SYSTEMATICS AND PHYLOGENY

Genetic diversity, population structure, and ancestry estimation in the *Antennaria rosea* (Asteraceae: Gnaphalieae) polyploid agamic complex

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Abstract *Antennaria rosea*, a polyploid agamic complex, originated from hybridization, polyploidization, and introgression of as many as eight diploid/tetraploid sexual *Antennaria* species in the Rocky Mountains of North America. The species shows tremendous variation within and among populations including phyllary coloration, number and arrangement of heads per flowering stalk, and leaf size/shape leading to the enumeration of many microspecies and a taxonomic challenge. A comprehensive evolutionary study of these populations would clarify the origins and evolution of *A. rosea* as a polyploid agamic complex. Using genetic markers developed for *Antennaria* species, genetic diversity, population structure, and ancestry estimation of 18 *A. rosea* populations distributed in the Rocky Mountains of the U.S.A. were studied. Comparatively, *A. rosea* populations distributed in the Northern Rocky Mountain region had higher levels of genetic diversity than the populations in the southern Rocky Mountain region. *Antennaria rosea* populations were divided into three genetically distinct clusters, and populations within each genetic cluster showed high correlations regarding geographic distribution and ancestry. Two of the putative parents, *A. microphylla* and *A. umbrinella*, were the major sources of parentage for *A. rosea* populations, and notably, these species were widely distributed and sympatric with most of the *A. rosea* populations from this study. A novel finding of this study was that each of the *A. rosea* populations had its origins from different sexual progenitors and, although *A. microphylla* and *A. umbrinella* were the major contributors to the parentage of most of the *A. rosea* clones sampled, the other six sexual species also contributed to a lesser degree to their origins. Our study also indicated that sympatry of parents contributes substantially to the origination of polyploid populations of *A. rosea*.

Keywords agamic complex; *Antennaria rosea*; apomixis; genetic diversity; microsatellites

Supporting Information may be found online in the Supporting Information section at the end of the article.

■ INTRODUCTION

Antennaria rosea Greene, one of the most morphologically diverse polyploid agamic complexes, occurs in diverse habitats from dry sagebrush steppes to moist alpine tundra and has a center of diversity in the Rocky Mountains of western North America (Bayer, 1989a). In North America, the species has a wide-ranging distribution from New Mexico and Southern California in the South to Alaska and the Northwest Territories in the North, and to the western shores of Hudson Bay in the East (Bayer, 1989a). The *A. rosea* complex also occurs sporadically along the shores of James Bay, north shore of Lake Superior, Gaspé Peninsula in eastern Quebec, and in western Newfoundland (Bayer, 1989a,b). Different ploidy levels including triploid ($2n = 42$), tetraploid ($2n = 56$), and pentaploid ($2n = 70$) cytotypes have been reported in the species, with tetraploid being the most prevalent form (Bayer & Stebbins, 1987). Being a dioecious gametophytic apomict, most *A. rosea* populations are composed entirely of pistillate clones, while staminate plants are extremely rare (Bayer, 1987; Bayer & Stebbins, 1987). Members of the *A. rosea* species complex have been identified by many authors as clones having rose-colored phyllaries (Greene, 1898; Piper, 1906; Rydberg, 1922; Tidestrom, 1925; Sharsmith, 1960); however, field studies have shown tremendous variation among populations for phyllary coloration ranging from white to pink, red or brown, as well as the number of heads per flowering stalk (Bayer, 1989a,b, 1990a,b). This variation among populations has led to the enumeration of many "microspecies" in *A. rosea* and other *Antennaria* Gaertn. polyploid agamic complexes. Microspecies can be defined as uniform populations with a hybrid constitution, mainly reproducing by uniparental methods, that are morphologically different from related uniform populations (Grant & Grant, 1971). The practice of giving microspecies the rank of a species has resulted in the listing of almost 400 published names in *Antennaria*, leading to a taxonomic conundrum (Bayer, 1987; IPNI, 2019). In the process, about 40 microspecies so closely similar to *A. rosea* that no taxonomic keys could be constructed for them, have been described (Bayer, 1989a,b). Earlier, Chmielewski & Chinnappa (1988) studied *A. rosea* populations in Northwestern Canada and suggested retaining the species status for those microspecies maintaining distinctive

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morphologies under controlled transplant studies. However, Anderson & Welsh (1974) had previously discarded the possibility of such recognition due to the lack of insufficient distinctness in them. Bayer (1989a,b), in agreement with Anderson, considered it unrealistic to recognize all the microspecies and concurred that the species should be considered as a single polyploid agamic complex. Bayer (1989b) proposed combining them into related groups of apomicts and recognizing them as an agamic species complex as has been exhibited in compilospecies groups such as *Bothriochloa* O.Kuntze, *Capillipedium* Stapf and *Dichanthium* Willemet (Harlan & De Wet, 1963; De Wet & Stalker, 1974). Evidence from morphological, isozyme, ecological, and phylogenetic studies indicates that the *A. rosea* complex evolved as a polyploid agamic complex from hybridization and introgression of as many as eight diploid or tetraploid sexual species followed by polyploidization event(s) (Bayer, 1989a, 1990c, 1991a; Bayer & Chandler, 2007). These eight sexual species are *A. aromatica* Evert, *A. corymbosa* E.E.Nelson, *A. marginata* Greene, *A. microphylla* Rydb., *A. pulchella* Greene, *A. racemosa* Hook., *A. rosulata* Rydb., and *A. umbrinella* Rydb. and have been recovered as closely related in previous phylogenetic analyses (Bayer, 1990c; Bayer & al., 1996; Thapa & al., 2020).

Apomixis, a process by which the formation of asexual seeds usually occurs via meiotically unreduced gametes, is rare among angiosperms, with only about 1.1% of genera harboring this characteristic (Carman, 1997). The process results in genetically invariant lineages, to which evolutionary forces, such as selection and genetic drift, may fail to act limiting their long-term evolutionary potential (Richards, 2003). However, apomixis is an important evolutionary feature that maintains desirable genotypes as one linkage group over generations, providing numerous ecological advantages (Harper, 1982; Hörandl & al., 2008) and plant breeding benefits (Hanna & Bashaw, 1987; Chen & al., 2018). In apomicts, the reduced or negated male functions (Noirot & al., 1997) can enhance reproductive success for the female increasing colonizing abilities (Van Dijk, 2007); however, as a tradeoff, apomicts may accumulate deleterious mutations (Comai, 2005; Hojsgaard & al., 2014) and experience poor adaptation to changing environments (Muller, 1964; Rodrigo & al., 2017). Heterozygosity, one of the parameters of genetic variability, is usually higher in some of the loci of apomictic populations compared to the related sexual species due to allopolyploidy during their genesis, but the total genotypic variation is often lower due to the absence of recombination and meiosis (Hörandl & Paun, 2007). Genetic variation in apomictic populations is dependent on a number of factors, including facultative sexuality of the apomicts leading to backcrossing with sexual relatives, mutations, and the involvement of divergent sexual ancestors in multiple evolutionary origins (Bayer & al., 1990; Gornall, 1999; Hörandl, 2004).

Antennaria was the first genus in which apomixis (gametophytic apomixis or agamospermy) was embryologically documented (Juel, 1900). This seminal work was followed up by Stebbins's studies (1932a,b) on apomixis in *Antennaria* in North America. Apomixis in *A. rosea* has never been studied cytologically, but it is assumed that it is an obligate apomict given its gynoecious nature and ability to set seed without pollen. Obligately apomictic *A. rosea* populations also develop clonal diversity through mutation; however, mutation alone cannot account for a large number of existing clones (Bayer & Chandler, 2007). If they are facultative apomicts, fertilization by compatible pollen from the sexual species in sympatry or parapatric distributions in *A. rosea* could generate new apomicts and clonal diversity (Bayer, 1989a, 1991a; Bayer & Chandler, 2007). Distribution ranges for the putative sexual parents of *A. rosea* overlap; for example, in west-central Montana, up to six sexual species, viz. *A. aromatica*, *A. corymbosa*, *A. microphylla*, *A. pulchella*, *A. racemosa*, and *A. umbrinella* (Bayer, 1987), can be found on a single mountain. However, these species are associated with specific habitats and communities, and are reproductively isolated (Bayer & al., 1991). *Antennaria rosea* populations are found in diverse habitats from dry steppe to alpine tundra and have wider geographic distribution compared to that of the sexual progenitors with whom they often have sympatric or parapatric coexistence (Bayer, 1990c, 1991a). When grown in proximity, *A. rosea* easily hybridizes with the sexual species and may develop a higher ecological amplitude compared to the putative sexual parents (Bayer & al., 1991). Bayer (1990b) demonstrated that clonal diversity is lower among *A. rosea* populations in the Pleistocene glaciated regions as compared to those distributed in the unglaciated regions; however, that study had a sampling bias with less sampling from the glaciated regions. The present distribution of the *A. rosea* population in the Pleistocene glaciated regions could be a result of the colonization of populations from south of the glacial margin after glacial retreat, or the remnants of populations from the unglaciated regions in Alaska and the Yukon Territory (Bayer, 1991a).

