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MOLECULAR SYSTEMATICS OF SELECTED GENERA OF SUBFAMILY MIMOSOIDEAE-FABACEAE

ZABTA K. SHINWARI, KHANSA JAMIL AND NADIA BATOOL ZAHRA

Department of Biotechnology, Quaid-i-Azam University, Islamabad-Pakistan

Abstract

Subfamily Mimosoideae-Fabaceae is of economic importance to local communities for its medicinal usage. It has commercial value, but the parts sold in the market are difficult to identify on the basis of morphological characters and therefore needs molecular systematics approaches. Hence, the utility of potential DNA barcodes for selected *Acacia* and *Albizia* species by using three cpDNA regions *rbcL, matK* and *trnH-psbA* was tested in this study. Our study suggests that the *rbcL* region can be used to identify these species and discriminate among them more effectively than *matK* and *trnH-psbA*. The latter regions proved to be less successful in sequencing particularly *trnH-psbA*. Therefore, *rbcL* is an improved and efficient tool for species identification of these medicinal plants and may be recommended for a broad series of subfamily Mimosoideae (Family: Fabaceae) plants, making it a potential DNA barcode for these taxa. Sequence data obtained from *rbcL* and *matK* also indicated that *Acacia* and *Albizia* are polyphyletic. The phylogenetic analysis on the basis of *rbcL* proved that *Acacia nilotica* and *Acacia nilotica* ssp. *hemispherica* are closely related as they form the sister groups.

Introduction

The subfamily Mimosoideae (which is sometimes treated as a distinct family, the Mimosaceae) belongs to the family Fabaceae. There are about 50-60 genera within Mimosoideae that are distributed throughout tropical, subtropical and warm-temperate regions of the world (Elias, 1981; Cowan, 1998; Mabberley, 2008). Bentham (1842) included three tribes Acacieae Dumort., Ingeae Benth. & Hook. f. and Mimoseae Bornn., in the Mimosoideae. *Acacia* Mill., and *Albizia* Durazz. are members of tribe Acacieae and Ingeae respectively. *Acacia* in the traditional sense is a large, polyphyletic, cosmopolitan genus which contains about 1380 species, of which many are cryptic sister species (Maslin *et al.,* 2003; Brown *et al.,* 2008)**.** Many *Acacia* species are difficult to differentiate from each other on the basis of morphological characters, resulting in some taxonomic confusion (Bentham, 1842; Wardill *et al.,* 2005). Morphological studies have suggested the tribe Acacieae and genus *Acacia* are artificial and have long been associated to tribe Ingeae. The only morphological character which is used to distinguish these two tribes is presence of free stamens in tribe Acacieae which are found to be fused in the form of a tube in tribe Ingeae. There are exceptions to this, with several species of Acacieae having stamens fused at the base (Cowan & Maslin, 1990). There are other important macro-morphological characters (foliage, pod, pollen, stipules, seed characters) being shared by these two tribes (Maslin *et al.,* 2003). The tribe Mimoseae shares the character state of free stamens with the Acacieae, but the Mimoseae has as many or twice as many stamens as petals while the Acacieae has numerous stamens (Vassal, 1981). These conflicting character states make a classification, based solely on morphological characters, difficult and unreliable. Relationships between lineages of tribe Acacieae and Ingeae are not well resolved. Sequence data produced by cpDNA regions has been used in many studies to study the phylogenetic relationships among the Acacia and Ingeae (Luckow *et al.,* 2003; Miller & Bayer, 2003; Lavin *et al.,* 2005).

The occurrence of invasive species of *Acacia* means identification is important for differentiating these species from rare or economically valuable species (Byrne *et al*., 2001; Kriticos *et al*., 2003; Midgley & Turnbull, 2003). The medicinal utility of species of *Acacia* and *Albizia* has been reported in many ethnobotanical studies (Summarized in Table 1).

DNA barcoding was proposed by Hebert *et al.,* (2003) as a means of identifying species. This method relies on the specific gene regions of the DNA sequence. A DNA barcode is a standardized, short (400-800 bp) and highly variable segment of DNA which is compared to a DNA sequence database for species identification, and it can accelerate the discovery of new species. There is only one gene, mitochondrial cytochrome C oxidase I (*COI* or *coxI*), which has been successfully applied to animals as a DNA barcode, but in land plants there are seven major plastid regions (*rbcL*, *matK*, *rpoB*, *rpoC1* genes and *trnH-psbA*, *atpF-atpH*, *psbK-psbI* spacers) which are being evaluated by CBOL (Consortium for the Barcode of Life) Plant Working Group, and they recommended the *rbcL* and *matK* as two-marker combination which is to be used as a core DNA barcode for plants (CBOL, 2009). The selection of *rbcL* and *matK* as a core barcode was based on the easy amplification, sequencing and alignment of the *rbcL* region and the better discriminatory power offered by the *matK* region due to its high rate of substitutions (Hollingsworth *et al.,* 2011). This characteristic of *matK* makes it an important gene for the evolutionary and systematic study in plants. It is still expected that a system which is made up of any one or a combination of plastid genes will not be successful in certain taxonomic groups that exhibit low amounts of plastid variation, while working well in other groups (Newmaster & Ragupathy, 2009). The sequence data of a query sample which is an unknown specimen is compared to a reference sequence generated from a well-identified and voucher specimen (Schori & Showalter, 2011). The difficulties rendered in plant barcoding have been debated in many studies (Chase *et al*., 2005; Shinwari *et al*., 1994, 1994a; Pennisi, 2007) but detailed studies have revealed barcoding to be a valuable tool for plant identification (Shinwari 1995, Newmaster *et al*., 2008; Kress & Erickson, 2008; Lahaye *et al*., 2008).

