RAD sequencing rejects a long-distance disjunction in *Stellaria* (Caryophyllaceae) and yields support for a new southern Rocky Mountains endemic

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**Abstract** Whereas the eastern North American–eastern Asian floristic connection represents one of the most widely studied biogeographical relationships in flowering plant evolution, connections between western North America and Asia have been comparatively rarely investigated, especially through genetic approaches. *Stellaria irrigua* is one of several plants that has been treated as an exceptionally dramatic example of a disjunction between floristically similar, high alpine biotas of the southern Rocky Mountains and south-central Siberia. We here employ numerous new field collections and ddRADseq data to test the hypothesis that *S. irrigua*—a species that has been known for over 180 years—represents a long-distance disjunction between the southern Rocky Mountains and central Asia. Extensive fieldwork, review and perusal of herbarium materials, and phylogenomic analyses indicate that *S. irrigua* is broadly distributed across an amphi-Beringian arc extending from southern and central Asia, east through Beringia, and south throughout mountainous regions of western North America. Sampled Asian populations formed two clades, and North American individuals all formed a clade embedded within this broader Asian lineage. *Stellaria irrigua* is, however, rendered non-monophyletic by a lineage that is embedded within the North American populations and is ecologically and morphologically distinctive from *S. irrigua*. The identity of this newly recognized lineage, which was in prior works attributed to *S. irrigua*, has been confused since plants of the former were first collected in the San Juan Mountains of Colorado in the late 1800s under *Arenaria* and *Alsine*. We provide a new name for this taxon, *Stellaria sanjuanensis*, a charismatic starwort of dry alpine scree slopes of the southern Rocky Mountains. Additionally, two lectotypes are designated, one holotype and one isotype are identified, and two new synonymies are proposed, to help stabilize the taxonomy and nomenclature of this long-confused species complex. A key to the starworts of the southern Rocky Mountains is also provided, and *Stellaria alsine* is reported as new to the region.

**Keywords** Altai; biogeography; Caryophyllaceae; disjunction; paraspecies; progenitor-derivative speciation; RADseq; southern Rocky Mountains; *Stellaria*

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

## INTRODUCTION

*Stellaria* L. (Caryophyllaceae) is a cosmopolitan genus of up to ~120 species (Morton, 2005). Species in this lineage occupy a broad range of habitats but are most diverse in upper montane environments and are typical elements of arctic-alpine floras worldwide, especially in western North America and eastern Asia, which represent two centers of diversity for the genus (Schischkin, 1970; Shilong & Rabeler, 2001; Morton, 2005; M. Sharples & E. Tripp, in revision). Whereas many among the species of *Stellaria* are restricted to one major phytogeographical area such as the Tibetan Plateau, New Zealand, or the southern Andes, others have been noted by prior authors for exhibiting long-distance biogeographical disjunctions. These include hypotheses of disjunctions between North America and South America (Morton, 2005), between Oceania and Patagonia (Webb & al., 1988), as well as between central Asia and the southern Rocky Mountains (Weber, 2003). One particularly marked example of long-distance disjunction in *Stellaria* figured starkly in a major biogeographical hypothesis put forward by Weber (2003) to explain floristic connections between central Asia and the southern Rocky Mountains. Connections first remarked upon by J.D. Hooker (1878). In his hypothesis, Weber (2003) proposed that this floristic connection comprises multitudes of once more widely ranging species that subsequently became isolated after the onset of the Pleistocene and consequent contraction of previously continuous northern, Tertiary, boreal habitats. Weber’s hypothesis is compelling but remains little explored by empirical, phylogenetic approaches, especially in comparison to the widely investigated, and much

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better understood, eastern Asian–eastern North American floristic connection (Li, 1952; Wen & al., 2010; Manos & Meireles, 2015).

Among the most notable of disjunctions between the mountains of central Asia and the southern Rocky Mountains is that of *Stellaria irrigua* Bunge (Weber, 1961, 2003; Morton, 2005). *Stellaria irrigua* was described in 1835 from the Altai Mountains of southern Siberia. All modern floristic treatments of the southern Rocky Mountains have recognized *S. irrigua* as a species that additionally inhabits alpine scree habitats present in southern portions of the southern Rocky Mountains (e.g., Douglas, 1992; Weber & Wittmann, 2012; Ackerfield, 2015). Other authors have, however, expressed skepticism as to whether populations in these two geographically disparate areas are in fact conspecific (Kozhevnikov, 1994; Morton, 2005), and the *Flora of China* considers *S. irrigua* to be Asian only (Shi-long & Rabeler, 2001).

Confusion surrounding *Stellaria irrigua* and its distribution stems from multiple sources. First, very little material of *S. irrigua* has been collected in Asia since the species was described over 180 years ago; for example, the largest repository of temperate Asian vascular plant material in the world—the Komarov Herbarium (LE)—contains only a single collection determined as *S. irrigua*, the holotype. As such, *S. irrigua* has been considered to be very rare in Asia. Second, the species is furthermore considered to be rare in North America; plants that have been attributed to this name are restricted to only a few mountain ranges in southern portions of the southern Rocky Mountains, namely the Elk, San Juan, and Sangre de Cristo Mountains of Colorado (and far northern New Mexico) where they occur on barren, dry alpine scree slopes of usually volcanic origin. Third, in Bunge’s (1835) original description of *S. irrigua*, he noted that plants occurred in “damp and mossy areas” (hence the specific epithet, “irrigated”), in marked contrast to the well-drained scree and tuff slopes on which plants occur in the southern Rocky Mountains. Finally, no molecular data have been generated with which to evaluate this proposed long-distance disjunction.

