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ABERRANT PLANT DIVERSITY IN THE PURGATORY WATERSHED OF SOUTHEASTERN COLORADO AND NORTHEASTERN NEW MEXICO

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ABSTRACT.—Despite a dearth of biological study in the area, the Purgatory Watershed concentrated in southeastern Colorado and northeastern New Mexico is home to a number of unique land formations and endemic organisms. At onetime nonarable land where Dust Bowl storms of the 1930s originated, the Purgatory Watershed is presently home to the Comanche National Grasslands, the Picketwire Canyonlands, and the expansive Piñon Canyon Maneuver Site. The Purgatory Watershed is composed of deep canyons, eroded mesas, and extensive intact shortgrass plains, and is located at a crossroads of the biodiversity of the Southern Rocky Mountains, Great Plains, and Chihuahuan Desert. Here we describe 2 anomalous populations of 2 plant species, prompted by observation of these and several additional, unrelated plants marked by morphologically aberrant forms in this watershed. Specifically, we described morphology of and generated sequence data for *Amorpha nana* (Fabaceae) and *Tetraneuris acaulis* (Asteraceae) to assess potential differences between Purgatory populations of these plants and populations from elsewhere across their ranges. Morphometric data from Purgatory and non-Purgatory populations of these 2 unrelated species were collected from specimens housed at herbaria. Similarly, molecular data from Purgatory and non-Purgatory populations of these 2 species, plus near outgroups, were generated from herbarium collections to reconstruct phylogenetic relationships within each species complex. Maximum likelihood bootstrap analysis recovered moderate support for a clade of aberrant *A. nana,* indicating the presence of a distinct Purgatory lineage of *A. nana,* which was also supported by our morphological data. In contrast, insufficient phylogenetic signal and morphological results in our *Tetraneuris* data set yielded unresolved relationships between aberrant and nonaberrant forms. The Purgatory Watershed is a biologically unique region hosting marked biodiversity in numerous groups, despite having been the focus of little prior research.

RESUMEN.—A pesar de la escasez de investigaciones biológicas en la zona, la Cuenca del Purgatorio (Purgatory Watershed) al sureste de Colorado y al noreste de Nuevo México, es hogar de una serie de formaciones de tierra únicas y de organismos endémicos. Así como en la década de 1930 las tierras no cultivables dieron origen a las tormentas de polvo Dust Bowl, el Purgatorio es, actualmente, hogar de las Praderas Nacionales Comanche, del Canyonlands Picketwire, y de la extensa zona del Piñon Canyon Maneuver. El Purgatorio está compuesto por profundos cañones, mesetas erosionadas y extensas llanuras de pastos cortos. Está situado entre la confluencia de una diversidad biológica formada por las montañas Rocosas del Sur, las Grandes Llanuras y el Desierto de Chihuahua. Describimos aquí dos poblaciones anómalas de dos especies de plantas, partiendo de la observación de éstas y de varias plantas adicionales, plantas no relacionadas con morfología anómala en esta cuenca. En especial, describimos la morfología y la secuencia de datos generados sobre *Amorpha nana* (Fabáceas) y *Tetraneuris acaulis* (Asteráceas) para evaluar las posibles diferencias entre las poblaciones de plantas del Purgatorio y poblaciones de otros lugares dentro de su rango. A partir de especímenes en herbarios, se colectaron datos morfométricos de poblaciones del Purgatorio y fuera del Purgatorio de dos especies no emparentadas (*Amorpha nana* y *Tetraneuris acaulis*). Del mismo modo, se obtuvieron datos moleculares, a partir de colecciones de herbarios, de poblaciones del Purgatorio y fuera del Purgatorio de estas dos especies y de otros grupos cercanos, para reconstruir las relaciones filogenéticas dentro de cada complejo de especies. Un análisis de remuestreo con reemplazo de máxima verosimilitud respaldó moderadamente la existencia de un clado de *A. nana* anómalos, indicando la presencia de un claro linaje del Purgatorio de *A. nana,* que también fue respaldado por nuestros datos morfológicos. Por el contrario, los escasos indicadores filogenéticos y los insuficientes resultados morfológicos en nuestro conjunto de datos de *Tetraneuris* reportaron relaciones no resueltas entre las formas anómalas y las no anómalas. El Purgatorio es una región biológicamente única que alberga una notable diversidad biológica en numerosos grupos, a pesar de haber sido un punto con escasa investigación previa.

The Purgatory Watershed (aka Purgatoire Watershed; henceforth, *the Purgatory*), west of Trinidad and south of La Junta in southeastern Colorado and northeastern New Mexico, is a remote area of over 0.5 million acres known for its rugged terrain of deep canyons, eroded mesas and tablelands, riparian zones, and extensive, intact shortgrass prairies. The landscape of much of the Purgatory is largely undeveloped and encompasses (1) much of the

