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Abelmoschus rhodopentalus var. *binhsonensis*: A New Variety of *Abelmoschus rhodopentalus* in Quang Ngai Province, Vietnam Based on Morphological and DNA Barcode Analyses

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Abstract. In this study, we identified Nghia Sam found in Quang Ngai province by morphological and DNA barcode analyses including *mat*K, *trn*L-F IGS, and ITS. The results showed that Nghia Sam possessed morphological characters of *Abelmoschus rhodopentalus* such as linear epicalyx (<9), free, persistent; tuberous root; short-stemmed shrub; pink flowers; capsule hairy, and apically dehiscent. In addition, Nghia Sam differed from *A. rhodopentalus* var. *rhodopentalus* in having unbranched tuberous roots, branches spreading close to the ground, and pink-spotted flowers. Phylogenetic analysis confirmed that Nghia Sam belongs to the same clade with *A. rhodopentalus*. Based on current taxonomic keys, Nghia Sam is proposed as a new variety: *A. rhodopentalus* var. *binhsonensis*. These findings contribute to clarifying the classification and conservation value of this rare native genetic resource.

Keywords: *Abelmoschus rhodopentalus*, new variety, Nghia Sam, Quang Ngai

1 Introduction

The genus *Abelmoschus* (family Malvaceae) is notable for its high medicinal potential, with key biological activities such as antioxidant, anti-inflammatory, and hypoglycemic effects, thereby offering considerable value in the prevention and supportive management of various diseases [1]. It comprises a significant group of predominantly herbaceous flowering plants and possesses a complex taxonomic history. Species of this genus were initially classified as a subgroup of Hibiscus in 1737 [2], and it was later established as an independent genus by Medikus in 1787. At present, only 13 species are formally recognized worldwide [3].

Nghia Sam is a rare plant species with significant value in traditional Vietnamese medicine, primarily recorded in Binh Son Commune, Quang Ngai Province. Preliminary morphological assessments indicate that this species exhibits many characteristic traits of the genus *Abelmoschus*, with a high degree of similarity to *Abelmoschus rhodopentalus*. However, notable differences in stem, leaves, tubers, and flower color suggest that it may represent a new variety within *A. rhodopentalus* [4]. Currently, no scientific records provide a comprehensive and precise description of this species.

In response to this gap, the present study aims to determine the scientific nomenclature of Nghia Sam through an integrated approach

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combining morphological and genetic analyses. DNA sequences from the nuclear ITS region and the chloroplast regions *mat*K and *trn*L-F IGS were analyzed to clarify the genetic relationship of this species [5, 6]. The findings of this study will not only establish the taxonomic identity and characteristics of *A. rhodopentalus* var. *binhsonensis* but also contribute to elucidating its biological value, supporting conservation efforts, and promoting the development of this valuable genetic resource.

2 Materials and methods

Materials

Fresh Nghia Sam plants—with roots, stems, leaves, flowers, and fruits—were gathered from five distinct sites in Quang Ngai Province (coordinates: 15.23743° N, 108.83895° E; 15.22284° N, 108.84563° E; 15.22281° N, 108.84563° E; 15.21022° N, 108.86713° E; 15.23053° N, 108.87375° E). Each specimen was carefully cleaned, labeled with collection details, and stored at 4–10 °C to preserve its freshness for precise morphological and genetic studies. Additionally, young leaves of *A. rhodopentalus* were collected from the same area for genetic analysis.

Methods

The study applied the traditional descriptive method to analyze the morphological characteristics of Nghia Sam. Species identification was based on morphological comparisons following to the works of Do Thi Xuyen (2005), Vo Van Chi (2012), and the species identification key by Misra et al. (2023) to propose an initial scientific name [7, 8, 9, 10].

