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PHYLOGENY OF TAMARICACEAE USING *psbA-trnH* NUCLEOTIDE SEQUENCES

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Abstract

Phylogenetic relationships within Tamaricaceae particularly for genus *Tamarix* were studied using chloroplast intergenic spacer sequences of *psbA-trnH*. A total of nine species (four collected from Pakistan and five retrieved from Gen Bank) were utilized for construction of generic tree while thirteen accessions representing other genera of this family were retrieved from GenBank and used to construct family tree. The trees were constructed using Bayesian inference analysis by BEAST software. A strong supported generic monophyly was observed followed by pairing of few species at strong support within the genera than the rest of the accessions. Our data has provided molecular evidence to the previously establishedmorphological treatments.

Key words: Phylogeny, Intergenic spacer, *psbA-trnH*, Tamaricaceae, Taxonomy.

Introduction

The use of sequence data in phylogenetics particularly from the chloroplast genome has found versatile applications in the field of plant molecular biology and evolution (Shinwari *et al.,* 1994a, 1994b; Zahra *et al.,* 2016; Shinwari *et al*., 2018). Among a number of coding regions used as informative tools in such discriminative studies, certain non-coding sequences have also proved to be potential biomarkers (Provan *et al.,* 2001; Hollingsworth *et al*., 2011). The photosystem II protein D1-transfer RNA Histidine *(psbA-trnH)* intergenic spacer is one such example and is also known as a supplemental barcode apart from the genes used as core barcodes *rbcL* and *matK* (Kress *et al.,* 2005; Chen *et al.,* 2010; Pang *et al.,* 2012; Jamil *et al.,* 2014; Shinwari *et al*., 2014). The variation in the intergenic spacer region between both coding genes is high enough to render its extensive use for differentiating species particularly at lower levels in different studies (Kress *et al.,* 2005; Chase *et al*., 2007; Yao *et al.,* 2009).

Tamaricaceae is a small plant family including 4 genera and 110 species worldwide; however it is represented by 4 genera and 35 species in Pakistan (Qaiser, 1982; Zhang, 2005). Tamaricaceae being native to Eurasia and Africa is distributed widely in temperate regions; mostly dry, salty sandy tracts along riparian habitats and maritime deserts (Gaskin, 2003a, 2003b). *Tamarix* is the largest genus containing 54 species worldwide (Baum, 1967, 1978), and comprising of 26 taxa in Pakistan (Qaiser, 1982; Naz *et al.,* 2013).

The taxonomical status of genus *Tamarix* has remained a subject of controversy since long. It is considered as one of the most difficult genus among angiosperms (Baum, 1967, 1978; Qaiser, 1981). The reasons behind such conflicts have always been the tiny, complex and confusing visible traits of its species that are indistinguishable in vegetative state prior flowering and fruiting (Crins, 1989). Morphological peculiarities of such type have led to misidentification and hence improper naming of *Tamarix* species by various botanists (Allred, 2002; Gaskin, 2003a). Certain closely related invasive *Tamarix* species are likely to form hybrids so as further leading to the taxonomic confusion (Gaskin & Schaal, 2002; Gaskin & Shafroth, 2005; Gaskin & Kazmer, 2009).

Previously the relationships of *Tamarix* were mostly based on the morphology of small disc in centre of flower being either androecial or nectary. Later, its nomenclature started to be based on an additional character i.e. branching of vernal or aestival racemes, which was considered as an unrealistic character by Baum (1967, 1978). Baum's work was complemented by Qaiser (1981) on *Tamarix* species from Pakistan. Systematic treatments based on the so far available evidences of its characters have not been sufficient enough to help withdraw the disagreements existing therein. The species placements based on deficient descriptions have only added to the dispute. Therefore, an attempt has been made in this study to analyze few species relationships of *Tamarix* (Tamaricaceae) using a rapidly evolving spacer *psbA-trnH* of the chloroplast genome and to assess its utility in estimating phylogenetic analyses which will also be of help in conservation of plant diversity (Shinwari & Qaiser, 2011).

Materials and Methods:

Species collection: Four species of genus *Tamarix* were collected from various regions of Pakistan. The chloroplast intergenic spacer region *psbA-trnH* nucleotide sequences of all available worldwide *Tamarix* representative members were retrieved from GenBank. Apart from *Tamarix* genus, members from other genera of family Tamaricaceae accessions were also retrieved from GenBank and utilized in the data analysis. The species with their names and genomic identifiers are represented in the Tables 1 and 2.

