

## Characterization of phenotypic diversity of a medicinal plant *Lunasia amara*

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**Abstract.** Cahyaningsih R. 2016. Characterization of phenotypic diversity of a medicinal plant *Lunasia amara*. *Nusantara Bioscience* 8: 245-251. Characterization of phenotypic diversity is an important first step in the conservation of plant species and in plant breeding for human purposes. *Lunasia amara* Blanco ('kemaitan', 'sanrego') is a medicinal plant native to Indonesia, used traditionally as an aphrodisiac. However, there is little knowledge about genetic diversity in this species, and even its taxonomic status remains under review. The study reported here describes and classifies phenotypic diversity among *Lunasia amara* and its relatives, focusing on vegetative characters assessed in the extensive collection of the species held in Indonesian Botanic Gardens. Based on results of a similarity analysis using SIMQUAL (Similarity for Qualitative Data) employing SM (Simple Matching Coefficients), the accessions exhibit low phenotypic diversity as indicated by high similarity coefficients. Nevertheless, the accessions could be divided into two not very distinctive groups. Based on PCA (Principal Component Analysis), none of the observed characters were of outstanding significance in partitioning the diversity among the accessions. Due to their high similarity value, and despite the wide geographic area represented in the accessions, the study lends support to the belief that they belong to a single taxon, *Lunasia amara* var. *amara*. For conservation of *Lunasia* spp, this study suggests further collection activity is not a priority. Studies on the quantitative content of medicinally active constituents as well as phylogenetic studies of molecular diversity would test this conclusion and support a plant breeding program to develop the usefulness of this plant for human purposes.

**Keywords:** *Lunasia amara*, morphology, phenotypic characterization, similarity indices

### INTRODUCTION

*Lunasia* is a named genus in the Rutaceae family that reputedly has only one dioecious species (*Lunasia amara* Blanco) with two varieties, namely *L. amara* var. *amara* and *L. amara* var. *babuyanica* (Hartley 1967). Nevertheless, the taxonomic status of the genus and its 15 unresolved species names is under review (The Plant List 2016) and the particular species *Lunasia amara* (Blanco) is listed as having several synonyms (Mansur 2001; The Plant List 2016). Variation in certain vegetative characters of *Lunasia* appears high but according to Hartley (1967) there is considerable overlap in this variation across its natural wide distribution (in Malesia and northern Australia), without distinguishing discontinuity in habitat. Thus, Hartley concludes that the evidence points to *Lunasia* belonging to a single variable species.

The Indonesian Botanic Gardens have more than 30 living collections of *Lunasia amara* and its relatives (later called *Lunasia* spp.) planted out in Bogor Botanic Garden (KRB), Cibodas Botanic Garden (KRC), Purwodadi Botanic Garden (KRP), and Eka Karya Botanic Garden (KRE). Mostly this collection has resulted from exploration. These plants became part of the collection of the Indonesian Botanic Gardens because it was recognized that *Lunasia amara* Blanco is a plant native to Indonesia that has long been use in traditional medicine, for such

things as treating body swelling and skin diseases (Heyne 1987), erectile dysfunction (Soekotjo 1994), snake venom, digestive problems, toxification (Mansur 2001), eye redness and itching (Mansur 2001 and Rahayu et al. 2006). Some researchers have revealed evidence for the effectiveness of this plant as an aphrodisiac (Arnida et al. 2003 and Zumrotun 2006), and in treating tuberculosis, *Mycobacterium tuberculosis* (Kishore et al. 2009), and some cancers (Zubair and Subehan 2010).

Characterization of plant species like *Lunasia* spp. is necessary in the development of programs of conservation and plant breeding. The results of studies of genetic diversity in *Lunasia* spp. can form the basis for action to conserve it and for future plant improvement endeavors. Distinctive morphological characteristics identified by phenotypic analysis as markers of discontinuities in genetic diversity levels and patterns, can serve taxonomic purposes, and be useful in planning specific conservation interventions and plant breeding actions (Tanksley 1983; Talhinhas et al. 2006). This current study aimed to (i) obtain genetic diversity information about the *Lunasia* collection in the Indonesia Botanic Gardens based on morphological markers, for use in conservation and plant breeding programs, and (ii) assist in clarifying the taxonomic status of species in *Lunasia* using morphological markers.

