POPULATION GENETIC ANALYSIS OF *ARGEMONE PLEIACANTHA* SUBSP. *PINNATISECTA* (SACRAMENTO PRICKLY POPPY, PAPAVERACEAE) AND RE-EVALUATION OF ITS TAXONOMIC STATUS

Sandy D. Cervantes
Department of Biology
New Mexico State University
Las Cruces, New Mexico 88003, U.S.A.

Phil Tonne
Natural Heritage New Mexico
UNM Biology Department
Albuquerque, New Mexico 87131, U.S.A.

Rajanikanth Govindarajulu
Department of Biology
New Mexico State University
Las Cruces, New Mexico 88003, U.S.A.

Patrick J. Alexander
Department of Biology
New Mexico State University
Las Cruces, New Mexico 88003, U.S.A.

C. Donovan Bailey
Department of Biology
New Mexico State University
Las Cruces, New Mexico 88003, U.S.A.
Author for Correspondence – dbailey@nmsu.edu

ABSTRACT

Argemone pleiacantha subsp. *pinnatisecta* (Sacramento Prickly Poppy) is a federally listed endangered member of Papaveraceae, known from a few small populations on the western slope of the Sacramento Mountains in Otero County, New Mexico. With the total number of established *A. pleiacantha* subsp. *pinnatisecta* apparently dwindling, and threats to the viability of the taxon coming from a variety of factors, the taxonomic status of this group is of interest to scientists and land managers. The objective of this study was to test whether *Argemone pleiacantha* subsp. *pinnasecta* is diagnosable at the level of species through the application of an AFLP-based molecular assessment of population-level variation. Results from principle coordinate and STRUCTURE analyses on 424 AFLP loci, taken from samples of *Argemone pleiacantha* subsp. *pinnatisecta* and related geographically proximate members of *Argemone*, identify that the Sacramento Prickly Poppy is a genetically unique population system. The results of the AFLP analysis, in combination with the geographic isolation and morphological differentiation, are consistent with applicable widely held concepts of plant species. A new combination, **Argemone pinnatisecta** (G.B. Ownbey) S.D. Cervantes & C.D. Bailey comb. et stat. nov., is presented.

RESUMEN

Argemone pleiacantha subsp. *pinnatisecta* (Sacramento Prickly Poppy) es una Papaveraceae de la lista federal de especies en peligro, conocida solo de pequeñas poblaciones en la ladera occidental de las montañas de Sacramento en el condado de Otero, Nuevo México. Con el número de individuos de *A. pleiacantha* subsp. *pinnatisecta* aparentemente disminuyendo, y con las amenazas para la viabilidad de este taxon provenientes de una variedad de factores, el estado taxonómico de este grupo es de interés. El objetivo de este estudio era probar si *A. pleiacantha* subsp. *pinnatisecta* es diagnosticable como especie a través de un estudio de variabilidad poblacional basado en datos de AFLP. Análisis de coordenadas principales y utilizando el programa STRUCTURE en 424 loci de AFLP tomados de muestras de *A. pleiacantha* subsp. *pinnatisecta* y otros miembros relacionados de *Argemone* geográficamente próximos, revelan que este taxon tiene un sistema genético único. Estos resultados, conjuntamente con el aislamiento geográfico y la diferenciación morfológica, son consistentes con conceptos frecuentemente aplicados y científicamente justificados para el reconocimiento de especies de plantas. Basado en esto, se presenta una nueva combinación, **Argemone pinnatisecta** (G.B. Ownbey) S.D. Cervantes & C.D. Bailey comb. et stat. nov.

INTRODUCTION

Our understanding of species has changed dramatically throughout the history of botanical nomenclature. Although disagreement persists, contemporary species concepts broadly agree in viewing species as morphologically and/or genetically discontinuous groups of populations (e.g., Nixon & Wheeler 1990) that are related to each other through common evolutionary history, with reproductive isolation playing a key role in creating discontinuity in sexual taxa (e.g., Dobzhansky 1935; Mayr 1942). This understanding derives in large part from a fusion of ideas from systematics, paleontology, cytology, and genetics, which became known as the "modern synthesis" (Huxley 1942). Previous authors, especially prior to Darwin's *On the Origin of Species*, largely viewed species as temporally unchanging, but not necessarily morphologically discontinuous, entities that were not connected through evolutionary history. Many treated species and infraspecific ranks merely as tools for naming natural variation, not as fundamental units of evolution.

Despite dramatic changes over time in how species are understood and delimited, it is common for non-specialists to assume that "species" in contemporary discussions reflect the application of a species concept that is consistent with modern evolutionary biology. However, the majority of the roughly 2.5 million described species (e.g., May 1988) and infraspecific taxa were published prior to the *On the Origin of Species*, much less works of the modern synthesis. Few authors for these taxa were aware that the species rank would one day represent a critical boundary in evolutionary biology.

Ideally the limits of all species would be determined consistently, based on contemporary species concepts, but the scale of such work and limited global investment in the field makes this level of study unlikely in the foreseeable future (e.g., Heywood 2001; Scotland et al. 2003). The historical and potentially arbitrary application of rank at and below the level of species may not impact many taxa. However, over-interpretation of ranks can be an acute problem in conservation, with critics often suggesting that threatened subspecies and varieties are less than "species" and therefore unworthy of protection. When taxa are threatened with global extinction, scientific assignments based on modern concepts of species are critical for our understanding of the taxon and for downstream conservation decisions (see Desalle & Amato 2004; Holsinger & Gottliebo 1991; Van Dyke 2008). This study was conducted to evaluate the species status of one such endangered taxon.

