

**INFRASPECIFIC VARIATION IN *HESPEROCYPARIS*
ABRAMSIANA: ISSRS AND TERPENOID DATA**

Robert P. Adams

Biology Department, Baylor University, Box 727
Gruver, TX 79040, USA
Robert_Adams@baylor.edu

Jim A. Bartel

U.S. Fish and Wildlife Service, Carlsbad Fish and Wildlife Office
6010 Hidden Valley Road, Suite 101
Carlsbad, CA 92011-4213

ABSTRACT

Five *Hesperocyparis* (*Cupressus*) *abramsiana* groves were analyzed by Inter-Simple Sequence Repeats (ISSRs). ISSRs analyses revealed geographical differentiation among the groves (= Silba's subspecies) with the Butano Ridge grove being the most distinct in both ISSRs and terpenoids. Combined ISSR and terpenoid data support the recognition of *Hesperocyparis. abramsiana* var. *abramsiana* and *Hesperocyparis abramsiana* var. *butanoensis* **Bartel & R. P. Adams, comb. nov.** The recognition of Silba's subsp. *locatellii*, *neolomondensis*, and *opleri* were not support by these data. *Phytologia* 91(2): 287-299 (August, 2009).

KEY WORDS: *Hesperocyparis* (= *Cupressus*) *abramsiana* var. *butanoensis*, *C. subsp. locatellii*, *C. subsp. neolomondensis*, *C. subsp. opleri*, ISSR, Inter-Simple Sequence Repeats, terpenes, DNA fingerprinting, systematics.

Hesperocyparis (= *Cupressus*, see Adams et al., 2009) *abramsiana* (C. B. Wolf) Bartel, widely known as Santa Cruz cypress, was listed (as *Cupressus abramsiana*) as an endangered species under the Endangered Species Act of 1973 (ESA) by the U.S. Fish and Wildlife Service (USFWS) in 1987. According to the ESA recovery plan developed for the species (USFWS 1998), *H. abramsiana* is restricted to five groves or populations (Fig. 1) that include a total of

5,100+ individuals that collectively occupy about 142 ha (356 acres) within a 24-km (15-mile) range in the Santa Cruz Mountains in Santa Cruz and San Mateo counties, California, USA. Using an ESRI shape file of the grove boundaries provided by the USFWS, McGraw (2007) clarified that the areal extent of the cypress groves depicted in the recovery plan actually include 41.28 ha (102.0 acres). McGraw (2007) estimated that the areal extent of the five groves totals only 25.87 ha (63.9 acres).

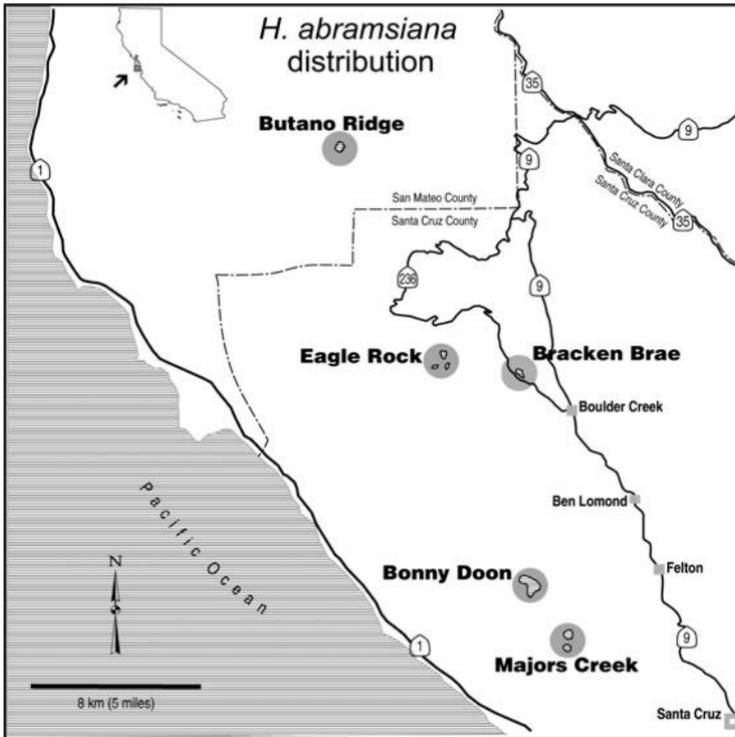


Figure 1. Distribution of *H. abramsiana* groves endemic to Santa Cruz and San Mateo counties, California, USA. Geographic limits of individual cypress stands within each grove, which are depicted in hatched polygons atop half-tone circles, were derived from Fig. 2 from McGraw (2007).

