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Short Communication: Phylogenetic analysis of mango (*Mangifera*) in Northern Sumatra based on gene sequences of cpDNA *trnL-F* intergenic spacer

FITMAWATI♥, SANDI PRATIWI HARAHAP, NERY SOFIYANTI
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Riau. Kampus Binawidya, Jl. HR Soebrantas Km 12,5 Pekanbaru 28293, Riau, Indonesia. Tel./Fax. +62-761-63273, ♥email: fitmawati2008@yahoo.com

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Abstract. *Harahap SP, Fitmawati, Sofiyanti N. 2017. Short Communication: Phylogenetic analysis of mango (*Mangifera*) in Northern Sumatra based on gene sequences of* cpDNA *trnL-F intergenic spacer. Biodiversitas 18: 715-719.* Northern Sumatra is an area with geographical variation. The environmental factors are affected on character plasticity such as found in Anacardiaceae family especially *Mangifera* genus. The character plasticity of *Mangifera* members raises a problem in determining clear boundaries between species based on morphological character. Therefore, a molecular approach is necessary to provide a specific character among *Mangifera* species. This study aimed to reconstruct the phylogeny among *Mangifera* members in Northern Sumatra based on the sequences of *trnL-F* intergenic spacer. All of the sequences were aligned by using Clustal W and Cladogram was reconstructed by using PAUP by Maximum Parsimony (MP) and Neighbour-Joining (NJ) methods. The MP cladogram produced two in groups i.e., clade I consist of *M. odorata*$_1$, *M. odorata*$_2$, *M. laurina*$_1$, *M. laurina*$_2$, *M. indica*, *M. zeylanica*, *M. quadrifida* and *Mangifera* sp. and clade II consisted of *M. foetida*$_1$ and *M. foetida*$_2$. Based on the NJ method, it was obtained *M. laurina*$_2$ which had the highest genetic distance among other members of *Mangifera*. The *trnL-F* intergenic spacer with a low sequences variation indicated that this region was highly conserved and had a low evolution rate in *Mangifera*.

Keywords: Northern Sumatra, *Mangifera*, molecular phylogeny, *trnL-F* intergenic spacer

INTRODUCTION

Mango (*Mangifera*) is the largest genus of Anacardiaceae family and grows in tropical regions. A mango tree can grow in a wide range of rainfall volumes and patterns. The most of *Mangifera* was distributed in the tropical region of Asia (India, Burma, Sri Lanka, Thailand, South Tropical China, Malaysia, Indonesia, Papua New Guinea, the Philippines and the Solomon Islands) (Kosterman and Bompard 1993). *Mangifera* distribution in Northern Sumatra consists of Nangroe Aceh Darussalam and North Sumatra provinces. Northern Sumatra has a unique geographic condition and *Mangifera* was well adapted in this region (Fitmawati 2015). Mango in Sumatra is well adapted to high rainfall volumes, and the flower is not easy to fall out and capable to fruit out of season (Fitmawati 2008), but in the last decade, *Mangifera* existence was threatened by deforestation and forest conversion which is as a source of germplasm.

In spite of the uniqueness of mango, a high character plasticity in *Mangifera* members raises a matter in a delimitation based on a morphology character. In the most current classification of *Mangifera*, it was described 58 species with 11 species in uncertain position by Kosterman and Bompard (1993). According to Mukherjee (1958), *Mangifera* origin comes from one ancestor, contradicted by Kosterman and Bompard (1993) stated that *Mangifera* origin comes from two different ancestors. Therefore, a molecular approach is necessary to provide a specific character among *Mangifera* members.

