JUNIPERUS MARITIMA, THE SEASIDE JUNIPER, A NEW SPECIES FROM PUGET SOUND, NORTH AMERICA

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ABSTRACT

Based on analyses of terpenoids, nrDNA and trnC-D SNPs as well as morphology and ecology, a new cryptic species, ***Juniperus maritima***, from the Puget Sound region is recognized. The species, previously included in *J. scopulorum*, is characterized by having seed cones that mature in one year (14-16 months), seeds usually exserted from the cone, obtuse scale leaf tips, usually reniform seed cones, scale leaves overlap less than 1/5 the length, and branchlets smooth and reddish-brown. Called the seaside juniper, it grows on rocky areas (rarely sand dunes) near the sea, in Puget Sound.

KEY WORDS: *Juniperus maritima*, Puget Sound, *J. scopulorum*, *J. virginiana*, cryptic species, terpenoids, nrDNA, trnC-trnD, SNPs.

The smooth leaf margined (40X) junipers in the western hemisphere are very widespread and are composed of the Caribbean *Juniperus*: *J. barbadensis* L., *J. bermudiana* L., *J. gracilior* Pilg. , *J. g.* var. *ekmanii* (Florin) R. P. Adams, *J. g.* var. *urbaniana* (Pilg. & Ekman) R. P. Adams, *J. lucayana* Britt., and *J. saxicola* Britt. & P. Wilson; the Mexican junipers: *J. blancoi* Mart. var. *blancoi*, *J. b*. var. *huehuentensis* R. P. Adams, S. Gonzales & M. G. Elizondo, and *J. mucronata* R. P. Adams and the Canada/ United States junipers: *J. horizontalis* Moench, *J. scopulorum* Sarg., *J. virginiana* L. and *J. v*. var. *silicicola* (Small) E. Murray (Adams, 2004).

Juniperus scopulorum and *J. virginiana* are weedy junipers that occupy millions of acres in the United States and Canada. Adams (1983) analyzed the leaf terpenoids of populations of *J. scopulorum*

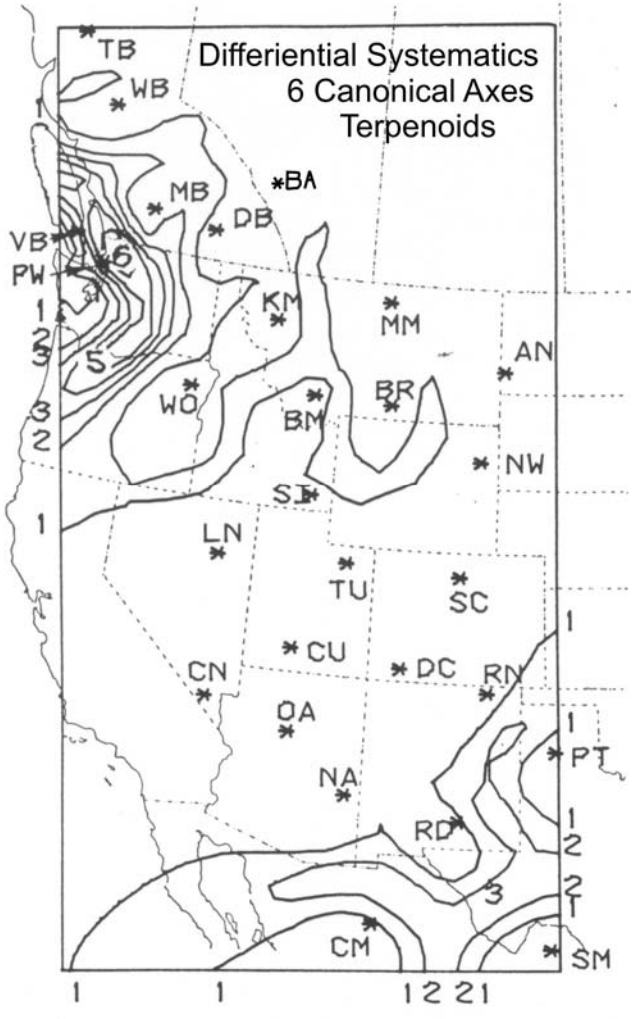

Figure 1. Contoured differentiation based on the first 6 canonical axes using leaf terpenoid data (from Adams, 1983). Areas with close contour lines are areas of high differentiation.

from throughout its range and found that much of the variation within putative *J. scopulorum* was due to differentiation in populations from Puget Sound from the balance of the range of *J. scopulorum* (Fig. 1). The differentiation of the two populations sampled in the Puget Sound (VB, Vancouver Isl., B.C.; PW, Whidbey Isl., WA) accounted for 50.2% of the variance among all 17 populations (Adams, 1983). It was hypothesized that the Puget Sound populations have been genetically isolated from the main, Rocky Mountain populations since the Pleistocene (or earlier) (Fig. 2). Notice (Fig. 2, A) that the Puget Sound