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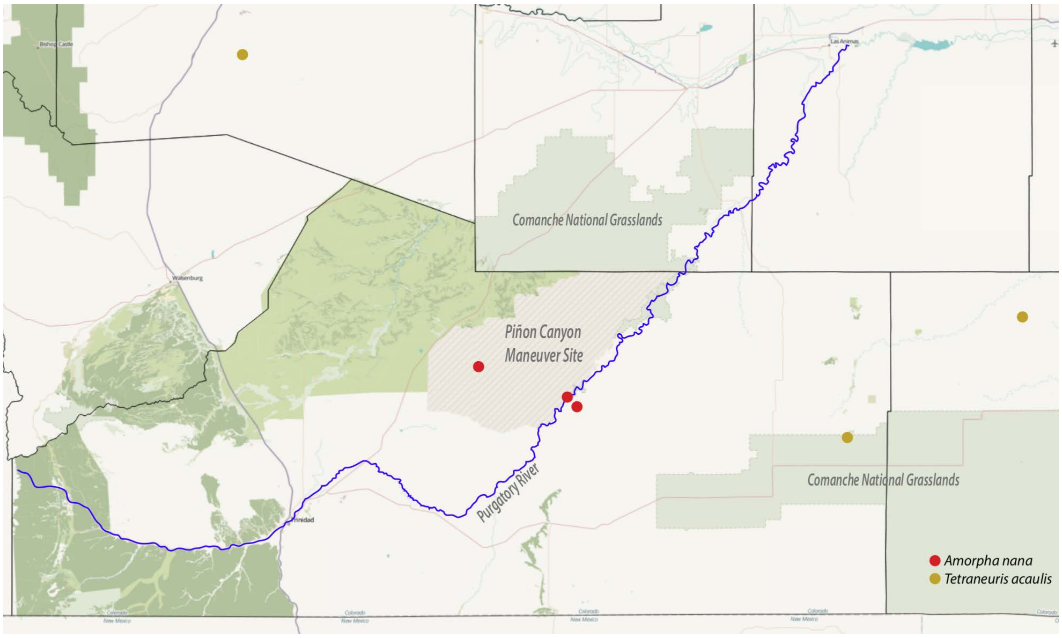

Fig. 1. Map of the Purgatory Watershed in southeastern Colorado and northeastern New Mexico. Collection sites of anomalous specimens of all 2 species are shown.

Comanche National Grasslands, including the Picketwire Canyonlands; (2) the Piñon Canyon Maneuver Site (PCMS), a U.S. Army base of 235,896 acres; and (3) a number of large private land holdings, some of these under conservation agreements (Fig. 1). A number of endemic organisms are found only in the Purgatory (Hazlett 2004), including plants such as *Frasera coloradensis* (C.M. Rogers) D.M. Post, *Herrickia horrida* Wooten & Standl., and *Solidago capulinensis* Cockerell & D.M. Andrews, and animals such as a morphologically distinct race of the rattlesnake *Sistrurus catenatus* Rafinesque (Campbell and Lamar 2004). The area is also home to other biodiversity discoveries in the state: 2 liverworts previously unknown to Colorado were collected recently in the Comanche Grasslands (A. Malone and E. Tripp, in preparation).

The present study was initiated based on observations made by one of us (D.A. Clark) with colleagues over the past 2 decades. These observations suggest that populations of several unrelated plant species are morphologically anomalous in the Purgatory compared to elsewhere across their ranges (Clark 1996). Here, we generated morphological and molecular data for plants of *Amorpha nana* Nutt. and *Tetraneuris acaulis* Greene from the Purgatory and compare these to data generated for populations of these 2 species from elsewhere across their ranges. We asked whether plants from the Purgatory exhibit unique morphologies and harbor unique nucleotide polymorphisms with respect to individuals of the species found elsewhere in their ranges.

METHODS

Study Area

Our area of study was contained within the Central Shortgrass Prairie Ecoregion that borders the Southern Rocky Mountain Ecoregion (TNC 2000, Stevens et al. 2008). This area is characterized by rolling plains that are dissected by streams, canyons, and buttes and is dominated by shortgrass and mixed grass prairie, riparian woodlands and shrublands, and juniper woodlands (Neely et al. 2006, Stevens et al. 2008). The Purgatory is further characterized by high winds that dehydrate soils and trigger dust storms, and the area as a whole is prone to decade-long periods of drought (Ruffner 1980). As such, biodiversity study of the Purgatory can be especially contingent upon weather patterns, which pose

Fig. 2. Landscape diversity within the Purgatory Watershed. A, B, C: terrain near the canyon reaches of a Purgatory River drainage on Chancellor Ranch. In C, the Purgatory River can be seen in the background. D, E, F: terrain representative of additional areas throughout the Comanche Grasslands.

general difficulties for biological inventories, including those of plant biota. The Purgatory on the whole is a biogeographical crossroads of diversity: organisms from the Southern Rocky Mountains to the west, the Great Plains to the east, and the Chihuahuan Desert to the south are found within this area, which comprises an intersection of these 3 vastly different ecosystems (Fig. 2; Clark 1996). To date, the Purgatory remains a biologically underexplored region of the Central Shortgrass Prairie, as it is isolated, rugged, and much neglected compared to the far more extensive scientific exploration of the nearby Southern Rocky Mountains (Inouye 2008, Elser et al. 2009, Forrest and Thomson 2011, Baldwin and Bender 2012).

TABLE 1. Morphometric data for aberrant and nonaberrant specimens of *Amorpha nana*. Aberrant specimens are highlighted.

