

***CAMELLIA SINENSIS* VAR. *MADOENSIS* (SECT. *THEA*, THEACEAE), A NEW TAXON FROM VIETNAM**

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Abstract

Camellia sinensis var. *madoensis* is described and illustrated as a new variety of *Camellia sinensis* (section *Thea*, *Theaceae*) from Xuan Loc Commune, Song Cau District, Phu Yen Province. The new variety is easily distinguishable from *C. sinensis* var. *sinensis* by style free ½ to the base. The ITS sequence of this variety is also different from that of *Camellia sinensis* and its other varieties, while the *matK* gene sequences are nearly identical among *Camellia* taxa.

Keywords: *Camellia sinensis*; ITS; Ma Do; *matK*; Section *Thea*; *Theaceae*; Vietnam.

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1. INTRODUCTION

The scientific name of tea is *Camellia sinensis* (L.) O. Kuntze. It is a shrub or small tree with broad evergreen leaves belonging to *Camellia* section *Thea* in the Theaceae (Pierre, 1887; Sealy, 1958). Several varieties of tea have been described and illustrated, including *C. sinensis* var. *sinensis*, *C. sinensis* var. *assamica* (Masters) Kitamura, *C. sinensis* var. *pubilimba* Hung T. Chang, and *C. sinensis* var. *dehungensis* (Hung T. Chang & B. H. Chen) T. L. Ming. It is distributed over countries and/or territories of Taiwan, China, northeast India, southern Japan, South Korea, Laos, Myanmar, Thailand, and Vietnam (Chang & Bartholomew, 1984; Ming & Bartholomew, 2007; Nguyen, 2017; Pham-Hoang, 1999; Sealy, 1958). All varieties of *C. sinensis* are used as tea. In recent surveys in July 2019 and October 2020, we collected specimens of tea, and after analyzing and comparing morphological characteristics and ITS sequences with other varieties of tea, we assert that this is a new taxon. The scientific name of this new taxon is *Camellia sinensis* var. *madoensis*, and its local name is Ma Do tea. The recognition of Ma Do tea as a new taxon helps to better understand the diversity of tea taxa and contributes to the scientific basis for conservation, development, and sustainable exploitation of this endemic plant resource.

2. MATERIALS AND METHODS

2.1. Materials

Materials for taxonomic treatment were individuals belonging to the natural Ma Do tea population and specimens collected from them. Three leaf samples from different individuals were taken as materials for phylogenetic analysis.

2.2. Methods

Taxonomic treatment was performed according to routine methods of comparing anatomical morphology.

Phylogenetic analysis was performed on the basis of the nuclear ITS1-5.8S rRNA-ITS2 sequence region and the chloroplast *matK* gene sequence.

DNA extraction from leaf samples was performed on the basis of the CTAB I protocol (Weising et al., 2005). The ITS1-5.8S rRNA-ITS2 sequence region was amplified using the primer pairs of ITS 5P (5'-GGA AGG AGA AGT CGT AAC AAG G-3') and ITS 8P (5'-CAC GCT TCT CCA GAC TAC A3') according to the method described in Möller and Cronk (1997). The *matK* gene sequence was amplified using the primer pairs *matK472F* (5'-CCC RTY CAT CTG GAA ATC TTG GTT C-3') and *matK1248R* (5'-GCT RTR ATA ATG AGA AAG ATT TCT GC-3') according to the method described in Yu et al. (2011). The primers used for amplification were also used for sequencing by the Sanger method.

Sequences obtained after sequencing were compared to find homologous sequences from GenBank for phylogenetic analysis using the BLAST tool. MEGA 5.1 software (Tamura et al., 2011) was used to align sequences, determine variable locations,

calculate genetic distances, and construct a dendrogram for phylogenetic relationships among the surveyed taxa.

