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STATUS OF *RESEDA PENTAGYNA* ABDALLAH & A.G. MILLER (RESEDACEAE) INFERRED FROM COMBINED NUCLEAR RIBOSOMAL AND CHLOROPLAST SEQUENCE DATA

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Abstract

The present study focuses on the status of *Reseda pentagyna* Abdallah & A.G. Miller (Resedaceae). The internal transcribed spacer (ITS) region of nuclear ribosomal DNA and chloroplast *trn*L-F gene of the questioned species were sequenced. The Basic Local Alignment Search Tool (BLAST) search showed maximum identity with *R. stenostachya.* The parsimony analysis of ITS, *trn*L-F and combined sequences data analyses revealed grouping of *Reseda* species consistent with established taxonomic sections of the genus, *R. pentagyna* showed proximity with *R. stenostachya* (100% bootstrap support), nested within the clade of section *Reseda.*

Introduction

The Resedaceae include six genera (i.e. *Caylusea* A. St.-Hil, *Ochradenus* Delile, *Oligomeris* Cambess., *Randonia* Coss., *Reseda* L. and *Sesamoides* Ortega) with approximately 85 species, and are widely distributed in the Old World, with a major center of species diversity in the Mediterranean basin (Martín-Bravo *et al*., 2007). The members of the family Resedaceae has been traditionally considered closely related to Capparaceae and Brassicaceae; however, the Angiosperm Phylogeny Group placed it under the order Brassicales (APG III, 2009).

The genus *Reseda* consists of approximately 65 species, mostly restricted to the Mediterranean basin, while four of them (i.e. *Reseda alba* L., *R. lutea* L., *R. luteola* L. and *R. phyteuma* L.) are distributed throughout the world (Martín-Bravo *et al*., 2007). The genus *Reseda* in Saudi Arabia is represented by seven species, *viz. R. alba*, *R. arabica* Boiss., *R. aucheri* Boiss., *R. lutea*, *R. muricata* C. Presl, *R. pentagyna* Abdallah & A.G. Miller and *R. sphenocleoides* Deflers (Chaudhary, 1999). Among these, *R. pentagyna* is endemic to Saudi Arabia, and reported to occur in Northern Hijaz mountain area, Wadi Sawawin and Tabuk of north western Saudi Arabia (Miller and Nyberg, 1994; Chaudhary, 1999; Llewellyn *et al*., 2010). *R. stenostachya* is the most closely allied taxon to the endemic *R. pentagyna* which differs from the latter by presence of only 3-4 toothed capsules as compared to the 5-6 toothed capsules in the latter.

In the last two decades, the internal transcribed spacer sequences of nuclear ribosomal DNA has gained much attention, not only because of its efficacy in carrying out phylogeny of the plants at lower taxonomic level, but also to be considered as the most trusted markers available for the DNA barcoding of the plants. Even after facing criticism of its utility, this marker stands parallel

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to the smartest genes available for the molecular phylogeny and plant DNA barcoding. Since the intrigued morphological similarities observed in between *R. pentagyna* and *R. stenostachya* (Miller and Nyberg, 1994; Chaudhary, 1999) we planned to carry out molecular phylogenetic analysis of internal transcribed spacer sequences (ITS) of nuclear ribosomal DNA and *trn*L-F sequences to confirm the species status of *R. pentagyna*.

Materials and Methods

The leaf material of *R. pentagyna* was collected from Wadi Sirr area of Saudi Arabia, and the taxonomic identification was confirmed through consultation of Flora of Saudi Arabia (Chaudhary, 1999) and protologue (Miller and Nyberg, 1994). Total genomic DNA was extracted using the DNeasy Plant Mini kit (QIAGEN, Valencia, CA, USA). The nuclear (internal transcribed spacer sequences of nuclear ribosomal DNA), and plastid (*trn*L-F) genes were amplified using AccuPower HF PCR PreMix (Bioneer, Daejeon, South Korea). The standard primers ITS (White *et al*., 1990) and *trn*L-*trn*L-F (Taberlet *et al*., 1991) were used for amplification and cycle sequencing. The amplified products were purified using PCR purification kit (SolGent, Daejeon, South Korea) prior to sequencing. The purified amplified products were sequenced using ABI PRISM 3730XL (Perkin-Elmer/Applied Biosystem, USA) following manufacturer's protocol. Each sample was sequenced in the sense and anti-sense direction. The nucleotide sequences of both the DNA strands (sense and anti-sense) were obtained and analyzed using Sequence Navigator (Perkin-Elmer/Applied Biosystems) to ensure accuracy of the base pair sequence.

