

**GEOGRAPHIC VARIATION AND SYSTEMATICS OF
JUNIPERUS PHOENICEA L. FROM MADEIRA AND THE
CANARY ISLANDS: SNPS FROM nrDNA and petN-psbM**

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ABSTRACT

SNPs from nrDNA and petN-psbM (cpDNA) were analyzed of *Juniperus phoenicea* from Madeira, Canary Islands, Morocco, and El Penon, Spain along with *J. p. var. turbinata* from the Tarifa sand dunes, Spain. The analysis of the 18 SNPs revealed that the Macaronesian and Moroccan plants are identical or nearly so to *J. p. var. turbinata* and quite differentiated from *J. phoenicea* var. *phoenicea*. In contrast, the leaf terpenoids showed that the oils of the Macaronesian plants are more similar to the Moroccan plants and not as similar to *J. phoenicea* or *J. p. var. turbinata*. At present, it seems prudent to treat the Madeira and Canary Island plants as *J. p. var. turbinata*. *Phytologia* 92(1):59-67 (April, 2010).

KEY WORDS: *Juniperus phoenicea*, *J. p. var. turbinata*, Cupressaceae, Madeira Island, Canary Islands, leaf essential oils, SNPs, nrDNA, petN-psbM.

Juniperus phoenicea L. is a very variable species of the Mediterranean region (Adams, 2008). The Canary Islands red-fruited, scale-leaf juniper was described as *J. canariensis* Guyot & Mathou,

(Trav. Lab. Forest. Toulouse T. 1 [3, 2]: 7. 1942) and later treated as *J. turbinata* Guss. subsp. *canariensis* (Guyot & Mathou) Rivas-Martinez et al. (Itinera Geobot. 7: 511. 1933). Farjon (2005) treated *J. canariensis* as a synonym of *J. phoenicea* var. *phoenicea* and recognized *J. phoenicea* var. *turbinata* (Guss.) Nyman as "restricted to littoral maritime habitats on rocks or sand dunes." in the Mediterranean in France, Spain, Portugal, Greece, Italy, Morocco, and Tunisia." Farjon (2005) treated the Macaronesian plants as *J. p.* var. *phoenicea*. Adams et al. (1996, 2002) showed that *J. p.* var. *turbinata* and *J. p.* subsp. *eu-mediterranea* Lebr. & Thiv. were the same taxon. Adams (2008, map p. 243,) treated the Madeira and the Canary Islands *J. phoenicea* as "var. *turbinata* ?"

RAPDs analysis (Adams et al., 2006) of *J. phoenicea* from sand and rock areas in Morocco, plants from Tenerife, Canary Islands and var. *turbinata*, Tarifa sand dunes, Spain showed (Fig. 1) that var. *phoenicea* (El Penon, Spain) was well resolved from the Morocco, Tenerife and var. *turbinata* populations. The Tenerife population accounted for about 14%

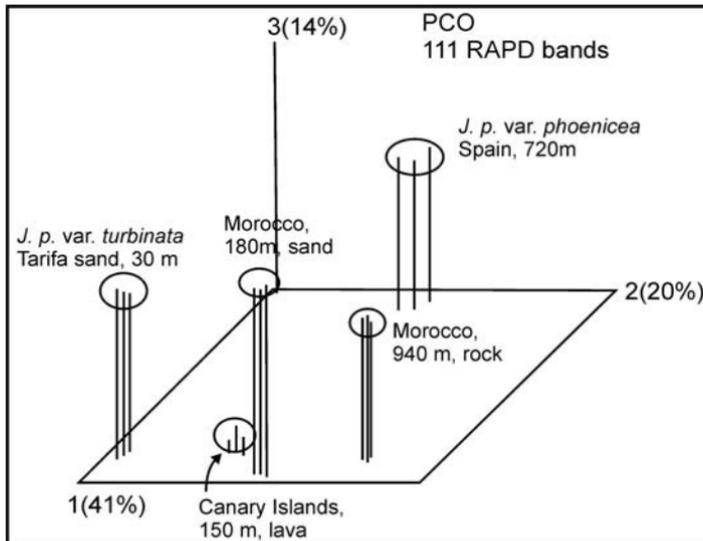


Figure 1. PCO ordination of *J. phoenicea* populations based on 111 RAPD bands. From Adams et al. (2006).

of the variance (Fig. 1). Although, the Canary Island plants are loosely associated with var. *turbinata*, they generally have large, round berries (seed cones), not turbinate-shaped.

