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In Silico Evaluation of *rbc*L, *mat*K, and *psb*A-*trn*H Regions on the Genus *Spatholobus* (Fabaceae)

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ABSTRACT

Spatholobus is a genus that belongs to the Fabaceae that is known to contain various bioactive compounds and distributed across Asia, including Indonesia. However, exploration of *Spatholobus* in Indonesia is still rare. Therefore, DNA barcoding is used to support the exploration and conservation of *Spatholobus* in Indonesia. However, there is no universal marker that can be used across all plant species. In addition, there are still few studies related to DNA barcoding within the genus *Spatholobus*. The purpose of this study was to evaluate the rbcL, matK, and intergenic spacer psbA-trnH regions in silico that can be used as DNA barcodes for the genus *Spatholobus*. This study began with a sequence search on the NCBI database including the rbcL, matK, and intergenic spacer psbA-trnH genes in the genus *Spatholobus* and *Phaseolus coccineus* as the outgroup. Each sequence was then aligned with ClustalW. Then, a phylogenetic tree was constructed using the Maximum-Likelihood (ML) with 1000× bootstrap. As a result, the rbcL, matK, and psbA-trnH regions can be used as markers for DNA barcoding in the genus *Spatholobus* with different specifications. The rbcL and matK can be used to distinguish *Spatholobus* at the genus level, while the psbA-trnH can be used to distinguish *Spatholobus* at the species level.

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INTRODUCTION

The genus *Spatholobus* is a plant from the Fabaceae family (Prasetyorini et al., 2022). This genus has been known as a traditional medicine that contains various bioactive compounds, such as terpenoids, alkaloids, flavonoids, and steroids. In addition, the content in this plant has been reported to have antioxidant, antibacterial, and anti-inflammatory activities (Rousdy et al., 2023; Yeni et al., 2023). Additionally, this plant has been widely studied as a plant that has anticancer activity (Hasna et al., 2022). This genus has 29 species distributed in the Asian region. Some of them, such as S. *suberectus* and S. *littoralis*, have been recognized by the people of Borneo, especially the Dayak tribe as traditional medicines (Wardah & Sundari, 2019).

The genus *Spatholobus* is very diverse in Indonesia. However, the most recognized and widely studied species are still limited to S. *suberectus* and S. *littoralis*, also known as Bajakah wood. In fact, *Spatholobus* species includes more species in Indonesia, including *S. albus*, *S. apoensis*, *S. auricomus*, *S. ferrugineus*, *S. gyrocarpus*, *S. hirsutus*, *S. latibractea*, *S. latistipulus*, *S. littoralis*, *S. macropterus*, *S. maingayi*, *S. multiflorus*, *S. oblongifolius*, *S. persicinus*, *S. ridleyi*, *S. sanguineus*, and *S. viridis*. Most of these species are distributed on the island of Borneo, although some species are also distributed in Sumatra and Java (POWO, 2024).

The lack of exploration of the genus *Spatholobus* could lead to insufficient attention and conservation efforts on *Spatholobus* in Indonesia. Therefore, *Spatholobus* in Indonesia requires conservation efforts through a genetic approach. Genetic conservation is carried out to maintain the existence of a population by enriching existing genetic variation, because genetic diversity can determine the success of the population to adapt. Furthermore, genetic conservation through the identification process can help in the management of endangered species. One of these identification methods is through the DNA barcoding approach (Handayani & Setia, 2021; Willi et al., 2022).

DNA barcoding is an approach to identify organism or taxonomic classification using one or a few standard loci, so that the species can be distinguished from another. One commonly used barcode is the *cytochrome oxidase* 1 (*CO*1) mitochondrial gene, as a standard barcode for animal DNA barcoding. However, *CO*1 is not suitable for DNA barcoding in plants due to the low rate of nucleotide substitution. Therefore, finding a standard barcode for plants is quite challenging. Researchers have proposed various barcodes for plants, either from the nucleus such as ITS1 and ITS2, or the coding and non-coding regions of plastids such as *rbc*L, *mat*K, *rpo*B, *psb*A-*trn*H, *trn*L-F,and others (Hollingsworth et al., 2011). The application of DNA barcoding in plants has been utilized in various fields, including for species identification (Nderitu et al., 2023), conservation of endangered and endemic plants (Rosario et al., 2019), and authentication of medicinal plants (Husin et al., 2018).

