BIODIVERSITAS
Volume 27, Number 3, March 2026
Pages: 12

ISSN: 1412-033X
E-ISSN: 2085-4722
https://doi.org/10.13057/biodiv/d270332

ITS DNA barcoding for species authentication of Thai arrowroot (*Maranta arundinacea*) and related Marantaceae

APHIDECH SANGDEE[1], THANWANIT THANYASIRIWAT[2], JUTHAPORN SAENGPRAJAK[1,♥]
[1]Department of Biology, Faculty of Science, Mahasarakham University. Kham Riang, Kantharawichai 44150, Maha Sarakham, Thailand.
Tel.: +66-437-54247, [♥]email: juthaporn.s@msu.ac.th
[2]Genome and Disease Research Unit, Department of Agriculture and Resources, Faculty of Natural Resources and Agro-Industry, Kasetsart University.
Vor Por Ror Or 366 Rd, Chiang Khruea, Mueang Sakon Nakhon, Sakon Nakhon 47000, Thailand

Manuscript received: 15 December 2025. Revision accepted: 29 March 2026.

Abstract. *Sangdee A, Thanyasiriwat T, Saengprajak J. 2026. ITS DNA barcoding for species authentication of Thai arrowroot* (Maranta arundinacea) *and related Marantaceae. Biodiversitas 27 (3): d270332. https://doi.org/10.13057/biodiv/d270332. Maranta arundinacea* (arrowroot) is an underutilized starch-producing crop whose taxonomic identification is often constrained by overlapping vegetative characters, pronounced phenotypic plasticity, and the frequent absence of diagnostic reproductive structures during dormancy. These factors complicate species identification and germplasm authentication within *Maranta* and related Marantaceae taxa. This study evaluated the effectiveness of nuclear ribosomal Internal Transcribed Spacer (ITS) DNA barcoding for identifying arrowroot and distinguishing closely related taxa in Thailand. ITS sequences were obtained from *Maranta* samples collected across several geographic regions, with *Goeppertia ornata* included for comparison. Phylogenetic relationships were inferred using maximum likelihood analysis. All *M. arundinacea* accessions showed high ITS sequence similarity and clustered into a single well-supported lineage, indicating genetic uniformity consistent with long-term vegetative propagation. In contrast, ITS variation enabled clear discrimination among other *Maranta* species that are difficult to distinguish morphologically. Phylogenetic analysis resolved distinct lineages within *Maranta* and recovered *G. ornata* as a separate lineage, supporting its classification as a distinct genus. These results demonstrate that ITS DNA barcoding is an effective tool for species authentication, phylogenetic analysis, and germplasm verification, and provide a molecular basis for future conservation and sustainable utilization of arrowroot resources in Thailand.

Keywords: DNA barcoding, genetic diversity, Marantaceae, species authentication, Thailand

INTRODUCTION

Maranta arundinacea L. (arrowroot or Indian arrowroot) is a perennial rhizomatous herb in the family Marantaceae (order Zingiberales) traditionally cultivated for its starch-rich underground organs (de Oliveira Guilherme et al. 2019; Brito et al. 2021). Although widely distributed in tropical regions, arrowroot remains botanically understudied and is often confused with morphologically similar taxa within Marantaceae, reflecting unresolved taxonomic boundaries and limited phylogenetic resolution (Luna et al. 2025a, 2025b). Under cultivation, the species is characterized by erect aerial shoots and fleshy fibrous rhizomes (Brito et al. 2021). However, reproductive structures are rarely produced, reducing the availability of floral characters important for classical taxonomy (Kress 1990; Da Costa Fernandes et al. 2023). Arrowroot starch is naturally gluten-free, easily digestible, and has a low glycemic index, supporting its use in specialized diets such as infant and geriatric nutrition (Malki et al. 2023). Despite its traditional value, its broader nutraceutical, industrial, and breeding potential remains insufficiently explored, although previous studies indicate genetic variability and crop improvement potential (Shintu et al. 2016; Malki et al. 2023).

Identification of *M. arundinacea* is difficult because vegetative traits often overlap among closely related species, while stem and rhizome architecture may appear similar across taxa (Brito et al. 2021; Malki et al. 2023). This problem is intensified by phenotypic plasticity driven by environmental conditions. Seasonal dormancy further complicates identification because aerial parts senesce and diagnostic foliar characters, such as venation patterns and pulvini, are lost (de Oliveira Guilherme et al. 2019). Infrequent flowering and limited seed production also restrict the use of reproductive characters traditionally employed for species delimitation (Kress et al. 2002; Da Costa Fernandes et al. 2023). These constraints reduce the reliability of morphology-based classification in Marantaceae and emphasize the need for complementary molecular approaches (Hollingsworth et al. 2011; Letsiou et al. 2024).

DNA barcoding is a method for plant species authentication by using species-specific nucleotide variation that is largely independent of environmental conditions, developmental stage, and physiological status. In plants, barcoding commonly uses nuclear and plastid loci, including *matK*, *rbcL*, and intergenic spacers (Hebert et al. 2003; Hollingsworth et al. 2011; Letsiou et al. 2024). Among available markers, the nuclear ribosomal Internal Transcribed Spacer (ITS) region has emerged as one of the most effective single-locus markers for species-level identification in angiosperms (Baldwin et al. 1995; Álvarez and Wendel 2003; Li et al. 2011). Its high copy number, short amplicon length, universal primer availability, and sequence variability enable reliable amplification and

discrimination among closely related taxa. Although multilocus barcoding is often recommended, ITS is particularly suitable for rapid and cost-effective species authentication because of its strong discriminatory power (Hollingsworth et al. 2011; Li et al. 2011; Le et al. 2025).

Recent phylogenetic studies have shown that ITS can clarify relationships within the *Maranta* clade and improve species delimitation where morphology alone is insufficient (Luna et al. 2025b). However, molecular authentication of underutilized starch crops remains uneven, especially in Southeast Asia, where genomic resources and reference data are still limited (Le et al. 2025). In Thailand, arrowroot is commonly cultivated in smallholder systems where local landraces are exchanged informally, increasing the risk of varietal misidentification and genetic erosion (Ieamkheng et al. 2024). Reliable molecular identification is therefore important for taxonomic accuracy, germplasm conservation, breeding programs, and the development of traceable value chains for functional starch products (Galimberti et al. 2013; Fanelli et al. 2021).

However, a clear ITS-based authentication framework for *M. arundinacea* in Thailand is still lacking, and ITS reference sequences remain limited for several *Maranta* species, constraining reliable phylogenetic placement of Thai accessions (Urumarudappa et al. 2022; Luna et al. 2025b). In addition, ITS representation in public databases such as GenBank is often uneven, which may reduce confidence in database-based identification and comparative analyses (Nilsson et al. 2006; Raja et al. 2017). To address these gaps, this study integrates morphological assessment with ITS sequence and phylogenetic analyses of arrowroot accessions collected across Thailand. It aims to establish an ITS-based barcoding framework for Thai arrowroot germplasm and related taxa, contribute new ITS reference sequences, and evaluate concordance between molecular and morphological evidence for species authentication. Specifically, the study tests whether Thai *M. arundinacea* accessions share a common ITS haplotype, whether ITS polymorphisms discriminate closely related *Maranta* species, and whether *Goeppertia ornata* forms a distinct lineage.

MATERIALS AND METHODS

Sampling site

Seven accessions of *Maranta*, representing four species, and one accession of *Goeppertia ornata* were collected between February and August 2023 from both wild and cultivated populations in Mahasarakham, Nakhon Ratchasima, Phitsanulok, Ubon Ratchathani, and Loei Provinces, Thailand (Figure 1). Sampling sites were selected based on the documented distribution of *Maranta* species in Thailand and available floristic records to represent both cultivated and natural populations (Hollingsworth et al. 2011; Da Costa Fernandes et al. 2023). Ethnobotanical information and previous botanical records were consulted to identify suitable sampling locations (Campanaro et al. 2019; Fanelli et al. 2021).

