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Anatomical variation in Cactaceae and relatives: Trait lability and evolutionary innovation[1]

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The cacti have undergone extensive specialization in their evolutionary history, providing an excellent system in which to address large-scale questions of morphological and physiological adaptation. Recent molecular phylogenetic studies suggest that (1) *Pereskia*, the leafy genus long interpreted as the sister group of all other cacti, is likely paraphyletic, and (2) Cactaceae are nested within a paraphyletic Portulacaceae as a member of the "ACPT" clade (Anacampseroteae, Cactaceae, *Portulaca*, and *Talinum*). We collected new data on the vegetative anatomy of the ACPT clade and relatives to evaluate whether patterns in the distributions of traits may provide insight into early events in the evolutionary transition to the cactus life form. Many traits had high levels of homoplasy and were mostly equivocal with regard to infraclade relationships of ACPT, although several characters do lend further support to a paraphyletic *Pereskia*. These include a thick stem cuticle, prominent stem mucilage cells, and hypodermal calcium oxalate druses, all of which are likely to be important traits for stem water storage and photosynthesis. We hypothesize that high lability of many putative "precursor" traits may have been critical in generating the organismal context necessary for the evolution of an efficient and integrated photosynthetic stem.

Key words: adaptation; Cactaceae; character evolution; homoplasy; Portulacaceae; Portulacineae; stem photosynthesis; vegetative anatomy.

Cactaceae are one of the most distinctive and immediately recognizable plant groups due to their unusual growth forms, which reflect adaptations that allow them to persist and flourish in extremely arid environments. Characteristics such as stem-based photosynthesis and a concomitant reduction of leaves, stem succulence, spines, and crassulacean acid metabolism (CAM) photosynthesis are all considered to be adaptations of cacti to water-limited environments (Gibson and Nobel, 1986), a supposition that is supported by the independent acquisition of different combinations of these traits in unrelated lineages that are also drought adapted (e.g., Euphorbiaceae, Agavaceae, Aizoaceae).

Cacti thus are an extremely useful system in which to examine questions surrounding the evolution of specialized morphologies and physiologies in plants, especially considering that within the group there is a range of variation in these traits. Such questions are best approached within an explicitly phylogenetic framework. In the case of Cactaceae, phylogenetic relationships both within and outside of the group have historically proven difficult to resolve by traditional morphology-based methods, but two recent breakthroughs in cactus molecular systematics now enable us to ask cogent evolutionary questions about the group. The first is that the genus *Pereskia* Mill., generally interpreted as the sister group to the rest of Cactaceae, appears to be paraphyletic (Nyffeler, 2002; Butterworth and Wallace, 2005; Edwards et al., 2005). The second is the finding that Cactaceae are nested within a paraphyletic Portulacaceae (Hershkovitz and Zimmer, 1997; Applequist and Wallace, 2001; Edwards et al., 2005; Nyffeler, 2007).

Phylogenetic context—Within Cactaceae, *Pereskia* has traditionally been viewed as the sister group to the remaining cacti, retaining many ancestral traits that have been lost in other species. Such traits include fully expanded photosynthetic leaves, a relatively nonsucculent stem, unspecialized wood, and a tree or shrub habit (Gibson and Nobel, 1986; Leuenberger, 1986; Mauseth and Landrum, 1997). Recent studies confirm this phylogenetic placement but further suggest that *Pereskia* as traditionally circumscribed is likely paraphyletic, with a mainly Caribbean/Central American/northern South American clade (hereafter, the northern clade) sister to a mainly Andean/southern South American clade (hereafter, the Andean/SSA clade) plus the remainder of Cactaceae (Edwards et al., 2005) (Fig. 1). Close examination of this *Pereskia* sensu lato reveals variation in some characters that may be taxonomically useful. Although no character perfectly distinguishes the two *Pereskia* clades, all northern clade species have precocious stem periderm onset and lack stem stomata, while most Andean/SSA clade species have delayed stem periderm (except *P. aculeata*) and possess stem stomata (except *P. nemorosa*). No *Pereskia* species have been reported to engage in significant photosynthetic carbon uptake through the stem (Nobel and Hartsock, 1986; Martin and Wallace, 2000; Edwards and Donoghue, 2006), but the presence of stem stomata and delayed periderm unites the Andean/SSA species with the most speciose cactus lineages, Opuntioideae and Cactoideae ("core cacti" of Edwards et al., 2005). The changes in these characters subsequent to the divergence of the northern *Pereskia* lineage from other cacti may

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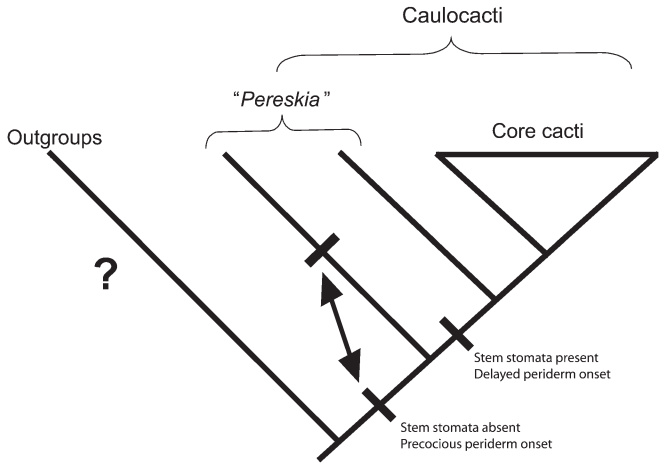

Fig. 1. Paraphyly of *Pereskia*, after Edwards et al. (2005). Note that interpretation of the timing and polarity of character state changes depends on states in outgroup taxa, as indicated by arrow.

have therefore been important preconditions for the evolution of the functionally leafless, stem-photosynthetic life form of the core cacti. However, assigning the polarity of transitions in periderm timing and stem stomata, as well as the evolutionary importance of changes in these characters, requires knowledge about these character states in outgroup taxa (Fig.1, arrow).

Molecular phylogenetic work in recent years has done much to elucidate relationships within Caryophyllales and has supported the association of Cactaceae with Portulacaceae, Didiereaceae, Basellaceae, and Hectorellaceae (i.e., suborder Portulacineae sensu Thorne, 1976) (Hershkovitz and Zimmer, 1997; Applequist and Wallace, 2001; Cuénod et al., 2002; Applequist et al., 2006; Nyffeler, 2007). Moreover, these molecular studies have clearly indicated that Portulacaceae are paraphyletic with respect to Cactaceae, Didiereaceae, and Hectorellaceae (Fig. 2), a result that was hinted at previously in some morphological studies (Chorinsky, 1931; Hershkovitz, 1993). Thus the nearest relatives of the cactus family appear to be three lineages of predominantly African, eastern American, and Australian taxa within Portulacaceae: *Talinum* Adans. (including *Talinella* Baillon), *Portulaca* L., and Anacampseroteae Nyananyo ex. G. D. Rowley (Nyffeler, 2007) (Fig. 2). Because a formal nomenclatural classification for this group has not yet been proposed, the clade consisting of these three portulacaceous lineages plus Cactaceae will hereafter be referred to by the working title ACPT (Anacampseroteae, Cactaceae, *Portulaca*, and *Talinum*).

Despite numerous molecular studies using chloroplast (*ndhF*: Applequist and Wallace, 2001; *mat*K and *ndhF*: Nyffeler, 2007), mitochondrial (*nad1*: Nyffeler, 2007), and nuclear markers (ITS: Hershkovitz and Zimmer, 1997; *phyC*: Edwards et al., 2005), the exact relationships among the four major clades of ACPT and within suborder Portulacineae as a whole have proven difficult to resolve. In ACPT, there is generally strong support across genes and methods of analysis for monophyly of the four major component clades of Cactaceae, Anacampseroteae, *Portulaca*, and *Talinum*, as well as for the ACPT clade itself. The nodes indicating infraclade relationships in ACPT, however, are less well resolved. In particular, the relationships among *Portulaca*, Anacampseroteae, and Cactaceae have yielded conflicting results (Applequist and Wallace, 2001; Edwards et al., 2005; Nyffeler, 2007).

Using this improved phylogenetic framework, we examined vegetative anatomical variation in Cactaceae and their nearest relatives. Anatomical characters are a particularly appropriate focus for investigations of the relationship between form, function, and adaptation at a macroevolutionary scale. Although vegetative anatomy in Cactaceae has been fairly well studied (see Mauseth, 2006, and references therein), it is less well characterized in Portulacaceae s.l. (but see Carlquist, 1998, for wood anatomy; Landrum, 2002). Furthermore, an explicit link between Cactaceae and their nearest portulacaceous relatives has generally not been considered in previous anatomical studies. We therefore examined vegetative anatomical characters within ACPT and related Portulacineae to (1) provide a general characterization of the anatomical variation within ACPT, (2) identify potential synapomorphic characters that would support any one of the alternative hypotheses for relationships within ACPT, and (3) examine trait patterns in the ACPT clade as they relate to the evolutionary transition to the cactus life form.

MATERIALS AND METHODS

Anatomical investigations—We investigated 34 taxa sampled broadly from all of the portulacaceous taxa within ACPT, as well as representatives of both *Pereskia* clades and *Pereskiopsis gatesii*, an opuntioid species (see Appendix 1 for taxa examined, nomenclatural authorities, and voucher numbers). Among outgroups of ACPT, we sampled *Portulacaria afra* and *Ceraria fruticulosa* from Didiereaceae and *Claytonia virginica*, *Phemeranthus teretifolius*, and *Parakeelya pickeringii* from Montiaceae. Some taxa were field-collected, but the majority originated from cultivated material, whether grown in commercial or institutional greenhouses (see Appendix 1 for details). The focus of this study was on anatomical characters of stems and leaves in all taxa examined. Roots could not be reliably obtained for all taxa and so were not observed as extensively. Hereafter, mention of characters relating to xylem, cortex, etc. will apply to stems only unless specifically stated otherwise.

Vegetative anatomical characters were investigated primarily through freehand sectioning of fixed material. Material was fixed in formalin-acetic acid-alcohol (FAA) (Ruzin, 1999) or 70% ethanol for at least 48 h prior to sectioning. Sections were stained with cresyl violet acetate (CVA) in a 15% ethanol solution before mounting in calcium chloride (Herr, 1992; Keating, 1996). CVA is a metachromatic dye that produces a striking and visually pleasing color contrast between cellulosic (staining pink to red) and lignified tissues (staining blue), although its use has not been widely adopted by botanists (Keating, 1996). For sections to examine calcium oxalate crystal structure, separate unstained sections were mounted in glycerin due to the tendency of calcium oxalate crystals and druses to erode over time in calcium chloride.

For stems, transverse, longitudinal radial, and longitudinal tangential sections were cut at nodes and internodes. To address ontogenetic variation within a single plant, whenever possible we obtained samples from stems in a state of primary growth, early secondary growth (i.e., shortly after the initiation of the vascular cambium) and mature growth from near the base of the stem. Stem epidermal peels were obtained at various ages as well. Mature leaves were sectioned transversely and leaf epidermal peels were obtained.

