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Phylogenomics Resolves the Relationships within *Antennaria* (Asteraceae, Gnaphalieae) and Yields New Insights into its Morphological Character Evolution and Biogeography

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Abstract—*Antennaria* are dioecious perennial herbs distributed mainly in the Holarctic Region, with their major center of diversity in the Rocky Mountains of Western North America. The genus comprises 33 known sexual diploid/tetraploid species and at least five polyploid agamic complexes which mostly reproduce by forming asexual seeds. We performed a phylogenetic reconstruction of the 31 sexually-reproducing *Antennaria* species using a novel target enrichment method that employs custom capture probes designed to work across Asteraceae. Both concatenated and coalescent-based analyses of DNA sequence data from hundreds of nuclear loci recovered *Antennaria* as a monophyletic group except for the long-disputed species, *Antennaria linearifolia*, which was recovered outside of the genus. *Antennaria* was further resolved into three distinct, major lineages. Analysis of ancestral state reconstruction of 12 taxonomically important morphological characters elucidated patterns of character evolution throughout the genus. Estimations of ancestral geographic ranges and molecular dating analyses demonstrated the Rocky Mountain region, including the Vancouverian Province, as the center of origin for the genus *Antennaria,* around 5.8 MYA. Subsequent dispersals of *Antennaria* into the Arctic and Appalachian provinces, Canadian provinces, and Eurasia took place roughly 3.2 MYA, 2.4 MYA, and 1.6 MYA, respectively. Biogeographical stochastic mapping indicated that 51.4% of biogeographical events were based on within-area speciation. The remaining 48.6% of the events were divided into two types of dispersals: 1) range expansion dispersals (anagenic, 37%), and 2) founder/jump dispersals (cladogenic, 11.6%). Our results provide a framework for future evolutionary studies of *Antennaria,* including speciation, origin(s) of polyploidy, and agamospermy in the genus.

Keywords—Apomixis, dioecy, Hyb-Seq, phylogeny, polyploidy.

Antennaria Gaertn. are dioecious (gynoecious) perennial, cespitose or stoloniferous herbs, with cuneate, elliptical, lanceolate or oblanceolate to linear basal leaves and generally linear cauline leaves. The flowering heads are discoid and are sexually dimorphic. This dimorphism is seen in the papery phyllaries, wide in the staminate plant, narrow in the pistillate; shape of the corolla, wide in the staminate and narrowly tubular in the pistillate. The pappus bristles of the staminate flowers are clavate, while those of the pistillate are barbellate. The achenes are ovoid with twin-hairs. The genus has mostly Holarctic distribution, except for three Andean South American species (*A. linearifolia* Wedd., *A. chilensis* J. Remy, and *A. sleumeri* Cabrera) and has a major center of diversity in the Rocky Mountains of North America. The dioecious nature, extensive hybridization, polyploidization, and the development of gametophytic apomixis, all have contributed to taxonomic complexity in the genus (Bayer and Stebbins 1987).

Gaertner (1791) first separated *Antennaria* as a genus unique from *Gnaphalium* L. based on its dioecious breeding system and unusual clavate pappus bristles of the staminate flowers. The genus consists of 33 known sexual species, including the recently discovered *A. sawyeri* R.J. Bayer and Figura (Bayer and Figura 2015) and the doubtfully-placed South American species, *A. linearifolia.* This South American species differs in morphology from the rest of the genus; having linear leaves and globose achenial trichomes (Bayer and Dillon 2019). The sexual *Antennaria* species are strictly diploid ($2n = 28$), except for six species which also have known tetraploid cytotypes; however, *A. stenophylla* (A. Gray) A. Gray has only tetraploids; diploids have not yet been reported (Table S1). Eighteen of these sexual species may have contributed to the formation of the polyploid agamic complexes by extensive hybridization and introgression (Bayer 2006; Bayer and Chandler 2007). These polyploid agamic complexes, which include sexual, facultative, or obligate agamospermous members, preserve the hybrid combinations by gametophytic apomixis (Bayer 1985a, 1990). Gametophytic apomixis produces many distinct polyploid races or microspecies, which were noted by botanists beginning in the late 1890s as separate species of *Antennaria*, causing taxonomic conundrums (Bayer and Stebbins 1982; Bayer 1987). The practice of giving species status to microspecies has resulted in the description of about 400 species of *Antennaria,* making a workable taxonomy difficult to attain (Bayer 1987).

Attempts have been made to explain the evolutionary history of some of the polyploid agamic complexes including *A. howellii* (= *A. neodioica* Greene; Bayer 1985a), *A. parlinii* (Bayer 1985b), *A. rosea* (Bayer 1989a, 1989b; Bayer and Chandler 2007) and others (Bayer 2006). However, less is known about the evolutionary relationships among the amphimictic, diploid/tetraploid species, some of which demonstrably contributed to the formation of agamic complexes. A robust phylogeny of these sexual species is necessary to study character evolution and biogeography in *Antennaria*, thus providing a framework for future evolutionary studies, including such topics as species diversification, evolution of polyploidy, and the origin(s) of agamospermy in the genus. For example, previous work has hypothesized that specific morphological traits were important in the evolutionary success of the "Catipes" group including their colonization and establishment into diverse habitats (Bayer et al. 1996).

The first phylogenetic hypothesis for the sexually reproducing species of *Antennaria* was based on a cladistic analysis of morphological character data (Bayer 1990); however, due to the shortage of suitable cladistically-informative characters, the topologies were not well resolved. Similarly, a phylogenetic tree (Bayer et al. 1996) inferred using DNA sequences from the nuclear ribosomal internal transcribed spacer (ITS) failed to recover a well-resolved tree due to the lack of adequate numbers of parsimony informative sites in the alignment. Those previous studies did, however, divide the genus *Antennaria* into two distinct clades, "Leontipes" and "Catipes," with "Leontipes" further divided into four and five distinct groups in the morphology and the ITS-based studies,

respectively. Also, direct comparison between those phylogenies could not be made as the trees were not well resolved and they also did not have the same sampling. Thus, studies aimed at understanding character evolution and biogeography, including dispersal and the major migrations of *Antennaria*, are hindered by a lack of resolution in the phylogeny.

Advances in next generation sequencing technologies coupled with novel computational analyses have facilitated the use of whole genomes or multi-locus sequence in phylogenetic studies. These advancements have helped evolutionary biologists to better resolve the history of species, providing improved understanding of lineage divergence while also reflecting time and speciation events (Liu et al. 2015). Targeted enrichment sequencing method, which captures loci of interest via in-solution hybridization, has been successfully used in many recent phylogenetic studies (Smith et al. 2013; Stull et al. 2013; Weitemier et al. 2014; Mandel et al. 2015; Zimmer and Wen 2015). The technique can be efficiently performed with degraded DNA from herbarium specimens (Enk et al. 2014), takes advantage of high specificity of the oligonucleotide probes (Cronn et al. 2012), and is cost efficient due to the multiplexing of indexed DNA libraries during sequence capture (Lemmon et al. 2012). The ability to assay efficiently hundreds of nuclear loci, as well as the chloroplast genome from the off-target reads, makes target enrichment sequencing a highly applicable method in the field of phylogenomics in many plant groups.

Here, we carried out phylogenomic analyses for sexual *Antennaria* (Asteraceae) species using targeted enrichment sequence data from both the conserved set of nuclear loci (Mandel et al. 2014) and off-target chloroplast reads (Mandel et al. 2015). By concentrating on the amphimictic, presumably non-hybrid species, we aimed to minimize some of the complications in phylogenetic reconstruction due to hybridization, agamospermy, and polyploidy. However, in future studies, we would like to include additional taxa, hybrid species as well as taxa from the polyploid agamic complexes, in order to understand the roles of hybridization and polyploidy in the evolution of *Antennaria.* A well-resolved phylogeny for the diploid/tetraploid sexual *Antennaria* species would be invaluable in the study of the origin of polyploid agamic complexes. The present study had the following objectives: 1) to resolve the phylogenetic relationship among 31 diploid/tetraploid sexual *Antennaria* species using nuclear and chloroplast genomic DNA sequence data, 2) to determine whether the doubtful *Antennaria* species, *A. linearifolia*, is a member of the ingroup *Antennaria* and to investigate the phylogenetic position of a newly discovered species, *A. sawyeri*, 3) to investigate the evolution of diagnostically important morphological characters in light of phylogenetic relationships among *Antennaria* species, and 4) to infer the biogeographical history of *Antennaria* species via model-testing and a dated phylogeny.

Materials and Methods

DNA Isolation and Library Preparation—Total DNA from 31 diploid/tetraploid sexual *Antennaria* species with one subspecies each for *A. pulcherrima*, *A. luzuloides*, and *A. friesiana* were considered for the study. This taxa sampling represented all known amphimictic species in the genus, except for *A. nordhagiana* Rune & Rønning and *A. sleumeri*. Similarly, four outgroups, *Mniodes argentea* (Wedd.) M.O. Dillon, *Gamochaeta alpina* (Poepp.) S.E. Freire & Anderb., *Facelis lasiocarpa* (Griseb.) Cabrera, and *Gamochaeta* Wedd. *sp.* (Appendix 1; Table S1), were also sampled in this study. The outgroups were selected based on Luebert et al. (2017) and we expected the taxa being close to *Antennaria* would help to check the monophyly of the genus. Also, in the phylogenetic tree by Nie et al. (2016), two of our outgroup taxa are placed in the clade sister to *Antennaria* species and from South America, excluding *Diaperia* Nutt., which is distributed in Northern Mexico and central and southern USA. Genomic DNA extracted from fresh leaves, dried leaves in silica gel, or from herbarium specimens using an Omega SQ DNA extraction kit (Omega Bio-tek, Norcross, Georgia) was considered for library preparation. Low quality DNA samples measured by NanoDrop spectrophotometer (Thermo Scientific, Waltham, Massachusetts) were cleaned with a QIAquick kit (QIAGEN, Valencia, California) and were quantified using the Qubit BR-assay (Life technologies, Carlsbad, California). Approximately 1μg of DNA was prepared in 60 μl of elution buffer/water by either diluting or concentrating via SAVANT Speed-Vac concentrator (SAVANT instruments, Farmingdale, New York) based on the original concentration. The sample was then sonicated in a Covaris machine (model S220; Covaris, Woburn, Massachusetts) with a program to generate fragment sizes of 400–500 bp. Using 55.5 μl of sonicated DNA, a genomic library for each sample was prepared using an NEBNext Ultra or NEBNext Ultra II library preparation kit (New England Biolabs, Ipswich, Massachusetts), following the manufacturer's protocol (Table S1), and selecting DNA in the range of 400–500 bp and using eight cycles of PCR amplification on the size-selected fragments. The barcoded libraries (NEBNext multiplex oligos) were quality checked with an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, California) and quantified using the Qubit HS assay.

Hybridization Based Target Enrichment—Libraries were used for target sequence capture using the MyBaits COS Compositae/Asteraceae 1Kv1 kit (Arbor Biosciences, Ann Arbor, Michigan) following manufacturer's protocol (v. 2.3.1) with the following modifications: nearly 500 ng of DNA in 6 μl of library was used in each MyBaits reaction when the capture was done individually. In the cases when four libraries were pooled, about 125 ng of DNA per sample for a total of 500 ng of DNA in 6μl was used for hybridizing with the baits for 36 hrs. For post-capture PCR amplification, the following conditions were set: 10 μl of DNA template from recovered capture, annealing temperature of 60°C, an elongation time of 45 sec, and 15 cycles of amplification. Target-enriched libraries were quantified using a KAPA library quantification kit (Kapa Biosystems, Boston, Massachusetts) and pooled for sequencing. Twenty-four samples were sequenced on an Illumina MiSeq sequencer (paired end, 150 bp) at the W. Harry Feinstone Center for Genomic Research, University of Memphis, Memphis, Tennessee, USA. The MiSeq controller software was set to trim adapters and deconvolute barcodes. Similarly, 10 samples were sequenced on an Illumina MiSeq sequencer (paired end, 150 bp) at Georgia Genomics and Bioinformatics Core at The University of Georgia, Athens, Georgia, and remaining four samples were sequenced on a Hiseq 2500 sequencer (paired end, 100 bp) at Macrogen, Korea (Table S1). The raw DNA sequence reads have been deposited in the Sequence Read Archive (SRA) at NCBI under BioProject accession number PRJNA5144045.