The most comprehensive phylogenetic analysis of *Stellaria* to date was that of Greenberg & Donoghue (2011), who sampled ca. 40 species including material of *S. irrigua* and its putative closest relative (*Stellaria umbellata* Turcz.) from Colorado but not from Asia. In the present study, we use RADseq data combined with new field collections, new field observations, and new insights from morphology and ecology to test the hypothesis that *S. irrigua* is widely disjunct between the southern Rocky Mountains and the Altai Mountains of southern Siberia. Under the phylogenetic expectations of this hypothesis, we would predict to recover the entity known as *Stellaria irrigua* in the southern Rocky Mountains to have a sister taxon relationship to *S. irrigua* from the Altai Mountains, or to be embedded within the broader Altai lineage. We additionally sampled several other species of *Stellaria* to gauge the efficacy of using RADseq data to more broadly resolve phylogenetic relationships within the genus. Our results suggest that RADseq likely represents a powerful and practical phylogenetic tool in Caryophyllaceae, and that a revised circumscription of the *S. irrigua* complex is warranted.

### MATERIALS AND METHODS

**Field, herbarium, and morphological study.** — To facilitate better understanding of Asian and North American material attributed to *Stellaria irrigua*, type material was studied at the LE Herbarium. Because of the age of the holotype of *S. irrigua* (early 19th century) as well as its depauperate condition, fieldwork to discover additional, extant populations of this species in Asia was conducted focusing on the Altai Mountains, particularly in the region where type material of this species originated (i.e., the mountains around the Chuya River valley). In North America, fieldwork in the southern Rocky Mountains was conducted to collect recent material of this species. On both continents, we attempted to study and collect as geographically and morphologically broad of a range of representative populations as possible. Besides this species, other *Stellaria* (and outgroup) samples suitable for molecular study were collected in the aforementioned regions and adjacent areas. Newly collected herbarium specimens were deposited at the following institutions: COLO, NY, and RM. We supplemented new field collections with sampling or study of existing herbarium materials at, or from, the following institutions: ALTB, BRIT, CAS, COLO, DAO, K, LE, MO, NDG, NSK, NY, P, RSA, US, and VLA (see Revised Taxonomic Concepts). Herbarium study included examination of type material of all names of species related to *S. irrigua*, including *Alsine polygonoides* Greene ex Rydb., *Stellaria gonomischa* Boivin, *Stellaria subumbellata* Edgew., *S. umbellata*, and *S. weberi* Boivin.

Because prior research (Mahdavi & al., 2012) along with our own studies suggested that seed coat morphology may be taxonomically informative in *Stellaria*, we used scanning electron microscopy to document seed morphologies of *S. irrigua* and close relatives from Asia and North America. Seeds were removed from mature capsules of fresh specimens and mounted onto JEOL aluminium SEM stubs, then coated with gold using a Cressington 108 Sputter Coater. Seed imaging was conducted using a JEOL JSM-6480 Scanning Electron Microscope housed in Ramaley Hall at the University of Colorado (Boulder).

**Molecular analysis.** — Double-digest restriction site-associated DNA sequencing (ddRADseq) was implemented to reconstruct phylogenetic relationships among 37 samples representing 14 species of *Stellaria* plus two very closely related outgroups based on Greenberg & Donoghue (2011). These two outgroups—*Cerastium beeringianum* Cham. & Schltdl. and *Pseudostellaria jamesiana* (Torr.) W.A.Weber & R.L.Hartm.—were selected in attempt to minimize locus dropout due to increased phylogenetic divergence among taxa (Eaton & al., 2017; Tripp & al., 2017), and we rooted all trees on the *P. jamesiana* branch. Our ingroup sampling of the *Stellaria irrigua* species complex included: 1 accession...
of *S. irrigua* from Asia, 5 accessions of *S. irrigua* from North America, and 17 accessions of collections originally attributed to *S. umbellata* or *S. subumbellata*, these from eastern Asia as well as western North America. The remaining 12 accessions represented a diversity of subclades of core *Stellaria* based on results of Greenberg & Donoghue (2011) and were included to assess the efficacy of using RAD loci in reconstructing relationships across the genus as well as to place results from our investigation of *S. irrigua* into a broader phylogenetic context. Voucher information of all samples included in our molecular analyses can be found in Appendix 1.

DNA extractions followed the CTAB protocol (Doyle & Doyle, 1987). A double-digest RADseq protocol adapted from Parchman & al. (2012) and further customized in Tripp & al. (2017) was used for library preparations and is briefly described here. Genomic DNA extractions containing an average of 150 ng/ml of DNA were subjected to double digestion with the restriction enzymes EcoRI and Msel. Custom-designed barcodes of variable length (7–10 bp) were ligated onto restriction fragments to facilitate multiplexing of 96 samples in pooled libraries. The barcoded restriction-ligation reaction products were stored at 4°C overnight. Products were then PCR amplified, samples were pooled, and the resultant products were run on 1% mixed half-and-half multipurpose molecular biology grade agarose/Invitrogen UltraPure 1000 high resolution agarose gels. DNA smears were size-selected by hand, targeting the 200–500 base pair region to improve comparison of homologous fragments across samples. Gel excisions were purified using QIAquick Gel Extraction Kits according to the manufacturer’s protocol. The end-product pooled libraries were submitted to the University of Colorado’s BioFrontiers Next-Generation Sequencing Facility (Boulder, Colorado), where they underwent a second round of size selection with BluePippin and were sequenced on an Illumina HiSeq 2000 with a V3 100-Cycle Single Read Sequencing Kit or a NextSeq 500 with a V2 High-Output 75-Cycle Single Read Sequencing Kit.

