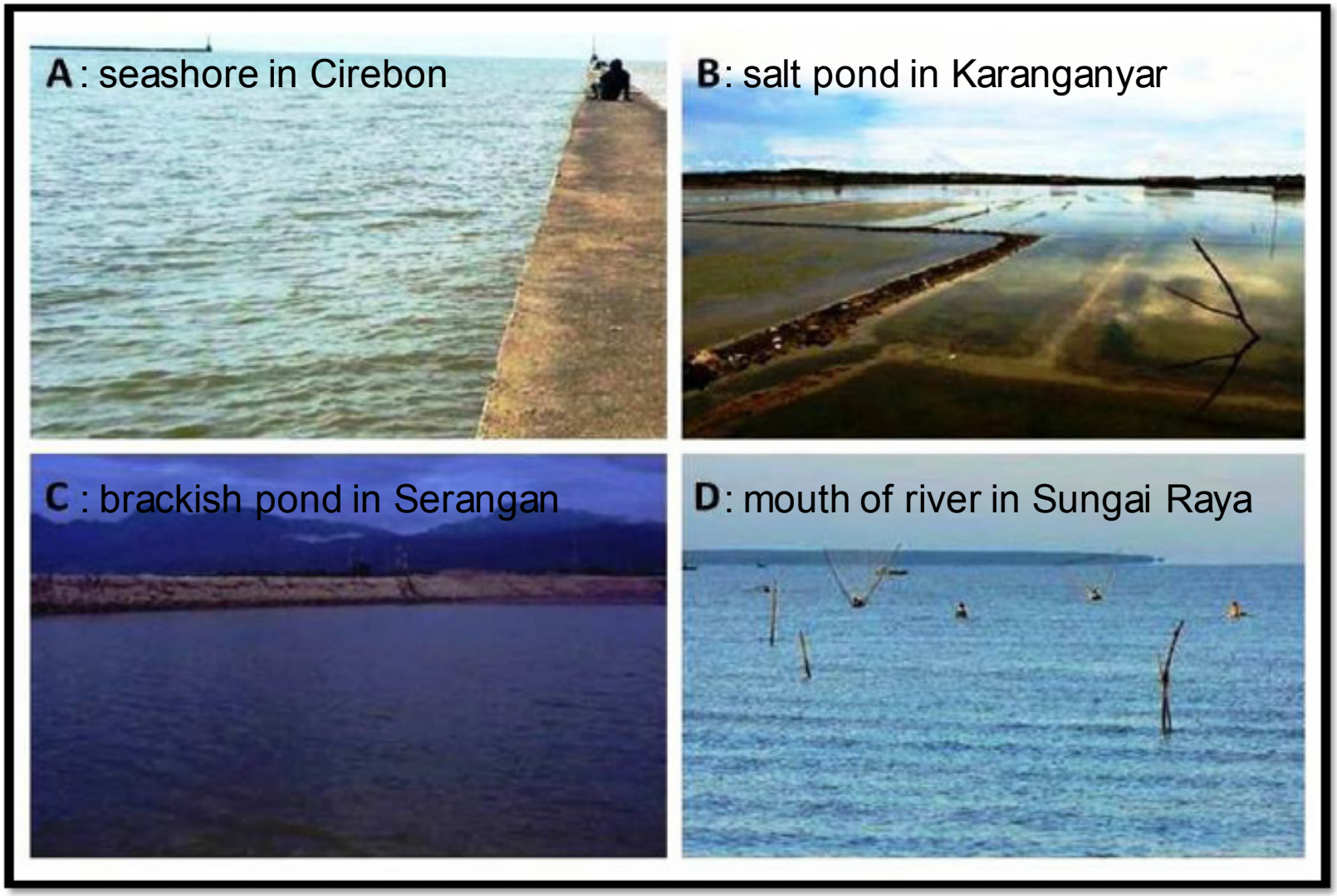

Fig. 2-2. Photos showing several types of habitats in the collection sites of *Mesopodopsis orientalis* (A: seashore in Cirebon, B: salt pond in Karanganyar, C:brackish pond in Serangan, and D: mouth of river in Sungai Raya).

2.2.2 DNA extraction, polymerase chain reaction amplification and DNA sequencing

Nucleotide sequence data of *COI* gene for specimens were obtained by applying two similar methods. The first method was implemented for 42 of 136 fine specimens. Total genomic DNA from a specimen was extracted using Qiagen DNeasy Tissue Kit (Qiagen), following the manufacturer's protocols; the final volume of the unquantitated DNA solution following extraction was 200 µl. Partial fragment in sequence of the *COI* gene was amplified by polymerase chain reaction (PCR) with the primer pairs LCO1490 and HCO2198 (Folmer *et al*., 1994). PCR reactions containing 0.5µl template solution, 2 mM $MgCl_2$, 2.5 mM dNTP, 10 pmol each primer, and 5U/µl Taq DNA polymerase (TaKaRa Ex Taq®) in 1X buffer provided by the manufacture were performed in 10-µl volumes in an iCycler thermal cycler (Bio-Rad) with an initial denaturation step at 94˚C for 2 min, followed by 35 cycles consisting of a denaturation step at 94˚C for 30 s, an annealing step at 50˚C for 30 s and an extension step at 72˚C for 60 s. PCR products were purified using AMPure kit (Agentcourt Bioscience). The forward and reverse sequences were obtained using a 3100 Avant Genetic Analyzer (Applied Biosystems; ABI).

The second method was carried out for the remaining 94 bad specimens. Whole body of a specimen was collapsed with a tungsten bead (six mm in diameter) and a Shake Master Auto ver. 2.0 (both from BioMedical Science). Genomic DNA from the

collapsed specimen was extracted using an automated DNA isolation system (Gene Prep Star PI-80X, KURABO), following the manufacturer's instructions. Using the same primer pair as the first method, PCR amplification of *COI* gene was performed in 10 μl reaction volumes, each containing approximately 10 to 50 ng DNA template, 0.5 U Taq DNA polymerase (Biotaq, Bioline), 1 × NH_4 buffer (Biotaq), 2.5 mM $MgCl_2$, 0.25 mM each dNTP and 0.25 μM of each primer. Thermal profiles on a C1000 thermal cycler (Bio-Rad) were as follows. Initial denaturation at 94 ˚C for 2 min was followed by 40 cycles of denaturation at 94 ˚C for 30 s, annealing at 55 ˚C for 30 s and extension at 72 ˚C for 60 s. A final extension at 72 ˚C was done for 5 min. PCR products were purified using AMPure XP kits (Agencourt Bioscience), following the manufacturer's protocols.

Cycle sequencing reactions of the forward and reverse sequences for the purified PCR products were conducted using a BigDye Terminator kit ver. 3.1 (ABI). After purifying the reaction products with CleanSeq kits (Agencourt Bioscience), following the manufacturer's protocols, these products were resolved on a 3130*xl* Genetic Analyzer (ABI).

2.2.3 Data analysis of DNA sequences

Both forward and reverse sequences of partial fragments of *COI* gene were assembled into a contig by visual inspection with the software SEQSCAPE ver. 2.6 (ABI), FINCH TV ver. 1.4.0 (Geospiza) and DNA BASER ver. 3.2 (Heracle BioSoft).

Haplotypes and their frequencies by the number of specimens were identified from all sequence data and variable nucleotide positions were examined among haplotype sequences. Genetic variability of haplotypes was evaluated by haplotype diversity (*h*; Nei and Tajima, 1981) and nucleotide diversity (π; Nei, 1987) using the software ARLEQUIN ver. 3.5.1.3 (Excoffier and Lischer, 2010). Haplotype diversity is a measure of the uniqueness of a particular haplotype in a given population. The haplotype diversity (H) is calculated as:

$$H = \frac{N}{N-1}\left(1-\sum_{i} x_i^2\right)$$ where *xi* is the (relative) haplotype frequency of each haplotype in the sample and *N* is the sample size. Nucleotide diversity is used to measure the degree of polymorphism within a population; a measure of genetic variation. The nucleotype diversity (π) is calculated as :

$$\pi = \sum_{ij} x_i x_j \pi_{ij} = 2 * \sum_{ij}^{n} \sum_{j=1}^{i=1} x_i x_j \pi_{ij}$$ where xi and xj are the respective frequencies of the ith and jth sequences, πij is the number of nucleotide differences per nucleotide site between the ith and jth sequences, and n is the number of sequences in the sample.

Phylogenetic relationships among haplotypes were investigated by constructing a maximum parsimony (MP) and a neighbor-joining (NJ) trees implemented in the software MEGA ver. 5.05 (Tamura *et al*., 2011). In the NJ analysis, the Kimura 2-parameter (K2P) model (Kimura, 1980) of nucleotide substitution was used to estimate

genetic distances. The robustness of phylogenies was assessed by bootstrap analysis consisting of 1,000 replicates (Felsenstein, 1985). The existence of genetic clades in the MP and NJ trees were determined based on bootstrap support probabilities and haplotypes in each clade were identified. Genetic variability of clades was assessed by h and π using ARLEQUIN. In addition, the most parsimonious unrooted haplotype network among haplotypes was also constructed using the Templeton-Crandall-Sing parsimony algorithm (TCS; Templeton *et al*., 1992) implemented in the software TCS ver. 1.21 (Clement *et al*., 2000). Sympatries of clades and their haplotypes in the network were compared with those of the MP tree.

Genetic diversity within population in a site was quantified from the number of haplotypes, h and π using ARLEQUIN. Genetic differentiations among populations were evaluated by average sequence divergence (d_A; Nei, 1987) and pairwise F_{ST} statistic, which is conventional index of population differentiation (Weir and Cockerham, 1984), using the same software. Statistical significance ($\alpha = 0.05$) for d_A and F_{ST} was tested with applying 10,000 permutations, followed by sequential Bonferroni corrections (Rice, 1989).

To elucidate phylogeographic population structure, haplotype compositions of populations were mapped on sites and dividing the populations into genetic groups was attempted with consideration for the phylogenetic relationships. An analysis of molecular variance (Excoffier *et al*., 1992) for F_{ST} was performed to hierarchically test

genetic heterogeneities among genetic groups, among populations within groups and within populations using ARLEQUIN with 10,000 permutations. Phylogeographic significance of the groups was also visually confirmed by constructing a neighbor-joining tree (Saitou and Nei, 1987) using F_{ST} implemented in MEGA ver. 5.05.

2.3 Results

2.3.1 Characteristics of haplotype sequences

A total of 458 base pairs (bp) of partial fragment sequences of *COI* region were determined for 136 specimens in 10 collection sites. All sequences were unambiguously aligned, revealing 37 haplotypes (Haps 01 to 37) defined from 38 variable sites (5.8 % in 458 bp) including 37 transitions and 3 transversions without insertions and deletions (Table 2-2). Haplotype diversity (h) and nucleotide diversity (π) were 0.8036 ± 0.0273 and 0.0089 ± 0.0049, respectively. Hap 01 (n = 51) most appeared, followed by Haps 32 (32) and 31 (6) in all haplotypes.

2.3.2 Phylogenetic tree and haplotype network

The maximum parsimony (MP) and neighbor-joining (NJ) trees were constructed with a total of 39 haplotypes including two ones, MOMC01 (GenBank accession number: AB451022) and MOTC01 (AB451027) that were obtained from specimens in Malaysia and Thailand, respectively (Hanamura *et al*., 2008b). The MP and NJ trees revealed two genetic clades, Clades 1 and 2, between which its divergence was significantly supported with 99% of bootstrap probability (Fig. 2-3; Appendix 2). In my study I only focused on MP tree. Clade 1 consisted of 27 haplotypes (Haps 01 to 27), while Clade 2 did of 10 ones (Haps 28 to 37). In Clades 1 and 2, h and π were 0.6703 ± 0.0581 and 0.0030 ± 0.0020, respectively.

Table 2-2. Haplotypes, their frequencies (the number of specimens; *n*) and variable positions in sequence (458 base pairs) of *COI* gene in *Mesopodopsis orientalis*. Dots indicate an identical nucleotide base.

No.	Haplotype (*n*)	Base pair (nucleotide) position																
		15	45	54	84	87	99	105	126	138	141	147	165	168	195	198	201	205
1	H01 (51)	G	C	T	G	G	T	G	T	T	T	T	A	T	T	T	T	T
2	H02 (1)	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
3	H03 (1)	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·
4	H04 (1)	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
5	H05 (1)	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·
6	H06 (1)	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
7	H07 (1)	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
8	H08 (1)	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·
9	H09 (1)	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
10	H10 (2)	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·
11	H11 (2)	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·
12	H12 (2)	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
13	H13 (1)	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·
14	H14 (4)	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·
15	H15 (1)	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·
16	H16 (2)	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
17	H17 (2)	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·
18	H18 (3)	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·
19	H19 (1)	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
20	H20 (1)	·	·	C	·	·	C	·	·	·	·	·	·	·	·	·	·	·
21	H21 (1)	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
22	H22 (1)	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
23	H23 (1)	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
24	H24 (1)	·	·	·	T	·	·	·	·	C	·	·	·	·	·	·	·	·
25	H25 (2)	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
26	H26 (1)	·	·	·	·	A	·	·	·	·	·	·	G	·	·	·	C	·
27	H27 (2)	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
28	H28 (1)	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
29	H29 (1)	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
30	H30 (2)	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
31	H31 (6)	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
32	H32 (32)	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
33	H33 (1)	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C
34	H34 (1)	A	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·
35	H35 (1)	A	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·
36	H36 (1)	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
37	H37 (1)	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

Table 2-2. Continued.

No.	Base pair (nucleotide) position																					
	216	222	240	252	258	261	267	285	291	304	315	324	327	354	360	411	417	421	429	435	453
1	T	T	A	A	G	T	C	C	T	G	T	G	T	C	A	T	A	T	G	T	G
2	·	·	·	G	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·
3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
4	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
5	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
6	·	·	·	·	A	·	·	·	·	·	·	·	·	T	·	·	G	·	·	·	·
7	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	C	·	·	·
8	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
9	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·
10	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
11	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	C	·
12	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
13	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
14	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·
15	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
16	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·
17	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
18	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
19	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·
20	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·
21	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
22	·	C	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·
23	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·
24	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·
25	·	·	G	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·
26	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
27	·	A	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·
28	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	A	·	·
29	·	·	·	·	·	·	·	T	C	·	·	·	·	·	·	·	·	·	A	·	A
30	·	A	·	·	·	·	·	T	C	·	·	·	·	G	·	·	·	·	A	·	A
31	·	A	·	·	·	·	·	·	C	·	·	·	·	G	·	·	·	·	A	·	A
32	·	A	·	·	·	·	·	·	C	·	·	·	·	G	G	·	·	·	A	·	A
33	·	A	·	·	·	·	·	·	C	·	·	·	·	G	·	·	·	·	A	·	A
34	·	A	·	·	·	·	·	·	C	A	·	·	·	G	G	·	·	·	A	·	A
35	·	A	·	·	·	·	·	·	C	·	·	·	·	G	·	·	·	C	A	·	A
36	·	A	·	·	·	·	·	·	C	A	·	·	·	G	·	·	·	C	A	·	A
37	·	A	·	·	·	·	·	·	C	·	·	·	·	G	·	·	·	C	A	·	A

