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A DNA-barcode for *Melia volkensii* Gürke (Meliaceae) and its phylogenetic relationship with some economically important relatives

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The study reports the first DNA-barcode and molecular phylogeny of the East African endemic tree species *Melia volkensii* using the standard two-locus plant barcoding genes (*rbc*L and *matK*). The two genes were amplified and the PCR products sequenced. Complete coding sequences were obtained for both genes. The edited and aligned sequences had lengths of 1371 bp for *rbc*L and 1524 bp for *mat*K. These DNA sequences were deposited into the DNA Data Bank of Japan (DDBJ) with cross-listing in the European Molecular Biology Labaratory (EMBL) and GenBank databases. The deposited gene sequences were then subjected to separate nucleotide BLASTs in NCBI's GenBank database. Out of 100 Blast results in which the query (*M. volkensii*) had 96–100 percentage similarity in nucleotide sequence for the *rbc*L gene and 90-100% similarity for the *mat*K gene, only 16 taxa had data for both *rbc*L and *mat*K genes. These 16 taxa were used for the phylogenetic analysis and comprised of 6, 9 and 1 taxa respectively from the families Meliaceae, Simaroubaceae and Rutaceae. The barcode allowed adequate discrimination of the taxa into their respective generic and species clades. Availability of a barcode for *M. volkensii* will ease identification of the species, provide more robust phylogenetic reconstructions and allow for better tracking of its exotic dispersal.

Key words: DNA barcoding, *matK*, *rbcL*, DDBJ/EMBL/NCBI Gene Databases, *Melia volkensii,* phylogeny.

INTRODUCTION

Melia volkensii (Gurke) is a hardwood tree species of high economic, ecological and germplasm value. It is endemic to the arid and semi-arid lands of East Africa and belongs to the mahogany family, Meliaceae (Orwa et al., 2009). Other members of the family known for their significant timber, pharmaceutical and conservation value

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are *Azadirachta indica* A. Juss. (Neem), *Melia azedarach* L. (Purple Lilac), *Swietenia macrophylla* (Big-leaf mahogany) and the *Khaya* species.

The primary objective of the study was to develop a DNA barcode sequence for *M. volkensii*. DNA barcoding is the use of nucleotide diversity within a short standardised region of DNA for identification of species (Hebert et al., 2003; Kuzmina et al., 2012; Vijayan and Tsou, 2015). DNA barcoding provides an automated species identification system that is quicker and more reliable than traditional taxonomic methods which rely on morphological characters (Newmaster and Ragupathy, 2009). DNA barcodes can not only resolve phylogenies of plant taxa but are also useful in ecological forensics such as the tracking of illegal trade in plant products (Kress et al., 2015). Other applications of a DNA barcode include monitoring of exotic dispersion, conservation impact assessments, authentication of parts used in preparation of herbal medicine and botanical pesticides, such as tree barks, fruits and leaves (Ferri et al., 2008; 2015; Kritpetcharat et al., 2011; Mankga et al., 2013; Mishra et al., 2016).

Until recently, DNA barcoding of plants was hampered by the lack of a standard region of DNA with sufficient universality, sequence quality and species discrimination power (Hollingsworth et al., 2011). The long search for a universal plant barcode culminated in the adoption of a two-locus barcode consisting of the phylogenetically conserved gene for the large subunit of the chloroplast enzyme ribulose-1,5-bisphosphate carboxylase/ oxygenase (rubisco), also known as *rbc*L, and the more rapidly evolving chloroplast gene for maturase K (*matk)* (Kress et al., 2009). The 2-locus combination of *rbc*L and *mat*K genes was adopted by the Consortium for the Barcode of Life Plant Working Group (CBOL, 2009) as the standard or core barcode for land plants.

The *rbc*L gene is a chloroplast gene of approximately 1400 bp that codes for the large subunit of rubisco, the enzyme that catalyzes carbon dioxide fixation in chloroplasts. The *mat*K gene, approximately 1500 bp, is located within a 2,400 bp group II intron of the chloroplast *trn*K gene which codes for the transfer RNA for lysine (Johnson and Soltis, 1994; Vogel et al., 1997; Steane, 2005; Hausner et al., 2006; Barthet and Hilu, 2007). It codes for maturase K, an enzymatic protein that allows the intron to remove itself for the two exons of the *trn*K gene to be spliced together.

A secondary objective of the study was to use the novel barcode sequences in a preliminary phylogenetic study of the Meliaceae and related families. A molecular phylogeny based on DNA barcoding could clarify evolutionary relationships between both the well-known and lesser known members of the family.

This study reports the first DNA barcode for *Melia volkensii*. The availability of such a barcode for the species is will enable faster and accurate identification of the species and a more robust reconstruction of phylogenetic relationships in the family. This will provide insights on the phylogenetic affinities between *M. volkensii,* well-known members of the family such as *A. indica*, *M. azederach* and *S. macrophylla* and the lesser known ones. Phylogenetic affinities at the family and generic levels could also reveal closely related families and genera for novel bio-prospecting for compounds of pharmaceutical and pesticidal importance similar to those found in some members of the Meliaceae.

MATERIALS AND METHODS

Plant materials and DNA extraction

DNA was extracted from shoot tips of 20 *M. volkensii* seedlings obtained from seeds collected from Mavuria provenance in Mbeere, Embu county, Eastern Kenya (Geo-reference 0° 46.379'S, 37° 39.308'E). DNA Extraction was done using the Cetyltrimethylammonium bromide (CTAB) method of Doyle and Doyle (1987), with slight modifications, which were addition of 10% sodium dodecyl sulphate to extraction buffer, centrifugation at 16,000g instead of 6,000g and washing of the DNA pellet with 70% ethanol instead of a mixture of 76% ethanol and 10mM ammonium acetate.

