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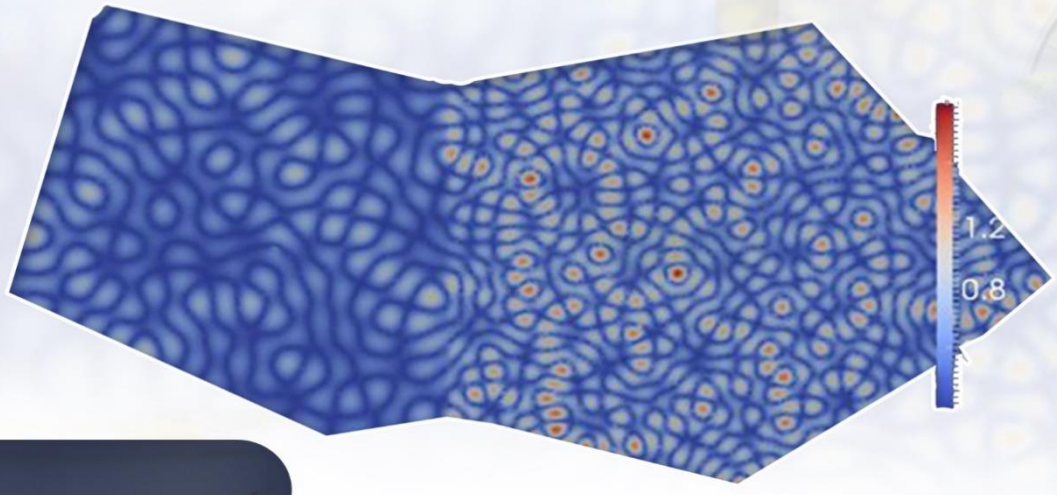

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The Phylogeography of Rhizophora in Peninsular Malaysia: High Genetic Variation between West and East

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A B S T R A C T

Geographic history of *Rhizophora* species and hybrid is effect on their genetic structure. In addition, determining population genetic variation is essential for phylogeography research. For this phylogeography investigation, Four natural population of *Rhizophora* were collected from five different regions in peninsular Malaysia for this phylogeography study. This study aims to evaluate the genetic diversity of *Rhizophora* species *namely Rhizophora apiculata* , *Rhizophora mucronata* , *Rhizophora stylosa* and hybrid *R.* × *lamarcki* and clarify their genetic structure of its populations using Nucleotide polymorphism in chloroplast intergenic spacer between trnL and trnF genes was studied.The populations of *Rhizophora* species and hybrids were divided into two groups: the east coast populations, which were represented by samples collected from Mersing, Tanjang Piai, and Sedili; and the west coast populations, which were represented by samples collected from Mersing, Tanjang Piai, and Sedili. The population of the west coast was the second cluster. This result was supported by UPGMA analysis using MEGA5 software.

1 Introduction

Rhizophora is a genus of tropical and subtropical coastal plants native to tropical and subtropical coastal areas from Africa's east coast through Asia, Australia, and most of the eastern Pacific Ocean's islands. East Pacific mangroves whose range naturally overlap only in small number of southern Pacific islands (Duke 2006). According to Tomlinson (1986) *Rhizophora mucronata* ranges from east Africa to the western Pacific further eastward in the Indo-Malayan region where it grows with *Rhizophora apiculata* and *Rhizophora stylosa*. But it becomes progressively less conspicuous element of mangrove floras as one moves eastward In Peninsular Malaysia; the most widely distributed species are *Rhizophora apiculata* and *Rhizophora mucronata* throughout mangrove forest areas. In the forestry and fishing industries, both species are economically important. Apart from these two economic species, Malaysian foresters are less familiar with *Rhizophora stylosa*. There is little information about *R. stylosa* in Malaysia. *R. stylosa* does not grow in all mangrove habitats in Peninsular Malaysia, and it can only be found in a few places. *Rhizophora stylosa* has a wide distribution in the Indo-Pacific region. However, its distribution is very localized and restricted in very specific sites in Peninsular Malaysia. *R. stylosa* is only found in Pulau Langkawi, Melaka and Johor (Kochummen, 1989). Others than these areas, Nasir & Yusmah. (2007) investigated the distribution of Rhizophora stylosa in Peninsular Malaysia and discovered it in Sg. Kurung Tengar, Perlis, Bagan Lalang, Sepang, Selangor, Pulau Besar, Melaka, Pulau Burung, a small rocky island off the coast of Port Dickson, and two sites in Sg. Mawar, Endau, Johor, Pulau Sibu and Pulau Tinggi, Both islands are located off the coast of Johor's Mersing..There are about 20 million hectares of mangroves in Asia, Oceania, Africa, the Americas and the Middle East (Saenger *et al*. 1983). Malaysia has about 6400 km2 of mangrove forest, of

