Title: Species identification of Indonesian agarwood using a DNA barcoding method

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Abstract

Agarwood is a type of resinous wood found in the trunks of *Aquilaria* and some other genera. It is widely used as an herbal medicine for sedation, detoxification, and treatment of stomachaches, as well as for incense sticks. However, the number of source plants is decreasing, and in 2005 they were added to Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). In order to identify source species of agarwood, we previously developed a DNA barcoding method using resin deposition sites. In this study, to identify additional agarwood source species, the barcoding method was applied to source plants and commercial agarwood samples collected from Sumbawa, Lombok, Sulawesi, and Kalimantan in Indonesia, a major agarwood producing country. In addition, the method was also applied to incense stick samples labeled as agarwood. As a result, several samples were identified as *Gyrinops*, which is not currently listed as an agarwood source plant in the Japanese standards for non-Pharmacopoeial crude drugs 2018 (Non-JPS 2018). From the viewpoint of securing future resources, these findings suggest that *Gyrinops* species should therefore be added to the list of agarwood source species.

Keywords

Aquilaria, *Gyrinops*, agarwood, DNA barcoding, Indonesia, incense sticks

Introduction

Agarwood is a type of resinous wood found in the trunks of *Aquilaria* and some other genera. It is widely used as an herbal medicine for sedation, detoxification, and treatment of stomachaches, as well as for incense sticks. However, the numbers of source plants in their native environments are decreasing, and in 2005 they were added to Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) [1]. Artificial production of resin and agarwood using cultivated trees is therefore increasing; however, the resulting quality varies and authentication of agarwood cannot be done based on appearance or component information. Identification of agarwood source species is therefore important. In our previous study, we developed a DNA barcoding method for identification of source species using resin deposition sites of agarwood obtained from markets in Japan, Indonesia, Thailand, and Vietnam [2]. DNA sequences of the *trn*L (UAA)-*trn*F (GAA) and *mat*K regions were subsequently obtained. However, the sequence data on GenBank does not cover all agarwood source species, and therefore, the origins of some samples could not be determined. According to the Japanese standards for non-Pharmacopoeial crude drugs 2018 (Non-JPS 2018), five species of *Aquilaria* have been designated as source species of agarwood [3]. However, in our previous study, *Gyrinops* species were also identified as sources of agarwood in the Japanese market [2]. Although *Gyrinops* species are not currently listed as source species of agarwood in the Non-JPS 2018, our findings suggest that they are used for medicinal

purposes in Japan. Moreover, due to decreasing resources, identification of additional source species on the market is important. In this study, the DNA barcoding method using *trn*L-*trn*F and *mat*K DNA sequences was applied to leaves, fruit, and wood from agarwood source plants and commercial agarwood collected in Sumbawa, Lombok, Sulawesi, and Kalimantan in Indonesia, a major agarwood producing country (Fig. 1). In addition, the method was also carried out using incense sticks labeled as agarwood.

Materials and methods

Plant materials and DNA extraction

Three samples were collected from Sumbawa, 13 from Lombok, 13 from Sulawesi, and 7 from Kalimantan (Table 1). Three commercial agarwood samples were also purchased in Sumbawa (Table 2), along with 8 incense stick samples (Table 3). Morphological images of the agarwood samples are shown in Fig. S1, and the incense sticks are shown in Fig. S2. The morphological characteristics of fruit collected from the Kalimantan samples are shown in Fig. 2. Voucher specimens were deposited in the Faculty of Mathematics and Natural Sciences, Mataram University, Indonesia, and the Experimental Station for Medicinal Plants at the Graduate School of Pharmaceutical Science, Kyoto University, Japan. Samples were crushed using ShakeMan (BioMedical Sciences, Japan) prior to DNA extraction using a DNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacturer's protocol.

Primer design and PCR amplification

PCR amplification was performed as in our previous study [2]. The *trn*L-*trn*F region was amplified using the forward primer A1 and reverse primer A2, and the *mat*K gene was amplified using the forward primer C1 and reverse primer C2 (Tables S1 and S2). The amplification enzyme was modified to KOD -Plus- Ver. 2 (Toyobo Co., Ltd., Japan).