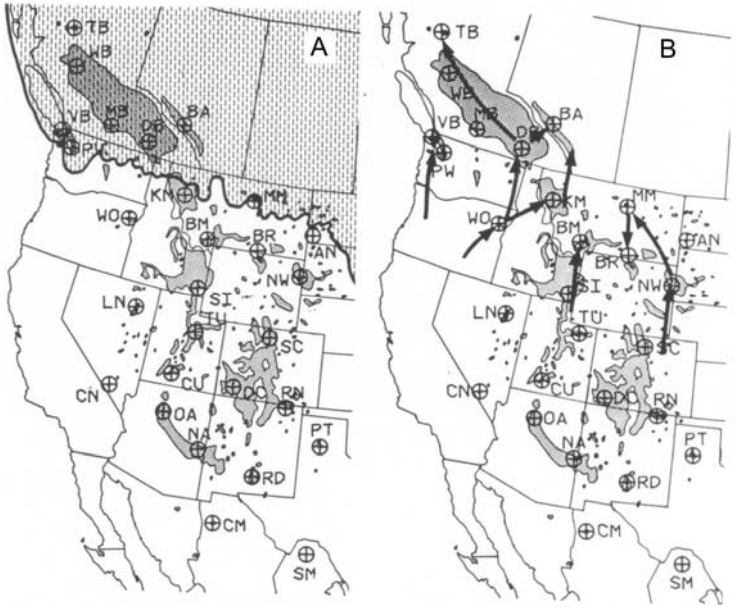

Figure 2. A. Maximal Wisconsin ice cover showing the extinction of local populations of *J. scopulorum*. B. Proposed refugia and recolonization following the Wisconsin (adapted from Adams, 1983).

populations were thought to have retreated to a refugium south of the their present distribution and that no common refugia are indicated for the Puget Sound populations and *J. scopulorum* from the Rocky Mountains (Fig. 2 B).

Recently, Schwarzbach et al. (2008), using combined ITS and trnC-D sequence data in their study of the phylogeny of *Juniperus*, found that an individual from Puget Sound came out in the clade with *J. virginiana*, not in the clade with *J. scopulorum*. This prompted the author to reexamine the terpenoid data (Adams, 1983). Figure 3 shows a PCO of the terpenoids. Four distinct entities are resolved: *J. horizontalis*, *J. scopulorum*, *J. virginiana*, and the Puget Sound populations. It should be noted that each stick represents the mean of 15 individuals (a total of 441 individuals analyzed for over 100 terpenoids, with the 30 terpenoids with the highest F ratios utilized for PCO). These data are robust and must be given significant weight in assigning the taxonomic position of the Puget Sound populations.

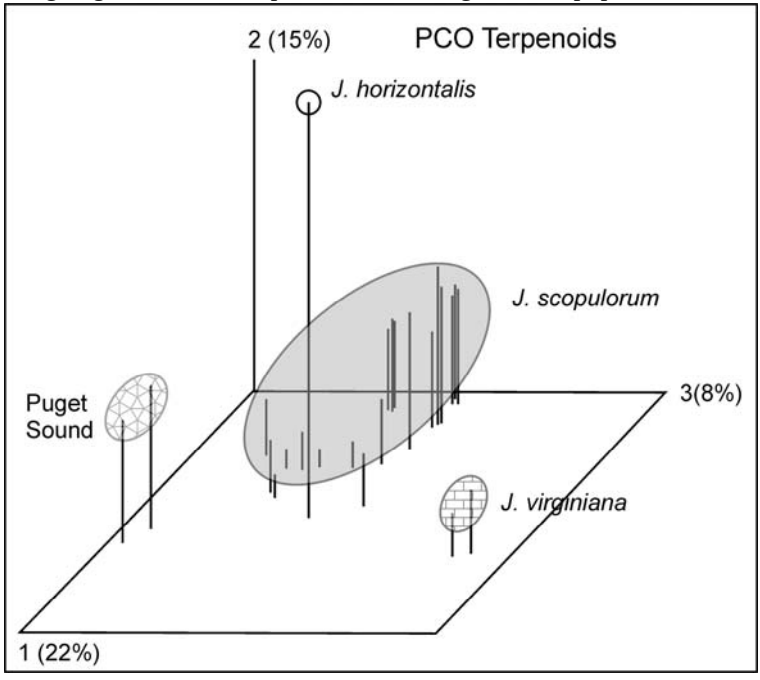

Figure 3. Principal coordinate ordination (PCO) utilizing terpenoid data from Adams (1983). Each of the sticks represents population mean of 15 individuals, except for the 2 Puget Sound populations that contained 8 and 13 samples.