DNA barcoding as a strategy in *Spatholobus* conservation efforts has several challenges. First, there is currently no universal marker that can be used in all plant species (Li et al., 2014). In addition, there has been limited research on DNA barcoding of the genus *Spatholobus*. Therefore, it is necessary to evaluate markers in the plastid region in silico, especially *rbc*L, *mat*K, and intergenic spacer *psb*A-*trn*H as an initial step to study the genetic diversity of *Spatholobus* in Indonesia. In silico DNA barcoding is considered an effective and applicable approach to initiate various studies, such as taxonomic conservation of biodiversity (Pratiwi et al., 2023).

MATERIALS AND METHODS

Data Mining

DNA sequences of the *rbc*L, *mat*K, and *psb*A-*trn*H genes belonging to the genus *Spatholobus* and the outgroup *Phaseolus coccineus* were retrieved from the NCBI nucleotide database (URL: http://www.ncbi.nlm.nih.gov). All the sequences were critically evaluated and low-quality sequences (ambiguous base N > 3%) were discarded (Suesatpanit et al., 2017). The exclusion criteria used in this analysis were as follows: first, no whole genome data exceeding 150,000 bp in length were used (Su'udi et al., 2023). Second, no sequences from unclear species were used (Suesatpanit et al., 2017). Third, no sequences from mitochondria were used. Finally, no sequences that are too short were used (Sundari et al., 2022).

Data Analysis

The sequences obtained were then aligned with Clustal W in MEGA X software (Thompson et al., 1994; Kumar et al., 2018). The sequences were then compared with each other to determine the differences. In addition, the mutation pattern of the sequences was also analyzed. Then, a phylogenetic tree was constructed using the Maximum-Likelihood (ML) algorithm with a bootstrap of 1000×. The fittest model for ML was selected based on Bayesian Information Criterion (BIC) score (Nei & Kumar, 2000). The phylogenetic tree was constructed using MEGA X software (Kumar et al., 2018).

RESULT AND DISCUSSION

Data mining of *Spatholobus* sp. nucleotide sequences in the NCBI database was performed using various exclusion criteria. The first criterion is that whole genome sequences longer than 150,000 base pairs were not used. Barcoding using whole genomes with excessively long sequences requires significant time and high-performance computing to manage large whole genome data, making it ineffective (Su'udi et al., 2023). In contrast, sequences that are too short were also not used. Based on previous studies, molecular markers with sequence lengths above 500 bp are more effective in identifying plant species (Sundari et al., 2022). Furthermore, as the loci used were derived from chloroplasts, sequences derived from mitochondria were not included in the analysis. Based on the results of data mining using NCBI, several *mat*K sequences were obtained from mitochondria, while *mat*K is a *maturase* K protein-

coding gene found in plant chloroplasts (Kalangi et al., 2014). Therefore, *mat*K sequence data from mitochondria were not used to avoid ambiguity in the results obtained. To ensure the precision of the data set, sequences with ambiguous species specifications, such as those that were only known as *Spatholobus* sp., were also excluded (Suesatpanit et al., 2017) (Table 1).

As previously outlined by Letsiou et al. (2024), barcoding studies of plant such as *Spatholobus* sp. generally use several specific loci derived from chloroplast that are known as genetic markers. Only four loci derived from chloroplast were found for the *Spatholobus* entry in NCBI, including *rbc*L, *mat*K, *psb*A-*trn*H, and *trn*L-*trn*F. The data mining results showed that there are only 24 sequences for the *rbc*L, 14 sequences for the *mat*K, and 14 sequences for the *psb*A-*trn*H. Furthermore, sequences from the *trn*L-*trn*F locus were also found, but the species is unclear (KF621116.1 *Spatholobus* sp.). Therefore, *trn*L-*trn*F was not included in the analysis. As a result of the selection and exclusion process based on these criteria, the accession number used for analysis was 17 for *rbc*L, 8 for *mat*K, and 14 for *psb*A-*trn*H (Table 1).

The Multiple Sequence Alignment (MSA) results on the *rbc*L sequences revealed 12 bases that differed among the species of *S. suberectus*, *S. gyrocarpus*, *S. parviflorus*, *S. pulcher*, and *S. acuminatus*. These differences are caused by point mutations. Point mutation is a specific type of mutation pattern that occurs in the DNA base sequence. According to Suprasanna et al. (2015), changes in the sequence of nitrogenous bases in DNA can lead to phenotypic changes (Suprasanna et al., 2015). The alignment results of the *rbc*L gene demonstrated intraspecies base differences with a low frequency, indicating a relatively high level of homology. The 24th, 243rd, 300th, and 375th nucleotides (the 1st position is the first nucleotide after trimming and alignment), highlighted in red to illustrate divergences within the same species (intraspecies). The results of the *rbc*L alignment also showed base differences between different species (interspecies) (Table 2).