Adams et al. (2009) reported on the volatile leaf oil compositions of populations of *J. phoenicea* from several islands in the Canarian archipelago and Madeira, and compared these oils with *J. p.* var. *phoenicea* (Iberian Peninsula, Spain) and var. *turbinata* (Tarifa sand dunes, Iberian Peninsula, Spain) oils. They found that the oils varied a little among the islands but Madeira, La Palma, Tenerife, and La Gomera do form a group (Fig. 2). However, the oils are also similar to Morocco (0.70) and less similar to var. *phoenicea* (Spain) and var. *turbinata* (Spain) (Fig. 2).

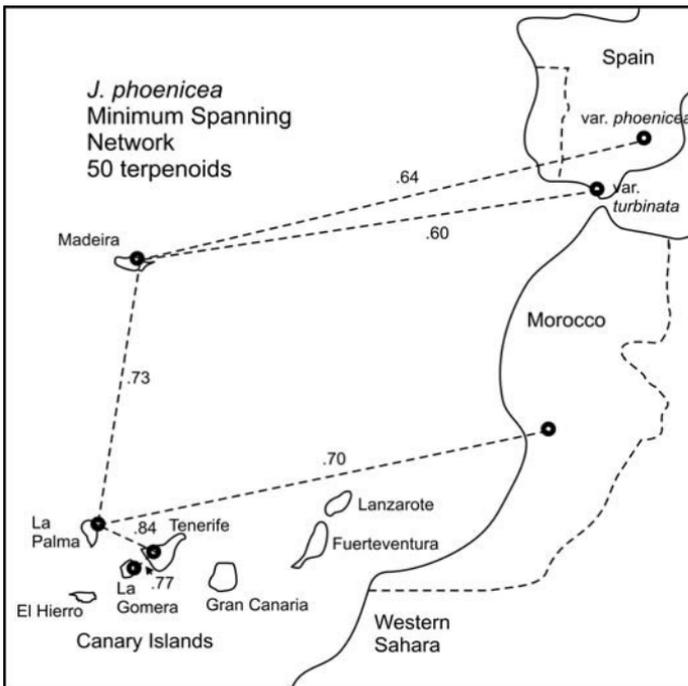


Figure 2. Minimum spanning network based on 50 terpenoids. From Adams et al. (2009).

The purpose of this paper is to report on analyses of SNPs from nrDNA and petN-psbM (cp DNA) and to compare the DNA data with the previous terpenoid analysis.

MATERIALS AND METHODS

Plant material (GenBank Accession: ITS, petN-psbM) - *J. phoenicea* Madeira Island: 1 km NE of Santana, 32° 48.822'N, 16° 52.627'W, ca 100 m, *R. P. Adams 11502, 11503*, cultivated at Botanic Garden in Funchal, putatively ex Madeira Island, 32° 39.08'N, 16° 47.14'W, ca 200 m, *R. P. Adams 11504*; Canary Islands: Tenerife, volcanic rock, ca. 150 m, *R. P. Adams 8147-8149*, La Palma Island, Santa Lucia, loose volcanic pumice, 28° 44.250'N, 17° 44.198'W, 283 m, *R. P. Adams 11514-11516*, La Gomera Island, volcanic rock, 28° 11.358'N, 17° 12.320'W, 370 m, *R. P. Adams 11528(GU197868, GU197871)-115230*; Spain, limestone soil, 25 km e. Guadahortuna, 720 m, El Penon, *R. P. Adams, 7077(GU197869, GU197873)-7079*; Morocco, red clay, 20 km sse Marrakech, 31° 21.033'N, 07° 45.893'W, 940 m, *R. P. Adams 9408-9410*; Iberian Peninsula, Spain, *J. phoenicea* var. *turbinata*: Tarifa sand dunes, 15 km w. of Tarifa, 30 m, 36° 04.996'N, 5° 42.104' W, *R. P. Adams, 7202(GU197870, GU197873)-7204*. Voucher specimens are deposited at the Herbarium, Baylor University (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 as per manufacturer's instructions.

PCR amplification ITS (nrDNA), petN-psbM 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-psbM) 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 MgCl₂ 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 PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the 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. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments and NJ trees were made using MAFFT (<http://align.bmr.kyushu-u.ac.jp/mafft/>). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009).

Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

Sequencing nrDNA revealed 15 nucleotide mutational events that included 3 indels and 3 mutations that occurred in only one individual among the taxa. The 3 single nucleotide changes were discarded from the SNPs leaving 12 nrDNA characters.

Sequencing petN-psbM revealed 8 nucleotide mutational events that included a 19 bp deletion in one of the plants from El Penon, Spain and 1 mutation that occurred in only one individual among the taxa. Discarding these 2 singular events provided 6 petN-psbM characters.

