

nrDNA and petN-psbM sequencing reveals putative *Juniperus oxycedrus* L. from Azerbaijan, Bulgaria, Cyprus and Israel to be *J. deltoides* R. P. Adams

Robert P. Adams

Baylor University, Biology Department, One Bear Place, #97388, Waco, TX 76798, USA,
Robert_Adams@baylor.edu

Vahid Farzaliyev

Deputy Director for Science, Central Botanical Garden, Azerbaijan National Academy of Science,
Badamdar shosse 40, Baku, Azerbaijan, AZ 1073.

Salih Gucel

Environmental Research Institute, Near East University, North Nicosia, Cyprus

Hagar V. Leschner

Collection Manager, The Herbarium (HUJ), The National Natural History Collections, The Hebrew
University, Edmond J. Safra Campus, Giv'at Ram, Jerusalem 91904 Israel

Tuğrul Mataraci

Tarabya Bayırı Cad., Prof. Sitesi C Blok D:1 Tarabya-Sarıyer, İstanbul, Turkey

Alexander N. Tashev

University of Forestry, Dept. of Dendrology, 10, Kliment Ochridsky Blvd., 1756 Sofia, Bulgaria

and

Andrea E. Schwarzbach

Department of Health and Biomedical Sciences, University of Texas - Rio Grande Valley,
Brownsville, TX 78520, USA.

ABSTRACT

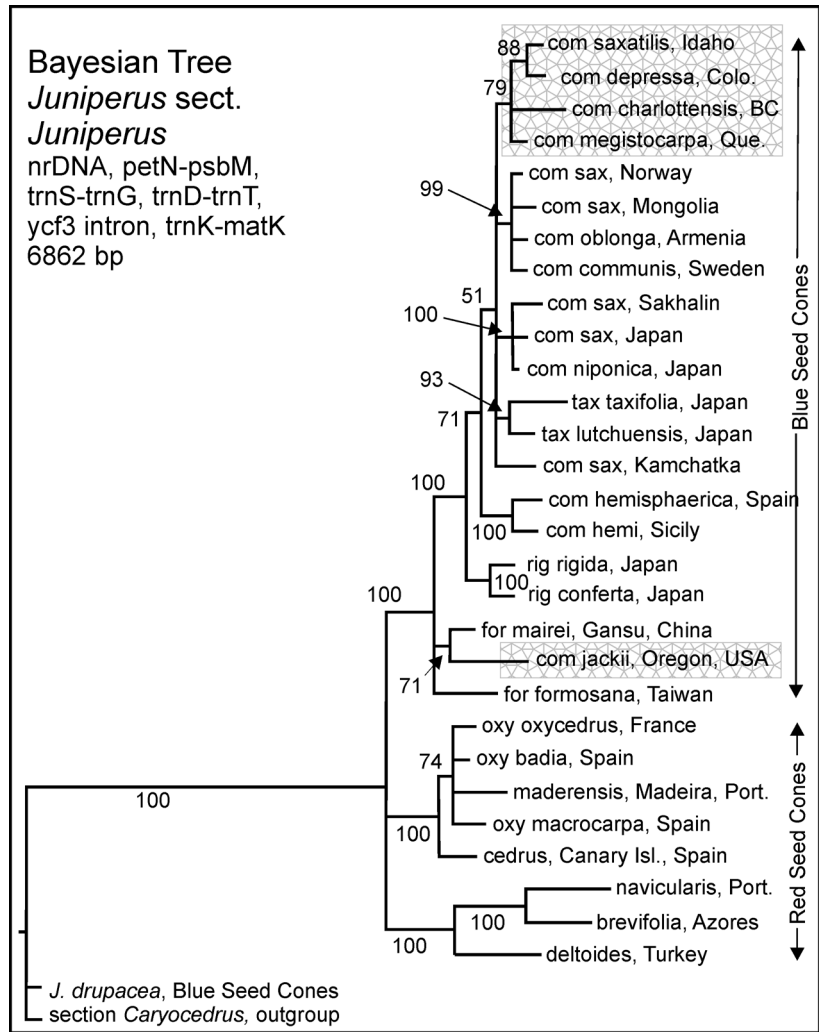
A Bayesian analysis based on nrDNA and petN-psbM (combined 2054 bp) of putative *J. deltoides* from Azerbaijan, Bulgaria, Cyprus and Israel confirmed that they are in the clade with *J. deltoides* and well resolved from *J. oxycedrus*. There is some minor variation in *J. deltoides* (only a one or two bp differences in the entire *J. deltoides* clade). This study supports previous studies that show *J. deltoides* occurs from central Italy, eastward through the Mediterranean to Azerbaijan as its eastern-most location. *Juniperus oxycedrus* appears to be confined to the western Mediterranean. The taxa can easily be recognized by the deltoid leaf bases and exposed leaf scars on the tips of seed cones of *J. deltoides* versus the tapered leaf bases and smooth seed cone tips of *J. oxycedrus*. Published on-line www.phytologia.org *Phytologia* 97(4): 286-290 (Oct 1, 2015). ISSN 030319430.