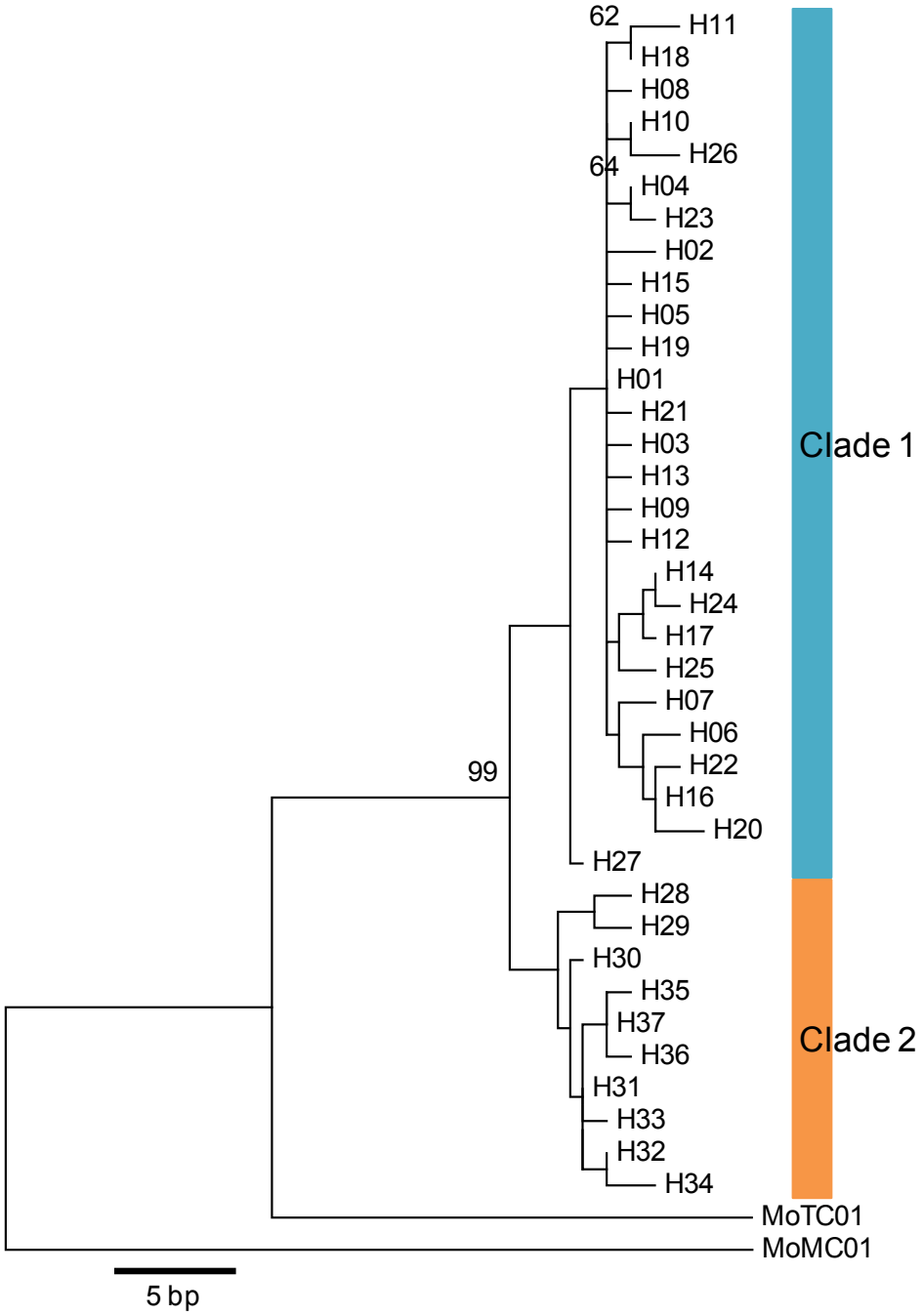

Fig. 2-3. Maximum parsimony tree of haplotypes of *COI* gene in *Mesopodopsis orientalis*. For each node bootstrap support is indicated; for the sake of clarity, only bootstrap values over 60 % are indicated. Haplotype labels correspond to Table 2-2. MoMC01 and MoTC01 show haplotypes in Malaysia and Thailand, respectively.

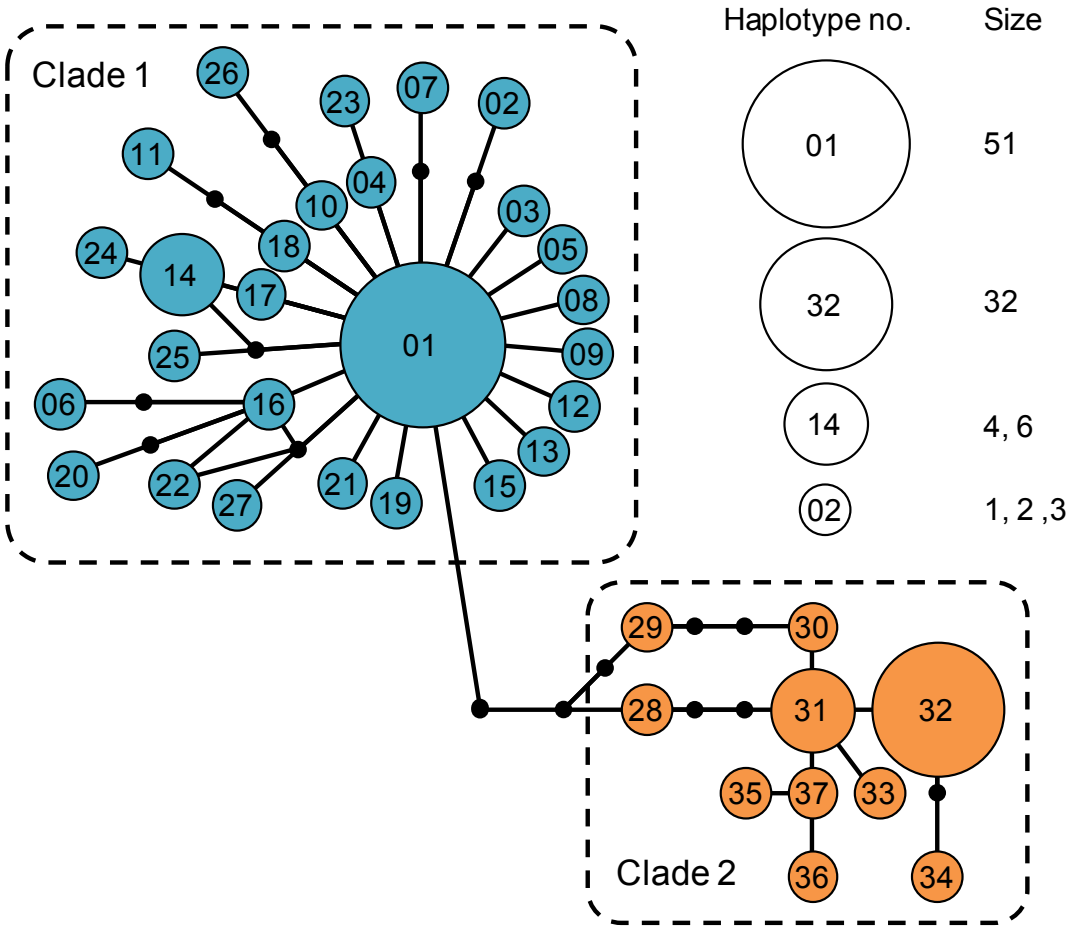

Fig. 2-4. The most parsimonious network of haplotypes of *COI* gene in *Mesopodopsis orientalis*. Each line between haplotypes indicates a single nucleotide substitution.

Two genetic clades formed in the most parsimonious network of haplotypes of COI gene in *M. orientalis* (Fig. 2-4) shows the relationship among them. However, the limited number of specimens analysed can not be made firm conclusions, and hence detailed molecular and morphological studies are needed to resolve the identity and evolutionary origin of this divergent lineage. A better taxonomic sampling of *M. orientalis* might be needed to reach a more detailed and correct view of the genealogy of the different clades within the species.

2.3.3 Genetic diversity and differentiation

Concerning genetic diversity within each local population, the number of haplotypes was polymorphic, with ranging from 2 (Jakarta) to 12 (Karanganyar) across the populations (Table 2-3). Values of h largely varied from 0.3744 (Serangan) to 1.0000 (Madura strait and Sungai Raya), while those of π moderately varied from 0.0024 (Cirebon) to 0.0090 (Perancak).

Regarding genetic differentiation among populations, average sequence differences (d_A) of 22 of a total 45 pairs were significant after sequential Bonferroni corrections (Table 2-4), with ranging from 0.0387 (the pair between Tegal and Sungai Raya). Many pairs (19/24 pairs) with significant d_A were observed between the populations of Jakarta to the Madura strait (population nos 1 to 6) and those of Guris to Sungai Raya (7 to 10). Besides, F_{ST} of a total of 20 of 45 pairs were significantly different from zero after sequential Bonferroni corrections (Table 2-4), with ranging

from 0.0393 (the pair between Cirebon and Tegal) to 0.8573 (the pair between Cirebon and Sungai Raya). Significant F_{ST} often occurred in pairs between Jakarta to the Madura strait and Guris to Sungai Raya (17/24 pairs) along with d_A.

2.3.4 Phylogeographic population structure

Haplotype compositions differed among populations (Table 2-3), although these populations were divided into two genetic groups (Fig. 2-5), Groups 1 and 2, based on the clade attributions of haplotypes observed in the MP tree (Fig. 2-3) and haplotype network (Fig. 2-4). The six populations from Jakarta to the Madura strait in Java and Madura Island (population nos. 1 to 6) belonged to Group 1 and predominated by haplotypes in Clade 1, while the four populations from Guris to Sungai Raya in Bali and Kalimantan Island (7 to 10) were categorized to Group 2 that was mainly occupied by haplotypes in Clade 2. However, NJ tree shows that H28 and H29 belong to Clade 1. In MP tree (Fig. 2-3) and haplotype network (Fig. 2-4), they also appear to be close to Clade 1.

The AMOVA to test significance of Groups 1 and 2 demonstrated that genetic differentiations were significantly observed at all hierarchical levels ($p < 0.01$; Table 2-5). The largest genetic variance accounting for 66.38 % in all the variances was found at the level of 'among groups (F_{ST} = 0.6961)', followed by 30.39 % of 'within populations (F_{CT} = 0.6638)' and 3.23 % of 'among populations within groups (F_{SC} = 0.0961)'. The topology of the neighbor-joining tree using F_{ST} (Table 2-4) also visually

and successfully illustrated two obvious genetic groups the among *M. orientalis* populations (Fig. 2-6) that corresponded to Groups 1 and 2 (Fig. 2-5).

Consequently a series of DNA analysis for haplotype sequences of *COI* gene showed the presence of a geographical barrier that separates the two distinct genetic groups across between Java-Madura and Bali-Kalimantan Island systems in Indonesian waters (Fig. 2-5).

Table 2-3. Genetic diversity within each local population of *Mesopodopsis orientalis* evaluated by number of haplotypes (N_H), haplotype diversity (h), nucleotide diverisy (π) and haplotype composition.

No.	Population (n)**	N_H	h	π	Haplotype composition
1	Jakarta (3)	2	0.6667 ± 0.3143	0.0029 ± 0.0030	Clade 1: H01 (2), H02 (1)
2	Cirebon (22)	8	0.5455 ± 0.1276	0.0024 ± 0.0018	Clade 1: H01 (15), H03 (1), H04 (1), H05 (1), H06 (1), H07 (1), H08 (1) Cade 2: H28 (1)
3	Tegal (16)	5	0.6750 ± 0.1159	0.0028 ± 0.0021	Clade 1: H01 (9), H09 (1), H10 (2), H11 (2), H12 (2)
4	Tuban (8)	3	0.6071 ± 0.1640	0.0071 ± 0.0047	Clade 1: H01 (5), H13 (1) Clade 2: H30 (2)
5	Karanganyar (33)	12	0.7557 ± 0.0754	0.0037 ± 0.0025	Clade 1: H01 (16), H14 (4), H15 (1), H16 (2), H17 (2), H18 (2), H19 (1), H20 (1), H21 (1), H22 (1), H23 (1) Clade 2: H31 (1)
6	Madura strait (3)	3	1.0000* ± 0.2722	0.0058 ± 0.0052	Clade 1: H01 (1), H18 (1), H24 (1)
7	Guris (11)	4	0.7636 ± 0.0833	0.0083 ± 0.0051	Clade 1: H01 (1), H25 (2) Clade 2: H31 (4), H32 (4)
8	Perancak (8)	4	0.6429 ± 0.1841	0.0090 ± 0.0057	Clade 1: H01 (1), H26 (1) Clade 2: H32 (5), H33 (1)
9	Serangan (29)	6	0.3744 ± 0.1130	0.0035 ± 0.0024	Clade 1: H01 (1), H27 (2) Clade 2: H29 (1), H31 (1), H32 (23), H34 (1)
10	Sungai Raya (3)	3	1.0000* ± 0.2722	0.0029 ± 0.0030	Clade 2: H35 (1), H36 (1), H37 (1)

* The value h = 1.0000 means the absence of any shared alleles.

** Population (n) corresponds to locality and the number of specimens in a collection site (Table 2-1).

Table 2-4. Genetic differentiation among populations of *Mesopodopsis orientalis* evaluated by average sequences differences (d_A; above diagonal) and F_{ST} (below diagonal). Values in yellow cells with diagonal lines indicate to be significant after sequential Bonferroni corrections.

No.	Population	1	2	3	4	5	6	7	8	9	10
1	Jakarta		0.0043	0.0417	0.2500	0.0578	0.0000	3.2000	3.5714	5.5493	7.0000
2	Cirebon	0.0367		0.0460	0.1748	0.0387	0.0043	2.9729	3.3371	5.2699	6.5952
3	Tegal	0.0354	0.0393		0.2917	0.0843	-0.0417	3.2417	3.5819	5.5909	6.9583
4	Tuban	0.0099	0.1399	0.1566		0.1638	0.2500	1.2682	1.5714	3.0751	4.2500
5	Karanganyar	0.0001	0.0227	0.0470	0.1077		-0.1847	2.8390	3.2428	5.1358	6.5022
6	Madura strait	0.0000	0.1657	0.0966	0.0553	-0.0199		3.0788	3.5714	5.5493	6.7778
7	Guris	0.4604	0.6086	0.5890	0.2610	0.5692	0.4492		-0.2286	0.2728	1.4727
8	Perancak	0.4822	0.6531	0.6268	0.2993	0.6115	0.4734	-0.0603		-0.0345	1.5714
9	Serangan	0.7778	0.7932	0.7898	0.6211	0.7568	0.7746	0.1343	0.0380		1.6987
10	Sungai Raya	0.8400	0.8573	0.8431	0.5879	0.7931	0.7722	0.2544	0.2637	0.5131	

Table 2-5. Results of AMOVA: degrees of freedom, sum of squares, variance components, percentage of variation and *F*-statistics in hierarchies.