DNA Extraction: Extraction of total genomic DNA from freeze-dried plant leaves was carried out using the standard CTAB method (Doyle & Doyle, 1987) with few modifications.

Table 1. Species names and accession numbers of collected plant samples.

S. #	Species	Accession numbers
1.	*Tamarixindica*	KC840657
2.	*Tamarixpassernioides*	KC840660
3.	*Tamarixpakistanica*	KC840658
4.	*Tamarixaphylla*	KC840661

Table 2. Species names and accession numbers of sequences retrieved from Genbank.

S. #	Species	Sequence	Accession numbers
1.	*Tamarixgallica*	*psbA-trnH*	KC584958
2.	*Tamarixchinensis*	*psbA-trnH*	GQ434941
3.	*Tamarixcanariensis*	*psbA-trnH*	EU531724
4.	*Tamarixamplexicaulis*	*psbA-trnH*	EU531725
5.	*Tamarixandrossowii*	*psbA-trnH*	EU240625
6.	*Myricariagermanica*	*psbA-trnH*	HQ680684
7.	*Myricariaprostrata*	*psbA-trnH*	EU240624
8.	*Myricariawardii*	*psbA-trnH*	EU240623
9.	*Myricariarosea*	*psbA-trnH*	EU240621
10.	*Myricarialaxiflora*	*psbA-trnH*	EU240620
11.	*Myricariapulcherrima*	*psbA-trnH*	EU240619
12.	*Myricariaplatyphylla*	*psbA-trnH*	EU240618
13.	*Myricariasquamosa*	*psbA-trnH*	EU240617
14.	*Myricariapaniculata*	*psbA-trnH*	EU240616
15.	*Myricariabracteata*	*psbA-trnH*	EU240615
16.	*Myricariaelegans* var. *tsetangensis*	*psbA-trnH*	EU240614
17.	*Myricariaelegans* var. *elegans*	*psbA-trnH*	EU240613
18.	*Reaumuriasongarica*	*psbA-trnH*	EU240626
19.	*Polygonumaviculare*	*psbA-trnH*	FJ503034

Primers and PCR: The *psbA-trnH* spacer region was amplified by manually designed forward and reverse primers of about 20-24 bp in length from consensus sequences of available spacer nucleotides using online software primer 3 input version 0.4.0 (Rosen & Skaletsky, 2000). Designed primers were synthesized from BIONEER (Korea) company. The obtained lyophilized primers were soaked in molecular grade water and stored at -20°C until further analysis. The 20 µL of PCR reaction volume contained 1.5 mM $MgCl_2$, 0.2 mM of dNTPs mix, 0.5 µM of each primer (forward and reverse), 1U of *Taq* polymerase (BIONEER, Korea) with supplied reaction buffer at 1X concentration and 50 ng/µL of DNA template. Thermo cycler conditions involved initial denaturation at 94°C for a minute, followed by 35 cycles of 94°C for 1 min, 50°C for 20 sec, 72°C for 1.5 min and final extension for 5 min at 72°C.

The PCR products were size fractionalized by electrophoresis through 1% (w/v) agarose gel in 1X TBE buffer (89mM Tris-Base, 89mM Boric acid, 20mM EDTA pH 8). Gels were stained with 5µL visualana (Molequle-on, New Zealand) and viewed in Gel documentation system (UVI Tech, UK). Successfully amplified PCR products of the spacer region were purified using PCR purification kit (BIONEER, Korea) following manufacturer instructions.

Sequencing: Purified PCR products were sequenced by commercial laboratory (BIONEER, Korea) and their electropherogram were analyzed for any errors or contiguous sequences. The nucleotide sequences were manually edited to reduce sequencing errors. The sequence data was deposited in GenBank and their accession numbers were recorded.

