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GENOME NOTE

REVISED The complete chloroplast genome of *Phyllanthus acidus***(L.) Skeels (Phyllanthaceae)**

[version 3; peer review: 1 approved, 4 approved with reservations]

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Hoang Dang Khoa Do ¹¹NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam²Faculty of Nursing and Medical Technology, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam**V3** First published: 31 Aug 2023, 12:1059
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<https://doi.org/10.12688/f1000research.140134.2>Latest published: 21 Nov 2025, 12:1059
<https://doi.org/10.12688/f1000research.140134.3>**Abstract**

Phyllanthus acidus (L.) Skeels (Phyllanthaceae) is a potential medicinal plant recognized for its sour and tart-tasting fruits. In this study, the chloroplast genome of *P. acidus* was sequenced, assembled, and characterized. The chloroplast genome size was 156,331 bp, and the overall GC content was 36.9%. Additionally, the chloroplast genome had a quadripartite structure consisting of a large single copy (LSC; 85,807 bp in length; GC content: 34.6%), a small single copy (SSC; 19,262 bp in length; GC content: 30.6%), and two inverted repeat regions (IR; 25,631 bp in length; GC content: 43.1%). A total of 113 unique genes were annotated in the chloroplast genome, including 79 protein-coding genes, 30 tRNAs, and four rRNAs. The phylogenetic analysis based on 79 protein-coding genes revealed the paraphyly of the *Phyllanthus* genus. These findings provided additional genetic information for further research on *P. acidus* and the cp genome in the Phyllanthaceae family.

Keywords

Malpighiales, Phyllanthaceae, phylogenetic relationships, plastome, star gooseberry

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version 3 (revision) 21 Nov 2025		✓ view		? view	? view
version 2 (revision) 16 Oct 2025		↑ ?	?		
version 1 31 Aug 2023	?				

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Author roles: **Nguyen HD:** Data Curation, Formal Analysis, Funding Acquisition, Writing – Original Draft Preparation; **Nguyen TDQ:** Formal Analysis, Investigation, Methodology, Writing – Original Draft Preparation; **Vu MT:** Conceptualization, Investigation, Methodology, Supervision, Writing – Review & Editing; **Do HDK:** Conceptualization, Methodology, Supervision, Validation, Writing – Review & Editing

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REVISED Amendments from Version 2

The revised manuscript was modified based on the comments of two new reviewers about the content of version 2 related to the aim of the study, the tool (OrganelleGenomeDRAW which is for illustrating the genome map) for annotating chloroplast genome, and the method to extract protein-coding genes for phylogenetic analysis. We revised the aim of the study and added more details about the methods of our manuscript (e.g., annotating, illustrating, and extracting sequences from chloroplast genomes). For the comment about phylogenetic analysis, we added an additional description about the phylogenetic result and refused to add discussion because of the regulation of Genome Note regulation. There is no change in title, figure, table, and authors of the manuscript.

Any further responses from the reviewers can be found at the end of the article

Introduction

Phyllanthus acidus (L.) Skeels, also known as star gooseberry, belongs to the Phyllanthaceae family and is commonly distributed in the wet tropical regions, including South Asia, Southeast Asia, Central Africa, the Caribbean region, Central America, and South America (POWO 2022). *P. acidus* has been traditionally used to treat various diseases, including inflammation, gastrointestinal problems, rheumatism, bronchitis, Alzheimer's, and hepatic diseases (Jain *et al.* 2011; Chakraborty *et al.* 2012; Srirama *et al.* 2012; Uddin *et al.* 2016). The leaves and roots of *P. acidus* also possess antidotal properties against viper venom (Jayvir 1998). Moreover, *P. acidus* could potentially alleviate hypertension (Leeya *et al.* 2010).

The chloroplast (cp) genome is highly effective in inferring phylogeny since it is predominantly maternally inherited, has a conserved structure and gene content, and has slow mutation frequency (Palmer *et al.* 1988). Additionally, the cp genomes provide essential data for examining population genetics, molecular identification, and genetic engineering (Powell *et al.* 1995; Daniell *et al.* 2016; Cao *et al.* 2022). Previously, a comparative chloroplast genomic study revealed the features of *Phyllanthus* chloroplast genomes and the non-monophyletic status of *Phyllanthus* species (Fang *et al.* 2023). Therefore, in the current study, we explored the characteristics of the *P. acidus* cp genome and its phylogenetic implication to gain more genomic information about the chloroplast genome evolution and to provide more data to resolve controversy of phylogenetic relationships within the *Phyllanthus* genus and closely related taxa in Phyllanthaceae.

