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Molecular phylogeny of *Juniperus* in Iran with special reference to the *J. excelsa* complex, focusing on *J. seravschanica*

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Abstract

A total of over 100 accessions representing 11 species of *Juniperus* in Iran using multiple DNA regions were included in phylogenetic analyses. Analyses of four plastid intergenic spacers (*pet*N-*psb*M, *trn*D-*trn*T, *trn*L-*trn*F, *trn*S-*trn*G) and nrDNA ITS sequences retrieved *Juniperus* in Iran as a monophyletic group with two clades corresponding to sections *Juniperus* and *Sabina*. Our data support the recognition of *J. communis*, *J. deltoides*, *J. foetidissima*, *J. polycarpos* var. *polycarpos* and var. *turcomanica*, *J. sabina* and *J. seravschanica* in Iran. Based on sequences from nrDNA ITS, plastid *pet*N-*psb*M and single copy nuclear gene *LHCA*, specimens from the SE Iran that were previously considered to be a part of the *J. excelsa* complex were shown to be *J. seravschanica*. Samples from NE Iran were found to be *J. polycarpos* var. *turcomanica* and specimens from NW Iran were shown to be *J. polycarpos* var. *polycarpos*. Plants belonging to the *J. excelsa* complex from SW Iran appear to be of hybrid origin between *J. polycarpos* from N Iran and *J. seravschanica* from SE Iran. None of the *Juniperus* samples from Iran were found to be *J. excelsa* s str., as compared with typical samples from Greece. The sequence data from nrDNA ITS, plastid *pet*N-*psb*M, *trn*D-*trn*T, *trn*L-*trn*F, *trn*S-*trn*G and single copy nuclear genes (*LHCA*4, *maldehy*, *myb*, *CnAIP*3 and *4CL*) were utilized in this study to identify Iranian samples R, U, K as *J. seravschanica*.

Keywords: *Juniperus*, *Juniperus excelsa* complex, nrDNA ITS, Plastid DNA, Single copy nuclear genes (SCNG)

Introduction

Juniperus L. is one of the most diverse genera of the conifers. A phylogenetic analysis indicates that *Juniperus* is a monophyletic genus with a close relationship to *Cupressus* and *Hesperocyparis* (Mao *et al.* 2010). The genus is composed of approximately 75 species in 3 monophyletic sections (Adams & Schwarzbach 2013): *Caryocedrus* with one species, *Juniperus* with 14 species and *Sabina* with 60 species. Six *Juniperus* species were reported from Iran (Assadi 1998): *Juniperus communis* L., *J. oblonga* M.Bieb. and *J. oxycedrus* L. (section *Juniperus*); *J. foetidissima* Willd., *J. sabina* L. and *J. excelsa* M.Bieb. (sect. *Sabina*).

The *Juniperus excelsa* complex is known as one of the most difficult taxonomic groups of *Juniperus* in Iran and adjacent regions (Assadi 1998). According to the latest study, this complex consists of three morphologically very similar species as: *J. excelsa*, *J. polycarpos* K.Koch (var. *polycarpos* and var. *turcomanica* R.P.Adams) and *J. seravschanica* Kom. (Hojjati & Adams unpublished).

Some populations of the *J. excelsa* complex have been studied. Based on isoenzyme data, Hojjati *et al.* (2009) recognized 3 major clusters as *J. polycarpos* var. *polycarpos*, *J. polycarpos* var. *turcomanica* and *J. seravschanica* of this complex in Iran. Adams & Shanjani (2011), using DNA sequence data, showed the juniper from the Elburz Mountain Range to be typical *J. polycarpos*, not *J. excelsa*. Subsequently, Adams & Hojjati (2012) and Adams *et al.* (2014) employing four DNA regions (nrDNA, *pet*N-*psb*M, *trn*D-*trn*T and *trn*S-*trn*G, 3,705 nucleotide sites) showed that the samples from NW Iran are *J. polycarpos* and samples from NE Iran are clearly *J. polycarpos* var. *turcomanica*, as are the samples from Fasa in SW Iran. The samples from nearby south-central Iran (Khabr protected area) are part

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of a clade with *J. polycarpos* var. *seravschanica*. A minimum spanning network revealed that the Kuhbanan trees are genetically diverse and differentiated from more typical *J. seravschanica*. Those studies revealed that junipers from the southern mountain ranges of Iran are very diverse especially in Khabr and Kuhbanan populations, so that a new taxon may be present in southern Iran. Nearly all samples from Kuhbanan were polymorphic in their nrDNA (ITS) chromatograms, implying either hybridization or incomplete lineage sorting.

Adams & Hojjati (2013) also analyzed leaf essential oils of *Juniperus* from southern Iran and recognized mainly two groups: high cedrol (cf. *J. excelsa*, *J. polycarpos* and *J. seravschanica*) and low cedrol (cf. *J. polycarpos* var. *turcomanica*). In additional research (Adams *et al.* 2016) reported probable hybrids, even in NW Iran. Hitherto, no comprehensive phylogenetic study on *Juniperus* taxa in Iran has been conducted using extensive taxon sampling and DNA fragments. In this study, we utilized 10 DNA regions including nrDNA ITS, four plastid and five nuclear single copy genes.

The main purposes of this study are: 1) to perform a molecular phylogenetic analysis on all *Juniperus* species in Iran; 2) to resolve the *J. excelsa* complex problems in Iran; and 3) to recognize hybrids in this complex in Iran.

Materials and methods

Plant materials

Some plant specimens were collected from their natural habitats in different parts of Iran and some leaf materials were taken from herbarium specimens (Table 2). Plant materials were preserved in plastic bags with silica gel until DNA extraction. Herbarium names followed Thiers (2016).

DNA isolation, PCR amplification, and sequencing

Total genomic DNA was extracted from dried or fresh leaf tissues using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA). The purity and quantity of genomic DNA were determined by using 0.8 % agarose gel electrophoresis. After isolation, samples were stored at -20°C prior to amplification. Five SCNG (Single Copy Nuclear Genes): type IV chlorophyll binding protein (*LHCA*4), malate dehydrogenase (*maldehy*), Myb transcription factor (*myb*), ABI3-interacting protein gene (*CnAIP*3), 4-coumarate CoA ligase (*4CL*) were used as they were informative in distinguishing *J. excelsa*, *J. polycarpos*, *J. p.* var. *turcomanica* and *J. seravschanica.* In addition, nrDNA ITS and four chloroplast intergenic spacers: *pet*N-*psb*M, *trn*D-*trn*T, *trn*L-*trn*F and *trn*S-*trn*G (Adams *et al.* 2009; Adams & Kauffmann 2010, Letelier *et al.* 2014) were amplified (Table1).

TABLE 1. Fragments used in this study with their source, size, primer sequences and PCR annealing temperature.

Region	Source	Size (bp)	primers	PCR annealing temperature(°C)
4CL	nucleus	700	4CL49F: AAA GAG CTC ATC AAA TAC AA 4CL814R: GAAGAGCTTCCAGCTCAG	60
CnAIP3	nucleus	1087	CnAIP3f431: CTG GCG AAG GTG GAT TTT T CnAIP3r1517: ACA TTG GAT CTT CCG TGG AG	60
ITS	nucleus	1100	ITSA: GGA AGG AGA AGT CGT AAC AAG G ITSB: CTT TTC CTC CGC TTA TTG ATA TG	50
LHCA4	nucleus	800	LHCA4F: GGA GCT AGT GAA CGG GAG GTG LHCA4R: GAA CGG GCC CTT TCC TGT TA	55
maldehy	nucleus	515	maldehyF8: GTG ATT GGG TGC TTG GTA CAC maldehyR531: AGT GGC ATC CAG TTT TTC CTT	60
myb	nucleus	950	mybF: TAA CCA GCT TTG CCC TCA G mybR: ATA CAA TTC GCG GCT ACC ATA	55
petN-psbM	Chloroplast	764	petN5F: AAC GAA GCG AAA ATC AAT CA psbM111R: AAA GAG AGG GAT TCG TAT GGA	50
trnD-trnT	Chloroplast	700	trnDf: ACC AAT TGA ACT ACA ATC CC trnTr: CTA CCA CTG AGT TAA AAG GG	50
trnL-trnF	Chloroplast	700	trnLf: CGA AAT CGG TAG ACG CTA CG trnFr: ATT TGA ACT GGT GAC ACG AG	50
trnS-trnG	Chloroplast	700	trnSf: GCC GCT TTA GTC CAC TCA GC trnGr: GAA CGA ATC ACA CTT TTA CCA C	50

TABLE 2. Accessions, locations, and herbarium numbers for plant material used in this study.

Accession	Location	Herbarium number	Accession Number	
J. communis var. *oblonga*	Armenia	8765 (BAYLU)	ITS	LC420870
			petN-psbM	LC420866
			trnD-trnT	LC420867
			trnL-trnF	LC420868
			trnS-trnG	LC420869
J. communis var. *oblonga*	Armenia	8766 (BAYLU)	ITS	LC420875
			petN-psbM	LC420871
			trnD-trnT	LC420872
			trnL-trnF	LC420873
			trnS-trnG	LC420874
J. communis	Eastern Rhodopes, Bulgaria	13730 (BAYLU)	ITS	LC420860
			petN-psbM	LC420856
			trnD-trnT	LC420857
			trnL-trnF	LC420858
			trnS-trnG	LC420859
J. communis	Eastern Rhodopes, Bulgaria	13731 (BAYLU)	ITS	LC420865
			petN-psbM	LC420861
			trnD-trnT	LC420862
			trnL-trnF	LC420863
			trnS-trnG	LC420864
J. communis 126 Bazargan	Bazargan, west Azerbaijan, Iran	101408(TARI)	ITS	LC420940
			petN-psbM	LC420936
			trnD-trnT	LC420937
			trnL-trnF	LC420938
			trnS-trnG	LC420939
J. communis 129 Veresk	Veresk region, Mazandaran road from Firouzkuh to Ghaemshahr, Iran	93625(TARI)	ITS	LC420950
			petN-psbM	LC420946
			trnD-trnT	LC420947
			trnL-trnF	LC420948
			trnS-trnG	LC420949
J. communis 130 Kurkhud	Kuh-e Kurkhud, between Bojnurd and Golestan forest, Khorassan, Iran	85587(TARI)	petN-psbM	LC420951
			trnD-trnT	LC420952
			trnL-trnF	LC420953
			trnS-trnG	LC420954
J. communis 175 Tabriz	Road Tabriz to Tehran, Azerbaijan, Iran	85352(TARI)	ITS	LC420945
			petN-psbM	LC420941
			trnD-trnT	LC420942
			trnL-trnF	LC420943
			trnS-trnG	LC420944
J. deltoides	*Turkey*	9430 (BAYLU)	ITS	LC420890
			petN-psbM	LC420886
			trnD-trnT	LC420887
			trnL-trnF	LC420888
			trnS-trnG	LC420889
J. deltoides	Turkey	9431 (BAYLU)	ITS	LC420895
			petN-psbM	LC420891
			trnD-trnT	LC420892
			trnL-trnF	LC420893
			trnS-trnG	LC420894
J. drupacea	Greece	8795 (BAYLU)	ITS	LC420930
			petN-psbM	LC420926
			trnD-trnT	LC420927
			trnL-trnF	LC420928
			trnS-trnG	LC420929
J. drupacea	Greece	8796 (BAYLU)	ITS	LC420935
			petN-psbM	LC420931
			trnD-trnT	LC420932
			trnL-trnF	LC420933
			trnS-trnG	LC420934

