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MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF AN ENDEMIC SPECIES FROM TAMIL NADU, INDIA: *WRIGHTIA INDICA* NGAN (APOCYNACEAE)

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Wrightia indica Ngan is an endemic Apocynaceae species collected from Sitheri hills of Dharmapuri district in the Southern Eastern Ghats of Tamil Nadu. Morphological and molecular identification of species was observed by analyzing the chloroplast DNA regions using ITS marker and generated sequences. The present study confirms the molecular sequence of *Wrightia indica* for the first time and voucher specimen deposited in NCBI. They allow us to infer the phylogenetic relationships among other taxa within the same genus and those of other genera detailed description, ecological notes, and photographs of the species are provided for a better understanding of this little known endemic taxa.

Keywords: DNA barcoding, Endemic, Morphology, Tamil Nadu, *Wrightia indica*.

The Apocynaceae was first explained as "Apocinae" (de Jussieu, 1789). They are commonly known as the Dogbane family (Gentianales) usually consisting of trees, shrubs, vines, relatives with white latex or hardly ever watery juice, smooth margined and opposite or whorled leaves. The flowers are large, colorful, and slightly fragrant with five contorted lobes in clusters (rarely solitary) and fruits are in pairs. The plants are mostly laticiferous and generate various alkaloids and carotenoids with more medicinal properties. Apocynaceae comprises of around 375 genera and 5100 species (Endress *et al*. 2007) in tropical and subtropical areas, with lower representation in temperate regions (Endress and Bruyns 2000, Potgieter and Albert 2001). The circle of acquaintance has been extended to five sub-families such as Apocynoideae, Asclepiadoideae, Periplocoideae, Rauvolfioideae and Secamonoideae (Wong *et al.* 2013).

It is interesting to note that, within the genus a number of widespread species occur in seasonal or evergreen forest and several other species within the local distribution, especially occurring on limestone. Their habitats are quite varied from rain forests, deciduous dry forests, savannas and sandy thickets on the strand (Middleton 2007a). Many of these taxa are local endemics from karst limestone habitats and several others are threatened due to quarrying for cement (Clements *et al*. 2006).

DNA based identification is a high through out technology that reduces processing time and can discriminate at species level without taxonomic expertise (Laube *et al*. 2010). DNA barcodes are short DNA sequences of a standardized portion of the genome that can be used in the identification and differentiation of a species. DNA barcoding supports species identification, discover vegetation, and floristic species surveys in addition to ecological forensics studies which are critical to biodiversity management. For the success of DNA barcode, the barcode loci must have sufficient information to differentiate between closely related plant species and discover new cryptic species. For herbal plant identification, matK, rbcL, trnH-psbA, ITS, trnL-F, 5S-rRNA, and 18S-rRNA have been used as successful DNA barcodes.

Plant taxa identification is an important issue in plant science where contemporary molecular markers help to overcome the limitation of classical species identification and

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recognition. Amplification of PCR products using primers can detect SNPs of targets taxa showing high specificity and accuracy for the molecular identification of plant species. The primary advantage of this technique is that amplification and authentication are combined and a plant species can be identified based on the amplification of a product which indicates the presence of a particular allele and vice versa (Wang *et al*. 2010). Characterization of plants with the use of morphological and molecular markers is an ideal approach for the conservation of plant genetic resources and genetic improvement (Rout and Mohapatha, 2008). Both molecular and morphological markers are valuable for the identification of distinct populations or genotypes for conservation optimum sites for germplasm collection and ongoing changes in the pattern of diversity over time. Thus the present study employs morphological and molecular techniques to characterize and confirm the identity of the less known endemic species *W.indica.*

MATERIALS AND METHODS

Chemicals and reagents: Extraction buffer of pH 8 consisting of 3% cetyltrimethylammonium bromide (CTAB) (w/v), 100 mM Tris-HCl, 2 M NaCl, 25 mM ethylenediaminetetraacetic acid (EDTA), 3% β-mercaptoethanol (v/v), and 3% polyvinylpyrrolidone (w/v) and was freshly prepared. Other reagents such as 24:1 chloroform:isoamyl alcohol (v/v), Tris-EDTA, 10 mM Tris-HCl (pH 8), 1 mM EDTA, isopropanol, 70% ethanol and all other chemicals were purchased from Alpha and HiMedia company.