Methods**Sample collection**

The *P. acidus* sample (young branches with leaves) was collected from Can Tho, Viet Nam (9°56'55.7"N, 105°30'16.0"E) and labeled with voucher number: NTT-2022.12.CR (contact person: Dr. Do Hoang Dang Khoa, dhdtkhoa@ntt.edu.vn). It was deposited at the NTT Hi-tech Institute, Nguyen Tat Thanh University. No specific permit was required to collect and study the species in Vietnam. The leaf sample was dried with silica gel and stored in a -80°C freezer until DNA extraction was conducted.

Data collection and analysis

The total genomic DNA extraction from the dried leaves was carried out using the Cetyltrimethylammonium bromide (CTAB) protocol (Doyle & Doyle 1987). The quality of genomic DNA samples was checked using gel electrophoresis and the NanoDrop One^C Spectrophotometer. The DNA samples that showed a clear band on agarose gel and had a 260/280 ratio between 1.8-2 and a 260/230 ratio between 2.0-2.2 were selected for conducting the next-generation sequencing step. Subsequently, the library was prepared with the NEBNext Ultra II DNA Library Prep Kit for Illumina (NEB, USA). The library was sequenced using the Illumina MiSeq platform to generate paired-end reads of 150 bp (Ktest Science Co. Ltd., Vietnam). The raw reads were qualified and filtered for low-quality reads (Q score < 20 and length < 100 bp) and reads containing primers or adapters using FastQC v0.12.1 and Trimmomatic v0.39 programs (Andrews 2010; Bolger *et al.* 2014). For the assembly of the cp genome, the NOVOPlasty v4.3.1 program was used (Dierckxsens *et al.* 2017). Preliminary annotation was conducted by Geseq with default parameters for land plant plastid and chloroplast land plant reference set (MPI-MP Reference Set) (Tillich *et al.* 2017). The complete annotation genome was illustrated using OrganellarGenomeDRAW v1.3.1 with default settings for circular plastid genome (Greiner *et al.* 2019). All 79 protein-coding regions in the cp genomes of *P. acidus* and 16 related taxa from the Phyllanthaceae were extracted and aligned for phylogenetic analysis using MUSCLE v5 program (Edgar 2004). The chloroplast genome of *Acalypha hispida* (Euphorbiaceae; Genbank accession no. NC_070339) was selected as an outgroup. A maximum likelihood phylogenetic tree was reconstructed using IQTREE with 1000 bootstrap replicates and GTRGAMMA substitution model (Nguyen *et al.* 2015). In addition, Bayesian inference method was applied to the aligned sequences under GTRGAMMA substitution model using MrBayes v3.2.7a with 1,000,000 generations and a discard of 25% sample (Ronquist *et al.* 2012).

Results

Approximately 349.8 MB of clean reads were obtained and used for completing the cp genome of *P. acidus*. The assembly process utilized 1,166,034 paired-end reads, resulting in an average coverage depth of 2,234.3X (Nguyen, Nguyen, Do, and Vu, 2023; Nguyen, Do, and Vu, 2023) (Supplementary Figure S1, Do 2025). The quadripartite cp genome of *P. acidus* (GenBank accession number OR050568) had a length of 156,331 bp and consisted of an LSC region of 85,807 bp, a SSC region of 19,262 bp, and a pair of IR regions of 25,631 bp (Figure 1). The overall GC contents of the genome was 36.9%, and the GC content of the LSC, SSC, and IR regions were 34.6%, 30.6%, and 43.1%, respectively. The cp genome of *P. acidus* contained a total of 130 genes, including 85 protein-coding regions, 37 tRNA genes, and eight rRNA genes (Table 1). Among 85 protein-coding genes, 17 genes contained introns, of which *ycf3* and *clpP* contained two introns (Supplementary Figure S2, Do 2025). In IR regions, a total of 19 genes were duplicated, including eight protein-coding regions (i.e., *rps19*, *rpl2*, *rpl23*, *ycf1*, *ycf2*, *ndhB*, *rps12*, and *rps7*),