Raw reads were first examined using FastQC (Andrews, 2010) to assess data quality and then demultiplexed and filtered using process_radtags, a module within the Stacks pipeline (Catchen & al., 2013). Flags -c and -q were set as additional filtering parameters, with an Illumina adapter mismatch of 3 allowed and both cut sites specified. FastQC was employed a second time to ensure high quality of data post-trimming. Resulting trimmed and demultiplexed reads were then used to create a RAD locus alignment for all samples in pyRAD v.3.0.66 via *de novo* assembly, with any low-quality bases further excluded from final alignments (Eaton, 2014). Select samples were separately aligned and visualized against the public *Silene latifolia* reference genome using SAMtools to ensure RAD loci were representative of Caryophyllaceae (Li & al., 2009), but *de novo* assembly was employed for final dataset assembly because it yields datasets with significantly less locus dropout (see Tripp & al., 2017 for comparison of *de novo* to reference-based assembly). Various exploratory parameters were implemented in pyRAD to yield a set of most optimal parameters for downstream analyses. In relation to this, to explore the effects of missing data on topology and support values, we ran additional series of analyses spanning varying thresholds of missing data. We produced alignments with varying amounts of missing data by altering the pyRAD parameter that only aligns RAD loci shared by X number of taxa in the input data files—the more loci needed to be shared between samples to be output, the less resultant missing data and the smaller the output alignment. Final parameters differing from the default included those pertaining to (1) clustering within samples (reads clustered at a similarity threshold of 0.88; minimum coverage of two reads to retain a locus) as well as (2) clustering between samples (0.88 similarity threshold and a minimum of four of 37 samples required to share a locus for retention in the final alignment).

Phylogenetic inference was conducted using RAxML v.8.2.9 on the concatenated, final output alignments under a GTRCAT model of sequence evolution (Stamatakis, 2014). To assess branch support, we implemented rapid bootstrapping analyses in RAxML with 100 replicates and exported the most highly supported replicate in each case. All bioinformatic tasks from demultiplexing up to phylogenetic inference were conducted on the JANUS supercomputer (subsequently replaced by SUMMIT) at the University of Colorado (Boulder). Raw phylogenomic sequence data, in the form of one fastq file for each individual/tip sampled, are available at https://www.ncbi.nlm.nih.gov, under SRA Accession #SRP149934.

### RESULTS

**Morphology and ecology.** — Bunge’s type specimen of *Stellaria irrigua* from Altai is composed of three juvenile, poorly developed individuals plus two disembodied flowers. The single mature fruit on this specimen is a capsule with valves that far exceed the sepals (Fig. 1C). Our study of newly collected field specimens plus existing herbarium materials from Asia and North America revealed numerous collections that share this and other features (see below) and thus can be confidently attributed to the name *S. irrigua*. These collections indicate that this species is far more common and geographically widespread than previously understood. In contrast, material from a previously unrecognized, monophyletic lineage from the southern Rocky Mountains (see Molecular Data below) bears capsules with valves that are equal or subequal to the sepals. Numerous additional morphological differences separate these two entities, as shown in Figs. 1–5 and 8 and as described in Table 1. Ecologically, plants of *S. irrigua* consistently inhabit (during their period of growth) poorly drained, moist substrata from upper montane mossy creaksides to damp areas of boreal subalpine forests, to late summer snow-holding arctic and alpine tundra, both in Asia and North America (e.g., Fig. 2B–D). In contrast, plants of the previously unrecognized entity are narrowly restricted and endemic to very dry, well-drained, exposed alpine scree slopes of the southern Rocky Mountains (Figs. 2A, 3). Early pressed material of three names relevant to this study—*Alpine...
polygonoides, S. irrigua, and S. umbellata—displays some of the morphological differences detailed in Table 1 (Fig. 4). Although populations of A. polygonoides have been previously attributed to S. irrigua (Weber, 1961), taxonomic, morphological, and ecological data in combination with molecular results presented here constitute strong evidence for a separately evolving lineage in the southern Rockies. Below, we propose the nomen novum Stellaria sanjuanensis (= Alsine polygonoides Greene ex Rydb.) to accommodate this lineage.

Molecular data. — RADseq data used in this study resolved phylogenetic relationships within the Stellaria irrigua complex as well as among samples of Stellaria with very high ML bootstrap support (Fig. 5). Our final alignment (that utilized nearly the maximum amount of phylogenomic data generated; suppl. Appendix S1) comprised 37 tips and contained 72,941 concatenated RAD loci with a total length of 6,128,319 nucleotides, 455,184 variable (SNP) sites, and the percent of missing data (undetermined N sites and gaps) in this alignment was 76%. Alignments of shorter lengths (ranging from 88,715 to 2,330,025 bases, and anywhere from ~15% to 52% missing data; Fig. 6; suppl. Appendices S2–S5) as well as alignments composed only of SNP data almost always resolved the same topologies as those inferred from our final concatenated locus alignment (Fig. 5), and these smaller alignments generally had comparable albeit slightly lower bootstrap support at some nodes (Fig. 6; SNP-inferred phylogenies not shown). All but two nodes were resolved at or near (two other nodes supported with values of 99% and 97%) 100% bootstrap support in the 76% missing data tree; these two nodes involved short branches and very closely related populations within species (Fig. 5).

Our data recovered two Asian clades plus one southern Rocky Mountains clade of S. irrigua, with the North American populations embedded within the broader Asian lineages (Fig. 5). Geographic structure within the Asian clades was limited: neither populations from the Altai Mountains nor the Sayan Mountains formed monophyletic groups (Fig. 5). Within the southern Rockies, S. irrigua as sampled occurs from the Park Range of northern Colorado to the South San Juan Mountains of southern Colorado; two accessions from the South San Juan Mountains formed a strongly supported clade (100% BS) whereas five populations from northern Colorado showed little geographic structure, although intrapopulation samples were recovered as monophyletic (Fig. 5).

Sister to the southern Rockies clade of Stellaria irrigua is the ecologically and morphologically distinctive lineage of S. sanjuanensis nom. nov. that phylogenetic data resolved as monophyletic with strong support (Fig. 5). Plants of this lineage are found from southwestern Colorado to northern portions of the Sangre de Cristo Mountains in New Mexico (Fig. 7). Despite extensive fieldwork in similar biomes and habitats in the Altai Mountains and elsewhere in central, southern, and eastern Asia, no representatives of S. sanjuanensis were found in that hemisphere. Likewise, new populations of this species have not been discovered during either authors’ various field trips throughout alpine areas of the mountainous western United States and Mexico, nor have specimens been seen at or from any institution outside of the distribution shown in
Table 1. Salient habitat and morphological differences between the southern Rocky Mountains endemic *Stellaria sanjuanensis* nom. nov. and the widespread eastern Asian–western North American *S. irrigua*.