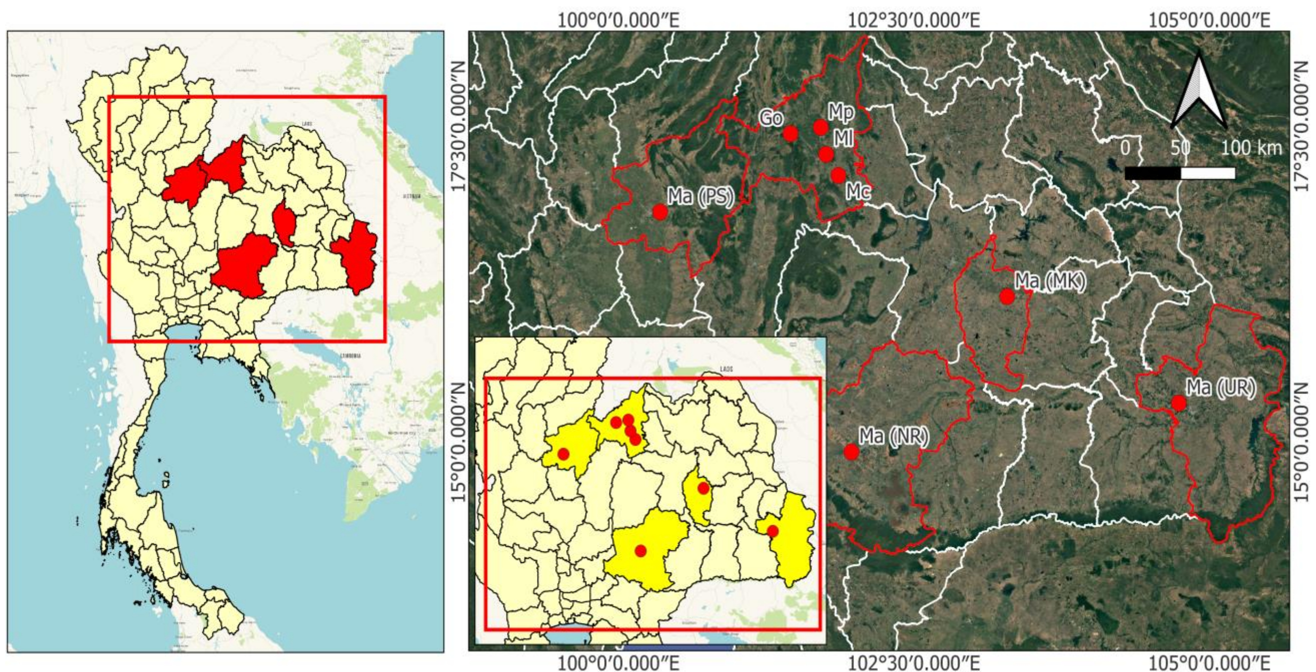

Figure 1. Geographic locations of sample collection sites in Thailand. Sampling sites include Mahasarakham, Nakhon Ratchasima, Phitsanulok, Ubon Ratchathani, and Loei Provinces, where *Maranta arundinacea*, *Maranta cristata*, *Maranta leuconeura*, *Maranta pohliana*, and *Goeppertia ornata* were collected. Map symbols are labeled with sample IDs that correspond to those listed in Table 1 and are defined in the figure legend

Sample collection

For each accession, geographic location, Global Positioning System (GPS) coordinates, and collection locality were recorded, and key vegetative morphological characteristics, including leaf shape, variegation, and rhizome form, were documented (Table 1, Figure 2). Whole rhizomes were collected and transplanted into soil under greenhouse conditions at the Department of Biology, Faculty of Science, Mahasarakham University, Thailand, for maintenance and observation. Taxonomic identification was conducted by Saengprajak using diagnostic keys from Woodson and Shery (1945), Lim (1978), and Wu and Kennedy (2002), supplemented with general ethnobotanical information obtained from local farmers to support species identification.

Plants were selected according to the following criteria: healthy and mature individuals, vegetative morphological characters consistent with species descriptions, and suitability for voucher specimen preparation (Chen et al. 2010; Hollingsworth et al. 2011). Because the study aimed to authenticate species using DNA barcoding rather than to assess population-level genetic variation, a single representative accession was collected from each locality (Hebert et al. 2003; Hollingsworth et al. 2011). Sampling across multiple provinces allowed representation of the morphological and geographic variation of Thai arrowroot and related species (Malki et al. 2023). *Ctenanthe glabra* (Körn.) Eichler was included as the outgroup for phylogenetic analysis, and its morphological characters were derived from a herbarium specimen (barcode K001250069; Table 2) because this taxon was not available for direct observation.

Voucher specimens were prepared and deposited in the Herbarium of the Faculty of Science, Mahasarakham University, Thailand. Young leaves (approximately five months old) were harvested from each accession for genomic DNA extraction. Each accession represents a single cultivated or wild clonal population from its locality, sampled to provide representative material for species identification rather than for regional genetic diversity analysis. All collections complied with local regulations and institutional policies in Thailand; the studied species are not protected under Thai law, and sampling was conducted with permission from landowners or local custodians where required.

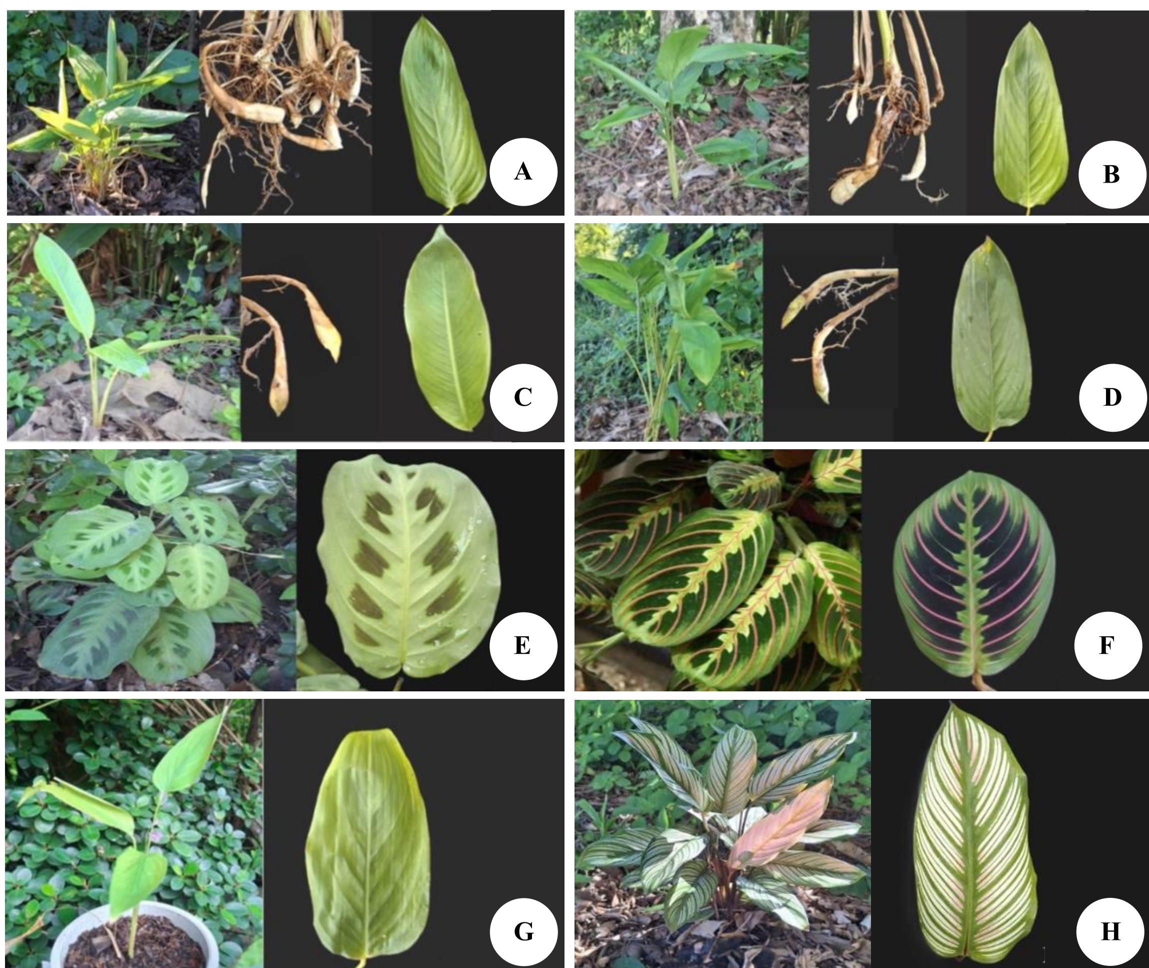

Figure 2. Morphological variation of *Maranta* and *Goeppertia* species examined in this study. A. *Maranta arundinacea* from Mahasarakham (Ma MK), B. *Maranta arundinacea* from Nakhon Ratchasima (Ma NR), C. *Maranta arundinacea* from Phitsanulok (Ma PS), D. *Maranta arundinacea* from Ubon Ratchathani (Ma UR), E. *Maranta cristata* (Mc), F. *Maranta leuconeura* (Ml), G. *Maranta pohliana* (Mp), and H. *Goeppertia ornata* (Go). Whole plants and representative leaves are shown

