January 2022

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

Committees: The Second International Conference on Genetic Resources and Biotechnology

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Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24th-25th of May 2021virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

Dr. Toto Hadiarto

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DNA barcoding of *Vatica bantamensis*, a critically endangered tree endemic to Banten, Indonesia

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Muhammad Rifqi Hariri, Iyan Robiansyah, Dipta Sumeru Rinandio, et al.

DNA Barcoding of *Vatica bantamensis*, a Critically Endangered Tree Endemic to Banten, Indonesia

Muhammad Rifqi Hariri[1, a)], Iyan Robiansyah[1], Dipta Sumeru Rinandio[1], Dodo[1], Desi Siti Sundari[2], Cecep H. Sukmawan[2] and Bayuntoro Ardi[2]

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Abstract. *Vatica bantamensis* is a member of the family Dipterocarpaceae endemic to Ujung Kulon National Parks in Banten Province, Indonesia. It is classified as a critically endangered species by IUCN Red List. Under this category, the species is considered to be facing an extremely high risk of extinction in the wild. The objectives of this study were to provide easy and accurate identification of *V. bantamensis* for the conservation and sustainable use of the species. In the present study, we conducted DNA barcoding of *V. bantamensis* using an *rbc*L marker. We used *V. rassak* as a comparison species since both species are morphologically closely related. A total of 606–608 bp nucleotides have been successfully amplified and sequenced. The dendrogram constructed using the neighbor-joining method with Tamura 3-parameter showed that *V. bantamensis* used in this study were clustered together with *V. rassak*. These two species were separated clearly against other *Vatica* species and two outgroup species, namely *Dryobalanops lanceolata* and *Shorea pinanga*, obtained from the NCBI database. The *rbc*L sequence can discriminate *V. bantamensis* from *V. rassak* in six different sites (136, 507, 522, 565, 566, and 567). This study demonstrated that the *rbc*L sequence could authenticate the samples of *V. bantamensis* and support the correct identification of this endangered plant species.

INTRODUCTION

The evaluation of threats to both individual species and overall biodiversity is critical to maximizing species survival through prioritizing and logical resource allocation. According to a comprehensive examination of extinction risk assessments, a total of 39% of plant species are endangered [1]. Once extinction debt and climate change are taken into consideration, the number of threatened species appears to be increasing. Habitat conversion and degradation due to the expansion of settlement and agricultural field, mining, and logging are considered the main threat to plant diversity, followed by unsustainable exploitation and climate change [2, 3]. These threats usually work synergistically [4, 5] and combined with other threats, e.g. invasive species, pollution, and pathogens.

Previous studies have shown that endemic plants with narrow geographic distribution face the most significant extinction risk from the threats mentioned above [6–9]. This is because species with small ranges tend to have the following attributes that make them vulnerable to environmental perturbations [10] such as: i) low number of localities, ii) small population number, iii) small population size and low genetic diversity, iv) experiencing over-exploitation, v) declining population size, vi) low reproductive ability, vii) requirement of specific ecological niche, and viii) growth that need relatively stable environmental conditions. Due to its crucial role, small size range becomes one of the critical criteria in the International Union for Conservation of Nature's (IUCN) Red List [11], the most widely used tool to assess the extinction risk of a species.

To conserve a narrow endemic plant species, it is vital to have an integrated conservation action that employs both in situ and *ex situ* approaches [12, 13]. One taxonomic impediment in both approaches is the correct identification of the species. The conventional species identification using morphological characteristics is subject to

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several caveats, including a lack of taxonomic expertise and high cost as well as considerable amount of time needed to sample and identify the plant material correctly, which is especially challenging in highly diverse ecosystem such as tropical forests [14–16]. DNA barcoding offers a more rapid and accurate species identification by non-specialists using DNA sequence comparison of specific gene regions [17, 18]. The method has helped to identify accurately many threatened endemic plant species, including 14 endemic plant species in Trinidad [19], *Decalepis* species in Peninsular India [20], and *Chamaecyparis formosensis* and *C. obtuse* var. *formosana* in Taiwan [21].

The chloroplast ribulose-1, 5-bisphosphate carboxylase/oxygenase or Rubisco large subunit gene (*rbc*L) is one of the approved barcodes for plants based on assessments of its levels of species discrimination, sequence quality, and recoverability [22]. The *rbc*L is the best-characterized gene among plastid regions, and therefore, primer design can be easily improved [23].