DNA was extracted from young Nghia Sam leaves using the CTAB method [11] with modifications. PCR was performed in a 20 µl reaction containing 1X Master Mix (Phu Sa Biochemical Co.), 0.5–1.0 pmol of each primer, and 80–100 ng of DNA. The ITS, *mat*K, and *trn*L-F IGS primer sets were used for species identification and genetic analysis. PCR amplification was carried out on an Aeris Thermal Cycler (ESCO, Singapore) with an initial denaturation at 95 °C for 3 min, followed by 30 cycles comprising denaturation at 95 °C for 30 s, primer-specific annealing at 58 °C for *trn*L-F, 56 °C for *mat*K, and 55 °C for ITS (30 s for each), extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. PCR products were mixed with GelRed nucleic acid stain (Biotium, USA) prior to loading and electrophoresed on a MultiSUB Midi system (Cleaver Scientific, UK) using 1% agarose gel, 1X TBE buffer, at 100V, 100mA for 70 min. DNA bands were visualized with an MS UVDI gel documentation system and compared with a DNA ladder for quality and size assessment.

Table 1. Sequences of primers used in the study

Gene	Primers	Seq (5′ – 3′)	Sources
*mat*K	3F_KIM-f	CGTACAGTACTTTTGTGTTTACGAG	[12]
	*mat*K_1R_kim	ACCCAGTCCATCTGGAAATCTTGGTCC	
ITS	ITS1	TCCGTAGGTGAACCTGCGG	[13]
	ITS4	TCCTCCGCTTATTGATATGC	
trnL-F IGS	E	GGTTCAAGTCCCTCTATCCC	[14]
	F	ATTTGAACTGGTGACACGAG	

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PCR products were sequenced at Apical Scientific, Malaysia, using the same primers as those used for amplification. Sequences were edited in MEGA 7 and verified for errors based on sequencing signals. Similarity and coverage of DNA sequences were assessed via BLAST-NCBI. Phylogenetic analysis of ITS, *mat*K, and *trn*L-F IGS regions was conducted using Maximum Likelihood (ML) with evolutionary distances calculated by the Tamura-Nei method. Bootstrap analysis (1,000 replicates) evaluated tree reliability. The final phylogenetic tree was processed using iTOL online software [15].

Morphological traits of *Abelmoschus* species were compared based on previous studies, encoded as binary or multistate characters. Data were standardized ($\mu = 0$, $\sigma = 1$) using YBAR in NTSYSpc 2.10e. A Neighbor-Joining tree based on Euclidean distances was constructed in the same software. Principal Component Analysis (PCA) was performed to assess trait relationships using the Eigen program in NTSYSpc [16].

3 Results and discussion

3.1 Morphological and location descriptions

Distribution — Binh Son Commune, Quang Ngai Province, Vietnam.

Habitat & Ecology — Species occurs both in the wild and under cultivation, typically in well-drained upland areas. Heliophilous, favoring humid climates with moderate to high atmospheric moisture. Substrates are generally sandy loam or light-textured soils, rich in organic matter and exhibiting good aeration. Commonly found along the margins of secondary forests, open slopes, fallow lands, and former agricultural plots. Phenology is seasonal, with flowering and fruiting recorded from April to October.

Description — Herbaceous, perennial, typically solitary. Stem short, much-branched, prostrate. Root system with a central, pale yellow, cylindrical tuber bearing numerous lateral roots (Fig 1A). Leaves simple, alternate; blade lanceolate to cordate, shallowly palmatilobate with crenate margins; petiole terete, green, both surfaces bearing stiff, white trichomes (Fig 1B). Flowers solitary in axillary position, actinomorphic, bisexual. Petals 5, free, with a narrow, dark red basal claw and a broadly ovate blade, white at base and red with conspicuous longitudinal veins (Fig 1C, D, F). Buds pre-anthesis are twisted counterclockwise (Fig 1G). Pedicel terete, slightly flared proximally, pubescent. Calyx gamosepalous in bud, splitting longitudinally at anthesis, lobes 2-fid, with 2–3 triangular teeth. Epicalyx of 7–10 free, linear segments (1 × 0.15 cm), green, 3-veined, externally pubescent; enclosing calyx in bud (prevalvate). Style filiform, white, glabrous, divided apically into 5 red arms ending in globose, pubescent stigmas. Staminal tube pink, 2–2.5 cm long, encasing the style and adnate to petal base (Fig 1 E); filaments mostly connate; anthers yellow, 1-celled, C-shaped, dorsifixed, longitudinally dehiscent (Fig 1H). Pollen spheroidal, ~120 µm, dark yellow. Ovary pyramidal, green, densely villous; 5 carpels, 5-locular, with 2 rows of ovules per locule (Fig 1I). Capsule pyramidal, slightly curved apically, green when young, turning dark with age, 5-ribbed, dehiscing loculicidally (Fig 1J, K). Seeds reniform, pale green when immature, black with brown concentric lines when mature, 0.22 × 0.32 cm (Fig 1L).