Table 1. *Maranta* accessions and one *Goeppertia* species collected in Thailand recorded in this study

Species collected	ID	Location	GPS coordinates	Voucher specimen	Distinctive characteristics		
					Leaf shape	Variegation	Rhizome form
Maranta arundinacea L.	Ma (MK)	Mahasarakham	16°09′25.5″N, 103°17′24.5″E	MSUT-8788	Broadly elliptic, symmetrical	Absent, uniformly green	Thick, fleshy, cylindrical rhizome
Maranta arundinacea L.	Ma (NR)	Nakhon Ratchasima	14°55′40.0″N, 101°58′24.5″E	MSUT-8789	Ovate to elliptic, apex acute	Absent, uniformly green	Thick, fleshy, cylindrical rhizome
Maranta arundinacea L.	Ma (PS)	Phitsanulok	16°49′31.6″N, 100°21′17.3″E	MSUT-8790	Narrowly ovate to lanceolate	Weak or absent, pale midrib only	Slender, creeping rhizome
Maranta arundinacea L.	Ma (UR)	Ubon Ratchathani	15°18′51.5″N, 104°44′31.7″E	MSUT-8791	Broadly ovate	Present, prominent green pattern	Short, compact rhizome
Maranta cristata Nees & Mart.	Mc	Loei	17°06′51.9″N, 101°51′46.9″E	MSUT-8792	Elliptic to ovate	Present, pale green to silvery markings	Moderately thick rhizome
Maranta leuconeura E.Morren	Ml	Loei	17°16′54.7″N, 101°45′31.6″E	MSUT-8793	Broadly elliptic	Strong, pink to light stripes on dark green lamina	Short, fibrous rhizome
Maranta pohliana Körn.	Mp	Loei	17°29′39.6″N, 101°42′49.6″E	MSUT-8794	Broadly elliptic, symmetrical	Absent, uniformly green	Thick, fleshy, cylindrical rhizome
Goeppertia ornata (Linden) Borchs.& S.Suárez	Go	Loei	17°26′45.0″N, 101°27′27.6″E	MSUT-8795	Ovate to elliptic, apex acute	Absent, uniformly green	Thick, fleshy, cylindrical rhizome

Table 2. Clade structure inferred from nuclear ribosomal ITS sequence analysis and corresponding diagnostic morphological characters of *Maranta* and related species

Characters	Clade					Outgroup
	I	II			III	
	Maranta arundinacea	***Maranta cristata***	***Maranta leuconeura***	***Maranta pohliana***	***Goeppertia ornata***	***Ctenanthe glabra***
Habit/rhizome	Erect herb, slender to moderately thick rhizome	Erect herb, tufted habit, short rhizome	Creeping to suberect herb, short rhizome	Erect to ascending herb, thick, well-developed rhizome	Erect herb, clustered habit, thick, well-developed rhizome	Erect herb, elongate, thick rhizome
Leaf sheath/petiole/ pulvinus	Sheath long and open, petiole elongate, pulvinus distinct	Sheath shorter, petiole moderate, pulvinus developed	Sheath short, petiole moderate, pulvinus prominent	Sheath narrow, petiole long, pulvinus distinct	Sheath smooth and pinkish, petiole slender, pulvinus reduced	Sheath narrow, petiole thick and glabrous, pulvinus small
Leaf blade (lamina)	Lanceolate to elliptic, uniformly green	Elliptic to ovate, variegation indistinct or absent	Ovate to elliptic, conspicuous variegation with pale midrib	Broadly elliptic, uniformly green or faintly patterned	Elliptic, green with distinct laminar patterning	Oblong-lanceolate, uniformly green, thick texture
Synflorescence/ inflorescence	Terminal or axillary thyrse, moderately branched	Compact thyrse, dense arrangement	Small thyrsoid clusters, loosely arranged	Branched synflorescences, well-developed	Few-flowered heads, colorful bracts present	Dense spike-like clusters, overlapping bracts
Flower: calyx/ corolla	Calyx narrow, corolla white to cream, tube short	Calyx short, corolla with broader lobes, tube short	Calyx narrow, corolla white, tube slender	Calyx persistent, corolla relatively large, tube moderate	Calyx not observed, corolla pink, tube elongate	Calyx not observed, corolla not observed, tube not observed (flowers not preserved)
Androecium/ staminodes	*Maranta*-type, inner staminodes cucullate	*Maranta*-type, outer staminodes broader	*Maranta*-type, staminodes petaloid and conspicuous	*Maranta*-type, staminodes enlarged and cucullate	*Goeppertia*-type, staminodes reduced	Staminodes not observed (flowers not preserved)

Note: Clade assignments (I-III and the outgroup) follow the phylogenetic topology inferred from ITS sequences as shown in Figure 4. Morphological characters of *Maranta* species were recorded directly from the studied accessions and, where necessary, supplemented with published identification keys (Woodson and Shery 1945; Lim 1978; Wu and Kennedy 2000). Morphological information for *Ctenanthe glabra* was compiled from a herbarium specimen deposited at the Royal Botanic Gardens, Kew (barcode K001250069); therefore, some characters were unavailable

Procedures

Genomic DNA extraction

Approximately 0.2 g of young leaf tissue from each accession was flash-frozen in liquid nitrogen and ground into a fine powder. Genomic DNA was extracted using the PureDireX Genomic DNA Isolation Kit (Plant) (BioHelix Co., Ltd., Taiwan) following the manufacturer's instructions. DNA integrity was assessed by electrophoresis on a 1.0% agarose gel in 1× TBE buffer, stained with Prime Juice™ Preloading Fluorescent Stain (BioHelix Co., Ltd., Taiwan), and visualized using a gel documentation system under UV illumination (GelDoc Go Gel Imaging System, Bio-Rad Laboratories, USA). DNA concentration and purity were determined using a NanoDrop Spectrophotometer (NND-1 NDL-PLUS-GL, Thermo Fisher Scientific Co., Ltd., USA), and all samples were adjusted to a final concentration of 50 ng/µL with nuclease-free water (Invitrogen, Thermo Fisher Scientific) prior to storage at -20°C until further use.

PCR amplification of the ITS region

The nuclear ribosomal ITS region was amplified using a PCR thermal cycler (Applied Biosystems Veriti, Applied Biosystems, Singapore). The primer pair ITS-5a (5′-CCTTATCATTTAGAGGAAGGA-3′) and ITS-4 (5′-TCCTCCGCTTATTGATATGC-3′), synthesized by U2Bio Co., Ltd. (Thailand), was used for amplification. ITS-4 was originally described by White et al. (1990), whereas ITS-5a represents a modified version of the ITS5 primer reported in previous studies (Mahadani et al. 2022). This primer pair was expected to produce an amplicon of approximately 800 bp and was used to amplify genomic DNA from eight accessions, including seven *Maranta* accessions (four species) and one *G. ornata*. PCR reactions were performed in a final volume of 25 µL containing 1× PCR buffer, 1.2 mM $MgCl_2$, 0.8 mM dNTPs, 0.2 µM of each primer, 1 U nano*Taq* hot-start DNA polymerase, and 50-100 ng of template DNA. All reagents were obtained from BioHelix Co., Ltd. (Taiwan), except for the primers, which were synthesized by U2Bio Co., Ltd. (Thailand). Nuclease-free water was added to adjust the final volume. The PCR program consisted of an initial denaturation at 94°C for 3 min; followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 56°C for 1 min, and extension at 72°C for 2 min; with a final extension at 72°C for 5 min (Chen et al. 2010; Da Costa Fernandes et al. 2023; Luna et al. 2025a). These PCR conditions were adopted from previously published ITS amplification protocols for angiosperms and related taxa (Chen et al. 2010; Fernandes et al. 2023) and were verified through preliminary amplification tests on Marantaceae samples. Initial trials confirmed that the selected parameters yielded clean, reproducible products without nonspecific amplification; therefore, no further extensive optimization was required. PCR products were analyzed by agarose gel electrophoresis under the same conditions described for genomic DNA. Negative (no-template) controls were included in each run, and all reactions were performed in duplicate to ensure reproducibility.

