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# Discovery of *Juniperus sabina* var. *balkanensis* R. P. Adams and A. N. Tashev in Macedonia, Bosnia-Herzegovina, Croatia and Central and Southern Italy and relictual polymorphisms found in nrDNA

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## ABSTRACT

Additional analyses of trnS-trnG and nrDNA from specimens from Bosnia-Herzegovina, southern and central, Italy, Croatia and Macedonia revealed the presence of *J. sabina* var. *balkanensis* in these areas west of the previously known populations in Greece, Bulgaria and western Turkey. Careful chromatogram analysis of eight (8) polymorphic sites in nrDNA revealed that nearly all of the populations of both var. *balkanensis* and var. *sabina* contained from 2 to 8 polymorphic sites. For these 8 heterozygous sites, two exclusive patterns were found in *J. sabina*. One type (GGACCCAG) was found in 16/62 plants and type 2 (ACGACAGT) was found in 4/62 plants. The majority of the plants examined (42/62) were heterozygous for 1 to 8 sites. These two nrDNA types appear to have arisen via hybridization with a *J. thurifera* ancestor. The two types appear in both v. *sabina* and v. *balkanensis* populations. Extant putative hybrids appear to have formed by crosses between present day type 1 and type 2 nrDNA. Published on-line www.phytologia.org *Phytologia 100(2): 117-127 (Jun 22, 2018).* ISSN 030319430.

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

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*Juniperus sabina* var. *balkanensis* R. P. Adams & A. N. Tashev appears to be a product of hybrid origin between *J. sabina* L. and an ancestor of *J. thurifera* (Adams et al. 2016). Subsequent backcrossing to *J. sabina* (var. *sabina*) is hypothesized to have resulted in the capture of ancestral '*J. thurifera* like' chloroplast by *J. sabina* var. *balkanensis*. In *Juniperus*, Terry et al. (2000) suggested that chloroplast capture was involved in the distribution of cp haplotypes in *J. osteosperma* in western North America. More recently, Adams (2015a, b) found widespread hybridization and introgression between *J. maritima* and *J. scopulorum* in the Pacific northwest, with introgression from *J. maritima* into *J. scopulorum* eastward into Montana. The disparity between cpDNA and nuclear markers (nrDNA and maldehy) suggested that cp capture had occurred. In *Pinus* and other conifers, Hipkins et al. (1994) concluded that "past hybridization and associated 'chloroplast capture' can confuse the phylogenies of conifers.' Bouille et al. (2011) found significant topological differences in phylogenetic trees based on cpDNA (vs. mtDNA sequences) in *Picea* that suggested organelle capture. *Juniperus sabina* L. is a smooth leaf-margined, multi-seeded juniper of the eastern hemisphere. It is very widely distributed from Spain through Europe to Kazakhstan, western China, Mongolia and Siberia (Fig. 1, Adams 2014). *Juniperus sabina* has a range that is discontinuous between Europe and central Asia; the species is generally a shrub less than 1 m tall and ranges up to 1-2 m wide. However, in the Sierra Nevada of Spain, *J. sabina* is a horizontal shrub.

