

# Comparative leaf and stem anatomy in selected species of Ruellieae (Acanthaceae) representative of all major lineages

Erin A. Tripp<sup>1</sup> & Mekbib Fekadu<sup>2</sup>

**Summary.** With more than 1,200 species, Ruellieae is a taxonomically and ecologically diverse tribe in the Acanthaceae. In recent years, numerous morphological and phylogenetic studies have contributed important new information about species belonging to this tribe, yet basic anatomical knowledge of lineages within Ruellieae is relatively scarce. The objective of the present study is to help close this anatomical knowledge gap through comparative leaf and stem anatomical study of 14 species representative of all seven subtribes within Ruellieae. We document relative conservatism in leaf and stem anatomy except that unifacial leaves characterise a few taxa and have evolved a minimum number of three times in the tribe. Cystoliths were found abundantly in both leaf and stem tissue; these were oriented in two different directions in leaves while in stems only one orientation was found. Finally, we discuss the putative presence of a stem and petiole endodermis in several taxa studied. These data serve as a starting point for further comparative anatomical studies within Ruellieae and other Acanthaceae.

**Key Words.** Cystolith, endodermis, petiole, unifacial.

## Introduction

The family Acanthaceae comprises >4000 species (230 genera) that are primarily tropical in distribution (Scotland & Vollesen 2000; McDade *et al.* 2000). It is among the 10 to 12 most diverse families of flowering plants (Tripp & McDade 2014a). This taxonomic diversity is partitioned among several major lineages (Scotland & Vollesen 2000; McDade *et al.* 2008): Nelsonioideae, Thunbergioideae, *Avicennia*, Acantheae, Barlerieae, Andrographideae, Whitfieldieae, Justicieae, and Ruellieae; Acanthaceae s.s. encompass the latter six tribes and contain >90% of total species diversity in the family (Tripp & McDade 2014a). Over the last decade, we have made a focused effort to better understand diversity and evolution in Ruellieae — a tribe of some 1,200+ species that inhabit primarily tropical to subtropical environments worldwide. These efforts include large-scale phylogenetic reconstruction and re-classification of the tribe (Tripp *et al.* 2013a), detailed study of phylogenetic relationships within large and / or difficult genera (Tripp 2007; Tripp *et al.* 2013b), fossil-based estimations of lineage divergence times (Tripp & McDade 2014a; Tripp & McDade 2014b), analysis of pollination system evolution (Tripp & Manos 2008), taxonomic contributions including regional treatments,

taxonomic novelties, and nomenclatural rearrangements (Tripp 2004; McDade & Tripp 2007a; McDade & Tripp 2007b; Schmidt-Lebuhn & Tripp 2009; Tripp *et al.* 2009; Tripp 2010; Tripp & Dexter 2012; Tripp & McDade 2012; Callmander *et al.* 2014; Tripp & Koenemann, *in press*), and anatomical study of genera of particular interest (Tripp & Fatimah 2012). Colleagues have similarly contributed important new knowledge of the tribe through varied studies (Daniel 1990; Scotland 1991; Ezcurra 1993; Manktelow 1996, 2000; Carine & Scotland 2002; Wasshausen & Wood 2003; Moylan *et al.* 2004a, 2004b; Schmidt-Lebuhn 2003; Vollesen 2006).

Despite marked morphological and ecological diversity within Ruellieae, there has been a surprising dearth of anatomical investigation in this tribe (but see Moylan *et al.* 2004b; Tripp & Fatimah 2012; Monteiro & Aoyama 2012). Although this statement applies to the vast majority of species across Acanthaceae, lineages other than Ruellieae have been a greater focus of anatomical study, especially Justicieae (e.g., Kuo-Huang & Yen 1996; O'Neill 2010; Paopun *et al.* 2011; Aoyama & Indriunas 2012; Muhaidat *et al.* 2012; Patil & Patil 2012; Amirul-Aiman *et al.* 2013). A lack of baseline anatomical information represents a conspicuous gap in a growing body of knowledge of this rich and important tribe within

Accepted for publication 28 October 2014. Published online 29 November 2014

<sup>1</sup> Department of Ecology and Evolutionary Biology and Museum of Natural History, University of Colorado, Boulder, Colorado 80309, U.S.A. e-mail: erin.tripp@colorado.edu

<sup>2</sup> Department of Plant Biology and Biodiversity Management, College of Natural Sciences, Addis Ababa University, PO Box 3434 Addis Ababa, Ethiopia. e-mail: mekbib\_fekadu@yahoo.com

Acanthaceae. This deficit impedes research on systematics and evolution of Ruellieae by reducing the number of information sources available with which to study family- and/or lineage-wide patterns.

The primary objective of the present study is to help close the anatomical knowledge gap in Ruellieae by conducting a comparative investigation of leaf and stem anatomy in several genera within the tribe. We aim to document general features of leaf and stem anatomy to explore similarities and differences between taxa. We give special attention to an interesting trait that characterises most Acanthaceae s.s. (i.e., all lineages except Acantheae) and one that has been the focus of several studies in prior anatomical works on Acanthaceae: cystoliths (Metcalf & Chalk 1950; Inamdar *et al.* 1990; Kuo-Huang & Yen 1996; Patil & Patil 2011; Stanfield 2013).

## Materials and methods

This study was conducted at Rancho Santa Ana Botanic Garden in Claremont, California. We sampled leaves, petioles, and stems using fresh material from 14 species in 14 genera that are cultivated at the RSABG greenhouses (Table 1). This sampling included representatives of all seven of the major lineages of Ruellieae (*sensu* Tripp *et al.* 2013a): Erantheminae, Hygrophilinae, Mimulopsinae, Petalidiinae, Ruelliinae, Strobilanthisinae, and Trichantherinae. Four of our 14 samples were derived from material originally wild collected by E. Tripp and colleagues whereas the remainder were acquired by E. Tripp from various other sources including botanic gardens (Marie Selby; Royal Botanic Gardens, Kew; University of Connecticut) and collections maintained by private individuals. The native ranges of these taxa include tropical Asia, tropical Africa, and the Neotropics, and span a diversity of environmental gradients from low to high altitude and from dry to wet habitats. All taxa

included in this study are represented by voucher specimens deposited at the RSA and/or COLO Herbaria.

