The relationship of *Monardella viminea* to closely related taxa based on analyses of ISSRs.

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Willowy Monardella [*Monardella linoides* A. Gray subsp. *viminea* (E. Greene) Abrams] is listed as an endangered species under both the Federal Endangered Species Act (Department of the Interior 1998) and the State of California (California Department of Fish and Game 1979). This taxon occurs in San Diego County, California, and adjacent Baja California, Mexico. A recent examination of Willowy Monardella (Elvin and Sanders 2003) resulted in a change of rank and circumscription. The taxon was elevated to species rank and the circumscription was narrowed, resulting in the recognition of two species, *M. viminea* and *M. stoneana* Elvin & Sanders. The newly circumscribed *M. viminea* is restricted to an area approximately 22.5 km wide by 11 km long in coastal San Diego County, mainly on Miramar Marine Corps Air Station as shown in Fig. 1. Thirty-one localities have been recorded in the California Department of Fish and Game's Natural Diversity Database (CNDDB) although some of those individual occurrences have been merged in the database. The newly described species *M. stoneana* is restricted to more inland regions of the county and adjacent Baja, and is currently known from 9 occurrences in the USA, spanning a 20 km wide by 6 km high region SE of Otay Reservoir. Given the isolated and undeveloped nature of the region and the restricted access (City of San Diego and Bureau of Land Management), it is likely that there are additional, undocumented occurrences of this taxon in the vicinity of Tecate Peak.

Both species are perennial members of the mint family (Lamiaceae) with pale purple flowers born during the summer months. Under the new circumscription parameters, *M. viminea* is restricted to coastal rocky drainages, occupying the areas just outside of the streambed on the sandy bench (Fig. 2). *Monardella stoneana* grows directly in the streambed among rocks and boulders (Fig. 3). Additional differences include leaf and stem indument (hair) density as illustrated in Fig. 4, gland density, and bract characters (Elvin and Sanders 2003).

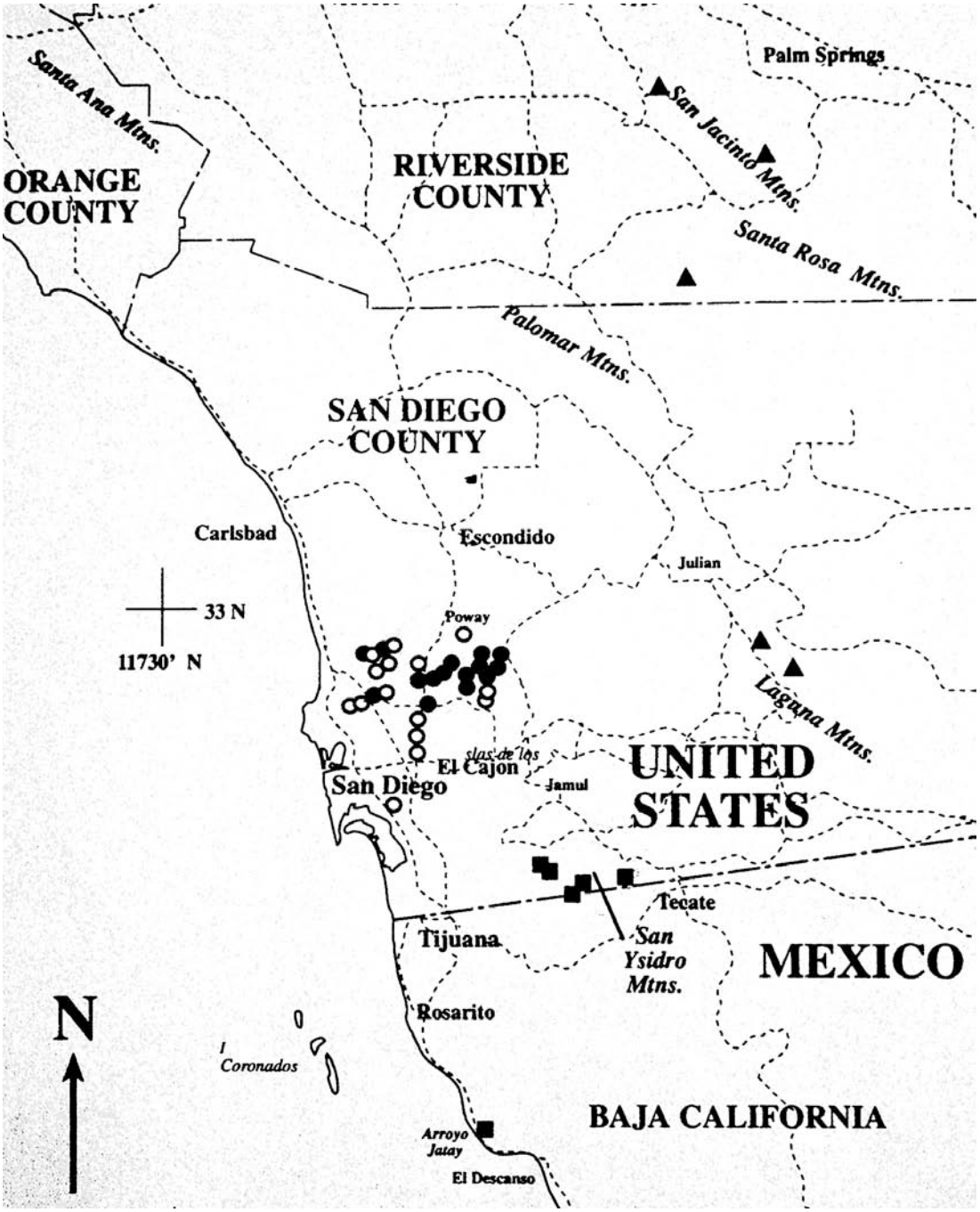

Figure 1. Distributions of *Monardella stoneana* ❍ (extirpated) ● (extant), *M. viminea* ■, and *M. linoides* subsp. *linoides* ▲ (representative samples only). Redrawn from Fig. 2 of Elvin and Sanders (2003).

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Figure 2. *Monardella viminea* habitat (left) and inflorescence (right).

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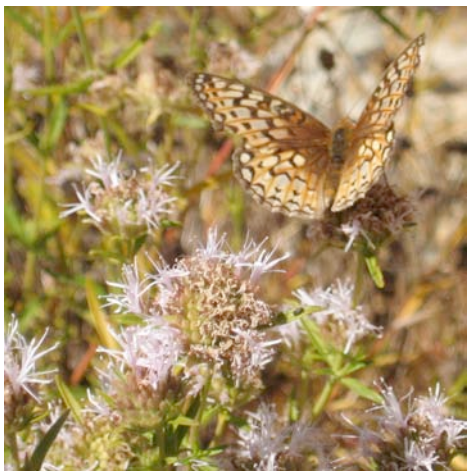

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Figure 3. *Monardella stoneana* habitat (left) and inflorescence (right)

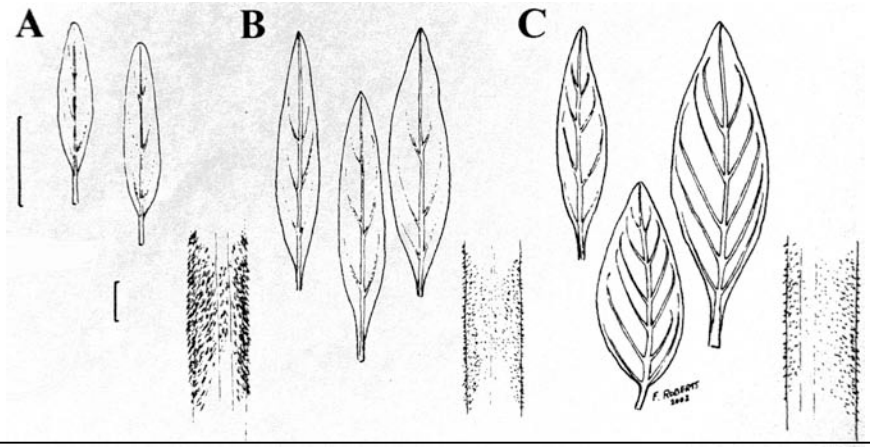

Figure 4. Pubescence details of the leaves (top) and stems (bottom) of *Monardella linoides* subsp. *linoides* (A), *M. viminea* (B), and *M. stoneana* (C). Leaf scale bar (top) = 1 cm, stem scale bar (bottom) = 1 mm. Reproduction of Fig. 3 from Elvin and Sanders (2003).

