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PHYLOGENY OF SOLANACEAE BASED ON MORPHOLOGICAL AND MOLECULAR DATA-USEFUL APPROACH FOR CLASSIFICATION

ISHRAT JAMIL[1*], ABID AZHAR[1], ZABTA KHAN SHINWARI[2,3] AND SYED IRTIFAQ ALI[1]

[1]*The Karachi Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi, Pakistan.*
[2]*Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan*
[3]*Pakistan Academies of Sciences, Islamabad-Pakistan.*
[*]*Corresponding author's email: ishratjamil.kibge@gmail.com*

Abstract

Data from morphological characters and sequence of chloroplast intergenic spacer region *ndh*F-*rpl*32 were analyzed for the same set of 18 species of Solanaceae and one outgroup *Ipomoea cairica*, a member of sister family Convolvulaceae. In the present study, *ndh*F-*rpl*32 intergenic spacer region was utilized to explore systematic relationships within Solanaceae. Sequence analysis revealed that the intergenic spacer *ndh*F-*rpl*32 was a hyper variable region in terms of nucleotide deletion compared to the other coding (*rbc*L, *mat*K) and non-coding region (*atp*β-*rbc*L) of the chloroplast genome. The result of separate parsimonious analysis revealed that the topology of both morphological and molecular phylogenetic trees were similar with respect to the division of subfamilies within Solanaceae but are in conflict with respect to the resolution of the tribal and intergeneric association within monophyletic subfamily Solanoideae. The sequence based parsimonious analysis results in few fully resolved clades within Solanoideae. Moreover, the Bayesian analysis provides the increased resolution within Solanoideae and for the rest of the tree.

Key words: Intergenic association, Molecular data, Morphological data, *ndh*F-*rpl*32, Bayesian inference, Parsimony analysis, subfamilial association.

Introduction

Plant systematics has been an area of research with constant advancements. Many datasets have been employed to infer the relationship at various taxonomic levels. However, focus has been on morphological and molecular characters (Chao *et al*., 2020; Freire *et al*., 2015; Bengtson *et al*., 2014). The morphological characters are frequently used in taxonomic studies as they are easily observable. Despite of holding phenotypic plasticity and high level of homoplasy, these are useful evidence, particularly at the specific and generic ranks. These evidences provide the basic language for plant identification, characterization, classification and relationships (Subrahmanyam, 2009; Givnish & Sytsma, 1992). Morphological data has also been used to reconstruct the phylogeny of plants (Pakso *et al*., 2016; Ghimire & Heo, 2014), particularly for the extinct species (Niklas and Crepet, 2020; Scotland *et al*., 2003; Donoghue & Sanderson, 1992). However, with the accessibility of new source of evidences, especially molecular characters, the understanding of resolved phylogeny is better now. Molecular data provide low level of homoplasy (Hilu *et al*., 2014; Avise, 2012; Dong *et al*., 2012; Givnish & Sytsma, 1992), large number of characters and relative unambiguity in scoring method (Sanderson & Donoghue, 1989). Although the output from molecular approach has improved greatly in both quality and quantity, it has also been recognized that both morphological and molecular methods have discrete advantages in phylogenetics (Bengtson *et al*., 2014; Marghali *et al*., 2014; Meireles *et al*., 2014; Wright & Hillis, 2014; Witt, 2004; Bousquet *et al*., 1992).

Phylogenetic analysis is used to classify genotypes into distinct group and help in the identification of unique genotypes (Sardar *et al*., 2021; Khan *et al*., 2019; Shinwari *et al*., 2018; Jan *et al*., 2017; Jan *et al*., 2016). The utility of different datasets has been exploited in the phylogeny of Solanaceae by using molecular markers (Hidayat *et al*., 2016; Bohs & Olmstead, 2001; Olmstead *et al*., 2008, 1999) as well as morphological characters (Särkinen *et al*., 2013; Knapp, 2002; Bohs, 2001; Estrada & Martinez, 1999; Knapp *et al*., 1998). The Solanaceae is an important family serving mankind with species having considerable economic importance as essential vegetables and fruits like potatoes, aubergines, tomatoes, peppers, etc.), as ornamentals and as medicinal plants (Edmonds, 2012; Knapp *et al*., 2004). This family is represented by 96 genera and around more than 3000 species all over the world (Hunziker, 2001; D' Arcy, 1991). In Pakistan, the family Solanaceae is represented by 14 genera and 52 species (Nasir, 1985). In the current study, an attempt was taken to study subfamilial relationships in 9 genera of Solanaceae representing 18 species from Pakistan using *ndh*F-*rpl*32 spacer sequence. No such study has been reported using morphological as well as molecular data on Pakistani members of Solanaceae to establish the associations via phylogenetic approach. There is some work done on pollen morphology on seven genera (Perveen & Qaiser, 2007), taxonomical and biochemical analyses on few medicinal species of Solanaceae from Pakistan (Yousaf *et al*., 2010, 2008, 2006) and our previous findings with *atp*β-*rbc*L spacer (Jamil *et al*., 2014). The objective of the present study is to investigate the usefulness of genetic and morphological characters in resolving infrafamilial relationship within the family Solanaceae.

Materials and Methods

Plant material: Total 18 representative species of Solanaceae and one species (*Ipomoea cairica*) of its sister family Convolvulaceae were collected from different localities of Karachi and interior Sindh during their flowering season.

Table 1. List of plant material used in this study along with their location, herbarium voucher number and GenBank accession number .