COS Loci Sequence Assembly—The demultiplexed sequence data were assessed for quality using FastQC v. 0.10.1 (Andrews et al. 2011) and processed following bioinformatic and phylogenetic workflows as described in Mandel et al. (2014). In brief, using PRINSEQ (v. 0.18.2; Schmieder and Edwards 2011), paired-end reads were quality trimmed removing short and low quality reads, and reformatted from FASTQ to FASTA format. The paired-end reads were then paired with script Pairfq (https://github.com/sestation/Pairfq) and shuffled using the program shuffleSequences_fasta.pl from the Velvet de novo sequence assembler package (Zerbino and Birney 2008). The shuffleSequences_fasta.pl interleaved the paired-end reads into one file whereas the remaining singles with missing pairs were saved in a separate file for the assembly. Both the files with interleaved paired-end reads and the un-paired reads were used in the genome assembly algorithm SPAdes (Bankevich et al. 2012) with K-mer values 99, 111, 115, and 127 to produce the contigs. Putative orthologs were identified and aligned using the PHYLUCE pipeline v. 0.1.0 (Faircloth et al. 2012) containing the following programs: match_contigs_to_probes.py, get_match_counts.py, get_fastas_from_match_counts.py, and seqcap_align_2.py. The program utilizes LASTZ to align tiled baits/probes of the COS loci (each tile 120 base pairs with 60 base pairs overlap) with the contigs of each species and retaining only the contigs that match only one COS locus. Thus, when two or more contigs match a single COS locus, these contigs are discarded leaving an empty COS locus in the data matrix for that particular taxon. Also, for each species, the total number of contigs matching a single locus was counted and a heat map created. This analysis was carried out to investigate the possible source of the paralogs in these species, i.e. arising from gene or genome duplication, and to compare

the paralog numbers among the *Antennaria* species and the outgroup taxa. The PHYLUCE pipeline also discards any contigs matching multiple COS loci, hence associating the contigs that are putative orthologs in a conservative manner.

Nucleotide Alignments and Phylogenetic Analysis—The retained putative orthologs of the COS loci were aligned in the PHYLUCE pipeline using MAFFT v. 7.029b aligner (Katoh et al. 2002) and the obtained orthologs were concatenated in the Geneious software v. R7.1.9 (Kearse et al. 2012). The final supermatrix of 756 concatenated genes was used to determine the best-fit model of nucleotide substitution in JModelTest 2.1.1 (Darriba et al. 2012) using the Akaike information criterion (AIC). The best fitting model, GTRGAMMAI, was used in phylogenetic inferences in both the maximum likelihood (ML) and Bayesian analyses. The ML phylogeny was executed in RAxML 8.1.3 (Stamatakis 2006) with 1000 bootstrap replicates, whereas Bayesian analyses were implemented in MrBayes Geneious plugin v. 3.2.6 (Huelsenbeck and Ronquist 2001) with the default Priors (brlenspr=unconstrained:gammadir(1.0,0.1,1.0,1.0)shapepr=exponential(10.0)). The Bayesian analysis was carried out with the following conditions in two simultaneous runs: 5×10^6 generations for MCMC analysis, sample MCMC analysis every 200 generations, and using 25% of the generations as burn-in. Convergence in the Bayesian analysis was assessed based on standard deviation of split frequencies value below 0.01. The supermatrix consisting of 756 concatenated genes had missing data, as many of the genes did not have representation of all taxa. To investigate impacts of missing data on phylogeny reconstruction, a smaller matrix consisting of 280 genes concatenated genes (each gene having nine or more taxa) was used in a separate ML analysis in RAxML 8.1.3 using the GTRGAMMAI model and 1000 bootstrap replicates.

To infer the phylogeny from multispecies coalescent (MSC) approach, Partition Finder v. 1.1.0 (Lanfear et al. 2012) in the RAxML version with rcluster search option (Lanfear et al. 2014) and the Corrected Akaike information criterion (AICc) was used to detect the best-fit nucleotide substitution models for each gene. In total, 656 gene trees (GTRGAMMAX, 407 genes; GTRGAMMAIX, 249 genes) from RAxML, each run with 500 bootstrap replicates, were used in MSC method-ASTRAL II (Mirarab and Warnow 2015), a gene-tree-based coalescent analysis, to produce a species tree with local posterior probability as support values. Unlike in the concatenation analysis which had representation of 756 genes, here we used only 656 gene trees as 100 COS loci had representation of only three taxa, and no gene trees were produced for them. Also, a comparison was made between the species tree built with gene trees inferred from the best-fit nucleotide substitution model (see above) and the species tree built with the gene trees inferred from only one nucleotide substitution model (GTRGAMMA). Similarly, as in the concatenation method, to investigate the influence of missing data in the phylogenetic inference, comparisons between species tree built with 338 gene trees with each tree/gene representing eight or more taxa (178 and 160 genes under GTRGAMMAX and GTRGAMMAIX model respectively) and species tree built with 656 gene trees (see above) was made. Here, since the smaller data matrix was produced, we considered genes with eight or more taxa compared to genes with nine or more taxa in the smaller data matrix of concatenation analysis (see above) so as to include an additional 58 gene trees in the analysis.

Off-target Chloroplast Sequence Assembly—Our capture procedure did not target chloroplast DNA; however, sequence reads from each sample were mapped to the published *Artemisia frigida* Willd. chloroplast genome (ref number NC_020607) in Geneious. Partial to nearly complete chloroplast genomes for all the *Antennaria* species and outgroups were assembled and the resulting consensus sequences were aligned to produce a data matrix. The chloroplast trees were estimated under GTRGAMMA model with 500 bootstrap replicates in RAxML and in MrBayes with the same parameters used to produce the nuclear tree.

Character Evolution—We reconstructed the ancestral states of 12 taxonomically important characters with the character states coded as follows: 1) base of a plant: herbaceous/lignescent or a woody caudex, 2) cauline leaf vs. basal leaf shape: monomorphic or dimorphic, 3) staminate plant height: about the same height as pistillate at maturity or much shorter than pistillate at maturity, 4) cauline leaf size: same size or larger than basal leaves or gradually reduced distally to less than half the length, 5) Flags (flat, scarious tips resembling the tips of phyllaries that occur at the apices of the upper cauline leaves): absent or present, 6) stolon growth form: short and erect or longer and horizontal, usually with roots at the tips, 7) purplish glands on upper stems and leaves: absent or present, 8) basal leaves: sessile or petiolate, 9) number of principal veins in basal leaves: one or many, 10) phyllary coloration: median phyllaries uniformly colored or colors distinctly zoned, multicolored, 11) capitulum shape of pistillate plant: narrow, subcylindrical or turbinate or campanulate, and 12) pappus type of staminate flower: Gnaphalioid type, barbellate to denticulate, or Antennarioid type, clavate. Morphological character state data for most of the taxa scored as distinct binary characters were obtained from an earlier publication (Bayer 1990). Outgroup taxa and *A. sawyeri* were scored from the herbarium specimens or the literature. We selected the characters either based on their significances in previous taxonomic treatments (Bayer and Stebbins 1982, 1987) or likely to be putative apomorphic character states for the distinct clades. These characters were analyzed by the parsimony reconstruction method as implemented in Mesquite 3.51 (Maddison and Maddison 2018). We used the "Trace Character History" option to reconstruct evolution of each character onto the maximum likelihood consensus tree. Characters states were treated as unordered.

Historical Biogeography—Inferences of ancestral ranges were estimated using the package BioGeoBEARS 0.2.1 (Matzke 2013) implemented in R 3.3.1 while comparing different models of ancestral-area reconstruction. The program offers a flexible likelihood-based framework to model range evolution along a dated phylogeny in the form of anagenetic or cladogenetic events among a set of discrete distribution areas. Anagenetic events include range expansion or contraction along a single branch of the phylogeny (A $<\rightarrow$ A+B, A and B representing discrete areas), whereas cladogenetic events refer to shifts that occur at the time of speciation involving sympatry (A $\rightarrow$ A, A) and jump-dispersal events (A $\rightarrow$ A, B). A dated phylogeny for *Antennaria* species was produced in software treePL v. 1.0 (Smith and O'Meara 2012) using the ML tree that had the same topology as that of the Bayesian tree. The program/algorithm implements the penalized likelihood method (Sanderson 2002), which utilizes tree branch lengths and age constraints without parametric distributions, and is efficient for large datasets. There are no fossils available for *Antennaria* to use for calibration in a molecular clock dating analysis. We therefore used three secondary calibration points/age constraints (Supplemental File S1) inferred from a larger Compositae molecular clock dating analysis (Mandel et al. 2019). Moreover, the ages for the origination of *Antennaria* species were inconsistent with the ages of the *Antennaria* nodes in the chronogram of the study by Nie et al. (2016). The treePL analysis was first primed to determine the best optimization parameters and the tree was then time-calibrated with the thorough setting. The random subsample and replicate cross validation analysis were conducted from 0.1 to 1000 to determine the best smoothing value.

We used three different models including dispersal–extinction–cladogenesis (DEC; Ree and Smith 2008), the likelihood version of DIVA (DIVALIKE; Ronquist 1997), and the BayArea likelihood version of the range evolution model (BAYAREALIKE; Landis et al. 2013) with and without inclusion of *j* parameter for controlling founder-event speciation. These models were compared using a log-likelihood ratio test, and the best-fitting model was selected based on the values of Akaike information criterion (AIC) and Akaike weights. Based on existing literature (Bayer 2006; Bayer and Stebbins 1987), all the taxa in the dated phylogenetic tree were coded as present or absent in seven Floristic provinces/regions of North America (Takhtajan 1986) including: 1) Arctic Province, 2) Canadian Province, 3) Rocky Mountain Region including Vancouverian Province, 4) Madrean Region including Great Basin Province and Californian Province, 5) North American Prairies Province, 6) Appalachian and Gulf Coastal Plain Province, and 7) Atlantic regions, and two broad geographic regions 1) South America and 2) Eurasia making a total of nine distribution areas (Table S2). In order to estimate the number and type of biogeographical events between each of the defined geographical regions, we used biogeographic stochastic mapping (BSM) under the best-fit BAYAREALIKE+*j* model (see Results) from 50 stochastic maps following Dupin et al. (2017).

Results

Raw reads and assembly information for the 38 taxa are given in Table S3 (Thapa et al. 2020). The minimum and maximum number of reads obtained were 379,052 and 20,319,222 for *A. pulchella* Greene and *A. dioica* (L.) Gaertn. respectively; on average, 3.6 million reads were generated per accession. Similarly, the minimum and the maximum number of contigs assembled from the reads were 2,281 and 76,011 for *A. carpatica* (Wahlenb.) Hook. and *A. argentea* Benth., respectively, and on average, 29,670 contigs per species were assembled from the SPAdes assembler. Altogether, 1061 genes or loci for each species were targeted; however, as the loci were discarded due to poor alignment and/or the retrieval of less

than three taxa per locus, 756 loci were retained for concatenation to produce the supermatrix. A minimum of three taxa represented 100 loci whereas a maximum of 33 taxa were retained for one locus. On average, 12 taxa were recovered for a single locus. The consensus sequence for the supermatrix was 352,045 bases long and the data matrix had 11,616 parsimony informative sites. Similarly, the lowest and the highest number of loci retained per species were 108 and 241 for species *A. carpatica* and the outgroup *Gamochaeta alpina* respectively; whereas, the average number of loci retained per species was 172. Also, copy number of each gene in all species was obtained by counting the number of contigs matching each gene/locus. Among all targeted 1061 genes, a maximum copy number of 81 was recorded for COS 346 in *A. flagellaris*. Similarly, four other higher copy numbers were 66, 60, 58, and 57 for genes COS 924, COS 1071, COS 479, and COS 419 from the species *A. linearifolia, A. racemosa* Hook., *A. suffrutescens* Greene, and *A. luzuloides* Torr., and *A. luzuloides* ssp. *aberrans* (E.E. Nelson) R.J. Bayer and Stebbins, respectively (Table S4).