For the molecular phylogenetic analysis, ITS and *trn*L-F sequences of a total of 36 related species of *Reseda* (comprising representative from all six sections i.e. *Glaucoreseda*, *Leucoreseda*, *Luteola*, *Neoreseda*, *Phyteuma* and *Reseda* as recognized by Martín-Bravo *et al*., 2007) were retrieved from GenBank (Table 1). According to Martín-Bravo *et al.* (2007) *Oligomeris* arose within the ranks of *Reseda*; hence, sequences of *Oligomeris* were retrieved from GenBank, and were used as outgroup in the phylogenetic analyses (Table 1). Sequence alignments were performed using Clustal X, version 1.81 (Thompson *et al*., 1997). Sequence alignments were subsequently adjusted manually using BioEdit (Hall, 1999). Gaps were treated as missing data in phylogenetic analyses. The voucher specimen (Chaudhary *et al.* 13704) of sequenced plant accession deposited at National Herbarium (RIY) of Saudi Arabia; and the generated sequences submitted in GenBank (Table 1). Maximum parsimony (MP) analysis was performed using PAUP* 4.0b10 (Swofford, 2002).

Results and Discussion

The combined length of ITS region (ITS1-5.8S-ITS2) in *Reseda pentagyna* was 634 bp. The ITS1 region was 261 bp (GC content 61%), the 5.8S gene was 162 bp (GC content 56%), and the ITS2 region was 211 bp (GC content 63%). The *trn*L-F sequence in *R. pentagyna* was 777 bp (GC content 33%). BLAST search of ITS sequence of *R. pentagyna* showed maximum identity (99%) with *R. stenostachya* followed by *R. aucheri* and *R. ellenbeckii* (95%), while *trn*L-F sequence showed maximum identity (100%) with *R. stenostachya* followed by *R. alphonsi*, *R. buhseana*, *R. gilgiana* and *R. sessilifolia* (97%). ITS sequence of *R. pentagyna* differs from *R. stenostachya* at position 67 and 75 in alignment, however, in *trn*L-F sequences, no base pair difference was observed in between sequence of *R. pentagyna* and *R. stenostachya*. Sequence characteristics and statistics of maximum parsimony trees derived from analyses of ITS, *trn*L-F and combined data are summarized in Table 2. The maximum parsimony tree derived from analysis of ITS and *trn*L-F sequence revealed comparatively week bootstrap support than combined analysis; and therefore, only the maximum parsimony trees topology derived from analysis of combined sequence data is discussed here.

Table 1. Plant accessions used for the molecular phylogenetic analysis of *Reseda pentagyna.*

