Species delimitation of *Melia dubia* Cav. from *Melia azedarach* L. complex based on DNA barcoding

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Species delimitation of *Melia dubia* Cav. from *Melia azedarach* L. complex based on DNA barcoding

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Abstract

The genus *Melia* L., which belongs to the 'Mahogany' family Meliaceae, is a source of important phytochemicals with marked medicinal properties. Species identification in *Melia* is complex due to the existence of overlapping morphological features. Though *Melia dubia* Cav. is listed as a synonym of *Melia azedarach* L., it is not clear from the available literature whether they are the same species or different, and the species complexity still remains unresolved. In the present study, ten accessions of *M. dubia* and *M. azedarach* were analysed by DNA barcoding using three chloroplast DNA markers (*rbcL*, *matK*, and *trnH-psbA*), and one nuclear marker (ITS2). Intra-specific divergence was not found in any of the four markers. However, the inter-specific divergence between *M. azedarach* and *M. dubia* ranged between 0.3% (*rbcL*) and 4.7% (ITS2) for individual markers, and for the combined dataset, it was 8.5%. Among the four markers, ITS2 was found to be the most suitable marker for differentiating *M. azedarach* and *M. dubia*. Parsimony analysis of the DNA barcoding data showed a clear segregation between *M. azedarach* and *M. dubia* as distinct monophyletic clades in all the markers, except the *rbcL* marker. Our results based on Bayesian analysis of the concatenated dataset strongly support the view that *M. dubia* be considered a distinct species; not a synonym of *M. azedarach*.

Keywords: *Melia azedarach*; Species complex; DNA barcodes; ITS2; Bayesian Phylogeny

Introduction

The genus *Melia* L. of Meliaceae (Mahogany family) contains hardwood deciduous tree species that are widely distributed in the tropical and subtropical regions of the world (Mabberley 1984). In Meliaceae, the species of *Melia* are distinguished by indumentum of stellate hairs in young plant parts, bi-tri-pinnate leaves, flowers on panicle inflorescence, 3-8-lobed or sometimes capitate stigma, 1-2-seeded drupe, and stony endocarp (Gamble 1915; Hajra et al. 1997). This genus is of great ecological, economic, and medicinal importance in the development of organic pesticides and fertilizers, agro-forestry, and herbal formulations. It is gaining importance due to a large demand for phytochemicals such as alkaloids, steroids, flavonoids, saponins, essential oils and limonoids, which have antimicrobial, insecticidal, and nematocidal properties (Kumar et al. 2003; Nagalakshmi et al. 2003; Kamaraj et al. 2010; Dharmalingam et al. 2014). Natural health products derived from *Melia* have been reported to be useful in treating diabetes, inflammation, and ulcers (Sharma and Singla 2013; Gopal et al. 2015). *Melia* is also in high demand for its fast-growing wood with good physical and mechanical properties, which are suitable for manufacturing facilities that make plywood, furniture and paper (Saravanan et al. 2013).

Despite its multipurpose values and importance, species level diversity within *Melia* has not yet been completely clarified. The taxonomic status of *Melia* is uncertain in Asia, Africa, America, Australia, and Europe owing to variable anatomical and morphological features, ecotypes, and karyotypes (Ghosh 1968; Mabberley 1984; Sharma et al. 2012). The genus *Melia* was first recognized in *Hortus cliffortianus*, and it consisted of two species, *M. azedarach* and *M. azadirachta* (Linnaeus 1738). Later, *M. azadirachta* was moved to a new genus (*Azadirachta*) based on the pinnate leaves (bipinnate in *Melia*) and the species was named as *A. indica* A. Juss (De Jussieu 1830). Species of *Melia* are native to the Indo-china regions, and they were introduced to several other countries for their timber value. *Melia*