According to Mark Elvin (pers. comm.) there are also differences in the composition or ratios of volatile chemicals produced by the different species of *Monardella* although those differences have never been quantified.

In addition to the change in rank and circumscription, the 2003 publication of Elvin and Sanders shifted the taxonomic affiliation of *M. viminea* from being closely allied with the *M. linoides* group to being closely allied with the *M. odoratissima* group. Both groups are members of *Monardella* section *Monardella*. The morphological data used to support the shift in taxonomic affiliation were presented clearly, but were not analyzed using phylogenetic or morphometric methods. Prior attempts to test the relationships proposed by Elvin and Sanders using independently generated molecular data (DNA sequence data; Prince 2005) were inconclusive, potentially due to the relatively recent origin of the taxa involved and appears to be a somewhat common problem for recently evolved taxa of southern California (L. Prince pers. obs.). The genus *Monardella* is estimated to be less than 5 million years old (B. Drew pers. comm.).

The change in rank and circumscription results in pressing management issues. If *M. viminea* is a distinct taxon from *M. stoneana*, additional action may be required to protect this species from future development impacts along coastal San Diego County. The narrower circumscription (and hence distribution) of *M. viminea* implies a more perilous future for this species due to fewer populations, intense development pressure throughout its range, and the limited area of suitable habitat in conservation. *Monardella viminea* is restricted to the vicinity of Miramar Marine Corps Air Station (MMCAS). Although the extant populations on generally on publicly held land (MMCAS, Department of Defense, City of San Diego, and San Diego County), almost half of the documented occurrences have been extirpated. Unfortunately, many of the remaining populations are isolated due to development and their habitat is under increased erosion threats due to

alterations in drainage patterns. This change, coupled with extensive habitat conversion to impervious or less permeable surfaces associated with development further threatens the species.

The goal of this study was to elucidate relationships among the geographically proximate members of the *M. linoides* and *M. odoratissima* groups using population genetics methods, namely *M. linoides* [subsps. *linoides*, *oblonga* (E. Greene) Abrams, and *stricta* (Parish) Epling], *M. viminea*, *M. stoneana*, and *M. australis* Abrams (of the *M. odoratissima* group) (but see Elvin and Sanders in press). Fluorescent ISSRs were employed because they require no up front information (unlike microsatellites) and provide a larger number of fragments than non-fluorescent methods. The specific goal was to test the hypotheses that *M. viminea* is distinct from *M. stoneana*, that *M. viminea* is distinct from *M. linoides*, and that *M. stoneana* and *M. viminea* are more closely related to *M. australis* than to *M. linoides*.

Materials and Methods

Plant Sampling— Leaf material was collected from thirty plants for two Southern California populations each of *Monardella viminea, M. stoneana, M. linoides subsp. linoides, M. linoides subsp. oblonga, M. linoides subsp. stricta*, and the hypothesized closest relative, *M. australis* as shown in Fig. 5. Potential sampling sites were identified from a review of CNDDB and Consortium of California Herbaria (CCH) database searches. Selected sampling sites were chosen based on a combination of criteria including accessibility, geography (from different drainages for taxa with restricted ranges such as *M. viminea*, *M. stoneana*, and *M. linoides oblonga*; from different mountain ranges for taxa with broad distribution ranges such as *M. linoides linoides* and *M. australis*,) and recency of last collection. Herbarium voucher and detailed information on population localities are provided in Table 1. Total genomic DNA was extracted from 1-3 silica-dried leaves using standard CTAB extraction methods (Doyle and Doyle 1987). All remaining leaf material for plants is housed in the permanent silica gel tissue collection and all DNA extractions are stored in the permanent freezer

(-80°C) collection of the molecular lab at Rancho Santa Ana Botanic Garden (RSABG). The *Monardella* taxa studied here are all perennial herbs. Any given population is expected to include a small number of genetically identical samples since stolon breakage may result in seemingly distinct individuals. Sampling strategies employed here was hyper dispersed in an attempt to avoid sampling clones.