Image capture and processing—All photographs were taken with a Nikon Coolpix 8700 (Nikon, Tokyo, Japan) mounted on a Martin Microscopes MM99 adapter (Martin Microscopes, Easley, South Carolina, USA). Most slides were viewed on an Olympus BX40 light microscope (Olympus, Tokyo, Japan). A Zeiss Universal microscope (Carl Zeiss, Oberkochen, Germany) was used to visualize calcium oxalate crystals and druses using polarized light filters. Images were processed using Photoshop 8.0 (Adobe Systems, San Jose, California). Processing was limited to reduction of image size, application of an unsharp mask filter, and adjustment of color levels. Original image files are available upon request from the first author.

Character evolution analyses—We generated measures of homoplasy for 14 stem characters that could be coded into discrete categories. Briefly, we calculated consistency indices (CI = minimum number of possible changes/

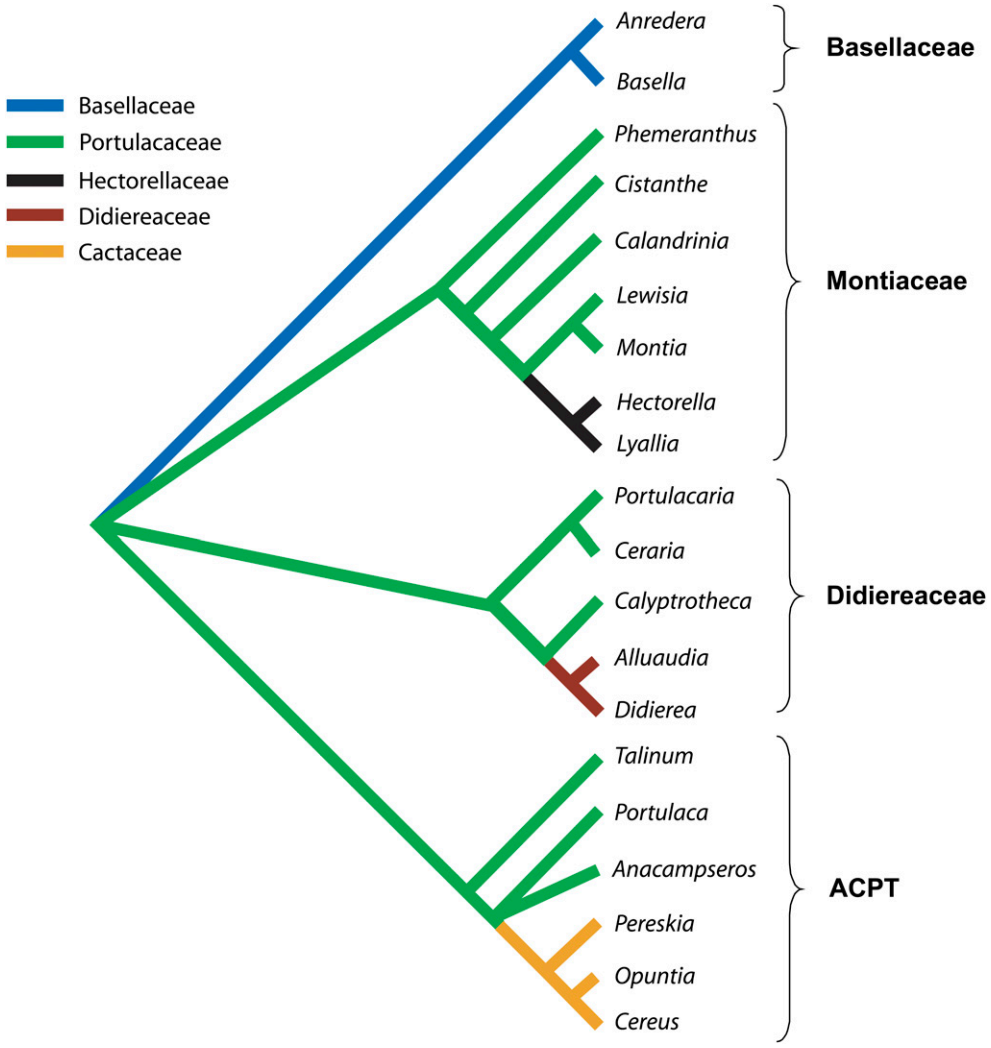

Fig. 2. Phylogenetic relationships in Portulacineae. Branch colors indicate traditional circumscription: blue = Basellaceae, green = Portulacaceae, black = Hectorellaceae, red = Didiereaceae, orange = Cactaceae. Brackets indicate recent reclassifications. Topology after Edwards et al. (2005), Applequist et al. (2006), Nyffeler (2007).

observed number of changes) for each character using the current best hypothesis of relationships within Portulacineae (e.g., based on Hershkovitz and Zimmer, 1997; Nyffeler, 2002; Butterworth and Wallace, 2005; Edwards et al., 2005; Nyffeler, 2007). Our phylogeny included several unresolved nodes, specifically (1) the relationships between Montiaceae, Didiereaceae, and the ACPT clade; (2) relationships between *Portulaca*, Anacampseroteae, and cacti; (3) relationships within *Anacampseros*; and (4) relationships within *Portulaca*. From the CI (measured using the program MacClade version 4.06, Sinauer, Sunderland, Massachusetts, USA), we calculated a homoplasy index (HI = 1 – CI). The nexus file used to generate CI values is available from the authors upon request.

We also estimated the degree of correlated evolution between a subset of stem characters we considered to be potentially important "developmental enablers" (sensu Donoghue, 2005) for the further evolution of stem photosynthesis and water storage in cacti. We employed a Bayesian reversible-jump Markov chain Monte Carlo (MCMC) approach (Pagel and Meade, 2006) as implemented in the program BayesTraits version 1.0 (available at website http://www.evolution.rdg.ac.uk). To account for topological uncertainties, we generated a set of 1000 trees with randomly resolved polytomies (using the program Mesquite version 2.01, Maddison and Maddison, 2006) and used this sample of trees for the Bayesian analyses. Briefly, for each pair of traits, we performed MCMC runs employing both a dependent (i.e., correlated) and independent (i.e., uncorrelated) model of trait evolution. For each analysis, we ran 5 050 000 iterations, discarding the first 50 000 as burn-in, and sampling every 100 iterations. We seeded the mean and variance of the gamma distribution of rate coefficents drawing from a uniform (0–10) hyperprior distribution. For each trait pair, we calculated a log BayesFactor statistic from the harmonic means of the likelihoods of both dependent and independent runs, estimated as log Bayes factor = 2 × [harmonic mean (dependent model) – harmonic mean (independent model)]. In general, values of log Bayes factor greater than 5 provide strong support for the dependent (correlated) model, and values of greater than 10 provide very strong support (Pagel and Meade, 2006).

RESULTS

Anatomical observations are summarized in Tables 1 and 2. General patterns are described next.

Periderm timing and position of initiation—Timing of periderm initiation has been identified previously as a character of interest in this study and here varies considerably in the outgroups of Cactaceae. Although the exact tissue age was unknown for many of the samples obtained here, in most cases, periderm onset is either essentially immediate (within the first

TABLE 1A. Stem characters of selected Portulacineae.

Taxon	Periderm location of onset	Periderm timing of onset	Phellem lignification	Stem stomata	Brachysclereids	Fusiform sclereids	Mucilage cells	Tannin cells
Phemeranthus teretifolius	Epidermis	Delayed	–	Brachyparacytic, transverse, sparse	–	–	Outer cortex, small cells	–
Parakeelya pickeringii	ND	Delayed	NA	Paracytic, longitudinal, dense	–	–	–	–
Claytonia virginica	ND	Delayed	NA	Brachyparacytic, longitudinal, dense	–	–	–	–
Portulacaria afra	Epidermis	Precocious	+	Parallelocytic, longitudinal, patchy/ sparse	–	Associated with phloem fiber cap, 2° phloem	Young outer cortex	Cortex, rays, pith, leaf epidermis
Ceraria fruticulosa	Epidermis	Precocious	+	–	–	Associated with phloem fiber cap	Outer cortex, small	Cortex, rays, pith
Talinum caffrum	ND	Delayed	ND	Paracytic, longitudinal, dense	–	–	Outer cortex	Cortex, pith, papillae
Talinum paniculatum	Epidermis	Delayed	–	Paracytic, longitudinal, moderately dense patches	–	Associated with phloem fiber cap	Pith, outer cortex, rare in mature stems	–
Talinum triangulare	Epidermis	Precocious	–	Paracytic, random, sparse	–	Associated with phloem fiber cap	Cortex, pith	Cortex, phloem rays, pith
Talinum portulacifolium	Outer cortex	Precocious	–	Parallelocytic, longitudinal, dense	–	Associated with phloem fiber cap, 2° phloem	Outer cortex, pith	Cortex, pith, leaf tip scale
Talinella pachypoda	Epidermis	Precocious	–	–	–	Associated with phloem fiber cap	Young cortex	Stem epidermis, papillae, leaf tip
Portulaca oligosperma	ND	Delayed	–	–	–	–	Cortex	–
Portulaca oleracea	Epidermis	Delayed	–	–	–	–	Young cortex	–
Portulaca grandiflora	Epidermis	Delayed	–	–	Root cortex, ovoid	–	Cortex, pith, often with druses	–
Portulaca amilis	Epidermis	Delayed	–	Paracytic, random, sparse	Pericycle, secondary phloem	–	Cortex, pith, often with druses	–
Portulaca pilosa	Epidermis	Delayed	In roots	Paracytic, random, sparse	Root cortex, pericycle	–	Cortex, pith, (small)	–
Talinopsis frutescens	Outer cortex, phloem, multiple	Precocious	+	–	–	–	Young outer cortex	–
Grahamia bracteata	Outer cortex	Precocious	+	–	Pith, cortex	–	–	–
Xenia vulcanensis	Epidermis, multiple	Precocious	–	Parallelocytic, longitudinal, moderate density	Rare in cortex	–	Cortex, pith, (small)	–
Anacampseros kurtzii	Epidermis	Precocious	+	ND	–	–	Cortex (small)	–
Talinaria coahuilensis	Epidermis	Precocious, persistent cuticle	–	–	Inner cortex, rays, pith	+	Outer cortex, larger in young stem	–
Anacampseros australiana	Epidermis	Precocious	–	–	–	–	Cortex, w/ large druses, larger in young stem	–
Anacampseros recurvata subsp. *buderiana*	NA	NA	NA	ND	–	–	–	–
Anacampseros quinaria subsp. *alstonii*	NA	NA	NA	ND	–	–	Cortex	–
Anacampseros rufescens	Epidermis	Precocious	–	Parallelocytic, transverse, moderate density	–	–	Young cortex	–
Anacampseros filamentosa	Epidermis	Precocious	–	–	–	–	–	–
Anacampseros lanceolata	Epidermis	Precocious	–	Parallelocytic, transverse, sparse	–	–	Outer cortex (small)	–
Pereskia guamacho	Outer cortex	Precocious	Sclereids	–	Cortex, primary phloem	–	–	–

TABLE 1A. Continued.