azedarach L. is widely distributed throughout the tropical, sub-tropical and warm temperate regions, and it is naturalized in Africa, Australia, India, China, southern Europe, the USA, Mexico, Central America, the Caribbean, and many Pacific islands. *Melia dubia* Cav. is distributed in south-eastern Asia, India, China, Japan, and Australia (Mabberley 1984; Husain and Anis 2009; Hasegawa et al. 2010). Species complexity exists in *Melia* due to the occurrence of cultivated varieties, and morphotypes, and therefore, the number of species in this genus remains to be conclusively determined. Inter-species differentiation between the *Melia* species in China is ambiguous, and it is not clear whether *M. toosendan* Siebold exists as a separate species (Liao et al. 2016). Gamble (1922) reported *M. azedarach* L., *M. birmanica* Kurz, *M. composita* Willd., and *M. indica* (A. Juss.) Brandis from India. Mabberley (1984) has reviewed the nomenclature of *Melia*, and treated all the cultivated varieties and morphotypes such as *M. sempervirens* (L.) Sw., *M. dubia* Cav., *M. composita* Willd., *M. toosendan* Siebold, *M. australasica* A. Juss., *M. volkensii* Gürke, *M. bukayun* Royle and *M. japonica* G. Don. as one species under *M. azedarach*. In the Flora of India (Hajra et al. 1997), *M. birmanica* and *M. composita* were synonymized under *M. dubia*, and *M. azedarach* and *M. dubia* were recognized as two distinct species. These two species could be clearly distinguished based on deep purple (lilac) honey-scented flowers in *M. azedarach*, and white inodorous flowers in *M. dubia*. The fruits of *M. azedarach* are globose-ellipsoid measuring 1.0 - 1.5 cm long, while that of *M. dubia* are ovoid-ellipsoid measuring 2.5 - 4.0 cm long (Table 1). Cavanilles (1789), Sasidharan and Sivarajan (1996), Nagalakshmi et al. (2001), and Murugesan et al. (2013) have also considered them as different species. In contrast, many studies have considered only *M. azedarach* as an accepted species (Dassanayake 1995; Ahmed et al. 2012; Dharmalingam et al. 2014; Lake 2015). The existing complexity of whether *M. azedarach* and *M. dubia* are the same or different species can be

resolved using DNA barcoding, which was reported to be efficient in species differentiation and identification of cryptic species (Ragupathy et al. 2009; Liu et al. 2016).

DNA barcoding uses short, standardized, universal DNA regions for species identification (Hebert et al. 2003). Differentiation of congeneric species by DNA barcoding requires higher inter-specific divergence than intra-specific divergence in all pair-wise species combinations (Vassou et al. 2015; Umapathy et al. 2015). Three chloroplast DNA barcode markers (*rbcL*, *matK* and *trnH-psbA*) and one nuclear DNA barcode marker (ITS2) have been widely used for DNA barcoding in plants (Chen et al. 2010; Hollingsworth 2011; Han et al. 2013; Sundar et al. 2016). We have collected ten accessions of *M. azedarach* and *M. dubia* from geographically distant places, and DNA barcoded by sequencing *rbcL*, *matK*, *trnH-psbA*, and ITS2 DNA barcode markers to understand the differentiation between the two species.

Materials and Methods

Plant Collection

Ten accessions each representing *M. azedarach* and *M. dubia* each were collected from eight different states in India (Table 2). Three accessions of *Azadirachta indica* collected from Tamil Nadu, India were included as an allied species. All the specimens were identified taxonomically and voucher specimens were deposited to the SRM University Herbarium.

DNA isolation, PCR amplification and DNA sequencing

Genomic DNA was extracted from 100 mg of fresh leaf tissue using the cetyl trimethyl ammonium bromide (CTAB) method (Saghai-Marooof et al. 1984) with minor modifications (Nithaniyal et al. 2014). The genomic DNA was checked on 0.8% Agarose gel and quantified. PCR amplification of the DNA barcodes was done using universal primer pairs for *rbcL* (Zhang et al. 2003; Fazekas et al. 2008), *matK* (Heckenhauer et al. 2016), *trnH-psbA* (Kress et al. 2005) and ITS2 (Chen et al. 2010). PCR reaction mixture contained 1X buffer

with 1.5 mM MgCl₂, 0.2 μM dNTPs, 5.0 pmol primers, 1 unit *Taq* DNA polymerase (GenetBio Inc., Korea) and 50 ng of genomic DNA. PCR amplification included initial denaturation at 95°C for 5 minutes, 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute, final extension at 72°C for 5 minutes, and hold at 4°C. The PCR amplified products were purified using EZ-10 Spin Column PCR Purification Kit (Bio Basic Inc. Ontario, Canada). Sequencing of the PCR products was carried out in our laboratory using Big-dye terminator chemistry and 3130xl Genetic Analyzer (Life Technologies, California, USA) by following the standard manufacturer's protocol. All of the collected accessions of *Melia* were successfully sequenced, even though they were reported to contain diverse metabolites; these can co-purify and irreversibly bind with nucleic acids to affect PCR amplification of the barcode markers (Rawat et al. 2016). Though the mononucleotide repeats were reported to cause problems in sequencing of *trnH-psbA* marker from plants (Devey et al. 2009), such problems were not encountered in *Melia*, probably due to smaller size of the repeats and low frequency of occurrence.