Argemone pleiacantha Greene subsp. *pinnatisecta* G.B.Ownbey (Sacramento Prickly Poppy) is a federally listed endangered member of Papaveraceae, known from a few small populations on the western slope of the Sacramento Mountains in Otero County, New Mexico (Fish & Wildlife Service 1989). In the most recent monograph of *Argemone*, Ownbey (1958) described the taxon from his own specimens and an 1899 Wooton collection. This geographically restricted (Fig. 1) taxon was distinguished from other *Argemone pleiacantha* Greene by the presence of simple bud prickles, paler yellow latex, and sparingly prickly capsules (Fig. 1).

In the introduction to the monograph, Ownbey (1958) explicitly provided a species concept consistent with numerous contemporary concepts. His definition focused on distinctive morphological traits and either geographic isolation or failure to intergrade when occurring in sympatry with other *Argemone*. The morphology and geographic isolation of *A. pleiacantha* subsp. *pinnatisecta* fits this definition; however, Ownbey subsequently recognized the taxon as a subspecies without any discussion of his reasoning. With the exception of Shinners' (1958) overlooked shift in rank to *A. pleiacantha* Greene var. *pinnatisecta* (G.B.Ownbey) Shinners, other taxonomic assessments have not been made since Ownbey's description.

Shinners' shift in rank to variety might easily be misconstrued as his viewing the taxon as a lesser entity than Ownbey. Similar interpretations, commonly applied by non-scientists in arguments against protecting subspecific taxa (e.g., Wilcove et al. 1993), demonstrate the danger of over-interpreting the differential use of ranks (particularly subspecies and varieties) as well as the risk of excluding plant varieties from the formal language in the Endangered Species Act (Wilcove et al. 1993). In actuality, Shinners' taxonomic modification simply reflected his opinion that "One only uses subspecies on the relatively uncommon occasion when it is desired to label a group of varieties." (Shinners 1958) and had nothing to do with isolation or uniqueness of the group.

With the total number of established *A. pleiacantha* subsp. *pinnatisecta* dwindling well below 1000 (Sivinski 1999; Tonne 2008), the unclear rank appropriate for the taxon (species, variety, or subspecies), and threats to its viability coming from aspects of reproductive biology (Sivinski 1992; Tonne 2008) as well a variety of human-related factors (e.g., water withdrawal and right-of-way development, flooding, off-roading, grazing, highway maintenance (Lightfoot and Sivinski 1994; Tonne 2008)), the taxonomic assignment of these plants is of considerable interest.

The objective of this study is to evaluate the genetic structure and distinctiveness of *Argemone pleiacantha* subsp. *pinnasecta* population systems through the application of an AFLP-based (Vos et al. 1995) molecular assessment of population-level genomic variation and to use this information to address the taxonomic status of these plants. AFLPs are randomly sampled genetic loci that, in combination with appropriate methods of analysis, have proven powerful in developing objective fine-scale assessments of population-level and