Using the ESA recovery plan and after visiting four of the five groves or populations, Silba (2003) subdivided *Cupressus abramsiana* into five subspecies with his descriptions of four new subspecies; *C. a.* subsp. *locatellii* Silba (restricted to the Eagle Rock grove in Santa Cruz County), *C. a.* subsp. *opleri* Silba (restricted to the Bracken Brae grove in Santa Cruz County), *C. a.* subsp. *neolomondensis* Silba (restricted to the Majors Creek grove in Santa Cruz County), and *C. a.* subsp. *butanoensis* Silba (restricted to the Butano Ridge grove in San Mateo County). According to Silba (2003), the nominate subspecies is restricted to the type locality of *H. abramsiana* (i.e., the Bonny Doon Grove in Santa Cruz County), which Wolf (1948) detailed as “on the southwest slope of Ben Lomond, a mountain 7/10 mi. east of the Bonnie Doon School, elevation 1600 feet.” Given the lack of a taxonomic key or a set of clear morphological characters to separate the subspecies, and the overall poor quality of the self-published article (e.g., orthographic errors, illegibility), Silba’s (2003) new subspecies seem to be morphologically indistinct and separated largely by collection locality.

Adams and Bartel (2009) examined the volatile leaf oils of *H. abramsiana* and Silba’s subsp. *butanoensis*, *locatellii*, *neolomondensis*, and *opleri* (Fig. 2). The leaf oils of these taxa appear to separate *H. abramsiana* into 2 groups (Fig. 2) composed of *butanoensis* and the remaining four groves. However, note that 3 trees of *butanoensis* had oils similar to *H. pygmaea* (Fig. 2, see also Table 1, Adams and Bartel, 2009). Adams and Bartel (2009) concluded that additional research was needed into the nature of infraspecific variation in *H. abramsiana*.

To gather additional genetic information about the validity of these subspecies, analyses using Inter-Simple Sequence Repeats (ISSRs) were conducted. The leaf samples utilized in the present study were taken from the same trees analyzed by Adams and Bartel (2009).

MATERIALS AND METHODS

Plant material - Specimens used in this study: *H. abramsiana*, Bonny Doon Grove, Santa Cruz Co., CA, Bartel 1598a-e; Butano Ridge Grove, (*butanoensis*), Pescadero Creek County Park,

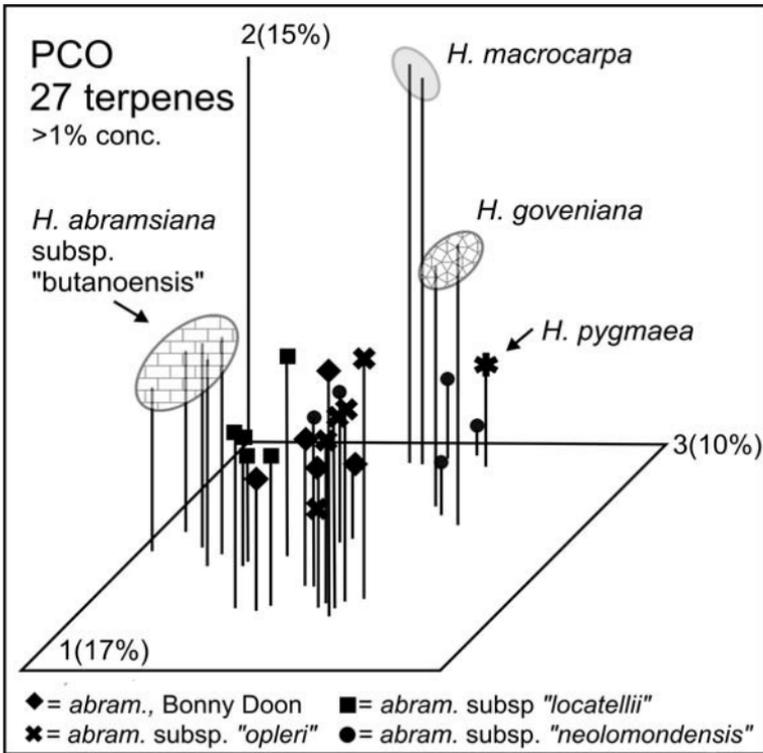


Figure 2. PCO of *H. abramsiana* and Silba's subspecies based on 27 terpenes. From Adams and Bartel (2009).

