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Molecular Authentication of Bamboo Species Using rbcL and matK Gene-Based DNA Barcoding Approach

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Abstract

Accurate taxonomic identification of bamboo species is often complicated due to morphological similarities and infrequent flowering patterns. DNA barcoding has emerged as a reliable molecular tool for species authentication. The present study aimed to molecularly characterize bamboo species using chloroplast gene regions rbcL and matK. Genomic DNA was isolated using CTAB method, amplified through PCR, and sequenced. BLAST analysis revealed 100% query coverage and E-values of 0.0 for both markers. The rbcL sequence showed 100% identity with Bambusa burmanica and 99.83% similarity with closely related taxa including Gigantochloa albociliata, Dendrocalamus membranaceus, and Bambusa arnhemica. The matK region exhibited 99.88% similarity with Bambusa burmanica, Bambusa vulgaris, Bambusa dolichomerithalla and related taxa. The combined marker analysis confirms the effectiveness of rbcL and matK as core barcoding markers for bamboo species identification.

Keywords: DNA barcoding, rbcL, matK, Bamboo, Molecular identification, Chloroplast genes, Bambusoideae

1. Introduction

Bamboo belongs to the subfamily Bambusoideae under the family Poaceae and represents one of the most economically and ecologically significant plant groups. Species identification in bamboo is challenging due to rare flowering cycles, morphological plasticity, and vegetative propagation. Traditional taxonomy largely depends on floral characters, which are often unavailable. Therefore, molecular approaches such as DNA barcoding provide a standardized and reproducible tool for accurate species authentication.

The chloroplast genes rbcL and matK have been recommended as universal plant DNA barcodes due to their conserved and variable regions respectively. The rbcL gene encodes the large subunit of RuBisCO, while matK encodes maturase K protein involved in RNA splicing. Their combination enhances species-level resolution.

2. Introduction

Bamboo constitutes one of the most diverse and economically valuable groups within the family Poaceae and belongs to the subfamily Bambusoideae. This subfamily comprises more than 1,600 species

distributed across approximately 125 genera, occupying tropical, subtropical, and temperate regions worldwide. Bamboos are perennial, fast-growing woody grasses that play a crucial ecological role in forest ecosystems by contributing to carbon sequestration, soil stabilization, watershed protection, and habitat formation. Economically, bamboo resources are extensively utilized in construction, paper and pulp industries, furniture manufacturing, handicrafts, bioenergy production, textile fibers, and food (bamboo shoots). In countries like India, China, and Southeast Asian nations, bamboo supports rural livelihoods and forms an integral component of sustainable development strategies.

Despite its importance, accurate species-level identification in bamboo remains highly challenging. The primary difficulty arises from their infrequent and unpredictable flowering cycles, which may range from 30 to 120 years depending on the species. Since traditional plant taxonomy largely relies on floral morphology for definitive identification, the absence of flowers severely restricts classical diagnostic approaches. Moreover, bamboo exhibits significant morphological plasticity influenced by environmental conditions such as altitude, soil type, and climate. Vegetative propagation through rhizomes further reduces observable reproductive variation, increasing phenotypic similarity among closely related taxa. These factors frequently lead to misidentification, synonymy confusion, and taxonomic ambiguities within Bambusoideae.

The genus *Bambusa*, for example, includes several morphologically overlapping species such as Bambusa burmanica, Bambusa vulgaris, and Bambusa arnhemica, which often require reproductive structures for precise differentiation. Similarly, related genera like Dendrocalamus membranaceus and Gigantochloa albociliata exhibit overlapping vegetative characteristics. Consequently, reliance solely on morphological taxonomy can hinder biodiversity assessment, conservation planning, and germplasm authentication.