Study sites and sample collection: Sitheri hills, one of the segments of Eastern Ghats of Tamilnadu falls under Pappireddipatti Taluk in Dharmapuri district with a latitude and longitude dimension of 11.8917°N and 78.5076°E respectively. The study site comprises of various kinds of vegetation namely evergreen, riparian, dry deciduous scrub, and thorn scrub forests. The mean annual temperature in the study area ranges from 12°C to 35°C during Mar-Jun and averages between 10°C to 25°C during Oct-Jan with an average rainfall of 200 mm annually.

Field survey: Survey and field visit were conducted in the Sitheri hills of Eastern Ghats from 2017-2018. The specimens were collected, poisoned, processed, and labelled based on standard herbarium method. Habit, habitat, diagnostic features of the species, ecological data, associated plants, phenology, and photographs were noted during the field visit. The collected plant specimens were authenticated by the Botanical Survey of India (BSI), Southern Circle, Coimbatore, India. The specimens were deposited at the Madras Herbarium located in the Botanical Survey of India, Southern circle, Coimbatore under the accession numbers MH177870-MH177872. The specimens were also deposited in the Bharathi Herbarium at the Department of Botany Bharathiar University, Coimbatore under the accession no: 007755.

Genomic DNA Isolation: Genomic DNA was isolated from the plant samples using a modified CTAB method (Khanuja, 1999). 200 mg of leaves were grounded with preheated extraction buffer and incubated at 60°C for one hr. Followed by incubation, samples were

Table1: PCR Reaction Mix

Reagents	**Reaction Volume (20 µL)**
Taq Buffer-10X (100 mM TrisHCl/ 500 mM KCl)	2 µL
$MgCl_2$-25 mM	2 µL
$dNTP_s$-100 mM	2 µL
Forward and reverse primers (10 pM)	0.5 µL each
DNA template	1 µL
Taq DNA polymerase (5 U/µL)	0.2 µL
Nuclease free water	Made to 20 µL

Table 2: PCR Primer Sequences and the Reaction Condition

S. No	Gene	Primer Sequences	Reaction Conditions
1	*ITS2*	F- 5'GAAGGAGAAGTCGTAACAAGG 3' R- 3'TCCTCCGCTTATTGATAT GC5'	95 ºC 5 min, 95 ºC 1 min 55 ºC 1 min, 72 ºC 1 min (30 cycles) 72 ºC 10 min

centrifuged at 10,000 rpm for 10 min. An equal volume of Chloroform: Isoamyl alcohol (24:1) was added and then centrifuged at 10,000 rpm for 10 min. To the aqueous solution, 0.6 volume of ice-cold isopropanol was added and incubated at 4°C for 1 hr. The samples were centrifuged at 10,000 rpm for 10 mins and the pellet thus obtained was washed with 70% ethanol and finally resuspended in TE Buffer. The samples were then loaded onto 1% agarose gel and the DNA bands were visualized using an Alpha imager EC®, USA. The DNA thus isolated was used as a template for PCR.

PCR Amplification and DNA sequencing: PCR was performed to amplify the specific DNA sequence using respective barcode candidate-specific primers. PCR was carried out in a thermal cycler, Eppendorf, Germany. All the reagents for PCR were procured from Fermentas, Germany. The PCR reaction mixture composition is shown in Table 1 and the list of specific PCR primers used and the conditions are given in Table 2. The PCR product was separated in 1% agarose gel and the bands were visualized in the gel documentation unit (Alpha Digidoc, USA).

