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Molecular Phylogeny and Pollen Evolution of Euphorbiaceae Tribe Plukenetieae

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Abstract—Tribe Plukenetieae (Euphorbiaceae, Acalyphoideae) is a pantropical lineage of mostly stinging, twining vines and lianas with diverse floral and pollen morphology. To elucidate generic relationships in the tribe and examine patterns of pollen morphology evolution, we conducted phylogenetic analyses of nuclear ribosomal ITS and plastid *psbA-trnH* DNA sequence and indel gap-scored data. We sampled all genera in subtribes Dalechampiinae and Tragiinae, and most in Plukenetiinae; species sampling was broad in the latter two subtribes. Our efforts produced a 2,207 character dataset of 154 terminals (representing ca. 93 species). Analyses of these data support the monophyly of each subtribe and weakly suggest Dalechampiinae (*Dalechampia*) is sister to Plukenetiinae + Tragiinae. Within Plukenetiinae, *Haematostemon* is resolved as sister to *Romanoa* + *Plukenetia*, and *Plukenetia* is divided into five subclades that mostly correspond to the current infrageneric classification. Tragiinae is resolved into an Old World lineage and a mostly New World lineage, and is divided into ten subclades also supported by floral and/or pollen morphology. Species-rich *Tragia* is recovered as para- or polyphyletic and intermixed with all other currently recognized Tragiinae genera. The recently segregated genera, *Bia*, *Ctenomeria*, and *Zuckertia*, are upheld, and *Gitara* is resurrected from *Acidoton*, resulting in two new combinations: ***Gitara nicaraguensis*** and ***Zuckertia manuelii***. Pollen aperture and exine morphology are largely correlated with phylogeny. The loss of pollen endopores is a potential synapomorphy of Plukenetiinae + Tragiinae, and we hypothesize that weakly defined apertures and inaperturate pollen originated independently four and three times, respectively.

Keywords—*Gitara*, internal transcribed spacer, *Plukenetia*, *psbA-trnH*, systematics, *Tragia*.

Tribe Plukenetieae (Benth.) Hutch. (Euphorbiaceae, Acalyphoideae) is a diverse pantropical lineage of ca. 17 genera and 350 species of twining vines and lianas, scandent to erect perennial herbs and subshrubs, and rarely shrubs and small trees (Gillespie 1994a; Webster 1994; Radcliffe-Smith 2001; Govaerts et al. 2015). Members of the tribe are distinguished in the family by their frequent twining habit, stinging hairs, and floral and pollen morphology. Taxa of economic or evolutionary interest that have been extensively studied include *Plukenetia volubilis* L. (Sacha Inchi), known for its omega-3 fatty acid oil-rich seeds (Wang et al. 2012), *Tragia involucrata* L., an urticant medicinal plant used in Ayurveda that has anti-microbial and anti-inflammatory properties (Perumal Samy et al. 2013), and *Dalechampia* Plum. ex L., known for its unique pseudanthial inflorescence and specialized pollination biology (Webster and Webster 1972; Webster and Armbruster 1991). Broader phylogenetic relationships in the tribe are poorly known outside of *Dalechampia* (Armbruster et al. 2009, 2013), and morphological, palynological, and molecular evidence suggests some genera are paraphyletic (Gillespie 1994a; Wurdack et al. 2005). Our paper presents the first comprehensively sampled molecular phylogeny of Plukenetieae and elucidates the generic relationships of its taxa and patterns of pollen morphology evolution.

Plukenetieae is differentiated in Acalyphoideae by the combination of apetalous flowers, valvate staminate sepal aestivation, unbranched styles, and frequent twining vine or liana habit (Webster 1994, 2014; Radcliffe-Smith 2001). Flowers are typically arranged in bisexual racemes or racemose thyrses with proximally located pistillate flowers (sometimes as a proximal pistillate branch), or in *Dalechampia* as a pseudanthial inflorescence of condensed unisexual pleiochasia subtended by two typically large involucral bracts (Fig. 1). Plukenetieae pollen is unusual in the subfamily for its considerable morphological variation. For example, aperture conditions range from tricolporate to tricolpate, weakly triporate, and inaperturate (Punt 1962; Gillespie 1994a; Nowicke and Takahashi 2002).

Plukenetieae is currently classified into subtribes Dalechampiinae (Müll. Arg.) G. L. Webster, Plukenetiinae Benth., and Tragiinae G. L. Webster (Webster 1994, 2014; Radcliffe-Smith 2001). Historically, Plukenetieae consisted of genera in Plukenetiinae and Tragiinae (Pax and Hoffmann 1919a, as Acalypheae subtribe Plukenetiinae; Hutchinson 1969; Webster 1975), but later included *Dalechampia* (previously in the monogeneric tribe Dalechampieae; Müller 1864, 1865; Pax and Hoffmann 1919b; Webster 1975) based on their shared twining habit, presence of stinging hairs, and elongate columnar styles (Webster 1994). The diagnostic characters of the three subtribes are given in Table 1.

Subtribe Dalechampiinae—Dalechampiinae is a monogeneric subtribe containing *Dalechampia* (Table 2), a pantropically distributed and species-rich genus (ca. 130 species) of clambering or twining vines and slender lianas, and in rare cases subshrubs. The genus is well known for its unique and specialized pseudanthial inflorescence (Fig. 1A), which contributes to a suite of resin-, fragrance-, and pollen-gathering insect pollination strategies (Armbruster 1984, 1993; Armbruster et al. 1989, 1992, 2009; Armbruster and Baldwin 1998).

Subtribe Plukenetiinae—Plukenetiinae is a small subtribe of five genera and ca. 27 species that can be subdivided into two informal groups: the small tree and shrub genera, *Astrococcus* Benth., *Angostylis* Benth., and *Haematostemon* Pax & K. Hoffm.; and the twining vine and liana genera, *Plukenetia* L. and *Romanoa* Trevis. (Table 2; Gillespie 1994a). Unlike the other subtribes, species of Plukenetiinae lack stinging hairs (Webster 1994, 2014). They are also characterized by simple unlobed leaves with basilaminar and/or scattered laminar glands, and diverse androecium and gynoecium morphology, particularly style shape and degree of connation (Gillespie 1993, 2007). *Plukenetia* (ca. 21 species) is the largest and only pantropically distributed genus in the

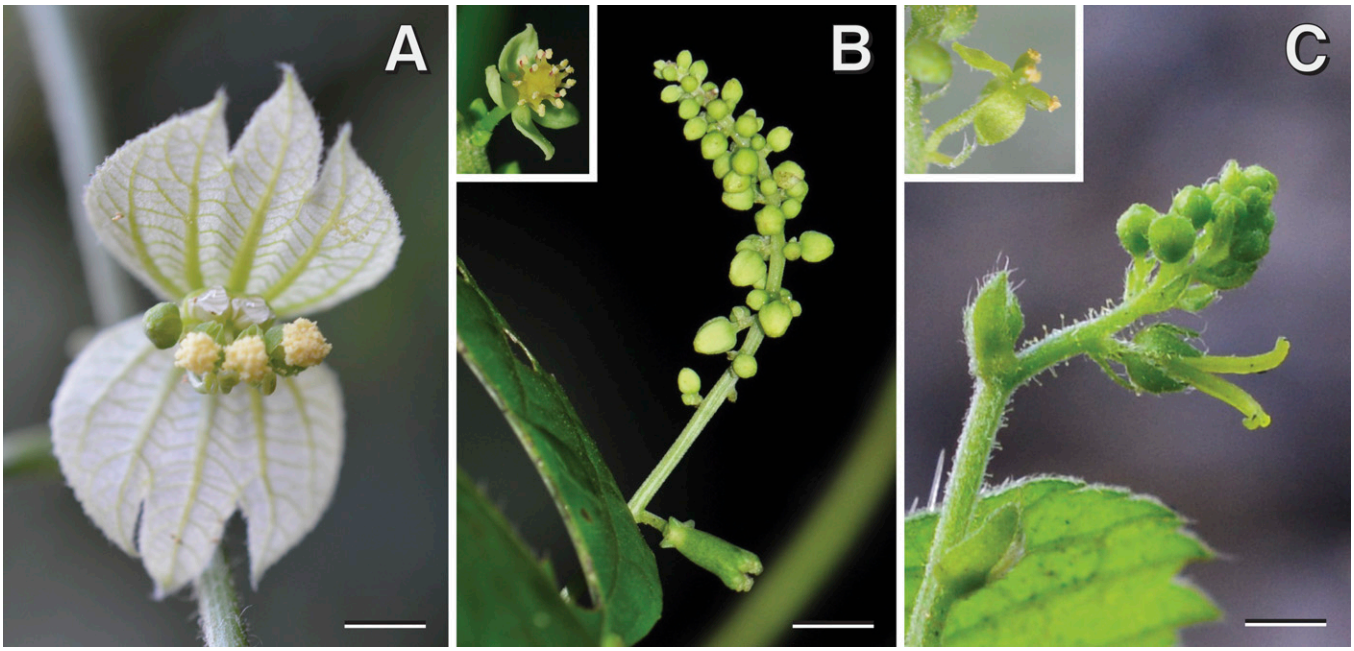

FIG. 1. Representative inflorescences for the subtribes of Plukenetieae. A. Dalechampiinae: pseudanthial inflorescence of *Dalechampia* sp. (*Medeiros and Cardinal-McTeague 562* R), composed of pistillate and staminate cymules and resiniferous glands subtended by two white involucral bracts (scale bar = 5 mm). B. Plukenetiinae: racemose thyrse of *Plukenetia stipellata* (*Cardinal-McTeague 8* CAN) with proximal pistillate flower and two- to three-flowered distal staminate cymules (scale bar = 5 mm). Inset, *Plukenetia volubilis* staminate flower. C. Tragiinae: raceme of *Tragia bahiensis* (*Medeiros and Cardinal-McTeague 561* R) with proximal pistillate flower and distal staminate flowers (scale bar = 3 mm). Inset, staminate flower.

subtribe (Table 2), and is unique in having four-carpellate ovaries and often winged or tuberculed fruits, compared to normally three-carpellate ovaries and unadorned fruits. *Plukenetia* was revised by Gillespie (1993, 2007) and includes three sections and two informal species groups (Table 3).

Subtribe Tragiinae—Tragiinae is the largest subtribe of Plukenetieae and comprises a diverse lineage of ca. 11 genera and 195 species (Table 2). Genera are characterized by their often-abundant stinging hairs and may be differentiated from other subtribes by the absence of stipels or laminar glands on their leaf blade bases (Table 1). Growth forms in Tragiinae are diverse and consist of scandent to erect herbs and subshrubs, twining vines, slender lianas, and rarely small to large shrubs (*Acidoton* Sw.). *Tragia* Plum. ex L. (ca. 150 species) is pantropically distributed and the sixth largest genus in Euphorbiaceae s. s. (sensu Wurdack et al. 2005; APG III 2009), following *Euphorbia* L., *Croton* L., *Acalypha* L., *Macaranga* Thouars, and *Jatropha* L. (Radcliffe-Smith 2001; Govaerts et al. 2015). The sectional classification of *Tragia* is presented in Table 3. Floral and pollen morphology suggest that *Tragia* is paraphyletic, with the other genera of Tragiinae embedded within it (Gillespie 1994a). Recently, three sections of *Tragia* were reinstated as genera, *Bia* Klotzsch (Webster 2007), *Zuckertia* Baill. (Medeiros et al. 2013), and *Ctenomeria* Harv. (Webster 2014), based on inferences from pollen morphology (Gillespie 1994a)

TABLE 1. Morphological characters for the subtribes of Plukenetieae.

Character	Dalechampiinae	Plukenetiinae	Tragiinae
Habit	Twining vines and slender lianas (subshrubs)	Twining vines and lianas (shrubs, small trees)	Twining vines and slender lianas, scandent or erect herbs and subshrubs (shrubs)
Stinging hairs	+	−	+
Leaves	Simple to palmately compound, unlobed to lobed	Simple, unlobed	Simple (palmately compound), unlobed to trilobed (deeply pinnately lobed)
Basilaminar or laminar leaf glands	−	+/−	−
Leaf stipels	+	+/−	−
Inflorescences	Pseudanthium of cymules subtended by two involucral bracts	Racemes, thyrses (fascicles)	Racemes (racemose thyrses with reduced cymules), sometimes with a proximal pistillate branch
Carpels	3	3 or 4	3
Styles	Entirely connate	Partly to entirely connate	Partly connate (entirely connate or mostly free)
Pollen shape	Subglobose to prolate	Suboblate to subglobose	Suboblate to globose
Pollen tectum	Reticulate	Perforate or reticulate	Perforate, rugulate, reticulate, or tectum absent
Pollen aperture	Tricolporate with endocingulate endopores	Tricolpate	Tricolpate (five-colpate), weakly tricolpate to triporate (irregularly aperturate), or inaperturate
Aperture margin	Even and smooth	Uneven or jagged (thickened)	Uneven or jagged

TABLE 2. Genera of tribe Plukenetieae sensu Webster (2014), with recognition of *Zuckertia* following Medeiros et al. (2013), and selected outgroups with total number of species, number of species sampled, and geographic distribution.

Taxon	Species number (species sampled)	Geographic distribution
Dalechampiinae		
Dalechampia Plum. ex L.	ca. 130 (4)	Pantropical (primarily New World)
Plukenetiinae		
Angostylis Benth.	1–2 (0)	Amazonian Brazil
Astrococcus Benth.	1 (0)	Amazonian Brazil and Amazonian Venezuela
Haematostemon Pax & K. Hoffm.	2 (1)	Guyana and Amazonian Venezuela
Plukenetia L.	ca. 21 (14)	Pantropical
Romanoa Trevis.	1 (1)	E Brazil, Paraguay, Bolivia
Tragiinae		
Acidoton Sw.	6 (2)	Central and South America, Hispaniola, and Jamaica
Bia Klotzsch	5 (2)	Costa Rica to South America
Cnesmone Blume	11 (4)	SE Asia
Ctenomeria Harv.	2 (1)	South Africa
Megistostigma Hook. f.	5 (2)	SE Asia
Pachystylidium Pax & K. Hoffm.	1 (1)	SE Asia
Platygyna P. Mercier	7 (1)	Cuba
Sphaerostylis Baill.	2 (1)	Madagascar
Tragia Plum. ex L.	ca. 150 (50)	Pantropical to warm temperate (primarily New World and Africa)
Tragiella Pax & K. Hoffm.	4 (3)	E and S Africa
Zuckertia Baill.	2 (1)	Mexico and Central America
Selected Outgroups		
Bernardia Houst. ex Mill.	ca. 70 (3)	North and South America
Caryodendron H. Karst.	4 (2)	Central and South America

and phylogenetic data (Wurdack et al. 2005). The remaining genera, *Acidoton*, *Cnesmone* Blume, *Megistostigma* Hook. f., *Pachystylidium* Pax & K. Hoffm., *Platygyna* P. Mercier, *Sphaerostylis* Baill., and *Tragiella* Pax & K. Hoffm., generally have been regarded as distinct, although their relationships within the suspected paraphyletic *Tragia* were unclear (Gillespie 1994a; Webster 1994, 2014).

Pollen Morphology Hypotheses—Pollen morphology is an informative taxonomic character in Euphorbiaceae and has been used extensively to guide the taxonomy of Plukenetieae (Webster 1975, 1994, 2007, 2014). Plukenetieae pollen is especially diverse in aperture condition and exine morphology (for pollen images see Punt 1962; Webster and Webster 1972; Gillespie 1994a, 1994b; Nowicke and Takahashi 2002) and is useful in differentiating among the subtribes (Table 1).