## MATERIALS AND METHODS

Observations of morphology were carried out on 35 accessions of *Lunasia*; including twenty-six labelled as *Lunasia amara*, two labelled as *L. amara* var. *costulata*, two as *L. amara* var. *genuina*, and five as 'yet to be determined' (Table 1). All observed accessions are part of the living collection of the Indonesian Botanic Gardens of Indonesian Institute of Sciences network; planted out in four separate garden locations, namely Bogor Botanic Garden, West Java (KRB), Purwodadi Botanic Garden, East Java (KRP), Eka Karya Botanic Garden, Bali (KRE), and Cibodas Botanic Garden, West Java (KRC).

Observations were carried out on 23 vegetative characters (Table 2). Generative characters could not be assessed on every accession due to differences in growth and stages of development (some accessions not having reached the flowering stage); thus reproductive characteristics of accessions were not included in this analysis of morphological diversity.

Data was analyzed according to the method described by Sulassih et al. (2013), who employed the SIMQUAL (Similarity for Qualitative Data) procedure within the statistical program NTSYSpc version 2.1 (Rohlf 2000), based on the determination of SM (Simple Matching Coefficient) outlined by Sneath and Sokal (1973). For each of the 23 characters a value of 0 or 1 was attributed to each of the 35 accessions based on the observation of the characters. For those characters with more than two possible variant classes (see Table 2), one of the classes was assigned the value 0 while the other classes were assigned the value 1. Thus all characters were defined as binary variables for the analysis.

A Principal Component Analysis (PCA) was then conducted according to the method described in Cahyaningsih (2011) in an attempt to determine the major factors that explain the groupings found among the 35 *Lunasia* spp. accessions.

**Table 1.** List of 35 accessions, of the dioecious genus *Lunasia*, in the living collection of Indonesian Botanic Gardens

Acc. No.	Vak.	Accession	Origin	Sex	Planted Loc.
E1	X.B.84	<i>Lunasia amara</i> Blanco	CA Gunung Wari Cycloops, Jayapura, Papua	-	KRE
E2	X.B.84a	<i>Lunasia amara</i> Blanco	CA Gunung Wari Cycloops, Jayapura, Papua	-	
E3	XII.CI.121	<i>Lunasia amara</i> Blanco	West Sumba, NTT	Female	
E4	XII.CI.121a	<i>Lunasia amara</i> Blanco	West Sumba, NTT	Male	
E5	XII.C.121b	<i>Lunasia amara</i> Blanco	West Sumba, NTT	Female	
E6	XIII.K.25	<i>Lunasia amara</i> Blanco	Buleleng, Bali	Female	
E7	XIII.K.25b	<i>Lunasia amara</i> Blanco	Buleleng, Bali	Male	
P1	XIV.C.7a	<i>Lunasia amara</i> Blanco	Kota Baru, South Kalimantan	Male	KRP
P2	XIV.C.7	<i>Lunasia amara</i> Blanco	Kota Baru, South Kalimantan	Female	
P3	XIV.B.83	<i>Lunasia amara</i> Blanco	Tanimbar Island, Maluku	Female	
P4	XIV.B.83a	<i>Lunasia amara</i> Blanco	Tanimbar Island, Maluku	Female	
P5	XIV.B.83b	<i>Lunasia amara</i> Blanco	Tanimbar Island, Maluku	Female	
P6	XIV.B.83c	<i>Lunasia amara</i> Blanco	Tanimbar Island, Maluku	Male	
P7	XVI.H.49	<i>Lunasia amara</i> Blanco	Biak Island, Papua	Female	
P8	XVI.H.49a	<i>Lunasia amara</i> Blanco	Biak Island, Papua	Female	
P9	XVI.H.49b	<i>Lunasia amara</i> Blanco	Biak Island, Papua	Male	
P10	XIV.B.93a	<i>Lunasia</i> sp.	Parigi, Central Sulawesi	-	
P11	XIV.B.93b	<i>Lunasia</i> sp.	Parigi, Central Sulawesi	Female	
P12	XIV.B.93c	<i>Lunasia</i> sp.	Parigi, Central Sulawesi	-	
P13	XIV.B.108	<i>Lunasia</i> sp.	Parigi, Central Sulawesi	Female	
P14	XIV.B.108a	<i>Lunasia</i> sp.	Parigi, Central Sulawesi	Male	
B1	III.F.76	<i>Lunasia amara</i> Blanco var. <i>genuina</i> Hochr.	Perbanyakan dari III.F.52; Minahasa, Sulawesi Utara	-	KRB
B2	III.F.103	<i>Lunasia amara</i> Blanco	Bone, Sulawesi Selatan	Female	
B3	III.F.103a	<i>Lunasia amara</i> Blanco	Bone, Sulawesi Selatan	-	
B4	III.F.103b	<i>Lunasia amara</i> Blanco	Bone, Sulawesi Selatan	Female	
B5	III.F.107	<i>Lunasia amara</i> Blanco var. <i>genuina</i> Hochr.	Manado, North Sulawesi	-	
B6	VII.E.153	<i>Lunasia amara</i> Blanco var. <i>costulata</i> Hochr.	Java	-	
B7	XXIV.A.189	<i>Lunasia amara</i> Blanco var. <i>costulata</i>	Java	Female	
B8	XXIV.A.190	<i>Lunasia amara</i> Blanco	Hulu Sungai Selatan, South Kalimantan	-	
B9	XXIV.A.272	<i>Lunasia amara</i> Blanco	Batudaka Island, Central Sulawesi	Male	
B10	XXIV.B.XX.21	<i>Lunasia amara</i> Blanco	Hulu Sungai Selatan, South Kalimantan	Female	
C1	VI.D.112	<i>Lunasia amara</i> Blanco	Cakke, South Sulawesi	Female	KRC
C2	VI.D.92	<i>Lunasia amara</i> Blanco	Supriori Mt., Papua	-	
C3	VI.D.92a	<i>Lunasia amara</i> Blanco	Supriori Mt., Papua	-	
C4	VI.D.98	<i>Lunasia amara</i> Blanco	CA Gunung Wari Cycloops, Jayapura, Papua	Male	