PCO using the 18 SNPs resulted in eigenroots that accounted for 58% (axis 1), 24% (axis 2) and 9% (axis 3) of the variance among the individuals. Ordination shows that the major trend was to separate *J. phoenicea* var. *phoenicea* (El Penon, Spain) from all other individuals (Fig. 3). The two natural trees from Madeira Island and 2 trees from La Gomera appear to share the same cpDNA petN-psbM in that they differ by 3 SNPs from the other Canary Island trees. It is interesting that var. *turbinata* (Tarifa sand dunes, Spain) is identical

with one Canary Islands tree (from Tenerife) and differs by only one SNP from most other Canary Island plants (Fig. 3). The *J. phoenicea* plant cultivated at Botanic Garden in Funchal (11504), putatively ex Madeira Island, differs by 6 SNPs from *J. phoenicea*, naturally growing on Madeira (Fig. 3). It seems likely that the cultivated plant actually came from the Canary Islands. Note that the 2 plants nearest to the 2 natural Madeira plants (Fig. 3) are from La Gomera. The 3 SNPs that separate these La Gomera plants from the bulk of the Canary Island plants are in the cpDNA region. So it may be that long distance dispersal between La Gomera and Madeira has caused this result.

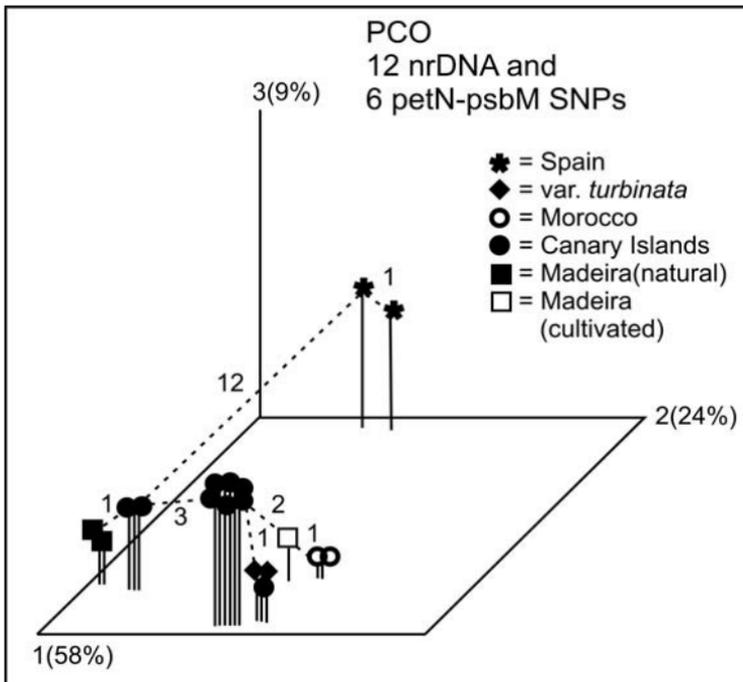


Figure 3. PCO based on 12 nrDNA and 6 petN-psbM SNPs. The numbers on the dashed lines are the number of SNPs in the minimum linkage network.

A minimum spanning network superimposed on a geographic map gives a little different perspective (Fig. 4). The large difference between *J. phoenicea* var. *phoenicea* (Spain) and *J. phoenicea* of the Canary Islands is clearly seen. The small or no differences between plants very widely separated is also apparent (cf. Morocco - cult. Madeira plant; Madeira (natural) - La Gomera; Tenerife plant - var. *turbinata*, Spain).