A molecular approach based on cpDNA has been used to study a phylogenetic relationship. One of the most frequent markers used by plant systematist is a *trnL-F* intergenic spacer. This non-coding region has a high rate of mutation, easily isolated, purified and cloned and relatively small in size. cpDNA is also suitable to investigate a phylogenetic relationship (Taberlet et al. 1991). The most of the structural mutation in cpDNA is small indel from 1-10 bp. (Vijeberg and Bachmann 1999). Another research about the use of *trnL-F* region in land plants and suitable to provide a phylogenetic information in Junacaeae (Drabkova 2004) and *Mangifera* had been conducted by Fitmawati and Hartana (2010) for *Mangifera laurina* and related species and by Dinesh et al. (2015) for *Mangifera indica* relatives. But for mango Sumatra, especially in Northern Sumatra, has not been done yet. This study aimed to reconstruct the phylogeny among *Mangifera* members in Northern Sumatra based on the gene sequences of the *trnL-F* intergenic spacer.

MATERIALS AND METHODS

Plant materials

Fresh leaf of mango used in this study for DNA extraction and specimens was collected from Nangroe Aceh Darussalam and North Sumatra. The list of plant material used could be seen in Table 1.

Table 1. Plant materials, their geographic origins, and codes used in this

Species	Origin	Coordinate	Code
Mangifera foetida Lour	Aceh Tengah, Aceh	N 04'08'12.7"; E 96'09'57.4''	*M. foetida*$_1$
Mangifera foetida Lour	Rantau Prapat, Labuhanbatu, N. Sumatra	N 02'55'15.5"; E 97'29'41.0''	*M. foetida*$_2$
Mangifera indica Lour	Aceh Besar, Aceh	N 05'20'44.8"; E 95'14'29.7''	*M. indica*
Mangifera laurina Bl	Takengon, Aceh Tengah, Aceh	N 04'36'18.6"; E 96'51'56.7''	*M. laurina*$_1$
Mangifera laurina Bl	Medan, N. Sumatra	N 03'38'14.4 "; E 97'41'53.8'';	*M. laurina*$_2$
Mangifera odorata Griff	Aceh Barat, Aceh	N 04'06'18.0"; E 96'11'55.6''	*M. odorata*$_1$
Mangifera odorata Griff	Medan, N. Sumatra	N 03'31'50.1"; E 98'37'12.0'';	*M. odorata*$_1$
Mangifera quadrifida Jack	Aceh Barat, Aceh	N 04'13'34.3"; E 96'04'00.6''	*M. quadrifida*
Mangifera sp.	Sipirok, Tapanuli Selatan, N. Sumatra	N 01'54'03.1"; E 99'23'57.6''	*Mangifera* sp.
Mangifera Zeylanica (Bl.) Hooker f.	Aceh Tenggara, Aceh	N 05'32'51.8"; E 95'20'02.5''	*M. zeylanica*

DNA extraction

The DNA was isolated from 2 g young leaf tissue at the terminal position by using a modified CTAB method of Doyle and Doyle (1987) and precipitated by using ethanol 96% about 24 h at 4^{o}C. Pellet was washed with 70% ethanol. DNA was stored in TE buffer at -20^{o}C until to be required.

DNA sequencing and cloning PCR products

The highest yield of polymerase chain reaction (PCR) products was achieved by using this following condition. The PCR reaction 50 µL consisted of 10-50 ng/µL genomic DNA, 10 pmol of each primer, Dream Taq Buffer 10x, and 2mM dNTP Mix. The PCR reaction was conducted according to Small et al. (2005) consisted of an activation step of denaturation 95^{o}C for 4 m, an annealing step of 52^{o}C for 1 m, and an extension step of 72^{o}C for 1 m 30s. The PCR mixture underwent for 35 cycles. The PCR products were run on 1.2% agarose gel electrophoresis at 110 volts for 30 minutes. The PCR products were sequenced at First Base Laboratories, Malaysia.

Phylogenetic analysis

DNA sequences of mangoes and out-group of *the trnL-F* region were aligned by using Clustal W Multiple Allignment in Bioedit. The phylogenetic tree was constructed with Maximum Parsimony (MP) and Neighbour Joining (NJ) by using PAUP* 40.b10 (Swofford 2002). The appearance of the phylogenetic tree was showed by using a tree view.