Interpreting pollen morphology variation has provided important hypotheses for the relationships of the Plukenetieae subtribes and genera (Gillespie 1994a). For example, the tricolporate aperture condition of *Dalechampia* is shared with the majority of genera in Acalyphoideae and is presumed to be plesiomorphic in the tribe. This suggests that Plukenetiinae plus Tragiinae is monophyletic and united by the absence of endopores (Gillespie 1994a). Aperture variation in Tragiinae provides hypotheses for the relationships of species groups

TABLE 3. Infrageneric classifications (including informal species groups) of *Plukenetia* (sensu Gillespie 1993, 2007) and *Tragia* (sensu Pax and Hoffmann 1919a, with modifications by: Miller and Webster 1967; Leandri 1971; Gillespie 1994b; Webster 2007, 2014). *Tragia* sects. *Leucandra* and *Ratiga* are regarded as synonyms of sect. *Tragia* (Miller and Webster 1967; Múlgura de Romero and Gutiérrez de Sanguinetti 1989), but are differentiated in our study for analytical purposes. Species circumscribed in *T.* sect. *Leptorhachis* (Klotzsch) Müll. Arg. (sensu Múlgura de Romero and Gutiérrez de Sanguinetti 1989) are included in sect. *Leucandra*. An informal group comprising the Australian species of *Tragia* is delineated here (previously considered in sect. *Leucandra*; Müller 1865; Pax and Hoffmann 1919a; Forster 1994).

Classification	Species number (species sampled)	Geographic distribution
Plukenetia	ca. 21 (14)	Pantropical
sect. *Angostylidium* Müll. Arg.	1 (1)	Tropical Central and West Africa
sect. *Hedraiostylus* (Hassk.) Müll. Arg.	3 (1)	S Africa and SE Asia
Madagascan species group	3 (2)	Madagascar
New World species group 2	7 (5)	Mexico to South America
sect. *Plukenetia*	7 (5)	Mexico to South America
Tragia	ca. 150 (50)	Pantropical to warm temperate (primarily New World and Africa)
sect. *Agirta* Baill.	5 (2)	Madagascar
Australian species group	3 (3)	Australia
sect. *Lassia* (Baill.) Müll. Arg.	2 (0)	Madagascar
sect. *Leucandra* (Klotzsch) Müll. Arg.	12 (3)	S U. S. A. to South America
sect. *Leptobotrys* (Baill.) Müll. Arg.	2 (2)	SE U. S. A.
subg. *Mauroya* Leandri	1 (0)	Madagascar
sect. *Monadelphae* L. J. Gillespie	1 (0)	Venezuela (Amazonas)
sect. *Ratiga* Müll. Arg.	5 (2)	Central to South America
sect. *Tagira* Müll. Arg.	82 (18)	Africa, Madagascar, S Asia
sect. *Tragia*	33 (20)	S U. S. A. to South America and Caribbean

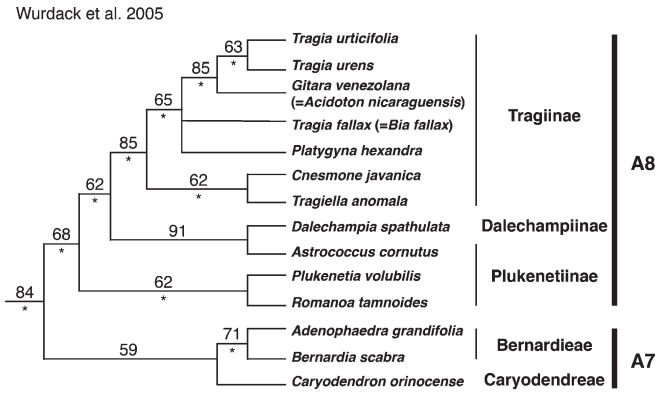

FIG. 2. Hypothesis for the relationships of Plukenetieae and its close relatives, Bernardieae and Caryodendreae, based on parsimony analysis of *trnL-F* data (previously the best taxon sampled dataset), modified from Wurdack et al. (2005). Numbers above branches are parsimony bootstrap percentages ≥ 50%; an asterisk (*) below branches indicates Bayesian posterior probabilities ≥ 95%.

and genera and strongly suggests that *Tragia* is paraphyletic (Gillespie 1994a). Specific hypotheses based on pollen morphology are addressed in the discussion.

Molecular Phylogenetic Hypotheses—Current molecular phylogenetic hypotheses for relationships in Plukenetieae are based on broad analyses of Euphorbiaceae, which sampled six to 11 representative species of the tribe (Wurdack et al. 2005; Tokuoka 2007). Although taxon sampling was limited, both studies strongly supported Plukenetieae as monophyletic and sister to tribes Bernardieae + Caryodendreae. Wurdack et al. (2005) also provided the first molecular evidence that *Tragia* is paraphyletic and that *Dalechampia* is embedded within Plukenetieae (Fig. 2).

Molecular phylogenetic analyses have also been conducted on *Dalechampia*, with a focus on evolutionary and ecological questions (Armbruster and Baldwin 1998; Armbruster et al. 2009, 2013). The most comprehensive phylogeny (Armbruster et al. 2009, 2013) recovered strong support for an early division in *Dalechampia*, resulting in two major lineages defined by the number of cymule branches in the male subinflorescence (four- vs. five-armed). Species relationships were mostly well resolved within each lineage, although their taxonomic significance or concordance with the sectional classification were not discussed.

In this paper, we present the first molecular phylogeny of Plukenetieae based on dense taxon sampling, with a focus on subtribes Plukenetiinae and Tragiinae, using DNA sequences of the nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) and plastid (cpDNA) *psbA-trnH* intergenic spacer regions, including *psbA-trnH* insertion/deletion (indel) gap-scored data. Our objectives are to (i) elucidate the relationships of the subtribes and genera of Plukenetieae, (ii) clarify generic circumscriptions and recommend taxonomic changes consistent with a phylogenetic classification based on molecular and morphological evidence, (iii) evaluate evolutionary hypotheses inferred from pollen morphology, and (iv) elucidate patterns of pollen aperture and exine evolution.

Materials and Methods

Taxon Sampling for Phylogenetic Analysis—We sampled a total of 154 accessions representing ca. 93 species of Plukenetieae and selected outgroups (taxonomy and voucher data are provided in Appendix 1). Sampling encompassed 16 species from three of five genera in Plukenetiinae (excluding *Astrococcus* and *Angostylis*, material not available) and 70 species from all 11 genera in Tragiinae, representing approximately 39% of their combined species diversity (Table 2). Sampling of the large genera *Plukenetia* and *Tragia* attempted to represent their sectional diversity and geographic distribution (Table 3). *Plukenetia* was sampled for 25 accessions (representing 14 species) and included at least one species from each of its three sections and two informal species groups. *Tragia* was sampled for 89 accessions (representing ca. 50 species) across seven sections/species groups (excluding *T.* sects. *Lassia* and *Monadelphae*, and *T.* subg. *Mauroya*, material not available). The remaining 14 genera were sampled for 31 accessions across 18 species (Table 2). In our study, sampling of Dalechampiinae was limited to four species of Madagascan *Dalechampia*, based on accessible material. We justify using only a few specimens given that the broader phylogeny of *Dalechampia* is already well known (Armbruster et al. 2009, 2013) and because the monophyly of the genus is strongly supported by its pseudanthial inflorescence synapomorphy. Five species of *Bernardia* Houst. ex Mill. (Bernardieae) and *Caryodendron* H. Karst. (Caryodendreae) were selected as outgroups to root the phylogeny, following the sister group relationships resolved by Wurdack et al. (2005).

DNA Extraction, Amplification, and Sequencing—Whole genomic DNA was extracted from herbarium or silica gel desiccated leaf material using a silica-based spin column method (Alexander et al. 2007) with a modified binding buffer (Starr et al. 2009). DNA was amplified on an Eppendorf EPGradientS Mastercycler using standard polymerase chain reaction (PCR) procedures; an initial denaturation period at 94°C for 3 min, then 34 cycles of (i) DNA denaturing at 94°C for 45 s, (ii) primer annealing at either 48°C (ITS) or 55°C (*psbA-trnH*) for 1 min, and (iii) polymerase extension at 72°C for 2 min (at 75% ramp-up speed), ending with a 5 min extension at 72°C. Most taxa were amplified in 15 μL reactions using Hot Start (HS) *Taq* DNA polymerase (BioShop, Burlington, Canada), with $MgCl_2$ concentrations optimized at 2.5 mM for ITS and 1.5 mM for *psbA-trnH*. HS reactions were supplemented with 1 M betaine (Sigma Aldrich, Oakville, Canada) for ITS and 0.27 mg/mL of bovine serum albumin (BioShop) for *psbA-trnH*, to improve amplification (Kreader 1996; Henke et al. 1997). Challenging samples were reattempted with Takara e2TAK DNA polymerase (Clontech Laboratories Inc., Mountainview, California) or *AccuPower Taq* PCR PreMix (Bioneer Inc., Alameda, California), following the manufacturer's instructions. The nrDNA ITS region (which in this study includes the partial 18S ribosomal RNA [rRNA] gene, the full ITS-1, 5.8S rRNA gene, and ITS-2 regions, and partial 26S rRNA gene) was amplified using KRC (Torrecilla and Catalán 2002) and AB102 (Douzery et al. 1999) primers, then sequenced using three primers, KRC, BMBCR (Lane et al. 1985), and ITS4 (White et al. 1990), to improve coverage. Samples that did not amplify at full length were reattempted in two shorter, overlapping regions using primer pairs BMBCR/ITS2 and ITS3/ITS4 (White et al. 1990). The cpDNA *psbA-trnH* intergenic spacer region was both amplified and sequenced using psbA (Sang et al. 1997) and $trnH^{GUG}$ primers (Tate and Simpson 2003; Shaw et al. 2005). PCR products were treated with an exonuclease I and shrimp alkaline phosphatase procedure (MJS Biolynx Inc., Brockville, Canada) followed by Sanger sequencing reactions with BigDye Terminator v3.1 chemistry (Applied Biosystems, Foster City, California). Sequence products were cleaned with a sodium acetate/ethanol precipitation then run on an ABI 3130*xl* Genetic Analyzer (Applied Biosystems) at the Laboratory of Molecular Biodiversity at the Canadian Museum of Nature. Sequence data were visualized, edited, and assembled using Geneious v6.1.5 (Biomatters Ltd., Auckland, New Zealand).

Nucleotide Alignments, Inversion Correction, Model Selection, and Gap Scoring—Sequences were aligned using the Geneious MAFFT plugin v7.017 (Katoh and Standley 2013) by implementing the auto-select algorithm with default parameters, followed by visualization and manual refinement in Geneious using a similarity criterion (Simmons 2004). The ITS alignment had high sequence divergence, numerous one to two base pair (bp) indels, and in general was most divergent among *Plukenetia* sequences. The *psbA-trnH* alignment had low sequence divergence and high indel variation. Irregular 29–59 bp stretches of unalignable non-homologous sequences were found in *psbA-trnH* accessions of *Plukenetia lehmanniana* (Pax and K. Hoffm.) Huft & L. J. Gillespie, and were excluded from our alignment. Also discovered was a 32 bp inversion in *psbA-trnH*, approximately 65–70 nucleotides downstream from the coding sequence of the *psbA* gene. Preliminary phylogenetic analyses indicated the inversion's conflicting sequence (relative to the alignment) was grouping 11 collectively unrelated terminals (Appendix 1)

into a long branched clade, suggesting the inversion originated in multiple lineages. Given that inversions are common in non-coding regions and known to be homoplasious in the stem loop of the *psbA* 3′ untranslated region (Štorchová and Olson 2007; Whitlock et al. 2010), we avoided the grouping of these samples by reverse-complementing the inverted sequence and treating the inversion event as a binary character (see Lehtonen et al. 2009 for additional discussion). Optimal models of molecular evolution for individual markers were determined using the Akaike information criterion (AIC; Akaike 1974) conducted through likelihood searches in jModeltest v2.1.4 at default settings (Darriba et al. 2012). Numerous indels in the *psbA-trnH* alignment were potentially phylogenetically informative and were gap scored using FastGap v1.2 (Borchsenius 2009). FastGap is an automated program that implements the "simple method" of gap scoring (Simmons and Ochoterena 2000) on large datasets and outputs the alignment with an appended binary matrix. The 32 bp inversion character was added to the *psbA-trnH* indel gap-scored matrix, and the Markov one-rate model (Mk1) was applied to the binary data during analyses (Lewis 2001). Data matrices are archived in the Dryad Digital Repository (http://datadryad.org).

Phylogenetic Analyses — Phylogenetic relationships were inferred using Bayesian inference (BI), maximum likelihood (ML), and maximum parsimony (MP) analyses. Prior to analyzing combined data, the ITS, *psbA-trnH*, and *psbA-trnH* + indel datasets were evaluated for incongruence using ML bootstrap analyses (Felsenstein 1985) in GARLI v2.0 (Zwickl 2006). For each of the three matrices, two independent searches were conducted for 500 bootstrap replicates, with models of molecular evolution set to GTR + I + Γ (the general time reversible model, with rate heterogeneity incorporated by calculating the proportion of invariant sites and modeling the rates of the remaining sites from a discrete approximation to a gamma distribution, as indicated by AIC in jModeltest) and Mk1 for binary data, allowing for parameter estimation and auto-optimization. Search runs were modified to terminate automatically after 1,000 generations without topological improvement or change in likelihood score (thresholds left at default). The best trees from each pseudoreplicate were summarized as 50% majority rule consensus trees in PAUP* v4.0b10 (Swofford 2002). The consensus trees for each dataset were then inspected for conflicting topologies using pairwise comparisons, with incongruence identified by branch conflicts with ≥ 85% maximum likelihood bootstrap percentage (MLBP). Since no supported topological conflicts were found, the remaining analyses were conducted on combined data, partitioned by ITS, *psbA-trnH*, and indel datasets.

Bayesian Markov chain Monte Carlo (MCMC) analyses were conducted in MrBayes v3.2.2 (Ronquist et al. 2012) on combined partitioned data, allowing for independent model estimation. Two independent runs of eight-chained searches were performed for 50 million generations, sampling every one-thousandth generation. The temperature factor was set to 0.025 (reduced from 0.2) to promote mixing between chains, while remaining parameters were left at default settings. Searches reached completion with an average standard deviation of split frequencies at 0.013131. To ensure independent runs had converged, we verified that potential scale reduction factors (PSRF) were close to 1.0 and that effective sample size (ESS) values of each parameter were > 2,000, as determined by Tracer v1.6 (Rambaut et al. 2014). A 10% burn-in was implemented before summarizing a maximum clade credibility tree and calculating Bayesian posterior probabilities (BPP).

Branch support was also assessed under MP and ML criteria using non-parametric bootstrapping. Parsimony analyses were conducted in PAUP* on a concatenated dataset with characters treated as unordered and equally weighted (Fitch 1971). One thousand bootstrap replicates were employed, each with 10 random-addition replicates, applying tree-bisection-reconnection (TBR) swapping, saving multiple shortest trees each step (Multrees), and with each random-addition replicate limited to 1,000 trees. Maximum likelihood bootstrapping was implemented in GARLI for 1,000 pseudoreplicates on combined and partitioned data with individually estimated models. Two independent searches were initiated from randomly assembled trees (changed from stepwise) and terminated after 2,000 generations with a stable topology or likelihood score (thresholds remained at default).