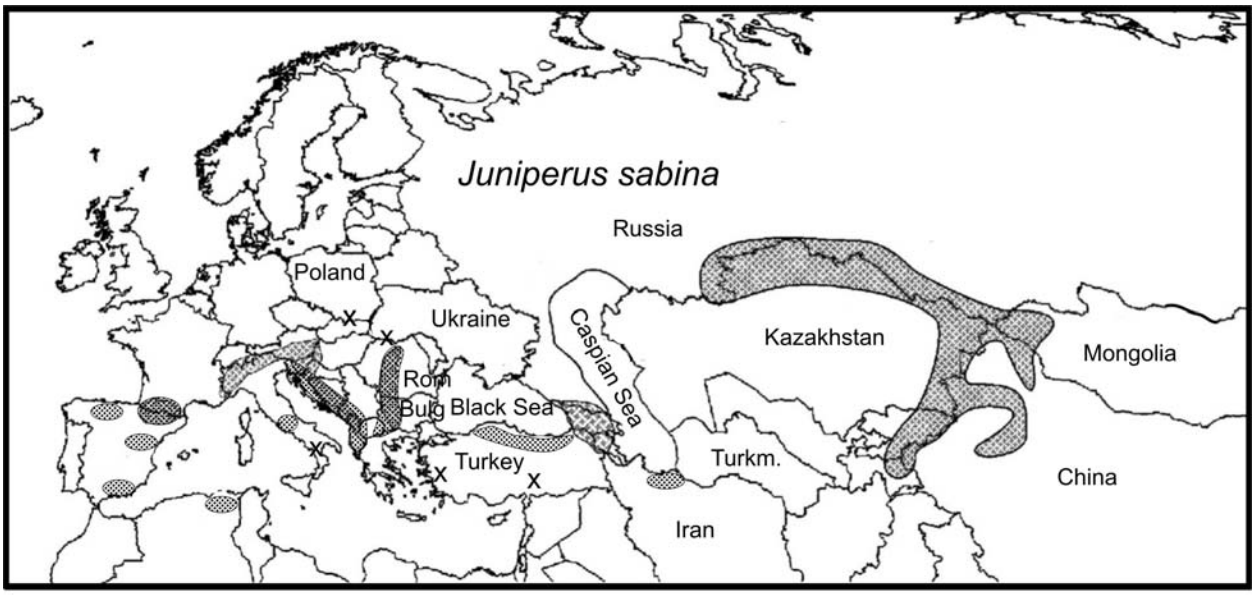


Fig. 1. Distribution (shaded areas) of *J. sabina*. x = outlying populations of *J. sabina*.

Adams et al. (2016) showed that nrDNA (ITS) did not resolve *J. sabina* populations due to the lack of sequence variation. However, their analyses (Adams et al., 2016) of cp DNA (petN-psbM, trnSG, trnDT, trnLF) revealed that *J. sabina* contained two kinds of cpDNA: typical *J. sabina* and that of *J. sabina* var. *balkanensis* cpDNA in a clade with *J. thurifera*. They recognized the taxon with the *J. thurifera* type cpDNA as a new variety: *J. s*. var. *balkanensis* R. P. Adams & A. N. Tashev. *Juniperus sabina* var. *balkanensis* was known only from sloping rocky limestone, at 1240 - 1630m, in the mountains of Bulgaria and northern Greece.

Subsequently, Adams et al. (2017), using samples from herbarium specimens, discovered two samples from far western Turkey that were *J. s*. var. *balkanensis*. However, numerous samples from

throughout Europe were confirmed to be *J. s.* var. *sabina* (Fig. 2). Due to the proximity of the Macedonia - Bosnia-Herzegovina - Croatia region to the populations of *J. s*. var. *balkanensis* in Greece, it seemed prudent to make additional collections and analyses of *J. sabina* plants from these areas to more precisely determine the distribution of *J. sabina* var. *balkanensis*.

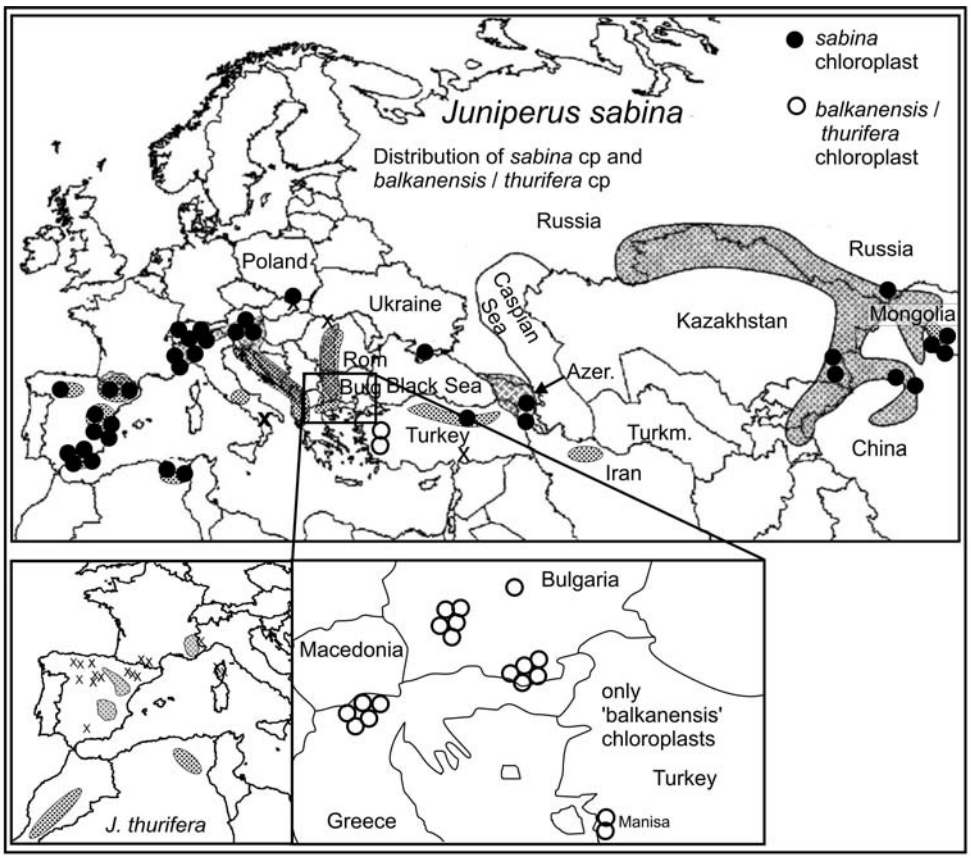


Figure 2. Distribution of *J. sabina* var. *balkanensis* and typical *J. sabina* chloroplast. The present day distributions of *J. thurifera* and var. *africana* (in north Africa) are shown in the insert on the lower left. (modified from Adams et al, 2017).