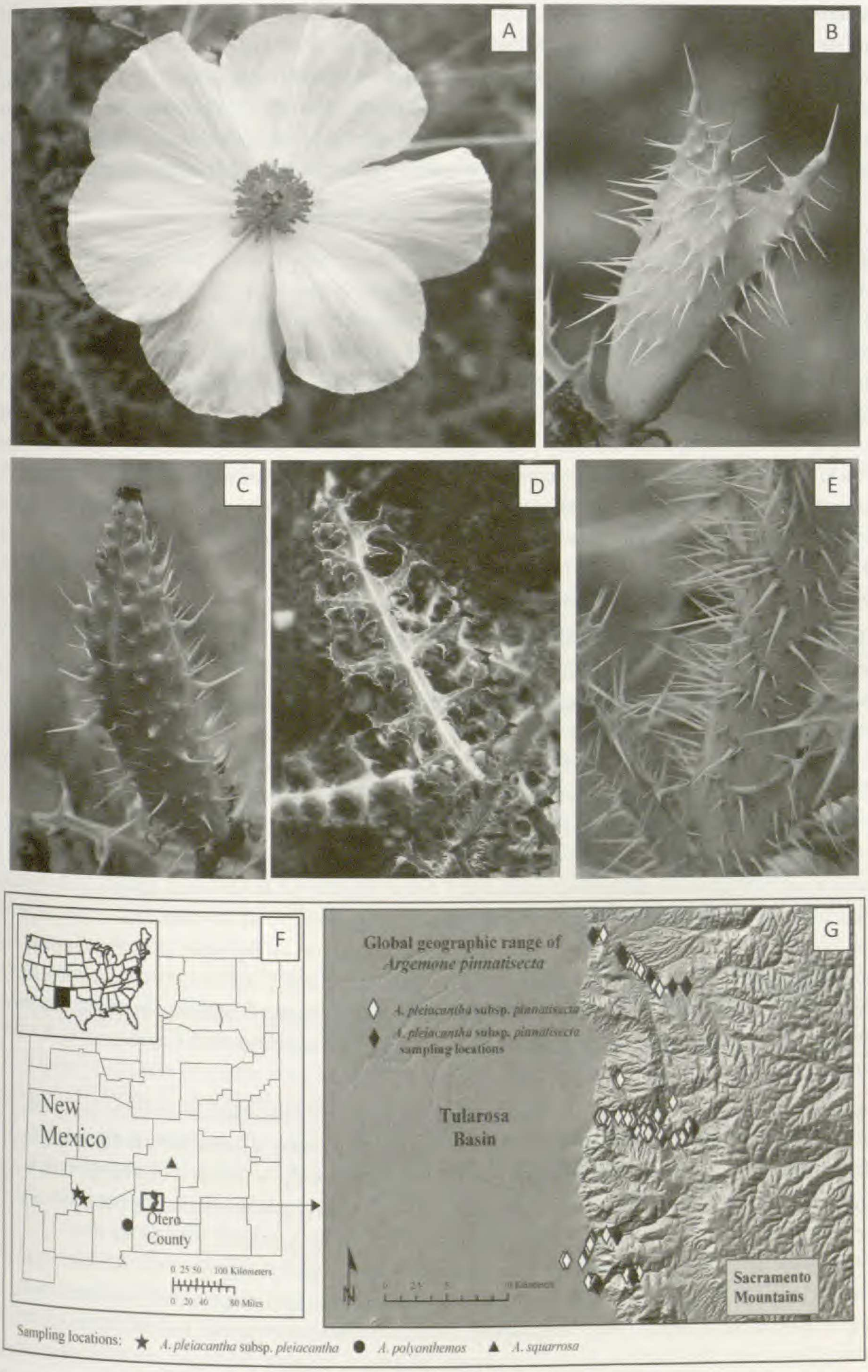

Fig. 1. Geography and Morphology. *Aregemone pleiacantha* subsp. *pinnatisecta* morphology (A–E). A) Flower and Bud, B) Bud, C) Capsule, D) cauline leaf, E) stem prickles. F) Geography of sampled material included in the study. G) The known geographic distribution of *A. pleiacantha* subsp. *pinnatisecta* (open diamonds) and the specific sampling of the species included (dark diamonds).

species-level variation in many plant and animal groups (e.g. Bacon & Bailey 2006; Baskauf & Burke 2009; Duminil et al. 2006; Martinez-Ortega et al. 2004; Routtu et al. 2007). For this study, AFLP profiles from *A. pleiacantha* subsp. *pinnatisecta* populations were compared to all geographically proximate species of *Argemone*. Results relating to the genetic isolation and differentiation of *A. pleiacantha* subsp. *pinnatisecta* are discussed to assess whether the taxon is a species based on scientifically accepted concepts of the species, or if these represent geographic, but not genetically distinct, populations of proximate species.

MATERIALS AND METHODS

Sampling.—Multiple collecting trips to *Argemone* localities in New Mexico were made between June and August of 2007. *Argemone pleiacantha* subsp. *pinnatisecta* were collected from 12 localities representing four canyon systems that run east to west across the Sacramento Mountains (Alamo, San Andres, Dog, and La Luz/Fresnal Canyons – Fig. 1 and Appendix 1). *Argemone pleiacantha* subsp. *pleiacantha* was collected from Kingston and Hillsboro, *A. polyanthemos* (Fedde) G.B.Ownbey from San Augustin Pass near Las Cruces, and *A. squarrosa* Greene from the eastern Sacramento Mountains (Fig. 1 and Appendix 1). The latter three taxa were sampled because they are the only other species of *Argemone* geographically proximate (within 150 miles) to *A. pleiacantha* subsp. p*innatisecta* and because they are among a number of possible close relatives to *A. pleiacantha* subsp. p*innatisecta* identified in a phylogenetic analysis of *Argemone* (Schwarzbach & Kadereit 1999). With the exception of the endangered *A. pleiacantha* subsp. *pinnatisecta*, which is sufficiently represented by specimens in the NMSU Dept. of Biology Herbarium (NMC – Thiers 2009), a voucher specimen was collected from each locality and deposited in NMC (Appendix 1).

DNA Extraction and AFLP Amplification.—DNA samples were extracted from each individual using the DNA extraction protocol of Alexander et al. (2007) eluting DNA into 10 mM Tris. DNA quantity and quality were evaluated on 0.7% agarose gels with a 100 bp DNA mass ladder standard (New England Biolabs). The restriction ligation (RL) and preselective amplifications followed a modified Vos et al. (1995) AFLP approached used by Bacon and Bailey (2006) and marketed by Applied Biosystems ("Plant Mapping Protocol" – P/N 402977 rev. E). In short, 50 ng of genomic DNA was digested overnight at 37°C with 1X T4 Ligase buffer (NEB), 0.046 M NaCl, 0.046 M BSA, 1 pM MseI Adapter pair, 10 pM *Eco*RI Adapter Pair, 1 U *Mse*I, 5 U *Eco*RI, and 67 U of T4 ligase (NEB). RLs were diluted to a final volume of 200 µL with 0.1 X TE. Preselective and selective amplifications included 1.5 mM $MgCl_2$ 0.1 M Tris-HCl pH 8.3, 0.5 M KCl, 0.25 µM of each primer, and ca. 2 U *Taq* in a 20 µL reaction containing 4 µL of dilute RL or preamplification product. Preselective amplifications applied single selective bases on each primer (A on *Eco*RI and C on *Mse*I) and selective primer combinations included *Eco*RI-AC/*Mse*I-CTA and *Eco*RI-TC/*Mse*I-CTA. Preselective and selective amplification cycling followed the ABI Plant Mapping Protocol. Selective amplification reactions, labeled with 5'FAM on the *Eco*RI primer, were run on a 3100 sequencer (Applied Biosystems) with a ROX500 standard (Applied Biosystems).

Data Analysis.—AFLP profiles were extracted from raw sequence files and converted to comparative allele presence/absence tables using GeneMapper 4.0 (Applied Biosystems). Alleles used in the analyses ranged from 100–500 bp. Runs on single individuals were considered to have failed if the number of fragments amplified was below the mean and standard deviation of fragments amplified across the population. In almost all cases, these failed runs correlated with low quality DNA and generated few or no peaks.