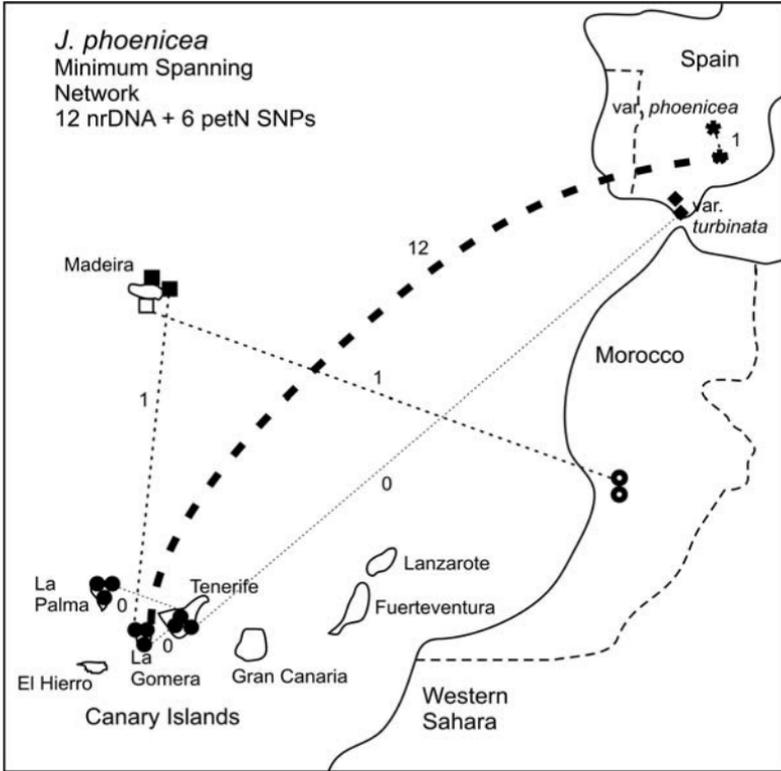


Figure 4. Minimum spanning network based on 12 nrDNA plus 6 petN-psbM SNPs. The numbers next the dashed lines are the number of SNPs separating the OTUs. Not all links are shown due to congestion on the small islands.

Both the terpenoids and SNPs clearly show that the Canary Island and Madeira *J. phoenicea* is quite different from *J. phoenicea* var. *phoenicea* (El Penon, Spain). Both data sets show a close affinity of plants from the Canary Island and Madeira to plants from Morocco. However, there is a marked difference in how the data depicts the relationship of *J. phoenicea* var. *turbinata* to Madeira and Canary Island junipers. The terpenoid data showed var. *turbinata* to be the least similar (0.60) to any island junipers. In contrast, the SNPs data showed var. *turbinata* to be indistinguishable from a tree on Tenerife and almost identical to most plants from the Canary Islands, Madeira and Morocco. Although the terpenoid data supports the recognition of *J. p.* subsp. *canariensis*, the SNPs analysis does not support it and favors the recognition of the Canary Islands and Madeira *J. phoenicea* trees as *J. p.* var. *turbinata*.

It seems probable that evolution is proceeding at different rates in the terpenoids than in the nrDNA and petN-psbM DNA. It may be that the terpenoids are under stronger adaptive selection pressure and reflect the differences in the survival ecology on the Tarifa sand dunes of Spain, versus the volcanic islands of Madeira and the Canary Islands. Certainly these habitats differ considerably in the environment, herbivores, insects and diseases faced by the junipers.

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LITERATURE CITED

- Adams R. P. 1975. Statistical character weighting and similarity stability. *Brittonia* 27: 305-316.
- Adams, R. P., 2008. Junipers of the world: The genus *Juniperus*. 2nd Ed., Trafford Publ., Vancouver, B.C., Canada.

- Adams, R. P., A. F. Barrero and A. Lara. 1996. Comparisons of the leaf essential oils of *Juniperus phoenicea* L., *J. phoenicea* subsp. *eu-mediterranea* Lebr. & Thiv. and *J. phoenicea* var. *turbinata* (Guss.) Parl. J. Essent. Oil Res. 8: 367-371.
- Adams, R. P. J. A. Bartel and R. A. Price. 2009. A new genus, *Hesperocypris*, for the cypresses of the new world. Phytologia 91: 160-185.
- Adams, R. P., S. Nguyen and N. Achak. 2006. Geographic variation in *Juniperus phoenicea* (Cupressaceae) from the Canary Islands, Morocco and Spain based on RAPDs analysis. Phytologia 88: 270-278.
- Adams, R. P., B. Rumeu, M. Nogales and S. S. Fontinha. 2009. Geographic variation and systematics of *Juniperus phoenicea* L. from Madeira and the Canary Islands: Analyses of volatile leaf oils. Phytologia 91: 40-53.
- Adams, R. P., R. N. Pandey, Serge Rezzi and Joseph Casanova. 2002. Geographic variation in the Random Amplified Polymorphic DNAs (RAPDs) of *Juniperus phoenicea*, *J. p.* var. *canariensis*, *J. p.* subsp. *eu-mediterranea*, and *J. p.* var. *turbinata*. Biochem. Syst. Ecol. 30: 223-229.
- Farjon, A. 2005. A monograph of Cupressaceae and *Sciadopitys*. Royal Botanic Gardens, Kew, London.
- Gaussen, H. 1968. Les Cupressaceae Fasc. X in Les Gymnospermes, Actuelles et Fossiles. Lab. Forest, Univ. Toulouse, France.
- Gower J. C. 1971. A general coefficient of similarity and some of its properties. Biometrics 27: 857-874.
- Gower J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 326-338.
- Veldman D. J. 1967. Fortran programming for the behavioral sciences. Holt, Rinehart and Winston Publ., NY.