## MATERIAL AND METHODS

*Specimens used in this and previous studies:* (species, popn. id., location, collection numbers): *J. chinensis*, CH, Lanzhou, Gansu, China, *Adams 6765-6767*; *J. sabina*: (SN), Sierra Nevada, Spain, *Adams 7197, 7199, 7200*; (PY), Pyrenees Mtns., Spain/ France border, *Adams 7573-7577*; (SW), Switzerland, *Adams 7611, 7612, 7614, 7615*; TS, Tian Shan Mtns., Xinjiang, China, *Adams 7836-7838*; Mongolia, Altai Mtns., *Adams 7585-7587*; Kazakhstan, Paniflor, *Adams 7811-7812*; Azerbaijan: *Adams 14316-14320*;

*J. davurica* (DV), 15 km se Ulan Bator, Mongolia, *Adams 7252, 7253, 7601*; *J. davurica* var. *arenaria* (AR) sand dunes, Lake Qinghai, Qinghai, China, *Adams 10347-10352*; river bank, Gansu, *J-Q. Liu and Adams 10354-10356*; *J. davurica* var. *mongolensis* (MS) sand dunes, 80 km sw Ulan Bator, Mongolia, *Adams 7254-7256*;

*Collections of taxon with non-J. sabina cpDNA in Adams, Schwarzbach and Tashev (2016): (acronyms used in Fig. 7)*

**Bulgaria and Greece**

B1-B5 Eastern Rhodopes. In protected site "Gumurdjinsky Snejnik", locality "Madzharsky Kidik". On limestone rocks above the upper border of a forest of *Fagus sylvatica* ssp. *moesiaca* with *Juniperus communis*. 41° 14' 44.7" N; 25° 15' 31.9" E. elev. 1270 m, 13 Aug. 2012, *Adams 13725-13729 (A. Tashev 2012-1-5*);

B6 Central Stara Planina (the Balkan). National Park "Central Balkan". Reserve "Sokolna". On a steep, rocky limestone slope, with *Sorbus aucuparia, S. aria, S. borbasii, Amelanchier ovalis, Carpinus orientalis, Sesleria latifolia, Pastinaca hirsute, Cephalanthera rubra, Laserpitium siler, Hieracium alpicola* etc. near a forest of *Fagus sylvatica.* 42º42'13.3" N,25º08'10.4" E, 1501 m , 22.08.2015. Bulgaria, *Adams 14721 (A. Tashev 2015 Balkan 1*;

B7-B9, Ba, Bb Rila Mountain, National Park "Rila". On the eco-path, "Beli Iskar", near river Beli Iskar, in a forest with *Pinus sylvestris, P. peuce, Picea abies, Abies alba, Juniperus communis, J. sibirica, Vaccinium myrtillus, Rosa canina, Sorbus aucuparia, Acer hyrcanum, Chamaespartium sagittale, Hypericum perforatum, Thymus* sp. etc. 42º14'26.5" N, 23º32'33.8" E, 1242 m, 24.06.2015. Bulgaria, *Adams 14722-14726 (A. Tashev 2015 Rila 1.1-1.3, 2.1-2.2)*;

G1-G5 Mt. Tsena, Greece, *Adams 14727-14731* (*A. Tashev 2015 So. 1-5 Tsena*);

**Turkey**

*14861* Turkey, Manisa. Spil Dağı Milli Parki (National Park) (Tas Suret), N38.55°, E 27.42°, ca 1250 m alt., leg. A. Boratyński, K. Boratyńska, 2005, TU_05/55, KOR 44573, female

*14934* Turkey, Manisa, Spil Dağı Milli Parki (National Park), N38°, 57', E 27° 41', 1024 m., *Tuğrul Mataraci 2016-1*

*14938* Turkey, Gŭmŭshane, Kŭrtŭn, Aktas village, Karakaya (Northeast Anatolia), 40° 36' 03" N, 38° 53' 21" E., 2376 m. Coll. *A. Kandemir 10745*.