Molecular methods

M. volkensii complete coding sequence for ribulose-1,5-carboxylase/oxygenase (rubisco) large subunit chloroplast gene (*rbcL*) was amplified by the PCR method. The expected fragment size was 1397bp (Fazekas et al., 2012). The primers used for *rbc*L gene were *rbclFayf* (5'TCCTTTTAGTAAAAGATTGGGCCGAG3') and *rbclFayr* (5'ATGTCACCACAAACAGAAACTAAAGC3') (Fay et al*.,* 1998). Primers were synthesised by Inqaba Biotec, South Africa. The reaction mixture contained 1 unit of MyTaq® DNA polymerase (Bioline, USA), 1x Mytaq buffer® (Bioline, USA) containing 3 mM $MgCl_2$ and 2 mM dNTPs; 0.4 µM forward and reverse primers, 1 µl of DNA template and brought to the total volume of 25 µl with nuclease-free water. Amplification was done on a MJ Research PTC-100 USA thermal cycler with the following conditions; initial denaturation at 95°C for 1 min , 40 cycles of at 95°C for 15 s (denaturation), 55°C for 15 s (annealing), 72°C for 1 min 30 s (extension), and a final extension at 72°C for 7 min.

Isolation of *M. volkensii* maturase-K chloroplast gene (*matK*) was also carried out in a 25 µl volume reaction. The expected fragment size was 1500 bp (Fazekas et al., 2012). The primers used were *Matk1f* (5'ACTGTATCGCACTATGTATCA3') and *Matk1r* (5'GAACTAGTCGGATGGAGTAG3'), also sourced from Inqaba Biotec South Africa. The reaction mixture contained 1 unit of MyTaq® DNA polymerase (Bioline, USA); 1x Mytaq® buffer (Bioline, USA) containing 3mM $MgCl_2$ and 2 mM dNTPs; 0.4 µM of forward and reverse primers, 1 µl of DNA template and brought to the total volume of 25 µl with nuclease-free water. Amplification was done on a MJ Research PTC-100 USA thermal cycler with conditions set at 95°C for 1 min, 20 cycles of 95°C for 15 s, 45°C for 15 s, 72°C for 1.5 min, followed by another 20 of cycles of 95°C for 15 s, 55°C for 15 s, 72°C for 1.5 min and a final extension at 72°C for 5 min.

PCR products were purified with EXO/SAP Amplicon purification kit (Affymetrix, Santa Clara, USA). Purified PCR products were sequenced by Inqaba Biotec South Africa using The BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems,USA) with ABI Prism 377 DNA sequencer (Applied Biosystems,USA). The same primers used for the PCR reactions were used in sequencing

reactions.

Database deposition and phylogenetic reconstruction

*M. volkensii rbc*L and *mat*K novel sequences were checked for quality and ambiguous nucleotides resolved in MEGA6 software suite (Tamura et al., 2013). Identical sequences were obtained for each gene. Processed sequences of the two genes were deposited in the DDBJ/EMBL/GenBank databases. They were assigned the following accession numbers: LC075516 for *rbc*L and LC075517 for *mat*K.

The sequences were then used to carry out two separate GeneBank nucleotide BLASTs. The first set of 100 Blast hits gave 96–100 percentage similarity in nucleotide sequence for the rbcL gene and 90-100% similarity for the matK gene between the query (*M. volkensii*) and the respective Genbank sequences of members of Meliaceae, Simaroubaceae and Rutaceae families. However, retrieved taxa having sequence data for both rbcL and matK genes were only 16, with the rest of the taxa having data for either rbcL or matK. Since the study intended to use both the barcoding genes separately and after concatenation, phylogenetic reconstruction was limited to the sequences of these 16 taxa. Sequence names, database codes, accession numbers, native distribution and uses of the selected species are listed in Table 1.

The retrieved database sequences were also checked for quality and ambiguous nucleotides resolved in MEGA6 software suite (Tamura et al., 2013). Multiple sequence alignments were performed in MEGA6 software suite using the MUSCLE algorithm (Edgar, 2004) and the aligned sequences used for phylogenetic reconstruction. The evolutionary history was inferred using the maximum likelihood method based on the General Time Reversible (GTR) model (Nei and Kumar, 2000). Initial trees for the heuristic search were obtained automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of pair-wise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree with the highest log likelihood was selected. A total of 1000 bootstrap replicates were performed (Felsenstein, 1985). Phylogenetic trees were edited in FigTree 1.4 (Rambaut, 2012).

RESULTS AND DISCUSSION

PCR amplification was 100% successful for both genes. Gel electrophoresis gave highly resolved bands of ≈ 1400 bp for *rbcL* and ≈ 1500 bp for *matK*, as expected (Figure 1). Sequencing success was 95% for both genes, with edited sequence lengths of 1371 bp for the *rbc*L gene and 1524 bp for *mat*K. These sequences were successfully deposited in the DDBJ/EMBL/GenBank databases and assigned the accession numbers LC075516 (*rbc*L) and LC075517 (*mat*K). To the best of our knowledge, they are the first barcode deposits for *M. volkensii* in these databases.