Pollen Morphology Terminology — Pollen aperture and exine terminology follows Walker and Doyle (1975), with Plukenetieae specific aperture terms from Gillespie (1994a) and general pollen morphology terms from Punt et al. (2007). Exine terminology varies among authors (e.g. Gillespie 1994a; Nowicke and Takahashi 2002) and is defined here to avoid confusion. Exine is the outer wall of a pollen grain and is composed of the foot layer (or nexine), columellae, and, usually, an upper roof of tectum; exine with a tectum is called tectate. Exine in Plukenetieae can be tectate-perforate (tectate with perforations less than the width of the adjoining unbroken tectum), semitectate (tectate with perforations greater than width of the intervening tectum), or intectate (without an upper roof of tectum and with columellae exposed). Tectal perforations can be described as foveolate (circular perforations ca. 0.5–1.5 μm diam; intermediate between punctate and foveolate according to Punt et al. 2007), punctate (minute circular perforations < 0.2 μm diam, following Gillespie 1994a), or fossulate (irregularly shaped grooves), or the tectum can be rugulate (with perforations between elongate and irregularly bent tectal elements called rugae). Semitectate exine is primarily characterized by reticulate patterning, with enlarged tectal perforations called lumina and the tectal reticulum called muri. Following Gillespie (1994a), we describe semitectate exine as coarsely reticulate if lumina are > 1μm, and finely reticulate if lumina are < 1μm (defined as microreticulate by Punt et al. 2007). In Plukenetieae, intectate exine is described as baculate (cylindrical rod-shaped) or clavate (club-shaped) columellae.

Results

Data Set Characteristics and Congruence — The combined matrix comprises 2,207 characters, of which 789 are variable and 635 are parsimony informative; the ITS, *psbA-trnH*, and *psbA-trnH* indel gap-scored partitions had aligned lengths of 1,000 bp, 1,038 bp, and 169 characters, respectively (Table 4). The ITS, *psbA-trnH*, and *psbA-trnH* + indel datasets produced similar tree topologies and did not recover strongly supported conflicts (≥ 85% MLBP) in incongruence assessments. Thus, the ITS and *psbA-trnH* + indel datasets were combined in remaining analyses. Tree topologies of combined data MP, ML, and BI analyses were congruent and did not have strongly supported clade conflicts. Bayesian and ML trees were better resolved and had higher support values than the MP tree.

Phylogenetic Reconstructions — The Bayesian maximum clade credibility tree is presented with MP, ML, and BI support values in Figs. 3A and 3B. Evidence of strong branch support was interpreted as ≥ 85% maximum parsimony

Table 4. Characteristics for individual and combined DNA sequence (ITS, *psbA-trnH*) and *psbA-trnH* indel gap-scored (including one hand-scored *psbA-trnH* inversion character) data sets.

	ITS	*psbA-trnH*	*psbA-trnH* indels	Combined ITS + *psbA-trnH* + *psbA-trnH* indels
Genome	nrDNA	cpDNA	cpDNA	Mixed
Number of accessions	143	138	138	154
Aligned length	1,000	1,038	169	2,207
Average length unaligned	855	544	n/a	n/a
% Missing data	4.07	0.57	n/a	10.87
Constant characters	526	883	0	1,409
Variable characters	474	155	169	798
Parsimony-informative characters (%)	429 (42.9%)	102 (9.8%)	104 (61.5%)	635 (28.8%)
Implemented model of evolution	GTR + I + Γ	GTR + I + Γ	Mk1	Partitioned

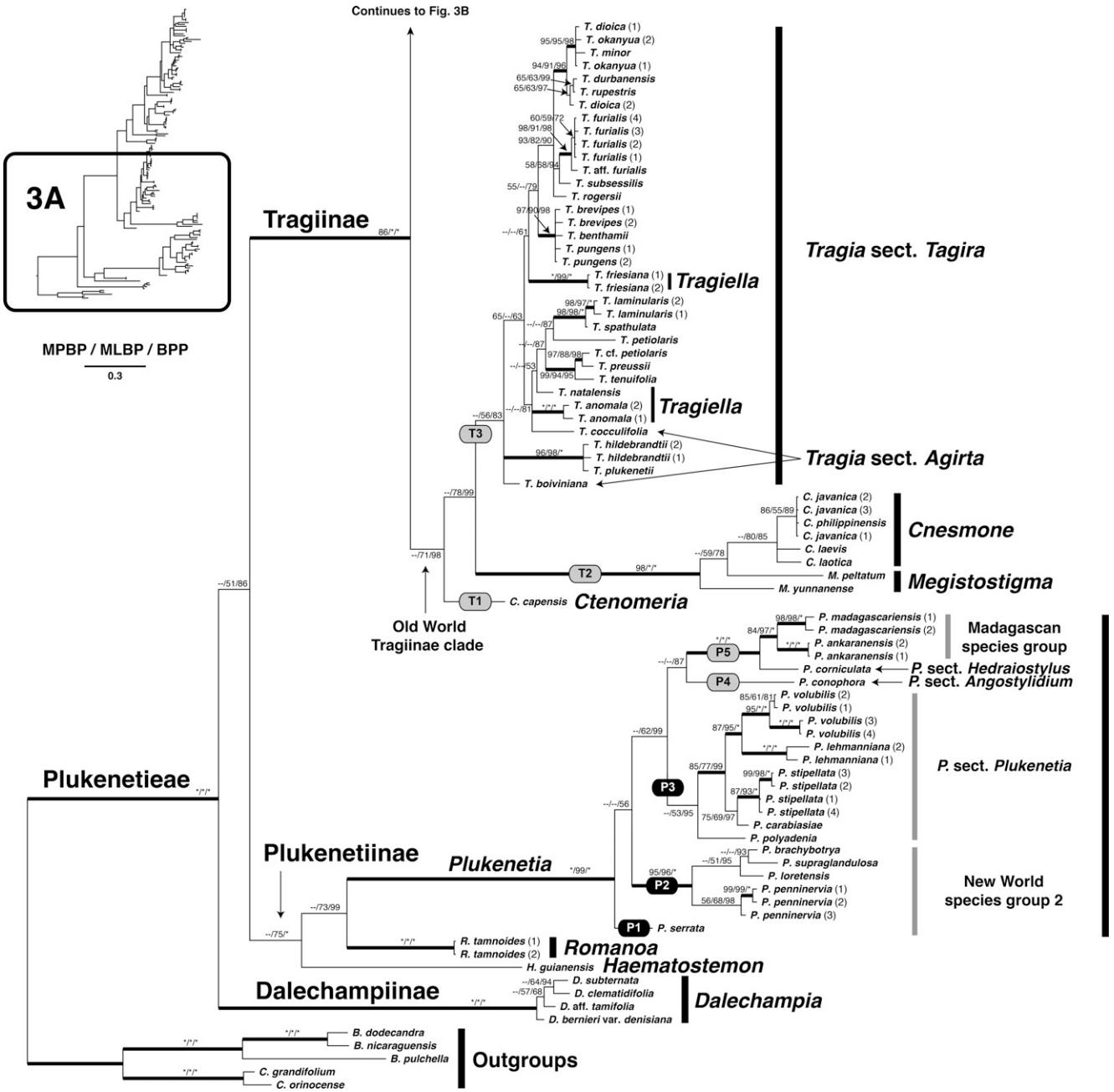

FIG. 3A. Bayesian inference (BI) maximum clade credibility tree based on combined and partitioned ITS, *psbA-trnH*, and indel data of Plukenetieae and selected outgroups. Support values > 50% are indicated on each branch for maximum parsimony (MP) and likelihood (ML) bootstrap analyses, and BI Markov chain Monte Carlo (MCMC) analysis, respectively (* indicates support values of 100%). Branches with strong support, interpreted as ≥ 85% MP and ML bootstrap percentages (MPBS and MLBS) and ≥ 95% Bayesian posterior probabilities (BPP), are in bold. Clades with Old World distribution are indicated by numbered grey boxes, clades with New World distribution by black boxes. Continued in Fig. 3B.

bootstrap percentage (MPBP) and MLBP, and ≥ 95% BPP, and is indicated by bold branches on the phylogeny.

All three subtribes were resolved as monophyletic, except in MP results, where genera of Plukenetiinae were collapsed into a polytomy with Tragiinae and Dalechampiinae. In ML and BI analyses, Dalechampiinae was resolved as the earliest diverging lineage and was sister to a poorly supported clade of Plukenetiinae + Tragiinae (Fig. 3A; MLBP = 51, BPP = 86).

Plukenetiinae and its genera were recovered as monophyletic (Fig. 3A; MLBP ≥ 75, BPP ≥ 99), although basal nodes of the subtribe were collapsed in MP trees. *Haematostemon* was resolved as the earliest diverging lineage and was sister to a moderately supported clade of *Romanoa* + *Plukenetia* (Fig. 3A; MLBP = 73, BPP = 99). *Plukenetia* was resolved into five subclades (P1–P5), which largely correlated with taxonomic section/species group concepts. Subclades were mostly strongly supported, although backbone support was poor (Fig. 3A). Subclades P1 and P2, together corresponding to New World species group 2, resolved in a functional polytomy with a weakly supported lineage of subclades P3–P5. The latter lineage included *P.* sect. *Plukenetia* (P3), *P.* sect. *Angostylidium* (P4), and *P.* sect. *Hedraiostylus* + the Madagascan species group (P5). Species relationships within

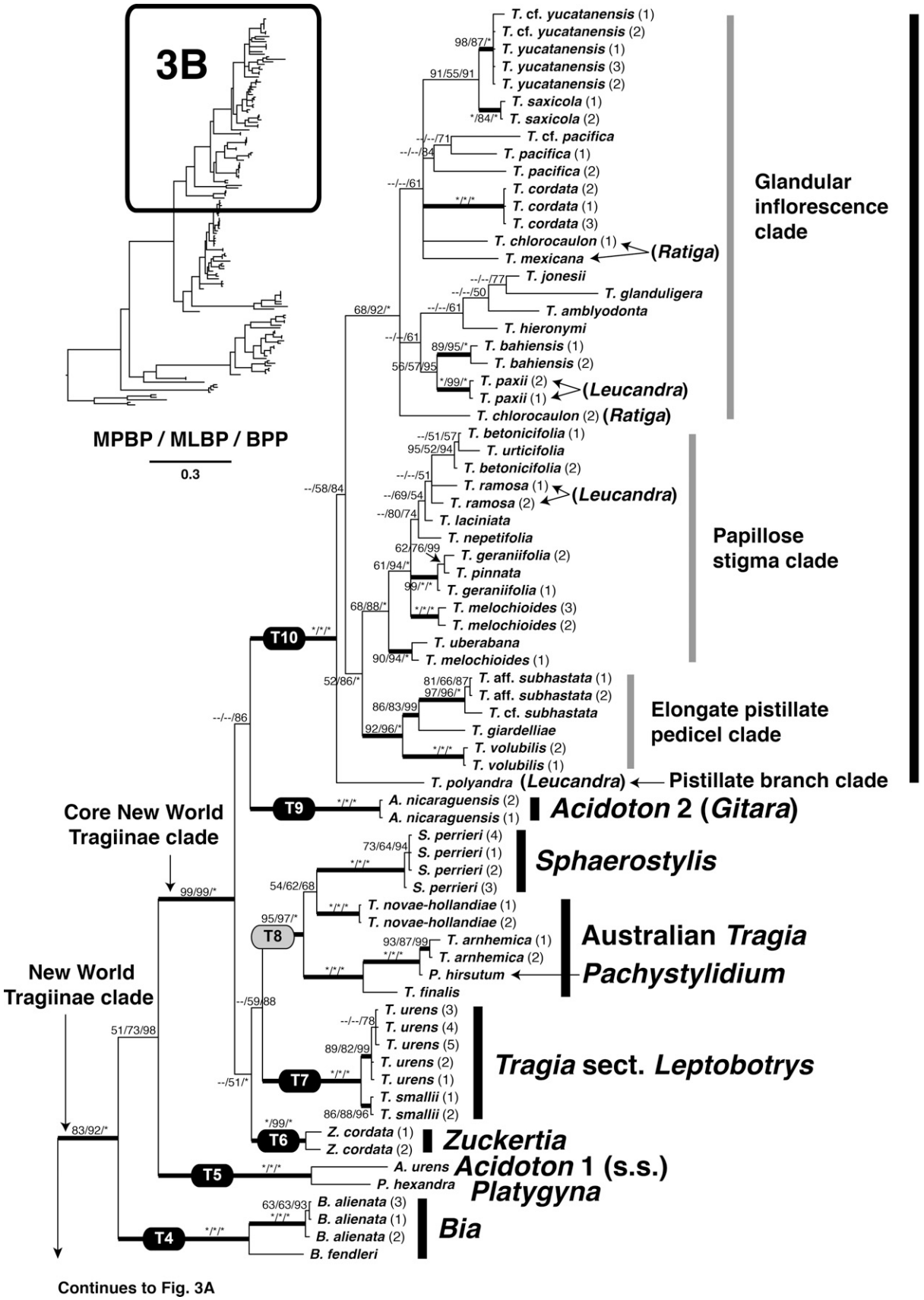

FIG. 3B. Continuation of Fig. 3A. Bayesian inference (BI) maximum clade credibility tree based on combined and partitioned ITS, *psbA-trnH*, and indel data of Plukenetieae and selected outgroups. Support values > 50% are indicated on each branch for maximum parsimony (MP) and likelihood (ML) bootstrap analyses, and BI Markov chain Monte Carlo (MCMC) analysis, respectively (* indicates support values of 100%). Branches with strong support, interpreted as ≥ 85% MP and ML bootstrap percentages (MPBS and MLBS) and ≥ 95% Bayesian posterior probabilities (BPP), are in bold. Clades with Old World distribution are indicated by numbered grey boxes, clades with New World distribution by black boxes.

the subclades had mostly strong support, except in subclade P2 (Fig. 3A).

Tragiinae was resolved as a monophyletic lineage with strong support (Fig. 3A; MPBP = 86, MLBP/BPP = 100), with the smaller genera nested throughout a para- and/or polyphyletic *Tragia*. Across the subtribe, ten subclades (T1–T10) were resolved with strong support (Figs. 3A, 3B), with the exception of subclade T3 (Fig. 3A; MLBP = 56, BPP = 83). These subclades can be divided into two lineages based on an early split in the subtribe: (i) an exclusively Old World clade comprised of T1–T3 (Fig. 3A; MLBP = 71, BPP = 98); and (ii) a primarily New World clade comprised of T4–T10 (Fig. 3B; MPBP = 83, MLBP = 92, BPP = 100).

Subclade relationships in the Old World Tragiinae clade (T1–T3) had moderate support (Fig. 3A; MLBP =78 BPP = 99), and suggest that T1 (*Ctenomeria*) was the earliest diverging lineage and sister to a clade of T2 (*Cnesmone* and *Megistostigma*) + T3 (*Tragia* sects. *Agirta* and *Tagira*, and *Tragiella*). Species relationships in subclades T2 and T3 had poor support.