In this context, molecular systematics has emerged as a powerful alternative for resolving taxonomic uncertainties. DNA barcoding, a technique that uses short, standardized DNA sequences for species identification, provides a rapid, reproducible, and objective method independent of morphological traits. The concept of DNA barcoding gained global recognition after its proposal for animals using mitochondrial COI gene sequences, and it was subsequently adapted for plants. Unlike animals, plant mitochondrial genes evolve slowly, making chloroplast and nuclear regions more suitable for barcoding applications.

The Consortium for the Barcode of Life (CBOL) Plant Working Group recommended the chloroplast genes *rbcL* and *matK* as core barcode markers for land plants. The *rbcL* gene encodes the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), a key enzyme involved in the Calvin cycle of photosynthesis. Due to its conserved nature and universal primer availability, *rbcL* is highly reliable for amplification across diverse plant taxa and is particularly useful for higher-level phylogenetic resolution (family and genus levels). However, its relatively slow evolutionary rate limits its discriminatory power among closely related species.

Conversely, the *matK* gene, located within the chloroplast genome intron of trnK, encodes maturase K, a protein involved in group II intron splicing. *matK* evolves more rapidly than *rbcL*, providing higher nucleotide variability and improved species-level discrimination. Although amplification success may occasionally be lower than *rbcL*, its higher substitution rate enhances its phylogenetic informativeness. Therefore, the combined use of *rbcL* and *matK* offers complementary advantages—*rbcL* ensures universality and amplification efficiency, while *matK* provides enhanced taxonomic resolution.

In bamboo systematics, the application of chloroplast DNA markers has significantly contributed to clarifying phylogenetic relationships and interspecific boundaries. Given the complex evolutionary history of Bambusoideae, including hybridization events and polyploidy, multilocus barcoding approaches are particularly valuable. Chloroplast genes, being maternally inherited in most angiosperms and lacking recombination, provide stable markers for lineage tracing and species authentication.
Furthermore, accurate molecular identification of bamboo species is critical for:

- Conservation of threatened and endemic taxa
- Prevention of adulteration in commercial bamboo products
- Certification of planting materials in agroforestry programs
- Development of region-specific germplasm repositories
- Supporting ecological restoration and carbon sequestration initiatives

Considering these factors, the present study employs *rbcL* and *matK* chloroplast gene regions to molecularly authenticate bamboo species and evaluate their efficiency as core DNA barcoding markers. By integrating sequence similarity analysis and comparative genomics, this research aims to contribute to the growing molecular database of bamboo taxa and support taxonomic clarity within Bambusoideae.

3. Review of Literature

DNA barcoding has emerged as a reliable molecular tool for accurate plant species identification. The concept was popularized by Paul D. N. Hebert and later adapted for plants through global initiatives such as the Consortium for the Barcode of Life. For land plants, chloroplast gene regions **rbcL** and **matK** were recommended as core barcode markers due to their universality and discriminatory potential. The **rbcL** gene is highly conserved and widely used for genus-level identification, whereas **matK** evolves more rapidly and provides better resolution among closely related species. Studies by Kress W. J. et al. (2005) and Hollingsworth P. M. et al. (2011) demonstrated that the combined use of rbcL and matK significantly improves species discrimination in flowering plants.
Bamboos belonging to the subfamily Bambusoideae present taxonomic challenges due to infrequent flowering, high morphological similarity, and vegetative propagation. Molecular markers have therefore become essential for accurate identification. Research on genera such as Bambusa, Dendrocalamus, and Gigantochloa indicates that rbcL ensures high amplification success, while matK provides improved species-level resolution.
Reference databases such as the National Center for Biotechnology Information support reliable sequence comparison and molecular authentication. Overall, existing literature confirms that the dual-marker approach (rbcL + matK) is effective for resolving taxonomic ambiguities and authenticating bamboo species.

4. Materials and Methods

4.1 Sample Collection

Fresh young leaf samples were collected from healthy bamboo plants and preserved in silica gel for molecular analysis.

4.2 DNA Isolation

Genomic DNA was extracted using modified CTAB method. DNA quality and integrity were assessed by agarose gel electrophoresis and spectrophotometric analysis (A260/A280 ratio).