Additionally, slight base differences were observed at the *mat*K locus within the same species (intraspecies). A single base difference was noted in *S. parviflorus* at position 561 (Table 3). Mutations that occur at the *mat*K locus are point mutations and insertion-deletion mutations (InDels). InDels, which are characterized by a gap (-), result from mismatches in the alignment of base sequences between species (Anzani et al., 2021). The results of the alignment using the *rbc*L and *mat*K indicate that these two loci are only applicable for *Spatholobus* studies at the genus level (interspecies).

The base sequence alignment in *Spatholobus* using the *psb*A-*trn*H locus identified the presence of InDel mutations and point mutations. Alignment results showed many base differences in nucleotide sequences within the same species (intraspecies), highlighted in red (Table 4). These results indicate that the *psb*A-*trn*H locus can be used for *Spatholobus* studies at the species level. Previous research has demonstrated the efficacy of the *psb*A-*trn*H locus for DNA barcoding in *Stelechocarpus*, indicating its potential as a DNA barcode at the species level (intraspecies) (Turhadi & Hakim, 2023)

Table 1. Exclusion accession number of *Spatholobus*

Exclusion criteria	Number of accession numbers (1)		
	***rbc*L**	***mat*K**	***psb*A-*trn*H**
Whole genome	6	6	0
Unclear species	0	1	0
Mitochondrial sequence	0	8	0
Sequence that are too short	1	1	0

Note: (1) Number of accession numbers that are not used

Table 2. Nucleotide differences in the *rbc*L locus of *Spatholobus*

Sample	Nucleotide Sequence											
	24	**81**	**96**	**168**	**169**	**197**	**203**	**243**	**300**	**303**	**306**	**375**
KP313866.1 *S. suberectus*	G	G	T	T	C	G	T	A	C	T	C	T
KP313865.1 *S. suberectus*	G	G	T	T	C	G	T	A	C	T	C	T
KP313864.1 *S. suberectus*	G	G	T	T	C	G	T	A	C	T	C	T
KP313863.1 *S. suberectus*	G	G	T	T	C	G	T	A	C	T	C	T
KP313862.1 *S. suberectus*	G	G	T	T	C	G	T	A	C	T	C	T
KP313861.1 *S. suberectus*	G	G	T	T	C	G	T	A	C	T	C	T
KF181528.1 *S. suberectus*	A	G	T	T	C	G	T	A	C	T	C	C
KP202396.1 *S. suberectus*	G	G	T	T	C	G	T	A	C	T	C	T
KP202395.1 *S. suberectus*	G	G	T	T	C	G	T	A	C	T	C	T
KP202394.1 *S. suberectus*	G	G	T	T	C	G	T	A	C	T	C	T
JF949991.1 *S. suberectus*	G	G	T	T	C	A	T	A	C	T	C	C
MG816933.1 *S. gyrocarpus*	G	G	C	C	T	A	A	A	C	G	T	C
MG816918.1 *S. gyrocarpus*	G	G	C	C	T	A	A	G	C	G	T	C
AB045825.1 *S. parviflorus*	G	G	T	T	A	A	T	G	A	A	C	C
AB925446.1 *S. parviflorus*	G	G	T	T	A	A	T	A	C	A	C	C
KF181563.1 *S. pulcher*	G	G	C	C	A	A	A	A	C	G	T	C
AB925736.1 *S. acuminatus*	G	C	C	C	C	A	A	A	C	G	C	C

*Red: intraspecies variation

Based on the MSA results, one of the factors that cause nucleotide sequence differences is transition and transversion (Monalisa & Lengkong, 2019). Transition itself is an exchange of bases between purines (A > G, G > A), or pyrimidines (C > T, T > C). Meanwhile, transversion is the exchange between purine bases and pyrimidine, or vice versa (A > C, A > T, G > C, G > T, C > A, C > G, T > A, T > G) (Hanifa et al., 2019). The overall mutation pattern showed a higher transition value compared to transversion.According to Rahayu et al (2019), transitions tend not to cause significant phenotypic changes. However, a higher transition value may indicate a mutation bias that could affect the accuracy of DNA barcodes. In the study conducted by Li et al. (2014), a low transition or transversion ratio was identified as a characteristic of a locus suitable for use as a DNA barcode in evolutionary studies.