The backbone topology of the mainly New World Tragiinae clade (T4–T10) had mostly strong support (Fig. 3B) and included a basal grade of T4 (*Bia*) and T5 (*Acidoton* group 1 + *Platygyna*), and a strongly supported clade of T6–T10, which we refer to as the core New World Tragiinae. Relationships in the core New World clade were poorly supported, but included two groupings. The first, supported in Bayesian analyses (Fig. 3B; MLBP = 51, BPP = 100), included T6 (*Zuckertia*), T7 (*Tragia* sect. *Leptobotrys*), and T8 (an Old World clade including *Pachystylidium*, *Sphaerostylis*, and Australian *Tragia*). The second, a functional polytomy (Fig. 3B; BPP = 86), included T9 (*Acidoton* group 2) and T10 (*T.* sect. *Tragia*, including *T.* sects. *Leucandra* and *Ratiga*). Species relationships in subclade T10 were well resolved, although most internal nodes were not strongly supported (Fig. 3B). *Tragia polyandra* Vell. was recovered as the earliest diverging species, and the remaining species resolved into three groups: (i) a strongly supported clade inclusive of *T. volubilis* (1) to *T.* aff. *subhastata* (1); (ii) a moderately supported clade (MPBP = 68, MLBP = 88, BPP = 100) inclusive of *T. melochioides* (1) to *T. betonicifolia* (1); and (iii) a moderately supported clade (MPBP = 68, MLBP = 92, BPP = 100) inclusive of *T. chlorocaulon* (2) and *T.* cf. *yucatanensis* (1) (Fig. 3B).

Discussion

The results presented here are the first comprehensively sampled phylogenetic analyses of Plukenetieae. The phylogeny of the tribe is largely consistent with current classifications (Table 2) and supports the monophyly of the three subtribes and most genera (excluding *Acidoton*, *Megistostigma*, *Tragia*, and *Tragiella*). Hypotheses that the smaller Tragiinae genera would be embedded within a paraphyletic *Tragia* (Gillespie 1994a; Webster 1994) are supported.

Here, we interpret the phylogeny and discuss taxonomic implications for generic and sectional circumscriptions in the tribe. We also propose minor taxonomic changes aimed towards a phylogenetic classification in Plukenetieae. A major generic revision is forthcoming and will incorporate the results of broadened molecular and morphological investigations. We also examine patterns of pollen aperture and exine evolution in the context of our phylogeny.

Tribal and Subtribal Relationships—The monophyly of Plukenetieae remained strongly supported with increased taxon sampling, although our outgroup selection is currently limited and biased toward this conclusion. While the relationships of Plukenetieae and Bernardieae + Caryodendreae have been strongly supported (Wurdack et al. 2005; Tokuoka 2007), it would be prudent to test this hypothesis with other putative close relatives, such as Adelieae and some genera of Acalypheae and Chrozophoreae (subclade A6, Wurdack et al. 2005). Frequent twining habit is one of the strongest synapomorphies of Plukenetieae, differentiating it from our sampled close relatives, *Bernardia* and *Caryodendron*, and other Acalyphoideae, which are shrubs, trees, and rarely herbs (Radcliffe-Smith 2001; Webster 1994, 2014).

The subtribes of Plukenetieae were monophyletic, with strong support for Dalechampiinae and Tragiinae, and moderate support for Plukenetiinae. Although our taxon sampling of Dalechampiinae is sparse (4 of ca. 130 species) and geographically limited to Madagascar, prior studies with much greater taxon sampling indicate its monophyly (Armbruster et al. 2009, 2013), as does its unique pseudanthial inflorescence. The strongly supported relationship of *Dalechampia* (Dalechampiinae) + *Astrococcus* (Plukenetiinae) embedded within the tribe (Fig. 1; Wurdack et al. 2005, Fig. 3) previously suggested Plukenetiinae was paraphyletic. In contrast, we found moderate support for a monophyletic Plukenetiinae (MLBS = 75; BPP =100), but cannot attest to the relationship of *Dalechampia* with *Astrococcus* since the latter was not sampled in our study (however, see the section below on Plukenetiinae small tree and shrub genera for further discussion of *Astrococcus*).

Relationships of the subtribes are currently poorly supported (MLBS = 51; BPP = 86) but suggest that Dalechampiinae is sister to Plukenetiinae + Tragiinae. This relationship agrees with pollen aperture hypotheses (Gillespie 1994a) that suggest Plukenetiinae and Tragiinae form a lineage based on the shared loss of endopores and gain of uneven/jagged aperture margins. Previous studies recovered part of Plukenetiinae (*Plukenetia* and *Romanoa*) as the earliest diverging lineage with moderate to low support (Fig. 1; Wurdack et al. 2005; Tokuoka 2007), but this may be an artifact of limited taxon and molecular sampling. Clarifying the relationships of the subtribes will be important to understanding character evolution in the tribe, particularly for the evolution of twining habit, stinging hairs, and *Dalechampia*'s pseudanthial inflorescence.

Generic Monophyly—Most Plukenetieae genera were recovered as monophyletic, including all genera sampled in Dalechampiinae (*Dalechampia*) and Plukenetiinae (*Haematostemon*, *Plukenetia*, and *Romanoa*). These results support the synonymization of *Eleutherostigma* (= *P. lehmanniana*) and *Vigia* (= *P. serrata*) with *Plukenetia* (Gillespie 1993), and reinforce the broadened circumscription of *Plukenetia* (Gillespie 1993, 2007). In Tragiinae, *Bia*, *Cnesmone*, *Sphaerostylis*, and *Zuckertia*, each resolved as a clade, but were nested throughout a large para-/polyphyletic *Tragia*, as were *Ctenomeria* and *Platygyna*, which were sampled for one accession each. Of the remaining genera, *Acidoton* was polyphyletic with species resolved in distant subclades T5 and T9, *Megistostigma* was a paraphyletic grade at the base of *Cnesmone* in subclade T2, and *Tragiella* was non-monophyletic and embedded within a poorly resolved paraphyletic *Tragia* sect. *Tagira* in subclade T3. *Pachystylidium* was sampled for one accession and was embedded within a paraphyletic group of Australian *Tragia* species in subclade T8.

These results support previous hypotheses that *Tragia* is paraphyletic and in need of revision (Gillespie 1994a; Webster 1994, 2014; Radcliffe-Smith 2001; Wurdack et al. 2005).

***Plukenetiinae*—**Plukenetiinae can be tentatively subdivided into a small tree and shrub group (*Haematostemon*) and a twining vine and liana group (*Plukenetia* and *Romanoa*) (Gillespie 1993, 1994a). It will be essential to include the other rare small tree and shrub genera, *Angostylis* and *Astrococcus*, in order to fully establish generic relationships in Plukenetiinae.

Plukenetiinae Small Tree and Shrub Genera—Our only sample of the Plukenetiinae small tree and shrub genera is *Haematostemon*, a rare genus with two species (one sampled here) from Amazonian Venezuela and Guyana (Pax and Hoffmann 1919a; Webster 2014). Among the small tree and shrub genera, *Astrococcus* and *Haematostemon* share four-parted staminate flowers and a unique pollen type (Gillespie 1994a), suggesting a close relationship. Our analyses resolved *Haematostemon* at the base of Plukenetiinae, sister to *Plukenetia* + *Romanoa* (Fig. 3A). Wurdack et al.'s (2005) analyses included only *Astrococcus* and resolved it in a clade with *Dalechampia* (Fig. 1; MPBS < 50, BPP = 91 based on *trnL-F*; MPBS = 94, BPP = 100 based on *rbcL* and *trnL-F*), which suggests there may be a discrepancy with the phylogenetic position of *Astrococcus*/*Haematostemon* between our studies. ITS data of *Astrococcus* (K. Wurdack, unpublished data) shares 92% sequence identity with *Haematostemon* compared with 72% with *Dalechampia spathulata* (data not shown), which suggests *Astrococcus* would likely resolve with *Haematostemon* if included in our analyses. *Angostylis*, a rare genus known only from a few collections, appears to be less derived than *Astrococcus* and *Haematostemon* in possessing numerous stamens (ca. 20 compared to four) and pollen that lacks thickened aperture margins and elongate exine chambers characteristic of the other two genera (Gillespie 1994a). The small tree and shrub genera are united by habit, pinnately veined oblanceolate leaves, and finely foveolate-rugulate pollen tectum (Gillespie 1994a), which suggests they form a natural group.

Plukenetiinae Twining Vine and Liana Genera—*Plukenetia* and *Romanoa* form a moderately supported clade in Plukenetiinae, united by twining vine and liana habit. *Romanoa* contains a single species distributed in eastern Brazil, Bolivia, and Paraguay (Radcliffe-Smith 2001; Jorgensen et al. 2014; Webster 2014) and is differentiated from *Plukenetia* by pistillate flowers with five sepals and three carpels (Gillespie 1993). By comparison, *Plukenetia* is a diverse pantropical genus with ca. 21 species (14 sampled here), and is distinct within the tribe in having pistillate flowers with four sepals and four carpels (Gillespie 1993, 2007; Webster 2014). Both genera have tricolpate pollen with jagged or uneven aperture margins, although irregularly foveolate and finely reticulate exine is specific to *Romanoa*, whereas *Plukenetia* has foveolate or coarsely reticulate exine (Gillespie 1994a).

***Plukenetia (Subclades P1–P5)*—**Subclade relationships within *Plukenetia* were weakly supported but hinted at three general groupings correlated with morphology and geographic distribution. The first is an unresolved, early diverging group corresponding to New World species group 2 (subclades P1 and P2) that is characterized by coarsely reticulate pollen exine (Gillespie 1994a). The remaining species form a weakly supported clade (MLBP = 62, BPP = 99) united by foveolate pollen exine that includes New World *P.* sect. *Plukenetia* (subclade P3) and the Old World *Plukenetia* lineages (subclades P4 and P5).

Plukenetia Subclade P1—Subclade P1 consists of *Plukenetia serrata*, a morphologically distinctive species found in southeast Brazil. Historically, *P. serrata* was accepted as a distinct genus, initially as *Fragariopsis scandens* A. St.-Hil., subsequently as the earlier described *Vigia serrata* Vell. (Webster 1994; Radcliffe-Smith 2001), based on having sessile anthers on an enlarged globose receptacle and fleshy fruits (Pax and Hoffmann 1919a). However, this taxon was combined with *Plukenetia* because these supposedly distinguishing androecial and fruit characteristics are found in other *Plukenetia* species (Gillespie 1993). Molecular evidence provides strong support that *P. serrata* belongs in *Plukenetia*, although its position is poorly supported and unresolved. Pinnate leaf venation, sessile anthers, entirely connate styles, and coarsely reticulate pollen exine strongly associate *P. serrata* with New World species group 2, although fleshy fruits, enlarged staminate receptacles, presence of leaf stipels, and several pistillate flowers (up to 10 per inflorescence vs. one) differentiate *P. serrata* from the other members of the group (Gillespie 1993).

Plukenetia Subclade P2—Subclade P2 includes the remaining members of New World species group 2 (six species excluding *P. serrata*, four sampled here), which are distributed from southern Mexico to Brazil and Bolivia (Gillespie 1993). They are differentiated from the other New World group, *P.* sect. *Plukenetia* (subclade P3), by mostly elliptic, pinnately veined leaves (cordiform and three-nerved at the base in *P. verrucosa* Smith; not sampled); sessile anthers (all or with an outer whorl of four to five stamens with filaments); entirely connate, columnar or globose styles; exclusively dry capsular fruits; and coarsely reticulate pollen tecta (Gillespie 1993, 1994a).

Plukenetia Subclade P3—Subclade P3 was only moderately supported but includes the strongly circumscribed *Plukenetia* sect. *Plukenetia* (seven species, five sampled here). Species of *P.* sect. *Plukenetia* are distributed from Mexico and the Lesser Antilles to Bolivia and Brazil and are differentiated by mostly cordate and palmately veined leaves (sometimes broadly ovate or three-nerved at the base), stamens with well-developed filaments, styles only partially fused into a cylindrical column, and pollen with foveolate exine (Gillespie 1993). Species relationships in subclade P3 (Fig. 3A) do not support the predicted close relationship of *P. stipellata* and *P. volubilis* (Gillespie 1993) and suggest that large, fleshy indehiscent fruits are not a synapomorphy of *P. lehmanniana* and *P. polyadenia*.

Plukenetia subclade P4—Subclade P4 contains *Plukenetia conophora* Müll. Arg. (the sole member of *P.* sect. *Angostylidium*), a distinctive species from tropical Central and West Africa traditionally cultivated for its oil-rich seeds. Morphologically, it is most similar to species of New World *P.* sect. *Plukenetia* in sharing stamens with well-developed filaments, partially connate cylindrical styles, and large indehiscent fruits (Gillespie 2007), although these similarities are possibly plesiomorphic for the P3–P5 clade.

Plukenetia subclade P5—Subclade P5 is a strongly supported lineage including *Plukenetia* sect. *Hedraiostylus* and the informal Madagascan species group. *Plukenetia* section *Hedraiostylus* contains three species (one sampled here) distributed in southern Africa (*P. africana* Sond. and *P. procumbens* Prain) and Southeast Asia (*P. corniculata* Sm.). They are differentiated by short styles (less than or equal to the length

of their ovaries) and small capsular fruits with lenticular seeds (Gillespie 2007). The Madagascan species group includes three species (two sampled here) that share an interesting combination of morphological characters found elsewhere in *Plukenetia*, for example, sessile anthers (similar to New World species group 2, except on an elongate rather than globose receptacle), medium sized fruits (intermediate between *P.* sects. *Angostylidium* and *Hedraiostylus*) with subglobose seeds, and ovate to suborbiculate leaf blades with three nerves at the base to weakly palmate venation (shared with most sections except New World species group 2) (Gillespie 1993, 2007). Although the Madagascan species group exhibits substantial interspecies variation, it seems to be united by elongate staminate receptacles (Gillespie 2007).

Tragiinae (Subclades T1–T10)—Tragiinae is divided into two lineages that correspond with geographic distribution, the Old World Tragiinae clade (subclades T1–T3) and the mostly New World Tragiinae clade (subclades T4–T10); these lineages have not been previously recognized, although they were recovered by Wurdack et al. (2005; Fig. 1). The resolution of a group of Old World species (subclade T8) within the New World Tragiinae clade was an unexpected discovery, and suggests that Tragiinae underwent multiple dispersal and/or migration events between the New and Old World regions.

Tragiinae Subclade T1—Subclade T1 contains *Ctenomeria*, a recently resurrected genus with two species (one sampled here) distributed in the east coast of southern Africa (Webster 2014). *Ctenomeria* was previously treated as a section of *Tragia* (Pax and Hoffmann 1919a), and can be distinguished from other Old World *Tragia* by numerous stamens (30–50), mostly free styles with papillose adaxial stigmatic surfaces, and pollen morphology. Pollen of *Ctenomeria* is weakly tricolpate with a finely and irregularly foveolate-reticulate tectum that extends continuously over an often-depressed and poorly defined colpus denoted by thinner sexine, and is unique in Plukenetieae (Gillespie 1994a).