**Table 2.** List of 23 vegetative characters, and the variants observed in the living *Lunasia* collection of the Indonesian Botanic Gardens

Character	Variant classes
Tree type	Tree; shrub; herb
Buttress	Present; absent
Bark	Rough; smooth
Sap	Present; absent
Spine	Present; absent
Stem form	Flat; cylinder
Leaf variation	Present; absent
Leaf division type	Single; compound
Leaf shape	Obovata; oblong-obovate
Leaf base	Rounded; shielding rounded; acute
Leaf tip	Acute-acuminate; rounded, acute-acuminate; rounded, acuminate; rounded, acute; acute; rounded; acuminate
Leaf edge	wavy; serrated-wavy; serrated
Leaf position	Spread; not spread
Oil dot	Present; absent
Leaf symmetry	Symmetrical; unsymmetrical
Leaf vein	pinnate; not pinnate
Gland	Present; absent
Domatia	Present; absent
Stipule	Present; absent
Maximum leaf length	Small (0-22 cm); moderate (23-44 cm); large (>45 cm)
Maximum leaf width	Small (0-10 cm); moderate (11-20 cm); large (>20 cm)
Maximum petiole length	Small (0-5 cm); moderate (6-10 cm); large (>11 cm)
Maximum secondary vein number (pair)	Small (0-10 cm); moderate (10-25 cm); large (>26 cm)

## RESULTS AND DISCUSSION

Careful observation of the 23 characters (listed in Table 2), revealed that distinctive variability was detectable in only eight of the characters, namely: leaf shape; leaf base; leaf tip; leaf edge; maximum leaf length; maximum leaf width; maximum petiole length; maximum secondary vein number. Present the PC values of the 8 characters in a table under the results and discussion section, while maintaining the complete table as in the appendix (Table S1-S2). There was no variability in the other 16 characters observed (see Table S1). This finding confirmed Hartley's (1967) report of minimal morphological differences across *Lunasia* spp. other than in leaf characteristics.

Figure 1 shows the results of the SIMQUAL analysis of data on the 23 vegetative binary variables recorded on the 35 *Lunasia* spp. accessions (see Table S2 for the similarity matrix upon which this analysis is based). The resulting dendrogram indicated that the accessions could be divided into two classes at the 0.85 similarity level, namely group I (0.90) and group II (0.87).

The dendrogram had a genetic matrix value of 0.74, meaning that the groupings among *Lunasia* spp. accessions

