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Micro-morphological and molecular characteristics of *Jasminum binhchauense*

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ABSTRACT

Jasminum binhchauense was recently described as a new species to science of which its specimen was found in Binh Chau-Phuoc Buu Nature Reserve, Vietnam. This study aims to characterize the DNA barcode and micro-morphological traits of *J. binhchauense* for the first time. As a result, the anatomical characteristics of the studied species were similar to those of *J. azoricum*. In addition, the *mat*K, *psa*B, and *psb*B regions of *J. binhchauense* were successfully amplified and sequenced and they were also deposited at the NCBI database with the accession numbers of PQ415075, PQ415076, and PQ415077, respectively. The pairwise alignment of the *mat*K, *psa*B, and *psb*B regions between *J. binhchauense* and *J. nervosum*, a morphologically resembled species, provided five, one, and two non-homologous locations, respectively.

KEYWORDS: Micro-morphology, Molecular markers, *Jasminum binhchauense*, Binh Chau-Phuoc Buu Nature Reserve

INTRODUCTION

Jasminum L., the largest genus belonging to the Oleaceae family, comprises over 200 accepted species widely found in subtropical and tropical zones like the Asian region or Pacific Islands (Chang *et al.*, 1996; Green, 1999; Newman *et al.*, 2007; Thanh *et al.*, 2023). In Vietnam, about 41 species, 1 variety, and 4 subspecies were also recorded by prior reports (Pham, 2000; Tran, 2003; Lee & Lee, 2013; Quang *et al.*, 2013, 2014, 2016, 2019, 2020; Thanh *et al.*, 2023). So many members of this genus include evergreen or deciduous shrubs which were grown for decoration in greenhouses, gardens, plant flowers, or smell flowers (Schmidt *et al.*, 2002; Singh, 2006). Notably, essential oils isolated from the flowers of the *Jasminum* plants have been reported to be used in the perfumery industry because of their special aroma and no synthesis by chemical pathways (Bose *et al.*, 2003).

With a large number of species, varieties, and subspecies, or cultivars, the accurate classification of the *Jasminum* species is inadequate and difficult once using the comparative morphological method. In 1970, Menninger demonstrated that the genus *Jasminum* included about 200 species (Menninger, 1970), another later report showed about 90 accepted species because many of these were synonyms, however (Muthukrishnan & Pappiah, 1980). Thus, the accurate classification using other taxonomic methods for *Jasminum* species is very important in their further research. Of these, the anatomical traits and the molecular markers are considered to play an important role in plant taxonomy. Therefore, the molecular and anatomical data contributes to distinguishing between morphologically resembled species within the genus (Dechbumroong *et al.*, 2018; Jeyarani *et al.*, 2018; de Melo Pereira *et al.*, 2023).

In 2023, *Jasminum binhchauense* B.H. Quang was described as a new species to science and its specimen was found in Binh Chau-Phuoc Buu Nature Reserve, Vietnam. The morphological characteristics of this species have been reported to differ from those of other related species by the following traits: branchlets; the elliptic or ovate to narrowly lanceolate leaf blade, the tomentose or pubescent calyx, many filiform lobes (Thanh *et al.*, 2023). To date, *J. binhchauense* is a rare plant and the

studies on this species are limited. The aim of this study, thus, is to characterize the DNA barcode and micro-morphological traits of *J. binhchauense*.

MATERIALS AND METHODS

Plant Materials

The specimen of *Jasminum binhchauense* was collected from Binh Chau-Phuoc Buu Nature Reserve, Xuyen Moc District, Ba Ria-Vung Tau Province, Vietnam. The vouchered specimen (NPN-1135BC) was deposited at the Herbarium of the University of Science, Vietnam National University-HCMC (PHH).

DNA Extraction, PCR Reaction and Sequencing Data Analysis

The total DNA isolated from the young leaves of *J. binhchauense* was performed using the CTAB 2X method with some modifications (Aboul-Maaty & Oraby, 2019). The three molecular markers, *mat*K, *psa*B, and *psb*B, were amplified using the Mastercycler PCR machine (Eppendorf, Germany) with the constituents of the PCR reaction as follows: (1) master mix (12.5 µL) (2) forward and reverse primer (1.25 µL of each primer), (3) deionized water (9.0 µL), (4) DNA sample (1.0 µL). PCR cycling conditions include 5 minutes at 95 °C; 35 cycles (1 minute at 95 °C, 1 minute at 55 °C and 1 minute 30 seconds at 72 °C); and a final extension at 72 °C for 10 minutes. PCR products were purified and sequenced at the LOCI Institute of Molecular Biology (Ho Chi Minh City, Vietnam). The *mat*K, *psa*B, and *psb*B regions were processed using the FinchTV and Seaview softwares. The Basic Local Alignment Search Tool (BLAST) on the NCBI (National Center Biotechnology Information) database was used to align these sequences to those of *Jasminum nervosum*, a morphologically resembled species with the studied plant.