Amplification by nested PCR

DNA samples from Lomb_12, Lomb_13, Sumb_m1, Sumb_m3, and IS_1 through IS_8 were also amplified using nested PCR as in our previous study [2]. To do so, forward primer B1 and reverse primer B2 were used for the *trn*L-*trn*F region, and forward primer D1 and reverse primer D2 were used for the *mat*K gene (Tables S1 and S2). The amplification enzyme was modified to KOD -Plus- Ver. 2 (Toyobo Co., Ltd.).

DNA sequencing and data analysis

PCR amplification products were separated on 0.5% agarose/TAE gel, purified using PCR clean-up gel extraction (Macherey-Nagel, Germany) and sequenced on a 3730×l DNA Analyzer using a BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Sequences were compared and aligned using DNASIS Pro version 2.09 (Hitachi Solutions Ltd., Japan).

Results

Identification of source species based on DNA sequences of the *trn*L-*trn*F region

Sequence information of the *trn*L-*trn*F region are shown in the Supplementary Material. The 47 samples (source plant, commercial, and incense stick samples) were classified into seven groups according to DNA sequences of their *trn*L-*trn*F regions (Tables 4-6) then a similarity search was carried out using GenBank. No matching sequences were found for samples Kali_1 through Kali_5. Species identification of remaining samples was carried out based on GenBank sequence data for *Gyrinops versteegii* (LC467530) [2], *G. moluccana* (KT726325) [4], *Aquilaria microcarpa* (LC467528) [2], *A. malaccensis* (LC467525) [2], *A. khasiana* (AY216744), and *A. beccariana* (KT726319) [5].

Genotype classification based on DNA sequences of the *mat*K gene

Sequence information of the *mat*K gene is shown in the Supplementary Material. The 47 samples were subsequently classified into two genotypes according to DNA sequences of their *mat*K genes (Tables 7, 8). Thirty-four samples, Sumb_1 through Sumb_3, Lomb_1 through Lomb_13, Sula_1, Sula_2, Sula_8 through Sula_13, Sumb_m1, Sumb_m2, and IS_1 through IS_8, were classified as genotype A, and the remaining 13 samples, Sula_3 through Sula_7, Kali_1 through Kali_7, and Sumb_m3, as genotype B (Table 8).

Classification based on the *trn*L-*trn*F and *mat*K regions

Source species were subsequently determined by combining sequencing information of the *trn*L-*trn*F

region and the genotype classifications based on the *mat*K gene as shown in Table 8.

Discussion

Identification of source species based on DNA sequences of the *trn*L-*trn*F region

Boundary lines for species distribution known as the Wallace Line and Weber Line run through Indonesia (Fig. 1). Shiou et al. (2018) carried out a comprehensive GenBank search to identify agarwood plant species in Indonesia [4]. As a result, in line with the species boundaries, they revealed that *Gyrinops* species are mainly found in eastern Indonesia and *Aquilaria* species in the western Indonesia.

In this study, of the samples collected on Sumbawa and Lombok, Sumb_1 through Sumb_3, Lomb_1 through Lomb_11, and Lomb_13 were identified as belonging to *G. versteegii*, while Lomb_12 was identified to be *G. moluccana* according to a similarity search of the *trn*L-*trn*F region in GenBank (Table 4). These results support previous studies whereby source plants collected from Lombok were found to be morphologically identical to *G. versteegii* [4, 6]. Sequence information of the *trn*L-*trn*F region of *G. moluccana* previously gave only one GenBank match (KT726325) [4], suggesting inhabitance of the Maluku Islands in Indonesia (Fig. 1). However, in this study, identification of sample Lomb_12 as *G. moluccana* also suggests that this species inhabits Lombok. Analysis of Kalimantan samples Kali_1 through Kali_5 revealed a single nucleotide polymorphism of *A.*

