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DNA BARCODING ANALYSIS OF PEPINA (*PLUKENETIA CORNICULATA* SM.) FROM RIAU, INDONESIA, USING FOUR DNA BARCODES

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SUMMARY

Pepina (*Plukenetia corniculata* Sm.) is a climbing woody plant belonging to the family Euphorbiaceae. However, for the said species, the DNA-related studies remain limited, particularly using DNA barcoding techniques. The following study aimed to characterize and verify the pepina from Riau using four DNA barcodes. The total DNA extraction took place, followed by polymerase chain reaction (PCR), electrophoresis, sequencing, and data analysis. The results revealed Pepina from Riau exhibited 100% similarity to the species *P. corniculata* based on *matK* and *ITS* sequences. However, based on the *rbcLb* and *trnL-trnL-trnF IGS* sequences, the similarity values of pepina from Riau with the species *P. volubilis* were 99.64% and 95.22%, respectively. The *ITS* and *trnL-trnL-trnF IGS* displayed the highest levels of substitution mutations and indels compared with the protein-coding DNA barcodes (*matK* and *rbcLb*). The most informative DNA barcode was *trnL-trnL-trnF IGS*, with 3.42% variable nucleotide sites. In summary, the pepina from Riau showed an identity as *P. corniculata* based on the *matK* and *ITS* sequences. Meanwhile, the *rbcLb* and *trnL-trnL-trnF IGS* sequences obtained in this study are the first released reports and can enrich the DNA barcode sequences of this species in the public GenBank database. Moreover, molecular identification of pepina can be successful using single-locus barcodes (*trnL-trnL-trnF IGS* and ITS or *matK*), as well as combinations of these loci.

Keywords: Pepina (*P. corniculata*), species, DNA barcode, *ITS, matK, rbcLb, trnL-trnL-trnF intergenic spacer,* similarity

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Key findings: The identity of Pepina (*P. corniculata*) can attain reliable confirmation using molecular DNA barcoding, either through single-locus or multi-locus approaches. Accurate taxonomic identification is essential to support both conservation strategies and sustainable utilization efforts.

INTRODUCTION

Pepina (*Plukenetia corniculata*) is a member of the family Euphorbiaceae, a traditional leafy vegetable in Southeast Asia. Its young shoots, leaves, and young fruits are edible vegetables, with its oil-rich seeds eaten as nuts having a similar flavor to peanuts (Cardinal-McTeague *et al*., 2019). Pepina is rich in several nutrients, particularly protein (5.27%), fatty acids (1.36%), dietary fiber (11.13%), sugar (2.80%), iron (>7.0 mg/100 g), and zinc (>2.0 mg/100 g) (Talang *et al*., 2023). It also contains essential amino acids (cysteine, tyrosine, threonine, and tryptophan), vitamin E, polyphenols, and minerals (Wang *et al*., 2018). Owing to this nutritional composition, pepina could offer health benefits, particularly in maintaining neural and cardiovascular functions (Feng *et al*., 2023).

Pepina typically has several local names, including pepina, pina-pina, and chumbai (in the Malaysian language). Pepina also has several taxonomic synonyms, such as *Pterococcus glaberrimus* Hassk., *P. corniculatus* (Sm.) Pax and K. Hoffm., and *Hedraiostylus glaberrimus* Hassk (Choo *et al*., 2022). Its natural habitat comprises open landscapes and tropical rainforests (Pradheep *et al*., 2015). In Riau Province, Indonesia, Pepina has distinct morphological characteristics, including a woody, twining liana habit with tendrils. The leaves are simple, oblong-ovate, arranged in alternate and opposite configurations along the tendrils. The leaves have a cordate base, a caudate apex, serrate margins, palmate venation with three primary veins, a smooth surface, and a dark green coloration, with a pinnate venation pattern. Pepina produces compound inflorescences arising from the tendril axils. Its fruits are star-shaped capsules containing 4–5 lobes, and each bearing seeds (Figure 1).

Although pepina has no current listing in the endangered species, its long-term survival faces threats from habitat degradation. Therefore, in support of conservation efforts, further in-depth studies are necessary to explore the existing, albeit limited, knowledge on the species. Molecular analysis using DNA barcoding is a valuable approach that can be applicable to recognizing the identity of pepina in this region. Besides that, the purpose of DNA barcoding analysis on pepina from Riau is to verify its scientific name, as several plant species have similar leaf and fruit morphology to the pepina from Riau. This verification is crucial to avoid incorrect sampling of pepina plants for use, particularly as herbal remedies.

DNA barcoding technology reached its first introduction from Hebert and colleagues in 2003 to identify the different organisms based on short DNA sequences (approximately 700–1500 bp), referred to as DNA barcodes (Hollingsworth *et al*., 2016; Roslim *et al*., 2023a). This technique aimed to overcome the limitations of conventional species identification, particularly when the specimens are incomplete, damaged, and lacking reproductive structures, making it difficult to distinguish morphologically (Waldchen *et al*., 2018). The DNA barcoding provides key advantages, including high accuracy, speed, and specificity in species identification (Raclariu *et al*., 2018).

An ideal DNA barcode can exhibit the highest interspecific variability while remaining conserved in certain regions to allow the design of universal primers; it also maintains high stability within the same species (Pagala and Nafiu, 2020). The DNA barcodes are typically orthologous sequences involved in essential biological processes such as photosynthesis and respiration. They display greater interspecific than intraspecific variations to achieve species-level resolution and are amenable to efficient processing using molecular tools and bioinformatic analyses. Potential barcode regions can result from the nuclear, chloroplast, and mitochondrial genomes (Khan *et al*., 2019).