Table 1. General information of the studied species and their reported medicinal uses.

Species	Common name	Parts used	Medicinal uses	Reference
Acacia modesta Wall	Phulai (in Pakistan)	Gum, bark	Used for indigestion, dysentery & tooth ache	Hussain *et al*., 2008; Jabeen *et al*., 2009
Acacia nilotica (L.) Willd. ex Delile	Gum arabic	Leaves, bark, pods	Used in inflammatory conditions of the respiratory, digestive & urinary tract, and useful in vomiting, diarrhea & dysentery	Shinwari *et al*., 2013
Albizia lebbeck (L.) Benth.	Lebbeck tree	Leaves, seeds	Decoction for hemorrhoids, malaria, peptic ulcer, intestinal worms, antifungal & antiviral	Taj *et al*., 2009
Albizia procera (Roxb.) Benth.	Silk tree	Bark, leaves	Bark is used for fish poison and considered useful in pregnancy and stomachache. Leaves are poulticed onto ulcers in India	Khatoon *et al*., 2013

In Pakistan, medicinal plants are used widely in the form of packaged medicine manufactured by herbal medicine industries and as raw herbs which are formulated by indigenous people in light of their indigenous knowledge. The raw material is collected from the wild and transported to national and international markets. The transportation chain, with many middlemen, results in increased events of misidentification and adulteration. In 2002 the global market for medicinal and aromatic plants was US $62 billion and is estimated to rise to US $5 trillion by 2050, indicating a global shift from an allopathic to a traditional healthcare system (Shinwari, 2010). Therefore, keeping the aforementioned situation in mind, there is a need for an effective identification system through barcoding these medicinal plants. Barcoding not only allows the pharmaceutical industry and consumers in Pakistan to authenticate the raw material but also provides reference sequences to the scientific community.

The objective of this study was to test whether the *rbcL* and *matK* genes could be used to correctly identify selected *Acacia* and *Albizia* species which are being used medicinally. The utility of *trnH-psbA* spacer for the authentication of these species was also studied. Further the data obtained from these cpDNA regions was analyzed to study the monophyletic/polyphyletic relationship among *Acacia* and *Albizia*.

Materials and Methods

Plant material: Selected species of *Acacia* and *Albizia* were collected from Islamabad and Karachi in Pakistan and identified morphologically by using the Flora of West Pakistan (Ali, 1973). The voucher samples were deposited in QAU and their information is given in Appendix I.

DNA isolation, amplification and sequencing: Genomic DNA was extracted from silica gel dried leaves by using a standard cetyltrimethylammonium bromide (CTAB) protocol (Doyle, 1991). Polymerase chain reaction (PCR) amplification of the *rbcL*, *matK* and *trnH-psbA* regions was carried out in a Veriti Thermal Cycler (Applied Biosystems, Carlsbad, California, USA) using the KAPA3G Plant PCR Kit (Kapa Biosystems, Woburn, Massachusetts, USA), as outlined in Schori *et al.,* (2013). Each reaction contained the KAPA3G Plant PCR Buffer (1× final concentration, includes dNTPs at 0.2 mM each), $MgCl_2$ (1.5 mM final concentration), 1 unit KAPA3G Plant DNA polymerase, primers at a final concentration of 0.3 µM each, template DNA and PCR-grade water to bring the volume to 50 µL. The following cycling parameters were used for *rbcL* : 95°C 10 min; 50 cycles: 95°C 20 s, 58°C 15 s, 72°C 90 s; 72°C 90 s. The *rbcL* primers 1F (Fay *et al.,* 1997) and 1460R (Fay *et al.,* 1998; Cuénoud *et al.,* 2002) were used in this experiment.

The same cycling parameters were performed using the *matK* 390F/1360R primers (Cuénoud *et al.,* 2002) with an annealing temperature of 50 °C for 40 cycles (Schori *et al.,* 2013). A touchdown program was carried out for *trnH-psbA* using the PsbAF/PsbHR primers (Sang *et al.*, 1997; Tate & Simpson, 2003), where the annealing temperature was 58°C for initial 11 cycles followed by touchdown to 48°C for 29 cycles (Schori *et al.,* 2013).

Appendix I. Voucher specimens of the species used in this study.

Sr. #	Species	Voucher specimen	Collection locality	Geographic coordinates
1.	*Acacia modesta*	MOSEL 250	Islamabad, Pakistan	33° 45′ 0″ N, 73° 8′ 0″ E
2.	*Albizia lebbeck*	MOSEL 251	Islamabad, Pakistan	33° 42′ 40.6″ N, 73° 7′ 55.47″ E
3.	*Albizia procera*	MOSEL 252	Islamabad, Pakistan	33° 42′ 40.6″ N, 73° 7′ 55.47″ E
4.	*Acacia nilotica*	MOSEL 253	Islamabad, Pakistan	33° 45′ 0″ N, 73° 8′ 0″ E
5.	*Acacia nilotica* subsp. *hemispherica*	MOSEL 254	Karachi, Pakistan	24° 50′ 37.52″ N, 66° 46′ 34.76″ E