Some nucleotide mutations can cause changes in the amino acids and the genomic sequence of chloroplast DNA. Changes in amino acids can affect the function of genes, thus changing their efficacy (Mahfut et al., 2019). Other research suggested that not all mutations can cause amino acid changes. Nonsense mutations can alter the genetic code that modify the amino acids produced. This can affect protein function due to changes in amino acid structure. Synonymous replacement mutations, on the other hand, only alter the genetic code without changing the amino acids produced. As a result, these mutations are usually neutral and do not significantly affect protein function because there is no change in amino acid structure, and they are not affected by natural selection (Sen et al., 2011).

The MSA results indicated that the conserved site in the *rbc*L mutation was 97.42%, while the variable site was 2.58%. The percentage of transitions in the *rbc*L mutation pattern was 0.44%, while the percentage of transversions was 0.22% (Table 5). The *psb*A-*trn*H mutation pattern showed that the conserved site at the *psb*A-*trn*H locus was 92.79%, while the percentage of variable sites was 7.21%.

Table 3. Nucleotide differences in the *mat*K locus of *Spatholobus*

Sample	Nucleotide sequence																										
	2	5	6	21	30	36	40	55	59-61	79	85-89	109	133	142	145	161	163-167	198-201	206	217	220-221	261	264-267	270	272	299
GU396724.1 *S. suberectus*	A	T	G	-	G	G	G	G	TTT	A	-	C	T	A	T	T	-	ATAT	C	A	CT	G	GAAA	G	A	T
GU396723.1 *S. suberectus*	A	T	G	-	G	G	G	G	AAA	A	-	C	T	A	T	T	-	ATAT	C	A	CT	G	GAAA	G	A	T
KX346984.2 *S. suberectus*	A	T	G	-	A	T	T	G	TTT	A	-	C	G	C	T	T	-	A—T	C	A	CT	G	GAAA	G	A	T
KX346983.2 *S. suberectus*	A	T	G	-	A	T	T	G	TTT	A	-	C	G	C	T	T	-	A—T	C	A	CT	G	GAAA	G	A	T
MT077510.1 *S. suberectus*	T	C	G	C	G	G	G	G	TTT	A	-	C	T	A	T	T	-	ATAT	C	A	CT	G	GAAA	G	A	T
MT077509.1 *S. suberectus*	T	C	G	C	G	G	G	G	TTT	A	-	C	T	A	T	T	-	ATAT	C	A	CT	G	GAAA	G	A	T
MT077508.1 *S. suberectus*	T	C	G	C	G	G	G	G	TTT	A	-	C	T	A	T	T	-	ATAT	C	A	CT	G	GAAA	G	A	T
MT077507.1 *S. suberectus*	T	C	G	C	G	G	G	G	TTT	A	-	C	T	A	T	T	-	ATAT	C	A	CT	G	GAAA	G	A	T
MT077506.1 *S. suberectus*	A	T	G	-	G	G	G	G	AAA	A	-	C	T	A	T	T	-	ATAT	C	A	CT	G	GAAA	G	A	T
HG005078.1 *S. suberectus*	A	T	G	-	A	T	T	G	TTT	A	-	C	G	C	T	T	-	A–T	C	A	CT	G	GAAA	G	A	T
MT077505.1 *S. pulcher*	A	T	T	-	G	G	G	C	AAA	A	CTTTT	A	G	C	G	A	TATGT	-	A	C	-	T	-	A	T	C
MT077504.1 *S. pulcher*	A	T	T	-	G	G	G	C	AAA	A	CTTTT	A	G	C	G	A	TATGT	-	A	C	-	T	-	A	T	C
MT077503.1 *S. pulcher*	A	T	T	-	G	G	G	C	AAA	A	CTTTT	A	G	C	G	A	TATGT	-	A	C	-	T	-	A	T	C
HG005110.1 *S. pulcher*	A	T	T	-	G	G	G	C	AAA	C	CTTTT	A	G	C	G	A	TATGT	-	A	C	-	T	-	A	T	C