*Samples new for this study: (with Lab Acc. ID = Adams xxxxx)*

**Bosnia-Herzogovian**

*Juniperus sabina* var. *balkanensis*, on calcareous, dolomite, between Mt. Cvrsnica and Mt. Cabulja, 43° 34' 18.09" N, 17° 30' 39.88" E., 1460m, 7 June 2017, Bosnia-Herzogovina, Coll. F. Bogunic & Sonja Siljak-Yakovlev, Lab Acc. Robert P. Adams *15277-15281.*

**Croatia**

*Juniperus sabina* var. *balkanensis*, Mt. Biokovo, Vosac, Strbina, 1200 m, NB! 4n. 43° 18' 34.9" N, 17° 02' 36.3" E., 1300m, 15 June 2017, Croatia, Coll. Sonja Siljak-Yakovlev, Lab Acc. Robert P. Adams *15282-15286.*

*Juniperus sabina* var. *balkanensis*, 44°32'36" N, 15°10'09" E, 1080 m, fall 2017, Country: Velebit, Croatia, Coll. Katarzyna Marcysiak, Lab Acc. Robert P. Adams *15343-15346*.

**Macedonia**

*Juniperus sabina* var. *balkanensis*, 41° 39' 18.16" N, 20° 44' 01.21" E., 4519 ft, Oct 2017, Macedonia, Mavrovo area. Coll. Katarzyna Marcysiak, Lab Acc. Robert P. Adams *15311-15315.*

*Juniperus sabina* var. *balkanensis*, 41° 35' 46.61" N, 20° 39' 04.94" E., 4755 ft, Oct 2017, Macedonia, Gaichnik area, Coll. Katarzyna Marcysiak, Lab Acc. Robert P. Adams *15316-15320.*

**Italy**

*Juniperus sabina* var. *balkanensis*, P.N. del Pollino (San Lorenzo Bellizzi, CS), Timpa di San Lorenzo, su roccia calcarea, leaves from herbarium specimen [in Pisa, (PI)], 39.91349° N, 16.28578° E, 1436 m, 18 Aug 2017, Calabria, Italy, Coll. Francesco Roma-Marzio et L. Peruzzi, Lab Acc. Robert P. Adams *15365*

*Juniperus sabina* var. *balkanensis*. Valle di Selva Romana (Pennapiedimonte, Chieti), pendii rupestri, 1600-1900 m, 42° 07' 41" N, 14° 07' 18" W., 5411 ft, 01 Aug 2002, Abruzzo, Italy, Coll. F. Conti (APP No. 1701), ex Fabrizio Bartolucci, received 14 Apr 2018, Lab Acc. *Robert P. Adams 15413*

*Juniperus sabina* var. *balkanensis*. Vallone di Pennapiedimonte (Pennapiedimonte, Chieti), pascoli, 800-900 m, 42° 09' 01" N, 14° 11' 02" W., 2017 ft, 05 May 1999, Abruzzo, Italy, Coll. F. Conti (APP No. 13142), ex Fabrizio Bartolucci, received 14 Apr 2018, Lab Acc. *Robert P. Adams 15414*

*Juniperus sabina* var. *balkanensis*. Gran Sasso - M.te Camicia, loc. il Gravone (Castelli, Teramo), pendii rupestri, 1700 m, 42° 26' 45" N, 13° 44' 06" W., 5484 ft, 02 Nov 1996, Abruzzo, Italy, Coll. F. Conti (APP No. 17471), ex Fabrizio Bartolucci, received 14 Apr 2018, Lab Acc. *Robert P. Adams 15415*

*Juniperus sabina* var. *balkanensis*. M. S. Domenico (Pizzoferrato, Chieti), 41° 55' 28" N, 14° 14' 12" W., 3967 ft, 17 June 1995, Abruzzo, Italy, Coll. F. Conti (APP No. 33835), ex Fabrizio Bartolucci, received 14 Apr 2018, Lab Acc. *Robert P. Adams 15416*

*Juniperus sabina* var. *balkanensis*. Scatafosse (Villavallelonga, L'Aquila), 41° 47' 55" N, 13° 41' 13" W., 5809 ft, 01 Aug 2001, Abruzzo, Italy, Coll. F. Conti (APP No. 50172), ex Fabrizio Bartolucci, received 14 Apr 2018, Lab Acc. *Robert P. Adams 15417*

Voucher specimens for all collections are deposited at Baylor University Herbarium (BAYLU), Herbarium (University of Forestry, Sofia, Bulgaria) and Herbarium Apenninicum (APP).

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$^{o}$ 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 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (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 $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and 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.).

