The Systematics and Evolution of Euphorbiaceae Tribe Plukenetieae

Warren Martin Cardinal-McTeague
môswa ᒪᐦᔕ· moose

Department of Biology, Faculty of Science, University of Ottawa
Research and Collections, Canadian Museum of Nature
Department of Botany, National Museum of Natural History, Smithsonian Institution

A dissertation submitted to the
Faculty of Graduate and Postdoctoral Studies
at the University of Ottawa

In partial fulfillment of the requirements for the degree of
Doctorate of Philosophy in Biology

Supervised by:
Dr. Lynn J. Gillespie

My people will sleep for one hundred years, but when they awake, it will be the artists who give them their spirit back.

—Louis Riel, 1885

Métis/Cree floral beadwork moccasin vamps on deer hide, illustrating sacha inchi (*Plukenetia volubilis* L.) flowers (left) and fruit (right).
—by Warren Cardinal-McTeague, Ottawa ON, 2018

Table of Contents

Dedication V

Acknowledgements VI

Abstract IX

Statement of Contributions X

List of Tables XI

List of Figures XIV

List of Appendices XXII

List of Supplemental Data XXIII

Chater 1: Introduction 1

Relevance 1

Background 2

Aims 7

Motivation and approach 7

Chapter 2: Molecular Phylogeny and Pollen Evolution of Euphorbiaceae Tribe Plukenetieae 10

Abstract 11

Background 12

Methods 18

Results 23

Discussion 27

Taxonomic Treatment 46

Chapter 3: Seed Size Evolution and Biogeography of *Plukenetia* (Euphorbiaceae), A Pantropical Genus with Traditionally Cultivated Oilseed Species 52

Abstract 53

Background 54

Methods 58

Results 65

Discussion 75

Conclusions 87

Supplemental data 88

Chapter 4: A Revised Sectional Classification of *Plukenetia* L. (Euphorbiaceae, Acalyphoideae) with Three New Taxa from South America 114

Abstract 115

Background 116

Methods 120

Results 123

Taxonomic Treatment 127

Chapter 5: Biome Shifts, Not Innovative Traits or Biogeography, Drive Diversification Rates of Pantropical Euphorbiaceae Vines (Tribe Plukenetieae) 172
Abstract 173
Background 174
Methods 177
Results 183
Discussion 191

Chapter 6: Conclusions 245

Literature Cited 249

Supplemental Data 270

Dedication

To my family
who's endless love and support
has brought me to this stage.

Marilyn Gloria Cardinal
Nora Rosalie Cardinal
John William Cardinal

James Warren McTeague
Eleanor Marie McTeague
James Hugh McTeague

Acknowledgements

I acknowledge that the land I stand and write these words on, and spent the last seven years living and studying on, is the unceded and unsurrendered territory of the Anishinaabe – Algonquin People of Ontario and Quebec. I thank them, and the other First Nation, Inuit, and Métis people I met in Ottawa/Gatineau, for their friendship, kindness, and community.

In Canada, the subject of education would be incomplete without discussion of the Indian Residential School system. Since before Confederation (1867) until 1996, Indigenous children were forcibly removed from their families, homes, and communities to attend church and government run Residential Schools. The aim was to prevent Indigenous children from learning or practicing their language, religion, and culture, and ultimately assimilate them into colonial society. Physical and sexual abuse, disease, and malnutrition were widespread in the Residential School system, and a minimum of 6,000 children perished in the care of the government and church. Over 150,000 children survived the Residential School system, and they and their descendants are still healing from the long-term effects of intergenerational trauma.

Readers of my dissertation are strongly encouraged to examine the report of the Truth and Reconciliation Commission of Canada (www.trc.ca) and support the legacy of the commission through their 94 Calls to Action. In particular, I feel strongly about calls (11) to end the backlog of First Nations students seeking post-secondary education through adequate Federal funding (which I interpret to include support for Inuit and Métis students), and (16) to support language rights by the creation of university and college degree and diploma programs in Indigenous languages. At some point we will have a discussion about how to incorporate Indigenous knowledge and methodologies into science. In the meantime, we must work to dismantle barriers to education and encourage, support, and include diversity of all people in academia.

It is with honour and privilege that I am the first member of the Métis/nêhiyaw(Plains Cree) side of my family to pursue an extensive post-secondary education and doctoral degree. This is a bittersweet honour to carry. The opportunities I received were impossible for previous generations of my family and community, in part due to racism, social/economic disparity, and mistrust of the colonial education system. I am grateful for the hard work of my mother, her parents, and their parents before them; everything they worked towards was for the betterment of their children. Because of their sacrifices, I am able to stand where I am today. I am hopeful that more Indigenous students will receive the same opportunities. Hiy hiy, kinanâskomitin.