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TABLE 2. (Continued)

Accession	Location	Herbarium number	Accession Number	
J. excelsa	Greece	8785 (BAYLU)	4CL	LC420805
			CnAIP3	LC420804
			ITS	LC420800
			LHCA4	LC420801
			maldehy	LC420802
			myb	LC420803
			petN-psbM	LC420796
			trnD-trnT	LC420797
			trnL-trnF	LC420798
			trnS-trnG	LC420799
J. excelsa	Greece	8786 (BAYLU)	4CL	LC420815
			CnAIP3	LC420814
			ITS	LC420810
			LHCA4	LC420811
			maldehy	LC420812
			myb	LC420813
			petN-psbM	LC420806
			trnD-trnT	LC420807
			trnL-trnF	LC420808
			trnS-trnG	LC420809
J. excelsa	Greece	14742 (BAYLU)	4CL	LC420825
			CnAIP3	LC420824
			ITS	LC420820
			LHCA4	LC420821
			maldehy	LC420822
			myb	LC420823
			petN-psbM	LC420816
			trnD-trnT	LC420817
			trnL-trnF	LC420818
			trnS-trnG	LC420819
J. excelsa	Bulgaria	13720 (BAYLU)	4CL	LC420835
			CnAIP3	LC420834
			ITS	LC420830
			LHCA4	LC420831
			maldehy	LC420832
			myb	LC420833
			petN-psbM	LC420826
			trnD-trnT	LC420827
			trnL-trnF	LC420828
			trnS-trnG	LC420829
J. excelsa	Bulgaria	13721 (BAYLU)	4CL	LC420845
			CnAIP3	LC420844
			ITS	LC420840
			LHCA4	LC420841
			maldehy	LC420842
			myb	LC420843
			petN-psbM	LC420836
			trnD-trnT	LC420837
			trnL-trnF	LC420838
			trnS-trnG	LC420839
J. excelsa	Turkey	9433 (BAYLU)	ITS	LC420850
			petN-psbM	LC420846
			trnD-trnT	LC420847
			trnL-trnF	LC420848
			trnS-trnG	LC420849
J. excelsa	Lebanon	14155 (BAYLU)	ITS	LC420855
			petN-psbM	LC420851
			trnD-trnT	LC420852
			trnL-trnF	LC420853
			trnS-trnG	LC420854

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TABLE 2. (Continued)

Accession	Location	Herbarium number	Accession Number	
J. excelsa 101 sarvestan	Sarvestan, Fars, Iran	46767 (TARI)	ITS	LC421059
			LHCA4	LC421060
			petN-psbM	LC421058
J. excelsa 102 Dehbakri	Deh-Bakri, Jiroft, Kerman, Iran	26949 (TARI)	ITS	LC421145
			LHCA4	LC421146
			petN-psbM	LC421144
J. excelsa 103 Genu	Kuh-e Genu, Bandar Abbas, Hormozgan, Iran	68105 (TARI)	ITS	LC421035
			LHCA4	LC421036
			petN-psbM	LC421034
J. excelsa 104 Hana	Bagh-e Hana, Bandar Abbas, Hormozgan, Iran	73687 (TARI)	ITS	LC421148
			LHCA4	LC421149
			petN-psbM	LC421147
J. excelsa 105 Saluk	Saluk, Khorassan, Iran	149(TMUH)	ITS	LC421168
			LHCA4	LC421169
			petN-psbM	LC421167
J. excelsa 107 Dena	Bijan, Kuh-e Dena, Esfahan, Iran	46156 (TARI)	ITS	LC421062
			LHCA4	LC421063
			petN-psbM	LC421061
J. excelsa 108 Abmalakh	Abmalakh, Kuh-e Dena, Fars	31380 (TARI)	ITS	LC421151
			LHCA4	LC421152
			petN-psbM	LC421150
J. excelsa 109 Savrz	Kuh-e Saverz, Dilegoon, Kohgilouyeh-Boirahmad	46409 (TARI)	ITS	LC421154
			LHCA4	LC421155
			petN-psbM	LC421153
J. excelsa 110 Sirachal	Sirachal, Karaj, Tehran, Iran	95113 (TARI)	ITS	LC421171
			LHCA4	LC421172
			petN-psbM	LC421170
J. excelsa 113 Urmia	Urmia, Azarbaijan, Iran	6887 (HWANRRC)	ITS	LC421218
			LHCA4	LC421219
			petN-psbM	LC421217
J. excelsa 114 Salmas	Salmas, Azarbaijan, Iran	7114 (HWANRRC)	ITS	LC421203
			LHCA4	LC421204
			petN-psbM	LC421202
J. excelsa 115 Maku	Maku, Azarbaijan, Iran	30488 (TARI)	ITS	LC421181
			LHCA4	LC421182
			petN-psbM	LC421180
J. excelsa 116 Bazargan	Bazargan, Azerbaijan, Iran	9480 (HWANRRC)	ITS	LC421210
			LHCA4	LC421211
			petN-psbM	LC421209
J. excelsa 117 Marand	Between Marand and Jolfa, Azarbaijan, Iran	29958 (TARI)	ITS	LC421215
			LHCA4	LC421216
			petN-psbM	LC421214
J. excelsa 119 Ardakan	Ardekan, Fars, Iran	2393 (TARI)	LHCA4	LC421141
			petN-psbM	LC421140
J. excelsa 120 Semnan	Kuh-e Sefid, Damghan to Shahroud, Semnan, Iran	82201(TARI)	LHCA4	LC421163
			petN-psbM	LC421162
J. excelsa 134 Barez	Baz mountains, Baghestan, Ghotbabad, Bandar Abbas, Hormozgan, Iran	50003 (TARI)	LHCA4	LC421143
			petN-psbM	LC421142
J. excelsa 136 Sabzkuh	Sabzkuh, Chahrmahal-e-Bakhtiari	57275(TARI)	LHCA4	LC421157
			petN-psbM	LC421156
J. excelsa 137 Khoy	Khoy, Azarbaijan, Iran	68916 (TARI)	LHCA4	LC421184
			petN-psbM	LC421183
J. excelsa 145 Firouzkuh	Firouzkuh, Iran	174(TMUH)	LHCA4	LC421161
			petN-psbM	LC421160
J. excelsa 146 Firouzkuh	Firouzkuh, Iran	150 (TMUH)	LHCA4	LC421159
			petN-psbM	LC421158
J. excelsa 152 Qushchi	Qushchi, Azerbaijan, Iran	8154 (HWANRRC)	LHCA4	LC421201
			petN-psbM	LC421200
J. excelsa 153 Urmia	Urmia, Azarbaijan, Iran	8152 (HWANRRC)	LHCA4	LC421221
			petN-psbM	LC421220

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TABLE 2. (Continued)

Accession	Location	Herbarium number	Accession Number	
J. excelsa 154 Urmia	Urmia, Azarbaijan, Iran	8151 (HWANRRC)	LHCA4	LC421223
			petN-psbM	LC421222
J. excelsa 155 Bazargan	Bazargan, Azerbaijan, Iran	4796 (HWANRRC)	LHCA4	LC421213
			petN-psbM	LC421212
J. excelsa 156 Salmas	Salmas, Azarbaijan, Iran	3759 (HWANRRC)	LHCA4	LC421206
			petN-psbM	LC421205
J. excelsa 157 Salmas	Salmas, Azarbaijan, Iran	4414 (HWANRRC)	LHCA4	LC421208
			petN-psbM	LC421207
J. excelsa 168 Hezarmasjed	Hezarmasjed mountains, Khorassan, Iran	95998 (TARI)	LHCA4	LC421177
			petN-psbM	LC421176
J. excelsa 169 Badramlou	Badramlou, Bojnurd, Khorassan, Iran	72900 (TARI)	LHCA4	LC421179
			petN-psbM	LC421178
J. foetidissima	Greece	5645 (BAYLU)	ITS	LC420900
			petN-psbM	LC420896
			trnD-trnT	LC420897
			trnL-trnF	LC420898
			trnS-trnG	LC420899
J. foetidissima	Greece	5646 (BAYLU)	ITS	LC420905
			petN-psbM	LC420901
			trnD-trnT	LC420902
			trnL-trnF	LC420903
			trnS-trnG	LC420904
J. foetidissima 123 Marzanabad	Marzanabad, Mazandaran, Iran	4.3(TMUH)	ITS	LC420979
			petN-psbM	LC420975
			trnD-trnT	LC420976
			trnL-trnF	LC420977
			trnS-trnG	LC420978
J. foetidissima 124 Asheghlu	Asheghlu, Aras river, Azarbaijan, Iran	73845(TARI)	ITS	LC420984
			petN-psbM	LC420980
			trnD-trnT	LC420981
			trnL-trnF	LC420982
			trnS-trnG	LC420983
J. foetidissima 171 Kaleibar	Kaleibar protected region, Azarbaijan, Iran	17035(TARI)	ITS	LC420989
			petN-psbM	LC420985
			trnD-trnT	LC420986
			trnL-trnF	LC420987
			trnS-trnG	LC420988
J. foetidissima 172 Arasbaran	Arasbaran protected area, Azarbaijan, Iran	20431(TARI)	ITS	LC420994
			petN-psbM	LC420990
			trnD-trnT	LC420991
			trnL-trnF	LC420992
			trnS-trnG	LC420993
J. oblonga 125 Arasbaran	Arasbaran protected area, Azarbaijan, Iran	81874(TARI)	ITS	LC420959
			petN-psbM	LC420955
			trnD-trnT	LC420956
			trnL-trnF	LC420957
			trnS-trnG	LC420958
J. oblonga 173 Kaleibar	Kaleibar, Azarbaijan, Iran	73957(TARI)	ITS	LC420964
			petN-psbM	LC420960
			trnD-trnT	LC420961
			trnL-trnF	LC420962
			trnS-trnG	LC420963
J. oblonga 174 Arasbaran	Arasbaran protected area, Azarbaijan, Iran	73863(TARI)	ITS	LC420969
			petN-psbM	LC420965
			trnD-trnT	LC420966
			trnL-trnF	LC420967
			trnS-trnG	LC420968