Two approaches were implemented to assess the number of genetically distinct clusters of individuals supported by the AFLP data irrespective of previously conceived notions of species or population limits. First, a principle coordinate analysis (PCO) employing Euclidean distances was run in MVSP ver. 3.131 (Kovach Computing Services). The first two coordinates were plotted to display the degree of differentiation among groups. This visual approach was augmented by the Bayesian statistical analysis presented by Pritchard et al. (2000). The application of STRUCTURE vers. 2.3.1 (Pritchard et al. 2000) tested relative likelihood support in the data for K genetic clusters (K=1–8) and the assignment of each individual to specific clusters under each value of K. The scoring of AFLP patterns in STRUCTURE followed the recommendation of Evanno et

al. (2005) in treating the unobserved alleles as missing data (i.e., for an individual with a presence allele at locus X, it is not possible to directly infer if the sample is homozygous dominant [1,1] or heterozygous [1,0]). STRUCTURE analyses included 10,000 burn-ins and MCMC replicates for each run, 10 replicate runs for each value of K, use of the admixture model, and allele frequencies set to independent, as recommended by Evanno et al. (2005). Other parameters were set to the software defaults. The inferred number of clusters best supported by the data was further tested through the application of ΔK (K=1–8) as applied by Evanno et al. (2005).

RESULTS

Of the 93 individuals from which DNA was extracted, 63 were successfully amplified using both selective primer combinations (30 *A. pleiacantha* subsp. *pinnatisecta*, 16 *A. pleiacantha* subsp. *pleiacantha*, 11 *A. squarrosa*, and 6 *A. polyanthemos*). Across these samples, AFLP reactions employing selective primer combinations *Eco*RI-AC/*Mse*I-CTA and *Eco*RI-TC/*Mse*I-CTA amplified 221 and 203 loci, respectively. Each primer combination amplified one fixed presence allele ("private") in every accession of *A. pleiacantha* subsp. *pinnatisecta* that was absent in all other sampled accessions.

Argemone squarrosa is considered polyploid based on chromosome counts from one locality (Ownbey 1958). When analyzed with diploid taxa in distance based analyses the increased number of fragments could generate artifactual results of concern to this study. However, individuals from the locality of *A. squarrosa* sampled for this study produced numbers of AFLP fragments well within the range for all other accessions amplified, reducing concerns with variance in fragment number. It is possible that there are both diploid and polyploid populations of the species and that we sampled diploid individuals.

Interspecific Analyses: PCO analysis (Fig. 2) identified three highly differentiated clusters. All accessions of *A. pleiacantha* subsp. *pinnatisecta* were recovered in a single cluster and the taxon showed greater differentiation from all other accessions than the other three taxa showed among one another. *Argemone squarrosa* and *A. polyanthemos* accessions displayed the lowest degree of differentiation between taxa (Fig. 2).

Results of the Ln P(D) in STRUCTURE supported K≥3 (Table 1). Pritchard et al. (2000) and Evanno et al. (2005) have documented likelihood values continuing to rise after passing the "true" K. This appears to be the case here, were the likelihood of K=3 is considerably higher than the values for K=1 or 2, but K continues to rise slightly and fluctuate above K=3. The application of Evanno et al.'s (2005) method found a 10 fold reduction in ΔK (from 15.95 to 1.61) between K=3 and K=4 (Table 1), identifying strong support for a K=3 conclusion (Evanno et al. 2005).

In all replications of the MCMC method with K>1, representatives of *A. pleiacantha* subsp. *pinnatisecta* were assigned to a single unique cluster, adding credence to the conclusion that these individuals represent a cohesive distinct group. With all values of K>1, no individual of *A. pleiacantha* subsp. *pinnatisecta* had less than 96.3% assignment to the same single cluster and no individuals from the other taxa show greater than 6.2% assignment to the *A. pleiacantha* subsp. *pinnatisecta* cluster (Table 1).

Intraspecific Differentiation: The sampling available for this study largely precluded a comprehensive analysis of intraspecific variation for *A. pleiacantha* subsp. *pinnatisecta*. Nonetheless, some preliminary analyses were run to test for signs of potential population differentiation. A PCO analysis restricted to *A. pleiacantha* subsp. *pinnatisecta* accessions displayed weak differentiation (Fig. 3) between accessions from canyons in the northern and southern portions of the range of the subspecies (see Discussion). The weakness of this differentiation is clear from the results in STRUCTURE, which failed to reject K=1 (Table 2) for the taxon. ΔK cannot be tested for K=1 and was not applied.

DISCUSSION

Taxonomic Status of the Sacramento Prickly Poppy.—The presence/absence pattern of randomly selected AFLP loci amplified from *A. pleiacantha* subsp. *pinnatisecta* and populations of other *Argemone* known within reasonable proximity of *A. pleiacantha* subsp. *pinnatisecta*, are consistent with the Sacramento Prickly