3. RESULTS

3.1. Taxonomic treatment

Camellia sinensis var. *madoensis* T. V. Nguyen, V. D. Luong & N. T. Le var. nov. (Figures 1, 2)

Type:—VIETNAM. Phu Yen Province: Song Cau District, Xuan Loc Commune, 400–500 m elevation, 22 July 2019, Nguyen Tran Vu, Luong Van Dung DL190702 (holotype, isotype, DLU); 10 October 2020, Nguyen Tran Vu, Luong Van Dung DL201001 (DLU)

Description: Small or medium trees, 4–8 (10) m tall, evergreen; young branches sparsely pubescent to glabrous, purple; older branches gray. Leaves stalked, broad elliptic, 7.0–10.5 cm long, 3.0–4.7 cm wide, lamina thin, coriaceous; apex acute to attenuate, base obtuse to broadly obtuse, margins regularly serrate; above dark green, shiny and glabrous, below light green and sparsely villous, generally on the midrib; midribs and lateral veins sunken above, protruding below; secondary venation pinnate with 7–10 pairs of veins; petiole stout, 0.5–0.7 cm long, slightly curved, round, purple in color, sparsely villous. Flowers solitary or 2–(3), axillary, nodding, 2.7–3.3 cm diameter; pedicel 1.2–1.5 mm long, thickened toward the apex, glabrous, pale blue-purple in color. Bracteoles 2–3, ovate, 1.8–2.0 mm long, 1.0–1.5 mm wide, pale blue, glabrous, ciliolate, deciduous. Sepals 5–6, broadly ovate to suborbicular, 3.0–4.5 mm long, 3.0–4.0 mm wide, pale green, glabrous, ciliolate, persistent. Petals 6, outer whorl 2, subglobose, 1.1–1.3 cm long, 0.8–1.0 cm wide, green-white, glabrous; inner whorl 4, broadly obovate, 1.4–1.7 cm long, 1.1–1.4 cm wide, white, glabrous; inner whorl petals united with outermost filaments 1.5–2.0 mm at the base. Androecium numerous stamens in 3–4 circles, filaments 0.8–2.2 cm long, light yellow, glabrous, outer filaments united 3.0–4.0 mm from the base and form a short cup; anthers yellow, about 1.5 mm. Gynoecium (2)–3 loculi, ovary ovate, 2.0–3.0 mm long, 2.0–2.5 mm wide, densely white tomentose; style 3, free $\frac{1}{2}$ to the base, 1.0–1.2 cm long, glabrous. Capsule globose to depressed globose, 1–3 locular, 2.0–3.5 cm in diameter, 2.0–2.5 cm high; seeds 1–2 per loculus, globose or hemispherical 1.4–1.8 cm long, 1.2–1.5 cm wide, glabrous; pericarp 2.0–2.5 mm thick, sparsely pubescent.

Phenology: Flowers from May to July and fruits from October to December.

Distribution and ecology: The distribution of this species is known from Ma Do Mountain, Xuan Loc Commune, Song Cau District, Phu Yen Province. It is located in secondary forests on rocky mountain slopes or in valleys.

Etymology: The specific epithet refers to the type location, Ma Do Mountain, Xuan Loc Commune, Song Cau District, Phu Yen Province, Vietnam.

Use: Leaves and buds are harvested and used as tea.

Note: The new taxon has many characteristics of section *Thea* (Linnaeus) Dyer in J. D. Hooker, such as pedicel thick, thickened toward the apex; bracteoles 2(or 3), caducous; sepals persistent; petals white, basally connate; stamens numerous, in 4 or 5 whorls, glabrous, outer filament whorl basally connate into a tube. Ovary 3–5-loculed; style apically 3–5-parted or lobed (Ming & Bartholomew, 2007; Sealy, 1958). The new taxon is easily distinguishable from *C. sinensis* var. *assamica* and *C. sinensis* var. *dehungensis* by ovary tomentose (vs. ovary glabrous at least apically); from *C. sinensis* var. *pubilimba* by sepals glabrous (vs. sepals outside pubescent); and from *C. sinensis* var. *sinensis* by style free ½ to the base (vs. style fused, apically 3-lobed). The known variants of *Camellia sinensis* can be distinguished using the following key.

