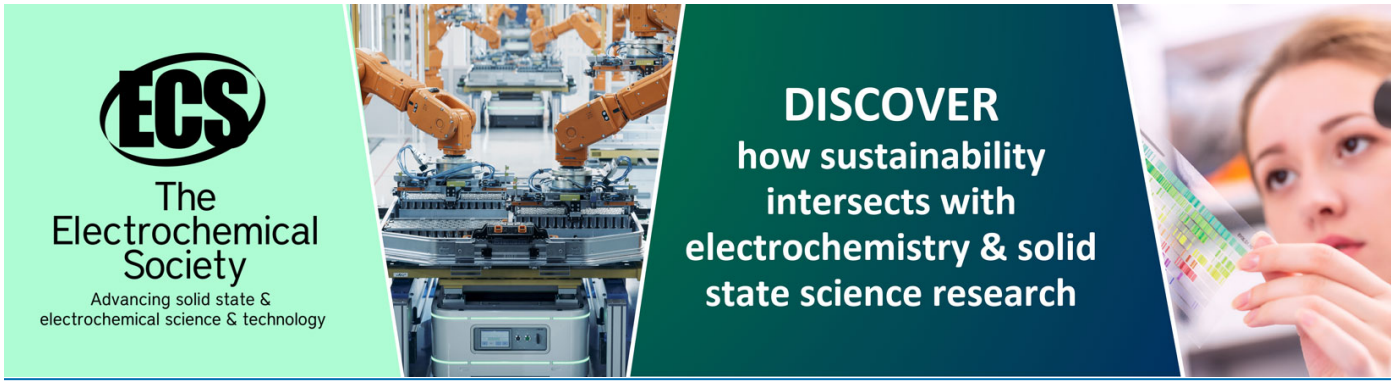
Phylogenetic Study of Genus *Mangifera* in Southern Sumatera Based on DNA Sequences of the *Internal Transcribed Spacer* (ITS) Region



Phylogenetic Study of Genus *Mangifera* in Southern Sumatera Based on DNA Sequences of the *Internal Transcribed Spacer* (ITS) Region

Fitmawati[1]*, M R Ningrum[1], R Mahatma[1] and F Suzanti[2]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Riau Kampus Binawidya Jl. HR. Soebrantas Km 12. 5 Pekanbaru, Riau, Indonesia
[2]Department of Biology, Faculty of Education, Universitas Riau Kampus Binawidya Jl. HR. Soebrantas Km 12. 5 Pekanbaru, Riau, Indonesia

*E-mail: fitmawati2008@yahoo.com

Abstract. The diversity of *Mangifera* in Southern Sumatra has not been thoroughly explored and studied. On the other hand, diversity of the existing *Mangifera* species is threatened with extinction due to the decline of their natural habitat in forest areas. This condition will automatically cause disappearance of *Mangifera* information, whereas the diversity has not been well identified and still being unclear upon its species stations. The purpose of this study was to reconstruct *Mangifera* relationship based on the sequences of nuclear ribosomal DNA (nrDNA) at the ITS region. DNA isolation of *Mangifera* leaf used CTAB method with slight modifications. DNA sequencing was conducted at First Base Laboratories, Malaysia. The reconstruction used PAUP program* Version 4.0b10 in both *maximum parsimony* (MP) and *neighbor joining* (NJ) methods. Cladogram by MP analysis formed a monophyletic clad with two main clads. The first clad consisted of *M. foetida1, M. foetida2, M. foetida3, M. odorata, M. kemanga1, M. indica1, M. indica2, M. zeylanica* and *M. lalijiwa*; and the second clad consisted of *M. torquenda, M. quadrifides* and *M. kemanga2*. Cladogram by NJ analysis strongly supported MP analysis and revealed that *M.indica2* has the longest genetic distance compared to other species.
Keywords: phylogenetic analysis, ITS, *Mangifera*, molecular marker, Southern Sumatera

1. Introduction

Mango is a potential fruit which has been long developed and become one of the leading tropical fruit favored by people in the world so that having the title as The Best Loved-Tropical [1]. One of the challenges of mango cultivation is to have a high level of genetic variety [2]. Kostermans and Bompard [3] stated that mangoes known to the general public as common mango fruits are the members of *Mangifera indica*. Other members of the *Mangifera* genus that can be consumed have lower quality in fruits and are commonly known as wild mangoes (mango relatives).

The natural habitat of the *Mangifera* genus is limited to the lowland of tropical rain forest [3]. However, *Mangifera* is able to grow in a wide range of conditions both in the tropics and subtropics [4]. The polyembrionic- *Mangifera* genus is originally from tropical Asia, most of them are found in Peninsular Malaysia, Sumatra, Java, Kalimantan and Sulawesi, with a high genetic diversity [3]; whereas, monoembrionic is originally from South Asia. Variation on fruits, flavors and names of

mangoes are easily found throughout Indonesia [5]. The availability of mango genetic diversity (*Mangifera*) in Indonesia is a high natural-potency that requires a deeper study and better understanding, and becomes an important aspect to actualize an improvement program of mango cultivation.