Taxon	Periderm location of onset	Periderm timing of onset	Phellem lignification	Stem stomata	Brachysclereids	Fusiform sclereids	Mucilage cells	Tannin cells
Pereskia portulacifolia	Outer cortex	Precocious	Sclereids	–	Cortex	–	Pith, young cortex	–
Pereskia quisqueyana	Epidermis	Precocious	Sclereids	–	NO	–	Young pith and cortex	–
Pereskia nemorosa	Epidermis	Delayed	Sclereids	–	–	2° phloem, aggregated	Cortex, pith, enlarged	–
Pereskia aculeata	Epidermis	Precocious	Sclereids	Brachyparacytic, longitudinal, moderate density	Cortex near nodes	1° and 2° phloem, aggregated	Pith, cortex, enlarged	–
Pereskia diaz-romeroana	ND	Delayed	ND	Paracytic, longitudinal, dense	–	Associated with phloem fibers	Pith, cortex, enlarged	–
Pereskia weberiana	Epidermis	Delayed	Brachy-sclereids	Paracytic, longitudinal, dense	Phellem	2° phloem, not aggregated	Pith, cortex, enlarged	–
Pereskiopsis gatesii	ND	Delayed	ND	Paracytic, longitudinal, dense	–	–	Pith, cortex, enlarged	–

few nodes of the shoot apex) or obviously delayed. Herbaceous taxa that lack stem periderm were here scored as delayed.

Periderm onset is delayed in the Andean/SSA *Pereskia* group, except *P. aculeata*, while all northern *Pereskia* species observed have precocious periderm formation. Anacampseroteae are characterized by precocious periderm onset, with the exception of *Anacampseros* sect. *Avonia*, in which stem periderm was not observed. Similarly, the herbaceous *Portulaca* generally lacks periderm in all but the oldest stem bases, while both precocious and delayed periderm initiation occur with no clear phylogenetic pattern in the *Talinum* clade.

Location of periderm onset also shows variation. In most Portulacineae observed, periderm initiates in the epidermis (Fig. 3A, B, green arrows). Exceptions occur in *Pereskia guamacho*, *P. portulacifolia*, *Talinopsis frutescens*, *Grahamia bracteata*, and *Talinum portulacifolium*, in which it initiates in the outer cortex, eventually cutting off the epidermis, hypodermis, and outermost cortical layers as rhytidome (Fig. 3C, D). Also noteworthy is the occurrence in most *Pereskia* species of stratified concentric bands of sclereids in the phellem layer of periderm (Fig. 3E). Lignified bands consisting of thinner-walled, flattened cells occur in the phellem of *Talinopsis frutescens* (Fig. 3F), *Grahamia bracteata*, and *Anacampseros kurtzii*, as well as in *Portulacaria afra* and *Ceraria fruticulosa.*

Stem and leaf stomata—Stem stomata occur in *Pereskiopsis* and all of the Andean/SSA *Pereskia* species observed here except *P. nemorosa* (Appendix S1A, see Supplemental Data with online version of this article). Stem stomata are lacking in the northern *Pereskia* clade and most Anacampseroteae, even on newly elongated green shoots prior to the precocious inception of periderm. However, they do occur in moderate densities on young shoots of *Xenia vulcanensis*, *Anacampseros rufescens*, and more sparsely in *A. lanceolata* (online Appendix S1B). Of the five *Portulaca* species examined, stomata are found at low densities on stems of *P. amilis* and *P. pilosa* alone. All species sampled in the *Talinum* clade have stem stomata, in a range of densities, except *Talinella pachypoda*. Outside of the ACPT clade, they were found in *Portulacaria afra* and the three observed representatives of Montiaceae.

Leaves of most taxa observed here, including those of Didiereaceae and Montiaceae, are amphistomatic, with essentially equal densities of stomata on their abaxial and adaxial surfaces, or equally around the entire leaf surface in the case of terete leaves. An exception to this was *Portulaca oligosperma*, in which stomata occurred, atypically for most leafy plants, on the adaxial leaf surface only. All observed taxa of ACPT and Didiereaceae have parallelocytic stomata, in which the stomate is laterally invested by a series of at least three alternating subsidiary cells that become increasingly larger progressing out from the guard cells (Fig. 4A). In contrast, the species of Montiaceae observed all have brachyparacytic stomata, in which two subsidiary cells surround but do not completely enclose the stomatal apparatus (Fig. 4B). Most Anacampseroteae and *Portulaca* sampled have transversely oriented leaf stomata, while in other taxa sampled here leaf stomatal orientation is more or less random.

Sclerenchyma—Sclereids of various forms were observed in stem cortex, pith, and phellem of many ACPT and Didiereaceae. They were not observed in leaves and were lacking in all species of Montiaceae examined.

TABLE 1B. Stem characters of selected Portulacineae continued.

Taxon	Hairs	Bristles	Calcium oxalate druses	Crystals	Extraxylary fibers	Xylem fibers	WBTs	Collenchyma	Epidermal papillae	Thickened cuticle
Phemeranthus teretifolius	–	–	–	–	–	–	–	–	Weak	–
Parakeelya pickeringii	–	–	–	–	–	–	–	–	Weak	–
Claytonia virginica	–	–	–	–	–	–	–	–	–	–
Portulacaria afra	–	–	Cortex, pith	–	Phloem fiber caps	+	–	Weak, 1–2 layers	+	–
Ceraria fruticulosa	–	–	Young cortex and pith, small	–	Phloem fiber caps	+	–	2 layers	–	–
Talinum caffrum	–	–	–	–	Primary phloem	–	–	Weak, 1–2 layers	+, multicellular at base	–
Talinum paniculatum	–	–	Pith, older cortex	–	Primary phloem	+	–	3–5 layers	YS only	–
Talinum triangulare	–	–	Cortex (rare), pith	–	+, thick-walled	+	–	1–2 layers	Stem trichomes	–
Talinum portulacifolium	–	–	Cortex, 2′ phloem, rays, pith	–	Phloem fiber caps	+	–	Weak in YS, 1–2 layers, cut off with rhytidome	YS only	–
Talinella pachypoda	–	–	Cortex, some in pith	–	Primary phloem	+	–	3–4 layers	Striated, some with tannins	–
Portulaca oligosperma	Multiseriate, reduced	–	–	–	–	–	–	–	Stem	–
Portulaca oleracea	Multiseriate, reduced	–	Pith, cortex	–	–	Base of stem	–	2–3 layers	Weak in young stem	–
Portulaca grandiflora	Triseriate, elongate, gland at tip	–	Pith, cortex	–	–	+	–	Weak, 1–2 layers	–	–
Portulaca amilis	Multiseriate at base, 2–3 seriate	–	Pith, cortex	–	–	–	–	2–3 layers	–	–
Portulaca pilosa	Multiseriate	–	Cortex, pith	–	–	Base of stem	–	1–2 layers	Weak in young stem	–
Talinopsis frutescens	Biseriate	–	–	–	–	+	–	–	–	–
Grahamia bracteata	Uniseriate, basal 2/3 lignified	Paired, lignified @ base	YS cortex @ node	Rare in primary pith	Hypodermis of YS	+	–	Weak in YS	–	–
Xenia vulcanensis	Multiseriate, reduced	–	–	–	Hypodermis of YS	–	–	–	YS	–
Anacampseros kurtzii	(2-)3-seriate	–	Sphero-crystals in cortex	–	–	–	+	–	–	–
Talinaria coahuilensis	Multiseriate	–	–	–	–	–	NO	–	–	–
Anacampseros australiana	2–4 seriate	–	Small in pith @ nodes, sphero-crystals in cortex with muc. cells	Rhomboid in cortex	–	–	Rays, pith	–	–	–
Anacampseros recurvata subsp. *buderiana*	Uniseriate	+	Cortex	Cortex	–	–	Rays, pith	–	–	–
Anacampseros quinaria subsp. *alstonii*	–	+	–	–	–	–	Rays, entirety of pith,	–	–	–
Anacampseros rufescens	2–3 seriate	+	Pith, rays	In mucilage cells of YS	–	–	Rays, pith	Weak in YS, 1 layer	–	–
Anacampseros filamentosa	2 seriate, also on leaf epidermis	+	Pith, rays	–	–	–	Rays, pith	–	ND	–
Anacampseros lanceolata	3 to multiseriate	–	Pith, rare in cortex	–	–	–	Rays, pith	–	–	–
Pereskia guamacho	Uniseriate	–	Hypodermis, cortex, rays, pith	Cortex, rays, pith of mature stem	+	+	–	–	Sparse	–

TABLE 1B. Continued.

Taxon	Hairs	Bristles	Calcium oxalate druses	Crystals	Extraxylary fibers	Xylem fibers	WBTs	Collenchyma	Epidermal papillae	Thickened cuticle
Pereskia portulacifolia	Uniseriate	–	Pith, rays, cortex	–	+	+	–	–	ND	–
Pereskia quisqueyana	NO	–	Cortex, phloem rays, pith	–	+	+	–	–	–	–
Pereskia nemorosa	Uniseriate, lignified	–	Cortex, pith, 2′ phloem	–	+	+	–	Weak, 4–5 layers	–	+
Pereskia aculeata	1 (2–3)-seriate	–	Hypodermis, pith, cortex	–	+	+	–	3–4 layers	–	–
Pereskia diaz-romeroana	Uniseriate	–	Hypodermis, cortex, pith	Rare in cortex mucilage cells	+	+	–	2(–3) layers	–	+
Pereskia weberiana	Uniseriate	–	Hypodermis, cortex, 2′ phloem, pith	Rhomboid, rectangular in pith	+	+	–	3–4 layers	–	+
Pereskiopsis gatesii	Uniseriate	–	Hypodermis, cortex, phloem	–	+	+	NO	3 layers	–	+

Notes: + = present; – = absent; ND = No data for the character in question, usually because material of the appropriate age was not available; NA =the character state does not apply; NO = not observed (i.e., instances in which an expected character was not seen despite being reported in the literature for the taxon in question); YS = young stem.

Aggregated isodiametric brachysclereids ("stone cells") occur in the outer cortex of *P. guamacho* and are also associated with the primary phloem of *P. guamacho* (Figs. 3E, 5A) and *P. portulacifolia*, both northern clade species. *P. diaz-romeroana* and *P. weberiana,* representatives of the Andean/SSA clade, lack cortical sclereids but have fusiform sclereids occurring singly or occasionally in aggregate in the secondary phloem (Fig. 5B). No sclereids were found in *P. quisqueyana.*

In Anacampseroteae, aggregations of stone cells were found infrequently in the cortex and pith of *Grahamia bracteata* (Appendix S1C, see Supplemental Data with online version of this article). In *Talinaria coahuilensis*, sclereids occur in a ring around vascular tissues as well as in rays and pith (Fig. 5C). In this species, sclereids occurring around the vascular cylinder tend to be more elongated, while those in the rays and pith tend to be isodiametric or only slightly elongated. Single brachysclereids were found rarely in the cortex of *Xenia vulcanensis*.

Thin-walled, elongated cells with lignified walls are associated with extraxylary fiber caps in *Talinum portulacifolium*, *T. triangulare*, and *Talinella pachypoda*, as well as *Portulacaria afra* and *Ceraria fruticulosa* (Fig. 5D). When viewed in longitudinal section, these sclereids are typically not fusiform but are squared-off and similar in shape to surrounding parenchyma cells. They do not appear to develop thicker secondary walls as do the sclereids of Anacampseroteae and *Pereskia*. Subelliptic brachysclereids with more or less thin walls occur in root cortex of *Portulaca amilis*, *P. pilosa*, and *P. grandiflora* (online Appendix S1D).