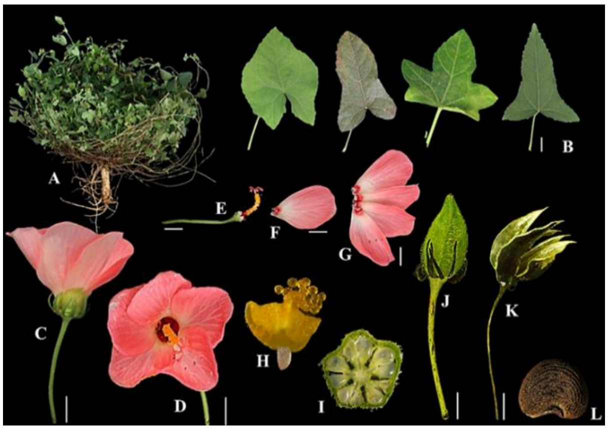

Fig. 1. A - General morphology of Nghia Sam; B - Leaf morphology; C, D - Flower morphology viewed horizontally and vertically; E - Stamens and pistils; F - Petals; G - twisted aestivation; H - Stamens (including filaments, anthers, and pollen grains); I - Ovary in cross-section; J - Young fruit; K - Mature fruit; L - Seeds

3.2 Comparison of the morphological characteristics of Nghia Sam with other species of the genus *Abelmoschus*.

To clarify the taxonomic position and characteristics of Nghia Sam, the morphological features of 11 species belonging to the genus *Abelmoschus* and Nghia Sam were evaluated. The characteristics focused on traits such as: stem length, stem morphology, root morphology, flower morphology, fruit morphology, and seed morphology. In addition, the morphological characteristics of *Hibiscus* × *rosa-sinensis* and *Hibiscus sabdariffa* were used as an outgroup to construct a phylogenetic tree.

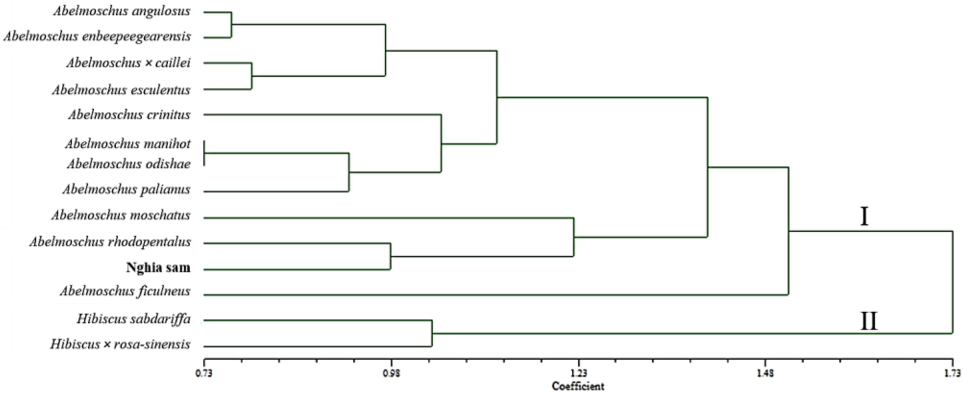

Fig. 2. Phylogenetic tree based on morphological characteristics of species of genus *Abelmoschus* and *Hibiscus*.