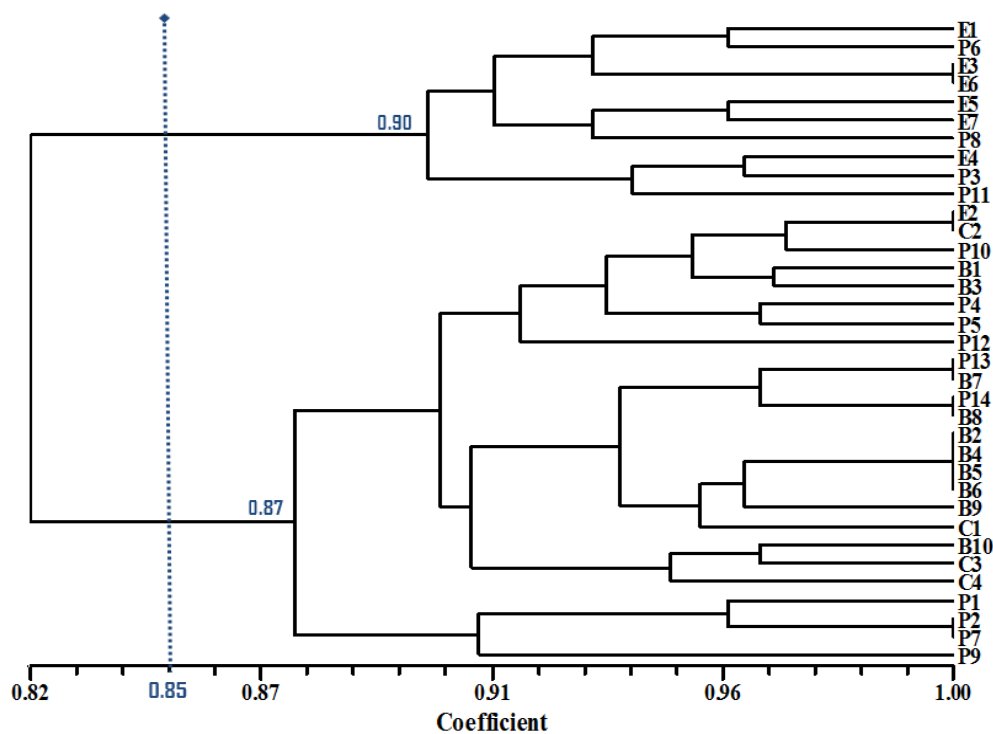
were quite suitably described by the analysis. Group I consisted of 10 accessions, namely three from East Nusa Tenggara (NTT), and two each from Maluku, Papua, and Bali. Group II consisted of 25 accessions, namely six from Papua, three from Maluku, four from South Sulawesi, four from Central Sulawesi, two from North Sulawesi, four from South Kalimantan, and two from Java.

Several accessions from different regions planted out in the same location had a similarity of 100% as indicated by Simple Matching Coefficients of 1 with other accessions growing in that same planting location (see Table S2). For example, accessions planted out in KRE but originating from NTT (E3) and from Bali (E6) had 100% similarity. Also, accessions originating from South Sulawesi (B2 and B4), North Sulawesi (B5), and Java (B6) planted out in KRB had 100% similarity, while two accessions planted out in KRP, namely one originating from South Kalimantan (P2) and one from Papua (P7) likewise exhibit 100% similarity.

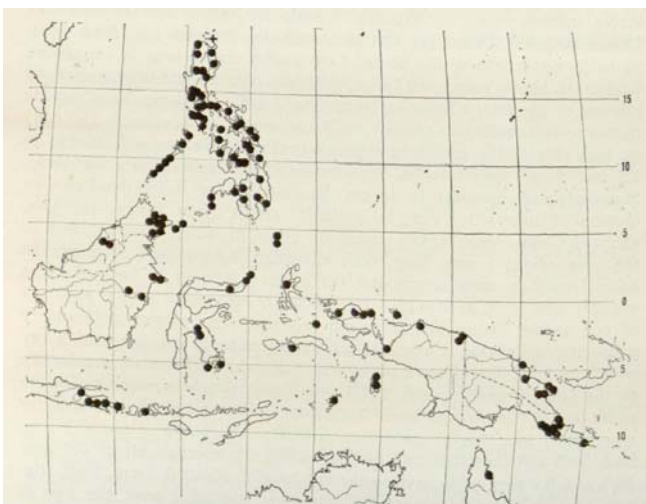
These similarities might perhaps have been interpreted as the result of the common environmental effect shared within the respective Botanical Gardens where the accessions had been planted out. However, 100% similarities were also observed between two accessions originating from the same province but planted out in different Botanical Gardens, namely E2 planted out in KRE and C2 planted out in KRC (Table S2). Moreover, Simple Matching Coefficients of 1 were also exhibited by accessions originating from two different provinces planted out in the different botanical garden can has a value of similarity coefficient 1, for example P14 originating from South Kalimantan planted in KRP matched with B7 originating from Java but planted in KRB.

It had been predicted that accessions collected from the same province, especially if from the same exact population, would have a similarity of 100%, but this was not generally found to be the case (Table S2). In the Group I cluster, the three accessions (E3, E4 and E5) from East Nusa Tenggara growing in the KRE Garden had a similarity coefficient of only 0.90, and the two accessions from Bali (E6 and E7) a coefficient of only 0.92. The accessions of Java origin (B6 and B7) growing in KRB has a similarity coefficient 0.93. Accessions from North Sulawesi (B1 and B5) had a similarity coefficient of 0.90; similarly, accessions from South Sulawesi (B2, B3, B4, and C1) also had a coefficient of 0.90. Meanwhile, the South Kalimantan accessions (P1, P2, B8 and B10) had a similarity coefficient of only 0.87.

