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RAPD DNA fingerprints and terpenoids: clues to past migrations of *Juniperus* in Arabia and east Africa

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Abstract. Random amplified polymorphic DNAs (RAPDs) and leaf volatile terpenoids were used to compare junipers from Abha, Saudi Arabia with *J. excelsa* from Greece and *J. procera* from Addis Ababa, Ethiopia. Both the RAPDs and terpenoids clearly identified the Abha juniper as *J. procera*. The migration and evolution of *J. excelsa* or pre-*J. excelsa* junipers southward from Asia Minor into Africa is discussed. A computer program, PCO3D, is now available for 3-D ordination of RAPDs data. In addition, this research supports the recognition of both *J. excelsa* and *J. procera* as separate species.

Key words: *Juniperus procera* – *Juniperus excelsa* – Cupressaceae – RAPDs – DNA – Terpenes – Taxonomy

Introduction

Juniperus procera Hochst. ex Endl. is the only species of the genus that grows naturally in the southern hemisphere.

The taxon was described by Hoschstetter and published by Endlicher in 1847 (Endlicher 1847) from material obtained in Ethiopia. Melville (1960) lists it as the only juniper in East Africa, where it occurs in the mountainous regions between altitudes of 1,500 and 2,500 m (Hall 1984) in Ethiopia, Kenya, Tanzania, Uganda, Malawi, Zaire, and Zimbabwe (Fig. 1). The Zimbabwe site is the southernmost native population of any known juniper (Kerfoot 1966). While *Juniperus procera* has been considered to be a "variant" (not formally treated as a variety) of *J. excelsa* M.-Bieb. from Europe (Exell and Wild 1960), it has more recently been treated as being conspecific with *J. excelsa* (Kerfoot 1975; Kerfoot and

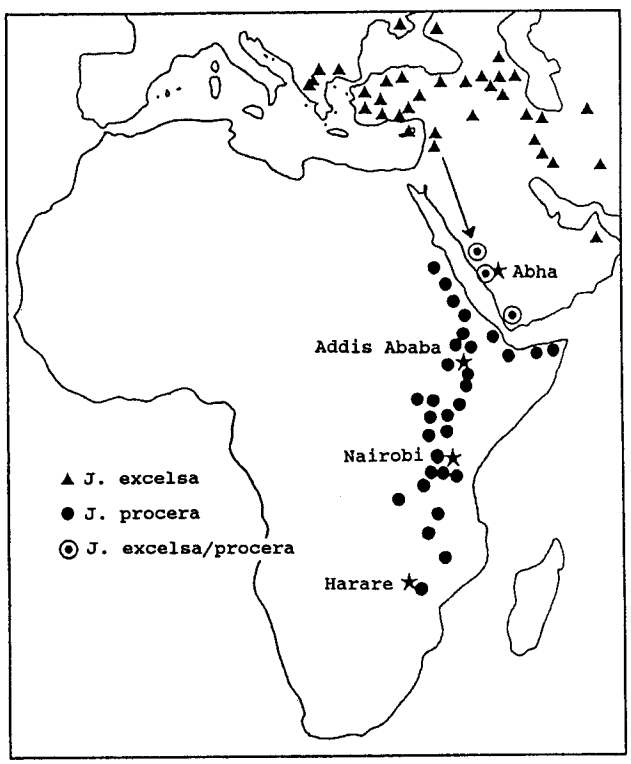

Fig. 1. Distribution of *J. excelsa*, *J. procera*, and the *J. excelsa/procera* junipers of the southwestern Arabian peninsula. The *Juniperus excelsa* distribution is based on Browicz (1982) and that of *J. procera*, on Kerfoot (1966). The *arrow* marks the proposed migration pathway for *J. excelsa* (or pre-*J. excelsa*) plants southward into east Africa (Kerfoot 1975)

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Lavranos 1984; Hall 1984). Kerfoot (1975) postulated that *J. procera* originated from *J. excelsa* in Asia Minor (Fig. 1) in the Mio-Pliocene during the period that *J. excelsa* expanded southward along the western mountains of the Arabian Peninsula, thence across the Red Sea to Ethiopia and southward along the East African rift mountains as far south as Zimbabwe (see Fig. 1). The junipers in the mountains of the southwestern Arabian peninsula would then represent a key link between *J. excelsa* in Asia Minor and the east African juniper (Fig. 1).

