DIVERSITY OF GANDARIA

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DIVERSITY OF GANDARIA (*BOUEA*) BASED ON MORPHOLOGICAL CHARACTERS IN INDONESIA

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SUMMARY

The major distribution of *Bouea* spp from the family *Anacardiaceae* is common in Malaysian region. The genus *Bouea* has three species *B. Marcophylla*, *B. oppositifolia*, and *B. poilanei*. Morphological variations were found in *Bouea* in various regions of Indonesia. This study aims to review the genetic diversity and grouping of *Bouea* spp in Indonesia using morphological markers. A total of 75 accessions of *B. macrophylla* and 30 accessions of *B. oppositifolia* obtained from 13 provinces in Indonesia observed with variations using 31 and 81 characters, respectively as morphological markers. Result of the observations were then cluster analyzed using the program NTSYS version 2.02 and confirmed using principal component analysis (PCA). Results revealed that morphological markers which can distinguish *Bouea* from other plants were opposite leaves pattern and purle seeds. Morphological markers that distinguished *B. macrophylla* with *B. oppositifolia* were leaf size, fruit size, fruit shape, fruit color, flesh color, and shape of the leaf axillary bud. Cluster analysis showed that *B. macrophylla* has similarity coefficient of 0.77 to 1.00 which is sub-divided in seven major groups with coefficient of 0.93. The *B. oppositifolia* has similarity coefficient between 0.49 to 1.00 and was sub-divided in five groups with major coefficient value of 0.85. The *B. oppositifolia* has higher similarity coefficient than *B. macrophylla* with respect to their morphology.

Key words: Bouea, morphological marker, cluster analysis, coefficient similarity

Key findings: The genetic diversity of genus Bouea in Indonesia has two species *Bouea oppositifolia* and *Bouea macrophyll*.

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INTRODUCTION

The genus *Bouea* is a member of the family *Anacardiaceae* (Ghazali and Mohammad, 2014). Most members of the *Bouea* still grown wildly and spread across Sumatera, Java, Borneo, Malaysia Peninsula, and Moluccas Island (Rifai, 1992). Hou (1975) reported that *Bouea* includes only two species, namely; *Bouea macrophylla* and *Bouea oppositifolia* based on fruit shape, leaf size, and bud size. The similar characteristic between both species is opposite leaf base, while distinguished characteristics are leaf size and shoots growing in leaf base. In some specimens, Harsono (2013) reported that wide range of morphological characteristics in leaf shape and

size and still possibility of reviewing their economic status. Moreover, it is also reported that there is a number of variations in shape, color, size, and skin spot of Gandari fruits.

In addition, Rehatta (2005) and Papilaya (2007) reported the presence of *Bouea* in Ambon with the characters of leaves similar to those of *B. macrophylla*, but having different fruit shape, size, and color. Indonesia is enriched with different kinds of Gandari fruits having different shapes (round and elliptical), tastes, weights and also with their own local identities such as *merinya* (Aceh), *haramania* (Padangbolak), *raman* fruit (Pekanbaru and Lampung), *kundang* fruits (Bengkalis Island, Riau), *Gondoria* (Batusangkar), *Gondorio* (Palembang), *Jatake* (Banten), Gandari (Bogor, Ambon and Karimun Java), and Ramania (Banjar Baru-Borneo) (Heyne, 1927; Hou, 1975; Harsono, 2013).

Based on the existing data, Ambon, Bogor and Borneo were popular for Gandaria, while Gandaria present in other regions has not been documented well. Moreover, in many cases the same names possibly referred to different Gandaria or, vice versa, the two different names are possibly intended for the same one Gandaria. For example, *merinya* fruits from Aceh have the same characteristics with Gandaria in Bogor, *gondoria* in Batusangkar, *jatake* in Banten and *Ramania* in South Borneo. This situation creates difficulties for the promotion of Gandaria trade with lack of their specific nomenclature and also for their germplasm maintenance.

Therefore, it is necessary to review taxonomic species and cultivar status in the genus *Bouea* as there is still a number of confusions in the limitation of species. The use of herbarium specimen, sites of the distribution of specimens, and several morphological characters is suggested that were previously not used by researchers. On the other hand, communities using Gandaria fruits generally with emphasis on the agronomic characteristics of fruits need clearer classification, so that the species of Gandaria can be sorted, selected, and nomenclature clarity too. The certainty with agronomic characters is also very important in the management of Gandaria germ plasms. Therefore, it is necessary to make a distinct classification system with the result of clustering of Gandaria cultivars only based on the agronomic characteristics of fruits. In this context, with the objective of genetic diversity study and to cluster the *Bouea* genus in Indonesia using morphological characters, the study was conducted from June 2013 to June 2015.

MATERIALS AND METHODS

The germplasms used in the study were *B. macrophylla* (75 accessions) and *B. oppositifolia* (30 accessions) (Table 1). The samples were collected from regions across Indonesia such as Aceh, North Sumatra, Riau, West-Sumatra, Jambi, South Sumatra, Lampung, West Java, Central Java, South Borneo, West Borneo, Ambon and Banten (Figure 1). The germplasms obtained mostly from wild condition and only few were deliberately cultivated.

Morphological observations done to determine the species status under the genus *Bouea* referred to the criteria used by Rifai (1992) and Vogel (1987). Some of the main characteristics used were presented in Table 2, while dendrogram was generated for the clustering of collected *Bouea* accessions. Documentation was done using digital camera. The clustering was carried out based on the diagnostic features of fruits and leaves as seen in Table 2.

Data analysis

The results of field observations on Gandaria specimens from all the sites of the study were classified and arranged in a form of matrix and then analyzed for similarity and difference to determine their cluster. Analysis of the clustering of inter-accession similarity was carried out using NTSYS ver. 2.02 for the clustering of SAHN by UPGMA (*Unweighted Pair Group Method with Arithmetic Average*). The clustering was carried out based on morphological characteristics with 21 diagnostic features such as leaf, fruit and seed shapes.