Because the ITS and trnC-D sequence data (Schwarzbach et al. 2008) fails to support a conspecific status of the Puget Sound population and *J. scopulorum*, it seemed prudent to make additional collections and analyze additional samples using several DNA methods.

The purpose of this paper is to compare ITS and trnC-D SNPs (single nucleotide polymorphisms) analyses of junipers from Puget Sound with *J. scopulorum* and *J. virginiana* with previous terpenoid, morphological and ecological data to determine the taxonomic status of the Puget Sound (seaside) juniper.

MATERIALS AND METHODS

Specimens used in this study are shown in table 1. Voucher specimens are deposited at BAYLU herbarium Baylor University.

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 using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA).

SNPs obtained from DNA sequencing

ITS and trnC-trnD amplifications were performed in 50 µl reactions using 10 ng of genomic DNA, 3 units Qiagen Taq polymerase, 5 µl 10x buffer (final concentration: 50 mM KCl, 10 mM Tris-HCl (pH 9), 0.01% gelatin and 0.1% Triton X-100), 1.75 mM $MgCl_2$, 20 µl Q solution (2X final), 400 µM each dNTP, 1.8 µM each primer and 4%(by vol.) DMSO.

Primers (5'-3'):

ITS: ITSA = GGA AGG AGA AGT CGT AAC AAG G;
ITSB = CTT TTC CTC CGC TTA TTG ATA TG.

ITSA and ITSB primers from Blattner (1999).

trnC-trnD: CDFor: CCA GTT CAA ATC TGG GTG TC
CDRev: GGG ATT GTA GTT CAA TTG GT

CDFor, CDRev primers from Demesure et al. (1995).

CD10F: AAA GAG AGG GAT TCG TAT GGA
CD3R: AAC GAA GCG AAA ATC AAT CA

CD10F and CD3R primers from Andrea Schwarzbach (per. comm.)

The following PCR conditions were used: MJ Research Programmable Thermal Cycler, 45 cycles, 94°C (1 min.), 50°C (1 min.), 72°C (1 min.), with a final step of 72°C (5 min.). The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 45 min.). The nrDNA primers (ITSA, ITSB) produced a band of approx. 1120 bp. The internal trnC-trnD primers, CD10F-CD3R produced a band of approx. 850 bp. In each case the band was excised and purified by use of a Qiagen QIAquick gel extraction kit.

The gel purified DNA band with the appropriate primer was sent to McLab Inc. for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments were done using Clustal W and then manually corrected. Indels were coded with a "-" for the first nucleotide and "I" for succeeding nucleotides such that an indel was treated as a single mutation event. Sequences were deposited in GenBank (table 1).

SNPs analyses

Aligned data sets (nrDNA and trnC-trnD) were analyzed by CLEANDNA (Fortran, R. P. Adams) to remove invariant data and nucleotides that only varied by a single polymorphism among individuals. Mutational differences were computed by comparing all SNPs, divided by the number of comparisons over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). A minimum spanning network was constructed by selecting the nearest neighbor for each taxon from the pair-wise similarity matrix, then connecting those nearest neighbors as nodes in the network (Adams, et al. 2003).

Table 1. Specimens collected, locations and GenBank accession numbers. All specimens deposited at BAYLU.

Taxon/collection #	Location	GenBank acc.
J. scopulorum,		
Adams 10895	Kamas, UT	ITS: EF608963-65
-10897		trnCD EF608988-90
J. virginiana		
Adams 6753-6755	Hewitt, TX	ITS: EF608980-82
		trnCD: EF609002-04
Adams 10230	Knoxville, TN	ITS: EF608973-75
-10232		trnCD: EF608996-98
J. v. var. *silicicola*		
Adams 9186-88	Ft. DeSoto Park,	ITS: EF608977-79
	Mullet Key, FL	trnCD: EF609009-11
J. maritima		
Adams 11056-58	Brentwood Bay (BB)	trnCD: EF608985-87
	Vancouver Isl., BC	
Adams 11061-63	Cowichan Bay (CB)	ITS: EF608968-70
	Vancouver Isl., BC	trnCD: EF608992,
		EF609007,
		EF608993
Adams 11064	Yellow Point (YP)	ITS: EF608984
	Vancouver Isl., BC	trnCD: EF608991
Adams 11065-66	Lesqueti Isl. (LS)	ITS: EF608967
	BC	trnCD: EF609000-01
Adams 11067-68	Friday Harbor (FH)	ITS: EF608971
	San Juan Isl., WA	trnCD: EF608994-95
Adams 11075	Whidbey Isl. (WI)	ITS: EF608983
	Cranberry L., WA	trnCD: EF609005
Adams 11076	Fidalgo Isl. (FI)	ITS: EF608972
	State Park, WA	trnCD: EF609006
Adams 11077-78	Skagit Isl. (SK), WA	ITS: EF608966,
		EF608976
		trnCD: EF609008,
		EF608999