Mucilage cells and cavities in leaves and stems—Mucilage-containing cells in leaves and stems are common throughout Portulacineae. These cells are often larger than surrounding parenchyma and contain mucilage that stains red in CVA. Of the taxa sampled here, the only ones lacking mucilage cells in leaf mesophyll are *Claytonia virginica* and *Parakeelya pickeringii*, both members of Montiaceae (*Phemeranthus*, the other sampled Montiaceae, does have leaf mucilage cells). Within ACPT, even taxa with relatively thin, nonsucculent leaves (e.g., *Talinum paniculatum*, *Pereskia quisqueyana*) have abundant mucilage cells.

In stems, mucilage cells were lacking in most Montiaceae but observed in most ACPT and Didiereaceae (Table 1), commonly in the outer cortex (Fig. 3A, white arrows). In most cases, however, they were not highly conspicuous components of stem ground tissues, occurring sparsely and/or ephemerally in young shoots only. However, in older stems of *Portulaca amilis, P. oligosperma,* the Andean/SSA *Pereskia* clade, and *Pereskiopsis,* large mucilage cells are retained and are a prominent feature of cortical and pith tissues (Fig. 6A, B).

Tannin cells—In addition to red-staining mucilage cells, amber-colored cells that stain blue in CVA were also commonly observed in stems of some taxa. We follow other authors in referring to these as tannin cells (Gibson and Nobel, 1986; Gibson, 1994; Landrum, 2002), although specific tests to determine their content were not performed. These cells were common in the cortex, rays, and pith of all Didiereaceae and *Talinum* species sampled except *T. paniculatum* and *Talinella pachypoda* (Figs. 3A, 6C). Viewed in longitudinal section, they occur in extensive columns of contiguous cells (online Appendix S1E, green arrow). Tannin cells were also found in the leaf epidermis of *P. afra* (Fig. 6D) and early-formed phellogen of *C. fruticulosa*. In *Talinella pachypoda*, tanniniferous cell contents occur in axil-

TABLE 2. Leaf characters of selected Portulacineae.

Taxon	Gross morphology	Venation	Mucilage cells	Tannin cells	Druses	Crystals	Stomata	WBTs	Epidermal papillae
Phemeranthus teretifolius	Succulent, terete	Pinnate	+	–	–	–	Brachyparacytic, amphi, random	–	–
Parakeelya pickeringii	Fleshy, lanceolate	Pinnate	–	–	See crystals	Rhomboidal, rectangular, ± aggregated	Brachyara- or parallelocytic, amphi, random	–	–
Claytonia virginica	Fleshy, lanceolate	Pinnate	–	–	–	–	Brachyparacytic, amphi, longitudinal	–	–
Portulacaria afra	Succulent, truncate	Palmate	+	+	Stellate, spherocrystals	–	Parallelocytic, amphi, transverse	–	–
Ceraria fruticulosa	Succulent, obovate	Palmate	+	+	Stellate, spherocrystals	–	Parellelocytic, amphi, transverse	–	–
Talinum caffrum	Fleshy, linear	Pinnate	+	Petiole	Stellate	–	Parallelo- to brachyparallelocytic, amphi, random	–	Margin, few on lamina
Talinum paniculatum	Thin, cuneate	Pinnate	Abundant in palisade layer	–	Stellate	–	Parallelocytic, amphi, random	–	Weak
Talinum triangulare	Fleshy, cuneate	Pinnate	+	+	Stellate, spherocrystals	–	Parallelocytic, amphi, random	–	Margin, midvein, weak elsewhere
Talinum portulacifolium	Fleshy, cuneate	Pinnate	+	–	Stellate, spherocrystals	–	Parallelocytic, amphi, random	–	Margin
Talinella pachypoda	Fleshy, elliptic	Pinnate	+	In scaly leaf tip	Stellate, spherocrystals	–	Parallelocytic, amphi (lower adaxial density), random	–	Margin, midvein, petiole
Portulaca oligosperma	Succulent, elliptic	Pinnate	+	–	See crystals	Rhomboidal, hexagonal, often ± aggregated	Parallelocytic, adaxial, transverse	–	–
Portulaca oleracea	Fleshy, obovate	Pinnate	Very small	–	Stellate in bundles, spherocrystals	–	Parallelocytic, amphi, transverse	–	–
Portulaca grandiflora	Succulent, cuneate	Pinnate	+	–	Stellate, spherocrystals	–	Brachyparallelocytic, amphi, weakly transverse	–	–
Portulaca amilis	Subterete, linear	Pinnate	+	–	Stellate, spherocrystals	–	Parallelocytic, amphi, transverse	–	–
Portulaca pilosa	Subterete, linear	Pinnate	Mostly abaxial	–	Spherocrystals	–	Parallelocytic, amphi, transverse	–	–
Talinopsis frutescens	Subterete, linear	Pinnate	+	–	Spherocrystals	–	Parallelocytic, amphi, transverse	–	–
Grahamia bracteata	Subterete, linear	Pinnate	+	–	See crystals	Rectangular, rhomboid, often ± aggregated	Parallelocytic, amphi, transverse	–	–
Xenia vulcanensis	Fleshy, ovate	Pinnate	+	–	Crystals often aggregate around organic material	Rectangular, rhomboid, often ± aggregated	Paracytic, amphi, random	–	Base, tip of lamina
Anacampseros kurtzii	Subterete, linear		+	–	Spherical, spherocrystals	Rectangular	Parallelocytic, amphi, weakly transverse	+	–
Talinaria coahuilensis	Fleshy, obtrullate	Pinnate	+	–	Spherocrystals	Rectangular, some hexagonal	Parallelocytic, amphi, transverse	+	–
Anacampseros australiana	Subterete, obtrullate	Pinnate	+	–	Stellate, spherocrystals	Square, rhomboid	Parallelocytic, amphi, transverse	+	–
Anacampseros recurvata subsp. *buderiana*	Reduced, reniform	ND	ND	–	Stellate	+	ND	+	–

TABLE 2. Continued.

Taxon	Gross morphology	Venation	Mucilage cells	Tannin cells	Druses	Crystals	Stomata	WBTs	Epidermal papillae
Anacampseros quinaria subsp. *alstonii*	Reduced, reniform	ND	ND	–	ND	ND	Parellelocytic, transverse	+	–
Anacampseros rufescens	Succulent, obtrullate	Pinnate	+	–	–	–	Parallelocytic, amphi, transverse	+	Tip, margin
Anacampseros filamentosa	Subterete, orbicular	Pinnate	+	–		Rhomboid, rectangular	Parallelocytic, amphi, random		Barbs prominent on broadly rounded tip
Anacampseros lanceolata	Subterete, oblanceolate	Pinnate	+	–	Spherocrystals	–	Parallelocytic, amphi, transverse	+	–
Pereskia guamacho	Fleshy, elliptic	Pinnate	+	–	Stellate	–	Parallelocytic, amphi, random	–	–
Pereskia portulacifolia	Fleshy, elliptic	Pinnate	+	–	Stellate, spherocrystals	–	Parallelocytic, amphi, longitudinal	–	–
Pereskia quisqueyana	Thin, elliptic	Pinnate	+	–	Stellate, spherocrystals	–	Parallelocytic, amphi, random	–	–
Pereskia nemorosa	Fleshy, elliptic	Pinnate	+	–	Stellate, spherocrystals	–	Parallelocytic, amphi, random	–	–
Pereskia aculeata	Fleshy, elliptic	Pinnate	+	–	Stellate, spherocrystals	–	Parallelocytic, amphi (lower adaxial density), random	–	–
Pereskia diaz-romeroana	Fleshy, elliptic	Pinnate	+	–	Stellate	Rare in mucilage cells	Parallelocytic, amphi, random	–	Margin
Pereskia weberiana	Fleshy, elliptic	Pinnate	+	–	Stellate, contain organic material	–	Parallelocytic, amphi, random	–	Margin
Pereskiopsis gatesii	Succulent, obtrullate	Palmate	+	–	Stellate	Hexagonal	Parallelocytic, amphi, longitudinal	–	–

Note: + = present; – = absent; Amphi = amphistomatic (stomata present in roughly equal densities on both sides of leaf).

