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Systematic position of *Phyllanthus talbotii* (Phyllanthaceae), a critically endangered species of Western Ghats, India

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Abstract

Phyllanthus talbotii Sedgw. (Phyllanthaceae) is endemic to Western Ghats regions of Goa and Karnataka, India and is critically endangered. For a long time, it was known only from the type collection and a subsequent untraceable collection and hence taxonomically not fully understood, though a recent treatment has placed it under subgenus *Eriococcus*. In the present study, attempts were made to confirm its position using morphological characters and sequences of nuclear internal transcribed spacer (ITS) of nuclear ribosomal DNA (nrDNA) and chloroplast *mat* K genes. Results from ITS and *mat* K phylogenetic analyses supported its placement in subgenus *Eriococcus*.

Keywords: CR plant, ITS, matK, Phyllanthaceae, Phyllanthus talbotii, phylogeny, systematic.

Introducion

Phyllanthus dominates tribe *Phyllantheae*, being the largest genus in the family Phyllanthaceae. The genus has a remarkable diversity of growth forms and floral morphology (Bancilhon, 1971). Webster (1956, 1957, 1958) divided the genus into eight subgenera and over 30 sections based on vegetative architecture and pollen morphology in addition to floral characters.

Phyllanthus talbotii Sedgw. (Phyllanthaceae) is a rare, critically endangered (CR) and endemic species of Western Ghats region of Goa and Karnataka. It was described by Sedgwick (1921) based on collections made in 1918 and 1919 from North Kanara, presently in Karnataka State, India and later it was again collected from Agumbe in adjacent Shimoga district (Raghavan, 1969). Known by a single untraceable collection after type, Singh and Kulkarni (1990) reported it as rare species. Though poorly known taxonomically, Balakrishnan and Chakrabarty (2007) placed it in subgenus *Eriococcus*.

The internal transcribed spacers (ITS) nrDNA sequences contain potential informative sites and been used as a useful molecular marker in studying phylogeny of many taxa (Lee *et al.*, 2006; Maria *et al.*, 2007; Juthatip *et al.*, 2010; Shi-Xiao *et al.*, 2011). The *mat*K gene also potentially contributes to plant molecular systematic and evolutionary studies (Steele & Vilgalys, 1994; Liang & Hilu, 1996; Kathriarachchi *et al.*, 2006; Samuel *et al.*, 2005).

The *mat*K gene with about 1500 base pairs (bp) is located within the intron of the chloroplast gene *trn*K. The present study is aimed at understanding the systematic position of *P. talbotii* using ITS and *mat*K gene sequences and also to provide an adequately detailed description which was not available in the literature.

Materials and Methods

Taxon sampling

Leaf samples were collected from naturally occurring healthy plant population from Saccordem (Longitude - E 074°09.647′ and Latitude - N 15° 24.822′; altitude 49 m), Goa [Goa University Herbarium 4011] and silica gel dried. Flowering twigs were collected and processed for herbarium and morphological study. Fresh and pickled specimens were studied and photographed under Leica EZ4D microscope with inbuilt camera.

Isolation of DNA and sequencing:

Silica gel dried leaf materials were used for the extraction of DNA. Total genomic DNA was isolated using CTAB method (Doyle & Doyle, 1990) with modification. Polymerase chain reaction was performed for the amplification of genes such as ITS and *mat*K by using universal random primers (Table 1). The PCR products were further purified using Exo-SAP-IT (GE Healthcare) treatment. The Exo-SAP treated PCR products were used for



Fig.1. Phyllanthus talbotii Sedgw.: a. Habitat; b. Habit; c. Staminate flowers; d. Pistillate flowers; e. Capsule; f. Seeds.

