

## The complete chloroplast genome of *Phyla nodiflora* (Linnaeus) Greene (1899) from the Verbenaceae family and its phylogenetic analysis

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### ABSTRACT

*Phyla nodiflora* (Linnaeus) Greene (1899) is a perennial creeping herb belonging to the family Verbenaceae. It has numerous pharmacological properties, including anti-dandruff, anti-inflammatory, anti-oxidant, anti-melanogenesis, anti-hypertensive, and anti-hyperuricemic properties. We generated the complete chloroplast genome sequence of *P. nodiflora* using Illumina paired-end sequencing data. The *P. nodiflora* chloroplast genome is 154,341 bp in length, containing a large single copy (LSC) region of 85,185 bp and a small single copy (SSC) region of 17,222 bp, separated by a pair of inverted repeats (IRs) of 25,967 bp. The genome contained 128 genes, including 86 protein-coding, 34 tRNA, and eight rRNA genes. Six genes had one intron, one gene had two introns, and the others did not have an intron. Overall GC content of the chloroplast genome was 39%, while those of LSC, SSC, and IR regions were 38.2%, 33.7%, and 44%, respectively. Phylogenetic analysis of the chloroplast genome revealed that *P. nodiflora* is closely related to the other species from Verbenaceae.

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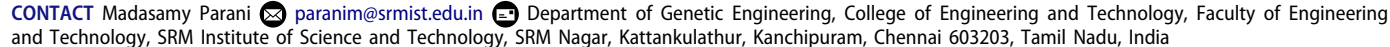
Chloroplast genome sequence; *Lippia nodiflora*; phylogeny tree; Poduthalai; Turkey tangle frog fruit


### Introduction

The genus *Phyla* includes five accepted species, namely *Phyla betulifolia* (Kunth) Greene, *Phyla cuneifolia* (Torr.) Greene, *Phyla lanceolata* (Michx.) Greene, *Phyla linearis* (Kunth) Tronc. & Lopez-Pal, and *Phyla nodiflora* (L.) Greene (O'Leary and Múlgura 2012). *P. nodiflora* is a perennial creeping herb with obovate leaves, entire at the base and serrate at the apex. Leaves are thinly pubescent. The flowers have white or purple petals with yellow in the center. Stems are decumbent, terete, and rooting at the nodes. *P. nodiflora* was reported to have flavonoids (Basu et al. 1969), steroids (Siddiqui et al. 2009), volatile phytochemicals (Elakovich and Stevens 1985), and other compounds (Lin et al. 2014). The plant is mainly used as an anti-dandruff (Fatima 2021), anti-bacterial, anti-cancer, hepatoprotective (Arumanayagam and Arunmani 2015), anti-melanogenesis (Yen et al. 2012), anti-inflammatory (Balakrishna et al. 1996), and anti-hyperuricemic (Cheng et al. 2015) activities. In this study, we report the chloroplast genome of *P. nodiflora* for the first time, which provides information for further taxonomic and DNA barcoding studies in this species.