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TABLE 2. (Continued)

Accession	Location	Herbarium number	Accession Number	
J. oxycedrus	France	9039 (BAYLU)	ITS	LC420880
			petN-psbM	LC420876
			trnD-trnT	LC420877
			trnL-trnF	LC420878
			trnS-trnG	LC420879
J. oxycedrus	France	9040 (BAYLU)	ITS	LC420885
			petN-psbM	LC420881
			trnD-trnT	LC420882
			trnL-trnF	LC420883
			trnS-trnG	LC420884
*J. oxycedrus*122 Mirzagol	Mirzagol, Khoy, Azarbaijan, Iran	30492(TARI)	ITS	LC420974
			petN-psbM	LC420970
			trnD-trnT	LC420971
			trnL-trnF	LC420972
			trnS-trnG	LC420973
J. polycarpos var. *polycarpos* F5 Fasa	Fasa, Fars, Iran	13756 (BAYLU)	ITS	LC421050
			LHCA4	LC421051
			petN-psbM	LC421049
J. polycarpos var. *polycarpos* Hashtjin	Hashtjin, Ardabil, Iran	12795 (BAYLU)	ITS	LC421195
			LHCA4	LC421196
			petN-psbM	LC421194
J. polycarpos var. *polycarpos* Lushan1	Lushan, Gilan, Iran	12603 (BAYLU)	ITS	LC421186
			LHCA4	LC421187
			petN-psbM	LC421185
J. polycarpos var. *polycarpos* Lushan1	Lushan, Gilan, Iran	12789 (BAYLU)	ITS	LC421189
			LHCA4	LC421190
			petN-psbM	LC421188
J. polycarpos var. *polycarpos* Lushan2	Lushan, Gilan, Iran	12604 (BAYLU)	ITS	LC421192
			LHCA4	LC421193
			petN-psbM	LC421191
J. polycarpos var. *polycarpos* Qushchi	Qushchi, Azerbaijan, Iran	12798(BAYLU)	ITS	LC421198
			LHCA4	LC421199
			petN-psbM	LC421197
J. polycarpos var. *polycarpos*	Azerbaijan	14162 (BAYLU)	4CL	LC420745
			CnAIP3	LC420744
			ITS	LC420740
			LHCA4	LC420741
			maldehy	LC420742
			myb	LC420743
			petN-psbM	LC420736
			trnD-trnT	LC420737
			trnL-trnF	LC420738
			trnS-trnG	LC420739
J. polycarpos var. *polycarpos*	Azerbaijan	14164 (BAYLU)	4CL	LC420755
			CnAIP3	LC420754
			ITS	LC420750
			LHCA4	LC420751
			maldehy	LC420752
			myb	LC420753
			petN-psbM	LC420746
			trnD-trnT	LC420747
			trnL-trnF	LC420748
			trnS-trnG	LC420749

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TABLE 2. (Continued)

Accession	Location	Herbarium number	Accession Number	
J. polycarpos var. *polycarpos*	Azerbaijan	14166 (BAYLU)	4CL	LC420765
			CnAIP3	LC420764
			ITS	LC420760
			LHCA4	LC420761
			maldehy	LC420762
			myb	LC420763
			petN-psbM	LC420756
			trnD-trnT	LC420757
			trnL-trnF	LC420758
			trnS-trnG	LC420759
J. polycarpos var. *polycarpos*	Azerbaijan	14167 (BAYLU)	4CL	LC420775
			CnAIP3	LC420774
			ITS	LC420770
			LHCA4	LC420771
			maldehy	LC420772
			myb	LC420773
			petN-psbM	LC420766
			trnD-trnT	LC420767
			trnL-trnF	LC420768
			trnS-trnG	LC420769
J. polycarpos var. *polycarpos*	Azerbaijan	14168 (BAYLU)	4CL	LC420785
			CnAIP3	LC420784
			ITS	LC420780
			LHCA4	LC420781
			maldehy	LC420782
			myb	LC420783
			petN-psbM	LC420776
			trnD-trnT	LC420777
			trnL-trnF	LC420778
			trnS-trnG	LC420779
J. polycarpos var. *polycarpos*	Azerbaijan	14171 (BAYLU)	ITS	LC420790
			petN-psbM	LC420786
			trnD-trnT	LC420787
			trnL-trnF	LC420788
			trnS-trnG	LC420789
J. polycarpos var. *polycarpos*	Armenia	8761 (BAYLU)	ITS	LC420795
			petN-psbM	LC420791
			trnD-trnT	LC420792
			trnL-trnF	LC420793
			trnS-trnG	LC420794
J. polycarpos var. *turcomanica* Baladae	Baladae, Mazandaran, Iran	12808 (BAYLU)	ITS	LC421174
			LHCA4	LC421175
			petN-psbM	LC421173
J. polycarpos var. *turcomanica* F1 Fasa	Fasa, Fars, Iran	12809 (BAYLU)	ITS	LC421038
			LHCA4	LC421039
			petN-psbM	LC421037
J. polycarpos var. *turcomanica* F2 Fasa	Fasa, Fars, Iran	12810 (BAYLU)	ITS	LC421041
			LHCA4	LC421042
			petN-psbM	LC421040
J. polycarpos var. *turcomanica* F3 Fasa	Fasa, Fars, Iran	13754 (BAYLU)	ITS	LC421044
			LHCA4	LC421045
			petN-psbM	LC421043
J. polycarpos var. *turcomanica* F4 Fasa	Fasa, Fars, Iran	13755 (BAYLU)	ITS	LC421047
			LHCA4	LC421048
			petN-psbM	LC421046
J. polycarpos var. *turcomanica* F6 Fasa	Fasa, Fars, Iran	13757 (BAYLU)	ITS	LC421053
			LHCA4	LC421054
			petN-psbM	LC421052
J. polycarpos var. *turcomanica* F7 Fasa	Fasa, Fars, Iran	13758 (BAYLU)	ITS	LC421056
			LHCA4	LC421057
			petN-psbM	LC421055

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TABLE 2. (Continued)

Accession	Location	Herbarium number	Accession Number	
J. polycarpos var. *turcomanica* Shahmirzad	Shahmirzad, Semnan, Iran	12799 (BAYLU)	ITS	LC421165
			LHCA4	LC421166
			petN-psbM	LC421164
J. polycarpos var. *turcomanica* U Kuhbanan	Kuhbanan, Kerman, Iran	12812 (BAYLU)	ITS	LC421106
			LHCA4	LC421107
			petN-psbM	LC421105
J. polycarpos var. *turcomanica* U1 Kuhbanan	Kuhbanan, Kerman, Iran	13759 (BAYLU)	ITS	LC421109
			LHCA4	LC421110
			petN-psbM	LC421108
J. polycarpos var. *turcomanica*, Bajgiran	Bajgiran, Khorassan, Iran	12802 (BAYLU)	4CL	LC420735
			CnAIP3	LC420734
			ITS	LC420730
			LHCA4	LC420731
			maldehy	LC420732
			myb	LC420733
			petN-psbM	LC420726
			trnD-trnT	LC420727
			trnL-trnF	LC420728
			trnS-trnG	LC420729
J. polycarpos var. *turcomanica*	Turkmenistan	8757 (BAYLU)	4CL	LC420695
			CnAIP3	LC420694
			ITS	LC420690
			LHCA4	LC420691
			maldehy	LC420692
			myb	LC420693
			petN-psbM	LC420686
			trnD-trnT	LC420687
			trnL-trnF	LC420688
			trnS-trnG	LC420689
J. polycarpos var. *turcomanica*	Turkmenistan	8758 (BAYLU)	4CL	LC420705
			CnAIP3	LC420704
			ITS	LC420700
			LHCA4	LC420701
			maldehy	LC420702
			myb	LC420703
			petN-psbM	LC420696
			trnD-trnT	LC420697
			trnL-trnF	LC420698
			trnS-trnG	LC420699
J. polycarpos var. *turcomanica*	Turkmenistan	8759 (BAYLU)	4CL	LC420715
			CnAIP3	LC420714
			ITS	LC420710
			LHCA4	LC420711
			maldehy	LC420712
			myb	LC420713
			petN-psbM	LC420706
			trnD-trnT	LC420707
			trnL-trnF	LC420708
			trnS-trnG	LC420709
J. polycarpos var. *turcomanica*	Turkmenistan	8760 (BAYLU)	4CL	LC420725
			CnAIP3	LC420724
			ITS	LC420720
			LHCA4	LC420721
			maldehy	LC420722
			myb	LC420723
			petN-psbM	LC420716
			trnD-trnT	LC420717
			trnL-trnF	LC420718
			trnS-trnG	LC420719

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TABLE 2. (Continued)