The BLASTs retrieved taxa belonging to three families: Meliaceae, Simaroubaceae and Rutaceae. This is in agreement with previous reports about the taxonomic proximity of these families (Wiart, 2006). However, most of the taxa had sequence data for either *rbc*L or *mat*K but not both. Therefore analysis was limited to the 16 closely related taxa which had sequences for both the *rbc*L and *mat*K genes. These consisted of 6 members of the Meliaceae family, 9 members of Simaroubaceae and 1 member of Rutaceae (Table 1). Consequently, phylogenetic reconstruction was severely constrained by the limited nature of the data retrieved from the databases. A more comprehensive molecular phylogeny of the Meliaceae will be possible only when more sequence data becomes available in these databases. Since the family Meliaceae consists of an estimated 51 genera and 550 species (Wiart, 2006), there is a vast scope for an expanded molecular phylogeny of the family.

The taxa included in the phylogenetic analysis had sequence percentage alignment scores of 96-99% for *rbc*L gene and 90-95% for the *mat*K gene (Table 1). This is in agreement with previous reports of higher discrimination power of *mat*K over *rbc*L for most plants (Li et al., 2011). This difference was also evident in the pair-wise distance matrices (Tables 2 and 3) and phylogenetic trees (Figures 2 and 3), with *mat*K giving larger genetic distances between the species than *rbc*L and the concatenated *rbcL* + *mat*K code giving intermediate distances (Table 4). This was expected as the *mat*K gene is reported to have a higher rate of mutation than the *rbc*L gene (Kress et al., 2009) and is thus more likely to reveal a greater amount of variation between species. The *rbc*L locus is generally more suitable for determination of evolutionary relationships at the generic level and above (Kress et al., 2005). On the other hand *mat*K has been more successful in resolving species relationships in several families (Johnson and Soltis, 1994; Hilu and Liang, 1997; Rohwer, 2000).

All the phylogenetic trees obtained with separate *rbcL*, *matK* sequences and with concatenated *rbcL* + *matK* sequences correctly resolved the 17 taxa into their respective familial clades with 100% bootstrap support (Figures 2, 3 and 4). In each family, the vast majority of branches also had high bootstrap values (> 90%). These barcoding genes also allowed adequate discrimination at generic and species levels, as seen in the clear resolution of the genus *Melia* (*M. volkensii* and *M. azederach*), genus *Swietenia* (*S. macrophylla* and *S. mahogany*), genus *Picrasma* (*P. javanica and P. quassioides*) and genus *Ailanthus* (*A. integrifolia*, *A. altissima* and *A. triphysa*). This suggests a possible use of the two barcoding genes, with additional empirical testing, in resolving taxa in the Meliaceae and related families up to the species level. This recommendation is supported by the findings of Kress et al. (2005) which showed that full-length sequences (>1 kb) of either gene can give enough sequence length to discriminate between species. The sequences obtained in this study were longer than 1kb and therefore met this criterion.

Despite the limited number of taxa used, the molecular phylogeny obtained in this study provides some useful insights into the evolutionary relationships between *M. volkensii* and the taxa that were included in the phylogeny. This is one of the suggested applications of a DNA barcode (Kress et al., 2015). The *M. volkensii*

Table 1. Species information and nucleotide BLAST alignment scores for *Melia volkensii* (DDBJ LC075516.1 and LC075517.1) and selected species.

Family	Species name (Common name)	Native distribution	Main uses	Similarity With *M. volkensii* (%)		Database/ Accession number	
				*rbc*L	*mat*K	*rbc*L	*mat*K
Meliaceae	*Melia azederach* L. (Purple lilac)	Indian subcontinent and South East Asia	Timber, medicinal, insecticidal, ornamental	99	99	GB/AY128234.1	GB/EF489117.1
	Azadirachta indica A. Juss. (Neem)	Indian subcontinent and South East Asia	Timber, medicinal, insecticidal, ornamental	99	97	GB/AY128214.1	GB/EF489115.1
	Toona sinensis (A.Juss.) M. Roem (Chinese mahogany)	Eastern and South Eastern Asia	Timber, medicinal, ornamental	97	94	EMB/FN599468.1	GB/JN680341.1
	Swietenia macrophylla King. (Honduran mahogany)	Mexico and South America	Timber, medicinal, ornamental	97	93	GB/U39080.2	GB/EF489114.1
	Swietenia mahogany (L.) Jacq. (West Indies mahogany)	Caribbean Islands and USA	Timber, medicinal, ornamental	97	93	EMB/FN599465.1	GB/EU042835.1
	Cipadessa baccifera (Roth) Miq.	India, Sri Lanka, Myanmar, China, Malaysia	Medicinal	96	94	GB/AY128225.1	GB/EF489116.1
Simaroubaceae	*Ailanthus integrifolia* Lam. (White Siris)	India, Indonesia, Malaysia, Papua New Guinea	Timber, Medicinal	96	91	GB/EU042981.2	GB/042843.1
	Ailanthus altissima (Mill. Swingle; (Tree of Heaven)	China and Taiwan	Timber, Medicinal, Ornamental	96	91	GB/KM360619.1	EMB/FM179922.1
	Ailanthus triphysa (Dennst.) Alston (White Siris)	India, Myanmar, Nepal	Timber, Medicinal, Ornamental	96	91	GB/EU042982.1	GB/EU042844.1
	Castela retusa Liebm.;	Mexico and Central America	Medicinal	96	90	GB/EU042992.1	GB/EU042854.1
	Picrasma quassioides (D,Don) Benn. (Quassia wood)	Eastern and South America; East Asia	Timber, Medicinal, Insecticidal	96	91	GB/EU043008.1	GB/EU042870.1
	Picrasma javanica Blume	India, Bangladesh, Java, Burma, Malaysia	Timber, Medicinal	96	91	GB/EU043011.1	GB/EU042873.1
	Nothospondias staudtii Engl.	West Africa and the DR Congo	Timber, Medicinal	96	91	GB/EU043004.1	GB/EU042866.1
	Holocantha emoryi A. Gray	South western USA	Medicinal	96	91	GB/EU043002.1	GB/EU042864.1
	Hannoa klaineana Pierre and Engl.	West and Central Africa	Timber, Medicinal	96	90	GB/EU042999.1	GB/EU042861.1
Rutaceae	*Choisya ternata* Kunth (Mexican Orange)	Mexico	Ornamental, Medicinal	96	91	GB/KM360716.1	GB/EF489104.1