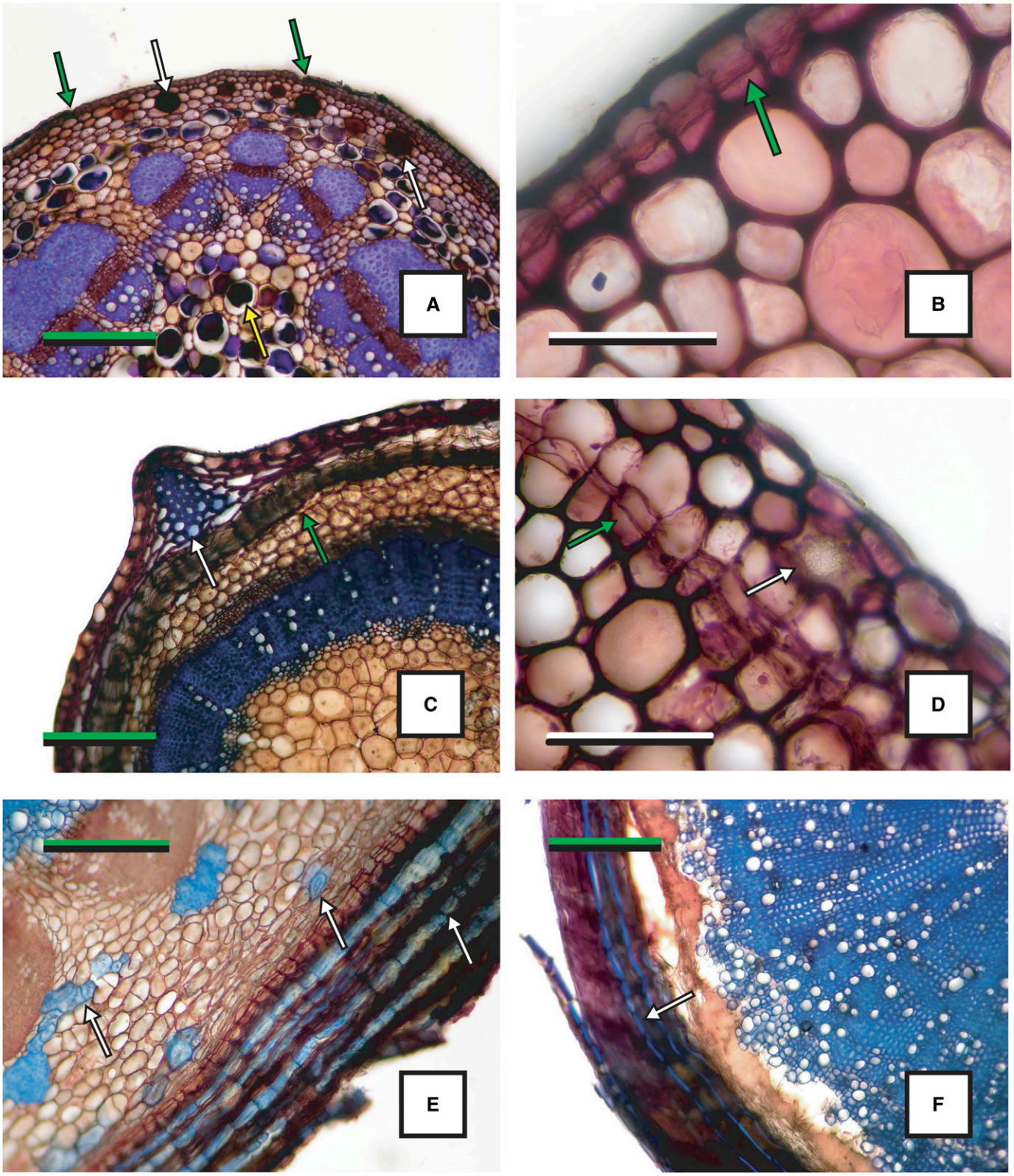

Fig. 3. Light micrographs of periderm features. Green arrows indicate location of periderm onset. (A) *Ceraria fruticulosa*, young stem transverse section (TS). White arrows indicate mucilage cells. (B) *Portulaca amilis,* stem TS near base of plant. (C) *Grahamia bracteata*, young stem TS. Note epidermis and outer cortex external to periderm. White arrow indicates subepidermal fibers. (D) *Pereskia guamacho*, young stem TS. White arrow indicates hypodermal druse, note location external to periderm. (E) *Pereskia guamacho*, mature stem TS. White arrows indicate sclereids in phellem, outer cortex, and pericycle. (F) *Talinopsis frutescens*, mature stem TS. White arrow indicates lignified cells in phellem. Green scale bar = 250 μm, white scale bar = 100 μm.

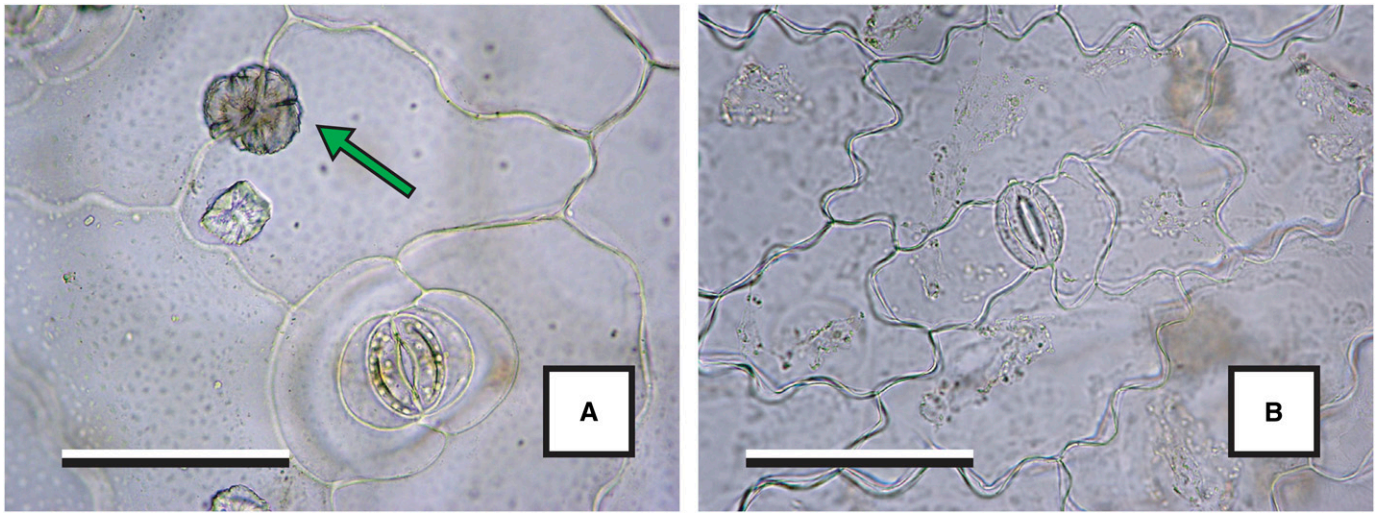

Fig. 4. Light micrographs of leaf epidermal characters. (A) *Portulaca oleracea*, leaf abaxial epidermis, arrow indicates spherocrystal druse. Stomatal subsidiary cells show parallelocytic arrangement. (B) *Parakeelya pickeringii*, leaf abaxial epidermis, paracytic subsidiary cells. Scale bar = 100 μm.

lary prophyll scales (see description in next section) (online Appendix S1F), in scarious leaf tips, and in stem epidermal papillae. Tannin cells were not observed in any taxa of *Portulaca*, Anacampseroteae, Cactaceae, or Montiaceae that were examined.

Nodal hairs/bristles—Cactaceae, Anacampseroteae, and *Portulaca* are characterized by the presence of distinctive hair- or bristle-like outgrowths, typically at nodes in the leaf axil, but in some taxa borne elsewhere on the stem or the leaf lamina. These structures are here interpreted as trichomes, as opposed to stipules, in that they grow from the epidermal layer, are not vascularized, and are noncaducous.

The hair-like trichomes range from one (uniseriate) to three or more (multiseriate) cells in width. In contrast, "bristles" are extremely wide, flattened multiseriate trichomes that are up to 20 or more cells in width. All *Portulaca*, Anacampseroteae, and Cactaceae observed have hairs at nodes (thus in the areoles of cacti), although they are highly reduced (i.e., not visible macroscopically) in *Portulaca oligosperma*, *P. oleracea*, and *Xenia vulcanensis*. The occurrence of bristles is more limited, being observed here in *Grahamia bracteata* and *Anacampseros* sect. *Anacampseros. Anacampseros* sect. *Avonia* has broad axillary scales that completely enclose the leaf distal to the subtending leaf and are lignified at the tip.

Talinum species have scarious scale-like structures in leaf axils, often paired, which can superficially appear similar to the hairs of other ACPT. However, closer examination reveals these structures to be the tips of prophylls, which may subsequently expand as full leaves (onlinen Appendix S1F and S1G). Indeed, the scale-like structures are typically still visible at the tips of fully expanded leaves in most *Talinum* (online Appendix S1H). We also observed vascularization leading to these axillary prophylls in *Talinum paniculatum*, which never occurs in the hairs or bristles of ACP.

Druses and crystals—Calcium oxalate druses (i.e., spherical crystal aggregations) and single crystals are widespread in all major subclades of ACPT as well as in Didiereaceae and *Parakeelya pickeringii*. Position of druses and crystals in stem and leaf tissues is highly variable even within individual plants and does not appear to carry a strong phylogenetic signal. An exception is found in *Pereskiopsis* and *Pereskia diaz-romeroana*, *P. weberiana*, *P. aculeata*, and *P. guamacho*, which are distinctive in consistently having druses immediately internal to the epidermal layer (Fig. 7A). In *P. guamacho* these druses are lost early in development, when they are cut off by the activity of periderm in the outer cortex (Fig. 3D).

Stem extraxylary fibers—Thick-walled phloem fiber caps (i.e., pericyclic aggregations of extraxylary fibers) occur in all species of Cactaceae, *Talinum*, and Didiereaceae examined here (Figs. 3A, E, 6B, C). Phloem fiber caps are lacking in *Portulaca,* Anacampseroteae, and Montiaceae (Figs. 3C, F, 6A), although there are short-lived subepidermal fibers in young stems of *Grahamia bracteata* (Fig. 3C).

Wide-band tracheids/wood characters—Short, barrel-shaped tracheids with wide helical or annular thickenings projecting into the cell lumen occurred in stems and leaves of all species of Anacampseroteae except *Talinopsis frutescens*, *Grahamia bracteata*, and *Xenia vulcanensis*. These wide-band tracheids (WBTs) (Bailey, 1964; Mauseth et al., 1995) always occur in xylem rays or in the ground tissue of the pith and are never associated with vessel elements in vascular bundles. Despite occurring in rays, they are usually oriented longitudinally in the stem. No WBTs were observed in *Portulaca*, *Talinum*, either *Pereskia* clade, or in any of the outgroups of ACPT that were examined. In Anacampseroteae, WBTs do not occur in *Talinopsis frutescens* and *Grahamia bracteata*, the only two species that have libriform xylem fibers. *Xenia* appears to have neither WBTs nor libriform fibers.

Epidermis—Papillae arising from the stem epidermis are a common feature in *Portulacaria afra* (Didiereaceae) and most *Talinum* species observed here and occur in a few other ACPT. These papillae are often outgrowths of a single epidermal cell, although in *Talinum triangulare*, *T. caffrum*, and less commonly *Portulacaria afra*, they are made up of two to four cells (Fig. 6C). They vary extensively among species in their lifespan, with some persisting in spite of extensive periderm