To ensure the successful amplification of the desired sequence, the PCR products were run on 1% agarose gel. PCR products were cleaned with the Wizard SV Gel and PCR Clean-Up System (Promega Corporation, Madison, Wisconsin, USA). The purified PCR products were sequenced at Ohio University's Genomics Facility and analyzed using an ABI 3130xl Genetic Analyzer (Applied Biosystems, Carlsbad, California, USA). Each sequencing reaction included 2 µL 5× buffer (Applied Biosystems), 0.5 µL dimethyl sulfoxide (DMSO ; Sigma), 0.5 µL BigDye (Applied Biosystems), 0.1 µL ThermoFidelase (Fidelity Systems, Gaithersburg, Maryland, USA), 10–40 ng template DNA, and PCR-grade water for a total volume of 8 µL. Cycle sequencing products were cleaned with the BigDye XTerminator Purification Kit (Applied Biosystems). For *rbcL*, external primers 1F and 1460R, and internal primers 636F and 724R (Fay *et al.,* 1997), were used for sequencing. For *matK* and *trnH-psbA* sequencing, the same primer pairs were used as for amplification.

Sequence alignment & data analysis: Sequences were assembled into contigs and generation of consensus sequences was performed using Geneious 6.1.6 (Biomatters Ltd., Auckland, New Zealand). Searches were performed using the BLAST megablast parameter search function, to compare the sequences to data in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Percent similarity was recorded for the closest matches (Table 2). Here the correct identification means that the highest BLAST % identity of the query sequence was from the expected species or the species belonging to the expected genera; ambiguous identification means that the highest BLAST % identity for a query sequence was found to match several genera of the expected family; incorrect identification means that the highest BLAST % identity of the query sequence was not from the expected species/expected genera/expected family.

Sequences were submitted to GenBank and their accession numbers are given in Appendix II. Sequence data of *rbcL*, *matK* and *trnH-psbA* for all the available *Acacia* and *Albizia* species in GenBank was downloaded (http://www.ncbi.nlm.nih.gov/genbank/). Analyses were conducted by using the selected sequences from GenBank and the sequences generated from our studied species (Appendix III for selected GenBank accessions). Sequence alignments were conducted on MUSCLE version 3.8.31 (Edgar, 2004). The final concatenated alignments using the primary barcoding loci *rbcL* and *matK* (1490 bp and 1621 bp) were analyzed separately through Geneious 6.1.6 by choosing Neighbor Joining (NJ) method and Jukes Cantor (JC) genetic distance model. Bootstrap support was accessed by 1000 replicates and *Calliandra* was taken as an outgroup.

Table 2. BLAST % identity of the studied species with the GenBank sequence data.

Species	Gene region	% Identity in GenBank
Acacia modesta	*rbcL*	95% to multiple *Acacia* species and other genera of Fabaceae
	matK	99% to a voucher of *Acacia modesta* and 98-99% to multiple *Acacia* species
	trnH-psbA	No sequence obtained
Acacia nilotica subsp. *hemispherica*	*rbcL*	99% to multiple *Acacia* species and other genera of Fabaceae
	matK	No PCR product obtained
	trnH-psbA	98% to 15 vouchers of *Acacia nilotica*, 90-97% to other multiple *Acacia* species
Acacia nilotica	*rbcL*	99% to multiple *Acacia* species and other genera of Fabaceae
	matK	No sequence obtained
	trnH-psbA	No sequence obtained
Albizia lebbeck	*rbcL*	97-99% to other genera of Fabaceae
	matK	100% to an *Albizia lebbeck* voucher and 99% to multiple *Acacia* species
	trnH-psbA	No sequence obtained
Albizia procera	*rbcL*	96-99% to other genera of Fabaceae
	matK	99% to multiple *Albizia* and *Acacia* species
	trnH-psbA	No sequence obtained

Appendix II. GenBank accession numbers of plant samples used in the present study.

Sr. #	Species	GenBank accession's		
		***rbc*L**	***matK***	***trnH-psbA***
1.	*Acacia modesta*	KC336419	KC689798	-
2.	*Albizia lebbeck*	KC417043	KC689799	-
3.	*Albizia procera*	KC417044	KC689800	-
4.	*Acacia nilotica*	KC417042	-	-
5.	*Acacia nilotica* subsp. *hemispherica*	KC417041	-	KF724863

Appendix III. Accession numbers of sequences retrieved from GenBank.

		rbcL	
AM234255.1	JF265248.1	JF265278.1	JX195517.1
EU213436.1	JF265250.1	JF265279.1	JX232068.1
EU213438.1	JF265256.1	JF265280.1	JX232086.1
GQ436354.1	JF265257.1	JF265281.1	JX232104.1
GQ436378.1	JF265260.1	JQ412305	JX232112.1
GU135162.1	JF265262.1	JQ591529.1	JX232128.1
HQ427141.1	JF265263.1	JQ591553.1	JX232132.1
JF265242.1	JF265274.1	JQ591567.1	JX232148.1
JF265243.1	JF265275.1	JQ591570.1	JX232157.1
JF265246.1	JF265276.1	JQ592106.1	JX856628.1
JF265247.1	JF265277.1	JX195516.1	Z70147.1
		matK	
AB504374.1	JF420003.1	JF270635.1	JQ412187.1
GU135096.1	JF270631.1	JF270637.1	JQ587495.1
HM020736.1	JF270632.1	JF419997.1	JQ587502.1
HM850600.1	JF270633.1	JF419999.1	JQ587507.1
HM850601.1	JF270634.1	JF420001.1	
HQ427295.1	JF270636.1	JF420007.1	