BLAST Analysis: BLAST (Basic Local Alignment Search Tool) is a commonly used method for identification (www.ncbi.nlm.nih.gov/BLAST/) of sequences that give the best alignments to all or only a part of the query sequence (Hebert *et al*. 2003). The obtained sequence when analyzed using BLAST tool, showed similarity to the ITS2 gene sequence of *Wrightia indica* plant available in the database.

Phylogenetic tree analysis: This method was used to construct phylogenetic trees. In the present study, trees constructed by Maximum likelihood method meet the requirements for species identifcation (Lahaye *et al*. 2008).

RESULTS

Morphological identification: In the present study, morphology and DNA barcoding was employed to confirm the identity of a little known endemic species *W.indica* Ngan in Ann. Missouri Bot. Gard. 52:140.1965.Fl. Tamil Nadu 2:80:1987.

Taxonomical treatment: Evergreen trees, 3-5 m tall, bark yellowish-brown; young branchlets yellowish pubescent, glabrate with age. Leaves opposite, petiole 25 mm long; lamina ovate, 5-10 × 3-4 cm, margin entire, apex acute to acuminate, membranous, glabrous above, puberulous below; lateral veins 8-12 pairs united near the margins of the lamina. Flowers actinomorphic, 5-merous, white or creamy or yellowish inside, 1.5-2 cm across in 15-31 flowered terminal aggregate dichasial cymes; pedicel 0.5-0.8 cm long, puberulous. Calyx 5 merous, ovate, connate, imbricate, obtuse, 2-3 mm long. Corolla white, subrotate, tube 4-6 mm long, lobes oblong to sub-obovate, 1-1.5 cm long; corona in 3 rows,