Sumatra had become one of the areas in Indonesia with a high diversity of *Mangifera* species [3]. Sumatra is one of the most diverse ecoregions in the world. The Collection of *Mangifera* on the island of Sumatra has started and pioneered by Kostermans and Bompart [3]. Exploration of *Mangifera* both wild and cultivated has been conducted in Central Sumatra since 2011-2013 [6]. However, exploration and survey of *Mangifera* diversity in southern Sumatra has never been done. Such activities are essential to be able to explain genetic diversity and determine the exact extent of the taxa on *Mangifera* species in southern Sumatra. The crops management of this fruit will be much more effective and efficient if their characterization is accurate so that a clear grouping can be established for reference to plant breeders, farmers, traders and researchers [7].

The decrease of *Mangifera*'s natural habitat in forest area is caused by expansion of oil palm plantations, deforestation, industrialization, habitat alteration and so on, causing Sumatran *Mangifera* species to be threatened [6]. Exploration, identification and rescue of *Mangifera*-germplasm resources must be raced with the rapid transfer of land functions before the disappearance of tens to hundreds of species of *Mangifera* in Sumatra. Conservation and cultivation should be undertaken to prevent this from happening. The first step in determining conservation strategies and cultivation techniques is the definition of a well-reconstructed evolutionary relationship of the *Mangifera* species [8].

Phylogenetic studies through molecular approaches can analyze the genetic relationships based on the evolutionary lines of each group of organisms and become one of the more comprehensive support of morphological characters [9]. Molecular-based phylogenetic studies can be performed using different molecular markers. Many of the molecular markers are used to identify *Mangifera* species such as AFLP markers from nuclear DNA [9], chloroplast DNA [11], DNA sequences such as trnL-F [12-14] to matK, rbcL [15] and ITS [16,17].

The DNA sequence of the ITS (*Internal Transcribed Spacer*) region was used in this study because of its effective use in solving phylogenetic cases of various taxa [18]. Over the past two decades, the DNA sequence of the ITS region is a sequence of nuclear ribosomal DNA (nrDNA) which can be utilized in tracing types of species that have a closer genetic relationship, discrimination in a variation, recognition of new species, and intraspecies and intrasub-species diversity [19].

DNA markers of the ITS region have a high number of copies of DNA, short size, high conservation areas, rapidly integrated evolution, and are classified as universal primers [18]. Therefore, the sequence of DNA of the ITS region is considered an appropriate tool for analyzing the diversity of *Mangifera* in southern Sumatra, which is part of the *Mangifera* dispersal region in Sumatra to obtain information in support of further research.

2. Materials and methods

2.1. Plant materials

This study used 13 species of *Mangifera* genus taken from three areas in southern Sumatra ie, 9 species taken from Bengkulu, 3 species from Lampung and 1 species from Palembang. Several species in this study used the same species to compare the ability of the ITS regional sequences in analyzing the phylogenetic relationship of the *Mangifera* genus at the cultivar level (table 1). Phylogenetic analysis involved two other genera as outgroup taken from Genebank [17] ie *Anacardium occidentale* L. and *Bouea macrophylla* Griff, with consecutive accession numbers AB071690 and AB071691 respectively.

Table 1. The list of *Mangifera* genus collections at Botanical Laboratory, Faculty of Mathematics and Natural Sciences – University of Riau, obtained from areas studied in the southern Sumatran and two outgroups from genebank.

Spesies Name	Section	Subgenus	Origin of Collection			NCBI Accession Number
			B	L	P	
***M. quadrifida* Jack.**	*Mangifera*	*Mangifera*	√			
***M. kemanga* Bl. (1)**	*Deciduae*	*Limus*	√			
***M. kemanga* Bl. (2)**	*Deciduae*	*Limus*			√	
***M. torquenda* Kosterm.**	*Mangifera*	*Mangifera*	√			
***M. laurina* Bl.**	*Mangifera*	*Mangifera*	√			
***M. odorata* Griff.**	*Perennis*	*Limus*		√		
***M. indica* L. (1)**	*Mangifera*	*Mangifera*		√		
***M. indica* L. (2)**	*Mangifera*	*Mangifera*	√			
***M. zeilanica* Hooker f.**	*Mangifera*	*Mangifera*	√			
***M. foetida* Lour. (1)**	*Perennis*	*Limus*	√			
***M. foetida* Lour. (2)**	*Perennis*	*Limus*	√			
***M. foetida* Lour. (3)**	*Perennis*	*Limus*	√			
***M. lalijiwa* Kosterm.**	*Mangifera*	*Mangifera*		√		
***Anacardium occidentale* L.**						AB071690
***Bouea macrophylla* Griff.**						AB071691

B: Bengkulu; L: Lampung; and P: Palembang

2.2. Work procedures

DNA was isolated from 2 grams of young leaf tissue using the modified CTAB method by [20] and precipitated using 96% of cold alcohol for 24 hours at 4°C. The pellets are washed with 70% alcohol and then the DNA is stored in a TE buffer at -20°C.

DNA amplification was performed under ITS conditions on PCR [15] using a primary set. DNA was amplified with primer of ITS *forward* (5'-GGA AGT AAA AGT CGT AAC AAG G-3') and *reverse* (5'-TCC TCC GCT TAT TGA TAT GC-3'). The PCR product was administered at electrophoresis of 1.2% agarose gel at 100 volts for 20 mins. The sequencing process was performed using *Single Pass* DNA with the same forward primer in the PCR process. PCR products were sent for sequencing at First BASE Laboratories through PT. Genetics Science Indonesia.