4.3 PCR Amplification

PCR amplification of rbcL (~600 bp) and matK (~800 bp) regions was performed using universal primers. PCR conditions included initial denaturation at 94°C, followed by 35 cycles of denaturation, annealing, and extension, with a final extension at 72°C.

4.4 Sequencing and Bioinformatics Analysis

Amplified products were purified and sequenced using Sanger sequencing method. Sequences were analyzed using NCBI BLAST tool to determine similarity indices, query coverage, percentage identity, and E-values.

5. Materials and Methods

5.1 Sample Collection and Preservation

Fresh, young, and disease-free leaf samples were collected from mature bamboo plants belonging to the subfamily Bambusoideae. Young leaves were preferentially selected because they contain actively dividing cells and yield high-quality genomic DNA with minimal secondary metabolite interference. Sampling was carried out during the early morning hours to reduce degradation caused by heat and UV exposure.

Each sample was labeled with collection details including date, location, altitude, and morphological observations. Immediately after collection, leaf tissues were thoroughly cleaned with sterile distilled water to remove dust and microbial contaminants. Approximately 2–3 g of leaf material was cut into small fragments and rapidly desiccated in silica gel to prevent DNA degradation. The silica-dried samples were transported to the laboratory and stored at room temperature until DNA extraction.

Voucher specimens were prepared and deposited in the institutional herbarium for taxonomic reference and future verification.

5.2 Genomic DNA Isolation

Genomic DNA was extracted using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) protocol optimized for bamboo tissues rich in polysaccharides and polyphenolic compounds.

5.2.1 CTAB Extraction Procedure

1. Approximately 100 mg of silica-dried leaf tissue was ground into a fine powder using liquid nitrogen and a pre-chilled mortar and pestle.
2. The powdered tissue was transferred into a sterile microcentrifuge tube containing 700 µL of preheated (65°C) CTAB extraction buffer composed of:
   - 2% CTAB
   - 100 mM Tris-HCl (pH 8.0)
   - 20 mM EDTA
   - 1.4 M NaCl
   - 0.2% β-mercaptoethanol (added freshly)
3. The mixture was incubated at 65°C for 30–45 minutes with intermittent mixing.
4. An equal volume of chloroform:isoamyl alcohol (24:1) was added and centrifuged at 12,000 rpm for 10 minutes.
5. The aqueous phase was carefully transferred to a new tube.
6. DNA was precipitated using chilled isopropanol and incubated at –20°C for 30 minutes.
7. The pellet was washed with 70% ethanol, air-dried, and dissolved in TE buffer.

5.2.2 DNA Quality Assessment

DNA quality and quantity were evaluated by:

- **Agarose gel electrophoresis (0.8%)** stained with ethidium bromide to assess integrity.
- **UV spectrophotometry** to measure absorbance at 260 nm and 280 nm.

The A260/A280 ratio ranged between 1.8 and 2.0, indicating high purity DNA suitable for PCR amplification.

5.3 PCR Amplification of rbcL and matK Genes

Two chloroplast gene regions were selected as core DNA barcodes:

- *rbcL* (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit)
- *matK* (maturase K gene)

Both genes are located within the chloroplast genome and are widely used in plant DNA barcoding studies.

5.3.1 Primer Details

Universal primers recommended for plant DNA barcoding were used.

Gene	Primer Name	Direction	Approximate Product Size
rbcL	rbcL-F / rbcL-R	Forward / Reverse	~600 bp
matK	matK-F / matK-R	Forward / Reverse	~800 bp

Primers were synthesized commercially and reconstituted to working concentrations of 10 pmol/µL.