Sequence data analysis: Multiple sequence alignment was performed using software Clustal W (Larkin *et al.*, 2007) and variable regions among the sequences were observed. The sequence data was partitioned in a way to construct two trees the first comprising of species representing genus *Tamarix* and the second including species from other genera of family Tamaricaceae. Phylogenetic trees were generated using Bayesian inference analysis with the Bayesian evolutionary analysis sampling trees (BEAST) version 1.7.5 software (Drummond *et al.*, 2012) coupled with MarKov chain Monte Carlo (MCMC). The general time reversible model of sequence evolution (GTR) was applied for spacer region as suggested being the best model for DNA substitution by the jModeltest software (Posada, 2008). The sequence data was partitioned as included and excluded group containing sequences of species under consideration and the out-group member respectively. The out group used was a member of the sister family Polygoneaceae, falling in the same order as that of Tamaricaceae. The chain length was set to run for $8x10^5$ generations and screened at every 10,000 runs whereas the trees were sampled every 200th generations. Out of the 4000 trees generated per chain the initial 40 (1%) were discarded as burnin, while posterior probabilities of the remaining trees were calculated. A lognormal relaxed uncorrelated clock model was selected to estimate using tree prior as speciation: Yule process (Gernhard, 2008). The resulting trees were visualized using software Fig Tree version 1.4 (Rambaut, 2012). In this way the evolutionary distinctness on the basis of intergenic spacer among the species of different genera of family Tamaricaceae were inferred and reported.

Results:

The genus tree generated from Bayesian analysis formed a single major clade at 100% Posterior Probability (PP). The members within the major clade were further separated into two clades. The first clade was supported at 89.09% PP grouping three Pakistani species *Tamarix indica*, *Tamarix pakistanica* and *Tamarix aphylla*. Similarly within the second group further subdivision into two sister clades were observed. One end of which at weak support grouped *Tamarix androssowii* and *Tamarix chinensis.* While, on the other end, linked a non-Pakistani *Tamarix amplexicaulis* and Pakistani *Tamarix passernioides* at 84.95% PP. Moreover, nested within this clade was the pair of *Tamarix canariensis* with *Tamarix gallica* depicting their close relationship at strong support of 91.39% PP (Fig. 1).

The family tree employing the *psbA-trnH* spacer region composed of in addition to Pakistani and worldwide *Tamarix* species, the sequences of taxa from other two genera of family Tamaricaceae namely *Myricaria* and *Reaumuria* along with the out group species. The spacer region was able to resolve phylogenetic relationships between species used in tree construction more clearly (Fig. 2).

The resulting Bayesian inference analysis using spacer region formed a major single clade at strong support of 100% PP against out group. This major clade further divided into two sub clades. One end of which extended into equally supported two sister clades at strong PP of 100% grouping all the members

representing the *Myricaria* genus in one group and all representatives of *Tamarix* genus in other group while the single representative of *Reamuria* genus formed a sister group to both these monophyletic clades. Furthermore, within the *Myricaria* clade, further two sister clades were observed, one of which combined two varieties of *Myricaria elegans* at 100% PP while the other linking all the rest of the members of the same genus at 96.24% PP. This sister clade further linked *Myricaria wardii* and *Myricaria rosea* in a single clade at 99.7% keeping *Myricaria prostrata* as its sister group at 90.13% PP. The other sub-sister clade at 99.77% PP support further formed clade at 91.09% PP connecting *Myricaria pulcherima* as sister group to *Myricaria squamosa*, *Myricaria bracteata*, *Myricaria platyphylla* and *Myricaria paniculata* at 96.92% PP. Similarly, within the *Tamarix* clade two subclades further strongly supported the grouping of *Tamarix gallica* and *Tamarix canariensis* at 99.95% PP and *Tamarix aphylla*, *Tamarix pakistanica* and *Tamarix indica* at 96.34% PP. A moderate support at 78.82% PP between *Tamarix passernioides* and *Tamarix amplexicaulis* was observed, however, the relationships of rest of the members of this genus remained poorly supported.