Studies based on morphology and isozyme markers have suggested that *A. rosea* has a complex evolutionary history involving multiple hybridizations and introgression among various putative sexual parents. However, the influence of these events on the genetic diversity and population structure in the complex has not been fully explored. Moreover, additional genetic markers would improve ancestry estimations, i.e., which parents contributed to which populations of *A. rosea*. In the present study, we analyzed genetic diversity, population structure, and ancestry estimation in 18 populations of *A. rosea* from the major centers of distribution across the species range in the Rocky Mountain region of the U.S.A. Ten populations were collected from the Northern Rocky Mountains and eight populations from the Southern Rocky Mountains, i.e., north and south of the Red Desert of Wyoming, respectively. All sampled individuals were genotyped using a collection of simple sequence repeat (SSR) markers developed for *Antennaria* species (Thapa & al., 2019). The resulting data allowed us to investigate the amount and distribution of genetic diversity within the sampled *A. rosea* populations and putatively assign sexual diploid/tetraploid progenitors to each population.

■ MATERIALS AND METHODS

Plant materials and genotyping. — Eight putative sexual diploid/tetraploid parent species were sampled from their respective geographic ranges. Five populations each for the putative parents, except four populations each for *A. aromatica*, *A. microphylla*, and *A. pulchella*, were utilized. Altogether, 276 individuals were assessed from 38 populations in eight species. Detailed information regarding the populations and the species is given in Appendix 1. Similarly, *A. rosea* populations were sampled from the principal range of the species distribution in the western United States with a focus on the Rocky Mountain region, where there is a great overlap of the putative parent's distributional ranges. The collection sites ranged from 36.398° to 48.290° in latitude and −113.379° to −106.001° in longitude with the elevation of the locations extending from 1346 to 3534 meters above sea level (Appendix 1). A total of 155 *A. rosea* individuals from 18 populations were assessed, and the herbarium voucher collections documenting the morphology of each sampled clone in each population were deposited in MEM. All DNA samples were genotyped with 12 microsatellite markers developed for *Antennaria* species (Thapa & al., 2019). In the present study, due to the presence of mixed ploidy levels in the study species and populations, some of the species/populations showed the amplification of more than two alleles for certain loci. Therefore, the microsatellites were scored as individual dominant loci as suggested in Rodzen & May (2002) and Rodzen & al. (2004). We thus adopted the approach of several other studies and converted our data to a binary format as dominant data (Samadi & al., 1999; Rodzen & May, 2002; Rodzen & al., 2004; Wu & al., 2015). All wet-lab protocols, including DNA extraction, PCR amplification, and genotyping protocols, followed Thapa & al. (2019). Briefly, total genomic DNA was extracted from silica-gel dried leaves or the herbarium specimens using the E.Z.N.A. SQ Plant DNA Kit from Omega Bio-Tek (Norcross, Georgia, U.S.A.), with a modification of the protocol adding PVP and ascorbic acid to the SQ1 buffer (10 ml SQ1 buffer, 100 mg PVP, 90 mg ascorbic acid). DNA samples were column purified using E.Z.N.A. Cycle Pure Kit from Omega Bio-Tek, and the following PCR conditions were used: 1.5 µl 10× buffer, 0.5 µl $MgCl_2$ 25 nM, 0.2 µl DNTPs 20 nM (5 nM of each), 0.35 µl forward primer at 5 nM, 0.35 µl reverse primer at 20 nM, 0.35 µl of oligonucleotide m13 containing one of the VIC, NED, FAM or PET fluorophores at 10 nM, 0.7 µl of *Taq* and 1.5 µl of DNA in a 15 µl reaction. A standard touch-down PCR protocol was used with the following thermal cycler conditions: 95°C initial denaturation for 3 min followed by 11 cycles of PCR to bolster primer annealing (30 s at 94°C, 30 s annealing with temperature decreasing by 1°C from 65°C to 55°C with each cycle, and 60 s at 72°C), followed by another 30 cycles of amplification (30 s at 94°C, 30 s at 55°C, and 60 s at 72°C), and a final extension at 72°C for 10 mins. PCR products were stained with GelRed (Biotium, Fremont, California, U.S.A.) and visualized on a 1% agarose gel. Amplified products were pooled into dilution plates, with each well containing 5 µl of the same sample amplified for four different loci having different fluorophores and diluted into a 30 µl volume. Each well in run plates was prepared with 1 µl of the pooled sample in 6.5 µl of GeneScan 500 LIZ Size Standard (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.) diluted at the rate of 200 µl of the size standard in 740 µl of Formamide. Genotyping was performed using capillary electrophoresis on an ABI 313070XL DNA Analyzer (Thermo Fisher Scientific) at the Molecular Resource Center, University of Tennessee, Memphis. The electropherograms were analyzed with GeneMarker v.2.6.3 (SoftGenetics LLC, State College, Pennsylvania, U.S.A., https://softgenetics.com) to score the variation in the markers.

Population genetic analyses and relatedness. — Measures of genetic diversity, including percentage of polymorphic loci, expected heterozygosity, multilocus genotype (MLG), Simpson's index, and number of private alleles were calculated across the 18 populations of *A. rosea* using GenAlEx v.6.5.02 (Peakall & Smouse, 2012) and R-package Poppr v.2.3.0 (Kamvar & al., 2014). Population structure was also investigated via analysis of molecular variance (AMOVA; Excoffier & al., 1992) as implemented in GenAlEx to detect genetic differentiation at three hierarchical levels: among different species (eight putative parents of *A. rosea*), among populations, and among different individuals within populations. Initially, the extent of differentiation among the eight putative parent species was estimated, followed by a pairwise analysis amongst the populations of the putative parent species, and finally among the 18 *A. rosea* populations. In all cases, the statistical analysis was obtained by performing 1000 permutations. Genetic relationships among the putative parent species and *A. rosea* populations were also investigated separately using principal coordinate analysis (PCoA) in GenAlEx. Based on multilocus genotypes of all the populations, a standard genetic distance matrix (Nei, 1978) was constructed for the PCoA analysis to graph the first two principal coordinates in two-dimensional space.

Population structure in *A. rosea* was investigated using the Bayesian model-based clustering algorithm implemented in the software package STRUCTURE v.2.3.4 (Pritchard & al., 2000). Based on their multi-locus genotypes, the individuals were assigned to K population genetic clusters, and for each individual, the proportion of membership in each cluster was estimated using the program CLUMPP v.1.1.2 (Jakobsson & Rosenberg, 2007) and visualized in Distruct v.1.1 (Rosenberg, 2004). The analysis was not influenced by the prior population information as the USEPOPINFO model was turned off. For each analysis, K = 1 to 18 population genetic clusters were evaluated with five runs per K value, and the probability values were averaged across runs for each cluster. For each run, the initial burn-in period was set to 200,000 with 1,000,000 MCMC iterations, and the admixture model assuming independent allele frequencies was selected for the analysis. The most likely number of clusters was then

determined using the Delta*K* method of Evanno & al. (2005). The program STRUCTURE assumes populations are in Hardy Weinberg equilibrium (HWE), and this assumption may be violated in obligate or nearly obligate apomictic populations such as those of *A. rosea* studied here. As an alternative method for evaluating population structure, we used discriminant analysis of principal components (DAPC), which uses the K-means clustering algorithm and is free of the assumptions regarding HWE and linkage disequilibrium (LD) (Jombart & al., 2010). During the analysis, we used the "find.clusters ()" function from the R-package adegenate v.2.1.1 (Jombart, 2008) and selected the axes based on principal components analysis and a discriminant analysis. We evaluated $K = 18$ groups with the Bayesian information criterion to determine the number of resolved genetic clusters. In order to investigate whether the separation of the *A. rosea* populations was potentially geography-driven, i.e., dispersal of the population is restricted, indicating isolation by distance (IBD), the correlation between geographic and genetic distances was investigated using Mantel tests in GenAlEx with 10,000 permutations in all 18 *A. rosea* populations. In order to investigate the genetic constitution of different *A. rosea* populations with respect to the different putative parent's gene pool, a mixed-stock analysis was performed following the approach of Mandel & al. (2011). The analysis estimated the contribution of the putative parent's gene pool to different *A. rosea* populations considered in this study. The assessment was carried out using the unconditional maximum likelihood (UML; Smouse & al., 1990) method based on the mixstock package v.0.8.4.2 (Bolker & al., 2003, 2007) in R programming language (R Development Core Team, 2011). The UML approach was preferred over the conditional maximum likelihood approach as it estimates the source frequencies independently, considering them not to be equal to the sampled frequencies, hence also allowing for sampling error (Bolker & al., 2003, 2007). For this analysis, the potential source gene pools were the populations of the putative parents of *A. rosea*. Altogether, 19 mixed-stock analyses were performed considering each *A. rosea* population as a separate mixed stock and also collectively taking all the 18 *A. rosea* populations together. For each analysis, 95% confidence intervals of the source contributions were obtained with 1000 non-parametric bootstrap resampling.

