

# Discovery of Juniperus sabina var. balkanensis R. P. Adams and A. N. Tashev in Albania and relictual polymorphisms found in nrDNA

Robert P Adams, Perla Farhat, Lulëzim Shuka, Sonja Siljak-Yakovlev

### ▶ To cite this version:

Robert P Adams, Perla Farhat, Lulëzim Shuka, Sonja Siljak-Yakovlev. Discovery of Juniperus sabina var. balkanensis R. P. Adams and A. N. Tashev in Albania and relictual polymorphisms found in nrDNA. Phytologia, 2018, 100 (3), pp.187-194. hal-03939425

## HAL Id: hal-03939425 https://hal.science/hal-03939425v1

Submitted on 14 Jan2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés. **Robert P. Adams** 

Biology Department, Baylor University, Utah Lab, 201 N 5500 W, Hurricane, UT, USA robert\_adams@baylor.edu

#### Perla Farhat

Laboratoire Caractérisation Genomique des Plantes, Université Saint-Joseph, Campus Sciences et Technologies, Mar Roukos, Mkalles, BP 1514, Riad el Solh, Beirut 1107 2050, Lebanon

#### Lulëzim Shuka

Department of Biology, Faculty of Natural Sciences, Tirana University, Bld. ZOG I, Albania

and

#### Sonja Siljak-Yakovlev

Ecologie, Systématique, Evolution, CNRS UMR 8079, AgroParisTech, Univ. Paris-Sud, Université Paris-Saclay, Bâtiment 360, 91405 Orsay Cedex France.

#### ABSTRACT

Additional analyses of trnS-trnG and nrDNA from specimens from Albania revealed the presence of *J. sabina* var. *balkanensis*. Careful chromatogram analysis of eight (8) polymorphic sites in nrDNA revealed that 6 of the 8 plants were polymorphic (RSRMYRK or RSRMARK) and apparently hybrids between type 1 GGACCCAG and type 2 (ACGACAGT) plants. Two plants were homozygous for the 8 sites and they both had the type 1 pattern (GGACCCAG). The type 2 pattern (ACGACAGT) was not found, but due to the presence of hybrids, it probably grows in the area. These two nrDNA types appear to have arisen via hybridization with a *J. thurifera* ancestor. The two types often are found in both v. *sabina* and v. *balkanensis* populations. Published on-line www.phytologia.org *Phytologia 100(2): 187-194 (Sep 22, 2018)*. ISSN 030319430.

**KEY WORDS:** *Juniperus sabina var. balkanensis, J. sabina*, distribution, nrDNA, trnS-trnG, chloroplast capture, ancient nrDNA heterozygotes.

Recently, Adams et al. (2018) reported on new populations of *J. sabina* var. *balkanensis* from Macedonia, Bosnia-Herzegovina, Croatia and central Italy (Fig. 1). As a part of this continuing study (Adams et al. 2016, 2017, 2018; Adams 2014), we now report on the confirmation of *J. sabina* var. *balkanensis* from Albania.

#### MATERIAL AND METHODS

Specimens used in this and previous studies: (species, popn. id., location, collection numbers): See Adams et al. (2018) for previously analyzed specimen locations. Albania: North-Eastern Albanian Alps, above the road from Ceren to Radomira village, 41° 49' 43.86" N; 20° 27' 43.05" E. ca. 1150 m and above the village of Ceren, on path to Sorokol, 41° 49' 37.01" N; 20° 28' 28.13" E. ca. 1430 m. Coll. *Lulëzim Shuka s.n.* 22 June 2018. Lab Acc. *Adams 15506-15513* (8 samples). Voucher specimens for all collections are deposited at Baylor University Herbarium (BAYLU). One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions. Amplifications were performed in 30  $\mu$ l reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15  $\mu$ l 2x buffer E (trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200  $\mu$ M each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl<sub>2</sub> according to the buffer used) 1.8  $\mu$ M each primer. See Adams, Bartel and Price (2009) for the ITS primers utilized. The primers for trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010). The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. 2.31 (Technelysium Pty Ltd.).