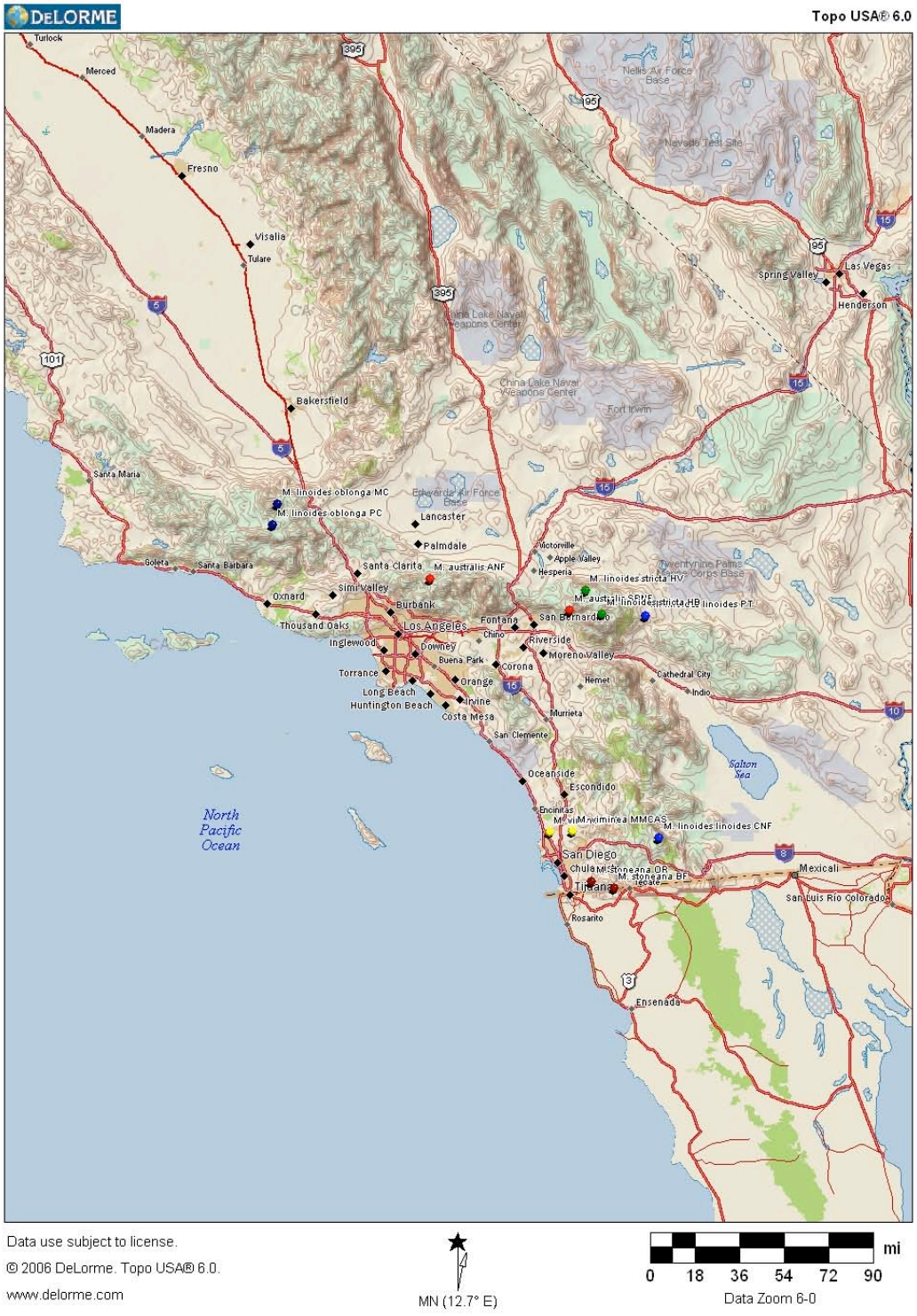

Figure 5. Location of *population collection sites for Monardella* included in this study. Bright blue = *M. l.* subsp. *linoides*, dark blue = *M. l.* subsp. *oblonga*, green = *M. l.* subsp. *stricta,* yellow = *M. viminea*, dark red = *M. stoneana*, and bright red = *M. australis.*

Table 1. Location of Southern California population collection sites for *Monardella* included in this study including voucher, GPS coordinates, and population size estimates.

Taxon	Location Abbrev.	Location Description	GPS Coord.	Pop. Size	Voucher
M. australis	ANF	Angeles National Forest. San Gabriel Mtns. Bare Mountain Canyon, SE of Fountainhead Spring. Soil sandy loam. Aspect: E. Elevation 2030 m.	N34.37718° W-118.02664°	~100 plants	Prince 516 RSA
M. australis	SBNF	San Bernardino National Forest. ~1.5 mi. east of Running Springs. Just downslope from the lookout tower. Soil sandy loam. Aspect: E. Elevation 2325 m.	N34.19458° W-117.04873°	>150 plants	Prince 517 RSA
M. linoides linoides	PT	Pioneertown. ~0.7 km NW of Pioneertown Rd crossing of main channel. Plants primarily in smaller drainage parallel to road. Soil sandy. Aspect: E. Elevation 1225 m.	N34.1608° W-116.5100°	36 plants	Prince and O'Brien 507 RSA
M. linoides linoides	CNF	Cleveland National Forest. Laguna Mtns. NE of Burnt Ranchera Campground. In rocky outcrop ca. 0.5 miles E. of Desert View Trail. Soil sandy loam. Aspect: NE. Elevation 1820 m.	N32.86531° W-116.41590°	~200 plants	Prince 515 RSA
M. linoides oblonga	PC	Los Padres National Forest. Pine Springs Campground. Along and in channel of seasonal stream bed. Soil sandy loam. Aspect: E. Elevation 1880 m.	N34.69028° W-119.13608°	>50 plants	Prince and Porter 522 RSA
M. linoides oblonga	MC	Los Padres National Forest. McGill Campground. Top slope of canyon South of campground. Soil sandy loam. Aspect: SE. Elevation 2200 m.	N34.81148° W-119.10375°	>50 plants	Prince and Porter 521 RSA
M. linoides stricta	HV	San Bernardino National Forest. Holcomb Valley. 0.5 KM W of Delmar Mountain Road. Loose gravelly slope. Aspect: SE. Elevation 2185 m.	N34.30825° W-116.93358°	>200 plants	Prince and O'Brien 508 RSA
M. linoides stricta	HB	San Bernardino National Forest. Heart Bar Campground vicinity. Rocky slope along dirt road. Aspect: NNW. Elevation 1970 m.	N34.16768° W-116.81594°	~100 plants locally, many more nearby	Prince and Vanderplank 558 RSA
M. viminea	CC	Carroll Canyon. Rescue population now housed at RSABG. Original locality Carroll Canyon, San Diego. Aspect: NE. Elevation 60 m. CNDDB EO: none?	N32.8940° W-117.1841°	>150 plants	Prince s.n. RSA
M. viminea	MMCAS	Miramar Marine Corps Air Station. West Sycamore Canyon. Growing on bench of drainage in sandy soil. Aspect: NE. Elevation 226 m. CNDDB EO: 21.	N32.90176° W-117.0232°	>30 plants	Fraga et al.1888 RSA
M. stoneana	OR	Otay Reservoir. In center of steep, bouldery canyon with seasonal water. Soil sandy. Aspect: W. Elevation 350 m. CNDDB EO: 3.	N32.6087° W-116.8897°	35 plants	Prince, Lauri, and Kauo 502B RSA
M. stoneana	BF	Border Fence. Gently sloping rocky/gravelly drainage. Growing directly in the drainage. Soil sandy. Aspect: WSW. Elevation 450 m. CNDDB EO: 1.	N32.57180° W-116.72963°	>250 plants	Prince, Snappcook, and Roblek 542 RSA

ISSR Amplification—Thirty-one samples representing 6 taxa were screened initially for 27 fluorescently labeled ISSR primers to identify the primers that produced the highest number of variable bands. Subsequently 4 primers were chosen for use on the entire sample set of 12 populations ranging from 30-31 individuals. The primers selected for use were 807-Fam: AGAGAGAGAGAGAGAGT, 809-Fam: AGAGAGAGAGAGAGAGG, 811-Vic: GAGAGAGAGAGAGAGAC, and 812-Vic: GAGAGAGAGAGAGAGAA. Products were amplified on an AB 9700 thermal cycler (Applied Biosystems, Foster City, California, USA) in 10 µl reaction volumes containing 1 µL 10X PCR Buffer (ammonium sulfate buffer), 0.5 µL dNTPs (2.5 mM each), 0.5 µL $MgCl_2$ (25 mM), 0.5 µL primer (20 µM), 0.5 µL DMSO, 20 ng of genomic DNA, and 0.25 units of Promega (Madison, Wisconsin, USA) GoTaq polymerase. Cycle parameters were as follows: 1 cycle at 94°C for 2 minutes, 35 cycles of 94°C for 30 seconds, 50°C for 30 seconds, 72°C for 1 minute 30 seconds, followed by a final extention cycle at 72°C for 5 minutes. Samples were multiplexed [2 µl of each fluorescently labeled polymerase chain reaction (PCR) product (807 + 811 or 809 + 812) co-loaded with 20 µl hi-di formamide and 0.5 µl custom Liz1200 size standard]. Products were electrophoresed on a 3130xl Genetic Analyzer (Applied Biosystems) using a custom electrophoresis module.

Data scoring and verification was conducted in GeneMapper v. 4.0 (Applied Biosystems). Although the GeneMapper software estimates the number and length of fragments produced in the ISSR amplifications, variance in the peak height and background between replicates requires all runs be rechecked and each fragment proofed as to presence or absence and fragment length. Only peaks with heights greater than 40 in at least one individual were scored, and only peaks with heights >20 were scored as present. All samples were amplified and run in triplicate. Only peaks that reproducibly produced scorable peaks (as described above) were analyzed. Background noise ranged from 5-7 with a few up to 10. Ambiguous peaks reflect real genetic differences but weak

amplification or may be due to Taq stutter (false or shadow peaks caused by *Thermus aquaticus* polymerase error). Peaks with height less than 20 were interpreted as Taq stutter and were not included in the final data matrix (see Fig. 6).