Morphological Characteristics

The scientific name of the studied species was determined using comparative morphology. The details of the reproductive and vegetative traits of the studied specimens were compared with those from prior studies (Pham, 2000; Tran, 2003; Lee & Lee, 2013; Quang *et al.*, 2013, 2014, 2016, 2019, 2020; Thanh *et al.*, 2023).

Anatomical Characteristics

The leaf blade, petiole and branchlet of *J. binhchauense* were cut into the thin slices. The Javel water was used to bleach these slices and then, they were stained by the iodine green-carmine. The samples were washed with fresh water several times and kept in 10% glycerol (Truong *et al.*, 2007). The Olympus BX53 Digital Upright Microscope was used to observe and take photographs of studied specimens.

RESULTS AND DISCUSSION

Jasminum binhchauense B.H. Quang. AJB 2023, 45(3): 35 (Figure 1)

Type: B.H. Quang, L.V. Son 100 (HN) Binh Chau-Phuoc Buu Nature Reserve, Xuyen Moc district, Ba Ria-Vung Tau Province, Vietnam.

Studied specimens: NPN-1135BC (PHH!), Binh Chau-Phuoc Buu Nature Reserve, Xuyen Moc District, Ba Ria-Vung Tau Province, Vietnam

Distribution: The species was only distributed in Binh Chau-Phuoc Buu Nature Reserve.

Micro-morphological Traits

Branchlets (Figure 2)

The cross-section is nearly round. The epidermis consists of a layer of rectangular cells, cellulose walls or sclerenchymatous when the branchlet is old, quite uniform, with many unicellular protective hairs on the epidermal surface. The angular collenchyma includes 2-4 layers of polygonal cells, and cellulose walls, quite uniform. The cortical parenchyma consists of 2-4 layers of polygonal cells, cellulose walls, and closely arranged. In the angular collenchyma and cortical parenchyma regions, there are many scattered sclerenchyma fibroblasts with very thick cellulose walls. The endoderm is absent. The sclerenchymatous pericycle consists of 4-6 layers of polygonal cells, very thick walls impregnated with lignin, quite uniform in size, and arranged haphazardly. The primary phloem is arranged in small clusters in a ring under the pericycle, each cluster has 3-6 layers of polygonal cells, cellulose walls, small and irregular in size. The secondary phloem contained 2-4 layers of rectangular cells, and cellulose walls, quite uniform in size, arranged radially. The secondary xylem with xylem vessels, evenly distributed throughout the secondary xylem area in a continuous posterior pattern, the parenchyma in the secondary xylem consists of polygonal cells, narrow, walls impregnated with lignin arranged radially. The primary xylem bundle is located below the secondary xylem, each primary xylem bundle consists of 1-3 polygonal xylem vessels, lignin-impregnated walls, and centrifugal differentiation. The medullary parenchyma comprised polygonal cells with cellulose walls, arranged haphazardly to create small intercellular spaces.

Leaf blade (Figure 3)

The leaf blade includes the midrib and the main lamina on both sides. Midrib: The upper surface is flat or slightly concave, and the lower surface is convex. The epidermis consists of a layer of rectangular cells, closely arranged. The lower epidermis has a thicker cuticle layer and is smaller in size than the upper epidermis. There are unicellular protective hairs mainly on the upper surface. The angular collenchyma includes 1-2 cell layers located under the upper epidermis and 5-7 cell layers above the lower epidermis, polygonal in shape, and irregular in size. The parenchyma consists of polygonal cells, and cellulose walls, irregular in size, surrounding the vascular bundles. The vascular bundle is arched with the xylem above and the phloem below. The xylem vessels are arranged in rows of 1-5 xylem vessels, they gradually enlarge towards the underside of the leaf. The xylem

Figure 1: *Jasminum binhchauense* B.H.Quang. a) Habit, b) Leaf shapes, c) Apex, adaxial and abaxial views of leaf, d) Petiole, e) Branchlets, f) Inflorescence, g) Top and bottom-up views of flower with corolla lobes, h) Longitudinal section of flower, i) External views of anther, j) Ovary with calices and k) Ovaries with transverse and longitudinal sections