Sequence Retrieval from GenBank Database

The GenBank database of NCBI (<https://www.ncbi.nlm.nih.gov/>) was searched for the presence of *M. azedarach* and *M. dubia* DNA barcode sequences. For *M. azedarach*, 31 sequences representing *rbcL*, *matK*, *trnH-psbA*, and ITS2 markers were retrieved. However, for *M. dubia*, only 2 sequences representing *rbcL* and *matK* markers were available. In addition, we have retrieved 110 barcode sequences of *rbcL*, *matK*, *trnH-psbA*, and ITS2 markers from closely related species from Meliaceae family to understand the species relationship. The sequences were included only if they were i) tagged with publication in refereed journals or unpublished data from scientists with good publication record in taxonomy, ii) placed in a clade that is appropriate to its taxonomic affiliation, and iii) showed

proper sequence alignment using MUSCLE algorithm (Edgar 2004) with 70% threshold. The sequences curated from GenBank database with other details such as voucher IDs and references are given in Table S1.

Phylogenetic Analysis

The sequences were manually edited for base calling errors using Sequence Scanner Software v1.0 (Applied Biosystems, CA, USA). Intra- and inter-species divergences were calculated using TaxonDNA v.1.6.2. (Meier et al. 2006). Divergence was calculated as per cent divergence = (Number of mismatched nucleotides / Total number of aligned nucleotides) x 100. The basic sequence statistics like conserved sites, variable sites and parsimony informative sites were calculated using PAUP*4.0b10 (Swofford 2002). Phylogenetic analysis was performed individually for each marker using parsimony method, and Bayesian approach was adopted for concatenated dataset.

Parsimony analysis: Heuristic search was conducted with 10000 random addition sequence replicates using Tree Bisection-Reconstruction (TBR) in PAUP. Bootstrap analysis was carried out with 100 bootstrap replicates, each with 200 random additions per replicate. The tree using 50% majority rule consensus was generated and viewed in FigTree ver. 1.3.1 (<http://tree.bio.ed.ac.uk/software/figtree>).

Bayesian analysis: Datasets were generated independently for four markers, and included as different partitions in the concatenated dataset. All four markers were combined considering the concordance at their topologies. Evolutionary model was set as mixed in MrBayes 3.2 (Ronquist et al. 2012), and Monte Carlo Markov Chain (MCMC) analysis was performed for 2M generations. Later, the parameters and trees were summarized into 50% majority rule consensus tree, and it was viewed in FigTree along with the branch support of posterior probabilities (PP) from the analysis.

Results

All the accessions of *Melia* collected for the current study were DNA barcoded using *rbcL*, *matK*, *trnH-psbA*, and ITS2 DNA barcoding markers. All the sequences were generated from vouchered specimen. In the case of the *rbcL* and *matK* markers, open reading frames were found to be intact, and there was no DNA sequence length variation between *M. azedarach* and *M. dubia* in either of the markers. For the ITS2 marker, good quality sequences were obtained for all the samples, and the DNA sequence length was 459 bp and 464 bp in *M. azedarach* and *M. dubia*, respectively. In the case of *trnH-psbA*, the maximum and the minimum size of 'A' or 'T' mononucleotide repeat was seven, and two, respectively. The mononucleotide repeat sequences did not affect sequencing, and the DNA sequence length was 377 bp and 378 bp in *M. dubia* and *M. azedarach*, respectively. All the DNA barcode sequences were obtained full-length for the target region data were submitted to the Barcode of Life Database (www.boldsystems.org) under the BOLD accession IDs SRM001018A to SRM001020J.

Entire barcode sequences were used to calculate divergences separately for individual markers and in combination for all the four markers. Intra-specific divergence was not observed in any of the four markers in *M. azedarach* and *M. dubia*. However, total inter-specific divergence between *M. azedarach* and *M. dubia* was found to be 8.5%, and the highest divergence was found in ITS2 (4.7%) followed by the *trnH-psbA* (2.0%), *matK* (1.5%), and *rbcL* (0.3%) markers. When the nucleotide composition of each marker was analyzed, the number of parsimony informative sites was also the highest in the ITS2 marker followed by the *trnH-psbA*, *matK* and *rbcL* markers (Table S2).