Determination of DNA sequences of ITS areas refers to Genebank *Mangifera* (NCBI) data from Yonemori *et al*. [17]. Data sequence in alignment used ClustalW, and then was checked manually with BioEdit. Reconstruction of phylogenetic tree utilized both PAUP* program version 4.0b10 [21] with Neighbour Joining (NJ) method using 100x bootstrap analysis.

3. Results

3.1. Multiple Alignment Analysis of DNA Sequence of Mangifera Genus at ITS Region.

The phylogenetic relationship studies of the 13 genera of Mangifera from southern Sumatra analyzed the comparison of nuclear ribosomal DNA (nrDNA) sequences of the ITS region. The studied area of ITS in the genus Mangifera was determined based on Genebank [17]. The sequencing of the nucleotide base of the ITS region produces a total length of 655-656 bp. The alignment of the entire Mangifera genus sequence encompasses the areas of ITS1, 5.8S and ITS2 produces a length of 656 bp (figure 1). Analysis of the sequence of ITS region in the in-group produced 78 informative sites for

uses in phylogenetic analyzes. Sequence alignment of the entire genus Mangifera and the studied out-group resulted in 664 bp with 99 informative sites for phylogenetic analysis (table 2). There are, 447 sites are constant and 118 characters are parsimony-uninformative, not showing any nucleotide base variation. Constant characters have the same nucleotide bases in all species while the informative character explains at least two different characters, thus the explanation of the variations on the site resulting from the evolutionary process.

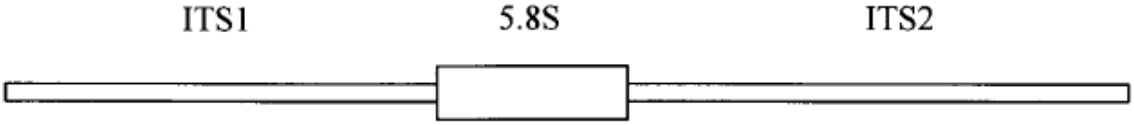


Figure 1. Position profile of ITS1, 5.8S and ITS2 genes in ribosomal DNA area.

The length of ITS1 was 264 pb and ITS2 ranged from 228-229 bp on the studied *Mangifera* species. The 5.8S gene region has no a long-base variation, all the studied *Mangifera* species have a length of 163 bp. According to [17] *Bouea macrophylla* as out-group taxa shows nearly the same length between ITS1 and ITS2 with *Mangifera* species as following: 264 bp and 225 bp respectively. *Anacardium occidentale* has a shorter length in the area of ITS1 (232 bp) and ITS2 (220 bp) than the other species studied. The length of the 5.8S gene region in the two out-groups has a small variation in length over *Mangifera* species (table 2).

The DNA base composition is expressed as G + C content, where the G + C content in all species analyzed has a mean of 61.74%. Under Chargaff's law, the content of G + C in the nucleotide sequence varies with a range of values 26% - 74% [22]. GC-rich DNA is more stable in structure compared to AT-rich DNA although other interacting factors affect the stability of DNA structures. The content of G + C shows a percentage of more than 50% in the area of ITS1, 5.8S and ITS2 genes for the entire genus *Mangifera* studied.

3.2. Phylogenetic Studies of the Mangifera Genus in Southern Sumatra

3.2.1. Study Analysis of Maximum Parsimony (MP). The results of the maximum parsimony (MP) analysis based on the sequence of ITS data are summarized in table 2. Based on criteria parsimony, it was obtained a cladogram with consistency index value (CI) = 0.87 and retention index (RI) = 0.84. The CI value is a parameter to measure the relative amount of homoplacy in phylogenetic trees. The value of CI = 1 if the character does not have homoplasi [23].

Homoplation is a character found among different taxa but not found in the same ancestor. Referring to Genebank data [17], the value of CI genus *Mangifera* studied in the ITS region was obtained with ITS1 (0.76) gene 5.8S (1.00) and ITS2 (0.96) (table 2). The Retention Index (RI) becomes a parameter to measure the proportion of sinapomorphs in trees as a measure of how well the sinapomorph character in describing phylogenetic trees. RI = 1 if the DNA characters are consistent with phylogeny [23]. The MP analysis is presented in two trees, a cladogram with branches representing the value of tree-branch strength with a 100X bootstrap replication (figure 2) and a cladogram with a nucleotide base character change (figure 3).

The phylogenetic tree of MP analysis separates the in-group into two main clans at branch number 26. Clad I consists of 10 members of the *Mangifera* genus with a bootstrap value of 66%. Clad II consists of 3 members of the *Mangifera* genus with a bootstrap value of 100%. MP analysis produces the shortest cladogram with the smallest number of changes. Hidayat and Pancoro [8] assumed that only the smallest nucleotide base changes are capable of providing a good explanation of the evolutionary process taking place.