RESULTS AND DISCUSSION

Analysis of the nrDNA (ITS) sequences revealed little variation among these essentially sibling species. One exception was individual 11076 from Fidalgo Island, WA that had a 67 bp deletion at position 399. The tree appeared to be morphologically similar to other trees in the area and it is assumed that this indel represents a single mutational event. A few single nucleotide mutations were found among individuals and removed from the data. This resulted in 18 SNPs among *J. scopulorum*, *J. virginiana*, *J. v.* var. *silicicola* and the Puget Sound (seaside) junipers. Factoring the associational matrix resulting in eigenroots that accounted for 55.4%, 24.8%, 6.1%, and 4.2% before they began to asymptote. Notice that two degrees of freedom (axes 1,2) accounted for 80.2% of the variance! This implies that there are only 3 groups (n-1 = 2).

Ordination of the individuals (Fig. 4) revealed three groups: *J. scopulorum*, *J. virginiana* (including var. *silicicola*) and the Puget Sound junipers. The minimum spanning network shows (Fig. 4) that the Puget Sound junipers are nearly equidistant between *J. scopulorum* (5 bp) and *J. virginiana* (4 bp). The ITS SNPs, although not plentiful, are fully congruent with the terpenoid and morphological data.

Analysis of the trnC-trnD cpDNA sequences proved to be difficult. Numerous indels and single mutational events were present. Figure 5 shows the variation encountered in the sequence length (1580 bp). This includes both nucleotide substitutions and single indels. NCBI blast of the region from CD10F to CD3R did not yield information on the nature of the conserved regions where these primers reside.

Each of the *J. v.* var. *silicicola* samples (3 indvs.) had a 254 bp deletion in the CD10F - CD3R region not found in any other samples. *Juniperus v.* var. *silicicola* is a coastal juniper from the sand foredunes of se United States. Analyses including *J. v.* var. *silicicola* samples in the data set showed it to be quite differentiated in its trnC-trnD sequence, so these were removed from further consideration for the trnC-trnD data.

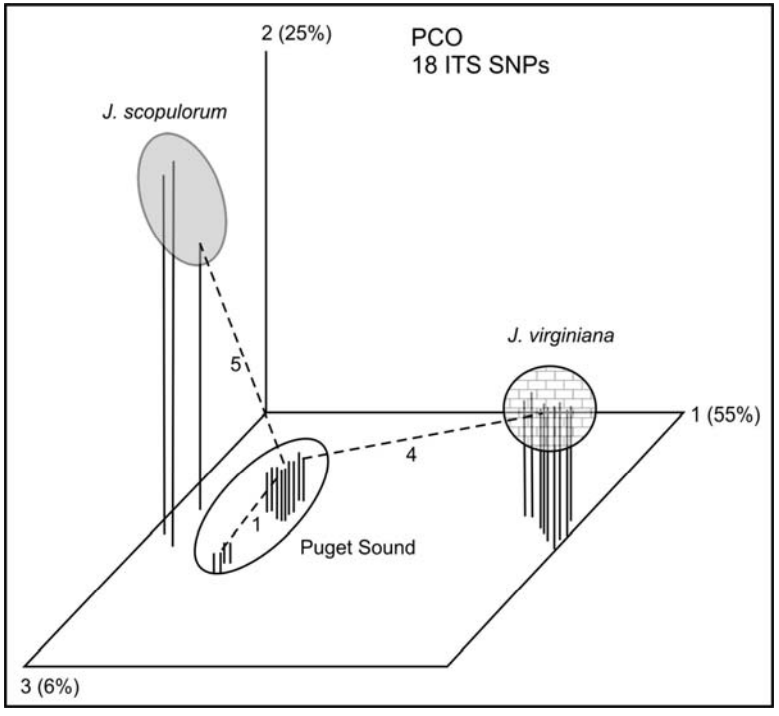

Figure 4. PCO ordination of based on 18 SNPs of ITS sequence data.

Juniperus scopulorum (3 indvs.) each had a 4 bp (TATA) insert at position 986, not shared with either *J. virginiana* or the Puget Sound junipers. *Juniperus virginiana* (6 indvs.) had an insert of 4 bp (TTTT) at position 262 not found in any other samples.

Four trees in the study had a 4 bp indel at position 712. These trees were from Friday Harbor (TATT, TATT) , Fidalgo Island (TAAT) and Whidbey Island (TAAT). The population from Fidalgo Island is only about 10 km north of the Whidbey Island population. However, the Skagit Island population, only 5 km east of the Whidbey Island population, did not have the indel.