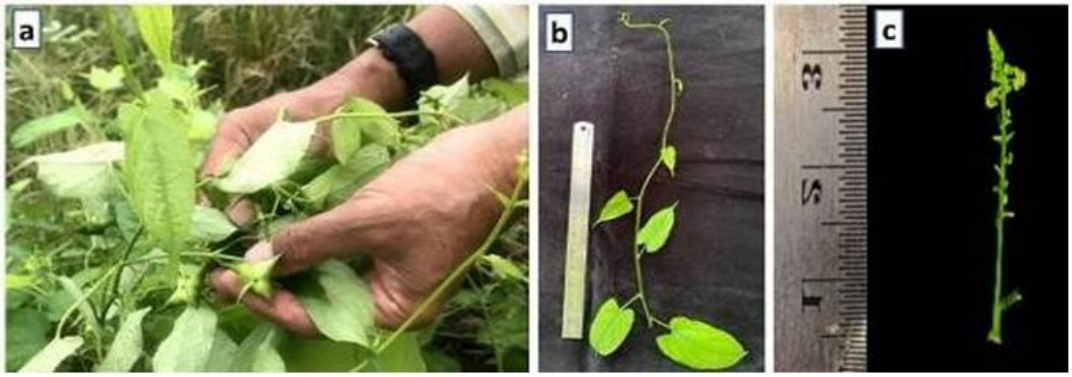

Figure 1. The morphology of pepina (*Plukenetia corniculata* Sm.) from Riau, Indonesia. a) Habitus, b) tendril, and c) flower.

Nuclear ribosomal DNA (rDNA) is one of the most widely used nuclear loci applied for plant DNA barcoding. This region encodes ribosomal RNAs that form the large subunit of the ribosome, occurring in multiple tandem repeats within the nuclear genome, and has a relatively low mutation rate. The spacer regions, such as the *internal transcribed spacer* (*ITS*) and the *external transcribed spacer* (*ETS*), have become recognizable within this locus (Letchuman, 2018). The *ITS* region, comprising two sub-regions, *ITS1* and *ITS2*, has a combined length of approximately 700 bp. *ITS1* appears between the *18S rRNA* and *5.8S rRNA* genes, whereas *ITS2* lies between the *5.8S rRNA* and *26S rRNA* genes (Roslim *et al*., 2023a).

The plants' chloroplast genome functionally divides into coding and non-coding regions (such as introns and intergenic spacers—IGS). Among the coding regions, *matK* and *rbcLb* have had documentation and recommendations from the Consortium for Barcode of Life (CBOL) as a universal DNA barcode candidate for crop plants, which has wide usage and provides better interspecific resolution and discrimination (CBOL Plant Working Group, 2009; Patwardhan *et al*., 2014). The *rbcL* gene encodes the rubisco protein, which plays a crucial role in photosynthesis. The *matK* encodes maturase K, a protein involved in pre-mRNA splicing. However, as compared to *matK*, the *rbcL* was substantially more conserved (Guo *et al*., 2016).

In addition to coding regions, the chloroplast genome IGS regions have a broad utilization as DNA barcodes in crop plants. The IGS regions are non-coding sequences located between the two genes. Compared with coding regions, IGSs tend to accumulate mutations more readily, resulting in higher mutation rates and greater interspecific variations. This recognizes them and serves as particularly useful for distinguishing closely related species and for plant identification using DNA barcoding. Shaw *et al*. (2007) further demonstrated that IGS regions exhibited greater sequence variability (4.12% ± 1.10%) than introns (3.09% ± 0.57%). The commonly used IGS regions include *trnL-trnL-trnF*, *trnK-matK-trnK, trnQ*(UUG)-*5'rps16*, *3'trnV*(UAC)-*ndhC*, *ndhF-rpl32*, *psbD-trnT*(GGU), *psbJ-petA*, *3'rps16–5'trnK*(UUU), *atpI-atpH*, *petL-psbE*, and *atpB*-psbH (Shaw *et al*., 2007; Kress, 2017).

In the chloroplast genome, the location of the *trnL-trnL–trnF IGS* is between the *tRNA-Leu* and *tRNA-Phe* genes. In past studies, the said region exhibited the highest rate of substitution mutation and considerable sequence variation (Mildawati *et al*., 2023). It has proven to be an effective technique for distinguishing cryptic species, with variability resulting from both substitutions and indels (Roslim *et al*., 2021). Universal primers targeting this region have successful development and wide application across the numerous plant taxa (Wang *et al*., 2019; Herman *et al*., 2023; Roslim *et al*., 2024;

Table 1. Four primer pairs used for the amplification of four DNA barcode sequences used in pepina (*Plukenetia corniculata* Sm.) (Roslim *et al*., 2021).