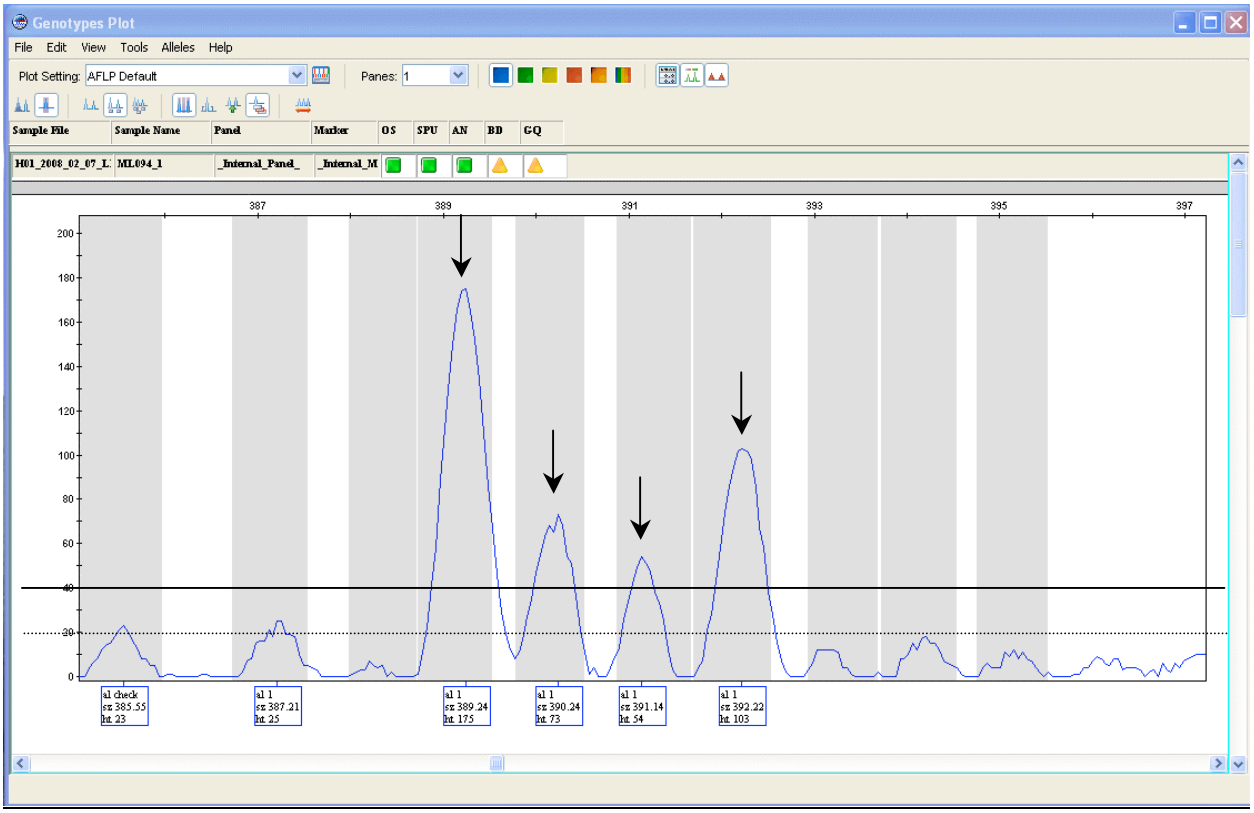

Figure 6. Typical peak profile of fluorescent ISSR data. Arrows indicate peaks scored as present. Solid black line indicates peak threshold for scoring any particular peak (=locus). Dashed black line indicates minimum peak height for scoring presence versus absence. Peaks below the dashed black line were considered noise or polymerase stutter and were not included in final data matrix.

Genetic Data Analyses—A binary matrix was generated and analyzed under standard and Bayesian criteria. The phylogenetic software package PAUP (Swofford 2002) was used to generate Neighbor Joining (NJ) distance dendrograms based on parsimony and unweighted pair group method with arithmetic mean (under both average distance and total distance) for individual samples (no a priori grouping based on taxonomy or population). Neighbor joining is a bottom-up

clustering method used for the construction of phylogenetic trees based on distance (http://en.wikipedia.org/wiki/Neighbor_joining). Branch support was estimated using 10,000 bootstrap replicates (NJ). A binary genetic distance matrix was generated and used in Principal Coordinates Analyses (PCoA or PCO) in GenAlEx (Peakall and Smouse 2006). Ancestry of samples was estimated using Bayesian methods for dominant data as described by Falush et al. (2007) in Structure v. 2.2 (Pritchard et al. 2007) (estimate λ, burn-in=50,000, run=100,000, # of populations=12, noadmix=0).

General population genetic diversity measure [proportion of polymorphic loci (*P*), Shannon's information index (*I*, Lewontin 1972), Nei's (1978) unbiased genetic identities (I_G,), and distances (*D*) were calculated using POPGENE v. 1.31 (Yeh et al. 1997). Population genetic structure (θ^B, an F_{ST} analog) was estimated for all pairwise population combinations using Bayesian criteria as implemented in HICKORY v1.1 (Holsinger and Lewis 2007). Values were estimated using the "f-free" model since estimates of *f* from dominant data can be unreliable (Holsinger et al. 2002). Mantel tests (Mantel 1967) for geographical structure were conducted in PASSaGE ver. 2 (Rosenberg 2009) on a population genetic distance (θ^B) versus population geographical distance matrix. Geographic distance was calculated using a Coordinate Distance Calculator online at http://boulter.com/gps/distance/.

RESULTS

Locus Frequencies–The number of scorable bands (loci) varied depending on primer. At least one fixed (present) band was present all samples, including the negative control. These false positive bands are a result of dye co-migration with the polymerase and were excluded from the final data matrix. All populations were fixed for the presence or absence of a large percentage of bands as shown in Table 2 (percentage of polymorphic loci), however there were no bands that were fixed

across all taxa, thus all bands were included in statistical analyses. The data matrix included 349 samples scored for 186 loci and was highly variable. Only one duplicate genotype was detected, in *M. linoides oblonga* from Pine Springs Campground. More than half of the populations displayed private alleles including all but one population of *M. linoides*. Private alleles were not detected for either population of *M. stoneana* nor *M. viminea*. The complete data matrix is provided in Appendix 1.

Table 2. Measures of genetic diversity in *Monardella*, pt. 1. SS=number of samples scored, NPo=number of polymorphic bands, NPc=number of common polymorphic bands (≥5% frequency), NPi=number of private bands.

Population	SS	NPo	NPc	NPi
M. australis ANF	29	79	69	0
M. australis SBNF	31	127	116	2
M. linoides linoides PT	27	62	52	0
M. linoides linoides CNF	23	58	49	1
M. linoides oblonga PC	31	81	68	1
M. linoides oblonga MC	29	107	97	1
M. linoides stricta HV	31	123	112	4
M. linoides stricta HB	31	106	97	1
M. viminea CC	31	77	71	0
M. viminea MMCAS	31	91	85	0
M. stoneana OR	31	44	44	0
M. stoneana BF	24	65	42	0
All populations	349	186	n/a	n/a

The relationship of the 349 samples is shown in the NJ dendrogram (Fig. 7) generated based on genetic distance conducted in PAUP. This method ignores a prior population and taxonomic assignments. Most of the samples cluster by taxon and population including all samples of *M. viminea*, however a few notable exceptions include the clustering of all *M. australis* samples from San Bernardino National forest with the two populations of *M. linoides stricta*, also from San Bernardino National Forest (Heart Bar and Holcomb Valley), and the diffuse relationship inferred for *M. linoides linoides* and *M. stoneana*. A more sophisticated analysis of individuals using PCoA is