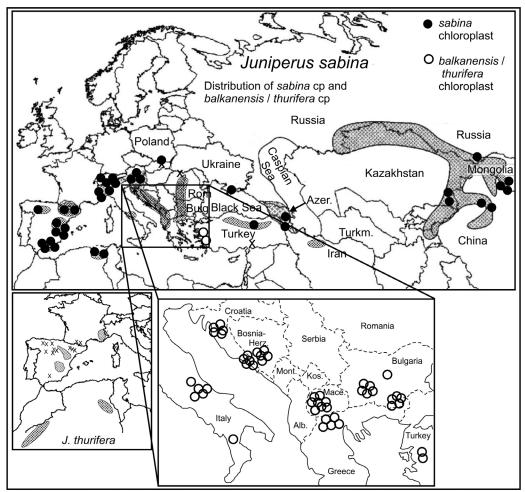


Figure 1. Distribution of *J. sabina* var. *balkanensis* and typical *J. sabina* chloroplast. The present day distributions of *J. thurifera* and var. *africana* (in north Africa) are shown in the insert on the lower left. (modified from Adams et al, 2018).

#### RESULTS

All 8 plants sampled in Albania were J. s. var. *balkanensis* based on cp DNA (Table 1). The revised distribution of v. *balkanensis* is shown in Figure 2. All known locations of v. *balkanensis* are in a relatively small geographical area.

Before discussing the nrDNA patterns, it should be noted that there are recent papers analyzing the inheritance of nrDNA in the Cupressaceae. Adams and Matsumoto (2016) analyzed 3 variable sites of nrDNA from synthetic crosses between *Cryptomeria japonica* cv. Haara and cv. Kumotooshi (= cv. Haara x Kumotooshi, ie., a backcross). They found that 3 of the 7 progeny had nrDNA very similar to that of the Haara x Kumo parent. In contrast, 4 of the 7 progeny had nrDNA exactly like the Haara parent. This appears to suggest that nrDNA polymorphisms can revert to that of a recurrent parent in the case of backcrossing.

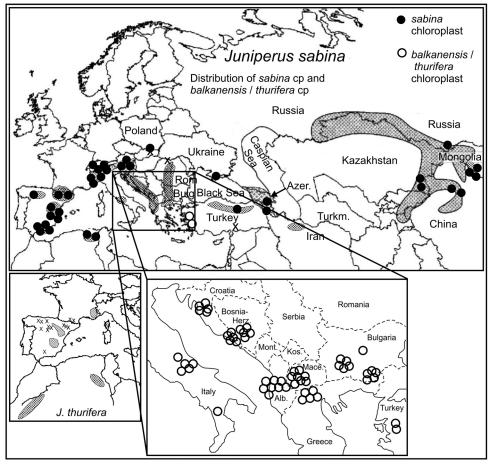


Figure 2. Revised distribution of *J. sabina var. sabina* (dark circles) and *J. s.* var. *balkanensis* (open circles) based on cp DNA (present study and from Adams et al. 2018)

Adams, Miller and Low (2016) examined 8 variable nrDNA sites in the parents (*Hesperocyparis arizonica*, *H. macrocarpa*), and their 18 artificial hybrid progeny. Each of the 18 hybrids were heterozygous for all 8 nrDNA sites. This study is very relevant to the present study, because *Hesperocyparis* (= *Cupressus* in the western hemisphere) is very closely related to *Juniperus* (Little et al. 2004, Terry, et al. 2012, Terry and Adams, 2015, Terry et al. 2016) and because there are no verified artificial hybrids of *Juniperus* available to the authors for the examination of the inheritance of nrDNA in *Juniperus*, the Adams, Miller and Low (2016) research on *Hesperocyparis* is surely applicable to *Juniperus*. So, we can confidently assume that nrDNA is inherited by complementation as found in *Hesperocyparis, Cryptomeria* and all other conifers. It should be noted that Adams, Miller and Low (2016) sequenced cp markers and confirmed that the cp genome is inherited via pollen (paternally inherited) in *Hesperocyparis* (and presumably *Juniperus*).