Table 2. Characteristics of the ITS region of *Mangifera* species in southern Sumatra and the combination with *outgroup*

	Length Range (nt)	Length Average (nt)	Length after *Alignment* (nt)	G+C Content (%)	Content Average G+C (%)	Number of Informative Sites	Tree Length*	CI*	RI*
***Mangifera* spp.**									
ITS1	264	264	264	63,64-67,80	65,41	35	52	0,78	0,89
5.8S rDNA	163	163	163	54,60-55,83	55,49	2	3	1,00	1,00
ITS2	228-229	228,30	229	53,71-61,40	59,55	41	96	0,94	0,94
Entire Sequence	655-656	655,23	656	58,84-62,14	60,90	78	152	0,88	0,91
***Mangifera* spp. + 2 taksa outgroup**									
ITS1	231-264	261,80	269	63,64-71,97	66,28	42	123	0,83	0,84
5.8S rDNA	162-163	162,93	166	54,60-55,83	55,60	5	9	1,00	1,00
ITS2	220-229	227,53	231	53,71-73,18	60,90	52	161	0,90	0,85
Entire Sequence	615-656	652,33	664	58,84-68,46	61,74	99	300	0,87	0,84

* Obtained from maximum parsimony-analyzed result in PAUD program
CI is the consistency index and RI is the retention index

The MP method uses a sequence of nucleotides or amino acids directly as a basis for reconstructing phylogeny trees [23]. Clad I is formed by the base difference at the order of 35, 72 (C-T), 89, 146, 203 (A-G), 251, 529 (G-C) and 630 (T-C). Clad I divides its members into two clans at branch number 23. In that branch *M. lalijiwa* separates itself from the other members by changing 6 nucleotide bases at 41 (CG), 61 (GT), 75, 480 (CT), 533 and 642 (CT). The next branch of *M. zeylanica* also brakes away at branch number 22 with the change of 2 nucleotide bases at the order of 201 (A-G) and 372 (C-T).

M. lalijiwa is closely related to *M. laurina* and *M. indica*. *M. lalijiwa* is distinguished from *M. laurina* and *M. indica* based on the characteristic of cherished leaves (*coriaceus*), streaks attached to the base of flower ornament [3]. Characteristics of *M. lalijiwa* with his close relatives are flower organs of 4-5 merous, the number of 1-2 fertile stamen, and seeds-not-labyrinth. Based on the shape of the tree canopy, mangoes are distinguished over the rounded headings possessed by *M. lalijiwa* and *M. laurina* while *M. indica* has a canopy form upward and laterally. *M. lalijiwa* has a dark green- colored leaf that is different from *M. laurina* and *M. indica* which has a light green leaf color to green. The reticulation pattern of *M. lalijiwa* is density with two branches of more than two, *M. indica* reticulation is bifurcated and sparse, while *M. laurina* has a tightly branched and multiples reticulation. Both *M. lalijiwa* and *M. indica* have a compound flower arrangement (*glomerulate*), while *M. laurina* has a non-glomerulate interest structure [2].

Branch number 21 places 9 *Mangifera* species in two different groups. The first group lies in branch number 18 which classifies *M. foetida1*, *M. foetida2*, *M. foetida3*, *M. odorata*, and *M. kemanga2* in the same branch with a bootstrap value of 88%. *M. kemanga2* is separated from the other four members. The second group lies in branch number 20 which classifies *M. indica1*, *M. indica2* and *M. laurina* with a bootstrap value of 99%. Clad II consists of 3 members of the *Mangifera* genus with a bootstrap value of 100%. These clad members consist of *M. kemanga1*, *M. quadrifida* and *M. torquenda* (figure 2 and 3).

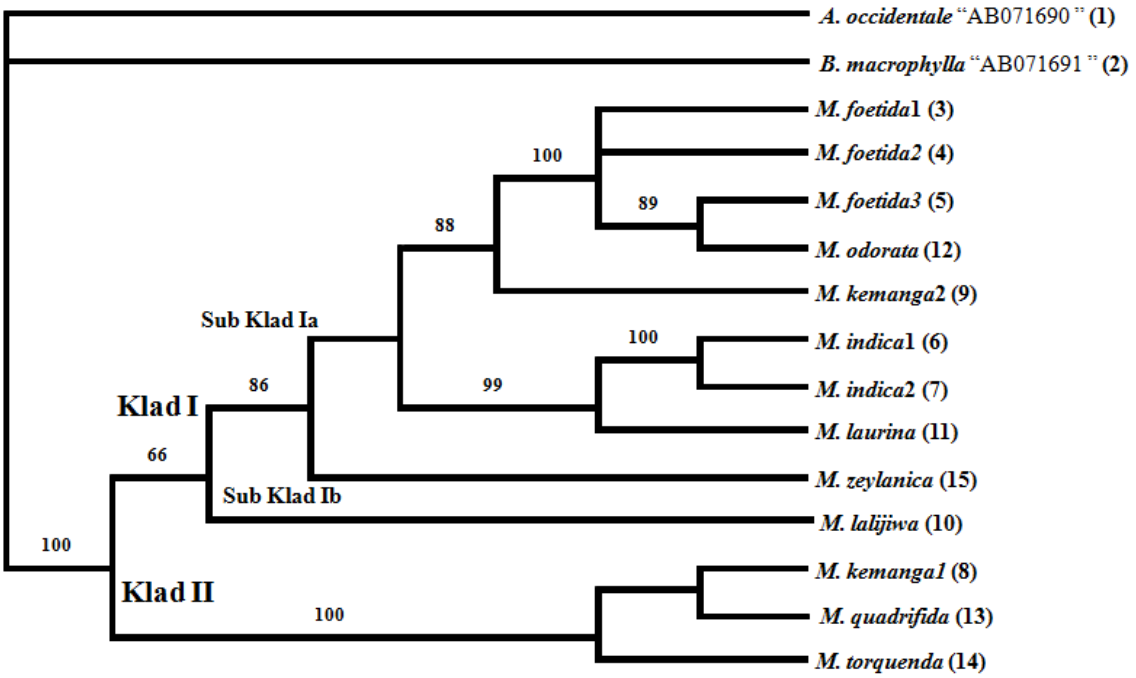


Figure 2. Phylogenetic tree based on maximum parsimony method. The number in the middle of the branch line shows the bootstrap value of 100X.