Results and Discussion

DNA was isolated from a total of five species including *Acacia modesta, Acacia nilotica, Acacia nilotica* subsp*. hemispherica* Ali & Faruqi, *Albizia lebbeck* and *Albizia procera*. Good quality DNA was obtained through the CTAB method and successfully used for amplifications. Our results showed obvious differences among the three barcoding loci with respect to amplification success, PCR product size and quality of generated sequences (Table 3). The *rbcL* and *trnH-psbA* regions were successfully amplified for all the five species with standard primers and PCR conditions. With the only exception of *Acacia nilotica* subsp. *hemispherica* which failed to amplify, the rest of the four species amplified for the *matK* region. Unlike *rbcL*, *matK* is more variable and may need custom primer design for different plants (Schori & Showalter, 2011).

All the PCR products of *rbcL* were sequenced successfully. We were able to generate about 1400 bp of *rbcL* for each species by using external and internal primers in cycle sequencing. Therefore, the lengths of the analysed *rbcL* sequences ranged from 1376 bp to 1474 bp. On the basis of our analysed *rbcL* region, we could distinguish *Acacia modesta* from *Acacia nilotica* and *Acacia nilotica* subsp*. hemispherica*. There were 23 nucleotides found in *Acacia modesta* which vary from *Acacia nilotica* and its subsp. *hemispherica.* However, *Acacia nilotica* and its subsp. *hemispherica* appeared to have completely identical nucleotides. *Albizia lebbeck* and *Albizia procera* can be distinguished from each other and from species of *Acacia* on the basis of their *rbcL* gene sequences. The number of variable nucleotides between *Albizia lebbeck* and *Albizia procera* was nine. In our study of *Acacia* and *Albizia*, we found that *rbcL* could be used to distinguish the selected species that are being used medicinally in the region. Newmaster & Ragupathy (2009) stated the efficacy of *rbcL* for distinguishing *Acacia* at the subgeneric and species level. Therefore, the *rbcL* region seems to be important for barcoding *Acacia.*

In the case of *matK,* for *Acacia* we could only obtain a sequence for *Acacia modesta. Albizia lebbeck* and *Albizia procera* were sequenced successfully and showed substitution of only six nucleotides. The substitution of nucleotides found in the *matK* region was lower than *rbcL* therefore *matK* appeared to be less a favourable candidate for barcoding these species. Newmaster & Ragupathy (2009) reported that all the three barcoding regions (*rbcL, matK, trnH-psbA*) could discriminate sister species within the *Acacia*.

Acacia nilotica subsp. *hemispherica* was the only species for which the *trnH-psbA* region was successfully sequenced. Assembling the *trnH-psbA* trace files into contigs was not always straightforward. A high frequency of mononucleotide repeats disrupted individual sequencing reads and resulted in unreliable sequences which could not be used. It has been suggested that this feature of *trnH-psbA* and other non-coding regions prevent their use in future large-scale barcoding projects, in which manual editing of sequences is necessarily kept to a minimum (Devey *et al.,* 2009). Kress & Erickson (2007) reported *trnH–psbA* as demonstrating good amplification across land plants with a single pair of primers and high levels of species discrimination. However, the difficulty given in obtaining the high quality bidirectional sequences was stated by CBOL (2009) as the primary limitation for this locus.