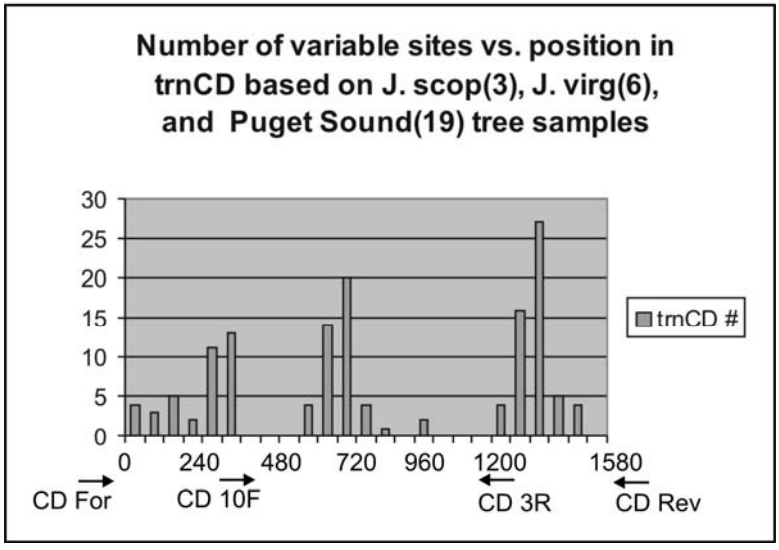

Figure 5. Frequency distribution of variable sites in the trnC-trnD region.

Principal Coordinates analysis of the association measures using 78 polymorphic SNPs from the trnC-trnD sequences produced three eigenroots before the eigenroots began to asymptote. These three eigenroots accounted for 25.8%, 14.6% and 11.6% of the variation among individuals. Three eigenroots implies that 4 groups are present in the data. However, ordination (Fig. 6) shows two principal groups: *J. scopulorum* and *J. virginiana* / Puget Sound individuals.

These two groups (axis 1) accounted for 26% of the variation among the individuals. There is a partial separation of the *J. virginiana* individuals (V, fig. 6), but it is incomplete. Considerable variation exists among the Puget Sound individuals, but a detailed examination failed to correlate their ordination with geography.

The trnC-trnD data seem similar to the trnL-trnF cp data from *J. occidentalis* Hook. var. *australis* (Vasek) A. & N. Holmgr.

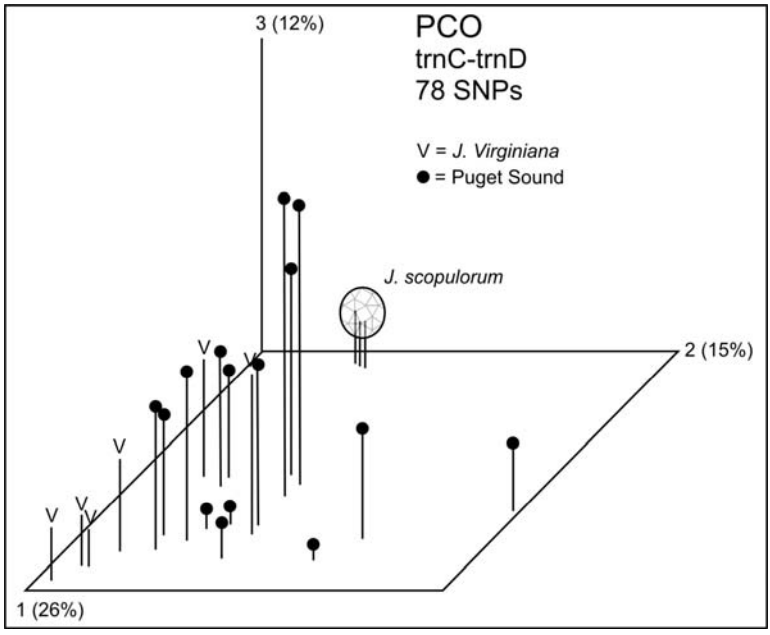

Figure 6. PCO ordination of *J. scopulorum*, *J. virginiana* and Puget Sound individuals based on 78 SNPs.

(now *J. grandis* R. P. Adams) and *J. osteosperma* (Torr.) Little from Terry et al. (2000). The latter workers found that a cp haplotype, a mutation at position 436 (at the 3' position of the *Tru 9I* restriction site), was invariant within *J. o.* var. *australis* (*J. grandis*), but varied clinally (with some notable exceptions) from the area of sympatry (w. Nevada) to Utah. However, several populations in UT, CO and WY, the farthest removed from *J. o.* var. *australis*, had high frequencies of the cp haplotypes. They considered three explanations: inheritance of ancestral polymorphism, intraspecific polymorphism, and hybridization between *J. occidentalis* var. *australis* and *J. osteosperma*. Of course, Vasek (1966) has already made a strong case for hybridization between these taxa based on morphological data. Terry et al. (2000) opted for the hybridization (and introgression) as the explanation with gene flow (via pollen) from *J. o.* var. *australis* to typical *J. osteosperma*. This

would be in agreement with the transfer of cpDNA via pollen from *J. o.* var. *australis*, but not the reverse flow. However, one can not rule out the persistence of ancestral cpDNA as another explanation. In any case, analysis of the trnL-trnF sequences gave a picture of incomplete separation between these morphologically well defined *Juniperus* species.

