Hybridization and introgression between *Juniperus communis* var. *saxatilis* and var. *hemispherica* in the Pyrenees Mountains, France.

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ABSTRACT

An investigation of variation between *J. communis* var. *hemispherica* and var. *saxatilis* in the Pyrenees revealed 5 SNPs and 1 indel (in nrDNA) that distinguished the varieties and revealed one var. *hemispherica* plant (15401) and 23 var. *saxatilis* plants. Plant 15401 had 3/5 informative sites typical of var. *hemispherica* and the other 2 bases were heterozygous, indicative of a backcross of var. *saxatilis* into var. hemispherica. Chloroplast (cp) petN-psbM did not clearly separate var. *hemispherica* and var. *saxatilis*. Although no plants of *J. c.* var. *hemispherica* was found, the presence of a backcross, indicates *J. c.* var. *hemispherica* either grows in the region or perhaps, long distance transfer of pollen. Published on-line www.phytologia.org *Phytologia 102(1): 9-13 (March 22, 2020).* ISSN 030319430.
KEY WORDS: *Juniperus communis* var. *saxatilis, J. c.* var. *hemispherica,* Pyrenees, hybridization, introgression, nrDNA, cpDNA, petN-psbM.

Juniperus communis L. is the only juniper species that grows in both the eastern and western hemispheres (Adams, 2014). It contains at least 10 varieties (Fig. 1) that are poorly resolved by nrDNA and cpDNA (Adams and Schwarzbach, 2013, Adams 2014). *Juniperus communis* var. *hemispherica* (J. & C. Presl.) Parl., described from a shrub growing on the flanks of Mt. Etna, Sicily, differs by 5 nrDNA SNPs and 1 indel (Adams and Schwarzbach, 2013) and seems one of the most distinct varieties (in its DNA, Fig. 1). Yet, the discovery of additional populations in Europe has been futile (Adams 2014). Prostrate juniper plants in Sierra Nevada, Spain, generally called var. *hemispherica*, not small shrubs as in Sicily. However, they do appear to be var. *hemispherica* (orange, Fig. 1).

To investigate the more northern range of *J. c.* var. *hemispherica*, we collected samples of horizontal, putative var. *hemispherica* from Pyrenees and examined nrDNA (ITS) and cp petN-psbM DNA sequences.

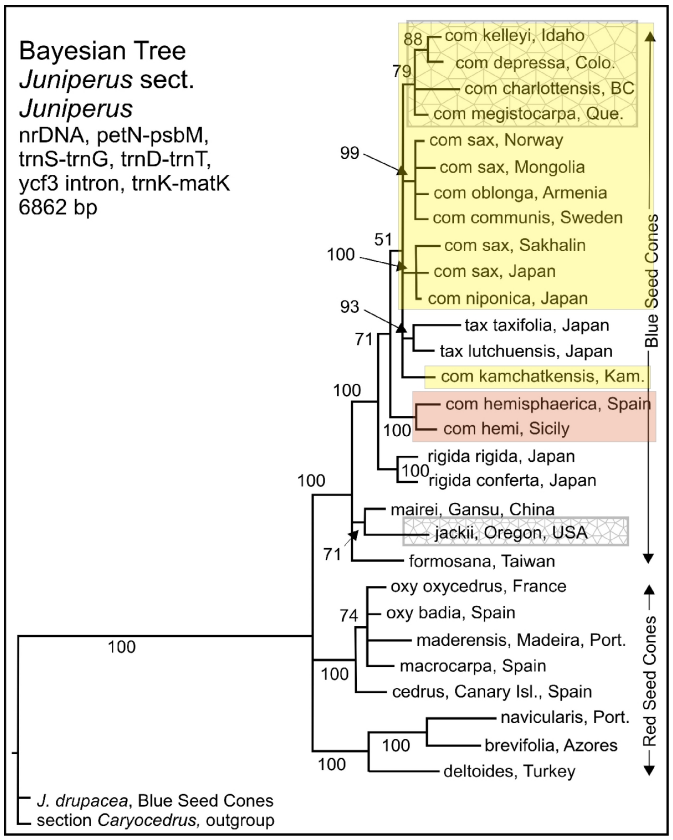

Figure 1. Bayesian tree of *Juniperus* sect. *Juniperus* (adapted from Adams and Schwarzbach, 2012).