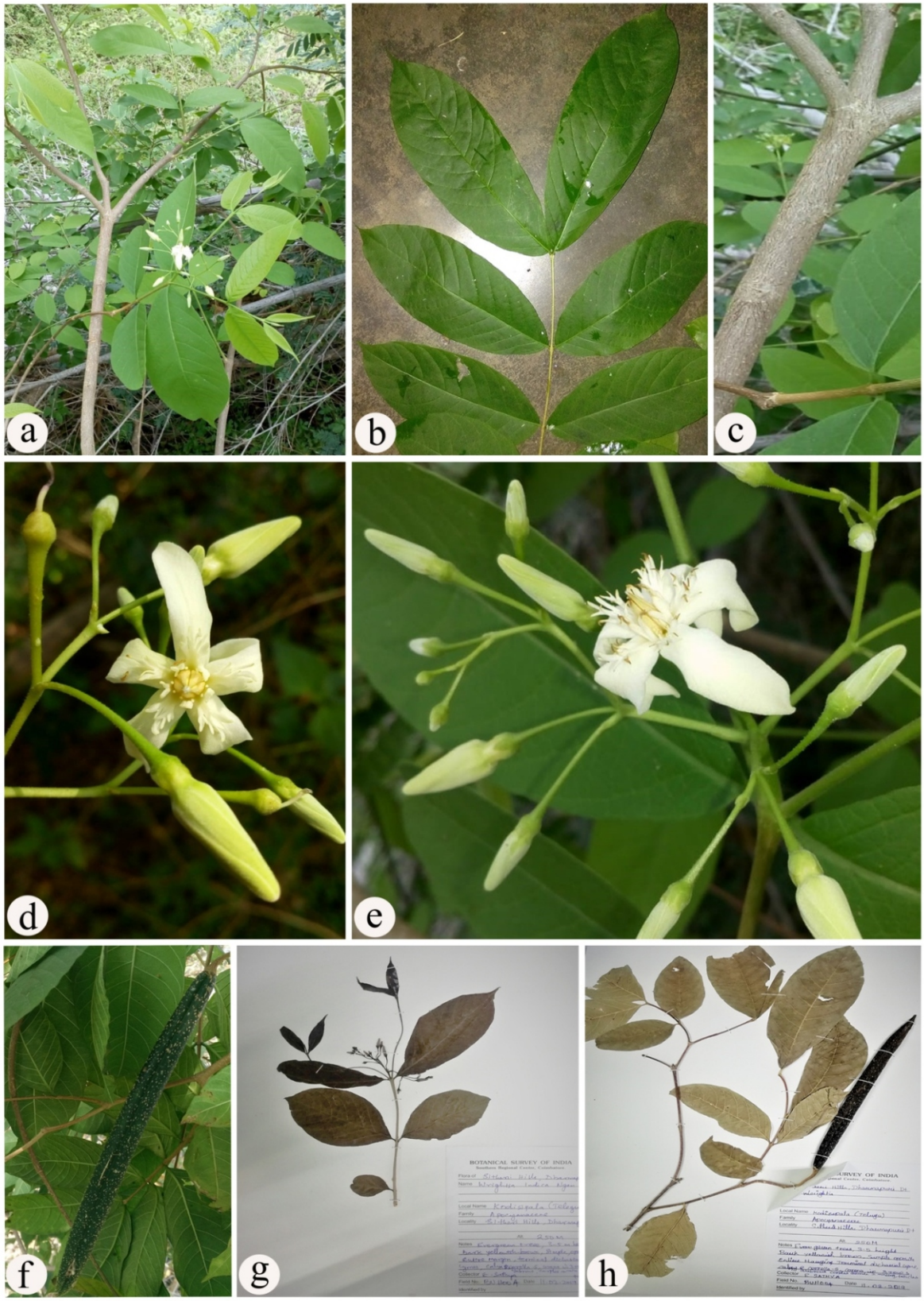

Figure 1: *Wrightia indica* **a.** Habit **b.** Leaf **c.** Stem **d.** Bud **e.** Flower **f.** Fruit **g.** herbarium flower **h.** Herbarium fruit

glabrous, outer row antipetalous 3 fids, 0.5-0.6 cm long, middle row alternipetalous, bifid, shorter than outer row, supplementary segments solitary simple. Stamens 5, inserted at the mouth of corolla tube; anthers sagittate, 6 mm long. Carpels connate, 2 mm long, ovary superior, oblong, many ovuled; style 5 mm long, stigma subcapitate; surrounded by anthers. Follicles are not observed(Fig.1).

Phenology: May to February.
Distribution : India Andhara Pradesh (Lalithamba A 2016) (Tamil Nadu, Coonoor ghat,Dharmapuri district (Ngan P T 1965) Endemic.

Ecology: A perennial endemic small tree grows in hillside forest in dry deciduous forest and also along the roadsides. It is found growing at an altitude of 11.8917°N and 78.5076°E. The elevation being 1097.3 m a. s.l. and the average annual rainfall is 900 mm (Vijayan *et al*. 2015). Associated species such as *Luisia pulniana, Kleinia grandiflora, Aegle marmelos, Andrographis paniculata, Santalum album, Hemidesmus indicus, Gloriosa superba, Sarcostemma intermedium, Eulophia graminea, Holarrhena antidysenterica, Capparis tomentosa, Capparis antidysenterica, Wrightia tinctoria, Pedalium murex, Abrus precatorius, Talinum portulacifolium, Vitex negundo, Bambusa sps are recorded*

Specimens examined: India, Tamil Nadu; Dharmapuri district, Sitheri hills, Sathya *et al.* accession number MH177870–MH177872;11/2/019 and Sathya *et al.* accession number (007755) Bharathiar University Herbarium.

Molecular Identification

DNA amplification and sequencing: *Wrightia indica* used to primers ITS2 and amplification of the sample DNA barcodes regions yielded 100% PCR act competence. Amplified samples were subjected to agarose gel electrophoresis and the result thus obtained are represented in Fig.2. PCR amplification for ITS2 primer resulted in 408bp in *Wrightia* sps. The amplicons of *ITS2* were purified and sequenced in Applied Biosystems 3730XL DNA Sequencer, Bioserve Technologies, Hyderabad, India. Sequence analysis was performed using codon code aligner software. The sequences were first assembled based on their names using the "Assemble in groups" option. The aligner software provides a quality-based consensus sequence rather than a majority-based consensus. But then, there might occur a few inconsistency which have to be cleared by manual editing. Hence, the raw sequences were subjected to manual editing where ever the base pairs are at discrepancies. The sequences were then compared using the "Compare contig" option using the built-in alignment algorithm "Muscle". The flanking primer sequences were deleted and the consensus sequvences were retrieved for further Blast analysis. Nucleotide sequvences data reported here have been submitted to the GenBank database with accession numbers (MG983523).