My doctoral program took seven years to complete, so bear with me, that's a long time with many moments and people to reflect on. First, I thank my supervisor, Dr. Lynn J. Gillespie, for her guidance, mentorship, patience, and understanding over the years. I came into your lab with much of the relevant experience, but you taught me how to write, conduct tropical field work, to pay attention to every detail, and about endless aspects of taxonomy and evolution, especially about Euphorbiaceae. You are a wealth of knowledge and a wonderful mentor.

Second, I thank the many people at the Canadian Museum of Nature who made the workplace and Ottawa/Gatineau feel like home. A big shout out goes to Paul Sokoloff for his ongoing friendship and support, both in and outside of the museum. Others include Roger Bull, Dr. Geoffrey Levin, and Dr. Jeff Saarela, for their helpful discussions. I had the pleasure of working alongside countless students and volunteers over the years, including (but not limited to): Neda Amiri, Dr. Alicia Alonso Mata, Katya Boudko, Dr. Sabina Donadio, Dr. Sharon Ebata, Claire Gilmour, Anna Ginter, Sam Godfrey, Simon Grafe, Frankie Janzen, Dr. Shan Leung, Dr. Étienne Léveillé-Bourret, Irene McKechnie, Alex Menna, Michel Paradis, Mary Ann Perron, Marianne Racine, and Cassandra Robillard. Thank-you for your friendship and company. I also thank the students and professors I met during the Organization for Tropical Studies (OTS) Tropical Plant Systematics field course (2014) and my Smithsonian Institution Predoctoral Fellowship (2015), with shout outs to Dr. Laura Frost and Erika Gardner. Lastly, special thanks go to Dr. Kenneth J. Wurdack for hosting my Predoctoral Fellowship, teaching me lab techniques, and for sharing his great enthusiasm and knowledge of the Euphorbiaceae family.

My experience in Ottawa/Gatineau would have been incomplete without the friendship and support of the Métis community. A big kinanâskomitin to Sahra MacLean, Brittany

Mathews, and Jaime Morse, who all happen to be from Alberta. Special thanks to Dr. Ashley (Az) Klymiuk, another Alberta Métis, for her ongoing friendships and mentorship. Although separated by distance, our online buddy system kept us motivated and on track (perhaps with the help of a few thousand screaming porgs). Endless thanks as well to my second family, Paul Sokoloff, David Carpenter, Jessica Freeborn, and Jeff Saarela.

Of course, my whole experience changed in the summer of 2014 when I met my loving partner, François-René Dussault. From the moment we met (and I invited myself on your trip to Paris!) we have been inseparable and have shared countless adventures. You taught me the importance of living life in the moment, enjoying a healthy home cooked meal, and begrudgingly making the bed each morning. Your love and support made a world of difference, especially during last stages of my degree. Je t'aime, et je promets d'apprendre à mieux parler français. We encourage readers to live life in the moment and consider registering as an organ donor (www.beadonor.ca).

Last but not least, this would not have been possible without the love and support of my family back in Alberta. I have been far from home for too long, but their unwavering encouragement, vocal pride, and boundless love have helped me continue through the challenges. They knew I had important work to get through, and I am grateful for their patience and understanding. To my mom and dad, my sisters and brother, my aunties and uncles, my grandma and grandpa, and my kokum and mushum, thank-you so much, I love you.

Funding for my research was provided by the Natural Sciences and Engineering Research Council of Canada's (NSERC) Alexander Graham Bell Canada Graduate Scholarships Master's (CGS M) and Doctoral (CGS D) Programs, a Systematics Research Graduate Supplement at the Canadian Museum of Nature, and Michael Smith Foreign Study Supplement for Washington, DC. I also received a Smithsonian Institution Predoctoral Student Fellowship held at the National Museum of Natural History under the supervision of Dr. Kenneth J. Wurdack, an Indspire Post-Secondary Education Bursary during a time in need, and generous travel grants and tuition scholarships from the University of Ottawa Faculty of Graduate and Postdoctoral Studies.