The fact that accessions originating from similar provenances have relatively low coefficients of similarity is presumably because the morphological characters (phenotype) of plant is not only controlled by genetic factors (genotype), but also by environmental factors, and the interaction between these factors ( $P = G + E + G \cdot E$ ; Falconer and Mackay 1996). Basically, the genetic diversity of *Lunasia* spp. as a whole appears to be narrow, because the similarity coefficient across all 35 accessions is high (0.82).



**Figure 1.** Dendrogram of similarity among 35 accessions of *Lunasia* spp. (See Table 1 for the identities of the accessions coded on the far right hand side of this dendrogram)



**Figure 2.** Distribution of *Lunasia amara* var. *amara* (dots) and var. *babuyanica* (Merr.) Hartley (plus sign) (according to Hartley 1967)

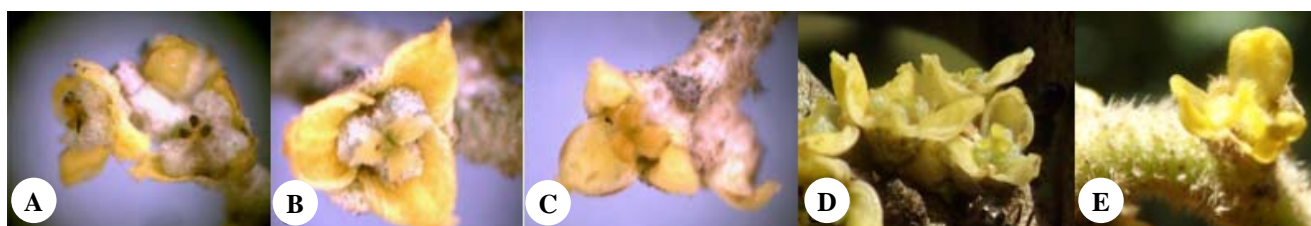
In accordance with the taxonomic study of Hartley (1967), our observations of vegetative characteristics on accessions assembled from a wide geographic range within Indonesia suggest that most likely all accessions grown in the Indonesian Botanic Gardens belong to a single taxon, *Lunasia amara* var. *amara*. Hartley (1967) proposed that a single species *Lunasia amara* distributed from eastern Java,

and Kalimantan, across the Wallace line through Sulawesi, the Philippines and Papua, to northeastern Australia, consisting of two distinguishable varieties - the *babuyanica* variety confined to the Babuyan Islands in the Philippines, and the *amara* variety comprising the rest of the distribution (Figure 2). There is nothing in our data that would contradict Hartley's assessment.

Hartley mentioned that the difference between the two varieties apart from their distribution is the appearance of their seed follicle. Variety *amara*'s follicle is transversely ribbed, with the ribs sometimes covered by vaguely defined trichomes on the lateral surfaces, otherwise smooth. On the other hand, the follicle of the *babuyanica* variety is densely covered by trichomes, appearing as scales up to 8 mm in length.

In our study, the female flowers of several observed accessions all had similar appearance (Figure 3). The small flowers, have white hairs, and consist of three yellow tepals and a gynoecium three yellow stigma parts. More observations, for other accessions as they come into flower are required and also as they produce seeds.

The results of the PCA analysis showed that none of the vegetative characters on their own could be considered as significantly contributing to partitioning of variation among the 35 accessions (Table S1). Variables that scored high on PC1 were leaf length and leaf width but it is very likely that environmental factors as much as genetic differences are involved in these variables.



**Figure 3.** Female flowers of *Lunasia* spp. at the Indonesian Botanic Gardens (Bogor Botanic Garden). A. *Lunasia amara*, B. *Lunasia amara* var. *costulata*, C. *Lunasia amara* var. *genuina*, D. *Lunasia* sp. KRP XIV.B.93a, E. *Lunasia* sp. KRP XIV.B.108a

The smaller the genetic distance among individuals in a population, the more uniform is expected to be the population. In this study, because of the low apparent genetic diversity in *Lunasia* spp., just one or two accessions representing each of I and group II would be adequate to include in any breeding program on the species (Sukartini 2007). But it must be emphasized that this is only with respect to variation in vegetative characteristics. An analysis of reproductive characters and especially of active chemical constituents in this medicinally useful plant could reveal differences in germplasm of greater significance for a plant breeding program.