The partial chloroplast genomes were assembled for all the species by mapping the reads to the 151,076 bp complete chloroplast genome of *Artemisia frigida* (reference number NC_020607) in the Geneious software. The maximum and minimum coverage ranges were 0–671 and 0–41 noted in *A. marginata* Greene, and *A. aromatica* Evert, respectively. The consensus sequence of the chloroplast matrix obtained by the alignment of the partially assembled chloroplast genomes was 195,787 nucleotides, which was reduced to 167,206 nucleotides after manually editing the matrix. In the matrix, *A. flagellaris* and *A. pulcherrima* ssp. *pulcherrima* had the maximum and minimum ungapped nucleotides of 159,204 and 108,156 bp, respectively (Table S5). The average ungapped bases for the study species was 142,285 nucleotides long, and the manually edited matrix had 2929 parsimony informative sites.

Concatenated (Supermatrix) Nuclear DNA Tree—Concatenated nuclear DNA trees produced by both ML and Bayesian analyses under nucleotide substitution model GRTGAMMAI had the same topology (Fig. 1). These analyses maximally supported the monophyly of *Antennaria* species with 100% bootstrap support values (BS) and 1 posterior probability (PP), except for the doubtful *Antennaria* species, *A. linearifolia*. The analyses also indicated strong support for most of the phylogenetic relationships in the genus. *Antennaria* species were resolved into three major monophyletic clades: 1) "Leontipes" (100% BS, 1 PP) consisting of seven species and one subspecies, 2) "Pulcherrimae" (91% BS, 1 PP) with five species and one subspecies, and 3) "Catipes" (93% BS, 1 PP) with 18 species and one subspecies. Earlier studies (Bayer 1990; Bayer et al. 1996) included the "Pulcherrimae" under the "Leontipes" group; we now redefine "Leontipes" s. s. to exclude the "Pulcherrimae." Relationships within the "Leontipes" clade were robustly supported except for the two nodes 1) resolving *A. flagellaris* sister to sister taxa *A. dimorpha* (Nutt.) Torr. and A. Gray and *A. stenophylla* (36% BS, 0.89 PP), and 2) *A. luzuloides* ssp. *aberrans* resolving as sister to a group consisting of *A. arcuata* Cronquist, *A. luzuloides* ssp. *luzuloides*, and *A. argentea* (59% BS, 0.99 PP). Similarly, our result maximally supported "Pulcherrimae" clade except for one node placing *A. lanata* (Hook.) Greene sister to sister subspecies *A. pulcherrima* (Hook.) Greene ssp. *eucosma* (Fernald and Wiegand) R.J. Bayer and *A. pulcherrima* ssp. *pulcherrima* (80% BS, 1 PP).

Relationships in the "Catipes" clade were robustly supported in the Bayesian analysis, however, moderate to weak support values were seen in the ML analysis for some of those relationships. Two out of six external nodes showed lower support values both in the ML and Bayesian analyses, resolving *A. pulchella* sister to *A. aromatica* (54% BS, 0.6 PP), and *A. rosulata* Rydb. sister to *A. marginata* (57% BS, 0.52 PP). For the internal nodes, all the relationships were well supported in the Bayesian analysis; however, in the ML analysis, six out of 12 nodes had weak bootstrap support values. Less resolved internal nodes included the placement of a "Suffrutescens" group sister to rest of the "Catipes" clade (55% BS) excluding *A. racemosa*, and resolution of two big groups within the "Catipes" clade, a "Corymbosa" group consisting of mostly western North American species and a "Neglecta" group consisting of mostly eastern North American and arctic-alpine species (67% BS). Also inferred was a node separating the "Corymbosa" group into two subclades (58% BS), and within those subclades, in one, a node splitting *A. densifolia* A.E. Porsild as a sister species to *A. chilensis* var. *magellanica* Reiche and *A. corymbosa* E.E. Nelson (67% BS), and in the other, resolution of *A. umbrinella* Rydb. as sister to the clade consisting of *A. rosulata*, *A. microphylla* Rydb., and *A. marginata* (66% BS). Similarly, in the "Neglecta" group, weak support was recovered for the resolution of a "Dioica" clade (*A.dioica, A. solitaria*, and *A. neglecta*) sister to a clade representing two other eastern North American species, *A. plantaginifolia* (L.) Hook. and *A. virginica*, and arctic species *A. friesiana* (Trautv.) Ekman ssp. *alaskana* (Malte) Hultén, *A. friesiana* ssp. *neoalaskana* (A.E. Porsild) R.J. Bayer and Stebbins, and *A. monocephala* DC. ssp. *monocephala* (33% BS).

The smaller data matrix consisting of concatenation of only 280 genes, with each gene having nine or more taxa, contained 145,650 nucleotide positions with 6,849 parsimony informative sites. Using the matrix, both ML and Bayesian analysis with GTRGAMMAI nucleotide substitution model produced a tree (Fig. S1, Thapa et al. 2020) with the same topology, except for *A. aromatica* and *A. dioica* resolving as sister taxa in the Bayesian analysis. However, relationships among taxa in the "Catipes" clade differed slightly compared to the tree produced with a bigger data matrix (Fig. 1). Three species, *A. suffrutescens, A. pulchella,* and *A. aromatica* were resolved differently; whereas there was a position change for a clade consisting of *A. umbrinella, A. rosulata, A. monocephala*, and *A. microphylla*. Different resolutions for *A. suffrutescens* and *A. pulchella* were robustly supported with BS support values of 96% and 98% respectively; however, changes in the other relationships showed soft incongruence having weak to moderate BS support values (Figs. 1, S1).

MSC-ASTRAL Tree—The ASTRAL tree (Fig. 1), in congruence with the concatenation tree, resolved *Antennaria* as a monophyletic group (1 local posterior support value, LPP) excluding *A. linearifolia*. Similarly, monophyly of all three major clades: "Leontipes," "Pulcherrimae," and "Catipes," were also maximally supported (0.94, 0.85, and 1 LPP, respectively). However, in the two analyses, there were some disagreements in the topology within those groups. Those incongruences, however, were weakly or moderately supported. For example, in the ASTRAL tree, regarding the "Leontipes" group, *A. dimorpha* was sister to sister taxa *A. flagellaris* and *A. stenophylla* (0.54 LPP), unlike *A. flagellaris* sister to a clade with *A. dimorpha* and *A. stenophylla* (36% BS, 0.89 PP) in the concatenation analysis. Also, in the same group, the ASTRAL tree placed *A. luzuloides* ssp. *aberrans* sister to *A. arcuata* (0.64 LPP), unlike in concatenated analysis where

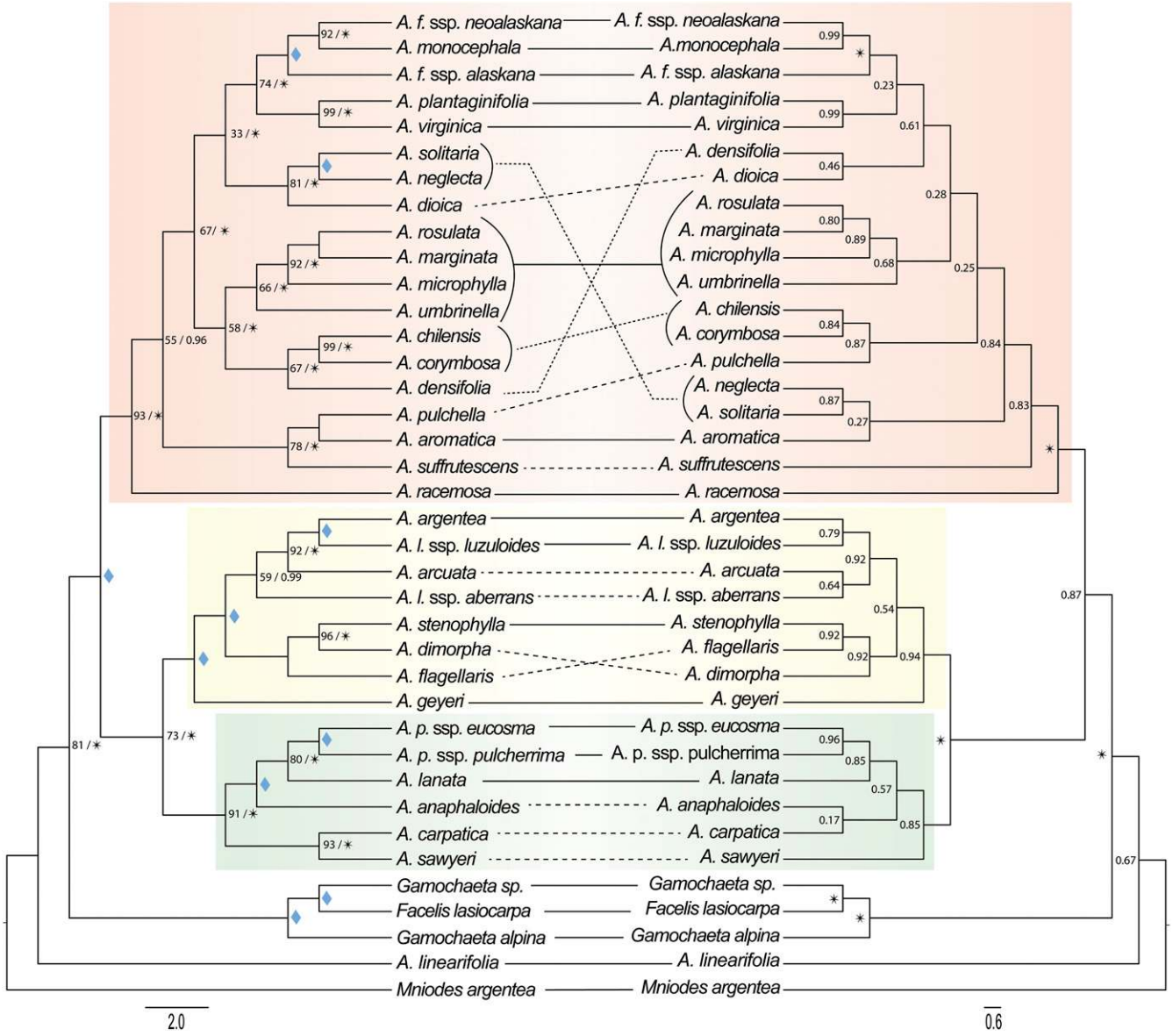


FIG. 1. Comparison of *Antennaria* phylogenies based on a concatenated analysis of nDNA sequence data (352045 bp, 756 genes) estimated using RAxML/MrBayes (same topology) with multispecies coalescence ASTRAL II inference based on 656 gene trees across 38 taxa. Nodes with a diamond indicate both bootstrap support values (BS) of 100% and the posterior probability values (PP) of 1.0. Asterisks indicate either BS of 100% or PP of 1.0. Numbers associated with nodes in the RAxML/MrBayes tree are the BS on the left obtained by RAxML, and PP on the right obtained by MrBayes, whereas numbers in the ASTRAL II tree are the local posterior probability support values. Broken lines indicate taxa position shifts within the clade.

A. luzuloides ssp. *aberrans* was sister to a group consisting of *A. arcuata* (59% BS, 0.99 PP) and the sister taxa *A. luzuloides* ssp. *luzuloides* and *A. argentea*. In addition, relationships within the "Pulcherrimae" group differed slightly between the concatenated and MSC analysis with regard to the positions of *A. carpatica*, *A. sawyeri*, and *A. anaphaloides* Rydb.