Aligning J. sabina and J. thurifera nrDNA sequences revealed that the taxa differ by SNPs at 22 sites, all in ITS1 or ITS2. Previously (Adams et al. 2018), a close examination of the nrDNA sequencing chromatograms revealed that generally, only 8 of the 22 sites contained heterozygous peaks. The 8 sites were (with position): 352(R), 391(S), 432(R), 606(M), 785(Y), 999(M), 1046(R), 1047(K). Two nrDNA types were found considering these 8 sites. Both of these types, type 1 (GGACCCAG) and type 2 (ACGACAGT), have been found in var. *balkanensis* and in var. *sabina* populations. Adams et al. (2018) reported the majority of the plants examined (42/62) were heterozygous for 1 to 8 sites, (Table 1). Note that 33/ 42 heterozygous individuals were heterozygous for all 8 sites. They reported the frequency of heterozygous sites was: 1,1,1,3,2,1,0,33 (for plants containing from 1 to 8 polymorphic sites, respectively).

In the Albania population, 2 plants (15508, 15511) were homozygous (for the 8 loci) and had the type 1 pattern: GGACCCAG. The other 6 plants were mostly heterozygous (Table 2). Of the 6 heterozygous plants, 15509 and 15510 were RSRYARK (7/8 heterozygous positions), and 15506, 15507, 15509, 15510, 15512, 15513 were RSRMYMRK (8/8 heterozygous positions) (Fig. 3). The type 2 ITS pattern (ACGACAGT, Adams et al. 2018) was not found among these 8 samples, but it surely must be present nearby, as the hybrids (between the 2 ITS types) were common.

Throughout the range of *J. sabina*, heterozygous plants are somewhat randomly distributed (Fig. 3). It is interesting that 4/4 Spain and 2/3 Switzerland plants were homozygous for the 8 sites. Only 2/7 plants from the far East were homozygous. Plants of var. *balkanensis* seem to be a bit more heterozygous (36/48 were heterozygous) than var. *sabina* (Fig. 3, Table 2).

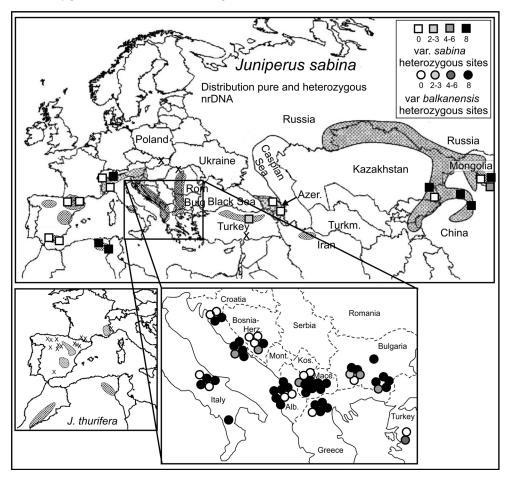


Figure 3. Distribution of homozygosity and heterozygosity among the 8 nrDNA polymorphic sites.

A more detailed examination of nrDNA type 1 (16/20) and type 2 (4/20) homozygous plants (Table 2) shows that both type 1 and type 2 plants were present in the Switzerland samples. One type 2 plant was found in Rila Mtn., Bulgaria and 2 were from Azerbaijan. All 16 type 1 nrDNAs share 4 bp sites with *J. thurifera*, and the four type 2 plants share 3 bp sites with *J. thurifera* (Table 2). It is strange that type 1 nrDNA contains 4 bp, typical of *J. thurifera* (in the 8 sites), and type 2 contain 3 different bp typical of *J. thurifera* (below and Table 2), but these are mutually exclusive in types 1 and 2.

most common sabina pattern (type 1)	GGACCCAG	4 sites in common with J. thurifera
7083, thurifera, France, Morocco <sup>2</sup>	AGGCTCGG	
2nd common sabina pattern (type 2)	ACGATAGT	4 sites in common with J. thurifera

Crossing type 1 (GGACCCAG) x type 2 (ACGACAGT) gives RSRMCMRK (see below), which differs at only site 5, from the putative hybrids (below and Table 1) of RSRMYMRK. Our data indicates that crossing between types 1 and 2 nrDNA types seem common, 46/70 plants were RSRMYMRK, however, none were RSRMCMRK! (the product of type 1 x type 2). These two nrDNA types may have arisen via hybridization with a *J. thurifera* ancestor and subsequent backcrossing to *J. sabina*.