The low apparent genetic diversity of *Lunasia* spp. is plausibly because of a propensity to pollinate across potential isolating geographic barriers and/or for free seed dispersal across such barriers. *In situ* studies of the mechanisms and agencies of pollination and seed dispersal would be required to test this hypothesis. This study of vegetative characters suggests that the current assembly of *Lunasia* collections in the Indonesian Botanic Gardens is adequate for conservation purposes. However, this hypothesis can only be tested by phylogenetic studies combining biochemical characters with reproductive morphological characters on the same collection of accessions of *Lunasia amara* var. *amara*, but hopefully including representative specimens of *L. amara* var. *babuyanica* as well as of outgroups related to *Lunasia*.

In conclusion, the *Lunasia* spp. collection assembled in the Indonesian Botanic Gardens has low genetic diversity based on a similarity analysis of 23 vegetative characteristics, despite some apparent morphological differences. Allegedly, this collection of accessions belongs to a single species *Lunasia amara* and the current study has tended to confirm this. So for the purpose of *Lunasia* species conservation, collections at the Gardens appear sufficiently representative: implementation of new exploration is not a priority. However, for the purposes of a plant breeding program of this species for medicinal purposes, an advanced characterization is required, especially in regard to content of medicinally active constituents and genetic diversity based on molecular characteristics. If the results of such an advanced characterization were to reveal hidden diversity amongst the accessions, procurement of further accessions through exploration could be justified. In the absence of such hidden diversity, mutagenesis to produce medicinally useful diversity might warrant consideration.

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## REFERENCES

- Adhiyanto E. 2001. Ecological Study on Several Aspects of Kemaitan (*Lunasia amara* Blanco) In Betiri National Park, East Java. [Thesis]. Department of Forest Resources Conservation, Faculty of Forestry, Institut Pertanian Bogor, Bogor.
- Arnida, A Imono, Donatus, and S Wahyuono. 2003. Isolation of the active fraction of aphrodisiac from wood sanrego (*Lunasia amara* Blanco). *Indon Pharmaceut Mag* 14 (4): 195-200. [Indonesian]
- Cahyaningsih R. 2011. Phenology Study of Flowering and Genetic Diversity of Castor (*Ricinus communis* L.) Using Morphology and Molecular Marker. [Thesis]. Graduate School. Institut Pertanian Bogor, Bogor. [Indonesian]
- Falconer DS and TFC Mackay. 1996. Introduction to quantitative genetic. 4<sup>th</sup> edition. Addison Wesley Longman, Essex, UK. 464 p.
- Hartley TG. 1967. A revision of the genus *Lunasia* (Rutaceae). *J Arnold Arboretum* 48: 460-475.
- Heyne K. 1987. Useful Plants Indonesia (Translation). Volume II. Foundation of Sarana Wana Jaya, Jakarta. [Indonesian]
- Kishore N, BB Mishra, V Tripathi, and VK Tiwari. 2009. Alkaloids as potential anti-tubercular agents. *Fitoterapia* 80: 149-163
- Mansur M. 2001. *Lunasia amara* Blanco In: van Valkenburg, J.L.C.H. and Bunyapraphatsara, N. (Editors). Plant Resources of South-East Asia No. 12 (2): Medicinal and poisonous plants 2. Backhuys Publisher, Leiden, The Netherlands.
- Pandin DS. 2010. Genetic diversity of Bali coconut (DBI) and Sawarna coconut (DSA) based on Random Amplified Polymorphic DNA markers. *Littri Journal* 16 (2): 83-89. [Indonesian]
- Rahayu M, S Sunarti, D Sulistiarini, and S Prawiroatmodjo. 2006. Utilization of Traditional Medicinal Plants by Local Communities in Wawonii Island, Southeast Sulawesi. *Biodiversitas* 7 (3): 245-250. [Indonesian]
- Rohlf FJ. 2000. NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System. Version 2.10. Exeter Software, New York.
- Soekotjo L. 1994. Isolation and Identification of Chemical Components of Extract Skin Stem Diethyl Ether of Sanrego Wood. [Hon. Thesis]. Department of Pharmacy, Faculty of Hasanuddin, Makassar. [Indonesian]

- Sukartini. 2007. Grouping of banana accession using morphological characters IPGRI. *J. Horticulture*. 17 (1): 26-33. [Indonesian]
- Sulassih, Sobir, Santosa E. 2013. Phylogenetic analysis of mangosteen (*Garcinia mangostana* L.) and its relatives based on morphological and inter simple sequence repeat (ISSR) markers. *Sabrao J Breed Genet* 45 (3): 478-490
- Talhinhas P, J Leitao, and J Neves-Martins. 2006. Collections of *Lupinus angustifolius* L. germplasm and characterization of morphological and molecular diversity. *Genet Resour Crop Evol* 53: 563-578.
- Tanksley SD. 1983. Molecular marker in plant breeding. *Plant Mol Biol Rep* 1 (1): 3-5.
- The Plant List. 2016. Version 1.1. Published on the Internet; <http://www.theplantlist.org/>. accessed 28th May
- Zubair MS, Subehan. 2010. Molecular Docking of Lunacridine from *Lunasia amara* to DNA: Its Inhibition And Interaction Study Correlated With The Cytotoxic Activity on P388 Murine Leukemia Cells. *Indon J Cancer Chemoprev* 1 (2): 108-117
- Zumrotun. 2006. Role of Sanrego (*Lunasia amara* Blanco) In Shortening Antler Cycle and Improving Sexual Libido Timor Deer (*Cervus Timorensis* De Blainville) Males. [Thesis]. Graduate School. Institut Pertanian Bogor. [Indonesian]

**Table S1.** Value of Principal Components (PC1 to PC8) accounting for 100% of the variability among 23 morphological characters examined in the *Lunasia* spp. collection of the Indonesian Botanic Gardens

Principal Component Analysis:								
Eigenanalysis of the Covariance Matrix								
	Eigenvalue	0.602	0.332	0.214	0.140	0.104	0.100	0.081
	Proportion	0.371	0.205	0.132	0.086	0.064	0.062	0.050
	Cumulative	0.371	0.576	0.708	0.794	0.858	0.920	0.970
Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Tree type	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Buttress	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Bark	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sap	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Spine	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Stem form	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Leaf variation	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Leaf division type	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Leaf shape	0.171	-0.073	0.004	-0.145	0.573	0.150	0.720	-0.274
Leaf base	-0.251	0.654	-0.184	0.052	-0.233	-0.504	0.393	-0.094
Leaf tip	0.062	0.428	0.394	0.699	0.336	0.194	-0.114	0.072
Leaf edge	0.376	0.553	0.143	-0.614	0.188	0.072	-0.337	-0.039
Leaf position	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Oil dot	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Leaf symmetry	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Leaf vein	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Gland	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Domatia	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Stipule	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Max. leaf length	-0.516	0.049	-0.033	-0.227	0.386	0.030	0.045	0.726
Max. leaf width	-0.539	0.200	-0.293	-0.049	-0.017	0.611	-0.138	-0.433
Max. petiol length	-0.240	-0.162	-0.086	0.037	0.535	-0.550	-0.410	-0.388
Maximum secondary vein number (pair)	-0.385	-0.096	0.834	-0.235	-0.182	-0.057	0.106	-0.210

**Table S2.** Similarity matrix (Simple Matching Coefficients) for the 35 *Lunasia* spp accessions, based on 23 observed characteristics