Table 1. List of *Bouea* accessions used in the study.

No.	Species	Province	Number of Accessions
1	*B. oppositifolia* (Roxb.) Adelb.	North Sumatra(SU)	5
2	*B. oppositifolia* (Roxb.) Adelb.	Riau (RI)	1
3	*B. oppositifolia* (Roxb.) Adelb.	Bangka Belitung Islands (BL)	19
4	*B. oppositifolia* (Roxb.) Adelb.	Bogor Botanical Garden (KR)	5
5	*B. macrophylla* Griffit.	Ambon (AM)	14
6	*B. macrophylla* Griffit.	Banten (BA)	8
7	*B. macrophylla* Griffit.	West Sumatera (BS)	5
8	*B. macrophylla* Griffit.	Bogor (BO)	13
9	*B. macrophylla* Griffit.	Jambi (JB)	2
10	*B. macrophylla* Griffit.	West Borneo (KB)	6
11	*B. macrophylla* Griffit.	South Borneo (KS)	18
12	*B. macrophylla* Griffit.	Palembang (PLB)	2
13	*B. macrophylla* Griffit.	Lampung (LP)	1
14	*B. macrophylla* Griffit.	Bangka Belitung (BL)	1
15	*B. macrophylla* Griffit.	Medan (MDN)	1
16	*B. macrophylla* Griffit.	Aceh (SN)	1
17	*B. macrophylla* Griffit.	Great Garden of Bogor (KR)	3
Total			105

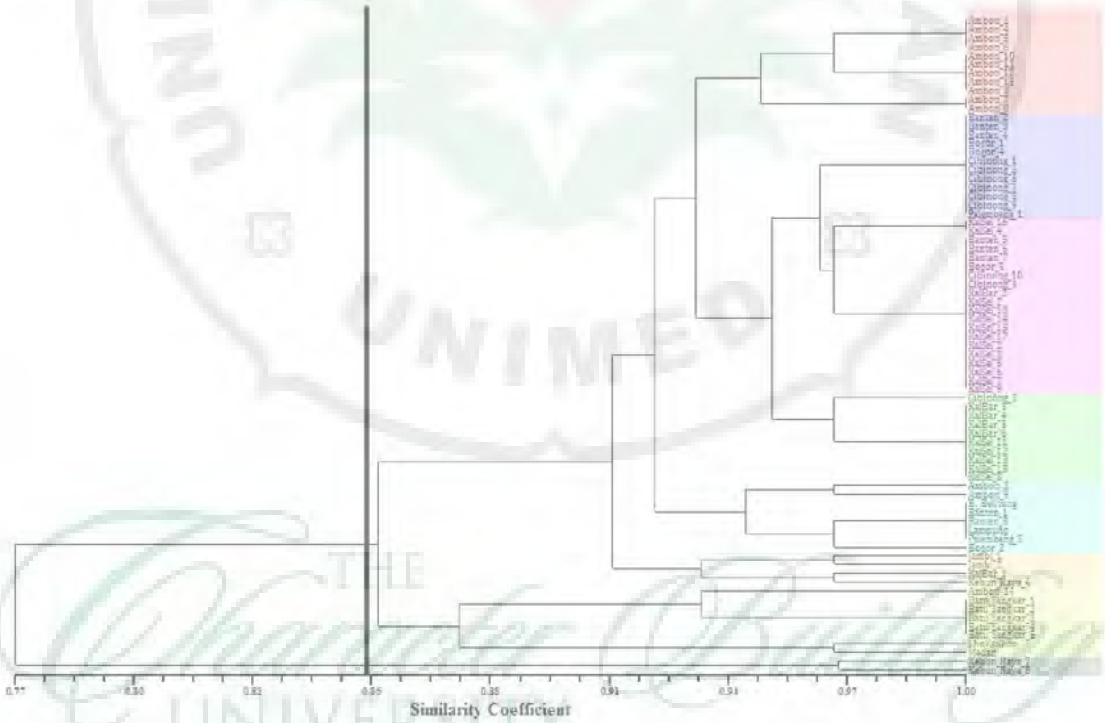

Figure 1. Dendrogram based on UPGMA from the morphological characteristics of *B. macrophylla*.

Table 2. Morphological characters used in the study.

No.	Morphological characters	Sub-characters	No. of characters
1	Trees	Dropping, medium	2
2	Crown	Dense, moderate	2
3	Leaf length	Very long, long, moderate, short	4
4	Leaf size	Very wide, wide, narrow	3
5	Leaf shape	Elliptic, obovate, oblong obovate, elliptic obovate, oblong, lanceolate	6
6	Leaf apex	Acuminate, acuminate obtuse, obtuse	3
7	Leaf base	Acute, acute obtuse, obtuse	3
8	Leaf apex size	Long, short, no leave apex	3
9	Adaxial color	Dark green, green	2
10	Abaxial color	Dark green, green	2
11	Adaxial appearance	Shine, dull	2
12	Abaxial appearance	Shine, dull	2
13	Leaf texture	Dull, fine, rough	3
14	Leaf appearance	Leaves flat, tortuous, convex	3
15	Petiole shape	Thin, round, square	3
16	Petiole size	Very long, long, short	3
17	Bud shape	Acute, round	2
18	Secondary bone	Many, few	2
19	Turpentine stone	No smell, finely smelt, strongly smelt	3
20	Fruit color	Red, yellow	2
21	Fruit shape	Round-oval, round, oval, bell	4
22	Fruit size	Small, big, very big	3
23	Flesh color	Red, yellow	2
24	Fruit taste	Sweet, sweet acid, astringent acid	3
25	Leaf type	Homofili, heterofili	2
26	Bud surface	Hairy, not hairy	2
27	Young stem surface	Hairy, not hairy	2
28	Seed color	Purple, white	2
29	Fruit type	Original, apparent	2
30	Leaf base	Opposite, dispersed	2
31	Flower type	Terminal, axillar	2
Total			81

RESULTS AND DISCUSSION

Morphological variation in accessions of *B. macrophylla* and *B. oppositifolia*

Based on the analysis of 81 morphological characters, variation in fruit color and shape, leaf size and structure, and shoot at bud were observed (Table 3). The data about morphological markers indicated that *B. macrophylla* and *B. oppositifolia* have morphological differences in their phenotypic characteristics of leaves, fruits, and stems. All *B. macrophylla* and *B. oppositifolia* accessions have opposite leaf base, pink seed and axial flower.