Taxa	GenBank Accession No.	
	ITS	*trn*L-F
Ingroup		
Sect. *Glaucoreseda*		
1. *Reseda battandieri* Pit.	DQ987183	DQ987045
2. *R. complicata* Bory	DQ987172	DQ987046
3. *R. glauca* L.	DQ987182	DQ987040
4. *R. gredensis* (Cutanda & Willk.) Müll.-Arg.	DQ987174	DQ987047
5. *R. virgata* Boiss. & Reut.	DQ987177	DQ987048
Sect. *Luteola*		
6. *R. luteola* L.	DQ987187	DQ987050
Sect. *Leucoreseda* Subsect. *Leucoreseda*		
7. *R. alba* L.	DQ987198	DQ987053
8. *R. attenuata* Ball	DQ987201	DQ987057
9. *R. gayana* Boiss.	DQ987205	DQ987055
10. *R. undata* L.	DQ987203	DQ987056
11. *R.valentina* Pau	DQ987207	DQ987059
Sect. *Leucoreseda* Subsect. *Erythroreseda*		
12. *R. suffruticosa* Loefl.	DQ987210	DQ987062
Sect. *Neoreseda*		
13. *R. ellenbeckii* Perkins	DQ987110	DQ986998
14. *R. telephiifolia* (Chiov.) Abdallah & de Wit	DQ987128	DQ986994
Sect. *Phyteuma*		
15. *R. alopecuros* Boiss.	DQ987139	DQ987028
16. *R. arabica* Boiss.	DQ987132	DQ987029
17. *R. collina* Müll.-Arg.	DQ987136	DQ987031
18. *R. diffusa* Ball	DQ987141	DQ987033
19. *R. inodora* Rchb.	DQ987142	DQ987030
20. *R. odorata* L.	DQ987133	DQ987026
21. *R. orientalis* (Müll.-Arg.) Boiss.	DQ987137	DQ987025
22. *R. phyteuma* L.	DQ987146	DQ987032
Sect. *Reseda*		
23. *R. alphonsi* Müll.-Arg.	DQ987108	DQ987005
24. *R. amblycarpa* Fresen.	DQ987125	DQ987001
25. *R. aucheri* Boiss.	DQ987123	DQ986989
26. *R. buhseana* Müll.-Arg.	DQ987119	DQ987004
27. *R. crystallina* Webb & Berthel.	DQ987088	DQ987021
28. *R. gilgiana* Perkins	DQ987114	DQ986999
29. *R. lanceolata* Lag.	DQ987099	DQ987015
30. *R. lutea* L.	DQ987094	DQ987018
31. *R. pentagyna* Abdallah & Miller	JX867260	JX867261
32. *R. sessilifolia* Thulin	DQ987127	DQ986995
33. *R. sphenocleoides* Deflers	DQ987117	DQ986993
34. *R. stenostachya* Boiss.	DQ987156	DQ987007
35. *R. stricta* Pers.	DQ987103	DQ987013
36. *R. urnigera* Webb	DQ987098	DQ987014
37. *R. viridis* Balf. f.	DQ987130	DQ986996
Outgroup		
38. *Oligomeris dipetala* (Aiton) Turcz.	DQ987168	DQ987037
39. *O. dregeana* (Müll. Arg.) Müll.-Arg.	DQ987166	DQ987038
40. *O. linifolia* (Vahl) J.F. Macbr.	DQ987165	DQ987039

Table 2. Summary of sequence characteristics and MP trees derived from analyses of ITS, *trn*L-F and combined data.

Characters	ITS	*trn*L-F	Combined data
Number of taxa included in analysis (including outgroup)	40	40	40
Sequence characteristics			
Length of sequenced	627-639	698-785	1325-1424
Aligned length	644	955	1622
Parsimony informative	92	146	433
Tree characteristics			
Number of trees	334	323	1299
Length	339	327	1305
CI (Consistency Index)	0.643	0.832	0.656
RI (Retention Index)	0.885	0.926	0.860
RC (Rescaled Consistency Index)	0.569	0.770	0.564
HI (Homoplasy Index)	0.478	0.318	0.446

The bootstrap strict consensus tree resulted from combined sequence data analysis has been shown in Fig. 1. The study revealed the grouping of *Reseda* species according to previously recognized taxonomic sections, which is consistent with earlier report (Martín-Bravo *et al*., 2007). Moreover, *R. pentagyna* nested within the clade of the section *Reseda*, and showed proximity (100% bootstrap support) with morphologically similar *R. stenostachya*. The ITS sequence of *R. pentagyna* (which was described based on 5-6 toothed capsule characters) differs from morphologically allied *R. stenostachya* (3-4 toothed capsule) at aligned position 67 (C in *R. pentagyna* but missing nucleotide in *R. stenostachya*) and 75 (C in *R. pentagyna*, T in *R. stenostachya*) possibly due to nucleotide polymorphism, a known features of ITS sequences of nrDNA.