Herbarium	Accession number	Leaflet color	Leaflet length (mm)	Margin pubescence	Number of leaflets per leaf
COLO	416321	Purple-brown/dark green	7.5–13.8	No	11–13
COLO	525500	Purple-brown/dark green	7.0–13.0	No	13–15
COLO	445036	Purple-brown/dark green	5.1–7.8	Yes	9–13
CS	106326	Purple-brown/dark green	5.0–9.5	Yes	9–13
CS	48801	Purple-brown/dark green	3.2–8.5	Yes	11–13
COLO	450750	Dark green/green	3.0–11.0	Yes	13–26
COLO	451195	Green/green	2.0–7.5	Yes	13–24
COLO	382418	Dark green/dark green	2.0–9.1	Yes	18–25
COLO	453030	Green/green	3.0–11.2	Yes	13–30
COLO	208969	Dark green/dark green	3.0–11.0	Yes	11–27
COLO	454044	Dark green/green	3.0–10.5	No	10–25
COLO	382465	Green/green	3.0–8.0	No	23–26
COLO	382028	Green/green	3.5–7.5	Yes	17–27
COLO	382464	Green/green	2.0–8.5	No	18–29
COLO	198127	Green/green	3.0–9.0	Yes	18–28
COLO	425761	Green/green	2.0–12.0	No	23–27
COLO	431666	Green/green	3.0–10.1	No	15–28
COLO	382466	Dark green/green	2.0–12.5	No	22–27
COLO	447916	Dark green/dark green	3.0–6.5	Yes	15–25
COLO	447305	Green/green	3.0–10.0	Yes	17–27
COLO	474108	Green/green	3.0–10.8	Yes	11–27
COLO	460024	Green/green	3.0–8.0	Yes	11–25
COLO	508247	Green/green	3.0–6.8	Yes	11–25

Study Systems

Amorpha nana is a perennial shrub found in rocky or sandy soils of prairies and hillsides across midwestern North America. Compared to non-Purgatory populations of *A. nana*, populations found in the Purgatory exhibit fewer leaflets per leaf and a brown to gray color on the abaxial surfaces of leaflets that is distinctly different from the solid green leaflet color on both surfaces typical of the species elsewhere throughout the Midwest. *Tetraneuris acaulis* is a perennial herb distributed across western North America and is found in open, dry hillsides and plains. This species typically exhibits inflorescences that contain both disc and ray flowers, but rayless populations are found in the Purgatory.

Morphometric Analysis

To gauge potential morphological uniqueness of Purgatory plants, we made quantitative and qualitative assessments of Purgatory and non-Purgatory specimens of each plant study system using collections housed in the University of Colorado (COLO) and Colorado State University (CS) herbaria (Tables 1, 2). For *Amorpha nana* we examined leaf morphology, leaflet color, number of leaflets per leaf, leaf margin pubescence, and leaflet length (5 Purgatory + 18 non-Purgatory collections). For *Tetraneuris acaulis* we examined head and leaf morphology, presence of ray flowers, receptacle width, leaf length, and leaf pubescence (7 Purgatory + 13 non-Purgatory collections). Outside of the accessions listed in Tables 1 and 2, we are not aware of any additional collections of these species from the Purgatory, further exemplifying the need for increased biological study of organisms from this region.

Data Set Construction

We built data sets for each study system (*Amorpha*, *Tetraneuris*) targeting 4 different sets of taxon sampling: (1) specimens of the target taxon from the Purgatory, (2) specimens of the target taxon from elsewhere in Colorado, especially those predicted to be most closely related to Purgatory populations because of geographical proximity, (3) specimens of the target taxon from other (non-Coloradan) portions of the range of the species, and (4) close relatives of the target taxon, as informed by prior published studies, with which to root our trees. With respect to (1), we sampled a minimum of 3 morphologically aberrant specimens collected from the Purgatory for each study

TABLE 2. Morphometric data for aberrant and nonaberrant specimens of *Tetraneuris acaulis*. Aberrant specimens are highlighted.

Herbarium	Accession number	Presence of ray flowers	Receptacle width at widest point (mm)	Maximum leaf length (mm)	Leaf pubescence
COLO	446981	No	11	40	Yes
COLO	525621	No	11	53	Yes
COLO	471420	No	14	39	Yes
COLO	471417	No	16	74	Yes
COLO	471413	No	14.5	34	Yes
COLO	76968	No	12.5	31	Yes
CS	93343	No	13.5	53	Yes
COLO	500107	Yes	13	27	Yes
COLO	442647	Yes	12	33	Yes
COLO	322392	Yes	13.5	48	Yes
COLO	525633	Yes	14.5	33	Yes
COLO	5520	Yes	13	51	Yes
COLO	541254	Yes	10.5	41	Yes
COLO	56056	Yes	11	45	Yes
COLO	455269	Yes	13	40	Yes
COLO	452013	Yes	11	52	Yes
COLO	197791	Yes	13.5	37	Yes
COLO	468664	Yes	13.5	51	Yes
COLO	517618	Yes	13	41	Yes
COLO	200912	Yes	15	22	Yes

system. Tissue samples were removed for subsequent DNA extractions from material housed at the COLO Herbarium at the University of Colorado's Museum of Natural History, following the herbarium's destructive sampling policy. We attempted to select recent collections that appeared to be in good states of preservation. A list of sampled specimens and associated Genbank accession numbers can be found in Appendix 1. Molecular aspects of this study were conducted in E. Tripp's molecular lab in Ramaley Hall at the University of Colorado–Boulder.

Amorpha Data Set

To estimate phylogenetic relationships between Purgatory and non-Purgatory populations of *Amorpha nana*, we sampled 3 specimens from the Purgatory that were morphologically divergent from non-Purgatory populations plus 17 non-Purgatory specimens (14 from elsewhere in Colorado and 3 from North Dakota). We additionally sampled one accession each of the closely related species *A. canescens* Pursh, *A. fruticosa* L., *A. georgiana* Wilbur, *A. paniculata* Torr. & A. Gray, and *A. schwerinii* C.K. Schneid. (based on Straub and Doyle 2014) to serve as outgroups. Four of these were collected in Colorado and the remaining 3 were collected in North Carolina and Louisiana (Appendix 1).