RESULTS AND DISCUSSION

Phylogenetic tree result

Multiple alignment analysis was performed by using Clustal X. The alignment of *trnL-F* comprised 433 characters. From these results, 199 characters were constant, 222 characters were parsimony uninformative, and nine characters were potentially parsimony informative. The reconstruction of a phylogenetic tree by using PAUP resulted in a consistency index (CI) of 0.97 and a retention index (RI) of 0.84. The phylogenetic tree as shown in Figure 1 was constructed by using Maximum Parsimony method with bootstrap 1000x. Neighbour Joining method was also used to show the difference of genetic distance and to analyze the sequences similarity among the samples.

Nucleotides composition claimed in G+C content which defined as a proportion of cytosine and guanine from whole nucleotides in the genome (Mesbah et al. 1989). The G+C content of *Mangifera* was approximately 36.4. From all sequences, a *gap* was found and assumed as insertion and deletion (indels). Insertion and deletion were defined as the process of mutations. From 10 kinds *Mangifera*, 15 deletions were found in these sequences (Table 2). Plastid DNA apparently does not undergo a recombination. The variation in cpDNA was commonly caused by a mutation of a single nucleotide in a long period (Fitmawati and Hartana 2008).

Table 2. Characteristic of *trnL-F* intergenic spacer region among *Mangifera* and out-group.

	Length range (nt)	Lenght mean (nt)	Alignment length (nt)	G+C content (%)	G+C mean (%)	Indels	No. of polymorphic site	No. of informative site	Tree length	CI	RI	RC
Mangifera spp.												
All sequences	410-427	418.5	433	35.1-37.7	36.4	15	20	9	21	0.8	0.85	0.68
Mangifera spp. + 1 taxa out-group												
All sequences	410-445	427.5	433	35.1-37.7	36.4	36	63	9	245	0.97	0.84	0.82

Noted: CI = Consistency index, RI = retention index, RC = rescaled consistency index

Table 3. Alignment of *trnL-F* DNA intergenic spacer sequences of ten mangoes and out-group