Using terpenoids Adams (1990a, b) recently found numerous differences between *J. excelsa* from Greece, and *J. procera* from Ethiopia and Kenya. He concluded that *J. procera* was in fact a separate species.

If the origin of *J. procera* was by migration of *J. excelsa* with concomitant or subsequent differentiation, then one might expect to detect this differentiation in a geographically intermediate population such as that found at Abha, Saudi Arabia.

The purpose of the investigation presented in this paper is to examine the random amplified polymorphic DNAs (RAPDs) and leaf oils of *J. procera* from east Africa and *J. excelsa* from northern Greece and compare these with the RAPDs and leaf oils of the juniper from Abha, Saudi Arabia.

Materials and methods

Specimens were collected from Addis Ababa, Ethiopia, 2,500 m, Adams *5292-5293*, *6184-6188*; 38 km w. of Nairobi, Kenya, 2,170 m, Adams, *5333-5335*; approximately 6 km s.w. of Kijabe, Kenya, 1,500 m, Adams *6007-6009*; near Abha, Saudi Arabia, H. F. Abulfatih *1–7* (=Adams *6190-619*). Voucher specimens are deposited at the Baylor University herbarium.

RAPDs analysis followed that described by Demeke et al. (1992). Fresh or silica gel-preserved leaves (0.2 g) were ground in liquid nitrogen and DNA extracted by the SDS protocol (Dellaporta et al. 1983). PVP (1% w/v)± was added to the extraction buffer. The polymerase chain reaction (PCR) was performed in a volume of 25 µl containing 50 m*M* TRIS-HCl (pH 9), 1.5 m*M* $MgCl_2$, 0.01% gelatin and 0.1% Triton X-100, 0.2 m*M* of each DNTPs, 0.36 µ*M* primers, 0.5 ng genomic DNA, and 1.0 unti of Taq DNA polymerase. A control PCR tube containing all of the components, with the exception of genomic DNA, was run with each primer to check for contamination. Inhibition of amplification was observed in some of the junipers (Demeke and Adams 1992) when the amount of DNA per PCR tube was greater than 1.0 ng. Reducing the amount of DNA to 0.5 ng (per 25 µl) resulted in amplification of all DNA samples. Ten-mer primers (Table 1) that gave several bright bands and did not have any false bands (in the controls) were used. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research). The thermal cycle used was 94°C (1.5 min) for initial strand separation, then 40 cycles of 37°C (2 min), 72°C (2 min) and 94°C (1 min). Two additional steps were used, 37°C (2 min) and 72°C (5 min), for final extension. Amplification products were analyzed by electrophoresis in 1.5% agarose gels and detected by staining with ethidium bromide. The gels were photographed under UV light with Polaroid film 667. pGEM DNA (Promega) was used as molecular size markers. The size of the bands and their intensity was scored as: 0=no band; 1=very, very faint, 2=very faint; 3=faint; 4=medium; 5=bright; 6=very bright band. Similarity measures were computed using absolute character state differences (Manhattan metric) divided by the maximum value for that character over all taxa (=Gower metric, Gower 1971; Adams 1975). Principal coordinate analysis (PCO) follows the procedure of Gower (1966). The software package for 3-D ordination of RAPDs, PCO3D, is available from RPA for PC compatible computers.

Table 1. List of primers used in this study

Code[a]	Sequence (5′–3′)	Code	Sequence (5′–3′)
116	TAC GAT GAC G	232	CGG TGA CAT C
143	TCG CAG AAC G	234	TCC ACG GAC G
153	GAG TCA CGA G	237	CGA CCA GAG C
204	TTC GGG CCG T	239	CTG AAG CGG A
212	GCT GCG TGA C	244	CAG CCA ACC G
223	GAT CCA TTG C	RC42	GCA AGT AGC T
227	CTA GAG GTC C		

[a] The numbered primers (166–244) were purchased from the University of British Columbia. RC42 was synthesized at the Plant Biotechnology Institute, NRC, Saskatoon, Canada

The volatile leaf oils were isolated by steam distillation of approximately 200 g of foliage suspended in a chamber above a boiling flask for 2 and 24 h to determine yields (von Rudloff 1967). Mass spectra (MS) were recorded with a Finnigan Ion Trap mass spectrometer (ITMS), model 800, directly coupled to a Varian 6,500 gas chromatograph, using a J & W DB5 column (see Adams 1990b, for operating conditions). Identifications were made using combined MS and retention times (Adams 1989).

