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Morphological variation and phylogenetic position of *Vatica rassak* (*Dipterocarpaceae*) based on the *trn*L-*trn*F region

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Abstract. *Vatica rassak* is one of the most exploited species of the genus *Vatica* for its wood and resin. The species exhibits high morphological variations across their range of distribution that crosses the border of the Wallace's line. To understand whether such variations may imply infra-specific variations related to the distribution pattern, it is necessary to study the morphological characteristics of *V. rassak* and determine its phylogenetic position within the genus. Morphological observations were carried out on 799 herbarium specimens of *V. rassak* deposited in Herbarium Bogoriense (BO). Data collected during the observation was used for a phenetic analysis. The evolutionary relationship of *V. rassak* within the genus *Vatica* was assessed by performing a phylogenetic analysis. The results of the phenetic analysis using 14 morphological characters showed a moderate range of morphological variations (similarity coefficient of 0.38–1.05). The spatial morphological variation deduced from the PCA analysis of the specimens examined showed almost the same grouping as in the phenetic analysis. Phylogenetic analysis based on *trnL-trn*F sequence suggested that *V. rassak* is included in a group comprised mostly *Vatica* Section Vatica. Section Sunaptea is a paraphyletic group.

Key words: morphology, phenetic, phylogenetic, variation, *Vatica rassak*

Introduction

Vatica rassak is a species of the genus *Vatica* Section Vatica of the *Dipterocarpaceae* family. The species is highly valued for its timber and resins (Martawijaya et al. 1981). This species is classified as class I commercial traded timber along with 52 other species based on the Decree of the Minister of Forestry No 163/Kpts-II/2003 and belongs to durable class III (Djarwanto et al. 2017). The stem bark of *V. rassak* has potential medicinal properties (Ito et al. 2002) evidenced by phytochemical studies conducted by Tanaka et al. (2000), who found resveratrol oligomers, vaticanols A, B, and C, as well as three known stilbenoids, resveratrol, piceid, and epsilon-viniferin.

The local and trade name of *V. rassak* is resak, but resak is also used to name other *Vatica* species, i.e., *V. oblongifolia* and *V. venulose*. *Vatica rassak* is distributed in Borneo, Sulawesi, Maluku, the Philippines, and Papua (Fig. 1) and the only species of *Dipterocarpaceae* whose distribution covers both the eastern and western parts of the Wallace's line. Wallacea line is an imaginary line that dividing the Malesian regions into two distint biogeographic regions representing the Sundaland and Sahul land. The Wallacea run through the Indonesian archipelago. It lies between Borneo and Sulawesi, and between Bali and Lombok. The global population of *V. rassak* is considered large enough, thus giving conservation status as the least concern based on the IUCN red list category and criteria (Barstow 2018). However, the occurrence of massive forest conversion in Indonesia and low natural regeneration caused a continuing decline in the natural populations. Hence, in Indonesia, *V. rassak* is considered a threatened species under category Vulnerable A4cd ver 3.1. at the national level (Kalima & Wardani 2020).

The existence of a species distribution that crosses the Wallace's line from West to East is interesting, especially

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Figure 1. Native distribution of *Vatica rassak*. (https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:321701-1)

from morphological and genetic aspects. It is unknown whether the distribution across the Wallace's line gives rise to morphological variations between the individual species in the western and eastern zones. A field observation in the West Papua region showed a variation of fruit and leaves (Nugroho et al. 2016). Observation of herbarium specimens also found variations of leaves and fruit on specimens across the distribution range. Thus, the boundaries of the species *V. rassak* may need to be redefined by examining the existing morphological variations. This study aims to determine: i) the morphological variations that exist in *V. rassak*; ii) the grouping pattern of *V. rassak* based on morphological variations, iii) whether the shape of the fruit is a diagnostic character in *V. rassak*; iv) the phylogenetic position of *V. rassak* in the genus *Vatica*.

To answer these questions, morphometric and phylogenetic studies were carried out. A morphometric study is a study that is based on morphological measurements, including length, width, and observations and measurements of plant parts (Purnawati 2015). Morphometrics in plants can be done on the stems, branches, leaves, flowers, and fruit. According to Tjitrosoepomo (1989), classification is carried out to study plant diversity by finding similarities based on the nature and characteristics of plants. Phylogenetic studies are described as the taxonomic classification of organisms based on evolutionary history, which is an integral part of systematic science and aims to determine the phylogeny of organisms based on the characteristics of these organisms (Mount 2001).

Several morphometric studies were carried out by various authors, such as *V. diospyroides* from Thailand (Srisawat et al. 2012), *Moehringia* complex sect, *Pseudomoehringia* from the Western Mediterranean (Lorite et al. 2018), *Euchiton traversii* complex (Flann et al. 2008), *Potamogeton compressus* (Kaplan & Marhold 2012), *Ixora* (*Rubiaceae*) (Mouly et al. 2016) and *Laretia* (*Apiaceae*) (Fernández et al. 2016). However, until now, no research has been carried out on the morphological variations of *V. rassak* in Indonesia. While many phylogenetic studies on *Dipterocarpaceae* have been carried out since 1996, namely Tsumura et al. (1996), Kajita et al. (1998), Dayanandan et al. (1999), Yulita et al. (2005), Cao et al. (2006) and Indrioko (2005). A recent phylogenomic study of *Dipterorpaceae* by Heckenhauer et al. (2017) reported that *Vatica* and *Cotylelobium* are sisters to *Upuna*, *Vateria*, and *Anisoptera.* The results of this study are expected to provide new/additional information about *V. rassak* for field identification and to evaluate the relationship of *V. rassak* with other *Vatica* species based on the *trn*L-*trn*F sequences.

Material and methods

Morphological observation

Selection of specimens. Morphological observations of *V. rassak* were carried out based on examining 799 herbarium specimens collected in the Herbarium Bogoriense (BO). Of the 799 specimens, only 32 specimens had complete leaves, seeds, and flowers and were used for morphometric measurement (Table 1). The herbarium collections used in this study came from Java and Kalimantan (representing the west of Wallace) and Sulawesi, Maluku, and Papua (representing the east of Wallace). Specimens from ex-situ conservation, such as botanic gardens and arboreta in Java, were omitted from observation so as not to provide bias during the analysis.

Table 1. Herbarium specimens with complete morphology used in this research.