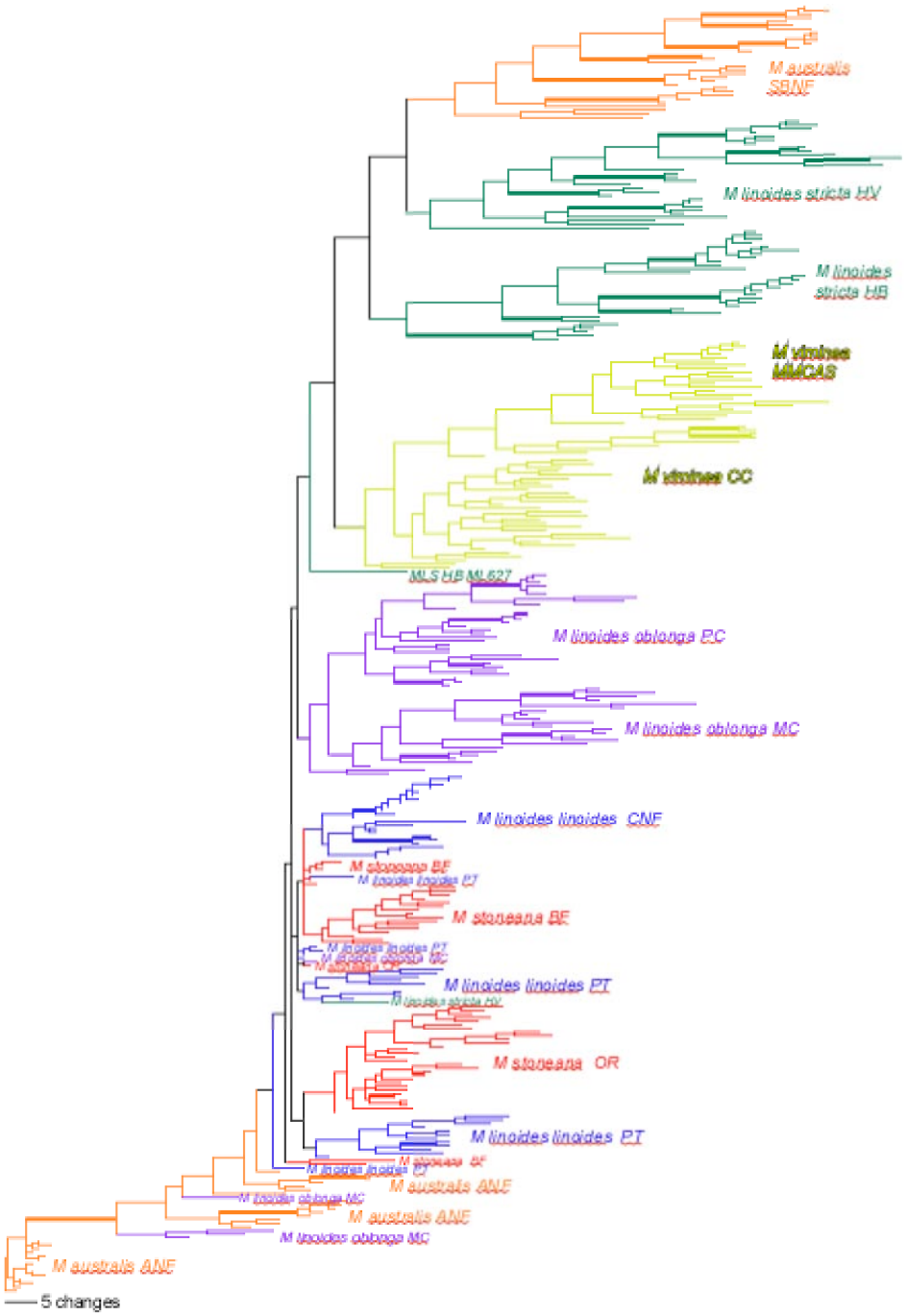

Figure 7. Neighbor Jioning dendrogram of *Monardella* ISSR data with branches color coded by species. Branch lengths proportional to number of changes. Bootstrap legend for internal branches: heavy weight ≥ 95%, medium weight ≥75% but <95%, light weight ≥50% but <75%, dashed <50%. *Monardella viminea* (yellow), *M. stoneana* (red), *M. australis* (orange), *M. linoides linoides* (blue), *M. linoides oblonga* (purple), *M. linoides stricta* (green). See Table 1 for population abbreviations.

shown in Fig. 8. Principal Coordinates Analysis (PCoA) analysis of *Monardella* ISSR data explains 63.7% of the variation in the first 3 axes. Data are first translated into a distance matrix and summarized into three-dimensional space (dimensions 2 and 3 plotted here). This plot also shows obvious clustering of samples according to both population and taxonomic assignment. Again, *M. linoides linoides* and *M. stoneana* are overlapping suggesting a very close relationship.

Finally, Bayesian analyses of the data based on individuals can be summarized in an estimate of ancestry using the software package STRUCTURE. The results depicted in Fig. 9 are based on an Admixture model where individuals may have mixed ancestry (i.e. might be of hybrid origin). This model was selected because *Monardella linoides* is reported to hybridize with other species, all the taxa sampled grow in relatively close proximity, and taxonomic identification is difficult. Figure 9 shows large amounts of structure in most populations except *M. australis* (ANF) and *M. linoides linoides* (CNF). *Monardella australis* (ANF) individuals share a large number of characters with samples of *M. linoides oblonga* (PC, MC), which are somewhat close geographically. *Monardella linoides linoides* from Cleveland National Forest share many similarities with but also many differences from the population in Pioneertown. Surprisingly, the only two populations that are very similar to each other are the two populations of *M. linoides oblonga*. Although the two populations are the closest geographically (~13.5 km apart) they do not seem significantly closer than the populations of *M. stoneana* (~14.5 km apart), *M. viminea* (~14.5 km apart) and *M. linoides stricta* (~18.75 km apart). Nor are populations of *M. linoides oblonga*, on average, less diverse than populations of other taxa (see *Genetic Diversity* results below).

Genetic Diversity–Dominant markers such as ISSRs, RAPDs, and AFLPs should not be analyzed using the same measures as co-dominant markers such as microsatellites and allozymes. Some scientific publications frequently report heterozygosity, gene diversity, and other indices that cannot be

