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Juniperus communis in Azerbaijan: analyses of nrDNA and cpDNA regions

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ABSTRACT

Juniperus 'pygmaea' from Azerbaijan was analyzed by DNA sequence data from nrDNA plus four cp DNA regions (4315 bp) and found in a clade with *J. communis 'oblonga'* (= *J. communis*) Armenia, not with *J. c.* forma *pygmaea* of Bulgaria. It seems prudent to not recognize this variant taxonomically but treat it as *J. communis*. Published on-line **www.phytologia.org** *Phytologia 97(1): 6-11 (Jan 2, 2015).* ISSN 030319430.
KEY WORDS: *Juniperus communis* forma *pygmaea, J. communis, J. oblonga, J. pygmaea,* Azerbaijan, nrDNA, cpDNA sequences, taxonomy.

Several taxa closely related to *Juniperus communis* are currently recognized (Askerov, 2005; Prilipko, 1961) in Azerbaijan (= nomenclature of Adams, 2014): *J. hemispherica* J. & C. Presl. (= *J. communis* var. *hemispherica* [J. & C. Presl.] Parl.); *J. oblonga* M.-Bieb. (= *J. communis* L.) and *J. pygmaea* M.-Bieb. In Azerbaijan, *Juniperus 'pygmaea'* grows as an upright to spreading shrub.

Recently, 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.

The leaves and seed cones of *J. 'pygmaea'* of Azerbaijan are quite similar to those of *J. c.* f. *pygmaea* of Bulgaria and *J. c*. var. *oblonga* of Armenia (Fig. 1).

Figure 1. Leaves and seed cones of *J. c.* f. *pygmaea,* Bulgaria *J. c.* f. *'pygmaea',* Azerbaijan and *J. c*. var. *oblonga*, Armenia.

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

MATERIALS AND METHODS

Plant material - 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° 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).

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 that four of the *J. 'pygmaea'* of Azerbaijan, are in a clade with *J. communis* '*oblonga*' of Armenia (= *J. communis*, Adams 2014). The fifth *J. 'pygmaea'* was polymorphic for two bp in its nrDNA and may be a hybrid.

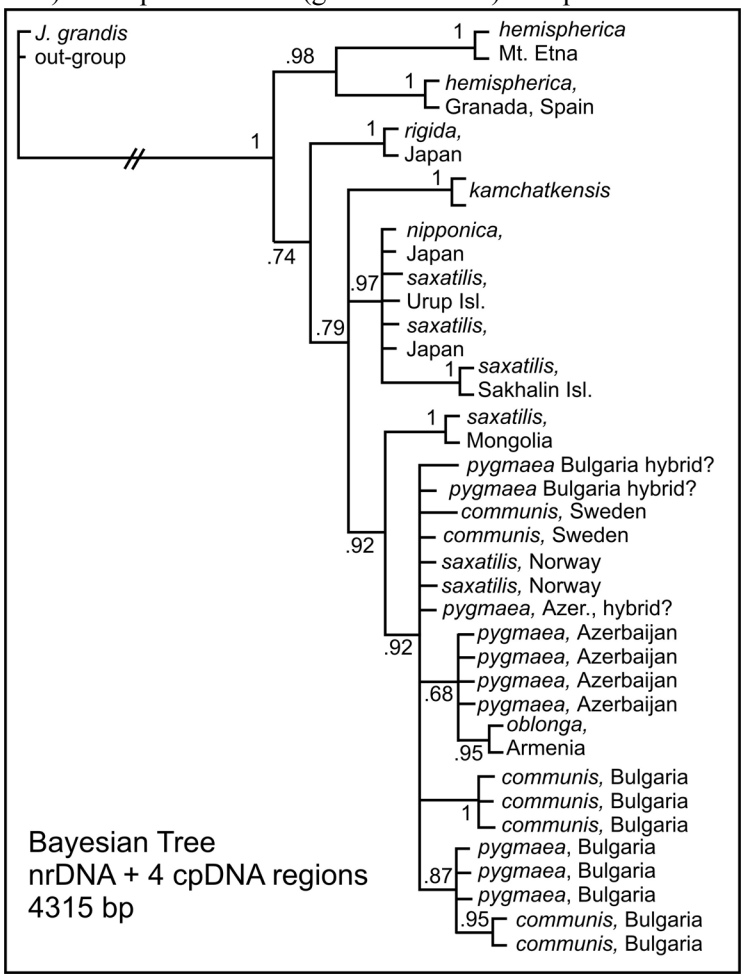

Figure 2. Bayesian tree of *Juniperus* sect. *Juniperus* taxa of the eastern hemisphere. Numbers at branch points are posterior probabilities. See text for discussion.

