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# The Identity of Wild Banana from Bengkulu Revealed by Internal Transcribed Spacer Region

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## ABSTRACT

Aura Annisa Rahma, Syaiful Azhar, Pandu Gusti Ananda, Isna Arofatun Nikmah, Muhammad Rifqi Hariri 2026. Identitas pisang liar dari Bengkulu berdasarkan wilayah Internal Transcribed Spacer. Floribunda 9(2) 34 – 44 — Pisang liar merupakan sumber daya genetik yang penting, namun banyak populasinya di Indonesia yang masih belum terdokumentasi dengan baik. Penelitian ini bertujuan mengidentifikasi spesimen pisang liar yang berasal dari Rejang Lebong, Bengkulu, Indonesia, berdasarkan pengamatan morfologi dan analisis molekuler menggunakan daerah internal transcribed spacer (ITS) DNA ribosomal inti. Karakterisasi morfologi mencakup karakter vegetatif dan generatif, sedangkan DNA genom diekstraksi dari jaringan daun kering dan diamplifikasi menggunakan primer ITS universal. Amplifikasi PCR menghasilkan fragmen DNA berukuran sekitar 900 pb yang selanjutnya disekuensing dan dianalisis menggunakan metode BLAST serta analisis filogenetik. Sekuen ITS menunjukkan tingkat kemiripan tertinggi dengan *Musa salaccensis* Zoll. ex Kurz, yaitu sebesar 99,70% dan 99,41% terhadap aksesi GenBank MK779039.1 dan FJ428072.1. Analisis filogenetik menempatkan spesimen Bengkulu dalam klad *M. salaccensis* dengan dukungan bootstrap yang kuat (94%). Karakter morfologi yang diamati juga sesuai dengan karakter *M. salaccensis*. Hasil penelitian ini mengonfirmasi bahwa spesimen pisang liar dari Bengkulu merupakan *Musa salaccensis* serta memberikan informasi penting bagi dokumentasi dan upaya konservasi keanekaragaman pisang liar di Indonesia.

Kata kunci: DNA barcoding; internal transcribed spacer; *Musa*; Sumatra.

Aura Annisa Rahma, Syaiful Azhar, Pandu Gusti Ananda, Isna Arofatun Nikmah, Muhammad Rifqi Hariri 2026. The Identity of wild banana from Bengkulu revealed by Internal Transcribed Spacer region. Floribunda 9(2) 34 – 44 — Wild bananas are important genetic resources, yet many populations in Indonesia remain poorly documented. This study identified a wild banana specimen from Rejang Lebong, Bengkulu, Indonesia, using morphological observations and

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molecular analysis of the nuclear ribosomal internal transcribed spacer (ITS) region. Morphological characterization included vegetative and reproductive traits, while genomic DNA was extracted from dried leaf tissue and amplified using universal ITS primers. PCR amplification produced an approximately 900 bp fragment, which was sequenced and analyzed using BLAST and phylogenetic methods. The ITS sequence showed the highest similarity to *Musa salaccensis* Zoll. ex Kurz, with 99.70% and 99.41% identity to GenBank accessions MK779039.1 and FJ428072.1, respectively. Phylogenetic analysis placed the Bengkulu specimen within the *M. salaccensis* clade with strong bootstrap support (94%). Morphological traits were also consistent with those of *M. salaccensis*. These results confirm that the wild banana specimen from Bengkulu is *M. salaccensis* and provide valuable information for the documentation and conservation of wild banana diversity in Indonesia.

Keywords: DNA barcoding; internal transcribed spacer; *Musa*; Sumatra

Bananas (*Musa* spp.) are among the most important fruit crops worldwide and play a significant role in food security, nutrition, and economic development in tropical and subtropical regions (Emmanuel et al. 2025). The genus *Musa* exhibits remarkable genetic diversity, encompassing both cultivated varieties and numerous wild relatives (Jenny et al. 2024). Wild banana species are valuable genetic resources because they possess traits such as disease resistance, environmental adaptability, and stress tolerance that can be utilized in breeding programs for crop improvement (Panwar et al. 2022).