Results and discussion

Figure 2 shows typical RAPDs bands for individuals of *J. procera*, Ethiopia (lanes 1–3), Abha (4–6), and *J. excelsa*, Greece (7–9). Notice the major 500-bp band shared by *J. procera* and the Abha junipers and the 390-bp band characteristic of *J. excelsa*. The computation of similarity measures using 121 RAPD bands and subsequent PCO ordination clearly reveals the pattern among these taxa (Fig. 3). The first three principal coordinates accounted for 54%, 14% and 9% of the total variance among the individuals. The first coordinate (54%) separates *J. excelsa* from *J. procera* (Fig. 3); the second axis (14%) separates *J. procera*, Ethiopia from *J. procera*, Abha, the thrid coordinate (9%) separates J. *procera* individuals. It should be noted that RAPDs can be used at various taxonomic levels from sub-generic to varieties in *Juniperus* (Adams and Demeke 1993). Several of the RAPD bands were conserved and are likely indicators of sectional differences (Adams and Demeke 1993). In any case, the RAPDs clearly indicate that the Abha plants are *J. procera*.

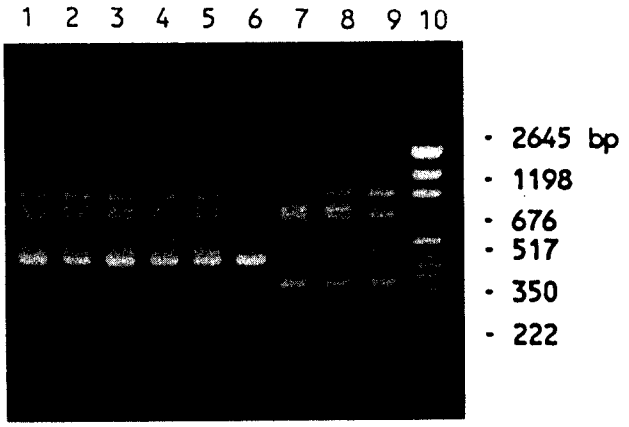

Fig. 2. RAPDs (primer 223) for juniper individuals. *Lanes 1–3 J. procera*, Ethiopia; *lanes 4–6* junipers from Abha, Saudi Arabia; *lanes 7–9 J. excelsa* Greece; *lane 10* molecular size markers. Note the 500 bp band uniting *J. procera*, Ethiopia and Abha and the 390-bp band characteristic of *J. excelsa*

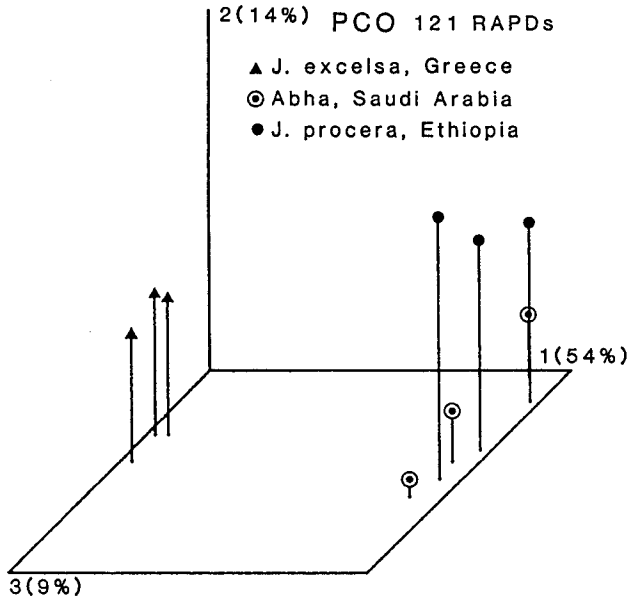

Fig. 3. PCO ordination of individuals of *J. excelsa*, Greece, juinipers at Abha, and *J. procera*, Ethiopia based on 121 RAPDs. The junipers from Abha are clearly *J. procera*