■ RESULTS

Genetic diversity. — The 12 microsatellite markers scored as individual dominant loci (see above) resulted in 108 variable sites. The expected heterozygosity (H_e) based on the converted dataset is bound between 0 and 0.5, with the maximum value possible when the band frequency (presence and absence of band) is equal, i.e., $p = q = 0.5$. The minimum and maximum H_e across the populations ranged from 0.037 to 0.074 for the populations of *A. rosulata* and *A. corymbosa* respectively, and the mean H_e for the populations of all putative parent species of *A. rosea* was 0.055 with the standard error of 0.0048. The minimum and maximum number of private alleles among the putative parents of *A. rosea* were recorded as 0 and 3 in *A. pulchella* and *A. racemosa* populations respectively (Table 1). For genetic differentiation at different hierarchical levels, the AMOVA analysis comprised the eight putative parent species of *A. rosea*; *A. aromatica* ($n = 23$), *A. corymbosa* ($n = 48$), *A. marginata* ($n = 35$), *A. microphylla* ($n = 30$), *A. pulchella* ($n = 22$), *A. racemosa* ($n = 43$), *A. rosulata* ($n = 41$), and *A. umbrinella* ($n = 34$) and showed the existence of genetic differences among and within species ($n = 276$, PhiPT = 0.437, $P < 0.001$) (Fig. 1). The PhiPT values among the populations for each putative parent ranged from 0.049 to 0.334, with the highest value occurring among *A. aromatica* populations and the lowest value among *A. racemosa* populations (Table 1). Inspection of the PCoA plot of the putative parent showed more resolution among the five diploid species, *A. corymbosa*, *A. microphylla*, *A. pulchella*, *A. racemosa*, and *A. rosulata* when compared to the known polyploids, *A. aromatica*, *A. marginata*, and *A. umbrinella*, which had more overlap with the other diploid putative parents. The percentages of

Table 1. PhiPT values among populations in the eight putative sexual parents of *Antennaria rosea*.

Species	Total populations	Total samples	Private alleles	PhiPT	*P* (rand ≥ data)	Mean H_e	Standard error
A. aromatica	4	23	1	0.334	0.001	0.053	0.007
A. corymbosa	5	48	1	0.156	0.001	0.074	0.007
A. marginata	5	35	1	0.228	0.001	0.045	0.005
A. microphylla	4	30	2	0.135	0.001	0.071	0.007
A. pulchella	4	22	0	0.328	0	0.050	0.006
A. racemosa	5	43	3	0.049	0.021	0.067	0.006
A. rosulata	5	41	1	0.110	0	0.037	0.005
A. umbrinella	5	34	1	0.304	0	0.045	0.005

PhiPT = estimated variation among populations / (estimated variation within populations + estimated variation among populations); Probability, *P* (rand ≥ data), for PhiPT is based on standard permutation across the full dataset. H_e, expected heterozygosity.

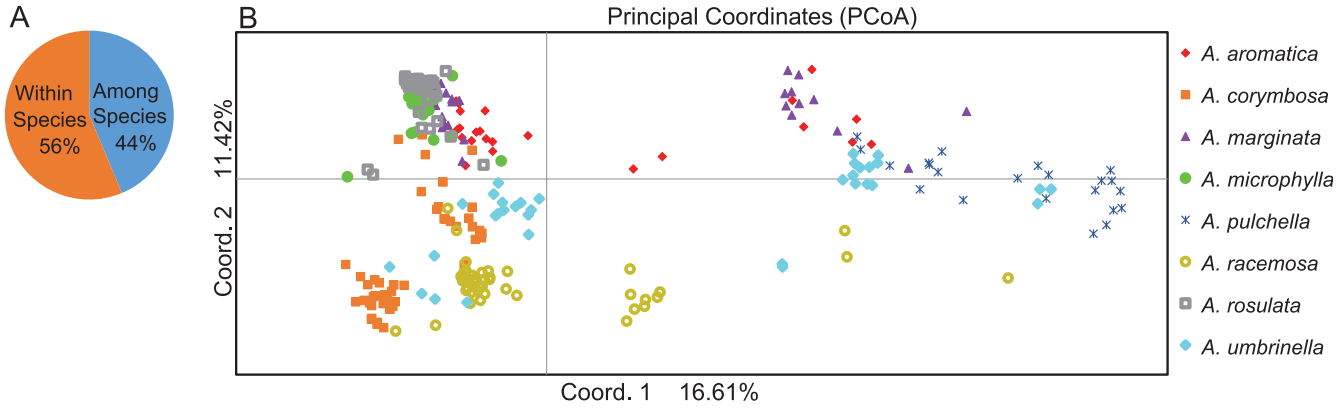

Fig. 1. Genetic diversity in eight sexual putative parents of *Antennaria rosea* based on 12 microsatellite markers scored as individual dominant loci resulting in 108 variable sites. **A**, AMOVA showing within and among species variations in the putative parents; **B**, Principal coordinate analysis (PCoA) resolving the individuals of the putative parent species in two principal coordinates.

the variation explained by the first two axes in the PCoA were 16.61 and 11.42 respectively (Fig. 1). *Antennaria corymbosa*, *A. microphylla*, and *A. rosulata* were separated from *A. pulchella* along coordinate one, whereas there was a separation of *A. aromatica*, *A. marginata*, *A. microphylla*, and *A. rosulata* from *A. corymbosa* and *A. racemosa* along coordinate two (Fig. 1).

The analysis of variance among *A. rosea* populations also showed high genetic differentiation among and within populations (n = 155, PhiPT = 0.478, $P < 0.001$) (Fig. 2). Out of 155 *A. rosea* individuals in 18 populations, 135 unique MLGs were identified with three MLGs shared across different populations; two of them were shared between populations R2 and R6, and one MLG was shared between R3 and R18 (suppl. Fig. S1). The Simpson's index for the populations ranged from 0.72 in R18 to 0.9 in three of the populations R9, R11, and R14 (Table 2). Expected heterozygosity (H_e) across the populations was 0.064, with the mean standard error of 0.003. Five out of eight Southern Rockies populations (R12, R11, R13, R15, R16) had comparatively low levels of expected heterozygosity, whereas only two out of ten Northern Rockies populations (R8, R4) had relatively lower expected heterozygosity. The highest and the lowest H_e values were recorded for R17 and R13 *A. rosea* populations, respectively (Table 2). As expected, populations with comparatively higher H_e usually had a higher percentage of polymorphic loci, and three of the populations (R12, R8, R1) also showed the presence of private alleles (Table 2). The percentages of variation explained by the first two coordinates in the PCoA plot with *A. rosea* populations were 16.22 and 11.18 respectively. The populations R7, R9, R15, and R16 were resolved from populations R4, R8, R11, R12, and R13 along coordinate two. Other populations, such as R2, R10, R14, were clustered based on coordinate one, whereas the populations, R1, R5, R17, and R18 were scattered over a large area (Fig. 2). Weak isolation by distance (IBD) was detected for the populations of *A. rosea* (suppl. Fig. S2); the

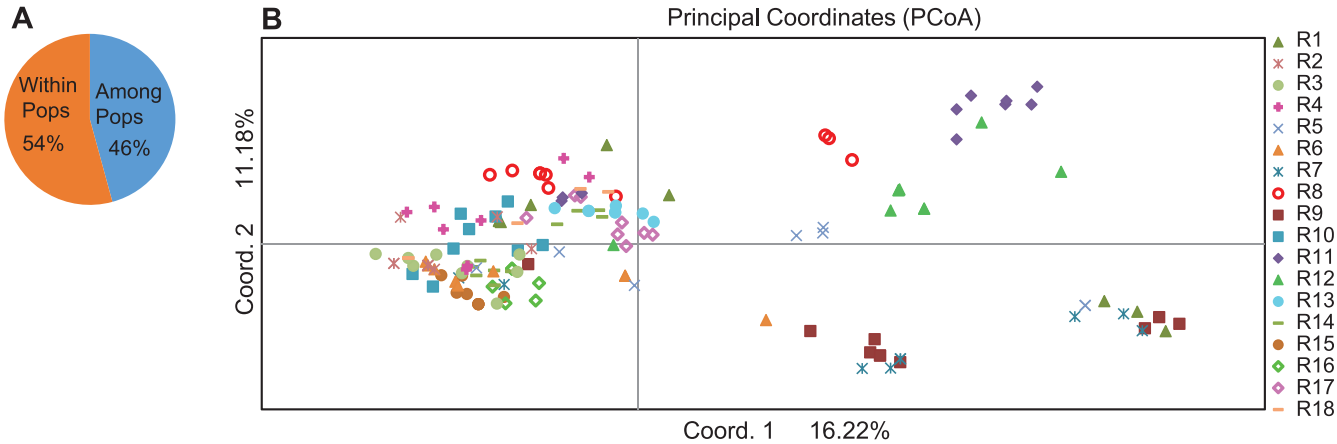

Fig. 2. Genetic diversity in 18 *Antennaria rosea* populations based on 12 microsatellite markers scored as individual dominant loci resulting in 67 variable sites. **A**, AMOVA showing within and among population variations in *A. rosea* populations; **B**, Principal coordinate analysis (PCoA) resolving the individuals of the populations in two principal coordinates. The 18 *A. rosea* populations are color-coded and shown alongside the figure.

finding was also corroborated by the fact that no strong geographical structuring of accessions was seen in the PCoA analysis (Fig. 2).