The second clad classifies *M. kemanga1* with *M. quadrifida* and *M. torquenda*. Kostermans and Bompard [3] classified *M. quadrifide* and *M. torquenda* into the *Mangifera* subgenus, which was considered more advanced than the *Limus* subgenus. Based on the morphological character of the

flower [5], *M. torquenda* and *M. quadrifida* have white flower and floral ornament (multiples 4) that are different from *M. kemanga*, based on the description of [3], which has pinkish-purple flowers and flower ornament (multiples of 5). Fitmawati *et al*. [12] explained that by using the trnL-F Intergenic Spacer marker of the *Magifera* type can be able to be grouped between *M. kemanga* with *M. laurina* and *M. odorata* in the same clad. Fitmawati *et al*. [16] placed *M. quadrifide* into separated clad from *M. torquenda* and *M. kemanga*. *M. quadrifida* emerged as the earliest species in Central Sumatra into one of the wild species found in the Sumatran Forest [2]. These findings remain as important notes, despite having the contradiction of the position of the *M. kemanga* species, in order to be a reference in the taxonomic studies of the type of *M. kemanga*.

3.2.2. Neighbor Joining Analysis Study (NJ). The results of neighbor joining (NJ) analysis are shown in (figure 3) with the length of the whole horizontal branch cladogram of 0.48721 that separates the in-groups into the two main clans ie clad I as first cluster and clad II as second clan. Clad I and Clad II are separated on branch number 27. The branches that make up Clad I have a length of 0.00221 while the Clad II is 0,02965. NJ analysis results a cladogram constructed based on the difference of nucleotide base pairs between two DNA sequences. According to [9], the changes that make up the different pairs of nucleotide bases show the evolutionary distance that occurs in a species.

The phylogenetic tree of the MP and NJ method shows the in-group species forming the same clad. The line on the NJ phylogenetic tree illustrates the proximity of evolutionary processes occurring between the studied in-group species. The longer the branching line, the further the evolution is; and the shorter the line, the closer the evolutionary distance [24].

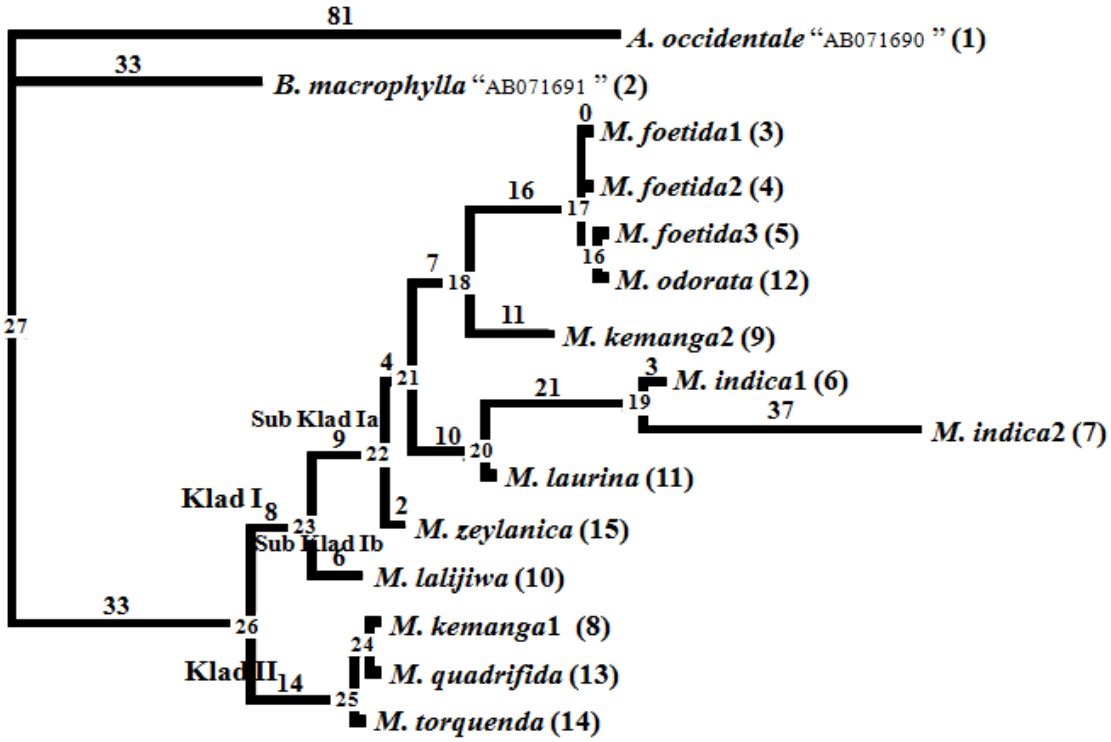


Figure 3. Phylogenetic tree with maximum parsimony method based on nucleotide base character change. The number in the middle of the branch shows the branch number, the number above the branch indicates the length of the branch based on the nucleotide base changes.