<table>
<thead>
<tr>
<th>Character</th>
<th><em>Stellaria sanjuanensis</em></th>
<th><em>Stellaria irrigua</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Habitat</td>
<td>Well-drained alpine screes and tuffs</td>
<td>Moist boreal and arctic-alpine substrata</td>
</tr>
<tr>
<td>Habit</td>
<td>Fleshy shoots; long-sprawling rhizomes</td>
<td>Not fleshy; plants solitary or densely clumped</td>
</tr>
<tr>
<td>Leaves</td>
<td>Purple/dark green</td>
<td>Light green/not conspicuously anthocyanic</td>
</tr>
<tr>
<td>Bracts</td>
<td>Indistinguishable from other leaves</td>
<td>Reduced, scarious</td>
</tr>
<tr>
<td>Inflorescence</td>
<td>Solitary or 2 flowers arising from same axil</td>
<td>Terminal, subumbellate cymes</td>
</tr>
<tr>
<td>Perianth</td>
<td>Petals ~2 mm, ~2/3 length to subequaling sepals</td>
<td>Petals absent to rarely minute, &lt;1 mm</td>
</tr>
<tr>
<td>Capsules</td>
<td>Subequaling sepals at maturity</td>
<td>Exceeding sepals at maturity</td>
</tr>
<tr>
<td>Seeds</td>
<td>Testa cells interleaved and ridged</td>
<td>Testa smooth</td>
</tr>
</tbody>
</table>

Fig. 2. Differences in habitat between *Stellaria sanjuanensis* nom. nov. and *S. irrigua*. A, Dry, exposed alpine screes representing the sole habitat in which the southern Rocky Mountains endemic *S. sanjuanensis* occurs. Several individuals are encompassed by each of the ellipses. B, *Stellaria irrigua* on damp, mossy screes in the Kazakh Altai Mountains fed by persistent snowmelt. Ellipse indicates a tuft of individuals. C, *Stellaria irrigua* on late-snowmelt portions of tundra, holding moisture throughout a typical growing season in northern Colorado (Niwot Ridge, September 2017). Note subumbellate cymes. D, *Stellaria irrigua* in damp, grassy areas at the base of rock faces in alps of the Chuya River valley, Altai Mountains, Russia. Ellipse indicates umbellate inflorescences of several individuals. — Photos: Mat Sharples.
Fig. 7. The distinctiveness of *S. sanjuanensis* is further highlighted by its geographical co-occurrence with plants of *S. irrigua*: the “SSJ” populations of *S. sanjuanensis* sampled here occur less than 20 air kilometers from our sampled “SSJ” populations of *S. irrigua*—both of these from the South San Juan Mountains—but are clearly not sister taxa (Figs. 5–7). Furthermore, the “SSJ” *S. irrigua* populations are embedded within a clade of *S. irrigua* populations from throughout northern Colorado (Figs. 5–7).

Other phylogenetic relationships among *Stellaria* and outgroups recovered in this study largely support the results of Greenberg & Donoghue (2011). Although our outgroup sampling was very limited, *Cerastium beeringianum* was sister to core *Stellaria* when trees were rooted using *Pseudostellaria jamesiana*, both of these sampled from Colorado. This result is similarly supported by our ongoing work in Caryophyllaceae, which includes a much broader sampling of outgroups (M. Sharples & E. Tripp, in revision). In the present study, petiolate members of *Stellaria* formed a clade with strong support: *Stellaria media* (L.) Vill. (Colorado)+*Stellaria nemorum* L. (Europe)+*Stellaria bungeana* Fenzl (Altai) (Figs. 5, 6). Our results additionally suggest that *Stellaria longipes* Goldie (Colorado) and *Stellaria longifolia* Muhl. ex Willd. (Colorado) are close relatives, and that *Stellaria brachypetala* Bunge (Altai) is also related to the former two. The Californian coastal endemic *Stellaria littoralis* Torr. is here placed phylogenetically for the first time as a close relative to the circumpolar, shoreline-inhabiting *Stellaria humifusa* Rottb. (Norway).

**DISCUSSION**

Across the range of *Stellaria irrigua*, there exists a clear phylogenetic distinction between Asian and North American
populations sampled here. This geographical separation of North American from Asian populations may be driving evolutionary divergence between populations on the two continents despite morphological and ecological cohesion. Nevertheless, our phylogenetic results in combination with morphological and ecological features shared among plants from these two distant localities support a broad eastern Asian–western North American connection in this Stellaria species complex. While Weber’s (2003) hypothesis posits such a connection, he also put forward the notion of subsequent extinction of intervening populations in oroboreal portions of a formerly much broader geographical range. In contrast to the latter, our results clearly establish the presence of a widespread and relatively abundant (rather than very rare) species. This species, S. irrigua, traverses a broad, amphi-Beringian oroboreal arc ranging more or less contiguously from cold elevations and latitudes throughout western North America to cold elevations and latitudes of eastern Asia, to as far west as the western Himalaya and Altai (Fig. 7). Himalayan plants have previously been referred to as S. subumbellata, a taxon that is not distinguishable from S. irrigua (see Revised Taxonomic Concepts). We recovered no phylogenetic evidence for a unique relationship between plants of the southern Rocky Mountains and the Altai Mountains: Altai plants were not specifically sister to any of the southern Rocky Mountains plants (but rather, were sister to either Sayan or Himalaya individuals sampled), and while the southern Rockies plants are indeed embedded within otherwise eastern Asian lineages, these eastern Asian plants include individuals from Altai, Himalaya, Sayan, and Kamchatka, with little phylogenetic-geographical structure recovered across these areas. The expanded geographical concept of S. irrigua presented here requires treatment of a later and more familiar name, S. umbellata, as a synonym of S. irrigua, which is supported by our study of type material, study of collections from across much of the geographical range of these plants, and molecular data (Figs. 1, 4, 5; see Revised Taxonomic Concepts). This name, S. umbellata, has been in common usage for over a century to represent the widespread eastern Asian–western North American species, but our results support the earlier name S. irrigua as applying to this taxon.