Following anatomical methods described in Tripp & Fatimah (2012) with slight modifications, fresh leaves, stems, and petioles were fixed in FPA (1:1:18 ratio of 37% formaldehyde : propionic acid : 70% ethanol) and then transferred to a 5% NaOH aqueous solution and held at 37 – 40 °C for 12 hrs; stems, leaves, and petioles were later rinsed thoroughly with distilled water to remove alkaline solution. Tissues were dehydrated by passing them through an alcohol series of 30%, 50%, 70%, 90%, and 95% (2 hrs per step). Samples were transferred to a 100% ethanol solution with 1% safranin overnight and then placed in 100% ethanol the next morning for 2 hrs. Leaf samples were then soaked in a 2:1 ratio of 100% ethanol : xylene (2 hrs), a 1:2 ratio of 100% ethanol : xylene (2 hrs), 100% xylene (2 hrs), 100% xylene (2 hrs), a 2:1 ratio of xylene to paraffin oil (2 hrs), a 1:2 ratio of xylene to paraffin oil (2 hrs), and then infiltrated via liquid paraffin (two 6 hr treatments).

After infiltration, leaf and stem samples were embedded with paraffin using a Leica Histoembedder. Following methods described in Columbus (1999), we prepared 10 µm sections on an American Optical Company Rotary Microtome (Spencer Model 820). Sections were affixed to slides over ~5 minutes on a warming plate and then stained using the staining series described in Ocampo & Columbus (2010), which is based on Sharman (1943). Slides were examined using microscopy equipped with SPOT software version 4.1.1.

## Results & Discussion

The overarching goal of the present study was to provide primary documentation of leaf and stem anatomy within genera of Ruellieae representative of all major lineages of the tribe (two additional species

**Table 1.** Species used during sampling for comparative leaf and stem anatomy of Ruellieae.

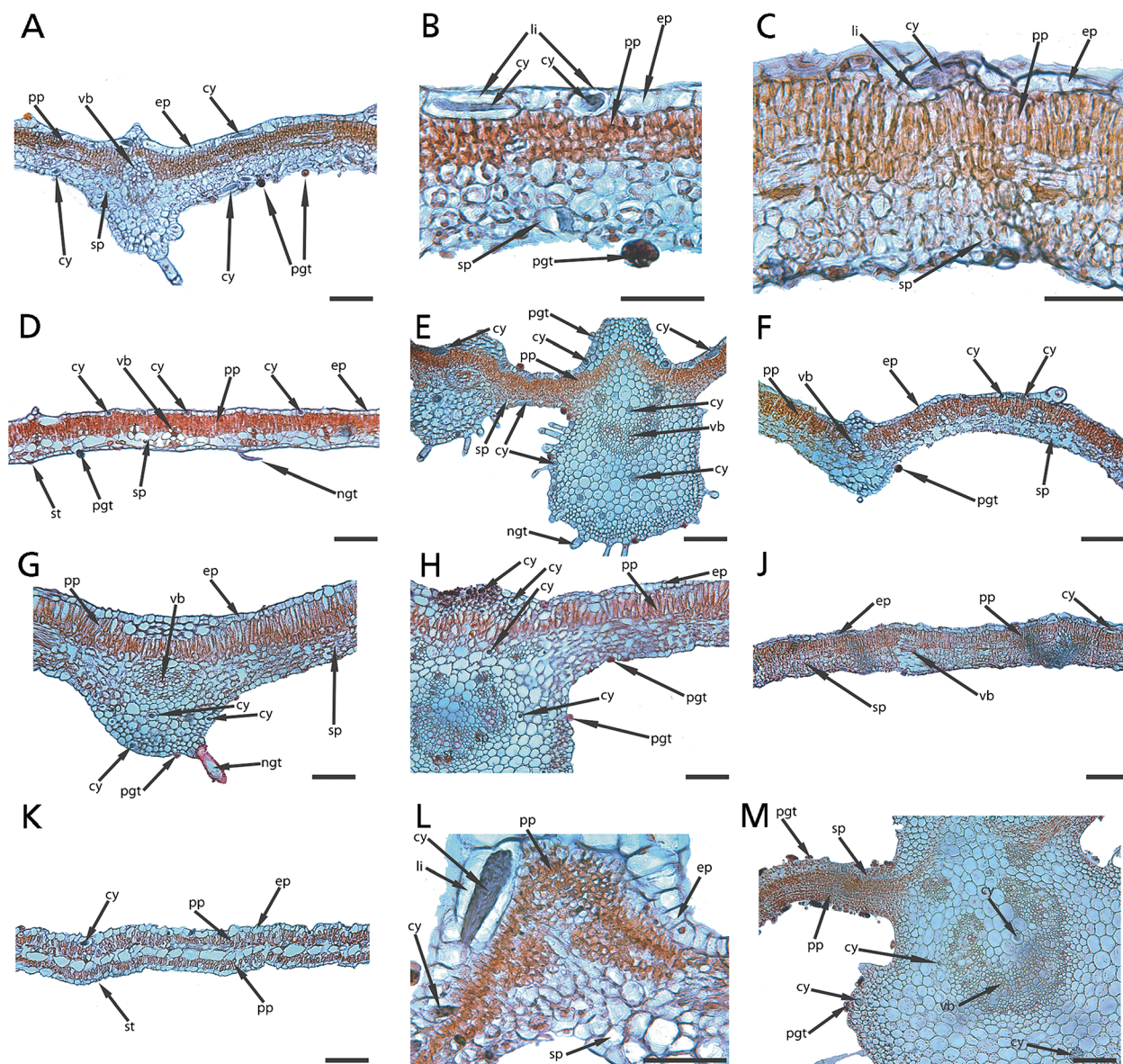
| Taxon   | Native Habitat      | Voucher                   |
|---|---------------------|---------------------------|
| <i>Brillantaisia owariensis</i> P. Beauv.               | Tropical Africa     | Tripp 192 (DUKE)          |
| <i>Duosperma longicalyx</i> (Deflers) Vollesen          | Tropical Africa     | Tripp & Ensermu 888 (RSA) |
| <i>Dyschoriste thunbergiiflora</i> Lindau               | Tropical Africa     | Tripp & Ly 937 (RSA)      |
| <i>Eranthemum</i> sp.                                   | Thailand            | Tripp 138 (DUKE)          |
| <i>Heteradelphina paulojaegeria</i> Heine               | Tropical Africa     | Tripp & Ly 939 (RSA)      |
| <i>Hygrophila schulli</i> M. R. Almeida & S. M. Almeida | Tropical Africa     | Tripp & Ensermu 927 (RSA) |
| <i>Louteridium costaricense</i> Radlk. & Donn. Sm.      | Central America     | Tripp & Ly 960 (RSA)      |
| <i>Petalidium englerianum</i> C. B. Clarke              | Tropical Africa     | Tripp & Dexter 778 (RSA)  |
| <i>Phaulopsis imbricata</i> Sweet                       | Tropical Africa     | Tripp & Ensermu 917 (RSA) |
| <i>Ruellia bignoniiflora</i> S. Moore                   | Tropical Africa     | Tripp & Ly 958 (RSA)      |
| <i>Ruellioopsis setosa</i> C. B. Clarke                 | Tropical Africa     | Tripp & Dexter 790 (RSA)  |
| <i>Sanchezia stenomacra</i> Leonard & L. B. Sm.         | Tropical S. America | Lujan 427 (RSA)           |
| <i>Strobilanthes</i> sp.                                | Thailand            | Lujan 429 (RSA)           |
| <i>Trichanthera gigantea</i> Humb. & Bonpl. ex Steud.   | Tropical S. America | Lujan 428 (RSA)           |