KEY WORDS: *Juniperus deltoides*, *J. oxycedrus*, SNPs, nrDNA, petN-psbM, plant geography.

In 2004, Adams described a new species, *J. deltoides* R. P. Adams from the eastern Mediterranean that was morphologically similar to *J. oxycedrus* L., except in having deltoid shaped leaves that were broad at the leaf base and with protruding leaf scar bracts on the end of the seed cones. Subsequently, Adams et al. (2005) found large differences in nrDNA sequences confirming the taxonomic decision. Adams et al. (2005) confirmed that trees previously identified as *J. oxycedrus* in Greece, Turkey and central Italy were, in fact, the new species, *J. deltoides*. Additional gene sequencing of nrDNA plus four cp genes confirmed the initial DNA work in the phylogeny of *Juniperus* (Adams and

Schwarzbach, 2013, Adams, 2014). Adams and Schwarzbach (2012) in a study of *Juniperus* sect. *Juniperus* based on nrDNA and five cp regions showed that *J. deltoides* was in a clade with *J. navicularis* and *J. brevifolia*, whereas, *J. oxycedrus* was in a clade with *J. cedrus*, *J. macrocarpa* and *J. maderensis* (Fig. 1).

Figure 1. Bayesian tree of *Juniperus* sect. *Juniperus* based on nrDNA and five cp regions. Notice that *J. deltoides* is in a well supported clade with *J. brevifolia* and *J. navicularis*, not in the clade with *J. oxycedrus*, *J. maderensis*, and *J. cedrus*. From Adams and Schwarzbach, 2012.



More recently, *J. oxycedrus* f. *yaltirikiana* Meral, Avci & Ziel., Turkey, has been shown (Adams and Mataraci, 2011) to be *J. deltoides* f. *yaltirikiana* (Meral, Avci & Ziel.) R. P. Adams and *J. oxycedrus* var. *spilinanus* Yalt., Elicin & Terzioglu was proved (Adams et al. 2010) to be *J. deltoides* var. *spilinanus* (Yalt., Elicin & Terzioglu) Terzioglu.

Adams and Tashev (2012) found that the volatile leaf oils of *Juniperus deltoides* in Bulgaria contained key components (cis-p-mentha-2,8-dien-1-ol, carvone, (2E)-decenal, α -copaene, α -copaen-11-ol, α -calacorene, cis-calamenen-10-ol, and cadalene that are absent in *J. oxycedrus* from France; indicating the