In this study, the ability of DNA barcoding to confirm the identity of the narrow endemic tree *Vatica bantamensis*, a member of the family Dipterocarpaceae, was assessed using *rbc*L. Found only in Ujung Kulon National Park, Indonesia, *V. bantamensis* is categorized as a critically endangered species by IUCN Red List and has a restricted geographic distribution with an extent of occurrence and area of occupancy of 8 km^2 [24]. The species was reported to be morphologically closely related to *V. rasssak* [25]. Therefore, we used *V. rassak* as a comparison species to test the discrimination capacity of *rbc*L. The objectives of this study were to provide easy and accurate identification of *V. bantamensis* for the conservation and sustainable use of the species. In order that it can help sustain the conservation and utilization of the species.

MATERIALS AND METHODS

Plant Sample Collection

Leaf samples of *V. bantamensis* were collected from Mount Payung (6°49'09.7"S, 105°15'53.0"E) in Ujung Kulon National Park, Banten Province, Indonesia. For DNA extraction and further barcoding analysis, we only used two samples collected from two different mature individuals. The samples were kept in plastic ziplock bags containing silica gel until they were transported to the Molecular Laboratory of the Bogor Botanic Gardens (BBG) and stored in the freezer (-80°C) for further analysis. For *V. rassak*, we used only one sample collected from the living collection of the BBG (XXV.B.126).

DNA Extraction, Amplification, and Sequencing

Total genomic DNA of three plant samples was extracted using GeneJET Plant Genomic DNA Purification Kit (Thermo Fisher Scientific, USA). The *rbc*L region was amplified using the universal primer of *rbc*La-F (5′-ATGTCACCACAAACAGAGACTAAAGC-3′) and *rbc*La-R (5′-GTAAAATCAAGTCCACCRCG-3′) [22]. PCR reaction was run using a 50 µl reaction mixture containing 10 ng of the DNA template, 1 µM each of forward and reverse primers, 25 µl of MyTaq™ HS Red Mix (Bioline, USA), and 17 µl distilled water. We used the following PCR conditions: 1 cycle (94°C for 5 min), 35 cycles (94°C for 15 sec, 52°C for 15 sec, and 72°C for 10 sec), and 1 cycle (72°C for 5 min). The PCR products were migrated on 1% agarose gel electrophoresis and visualized through Bio-Rad GelDoc. We then sent these PCR products to 1st Base (Singapore Science Park II, Singapore) for DNA sequencing.

Data Analysis

The *rbc*L sequences were constructed into contig sequence using CodonCode Aligner 9.0.1 (CodonCode Corporation, Centerville, Massachusetts, USA; http://www.codoncode.com/aligner/) and then submitted to NCBI BLAST to determine the homology level (https://blast.ncbi.nlm.nih.gov/). The Automated Barcode Gap Discovery (ABGD) analysis was carried out to distinguish the species divergence through the ABGD website (https://bioinfo.mnhn.fr/abi/public/abgd/) using JC69 Jukes-Cantor measure of distance [26]. Two assessment methods were used to prove the correct identification of the obtained *rbc*L sequences, namely the sequence similarity and phylogenetic tree methods. In the sequence similarity method, the query sequences were identified correctly when the E value was 0 and showing a percent identity more than equal to 96% with a species in the reference database. For the phylogenic tree method, we used MEGA X [27] to construct a phylogenetic tree using the neighbor-joining (NJ) method and the Tamura 3-parameter model with 1,000 bootstrap replicates [28–30]. There

were a total of 579 positions in the final dataset. The species identification was considered successful if the monophyletic group of samples representing a species was formed with bootstrap support of more than 70%.

RESULTS AND DISCUSSIONS

PCR Amplification

The *rbc*L sequences were successfully amplified from the three samples used in this study. The length of the products from the two samples of *V. bantamensis* and one sample of *V. rassak* were 606, 607, dan 608 bp, respectively (Fig. 1). Most of the *rbc*L sequences have the length range of 501–1,000 bp with the full-length around 2,500 bp [31]. However, the length would be around 600 bp for the most commonly used primers [32].