Our best-estimate RADseq phylogeny supports a scenario of in situ speciation in southern ranges of the southern
Rocky Mountains of *Stellaria sanjuanensis* from a more widespread ancestor, *S. irrigua*. Mechanisms of speciation of the former from the latter are likely to have included ecological isolation of *S. sanjuanensis* when plants reached alpine scree slopes of volcanic southern Colorado, after having migrated eastward from eastern Asia and southward into southern portions of western North America. We hypothesize that this migration occurred via Beringia given the contemporary presence of *S. irrigua* there, and that this likely happened during the Pleistocene given the lack of arctic tundra habitat at present-day arctic latitudes during the Tertiary (Fig. 7) (Basinger & al., 1994). Future molecular dating work across all of *Stellaria* will help address this hypothesis (M. Sharples & E. Tripp, in prep.). Despite the close phylogenetic affinity of *S. sanjuanensis* and *S. irrigua*, the southern Rockies endemic is sufficiently distinctive from *S. irrigua* to warrant recognition at the species level. Though this recognition renders *S. irrigua* paraphyletic, *S. sanjuanensis* has never been confused in the literature for well over a hundred years, and we provide evidence that these two lineages are evolving under distinct trajectories. Central to this evidence is the narrow ecology of, and hence dispersal limitations to, *S. sanjuanensis* (i.e., restricted to mostly volcanic substrata above treeline), as well as likely reproductive isolation of species of *Stellaria* that have divergent floral morphologies (i.e., one petalous and the other apetalous; M. Sharples, unpub. data). Our recognition of *S. sanjuanensis* as a distinct species is further vindicated by the fact that both Porsild and Hultén could not identify the endemic southern Rocky Mountains scree-restricted taxon as anything they had seen in *Stellaria* anywhere before, including *S. umbellata* (Weber, 1961). This narrow endemic was first described as *Alsine polygonoides* (by Greene in Rydberg, 1906), but this name has rarely appeared in subsequent literature, perhaps in part because it is an illegitimate name (Jessen, 1879; Prantl, 1884; Sprague, 1920).

Paraphyletic taxa above the rank of species are commonplace and are often, implicitly accepted by scientists who teach such examples in the classroom. At a broad scale, a few common examples include prokaryotes (without inclusion of eukaryotes), reptiles (rendered paraphyletic by lack of...

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**Fig. 5.** Maximum likelihood phylogeny of the *Stellaria irrigua* complex and additional relatives as inferred from RAD loci. The new name *S. sanjuanensis* is here represented by five individuals from the South San Juan Mountains and nearby Sangre de Cristo Mountains that form a well-supported, monophyletic group nested within a paraphyletic assemblage of Asian and North American populations of *S. irrigua*. All branches have 97% or higher bootstrap support (32 out of 36 nodes resolved with 100% support) except for two nodes marked by circles (lowermost: 82% in the *S. sanjuanensis* clade; uppermost: 87% in one Asian *S. irrigua* clade). Boxed populations of SSJ *S. irrigua* are sympatric with *S. sanjuanensis*, further supporting the monophyly and distinctiveness of *S. sanjuanensis*. Abbreviations: ALT, Altai Mountains; HM, Himalaya; ID, Idaho; KM, Kamchatka; NCO, northern Colorado Mountains; SoRo, southern Rocky Mountains; SSJ, South San Juan Mountains; SY, Sayan Mountains. *Inset*, petal differences between *S. sanjuanensis* (A) and *S. irrigua* (B) represent one of several morphological features distinguishing these two taxa (Table 1). Yellow lines in A run parallel with and indicate extent of a single bifid petal. B shows a flower entirely absent of petals at a similar developmental stage to that in A. Well-developed petals most likely reappeared in the most recent common ancestor of the clade containing all accessions of *S. sanjuanensis*, as depicted. — Photos: Mat Sharples.
Fig. 6. Cladograms inferred from alignments with lower missing data thresholds than those presented in Fig. 5. Percent missing data per alignment is as follows. A, 15.86%; B, 25.36%; C, 44.38%; D, 52.31%. Topologies and topological layouts are identical to those in Fig. 5 except in these few examples of incongruencies: A and B infer a relationship of *Stellaria longifolia* as sister to *S. brachypetala*, as opposed to *S. longifolia* as sister to *S. brachypetala*+*S. peduncularis*+*S. longipes*; in A, one specimen of *S. sanjuanensis* is more closely related to a different specimen of that species from the San Juan Mountains; and the exact position of the *S. irrigua* specimen from Krasnoyarsk Krai (= *Stellaria irrigua* SY1) slightly differs in both the 15% and 25% alignments. The relatively poor support in the *S. sanjuanensis* clade in A is likely solely attributable to generally lower data amounts for those samples (see SRA data for comparison of file sizes). That is, when we raised the minimum number of taxa sharing a locus in the final alignment used to infer this phylogeny, these samples lost a significant number of their locus representatives in the alignment. As the most significant topological incongruency involves *S. longifolia* and close relatives, hybridization or other phenomena may be at work in the evolutionary history of this group (see Discussion).
of inclusion of avians), bony fishes (without inclusion of tetrapods), algae, the ANITA Grade in flowering plants, bryophytes, and lichens (Raven & al., 2005; Urry & al., 2016). On a more narrow scale, and within flowering plants, examples include genera of Hawaiian lobelia (Givnish & al., 2009), Ranunculus subg. Auricomus (Hörandl & Emadzade, 2012), Hawaiian mints (Welch & al., 2016), and numerous other higher taxonomic ranks within angiosperms (Willner & al., 2014). However, despite advocacy for monophyly of lineages at the rank of species by numerous practicing systematists, paraphyletic species oftentimes reflect evolutionary processes (Crisp & Chandler, 1996; Hörandl, 2006). In such instances, sophisticated methods of phylogenetic inference such as coalescence-based approaches aid in the recognition and understanding of paraphyletic lineages (Harrington & Near, 2011), and such an approach may be appropriate in the future within certain lineages of starworts. Lineages that can readily hybridize—thereby obscuring true species trees—are also regularly still recognized at the rank of species (Hörandl, 2006); many species shown to have experienced recent introgression are customarily still recognized with binomials (e.g., within Vaccinium: Beeler & al., in press). Although we did not explicitly test for introgression in this study, if hybridization between S. irrigua and S. sanjuanensis were prevalent, we would expect the sympatric populations of S. irrigua in the South San Juan Mountains to be more closely related to S. sanjuanensis than they are, and we would expect intermediate taxa between the two lineages to be represented in herbarium collections.