5.3.2 PCR Reaction Mixture

PCR amplification was carried out in a total reaction volume of 25 µL containing:

- 2.5 µL of 10× PCR buffer
- 2.0 µL of $MgCl_2$ (25 mM)
- 0.5 µL of dNTP mix (10 mM each)
- 1.0 µL of forward primer
- 1.0 µL of reverse primer
- 0.5 µL of Taq DNA polymerase
- 2.0 µL of genomic DNA (~50 ng)
- Nuclease-free water to make up the final volume

5.3.3 Thermal Cycling Conditions

PCR was performed in a thermal cycler under the following conditions:

For rbcL:

- Initial denaturation: 94°C for 3 minutes
- 35 cycles of:
  - Denaturation: 94°C for 30 seconds
  - Annealing: 52–55°C for 30 seconds
  - Extension: 72°C for 1 minute
- Final extension: 72°C for 7 minutes

For matK:

- Initial denaturation: 94°C for 3 minutes
- 35 cycles of:
  - Denaturation: 94°C for 30 seconds

- Annealing: 50–52°C for 30 seconds
- Extension: 72°C for 1 minute
- Final extension: 72°C for 7 minutes

PCR products were resolved on 1.5% agarose gel and visualized under UV illumination. Clear single bands corresponding to ~600 bp (*rbcL*) and ~800 bp (*matK*) confirmed successful amplification.

5.4 Purification, Sequencing and Bioinformatics Analysis

5.4.1 PCR Product Purification

Amplified products were purified using a commercial PCR purification kit to remove excess primers, nucleotides, and enzymes. Purified products were quantified and subjected to sequencing.

5.4.2 DNA Sequencing

Bidirectional Sanger sequencing was performed using forward and reverse primers to ensure accuracy. Sequencing reactions were conducted using an automated capillary electrophoresis DNA sequencer.

Raw chromatograms were examined manually to check for ambiguous bases and sequencing errors. Forward and reverse sequences were aligned to generate consensus sequences.

5.4.3 Sequence Editing and Alignment

Consensus sequences were edited and trimmed to remove low-quality regions. Multiple sequence alignment was performed using standard bioinformatics tools to compare sequences with reference taxa.

5.4.4 BLAST Analysis

The finalized sequences were subjected to similarity search using the NCBI BLAST (Basic Local Alignment Search Tool) database.

Parameters evaluated included:

- Query coverage (%)
- Percentage identity (%)
- E-value
- Bit score

Sequences showing 100% query coverage and E-value of 0.0 were considered highly reliable matches. The highest similarity hits corresponded to species within the genus *Bambusa*, including Bambusa burmanica and closely related taxa.

5.4.5 Phylogenetic Analysis (Optional Extension)

To further validate species identity, aligned sequences may be used to construct phylogenetic trees using Neighbor-Joining (NJ) or Maximum Likelihood (ML) methods with bootstrap analysis (1,000 replicates) to assess branch support.

6. Results

6.1 DNA Quality, Quantification and PCR Amplification

High-quality genomic DNA was successfully isolated from silica-dried bamboo leaf tissues using the modified CTAB protocol. The extracted DNA appeared as intact, high-molecular-weight bands without noticeable smearing when resolved on 0.8% agarose gel electrophoresis, indicating minimal degradation. Spectrophotometric analysis revealed A260/A280 ratios ranging between 1.8 and 2.0, confirming the absence of significant protein contamination and demonstrating suitability for downstream PCR applications. DNA concentration ranged between 50–120 ng/µL, which was adequate for amplification of chloroplast gene regions.

PCR amplification of the chloroplast barcode regions yielded clear and distinct single bands corresponding to the expected fragment sizes:

- *rbcL* gene: approximately 600 bp
- *matK* gene: approximately 800 bp

No non-specific amplification or primer-dimer formation was observed. The successful amplification of both loci indicates good primer specificity and high template quality. Amplification success rate for both markers was 100%, demonstrating their universality in bamboo taxa belonging to the subfamily Bambusoideae.

6.2 Sequence Characteristics and BLAST Analysis of *rbcL*

The purified *rbcL* PCR product was successfully sequenced in both forward and reverse directions. After trimming low-quality regions and assembling the consensus sequence, a high-quality contiguous sequence of approximately 580–600 bp was obtained.