Tetraneuris Data Set

We sampled 5 rayless and 9 rayed specimens of *Tetraneuris acaulis*. Four of the 5 rayless specimens were collected in the Purgatory in Las Animas and Baca Counties, and one was collected at the northern edge of the Purgatory in Pueblo County. We sampled rayed specimens from throughout Colorado including from the Purgatory. We additionally sampled the closely related species *T. scaposa* Greene, *T. ivesiana* Greene, *T. torreyana* (Nutt.) Greene, *Rydbergia grandiflora* (Torr. & A. Gray) Greene, and *R. brandegei* (Porter ex A. Gray) Rydb. (based on Bierner and Turner 2003) to serve as outgroups; these were all collected in Colorado (Appendix 1).

Marker Sampling and Laboratory Methods

Genomic DNA was extracted following the CTAB extraction procedure (Doyle and Doyle 1987). Polymerase chain reaction (PCR) was used to amplify the nuclear ribosomal DNA (nrDNA) region ITS1+*5.8S*+ITS2 using ITS-4 and ITS-5 primers (White et al. 1990) for the *Amorpha* data set. PCR was used to amplify the chloroplast DNA (cpDNA) intergenic spacer *psbA-trnH* using the psbA (Sang et al. 1997) and trnH (Tate and Simpson 2003) primers for the *Tetraneuris* data set. We initially attempted amplification of both loci for both data sets but were unsuccessful. PCR reactions

TABLE 3. Information for molecular data used in this study. Information about the *Amorpha* data set pertains to the ITS1+5.8S+ITS2 sequence alignment and information about the *Tetraneuris* data set pertains to the *psbA-trnH* sequence alignment.

	Amorpha	*Tetraneuris*
Number of accessions	27	25
Aligned length	725	535
Number of variable characters	9	33
Number of parsimony informative characters	7	24
Pairwise divergence (all taxa)	0.0000–0.0115	0.0000–0.0627
Pairwise divergence (ingroup only)	0.0000–0.0034	0.0000–0.0151
Model	HKY+I	F81+Γ
State frequencies	Empirical: A = 0.19294, C = 0.30664, G = 0.27816, T = 0.22226	Empirical: A = 0.29892, C = 0.13994, G = 0.15957, T = 0.40157
Proportion of invariable sites	0.948893	0.348628
Rates at variable sites	Equal	Gamma, shape = 0.009219 (4 categories, mean)

were run in 25-μL quantities using 2.5 μL TopTaq 10× Buffer, 1 μL $MgCl_2$ (25 mM), 2 μL dNTP (2.5 mM), 1 μL of each primer (10 mM), 0.125 μL TopTaq polymerase, approximately 25 ng DNA template, and sterile water to bring the PCR mix to 25 μL. We utilized the following touchdown PCR protocol: 94 °C (3 min), 7 cycles of [94 °C (10 s), 65 °C (30 s), and 72 °C (1 min)], 16 cycles of [94 °C (10 s), 65 °C to 1 °C every cycle (30 s), and 72 °C (1 min)], 17 cycles of [94 °C (10 s), 50 °C (30 s), and 72 °C (1 min)], 72 °C (7 min).

Phylogenetic Analyses

PCR products were sequenced bidirectionally at Quintara Biosciences (San Francisco, CA). Sequences were edited in-house using Geneious 7 (Kearse et al. 2012), then manually aligned using PhyDE (Müller et al. 2010). Indels were not excluded or recoded for any matrices. JModelTest 2.0 (Guindon and Gascuel 2003, Darriba et al. 2012) was used to find the best-fitting models of evolution for each data set, and the Akaike information criterion (Akaike 1974) was used to choose the best estimated model for each data set. The HKY+I (Hasegawa et al. 1985) and F81+Γ (Felsenstein 1981) models were selected as the best-fitting models for the *Amorpha* and *Tetraneuris* data sets, respectively. Maximum likelihood (ML) methods, conducted in Garli 0.951 (Zwickl 2006), were used to infer phylogenies. Descriptive information for molecular data is provided in Table 3. To assess branch support, 100 ML bootstrap replicates were conducted for each data set, and a 50% majority-rule consensus tree was constructed from resulting trees. Maximum likelihood phylogenies were visualized in PAUP* 4.0a146 (Swofford 2002). All data sets were deposited into TreeBASE (https://www.treebase.org; study number 21328).

RESULTS

Morphological Results

Morphological data for Purgatory and non-Purgatory populations of *Amorpha nana* are summarized in Table 1. All 5 specimens from the Purgatory exhibited a purple-brown color on the adaxial side of leaflets, with a dark green color on the abaxial side. Non-Purgatory specimens are green on both sides of leaflets (12 specimens), dark green on both sides of leaflets (3 specimens), or dark green on the adaxial side with green on the abaxial side of leaflets (3 specimens). Plants of *A. nana* from the Purgatory exhibited leaflet lengths ranging from 3.2 mm (1 specimen) to 13.8 mm (1 specimen), while non-Purgatory specimens exhibited leaflet lengths ranging from 2.0 mm (5 specimens) to 12.5 mm (1 specimen). Three of the 5 Purgatory specimens and 12 of the 18 non-Purgatory specimens exhibited leaflet margin pubescence while the rest did not. Purgatory specimens exhibited 9 (2 specimens) to 15 (1 specimen) leaflets per leaf, and non-Purgatory specimens exhibited between 10 (1 specimen) and 28 (2 specimens) leaflets per leaf.