Tragiinae Subclade T2—Subclade T2 unites the Southeast Asian genera *Cnesmone* (11 species, four sampled here) and *Megistostigma* (five species, two sampled here), which are distinguished from other Tragiinae genera by their cup-like staminate calyx tube and stout stamens with enlarged apiculate anther connectives (Webster 2014). They differ from each other principally in style morphology, with thick, nearly free styles and papillose adaxial stigmatic surfaces in *Cnesmone*, and massively globose or clavate styles and non-papillose stigmas in *Megistostigma* (Airy Shaw 1969; Webster 1994; Qiu and Gillespie 2008). Both genera have weakly tricolpate pollen with apertures covered with dense sexine islands, which is a unique combination in Tragiinae (Gillespie 1994a). However, pollen of *Megistostigma* has additional variability, ranging from weakly tricolpate to irregularly aperturate, and sometimes inaperturate, and often exhibits a combination of these aperture types within a single specimen (Gillespie 1994a). Our results suggest that *Megistostigma* is paraphyletic, which supports previous doubts about the delineation of *Cnesmone* and *Megistostigma* primarily based on differences in style morphology (Gillespie 1994a; L. J. Gillespie, unpubl. data). Additional species should be sampled before deciding whether or not to combine *Megistostigma* under the earlier described genus *Cnesmone*. *Pachystylidium* was thought to be a close relative of *Cnesmone* and *Megistostigma* based on similarities in pollen morphology and Indomalaysian distribution (Gillespie 1994a), but instead resolved in the distantly related subclade T8.

Tragiinae Subclade T3—Subclade T3 is comprised of a large paraphyletic *Tragia* sect. *Tagira* intermixed with species of *Tragiella* and *Tragia* sect. *Agirta*. Based on our phylogeny, the circumscriptions of *T.* sects. *Agirta* and *Tagira*, and *Tragiella* are not supported (Fig. 3A), although resolution and node support need improvement before taxonomic boundaries are revised.

Tragia sect. *Tagira* is a diverse and species-rich lineage (ca. 82 species, 18 sampled here) broadly distributed in Africa, Madagascar, and South/West Asia, with its highest diversity in dry regions of Africa (Pax and Hoffmann 1919a; Radcliffe-Smith 1987). Species of *T.* sect. *Tagira* are united by pinnatifid or highly dissected pistillate sepals, and to a lesser extent by partially connate styles and staminate flowers with well-developed filaments (Pax and Hoffmann 1919a). *Tragiella* is a small African genus (four species, three sampled here) that is morphologically similar to *T.* sect. *Tagira*, and is primarily differentiated by conical, funnel-shaped, or globose connate styles (Pax and Hoffmann 1919a; Webster 2014). *Tragiella* and *T.* sect. *Tagira* share tricolpate pollen with scattered apertural sexine islands and coarsely reticulate tecta (Gillespie 1994a), which would support combining these taxa.

Tragia sect. *Agirta* is a small lineage (ca. five species, two sampled here) endemic to Madagascar. It is differentiated from other African Tragiinae by unlobed pistillate sepals and subsessile introrse anthers (Baillon 1858; Pax and Hoffmann 1919a; Leandri 1938b), which suggests that *T.* sect. *Agirta* might have resolved separately from *T.* sect. *Tagira* and *Tragiella*. Instead, poorly supported relationships imply that the origin of *T.* sect. *Agirta* is closely associated with or within the mainland African lineage. We suspect that the non-monophyly of *T.* sect. *Agirta* is an artifact of limited taxon sampling for the section and non-overlapping marker coverage of *T. boiviniana* and *T. cocculifolia* (Appendix 1).

Tragiinae Subclade T4—Subclade T4 consists of *Bia*, a recently resurrected segregate of *Tragia* with five species (two sampled here) distributed in Central and South America (Webster 2007; Medeiros et al. 2013). *Bia* was previously treated as a section of *Tragia* (Pax and Hoffmann 1919a; Múlgura de Romero and Gutiérrez de Sanguinetti 1989) but was revalidated based on molecular evidence that showed *T. fallax* (sect. *Bia*) was not most closely related to other sampled *Tragia* (Fig. 1; Wurdack et al. 2005 in *trnL-F* analyses only; Webster 2007). *Bia* is distinguished by staminate flowers with disk glands, numerous (8–20) stamens, and inaperturate pollen, whereas *Tragia* lacks staminate disk glands, has typically three stamens (rarely two or up to 23), and tricolpate or weakly triporate/tri-aperturate pollen (Gillespie 1994a; Webster 2007). Additionally, *Bia* possesses a distinctive inflorescence comprised of a primary staminate axis and proximal pistillate branch with multiple (5–20) pistillate flowers, which is sometimes inadequately described as a "bifurcating" inflorescence (e.g. Webster 2007, 2014; Medeiros et al. 2013). This inflorescence type is shared with *Zuckertia*, and was used as morphological evidence to recombine *Zuckertia* (at that time, also a section of *Tragia*) as a section of *Bia* (Webster 2007). *Zuckertia* forms the distantly related subclade T6, and is differentiated from *Bia* by several morphological features (see T6 for further discussion).

Tragiinae Subclade T5—Subclade T5 includes taxa endemic to the Greater Antilles, *Acidoton* group 1 and *Platygyna*, that are united by having globose inaperturate pollen with rugulate or reticulate tecta with broad rugae/muri, which is a unique combination in Plukenetieae (Gillespie 1994a).

Platygyna contains seven species endemic to Cuba; *P. hexandra* (Jacq.) Müll. Arg., the species sampled here, is widespread, whereas the remaining species are narrowly distributed in eastern Cuba (Liogier 1952; Borhidi 1972). *Platygyna* is distinguished by characteristic oblong leaves with dentate margins and staminate flowers with 3–14 short stamens on a hairy (rarely glabrous) subglobose to convex receptacle (Pax and Hoffmann 1919a; Webster 1994, 2014).

Acidoton contains six species of shrubs (two sampled here) distributed in the Caribbean and Central and northwestern South America. Species of *Acidoton* have staminate flowers with 20–60 stamens attached to a usually glabrous, convex, planar, or semi-globose receptacle (absent in *A. nicaraguensis* [Hemsl.] G. L. Webster), and anther connectives with tufts of minute stinging hairs (Webster 1967; Webster 1994, 2014). *Acidoton* 1 is likely to contain all of the Caribbean species with inaperturate pollen (*Acidoton* pollen type 2 in Gillespie 1994a) and can be classified into species of large shrubs endemic to Jamaica (*A.* sect. *Acidoton*, *A. urens* Sw.) or smaller shrubs endemic to Haiti and the Dominican Republic (*A.* sect. *Micracidoton* Ule, ca. four species). The remaining species, *A. nicaraguensis* (*Acidoton* group 2), is found only on the mainland, and was recovered separately in subclade T9, which strongly suggests that the Caribbean and mainland species should be divided into two genera.

Tragiinae Subclade T6—Subclade T6 delineates *Zuckertia*, a recently resurrected segregate genus with two species (one sampled here) distributed in Mexico and Central America. *Zuckertia* was previously treated as a section of *Tragia* (Müller 1865; Pax and Hoffmann 1919a) and subsequently as a section of *Bia* (Webster 2007), neither of which is supported by our phylogeny. *Zuckertia* was associated with *Bia* because they share inflorescences with a primary staminate axis and proximal pistillate branch with multiple flowers (Webster 2007). *Zuckertia* differs by having often large (≥ 20 cm), cordate, and sometimes three-lobed leaf blades, staminate flowers that lack disk glands and have numerous (30–40) stamens, and tricolpate pollen that is free of apertural sexine islands and has a finely reticulate tectum, whereas *Bia* has smaller (5–15 cm), ovate to lanceolate, unlobed leaf blades, staminate flowers with disk glands and fewer (8–20) stamens, and inaperturate pollen with a finely reticulate or foveolate-fossulate tectum (Pax and Hoffmann 1919a; Gillespie 1994a; Webster 2007; Medeiros et al. 2013). A second species associated with *Zuckertia* was recently described from the Sierra Madre del Sur, Mexico (Steinmann and Ramírez-Amezcua 2013) and is discussed in the taxonomic treatment.

Tragiinae Subclade T7—Subclade T7 contains *Tragia* sect. *Leptobotrys*, a small group of two species (both sampled) distributed in the southeastern United States. Species of *T.* sect. *Leptobotrys* are most clearly differentiated from other New World *Tragia* by having two stamens rather than three or more (Pax and Hoffmann 1919a; Miller and Webster 1967; Gutiérrez de Sanguinetti and Múlgura de Romero 1986), and by weakly triporate pollen with poorly defined circular apertures covered with fragmented sexine (Gillespie 1994a). This pollen type closely resembles those of Old World taxa resolved in the sister subclade T8 (described below). Phylogeny and pollen associations suggest that *T.* sect. *Leptobotrys* is distinct from other North American *Tragia* and could be treated as a separate genus.

Tragiinae Subclade T8—Subclade T8 is a heterogeneous group that includes all three Australian *Tragia* species (sampled here), the Southeast Asian genus *Pachystylidium* (one species, sampled here), and the Madagascan genus *Sphaerostylis* (two species, one sampled here). *Pachystylidium hirsutum* and *T. novae-hollandiae* (the only Australian species of *Tragia* known at the time) were thought to be closely related because they share subsessile anthers, triporate pollen with weakly defined apertures covered in dense fragments of sexine, and adjacent geographic distributions (Airy Shaw 1969; Gillespie 1994a). However, their association with the Madagascan *Sphaerostylis perrieri* Leandri was unanticipated. Upon closer investigation, we found that species of subclade T8 have staminate flowers with typically four or five unlobed sepals and subsessile anthers on a glabrous and sometimes raised receptacle (Pax and Hoffmann 1919a; Leandri 1938a; Airy Shaw 1969; Forster 1994, 1997; Li and Gillespie 2008), which may be synapomorphies for the lineage.

Tragiinae Subclade T9—Subclade T9 denotes *Acidoton* group 2, which includes only the widespread Central and northwestern South American species *A. nicaraguensis*. *Acidoton nicaraguensis* was originally described as *Gitara*, but was synonymized with *Acidoton* based on sharing shrub habit, numerous stamens (20–60), and minute stinging hairs on their anther connectives (Webster 1967), although they have notably different pollen morphology (Gillespie 1994a). Pollen of *A. nicaraguensis* is tricolpate with narrow apertures and small scattered islands of apertural sexine and a finely and irregularly foveolate-reticulate tectum, whereas *Acidoton* group 1 is inaperturate with a rugulate tectum. Recognition of *Gitara* is supported by our phylogeny and pollen morphology differences, and is discussed further in the taxonomic treatment.

Tragiinae Subclade T10—Subclade T10 contains the majority of New World *Tragia*, including *T.* sects. *Tragia* (ca. 33 species, 20 sampled here), *Leucandra* (12 species, three sampled here), and *Ratiga* (five species, two sampled here) sensu Pax and Hoffmann (1919a). Each section is distributed in both North and South America and they are likely united by tricolpate pollen with scattered apertural sexine islands and intectate-baculate exine (Gillespie 1994a).

Species placed in *T.* sects. *Leucandra* and *Ratiga* (sensu Pax and Hoffmann 1919a) are labeled on the phylogeny (Fig. 3B); both sections were found to be non-monophyletic and embedded in sect. *Tragia*. The combination of these three sections is now supported by gross morphology (Miller and Webster 1967; Múlgura de Romero and Gutiérrez de Sanguinetti 1989), pollen (Gillespie 1994a), and molecular evidence (Fig. 3B), and we recognize the clade as *Tragia* sect. *Tragia* s. l. *Tragia* section *Ratiga* was differentiated by introrse anthers and incurved stamen orientation (Pax and Hoffmann 1919a) and a more resolved and strongly supported phylogeny might show it to be a cohesive species group within *T.* sect. *Tragia* s. l. *Tragia* section *Leucandra* was defined by having 4–20 stamens (Pax and Hoffmann 1919a) but has not been considered a section worthy of recognition since stamen number is a weak taxonomic character (Miller and Webster 1967). Species with 4–20 stamens are found in at least three different lineages in subclade T10 (see species labeled *Leucandra* in Fig. 3B), which supports *T.* sect. *Leucandra* is an artificial group.

Subclade T10 was resolved into four novel lineages that correspond with reproductive character variation. The earliest diverging lineage includes *T. polyandra*, a species defined by high and variable stamen number (17–23) and multiple (two to four) pistillate flowers on a short proximal pistillate branch. *Tragia polyandra* belonged to the recently resurrected *T.* sect. *Leptorhachis* (Klotzsch) Müll. Arg. (not recognized by Gillespie 1994a), which was recircumscribed by Múlgura de Romero and Gutiérrez de Sanguinetti (1989) to include species that have staminate flowers with 6–22 stamens (without disk glands) and inflorescences that frequently have a short proximal pistillate branch with two to four pistillate flowers (sometimes only one flower on a primary raceme axis). *Tragia* section *Leptorhachis* (sensu Múlgura de Romero and Gutiérrez de Sanguinetti 1989) included a subset of species that were previously placed in *T.* sect. *Leucandra*, including *T. paxii* Lourteig & O'Donnell in our phylogeny; although, with 6–10 stamens and only a single proximal pistillate flower, *T. paxii* is more similar to the remaining species of *T.* sect. *Tragia* s. l. The revised *T.* sect. *Leptorhachis* may have taxonomic value if it is amended to only include species with short proximal pistillate branches containing two to four pistillate flowers, which might characterize this early diverging lineage.

The three remaining lineages of subclade T10 are putatively united by inflorescences with one (or rarely two) proximal pistillate flowers. The first is a small, strongly supported clade distinguished by elongate pistillate pedicels, and comprising the *T. volubilis* L. species complex and *T. giardelliae* (Pax and Hoffmann 1919a; Múlgura de Romero and Gutiérrez de Sanguinetti 1989). Species in the elongate pistillate pedicel clade have two to three stamens (rarely four or five), smooth to undulate stigmatic surfaces, and lack glandular trichomes. The remaining two clades are large, moderately supported, and defined by papillose adaxial stigmatic surfaces or inflorescences with stipitate-glandular trichomes. Species in these two clades typically have three stamens, although four or more stamens are also present (e.g. 6–10 stamens in *T. paxii* and *T. ramosa* Torr.). The defining characters of these two clades appear to be mostly mutually exclusive: species of the papillose stigma clade do not have stipitate-glandular trichomes on their inflorescences, and the glandular inflorescence clade mostly exhibits smooth to undulate (rarely subpapillose) adaxial stigmatic surfaces.

Previous sectional classifications of New World *Tragia* have not considered species group boundaries based on pistillate pedicel length, stigma morphology, or glandular trichomes (Pax and Hoffmann 1919a; Lourteig and O'Donnell 1941; Miller and Webster 1967; Múlgura de Romero and Gutiérrez de Sanguinetti 1989), although these characters were commonly used in dichotomous keys. We anticipate that these four species groups will be supported following further taxon sampling, and that these reproductive characters have good potential to outline a revised infrageneric classification.

Remarks on Tragiinae—Our phylogeny reveals that *Tragia*, as currently circumscribed, is para- and/or polyphyletic and intermixed with all other Tragiinae genera, and that a major revision is required to provide a generic classification that reflects monophyly and evolutionary history. One possibility is that we convert all the genera of Tragiinae into synonyms of *Tragia* and develop a broad subgeneric classification that emphasizes morphological diversity and phylogeny in the subtribe (e.g. Lowry et al. 2013). However, creating a large heteromorphic genus is not desirable, given that many Tragiinae genera form strongly supported lineages supported by morphology (e.g. *Acidoton* groups 1 and 2, *Bia*, *Ctenomeria*, *Platygyna*, and *Zuckertia*), and that *Tragia* could be easily divided into monophyletic genera based on existing or revised taxonomic sections (e.g. *T.* sects. *Leptobotrys* and *Tragia* s. l.). We believe that revising the genera of Tragiinae and dividing *Tragia* into smaller genera will result in the most functional classification for the subtribe, but are exploring additional taxon sampling, molecular markers, and morphological characters before enacting such significant changes.