No.	Herbarium no.	Collection no.	Location
1	BO-1264576	No.13956	Kalimantan
2	BO-1265932	No. b.b. 29662	Kalimantan
3	BO-1399700	P.K.1429	Kalimantan
4	BO-1858518	2903	Kalimantan
5	BO-1245881	No. 6127	Kalimantan
6	BO-1263320	No.29661	Kalimantan
7	BO-1266001	No.6826	Papua
8	BO-1263495	No.31979	Papua
9	BO-1263496	Kep.76044	Papua
10	BO-1263548	No.b.b. 30935	Papua
11	BO-1260599	No. BW.5365	Papua
12	BO-1263494	LAE62168	Papua
13	BO-1263637	5009	Papua
14	BO-1263547	No.b.b.30935	Maluku
15	BO-1261272	No.VIIB54	Culta Hort. Bogor
16	BO-1932172	A.H.5023	Maluku
17	BO-1263640	No.b.b.33.745	Maluku
18	BO-1263555	No.b.b.30.397	Maluku
19	BO-1264574	No.21646	Kalimantan
20	BO-111658	No.b.b.21.b79	Sulawesi
21	BO-1474544	No.H.W.10718	Papua
22	BO-1474545	No.H.W.10718	Papua
23	BO-0014264	No.6508	Papua
24	BO-1263621	S.H.No: A.89	Kalimantan
25	BO-111637	No.Profest: Cal/III-12	Sulawesi
26	BO-1265409	No.Profest: Cal/III-12	Sulawesi
27	BO-0014517	TFB3799	Papua
28	BO-1266002	No.28138	Papua
29	BO-1260619	No.5340	Papua
30	BO-1258996	No.b.b.2169	Kalimantan
31	BO-1359521	PK1670	Kalimantan
32	BO-1257771	No.b.b.22686	Kalimantan

Selection of characters. The selection of morphological characters and the descriptions of plant organs was carried out based on the morphological study of *Dipterocarpaceae* conducted by Yulita (2001). The macro characters, i.e., seed shape, leaf basal, leaf apex, number of secondary leaf nerves, domatia, and stipule scars, were directly observed without a microscope. The micro characters, such as leaf and seed surfaces, were observed under a light microscope EUROMEX Holland Microscope. Data on quantitative characters such as leaf length, leaf width, petiole length, seed length, seed width, and seed calyx length were measured using a ruler. Leaf color characters in herbarium material were not included in the analysis because the leaf color measurements have a high subjectivity value (Chandler & Crisp 1998). A total of 14 morphological characters were selected from leaves and fruit (Table S1), with the description of each character as follows:

1. Leaf length

   Leaf length is measured at the longest leaf part (Fig. 2a) with three- character states:

   0 = leaf length less than 20 cm
   1 = leaf length between 20 cm to 25 cm
   2 = leaf length is more than 25 cm

2. Leaf width

   Leaf width measurements were made at the widest part of the leaf (Fig. 2b) with three character states:

   0 = leaf width less than 7 cm
   1 = leaf width between 7 to 8 cm
   2 = leaf width more than 8 cm

3. Number of secondary leaf nerves

   The number of secondary leaf nerves is often used as a character that distinguishes individuals within a species (Ashton 1982) with three character states:

   0 = number of secondary leaf nerves less than 14
   1 = number of secondary leaf nerves between 14 and 17
   2 = number of secondary leaf nerves more than 17

4. Petiole length

   The petiole length of *V. rassak* has three character states (Fig. 2c):
   0 = petiole length less than 1 cm
   1 = petiole length between 1 and 2 cm
   2 = petiole length more than 2 cm

5. The texture of tertiary leaf nerves

   The tertiary nerves in *V. rassak* have two character states (Fig. 3):
   0 = non-prominent
   1 = prominent

6. The shape of the leaf base

   Two characters are stated for the leaf base, cuneate, and subcordate (Fig. 4).
   0 = cuneate (Fig. 4A)
   1 = subcordate (Fig. 4B)

Figure 2. Measurement of leaf size: leaf length (a), leaf width (b) and petiole length (c).

Table 2. Sample species used for molecular study.

No.	Name	GenBank accession number	Location	Reference
1	*Vatica rassak* 3	PQ824224	Fef, West Papua	**This study**
2	*Vatica rassak* 12	PQ824225	Papua	**This study**
3	*Vatica endertii*	KY972875	Temburong, Brunei Darussalaam	Heckenhauer et al. (2017)
4	*Vatica endertii*	KY972874	Temburong, Brunei Darussalaam	Heckenhauer et al. (2017)
5	*Vatica oblongifolia* subsp. *oblongifolia* 1	KY972892	Temburong, Brunei Darussalaam	Heckenhauer et al. (2017)
6	*Vatica oblongifolia* subsp. *oblongifolia* 2	KY972891	Temburong, Brunei Darussalaam	Heckenhauer et al. (2017)
7	*Vatica dulitensis* 1	KY972883	Temburong, Brunei Darussalaam	Heckenhauer et al. (2017)
8	*Vatica dulitensis* 2	KY972882	Temburong, Brunei Darussalaam	Heckenhauer et al. (2017)
9	*Vatica vinosa* 1	KY972897	Temburong, Brunei Darussalaam	Heckenhauer et al. (2017)
10	*Vatica vinosa* 2	KY972896	Temburong, Brunei Darussalaam	Heckenhauer et al. (2017)
11	*Vatica micrantha* 1	KY972887	Temburong, Brunei Darussalaam	Heckenhauer et al. (2017)
12	*Vatica micrantha* 2	KY972886	Temburong, Brunei Darussalaam	Heckenhauer et al. (2017)
13	*Vatica chinensis*	KY972876	Unknown	Heckenhauer et al. (2017)
14	*Vatica harmandiana*	KY972881	Thailand	Heckenhauer et al. (2017)
15	*Vatica sarawakensis* 1	KY972895	Temburong, Brunei Darussalaam	Heckenhauer et al. (2017)
16	*Vatica sarawakensis* 2	KY972894	Temburong, Brunei Darussalaam	Heckenhauer et al. (2017)
17	*Dipterocarpus confertus* 1	KY972722	Temburong, Brunei Darussalaam	Heckenhauer et al. (2017)
18	*Dipterocarpus confertus* 2	KY972721	Temburong, Brunei Darussalaam	Heckenhauer et al. (2017)

7. Leaf apex

   V. rassak has two types of leaf apex (Fig. 5):

   0 = acute
   1= acuminate

8. Presence of domatia

   Domatia is a gland that is usually present in the leaf base attached to the midrib (Fig. 6). The scoring is based on the presence or absence of domatia:

   0 = absent
   1 = present

9. Presence of stipule scar

   The stipule scar can be used as a diagnostic character for an *infra-generic* level of the *Dipterocarpaceae* (Yulita 2001). The scoring is based on the presence or absence of a stipule scar.