were studied for petiolar anatomy). These data enable exploration of similarities and differences among genera.

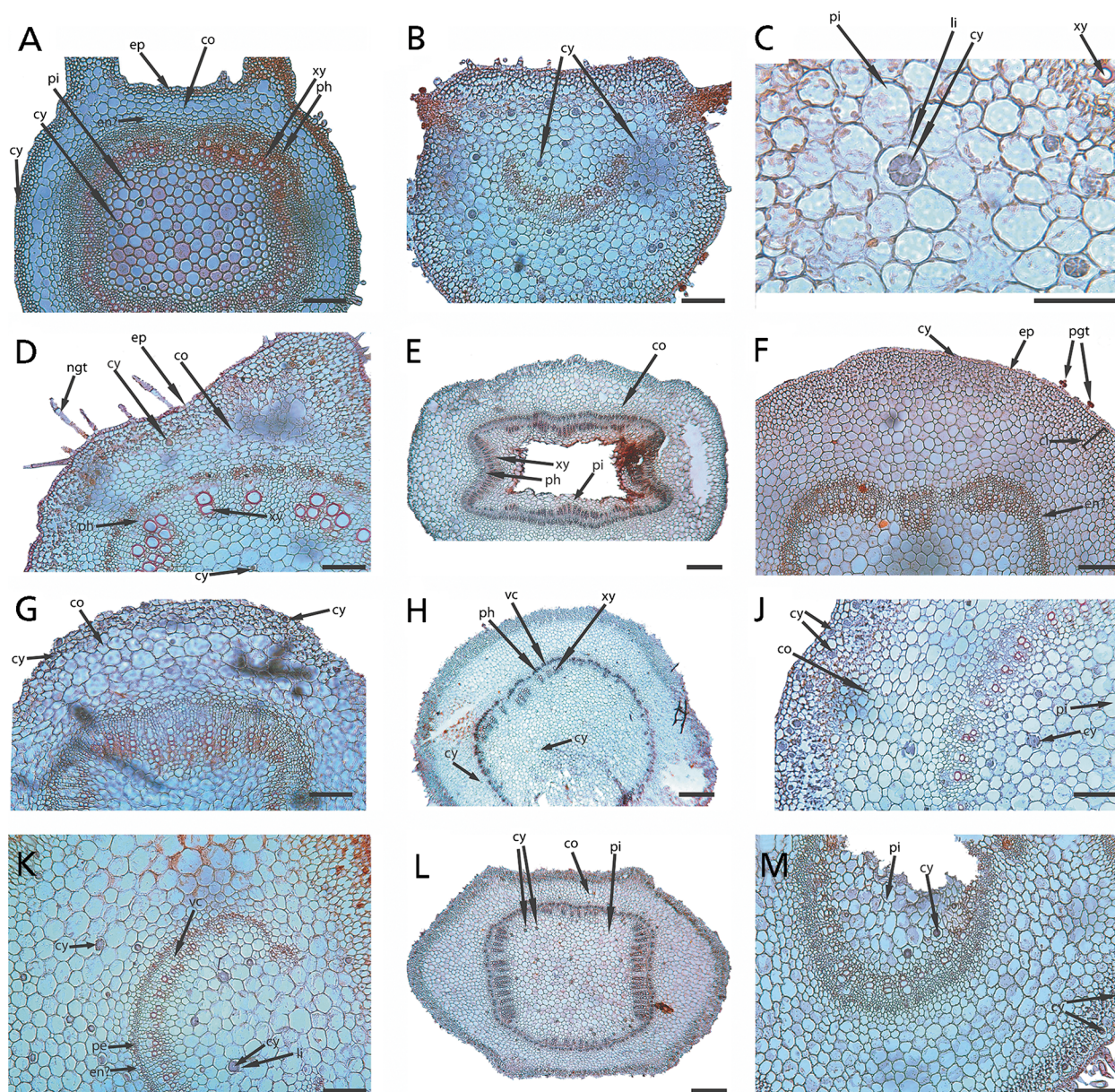
**Comparative Leaf Blade Anatomy.** We sampled 11 of the 14 species of Ruellieae for comparative leaf blade (hereafter, leaves) anatomical study. Our results demonstrate that among the studied taxa, leaf anatomy is relatively uniform and conservative across subtribes: (1) cystoliths were observed in both the upper and lower epidermis but were in general more abundant in the former (Figs 1D, 1F); (2) cystoliths additionally occur proximal to vascular bundles in several taxa (e.g., Figs 1E, 1G, 1H, 1M); (3) cystoliths were oriented both parallel to the plane (of the long axis of the leaf as well as perpendicular to it (e.g., Figs 1D, 1G, 1H, 1K vs Figs 1C, 1J, 1L)); (4) both non-glandular trichomes (Fig. 1E) and sessile peltate glandular trichomes were seen, but the latter were more common on abaxial leaf surfaces (e.g., Figs 1B, 1D, 1F, 1M; see also Tripp & Fatimah 2012 and Tripp *et al.* 2013a for discussion of these structures); (5) most taxa had a well differentiated, uniseriate upper and lower epidermis, with the upper generally thicker than the lower (e.g., Figs 1A, 1F); (6) most taxa had tightly packed palisade parenchyma consisting generally of two layers (more rarely, three layers), with more elongated cells in the upper layer and more spherical cells in the lower layer(s) (e.g., Figs 1C, 1D, 1G); (7) most taxa had 3 or 4 layers (more rarely, two layers) of nearly isodiametric spongy parenchymatic cells, with large intercellular cavities (e.g., Figs 1A, 1B, 1C, 1D); and (8) most vascular bundles were surrounded by enlarged bundle sheath cells (e.g., Figs 1D, 1F, 1J). This general morphology applies to the leaves of numerous other Acanthaceae that have been studied at the anatomical level (e.g., several *Barleria* spp. and *Lepidagathis* spp., Ahmad 1975; *Thunbergia laurifolia* Lindl., Paopun *et al.* 2011; *Ruellia prostrata* Poir., Iyyappan & Mounnissamy 2011; *Satanocrater* spp., Tripp & Fatimah 2012; *Justicia brandegeana* Wassh. & L. B. Sm., Aoyama & Indriunas 2012; *Ruellia furcata* (Nees) Lindau, Monteiro & Aoyama 2012). The most noticeable difference observed in leaves of Ruellieae is the presence of unifacial leaves in *Ruellioopsis setosa* C. B. Clarke vs bifacial leaves in all other taxa (see, however, Fig. 1M [*Trichanthera gigantea* Humb. & Bonpl. ex Steud.] in which the mesophyll is not noticeably differentiated; limited number of sections prevented our further assessment of whether this holds true in regions further away from the midrib). Unifacial leaves also characterise some species in the genus *Satanocrater* (Tripp & Fatimah 2012), and unpublished data [E. Tripp, manuscript in prep.] also indicate that species of *Petalidium* have unifacial leaves). Given that *Ruellioopsis* and *Petalidium* belong to the subtribe Petalidiinae (but are not sister taxa)