Population structure and ancestry estimation. — With regard to the population structure of the 18 *A. rosea* populations, the Delta*K* method of Evanno & al. (2005) provided support for the presence of three genetically distinct clusters (i.e., $K = 3$), which roughly distinguished the population clusters distributed in the Southern Rockies, Northern Rockies and those having a much wider distribution occupying both the Northern and Southern Rockies. The percentage membership in each cluster for all individuals and the proportion memberships of each population into the three clusters is shown in Fig. 3. For the DAPC, the value of the Bayesian information criterion decreased with each increase in the value of *K*, and the best explained value of *K* was inconclusive (suppl. Fig. S3). Therefore, the same *K* value, i.e., $K = 3$, identified from the STRUCTURE analysis, was set in the DAPC for further analysis. DAPC revealed a pattern consistent with STRUCTURE analyses while similarly categorizing each individual from the 18 populations into one of the three clusters (Fig. 3B). The mixed-stock analysis revealed that most of the source contributions for the *A. rosea* populations (analyzed as one mixture as well as the 18 populations separately) were from *A. microphylla* and *A. umbrinella* (Fig. 4, suppl. Fig. S4). *Antennaria microphylla* is a diploid species, whereas *A. umbrinella* is known to have diploid/tetraploid cytotypes (Bayer, 2006). The estimated UML source contributions were consistently higher for these two species, and they were the highest major source contributors in 11 out of 18 *A. rosea* populations (Fig. 5, suppl. Fig. S4). However, overlaps in the bootstrap confidence intervals (95%) between them and also with that of other major source species were also seen (Fig. 4, suppl. Fig. S4). In total, *A. microphylla* was the major source contributor for 11 populations, followed by *A. umbrinella* and *A. marginata* with three populations each, and *A. aromatica* contributing to one population (Fig. 6, suppl. Fig. S4). Pie charts showing the proportion of source contribution of the putative parent species in each population in the collection sites are shown in Fig. 5.

■ DISCUSSION

Genetic diversity. — Genetic diversity in agamospermous populations, which is generally expressed at two major levels, (i) heterozygosity at many loci in each individual and

Table 2. Genetic diversity statistics for 18 *Antennaria rosea* populations.

P. No.	Coll. No.	Rocky Mountains	*n*	%P	MLG	H_e	PA	Simpson's index	G. cluster	SDI	Parentage
R1	MT-04014	Northern	8	44.78%	8	0.094	2	0.864	3	5	umb/mic
R2	MT-04020	Northern	9	32.84%	6	0.073	0	0.790	3	6	mic/umb
R3	MT-04029	Northern	9	35.82%	9	0.080	0	0.889	3	6	mic/umb
R4	MT-04031	Northern	8	23.88%	8	0.049	0	0.875	3	6	mic/umb
R5	MT-04011	Northern	7	28.36%	7	0.067	0	0.844	3	6	mic/umb
R6	MT-04008	Northern	9	34.33%	8	0.082	0	0.864	3	6	mic/umb
R7	MT-04035	Northern	9	40.30%	8	0.065	0	0.864	1	6	umb/cory
R8	MT-04026	Northern	10	13.43%	9	0.032	2	0.880	2	6	aro/mar
R9	WY-04003	Northern	10	35.82%	10	0.065	0	0.900	1	6	umb/cory
R10	WY-04012	Northern	9	34.33%	9	0.082	0	0.889	3	6	mic/umb
R11	UT-04002	Southern	8	14.93%	10	0.036	0	0.900	2	4	mic/roz
R12	UT-04001	Southern	8	20.90%	7	0.047	1	0.840	3	4	mic/umb
R13	CO-07002	Southern	8	10.45%	6	0.024	0	0.812	2	6	mar/pul
R14	UT-17001	Southern	10	29.85%	10	0.073	0	0.900	2	6	mic/roz
R15	CO-07010	Southern	9	11.94%	6	0.030	0	0.741	3	6	mic/umb
R16	CO-17007	Southern	6	23.88%	5	0.052	0	0.778	3	6	mic/umb
R17	NM-17003	Southern	8	40.30%	8	0.101	0	0.875	2	5	mar/roz
R18	AZ-17009	Southern	5	43.28%	4	0.100	0	0.720	2	5	mar/mic

population number (P. No.), collection number (Coll. No.), number of individuals sampled (*n*), percentage of polymorphic loci (%P), multilocus genotype (MLG), expected heterozygosity (H_e), private alleles (PA), genetic cluster assigned based on Structure analysis (G. cluster), sexual diversity index (SDI), *A. aromatica* (aro), *A. corymbosa* (cory), *A. marginata* (mar), *A. microphylla* (mic), *A. pulchella* (pul), *A. racemosa* (race), *A. rosulata* (roz), and *A. umbrinella* (umb)

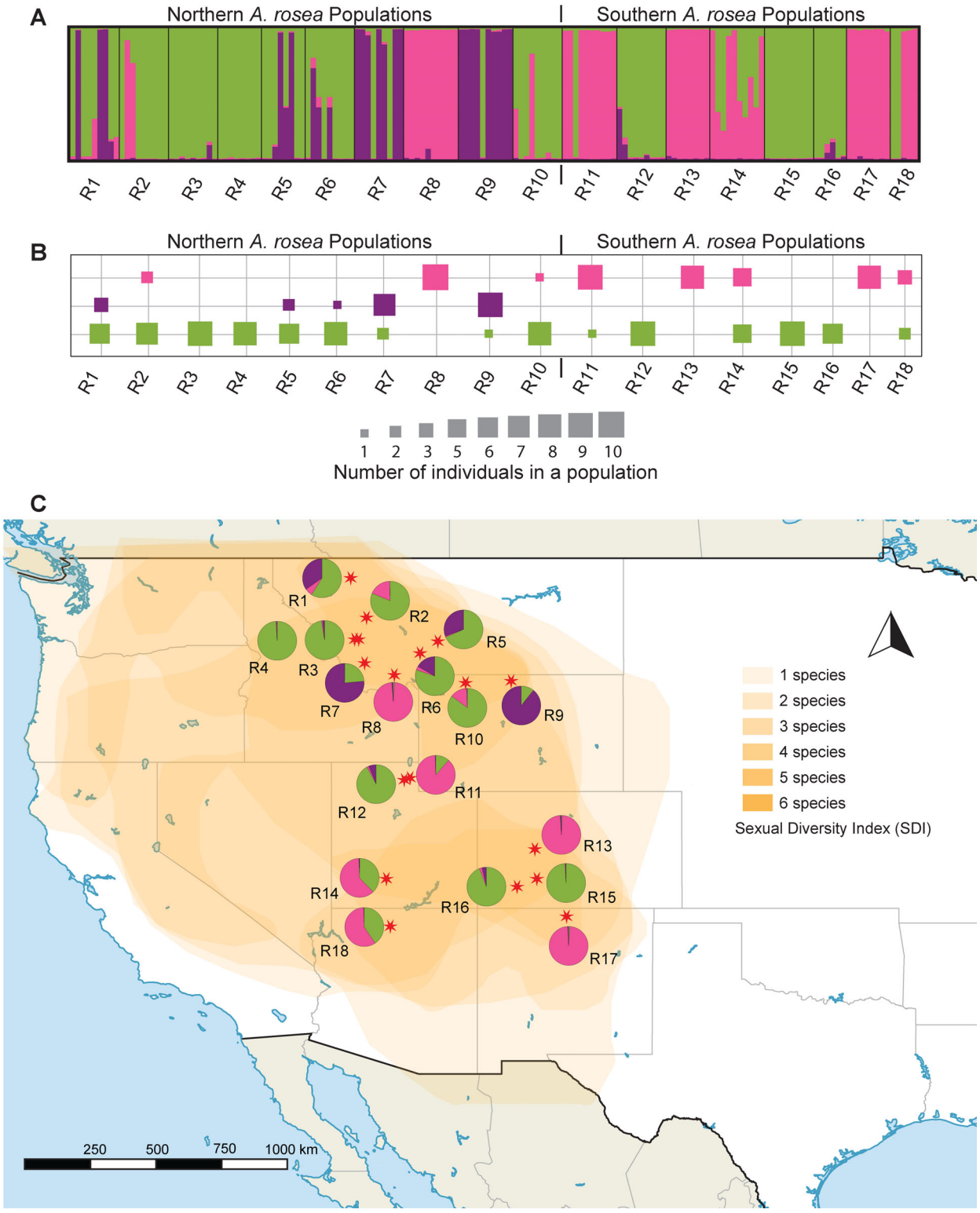

Fig. 3. Genetic structure of *Antannaria rosea* populations. **A**, STRUCTURE plot of 155 individuals belonging to 18 *A. rosea* populations (red stars in C) with $K = 3$ clusters (violet, cluster 1; pink, cluster 2; light green, cluster 3). The Y-axis shows the proportion of membership in the various clusters. The analysis is based on the 12 microsatellite markers with each marker scored as an individual dominant locus. The vertical black bars have been included as visual separators between the *A. rosea* populations. **B**, DAPC plot of 155 individuals belonging to 18 *A. rosea* populations assigned to three genetic clusters (violet, cluster 1; pink, cluster 2; light green, cluster 3) identified by the STRUCTURE program. **C**, Map of the collection sites of 18 *A. rosea* populations considered in this study. The sexual diversity index (SDI) is indicated by shading and reflects the number of sexual progenitor species that occur in areas across the study range. The pie chart alongside each population indicates its proportion memberships in the three genetic clusters by the STRUCTURE analysis.