Morphological characteristics of *B. macrophylla* and *B. oppositifolia* based on the characters of leaves were presented in Table 4. The leaves of *B. macrophylla* were very long (60 accession), long (13 accessions) and moderate (2 accessions), while those of *B. oppositifolia* were generally moderate (15 accessions), short (10 accessions) and long (5 accessions). The leaves of *B. macrophylla* were very wide (48 accessions) and wide (27 accessions), while those of *B. oppositifolia* were generally narrow (22 accessions) and wide (8 accessions). Majority of the leaves of *B. macrophylla* were lanceolate in shape (73 accessions) and few were obovate (2 accessions). However, *B. oppositifolia* has variation in oval/elliptic shape

Table 3. Morphological marker variations in *Bouea* fruits, leaves and buds.

Morphological markers	Groups				
	1	2	3	4	5
Fruits					
Leaves					
Buds					

(1 accession), obovate (1 accession), oblong obovate (5 accessions), elliptic obovate (8 accessions) and oblong (15 accessions). Genus *Bouea* has a wide range of variability for morphologoical traits especially leaf characters (Harsono 2013), and the said idea was also supported by Hou (1975) who reported that *B. macrophyla* has high intra-sepcific variations for various traits compared *B. oppositifolia*. These variations might be due to selection pressure of cultivation for a long period. Besides Rehatta (2005) and Papilaya (2007) have reported that *Bouea* from Ambon and *B. macrophyla* have similar leaf characters; however, different in size, shape and colour of fruits.

The upper epidermis surfaces of *B. macrophylla* (56 accessions) and *B. oppositifolia* (23 accessions) were largely dark green in color, while those of *B. macrophylla* (19 accessions) and *B. oppositifolia* (7 accessions) were green. The lower surfaces of *B. macrophylla* (65 accessions) and *B. oppositifolia* (28 accessions) were mostly green in color, while 10 accessions of *B. macrophylla* and two of *B. oppositifolia* were dark green in color. The apex size was entirely long for *B. macrophylla*, while that of *B. oppositifolia* showed variation, i.e., long (6 accessions), short (23 accessions), and no apex (1 accession).

The leaf apex for all accessions from *B. macrophylla* had acuminate-shape, while *B. oppositifolia* showed variation, i.e. acuminate (17 accessions), acuminate obtuse (12 accessions) and obtuse (1 accession). Rough leaf texture was noticed for most of the accessions. All *B. macrophylla* accessions had convex leaves, while most of the leaves of *B. oppositifolia* were flat (26 accessions) and convex (4 accessions). The leaf petiole in *B. macrophylla* accessions had round and long size, while *B. oppositifolia* accessions had thin and short (22 accessions) and round and long (8 accessions). The 17 accessions from *B. oppositifolia* and mostly all accessions of *B. macrophylla* had homofili leaves, while 13 accessions from *B. oppositifolia* had heterofili leaves.

The dropping tree characteristics were noticed in *B. macrophylla* (64 accessions) and *B. oppositifolia* (KR1, KR2, KR5, and KR6), while medium trees were noticed in *B. macrophylla* (11 accessions) and *B. oppositifolia* (26 accessions) (Table 5). Mostly *B. macrophylla* (67 accessions) and *B. oppositifolia* (9 accessions) were dense crown while moderate crown seen in most *B. oppositifolia* (21 accessions) and *B. macrophylla* (8 accessions). Most of the accessions from *B. macrophylla* and 22 accessions from *B. oppositifolia* noticed no hairy young stem, while 8 accessions from *B. oppositifolia* had hairy young stem.

Table 4. Morphological characteristics of *B. macrophylla* and *B. oppositifolia* accessions based on the characters of leaves.

No.	Characters of leaves	Sub-characters	Accessions	
			B. macrophylla	*B. oppositifolia*
1	Leaf length	Very long	60	0
		Long	13	5
		Moderate	2	15
		Short	0	10
2	Leave size	Very wide	40	0
		Wide	27	8
		Narrow	0	22
3	Leaf shape	Elliptic	0	1
		Obovate	0	1
		Oblong Obovate	0	5
		Elliptic Obovate	2	8
		Oblong	0	15
		Lanceolate	73	0
4	Adaxial leaf color	Dark green	56	23
		Green	19	7
5	Abaxial leaf color	Dark green	10	2
		Green	65	28
6	Adaxial leaf surface	Shine	70	24
		Dull	5	6
7	Abaxial leaf surface	Shine	0	3
		Dull	75	27
8	Leaf surface texture	Fine	0	2
		Rough	75	28
9	Leaf surface shape	Convex	74	4
		Flat	0	26
		Tortuous	1	0
10	Leaf petiole shape	Thin	0	22
		Round	75	8
		Square	0	0
11	Leaf petiole size	Very long	1	0
		Long	75	7
		Short	0	23
12	Secondary bone	Many	0	10
		Few	75	20
13	Leaf landscape	Homofili	75	17
		Heterofili	0	13
14	Leaf position	Opposite	73	30

Table 5. Morphological characters based on the properties of trees.