M. foetida is associated with *M. odorata* forming a monophyletic group and is associated with *M. kemanga2*, the group of *M. indica1*, *M. indica2*, *M. laurina* and is connected with *M. zeylanica* in sub Clad Ia (figure 4), it is same as the tree on Maximum parsimony analysis (figure 2 and 3). The same

pattern occurred between both the neighbor-joining and the parsimony tree, where they both also separate *M. kemanga1* with *M. kemanga2* on different main clad, while both are collections of the same species.

The in-group species in Clad I consists of 10 *Mangifera* species, separating *M.lalijiwa* from the other group on branch number 24 with the length of 0.01314. NJ phylogenetic tree shows that *M. lalijiwa* species emerged as ancestors in Clad Ib, with shorter branching lines. *M. lalijiwa* evolved more slowly than other species in Clad I. Konsterman and Bompard [3] classified *M. lalijiwa* into the *Mangifera* section within *Mangifera* subgenus along with *M. indica*, *M. laurina* and *M. zeylanica*. The four types of *Mangifera* are unified by the characteristic equations of flower characters that possess 5 flower ornaments, 5 stamen and 4 staminodia. The short branch of *M. lalijiwa* shows that the species has a shorter and more primitive evolutionary distance compared to other *Mangifera* species on Clad I.

The *M. indica2* species is the most modern offspring with more advanced characters based on the sequence of ITS regions when compared to other *Mangifera* species. *M. indica1* with *M. indica2* is the same species but has different branch lengths where *M. indica1* has a shorter branch length. This event can occur because both types come from different places. *M. indica1* is a collection of Botanical Laboratory taken from Lampung city while *M. indica2* was taken from Palembang (table 1). The collection location can be an affecting factor on genetic diversity. Cultivation selection pressures can also be a determinant factor of the differences between them.

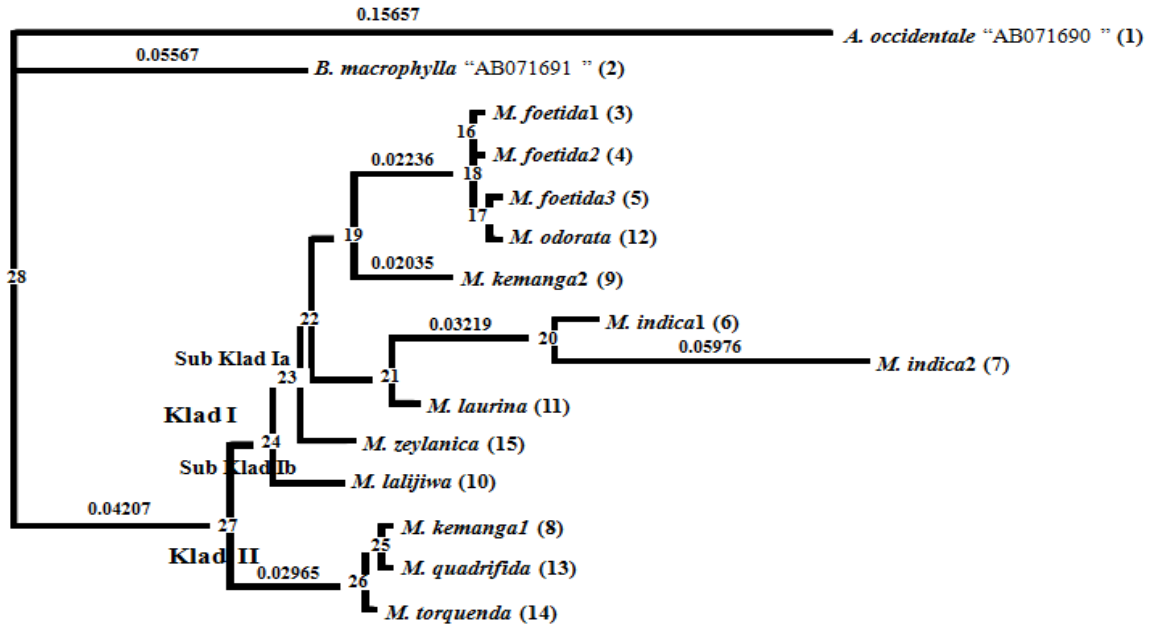


Figure 4. Phylogenetic tree based on Neighbor Joining method with genetic distance of HKY85 model.

The genetic distance matrix in table 3 shows the relationship between *M. indica2* with *M. kemanga1*, *M. torquenda* and *M. quadrifida* far enough compared to the others when viewed from its matrix value, that is, it has 0.16254, 0.16266 and 0.16067 respectively. The lowest genetic distance matrix with value 0.0000 was obtained between *M. foetida1* and *M. foetida2* indicating that the genetic relationship between the two is very close and can even be identical. A close enough relationship is also obtained in *M. foetida1* with *M. foetida3* and *M. foetida2* with *M. foetida3* which have a matrix of 0.00256. The value of matrix of genetic distance obtained is quite interesting where *M. quadrifide* with *M. torquenda* have a value of 0.00153, smaller than the matrix value between *M. foetida1* with *M. foetida3* and *M. foetida2* with *M. foetida3*, whereas both are different species. *M. foetida1*, *M. foetida2* and *M. foetida3* are the same species, but they are different cultivars.

