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MOLECULAR PHYLOGENY OF SAUDI ARABIAN *TETRAENA* MAXIM. AND *ZYGOPHYLLUM* L. (ZYGOPHYLLACEAE) BASED ON PLASTID DNA SEQUENCES

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Abstract

In order to provide a basis for better understanding of phylogenetic relationships of Saudi Arabian *Tetraena* Maxim. and *Zygophyllum* L., 44 specimens representing seven taxa, were reconstructed based on chloroplast DNA data of *rbc*L and *trn*L-F. The combined chloroplast (*rbc*L and *trn*L-F) contributed more phylogenetically informative characters than in individual regions. Phylogenetic analysis of the combined chloroplast (*rbc*L and *trn*L-F) and in individual regions based on both of Maximum Parsimony and Bayesian criteria showed that the Saudi Arabian species of *Tetraena* and *Zygophyllum* were monophyletic. *Zygophyllum fabag* L. was nested in one clade with *Z. xanthoxylum* (Bunge) Engl. (Asian species), and all taxa of *Tetraena* were distributed in other clades.

Introduction

The widespread family Zygophyllaceae includes five subfamilies *viz*. Zygophylloideae, Tribuloideae, Seetzenioideae, Larreoideae and Morkillioideae (Sheahan and Chase, 2000; Beier *et al*., 2003; Bellstedt *et al*., 2008). The *Zygophyllum* L. and *Tetraena* Maxim. belong to Zygophylloideae along with *Fagonia* L., *Augea* Thunb., *Roepera* (A. Juss.) Engl. and *Melocarpum* (Engl.) Beier & Thulin (Beier *et al*., 2003; Bellstedt *et al*., 2008).

The only detailed examination of the systematics of *Zygophyllum* and *Tetraena* taxa have focused on morphological and anatomical characters (El-Hadidi, 1977, 1980; Boulos, 1978; Engler, 1931; Hosny, 1988; Hussein *et al.*, 2009; Ma and Zhang, 1990; Takhtajan, 1987; Thulin, 1993; Van Huyssteen, 1937; Van Zyl, 2000). In contrast, a few studies have used molecular markers to the phylogenetic relationships of the intergeneric of Zygophyllaceae (Sheahan and Chase, 1996, 2000; Beier *et al*., 2003) or to infer the relationships within the genus *Zygophyllum* (Bellstedt *et al*., 2008; Hammad and Qari, 2010). Sheahan and Chase (1996) studied the phylogenetic relationships of Zygophyllaceae based on morphology, anatomy and the *rbc*L DNA sequence. Sheahan and Chase (2000) investigated the phylogenetic relationships of 36 taxa of Zygophyllaceae including 15 species of *Zygophyllum* L. from Africa, Australia, and south western Asia using nucleotide sequences of the plastid gene *rbc*L and non-coding *trn*L-F and found *Zygophyllum* as polyphyletic. They showed that the *Zygophyllum fabago* L. (the type species of *Zygophyllum*) nested with another Asian species *Z. xanthoxylum* (Bunge) Engl., whereas *Z. simplex* L. placed in a strong clade with the genus *Tetraena* and other *Zygophyllum* species, *viz. Z. album* L. f., *Z. coccineum* L., *Z. cylindrifolium* Schinz and *Z. decumbens* Delile (the last three are

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distributed in Saudi Arabia). The study indicated that *Tetraena* is nested within the large and variable *Zygophyllum* and reported that the *Z. simplex* is sister to *Tetraena.*

Beier *et al.* (2003) investigated the phylogenetic relationships of Zygophyllaceae using *trn*L plastid DNA sequences and morphological data for 43 species of Zygophylloideae including the genera *Zygophyllum, Fagonia*, *Augea* and *Tetraena* which represent most of the morphological and geographical variations in the subfamily Zygophylloideae. They reported that the subfamily Zygophylloideae is monophyletic, whereas the genus *Zygophyllum* is paraphyletic, since this genus was spontaneously distributed with the genera of *Augea*, *Tetraena* and *Fagonia*. Based on the results of this study, Beier *et al.* (2003) produced a new classification for genera *Tetraena* and *Zygophyllum*, and transferred 35 species from genus *Zygophyllum* to genus *Tetraena* as new combinations. Later, Bellstedt *et al.* (2008) assessed the phylogenetic relationships of 53 species of *Zygophyllum* in southern Africa employing the sequences of *rbc*L and *trn*L-F regions. They included the published sequences of the same genes for other species from different regions and the results supported the subdivision of the genus *Zygophyllum* into subgenera *Agrophyllum* and *Zygophyllum*. They found relatively similar results by conducting the same methods to study the relationships of *Zygophyllum* and *Tetraena* species (cpDNA sequences) and similar morphological characteristics (i.e. capsule dehiscence, seed attachment and the presence of spiral threads in the seed mucilage). These species are known from Africa and Asia. Bellestedt *et al.* (2008) did not agree with Beier *et al*. (2003) for the new classification of *Tetraena* and *Zygophyllum.* However, many authors agreed with this transfer and used the combinations proposed by Beier *et al*. (2003) as valid in their works, including Alzahrani (2017), Alzahrani and Albokhari (2017a, b), Azevedo (2014), Ghazanfar and Osborne (2015), Louhaichi *et al*. (2011), Mosti *et al.* (2012), Norton *et al.* (2009), Sakkir *et al*. (2012).