Correlation of Pollen Morphology with Molecular Phylogeny—Relationships in Plukenetieae predicted by pollen morphology (Gillespie 1994a) were mostly congruent with the molecular phylogeny (Fig. 4). Most molecular clades can be defined by a combination of aperture and exine condition, with the exception of subclades P1 and P2, which both have tricolpate pollen with uneven aperture margins and coarsely reticulate tecta, but are currently poorly resolved (Fig. 3A; shown as a polytomy in Fig. 4 when poorly supported branches are collapsed).

Exine Morphology Evolution—Exine condition in Plukenetieae is diverse (Punt 1962; Gillespie 1994a; Nowicke and Takahashi 2002) and does not appear to exhibit clear patterns of variation (Fig. 4). Tectate-perforate exine is the most common condition in Plukenetieae (Gillespie 1994a), and is observed in nine lineages based on seven distinct morphology types. Punctate tectum is found in two distant lineages: *Cnesmone* and *Megistostigma* (T2), and a clade comprising *Tragia* sect. *Leptobotrys* (T7) and *Pachystylidium* and *T. novae-hollandiae* (T8). Foveolate tectum is observed in *Plukenetia* subclades P3–P5, as well as *Haematostemon* and *Romanoa* but with some modification: fossulate-foveolate in *Romanoa* (appearing only foveolate in Nowicke and Takakashi 2002; punctate by their definition), and finely foveolate-rugulate in *Haematostemon* (interpreted as 'microcrotonoid' by Nowicke and Takahashi 2002). *Bia alienata* Didr. (T4) has a foveolate-fossulate tectum similar to *Romanoa*, although other species of *Bia* (not sampled) are semitectate and finely reticulate (Gillespie 1994a). *Acidoton urens* (*Acidoton* group 1, subclade T5) and some species of *Platygyna* (not sampled here) have rugulate tecta with broad rugae (Gillespie 1994a, Figs. 33–35, 46), whereas other species of *Platygyna* (e.g. *P. hexandra*, subclade T5) have tectate-perforate reticulate exine with broad muri that are wider than the lumina (Gillespie 1994a, Figs. 44–45). Our results support the homology between the broad muri and rugae of *Acidoton* group 1 and *Platygyna*. *Acidoton nicaraguensis* (*Acidoton* group 2, subclade T9) is characterized by a finely and irregularly foveolate-reticulate tectum, which is similar to the distantly related *Ctenomeria* (T1).

Semitectate exine is also common in Plukenetieae and is observed in each of the subtribes (Fig. 4). Coarsely reticulate tecta characterize three disparate lineages: *Dalechampia*, *Plukenetia* subclades P1 and P2, and Old World Tragiinae subclade T3, at least in *Tragiella* and *Tragia* sect. *Tagira* (pollen of *T.* sect. *Agirta* is not known). Nowicke and Takahashi (2002) examined different specimens of *Tragiella* and *T.* sect. *Tagira* and determined some samples were finely reticulate. Semitectate exine with a finely reticulate tectum is found in *Zuckertia* (T6), as well as *Bia lessertiana* Baill. (not sampled here) (Gillespie 1994a).

Intectate baculate or clavate exine is unique to *Tragia* sect. *Tragia* s. l. (T10). This distinctive exine appears to be a synapomorphy that unites species previously attributed to sects.

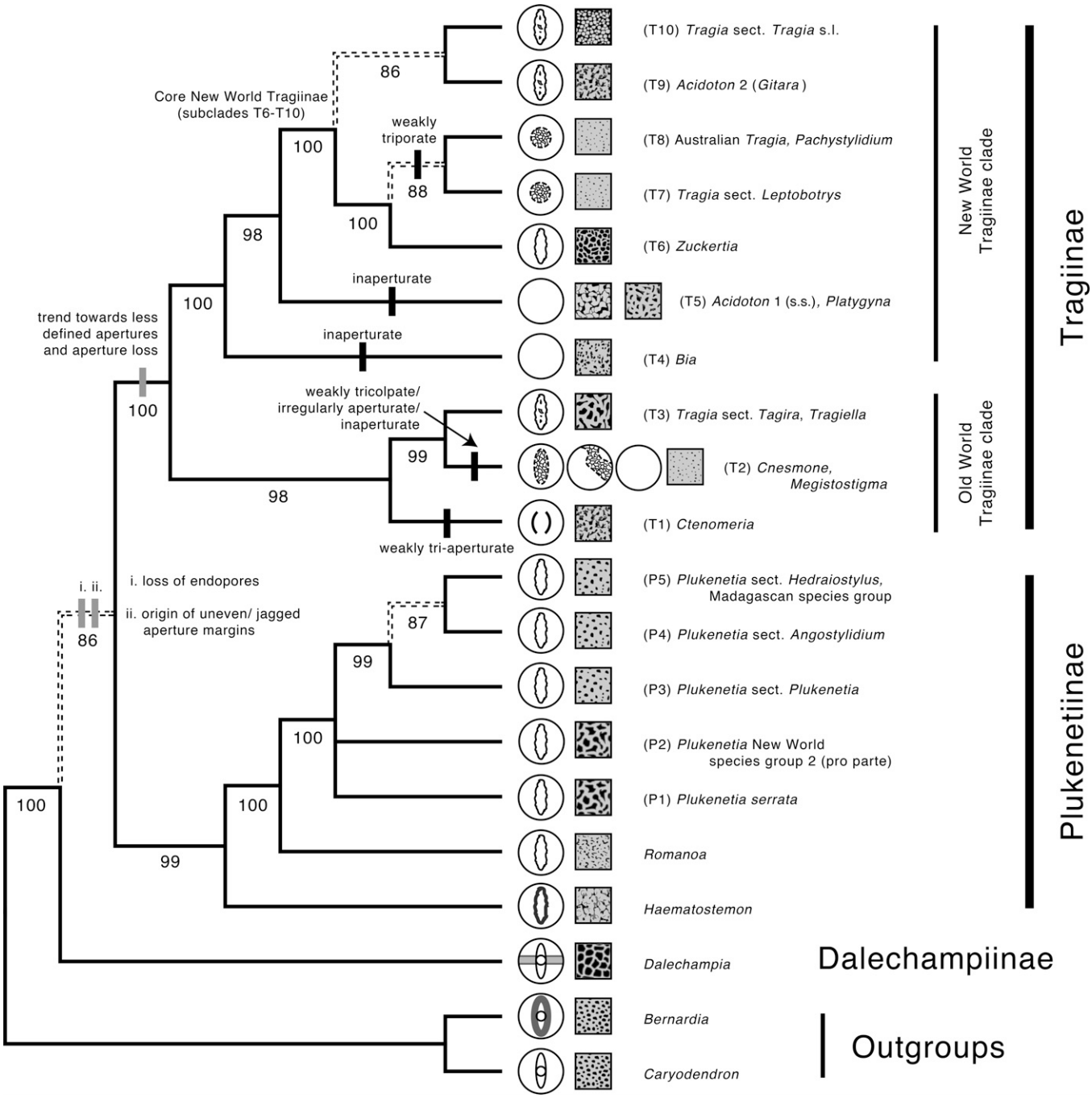

FIG. 4. Summary cladogram of the relationships recovered by Bayesian analysis of combined and partitioned ITS, *psbA-trnH*, and indel data of Plukenetieae and selected outgroups, with schematic pollen aperture and exine illustrations. Bayesian posterior probabilities (BPP) are specified below each branch; strongly supported branches (BPP ≥ 95) are indicated by solid lines, moderately supported branches (BPP = 85–94) by dashed lines, and poorly supported clades (BPP ≤ 84) were collapsed. Aperture and exine conditions are referenced from Gillespie (1994a) for Plukenetiinae and Tragiinae, Nowicke and Takahashi (2002) for *Dalechampia*, and Nowicke et al. (1999) for *Bernardia* and *Caryodendron*. Circles depict a single pollen aperture in equatorial view, and do not reflect features such as pollen shape, size, or exine. Squares depict exine condition with light grey for tectum and black for empty spaces (i.e. perforations, lumina, absence of tectum). Aperture and exine states are as follows (exine abbreviations: TP = tectate perforate; Sem. = semitectate; In. = intectate): *Caryodendron*: tricolporate and TP (foveolate) to Sem. (finely reticulate); *Bernardia*: tricolporate with a margo and TP (foveolate) to Sem. (finely reticulate); *Dalechampia*: tricolporate with an endocingulate endopore and Sem. (coarsely reticulate); *Haematostemon*: tricolpate with thickened margins sometimes covered in an unbroken granulate sexinous membrane and TP (finely foveolate-rugulate); *Romanoa*, tricolpate and TP (fossulate-foveolate); P1 and P2: tricolpate and Sem. (coarsely reticulate); P3–P5: tricolpate and TP (foveolate); T1: weakly tri-aperturate with apertures denoted by elliptic zones of thin and often-depressed sexine and TP (finely and irregularly foveolate-reticulate); T2: weakly tricolpate or irregularly aperturate with apertures densely covered with sexine islands, sometimes inaperturate, and TP (punctate); T3: tricolpate with scattered apertural sexine islands and Sem. (coarsely reticulate, sometimes finely reticulate); T4: inaperturate and TP (foveolate-fossulate); T5: inaperturate and TP (rugulate with broad rugae, or reticulate with broad muri); T6: tricolpate and Sem. (finely reticulate); T7 and T8: weakly triporate with apertures densely covered with sexine islands and TP (punctate); T9: tricolpate with scattered apertural sexine islands and TP (finely and irregularly foveolate-reticulate); and T10: tricolpate with scattered apertural sexine islands and In. (baculate or clavate). Note that tricolpate pollen in Plukenetiinae and Tragiinae has uneven or jagged aperture margins.

Tragia, *Leucandra*, and *Ratiga* (Gillespie 1994a), including sect. *Leptorhachis* sensu Múlgura de Romero and Gutiérrez de Sanguinetti (1989).

Our sampled close relatives of Plukenetieae, *Bernardia* and *Caryodendron*, are characterized by tectate-perforate foveolate to semitectate finely reticulate exines (described as punctate, deeply punctate, or microreticulate by Nowicke et al. 1999). Their exine conditions are hard to categorize since they are intermediate between foveolate and finely reticulate, as defined here (Nowicke et al. 1999, Figs. 117–154).

It is difficult to evaluate the evolutionary history of Plukenetieae exine due to the breadth of variation and absence of obvious patterns, and it is premature to do formal reconstructions until the phylogeny is more robust. One hypothesis is that a foveolate to finely reticulate exine condition (similar to *Bernardia* and *Caryodendron*) was ancestral in Plukenetieae. This would support some exines as symplesiomorphic (e.g. P3–P5 and T6) with a few exine types as closely related conditions (e.g. finely foveolate-rugulate in *Haematostemon*; fossulate-foveolate in *Romanoa*; irregularly foveolate and finely reticulate in T1 and T9; and irregularly foveolate-fossulate in T4). This hypothesized ancestral condition would suggest that several exine conditions are derived (e.g. coarsely reticulate in *Dalechampia*, P1 and P2, and T3; rugulate or reticulate with broad rugae/muri in T5; punctate in T2, T7, and T8; and intectate baculate or clavate in T10).

Pollen Aperture Evolution—Pollen apertures in Plukenetieae exhibit a trend towards less defined apertures and aperture loss (Gillespie 1994a), and do not appear to be correlated with exine morphology (Fig. 4). Tricolporate pollen appears to be symplesiomorphic in the tribe and is found in *Dalechampia* and the putative sister tribes Bernardieae and Caryodendreae. Most Acalyphoideae are also characterized by tricolporate pollen, although other potential close relatives of Plukenetieae (tribes Adelieae and Chrozophoreae pro parte of subclade A6; Wurdack et al. 2005) share a mix of tricolporate (e.g. *Caperonia* A. St.-Hil., *Chiropetalum* A. Juss., and *Philyra* Klotzsch) and tricolpate (e.g. *Adelia* L., *Argythamnia* P. Browne, *Ditaxis* Vahl ex A. Juss., *Lasiocroton* Griseb., and *Leucocroton* Griseb.) aperture conditions (Nowicke et al. 1999, Takahashi et al. 2000). Our results suggest there may have been a single transition to the absence of endopores in the ancestor of Plukenetiinae and Tragiinae, although node support is currently low.

Tricolpate pollen in Plukenetieae is characterized by uneven or jagged aperture margins, which has been hypothesized as a second synapomorphy of Plukenetiinae and Tragiinae (Gillespie 1994a). *Haematostemon* and the other Plukenetiinae small tree/shrub genera (*Angostylis* and *Astrococcus*, not sampled) are distinct in having uneven and granular or gemmate aperture margins, which are thickened in *Astrococcus* and *Haematostemon* (Gillespie 1994a).

Most lineages in Tragiinae with tricolpate pollen are also characterized by scattered apertural sexine islands. Their presence coincides with a shift in Tragiinae towards less defined apertures and inaperturate pollen, and is hypothesized to be the plesiomorphic condition of the subtribe (Gillespie 1994a). Two distant lineages in Tragiinae are characterized by tricolpate pollen with scattered sexine islands: (i) the weakly supported clade comprised of *Acidoton* group 2 (T9) and *Tragia* sect. *Tragia* s. l. (T10); and (ii) *T.* sect. *Tagira* of subclade T3 (Fig. 4). Currently, it is unclear if *Tragiella* (T3) possesses apertural sexine islands since prior pollen SEMs were imaged from acetolyzed grains, which eliminates the aperture membrane (Gillespie 1994a; Nowicke and Takahashi 2002). The absence of sexine islands in *Zuckertia* (T6) previously supported ideas that it was one of the least derived members of Tragiinae (Gillespie 1994a), although its embedded placement suggests this could also be secondary loss.

Pollen with apertures densely covered with sexine islands is considered to have weakly defined apertures. This condition was thought to have originated once from tricolpate pollen with scattered apertural sexine islands, initially as densely covered colpi, then evolving into weakly porate or circular apertures and apertures of irregular shape (Gillespie 1994a, Fig. 78). Contrary to this idea, we infer that apertures densely covered with sexine islands evolved in two separate lineages, one with elliptic and irregular shaped apertures in subclade T2 (*Cnesmone* and *Megistostigma*), and another with weakly porate or circular shaped apertures in the ancestor of subclades T7 (*Tragia* sect. *Leptobotrys*) and T8 (represented by *Pachystylidium* and *T. novae-hollandiae*).