Species	The DNA sequences
Bouea	AACTAATTAATCAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGCCAATATCAATACTGGCAACAAT
M. sp	CCATTTCTC-TCAGCTGAGCTATCCCGACCATTA---CCGACGCATCATTTTCATTTTACTAGATGGTTGGGT
M. lau$_1$	TATCCTCTC-TCAGCTGAGCTATCCCGACCATTA---CCGACGCATCATTTTCATTTTACTAGATGGGTTGGG
M. lau$_2$	ACTCCTCTC-TCAGCTGAGCTATCCCGACCATTA---CCGACGCATCATTTTCATTTTACTAGATGGGTTGGG
M. odo$_1$	AACTAGCTC-TCAGCTGAGCTATCCCGACCATTA---CCGACGCATCATTTTCATTTTACTAGATGGGTTGGG
M. odo$_2$	GACTAGCTC-TCAGCTGAGCTATCCCGACCATTA---CCGACGCATCATTTTCATTTTACTAGATGGGTTGGG
M. zey	TCTCTGCTC-TCAGCTGAGCTATCCCGACCATTA---CCGACGCATCATTTTCATTTTACTAGATGGGTTGGG
M. qua	CTCTGGCTC-TCAGCTGAGCTATCCCGACCATTA---CCGACGCATCATTTTCATTTTACTAGATGGGTTGGG
M. ind	ATCTTGCTC-TCAGCTGAGCTATCCCGACCATTA---CCGACGCATCATTTTCATTTTACTAGATGGGTTGGG
M. foe$_1$	AGCTC-TCAGCTGAGCTATCCCGACCATTA---CCGACGCATCATTTTCATTTTACTAGATGGGT
M. foe$_2$	AGCTC-TCAGCTGAGCTATCCCGACCATTA---CCGACGCATCATTTTCATTTTACTAGATGGGT
Bouea	GAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAAATC
M. sp	CGATGTCAATTAAAAAAGACGCAATTCAGTATTAAAAGTCTTGGACGGGAAATTGCATTT---CTTGAATAT
M. lau$_1$	TCGATGTCAATTAAAAAAGACGCAATTCAGTATTAAAAGTCTTGGACGGGAAATTGCATTT---CTTGAATAT
M. lau$_2$	TCGATGTCAATTAAAAAAGACGCAATTCAGTATTAAAAGTCTTGGACGGGAAATTGCATTT---CTTGAATAT
M. odo$_1$	TCGATGTCAATTAAAAAAGACGCAATTCAGTATTAAAAGTCTTGGACGGGAAATTGCATTT---CTTGAATAT
M. odo$_2$	CGATGTCAATTAAAAAAGACGCAATTCAGTATTAAAAGTCTTGGACGGGAAATTGCATTT---CTTGAATAT
M. zey	TCGATGTCAATTAAAAAAGACGCAATTCAGTATTAAAAGTCTTGGACGGGAAATTGCATTT---CTTGAATAT
M. qua	TCGATGTCAATTAAAAAAGACGCAATTCAGTATTAAAAGTCTTGGACGGGAAATTGCATTT---CTTGAATAT
M. ind	TCGATGTCAATTAAAAAAGACGCAATTCAGTATTAAAAGTCTTGGACGGGAAATTGCATTT---CTTGAATAT
M. foe$_1$	TGGGTCGATGTCAATTAAAAAAGACGCAATTCAGTATTAAAAGTCTTGGACGGG---AAATTGCA
M. foe$_2$	TGGGTCGATGTCAATTAAAAAAGACGCAATTCAGTATTAAAAGTCTTGGACGGG---AAATTGCA
Bouea	CCCCCAAAAAGGGCCCATTTAACTCCCTAACGATTTATCCTATGT-TAGTGGTTCCAATTTCGTTATGTTC
M. sp	TCAAAAAGAAGACTTTGTAAGTTTCAGTATGAGCAATGAGATGGATTGTGAATC-ATTCACATGGAGATTC
M. lau$_1$	TCAAAAAGAAGACTTTGTAAGTTTCAGTATGAGCAATGAGATGGATTGTGAATC-ATTCACATGGAGATTC
M. lau$_2$	TCAAAAAGAAGACTTTGTAAGTTTCAGTATGAGCAATGAGATGGATTGTGAATC-ATTCACATGGAGATTC
M. odo$_1$	TCAAAAAGAAGACTTTGTAAGTTTCAGTATGAGCAATGAGATGGATTGTGAATC-ATTCACATGGAGATTC
M. odo$_2$	TCAAAAAGAAGACTTTGTAAGTTTCAGTATGAGCAATGAGATGGATTGTGAATC-ATTCACATGGAGATTC
M. zey	TCAAAAAGAAGACTTTGTAAGTTTCAGTATGAGCAATGAGATGGATTGTGAATC-ATTCACATGGAGATTC
M. qua	TCAAAAAGAAGACTTTGTAAGTTTCAGTATGAGCAATGAGATGGATTGTGAATC-ATTCACATGGAGATTC
M. ind	TCAAAAAGAAGACTTTGTAAGTTTCAGTATGAGCAATGAGATGGATTGTGAATC-ATTCACATGGAGATTC
M. foe$_1$	TCAAAAAGAAGACTTTGTAAGTTTCAGTATGAGCAATGAGATGGATTGTGAATC-ATTCACATGGAGATT
M. foe$_2$	TCAAAAAGAAGACTTTGTAAGTTTCAGTATGAGCAATGAGATGGATTGTGAATC-ATTCACATGGAGATT