Primers	Sequence (5'→3')	Ta (°C)	Regions
matK-413f-1	TAATTTACRATCAATTCATTCAATATTTCC	47.5	*Maturase K*
matK-1227r-3	GARGATCCRCTRTRATAATGAAAAAGATTT		
*rbcLb*_SF	AGACCTTTTTGAAGAAGGTTCTGT	56.6	*Ribulose-1,5-bisphosphate carboxylase large subunit*
*rbcLb*_SR	TCGGTCAGAGCAGGCATATGCCA		
B49873_F3	GGTTCAAGTCCCTCTATCCC	49.5	*trn*L*(UAA) 3'exon-trn*F *intergenic spacer*
A50272_R3	ATTTGAACTGGTGACACGAG		
FP_ITS5_F	GAAAGTAAAAGTCGTAACAAGG	47.0	*Internal transcribed spacer*
FP_ITS4_R	TCCTCCGCTTATTGATATGC		

Khairi *et al*., 2025). Based on the above discussion, the presented study sought to analyze the four DNA barcodes (*matK*, *rbcLb*, *ITS*, and *trnL-trnL–trnF IGS*) and their combinations to confirm the identity of pepina originating in Riau, Indonesia. Furthermore, the analysis aimed to provide an observable molecular characterization for species validation and contribute additional barcode sequences of pepina to public databases.

MATERIALS AND METHODS

Plant material

Young and fresh leaves of pepina (*P. corniculata*) underwent collection from the Air Putih Village, Tuah Madani Subdistrict, Pekanbaru, Indonesia. Determining the scientific name of pepina from Riau relied on public information, stating that pepina is one of the local names for *Plukenetia corniculata* (https://plantamor.com/species/profile/plukenetia/corniculata). Therefore, this research aimed to verify the scientific name of pepina. The sample is quite representative of pepina from Riau because there are three plant replicates, with the molecular markers not influenced by the environment. The name pepina is common from the local usage. Therefore, this research continued to verify its scientific name. Total genomic DNA extraction came from three individual plants following the manufacturer's protocol using the Plant Genomic DNA Mini Kit (Geneaid, Taiwan) in the Genetics and Molecular Biology Laboratory, University of Riau, Indonesia. Approximately 0.2 g of leaf tissue, cut into small pieces with sterile scissors, sustained grinding into fine powder in liquid nitrogen using a mortar and pestle before being transferred into a 1.5 mL microtube. The extracted DNA then served as a template for PCR amplification. The primers used in this study are available in Table 1.

PCR amplification

The PCR reactions had their preparation in a total volume of 50 µL following the manufacturer's instructions (Thermo Scientific), comprising 1× PCR buffer (+Mg^{2+}), 0.1 mM dNTPs, 0.4 µM forward primer, 0.4 µM reverse primer, 1.5 U DreamTaq DNA polymerase, 1 µL genomic DNA, and nuclease-free water. Amplification consisted of the following procedure. An initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 45 s. Annealing was at 47.5 °C for *matK,* 49.5 °C for *trnL-trnF IGS* (Khairi *et al*., 2025), 56.6 °C for *rbcLb*, and 47 °C for ITS (Roslim *et al*., 2025), with each for 45 s, and an extension at 72 °C for 1 min. A final extension ensued at 72 °C for 10 min.

PCR product visualization and sequencing

The PCR products' analysis ensued on agarose gel electrophoresis. A mixture of 2 µL PCR product and 2 µL loading dye, as loaded into the wells, had a DNA ladder prepared similarly before loading into the first well. Electrophoresis ran at 50 V for 45 min, with bands visualized under UV light using a UV transilluminator and documented with a UV-filtered camera. Successful PCR products (40 µL), along with 30 µL of forward and reverse primers, incurred sealing with parafilm before

sending to PT Genetika Science Indonesia. Meanwhile, sequencing proceeded at First Base Laboratories, Malaysia.

Data analysis

The obtained DNA sequences attained first editing using BioEdit version 7.0. Sequence similarity searches, when performed, used the BLASTn (Basic Local Alignment Search Tool nucleotide) program available at the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST). From the BLASTn results, the top 10 closest species, together with the three sample sequences, entailed subsequent analysis in MEGA version 11 to construct phylogenetic trees. Phylogenetic reconstruction succeeded in using both single-locus and multi-locus DNA barcode datasets.

RESULTS AND DISCUSSION

DNA barcode sequences' analysis

In pepina (*P. corniculata*), the DNA barcoding was successful in carrying out the four different DNA barcodes, i.e., *matK*, *rbcLb*, *ITS*, and *trnL-trnL–trnF IGS*. Gel electrophoresis confirmed and revealed the single DNA bands with approximate sizes of 900 bp for *matK*, 850 bp (*rbcLb*), 700 bp (*ITS*), and 1100 bp (*trnL-trnL–trnF IGS*) (Figure 2). These distinct bands underwent subsequent use for sequencing. The obtained sequences were 873 bp (*matK*), 840 bp (*rbcLb*), 715 bp (*ITS*), and 1067 bp (*trnL-trnL–trnF IGS*) in length after quality trimming. All the sequences reached their deposit in the GenBank database with the following accession numbers: OR898304, OR898305, and OR898304 for *matK*; OR898310, OR898311, and OR898312 for *rbcLb*; OR898316, OR898317, and OR898318 for *ITS*; and PP964911, PP964912, and PP964913 for *trnL-trnL-trnF IGS*. The four-barcode sequences with average nucleotide composition showed the highest frequencies of adenine (A, 29.3%) and thymine (T, 30.6%) as compared with cytosine (C, 20.7%) and guanine (G, 19.4%) (Table 2).