   0 = absent
   1 = present

10. Seed shape

    Three characters states were observed for seed shape (Fig. 7):
    0 = oblong with attenuate tip (Fig. 7A)
    1 = obovoid (Fig. 7B)
    2 = ovoid (Fig. 7C)

11. Fruit surface

    According to Ashton (1982), the fruit surface of *V. rassak* has two kinds: verruculose and wrinkled (rugulose). The surface character of rugulose seeds (Fig. 8A) has a smoother texture than verruculose (Fig. 8B), which has a slightly raised and rough surface. The two characters states for seed surfaces are:

    0 = rugulose
    1 = verruculose

12. Fruit length

    Seed length measurements were made at the longest part of the seed (Fig. 9a). Three character states were observed:

    0 = seed length less than 3.5 cm
    1 = seed length is more than 3.5 cm and less than 4.7 cm
    2 = seed length is more than 4.7 cm

13. Fruit width

    Seed width measurements were made at the widest part of the seed (Fig. 9b) with three character states:

    0 = seed width less than 2.5 cm
    1 = seed width between 2.5 cm and 3 cm
    2 = seed width is more than 3 cm

14. Fruit calyx length

    Vatica rassak has several shapes of calyx lobes, i.e., deltoid, conical (acute), incrassate, reflexed, and recurved (Ashton 1982), with different sizes. Three character states of the length of calyx lobes are recognized:
    0 = length of calyx less than 0.9 cm
    1 = length of calyx between 0.9 cm and 1.2 cm
    2 = length of calyx more than 1.2 cm

Molecular study. Sixteen samples, consisting of nine *Vatica* spp. and *Dipterocarpus confertus* as an outgroup, were selected in this study (Table 2). Samples for molecular work consisted of silica dried samples collected from West Papua (Nugroho et al. 2016) and small cuts of leaf from herbarium specimens.

Isolation of DNA, amplification, and sequencing. Specimens from the herbarium collection were pre-treated before proceeding with the DNA isolation. Approximately 10 mg dried leaf was incubated in 1X CTAB-PVP in a 37°C water bath overnight to eliminate residual chemicals existed during the processing of herbarium specimens. The total DNA was isolated using a Genomic DNA Mini Kit (Plant) from the Geneaid following the manufacturer's protocol. Amplification of the *trn*L-*trn*F region by the PCR technique was conducted using a universal pair primer of forward primer 'c' (CGAAATCGGTAGACGCTACG) and reverse primer

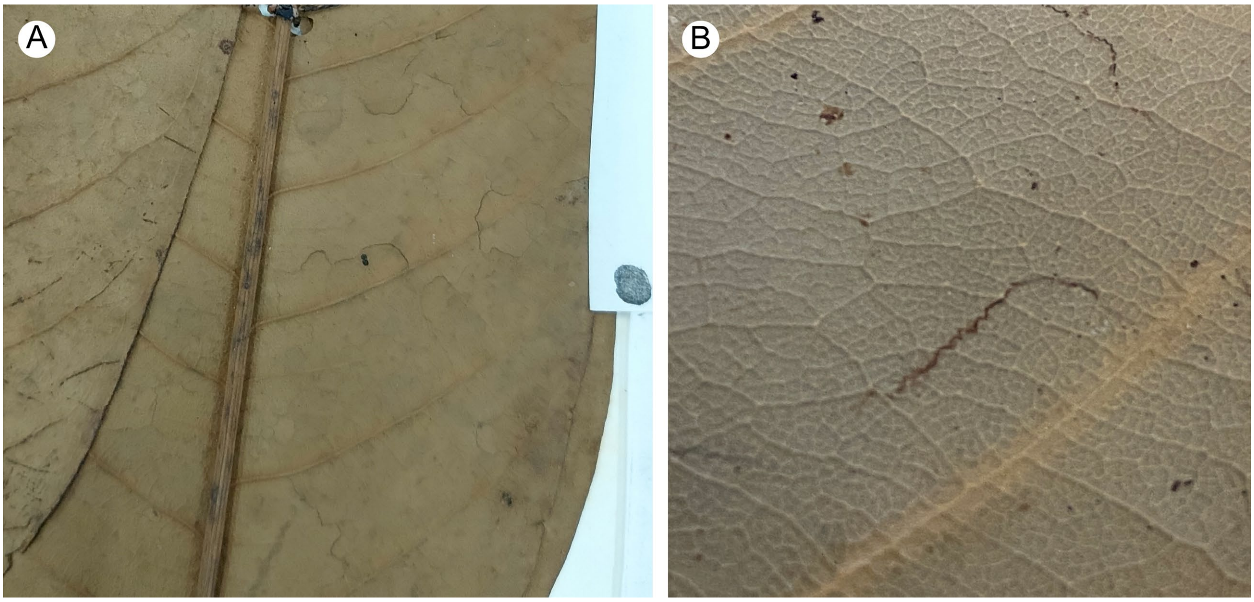

Figure 3. Type of tertiary leaf nerves in *V. rassak*: *non-prominent* (A) and *prominent* (B).