Hierarchy	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	*F*-statistics
Among groups	1	136.32	2.0897	66.38	F_{ST} = 0.6961**
Among populations within groups	8	17.13	0.1017	3.23	F_{SC} = 0.0961**
Within populations	126	120.56	0.9568	30.39	F_{CT} = 0.6638**
Total	135	274.01	3.1482		

** indicates significantly different ($p < 0.01$). The test revealed significant heterogeneity among Indonesian waters.

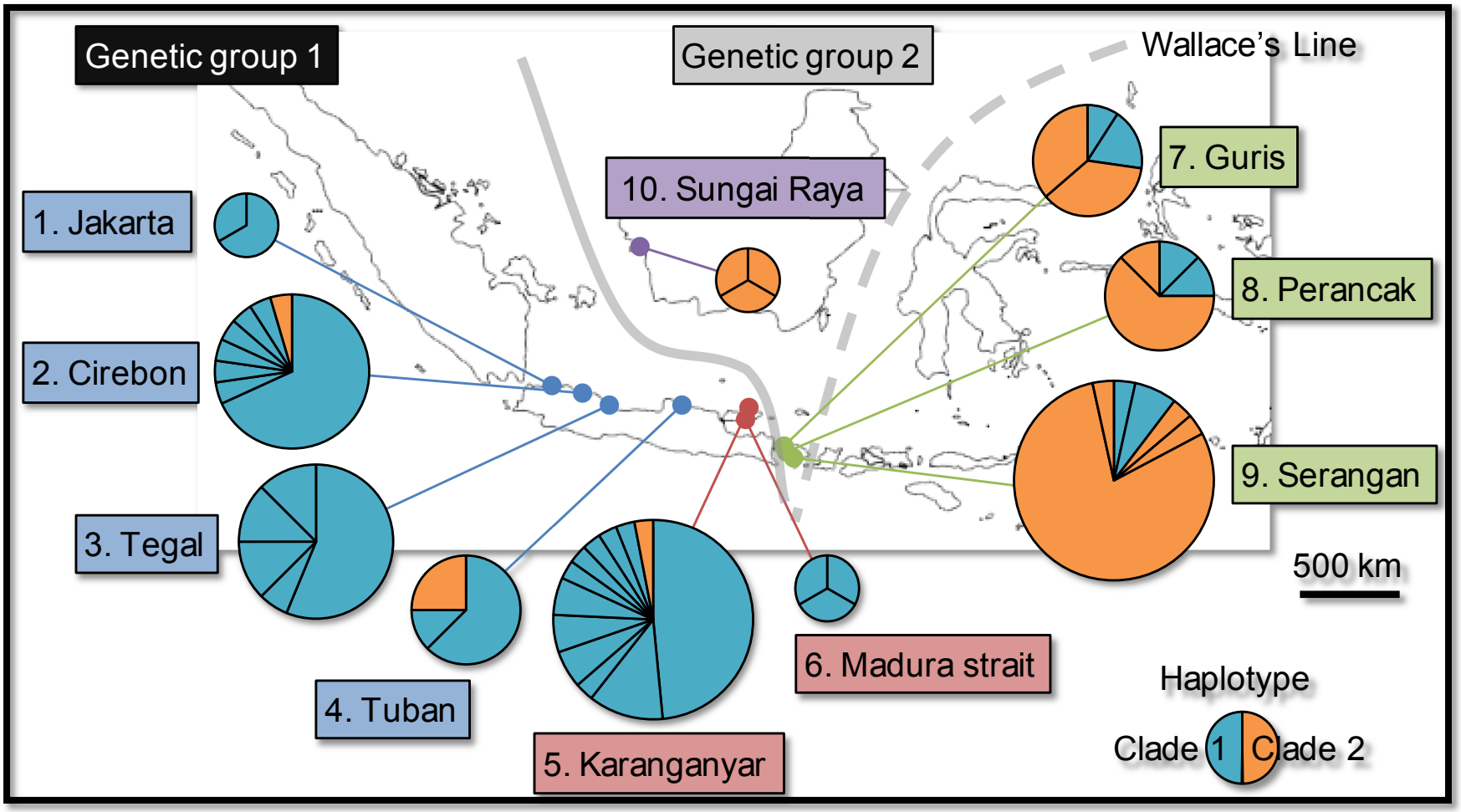

Fig. 2-5. Geographical distribution of haplotypes in populations of *Mesopodopsis orientalis* including haplotype compositions. Circle sizes of populations indicate the number of specimens. Thick unbroken light purple color line indicates the geographical barrier. Compositions of haplotypes are shown in Table 2-3.

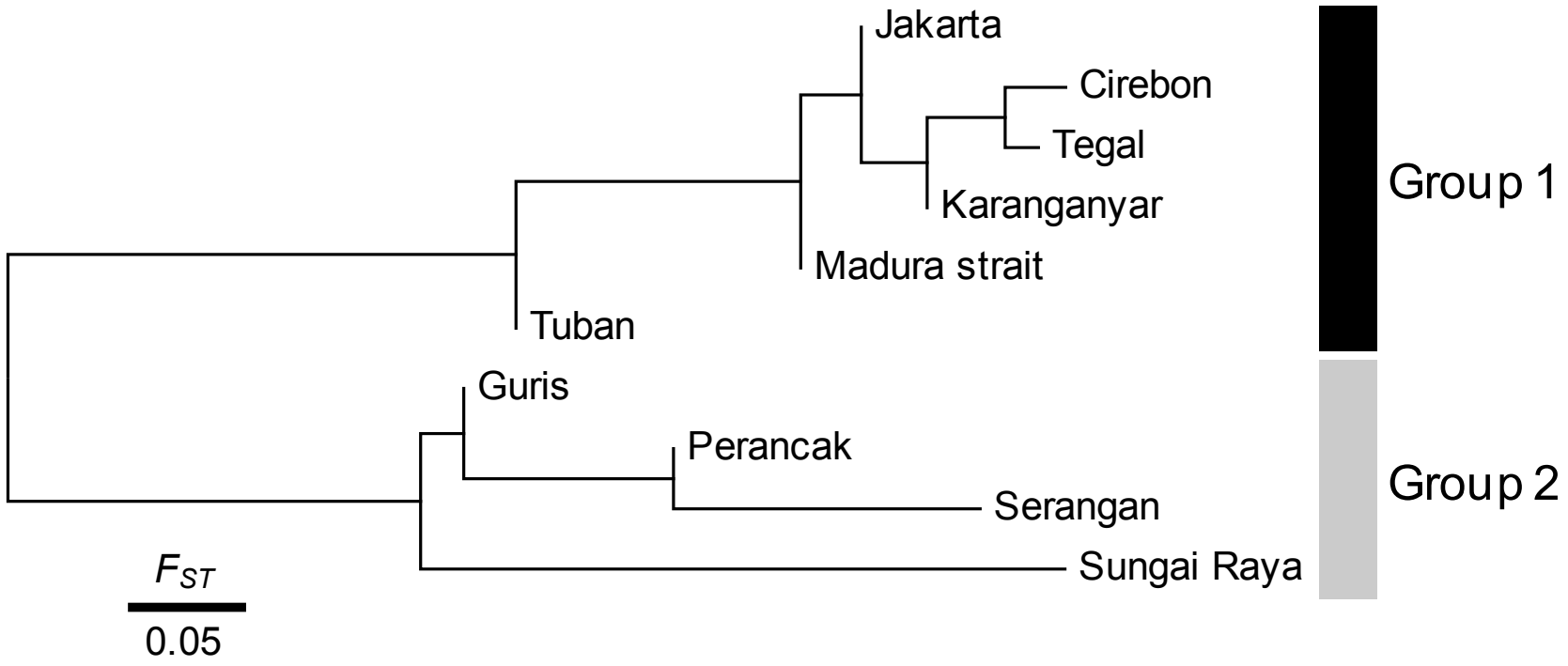

Fig. 2-6. Neighbor-joining tree of populations using F_{ST} in Table 2-4.

2.4 Discussion

Characterization of genetic properties of *Mesopodopsis orientalis* in this study is reviewed and compared with those in Malaysia and Thailand along with other relative species (e.g. *M. tenuipes*, *M. slabberi*, and *M. wooldridgei*) (Hanamura *et al*., 2008b; see Appendix 1). Concerning the genetic properties of *M. orientalis* population in Indonesia to the populations in Malaysia and Thailand, it can be inferred that Indonesian population was inherited together with Malaysia's and Thailand's because of genetic linkage, though each has its own characteristic of haplotype sequences .

Fig. 2-3 shows that haplotypes of *M. orientalis* population from Indonesia differed from those of Malaysia and Thailand, and formed two different clades in the phylogenetic tree. However, it is possible that the populations of four clades (Malaysia's, Thailand's and two Indonesian clades) could be a complex of cryptic species (Knowlton *et al.,*1993, Knowlton, 2000), as noted in several previous studies by Knowlton and Weight (1998) and Remerie *et al*. (2006). Remerie *et al*. (2006) said that the discovery of cryptic species is common in the marine invertebrates.

Furthermore, the MP tree (Fig. 2-3) shows that *M. orientalis* population from Indonesia was closer to Thailand's. It revealed that Indonesian clades and Thailand's may share a common ancestral origin or be a sister group (see Remerie

et al., 2006), in which is totally different from the Malaysian's, and the Malaysian's might follow a different evolutionary path. The close relationship between Indonesian clade and Thailand's might be attributed to the geographical distribution of the clades based on evolutionary process of Indonesian population reffering to the Pleistocene glacial period. In this case, allopatric speciation occured through the glacial period.

During glacial period, the glaciations made the sea level arose and covered many areas including Sundaland, a biogeographical region of Southeastern Asia which encompasses the Sunda shelf (Fleminger, 1986; Voris, 2000; Sathiamurthy and Voris, 2006). The animations of marine transgression (the rising of sea level) in Indo Pasific sea and Sunda shelf sea to see how the sea water covered these areas can be seen at https://www.eeb.ucla.edu/Faculty/Barber/Animations.htm. (Barber, 2013).

Concerning the phylogeographical distribution to the closeness of Indonesian *M. orientalis* population with Thailand's, sampling sites were considered as another reason. Sample of Thailand' clade was from the Gulf of Thailand which is located in western South China Sea at the same surroundings as studied sites, while Malaysia's was from the west coast of Peninsular Malaysia which is located in the Andaman Sea. Continued molecular studies of *M. orientalis* with a more complete geographic sampling of Malaysia and Thailand,

even more of Indonesia, will undoubtedly yield more insights into the phylogeographic patterns and cryptic speciation of this ecologically important key species.

The presence of two distinct genetic groups of *M. orientalis* in Indonesian waters indicated a geographical barrier across between Java-Madura and Bali-Kalimantan Island systems. The concept arises and influenced by theory of Pleistocene glacial period. Allegedly, the same ancestor of these two groups inhabited 2 rivermouths of huge palaeo rivers on the Sunda shelf, so called the East Sunda River and the North Sunda River . The East Sunda River ran into the sea near Bali, across what is now the Java sea, and the North Sunda River ran into the sea north-east of Natuna Island, flowing from the north-east coast of Sumatra and joining the large Kapuas river from Kalimantan/Borneo (Voris, 2000; Sathiamurthy and Voris, 2006). In this hypothesis, wind (monsoon) and water current had slight influence to their dispersal.

Another hypothesis, the ancestor of these two groups inhabited the rivermouth of the East Sunda River, and when the sea level arose at glacial period the population spreaded to Bali, Kalimantan, Java and Madura islands forming allopatric speciation. The map showing palaeo river systems or submerged rivers on Sunda Land can be seen at Fig. 2-7. These hypotheses can also be applied to the closeness of Indonesian *M. orientalis* population with Thailand's.

The establishment and evolutional process of the detected genetic Groups 1 and 2 were estimated using a general molecular clock for crustacean and comparing with other *M. orientalis* populations in the other neighbouring countries (Hanamura *et al.*, 2008b). The divergence rate of Groups 1 and 2 shows a reasonable evidence that evolutionary process between these two groups may have taken around 0.22 – 14 Myr or occurred during middle Miocene to late Pleistocene, as almost the same as a general molecular clock which ranges from 0.5 to 6.0 % / Myr (Knowlton and Weight 1998; Schubart *et al*., 1998; Audzijonyte *et al.*, 2005, 2006, 2008; Remerie *et al.*, 2009).

Considering these estimated divergence rates of *M. orientalis* populations in southeastern Asian waters, it is likely that the species has originated in and colonized the brackish waters of these tropical areas, being divergent to many genetic types since the Miocene. Since *M. orientalis* is a true brackish species, populations seems to have been more strongly isolated from one another in comparison with coastal mysids. The species is one of the best material to understand the evolution of marine invertebrates in tropical Asian waters, partly due to its broad distribution and high abundance in the area (Hanamura *et al.,* 2008a, b).

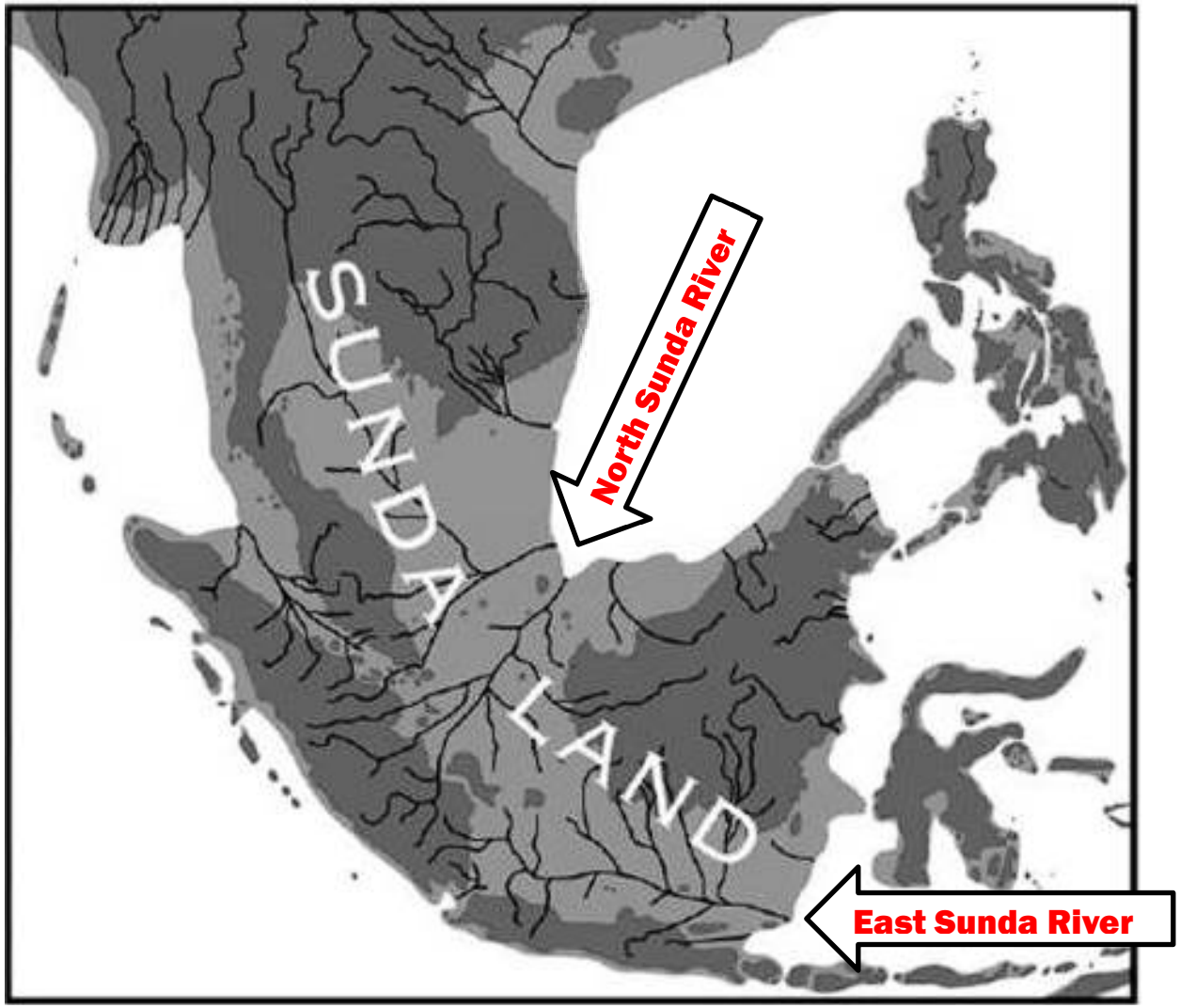

Fig. 2-7. Chart of submerged river valley on Sunda Land; palaeo river systems (After Voris, 2000; Sathiamurthy and Voris, 2006 with permission from Harold K. Voris, Ph.D., Field Museum of Natural History (http://fieldmuseum.org/users/harold-k-voris).