San Mateo Co., CA, *Bartel 1605a-e*; Eagle Rock Grove, (*locatellii*), Santa Cruz Co., CA, *Bartel 1599a-e*; Majors Creek Grove, (*neolomondensis*), Wilder Ranch State Park, Santa Cruz Co., CA, *Bartel 1604a-e*; Bracken Brae Grove, (*opleri*), Santa Cruz Co., CA, *Bartel 1600a-e*; *H. goveniana*, SFB Botanical Reserve, Monterey Co., CA, *Bartel 1596a-e*; *H. pygmaea*, Casper Little Lake Rd., CA, *Bartel 1603a-e*. Bartel specimens are held in his personal herbarium in Carlsbad, CA.

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 using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia, CA). ISSR primers were purchased from the University of British Columbia (5'-3' seq., annealing temperature used): 807: AGA GAG AGA GAG AGA GT (50°C), 808: AGA GAG AGA GAG AGA GC (50°C), 811: GAG AGA GAG AGA GAG AC (50°C), 812: GAG AGA GAG AGA GAG AA (50°C), 836: AGA GAG AGA GAG AGA GYA (54°C), 840: GAG AGA GAG AGA GAG AYT (54°C), 841: GAG AGA GAG AGA GAG AYC (54°C), 847: CAC ACA CAC ACA CAC ARC (58°C), 881: GG TGG GGT GGG GTG (50°C), 886: VDV CTC TCT CTC TCT CT (50°C), 887: DVD TCT CTC TCT CTC TC (54°C), 895: AGA GTT GGT AGC TCT TGA TC (50°C).

PCR conditions and numerical methods - see Adams and Bartel, 2009.

RESULTS AND DISCUSSION

The 12 ISSR primers resulted in 89 scoreable bands among the taxa. A minimum spanning network was constructed using the matrix of associations and is shown in figure 3. Individuals of each of the five groves of *H. abramsiana* cluster together. *Hesperocyparis goveniana* and *H. pygmaea* are well resolved (Fig. 3). However, the Butano Ridge grove actually clustered after *H. pygmaea* clusters (Fig. 3).

PCO of the association matrix removed six significant eigenroots accounting for: 13.74, 11.84, 10.51, 7.89, 7.70 and 6.67% of the variance among individuals. Ordination shows a similar pattern (Fig. 4) with *H. goveniana* and *H. pygmaea* being distinct from *H. abramsiana*. Most of the *H. abramsiana* groves are not resolved.

Removing *H. goveniana* and *H. pygmaea* from the data set and running a PCO focuses on the *H. abramsiana* groves. Ordination shows that all five *H. abramsiana* groves are resolved (Fig. 5). A minimum spanning network (dashed lines, Fig. 5) shows that Butano Ridge population is the most divergent grove (0.730). While three groves consist of a single largely distinct stand of *H. abramsiana*, the