Table 3. Matrix of genetic relationship distance in Neighbor Joining method using HKY85 model

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1 *A.occidentale*	-														
2*B.macrophylla*	0.21224	-													
3 *M.foetida1*	0.23495	0.12724	-	*											
4 *M.foetida2*	0.23495	0.12724	0.00000	-											
5 *M.foetida3*	0.22998	0.13118	0.00306	0.00306	-										
6 *M.indica1*	0.27140	0.16962	0.09138	0.09138	0.09138	-									
7*.M.indica2*	0.33628	0.22446	0.14516	0.14516	0.14325	0.06365	-								
8*.M.kemanga1*	0.22293	0.13136	0.06238	0.06238	0.06579	0.10742	**0.16254**	-							
9*.M.kemanga2*	0.24690	0.14226	0.04261	0.04261	0.04591	0.06708	0.11733	0.07767	-						
10*.M.lalijiwa*	0.22518	0.12179	0.05410	0.05410	0.05410	0.07230	0.12838	0.04601	0.04900	-					
11*.M.laurina*	0.23444	0.13123	0.05242	0.05242	0.05242	0.03596	0.09420	0.07101	0.04082	0.03930	-				
12*.M.odorata*	0.22960	0.13315	0.00460	0.00460	0.00153	0.09317	0.14515	0.06750	0.04757	0.05579	0.05409	-			
13*.M.quadrifida*	0.22328	0.13159	0.06244	0.06244	0.06585	0.10752	**0.16266**	0.00153	0.07775	0.04607	0.07109	0.06757	-		
14*.M.torquenda*	0.22079	0.12959	0.06074	0.06074	0.06414	0.10569	**0.16067**	0.00153	0.07600	0.04440	0.06936	0.06585	0.00153	-	
15*.M.zeilanica*	0.22182	0.11444	0.04265	0.04265	0.04596	0.05898	0.11400	0.05415	0.03770	0.02645	0.02494	0.04763	0.05421	0.05253	-

Description: The bold number represents the high-relative species range matrix, the star with the low genetic relationship matrix.

4. Discussion

The MP analysis using the DNA sequence of the ITS region can reveal a very close relationship between *M. foetida* and *M. odorata*, where *M. foetida3* is relatively closer to *M. odorata* than *M. foetida1* and *M. foetida2* (figure 2). Three *M. foetida* cultivars lie in the same group and have a slight variation of sequences among the cultivars. Konsterman and Bompard [3] classified *M. foetida* and *M. odorata* into the *Limus* subgenus based on the floral disk equation and they are equal in the Perennes section because of the non-deciduous characteristic similarities. Subgenus *Limus* is one of two subgenus grouped by [3] based on multiples of flower organs and floral disk shape. The proximity of phylogenetic relationships was both supported by previous studies that also used the DNA sequences of the ITS region [16] and Thailand [17]. This study reinforces the theory of *M. odorata*'s proposal as a hybrid of *M. foetida* and *M. Indica* [25], although this proposal was denied [3] which states that *M. odorata*'s reticulation differs from *M. indica* and *M. Foetida;* and *M. odorata* are not intermediates between the two species.

The proximity of *M. indica* and *M. laurina* is shown in branch number 20 supported by the opinion of [17] using the ITS sequence against 14 *Mangifera* species in Thailand, classifying 7 *M. indica* cultivars that clumped into one klad with *M. laurina*. According to [3] based on the morphological characters, *M. indica* and *M. laurina* have floral disks that are cushion-like with the number of fertile stents 1. The character that distinguishes this species is that *M. indica* has a glomerulate flower, hairy at the flowering branch whereas in the species *M. laurina* flowers are not glomerulate.

The closeness between *M. indica* and *M. laurina* was also suggested by [16] using the ITS sequence of 10 *Mangifera* species in Central Sumatra. They revealed that *M. indica* as a monophyletic group with *M. laurina* and *M. kemanga*, although the status of *M. kemanga* is still controversial. In this research a unique pattern is formed where 2 species of *M. kemanga* were collected from 2 different places ie Bengkulu (*M. kemanga1*) and Palembang (*M. kemanga2*) clustering on different clusters. In the first clad *M. kemanga2* grouped with 3 cultivars *M. foetida* and *M. Odorata* [3]. Grouped the three types into the subgenus *Limus*. *M. odorata*, *M. foetida* and *M. kemanga* are classified in subgenus *Limus* (more primitive), but these three species belong to different sections. *M. kemanga* is a *Deciduae* section that has bractea covering young leaves while *M. odorata* and *M. Foetida* do not have bractea and included into section *Perennes*.

Three species of *M. foetida* studied in this study are 3 types of infraspesies differentiated by different fruit characters. *M. foetida1* is *Macang susu* with a special character in which the fruit has a mamma gland-like bulge on the fruit tip. An oval *Macang* is a specimen of *M. foetida2* which has a longer-fruit character so that the shape of the fruit is slightly oval than *M. foetida* in general. *M. foetida3* with the local name is *Macang lado*, with a sweet taste and has a smaller size than the *Macang* commonly known by the community. Kosterman and Bompard [3] described *M. foetida* as a fruit that has a rounded shape, dirty green with brown spots and a lot of fiber.

The ITS region has a high sequence variation because it is a non-coding region that has a higher mutation rate than the coding region [26]. However, the location of the ITS region in the nucleotide sequence of rnDNA makes the area highly conserved so that this sequence has a smaller variation at the cultivar level of the *M. foetida* species in *Mangifera* genus. Existing matrix values provide clues that the genetic markers of the ITS region have lower variations at the level of infraspesies of *M. foetida* which was being analyzed. Unlike the matrix values between *M. kemanga1* and *M. kemanga2* which has a value of 0.07767. The value is quite distant when compared to *M. kemanga1* with *M. quadrifida* and *M. kemanga1* with *M. Torquenda*, which have a smaller matrix value of 0.00153. It means that the variation of DNA sequence of ITS regions between *M. kemanga* is very high. *M. kemanga1* and *M. kemanga2* are the same species. *M. kemanga1* is a collection of Botanical Laboratories taken from Lampung while *M. kemanga2* comes from Palembang.