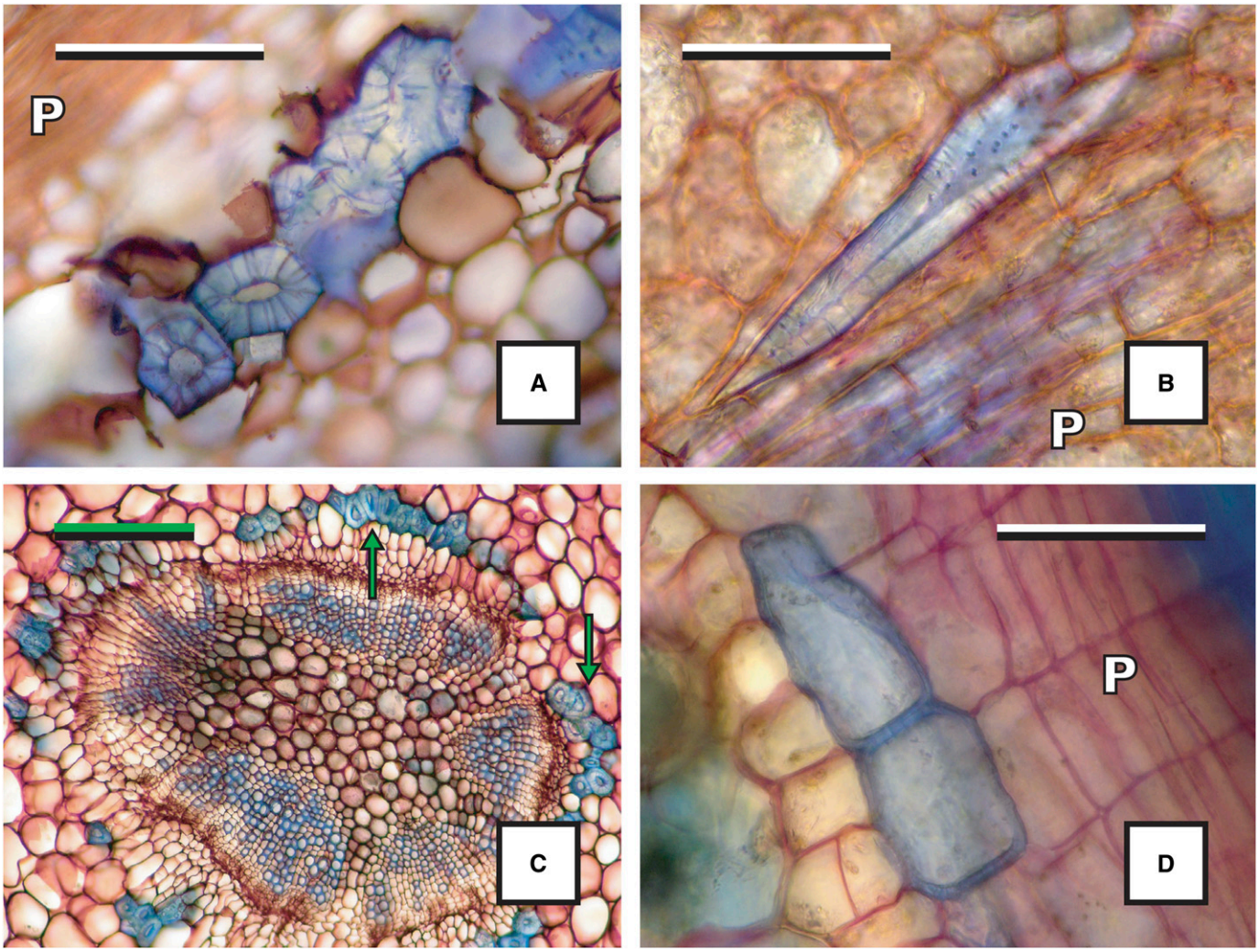

Fig. 5. Light micrographs of sclereids. (A) *Pereskia guamacho*, stem longitudinal section, brachysclereids in cortex. (B) *Pereskia diaz-romeroana*, stem radial section (RS), single fusiform sclereid. (C) *Talinaria coahuilensis,* stem transverse section, sclereids form a near-continuous ring around pericycle. (D) *Talinum portulacifolium*, stem RS, thin-walled elongate sclereids associated with extraxylary fiber caps. Green scale bar = 250 μm, white scale bar = 100 μm. P = phloem.

(*Portulacaria afra*, *Portulaca oligosperma*, and some *Talinum* species), while others are ephemeral (other sampled *Portulaca* species). Papillae are generally lacking in the Anacampseroteae species observed here, occurring only on very young stems and bases of the leaf lamina in *Xenia vulcanensis* (online Appendix S1B). Although stem epidermal papillae were not found in *Anacampseros* sect. *Anacampseros*, elongate epidermal papillae were observed at the leaf tip in *A. rufescens* and over the entire lamina in *A. filamentosa*. Papillae also were observed on leaf margins of numerous *Talinum* species. Epidermal papillae were not observed on stems of either *Pereskia* group, although they did occur on leaf margins of *P. weberiana* and *P. diaz-romeroana*.

Pereskiopsis gatesii and three of the Andean/SSA *Pereskia* species, *P. diaz-romeroana*, *P. nemorosa*, and *P. weberiana*, are unique among the taxa observed here in possessing a distinctly thickened stem cuticle that stains purple in CVA (Fig. 7A, B). In *P. diaz-romeroana, P. nemorosa,* and *P. weberiana* the cuticle is intercalated between the lateral walls of the epidermal cells.

Leaves—Leaves in taxa observed here are typically pinnately veined, i.e., with a more or less prominent midvein joined along its length by secondary veins. *Pereskiopsis*, *Ceraria*, and *Portulacaria* have palmately veined leaves.

Leaves of all taxa observed in this study have little differentiation between palisade and spongy layers of mesophyll tissues, with the exception of *Claytonia virginica*. In general, taxa with fleshy, bifacial leaves (*Talinum*, *Pereskia*, some Anacampseroteae) have greater mesophyll differentiation than those with more succulent, subterete to terete leaves.

Character evolution analyses—In general, many traits exhibited very high levels of homoplasy (Table 3). The two most labile traits were the presence of stem stomata and the timing of periderm formation, each having arisen multiple times across all of Portulacineae. Several characters were less labile. Axillary hairs, for example, appear to have arisen once, along the stem to the ACP clade, and were subsequently lost within *Anacampseros* sect. *Avonia*. Similarly, a thickened stem cuticle is likely to have arisen once in the caulocactus

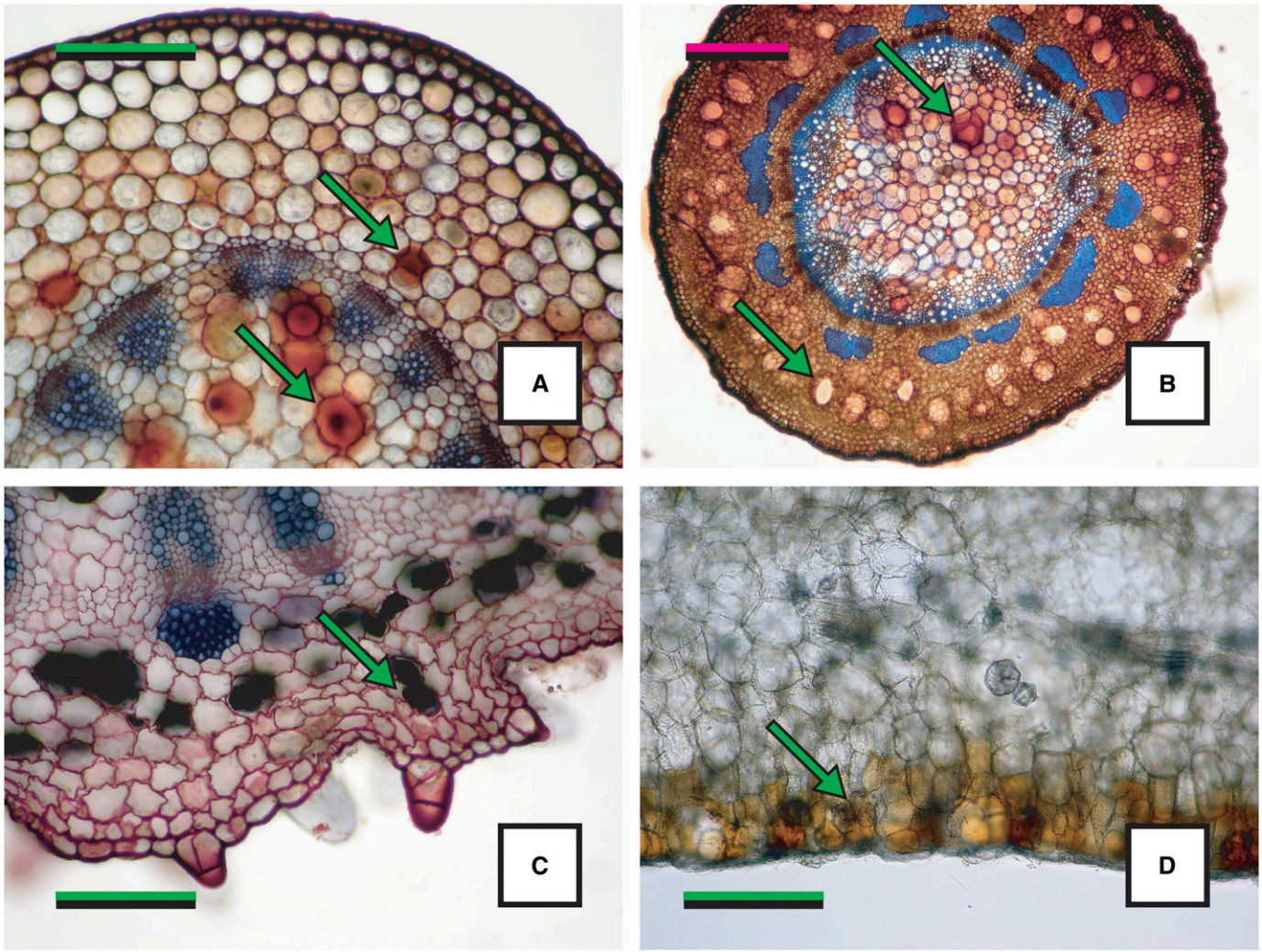

Fig. 6. Light micrographs of mucilage and tannin cells. (A) *Portulaca amilis*, stem transverse section (TS), arrows indicate large mucilage cells in cortex and pith. (B) *Pereskia diaz-romeroana*, stem TS, arrows indicate mucilage cells in cortex and pith. (C) *Talinum caffrum*, stem TS, arrows indicate tannin cells in cortex; note mutiple cell divisions within papillae. (D) *Portulacaria afra*, leaf TS, arrow indicates epidermal tannin cells, which are amber colored without staining. Purple scale bar = 500 μm, green scale bar = 250 μm.

clade, and to have been subsequently lost in *Pereskia aculeata*.

Measures of homoplasy have been generally shown to be positively correlated with the number of taxa on the tree (Sanderson and Donoghue, 1989) and negatively correlated with the number of states to which characters may be assigned (Donoghue and Ree, 2000). The first issue is not a concern because all HI values were generated using the same tree. While some of our stem characters had two states and others three, the three-state characters (stem sclereids and mucilage cells) had among the highest HI values; therefore, we do not think their homoplasy was significantly underestimated.

Our Bayesian analyses also provided little evidence for significantly correlated evolution between many of these labile traits (Table 4). There were four notable exceptions: there is strong evidence for a correlation between delayed periderm and a thickened cuticle; between hypodermal druses and a thickened cuticle; between hypodermal druses and stem stomata; and very strong evidence for a correlation between delayed periderm and enlarged, persistent mucilage cells. The two most labile stem traits, delayed periderm and stem stomata, had nearly the lowest support for any correlation between them, with a log Bayes factor of −1.00.

DISCUSSION

Morphological evidence for relationships in ACPT and greater Portulacineae—No clear morphological synapomorphies for the ACPT clade were identified in this study. However, the shared presence of parallelocytic leaf stomata, tannin cells, and pericyclic sclereids in both ACPT and Didiereaceae suggests a close relationship between these two clades, as hinted in at least one study (Nyffeler, 2007). These characters have been reported from other Didiereaceae that were not sampled here (Gibson, 1994), and, as far as is known, are lacking in Montiaceae and Basellaceae (Metcalfe and Chalk, 1950; Sperling, 1987; Carlquist, 1999) (Table 5). If ACPT and Didiereaceae are ultimately shown to be sister taxa, these characters may be considered potential synapomorphies of such a clade.