Indonesia is recognized as one of the centers of diversity for bananas, harboring a rich collection of wild and cultivated *Musa* species (Ahmad et al. 2020). Among its regions, Bengkulu Province on the western coast of Sumatra is characterized by diverse ecosystems that support the growth of various wild banana populations. However, the taxonomic identity of many wild bananas in this region remains poorly understood due to morphological similarities among species and the influence of environmental factors on phenotypic characteristics. Accurate species identification is essential for biodiversity conservation, germplasm management, and the effective utilization of genetic resources.

Traditional classification of bananas has largely relied on morphological traits, including plant stature, leaf characteristics, inflorescence structure, and fruit morphology (Nelson et al. 2006). Nevertheless, these characteristics are often insufficient for distinguishing closely related species, particularly when phenotypic variation occurs within populations. Molecular approaches have therefore become increasingly important for resolving taxonomic uncertainties and elucidating genetic relationships among banana species (Dhivya et al. 2020).

The internal transcribed spacer (ITS) region of nuclear ribosomal DNA is one of the most widely used molecular markers in plant systematics and phylogenetic studies (Devi et al. 2022). The ITS region evolves relatively rapidly and provides sufficient sequence variation for species-level discrimination (Qin et al. 2017). Consequently, ITS analysis has proven effective in identifying plant taxa and assessing evolutionary relationships among closely related species.

This study aims to determine the identity of wild banana accessions collected from Bengkulu using sequences of the ITS region. By integrating molecular data with taxonomic analysis, the research is expected to contribute to a better understanding of wild banana diversity in Bengkulu and provide valuable information for conservation and future banana breeding efforts.

## MATERIALS AND METHODS

### Materials

One plant specimen used in this study was collected from a natural population of wild banana growing in Rejang Lebong Regency, Bengkulu Province, Indonesia (Figure 1). The sampling site was selected based on the presence of healthy and well-established wild banana individuals. For molecular analysis, young and healthy leaf

tissue was chosen because it generally contains high-quality genomic DNA and lower concentrations of secondary metabolites that may interfere with DNA extraction (Bailey et al. 2022). An intact leaf showing no signs of disease, herbivore damage, chlorosis, necrosis, or mechanical injury was carefully selected from the sampled individual. Approximately 2 to 5 cm² of leaf tissue was excised using sterilized cutting tools to minimize contamination.




**Figure 1.** Morphological characters of wild banana collected from Rejang Lebong, Bengkulu, Indonesia. (A) Flowering plant exhibiting characteristic inflorescence and bracts. (B) Fruiting infructescence with developing fruits. (C) Inflorescence morphology and sequential fruit developmental stages (1-5) from immature to mature fruits.

Immediately after collection, the leaf sample was placed into a specialized paper envelope commonly referred to as a "tea bag," following the protocol described by Aprilianingsih et al. (2022). The tea bag method facilitates rapid dehydration of plant tissues and helps preserve DNA integrity during transportation and storage. The tea bag containing the leaf material was then immersed in silica gel desiccant to accelerate drying and prevent DNA degradation caused by moisture. Silica gel was periodically monitored and replaced when necessary to maintain effective desiccation.

The dried leaf sample was transported to the laboratory and stored under dry conditions until DNA extraction was performed. The preserved tissue served as the source material for subsequent genomic DNA isolation, amplification of the internal transcribed spacer (ITS) region, and molecular identification of the wild banana specimen from Bengkulu.

**Morphological Observation**

Morphological observations were conducted directly in the field prior to sample collection to ensure that the selected individual accurately represented the general characteristics of the wild banana population occurring at the study site. Several diagnostic morphological traits were examined. Particular attention was given to identifying any unusual phenotypic variations, disease symptoms, or physical damage that could influence taxonomic interpretation. The selected specimen exhibited normal growth and morphological characteristics consistent with the surrounding population and was therefore considered suitable for molecular identification.