S. #	Genus	Species	Locality	Specimen voucher No.	GenBank accession No.
1.	*Solanum*	*Solanum nigrum* L.	Department of Visual studies, Univrsity of Karachi	G. H. No. 86479	KF055419
		Solanum tuberosum L.	Gulshan e Maymar, Karachi	G.H. No. 86582	KF055430
		Solanum surattense Burm	Department of Physiology, University of Karachi	G. H. No. 86480	KF055420
		Solanum melongena L.	KIBGE, University of Karachi	G. H. No. 86485	KF055421
		Solanum forskalii Dunal	Safari park, Karachi	G. H. No. 86534	KF055422
		Solanum incanum L.	Gharo, Sindh	G. H. No. 86531	KF055426
2.	*Lycopersicon*	*Lycopersicon esculentum* Miller.	Center for plant conservation, University of Karachi	G. H. No. 86481	KF055428
3.	*Capsicum*	*Capsicum annuum* L.	NARC, Islamabad	G.H. No. 86538	KJ652196
		Capsicum frutescens L.	Shah Faisal Colony, Karachi	G. H. No. 86480	KJ652195
4.	*Physalis*	*Physalis divaricata* D.Don	Department of Botany, University of Karachi	G. H. No. 86474	KF055425
5.	*Withania*	*Withania coagulans* (Stocks) Don	Center for plant conservation, University of Karachi	G. H. No. 86484	KJ652197
		Withania somnifera (L).	Department of Physics, Univer sity of Karachi	G. H. No. 86476	KF055418
6.	*Lycium*	*Lycium edgeworthii* (Dunal)	Center for plant conservation, University of Karachi	G. H. No. 86533	KJ652198
7.	*Datura*	*Datura stramonium* L.	Center for plant conservation, University of Karachi	G. H. No. 86475	KF055423
		Datura inoxia Miller	Muskan site, University of Karachi	G. H. No. 86478	KF055424
8.	*Nicotiana*	*Nicotiana tabacum* L.	Center for plant conservation, University of Karachi	G. H. No. 86537	KF055431
9.	*Cestrum*	*Cestrum nocturnum* L.	Center for plant conservation, University of Karachi	G. H. No. 86535	KF055427
		Cestrum diurnum L.	Center for plant conservation, University of Karachi	G. H. No. 86532	KJ652194
10.	*Ipomoea*	*Ipomoea cairica* (L.) Sweet	Shah Faisal Colony, Karachi	G.H. No. 86551	KJ652199

Identification of plants on morphological basis: All collected species were identified with the help of Floras and authentic herbarium specimens. For morphological studies, total 69 characters were analyzed out of which, 18 vegetative, 41 floral, 5 fruit and 5 seed characters were studied with the help of light and stereo microscope. For every species, three replicates, collected from different localities, were studied. Herbarium sheets were prepared for the identified species and deposited to center for plant conservation, Herbarium University of Karachi. The plant materials, their locations, herbarium voucher number and GenBank accession numbers are listed in Table 1.

Phylogenetic tree reconstruction using morphological data: Each character state was converted into numerical key (1-9) and a data matrix was prepared that was later used to generate nexus file (Table 1 of the supplementary data). Maximum parsimony was used to find the most parsimonious trees. The trees were rooted using outgroup *Ipomoea cairica* a member of sister family Convolvulaceae. The cladogram was constructed by using PAUP* version 4.0b10 (Swofford, 2002) using heuristics search with 1000 replicates. The consistency index (CI) (Kluge & Farris, 1969) and retention index (RI) (Farris, 1989) were calculated to measure the level of homoplasy.

DNA extraction, PCR amplification and sequencing: Total genomic DNA was extracted from fresh leaf samples according to modified Cetyl trimethyl ammonium bromide (CTAB) method (Doyle & Doyle, 1987). The chloroplast non-coding region *ndh*F-*rpl*32 was amplified using the family specific primers, designed with the help of primer3 software version 0.4.0 (Rozen & Skaletsky, 2000). For amplification of *ndh*F-*rpl*32 spacer region, 20 µL PCR reaction included 1X PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.3 µM of each primer (Forward, 5' TTCCGATTCACCGGATCTTA 3' and reverse, 5' ACTCATTGTTATAGCTGGAT 3'), 1.25 units of *Taq* DNA Polymerase, 100 ng DNA template and an appropriate amount of Milli-Q water was prepared. The following steps were fed in thermal cycler machine; initial denaturation at 94°C for 4 minute and final denaturation at 94°C for 30 seconds, annealing at 54°C for 1 minute, extension at 72°C for 1.5 minutes and after 35 cycles final extension at 72°C for 7 minutes. Program was run for 35 cycles. The amplicons were checked on 1% agarose gel electrophoresis and, after purification with a column based PCR purification kit (Bioneer, Korea), sent for direct sequencing at Macrogen (Korea).

Sequence editing and alignment: Each sequence was analyzed using BLASTn, Local Alignment Search Tool, (Altschul *et al*., 1990). Sequences were edited manually to resolve any discrepancy. Multiple sequence alignment (MLA) was performed for all sequences using Multalin software (Corpet, 1988). The refined sequences were deposited to NCBI Genbank and accession numbers were recorded.

Phylogenetic tree reconstruction using molecular data: The relationship between different species of Solanaceae was studied by maximum parsimony method in PAUP* version 4.0b10 (Swofford, 2002) using heuristics search with 1000 replicates, random stepwise addition of sequence and tree-bisection-reconnection (TBR) branch swapping algorithm. Clades robustness was estimated using 1000 bootstrap replicates (Felsenstain, 1985). *Ipomoea cairica* from a sister family Convolvulaceae was used as outgroup in this study. Bayesian Inference (BI), another character based method, based on the probability value, was used for further corroboration by using BEAST, v1.8.0 (Drummond & Rambaut, 2007). Using jmodeltest-2.1.3 software (Posada, 2008), the best model of nucleotide substitution HKY was identified for sequence data. Phylogenetic analysis was performed in BEAST software by applying the selected model. Length of chain was set as 800,000 MCMC generations. At every 10,000 generation, trees were screened and saved at every 200 generations. The burn-in value was set as 40 to discard the initial portion of a Markove chain sample to minimize the effect of initial value on posterior inference. The resulting tree was analyzed on Fig tree software version 1.4 (Rambaut, 2012).

Sequence variability using PAUP*: To find out the genetic variability of *ndh*F-*rpl*32 compared with other coding and non-coding regions (*rbc*L, *mat*K and *atp*β-*rbc*L), multiple sequence alignment of the same 18 species of Solanaceae was done for each set by ClustalW and aligned files were analyzed by PAUP* version 4.0b10 (Swofford, 2002).

Results

For the reconstruction of morphology-based tree morphological characters were observed. 69 morphological characters were used for phylogeny. Morphological and molecular characters information is given in (Table 2).

The phylogenetic tree based on morphological characters resulted three major lineages and each lineage may represent a subfamily (Fig. 1). The first lineage representing subfamily Solanoideae was supported with 50% bootstrap support and comprised of a large clade. The second lineage was supported by receiving 62% bootstrap value displaying *Nicotiana tabacum* as a representation of subfamily Nicotianoideae. The third lineage with 64% support formed a clade of *Cestrum* to represent subfamily Cestroideae. Within the first large clade, 6 unresolved lineages were observed. The first lineage with 56% support clustering the 6 species of *Solanum* and one species of *Lycopersicon* in a clade I. Within clade I, *Solanum surattense*, *Solanum melongena, Solanum forskalii* and *Solanum incanum* were clustered together in a subclade by receiving 98% support. *Solanum forskalii* and *Solanum incanum* showed close association with 80% internal support. The species of genus *Capsicum* were grouped in a clade with moderate support (78% bootstrap), species of *Withania* in other clade with 94% support and species of *Datura* in separate clade with 100% support. The association of subfamily Cestroideae with other subfamilies was found unresolved, similarly the association of all the investigated genera present in subfamily Solanoideae was also unresolved.