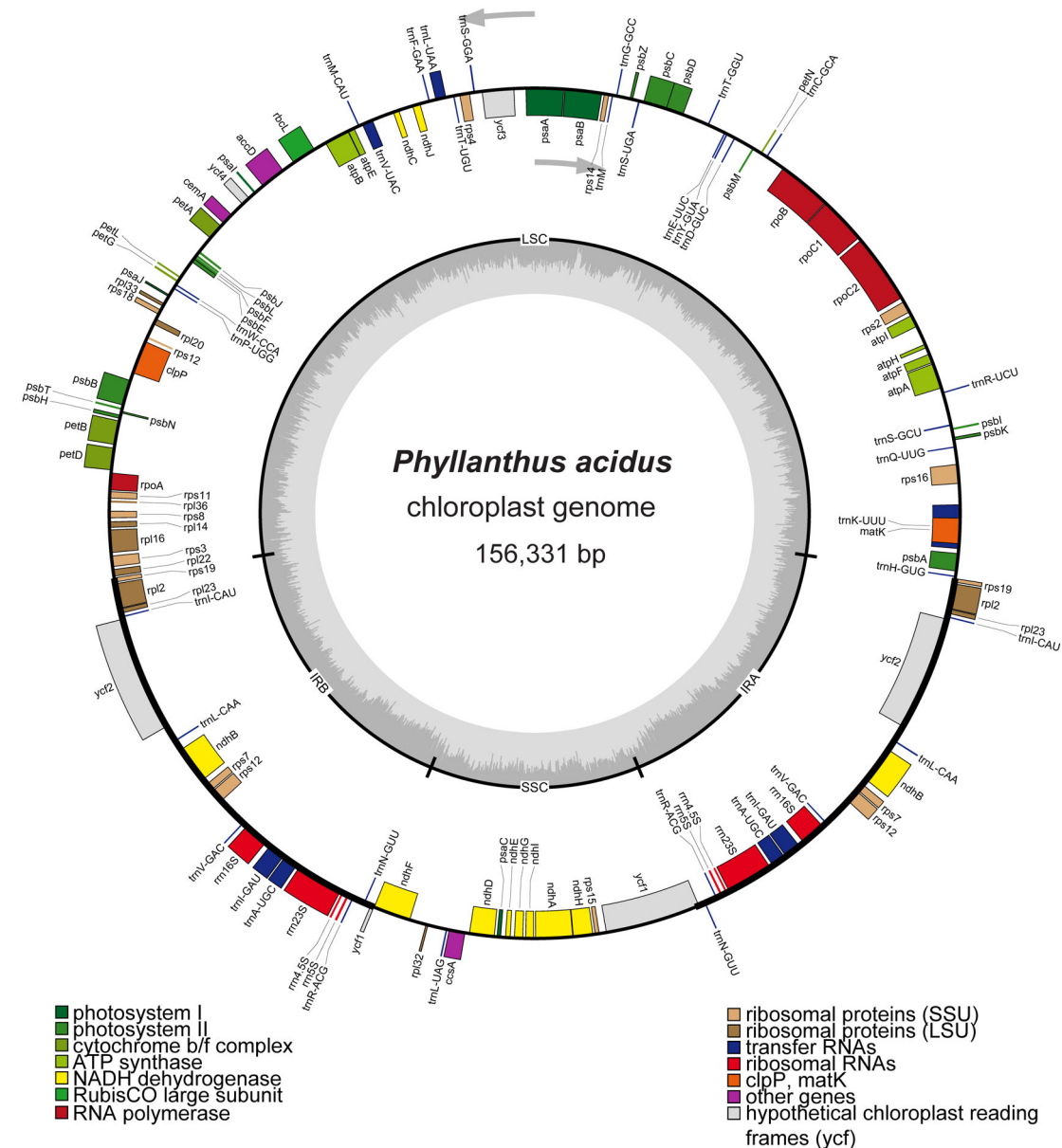


Figure 1. Map of the cp genome of *Phyllanthus acidus*. Genes located inside the circle are transcribed in a clockwise direction, while genes outside the circle are transcribed counterclockwise. The inner circle depicted in dark gray represents the GC content, while the light-gray circle represents the AT content of the genome. LSC: large single copy; SSC: small single copy; IRA/IRB: inverted repeat regions.

Table 1. List of genes in the chloroplast genome of *Phyllanthus acidus*.