and that *Satanocrater* belongs to the subtribe Ruelliinae, which is not sister to Petalidiinae (Tripp *et al.* 2013a), unifacial leaves have clearly evolved a minimum of three times in Ruellieae.

**Comparative Stem and Petiole Anatomy.** We sampled 9 of the 14 species of Ruellieae for comparative stem anatomical study, and two of the 14 species for preliminary exploration of petiolar anatomy. Similar to leaf anatomy, our results demonstrate that among the studied taxa, stem (and petiolar) anatomy is relatively uniform and conservative across subtribes: (1) some species showed distinct longitudinal ridges (and associated stem or petiolar furrows), typical of external morphology of many Ruellieae (Figs 2A, 2B, 2D, 2L); (2) stems were observed to be quite rigid despite the herbaceous habits (some species such as *Brillantaisia owariensis* P. Beauv., *Dyschoriste thunbergiiflora* Lindau, *Louteridium chartaceum* Leonard, *Sanchezia stenomacra* Leonard & L. B. Sm. reach 2–3 m in height), perhaps owing to ample collenchyma tissue that enhances rigidity (O'Neill 2010; Leroux 2012); (3) stems of most species were surrounded by a uniseriate epidermis (e.g., Figs 2A, 2F, 2J); (4) internal to the epidermis was a prominent layer of angular collenchyma composed of 2–6 cell layers in at least some taxa (e.g., Fig 2F); (5) internal to the collenchyma (where present) was a well differentiated layer of parenchymatous cortex followed by the vascular tissue; (6) from external to internal portions, stem vasculature consisted of a putative endodermis of differentiated cells (e.g., Figs 2A, 2F; also putatively present in both petiolar sections: see Figs 2B, 2K) and collateral vascular bundles of phloem conductive elements, the vascular cambium, and xylary conductive elements (e.g., Fig. 2A), these ranging in size depending on species (e.g., note relatively large xylem elements in Fig. 2D compared to Figs 2F and 2G); (7) all species possessed large, thin-walled cells comprising the parenchymatous pith in stem centres; (8) cystoliths were found to occur in all stem and petiole tissue layers except the vascular bundle, i.e., the epidermis, the cortex (including collenchyma), and the pith, with no apparent bias in location (e.g., Figs 2A, 2B, 2D, 2J); and (9) cystoliths were always oriented parallel to the long axis of the stem (e.g., Figs 2A, 2B, 2C, 2D, 2J, 2K, 2M), in contrast to leaf cystoliths that displayed two different orientations. The above-described general morphology applies to the stems of other Acanthaceae that have been studied at the anatomical level (e.g., *Ruellia ciliosa* Pursh: Holm 1907; several taxa: Metcalfe & Chalk 1950; *Justicia brandegeana*: O'Neill 2010). Among the more unexpected structures documented in this study was the putative presence of an endodermis that surrounds the stem (and petiolar) vasculature of several species. Stem endodermises are relatively underappreciated compared to the widespread occur-