Tetraena is represented in Saudi Arabia by six species, two subspecies and six varieties, while genus *Zygophyllum* is represented by a single species, namely *Z. fabago* (Beier *et al*., 2003; Alzahrani, 2017; Alzahrani and Albokhari, 2017a, b;). Saudi Arabian *Tetraena* and *Zygophyllum* have never been included in the published phylogenetic studies. The only two studies have used RAPD markers data to study genetic variation among and within populations of some Saudi Arabian *Zygophyllum* taxa (Al-Arjany, 2011; Hammad and Qari, 2010). Hammad and Qari (2010) studied the genetic diversity of 12 populations of *Zygophyllum coccineum*, *Z. album* and *Z. aegyptium* A.I. Hosny which were collected from various locations in Egypt and Saudi Arabia using RAPD markers employing five random primers. They found that *Zygophyllum coccineum* revealed higher levels of genetic variation and more unique alleles than the other species and *Z. aegyptium* is genetically closely related to *Z. album* Later, Al-Arjany (2011) studied the molecular taxonomy of *Zygophyllum simplex* and *Z. migahidii* using of random PCR (RAPD) technology to analyse phylogenetic relationships between both species and found that these species are closely allied to each other. In the present study, phylogenetic relationships of 43 individual specimens of Saudi Arabian *Tetraena* and *Zygophyllum* species were reconstructed using combined DNA sequences data from the *rbc*L and the *trn*L-F regions.

Materials and Methods

Selection of ingroup and outgroup

Leaf material for 37 individual specimens of Saudi Arabian *Tetraena* representing six taxa were sampled in the field and from herbarium specimens listed in Tables 1 and 2. Collected specimens were deposited in KAUH (King Abdulaziz University Herbarium, Jeddah, Saudi Arabia). Sequenced data of the 10 *Tetraena* and *Zygophyllum* sequenced by Bellstedt *et al.* (2008) for the two regions (*rbc*L and *trn*L-F) were obtained from GenBank (Table 3). Three sequences of

Table 1. Accessions of Saudi Arabian *Tetraena* collected and used for the molecular study.