Accession	Location	Herbarium number	Accession Number	
J. sabina	Azerbaijan	14316 (BAYLU)	4CL	LC420915
			CnAIP3	LC420914
			ITS	LC420910
			LHCA4	LC420911
			maldehy	LC420912
			myb	LC420913
			petN-psbM	LC420906
			trnD-trnT	LC420907
			trnL-trnF	LC420908
			trnS-trnG	LC420909
J. sabina	Azerbaijan	14317 (BAYLU)	4CL	LC420925
			CnAIP3	LC420924
			ITS	LC420920
			LHCA4	LC420921
			maldehy	LC420922
			myb	LC420923
			petN-psbM	LC420916
			trnD-trnT	LC420917
			trnL-trnF	LC420918
			trnS-trnG	LC420919
J. sabina 121 Tuskastan	Tuskastan, Golestan, Iran	101434 (TARI)	petN-psbM	LC420995
			trnD-trnT	LC420996
			trnL-trnF	LC420997
			trnS-trnG	LC420998
J. seravschanica K1 Khabr	Kuh-e Khabr, Kerman, Iran	13768 (BAYLU)	ITS	LC421000
			LHCA4	LC421001
			petN-psbM	LC420999
J. seravschanica K2 Khabr	Kuh-e Khabr, Kerman, Iran	13769 (BAYLU)	4CL	LC421011
			CnAIP3	LC421010
			ITS	LC421006
			LHCA4	LC421007
			maldehy	LC421008
			myb	LC421009
			petN-psbM	LC421002
			trnD-trnT	LC421003
			trnL-trnF	LC421004
			trnS-trnG	LC421005
J. seravschanica K3 Khabr	Kuh-e Khabr, Kerman, Iran	13770 (BAYLU)	ITS	LC421013
			LHCA4	LC421014
			petN-psbM	LC421012
J. seravschanica K4	Kuh-e Khabr, Kerman, Iran	13771 (BAYLU)	ITS	LC421016
			LHCA4	LC421017
			petN-psbM	LC421015
J. seravschanica K5 Khabr	Kuh-e Khabr, Kerman, Iran	13772 (BAYLU)	ITS	LC421019
			LHCA4	LC421020
			petN-psbM	LC421018
J. seravschanica K6 Khabr	Kuh-e Khabr, Kerman, Iran	12815 (BAYLU)	ITS	LC421022
			LHCA4	LC421023
			petN-psbM	LC421021
J. seravschanica K7 Khabr	Kuh-e Khabr, Kerman, Iran	12816 (BAYLU)	4CL	LC421033
			CnAIP3	LC421032
			ITS	LC421028
			LHCA4	LC421029
			maldehy	LC421030
			myb	LC421031
			petN-psbM	LC421024
			trnD-trnT	LC421025
			trnL-trnF	LC421026
			trnS-trnG	LC421027

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TABLE 2. (Continued)

Accession	Location	Herbarium number	Accession Number	
J. seravschanica R1 Rabor	Rabor, Kerman, Iran	13773 (BAYLU)	ITS	LC421112
			LHCA4	LC421113
			petN-psbM	LC421111
J. seravschanica R2 Rabor	Rabor, Kerman, Iran	13774 (BAYLU)	4CL	LC421123
			CnAIP3	LC421122
			ITS	LC421118
			LHCA4	LC421119
			maldehy	LC421120
			myb	LC421121
			petN-psbM	LC421114
			trnD-trnT	LC421115
			trnL-trnF	LC421116
			trnS-trnG	LC421117
J. seravschanica R3 Rabor	Rabor, Kerman, Iran	13775 (BAYLU)	ITS	LC421125
			LHCA4	LC421126
			petN-psbM	LC421124
J. seravschanica R4 Rabor	Rabor, Kerman, Iran	13776 (BAYLU)	4CL	LC421136
			CnAIP3	LC421135
			ITS	LC421131
			LHCA4	LC421132
			maldehy	LC421133
			myb	LC421134
			petN-psbM	LC421127
			trnD-trnT	LC421128
			trnL-trnF	LC421129
			trnS-trnG	LC421130
J. seravschanica R5 Rabor	Rabor, Kerman, Iran	13777 (BAYLU)	ITS	LC421138
			LHCA4	LC421139
			petN-psbM	LC421137
J. seravschanica U2 Kuhbanan	Kuhbanan, Kerman, Iran	13760 (BAYLU)	ITS	LC421068
			LHCA4	LC421069
			petN-psbM	LC421067
J. seravschanica U3 Kuhbanan	Kuhbanan, Kerman, Iran	13761 (BAYLU)	ITS	LC421071
			LHCA4	LC421072
			petN-psbM	LC421070
J. seravschanica U4 Kuhbanan	Kuhbanan, Kerman, Iran	13762 (BAYLU)	ITS	LC421074
			LHCA4	LC421075
			petN-psbM	LC421073
J. seravschanica U5 Kuhbanan	Kuhbanan, Kerman, Iran	13763 (BAYLU)	4CL	LC421085
			CnAIP3	LC421084
			ITS	LC421080
			LHCA4	LC421081
			maldehy	LC421082
			myb	LC421083
			petN-psbM	LC421076
			trnD-trnT	LC421077
			trnL-trnF	LC421078
			trnS-trnG	LC421079
J. seravschanica U6 Kuhbanan	Kuhbanan, Kerman, Iran	13764 (BAYLU)	ITS	LC421087
			LHCA4	LC421088
			petN-psbM	LC421086
J. seravschanica U7 Kuhbanan	Kuhbanan, Kerman, Iran	13765 (BAYLU)	ITS	LC421090
			LHCA4	LC421091
			petN-psbM	LC421089
J. seravschanica U8 Kuhbanan	Kuhbanan, Kerman, Iran	13766 (BAYLU)	ITS	LC421093
			LHCA4	LC421094
			petN-psbM	LC421092

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TABLE 2. (Continued)

Accession	Location	Herbarium number	Accession Number	
J. seravschanica U9 Kuhbanan	Kuhbanan, Kerman, Iran	13767 (BAYLU)	4CL	LC421104
			CnAIP3	LC421103
			ITS	LC421099
			LHCA4	LC421100
			maldehy	LC421101
			myb	LC421102
			petN-psbM	LC421095
			trnD-trnT	LC421096
			trnL-trnF	LC421097
			trnS-trnG	LC421098
J. seravschanica Ux Kuhbanan	Kuhbanan, Kerman, Iran	12813 (BAYLU)	ITS	LC421065
			LHCA4	LC421066
			petN-psbM	LC421064
J. seravschanica	Pakistan	8483 (BAYLU)	4CL	LC420675
			CnAIP3	LC420674
			ITS	LC420670
			LHCA4	LC420671
			maldehy	LC420672
			myb	LC420673
			petN-psbM	LC420666
			trnD-trnT	LC420667
			trnL-trnF	LC420668
			trnS-trnG	LC420669
J. seravschanica	Pakistan	8484 (BAYLU)	4CL	LC420685
			CnAIP3	LC420684
			ITS	LC420680
			LHCA4	LC420681
			maldehy	LC420682
			myb	LC420683
			petN-psbM	LC420676
			trnD-trnT	LC420677
			trnL-trnF	LC420678
			trnS-trnG	LC420679
Juniperus seravschanica	Kazakhstan	8224 (BAYLU)	4CL	LC420645
			CnAIP3	LC420644
			ITS	LC420640
			LHCA4	LC420641
			maldehy	LC420642
			myb	LC420643
			petN-psbM	LC420636
			trnD-trnT	LC420637
			trnL-trnF	LC420638
			trnS-trnG	LC420639
Juniperus seravschanica	Kazakhstan	8225 (BAYLU)	4CL	LC420655
			CnAIP3	LC420654
			ITS	LC420650
			LHCA4	LC420651
			maldehy	LC420652
			myb	LC420653
			petN-psbM	LC420646
			trnD-trnT	LC420647
			trnL-trnF	LC420648
			trnS-trnG	LC420649
Juniperus seravschanica	Kazakhstan	8226 (BAYLU)	4CL	LC420665
			CnAIP3	LC420664
			ITS	LC420660
			LHCA4	LC420661
			maldehy	LC420662
			myb	LC420663
			petN-psbM	LC420656
			trnD-trnT	LC420657
			trnL-trnF	LC420658
			trnS-trnG	LC420659

TARI: Herbarium of Research institute of Forests & Rangelands, HWANRR: Herbarium of West Azerbaijan Natural Resource Research Center, BAYLU: Baylor University Herbarium, Robert P. Adams, specimens, TMUH: Tarbiat Modares University Herbarium.

DNA amplification was performed in a 30 µl volume containing 9 µl genomic DNA (4 ng/µl), 21 µl master mix containing 15 µl 2x buffer (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl2 according to the buffer used), 1.8 µM each primer and 1.0 unit Epi-Centre Fail-Safe Taq polymerase. The reaction mixtures were amplified in a DNA Thermal Cycler. The PCR reaction was subjected to agarose gel electrophoresis (1.5% agarose, 82 v., 40 min.). The expected molecular weight band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band, with the appropriate primer, was sent to McLab Inc. (South San Francisco) for sequencing.

Alignment and phylogenetic analyses

Each individual data set was aligned using the web-based version of MUSCLE (Edgar 2004; at http://www.ebi.ac.uk/Tools/msa/muscle/) under default parameters followed by manual adjustment. Sequences of all data sets showed length variation (because of noncoding region) and it was necessary to introduce insertions/deletions in the alignment. Positions of indels were treated as missing data for all datasets. Phylogenetic analyses were performed based on the maximum parsimony (MP) and maximum likelihood (ML) methods as well as Bayesian inference (BI). Parsimony analyses were conducted using PAUP* version 4.0b10 (Swofford 2002). The heuristic search option was employed for each dataset, using tree bisection-reconnection (TBR) branch swapping, with 100 replications of random addition sequence and an automatic increase in the maximum number of trees. Branch support values were calculated using a full heuristic search with 1,000 bootstrap replicates (Felsenstein 1985) each with simple addition sequence. In Bayesian method, Models of sequence evolution were selected using the program MrModeltest version 2.3 (Nylander 2004) based on the Akaike information criterion (AIC) (Posada & Buckley 2004). This program indicated GTR+I for almost all nrDNA ITS, single copy nuclear genes and plastid DNA as the best model for nucleotide substitution. The program MrBayes version 3.1.2 (Ronquist & Huelsenbeck 2003) was used for the Bayesian phylogenetic analyses. Posteriors on the model parameters were estimated from the data, using the default priors. The analysis was carried out with 10 million generations, using the Markov chain Monte Carlo (MCMC) search. MrBayes performed two simultaneous analyses starting from different random trees (Nruns = 2) each with four Markov chains and trees sampled at every 1000 generations. The first 25% trees were discarded as the burn-in. The remaining trees were then used to build a 50% majority rule consensus tree accompanied with posterior probability (PP) values. Maximum likelihood (ML) analyses were performed for the datasets in the program raxmlGUI (Silvestro & Michalak 2011). The model of evolution employed for each dataset is the same as that of Bayesian analyses. Parametric bootstrap values for ML were calculated in raxmlGUI based on 1000 replicates with one search replicate per bootstrap replicate. Tree visualization was carried out using TreeView version 1.6.6 (Page 2001).