The parsimony trees constructed using DNA barcode data showed a clear segregation between *M. azedarach* and *M. dubia* as distinct monophyletic clades in all the markers, except the *rbcL* marker (Fig. S1). The phylogenetic tree constructed using the *rbcL* marker

contained 33 sequences representing *M. azedarach*, *M. dubia*, and *M. volkensii*. *M. azedarach* formed a separate clade, which consisted of the accessions from Australia, Austria, Egypt, India, Korea, South Africa, and the United States (Fig. S1a). Accessions of *M. dubia* from India and Austria formed another distinct clade. *M. volkensii* from Kenya was differentiated from the *M. azedarach* cluster albeit with very low genetic divergence. The phylogenetic tree constructed using the *matK* marker contained 26 sequences representing *M. azedarach*, *M. dubia*, and *M. volkensii*. In this tree, the *M. azedarach* clade was represented by the accessions from Austria, South Africa, Switzerland, and the USA (Fig. S1b). The *M. dubia* clade was distinct and included accessions from India and Austria. *M. volkensii* from Kenya was clearly differentiated from the *M. azedarach* cluster, which formed the basal clade for *M. azedarach* and *M. dubia* at 99% bootstrap support. In the case of *trnH-psbA* marker, the phylogenetic tree was constructed using 25 sequences representing *M. azedarach*, and *M. dubia* (Fig. S2a). *M. dubia* species were clearly differentiated (99% bootstrap support) from *M. azedarach*, which formed a separate clade consisting of the accessions from India, China, Korea, and the USA. The phylogenetic tree constructed with the ITS2 marker contained 28 sequences representing *M. azedarach* and *M. dubia*. The accessions of these two species formed distinct clades with 79% bootstrap support. However, the accessions of *M. azedarach* from India and Korea formed an internal clade. The accessions of *M. azedarach* and *M. azedarach* var. *toosendan* from China formed a clade within *M. azedarach* with 99% bootstrap support (Fig. S2b). *A. indica* stood as a sister to the genus *Melia* in all the four markers. In the Bayesian analysis based on the concatenated dataset of all four markers, one clade with the accessions of *M. azedarach* and another clade with the accessions of *M. dubia* along with the polytomy of the lone accession of *M. volkensii* from Kenya were observed (Fig. 1). The branch support with high PP value (100%) clearly showed that *M. azedarach* and *M. dubia* are distinct lineages. An accession of *M. dubia* reported from Austria was

clustered within the *M. dubia* clade that was formed by the accessions from India. Two different lineages were observed within the *M. azedarach* clade. *Cipadessa baccifera* was used as an outgroup in both parsimony and Bayesian analysis.

Discussion

We observed that *M. azedarach* and *M. dubia* have morphologically distinguishable characters, but information on their genetic variations is lacking. Recently, DNA barcoding has been extensively utilized to confirm species level identification and to resolve taxonomically complex groups based on genetic divergence (Song et al. 2009; Ragupathy et al. 2009; Pang et al. 2011; Nithaniyal and Parani 2016). Therefore, DNA barcoding was employed as a tool to resolve species complexity between *M. azedarach* and *M. dubia*. An ideal DNA barcode region should have lower variability within a species than between the congeneric species (Taberlet et al. 2007). Therefore, three chloroplast markers and one nuclear marker, which were reported as efficient DNA barcodes for species differentiation, were used in this study.

Ten accessions of *M. azedarach* and *M. dubia* were collected from geographically distant locations and they showed fidelity to the respective species as indicated by low level of intra-specific and high level of inter-specific divergence. Even though all the four markers were able to differentiate the two species, considering the level of divergence, ITS2 was found to be the most promising DNA barcode for species differentiation. Earlier studies on taxonomic classification of various congeneric species have also reported ITS2 as the best candidate for species identification with high genetic divergence (Coleman 2003; Yao et al. 2010; Dong et al. 2011; Umapathy et al. 2015). In addition, there was also length variation between the two species with regard to *trnH-psbA* and ITS2 markers. Combining all the four DNA barcode markers, *M. azedarach* and *M. dubia* showed 8.5% inter-specific divergence, which is unlikely if they belong to the same species. In the parsimony analysis using the data