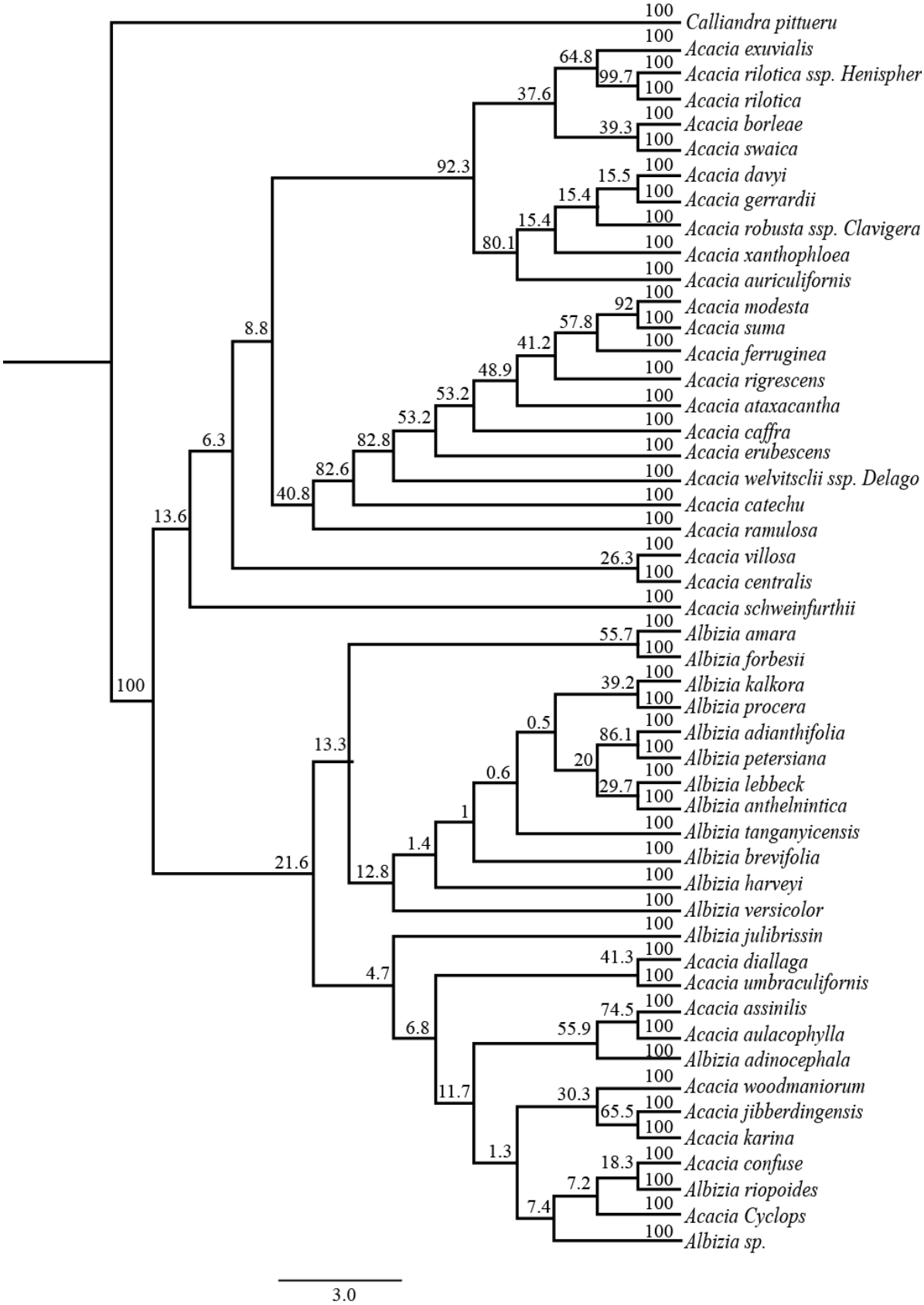

Fig. 1. NJ tree of genetic distance (JC) for *Acacia* and *Albizia* based on *rbcL*. Numbers above branches correspond to bootstrap support. *Calliandra pittieri* taken as an outgroup is sister taxa of *Albizia.*