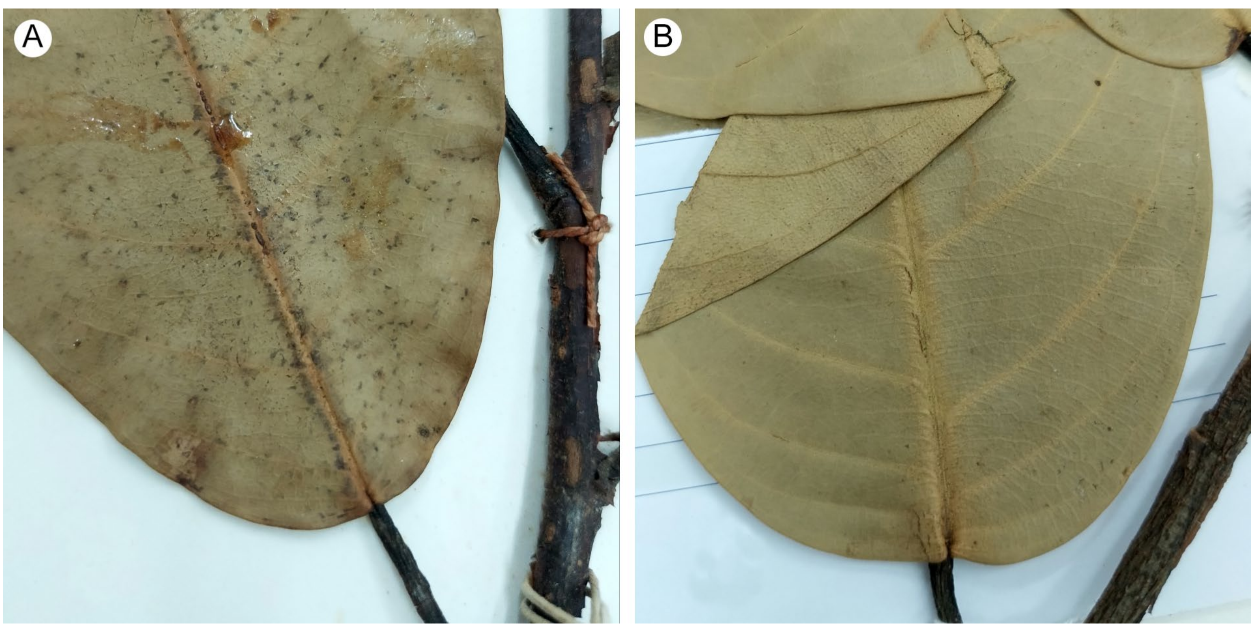

Figure 4. The shape of the leaf base observed in *V. rassak*: *cuneate* (A) and *subcordate* (B).

'f' (ATTTGAACTGGTGACACGAG) (Taberlet et al. 1991). The PCR mixture of a total volume of 12.5 µL consisted of 0.375 µL each of forward and reverse primer (5 pmol each), 0.25 µL Taq DNA polymerase, 2.5 µL of dNTP, 6.25 µL PCR Buffer, 1.75 µL of nuclease free water, 1 µL of DNA template (10 ng/µL). The reaction was performed in Sedi G Thermo Cycler (Wealtec) with the optimum condition comprising a pre-denaturation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 10 min.

The amplified bands were visualized on 1.5% agarose stained with GelRed. Electrophoresis was executed with 50 V for 60 min in 1× TBE buffer and the target *trn*L-*trn*F bands were visualized under UV light using Atto Bioinstrument. Sanger sequencing was used to sequence the amplicons at the First Base Company, Singapore.

Data analysis

Morphology

The selected characters consisted of quantitative and qualitative characters. The quantitative (including continuous) characters were standardized to minimize the bias due to a wide measurement range. The standardization method used the transformation of log-n because it is the most effective measure for taxonomic variables (Chandler & Crisp 1998).

The scored data matrix was used for cluster analysis using the *Unweighted Pair Group Method with Arithmethical average* (UPGMA) and Principal Component Analysis (PCA) available in NTsys-pc *version* 2.02. The similarity was calculated using SIMINT (*Similarity for Interval Data*) with the DIST (*Distance*) coefficient. The SIMINT matrix was used to build a dendrogram through SAHN using the clustering method.

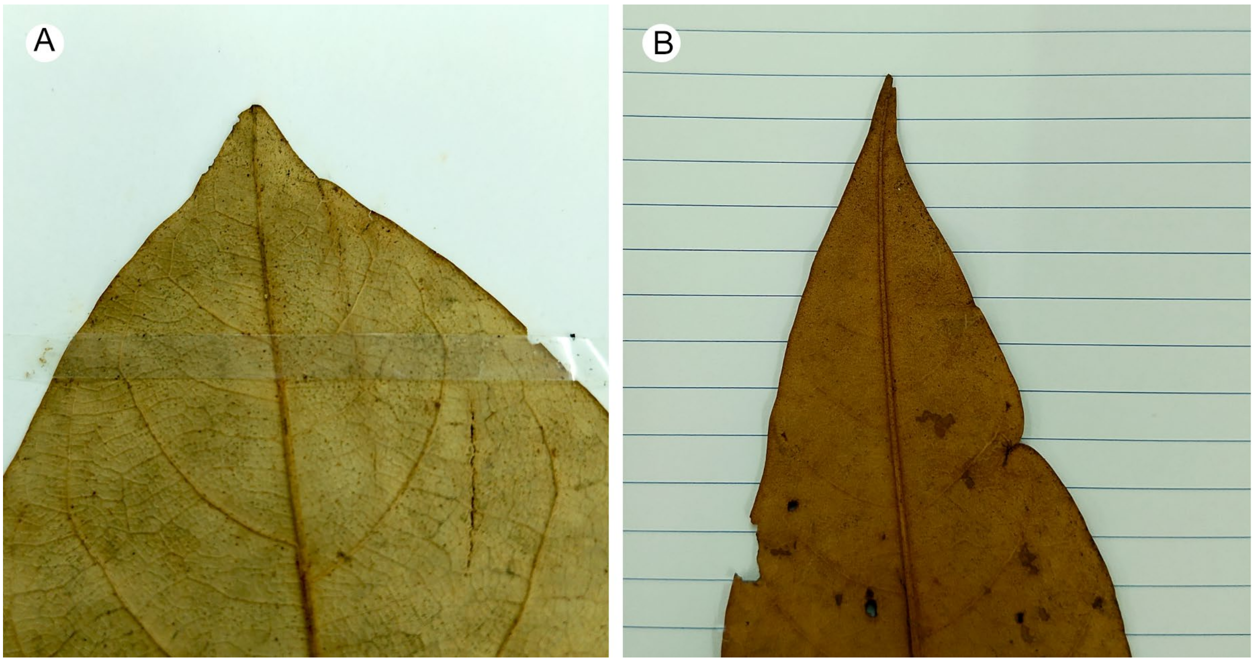

Figure 5. The shape of the leaf apex: acute (A) and acuminate (B).

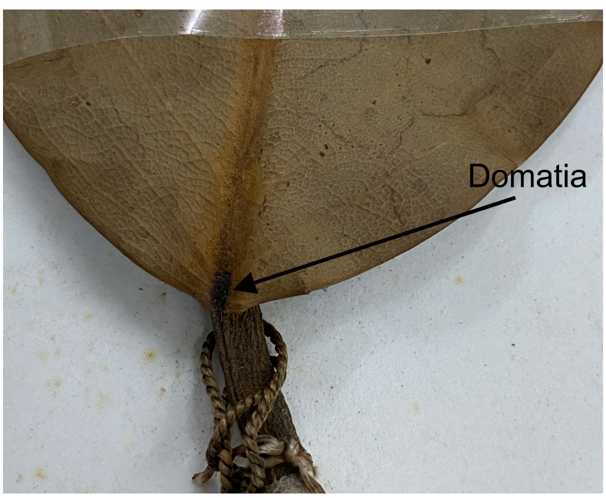

Figure 6. Domatia.