The *ndh*F-*rpl*32 spacer sequence parsimonious tree revealed three lineages against outgroup *Ipomoea cairica* (Fig. 2). First lineage which was unresolved presented a subfamily Cestroideae, the second lineage with 68% BS included the subfamily Solanoideae; the third branch with 86% BS comprised of subfamily Nicotianoideae. The sequence based parsimonious tree resolved inter specific association within the genus *Solanum* but remained unresolved in the morphological character based tree. Similarly, DNA sequence-based tree also resolved the intergeneric association between *Withania* and *Physalis*. However, other relationships were not determined using parsimony approach. The connections between Solanaceae were also explored and confirmed by Bayesian approach.

The Bayesian inference method based tree revealed that all the members of Solanaceae were clustered in a major clade against the outgroup (Fig. 3). The resulted tree was divided into three descents. First division with full posterior probability (PP) contained two species of *Cestrum*. Second lineage with 0.75 PP separating the *Nicotiana tabacum* as a subfamily. Third branch with 0.91 PP was divided into two clades; the lower clade contained two species of *Datura* and the upper clade was further divided into two sub clades in which the lower one bifurcated into two groups. The one group comprised of species of *Solanum* and *Lycopersicon* whereas in the other group, species of *Capsicum* is showed weak association (0.61 PP) with *Lycium edgeworthii*. In the upper sub clade, close association between *Withania* and *Physalis* was observed.

A comparison was made to check the genetic variability between the different coding and non-coding region of the chloroplast genome. It was observed from multiple sequence alignment that *ndh*F-*rpl*32 region has more insertions/deletions (InDels) when compared to other coding and non-coding region of the chloroplast genome. Data from PAUP* revealed that *ndh*F-*rpl*32 had more (46.63%) variable and parsimony informative (23.1%) characters as compared to the other regions (Table 3).

Discussion

The analysis based on morphological characters resulted with low resolution at subfamilial level. Low consistency index (0.58) revealed that the morphological data had many homoplastic characters. In this analysis as many as possible morphological characters, presumed to be phylogenetically informative or important for taxonomic point of view were used (Wiens, 2004). However, these characters were not helpful in resolving the tribal and intergeneric association within Solanaceae. This may be due to some known problems associated with these characters like character coding, conceptualization and homology assessment (Scotland *et al*., 2003). Ex estipulate leaf and many floral characters like calyx

aestivation, number of lobes, margin, corolla aestivation, petal fusion, number of stamens, bithecous anther, bicarpellary ovary, syncarpous ovary and arrangement of stamen were found to be constant in most of the studied species. On the contrary, size of the plant, petiole, lamina, calyx, anther, style, fruit, and seeds were different in almost all species under investigation thus providing no information in tree reconstruction. The variable and parsimoniously informative characters were type of inflorescence, flower color, shape, anther arrangement, color, shape and type of dehiscence, anther attachment to the filament, type of fruit, color, shape, seed color, and shape. The characters of leaf were most important and helpful in species delimitation.

Within Solanaceae, both morphological and molecular characters based trees identified three subfamilies; Solanoideae, Cestroideae and Nicotianoideae. In the morphological tree, the association of Solanoideae and Nicotianoideae was clear however, the connection of Cestroideae could not be resolved whereas molecular tree resolved relationship among all three investigated subfamilies. The existence of these subfamilies is in accordance with molecular based classification of Solanaceae (Martins & Barkman, 2005; Olmstead *et al*., 2008, 1999). Conversely, according to the traditional classification (Hunziker, 2001; D' Arcy, 1991), there are two subfamilies within Solanaceae; Solanoideae and Cestroideae. According to our data, the morphological characters that separate *Nicotiana tabacum* as representative of subfamily Nicotianoideae were the type of inflorescence that is axillary and terminal, compact corymboid panicle cyme and sessile leaves.