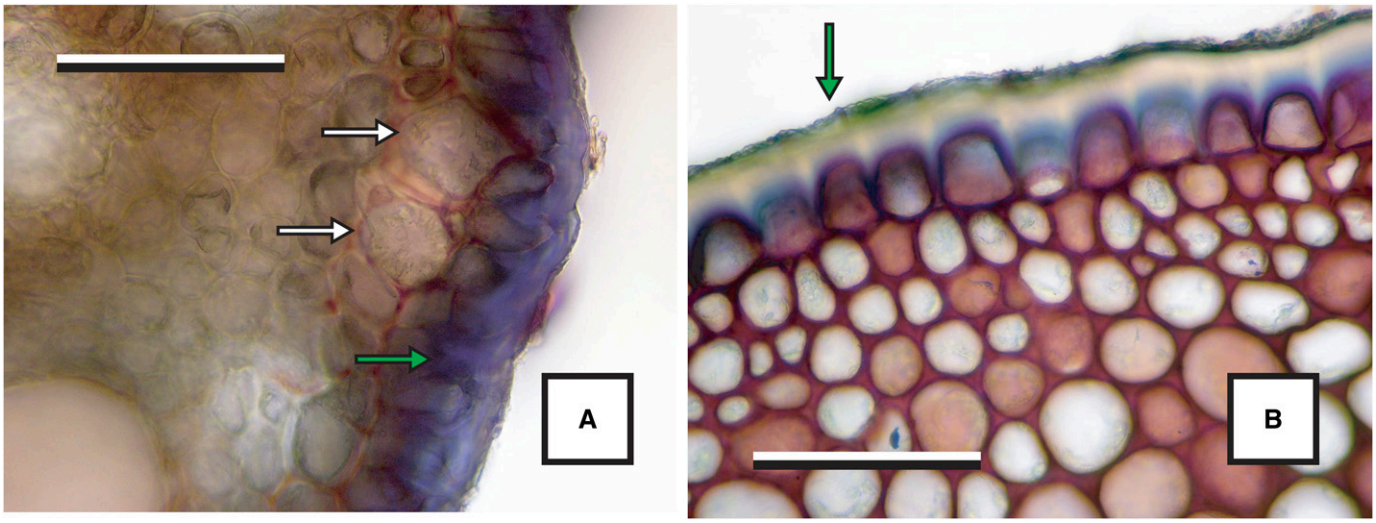

Fig. 7. Light micrographs of stem cuticle. (A) *Pereskia diaz-romeroana*, stem transverse section (TS), green arrow indicates cuticle, white arrows indicate hypodermal druses. B. *Pereskia nemorosa*, stem TS, green arrow indicates cuticle. Scale bar = 100 μm.

Within ACPT, the clade of Anacampseroteae, *Portulaca*, and Cactaceae is supported by the presence of nodal trichomes and bristles. Relationships among these three lineages at this point, however, are uncertain and are here treated as a polytomy (Edwards et al., 2005; Nyffeler, 2007). The morphological characters studied here similarly fail to provide satisfactory evidence to support one phylogenetic hypothesis over another for relationships within the ACP grouping. In common with the northern *Pereskia* clade, and in contrast with *Portulaca*, the ancestral condition for stems in Anacampseroteae is inferred as fibrous-woody and nonsucculent, with precocious periderm initiating in the outer cortex (see Table 1, *Talinopsis frutescens* and *Grahamia bracteata*), which could be interpreted as supporting an affinity between Cactaceae and Anacampseroteae. However, stem woodiness and position and timing of periderm onset vary considerably across all of Portulacineae, and within well-defined clades, suggesting that these characters may not be phylogenetically informative. Wide-band tracheids occur in both Anacampseroteae and Cactaceae, although their phylogenetic distribution indicates that they are derived independently in both clades and likely evolved in parallel (Mauseth, 2004; Landrum, 2006).

TABLE 3. Homoplasy indices for measured stem traits in selected Portulacineae.

Trait	Homoplasy index
Presence of stem stomata	0.87
Delayed periderm formation	0.83
Location of periderm onset	0.75
Presence of sclereids	0.75
Enlarged stem mucilage cells	0.75
Presence of xylary fibers	0.75
Woody vs. herbaceous habit	0.75
Presence of epidermal papillae	0.71
Presence of tannin cells	0.67
Presence of hypodermal druses	0.67
Presence of axillary hairs	0.5
Presence of phloem fiber caps	0.5
Presence of wide band tracheids	0.5
Thickened stem cuticle	0.5

Characters supporting a possible phylogenetic association between Anacampseroteae and *Portulaca* include transversely oriented leaf stomata, a lack of extraxylary fibers, and a base chromosome number of $x = 9$ (but also $x = 8$ in some *Portulaca*) (Goldblatt and Johnson, 1979). Again, however, all these characters are labile in the larger phylogenetic context (Table 5). The reduced, herbaceous habit in *Portulaca* and *Anacampseros* appears to be convergent and is not evidence of a close relationship of these two clades.

Calcium oxalate druses distributed in the hypodermis have previously been reported from many Cactaceae, including both *Pereskia* lineages (Metcalfe and Chalk, 1950; Mauseth and Landrum, 1997; Mauseth, 2005). Their absence in the portulacaceous clades of ACPT observed here indicates that they may be a synapomorphy of Cactaceae.

Evolution in Cactaceae—The core cacti represent a relative extreme in plant morphological evolution; leaves are highly reduced, and stems have been modified for the dual purposes of water storage and photosynthetic carbon uptake. Reconstructing the evolution of the cactus life form will therefore have general implications for our understanding of major morphological transitions in plants. A paraphyletic *Pereskia* provides a starting point for analyzing the evolution of the core cacti from leafy, nonsucculent ancestors (Fig. 1). The presence of delayed periderm and stem stomata unites the Andean/SSA *Pereskia* clade with core cacti. While stems of many plants may photosynthetically recycle respiratory carbon dioxide under periderm and in the absence of stem stomata (Pfanz et al., 2002), these coincident character changes were clearly necessary precursors for the evolution of the stem as the major photosynthetic organ. Edwards et al. (2005) tentatively named the clade comprising the core cacti + Andean/SSA *Pereskia* clades the "caulo cacti" to highlight these early and potentially important stem developments (Fig. 1).

The current study, however, demonstrates that both of these characters are highly variable in Portulacineae (Tables 3, 5). Stem stomata of various densities occur at least ephemerally in most *Talinum* species observed and also appear in *Portulaca*, Anacampseroteae, Didiereaceae, and Montiaceae. Many Portu-

TABLE 4. Results from Bayesian analyses of correlated evolution of stem characters in selected Portulacineae.

Trait–trait correlation	Log Bayes factor
Stem stomata + enlarged mucilage cells	−1.96
Stem stomata + hypodermal druses	5.16*
Stem stomata + woody vs. herbaceous habit	0.76
Stem stomata + thickened cuticle	3.60
Stem stomata + delayed periderm	−1.00
Delayed periderm + enlarged mucilage cells	12.17**
Delayed periderm + hypodermal druses	4.36
Delayed periderm + woody vs. herbaceous habit	0.84
Delayed periderm + thickened cuticle	8.45*
Enlarged mucilage cells + woody vs. herbaceous habit	0.32
Hypodermal druses + woody vs. herbaceous habit	4.56
Hypodermal druses + thickened stem cuticle	8.84*

Notes: *Strong evidence for correlated evolution, **very strong evidence for correlated evolution

lacineae also have delayed or absent stem periderm, especially in *Portulaca* and Montiaceae. Furthermore, the specific combination of delayed or no periderm and fairly dense stem stomata occurs in many taxa (e.g., *Talinum caffrum*, *Claytonia virginica*; Table 1; although they are not significantly correlated across the tree, Table 4). The apparent homoplasy of these traits across Portulacineae makes it currently difficult to infer whether they were lost in the northern *Pereskia* clade or gained in the caulocactus clade. However, neither the uncertainty about the polarity of these changes nor their observed lability should exclude these traits from a discussion of their importance in early cactus evolution. The activity and significance of a given character needs to be considered in its context, both in terms of the other characters occurring within the organism, as well as the ecological setting in which that organism is found (de Queiroz, 2002; Donoghue, 2005). For example, almost all examples of stem stomata and delayed periderm observed here outside of the caulocactus clade are found in reduced, herbaceous plants. It is likely that in the cacti, it was the organismal context of the change in these stem traits, i.e., their occurrence in a perennial, woody plant, that was critical in the evolution of the stem as a long-lived, photosynthetic organ.

Along these lines, a further question would be whether other stem characters can be identified that may have interacted with delayed periderm and stem stomata in the evolution of cacti. These characters may also have changed between the two *Pereskia* lineages, or they may be invariant, their importance being based in their interaction with changes in the previously identified epidermal characters. If such characters can be found and are lacking in outgroup taxa, then they may be candidates for important components of complex morphological and physiological innovation in cacti and would be worthy of further investigation.

Three characters identified here are potential candidates for such a role, and each carries physiological implications for stem water storage and/or photosynthesis. They have been mentioned by previous authors, but not in the context of the hypothesis of a paraphyletic *Pereskia*.

The first of these is the development of a thick stem cuticle seen in the Andean/SSA *Pereskia* clade and the core cacti (Fig. 7A, B). Such a cuticle is qualitatively different from those seen in either leaves or stems of other taxa here, including young preperiderm shoots of the northern *Pereskia* lineage, and has been shown in core cacti to be an important factor in the ability of the stem to store water (Gibson and Nobel, 1986). Stems of the Andean/SSA *Pereskia* clade possess an only slightly succulent cortex when compared with core cacti, and therefore the evolution of a thickened cuticle appears to have preceded that of major stem succulence. It is noteworthy that the only Andean/SSA *Pereskia* species seen here to lack a thick cuticle, *P. aculeata*, is also the only species in this group with precocious periderm onset. If the cuticle is secreted by the epidermis (Esau, 1977), then it follows that precocious bark formation would disrupt the epidermis before a cuticle could form, making delayed periderm essentially a requirement for the development of a thickened cuticle. Indeed, the strong statistical support for their correlated evolution (Table 4) further suggests that these two traits may be mechanistically linked. This relationship illustrates the importance of delayed periderm as a potentially key developmental enabler (Donoghue, 2005) for subsequent stem evolution in the cacti.

A second character of interest is the development of greatly enlarged, persistent mucilage cells or cavities in the cortex and

TABLE 5. Summary of character states in Portulacineae.