No.	Tree characteristics	Sub-characters	Accessions	
			B. macrophylla	*B. oppositifolia*
1	Tree	Dropping	64	4
		Medium	11	26
2	Crown	Dense	67	9
		Moderate	8	21
3	Young stem surface	Hairy	0	8
		Not hairy	75	22

Mostly, 73 accessions of *B. macrophylla* and 28 accessions of *B. oppositifolia* had round buds while 2 accessions each from both species were accumate buds. All *B. macrophylla* accessions and 20 accessions of *B. oppositifolia* had few secondary bones, while 10 accessions of *B. oppositifolia* noticed with many secondary bones. All *B. macrophylla* accessions noticed no hairy buds, while out of 30, 15 accessions had hairy buds and remaining had no hairy buds in case of *B. oppositifolia*. Almost all *B. macrophyla* accessions and 6 accessions of *B. oppositifolia* had mild smell of turpentine while only 2 accessions of *B. oppositifolia* had strong smell of turpentine and remaining accessions noticed with no other smell. The color of fruits in all *B. macrophylla* accessions was yellow while most of the *B. oppositifolia* accessions (22) were red and few (8) were yellow (Table 6). The round shaped fruits with bigger size and yellow flesh were noticed for all *B. macrophylla* accessions and 8 accessions of *B. oppositifolia* while oval shaped fruits with smaller size and red flesh characteristics were found for 22 accessions of *B. oppositifolia*.

Based on the similarity value, the lowest similarity value is 0.11 or 11% between BL16 and KR5 (Tables 7 and 8). Based on morphological character, *B. oppositiolia* have a few differences in morphological character for example, an acute bud, red peel and have an excellent taste. On the other hand the highest similarity value is 0.96 or 96% between BL13 and BL20. Based on morphological character the different between BL13 and BL20 is the different size in leaf.

Cluster analysis

Bouea macrophylla

The data obtained from the morphological markers were used for cluster analysis using NTSYS program version 2.02. The dendrogram was made based on the similarity using a Dice Coefficient method. The cluster analysis was performed by clustering the data obtained from dendrogram. Approximately 75 accessions of *B. macrophylla*, 30 accessions of *B. oppositifolia* and 2 out-groups (*M. indica* and *A. occidentale*) had similarity coefficient ranging from 0.77 to 1.00 and clustered in 7 main groups at a similarity coefficient of 0.93. otherwise dissimilarity value ranging 0-23%.

Table 6. Morphological characteristics of *B. macrophylla* and *B. oppositifolia* based on the characters of fruits.

No.	Characteristics of fruits	Sub-characters	Accessions	
			B. macrophylla	*B. oppositifolia*
1	Fruit color	Yellow	75	8
		Red	0	22
2	Fruit shape	Oval round	0	22
		Round	75	8
		Oval	0	0
		Bell	0	0
3	Fruit size	Very big	0	0
		Big	75	8
		Small	0	22
4	Flesh color	Red	0	22
		Yellow	75	8
5	Fruit taste	Sweet	14	4
		Sweet acid	61	26
		Astringent acid	0	0

Table 7. Similarity coefficient of 36 *B. macrophylla* accessions based on the morphological characteristics.