Table 4. Nucleotide differences in the *psb*A-*trn*H locus of *Spatholobus*

Sample	Nucleotide Sequence																														
	1	7	19-21	51-56	57	116	135	141	155	185	186	197	214	239	282	290	325	346	365	373	397-399	401-403	422	423-427	447	450	497	500	506	534	545	561	568
EU106113.1 Spatholobus parviflorus	G	C	---	CTATTC	C	C	G	C	A	C	C	A	C	C	A	G	A	G	C	G	ACC	---	G	GGCTA	C	T	A	T	T	C	T	G	C
EU106112.1 Spatholobus parviflorus	G	C	---	CTATTC	C	C	G	C	A	C	C	A	C	C	A	G	A	G	C	G	ACC	---	G	GGCTA	C	T	A	T	T	C	T	G	C
LC080907.1 Spatholobus parviflorus	G	C	---	CTATTC	C	C	G	C	A	C	C	A	C	C	A	G	A	G	C	G	ACC	---	G	GGCTA	C	T	A	T	T	C	T	-	C
AB924834.1 Spatholobus parviflorus	G	C	---	CTATTC	C	C	G	C	A	C	C	A	C	C	A	G	A	G	C	G	ACC	---	G	GGCTA	C	T	A	T	T	C	T	G	C
HG004992.1 Spatholobus pulcher	G	C	---	-----	T	A	T	A	G	T	T	A	A	T	G	G	A	G	T	G	ACC	---	G	GGCTA	C	C	A	A	T	C	C	G	C
HG004955.1 Spatholobus suberectus	T	C	---	-----	T	A	T	A	G	T	C	A	A	T	G	A	A	G	T	G	ACC	---	G	GGCTA	C	C	A	A	T	C	C	G	C
AB925106.1 Spatholobus acuminatus	T	A	TTT	CTATTC	C	C	T	C	G	T	C	C	C	T	G	G	A	T	T	A	ACC	---	A	GGCTA	C	T	G	A	G	C	T	G	A
MG816804.1 Spatholobus littoralis	T	C	NNN	-----	C	A	T	A	G	T	C	A	A	T	G	G	T	G	T	G	NNN	CCA	N	NNNNN	T	C	A	A	T	A	C	G	C

Note: *Red: intraspecies variation

The percentage of transitions obtained from the alignment results using the *psb*A-*trn*H locus was 0.7%, while the percentage of transversions was 2.44% (Table 7). From the alignment using the *mat*K locus, it was determined that the conserved site in the *mat*K mutation was 93.19%, while the variable site was 4.82%. The *mat*K mutation pattern showed a transition percentage of 1.02% while for transversion 0.85% (Table 6).

A high percentage of variable sites in a locus indicates that the locus meets the principle of minimalism, which is an effective means of describing genetic variation between taxa both interspecies and intraspecies. The findings of this study are consistent with previous research, which indicates that *psb*A-*trn*H has a higher variable site in comparison to *mat*K and rbcL. The results indicate that the *psb*A-*trn*H region is an effective locus for identifying both interspecies and intraspecies kinship relationships. The three loci used in this study have a high percentage of conserved sites. A very small percentage of conserved sites represents a weakness of DNA barcoding, as it led to bias in the phylogenetic tree produced (Sumarlina & Napitupulu, 2022).

Phylogenetic trees of the genus *Spatholobus* were constructed with different models. The fittest model for *rbc*L was K2+G, while the fittest model for *mat*K and *psb*A-*trn*H was T92. Construction of a phylogenetic tree in the genus *Spatholobus* showed clade differences among the markers *rbc*L, *mat*K, and *psb*A-*trn*H (Figure 1). These results showed that *rbc*L, *mat*K, and *psb*A-*trn*H successfully grouped the same species in a separate clade. The phylogenetic tree based on the *rbc*L marker showed five clades formed, namely S. *suberectus*, S. *parviflorus*, S. *acuminatus*, S. *pulcher*, and S. *gyrocarpus* (Figure 1A). The phylogenetic tree based on *mat*K markers also showed five clades formed, namely S. *parviflorus*, S. *suberectus*, S. *pulcher*, S. *acuminatus*, and S. *littoralis* (Figure 1C). Meanwhile, the phylogenetic tree based on *psb*A-*trn*H markers only showed two clades formed, namely S. *suberectus* and S. *pulcher* (Figure 1B).

A phylogenetic tree is a diagram that illustrates the relationship of living things based on their evolution. The branches on a phylogenetic tree represent different levels of sequence. The closer the relationship in the phylogenetic tree, the lower the genetic distance between individuals and the higher the genetic similarity. Therefore, closely related individuals will be grouped in the same clade (Anzani et al., 2021).

Table 5. Mutation pattern of *rbc*L

	A	T	G	C
A	**26,24**	0,22(2)	0,22(1)	0,00(2)
T	0,00(2)	**29,25**	0,00(2)	0,00(1)
G	0,00(1)	0,00(2)	**22,15**	0,00(2)
C	0,00(2)	0,22(1)	0,00(2)	**21,29**

(1) Transition
(2) Transversion

Table 6. Mutation pattern of *mat*K

	A	T	G	C
A	**30,84**	0,17(2)	0,17(1)	0,17(2)
T	0,00(2)	**39,75**	0,17(2)	0,34(1)
G	0,17(1)	0,17	**12,51**	0,00(2)
C	0,17(2)	0,34(1)	0,00(2)	**14,91**