Between the MSC and concatenation analyses, different relationships were resolved for five taxa in the "Catipes" clade (Fig. 1). In the MSC analysis, *A. suffrutescens* (0.83 LPP) was sister to the rest of the species in the clade leaving *A. racemosa,* unlike in the concatenated analysis where *A. suffrutescens* along with sister taxa *A. aromatica* and *A. pulchella* formed a group sister to the rest of the clade (55% BS, 1 PP), except *A. racemosa.* Incongruence in the two types of analyses was also seen in the placement of two eastern species and sister taxa *A. neglecta* Greene and *A. solitaria* Rydb. In the concatenated analysis, these taxa were sister to *A. dioica* (81% BS, 1 PP), a Eurasian species, forming a clade in the large "Neglecta" group; whereas, in the MSC analysis, they formed a group with *A. aromatica* (0.27 LPP), a species prevalent in the northern Rocky Mountains. Similarly, incongruence was seen regarding the placement of two other species, *A. pulchella* and *A. densifolia*. In the ASTRAL tree, *A. pulchella* formed a group with *A. chilensis* var. *magellanica* and *A. corymbosa* (0.87 LPP), whereas in the concatenated analysis, *A. pulchella* was sister to *A. aromatica* (54% BS, 0.6 PP) in a group including *A. suffrutescens*. Likewise, *A. densifolia*, which was sister to *A. dioica* (0.46 LPP) in the MSC analysis formed a group with sister species, *A. chilensis* var. *magellanica* and *A. corymbosa* (67% BS, 1 PP) in the concatenated analysis.

The ASTRAL tree (Fig. S2) generated using only 338 gene trees (178 and 160 genes under GTRGAMMA and

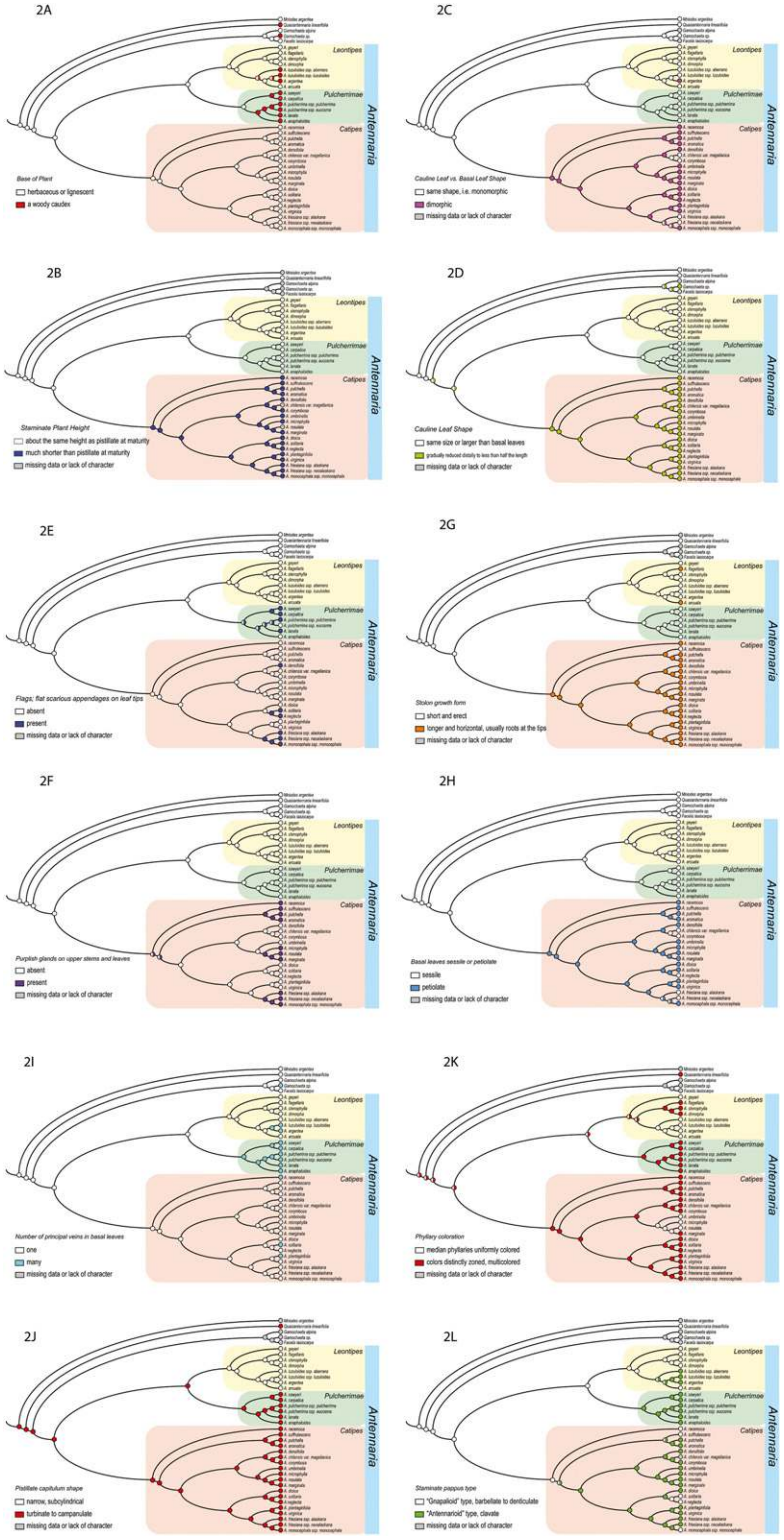


FIG. 2. Maximum parsimony ancestral state reconstruction for 12 taxonomically important characters for *Antennaria* and outgroup taxa optimized onto the RAxML /MrBayes (same topology) phylogenetic tree.

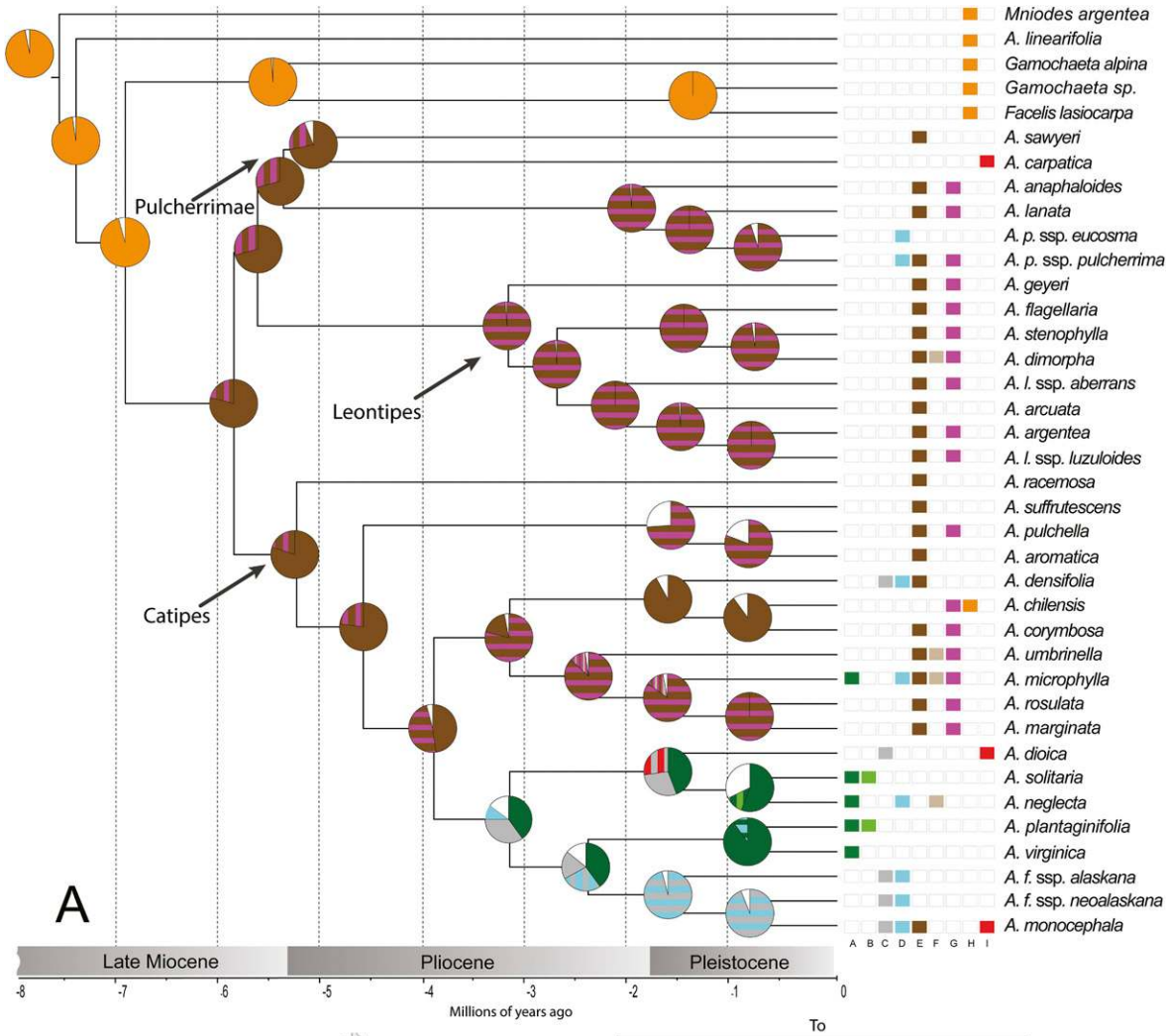
GTRGAMMAI models, respectively) with each tree/gene representing eight or more taxa showed no difference in the topology with the ASTRAL tree drawn with 656 genes (Fig. 1), except for the slightly lower LPP support values in the former tree. However, the ASTRAL tree (Fig. S3), drawn from 656 gene trees under one nucleotide substitution model (GTRGAMMA), i.e. without running the Partition Finder, showed a slightly different topology compared to the ASTRAL tree produced from the gene trees built with best-fit models of nucleotide substitution (see Methods). The former tree did not resolve the "Leontipes" and the "Catipes" clades as monophyletic, and there were some topology changes in the

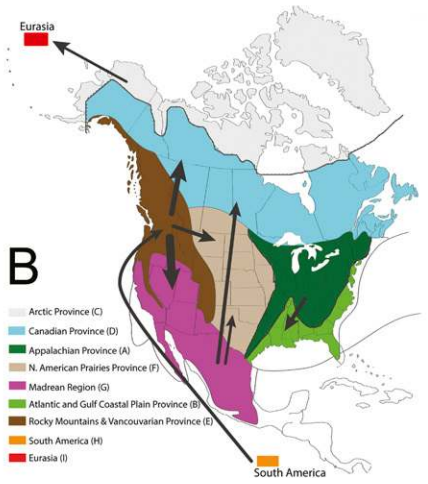
"Catipes" clade regarding the positions of *A. pulchella, A. aromatica, A. dioica, A. densifolia*, and sister taxa *A. solitaria* and *A. neglecta*.

Chloroplast Tree—The chloroplast tree (Fig. S4) was produced in RAxML with 500 bootstrap replicates and GTRGAMMA nucleotide substitution model based on JModelTest 2.2.1. The same matrix was also used in MrBayes with the same nucleotide substitution model to produce the Bayesian tree (Fig. S5). In both the analyses, *Antennaria* was monophyletic (100% BS, 1 PP) excluding the doubtful *Antennaria* species *A. linearifolia* which was grouped with the outgroup species. In the Bayesian analysis, *A. linearifolia* formed a clade with *Gamochaeta sp.* and sister species *F. lasiocarpa* and *G. alpina* (0.92 PP), but in the maximum likelihood analysis, it appeared as sister to *Gamochaeta sp.* (85% BS). In both the ML and the Bayesian chloroplast trees, the "Leontipes" clade was monophyletic; however, the relationships of the taxa within the clade differed between the two chloroplast trees, but were not robustly supported in both analyses. Also, members of the "Pulcherrimae" clade grouped with the "Catipes" clade forming a weakly supported monophyletic group (Figs. S4, S5).

