Juniperus communis in Morocco: analyses of nrDNA and cpDNA regions

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ABSTRACT

Juniperus communis from Morocco was analyzed by DNA sequence data from nrDNA plus four cp DNA regions (4315 bp) and found to be in a clade with other *J. communis* from Europe. The sub-alpine, prostrate *J. communis* plants of Morocco, each only contained a single SNP and a one bp indel (deletion) in the trnSG sequence data for a total of 2 MEs (mutation events). The Moroccan prostrate *J. communis* appear to be a variant of *J. communis* of Europe and central Asia and not part of the var. *hemispherica* group. Published on-line **www.phytologia.org** *Phytologia 97(2): 123-128 (April 1, 2015).* ISSN 030319430.

KEY WORDS: *Juniperus communis* forma *pygmaea, J. communis, J. oblonga, J. pygmaea,* Azerbaijan, nrDNA, cpDNA sequences, taxonomy.

Juniperus communis is a circumboreal species with perhaps the largest distribution of any conifer. Its habit ranges from upright trees (in Europe) to shrubs, to prostrate shrubs (Adams, 2014). In spit of the variation in habit, few differences in its DNA have been found (see Adams, 2014 for discussion).

Adams and Tashev (2013) compared the leaf essential oils of *J. communis, J. pygmaea* and *J. sibirica* from Bulgaria with the oils of *J. communis* of Sweden and *J. saxatilis* of Switzerland. From their analysis, the oils do not ordinate *J. communis, J. pygmaea* and *J. sibirica* from Bulgaria into separate groups, but they are generally interspersed. Additional research (Adams, Tashev and Schwarzbach, 2014) using DNA sequences from nrDNA and four cp regions gave no clear separation of *'pygmaea'* from *J. communis* and *J. c.* var. *saxatilis*. They concluded that the shrubby habit is likely controlled by only a few genes and recognized the taxon as *J. communis* f. *pygmaea* (K. Koch) R. P. Adams and A. N. Tashev.

Recently, Adams et al. (2015) compared the DNA sequences of putative *J. communis 'pygmaea'* from Azerbaijan and found it in a clade with *J. communis 'oblonga'* from Armenia, not with *J. communis* forma pygmaea of Bulgaria. They elected to not recognize this variant, but to treat it as *J. communis*.

While doing routine field work one of the authors (MR) found and collected samples from unusual, prostrate *J. communis* plants growing at 3000 m in the Atlas Mtns. of Morocco (Fig. 1). Examination of the specimens and photos by senior author (RPA), raised the question if these might be part of var. *hemispherica* as found in Mt. Etna, Sicily and the Sierra Nevada, Spain.

Figure 1. Habitat of *J. communis* in the high Atlas Mtns., Morocco (3000m). Notice the plants are very prostrate. This may be due to winter temperatures.

The purpose of this study was to compare data from nrDNA and four cpDNA regions of *J. communis* from Morocco with other members of *Juniperus* sect. *Juniperus* from the eastern hemisphere to determine the taxonomic affinity of *J. communis* from Morocco.

MATERIALS AND METHODS

Plant material - Morocco: *J. communis, Adams Lab acc 14222-14226, (M. Rhanem ns),* common on exposed slopes, prostrate plants, High Atlas Mtns., 32° 31.5' N; 4° 57' W. elev. 3000m, Morocco. Bulgaria, *J. communis* var. *communis*, *Adams Lab acc 13730-31, 14058-60, (Alex Tashev, 2012-JC1-5)*, Eastern Rhodopes, in protected site "Gumurdjinsky Shezhnik", locality "Madzharsky Kidik". On limestone rocks above the upper border of a forest of *Fagus sylvatica* ssp. *moesiaca,* 41° 14' 44.7" N; 25° 15' 31.9" E. elev. 1270 m. *J. communis* f. *pygmaea*, *Adams Lab acc. 13734-35, 14064-66,(Alex Tashev, 2012-JP1-5)*, Central Rhodopes. Mursalitza part, locality "Piramidata". On high-mountain meadow, on a limestone rock near a forest of *Pinus sylvestris* together with *Picea abies,* 41° 40' 22.8" N; 24° 26' 36.6" E. elev. 1756 m.
J. communis var. *saxatilis* - Bulgaria, *Adams Lab Acc. 13732-33, 14061-63,(Alex Tashev, 2012-JSI1-5),* Vitosha Region. Nature Park "Vitosha". Above the hut "Aleco" near the alpine timber line formed by a forest of *Picea abies*. On silicate rock together with *Vaccinium myrtillus, V. uliginosum, Ribes petraeum, Rubus idaeus, Calamagrostis arundinacea, Festuca valida (*Bulgarian endemic*),* 42° 34' 52.1" N; 23° 17' 28.0" E. elev. 1848 m.
J. 'pygmaea' - Azerbaijan, shrubs, 0.5 - 1m tall, with *J. sabina*, on rocks in mountains. 41° 11.790' N; 48° 15.313' E. elev. 1649m *Adams Lab acc. 14321-14325* (*V. Farzaliyev 1-5*) 6 Jun 2014.