microcarpa, with (T) at 291 base pair (bp) rather than (G). The source species could therefore not be determined using sequence information of the *trn*L-*trn*F region alone (Table 4). However, since the fruit morphology of Kali_5 was similar to that of *A. microcarpa*, it is believed to be very closely related. Meanwhile, although samples Kali_6 and Kali7 were thought to be *A. beccariana* based on their sequence information, their fruit differed (Fig. 2), with the fruit form of Kali_6 similar to that of *A. malaccensis*. Thus, because the *trn*L-*trn*F region is located on chloroplast DNA, which is likely to be conserved via maternal inheritance, Kali_6 is thought to have been derived from hybridization between *A. malaccensis* and *A. beccariana*. To confirm this, establishment of a DNA barcoding method using nuclear gene of, for example, internal transcribed spacer (ITS) regions from agarwood source species is required in the future. The 13 samples collected on Sulawesi were classified into four groups according to DNA sequences of their *trn*L-*trn*F regions (Table 4). These results suggest that various agarwood source species inhabit Sulawesi. Sulawesi Island is located within the Wallacea region, which exists between the Wallace and Weber lines (Fig. 1). Agarwood plants from western and eastern Indonesia are therefore thought to inhabit this region, consistent with the DNA sequencing classification. Of the commercial agarwood samples collected from Sumbawa markets, sample Sumb_m1 was identified as *A. microcarpa*, while Sumb_m2 and Sumb_m3 were thought to belong to *G. moluccana* and *A. malaccensis*, respectively (Table 5). Because the source species of these samples differed, production of commercial agarwood on Sumbawa is thought to use agarwood

collected locally as well as from other islands in Indonesia. Meanwhile, although the incense stick samples (IS_1 through IS_8) were purchased from different countries, all showed the same sequence pattern (Table 6), confirming that they were all made from agarwood.

Classification of genotypes based on DNA sequences of the *mat*K gene

Of the matK gene patterns shown in Table 7, *A. crassna*, *A. hirta*, and *A. subintegra*, which represent genotype A, are usually found in Vietnam and Thailand [5, 6], while *G. versteegii* and *G. caudate*, also representing genotype A, are usually found in eastern Indonesia and Papua New Guinea [4, 5, 7]. Meanwhile, *A. malaccensis, A. beccariana*, and *A. microcarp*a, which represent genotype B, are usually found in western Indonesia and in Malaysia [4, 5], while *A. sinensis*, representing genotype C, is usually found in China [5]. DNA sequences of the matK gene obtained in this study were classified into two genotypes, A and B, with (C) at 358 bp indicating genotype A and (T) at 358 bp indicating genotype B. Samples collected from Sumbawa and Lombok (Sumb_1 through Sumb_3 and Lomb_1 through Lomb_13) were genotype A, while those collected from Kalimantan (Kali_1 through Kali_7) were genotype B. These results are consistent with the known habitat distribution [4–7]; however, of the samples collected on Sulawesi (Sula_1 through Sula_13), Sula_1, Sula_2, and Sula_8 through Sula_13 represented genotype A, while Sula_3 through Sula_7 were genotype B. Although Sulawesi, Sumbawa, and Lombok all fall similarly within Wallacea region, their history of continental migration may differ, resulting in differences in the distribution of agarwood source

plants (Fig. 1). Of the commercial agarwood samples collected from Sumbawa markets, Sumb_m1 and Sumb_m2 represented genotype A, while Sumb_m3 was genotype B, supporting the DNA sequencing results of the *trn*L-*trn*F region. Meanwhile, the incense stick samples (IS_1 through IS_8) were all genotype A, also consistent with sequencing patterns of the *trn*L-*trn*F region.

Source species identification based on DNA sequences of the *trn*L-*trn*F region and *mat*K gene

The source species of Lomb_12, Sula_8 through Sula_13, and Sumb_m2 could not be determined because sequence information of the *mat*K gene in *A. khasiana* and *G. moluccana* does not exist in GenBank (Table 8). To do so, voucher specimens of *A. khasiana* and *G. moluccana* are therefore required.

Sumb_m1 and IS_1 through IS_8 showed identical sequences in both regions, and were also identical to those of *A. microcarpa* in the *trn*L-*trn*F region; however, according to the matK gene, they were classified as genotype A. Due to these conflicting findings, the source species could not be determined (Table 8). Our previous report suggested similar difficulties in identifying all agarwood source plants using current genetic data in GenBank [2]. Furthermore, the *trn*L-*trn*F region alone, which is most frequently used, is thought to be insufficient for identification of agarwood species.