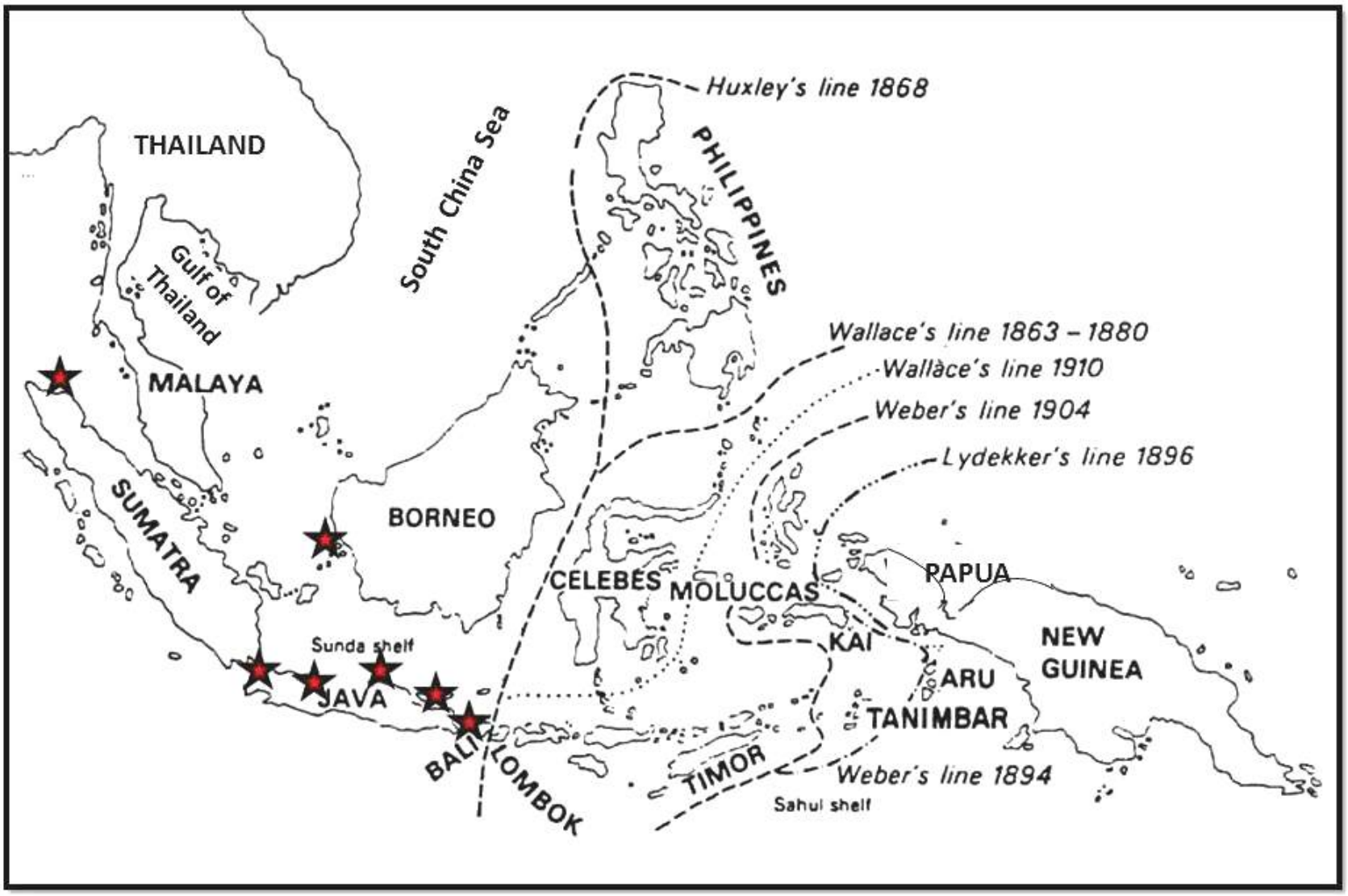

Fig. 2-8. Map of the dDistribution of *Mesopodopsis orientalis* (★) in western Indonesian waters (Mantiri *et al*., unpublished).

Wallace line has been considered to be a barrier separating various marine faunal communities in the Coral triangle zone (Wallace, 1914). In my study, the distribution of *M. orientalis* population (Fig. 2-8) is in the western site of Wallace line boundary; at the western part of Indonesia; on the southeast asia region, and categorized by Wallace as oriental fauna or asiatic fauna (Adminsite, 2012). Future research subjects for elucidating genetic diversity and population structure, proposing effective sampling strategies, and many more data are encouraged to do for populations, particularly in Sumatra, Kalimantan, Sulawesi, etc.

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Chapter 3

FISHERIES ON *MESOPODOPSIS* (MYSIDA: MYSIDAE) AND *ACETES* (DECAPODA: SERGESTIDAE) IN INDONESIA

3.1 Introduction

Pelagic crustaceans such as mysids and *Acetes* are important organisms in coastal and estuarine food webs and for humans as food, in particular in Asian countries (Omori, 1975, 1978; Mauchline, 1980). For example, the following species of mysids are harvested as food for humans or live food for cultured aquatic organisms in these areas such as Japan: *Neomysis awatschensis*, *N. japonica*, *Orientomysis mitsukurii* (*Acanthomysis mitsukurii*) (Murano, 1963; Mauchline, 1980; Toda *et al*., 1982; Hanamura, 2001) and India: *Mesopodopsis orientalis*, *M. zeylanica*, *Gangemysis assimilis* (Mauchline, 1980; Jadhav and Josekutty, 2003; Paul and Josekutty, 2005). The annual catch of *N. awatschensis* in the Lake Kasumigaura, eastern Japan, during 1954–1980, reached up to 2,000 metric tons in wet weight and was composed of 10–20% of the total fisheries landings from that lake (Toda *et al*., 1982). In Mumbai, India, the fishery of *M. orientalis* is lucrative and regularly conducted by local fishermen, and the overall catch reached 1,250 kg/month in 2004 (Paul and Josekutty, 2005).

The pelagic shrimp *Acetes* has also been commercially harvested in Asian countries such as in China, Korea, Japan, Vietnam, Indonesia, Thailand, Malaysia, and India for over 200 years (Koba, 1941; Omori, 1975; Xiao and Greenwood, 1993). The following eight species are captured on fishery grounds in Asia: *Acetes chinensis, A. japonicus, A. indicus, A. erythraeus, A. serrulatus, A. intermedius, A. sibogae sibogae,* and *A. vulgaris* (Omori, 1975). According to Omori (1975), seven of the *Acetes* species, other than *A. chinensis*, are distributed in Indonesia. Omori confirmed that *A. intermedius* and *A. vulgaris* were seen on markets in Jakarta and Pelabuhan Ratu, Indonesia. Although the annual catch of *Acetes* was not addressed in Indonesia, the world catch of *Acetes* was recorded to be at least 170,000 tons per year (Omori, 1975).

In Indonesia, fisheries of mysids and *Acetes* have been intensively continued since 1930s (Djajadiredja and Sachlan, 1956; Omori, 1975). Locally fresh or dried mysids and *Acetes* are called “jembret” and “rebon”, respectively. Both “jembret” and “rebon” are processed for making fermented shrimp paste called “terasi”. This product is commercially important in Indonesia to make chili sauce (= “sambal terasi” in Indonesian) or flavor cooking. “Rebon” is also utilized to make shrimp sauce (= “petis”).

The purpose of this study is to get detailed information about fisheries of the mysid *Mesopodopsis* and the shrimp *Acetes* in Indonesia, because the current status

of these fisheries has never been addressed. In addition, I would like the Indonesian Government to provide the fisheries statistics of these crustaceans in consideration of the economic importance. This paper deals with the current fisheries status of these organisms such as target species at each locality, kinds of gears employed by fishermen, the amount of catches, and the processing to make shrimp paste “terasi” on the basis of my field samplings and interview assessments as well as fisheries statistics of the Ministry of Marine Affairs and Fishery, Indonesia.

3.2 Materials and Methods

3.2.1 Sampling

To find out the fishery species of mysids and *Acetes* in Indonesia and species composition in shrimp paste "terasi", samples were obtained either by field sampling or by buying fresh and dried materials and shrimp paste "terasi" at various local markets in Indonesia. Field samplings were carried out by fishermen in December 2008, June and July 2009, and June and July 2010 at fishing grounds in the mouth of rivers, seashores, shallow water, brackish and salt culture ponds, and lakes across Indonesia (Fig. 3-1, Table 3-1). Additional samples examined were provided by local fishermen during other fishing seasons. Some fresh and dried materials and shrimp paste were bought at traditional and modern markets and processing industries.

Fresh samples were fixed with 70% ethanol immediately after capture. In the laboratory, mysids and *Acetes* were identified to species level using a dissecting microscope (Olympus SZ60), following the identification keys of Hanamura *et al*. (2008) and Omori (1975), respectively. Surface water temperatures and salinity were simultaneously recorded at some collection sites with a salinometer (YSI Model 556 MPS).

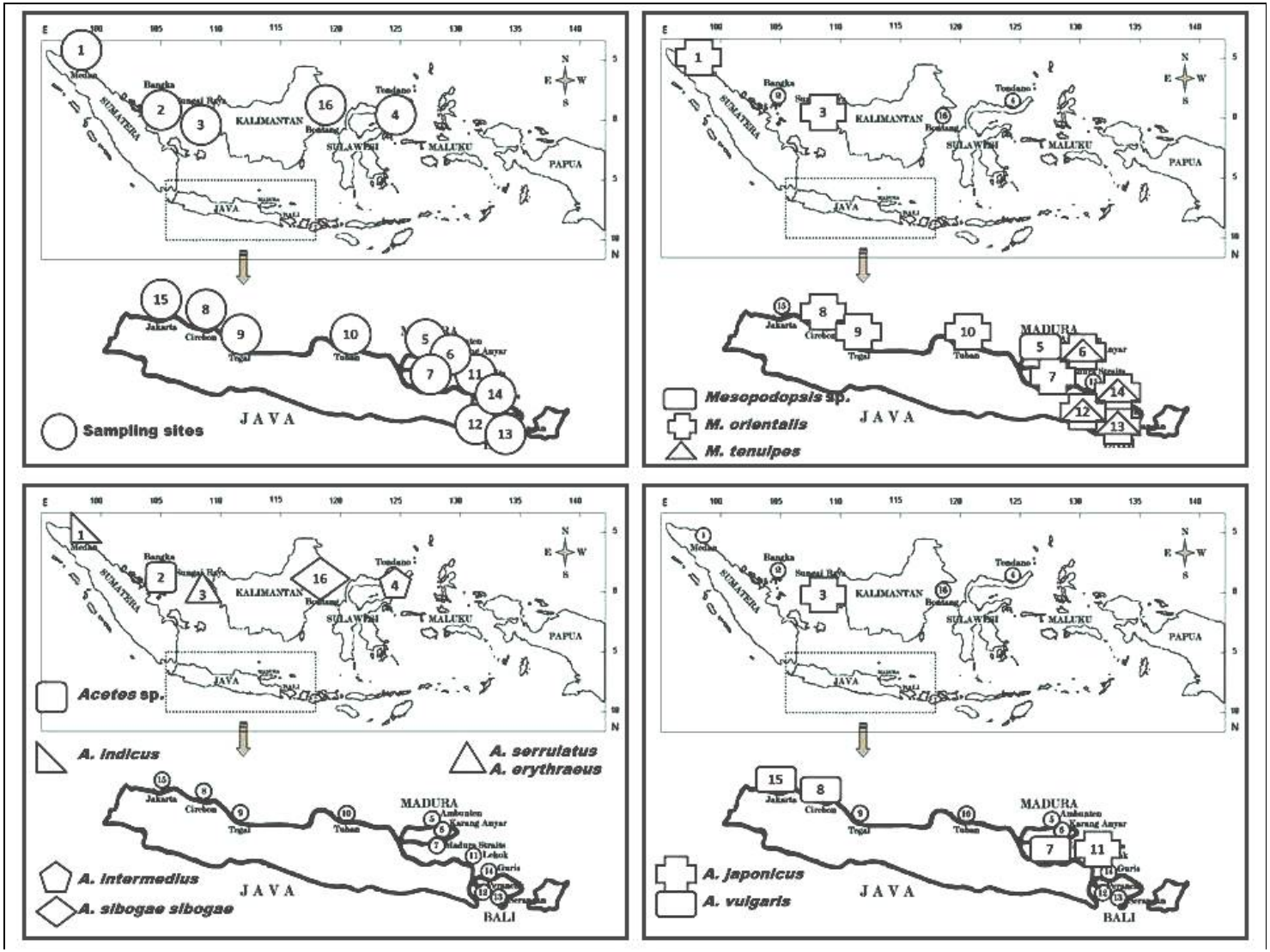

Fig. 3-1. Map of study area and fishery sites for mysid *Mesopodopsis* and shrimp *Acetes* in Indonesia (After Mantiri *et al*., 2012 with permission from Graduate School of Kuroshio Science, Kochi University) (see Table 3-1 for sampling sites name).

Table 3-1. Sampling sites and species of mysid *Mesopodopsis* and shrimp *Acetes* in Indonesia. Abbreviation: A, *Acetes;* M, *Mesopodopsis*.

Sta.	Locality	Date/Time	Remarks	Temp. °C	Sal. ‰	Species	Type of sample
1	Medan, Sumatera Is. 03°40.000' N 98°38.000' E	July 2010 Day time	Market	-	-	*A. indicus* *M. orientalis*	Dried materials
2	Bangka, Bangka-Belitung Is. 02°00.000' S 105°50.000' E	June 2009 Day time	Market	-	-	*Acetes* sp.	Shrimp paste
3	Sungai Raya, Kalimantan Is. 00°42.448' N 108°52.260' E	08 July 2010 04:00	Mouth of river (sandy and muddy bottom)	28.0°	18.0	*A. japonicas* *A. serrulatus* *A. erythraeus* *M. orientalis*	Fresh materials
4	Tondano, Sulawesi Is. 01°35.000' N 124°54.000' E	20 December 2008/04:00	Lake (4,278 ha) Market	23.4°	0.3	*A. intermedius*	Fresh & dried materials
5	Ambunten, Madura Is. 07°30.000' S 114°00.000' E	05 July 2010 Day time	Processing factory	-	-	*Mesopodopsis* sp.	Shrimp paste
6	Karanganyar, Madura Is. 07°38.000' S 113°37.000' E	12 July 2010 07:00	Salt pond (1 ha) Market	27.3°	25.1	*M. orientalis* *M. tenuipes*	Fresh materials Shrimp paste

Table 3-1. Continued.

7	**Madura strait, Madura Is. 07°38.533' S 113.33.417°'E**	**11 July 2010 10:00**	**Shallow seawater Market**	**30.0°**	**32.4**	***A. vulgaris* *M. orientalis***	**Fresh materials Shrimp paste**
8	**Cirebon, Java Is. 06°42.561' S 108°34.310' E**	**05 July 2009 14:00 25 June 2010 05:00**	**Seashore (sandy and muddy bottom) Market**	**30.4°**	**25.3**	***A. vulgaris* *M. orientalis***	**Fresh & dried materials Shrimp paste**
9	**Tegal, Java Is. 06°50.681' S 109°08.506' E**	**03 July 2009 23:00**	**Shallow seawater (sandy and muddy bottom)**	**29.8°**	**30.4**	***M. orientalis***	**Fresh materials**
10	**Tuban, Java Is. 06°51.435' S 112°01.665' E**	**01 July 2009 13:00**	**Seashore (sandy and muddy bottom)**	**27.9°**	**29.8**	***M. orientalis***	**Fresh materials**
11	**Lekok, Java Is. 07°39.360' S 112°59.175' E**	**30 June 2009 Day time**	**Shallow seawater (sandy and muddy bottom) Home industry**	**29.2°**	**29.2**	***A. japonicus***	**Dried materials Shrimp paste**
12	**Perancak, Bali Is 08°24.361' S 109°08.506' E**	**03 June 2010 19:00**	**Shrimp brackish culture pond (2500m²)**	**28.0°**	**25.6**	***M. orientalis* *M. tenuipes***	**Fresh materials**

Table 3-1. Continued.