Morphological data for Purgatory and non-Purgatory populations of *Tetraneuris acaulis* are summarized in Table 2. All 7 Purgatory specimens lacked ray flowers, while all 13

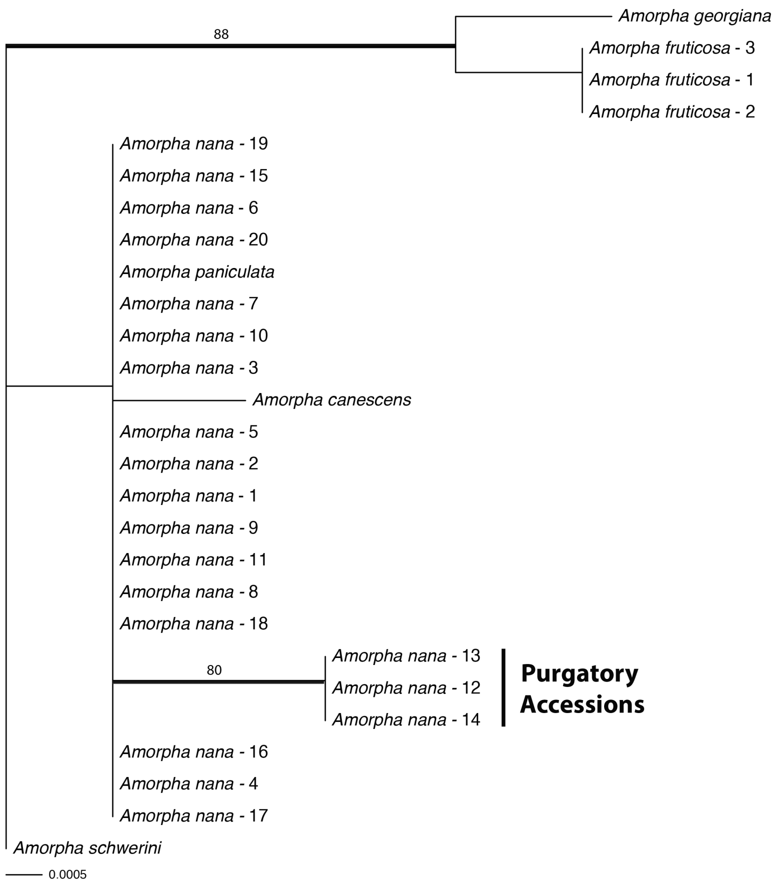

Fig. 3. Most likely tree derived from maximum likelihood analysis of the *Amorpha* data set. Bootstrap values greater than 70 are included and highlighted. Morphologically aberrant specimens from the Purgatory Watershed are *Amorpha nana* 12–14.

non-Purgatory specimens exhibited ray flowers. Receptacle width at its widest point for Purgatory specimens ranged from 11 mm (2 specimens) to 16 mm (1 specimen), with an average maximum width of 13.2 mm. Non-Purgatory specimens exhibited maximum receptacle widths ranging from 11 mm (2 specimens) to 14.5 mm (1 specimen), with an average maximum width of 12.8 mm. Maximum leaf length for Purgatory specimens ranged from 31 mm to 74 mm, with an average maximum leaf length of 46.2 mm. Non-Purgatory specimens exhibited maximum leaf lengths between 22 mm and 51 mm, with an average maximum leaf length of 40.1 mm. All specimens from both Purgatory and non-Purgatory populations exhibited some level of leaf pubescence.

Phylogenetic Results

Moderate bootstrap support (80%) indicated that 3 Purgatory accessions of *A. nana* (accessions 12–14) formed a clade that is distinctive from other populations of this species from elsewhere in Colorado and North America (Fig. 3). This result was driven primarily by the presence of 3 single nucleotide polymorphisms (SNPs). The remaining 17 accessions of *A. nana* (all from areas outside the Purgatory) formed an unsupported clade that included one accession of *A. canescens* and *A. paniculata.* Finally, 3 accessions of *A. fruticosa* formed an unsupported clade that was sister to *A. georgiana;* together this clade was strongly supported (88% bootstrap). This tree was rooted using *A. fruticosa* (accessions 1–3), *A. canescens, A. paniculata,* and *A. schwerinii* as the outgroup.