Data analyses

Sequence analysis

PCR amplicons of approximately 800 bp were purified and subjected to bidirectional Sanger sequencing in triplicate using the original amplification primers to minimize sequencing artifacts. Sequencing was performed by U2Bio Co., Ltd. (Thailand) on an ABI 3730xl DNA Analyzer (Applied Biosystems, Thermo Fisher Scientific, USA). Replicate reads showed no consistent base-call disagreements, and no heterozygous or ambiguous sites were detected after quality trimming, indicating that ITS sequences were homogeneous within individuals (Li et al. 2011; Nilsson et al. 2012). Forward and reverse chromatograms were assembled and edited in BioEdit version 7.2.5 (Ibis Biosciences, USA), and low-quality ends and poorly aligned regions were trimmed to reduce missing data (Hall 1999; Kumar et al. 2018). The initial PCR amplicons were approximately 800 bp in length. After sequence editing and quality trimming, the resulting sequences ranged from 552 to 659 bp. Following multiple sequence alignment and removal of poorly aligned regions, a final dataset of 503 bp was used for phylogenetic analysis (Hollingsworth et al. 2011). Consensus sequences were generated for each accession and verified using BLASTn searches against the NCBI nucleotide database (Altschul et al. 1990). Sequence identifications were considered reliable when the top BLASTn hit corresponded to the expected species or genus; otherwise, results were treated as ambiguous (Nilsson et al. 2014; Raja et al. 2017).

BLASTn comparisons showed that all accessions matched reference sequences of their respective taxa with 98.2-100% sequence similarity and complete (100%) query coverage (Table 3). The estimated Standard Error (SE) of similarity values was minimal (0.00-0.52), indicating highly consistent sequence matches across accessions. All ITS sequences generated in this study have been deposited in GenBank (NCBI, USA) under accession numbers PP514351-PP514712 and PX576648-PX576651, corresponding to the samples listed in Table 3. ITS sequences for the seven *Maranta* accessions and one *G. ornata* were generated in this study. In contrast, ITS sequences of the outgroup (*C. glabra*) and additional comparative taxa used in phylogenetic reconstruction were retrieved from the NCBI GenBank database. Reference sequences were selected based on the following criteria: (i) correct taxonomic annotation, (ii) high sequence quality with minimal ambiguous bases, (iii) adequate length and coverage of the ITS region, and (iv) availability of accession information and associated metadata (Nilsson et al. 2006, 2014; Hollingsworth et al. 2011). Where possible, sequences from published studies and representative geographic origins were preferentially selected (Hebert et al. 2003; Letsiou et al. 2024). Accession numbers are provided in Table 2.

Phylogenetic analysis

Sequence similarity and coverage were calculated based on the proportion of identical nucleotides and aligned sequence length, respectively, and are summarized in Table 3. After trimming, the final multiple sequence alignment used for phylogenetic analysis comprised 503 bp. The

Standard Error (SE) of the match proportion was estimated using the binomial standard error formula described by Newcombe (1998). Multiple sequence alignments were generated using Clustal Omega v1.2.4 (EMBL-EBI, UK) (Sievers et al. 2011). Phylogenetic analyses were conducted in MEGA version 11 (Tamura et al. 2021) using the Maximum Likelihood (ML) method. The best-fitting nucleotide substitution model (Kimura 2-Parameter, K2P) was selected based on the Bayesian Information Criterion (BIC) implemented in MEGA11. Gaps and Insertion-Deletion events (InDels) were treated as missing data. Branch lengths were estimated as the expected number of nucleotide substitutions per site, representing genetic divergence among taxa (Nei and Kumar 2000). Node support was assessed using 1,000 bootstrap replicates. Following Kress et al. (2002), nodes with bootstrap values <50% were not interpreted, whereas values ≥70% were considered to indicate strong support (Hillis and Bull 1993).

RESULTS AND DISCUSSION

Molecular analysis of ITS sequences

The nuclear ribosomal ITS region was used to assess molecular variation among seven *Maranta* accessions and one *G. ornata* included in this study. High-quality PCR amplification yielded clear ~800 bp ITS products in all 8 accessions (Figure 3). Following editing and trimming of ambiguous ends, sequence lengths ranged from 552-659 bp. BLASTn comparisons confirmed accurate species identification, with all accessions matching reference sequences of their respective taxa with 98.2-100% sequence similarity and complete (100%) query coverage (Table 3). Standard Error (SE) values were minimal (0.00-0.52), supporting the reliability of these identifications and confirming that the ITS region provides sufficient discriminatory power for these taxa. After multiple sequence alignment and removal of poorly aligned regions, a final dataset of 503 bp was retained for subsequent phylogenetic analysis.

A notable exception was observed for *M. cristata*, for which the ITS sequence showed 100% similarity to *M. leuconeura* in BLAST searches. Because ITS reference sequences of *M. cristata* are currently absent or extremely limited in public databases, ITS alone cannot provide unambiguous BLAST-based identification for this species. This result reflects a limitation of reference coverage rather than a conflict in specimen identification, and the observed discrepancy between BLAST similarity and phylogenetic placement is most likely attributable to incomplete representation of ITS reference sequences in public databases.

All four *M. arundinacea* accessions showed ≥98.2% similarity to the reference sequence (GenBank JQ341260), indicating high ITS sequence conservation across geographically separated Thai populations. This molecular homogeneity is consistent with the species' morphological uniformity and reflects genetic uniformity among the sampled accessions. For other *Maranta* species, ITS sequence similarity results should be interpreted with caution. The ITS sequence of *M. cristata* showed 100% similarity to *M. leuconeura* in BLAST searches; however, this does not constitute unambiguous species identification. To our knowledge, reference ITS sequences of *M. cristata* are currently unavailable in GenBank. Therefore, the closest match to *M. leuconeura* likely reflects limitations of the reference database rather than true species identity. In contrast, the ITS sequences of *M. leuconeura* and *M. pohliana* matched their respective reference sequences with 100% similarity. Similarly, *G. ornata* showed 100% similarity to *Goeppertia* accessions, supporting its genetic distinctiveness relative to *Maranta*.