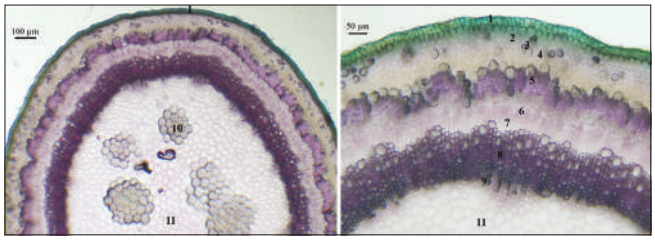

Figure 2: The cross-section of branchlets. 1-epidermis, 2-angular collenchyma, 3-sclerenchyma fiber, 4-cortical parenchyma, 5-sclerenchymatous pericycle, 6-primary phloem, 7-secondary phloem, 8-secondary xylem, 9-primary xylem, 10-cluster of sclerenchyma fibers, 11-medullary parenchyma

parenchyma consists of polygonal cells, and cellulose walls, sometimes impregnated with lignin, located between the xylem vessel rows. The primary phloem includes 2-4 layers of irregular, polygonal cells with cellulose walls. Main lamina: The upper and lower epidermis consists of a single layer of rectangular cells with cellulose walls. The lower epidermis is smaller than upper the epidermis and they has several secretory hairs (unicellular angle, multicellular head) while the upper epidermis has some unicellular protective hairs. The mesophyll has an asymmetrical heterophylly with chlorenchyma on the upper surface and spongy parenchyma on the lower surface. The chlorenchyma includes 3-4 layers of short rectangular cells, cellulose walls, quite uniform, closely packed, and perpendicular to the upper epidermis. The spongy parenchyma consists of polygonal cells with cellulose walls, arranged haphazardly to create small intercellular spaces. There are small vascular bundles arranged in rows in the mesophyll region.

Petiole (Figure 4)

The upper surface is concave and the lower surface is convex, and its structures are similar to the midrib of the leaf blade. The

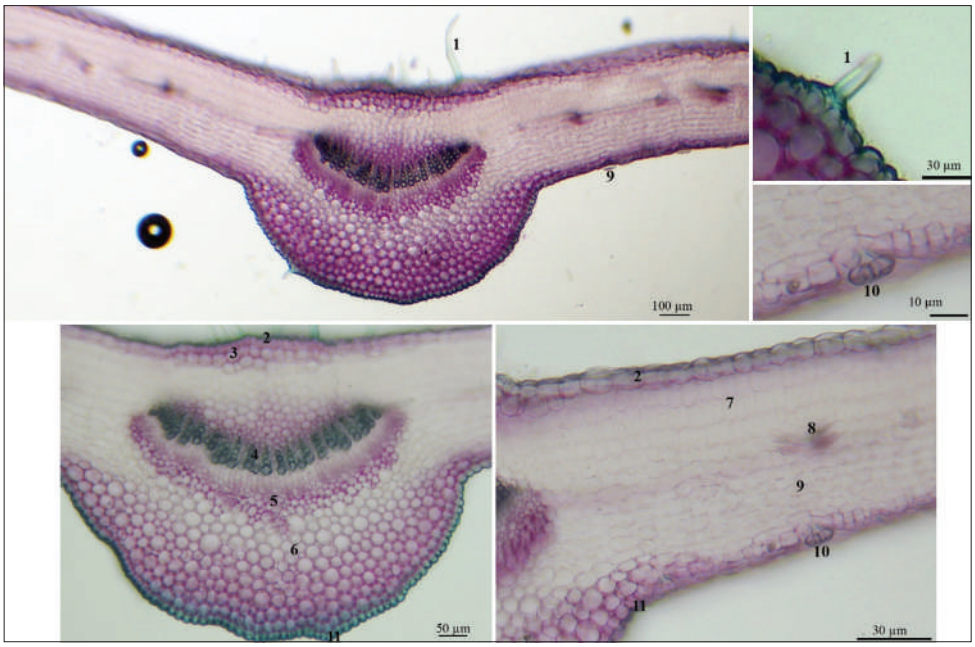

Figure 3: The cross-section of leaf blade. 1-unicellular protective hairs, 2-upper epidermis, 3-angular collenchyma, 4-xylem, 5-phloem, 6-parenchyma, 7-chlorenchyma, 8-vascular bundle, 9-spongy parenchyma, 10-secretory hairs (unicellular angle, multicellular head), 11-lower epidermis