from individual markers, the accessions of *M. azedarach* and *M. dubia* bifurcated into two distinct clades in all the markers, except the *rbcL* marker. No accession from *M. azedarach* was intermixed with the clades of *M. dubia* and *vice versa*. Bayesian analysis of the concatenated dataset clearly resolved *M. azedarach* and *M. dubia* as distinct lineages and there was an unresolved polytomy of *M. dubia* with *M. volkensii* in the root of the genus *Melia*. This may be due to a poor contribution of the *rbcL* marker (less divergent) in the absence of retrieved data for *trnH-psbA* and ITS2 markers. But the clades are supported with high PP values compared the independent datasets. Within the *M. azedarach* clade, there are two lineages, which may be due to sharing of native and introduced germplasms. But one cannot derive such relationships conclusively from the current study with limited data from world populations. An earlier study based on SRAP markers also reported the existence of significant intra-specific genetic polymorphism in the *M. azedarach* populations from China (Liao et al. 2016). These observations indicated genetic complexity within *M. azedarach*, which requires further study.

Species differentiation in *Melia* is difficult when it is only based on vegetative characters. Accurate methods for selection of species for agro-forestry, silviculture and medicinal uses are necessary. The present study shows that the ITS2 DNA barcode marker could be used as a species-specific marker for confirming species identity. In addition, the DNA barcodes will be much more helpful in authentication of *Melia*-derived materials that are traded, including raw drugs and commercial timber.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figure caption

Fig. 1: Bayesian phylogeny of genus *Melia* based on concatenated dataset of four markers using MrBayes 3.2. Highlight with color shows the cladogenesis of both *Melia azedarach* clade (Clade 1) and the clade with *Melia dubia* and *M. volkensii* (Clade 2). The posterior probabilities values in percentage are shown at the nodes. The arrow indicates the created polytomy of the *Melia dubia* accessions with the *M. volkensii* accession.

Draft

Table 1. Morphological characters of *Melia azedarach* L. and *Melia dubia* Cav.

Characters	<i>Melia azedarach</i>	<i>Melia dubia</i>
Habit	Deciduous tree, to 11 m tall	Deciduous tree, to 25 m tall
Bark	Dark brown, thick and deeply exfoliate	Dark brown, smooth, exfoliating in thin narrow strips
Branchlets	Scurfy-tomentose, glabrous when mature	Scurfy-tomentose, glabrous when mature
Leaves	2-3-pinnate, 30-90 cm long	2-3-pinnate, >1 m long
Pinnae	3-8 pairs, usually opposite, 20-30 cm	3-8 pairs, usually opposite, 20-30 cm
Leaflets shape and size	5-9 on each pinna, ovate-lanceolate or elliptic-lanceolate, oblique at base, toothed or lobed at margins, acuminate at apex, 2.5-5 x 1-2.5 cm	5-13 on each pinna, ovate to lanceolate, oblique at base, serrulate or entire at margins, acuminate at apex, 3-12 x 1.5-4 cm
Secondary nerves	8-15 pairs	6-16 pairs
Petiолules	c.5 mm long, longer in terminal leaflet	c.5 mm long, longer in terminal leaflet
Inflorescence	Panicle, 15-20 cm long, axillary	Panicle, 12-20 cm long, axillary
Flowers*	Deep purple (lilac), honey-scented	White, inodorous
Pedicle	Slender, to 7 mm long	Slender, to 7 mm long
Calyx	5-lobed; lobes ovate-oblong, pubescent without	5-lobed; lobes ovate-oblong, Tomentose
Corolla	5, linear-oblong, deflexed, c.8 mm long, pubescent without when young, glabrous	5, linear-spathulate, concave, c.5 mm long, pubescent on both surfaces
Staminal column	0.5-1 cm long, cylindric, 20-30-toothed, purple.	0.5-1 cm long, gibbous at base, 10-toothed, nearly glabrous within, white
Anther	Oblongoid, shorter than lobe, apiculate, pubescent	Oblongoid, at mouth of tube, apiculate, pubescent
Ovary	Ovoid-subglobose, glabrous; 3-loculed; ovules 2 in each cell	Ovoid-subglobose, glabrous; 3-loculed; ovules 2 in each cell
Style	Cylindric, clavate at apex, longer than staminal tube, apiculate	Cylindric, clavate at apex, longer than staminal tube
Stigma	3-5-fid, truncate	3-5-fid, truncate
Drupe*	Globose-ellipsoid, 1-1.5 x 0.8-1 cm, bright yellow when ripe, 1-seeded	Ovoid-ellipsoid, 2.5-4 x 2-2.5 cm, yellowish when ripe, pendulous, 1-4-seeded