Conclusions

The results of this study revealed the distribution of agarwood source plants on four islands in Indonesia, supporting the previous study of Shiou et al. (2018), whereby *Gyrinops* species were found to inhabit mainly eastern Indonesia and *Aquilaria* species were found to inhabit mainly western Indonesia, separated by the Wallace boundary line [4]. However, because the agarwood plants collected from Sulawesi were classified into various species, the Wallace Line alone cannot be used to classify habitat distribution. In this study, DNA was also extracted from incense sticks labeled as agarwood, and their DNA barcoding regions were successfully amplified. These findings suggest that this method can therefore be applied not only to the resin-containing sites, which is considered difficult to extract DNA from, but also to processed products. However, because samples Kali_6 and Kali_7 had identical DNA sequences in both regions but differing fruit forms, establishment of a DNA barcoding method using nuclear genes such as the ITS region are also required in the future. In addition, based on the commercial agarwood samples from Sumbawa, it is thought that commercial production uses agarwood obtained both locally and from other islands in Indonesia. Agarwood products obtained from various source species are therefore thought to be distributed around the world. According to the Non-JPS 2018, five species of *Aquilaria*, *A. agallocha*, *A. crassna*, *A. malaccensis*, *A. sinensis*, and *A. filaria*, are currently designated as source species of agarwood [3]. However, our results suggest that agarwood produced from *A. microcarpa*, *A. beccariana*, and *Gyrinops* species also exist in the Japanese market. In addition, because all agarwood source species are classified in Appendix II of

CITES, it is recommended that certain species of *Gyrinops* also be added as source plants of herbal medicinal “Agarwood” under Non-JPS 2018, from the viewpoint of securing future resources. Moreover, sequence information lacking in GenBank should also be determined in the future using additional specimens.

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Tables

Table 1 Details of the agarwood samples collected from source species in Sumbawa, Lombok, Sulawesi, and Kalimantan

Collection site	Sample no.	Part used	Collection date
Sumbawa	Sumb_1	Leaf	January, 2018
	Sumb_2	Fruit	January, 2018
	Sumb_3	Agarwood (branch)	January, 2018
Lombok	Lomb_1	Leaf	January, 2018
	Lomb_2	Leaf	January, 2018
	Lomb_3	Leaf	January, 2018
	Lomb_4	Leaf	January, 2018
	Lomb_5	Leaf	January, 2018
	Lomb_6	Leaf	January, 2018
	Lomb_7	Leaf	January, 2018
	Lomb_8	Leaf	January, 2018
	Lomb_9	Fruit	January, 2018
	Lomb_10	Fruit	January, 2018
	Lomb_11	Fruit	January, 2018
	Lomb_12	Agarwood (chunk)	January, 2018
	Lomb_13	Agarwood (branch)	January, 2018
Sulawesi	Sula_1	Leaf	July, 2018
	Sula_2	Leaf	July, 2018
	Sula_3	Leaf	July, 2018
	Sula_4	Leaf	July, 2018
	Sula_5	Leaf	July, 2018
	Sula_6	Leaf	July, 2018
	Sula_7	Leaf	July, 2018
	Sula_8	Leaf	July, 2018
	Sula_9	Leaf	July, 2018
	Sula_10	Leaf	July, 2018
	Sula_11	Leaf	July, 2018
	Sula_12	Leaf	July, 2018
	Sula_13	Leaf	July, 2018

Kalimantan	Kali_1	Leaf	November, 2018
	Kali_2	Leaf	November, 2018
	Kali_3	Leaf	November, 2018
	Kali_4	Leaf	November, 2018
	Kali_5	Fruit	November, 2018
	Kali_6	Fruit	November, 2018
	Kali_7	Fruit	November, 2018

1

2 Table 2 Details of the commercial agarwood samples collected from markets in Sumbawa

Market location	Sample no.	Sample type	Purchase date
Sumbawa	Sumb_m1	chip	January, 2018
	Sumb_m2	chunk	January, 2018
	Sumb_m3	small pieces	January, 2018

3

4 Table 3 Details of the incense stick samples bought from markets in China, Taiwan and Japan; one

5 sample of unknown origin was also included

Sample no.	Market location
IS_1	China
IS_2	China
IS_3	China
IS_4	China
IS_5	Taiwan
IS_6	Taiwan
IS_7	Japan
IS_8	Unknown