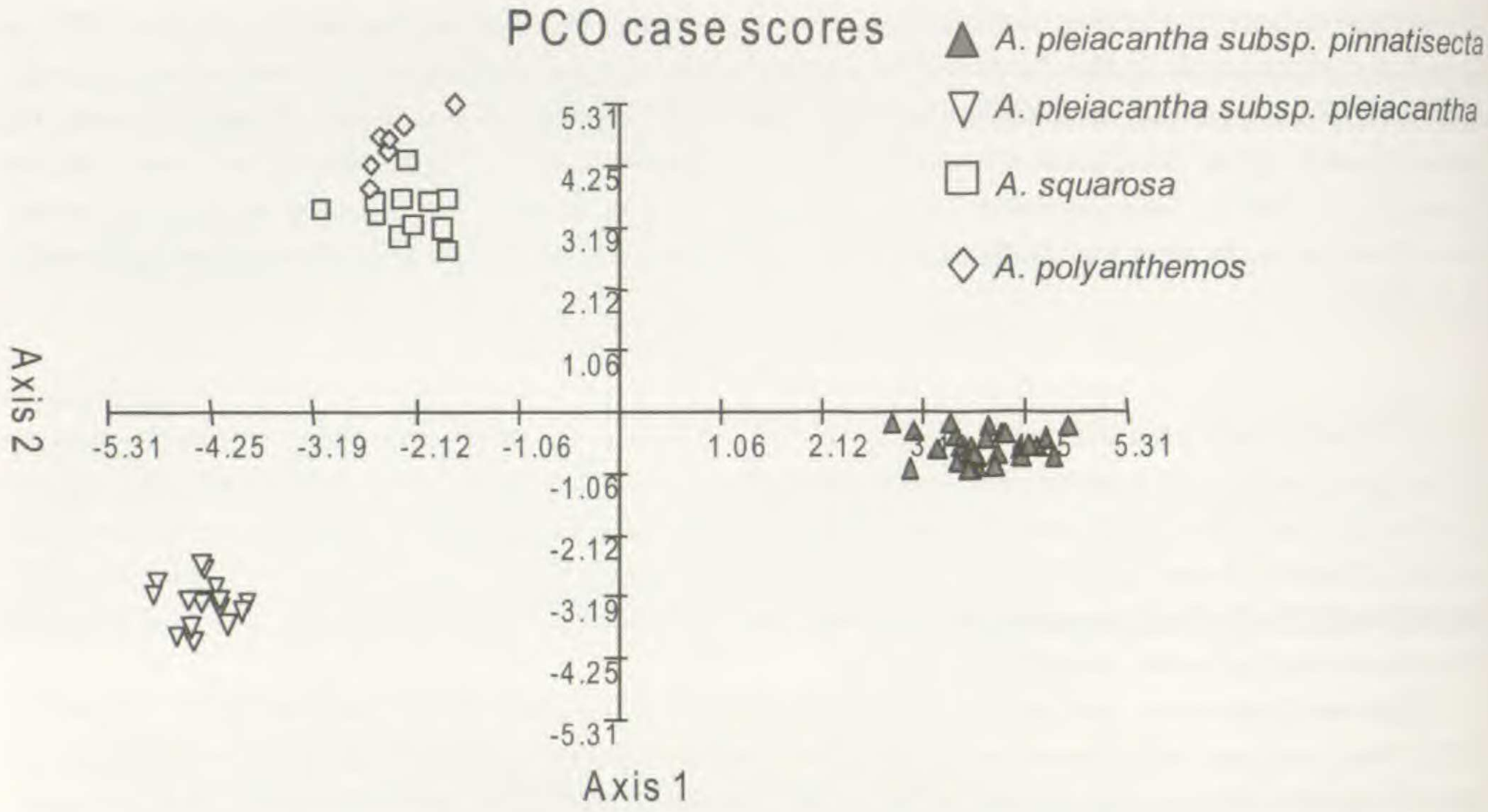

Fig. 2. Interspecific PCO analysis applying Euclidean distances calculated from AFLP data representing 424 loci.

Table 1. Results from the interspecific STRUCTURE analyses with values of K ranging from 1–8. Abbreviations: K - number of distinct groups applied, Ln - log probability, ΔK - rate of change in the log probability between successive values of K.

K=	1	2	3	4	5	6	7	8
mean Ln D(K)	-12945.75	-10190.29	-8810.68	-8748.87	-8439.63	-8509.82	-8534.8	-8504.54
SD Ln D(K)	1.40	67.06	81.46	142.99	239.03	193.04	353.11	199.96
ΔK	NA	20.53	15.95	1.61	1.42	0.188	0.156	NA
SPP CLUSTER - of min. assignment each SPP individual	NA	0.963	0.985	0.985	0.99	0.985	0.974	0.982
SPP CLUSTER - max. assignment of any non-SPP individual	NA	0.062	0.023	0.018	0.029	0.017	0.012	0.005

Poppy being genetically cohesive and reproductively isolated. In particular, the identification of two fixed (private) allelic differences present in all sampled *A. pleiacantha* subsp. *pinnatisecta*, which are absent from the other samples, suggests that the taxon has been reproductively isolated from these other *Argemone* for sufficient time to have: 1) developed unique fixed genetic traits not found in other taxa, or 2) that other geographically and phylogenetically proximate *Argemone* have lost traits that were once common to more species. Furthermore, the fixed allelic differences at two of the 424 loci are not the only differentiating signal loci found in the genetic dataset. Isolation at the level of species is further identified by the sum of allelic frequencies investigated through PCO (Fig. 2) and STRUCTURE (Table 1).