The results further revealed that the pepina collected from Riau, Indonesia, exhibited 100% similarity with the species *P. corniculata,* based on the *matK* and *ITS* sequences (Table 3). However, the *rbcLb* and *trnL-trnL-trnF IGS* sequences' analysis disclosed the highest similarity with the species *P. volubilis* (99.64% and 95.22%,

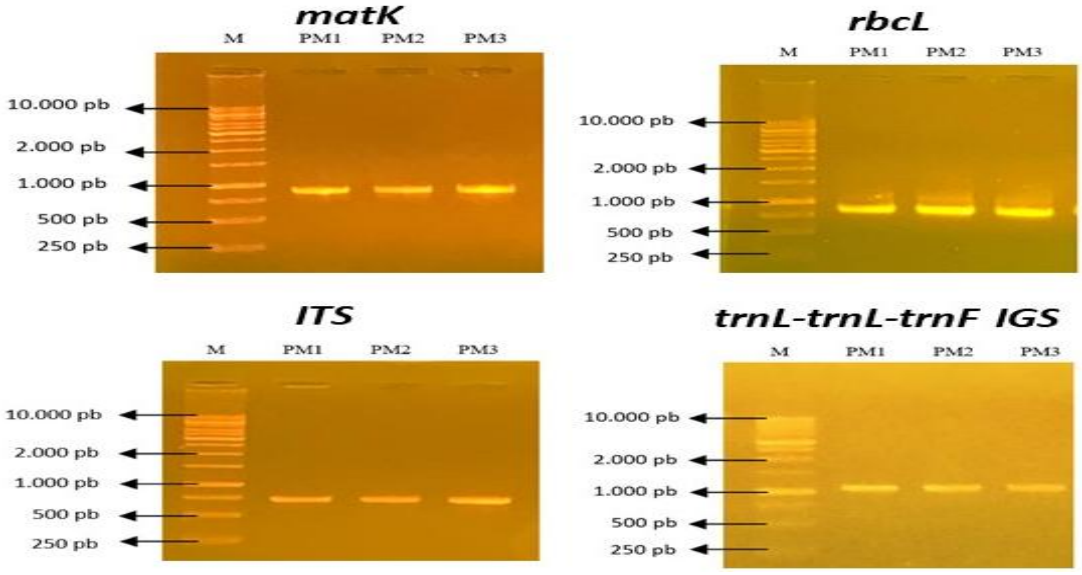

Figure 2. The DNA band profiles of four DNA barcodes on pepina (*Plukenetia corniculata* Sm.). M: 1 kb DNA ladder (Thermo Scientific).

Table 2. Average nucleotide base frequencies (%) in the four DNA barcode sequences analyzed in pepina (*Plukenetia corniculata* Sm.).

No.	DNA region	Base Frequency Average (%)			
		T/U	C	A	G
1	*matK*	17.9	31.4	20.4	30.3
2	*rbcLb*	29.3	17.9	35.4	17.3
3	*trnL-trnL-trnF IGS*	36.0	15.2	33.3	15.5
4	*ITS*	39.4	18.1	28.0	14.5
Means		30.6	20.7	29.3	19.4

Table 3. Results of BLASTn analysis of the four DNA barcode sequences in pepina (*Plukenetia corniculata* Sm.).

No.	DNA Barcode	Top Accession	Query Cover (%)	E-Value	Identity (%)	Acc. Number
1	*matK*	*Plukenetia corniculata*	100	0.0	100.00	MF502723.1
2	*rbcLb*	*Plukenetia volubilis*	99	0.0	99.64	NC_058006.1
3	*trnL-trnL-trnF IGS*	*Plukenetia volubilis*	100	0.0	95.22	NC_058006.1
4	*ITS*	*Plukenetia corniculata*	98	0.0	100.00	MF502525.1

Table 4. Nucleotide differences, mutations, and number of critical nucleotides in the four analyzed DNA barcode sequences in pepina (*Plukenetia corniculata* Sm.).

No.	Region	Variation		Indels Mutation		Substitution Mutation		Critical Nucleotide	
		(bp)	(%)	(bp)	(%)	(bp)	(%)	(bp)	(%)
1.	*matK*	26	3.07	0	0.00	26	3.07	3	0.35
2.	*rbcLb*	38	4.52	0	0.00	38	4.52	2	0.24
3.	*trnL-trnL-trnF IGS*	250	21.37	181	15.47	69	5.90	40	3.42
4.	*ITS*	158	21.29	49	6.60	109	14.69	2	0.27
Means		118	12.56	57.5	5.52%	60.5	7.04%	11.75	1.07

respectively). The query cover ranged from 98% to 100%, and the E-value was 0.00 (Table 3). These results suggested that pepina from Riau appeared closely associated with the species *P. volubilis*, which also received support from their relatively similar leaf and fruit morphology. According to Kodahl and Sorensen (2021), the species *P. volubilis* is a member of the family Euphorbiaceae, locally known as 'sacha inchi,' producing star-shaped fruits with five lobes.

The BLASTn analysis further confirmed that the pepina obtained from Riau, Indonesia, could be identifiable as *P. corniculata* based on the *matK* and *ITS* sequences (Table 3). Moreover, the study revealed that the markers *rbcLb* and *trnL-trnL-trnF I*GS sequences for *P. corniculata* were still unavailable in the GenBank; thus, the sequences generated here represent the first report for these markers in *P. corniculata*. Cock *et al*. (2015) studied several parameters by using BLASTn analysis, including E-value, max score, total score, query cover, and percentage identity, and all together determined the degree of homology between the sample sequence and those deposited in the GenBank. Roslim *et al*. (2016) mentioned that in establishing the taxonomic status of a plant species, a species could become identical if it emerges with 100% identity, 100% query cover, and an E-value of 0.0.