6

7 Table 4 SNPs in the *trn*L-*trn*F IGS region amplified from DNA extracted from the source species

8 samples

Sample no.	SNP								Species identification
	159	291	301	327	328	339	368	381	
Sumb_1	A	T	T	T	-	T	C	A	*G. versteegii*
Sumb_2	A	T	T	T	-	T	C	A	*G. versteegii*
Sumb_3	A	T	T	T	-	T	C	A	*G. versteegii*
Lomb_1	A	T	T	T	-	T	C	A	*G. versteegii*
Lomb_2	A	T	T	T	-	T	C	A	*G. versteegii*
Lomb_3	A	T	T	T	-	T	C	A	*G. versteegii*
Lomb_4	A	T	T	T	-	T	C	A	*G. versteegii*
Lomb_5	A	T	T	T	-	T	C	A	*G. versteegii*
Lomb_6	A	T	T	T	-	T	C	A	*G. versteegii*
Lomb_7	A	T	T	T	-	T	C	A	*G. versteegii*
Lomb_8	A	T	T	T	-	T	C	A	*G. versteegii*
Lomb_9	A	T	T	T	-	T	C	A	*G. versteegii*
Lomb_10	A	T	T	T	-	T	C	A	*G. versteegii*
Lomb_11	A	T	T	T	-	T	C	A	*G. versteegii*
Lomb_12	A	T	T	T	T	T	C	A	*G. moluccana*
Lomb_13	A	T	T	T	-	T	C	A	*G. versteegii*
Sula_1	A	T	T	T	-	T	C	A	*G. versteegii*
Sula_2	A	T	T	T	-	T	C	A	*G. versteegii*
Sula_3	A	T	T	-	-	G	C	A	*A. microcarpa*
Sula_4	C	T	T	T	-	G	C	A	*A. malaccensis*
Sula_5	A	T	T	-	-	G	C	A	*A. microcarpa*
Sula_6	A	T	T	-	-	G	C	A	*A. microcarpa*
Sula_7	A	T	T	-	-	G	C	A	*A. microcarpa*
Sula_8	A	T	T	-	-	T	C	A	*A. khasiana*
Sula_9	A	T	T	-	-	T	C	A	*A. khasiana*
Sula_10	A	T	T	-	-	T	C	A	*A. khasiana*
Sula_11	A	T	T	-	-	T	C	A	*A. khasiana*
Sula_12	A	T	T	-	-	T	C	A	*A. khasiana*
Sula_13	A	T	T	-	-	T	C	A	*A. khasiana*
Kali_1	A	G	T	-	-	G	C	A	Unknown
Kali_2	A	G	T	-	-	G	C	A	Unknown
Kali_3	A	G	T	-	-	G	C	A	Unknown
Kali_4	A	G	T	-	-	G	C	A	Unknown
Kali_5	A	G	T	-	-	G	C	A	Unknown

Kali_6	C	T	T	-	-	G	C	A	*A. beccariana*
Kali_7	C	T	T	-	-	G	C	A	*A. beccariana*

SNP single nucleotide polymorphism, *IGS* intergenic spacer

Table 5 SNPs in the *trn*L-*trn*F IGS region amplified from DNA extracted from commercial agarwood samples

Sample no.	SNP								Species identification
	159	291	301	327	328	339	368	381	
Sumb_m1	A	T	T	-	-	G	C	A	*A. microcarpa*
Sumb_m2	A	T	T	T	T	T	C	A	*G. moluccana*
Sumb_m3	C	T	T	T	-	G	C	A	*A. malaccensis*

SNP single nucleotide polymorphism

Table 6 SNPs in the *trn*L-*trn*F IGS region amplified from DNA extracted from the incense stick samples

Sample No.	SNPs								Species identification
	159	291	301	327	328	339	368	381	
IS_1	A	T	T	-	-	G	C	A	*A. microcarpa*
IS_2	A	T	T	-	-	G	C	A	*A. microcarpa*
IS_3	A	T	T	-	-	G	C	A	*A. microcarpa*
IS_4	A	T	T	-	-	G	C	A	*A. microcarpa*
IS_5	A	T	T	-	-	G	C	A	*A. microcarpa*
IS_6	A	T	T	-	-	G	C	A	*A. microcarpa*
IS_7	A	T	T	-	-	G	C	A	*A. microcarpa*
IS_8	A	T	T	-	-	G	C	A	*A. microcarpa*