Groups of genes	Name of genes
Ribosomal RNAs	<i>rrn4.5(2x), rrn5(2x), rrn16(2x), rrn23(2x)</i>
Transfer RNAs	<i>trnA_UGC*(2x), trnC_GCA, trnD_GUC, trnE_UUC, trnF_GAA, trnG_UCC*, trnG_GCC, trnH_GUG, trnI_GAU*(2x), trnK_UUU*, trnL_CAA(2x), trnL_UAA*, trnL_UAG, trnM_CAU, trnM_CAU(2x), trnM_CAU, trnN_GUU(2x), trnP_UGG, trnQ_UUG, trnR_ACG(2x), trnR_UCU, trnS_GCU, trnS_GGA, trnS_UGA, trnT_GGU, trnT_UGU, trnV_GAC(2x), trnV_UAC*, trnW_CCA, trnY_GUA</i>
Photosystem I	<i>psaA, psaB, psaC, psaI, psaj</i>
Photosystem II	<i>psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbM, psbN, psbT, psbZ</i>
Cytochrome	<i>petA, petB*, petD*, petG, petL, petN</i>
ATP synthases	<i>atpA, atpB, atpE, atpF*, atpH, atpI</i>
Large unit of Rubisco	<i>rbcL</i>
NADH dehydrogenase	<i>ndhA*, ndhB*(2x), ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK</i>
ATP-dependent protease subunit P	<i>clpP*</i>
Envelop membrane protein	<i>cemA</i>
Large units of ribosome	<i>rpl2*(2x), rpl14, rpl16*, rpl20, rpl22, rpl23(2x), rpl32, rpl33, rpl36</i>
Small units of ribosome	<i>rps2, rps3, rps4, rps7(2x), rps8, rps11, rps12*(2x), rps14, rps15, rps16*, rps18, rps19 (2xa)</i>
RNA polymerase	<i>rpoA, rpoB, rpoC1*, rpoC2</i>
Initiation factor	<i>infA</i>
Other genes	<i>accD, ccsA, matK</i>
Hypothetical proteins and conserved reading frames	<i>ycf1(2xa), ycf2(2x), ycf3*, ycf4</i>

*Gene with introns; 2x – duplicated gene in IR region; 2xa – incomplete duplicated gene in IR region.

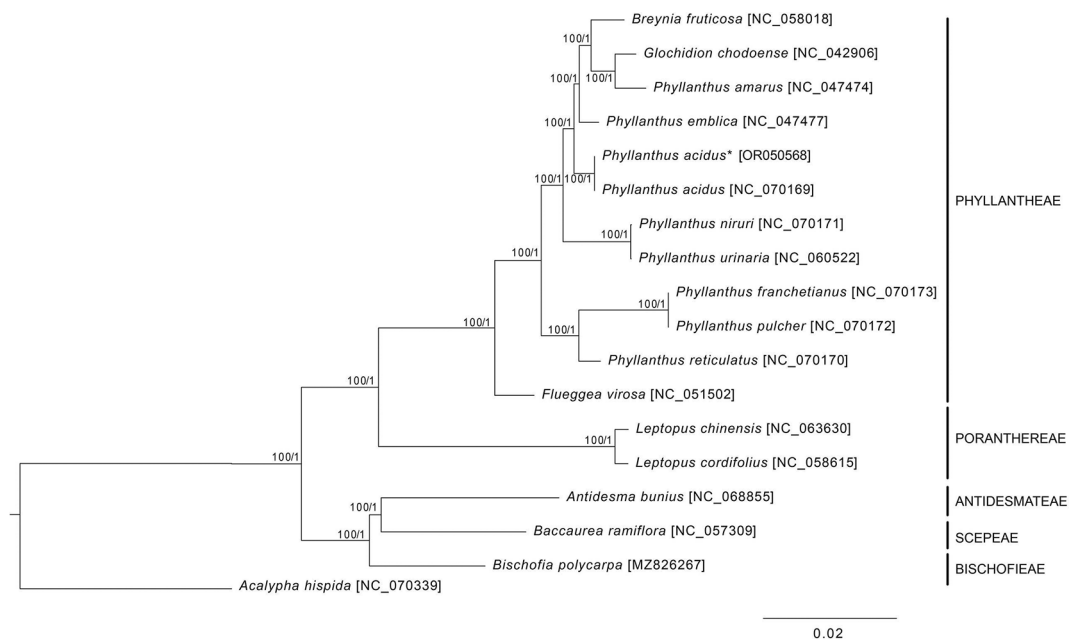


Figure 2. The phylogenetic tree of *Phyllanthus acidus* and related species based on the 79 protein-coding sequences of cp genome. The asterisk indicates *P. acidus* sequenced in this study. The numbers next to each node are bootstrap values and posterior probability inferred from maximum likelihood and Bayesian inference methods.

seven tRNAs (*trnI_CAU*, *trnL_CAA*, *trnV_GAC*, *trnI_GAU*, *trnA_UGC*, *trnR_ACG*, and *trnN_GUU*), and four rRNAs (*rrn16S*, *rrn23S*, *rrn4.5S*, and *rrn5S*). Notably, *rps19* and *ycf1* duplications were incomplete. The phylogenetic analysis revealed a paraphyly of *Phyllanthus* species, in which *Breynia fuitcosa* and *Glochidion chodoense* formed a clade with *Phyllanthus amarus* (Figure 2). Therefore, more genomic data and samples of *Phyllanthaceae* species are required for further phylogenetic studies.