barcode could also be useful in aiding identification of the species and its products, enabling more detailed phylogenetic reconstructions and the tracking of its exotic dispersion. However, for application of the *mat*K + *rbc*Lplant barcode in a more comprehensive study of the Meliaceae, there is an urgent need for sequencing of the *rbc*L and *mat*K genes for all the estimated 550 species of the Meliaceae and deposition of the data

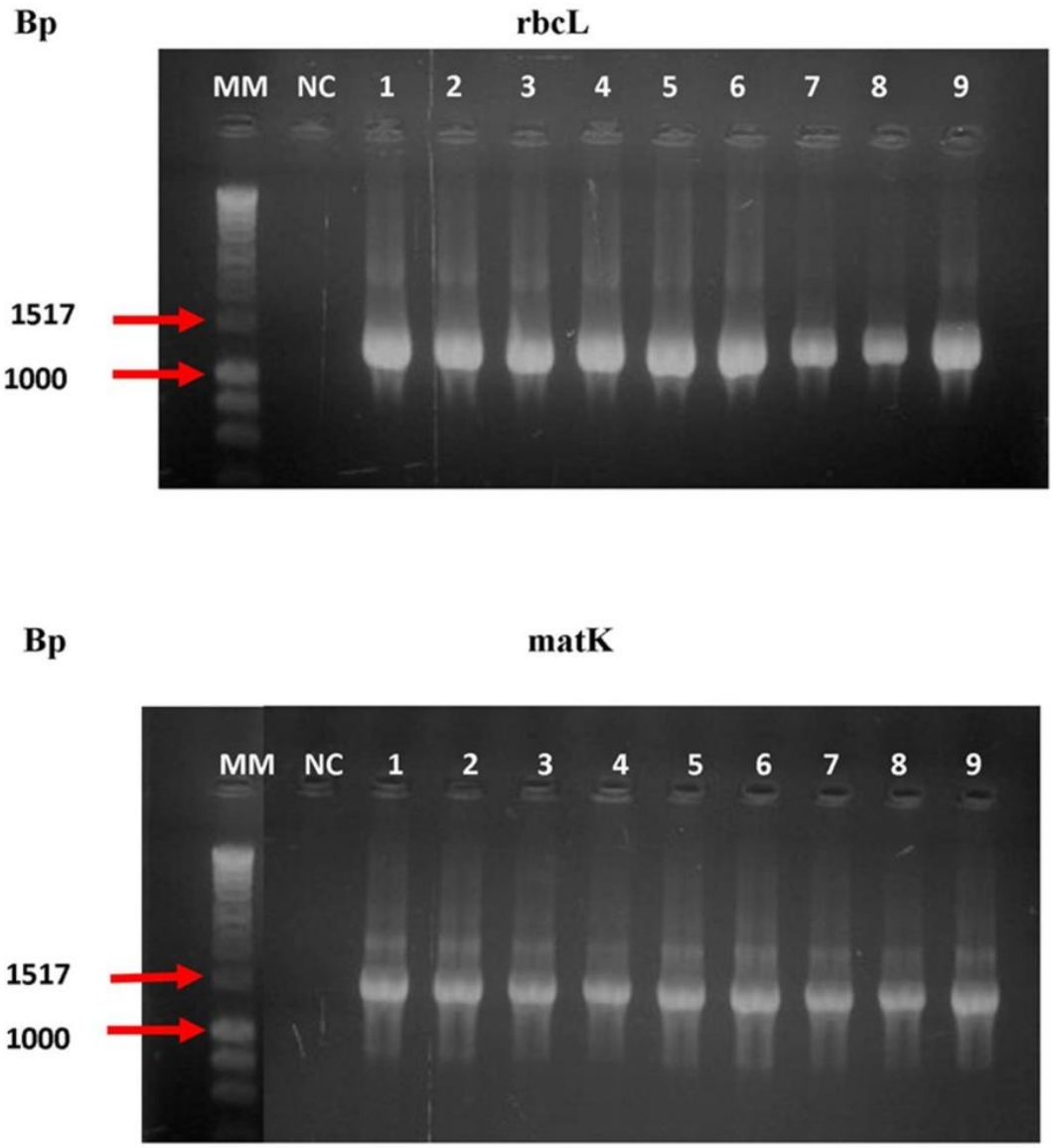

Figure 1. Agarose gel profiles of the isolated chloroplast *rbc*L and *mat*K genes. MM= 1kb ladder, NC= negative control, 1-9 = some of the DNA samples used.

in DNA Databases.

Conclusions and recommendations

The plant barcoding genes *rbc*L and *mat*K managed to resolve selected taxa up to the species level. A partial molecular phylogeny of the Meliaceae and closely related famlilies was obtained. The main limiting factor was the lack of complete data on *rbc*L and *mat*K sequences in the DNA repositories for members of these families. This calls for accelerated deposition of more sequence data in order to fill the huge gaps in the DNA libraries. Such data can also be used in future Bayesian inferences.