Abstract

The aim of this dissertation is to study the systematics and evolution of Euphorbiaceae vines (tribe Plukenetieae), a diverse pantropical lineage (~365 species and 18 genera) composed of three morphologically distinct subtribes, Dalechampiinae, Plukenetiinae, and Tragiinae. Through the course of my research I largely resolved the evolutionary history of Plukenetieae and made broader contributions to the study of pollen and seed evolution, pantropical biogeography, and plant diversification. In chapter two I developed the first well-sampled molecular phylogeny for Plukenetieae (154 terminals, ~93 species, 2,207 character dataset composed of ITS and *psbA-trnH* with indel gap-scored data), and determined baseline species group relationships of the tribe. Molecular phylogeny largely agreed with pollen morphology hypotheses and confirmed that the large genus *Tragia* was para- and/or polyphyletic and should be split into smaller genera. Analysis of pollen morphology revealed a trend towards aperture reduction and loss in Tragiinae, with four origins of weakly defined apertures and up to three origins of inaperturate pollen. In chapter three, I studied the seed size evolution of *Plukenetia*, a pantropical genus with large edible oil-rich seeds, by developing a near-exhaustive phylogeny (83 terminals, 20 of ~24 species, 5,069 bp dataset of ETS, ITS, *KEA1* introns 11 and 17, *TEB* exon 17, *matK*, *ndhF*) and conducting ancestral state estimation and phylogenetic regression. Seed size evolution in *Plukenetia* was dynamic and associated with competing selective pressures of plant size, fruit type (and inferred dispersal syndrome), and seedling ecology. In chapter four I presented a revised sectional classification of *Plukenetia* based on phylogeny and morphological evidence, including three new taxa from South America. Chapters three and five included biogeographical investigations on *Plukenetia* and Plukenetieae. Analyses revealed that pantropical disjunct distributions arose one to three times in each subtribe via periodic long-distance dispersals from the Oligocene to the Pliocene, most often from South America to Africa and then Southeast Asia. Lastly, in chapter five, I developed an improved phylogeny for Plukenetieae (289 terminals, ~109 species, 5,160 bp dataset of ETS, ITS, *KEA1* intron 11, *TEB* exon 17, *matK*, *ndhF*) to study the influence of innovative traits (twining growth form, stinging hair defences, and pseudanthial inflorescences) on diversification in the tribe. However, increased diversification was not associated with innovative traits. Instead, diversification was associated with clades that shifted into drier open habitats, aided by habitat expansion following the Late Miocene cooling period.

Statement of Contributions

I, Warren Cardinal-McTeague, contributed to the majority of the work performed for the completion of this dissertation. This includes the DNA sequencing lab work (extractions, amplification, sequencing, clean-up/editing), phylogenetic analyses, biogeographic and evolutionary analyses, roughly half of the seed size measurements, and analysis of herbarium specimens for sectional and new species descriptions. I wrote the four first-author papers included in this dissertation: Chapter 2 has been published (Cardinal-McTeague & Gillespie, 2016), Chapter 3 is under revised submission (Cardinal-McTeague *et al.*, in review), Chapter 4 is nearly ready for submission (Cardinal-McTeague & Gillespie, in prep), and Chapter 5 is in its first draft (Cardinal-McTeague *et al.*, in prep).

My PhD supervisor and external researchers also contributed to this work. Dr. Lynn J. Gillespie contributed to the overall development, direction, and review of each manuscript in the dissertation. Dr. Kenneth J. Wurdack hosted me during a three month Smithsonian Institution Predoctoral Fellowship from September to December 2015, providing invaluable laboratory training, and contributed to the project development and data collection of rare and challenging specimens of Chapters 3 and 5. Dr. Erin. M. Sigel, a former Postdoctoral Fellow at the Smithsonian Institution, developed the modified transcriptome/genome data-mining pipeline LITE BLUE DEVIL v0.3 used in Chapter 3. All contributors provided helpful comments on the manuscripts they co-authored, and approved the final content prior to submission.

List of Tables

Table 1.1 Species number, growth form, and geographic distribution of genera in Euphorbiaceae tribe Plukenetieae.

Table 2.1 Morphological characters for the subtribes of Plukenetieae.

Table 2.2 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.

Table 2.3 Infrageneric classifications (including informal species groups) of *Plukenetia* (sensu Gillespie, 1993, 2007) and *Tragia* (sensu Pax & Hoffmann, 1919a, with modifications by: Miller & Webster, 1967; Leandri, 1971; Gillespie, 1994a; Webster, 2007, 2014). *Tragia* sects. *Leucandra* and *Ratiga* are regarded as synonyms of sect. *Tragia* (Miller & Webster, 1967; Múlgura de Romero & 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 & 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 & Hoffmann, 1919a; Forster, 1994).

Table 2.4 Characteristics for individual and combined DNA sequence (ITS, *psbA-trnH*) and *psbA-trnH* indel gap-scored (including one hand-scored *psbA-trnH* inversion character) datasets.

