


## The complete chloroplast genome of *Vitex trifolia* L. (Lamiaceae)

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### ABSTRACT

The three-leaved chaste tree (*Vitex trifolia*) is a medicinal and ornamental plant widely distributed from East Africa to the Pacific but has no complete chloroplast genome sequence. We assembled and characterized the *V. trifolia* accession from the germplasm collection of the Institute of Crop Science, University of the Philippines Los Baños. The complete plastome sequence is 154,444-bp long with 131 coding genes comprising 87 mRNA genes, 36 tRNA genes, and 8 rRNA genes. A phylogenetic analysis of the assembled genome, together with nine other Lamiaceae species, identified *V. rotundifolia* as its closest relative with available complete cpDNA sequence. The clustering also supports the genotypic similarity of the species belonging to *trifolia* group of the genus *Vitex*.

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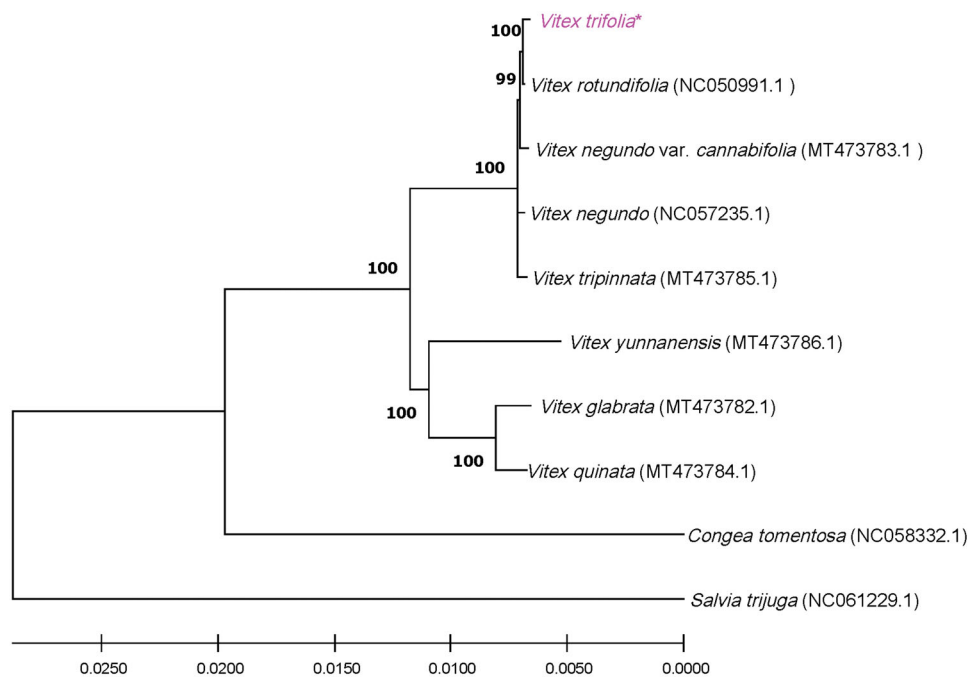
Verbenaceae; Viticoideae;  
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three-leaved chaste tree

*Vitex trifolia* Linnaeus, 1753 (Lamiaceae), is known for its medicinal and ornamental uses. It has a wide native range from East Africa to the Pacific (POWO 2022). It is under the subfamily Viticoideae which is close to the subfamily Symphorematoideae based on plastome phylogenomics (Zhao et al. 2021). Commonly known as the three-leaved chaste tree, it is delineated from other chaste tree species under the same genus through commonly observed trifoliate leaves with sessile to subsessile terminal leaflets, sessile lateral leaflets with visible secondary veins, shrubs to small trees growth habit, and a fruiting calyx that partly encloses its fruit (de Kok 2019). It has antibacterial (Natheer et al. 2012), anthelmintic (Thenmozhi et al. 2013), anti-cancer (Vasanthi, Radhjayalakshmi, and Nasrin 2014), anti-inflammatory (Goverdhan and Bobbala 2009), and anti-asthmatic (Wahyuono et al. 2009) properties. In the Philippines, it is often called 'lagunding dagat' or 'lagundi' – a popular medicinal crop in the country, that is often attributed to the species name *Vitex negundo* L. Despite its importance and distribution, there is still no published complete chloroplast genome sequence; hence, in this study, we assembled, annotated, and characterized the complete plastome of *V. trifolia*.