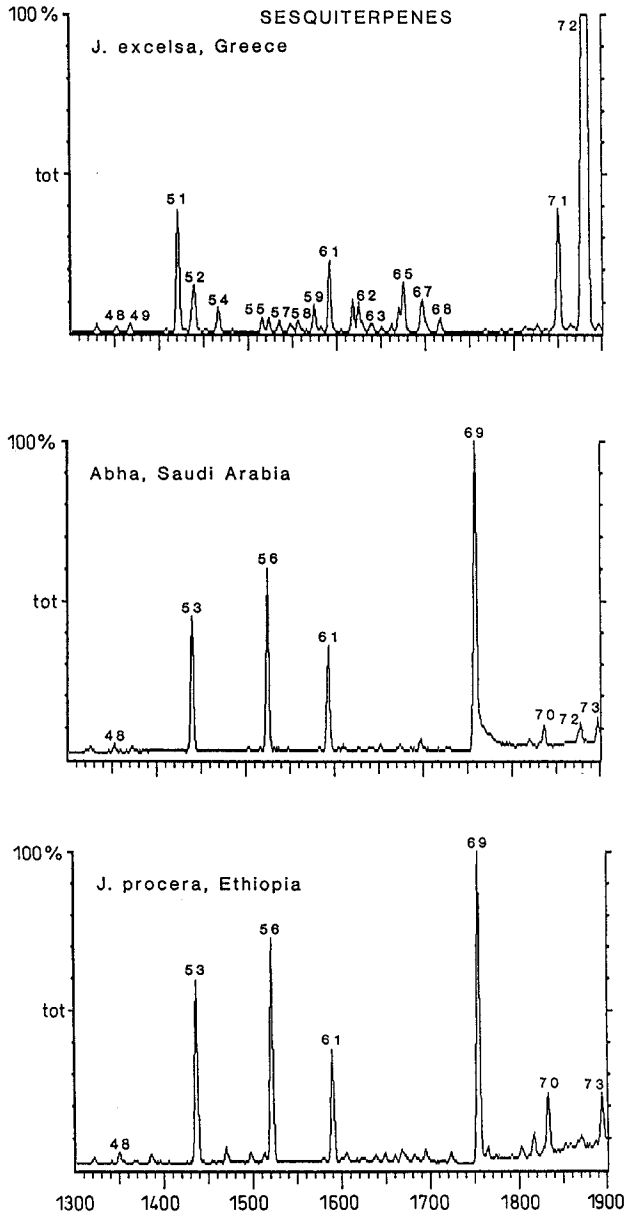

Fig. 4. Sesquiterpenes from the volatile leaf oils of *J. excelsa*, Greece, junipers at Abha, and *J. procera*, Ethiopia. The junipers from Abha are clearly *J. procera*. The peak numbers refer to Table 2, where complete analyses are reported

The volatile leaf oil of *J. procera* from east Africa is complex but dominated by α-pinene, 3-carene and diterpenes such as *cis*- and *trans*-totarol, abietadiene, and *cis*-abietal. Moderate amounts of the eudesmols are present, as well as elemol, along with lesser quantities of β-pinene, myrcene, β-phellandrene, and terpinolene (Table 2). Numerous compounds are present in small concentrations (Table 2). Cedrol, the major component of the wood oil (Adams 1990b), is either missing or present in trace amounts in *J. procera* (Table 2). The oils of *J. procera* are very different from the Grecian *J. excelsa*. It is noteworthy that *J. excelsa* has a great diversity of oxygenated terpenes and sesquiterpene hydrocarbons; these are absent in every population of *J. procera* examined. Cedrol, the major component of the leaf oil of *J. excelsa*, is missing or found in only trace amounts in *J. procera*. Limonene, another major component in *J. excelsa*, is also present in quite low quantities in *J. procera* (Table 2).

The sesquiterpenes are particularly different between the taxa in this case. Figure 4 shows the sesquerpenes for *J. excelsa*, Greece, Abha plants, and *J. procera*, Ethiopia.

Table 2. Volatile leaf oil compositions of *Juniperus excelsa* leaf oil from Greece, the junipers from Abha, Saudi Arabia, and *J. procera* from Ethiopia and Kenya