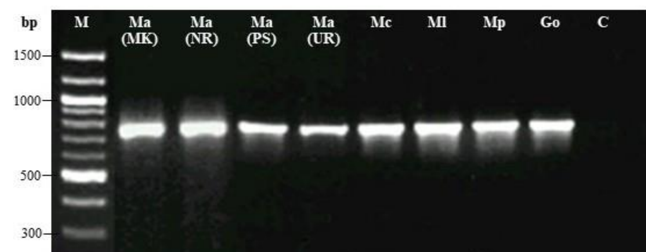

Figure 3. PCR amplification of the ITS region. M: 100 bp molecular size marker, lanes 1-8: *Maranta* accessions (Ma, Mc, Ml, and Mp) and *Goeppertia ornata* (Go) as listed in Table 1, C: negative control

Table 3. Sequence similarity results based on the ITS region, showing the closest matches of *Maranta arundinacea* and related species retrieved from the NCBI database

Sample	Sample ID	Accession number	Closest match (GenBank)	Match accession no.	Aligned length (bp)	Similarity	% Match	Coverage (%)	SE
Maranta arundinacea	Ma (MK)	PP514712	*Maranta arundinacea*	JQ341260	644	644/649	99.0	100	0.39
Maranta arundinacea	Ma (NR)	PP514713	*Maranta arundinacea*	JQ341260	638	638/640	99.7	100	0.22
Maranta arundinacea	Ma (PS)	PP514715	*Maranta arundinacea*	JQ341260	659	659/671	98.2	100	0.52
Maranta arundinacea	Ma (UR)	PP514351	*Maranta arundinacea*	JQ341260	649	649/654	99.2	100	0.35
Maranta cristata	Mc	PX576649	*Maranta leuconeura*	MF796520	552	552/552	100.0	100	0.00
Maranta leuconeura	Ml	PX576650	*Maranta leuconeura*	MF796520	552	552/552	100.0	100	0.00
Maranta pohliana	Mp	PX576648	*Maranta pohliana*	PP726691	643	643/643	100.0	100	0.00
Goeppertia ornata	Go	PX576651	*Goeppertia* sp.	MW027320	650	650/650	100.0	100	0.00

Note: The reference sequences obtained from GenBank included *Maranta arundinacea* from Denmark (JQ341260), and *Maranta arundinacea* from Brazil (ON239732). In addition, *Ctenanthe glabra* from Brazil (PP726680) was selected as the outgroup

ITS polymorphisms and species-level discrimination

Sequence alignment of the ITS region revealed several species-specific polymorphic nucleotide positions among *Maranta* taxa, including fixed substitutions and insertion-deletion events (Table 4). No polymorphic sites were detected among the four *M. arundinacea* accessions, indicating complete sequence identity across the analyzed ITS region and strong intraspecific conservation.

In contrast, pairwise comparisons among species revealed clear diagnostic differences. Approximately 16-21 fixed substitutions distinguished *M. arundinacea* from the other *Maranta* taxa, with *M. pohliana* additionally characterized by a single indel. *Maranta cristata* and *M. leuconeura* differed by only 6-7 substitutions and showed no indels, which explains their identical BLAST matches despite the presence of diagnostic polymorphic sites in the alignment. More broadly, *M. pohliana* was consistently differentiated from the other species by multiple unique substitutions together with at least one indel event. Together, these species-specific polymorphic characters demonstrate that the ITS region provides sufficient diagnostic signal for reliable species-level discrimination within the sampled *Maranta* taxa, even in cases where reference database coverage is incomplete.

ITS sequence divergence and substitution patterns

ITS sequence divergence analyses revealed very low levels of genetic variation within species and markedly higher divergence among species (Table 5). Intraspecific genetic distances ranged from 0.000 to 0.004 (mean = 0.001), indicating pronounced genetic uniformity; notably, *M. arundinacea* exhibited effectively zero intraspecific divergence, consistent with the absence of polymorphic sites observed in Table 4. In contrast, interspecific distances ranged from 0.009 to 0.096 (mean = 0.049). Importantly, the minimum interspecific distance exceeded the maximum intraspecific distance, supporting the presence of a clear barcoding gap within the current dataset and indicating the potential of the ITS region for species discrimination within *Maranta*.

Substitution pattern analysis further showed that nucleotide changes in the ITS region were dominated by transitions, particularly A→G and T→C substitutions (Table 6). This transition bias is characteristic of nuclear ribosomal markers and reflects underlying mutation tendencies, contributing to observed sequence variation in ITS regions. The predominance of transitions reflects common mutational patterns in non-coding regions and may facilitate the accumulation of sequence variation useful for phylogenetic inference. Many of the diagnostic nucleotide differences identified among *Maranta* species in Table 4 correspond to such transition-type substitutions, indicating that the observed substitution bias contributes directly to the generation of species-informative barcode characters in the ITS region.

Phylogenetic analysis

Maximum likelihood phylogenetic analysis resolved two major lineages corresponding to *Maranta* and *Goeppertia* (Figure 4). Within *Maranta*, three well-defined clades were recovered. All Thai *M. arundinacea* accessions clustered with the Danish reference sequence, forming a strongly supported monophyletic clade with very short branch lengths (BS = 100%). A second clade comprised *M. cristata* and *M. leuconeura*, which formed a closely related sister group with strong bootstrap support (BS = 100%). A third, distinct clade consisted of *M. pohliana*, which was separated from the former pair with moderate bootstrap support (BS = 68%). *Goeppertia ornata* was consistently resolved as an outgroup relative to *Maranta*, with moderate support for the generic split (BS = 64%) and substantially longer branch lengths, supporting its separation at the genus level.

Overall, the combined evidence from BLAST similarity, diagnostic polymorphisms, substitution patterns, and phylogenetic topology supports the utility of ITS for species identification and discrimination among closely related *Maranta* taxa, as well as for providing insights into the phylogenetic position of *Goeppertia*.

Discussion

Accurate species identification is fundamental to biodiversity conservation, germplasm management, and the sustainable utilization of economically important crops (Hebert et al. 2003; Hollingsworth et al. 2016; Saengprajak et al. 2024), yet remains challenging in morphologically plastic taxa such as *Maranta*. These results are consistent with previous DNA barcoding frameworks and support the use of ITS for species authentication in *Maranta* and related taxa. While vegetative morphology has traditionally been used for arrowroot identification, its plasticity and environmental sensitivity limit reliable species delimitation (Hollingsworth et al. 2011; Mayo 2022), underscoring the value of molecular diagnostics for taxa with reduced or seasonally unavailable diagnostic structures (Da Costa Fernandes et al. 2023; Luna et al. 2025a). Within a DNA barcoding framework, the combined evidence of a clear barcoding gap, fixed diagnostic polymorphisms, and congruent phylogenetic clustering supports the effectiveness of ITS for species authentication in the taxa examined (Kress et al. 2002; Hebert et al. 2003; Hollingsworth et al. 2011). Accordingly, the use of a single nuclear marker (ITS) was considered sufficient for the primary objective of species authentication within the scope of this study. Although plastid markers such as *matK* and *rbcL* are commonly used in plant barcoding, their relatively low sequence variability may limit resolution among closely related species (Dong et al. 2012). Therefore, ITS was selected as a practical marker for establishing a baseline identification framework in this study. Consistency between edited sequence lengths (552-659 bp) and the final aligned dataset (503 bp) further supports the reliability of sequence processing and subsequent phylogenetic inference. However, intraspecific divergence was assessed only for *M. arundinacea* due to the availability of multiple accessions, whereas other species were represented by a single accession. Therefore, although a barcoding gap was observed, a more robust evaluation would require multiple accessions per species.

Table 4. Polymorphic nucleotide positions in the ITS region among *Maranta* species and related taxa

Nucleotide number	**2**	**3**	**4**	**6**	**20**	**23**	**30**	**32**	**35**	**40**	**42**	**46**	**53**	**54**	**61**	**62**	**102**	**107**	**113**	**114**	**115**	**118**	**122**	**128**	**131**	**135**	**137**	**138**	**139**	**312**	**314**	**315**	**330**	**333**	**335**	**336**
Common sequence	**C**	**T**	**G**	**A**	**T**	**C**	**A**	**G**	**G**	**T**	**C**	**A**	**C**	**C**	**G**	**C**	**A**	**A**	**G**	**A**	**G**	**C**	**C**	**C**	**C**	**T**	**C**	**C**	**T**	**C**	**T**	**C**	**A**	**C**	**G**	**C**
JQ341260 *Maranta arundinacea**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Maranta arundinacea (NR)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Maranta arundinacea (PS)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Maranta arundinacea (MK)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Maranta arundinacea (UR)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Maranta pohliana	.	C	.	.	C	T	.	C	.	.	.	C	T	T	A	.	G	G	C	T	T	.	.	A	.	C	T	.	.	.	C	.	.	.	A	A
Maranta cristata	.	.	.	.	.	.	C	.	C	C	T	C	.	T	.	T	G	G	C	.	.	T	T	T	A	.	.	T	G	T	.	A	T	T	.	.
Maranta leuconeura	T	.	A	T	.	.	C	.	C	C	T	C	.	T	.	T	G	G	C	.	.	T	T	T	A	.	.	T	G	T	.	A	T	T	.	.