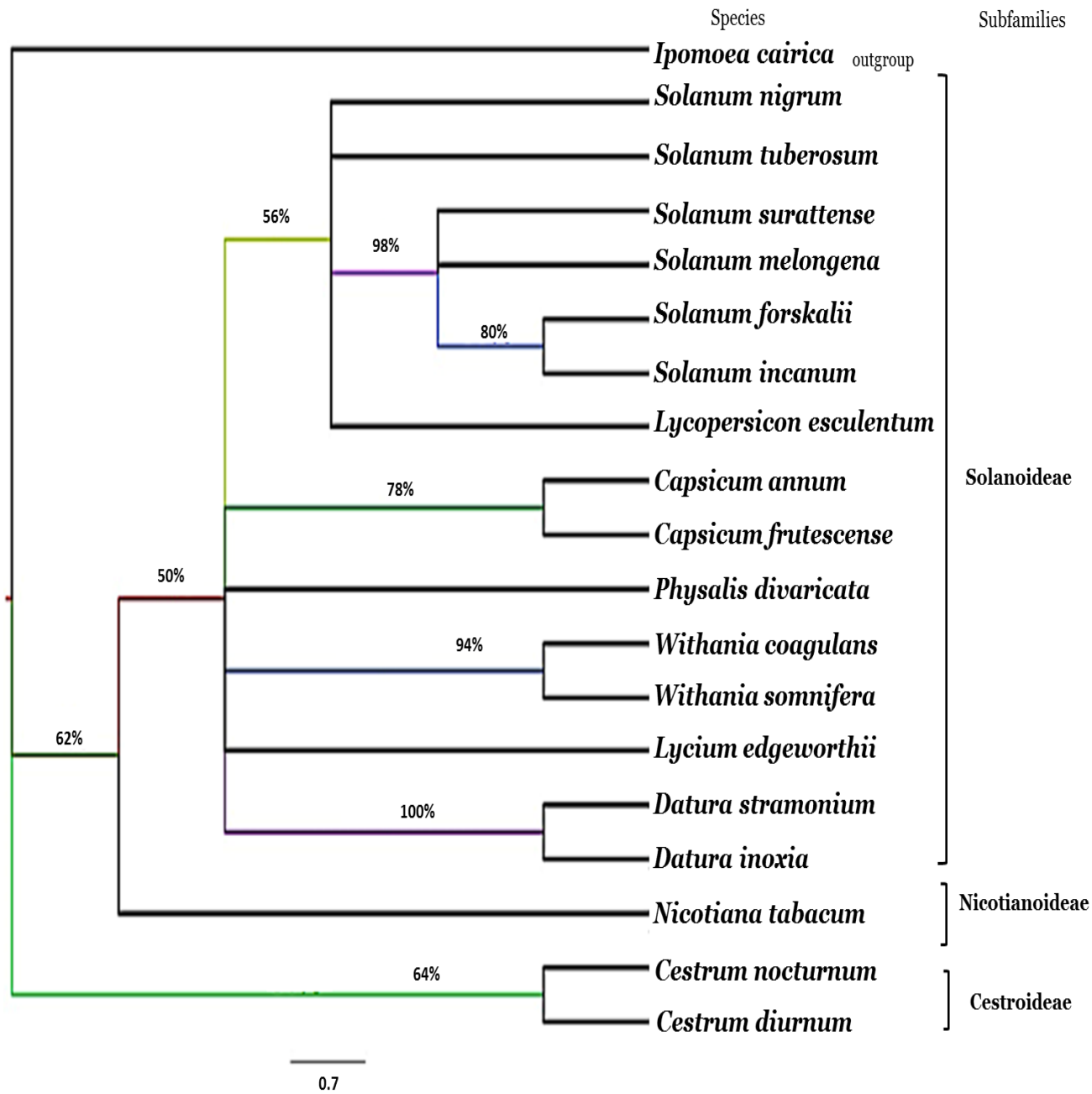

Fig. 1. Majority rule consensus most parsimonious tree derived from morphological characters for Solanaceae members. Numbers above branches indicate Bootstrap value (BS).

There was a discrepancy between morphological and molecular character based tree in resolving the tribal and intergeneric relationship. In morphological character based tree, the association among the tribes as well as genera was not clear whereas these associations were resolved in molecular tree. The reason may be the large number of characters, low level of homoplasy and relative unambiguity in scoring method of molecular data (Michael & Richard, 2000; James & Kenneth, 1994;) that may help in representing the clear picture of the relationships. Based on molecular data, within Solanoideae five tribes, Datureae, Solaneae, Lycieae, Capsiceae, and Physaleae were observed whereas morphological data also established existence of these tribes however, their association was not conclusive.

This study demonstrates that the spacer region *ndh*F-*rpl*32 is a good marker to resolve the association at subfamilial level within Solanaceae. The spacer region recognized the weak association between tribe Lycieae and Capsiceae whereas in our previous study (Jamil *et al*., 2014) which was based on other spacer region *atp*β-*rbc*L, the Lycieae was found to be associated with *Nicotiana tabacum*. Current study also took advantage of Bayesian Inference based on best substitution model, which in this case was calculated as Hasegawa, Kishino and Yano (HKY). Bayesian is the most prominent among all advanced phylogeny methods because it has computational advantages by estimating the probability that a given hypothesis is true, given the observations and model assumptions (Goloboff & Pol, 2005). However, the parsimony analysis is not based on nucleotide substitution model. Instead, it relies on the assumption that the minimum evolutionary changes occurred in the observed data (Albert, 2005). Some previous studies (Yani *et al*., 2016; Scarcelli *et al*., 2011; Miller *et al*., 2009; Timme *et al*., 2007) have shown the utility of *ndh*F-*rpl*32 in phylogenetics. When compared *ndh*F-*rpl*32 intergenic spacer with other coding (*rbc*L, *mat*K) and non-coding (*atp*β-*rbc*L) region, it was found to be highly variable sequence. Presence of high variability in this region indicates that *ndh*F-*rpl*32 is a good marker to infer phylogenetic relationship at lower taxonomic level.