Compound[a]	Percentage total oil			
	J. excelsa		*J. procera*	
	Greece	Abha	Ethiopia	Kenya
1. (*E*)-2-Hexenal	0.1	T	0.2	0.2
2. Tricyclene	0.1	T	T	T
3. α-Thujene	T	T	T	T
4. α-Pinene	22.5	23.5	28.1	26.3
5. α-Fenchene	0.2	1.0	1.5	1.1
6. Camphene	0.5	0.5	T	T
7. Thuja-2,4(10)-diene	0.1	–	–	–
8. Verbenene	T	T	0.2	0.1
9. Sabinene	T	0.1	0.4	T
10. 1-Octen-3-ol	–	–	0.5	T
11. β-Pinene	0.6	3.1	4.4	4.8
12. Myrcene	1.9	3.4	4.7	3.8
13. α-Phellandrene	0.1	T	T	–
14. Car-3-ene	2.3	30.8	29.6	20.5
15. α-Terpinene	0.1	T	0.1	T
16. *o*-Cymene	–	T	T	–
17. *p*-Cymene	0.4	T	T	T
18. Sylvestrene	–	0.3	0.4	0.3
19. Limonene	22.7	1.0	0.7	T
20. β-Phellandrene	T	1.1	2.1	3.0
21. 1,8-Cineole	–	–	–	T
22. *trans*-Ocimene	T	T	T	T
23. *Γ-Terpinene*	0.6	0.2	0.2	0.2
24. Terpinolene	0.9	3.3	4.1	2.9
25. Linalool	–	0.8	1.0	0.5
26. endo-Fenchol	0.2	–	–	–
27. 1,3,8-*p*-Menthatriene	–	T	0.1	T
28. *cis*-*p*-Menth-2-en-1-ol	–	T	T	–
29. *cis*-Pinene hydrate	T	–	–	–
30. α-Campholenal	0.1	T	T	–
31. *trans*-Pinocarveol	0.2	–	–	T
32. Camphor	0.5	T	0.4	0.1
33. Borneol	T	T	0.4	0.2
34. Nonanol	–	–	T	T
35. 4-Terpineol	0.2	T	0.2	T
36. Naphthalene	T	T	–	–
37. *p*-Cymen-8-ol	T	T	0.1	T
38. α-Terpineol	T	0.3	0.4	0.2
39. Myrtenol	T	–	–	–
40. Verbenone	0.1	–	–	–
41. *trans*-Carveol	0.1	–	–	–
42. endo-Fenchyl acetate	0.3	–	–	–
43. Piperitone	T	–	–	–
44. RT1068	0.2	0.7	0.5	0.2
45. Bornyl acetate	0.4	0.3	1.2	0.3
46. Carvacrol	T	–	–	–
47. RT1172	3.3	–	–	–
48. β-Bourbenene	T	0.1	T	T
49. β-Cubebene	0.1	–	–	–
50. α-Cedrene	T	–	–	–
51. 1,7-di-epi-Cedrene	1.7	–	–	–
52. β-cedrene	0.9	–	–	–
53. Caryophyllene	–	2.1	1.0	0.5
54. Thujopsene	0.4	–	–	–
55. α-Cadinene	0.2	–	–	–
56. α-Humulene	0.2	2.5	1.3	0.8
57. *cis*-β-Farnesene	0.2	–	–	–
58. β-Acoradiene	0.1	–	–	–
59. β-Cadinene	0.4	–	–	–
60. *Γ*-Muurolene	T	–	–	–
61. Germacrene D	0.9	1.6	0.7	0.4
62. Valencene	0.5	–	–	–
63. α-Muurolene	0.2	–	–	–
64. α-Alaskene	0.3	–	–	–
65. *Γ*-cadinene	0.8	–	–	–
66. *cis*-Calamenene	T	–	–	–
67. δ-Cadinene	0.7	–	–	–
68. *trans*-*Γ*-Bisabolene	0.2	–	–	–
69. Elemol	–	5.5	1.8	2.2
70. Caryophyllene oxide	–	0.4	0.4	0.3
71. RT1854	2.0	–	–	–
72. Cedrol	28.1	0.4	–	0.2
73. Humulene epoxide II	T	0.4	0.3	0.3
74. Cubenol	0.6	–	–	–
75. *Γ*-Eudesmol	–	0.7	0.4	0.3
76. τ-Cadinol	T	–	–	–
77. τ-Muurolol	T	–	–	–
78. Torreyol	T	–	–	–
79. β-Eudesmol	–	1.1	0.6	0.6
80. α-Eudesmol	–	2.6	1.2	1.3
81. α-Cadinol	T	–	–	–
82. RT2033	0.6	–	–	–
83. (Elemol acetate)	–	0.6	0.6	0.6
84. 8-α-Acetoxyelemol	–	1.2	0.6	0.8
85. epi-13-Manool	–	–	–	0.2
86. Manoyloxide	–	0.4	2.1	1.9
87. Abietatriene	–	0.1	0.1	0.6
88. Abietatdiene	–	0.7	1.9	6.2
89. RT2937	–	–	–	9.0
90. RT3080	–	–	–	0.3
91. *cis*-Totarol	–	T	–	T
92. *cis*-Abietal	0.1	3.7	0.3	0.9
93. RT3282	–	–	–	0.3
94. *trans*-Totarol	–	2.5	0.8	4.6
95. *trans*-Ferruginol	–	0.8	T	0.9