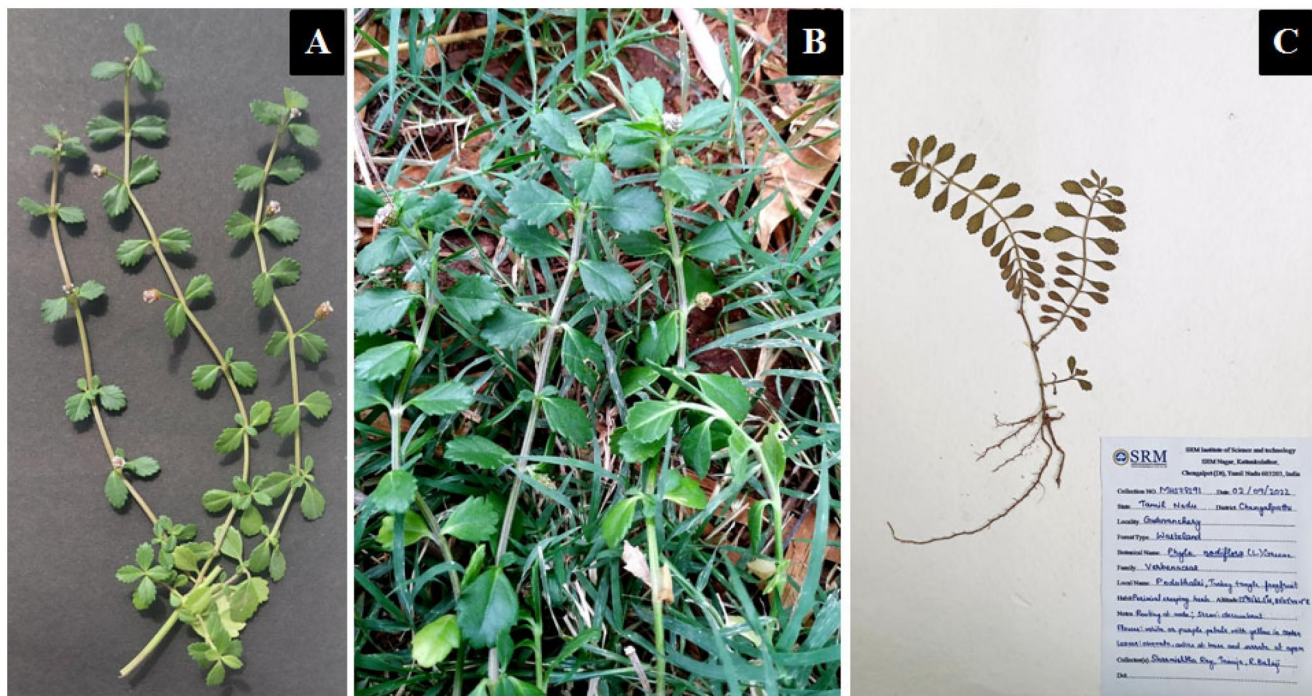
### Materials and methods

Plant material of *P. nodiflora* was collected from wasteland and roadside at Guduvanchery, Chengalpattu District, Tamil Nadu, India (GPS coordinates: 12°51'16.1"N 80°03'49.4"E; Figure 1). The voucher specimen (MH178191) was deposited in the Madras Herbarium, Botanical Survey of India (BSI), Southern Regional Centre, Coimbatore, Tamil Nadu, India, (<https://bsi.gov.in/regional-centres/en?rcu=133>, Dr. M. U. Sharief, Scientist-E and Head of Office, email: [sc@bsi.gov.in](mailto:sc@bsi.gov.in)). Total genomic DNA from *P. nodiflora* was extracted using the CTAB method (Doyle and Doyle 1987), with some modifications (Balaji and Parani 2022). According to manufacturer protocol, a whole-genome DNA sequencing library was constructed using the Nextera XT Library Prep Kit (Cat. No. FC-131-1024). The library was sequenced on the Illumina Novoseq 6000 platform (Illumina Inc., San Diego, CA, USA) with a paired-end sequencing length of 150 bp. Paired-end sequencing generated 4.68 Gb of data with q30 bases of more than 87%. The chloroplast genome assembly was done using NovoPlasty v.4.3.2 (Dierckxsens et al. 2017) and GetOrganelle v1.7.7.0 (Jin et al. 2020), with ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbcl*) gene from *Nicotiana tabacum* (GenBank accession no. NC\_001879.2) as a

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**Figure 1.** *Phyla nodiflora* plant used in this study. A. Collected sample, B. Habitat, and C. Digital herbarium (MH178191). The features that differentiate *P. nodiflora* from the other species of this genus are obovate leaves with sub-obtuse apex, pinnate venation prominent abaxially, serrate leaf margin, spherical-shaped fruit and rooting at the nodes (O'Leary and Múlgura 2012). These images were taken by authors (Ray Sharmishtha and Tanuja). The plant sample was collected from Guduvanchery, Chengalpattu District, Tamil Nadu, India (GPS coordinates: 12°51'16.1"N 80°03'49.4"E) on 9 September 2022.

seed sequence. The assembled *P. nodiflora* chloroplast genome was annotated with GeSeq (Tillich et al. 2017). The predicted transfer RNAs (tRNAs) were confirmed by tRNAscan-SE 2.0 (Lowe and Chan 2016). In addition, the CPGView ([www.1kmpg.cn/cpgview/](http://www.1kmpg.cn/cpgview/); Liu et al. 2023) was applied to structures to visualize the intron-containing genes. The sequencing depth of assembled chloroplast genome was done by aligning to the raw reads using a BWA aligner (Li and Durbin 2009). The bam file was viewed using the software Qualimap for coverage map (Fernando et al. 2012). A maximum likelihood tree of molecular distance analysis was performed using 1000 bootstrap replicates with the (GTR + G) model in MEGA version 11.0.13 (Tamura et al. 2021) based on the alignments created using the MAFFT program (Katoh and Standley 2013).