BLAST Analysis: Molecular markers represent a potentially cheaper and more exact alternative to chemical analysis, as DNA markers are unique nucleotide sequences and not affected by any environmental or physiological conditions. Their analysis can provide rapid and accurate monitoring of multiple types of samples including fresh plants, dried medicinal parts, and processed foods (Joshi *et al*. 2004, Ma *et al*. 2014, Vassou *et al*. 2015)."BLASTN" nucleotide analysis option was used to compare the sequences of uncertain plant species against the nucleotide database.The plant species were taxonomically proved and identified as *W. indica* Ngan sequences submitted to NCBI. Therefore the main focus of DNA barcoding is the discovery of universal sequences of regions that contain considered sequences as well as diversity across species. The tree showed a generally phylogenetic classification on the genetic

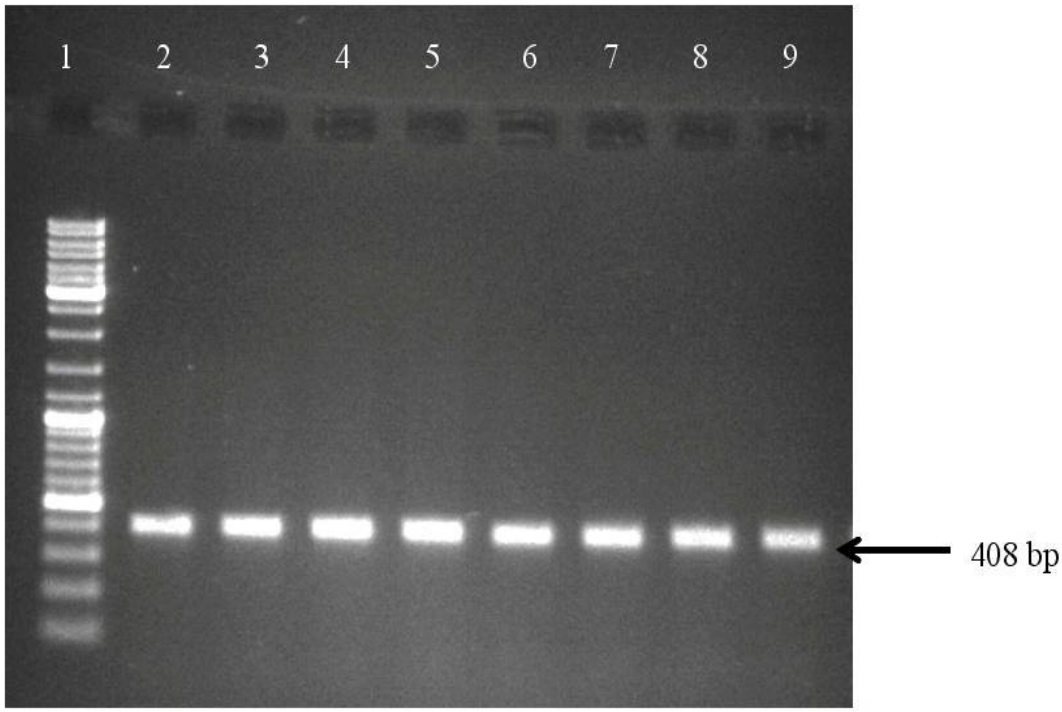

Figure 2: Genomic DNA Isolation.

level.