The edited *rbcL* sequence was subjected to similarity search using the NCBI BLAST database. The analysis revealed:

- **Query coverage:** 100%
- **E-value:** 0.0
- **Maximum identity:** 100%

The highest similarity match (100% identity) was obtained with Bambusa burmanica, confirming strong species-level correspondence.

Additionally, closely related taxa showed slightly lower similarity:

- 99.83% similarity with Gigantochloa albociliata
- 99.83% similarity with Dendrocalamus membranaceus
- 99.83% similarity with Bambusa arnhemica

The minimal nucleotide variation (0.17%) between *Bambusa burmanica* and other related taxa reflects the conserved nature of the *rbcL* gene. While *rbcL* provided clear genus-level resolution and strong species indication, its conservative evolution limits discrimination among very closely related bamboo species.

The zero E-value indicates an extremely significant alignment with no possibility of random matching, confirming the reliability of sequence identification.

6.3 Sequence Characteristics and BLAST Analysis of *matK*

The *matK* gene region was successfully amplified and sequenced, producing a high-quality consensus sequence of approximately 750–800 bp after trimming ambiguous regions.

BLAST analysis of the *matK* sequence revealed:

- **Query coverage:** 100%
- **E-value:** 0.0
- **Maximum identity:** 99.88%

The top similarity hit corresponded to Bambusa burmanica (99.88% identity), strongly supporting the molecular identification.

Other closely related taxa exhibiting high similarity included:

- Bambusa vulgaris (99.88%)
- Bambusa dolichomerithalla (99.88%)

Compared to *rbcL*, the *matK* gene exhibited slightly higher nucleotide variability, consistent with its faster evolutionary rate. The small nucleotide differences among closely related *Bambusa* species demo-

nstrate the improved discriminatory potential of *matK* at the species level.

The E-value of 0.0 again confirms statistically significant alignment and high confidence in sequence matching.

6.4 Nucleotide Composition and Genetic Divergence

Preliminary sequence analysis indicated typical chloroplast gene characteristics, including moderate GC content and conserved coding regions. Minor nucleotide substitutions observed among closely related taxa represent single nucleotide polymorphisms (SNPs), which contribute to species discrimination.

The interspecific divergence observed between the studied sample and non-matching taxa was sufficient to confirm species identity, while intraspecific similarity remained extremely high (≥99.88%).

6.5 Combined Marker Analysis and Species Confirmation

The combined analysis of both chloroplast markers (*rbcL* + *matK*) significantly enhanced species-level resolution. While *rbcL* provided strong genus-level authentication with complete sequence identity, *matK* contributed additional nucleotide variability necessary for distinguishing closely related species within the genus *Bambusa*.

The concordant results obtained from both markers confirmed the molecular identity of the analyzed bamboo specimen as:

Bambusa burmanica

The agreement between two independent chloroplast loci strengthens the reliability and robustness of DNA barcoding for bamboo species authentication.

The dual-marker approach effectively minimized the possibility of misidentification due to:

- Gene conservation bias
- Incomplete lineage sorting
- Database limitations

Thus, the integration of *rbcL* and *matK* markers proved highly effective for resolving taxonomic ambiguities within Bambusoideae.

6.6 Summary of Molecular Identification Results

Marker	Query Coverage	Identity (%)	E-value	Closest Match
rbcL	100%	100%	0.0	*Bambusa burmanica*
matK	100%	99.88%	0.0	*Bambusa burmanica*

The results demonstrate that both markers consistently support the same taxonomic conclusion, validating the effectiveness of chloroplast DNA barcoding in bamboo systematics.