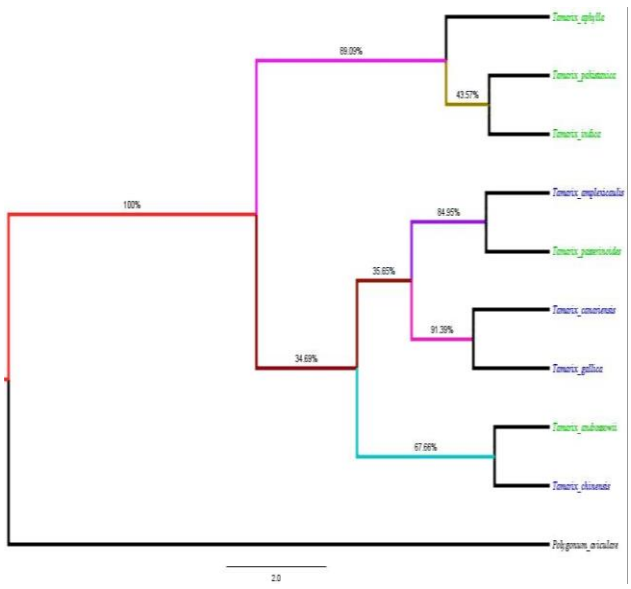

Fig. 1. Bayesian inference based tree of *psbA-trnH* spacer region using *Tamarix* species from the world.
Bayesian inference analysis with percent posterior probabilities: Branch width and colors subjected to posterior probability percentages, species from Pakistan shown in green color.

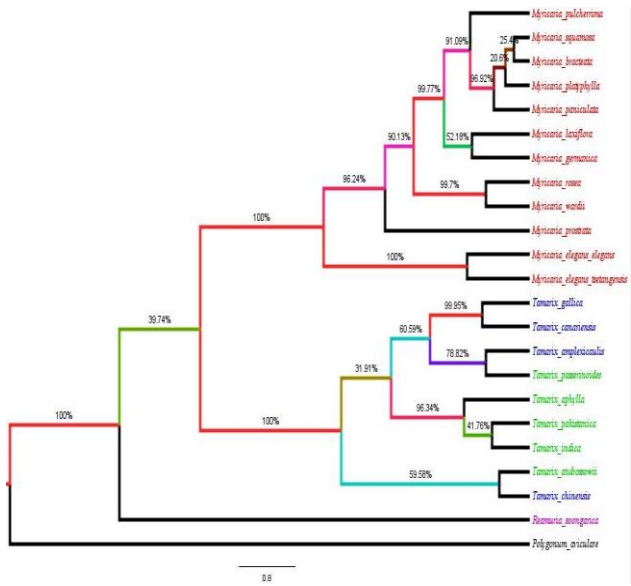

Fig. 2. Bayesian inference based tree of *psbA-trnH* spacer region using *Tamarix* and species from other genera of family Tamaricaceae.
Bayesian inference analysis with percent posterior probabilities: branch width and colors subjected to posterior probability percentages, species from Pakistan shown in green color.

Discussion

A strong supported generic monophyly was observed in both the genus and family tree using the intergenic spacer region. There were no topological variations among the generated Bayesian trees showing similar relationships in both the trees and yet more clarified connections in the family tree as compared to the genus tree probably due to increased number of accessions.

The formation of three distinct subclades in *Tamarix* between certain species may suggest intra-specific variation and series within the genus based on shared morphological characters different than those suggested by Baum (1967, 1978) as well as sequential differences at molecular level based on their biogeography not reported earlier. The topologies that we have attained in our analysis deviate from the morphological sections previously established by Baum (1967, 1978) and since to the best of our knowledge, there is not much work on the molecular side we do not find related supporting evidences to further illustrate our point.

The grouping of three species from our region in a single clade is supported by some shared external characters. *Tamarixpakistanica*, *Tamarixindica*resemble each other in having pseudovaginate leaves and papillose rachis but differ in other characters such as the thickness of racemes, rachis being more or less papilose and dissimilar disc shapes, while *Tamarix aphylla* linked as sister group share the same type of androecial disc as found in *Tamarix indica*. Leaf shapes and disc types found in *Tamarix* are considered reliable characters for distinction (Qaiser, 1981). Our analysis has provided molecular evidence to these morphological traits and also suggested some sort of sequence similarity based on geographical distribution of these species.