PCR Amplification of ITS2 region from *Wrightia indica*

Lane 1: 10 kb ladder

Lane 2-9: amplified products

>*Wrightia indica*

TTCCTCCGCTTATTGATATGCTTAAACT
CAGCGGGTAGCCCCGCCTGACC
TGGGGTCGCGGTCGTGGAGAGCGCTC
CTCGCAAGGGGCTCGCTCGCCTCA
GGGTCTGGCGGTCTCCTGGCTCGCGGG
GCGCGCGCACGACTCGCTTTCGA
GTTGAGGGTTTCAACCACCACTCGTCG
TGACGTCCGCGGCTAGGGACCCG
TGTTTAGGCCGGCCGCGCGAAGGCAC
GGGAGGCCAGTTTCCGCCCTCACC
CGCGGTCCTCGTCCCGTTGGGGGCGAG
GAGTGGGGAGGGCGACGTGATAC
GTGACGCCCAGGCAGGCGTGCCCTTG
GCCTGATGGCCTCGGGCGCAACTT
GCGTTCAAAAACTCGATGGTTCACGGG
ATTCTGCAATTCACACCAAGTAT
CGC

W.indica was deposited in NCBI gene sequence bank and assigned with accession no: MG983523 for further reference and consultations.

Evolutionary analysis by Maximum Likelihood method: The evolutionary history was inferred by using the Maximum Likelihood method and the Kimura 2-parameter model (Kimura 1980). The tree with the highest log likelihood (-2811.08) is shown Fig 3. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 18.9823)). This analysis involved 6 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions with less than 95% site coverage were eliminated, i.e., fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any

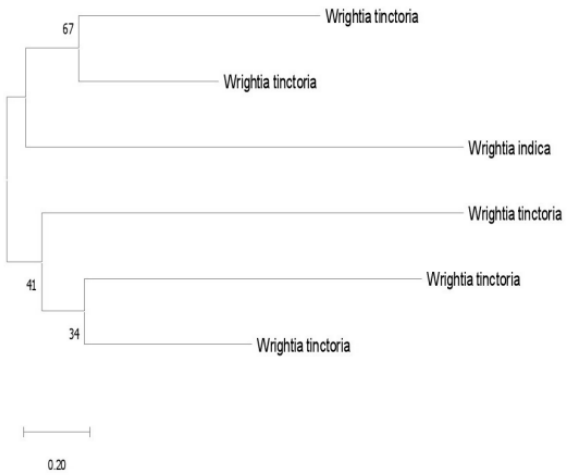

Figure 3: Phylogenetic tree species of *Wrightia*

position (partial deletion option). There were a total of 351 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al*. 2018). The phylogenetic trees of *W.indica,* and *W.tinctoria* are shown in Fig.3.

Phylogenetic analysis also revealed that distinct clusters showis the high resolution at the species level (Kim *et al*. 2016). The phylogenetic tree was constructed by applying the likelihood method. From these phylogenetic analyses, we confirm that the identification of the *Wrightia indica* can be achieved using sequence variability in this ITS sequences. The phylogenetic tree of the *Wrightia indica* derived from ITS sequences is shown in Fig. 3. Revised morphology and molecular data in phylogenetic fertenity in the genus *Wrightia.*

The phylogenetic tree was constructed by Maximum Likelihood method. 2 species of *Wrightia* using ITS Region for the 2 species of *Wrightia* include *W.tinctoria, W.tinctoria, W.indica, W.tinctoria, W.tinctoria, W.tinctoria.*

DISSCUSSION

During floristic exploration in various plants of Eastern Ghats of Tamil Nadu, few specimens of *Wrightia* were critically examined, collected from Coonoor Nilgiris in the year (1965) was identified as the genus *Wrightia* (Ngan 1965). *Wrightia indica* Ngan is an endemic species. A specimen is stored under the name of *Wrightia rothii* G. Donin in the Natural history museum (BM). The species was first collected by Ngan, followed by Yeshoda 189 (NY) collector from Dharmapuri district Tamil Nadu. As per the recent distributional record of Andhra Pradesh, it is reported as endemic (Lalithabhama 2016). International Union for the Conservation of Nature (IUCN) Red List categories and the global starting system species located in hotspots of high conservation value should be inventoried to assess the distribution and population status of endemics (Bakshcomeau *et al*. 2016). This species is considered as rare and endemic to Tamil Nadu.