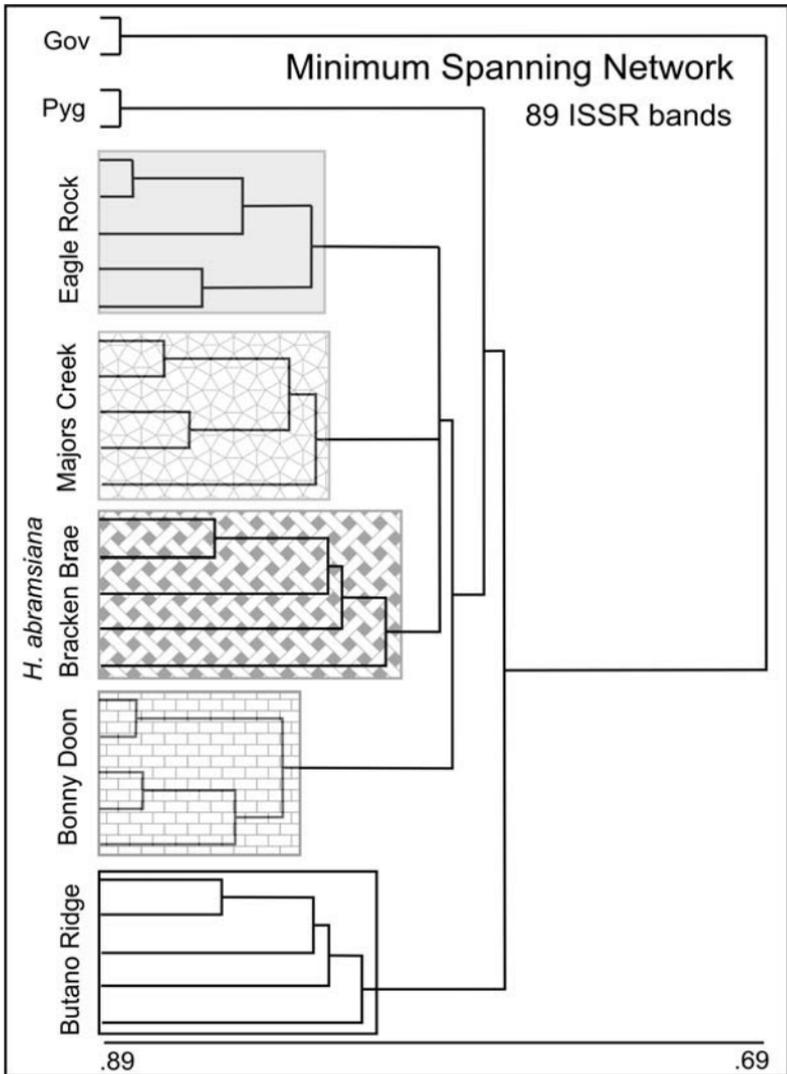


Figure 3. Minimum spanning network based on 89 ISSR bands. Gov = *H. goveniana*, Pyg = *H. pygmaea*.

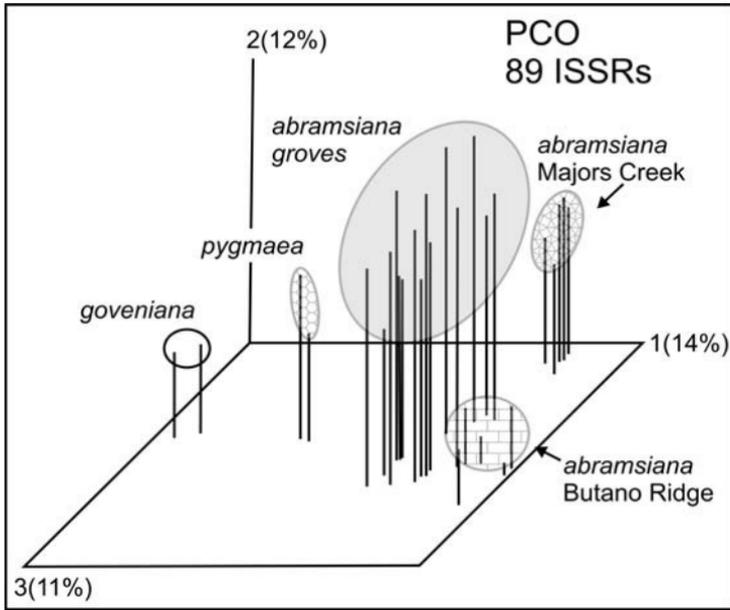


Figure 4. PCO based on 89 ISSR bands.

Eagle Rock and Majors Creek groves are made up of three and two stands respectively (USFWS 1998, McGraw 2007). Though Silba (2003) used the groves or populations as identified in the ESA recovery plan to delimit his five subspecies, he also included within *C. a.* subsp. *neolomondensis* two collections (*B-256* and *B-257*) from “a small grove of less than a dozen trees” within the Bonny Doon Ecological Reserve. This collection site apparently is between the Bonny Doon and Majors Creek groves, yet west of Laguna Creek and across Martin Road from the largest cypress stand and type locality. Much of the ecological reserve, including most of the cypress grove, burned in June 2008 in the 210-ha (520-acre) Martin Fire.