Conflict of interest

The authors have not declared any conflict of interests.

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Table 2. Estimates of genetic distance between sequences using *rbc*L alone, based on the number of base substitutions per site. Standard error estimate(s) are shown above the diagonal and were obtained by a bootstrap procedure (1000 replicates).

		Rubisco (*rbc*L)																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	*Melia_volkensii*_{Meliaceae}		0.002	0.003	0.005	0.005	0.005	0.005	0.005	0.006	0.005	0.006	0.006	0.005	0.006	0.006	0.006	0.006
2	*Melia_azedarach*_{Meliaceae}	0.004		0.003	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.005
3	*Azadirachta_indica*_{Meliaceae}	0.013	0.012		0.004	0.004	0.005	0.005	0.005	0.006	0.005	0.005	0.005	0.005	0.006	0.005	0.006	0.005
4	*Swietenia_macrophylla*_{Meliaceae}	0.029	0.028	0.024		0.002	0.001	0.004	0.004	0.005	0.004	0.005	0.005	0.004	0.005	0.005	0.006	0.005
5	*Toona_sinensis*_{Meliaceae}	0.026	0.027	0.023	0.007		0.002	0.004	0.004	0.005	0.005	0.004	0.005	0.004	0.005	0.005	0.005	0.005
6	*Swietenia_mahagoni*_{Meliaceae}	0.029	0.030	0.027	0.003	0.006		0.004	0.004	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
7	*Picrasma_quassioides*_{Simaroubaceae}	0.037	0.037	0.034	0.027	0.025	0.027		0.001	0.004	0.005	0.004	0.004	0.004	0.004	0.004	0.006	0.004
8	*Picrasma_javanica*_{Simaroubaceae}	0.037	0.038	0.034	0.026	0.024	0.027	0.003		0.004	0.005	0.004	0.004	0.004	0.004	0.004	0.006	0.004
9	*Castela_retusa*_{Simaroubaceae}	0.039	0.040	0.040	0.032	0.030	0.032	0.022	0.023		0.006	0.005	0.005	0.005	0.003	0.005	0.007	0.005
10	*Choisya_ternata*_{Rutaceae}	0.041	0.040	0.038	0.028	0.030	0.031	0.034	0.034	0.039		0.005	0.005	0.005	0.006	0.005	0.006	0.005
11	*Ailanthus_integrifolia*_{Simaroubaceae}	0.040	0.040	0.037	0.031	0.030	0.032	0.023	0.023	0.027	0.037		0.002	0.003	0.005	0.002	0.006	0.004
12	*Ailanthus_altissima*_{Simaroubaceae}	0.040	0.041	0.038	0.033	0.031	0.034	0.023	0.024	0.029	0.038	0.004		0.003	0.005	0.002	0.006	0.004
13	*Nothospondias_staudtii*_{Simaroubaceae}	0.041	0.040	0.035	0.030	0.030	0.033	0.023	0.025	0.028	0.039	0.017	0.017		0.005	0.004	0.006	0.003
14	*Holacantha_emoryi*_{Simaroubaceae}	0.042	0.040	0.040	0.033	0.032	0.034	0.024	0.027	0.009	0.041	0.029	0.028	0.028		0.005	0.007	0.005
15	*Ailanthus_triphysa*_{Simaroubaceae}	0.042	0.043	0.040	0.034	0.033	0.034	0.024	0.026	0.030	0.039	0.004	0.006	0.019	0.030		0.007	0.004
16	*Cipadessa_baccifera*_{Meliaceae}	0.043	0.044	0.039	0.037	0.036	0.038	0.045	0.043	0.049	0.045	0.052	0.054	0.051	0.052	0.056		0.006
17	*Hannoa_klaineana*_{Simaroubaceae}	0.047	0.047	0.043	0.036	0.034	0.037	0.028	0.030	0.033	0.041	0.020	0.020	0.017	0.034	0.020	0.053	

Table 3. Estimates of genetic distance between sequences using *mat*K alone, based on the number of base substitutions per site. Standard error estimate(s) are shown above the diagonal and were obtained by a bootstrap procedure (1000 replicates).

		Maturase K (*mat*K)																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	*Melia_volkensii*_{Meliaceae}		0.003	0.005	0.008	0.009	0.009	0.009	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.012
2	*Melia_azedarach*_{Meliaceae}	0.015		0.005	0.007	0.009	0.009	0.009	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.012
3	*Azadirachta_indica*_{Meliaceae}	0.035	0.028		0.008	0.008	0.009	0.009	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.011	0.012
4	*Cipadessa_baccifera*_{Meliaceae}	0.060	0.054	0.055		0.007	0.008	0.008	0.009	0.009	0.010	0.010	0.010	0.010	0.010	0.010	0.011	0.011
5	*Toona_sinensis*_{Meliaceae}	0.064	0.062	0.059	0.046		0.003	0.003	0.009	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.011	0.011
6	*Swietenia_mahogani*_{Meliaceae}	0.067	0.064	0.062	0.051	0.010		0.001	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.011	0.011	0.011
7	*Swietenia_macrophylla*_{Meliaceae}	0.069	0.065	0.063	0.052	0.011	0.003		0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.011	0.011	0.011

Table 3. Contd.