Sl. No.	Vr. No.	Taxa	Location	Coordinates	GenBank accession *rbc*L	GenBank accession *trn*L-F
1.	109	*Tetraena propinqua* ssp. *propinqua*	Shuaibah	20° 52’ 23" N 39° 22’ 16" E	MG664288	MG664319
2.	110	*T. alba* var. *alba*	Shuaibah	20° 52’ 23" N 39° 22’ 16" E	MG664308	MG664339
3.	111	*T. coccinea*	Shuaibah	20° 52’ 23" N 39° 22’ 16" E	MG664302	MG664333
4.	117	*T. coccinea*	North of Jeddah	21° 50’ 23" N 39° 07’ 05" E	MG664303	MG664334
5.	120	*T. coccinea*	South of Alleith	19° 56’ 15" N 40° 31’ 17" E	MG664304	MG664335
6.	128	*T. coccinea*	Between Rabigh and Yanbu	23° 59’ 20" N 38° 16’ 02" E	MG664305	MG664336
7.	130	*T. coccinea*	Umluj	24° 33’ 20" N 37° 25’ 23" E	MG664306	MG664337
8.	133	*T. propinqua*ssp.*propinqua*	Umluj	24° 59’ 05" N 37° 17’ 09" E	MG664289	MG664320
9.	137	*T. propinqua*ssp. *propinqua*	Umluj	24° 58’ 19" N 37° 17’ 03" E	MG664290	MG664321
10.	138	*T. alba* var. *arabica*	Umluj	24° 58’ 19" N 37° 17’ 03" E	MG664310	MG664341
11.	139	*T. alba* var. *alba*	Umluj	24° 58’ 19" N 37° 17’ 03" E	MG664309	MG664340
12.	142	*T. decumbens*	30 km South of Umluj	24° 45’ 06" N 37° 19’ 56" E	MG664307	MG664338
13.	143	*T. propinqua*ssp*migahidii*	WadiTarabah	23° 20' 10" N 12° 41’ 47" E	MG664286	MG664317
14.	145	*T. propinqua*ssp*migahidii*	Al-Qaeid road - Hail	27° 41’ 18" N 41° 44’ 38" E	MG664287	MG664318
15.	146	*T. simplex*	Alnuqrah - PrinceAbdul AzizbinMuqrin road-Hail	27° 27’ 27" N 41° 38’ 59" E	MG664281	MG664312
16.	D1	*T. simplex*	Dhalam - Taif-Riyadh road	22° 12’ 10" N 41° 24’ 19" E	MG664280	MG664311
17.	D5	*T. propinqua*ssp*migahidii*	Alkhasrah- Taif-Riyadh road	23° 24’ 59" N 43° 43’ 27" E	MG664282	MG664313
18.	D7	*T. propinqua*ssp*migahidii*	Khurais Road-150km beforeAl Ahsa	25° 11’ 47" N 48° 19’ 12" E	MG664283	MG664314
19.	D13	*T. hamiensis*var. *mandavillei*	Khurais - Al Ahsa road	25° 13’ 55" N 48° 36’ 16" E	MG664299	MG664330
20.	D16	*T. hamiensis*var. *qatarensis*	Al Ahsa - Qatar road	25° 16’ 29" N 49° 41’ 07" E	MG664295	MG664326
21.	D18	*T. hamiensis*var. *hamiensis*	Al Ahsa - Qatar road	25° 16’ 30" N 49° 41’ 09" E	MG664291	MG664322
22.	D19	*T. hamiensis*var. *hamiensis*	Al Ahsa - Qatar road - 25 km beforeSalwa	24° 49’ 54" N 50° 40’ 25" E	MG664292	MG664323
23.	D20	*T. hamiensis*var. *qatarensis*	Al Ahsa - Qatar road	24° 48’ 40" N 50° 44’ 26" E	MG664296	MG664327
24.	D21	*T. hamiensis*var. *qatarensis*	Al Ahsa - Qatar road	24° 48’ 40" N 50° 44’ 26" E	MG664297	MG664328
25.	D22	*T. hamiensis*var. *qatarensis*	Al Ahsa - Qatar road	24° 48’ 40" N 50° 44’ 26" E	MG664298	MG664329
26.	D24	*T. hamiensis*var. *hamiensis*	Al Ahsa - Qatar road -10 km before Alaudaidah	24° 27’ 32" N 51° 02’ 52" E	MG664293	MG664324
27.	D25	*T. hamiensis*var. *mandavillei*	Al Ahsa - Qatar road -10 km before Alaudaidah	24° 27’ 32" N 51° 02’ 52" E	MG664300	MG664331
28.	D27	*T. propinqua*ssp*migahidii*	Al Ahsa - Dammam road	25° 37’ 33" N 49° 32’ 12" E	MG664284	MG664315
29.	D28	*T. hamiensis*var. *hamiensis*	Al Ahsa - Dammam road	25° 37’ 33" N 49° 31’ 11" E	MG664294	MG664325
30.	D29	*T. propinqua*ssp*migahidii*	Shedgum-next tothe cement factory-Al Ahsa-Dammam road	25° 40’ 07" N 49° 30’ 31" E	MG664285	MG664316
31.	D30	*T. hamiensis*var. *mandavillei*	Shedgum-next tothe cement factory-Al Ahsa-Dammam road	25° 40’ 07" N 49° 30’ 31" E	MG664301	MG664332

the two regions (*rbc*L and *trn*L-F) from *Fagonia*, the most closely related genus to *Tetraena* and *Zygophyllum*, were downloaded from GenBank to use as the out-group (Table 3). Out-group choice was based on previous work on the genus *Zygophyllum* (Bellstedt *et al.*, 2008) and work on the sisters' genera to *Tetraena* and *Zygophyllum*, which is *Fagonia.*

Table 2. Herbarium specimens used in the present study for phylogenetic analyses.

No.	Taxa	Collection number	Collector's name	Date	Country	Herbarium
1.	*Tetraena hamiensis* var. *hamiensis* E4	M. 8153	Miller *et al*.	13/2/1989	Yemen	E
2.	*T. hamiensis* var. *hamiensis* E10	MTA 155	Abdullah M.	9/5/2012	Kuwait	E
3.	*T. hamiensis* var. *qatarensis* E9	21/2	Munton	21/1/1985	Oman	E
4.	*T. hamiensis* var. *qatarensis* K8	2	Vujo, K. J.	4/ 1979	Bahrain	K
5.	*T. hamiensis* var. *qatarensis* K9	10953	Boules, L.	29/3/1977	Qatar	K
6.	*T. propinqua* ssp. *migahidii* E6	6731	S. Collenette	27/4/1988	Saudi Arabia	E

Table 3. Sequences obtained from GenBank and previously used in the analysis of *Tetraena* and *Zygophyllum* plants (After Bellstedt *et al.*, 2008).