Exemplar specimens: *J. communis* var. *communis,* Stockholm, Sweden, *Adams 8167 (7846-7848); J. communis* var. *saxatilis,* Switzerland, *Adams 11164 (7618-7621)*. Voucher specimens deposited in 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^{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. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. R6-1 (Biomatters. Available from http://www.geneious.com/) and the MAFFT alignment program. Further analyses utilized the Bayesian analysis software Mr. Bayes v. 3.1 (Ronquist and Huelsenbeck, 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall, 1998) and Akaike's information criterion. Minimum spanning networks were constructed from mutational events (ME) data using PCODNA software (Adams et al., 2009; Adams, 1975; Veldman, 1967).

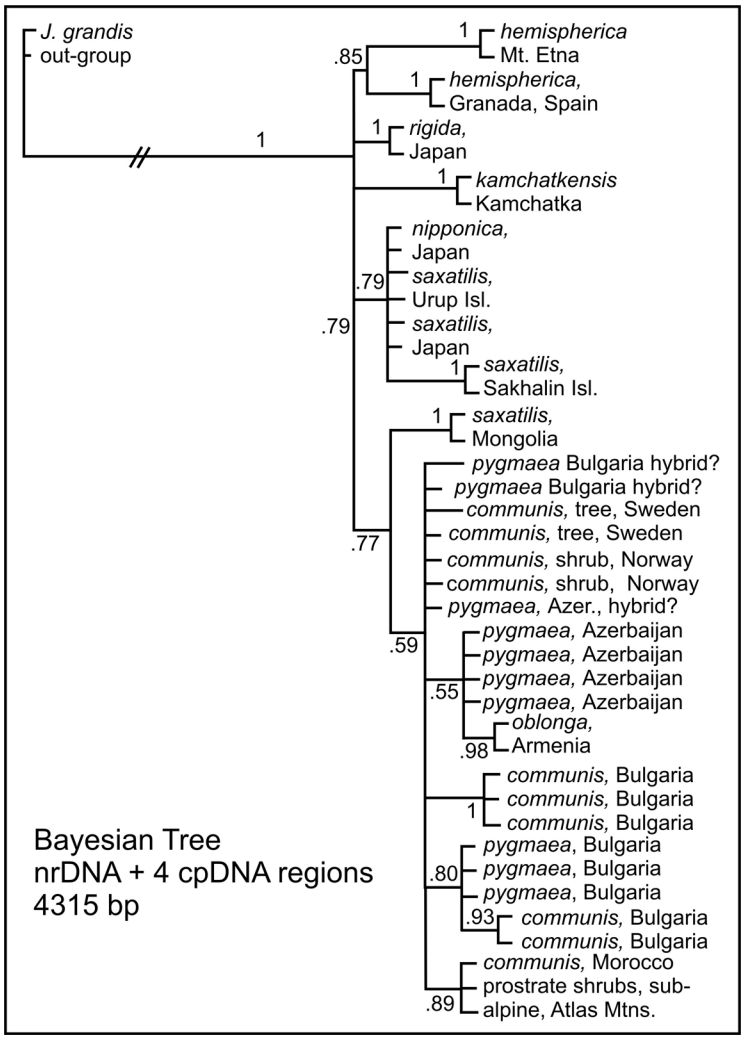

Figure 2. Bayesian tree of *Juniperus communis* taxa of the eastern hemisphere. Numbers at branch points are posterior probabilities. See text for discussion.