SNP single nucleotide polymorphism, *IGS* intergenic spacer

Table 7 Genotype classification based on alignment of the *matK* gene

Genotype of the *mat*K gene	SNPs						Classification of agarwood origin species based on each genotype
	156	265	294	358	371	406	
A	C	C	G	C	C	A	*A. crassna, A. hirta, A. subintegra, G. versteegii, G. caudata*
B	C	C	G	T	C	A	*A. malaccensis, A. beccariana, A. microcarpa*
C	A	T	C	C	A	C	*A. sinensis*

Classification of each species was based on GenBank sequence data: *A. crassna* (LC467499) [2], *A. hirta* (KX424695), *A. subintegra* (KU244050) [6], *G. versteegii* (LC467513) [2], *G. caudata* (MF443403) [9], *A. malaccensis* (LC467508) [2], *A. beccariana* (FJ572802), *A. microcarpa* (LC467511) [2], and *A. sinensis* (LC467505) [2].

Table 8 Species identification based on sequences of the *trn*L-*trn*F and *mat*K regions

Sample no.	Species identification based on the ***trn*L-*trn*F** region	Genotyping of the ***mat*K** gene	Species identification based on **both regions**
Sumb_1	*G. versteegii*	**A**	*G. versteegii*
Sumb_2	*G. versteegii*	**A**	*G. versteegii*
Sumb_3	*G. versteegii*	**A**	*G. versteegii*
Lomb_1	*G. versteegii*	**A**	*G. versteegii*
Lomb_2	*G. versteegii*	**A**	*G. versteegii*
Lomb_3	*G. versteegii*	**A**	*G. versteegii*
Lomb_4	*G. versteegii*	**A**	*G. versteegii*
Lomb_5	*G. versteegii*	**A**	*G. versteegii*
Lomb_6	*G. versteegii*	**A**	*G. versteegii*
Lomb_7	*G. versteegii*	**A**	*G. versteegii*
Lomb_8	*G. versteegii*	**A**	*G. versteegii*
Lomb_9	*G. versteegii*	**A**	*G. versteegii*
Lomb_10	*G. versteegii*	**A**	*G. versteegii*
Lomb_11	*G. versteegii*	**A**	*G. versteegii*

Lomb_12	*G. moluccana*	**A**	Unknown
Lomb_13	*G. versteegii*	**A**	*G. versteegii*
Sula_1	*G. versteegii*	**A**	*G. versteegii*
Sula_2	*G. versteegii*	**A**	*G. versteegii*
Sula_3	*A. microcarpa*	**B**	*A. microcarpa*
Sula_4	*A. malaccensis*	**B**	*A. malaccensis*
Sula_5	*A. microcarpa*	**B**	*A. microcarpa*
Sula_6	*A. microcarpa*	**B**	*A. microcarpa*
Sula_7	*A. microcarpa*	**B**	*A. microcarpa*
Sula_8	*A. khasiana*	**A**	Unknown
Sula_9	*A. khasiana*	**A**	Unknown
Sula_10	*A. khasiana*	**A**	Unknown
Sula_11	*A. khasiana*	**A**	Unknown
Sula_12	*A. khasiana*	**A**	Unknown
Sula_13	*A. khasiana*	**A**	Unknown
Kali_1	Unknown	**B**	Unknown
Kali_2	Unknown	**B**	Unknown
Kali_3	Unknown	**B**	Unknown
Kali_4	Unknown	**B**	Unknown
Kali_5	Unknown	**B**	Unknown
Kali_6	*A. beccariana*	**B**	*A. beccariana*
Kali_7	*A. beccariana*	**B**	*A. beccariana*
Sumb_m1	*A. microcarpa*	**A**	Unknown
Sumb_m2	*G. moluccana*	**A**	Unknown
Sumb_m3	*A. malaccensis*	**A**	*A. malaccensis*
IS1	*A. microcarpa*	**A**	Unknown
IS2	*A. microcarpa*	**A**	Unknown
IS3	*A. microcarpa*	**A**	Unknown
IS4	*A. microcarpa*	**A**	Unknown
IS5	*A. microcarpa*	**A**	Unknown
IS6	*A. microcarpa*	**A**	Unknown
IS7	*A. microcarpa*	**A**	Unknown
IS8	*A. microcarpa*	**A**	Unknown