The phylogenetic tree results reveal two distinct groups corresponding to two major branches: Branch I comprises species of the genus *Abelmoschus*, while Branch II includes species of the genus *Hibiscus* (Fig 2). Nghia Sam belongs to Branch I and clusters within a smaller sub-branch alongside *A. rhodopentalus*. This indicates that Nghia Sam is a species of *Abelmoschus*, exhibiting the typical morphological characteristics of this genus. Among *Abelmoschus* species, Nghia Sam shares the highest similarity with *A. rhodopentalus*. This species is distributed in Vietnam and exhibits morphological traits similar to those of Nghia Sam, with three key distinguishing features: unbranched tuberous roots, the coloration of the central flower spot, and branching pattern. Specifically, *A. rhodopentalus* has pink flowers lacking a red spot in the center, whereas Nghia Sam possesses this red central spot. Additionally, *A. rhodopentalus* displays upright branches, while Nghia Sam's branches tend to spread along the ground. Geographically, both species are found in Quang Ngai and thrive under similar environmental conditions [4]. This further reinforces the hypothesis of a close genetic relationship between Nghia Sam and *A. rhodopentalus*.

3.3 Comparison of genetic characteristics of Nghia Sam and *A. rhodopentalus* through *mat*K, ITS, *trn*L-F IGS gene region sequences

In this study, samples of *A. rhodopentalus* and Nghia Sam were collected from Binh Son Commune, Quang Ngai Province to explore their genetic relationships. PCR amplification and agarose gel electrophoresis successfully generated clear DNA bands of approximately 700 bp, 500 bp, and 850 bp for the ITS, trnL-F, and matK primer pairs in both samples (Fig 3). These products exhibited high specificity and quality, meeting the criteria for reliable DNA sequencing.

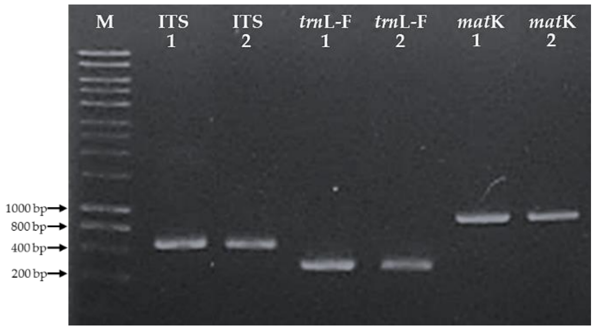

Fig. 3. Agarose gel electrophoresis of PCR products amplified with ITS, *trn*L-F, and *mat*K primers. Lane 1: Nghia Sam; Lane 2: *A. rhodopentalus*.

Subsequent sequence analysis of the *mat*K, *trn*L-F IGS, and ITS regions using BLAST confirmed that Nghia Sam belongs to the genus Abelmoschus. Furthermore, phylogenetic analysis revealed that Nghia Sam and *A. rhodopentalus* consistently clustered together within a well-supported clade (Fig. 4), demonstrating their close genetic relationship and clear distinction from other species in the genus.

The stability of this clustering pattern, together with the low genetic divergence between the two, suggests a shared genetic origin and provided no molecular evidence to justify their recognition as separate species. These results support the conclusion that Nghia Sam and *A. rhodopentalus* represent the same taxonomic entity, with the morphological differences observed in Nghia Sam likely reflecting intraspecific variation rather than species-level divergence.

Based on the comparison of morphological characteristics and sequences of characteristic gene regions, it can be preliminarily concluded that *A. rhodopentalus* and Nghia Sam are two different varieties of the same species. The proposed scientific name for Nghia Sam is *Abelmoschus rhodopentalus* var. *binhsonensis*, while the species *A. rhodopentalus* was previously identified as *Abelmoschus rhodopentalus* var. *rhodopentalus*.

Based on classification data from Flora of Vietnam [17], Plants of the World Online [3], and taxonomic keys for *Abelmoschus* described by Sutar [18] and Misra [10], the identification key for Nghia Sam is as follows:

1. Epicalyx linear, free, not enclosing flower buds, persistent in fruit, fewer than 9; tuberous root; small shrub, short stem; flowers pink; capsule covered with long, soft hairs, dehiscing at the apex *Abelmoschus rhodopentalus*

a. Branched tuberous root; erect branches; deeply lobed leaves with five distinct lobes; uniformly pink flowers, no spots at the center *Abelmoschus rhodopentalus* var. *rhodopentalus*

b. Unbranched tuberous root; branches spreading close to the ground; shallowly lobed leaves, usually with three or five lobes; pink flowers with a red spot at the center *Abelmoschus rhodopentalus* var. *binhsonensis*