No.	Character	Basellaceae	Montiaceae	Didiereaceae	*Talinum*	*Portulaca*	Anacampseroteae	*Pereskia* 'Northern clade'	*Pereskia* 'Andean/SSA clade'	Opuntioideae	Cactoideae
1.	Periderm onset: location	1	0	0	0/1	0	0/1	1	0	0	0
2.	Periderm onset: timing	?	1	0	0/1	1	0	0	1	1	1
3.	Tuberous roots	1	0/1	0/1	1	0/1	1	0/1	0/1	0/1	0/1
4.	Stem stomata	?	1	0/1	0/1	0	0/1	0	1	1	1
5.	Hypodermal druses	0	0	0	0	0	0	0/1	1	1	0/1
6.	Stem mucilage cells	?	0/1	1	1	1/2	1	0/1	2	2	2
7.	Phloem/cortical sclereids	0	0	1	1	0/1	0/2	2	2	0	0
8.	Tanniniferous cells	0	0	1	1	0	0	0	0	0	0/1
9.	Leaf stomata type	0	0	1	1	1	1	1	1	1	?
10.	Leaf stomata orientation	?	2	1	0	1	1	0	0	2	?
11.	Wide-band tracheids	0	0	0/1	0	0	0/1	0	0	1	1
12.	Stem cuticle	0	0	0	0	0	0	0	1	1	1

Notes: ? = No data for the character in question. 1. Periderm onset: location: 0 = epidermis, 1 = outer cortex. 2. Periderm onset: timing: 0 = precocious, 1 = delayed/suppressed. 3. Tuberous roots: 0 = absent, 1 = present. 4. Stem stomata: 0 = absent or sparse, 1 = present. 5. Hypodermal druses: 0 = absent, 1 = present. 6. Stem mucilage cells: 0 = absent, 1 = ephemeral, 2 = persistent. 7. Phloem/cortical sclereids: 0 = absent, 1 = thin-walled, 2 = thick-walled. 8. Tanniniferous cells: 0 = absent, 1 = present. 9. Leaf stomata subsidiary cell type: 0 = paracytic, 1 = parallelocytic. 10. Orientation of leaf stomata: 0 = random, 1 = transverse, 2 = longitudinal. 11. Wide-band tracheids: 0 = absent, 1 = present. 12. Stem cuticle: 0 = nonthickened, 1 = thickened

pith of all Andean/SSA *Pereskia* species observed in this study (Fig. 6B, Table 5). In contrast, stem mucilage cells in the northern *Pereskia* species are generally lacking or are poorly developed (Table 5). Similarly, although mucilage cells are common in leaves of Portulacineae, they are typically ephemeral and/or poorly developed in stems and are often no larger than surrounding parenchyma cells (Fig. 3A). Outside of the Andean/SSA *Pereskia* group, the largest stem mucilage cells seen in this study are in *Portulaca amilis* (Fig. 6A), but these are still a qualitatively less prominent component of stem tissues than in the former taxa. Chemically, the mucilage found in cacti consists of complex fibrous polysaccharides that may contain up to 30 000 sugar subunits in a single molecule (Gibson and Nobel, 1986). Mucilage cells have been demonstrated to serve as effective reservoirs for water molecules due to the chemical affinities of these polysaccharides (Gibson and Nobel, 1986). The distribution of mucilage cells in leaves of most Portulacineae correlates well with the role of leaves as the major water-storing organs in these taxa, and it follows that the gain of enlarged mucilage cells in stems of the Andean/SSA *Pereskia* group may also have been important in the assumption of water storage by stems. Interestingly, the correlation between mucilage cells and delayed periderm is the strongest of our data set (Table 4), suggesting a possible functional link between these two traits. Because suberized cork tissue mitigates water loss in perennating stems, a delay in periderm onset may require the development of some small degree of cortical water storage to buffer evaporative loss from the stem surface, which could be provided by the gain of cells with copious mucilage. This functional analogy between periderm and mucilage is supported by the presence of ephemeral mucilage cells in young stems of many taxa with early periderm onset, which are subsequently lost as the stem matures (Fig. 3A).

A third character that may have played a role in the evolution of the cactus stem as the major photosynthetic organ is the distribution of calcium oxalate druses in the hypodermal layer. It has been demonstrated that an excess of calcium ions in the vicinity of guard cells results in stomatal closure (de Silva et al., 1985) and that formation of druses serves to prevent calcium from interfering with stomatal function (Ruiz and Mansfield, 1994). The ability to sequester calcium ions in this manner may be relevant for the correct functioning of stem stomata. It is interesting to consider the strong support for the correlated evolution of hypodermal druses and stem stomata in our data set (Table 4); currently, however, there is not much evidence that these traits are functionally linked, because stem stomata in the Andean/SSA *Pereskia* lineage do not appear to be heavily involved in gas exchange (Martin and Wallace, 2000; Edwards and Donoghue, 2006). An alternative explanation for a functional role of hypodermal druses may be as a deterrent to herbivory, which would be important in plants that are lacking the protective functions of an early periderm. However, the correlation between periderm timing and hypodermal druses is weaker in our data set (Table 4). It also bears noting that hypodermal druses were found in both *Pereskia* lineages, although the onset of periderm in the cortex in *P. guamacho* means that they are soon lost with the outer cortex and epidermis, emphasizing that the interaction between characters may be most useful from a functional standpoint.

The results obtained here underscore the importance of sampling multiple ontogenetic stages when examining anatomical characters. Many important characters, such as mucilage cells, epidermal papillae, and calcium oxalate druses occurred ephemerally in younger tissues and were often subsequently either lost or greatly altered during ontogeny. Failure to sample a wide range of ontogenetic states would result in an erroneous interpretation of character distributions. Ontogenetic variability in the occurrence of many characters also raises the potential of examining heterochronic shifts in the timing of developmental events as a mechanism of morphological evolution in the ACPT clade.

A link between trait lability and evolutionary innovation?—In summary, morphological and anatomical characters show complex patterns of variation both within ACPT and Portulacineae as a whole. Characters such as leaf succulence, stem woodiness, and periderm timing, to name a few, are highly variable both within and between clades (Tables 3, 5), making interpretation of character evolution and assessment of morphological support for phylogenetic hypotheses both challenging endeavors. However, the distribution of a number of stem traits supports a paraphyletic *Pereskia*, and furthermore, all of these characters are potentially significant for the development of the stem as a photosynthetic, water-storing organ. The apparent lability of these characters underscores the importance of considering whole organismal context when assigning functional or evolutionary significance to a trait: although all of these traits appear in other lineages across the Portulacineae, it is only in the caulo cacti that we find the combination of a perennial habit, delayed periderm, stem stomata, enlarged stem mucilage cells, a thickened cuticle, and hypodermal calcium oxalate druses. We hypothesize that it is this particular combination of traits, rather than any one in isolation, that provided the necessary organismal setting for the further evolution of the cactus stem.

Many of these traits have unknown adaptive significance when considered in isolation; it is not immediately obvious, for example, what sort of competitive advantage delayed periderm formation brings to a plant with no photosynthetic stem tissue. Similarly, it is not clear what sort of significant costs delayed periderm would present. If many of these traits are more or less adaptively neutral in isolation and are also evolving independently from one another, then it follows that higher evolutionary lability in these traits will increase the likelihood that one lineage will evolve the "right" character states in all of them, thus providing the organismal context that would promote further innovation and eventual functional integration. It is important to note that while these traits are labile across our sample of Portulacineae, they are all highly conserved within the leafless, stem succulent cacti (Gibson and Nobel, 1986; Mauseth and Landrum, 1997; Mauseth, 2005, 2006), indicating that they have become integrated components of the stem photosynthetic and water storage systems. Similar patterns of variation in the tempo and mode of trait evolution during a major morphological transition have been previously reported: for example, the great variation in floral phyllotaxis in ANITA grade and magnoliid angiosperms as compared to the highly conserved whorl found in monocots and eudicots (Endress, 1987; see also Donoghue, 1989, and references therein), or the variation in embryo sac type among the deepest lineages of angiosperms (Friedman, 2006). High trait lability at the base of evolutionary radiations has been noted in the metazoa as well (Simpson, 1944; Gould, 1989; Foote, 1992). These examples highlight Riedl's notion of burden (Riedl, 1978; Donoghue, 1989) and that the likelihood of character change is dynamic and highly dependent upon that character's integration (and resulting burden) with other traits.

Homoplasy has been extensively discussed within the context of key innovations, but primarily as an indicator of adaptation (i.e., the repeated evolution of a certain structure in response to a certain habitat illustrates the adaptive nature of that structure) (Armbruster, 1996), or as outlined earlier, as a simple observation that homoplasy of traits may decrease over time as they become fundamentally integrated into a novel structure or pathway. The notion that homoplasy may itself serve as an engine of morphological novelty has only been discussed in a very general way (see Sanderson and Hufford, 1996). Here we propose a potential mechanism: in the sense that any "key innovation" is in reality a sum of many traits, assembling the right combination of those traits in one organism is an essential first step in their integration into a novel structure or function. We suggest that there may be an optimal rate of evolution for these traits that will maximize the likelihood of this initial assembling of characters. In the case of cacti, two prominent developmental enabler traits, stem stomata and delayed bark formation, had the highest lability of any traits in our data set. While we do not currently know the mechanisms that generated high lability in these traits, we hypothesize that initially elevated rates of trait evolution may promote the accumulation of greater combinations of new characters in lineages, which in turn may be a primary driver of evolutionary innovation.

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APPENDIX 1. Taxon names, nomenclatural authorities, and voucher numbers used in this study.

Taxon	Voucher	Notes
Phemeranthus teretifolius (Pursh) Raf.	Ogburn 4, MO	
Parakeelya pickeringii (A. Gray) Hershkovitz	P.I. Forster 16465A, BRI	
Claytonia virginica L.	Ogburn 32, MO	
Portulacaria afra Jacq.	Ogburn 27, MO	Cult.
Ceraria fruticulosa Pearson & Stevens	Ogburn 37, MO	Cult.
Talinum caffrum (Thunb.) Eckl. & Zeyh.	Ogburn 21, MO	Cult.
Talinum paniculatum (Jacq.) Gaertn.	Ogburn 16, MO	Cult.
Talinum triangulare (Jacq.) Willd.	Ogburn 12, MO	Cult.
Talinum portulacifolium (Forssk.) Asch. ex Schweinf.	Ogburn 23, MO	Cult.
Talinella pachypoda Eggli	Ogburn 22, MO	Cult.
Portulaca oligosperma F. Muell.	P.I. Forster 12960, BRI	
Portulaca oleracea L.	Ogburn 18, MO	
Portulaca grandiflora Hook.	Ogburn 14, MO	Cult.
Portulaca amilis Speg.	Ogburn 11, MO	
Portulaca pilosa L.	Ogburn 10, MO	
Talinopsis frutescens A. Gray	Ogburn 31, MO	Cult.
Grahamia bracteata Gillies ex Hook.	Ogburn 25, MO; Leuenberger, Arroyo-Leuenberger, and Eggli 4184, B	Cult.
Xenia vulcanensis (Añon) Gerbaulet	Leuenberger 3534, B	Cult.
Anacampseros kurtzii Bacigalupo	Leuenberger, Arroyo-Leuenberger, and Eggli 4217, B	Cult.
Talinaria coahuilensis (S. Watson) P. Wilson	Ogburn 35, MO	Cult.
Anacampseros australiana J.M. Black	Menkins DOP12, BRI	
Anacampseros recurvata subsp. *buderiana* (Poelln.) Gerbaulet	Ogburn 29, MO	Cult.
Anacampseros quinaria E. Mey. ex Fenzl	Ogburn 17, MO	Cult.
Anacampseros rufescens (Harv.) Sweet	Ogburn 13, MO	Cult.
Anacampseros filamentosa (Haw.) Sims	Ogburn 36, MO	Cult.
Anacampseros lanceolata (Harv.) Sweet	Ogburn 38, MO	Cult.
Pereskia guamacho F.A.C. Weber	Edwards 18,19, YU	
Pereskia portulacifolia (L.) DC.	Edwards 11, YU	
Pereskia quisqueyana Alain	Edwards 7, 8, YU	
Pereskia nemorosa Rojas Acosta	J.C. Solomon 6918, MO	
Pereskia aculeata Mill.	Ogburn 34, MO	Cult.
Pereskia diaz-romeroana Cárdenas	Edwards 128, YU	
Pereskia weberiana K. Schum.	Edwards 137, YU	
Pereskiopsis gatesii Baxter	Ogburn 24, MO	Cult.

Notes: MO = Missouri Botanical Garden; BRI = Queensland Herbarium, Brisbane; B = Berlin Botanical Garden; YU = Yale University Herbarium; cult. = cultivated material.