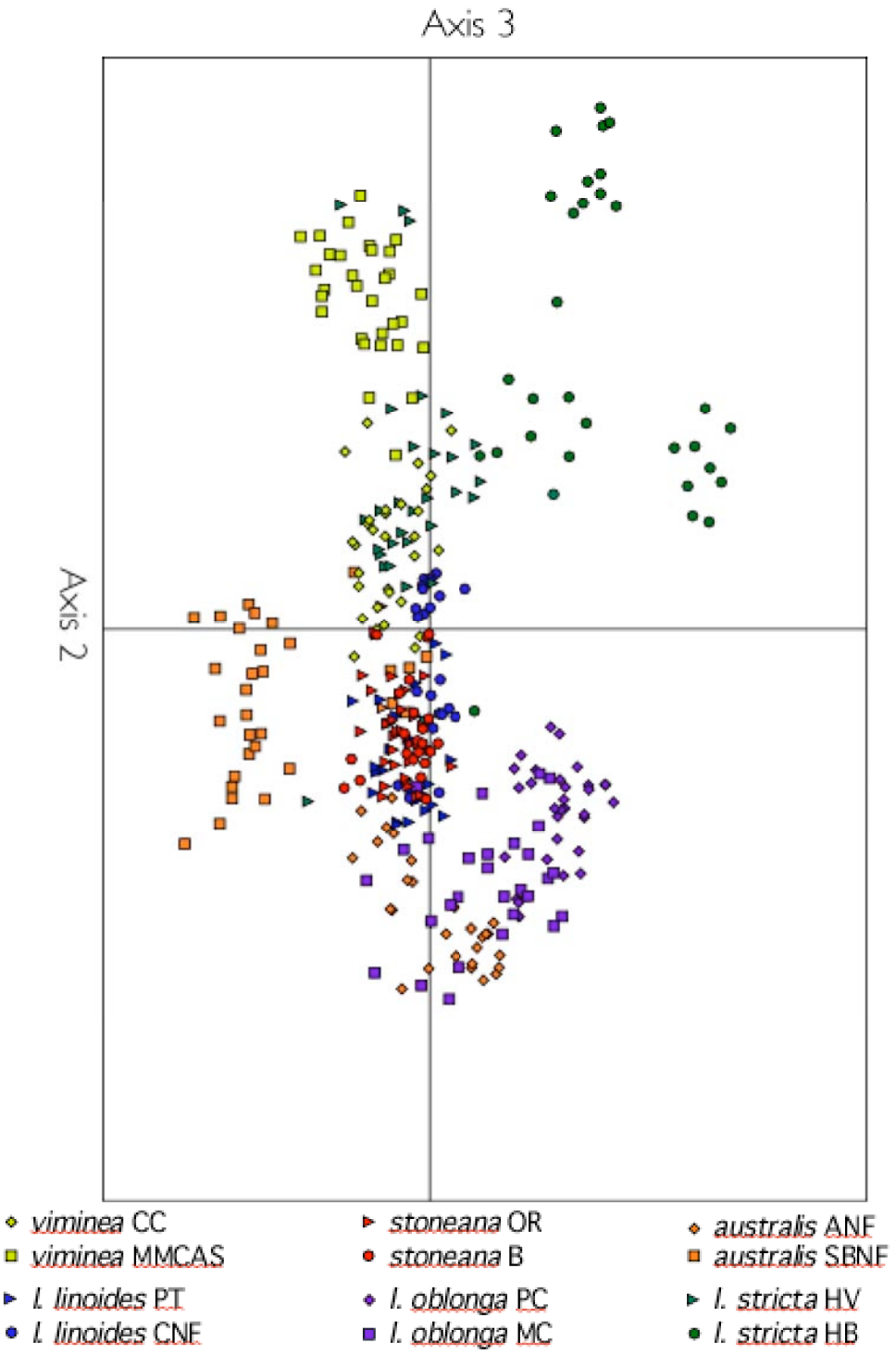

Figure 8. Principal Coordinates Analysis (PCoA or O) results for analysis of *Monardella* ISSR data explaining 63.7% of the variation. Data are translated into a distance matrix and summarized into three-dimensional space (two dimensions plotted here). See Table 1 for population abbreviations.

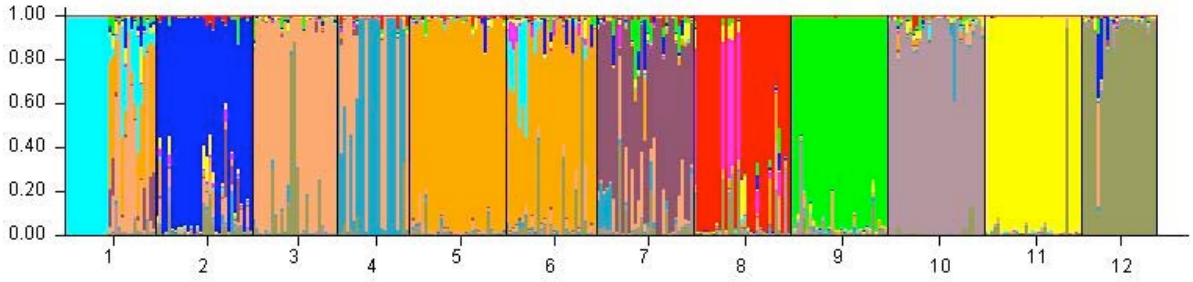

Figure 9. Inferred ancestry of *Monardella* samples based on ISSR distance data under Bayesian criteria (burn-in 50,000 generations). Populations listed on the X axis are:
1=*M. australis* ANF; 2=*M. australis* SBNF, 3=*M. linoides linoides* PT; 4=*M. linoides linoides* CNF; 5=*M. linoides oblonga* PC; 6=*M. linoides oblonga* MC; 7=*M. linoides stricta* HV; 8=*M. linoides stricta* HB; 9=*M. viminea* CC; 10=*M. viminea* MMCAS; 11=*M. stoneana* OR; 12=*M. stoneana* BF. See Table 1 for population abbreviations.

calculated from dominant data in which heterozygosity and homozygosity are unknown (Culley s.d.).

Population genetic diversity measures are shown in Table 3. The proportion of polymorphic loci was lowest in the two populations of *M. stoneana* (23.7% in OR and 35.0% in BF) and highest in *M. australis* (SBNF 68.3%) and *M. linoides stricta* (66.1% HV). Both populations of *M. viminea* were intermediate at 41.4% (CC) and 48.9% (MMCAS). Both Nei's (1973) gene diversity (h) and Shannon's information index (*I*) (Lewontin 1972), were lowest in populations of *M. stoneana* (h = 0.0499 to 0.0641; I = 0.0890 to 0.1031) and highest in *M. linoides stricta* (h = 0.1543 to 0.1644; I = 0.2414 to 0.2618). *Monardella viminea* is somewhat intermediate with h = 0.0968 to 0.1199 and I = 0.1565 to 0.19896. Gene diversity measures were similar under distance (h) and Bayesian estimates (hs) although Bayesian estimates were consistently slightly higher under the f free model (Table 3).

Recent studies show that values for dominant markers (AFLPs, ISSRs, RAPDs) are not directly comparable to values for co-dominant markers (microsatellites, allozymes) (Zhang and Yang 2008). Levsen et al. (2008) found that values for dominant markers are consistently lower

than for co-dominant markers for the same samples. A number of diversity measures based on analyses of dominant data for other rare and endemic perennial plants are provided in Table 5. Values presented here (% polymorphic loci, h, I) for individual populations are similar to those reported in Table 5, but species values reported here are lower. That difference is likely due to the small sample size (2 populations per taxon) employed in this study.

Results based UPGMA clustering using Nei's unbiased genetic distance (1978) based upon population partitioning are somewhat confusing because populations do not necessarily cluster along taxonomic designation. This may be due to limited population sampling per taxon, limited character sampling, or homoplasy. Additional study would be required to identify the exact cause. The dendrogram in Fig. 10 (based on partitioning by taxonomy) indicates *M. viminea* is most similar to *M. linoides stricta*, not *M. stoneana,* and is consistent with the results shown in the NJ tree (Fig 7) and the PCoA analysis (Fig 8).

Several recent studies have found a strong geographic bias to genetic data. This is consistent with expectations for pollinator ranges and associated geneflow. Unexpectedly, this geographic bias sometimes overwhelms taxonomic identity (Percy et al. 2008). This may be due to a variety of factors including introgression, incomplete lineage sorting, or an inaccurate taxonomy. To test whether genetic differentiation is correlated with geographic distance rather than taxonomic identity, Mantel tests were conducted. The results show a modest (R=0.118) but significant (P = 0.001) relationship.

Table 3. Measures of genetic diversity in *Monardella*, pt. 2. P=Percent polymorphic bands, h=gene diversity (standard) ± standard deviation, hs=gene diversity ± standard deviation, I=Shannon Index ± standard deviation.