Taxa	GenBank accession for *rbc*L	GenBank accession for *trn*L-F
Fagonia cretica L. (out group)	AJ133855	AJ387942
F. indica Burm.f. (out group)	Y15018	AJ387943
F. luntii Baker (out group)	AJ133856	AJ387944
Tetraena mongolica Maxim.	Y15027	AJ387959
Zygophyllum album L.f.	AJ133861	AJ387963
Z. coccineum L.	AJ133863	AJ387965
Z. decumbens Delile	AJ133865	AJ387967
Z. decumbens Delile var. *decumbens*	EF655991	EF 656011
Z. fabago L.	Y15030	AJ387968
Z. sessilifolium L.	EF655997	EF656047
Z. simplex L.	EF655984	EF 656004
Z. simplex L.	Y15031	AJ387974
Z. xanthoxylum Engl.	AJ133872	AJ387975

DNA extraction

Leaf material from field-collected plants and herbarium specimens (Tables 1 & 2) were used for DNA extraction. Leaves were dried and stored in small polythene bags at -20°C. Total genomic DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN) following the manufacturer's protocol. The isolated DNA was stored at -20°C until further use.

Choice of molecular markers

The phylogenetic relationship of Saudi Arabian *Tetraena* and *Zygophyllum* taxa was clarified using two different chloroplast regions (*rbc*L and *trn*L-F regions) based on results from the previous work on *Tetraena* and *Zygophyllum* (Beier *et al*., 2003; Bellstedt *et al*., 2008).

DNA amplification

The DNA template amplified using PCR (Polymerase Chain Reaction). The PCR used different primers to amplify the *rbc*L and the *trn*L-F chloroplast DNA (cpDNA) regions. The PCR amplifications for each region were carried out in 25 µl reactions using 2 µl of template DNA, 12.5 µl 2x BioMix (Bioline), 2 µl of each primer [1-10 mM] and, 6.5 µl of distilled water. The *rbc*L gene was amplified using the forward primer 20bp at 1F (5'- ATGTCACCACAAACAG AAAC-3') and reverse primer 26bp at 1460R (5'- TCCTTTTAGTAAAAGATTGGGCCGAG-3') based on Savolainen *et al*. (2000a, b). The PCR conditions for the *rbc*L amplification used the protocol as outlined in Bellstedt *et al*. (2008), with some modifications for some accessions. The reaction condition was 5 min at 94°C, followed by 30 cycles of denaturation at 94°C for 30s, annealing temperature at 50-53°C for 50s, extension at 72°C for 60s, followed by a final extension for 6 min at 72°C. The *trn*L-F region was amplified using the forward primer 20 bp at c (5'-CGAAATCGGTAGACGCTACG-3') and reverse primer f (5'-ATTTGAACTGGTGACACGAG-3') based on Taberlet *et al*. (1991). The PCR conditions for the *trn*L-F amplifications were used the following program based on Bellstedt *et al*. (2008) which included 5 min at 94°C, followed by 35 cycles of denaturation at 94°C for 60s, annealing temperature at 55°C for 60s, extension at 72°C for 90s, followed by a final extension for 6 min at 72°C.

PCR product purification and sequences

PCR reactions used an automatic sequencer ABI3730XL (Macrogen Sequencing System, Korea) for purification and sequencing. For each sequence, the complementary bi-directional sequence strands were trimmed and assembled into a contig and manually edited using SeqMan software 6.1, Lasergene DNAStar 6.1 Windows 32 (DNAStar Corporation, Madison, WI, USA). All sequences were aligned automatically by BioEdit v.7.0.4.1 (Hall, 1999) or Clustal X (Thompson *et al*., 1997) followed by extensive manual adjustments. The two alignments were combined in one matrix using MacClade v. 4.07 (Maddison and Maddison, 2003).

Phylogenetic analyses

Maximum Parsimony: Separate analyses of *rbc*L and *trn*L-F data, and of combined chloroplast (*rbc*L and *trn*L-F) data were performed to infer relationships of Saudi Arabian taxa of *Tetraena* and *Zygophyllum* using the Maximum Parsimony approach, implemented with the computer program PAUP* 4.06 b10 for 32-bit Microsoft Windows XP (Swofford, 2001). Bootstrap support analysis (Felsenstein, 1985; Felsenstein and Kashino, 1993) was implemented in PAUP* 4.06 (Swofford, 2001) to estimate the support value of individual and combined data sets with 1000 pseudoreplicates of the data using the heuristic search strategy.