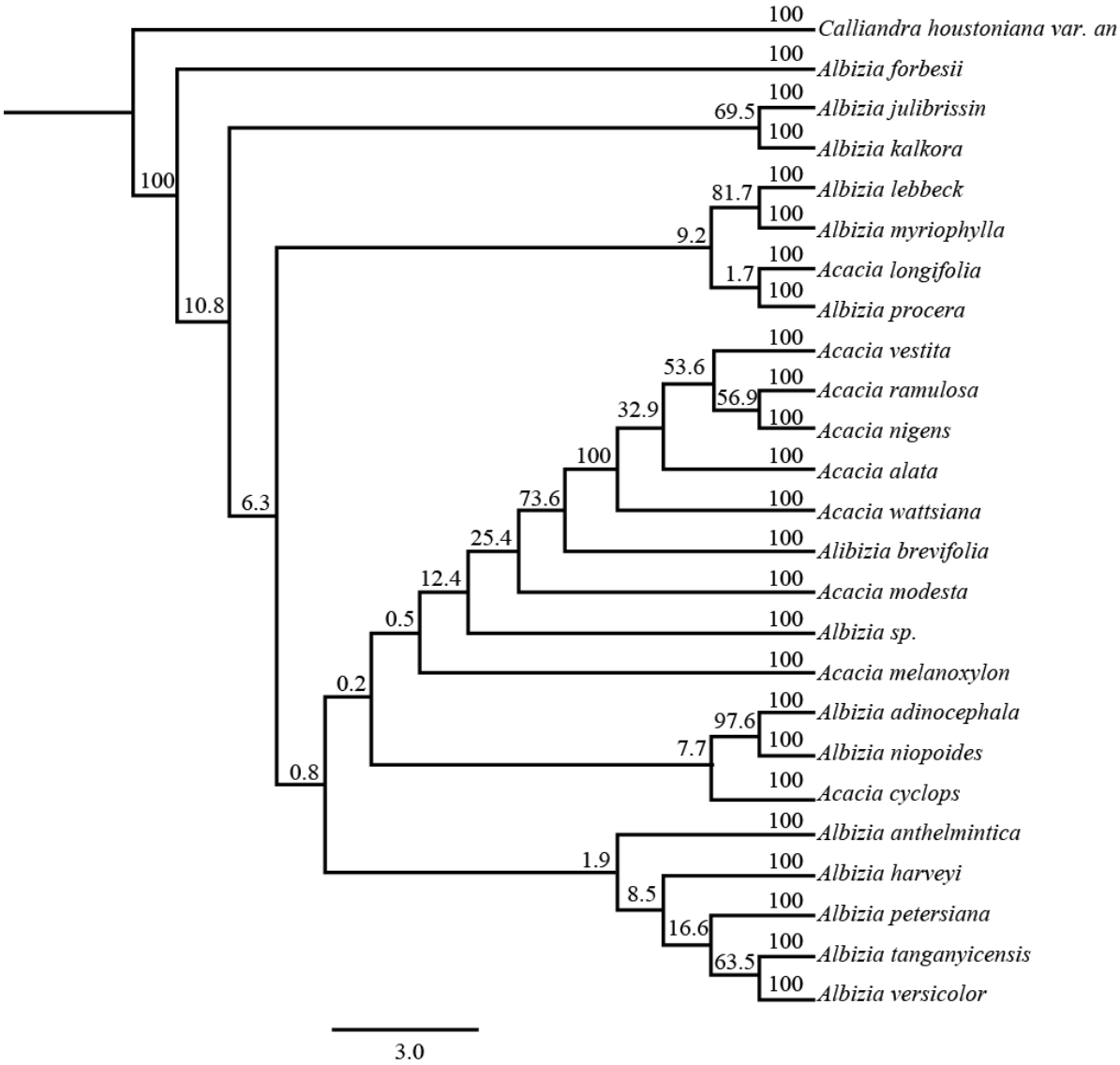

Fig. 2. NJ tree of genetic distance (JC) for *Acacia* and *Albizia* based on *matK*. Numbers above branches correspond to bootstrap support. *Calliandra houstoniana* var. *anomala* taken as an outgroup is sister taxa of *Albizia.*

Table 3. Summary of the successful amplification and sequencing of the studied species from three candidate barcoding regions.

	Species	*rbcL*	*matK*	*trnH-psbA*
Amplification success	*Acacia modesta*	Amplified	Amplified	Amplified
	Acacia nilotica subsp. *hemispherica*	Amplified	Not Amplified	Amplified
	Acacia nilotica	Amplified	Amplified	Amplified
	Albizia lebbeck	Amplified	Amplified	Amplified
	Albizia procera	Amplified	Amplified	Amplified
Sequencing success	*Acacia modesta*	Sequenced	Sequenced	Not Sequenced
	Acacia nilotica subsp. *hemispherica*	Sequenced	Not Sequenced	Sequenced
	Acacia nilotica	Sequenced	Not Sequenced	Not Sequenced
	Albizia lebbeck	Sequenced	Sequenced	Not Sequenced
	Albizia procera	Sequenced	Sequenced	Not Sequenced
Sequence length	*Acacia modesta*	1376 bp	826 bp	-
	Acacia nilotica subsp. *hemispherica*	1408 bp	-	439 bp
	Acacia nilotica	1474 bp	-	-
	Albizia lebbeck	1472 bp	825 bp	-
	Albizia procera	1434 bp	863 bp	-

DNA barcoding has also helped to revive the taxonomy. The objective of our evolutionary analysis was to test the monophyly of *Acacia* and *Albizia* using the DNA sequence data from the *rbcL* and *matK* chloroplast region. Our analysis showed that *Acacia* and *Albizia,* both of them, appeared to be polyphyletic. The results presented here show that genus *Acacia* and tribe Acacieae are polyphyletic and agree with data reported by Miller & Bayer (2000) from nuclear *Histone H3*. Sequence analysis of the chloroplast *trnK* intron, including the *matK* coding region and flanking noncoding regions, indicate that neither the tribe Acacieae nor the genus *Acacia* are monophyletic (Miller & Bayer, 2001). The large, variable genus *Albizia* was reported polyphyletic by Brown *et al.,* (2008) on the basis of nuclear DNA regions (*ITS* and *ETS*). Bentham (1842) originally described the tribe Acacieae as non-monophyletic, containing taxa which are currently placed in both Acacieae and Ingeae. This is supported by the fact that there is only one morphological character which separates the Acacieae and Ingeae that is the presence of free stamens in Acacieae and fused stamens in Ingeae. Although this character is even not consistent throughout and exceptions are found (Chappill & Maslin, 1995). Therefore, a set of morphological character changes is required to separate the tribes.

These findings suggest that retention of Ingeae and Acacieae is not warranted until a reclassification is carried out for all the tribes of Mimosoideae. A large scale molecular and morphological analysis of the entire subfamily Mimosoideae, especially the tribe Mimoseae, is needed. The Ingeae and Acacieae are thought to be derived from a paraphyletic Mimoseae (Pohil *et al.,* 1981), and analyses of the three tribes together will shed light on the phylogeny and morphological character state changes in the Mimosoideae (Figs. 1 and 2).

Conclusion

In this study, *rbcL, matK* and *trnH-psbA* were examined for their usefulness in identifying the selected medicinal species of *Acacia* and *Abizia*. Our findings show that the *rbcL* region can be used to identify these species and discriminate among them more effectively than *matK* and *trnH-psbA*. The latter regions proved to be less successful in sequencing particularly *trnH-psbA*. Hence, *rbcL* is an improved and efficient tool for species identification of these medicinal plants and may be recommended for broad series of subfamily Mimosoideae (Family: Fabaceae) plants, making it a potential DNA barcode for these taxa. DNA sequence data from chloroplast *rbcL* and *matK* shows that *Acacia* and *Albizia* are polyphyletic. More sequence data and increased sampling will be required to further investigate and elucidate the evolutionary relationships.

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References

Ali, S.I. 1973. Mimosaceae, pp. 1-41. In: *Flora of West Pakistan*. (Eds.): E. Nasir & S.I. Ali. Department of Botany, University of Karachi, Karachi, Pakistan.

Bentham, G. 1842. Notes on Mimoseae, with a synopsis of species. *London J. Bot*., 1: 318-528.

Brown, G.K., D.J. Murphy, J.T. Miller and P.Y. Ladiges. 2008. *Acacia s.s.* and its relationship among tropical legumes, Tribe Ingeae (Leguminosae: Mimosoideae). *Syst. Bot*., 33: 739-751.