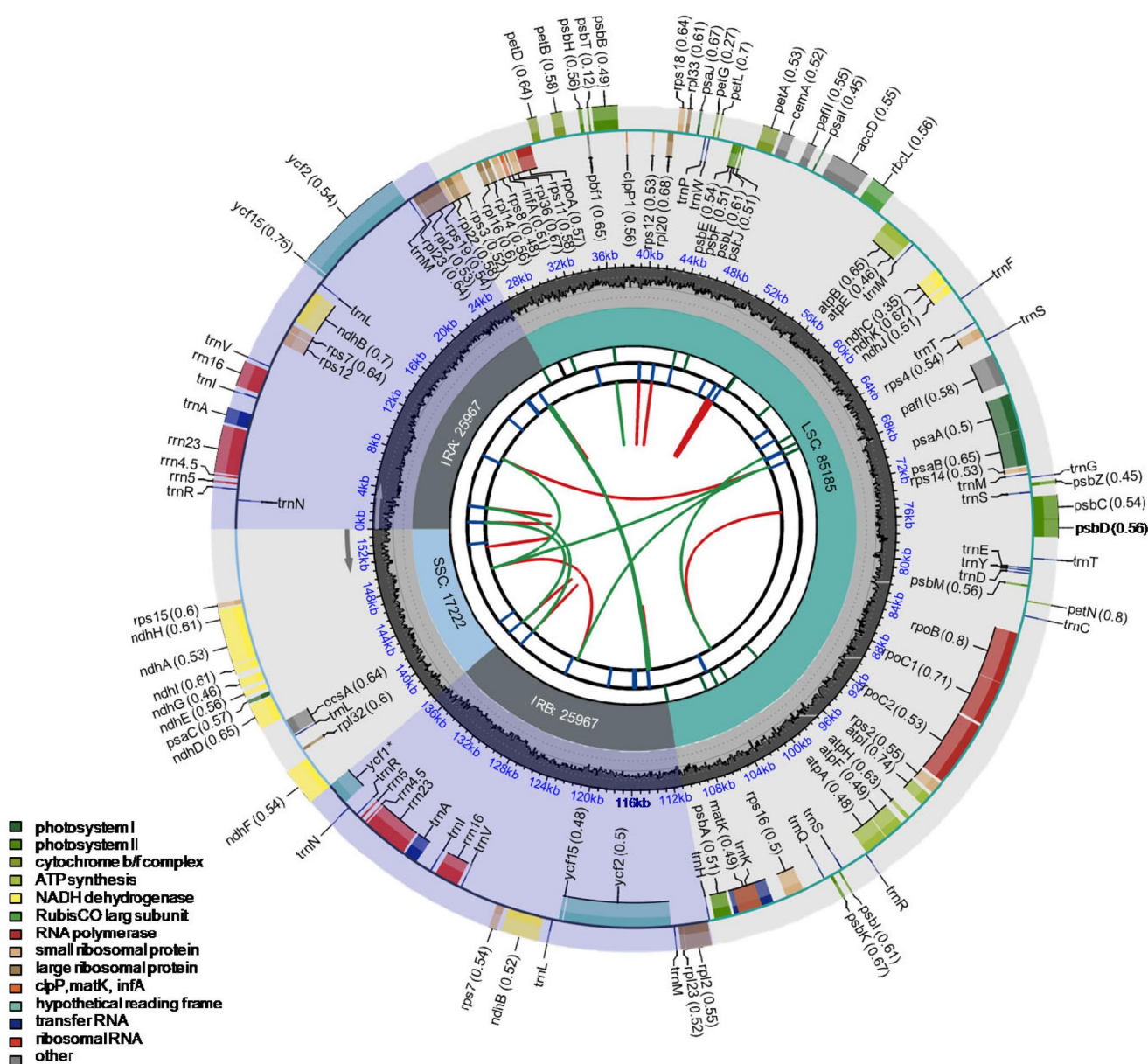
## Results and discussion

The chloroplast genome of *P. nodiflora* was 154,341 bp with a mean organelle coverage of 378× (Figure 2). The chloroplast genomes assembled using NOVOPlasty and GetOrganelle were 100% identical. It had a typical quadripartite structure, including a large single copy (LSC) region of 85,185 bp, a small single copy (SSC) region of 17,222 bp, and a pair of inverted repeats (IRs) of 25,967 bp. The chloroplast genome contained 86 protein-coding genes, 34 tRNA genes, and eight rRNA genes. Six genes (*atpF*, *ndhA*, *ndhB*, *rpl2*, *rpoC1*, *rps16*) were single-intron genes, and one gene (*pfal*) had two introns (Supplemental Figures 1 and 2). A Chloroplast genome of comparable size (154,310 bp) was reported from *Lippia organoides*, which is closely related to *P. nodiflora*. *L.*

*organoides* contained the same number of rRNA genes, but the number of protein-coding genes (88) and tRNA genes differed in small numbers compared to *P. nodiflora* (Sarzi et al. 2019). This comparative analysis highlights the structural similarities and differences between these closely related species within the broader context of phylogenetic relationships. The overall GC content was 39%, while LSC, SSC, and IR regions were 38.2%, 33.7%, and 44%, respectively. The average sequencing depth of the bases in the assembled genome was between 52× and 5644×, with a mean of 2041.3× (Supplementary Figure 3). The complete chloroplast genome of *P. nodiflora* with annotations was submitted to GenBank (Accession No. OQ673174). The raw reads were deposited in the GenBank Sequence Read Archive (Accession No. SRR23875832).