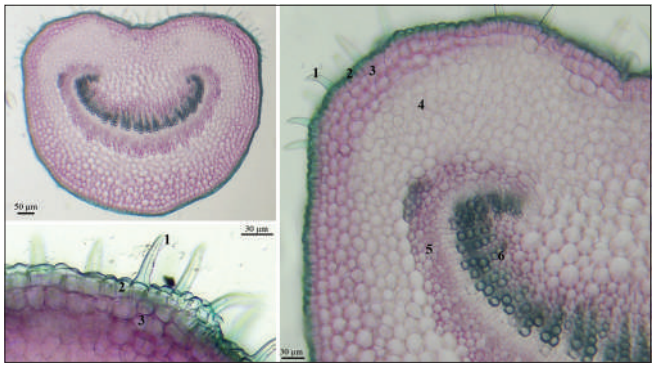

Figure 4: The cross-section of petiole. 1-unicellular protective hairs, 2-epidermis, 3-angular collenchyma, 4-parenchyma, 5-phloem, 6-xylem

epidermis of the petiole has many unicellular protective hairs, mainly on the upper surface.

Molecular Characteristics

In the current study, the *mat*K, *psa*B, and *psb*B regions of *J. binhchauense* were successfully amplified and sequenced in which the final lengths of these regions were 759, 660, and 661 bps, respectively. All sequences were also deposited at the NCBI database with the accession numbers of PQ415075, PQ415076, and PQ415077, respectively. In 2023, *J. binhchauense* was described as a new species to science and its specimen was found in Binh Chau-Phuoc Buu Nature Reserve, Vietnam. The morphological characteristics of this species have been reported to closely relate to *J. nervosum* and *J. ledangense* but differ from the morphologically allied species by the following traits: branchlets; the elliptic or ovate to narrowly lanceolate leaf blade, the tomentose or pubescent calyx, many filiform lobes (Thanh *et al*., 2023). More recently, the complete chloroplast genome of *J. nervosum* was also sequenced and deposited at the NCBI database with the accession numbers of NC_085477.1 by Le and Do (2024) whereas no information about DNA sequences of *J. ledangense* has been recorded so far. In this study, thus, *mat*K, *psa*B, and *psb*B regions of *J. binhchauense* were aligned to those of *J. nervosum* from the previous report (Le & Do, 2024). As a result, the pairwise alignment of the *mat*K region between *J. binhchauense* and *J. nervosum* provided 5 non-homologous (Figure 5a). Additionally, in Figure 5b there were only one non-homologous within the *psa*B region of two species were recorded while two non-homologous locations were found in the *psb*B region (Figure 5c).

DISCUSSION

The anatomical traits of the stem, petiole, and leaf blade of *J. azoricum* were also demonstrated by prior report (de Melo Pereira *et al*., 2023). Overall, the micro-morphological characteristics of the leaf blade of *J. binhchauense* were similar to those of *J. azoricum* in having: Leaf blade includes the midrib and the main lamina on both sides; The upper surface of the midrib is flat or slightly concave, the lower surface is convex; the epidermis consists of a layer of rectangular cells, closely arranged, the lower epidermis has a thicker cuticle layer and is smaller in size than the upper epidermis; the vascular bundle is arched with xylem above and phloem below, the xylem vessels are arranged in rows of 1-5 xylem vessels, they gradually enlarge towards the underside of the leaf; the upper and lower epidermis of main lamina consists of a single layer of rectangular cells with cellulose walls. However, the species from this study can be distinguished from *J. azoricum* in having: the epidermis has scattered unicellular protective hairs; the spongy parenchyma