most common sabina pattern (type 1)	GGACCCAG
2nd common sabina pattern (type 2)	A C G A T A G T
cross between J. sabina type1 x type2	R S R M Y M R K
Putative 'hybrid' pattern (table 1)	R S R M Y M R K

At our present level of understanding, the distributions of *J. s.* var. *balkanensis* and *J. thurifera* do not appear to overlap, negating modern hybridization. However, there were large changes in plant distributions in the Pleistocene and earlier, it seem probable that *J. thurifera*-like ancestors were sympatric with *J. sabina*, and presenting opportunities for chloroplast capture from *J. thurifera*.

#### ACKNOWLEDGEMENTS

This research was supported with funds provided by Baylor University on Project 0324512 to RPA.

#### LITERATURE CITED

Adams, R. P. 2014. The junipers of the world: The genus Juniperus. 4th ed. Trafford Publ., Victoria, BC.

Adams, R. P., J. A. Bartel and R. A. Price. 2009. A new genus, *Hesperocyparis*, for the cypresses of the new world. Phytologia 91: 160-185.

- Adams, R. P. and M. E. Kauffmann. 2010. Geographic variation in nrDNA and cp DNA of *Juniperus californica, J. grandis, J. occidentalis* and *J. osteosperma* (Cupressaceae). Phytologia 92: 266-276.
- Adams, R. P. and Asako Matsumoto. 2016. Inheritance of nrDNA in artificial hybrids of *Cryptomeria japonica* cv. *Haara* and *C. japonica* cv. *Kumotoshi*. Phytologia 98: 37-41.
- Adams, R. P., M. Miller and C. Low. 2016. Inheritance of nrDNA in artificial hybrids of *Hesperocyparis* arizonica x H. macrocarpa. Phytologia 98: 277-283.
- Adams, R., A. E. Schwarzbach and A. N. Tashev. 2016a. Chloroplast capture in *Juniperus sabina* var. *balkanensis* R. P. Adams and A. N. Tashev, from the Balkan peninsula: A new variety with a history of hybridization with *J. thurifera*. Phytologia 98: 100-111.

- Adams, R. P., A. Boratynski, T. Mataraci, A. N. Tashev and A. E. Schwarzbach. 2017. Discovery of *Juniperus sabina* var. *balkanensis* R. P. Adams and A. N. Tashev in southwestern Turkey. Phytologia 99: 22-31.
- Adams, R. P., A. Boratynski, K. Marcysiak, F. Roma-Marzio, L. Peruzzi, T. Mataraci, A. N. Tashev and S. Siljak-Yakovlev. 2018. Discovery of *Juniperus sabina* var. *balkanensis* R. P. Adams & Tashev in Macedonia, Bosnia-Herzegovina, Croatia and Central and southern Italy and relictual polymorphisms found in nrDNA. Phytologia 100: 117-127.
- Little, D. P., A. E. Schwarzbach, R. P. Adams and C-F. Hsieh. 2004. The circumscription and phylogenetic relationships of Callitropsis and the newly described genus *Xanthocyparis* (Cupressaceae). Am. J. Bot. 91: 1872-1881.
- Terry, R. G., J. A. Bartel and R. P. Adams. 2012. Phylogenetic relationships among the new world cypresses (Hesperocyparis; Cupressaceae): evidence from noncoding chloroplast DNA sequences. Plant Syst. Evol. DOI 10.1007/s00606-012-0696-3.
- Terry, R. G. and R. P. Adams. 2015. A molecular re-examination of phylogenetic relationships among Juniperus, Cupressus, and the Hesperocyparis-Callitropsis-Xanthocyparis clades of Cupressaceae. Phytologia 97: 67-75.
- Terry, R. G., M. I. Pyne, J. A. Bartel and R. P. Adams. 2016. A molecular biogeography of the New World cypresses (Callitropsis, Hesperocyparis; Cupressaceae). Plant Syst. Evol. DOI 10.1007/s00606-016-1308-4.