Fixed allelic differences are the explicit delimiting factor in the "phylogenetic species concept" (Davis & Nixon 1992; Nixon & Wheeler 1990), are consistent with the principles of the more widely known "biological species concept" (Dobzhansky 1935; Mayr 1942), and the overall pattern corroborates Ownbey's (pg. 9, 1958) suggestion that that the most important form of speciation in *Argemone* is "... geographic isolation leading to the accumulation of genetic differences in isolated populations." Thus, for the Sacramento Prickly Poppy the combination of genetics, morphology, and geography identify extensive reproductive isolation

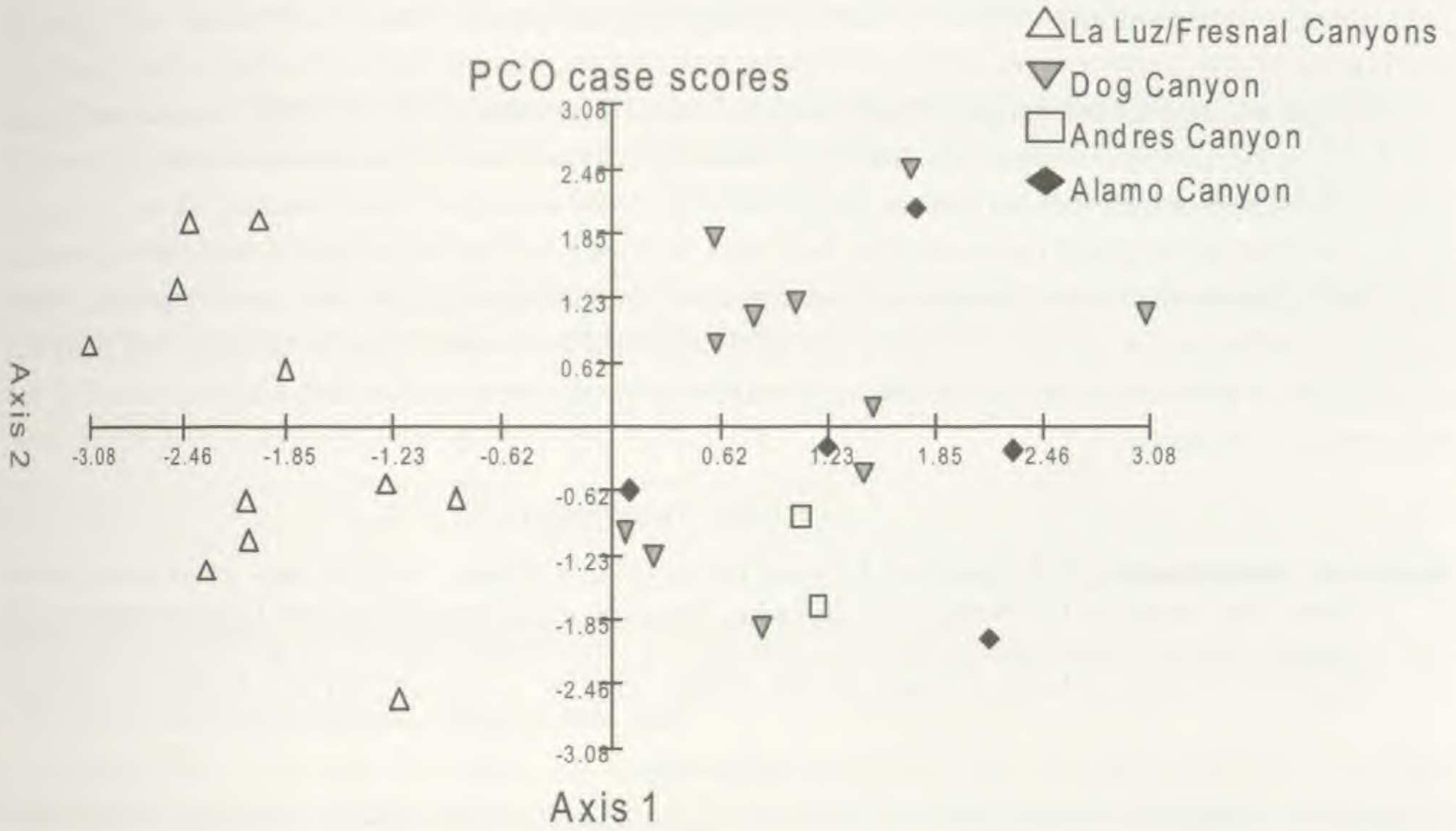

Fig. 3. Intraspecific PCO analysis of *A. pleiacantha* subsp. *pinnatisecta* applying Euclidean distances calculated from AFLP data representing 424 loci.

Table 2. Results from the intraspecific STRUCTURE analyses for *Argemone pleiacantha* subsp. *pinnatisecta* with values of K ranging from 1–8. Abbreviations: K - number of distinct groups applied, Ln - log probability, ΔK – rate of change in the log probability between successive values of K.

K=	1	2	3	4	5	6	7	8
Mean Ln D(K)	-3161.3	-3166.8	-3256.5	-3382.1	-3389.5	-3299.1	-3689.3	-3253.6
SD Ln D(K)	0.81	2.47	281.40	521.27	704.81	510.24	848.12	340.44

from other proximate *Argemone* as well as continued intraspecific contact. These patterns are consistent with specific status under widely accepted concepts of eukaryotic sexual species.

Intraspecific Differentiation.—Analyses of the available *A. pleiacantha* subsp. *pinnatisecta* data set did not reject K=1 for the taxon. However, the PCO analysis of *A. pleiacantha* subsp. *pinnatisecta* (Fig 3) provides evidence for weak divergence of populations found in different canyon systems. The Fresnal and La Luz canyon accessions tend to cluster in one cohort and the three remaining canyon systems into another. Clearly there is no strong population structure on the level of species differentiation, but these preliminary results are of significance to future management practices. Most importantly, resource managers should not assume that genetic variation in the Sacramento Prickly Poppy is randomly distributed across the species' range.