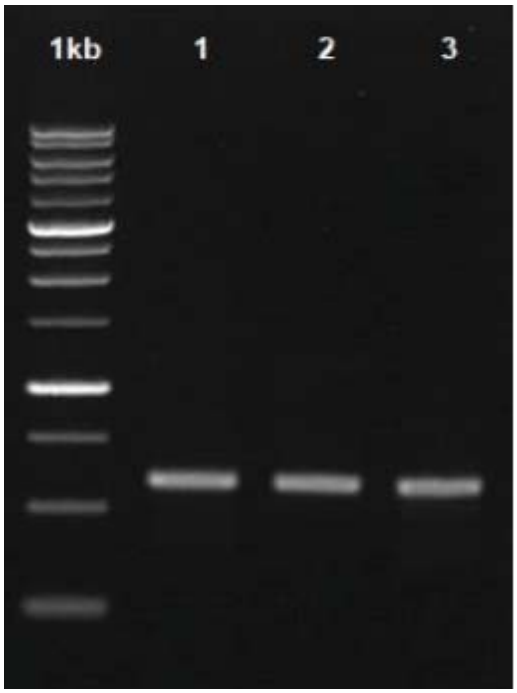

FIGURE 1. The amplified *rbc*L sequence from the samples. 1 = *Vatica bantamensis* 4.1, 2 = *V. bantamensis* 14.7, 3 = *V. rassak*; using 1 kb ladder as a comparison.

Molecular Identification and Barcode Gap Analysis

In this study, molecular identification was carried out to evaluate the species determination accuracy by comparing *rbc*L sample sequences to those deposited in the NCBI and BOLD databases. A total of 15 sequences were used to construct phylogenetic trees consisting of two *V. bantamensis* samples from Mount Payung and one *V. rassak* from the BBG' living collection. The other 12 sequences consisted of nine members of the *Vatica* species as the ingroup and two other species as an outgroup, namely *Dryobalanops lanceolata* and *Shorea pinanga* (Table 1). The ingroup was collected from the tribe Dipterocarpeae and the outgroup was from the tribe Shoreae. All the compared sequences from *Vatica* and *Dryobalanops* were extracted from the NCBI database. Meanwhile, *Shorea* sequence was from the BOLD database.

TABLE 1. Sequence length variation of *rbc*L region within observed samples compared to NCBI and BOLD databases.

No.		Species	Accession number	Sequence length (bp)
Observed samples				
Ingroup	1	*Vatica bantamensis* 4.1	-	606
	2	*Vatica bantamensis* 14.7	-	607
Outgroup	3	*Vatica rassak*	-	608
NCBI and BOLD databases				
Ingroup	4	*Vatica coriacea*	KY973265.1	697
	5	*Vatica endertii*	KY973261.1	697
	6	*Vatica dulitensis*	KY973269.1	697
	7	*Vatica diospyroides*	KY973267.1	697
	8	*Vatica mangachapoi*	MK030577.1	660
	9	*Vatica micrantha*	KY973273.1	697
	10	*Vatica oblongifolia* subsp. *multinervosa*	KY973277.1	697
	11	*Vatica odorata* subsp. *mindanensis*	KY973280.1	697
	12	*Vatica ridleyana*	KJ594929.1	553
	13	*Vatica vinosa*	KY973283.1	697
Outgroup	14	*Dryobalanops lanceolata*	KY973130.1	697
	15	*Shorea pinanga*	BABRU123-14	697

The ABGD analysis retrieved no recursive partition but two initial partitions with all *V. bantamensis* and *V. rassak* samples clustered in one clade separated from the other *Vatica* species. The values of intraspecific divergence (P) suggested the compared sequences of *V. bantamensis*, *V. rassak*, and the other *Vatica* species were split into two different lineages by the value of barcode gap distance of 0.548 represented in Fig. 2 and Table 2.