The five groves range from about 2.2 to 25.7 km (1.4 to 16.1 miles) apart, though the populations generally fall within three watershed basins. Going south to north, the Bonny Doon and

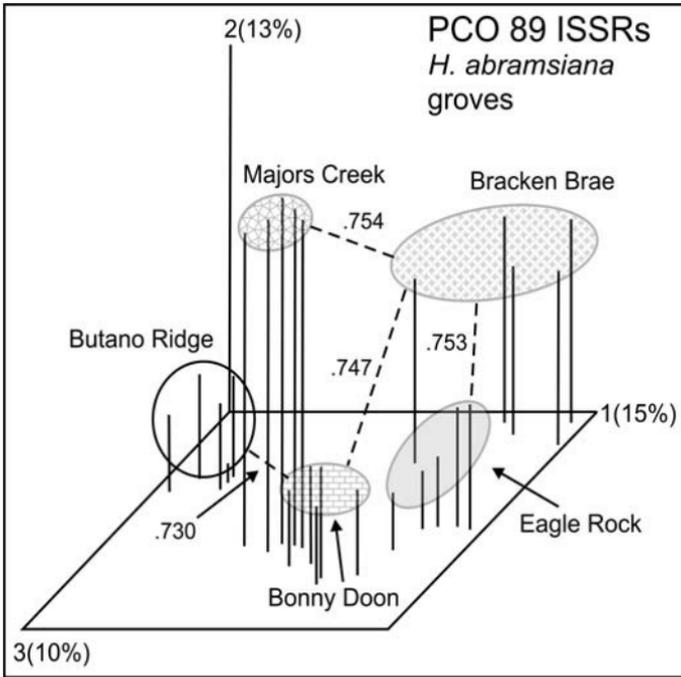


Figure 5. PCO based on 89 ISSR bands analyzing five *H. abramsiana* groves.

Majors Creek groves lie between Mill Creek (a tributary to San Vicente Creek) and Majors Creek with Laguna Creek bisecting both groves. The Eagle Rock and Bracken Brae groves largely fall within the watershed of Boulder Creek and its tributary Jamison Creek. The four southernmost groves drain generally south toward Santa Cruz or immediately to the northwest of Monterey Bay. However, the Butano Ridge grove, well isolated from the other groves, is located within the Butano Creek watershed, which drains west and empties into the estuary in Pescadero Creek State Beach. With this hydrology in mind, the four southernmost groves are very similar (0.747 - 0.754), while the divergence of the Butano Ridge grove (0.730) correlates with its relative isolation from the other populations.

In summary, both the terpenoids (Fig. 2) and ISSRs (Figs. 3-6) data show that the Butano Ridge grove is differentiated from the four southernmost *H. abramsiana* groves. Little variation was found among the four groves in the terpenoids (Fig. 2). The three individuals (Fig. 2) that had leaf oils similar to *H. pygmaea* were not similar to *H. pygmaea*