- 1a. Ovary densely tomentose.....2
- 1b. Ovary glabrous at least apically.....4
- 2a. Sepals outside white pubescentvar. *pubilimba*
- 2b. Sepals outside glabrous.....3
- 3a. Style free ½ to the base.....var. *madoensis*
- 3b. Style fused, apically 3-lobed.....var. *sinensis*
- 4a. Leaf blade abaxially densely spreading villous along midvein.....var. *assamica*
- 4b. Leaf blade abaxially appressed pubescent.....var. *dehungensis*

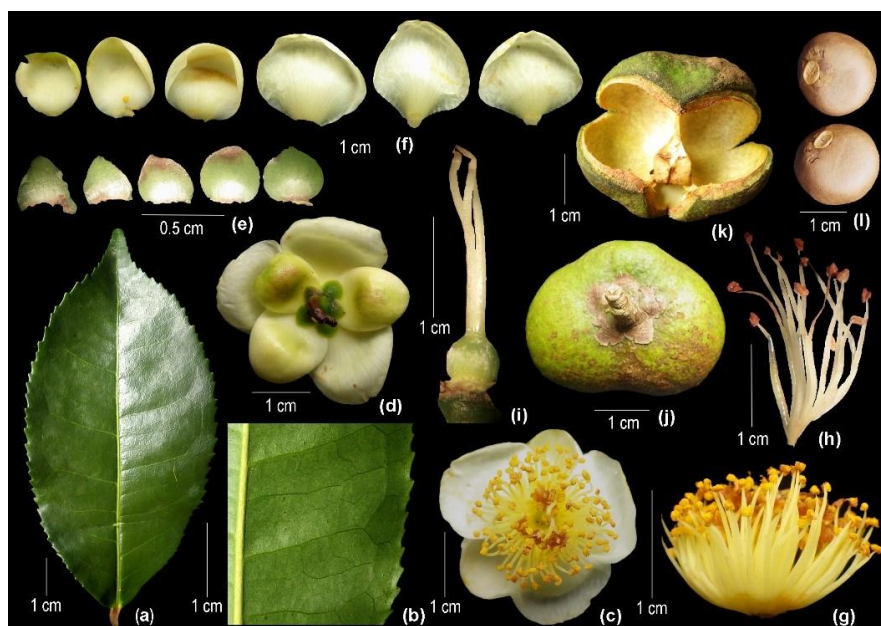


Figure 1. *Camellia sinensis* var. *madoensis* T. V. Nguyen, V. D. Luong & N. T. Le

Notes: (a) Leaf (adaxial view); (b) Venation detail of leaf (abaxial view); (c) Flower (top view); (d) Flower (back view); (e) Sepals; (f) Petals; (g) Androecium; (h) Stamens; (i) Gynoecium; (j) Fruit; (k) Capsule after dehiscence; (l) Seeds.

Source: Photos by Luong Van Dung.



Figure 2. *Camellia sinensis* var. *madoensis* T. V. Nguyen, V. D. Luong & N. T. Le

Notes: (a) Natural plant; (b) Leader of field trip that recorded Ma Do tea; (c) Shoots and leaves;
(d) Flower buds; (e) Flowers; (f), (g) Fruit.

Source: Photos by Luong Van Dung.

3.2. Phylogenetic analysis

The sequencing results showed that the ITS1-5.8S rRNA-ITS2 region sequences of three Ma Do tea samples were identical, and the length of the clear region was 669 bp.

The results of a sequence comparison on GenBank using the BLAST tool showed that the 100 sequences most nearly identical (94.84–99.19%) to ITS1-5.8S rRNA-ITS2 sequences of Ma Do tea were homologous sequences of the taxa belonging to the *Camellia* genus, including *C. sinensis*, *C. sinensis* var. *sinensis*, *C. sinensis* var. *assamica*, *C. chekiangoleosa*, *C. tachangensis* var. *remotiserrata*, and *C. liberistamina*.