13	**Serangan, Bali Is. 08°50.681' S 115°14.575' E**	**01 June 2010 05:00**	**Shrimp brackish culture pond (2500m²)**	**26.1°**	**26.3**	***M. orientalis M. tenuipes***	**Fresh materials**
14	**Guris, Bali Is. 08°25.556' S 114°34.420' E**	**04 June 2010 18:00**	**Fish brackish culture pond (5000m²)**	**28.3°**	**26.2**	***M. orientalis M. tenuipes A. vulgaris***	**Fresh materials**
15	**Jakarta, Java Is. 06°09.000' S 106°49.000' E**	**June 2009 Day time**	**Coastal area, brackish pond Market**	**28.0°**	**25.9**	***M. orientalis***	**Dried materials Fresh materials**
16	**Bontang, Kalimantan Is. 00°10.000' N 117°30.000' E**	**June 2010 Day time**	**Coastal area Market**	**30.0°**	**31.4**	***A. sibogae sibogae Acetes* sp.**	**Dried materials Shrimp paste**

3.2.2 Interview assessments and fisheries statistics

To describe the mysids and *Acetes* fisheries and processing procedures, I tried to obtain data during a 3-year investigation as accurate information as possible from 17 interviewees from different fields and localities such as governmental officers, owners of processing factories, fishermen, teachers and local people (Table 3-2). Inquiries were fishing grounds, fishing seasons and time, fishing gears, prices (fresh and dry organisms, shrimp paste), food process, and environmental conditions. I visited a home factory in Lekok, Java (Sta. 11) on 30 June 2009 and a traditional processing factory in Ambunten, Madura, Java (Sta. 5) on 5 July 2010. The factory owners and fishermen answered my inquiries concerning fisheries of mysids and *Acetes*, and showed how they collected these pelagic crustaceans and processed them to produce shrimp paste. Additional interviews were carried out with several residents living near fishing grounds and officers belonging to the regional offices of marine affairs and fisheries in Lekok, Bali, and Kalimantan. Since the present study largely depended on comments of interviewees, I tried to obtain as accurate information as possible from other people during the 3-year investigation. In this study, currency conversion from Indonesian Rupiah (IDR) to US dollar (US$) were made to express the economic aspects using a factor of US$1=IDR 9,000.

The Ministry of Marine Affairs and Fisheries Indonesia provided me with official data concerning the amount and price of mysids and *Acetes* products. The owner of a traditional processing factory in Madura and a fisherman in Kalimantan (Sta. 3) also gave me some additional information about the amount of catch. There is no detailed and complete statistics available at the national level and it is not recorded in FAO statistics. It is difficult to collect such data because there is no awareness from local people or fishermen.

Table 3-2. People for interviews on fisheries of mysids and *Acetes*.

Sta.	Interviewees	Office Name/Address	Position	Inquiries
1	(a) Mr. Simanjuntak	Medan, Sumatera Is.	Local people	Fishing grounds
2	(b) Mr. Ucok	Bangka Is.	Local people	Fishing grounds
3	(c) Mr. Kris Handoko, A.Pi.,MT	Regional Office for Marine and Coastal Resmyce Management, Pontianak Directorate General of Marine Coastal and Small Islands Affairs Ministry of Marine Affairs and Fisheries, Republic of Indonesia Jl. Sultan Abdurrachman No 115 Pontianak Kalimantan Barat	Head of Conservation and Utilization Section & researcher	Environmental condition Fishing grounds Fishing seasons & time Fishing gears Price of fresh organisms

Table 3-2. Continued.

3	**(d) Mr. Muchtar**	**Sungai Raya, Kalimantan Is.**	**Fisherman**	**Fishing grounds Fishing seasons & time Fishing gears Price of fresh organisms**
4	**(e) Mr. Lukas Pelengkahu, alm.**	**Tondano, Sulawesi Is.**	**Local people**	**Fishing grounds Fishing seasons & time Fishing gears**
5	**(f) Mr. Ahmad**	**Traditional shrimp paste (terasi) processing factory Jl. Raya Ambunten, Sumenep, Madura**	**Owner**	**Fishing grounds Fishing seasons & time Fishing gears Prices (fresh & dry organisms, shrimp paste) Food process**
5	**(g) Mr. Abdu Samad Ahmady**	**Sumenep, Madura Is.**	**School teacher**	**Fishing grounds Fishing seasons & time Fishing gears Food process**
5-7	**(h) Mr. Novianto Samad**	**Sumenep, Madura Is.**	**Promoter of shrimp paste**	**Price of shrimp paste**
8	**(i) Mr. Ismail**	**Mataram, Lombok Is.**	**Local people**	**Fishing grounds**

Table 3-2. Continued.

8	**(j) Mr. Udin**	**Padak Guar, Lombok Is.**	**Fisherman**	**Fishing grounds Fishing seasons & time Fishing gears**
9	**(k) Mr. Triyoga**	**Cirebon, Java Is.**	**Head of High schools**	**Fishing grounds Fishing seasons & time Fishing gears**
9	**(l) Ms. Ijah**	**Cirebon, Java Is.**	**Shrimp paste maker & Fisherwoman**	**Fishing grounds Fishing seasons & time Fishing gears Food process**
12	**(m)Mr. Asep S**	**Local Office for Ministry of Marine Affairs and Fisheries, Lekok Jl. Raya Lekok, Pasuruan, Jawa Timur**	**Field instructor**	**Fishing grounds Fishing seasons & time Fishing gears**
12	**(n) Ms. Sarmini**	**Home industry for shrimp paste (terasi) Jl. Tambak, Lekok Pasuruan**	**Owner**	**Fishing grounds Fishing seasons & time Fishing gears Prices (fresh & dry organisms, shrimp paste) Food process**

Table 3-2. Continued.

13	**(o) Mr. Komang**	**Local Office for Ministry of Marine Affairs and Fisheries, Southeast Asia Center for Ocean Research and Monitoring (SEACORM)** **Perancak, Jembrana Bali Barat**	**Field instructor**	**Fishing grounds** **Fishing time** **Fishing gears**
14	**(p) Mr. I made**	**Serangan, Bali Is.**	**Seahorse culturist & Public organisator**	**Fishing grounds** **Fishing time** **Fishing gears** **Prices (fresh organisms)**
15	**(q) Mr. Saleh**	**Regional Office for Ministry of Marine Affairs and Fisheries, Bali** **Jl. Pattimura No.77 Denpasar, Bali**	**Staff & researcher**	**Fishing grounds** **Target organisms**

3.3 Results and Discussion

3.3.1 Fishing grounds and gears

Mysids and *Acetes* were distributed on sandy and muddy bottoms of shallow and calm waters, at river mouths, and brackish water culture ponds (Fig. 3-2). They were mainly caught from the shoreline at depths of 10–20 m, along the coast. Brackish and salt ponds are mostly utilized for fisheries of mysids. In Bali, brackish ponds (Sta. 12–14 in Table 3-1) were occupied mostly by *Mesopodopsis orientalis*. This species occurred at high abundance in salt ponds in Madura (Sta. 6), whereas *Acetes* preferably occurred at shallow seawaters and lakes. The fishing grounds were located in the innermost parts of the sea, lakes and ponds because these organisms tended to aggregate near the edges of these grounds. The surface water temperature ranged from 23.4°C in December (Sta. 4) to 30.4°C in July (Sta. 8), and salinity from 0.3 in the lake Tondano (Sta. 4) to 32.4 in the shallow seawater of the Madura Strait (Sta. 7). The transparency at all stations was about 1 m and mysid swarms could be seen at most locations. In the previous studies (Djajadiredja and Sachlan, 1956; Omori, 1975, 1978; Mauchline, 1980; Christensen, 1983; Xiao and Greenwood, 1993; Chan, 1998), these edible crustaceans were also collected from mangrove swamps and brackish culture ponds of penaeid shrimps, where the salinity fluctuated seasonally, ranging from 1.5–35.0 (Chan, 1998), and the tidal range was considerable (Omori, 1975).

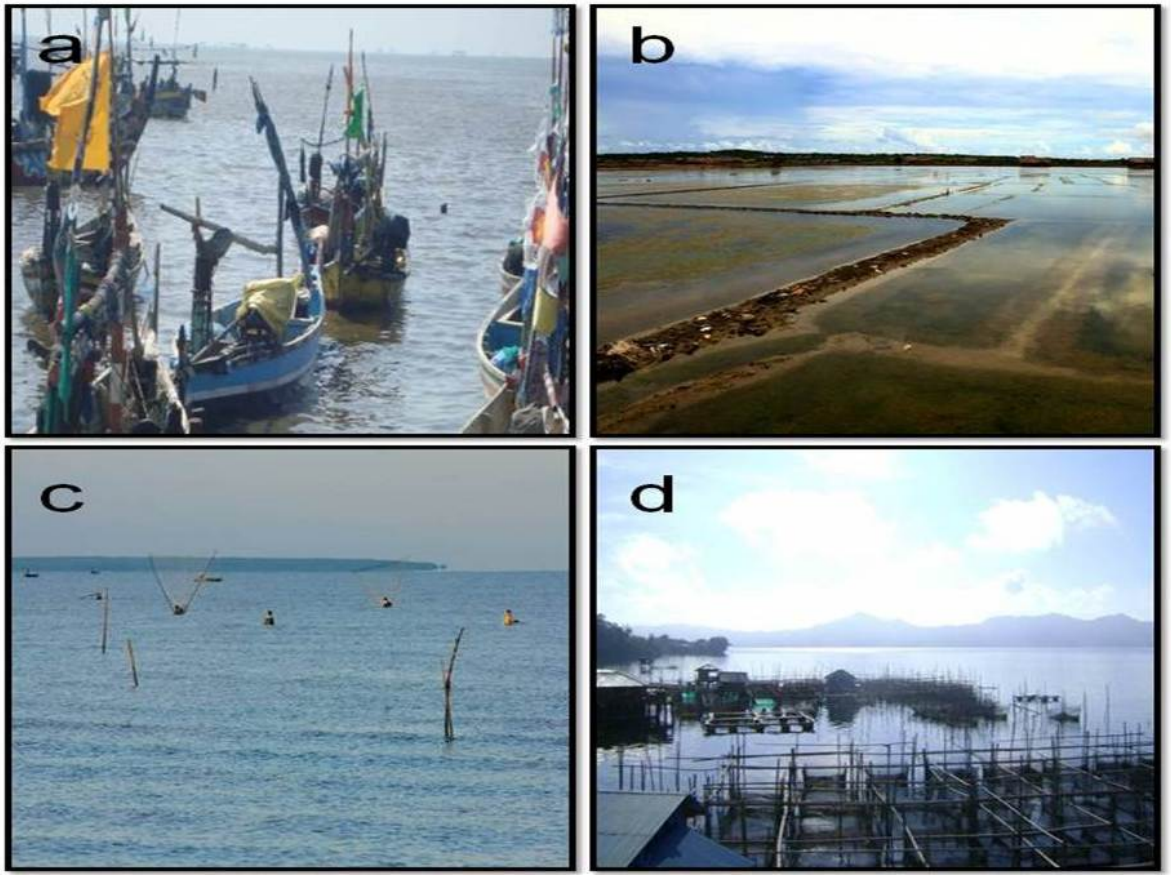

Fig. 3-2. Some fishing grounds of mysid *Mesopodopsis* and shrimp *Acetes* in Indonesia (After Mantiri *et al*., 2012 with permission from Graduate School of Kuroshio Science, Kochi University). a) Shallow seawater (sandy and muddy bottoms) of Lekok, East Java (Sta. 11), b) Salt pond (10000m^2 = 1 ha in Table 3-1) of Karanganyar, Madura Is. (Sta. 6), c) Mouth of river (sandy and muddy bottom) of Sungai Raya, West Kalimantan (Sta. 3), and d) Lake Tondano (4,278 ha) North Sulawesi (Sta. 4).

Fishing gears for mysids and *Acetes* varied depending on the types of fishing grounds (Fig. 3-3, Table 3-3). For example, boat-seine was used in deeper water at 10–20m depths and push-net was employed at seashore, whereas lift-net and scoop-net with a long handle were utilized in brackish ponds close to seashore and in lakes at a depth of 6m. The fishing gears for these organisms were relatively simple and usually operated manually.

Lift-net (Fig. 3-3a) called "bagan" usually needed a light to attract mysids and *Acetes* at night. It was used in shallow waters and brackish ponds of Java (Sta. 8-11), Madura (Sta. 5, 7), Bali (Sta. 12-14), and in lakes of Sulawesi (Sta. 4). "Bagan" was used for the first time in South Sulawesi by Makassar and Bugis fishermen in the early 1950s (Hakim, 2010) and then spreads widely in Java, Sumatra and other places (Table 3-2 : m). The size of this gear varied depending on the place where it was set up. In Pelabuhan Ratu, west Java, it was set at a depth of 10–20 m with the net size about 20 m long and 8 m wide (Omori, 1975). In Bali, "bagan" was set at the edge of a pond, around 1 m deep with a net size of 1 m x 1 m and a mesh size of 3 mm (present study).

A boat-seine or surrounding net (Fig. 3-3b), called "pajeng bering" or "odeng mayangan" in Madura, was used in deeper waters about 1 km from the shoreline or at a depth of 10–20 m in Java (Sta. 8–11) and Madura (Sta. 5, 7). The size of boat seine was 40 m in length and 2 m in width with a mesh size of 3 mm.

To operate the nets, usually fishermen employed one or two boats, but nowadays they have used only one boat due to high prices of fuel. The nets with buoys were dropped into the water and the boat started to move ahead in a circular path. After completing the circle in less than 5 minutes, the fishermen retrieved the buoy in order to take aboard the net. The bottom of the net was gradually being drawn tight so that the organisms were kept still inside the net.

Push-net (Fig. 3-3c) was used in seashores of Java (Sta. 8–11), Madura (Sta. 5), and Kalimantan (Sta. 3). In Sungai Raya, west Kalimantan a single type of gear, push-nets was in use by fishermen to catch mysids and *Acetes*. Push-nets, called "sudu" in Kalimantan and "odeng sottal" in Madura, were operated at seashore by one or two persons who are capable to push the net in the water against the flow of tides. The gear was 3 m long and 2 m wide with mesh sizes of 1 mm.

Scoop-net (Fig. 3-3d,e), called "serok", had two types with a long or short handle, and was used depending on the types of fishing grounds. For example in Madura (Sta. 5), scoop-net with a long handle called "odeng soddu" was used in the middle area of seawater where the water reached around the neck of an adult. These nets were also used in most brackish ponds in Java (Table 3-2 : m) and Bali (Sta. 12–14), and in salt ponds of Madura (Sta. 6) to catch the crustaceans at the middle part of the pond. On the other hand the short-handle net was used just near

the edge of the brackish (Sta. 12–14) and salt ponds (Sta. 6) and in lake Tondano of North Sulawesi (Sta. 4). Scoop-nets were operated easily by dragging or pulling in the water using the handle. The size of the long-handle net was 2 m in length and 30 cm in diameter with mesh sizes of 3 mm, whereas the short-handle net was 2 m in length and 30 cm in diameter with mesh sizes of 1 mm.

In other Southeast Asian countries, similar types of fishing gears were used by fishermen for catching mysids and *Acetes* (Omori, 1975; Xiao and Greenwood, 1993; Jadhav and Josekutty, 2003; Paul and Josekutty, 2005; Dineshbabu *et al.*, 2006). In China, larger set-nets called "hole-in-belly" are commonly employed (Xiao and Greenwood, 1993).