Data availability

NCBI Short Read Archive (SRA): DNA-seq of *Phyllanthus acidus*. Accession number SRR24772537; <https://www.ncbi.nlm.nih.gov/sra/SRR24772537> (Nguyen, Nguyen, Do, and Vu, 2023).

NCBI Assembly database: *Phyllanthus acidus* chloroplast, complete genome. Accession number OR050568; <https://www.ncbi.nlm.nih.gov/nucleotide/OR050568> (Nguyen, Do, and Vu, 2023).

Extended data

Figshare: Supplementary Files. <https://doi.org/10.6084/m9.figshare.30354073> (Do 2025).

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0)

Acknowledgements

The authors thank to anonymous reviewers for their helpful comments to improve the quality of this manuscript.

References

- Andrews S: **FastQC: A Quality Control Tool for High Throughput Sequence Data**. 2010.
- Bolger AM, Lohse M, Usadel B: **Trimomatic: a flexible trimmer for Illumina sequence data**. *Bioinformatics*. 2014; **30**(15): 2114–2120.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Cao D-L, et al.: **Application of chloroplast genome in the identification of Traditional Chinese Medicine Viola philippica**. *BMC Genomics*. 2022; **23**(1): 540.
[Publisher Full Text](#)
- Chakraborty R, et al.: **Antiinflammatory, antinociceptive and antioxidant activities of Phyllanthus acidus L. extracts**. *Asian Pac. J. Trop. Biomed*. 2012; **2**(2): S953–S961.
[Publisher Full Text](#)
- Daniell H, et al.: **Chloroplast genomes: diversity, evolution, and applications in genetic engineering**. *Genome Biol*. 2016; **17**(1): 134.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Dierckxens N, Mardulyn P, Smits G: **NOVOPlasty: De novo assembly of organelle genomes from whole genome data**. *Nucleic Acids Res*. 2017; **45**(4): gkw955.
[Publisher Full Text](#)
- Do KHD: Supplementary Files. [Dataset]. *figshare*. 2025.
[Publisher Full Text](#)
- Doyle JJ, Doyle JL: **A rapid DNA isolation procedure for small quantities of fresh leaf tissue**. *Phytochem. Bull*. 1987; **19**: 11–15.
- Edgar RC: **MUSCLE: multiple sequence alignment with high accuracy and high throughput**. *Nucleic Acids Res*. 2004; **32**(5): 1792–1797.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Fang H, et al.: **Application of chloroplast genome in the identification of Phyllanthus urinaria and its common adulterants**. *Front. Plant Sci*. 2023; **13**: 1099856.
[Publisher Full Text](#)
- Greiner S, Lehwark P, Bock R: **OrganellarGenomeDRAW (OGDRAW) version 1.3.1: expanded toolkit for the graphical visualization of organellar genomes**. *Nucleic Acids Res*. 2019; **47**(W1): W59–W64.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Jain NK, et al.: **Effects of Phyllanthus acidus (L.) Skeels fruit on carbon tetrachloride-induced acute oxidative damage in livers of rats and mice**. *Zhong Xi Yi Jie He Xue Bao = Journal of Chinese Integrative Medicine*. 2011; **9**(1): 49–56.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Jayvir A: **Nature heals, a glossary of selected indigenous medicinal plants of India**. *Indian J. Pharm*. 1998; **30**(2): 126.
- Leeya Y, et al.: **Hypotensive activity of an n-butanol extract and their purified compounds from leaves of Phyllanthus acidus (L.) Skeels in rats**. *Eur. J. Pharmacol*. 2010; **649**(1–3): 301–313.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Nguyen L-T, et al.: **IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies**. *Mol. Biol. Evol*. 2015; **32**(1): 268–274.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Nguyen DH, Nguyen TDQ, Do KHD, et al.: **DNA-seq of Phyllanthus acidus**. NCBI Short Read Archive. [Dataset]. 2023.
[Reference Source](#)
- Nguyen DH, Do KHD, Vu TM: **Phyllanthus acidus chloroplast, complete genome**. [Dataset]. *GenBank*. 2023.
[Reference Source](#)
- Palmer JD, et al.: **Chloroplast DNA variation and plant phylogeny**. *Ann. Mo. Bot. Gard*. 1988; **75**: 1180–1206.
[Publisher Full Text](#)
- Powell W, et al.: **Polymorphic simple sequence repeat regions in chloroplast genomes: applications to the population genetics of pines**. *Proc. Natl. Acad. Sci*. 1995; **92**(17): 7759–7763.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- POWO: **Plants of the World Online**. Facilitated by the Royal Botanic Gardens, Kew. 2022.
- Ronquist F, et al.: **MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space**. *Syst. Biol*. 2012; **61**(3): 539–542.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Srirama R, et al.: **Hepatoprotective activity of Indian phyllanthus**. *Pharm. Biol*. 2012; **50**(8): 948–953.
[Publisher Full Text](#)
- Tillich M, et al.: **GeSeq – versatile and accurate annotation of organelle genomes**. *Nucleic Acids Res*. 2017; **45**(W1): W6–W11.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Uddin MS, et al.: **Neuroprotective Effect of Phyllanthus acidus L. on Learning and Memory Impairment in Scopolamine-Induced Animal Model of Dementia and Oxidative Stress: Natural Wonder for Regulating the Development and Progression of Alz**. *Adv. Alzheimer's Dis*. 2016; **05**(02): 53–72.
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Version 3