The highest levels of DNA variation were evident in the non-coding sequences of the markers *trnL-trnL-trnF IGS* (21.37%) and *ITS* (21.29%) (Table 4). Generally, the DNA sequence variation arises from mutation, including insertions-deletions (indels) and substitutions. However, the indel mutations prevailed exclusively in the non-coding sequences, with frequencies of 15.47% in the DNA barcode *trnL-trnL-trnF IGS* and 6.60% in

ITS, while no indels surfaced in the coding regions of the DNA barcodes *matK* and *rbcLb*. In contrast, the substitution mutations occurred in both coding and non-coding regions; however, the highest frequency observed was in non-coding sequences, particularly the DNA barcodes *ITS* (14.69%) and *trnL-trnL-trnF IGS* (5.90%), compared with the coding sequences of *rbcLb* (4.52%) and *matK* (3.07%) (Table 4).

Mutations generally cause variations in DNA that can generate subsequent variations among the species, which proved valuable for phylogenetic analysis and species identification (Roslim and Herman, 2017). Indel mutations, in particular, caused the differences in DNA sequence length among the species. Insertions enhanced the size of DNA sequences, while mutations caused by deletions reduced the DNA size (Sehn, 2015). Indels were rarely evident in protein-coding regions, such as DNA barcodes *matK* and *rbcLb*, because of the disruption of the reading frame of codons and produced non-functional proteins. Similarly, substitution mutations also occurred less frequently in coding sequences, which may alter the resulting protein, especially when the mutation affects the first or second base of the codon. However, if a substitution occurs at the third base of the codon, the protein produced will usually be functional due to redundancy of the genetic code.

In pepina, the crucial and diagnostic nucleotides were most frequently prevalent in the DNA barcode *trnL-trnL-trnF IGS* region with 40 bp (3.42%), compared with three other DNA barcodes: *matK* (3 bp, 0.35%), *ITS* (2 bp, 0.27%), and *rbcLb* (2 bp, 0.24%) (Table 4). Thus, among the four DNA barcodes examined, the single locus *trnL-trnL-trnF IGS,* followed by *ITS*, showed the most robust potential as a molecular barcode in the identification of pepina collected from Riau, Indonesia. It harbors the highest proportion of sequence variations, indel mutations, and diagnostic nucleotides. Previous studies have also highlighted the potential of the DNA barcode *trnL-trnL-trnF IGS* in DNA barcoding of the species *Cleome gynandra* (Roslim *et al*., 2023b), within the families Solanaceae and Fabaceae (Herman *et al*., 2023), and *Kalanchoe* sp. (Herman, 2024), however, not in the genus *Calamus* (Khairi *et al*., 2025).

Phylogenetic tree analysis

Consistent with the BLASTn results (Table 3), the phylogenetic tree showed the three studied pepina individuals clustered together with the species *P. corniculata* based on *ITS* and *matK* DNA barcode sequences, with bootstrap values of 100% and 94%, respectively (Figure 3). In contrast, based on the DNA barcodes *rbcLb* sequences, the three individuals formed a distinct group closely related to *P. volubilis*. These results also enunciated that the DNA barcode *rbcLb* sequence was less effective in precisely distinguishing the genus *Plukenetia* from other genera examined, compared with the DNA barcode *trnL-trnL-trnF IGS*. This limitation was likely due to the lower DNA variation and slower mutation rate of *rbcLb* than the three other DNA barcodes analyzed (Duan *et al*., 2019); hence, the ability of the *rbcLb* sequence to differentiate species is very low.

The main differences between *P. corniculata* and *P. volubilis* lie in their region of origin, the physical characteristics of the fruit, and their uses. *Plukenetia volubilis* is popular for its smooth, star-shaped fruit, while *P. corniculata* has a more "horned" or winged fruit shape, as its name suggests (corniculata means horned). Both are climbing plants in the Euphorbiaceae family, but *P. volubilis* has a long, cylindrical column in its flower structure compared with the local species.

The organism groups that share similar morphological traits tended to have a close evolutionary relationship (Astarini *et al*., 2021). Such similarities seemed to be an inheritance from a common ancestor, ultimately forming a monophyletic group. In the presented study, the construction of the phylogenetic tree succeeded in using the neighbor-joining method with 1000 bootstrap replications. Priyadi *et al*. (2022) reported that employing 1000 bootstrap replications aimed to produce a more accurate phylogenetic tree. High bootstrap values revealed the species in comparison share a close evolutionary relationship.