(ii) multilocus genotypic diversity, i.e., the occurrence of distinct individual genotypes within and among populations (Hörandl & Paun, 2007), differs fundamentally from their sexual relatives (Paun & al., 2006). Traditionally, two major processes that influence the levels of heterozygosity in apomictic populations include polyploidization, especially allopolyploidization (Gornall, 1999), and independent evolution/mutation of different alleles of a locus (Birky, 1996). However, recent studies have also suggested the roles of climate change (Wan & al., 2016) and somatic mutations (Barrett, 2015) in affecting levels of genetic diversity. Hörandl & Paun (2007) summarized the work by different authors (Bayer & Crawford, 1986; Hughes & Richards, 1989; Yahara, 1990; Bayer, 1991a; Noyes & Soltis, 1996; Hörandl & al., 2000), highlighting the significance of allopolyploidy compared to autopolyploidy for leading to elevated levels of heterozygosity in apomicts. A similar conclusion was reached in earlier studies (Bayer & Crawford, 1986; Bayer, 1991b) that demonstrated that *A. howellii* Greene (≡ *A. neodioica* Greene), a polyploid agamic complex consisting of as many as four sexual parents, *A. neglecta* Greene, *A. plantaginifolia* (L.) Richardson, *A. racemosa* and *A. virginica* Stebbins, displayed higher levels of heterozygosity compared to the *A. friesiana* species which consists of a mixture of diploids, autopolyploids, and allopolyploids.

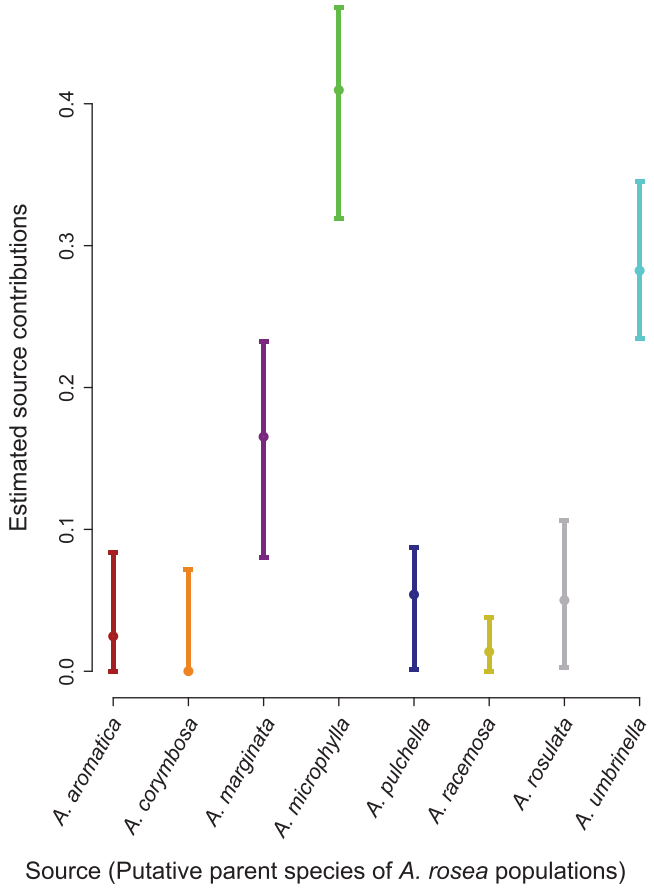

Fig. 4. Unconditional maximum likelihood (UML) estimation of source contributions of eight putative parent species to all 18 *Antennaria rosea* populations taken together. Source contribution estimates for each *A. rosea* population are shown in supplementary Fig. 4. Error bars indicate 95% confidence intervals as determined by 1000 bootstrap replicates.

As studies have suggested that *A. rosea* is a polyploid agamic complex consisting of as many as eight sexual putative parents (see above), higher levels of heterozygosity at many loci could be expected. The results of this study indicate that populations of *A. rosea* harbor approximately 62% of the allelic diversity (SSR loci converted into the binary format as dominant data) present in all the eight sexual putative parents combined. The minimum, maximum and the mean expected heterozygosity values were 0.024, 0.101, and 0.064, respectively (Table 2). Earlier, genetic diversity among 33 *A. rosea* populations estimated based on allozyme variation from 19 loci (Bayer, 1989a) indicated a mean observed heterozygosity of 0.229. However, the choice of genetic marker influences the variation described for each locus so that measures of genotypic diversity are usually highly dependent on the type of marker used (Lushai & al., 2003). Also, the degree of genetic diversity is directly proportional to the number of loci in the marker system (Hörandl & al., 2000). Given this, absolute values of diversity and differentiation calculated on data from dominant and co-dominant markers are generally not comparable (Gaudeul & al., 2004). We scored the microsatellite markers as independent dominant loci, such that two individuals carrying a band of the same size, or the two individuals lacking the band of same size would be considered to carry the same allele at the locus. Due to this methodological approach, estimated H_e is not considered a true measure of heterozygosity and thus comparable to different studies; however, the values are useful for comparing genetic diversity among the populations studied here. The mean H_e for the populations of eight putative parent species was 0.055, whereas for the *A. rosea* populations, the value was slightly higher, 0.064. However, a Wilcoxon Rank Sum Test performed ($W = 56.5$, p-value = 0.4043) between the two datasets failed to reject the null hypothesis supporting the notion that H_e does not differ between *A. rosea* populations and the populations of putative parents of *A. rosea*.

The comparison of the genetic diversity among *A. rosea* populations in this study indicated that genetic variability was relatively higher in Northern Rocky Mountain populations (8 out of 10 populations with genetic diversity >0.06) when compared to the Southern Rocky Mountain populations (3 out of 8 populations with genetic diversity >0.06) (Table 2). The two Southern Rocky Mountain populations, R12 and R11, which were in sympatry with the least number of putative sexual parents, four for each population, were among the populations having low levels of genetic diversity (Table 2). Earlier, in a similar study, Bayer (1990c) in western Montana found that *A. rosea* populations in sympatry with fewer putative sexual progenitors, or at greater distances from the center of the sexual diversity, produced fewer apomicts compared to the populations in sympatry with more sexual species, or near the center of sexual diversity. Similar results were also obtained by Hörandl & al. (2001) in their study of *Ranunculus*

auricomus L. in which the mean genotypic diversity was higher in sympatric sexual-apomictic systems compared to the allopatric ones. Other apomictic species that corroborate the results include *Taraxacum* sect. *Ruderalia* Kirschner & al. and *Bohemeria spicata* (Thunb.) Thunb. (reviewed in Hörandl & Paun, 2007). These results suggest that backcrossing with sexual relatives could be one of the sources of genetic diversity in apomictic species. Here, six out of eight putative parents of *A. rosea*, viz. *A. aromatica*, *A. corymbosa*, *A. microphylla*, *A. racemosa*, *A. pulchella*, and *A. umbrinella*, having major distribution centers in the Northern Rocky Mountains may have contributed to the relatively higher genetic variability in the sympatric *A. rosea* populations. Regarding multilocus genotypic diversity, only three MLGs were shared among the 18 *A. rosea* populations studied here (suppl. Fig. S1). Note the *A. rosea* populations are geographically separated and are sympatric with different combinations of the putative parent species, a notion that supports the hypothesis of multiple independent origins of the populations.

Bayer (1990c) also found that *A. rosea* populations in the regions glaciated by continental glaciers during the Pleistocene epoch harbored less genetic diversity and had fewer clones per population compared to *A. rosea* populations from the unglaciated regions. Similarly, while studying clonal diversity of *A. rosea* populations from the subarctic Alaska and Yukon Territory, Bayer (1991a) found that clonal diversity is negatively correlated with latitude, longitude, and elevation of the sites. As putative sexual parents of *A. rosea* are not found in the subarctic Alaska and Yukon Territory, *A. rosea* populations in these regions, which might be recent migrations from unglaciated regions or glacial refugia, do not have the opportunity to backcross with sexual relatives and therefore may demonstrate lower levels of clonal diversity. In this present study, all the *A. rosea* populations were sampled from the regions sympatric with putative sexual parents of *A. rosea*. In the future, studies comparing genetic diversity between the *A. rosea* populations from previously unglaciated and glaciated regions could lend evidence to support this hypothesis.