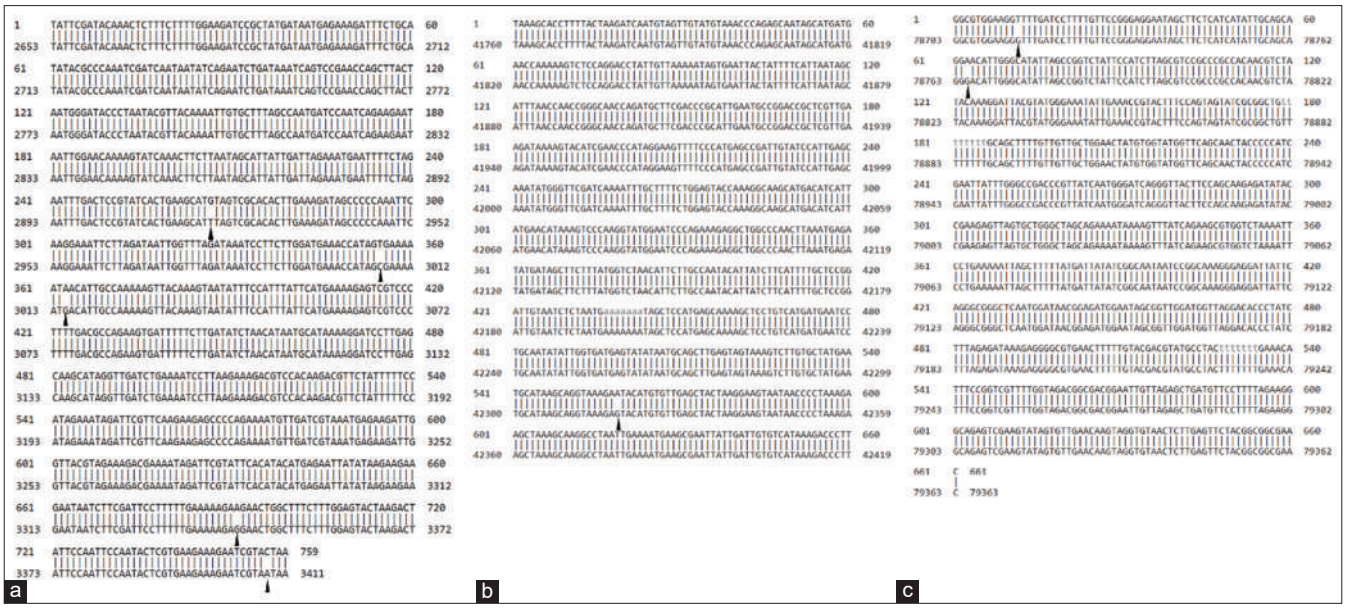

Figure 5: Pairwise sequence alignment of three studied regions between *Jasminum binhchauense* and *Jasminum nervosum*. a) *mat*K, b) *psa*B and c) *psb*B, the upper rows belong to *J. binhchauense* while lower rows are included to *J. nervosum*

consists of polygonal cells, arranged haphazardly to create small intercellular spaces (the unicellular protective hairs are absent; the spongy parenchyma consists of polygonal or round cells, closely arranged and does not create small intercellular spaces in *J. azoricum*). In addition, the cross-section of the petiole of both species was concave on the upper surface and convex on the lower one. Meanwhile, the epidermis of the petiole of *J. binhchauense* has many unicellular protective hairs, mainly on the upper surface while the unicellular protective hairs are absent in *J. azoricum* (de Melo Pereira *et al.*, 2023).

Studies provided the importance of using the DNA barcodes to classify and trace the evolution of *Jasminum* genus. For instance, Jeyarani *et al.* (2018) used chloroplast regions (*mat*K, *trn*H-*psb*A, and *trn*L-F), and nrDNA (ITS) to establish the phylogenetic tree of 22 species and one variety of *Jasminum* in India. Dechbumroong *et al.* (2018) showed the specific multiplex PCR based on the ITS2 region to identify the medicinal plants from Thailand such as *Jasminum adenophyllum*, *Jasminum sambac*, *Jasminum* sp. Based on ITS region, Mai *et al.* (2020) distinguished the two subspecies of *Jasminum annamense* collected from Vietnam, including *J. annamense* subsp. *glabrescens* and *J. annamense* subsp. *annamense*. Anakha and Hari (2021) used two DNA barcodes, *trn*H-*psb*A and *mat*K, to distinguish two *Jasminum* plants grown in India, including *J. auriculatum* and *J. fluminense*. The nrITS and *rbc*L regions of *Jasminum grandiflorum* were also successfully sequenced by Kumar *et al.* (2022).

Xu *et al.* (2024) established the phylogenetic tree to provide the evolutionary patterns and phylogenetic relationships among 7 *Jasminum* species (*J. sambac*, *J. multiflorum*, *J. floridum*, *J. dichotomum*, *J. nudiflorum J. odoratissimum*, and *J. auriculatum*) and other plants in the family Oleaceae using 12 regions of chloroplast genomes. Recently, the complete chloroplast genome belonging to the genus *Jasminum* was also sequenced and compared with the large single-copy sequences from other related species, including *J. sambac* (Qi *et al.*, 2020) and *J. nudiflorum* (Lee *et al.*, 2007), *J. multiflorum*, and *J. nervosum* (Le & Do, 2024). In 2014, Banfi transferred some *Jasminum* species with special characteristics, yellow flowers and alternate leaves, to a new genus *Chrysojasminum* (Banfi, 2014). More recently, the complete chloroplast genome of *Chrysojasminum subhumile* was also investigated and based on the phylogenetic tree, *C. subhumile* was grouped with other species of the genus *Jasminum* (Ling *et al.*, 2023).

CONCLUSION

The current report demonstrated the DNA barcode and micro-morphological traits of *J. binhchauense*. The results from this study not only have scientific significance in supplement knowledge about this new species, but also potential applications in the field of the pharmaceutical field.

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