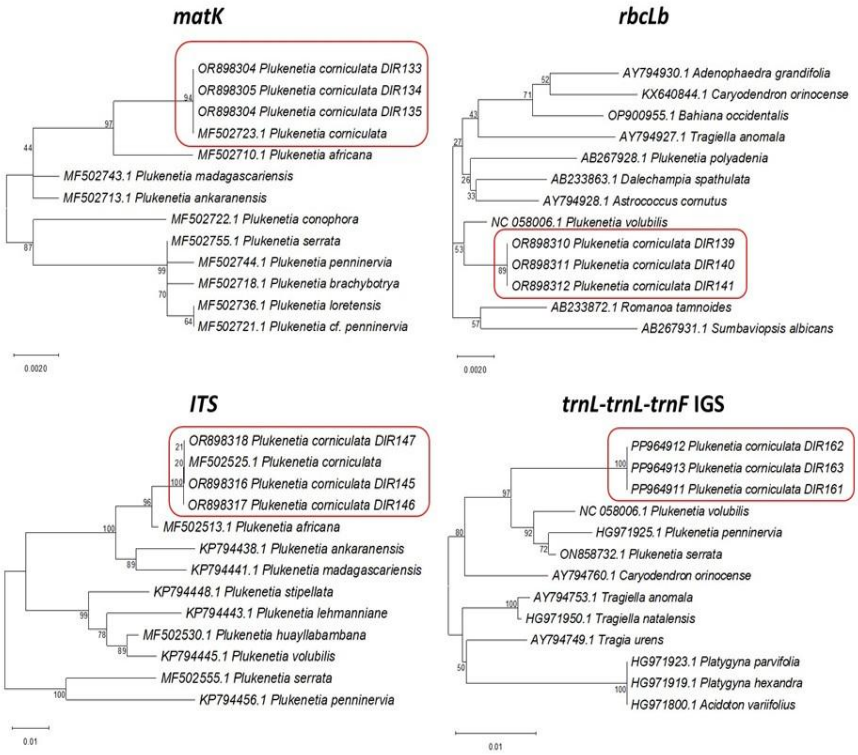

Figure 3. Dendrogram constructed using the Neighbor-Joining method with 1000 bootstrap replicates, based on *matK*, *rbcLb*, *ITS*, and *trnL-trnL-trnF IGS* sequences in pepina (*Plukenetia corniculata* Sm.) and other accessions studied.

The construction of the phylogenetic tree using combined DNA barcodes of two loci (*ITS* + *trnL-trnL-trnF IGS*), three loci (*ITS* + *trnL-trnL-trnF IGS* + *matK*), and four loci (*ITS* + *trnL-trnL-trnF IGS* + *matK* + *rbcLb*) showed the multi-locus combinations consistently grouped the studied individuals into the same cluster, appearing distinct from the other analyzed accessions. The results further revealed that in addition to single-locus markers, the use of multi-locus DNA barcodes could also be effective for barcoding analysis in pepina (Figure 4).

Euphorbiaceae is a diverse and economically important family, comprising approximately 340 genera and 8000–9000 species (Wurdack *et al*., 2004). One of its genera is *Plukenetia*, which belongs to the tribe Plukenetieae. *Plukenetia* comprises about 25 species, characterized by non-stinging twining lianas, vines, and rarely subshrubs. Based on DNA barcoding analyses using *ETS*, *ITS*, *KEA1* introns 11 and 17, *TEB* exon 17, *matK*, and *ndhF* sequences, reports stated the genus *Plukenetia* could undergo division into two major groups: the palmately veined clade and the pinnately veined clade. The palmately veined clade comprises four sections, *Madagascariensis*, *Hedraiostylus*, *Angostylidium*, and *Plukenetia*, while the pinnately veined clade consists of two sections, *Penninerviae* and *Fragariopsis*. Pepina (*P. corniculata*) and *P. africana* emerged as members of sect. *Hedraiostylus* (Cardinal-McTeague and Gillespie, 2020); then in the presented study, both species resulted in being closely associated based on *matK* and *ITS* sequences (Figure 3). Regarding these results, identification of *Plukenetia* species was effective using *matK*, *ITS*, and *trnL-trnL-trnF IGS* sequences. Furthermore, supporting conservation efforts is essential for pepina.