## RESULTS

All the plants sampled in Macedonia, Bosnia-Herzegovina, Croatia and Calabria and Abruzzo areas of Italy were *J. s*. var. *balkanensis* based on cp DNA (Table 1). The revised distribution of v. *balkanensis* is shown in Figure 3. 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. 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

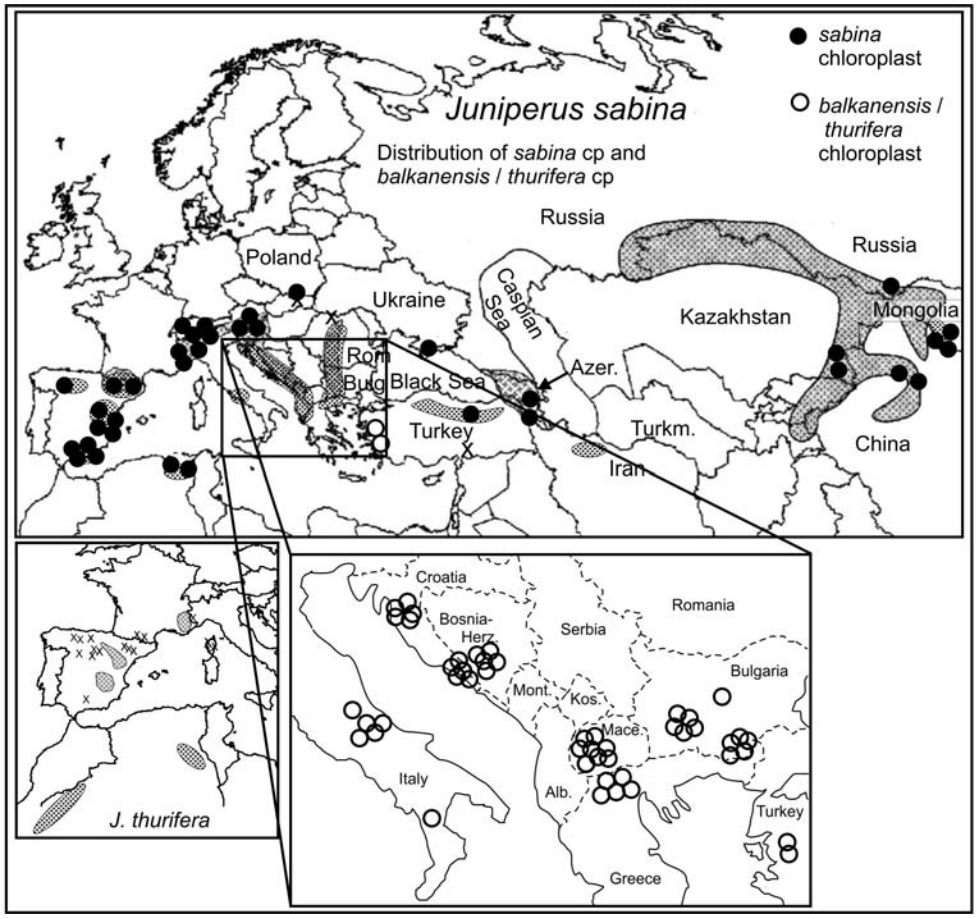


Figure 3. Revised distribution of *J. sabina var. sabina* (dark circles) and *J. s.* var. *balkanensis* (open circles) based on cp DNA (present study and from Adams et al. 2017)

nrDNA in *Juniperus*, the Adams, Miller and Low (2016) research on *Hesperocyparis* stands as a proxy for the inheritance of nrDNA in *Juniperus* and thus, their study in *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. However, a close examination of the nrDNA sequencing chromatograms revealed that 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. One nrDNA type (GGACCCAG) was found in 16/62 plants and type 2 (ACGACAGT) was found in 4/62 plants. 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. The frequency of heterozygous sites was: 1,1,1,3,2,1,0,33 (for plants containing from 1 to 8 polymorphic sites, respectively). Heterozygous plants are somewhat randomly distributed (Fig. 4). 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 (only 12/48 were homozygous, Fig. 4).