**Fig. 1.** Transverse sections through leaves of 11 species of Ruellieae, with adaxial surfaces towards top of plate and abaxial surfaces towards bottom. **A** *Brillantaisia owariensis*, showing section through midrib, clearly differentiated upper and lower mesophyll, cystoliths in upper and lower epidermises, and peltate glandular trichomes on abaxial surface. **B** *Brillantaisia owariensis*, showing details of cystoliths and lithocysts, palisade and spongy parenchyma, and abaxial peltate glandular trichome. **C** *Duosperma longicalyx*, showing section through portion of leaf blade with a cystolith and lithocyst, the upper epidermis with waxy coating, palisade parenchyma, and spongy parenchyma. **D** *Dyschoriste thunbergiiflora*, showing section through portion of leaf blade with abundant cystoliths embedded in upper epidermis, clearly differentiated palisade and spongy parenchyma, and abaxial stomata, peltate glandular trichomes, and non-glandular trichomes; cystolith on abaxial surface has been broken free from lithocyst and is protruding beyond surface of blade. **E** *Eranthemum* sp., showing section through midrib and nearby secondary vein, abundant cystoliths embedded in upper epidermis as well as near and below vasculature. **F** *Heterodelphia paulojaegeria*, showing section through portion of leaf blade with clearly differentiated upper and lower mesophyll cells, cystoliths embedded in upper epidermis, and peltate glandular trichomes on abaxial surface. **G** *Louteridium costaricense*, showing section through secondary vein, cystoliths embedded in spongy mesophyll, and uniseriate non-glandular trichomes on abaxial surface. **H** *Louteridium costaricense*, section through midrib showing abundant cystoliths towards adaxial surface and abundant peltate glandular trichomes towards adaxial surface. **I** *Ruellia bignoniiflora*, section through portion of leaf blade showing weakly differentiated mesophyll layers [palisade and spongy parenchyma], and lithocyst with cystolith embedded just below upper epidermis. **K** *Ruelliopsis setosa*, section through portion of leaf blade showing a distinctive unifacial anatomy with conspicuous layer of enlarged, hyaline cells in between two palisade layers. **L** *Strobilanthes* sp., section through portion of leaf blade showing large cystolith oriented perpendicular to long axis of blade and clearly differentiated palisade and spongy parenchyma. **M** *Trichanthera gigantea*, showing section through midrib with large cystoliths oriented parallel to long axis of blade, abundant peltate glandular trichomes on both adaxial and abaxial surfaces, and mesophyll cells that are not noticeably differentiated. Abbreviations: cy=cystolith, ep=epidermis, li=lithocyst, ngt=non-glandular trichome, pgt=peltate glandular trichome, pp=palisade parenchyma, sp=spongy parenchyma, st=stomata, vb=vascular bundle. Scale bars = 250 µM.



**Fig. 2.** Transverse sections through stems (9 species) and petioles (2 species) of Ruellieae. **A** *Dyschoriste thunbergiiflora* stem [winged], showing definitive single layer of end-to-end cells functioning putatively as an endodermis (including thickening of radial walls), cystoliths oriented parallel to long axis of stem embedded in pith as well as outer epidermis. **B** *Eranthemum* sp. petiole, showing massive number of cystoliths oriented parallel to long axis of petiole, these scattered throughout tissue. **C** *Eranthemum* sp. stem, showing detail of cystolith inside of lithocyst embedded in pith. **D** *Heteradelphina paulojaegeria* stem, showing enlarged xylary tissue with respect to smaller phloem tissue, a well differentiated epidermis with uniseriate non-glandular trichomes, and cystoliths embedded in both the cortex and pith. **E** *Hygrophila schullii* stem, showing vascular tissue and irregular cortex cells. **F** *Petalidium englerianum* stem, showing peltate glandular trichomes on epidermis, collenchyma cells with unevenly thickened walls below epidermis, a single layer of end-to-end cells functioning putatively as an endodermis but without evidence of a Casparian strip, and a cortex consisting of alternating small and large cells arranged in a honeycomb pattern. **G** *Ruelliopsis setosa* stem, showing concentration of cystoliths among epidermal cells. **H** *Phaulopsis imbricata* stem, showing cystoliths embedded in both the cortex and pith. **J** *Sanchezia stenomacra* stem, showing an abundance of cystoliths, especially concentrated in outer 3 to 5 cell layers of cortex but also abundant in pith. **K** *Strobilanthes* sp. petiole, showing a single layer of cells functioning putatively as an endodermis (but again without evidence of a Casparian strip) and abundant cystoliths oriented parallel to long axis of petiole. **L** *Trichanthera gigantea* stem [sub-quadrangular], showing non-differentiated cortex and pith cells and abundance of cystoliths inside pith. **M** *Trichanthera gigantea* stem, showing cystoliths encased in much larger lithocysts. Abbreviations: cl=collenchyma, co=cortex, cy=cystolith, en?=putative endodermis, ep=epidermis, li=lithocyst, ngt=non-glandular trichome, pgt=peltate glandular trichome, ph=phloem, pi=pith, pp=palisade parenchyma, sp=spongy parenchyma, st=stomata, xy=xylem. Scale bars = 250  $\mu$ M.

rence of an endodermis in roots (Lersten 1997). Yet, over a century ago, Holm (1907) documented an endodermis in the stems of two species of Acanthaceae, one of which was in the tribe Ruellieae (i.e., *Ruellia ciliosa*). More recently, Remadevi *et al.* (2006) documented a stem endodermis in 28 species of Acanthaceae. In 2010, O'Neill used staining techniques to demonstrate the presence of a lipid-rich layer in a species of Acanthaceae, most likely suberin-containing, located just outside of stem vascular bundles; he concluded that this finding was likely representative of an endodermis in the studied taxon. In the present study, we did not employ staining methods to detect an endodermis in these tissues. However, several taxa in this study shared the presence of a thin layer of cells that is anatomically clearly differentiated from both the vascular tissue and parenchyma cells immediately adjacent to it (i.e., internal and external to it), as seen in Fig. 2A. We thus conclude that these anatomical sections provide some preliminary evidence consistent with previous findings of an endodermis in above-ground tissue in Acanthaceae.