Results

The general phylogenetic view of *Juniperis* in Iran

Both chloroplast data Bayesian tree based on 3422 nucleotide sites (including 93 potentially parsimony-informative sites) for four plastid fragments including *pet*N-*psb*M, *trn*D-*trn*T, *trn*L-*trn*F and *trn*S-*trn*G (Fig. 1) and nrDNA ITS Bayesian tree (Fig 2) based on 1293 nucleotide sites (including 80 potentially parsimony-informative sites), show the distinction of the ingroup from *J. drupacea* (outgroup) with strong support. There are two major clades of ingroup taxa.

One clade contains *J. communis* subsp. *hemisphaerica*, *J. oblonga*, *J. oxycedrus* and *J. deltoides* which belong to section *Juniperus*. The *J. communis* clade in both trees includes Iranian specimens of *J. communis* subsp. *hemisphaerica* and *J. oblonga* with a strong support. The *J. oxycedrus* clade contains samples from France with a strong support, but the Iranian specimen labeled as *J. oxycedrus* is strongly linked with the *J. deltoides* clade from Turkey. Clearly this shows that '*J. oxycedrus*' in Iran should be referred to as *J. deltoides*.

The next clade that contains other *Juniperus* species of Iran is section *Sabina*. *Juniperus foetidissima* plants from Iran are in a clade with *J. foetidissima* samples from Greece in a strong monophyletic group. The *J. sabina* sample from Iran groups with *J. sabina* samples from Azerbaijan in a strongly supported clade (Fig. 2). *Juniperus seravschanica* samples from S Iran, Kazakhstan and Pakistan form a strong clade. *Juniperus polycarpos* var. *turcomanica*, *J. polycarpos* var. *polycarpos* and *J. excelsa* are located in clades with high support as monophyletic groups (Fig. 1).

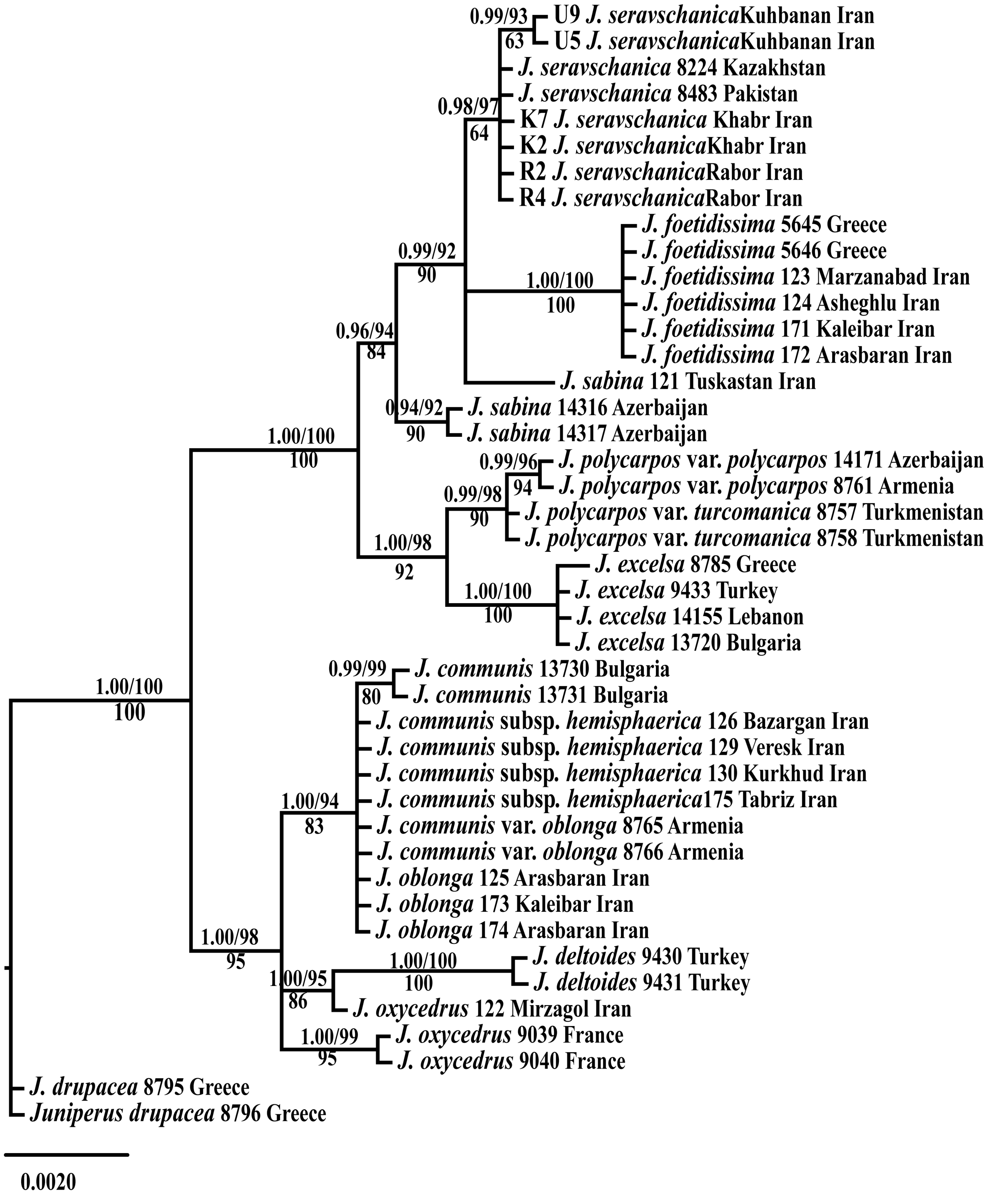

FIGURE 1. Fifty percent majority rule consensus Bayesian tree based on combined plastid *pet*N-*psb*M, *trn*D-*trn*T, *trn*L-*trn*F and *trn*S-*trn*G intergenic spacer fragments data. Numbers above branches are Bayesian posterior possibilities (before slashes) and maximum likelihood bootstrap support values (after slashes), while numbers under branches are maximum parsimony bootstrap support values. Values < 50 % are not shown. CI= 0.850 RI= 0.971.

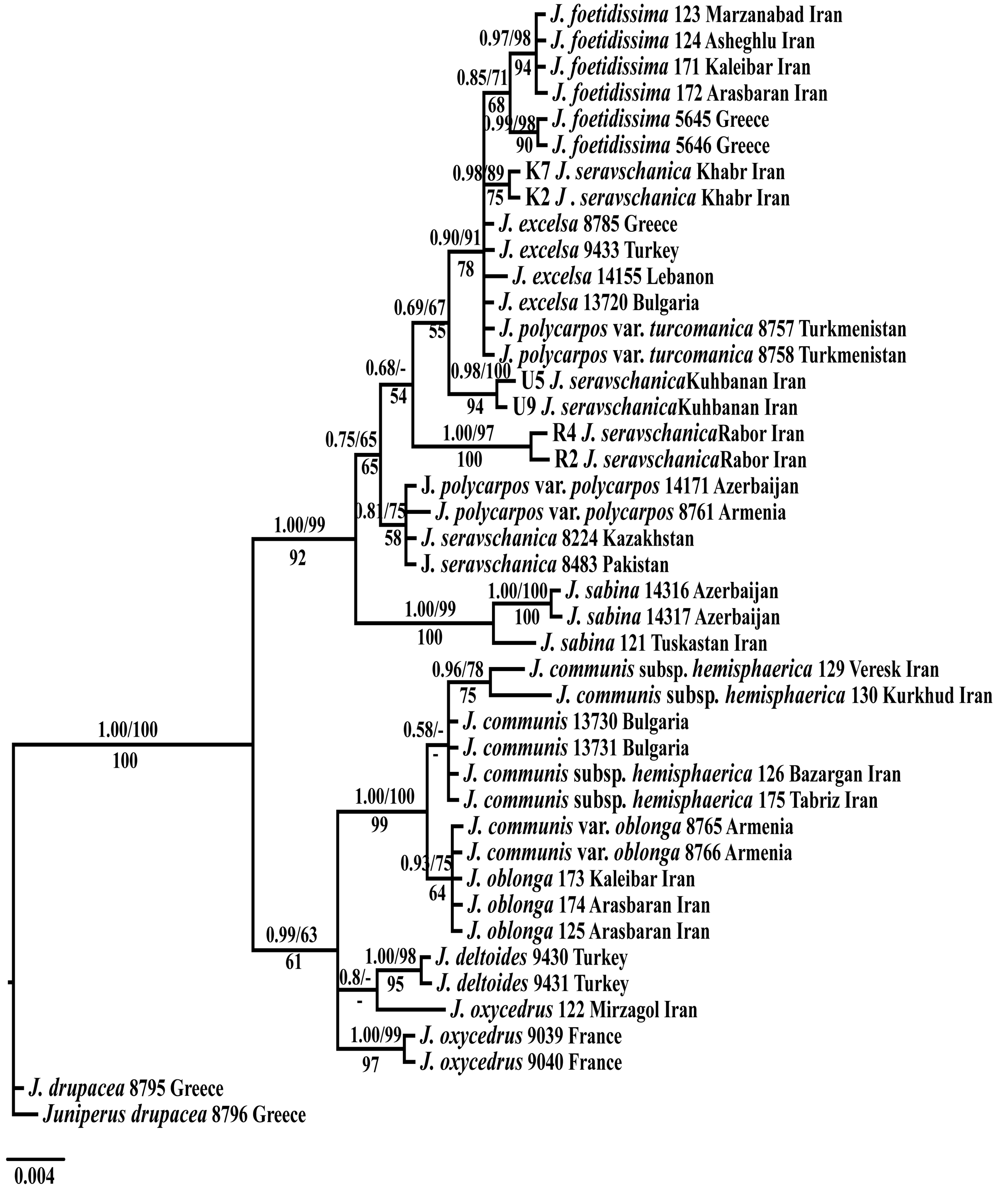

FIGURE 2. Fifty percent majority rule consensus Bayesian tree based on nrDNA ITS data. Numbers above branches are Bayesian posterior possibilities (before slashes) and maximum likelihood bootstrap support values (after slashes), while numbers under branches are maximum parsimony bootstrap support values. Values < 50 % are not shown. CI= 0.833 RI= 0.965.