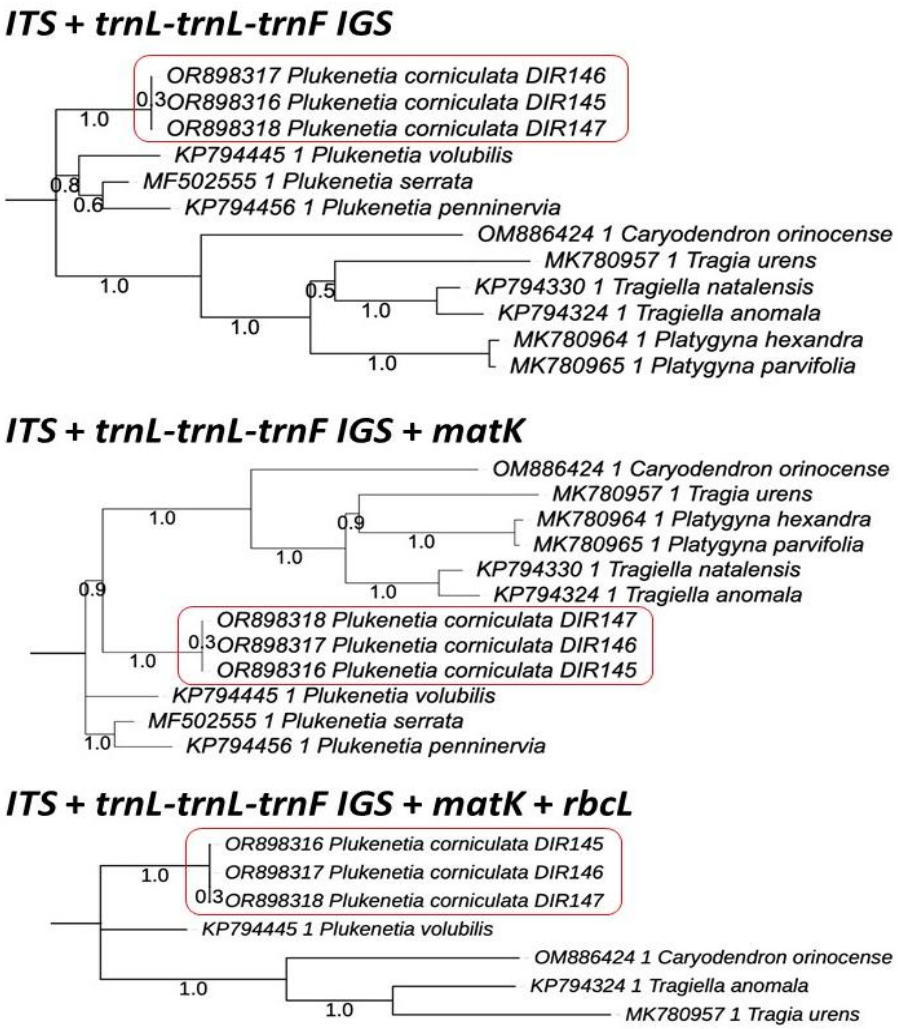

Figure 4. The phylogenetic tree of the concatenated sequence based on Maximum-likelihood method. The number in each node is the bootstrap support value in pepina (*Plukenetia corniculata* Sm.) and other accessions studied.

Although pepina has no current classification as an endangered species, habitat degradation could threaten the long-term survival of pepina. Its sustainable management and harvesting are essential for species conservation in the future.

CONCLUSIONS

DNA barcoding analysis using DNA barcodes *matK* and *ITS* sequences successfully confirmed the pepina from Riau as *Plukenetia corniculata*. In this study, the DNA barcodes *rbcLb* and *trnL-trnL-trnF IGS* sequences obtained in pepina were the first reports in the public GenBank database. Among the DNA barcodes tested, the highest level of DNA variation, indel mutations, and the crucial and diagnostic nucleotides were evident in the DNA barcode *trnL-trnL-trnF IGS*, followed by *ITS*. In contrast, the DNA barcode *rbcLb* sequences were unable to distinguish *Plukenetia* completely from other genera examined. Molecular identification of pepina can therefore be successful using single-locus barcodes, such as trnL-*trnL-trnF IGS*, *ITS*, and *matK*, as well as multi-locus combinations.

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REFERENCES

Astarini IA, Ardiana SA, Putra ING, Pertiwi PD, Sembiring A, Yusmalinda A, Al Malik D (2021). Genetic diversity and phylogenetic of longtail tuna (*Thunnus tonggol*) landed in Pabean Fish Market, Surabaya. *Musamus Fish. Marine J.* 3(2): 107–115.

Cardinal-McTeague WM, Gillespie LJ (2020). A revised sectional classification of *Plukenetia* L. (Euphorbiaceae, Acalyphoideae) with four new species from South America. *Syst. Bot.* 45(3): 507–536.

Cardinal-McTeague WM, Wurdack KJ, Sigel EM, Gillespie LJ (2019). Seed size evolution and biogeography of *Plukenetia* (Euphorbiaceae), a pantropical genus with traditionally cultivated oilseed species. *BMC Evol. Biol.* 19: 29.

CBOL (Consortium for Barcode of Life) Plant Working Group (2009). A DNA barcode for land plants. *PNAS USA* 106: 12794–12797.

Choo LM, Ng XY, Lua HK (2022). Rediscovery and lectotypification of a native vine, *Plukenetia corniculata* Sm. (Euphorbiaceae), in Singapore. *Nat. Singapore* 15: e2022001.

Cock PJ, Chilton JM, Grüning B, Johnson JE, Soranzo N (2015). NCBI BLAST+ integrated into galaxy. *Gigascience* 4(1): 39.

Duan H, Wang W, Zeng Y, Guo M, Zhou Y (2019). The screening and identification of DNA barcode sequences for *Rehmannia*. *Sci. Rep.* 9: 17295. https://doi.org/10.1038/s41598-019-53752-8

Feng C, Zhu Y, Su Q, Zhou X, Chen W, Tao Y, Chen M, He H, Pan B, Xu Z, Fu Q (2023). Phenology and reproductive biology of *Plukenetia corniculata* S., a traditional wild vegetable of Southeast Asia. *Res. Sq*. 1–21.

Guo Y-Y, Huang L-Q, Liu Z-J, Wang X-Q (2016). Promise and challenge of DNA barcoding in *Venus slipper* (Paphiopedilum). *PloS One* 11(1): e0146880.

Herman (2024). Molecular characterization of sidingin plant (*Kalanchoe* sp.) from Riau using four DNA barcodes. *Biota* 9(2): 164–171.

Herman, Khairi HA, Wansyah AR, Asmania, Utami RI, Anzaelina DN, Oktaviano Z, Lestari W, Adiwirman, Roslim DI (2023). The ability of *matk* and *trnL-trnL-trnF intergenic spacer* to discern certain species accessions of Solanaceae and Fabaceae. *SABRAO J. Breed. Genet.* 55(1): 97–106.

Hollingsworth PM, Li DZ, van der Bank M, Twyford AD (2016). Telling plant species apart with DNA: From barcodes to genomes. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 371:20150338. doi: 10.1098/rstb.2015.0338

Khairi HA, Roslim DI, Vitovang AD, Hariani M, Herman, Lestari W, Adiwirman, Hasibuan ARW, Novita L, Zulaeha S, Hindaningrum IF (2025). Molecular characterization of water rattan palm (*Calamus angustifolius* Griff.) from Kampar River, Riau Province, Indonesia using *matK* and *trnL-trnL-trnF* intergenic spacer. *Jordan J. Biol. Sci.* 18(2): 253–263.