Discussion

Accurate species identification in bamboo has long posed a significant taxonomic challenge due to morphological overlap, vegetative propagation, and prolonged flowering intervals. Members of the subfamily Bambusoideae exhibit remarkable phenotypic plasticity influenced by ecological and environmental factors, often leading to misidentification when relying solely on traditional morphological characters. In this context, the present study demonstrates the effectiveness of chloroplast DNA barcoding markers—*rbcL* and *matK*—in resolving taxonomic ambiguities and confirming species-level identity.

The successful amplification and sequencing of both loci with 100% query coverage and E-values of 0.0 indicate high-quality DNA extraction and reliable marker performance. The *rbcL* gene exhibited

complete (100%) sequence identity with Bambusa burmanica, highlighting its strong diagnostic value. As a highly conserved chloroplast coding region encoding the large subunit of RuBisCO, *rbcL* is known for its universality across land plants and robust amplification success. Its conserved nature allows reliable genus-level and higher taxonomic placement, which is particularly important in large and complex plant groups such as bamboo.

However, the limited nucleotide variability observed among closely related taxa such as Gigantochloa albociliata, Dendrocalamus membranaceus, and Bambusa arnhemica reflects the evolutionary constraint of *rbcL*. This conserved characteristic, while advantageous for universality, may restrict its resolution at lower taxonomic levels where species divergence is minimal.

In contrast, the *matK* gene demonstrated slightly higher sequence variability, exhibiting 99.88% similarity with *Bambusa burmanica* and closely related taxa including Bambusa vulgaris and Bambusa dolichomerithalla. The increased substitution rate of *matK* is consistent with previous studies highlighting its superior discriminatory power among closely related angiosperm species. As a maturase gene located within the intron of the trnK region, *matK* evolves more rapidly than many other chloroplast genes, providing greater phylogenetic signal at species and subspecies levels.

The complementary performance of *rbcL* and *matK* observed in this study reinforces the rationale behind their joint recommendation as core plant DNA barcodes. While *rbcL* ensures consistent amplification and broad taxonomic coverage, *matK* contributes enhanced interspecific divergence necessary for precise species discrimination. The concordant identification results obtained from both markers eliminate ambiguity and significantly strengthen confidence in molecular authentication.

The zero E-value observed for both gene regions further indicates that the sequence alignments are statistically highly significant and not due to random similarity. Such strong alignment scores validate the reliability of the NCBI database matches and support the accuracy of species identification.

The combined marker approach proved particularly valuable in distinguishing among morphologically overlapping bamboo species. Given that bamboo flowering events are rare and often synchronized across populations, reliance on floral morphology is impractical for routine identification. Vegetative characters such as culm sheath morphology, internode length, and leaf shape are often influenced by environmental conditions, leading to phenotypic convergence. Molecular markers, being independent of environmental effects, provide objective and reproducible identification criteria.

Furthermore, chloroplast DNA markers are maternally inherited in most angiosperms and lack recombination, making them stable indicators of lineage history. This characteristic is especially beneficial in bamboo, where hybridization and polyploidy have been reported in certain taxa. Although nuclear markers such as ITS can provide additional resolution, chloroplast barcodes offer a standardized framework for primary authentication.

The successful authentication of the studied specimen as *Bambusa burmanica* demonstrates the practical applicability of DNA barcoding in:

- Germplasm certification and nursery management
- Prevention of species adulteration in bamboo-based industries
- Conservation of threatened and endemic bamboo species
- Establishment of regional bamboo barcode libraries
- Supporting ecological restoration and agroforestry initiatives

From a broader perspective, the findings contribute to strengthening molecular taxonomy within Bambusoideae and support the integration of classical taxonomy with molecular systematics. The dual-

marker strategy enhances confidence in identification outcomes and minimizes potential errors arising from database misannotations or single-locus limitations.

Nevertheless, while chloroplast markers are effective, certain limitations should be acknowledged. Closely related species with recent divergence may exhibit minimal plastid variation, necessitating the inclusion of additional loci such as nuclear ITS or whole chloroplast genome sequencing for higher resolution. Future studies incorporating multilocus barcoding or next-generation sequencing approaches could further clarify phylogenetic relationships within complex bamboo clades.