To confirm the phylogenetic positions of *P. nodiflora* in the Verbenaceae, the complete chloroplast genome of *P. nodiflora* (OQ673174) and the other species of the Verbenaceae family, namely *Duranta erecta* (MW525381.1), *Aloysia citrodora* (ON641356.1), *Lantana camera* (MN885891.1), *Lippia organoides* (MK248831.1), *Glandularia tenera* (MW538952.1), and *Verbena officinalis* (MW328640.1) were downloaded from NCBI database. *Ocimum gratissimum* (MW348919.1) from Lamiaceae, *Nicotiana tabacum* (Z00044.2), and *Withania somnifera* (MK142783.1) from the Solanaceae family were used as outgroups. The phylogenetic analysis fully resolved *P. nodiflora* in a clade with *Lantana camera* and *Lippia organoides* within the Verbenaceae family (Figure 3). A phylogenetic tree constructed based on the polymorphisms in ETS, ITS, trnL/rpl32, PHOTII, and seven pentatrichoepptide repeat (PPR)





**Figure 2.** Circular map of the chloroplast genome of *Phyla nodiflora*. From the center going outward, the first circle shows the distribution of the repeats connected with red (the forward direction) and green (the reverse direction) arcs. The second circle displays the tandem repeats marked with short bars. The third circle shows the LSC, SSC, IRA, and IRb regions. The fourth circle shows the percentage of GC content. The next circle shows the genes having different colors based on the functional groups. The functional classification is shown at the bottom left. Genes inside the circle are transcribed in a clockwise direction, and those outside are in a counter-clockwise direction.

genes for the members of *Lantana*, *Lippia*, *Aloysia*, *Nashia*, *Phyla*, *Burroughsia*, and *Acantholippia* genera of Verbanaceae also showed that *Phyla*, *Lippia*, and *Lantana* were closely related (Lu-Irving et al. 2021). The complete chloroplast genome of *P. nodiflora* can be subsequently used for phylogenetic analysis, DNA barcoding, and molecular marker studies to authenticate the herbal product of *P. nodiflora*.