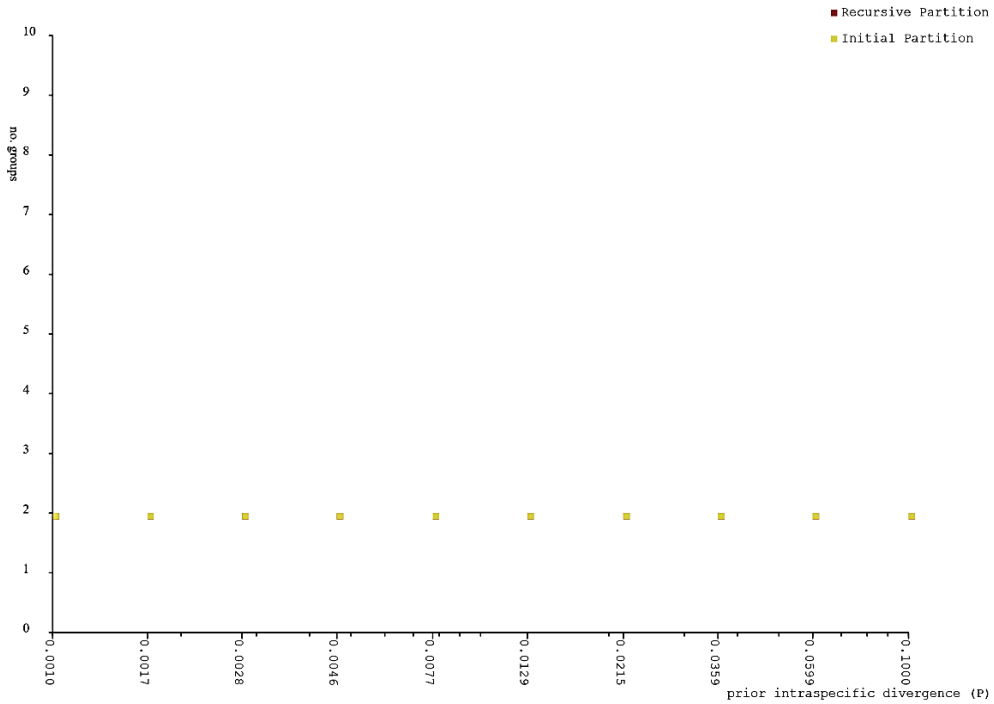

FIGURE 2. Results of the ABGD analysis showing the two partitions (initial) which suggested all representatives of *Vatica bantamensis* and *V. rassak* samples rendered monospecific.

TABLE 2. The specific composition of the two group hypotheses derived from ABGD analysis of *Vatica* species.

Group	Two group hypothesis species composition
1	*Vatica bantamensis* 14.7
	V. bantamensis 4.1
	V. rassak KRB
2	*V. vinosa* KY973283.1
	V. oblongifolia subsp. *multinervosa* KY973277.1
	V. endertii KY973261.1
	V. odorata subsp. *mindanensis* KY973280.1
	V. dulitensis KY973269.1
	V. diospyroides KY973267.1
	V. ridleyana KJ594929.1
	V. micrantha KY973273.1
	V. mangachapoi MK030577.1
	V. coriacea KY973265.1

Phylogenetic Tree

The topology tree constructed from *rbc*L sequences showed that all *Vatica* species and the outgroups are clustered simultaneously into their respected tribe as monophyletic clade. A group of species is monophyletic if all of the species present in the branches come from one common ancestor [33]. *D. lanceolata* and *S. pinanga* are classified in the tribe Shoreae and all *Vatica* species in the tribe Dipterocarpeae (Fig. 3). This is in accordance with the study carried out by de Melo Moura *et al*. [34] that stated *Vatica*, *Anisoptera*, and *Dryobalanops* are sister to *Shorea*, so that *D. lanceolata* and *S. pinanga* is a best choice of outgroup used in this research.

Within the clade of Dipterocarpeae, *V. bantamensis* is closely related to *V. rassak* supported by solid bootstrap value of 100. Both are closely related to *V. ridleyana* which has the most similar sequence compared to the other *Vatica* as shown in the Table 3. This is consistent with the research of [35, 36] using chloroplast DNA analysis which resulted that the observed species, namely *S. johorensis* and *S. parvifolia* formed a separate group and constitute monophyletic groups.

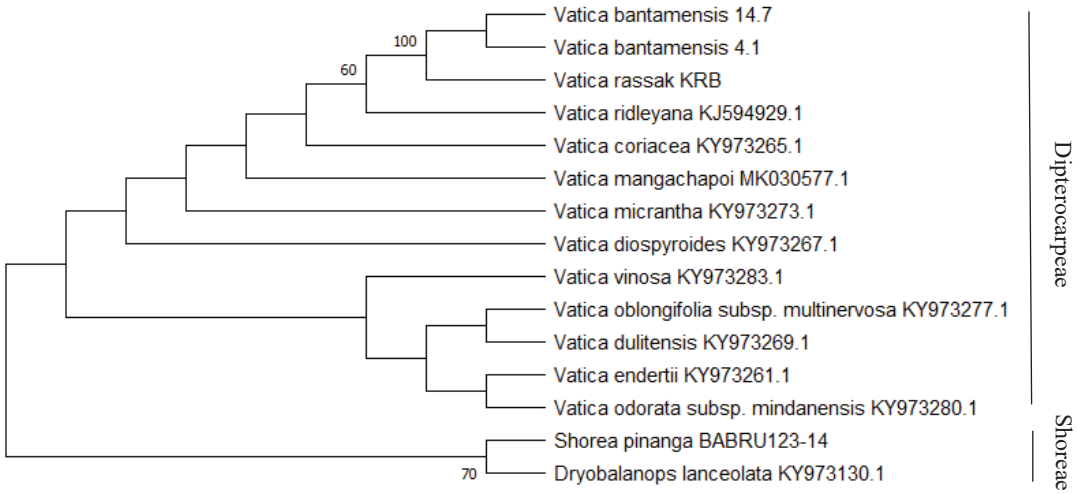

FIGURE 3. Dendrogram obtained from a neighbor-joining (NJ) method with Tamura 3-parameter evolution model through 1,000 bootstrap replications based on *rbc*L gene sequence has clearly stated the relationship and separated *Vatica* species with *Dryobalanops lanceolata* and *Shorea pinanga.*