Bayesian analysis

The *rbc*L and *trn*L-F and combined chloroplast (*rbc*L and *trn*L-F) were analysed to infer relationships of Saudi Arabian *Tetraena* and *Zygophyllum* plants using Bayesian inference (Mau *et al*., 1999; Rannala and Yang, 1996) of the separate and combined data. Bayesian analysis used the Markov Chain Monte Carlo (mcmc) simulation programme, MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). The best fit model of molecular evolution for each individual and combined data set was selected using the Akaike Information Criterion (AIC), calculated with MrModeltest 2.2 (Nylander, 2004). The general time reversible model with

gamma and proportion of invariable sites of (GTR+I+G) was selected for all partitions as the best fit model. Five million generations were performed and 5000 trees were saved (sampling one tree per 1000 generations). Runs were repeated twice to confirm results, and typically 0.25% (c. 1250 trees) of the samples were discarded as burn-in. Majority rule consensus trees were constructed from the remaining trees to obtain posterior probabilities using PAUP* programme.

Results and Discussion

Parsimony analyses

The characteristics obtained by Parsimony Analyses of the individual and combined datasets for the taxa are summarizes in Table 4.

The *trn*L-F Parsimony analysis of 44 sequences yielded 100 of most parsimonious trees. All trees were saved and the strict consensus was generated (not shown). The *rbc*L Parsimony analysis of 40 sequences yielded 100 of the most parsimonious trees. All trees were saved and the strict consensus was generated (not shown). In case of combined cpDNA, the aligned matrix of combined chloroplast (*rbc*L and *trn*L-F) sequences was 2505 bp in length. Parsimony analysis of 44 sequences produced 100 of the most parsimonious trees. All trees were saved and the strict consensus was generated (Fig. 1).

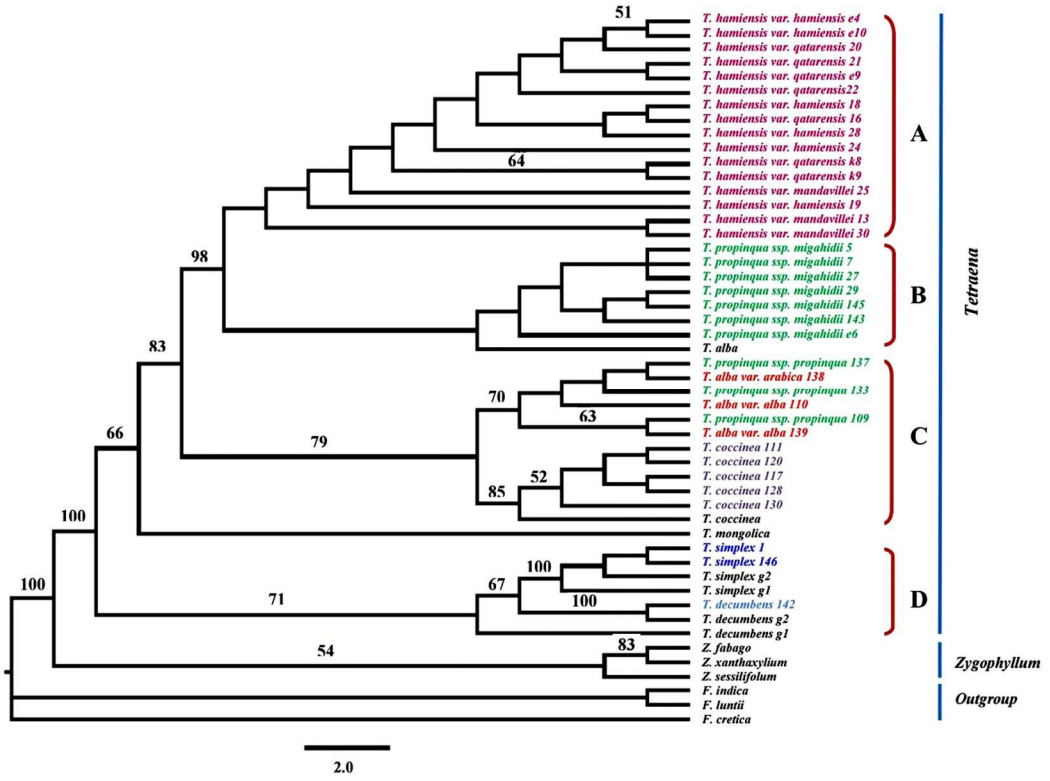

Fig. 1. One of 100 most equally parsimonious trees from analysis of the combined chloroplast of *rbc*L and *trn*L-F data set, using maximum parsimony for 43 Saudi Arabian *Tetraena* and one *Zygophyllum* accessions. Numbers above nodes are bootstrap (BS) support percentage values for clades supported above a 50% bootstrap value from 100000 replicates. Sequences of Saudi taxa are indicated with different colours and clades are indicated in letters.