Table 2.5 Distinguishing characters for *Gitara* and the sections of *Acidoton*.

Table 3.1 Molecular dataset characteristics.

Table 3.2 Summary of seed measurements for *Plukenetia*, *Romanoa*, and *Haematostemon* (min–max [mean]). Size categories: (S) small, 25–125 mm^3; (M) medium, 125–500 mm^3; (L) large, 500–3,000 mm^3; (XL) extra-large, 3,000–13,000 mm^3; (Max) maximum, 26,000–38,000 mm^3. † = measurements based solely on the species description; n/a = seeds not known.

Table 3.3 Results of phylogenetic regression analysis using the threshold model.

Table 3.4 Diagnostic characters of the high elevation species complex (*Plukenetia carolis-vegae*, *P.* cf. *carolis-vegae*, *P. huayllabambana*) and *P. volubilis*. Most characters were updated from species descriptions; † = unverified and potentially inaccurate.

Table 3.5 Traits associated with seed size for species of *Plukenetia* and outgroup genera (arranged by size). DDF = dry deciduous forest; ERF = evergreen rain forest; SMF = seasonal moist forest.

Table 3.S1 Accession table for vouchers used in phylogenetic analyses.

Table 3.S2 Accession table for genomes/transcriptomes used in data-mining analyses.

Table 3.S3 List of molecular markers, primer sequence, and amplification/sequencing protocols.

Table 3.S4 Biogeographical distribution matrix (A = Mexico to NW South America, B = Amazon/Guiana, C = Atlantic Forest, D = Tropical Africa, E = Madagascar, F = SE Asia).

Table 3.S5 Manual dispersal matrix between biogeographical areas (adjacent = 1.0, short-distance dispersal = 0.5, long-distance dispersal = 0.1).

Table 3.S6 Trait matrix used in phylogenetic regression analysis with seed size. Plants size (0 = slender stems; 1 = robust to thick stems), seedling ecology (0 = light gap or forest edge; 1 = shade avoidance, canopy liana strategy), fruit type/ hypothesized dispersal mechanism (0 = dry, dehiscent/ scatter hoarding rodents; 1 = fleshy, indehiscent/ mammal predation), biome (0 = wet dominant; 1 = dry component), fire tolerance (0 = non-fire adapted; 1 = fire adapted).

Table 3.S7 The best evolutionary model for seed size based on ΔAIC and Akaike weights (w_i).

Table 4.1 Generic and sectional classifications of *Plukenetia* and *Romanoa* (NWSG2 = New World species group "2"; [a] = genera described by Pax & Hoffmann, 1919a).

Table 4.2 Revised sectional classification of *Plukenetia* with species number, distribution, habitat, and growth forms of each section, including *Romanoa*.

Table 4.3 Morphological differences between the sections of *Plukenetia*, including *Romanoa*.

Table 5.1 Genera of tribe Plukenetieae and its sister group (including tribes Bernardieae, Caryodendreae, and *Avellanita*).

Table 5.2 Molecular datasets and their characteristics.

Table 5.3 Results of the approximately unbiased (AU) test examining if enforced competing gene tree backbone topologies are significantly worse than the unconstrained Concatenated dataset.

Table 5.4 Best fitting models of trait-dependent diversification for growth form, plant defence, and inflorescence type, based on ΔAIC and Akaike weights (w_i). Traits with multiple models of good fit (ΔAIC < 2) are shown. Models used for 'MarginRecon' analysis are

indicated by an asterisk (*); note that reconstructions under CID-4 are currently not permitted, so the second best model was used for inflorescence type.

Table 5.S1 Accession table with voucher information for taxa used in phylogenetic analyses.

Table 5.S2 Biogeographical distribution matrix for BIOGEOBEARS analyses of Plukenetieae (A = Central + South America, B = North America, C = Caribbean, D = Africa + South Arabia, E = Madagascar, F = South + Southeast Asia; G = Australasia).

Table 5.S3 Manual dispersal matrix between biogeographical areas for two time periods for BIOGEOBEARS analyses of Plukenetieae (adjacent = 1.0, short-distance dispersal = 0.5, long-distance dispersal = 0.1).

Table 5.S4 Proportion of extant species sampled per designated terminal for BAMM analysis of Plukenetieae.

Table 5.S5 Trait matrix for HISSE analysis of Plukenetieae: Growth form (0 = non-twining; 1 = twining), plant defences (0 = non-stinging; 1 = stinging), pseudanthial inflorescences (0 = non-pseudanthial; 1 = pseudanthial). Accessions with a number were randomly added to the BEAST maximum clade credibility tree to balance undersampled clades (see methods).