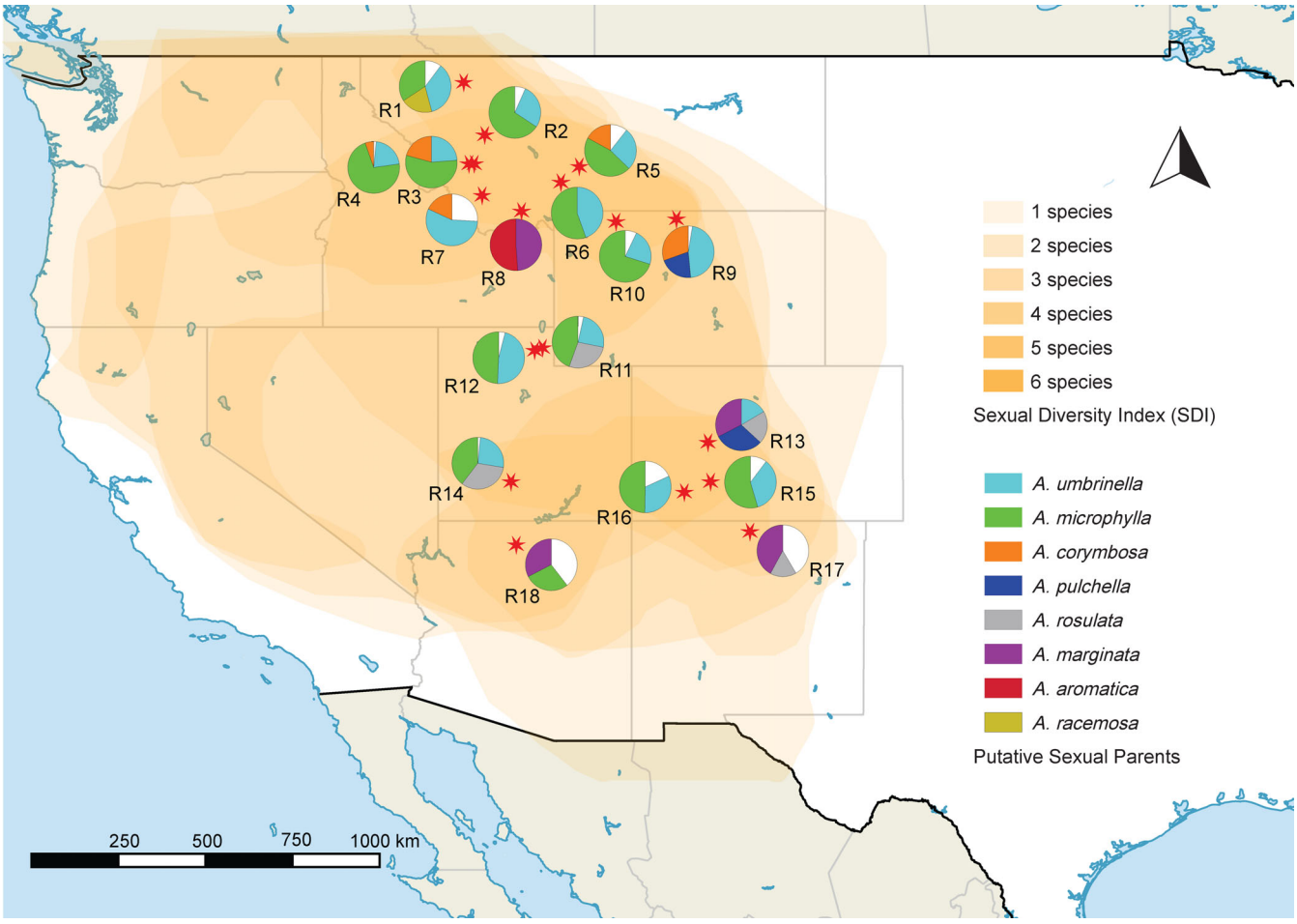

Fig. 5. Ancestry estimation in *Antennaria rosea* populations. Red stars indicate the collection sites of the 18 *A. rosea* populations considered in this study. The sexual diversity index (SDI) is indicated by shading and reflects the number of sexual progenitor species that occur in areas across the study range. Pie chart alongside each population indicates the proportion of source contribution of the putative parent species in each population from the unconditional maximum likelihood (UML) analysis. The proportion of source contribution of 5% or more from each putative parent species is only shown in each pie chart. Less than 5% contribution from each putative parent species is added in a white sector of the pie chart.

Population structure. — Genetic contribution from different sexual parents, backcrossing, and development of agamospermy all make population structure and genetic clustering difficult. The results of the STRUCTURE analysis of the *A. rosea* populations in this study supported three genetically distinct clusters. Populations in each genetic cluster showed high correlation among themselves regarding both the geographic distribution and the ancestry. For example, genetic cluster one consisted of two populations that were both from the Northern Rocky Mountains and had major contributions from *A. umbrinella* and *A. corymbosa* in the parentage (Table 2, Fig. 5). Genetic cluster two consisted of six populations, five populations distributed in the Southern Rocky Mountains and one population from the Northern Rockies, and these populations had major contributions from *A. marginata* and/or *A. rosulata*, which are only distributed in the Southern Rocky Mountains. The remaining 10 populations were grouped in genetic cluster three, of which three were distributed in the Southern Rocky Mountains and seven were from the Northern Rocky Mountains. All the populations from genetic cluster three received major contributions from *A. microphylla* and *A. umbrinella*, with *A. microphylla* as the highest contributor in the nine populations, while *A. umbrinella* being the highest contributor in one of the populations (Table 2, Fig. 5). We also carried out the DAPC analysis to analyze any possible effects of HWE and/or LD in the population structure of highly apomictic *A. rosea* populations. DAPC analysis, which relaxes the assumptions about HWE and LD, produced results in agreement with the STRUCTURE program (Fig. 3B). Although the K-means clustering method in DAPC tends to produce biased inference with markers having limited information, such as dominant markers and co-dominant markers with unknown dosage and weak population differentiation (Stift & al., 2019), no such results were seen in the analysis.

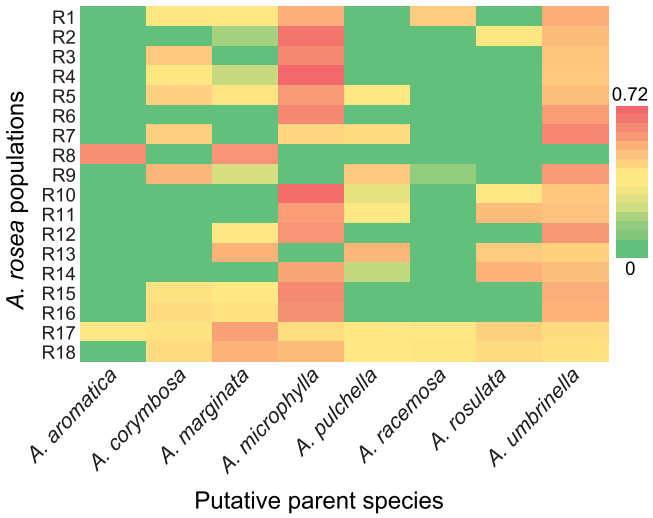

Fig. 6. Heat map showing estimated source contributions from the eight sexual putative parents to the 18 *Antennaria rosea* populations based on unconditional maximum likelihood (UML) analysis using the "mixstock" package in R. Color temperature indicates the source contributions, with warmer colors indicating higher contribution and cooler colors representing less contribution. The figure legend shows the range of contribution value and the complete data matrix for the generation of the heatmap is given in supplementary Table 1.

Ancestry estimation. — Much research has been carried out regarding the involvement of diploid/tetraploid sexual species in the origin of the polyploid agamic complex, *A. rosea*. Based on the analysis of morphological data, Bayer (1990b, 1997) found the significant involvement of six species, *A. aromatica*, *A. corymbosa*, *A. microphylla*, *A. pulchella*, *A. racemosa*, and *A. umbrinella*, in the parentage of *A. rosea* populations. However, overlapping of different morphological characters unique to putative sexual parents in different populations of *A. rosea* and the possibility of extinction of the sexual ancestors involved in the parentage made it difficult to trace their evolutionary history. The involvement of these putative sexual progenitors in the parentage of *A. rosea* populations is also corroborated by data from ecological studies and molecular markers (Bayer, 1987, 1989a; Bayer & Chandler, 2007). These studies have also suggested that facultatively apomictic clones of *A. rosea* can produce new apomicts by hybridizing with a compatible pollen donor present in close proximity. These new apomicts are identified by their suites of morphological characters resembling that of the putative sexual parents in sympatry or parapatric habitats.