MATERIAL AND METHODS

Specimens used in this study:
Juniperus communis var. *hemispherica*:
France:
horizontal plants, on limestone. Opoul, Pyrénées-orientales, France, Mount Montoulier de Périllos, 42.907° N 02.943° E, 600 m, Feb. 2018, coll. *Marc Espeut, ns 1,2,3,4*, 23 Lab Acc. *Robert P. Adams 15401,15402,15403,15404,*
horizontal plants, on limestone. Opoul, Pyrénées-orientales, France, Mount Montoulier de Périllos, 42° 54' 42.82" N, 2° 50' 37.47" W. 630 m, 8 March 2019, Coll. Marc Espeut ns 1-20, Lab Acc. Robert P. Adams 15581-15600(20), all horizontal, except 15598 was a sub-shrub.
Spain:
prostrate to 0.5m tall, with *J. sabina*. Sierra Nevada, s. of Granada, Spain. 37˚ 06' 17" N 3˚ 24' 51" W, 2100m, 20 Oct. 1993.Coll. *Robert P. Adams 7194-7195.*
prostrate to 0.2m tall x 3-5 m wide, abundant at Ski area, Sierra Nevada, s. of Granada, Spain. 37° 06' 02.54" N, 03° 24' 00.55" W 2024 m, 6 June 2019, Coll. *Robert P. Adams 15702-15703*

See Adams and Schwarzbach. (2013) for previously analyzed specimen locations.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20º 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 (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 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.).

RESULTS

Analyses of 24 putative var. *hemispherica* plants revealed one var. *hemispherica* (15401) plant and 23 var. *saxatilis* plants (Table 1). Plant 15401 had 3/5 nucleotides typical of var. *hemispherica* and 2 sites that were heterozygous (purple, Table 1), indicative of its being a backcross of var. *saxatilis* into var. *hemispherica*. Data for *J. communis* var. *communis* (Sweden) and *J. c.* var. *saxatilis* (Norway) were included the analyses and it is interesting that they are not distinguished from *J. c*. var. *saxatilis* of Pyrenees (Table 1). The cp petN-psbM data did not clearly separate var. *hemispherica* and var. *saxatilis* (Table 1). Analyses of trnS-trnG, trnD-trnT, and trnL-trnF failed to find SNPs that distinguish var. *hemispherica* and var. *saxatilis* (Adams unpublished).

Two of the samples of var. *hemispherica* from Sierra Nevada (7194, 7195) have an A at site 1149 suggesting they are backcrossed from var. *saxatilis* (Table 1). They also have a T at cp petN-psbM site 305, that is common in the var. *saxatilis* from the Pyrenees. Two plants (15589, 15590, Table 1) are heterozygous at site 305 (W = A/T), but perhaps with is just a local mutation.

Table 1. SNPs and indels from nrDNA (ITS) and cp petN-psbM for *J. communis* from the Pyrenees. The 7 bp duplication-insert was not found in *Juniperus* sect. *Juniperus*, and appears to be unique to the Pyrenees. nrDNA sites highlighted in yellow are informative sites between var. *hemispherica* and var. *saxatilis* (and var. *communis*). Site values highlighted in Gold are unusual mutations, but not informative to distinguish known taxa.