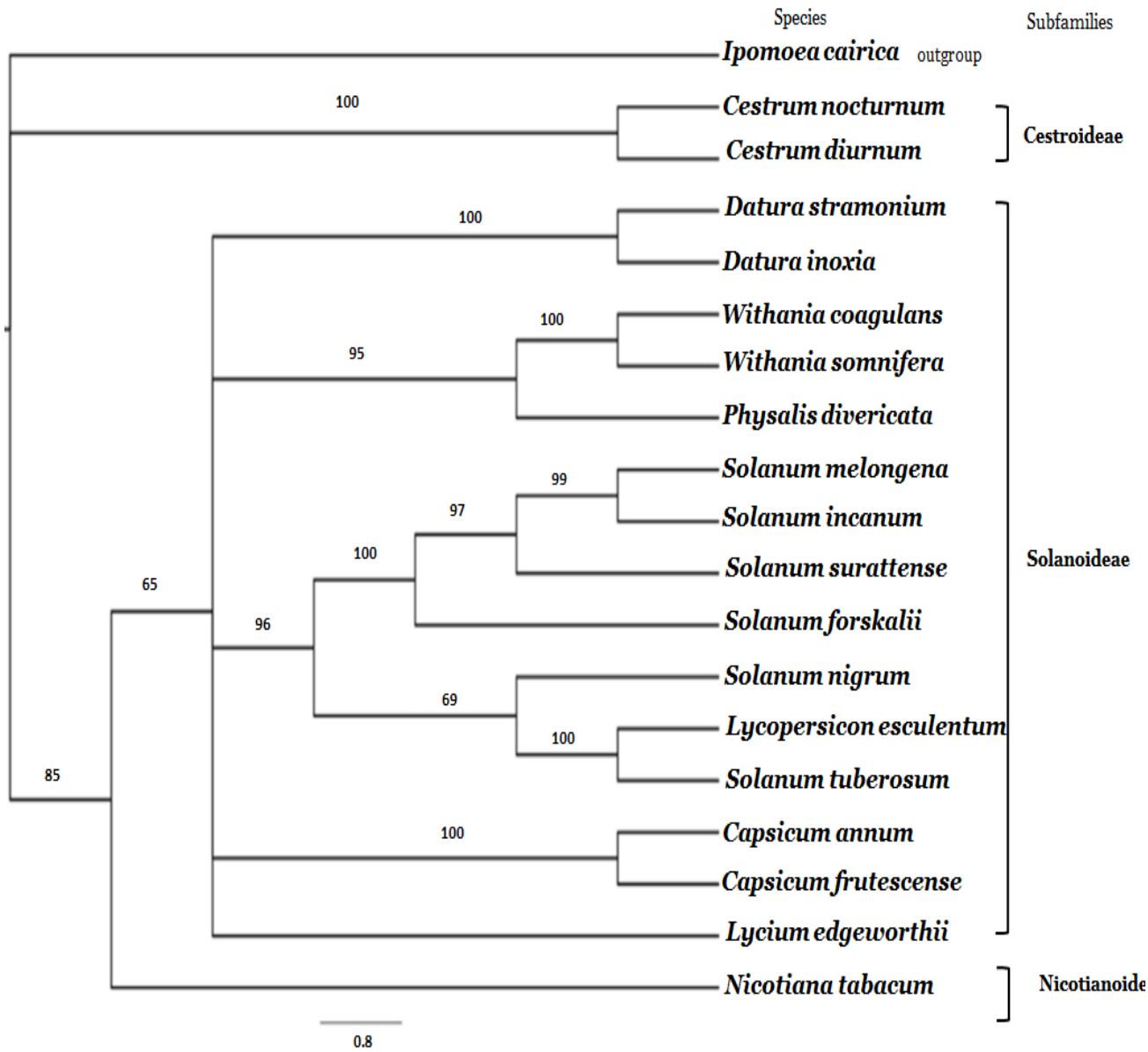

Fig. 2. Majority rule consensus most parsimonious tree derived from sequence data from chloroplast region, *ndh*F-*rpl*32 intergenic spacer. Numbers above branches indicate Bootstrap value (BS).

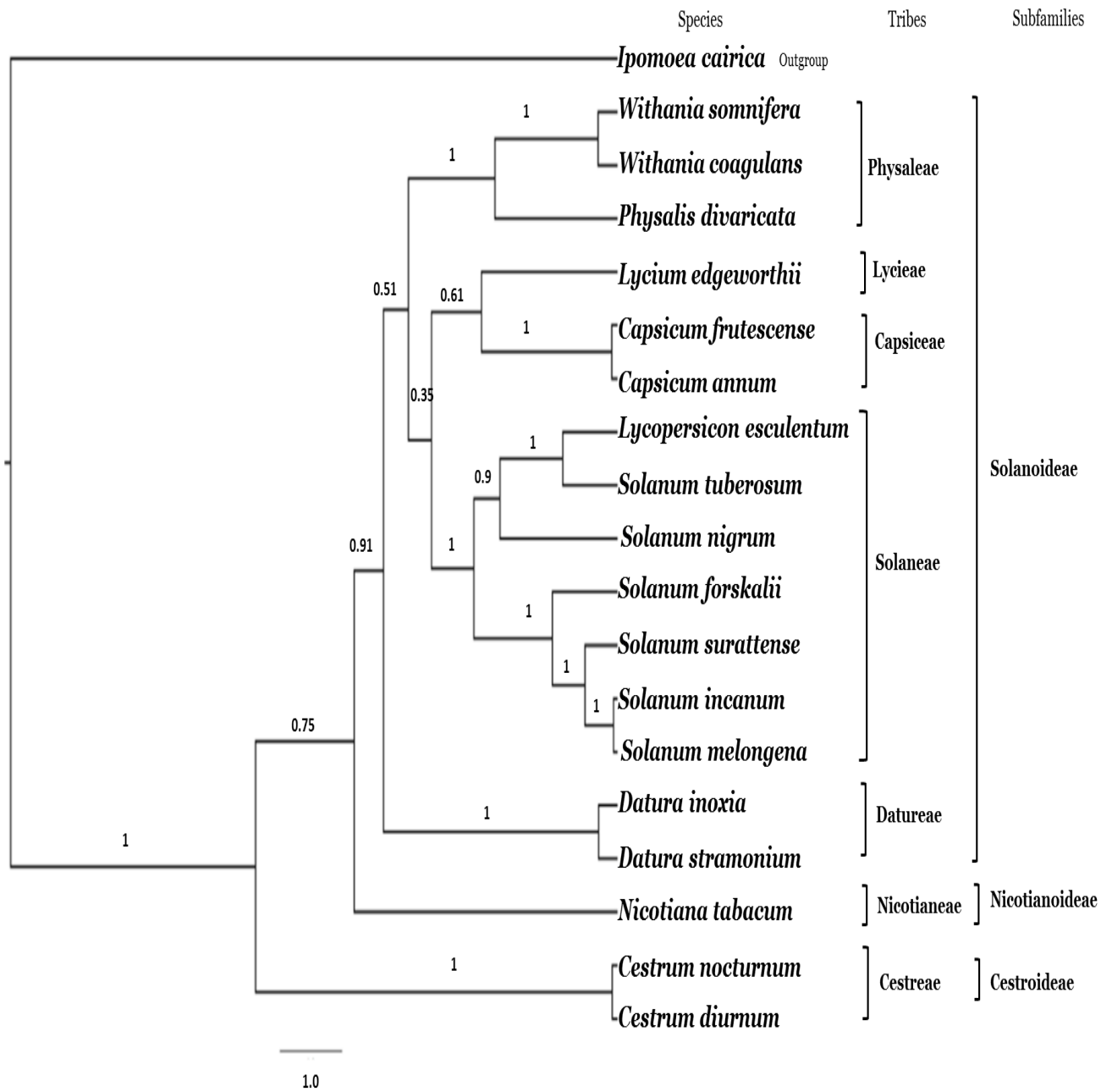

Fig. 3. Solanaceae phylogenetic tree based on Bayesian inference from *ndh*F-*rpl*32 intergenic spacer sequences using *Ipomoea cairica* as outgroup. Numbers above branches representing the posterior probabilities.

Table 2. Morphological and molecular characters information used in the reconstruction of tree.

	Total characters			Tree length	CI	RI
	Constant	Variable				
		Uninformative	Informative			
Morphology	10	9	50	253	0.58	0.53
Molecular	412	182	178	543	0.8	0.7

Table 3. Sequence length and variability in characters of different chloroplast regions.

Chloroplast regions	Sequence length	Constant characters (%)	Variable characters (%)	No. of parsimony informative sites (%)	No. of parsimony uninformative sites (%)
*ndh*F-*rpl*32	772	53.37	46.63	23.1	23.6
*atp*β-*rbc*L	879	58.70	41.30	14.1	27.2
*mat*K	910	76.70	23.30	8.6	14.7
*rbc*L	1447	85.90	14.10	3.9	10.2

Supplementary Table 1. Numerical keys assigned to morphological characters observed in Solanaceae.