Target gene	Primer name	Sequence (5'→ 3')	Reference
ITS	ITS5-F	GGAAGTAAAAGTCGTAACAAGG	White <i>et al.,</i> 1990
	ITS4-R	TCCTCCGCTTATTGATATGC	
mat K	3F KIM-F	CGTACAGTACTTTTGTGTTTACGAG	CBOL Plant Working Group
	1R KIM-R	ACCCAGTCCATCTGGAAATCTTGGTTC	(http://www.barcoding.si.edu/ plant_working_group.html)

Table 1. Primers used for sequencing of ITS and *mat* K genes.

gene sequencing. Both the genes were sequenced using the Big Dye terminator v3.1 sequencing kit and ABI 3730 DNA Analyzer (Applied Biosystem, USA). DNA isolation, amplification and Gene sequencing was carried out at Rajiv Gandhi Center for Biotechnology, Regional Facility for DNA fingerprinting, Thiruvananthapuram, Kerala, India. The sequences are deposited at NCBI (ITS: Acc. No.KC414630; *mat*K: Acc. No. KC514101).

Sequence Analysis

The sequence analysis was carried out using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1. The ITS and *mat*K sequences were blast with highly similar sequences of related species. The maximum identical sequences to *P. talbotii* were retrieved from NCBI in FASTA format and aligned with the query sequences.

Sequence alignment and construction of phylogenetic tree

For ITS, indent value was taken as 83% and above and for *mat*K 95% and above. The sequence analysis involved *Phyllanthus* nucleotide sequences of 13 species for ITS and 15 species for *mat*K along with query sequences. The sequence alignment was carried out using clustalW (ver. 1.6) and improved by visual treatment (Kelchner, 2000). Further analysis was carried out using the Maximum Likelihood tree model (Tamura *et al.*, 2004, 2011). The confidence limit (bootstrap percentage) for clades was assessed by performing 1000 replicates of bootstrapping (Felsenstein, 1985). The individual Maximum likelihood analyses were performed using MEGA (version 5.05) software.



Fig 2. Maximum likelihood tree using ITS gene sequences of *Phyllanthus talbotii* (Bootstrap percentage indicated on side). Asterisks' indicate that the position of these species is not known.

Results and Discussion

Taxonomc description

Phyllanthus talbotii Sedgw., J. Indian Bot. Soc. 1: 124, f. 2. 1921 (as 'talboti'); Singh & Kulkarni in Red Data Book of Indian Pl. 3: 124. 1990; Balakrishnan & Chakrabarty, Family Euphorbiaceae in India, 374. 2007. Fig. 1

A monoecious subshrub, up to 150 cm high; stem terete, suffrutescent, brown, often with vertical streaks with prominent nodes; branchlets up to 25 cm long, with up to 100 leaves, hirsute. Stipules triangular to deltoid, $c. 1.5 \times 1$ mm, margin white or brown, acuminate at apex. Petiole c. 0.5 mm long; lamina oblong, up to 5.9 × 3 mm, rounded at base, entire, acute or slightly apiculate at apex, mid vein prominent, secondary veins 4 or 5-pairs, often invisible, sparsely hairy on both surfaces. Staminate flowers: axillary, solitary or in clusters of 2 or 3, c. 2.5×2 mm. Bracts ovate, shorter than stipule; bracteoles subulate. Pedicels capillary, c. 2 mm long, slightly dilated at apex. Sepals 4, obovate to elliptic, c. 2 × 1.3 mm, acute to rounded at apex, green in the middle with a broad white wavy margin, one-veined. Disc of 4 distinct units, yellow. Staminal column c. 0.7 mm long; anthers 4, in two pairs, c. 0.5 mm across, yellow. Pistillate flowers: solitary, axillary on the same branchlet as staminate flowers, c. 3 mm across when open. Bracteoles lanceolate to ovate, up to 1 mm long. Pedicel *c*. 2 mm long, dilated at apex. Sepals 6, ovate to elliptic, $1.5-2 \times 1$ mm, entire, acute to acuminate at apex, greenish in the middle and white to creamy on either side. Disk yellow, wavy, slightly 5-lobed. Ovary 3-lobed, *c*. 0.5×1 mm; styles 3, each distinctly forked, horizontal, adpressed to ovary. Fruiting pedicel *c*. 3 mm long, dilated at apex; fruiting calyx reflexed. Capsule subglobose, *c*. 3×4 mm, distinctly 3-lobed, hirsute; cocci 3; seeds 2 in each cocci, *c*. 1.8×1 mm, 3-angled, curved on dorsal side, smooth brown in colour.