Table 1. Survey of *J. sabina* and classification based on ITS and trnS-trnG sequences. Putative hybrids and backcrosses [*J. sabina* x ancestor of *J. thurifera*] based on ITS polymorphisms are in **bold**.

				170
	trnS-trnG ITS		polymorphic sites <sup>1</sup>	ITS
coll. #, location	classification	classif.	1 2 3 4 5 6 7 8	#poly/
	(ie. cp genome)			8 sites
14861 Spil Daği, Turk., Boratynski	v. balkanensis	sabina	RGRCYCRG	4
14934 Spil Dagi, Turkey, Mataraci	v. balkanensis	sabina	GGACCCAG	0
13725 eastern Rhodopes, Bulgaria	v. balkanensis	sabina	GGAMYMRK	5
13726 eastern Rhodopes, Bulgaria	v. balkanensis	sabina	RSRMYMRK	8
13727 eastern Rhodopes, Bulgaria	v. balkanensis	sabina	RSRMYMRK	8
13728 eastern Rhodopes, Bulgaria	v. balkanensis	sabina	RSRMYMRK	8
13729 eastern Rhodopes, Bulgaria	v. balkanensis	sabina	RSRMYMRK	8
14721 Sokolna reserve, Bulgaria	v. balkanensis	sabina	RSRMYMRK	8
14722 Rila Mtn., Bulgaria	v. balkanensis	sabina	GGACYCAG	1
14723 Rila Mtn., Bulgaria	v. balkanensis	sabina	ACGATAGT	0
14724 Rila Mtn., Bulgaria	v. balkanensis	sabina	RGACYMAG	3
14725 Rila Mtn., Bulgaria	v. balkanensis	sabina	RSRMYMRK	8
14726 Rila Mtn., Bulgaria	v. balkanensis	sabina	RSRMYMRK	8
14727 Tsena Mtn., Greece	v. balkanensis	sabina	RSRMYMRK	8
14728 Tsena Mtn., Greece	v. balkanensis	sabina	GGACCCAG	0
14729 Tsena Mtn., Greece	v. balkanensis	sabina	RSRMYMRK	8
14730 Tsena Mtn., Greece	v. balkanensis	sabina	RSRMYMRK	8
14731 Tsena Mtn., Greece	v. balkanensis	sabina	RSRMYMRK	8
15311 Mavrovo, Macedonia	v. balkanensis	sabina	RSRMYMRK	8
15312 Mavrovo, Macedonia	v. balkanensis	sabina	RSRMYMRK	8
15313 Mavrovo, Macedonia	v. balkanensis	sabina	GGACCCAG	0
15314 Mavrovo, Macedonia	v. balkanensis	sabina	RSRMYMRK	8
15315 Mavrovo, Macedonia	v. balkanensis	sabina	RSRMYMRK	8
15316 Gaichnik, Macedonia	v. balkanensis	sabina	RSRMYMRK	8
15317 Gaichnik, Macedonia	v. balkanensis	sabina	RSRMYMRK	8
15318 Gaichnik, Macedonia	v. balkanensis	sabina	RSRMYMRK	8
15319 Gaichnik, Macedonia	v. balkanensis	sabina	GGACCCAG	0
15320 Gaichnik, Macedonia	v. balkanensis	sabina	RCRATMRK	5
15277 Mt. Cabulja, Bosnia-Herze.	v. balkanensis	sabina	RSRAYMRK	6
15278 Mt. Cvsnica, Bosnia-Herze.	v. balkanensis	sabina	RSRMYMRK	8