**DNA Extraction, PCR Amplification, and Sequencing**

Genomic DNA was extracted from silica gel-dried leaf tissue using the Plant Genomic DNA Kit (Tiangen Biotech Co., Ltd., Beijing,

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China) according to the manufacturer's instructions. The quality and quantity of the extracted DNA were assessed prior to downstream molecular analyses. The isolated DNA was subsequently used as a template for amplification of the internal transcribed spacer (ITS ) region of nuclear ribosomal DNA.

Amplification of the ITS region was performed using the universal ITS primers described by Sun et al. (1994). Polymerase chain reaction (PCR) was conducted in a total reaction volume of 50 µL following the protocol of Aprilianingsih et al. (2022). Each reaction contained approximately 10 ng of genomic DNA template, 1 µM of each forward and reverse primer, 25 µL of MyTaq™ HS Red Mix (Bioline, USA), and 17 µL of nuclease-free water. All PCR reactions were prepared under sterile conditions to minimize the risk of contamination.

PCR amplification was carried out in a thermal cycler under the following conditions: an initial denaturation step at 95 °C for 3 min, followed by 35 amplification cycles consisting of denaturation at 95 °C for 30 s, primer annealing at 58 °C for 45 s, and extension at 72 °C for 45 s. The amplification process was completed with a final extension step at 72 °C for 5 min to ensure complete synthesis of all PCR products.

The amplified products were verified by electrophoresis on a 1% agarose gel prepared in an appropriate electrophoresis buffer. DNA bands were separated under a constant electrical current and subsequently visualized under ultraviolet illumination. Gel images were captured and documented using a GelDoc UV transilluminator (Bio-Rad, USA). Successful amplification was confirmed by the presence of a distinct DNA fragment corresponding to the expected size of the ITS region.

Following verification, PCR products exhibiting clear and specific amplification were purified and submitted for bidirectional DNA sequencing. Sequencing services were provided by 1st BASE through PT Genetika Science Indonesia. The resulting nucleotide sequences were used for subsequent sequence assembly, editing, and molecular identification analyses of the wild banana specimen from Bengkulu.

**Sequence Assembly and Phylogenetic Analysis**

The nucleotide sequences obtained from forward and reverse sequencing reactions were manually inspected to ensure sequence quality and accuracy. The forward and reverse reads were subsequently assembled into a consensus contig sequence using the ClustalW algorithm, a widely applied multiple sequence alignment method that facilitates the generation of high-quality consensus sequences from overlapping DNA fragments. Ambiguous nucleotide positions, if present, were carefully examined and corrected based on chromatogram quality before further analyses.

The assembled ITS sequence was used as a query in the Basic Local Alignment Search Tool (BLAST) available through the National Center for Biotechnology Information (NCBI) database (https://blast.ncbi.nlm.nih.gov/). BLAST analysis was conducted to determine sequence similarity and identify closely related taxa based on homology with publicly available nucleotide sequences. The percentage identity, query coverage, and E-value obtained from the BLAST search were used as preliminary indicators of species affiliation and genetic relatedness (Hung and Weng, 2016).

To infer the phylogenetic position of the Bengkulu wild banana specimen, twenty representative ITS sequences of *Musa* species showing high similarity to the query sequence were retrieved from GenBank based on the BLAST results (Table 1). These sequences were selected to represent closely related taxa and to provide a robust framework for phylogenetic reconstruction. In addition, sequences of *Ensete ventricosum* (Welw.) Cheesman (GenBank accession number FJ428078.1) and *Musella lasiocarpa* (Franch.) C.Y. Wu ex H.W. Li (GenBank accession number AY673072.1) were included as outgroup taxa to root the phylogenetic tree and facilitate interpretation of evolutionary relationships within the genus *Musa*.