The genus *Wrightia* is mainly distributed in the tropical region in Asia. The genus *Wrightia* comprises 23 species distributed in the tropical world (Mabberly 2008). Ngan (1965) working on the revision of the genus *Wrightia* has reported 23 species and 10 variety in India. Recently a few new species have been described (Forster 1993, Bahadur and Bennet 1978, Middleton and Santisuk 2001, Middleton 2007a, Middleton 2013; Middleton 2010).

The present study deals with a species endemic to Eastern Ghats from the Apocyanaceae family. *Wrightia dolichocarpa* Bahadur & Bennet and *Wrightia indica* Ngan were reported to be endemic to India (Nayar 1996). The rbcL sequenced data of *W.arborea* was submitted in EMBL (Sennblad *et al.* 1998). In the present study specific aspects, such as the morphological and molecular characters and the environment with the distribution in the studied area were included. Along with these, DNA barcode of this species based on sequence variation in this blast will help in the identification of this species.

These data would also be helpful in the conservation of the species using biotechnological approaches because endemic plant species are of genetic importance resources of any nation. Emerging advances in

DNA barcoding coupled with next-generation sequencing and high-resolution melting curve analysis have paved the way for successful species-level resolution recovered from finished herbal products. The study reported in DNA barcode regions, rDNA-ITS, matK, and rbcL, can be used to discriminate between the four *Paeonia* species (Kim *et al.,* 2016). Furthermore, species-specific marker nucleotides identified in this study will help to accurately authenticate each species and standardize the quality of the two traditional herbal medicines, Paeoniae Radix and Moutan Radicis Cortex (Kim *et al.* 2016).

CONCLUSION

Wrightia indica (family: Apocyanaceae) is an important medicinal and endemic plant of Eastern Ghats. Phylogenetic study and barcoding are very important for this species. DNA-based molecular markers are more reproducible when compared to other species for identification and authentication. Nuclear and chloroplast DNA markers are currently used for the development of DNA barcoding and phylogenetic studies.

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REFERENCES

Bahadur K N and Bennet S S R 1978 A New Species of *Wrightia* R. Br. (Apocynaceae) from India. *Indian Forester* **104** 1 32-35.

Baksh-Comeau Y S, Maharaj S S, Adams C D, Harris SA, Filer DL and Hawthorne WD 2016 An annotated checklist of the vascular plants of Trinidad and Tobago with analysis of vegetation types and botanical hotspots. *Phytotaxa* **250** 1 1-431.

Clements R, Sodhi NS, Schilthuizen M and Ng PK 2006 Limestone karsts of Southeast Asia: imperiled arks of biodiversity. *Bioscience* **56** 733-742.

De Jussieu AL 1789 *Genera plantarum*. Viduam Herissant, Paris. 498

Endress ME and Bruyns PV 2000 A revised classification of the Apocynaceae sl. *Bot Rev* **66** 1 1-56.

Endress ME, Liede-Schumann S and Meve U 2007 Advances In Apocynaceae: The Enlightenment, an Introduction *Ann Missouri Bol Garden* **94** 2 259-267.

Forster PI 1993 Resurrection of *Wrightia versicolor* ST Blake (Apocynaceae). *Austrobaileya* 109-112.

Hebert PDN, Cywinska A, Ball SL and deWaard JR 2003 Biological identifications through DNA barcodes. *Proc Biol Sci* 270 313-321.

Joshi K, Chavan P, Warude D and Patwardhan B 2004 Molecular markers in herbal drug technology. *Curr Sci* 159-165.

Khanuja SPS, Shasany AK, Darokar MP and Kumar S 1999 Rapid isolation of DNA from dry and fresh samples of plants producing large amounts of secondary metabolites and essential oil. *Plant Mol Biol Rep* **17** 1-7

Kim WJ, Ji Y, Choi G, Kang YM, Yang S and Moon BC 2016 Molecular identification and phylogenetic analysis of important medicinal plant species in genus *Paeonia* based on rDNA-ITS, matK, and rbcL DNA barcode sequences. *Genet Mol Res* **15** 10 4238.