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which 5000 km2 are found in Sabah and Sarawak and in Peninsular Malaysia about 1400 km2 (Mansor *et al*. 2005). Of the total mangrove areas in Malaysia, Sabah covers 57%, followed by Sarawak 26% and Peninsular Malaysia has 17%. Peninsular Malaysia has about 86,545 ha of mangrove areas gazetted as forest reserves, whereas Perak has the largest area (47.8%), followed by Johor (20.6%), Selangor (17.3%) and Kedah (9.2%) (Wan Juliana et al. 2007).

Nucleic acid variation can be described most directly by using the nucleotides themselves as discrete characters, each with four possible states. Sequencing can identify very modest levels of variation since nucleotides are the basic units of genetic information in all organisms. Virtually any taxon's nuclear and organelle genomes can be sequenced; just little amounts of DNA or RNA are required, and fresh or stored DNA can be used (Avise 1994).The universal primers of the *trn*L–*trn*F spacer were designed by Taberlet *et al*. (1991), and are now widely used to survey the intraspecific genetic variation and to construct phylogenetic trees for various plant species (Fujii et al. 1995;Wang *et al*. 1999).

2 Materials and Methods

2.1 Plant Materials

Leaves were obtained from 20 trees of *R. apiculata*, *R. mucronata*, *R. stylosa and R.* × *lamarcki* from different localities in peninsular Malaysia. The locations for each site are listed in table 1 and showed in Figure1.

2.2 Genomic DNA Extraction

Genomic DNA was isolated using DNeasy Plant Mini Kit method protocol. Approximately 40 mg of leaf tissue was ground to a powder in liquid nitrogen with a mortar and pestle. The powder was combined with 400 ml RNase stock solution and incubated for 10 minutes at 65 C°. the mixture was centrifuged at 10000 rpm. The lysate was transferred into QIA Shredder Mini spin column placed in a 2 ml collection tube and centrifuged for 4 min at 10000 rpm. Without disrupting the cell-debris pellet, the flow through fraction was transferred to a new tube. 1.5 volume of buffer AP3 ⁄E was added and mixed. 650 ml of the previous step's mixture, including any precipitate that may have formed, was placed in a 2 ml collection tube with a DNeasy Mini spin column. Centrifuged for 1 min at 8000 rpm and discarded the through reuse the collection tube in next step. The last step was repeated with the remaining sample and discarded the flow-through and collection tube. The DNeasy Mini spin column was placed into a new 2ml collection tube. 500 µl Buffer AW was added centrifuged for 1 min at 8000 rpm and discarded the flow-through and reuse the collection tube in next step. 500 µl Buffer AW was added to DNeasy Mini spin column and centrifuged for 4 min at 10000 rpm. The flow-through was discarded and then centrifuged for 1 min at 10000 rpm. The DNeasy Mini spin column was transferred to 1.5 ml micro centrifuge tube. And then 100 µl of Buffer AE was added directly onto DNeasy membrane and incubated for 20 min and then centrifuged for 1 min at 8000 rpm. The Buffer AE was added onto DNeasy membrane and then incubated for one night.