**Table 1.** Sequence length variation of ITS region of wild banana (*Musa salaccensis*) from Bengkulu compared to NCBI database

| No | Species | Accession No | Sequence Length (bp) |
|---|---|---|---|
| Observed specimen | | | |
| 1 | *Musa salaccensis* Bengkulu | This study | 734 |
| NCBI Database Sequences | | | |
| Ingroup | | | |
| 1 | *Musa violascens* | FJ428071.1 | 624 |
| 2 | *Musa salaccensis* | FJ428072.1 | 673 |
| 3 | *Musa monticola* | FJ428073.1 | 627 |
| 4 | *Musa hirta* | FJ428074.1 | 682 |
| 5 | *Musa campestris* | FJ428076.1 | 670 |
| 6 | *Musa paracoccinea* | FJ428077.1 | 675 |
| 7 | *Musa coccinea* | KY214932.1 | 1158 |
| 8 | *Musa beccarii* | LC610753.1 | 6532 |
| 9 | *Musa textilis* | LC610767.1 | 6801 |
| 10 | *Musa paracoccinea* | MK779037.1 | 675 |
| 11 | *Musa salaccensis* | MK779039.1 | 666 |
| 12 | *Musa gracilis* | MK779040.1 | 665 |
| 13 | *Musa lokok* | MK779041.1 | 665 |
| 14 | *Musa johnsii* | MK779043.1 | 665 |
| 15 | *Musa lolodensis* | MK779044.1 | 669 |
| 16 | *Musa jackeyi* | MK779045.1 | 665 |
| 17 | *Musa maclayi* subsp. *maclayi* | MK779046.1 | 672 |
| 18 | *Musa troglodytarum* | MK779047.1 | 665 |
| 19 | *Musa peekelii* subsp. *angustigemma* | MK779048.1 | 669 |
| 20 | *Musa barioensis* | MK779049.1 | 667 |
| Outgroup | | | |
| 1 | *Ensete ventricosum* | FJ428078.1 | 683 |
| 2 | *Musella lasiocarpa* | AY673072.1 | 865 |

All sequences were aligned using the ClustalW algorithm implemented in MEGA version 11 (Tamura et al. 2021). The resulting alignment was manually inspected and adjusted when necessary to optimize positional homology among nucleotide sites. Phylogenetic relationships were reconstructed using the Maximum Likelihood (ML) method, which estimates the most probable evolutionary tree based on the observed sequence data. The Tamura 3-parameter (T92) substitution model with a gamma distribution and five discrete rate categories was selected as the best fitting model to account for variation in nucleotide substitution rates and evolutionary heterogeneity across sites (Blasques et al. 2022; Ahamad et al. 2023).

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The reliability and statistical support of the inferred phylogenetic relationships were evaluated through bootstrap analysis with 1,000 replicates. Bootstrap values were mapped onto the resulting phylogenetic tree to assess the confidence level of individual branches, with higher values indicating stronger support for the corresponding clades (Lemoine et al. 2018). All phylogenetic analyses, including sequence alignment, model implementation, tree reconstruction, and bootstrap testing, were conducted using MEGA version 11 (Tamura et al. 2021).

## RESULT AND DISCUSSION

### Molecular Identification

Genomic DNA extracted from the wild banana specimen collected in Bengkulu was successfully amplified using the universal ITS primer pair described by Sun et al. (1994). Agarose gel electrophoresis revealed a single, distinct DNA band with an approximate size of 900 bp (Figure 2), indicating successful amplification of the target region and the absence of substantial non-specific amplification. The presence of a clear and well-defined PCR product also suggested that the extracted genomic DNA was of sufficient quality for downstream sequencing and phylogenetic analyses.

The amplified fragment corresponded to the nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) region, which comprises the ITS1 spacer, the 5.8S rDNA gene, and the ITS2 spacer (Aprilianingsih et al. 2022). This marker has been extensively utilized in plant molecular systematics because it combines highly conserved coding regions with rapidly evolving spacer sequences, making it suitable for species identification and phylogenetic reconstruction at the interspecific and intraspecific levels.