The relationship between *Tamarix passernioides* from our region with that of a non-Pakistani *Tamarix amplexicaulis* may be based on their general habitat, global distribution or from some morphological or molecular evidences that were to the best of our knowledge not reported earlier. Whereas, the link between the third supported pair (*Tamarix gallica* and *Tamarix canariensis*), can be discussed in the light of previous studies. According to Baum (1967, 1978) sectional classification of morphological evidences, both *Tamarix gallica* and *Tamarix canariensis* were placed in the same

section (Tamarix) but in different series (Gallicae) and (Leptostachyae) due to narrow racemes, no papillae vs younger papillose growth respectively. Baum also declared that both these species are indistinct while considering their aestival flower branches as compared to the vernal forms of racemes in which there was difference in the petal shapes and surfaces of rachis between these two species. However, Crins (1989) observed coinciding morphological traits among both the species and recommended reconsideration of their relationships. Morphologically, both these species can be identified from other *Tamarix* species on the basis of shared characters such as pentamerous flowers, sessile leaves and androecial disc type being synlophic but yet these characters are not helpful enough to distinguish them from each other. Gaskin & Schaal (2003) were not able to distinguish between both these species at molecular level using chloroplast and nuclear DNA as markers. The incongruent chloroplast and nuclear evolutionary histories in Gaskin and Schaal analysis suggested possible introgressions of these species most likely to form either with each other or with *Tamarix ramossisima* making them indistinct appearance wise as well as genetically (Gaskin, 2003a). The link between *Tamarix gallica* and *Tamarix canariensis* as discrete entities in our analysis is supported because it is based on a more rapidly evolving spacer the *psbA-trnH* a non-coding segment of the chloroplast DNA. Moreover, since *psbA-trnH* is one of the putative core genes qualifying as DNA barcodes (Yao *et al.,* 2009) due to the phylogenetically informative variation it accounts at species level (Hamilton *et al.,* 2003), we propose that our data has supported and resolved the identities of these species being distinct at sequence level and has designated them as closely related other than being hybrids.

The *Myricaria* clade indicated that *Myricaria elegans* var. *elegans* and var. *tsetangensis* formed a strongly supported separate single basal clade that served as a sister group to rest of the *Myricaria* species. On morphological basis this species is different from rest of the *Myricaria* species in having sessile stigmas and ten stamens like any other *Myricaria* species but the stamens are not monadelphous instead distinct like those found in *Tamarix* with shorter styles (Gaskin *et al.,* 2004; Zhang *et al.,* 2006). The strong supported placement of *Myricaria elegans* varietal forms within the *Myricaria* clade at a much basal position in the tree suggested it has resulted from a much primitive process of evolution. In our analysis the species under consideration lies at a position within the *Myricaria* clade near *Tamarix* and may be proposed as a transit in the course of evolution but not a hybrid. In either case, *Myricaria elegans* is more closely related to *Myricaria* genus as suggested by its retention within the *Myricaria* clade. Therefore, in this regard our results coincide to those published lately suggesting these variants of the same species as an evolutionary link between both the closely aligned genera (Wang *et al.,* 2009). The rest of the species relationships of *Myricaria* genus from our analysis, were also observed previously while using the same spacer sequence (*psbA-trnH*) in addition to nuclear ribosomal internal transcribed spacer (ITS) sequences (Wang *et al.,* 2009).

So far the utility of the intergenic spacer region to assess phylogenetic relationships in our analysis proved to be good enough (Jabeen *et al.,* 2012). Particularly for the genus *Tamarix*, a better resolution was observed as at least three pairs of species were well established with strong support while more highly supported relationships were observed in the *Myricaria* genus. The non-coding intergenic spacer region accumulates high sequence variations useful enough to embark evolutionary relationships particularly below genus level (Hamilton *et al.,* 2003; Hao *et al.,* 2010) which is concordant to the strongly supported clades observed at species level in the *psbA-trnH* phylogeny of our analysis. It can be concluded that our data is concordant to the morphological treatments and has basically provided molecular evidence. In future, there is a need to increase the sample size of *Tamarix* species to get further resolved relationships. Moreover, there is a need to combine molecular data from other plant genomes such as nuclear and mitochondrial or such molecular segments within these genomes as well as within the chloroplast that evolve more rapidly so as to get improved phylogenies.

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References

Allred, K.W. 2002. Identification and taxonomy of *Tamarix* (Tamaricaceae) in New Mexico. *Desert Plants.*, 18: 26-32.

Baum, B.R. 1967. A monograph of the genus *Tamarix.* Thesis Jerusalem.

Baum, B.R. 1978. The Genus *Tamarix.* Israel Academy of Science and Humanities, Jerusalem, pp. 209.