Reviewer Report 07 January 2026

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Sunil Kumar Sahu 

BGI-Shenzhen, Shenzhen, China

This Genome Note is technically sound and presents a well-covered *Phyllanthus acidus* plastome, accompanied by open data and a straightforward phylogenetic analysis that agrees with previous plastome-based inferences for Phyllanthaceae. The study has clear potential value, both because *P. acidus* is medicinally important and because the clade itself is taxonomically challenging. That said, several aspects still need clarification or correction before the manuscript can be considered fully robust. In particular, the rationale for generating a new plastome despite the existence of an earlier *P. acidus* assembly needs to be explicitly stated, along with any improvements your dataset contributes. My specific comments and questions are below:

- What is the explicit justification for re-sequencing *P. acidus* given the available GenBank plastome (N C_070169)? Please indicate whether your assembly provides concrete improvements or corrections (for example, IR boundary resolution, the extent of *ycf1/rps19* pseudogenization, or annotation differences).
- The manuscript reports inconsistent gene counts: the Abstract lists 113 unique genes (79 PCGs, 30 tRNAs, 4 rRNAs) whereas the Results and Table 1 suggest 130 total genes (85 PCGs, 37 tRNAs, 8 rRNAs). Please clarify which numbers refer to unique genes versus totals including IR duplicates, and ensure Table 1 reflects accurate gene names and counts.
- Table 1 contains several typographical issues (for example, Cyrillic characters in *rpo*-genes, duplicated *trnM-CAU*, and the inclusion of "*rps1*"). Please correct these using your GeSeq output, and explicitly indicate that *rps12* is a trans-spliced gene.
- Please specify the seed sequence and parameters used in NOVOPlasty, and describe how you validated genome circularization and IR/SC junction accuracy (for example, via read-mapping coverage, junction-spanning reads, or targeted PCR).
- For the phylogenetic analyses, please report the exact models used in IQ-TREE and MrBayes, using each program's proper nomenclature. Indicate whether you performed model selection (e.g., ModelFinder), partitioning (gene or codon level), alignment trimming or masking, or any saturation testing.
- If you have compared your plastome directly to NC_070169 (e.g., pairwise divergence, structural differences, annotation discrepancies), please summarize the key findings. If not, a brief

comparison would strengthen the manuscript.

Overall, the work is promising and close to solid, but addressing these issues will substantially improve clarity, reproducibility, and the manuscript's contribution to plastome resources for *Phyllanthus*.

Are the rationale for sequencing the genome and the species significance clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?

Yes

Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Plant genomics and evolution

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 11 December 2025

<https://doi.org/10.5256/f1000research.190830.r435864>

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Yoshinori Fukasawa 

Center for Bioscience Research and Education Utsunomiya University, Tochigi, Japan

I agree with the other reviewers. The manuscript clearly describes the importance of *P. acidus* as a species and provides a technically sound chloroplast assembly with appropriate sequencing and annotation procedures. These strengths are appreciated.

However, there is already an existing *P. acidus* chloroplast genome available in NCBI, and the Genome Note guidelines require authors to explain "*why and how the genomes were sequenced*." Simply removing the earlier incorrect statement ("has not been studied") or adding comments on

the species' economic or medicinal value does not address the core issue raised by the reviewer. The manuscript should explicitly clarify the rationale for resequencing the chloroplast genome despite the presence of a previously published assembly—e.g., whether the new (meta)data provide validation, improved quality, structural corrections, or broader comparative relevance.

Minor technical corrections

Additionally, several tool names should be standardized (e.g., GeSeq, IQ-TREE), and the substitution model should match the nomenclature actually used in IQ-TREE or MrBayes, as "GTRGAMMA" is not a conventional model designation in those programs.

Are the rationale for sequencing the genome and the species significance clearly described?

Partly

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?