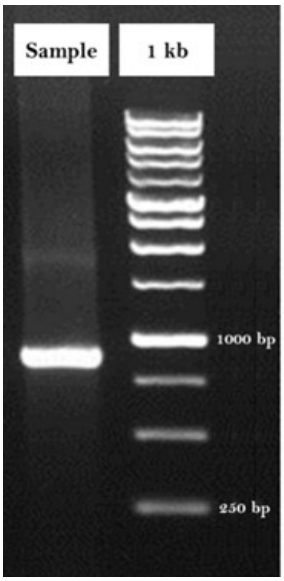



**Figure 2.** The amplified ITS sequence from the wild banana (sample) showing a band size nearly 900 bp.

The observed amplicon size of approximately 900 bp is consistent with previous studies employing the same primer pair in members of the family *Musa*ceae and other angiosperm taxa. Amplification using the ITS primers developed by Sun et al. (1994) has been reported to generate fragments ranging from 573 to 1,517 bp depending on the taxonomic group under investigation (Hariri et al. 2021a; Hariri et al. 2021b; Irsyam et al. 2021; Aprilianingsih et al. 2022). The variation in fragment length primarily reflects differences in the size of the ITS1 and ITS2 spacer regions among species and genera.

According to Cheng et al. (2016), the complete ITS region, including ITS1, 5.8S rDNA, and ITS2, typically ranges from approximately 900 to 1,500 bp in higher plants. The amplification result obtained in the present study falls within this expected range, confirming that the target ITS region was successfully recovered. Previous studies have also demonstrated that ITS1 and ITS2 exhibit moderate length variation among plant lineages due to insertion and deletion events that accumulate during evolutionary divergence (Jobst et al. 1998). Such variation contributes to the discriminatory power of the ITS marker and enhances its utility in resolving taxonomic relationships among closely related species.

The successful amplification of the ITS region provided a reliable molecular marker for subsequent sequence analysis, homology searches using BLAST, and phylogenetic reconstruction. The resulting sequence data were therefore considered suitable for elucidating the taxonomic identity and evolutionary relationships of the wild banana specimen from Bengkulu.

The assembled ITS sequence obtained from the wild banana specimen collected in Bengkulu was subjected to BLAST analysis against the nucleotide database of the National Center for Biotechnology Information (NCBI) to determine its taxonomic identity. The BLAST results revealed that the sequence shared the highest similarity with *M. salaccensis*, particularly with accessions MK779039.1 and FJ428072.1, exhibiting sequence identities of 99.70% and 99.41%, respectively (Table 2). These exceptionally high identity values indicate a very close genetic relationship between the Bengkulu specimen and previously reported *M. salaccensis* accessions, strongly supporting its identification as *M. salaccensis*.

**Table 2.** Top 10 BLAST result of *Musa* sp. based on percent of identity value

| BLAST result | Accession No | % ID | E value | Query Cover (%) |
|---|---|---|---|---|
| *Musa salaccensis* | MK779039.1 | 99.70 | 0 | 90 |
| *Musa salaccensis* | FJ428072.1 | 99.41 | 0 | 91 |
| *Musa lokok* | MK779041.1 | 98.52 | 0 | 90 |
| *Musa gracilis* | MK779040.1 | 98.20 | 0 | 90 |
| *Musa gracilis* | KT257616.1 | 98.06 | 0 | 91 |
| *Musa troglodytarum* | CP097507.1 | 97.82 | 0 | 100 |
| *Musa textilis* | LC610767.1 | 97.68 | 0 | 100 |
| *Musa beccarii* | LC610753.1 | 97.14 | 0 | 100 |
| *Musa troglodytarum* | CP097508.1 | 97.14 | 0 | 100 |
| *Musa troglodytarum* | CP097511.1 | 95.92 | 0 | 100 |

In molecular taxonomy, sequence identity values exceeding 98% are generally considered strong evidence for species-level identification when using the ITS region (Antil et al. 2023). The high degree of similarity observed in this study demonstrates that the ITS sequence of the Bengkulu specimen is highly conserved relative to authenticated *M. salaccensis* sequences deposited in GenBank. Furthermore, the BLAST search returned only representatives of the genus *Musa* as the closest matches, confirming that the obtained sequence belongs to

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the genus *Musa*. The absence of closely matching sequences from other genera within *Musa*ceae further strengthens the reliability of the molecular identification.

To further investigate the evolutionary placement of the specimen, a phylogenetic analysis was conducted using the Maximum Likelihood method based on ITS sequence data. The resulting phylogenetic tree (Figure 3) showed that the Bengkulu specimen clustered within the *M. salaccensis* clade together with reference sequences obtained from GenBank. This grouping was supported by a bootstrap value of 94%, indicating strong statistical confidence in the inferred relationship. Bootstrap values above 70% are generally regarded as evidence of well-supported phylogenetic groupings, while values exceeding 90% indicate a highly reliable clade structure (Simon, 2022).