This appears to be the case for trnC-trnD cp data for *J. virginiana* and the Puget Sound junipers. The trnC-trnD PCO (Fig. 6) stands in contrast to the terpenoid data (Fig. 3) and ITS data (Fig. 4).

A striking aspect of the Puget Sound, seaside junipers is their habitat. They all grow at the seaside (or lakeside) on granite or sand (Fig. 7). This is a very different kind of habitat than that found in *J. scopulorum* and *J. virginiana*. *Juniperus scopulorum* grows on dry, rocky mountainous soils. *Juniperus virginiana* is more cosmopolitan, growing in limestone areas as well as deep soils. Both *J. scopulorum* and *J. virginiana* are weedy junipers that invade old fields and disturbed roadsides. In contrast, the seaside juniper is not weedy and usually appears as if it is relictual (i.e., older trees, with few or no seedlings). The Puget Sound juniper's habitat seems to be very restricted and has only been collected in a few locations (Fig. 7). The Puget Sound climate is very different than the Rocky Mountain or the eastern US climates, having a mild, wet regime. In short, the Puget Sound juniper has evolved physiological genes to facilitate its growth in such an environment.

Is the Puget Sound, juniper a distinct species? Ownbey (1950) has provided us with a very practical species definition. He emphasizes that species are natural groups, characterized by: 1. a combination of distinctive morphological features (and/or chemical/ DNA features, *my addition*); 2. The taxa are reproducing under natural conditions; and 3. There is not free gene exchange between the taxa concerned.

How can we apply the 'Ownbey species concept' to the present taxonomic problem?

1. The taxa are natural groups, characterized by a combination of distinctive morphological features (and/or chemical/ DNA features, *my addition*).

Recently, Issakainen (1999) wrote "We easily forget that different parts of a single organism's genome may have a different evolutionary history." We might modify his statement to read "different parts of the genome may be under differential selection pressure." We, as taxonomists, have relied on morphology as the deciding data for the recognition of species, varieties, and indeed most of our nomenclatural taxa. This is only natural, as the morphology is "what you see." The morphology is a product of the plant's genes plus the environment. The genes are composed of DNA and in tomato the genome size is about 700,000,000 base pairs (bp) versus 4,000,000 bp in *E. coli* and 230,000,000,000 bp in man (Brown, 1986) and these appear to represent 20,000 to 30,000 genes (Somerville and Somerville, 1999). The amount of the genome that we see in the morphology is not known precisely. But, in an interesting study of two species of goldenrod (*Solidago*), Charles and Goodwin (1953) made the following estimates for the minimum number of genes for several key taxonomic characters:

Character	Minimum number of genes
leaf margins: entire vs. serrate	7
leaf surface: glabrous to pubescent	6
leaf thickness	6
basal leaves: length	8
leaf cuticle: degree of sculpturing	5
stomatal apparatus: length	3

Thus, for these 6 key characters separating *S. sempervirens* and *S. rugosa*, they estimated that the species differed by a minimum of 35 genes. How many DNA base pairs this represents is unknown.

Irving and Adams (1973) applied these methods to estimate the minimum number of genes controlling monoterpenes in *Hedeoma*. They found that 20 monterpenoids were inherited by from 1 to 7 genes, with an average of 1.95 genes per compound. Thus, these 20 monoterpenoids appeared to be inherited by a minimum of 39 genes. Again a small sample of the total genome.

If *Solidago* and *Hedeoma* have 20,000 to 30,000 genes as commonly expected in plants (Somerville and Somerville, 1999), then the *Solidago* morphology and *Hedeoma* monoterpenes are small samples of these genomes. Somerville and Somerville (1999) show that, in *Arabidopsis*, 54% of the genes can be assigned a known function. Although they did not show morphology *per se*, they did show that of the genes with known function, approximately 5% control cell structure and 6% code for secondary metabolism in *Arabidopsis*.

For the case of the seaside (Puget Sound) juniper, the taxon is distinct from both *J. scopulorum* and *J. virginiana* in its terpenoids and ITS sequences. It is also differentiated in its physiology, enabling it to grow in a habitat foreign to both *J. scopulorum* and *J. virginiana.* Clearly the Puget Sound juniper (seaside juniper) is characterized by a combination of terpenoid, ITS DNA and physiological traits, these independent of those relating to morphology.