TABLE 3. Genetic distance generated using Tamura-3 parameter among *Vatica* species, *Dryobalanops lanceolata*, and *Shorea pinanga* inferred from *rbc*L sequence.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1														
2	0.0000													
3	0.0106	0.0106												
4	1.1122	1.1146	1.1266											
5	1.1295	1.1319	1.1440	0.0070										
6	1.1425	1.1449	1.1568	0.0070	0.0106									
7	1.1233	1.1257	1.1375	0.0070	0.0088	0.0035								
8	1.1418	1.1442	1.1464	0.0070	0.0106	0.0035	0.0035							
9	1.1194	1.1219	1.1340	0.0035	0.0035	0.0070	0.0053	0.0070						
10	1.1150	1.1174	1.1296	0.0018	0.0053	0.0088	0.0070	0.0088	0.0018					
11	1.1324	1.1349	1.1457	0.0088	0.0124	0.0018	0.0053	0.0053	0.0088	0.0106				
12	1.1122	1.1122	1.1012	0.0108	0.0108	0.0144	0.0126	0.0126	0.0072	0.0090	0.0163			
13	1.1260	1.1284	1.1404	0.0088	0.0088	0.0035	0.0035	0.0053	0.0053	0.0071	0.0018	0.0126		
14	1.1576	1.1600	1.1721	0.0177	0.0141	0.0141	0.0159	0.0177	0.0141	0.0159	0.0159	0.0218	0.0142	
15	1.1324	1.1349	1.1467	0.0213	0.0177	0.0141	0.0159	0.0177	0.0177	0.0196	0.0124	0.0255	0.0106	0.0106

1 = *V. bantamensis* 4.1, **2** = *V. bantamensis* 14.7, **3** = *V. rassak* KRB, **4** = *V. coriacea* KY973265.1, **5** = *V. diospyroides* KY973267.1, **6** = *V. dulitensis* KY973269.1, **7** = *V. endertii* KY973261.1, **8** = *V. odorata* subsp. *mindanensis* KY973280.1, **9** = *V. micrantha* KY973273.1, **10** = *V. mangachapoi* MK030577.1, **11** = *V. oblongifolia* subsp. *multinervosa* KY973277.1, **12** = *V. ridleyana* KJ594929.1, **13** = *V. vinosa* KY973283.1, **14** = *D. lanceolate* KY973130.1, **15** = *S. pinanga* BABRU123-14

Sequence Similarity

The alignment results of *rbc*L sequences of *V. bantamensis* and *V. rassak* showed that there were nucleotide base substitutions consisted of two transitions (136 and 522; shown in blue color) and four transversions (507, 565, 566, and 567; shown in red color) (Table 4). According to [37, 38], *rbc*L has low interspecies variability but extremely high intraspecies variability that makes *rbc*L cannot classify similar species among closely related species within the genus. They believe that the low performance of *rbc*L is due to the low mutation rate of bases in the plastid DNA markers. However, the interesting result in this study showed that the *rbc*L sequence could distinguish *Vatica* species and separated them from the outgroup, as shown in Fig. 3. Both *V. bantamensis* and *V. rassak* also be distinguished clearly based on the nucleotide variations shown in Table 4.

TABLE 4. Nucleotide base substitution in *rbc*L sequence of *Vatica bantamensis* and *V. rassak*.

Species	Variations of nucleotide bases					
	136	**507**	**522**	**565**	**566**	**567**
Vatica bantamensis 4.1	T	G	C	G	T	G
V. bantamensis 14.7	T	G	C	G	T	G
V. rassak	C	T	T	T	G	T

CONCLUSION

The barcode gap analysis showed two different lineages of *Vatica* species that clearly separated *V. bantamensis* and *V. rassak* from the rest of *Vatica* species. Also, the reconstruction of the phylogenetic tree based on the *rbc*L gene showed that there were two monophyletic groups. The first group consisted of all the ingroup *Vatica* species and the second group consisted of the outgroup, namely *D. lanceolata* and *S. pinanga*. Based on the transitions and

transversions in the *rbc*L sequence of *V. bantamensis* and *V. rassak*, both species can be distinguished easily from their respective six nucleotide sites.

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