(1) Transition
(2) Transversion

Table 7. Mutation pattern of *psb*A-*trn*H

	A	T	G	C
A	**31,88**	0,69(2)	0,35(1)	0,35(2)
T	0,35(2)	**45,05**	0,35(2)	0,00(1)
G	0,00(1)	0,35(2)	**11,78**	0,00(2)
C	0,35(2)	0,35(1)	0,00(2)	**8,32**

(1) Transition
(2) Transversion

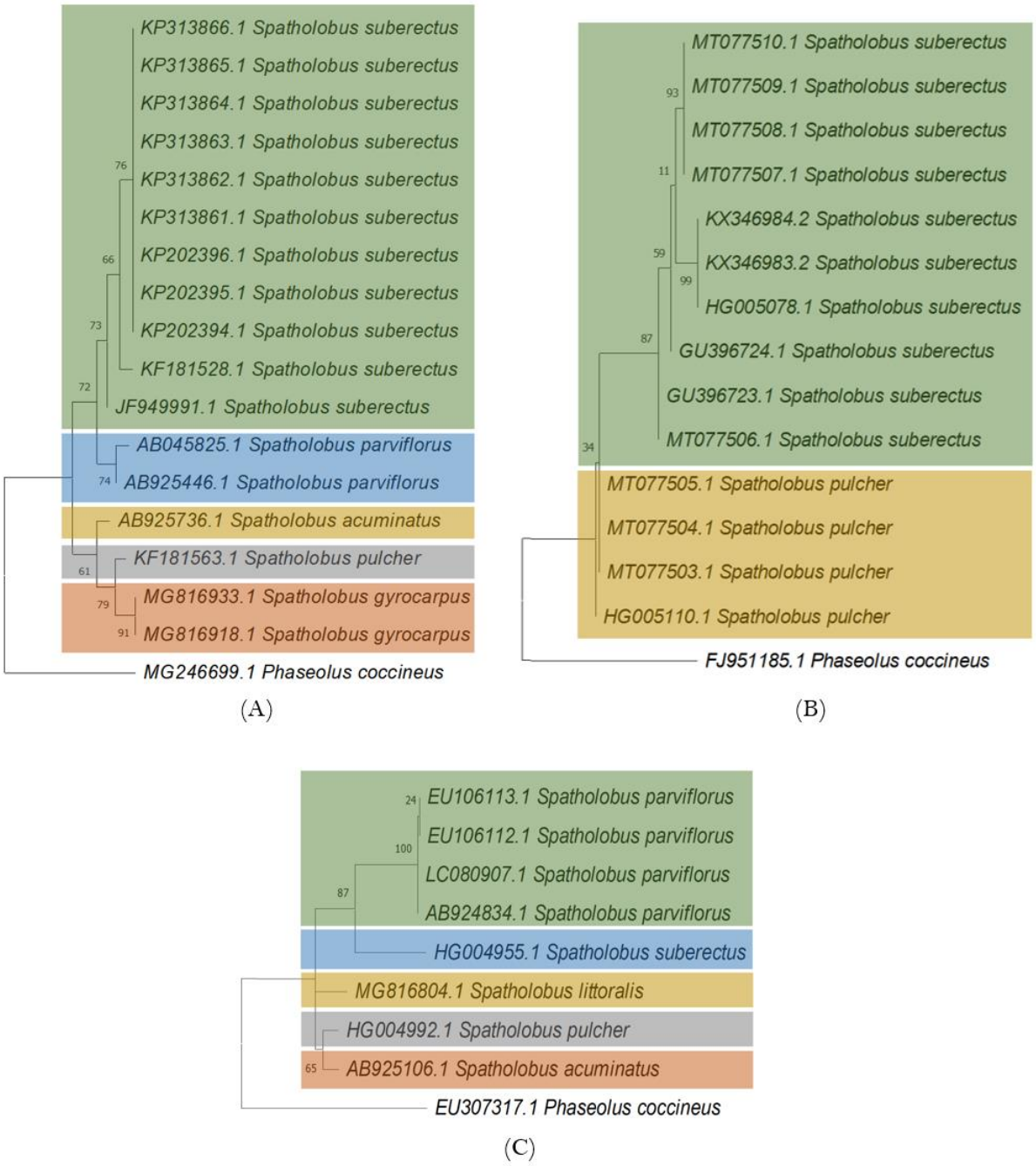

Figure 1. Phylogenetic tree of genus *Spatholobus*. (A) based on *rbc*L, (B) based on *psb*A-*trn*H, and (C), based on *mat*K. The number next to the node shows bootstrap value.