15279 Mt. Cabulja, Bosnia-Herze.	v. balkanensis	sabina	GGACCCAG	0
15280 Mt. Cabulja, Bosnia-Herze.	v. balkanensis	sabina	GGACCCAG	0
15281 Mt. Cabulja, Bosnia-Herze.	v. balkanensis	sabina	GGACCCAG	0
15282 Mt. Biokovo, Croatia	v. balkanensis	sabina	RSRMYMRK	8
15283 Mt. Biokovo, Croatia	v. balkanensis	sabina	GGACYMRK	4
15284 Mt. Biokovo, Croatia	v. balkanensis	sabina	RSRMYMRK	8
15285 Mt. Biokovo, Croatia	v. balkanensis	sabina	RSRMYMRK	8
15286 Mt. Biokovo, Croatia	v. balkanensis	sabina	RSRMYMRK	8
15343 Velebit, Croatia	v. balkanensis	sabina	GGACCCAG	0
15344 Velebit, Croatia	v. balkanensis	sabina	GGACCCAG	0
15345 Velebit, Croatia	v. balkanensis	sabina	RSRMYMRK	8
15346 Velebit, Croatia	v. balkanensis	sabina	RSRMYMRK	8
15365 Calabria area, southern Italy	v. balkanensis	sabina	RSRMYMRK	8
15413 Abruzzo area, central Italy	v. balkanensis	sabina	RSRMYMRK	8
15414 Abruzzo area, central Italy	v. balkanensis	sabina	RSRMYMRK	8
15415 Abruzzo area, central Italy	v. balkanensis	sabina	GGACCCAG	0
15416 Abruzzo area, central Italy	v. balkanensis	sabina	GGACCCAG	0
15414 Abruzzo area, central Italy	v. balkanensis	sabina	RSRMYMRK	8
15506 Ceren, Albania	v. balkanensis	sabina	RSRMYMRK	8
15507 Ceren, Albania	v. balkanensis	sabina	RSRMYMRK	8
15509 Ceren, Albania	v. balkanensis	sabina	RSRAYMRK	7
15510 Ceren, Albania	v. balkanensis	sabina	RSRAYMRK	7
15512 Ceren, Albania	v. balkanensis	sabina	RSRMYMRK	8
15513 Ceren, Albania	v. balkanensis	sabina	RSRMYMRK	8
15508 Ceren, Albania	v. balkanensis	sabina	GGACCCAG	0
15511 Ceren, Albania	v. balkanensis	sabina	GGACCCAG	0
7612 Switzerland	v. sabina	sabina	GCACCCAG	0
7614 Switzerland	v. sabina	sabina	ACGATAGT	0
14938 northeast Turkey Kandemir	v. sabina	sabina	RGRCTAGT	2
14316 Azerbaijan	v. sabina	sabina	ACGATAGT	0
14317 Azerbaijan	v. sabina	sabina	ACGATAGT	0
7811 Kazakhstan, Paniflor	v. sabina	sabina	RSRMYMRK	8
7812 Kazakhstan, Paniflor	v. sabina	sabina	GGACCCAG	0
7585 Mongolia, Altair Mtns.	v. sabina	sabina	RSRMYMRK	8
7586 Mongolia, Altair Mtns	v. sabina	sabina	ACGAYMRK	4
7587 Mongolia, Altair Mtns	v. sabina	sabina	GGACCCAG	0
7836 China, Heaven Lake, Xinjiang	v. sabina	sabina	RSRMYMRK	8
7837 China, Heaven Lake, Xinjiang	v. sabina	sabina	RSRMYMRK	8
most common sabina pattern	Туре 1		G G A C C C A G	Type 1
7083, thurifera, France <sup>2</sup>			AGGCTCGG	0
9420, thurifera v. africana, Morocco <sup>2</sup>			A G <b>G C</b> T C G G	0
2nd most common sabina pattern	Туре 2		A C G A T A G T	Type 2

<sup>1</sup>Eight polymorphic sites (1-8): R352, S391, R432, M606, Y785, M999, R1046, K1047.
350 xTGTCGGAG; 391 xGAGGTCCG; 432 xTCGTGTGC; 606 CGACAAGAx;
785(105) xCCAAAAGA; 999(333) xGCGAGGAG; 1046(392) xNGCGGTCGG;1047 xGCGGTCGG
<sup>2</sup>This pattern, A G G C T C G G, was also found in ALL *J. thurifera* samples examined, to date (14 *J. thurifera* samples from Corse, Morocco, France and Spain, Adams, et al. 2018)