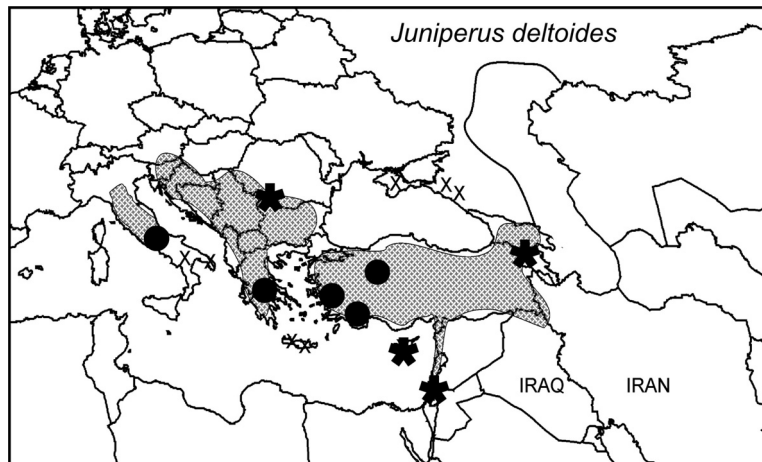


Fig. 2. The distribution of *J. deltoides*. Stars show the locations of newly sampled populations included in this study.

presence of *J. deltooides* in Bulgaria.

The purpose of the present study was to examine additional populations of putative *J. deltooides* in Azerbaijan, Bulgaria, Cyprus and Israel by nrDNA and petN-psbM sequencing to confirm the presence of *J. deltooides* in these regions.

MATERIALS AND METHODS

Plant material: *J. deltooides*, Adams 9430-9432, Turkey; Adams (Salih Gucel ns) 14544-14547; Cyprus; Adams 9436-9438, Archova, Greece; Adams 9445-9447, Riano, Italy; Adams (Tashev ns) 13126-13130), Bulgaria; Adams (Leshner ns) 14463-14465, Israel; Adams (Farzaliyev ns) 14466-14468, Azerbaijan; *J. d.* var. *spilinanus*, Adams (Mataraci ns) 12064-12066, Turkey. *J. d.* f. *yaltirikiana*, Adams (Mataraci ns) 12393-12395, Zonguldak Prov., Turkey. Voucher specimens are deposited at Baylor University (BAYLU). Vouchers for other locations are reported in previous Adams, et al. papers (cited above).

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.

DNA Amplifications and purification: see Adams, Bartel and Price (2009) and Adams and Kauffmann (2010). 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. R7 (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.

RESULTS AND DISCUSSION

Sequencing nrDNA (ITS1 and ITS2 and flanking regions) gave 1274 bp of data and sequencing petN-psbM (spacer plus flanking regions) yielded 780 bp, for a total of combined of 2054 bp of data. A Bayesian analysis (Fig. 3) shows that all the putative *J. deltooides*, new to this study (Azerbaijan, Bulgaria, Cyprus and Israel), are in the clade with *J. deltooides* and well resolved from *J. oxycedrus*. There is some minor variation in *J. deltooides* (only a one or two bp differences in the entire *J. deltooides* clade).

As previously shown (Fig. 1), *J. deltooides* is part of the *J. navicularis* - *J. brevifolia* - *J. deltooides* clade. In contrast, *J. oxycedrus* is part of the *J. oxycedrus* - *J. macrocarpa* - *J. maderensis* - *J. cedrus* clade.

It is interesting to note that colonization of the Azores (*J. brevifolia*) appears to have come from ancestors similar to *J. navicularis* on the continent (Portugal) (Fig. 3). Whereas, colonization of the Canary Islands (*J. cedrus*) likely came from ancestors similar to *J. maderensis* (Madeira) or *J. oxycedrus* (Portugal, Spain or Morocco) (Fig. 3).