S. #	Characters	Observed forms
1.	Life form	annual (1), biennial (2), perennial (3)
2.	Habit	herb (1), shrub (2)
3.	Prickles on plant	absent (0), present (1)
4.	Stem indument	pubescent (1), glabrous (2), sub-glabrous (3), glabrous to subglabrous (4),
5.	Type of hair on stem	absent (0), simple (1), stellate (2)
6.	Leaf type	simple (1), compound (2)
7.	Phylotaxy	alternate (1)
8.	Sessile/Petiolate	petiolate (1), sessile (2)
9.	Petiole indument	pubescent (1), glabrous (2)
10.	Lamina color	green (1), yellowish green (2), grayish green (3)
11.	Lamina shape	ovate (1), elliptic to ovate (2), elliptic -oblong (3), elliptic (4), elliptic ovate to elliptic lanceolate (5), elliptic -ovate (6), oblong to elliptic oblong (7), elliptic to lanceolate (8), ovate to orbicular (9)
12.	Lamina indument	pubescent (1), glabrous (2), subglabrous (3)
13.	Type of hairs on lamina	absent (0), simple (1), stellate (2), glandular (3)
14.	Leaf margin	entire (1), sinuate to dentate (2), sinuate to lobed (3), entire to sinuate (4), sinuate (5), dentate (6), sinuate-dentate to subentire (7), sinuate to deeply lobed (8)
15.	Leaf apex	acuminate (1), acute (2), obtuse (3)
16.	Leaf base	cuneate (1), cordate (2), subcordate (3), oblique (4), cordate to oblique (5), cuneate to oblique (6)
17.	Prickles on leave	absent (0), present (1)
18.	Stipules	absent (0), pseudo stipule (1)
19.	Type of inflorescence	axillary umbellate cyme (1), terminal paniculate cyme (2), solitary axillary (3), axil lary cyme (4), cluster of two or more (5), axillary and terminal compact penicle cyme (6), terminal and axillary recame (7), axillary (8), 1 to few flowered cyme (9)
20.	Number of flowers	3-8 (1), 2-4 (2), 1-5 (3), 2-6 (4), 2-20 (5), 1 (6), 2-5 (7), many (8), 1-4 (9)
21.	Flower color	white (1), yellow (2), greenish yellow (3), pink (4), purple (5)
22.	Flower shape	rotate (1), campanulate (2), funnel-shaped (3), tubular (4), Infundibuliform to tubular(5)
23.	Flower stalk	pedicellate (1), subsessile (2)
24.	Flower sex	bisexual (1), bisexual and male (2)
25.	Flower Bract	ebracteate (1), bracteates (2)
26.	Flower symmetry	actinomorphic (1), zygomorphic (2)
27.	Calyx aestivation	absent (0), valvate (1)
28.	Calyx fusion	gamosepalous (1), polysepalous (2)
29.	Calyx shape	absent (0), campanulate (1), tubular (2), tubular to funnel shaped (3)
30.	Calyx indument	pubescent (1), glabrous (2)
31.	Prickles on calyx	absent (0), present (1)
32.	Number of lobes	5 lobes (1), 5 sepals (2)
33.	Lobes shape	obtuse (1), lanceolate (2), triangular (3), ovoid (4), acute (5), ovate (6)

Supplementary Table 1. (Cont'd.).

S. #	Characters	Observed forms
34.	Lobe apex	obtuse (1), acute (2), acuminate (3), apiculate (4), obtuse to acute (5)
35.	Lobe margin	entire (1)
36.	Corolla aestivation	valvate (1)
37.	Corolla fusion	gamopetalous (1)
38.	Corolla indument	pubescent (1), subglabrous (2), glabrous (3)
39.	Prickles on corolla	absent (0), present (1)
40.	Number of corolla lobes	5 (1), 10 (2)
41.	Corolla lobe shape	triangular (1), obtuse (2)
42.	Corolla lobe apex	acute (1), obtuse (2), acuminate (3), obtuse or acute (4)
43.	Corolla lobe margin	entire (1)
44.	Number of stamens	5 (1)
45.	Anther arrangement	absent (0), connivent (1), non-connivent (2)
46.	Anther color	yellow (1), white (2), bluish (3), brown (4)
47.	Anther shape	elongated (1), ovate to lanceolate (2), oblong (3), ovoid (4), oblong to elliptic (5), ovate (6)
48.	Anther dehiscence type	by two apical pores (1), by longitudinal slit (2)
49.	Anther type based on theca	bithecous (1)
50.	Anther attachment	basifixed (1), dorsifixed (2)
51.	Anther adhesion	near the mouth of corolla tube (1), near the base of corolla tube (2)
52.	Stamen arrangement	alternate with petal (1)
53.	Stamen: exserted/ inserted	exserted (1), inserted (2), sub-inserted (3), sub-exserted (4)
54.	Style indument	pubescent at the base (1), glabrous (2), glabrescent (3)
55.	Stigma shape	capitate (1), sub-capitate (2), bilobed (3)
56.	Ovary texture	glabrous (1), glabrescent (2), pubescent (3)
57.	Ovary Bicarp/ monocarp	bicarpellary (1)
58.	syncarpous	syncarpous (1)
59.	Bi or tetra locular	bilocular (1), bilocular to tatralocular (2)
60.	Type of fruit	berry (1), capsule (2)
61.	Color of fruit	black (1), green (2), yellow (3), purple (4), red (5), green or red (6), orange (7), brown (8), white (9)
62.	Shape of fruit	globose (1), ovoid-subglobose or elongated (2), subglobose to ovoid (3), oblong to ovoid (4), subglobose (5)
63.	Fruit texture	pubescent (1), glabrous (2)
64.	Number of seeds	few (1), many (2)
65.	Fruit covering	necked (1), enclosed (2)
66.	Color of seed	dark brown (1), light yellow (2), brownish yellow (3), brown (4), black (5)
67.	Shape of seed	discoid (1), obvate to oblong (2), subreniform (3), reniform (4), ovoid (5), elliptical (6)
68.	Seed texture	reticulate-faveolate (1), reticulate (2), faveolate (3), reticulate-rugose (4), reticulate-undulate (5), ruminate (6), hairy (7)
69.	Spherical/ compressed	compressed (1), not flattened (2)

Conclusions

Molecular and morphological, both data established that Solanaceae is divided into three subfamilies: Solanoideae, Cestroideae and Nicotianoideae and five tribes within Solanoideae namely Solaneae, Capsiceae, Physaleae, Datureae and Lycieae. However, molecular data (*ndh*F-*rpl*32) more robustly resolved and support the inferred association. In future, there is a need to increase the number of species, use of coding and noncoding region of chloroplast and other genome such as nuclear and mitochondrial to get improved phylogenetic association.