The *Juniperus excelsa* complex

The Bayesian combined tree based on 2956 nucleotide sites (including 52 potentially parsimony-informative sites) of nrDNA ITS, plastid *pet*N-*psb*M (found as the most informative plastid fragment in this complex by screening) and the single copy nuclear gene, *LHCA*4 (found as the most informative *SCNG* fragment in this complex by screening) was utilized to examine the variation in the *J. excelsa* complex in Iran (Fig. 3). This tree shows *J. seravschanica* as a well-supported clade that contains *Juniperus* samples from SE Iran. *Juniperus excelsa* specimens from Greece are located in a distinct clade with a strong support. However, that clade does not contain any plants from Iran, supporting the conclusion of Adams *et al.* (2014) that *J. excelsa* does not occur in Iran. Putative *Juniperus turcomanica* samples from SW Iran are revealed as polytomy due to apparent hybridization (Adams *et al.* 2016). *Juniperus polycarpos* samples from NW Iran do not form a clade, and again this is likely due to hybridization (see Adams *et al.* 2016). *Juniperus* samples from SW Iran (group X) form a polytomy.

The Bayesian tree based on plastid *pet*N-*psb*M (Fig. 4) shows the *J. excelsa* clade and a clade with low support that contains *J. seravschanica* and samples from SE Iran.

The Bayesian tree based on single copy nuclear gene *LHCA*4 (Fig. 5) shows a weakly supported clade that contains *J. seravschanica* and samples from SE and SW Iran.

Juniperus seravschanica

The most robust analysis of Iranian junipers utilized all our molecular data (cpDNA, nrDNA, 6 SCNGs). Figure 6 shows that *J. excelsa*, *J. polycarpos*, *J. polycarpos* var. *turcomanica* and *J. seravschanica* resolve into 4 groups. *Juniperus excelsa*, *J. polycarpos*, and *J. seravschanica* are in well supported clades (Fig. 6). As previously mentioned, no samples of *J. excelsa* were found in Iran. It should be noted that, although all *J. polycarpos* var. *turcomanica* samples are grouped together, they are not in a distinct clade. This is because of the incongruence between their cpDNA and nuclear DNA, suggestive that this taxon is of hybrid origin. Putative *J. seravschanica* from Khabr, Kuhbanan and Rabor are clearly nested with typical *J. seravschanica* (Fig. 6), and this supports the recognition of *J. seravschanica* as a distinct species in the flora of Iran. Putative *J. polycarpos* var. *turcomanica* from Bajgiran is in the group with *J. polycarpos* var. *turcomanica* from Turkmenistan (Fig. 6).

Discussion

The species composition of *Juniperus* in Iran

As shown in Figs. 1 and 2, Iranian *Juniperus* species belong to two sections: sect. *Juniperus*, with *J. communis*, *J. oblonga* (treated as *J. communis* var. *oblonga* by Adams 2014), *J. oxycedrus* and *J. deltoides*; and sect. *Sabina*, which contains the other Iranian *Juniperus* species. These two sections are differentiated from each other by leaf type, seed number and seed cone color (Adams 2014).

Juniperus communis subsp. *hemisphaerica* and *J. oblonga* has been reported from Iran in the Flora of Iran (Assadi 1998). In this study, we analyzed *J. communis* samples from NW, N and NE Iran and *J. oblonga* from NW Iran. In our results, (Fig. 2) Iranian samples of *J. communis* are placed in a clade with *J. communis* from Bulgaria. Likewise, Iranian samples of *J. oblonga* along with two accessions of *J. communis* var. *oblonga* from Armenia formed a sister-group. Adams (2014) compared DNA from *J. communis* subsp. *hemisphaerica* from the type locality on Mt. Etna, Sicily and near Granada, Spain with all *J. communis* taxa world-wide. He reported that *J. communis* subsp. *hemisphaerica* was found only from the locations in Sicily and Spain. It seems most likely that the shrub form of *J. communis* in Iran belongs to *J. communis* var. *hemisphaerica*. This question is being researched in a separate study (in progress).

Juniperus oxycedrus has been reported from NW and NE Iran but no *J. oxycedrus* has been found in field trips. In fact, the presence of *J. oxycedrus* in Iran seems unlikely because it is a Mediterranean element (Assadi 1998). In this study a herbarium specimen identified as *J. oxycedrus* from NW Iran was placed in a clade with *J. deltoides* from Turkey. Moreover, morphological characters (Adams 2014) such as seed cones with raised cone scale tips, the position of the stomatal bands on the leaves so that the two white stomatal bands are not sunken but smooth to the leaf surface, and leaves with base of attachment nearly as wide as the blade confirm that this specimen is *J. deltoides*. It is also confirmed by the geographical distribution pattern: *Juniperus deltoides* was recognized as a new *Juniperus* species from the Mediterranean (Adams 2004) that occurs from Italy eastward through Turkey into the Caucasus Mts. and Iran, whereas *J. oxycedrus* var. *oxycedrus* grows west of central Italy into France, Spain, Portugal and Morocco (Adams *et al.* 2005). Also, nrDNA and *pet*N-*psb*M sequencing reveals putative *J. oxycedrus* from Azerbaijan, Bulgaria, Cyprus and Israel to be *J. deltoides* (Adams *et al.* 2015).

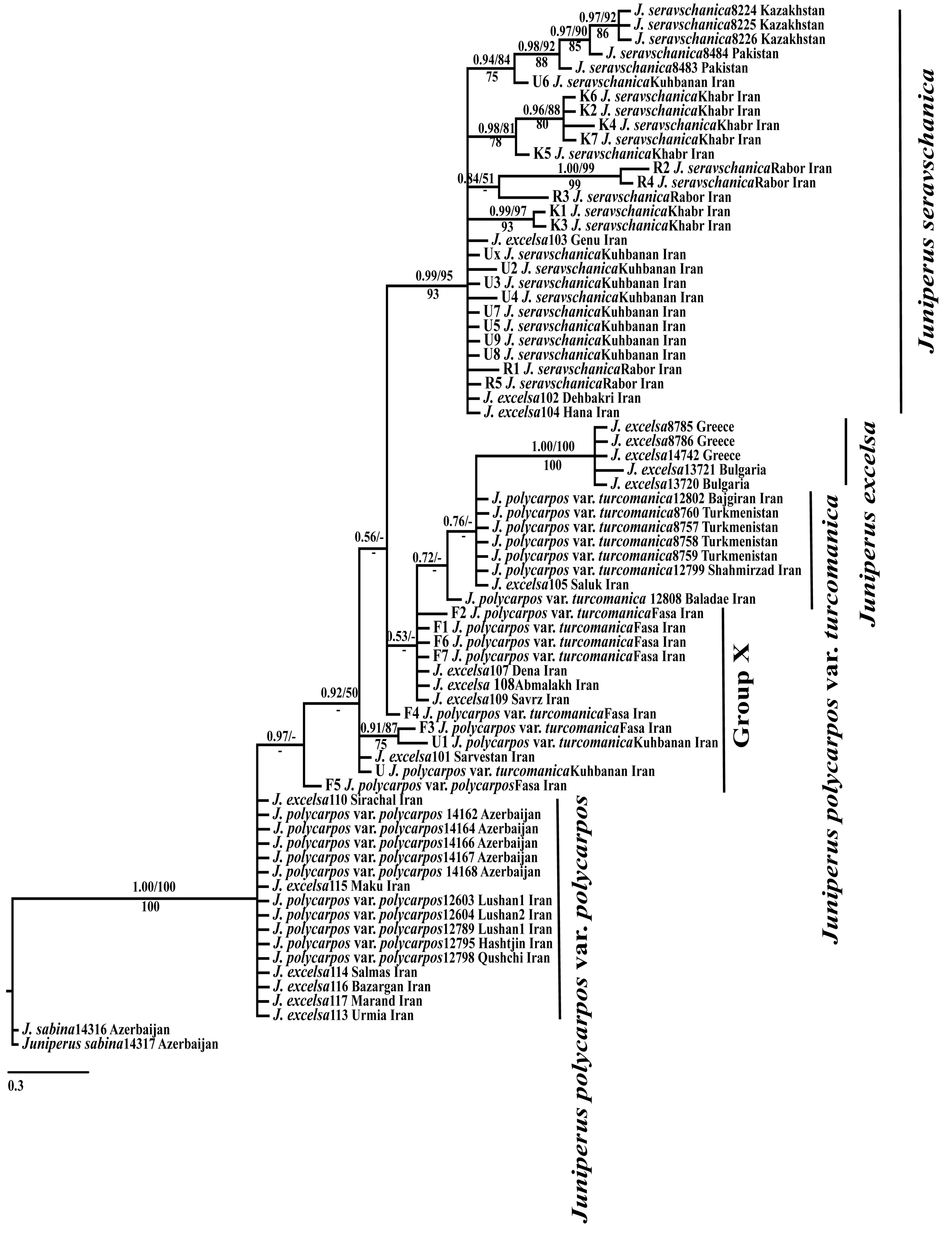

FIGURE 3. Fifty percent majority rule consensus Bayesian tree based on nrDNA ITS, plastid *pet*N-*psb*M intergenic spacer and single copy nuclear gene *LHCA4* combined data. Numbers above branches are Bayesian posterior possibilities (before slashes) and maximum likelihood bootstrap support values (after slashes), while numbers under branches are maximum parsimony bootstrap support values. Values < 50 % are not shown. CI= 0.654 RI= 0.933.