Nucleotide number (Cont.)	**340**	**342**	**344**	**350**	**368**	**380**	**395**	**396**	**397**	**398**	**402**	**412**	**423**	**434**	**435**	**445**	**452**	**454**	**460**	**463**	**464**	**466**	**467**	**468**	**470**	**471**	**479**	**489**	**492**	**493**	**495**	**499**	**500**	**501**	**502**	**503**
Common sequence (Cont.)	C	G	T	G	C	T	A	G	C	G	C	T	A	T	G	A	C	T	C	G	G	C	G	T	A	T	T	A	C	C	C	C	G	G	G	A
JQ341260 *Maranta arundinacea**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Maranta arundinacea (NR)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	T	G	.	.	A	C	C
Maranta arundinacea (PS)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	T	G	.	.	A	C	C
Maranta arundinacea (MK)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	T	G	.	.	A	C	C
Maranta arundinacea (UR)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	T	G	.	.	A	C	C
Maranta pohliana	.	.	.	.	.	.	.	.	T	T	T	.	T	C	A	G	A	A	T	C	.	.	A	.	G	C	A	G	T	T	G	.	.	A	C	C
Maranta cristata	T	C	C	T	T	C	T	A	.	.	.	G	.	C	A	.	T	.	.		T	A	C	C	.	C	A	G	T	T	G	.	.	A	C	C
Maranta leuconeura	T	C	C	T	T	C	T	A	.	.	.	G	.	C	A	.	T	.	.		T	A	C	C	.	C	A	G	T	T	G	G	T	A	.	T

Note: The alignment includes nine ITS sequences, comprising GenBank reference sequences and sequences generated in this study (asterisk indicates GenBank accessions). Numbers at the top denote nucleotide positions in the aligned sequences, and the consensus sequence is shown in the first row. Dots (.) indicate identity with the consensus sequence, whereas letters indicate nucleotide substitutions

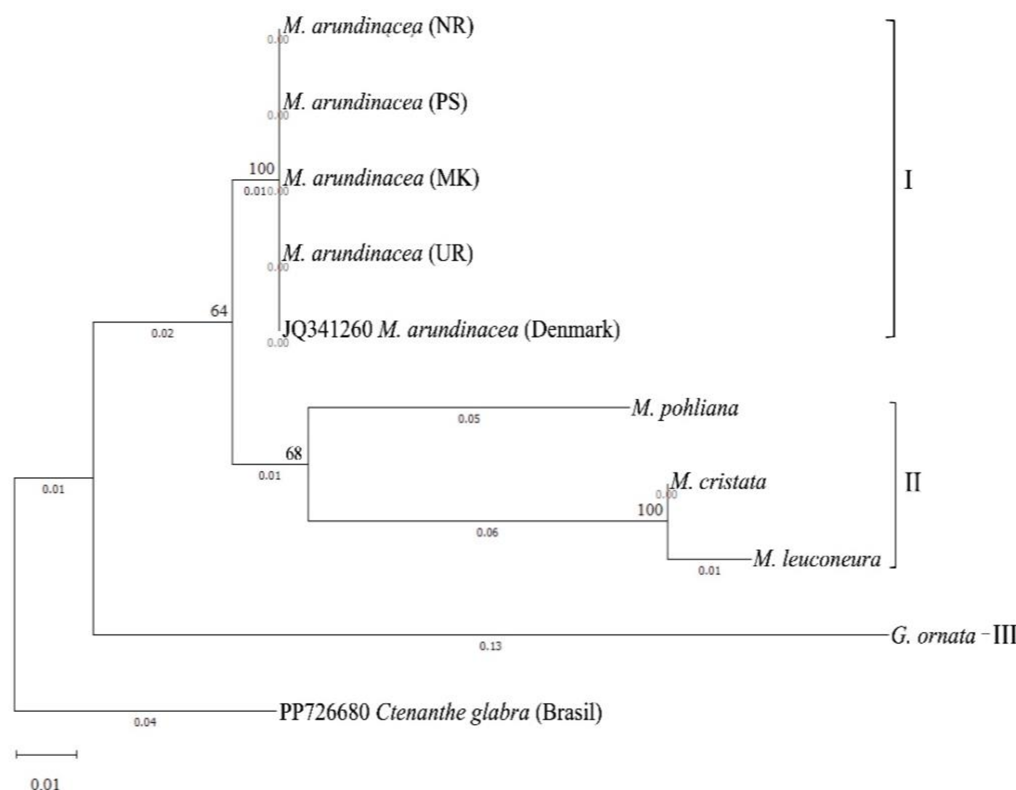

Figure 4. Phylogenetic tree with 1000 bootstrap replicates based on the ITS region of *Maranta arundinacea* collected from four provinces in Thailand, together with *Maranta pohliana, Maranta cristata, Maranta leuconeura*, and *Goeppertia ornata*, as well as the reference sequence of *Maranta arundinacea* (Denmark), constructed using Maximum Likelihood (ML) analysis. *Ctenanthe glabra* (Brazil) was designated as the outgroup

Table 5. Estimates of intraspecific and interspecific evolutionary divergence based on nuclear ribosomal ITS sequences

Parameter	ITS
Range of intraspecific distance	0.000-0.004
Range of interspecific distance	0.009-0.096
Mean intraspecific distance	0.001
Mean interspecific distance	0.049

Note: Genetic distances were calculated in MEGA version 11 using the Kimura 2-Parameter (K2P) model with pairwise exclusion of gaps, intraspecific and interspecific values were derived from within- and between-species comparisons, respectively

Table 6. Nucleotide substitution patterns (%) in the ITS regions

From/To	ITS			
	A	T	C	G
A	-	5.09	7.14	**13.33**
T	5.07	-	**15.91**	8.38
C	5.07	**11.34**	-	8.38
G	**8.07**	5.09	7.14	-

Note: Substitution patterns and rates were estimated using the Tamura-Nei model (1993). Rates of different transitional substitutions are shown in bold and those of transversionsal substitutions are shown in italics

A key result of this work is the exceptionally high ITS sequence conservation observed among Thai *M. arundinacea* accessions, indicating limited intraspecific divergence across geographically separated populations. All specimens clustered closely with the Denmark reference accession (JQ341260) and exhibited uniformly short branch lengths, indicating minimal genetic divergence across regions. Comparable patterns of shallow branch lengths and strong clustering in ITS-based phylogenies of *Maranta* have been attributed to predominant vegetative propagation and restricted sexual recombination (Da Costa Fernandes et al. 2023; Luna et al. 2025a). In line with large-scale DNA barcoding studies, the molecular homogeneity observed here suggests low intraspecific ITS variation in arrowroot, most plausibly explained by clonal propagation and human-mediated dissemination of genetically uniform planting material (McKey et al. 2010; Li et al. 2011; Samarina et al. 2025). Together, these findings indicate that ITS functions as a useful marker for species authentication in *M. arundinacea* within the sampled accessions, and suggest that clonal cultivation history may contribute to the observed genetic cohesion. While clonal propagation and human-mediated dissemination provide a plausible explanation for this genetic uniformity, alternative scenarios such as recent introductions, demographic bottlenecks, or the limited geographic sampling of the present study may also contribute to the observed pattern (Ingvarsson and Dahlberg 2019).

The current dataset does not allow these mechanisms to be distinguished, and broader geographic sampling together with multilocus or genome-wide analyses would be required to evaluate their relative importance.

In contrast to the genetic uniformity observed in *M. arundinacea*, *M. cristata*, *M. leuconeura*, and *M. pohliana* exhibited diagnostic ITS polymorphisms that supported species-level discrimination when interpreted alongside phylogenetic placement and morphological evidence. Maximum likelihood analysis resolved these taxa into distinct lineages largely congruent with morphological expectations, demonstrating that ITS captures evolutionary relationships within *Maranta* despite overlapping vegetative traits. Similar patterns of species-level resolution using ITS have been reported previously (Da Costa Fernandes et al. 2023), and the close affinity between *M. cristata* and *M. leuconeura*, together with the distinct placement of *M. pohliana*, is consistent with multilocus phylogenetic analyses of Marantaceae (Luna et al. 2025a).