Species endemic to islands recently derived via dispersal from mainland populations should recover the same phylogenetic patterns as those recovered here: a monophyletic descendant embedded within an ancestral lineage that is more widespread, yet rendered paraphyletic, by the island dispersal event, which may be followed by reproductive isolation and peripatric speciation (e.g., Valtueña & al., 2017). In the case of Stellaria sanjuanensis, present-day geographical isolation seems less at work in maintaining species boundaries than do edaphic and other forms of ecological isolation; this system is thus consistent with models of parapatric rather than peripatric speciation, though the southern Rocky Mountains may be

Fig. 7. Geographical distributions of Stellaria irrigua (widespread) and S. sanjuanensis nom. nov. (endemic to southern Rocky Mountains). Triangles represent digitized as well as newly collected populations of S. irrigua, while stars represent all currently known populations of S. sanjuanensis (SEINet, 2013–; GBIF Secretariat, 2017). Blue triangles (Asia), green triangles (North America), or orange triangles (southern Colorado) represent individuals of S. irrigua included in phylogenetic analyses; the orange triangles represent sampled S. irrigua individuals that are broadly sympatric with S. sanjuanensis. Inset, distribution of both species in Colorado and far northern New Mexico. Red stars represent individuals of S. sanjuanensis included in phylogenetic analyses. Note sympathy of the sampled populations of the two species in the South San Juan Mountains of southern Colorado. The worldwide map was constructed with ArcGIS v.10.5; the inset map was constructed with D-Maps (https://d-maps.com/carte.php?num_car=19882).
considered its own sort of terrestrial “sky island”. Related and not necessarily mutually exclusive modes of speciation that yield paraphyletic species include progenitor-derivative (i.e., budding) speciation, centrifugal speciation, and catastrophic speciation (Anacker & Strauss, 2014). In an empirical study in the Helianthus petiolaris complex, Rieseberg & Brouillet (1994) speculated that progenitor-derivative speciation is a rather important process in plants. They and others have further hypothesized that a very high percentage of plant species (upwards of 25%–50%) may be expected to have paraphyletic origins through a wide array of speciation processes; consequently, these authors argue that strictly monophyletic plant species are untenable (Crisp & Chandler, 1996; Hörandl, 2006). Other examples of paraphyletic species-rank taxa, sometimes referred to as “paraspecies”, can be found outside of plants, such as in Anas, Bufo, Nesticus, Peromyscus, and Ursus (Avise, 2000; Crandall & al., 2000). Rejecting paraphyletic species can furthermore have negative conservation implications (Crandall & al., 2000).

Indeed, the case of Stellaria sanjuanensis closely resembles that of Pozoa volcanica Mathias & Constance (Apiaceae) in relation to P. coriacea Lag. in the southern Andes, with the former comprising a monophyletic, volcanic, edaphic endemic lineage embedded within a paraphyletic assemblage of P. coriacea, a characteristic phylogenetic pattern of progenitor-derivative speciation (López & al., 2012). The California Floristic Province genus Layia similarly reflects progenitor-derivative speciation: the widespread L. glandulosa (Hook.) Hook. & Arn. is rendered paraphyletic by evolution of the monophyletic edaphic endemic L. discoidea D.D.Keck nested within it (Baldwin, 2005). Such speciation patterns have been found to be extremely widespread in angiosperm evolution (e.g., Crisp & Chandler, 1996; Perron & al., 2000; Jaramillo-Correa & Bousquet, 2003; Grossenbacher & al., 2014; Chung & al., 2015). Here, we present strong evidence demonstrating progenitor-derivative speciation in Stellaria using RADseq data. This mode of speciation may be very common across other systems characterized by narrowly endemic taxa: signatures of progenitor-derivative speciation were phylogenetically widespread in a meta-analysis on plants from the California Floristic Province (Anacker & Strauss, 2014). We hypothesize that further work exploring origins of range-restricted, high-elevation species in the southern Rocky Mountains may reveal these mountains as an under-recognized region of phylogenetically widespread progenitor-derivative speciation.

The purported disjunction of Stellaria irrigua was the sole, improbable example of a plant species found only in Altai and in the southern Rocky Mountains and was hypothesized to be particularly representative of a deep Tertiary-Quaternary vicariance event affecting multiple members of the floras from both regions (Weber, 2003). Our results indicate that previous skepticism towards the hypothesized long-distance disjunction between the southern Rockies and Altai was not unwarranted (Kozhevnikov, 1994; Czerepanov, 1995; Morton, 2005). Indeed, the likelihood of a range-restricted, narrowly adapted, and narrowly distributed species in the southern Rockies is inherently greater than that of a singularly peculiar intercontinental disjunction between this region and elsewhere. The alpine life zone of the southern Rocky Mountains alone is already reported to harbor some 25 to 26 endemic taxa (Fowler & al., 2014), likely an underestimate and perhaps a reflection of the highly circumscribed niches of species adapted to this region. Thus, in light of results herein presented in combination with prior knowledge of a relatively high number of endemic species reported from an ecosystem inherently inhospitable to many other lineages, it may be expected that future phylogenetic studies on alpine plants in western North America may similarly reveal evidence of overlooked speciation in such ecosystems.