Bouea	TCTCA--TTCATCCTACTCTTTTCCATTT-------GTATCCGAGCAGAATTTTTTCTCTTATCATACACAA
M. sp	CTTGCTCCAAAGTGTTCATTTCTACGTGTATCCTATATACCACACGACTTGTATATGATAAGAGAAAAAATT
M. lau$_1$	CCTGCTCCAAAGTGTTCATTTCTACGTGTATCCTATATACCACACGACTTGTATATGATAAGAGAAAAAATT
M. lau$_2$	CCTGCTCCAAAGTGTTCATTTCTACGTGTATCCTATATACCACACGACTTGTATATGATAAGAGAAAAAATT
M. odo$_1$	CTTGCTCCAAAGTGTTCATTTCTACGTGTATCCTATATACCACACGACTTGTATATGATAAGAGAAAAAATT
M. odo$_2$	CTTGCTCCAAAGTGTTCATTTCTACGTGTATCCTATATACCACACGACTTGTATATGATAAGAGAAAAAATT
M. zey	CCTGCTCCAAAGTGTTCATTTCTACGTGTATCCTATATACCACACGACTTGTATATGATAAGAGAAAAAATT
M. qua	CTTGCTCCAAAGTGTTCATTTCTACGTGTATCCTATATACCACACGACTTGTATATGATAAGAGAAAAAATT
M. ind	CCTGCTCCAAAGTGTTCATTTCTACGTGTATCCTATATACCACACGACTTGTATATGATAAGAGAAAAAATT
M. foe$_1$	CCTTGCTCCAAAGTGTTCATTTCTACGTGTATCCTATATACCACACGACTTGTATATGATAAGAGAAAAAAT
M. foe$_2$	CCTTGCTCCAAAGTGTTCATTTCTACGTGTATCCTATATACCACACGACTTGTATATGATAAGAGAAAAAAT
Bouea	GTCGTGTGGTATATAGGATACACGTAGAAATGAACACTTTGGAGCAAGGAATCTCCATGTGAATGATTCAC
M. sp	CTGCTCGGATAC-GTTTGTAAATGGAAAAGAGTAGGATGAATGA--GAAACATAACGAAATTGGAACCGCT
M. lau$_1$	CTGCTCGGATAC-GTTTGTAAATGGAAAAGAGTAGGATGAATGA--GAAACATAACGAAATTGGAACCGCT
M. lau$_2$	CTGCTCGGATAC-GTTTGTAAATGGAAAAGAGTAGGATGAATGA--GAAACATAACGAAATTGGAACCGCT
M. odo$_1$	CTGCTCGGATAC-GTTTGTAAATGGAAAAGAGTAGGATGAATGA--GAAACATAACGAAATTGGAACCGCT
M. odo$_2$	CTGCTCGGATAC-GTTTGTAAATGGAAAAGAGTAGGATGAATGA--GAAACATAACGAAATTGGAACCGCT
M. zey	CTGCTCGGATAC-GTTTGTAAATGGAAAAGAGTAGGATGAATGA--GAAACATAACGAAATTGGAACCGCT
M. qua	CTGCTCGGATAC-GTTTGTAAATGGAAAAGAGTAGGATGAATGA--GAAACATAACGAAATTGGAACCGCT
M. ind	CTGCTCGGATAC-GTTTGTAAATGGAAAAGAGTAGGATGAATGA--GAAACATAACGAAATTGGAACCGCT
M. foe$_1$	CTGCTCGGATAC-GTTTGTAAATGGAAAAGAGTAGGATGAATGA--GAAACATAACGAAATTGGAACCGCT
M. foe$_2$	CTGCTCGGATAC-GTTTGTAAATGGAAAAGAGTAGGATGAATGA--GAAACATAACGAAATTGGAACCGCT
Bouea	AATCCATCTCATTGCTCATACTGAAACTTACAAAGTCTTCTTTTTGAATATTCAAGAAATGCAATTTCCCGT
M. sp	AACATAGGATAAATCGTTAGGGAGTTAAATGAGCCCTTTTTGGGGGGATTTGGGGATAGAGGGACTTGAACC
M. lau$_1$	AACATAGGATAAATCGTTAGGGAGTTAAATGGGCCCTTTTTGGGGGGATTTGGGGATAGAGGGACTTGAACCA
M. lau$_2$	AACATAGGATAAATCGTTAGGGAGTTAAATGGGCCCTTTTTGGGGGGATTTGGGGATAGAGGGACTTGAACCA
M. odo$_1$	AACATAGGATAAATCGTTAGGGAGTTAAATGAGCCCTTTTTTGGGGGATTTGGGGATAGAGGACCTTGAACCA
M. odo$_2$	AACATAGGATAAATCGTTAGGGAGTTAAATGAGCCCTTTTTTGGGGGATTTGGGGATAGAGGGACTTGAACCA
M. zey	AACATAGGATAAATCGTTAGGGAGTTAAATGGGCCCTTTTTGGGGGGATTTGGGGATAGAGGGACTTGAACCA
M. qua	AACATAGGATAAATCGTTAGGGAGTTAAATGAGCCCTTTTTGGGGGGATTTGGGGATAGAGGAACTTGAACCA
M. ind	AACATAGGATAAATCGTTAGGGAGTTAAATGGGCCCTTTTTGGGGGGATTTGGGGATAGAGGAACTTGAACCA
M. foe$_1$	AACATAGGATAAATCGTTAGGGAGTTAAATGAGCCCTTTTTTGGGGGATTTGGGGATAAA----------
M. foe$_2$	TAGGATAAATCGTTAGGGAGTTAAATGAGCCCTTTTTTGGGGGATTTGGGGATA----------