Kimura M, 1980 A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16** 111-120.

Kumar S, Stecher G, Li M, Knyaz C, and Tamura K 2018 MEGA X: Molecular Evolutionary Genetics Analysis across

computing platforms. *Mol Biol Evol* **35** 1547-1549.

Lalithamba A 2016 The New Distributional Record of *Wrightia indica* Ngan, (Apocynaceae) from Velugonda Hills of Nellore District, Andhra Pradesh, India. *J Indian Bot Soc* **95** 76-77.

Laube I, Hird H, Brodmann P, Ullmann S, Schöne-Michling M, Chisholm J and Broll H 2010 Development of primer and probe sets for the detection of plant species in honey. *Food Chem* **118 4** 979-986.

Lahaye R, VanderBank M and Bogarin D 2008 sDNA bareoding the foras of biodiversity hotspots. *PNAS* **105** 2923–2928

Ma C, Li Y, Li M, Deng G, Wu X, Zeng J and Liu X. 2014 MicroRNA-124 negatively regulates TLR signaling in alveolar macrophages in response to mycobacterial infection. *Molimmunol* **62** 1 150-158.

Mabberley DJ 2008 *Mabberley's Plant-Book - A portable dictionary of the vascular plants, their classification and uses.* Third edition. Cambridge University Press. Cambridge.

Middleton DJ 2007a. A new species of *Wrightia* (Apocynaceae: Apocynoideae) from Thailand. *Thai Forest Bull* **35** 80-85.

Middleton DJ 2007b. Apocynaceae (subfamilies Rauvolfioideae and Apocynoideae). *Flora Malesiana-Series 1 Spermatophyta* **18** 1 1-152.

Middleton DJ 2010 Three new species of *Wrightia* (Apocynaceae: Apocynoideae) from Thailand. Gard Bull (Singapore) **61** 129-138.

Middleton DJ 2013 *Wrightia calcicola* (Apocynaceae: Apocynoideae), a new species from Thailand. *Thai Forest Bull* **41** 81-84.

Middleton DJ and Santisuk T 2001 A new species of *Wrightia* (Apocynaceae: Apocynoideae) from Thailand. *Thai Forest Bull Bot* **29** 1-10.

Nayar MP 1996 *Hot spots of endemic plants of India, Nepal and Bhutan.* Tropical Botanic garden and research Institute Botany 252.

Ngan PT 1965 A revision of the genus *Wrightia* (Apocynaceae). *Ann Missouri Bot Garden* **52** 2 114-175.

Potgieter K and Albert VA 2001 Phylogenetic relationships within Apocynaceae s.l. based on trnL intron and trnL-F spacer sequences and propagule characters. *Annals Missouri Bot Garden* 523-549.

Rout GR and Mohapatra A 2006 Use of molecular markers in ornamental plants: a critical reappraisal. *Eur J Hortic* 53-68.

Sennblad B, Endress ME and Bremer B 1998 Morphology and molecular data in phylogenetic fraternity: the tribe *Wrightieae* (Apocynaceae) revisited *Am J Bot* **85**8 1143-1158.

Vassou SL, Kusuma G and Parani M 2015 DNA barcoding for species identification from dried and powdered plant parts: a case study with authentication of the raw drug market samples of *Sida cordifolia*. *Gene* **559** 1 86-93.

Vijayan A, Santhakumar S, Vivekraj P and Kalavathy S 2015 Parasitism of host trees by the Loranthaceae in the region of Sitheri Hills (Eastern Ghats), Tamil Nadu, India. *Bull Env Pharmaco Life Sci* **4** 104-109.

Wong SK, Lim YY and Chan EW 2013 Botany, uses, phytochemistry and pharmacology of selected Apocynaceae species. *Rev Pharma Comm* 3 3 2.