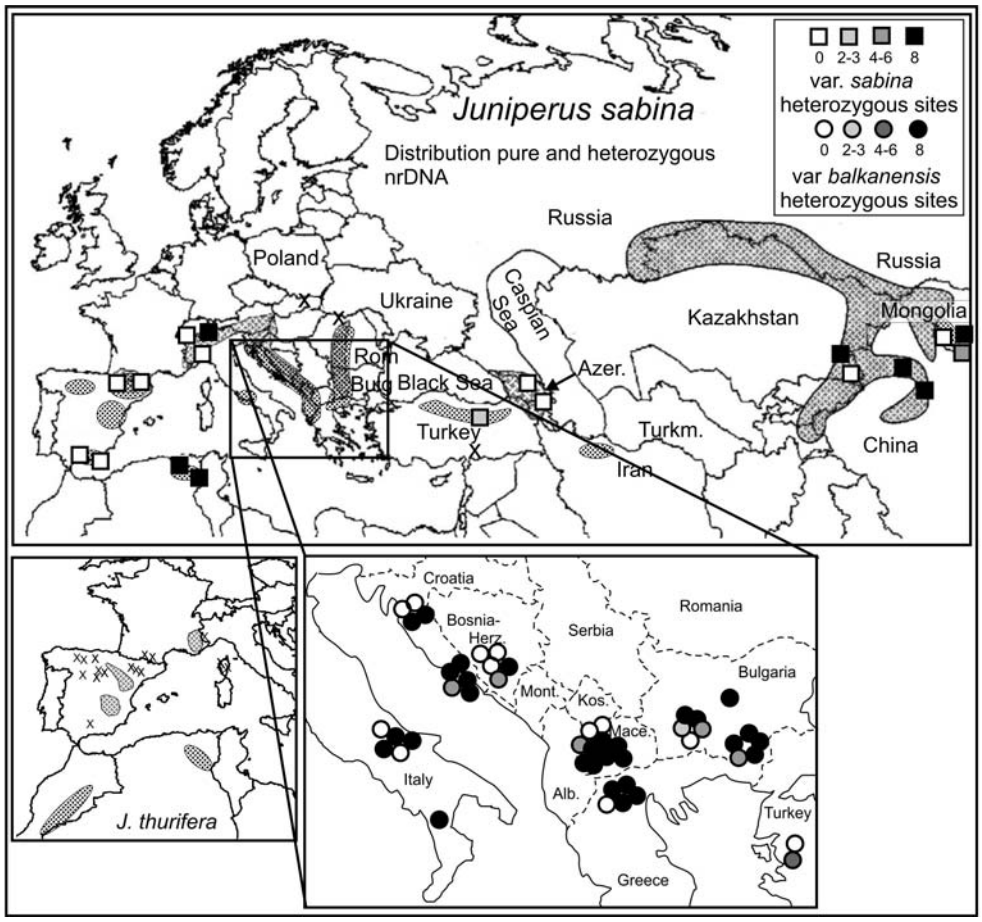


Figure 4. Distribution of homozygosity and heterozygosity among the 8 nrDNA polymorphic sites.

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 2 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) | G **G** A **C** *C* **C** A **G** | 4 sites in common with *J. thurifera* |
| **7083, thurifera, France, Morocco[2]** | **A G G C** *T* **C G G** | |
| 2nd common sabina pattern (type 2) | **A** C **G** A *C* A **G** T | 3 sites in common with *J. thurifera* |

Crossing type 1 (GGACCCAG) x type 2 (ACGA*C*AGT) gives RSRM*C*MRK (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, 42/62 plants were RSRMYMRK, however, none were RSRM*C*MRK! (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) | G G A C C C A G |
| 2nd common sabina pattern (type 2) | A C G A C A G T |
| cross between *J. sabina* type1 x type2 | R S R M C 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

Thanks of A. Kandemir and F. Bogunic for the specimens of *J. sabina* respectively from Aktas village, northern Turkey and Mt Cvrsnica, Bosnia and Herzegovina. This research was supported with funds provided by Baylor University.

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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**.

| coll. #, location | trnS-trnG classification (ie. cp genome) | ITS classif. | polymorphic sites[1] 1 2 3 4 5 6 7 8 | ITS #poly/ 8 sites |
|---|---|---|---|---|
| **14861 Spil Dağı, Turk., Boratynski** | **v. *balkanensis*** | ***sabina*** | **R G R C Y C R G** | **4** |
| 14934 Spil Dagi, Turkey, Mataraci | v. *balkanensis* | *sabina* | G G A C C C A G | 0 |
| **13725 eastern Rhodopes, Bulgaria** | **v. *balkanensis*** | ***sabina*** | **G G A M Y M R K** | **5** |
| **13726 eastern Rhodopes, Bulgaria** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **13727 eastern Rhodopes, Bulgaria** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **13728 eastern Rhodopes, Bulgaria** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **13729 eastern Rhodopes, Bulgaria** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **14721 Sokolna reserve, Bulgaria** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **14722 Rila Mtn., Bulgaria** | **v. *balkanensis*** | ***sabina*** | **G G A C Y C A G** | **1** |
| 14723 Rila Mtn., Bulgaria | v. *balkanensis* | *sabina* | A C G A T A G T | 0 |
| **14724 Rila Mtn., Bulgaria** | **v. *balkanensis*** | ***sabina*** | **R G A C Y M A G** | **3** |
| **14725 Rila Mtn., Bulgaria** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **14726 Rila Mtn., Bulgaria** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **14727 Tsena Mtn., Greece** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| 14728 Tsena Mtn., Greece | v. *balkanensis* | *sabina* | G G A C C C A G | 0 |
| **14729 Tsena Mtn., Greece** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **14730 Tsena Mtn., Greece** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **14731 Tsena Mtn., Greece** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **15311 Mavrovo, Macedonia** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **15312 Mavrovo, Macedonia** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| 15313 Mavrovo, Macedonia | v. *balkanensis* | *sabina* | G G A C C C A G | 0 |
| **15314 Mavrovo, Macedonia** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **15315 Mavrovo, Macedonia** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **15316 Gaichnik, Macedonia** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **15317 Gaichnik, Macedonia** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **15318 Gaichnik, Macedonia** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| 15319 Gaichnik, Macedonia | v. *balkanensis* | *sabina* | G G A C C C A G | 0 |
| **15320 Gaichnik, Macedonia** | **v. *balkanensis*** | ***sabina*** | **R C R A T M R K** | **5** |
| **15277 Mt. Cabulja, Bosnia-Herze.** | **v. *balkanensis*** | ***sabina*** | **R S R A Y M R K** | **6** |
| **15278 Mt. Cvsnica, Bosnia-Herze.** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| 15279 Mt. Cabulja, Bosnia-Herze. | v. *balkanensis* | *sabina* | G G A C C C A G | 0 |
| 15280 Mt. Cabulja, Bosnia-Herze. | v. *balkanensis* | *sabina* | G G A C C C A G | 0 |
| 15281 Mt. Cabulja, Bosnia-Herze. | v. *balkanensis* | *sabina* | G G A C C C A G | 0 |
| **15282 Mt. Biokovo, Croatia** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **15283 Mt. Biokovo, Croatia** | **v. *balkanensis*** | ***sabina*** | **G G A C Y M R K** | **4** |
| **15284 Mt. Biokovo, Croatia** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **15285 Mt. Biokovo, Croatia** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **15286 Mt. Biokovo, Croatia** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| 15343 Velebit, Croatia | v. *balkanensis* | *sabina* | G G A C C C A G | 0 |
| 15344 Velebit, Croatia | v. *balkanensis* | *sabina* | G G A C C C A G | 0 |
| **15345 Velebit, Croatia** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **15346 Velebit, Croatia** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |

| | | | | |
|---|---|---|---|---|
| **15365 Calabria area, southern Italy** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **15413 Abruzzo area, central Italy** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **15414 Abruzzo area, central Italy** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **15415 Abruzzo area, central Italy** | **v. *balkanensis*** | ***sabina*** | G G A C C C A G | 0 |
| **15416 Abruzzo area, central Italy** | **v. *balkanensis*** | ***sabina*** | G G A C C C A G | 0 |
| **15414 Abruzzo area, central Italy** | **v. *balkanensis*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **13167 Algeria** | **v. *sabina*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **13168 Algeria** | **v. *sabina*** | ***sabina*** | **R S R M Y M R K** | **8** |
| 7197 Sierra Nevada, Granada, Spain | v. *sabina* | *sabina* | G G A C C C A G | 0 |
| 7199 Sierra Nevada, Granada, Spain | v. *sabina* | *sabina* | G G A C C C A G | 0 |
| 7573 Sallent deGallego, Spain | v. *sabina* | *sabina* | G G A C C C A G | 0 |
| 7574 Sallent deGallego, Spain | v. *sabina* | *sabina* | G G A C C C A G | 0 |
| **7611 Switzerland** | **v. *sabina*** | ***sabina*** | **R S R M Y M R K** | **8** |
| 7612 Switzerland | v. *sabina* | *sabina* | G G A C C C A G | 0 |
| 7614 Switzerland | v. *sabina* | *sabina* | A G G A T A G T | 0 |
| **14938 northeast Turkey Kandemir** | **v. *sabina*** | ***sabina*** | **R G R C T A G T** | **2** |
| 14316 Azerbaijan | v. *sabina* | *sabina* | A C G A C A G T | 0 |
| 14317 Azerbaijan | v. *sabina* | *sabina* | A C G A C A G T | 0 |
| **7811 Kazakhstan, Paniflor** | **v. *sabina*** | ***sabina*** | **R S R M Y M R K** | **8** |
| 7812 Kazakhstan, Paniflor | v. *sabina* | *sabina* | G G A C C C A G | 0 |
| **7585 Mongolia, Altair Mtns.** | **v. *sabina*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **7586 Mongolia, Altair Mtns** | **v. *sabina*** | ***sabina*** | **A C G A Y M R K** | **4** |
| 7587 Mongolia, Altair Mtns | v. *sabina* | *sabina* | G G A C C C A G | 0 |
| **7836 China, Heaven Lake, Xinjiang** | **v. *sabina*** | ***sabina*** | **R S R M Y M R K** | **8** |
| **7837 China, Heaven Lake, Xinjiang** | **v. *sabina*** | ***sabina*** | **R S R M Y M R K** | **8** |
| most common sabina pattern | | | G **G** A C C C A G | 0 |
| 7083, thurifera, France[2] | | | A **G** G C T C G G | 0 |
| 9420, thurifera v. africana, Morocco[2] | | | A G G C T C G G | 0 |
| 2nd most common sabina pattern | | | A C G A C A G T | 0 |
| | | | | |

[1]Eight polymorphic sites (1-8): R352, S391, R432, M606, Y785, M999, R1046, K1047.
[2]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, unpublished)