		nrDNA (ITS) sites[1]										cp DNA (petN-psbM) sites**	
acc. no.	nrDNA classif.	Indel[2]	S1	S2	S3[2]	S4[2]	S5[2]	S6	S7	S8[2]	S9[2]	cp S1[2]	7 bp cp Indel
		205	277	305	400	404	414	428	602	642	1149	305	535
9045	hemi Sicily	TTT	C	T	A	C	C	C	A	T	G	C	GTAATTAC - - - - - - -
9046	hemi Sicily	TTT	C	T	A	C	C	C	A	T	G	C	GTAATTAC - - - - - - -
15702	hemi S Nev	TTT	C	T	A	C	C	C	A	T	G	C	GTAATTAC - - - - - - -
15703	hemi S Nev	TTT	C	T	A	C	C	C	A	T	G	C	GTAATTAC - - - - - - -
7194	hemi BC,t1 Sax S Nev	TTT	G	T	A	C	C	C	A	T	A	T	GTAATTAC - - - - - - -
7195	hemi BC,t1 Sax S Nev	TTT	G	T	A	C	C	C	A	T	A	T	GTAATTAC - - - - - - -
15401	hemi BC,t2 sax Pyr	TTT	C	T	A	C	C	C	A	Y	R	C	GTAATTAC - - - - - - -
11206	sax,Nor.	---	C	T	G	T	T	C	A	C	A	C	GTAATTAC - - - - - - -
11207	sax,Nor.	---	C	T	G	T	T	C	A	C	A	C	GTAATTAC - - - - - - -
7846	com,Swed	---	C	T	G	T	T	C	A	C	A	C	GTAATTAC - - - - - - -
7847	com,Swed	---	C	T	G	T	T	G	A	C	A	C	GTAATTAC - - - - - - -
15583	sax Pyr	---	C	T	G	T	T	C	A	C	A	C	GTAATTAC - - - - - - -
15586	sax Pyr	---	C	T	G	T	T	C	A	C	A	C	GTAATTAC - - - - - - -
15587	sax Pyr	---	C	T	G	T	T	C	A	C	A	C	GTAATTAC - - - - - - -
15589	sax Pyr	---	C	W	G	T	T	C	A	C	A	C	GTAATTAC - - - - - - -
15595	sax Pyr	---	C	T	G	T	T	C	A	C	A	C	GTAATTAC - - - - - - -
15598	sax Pyr	---	C	T	G	T	T	C	A	C	A	C	GTAATTAC - - - - - - -
15592	sax Pyr	---	C	T	G	T	T	C	A	C	A	C	GTAATTAC - - - - - - -
15581	sax Pyr	---	C	T	G	T	T	C	A	C	A	T	GTAATTAC - - - - - - -
15582	sax Pyr	---	C	T	G	T	T	C	A	C	A	T	GTAATTAC - - - - - - -
15584	sax Pyr	---	C	T	G	T	T	C	A	C	A	T	GTAATTAC - - - - - - -
15585	sax Pyr	---	C	T	G	T	T	C	A	C	A	T	GTAATTAC - - - - - - -
15588	sax Pyr	---	C	T	G	T	T	C	A	C	A	T	GTAATTAC - - - - - - -
15403	sax Pyr	---	C	T	G	T	T	C	A	C	A	T	GTAATTAC - - - - - - -
15591	sax Pyr	---	C	T	G	T	T	C	A	C	A	T	GTAATTAC - - - - - - -
15594	sax Pyr	---	C	T	G	T	T	C	A	C	A	T	GTAATTAC - - - - - - -
15596	sax Pyr	---	C	T	G	T	T	C	A	C	A	T	GTAATTAC - - - - - - -
15597	sax Pyr	---	C	T	G	T	T	C	A	C	A	T	GTAATTAC - - - - - - -
15599	sax Pyr	---	C	T	G	T	T	C	A	C	A	T	GTAATTAC - - - - - - -
15600	sax Pyr	---	C	T	G	T	T	C	A	C	A	T	GTAATTAC - - - - - - -
15402	sax Pyr	---	C	T	G	T	T	C	G	C	A	C	GTAATTACTAATTAC
15404	sax Pyr	---	C	T	G	T	T	C	G	C	A	C	GTAATTACTAATTAC
15590	sax Pyr	---	C	W	G	T	T	C	A	C	A	C	GTAATTACTAATTAC
15593	sax Pyr	---	C	T	G	T	T	C	A	C	A	C	GTAATTACTAATTAC

[1]Indel 205: xxxTGCTGGACGG; S1,277:xGTGGATTCCC; S2,305:xCGGGCGCAAA; S3,400: GGACGTCCGx; S4,404: GGACGTCCGNGGCCx;S5,414: xTGAGATTT; S6,428: xTCGGTCGTG; S7,602: xCGACTCTCCC; S8,642: XGGGGCGGGG; S9,1149: xTCTTTGGTG.

**cp S1,305: xGAACCATAC; site 536:GTAATTACxxxxxxx, where xxxxxxx = TAATTAC or TATTACT (7bp insert).

[2]informative sites

The 7 bp duplication-insert at site 535 in cp petN-psbM (15402, 15404, 14409, 15593, Table 1) is not common in these Pyrenees data. The duplication-insert was not found in any juniper species in a search of *Juniperus* sect. *Juniperus*, (Adams and Schwarzbach, 2013) and appears to be unique to the Pyrenees population.

The discovery and verification of the presence of a backcross of var. *saxatilis* into var. *hemispherica* in the Pyrenees expands our knowledge of the range of *J. communis* var. *hemispherica*. Although it appears to be a small semi-globose shrub (hence the name *hemispherica*) near the type locality in Sicily (Fig. 2), it also grows as a nearly prostrate shrub on Mt. Etna, Sicily (Fig. 3). In the Sierra Nevada it is a very prostrate shrub (Fig. 4) and a nearly prostrate shrub in the Pyrenees, France (Fig. 5). Additional population research is needed to better understand its distribution.

Figure 2. *J. c.* var. *hemispherica* as a shrub at type locality, slopes of Mt. Etna, Sicily.

Fig. 3. *J. c*. var. *hemispherica*, spreading shrub, at a second location on the slopes of Mt. Etna. Photo from Pietro Miniscale.

Fig. 4. *J. c.* var. *hemispherica* as a very prostrate shrub, Sierra Nevada, Spain.

Fig. 5. *J. c.* var. *hemispherica* is a nearly prostrate shrub in the Pyrenees, France.

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