2. The taxa are reproducing themselves under natural conditions.

Of immediate concern upon examining the Puget Sound juniper, was that it might be an escaped cultivar of *J. virginiana*. *Juniperus virginiana* was (and continues to be) commonly cultivated by settlers moving westward in the United States. It is a very common ornamental tree found at homesteads, cemeteries and parks in the central and western United States. Several groups of early immigrants came to the Pacific Northwest. Likely, the earliest were the Spanish and Portuguese sailors and explorers. It is extremely unlikely that these explorers, who apparently did not build permanent settlements in the Pacific Northwest would have brought *J. virginiana* for cultivation. The most likely group of settlers were the Anglos from the eastern United States who used the Oregon Trail to migrate to the Pacific Northwest between 1841 and 1869. Apparently, Hudson Bay trappers and Russians visited Puget Sound as early as 1830 (Steve Erickson, pers. comm.). So any junipers older than 176 years old (in 2006) would have pre-dated the earliest known Anglo settlers.

Although juniper growth rings are not reliable in desert regions due to lack of rings in dry years, the precipitation of Puget Sound is very consistent with a wet season each year. Therefore, the

growth rings should be a very good measure of the age of junipers in the area. In 2006, the author cored several very large junipers in Puget Sound. Table 2 shows the growth rings varied from 86 to 210 rings. A linear exploitation gives values over 400 yr. Most of the cores had uniform ring spacing for the region scored, except for 11070, Lesqueti

Table 2. Estimated ages and sizes of junipers in the Puget Sound area.

Tree and Location	trunk radius	# rings counted	% radius counted	approx. age
11065, Yellow Point, BC	22.8 cm	128 in 22.8 cm	100%	128 yr.
11061, Cowichan Bay, BC	35.5 cm	167 in 20.8 cm	58.6% ca. 285 yr.	> 167 yr.
11065, Lesqueti Island, BC	35 cm	163 in 29 cm	82.9%	> 163 yr. ca. 196 yr.
11070, Lesqueti Island, BC	64 cm	210 in 11cm	17.2%	> 210 yr ca. 400- 500
11067, Friday Harbor, San Juan Isl.	40 cm in 24 cm	86	60% ca. 140 yr.	> 86 yr.
11072, English Camp, San Juan Isl.	106.7	92 in 18 cm	33.7%	> 92 yr. ca. 273 yr.
11077, Skagit Island, WA	118.6 cm	140 in 20 cm	33.7%	>140 yr. ca. 415 yr.

Island, that had very compressed rings in the 11 cm that was scoreable. It is clear that the seaside juniper predates Anglo settlement and the taxon is naturally occurring. In addition, high genetic variation between the seaside junipers, argues against the introduction by settlers. Recent introduction would have produced a genetic bottleneck effect that is not present in these populations. Although there is almost universal damage to the seed cones by insects, resulting in exserted seeds, the seaside juniper is reproducing itself under natural conditions.

3. There is not free gene exchange between the taxa.

The nearest population of *J. scopulorum* is about 140 km east of Puget Sound at Ross Lake, BC. The nearest population of *J. virginiana* is in central Nebraska, several thousand km to the east. It seems unlikely that gene flow is currently occurring between the seaside juniper and either *J. scopulorum* or *J. virginiana*.

In summary, the seaside juniper of Puget Sound is an entity that is genetically defined (primarily by its chemistry and DNA sequences), reproducing itself under natural conditions and is not interbreeding with other juniper species. Because of this, I recognize it as a new species as follows:

Juniperus maritima R. P. Adams **sp. nov.** Type: Canada, BC, Vancouver Island, Brentwood Bay, Lat 48° 34.794' N; Long 123° 20.211' W, elev. 5 m., 29 May 2006, *R. P. Adams 11056* (HOLOTYPE: BAYLU, ISOTYPE: V).

A *J. scopulorum* similis sed differt strobilis seminiferis in 14-16 menses maturescentibus, seminibus plerumque ex strobilo exsertis, et apicibus foliorum squamiformium obtusis. Differt a *J. virginiana* strobilis seminiferis majoribus (6-8 mm) saepe reniformibus, seminibus plerumque ex strobilo exsertis, foliis squamiformibus minus quam 1/5 longitudinis imbricatis, et ramulis laevibus porphyreis.

This species is similar to *J. scopulorum* but differs in that the seed cones mature in 1 year (14-16 months), seeds are usually exserted from the cone, and the scale leaf tips are obtuse (Table 3). It differs

from *J. virginiana* in having larger seed cones (6-8 mm) that are often reniform, seeds usually exserted from the cone, scale leaves overlap less than 1/5 the length, and branchlets are smooth and reddish-brown.

Table 3. Morphological comparison of *J. maritima*, *J. scopulorum* and *J. virginiana*.