The genetic distances between Ma Do tea and *Camellia sinensis* varieties, including *C. sinensis* var. *sinensis*, *C. sinensis* var. *assamica*, and other *Camellia* taxa, range from 0.0081 to 0.0195 based on the ITS region. The genetic distances were calculated using MEGA 5.1 software. The phylogenetic relationship dendrogram was constructed on the basis of the genetic distances using the maximum likelihood method, with *Stewartia rostrata* as the outgroup (Figure 3).

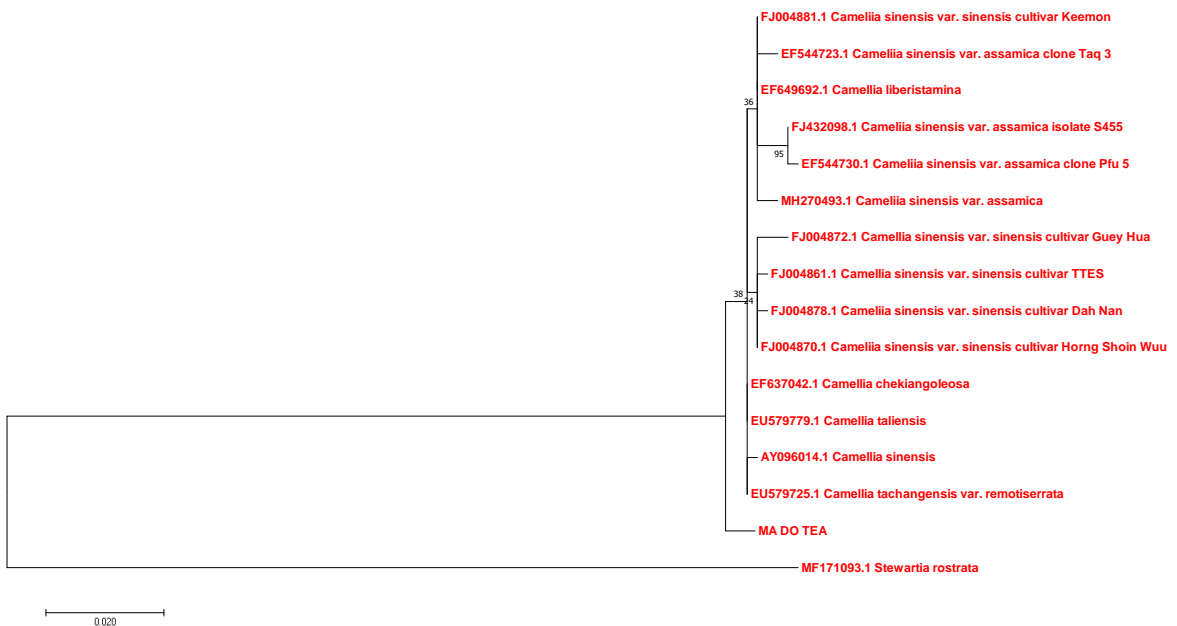


Figure 3. Phylogenetic relationship between Ma Do tea and other *Camellia* taxa based on the ITS sequence using the maximum likelihood method

The sequencing results showed that the *matK* gene sequences of three Ma Do tea samples were identical, and the length of the clear region was 860 bp. A sequence comparison on GenBank using the BLAST tool showed that the 100 sequences most nearly identical (99.88–100.00%) with the *matK* gene sequences of Ma Do tea were homologous sequences of the taxa belonging to the *Camellia* genus, including *Camellia sinensis*, *C. sinensis* var. *sinensis*, *C. sinensis* var. *assamica*, *C. sinensis* var. *lasiocalyx*, *C. atrothea*, and *C. ptilophylla*.