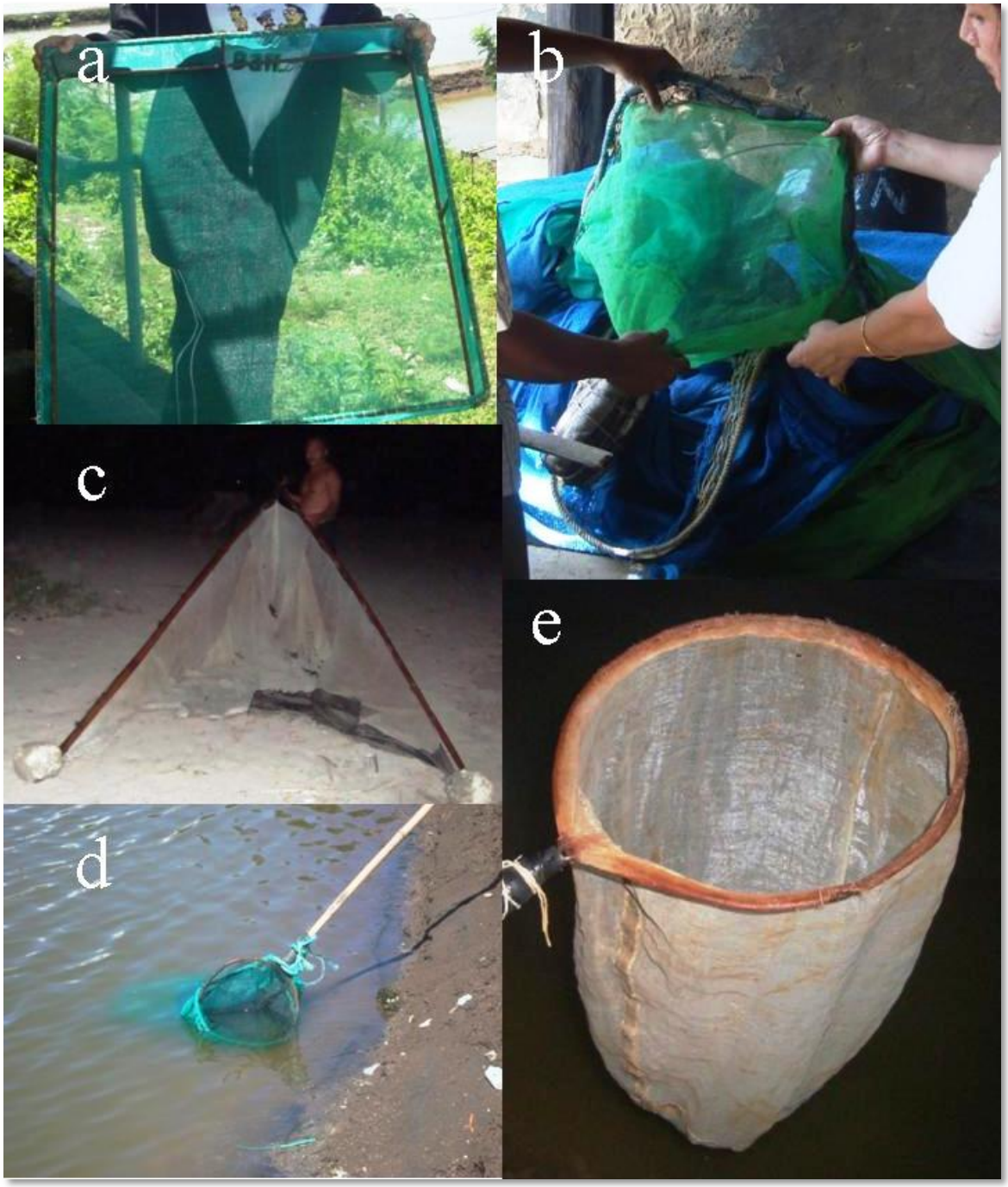

Fig. 3-3. Fishing gears of mysid *Mesopodopsis* and shrimp *Acetes* in Indonesia (After Mantiri *et al*., 2012 with permission from Graduate School of Kuroshio Science, Kochi University) . a) Lift-net (Sta. 4–14), b) Boat-seine (Sta. 5, 7–11), c) Push-net (Sta. 3 and 5–11), d) Scoop-net with long handle (Sta. 5–14), and e) Scoop-net with short handle (Sta. 4–14).

Table 3-3. Descriptions of fishing gears for mysid *Mesopodopsis* and shrimp *Acetes* in Indonesia.

Gear	Local name	Size	Type of fishing ground	Locality (Sta.)
Lift-net	"Bagan"	Mesh size : 3 mm Net length : 1 m Net width : 1 m	Brackish culture pond Shallow water 6-20m depth Lake	Java (8-11), Madura (5, 7), Bali (12-14), Sulawesi (4)
Boat-seine / surrounding net	"Pajeng bering" (Madura)	Mesh size : 3 mm Net length : 40 m Net height : 2 m	Shallow water 10-20m depth	Java (8-11), Madura (5, 7)
Push-net	"Odeng sottal" (Madura) "Sudu" (Kalimantan)	Mesh size : 1 mm Net length : 3 m Net width : 2 m	Seashore < 1m depth	Java (8-11), Madura (5), Kalimantan (3)
Scoop-net with long handle	"Odeng soddu" (Madura) "Serok" (Bali)	Mesh size : 3 mm Net length : 2 m Net diameter : 30 cm	Brackish culture pond Seashore 6m depth	Java (8-11), Madura (5-6), Bali (12-14)
Scoop-net with short handle	"Odeng soddu" (Madura) "Serok" (Bali)	Mesh size : 1 mm Net length : 30 cm Net diameter: 30 cm	Brackish culture pond Lake	Java (8-11), Madura (6), Bali (12-14), Sulawesi (4)

3.3.2 Fishing season and time

The fishery species of pelagic crustaceans in Indonesia and the season and the time of fishing are summarized in Table 3-1. *Acetes* was caught to a certain amount throughout the year in Indonesia, although its peak fishing season varied depending on the location of the fishing grounds and the alternation of the West and East monsoons (Table 3-2 : q). During my collection, *A. japonicus* was fished in shallow water of Lekok (Sta. 11) in June 2009, *A. vulgaris* at seashore of Cirebon (Sta. 8) in June 2010 and *A. japonicus, A. serrulatus*, and *A. erythraeus* at the mouth of the river in Kalimantan (Sta. 3) in July 2010. According to the owner of a processing factory and local people in Madura, June and July were not good seasons for fishing *Acetes* in Ambunten, Madura (Sta. 5), because of the strong wind at the sea, while February-March was the best for these crustaceans.

There is no exact information regarding the peak season of mysid fisheries in Indonesia. During my collection in July 2009, *Mesopodopsis orientalis* was caught at seashore of Cirebon (Sta. 8). In June 2010, swarms of this species and *M. tenuipes* were found in brackish culture ponds in Bali (Sta. 12–14) and in July 2010 in a salt pond in Madura (Sta. 6). According to local people these organisms were available in brackish and salt ponds throughout the year (Table 3-2 : h).

Fishing seasons for *Acetes* are different at localities in every country and are largely determined by monsoons (Omori, 1975; Xiao and Greenwood, 1993). For

example, in Thailand, the fishing season in the Gulf of Thailand is determined by southwest and northeast monsoons, whereas in other localities fishing takes place throughout the year (Xiao and Greenwood, 1993). The main fishing season in Pelabuhan Ratu (west Java, Indonesia) was in the transitional period between the West and East monsoons, from the end of March to June, while fishing was not carried out off Pelabuhan Ratu during the West monsoon, from December to February, because of strong landward winds (Omori, 1975). Djajadiredja and Sachlan (1956) stated that in Indonesia, the availability of *Mesopodopsis* and *Acetes* coincided with periods of high tides during the months of April–June and November–January. The fishing season for *Acetes* was during September to October in Murdeswar Bay of India (Dineshbabu *et al.*, 2006). Dineshbabu *et al.* (2006) stated that the regularity and success of the fishery depends on the magnitude and patterns of the currents. In Mumbai, India, the fishing season of *Mesopodopsis zeylanica* and *M. orientalis* was during April to May (Jadhav and Josekutty, 2003; Paul and Josekutty, 2005).

Fishing time for *Mesopodopsis* and *Acetes* was either day or nighttime at high tides. In the present study, the best time for fishing was during darkness, either at dusk or dawn. In Kalimantan, fishing time started from dawn, around 04:00 until 10:00 am. According to Mr. Made (Table 3-2 : p) who run a seahorse aquaculture, in Bali fishing time for *M. orientalis* and *M. tenuipes* in brackish

culture ponds was usually at dawn around 04:00. He usually buys mysids from the fishermen at around 05:00. According to fishermen and local people in Cirebon (Table 3-2 : l) and Madura (Table 3-2 : g), at the peak season, fishermen went fishing at daytime whenever they saw the water color turned to reddish or brownish indicating mysid swarms. The fishing activity was carried out by fisherman's family members, especially for fishing in shallow waters without a boat.

3.3.3 Catches and species

The catches and values of a mixture of mysids and *Acetes* ("rebon") fluctuated year-by-year (Table 3-4 and 3-5). There was much greater annual catch in the sea in 1954 than in brackish ponds during 1999–2005. The catch of "rebon" in Madura (Madura Strait; Sta.7) was the highest among all locations at the study sites where 50 kg/day were yielded. Madura Island has a reputation for a long tradition of shrimp paste "rebon" preparation. According to the owner of a traditional processing factory in Madura (Sta. 5), the catch in a peak fishing season from February to March was about 4 tons/day with a value of IDR 15,300,000/ton (1,700 US$/ton). The catch in Sungai Raya, east Kalimantan (Sta. 3) from March to August was 20 kg/day on the average (Table 3-2 : d), and the catch in other sites (Sta. 6, 8–10, 12–14) was less than 1 kg/day.

Table 3-1 shows the fisheries species belonging to mysids and *Acetes* in Indonesia found in the present study. No dried or fresh mysids were found to be on sale alone without being mixed with *Acetes*. A small number of the mysid *Mesopodopsis orientalis* were found to be sold being mixed with *A. vulgaris* in my collection of fresh "rebon" from Cirebon (Sta. 8), whereas in Sungai Raya, east Kalimantan (Sta. 3) *M. orientalis* were found to be sold being mixed with *A. japonicus*, *A. serrulatus*, and *A. erythraeus* in 2010. *Acetes vulgaris* was also found being mixed with *M. orientalis* in my collection of fresh "jembret" from Cirebon (Sta. 8) in 2009.

Acetes vulgaris was identified from dried materials (as "rebon") bought at supermarkets in Jakarta (Sta. 15), and *A. japonicus* in the collection of dried materials provided by the owner of a home factory in Lekok (Sta. 11) in 2009. In 2010, *A. sibogae sibogae* was identified from dried materials from Bontang, east Kalimantan (Sta. 16), and in my collection of dried materials from Medan (Sta. 1) *M. orientalis* and *A. indicus* were found being mixed together (Fig. 3-4a). *Mesopodopsis tenuipes* was also mixed with *M. orientalis* at Sta. 6 and 12–14.

I identified *Acetes* sp. mixed with *Mesopodopsis* sp. in the composition of "terasi" from the Madura Strait (Sta. 7). In the composition of "terasi" I found *Acetes* sp. from Bangka, Sumatra (Sta. 2) and from Bontang, Kalimantan (Sta. 16). I could not identify them at species level, due to fragmentation of specimens. Since

I could identify the intact specimen at its material, I found that “terasi” from Madura (Sta. 6, 7) was exclusively composed of *M. orientalis* (Fig. 3-4b, c).

Table 3-4. Annual catch and value of mysid *Mesopodopsis* and shrimp *Acetes* ("rebon") in Indonesia.

DATA	1954*	1999**	2000**	2001**	2002**	2003**	2004**	2005**
Production (metric ton)	1,826	90	544	610	415	700	315	172
Value (US$)	328.7	96.3	1,867.7	332.7	499.1	463.3	266.8	230.2

***Rebon from the sea, based on Djajadiredja and Sachlan (1956).**
****Rebon from brackish ponds, based on Ministry of Marine Affairs and Fisheries Indonesia interview (2010).**

Table 3-5. Local annual catch (ton) and value (USD) of mysids and *Acetes* ('rebon') in Indonesia.

Locality	1954*	1999**	2000**	2001**	2002**	2003**	2004**	2005**
Sumatera :								
Nangro Aceh Darussalam,		-	-	-	207 / 350. 0	-	-	-
Lampung		-	430 / 1,194.2	479 / 183.8	-	-	-	-
Java :								
Banten,								
West Java,	29 / 6.7	-	-	-	-	-	-	8 / ?
Central Java,	515 / 89.6	-	-	-	1 / 1.4	-	-	-
East Java	193 / 33.4	6 / 2.4	22 / 63.7	67 / 66.3	187 / 122.4	131 / 94.8	82 / 66.5	111 / 125.7
Madura Island	1,089 / 199.0	-	-	-	-	-	1 / 1.1	42 / 92.2
Bali & West Nusa Tenggara	-	-	-	2 / 1.3	20 / 25.2	177 / 24.9	-	-
Kalimantan :								
West Kalimantan	-	-	-	-	-	-	89 / ?	-
East Kalimantan	-	-	-	-	-	-	-	-
Sulawesi:								
South Sulawesi		84 / 93.8	92 / 100.6	62 / 81.3	-	129 / 114.2	-	-

* Rebon from the sea, based on Djajadiredja and Sachlan (1956).

****Rebon from brackish ponds, based on Ministry of Marine Affairs and Fisheries Indonesia interview (2010).**

Fig. 3-4. Some examples of "terasi" sold in Indonesia (After Mantiri *et al*., 2012 with permission from Graduate School of Kuroshio Science, Kochi University). a) Materials for terasi (Sta. 1), dried *A. indicus* mixed with *M. orientalis*, b) & c) pictures of mysid fragments in the shrimp paste (terasi) made of *M. orientalis* only (Sta. 6), d) & e) samples of shrimp paste (terasi) made of "rebon" (Sta. 2 & 8).

3.3.4 Utilization and processing of mysids and *Acetes*

In Indonesia, several types of "rebon" were usually on sale at markets; raw or fresh materials, dried in the sun materials, and fermented with salt (shrimp paste "terasi" and shrimp sauce "petis"). Most of the products were sold at modern markets as dried materials and shrimp paste ("terasi"), while fresh materials were found only at a traditional market in Cirebon in June 2010 (Sta. 8). "Terasi" is very popular in Indonesia, because it gives more taste and strong flavor to cooked food. Most people especially in Java use "terasi" for making "sambal terasi" (a chili sauce with terasi) and eat it with raw vegetables and fried chicken/fish, because it makes a good appetite. According to Omori (1975), dried *Acetes* and fermented shrimp paste and shrimp sauce made of *Acetes* are highly desired by people in Asian countries.

During my study, there were three types of shrimp paste, i.e. (1) a mixture of mysids and *Acetes*, (2) exclusive of *Acetes*, and (3) mysids alone. According to the owners of "terasi" home factory in Lekok (Sta. 11) and a "terasi" traditional processing factory in Ambunten, Madura (Sta. 5), the main material for making terasi was *Acetes* but was partly mixed with mysids. However, in Madura (Sta. 5) if *Acetes* is unavailable for making terasi, then they produce "terasi" from the mysid *M.*

orientalis and *M. tenuipes* taken from salt ponds (Sta. 6) or *M. orientalis* from the Madura Strait (Sta. 7). The owner of the traditional processing factory further said that "terasi" made of mysids was tastier than if made of *Acetes* alone.

Fig. 3-5 shows the manufacturing process of "terasi" based on the explanation of the owners of a "terasi" home factory in Lekok (Sta. 11) and a "terasi" traditional processing factory in Madura (Sta. 5). The process of fermentation, crashing, and drying can be repeated 2 or 3 times before pressing it into a hard mass, to get a good "terasi". The product remains in good condition for a long time. The longer it is kept the better it tastes.

3.3.5 Economical aspects

The selling price at markets for "rebon" and "terasi" varied depending on the quality and the locality of the products. Usually the price at traditional markets was different according to the localities. Dry and fresh "rebon" were sold at markets for IDR 15,000/ kg (US$ 1.7/ kg) in Sumenep, Madura (Sta. 5), IDR 10,000/ kg (US$ 1.1/ kg) in Medan (Sta. 1), Cirebon (Sta. 8) and Jakarta (Sta. 15), and IDR 5,000/ kg (US$ 0.6/ kg) in Tondano (Sta. 4), Lekok (Sta. 11) and Bontang (Sta. 16).

The selling price of “terasi” of high quality was IDR 30,000/ kg (US$ 3.3/ kg) in Sumenep, Madura (Sta. 5) and IDR 40,000/ kg (US$ 4.4 /kg) in Bangka (Sta. 2, Fig. 3-4d). On the other hand, the price for low quality of “terasi” was IDR 15,000/ kg (US$ 1.7/ kg) in Madura, IDR 20,000/ kg (US$ 2.2/ kg) in Bangka, and IDR 3,000–4,000/ kg (US$ 0.3–0.4/ kg) in Pelabuhan Ratu, Sukabumi, Bogor, Cianjur and Cirebon. At modern markets “terasi” was only sold in small sachets or in one pack containing 20 sachets; for Mama Suka product (Fig. 3-4e) the price was IDR 10,000/ pack of 20 sachets (US$ 1.1/ pack).