The germplasm collection of *V. trifolia* was conserved at the field genebank of the Crop Breeding and Genetic Resources Division, Institute of Crop Science, University of the Philippines Los Baños, Laguna, Philippines with a type locality at Bingawan, Iloilo, Philippines (11° 13' 58.79338" N, 122° 34' 2.03563" E). The voucher specimen (ICROPS 19332) was deposited in the Philippine Herbarium of Cultivated Plants of the Institute of Crop Science, University of the Philippines Los

Baños, Laguna, Philippines (<https://cafs.uplb.edu.ph/icrops/>, Renerio P. Gentallan Jr., [rpgentallan@up.edu.ph](mailto:rpgentallan@up.edu.ph)). Fresh leaves of *V. trifolia* were extracted using a slightly modified CTAB protocol of Doyle and Doyle (1978). The DNA sample was sent to NovogeneAIT Genomics Singapore PTE LTD, Singapore, for sequencing using HiSeq-PE150 platform (Illumina Inc., San Diego, CA, USA). This generated 3.5 Gbp of 150-bp paired-end reads which were subsequently filtered to produce 23,246,368 cleaned reads. The GetOrganelle v1.7.5+ software (Jin et al. 2020) was used to assemble the chloroplast genome, generating a circular genome that was subsequently annotated and mapped using CPGAVAS2 (Shi et al. 2019) and was visualized using OGDRAW (Greiner, Lehwark, and Bock 2019). The assembled chloroplast genome sequence was submitted to GenBank with the accession number OM868083.

We assembled a complete chloroplast genome (cpDNA) of *V. trifolia* with a sequence length of 154,444 bp. It follows the characteristic quadripartite circular structure of a cpDNA exhibiting a pair of inverted repeat (IRs) regions of 25,687 bp each, a short single-copy region of 17,922 bp (SSC), and a long single-copy (LSC) region of 85,148 bp. The base composition of the genome is 30.5% A, 31.3% T, 18.8% G, and 19.4% C, yielding a GC content of 38.3% which is similar to *V. rotundifolia* (NC050991.1); however, the *V. trifolia* plastome is 74 bp longer (Jo et al. 2021). *V. trifolia* has longer LSC and SSC regions compared to *V. rotundifolia*, but they are equal in length for the inverted repeat regions. It encodes 131 genes, comprising 87 mRNA genes, 36 tRNA genes, and 8 rRNA genes. Among these are 45 genes for photosynthesis, 28



**Figure 1.** Phylogenetic tree reconstructed using maximum likelihood (ML) method based on complete chloroplast genome sequences of the 10 Lamiaceae species with *Salvia trijuga* as the outgroup. Numbers above the lines represent ML bootstrap values (>99%).

genes for self-replication, 7 unknown conserved open reading frames (*ycf1* x 2, *ycf15* x 2, *ycf2* x 2, *ycf4*) and 6 other genes (*accD*, *ccsA*, *cemA*, *clpP*, *infA*, *matK*).

Complete chloroplast DNA sequences were downloaded from the NCBI database of Lamiaceae species, comprising seven *Vitex* species and two other species from other genera. The downloaded sequences, together with the assembled genome sequence of *V. trifolia*, were aligned using MAFFT (Kato and Standley 2013). Phylogenetic analysis of the downloaded sequences was done using MEGA-X (Kumar et al., 2018) which generated a Maximum Likelihood (ML) tree using Jukes-Cantor model (Jukes and Cantor 1969) with 1,000 bootstrap replicates. The analysis indicated that *V. trifolia* is closely related to *V. rotundifolia* (Figure 1). The close relationship between the two *Vitex* species has been observed in their nomenclatural history as *V. rotundifolia* was previously subsumed under *V. trifolia* subsp. *littoralis* Steenis. The observed topology also supports the *trifolia* group of the genus *Vitex* where *V. rotundifolia* and *V. negundo* also belong (de Kok 2007), which provides additional insights into the evolutionary relationship of species in the genus *Vitex*.

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## Author contributions

All authors agree to be accountable for all aspects of the work. Renerio P. Gentallan Jr. conceptualized the study, performed the experiments, analyzed the data, and wrote the paper; Kristine Joyce Quiñones,

Michael Cedric Bartolome, Roselle Madayag, Jessabel B. Magtoltol, Reneliza Cejalvo, Bartimeus Alvaran, Juan Rodrigo Vera Cruz and Bartimeus Alvaran planted, collected and prepared the germplasm, pressed the herbarium samples, reviewed literature, performed experiments, edited the paper; Teresita H. Borromeo, Nestor Altoveros, Leah Endonela, and Antonio Lalusin helped conceptualized the study, validated the design of the experiment and the data presented, reviewed drafts of the paper.

## Ethical approval

The collection of plant material was carried out in accordance with guidelines provided by the authors' institution (Institute of Crop Science, College of Agriculture and Food Science, University of the Philippines Los Baños).

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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## Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov> under the accession no. OM868083. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA824823, SRR18689873, and SAMN27479778, respectively

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