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

| coll. #, location | trnS-trnG classification | ITS classif. | nrDNA type | polymorphic sites[1] 1 2 3 4 5 6 7 8 | # sites in common with *J. thurifera* |
|---|---|---|---|---|---|
| 14934 Spil Dagi, Turkey, Mataraci | v. *balkanensis* | *sabina* | 1 | G G A C C C A G | 4 |
| 14728 Tsena Mtn., Greece | v. *balkanensis* | *sabina* | 1 | G G A C C C A G | 4 |
| 15313 Mavrovo, Macedonia | v. *balkanensis* | *sabina* | 1 | G G A C C C A G | 4 |
| 15319 Gaichnik, Macedonia | v. *balkanensis* | *sabina* | 1 | G G A C C C A G | 4 |
| 15279 Mt. Cabulja, Bosnia-Herze. | v. *balkanensis* | *sabina* | 1 | G G A C C C A G | 4 |
| 15280 Mt. Cabulja, Bosnia-Herze. | v. *balkanensis* | *sabina* | 1 | G G A C C C A G | 4 |
| 15281 Mt. Cabulja, Bosnia-Herze. | v. *balkanensis* | *sabina* | 1 | G G A C C C A G | 4 |
| 15343 Velebit, Croatia | v. *balkanensis* | *sabina* | 1 | G G A C C C A G | 4 |
| 15344 Velebit, Croatia | v. *balkanensis* | *sabina* | 1 | G G A C C C A G | 4 |
| 7197 Sierra Nevada, Granada, Spain | v. *sabina* | *sabina* | 1 | G G A C C C A G | 4 |
| 7199 Sierra Nevada, Granada, Spain | v. *sabina* | *sabina* | 1 | G G A C C C A G | 4 |
| 7573 Sallent deGallego, Spain | v. *sabina* | *sabina* | 1 | G G A C C C A G | 4 |
| 7574 Sallent deGallego, Spain | v. *sabina* | *sabina* | 1 | G G A C C C A G | 4 |
| 7812 Kazakhstan, Paniflor | v. *sabina* | *sabina* | 1 | G G A C C C A G | 4 |
| 7587 Mongolia, Altair Mtns | v. *sabina* | *sabina* | 1 | G G A C C C A G | 4 |
| 14723 Rila Mtn., Bulgaria | v. *balkanensis* | *sabina* | 2 | A C G A T A G T | 3 |
| **7612 Switzerland** | **v. *sabina*** | ***sabina*** | **1** | **G G A C C C A G** | **4, Note Type 1!** |
| **7614 Switzerland** | **v. *sabina*** | ***sabina*** | **2** | **A G G A T A G T** | **3, Note Type 2!** |
| 14316 Azerbaijan | v. *sabina* | *sabina* | 2 | A C G A C A G T | 3 |
| 14317 Azerbaijan | v. *sabina* | *sabina* | 2 | A C G A C A G T | 3 |
| | | | | bases in common (**bold**) w *thruifera* | |
| most common sabina pattern(type 1) | | | 1 | G **G** A **C** *C* **C** A **G** | 4 sites in common |
| **7083, thurifera, France, Morocco[2]** | | | | **A G G C** *T* **C G G** | 8 |
| 2nd common sabina pattern(type 2) | | | 2 | **A** C **G** A *C* A **G** T | 3 sites in common |
| 14861 Spil Dağı, Turk., Boratynski | v. *balkanensis* | *sabina* | | R G R C Y C R G | 4 |
| 13725 eastern Rhodopes, Bulgaria | v. *balkanensis* | *sabina* | | G G A M Y M R K | 5 |
| 14722 Rila Mtn., Bulgaria | v. *balkanensis* | *sabina* | | G G A C Y C A G | 1 |
| 14724 Rila Mtn., Bulgaria | v. *balkanensis* | *sabina* | | R G A C Y M A G | 3 |
| 15320 Gaichnik, Macedonia | v. *balkanensis* | *sabina* | | R C R A T M R K | 5 |
| 15277 Mt. Cabulja, Bosnia-Herze. | v. *balkanensis* | *sabina* | | R S R A Y M R K | 6 |
| 15283 Mt. Biokovo, Croatia | v. *balkanensis* | *sabina* | | G G A C Y M R K | 4 |
| 14938 northeast Turkey Kandemir | v. *sabina* | *sabina* | | R G R C T A G T | 2 |
| 7586 Mongolia, Altair Mtns | v. *sabina* | *sabina* | | A C G A Y M R K | 4 |
| | | | | | |
| most common sabina pattern(type 1) | | | 1 | G G A C C C A G | |
| 2nd common sabina pattern(type 2) | | | 2 | A C G A C A G T | |
| cross between *J. sabina* type1 x type2 | | | 1x2 | R S R M C M R K | |
| Putative 'hybrid' pattern (table 1) | | | | R S R M Y M R K | |

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