Growing demands for commodities made of mysids and *Acetes* seem to have been getting remarkable in Indonesia. For example, the economical importance of the crustacean products in Berau, east Kalimantan, has been increasing, because of exporting “terasi” to Lombok and other places (Kaltim Post, 2010). “Terasi” exported to Lombok by one factory owner approximately reached up to about 400 tons/month, corresponding to the value of IDR 50,000,000 (US$ 5,555.6). Table 3-4 and 3-5 show that the production from the sea was higher than from the brackish ponds. However, these data seemed to be too insufficient in consideration of my interview in the present study (see “Catches and species”). According to Hutomo *et al.* (2009) or more

recent and reliable data, the total marine capture production in Indonesia in 2007 was about 4.73 million tones with estimated value of IDR 48.4 trillion (US$ 53,777,778). Unfortunately the data did not separate between the respective percentages of mysids and *Acetes*.

Since mysids and *Acetes* are important organisms in coastal and estuarine food webs at lower trophic levels (Omori, 1975; Mauchline, 1980), these crustaceans can influence the production of marine animals at higher trophic level. As mentioned in the introduction, these crustaceans are economically important for fisheries in Asian countries. Unfortunately fisheries statistics are not sufficient in many southeastern Asian countries, which makes a sustainable management difficult. A long-term monitoring of catches of these marine organisms is urgently required in order to maintain sustainable catches from coastal waters.

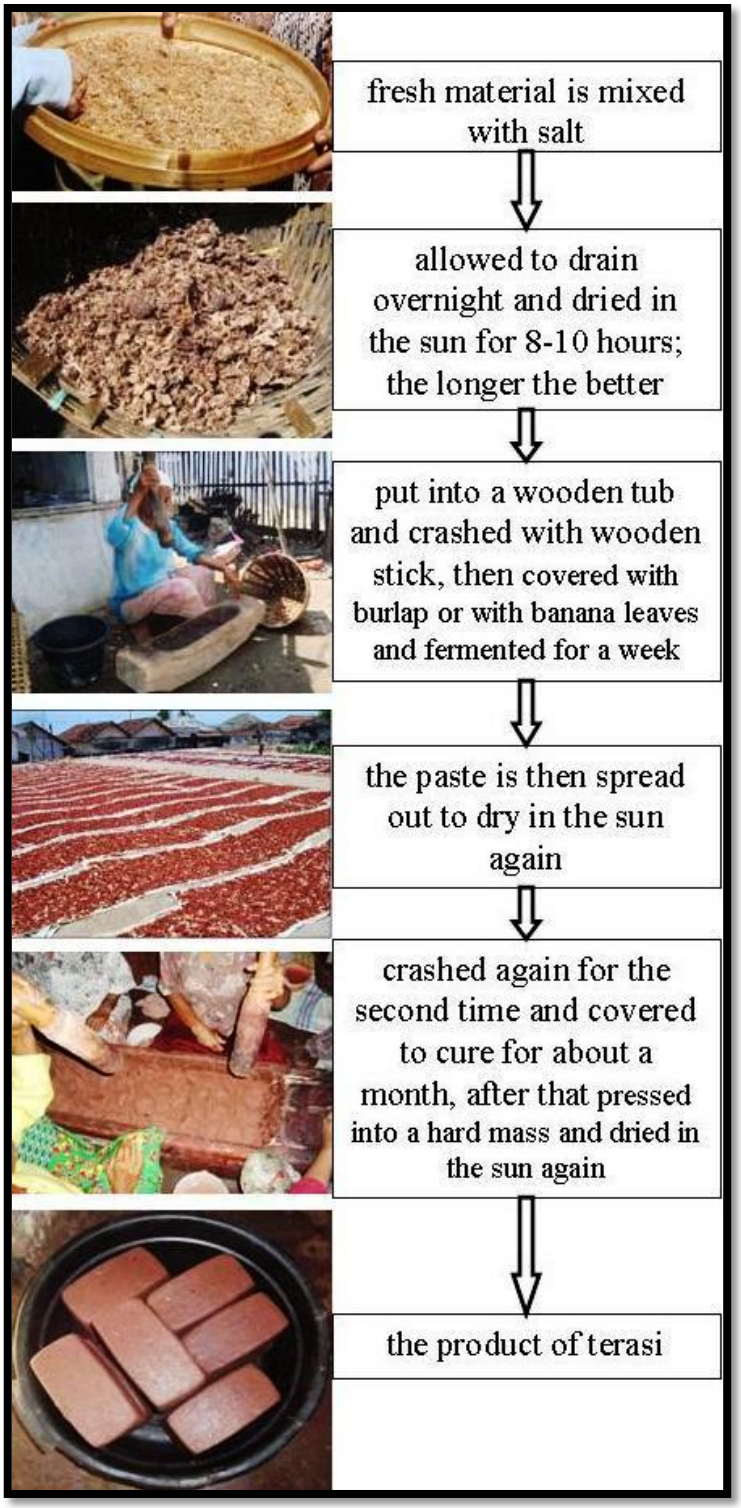

Fig. 3-5. The process of how to make shrimp paste ("terasi") in Indonesia (Sta. 5, 11; Table 3-2: h, n) (After Mantiri *et al*., 2012 with permission from Graduate School of Kuroshio Science, Kochi University).

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Chapter 4

GENERAL DISCUSSION

In Indonesia the mysid *Mesopodopsis orientalis* is an economically important species for fisheries along with the pelagic shrimp *Acetes*. In addition it must be ecologically important, due to the extremely high abundance in the southwestern Asian waters including Indonesia (Hanamura *et al.*, 2008; Mantiri *et al.*, unpublished). *Mesopodopsis orientalis* has been so far recorded from India, Singapore, Indonesia, Malaysia, Thailand, and the Philippines, and been suggested to be highly differentiated in these waters (Hanamura *et al*., 2008; unpublished) as proposed for other crustaceans (Fleminger, 1986; Bruyn *et al.*, 2004). Since Wallacea and its neighbouring waters are considered to have caused remarkably genetic differentiations of coastal and brackish organisms (Fleminger, 1986), such evolutionary scenarios may be applicable to the differentiation of *M. orientalis*.

I have first succeeded in detecting the genetic diversity in the Indonesian populations of *M. orientalis* in this study, and suggested a presumed differential pattern due to the last transgression after the end of the Pleistocene. I proposed that the ancient river systems located between Borneo and Java Islands seem to have played an important role in the genetic differentiation (see Voris, 2000; Sathiamurthy and Voris, 2006). Since it has a restricted distribution in brackish waters in the present, its newly colonized populations might have been more

strongly and rapidly isolated from others than in coastal organisms, causing the relatively rapid divergence. More extensive genetic analyses of *M. orientalis* collected from other Indonesian waters may enhance my hypothesis.

For sustainable utilization of fisheries targets we have to employ two strategies: (1) annual catch must be regulated in consideration of the abundance of a target species; and (2) easy introduction of a different population into a new wild habitat should be prohibited. Fisheries statistics of pelagic shrimps and mysids in Indonesia should be annually prepared, but no new information has been available since 2006. Since live specimens of *M. orientalis* are utilized as a prey item for aquaculture of fish such as sea horses in tropical Asian waters (Mantiri, personal observation; Khwanruan Srinui, personal communication), it is likely that it is accidentally introduced into a new habitat. In this case we must think of its genetic diversity and avoid such accidental introduction, because each haplotype is adapted to its original habitat. Since predominant mysids are regarded as a bioengineer (Roast *et al*., 2004), a newly colonized population of mysids might become an invasive alien to cause drastic changes in the ecosystem like *Limnomysis benedeni* in the European waters (Semenchenko *et al*., 2007).

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Appendix 1. Variable nucleotide positions observed in the 458-base-pair segment of the mitochondrial COI gene of the *Mesopodopsis orientalis* and *Mesopodopsis tenuipes* in Malaysia and Thailand (Hanamura *et al.*, 2008b; Chapter 2).

Haplotype (no. individuals)		Nucleotide position																																							
		6	9	13	15	21	24	27	30	31	33	36	39	42	45	48	51	54	64	66	78	81	84	87	90	96	102	108	111	114	117	123	126	133	135	138	141	144	147	150	153
1	MOMC01(23)	A	G	C	A	A	C	T	G	A	C	C	T	A	T	T	T	G	A	T	T	G	G	A	T	A	T	T	G	C	A	G	A	T	A	T	G	T	C	A	A
2	MOMC02(20)	·	·	T	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·
3	MOMC03(1)	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·
4	MOMC04(1)	T	·	T	·	·	·	·	·	·	T	·	·	G	·	·	·	·	·	·	·	·	·	G	·	·	C	·	·	T	·	·	T	·	·	·	·	·	·	G	·
5	MOMC05(1)	·	·	T	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·
6	MOTC01(2)	T	T	T	·	G	·	·	A	·	T	T	C	C	C	A	·	T	·	·	·	·	·	G	·	·	C	·	T	T	·	A	T	C	·	·	T	·	·	G	T
7	MOTC02(2)	T	T	T	·	G	·	·	A	·	T	T	C	T	C	A	·	T	·	·	·	·	·	G	·	·	C	·	T	T	·	A	T	C	·	·	T	·	T	G	T
8	MOTC03(1)	T	T	T	·	G	·	·	A	·	T	T	C	C	C	A	·	T	·	·	·	·	·	G	·	·	C	·	T	T	·	A	T	C	·	·	T	·	T	G	T
9	MOTC04(1)	T	T	T	·	G	·	·	A	·	T	T	C	C	C	A	·	T	·	·	·	·	·	G	·	·	C	·	T	T	·	A	T	C	·	·	T	·	T	G	T
10	MOTC05(1)	T	T	T	·	G	·	·	A	·	T	T	·	C	C	A	·	T	·	·	·	·	·	G	·	·	C	·	T	T	·	A	T	C	·	·	T	·	T	G	T
11	MOTC06(1)	T	T	T	·	G	·	·	A	·	T	T	C	C	C	A	·	T	·	·	·	·	·	G	·	·	C	·	T	T	·	A	T	C	·	·	T	·	T	G	T
12	MOTC07(1)	T	T	T	·	G	·	·	A	·	T	T	C	C	C	A	·	T	·	·	·	·	·	G	·	·	C	·	T	T	·	A	T	C	·	·	T	·	T	G	T
13	MOTC08(1)	T	T	T	·	G	·	·	A	·	T	T	C	C	C	A	·	T	·	·	·	·	·	G	·	·	C	·	T	T	·	A	T	C	·	·	T	·	T	G	T
14	MOTC09(1)	T	T	T	·	G	T	·	A	·	T	T	C	C	C	A	·	T	·	·	·	·	·	G	·	·	C	·	T	T	·	A	T	C	·	·	C	·	T	G	T
15	MOTC10(1)	T	T	T	·	G	·	·	A	·	·	T	·	C	C	G	·	C	·	·	·	·	·	G	·	·	·	·	T	T	·	A	T	C	·	·	C	·	T	G	T
16	MOTC11(1)	T	T	T	·	G	·	·	A	·	T	T	C	C	C	A	·	T	·	·	·	·	·	G	·	·	C	·	T	T	·	A	T	C	·	·	T	·	T	G	T
17	MOTC12(1)	T	T	T	·	G	·	·	A	·	T	T	C	C	C	A	·	T	·	·	·	·	·	G	·	·	C	·	T	T	·	A	C	C	·	·	T	·	T	G	T
18	MOTC13(1)	T	T	T	·	G	T	·	A	·	T	T	C	C	C	A	·	T	·	·	·	·	·	G	·	·	C	·	T	·	·	A	T	C	·	·	T	·	T	G	T
19	MOTC14(1)	T	T	T	·	G	·	·	A	·	T	T	C	C	C	A	·	T	·	·	·	·	·	G	·	·	C	·	T	T	·	A	C	C	·	·	T	·	T	G	T
20	MOTC15(1)	T	T	T	·	G	·	·	A	·	T	T	C	C	C	A	·	T	·	·	·	·	·	G	·	·	C	·	T	·	·	A	T	C	·	·	C	·	T	G	T
21	MOTC16(1)	T	T	T	·	G	·	·	A	·	T	T	C	C	C	A	·	T	·	·	·	·	·	G	·	·	C	·	T	T	·	A	T	·	·	·	T	·	T	G	T
22	MOTC17(1)	T	T	T	·	G	·	·	A	·	T	T	C	C	C	A	·	T	·	·	·	·	·	G	·	·	C	·	T	T	·	A	T	C	·	·	T	·	T	G	T
23	MTMC01(17)	T	T	·	T	G	T	C	·	·	·	·	·	T	G	G	G	T	·	·	C	·	T	G	·	·	A	C	A	·	G	C	T	C	·	A	·	·	T	·	T
24	MTMC02(1)	T	T	·	T	·	T	C	·	·	·	·	·	T	G	G	G	T	·	·	C	·	T	G	·	·	G	C	A	·	G	C	T	C	·	A	·	·	T	·	T
25	MTMC03(1)	T	T	·	T	G	T	C	·	·	·	·	·	T	G	G	G	T	G	·	C	·	T	G	·	·	G	C	A	·	G	C	T	C	·	A	·	·	T	·	T
26	MTMC04(1)	T	T	·	T	G	T	C	·	·	T	·	·	T	G	G	G	T	G	·	C	·	T	G	·	G	G	C	A	·	G	C	T	C	·	A	·	C	T	·	T
27	MTMC05(1)	T	T	·	T	G	·	C	·	·	·	·	·	T	G	G	G	T	·	·	C	·	T	G	·	·	G	C	A	·	G	C	T	C	·	A	·	·	T	·	T
28	MTMC06(1)	T	T	·	T	G	T	C	·	·	·	·	·	T	G	G	G	T	·	·	C	·	T	G	·	·	A	C	A	·	G	C	T	C	·	A	·	·	T	·	T
29	MTMC07(1)	T	T	·	T	G	T	C	·	·	·	·	·	T	G	G	G	T	G	·	C	·	T	G	·	·	G	C	A	·	G	C	T	C	·	A	·	·	T	·	T
30	MTMC08(1)	T	T	·	T	G	T	C	·	·	·	·	·	T	G	G	G	T	G	·	C	·	T	G	·	·	G	C	A	·	G	C	T	C	·	A	·	C	T	·	T
31	MTMC09(1)	T	T	·	T	G	T	C	·	·	·	·	·	T	G	G	G	T	·	·	C	·	T	G	·	·	A	C	A	·	G	C	T	C	·	A	·	·	T	·	T
32	MTMC10(1)	T	C	·	T	G	T	C	·	·	T	·	·	T	G	G	G	T	G	·	C	·	T	G	·	·	G	C	A	·	G	C	T	C	·	A	·	·	T	·	T
33	MTMC11(1)	T	T	·	T	G	T	C	·	·	·	·	·	T	G	G	G	T	G	·	C	·	T	G	·	·	G	C	A	·	G	C	T	C	·	A	·	·	T	·	T
34	MTMC12(1)	T	T	·	T	G	T	C	·	·	·	·	·	T	G	G	G	T	·	·	C	·	T	G	·	·	A	C	A	·	G	C	T	C	·	A	·	·	T	·	T
35	MTMC13(1)	T	T	·	T	G	T	C	·	·	·	·	·	T	G	G	G	T	·	·	C	·	T	G	·	·	A	C	A	·	G	C	T	C	·	A	·	·	T	·	T
36	MTMC14(1)	T	T	·	T	G	T	C	·	G	·	·	·	T	G	G	G	T	·	·	C	·	T	G	·	·	A	C	A	·	G	C	T	C	·	A	·	·	T	·	T
37	MTMC15(1)	T	T	·	T	G	T	C	·	·	·	·	·	T	G	G	G	T	·	·	C	·	T	G	·	·	A	·	A	·	G	C	T	C	·	A	·	·	T	·	T

Appendix 1. Continued.