Byrne, M., G. Tischler, B. Macdonald, D.J. Coates and J. McComb. 2001. Phylogenetic relationships between two rare acacias and their common, widespread relatives in south-western Australia. Conserv. *Genet*., 2: 157-166.

CBOL Plant Working Group. 2009. A DNA barcode for land plants. *P. Natl. Acad. Sci. USA*. 106: 12794-12797.

Chappill, J.A. and B.R. Maslin. 1995. A phylogenetic assessment of tribe Acacieae, pp. 77-99. In: *Advances in legume systematics 7 Phylogeny*. (Eds.): M. Crisp and J.J. Doyle. Royal Botanic Gardens, Kew, Richmond, Surrey, UK.

Chase, M.W., N. Salamin, M. Wilkinson, J.M. Dunwell, R.P. Kesanakurthi, N. Haidar and V. Savolainen. 2005. Land plants and DNA barcodes: Short-term and long-term goals. *Philos. Trans. R. Soc. B*., 360: 1889-1895.

Cowan, R.A. 1998. Mimosaceae (excl. *Acacia*), Caesalpiniaceae. *Flora of Australia*. 12 (ABRS/CSIRO Publishing).

Cowan, R.S. and B.R. Maslin. 1990. *Acacia* Miscellany 2. Species related to *A. deltoidea* (Leguminosae: Mimosoideae: Section *Plurinerves*) from Western Australia. *Nuytsia*, 7: 201-208.

Cuénoud, P., V. Savolainen, L.W. Chatrou, M. Powell, R.J. Grayer and M.W. Chase. 2002. Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid *rbcL* , *atpB* , and *matK* DNA sequences. *Am. J. Bot*., 89: 132-144.

Devey, D.S., M.W. Chase and J.J. Clarkson. 2009. A stuttering start to plant DNA barcoding: microsatellites present a previously overlooked problem in non-coding plastid regions. *Taxon*., 58: 7-15.

Doyle, J.J. 1991. DNA protocols for plants, pp. 283-293. In: *Molecular techniques in taxonomy*. (Eds.): G. Hewitt, A.W.B. Johnson, and J.P.W. Young. NATO ASI Series H, Cell Biology 57. Springer-Verlag, Berlin, Germany.

Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res*., 32: 1792-97.

Elias, T.S. 1981. Mimosoideae, pp. 143-151. In: *Advances in Legume Systematics*. (Eds.): R.M. Polhill and P.H. Raven. Part 1. Royal Botanic Gardens, Kew, London.

Fay, M.F., C. Bayer, W.S. Alverson, A.Y. DeBruijn and M.W. Chase .1998. Plastid *rbcL* sequence data indicate a close affinity between *Digodendron* and *Bixa*. *Taxon*., 47: 43-50.

Fay, M.F., S.M.S. Wensen and M.W. Chase. 1997. Taxonomic affinities of *Medusagyne oppositifolia* (Medusagynaceae). *Kew Bulletin*, 52: 111-120.

Hebert, P.D.N., A. Cywinska, S.L. Ball and J.R. deWaard. 2003. Biological identifications through DNA barcodes. *Proc. Biol. Sci*., 270: 313-321.

Hollingsworth, P.M., S.W. Graham and D.P. Little. 2011. Choosing and using a plant DNA Barcode. *PLoS ONE*. 6(5): e19254.

Hussain, K., A. Shahazad and S.Z. Hussain. 2008. An ethnobotanical survey of important wild medicinal plants of Hattar District. Haripur, Pakistan. *Pak. J. Bot*., 42: 3747-3753.

Jabeen, A., M.A. Khan, M. Ahmad, M. Zafar and F. Ahmad. 2009. Indigenous uses of economically important flora of Margallah Hills National Park, Islamabad, Pakistan. *Afr. J. Biotechnol*., 8: 763-784.

Khatoon, M., E. Islam, R. Islam, A.A. Rahman, K. Alam, P. Khondkar, M. Rashid and S. Parvin. 2013. Estimation of total phenol and *in vitro* antioxidant activity of *Albizia procera* leaves. *BMC. Res. Notes*, 6: 121.

Kress, W.J. and D.L. Erickson. 2007. A two-locus global DNA barcode for land plants: the coding rbcL gene complements the non-coding trnH-psbA spacer region. *PLoS ONE*. 6: e508.

Kress, W.J. and D.L. Erickson. 2008. DNA barcodes: genes, genomics, and bioinformatics. *Proc. Nat. Acad. Sci., USA*. 105: 2761-2762.

Kriticos, D.J., R.W. Sutherst, J.R. Brown, S.W. Adkins and G.F. Maywald. 2003. Climate change and the potential distribution of an invasive alien plant: *Acacia nilotica* ssp. *indica* in Australia. *J. App. Ecol*., 40: 111-124.

Lahaye, R., M. van der Bank, D. Bogarin, J. Warner, F. Pupulin, G. Gigot, O. Maurin, S. Duthoit, T.G. Barraclough and V. Savolainen. 2008. DNA barcoding the floras of biodiversity hotspots. *Proc. Nat. Acad. Sci., USA*. 105: 2923-2928.

Lavin, M., P.S. Herendeen and M.F. Wojciechowski. 2005. Evolutionary rates analysis of the Leguminosae implicates a rapid diversification of lineages during the tertiary. *Syst. Biol*., 54: 575-594.