Character Evolution—Our results of the morphological character mapping study distinguished ancestral and derived state for 12 taxonomically important characters showing their evolutionary trends in *Antennaria* (Fig. 2). Herbaceous or lignescent plant bases were apparently ancestral characters in *Antennaria* (Fig. 2A), and the woody caudex (the derived state) arose independently two separate times in the genus with one reversal in the "Leontipes" clade (Fig. 2A). Staminate plants that are about the same height as pistillate plants at maturity was the ancestral state, with a shift to the derived state occurring in the "Catipes" clade (Fig. 2B). Cauline leaf and basal leaves appearing monomorphic was the ancestral state, while dimorphic ones were derived in "Catipes" (with losses in three taxa) and also appeared independently in *A. argentea* ("Leontipes" group; Fig. 2C). Similarly, cauline leaves of similar size was an ancestral feature in *Antennaria*, with the derived state, cauline leaves becoming gradually reduced upward, found exclusively in all "Catipes" taxa (Fig. 2D). Lack of flags is the ancestral state in *Antennaria,* and they appeared independently in the most members of "Pulcherrimae" (Fig. 2E) and in several members of "Catipes" (viz. *A. densifolia, A. solitaria, A. neglecta, A. friesiana*, and *A. monocephala*). Like flags, purplish glands on the upper cauline stems is a derived key taxonomic feature in *Antennaria* (Fig. 2F). It is found in four groups in the "Catipes" clade. The distribution of the character is most easily explained by acquisition of the character in the ancestor of "Catipes" and subsequent loss in three groups, the "Densifolia" group, the "Dioica" group, and the "Plantaginifolia" group (Fig. 2F). The presence of long humifuse stolons that root at the tips is a derived feature in *Antennaria* (Fig. 2G), occurring in all "Catipes," except *A. suffrutescens*. The character was also apparently independently evolved in *A. flagellaris* and *A. arcuata* of the "Leontipes" clade (Fig. 2G). Sessile basal leaves are ancestral in *Antennaria* with petiolate ones being derived (Fig. 2H) in "Catipes"; however, within the clade they have seemingly been lost through reversal by *A. corymbosa, A. chilensis, A. neglecta,* and *A. friesiana* (Fig. 2H). The "Pulcherrimae" are easily recognized by the presence of many primary parallel veins in their basal leaves, which is a derived feature in *Antennaria* (Fig. 2I). The character has also arisen independently in a few taxa outside the "Pulcherrimae" group (Fig. 2I). The evolutionary directionality of the pistillate capitulum shape in *Antennaria* is from turbinate-campanulate-shaped to narrow subcylindrical heads (Fig. 2J). The turbinate-campanulate heads are found exclusively in "Catipes" and "Pulcherrimae," while "Leontipes" have narrowly subcylindrical ones (Fig. 2J). Multicolored phyllaries with distinct banded zones of colors are very common in *Antennaria* (Fig. 2K) and are of great taxonomic utility. They are present in all species of *Antennaria* except *A. geyeri*, a "Luzuloides" group, *A. umbrinella, A. rosulata,* and *A. microphylla* (Fig. 2K). As with pistillate head shape, the directionality of this character is hard to ascertain. It should, however, be noted that these characters (zoned colors and turbinate-campanulate heads) are also found in doubtful *Antennaria* species, *A. linearifolia* (Fig. 2J–K). Pappus bristles of staminate plants in *Antennaria* can be of the "Gnaphaloid" type (barbellate-denticulate) or the *Antennaria* type (clavate, an allusion to an insect antenna; Fig. 2L). The hallmark, name-bearing character of *Antennaria* is the antenna-shaped pappus bristle of staminate flower (Fig. 2L), however, not all species possess this feature. It has been lost in several species of the "Leontipes" group, and independently in *A. racemosa, A. suffrutescens, A. solitaria, A. neglecta*, and *A. monocephala* of "Catipes" (Fig. 2L). The doubtful *Antennaria* species, *A. linearifolia*, now grouped with the outgroup taxa in this study, has "Gnaphaloid" type bristles suggesting that the character arose within an early clade of *Antennaria*.

Historical Biogeography—Evaluation of the six biogeographical models in BioGeoBEARS supported the BayArea+*j* model. With the lowest AIC score and the highest $AICc_{wt}$ value (Table S6), the model best explained the historical biogeography of the genus. The model has parameters for anagenic range evolution in the form of range expansion and contraction, both narrow and wide spread form of sympatry, but not for vicariant speciation. Based on the analysis, *Antennaria* most likely evolved in the Rocky Mountain region including the Vancouverian Province (0.79 probability, hereafter probabilities given in parentheses) of North America around 5.8 MYA. Next, by 3.9 MYA, the genus had dispersed into the adjacent Madrean Region. Following this, there were multiple dispersals into the Canadian, Arctic, and Appalachian provinces taking place roughly 3.2 MYA (Fig. 3A). More recent dispersals noted were into North American Prairies Province, Eurasia, and Atlantic and Gulf Coastal Plain Province together with South America, in 2.4 MYA, 1.5 MYA, and 0.8 MYA, respectively. BSM analyses indicate that 51.4% of the biogeographical events were based on within-area speciation and the remaining 48.6% of the events were divided into two types of dispersals: 1) range expansion dispersals (anagenic, 37%), and 2) founder/jump dispersals (cladogenic, 11.6%) (Table S7). Two areas/ranges with greater within-area speciation were 1) the Rocky Mountain + Madrean regions and 2) Rocky Mountain region, whereas areas involving the Appalachian region, Canadian province, and North American Prairies province had lower within-area speciation (data not shown). Regarding total dispersal events, the highest number of dispersals was from the Rocky Mountains to the Madrean province (Fig. 3B), especially in both the "Leontipes" and the "Pulcherrimae" clades, and some groups in the "Catipes" (Fig. 3A, B). The analysis also showed that the Rocky Mountain region including the Vancouverian province was the major source for the dispersal events (10.02 of 24.42 events, 41.03%), whereas the





To

From	A	B	C	D	E	F	G	H	I	Total	%
A	0	1.7	0.36	0.84	0.02	0.9	0	0	0.28	4.1	14.38
B	0.36	0	0.02	0.02	0	0.04	0	0	0.02	0.46	1.61
C	0.68	0.06	0	1.02	0.62	0	0.02	0.04	1.04	3.48	12.20
D	0.46	0.18	0.68	0	0.44	0.36	0.04	0	0.46	2.62	9.19
E	0.6	0	0.86	2.22	0	1.54	3.46	0.36	0.98	10.02	35.13
F	0.22	0.02	0	0.32	0.02	0	0.02	0	0	0.6	2.10
G	0.58	0.02	0.28	1.48	0.3	1.16	0	0.74	0.22	4.78	16.76
H	0.08	0.06	0.06	0.04	0.98	0.08	0.34	0	0.02	1.66	5.82
I	0.1	0.04	0.36	0.02	0.24	0.02	0	0.02	0	0.8	2.81
Total	3.08	2.08	2.62	5.96	2.62	4.1	3.88	1.16	3.02	28.52	
%	10.80	7.29	9.19	20.90	9.19	14.38	13.60	4.07	10.59		

FIG. 3. Biogeography of *Antennaria*. A. Diagrams depicting estimation of *Antennaria* and outgroup taxa historical biogeography using BAYAREALIKE+*j* model in BioGeoBEARS. Pie diagrams at each node illustrate the single or combination of geographical regions inferred to have been occupied by ancestral taxa. Sector color in pie chart indicates ancestral region, with the sector size being the probability of that region or combination of regions. Combinations of regions are indicated by hatching of colors. White sectors portray the sum of origins in regions with individual probabilities less than 10%. Present distributions of individual species are indicated by colored boxes. B. Results from 50 biogeographic stochastic mapping (BSM) under BAYAREALIKE+*j* model in BioGeoBEARS. Total number of dispersals, including range expansion and founder dispersals, averaged across 50 BSMs are presented in the table. Color temperature indicates the frequency of dispersal events. Sum and corresponding percentage of the dispersal events involving each area from the source to the destination are shown towards the end of the rows and columns respectively. Map on the left is color coded with seven Floristic provinces/regions of North America (Takhtajan 1986) and two broad geographic regions representing present distribution areas. Arrows which are scaled show the main dispersal routes.

Canadian province formed the largest destination (5.12 of 24.42 events, 20.97%; Fig. 3B).

Discussion

Comparison Between Concatenation and MSC Analysis—In our study, concatenation and MSC analyses produced comparable results retaining most of the clades; however, there was some discordance, especially in the shallower relationships. Since the concatenation and MSC analyses are based on 756 and 656 genes respectively, we also built a tree using the same 656 genes from the MSC in concatenation analysis, but there was no difference in the tree topology whether using the concatenated matrix of 756 or 656 genes. Elevated levels of discordance among the gene trees in multilocus sequence data is likely a major reason for incongruent trees between coalescent-based and concatenation analyses (Edwards 2009). When analyzing mixture of ploidies, polyploidy and the presence of paralogs might lead to incongruence in phylogeny reconstruction; however, the two important sources of incongruence among the gene trees are hybridization and ILS. In our study, taxon sampling for sequencing was carried out based on morphological characteristics to exclude the individual with intermediate looking morphology signaling hybrid origin; however, we cannot rule out ancient hybridization in the genus during speciation as a potential source of the incongruencies seen among the gene trees. Also, as hybridization usually occurs among closely related species where ILS may also be high, disentangling the effects of these two processes in gene tree incongruence can be difficult (Yu et al. 2012). Inspection of each locus/gene and comparison with the species distribution might be helpful in investigating the possible source of discordance in the gene trees (Peters et al. 2007). Here, manually analyzing each gene tree for variability was not very helpful as there were some missing taxa in them, and with few parsimony informative sites per gene, the gene trees were also not well resolved. When some of the variable gene trees were analyzed, they showed no dominant topology, thus potentially supporting the occurrence of ILS. This may indicate rapid radiation and/or high levels of reticulate evolution in *Antennaria,* as has been suggested in other groups (Stephens et al. 2015). Also, the MSC analysis in the ASTRAL tree revealed a quartet score of 0.66 implying that 66% of the quartet trees induced by the gene trees are in the species trees, further supporting a moderate level of ILS in the genus.

According to Springer and Gatesy (2016), missing data in coalescence analysis produces erroneous gene trees, which later influences the topology in the species tree. Also, nonrandom missing data is a problem in concatenation analyses, especially ML, which is exacerbated with higher gene rate heterogeneity and ILS (Xi et al. 2015). In our study, many of the loci/genes did not have representation for all taxa, as many sequenced loci were dropped while assigning orthology in a conservative PHYLUCE pipeline, which retained the contigs on a one to one basis, when matching them to the probes. Out of 756 loci retained for the analysis, 100 and 102 loci had only representation of three and four taxa respectively, whereas the average number of taxa represented by a locus was 12. Here, the MSC analysis, using both 656 (Fig. 1) or 338 gene trees (Fig. S2) produced the same phylogeny; however, the study demonstrated the importance of modeling each gene matrix for a suitable nucleotide substitution model for the analysis. For instance, the species tree built from the gene trees modeled for the best-fit nucleotide substitution slightly differed from the species tree built from the gene trees modeled by only GTRGAMMA model (Figs. 1 and S3, respectively) and shared more similarities with the concatenated tree. Importantly, the latter did not support the monophyly of the "Leontipes" group, and also there were some topology changes in the "Catipes" clade. Regarding concatenation analyses, the missing data or the number of genes considered brought a few topology changes. The trees recovered with all 756 genes (Fig. 1) versus the tree with 280 genes (Fig. S1) produced a slightly different topology in the "Catipes" clade; however, these changes were not always supported by strong support values.