8	*Picrasma_javanica*_{Simaroubaceae}	0.087	0.082	0.082	0.069	0.072	0.074	0.075		0.002	0.007	0.007	0.007	0.006	0.007	0.009	0.008	0.008
9	*Picrasma_quassioides*_{Simaroubaceae}	0.088	0.083	0.083	0.070	0.072	0.075	0.075	0.004		0.007	0.007	0.007	0.007	0.008	0.009	0.008	0.008
10	*Ailanthus_triphysa*_{Simaroubaceae}	0.091	0.087	0.087	0.078	0.076	0.077	0.078	0.044	0.046		0.004	0.008	0.004	0.006	0.010	0.009	0.006
11	*Ailanthus_integrifolia*_{Simaroubaceae}	0.094	0.090	0.090	0.080	0.076	0.078	0.079	0.044	0.047	0.017		0.008	0.004	0.006	0.010	0.009	0.006
12	*Holacantha_emoryi*_{Simaroubaceae}	0.094	0.088	0.090	0.083	0.082	0.086	0.085	0.051	0.052	0.061	0.059		0.008	0.008	0.010	0.004	0.009
13	*Ailanthus_altissima*_{Simaroubaceae}	0.094	0.090	0.089	0.078	0.074	0.076	0.076	0.044	0.046	0.017	0.017	0.058		0.006	0.010	0.009	0.006
14	*Nothospondias_staudtii*_{Simaroubaceae}	0.094	0.091	0.092	0.080	0.078	0.082	0.083	0.051	0.051	0.035	0.035	0.059	0.036		0.010	0.008	0.006
15	*Choisya_ternata*_{Rutaceae}	0.094	0.087	0.089	0.081	0.077	0.081	0.082	0.072	0.071	0.079	0.080	0.082	0.080	0.080		0.011	0.011
16	*Castela_retusa*_{Simaroubaceae}	0.100	0.094	0.091	0.091	0.090	0.093	0.094	0.057	0.058	0.065	0.065	0.022	0.066	0.064	0.090		0.010
17	*Hannoa_klaineana*_{Simaroubaceae}	0.102	0.098	0.099	0.089	0.084	0.086	0.087	0.053	0.055	0.037	0.035	0.067	0.038	0.037	0.087	0.074	

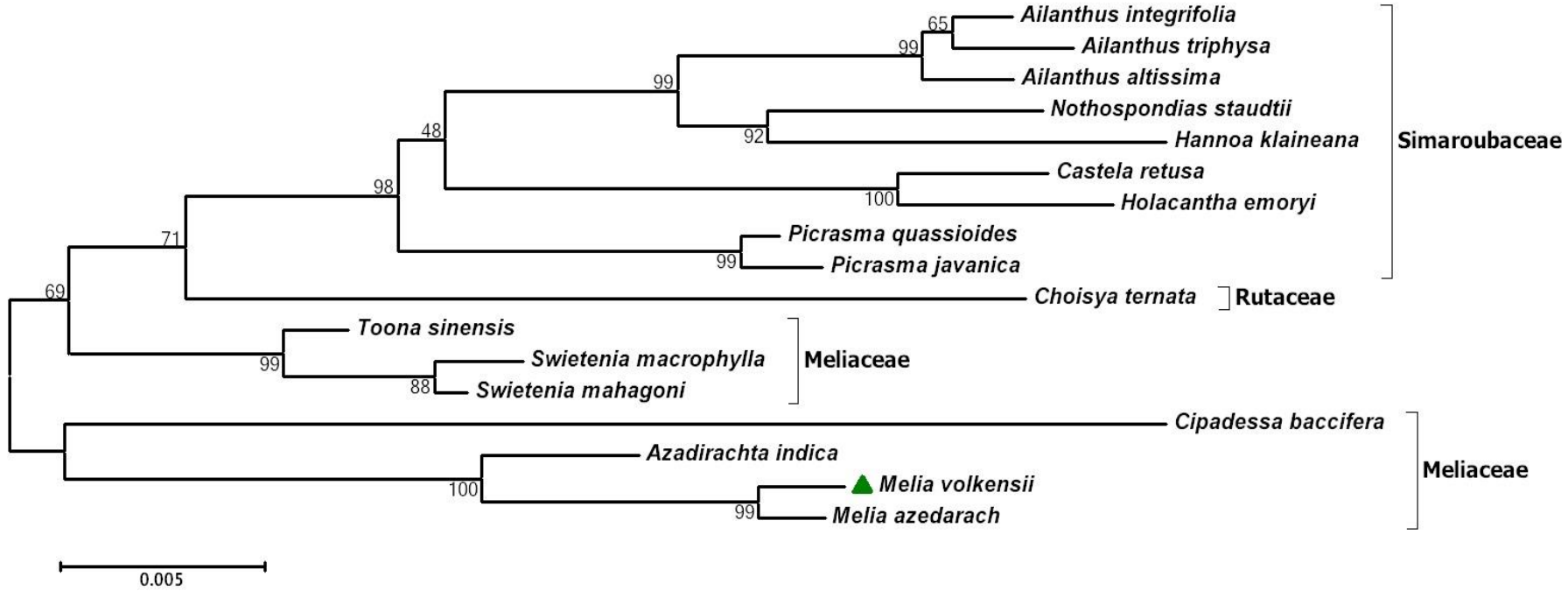

Figure 2. Maximum Likelihood phylogenetic tree for *Melia volkensii* and 16 closely related species based on *rbc*L gene, with 1000 bootstraps. Bootstrap support values are shown at nodes. Scale = number of substitutions per site.