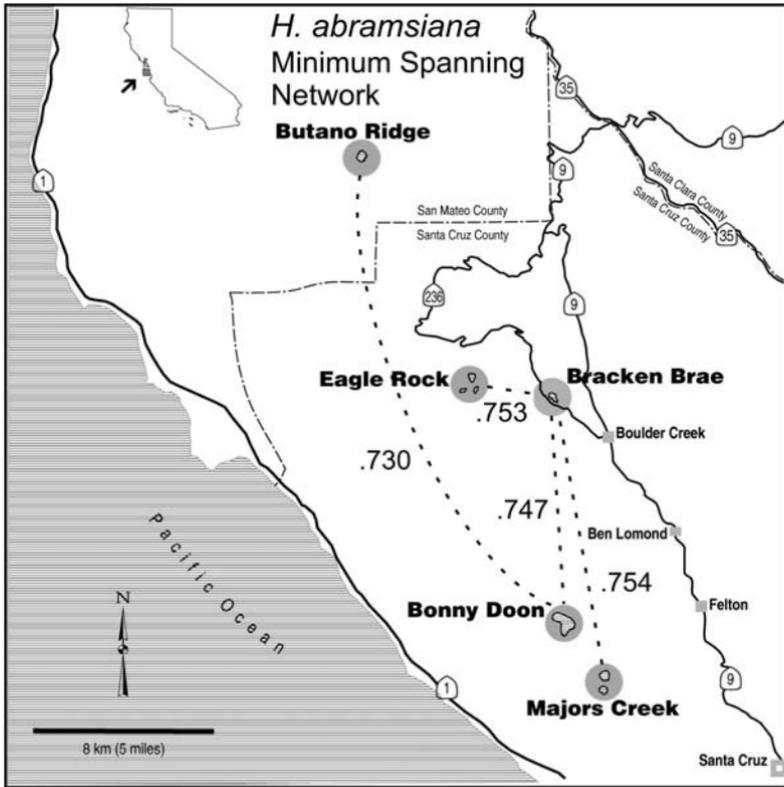


Figure 6. Minimum spanning network (dotted lines) superimposed on a geographic map of *H. abramsiana* groves.

in their ISSRs (Figs. 4-6). None of the subspecies proposed by Silba (2003) are supported by terpenoid or DNA data, except the Butano Ridge grove. This taxon is recognized as a variety:

Hesperocyparis abramsiana (C. B. Wolf) Bartel var. *butanoensis* (Silba) Bartel & R. P. Adams, **comb. nov.**

Basionym: *Cupressus abramsiana* C. B. Wolf subsp. *butanoensis* Silba, J. Intl. Conifer Pres. Soc. 10: 34. 2003.

Type: On sandstone slope, Butano Ridge, Santa Cruz mountains, north of Big Basin. (The cypress area includes the common corner of sections 11, 12, 13, 14 of township 8 South, Range 4 West, Mount Diablo Base and Meridian, Santa Cruz Quadrangle.), San Mateo County, 1 Sep 1951, C. McMillan 1620 (with P. McMillan, R. Bacigalupi, L. Heckard and H. Dutton) (holotype - NY).

McMillan (1952) reiterated the above locality the holotype with his description that a "trek of approximately one-quarter mile down the slope leads directly into the cypress area, which probably includes the common corner of sections 11,12,13, and 14, of Township 8 South, Range 4 West, Mount Diablo Base and Meridian, Santa Cruz quadrangle." However, the grove does not occur near this common corner of sections, but rather the cypresses are largely centered at N 37° 14.512', W 122° 15.127' on land managed by Pescadero Creek County Park.

McMillan (1952), in his article reporting the discovery of a third *H. abramsiana* grove, the Butano Ridge grove, noted that a "striking difference among the [then three known] populations is to be found in the size of the female cone." After randomly sampling 100 seed cones from each grove, McMillan (1952) reported that the average length of the Butano Ridge grove was 28 mm, Eagle Rock grove was 24 mm, and Bonny Doon grove was 21 mm. With the exception of the Bracken Brae grove (n=11), we measured 45 to 70 cones per grove to validate and update McMillan's data (Table 1). Not only were seed cones from Butano Ridge consistently longer than the other four groves, but the cones also were consistently wider with only four cones from the other groves falling within the measured range of the Butano Ridge grove cones.

Table 1. Grove-by-grove comparison of *H. abramsiana* seed cones.

	Butano Ridge	Eagle Rock	Bracken Brae	Bonny Doon	Majors Creek
mean cone length (mm)	27.0	22.1	16.7	22.0	19.9
mean cone width (mm)	25.5	20.0	16.1	18.8	18.3
mean number of scale pairs per cone	4.7	4.2	3.6	4.3	4.4

Like cone size, the number of scales per cone pairs varies from cone to cone. McMillan (1952) reported that the Butano Ridge grove averaged 5.2 scale pairs per cone with 5 and 6 pairs “common.” While 48% of our sampled cones had 5 and 6 scale pairs, the remaining cones had 4 pairs per cone. In contrast to Butano Ridge, McMillan (1952) reported that the Bonny Doon and Eagle Rock groves “were predominantly of 8-scaled [4-paired] cones, although the average number for the hundred cones was found to be 8.5 [4.3 pairs] and 8.7 [4.4 pairs] respectively.” However, because we found that the Bonny Doon (31%) and Major Creek (45%) groves often have 5 scale pairs, only cone width and length (especially the former measurement) appear to reliably differentiate the Butano Ridge grove from the four other groves.