CONCLUSION

The *rbc*L, *mat*K, and *psb*A-*trn*H regions can be used as markers for DNA barcoding in the genus *Spatholobus* with different specifications. The *rbc*L and *mat*K can be used to distinguish *Spatholobus* at the genus level, while the *psb*A-*trn*H can be used to distinguish *Spatholobus* at the species level.

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REFERENCES

Anzani, A. N., Martiansyah, I., & Yuliani, N. (2021). Studi *In Silico* DNA *Barcoding* Pada Bunga Soka (*Ixora*). *Prosiding Biologi Achieving the Sustainable Development Goals with Biodiversity in Confronting Climate Change*, *7*(1), 168-177. https://doi.org/10.24252/psb.v7i1.23693

Handayani, H., Setia,T. M. (2021). Konservasi Genetika Badak Sumatera di Indonesia. *Bio-Sains*, 1(1), 19-25. https://doi.org/10.34005/bio-sains.v1i1.1566

Hanifa, Y. R., Pujiyanto, S., Ferniah, R. S., & Kusumaningrum, H. P. (2021). Identifikasi Molekuler Jeruk Nipis Tegal Berdasarkan Fragmen Gen 18s Ribosomal RNA. *Jurnal Bioteknologi & Biosains Indonesia*, *8*(2), 244-254. https://ejurnal.bppt.go.id/index.php/JBBI/article/view/4883/4188

Hasna, L. Z., Sehkhaemi, P., & Aviciena, M. A. (2022). Review: Akar Kayu Bajakah dan Manfaatnya untuk Kesehatan. *FoodTech: Jurnal Teknologi Pangan*, *4*(1), 32-39. http://dx.doi.org/10.26418/jft.v4i1.56637

Hollingsworth, P. M., Graham, S. W., Little, D. P. (2011). Choosing and Using a Plant DNA Barcode. *PLoS ONE*, *6*(5), e19254. https://doi.org/10.1371/journal.pone.0019254

Husin, C. N. C., Naim, D. M., Ahmad M. I. (2018). Authentication of a selected medicinal plants using DNA barcoding technique. *SHS Web of Conference*, *45*, 05004. https://doi.org/10.1051/shsconf/20184505004

Kalangi, C., Kamu, V. S., Kumaunang, M. (2014). *Barcode* DNA Tanaman Leilem (*Clerodendrum minahassae* L.) Berdasarkan Gen *mat*K. *Jurnal MIPA UNSTRAT Online*, *3*(2), 108-112. https://doi.org/10.35799/jm.3.2.2014.5861

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, *35*(6), 1547-1549. https://doi.org/10.1093/molbev/msy096

Letsiou, S., Madesis, P., Vasdekis, E., Montemurro, C., Grigoriou, M. E., Skavdis, G., Moussis, V., Koutelidakis, A. E., & Tzakos, A. G. (2024). DNA Barcoding as a Plant Identification Method. *Applied Sciences*, *14*(4), 1415. https://doi.org/10.3390/app14041415

Li, X., Yang, Y., Henry, R. J., Rossetto, M., Wang, Y., & Chen, S. (2014). Plant DNA barcoding: from gene to genome. *Biological Reviews*, *90*(1), 157-166. https://doi.org/10.1111/brv.12104

Mahfut., Daryono, B. S., Indrianto, A., & Sumowiyarjo, S. (2019). Plant-Virus Interaction on Orchids Infected Odontoglossum ringspot virus (ORSV) in Bogor Botanical Garden, Indonesia. *International Conference on Science Technology*, (May). http://dx.doi.org/10.4108/eai.2-5-2019.2284701

Nderitu, K. W., Ager, E., Mecha, E., & Nyachieo, A. (2023). DNA Barcoding Using *ITS*2 and *rbc*L Markers for Solanaceae Species Identification. *East African Medical Journal*, *100*(1), 5567-5574. https://www.ajol.info/index.php/eamj/article/view/242644

Nei, M. & Kumar, S. (2000). *Molecular Evolution and Phylogenetics*. Oxford University Press.

POWO. (2024). *Spatholobus* Hassk. Accessed through: Plants of The World Online at: https://powo.science.kew.org/.