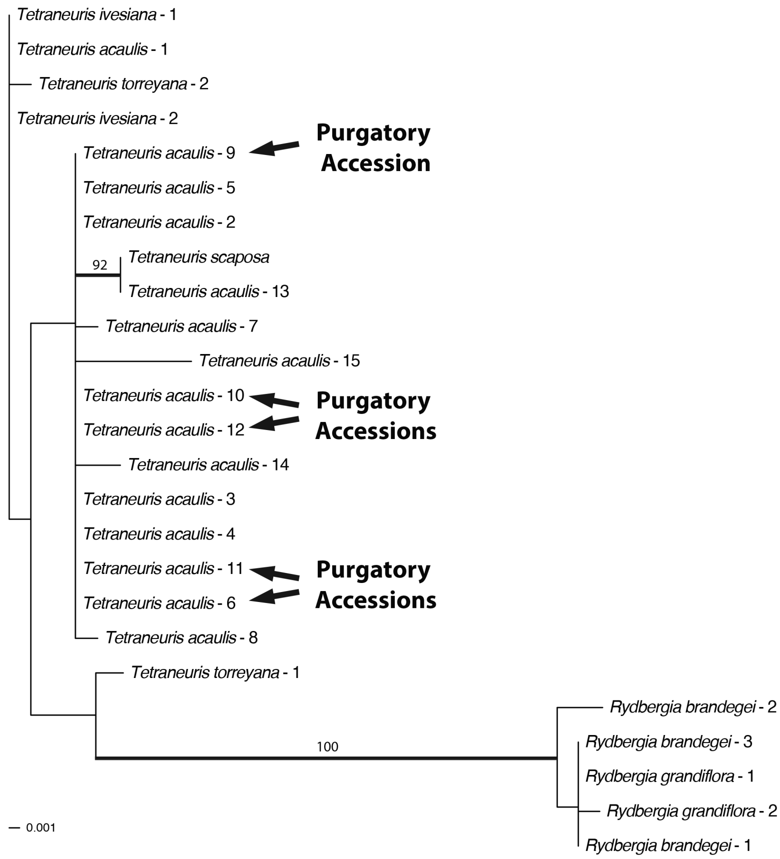

Fig. 4. Most likely tree derived from maximum likelihood analysis of the *Tetraneuris* data set. Bootstrap values greater than 70 are included and highlighted. Morphologically aberrant (rayless) specimens from the Purgatory Watershed are *Tetraneuris acaulis* 6, 9–12.

The 5 rayless Purgatory specimens of *Tetraneuris acaulis* (accessions 7, 10–13) formed an unsupported clade with all but 2 rayed non-Purgatory specimens of *T. acaulis* (accessions 2–20) (Fig. 4). Strong bootstrap (92%) supported a sister group relationship between *T. scaposa* (from Yuma Co., CO) and *T. acaulis*-13 (from Baca Co., CO). Three accessions of *Rydbergia brandegei* and 2 accessions of *R. grandiflora* formed a strongly supported clade (100% bootstrap) that was together sister to one accession of *Tetraneuris torreyana* (accession 1), but this latter relationship was not supported. The remaining samples (*T. ivesiana*-2, *T. ivesiana*-1, *T. acaulis*-1, and *T. torreyana*-2) formed a polytomy at the base of the tree. This tree was rooted using *T. torreyana* (accessions 1 and 2), *T. ivesiana* (accessions 1 and 2), *T. scaposa, Rydbergia brandegei* (accessions 1–3), and *R. grandiflora* (accessions 1 and 2) as the outgroup.

DISCUSSION

Amorpha nana

Both morphological and molecular results from this study support a Purgatory lineage of *A. nana* that is distinct from those found elsewhere in the range of this species. While the presence or absence of leaflet margin pubescence is clearly not diagnostic of a Purgatory lineage, the other leaflet characteristics we investigated (color, length, and number per leaf) support a distinct Purgatory lineage of *A. nana.* Adaxial surfaces of leaflets in all Purgatory specimens were a purple-brown color, which was not seen in non-Purgatory specimens. Additionally, leaflets were generally larger and there were fewer leaflets per leaf for the Purgatory specimens compared to non-Purgatory specmiens. It is possible that environmental conditions of the Purgatory have selected for these leaflet characteristics (e.g., these characteristics may be driven by environmental plasticity rather than shared evolutionary history), but further study is needed to assess this hypothesis.

Phylogenetic analysis of *Amorpha* suggests that Purgatory populations of *Amorpha nana* form a supported clade distinctive from non-Purgatory populations of *A. nana.* Thus, Purgatory populations of *A. nana* exhibit a molecular signature consistent with the hypothesis that this lineage may be separately evolving from other populations of this species elsewhere across its range. These data add to prior evidence from a number of endemic plant and animal species from the Purgatory documenting the biological significance of this area. We think it is likely that, in addition to *A. nana,* morphologically divergent populations of other plants in the Purgatory may similarly show molecular signatures of divergence from other (non-Purgatory) populations. Following additional research, revisions to taxonomy including the recognition of newly identified, distinctive lineages may be needed. Compared to other areas of western North America with a much greater density of botanists and far higher overall levels of field activity (e.g., the Front Range of Colorado or southern California), the Purgatory is a dramatically understudied area and biodiversity here may be richer than previously thought.

Tetraneuris acaulis

In contrast to the above study, morphological and molecular data from the *Tetraneuris* study yielded inconclusive results. Morphologically, we were unable to recover any distinctions between Purgatory and non-Purgatory specimens based on receptacle width, maximum leaf length, or leaf pubescence. The only morphological character unique to Purgatory specimens was the absence of ray flowers, whereas all non-Purgatory specimens exhibited ray flowers.

Our phylogenetic study of *Tetraneuris acaulis* yielded Purgatory (rayless) plants that were members of a large polytomy that included non-Purgatory (rayed) plants. Given the largely unresolved nature of the topology, this result does not support or refute a hypothesis that populations of this species in the Purgatory may be part of a distinctive, separately evolving lineage. It is nevertheless interesting that only Purgatory specimens lack ray flowers. Further study using larger or more informative data sets is required to understand biogeographical and evolutionary affinities of Purgatory *T. acaulis* to other populations of this species. Specifically, Next-Generation (NGS) sequence data such as RADseq loci may better inform relationships and support for these relationships (McCormack et al. 2013, Andrews et al. 2016, Tripp et al. 2017) as well as patterns of gene expression that may explain morphological diversity in this group.