AM1	AM12	AM14	AM3	AM5	AM7	AU	BA1	BA2	BA4	BA5	BL17	BS1	BS3	BS5	JB1	JB2	BO1	KB1	KB4	KB6	KR4	KS1	KS10	KS12	KS14	KS2	KS3	KS4	KS5	KS6	KS8	LP	BO2	PLB	BO3
1,00																																			
0,96	1,00																																		
0,89	0,93	1,00																																	
0,96	1,00	0,93	1,00																																
1,00	0,96	0,89	0,96	1,00																															
0,96	0,93	0,85	0,93	0,96	1,00																														
0,70	0,74	0,81	0,74	0,70	0,74	1,00																													
0,85	0,89	0,81	0,89	0,85	0,89	0,85	1,00																												
0,89	0,93	0,85	0,93	0,89	0,85	0,81	0,96	1,00																											
0,89	0,93	0,85	0,93	0,89	0,85	0,81	0,96	1,00	1,00																										
0,93	0,96	0,89	0,96	0,93	0,89	0,78	0,93	0,96	0,96	1,00																									
0,85	0,89	0,81	0,89	0,85	0,89	0,85	1,00	0,96	0,96	0,93	1,00																								
0,81	0,85	0,93	0,85	0,81	0,78	0,89	0,81	0,85	0,85	0,89	0,81	1,00																							
0,81	0,85	0,93	0,85	0,81	0,78	0,89	0,81	0,85	0,85	0,89	0,81	1,00	1,00																						
0,81	0,85	0,93	0,85	0,81	0,78	0,89	0,81	0,85	0,85	0,89	0,81	1,00	1,00	1,00																					
0,81	0,85	0,78	0,85	0,81	0,78	0,89	0,89	0,93	0,93	0,89	0,89	0,85	0,85	0,85	1,00																				
0,85	0,89	0,81	0,89	0,85	0,81	0,85	0,93	0,96	0,96	0,93	0,93	0,81	0,81	0,81	0,96	1,00																			
0,89	0,93	0,85	0,93	0,89	0,85	0,81	0,96	1,00	1,00	0,96	0,96	0,85	0,85	0,85	0,93	0,96	1,00																		
0,85	0,89	0,81	0,89	0,85	0,81	0,85	0,85	0,89	0,89	0,93	0,85	0,89	0,89	0,89	0,96	0,93	0,89	1,00																	
0,96	0,93	0,85	0,93	0,96	0,93	0,74	0,89	0,93	0,93	0,96	0,89	0,85	0,85	0,85	0,85	0,89	0,93	0,89	1,00																
0,96	0,93	0,85	0,93	0,96	0,93	0,74	0,89	0,93	0,93	0,96	0,89	0,85	0,85	0,85	0,85	0,89	0,93	0,89	1,00	1,00															
0,89	0,93	0,85	0,93	0,89	0,85	0,81	0,89	0,93	0,93	0,96	0,89	0,93	0,93	0,93	0,93	0,89	0,93	0,96	0,93	0,93	1,00														
0,93	0,96	0,89	0,96	0,93	0,89	0,78	0,93	0,96	0,96	1,00	0,93	0,89	0,89	0,89	0,89	0,93	0,96	0,93	0,96	0,96	0,96	1,00													
0,93	0,96	0,89	0,96	0,93	0,89	0,78	0,93	0,96	0,96	1,00	0,93	0,89	0,89	0,89	0,89	0,93	0,96	0,93	0,96	0,96	0,96	1,00	1,00												
0,96	0,93	0,85	0,93	0,96	0,93	0,74	0,89	0,93	0,93	0,96	0,89	0,85	0,85	0,85	0,85	0,89	0,93	0,89	1,00	1,00	0,93	0,96	0,96	1,00											
0,93	0,96	0,89	0,96	0,93	0,89	0,78	0,93	0,96	0,96	1,00	0,93	0,89	0,89	0,89	0,89	0,93	0,96	0,93	0,96	0,96	0,96	1,00	1,00	0,96	1,00										
0,93	0,96	0,89	0,96	0,93	0,89	0,78	0,93	0,96	0,96	1,00	0,93	0,89	0,89	0,89	0,89	0,93	0,96	0,93	0,96	0,96	0,96	1,00	1,00	0,96	1,00	1,00									
0,93	0,96	0,89	0,96	0,93	0,89	0,78	0,93	0,96	0,96	1,00	0,93	0,89	0,89	0,89	0,89	0,93	0,96	0,93	0,96	0,96	0,96	1,00	1,00	0,96	1,00	1,00	1,00								
0,89	0,93	0,85	0,93	0,89	0,93	0,81	0,96	0,93	0,93	0,96	0,96	0,85	0,85	0,85	0,85	0,89	0,93	0,89	0,93	0,93	0,93	0,96	0,96	0,93	0,96	0,96	0,96	1,00							
0,93	0,96	0,89	0,96	0,93	0,89	0,78	0,93	0,96	0,96	1,00	0,93	0,89	0,89	0,89	0,89	0,93	0,96	0,93	0,96	0,96	0,96	1,00	1,00	0,96	1,00	1,00	1,00	0,96	1,00						
0,93	0,96	0,89	0,96	0,93	0,89	0,78	0,93	0,96	0,96	1,00	0,93	0,89	0,89	0,89	0,89	0,93	0,96	0,93	0,96	0,96	0,96	1,00	1,00	0,96	1,00	1,00	1,00	0,96	1,00	1,00					
0,96	0,93	0,85	0,93	0,96	0,93	0,74	0,89	0,93	0,93	0,96	0,89	0,85	0,85	0,85	0,85	0,89	0,93	0,89	1,00	1,00	0,93	0,96	0,96	1,00	0,96	0,96	0,96	0,93	0,96	0,96	1,00				
0,85	0,89	0,81	0,89	0,85	0,89	0,85	1,00	0,96	0,96	0,93	1,00	0,81	0,81	0,81	0,89	0,93	0,96	0,85	0,89	0,89	0,89	0,93	0,93	0,89	0,93	0,93	0,93	0,96	0,93	0,93	0,89	1,00			
0,81	0,85	0,85	0,85	0,81	0,85	0,89	0,96	0,93	0,93	0,89	0,96	0,85	0,85	0,85	0,85	0,89	0,93	0,81	0,85	0,85	0,85	0,89	0,89	0,85	0,89	0,89	0,89	0,93	0,89	0,89	0,85	0,96	1,00		
0,85	0,89	0,81	0,89	0,85	0,89	0,85	1,00	0,96	0,96	0,93	1,00	0,81	0,81	0,81	0,89	0,93	0,96	0,85	0,89	0,89	0,89	0,93	0,93	0,89	0,93	0,93	0,93	0,96	0,93	0,93	0,89	1,00	0,96	1,00	
0,93	0,96	0,89	0,96	0,93	0,89	0,78	0,93	0,96	0,96	1,00	0,93	0,89	0,89	0,89	0,89	0,93	0,96	0,93	0,96	0,96	0,96	1,00	1,00	0,96	1,00	1,00	1,00	0,96	1,00	1,00	0,96	0,93	0,89	0,93	1,00

Table 8. Similarity coefficient of 23 *B. oppositifolia* accessions based on the morphological characteristics.

Aksesi	SU1	SU2	SU3	SU4	BL1	BL3	BL4	BL5	BL6	BL7	BL8	BL13	BL14	BL15	BL16	BL18	BL20	KR1	KR2	KR3	KR5	KR6	RI
SU1	1,00																						
SU2	1,00	1,00																					
SU3	1,00	1,00	1,00																				
SU4	0,81	0,81	0,81	1,00																			
BL1	0,41	0,41	0,41	0,30	1,00																		
BL3	0,41	0,41	0,41	0,30	0,93	1,00																	
BL4	0,52	0,52	0,52	0,70	0,56	0,56	1,00																
BL5	0,52	0,52	0,52	0,33	0,89	0,89	0,56	1,00															
BL6	0,59	0,59	0,59	0,56	0,74	0,74	0,78	0,78	1,00														
BL7	0,67	0,67	0,67	0,56	0,74	0,74	0,78	0,78	0,93	1,00													
BL8	0,44	0,44	0,44	0,33	0,93	0,85	0,56	0,89	0,78	0,78	1,00												
BL13	0,44	0,44	0,44	0,33	0,96	0,96	0,59	0,93	0,78	0,78	0,89	1,00											
BL14	0,52	0,52	0,52	0,41	0,89	0,89	0,67	0,85	0,70	0,78	0,81	0,93	1,00										
BL15	0,67	0,67	0,67	0,63	0,67	0,67	0,85	0,70	0,93	0,93	0,70	0,70	0,78	1,00									
BL16	0,41	0,41	0,41	0,30	0,85	0,85	0,56	0,81	0,67	0,74	0,78	0,89	0,89	0,67	1,00								
BL18	0,67	0,67	0,67	0,56	0,74	0,74	0,81	0,70	0,85	0,93	0,70	0,78	0,85	0,93	0,74	1,00							
BL20	0,44	0,44	0,44	0,33	0,93	0,93	0,59	0,89	0,78	0,78	0,93	0,96	0,89	0,70	0,85	0,78	1,00						
KR1	0,41	0,41	0,41	0,30	0,59	0,59	0,52	0,56	0,67	0,74	0,63	0,56	0,56	0,67	0,67	0,67	0,56	1,00					
KR2	0,44	0,44	0,44	0,26	0,89	0,81	0,52	0,85	0,63	0,70	0,81	0,85	0,85	0,63	0,81	0,70	0,81	0,63	1,00				
KR3	0,48	0,48	0,48	0,67	0,59	0,59	0,96	0,59	0,81	0,81	0,59	0,63	0,63	0,81	0,59	0,78	0,63	0,56	0,56	1,00			
KR5	0,56	0,56	0,56	0,67	0,19	0,11	0,56	0,19	0,33	0,33	0,19	0,15	0,22	0,41	0,11	0,37	0,15	0,30	0,30	0,52	1,00		
KR6	0,56	0,56	0,56	0,67	0,19	0,11	0,56	0,19	0,33	0,33	0,19	0,15	0,22	0,41	0,11	0,37	0,15	0,30	0,30	0,52	1,00	1,00	
RI	0,78	0,78	0,78	0,81	0,26	0,19	0,52	0,30	0,37	0,44	0,30	0,22	0,30	0,44	0,26	0,44	0,22	0,41	0,37	0,48	0,70	0,70	1,00

The seven groups i.e., 11 accessions from group 1 (Ambon), 42 accessions from group 2 (Banten, Cibinong, South Borneo, Bogor and West Borneo), 8 accessions from group 3 (Ambon, Bangka Belitung, Banten, Lampung, Palembang, and Bogor), 4 accessions from group 4 (Jambi, West Borneo, and Bogor Botanical Garden (KR4)), 6 accessions from group 5 (Batu Sangkar, West Sumatera and Ambon (AM14)), 2 accessions from group 6 (Lhoksukon, Aceh and Medan) and finally 2 accessions from Bogor Botanical Garden (KR7 and KR8) as a group 7 (Figure 2). The highest similarity coefficient of 0.96 was found in many accessions of *B. macrophylla* showed low diversity based on the morphological markers. Because of 42 accessions, group 2 can still be divided into two sub-groups. Group 1 consisting of 32 accessions from Banten and Cibinong with a similarity coefficient of 0.96, consisted of those from Banten, Bogor, Cibinong, Palembang, South Borneo and West Borneo. Group 2 consisted of 10 accessions from Cibinong, West Borneo, and South Borneo.

B. macrophyla is one of most cultivated specie because of its edible fruit and high economic value (Papilaya, 2007). *Bouea* was originated at Western Malesiana. In Ambon Island, Indonesia, *Bouea* was discoured and brought by the traders for plantation, and the same way introduced in Banten and Kalsel, Indonesia, and this idea was supported by grouping showed in Figure 2. In a few groups, there are combination of Bouea's origin which might be due to exchnage of seeds through human migration.

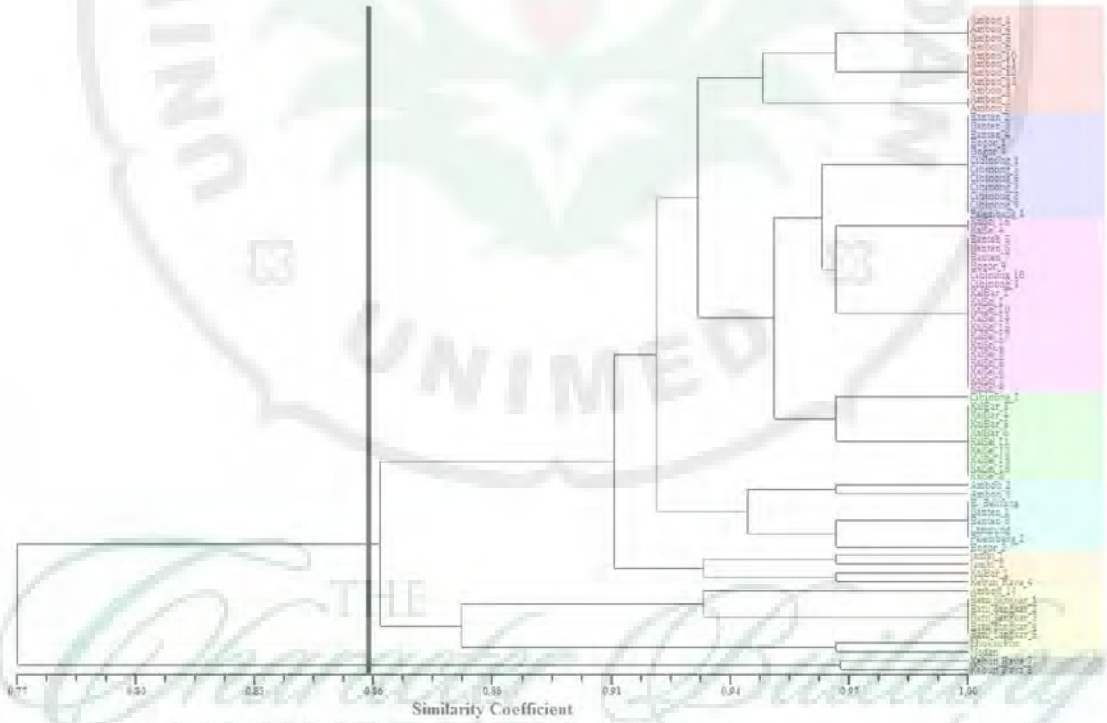

Figure 2. Dendrogram based on UPGMA from the morphological characteristics of *B. macrophylla*.