Bayesian analyses

The best fitting model retrieved by MrModeltest as the most likely evolutionary model for all individual and combined data sets was the GTR+I+G model. Majority rule consensus trees were derived from 5000 trees from each analysis of the separate trnL-F (not shown) and rbcL (not shown) partitions and from combined chloroplast (Fig. 2) data sets. Burn-in was reached after 1250 generations for all partitions and for the combined matrix.

The represent study represents the first molecular phylogenetic study of the genus *Tetraena* and *Zygophyllum* in Saudi Arabia. Maximum Parsimony analysis and Bayesian criteria of the individuals and combined dataset of the *rbc*L and the *trn*L-F chloroplast DNA sequences used to study the phylogenetic relationships of *Tetraena* and *Zygophyllum* taxa in Saudi Arabia. The most notable similarity with respect to the individual and combined analysis regarding the overall topologies of the Maximum Parsimony and Bayesian trees are quite similar.

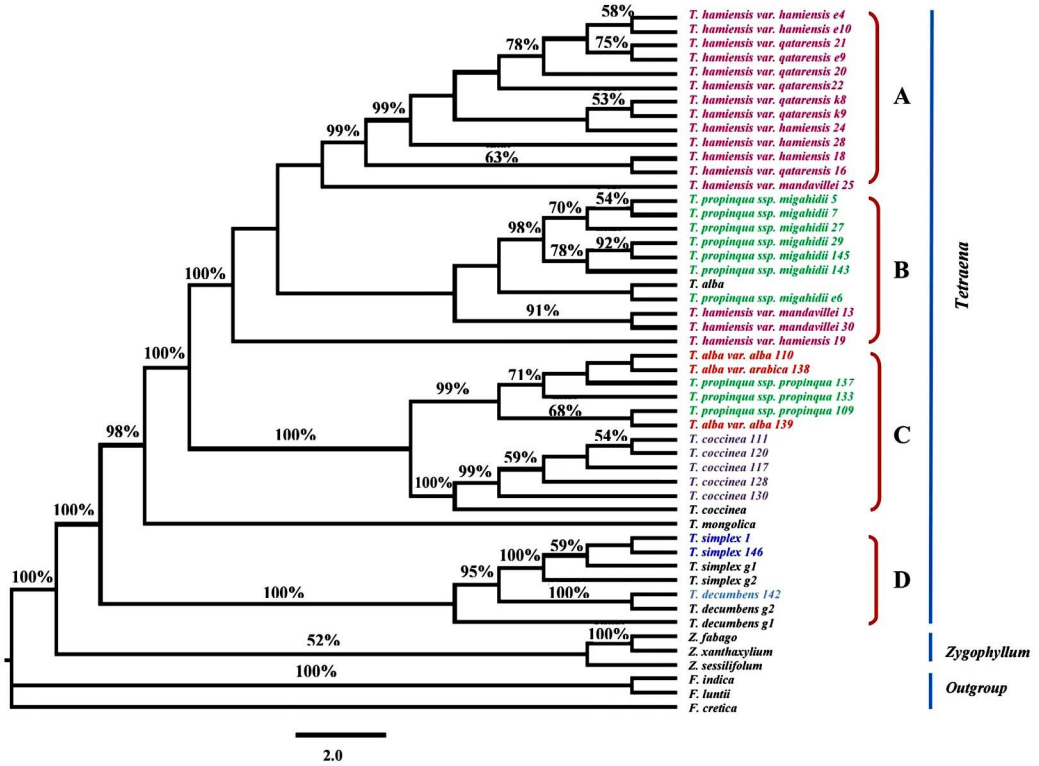

Fig. 2. Majority-rule consensus tree of the Bayesian inference based on the combined chloroplast *rbc*L and *trn*L-F data set of 43 Saudi Arabian *Tetraena* and one *Zygophyllum* accessions. Posterior probability values of the nodes are indicated above the branches. Sequences of Saudi taxa are indicated with different colours and clades are indicated in letters

Each of the *Tetraena* and the *Zygophyllum* genera appear as a monophyletic group with strong support in all phylogenies. In all phylogenetic analysis, the sequences of *T. mongolica* Maxim. (the type species of *Tetraena*), downloaded from GenBank, were nested within the rest of Saudi Arabian *Tetraena*. This finding agrees with Sheahan and Chase (2000) and supports the new classification of Beier *et al.* (2003). *Z. fabago* and *Z. xanthoxylum* (Asian species) samples that

were downloaded from GenBank are nested together in one clade as monophyletic group in all phylogenies of the Maximum Parsimony and Bayesian analysis (Figs 1 & 2). Molecular phylogenetic results of the *rbc*L and *trn*L-F individually or in combination datasets analysis in this study suggested that *Z. fabago* species is differing from other samples. Moreover, the strong agreement of the findings of the morphological studies (Alzahrani, 2017; Alzahrani and Albokhari, 2017a, b;) and molecular phylogenetic analysis in this study support the classification of Beier *et al*. (2003) to separate *Tetraena* and *Zygophyllum* plants into two genera. Molecular

Table 4. Characteristics of the individual and combined datasets from Parsimony analysis.