Here, in the concatenation analysis, even though long loci having high sequence variation could have been influential, the analysis may have accommodated the effects of moderate levels of ILS and/or bypassed low amounts of phylogenetic signal and any methodological artifacts that would be present in an MSC analysis (Springer and Gatesy 2016). The concatenated analysis might also have garnered some "hidden support" to produce highly resolved trees (Gatesy and Springer 2014). On the other hand, our use of the MSC analysis may have effectively addressed ILS and any elevated levels of nucleotide substitution in the genes (Goremykin et al. 2010) when compared to concatenation (Xi et al. 2013, 2014). Therefore, the concatenated and MSC analyses are considered as complementary, and the findings of the relationships are discussed below.

Evolutionary Relationships Within Antennaria—Formal taxonomic sections have been described for *Antennaria*, which included a few early infrageneric groups that were described in the nineteenth century (De Candolle 1837; Von Mueller 1855; Gray 1861) and are now parts of related genera such as *Anaphalis* DC., *Ewartia* Beauverd, *Mniodes* (A.Gray) Benth., and *Parantennaria* Beauverd. Earlier, while studying the flora of the Rocky Mountains of North America, Rydberg (1922) devised 11 groups for *Antennaria* species ("Racemosae," "Alpinae," "Roseae," "Nardinae," "Aridae," "Apricae," "Campestres," "Rosulatae," "Argenteae," "Pulcherrimae," and "Dimorphae"); however, as the ranks for these groups were not indicated, the divisions do not hold any nomenclatural significance (Bayer 1990). Similarly, for the nine Eurasian *Antennaria* species, Borissova (1959) has devised two informal sections ("Catipes" and "Urolepis") and five series ("Dioicae," "Monocephalae," "Alpinae," "Sibiricae," and "Carpaticae"). Formal ranks as subgenera, sections, series, etc., within the genus are not established yet; however, some of these names have been used for monophyletic groups in *Antennaria* in the later phylogenetic studies (Bayer 1990; Bayer et al. 1996). Based on morphological analysis, Bayer (1990) divided *Antennaria* into five taxonomic groups; "Geyeriae," "Argenteae," "Dimorphae," "Pulcherrimae," and "Catipes" (including subgroups "Umbrinellae," "Alpinae," and "Dioicae"). Using internal transcribed spacer regions (ITS), Bayer et al. (1996) rearranged *Antennaria* species into six monophyletic groups of equal rank, adding one more group "Arcuatae" to the "Geyeriae," "Argenteae," "Dimorphae," and "Pulcherrimae" groups within a larger group "Leontipes." Unravelling the nomenclatural complexities of these early names will be dealt with in a later publication, so for the purposes of this work, the informal species groups will still be used.

The position of *A. linearifolia* outside of the genus *Antennaria* in this study is consistent with the recent phylogeny of the

"Lucilia" group in the tribe Gnaphalieae (Luebert et al. 2017). Based on these findings, *A. linearifolia* has been moved to a new monotypic genus as *Quasiantennaria linearifolia* (Wedd.) R.J. Bayer & M.O. Dillon (Bayer and Dillon 2019). Earlier cladograms using morphological data (Bayer 1990) and ITS (strict consensus tree; Bayer et al. 1996) established only "Catipes" as monophyletic; however, in the present study, monophyly for these three broad groups is maximally supported (Fig. 1). In both the previous phylogenetic studies (Bayer 1990; Bayer et al. 1996), *A. geyeri* A. Gray was sister to the rest of the *Antennaria* species. Here, in both the concatenated and MSC analyses, *A. geyeri* A. Gray is sister to the rest of the "Leontipes" clade with many ancestral characters. It resembles *Anaphalis*, one of the few Gnaphalieae genera in the northern hemisphere, showing a tendency towards polygamodioecy (having bisexual and staminate flowers on some plants, and bisexual and pistillate flowers on others) and lacking basal leaves (Bayer et al. 1996). Therefore, its resolution as an early diverging lineage to rest of the "Leontipes" clade was as expected.

The "Dimorpha" subclade retains all the three species from the morphology-based cladogram (Bayer 1990); however, in the ITS based strict consensus tree (Bayer et al. 1996), *A. stenophylla* of the group appeared in the "Argentea" group. It was interesting that our result was comparatively more consistent with the earlier phylogeny based on the morphological data than the phylogeny from the ITS/ETS sequences. One of the reasons could be due to the fewer number of parsimony informative characters in the data matrix of the ITS sequences. The three morphologically similar species of the "Dimorpha" subclade are the most xerophytic among all *Antennaria* species, grow at low elevations, and flower earlier than any other species in the western cordillera. They all possess the morphological synapomorphy of dark colored phyllary tips (Bayer 1990), although this is a fairly homoplasious character, appearing in several other lineages, e.g. "Pulcherrimae." The "Argentea" subclade, including four taxa, *A. luzuloides* ssp. *aberrans*, *A. luzuloides* ssp. *luzuloides*, *A. arcuata,* and *A. argentea,* forms a morphologically cohesive group. They are distributed in the northwestern part of the United States and adjacent Canada, and as morphological synapomorphies, bear pubescent stolons and tomentose phyllary bases (Bayer 1990). In this study, relationship between *A. argentea* and *A. luzuloides* ssp. *aberrans* slightly differs between the concatenation and MSC analyses. There is some disagreement regarding the species status of *A. luzuloides* ssp. *aberrans.* Most recently, it has been regarded at the infraspecific level within *A. luzuloides*; Bayer and Stebbins (1993) ranked it as a subspecies of *A. luzuloides,* but Cronquist (1994) as a variety of *A. luzuloides* (viz. *A. luzuloides* var. *microcephala* (A. Gray) Cronquist). Some authors have recognized it as a distinct species (Sharsmith 1960; Jepson 1925) as *A. microcephala* A. Gray, while others have synonymized *A. microcephala* (*A. luzuloides* ssp. *abberans*) within *A. luzuloides* (Munz 1974; Cronquist 1955). These two taxa show some dissimilarity as *A. luzuloides* ssp. *aberrans* Torrey & A. Gray has 10–30 heads arranged in racemiform or paniculiform arrays, have phyllaries proximally green but distally white, and has narrower distribution in California, Nevada, and Oregon in moist open meadows and drainages. Conversely, *A. luzuloides* ssp. *luzuloides* possesses 10–110 + heads arranged in corymbiform arrays, have phyllaries proximally golden brown and distally white, and has comparatively a wider distribution occurring in semi-dry meadows and extending up to the southern part of British Columbia and Alberta in the north and to South Dakota in the east (Bayer 2006). In the present study, these two taxa do not hold the sister relationship within the same "Argentea" group, and further studies will be needed to investigate whether these two taxa should have a status of separate species restored. Apart from *A. luzuloides* ssp. *aberrans* not included in the study then, the "Argentea" group retained all the three species, *A. arcuata*, *A. argentea*, and *A. luzuloides* ssp. *luzuloides* similar to results in the morphology-based cladogram (Bayer 1990). However, in the ITS based strict consensus tree (Bayer et al. 1996), "Argenteae" group had *A. argentea* and *A. luzuloides* ssp. *luzuloides* as in this study, and *A. stenophylla* from the "Dimorphae" group, however *A. arcuata* was resolved as a monotypic group. The "Argentea" group is characterized by having very wooly stolons, and comparatively numerous, small heads with light colored phyllaries (zones of white, golden brown, or green). The group is restricted to the northern Rockies and Cascades, with all but *A. luzuloides* ssp. *luzuloides* having notably narrow ranges (Bayer 2006).

All five species of the "Pulcherrimae" clade in this study share a unique character of having a woody caudex at the base of the plant, consisting of many parallel principle veins (Bayer 1990), and having a wider distribution in the western United States with *A. pulcherrima* extending its range across the boreal region of North America from Newfoundland to Alaska. The group is represented across Eurasia from the Pyrenees to the Kamchatka Peninsula by *A. carpatica* and *A. villifera* Boriss. (Borissova 1959; Bayer 2006). The earlier morphology based phylogeny (Bayer 1990) recovered seven species for the group, five out of six taxa retained for the group in this analysis, except for the recently discovered *A. sawyeri*, and two additional species *Q. linearifolia*, now shown to be outside of the *Antennaria* monophyly, and *A. villifera*, a likely synonym for *A. carpatica*, not included in the present study. Both of these analyses retain almost the same taxa in the clade; however, relationships within the subclades could not be compared, as the former study did not have ample resolution in the tree. In the ITS based phylogeny (Bayer et al. 1996), only three taxa, *A. lanata*, *A. anaphaloides,* and *A. pulcherrima* ssp. *pulcherrima* formed the "Pulcherrimae" group, as *A. sawyeri* was not discovered, the taxon *A. pulcherrima* ssp. *eucosma* was not included in that study, and *A. carpatica* fell within the "Catipes" clade as sister taxon to *A. dioica*. However, Bayer et al. (1996) were suspicious regarding the DNA source for *A. carpatica* as it was collected in the Swiss Alps where hybridization between *A. carpatica* and *A. dioica* is common (Urbanska-Worytkiewicz 1968). As the morphology of *A. carpatica* resembles members of the "Pulcherrimae" group (Bayer 1990; Bayer et al. 1996), and also both the morphology-based cladogram (Bayer 1990) and the present analyses retain it under "Pulcherrimae" group, the position of *A. carpatica* in the group seems certain. Also of interest is the sister relationship of the two species of *A. pulcherrima*, ssp. *pulcherrima* and ssp. *eucosma*. This supports Bayer's (2004) suggestion of the close relationship between the two taxa and the reduction of *A. eucosma* Fernald and Wiegand to a subspecies based on the lack of sufficient characteristics to separate it reliably from *A. pulcherrima*.

Earlier, based on morphology, Bayer (1990) divided the members of the "Catipes" clade into three groups; a monotypic group Umbrinellae sister to the bigger groups Alpinae and Dioicae. In Bayer (1990), *A. umbrinella* differed from the two

bigger groups in having unpigmented corollas. Similarly, principal synapomorphies for the group "Alpinae" were dark colored phyllaries and short cauline stems, with most of the members also having flags on the upper cauline leaves and purple and brown glandular hairs in the upper stem and cauline leaves. In contrast, the "Dioicae" group had all members with light colored phyllaries, except dark green and black, and had a few other weak characters delineating them. However, these two groups "Dioicae" and "Alpinae" were determined to be unnatural and polyphyletic in the study using ITS and also in the tree produced by re-evaluation of morphological data (Bayer et al. 1996). Later, it was suggested that the "Dioicae" and "Alpinae" group names be abandoned as unnatural (Bayer and Chandler 2007). In the present study too, both in the concatenation and MSC analyses, monophyly for the two groups "Dioicae" and "Alpinae" is not supported, reinforcing the contention that these groups should no longer be recognized. In the concatenated analysis, four distinct groups are noted, *A. racemosa* at the base, a smaller "Suffrutescens" group with three taxa, and the two larger groups: the "Densifolia" group consisting mostly of species from the Rocky Mountains and western North America, and the "Dioica" group, consisting of species from the eastern United States, Alaska and the Yukon territory, and also Eurasia. However, in the MSC analysis, six subgroups are formed: monotypic groups *A. racemosa* and *A. suffrutescens*, two smaller "Aromatica" and "Pulchella" groups, and two comparatively larger "Umbrinella" and "Dioica" groups. Some of the nodes in the "Catipes" clade are weakly supported in ML analysis; however, in Bayesian analysis, only two of the external nodes resolving two sister taxa, *A. pulchella* with *A. aromatica* and *A. rosulata* with *A. marginata*, have low support values (0.6 and 0.5 PP respectively). Also, between the MSC and concatenation analyses, there are topology changes for five species: *A. pulchella*, *A. densifolia*, *A. suffrutescens,* and sister species *A. neglecta* and *A. solitaria*; however, most of the topology changes are not supported by strong support values showing the instances of soft incongruence.