Yes

Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioinformatics, genome assembly, and computational genomics.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 08 December 2025

<https://doi.org/10.5256/f1000research.190830.r434949>

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Qiang Li 

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I have finished reading the revised manuscript. The article reads very well, and I have no further comments.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 03 November 2025

<https://doi.org/10.5256/f1000research.189635.r426288>

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Shiou Yih Lee

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The manuscript describes on the characterisation of the chloroplast genome sequence of *Phyllanthus acidus*. My comments are as follows:

1. There is one chloroplast genome sequence of *P. acidus* in NCBI. The problem statement and objective of this study are not very relevant.
2. How did the authors extract the protein-coding sequences from the chloroplast genome sequence for phylogenetic analysis?
3. As there is another record of *P. acidus* in NCBI GenBank, no discussion or comparison was conducted between that and the one from this study. The placement of *P. acidus* of this study in the phylogenetic tree was not described in the Results. There is no discussion on the findings from the phylogenetic analysis.

Are the rationale for sequencing the genome and the species significance clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?

Yes

Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: forestry, molecular phylogenetics and evolutionary

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 04 Nov 2025

Hoang Dang Khoa Do

Dear Reviewer,

Thank you very much for your comments and suggestions.

We revised the manuscript based on your suggestions.

Please check our responses as mentioned below.

The manuscript describes on the characterisation of the chloroplast genome sequence of *Phyllanthus acidus*. My comments are as follows:

1. There is one chloroplast genome sequence of *P. acidus* in NCBI. The problem statement and objective of this study are not very relevant.

Response: Thank you very much for your comment. In the original version, we did not mention as "The current study explored the characteristics of the *P. acidus* cp genome which has not been studied" which was added in the revised version. We are so sorry for the wrong information. We revised this information in the newest version of our manuscript.

2. How did the authors extract the protein-coding sequences from the chloroplast genome sequence for phylogenetic analysis?

Response: Thank you very much for your question. We added the program for extracting protein-coding genes which is Geneious Prime v2023.0 in the revised manuscript.

3. As there is another record of *P. acidus* in NCBI GenBank, no discussion or comparison was conducted between that and the one from this study. The placement of *P. acidus* of this study in the phylogenetic tree was not described in the Results. There is no discussion on the findings from the phylogenetic analysis.

Response: Thank you very much for your comment. We submitted our manuscript as a Genome Note type which requires "brief descriptions of genome sequences that include details of why and how the genomes were sequenced; they do not include any analyses or conclusions.". Therefore, we focused on description of our target genome and did not do comparative genomic analyses. In addition, in Genome Note type, the regulation does not allow a Discussion part in the manuscript (<https://f1000research.com/for-authors/article-guidelines/genome-notes>). Therefore, we did not discuss in depth about phylogenetic analysis in our manuscript. We added more details about phylogenetic relationship between *Phyllanthus acidus* in the main text.

Thank you.

Competing Interests: We do not have any conflicts of interest.

Reviewer Report 03 November 2025

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**Qiang Li** ¹ Nanjing Forestry University, Nanjing, China² Anhui Normal University, Wuhu, Anhui, China³ Nanjing Forestry University, Nanjing, China⁴ Anhui Normal University, Wuhu, Anhui, China

Phyllanthus is one of the taxonomic difficulties genera of Phyllanthaceae. It contains approximately 800 species, mainly distributed in the tropical and subtropical regions of the world, and a few are in the northern temperate zone. Previous studies have demonstrated that *Phyllanthus* is a paraphyletic group. Several solutions have been proposed for dividing *Phyllanthus* species. Some researchers classified previously established classifications into *Phyllanthus* sensu lato, such as (Kathriarachchi et al. 2006), who placed *Breynia*, *Glochidion* and *Synostemon* into *Phyllanthus*. Others split *Phyllanthus* into several monophyletic genera. However, these taxonomic evidence lack support from genomics. Therefore, we need to obtain more genomic data of this genus to better resolve the taxonomic controversies within it. I believe this is also the most important significance of this manuscript reporting the chloroplast genome of *Phyllanthus acidus*.

The primary objective of this manuscript is to assemble and provide a basic description of the *Phyllanthus acidus* genome. The methods and content are straightforward and easy to follow. I have two main concerns that I hope the authors will address to enhance the quality of the manuscript.

[1] I agree with the previous reviewer's opinion that the introduction lacks the significance of studying the chloroplast genome of this species. I suggest that the authors could add a concise introduction. For example, there is controversy over the broad and narrow senses of the genus *Phyllanthus*, and this molecular phylogenetic debate lacks evidence from genomics. Therefore, the genome sequenced in this study lays the foundation for resolving the taxonomic controversy of the genus *Phyllanthus* in the future.

[2] OrganellarGenomeDRAW requires a reference genome for annotating chloroplast genomes, and the authors should specify which species' chloroplast genome was used as the reference.