Phylogenetic information	*rbc*L	*trn*L	Combined cpDNA
Number of accession	46	50	50
Aligned length	1434	1071	2505
No. of constant characters	1286	792	2078
No. of variable characters	56	134	190
No. of informative characters	92	145	237
No. of most equally maximum Parsimony trees	100	100	100
Length of shortest trees (steps)	194	457	673
Consistency index (CI)	0.8144	0.7287	0.7296
Retention index (RI)	0.9032	0.8041	0.8189
Rescaled consistency index (RC)	0.7356	0.5859	0.5974

phylogenetic of the cpDNA analysis divided Saudi Arabian *Tetraena* plants into six groups: *T. hamiensis* (Schweinf.) Beier & Thulin, *T. propinqua* (Decne.) Ghazanfar & Osborne, *T. alba* (L. f.) Beier & Thulin, *T. coccinea, T. simplex* (L. f.) Beier & Thulin, and *T. decumbens* (Delile) Beier & Thulin.

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References

Al-Arjany, K.M. 2011. Molecular taxonomic perspective and eco-physiological variations of some species of *Tribulus, Zygophyllum* and *Fagonia* genera of family Zygophyllaceae in Saudi Arabia. Master dissertation, King Saud University, Saudi Arabia.

Alzahrani, D.A. 2017. Systematic studies on the Zygophyllaceae of Saudi Arabia: Two new subspecies combination in *Tetraena* Maxim. Saudi J. Biol. Sci. DOI: 10.1016/j.sjbs.2016.12.022.

Alzahrani, D.A., and Albokhari, E.J. 2017a. Systematic studies on the Zygophyllaceae of Saudi Arabia: a new variety and new variety combination in *Tetraena*. Saudi J. Biol. Sci. **24**: 1574–1579.

Alzahrani, D.A. and Albokhari, E.J. 2017b. Systematic studies on the Zygophyllaceae of Saudi Arabia: new combinations in *Tetraena* Maxim. Turk. J. Bot. **41**: 96–106.

Azevedo, L.B. 2014. Development and application of stressor-response relationships of nutrients. Ph.D. Thesis, Radboud University Nijmegen, the Netherlands.

Beier, B.A., Chase, M.W. and Thulin, M. 2003. Phylogenetic relationships and taxonomy of subfamily Zygophylloideae (Zygophyllaceae) based on molecular and morphological data. Plant Syst. Evol. **240**: 11–39.

Bellstedt, D.U., Van Zyl, L., Marais, E.M., Bytebier, B., de Villiers, C.A., Makwarela, A.M. and Dreyer, L.L. 2008. Phylogenetic relationships, character evolution and biogeography of southern African members of *Zygophyllum* (Zygophyllaceae) based on three plastid regions. Mol. Phylogenet. Evol. **47**: 932-949.

Boulos, L. 1978. Materials for a Flora of Qatar. Webbia **32**: 369–396.

El-Hadidi, M.N. 1977. Two new *Zygophyllum* species from Arabia. Publications from Cairo University Herbarium **7**&**8**: 327-329.

EI-Hadidi, M.N. 1980. On the taxonomy of *Zygophyllum* section *Bipartita.* Kew Bull. **35**: 335–340.

Engler, A. 1931. Zygophyllaceae. *In*: Engler A., Prantl K. (2nd ed.) *Die Naturlichen Pflanzenfamilien* **19**: 144-184. Engelmann, Leipzig.

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

Felsenstein, J. and Kashino, H. 1993. Is there something wrong with the bootstrap on phylogenies? A reply to Hillis and Bull. Syst. Biol. **42**: 193–199.

Ghazanfar, S.A. and Osborn, J. 2015. Typification of *Zygophyllum propinquum* Decne. and *Z. coccineum* L. (Zygophyllaceae) and a key to *Tetraena* in SW Asia. Kew Bull. **70**: 38.

Hall, T.A. 1999. BioEdit, a user-friendly biological sequence alignment editor and analysis program for Windows. Nucleic Acids Symp. Ser. **41**: 95–98.