The genetic distances between Ma Do tea and *Camellia sinensis* and its varieties, including *C. sinensis* var. *sinensis*, *C. sinensis* var. *assamica*, and *C. sinensis* var. *lasiocalyx*, range from 0.0000 to 0.0012 on the basis of the *matK* gene. Ma Do tea cannot be identified from several sources belonging to *Camellia sinensis* and *C. sinensis* var. *sinensis*. Based on these genetic distances, a phylogenetic relationship dendrogram was constructed using the maximum likelihood method with *Stewartia rostrata* as the outgroup (Figure 4).

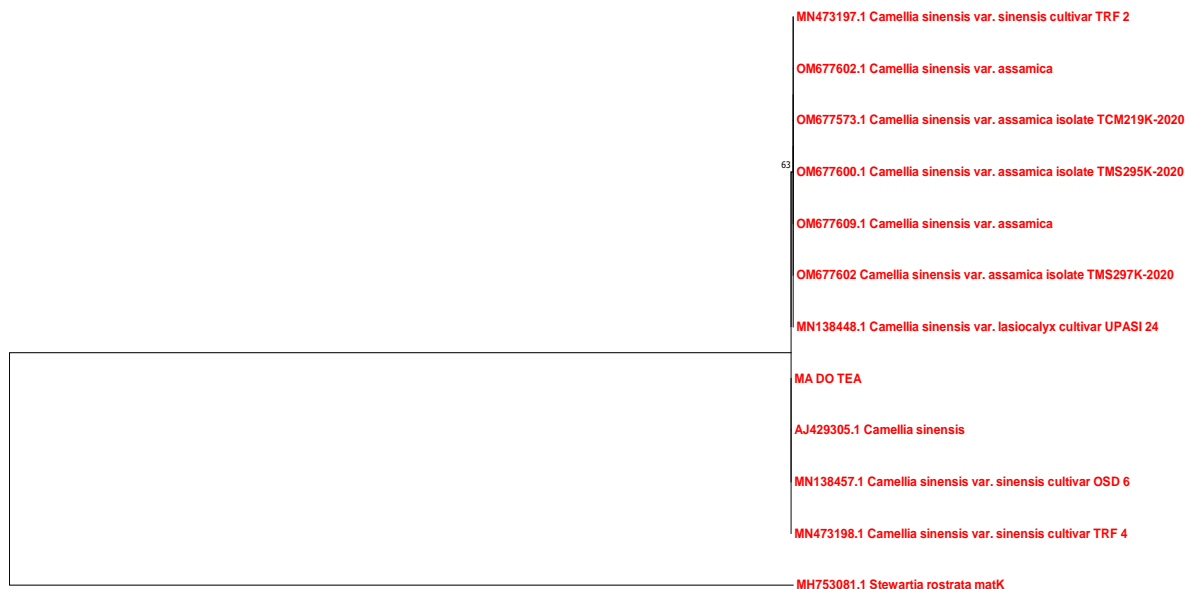


Figure 4. Phylogenetic relationship between Ma Do tea and other *Camellia* taxa based on the *matK* gene sequence using the maximum likelihood method

The results of phylogenetic analysis show that the ITS region and *matK* gene sequences of Ma Do tea are closely grouped with *Camellia* taxa, particularly *C. sinensis* and its varieties. However, based on the sequence of the ITS region, Ma Do tea has certain differences with the investigated *Camellia* taxa, and this supports the identification of Ma Do tea as a separate taxon that does not overlap with published varieties of *Camellia sinensis*, such as *C. sinensis* var. *sinensis* or *C. sinensis* var. *assamica*. Based on the *matK* gene sequence, Ma Do tea is identical to several sources of *C. sinensis* var. *sinensis* released on GenBank.

Combining the morphological data (especially the style characteristics) and the molecular data (especially the sequence of the ITS region), it is possible to identify Ma Do tea as a new variety of *C. sinensis*.

4. CONCLUSIONS

Ma Do tea possesses its own morphological and molecular characteristics that are different from those of other varieties of *Camellia sinensis* varieties. This is the scientific basis to identify this tea as a new taxon from Vietnam with the scientific name of *Camellia sinensis* var. *madoensis*.

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