McMillan (1952) noted that seed color (dark brown to dull black), presence of glaucous seeds, foliage texture (fine versus coarse), and foliage color (dark green versus yellow green) failed to differentiate any of the then three known groves. With the inclusion of the Bracken Brae and Majors Creek groves, our observations confirm that these morphological characters do not consistently differentiate any of the five groves from one another. While the average number of cotyledons per seedling clearly separated the Butano Ridge and Eagle

Rock groves from the Bonny Doon grove (McMillan 1953), additional work is needed to determine whether this character has any taxonomic merit for *H. abramsiana* groves.

ACKNOWLEDGEMENTS

This research supported in part with funds from U. S. Fish and Wildlife Service, Grant 814307J011. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service. Thanks to Tonya Yanke for lab assistance and thanks to Tim Hyland (State Parks Resources Ecologist at Henry Cowell Redwoods State Park) and Mark Schneider (Parks Ranger at Pescadero Creek County Park) for providing access to the cypress groves.

LITERATURE CITED

- Adams, R. P. 2008. Junipers of the world: The genus *Juniperus*. 2nd Edition. Trafford Publ., Vancouver, B.C., Canada.
- Adams, R. P. and J. A. Bartel. 2009. Geographic in the leaf essential oils of *Hesperocyparis (Cupressus) abramsiana*, *C. goveniana* and *C. macrocarpa*: Systematic implications. *Phytologia* 91(2): 226-243.
- Adams, R. P., J. A. Bartel and R. A. Price. 2009. A new genus, *Hesperocyparis*, for the cypresses of the western hemisphere. *Phytologia* 91(1): 160-185.
- Adams, R. P. and J. A. Bartel. 2009. Intraspecific variation in *Hesperocyparis goveniana* and *H. pygmaea*: ISSRs and terpenoid data. *Phytologia* 91(2): 277-286.
- Bartel, J. A., R. P. Adams, S. A. James, L. E. Mumba and R. N. Pandey. 2003. Variation among *Cupressus* species from the western hemisphere based on random amplified polymorphic DNAs. *Biochem. Syst. Ecol.* 31: 693-702.
- Gower, J. C. 1971. A general coefficient of similarity and some of its properties. *Biometrics* 27: 857-874.
- Gower, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* 53: 326-338.

- Little, D. P., A. E. Schwarzbach, R. P. Adams and C-F. Hsieh. 2004. The circumscription and phylogenetic relationships of *Callitropsis* and the newly described genus *Xanthocyparis* (Cupressaceae). Amer. J. Bot. 91: 1872-1881.
- Little, D. P. 2006. Evolution and circumscription of the true cypresses (Cupressaceae, *Cupressus*). Syst. Bot. 31: 461-480.
- McGraw, J. 2007. Distribution, abundance, size structure and conservation status of three populations of the endangered Santa Cruz cypress (*Callitropsis abramsiana*). Unpublished reported prepared for U.S. Fish and Wildlife Service, Ventura, California. 29 pp. + appendices.
- McMillan, C. 1952. The third locality for *Cupressus abramsiana* Wolf. Madrono 11: 189-194.
- McMillan, C. 1953. Variation in seedlings of *Cupressus abramsiana* Wolf. Madrono 12: 28-30.
- Rieseberg, L. H. 1996. Homology among RAPD fragments in interspecific comparisons. Mol. Ecol. 5: 99-105.
- Silba, J. 2003. Field observations of *Cupressus* in central and coastal California, July 2002 to January 2003. J. Intl. Conifer Pres. Soc. 10: 1-49.
- U.S. Fish and Wildlife Service. 1987. Endangered and threatened wildlife and plants; Determination of endangered status for *Cupressus abramsiana* (Santa Cruz cypress). Federal Register 52: 675-679.
- U.S. Fish and Wildlife Service. 1998. Recovery plan for the Santa Cruz cypress (*Cupressus abramsiana*). U.S. Fish and Wildlife Service, Portland, Oregon. 51 pp. + appendices.
- Veldman, D. J. 1967. Fortran programming for the behavioral sciences. Holt, Rinehart and Winston Publ., NY.
- Wolf, C. B. 1948. The new world cypresses - Part I: Taxonomic and distributional studies of the new world cypresses. Aliso 1: 1-250.