Chase, M.W., R.S. Cowan, P.M. Hollingsworth, C. Van-den-Berg, S. Madrinan, G. Petersen, O. Seberg, T. Jorgsensen, K.M. Cameron, M. Carine, N. Pedersen, T.A.J. Hedderson, F. Conrad, G.A. Salazar, J.E. Richardson, M.L. Hollingsworth, T.G. Barraclough, L. Kelly and M. Wilkinson. 2007. A proposal for a standard protocol to barcode all land plants. *Taxon.*, 56: 295-299.

Chen, S.L., H. Yao, J.P. Han, C. Liu, J.Y. Song, L.C. Shi, Y.J. Zhu, X.Y. Ma, T. Gao, X.H. Pang, K. Luo, Y. Li, X.W. Li, X.C. Jia, Y.L. Lin and C. Leon. 2010. Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS ONE.*, 5: e8613.

Crins, W.J. 1989. The Tamaricaceae in the southeastern United States. *J. Arnold Arbor.*, 70: 403-425.

Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissues. *Phytochem. Bull.*, 19: 11-15.

Drummond, A.J., M.A. Suchard, D. Xie and A. Rambaut. 2012. Bayesian phylogenetics with BEAUti and the BEAST v1.7. *Mol. Biol. Evol.*, 29: 1969-1973.

Gaskin, J.F. 2003a. Molecular systematics and the control of invasive plants: A case study of *Tamarix* (Tamaricaceae). *Ann. Missouri Bot. Gard.*, 90: 109-118.

Gaskin, J.F. 2003b. Tamaricaceae. In: *The families and genera of vascular plants*. (Eds.): Kubitzki, K. and C. Bayer. V. Springer-Verlag, Berlin, Germany, pp. 363-368.

Gaskin, J.F. and B.A. Schaal. 2002. Hybrid *Tamarix* widespread in U.S. invasion and undetected in native Asian range. *PNAS*., 99: 11256-11259.

Gaskin, J.F. and B.A. Schaal. 2003. Molecular phylogenetic investigation of U.S. invasive *Tamarisk. Syst. Bot*., 28: 86-95.

Gaskin, J.F. and D.J. Kazmer. 2009. Introgression between invasive saltcedars (*T. chinensis* & *T. ramosissima*) in the USA. *Biol. Invasions*., 11: 1121-1130.

Gaskin, J.F. and P.B. Shafroth. 2005. Hybridization of *T. ramosissima* and *T. chinensis* (saltcedars) with *T. aphylla* (Athel) (Tamaricaceae) in the southwestern USA determined from DNA Sequence data. *Madroňo*., 52: 1-10.

Gaskin, J.F., F. Ghahremani-Nejad, D.Y. Zhang and J.P. Londo. 2004. A systematic overview of Frankeniaceae and Tamaricaceae from nuclear rDNA and plastid sequence data. *Ann. Missouri Bot. Gard.*, 91: 401-409.

Gernhard, T. 2008. The conditioned reconstructed process. *J. Theor. Biol*., 253: 769-778.

Hamilton, M.B., J.M. Braverman and D.F. Soria-Hernanz. 2003. Patterns and relative rates of nucleotide and insertion/deletion evolution at six chloroplast intergenic regions in new world species of the Lecythidaceae. *Mol. Biol. Evol*., 20: 1710-1721.

Hao, D. C., S.L. Chen and P.G. Xiao. 2010. Sequence characteristics and divergent evolution of the chloroplast *psbA-trnH* noncoding region in gymnosperms. *J. Appl. Genet.*, 51: 259-273.

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

Jabeen A., B. Guo, B.H. Abbasi, Z.K. Shinwari and T. Mahmood. 2012. Phylogenetics of selected *Mentha*species on the basis of *rps*8, *rps*11 and *rps*14 chloroplast genes. *J. Med. Plant Res.*, 6: 30-36

Jamil, I., S. Qamarunnisa, A. Azhar, Z.K. Shinwari, S.I. Ali and M. Qaiser. 2014. Subfamilial relationships within Solanaceae as inferred from a*tpβ-rbc*L intergenic spacer. *Pak. J. Bot.*, 46: 585-590.

Kress, W.J., K.J. Wurdack, E.A. Zimmer, L.A. Weigt and D.H. Janzen. 2005. Use of DNA barcodes to identify flowering plants. *PNAS*., 102: 8369-8374.