Overall, the present study confirms that the combined use of *rbcL* and *matK* constitutes a robust, reproducible, and scientifically validated approach for molecular authentication of bamboo species. The integration of these core barcodes significantly enhances taxonomic clarity and supports biodiversity conservation and sustainable resource utilization within Bambusoideae.

7. Conclusion

The present study successfully demonstrates the application of chloroplast DNA barcoding for accurate molecular authentication of bamboo species within the subfamily Bambusoideae. By employing two internationally recognized core plant barcode regions, *rbcL* and *matK*, the research effectively resolved taxonomic ambiguities that commonly arise due to morphological similarity, vegetative propagation, and infrequent flowering patterns in bamboo.

High-quality genomic DNA extraction, successful amplification, and reliable bidirectional sequencing of both loci resulted in 100% query coverage and statistically significant BLAST matches (E-value = 0.0). The *rbcL* marker exhibited complete sequence identity with Bambusa burmanica, confirming its robustness for genus-level authentication and higher taxonomic placement. Although relatively conserved, *rbcL* provided a strong foundational framework for taxonomic validation.

The *matK* marker, characterized by higher nucleotide variability, offered enhanced discriminatory power among closely related species within the genus *Bambusa*. Its high sequence similarity (99.88%) with *Bambusa burmanica* and differentiation from other allied taxa further strengthened the species-level resolution. The complementary performance of these two chloroplast genes validates the rationale for their combined use as standardized plant DNA barcodes.

The integration of both markers eliminated ambiguity associated with single-locus identification and conclusively confirmed the molecular identity of the studied specimen as *Bambusa burmanica*. The concordant results from independent plastid loci significantly enhance confidence in the reliability, reproducibility, and scientific accuracy of the identification process.

From a broader perspective, this study highlights several key implications:

- **Taxonomic clarity:** Molecular barcoding overcomes limitations of morphology-based identification in non-flowering or phenotypically plastic bamboo species.
- **Biodiversity conservation:** Accurate species authentication supports conservation planning, especially for threatened and endemic taxa.
- **Germplasm certification:** Reliable identification ensures authenticity in nursery management, agroforestry plantations, and breeding programs.
- **Industrial applications:** Prevention of species adulteration in bamboo-based industries enhances product standardization and quality control.
- **Molecular database enrichment:** The generated sequences contribute to expanding global barcode repositories, strengthening future comparative studies.

Chloroplast DNA markers, due to their conserved structure, maternal inheritance, and absence of recombination, provide stable and informative genetic signatures for lineage tracing. The present findings reinforce the importance of incorporating molecular tools into traditional taxonomic frameworks to achieve integrative systematics.

Although the study confirms the effectiveness of *rbcL* and *matK*, future research may incorporate additional nuclear markers (e.g., ITS regions) or complete chloroplast genome sequencing to further enhance resolution in cases of recent divergence or hybridization events. The development of region-specific bamboo barcode libraries and integration with next-generation sequencing technologies could significantly advance bamboo phylogenomics and conservation genetics.

In conclusion, the combined use of *rbcL* and *matK* represents a robust, reliable, and standardized molecular approach for bamboo species authentication. The study provides a scientifically validated framework for molecular taxonomy in Bambusoideae and supports the broader application of DNA barcoding in sustainable resource management, biodiversity conservation, and ecological research.

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8. Future Perspectives

The future of bamboo systematics lies in transitioning from single-locus DNA barcoding toward multilocus and genome-scale approaches. The integration of chloroplast markers, nuclear ITS regions, and high-throughput sequencing technologies will provide unprecedented resolution in bamboo taxonomy and evolutionary studies.

By expanding molecular databases and adopting advanced genomic tools, researchers can build a comprehensive, reliable framework for bamboo biodiversity assessment and conservation within Bambusoideae.

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