In addition to the above patterns regarding Purgatory populations, our *Amorpha* phylogeny suggests that *Amorpha paniculata, Amorpha canescens,* and non-Purgatory *Amorpha nana* may be more closely related to one another than Purgatory and non-Purgatory *A. nana* are to each other. However, we cannot make more definitive assessments regarding these relationships because we obtained sequences for only one accession each of *A. paniculata* and *A. canescens* and because this backbone is unresolved and unsupported. Similarly, the polytomy formed at the base of the tree by *Tetraneuris acaulis*-1, *T. ivesiana,* and *T. torreyana*-2, in addition to the large polytomy containing the bulk of *T. acaulis* accessions, was unsupported, likely attributable to our use of only one locus (*psbA-trnH*) for this data set. While we predict that both of these species are relatively recently evolved based on climate and comparatively high numbers of endemics in the region, future studies investigating a hypothesis of neoendemism in the Purgatory would ideally employ approaches such as that proposed by Mishler et al. (2014), which will require more robust taxon sampling and use of more powerful sequence data.

Other Aberrant Plant Populations in the Purgatory

In addition to the 2 plant groups we studied, additional lineages including *Potamogeton* (Potamogetonaceae), *Oonopsis* (Asteraceae), and *Opuntia* (Cactaceae) represent candidates for future investigation of biodiversity and diversification in the Purgatory. An aberrant form of *Potamogeton diversifolius* with up to 14 veins per floating leaf exists in the Purgatory, while the typical *P. diversifolius* found elsewhere exhibits only between 3 and 7 veins per leaf. The typical variety of *Oonopsis foliosa* (A. Gray) Greene occurs in Wyoming and Colorado while another variety, *O. foliosa* var. *monocephala* (A. Nelson) Kartesz & Gandhi, is found only in and around the Purgatory. Whereas the typical variety has a radiate head consisting of disc and ray flowers, *O. foliosa* var. *monocephala* has a discoid head and lacks ray flowers, similar to the *Tetraneuris* system we described in this study. The 2 varieties were once thought to be geographically disjunct, but specimens with intermediate head morphologies at the eastern edge of the PCMS in the Purgatory, and the presence of meiotic chromosome-pairing anomalies in these intermediate plants, are consistent with a possible hybrid origin of these morphological and geographical intermediates (Hughes and Brown 2004). Additionally, *Opuntia polyacantha* Haw. is a widespread cactus found throughout western North America. One variety, *O. polyacantha* var. *trichophora* (Engelm. & J.M. Bigelow) J.M. Coult., exhibits peculiar long, soft, hairlike spines and exists in abundance in the Purgatory. The presence of a large number of endemics found in the Purgatory indicates there may also be high levels of species richness and threatened species (Orme et al. 2005, Lamoreux et al. 2006) in the region. Delimiting highly endemic regions is one strategy to prioritize areas for conservation (Kerr 1997), and the Purgatory is an ideal candidate for conservation based on the results of our study and the number of previously identified endemics found here.

Our study relied on observations made in the field and on herbarium specimens to study 2 species complexes found in the Purgatory and across western North America. By utilizing both recent and decades-old collections, we were able to obtain morphometric and molecular data from *Amorpha nana* and *Tetraneuris acaulis,* populations of which in the Purgatory exhibit anomalous morphological features. Without the COLO and CS herbaria, initiation of this study would have never occurred, and it is likely that investigation of museum collections will result in similar studies and findings in the future (Bebber et al. 2010, Lepis et al. 2011, Yang et al. 2012). The Purgatory is a region of biological interest comprising a unique climate and landscape, a number of endemics, and morphologically unique populations of species complexes. Further study of the Purgatory is clearly needed. We hope this study serves to increase scientific interest in the Purgatory, where biogeographical and population genetics approaches may yield interesting results regarding the biodiversity of the area and aid in future conservation efforts of the native plant and animal species of Colorado and New Mexico.

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APPENDIX 1. Sampled specimens and associated GenBank accession numbers.

Amorpha Data Set

Amorpha nana-1 Nutt., *Cooper s.n.* [COLO], Boulder Co. CO, ITS: MF536005. *Amorpha nana*-2 Nutt., *Weber & Foster 17843* [COLO], Boulder Co. CO, ITS: MF536016. *Amorpha nana*-3 Nutt., *Cooper s.n.* [COLO], Boulder Co. CO, ITS: MF536018. *Amorpha nana*-4 Nutt., *Cooper s.n.* [COLO], Boulder Co. CO, ITS: MF536019. *Amorpha nana*-5 Nutt., *Cooper s.n.* [COLO], Boulder Co. CO, ITS: MF536020. *Amorpha nana*-6 Nutt., *Maley 510* [COLO], Boulder Co. CO, ITS: MF536021. *Amorpha nana*-7 Nutt., *Weber s.n.* [COLO], Boulder Co. CO, ITS: MF536022. *Amorpha nana*-8 Nutt., *Lederer 4122* [COLO], El Paso Co. CO, ITS: MF536023. *Amorpha nana*-9 Nutt., *Cooper s.n.* [COLO], Boulder Co. CO, ITS: MF536024. *Amorpha nana*-10 Nutt., *Hogan 1518* [COLO], Boulder Co. CO, ITS: MF536006. *Amorpha nana*-11 Nutt., *Hogan 1765* [COLO], Boulder Co. CO, ITS: MF536007. *Amorpha nana*-12 Nutt., *Clark et al. 2634* [COLO], Las Animas Co. CO, ITS: MF536008. *Amorpha nana*-13 Nutt., *Yeatts 2105* [COLO], Las Animas Co. CO, ITS: MF536009. *Amorpha nana*-14 Nutt., *Cushman s.n.* [COLO], Las Animas Co. CO, ITS: MF536010. *Amorpha nana*-15 Nutt., *Nelson 568* [COLO], Jefferson Co. CO, ITS: MF536011. *Amorpha nana*-16 Nutt., *Hazlett 11045* [COLO], Jefferson Co. CO, ITS: MF536012. *Amorpha nana*-17 Nutt., *Baumeister & Fair 73* [COLO], El Paso Co. CO, ITS: MF536013. *Amorpha nana*-18 Nutt., *Harner White Ecol. Consult. s.n.* [COLO], Oliver Co. ND, ITS: MF536014. *Amorpha nana*-19 Nutt., *Regenhardt & Pye s.n.* [COLO], Mercer Co. ND, ITS: MF536015. *Amorpha nana*-20 Nutt., *Clarke s.n.* [COLO], Oliver Co. ND, ITS: MF536017. *Amorpha canescens* Pursh, *Clark & Crawford 432* [COLO], Las Animas Co. CO, ITS: MF536000. *Amorpha fruticosa*-1 L., *Clark 617* [COLO], Las Animas Co. CO, ITS: MF536001. *Amorpha fruticosa*-2 L., *Kelso et al. s.n.* [COLO], El Paso Co. CO, ITS: MF536002. *Amorpha fruticosa*-3 L., *Rondeau et al. 01-05* [COLO], Larimer Co. CO, ITS: MF536003. *Amorpha georgiana* Wilbur, *Leonard & Moore 1720* [COLO], Columbus Co. NC, ITS: MF536004. *Amorpha paniculata* Torr. & A. Gray, *Thomas & Lab 93725* [COLO], Franklin Parish LA, ITS: MF536025. *Amorpha schwerini* C.K. Schneid., *Leonard s.n.* [COLO], Davidson Co. NC, ITS: MF536026.