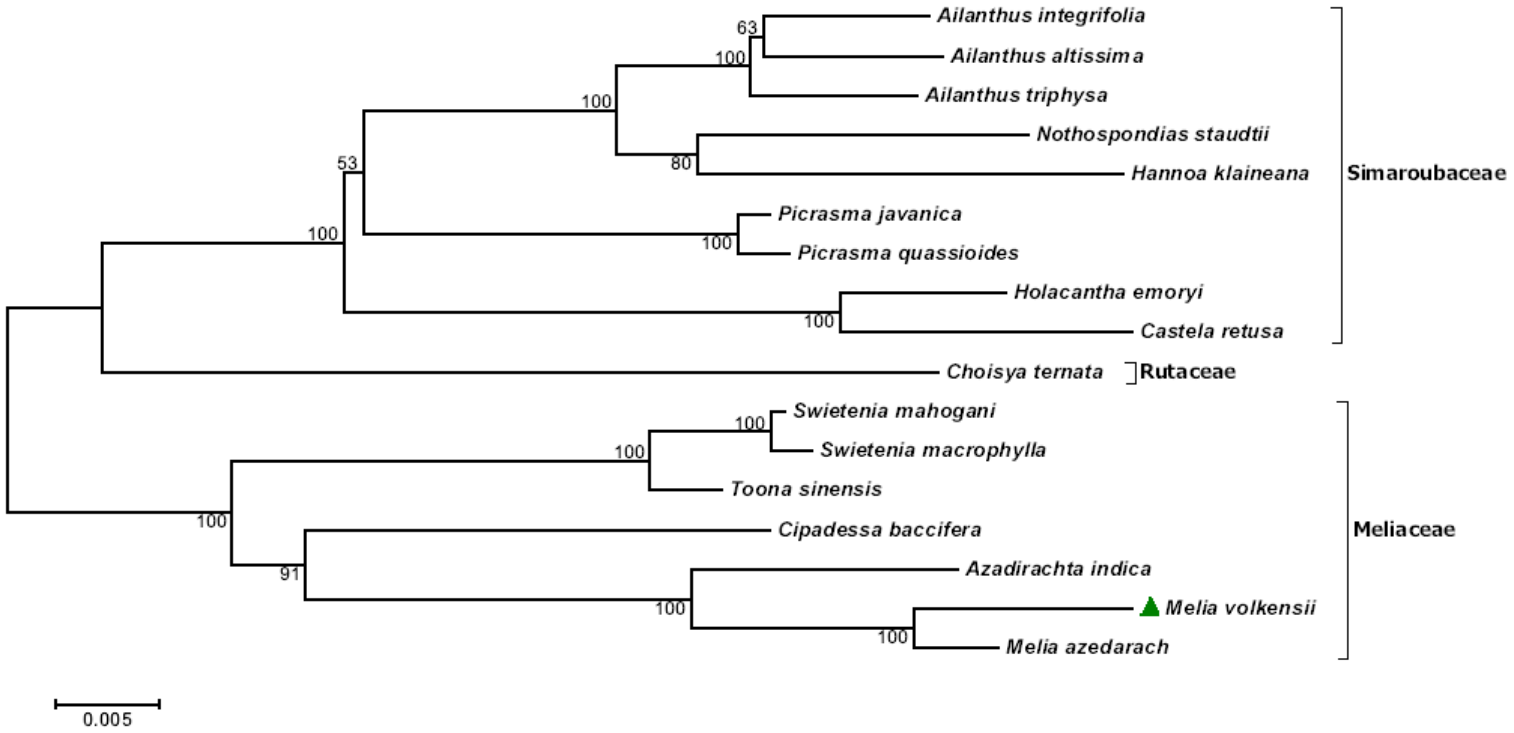

Figure 3. Maximum Likelihood phylogenetic tree for *Melia volkensii* and 16 closely related species based on *mat*K gene, with 1000 bootstraps. Bootstrap support values are shown at nodes. Scale = number of substitutions per site.

Table 4. Estimates of genetic distance between sequences using *rbc*L + *mat*K concatenated sequences, based on the number of base substitutions per site. Standard error estimate(s) are shown above the diagonal and were obtained by a bootstrap procedure (1000 replicates).

		***rbc*L + *mat*K concatenated**																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	*Melia_volkensii*_{Meliaceae}		0.002	0.003	0.004	0.004	0.004	0.004	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
2	*Melia_azedarach*_{Meliaceae}	0.010		0.002	0.004	0.004	0.004	0.004	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
3	*Azadirachta_indica*_{Meliaceae}	0.024	0.020		0.004	0.004	0.004	0.004	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
4	*Cipadessa_baccifera*_{Meliaceae}	0.052	0.049	0.047		0.004	0.004	0.004	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
5	*Toona_sinensis*_{Meliaceae}	0.046	0.045	0.042	0.041		0.002	0.002	0.004	0.004	0.005	0.004	0.005	0.004	0.004	0.004	0.005	0.004
6	*Swietenia_mahogani*_{Meliaceae}	0.049	0.048	0.045	0.045	0.008		0.001	0.005	0.004	0.005	0.004	0.005	0.004	0.005	0.004	0.005	0.004

Table 4. Contd.