Khan SA, Baeshen MN, Ramadan HA, Baeshen NA (2019). *ITS2*: An ideal DNA barcode for the arid medicinal plant *Rhazya stricta*. *Pharmaceut. Med.* 33(1): 53–61.

Kodahl N, Sorensen M (2021). Sacha inchi (*Plukenetia volubilis* L.) is an underutilized crop with a great potential. *Agronomy* 11(6): 1066.

Kress WJ (2017). Plant DNA barcodes: Applications today and in the future. *J. Syst. Evol*. 55(4): 291–307.

Letchuman S (2018). Short introduction of DNA barcoding. *Int. J. Res.* 5(4): 673–686.

Mildawati, Sobir, Sulistijorini, Chikmawati T (2023). Phylogeny of *Davallia* (Davalliaceae) from Sumatera and Mentawai Island, Indonesia: Evidence from *trnL-F intergenic spacer*. *Biodiversitas* 24(8):4589–4596.

Pagala MA, Nafiu LO (2020). Teknologi Biomarka Molekuler. Kendari: Universitas Halu Oleo Press.

Patwardhan A, Ray S, Roy A (2014). Molecular markers in phylogenetic studies - A review. *J. Phylogenet. Evol. Biol*. 2: 131.

Pradheep K, Rathi RS, Soyimchiten, Nayar ER (2015). Meetha patta (*Plukenetia corniculata* Sm.): A new report of leafy vegetable crop from Northeastern region of India. *Genet. Resour. Crop Evol*. 62: 1113–1120.

Priyadi A, Asih NPS, Erlinawati I (2022). Reconstructing phylogenies of *Alocasia* spp.

(Araceae) distributed in Indonesia for conservation prioritization. *J. Trop. Life Sci*. 12(3): 317–323.

Raclariu AC, Heinrich M, Ichim MC, Deboer H (2018). Benefits and limitations of DNA barcoding and metabarcoding in herbal product authentication. *Phytochem. Anal*. 29 (2): 123–128.

Roslim DI, Fauzan M, Taufik ME, Al Khairi H, Herman, Safarrida A, Lestari W, Sofiyanti N, Effendi A, Novita L, Reninta R, Altuhaish AAF (2025). Evaluation of maman plant from Riau with *rbcl, ITS*, and their combination as DNA barcodes. *SABRAO J. Breed. Genet.* 57(3): 1070–1080.

Roslim DI, Herman (2017). Application of *rps16* intron and *trnL-trnF Intergenic Spacer* sequences to identify Rengas clone Riau. *Biosaintifika* 9(2): 209–216.

Roslim DI, Herman, Adiwirman, Lestari W (2023a). Barkoding DNA. Yogyakarta: Penerbit KBM Indonesia.

Roslim DI, Herman, Budiono DYF, Cahyati IE, Lestari W, Novita L, Priyadi A (2024). Analysis of *trnL-trnL-trnF intergenic spacer* and *matK* sequences combined with morphological observations showed pucuk seminyak from Riau is *Champereia manillana var*. *manillana* Merr. *J. Trop. Life Sci*. 14(3): 575–584.

Roslim DI, Herman, Putri SR, Furqoni AT, Budiono DYF, Altuhaish AAF, Lestari W (2023b). Verification of Maman (*Cleome gynandra* (L.) Briq.) from Riau based on *matK* and *trnL-trnL-trnF intergenic spacer*. *Biosaintifika* 15(3): 296–305.

Roslim DI, Khumairoh S, Herman (2016). Confirmation of tuntun angin (*Elaeocarpus floribundus*) taxonomic status using *matK* and *ITS* sequences. *Biosaintifika* 8(3): 392–399.

Roslim DI, Putra YO, Dewi YM, Bago Y, Sitohang H, Herman, Fitmawati, Sofiyanti N (2021). First record of five DNA barcodes on Nothospecies Cocor Bebek (*Kalanchoe* × *laetivirens*). *SABRAO J. Breed. Genet*. 53(2): 263–377.

Sehn JK (2015). Chapter 9: Insertions and Deletions (Indels). Shashikant Kulkarni and John Pfeifer (Eds.), Clinical Genomics. Academic Press. pp. 129–150.

Shaw J, Lickey EB, Schilling EE, Small RL (2007). Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. *Am. J. Bot.* 94: 275–288.

Talang H, Yanthan A, Rathi RS, Pradheep K, Longkumer S, Imsong B, Singh LH, Assumi RS, Devi MB, Vanlalruati, Kumar A, Ahlawat SP, Bhatt KC, Bhardwaj R (2023). Nutritional evaluation of some potential wild edible plants of North Eastern region of India. *Front. Nutr.* 10:1052086.

Waldchen J, Rzanny M, Seeland M, Mäder P (2018). Automated plant species identification — Trends and future directions. *PLoS Comput. Biol*. 14(4): 1–19.

Wang L, Jiang Y, Shi Q, Wang Y, Sha L, Fan X, Kang H, Zhang H, Sun G, Zhang L, Shou Y (2019). Genome constitution and evolution of *Elytrigia lolioides* inferred from Acc1 EF-G ITS TrnL-F sequences and GISH. *BMC Plant Biol*. 19: 1–14.

Wang S, Zhu F, Kakuda Y (2018). Sacha inchi (*Plukenetia volubilis* L.): Nutritional composition, biological activity and uses. *Food Chem*. 265(2): 316–328.

Wurdack KJ, Hoffmann P, Samuel R, de Bruijn A, van der Bank M, Chase MW (2004). *Am. J. Bot.* 91(11): 1882–1900.