5. Conclusion

Phylogenetic studies of 13 types of mangoes from the *Mangifera* genus in southern Sumatra using DNA sequences of ITS regions results a monophyletic group based on the maximum parsimony method. The Cladogram formed two main clans separating *M. torquenda*, *M. quadrifida* and *M. kemanga2* from 10 other *Mangifera* species. This analysis shows a close relationship between *M. foetida* and *M. odorata* and is relatively close to *M. kemanga1*. *M. indica2* formed a sister group with *M. indica2* and was separated from *M. laurina* which forms a monophyletic group. Together with *M. zeylanica* the nine species separated into subclad. It was separated from *M. lalijiwa* on Clad I. Clad II was formed on *M. torquenda*, *M. quadrifida* and *M. kemanga2*. Cladogram by NJ analysis strongly supported MP analysis and revealed that *M.indica2* has the longest genetic distance compared to other species.

Acknowledgements

This research was supported by Directorate General of Higher Education of Indonesia through Hibah Kompetensi 2017. The authors thank all parties involved in this study.

References

[1] Oktavianto Y S and Suryanto A 2015 *Jurnal Produksi Tanaman* **3** 91-97
[2] Fitmawati 2008 *Biosistematika Mangga Indonesia* [*dissertation*] (Bogor: Bogor Agricultural University)
[3] Kostermans A J G H and Bompard J M 1993 The mangoes: their botany, nomenclature, horticulture and utilization. In: Litz RE, editor. *The Mango: Botany, Production and Uses. 2nd Edition* (Massachussets: CABI)
[4] Gajera H P, Bambharolia R P, Rinkal K D, Patel S V and Golakiya B A 2013 *Plant Syst Evol* **300** 1011–1020
[5] Fitmawati, Hartana A and Bambang S P 2009 *J Agron. Indonesia* **37** 130 – 137
[6] Fitmawati, Suwita A, Sofiyanti N and Herman 2013 *Floribunda* **4** 169-179
[7] Fitmawati, Hartana A and Bambang S P 2010 *Journal of Breeding and Genetics* **42** 83–94
[8] Hidayat T and Pancoro A 2008 *Jurnal AgroBiogen.* **4** 35-40.
[9] Hidayat T and Pancoro A 2006 *Kursus Singkat Aplikasi Perangkat Lunak PAUP dan Mr Bayes untuk Penelitian Filogenetika Molekuler* (Bandung: ITB)
[10] Eiadthong W, Yonemori K, Kanzaki S *et al*. 2000 *J Am Soc Horti Sci.* **125** 160-164
[11] Eiadthong W, Yonemori K, Sugiura A, Utsunomiya N and Subhadrabandhu S 1999 *Scientia Horticulturae* **80** 145-155
[12] Fitmawati[a], Fauziah R, Hayati I, Sofiyanti N, Inoue E and Mtra D D 2017 *Biodiversitas* **18** 1035-1040
[13] Fitmawati[b], Sandi P.H and Sofiyanti N 2017 *Biodiversitas* **18** 715-719
[14] Fitmawati and Hartana A 2010 *HAYATI Journal of Biosciences* **17** 9-14
[15] Fitmawati[a], Juliantari E and Sofiyanti N 2017 *Applied Science and Technology* **1** 127-131
[16] Fitmawati[b], Hayati I and Sofiyanti N 2016 *Biodiversitas* **17** 653-656
[17] Yonemori K, Honsho C, Kanzaki S, Eiadthong W and Sugiura A 2002 *Plant Systematics and Evolution* **231** 59-75
[18] Baldwin B G, Sanderson M J, Porter J M, Wojciechowski M F, Campbell C S and Donoghue M J 1995 *Annals of the Missouri Botanical Garden* **82** 247-277
[19] Ali M A, Joongku L, Soo-Yong K, Sang-Hong P and Fahad M A A 2015 *Bangladesh J. Plant Taxon* **22** 1–8
[20] Doyle J J and Doyel J L 1987 *Phytochemical Bulletin* **19** 11-15
[21] Swofford D L 2000 PAUP*, *Phylogenetic Analysis Using Parsimony* (*and other methods). Version 4.0b10. (Massachusetts, USA : Sinauer Associates)
[22] Yuwono T 2005 *Biologi Molekular* (Jakarta: Erlangga).

[23] Nei M and Kumar S 2000 *Molecular Evolution and Phylogenetics* (New York: Oxford University Press)
[24] Fitmawati, Zulkifli M A and Sofiyanti N 2015 Spatial distribution of mango (*Mangifera*) in East Sumatra based on land cover and altitude In: Widodo P (ed.) *Proceeding of the 5th International Conference on Plant Diversity* (Purwokerto: Universitas Jenderal Soedirman)
[25] Hou D 1978 *Mangifera Flora Malesiana I* **8** 423-440
[26] James S A, Collins M D and Robert I N 1996 *International Journal of Systematic Bacteriology* **46** 189-194 26