RESULTS AND DISCUSSION

Sequencing nrDNA (ITS) and four cp-regions (petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF) yielded 4315 bp of data. The Bayesian consensus tree (Fig. 2) revealed the sub-alpine, prostrate *J. communis* plants of Morocco are in the clade with *J. communis* from Europe. They are not in the clade with var. *hemispherica* (Spain and Sicily). Our initial thought that these plants might be a southern extension of the plans in Spain was incorrect.

To examine the magnitude of the differences, a minimum spanning network was constructed (Fig. 3). *Juniperus communis*, eastern hemisphere, is divided into three groups: *J. communis*, Europe, *J. communis*, Japan and far east, and *J. c.* var. *hemispherica*, the latter divided among Mt. Etna, Sicily (type locality) and Sierra Nevada, Granada, Spain. All the samples of *J. 'pygmaea'* of Azerbaijan, are tightly grouped with *J. communis* from Europe (Fig. 3). Interestingly, the *J. communis 'oblonga'* of Armenia differs by 3 MEs (indels in this case) from *J. communis* of Sweden. The sub-alpine, prostrate *J. communis* plants of Morocco each only contained a single SNP and a one bp indel (deletion) in the trnSG sequence data for a total of 2 MEs (mutation events). The Moroccan prostrate *J. communis* appear to be a variant of *J. communis* of Europe and central Asia (Fig. 3) and not part of the var. *hemispherica* group.

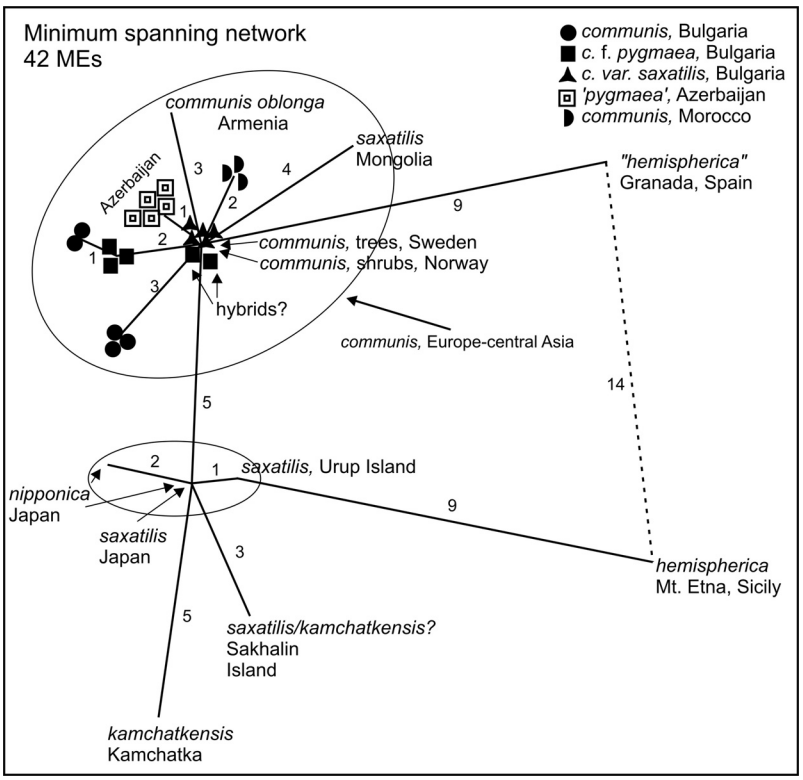

Figure 3. Minimum spanning network of *J. communis* and its varieties based on 42 MEs (mutational events = SNPs + indels). Numbers next to the links are the number of MEs. The dashed line is the second shortest link between the *hemispherica* taxa.

In summary, the sub-alpine, prostrate *J. communis* plants of Morocco do not appear, at this stage of research to be significantly different from *J. communis* of Europe to warrant it recognition as a distinct variety, in spite of the fact that typical *J. communis* are trees or shrub-trees. Transplant studies will likely be needed to ascertain if the prostrate habit is genetic or environmentally induced.

Clearly there considerable differences in habit of *J. communis* in various regions (Fig. 4) that may be due to only a few genes, as we are finding few DNA differences in our studies.