Inaperturate pollen was hypothesized to have evolved at least twice in Tragiinae, once via apertures densely covered with sexine islands in Old World *Megistostigma*, and one or more times in the New World genera *Acidoton* 1 (pollen type 2), *Bia*, and *Platygyna* (Gillespie 1994a, Fig. 78). It was suggested that inaperturate pollen of *Acidoton* 1 and *Platygyna* shared an origin, with an independent origin in *Bia*. Our phylogeny supports this hypothesis and suggests that inaperturate pollen of New World genera evolved under two possible scenarios: (i) through two independent transitions in T4 (*Bia*) and T5 (*Acidoton* 1 and *Platygyna*); or (ii) by a single transition in the ancestor of the New World Tragiinae clade followed by a reversal to tricolpate pollen in the core New World Tragiinae (T6–T10). The loss and recovery of pollen apertures seems like a complicated evolutionary hypothesis, but may be plausible if the phenotypic expression of apertures were influenced by a single gene, such as INAPERTURATE POLLEN1 described in *Arabidopsis thaliana* (Dobritsa and Coerper 2012). Our phylogeny confirms the separate origin of inaperturate pollen in *Megistostigma* (T2). We should note that *Megistostigma* pollen is most often weakly tricolpate or with irregularly shaped apertural areas (with apertures denoted by dense sexine islands), and is sometimes inaperturate in only some species (Gillespie 1994a). Furthermore, individual specimens of *Megistostigma* often contain a range of aperture types (Gillespie 1994a; Nowicke and Takahashi 2002), and the extent of variation within each species requires further investigation. Together this suggests that inaperturate pollen evolved at least two to three times in Tragiinae.

Tragiinae contains two other forms of weakly defined apertures that were suggested to have evolved independently of other reduced aperture types (Gillespie 1994a). *Ctenomeria* (T1) has weakly tri-aperturate pollen denoted by elliptical zones of thin and often-depressed sexine, which appear to be distinct from pollen with weakly defined apertures and inaperturate pollen observed in the neighbouring subclade T2 (Fig. 4). *Tragia* subg. *Mauroya* (not sampled here) has weakly tri-aperturate pollen with broad circular apertural areas covered with thin strands of sexine, unlike any other aperture condition in Plukenetieae (Gillespie 1994a) and likely independently derived within or near the African and Madagascan taxa of subclade T3.

To summarize, it was initially estimated that there were three origins of weakly aperturate pollen (based on three different morphologies) and at least two origins of inaperturate pollen (Gillespie 1994a, Fig. 78). Based on a re-examination of pollen data in the context of our phylogeny, we suggest that weakly aperturate pollen emerged four times in Tragiinae: (i) *Ctenomeria* (T1); (ii) *Cnesmone* and *Megistostigma* (T2); (iii) the putative ancestor of T7 (*Tragia* sect. *Leptobotrys*) and T8 (confirmed in *Pachystylidium* and *T. novae-hollandiae*); and (iv) *T.* subg. *Mauroya* (not sampled here). Inaperturate pollen is hypothesized to have evolved up to three times in *Megistostigma* pro parte (T2), *Bia* (T4), and the putative ancestor of *Acidoton* 1 and *Platygyna* (T5).

Tragiinae exhibits several parallel transitions from tricolpate pollen to inaperturate and intermediate weakly defined aperture states. This suggests that the subtribe may be a useful system to explore the underlying mechanisms of aperture reduction and selective pressures for inaperturate pollen, which is a rare and poorly understood condition of eudicots (Furness 2007; Matamoro-Vidal et al. 2012). Character optimization on a better resolved phylogeny would help elucidate putative ancestral states for the pollen of Tragiinae, and help quantify the number of transitions to weakly defined aperture and inaperturate conditions.

***Towards a Revised Phylogenetic Classification of Plukenetieae*—**The combination of molecular and pollen morphology data strongly supports the para- and/or polyphyly of *Tragia* and the potential to divide the genus into smaller monophyletic genera. Recently resurrected genera, *Bia*, *Ctenomeria*, and *Zuckertia*, are supported as evolutionarily distinct lineages and should be maintained at their current rank, resulting in a new combination for a recently described species associated with *Zuckertia*. In addition, we find sufficient molecular, pollen, and floral morphology evidence to reinstate the Central and northwestern South American species *Acidoton nicaraguensis* (*Acidoton* group 2, subclade T9) as *Gitara*, which necessitates another new combination.

Taxonomic Treatment

Gitara Pax & K. Hoffm., in H. G. A. Engler (ed.), Pflanzenr., IV, 147, XVII (Heft 85): 187. 1924.—TYPE: *Gitara venezolana* Pax & K. Hoffm. 1924 = *Cleidion? nicaraguense* Hemsl. 1883.

1. **Gitara nicaraguensis** (Hemsl.) Card.-McTeag. & L. J. Gillespie, comb. nov. *Cleidion? nicaraguense* Hemsl., Biol. Cent.-Amer., Bot. 3: 130. 1883. *Acidoton nicaraguensis* (Hemsl.) G. L. Webster, Ann. Missouri Bot. Gard. 54: 191. 1967.—TYPE: NICARAGUA. Chontales: 1867–8, *Tate 352(455)* (holotype: K!; isotype: BM!).

Gitara venezolana Pax & K. Hoffm., in H. G. A. Engler (ed.), Pflanzenr., IV, 147, XVII (Heft 85): 187. 1924. *Acidoton venezolanus* (Pax & K. Hoffm.) G. L. Webster, Ann. Missouri Bot. Gard. 54: 191. 1967.—TYPE: VENEZUELA. Carabobo: Guaramales, camino de El Palito o San Felipe, en selvas, 15–29 May 1920, *Pittier 8836* (holotype: VEN!; isotype: US!).

Gitara panamensis Croizat, J. Arnold Arbor. 26: 192. 1945.—TYPE: PANAMA. Darién: Hills between Pinogana and Yavisa, 17 Apr 1914, *Pittier 6543* (holotype: A; isotypes: K!, US!).

Taxonomic Discussion*—Gitara* has experienced a brief, but complicated, taxonomic history. *Gitara venezolana* was first described as a shrubby segregate of *Tragia* from Venezuela (Pax and Hoffmann 1924). A second species (*G. panamensis*) was described from Central America and was distinguished by leaf shape and venation differences and smaller staminate flowers (Croizat 1945). *Gitara* was then synonymized with the Caribbean genus *Acidoton* (Webster 1967) based on their shrub habit (these are the only shrub taxa in Tragiinae), large number of stamens (ca. 20–60), and tufts of small stinging hairs on their anther connectives (a putative generic synapomorphy). *Cleidion? nicaraguense* (Hemsley 1883) was also determined to be the same taxon as *G. panamensis* and has priority as the type of the Central American species. As such, new combinations were made for the Central American (*A. nicaraguensis*) and South American (*A. venezolanus*) species, although it was suspected they were conspecific (Webster 1967). These taxa have since been synonymized (Webster 1994, 2014; Gillespie 1999). Radcliffe-Smith (2001) recognized *Gitara* based on differences in palynology, geographic distribution, and 'gestalt,' although most recent treatments have continued to accept *A. nicaraguensis* (González 2010; Webster 2014).

Gitara is distinguished from *Acidoton* group 1 (hereafter referred to as *Acidoton* s. s.) by pollen morphology, staminate flower differences, and non-overlapping geographic range (Table 5). Shrub habit, once presumed to be a uniting character of these genera (Webster 1967), is now implied to have evolved independently. We question if anther connective stinging hairs are unique to *Acidoton* s. s. and *Gitara* since we have observed similar minute hairs on specimens of *Bia*, *Ctenomeria*, *Platygyna*, and *Zuckertia*. Based on floral characters, *Gitara* is differentiated by 20–35 stamens with minute anthers (0.2–0.4 mm) attached to an inconspicuous receptacle, whereas *Acidoton* s. s. has ca. 30–55 stamens with larger anthers (1–2 mm) attached to a conspicuous convex glabrous receptacle.

We recognize *Gitara nicaraguensis* as a single widespread species and provide a new combination based on the earliest recorded type.

Zuckertia Baill., Étude Euphorb.: 495. 1858. *Tragia* sect. *Zuckertia* (Baill.) Müll. Arg., Linnaea 34: 178. 1865. *Bia* sect. *Zuckertia* (Baill.) G. L. Webster, Contr. Univ. Michigan Herb. 25: 237. 2007.—TYPE: *Zuckertia cordata* Baill. 1858.

1. Zuckertia cordata Baill., Étude Euphorb.: 495. 1858. *Tragia bailloniana* Müll. Arg., Linnaea 34: 178. 1865. *Bia cordata* (Baill.) G. L. Webster, Contr. Univ. Michigan Herb. 25: 237. 2007.—TYPE: MEXICO. Tabasco: Cerros de Teapa, *J. Linden s. n.* (holotype: P!).

2. **Zuckertia manuelii** (V. W. Steinm. & Ram.-Amezcua) Card.-McTeag. & L. J. Gillespie comb. nov. *Bia manuelii* V. W. Steinm. & Ram.-Amezcua, Revista Mex. Biodivers. 84: 747. 2013.—TYPE: MEXICO. Michoacán: Municipio de Coalcomán, 34 km al sur de Coalcomán y 2.4 km al sur de río Ocorla sobre el camino a San José de la Montaña, 18°35′52″ N, 103°08′45″ W, 1,108 m, 29 Aug 2008, *Steinmann et al. 6326* (holotype: IEB; isotypes: ARIZ, MEXU, MICH).

Taxonomic Discussion*—Zuckertia cordata* was initially described as a monotypic genus (Baillon 1858), but was reclassified as a section of *Tragia* and given the replacement

Table 5. Distinguishing characters for *Gitara* and the sections of *Acidoton*.

Character	*Gitara* Pax & K. Hoffm.	*Acidoton* Sw. sect. *Acidoton*	*Acidoton* sect. *Micracidoton* Urb.
Geographic distribution	Central and NW South America	Caribbean (Jamaica)	Caribbean (Hispaniola)
Included species	*G. nicaraguensis*	*A. urens*	*A. haitiensis*, *A. lanceolatus*, *A. microphyllus*, *A. variifolius*
Habit	Shrubs 1–5 (7) m	Shrubs 3–6 m	Shrubs 1–1.5 m
Leaf size	Large, 10–20 cm	Large, 8–10 cm	Small, 0.5–2(–4.5) cm
Leaf margin	Serrate towards apex	Entire, sometimes with 1–2 large teeth or small acute lobes	Entire or rarely minutely remotely toothed
Staminate receptacle	Inconspicuous	Thick and elevated, convex upper surface	Short and fleshy, semi-globose or planar upper surface
Stamen number	15–25 (35)	30–60	24–44
Anthers	Oblong, 0.2–0.4 mm long, thecae remain parallel post dehiscence	Narrowly oblong, 1.0–2.0 mm long, thecae often spreading post dehiscence	(Measurements not available)
Pollen shape	Suboblate	Globose	Globose
Pollen apertures	Tricolpate with narrow apertures and scattered sexine islands	Inaperturate	Inaperturate
Pollen tectum	Finely and irregularly foveolate-reticulate	Rugulate with broad rugae	Rugulate with broad rugae

name *T. bailloniana* (Müller 1865), which would become rooted in the scientific literature (Pax and Hoffmann 1919a; Webster and Huft 1988; Gillespie 1994a; Burger and Huft 1995; Radcliffe-Smith 2001; González 2010). *Tragia* sect. *Zuckertia* was briefly treated as a section of *Bia* (Webster 2007) and then reinstated as a distinct genus (Medeiros et al. 2013).

Around the same time that *Zuckertia* was resurrected, a new species of *Bia* sect. *Zuckertia* was described from Mexico (Steinmann and Ramírez-Amezcua 2013). *Bia manuelii* has inflorescences with a primary staminate axis and proximal pistillate branch consisting of a short spike of two to four pistillate flowers, which was thought to be homologous with the elongate pistillate branches found in *Bia* and *Zuckertia* (Steinmann and Ramírez-Amezcua 2013). This species is associated with *Zuckertia* in having no staminate disk glands, similar tricolpate pollen with a finely reticulate tectum, and overlapping distribution in Mexico, whereas its stamen number (17–24) is closer in range to *Bia* (8–20) than *Zuckertia* (30–40) (Webster 2007; Steinmann and Ramírez-Amezcua 2013). We agree that similarities in pollen morphology and lack of staminate disk glands support *Bia manuelii* as a member of *Zuckertia*. As such, we recognize two species of *Zuckertia*, adjust the definition of the genus to allow for an increased range in stamen number (17–40) and for proximal pistillate branches to be either elongate or short, and provide a new combination for this species.

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Appendix 1. List of species and vouchers used in this study, arranged by: ***Species*** Authority. COUNTRY. *Collector & Number* (Herbarium Code), nrITS, and *psbA-trnH* GenBank accession numbers. All sequences are new GenBank submissions from this study. Sequences not obtained are indicated by an em dash (—). *psbA-trnH* GenBank accessions that possess the inversion are indicated by an asterisk (*).

Acidoton urens Sw. JAMAICA. *Thorne 48242* (MO), KP794316, —. ***Bernardia dodecandra*** (Sessé ex Cav.) McVaugh. BELIZE. *Rees 176* (MO), KP794418, KP794459. ***B. nicaraguensis*** Standl. & L. O. Williams. NICARAGUA. *Stevens 30168* (MO), KP794419, KP794460. ***B. pulchella*** (Baill.) Müll. Arg. BRAZIL. *Kegler 1333* (MO), KP794420, KP794461. ***Bia alienata*** (1) Didr. BOLIVIA. *Carretero 1237* (MO), KP794320, KP794494*. ***B. alienata*** (2). PARAGUAY. *Pérez 352* (MO), KP794318, KP794492*. ***B. alienata*** (3). PARAGUAY. *Zardini 11174* (MO), KP794319, KP794493*. ***B. fendleri*** Müll. Arg. BOLIVIA. *Beck 8253* (MO), KP794321, KP794495*. ***Caryodendron grandifolium*** (Müll. Arg.) Pax. ECUADOR. *Korning 8502* (MO), KP794421, KP794462. ***C. orinocense*** Karst. BOLIVIA. *Gentry 70541* (MO), KP794422, KP794463. ***Cnesmone javanica*** (1) Blume. THAILAND. *Chamchumroon 2019* (L), KP794430, KP794498. ***C. javanica*** (2). THAILAND. *Larsen 44027* (MO), KP794427, KP794496. ***C. javanica*** (3). THAILAND. *Larsen 44147* (MO), KP794428, KP794497. ***C. laevis*** (Ridl.) Airy Shaw. THAILAND. *Smitinand 1180* (L), —, KP794499. ***C. laotica*** (Gagnep.) Croizat, THAILAND. *van Beusekom 4154* (L), KP794431, —. ***C. philippinensis*** (Merr.) Airy Shaw. PHILIPPINES. *Fenix 30010* (L), KP794429, —. ***Ctenomeria capensis*** (Thund.) Harv. ex Sond. SOUTH AFRICA. *Hugo 2107* (MO), KP794322, KP794562*. ***Dalechampia bernieri*** var. ***denisiana*** Leandri. MADAGASCAR. *Gillespie 10675* (CAN), KP794424, KP794501*. ***D. clematidifolia*** Bojer ex Baill. MADAGASCAR. *Gillespie 10815* (CAN), KP794426, KP794503*. ***D. subternata*** Mull. Arg. MADAGASCAR. *Gillespie 10810* (CAN), KP794425, KP794502*. ***D.*** aff. ***tamifolia*** Lam. MADAGASCAR. *Gillespie 10658* (CAN), KP794423, KP794500*. ***Gitara nicaraguensis*** (1) (Hemsl.) Card.-McTeag. & L. J. Gillespie. ECUADOR. *Korning 47437* (MO), KP794398, —. ***G. nicaraguensis*** (2). PANAMA. *Ibañez 2038* (MO), KP794397, KP794512. ***Haematostemon guianensis*** Sandwith. GUYANA. *Wurdack 4350* (US), KP794434, KP794464. ***Megistostigma yunnanense*** Croizat. THAILAND. *Larsen 46354* (L), KP794433, —. ***M. peltatum*** (J. J. Sm.) Croizat. THAILAND. *Maxwell 87-462* (L), KP794432, —. ***Pachystylidium hirsutum*** (Blume) Pax & K. Hoffm. THAILAND. *Keer 17893* (L), KP794408, —. ***Platygyna hexandra*** (Jacq.) Müll. Arg. CUBA. *Acevedo-Rodríguez 5566* (NY), KP794317, KP794504*. ***Plukenetia ankaranensis*** (1) L. J. Gillespie. MADAGASCAR. *Gillespie 10697* (CAN), KP794438, KP794470. ***P. ankaranensis*** (2). MADAGASCAR. *Lees s. n.* (CAN), KP794437, KP794469. ***P. brachybotrya*** Müll. Arg. BOLIVIA. *Araujo-M. 1722* (MO), KP794452, KP794473. ***P. carabiasiae*** J. Jiménez Ram. MEXICO. *Meave 1550* (MO), —, KP794480. ***P. conophora*** Müll. Arg. CAMEROON. *Nemba 434* (MO), KP794457, KP794472. ***P. corniculata*** Sm. BANGLADESH. *Huq 10780* (MO), KP794439, KP794471. ***P. lehmanniana*** (1) (Pax & K. Hoffm.) Huft & L. J. Gillespie. ECUADOR. *Acevedo-Rodríguez 1658* (MO), KP794443, KP794482. ***P. lehmanniana*** (2). ECUADOR. *Zak 3401* (MO), KP794442, KP794481. ***P. loretensis*** Ule. PERU. *Grandez 19608* (US), KP794453, KP794474. ***P. madagascarensis*** (1) Leandri. MADAGASCAR. *Gillespie 4175* (CAN), KP794441, KP794467. ***P. madagascarensis*** (2). MADAGASCAR. *Villiers 4899* (MO), KP794440, KP794468. ***P. penninervia*** (1) Müll. Arg. BELIZE. *Atha 1001* (MO), KP794455, KP794476. ***P. penninervia*** (2). MEXICO. *Martínez 10527* (MO), KP794456, KP794477. ***P. penninervia*** (3). PANAMA. *McPherson* 8461 (MO), KP794454, KP794475. ***P. polyadenia*** Müll. Arg. GUYANA. *Wurdack 5288* (US), —, KP794483. ***P. serrata*** (Vell.) L. J. Gillespie. BRAZIL. *Peixoto 4154* (MO), KP794458, KP794479.