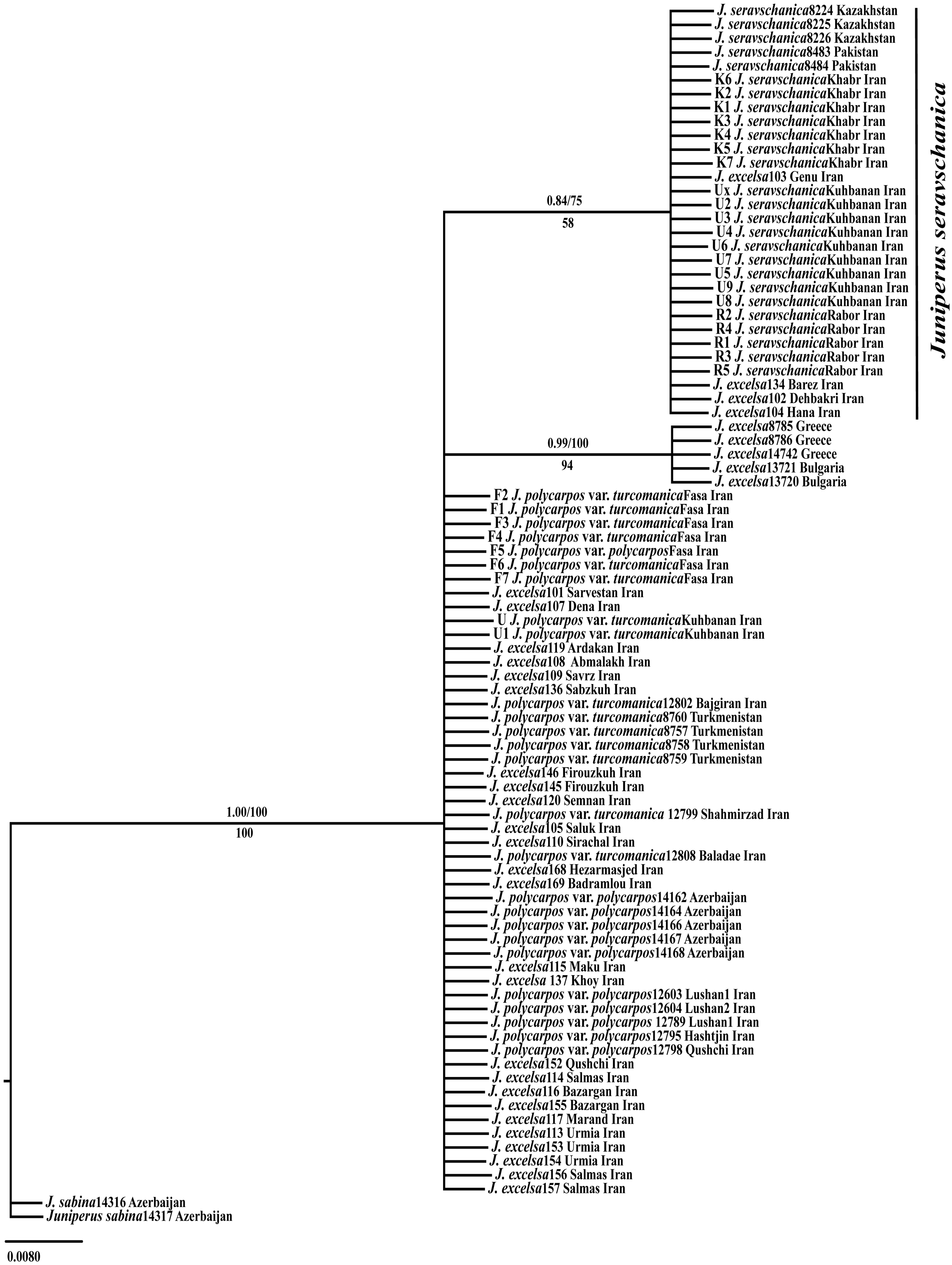

FIGURE 4. Fifty percent majority rule consensus Bayesian tree based on plastid *pet*N-*psb*M intergenic spacer data. Numbers above branches are Bayesian posterior possibilities (before slashes) and maximum likelihood bootstrap support values (after slashes), while numbers under branches are maximum parsimony bootstrap support values. Values < 50 % are not shown. CI= 1.000 RI= 1.000.

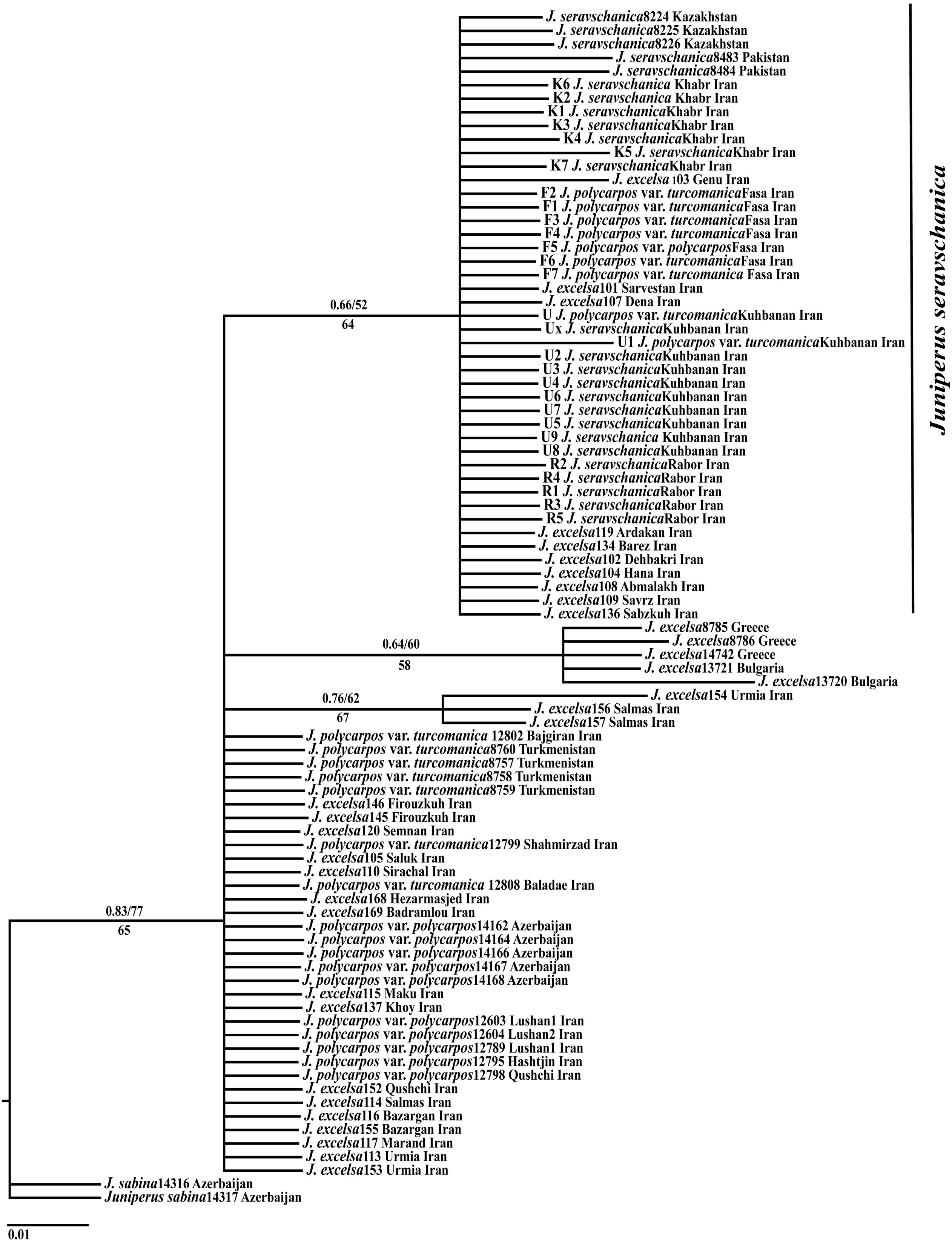

FIGURE 5. Fifty percent majority rule consensus Bayesian tree based on single copy nuclear gene *LHCA4* data. Numbers above branches are Bayesian posterior possibilities (before slashes) and maximum likelihood bootstrap support values (after slashes), while numbers under branches are maximum parsimony bootstrap support values. Values < 50 % are not shown. CI= 0.909 RI= 0.989.