7	*Swietenia_macrophylla*_{Meliaceae}	0.050	0.047	0.044	0.045	0.010	0.003		0.005	0.005	0.005	0.004	0.005	0.005	0.004	0.004	0.005	0.004
8	*Picrasma_javanica*_{Simaroubaceae}	0.063	0.061	0.059	0.056	0.049	0.051	0.051		0.001	0.004	0.004	0.004	0.003	0.004	0.004	0.004	0.004
9	*Picrasma_quassioides*_{Simaroubaceae}	0.063	0.061	0.059	0.058	0.050	0.052	0.052	0.004		0.004	0.004	0.004	0.003	0.004	0.004	0.004	0.004
10	*Ailanthus_triphysa*_{Simaroubaceae}	0.068	0.066	0.064	0.067	0.055	0.057	0.057	0.035	0.036		0.002	0.004	0.002	0.003	0.004	0.004	0.003
11	*Ailanthus_integrifolia*_{Simaroubaceae}	0.068	0.066	0.065	0.066	0.054	0.056	0.056	0.034	0.035	0.011		0.004	0.002	0.003	0.004	0.004	0.003
12	*Holacantha_emoryi*_{Simaroubaceae}	0.069	0.065	0.066	0.068	0.058	0.061	0.060	0.040	0.039	0.046	0.044		0.004	0.004	0.005	0.003	0.004
13	*Ailanthus_altissima*_{Simaroubaceae}	0.068	0.066	0.065	0.066	0.053	0.055	0.055	0.034	0.035	0.012	0.011	0.044		0.003	0.005	0.004	0.003
14	*Nothospondias_staudtii*_{Simaroubaceae}	0.068	0.066	0.064	0.066	0.055	0.058	0.058	0.038	0.038	0.027	0.027	0.044	0.027		0.004	0.004	0.003
15	*Choisya_ternata*_{Rutaceae}	0.069	0.064	0.065	0.063	0.054	0.057	0.056	0.054	0.053	0.060	0.059	0.062	0.060	0.060		0.005	0.004
16	*Castela_retusa*_{Simaroubaceae}	0.070	0.068	0.066	0.070	0.061	0.064	0.064	0.041	0.041	0.049	0.047	0.016	0.048	0.047	0.066		0.004
17	*Hannoa_klaineana*_{Simaroubaceae}	0.075	0.073	0.071	0.072	0.060	0.062	0.062	0.041	0.042	0.029	0.028	0.051	0.029	0.027	0.065	0.054	

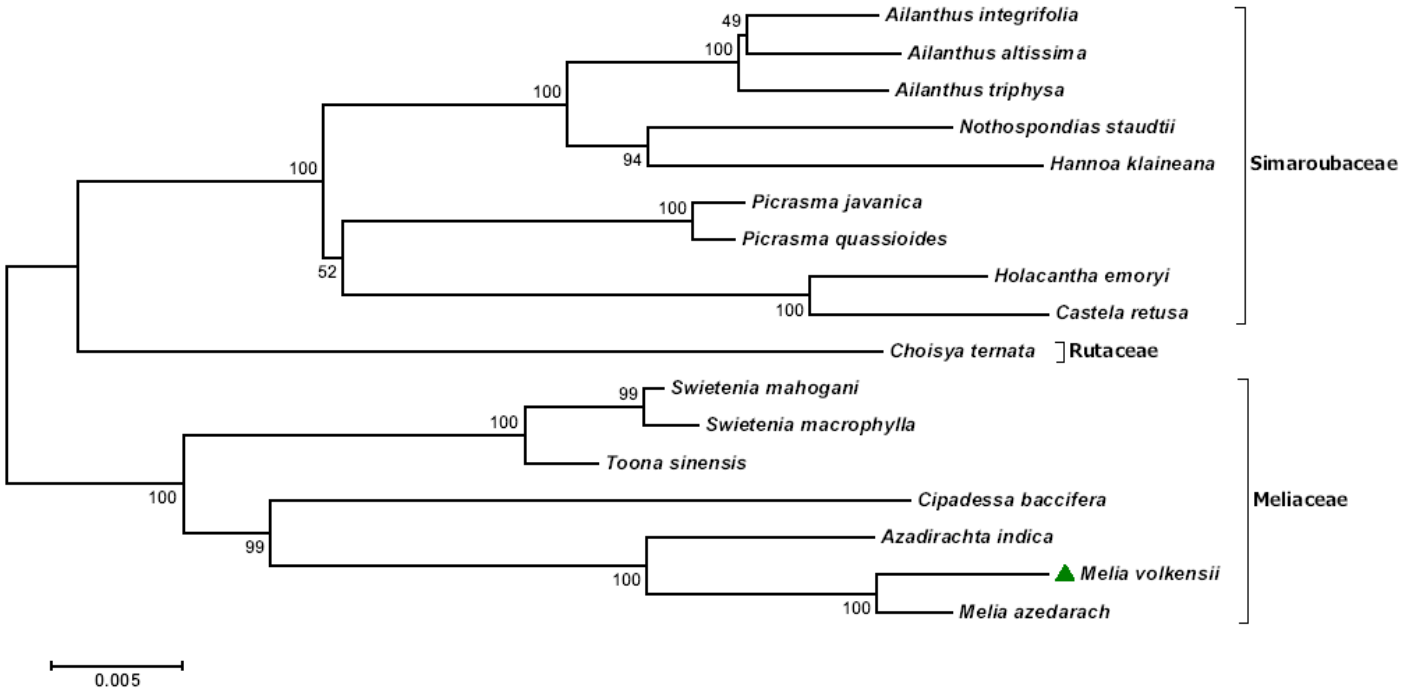

Figure 4. Maximum Likelihood phylogenetic tree for *Melia volkensii* and 16 closely related species based on *rbc*L + *mat*K concatenated genes, with 1000 bootstraps. Bootstrap support values are shown at nodes. Scale = number of substitutions per site.

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