Flowering & Fruiting: August – November.

Specimens examined: INDIA. Goa, North Goa District, Sanguem, 23.9.1997, V. Joshi & S. Rajkumar 1022, 1070; Selaulim dam site, 30.8.2010, S.S. Naik 4001, 4002, Collem stream, 11.9.2010, S.S. Naik, 4003, 4004, Waddem stream, 12.10.2010, S.S. Naik, 4005, 4006, 4007; Dudhsagar waterfalls, 11.9.2010, S.S. Naik, 4008, 4009, 4010. (All the specimens are deposited at Goa University Herbarium).

Morphologically, *P. talbotii* with the characters such as woody shrub, 4 sepals in staminate flowers, connate filaments and 6 sepals in pistillate flowers place it in subgenus *Eriococcus* of *Phyllanthus*, as proposed by Balakrishnan and Chakrabarty (2007).

Systematic position using molecular phylogeny

The ITS and *matK* based phylogenetic studies



Fig 3. Maximum likelihood tree using *mat* K gene sequences of *Phyllanthus talbotii* (Bootstrap percentage indicated on side). Asterisks' indicate that the position of these species is not known.

have contributed largely for understanding the evolutionary and ancestral relationships within the family Phyllanthaceae and specifically within the genus *Phyllanthus*. Shi-Xiao *et al.* (2011) used nuclear ITS sequences to disentangle *Phyllanthus reticulatus* Poir. in Africa. Maria *et al.* (2007) reported the use of ITS and *mat*K gene sequences to solve the taxonomic position of *Andrachne cuneifolia* Britton. Kathriarachchi *et al.* (2006) studied the phylogenetic relationship within the tribe Phyllantheae using ITS and *mat*K sequences of 95 species.

Phyllanthus talbotii is taxonomically an insufficiently known species till Balakrishnan and Chakrabarty (2007) placed it in the subgenus Eriococcus. The earlier works such as Webster (1956, 1957, 1958) and Kathriarachchi (2006) never dealt this species due to its narrow endemic nature. The phylogenetic tree constructed (Fig. 2) using ITS gene sequences shows the species nesting in a distinct clade along with P. cinereus Müll. Arg., P. pulcher (Baill.) Wall. ex Müll. Arg., P. sikkimensis Müll. Arg., and P. hainanensis Merr. which are members of the subgenus Eriococcus (Kathriarachchi et al., 2006; Balakrishnan & Chakrabarty, 2007; Juthatip et al., 2010). This clade is supported by 100% boot strap value. The sub-clade formed by P. cinereus and P. talbotii is supported by 71% bootstrap value, though geographically these are the closely distributed species among the species studied. Tree constructed using mat K sequences (Fig. 3) also supported the grouping of *P. talbotii* with *P. cinereus* and *P. pulcher* along with three more species with 100% boot strap value thus providing additional support for placing P. talbotii in the subgenus Eriococcus. As in ITS sequence, within the clade, bootstrap value supporting the subclade formed by P. talbotii and P. cinereus is low (65%). However, the systematic position of P. talbotii in subgenus Eriococcus is well supported by both ITS and mat K sequences.

Conclusion

The position of *P. talbotii*, a less known endemic and Critically Endangered (CR) species, in subg. *Eriococcus* has been confirmed by present morphological and molecular studies. Analyses of its closely related endemic species in the Western Ghats will certainly strengthen the knowledge on the group.