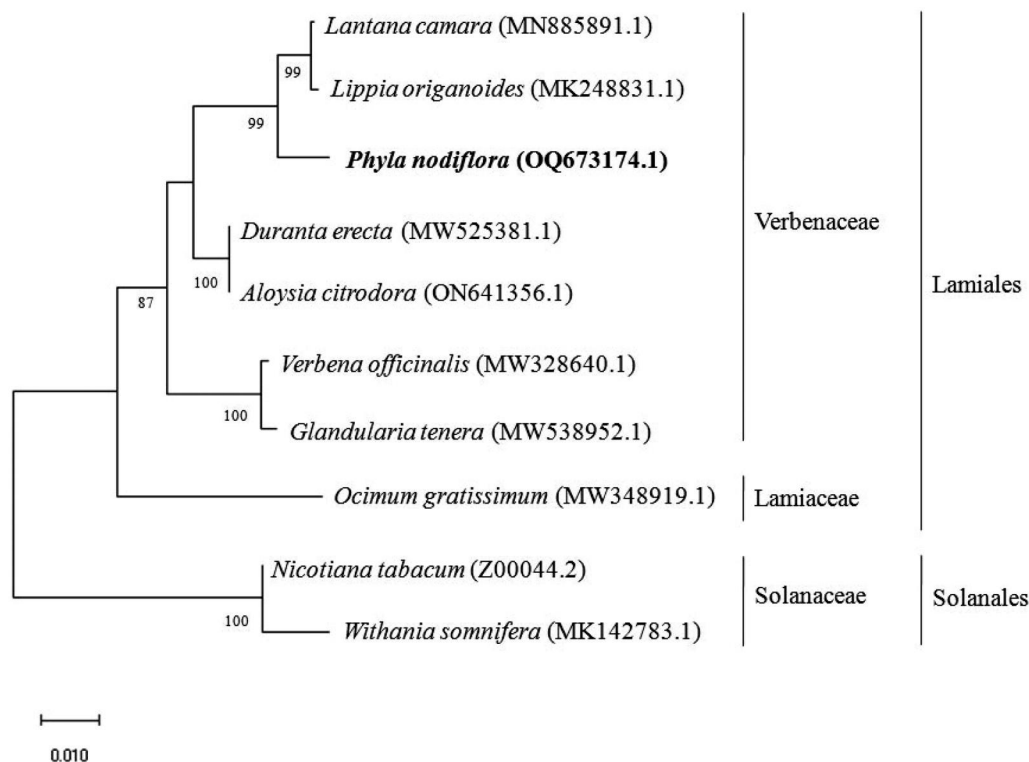
## Conclusion

This study assembled and annotated the complete chloroplast sequence of *Phyla nodiflora* for the first time. *P.*

*nodiflora* and other species were entirely resolved in a clade by the phylogenetic analysis within the Verbanaceae family. Following that, phylogenetic analysis, DNA barcoding, and molecular marker studies can be used to confirm the authenticity of the herbal products derived from *P. nodiflora*.

## Ethical approval

No permissions were required for the sample collection of *Phyla nodiflora* L. because it is widely distributed in the wastelands and roadsides in tropical regions. The plant species was collected from Guduvanchery, Chengalpattu District, Tamil Nadu, India (GPS coordinates: 12°51'16.1"N 80°03'49.4"E).



**Figure 3.** Phylogenetic tree constructed by maximum likelihood analysis based on complete chloroplast genome sequences, including the chloroplast genome of *P. nodiflora* (OQ673174). The bootstrap support values are shown on the nodes. The chloroplast genome sequences used for the phylogenetic analysis were *Ocimum gratissimum* MW348919.1 (Balaji et al. 2021), *Duranta erecta* MW525381.1 (Song et al. 2021), *Aloysia citrodora* ON641356.1, *Verbena officinalis* MW328640.1 (Yue et al. 2021), *Phyla nodiflora* OQ673174.1, *Lantana camara* MN885891.1, *Lippia origanoides* MK248831.1 (Sarzi et al. 2019), *Nicotiana tabacum* Z00044.2 (Shinozaki et al. 1986), and *Withania somnifera* MK142783.1 (Mehmood et al. 2020).

### Author contributions

Ray Sharmishtha, Tanuja, and R. Balaji collected the specimen material, conducted the experiment, analyzed the sequence data, and drafted the paper. M. Parani contributed to the conception and design of this work. All the authors carefully read, revised, and approved the final manuscript to be published. We thank Dr. Senthilkumar Umapathy for his help in the authentication of plant material.

### Disclosure statement

The authors reported no potential competing interests.

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### Data availability statement

The genome sequence data supporting the findings of this study are available in NCBI under the accession number OQ673174 (<https://www.ncbi.nlm.nih.gov/nuccore/OQ673174.1>).

The associated next-generation sequencing data files are available from the BioProject, Bio-Sample, and SRA submission under the accession numbers PRJNA945246, SAMN33776799, and SRR23875832, respectively.

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