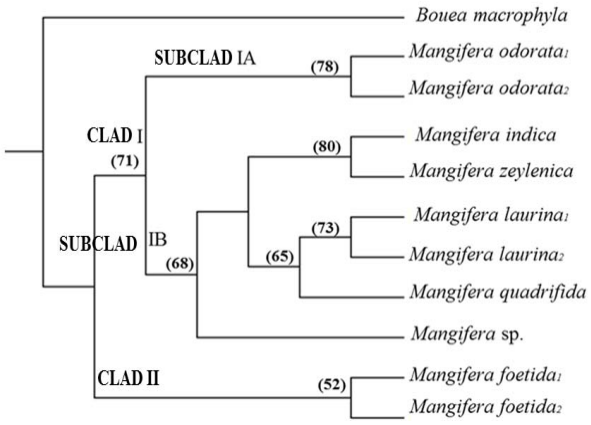

Figure 1. Cladogram of *Mangifera* based on *trnL-F* sequences with Maximum Parsimony Method with a bootstrap value above the branch.

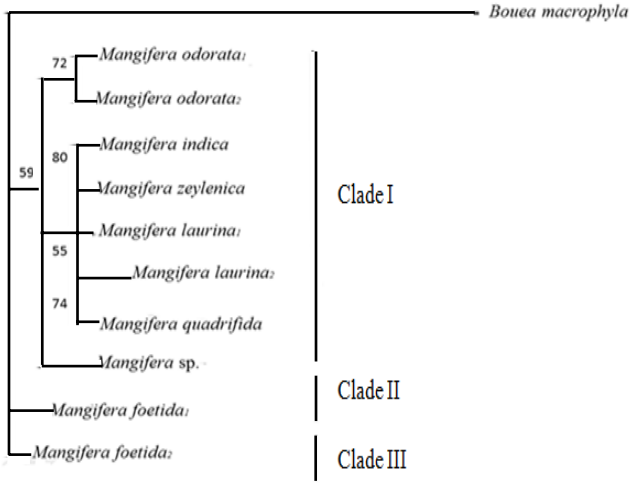

Figure 2. The cladogram of *Mangifera* based on *trnL-F* sequences with a Neighbour-Joining method. The number of branches indicated bootstrap values 100x