Population	P	h	hs	I
M. australis ANF	41.40	0.1117 ± 0.1711	0.1269 ± 0.0062	0.1731 ± 0.2483
M. australis SBNF	68.28	0.1712 ± 0.1708	0.1948 ± 0.0135	0.2722 ± 0.2448
***M. australis* mean**	**77.96**	**0.1658 ± 0.1543**		**0.2717 ± 0.2225**
M. linoides linoides PT	33.33	0.0594 ± 0.1080	0.0792 ± 0.0084	0.1024 ± 0.1720
M. linoides linoides CNF	30.11	0.0633 ± 0.1264	0.0825 ± 0.0064	0.1042 ± 0.1905
***M. linoides linoides* mean**	**50.00**	**0.0723 ± 0.1119**		**0.1284 ± 0.1740**
M. linoides oblonga PC	43.55	0.0992 ± 0.1546	0.1144 ± 0.0071	0.1590 ± 0.2279
M. linoides oblonga MC	57.53	0.1216 ± 0.1460	0.1460 ± 0.0131	0.2016 ± 0.2184
***M. linoides oblonga* mean**	**72.58**	**0.1211 ± 0.1390**		**0.2073 ± 0.2029**
M. linoides stricta HV	66.13	0.1644 ± 0.1694	0.1876 ± 0.0133	0.2618 ± 0.2443
M. linoides stricta HB	56.45	0.1543 ± 0.1755	0.1744 ± 0.0105	0.2414 ± 0.2560
***M. linoides stricta* mean**	**84.41**	**0.1801 ± 0.1530**		**0.2954 ± 0.2152**
M. viminea CC	41.40	0.0968 ± 0.1486	0.1140 ± 0.0083	0.1565 ± 0.2220
M. viminea MMCAS	48.85	0.1199 ± 0.1669	0.1351 ± 0.0065	0.1896 ± 0.2433
***M. viminea* mean**	**59.14**	**0.1247 ± 0.1602**		**0.2024 ± 0.2329**
M. stoneana OR	23.66	0.0641 ± 0.1250	0.0783 ± 0.0061	0.1031 ± 0.1945
M. stoneana BF	34.95	0.0499 ± 0.0981	0.0690 ± 0.0074	0.0890 ± 0.1557
***M. stoneana* mean**	**46.24**	**0.0680 ± 0.1162**		**0.1184 ± 0.1789**
All populations	100.00	0.1457 ± 0.1040	0.1218 ± 0.0066	0.2620 ± 0.1476

Table 4. Nei's unbiased measures of genetic identity and genetic distance in *Monardella* (Nei 1978). Nei's unbiased genetic identity (above diagonal) and genetic distance (below diagonal).

	Ma ANF	Ma SBNF	Mll PT	Mll CNF	Mlo PC	Mlo MC	Mls HV	Mls HB	Mv CC	Mv MMCAS	Ms OR	Ms BF
Ma ANF	****	0.9487	0.9654	0.9425	0.9561	0.9697	0.9474	0.9308	0.9478	0.9215	0.9540	0.9601
Ma SBNF	0.0527	****	0.9696	0.9530	0.9533	0.9617	0.9579	0.9477	0.9501	0.9257	0.9526	0.9657
Mll PT	0.0352	0.0309	****	0.9776	0.9716	0.9822	0.9704	0.9558	0.9761	0.9486	0.9777	0.9927
Mll CNF	0.0592	0.0482	0.0227	****	0.9577	0.9650	0.9621	0.9484	0.9628	0.9447	0.9591	0.9779
Mlo PC	0.0449	0.0479	0.0288	0.0432	****	0.9772	0.9536	0.9561	0.9575	0.9312	0.9585	0.9716
Mlo MC	0.0307	0.0391	0.0180	0.0356	0.0230	****	0.9625	0.9528	0.9636	0.9366	0.9677	0.9790
Mls HV	0.0540	0.0430	0.0301	0.0386	0.0475	0.0382	****	0.9537	0.9596	0.9364	0.9543	0.9677
Mls HB	0.0717	0.0537	0.0453	0.0530	0.0449	0.0483	0.0474	****	0.9528	0.9408	0.9425	0.9543
Mv CC	0.0536	0.0512	0.0242	0.0379	0.0434	0.0371	0.0413	0.0483	****	0.9654	0.9699	0.9780
Mv MMCAS	0.0818	0.0772	0.0528	0.0569	0.0712	0.0655	0.0657	0.0610	0.0352	****	0.9444	0.9523
Ms OR	0.0471	0.0486	0.0225	0.0418	0.0424	0.0328	0.0467	0.0592	0.0306	0.0572	****	0.9793
Ms BF	0.0408	0.0349	0.0073	0.0223	0.0288	0.0212	0.0328	0.0468	0.0223	0.0489	0.0209	****

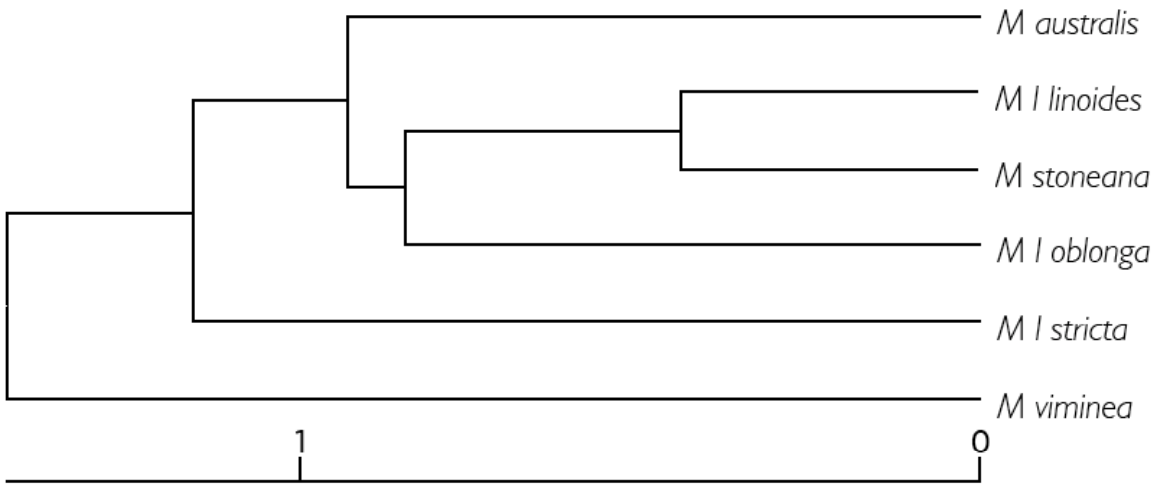

Figure 10. UPGMA dendrogram of relationships among *Monardella viminea* and closely related taxa based on ISSR data.

Discussion

This study tests taxonomic hypotheses based on morphological data used to support the recognition of a newly described taxon *M. stoneana* and to test the shift in taxonomic affiliation of *Monardella viminea*. Molecular data representing the entire genome (ISSRs) were much more variable than DNA sequence data used in earlier phylogenetic studies. Genetic diversity within species was similar to recorded data for other rare perennials, suggesting the limited sampling of this study was sufficient to detect population level differences within species. Proportion of polymorphic loci was also similar to other published studies.

The goal of this study was to elucidate relationships among the geographically proximate members of the *M. linoides* and *M. odoratissima* groups. Results of analyses of individuals, populations, and taxa varied in their ability to elucidate those relationships. Specificially, *M. viminea* and the newly described *M. stoneana* are part of the *M. odoratissima* group, yet fail to cluster with both populations of *M. australis* in any of the analyses (NJ, Fig. 7; PCoA, Fig. 8; UPGMA, Fig. 10).

Table 5. Diversity measures for a variety of perennial plants based on dominant marker data.