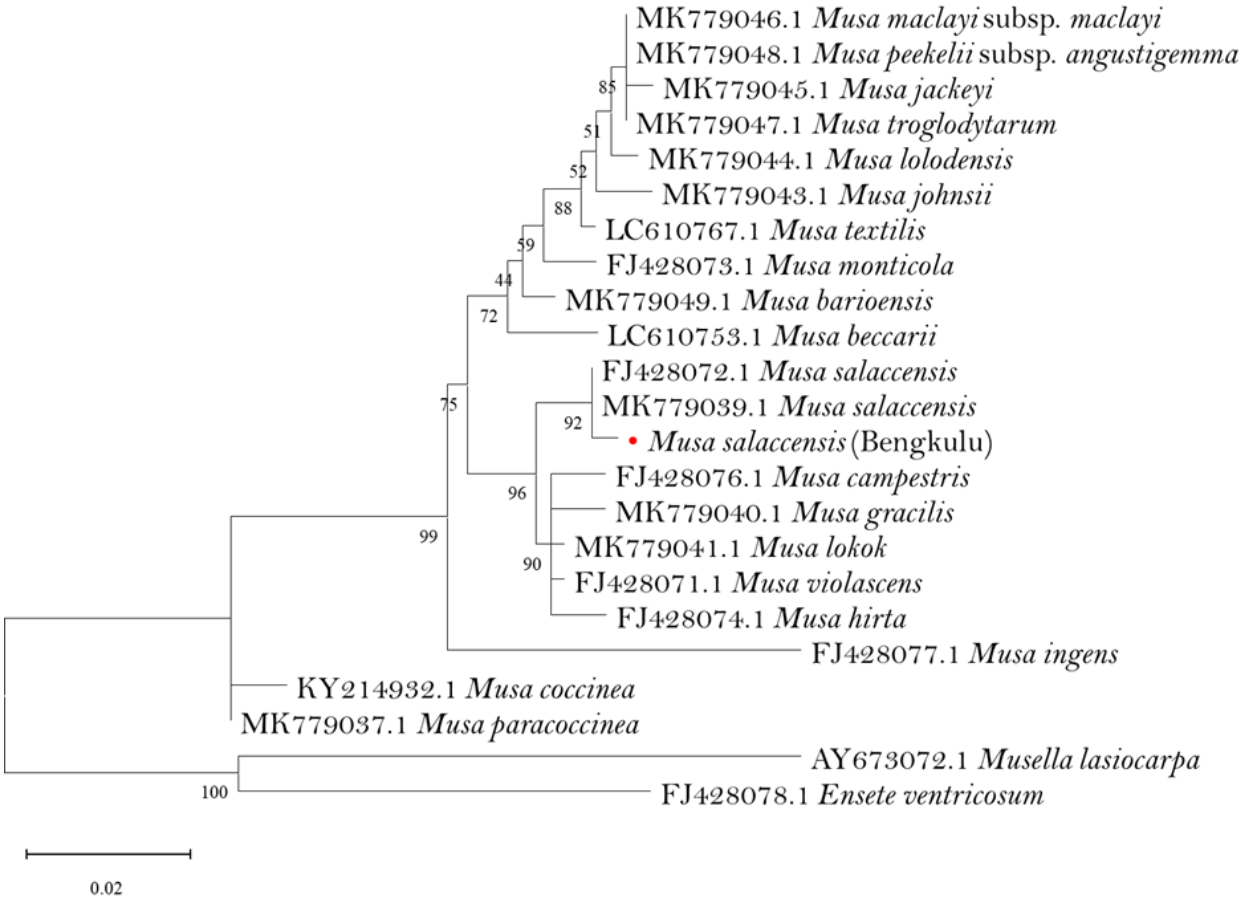



**Figure 3.** Dendrogram generated from a Maximum Likelihood method using Tamura3-parameter evolution model with +5 gamma distribution and 1000 bootstrap replications based on ITS sequence.