**Cystoliths.** One of the most intriguing anatomical features of most Acanthaceae (i.e., all lineages of Acanthaceae s.s. except the tribe Acantheae; see McDade *et al.* 2008 for a phylogenetic overview) are the cystoliths. Cystoliths are calcium carbonate or calcium oxalate crystals that are otherwise absent from Lamiales but present in a limited number of unrelated families such as Cannabaceae, Urticaceae and Moraceae (e.g., Philpott 1953; Okazaki *et al.* 1986; Watt *et al.* 1987; Setoguchi *et al.* 1989; Kuo-Huang & Yen 1996; Wu & Kuo-Huang 1997; Scotland & Vollesen 2000). Data presented here (see figure Citations above) demonstrate three general patterns with respect to cystolith distribution and orientation in Ruellieae. (1) Consistent with prior findings, we documented cystoliths in both leaf and stem tissue (Metcalf & Chalk 1950; Moylan *et al.* 2004b; O'Neill 2010; Patil & Patil 2011), although some studies have failed to find cystoliths in stem pith tissue (Holm 1907; note that in this study, cystoliths were not evident in the stem section of *Hygrophila schulli* M. R. Almeida & S. M. Almeida [Fig. 2E], a result that we suspect may be attributable to a limited number of sections made for this species rather than the true absence of stem cystoliths). (2) Leaf cystoliths were found to be oriented in two different directions compared to stem (and petiolar) cystoliths, which were only oriented in one direction (Fig. 1 vs Fig. 2). The two different orientations of cystoliths in leaves, which appears to be governed by their proximity to the midrib (herein documented as well as in Kuo-Huang & Yen 1996), may signify two different functions of these structures in the leaf. For example, one hypothesis is that cystoliths serve a

structural function proximal to midribs whereas elsewhere in the leaf blade, cystoliths serve to scatter light and reduce photoinhibition, shifting light from photon-saturated upper mesophyll cells to light-limited lower mesophyll cells (Gal *et al.* 2012). This leaves open the question as to their original function or adaptive value, if any, during their early appearance(s) evolutionarily within Acanthaceae. (3) In leaf tissues, cystoliths occur in both epidermises as well as proximal to vascular bundles but were more abundant in the upper epidermis and near bundles than in the lower epidermis (Fig. 1). (4) In stem tissue, cystoliths were commonly found in the epidermis, the cortex, and the pith, with no apparent bias in terms of relative abundance in these different regions. Although not studied here, cystoliths have also been documented in the wood of species of Acanthaceae that have secondary growth — a relatively uncommon phenomenon with respect to the distribution of cystoliths elsewhere in vegetative portions of a plant (Carlquist 2001). The functional and/or adaptive significance of cystoliths has long been debated, but little light has been shed on resolving the question (Esau 1953; Watt *et al.* 1987; Setoguchi *et al.* 1989; Kuo-Huang & Yen 1996; Bauer *et al.* 2011; Stanfield 2013).

According to Metcalfe & Chalk (1950), the nature and distribution of cystoliths is of paramount importance in recognising genera and species. Other comparative studies such as Ahmad (1975) and Patil & Patil (2011) have discussed the taxonomic importance of cystoliths. However, we caution that there has never been a comprehensive investigation — and one taking phylogeny into consideration — of the taxonomic utility of cystoliths. In particular, structural diversity of cystoliths as seen in the present investigation was relatively minimal, suggesting limited utility of this character in taxonomic systems beyond the major split of “the cystoliths-bearing clade” (all Acanthaceae s.s. except for the tribe Acantheae). This opinion of limited taxonomic utility was similarly echoed in Inamdar *et al.* (1990). The present investigation serves to further our knowledge about the morphology and distribution of cystoliths in tissues of Acanthaceae, in particular, of plants in the large tribe Ruellieae.

## Conclusions

In this comparative anatomical study of the taxonomically and ecologically rich tribe Ruellieae, we have documented primary leaf and stem anatomy of genera representative all major subtribes *sensu* Tripp *et al.* (2013a). We documented a general conservatism in anatomical structures with the exception of the presence of unifacial leaves, which have evolved a minimum of three times in Ruellieae. Cystoliths were observed in all our studied species and appear to be as common in stem tissue as in leaf tissue of Ruellieae.

These data serve as a starting point for further comparative anatomical studies within Ruellieae.

### Acknowledgements

We thank Rancho Santa Ana Botanic Garden for welcoming our research in their anatomy lab. We are grateful to Travis Columbus (RSABG) for helpful discussions on anatomy and Manual Lujan (RSABG) for assistance in preparing vouchers from living material. Two anonymous reviewers contributed important comments that improved this manuscript. Finally, we thank the National Science Foundation (NSF-DEB Award #0919594 and NSF-DEB Award #1354963 to Erin Tripp and Lucinda McDade) for funding this research and for funding the second author's participation in RSABG's Summer Research Institute training program.