Conclusion and Future Research.—Ownbey's (1958) reasoning for having described the Sacramento Prickly Poppy at the subspecific rank contradicts both his own description of what constitutes a species and the genetic data generated from this study. *Argemone pleiacantha* subsp. *pinnatisecta* is geographically isolated, morphologically distinct, and genetically unique. These features are consistent with contemporary concepts of plant species and are applied here as the scientific evidence behind an elevation in taxonomic rank to species.

This study incorporated taxa growing within 150 miles of *Argemone pleiacantha* subsp. *pinnatisecta* in the southwestern "sky island" system. This distance was selected based on an evaluation of taxa that could conceivably interbreed with *A. pleiacantha* subsp. *pinnatisecta* and what was feasible for the study. Future

assessments of species limits within *Argemone* will hopefully address the limits of all species in the group. Of potential relevance to the understanding of *A. pleiacantha* subsp. *pinnatisecta* may be the comparison of three morphologically similar but geographically disjunct taxa (*A. arizonica* G.B. Ownbey, *A. gracilenta* Greene, and *A. pleiacantha* subsp. *ambigua* G.B. Ownbey). Since these taxa are only known from localities at least 300 miles distant, across inhospitable habitat, recent historic or contemporary interbreeding with *A. pleiacantha* subsp. *pinnatisecta* is largely inconceivable, but more inclusive comparative studies may shed light on the origin of *A. pleiacantha* subsp. *pinnatisecta* and aspects of the phylogeographic history of *Argemone*. Further analyses of infraspecific genetic variability and geographic differentiation incorporating greater population sampling for *A. pleiacantha* subsp. *pinnatisecta* should also be carried out to develop an appropriate fine scale assessment of variation.

TAXONOMIC TREATMENT

Argemone pinnatisecta (G.B. Ownbey) S.D. Cervantes & C.D. Bailey, comb. et stat. nov. *Argemone pleiacantha* Greene subsp. *pinnatisecta* G.B.Ownbey, Mem. Torrey Bot. Club 21:99, 1958; *Argemone pleiacantha* Greene var. *pinnatisecta* (G.B. Ownbey) Shinners, Southw. Naturalist 3:213–214. 1958. TYPE: U.S.A. NEW MEXICO: Otero Co.: 9.6 mi W of Cloudcroft, 6600 ft, 12 Aug 1953, *G.B. Ownbey & Findley 1754* (HOLOTYPE: MIN; ISOTYPES: ARIZ, BM, CAS, COLO, F, GH, MIN, RM, RSA, UC, UNM!, US)

APPENDIX 1

Sampling Information. For each taxon different collecting localities include location, centroid of population latitude, centroid of population longitude, collector and number, date, and number of samples taken.

Argemone pleiacantha* subsp. *pinnatisecta—Sacramento Mtns. Fresnal Canyon, 32.9698,-105.9010, *Phil Tonne & Bob Sivinski*, 2 Samples. Sacramento Mtns. Dog Bench, 32.7550, -105.8872, *Phil Tonne & Bob Sivinski*, 8 Samples. Sacramento Mtns. Fresnal Canyon, 32.9548, -105.8748, 14 Aug 2007, *Phil Tonne & Bob Sivinski*, 2 Samples. Sacramento Mtns. Fresnal Canyon, 32.9665, -105.8978, 14 Aug 2007, *Phil Tonne & Bob Sivinski s.n.*, 1 sample. Sacramento Mtns. Dog Bajada, 32.7503, -105.9191, 14 Aug 2007, *Phil Tonne & Bob Sivinski s.n.*, 5 samples. Sacramento Mtns. La Luz, 32.9817, -105.9257, 14 Aug 2007, *Phil Tonne & Bob Sivinski s.n.*, 1 Sample. Sacramento Mtns. Fresnal Canyon, 32.9480, -105.8428, 14 Aug 2007, *Phil Tonne & Bob Sivinski s.n.*, 4 Samples. Sacramento Mtns. La Luz, 32.9817, -105.9257, 14 Aug 2007, *Phil Tonne & Bob Sivinski s.n.*, 2 Samples. Sacramento Mtns. San Andres Canyon, 32.7826, -105.9013, 15 Aug 2007, *Phil Tonne & Bob Sivinski s.n.*, 2 Samples. Sacramento Mtns. San Andres Canyon, 32.7826, -105.9013, 15 Aug 2007, *Phil Tonne & Bob Sivinski s.n.*, 1 Sample. Sacramento Mtns. Fresnal Canyon, 32.9476, -105.8553, 14 Aug 2007, *Phil Tonne & Bob Sivinski s.n.*, 4 Samples. Sacramento Mtns. Upper Alamo Canyon, 32.8537, -105.8348, 14 Aug 2007, *Phil Tonne & Bob Sivinski s.n.*, 8 Samples. ***A. pleiacantha* subsp. *pleiacantha***—Hillsboro, NM, 32.8249, -107.5421, 19 Jun 2007, *Sandy Cervantes 2*, 14 Samples. Kingston, NM, 32.9187, -107.6873, 19 Jun 2007, *Sandy Cervantes 1*, 13 samples. ***A. polyanthemos***—Organ Mtns, leaf samples taken from plants along I10 - collected from 0.5 mi W of White Sands Missile Range entrance, 32.4381, -106.4866, 26 Jun 2007, *Sandy Cervantes 3*, 13 samples. ***A. squarrosa*** - Lincoln, NM 33.5340, -105.4964, 27 Jun 2007, *Sandy Cervantes 4*, 13 Samples.