The phylogenetic reconstruction also clearly separated the Bengkulu specimen and other *Musa* species from the outgroup taxa, *Ensete ventricosum* and *Musella lasiocarpa*. The distinct placement of these outgroups confirms the effectiveness of the ITS marker in discriminating among genera within the family *Musa*ceae and demonstrates the robustness of the phylogenetic analysis performed in this study.

The clustering pattern observed in the phylogenetic tree reflects the underlying genetic distances among the analyzed taxa. Species with lower genetic distances are expected to share a more recent common ancestor and therefore cluster together within

the same clade. The close association between the Bengkulu specimen and reference sequences of *M. salaccensis* indicates minimal genetic divergence, suggesting that they belong to the same species. According to Clement and Donoghue (2012), species that exhibit low genetic distances generally possess close evolutionary relationships and are often grouped within the same phylogenetic lineage. Consequently, both the BLAST similarity analysis and phylogenetic reconstruction consistently support the conclusion that the wild banana specimen collected from Bengkulu belongs to *Musa salaccensis* Zoll. ex Kurz.

**Taxonomic treatment**

***Musa salaccensis*** Zoll. ex Kurz, J. Agric. Soc. India 14: 301 (1867). — TYPE: Indonesia, West Java, Mt. Salak (lost?); Lectotype: Indonesia, West Java, Bogor, culta, *Kurz s.n.* (lecto CAL! 469332, designated by Veldkamp & Sulistyaningsih (2015)).

Perennial herbs, 5.0—6.0 m tall, with a short underground stem (corm) reaching 8.0—10.0 cm in diam. Roots adventitious. Pseudostem consisting of overlapping leaf-sheaths which are tightly rolled round each other to form a rigid bundle, green to brownish. Leaf-blades broadly lanceolate, apex truncate, base asymmetric, with a strong midrib and well-marked pinnately arranged, parallel veins; petiole relatively long, 88.0—90.2 cm. Inflorescence terminal, erect, ca. 17 cm long. Bract ovate, pointed, purplish white and slightly green apex, 10.0—12.0 × 4.0—4.5 cm. Female flowers develop proximally, 4—5 per bract. Male flowers at the distal end of the inflorescence, 2—4 per bract, uniseriate, compound tepal linear, free tepal lanceolate, transparent. Fruits 2-4 per hand, 2.5—11.0 cm long, slightly curved, cylindrical. Seeds not observed.

**Note**. Field observations showed that the interval between bract emergence and complete fruit maturity was approximately 45 to 60 days. The species reproduces both sexually through mature seeds and vegetatively through rhizomatous suckers. Numerous offspring were found around mature plants, promoting colony development and long-term population persistence.

This species is primarily associated with primary and secondary forests at elevations of 900 to 1,300 m above sea level. It is widely distributed across Sumatra and Java, where it commonly occurs in colonies of varying sizes. Smaller colonies typically consisted of two to three individuals, while larger colonies contained up to 27 to 30 individuals. Colony clumps generally occupied areas about 4 to 5 m in diameter and were accompanied by abundant juvenile suckers, indicating ongoing regeneration and recruitment within the population.

## CONCLUSION

Morphological observations and molecular analyses based on the ITS region successfully revealed the identity of the wild banana specimen collected from Rejang Lebong, Bengkulu. The specimen exhibited morphological characteristics consistent with those previously reported for *M. salaccensis*. Phylogenetic reconstruction using the Maximum Likelihood method placed the Bengkulu specimen within the *M. salaccensis* clade with strong bootstrap support (94%). The congruence between morphological and molecular evidence confirms that the wild banana specimen from Bengkulu is *M. salaccensis*.

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involved in field sampling, laboratory analyses, and data collection that contributed to the successful completion of this study.

## DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES

While preparing this manuscript, the author(s) used ChatGPT-5 to improve the language, enhance the conciseness of the text, and ensure grammatical accuracy. The author (s) critically reviewed, verified, and revised the resulting output and take full responsibility for the accuracy, integrity, and content of the published article.

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