### References

- Ahmad, K. J. (1975). Cuticular studies in some species of *Lepidagathis* and *Barleria*. *Bot. Gaz.* 136: 129 – 135.
- Amirul-Aiman, A. J., Noraini, T., Nurul-Aini, C. A. C. & Ruzi, A. R. (2013). Petal anatomy of four *Justicia* (Acanthaceae) species. *AIP Conference Proceedings* 1571: 368 – 371.
- Aoyama, E. M. & Indriunas, A. (2012). Leaf anatomy of *Justicia brandegeana* Washh. & L. B. Sm. (Acanthaceae). *Commun. Pl. Sci.* 2: 37 – 39.
- Bauer, P., Elbaum, R. & Weiss, I. M. (2011). Calcium and silicon mineralization in land plants: transport, structure and function. *Pl. Sci.* 180: 746 – 756.
- Callmander, M. W., Tripp, E. A. & Phillipson, P. B. (2014). A new name in *Ruellia* L. (Acanthaceae) for Madagascar. *Candollea* 69: 81 – 83.
- Carine, M. A. & Scotland, R. W. (2002). Classification of Strobilanthinæ (Acanthaceae): Trying to classify the unclassifiable? *Taxon* 51: 259 – 279.
- Carlquist, S. (2001). *Comparative Wood Anatomy: Systematic, Ecological, and Evolutionary Aspects of Dicotyledon Wood*. Springer-Verlag, Berlin.
- Columbus, J. T. (1999). Morphology and leaf blade anatomy suggest a close relationship between *Bouteloua aristidoides* and *B. (Chondrosium) eriopoda* (Graminae: Chloridoideae). *Syst. Bot.* 23: 467 – 478.
- Daniel, T. F. (1990). New, recognised, and little-known Mexican species of *Ruellia* (Acanthaceae). *Contr. Univ. Mich. Herb.* 17: 139 – 162.
- Esau, K. (1953). *Plant Anatomy*. John Wiley & Sons, Inc., New York.
- Ezcurra, C. E. (1993). Systematics of *Ruellia* (Acanthaceae) in Southern America. *Ann. Missouri Bot. Gard.* 80: 787 – 845.
- Gal, A., Brumfeld, V., Weiner, S., Addadi, L. & Oron, D. (2012). Certain biominerals in leaves function as light scatterers. *Advanced Materials* 24: OP77 – O83.
- Holm, T. (1907). *Ruellia* and *Dianthera*: an anatomical study. *Bot. Gaz.* 43: 308 – 329.
- Inamdar, J. A., Chaudhari, G. S. & Ramana Rao, T. V. (1990). Studies on the cystoliths of Acanthaceae. *Feddes Repert.* 101: 417 – 424.
- Iyyappan, C. & Mounnissamy, V. M. (2011). Microscopical study of plant “*Dipteracanthus prostratus* Poir — Acanthaceae”. *Int. J. Pharm. Bio. Sci.* 2: 358 – 366.
- Kuo-Huang, L. L. & Yen, T. B. (1996). The development of lithocysts in the leaves and sepals of *Justicia procumbens* L. *Taiwania* 42: 17 – 26.
- Leroux, O. (2012). Collenchyma: a versatile mechanical tissue with dynamic cell walls. *Ann. Bot.* 110: 1083 – 1098.
- Lersten, N. S. (1997). Occurrence of endodermis with a casparian strip in stem and leaf. *Bot. Rev. (Lancaster)* 63: 265 – 272.
- Manktelow, M. (1996). *Phaulopsis* (Acanthaceae) — a monograph. *Symb. Bot. Upsal.* 31: 1 – 183.
- (2000). The filament curtain: a structure important to systematics and pollination biology in the Acanthaceae. *Bot. J. Linn. Soc.* 133: 129 – 160.
- McDade, L. A., Daniel, T. F., Masta, S. E. & Riley, K. M. (2000). Phylogenetic relationships within the tribe Justicieae (Acanthaceae): evidence from molecular sequences, morphology, and cytology. *Ann. Missouri Bot. Gard.* 87: 435 – 458.
- & Tripp, E. A. (2007a). Synopsis of Costa Rican *Ruellia* L. (Acanthaceae), with descriptions of four new species. *Brittonia* 59: 199 – 216.
- & ——, with assistance from T. F. Daniel (2007b). Acanthaceae of La Selva Biological Station, Costa Rica. In: *La Flora Digital de La Selva*. pdf available at <http://clade.acnatsci.org/mcdade/>
- , Daniel, T. F. & Kiel, C. A. (2008). Toward a comprehensive understanding of phylogenetic relationships among lineages of Acanthaceae s.l. (Lamiales). *Amer. J. Bot.* 95: 1136 – 1152.
- Metcalfe, C. R. & Chalk, L. (1950). *Anatomy of Dicotyledons* (First ed., Vol. 1). Oxford University Press, London.
- Monteiro, M. M. & Aoyama E. M. (2012). Effects of different light conditions on leaf anatomy of *Ruellia furcata* (Nees) Lindau (Acanthaceae). *Commun. Pl. Sci.* 2: 45 – 47.
- Moylan, E. C., Bennett, J. R., Carine, M. A., Olmstead, R. G. & Scotland, R. W. (2004a). Phylogenetic relationships among *Strobilanthes* s.l. (Acanthaceae): evidence from ITS nrDNA, *trnL-F* cpDNA, and morphology. *Amer. J. Bot.* 91: 724 – 735.
- , Rudall, P. J. & Scotland, R. W. (2004b). Comparative floral anatomy of Strobilanthinæ (Acanthaceae), with particular reference to internal partitioning of the flower. *Pl. Syst. Evol.* 249: 77 – 98.
- Muhaidat, R., McKown, A. D., Khateeb, W. A., Al-Shreideh, M., Domi, Z. B., Hussein, E. & El-Oqlah,