Haplotype (no. individuals)		Nucleotide position																																					
		6	9	13	15	21	24	27	30	31	33	36	39	42	45	48	51	54	64	66	78	81	84	87	90	96	102	108	111	114	117	123	126	133	135	138	141	144	147	150	153
38	MTMC16(1)	T	T	•	T	G	T	C	•	•	T	•	•	T	G	G	G	T	G	•	C	•	T	G	•	•	G	C	A	•	G	T	T	C	•	A	•	C	T	•	T
39	MTMC17(1)	T	T	•	T	G	T	C	•	•	•	•	•	T	G	G	G	T	•	•	C	•	T	G	G	•	A	C	A	•	G	C	T	C	•	A	•	•	T	•	T
40	MTMC18(1)	T	C	•	T	G	T	C	•	•	T	•	•	T	G	G	G	T	G	•	C	•	T	G	•	•	G	C	A	•	G	C	T	C	•	A	•	C	T	•	T
41	MTMC19(1)	T	T	•	T	G	T	C	•	•	T	•	•	T	G	G	G	T	G	•	C	•	T	G	•	•	G	C	A	•	G	C	T	C	•	A	•	C	T	•	T
42	MTMC20(1)	T	T	•	T	G	T	C	•	•	•	•	•	T	G	G	G	T	G	•	•	•	T	G	•	•	G	C	A	•	G	C	T	C	•	A	•	•	T	•	T
43	MTMC21(1)	T	T	•	T	G	T	C	•	•	T	•	•	T	G	G	G	T	G	•	C	•	T	G	•	•	G	C	A	•	G	C	T	C	•	A	•	C	T	•	T
44	MTMC22(1)	T	T	•	T	G	T	C	•	•	•	•	•	T	G	G	G	T	G	•	C	•	T	G	•	•	G	C	A	•	G	C	T	C	•	A	•	•	T	•	T
45	MTMC23(1)	T	T	•	T	G	T	C	•	•	T	•	•	T	G	G	G	T	G	•	C	A	T	G	•	•	G	C	A	•	G	C	T	C	•	A	•	C	T	•	T
46	MTMC24(1)	T	T	•	T	G	T	C	•	•	•	•	•	T	G	G	G	T	G	•	C	•	T	G	•	•	G	C	A	•	G	C	T	C	•	A	•	•	T	•	T
47	MTMC25(1)	T	T	•	T	G	T	C	•	•	T	•	•	T	G	G	G	T	G	•	C	•	T	G	•	•	G	C	A	•	G	C	T	C	•	A	•	C	T	•	T
48	MTMC26(1)	T	C	•	T	G	T	C	•	•	•	•	•	T	G	G	G	T	G	•	C	•	T	G	•	•	G	C	A	•	G	C	T	C	•	A	•	•	T	•	T
49	MTMC27(1)	T	T	•	T	G	T	C	•	•	•	•	•	T	G	G	G	T	•	•	C	•	T	G	•	•	G	C	A	•	G	C	T	C	•	A	•	•	T	•	T
50	MTMC28(1)	T	T	•	T	G	T	C	•	•	T	•	•	T	G	G	G	T	G	•	C	•	T	G	•	•	G	C	A	•	G	C	T	C	•	A	•	C	T	•	T
51	MTMC29(1)	T	T	•	T	G	T	C	•	•	T	•	•	T	G	G	G	T	G	•	C	•	T	G	•	•	G	C	A	•	G	C	T	C	•	A	•	C	T	•	T
52	MTMC30(1)	T	T	•	T	G	T	C	•	•	•	•	•	T	G	G	G	T	G	•	C	•	T	G	•	•	G	C	A	•	G	C	T	C	•	A	•	•	T	•	T
53	MTMC31(1)	T	T	•	T	G	T	C	•	•	•	•	•	T	G	G	G	T	•	•	C	•	T	G	•	•	A	C	A	•	G	C	T	C	•	A	•	•	T	•	T
54	MTMC32(1)	T	T	•	T	G	T	C	•	•	•	•	•	T	G	G	G	T	•	•	•	•	T	G	•	•	A	C	A	T	G	C	T	C	•	A	•	•	T	•	T
55	MTMC33(1)	T	T	•	T	G	T	C	•	•	•	•	•	T	G	G	G	T	•	•	C	•	T	G	•	•	A	C	A	•	G	C	T	C	•	A	•	•	T	•	T
56	MTMC34(1)	T	T	•	T	G	T	C	•	•	T	•	•	T	G	G	G	T	G	•	C	•	T	G	•	•	G	C	A	•	G	C	T	C	•	A	•	C	T	•	T
57	MTTC01(2)	T	T	•	T	G	•	C	•	•	•	•	•	T	G	G	G	T	•	C	•	•	T	G	•	•	G	C	A	•	G	C	T	•	•	A	•	•	T	•	T
58	MTTC02(2)	T	T	•	T	G	•	C	•	•	•	•	•	T	G	G	G	T	•	C	•	•	T	G	•	•	G	C	A	•	G	C	T	•	•	A	•	•	T	•	T

Appendix 1. Continued.

Haplotype	156	159	162	165	168	171	174	177	180	186	189	192	195	199	201	204	205	208	210	211	213	214	216	219	222	225	228	231	234	237	240	243	246	252	255	258	261	264	267	270	271	273	279	280
1	T	T	T	A	T	T	A	G	C	T	C	T	T	C	T	G	T	C	T	T	A	C	T	G	G	A	G	G	A	G	A	T	T	A	G	T	T	T	T	T	T	C	T	T
2	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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6	.	.	.	G	C	.	.	A	T	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	C	.	.	.	G	.	A	C	.	.	T	.	C
7	.	.	.	G	C	.	.	A	T	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	C	.	.	.	G	.	A	C	.	.	T	.	C
8	.	.	.	G	C	.	.	A	T	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	C	.	.	.	G	.	A	C	.	.	T	.	C
9	.	.	.	G	C	.	.	A	T	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	C	.	.	.	G	.	G	C	.	.	T	.	C
10	.	.	.	G	C	.	.	A	T	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	C	.	.	.	G	.	A	C	.	.	T	.	C
11	.	.	.	G	C	.	.	A	T	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	C	.	.	.	G	.	A	C	.	.	T	.	C
12	.	.	.	G	C	.	.	A	T	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	C	.	.	.	G	.	A	C	.	.	T	.	C
13	.	.	.	G	C	.	.	A	T	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	C	.	.	.	G	.	A	C	.	.	T	.	C
14	.	.	.	G	C	.	.	A	T	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	C	.	.	.	G	C	A	C	.	.	T	.	C
15	.	.	.	G	C	.	G	A	T	.	T	.	.	.	.	.	.	.	.	.	.	.	.	A	T	.	.	.	.	T	C	.	.	.	.	G	.	A	C	.	.	T	C	.
16	.	.	.	G	C	.	.	A	T	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	A	T	C	.	.	.	G	C	A	.	.	.	T	.	C
17	.	.	.	G	C	C	.	A	T	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	C	.	.	.	G	.	A	A	.	.	T	.	C
18	.	.	.	G	C	.	.	A	T	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	C	.	.	.	G	.	A	C	.	.	T	.	C
19	.	.	.	G	C	.	.	A	T	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	C	.	.	.	G	.	A	C	.	.	T	.	C
20	.	.	.	G	C	.	.	A	T	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	C	.	.	.	G	.	A	C	.	.	T	.	C
21	.	.	.	G	C	.	.	A	T	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	C	.	.	.	G	.	A	C	.	.	T	.	C
22	.	.	.	G	C	.	.	A	T	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	C	.	.	.	G	.	A	C	.	.	T	.	C
23	.	G	G	G	C	.	.	.	T	G	T	A	G	T	G	A	.	T	G	C	T	T	A	T	.	G	A	A	.	.	.	G	G	G	T	.	C	A	G	A	G	T	.	A
24	.	G	G	.	C	.	.	.	T	G	T	A	G	T	G	.	.	T	G	C	T	T	A	T	.	G	A	A	.	.	.	G	G	G	T	.	C	A	G	A	G	T	.	A
25	.	.	G	G	C	.	.	.	T	G	T	A	G	T	G	A	.	T	G	C	T	T	A	T	.	G	A	A	.	.	.	G	G	G	T	.	.	A	G	A	G	T	.	A
26	.	G	G	G	C	.	.	.	T	G	T	A	G	T	G	A	.	T	G	C	T	T	A	A	.	G	A	A	.	.	.	G	G	G	T	C	C	A	G	A	G	T	.	A
27	.	G	G	G	C	.	.	.	T	G	T	A	G	T	G	A	.	T	G	C	T	T	A	T	.	G	A	A	.	.	.	G	G	G	T	.	C	A	G	A	G	T	.	A
28	.	G	G	G	C	.	.	.	T	G	T	A	G	T	G	A	.	T	G	C	T	T	A	T	.	G	A	A	.	.	.	G	G	G	T	.	C	A	G	A	G	T	.	A
29	.	.	G	G	C	.	.	.	T	G	T	A	G	T	G	A	.	T	G	C	T	T	A	T	.	G	A	A	.	.	.	G	G	G	T	.	.	A	G	A	G	T	.	A
30	.	G	G	G	C	.	.	.	T	G	T	A	G	T	G	A	.	T	G	C	T	T	A	A	.	G	A	A	.	.	.	G	G	G	T	.	C	A	G	A	G	T	.	A
31	.	G	G	G	C	.	.	.	T	G	T	A	G	T	G	A	.	T	G	C	T	T	A	T	.	G	A	A	G	.	.	G	G	G	T	.	C	A	G	A	G	T	.	A
32	.	G	G	G	C	.	.	.	T	G	T	A	G	T	G	A	.	T	G	C	T	T	A	T	.	G	A	A	.	.	.	G	G	G	T	.	.	A	G	A	G	T	.	A
33	.	.	G	G	C	.	.	.	T	G	T	A	G	T	G	A	.	T	G	C	T	T	A	T	.	G	A	A	.	.	.	G	G	G	T	.	.	A	G	A	G	T	.	A
34	C	G	G	G	C	.	.	.	T	G	T	A	G	T	G	A	.	T	G	C	T	T	A	T	.	G	A	A	.	.	.	G	G	G	T	.	C	A	G	A	G	T	.	A
35	.	G	G	G	C	.	.	.	T	G	T	A	G	T	G	A	.	T	G	C	T	T	A	T	.	G	A	A	.	.	.	G	G	G	T	.	C	A	G	A	G	T	.	A
36	.	G	G	G	C	.	.	.	T	G	T	A	G	T	G	A	.	T	G	C	T	T	A	T	.	G	A	A	.	.	.	G	G	G	T	.	C	A	G	A	G	T	.	A
37	C	G	G	G	C	.	.	.	T	G	T	A	G	T	G	A	.	T	G	C	T	T	A	T	.	G	A	A	.	.	.	G	G	G	T	.	C	A	G	A	G	T	.	A

Appendix 1. Continued.

Haplo-type	Nucleotide position																																										
	156	159	162	165	168	171	174	177	180	186	189	192	195	199	201	204	205	208	210	211	213	214	216	219	222	225	228	231	234	237	240	243	246	252	255	258	261	264	267	270	271	273	279	280
38	•	G	G	G	C	•	•	•	T	G	T	A	G	T	G	A	•	T	G	C	T	T	A	A	•	G	A	A	•	•	•	G	G	G	T	•	C	A	G	A	G	T	•	A
39	•	G	G	G	C	•	•	•	T	G	T	A	G	T	G	A	•	T	G	C	T	T	A	T	•	G	A	A	•	•	•	G	G	G	T	•	C	A	G	A	G	T	•	A
40	•	G	G	G	C	•	•	•	T	G	T	A	G	T	G	A	•	T	G	C	T	T	A	A	•	G	A	A	•	•	•	G	G	G	T	•	C	A	G	A	G	T	•	A
41	•	G	A	G	C	•	•	•	T	G	T	A	G	T	G	A	•	T	G	C	T	T	A	A	•	G	A	A	•	•	•	G	G	G	T	•	C	A	G	A	G	T	•	A
42	•	•	G	G	C	•	•	•	T	G	T	A	G	T	G	A	•	T	G	C	T	T	A	T	•	G	A	A	•	•	•	G	G	G	T	•	•	A	G	A	G	T	•	A
43	•	G	G	G	C	•	•	•	T	G	T	A	G	T	G	A	•	T	G	C	T	T	A	A	•	G	A	A	•	•	•	G	G	G	T	•	C	A	G	A	G	T	•	A
44	•	•	G	G	C	•	•	•	T	G	T	A	G	T	G	A	C	T	G	C	T	T	A	T	•	G	A	A	•	•	•	G	G	G	T	•	•	A	G	A	G	T	•	A
45	•	G	G	G	C	•	•	•	T	G	T	A	G	T	G	A	•	T	G	C	T	T	A	A	•	G	A	A	•	•	•	G	G	G	T	•	C	A	G	A	G	T	•	A
46	•	G	G	G	C	•	•	•	T	G	T	A	G	T	G	A	•	T	G	C	T	T	A	T	•	G	A	A	•	•	•	G	G	G	T	•	•	A	G	A	G	T	•	A
47	C	G	G	G	C	•	•	•	T	G	T	A	G	T	G	A	•	T	G	C	T	T	A	A	•	G	A	A	•	•	•	G	G	G	T	•	C	A	G	A	G	T	•	A
48	•	G	G	G	C	•	•	•	T	G	T	A	G	T	G	A	•	T	G	C	T	T	A	T	•	G	A	A	•	•	•	G	G	G	T	•	•	A	G	A	G	T	•	A
49	•	G	G	•	C	•	•	•	T	G	T	A	G	T	G	A	•	T	G	C	T	T	A	T	•	G	A	A	•	•	•	G	G	G	T	•	C	A	G	A	G	T	•	A
50	•	G	G	G	C	•	•	•	T	G	T	A	G	T	G	A	•	T	G	C	T	T	A	A	•	G	A	A	•	•	•	G	G	G	T	•	C	A	G	A	G	•	•	A
51	•	G	G	G	C	•	•	•	T	G	T	A	G	T	G	A	•	T	G	C	T	T	A	A	•	G	A	A	•	•	•	G	G	G	T	A	C	A	G	A	G	T	•	A
52	•	G	G	G	C	•	•	•	T	G	T	A	G	T	G	A	•	T	G	C	T	T	A	T	•	G	A	A	•	•	•	G	G	G	T	•	•	A	G	A	G	T	•	A
53	•	G	G	G	C	•	•	•	T	G	T	A	G	T	G	A	•	T	G	C	T	T	A	T	•	G	A	A	•	•	•	G	G	G	T	•	C	A	G	A	G	T	•	A
54	•	G	G	G	C	•	•	•	T	G	T	A	G	T	G	A	•	T	G	C	T	T	A	T	•	G	A	A	•	•	•	G	G	G	T	•	C	A	G	A	G	T	•	A
55	•	G	G	G	C	•	•	•	T	G	T	A	G	T	G	A	•	T	G	C	T	T	A	T	•	G	A	A	•	•	•	G	G	G	T	•	C	A	G	A	G	T	•	A
56	•	G	G	G	•	•	•	•	T	G	T	A	G	T	G	A	•	T	G	C	T	T	A	A	•	G	A	A	•	•	•	G	G	G	T	C	C	A	G	A	G	T	•	A
57	•	G	G	G	C	•	•	•	T	G	T	A	G	T	G	A	•	T	G	C	T	T	A	T	•	G	A	A	•	•	•	G	G	G	T	•	C	C	G	A	G	T	•	A
58	•	G	G	G	C	•	•	•	T	G	T	A	G	T	G	A	•	T	G	C	T	T	A	T	•	G	A	A	•	•	•	G	G	G	T	•	C	C	G	A	G	T	•	A

Appendix 2. Illustration of neighbor-joining (NJ) tree of haplotypes of *COI* gene in *M. orientalis*. Haplotype labels correspond to Table 2-2. MoMC01 and MoTC01 show haplotypes in Malaysia and Thailand, respectively.