Fig. 8. A, Seeds of Stellaria sanjuanensis nom. nov. (M. Sharples 1367, Colorado) have testae that are distinctly interleaved and ridged. B, Seeds of S. irrigua lack visible testa boundaries (M. Sharples & S. Smirnov 1250, Altai).
Despite evidence here presented, there exists a number of other plant species that serve as candidates for long-distance range disjunctions between central or eastern Asia and the southern Rocky Mountains. These study systems, which require expanded investigation that in part incorporates high-resolution phylogenetic information, include Artemisia laciniata Wild., Eutrema edwardsii R.Br., E. salsugineum (Pall.) Al-Shehbaz & Warwick, and Gentiana algida Pall. (Weber, 2003; Wang & al., 2015). In some of these instances, immense stretches of inhospitable continental interiors separate putatively conspecific populations (though present in the Arctic) between central Asia and the southern Rockies; in other instances, a number of northern and temperate stations exist between the two geographical extremes. However, none of the hypothesized disjunctions is as dramatic as that proposed between the two geographical extremes. However, none of the hypothesized disjunctions is as dramatic as that proposed for Stellaria irrigua, which our investigation has rejected. Phylogenetic knowledge combined with field studies will help to more broadly characterize floristic histories within and amongst these two regions as well as to provide more robust tests of Weber’s (2003) as yet still underexplored hypothesis.

To our knowledge, the present study represents the first use of RAD loci to resolve phylogenetic relationships within Caryophyllaceae (here, specifically in tribe Alsineae cf. Harbaugh & al., 2010). Our results confirm the utility of these data for phylogenetic inference among closely related species of flowering plants (e.g., Hipp & al., 2014; Hou & al., 2015, 2016; Massatti & al., 2016; Eaton & al., 2017; Tripp & al., 2017). Specifically, RAD loci utilized in our study resolved population- to species-level relationships, although the latter was not an explicit goal of our investigation and thus outgroup inclusion was minimal—sampling was geared primarily towards ingroup representation. Furthermore, samples of different individuals from the same populations of Stellaria irrigua were unambiguously always recovered as monophyletic (i.e., Stellaria irrigua_ALT1 & 2, Stellaria irrigua_ALT-Chuya1 & 2, Stellaria irrigua_NCO1 & 2, and Stellaria irrigua_NCO4 & 5). In view of these results, RADseq represents a powerful tool for investigating questions across multiple phylogenetic scales in Caryophyllaceae. Given numerous groups within the family have been challenged with robust phylogenetic resolution and taxonomic placement as a function of convergent or parallel evolution of non-sister taxa upon very similar morphologies, including within Minuartia and relatives (Dillenberger & Kaderet, 2014) as well as within Stellaria itself (Greenberg & Donoghue, 2011), RAD sequencing may prove a fruitful approach to forthcoming phylogenetic reconstruction and taxonomic classification in the family, which have heretofore involved morphology or DNA alignments of relatively few loci (e.g., Scheen & al., 2004; Harbaugh & al., 2010; Greenberg & Donoghue, 2011). Although prior studies have expressed some concern over inflated bootstrap values and phylogenetic incongruence associated with data concatenation (e.g., Lambert & al., 2015; Fernández-Mazuecos & al., 2018), our confidence in the phylogenetic relationships recovered with these data further derives from salient macro-morphological and ecological differences, the geographical patterns inferred, and the generally high level of congruence with the primary extant phylogenetic hypothesis of Stellaria (Greenberg & Donoghue, 2011).

Our RAD sequencing analyses across a diversity of subclades within Stellaria suggest that these data are likely to resolve a more extensive set of relationships across the genus. As mentioned, the petiolate S. bungeana, S. media, and S. nemorum form a strongly supported clade, in agreement with results from prior investigation (Greenberg & Donoghue, 2011). Our results also place the S. longipes group with some increased resolution. We demonstrate for the first time that S. longifolia may be phylogenetically distinct from, yet closely related to, S. longipes, a species with which the former is thought to hybridize over evolutionary time scales (Chinnappa & al., 2005). Previously recognized closer relatives of S. longipes, such as S. peduncularis Bunge (Altai), may ultimately be supported as regional variants of S. longipes pending further sampling of S. longipes across its distribution; as S. longipes is circumboreal, widespread, morphologically plastic, and replete with localized synonyms, much expanded sampling of this species across its range is needed. Stellaria brachypetala, although a member of the putatively hybridizing S. longipes–S. longifolia species complex, is supported by morphology and phylogeny as a distinctive species rather than as a regional variant or synonym of either entity. We also demonstrate that the circumboreal S. borealis, S. humifusa, and the California endemic S. littoralis share a more recent common ancestor with core ingroup taxa sampled here than with S. longipes and relatives, which conflicts with previous results suggesting that the former three are part of the same clade as the S. longipes complex (Greenberg & Donoghue, 2011). In contrast to suggestions by Morton (2005), S. littoralis of California is not at all closely related to S. dichotoma L. from the steppes of Asia, a taxon that most likely should be transferred to a different genus based on phylogenetic and morphological characteristics (M. Sharples & E. Tripp, unpub. data). Further work will more clearly elucidate phylogenetic relationships among these and other species of Stellaria using a greatly expanded RAD locus database.

### REVISED TAXONOMIC CONCEPTS


**Note.** – See below regarding Tiehm’s (1989) erroneous lectotypification of this name.
Geographical and ecological notes. – As currently understood, *Stellaria sanjuanensis* is narrowly restricted to dry, exposed alpine scree slopes of usually volcanic origin in the San Juan, Elk, and Sangre de Cristo Mountains of Colorado, extending into more southerly portions of the Sangre de Cristo Mountains in far northern New Mexico (Figs. 3, 7). Oddly, it is not known from the Sawatch Mountains, with ostensibly suitable habitat less than 10 air kilometers from vouched localities in the Elk Mountains across the Taylor River valley.