The *J. 'pygmaea'* plants of Azerbaijan are not in a clade with typical *J. communis* f. *pygmaea* of Bulgaria.

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. Because the two polymorphic bp were removed from the nrDNA of the *J. 'pygmaea'*, these five samples show no variation. The two samples of *J. communis* f. *pygmaea* of Bulgaria, next to *J. communis* of Sweden in Fig. 3, appear to be hybrids.

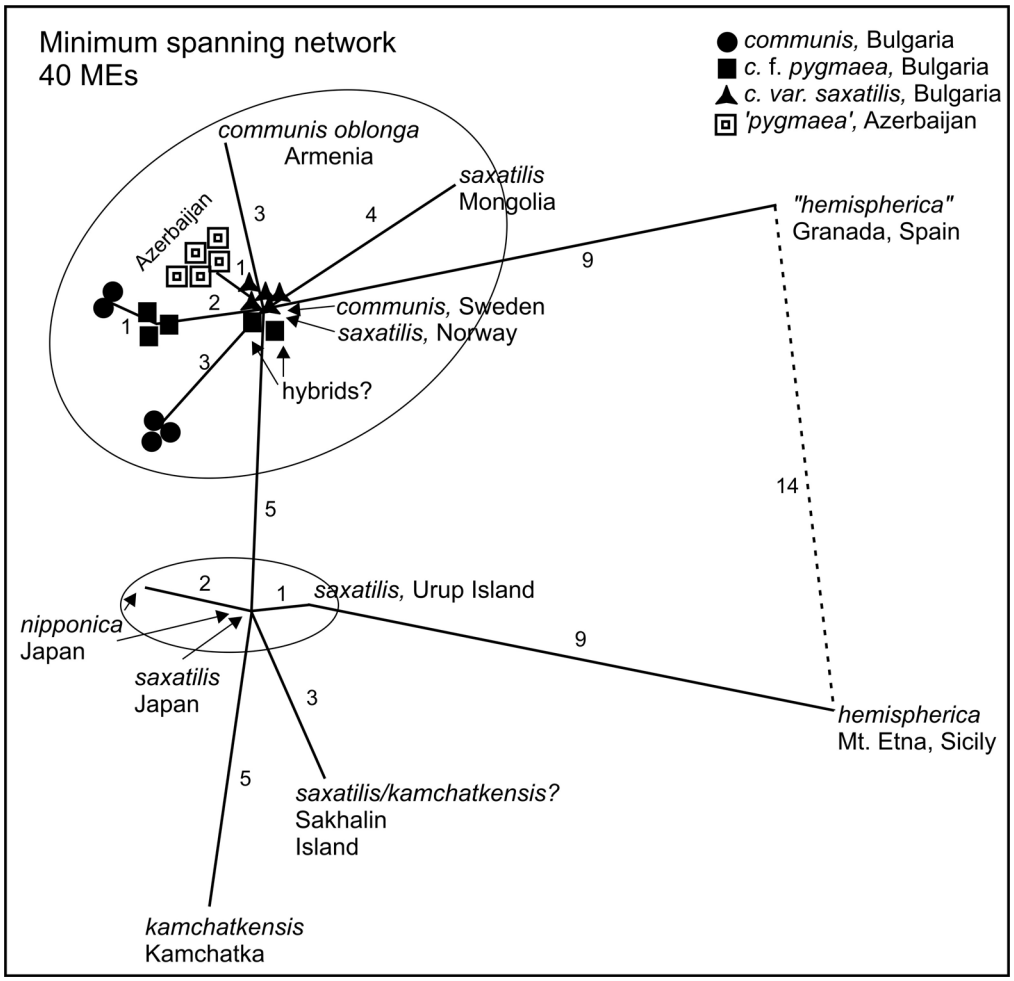

Figure 3. Minimum spanning network of *J. communis* and its varieties based on 40 MEs (mutational events = SNPs + indels). Numbers next to the links are the number of MEs. The dashed line is the second shortest link between the *hemispherica* taxa.

Examination of the habits of *J. communis* f. *pygmaea* of Bulgaria, *J. communis 'oblonga'* of Armenia compared to *J. 'pygmaea'* of Azerbaijan (Fig. 4) is useful. The habit of *J c.* f. *pygmaea* of Bulgaria, is a compact shrub with rigid branchlets. In contrast, *J. 'pygmaea'* of Azerbaijan, is a large, open shrub with weeping branchlets and is quite similar to *J. communis 'oblonga'* of Armenia. *Juniperus 'pygmaea'* differs from *J. communis* in Spain and Hungary in having weeping, versus erect, foliage (Fig. 4). However, the flaccid (weeping) branchlets may be controlled by only a few genes (as suggested by the lack of DNA differences). The wide range of plant habits is shown in the Hungary population of *J. communis* (Fig. 4) with upright trees, shrub-trees, and shrubs as apical growth is differentially expressed.