	J. maritima	*J. scopulorum*	*J. virginiana*
seed cones mature	1 yr (14-16 mos.)	2 years	1 year
seed cone diam.	6-8 mm	6-9 mm	3-6(7) mm
seed cone shape	globose to reniform	globose to reniform	ovoid
seeds per cone	(1) 2	(1) 2 (3)	1-2 (3)
exserted seeds	ubiquitous	rare	rare
scale leaf overlap	< 1/5 length	< 1/5 length	> 1/4 length
scale leaf tips	obtuse	acute to obtuse	acute
branchlets (6-15mm, diam.)	smooth, reddish-brown	smooth, bright reddish-brown	brown with persistent old leaves

Junipers maritima is known only from the Puget Sound area (Fig. 7). It is usually found in rocky areas, often within meters of the water. However, a population exists on coastal sand dunes near Cranberry Lake, Whidbey Island, WA. No other population has been found on sand, so that site is likely atypical.

Population Status

The Lesqueti Island population (LS, Fig. 7) is in a nature reserve and consists of hundreds of trees. It appears to be a robust population and not threatened.

The Yellow Point population (YP, Fig. 7) at Yellow Point Resort, private land, has tens of trees that appear to be reproducing, but development and human impact at the resort threatens it.

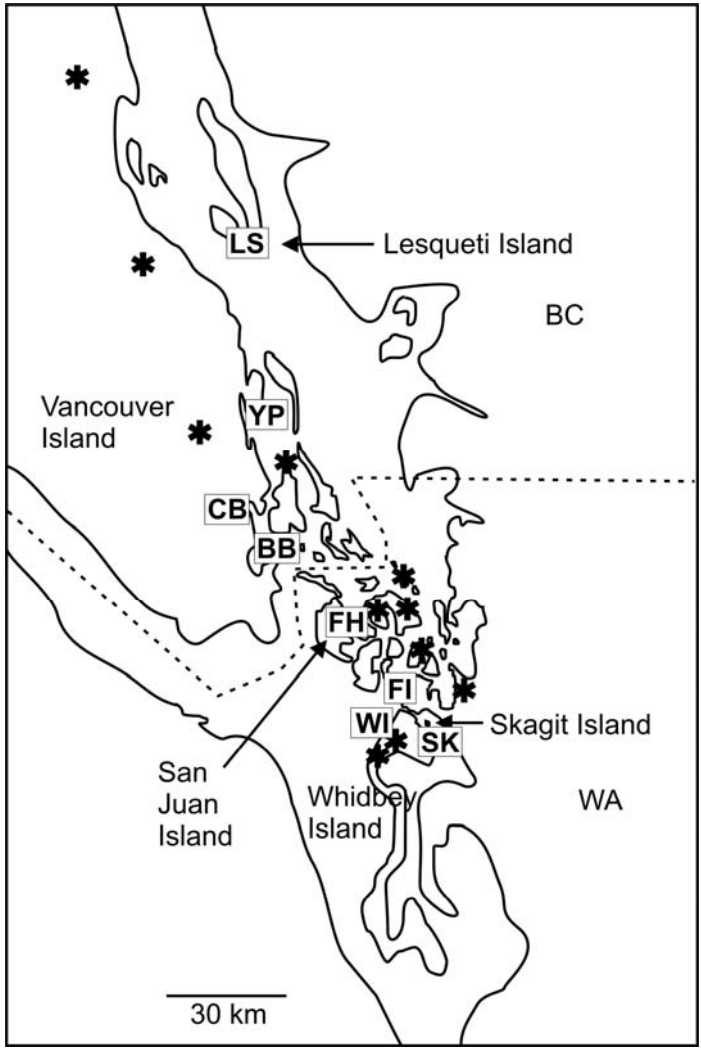

Figure 7. Distribution of *Juniperus maritima* based on Adams field collections (acronyms) and herbarium specimens (stars) from V, WS, and WTU.

The Cowichan Bay population (CB, Fig. 7) is on private land. Approximately 10 trees were seen. No seedlings or saplings were observed.

The Brentwood Bay population (BB, Fig. 7) consists of 6 mature trees on seaside granite. It is at the north end of the Tsartlit Reserve and is protected from development.

The Friday Harbor plants are found chiefly on rocks at the Univ. of Washington Marine Station (8-10 trees) and at the NPS, English Camp (6 old, mature trees) on the opposite side of San Juan Island. These sites are protected from development.

The Fidalgo Island, Washington State Park, Anacortes, WA was the most robust population examined with hundreds of trees of various ages. It is in a protected park and its future looks secure.

On Whidbey Island, a natural population was found on coastal sand dunes in Deception Pass Park (near Cranberry Lake). There are 10-20 trees, all very stunted from constant ocean winds and salt spray. Some age differences were observed. The site is in a park and protected from cutting. However, beach use and a large storm could threaten this population. Several other seaside junipers appear to have been planted at houses in the interior of Whidbey Island and are growing well in deep soil.

About 10 individuals were seen on Skagit Island, ranging from very old to young saplings. Skagit Island is a protected area so, aside from fires, this little population appears stable.

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