Prasetyorini, B. E., Kusumawardani, A., Fitriani, F., Oktriana, P., Amelinda, N., & Ramadhani, A. N. (2022). Analisis *In Silico* Senyawa Aktif Batang Kayu Bajakah (*Spatholobus Littoralis Hassk*) Sebagai Terapi Psoriasis. *Herb Medicine Journal*, *5*(1), 26-35. http://dx.doi.org/10.30595/hmj.v5i2.12744

Pratiwi, A., Kinasih, A., Meidianing, M. I., Kurniawan, F. Y., Semiarti, E. (2023). In Silico Approach for DNA Barcoding using Phylogenetic Analysis of *Coelogyne* spp. based on the *mat*K, *rpo*C1, *rbc*L and nrDNA Markers. *Journal of Tropical Biodiversity and Biotechnology*, *8*(3), 1-14. https://doi.org/10.22146/jtbb.73130

Rahayu, D. A., Nugroho, E. D., & Listyorini, D. (2019). DNA barcoding ikan introduksi khas Telaga Sari, Kabupaten Pasuruan. *Biotropika: Journal of Tropical Biology*, *7*(2), 51-62. https://doi.org/10.21776/ub.biotropika.2019.007.02.2

Rosario, L. H., Padilla, J. O. R., Martinez, D. R., Grajales, A. M., Reyes, J. A. M., Feliu, G. J. V., Ee, B. V., & Siritunga, D. (2019). DNA Barcoding of the Solanaceae Family in Puerto Rico Including Endangered and

Endemic Species. *Journal of the America Society for Horticultural Science*, *144*(5), 363–374. https://doi.org/10.21273/JASHS04735-19

Rousdy, D. W., & Wardoyo, E. R. P. (2023). In Vitro Antiinflammatory Activity of Bajakah (*Spatholobus littoralis*) Stem Extract. *Biosaintifika: Journal of Biology & Biology Education*, *15*(2), 150-157. https://doi.org/10.15294/biosaintifika.v15i2.36227

Sen, L., Fares, M. A., Liang, B., Gao, L., Wang, B., Wang, T., & Su, Y. J. (2011). Molecular Evolution Of *rbc*L In Three Gymnosperm Families: Identifying Adaptive And Coevolutionary Patterns. *Biology Direct*, *6*(29). https://doi.org/10.1186/1745-6150-6-29

Suesatpanit, T., Osathanunkul, K., Madesis, P., & Osathanunkul, M. (2017). Should DNA sequence be incorporated with other taxonomical data for routine identifying of plant species? *BMC Complementarty and Alternative Medicine*, *17*, 437. https://doi.org/10.1186/s12906-017-1937-3

Sumarlina, & Napitupulu, T. S. (2023). Evaluasi Aplikasi DNA Barcode Lokus *psb*A-*trn*H pada Genus *Momordica*. *Jurnal Biotek*, *11*(2), 182-195. https://doi.org/10.24252/jb.v11i2.37914

Sundari, S., Mas'ud, A., Arumingtyas, E. L., & Wahyudi, D. (2022). Using Short Sequence *mat*K Gene as Barcode DNA For Identification Of *Durio* sp. In Ternate Island. *Jurnal Biosilampari*, *5*(1), 50-56. https://doi.org/10.31540/biosilampari.v5i1.1528

Suprasanna, P., Mirajkar, S. J., & Bhagwat, S. G. (2015). *Induced Mutation and Crop Improvement, Plant Biology and Biotechnology: Plant Diversity, Organization, Function and Improvement*. Springer.

Su'udi, M., Ramadany, Z., Rohimah, S., Arum, A. Y., Setyati, D., & Ulum, F. B. 2023. Peran Mini-Barcode Internal Transcribed Spacer 2 (ITS2) untuk Identifikasi Molekuler Spesies Anggrek. *Indonesian Journal of Biotechnology and Biodiversity*, *7*(1), 34-45. https://doi.org/10.47007/ijobb.v7i1.166

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, *22*(22), 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Turhadi, & Hakim, L. (2023). Evaluasi Lokus Kloroplas untuk DNA Barcoding pada Marga *Stelechocarpus* (Annonaceae) Secara *In-Silico*. *Agro Bali: Agricultural Journal*, *6*(1), 56-64. https://doi.org/10.37637/ab.v6i1.1105

Wardah, & Sundari, S. (2019). Ethnobotany Study of Dayak Society Medicinal Plants Utilization in Uut Murung District, Murung Raya Regency, Central Kalimantan. *IOP Conference Series: Earth and Environmental Science*. 298, 012005. https://iopscience.iop.org/article/10.1088/1755-1315/298/1/012005

Yeni, L. F., Kamelia, N., & Chaerani. (2023). Antibacterial Potential of West Kalimantan Local Bajakah (*Spatholobus suberectus*) Ethanol Extract against *Micrococcus luteus*. *Quagga: Jurnal Pendidikan dan Biologi*, *15*(2), 193-200. https://doi.org/10.25134/quagga.v15i2.27