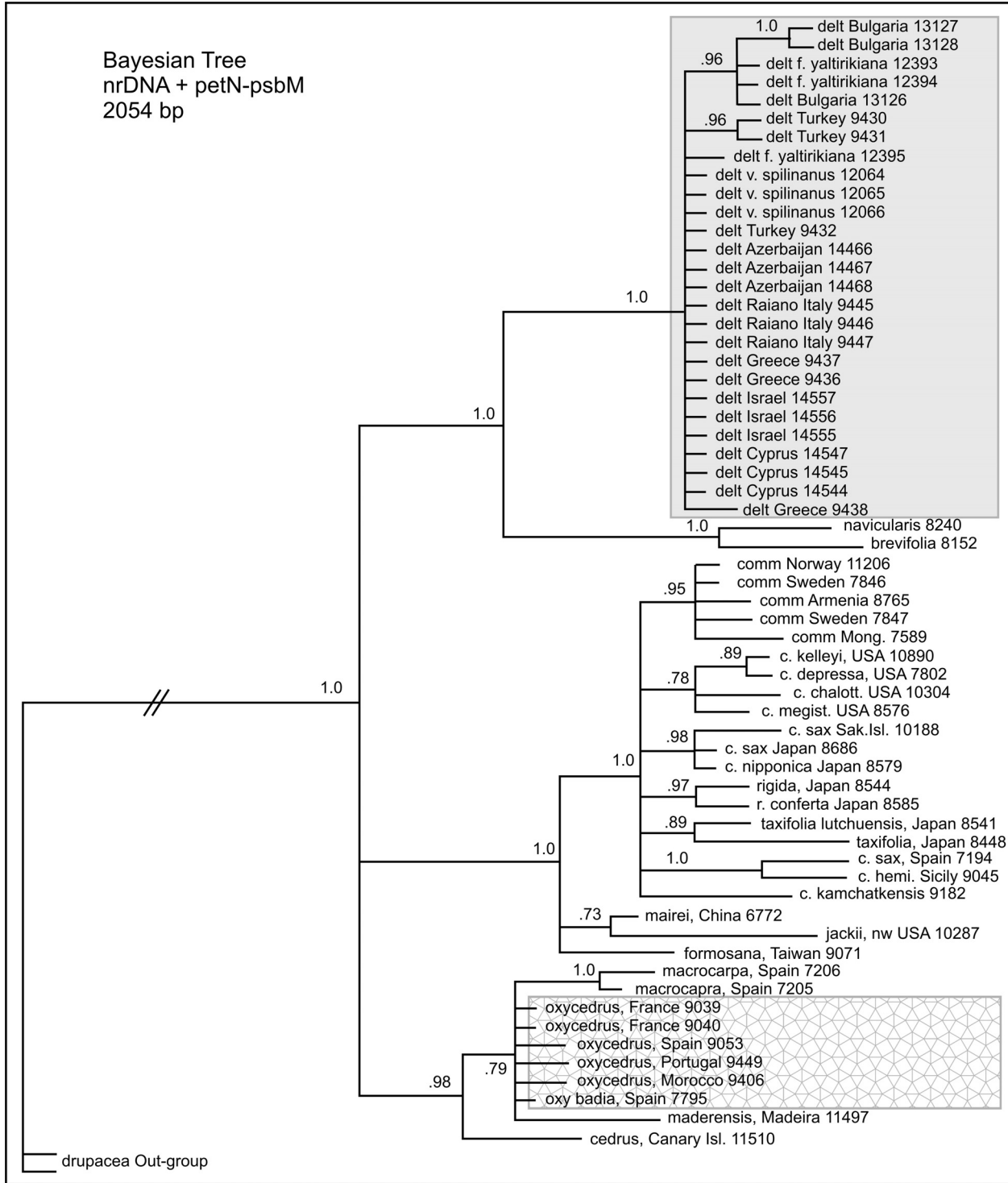


Figure 3. Bayesian tree based on nrDNA and petN-psbM sequences (2054 bp). Numbers at the branch points are posterior probabilities. The shaded area is *J. deltooides* (top right) and the cross hatched area is *J. oxycedrus* (lower right).

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