Figure legends

1 Fig. 1 Geographical separation of Indonesia based on the Wallace Line and Weber Line

2 Fig.2 Fruit forms of the Kalimantan samples used in this study

Fig. 1

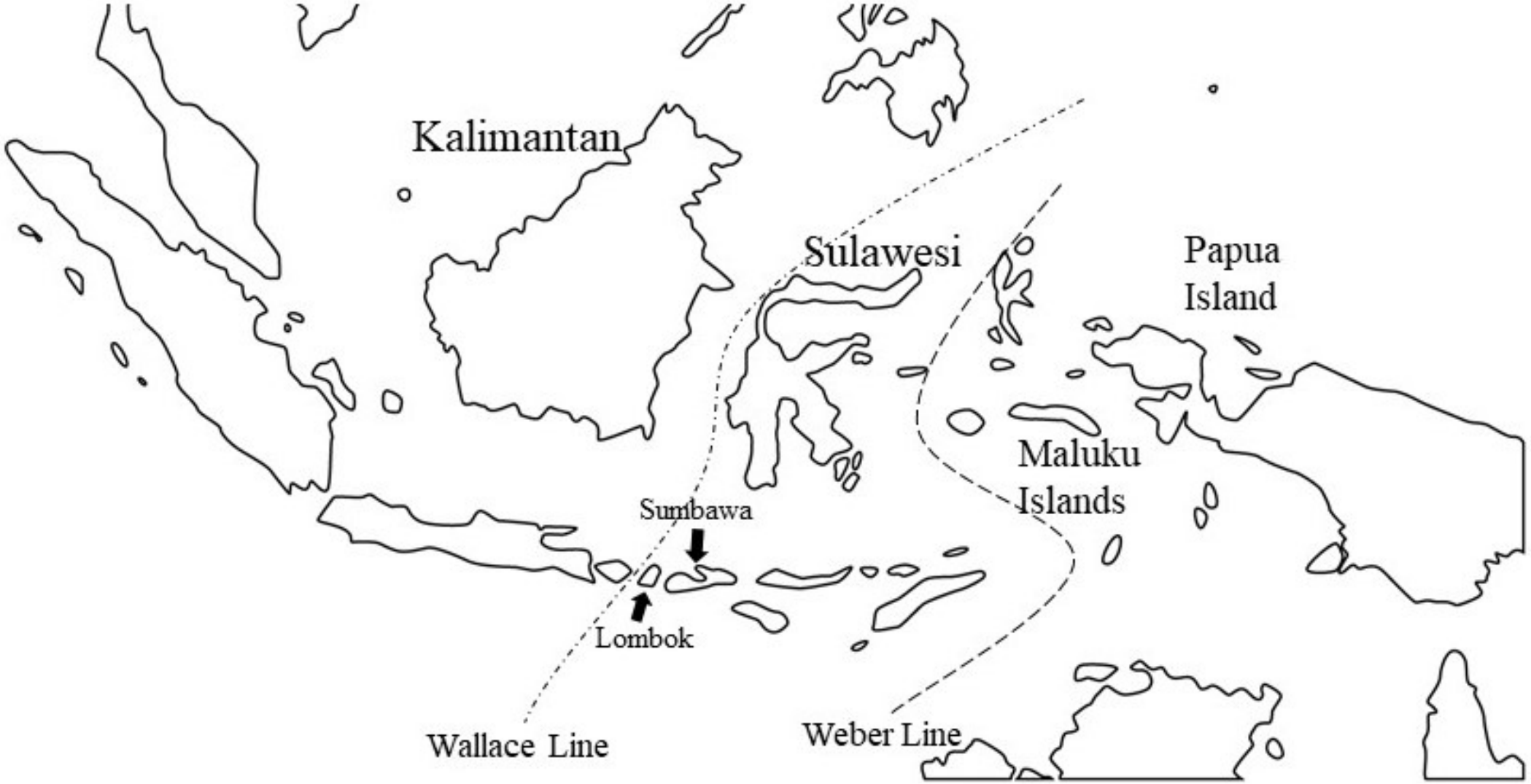
Kalimantan
Sulawesi
Papua
Island
Maluku
Islands
Sumbawa
Lombok
Wallace Line
Weber Line

Fig.2

Kali_5

Kali_6

Kali_7