P. stipellata (1) L. J. Gillespie. COSTA RICA. *Aguilar 8193* (MO), KP794448, KP794485. ***P. stipellata*** (2). COSTA RICA. *Liesner 3088* (MO), KP794451, KP794487. ***P. stipellata*** (3). COSTA RICA. *Morales 5342* (MO), KP794450, KP794484. ***P. stipellata*** (4). NICARAGUA. *Urbina 1155* (MO), KP794449, KP794486. ***P. supraglandulosa*** L. J. Gillespie. SURINAME. *Acevedo-Rodríguez 6022* (NY), —, KP794478. ***P. volubilis*** (1) L. BOLIVIA. *Nee 55162* (MO), KP794445, KP794489. ***P. volubilis*** (2). BOLIVIA. *Parada 206* (MO), KP794444, KP794488. ***P. volubilis*** (3). ECUADOR. *Burnham 1640* (MO), KP794446, KP794490. ***P. volubilis*** (4). PERU. *Bell 93-546* (US), KP794447, KP794491. ***Romanoa tamnoides*** (1) (A. Juss.) Radcl.-Sm. BOLIVIA. *Raes 177* (MO), KP794435, KP794465. ***R. tamnoides*** (2). BOLIVIA. *Raes 211* (MO), KP794436, KP794466. ***Sphaerostylis perrieri*** (1) Leandri. MADAGASCAR. *Gillespie 10738* (CAN), KP794413, KP794505. ***S. perrieri*** (2). MADAGASCAR. *Gillespie 10739* (CAN), KP794414, —. ***S. perrieri*** (3). MADAGASCAR. *Gillespie 10742* (CAN), KP794415, KP794506. ***S. perrieri*** (4). MADAGASCAR. *Labat 3441* (MO), KP794412, —. ***Tragia amblyodonta*** (Müll. Arg.) Pax & K. Hoffm. U. S. A. *B. L. 98-372* (MO), KP794376, KP794548. ***T. arnhemica*** (1) P. I. Forst. AUSTRALIA. *Brennan 2016* (DNA), KP794410, KP794513. ***T. arnhemica*** (2). AUSTRALIA. *Russel-Smith 5235* (DNA), KP794409, —. ***T. bahiensis*** (1) Müll. Arg. PARAGUAY. *Zardini 52840* (MO), KP794378, KP794542. ***T. bahiensis*** (2). PARAGUAY. *Zardini 54517* (MO), —, KP794543. ***T. benthamii*** Baker, TANZANIA. *Bidgood 4648* (MO), KP794328, KP794588. ***T. betonicifolia*** (1) Nutt. U. S. A. *Smith 3940* (MO), KP794364, KP794553. ***T. betonicifolia*** (2). U. S. A. *Summers 9529* (MO), KP794363, KP794552. ***T. boiviniana*** Müll. Arg. MADAGASCAR. *Birkinshaw 21* (MO), —, KP794579. ***T. brevipes*** (1) Pax. TANZANIA. *Bidgood 4686* (MO), KP794325, KP794565. ***T. brevipes*** (2). UGANDA. *Rwaburindore 4488* (MO), KP794329, KP794566. ***T. chlorocaulon*** (1) Baill. BRAZIL. *Dawson 15122* (MO), KP794390, KP794519. ***T. chlorocaulon*** (2). BRAZIL. *Irwin 15858* (MO), KP794391, KP794518. ***T. cocculifolia*** Prain. MADAGASCAR. *Razanatsoa 250* (MO), KP794331, —. ***T. cordata*** (1) Michx. U. S. A. *McDaniel 28890* (MO), KP794395, KP794529. ***T. cordata*** (2). U. S. A. *Thomas 76208* (CAN), KP794394, KP794528. ***T. cordata*** (3). U. S. A. *Thomas 171769* (MO), KP794396, KP794530. ***T. dioica*** (1) Sond. NAMIBIA. *Seydel 5539* (MO), KP794335, KP794568. ***T. dioica*** (2). NAMIBIA. *Volk 6173* (MO), KP794332, KP794567. ***T. durbanensis*** Kuntze. SOUTH AFRICA. *Balsinhas 3095* (MO), KP794333, KP794569. ***T. finalis*** P. I. Forst. AUSTRALIA. *Gillespie 7390* (CAN), KP794411, KP794514. ***T. furialis*** (1) Bojer ex Prain. MADAGASCAR. *Gillespie 10648* (CAN), KP794342, KP794575. ***T. furialis*** (2). MADAGASCAR. *Nusbaumer 2725* (MO), KP794343, KP794574. ***T. furialis*** (3). MAYOTTE. *Barthlet 171* (MO), KP794341, KP794573. ***T. furialis*** (4). TANZANIA. *Kayombo 5098* (MO), KP794340, KP794572. ***T.*** aff. ***furialis*** Bojer ex Prain. TANZANIA. *Lovett 3707* (MO), KP794344, KP794589. ***T. geraniifolia*** (1) Klotzsch ex Baill. ARGENTINA. *Krapovickas 20133* (MO), KP794368, KP794549. ***T. geraniifolia*** (2). PARAGUAY. *Peña-Chocarro 2240* (MO), KP794370, KP794550. ***T. giardelliae*** M. M. Gut. & Múlgura. ARGENTINA. *Guaglianone 2994* (MO), KP794360, KP794523. ***T. glanduligera*** Pax & K. Hoffm. MEXICO. *Tenorio 21300* (MO), —, KP794540. ***T. hieronymi*** Pax & K. Hoffm. PARAGUAY. *Krapovickas 45464* (MO), —, KP794531. ***T. hildebrandtii*** (1) Müll. Arg. TANZANIA *Abdallah 96/157* (MO), KP794355, KP794577. ***T. hildebrandtii*** (2). TANZANIA. *Abeid 575* (MO), —, KP794576. ***T. jonesii*** Radcl.-Sm. & Govaerts. MEXICO. *Felger 85-909* (MO), KP794377, KP794539. ***T. laciniata*** (Torr.) Müll. Arg. MEXICO. *Yatskievych 11-59* (MO), —, KP794561. ***T. laminularis*** (1) Müll. Arg. CÔTE D'IVOIRE. *Gautier-Béguin 592* (MO), KP794349, KP794582. ***T. laminularis*** (2). CÔTE D'IVOIRE. *Gautier-Béguin 959* (MO), KP794348, KP794581. ***T. melochioides*** (1) Griseb. ARGENTINA. Zuloaga 4896 (MO), —, KP794558. ***T. melochioides*** (2). BOLIVIA. *Coca 50* (MO), KP794373, KP794556. ***T. melochioides*** (3). BOLIVIA. *Lozano 1784* (MO), KP794372, KP794555. ***T. mexicana*** Müll. Arg. BELIZE. *Peña 1029* (MO), KP794393, KP794541. ***T. minor*** Sond. SOUTH AFRICA. *Balsinhas 3631* (MO), KP794338, KP794586. ***T. nepetifolia*** Cav. MEXICO. *García 446* (MO), KP794366, KP794527. ***T. novae-hollandiae*** (1) Müll. Arg. AUSTRALIA. *Bower S3* (CAN), KP794416, KP794515. ***T. novae-hollandiae*** (2). AUSTRALIA. *Gillespie 7394* (CAN), KP794417, KP794516. ***T. okanyua*** (1) Pax. BOTSWANA. *Long 386* (MO), KP794336, KP794590. ***T. okanyua*** (2). ZAMBIA. *Schmidt 2323* (MO), KP794337, KP794580. ***T. pacifica*** (1) McVaugh. EL SALVADOR. *Rosales 672* (MO), KP794389, KP794526. ***T. pacifica*** (2). EL SALVADOR. *Sandoval 1777* (MO), KP794388, KP794525*. ***T.*** cf. ***pacifica*** McVaugh. MEXICO. *Castrejón 753* (MO), KP794392, KP794524. ***T. paxii*** (1) Lourteig & O'Donnell. ARGENTINA. *Johnson 920* (MO), KP794380, KP794545. ***T. paxii*** (2). ARGENTINA. *Múlgura 2307* (MO), KP794379, KP794544. ***T. petiolaris*** Radcl.-Sm. TANZANIA. *Kuchar 25067* (MO), KP794354, KP794584. ***T.*** cf. ***petiolaris*** Radcl.-Sm. UGANDA. *ATBP 624* (MO), KP794351, KP794585. ***T. pinnata*** (Poir.) A. Juss. ARGENTINA. *Tressens 2202* (MO), KP794369, KP794551. ***T. plukenetii*** Radcl.-Sm. UGANDA. *Rwaburindore 5745* (MO), KP794356, KP794578. ***T. polyandra*** Vell. ARGENTINA. *Múlgura 2061* (MO), KP794375, KP794517. ***T. preussii*** Pax. CAMEROON. *Nkongmeneck 1400* (MO), KP794352, KP794592. ***T. pungens*** (1) (Forssk.) Müll. Arg. ETHIOPIA. *De Wilde 6406* (MO), KP794327, KP794593. ***T. pungens*** (2). SOMALIA. *Bally 16026* (MO), KP794326, KP794594. ***T. ramosa*** (1) Torr. U. S. A. *Hill 18339* (MO), KP794371, KP794559. ***T. ramosa*** (2). U. S. A. *Ricketson 4600* (MO), KP794367, KP794560. ***T. rogersii*** Prain. SOUTH AFRICA. *Ellery 92/96* (MO), KP794339, KP794595. ***T. rupestris*** Sond. SOUTH AFRICA. *Venter 9666* (MO), KP794334, KP794596. ***T. saxicola*** (1) Small. U. S. A. *Conell 40056* (MO), KP794386, KP794537. ***T. saxicola*** (2). U. S. A. *Kral 51842* (MO), KP794387, KP794538. ***T. smallii*** (1) Shinners. U. S. A. *McDaniel 28193* (MO), KP794401, —. ***T. smallii*** (2). U. S. A. *McDaniel 28698* (MO), KP794402, —. ***T. spathulata*** Benth. TOGO. *Breteler 7083* (MO), KP794350, KP794583. ***T.*** aff. ***subhastata*** (1) Poepp. BOLIVIA. *Serrano 6929* (MO), KP794358, KP794522. ***T.*** aff. ***subhastata*** (2). BOLIVIA. *Serrano 6965* (MO), KP794357, KP794521. ***T.*** cf. ***subhastata*** Poepp. BOLIVIA. *Serrano 5264* (MO), KP794359, KP794520. ***T. subsessilis*** Pax. TANZANIA. *Vollesen 96/44* (MO), KP794345, KP794591. ***T. tenuifolia*** Benth. CÔTE D'IVOIRE. *Gautier 2266* (MO), KP794353, —. ***T. uberabana*** Müll. Arg. ARGENTINA. *Zuloaga 5785* (MO), KP794374, KP794557. ***T. urens*** (1) L. U. S. A. *Anderson 7188* (MO), KP794407, —. ***T. urens*** (2). U. S. A. *Kral 41155* (MO), KP794406, KP794509. ***T. urens*** (3). U. S. A. *Thomas 55051* (CAN), KP794403, KP794507. ***T. urens*** (4). U. S. A. *Thomas 100075* (MO), KP794404, —.***T. urens*** (5). U. S. A. *Thomas 124328* (MO), KP794405, KP794508. ***T. urticifolia*** Michx. U. S. A. *Thomas 97259* (CAN), KP794365, KP794554. ***T. volubilis*** (1) L. COSTA RICA. *Hammel 19108* (MO), KP794362, KP794547. ***T. volubilis*** (2). NICARAGUA. *Stevens 29658* (MO), KP794361, KP794546. ***T. yucatanensis*** (1) Millsp. GUATEMALA. *Christenhusz 5678* (MO), KP794384, KP794534. ***T. yucatanensis*** (2). MEXICO. *Álvarez 4398* (MO), KP794382, KP794536. ***T. yucatanensis*** (3). MEXICO. *Lira 420* (MO), KP794385, KP794535. ***T.*** cf. ***yucatanensis*** (1) Millsp. GUATEMALA. Wallnöfer 5898 (MO), KP794381, KP794532. ***T.*** cf. ***yucatanensis*** (2). MEXICO. *Álvarez 6623* (MO), KP794383, KP794533. ***Tragiella anomala*** (1) (Prain) Pax & K. Hoffm. TANZANIA. *Luke 11120* (MO), KP794324, KP794564. ***T. anomala*** (2). TANZANIA, *Mwasumbi 16202* (MO), KP794323, KP794563. ***T. friesiana*** (1) (Prain) Pax & K. Hoffm. ZAMBIA. *Nkhoma 73* (MO), KP794346, KP794570. ***T. friesiana*** (2). ZAMBIA. *Nkhoma 74* (MO), KP794347, KP794571. ***T. natalensis*** (Sond.) Pax & K. Hoffm. TANZANIA. *Massawe 519* (MO), KP794330, KP794587. ***Zuckertia cordata*** (1) Baill. COSTA RICA. *González 1045* (MO), KP794399, KP794510. ***Z. cordata*** (2). MEXICO. *Calzada 1544* (MO), KP794400, KP794511.