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References

Albert, V.A. 2005. Parsimony and phylogenetics in the genomic age. In: *Parsimony, Phylogeny, and Genomics.* (Ed.): V.A. Albert. pp. 1-11. Oxford University Press, New York.

Altschull, S.F., W. Gish, W. Miller, E.W. Myers and D.J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol*., 215: 403-410.

Avise, J.C. 2012 Molecular markers, natural history and evolution. *Springer Science and Business Medi*a., pp. 8.

Bengtson, A., A.A. Anderberg and P.O. Karis. 2014. Phylogeny and evolution of the South African genus *Metalasia* (Asteraceae–Gnaphalieae) inferred from molecular and morphological data. *Bot. J. Linn. Soc.*, 174: 173-198.

Bohs, L. 2001. Revision of *Solanum* section *Cyphomandropsis* (Solanaceae). *Syst. Bot. Monogr*., 61: 1-85.

Bohs, L. and R. Olmstead. 2001. A reassessment of *Normania* and *Triguera* (Solanaceae). *Plant Sys. Evol*., 228: 33-48.

Bousquet, J., S. Strauss and P. Li. 1992. Complete congruence between morphological and *rbc*L based molecular phylogenies in birches and related species (Betulaceae). *Mol. Biol. Evol*., 9: 1076-1088.

Chao, C.T., B.H. Huang, J.T. Chang and P.C. Liao. 2020. Taxonomic notes on *Scutellaria taipeiensis* (Lamiaceae) from morphological and molecular data. *Phyto Key.*, 140: 33-45.

Corpet, F. 1988. Multiple sequence alignment with hierarchical clustering. *Nucl. Acids Res*., 16: 10881-10890.

D'Arcy, W.G. 1991. The Solanaceae since 1976, with a review of its biogeography. In: *Solanaceae III. Taxonomy, Chemistry, evolution*. (Eds.): Hawkes, J.G., R.N. Lester, M. Nee & N. Estrada, pp. 75-137. Royal Botanic Garddens, Kew and Linnean Soc, London.

Dong, W., J. Liu., J. Yu., L. Wang. and S. Zhou. 2012. Highly variable chloroplast markers for evaluating plant phylogeny at low taxonomic levels and for DNA barcoding. *PLoS One*., 7: 1-9.

Donoghue, M.J. and M.J. Sanderson. 1992. The suitability of molecular and morphological evidence in reconstructing plant phylogeny. In: *Molecular systematics in plants*, (Eds.): Soltis, P.S., D.E. Soltis & J.J. Doyle, pp. 340-368. Chapman and Hall, New York.

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

Drummond, A.J. and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *B.M.C. Evol. Biol.*, 7: 214.

Edmonds, J.M. 2012. Solanaceae In: Beentje H.J. editor. Flora of East Africa. Royal Botanic Gardens, Kew, UK, pp 1-240.

Estrada, E. and M. Martinez. 1999. *Physalis* L.(Solanoideae: Solaneae) and allied genera. In: *A morphology-based cladistic analysis. Solanaceae IV. Advances in biology and utilization*. (Eds.): Nee, M., D.E. Symon, R.N. Lester and J.P. Jessop, pp. 139-159, Royal Botanical Gardens, Kew, London.

Farris, J.S. 1989. The retention index and the rescaled consistency index. *Cladistics*., 5: 417-419.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39: 783-791.

Freire, S.E., M.A. Chemisquy, A.A. Anderberg, S.G. Beck, R.I. Meneses, B. Loeuille and E. Urtubey. 2015. The *Lucilia* group (Asteraceae, Gnaphalieae): phylogenetic and taxonomic considerations based on molecular and morphological evidence. *Plant Syst. Evol*., 301: 1227-1248.

Ghimire, B. and K. Heo. 2014. Cladistic analysis of Taxaceae s.l. *Plant. Syst. Evol*., 300: 217-223.

Givnish, T. and K. Sytsma. 1992. Chloroplast DNA restriction site data yield phylogenies with less homoplasy than analyses based on morphology or DNA sequences. *Amer. J. Bot.*, 79: 145.

Goloboff, P.A. and D. Pol. 2005. Parsimony and Bayesian phylogenetics. In: *Parsimony, Phylogeny, and Genomics,* (Ed.): Albert, V.A. pp. 148-159. Oxford University Press, New York.

Hidayat, T., D. Priyandoko., D.K. Islami and P.T. Wardiny. 2016. Molecular phylogenetic analysis of Indonesia Solanaceae based on DNA sequences of internal transcribed spacer region. *AIP Conference Proceeding.*, 1708: 030003-1–030003-5.

Hilu, K.W., C.M. Black and D. Oza. 2014. Impact of gene molecular evolution on phylogenetic reconstruction: A Case Study in the Rosids (Superorder Rosanae, Angiosperms). *PLoS One.,* 9: 1-12.

Hunziker, A. 2001. The genera of Solanaceae illustrated, arranged according to a new system, Gantner, ARG. Ruggell, 500.

James, F.S. and J.S. Kenneth. 1994. Molecules and morphology: congruence of data in *Columnea* (Gesneriaceae). *Plant Syst. Evol.*, 193: 37-52.

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

Jan, S.A., Z.K. Shinwari and M.A. Rabbani. 2016. Determining genetic divergence among *Brassica rapa* ecotypes through electrophoretic mobility of total seed proteins. *J. Anim. Plant Sci.*, 26: 1758-1764.

Jan, S.A., Z.K. Shinwari, M.A. Rabbani, H. Khurshid, M.I. Ibrahim, M. Adil and M. Ilyas. 2017. Comparison of electrophoretic protein profiles of *Brassica rapa* sub-species brown sarson through SDS-PAGE method. *Genetika.*, 49: 95-104.

Khan, I., Z.K. Shinwari, N.B. Zahra, S.A. Jan, S. Shinwari and S. Najeebullah. 2019. DNA barcoding and molecular systematics of selected species of family Acanthaceae. *Pak. J. Bot.*, 52(1): 205-212.

Kluge, A.G. and J.S. Farris. 1969. Quantitative phyletics and the evolution of *anurans*. *Syst Biol*., 18: 1-32.

Knapp, S. 2002. Tobacco to tomatoes: a phylogenetic perspective on fruit diversity in the Solanaceae. *J. Exp. Bot.*, 53: 2001-2022.

Knapp, S., L. Bohs., M. Nee and D.M. Spooner. 2004. Solanaceae a model for linking genomics with biodiversity. *Comp. Func. Genom.*, 5: 285-291.

Knapp, S., V. Persson and S. Blackmore. 1998. A phylogenetic conspectus of the tribe Juanulloeae. *Ann. Mol. Bot. Gard.*, 84: 67-89.