A complete knowledge of the ploidy levels in the *Antennaria* is still lacking; however, studies by Bayer (1984, 1987) and Bayer and Stebbins (1987) demonstrated that levels of high polyploidy (hexaploid and above) are restricted to "Catipes" compared to the "Leontipes" clade with only diploid/tetraploid cytotypes. One of the reasons for the "Catipes" clade to become recalcitrant to resolution could be the occurrence of high level of polyploidy, a case that can aggravate paralog issues (Lemmon and Lemmon 2013). Also, the retrieved copy number for each targeted gene for all the species (Table S4; Fig. S6) showed higher values for the *Antennaria* species, especially in some members of the "Catipes "clade, compared to the outgroups. In the clades with high copy numbers of genes, paralogs may be co-enriched by a single bait making de novo assembly further difficult (Nicholls et al. 2015). However, widespread polyploidy could be one of major reasons for the success of the "Catipes" clade (Bayer and Chandler 2007). Gene duplication may influence lineage diversification by providing raw genetic materials for adaptative evolution (Crow and Wagner 2005); hence, occurrence of polyploidy in the clade may have enhanced their ability to grow in diverse environmental conditions covering a large geographic area (Bayer and Chandler 2007). For example, many of the species in this group are established as specialized edaphic endemics; *A. aromatica* and *A. densifolia* are found on limestone talus, *A. suffrutescens* grows only on serpentine soil (Bayer 1989a, 1992), whereas others have large geographic ranges spreading across continents (e.g. *A. dioica*, *A. neglecta*, and *A. microphylla*).

In this study, evolutionary relationships among the sexual *Antennaria* species based on chloroplast data was not well resolved. One of the reasons for poor resolution or support values seen in the chloroplast trees could be the presence of fewer parsimony informative characters in the chloroplast DNA data matrix (2929) compared the nuclear DNA data matrix (11,616 nt positions). However, the resolution was enough to resolve *Antennaria* and the "Leontipes" within *Antennaria* as monophyletic groups. The relationships of the taxa within the two chloroplast trees produced by maximum likelihood and Bayesian analyses differed slightly but were in high disagreement with the nuclear trees. Owing to the different evolutionary history, discordance between nuclear and chloroplast phylogenies is not uncommon (Soltis and Kuzoff 1995; Bruun-Lund et al. 2017; Herrando-Moraira et al. 2019).

Character Evolution in Antennaria—The two clades, "Leontipes" and "Pulcherrimae," are considered as unspecialized groups having characters comparatively closer to ancestral states. These characters include: consisting of mainly diploid species, except for known tetraploids in *A. dimorpha, A. stenophylla,* and *A. pulcherrima* ssp. *pulcherrima* (Bayer and Stebbins 1987; Table S1), reproducing only by amphimixis, producing no stolons (Fig. 2G; two exceptions are *A. arcuata* and *A. flagellaris*), and showing less distinct sexual dimorphism. On the other hand, "Catipes" shows specialization; diploid and frequent tetraploid or hexaploid amphimicts giving rise to the polyploid agamic complexes in the genus. It is difficult to envision an adaptive significance of many of the specialized character traits that were investigated in this study; however, the adaptive impacts of some traits are recognizable. In "Catipes" aggressive stolons form a mat-like growth (Fig. 2G); this is obviously an effective way for individuals to spread vegetatively and persist for perhaps many decades. Stolons also independently evolved in *A. flagellaris* and *A. arcuata* of the "Leontipes" clade (Fig. 2G); however, stolons of these species appear to be different, and may be non-homologous. *Antennaria flagellaris* stolons are actually runners with no leaves along the stolon. The "Catipes" also show distinct sexual dimorphism in floret and head morphology, as well as overall height differences between staminate and pistillate individuals (Fig. 2B) (Bayer 1990; Bayer et al. 1996), features that benefit pollination success and the taller pistillate plants are better able to have their fruits exposed to passing winds to aid in dispersal (Pickup and Barrett 2011). The cauline leaves of "Catipes" are of a markedly different shape from the basal leaves, and decline in size up the stem unlike in "Leontipes" and "Pulcherrimae" where the cauline leaves are the same shape as the basal leaves (Fig. 2C–D). The basal leaves of the "Catipes" clade have distinct petioles, while those of the "Leontipes" are generally sessile (Fig. 2H). All species of the "Pulcherrimae" and many species of the "Leontipes" arise from woody caudices, while in "Catipes" there are herbaceous caudices (Fig. 2A). Two exceptions to this are *A. suffrutescens* ("Catipes") and *A. geyeri* ("Leontipes"), which are distantly related, small, suffruticose shrubs lacking stolons (Bayer 2006). Woody caudices seem to be the alternative to aggressive stolons and may also be a secondary mechanism that assists in maintaining the perennial habit. The "Catipes" and "Pulcherrimae" clades do have one strong morphological trait:

possession of turbinate heads, while those of "Leontipes" are narrowly subcylindrical (Fig. 2J). It is obviously a strong feature that defines the three major lineages in the genus. This may be related to a clutch size tradeoff (Levin and Turner 1977) as those species with the turbinate heads tend to have many florets, but fewer heads per capitulescence while those taxa with narrowly subcylindrical heads have fewer florets per head, but many more heads.

Flags are an important taxonomic character in *Antennaria* because they are consistently present or absent within a species. Flags are common but not universally found in both the "Catipes" and "Pulcherrimae" clades, however they are absent in all "Leontipes" species (Fig. 2E). In fact, presence/absence of flags is the key character used to separate the subspecies of *A. pulcherrima* (Fig. 2E). In "Catipes," flags unite the arctic alpine species of *A. friesiana* and *A. monocephala*, as well as the eastern temperate species, *A. solitaria* and *A. neglecta* (Fig. 2E). Flags are almost universally associated with dark (brown or black) phyllary colors and may be part of a flower developmental adaptation whereby dark phyllaries absorb more sunlight which warms up the developing heads, hastening their growth in the short growing season in montane and alpine environments (Mu et al. 2010). Purple moniliform glands that appear on the upper cauline stem are a character that is found in four lineages within the "Catipes" clade (Fig. 2F); the monotypic "Racemosa" group, the "Aromatica" group (*A. aromatica, A. suffrutescens*, and *A. pulchella*), the "Microphylla" group (*A. microphylla*, *A. rosulata*, and *A. marginata*), and the "Arctic-Alpine" group (*A. friesiana* and *A. monocephala*). These hairs bear a strong odor of citronella in *A. aromatica*, *A. marginata*, and *A. racemosa;* one could postulate that these hairs produce anti-herbivory substances. The "Pulcherrimae" group is well-defined by possessing three or more parallel primary veins in its basal leaves (Fig. 2I). This feature has also arisen independently in *A. luzuloides* ("Leontipes") and in three species of forest dwelling members of the "Catipes" group, *A. racemosa, A. plantaginifolia,* and *A. solitaria* (Fig. 2I). Most species of *Antennaria* grow in open, sun-exposed habitats and the larger, broader leaves of *A. racemosa, A. plantaginifolia,* and *A. solitaria* are likely an adaptation to low light conditions found in the forest margins where they occur. Species such as *A. racemosa, A. marginata, A. corymbosa, A. suffrutescens, A. plantaginifolia,* and *A. solitaria*, which grow in forests and forest margins, have glabrous or floccose glabrescent adaxial leaf surfaces. This is another adaptation to lower light conditions. Zoned phyllary coloration, i.e. phyllaries with different colors in marked bands in them, is a feature of most *Antennaria* species and the different color patterns are important taxonomic characters at the species level in the genus (Fig. 2K; Bayer 2006). Zoned phyllaries are conspicuously lacking in the many of the "Leontipes" species and a few species of the "Catipes," viz. *A. umbrinella*, *A. rosulata*, and *A. microphylla*. Phyllary color patterns are undoubtedly a character involved in pollination in amphimictic species and is a strong key taxonomic character for many. The clavate antenna-like pappus bristles of staminate florets, from which the generic name is derived, are prevalent in the genus, but not universally so. They are conspicuously lacking in many of the "Leontipes" species, which have the "Gnaphaloid" barbellate-denticulate pappus common throughout most of the Gnaphalieae. Additionally, the antenna-like bristles have been apparently lost by several members of the "Catipes" group, viz. *A. microphylla*, *A. rosulata*, and *A. umbrinella* (Fig. 2L). The outgroup taxon, *Q. linearifolia*, has "Gnaphaloid" type bristles suggesting that the character arose within an early clade of *Antennaria*. The clavate pappus bristles seem to be more prevalent in species with broad turbinate staminate heads, while the pistillate heads and narrowly subcylindrical heads have the thin, delicate barbellate-denticulate bristles. It may be a "packaging" problem, the space-demanding clavate bristles require bigger heads to house their larger size, while the smaller, narrower heads cannot. To summarize, the specialized morphological character traits are more common in, indeed often restricted to, the "Catipes" and "Pulcherrimae" groups.

Biogeography of Antennaria Species—The following phytogeographic scenario is offered. Dispersal of the ancestor of the *Antennaria* crown group from South America to the Rocky Mountain region occurred about 6.8 MYA, when suitable habitats became available in the western part of the North America (Leopold and Denton 1987). The time period for the origination of the genus in this study is concurrent with the study of Gnaphalieae tribe by Nie et al. (2016). Nie et al. (2016) also found that the late Miocene-Pliocene was the major period for the global expansion of Gnaphalieae lineages, and also hypothesized the origination of North American Gnaphalieae from their ancestors in the Mediterranean region (Filago group) and South America (Lucilia group). It has long been hypothesized that birds migrating from South America to North America are vectors for plant diaspores (Cruden 1966; Carlquist 1967; Raven 1972). Most amphitropical dispersals between North and South America have occurred in plants growing in similar semi-arid habitats (Raven 1972). This may be the result of the fact that migratory bird flight paths lie over semi-arid regions on both continents (Cruden 1966; Raven 1972). The ancestor of the crown group of *Antennaria* would likely have been heterogamous, as successful long-distance dispersal in dioecious species would be very unlikely. The fact that *A. geyeri* ("Leontipes" group) is often subdioecious (central florets are hermaphroditic, Bayer and Stebbins 1993) and that sexual dimorphism is poorly evolved (see Fig. 2 in Bayer and Stebbins 1993), can be used as evidence in support of the evolution of dioecy in *Antennaria* as occurring from a heterogamous ancestor via subdioecy (See Ross 1982 for a discussion of evolution of subdioecy). The independent evolution of dioecy in several lineages of the Gnaphalieae has been documented by phylogenetic analyses of the tribe (Ward et al. 2009; Nie et al. 2016; Bayer and Dillon 2019). Aridity increased as the Miocene gave way to the Pliocene (~5.2 MYA), especially as the result of the continual rising of the Cascade (Leopold and Denton 1987) and Sierra Nevada ranges (Chabot and Billings 1972). Consequently, as aridity increased in western North America during the Pliocene (2–5 MYA), the newly evolved group migrated (Fig. 3) into the Great Basin of the Madrean region and Columbia Plateau region, where the least specialized *Antennaria* group ("Leontipes" clade), still occurs today in open forest habitats at relatively low elevations. Other Asteraceae with affinities to the Madrean region that were adapted to xeric conditions had expanded their ranges into this area during the Miocene as well (Taggart and Cross 1980; Taggart et al. 1982; Leopold and Denton 1987; Wolfe 1987), therefore appropriate habitats for the early *Antennaria* groups, the montane zone with *Pinus ponderosa* and *Artemisia* steppe, were likely present in the Columbia Basin and Great Basin in the late Miocene (Taggart and Cross 1980; Leopold and Denton 1987).

The "Pulcherrimae" and "Leontipes" clades diverged from each other early (~5.6 MYA, Fig. 3) and while species in the "Leontipes" clade have not expanded their ranges beyond the Rocky Mountain and Madrean region (except *A. dimorpha* is currently in the western part of the N. American Prairie Province), the "Pulcherrimae" have expanded beyond. About 2 MYA, *A. pulcherrima* s. l. expanded into the Canadian Region and Atlantic Canada, and *A. carpatica* crossed Beringia and is currently endemic exclusively to Eurasia (Fig. 3). Land bridges have existed intermittently across Beringia from the Mesozoic up until as recently as 13,000 yr B. P. (Matthews 1979) and so *Antennaria* could have migrated to Eurasia at any time from the Late Miocene through to the to the end of Pleistocene.

The "Catipes" clade diverged from "Leontipes" and "Pulcherrimae" about 5.8 MYA, but did not expand its range outside the Madrean/Rocky Mountain Province until about 3.2 MYA (Fig. 3). The ancestor of the *A. neglecta, A. solitaria, A. plantaginifolia*, and *A. virginica* group moved eastward into mixed deciduous/conifer forests of the Appalachian and Atlantic and Gulf Coastal Plain Provinces below the established terminus of the last Wisconsinan glaciation. *Antennaria neglecta* expanded its range into the N. American Prairie Province and the Canadian Province. Our results indicate that the lineage containing *A. friesiana* s. l. and *A. monocephala* had already moved north into the Canadian and Arctic Provinces about 3.2 MYA (9.6), before Pleistocene glaciation about 2.58 MYA (Fig. 3). Hence, the present-day distribution of the clade in the alpine region could be the survivors of the descendants in refugia or the recent migrants after the Pleistocene glaciation. These species along with *A. densifolia*, *A. dioica*, and *A. microphylla* may once have had much wider distributions in the Canadian/Arctic Provinces. Also, their current distribution overlays regions of Alaska, Yukon, Alberta, and British Columbia that were unglaciated during the Wisconsinan glacial maxima (Matthews 1979; Bayer 1989a; Bayer and Stebbins 1993). *Antennaria dioica* expanded its range from the Aleutian Islands, across continental Eurasia, to the British Isles (Borissova 1959). *Antennaria chilensis* is likely a segregate clone of the *A. rosea* complex (Bayer and Chandler 2007) and dispersed to South America following formation of the complex in North America.

Present-day distribution of *Antennaria* shows that nearly all sexual diploid/tetraploids (the focus of this study), occur primarily in unglaciated regions, be they former glacial refugia in Alaska (Beringian refugium), Atlantic Canada, the ice-free corridor in western Alberta (Packer and Vitt 1974), or the area of the United States that was below the Wisconsinan glacial maximum. Three centers of diversity for the "Catipes" exist; the western United States is a primary center, while the Alaska-Yukon area and the eastern United States are secondary (Bayer and Stebbins 1987). The polyploid sexual and agamic complexes, such as *A. alpina*, *A. howellii*, *A. parvifolia*, and *A. rosea* were the most recent to evolve as they were derived from the sexual progenitors of section "Catipes" (Bayer 1987) and have colonized the previously glaciated regions of North America and Eurasia. The overwhelming success of the "Catipes" seems to be correlated with the high incidence of polyploidy and agamospermy in certain species of this group.

Summary—Hybridization, polyploidization, and development of agamospermy have complicated reconstructing interrelationships among species in *Antennaria* such that morphology-based markers (Bayer 1990) and ITS sequences (Bayer et al. 1996) have only managed to produce low resolution phylogenetic trees with conflicting topologies. Here, we used a target enrichment-based approach (Mandel et al. 2014) designed to capture a conserved set of nuclear loci across a wide range of Asteraceae species to draw a clearer picture of the phylogenetic relationship in the genus. Results from both the concatenation and coalescence approaches concurred in resolving most of the relationships of diploid/tetraploid sexually reproducing *Antennaria* species. Three major clades in *Antennaria*, "Leontipes", "Pulcherrimae", and the "Catipes" were identified. Based on this backbone phylogeny, evolutionary trends in 12 taxonomically important morphological characters for various clades of *Antennaria* were studied, and the phylogeny will be helpful for studying the evolution of other important morphological, anatomical, and physiological characters in the genus. The study also supported the earlier findings (Bayer 1990; Bayer et al. 1996) that *Antennaria* most likely evolved in the Rocky Mountain region including the Vancouverian province of North America around 5.8 MYA, and subsequently dispersed into the Arctic region, Appalachian province, Canadian provinces and Eurasia, primarily by range expansion dispersals. The study based on hundreds of nuclear loci presents the most resolved phylogeny of the sexual *Antennaria* species to date. The phylogenetic study along with results from character evolution and biogeography will be helpful for further evolutionary studies in the genus. Especially, the study will be valuable in the investigation of different *Antennaria* polyploid agamic complexes, including their origination, phylogenetic relationships, and the development of agamospermy in them.

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Author Contributions

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Appendix 1. Specimen Voucher Data. Presented are taxon, cytology (ploidy level(s), ! = direct count on collection), location, collectors, collector(s) numbers, location of voucher specimen (herbarium acronym), [sequence accession number].

Antennaria anaphaloides, (2*x*), USA, Montana, Choteau Co., *Bayer, Purdy, and Newby, MT-92005*, (ALTA), [SRR8612195]; *A. arcuata*, (2*x*), USA, Wyoming, Fremont Co., Mormon Spring, *Bayer, Purdy, and Minish, WY-90022*, (ALTA), [SRR8612196]; *A. argentea*, (2*x*), USA, Oregon, Grant Co., Aldrich Mountains, *Bayer, Francis, and Minish, OR-91004*, (ALTA), [SRR8612197]; *A. aromatica*, (2*x*!, 4*x*, 6*x*), USA, Montana, Carbon Co., Beartooth Plateau, *Bayer, Ainouche and Ainouche, and Starr, MT-96046*, (ALTA), [SRR8612198]; *A. carpatica*, (2*x*, 4*x*), Russian Federation, Ural Mountains, *Skvortsov, Filin, and Isakova*, 10694, (US), [SRR8612199]; *A. chilensis var. magellanica*, (4*x*!), Argentina, Tierra del Fuego, Isla Grande, *Bayer and Chandler, ARG-02073*, (CANB, LP, F, MO, CHR), [SRR8612200]; *A. corymbosa*, (2*x*!), USA, Montana, Beaverhead Co., *Bayer and Lebedyk,*

M-508, (ALTA), [SRR8424623]; *A. densifolia*, (2*x*!), USA, Montana, Granite Co., Goat Flat, *Bayer, DeLuca, and Lebedyk, MT-725*, (ALTA), [SRR8612201]; *A. dimorpha*, (2*x*, 4*x*), USA, Nevada, Elko Co., Independence Mtns., *Bayer, Purdy, and Minish, NV-90007*, (ALTA, RM), [SRR8612202]; *A. dioica*, (2*x*), France, Dept. of Savoie, Col de Balme, *Bayer, Ainouche and Ainouche, Misset, Jory, Charpin, and Urbanska, FR-02031*, (CANB), [SRR8612203]; *A. flagellaris*, (2*x*), USA, Oregon, Crook Co., Ochocho Mountains, *Bayer, Francis, and Minish, OR-91006*, (ALTA, RM), [SRR8612204]; *A. friesiana* ssp. *alakana*, (2*x*, 4*x*), USA, Alaska, Brooks Mountains, Dalton Hwy., *Bayer, Jonsell, Marvin, Purdy, and Urbanska AK-89056*, (ALTA, RM), [SRR8612224]; *A. friesiana* spp. *neoalakana*, (4*x*), Canada, Northwest Territories, District of MacKenzie, Richardson Mountains, *Bayer, Purdy, and Marvin, NWT-89029*, (ALTA), [SRR8612225]; *A. geyeri*, (2*x*), USA, Oregon, Deschutes Co., *Bayer, Francis, and Minish, OR-91008*, (ALTA), [SRR8612226]; *A. lanata*, (2*x*), Canada, Alberta, Edson Forest Reserve, Cardinal Divide, *Bayer and Purdy, AB-90005*, (ALTA), [SRR8612227]; *A. luzuloides* ssp. *aberrans*, (2*x*), USA, Oregon, Umatilla Co., Blue Mountains, *Bayer, Francis, and Minish, OR-91001*, (ALTA), [SRR8612221]; *A. luzuloides* ssp. *luzuloides*, (2*x*), USA, Washington, Spokane Co., *Bayer, Purdy, and Minish, WA-90001*, (ALTA), [SRR8612222]; *A. marginata*, (2*x*!, 4*x*, 6*x*), USA, Arizona, Mohave Co., Hualapai Mountains, *Bayer, DeLuca, and Lebedyk, AZ-705*, (ALTA), [SRR8612223]; *A. microphylla*, (2*x*), USA, Montana, Granite Co., Sapphire Mountains, *Bayer, Purdy, and Newby, MT-92065*, (ALTA), [SRR8612229]; *A. monocephala* ssp. *monocephala*, (2*x*), Canada, Yukon Territory, Keno Hill, *Bayer, Purdy, and Marvin, YK-89052*, (ALTA), [SRR8612230]; *A. neglecta*, (2*x*), USA, Ohio, Delaware Co., *Bayer and Stebbins, BPN-56*, (OS), [SRR8612206]; *A. plantaginifolia*, (2*x*), USA, Kentucky, Magoffin Co., *Bayer, PV-282*, (OS), [SRR8612205]; *A. pulchella*, (2*x*!), USA, California, Inyo Co., Evolution Peaks, *Bayer, DeLuca, and Lebedyk, CA-724*, (ALTA), [SRR8612208]; *A. pulcherrima* ssp. *eucosma*, (4*x*), Canada, Newfoundland, Cape St. George, *Rouleau and Rast, 11035*, (MT), [SRR8612207]; *A. pulcherrima* ssp. *pulcherrima*, (2*x*, 4*x*), USA, Colorado, Elk Mountains, *Bayer, Francis, and Minish, CO-91012*, (ALTA, RM), [SRR8612210]; *A. racemosa*, (2*x*), USA, Montana, Flathead Co., Baldhead Mountain, *Bayer, Bilodeau, and Lebedyk, MT-895*, (ALTA, RM, DAO), [SRR8612209]; *A. rosulata*, (2*x*), USA, Arizona, Apache Co., Chuska Mountains, *Bayer, Bilodeau, and Lebedyk, AZ-800*, (ALTA, RM, DAO), [SRR8612212]; *A. sawyeri*, (2*x*!), USA, California, Trinity Co., Klamath Mountains, *Figura, 170*, (HSC), [SRR8612211]; *A. solitaria*, (2*x*), USA, Tennessee, Wayne Co., Collinwood, *Liu and Munch, ASOL-9106*, (ALTA), [SRR8612214]; *A. stenophylla*, (2*x*), USA, Washington, Lincoln Co., *Bayer, WA-94002*, (ALTA, WS), [SRR8612213]; *A. suffrutescens*, (2*x*), USA, California, Humboldt Co., *Bayer, Francis, and Minish, CA-91002*, (ALTA), [SRR8612217]; *A. umbrinella*, (2*x*, 4*x*), USA, Wyoming, Sublette Co., Wind River Range, *Bayer, DeLuca, and Lebedyk, WY-700*, (ALTA, RM, CAN), [SRR8612218]; *A. virginica*, (2*x*, 4*x*), USA, Grant Co., *Bruner and Giust, WV-94002*, (ALTA), [SRR8612215]; *Mnoides argentea* (Wedd.) M. O. Dillon, (unknown), Argentina, Jujuy Province, Yavi, *Bayer and Chandler, ARG-02037*, (CANB, LP, F, MO, CHR), [SRR8612216]; *Facelis lasiocarpa*, (unknown), Argentina, Mendoza Province, Tunuyan, Andes Range, *Bayer and Chandler, ARG-02049*, (CANB, LP, F, MO, CHR), [SRR8612228]; *Gamochaeta alpina*, (unknown), Argentina, Tierra del Fuego, Isla Grande, Garibaldi Pass, *Bayer and Chandler, ARG-02080*, (CANB, LP, F, MO, CHR), [SRR8612231]; *Gamochaeta sp.*, ($n = 12$), Argentina, Jujuy Province, Yavi, *Bayer and Chandler, ARG-02029A*, (CANB, LP, MO), [SRR8612219]; *Quasiantennaria linearifolia*, (2*x*), Peru, Dept. La Libertad, Prov. Pataz, near Laguna Huascacocha, Sagástegui, *Zapata, Rodríguez and Medina, 17317*, (HAO), [SRR8612220].