Tetraneuris Data Set

Tetraneuris acaulis-1 Greene, *Harner s.n.* [COLO], Delta Co. CO, psbA-trnH: MF535915. *Tetraneuris acaulis*-2 Greene, *Weber & Wittmann 18625* [COLO], Baca Co. CO, psbA-trnH: MF535922. *Tetraneuris acaulis*-3 Greene, *Clark et al. 5* [COLO], Las Animas Co. CO, psbA-trnH: MF535923. *Tetraneuris acaulis*-4 Greene, *C.M. Rogers 4541* [COLO], Las Animas Co. CO, psbA-trnH: MF535924. *Tetraneuris acaulis*-5 Greene, *Kuhn et al. 6968* [COLO], Las Animas Co. CO, psbA-trnH: MF535925. *Tetraneuris acaulis*-6 Greene, *Clark 2316* [COLO], Baca Co. CO, psbA-trnH: MF535926. *Tetraneuris acaulis*-7 Greene, *Clark 2357* [COLO], Las Animas Co. CO, psbA-trnH: MF535927. *Tetraneuris acaulis*-8 Greene, *Archibald a226* [COLO], Las Animas Co. CO, psbA-trnH: MF535928. *Tetraneuris acaulis*-9 Greene, *Clark 643* [COLO], Las Animas Co. CO, psbA-trnH: MF535929. *Tetraneuris acaulis*-10 Greene, *Clark 649* [COLO], Las Animas Co. CO, psbA-trnH: MF535916. *Tetraneuris acaulis*-11 Greene, *Clark 645* [COLO], Las Animas Co. CO, psbA-trnH: MF535917. *Tetraneuris acaulis*-12 Greene, *Parker & McClintock 6995* [COLO], Pueblo Co. CO, psbA-trnH: MF535918. *Tetraneuris acaulis*-13 Greene, *Dowell et al. 7.3* [COLO], Baca Co. CO, psbA-trnH: MF535919. *Tetraneuris acaulis*-14 Greene, *Lum & Wilson 4045* [COLO], Park Co. WY, psbA-trnH: MF535920. *Tetraneuris acaulis*-15 Greene, *Jones 38* [COLO], Fall River Co. SD, psbA-trnH: MF535921. *Tetraneuris scaposa* Greene, *Wittmann 1581* [COLO], Yuma Co. CO, psbA-trnH: MF535932. *Tetraneuris ivesiana*-1 Greene, *Clark & Hogan 1964* [COLO], Gunnison Co. CO, psbA-trnH: MF535930. *Tetraneuris ivesiana*-2 Greene, *Harner s.n.* [COLO], Delta Co. CO, psbA-trnH: MF535932. *Tetraneuris torreyana*-1 (Nutt.) Greene, *O'Kane & Neely 2661* [COLO], Moffat Co. CO, psbA-trnH: MF535933. *Tetraneuris torreyana*-2 (Nutt.) Greene, *Hogan et al. 3994* [COLO], Gunnison Co. CO, psbA-trnH: MF535934. *Rydbergia grandiflora*-1 (Torr. & A. Gray) Greene, *Hartman & Nelson 71982* [COLO], Jackson Co. CO, psbA-trnH: MF535913. *Rydbergia grandiflora*-2 (Torr. & A. Gray) Greene, *Hogan 3925* [COLO], Clear Creek Co. CO, psbA-trnH: MF535914. *Rydbergia brandegei*-1 (Porter ex A. Gray) Rydb., *Hogan 4532* [COLO], Fremont Co. CO, psbA-trnH: MF535910. *Rydbergia brandegei*-2 (Porter ex A. Gray) Rydb., *Hogan 3327* [COLO], Alamosa Co. CO, psbA-trnH: MF535911. *Rydbergia brandegei*-3 (Porter ex A. Gray) Rydb., *Elliott & Hartman 11043* [COLO], Huerfano Co. CO, psbA-trnH: MF535912.