Taxonomic and nomenclatural notes. – The holotype and isotype cited above (originally identified by M.E. Jones as “*Arenaria saxosa* Gray var.”) represent the earliest known collections of *Stellaria sanjuanensis*. The locality information on these specimens (C.F. Baker & al. 515) matches verbatim that referenced in the protologue of *Alpine polygonoides*. Digitized herbarium specimens (viewable on https://island.plants.org) of a different collection, C.F. Baker 307 (K barcode K000742072!, MO barcode MO-216575!, NDG barcode NDG16455!, NY barcode NY03423232!, US barcode US0610728!), represent material used by Tiehm (1989: 153) in attempt to lectotypify the name *A. polygonoides*. However, C.F. Baker 307 was collected “Near Pagosa Peak”—dozens of kilometers to the east of Little Kate Basin—and thus cannot be considered original material. Tiehm’s lectotypification is thus in conflict with the protologue and must therefore be revised following Article 9.19 in Turland & al. (2018). Because the epithet “polygonoides” is occupied in *Stellaria* (Jessen, 1879), a new name is proposed here.


Additional syntype: Tibet, Nubra, alt. 11–15,000 ft., T. Thompson s.n. (K barcode K000726353).


Geographical and ecological notes. – Stellaria irrigua is widespread in boreal and tundra habitats across the mountains of western North America and eastern Asia, including suitable habitats at high northern latitudes (i.e., ≥71°N). Unlike St. sannjuanensis, which occupies dry, well-drained alpine volcanic and sedimentary tuffs and screes below 39°N, S. irrigua is not found on substrata that lack consistent moisture throughout the majority of the growing season.

Taxonomic notes. – The type material of Stellaria irrigua is immature and indistinguishable from early developmental stages and type material of St. umbellata (Fig. 4). In comparing the protologues of S. irrigua (from Altai) and S. umbellata (from the geographically proximal Sayan Mountains near Lake Baikal), the descriptions are for the most part identical where they overlap in characters (except for one feature pertaining to minute to absent petals). Indeed, Turczaninow (1842) noted that his S. umbellata was very similar to Bunge’s S. irrigua. Observations by the first author in North America and Asia indicate that plants of S. irrigua sometimes have, but more often lack, minute petals. Thus, this character appears to be variable across the full range of the species.

Stellaria subumbellata Edgew. is here placed into synonymy with S. irrigua due to having overlapping morphological and habitat characteristics with Stellaria irrigua, distinct only in being geographically situated in the Himalaya. Our phylogenetic results (Fig. 5: “Stellaria_irrigua_HM”) place the entity from the Himalaya as embedded within S. irrigua, and the protologue (Edgeworth & Hooker, 1874) itself notes that the new entity was “very near the Baikal S. umbellata, Turcz.”.

Nomenclatural notes. – Although the name Stellaria umbellata has been used broadly for many decades and on two different continents, the name S. irrigua is validly published and has priority. A formal proposal to conserve the name S. umbellata would be needed to allow retaining S. umbellata.


Key to the native montane Stellaria species of the southern Rocky Mountains

1. Leaves approximately or more than three times as long as wide, linear to very narrowly lanceolate or elliptic; petals equaling or exceeding sepals; bracts scarious ...............2
2. Leaves less than three times as long as wide, lanceolate, elliptic, or ovate; petals equaling sepals, shorter than sepals, or lacking; bracts leafy or scarious ...............3
3. Inflorescence terminal; flowers often solitary or sometimes few ............................................................................. Stellaria longipes
4. Inflorescence axillary; flowers in compound cymes .......... Stellaria alsine
5. Inflorescence terminal, umbelletum and often further branched (= subumbellate); flowers three to numerous; petals absent to sporadically minute (<1 mm) ............... Stellaria irrigua
6. Inflorescence axillary; flowers solitary or 2–5 in cymes, these cymes sometimes secondarily branched; petals present and up to 2/3 length of sepals ........... Stellaria alsine

RADseq rejects disjunction in Stellaria
6. Creeping/sprawling habit; leaves broadly elliptic to widely ovate, of similar width and length or slightly longer than wide, often with tiny petioles; flowers solitary and axillary; petals lacking..........................Stellaria obusa

6. Plants not creeping/sprawling; leaves lanceolate to elliptic, longer than wide, epetiolate; flowers solitary and axillary or multiple in terminal cymes; petals present or lacking........................................................................................................

7. Plants fleshy; leaves tinged to mostly deeply maroon; internodes generally greater than length of leaves; flowers ± solitary and axillary; petals lacking....................................Stellaria sanjuanensis

7. Plants not fleshy; leaves green; internodes generally greater than length of leaves; flowers solitary and axillary or multiple in terminal cymes; petals up to 2/3 length of sepals or absent..........................Stellaria borealis complex

Note. – Stellaria alsine Grimm has not been previously reported from Colorado, or indeed the Rocky Mountains. Thus the report here is novel, with the closest known stations being in Minnesota or Washington state (Morton, 2005). The only known occurrence of Stellaria alsine in Colorado to date is from the following collection event. It is presumed native due to the high-quality habitat indicated on the collection label, but it possibly alternatively represents an anthropogenic introduction. Boulder County, 2 miles north of Nederland, peaty fen in broad gently sloping valley, 8400 ft., growing in dense clumps in rivelets, 29 Jun 1999, N. Lederer & Bill Jennings 99-60 (COLO barcode 00325092!; KHD barcode 00001368!)

AUTHOR CONTRIBUTIONS

MTS conceived of the study and conducted field, herbarium, lab, and bioinformatic work. EAT provided material/laboratory support and offered phylogenetic and taxonomic guidance. Both investigators wrote the manuscript. — EAT, https://orcid.org/0000-0001-9340-8723

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LITERATURE CITED


Prantl, K. 1884. Eugen Ulmer. Stuttg.:


