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The Phylogeny of *Errazurizia* (Fabaceae: Amorpheae) and Description of the New Monotypic Genus *Pictarena*

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Abstract*—Errazurizia* (Fabaceae) is a genus comprised of four species of New World desert shrubs with an ambiguous evolutionary history. Prior studies determined the North American species of *Errazurizia* were polyphyletic and the relationship of *E. rotundata* with other genera in the tribe Amorpheae remained undetermined. The sole South American species, which is also the type species, has never been included in a molecular study. We inferred the phylogenetic relationships of *Errazurizia* and six closely related genera using data from the cpDNA genome and nrDNA cistron from reference guided assemblies. Maximum likelihood and Bayesian analyses found two of the North American and the South American species were a monophyletic group, but that *E. rotundata* was sister to the monotypic genus *Parryella*. Gland and pollen surface characters confirm the close relationship between *P. filifolia* and *E. rotundata*. Cytonuclear discordance yielded partially incongruent tree topologies, and while the cpDNA phylogeny indicated a monophyletic *Amorpha* was sister to the *E. rotundata* and *P. filifolia* clade, the nrDNA cistron phylogeny recovered a paraphyletic *Amorpha*, with *A. californica* sister to the *E. rotundata* and *P. filifolia* clade. Molecular and morphological evidence support the elevation of *E. rotundata* to its own monotypic genus, ***Pictarena***. The new genus ***Pictarena*** is defined by subsessile, suborbicular leaflets, mammiform leaflet glands, spicate inflorescences, and flowers with either all petals absent or rarely with a banner petal. Elevating *E. rotundata* to ***Pictarena*** resolves the confusing classification of the species, an imperiled endemic known only from four localities in northern Arizona and lends support for continued conservation.

***Keywords*—**cpDNA, Fabaceae, next-generation sequencing, nrDNA cistron, reference-guided assembly.

Errazurizia Philippi is a genus of four species of aromatic shrubs that occur in the desert regions in western North and South America. *Errazurizia* is in the papilionoid tribe Amorpheae, a tribe characterized by epidermal glands, simple basifixed hairs, and a single-seeded indehiscent pod (Barneby 1977). *Errazurizia* is part of the amorphoid lineage (McMahon and Hufford 2004) along with *Eysenhardtia* Kunth, *Amorpha* L., *Parryella* Torr. & A.Gray, and *Apoplanesia* C.Presl, which all lack the ancestral papilionoid floral form and instead have nearly regular flowers (or a reduced number of petals) and exposed androecia. The amorphoid clade is thought to have originated in the Miocene around 6.8–17.4 million years ago (Lavin et al. 2005).

The genus *Errazurizia* is characterized by short shrubs (< 1 m) with densely pubescent foliage, large glands, spicate inflorescences, large ellipsoid fruits with persistent calices, and flowers with five yellow to maroon petals, except for *E. rotundata* (Wooton) Barneby which either lacks petals or has a banner petal. *Errazurizia* has an amphitropic disjunct distribution, with species distributed in the deserts of North and South America (Fig. 1). The type species, *E. multifoliolata* (Clos) I.M.Johnst., occurs at the southern edge of the Atacama Desert in the Atacama and Antofagasta regions in Chile. *Errazurizia benthamii* I.M.Johnst. is endemic to the Pacific side of Baja California Sur, Mexico in the Baja Californian Desert. *Errazurizia megacarpa* I.M.Johnst. is more widely distributed along the Gulf of California coasts of Sonora and the Baja California Peninsula in the Sonoran and Baja Californian deserts. *Errazurizia rotundata* is endemic to the Little Colorado River watershed in the Arizona/New Mexico Plateau cold deserts in northern Arizona.

Errazurizia was first described by Philippi (1872) for *E. glandulifera* Phil., synonymous to the previously described *Psoralea multifoliolata* Clos (Gay 1846). *Errazurizia multifoliolata* was also formerly included in *Dalea* L. (Reiche 1898) and *Parosela* Cav. (Macbride 1922) before eventually being transferred to *Errazurizia*, the genus with priority, by I. M. Johnston (1924) along with *E. megacarpa* and *E. benthamii*. The two Mexican species, formerly recognized in *Dalea* (Brandegee 1889), *Psorobatus* Rydb. (Rydberg 1919), and *Parosela* (Standley 1922), were reclassified in *Errazurizia* with *E. multifoliolata* due to the shared characters of regular floral symmetry and thick, nearly clawless petals (Johnston 1924). *Errazurizia rotundata* was originally placed in *Parryella* (Wooton 1898) due to similar floral morphology with *P. filifolia*, specifically exserted stamens and the lack of petals. The broader range of *P. filifolia* across the Colorado Plateau also overlaps with *E. rotundata* and the two species sometimes co-occur. *Errazurizia rotundata* was reclassified by Barneby (1962), however, due to the resemblance with other *Errazurizia* spp., namely *E. benthamii*. *Errazurizia rotundata* shares several characters with *Errazurizia* spp. that *Parryella* lacks, including a short habit, tomentose foliage, suborbicular leaflets with prominent glands, spikes with short peduncles, deeply campanulate calices with proximally pubescent teeth, petal(s) inserted on the hypanthium rim, and large fruits with a marcescent calyx (Barneby 1962).

Recent phylogenetic studies (McMahon and Hufford 2004; McMahon 2005; Straub and Doyle 2014) have sampled the North American species of *Errazurizia* and recovered *E. megacarpa* and *E. benthamii* in a clade, but *E. rotundata* was distantly related. In a study of Amorpheae (McMahon and Hufford 2004), ITS data supported *E. rotundata* as sister to *Amorpha*, whereas *trnK-matK* data and the combined dataset reconstructed a polytomy that included *Amorpha*, *E. rotundata*, and a clade of *Errazurizia* and *Eysenhardtia*. McMahon (2005), using a combined dataset of *trnK*, ITS, and nuclear gene *CNGC4*, found *E. rotundata* was sister to *Parryella filifolia* Torr. & A.Gray and *A. californica* was sister to the clade but with low support. A study of the phylogeny of *Amorpha* (Straub and Doyle 2014) recovered a polytomy among *E. rotundata*, *P. filifolia*, and *Amorpha* with nuclear *CNGC5* data, *minD* data, and a combined dataset of noncoding plastid regions *trnD-trnT*, *trnH-psbA*, and *petN-psbM*. *Errazurizia multifoliolata* has never

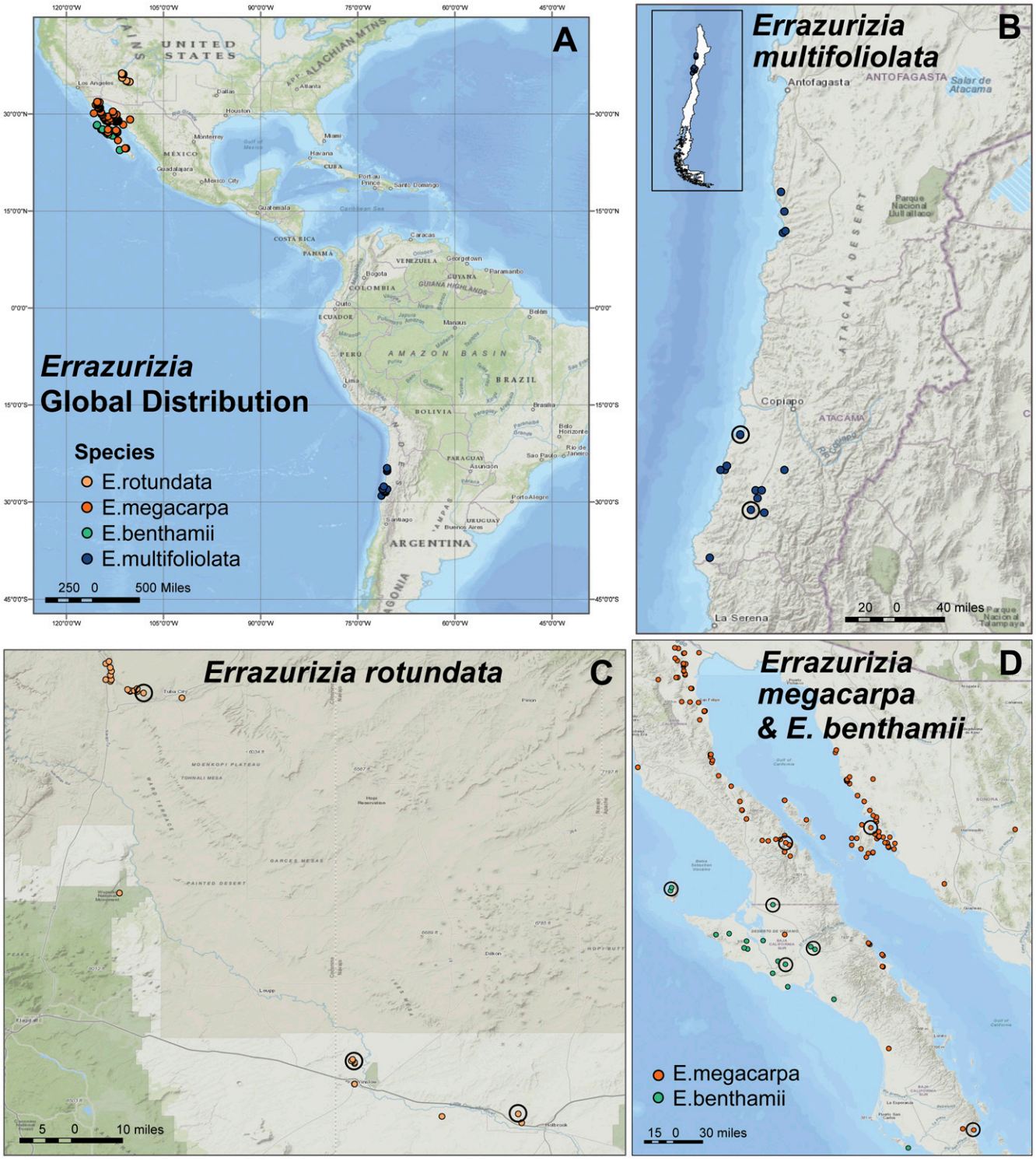

FIG. 1. Distribution of *Errazurizia*. A. New World distribution of the four species. B. Distribution of *E. multifoliolata* in the Atacama Region and Antofagasta Region, Chile. Circles indicate sampled localities. C. Distribution of *E. rotundata* in northern Arizona, USA. D. Distribution of *E. megacarpa* (orange) and *E. benthamii* (green) in Baja California, Baja California Sur, and Sonora, Mexico.

been included in a phylogenetic study and its relationship to the other *Errazurizia* species remains uncertain.

The ambiguous relationships of the species of *Errazurizia* have been a persistent issue in understanding the evolutionary history in the amorphoid clade. Addressing the evolutionary history of *Errazurizia* is long overdue and necessary to not only clarify our understanding of the relationships of *Errazurizia* species but also among the amorphoid genera. To achieve a comprehensive understanding of the phylogenetic relationships of *Errazurizia*, a larger molecular dataset and widespread sampling was imperative. Genome skimming from next-generation sequencing has been a useful method to obtain more data from high-copy genetic sources for phylogenetic purposes (Straub et al. 2012; Ripma et al. 2014; Egan and

Vatanparast 2019). The success of genome skimming in previous phylogenetic studies (Mabry and Simpson 2018; Schafran et al. 2018; Nauheimer et al. 2019) suggests that next-generation sequencing will provide the larger dataset required to infer the phylogeny of *Errazurizia*. The current study used next-generation sequencing and reference-guided assembly to isolate the cpDNA genome and nrDNA cistron for phylogenetic analyses of *Errazurizia* and related genera. The aims of our study were to 1) test the monophyly of *Errazurizia* by inclusion of *E. multifoliolata*, 2) determine the evolutionary relationships of *E. rotundata* and other genera in the amorphoid clade, and 3) identify key morphological features that support generic boundaries.

Materials and Methods

Specimen Collection—To test the monophyly of *Errazurizia*, the four species were sampled from 2–4 localities broadly distributed across their known ranges (Fig. 1). *Parryella filifolia*, *Apoplanesia paniculata* C.Presl, three species of *Eysenhardtia*, and four species of *Amorpha* were included to infer the placement of *E. rotundata* in the amorphoid clade (Appendix 1). Two species of *Dalea* and two species of *Psorothamnus* Rydb. were included as outgroups (Appendix 1). Specimens were field collected in the Navajo Nation, Arizona, California, and New Mexico under the following permits: Navajo Nation Department of Fish and Wildlife permit #1155, United States Forest Service Region 3 RO-307. Leaf material was dried in silica gel for molecular analyses. Stems, leaves, and inflorescences were collected for morphological comparison, preserved in either a 1:3:1 FAA solution of formalin, 70% ethanol, and acetic acid, or in 70% ethanol. Specimens from the Atacama Region in Chile were gifted from ULS (Thiers 2020). Voucher specimens are housed at ASC and ULS (Thiers 2020). Species exclusive to Mexico and outside of the southwestern United States were provided by ARIZ, ETSU, HCIB, and SD (Thiers 2020). Included species and accession information are detailed in Appendix 1.

DNA Extraction—DNA was extracted from dried leaflet material using a modified sorbitol protocol (Štorchová et al. 2000). *Parryella filifolia*, *Amorpha* spp., and *Errazurizia rotundata* were difficult to extract and the protocol was amended as follows. The first sorbitol buffer solution was substituted with a CTAB extraction buffer (Doyle and Doyle 1987) warmed to 65°C, with 2% polyvinylpyrrolidone (PVP-40) added, as suggested by Straub and Doyle (2014). Centrifugation between the sorbitol and lysis buffer addition were omitted, and instead 650 µL of the modified extraction buffer was added twice to the sample split into two 1.5 mL tubes. Sample purity was evaluated with the NanoDrop spectrophotometer (Thermo Scientific, Carlsbad, CA). If 260/280 absorbance values were below 1.8 the sample was cleaned using the Monarch PRC & DNA Cleanup Kit (New England Biolabs, Inc., Ipswich, MA). Samples were assessed for size of DNA fragments using 0.7% gel electrophoresis. The final samples were quantified with the Qubit fluorometer (Thermo Scientific, Carlsbad, CA).

Library Preparation—Genome skimming techniques were employed to capture the cpDNA genome and complete nrDNA cistron. Total DNA was fragmented into 200–300 base-pair (bp) fragments with NEBNext dsDNA Fragmentase (New England Biolabs, Inc., Ipswich, MA). Fragmentation was omitted if the extracted DNA was degraded or already fragmented. Libraries were built with the NEBNext Ultra II DNA Library Prep kit (New England Biolabs, Inc., Ipswich, MA) following the Version 5.0 protocol. Fragments were size selected with 18% magnetic carboxylate beads (MilliporeSigma, St. Louis, MO) for 250 bp fragments when starting DNA exceeded 100 ng of DNA; otherwise, the sample was not size selected. During primer ligation, the reactions were run for 8–11 PCR cycles. Following library preparation, samples were assessed with AATI Fragment Analyzer (Agilent, Santa Clara, CA). If samples contained adaptor contamination (peak at ~120 bp), remaining stock of the adaptor-ligated sample was cleaned using a 0.9 × bead cleanup before repeating the primer ligation step in the NEB protocol. If samples contained primer contamination (peak at ~60 bp), the final product was cleaned using an additional 0.9 × bead cleanup. Sequence data were generated using the Illumina MiSeq platform for 2 × 250 bp paired-end read fragments at the Northern Arizona University Environmental Genetics and Genomic Laboratory. One sample of *Errazurizia rotundata* (29) was included in a preliminary MiSeq run of 2 × 75 bp paired-end reads before the larger run.

Alignment and Phylogenetic Analyses—Reads were demultiplexed by the unique barcodes assigned during library preparation. Barcodes, duplicates, and short reads (< 20 bp) were removed with the BBDuk plugin in Geneious (Kearse et al. 2012). The cpDNA genome and nrDNA cistron were reassembled in five medium-low sensitivity iterations of the reference-guided assembly pipeline in Geneious 10.0 (Kearse et al. 2012). The cpDNA genome of *Dalbergia hainanensis* Merr. & Chun (GenBank ID: NC_036961) was used for reference guided assembly of the plastome. *Dalbergia hainanensis* is a member of Dalbergieae, which is sister to Amorpheae (Cardoso et al. 2013), and at the time of sequencing was the closest relative to Amorpheae with an annotated chloroplast genome. To evaluate the reference-guided assembly to *D. hainanensis*, one sample of *Errazurizia multifoliolata* was mapped to the reference and the resulting consensus sequence used as a reference sequence to test for better read mapping accuracy; little difference was seen between the two references, therefore all samples were mapped to the *D. hainanensis* genome for consistency. The complete nrDNA cistron sequence of *Glycyrrhiza uralensis* Fisch. ex DC. (GenBank ID: KX530461) served as a reference for the high-copy nuclear ribosomal region. Consensus sequences were assembled from bases matching at least 60% of the total bases at each site, otherwise the site was designated the corresponding IUPAC ambiguous code or, in instances of < 7 coverage, the site was called "N" (Straub et al. 2012).

Consensus sequences were aligned using MAFFT v. 7.402 (Katoh and Standley 2013) on the Cipres Science Gateway (Miller et al. 2010). To evaluate if gaps and ambiguous sites in the consensus sequences influenced topology and branch support in the cpDNA phylogeny, we tested an additional alignment with gaps and ambiguous sites masked in Geneious (Kearse et al. 2012) as used in other phylogenetic studies (Ripma et al. 2014; Mabry and Simpson 2018). The cpDNA genome and nrDNA cistron were not concatenated due to the large difference in the number of bp and previous results that identified substantial cytonuclear discordance (McMahon and Hufford 2004; Straub and Doyle 2014; Lee-Yaw et al. 2019, Dodsworth et al. 2020). The general time-reversible model with invariable sites and gamma distribution (GTR + I + G) was used for both datasets based on the corrected Akaike information criterion (AICc) scores from jModelTest v. 2.1.6 (Posada 2008). Information on variable and phylogenetically informative sites were determined in PAUP* v. 4.0 (Swofford 2002).

Maximum likelihood analyses were performed with the plugin for RAxML v. 8.2.12 (Stamatakis 2014) in Geneious (Kearse et al. 2012) to generate the best tree with statistical support assessed in 10,000 bootstrap replicates. Bayesian posterior probabilities were estimated using MrBayes v. 3.2.7a (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) on Cipres (Miller et al. 2010) to runs of five million generations of the Markov Chain Monte Carlo, sampled every 1000 generations with the first 25% of trees discarded as burn-in. Convergence was assured by the standard deviation of split frequencies reaching < 0.01 and burn-in was assessed in Tracer v1.7.1 (Rambaut et al. 2018).

Imaging and Ancestral Character State Estimation—Petal number, gland shape, and pollen exine were selected for evaluation in the amorphoid clade. Petal number in the amorphoid genera changes through an interesting morphological shift from petal suppression and loss (McMahon and Hufford 2004, 2005). Although glands are an important synapomorphy in Amorpheae, characterization beyond developmental comparisons (Turner 1986) and general interpretations was lacking in the literature and required investigation. Pollen morphology (Ferguson 1990) has compelling patterns across the Amorpheae and may have implications to the evolutionary history of the group.

To investigate the microcharacters, stem glands, leaflet glands, and pollen exine were examined through scanning electron microscopy (SEM) of species of *Errazurizia*, *Parryella*, and *Amorpha*. Samples preserved in FAA were transferred to 70% ethanol for long-term storage. Leaves and stems were transferred through an ethanol dehydration series and ethanol was removed with the Pelco Critical Point Dryer. Air-dried pollen grains were imaged without ethanol dehydration. Samples were coated in gold/palladium using the Denton Vacuum Desk II Cold Sputter Etch unit. Samples were imaged at the Imaging Histology Core Facility at Northern Arizona University on the Zeiss Supra 40VP Scanning Electron Microscope using the Everhart-Thornley secondary electron detector. Images were captured at 4–5 kV with a working distance of 0–3 mm. Leaflet surfaces were imaged at 24×–29×. Leaflet and stem glands were imaged at 200×–264 × and at 495 × for *Amorpha californica* Nutt. due to its smaller size. Pollen was imaged at 2170×–3490×. Additional light microscopy images of *E. rotundata* flowers were captured using a Keyence VHX-2000 digital microscope.

Chromosomes were imaged and counted to evaluate the number and ploidy level of *Errazurizia rotundata* and *Parryella filifolia*. Germinated seeds were incubated at room temperature in 0.05% colchicine for 2.5 hr and then stored overnight at 4°C in 3:1 absolute ethanol and glacial acetic

acid. Root radicles were incubated in 1 M HCl at 60°C for 3 min, rinsed with water, and stained in 2% aceto-orcein for 1 hr. The root cap was removed and the root tip was squashed on a slide with a drop of 9:1 solution of 45% acetic acid and glycerol. Cells were viewed at 1000 × magnification.

To evaluate evolutionary patterns, we estimated the ancestral character states of petal number, stem gland shape, and pollen exine in the amorphoid clade (Appendix 2). Character states were identified from SEM, light microscopy, and species descriptions (Wilbur 1975; Barneby 1977; Turner 1986; Ferguson 1990). Petal number was characterized as maximum petal number (one) for the variable *Errazurizia rotundata* because of the apparent difference in petal suppression versus complete loss as seen in the floral ontogeny of *Parryella filifolia* (McMahon and Hufford 2005). Stem, leaf rachis, and peduncle glands were uniform within all species (if present) and were characterized into three main shapes: prickle, spherical, and pustulose. Pollen exine was either perforate or reticulate. Using the cpDNA maximum likelihood phylogeny we tested the fit using two models, an equal character transition rates model and an all-rates-different model, with the GEIGER package (Pennell et al. 2014) in R (R Development Core Team 2005). The log-likelihood scores of the models were statistically evaluated with a χ^2 distribution; if the log-likelihood score of the all-rates-different model was not significantly closer to zero, the simpler equal-rates model was used to infer ancestral states at nodes. Models were implemented in a maximum likelihood ancestral character estimation using the APE package (Paradis and Schliep 2019) in R (R Development Core Team 2005).

Results

Data—Sequence data are available in the NCBI Short Read Archive under the BioProject PRJNA743607. Supplementary data are available in the Dryad Repository (Becklund and Ayers 2022).

Alignments—The number of raw reads ranged from 279,304 to 2,262,388 (median = 1,053,460) and 4,890,162 for sample 29 (Supplemental Table S1; Becklund and Ayers 2022). After duplicate and short read removal, reads ranged from 274,704 to 2,336,760 (median = 1,035,740) and 4,830,266 for sample 29 (Supplemental Table S1). For the cpDNA genome alignment, 0.9–23.49% of the total cleaned reads were mapped to the *Dalbergia hainanensis* reference genome. Sample read coverage was variable but substantial, with mean sequencing depth ranging from 14–333 (median = 53) reads per sample, covering 98.6–100% of the reference genome (Supplemental Table S1). The number of ambiguous characters and gaps per sample ranged from 503–3,061 total bp or 0.32–1.97% of the total sequence length. Gaps varied in size and position, the largest being a 100–250 bp gap between the *ndhI* and *ndhG* genes of the small single-copy region. Individual consensus sequences of the plastome for the 34 samples varied in length from 155,256–156,123 bp. The final cpDNA alignment consisted of 34 taxa and 177,711 bp with 21,136 variable characters (11.9%) and 12,914 phylogenetically informative characters (7.3%).

For the nrDNA cistron alignment, 675–30,285 (median = 3,916) reads were mapped to the *Glycyrrhiza uralensis* reference sequence (Supplemental Table S2). Mean sequencing depth ranged from 21–602 (median = 113) reads per sample and covered 98.6–100% of the reference sequence. Resulting consensus sequences varied in length from 5,929–5,950 bp. The number of gaps and ambiguous characters per sample ranged from 0–136 bp or 0–2.29% of the total sequence length. The final nrDNA cistron alignment consisted of 34 taxa and 5,912 bp with 569 variable characters (9.1%) and 383 phylogenetically informative characters (6.5%).

Phylogenetic Relationships—Maximum likelihood and Bayesian analyses produced the same topology within each dataset but were incongruent between datasets. Bayesian posterior probabilities (PP) yielded high support (> 0.98) for all nodes, whereas maximum likelihood bootstrap support (BS) values revealed nodes with the most uncertainty. In the cpDNA genome phylogeny (Fig. 2) *Errazurizia multifoliolata*, *E. megacarpa*, and *E. benthamii* formed a clade, *Errazurizia* sensu stricto (s.s.), with high support (BS = 100, PP = 1.0). *Errazurizia multifoliolata* was sister to the clade of *E. megacarpa* and *E. benthamii*. *Errazurizia* s.s. was sister to *Eysenhardtia*, and together formed a clade that was sister to the rest of the amorphoids. *Apoplanesia paniculata* was sister to the clade comprised of *Amorpha, E. rotundata*, and *Parryella*. *Errazurizia rotundata* was sister to *P. filifolia* with high support (BS = 92, PP = 1.0) and together formed a clade sister to *Amorpha* with high support (BS = 100, PP = 1.0). The weakest relationship was between *A. californica* and the rest of the sampled *Amorpha* species (BS = 66, PP = 1.0); however, in the maximum likelihood analysis of the additional cpDNA alignment with gaps and ambiguous sites masked, *A. californica* was sister to the rest of *Amorpha* with high support (BS = 100) (Supplemental Fig. S3; Becklund and Ayers 2022). All species were highly supported as monophyletic except *A. fruticosa* L. and *A. canescens* Pursh, each with a sample more closely related to the other species.

Results of maximum likelihood and Bayesian analyses of the nrDNA cistron (Fig. 3) were similar to the cpDNA dataset and included strong support for the monophyly of *Errazurizia* s.s. and the sister relationship between *E. rotundata* and *Parryella filifolia*. However, *Amorpha* was paraphyletic with the inclusion of the *P. filifolia* and *E. rotundata* clade. *Amorpha californica* was sister to the *P. filifolia* and *E. rotundata* clade with high support (BS = 94, PP = 1.0), forming a clade that was sister to the rest of the sampled *Amorpha* spp. Relationships incongruent with the cpDNA phylogeny were also found between species in *Amorpha* and *Eysenhardtia*.

Morphology and Ancestral Character Estimations—Microcharacters from SEM (Fig. 4) revealed patterns in support of the newly inferred phylogenetic relationships. The tessellated, multicellular stem glands were prominent in all groups. *Errazurizia multifoliolata* had large, spherical glands with prominent mammiform cells. *Errazurizia megacarpa* and *E. benthamii* shared spherical-mammiform glands with blunt, prickle-shaped cells. In contrast, *E. rotundata*, *Parryella filifolia*, and *Amorpha californica* had prickle-shaped glands comprised of flattened, smooth cells. Abaxial leaflet surfaces exhibited a range in pubescence, from sparsely hairy in *P. filifolia* to densely tomentose in *E. megacarpa*. Glands on the abaxial leaflet surface were hemispherical in *Errazurizia* s.s., mammiform in *E. rotundata*, and punctate in *P. filifolia* and *A. californica.* The individual cells of the glands were flattened in all species except *E. multifoliolata*, which had mammiform cells. The tricolporate pollen grain exines were perforate in *Errazurizia* s.s. and reticulate with larger and deeper depressions in *E. rotundata, P. filifolia*, and *A. californica*. Chromosome counts from the root tips were $2n$ = ca. 16 in *E. rotundata* and $2n$ = 16–18 in *P. filifolia*. Both species were diploid.

For ancestral character estimations, the all-rates-different model was selected for petal number (–lnL = 8.89; d.f. = 1; $p < 0.05$) and gland shape (–lnL = 14.18; d.f. = 1; $p < 0.05$) and the equal-rate model for pollen exine (–lnL = 6.78; d.f. = 1; $p > 0.05$). The ancestral petal number (Fig. 5) for the amorphoid clade was five, transitioning to one by the most recent common ancestor (MRCA) of *Errazurizia rotundata*, *Parryella*,

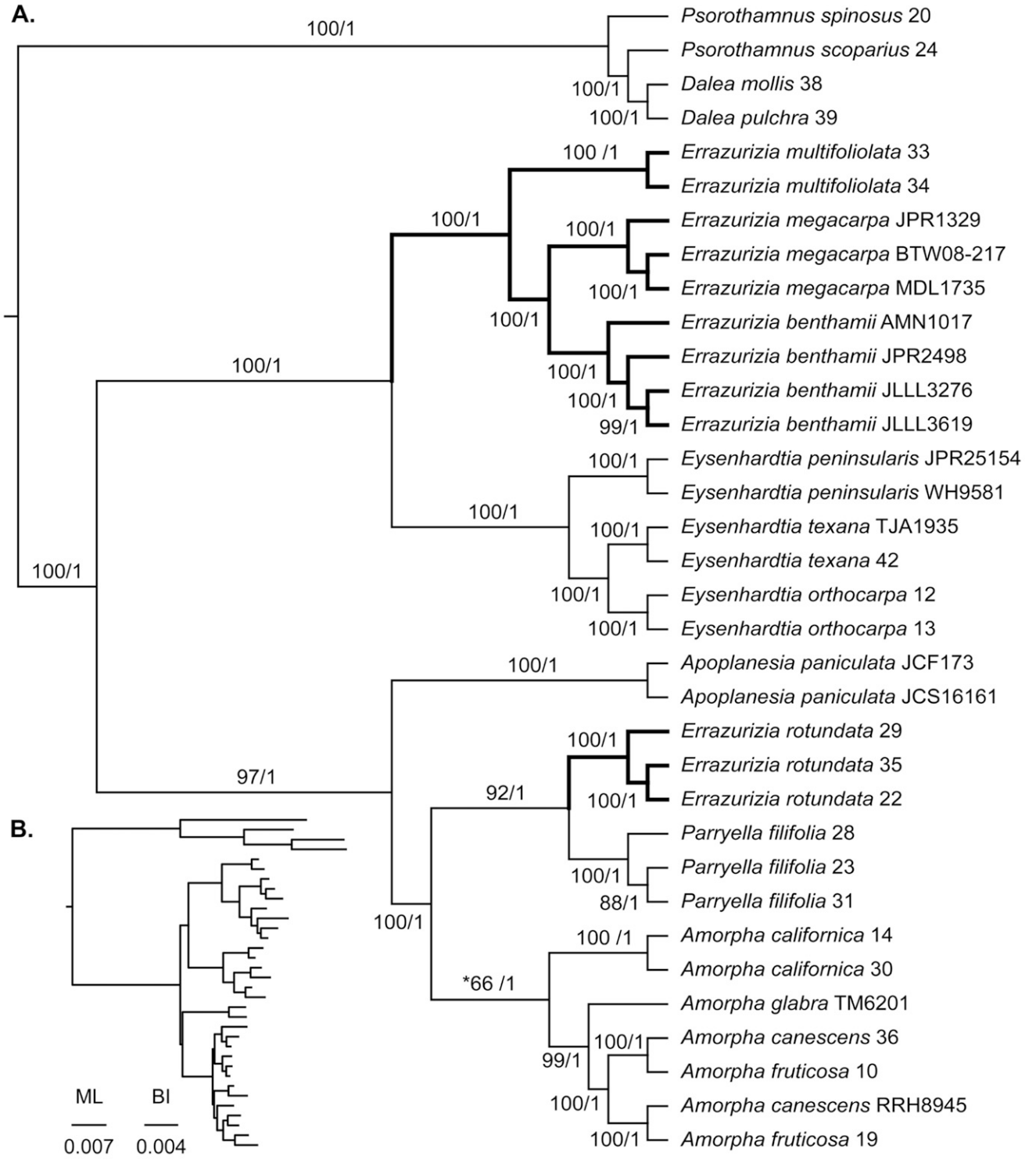

FIG. 2. Phylogeny of *Errazurizia* and the other amorphoid genera based on cpDNA genome dataset. A. Maximum likelihood (ML) cladogram. Branches of *Errazurizia* spp. are bolded. Branch support is displayed as BS/PP. The star indicates the branch with low ML support but with 100% BS support in the dataset stripped of gaps and ambiguous sites (Fig. S3). B. Phylogram of cpDNA genome dataset, scale bars represent 0.007 substitutions per site (ML) and 0.004 substitutions per site (Bayesian inference, noted as BI). Notations after species names are collector initials and collection number; specimens collected for this study are represented by collection number only (Appendix 1).

and *Amorpha*, and to no petals within *Parryella*. Prickle-shaped glands were likely the ancestral state of the amorphoids, transitioning to spherical by the MRCA of *Errazurizia* and to pustulose by the MRCA of *A. glabra*, *A. canescens*, and *A. fruticosa* and in *Apoplanesia paniculata*. Perforate pollen exines were estimated to be the ancestral state for the amorphoid clade, transitioning to reticulate by the MRCA of *E. rotundata*, *Parryella*, and *Amorpha*.

Light microscopy images (Fig. 6) revealed specimens of *Errazurizia rotundata* with ten stamens regardless of banner petal presence. One *E. rotundata* specimen exhibited two additional petaloid appendages in the position of the wing

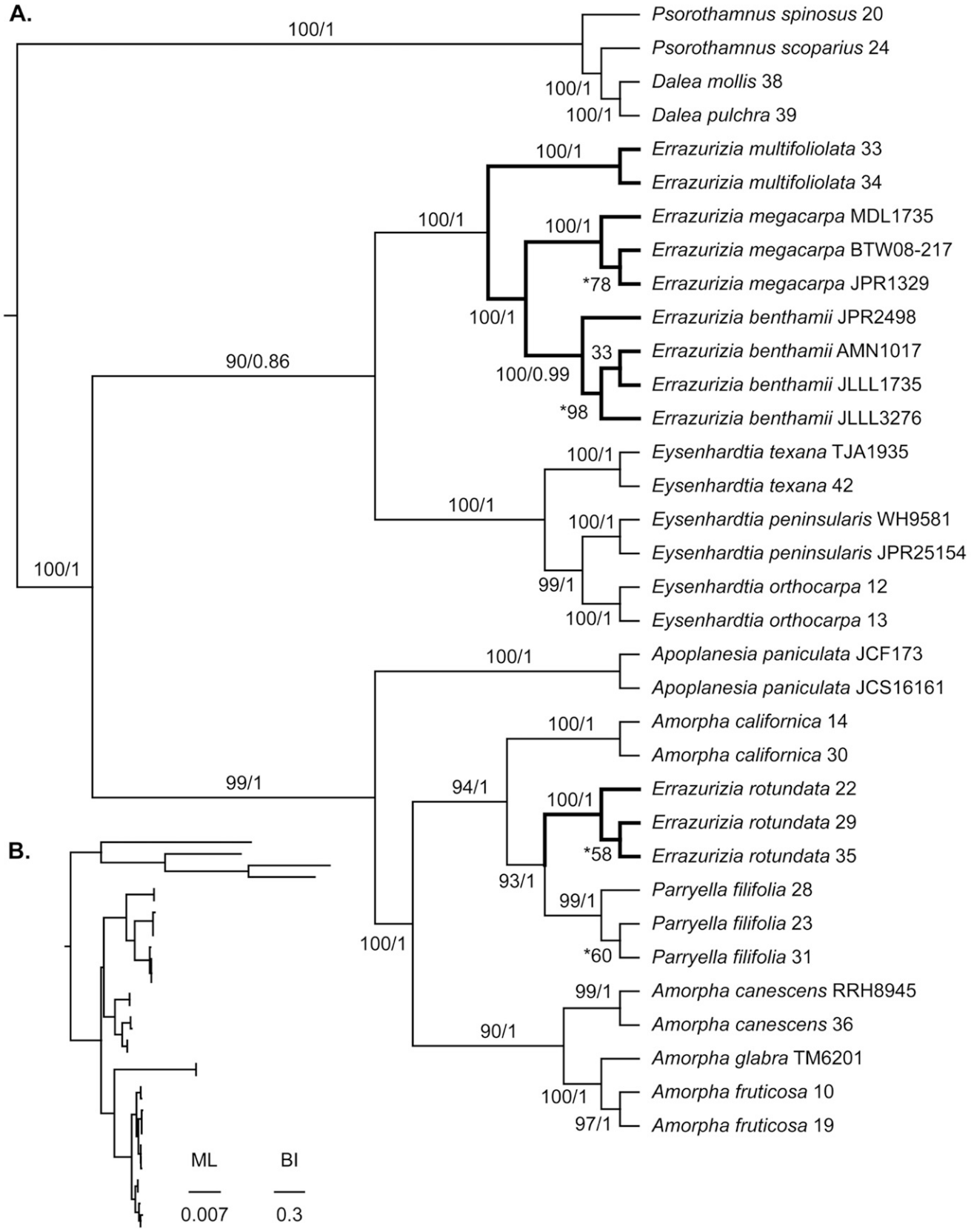

FIG. 3. Phylogeny of *Errazurizia* and the other amorphoid genera based on nrDNA cistron dataset. A. Maximum likelihood cladogram. Branches of *Errazurizia* spp. are bolded. Branch support is displayed as BS/PP. The stars indicate branches with Bayesian PP under 0.5 or a polytomy. B. Phylogram of nrDNA dataset, scale bars represent 0.007 substitutions per site (ML) and 0.3 substitutions per site (BI). Notations after species names are collector initials and collection number; specimens collected for this study are represented by collection number only (Appendix 1).

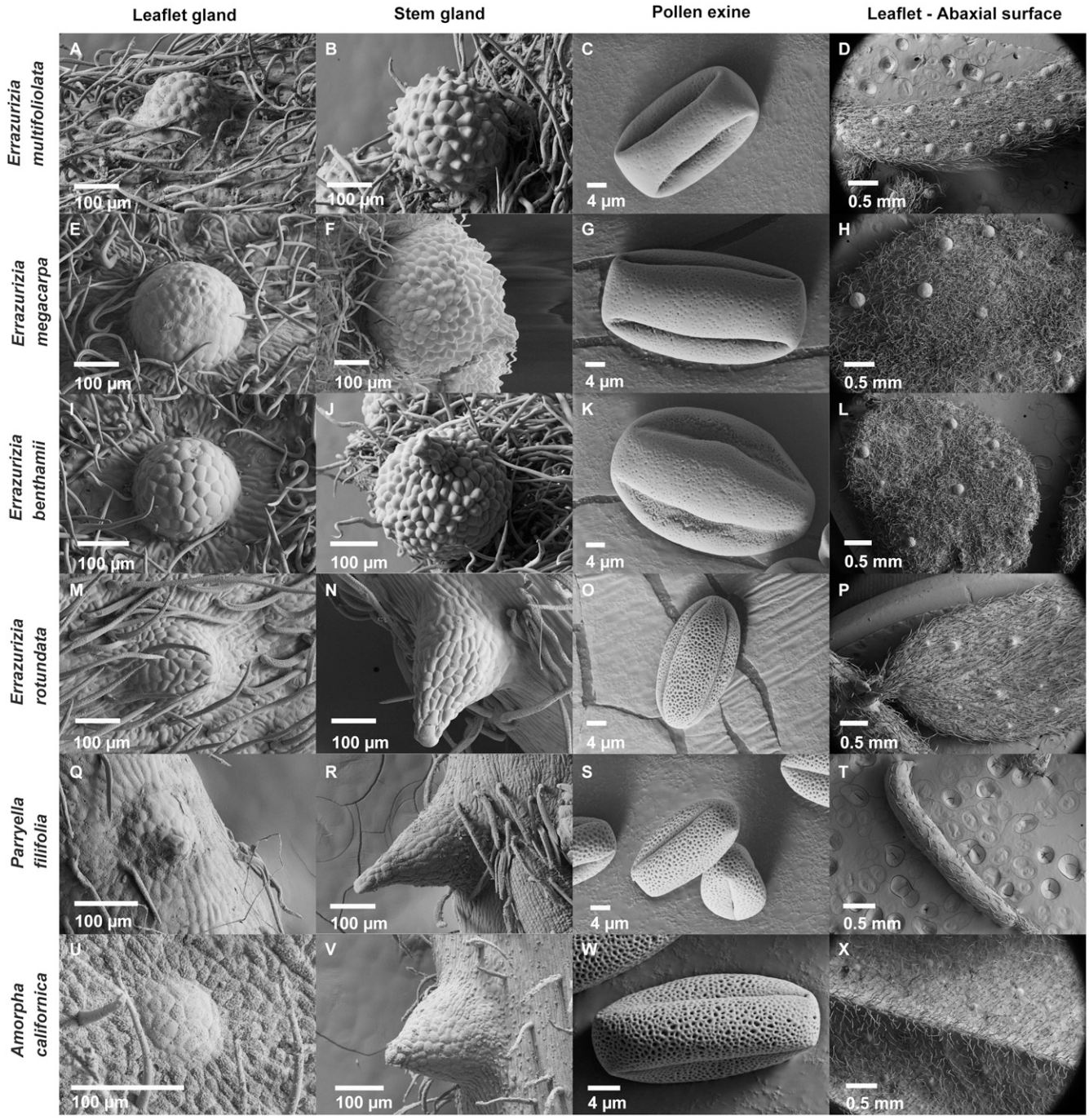

FIG. 4. SEM images of leaflet gland, stem gland, pollen exine, and abaxial leaflet surface. A–D. *Errazurizia multifoliolata*. E–H. *E. megacarpa*. I–L. *E. benthamii*. M–P. *E. rotundata*. Q–T. *Parryella filifolia*. U–X. *Amorpha californica*. Scale bars for each character are indicated.

petals, but only had eight of ten stamens (Fig. 6). Two other specimens also had varying numbers of petaloid appendages in place of fertile stamens.

DISCUSSION

The Amorphoid Clade and Errazurizia s.s.—*Errazurizia multifoliolata*, *E. megacarpa*, and *E. benthamii* form a clade that excludes the most recent addition, *E. rotundata*, confirming the work of Johnston (1924). Barneby (1977) originally placed *E. multifoliolata* and *E. megacarpa* into *E.* section *Errazurizia* based on the presence of pliant stems, many-flowered spikes, and a bent and glandular style but molecular and morphological data support the Mexican taxa *E. megacarpa* and *E. benthamii* as sister species. The prominent, hemispherical leaflet glands and spherical stem glands are synapomorphies in *Errazurizia* spp. and are additional traits to delineate the genus from other amorphoids.

Though previous studies (McMahon and Hufford 2004; McMahon 2005) found *Apoplanesia paniculata* was sister to the clade comprised of *Errazurizia* s.s. and *Eysenhardtia*, we determined that it was sister to the clade of *Amorpha*, *E. rotundata*, and *Parryella*. *Apoplanesia paniculata* shares characters with *Errazurizia* s.s. and *Eysenhardtia*, such as having five petals

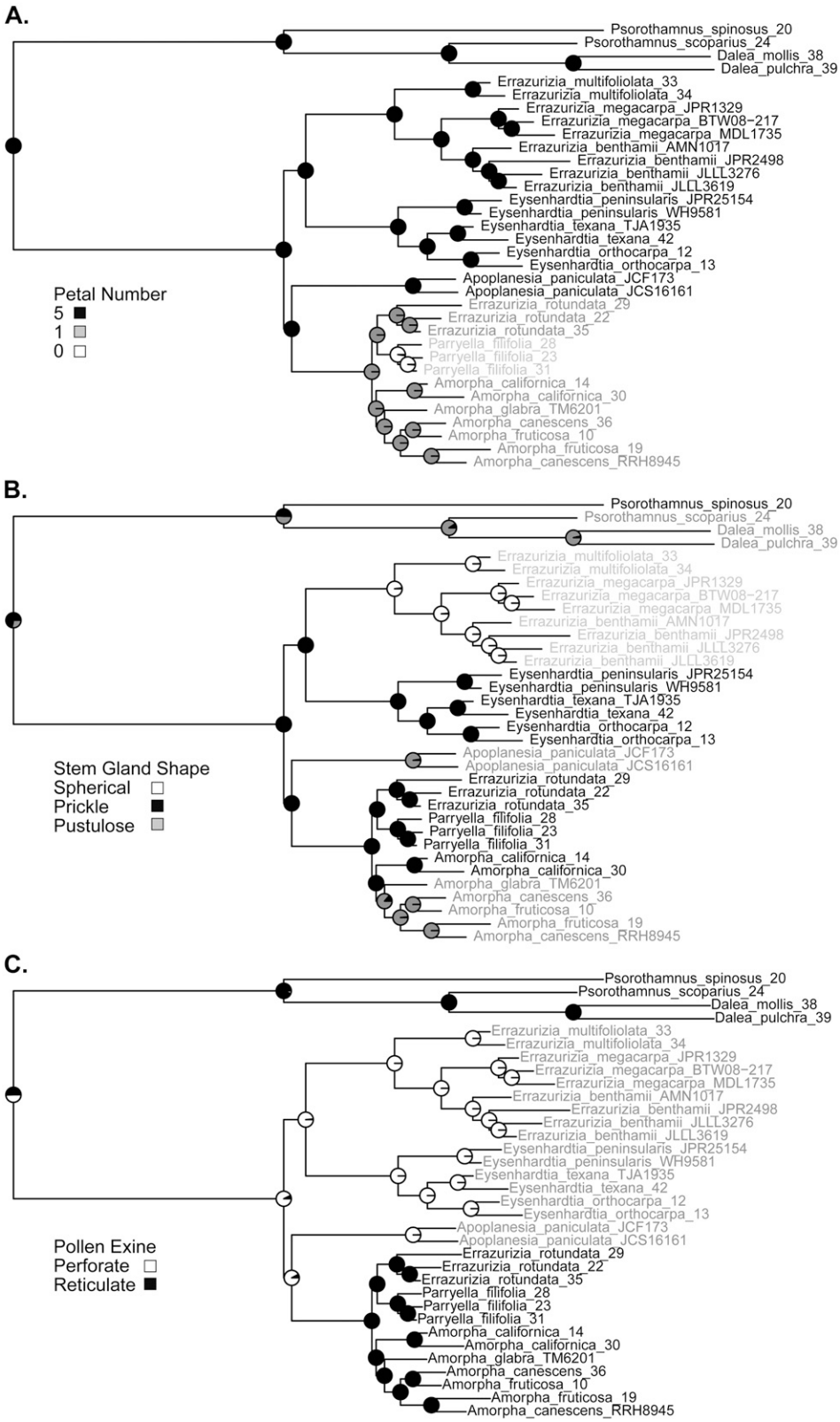

FIG. 5. Ancestral character state estimations using the cpDNA phylogram. A. Petal number using all-rates-different model. B. Gland shape using all-rates-different model. C. Pollen exine using equal-rates model.

and perforate pollen exine, but shares few traits with *Amorpha*, *E. rotundata*, and *Parryella*. Ancestral character estimations, however, revealed five petals and perforate pollen exine were ancestral traits in the amorphoids, indicating changes in the two character states likely happened after the lineage of *A. paniculata* diverged, thus providing a plausible explanation for the lack of shared characters with the clade of *Amorpha*, *E. rotundata*, and *Parryella*.

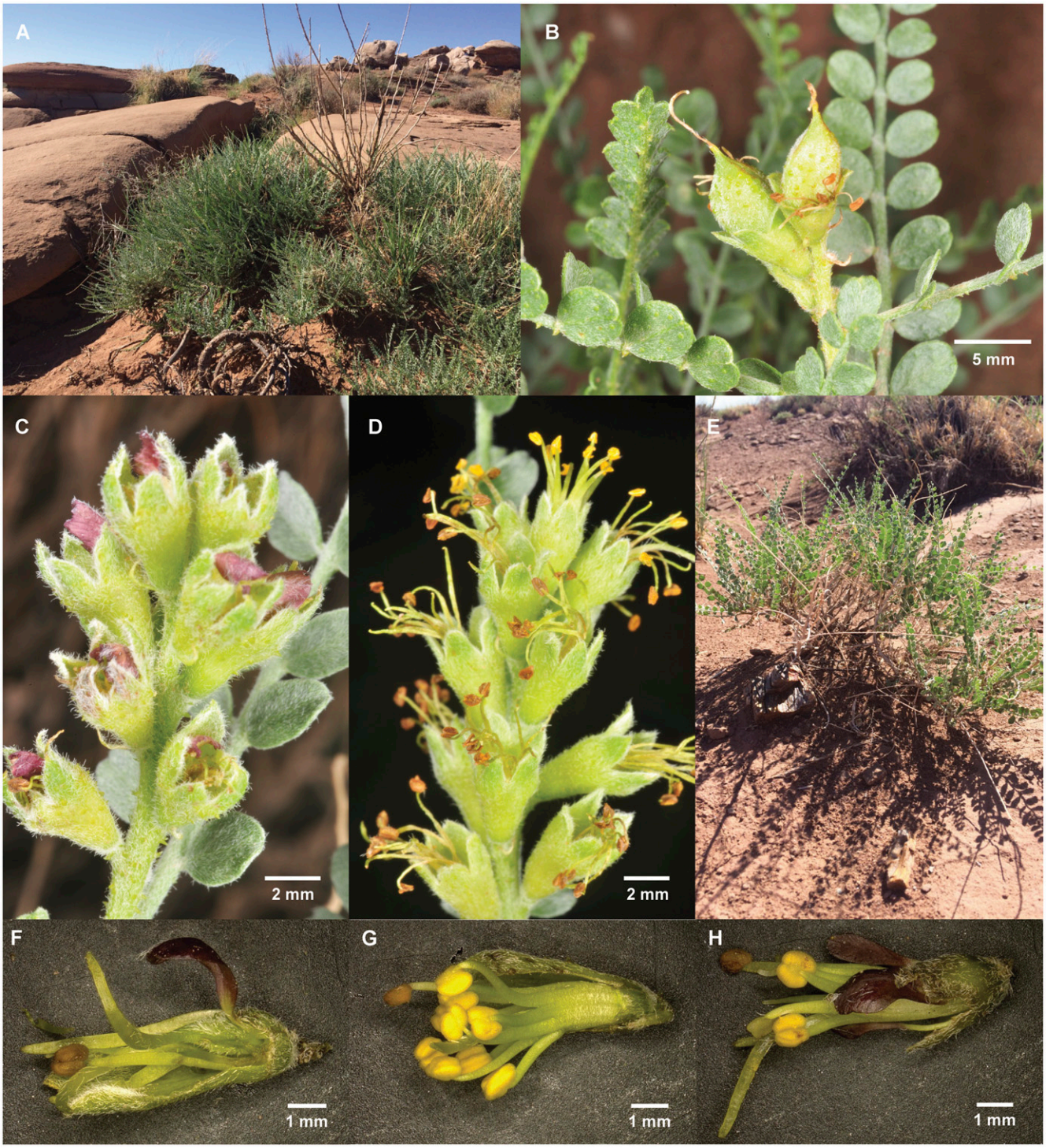

FIG. 6. Photographic and light microscopic images of *Pictarena rotundata*. A. Plant habit. B. Foliage, glands, and ripening fruit. C. Inflorescence with flowers with one petal. D. inflorescence with flowers with no petals. E. Closeup of plant habit. F. Flower with calyx removed and petal bent upwards to show attachment. G. Flower without petal, calyx removed showing fused filaments. H. Flower with a banner petal, two petaloid appendages, and eight stamens.

Ancestral character state estimations revealed petal number, gland shape, and pollen exine are further evidence that *Errazurizia rotundata* is not closely related to the other *Errazurizia* species and support the sister relationship between *E. rotundata* and *Parryella filifolia*. Pollen patterns align with previously described pollen attributes in *E. rotundata* and *P. filifolia* (Mahler 1965, as cited in Barneby 1977). An important morphological shift from five petals to a single petal and from perforate to reticulate pollen occurred by the MRCA of *Amorpha*, *E. rotundata*, and *Parryella*, after which further character state changes occurred in a period of rapid diversification, evidenced by the short branch lengths of the cpDNA and nrDNA phylogenies. Prickle-shaped glands occur throughout the amorphoids and were recovered as the likely ancestral state. Due to incomplete sampling of *Amorpha* and *Eysenhardtia*, however, genera with potentially polymorphic

gland states are inadequately represented and a more complete sampling is necessary to determine the ancestral states of the internal nodes. Gland patterns in the early diverging lineages could not be inferred; within *Dalea*, a genus of 165 + spp., gland shape undoubtedly varies, and *Marina* Liebm. lacks representation in the current phylogeny.

Cytonuclear Discordance—The incongruency of the cpDNA and nrDNA phylogenies is unsurprising. Cytonuclear discordance is common in other plant groups with rapid radiations (Lee-Yaw et al. 2019; Dodsworth et al. 2020). The presence of short branch lengths in both datasets suggests a pattern of rapid diversification between *Amorpha* and the clade of *Errazurizia rotundata* and *Parryella*, a trend seen in other groups in the family (Cardoso et al. 2013; Koenen et al. 2020). A possible explanation is incomplete lineage sorting among the cpDNA and nrDNA markers (Straub and Doyle 2014) and lack of variation in the nrDNA cistron dataset. The nrDNA cistron exhibited the least amount of divergence with shortest branch lengths and had fewer phylogenetically informative sites (6.5%), which could be attributed to several processes, such as concerted evolution decreasing variability of the nrDNA cistron (Álvarez and Wendel 2003). Issues with phylogenetically informative sites of the ITS marker have been identified in other legume studies (Lavin et al. 2005) and the shorter branches found in the amorphoid clade in comparison to the daleoids is consistent with findings in the previous phylogeny of the tribe (McMahon and Hufford 2004). Other potential causes of cytonuclear discordance include a whole genome duplication event or hybridization. Barneby (1977) suggested that an ancient hybridization event between *Psorothamnus* and *Parryella* led to the formation of *E. rotundata* based on *Psorothamnus*-like pods and *Parryella*-like apetalous flowers. Hybrid origin involving *Psorothamnus* is unsupported phylogenetically, but short branch lengths and the non-monophyly in *Amorpha* could indicate a hybridization event between *Amorpha* and *P. filifolia* in the origin of *E. rotundata*.

Reclassifying Errazurizia rotundata—*Errazurizia rotundata* requires reclassification to reflect its evolutionary history. Based on morphological and phylogenetic evidence the species should be elevated as a monotypic genus, *Pictarena* Becklund gen. nov. The genus is characterized by the pertinent characters noted by Barneby (1962) in the removal of *E. rotundata* from *Parryella* and inclusion in *Errazurizia*: suborbicular leaflets with prominent glands, spikes of 15 flowers or fewer, a banner with a polymorphic presence or absence, a partially fused filament tube, and a large, ellipsoid pod. Phylogenetic analyses presented here confirm *E. rotundata* is sister to *Parryella filifolia* and both are closely related to *Amorpha*. However, the evidence of important morphological characters, specifically petal presence, and phylogenetic relationships complicate the inclusion of *E. rotundata* into *Parryella* as well as the inclusion of *E. rotundata* and *Parryella* into *Amorpha*.

Errazurizia rotundata and *Parryella filifolia* differ from *Amorpha* in inflorescence and floral traits that argue against their inclusion into *Amorpha*. Although *Amorpha*, *Parryella*, and *Errazurizia rotundata* have ten exserted stamens and operculate and reticulate pollen, *Parryella* and *Amorpha* have racemes or panicles with many (30–90+) small flowers (versus 15 or fewer in *E. rotundata*) and small, oblique fruit (versus large ellipsoid fruit in *E. rotundata*) (Wilbur 1975). More importantly, *Amorpha* and *Parryella* are delimited by the presence or absence of a banner (present in *Amorpha*; absent in *Parryella*), while *E. rotundata* is polymorphic for that feature. Studies of floral development determined the petal suppression in *Amorpha* is fundamentally different from petal absence in *Parryella* (McMahon and Hufford 2005), while petal development has not been evaluated in *E. rotundata*. Petal morphology in *E. rotundata* is unique in the tribe and differs from *Amorpha* species in that it is thickened and does not extend much beyond the calyx or envelop the androecium, a diagnostic character in *Amorpha* (Wilbur 1975). The presence of ten stamens regardless of petal presence demonstrates the petal in *E. rotundata* is not an aberrant petaloid staminode, despite the rare occurrence (Fig. 6). Petal loss is documented in other papilionoid tribes (Ireland 2005; Pennington et al. 2005; Cardoso et al. 2013) and presence or absence of petals is a diagnostic character used to delineate genera in Amburaneae (Kirkbride 2005), Swartzieae (Torke and Schall 2008; Pinto et al. 2012), and Sophoreae (Tucker 1990). *Swartzia* Schreb., a genus comprised of apetalous or single-petaled species, is an exception to the use of petals to delimit genera. However, the $>$ 180 species of *Swartzia* are separated into series that have yet to be the focus of phylogenetic studies (Torke and Schall 2008).

In addition to the floral differences among *Amorpha*, *Parryella*, and *Errazurizia rotundata*, the uncertainty of whether *Amorpha* is monophyletic further impedes the addition of the taxa into *Amorpha*. Published molecular phylogenies of *Amorpha* were incongruent, and either reconstructed a monophyletic or polyphyletic genus based on the molecular markers used (McMahon and Hufford 2004; Straub and Doyle 2014). Our nrDNA analyses reconstructed *Amorpha* as paraphyletic, with *A. californica* more closely related to *E. rotundata* and *P. filifolia*. The cpDNA dataset recovered two polyphyletic species, *A. canescens* and *A. fruticosa*, each with an individual that was sister to the other species. Uncertainty in the relationships of *Amorpha* species could be attributed to the lack of variation found in the nrDNA cistron dataset. Phylogenetic uncertainty could also be a result of the low sequencing depth in one sample each of *A. canescens* (mean = 17) and *A. fruticosa* (mean = 14). Another likely explanation is hybridization of the *Amorpha* species and chloroplast capture, as *A. fruticosa* is a known polyploid that was polyphyletic in a previous study (Straub and Doyle 2014). The inclusion of *P. filifolia* and *E. rotundata* in *Amorpha* is premature due to the remaining uncertainty of species' relationships in *Amorpha*. *Amorpha* requires a deeper sampling of genetic markers (e.g. a phylogenomic approach) and a broader sampling of species to determine interspecific relationships.

The transfer of *Errazurizia rotundata* back into *Parryella* is similarly restricted by morphology. *Errazurizia rotundata* and *Parryella* have substantial differences in leaf, inflorescence, and floral traits that led to the removal of *E. rotundata* from the *Parryella* (Barneby 1962; Heil et al. 2013). *Errazurizia rotundata* is delimited from *Parryella* by subsessile and suborbicular leaflets with mammiform glands versus pedicellate, filiform leaflets and punctate glands; spikes with 15 flowers or less versus spicate racemes or panicles with 30–90 flowers; a large calyx with proximally pubescent teeth versus a small, glabrous calyx; an androecium fused up to half the length versus an androecium fused only at the base; and a large, ellipsoid pod versus a small, oblique pod. Although our analyses support a sister relationship between *E. rotundata* and *P. filifolia*, a potential hybrid origin for *E. rotundata* involving *Parryella* and a member of the amorphoid clade was beyond the scope

of this study. Presently, the morphological and genetic differences between the three taxa are substantial and any recombination of *E. rotundata* into *Parryella* or *E. rotundata* and *Parryella* into *Amorpha* would be premature and would only serve to prolong an apparent artificial classification.

As surmised by Barneby (1977), we confirm that *Errazurizia rotundata* should be elevated as the new monotypic genus *Pictarena* based upon the phylogenetic relationships and morphology presented here. The following descriptions are adapted from Barneby's (1977) description of *E. rotundata*.

Taxonomic Treatment

Pictarena Becklund, gen. nov. Type: USA. *Parryella rotundata* Wooton, Bull. Torrey Club 25: 457, 1898.

Habit shrubs. **Stems** erect, many branched from a tortuously woody base, pubescent with prominent mammiform- or prickle-shaped glands. **Leaves** alternate, imparipinnate, stipulate, densely pubescent, with mammiform- or prickle-shaped glands; leaflets subsessile, estipellate, blade suborbicular, margins entire, with mammiform glands on abaxial surface. **Inflorescences** spicate, terminal. **Flowers** bisexual; calyx vase-shaped, 10-nerved, the five teeth proximally pubescent, glandular, marcescent; petals reduced to banner only or absent, inserted on the hypanthium when present, yellow or maroon, not much extended past calyx teeth; androecium 10, monadelphous, glandless, exserted past calyx teeth, the filaments united past the hypanthium, anthers yellow; style often persistent in fruit; ovules 2, one maturing. **Fruits** indehiscent, ellipsoid, single-seeded, papery at maturity, glandular; seed ellipsoid.

Distribution and Habitat—The species occurs in the United States, in Arizona, in Coconino and Navajo counties (Fig. 1C).

Etymology—*Pictarena* comes from the Latin "pictus", painted, and "arena", sand or desert, and is in reference to the Painted Desert in the Little Colorado River region, the known range of this narrow endemic.

Pictarena rotundata (Wooton) Becklund, comb. nov. *Errazurizia rotundata* (Wooton) Barneby, Leafl. West. Bot. 9: 210, 1962. *Parryella rotundata* Wooton, Bull. Torrey Club 25: 457, 1898. Type: USA. Arizona, Coconino County, Near Winslow *Wooton s.n.* 1892 (holotype: US!).

Stems to 3.5 dm tall, grayish-brown, up to 1 cm thick; young stems cinereous or canescent, densely pilosulous-strigulose, hairs sub-appressed and narrowly ascending, 0.3–0.4 mm long; glands orange or livid. **Leaves** 3–14 cm long; stipules subulate or narrowly triangular, 0.5–2.5 mm long, dimorphic, glandular at base and apex with one or more grain-like glands, deciduous, purplish-brown, and distally glabrate; rachis narrowly green-margined, dorsoventrally flattened, stiffly marcescent; leaflets in 14–30 pairs, gradually diminishing in size towards distal end, larger leaflets pinnately nerved, the terminal leaflet smallest and elevated beyond the last pair; blades suborbicular to oblong-obovate, 1–11.5 mm long, dorsally keeled; apex obtuse or shallowly emarginate, minutely gland-apiculate. **Inflorescences** sessile or pedunculate, peduncles to 1 cm long, the spike axis 4–15 mm long, distally glabrate, and glandular; bracts deciduous, glandular. **Flowers** 6–15; calyx 5–6.5 mm long, pilosulous abaxially; calyx tube 3.5–4.2 mm long, prominently 10-ribbed, glandular, the glands 2–5 between the ribs in an irregular row; teeth oblong-obovate, 1–2.3 mm long, nearly equal in length or the dorsal one slightly longer, the apex obtuse, or gland-apiculate, densely pilosulous internally, at anthesis erect, in fruit recurved; hypanthium 1.4–2 mm deep; banner (if present) oblanceolate, 5–5.4 mm long, 1.5–2.1 mm wide, pale yellow fading reddish-maroon, apex subacute to emarginate, thinly pubescent dorsally above middle; androecium (7) 8.5–12 mm long; filaments united into a tubular sheath, 3.5–4 mm long, free for (3.5) 5–8 mm distally, greenish-yellow, the connective glandless and sometimes present atop the maturing fruit; anthers 0.8–1.3 mm long, minutely pubescent; style thinly pilosulous, the stigma lateral; ovules not exactly collateral. **Fruits** ellipsoid or obovoid-ellipsoid, slightly compressed, 9–11 mm long; base cuneate; apex abruptly contracted into the persistent thick-carinate style-base; valves rugulose densely strigulose, glandular, the glands grain-like, red or livid; seed (4.6) 5–7 mm long, (2.7) 3–3.7 mm wide in profile, 2–2.5 mm thick, sub-compressed, testa reddish-brown and lustrous. Figure 6.

Distribution and Habitat—USA. Endemic to four known localities in the Little Colorado River watershed in the Chinle, Moenkopi, and Glen Canyon Group sandstones, and Holocene surficial deposits. Often found along drainages or on rimrock and ledges of cliffs. Localities are almost exclusive to the Navajo Nation.

Etymology—The specific epithet, *rotundata*, refers to the rounded leaflets.

Notes—Flowering April to May and/or August to September; fruiting in June and/or September-October. Flowering is dependent on late fall, winter, and summer rains. $2n$ = ca. 16. *Pictarena rotundata* is classified as imperiled (G2) by the United States and Arizona (Laurenzi and Spence 2013; NatureServe 2016) and endangered (Group 3) by the Navajo Nation Endangered Species List (Mikesic and Roth 2008). The common name is roundleaf dunebroom.

***Additional Specimens Examined*—USA.** —Arizona: Coconino County: Winslow, *L.E. Becklund 22* (ASC, NAVA), *R.K. Gierisch 3936* (ARIZ, ASC, ASU); Moenave, *L.E. Becklund 29* (ASC, NAVA), *C.F. Deaver 5907* (ARIZ, ASC, ASU, NY), *L.T. Green 8084* (ARIZ, MNA, NAVA) *A. Hazelton 1661* (NAVA), *D. Roth 1857* (ASC, NAVA); Willow Springs, *J. Beasley 970* (ASC). Navajo County: Holbrook, *L.E. Becklund 35, 43* (ASC); Apache Butte, *B. Hevron 1117* (ASC, NAVA).

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Author Contributions

LEB was responsible for most of the field work, gathering the morphological and molecular data sets, completing all analyses, and writing the draft manuscript based on a Master's thesis submitted to Northern Arizona University. TJA provided guidance throughout the project, contributed to the field work, and reviewed the manuscript and taxonomic descriptions.

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Appendix 1. Vouchers and GenBank accession numbers of samples included in this study. Order of data are species, sample ID, collector and voucher number, herbarium, NCBI Short Read Archive accession number.

Ingroup: *Amorpha californica*, 14, *L.E. Becklund 14*, ASC, SAMN20056860; *Amorpha californica*, 30, *L.E. Becklund 30*, ASC, SAMN20056861; *Amorpha canescens*, 36, *L.E. Becklund 36*, ASC, SAMN20056862; *Amorpha canescens*, RRH8945, *R. R. Halse 8945*, ARIZ, SAMN20056863; *Amorpha fruticosa*, 10, *L.E. Becklund 10*, ASC, SAMN20056864; *Amorpha fruticosa*, 19, *L.E. Becklund 19*, ASC, SAMN20056865; *Amorpha glabra*, TM6201, *T. McDowell 6201*, ETSU, SAMN20056866; *Apoplanesia paniculata*, JCF173, *J.C. Flores 173*, ARIZ, SAMN20056867; *Apoplanesia paniculata*, JCS16161, *J.C. Soto N. 16161*, ARIZ, SAMN20056868; *Errazurizia benthamii*, JLLL3276, *J.L. Leon de la Luz 3276*, SD, SAMN20056871; *Errazurizia benthamii*, JPR2498, *J.P. Rebman 2498*, HCIB, SAMN20056872; *Errazurizia benthamii*, AMN1017, *A.M. Narvaez 1017*, HCIB, SAMN20056873; *Errazurizia benthamii*, JLLL3619, *J.L. Leon de la Luz 3619*, HCIB, SAMN20056874; *Errazurizia megacarpa*, JPR1329, *J.P. Rebman* 1329, SD, SAMN20056875; *Errazurizia megacarpa*, BTW08-217, *B.T. Wilder 08–217*, ARIZ, SAMN20056876; *Errazurizia megacarpa*, MDL1735, *M. Dominguez L. 1735*, HCIB, SAMN20056877; *Errazurizia multifoliolata*, 33, *L.E. Becklund 33*, ASC, SAMN20056878; *Errazurizia multifoliolata*, 34, *L.E. Becklund 34*, ASC, SAMN20056879; *Errazurizia rotundata*, 22, *L.E. Becklund 22*, ASC, SAMN20056880; *Errazurizia rotundata*, 29, *L.E. Becklund 29*, ASC, SAMN20056881; *Errazurizia rotundata*, 35, *L.E. Becklund 35*, ASC, SAMN20056882; *Eysenhardtia orthocarpa*, 12, *L.E. Becklund 12*, ASC, SAMN20056883; *Eysenhardtia orthocarpa*, 13, *L.E. Becklund 13*, ASC, SAMN20056884; *Eysenhardtia peninsularis*, JPR25154, *J.P. Rebman 25154*, SD, SAMN20056885; *Eysenhardtia peninsularis*, WH9581, *W. Hodgson 9581*, SD, SAMN20056886; *Eysenhardtia texana*, TJA1935, *T.J. Ayers 1935*, ASC, SAMN20056887; *Eysenhardtia texana*, 42, *L.E. Becklund 42*, ASC, SAMN20056888; *Parryella filifolia*, 23, *L.E. Becklund 23*, ASC, SAMN20056889; *Parryella filifolia*, 28, *L.E. Becklund 28*, ASC, SAMN20056890; *Parryella filifolia*, 31, *L.E. Becklund 31*, ASC, SAMN20056891.

Outgroup: *Dalea mollis*, 38, *L.E. Becklund 38*, ASC, SAMN20056869; *Dalea pulchra*, 39, *L.E. Becklund 39*, ASC, SAMN20056870; *Psorothamnus scoparius*, 24, *L.E. Becklund 24*, ASC, SAMN20056892; *Psorothamnus spinosus*, 20, *L.E. Becklund 20*, ASC, SAMN20056893.

Appendix 2. Character states for ancestral character state estimations. Order of data are species_sampleID, petal number 0 or 1 or 5, gland shape 0 = prickle or 1 = spherical or 2 = pustulose, pollen exine 0 = perforate 1 = reticulate.

*Amorpha_californica*_14, 1, 0, 1; *Amorpha_californica*_30, 1, 0, 1; *Amorpha_canescens*_36, 1, 2, 1; *Amorpha_canescens*_RRH8945, 1, 2, 1; *Amorpha_fruticosa*_10, 1, 2,1; *Amorpha_fruticosa*_19, 1, 2, 1; *Amorpha_glabra*_TM6201, 1, 2, 1; *Apoplanesia_paniculata*_JCF173, 5, 2, 0; *Apoplanesia_paniculata*_JCS16161, 5, 2, 0; *Dalea_mollis*_38, 5, 2, 1; *Dalea_pulchra*_39, 5, 2, 1; *Errazurizia_benthamii*_JLLL3276, 5, 1, 0; *Errazurizia_benthamii*_JPR2498, 5, 1, 0; *Errazurizia_benthamii*_AMN1017, 5, 1, 0; *Errazurizia_megacarpa*_JPR1329, 5, 1, 0; *Errazurizia_megacarpa*_BTW08-217, 5, 1, 0; *Errazurizia_megacarpa*_MDL1735, 5, 1, 0; *Errazurizia_benthamii*_JLLL3619, 5, 1, 0; *Errazurizia_multifoliolata*_33, 5, 1, 0; *Errazurizia_multifoliolata*_34, 5, 1, 0; *Errazurizia_rotundata*_22, 1, 0, 1; *Errazurizia_rotundata*_29, 1, 0, 1; *Errazurizia_rotundata*_35, 1, 0, 1; *Eysenhardtia_orthocarpa*_12, 5, 0, 0; *Eysenhardtia_orthocarpa*_13, 5, 0, 0; *Eysenhardtia_peninsularis*_JPR25154, 5, 0, 0; *Eysenhardtia_peninsularis*_WH9581, 5, 0, 0; *Eysenhardtia_texana*_TJA1935, 5, 0, 0; *Eysenhardtia_texana*_42, 5, 0, 0; *Parryella_filifolia*_23, 0, 0, 1; *Parryella_filifolia*_28, 0, 0, 1; *Parryella_filifolia*_31, 0, 0, 1; *Psorothamnus_scoparius*_24, 5, 2, 1; *Psorothamnus_spinosus*_20, 5, 0, 1.