Taxon	Rarity[1]	Data Type	% Polymorphic Markers P=population S=species	Population Diversity Values[2] P=population S=species	Reference
Borderea chouardii	Eg	RAPD	P: 30-43 S: 48	h(S)=0.14 h(P)=0.11-0.15	Segarra-Moragues et al. 2005
Clintonia udensis		ISSR	P: 11.9-59.5 S: 98.8	I=0.69	Wang and Zhao 2007
Eremosparton songoricum		ISSR	91.7	I(P)=0.224 I(S)=0.319	Liu et al. 2007
Gentiana atuntsiensis	Ed	ISSR	P: 33.5-46.6 S: 88.4	I(P)=0.225 I(S)=0.324 Φ_{ST}= 0.232	Zhang et al. 2007
Gentiana striolata	Ed	ISSR	P: 40.4-52.5 S: 91.5	I(P)=0.274 I(S)=0.391 Φ_{ST}= 0.226	Zhang et al. 2007a
Isoetes yunguiensis	Ed	RAPD	P: 3.4-33.9 S: 62.8	θ^B(ave)=0.742	Chen et al. 2007a
Lasthenia conjugens	Ed	ISSR	P: 68.2-86.9	I(S)=0.390 h(S)=0.243 Hs(S)=0.193 θ^B(ave)=0.124	Ramp Neale et al. 2008
Nelumbo nucifera	C	ISSR	P: 35.8 S: 90.0	I(P)=0.165 I(S)=0.383	Han et al. 2007
Physaria bellii	R	ISSR	62.9	h=0.22	Kothera et al. 2007
Potentilla ikonnikovii	R, Ed	RAPD	P: 18.5-83.8	Φ_{ST}=0.68	Wesche et al. 2006
Primula apennina	Ed	ISSR	P: 42-82 S: 97	h(P)=0.204 h(S)=0.242 I(P)=0.319 I(S)=0.381	Crema et al. 2009
Rhodiola chrysanthemifolia	Ed	ISSR	P: 22.0-48.8 S: 89.7	I(P):0.083-0.241	Xia et al. 2007
Sagittaria natans	Eg, C	ISSR	S: 48.9	h(P)=0.016-0.129 h(S)=0.150	Chen et al. 2007b
Sagittaria trifolia	W, C	ISSR	S: 32.6	h(P)=0.017-0.068 h(S)=0.082	Chen et al. 2007b
Swertia przewalskii	Eg	ISSR	P: 47-53 S: 96	I (P): 0.22-0.28 θ^B(f-free)=0.389	Zhang et al. 2007b
Swertia przewalskii	Eg	RAPD	P: 47-59 S: 94	I (P): 0.24-0.30 θ^B(f-free)=0.431	Zhang et al. 2007b
Viola flettii	Ed	ISSR	P: 30.0-56.7	Φ_{ST}(P)=0.367-0.759 Φ_{ST}(S)=0.445	McCreary 2005

[1]C=clonal, Ed=endemic, Eg=endangered, R=rare, W=widespread.

[2]h=Nei's Diversity, Hs=Diversity (Bayesian); I=Shannon's Index, Φ_{ST}=FST analog, θ^B=FST analog (Bayesian).

These data suggest *M. stoneana* is most closely related to *M. linoides linoides*, and *M. viminea* is most closely related to *M. linoides stricta*. This is surprising given the geographic distribution of those taxa. *Monardella viminea* is a coastal San Diego County taxon while *M. linoides stricta* is distributed in the San Gabriel and San Bernardino Mountains (Jokerst 1993; but see Elvin and Sanders in press). Similarly, *M. stoneana* is restricted to inland San Diego County and adjacent Baja while its closest relative, *M. linoides linoides*, is distributed as far north as the Little San Bernardino mountains (Elvin and Sanders in press). It is clear that *M. stoneana* and *M. viminea* are not each other's closest relatives, and are genetically distinct. This finding has been confirmed by subsequent comparative DNA sequence analyses involving large numbers of genic regions (Prince unpublished). Although genetic differentiation was correlated with geographic distance on an individual sample, that finding does not seem to be related to the two taxa in question. *Monardella stoneana* and *M. viminea* populations occur within 35-55 km of each other, closer than most other taxa. The correlation is likely due to geographical and genetically close individuals of the same taxon.

Many of the more confusing and interesting findings are the taxonomic muddiness of *M. australis*, *M. linoides linoides*, and the relationship of *M. linoides linoides* to *M. stoneana*. Results presented here suggest the SBNF population of *M. australis* is more closely related to *M. linoides stricta* than to the other population of *M. australis*. The population of *M. australis* in question grows in close proximity to the *M. linoides stricta* populations sampled here. The Jepson manual (Jokerst 1993) states that *M. australis* and *M. linoides stricta* hybridize. It is possible that these results support that hypothesis. Alternatively, we may simply have a poor understanding of the taxonomic boundaries of these taxa. The most recent treatment of Southern California *Monardella* (Elvin and Sanders in press) does not recognize *M. linoides stricta*, stating "The type specimen for *M. linoides* A. Gray var. *stricta* Parish and *M. epilobioides* Greene represent introgressant individuals that are intermediate in characters between *M. linoides* subsp. *erecta* and *M. australis* subsp. *australis*." If the

samples included in this study are actually *M. australis*, then the material of *M. australis* from the San Gabriel Mountains may require closer examination as it falls closer to *M. linoides oblonga*, an endemic of the Mount Pinos region in the Los Padres National Forest. The relationship of *M. linoides linoides* to *M. stoneana* is also unclear. In both PCoA and NJ analyses individuals of the two taxa intermix/overlap. Unpublished comparative DNA sequence analyses (Prince unpublished) clearly distinguish *M. stoneana* from *M. linoides*. Additional population level sampling of both taxa may be necessary.

The rank of taxonomic recognition and circumscription of *M. viminea* was recently challenged (Elvin and Sanders 2003) based on a re-evaluation of gross morphology. Analyses of molecular data presented here support the recognition of two taxa, *M. viminea* and *M. stoneana*., but are inconclusive with regard to taxonomic rank. *Monardella australis* was used as the outgroup and an example of a species in this closely related group. The variation detected in M. australis populations cannot be used as a proxy for species level variation because of the unexpected non-monophyly of *M. australis* samples. Based on genetic distance as shown in the dendrogram of Fig. 10, *M. viminea* is the most different taxon, thus could reasonably recognized at the species level. Similarly, although not shown here, comparative DNA sequence analyses (Prince unpublished) clearly distinguish *M. stoneana* from *M. linoides* (3 subspecies sampled), supporting the recognition of this taxon at the species level as well. The overlap (PCoA, NJ tree) of *M. stoneana* and *M. linoides linoides* may be due to ongoing gene flow between these two taxa.

This study provides preliminary information on population diversity for a number of locally rare and threatened taxa. While the goals of the study were met with limited data, this work forms the foundation for future work on the genetic structure of *M. viminea* and *M. stoneana*. Collection of data from additional populations will allow for inference of gene flow, bottlenecks, and overall genetic trajectory of the species. The recent description of several additional taxa in

the *linoides* group, and the numerous taxonomic changes proposed by Elvin and Sanders (in press) suggests there are a large number of hypotheses still to be tested. Population genetics approaches are invaluable, but rely on knowing closest relatives for comparison. The inclusion of distantly related taxa can lead to erroneous topologies due to excessive homoplasy. Based on the data presented here, it is unclear what the taxon *M. australis* is. It may not be a close relative of either *M. stoneana* or *M. viminea*. Future work should focus on an overall *Monardella* phylogeny. Once a stable phylogeny is established, research within the genus can be focused on monophyletic clades that include small numbers of closely related taxa.

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