	E1	E2	E3	E4	E5	E6	E7	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	C1	C2	C3	C4							
E1	1																																									
E2	0.82	1																																								
E3	0.93	0.82	1																																							
E4	0.90	0.82	0.90	1																																						
E5	0.91	0.82	0.91	0.90	1																																					
E6	0.93	0.82	1.00	0.90	0.91	1																																				
E7	0.91	0.82	0.91	0.90	0.96	0.91	1																																			
P1	0.82	0.87	0.82	0.82	0.82	0.82	0.82	1																																		
P2	0.82	0.87	0.82	0.82	0.82	0.82	0.82	0.96	1																																	
P3	0.90	0.82	0.90	0.96	0.90	0.90	0.90	0.82	0.82	1																																
P4	0.82	0.93	0.82	0.82	0.82	0.82	0.82	0.87	0.87	0.82	1																															
P5	0.82	0.93	0.82	0.82	0.82	0.82	0.82	0.87	0.87	0.82	0.96	1																														
P6	0.96	0.82	0.93	0.90	0.91	0.93	0.91	0.82	0.82	0.90	0.82	0.82	1																													
P7	0.82	0.87	0.82	0.82	0.82	0.82	0.82	0.96	1.00	0.82	0.87	0.87	0.82	1																												
P8	0.91	0.82	0.91	0.90	0.93	0.91	0.93	0.82	0.82	0.90	0.82	0.82	0.91	0.82	1																											
P9	0.82	0.87	0.82	0.82	0.82	0.82	0.82	0.91	0.91	0.82	0.87	0.87	0.82	0.91	0.82	1																										
P10	0.82	0.97	0.82	0.82	0.82	0.82	0.87	0.87	0.82	0.93	0.93	0.82	0.87	0.82	0.87	1																										
P11	0.90	0.82	0.90	0.94	0.90	0.90	0.82	0.82	0.94	0.82	0.82	0.90	0.82	0.90	0.82	0.82	1																									
P12	0.82	0.92	0.82	0.82	0.82	0.82	0.82	0.87	0.87	0.82	0.92	0.92	0.82	0.87	0.82	0.87	0.92	0.82	1																							
P13	0.82	0.90	0.82	0.82	0.82	0.82	0.87	0.87	0.82	0.90	0.90	0.82	0.87	0.82	0.87	0.90	0.82	0.90	0.82	0.82	1																					
P14	0.82	0.90	0.82	0.82	0.82	0.82	0.87	0.87	0.82	0.90	0.90	0.82	0.87	0.82	0.87	0.90	0.82	0.90	0.82	0.90	0.96	1																				
B1	0.82	0.95	0.82	0.82	0.82	0.82	0.82	0.87	0.87	0.82	0.93	0.93	0.82	0.87	0.82	0.87	0.95	0.82	0.92	0.90	0.90	1																				
B2	0.82	0.90	0.82	0.82	0.82	0.82	0.82	0.87	0.87	0.82	0.90	0.90	0.82	0.87	0.82	0.87	0.90	0.82	0.90	0.94	0.94	0.90	1																			
B3	0.82	0.95	0.82	0.82	0.82	0.82	0.82	0.87	0.87	0.82	0.93	0.93	0.82	0.87	0.82	0.87	0.95	0.82	0.92	0.90	0.90	0.97	0.90	1																		
B4	0.82	0.90	0.82	0.82	0.82	0.82	0.82	0.87	0.87	0.82	0.90	0.90	0.82	0.87	0.82	0.87	0.90	0.82	0.90	0.94	0.94	0.90	1.00	0.90	1																	
B5	0.82	0.90	0.82	0.82	0.82	0.82	0.82	0.87	0.87	0.82	0.90	0.90	0.82	0.87	0.82	0.87	0.90	0.82	0.90	0.94	0.94	0.90	1.00	0.90	1.00	1																
B6	0.82	0.90	0.82	0.82	0.82	0.82	0.82	0.87	0.87	0.82	0.90	0.90	0.82	0.87	0.82	0.87	0.90	0.82	0.90	0.94	0.94	0.90	1.00	0.90	1.00	1.00	1															
B7	0.82	0.90	0.82	0.82	0.82	0.82	0.82	0.87	0.87	0.82	0.90	0.90	0.82	0.87	0.82	0.87	0.90	0.82	0.90	1.00	0.96	0.90	0.94	0.90	0.94	0.94	0.94	1														
B8	0.82	0.90	0.82	0.82	0.82	0.82	0.82	0.87	0.87	0.82	0.90	0.90	0.82	0.87	0.82	0.87	0.90	0.82	0.90	0.96	1.00	0.90	0.94	0.90	0.94	0.94	0.96	1														
B9	0.82	0.90	0.82	0.82	0.82	0.82	0.82	0.87	0.87	0.82	0.90	0.90	0.82	0.87	0.82	0.87	0.90	0.82	0.90	0.94	0.94	0.90	0.96	0.90	0.96	0.96	0.96	0.94	0.94	1												
B10	0.82	0.90	0.82	0.82	0.82	0.82	0.82	0.87	0.87	0.82	0.90	0.90	0.82	0.87	0.82	0.87	0.90	0.82	0.90	0.91	0.91	0.90	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91				
C1	0.82	0.90	0.82	0.82	0.82	0.82	0.82	0.87	0.87	0.82	0.90	0.90	0.82	0.87	0.82	0.87	0.90	0.82	0.90	0.94	0.94	0.90	0.95	0.90	0.95	0.95	0.95	0.94	0.94	0.95	0.91	1										
C2	0.82	1.00	0.82	0.82	0.82	0.82	0.82	0.87	0.87	0.82	0.93	0.93	0.82	0.87	0.82	0.87	0.97	0.82	0.92	0.90	0.90	0.95	0.90	0.95	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	1				
C3	0.82	0.90	0.82	0.82	0.82	0.82	0.82	0.87	0.87	0.82	0.90	0.90	0.82	0.87	0.82	0.87	0.90	0.82	0.90	0.91	0.91	0.90	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.96	0.91	0.90	1	
C4	0.82	0.90	0.82	0.82	0.82	0.82	0.82	0.87	0.87	0.82	0.90	0.90	0.82	0.87	0.82	0.87	0.90	0.82	0.90	0.91	0.91	0.90	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.95	0.91	0.90	0.95	1