Although the inferred topology broadly agrees with morphology, the statistical support varied among nodes. The strong bootstrap support for the *M. arundinacea* clade confirms its genetic coherence within the sampled material. In contrast, the placement of *M. pohliana* received only moderate support, indicating that its inferred position should be interpreted cautiously and may benefit from confirmation using additional loci. Likewise, the separation between *Goeppertia* and *Maranta* was supported at a moderate level, consistent with their recognized generic distinction but suggesting that deeper phylogenetic relationships within Marantaceae may require multilocus data. Taken together, these results support the utility of ITS for species-level discrimination in *Maranta*; however, relationships supported by moderate bootstrap values should be interpreted cautiously.

Notably, the ITS sequence obtained from the specimen identified as *M. cristata* showed its closest BLAST match to *M. leuconeura* rather than to a conspecific reference. This result reflects limited reference coverage rather than misidentification, as ITS sequences for this species are currently underrepresented in public databases (Nilsson et al. 2006, 2014). Consequently, similarity-based identification using BLAST may lead to misidentification when reference coverage is incomplete (Altschul et al. 1990; Raja et al. 2017). Differences between similarity-based identification and phylogenetic inference should therefore be interpreted in the context of database completeness and marker resolution. In this study, the placement of the specimen within a lineage closely related to, but distinct from, *M. leuconeura*, together with diagnostic morphological characters, supports its identification as *M. cristata*. Nevertheless, ITS data should be interpreted alongside morphological evidence and, where possible, complemented with plastid or multilocus markers to improve species delimitation.

Beyond taxonomic clarification, these findings have practical implications for germplasm authentication, conservation planning, and the development of traceable starch resources (Engels and Ebert 2021; Samarina et al. 2025). The ITS sequences generated in this study, together with well-documented accession metadata, provide a reference framework for developing species-specific molecular diagnostics in *Maranta* (Paran and Michelmore 1993; Zhu et al. 2022). Fixed ITS polymorphisms distinguishing *M. arundinacea* from related taxa have the potential to be translated into rapid PCR-based assays or sequence-characterized markers for routine verification of planting material and germplasm identity. Such molecular identification supports the maintenance of genetic resources and the emerging economic potential of arrowroot as a functional starch crop. In the Thai context, these tools may contribute to nursery certification, supply chain verification, and germplasm documentation. However, these applications were not directly evaluated in the present study.

Despite these strengths, several limitations of this study should be acknowledged. Sampling was limited in both accession number and geographic coverage within Thailand, and each accession represents a single cultivated or wild clonal population rather than comprehensive representation of regional diversity. Importantly, the sampling design was intended for species authentication rather than population-level genetic analysis. Therefore, the observed ITS uniformity in *M. arundinacea* should be interpreted cautiously and should not be taken as evidence of nationwide genetic homogeneity. In addition, molecular inference relied on a single nuclear marker (ITS), which limits resolution of population structure and deeper evolutionary relationships. Public reference coverage also remains incomplete for some taxa. Future studies should therefore combine broader sampling with plastid barcodes and multilocus or genome-wide approaches to provide a more comprehensive understanding of genetic diversity and evolutionary dynamics.

In conclusion, this study indicates that nuclear ribosomal ITS barcoding can serve as a useful first-line molecular tool for species authentication among the studied *Maranta* taxa in Thailand. All *M. arundinacea* accessions showed high sequence similarity and formed a well-supported lineage, suggesting limited detectable genetic variation consistent with vegetative propagation. In contrast, ITS variation enabled discrimination among other *Maranta* species that are difficult to distinguish morphologically, while *G. ornata* was recovered as a distinct lineage separate from *Maranta*. Within the scope of the present dataset, these findings support the potential utility of ITS barcoding for arrowroot identification and germplasm verification. However, further studies incorporating broader sampling and additional molecular markers are needed to confirm these patterns and strengthen their practical basis for future conservation-oriented management.

ACKNOWLEDGEMENTS

This study was financially supported by Mahasarakham University, Thailand. The authors also wish to thank the Department of Biology, Faculty of Science, Mahasarakham University, for providing laboratory facilities and technical support.

REFERENCES

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215 (3): 403-410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Álvarez I, Wendel JF. 2003. Ribosomal ITS sequences and plant phylogenetic inference. Mol Phylogenet Evol 29 (3): 417-434. https://doi.org/10.1016/S1055-7903(03)00208-2.

Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF, Campbell CS, Donoghue MJ. 1995. The ITS region of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny. Ann Missouri Bot Gard 82 (2): 247-277. https://doi.org/10.2307/2399880.

Brito V, Nascimento R, Narcisa-Oliveira J, Joffer N, Fattori A, Cereda M, Oliveira C, Costa R, Tiburtino-Silva L, Maciel J. 2021. Arrowroot (*Maranta arundinacea* L.): Botany, horticulture, and uses. In: Warrington I (eds). Horticultural Reviews. John Wiley & Sons, Inc., Hoboken, New Jersey. https://doi.org/10.1002/9781119750802.ch4.

Campanaro A, Tommasi N, Guzzetti L, Galimberti A, Bruni I, Labra M. 2019. DNA barcoding to promote social awareness and identity of neglected, underutilized plant species having valuable nutritional properties. Food Res Intl 115: 1-9. https://doi.org/10.1016/j.foodres.2018.07.031.

Chen S, Yao H, Han J, Liu C, Song J, Shi L, Zhu Y, Ma X, Gao T, Pang X, Luo K, Li Y, Li X, Jia X, Lin Y, Leon C. 2010. Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. PLoS One 5 (1): e8613. https://doi.org/10.1371/journal.pone.0008613.

Da Costa Fernandes G, Luna NK, Fraga E, Barros MC, Chase MW, Pessoa EM. 2023. Molecular phylogenetics of *Maranta* (Marantaceae: Zingiberales): Non-monophyly and support for a wider circumscription. Bot J Linn Soc 202 (2): 181-194. https://doi.org/10.1093/botlinnean/boac064.

de Oliveira Guilherme D, Branco FP, Madeira NR, Brito VH, de Oliveira CE, Jadoski CJ, Cereda MP. 2019. Starch valorization from corms, tubers, rhizomes, and roots crops: The arrowroot (*Maranta arundinacea* L.) case. In: Clerici MTPS, Schmiele M (eds). Starches for Food Application. Chemical, Technological and Health Properties. Academic Press, London. https://doi.org/10.1016/B978-0-12-809440-2.00005-8.

Dong W, Liu J, Yu J, Wang L, Zhou S. 2012. Highly variable chloroplast markers for evaluating plant phylogeny at low taxonomic levels and for DNA barcoding. PLoS One 7 (4): e35071. https://doi.org/10.1371/journal.pone.0035071.

Engels JMM, Ebert AW. 2021. A critical review of the current global ex situ conservation system for plant agrobiodiversity. I. History of the development of the global system in the context of the political/legal framework and its major conservation components. Plants 10 (8): 1557. https://doi.org/10.3390/plants10081557.

Fanelli V, Mascio I, Miazzi MM, Savoia MA, De Giovanni C, Montemurro C. 2021. Molecular approaches to agri-food traceability and authentication: An updated review. Foods 10 (7): 1644. https://doi.org/10.3390/foods10071644.

Galimberti A, De Mattia F, Losa A, Bruni I, Federici S, Casiraghi M, Martellos S, Labra M. 2013. DNA barcoding as a new tool for food traceability. Food Res Intl 50 (1): 55-63. https://doi.org/10.1016/j.foodres.2012.09.036.

Mayo SJ. 2022. Plant taxonomic species and their role in the workflow of integrative species delimitation. Kew Bull 77: 1-26. https://doi.org/10.1007/s12225-022-10002-x.

Hall TA. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41: 95-98.

Hebert PDN, Cywinska A, Ball SL, deWaard JR. 2003. Biological identifications through DNA barcodes. Proc Biol Sci 270 (1512): 313-321. https://doi.org/10.1098/rspb.2002.2218.

Hillis DM, Bull JJ. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst Biol 42 (2): 182-192. https://doi.org/10.1093/sysbio/42.2.182.

Hollingsworth PM, Graham SW, Little DP. 2011. Choosing and using a plant DNA barcode. PLoS One 6 (5): e19254. https://doi.org/10.1371/journal.pone.0019254.