Larkin, M.A., G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson and D.G. Higgins. 2007. CLUSTAL W and CLUSTAL X version 2.0. *Bioinformatics*, 23: 2947-2948.

Naz, F., S. Qamarunnisa, Z.K. Shinwari, A. Azhar and S.I. Ali. 2013. Phytochemical investigations of *Tamarixindica* Willd. And *Tamarix passernioides* Del Ex Desv. leaves from Pakistan. *Pak. J. Bot*., 45: 1503-1507.

Pang, X., C. Liu, L. Shi, R. Liu, D. Liang, H. Li, S.S. Cherny and S. Chen. 2012. Utility of the *trnH–psbA* intergenic spacer region and its combinations as plant DNA barcodes: a meta- analysis. *PLoS ONE*., 7: e48833.

Posada, D. 2008. jModel Test: Phylogenetic model averaging. *Mol. Biol. Evol*., 25: 1253-1256.

Provan, J., W. Powell and P.M. Hollingsworth. 2001. Chloroplast microsatellites: new tools for studies in plant ecology and evolution. *Trends Ecol Evol.*, 16: 142-147.

Qaiser, M. 1981. The genus *Tamarix* Linn. (Tamaricaceae) in Pakistan. *Pak. J. Bot.*, 13: 107-158.

Qaiser, M. 1982.Tamaricaceae.In: *Flora of Pakistan*,(Eds.): E. Nasir and S.I. Ali, 14: 1-65 Karachi.

Rambaut, A. 2012. Fig Tree (release 1.4.0). http:// tree.bio.ed.ac.uk/software.

Rozen, S. and H.J. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols, Methods in Molecular Biology*. (Eds.): Krawetz, S. and S. Misener. Humana Press, Totowa, NJ, pp. 365-386.

Shinwari, Z.K. and M. Qaiser. 2011. Efforts on conservation and sustainable use of medicinal plants of Pakistan. *Pak. J. Bot*., 43: 5-10.

Shinwari, Z.K., K. Jamil and N.B. Zahra. 2014. Molecular systematics of selected genera of family Fabaceae. *Pak. J. Bot.*, 46: 591-598

Shinwari, Z.K., R. Terauchi and S. Kawano. 1994a. Molecular systematics of Liliaceae-Asparagoideae-polygonatae. RFLP analysis of cpDNA in several species of asiaticdisporum species. *Plant Species Biol*., 9: 11-18.

Shinwari, Z.K., R. Terauchi, F.H. Utech and S. Kawano. 1994b. Recognition of the new world disporum section prosartes as prosartes (Liliaceae) based on the sequence data of the *rbcL* gene. *Taxon*., 43: 353-366.

Shinwari, Z.K., S.A. Jan, A.T. Khalil, A. Khan, M. Ali, M. Qaiser and N.B. Zahra. 2018. Identification and phylogenetic analysis of selected medicinal plant species from Pakistan: DNA barcoding approach. *Pak. J. Bot*., 50: 553-560.

Wang, Y., L.I.U. Yifei, L.I.U. Songbai and H. Huang. 2009. Molecular phylogeny of *Myricaria* (Tamaricaceae): Implications for taxonomy and conservation in China. *Bot. Stud.*, 50: 343-352.

Yao, H., J.Y. Song, X.Y. Ma, C. Liu, Y. Li, H.X. Xu, J.P. Han, L.S. Duan and S.L. Chen. 2009. Identification of *dendrobium* species by a candidate DNA barcode sequence: the chloroplast *psbA-trnH* intergenic region. *Planta Med.*, 75: 667-669.

Zahra, N.B., Z.K. Shinwari and M. Qaiser. 2016. DNA Barcoding: A Tool for standardization of herbal medicinal products (Hmps) of Lamiaceaefrom Pakistan. *Pak. J. Bot.*, 48: 2167-2174

Zhang, D., Y. Zhang, J.F. Gaskin and Z. Chen. 2006. Systematic position of *Myrtama* Ovcz. & Kinz.based on morphological and nrDNA ITS sequence evidence. *Chin. Sci. Bull.*, 51: 117-123.

Zhang, D.Y. 2005. Discussion on some systematical problems of Tamaricaceae. *Acta. Bot. Yunnanica.*, 27: 471-478.

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