Earlier studies based on morphology and molecular markers (isozymes, AFLP) were inconsistent regarding the involvement of eight sexual putative parents in the origin of *A. rosea* populations. The exact number of sexual parents and the extent of their involvement in parentage of different *A. rosea* populations in these studies differed slightly; however, a significant involvement of *A. microphylla* and *A. umbrinella* was always noted in those studies. There is much overlap in the distribution range of *A. microphylla* and *A. umbrinella*, and they also occur in similar elevations; however, the habitat for the two species differs as the former is mostly found in floodplains or moist alkaline depressions, whereas the latter is prevalent in drier sagebrush-covered hillsides (Bayer & al., 1991). Moreover, the greatest amount of habitat overlap for *A. rosea* is with *A. microphylla* and *A. umbrinella* (Bayer & al., 1991). The present study corroborates these findings as the estimated UML source contribution value for all the *A. rosea* populations taken together was the highest for *A. microphylla* (0.41), followed by *A. umbrinella* (0.28), with *A. marginata* (0.17) being the third major contributor (Fig. 4). Ten out of 18 studied *A. rosea* populations had *A. microphylla* and *A. umbrinella* as the first two major contributors (suppl. Fig. S4). Along with the genetic data presented in this study, the morphological diversity of the *A. rosea* populations supports the notion of multiple origins. Instances of multiple origins of hybrid taxa, having diploid or polyploid cytotypes, are not uncommon in nature, as has been documented in earlier studies (Levin, 2001; Schwarzbach & Rieseberg, 2002; Wallace, 2003). Results of the present study also showed that the putative parent species *A. microphylla* contributed greatly to the parentage of two *A. rosea* populations, R4 and R10 with

UML values 0.72 and 0.70, respectively. Observation of the gross morphology of the herbarium vouchered individuals from these populations, particularly R10, shows a close similarity to *A. microphylla*, indicating that they could conceivably be the autopolyploid agamospermous forms of *A. microphylla*, which, so far, is known to have only sexual diploid ($2n = 28$) cytotypes (Bayer, 2006). This finding corroborates the study by Bayer (1989a), where close morphological resemblance was detected between some of the segregates of *A. rosea* with one of their sexual putative parents. More genetic analyses, chromosome counts, and morphological studies would be useful to further substantiate this finding.

■ CONCLUSIONS

Antennaria rosea, one of the most morphologically diverse polyploid agamic complexes, has a center of diversity in the Rocky Mountains of western North America (Bayer, 1989a, b). Tremendous variation among *A. rosea* populations, including phyllary coloration, number of heads per flowering stalk, arrangement of heads in capitulescences, leaf size/shape, etc., has led to the enumeration of many microspecies leading to a taxonomic challenge (Bayer, 1987). Evidence from morphological, isozyme, ecological, and phylogenetic studies indicates that the *A. rosea* polyploid agamic complex evolved from hybridization and introgression of as many as eight diploid to tetraploid sexual *Antennaria* species followed by polyploidization event(s) and continued introgression. A comprehensive study of genetic diversity, population structure, and ancestry estimation in *A. rosea* populations would be helpful to understand the evolution of these polyploid agamic complexes. Using genetic markers developed for *Antennaria* species, genetic diversity, population structure, and ancestry estimation of 18 *A. rosea* populations distributed in the Northern and Southern Rocky Mountains of the U.S.A. were studied. Results showed the major involvement of two putative sexual parent species, *A. microphylla* and *A. umbrinella*, widely distributed and highly sympatric to *A. rosea* in their parentage. It was also demonstrated that the six other diploid/tetraploid species (viz. *A. aromatica*, *A. corymbosa*, *A. marginata*, *A. pulchella*, *A. racemosa*, and *A. rosulata*) may be involved in the ancestry of some of the clones; this is particularly convincing for *A. marginata* and *A. rosulata*. *Antennaria rosea* populations were divided into three genetically distinct clusters, with populations in each genetic cluster showing high correlation among them regarding geographic distribution and ancestry. Comparatively, *A. rosea* populations in the Northern Rocky Mountain regions had higher levels of genetic diversity than in the Southern Rocky Mountain regions. Recently developed genetic markers for *Antennaria* species utilized in this study were helpful for investigating the genetic diversity, population structure, and parentage analysis for 18 *A. rosea* populations. Sampling more *A. rosea* populations in those regions would be helpful to substantiate the findings in the present study. Also, it would be interesting to extend the present study to the *A. rosea* populations distributed in the Pleistocene glaciated regions with no putative sexual parents in sympatry, and also in the investigation of other *Antennaria* polyploid complexes such as *A. parvifolia* Nutt, *A. howellii* Greene, *A. parlinii* Fernald, and *A. alpina* (L) Gaertn.

■ AUTHOR CONTRIBUTIONS

The experimental design was conceived by RT, RJB & JRM. The field work and experimental work was carried out by RT & RJB. RT, RJB & JRM performed the data analysis. RT, RJB & JRM were involved in the writing of the manuscript. RT compiled all the figures. An earlier version of this article is part of the Ph.D. thesis of RT. — RT, https://orcid.org/0000-0002-4730-1076; RJB, https://orcid.org/0000-0002-7827-5886; JRM, https://orcid.org/0000-0003-3539-2991

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Appendix 1. Voucher details of taxa/population sampled.

Taxon name + taxonomic authority (population name), collection place (country/state/county/place of collection), latitude in decimal degrees, longitude in decimal degrees, elevation in meters, collection date (mm/dd/yyyy), *collectors*, *collection number*.