Bentham and Hooker (1862) reported *Reseda* as a polymorphic genus with not more than 30 existing species. Latter, Abdallah and de Wit (1978) updated the list with some addition, and emphasized the need of experimental taxonomical research to get a strong support for the delimitation of species. Muller (1864) also described the variations in the morphology of leaf blades of *Reseda* that might be arranged in various manners and could be entire, crenate to ternately or pinnately (or rarely bi-pinnately) lobed. The occurrence of brachycarpous or macrocarpous capsules in *Reseda* is a known feature (Muller, 1864). Under various ecological conditions, plants may show certain morphological changes, *viz. R. lutea* shows change in the proximity of the veins in the lamina (Abdallah and de Wit, 1978). Further, the emergence of indumentums depends more or less on the moisture content present in the plant. In dry condition, these hairs can shrink, flatten or curl; while in wet conditions, they appear as blisters, or a scabrid, or muricated surface. As variations in fruit size within the same species usually do occur, therefore it cannot be taken as a strong taxonomic character for species level delimitation (Donald, 1988); and thus, the wide degree of variation in quantitative fruit-spine characters limits their use taxonomically. The proximity of questioned sequenced material with *R. stenostachya* in the MPTs indicates the quantitative differences of tooth characters or the variable trait which limits its use in species delimitation; therefore, we herein propose the merger of *R. pentagyna* into *R. stenostachya*.

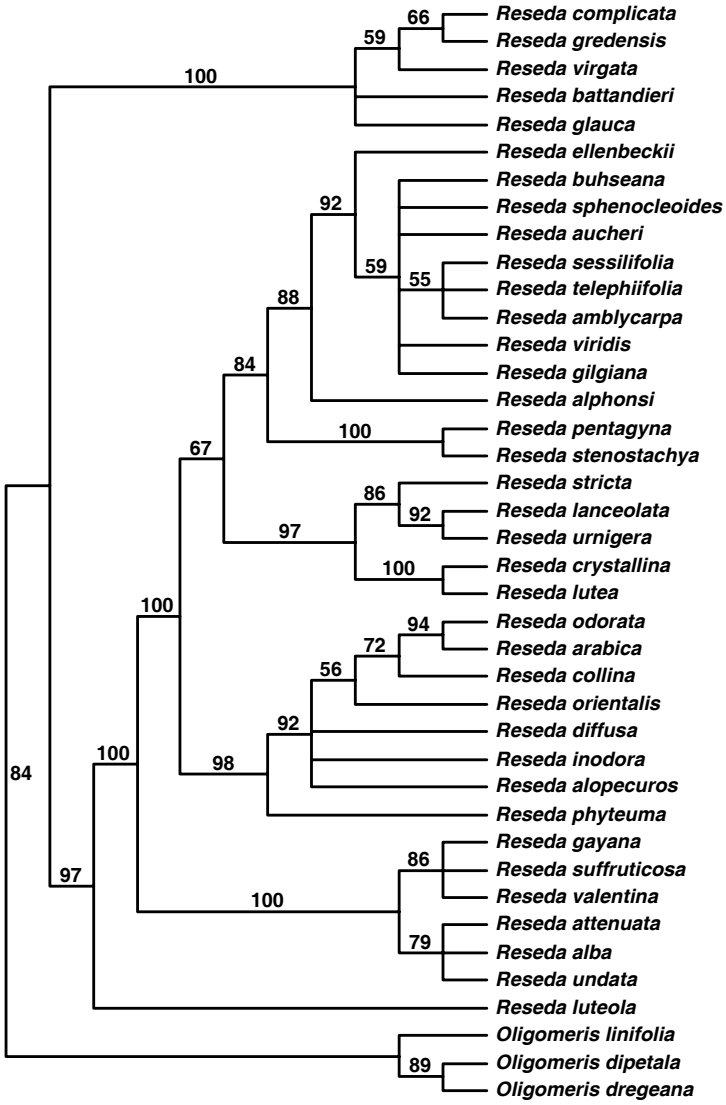

Fig. 1. Bootstrap strict consensus tree inferred from combined sequence data analysis of internal transcribed spacer (ITS) sequence of nuclear ribosomal DNA and *trn*L-F region. The Bootstrap strict consensus tree of 1299 maximally parsimonious trees (MPTs) with a total length of 1305 steps, a consistency index (CI) of 0.656, a homoplasy index (HI) of 0.446, rescaled consistency index (RC) of 0.564 and a retention index (RI) of 0.860. Bootstrap values greater than 50% in 1000 bootstrap replicates are shown above lines.

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