Luckow, M., J.T. Miller, D.J. Murphy and T. Livshultz. 2003. A phylogenetic analysis of the Mimosoideae (Leguminosae) based on chloroplast DNA sequence data, pp. 197-220. In: *Advances in legume systematic*. (Eds.): B.B. Klitgaard and A. Bruneau. Part 10, Higher Level Systematics. Royal Botanic Gardens, Kew, UK.

Mabberley, D.J. 2008. Mabberley's Plant-Book.: *A portable dictionary of plants, their classification and uses*. Third edition, Cambridge University Press.: 7-18: 1-1021.

Maslin, B.R., J.T. Miller and D.S. Seigler. 2003. Overview of the generic status of *Acacia* (Leguminosae: Mimosoideae). *Austral. Syst. Bot*., 16: 1-18.

Midgley, S.J. and J.W. Turnbull. 2003. Domestication and use of Australian Acacias: an overview. *Austral. Syst. Bot*., 16: 89-102.

Miller, J.T. and R.J. Bayer. 2000. Molecular phylogenetics of *Acacia* (Fabaceae: Mimosoideae) based on the chloroplast *trn*K/*mat*K and nuclear histone H3-D DNA sequences, pp. 181-200. In: *Advances in legume systematics*, part 9. (Eds.): P.S. Herendeen and A. Bruneau. Royal Botanic Gardens, Kew, UK.

Miller, J.T. and R.J. Bayer. 2001. Molecular phylogenetics of *Acacia* (Fabaceae: Mimosoideae) based on the chloroplast *mat*K coding sequence and flanking *trn*K intron spacer regions. *Am. J. Bot*., 88: 697-705.

Miller, J.T. and R.J. Bayer. 2003. Molecular phylogenetics of *Acacia* subgenera *Acacia* and *Aculeiferum* (Fabaceae: Mimosoideae), based on the chloroplast *mat*K coding sequence and flanking *trn*K intron spacer regions. *Aust. Syst. Bot*., 16: 27-33.

Newmaster, S.G. and S. Ragupathy. 2009. Testing plant barcoding in a sister species complex of pantropical *Acacia* (Mimosoideae, Fabaceae). *Mol. Ecol. Resour*., 9: 172-180.

Newmaster, S.G., A. Fazekas, R. Steeves and J. Janovec. 2008. Testing candidate plant barcode regions in the Myristicaceae. *Mol. Ecol. Resour*., 8: 480-490.

Pennisi, E. 2007. Taxonomy. Wanted: A barcode for plants. *Science*, 318: 190-191.

Pohill, R.M., P.H. Raven and C.H. Stirton. 1981. Evolution and systematics of the Leguminosae, pp. 1-26. In: *Advances in legume systematics, Part 1*. (Eds.): R.M. Pohill and P.H. Raven. Royal Botanic Gardens, Kew, Richmond, Surrey, UK.

Sang, T., D.J. Crawford and T.F. Stuessy. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *Am. J. Bot*., 84: 1120-1136.

Schori, M. and A.M. Showalter. 2011. DNA barcoding as a means for identifying medicinal plants of Pakistan. *Pak. J. Bot*., 43: 1-4. Special Issue, December, 2011 (Medicinal Plants: Conservation & Sustainable Use).

Schori, M., M. Appel, A. Kitko and A.M. Showalter. 2013. Engineered DNA polymerase improves PCR results for plastid DNA. *Applications in Plant Sciences*, 1: 1-7.

Shinwari, Z.K. 1995. Congruence between morphology and molecular phylogeneties in Prosartes (Liliaceae). *Pak. J. Bot*., 27(2): 361-369.

Shinwari, Z.K. 2010. Medicinal plants research in Pakistan. *J. Med. Plant. Res*., 4: 161-176.

Shinwari, Z.K., M. Salima, R. Faisal, S. Huda and M. Asrar. 2013. Biological screening of indigenous knowledge based plants used in diarrheal treatment. *Pak. J. Bot*., 45: 1375-1382.

Shinwari, Z.K., R. Terauchi and S. Kawano. 1994a. Phylogenetic relationships among genera in the Liliaceae-Asparagoideae-Polygonatae sensu lato inferred from rbcL gene sequence data. *PI. Systematic & Evolution*, 192: 263-277.

Shinwari, Z.K., R. Terauchi, F.H. Utech and S. Kawano. 1994. Recognition of the New World Disporum Section Prosartes as Prosartes (Liliaceae) based on the sequence data of the rbcL gene. *Taxon*, 43(3): 353-366.

Taj, S., S.M. Wazir, M. Subhan, M. Hassan, S.U. Khan and M. Kamal. 2009. Some of the ethnobotanically important plants of Godi Khel and its outskirts hilly areas, district Karak, Pakistan. *Pak. J. Pl. Sci*., 15: 39-43.

Tate, J.A. and B.B. Simpson. 2003. Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. *Syst. Bot*., 28: 723-737.

Vassal, J. 1981. Acacieae, pp. 169-171. In: *Advances in legume systematics, Part 1*. (Eds.): R.M. Pohill and P.H. Raven. Royal Botanic Gardens, Kew, Richmond, Surrey, UK.

Wardill, T.J., G.C. Graham, M. Zalucki, W.A. Palmer, J. Playford and K.D. Scott. 2005. The importance of species identity in the biocontrol process: identifying the subspecies of *Acacia nilotica* (Leguminosae: Mimosoideae) by genetic distance and the implications for biological control. *J. Biogeogr*., 32: 2145-2159.

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