Bouea oppositifolia

The cluster analysis of *B. oppositifolia* was also carried out by clustering the genotypes obtained in dendrogram. Thirty accessions had similarity coefficients ranging from 0.49 to 1.00 and clustered into 5 groups based on the similarity coefficient of 0.84. otherwise have dissmilirity value ranging from 0- 51% . Six accessions from North Sumatra and Riau (group 1), 2 accessions from Bogor Botanical Garden (KR5 and KR6) (group 2), 12 accessions from Bogor Botanical Garden (KR2) and Bangka Belitung (group 3), 9 accessions from Bogor Botanical Garden (KR3) and Bangka Belitung (group 4) and 1 accession from Bogor Botanical Garden (KR1) consisted as group 5. The highest similarity coefficient of 0.96 was found in accessions from Bangka, Belitung (BL1 and BL13, BL3 and BL13, BL13 and BL20).

The combined data on the morphological markers of *B. macrophylla* and *B. oppositifolia* were reanalyzed by using NTSYS version 2.0. Results showed that the combined data had similarity coefficients ranging from 0.37 to 1.00 and could be classified into three groups. Group 1 consisted of 75 accessions from *B. macrophylla*, Group 2 consisted of 30 accessions from *B. oppositifolia*, and Group 3 consisted of out group including *M. indica* and *A. occidentale*.

The *B. oppositifolia* originating from Bogor Botanical Garden (B1) had the typical characteristics of elliptic leaves, while those from Bangka Belitung (BL19) had obovate leaves and obtuse-shaped apex. *B. opposifolia* from Bogor Botanical Garden (KR1, GG5, and GG6) had the typical characteristics of shining abaxial surface and similarly from north Sumatra, Riau and Bogor Botanical Garden (KR5, GG6) were characterized by oblong-shaped leaf, while those from Bangka, Belitung and Bogor Botanical Garden (KR2, GG3) were characterized by elliptic/ obovate- and oblong/obovate-shaped leaves. The elliptic-shaped leaves were noticed from Bogor Botanical Garden (KR1). *B. macrophylla* from Aceh, West Sumatra, Jambi, South Sumatra, Bangka Belitung Island (BL17), Banten, West Java, West Borneo, South Borneo, and Moluccas were characterized by lanceolate-shaped leaves. The greenish yellow color of ripe fruits was owned by *B. oppositifolia* from North Sumatra and Riau and the red color of ripe fruits from Bangka Belitung Island and Bogor Botanical Garden (KR1, GG2 and GG3). *B. macrophylla* from Aceh, West Sumatra, Jambi, South Sumatra, Lampung, Bangka Belitung Islands (BL17), Banten, West Java, West Borneo, South Borneo, and Moluccas had yellow to bright red skin of ripe fruits.

The *B. oppositifolia* from Bogor Botanical Garden (KR2) was included into the group of Bangka Belitung. The group had the morphological characteristics of heterofili leaves, i.e. elliptic and obovate, accumate leaf apex, and no leaf apex, hairy bud and young stem, acuminate bud apext such as lanceolate, small fruit size (2.1-3.4 cm), red ripe fruit color, red ripe flesh color, and sweet acidic taste.

The *B. oppositifolia* from Bogor Botanical Garden (KR3) also was separated from the group of Bogor Botanical Garden together with that of Bangka Belitung. *B. oppositifolia* from Bogor Botanical Garden (KR3) was characterized by only- one-leave shape, i.e. oblong, acute leaf apex, very obvious leave bones, dark green leaf color, sufficiently large leaf size (10.1-18.7 cm), hairy bud and young stem, acute bud, red ripe fruit color, red ripe flesh color, and sweet acidic taste. Detection of variability among germplasm for selected species will provide insight into the genom evolution, origin of cultivated species, and current level of diversity in modern agriculture cultivars (Kaewpongumpai *et al*., 2016).

Results indicated by dendrogram were reconfirmed using the principal component analysis (PCA) in terms of evaluating the distribution of accessions in a two-dimensional space (Figure 3). PCA clustered the access based on the data obtained from the morphological markers. The results of PCA analysis in Figures 4 and 5 showed that there was similarity in clustering in the dendrogram of UPGMA. PCA confirmed the profiles of morphological data on similarity at X-axis ranging of -0.05 to 0.48% and at Y-axis ranging from -0.44 to 0.10. The results of PCA showed that there was a significant relationship between the data obtained using the morphological ones and those observed (*B. macrophylla, B. oppositifolia, M.*

indica, and *A. occidentale*). The clear cluster was noticed for each type in Figure 5. Column 1 was filled by out group, i.e. *M. indica* and *A. occidentale*, column 2 was filled by samples of *B. oppositifolie* from Bogor Botanical Garden (KR5), column 3 was filled by samples of *B. macrophylla*, column of 5 was filled by *B. oppositifolia* from North Sumatra, column of 6 was filled by *B. macrophylla* from Great Garden of Bogor (KR7), and column 7 was filled by *B. oppositifolia*. The results were also confirmed by variation appeared in *B. oppositifolia* of three columns in the results of PCA analysis. They showed that morpholigical markers can distinguish the species in the genus *Bouea* and its out-group.