2.3 Polymerase Chain Reaction (PCR)

The region was amplified by the Polymerase Chain Reaction (PCR). The universal primers for amplification of the *trn*L-*trn*F spacer were those of Taberelt et al. (1991). PCR reaction mixtures (25 µl) contained approximately 1 µl of total DNA, 0.5 µl of each primer, 3 µl $Mgcl_2$, 1µL DNTp and 0.2 µl of Taq polymerase. Amplifications were carried out using 30 cycles for 1 min at 94 Cº, 1 min at 51 Cº or 48 Cº, and 2 min at 72 Cº the PCR products were excised from agarose gel under the long wave UV light. The PCR products were purified with GF-1(PCR clean- up kit)

2.4 Alignment of DNA Sequences and Data Analysis.

For aligned the DNA sequences, we used MEGA5 program. Clustering was done following unweighted pair group with arithmetic mean average (UPGMA) method MEGA5 software.

Table (1). List of specimens and collection sites of each species.

Species		Specimen
R.apiculata	AJ1	Johor, Mersing
	AL1	Kedah, Pulau Langkawi
	AS1	Johor, Sedili
	AM1	Melaka, Sungai linggi
	AT1	Melaka, Port Dickson, Cape Rachado Beach
R. mucronata	ML2	Kedah, Pulau Langkawi.
	MS2	Johor, Sedili,
	MO	Johor, Mersing
	MM2	Melaka, Sungai Linggi
	MP2	Selangor, Morib
R. stylosa	SL3	Kedah, Pulau Langkawi.
	SM3	Melaka, Port Dickson, Cape Rachado Beach
	SP3	N. Sembilan, Port Dickson, Teluk Tanjung
	SJ3	Johor, Mersing
	ST3	Johor, Tanjung Piai
R. lamarckii	LL4	Kedah, Pulau Langkawi
	LS4	N. Sembilan, Port Dickson, Teluk Tanjung Pelandok
	LM4	Melaka, Port Dickson, Cape Rachado Beach Resort
	LT4	Johor, Tanjung Piai
	LJ4	Johor, Mersing

Figure (1). The locality of collections sites.

3 Results

In *Rhizophora apiculata*, clustering based on genetic distances showed that the populations of *Rhizophora apiculata* are grouped into two distinct clusters. The three populations of *R. apiculata*, Sungai linggi (AM1), Cape Rachado Beach Resort, (AT1) and Mersing (AJ1) formed the first cluster wherein AM1 (Sungai linggi) and AT1 (CapeRachado Beach Resort population) was clustered to AJ1 (distance 0.017) and formed the second cluster a long with AS1 (Sedili population) at 0.021 and formed a node with AL1 (Langkawi population). There were clear relationship between AM1 (Sungai linggi population) and AT1 (Cape Rachado Beach Resort population). Whereas, Sungai linggi and Cape Rachado Beach Resort population had shorter genetic distance (Figure 2).

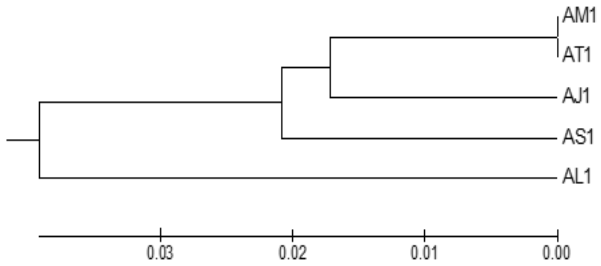

Figure (2). Dissimilarity coefficient UPGMA dendrogram of *R. apiculata* based on the G_{ST} of the *trnL-trnF* spacer of *cpDNA.*

Rhizophora mucronata populations are grouped into three distinct clusters. MS2 (Sedili population), MO2 (Mersing population) formed a first cluster wherein MS2 was clustered to MO2 (distance of 0.014) and formed second cluster along with ML2 (Langkawi population) at 0.019 and MM2 (Sungai linggi population) formed a third cluster with MP2 (Morib population) at 0.014. The two groups of populations formed a node at 0.026. Sungai Linggi and Morib population with Sedili and Mersing population had a same and a shorter genetic distance (Figure 3).