ACKNOWLEDGMENTS

This research was completed in partial fulfillment of undergraduate honors thesis requirements (S.D. Cervantes) at New Mexico State University. We are grateful to Robert Sivinski for collecting leaf material under permit TE820730-0 and Fabian Michelangeli for his assistance with the Spanish abstract. We also thank Julie McIntyre, Brook Milligan, Tom Parchman, Richard Spellenberg, Lillis Urban, and an anonymous reviewer for helpful discussions on the topic and/or manuscript. Funding for this project came from the Howard Hughes Medical Institute funded NMSU Undergraduate Scholars Program and NSF grants DEB0817033 & EF0542228 (to CDB).

REFERENCES

ALEXANDER, P.J., G. RAJANIKANTH, C. BACON, AND C.D. BAILEY. 2007. Rapid inexpensive recovery of high quality plant DNA using a reciprocating saw and silica-based columns. Molec. Ecol. Notes 7:5–9.

BACON, C.D. AND C.D. BAILEY. 2006. Taxonomy and conservation: a case study from *Chamaedorea alternans*. Ann. Bot. 98:775–763.

BASKAUF, C.J. AND J.M. BURKE. 2009. Population genetics of *Astragalus bibullatus* (Fabaceae) using AFLPs. J. Heredity 100:424–431.

Davis, J.I. and K.C. Nixon. 1992. Populations, genetic variation, and the delimitation of phylogenetic species. Syst. Biol. 41:421–435.

Desalle, R. and G. Amato. 2004. The expansion of conservation genetics. Nat. Rev. Genetics 5: 702–712.

Dobzhansky, T.H. 1935. A critique of the species concept in biology. Philos. Sci. 2:344–355.

Duminil, J., H. Caron, I. Scotti, C. Saint-Omer, and R.J. Petit. 2006. Blind population genetics survey of tropical rainforest trees. Molec. Ecol. 15:1305–1313.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molec. Ecol. 14:2611–2620.

Fish & Wildlife Service. 1989. Final rule to determine *Argemone pleiacantha* ssp. *pinnatisecta* (Sacramento prickly poppy) to be an Endangered Species. Fed. Reg. 52 No. 115.

Heywood, V. 2001. Floristics and monography - an uncertain future. Taxon 50:311–330.

Holsinger, K.E. and L.D. Gottlieb. 1991. Conservation of rare and endangered plants. In: Genetics and conservation of rare plants, D. A. I. Falk, and K. E. Holsinger, eds. Oxford University Press, U.S.A. Pp. 198–208.

Huxley, J. 1942. Evolution: The Modern Synthesis. Allen & Unwin Ltd., London.

Lightfoot, K. and R. Sivinski. 1994. Sacramento Mountain Poppy (*Argemone pleiacantha* subsp. *pinnatisecta*) Recovery Plan. US Fish and Wildlife Service, Albuquerque, NM.

Martinez-Ortega, M.M., L. Delgado, D.C. Albach, J.A. Elena-Rossello, and E. Rico. 2004. Species boundaries and phylogeographic patterns in cryptic taxa inferred from AFLP Markers: *Veronica* subgen. *Pentasepalae* (Scrophulariaceae) in the Western Mediterranean. Syst. Bot. 29:965–986.

May, R.M. 1988. How many species are there on earth? Science Washington D C 241:1441–1449.

Mayr, E. 1942. Systematics and the origin of species. Columbia University Press, New York City.

Nixon, K.C. and Q.D. Wheeler. 1990. An amplification of the phylogenetic species concept. Cladistics 6:211–224.

Ownbey, G.B. 1958. Monograph of the genus *Argemone* for North America and the West Indes. Mem. Torrey Bot. Club 21:1–159.

Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Routtu, J., A. Hoikkala, and M. Kankare. 2007. Microsatellite-based species identification method for *Drosophila virilis* group species. Hereditas 144:213–221.

Schwarzbach, A.E. and J.W. Kadereit. 1999. Phylogeny of prickly poppies, *Argemone* (Papaveraceae), and the evolution of morphological and alkaloid characters based on ITS nrDNA sequence variation. Pl. Syst. Evol. 218:257–279.

Scotland, R., C.E. Hughes, C.D. Bailey, and A. Wortley. 2003. The big machine and the much-maligned taxonomist. Syst. Biodivers. 1:139–143.

Shinners, L.H. 1958. New varietal names in *Argemone* (Papaveraceae). Southw. Naturalist 3:213–214.

Sivinski, R. 1992. *Argemone pleiacantha* subsp. *pinnatisecta* seed germination study. U.S. Fish and Wildlife Service, Albuquerque, NM.

Sivinski, R. 1999. Sacramento Prickly Poppy habitat study. 1998–1999 Progress Report. (Section 6 Segment 13) Progress Report to the US Fish and Wildlife Service.

Thiers, B. 2009. Index herbariorum: a global directory of public herbaria and associated staff. In: http://sciweb.nybg.org/science2/IndexHerbariorum.asp

Towne, P. 2008. Results of Sacramento Prickly Poppy studies, surveys, and monitoring 2006–2007. Natural Heritage New Mexico.

Van Dyke, F. 2008. Conservation biology: Foundations, Concepts, Applications, 2nd edition. McGraw Hill, New York.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucl. Acids Res. 23:4407–4414.

Wilcove, D.S., M. McMillan, and K.C. Winston. 1993. What exactly is an endangered species? An analysis of the U.S. Endangered Species List: 1985–1991. Conservation Biol. 7:87–93