Antennaria aromatica Evert (aro_7), U.S.A./Montana/Carbon Co./Custer National Forest, 45.027, −109.407, 2930, 07/20/2004, *R.J. Bayer, G. Chandler & B. Robertson, MT-04001* (MEM); ***Antennaria aromatica*** Evert (aro_14), U.S.A./Montana/Gallatin Co./Gallatin National Forest, 45.903, −110.967, 2495, 07/22/2004, *R.J. Bayer, G. Chandler & B. Robertson, MT-04005* (MEM); ***Antennaria aromatica*** Evert (aro_19), U.S.A./Montana/Cascade Co./Lewis and Clark National Forest, 46.834, −110.684, 2410, 07/23/2004, *R.J. Bayer, G. Chandler & B. Robertson, MT-04012* (MEM); ***Antennaria aromatica*** Evert (aro_22), U.S.A./Montana/Teton Co./Lewis and Clark National Forest, 47.914, −112.698, 2045, 07/25/2004, *R.J. Bayer, G. Chandler & B. Robertson, MT-04017* (MEM); ***Antennaria corymbosa*** E.E.Nelson (cory_3), U.S.A./Wyoming/Sheridan Co./Big Horn National Forest, 44.43, −107.756, 2810, 07/17/2004, *R.J. Bayer, G. Chandler & B. Robertson, WY-04001* (MEM); ***Antennaria corymbosa*** E.E.Nelson (cory_30), U.S.A./Montana/Granite Co./Deerlodge National Forest, 46.224, −113.249, 2020, 07/28/2004, *R.J. Bayer, G. Chandler & B. Robertson, MT-04028* (MEM); ***Antennaria corymbosa*** E.E.Nelson (cory_43), U.S.A./Colorado/Gunnison Co./Gunnison National Forest, 39.024, −107.053, 3182, 07/03/2007, *R.J. Bayer, G. Chandler & B. Robertson, CO-07001* (MEM); ***Antennaria corymbosa*** E.E.Nelson (cory_45), U.S.A./Colorado/Gunnison Co./Gunnison National Forest, 38.834, −106.418, 3534, 07/05/2004, *R.J. Bayer, G. Chandler & B. Robertson, CO-07003* (MEM); ***Antennaria corymbosa*** E.E.Nelson (cory_50), U.S.A./Colorado/Gunnison Co./Gunnison National Forest, 38.833, −107.100, 3048, 07/06/2004, *R.J. Bayer, G. Chandler & B. Robertson, CO-07008* (MEM); ***Antennaria marginata*** Greene (mar_59), U.S.A./New Mexico/Sante Fe Co./Sante Fe National Forest, 36.88, −107.002, 2131, 07/04/2017, *R.J. Bayer, R. Thapa, N.P. Prather & S.M. Ballou*, *NM-17004* (MEM); ***Antennaria marginata*** Greene (mar_60), U.S.A./New Mexico/Taos Co./Carson National Forest, 36.378, −105.477, 2477, 07/05/2017, *R.J. Bayer, R. Thapa, N.P. Prather & S.M. Ballou*, *NM-17007B* (MEM); ***Antennaria marginata*** Greene (mar_69), U.S.A./Arizona/Mohave Co./Hualapai Mountain, 35.098, −113.886, 1989, 07/08/2017, *R.J. Bayer, R. Thapa, N.P. Prather & S.M. Ballou*, *AZ-17001* (MEM); ***Antennaria marginata*** Greene (mar_71), U.S.A./Arizona/Coconino Co./Kaibab National Forest, 36.398, −112.041, 2666, 07/09/2017, *R.J. Bayer, R. Thapa, N.P. Prather & S.M. Ballou*, *AZ-17004* (MEM); ***Antennaria marginata*** Greene (mar_76), U.S.A./Arizona/Coconino Co./Coconino National Forest, 34.986, −111.460, 2287, 07/11/2017, *R.J. Bayer, R. Thapa, N.P. Prather & S.M. Ballou*, *AZ-17011* (MEM); ***Antennaria microphylla*** Rydb. (mic_46), U.S.A./Colorado/Gunnison Co./Gunnison National Forest, 38.834, −106.418, 3534, 07/05/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *CO-07004* (MEM); ***Antennaria microphylla*** Rydb. (mic_53), U.S.A./Colorado/Saguache Co./Gunnison National Forest, 38, −106.000, 3292, 07/08/2007, *R.J. Bayer, G. Chandler & B. Robertson*, *CO-07011* (MEM); ***Antennaria microphylla*** Rydb. (mic_65), U.S.A./Colorado/Conejos Co./Rio Grande National Forest, 37.15, −106.416, 2687, 07/06/2017, *R.J. Bayer, R. Thapa, N.P. Prather & S.M. Ballou*, *CO-17002* (MEM); ***Antennaria microphylla*** Rydb. (mic_66), U.S.A./Colorado/Saguache Co./Gunnison National Forest, 38.221, −106.607, 2879, 07/07/2017, *R.J. Bayer, R. Thapa, N.P. Prather & S.M. Ballou*, *CO-17004* (MEM); ***Antennaria pulchella*** Greene (pul_78), U.S.A./California/Inyo Co./Inyo National Forest, 36.483, −118.217, 3231, 07/12/1987, *R.J. Bayer, R. Deluca & D. Lebedyk, CA-700* (MEM); ***Antennaria pulchella*** Greene (pul_79), U.S.A./California/Inyo Co./Inyo National Forest, 36.75, −118.367, 3225, 07/13/1987, *R.J. Bayer, R. Deluca & D. Lebedyk, CA-707* (MEM); ***Antennaria pulchella*** Greene (pul_80), U.S.A./California/Mono Co./Inyo National Forest, 37.95, −119.300, 3078, 07/14/1987, *R.J. Bayer, R. Deluca & D. Lebedyk, CA-720* (MEM); ***Antennaria pulchella*** Greene (pul_81), U.S.A./California/Inyo Co./Inyo National Forest, 37.183, −118.633, 3170, 07/16/1987, *R.J. Bayer, R. Deluca & D. Lebedyk, CA-724* (MEM); ***Antennaria racemosa*** Hook. (race_15), U.S.A./Montana/Gallatin Co./Bridger Mountains, 45.892, −110.894, 1881, 07/22/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *MT-04006* (MEM); ***Antennaria racemosa*** Hook. (race_17), U.S.A./Montana/Park Co./Gallatin National Forest, 46.162, −110.438, 1938, 07/23/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *MT-04010* (MEM); ***Antennaria racemosa*** Hook. (race_21), U.S.A./Montana/Flathead Co./Flathead National Forest, 48.219, −113.329, 1554, 07/24/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *MT-04015* (MEM); ***Antennaria racemosa*** Hook. (race_25), U.S.A./Montana/Deerlodge Co./Deerlodge National Forest, 46.239, −112.586, 2095, 07/26/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *MT-04022* (MEM); ***Antennaria racemosa*** Hook. (race_39), U.S.A./Montana/Beaverhead Co./Beaverhead National Forest, 45.407, −112.897, 2391, 07/30/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *MT-04037* (MEM); ***Antennaria rosea*** Greene (R1), U.S.A./Montana/Flathead Co./Flathead National Forest, 48.290, −113.379, 1587, 07/23/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *MT-04014* (MEM); ***Antennaria rosea*** Greene (R2), U.S.A./Montana/Powell Co./Helena National Forest, 46.943, −112.833, 1346, 07/26/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *MT-04020* (MEM); ***Antennaria rosea*** Greene (R3), U.S.A./Montana/Granite Co./Deerlodge National Forest, 46.224, −113.249, 2020, 07/28/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *MT-04029* (MEM); ***Antennaria rosea*** Greene (R4), U.S.A./Montana/Deerlodge Co./Deerlodge National Forest, 46.209, −113.252, 1979, 07/28/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *MT-04031 (MEM)*; ***Antennaria rosea*** Greene (R5), U.S.A./Montana/Park Co./Shields River Rd., 46.165, −110.426, 1968, 07/23/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *MT-04011* (MEM); ***Antennaria rosea*** Greene (R6), U.S.A./Montana/Gallatin Co./Gallatin National Forest, 45.764, −110.854, 1740, 07/22/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *MT-04008* (MEM); ***Antennaria rosea*** Greene (R7), U.S.A./Montana/Beaverhead Co./Beaverhead National Forest, 45.384, −112.912, 2958, 07/30/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *MT-04035* (MEM); ***Antennaria rosea*** Greene (R8), U.S.A./Montana/Madison Co./Beaverhead National Forest, 44.985, −111.862, 2958, 07/27/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *MT-04026* (MEM); ***Antennaria rosea*** Greene (R9), U.S.A./Wyoming/Bighorn Co./US highway 14A, 44.805, −107.893, 2958, 07/19/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *WY-04003* (MEM); ***Antennaria rosea*** Greene (R10), U.S.A./Wyoming/Park Co./Shoshone National Forest, 44.763, −109.428, 1903, 07/20/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *WY-04012* (MEM); ***Antennaria rosea*** Greene (R11), U.S.A./Utah/Rich Co./Wasatch National Forest, 41.48, −111.370, 2958, 07/17/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *UT-04002* (MEM); ***Antennaria rosea*** Greene (R12), U.S.A./Utah/Weber Co./Wasatch National Forest, 41.41, −111.523, 2634, 07/17/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *UT-04001* (MEM); ***Antennaria rosea*** Greene (R13), U.S.A./Colorado/Gunnison Co./Gunnison National Forest, 39.024, −107.053, 2958, 07/03/2007, *R.J. Bayer, G. Chandler, C. Blanchfield & B. Robertson*, *CO-07002* (MEM); ***Antennaria rosea*** Greene (R14), U.S.A./Utah/Garfield Co./Dixie National Forest, 38.045, −112.187, 2958, 07/10/2017, *R.J. Bayer, R. Thapa, N.P. Prather & S.M. Ballou*, *UT-17001* (MEM); ***Antennaria rosea*** Greene (R15), U.S.A./Colorado/Hinsdale Co./Gunnison National Forest, 38, −107.000, 3292, 07/08/2007, *R.J. Bayer, G. Chandler, C. Blanchfield & B. Robertson*, *CO-07010* (MEM); ***Antennaria rosea*** Greene (R16), U.S.A./Colorado/San Juan Co./San Juan National Forest, 37.734, −107.715, 3213, 07/07/2017, *R.J. Bayer, R. Thapa, N.P. Prather & S.M. Ballou*, *CO-17007* (MEM); ***Antennaria rosea*** Greene (R17), U.S.A./New Mexico/Rio Arriba Co./Carson National Forest, 36.705, −106.001, 2958, 07/04/2017, *R.J. Bayer, R. Thapa, N.P. Prather & S.M. Ballou*, *NM-17003* (MEM); ***Antennaria rosea*** Greene (R18), U.S.A./Arizona/Coconino Co./Kaibab National Forest, 36.398, −112.041, 2666, 07/09/2017, *R.J. Bayer, R. Thapa, N.P. Prather & S.M. Ballou*, *AZ-17009* (MEM); ***Antennaria rosulata*** Rydb. (roz_58), U.S.A./New Mexico/Arriba Co./Carson National Forest, 36.655, −106.042, 2632, 07/04/2017, *R.J. Bayer, R. Thapa, N.P. Prather & S.M. Ballou*, *NM-17002* (MEM); ***Antennaria rosulata*** Rydb. (roz_63), U.S.A./Colorado/Conejos Co./Rio Grande National Forest, 37.15, −106.416, 2687, 07/06/2017, *R.J. Bayer, R. Thapa, N.P. Prather & S.M. Ballou*, *CO-17001* (MEM); ***Antennaria rosulata*** Rydb. (roz_67), U.S.A./Colorado/Saguache Co./Gunnison National Forest, 38.221, −106.607, 2879, 07/06/2017, *R.J. Bayer, R. Thapa, N.P. Prather & S.M. Ballou*, *CO-17005* (MEM); ***Antennaria rosulata*** Rydb. (roz_74), U.S.A./Utah/Garfield Co./Dixie National Forest, 38.039, −112.197, 3065, 07/10/2017, *R.J. Bayer, R. Thapa, N.P. Prather & S.M. Ballou*, *UT-17002* (MEM); ***Antennaria rosulata*** Rydb. (roz_75), U.S.A./Arizona/Coconino Co./Coconino National Forest, 34.986, −111.460, 2174, 07/11/2017, *R.J. Bayer, R. Thapa, N.P. Prather & S.M. Ballou*, *AZ-17010* (MEM); ***Antennaria umbrinella*** Rydb. (umb_6), U.S.A./Wyoming/Bighorn Co./US highway 14A, 44.805, −107.893, 2710, 07/19/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *WY-04004* (MEM); ***Antennaria umbrinella*** Rydb. (umb_12), U.S.A./Montana/Gallatin Co./Gallatin National Forest, 45.093, −111.208, 1986, 07/21/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *MT-04003* (MEM); ***Antennaria umbrinella*** Rydb. (umb_23), U.S.A./Montana/Teton Co./Lewis and Clark National Forest, 47.914, −112.698, 2045, 07/25/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *MT-04018* (MEM); ***Antennaria umbrinella*** Rydb. (umb_29), U.S.A./Montana/Madison Co./Beaverhead National Forest, 45.075, −111.868, 2570, 07/27/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *MT-04027* (MEM); ***Antennaria umbrinella*** Rydb. (umb_36), U.S.A./Montana/Madison Co./Beaverhead National Forest, 45.229, −112.912, 1950, 07/30/2004, *R.J. Bayer, G. Chandler & B. Robertson*, *MT-04034* (MEM).