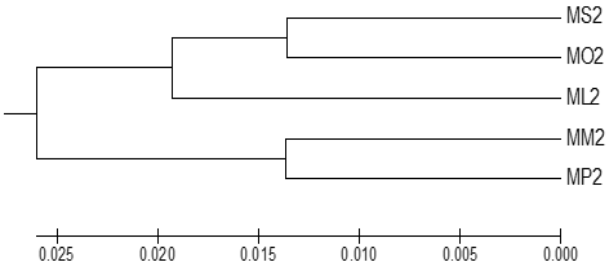

Figure (3). Dissimilarity coefficient UPGMA dendrogram of R. *mucronata* based on the G_{ST} of the trnL-trnF spacer of cpDNA.

R. stylosa populations are grouped into three distinct clusters. That of, SP3 (Cape Rachado Beach Resort) population and SM3 (Teluk Tanjung Pelandok) population formed a first cluster wherein SP3 was clustered to SM3 (distance of 0.025) and SJ3 (Mersing population) was clustered with ST3 (Tanjung Piai population) at 0.005. The two groups of populations formed a node at 0.031 and formed a third cluster along with SL3 (Langkawi population). Tanjung Piai and Mersing populations had shorter genetic distance than those of the Cape Rachado Beach Resort and Teluk Tanjung Pelandok populations (Figure 4).

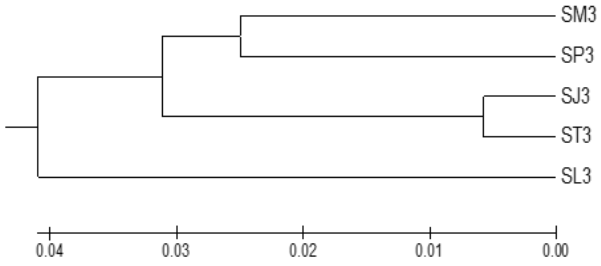

Figure (4). Dissimilarity coefficient UPGMA dendrogram of *R. stylosa* based on the G_{ST} of the trnL-trnF spacer of cpDNA.

The population of natural hybrid (*R.* × *Lamarckii*) grouped into two distinct clusters. LS4 (Teluk Tanjung Pelandok population) and LM4 (Cape Rachado Beach Resort population) formed a first cluster with LL4 (Langkawi population) and formed second cluster a long with LT4 (Tanjung Piai population) and LJ4 (Mersing population) (Figure 5).

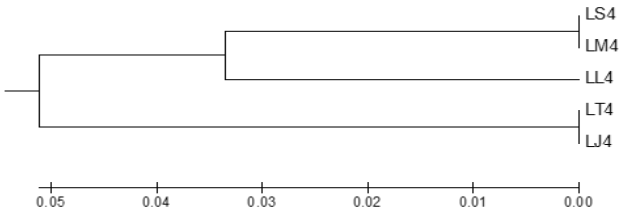

Figure (5). Dissimilarity coefficient UPGMA dendrogram of *R* × *lamarckii* based on the G_{ST} of the *trn*L-*trn*F spacer of cpDNA.

4 Discussion

For population studies, samples were collected from different geographically locations from east and west coast of Peninsular Malaysia. There were high genetic variation in east and west coasts of Peninsular Malaysia for population for all *Rhizophora* species and hybrid. The east coast population is represented by samples collected from Mersing, Tanjang Piai and Sedili while the west coast populations are represented by Cape Rachado Beach Resort, Teluk Tanjung Pelandok, Sungai linggi and Morib. This result is consistent with Huang et al. (1999) who had found a relatively high genetic variation in both populations of east and west coasts of Malay Peninsula of *Rhizophoraceae* using isozyme analysis.

In this study, it was noticed that the UPGMA analysis based on genetic distances could reveal in all *Rhizophora* taxa, there are high genetic variation between both west and east coasts population of *Rhizophora*.

5 Conclusions

The geographic history is another factor that may have influenced genetic differentiation in this area. In this study, from the result of data analysis the geographical haplotype distribution reveals significant discrimination between eastern and western Malay Peninsula populations.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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