[a] Compounds are listed in order of their elution from a DB5 (=SE54) column. Compounds in parentheses are tentativley identified. Compositional values less than 0.1% are denoted as traces (T) and counted as 0.05% in computing the totals. Unidentified constituents smaller than 0.5% are not reported. See Adams (1990b) for mass spectra of the unknown compounds

The plants from Abha clearly show the sesquiterpene pattern of *J. procera* (Fig. 4).

In conclusion, on the basis of their DNA and terpenoids, the plants at Abha are clearly *J. procera*. Morphologically, the junipers at Abha are very similar in appearance to *J. excelsa*. A key character used to separate the species is the number of seeds per female cone: 5–6 in *J. excelsa* (Franco 1964); 1–4 in *J. procera* (Exell

and Wild 1960). However, because the female cones from junipers from Abha contained 2–5 seeds per cone, unequivocal morphological identification is difficult. It appears that *J. excelsa* and *J. procera* are sibling species. Their divergence and continued separation has led to numerous RAPDs and terpenoid differences. *Juniperus procera* appears to have a unique, common gene pool and is maintaining these gene combinations. The population of *J. procera* at Abha shows some differentiation as would be expected. Clearly, the barrier to past and present migration of *J. excelsa* southward is the wide geographic disjunction between Asia Minor and the mountains in the southwestern Arabian peninsula.

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References

Adams RP (1975) Statistical character weighting and similarity stability. Brittonia 27:305–316

Adams RPO (1989) Identification of essential oils by ion trap mass spectroscopy. Academic Press, New York

Adams RP (1990a) The volatile leaf oils of *Juniperus excelsa* M.-Bieb. from a native stand in Greece versus *J. excelsa* cultivated at Kew, London. J Ess Oil Res 2:45–48

Adams RP (1990b) *Juniperus procera* of east Africa: volatile leaf oil composition and putative relationship to *J. excelsa*. Biochem Syst Ecol 18:207–210

Adams RP, Demeke T (1993) Systematic relationships in *Juniperus* based on random amplified polymorphic DNAs (RAPDs). Taxon 42:1–19

Browicz K (1982) Chorology of trees and shrubs in south-west Asia and adjacent regions, vol 1. Polish Sci Publ, Warsaw

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: version II. Plant Mol Biol Rep 1:19–21

Demeke T, Adams RP (1992) The effects of plant polysaccharides and buffer additives on PCR. Biotechniques 12:332–334

Demeke T, Adams RP, Chibbar R (1992) Potential taxonomic use of random amplified polymorphic DNA (RAPDs): a case study in Brassica. Theor Appl Genet 84:990–994

Endlicher SL (1847) Synopsis confierarum. Scheitlin & Zollikofer, Sankt Gallen

Exell AW, Wild H (1960) *Cupressacene*. In: Exell AW, Wild H (eds) Flora Zambesiaca, vol 1, part 1. Crown Agents for Overseas Govt, London, p 88

Franco JA (1964) *Juniperus*. In: Tutin TG, Heywood VH, Valentine VH, Walters SM, Webb DA (eds) Flora Europaea, vol 1. Cambridge University Press, Cambridge, p 38

Gower JC (1966) Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53:315–328

Gower JC (1971) A general coefficient of similarity and some of its properties. Biometrics 27:857–874

Hall JB (1984) *Juniperus excelsa* in Africa: a biogeographical study of an Afromontane tree. J Biogeogr 11:47–61

Kerfoot O (1966) Distribution of coniferae: the cupressaceae in Africa. Nature 5065:961

Kerfoot O (1975) Origin and speciation of the cupressaceae in Sub-Sahara Africa. Boissiera 24:145–150

Kerfoot O, Lavranos JJ (1984) Studies on the flora of Arabia. X. *J. phoenicea* L. and *J. excelsa* M. Bieb. Notes R Bot Gard Edninburgh 41:483–489

Melville R (1960) *Gymnospermae*. In: Turrill WB, Milne-Redhead E (eds) *Flora of Tropical East Africa*. Crown Agents for Overseas Govt, London, p 1

Von Rudloff E (1967) Chemosystematic studies in the genus *Picea* Pinaceae). Can J Bot 45:891–901