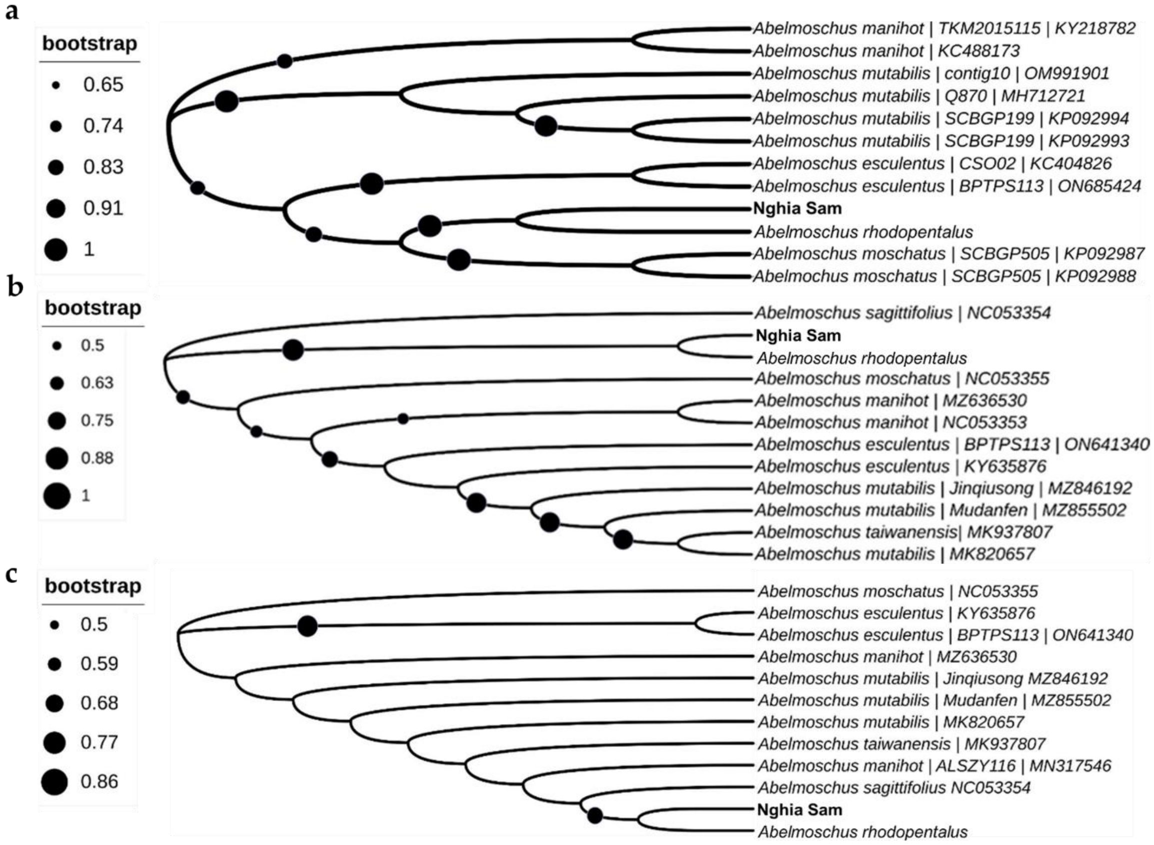

Fig. 4. Phylogenetic relationship of *Abelmoschus* species. (a) ITS, (b) *trn*L-F IGS, (c) *mat*K.

4 Conclusions

The combination of morphological characteristics and molecular data provides clear scientific evidence for the recognition of Nghia Sam as a new subspecies: *Abelmoschus rhodopentalus* var. *binhsonensis*. This finding not only contributes to refining the taxonomy of the genus *Abelmoschus*, but also sheds light on the value of a plant species historically documented in Quang Ngai. The re-identification of this plant through modern scientific approaches carries both taxonomic significance and potential for reviving indigenous knowledge associated with valuable genetic resources. Moreover, the study offers a new perspective for exploring regional plant diversity, phylogenetic relationships, and the sustainable utilization of *Abelmoschus* species.

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