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Literature Cited

- Balakrishnan, N.P. & T. Chakrabarty 2007. *The Family Euphorbiaceae in India*. Bishen Singh Mahendra Pal Singh, Dehra Dun.
- Bancilhon, L. 1971. Contribution a' l'e'tude taxonomique du genre *Phyllanthus* (Euphorbiace'es). *Boissiera* 18: 9–81.
- **CBOL** Plant Working Group (http://www. barcoding.si.edu/plant_working_group.html)
- **Doyle, J.J. & J.L. Doyle 1990.** A rapid total DNA preparation procedure for fresh plant tissue. *Focus* **12**: 13–15.
- Felsenstein, J. 1985. Confidence limits on phylogenetics: an approach using bootstrap. *Evolution* **39**: 783–791.
- Juthatip, M., Suchada S., Nijsiri R. & H. Mizukami 2010. Molecular Phylogenetic Analysis of *Phyllanthus* Species in Thailand and the Application of Polymerase Chain Reaction-Restriction Fragment Length Polymorphism for *Phyllanthus amarus* Identification. *Biol. Pharm. Bull.* 33(10) 1723–1727.
- Kathriarachchi, H., Samuel R., Hoffmann, P., Mlinarec, J., Wurdack, K.J., Ralimanana, H., Stuessy, T.F. & M.W. Chase 2006. Phylogenetics of tribe Phyllantheae (Phyllanthaceae; Euphorbiaceae sensu lato) based on nr ITS and plastid matK sequence data. Am. J. Bot. 93 (4): 637–655.
- Kelchner, S.A. 2000. The evolution of non-coding chloroplast DNA and its application in plant systematics. *Ann. Mo. Bot. Gard.* 87: 482–498.
- Lee, S.K., Li, P.T., Lau, D.T., Yung, P.P., Kong, R.Y. & W.F. Fong 2006. Phylogeny of medicinal *Phyllanthus* species in China based on nuclear ITS and chloroplast *atpB- rbcL* sequences and multiplex PCR detection analysis. *Planta Med.* 72: 721–726.
- Liang, H., & K.W. Hilu 1996. Application of the *mat*K gene sequences to grass systematics. *Can. J. Botany.* 4: 125–134.
- Maria, S.V., Petra, H., Hashendra, K., Duane, A.K. & W.C. Mark 2007. Andrachne cuneifolia (Phyllanthaceae; Euphorbiaceae s.l.) is a Phyllanthus. Biol. J. Linn. Soc., 155: 519–525.

Raghavan, R.S. 1969. Flora of Agumbe and Tirthahalli

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area, Shimoga district. Ph. D. thesis, University of Pune, India.

- Samuel, R., Kathriarachchi, H., Hoffmann, P., Barfuss, M.H.J., Wurdack, K.J.C., Davis, C. & M.W. Chase 2005. Molecular phylogenetics of Phyllanthaceae: evidence from plastid *matK* and nuclear PHYC sequences. *American J. Bot.* 92: 132–141.
- Sedgwick, L.J. 1921. New Bombay species. J. Indian Bot. 2: 124
- Shi-Xiao, L., Hans-Joachim, E., Dianxiang, Z. & S.S. Renner 2011. Nuclear ITS Sequences Help Disentangle *Phyllanthus reticulatus* (Phyllanthaceae), an Asian Species not Occurring in Africa, but Introduced to Jamaica. *Syst. Bot.* 36(1): 99–104.
- Singh, N.P. & B.G. Kulkarni 1990. Phyllanthus talbotii Sedg. In: Nayar MP, Sastry ARK (eds) Red Data Book of Indian plants, Volume 3. Botanical Survey of India Calcutta, pp. 124– 125.
- Steele, K.P. & R. Vilgalys 1994. Phylogenetic analyses of Polemoniaceae using nucleotide sequences of the plastid gene *mat*K. *Syst. Bot.* 19: 126–142.
- Tamura, K., Nei, M. & S. Kumar 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences (USA)* 101: 11030– 11035.

- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & S. Kumar 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Mol. Biol. Evol.* (doi: 10.1093/molbev/msr121).
- Webster, G.L. 1956. A monographic study of the West Indian species of *Phyllanthus J. Arnold Arbor.* 37: 9–122, 217–268, 340–359.
- Webster, G.L. 1957. A monographic study of the West Indian species of *Phyllanthus J. Arnold Arbor.* 38: 51–80, 170–198, 295–373.
- Webster, G.L. 1958. A monographic study of the West Indian species of *Phyllanthus J. Arnold Arbor.* 39: 49–100, 111–212.
- White, T.J., Bruns, T., Lee, S. & J.W. Taylor 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications, (Ed.), Innis, M.A., Gelfand, D.H., Sninsky, J.J. & T.J. White. *Academic Press, Inc., New York*, pp. 315–322.

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