	trnS-trnG	ITS	nrDNA	polymorphic sites <sup>1</sup>	# sites in common
coll. #, location	classification	classif.	type	1 2 3 4 5 67 8	with J. thurifera
14934 Spil Dagi, Turkey, Mataraci	v. balkanensis	sabina	1	GGACCCAG	4
14728 Tsena Mtn., Greece	v. balkanensis	sabina	1	GGACCCAG	4
15313 Mavrovo, Macedonia	v. balkanensis	sabina	1	GGACCCAG	4
15319 Gaichnik, Macedonia	v. balkanensis	sabina	1	GGACCCAG	4
15279 Mt. Cabulja, Bosnia-Herze.	v. balkanensis	sabina	1	GGACCCAG	4
15280 Mt. Cabulja, Bosnia-Herze.	v. balkanensis	sabina	1	GGACCCAG	4
15281 Mt. Cabulja, Bosnia-Herze.	v. balkanensis	sabina	1	GGACCCAG	4
15343 Velebit, Croatia	v. balkanensis	sabina	1	GGACCCAG	4
15344 Velebit, Croatia	v. balkanensis	sabina	1	GGACCCAG	4
15508 Ceren, Albania	v. balkanensis	sabina	1	GGACCCAG	4
15508 Ceren, Albania	v. balkanensis	sabina	1	GGACCCAG	4
7197 Sierra Nevada, Granada, Spain	v. sabina	sabina	1	GGACCCAG	4
7199 Sierra Nevada, Granada, Spain	v. sabina	sabina	1	GGACCCAG	4
7573 Sallent deGallego, Spain	v. sabina	sabina	1	GGACCCAG	4
7574 Sallent deGallego, Spain	v. sabina	sabina	1	GGACCCAG	4
7812 Kazakhstan, Paniflor	v. sabina	sabina	1	GGACCCAG	4
7587 Mongolia, Altair Mtns	v. sabina	sabina	1	GGACCCAG	4,
7612 Switzerland	v. sabina	sabina	1	GGACCCAG	4 Note Type 1!
7614 Switzerland	v. sabina	sabina	2	ACGATAGT	3, Note Type 2!
14723 Rila Mtn., Bulgaria	v. balkanensis	sabina	2	ACGATAGT	3
14316 Azerbaijan	v. sabina	sabina	2	ACGATAGT	3
14317 Azerbaijan	v. sabina	sabina	2	ACGATAGT	3
				bases in common	
				(bold) w thruifera	
most common sabina pattern(type 1)			1	GGACCCAG	4 sites in common
7083, thurifera, France, Morocco <sup>2</sup>				AGGCTCGG	8
2nd common sabina pattern(type 2)			2	<b>A</b> C <b>G</b> A <i>C</i> A <b>G</b> T	3 sites in common
14861 Spil Daği, Turk., Boratynski	v. balkanensis	sabina		RGRCYCRG	4
13725 eastern Rhodopes, Bulgaria	v. balkanensis	sabina		G G A M Y M R K	5
14722 Rila Mtn., Bulgaria	v. balkanensis	sabina		GGACYCAG	1
14724 Rila Mtn., Bulgaria	v. balkanensis	sabina		RGACYMAG	3
15320 Gaichnik, Macedonia	v. balkanensis	sabina		R C R A T M R K	5
15277 Mt. Cabulja, Bosnia-Herze.	v. balkanensis	sabina		RSRAYMRK	6
15283 Mt. Biokovo, Croatia	v. balkanensis	sabina		G G A C Y M R K	4
14938 northeast Turkey Kandemir	v. sabina	sabina		RGRCTAGT	2
7586 Mongolia, Altair Mtns	v. sabina	sabina		A C G A Y M R K	4
most common sabina pattern)			Type 1	GGACCCAG	
2nd common sabina pattern			Type 2	A C G A T A G T	
cross between J. sabina type1 x type2			1x2	R S R M Y M R K	
Putative 'hybrid' pattern (table 1)				R S R M Y M R K	

Table 2. *Juniperus sabina* classified based on ITS homozygous for all 8 polymorphic sites. Putative hybrids (heterozygous for the 8 polymorphic sites) were excluded.

<sup>1</sup>Eight polymorphic sites (1-8): R352, S391, R432, M606, Y785, M999, R1046, K1047. <sup>2</sup>This pattern, AGGCTCGG, was found in all *J. thurifera* samples examined, to date (14 *J. thurifera* samples from Corse, Morocco, France and Spain, Adams, et al. 2018)