Hollingsworth PM, Li D-Z, 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. https://doi.org/10.1098/rstb.2015.0338.

Ieamkheng S, Santibenchakul S, Poonpaerdchon S, Soem B, Sooksawat N. 2024. Growth performance and starch yield potential of arrowroot (*Maranta arundinacea*) from various locations in Thailand. Biodiversitas 25 (10): 3750-3757. https://doi.org/10.13057/biodiv/d251037.

Ingvarsson PK, Dahlberg H. 2019. The effects of clonal forestry on genetic diversity in wild and domesticated stands of forest trees. Scand J For Res 34 (5): 370-379. https://doi.org/10.1080/02827581.2018.1469665.

Kress WJ, Prince LM, Williams KJ. 2002. The phylogeny and a new classification of the gingers (Zingiberaceae): Evidence from molecular data. Am J Bot 89 (10): 1682-1696. https://doi.org/10.3732/ajb.89.10.1682.

Kress WJ. 1990. The phylogeny and classification of the Zingiberales. Ann Missouri Bot Gard 77 (4): 698-721. https://doi.org/10.2307/2399669.

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

Le TTM, Jin L, Wang R-J, Deng Y-F, Yan H-F, Ge X-J. 2025. A DNA barcode reference library of native seed plants in the Guangdong-Hong Kong-Macao Greater Bay Area. Sci Data 12: 1505. https://doi.org/10.1038/s41597-025-05833-9.

Letsiou S, Madesis P, Vasdekis E, Montemurro C, Grigoriou ME, Skavdis G, Moussis V, Koutelidakis AE, Tzakos AG. 2024. DNA barcoding as a plant identification method. Appl Sci 14 (4): 1415. https://doi.org/10.3390/app14041415.

Li D-Z, Gao L-M, Li H-T, Wang H, Ge X-J, Liu J-Q, Chen Z-D, Zhou S-L, Chen S-L, Yang J-B, Fu C-X, Zeng C-X, Yan H-F, Zhu Y-J, Sun Y-S, Chen S-Y, Zhao L, Wang K, Yang T, Duan G-W. 2011. Comparative analysis of a large dataset indicates that ITS should be incorporated into the core barcode for seed plants. Proc Natl Acad Sci USA 108 (49): 19641-19646. https://doi.org/10.1073/pnas.1104551108.

Lim SC. 1978. Marantaceae. In: Whitmore TC (eds). Tree Flora of Malaya. Department of Forestry, Kuala Lumpur.

Luna NKM, Coutinho TS, Chase MW, Felix LP, Pessoa EM. 2025a. Molecular phylogenetics and morphological analyses support *Dolichopoda*, a new neotropical genus of Marantaceae (Zingiberales). Plants 14 (22): 3486. https://doi.org/10.3390/plants14223486.

Luna NKM, Fernandes GC, Christenhusz MJM, Pessoa EM. 2025b. Taxonomic notes *Maranta* (Marantaceae): A new combination and an updated infrageneric classification for the genus. Phytotaxa 684 (1): 135-138. https://doi.org/10.11646/phytotaxa.684.1.9.

Mahadani P, Chakrabarti S, Pal R, Jain SK, Sevanthi AM. 2022. Application of DNA barcoding tool for authentication of traditionally important medicinal *Dendrobium* (Orchidaceae) species from Sikkim Himalaya. S Afr J Bot 146: 905-911. https://doi.org/10.1016/j.sajb.2022.04.013.

Malki S, Sivalingam S, Wijesinghe A, Ratnayake K, Gimhani R. 2023. Arrowroot *(Maranta arundinacea*): Variation in morphological and yield traits across Sri Lanka's agro-climatic zones and genetic diversity assessment. Adv Biosci Bioeng 11 (3): 76-84. https://doi.org/10.11648/j.abb.20231103.17.

McKey D, Elias M, Pujol B, Duputié A. 2010. The evolutionary ecology of clonally propagated domesticated plants. New Phytol 186 (2): 318-332. https://doi.org/10.1111/j.1469-8137.2010.03210.x.

Nei M, Kumar S. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, Oxford.

Newcombe RG. 1998. Two-sided confidence intervals for the single proportion: Comparison of seven methods. Stat Med 17 (8): 857-872. https://doi.org/10.1002/(sici)1097-0258(19980430)17:8<857::aid-sim777>3.0.co;2-e.

Nilsson RH, Hyde KD, Pawłowska J et al. 2014. Improving ITS sequence data for identification of plant pathogenic fungi. Fungal Divers 67: 11-19. https://doi.org/10.1007/s13225-014-0291-8.

Nilsson RH, Ryberg M, Kristiansson E, Abarenkov K, Larsson K-H, Kõljalg U. 2006. Taxonomic reliability of DNA sequences in public sequence databases: A fungal perspective. PLoS One 1 (1): e59. https://doi.org/10.1371/journal.pone.0000059.

Nilsson RH, Tedersoo L, Abarenkov K, Ryberg M, Kristiansson E, Hartmann M, Schoch CL, Nylander JAA, Bergsten J, Porter TM, Jumpponen A, Vaishampayan P, Ovaskainen O, Hallenberg N, Bengtsson-Palme J, Eriksson KM, Larsson K-H, Larsson E, Kõljalg U. 2012. Five simple guidelines for establishing basic authenticity and reliability of newly generated fungal ITS sequences. MycoKeys 4: 37-63. https://doi.org/10.3897/mycokeys.4.3606.

Paran I, Michelmore RW. 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85: 985-993. https://doi.org/10.1007/BF00215038.

Raja HA, Miller AN, Pearce CJ, Oberlies NH. 2017. Fungal identification using molecular tools: A primer for the natural products research community. J Nat Prod 80 (3): 756-770. https://doi.org/10.1021/acs.jnatprod.6b01085.

Saengprajak J, Thaewnongiw B-O, Phetsom J, Sangdee A, Atichart P. 2024. Development of ISSR-Derived SCAR marker for rapid and accurate authentication of West Indian arrowroot (*Maranta arundinacea* L.). Asia Pac J Sci Technol 29 (6): 1-8. https://doi.org/10.14456/apst.2024.93.

Shintu PV, Radhakrishnan VV, Mohanan KV. 2016. A study of the genetic variability of West Indian Arrowroot (*Maranta arundinacea* L.) in Kerala State of India. Agric For Fish 5 (5): 186-190. https://doi.org/10.11648/j.aff.20160505.17.

Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD, Higgins DG. 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 7: MSB201175. https://doi.org/10.1038/msb.2011.75.

Tamura K, Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10 (3): 512-526. https://doi.org/10.1093/oxfordjournals.molbev.a040023.

Tamura K, Stecher G, Kumar S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis version 11. Mol Biol Evol 38 (7): 3022-3027. https://doi.org/10.1093/molbev/msab120.

Urumarudappa SKJ, Tungphatthong C, Jaipaew J, Pornputtapong N, Pakdeesattayapong D, Vimolmangkang S, Sukrong S. 2022. Development of a DNA barcode library of plants in the Thai Herbal Pharmacopoeia and Monographs for authentication of herbal products. Sci Rep 12: 9624. https://doi.org/10.1038/s41598-022-13287-x.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds). PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego. https://doi.org/10.1016/B978-0-12-372180-8.50042-1.

Woodson RE, Shery RW. 1945. Marantaceae. In Flora of Panama. Ann Missouri Bot Gard 32: 81-105.

Wu D, Kennedy H. 2000. Marantaceae. In: Wu ZY, Raven PH (eds). Flora of China. Vol. 24. (Flagellariaceae through Marantaceae). Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.

Samarina LS, Koninskaya NG, Shkhalakhova RM, Simonyan TA, Kuzmina DO. 2025. DNA-barcoding for cultivar identification and intraspecific diversity analysis of agricultural crops. Intl J Mol Sci 26 (14): 6808. https://doi.org/10.3390/ijms26146808.

Zhu S, Liu Q, Qiu S, Dai J, Gao X. 2022. DNA barcoding: An efficient technology to authenticate plant species of traditional Chinese medicine and recent advances. Chin Med 17: 112. https://doi.org/10.1186/s13020-022-00655-y.