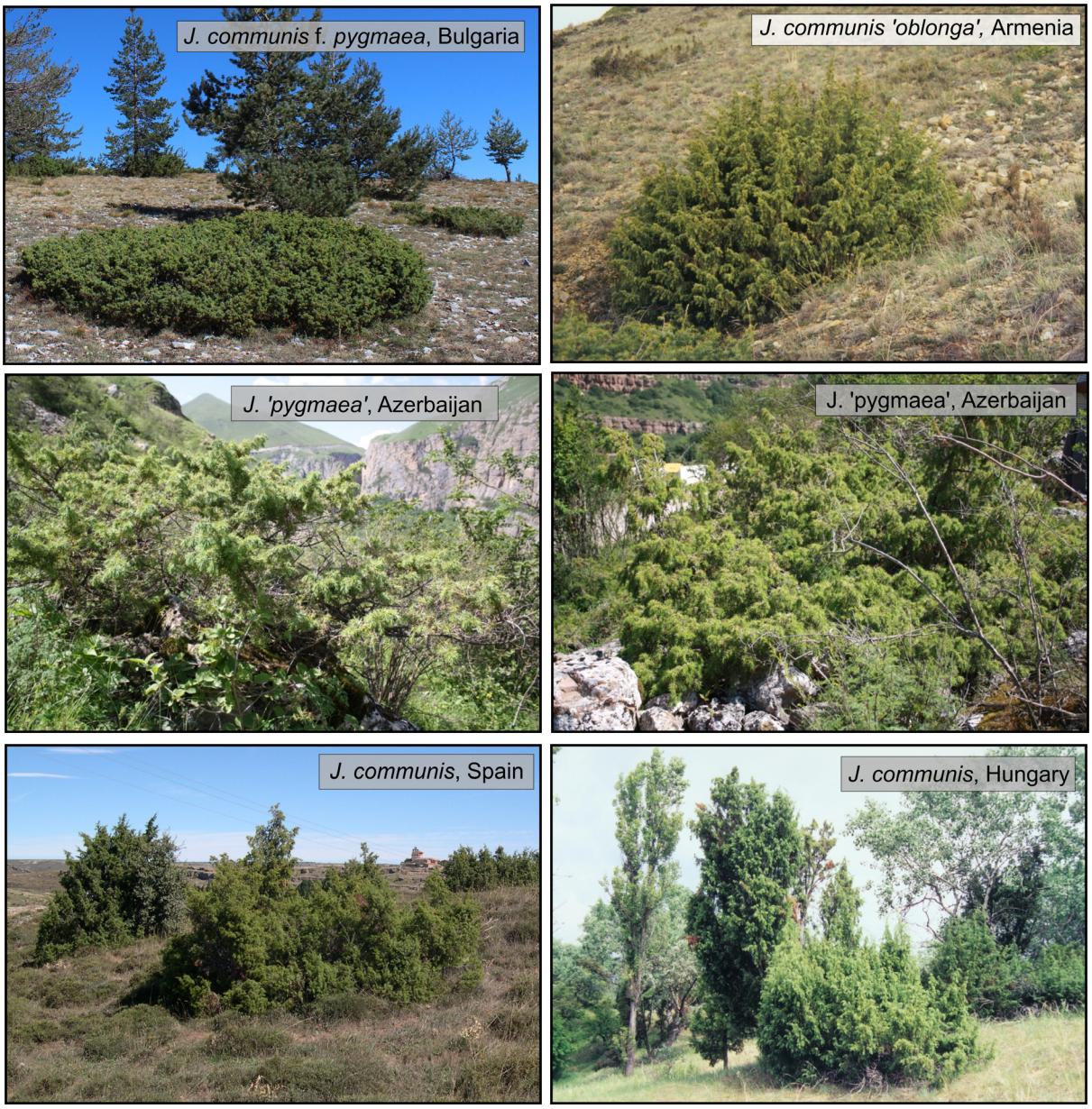

Figure 4. Plant habits of *J communis* f. *pygmaea*, Bulgaria, *J. communis 'oblonga',* Armenia compared to *J. 'pygmaea'*, Azerbaijan and *J. communis*, Spain and Hungary.

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