Molecular analyses

Chromas Pro (Technelysium, South Brisbane, Australia) was used to assemble the *trn*L-*trn*F sequence (Genetyx Co., Japan). The forward and reverse sequences were observed to ensure no mismatch in the consensus produced. MEGA 10.0 software was used to evaluate the nucleotide composition of the *trn*L-*trn*F gene (Kumar et al. 2016). The homology and identity of samples were examined using BLAST nucleotide on GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The data from the GenBank were downloaded in FASTA format and aligned using Geneious.

The phylogenetic tree was constructed using the Maximum Likelihood method based on the Kimura 2-parameter model (K2P) (Kimura 1980; Kumar et al. 2016). The bootstrap consensus tree was inferred from 1,000 replicates (Felsenstein 1985). The branches with less than 50% bootstrap value were collapsed. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances. They were estimated using the Maximum Composite Likelihood (MCL) approach, and their topology was selected using a superior log-likelihood value. Furthermore, a discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 1.7815)). Bootstrap support (BS) values of ≥85%, 75–84%, and 50–74% were considered to be strongly, moderately, and weakly supported; and values <50% were not indicated (Devecchi et al. 2018).

Results

Morphological variation of Vatica rassak

The results of the observations from the herbarium specimens confirmed the distribution of *V. rassak* covers Kalimantan, Sulawesi, Maluku (Bacan, Ternate, and Halmahera), and Papua as reported by Ashton (1982). Among 14 morphological characters observed, nine characters originated from the leaf and five are from the fruit (Table S1).

Leaf shape can be deduced from leaf length and width (Table S1), which varies across regions. Specimens from Kalimantan tend to have longer leaves (>25 cm) than those from Sulawesi, Maluku and Papua. The narrow leaf (<7 cm) and broad (>8 cm) leaf widths were dominated by specimens originating from Papua and Maluku, while Kalimantan's specimens have leaf sizes in between (Table S1). This observation concluded that the Kalimantan's specimens representing west Wallace have longer leaf sizes than specimens from east Wallace. It seemed that leaf shape and size had randomly occurred across the region.

The specimens from Kalimantan have a relatively larger number (more than 17) of secondary leaf nervations and a longer petiole (>2 cm) than those of Sulawesi, Maluku,

Figure 7. Seed shape of *V. rassak*: oblong with attenuate tip (A), ovoid (B), and ovoid (C).

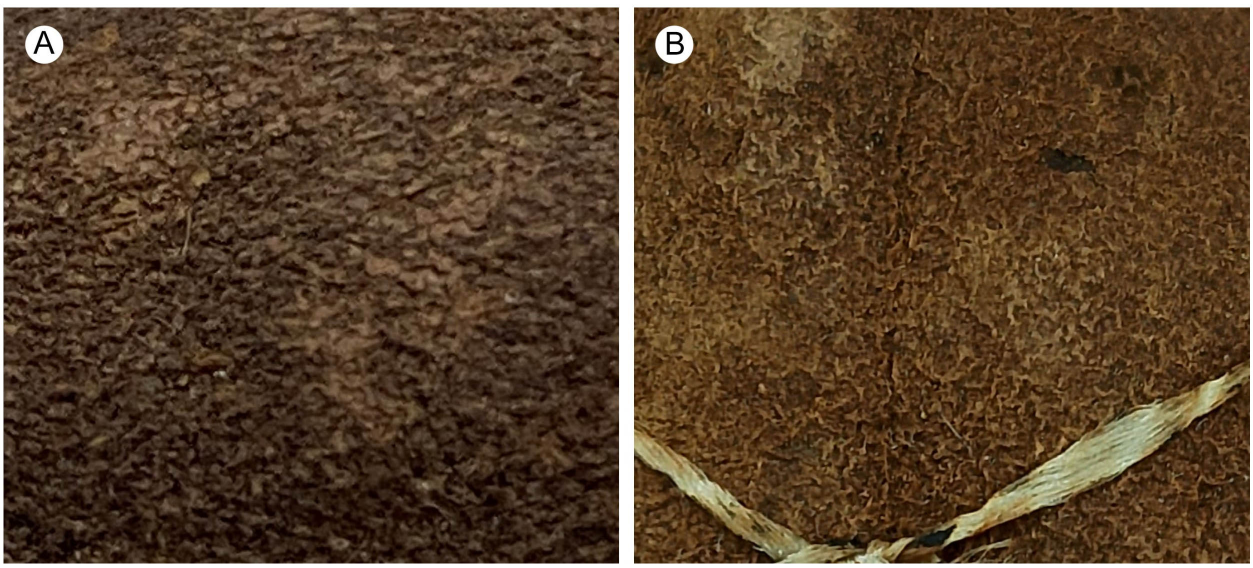

Figure 8. The texture of fruit surface on *V. rassak*: rugulose (A) and verruculose (B).

and Papua's specimens (Table S1). As for the character of the leaf base, the Kalimantan and Sulawesi specimens tend to have round to subcordate bases, while the majority of specimens from Papua have subcordate leaf bases. Meanwhile, the shape of the leaf apex randomly exists in all specimens (Table S1).

This study also examined variations on domatia and stipule scars, which were possessed by the majority of the specimens. However, the two characters were randomly present across the specimens examined and did not follow any particular pattern of geographic distribution.

Vatica rassak has three types of fruit: oblong with attenuate tip, obovoid, and ovoid, with the oblong with attenuate seed shape (Fig. 7A) being the most common form found in 59 specimens from 77 specimens observed and found in all regions. This fruit type was found in Kalimantan, Maluku, and Papua. The obovoid type (Fig. 7B) was found on 15 specimens from Sulawesi and Kalimantan. The ovoid seeds (Fig. 7C) were only found in 3 specimens from Kalimantan. Hence, Kalimantan has more variation in seed shapes than other regions. The texture of the fruit surface is almost in accordance with biogeographic delineation. Both regulose and verruculose texture can be found on specimens from all regions, but specimens from Kalimantan have mainly rugulose texture (Table S1). The other fruit characters, the fruit size (length and width) and fruit calyx length are randomly present on all specimens.

Grouping analysis of *Vatica rassak*

The cluster analysis results (Fig. 10) showed two main clusters (clusters I and II) with a coefficient of 1.05. The clustering tends to be grouped based on the shape of the seeds.

Cluster I consisted of *V. rassak* with prominent characteristics of obovoid seeds and other characteristics such as leaf surface, leaf basal, seed surface, leaf apex, stipule scar, and domatia. In cluster I, there is a variation where the seeds *V. rassak* are round in shape (ovoid) from sub-cluster IA, forming a separate group and separating from sub-cluster IB, which is a *V. rassak* with seed shape which is dominantly obovoid. However, the two clusters are generally grouped in cluster I with a morphological distance coefficient of 1.03.