Marghali, S., N. Zitouna., M. Gharbi., H. Chennaoui-Kourda and N. Trifi-Farah. 2014. Morphological and molecular characters: Congruence or conflict in the phylogeny of *Sulla* species?. *Aust. J. Crop. Sci*., 8: 148-158.

Martins, T.R. and T.J. Barkman 2005. Reconstruction of Solanaceae phylogeny using the nuclear gene SAMT. *Syst. Bot*., 30: 435-447.

Meireles, J.E., A.M.G.A. Tozzi and M. Lavin. 2014. A phylogenetic analysis of molecular and morphological data reveals a paraphyletic *Poecilanthe* (Leguminosae, Papilionoideae). *Syst. Bot*., 39: 1142-1149.

Michael, J.D. and H.R. Richard. 2000. Homoplasy and developmental constraint: A model and an example from plants. *Amer Zool.*, 40: 759-769.

Miller, J.S., A. Kamath and R.A. Levin. 2009. Do multiple tortoises equal a hare? The utility of nine non-coding plastid regions for species-level phylogenetics in tribe Lycieae (Solanaceae). *Syst. Bot*., 34: 796-804.

Nasir, J. 1985. Solanaceae In: *Flora of Pakistan*, (Eds.): Ali, S.I. and E. Nasir. 168: 1-61. Pak. Agric. Research Council, Islamabad.

Niklas, K.J. and W.L. Crepet. 2020. Morphological (and not anatomical or reproductive) features define early vascular plant phylogenetic relationships. *Amer. J. Bot*., 107: 477-488.

Olmstead, R.G., J.A.,Sweere., R.E. Spangler., L. Bohs and J.D. Palmer. 1999. Advances in Biology and Utilization phylogeny and provisional classification of the Solanaceae based on chloroplast DNA, pp. 111-137. In: (Eds.): Nee, M., D. Symon, R.N. Lester & J. Jessop. Solanaceae IV, Royal Botanic Gardens, Kew, London.

Olmstead, R.G., L. Bohs., H.A. Migid., E. Santiago-Valentin., V.F. Garcia and S.M. Collier. 2008. A molecular phylogeny of the Solanaceae. *Taxon*, 57: 1159-1181.

Pakso, M.Y., E. Sevindik and F. Coşkun. 2016. Phylogenetic relationships based on morphological and anatomical characters on *Ricotia* L. genus (Brassicaceae) growing in Turkey. *Bangl. J. Bot.*, 45: 971-977.

Perveen, A. and M. Qaiser. 2007. Pollen morphology of family Solanaceae from Pakistan. *Pak. J. Bot*., 39: 2243-2256.

Posada, D. 2008. jModelTest phylogenetic model averaging. *Mol. Biol. Evol*., 25: 1253-1256.

Rambaut, A. 2012. FigTree, version 1.4.0. available online from http://tree.bio.ed.ac.uk/software

Rozen, S. and H.J. Skaletsky. 1998. Primer 3. Code available at http://www-genome.wi.mit.edu/genome_software/ other/ primer3.html.

Sanderson, M.J. and M.J. Donoghue. 1989. Patterns of variation in levels of homoplasy. *Evolution*, 43: 1781-1795.

Sardar, A., A.H. Shah, B.H. Shah, Z.K. Shinwari, S.A. Jan, U. Khan and M.A. Nawaz. 2021. Molecular analyses of selected tea genotypes irradiated with gamma rays. *Pak. J. Bot*., 53: 1737-1742.

Särkinen, T., L. Bohs, R.G. Olmstead and S. Knapp. 2013. A phylogenetic framework for evolutionary study of the nightshades (Solanaceae): A dated 1000-tip tree. B.M.C. *Evol. Biol*., 13: 214-228.

Scarcelli, N., A. Barnaud, W. Eiserhardt, U.A. Treier, M. Seveno, A. d'Anfray, Y. Vigouroux and J.C. Pintaud. 2011. A set of 100 chloroplast DNA primer pairs to study population genetics and phylogeny in monocotyledons. *PLoS One*., 6: 1-8.

Scotland, R.W., R.G. Olmstead and J.R. Bennett. 2003. Phylogeny reconstruction: the role of morphology. *Syst Biol*., 52: 539-548.

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.

Subrahmanyam, N.S. 2009. Modern Plant Taxonomy 1E. Vikas Publishing House Pvt Ltd., New Delhi, 23pp.

Swofford, D. 2002. PAUP*: phylogenetic analysis using parsimony (* and other methods) Sinauer. Sunderland, Massachusetts, USA.

Timme, R.E., J.V. Kuehl., J.L. Boore and R.K. Jansen. 2007. Comparative analysis of the *Lactuca* and *Helianthus* (Asteraceae) plastid genomes: identification of divergent regions and categorization of shared repeats. *Amer. J. Bot.*, 94: 302-313.

Wiens, J.J. 2004. The role of morphological data in phylogeny reconstruction. *Syst. Biol*., 53: 653-661.

Witt, C.C. 2004. Rates of molecular evolution and their application to Neotropical avian biogeography: Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in The Department of Biological Sciences by Christopher C. Witt BA, College of the Atlantic

Wright, A.M. and D.M. Hillis. 2014. Bayesian analysis using a simple likelihood model outperforms parsimony for estimation of phylogeny from discrete morphological data. *PLoS One*., 9: 1-6.

Yani, Hu., Z. Zongwen., W. Bin., G. Jia and Li. Yanqin. 2016. Genetic relationships of buckwheat species based on the sequence analysis of ITS and *ndh*F-*rpl*32. *Biodiv. Sci.*, 24: 296-303.

Yousaf, Z., S. Masood., Z.K. Shinwari., M.A. Khan and A. Rabani. 2006. Evaluation of taxonomic status of medicinal species of the genus *Solanum* and *Capsicum* based on poly acrylamide gel electrophoresis. *Pak. J. Bot.*, 38: 99-106.

Yousaf, Z., S. Masood., Z.K. Shinwari., M.A. Khan and A. Rabani. 2008. Evaluation of taxonomic status of medicinal Species of the genus *Hyoscyamous*, *Withania*, *Atropa* and *Datura* based on poly acrylamide gel electrophoresis. *Pak. J. Bot*., 40: 2289-2297.

Yousaf, Z., Z.K. Shinwari and M.A. Khan. 2010. Phenetic analysis of medicinally important species of the genus *Solanum* from Pakistan. *Pak. J. Bot.*, 42: 1827-1833.

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