In summary, *J. 'pygmaea'* of Azerbaijan, is the same taxon as *J. communis 'oblonga'* of Armenia and not *J. communis* f. *pygmaea* of Bulgaria. It should be treated at *J. communis* in Azerbaijan.

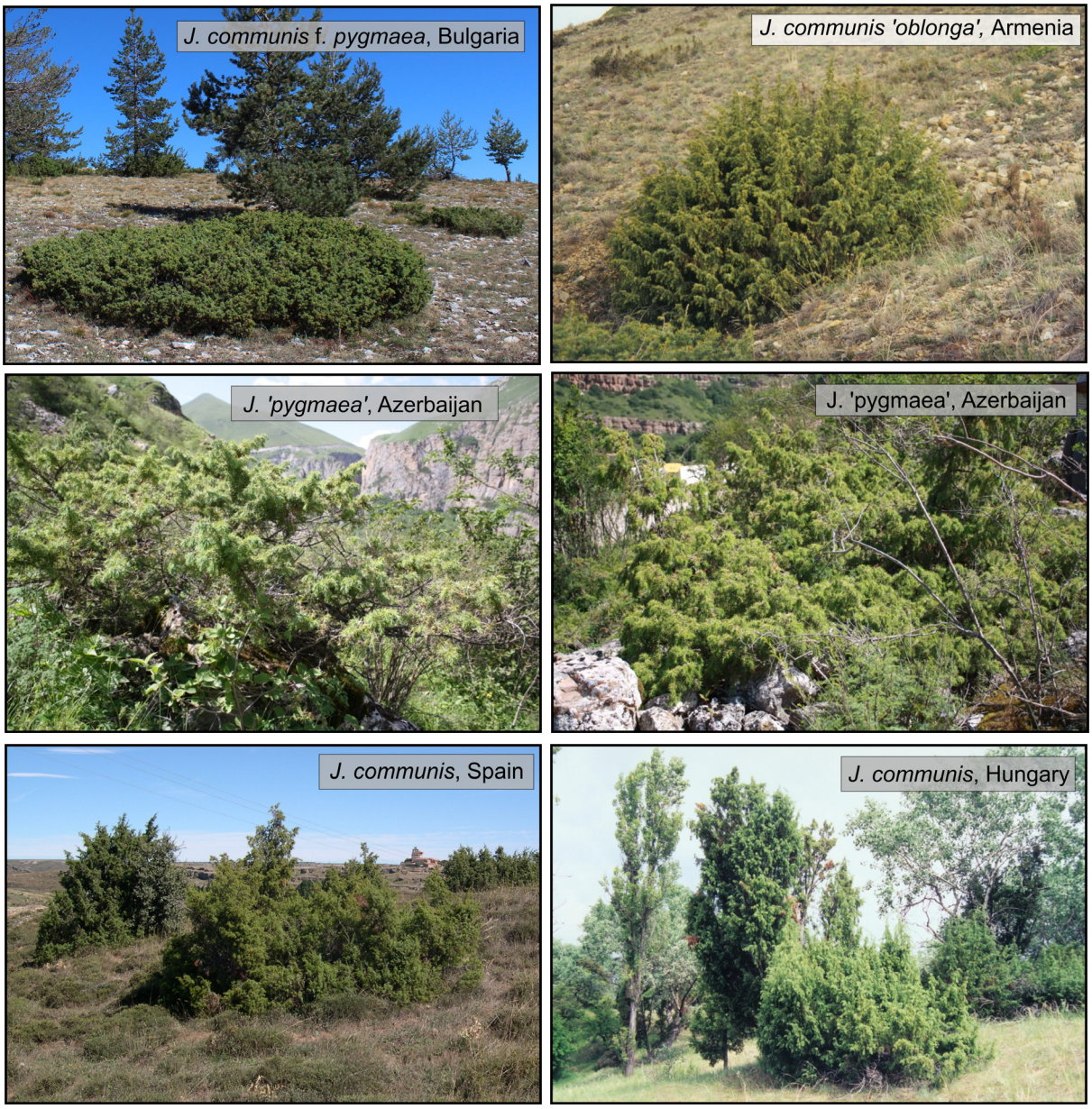

Figure 4. Plant habits of *J communis* f. *pygmaea*, Bulgaria, *J. communis 'oblonga',* Armenia compared to *J. 'pygmaea'*, Azerbaijan and *J. communis*, Spain and Hungary.

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