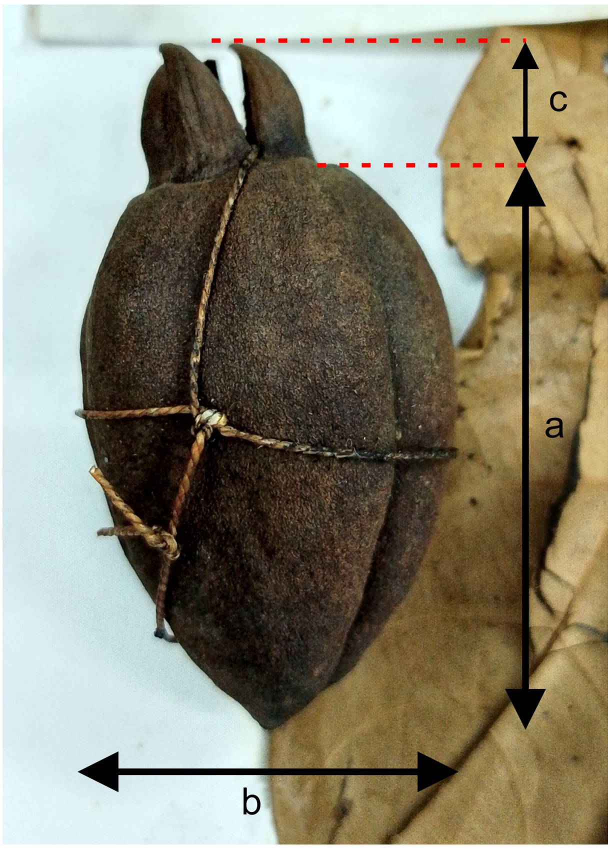

Figure 9. Measurement of fruit and fruit calyx: seed length (a), seed width (b) and calyx length (c).

Cluster II consisted of *V. rassak* with the most dominant seed shape being oblong with attenuate tip and having prominence, which grouped into one cluster, namely the character of leaf width and length of the calyx. *V. rassak* sample no 21 Sub-cluster IIA from Sulawesi was separated from other *V. rassak* with a morphological distance coefficient of 0.95. This specimen of *V. rassak* has an ovoid seed character, but the apex is not tapered like most of the dominant ovoid seeds, but blunt.

The distribution pattern of 32 *V. rassak* specimens based on morphological characters by PCA analysis showed the same grouping as in the UPGMA dendrogram (Fig. 11). The grouping of 32 specimens of *V. rassak* analyzed based on morphological variations did not appear to be related to geographic origin, but mixed between specimens from one area to another.

Phylogenetic analysis of *Vatica* spp.

The result from phylogenetic analysis suggested that the genus *Vatica* formed a group with weak support from the bootstrap (A, BS < 50%, Fig. 12). The group *Vatica* consisted of gradual groupings with *V. harmandiana* (Section Sunaptea) located at the basal lineage. Taxa included in groups B and C consisted of species from both sections with mostly unresolved branches with moderate support from the bootstrap (Fig. 12). Group B consisted of a group of *V. chinensis* (Section Vatica) with unresolved branches that are the sister of the remaining *Vatica* spp. included in this study (Group C). Group C is the main group of *Vatica* spp. where eight species are unresolved position, including *V. rassak*. Most are the unresolved *Vatica* spp. belong to Section Vatica. Three species within the Group C, i.e., *V. endertii*, *V. oblongifolia subsp. oblongifolia*, and *V. dulitensis* form a separate group.

Discussion

Morphological variation

Morphological characters are still the primary important characters to identify and describe particular taxa. This is because of the ease of determining morphological characters, the large number of variations, and the ease of use of herbarium and fossil collections. The herbarium specimens are an important source of information in studying morphological variations, including understanding the process of plant evolution (Espinosa & Castro 2018). Morphological characters can be described through

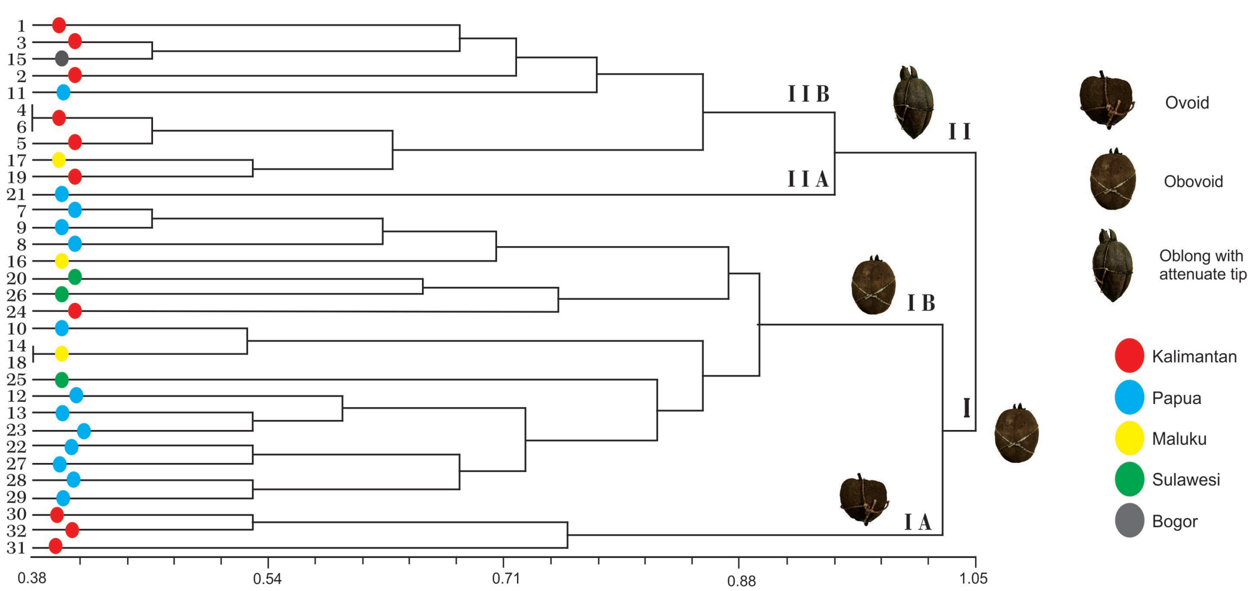

Figure 10. A UPGMA dendogram showing clustering of samples of Vatica rassak. The coefficient of similarity is shown as the X axis. Colored dots are the origin of samples.