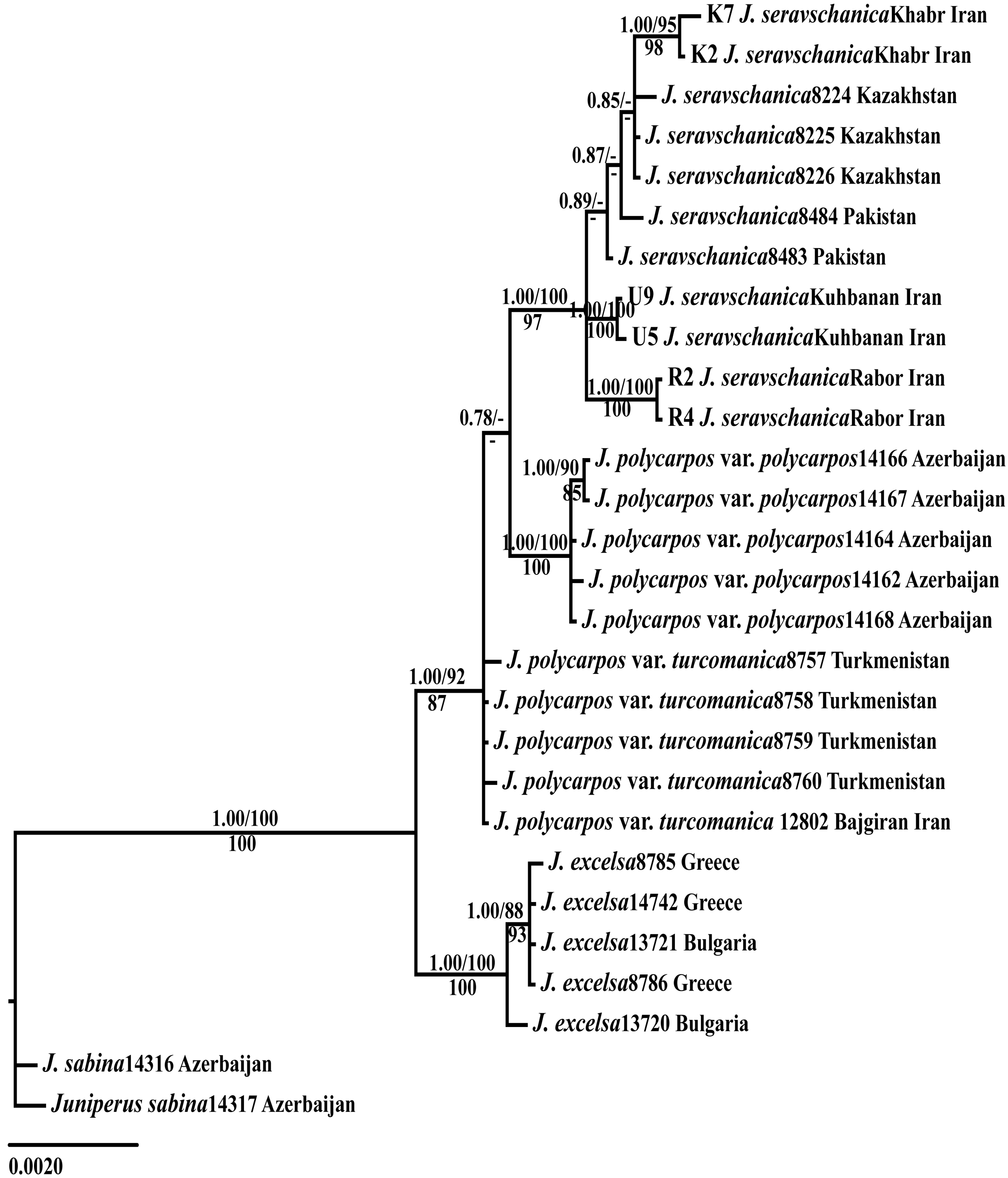

FIGURE 6. Fifty percent majority rule consensus Bayesian tree based on nrDNA ITS, plastid *pet*N-*psb*M, *trn*D-*trn*T, *trn*L-*trn*F, *trn*S-*trn*G intergenic spacers and single copy nuclear genes (*LHCA4*, *maldehy*, *myb*, *CnAIP3* and *4CL*) combined data. Numbers above branches are Bayesian posterior possibilities (before slashes) and maximum likelihood bootstrap support values (after slashes), while numbers under branches are maximum parsimony bootstrap support values. Values < 50 % are not shown. CI= 0.813 RI= 0.938.

Our results confirm the existence of *J. sabina* in N and NW Iran. However, the plastid Bayesian combined tree (Fig. 1) is incompatible with nrDNA ITS Bayesian tree (Fig 2) on the position of the Iranian specimen of *J. sabina*. In the plastid tree, this specimen is distinct from the reference specimens of *J. sabina* but in the ITS tree all specimens of *J. sabina* are placed in a strongly supported clade. This incompatibility suggests that a chloroplast capture via hybrid origin has occurred in the Iranian specimen of *J. sabina*. This question is being researched in a separate study (in progress).

The *Juniperus excelsa* complex

The present molecular analyses suggested that 24 individual specimens from SE Iran, which were considered members of the broadly defined *J. excelsa* by Assadi (1998), formed their own lineage distinct from the typical *J. excelsa*. Those samples are morphologically more similar to *J. seravschanica,* which is characterized mainly by the thickest ultimate branchlets. Other diagnostic characters are: seed cones 8–10 mm, scale leaves with clear, ellipsoid glands, often ruptured, with a clear exudate (Adams 2014). Moreover, some representatives (R2, R4, U5, U9, K2, K7) of this complex that were thought to be new taxa (Adams *et al.* 2014) were analyzed in this study utilizing additional DNA sequences. Our phylogenetic analyses of 8428 nucleotide sites of nrDNA ITS, four plastid intergenic spacers (*pet*N-*psb*M, *trn*D-*trn*T, *trn*L-*trn*F and *trn*S-*trn*G) and five single copy nuclear genes (*LHCA*4, *maldehy*, *myb*, *CnAIP*3 and *4CL*) show that these individuals from Iran belong to the *J. seravschanica* clade and the differences are just at the inter- and/ or infra-population level (Fig. 6).

The *Juniperus* plants from NE Iran are recognized as *J. polycarpos* var. *turcomanica* with thin ultimate branchlets. The *Juniperus* plants from NW Iran are recognized as *J. polycarpos* var. *polycarpos* with ultimate branchlets being thicker than found in var. *turcomanica*. These results are consistent with the findings of previous studies on the Iranian *J. excelsa* complex based on isoenzyme, essential oils and DNA sequencing data (Hojjati *et al.* 2009; Adams & Shanjani 2011; Adams & Hojjati 2012, 2013; Adams *et al.* 2014). Accordingly, there are no *Juniperus* samples from Iran that should be recognized as *J. excelsa. Juniperus excelsa* is distinguished from *J. polycarpos* in having very thin ultimate branchlets (vs. thicker branchlets in *J. polycarpos*). Other diagnostic characters to distinguish these taxa, such as scale-leaf size and seed cone size, are discussed in Adams (2014).

The major unresolved problem in the Iranian *Juniperus* is the nature of samples from SW Iran (group X). These junipers are morphologically very diverse. In fact, Adams & Hojjati (2012) recognized a population from Fasa, SW Iran, as *J. polycarpos* var. *turcomanica*. Analyses of leaf essential oils data indicated that the low cedrol trees (three trees from Kuhbanan and 2 trees from Fasa but none of the samples from Khabr or Rabor) were loosely grouped with the samples of *J. polycarpos* var. *turcomanica* (Adams & Hojjati 2013). Furthermore, Adams *et al.* (2014) recognized the plants from Fasa and two samples from Kuhbanan as *J. polycarpos* var. *turcomanica.* However, the minimum spanning network analysis of nrDNA ITS and plastid sequences in that study indicated that an individual from Fasa (F5) might be a hybrid. Also based on the nrDNA ITS sequences, Adams *et al.* (2014) indicated that most of the trees from Kuhbanan appear to be hybrids. Clearly the dynamics of *Juniperus* taxa in S Iran are complex and implied either hybridization or incomplete lineage sorting. We utilized *LHCA4,* a single copy nuclear gene, to address the evolutionary history of the group X. In the *LHCA*4 tree (Fig. 5) group X is placed in the *J. seravschanica* clade, but in plastid *pet*N-*psb*M tree group X is outside that clade. Although these clades are weakly supported, the geographical distributions of the species infer the hybrid nature for group X. The Bayesian tree based on concatenated nrDNA ITS, *pet*N-*psb*M and *LHCA*4 sequences does not group clearly group X with any species (Fig. 3). However, they are morphologically more similar to *J. seravschanica* and *J. polycarpos*. Our examination of Figs. 4 and 5 suggests a hybrid nature for group X because it is grouped differently in plastid and *LHCA*4 trees.

The examination of habitats of the taxa reveals that *J. seravschanica* is distributed in Central Asia, Pakistan to SE Iran with an arid climate, *J. polycarpos* var. *turcomanica* grows in mesic areas of the Elburz and Kopet mountains of Iran and Turkmenistan, *J. polycarpos* var. *polycarpos* occurs in the Caucasus and NW Iran with a temperate climate, and *J. excelsa* is distributed in Europe with a Mediterranean climate.

Hence, three species of this complex are clearly distributed in three very different climates. Thus, the existence of these species in SW Iran seems unlikely because the climate in SW Iran is different from climates in SE and N Iran. In fact, the climate is somewhat intermediate between climates in SE and N Iran. The intermediate climate in SW Iran would provide an intermediate habitat for group X of junipers that are hybrids (or derived from hybrids) between *J. polycarpos* from N Iran and *J. seravschanica* from SE Iran as supposed. Fig. 7 shows a distribution pattern of the *J. excelsa* complex members in Iran.

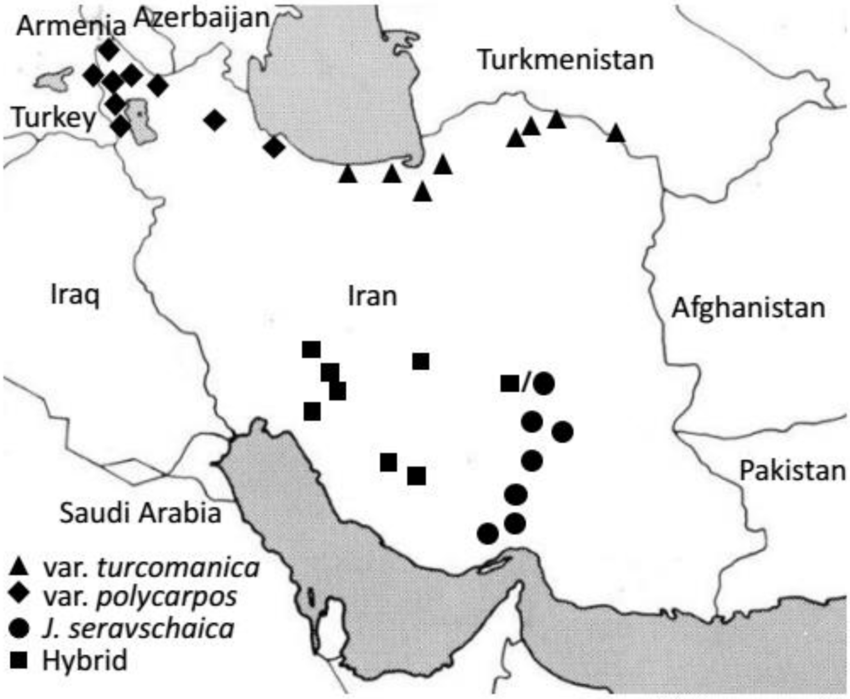

FIGURE 7. Distribution of *Juniperus polycarpos* var. *polycarpos*, *J. polycarpos* var. *turcomanica*, *J. seravschanica* and hybrids in Iran.

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