*Key morphological characters based on Hajra et al. (1997)

Table 2. Details of accessions of *Melia azedarach* L., *Melia dubia* Cav., and *Azadirachta indica* A. Juss. collected from different part of India

Voucher ID	Name of the species	District	State	BOLD accession No.	GPS Coordinates
B0093A	<i>M. azedarach</i>	Chennai	Tamil Nadu (TN1)	SRM001018A	12°51'N, 80°03'E
B0093B	<i>M. azedarach</i>	Cuddalore	Tamil Nadu (TN2)	SRM001018B	11°32'N, 79°28'E
B0093C	<i>M. azedarach</i>	Villupuram	Tamil Nadu (TN3)	SRM001018C	11°47'N, 79°24'E
B0093D	<i>M. azedarach</i>	West Godavari	Andhra Pradesh (AP)	SRM001018D	16°39'N, 81°31'E
B0093E	<i>M. azedarach</i>	Thrissur	Kerala (KL)	SRM001018E	8°32'N, 76°55'E
B0093F	<i>M. azedarach</i>	Kolhapur	Maharashtra (MH)	SRM001018F	16°42'N, 74°16'E
B0093G	<i>M. azedarach</i>	Amroha	Uttar Pradesh (UP)	SRM001018G	28°50'N, 78°14'E
B0093H	<i>M. azedarach</i>	Howrah	West Bengal (WB)	SRM001018H	22°33'N, 88°17'E
B0093I	<i>M. azedarach</i>	Indore	Madhya Pradesh (MP)	SRM001018I	22°45'N, 75°53'E
B0093J	<i>M. azedarach</i>	Hassan	Karnataka (KA)	SRM001018J	13°00'N, 77°26'E
B1080A	<i>M. dubia</i>	Chennai	Tamil Nadu (TN1)	SRM001020A	12°49'N, 80°02'E
B1080B	<i>M. dubia</i>	Chennai	Tamil Nadu (TN2)	SRM001020B	12°49'N, 80°02'E
B1080C	<i>M. dubia</i>	Chennai	Tamil Nadu (TN3)	SRM001020C	12°49'N, 80°02'E
B1080D	<i>M. dubia</i>	West Godavari	Andhra Pradesh (AP)	SRM001020D	16°32'N, 80°34'E
B1080E	<i>M. dubia</i>	Thrissur	Kerala (KL)	SRM001020E	8°32'N, 76°55'E
B1080F	<i>M. dubia</i>	Kolhapur	Maharashtra (MH)	SRM001020F	16°42'N, 74°15'E
B1080G	<i>M. dubia</i>	Amroha	Uttar Pradesh (UP)	SRM001020G	28°50'N, 78°14'E
B1080H	<i>M. dubia</i>	Howrah	West Bengal (WB)	SRM001020H	22°33'N, 88°17'E
B1080I	<i>M. dubia</i>	Indore	Madhya Pradesh (MP)	SRM001020I	22°45'N, 75°53'E
B1080J	<i>M. dubia</i>	Hassan	Karnataka (KA)	SRM001020J	13°00'N, 77°26'E
B0103A	<i>A. indica</i>	Chennai	Tamil Nadu (TN1)	SRM001019A	12°49'N, 80°02'E
B0103B	<i>A. indica</i>	Cuddalore	Tamil Nadu (TN2)	SRM001019B	11°32'N, 79°28'E
B0103C	<i>A. indica</i>	Villupuram	Tamil Nadu (TN3)	SRM001019C	11°47'N, 79°24'E

Fig. 1: Bayesian phylogeny of genus *Melia* based on concatenated dataset of four markers using MrBayes 3.2. Highlight with color shows the cladogenesis of both *Melia azedarach* clade (Clade 1) and the clade with *Melia dubia* and *M. volkensii* (Clade 2). The posterior probabilities values in percentage are shown at the nodes. The arrow indicates the created polytomy of the *Melia dubia* accessions with the *M. volkensii* accession.

262x329mm (300 x 300 DPI)