Phylogenetic analyses of *Mangifera*

From the result of Parsimony analysis based on the sequences data of *trnL-F* intergenic spacer of *Mangifera* in Northern Sumatra, it was obtained a cladogram with the first clade consisted of several *Mangifera*, i.e. *M. odorata*$_1$, *M. odorata*$_2$, *M. indica*, *M. zeylanica*, *M. laurina*$_1$, *M. laurina*$_2$, *M. quadrifida* and *Mangifera* sp., and clade II consisted of *M. foetida*$_1$ and *M. foetida*$_2$ only. Based on Neighbour-Joining analysis which showed differences, Neighbour Joining tree showed tree clades in which the first clad consisted of *M. odorata*$_1$, *M. odorata*$_2$, *M. indica*, *M. zeylanica*, *M. laurina*$_1$, *M. laurina*$_2$, *M. quadrifida* and *Mangifera* sp., the second clade consisted of *M. foetida*$_1$ only, and the third clade consisted of *M. foetida*$_2$ only. Both of this *M. foetida* were separated and formed each clade.

A phylogenetic analysis was conducted by using a Maximum Parsimony method to get a phylogenetic tree, in which the tree was a monophyletic tree with two main clades. Clade I and Clade II were separated because of the different nucleotide in 4, 420, 422 (A-G), 425 (C-T), 426 and 427 (C-G). The first clade consisted of two subclades, i.e. subclade IA and subclade IB, while the second clade consisted of just *M. foetida*$_1$ and *M. foetida*$_2$. Subclade IA consisted of just *M. odorata*$_1$ and *M. odorata*$_2$ which joined into a sister group with a different nucleotide in 218 (C-T) and 418 (G-A). Both of these *Mangifera* came from two different regions, because of just a few sample was inadequate to show a diversification.

The subclade IA, *M. odorata* among *M. foetida* and another *Mangifera*, showed a unique position by became an intermediate of subgenus *Limus* and *Mangifera*. This species belonged to subgenus *Limus* according to Kosterman and Bompard (1993). It suggested that *M. odorata* was a hybrid from *M. foetida* and *M. odorata* (Hou 1978), and another molecular evidence by Eiadthong and Yonenomori (2000) based on AFLP analysis also suggested that this *Mangifera* was a natural hybrid from *M. foetida* and *M. indica*.

The subclade IB consisted of *M. indica*, *M. zeylanica*, *M. laurina*, *Mangifera* sp. and *M. quadrifida.* From this subclade IB, *M. indica* joined to *M. zeylanica* that formed a sister group with a similarity of morphological character has a cushion-like floral disk and the number of the fertile stamen is just one, it was very rarely (Kosterman and Bompard 1993). Based on a morphological character of *M. indica* and *M. zeylanica* showed some similarities by grouping into a sister group (Fitmawati et al. 2013) and so does by *trnL-F* sequences. In subclade IB, *Mangifera* sp. was separated from *M. quadrifida* and *M. laurina*. *Mangifera* sp. was assumed as a new species.

Based on Neighbour-Joining method (Figure 2), *Mangifera* formed three clades. *Mangifera laurina*$_2$ had the longest branch and this species was assumed as a species which underwent a highest changing character. Other molecular evidence was conducted by Fitmawati and Hartana (2010) and showed that *M. laurina* had the longest branch, but the earlier occurrence showed that *M. foetida* was earlier than others *Mangifera*. *Mangifera foetida* was suggested as a progenitor from other *Mangifera*. Based on the morphological characters, *M. foetida* was more primitive than *M. laurina*. Fitmawati (2015) classified *M. foetida* as a wild type and *M. laurina* was a semi-wild type. This study demonstrated that *trnL-F* region clearly separated *M. foetida* which belonged to subgenus *Limus* and subgenus *Mangifera*, and showed a unique position of *M. odorata* which presumed as a hybrid from *M. foetida* and *M. indica.*

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