References

1. Nguyen H, Nguyen T, Vu M, Do H: The complete chloroplast genome of *Phyllanthus acidus* (L.) Skeels (Phyllanthaceae). *F1000Research*. 2025; **12**. [Publisher Full Text](#)

Are the rationale for sequencing the genome and the species significance clearly described?

Partly

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?

Yes

Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: My research areas primarily encompass speciation, phylogenetics, and the conservation of endangered plants. Currently, I mainly utilize chloroplast genomes, mitochondrial genomes, and nuclear genomes to address evolutionary questions.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 03 Nov 2025

Hoang Dang Khoa Do

Dear Reviewer,
Thank you very much for your helpful comments.
We revised the manuscript based on your comments.
Please check our response as mentioned below.

The primary objective of this manuscript is to assemble and provide a basic description of the *Phyllanthus acidus* genome. The methods and content are straightforward and easy to follow. I have two main concerns that I hope the authors will address to enhance the quality of the manuscript.

[1] I agree with the previous reviewer's opinion that the introduction lacks the significance of studying the chloroplast genome of this species. I suggest that the authors could add a concise introduction. For example, there is controversy over the broad and narrow senses of the genus *Phyllanthus*, and this molecular phylogenetic debate lacks evidence from genomics. Therefore, the genome sequenced in this study lays the foundation for resolving the taxonomic controversy of the genus *Phyllanthus* in the future.

Response: Thank you very much for your suggestion. We revised the second part of the Introduction with an adding of a previous comparative genomic analysis based on complete chloroplast genomes of *Phyllanthus* which indicated a non-monophyletic status of *Phyllanthus* species. In addition, we modified the aims of the current study as "Therefore, in the current study, we explored the characteristics of the *P. acidus* cp genome and its phylogenetic implication to gain more genomic information about the chloroplast genome

evolution and to provide more data to resolve controversy of phylogenetic relationships within the *Phyllanthus* genus and closely related taxa in *Phyllanthaceae*."

[2] OrganellarGenomeDRAW requires a reference genome for annotating chloroplast genomes, and the authors should specify which species' chloroplast genome was used as the reference.

Response: Thank you very much for your suggestion. We checked the paper (<https://doi.org/10.1093/nar/gkz238>) and the website (<https://chlorobox.mpimp-golm.mpg.de/OGDraw.html>) of OGDRAW and we did not find any regulation to select a reference genome for annotation chloroplast genome before drawing the chloroplast genome map. The input of OGDRAW is an annotated file (a GenBank flat file) of chloroplast genome which included all information of genes. Then OGDRAW will converts annotations in GenBank format to graphical maps.

For annotating chloroplast genome using GESEQ, there is an option for selecting the reference genome. We selected the default reference set of the Geseq which is "MPI-MP Reference Set chloroplast land plants (CDS + rRNA)". We did not use a reference genome from *Phyllanthus* species because we would like to compare our sequence to the land plant database. The more annotation information we compare, the more accuracy of chloroplast genome annotation we get.
Thank you.

Competing Interests: No competing interests were disclosed.

Version 1

Reviewer Report 22 January 2024

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This is a good genome note on the sequencing and assembly of the plastome of *Phyllanthus acidus*. However, the paper lacks enough details on the reasons behind investigating the species (i.e.,

limited genetic and genomic resources? problems with identification?).

The protocol on sequencing and assembly is appropriate and is clearly described in the paper. To improve data transparency, I suggest including supplementary data such as depth of coverage/read mapping depth and the list of genes which are hard to annotate (i.e., cis-splicing/trans-splicing genes). In addition, I would also like to suggest the use of at least two different methods of phylogenetic analyses (e.g., Maximum likelihood and Bayesian inference) and to indicate the statistical support values [of the two methods] in the tree.

Lastly, to improve readability of the paper I would like to recommend checking for grammatical errors.

Are the rationale for sequencing the genome and the species significance clearly described?

Partly

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?

Yes

Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular characterization of different plant species i.e. Philippine endemic medicinal plants, strawberry varieties planted in the Philippines, genetics of disease resistance cultivated rice, soilless culture of leafy and fruiting vegetables

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 12 Oct 2025

Hoang Dang Khoa Do

Dear Reviewers,

Thank you very much for your helpful comments.

1/ We added the reason for conducting genomic research about *Phyllanthus acidus* in the Introduction as "which has not been studied".

2/ We added the information of depth of coverage/read mapping depth and cis-splicing/trans-splicing genes as supplementary files.

3/ We revised the phylogenetic tree with the Bayesian inference method.
Best regards.

Competing Interests: No competing interests were disclosed.

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