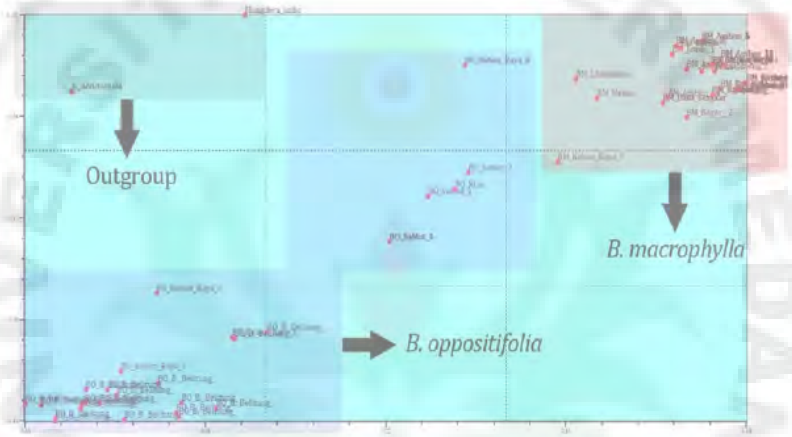

Figure 3. 2D plot of principal component analysis, indicating a relationship between 30 *B. oppositifolia* accessions and 75 *B. macrophylla* accessions as well as 2 out-groups (*M. indica* and *A. occidentale*) based on the morphological markers.

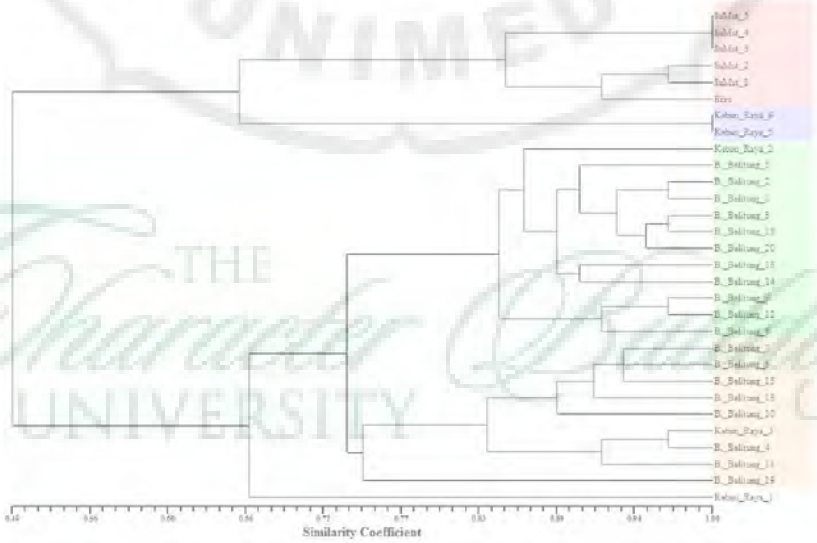

Figure 4. Dendrogram based on UPGMA from the morphological characteristics of *B. oppositifolia*.

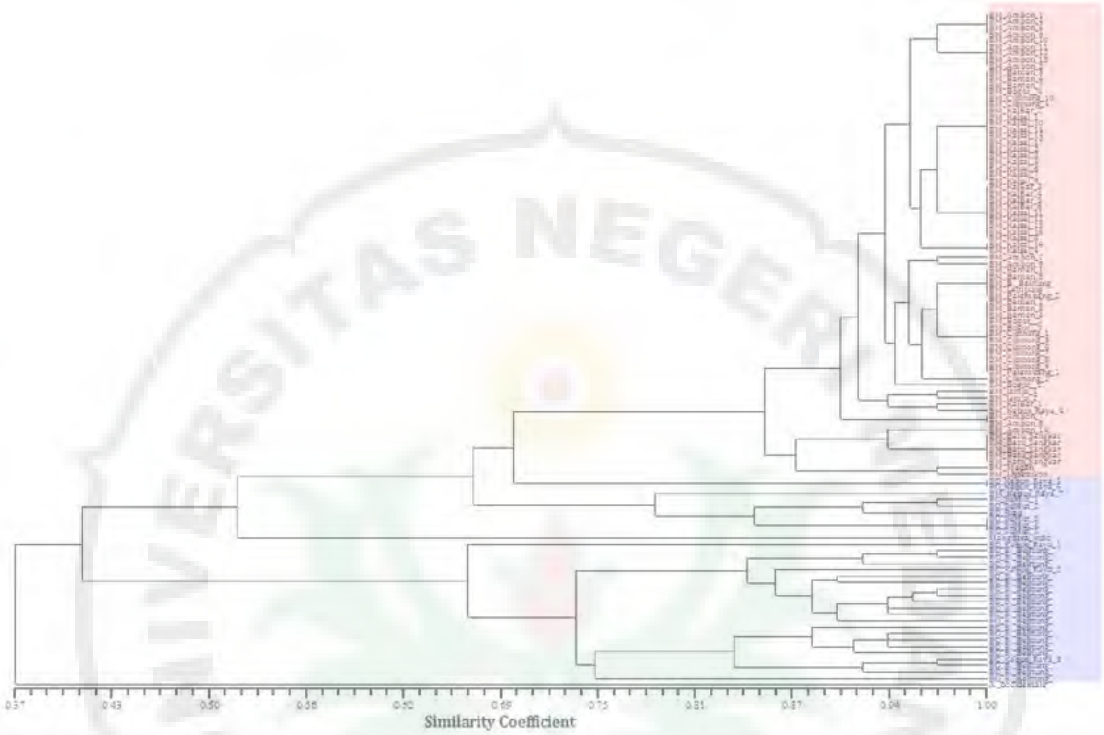

Figure 5. Dendrogram based on UPGMA from the morphological markers of *B. oppositifolia* (blue) and *B. macrophylla* (red).

CONCLUSION

The morphological markers that differentiate *Bouea* from other plants were opposite leaves and purple colored seeds. Similarly, based on the observations, morphological markers like leaf size, fruit size, fruit shape, fruit color, flesh color, leaf axillary buds shape clearly differentiate the accessions of *B. macrophylla* and *B. oppositifolia*. The cluster analysis on *B. Macrophylla* from Indonesian origin yielded similarities of coefficient between 0.77-1.00 and grouped into seven main groups on the similarity coefficient 0.93. Similarly, cluster analysis on *B. oppositifolia* Indonesia yielded coefficients of similarity between 0.49-1.00 and grouped into five main groups on the similarity coefficient of 0.84.

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