- A. (2012). Full assessment of C<sub>4</sub> photosynthesis in *Blepharis attenuata* Napper (Acanthaceae) from Jordan: evidence from leaf anatomy and key C<sub>4</sub> photosynthetic enzymes. *Asian J. Pl. Sci.* 2012: 1 – 11.
- Ocampo, G. & Columbus, J. T. (2010). Molecular phylogenetics of suborder Cactineae (Caryophyllales), including insights into photosynthetic diversification and historical biogeography. *Amer. J. Bot.* 97: 1827 – 1847.
- Okazaki, M., Setoguchi, H., Aoki, H. & Suga, S. (1986). Application of soft x-ray microradiography to observation of cystoliths in the leaves of various higher plants. *Bot. Mag. (Tokyo)* 99: 281 – 287.
- O'Neill, C. S. (2010). Anatomy of the shrimp plant, *Justicia brandegeana* (Acanthaceae). *Studies by Undergraduate Researchers at Guelph* 3: 41 – 47.
- Paopun, Y., Umrung, P., Thanomchat, P. & Kongpakdee, C. (2011). Epidermal surface and leaf anatomy of *Thunbergia laurifolia* Lindl. *J. Microsc. Soc. Thailand* 4: 61 – 64.
- Patil, A. M. & Patil, D. A. (2011). Occurrence and significance of cystoliths in Acanthaceae. *Curr. Bot.* 2: 1 – 5.
- & —— (2012). Petiolar anatomy of some hitherto unstudied Acanthaceae. *J. Exp. Sci.* 3: 5 – 10.
- Philpott, J. (1953). A blade tissue study of leaves of forty-seven species of *Ficus*. *Bot. Gaz.* 115: 15 – 35.
- Remadevi, S., Vinesh, R. & Binoj Kumar, M. S. (2006). Stem anatomy of Acanthaceae and its taxonomic significance. *J. Econ. Taxon. Bot.* 30: 453 – 468.
- Schmidt-Lebuhn, A. (2003). A taxonomic revision of the genus *Suessenguthia* Merxm. (Acanthaceae). *Candollea* 58: 101 – 128.
- & Tripp, E. A. (2009). *Ruellia saccata* (Acanthaceae), a new species from Bolivia. *Novon* 19: 515 – 519.
- Scotland, R. W. (1991). A systematic analysis of pollen morphology of Acanthaceae genera with contorted corollas. *Pollen & Spores* 44: 269 – 289.
- & Vollesen, K. (2000). Classification of Acanthaceae. *Kew Bull.* 55: 513 – 589.
- Setoguchi, H., Okazaki, M. & Suga, S. (1989). Calcification in higher plants with special references to cystoliths. Pp. 409 – 418 In: *Origin, Evolution, and Modern Aspects of Biomineralization in Plants and Animals*. Plenum Press, New York.
- Sharman, B. C. (1943). Tannic acid and iron alum with safranin and orange G in studies of the shoot apex. *Stain Technol.* 18: 105 – 111.
- Stanfield, R. (2013). *The physiological significance of calcium carbonate cystoliths: can these structures sequester heavy metals?* M.A. Thesis, California Polytechnic State University, Pomona.
- Tripp, E. A. (2004). The current status of *Ruellia* (Acanthaceae) in Pennsylvania: two endangered/threatened species. *Bartonia* 62: 55 – 62.
- (2007). Evolutionary relationships within the species-rich genus *Ruellia* (Acanthaceae). *Syst. Bot.* 32: 628 – 649.
- (2010). Taxonomic revision of *Ruellia* sect. *Chiropterophila* (Acanthaceae): a lineage of rare and endemic species from Mexico. *Syst. Bot.* 35: 629 – 661.
- , Daniel, T. F., Lendemer, J. C. & McDade, L. A. (2009). New molecular and morphological insights prompt transfer of *Blechnum* to *Ruellia* (Acanthaceae). *Taxon* 58: 893 – 906.
- , ——, Fatimah, S. & McDade, L. A. (2013a). Phylogenetic relationships within Ruellieae (Acanthaceae), and a revised classification. *Int. J. Pl. Sci.* 174: 97 – 137.
- & Dexter, K. G. (2012). Taxonomic novelties in Namibian *Ruellia* (Acanthaceae). *Syst. Bot.* 37: 1023 – 1030.
- & Fatimah, S. (2012). Comparative anatomy, morphology, and molecular phylogenetics of the African genus *Satanocrater* (Acanthaceae). *Amer. J. Bot.* 99: 967 – 982.
- , ——, Darbyshire, I. & McDade, L. A. (2013b). Origin of African *Physacanthus* (Acanthaceae) Via Wide Hybridization. *PLoS ONE* 8: e55677.
- & Koenemann, D. M. (in press). Nomenclatural synopsis of the genus *Sanchezia* (Acanthaceae). *Novon*.
- & Manos, P. S. (2008). Is floral specialization an evolutionary dead-end? Pollination system evolution in *Ruellia* (Acanthaceae). *Evolution* 62: 1712 – 1737.
- & McDade, L. A. (2012). New synonymies for *Ruellia* (Acanthaceae) of Costa Rica and notes on other neotropical species. *Brittonia* 64: 305 – 317.
- & —— (2014a). A rich fossil record yields calibrated phylogeny for Acanthaceae (Lamiales) and evidence for marked biases in timing and directionality of intercontinental disjunctions. *Syst. Biol.* 63 (5): 660 – 684.
- & —— (2014b). Time-calibrated phylogenies of hummingbirds and hummingbird-pollinated plants reject hypothesis of diffuse co-evolution. *Aliso* 31: 89 – 103.
- Vollesen, K. (2006). Taxonomic revision of the genus *Duosperma* (Acanthaceae). *Kew Bull.* 61: 289 – 351.
- Wasshausen, D. C. & Wood, J. R. I. (2003). The genus *Dyschoriste* (Acanthaceae) in Bolivia and Argentina. *Brittonia* 55: 10 – 18.
- Watt, W. M., Morrell, C. K., Smith, D. L. & Steer, M. W. (1987). Cystolith development and structure in *Pilea cadierei* (Urticaceae). *Ann. Bot.* 60: 71 – 84.
- Wu, C. C. & Kuo-Huang, L. L. (1997). Calcium crystals in the leaves of some species of Moraceae. *Bot. Bull. Acad. Sin.* 38: 97 – 104.