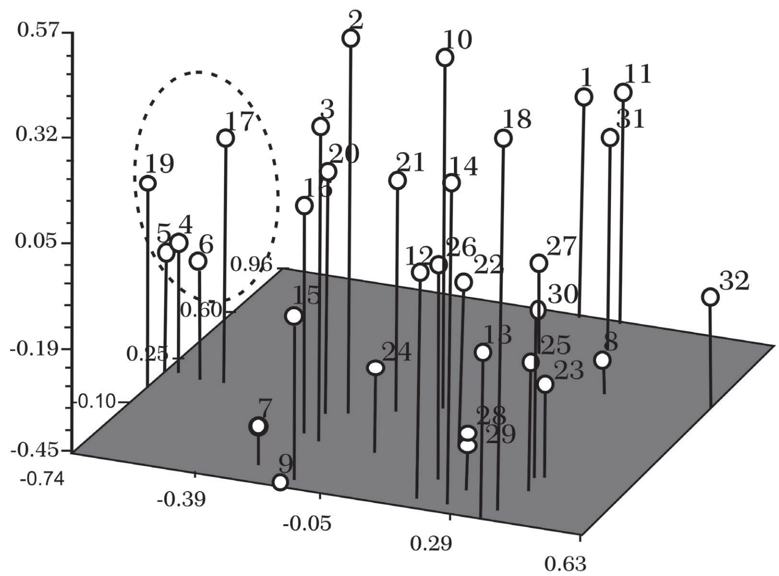

Figure 11. A 3-dimensional PCA diagram of 32 specimens of *Vatica rassak*. Accession number as in Table 1. The circle showing the group corresponds to the cluster diagram in Fig 10.

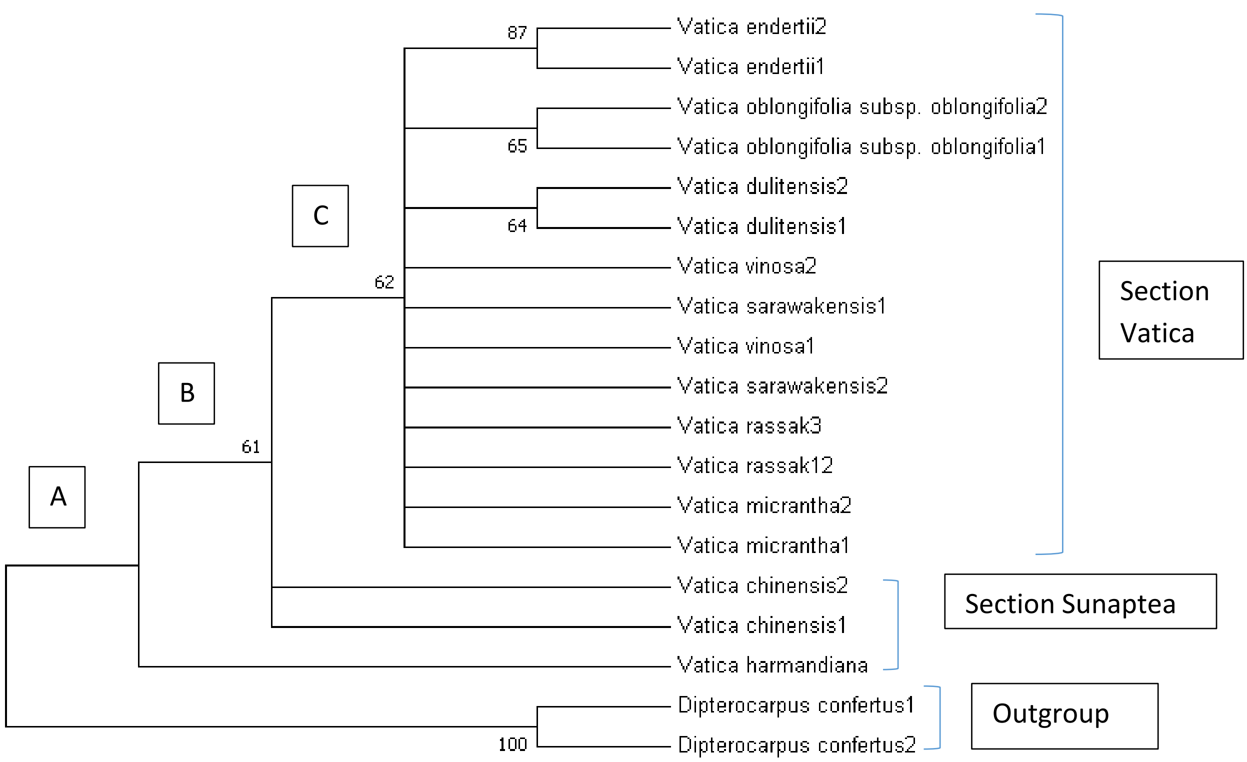

Figure 12. A phylogenetic tree of *Vatica* spp. based on the *trn*L-*trn*F region using the Maximum Likelihood model. Bootstrap support of > 50% was written on the branch. A: the ingroup, B: Section Sunaptea, C: Section Vatica.

measurement, counting, or scoring, known as morphometrics (Bookstein 1982).

This study demonstrated that morphological traits in *Vatica rassak* exhibit variations between specimens from the east and west Wallace regions, with notable differences observed in leaf and fruit characteristics. Characters were present almost in accordance with the biogeographic origin, such as leaf size, fruit shape and texture, while other characters were distributed randomly across the regions (domatia, stipule scars and length of fruit calyx).

Vatica rassak is the only *Diperocarpacae* species whose natural distribution crosses the eastern and western boundaries of the Wallace line (Ashton 1982). Although in the early Tertiary period, Sulawesi was isolated from Kalimantan near the Makassar Strait, resulting in strong biogeographical differences between Kalimantan and Sulawesi. This species is abundant in riverside areas, and the seeds were likely dispersed by water. The reduced size of the fruit calyx does not allow dispersal by wind as with many other Dipterocarps winged species. Indrioko

(2005) reported that *V. rassak* from Maluku shows similarities in character with *V. venulosa* and *V. pauciflora*, originating from Sumatra (Sundaland) (Indrioko 2005). Further examinations of the morphological variations of those three *Vatica* species may be needed.