Hammad, I. and Qari, S.H. 2010. Genetic diversity among *Zygophyllum* (Zygophyllaceae) populations based on RAPD analysis. Genet. Mol. Res. **9**: 2412–2420.

Hosny, A.I. 1988. Genus *Zygophyllum* L. in Arabia. Taeckholmia **11**: 19–32.

Huelsenbeck, J.P. and Ronquist, F. 2001. MrBayes: Bayesian inference in phylogenetic trees. Bioinformatics **17**: 754–755.

Hussein, S.R., Kawashty, S.A., Tantawy, M.E. and Saleh, N.A. 2009. Chemosystematic studies of *Nitraria retusa* and selected taxa of Zygophyllaceae in Egypt. Plant Syst. Evol. **277**: 251–264.

Louhaichi, M., Salkini, A.K., Estita, H.E. and Belkhir, S. 2011. Initial assessment of medicinal plants across the Libyan Mediterranean coast. Adv. Environ. Biol. **5**: 359-370.

Ma, Y. and Zhang, S. 1990. Study on the systematic position of *Tetraena*. Acta. Phytotax. Sin. **28**: 89–95.

Maddison, D.R. and Maddison, W.P. 2003. MacClade V. 4.07: Analysis of phylogeny and character evolution. Sinauer Associates, Sunderland, MA.

Mau, B., Newton, M.A. and Larget, B. 1999. Bayesian phylogenetic inference via Markovchain Monte Carlo methods. Biometrics **55**: 1–12.

Mosti, S., Raffaelli, M. and Tardelli, M. 2012. Contribution to the Flora of Central-Southern Dhofar (Sultanate of Oman). Webbia **67**: 65–91.

Norton, J., Abdul Majid, S., Allan, D., AlSafran, M., Böer, B. and Richer, R. 2009. An Illustrated Checklist of the Flora of Qatar. Browndown Publications, Gosport, UK. 67 pp.

Nylander, J. 2004. MrModeltest Q. Uppsala: Evolutionary Biology Centre, Uppsala University.

Rannala, B. and Yang, Z. 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. J. Mol. Evol. **43**: 304–311.

Ronquist, F. and Huelsenbeck, J.P. 2003 MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19**: 1572–1574.

Sakkir, S., Kabshawi, M. and Mehairbi, M. 2012. Medicinal plants diversity and their conservation status in the United Arab Emirates (UAE). J. Med. Plants Res. **6**: 1304–1322.

Savolainen, V., Fay, M.F., Albachi, D.C., Backlund, A., Van der Bank, M., Cameron, K.M., Johnson, S.A., Lledo, M.D., Pintaud, J.C., Powell, M., Sheahan, M.C., Soltis, D.E., Soltis, P.S., Weston, P., Whitten, W.M., Wurdack, K.J. and Chase, M.W. 2000a. Phylogeny of the eudicots: a nearly complete familial analysis based on *rbc*L gene sequences. Kew Bull. **55**: 257–309.

Savolainen, V., Chase, M.W., Hoot, S.B., Morton, C.M., Soltis, D.E., Bayer, C., Fay, M.F., De Bruijn, A.Y., Sullivan, S. and Qiu, Y. 2000b. Phylogenetics of flowering plants based on combined analysis of plastid *atp*B and *rbc*L gene sequences. Syst. Biol. **49**: 306–362.

Sheahan, M.C. and Chase, M.W. 1996. A phylogenetic analysis of Zygophyllaceae based on morphological, anatomical and *rbc*L DNA sequence data. Bot. J. Linn. Soc. **122**: 279–300.

Sheahan, M.C. and Chase, M.W. 2000. Phylogenetic relationships within Zygophyllaceae based on DNA sequences of three plastid regions, with special emphasis on Zygophylloideae. Syst. Bot. **25**: 371–384.

Swofford, D. 2001. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.06 b10. Sinauer, Sunderland, Massachusetts, USA.

Taberlet, P., Gielly, L., Pautou, G. and Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. **17**: 1105–1109.

Takhtajan, A.L. 1987. Flowering Plant. Komarov Botanical Institute, Russia.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. 1997. The CLUSTAL_X windows interface, flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **25**: 4876–4882.

Thulin, M. 1993. Zygophyllaceae *In*: Thulin, M. (Ed.) Flora of Somalia. Royal Botanical Gardens, Kew **1**: 176–189.

Van Huyssteen, D.C. 1937 Morphologisch-systematische studien über die gattung *Zygophyllum*. Dissertation. Berlin.

Van Zyl, L. 2000. A systematic revision of *Zygophyllum* in the southern African region. Ph.D. thesis, University of Stellenbosch, Stellenbosch.

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