The distribution of *V. rassak*, influenced by geological distribution, is also thought to influence the grouping pattern of morphological variations. Mouly et al. (2016) studied seed morphology and texture information combined from alcohol-preserved specimens, and field notes reported that grain texture varied in response to environmental elevation. A similar study involving environment and climatic factors that contributed to the morphological variation of *V. rassak* is important to carry out since *V. rassak* has large morphological variations. Our present study showed that specimens from Kalimantan have the most morphological variations. Tropical forests in Borneo (Brunei Darussalam, Kalimantan, Sabah and Sarawak) are considered one of the twelve mega biodiversity centers in the world (Tan et al. 2009). Many plant groups have their center of diversity on the island, including *Dipterorcarpaceae*. The morphological diversity in the tropics is thought to be the result of large, frequent changes over time in habitat distribution and geographic boundaries. However, it is uncertain that species with the same habitat and geography will respond identically from flower bud initiation to light sensitivity, water stress, mycorrhizal invasion, seed predation, or sharing the same pollinators and seed vectors (Ashton 1982). In Borneo, *V. rassak* grows well in the lowland forest. The natural habitat of *V. rassak* are areas along river banks, swamp forests, primary forests, and secondary forests. It grows in sandy or loamy soil conditions and typically thrives at altitudes not exceeding 400 meters above sea level. This is supported by Slik's (2009) report indicating that *V. rassak* is known to grow at altitudes ranging from sea level up to 400 meters above sea level (m a.s.l.).

In addition, the hermaphrodite inflorescence of *V. rassak* is also a factor in the morphological variation, as found in many other *Dipterocarpaceae* species. Dipterocarp flowers are hermaphrodites pollinated by various insects as vectors (Appanah 1981; Appanah & Chan 1981; Momose et al. 1998; Corlett 2004). Plants with out-crossing reproductive biology provide a greater opportunity to increase the heterozygosity value in their population (Syamsuardi 2015), thus contributing to the high variations observed.

Phenetic analysis

Phenetic analysis in this study aims to determine the pattern of grouping based on the similarity of morphological characters. The grouping of specimens into one cluster indicates a high level of similarity in their morphological characters, as indicated by the similarity coefficient. The observed morphological distances of similarity coefficients ranged from 0.38 to 1.05 (Fig. 11). This variability span of 0.67 indicates that the morphological variation in Vatica rassak is moderate.

The x-axis (PC1), y-axis (PC2), and z-axis (PC3) represent the proportions of variance explained by the first, second, and third principal components, respectively. PC1 captured the largest variation in the dataset, followed by PC2 and PC3, which also account for additional, distinct variations. Points with higher variance indicate greater similarity among the corresponding individuals, resulting in closer clustering within the dataset.

Several taxa can be consistently identified based on the origin of the specimens, such as the taxa *Potamogeton compressus* (Kaplan & Marhold 2012), but not for *V. rassak*. Hence, the possible grouping of *V. rassak* is based on the most prominent character. Several informative character parameters used in this study were not sufficiently informative to separate the specimens into distinct groups. This may to be due to the selection of prominent characters from only two organs, leaf and fruit. Other parameters, such as floral organs, need further examination (Kaewmuangmoon et al. 2010). According to Jones & Luchsinger (1986), reproductive organs show more specific and consistent variations in a plant, such as pistil, number of stamens, and completeness of flowers. In addition, pollen generative organs have exine surfaces with distinctive ornaments to provide accurate morphological data. Meanwhile, Candolle (1868, 1824) in Appanah & Chan (1981) emphasized the importance of the number of stamens and their position in the petals to separate the genus *Dipterocarpus.*

Phylogenetic analysis

A detailed examination of the phylogenetic tree suggests that the phylogenetic position of *V. rassak* remained unresolved. Several collapsed branches observed in the main group (B) of Section Vatica may be due to the low number of nucleotide variations obtained, thus additional analysis of more variative nucleotide composition is needed. The inclusion of three species of Section Sunaptea within the main group comprised mainly taxa from Section Vatica therefore suggests that Section Sunaptea is a paraphyletic group. The monophyly of Section Vatica needs further confirmation by including more parsimony informative characters derived from other genes/regions or other non-molecular characters, as well as inclusion of more *Vatica* species into the analysis.

In their study, Dayanandan et al. (1999) showed that the genus *Vatica* was monophyletic in the resulting phylogenetic tree. The clades of the genera *Anisoptera*, *Vatica*, and *Cotylelobium* are monophyletic groups, even though only the clade *Cotylelobium* received a strong BS value (93%). Kajita et al. (1998), who carried out the phylogenetic study using matK, IGS of *trn*L-*trn*F, and intron *trn*L, also reported that *Vatica* formed a monophyletic group with a group consisting of *Anisoptera*, *Cotylelobium*, and *Upuna*, with a close relationship between *Anisoptera* and *Vatica*. Another phylogenetic study by Indrioko (2005) suggested the monophyly of infrageneric division of *Vatica* revealed by Section Vatica (except for *Vatica bella*) with strong branch support (BS value of 94%). Hence, a sectional division of *Vatica* may be possible to form a natural group. The tendency of natural grouping within Dipterocarps has also been shown in Tengkawang group (*Shorea* section *Pachycarpae*)

(Yulita 2016), both the morphological and molecular data showed the same trend.

The subdivisions of the *Vatica* genus have been modified by several authors. According to Cao (2006), the *Vatica* genus is characterized by fruit sepals, scattered resin canals, and the number of chromosomes x = 11. Several species or groups of *Vatica* are categorized as separate sections, including Sunaptea, Euvatica, Isauxis, Retinodendron, and Pachynocarpus (Maury-Lechon & Curtet 1998). Ashton (1982) reduced the classification of the infrageneric genus *Vatica* into two parts, mainly based on the lobes of the fruit petals, namely Section Vatica and Section *Sunaptea*. Section Vatica is characterized by the same calyx lobes (including Section *Isauxis*, *Retinodendron*, and *Pachynocarpus*). Section *Sunaptea* is characterized by unequal fruiting lobes, two lobes longer than the other three (including Section *Euvatica*). However, the flower, leaf and woody structures of *Vatica* are almost identical, and the general division cannot be clearly defined (Symington 1943) as supported by this present study.

Conclusion

Morphological variation, assessed using 14 characters, exhibited similarity coefficients with morphological distances ranging from 0.38 to 1.05, indicating a variability span of 0.67. The resulting groupings are not related to geographic origin. Variation in fruit shape was not a diagnostic character for the infrageneric division of *Vatica*. Molecular phylogenetic results confirm that *V. rassak* is in group of Section Vatica with an unresolved position. Both morphometric and phylogenetic analysis suggest that *Vatica* species included in this study cannot be delineated based on phytoregions nor according to the present infra-generic classification (Ashton 1982).

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No potential conflict of interest was reported by the author(s).

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Supplementary electronic materials

Table S1. Summary of characters, herbarium samples and distribution of each sample. Download file

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