Table 5.S6 The fit of alternative models of trait-dependent diversification in Plukenetieae, with best models selected by ΔAIC and Akaike weights (w_i) in bold.

List of Figures

Figure 1.1 Representative habit (a–c) and inflorescence (d–f) diversity for the subtribes of Plukenetieae. Dalechampiinae: (a) stinging twining vines, (d) pseudanthial inflorescence composed of pistillate and staminate cymules and resiniferous glands subtended by two white involucral bracts (*Dalechampia* sp.; *Medeiros & Cardinal-McTeague 562*, R). Plukenetiinae: (b) non-stinging twining vines and slender lianas, (e) racemose thyrse with a proximal pistillate flower and two to three flowered distal staminate cymules in bud (*Plukenetia volubilis* L.; *Wurdack s.n.*, US). Tragiinae: (c) stinging twining vines and subshrubs, (f) raceme with proximal pistillate flower and distal staminate flowers (*Tragia volubilis* L.; *Medeiros & Cardinal-McTeague 556*, R).

Figure 2.1 Representative inflorescences for the subtribes of Plukenetieae. (a) Dalechampiinae: pseudanthial inflorescence of *Dalechampia* sp. (*Medeiros & 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 & Cardinal-McTeague 561*, R) with proximal pistillate flower and distal staminate flowers (scale bar = 3 mm). Inset, staminate flower.

Figure 2.2 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%.

Figure 2.3A 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 (* indicate 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. 2.3B.

Figure 2.3B Continuation of Fig. 2.3A. 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 (* indicate 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.

Figure 2.4 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 (1994b) for Plukenetiinae and Tragiinae, Nowicke & 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.

Figure 3.1 Seed size variation in *Plukenetia* and *Romanoa*. (a) *Plukenetia polyadenia* (*van der Werff 16350*, MO); (b–c) *P. huayllabambana* (*Gruhn 84*, MO); (d–e) *P. volubilis* (*Nee 35694*, MO); (f–g) *P. stipellata* (*Zambraver 1047*, MO); (h) *P. lehmanniana* (*Daniel 4943*, US); (i–j). *P. africana* (*Bartsch 1859*, MO); (k–l) *P. supraglandulosa* (*Granville 10783*, US); (m–n) *P. loretensis* (*Krukoff 1031*, MO); (o–p) *P. verrucosa* (*Barrabé & Crozier 145*, US); (q–r) *Romanoa tamnoides* (*Zardini & Cardozo 44939*, MO). Dual orientations (face and side) for most species to show flattened lenticular shape. Source: Vouchers in parentheses. Single photograph taken with a Nikon D800.

Figure 3.2 Bayesian maximum clade credibility tree based on the combined seven marker (cpDNA and nDNA), 83 accession dataset for *Plukenetia* and Plukenetiinae outgroups. Maximum parsimony bootstrap percentage (MPBP) and Bayesian posterior probabilities (PP) support values > 50% are indicated on each branch. Branches in bold indicate strong support (≥ 85 MPBP and ≥ 0.95 PP). Subclade numbering system (P1–P5) follows Cardinal-McTeague & Gillespie (2016). Inset, leaf clearings demonstrating pinnately-veined (*P. supraglandulosa*, *Granville 3626*, CAY) and palmately-veined (*P. stipellata*, *Gillespie 413*, US) leaf architecture.

Figure 3.3 Ancestral range estimation on the *Plukenetia* BEAST chronogram using BIOGEOBEARS (DEC+J model). Areas of tip species are shown left of taxa names, color-coded for the six biogeographical areas depicted on the map inset. Boxes at each node and corner are color-coded for the area (or combined area, up to two allowed) with the highest maximum likelihood probability. Pie charts indicate the probability of each area and are included when there is < 75% confidence for a single area. Numbers at each node indicate mean age estimates, blue bars the 95% highest posterior density (HPD) confidence interval, and yellow bars the 95% HPD of calibrated nodes estimated under constraint. Arrows on the map indicate the general direction of range movement in *Plukenetia*; dashed lines indicate area reversals.

Figure 3.4 Ancestral state estimation of $\log_{10}$(estimated seed volume) mapped on the modified *Plukenetia* BEAST chronogram using the ContMap function of PHYTOOLS.

Figure 3.5 Traitgram of $\log_{10}$(estimated seed volume) mapped on the modified *Plukenetia* BEAST chronogram using the phenogram function of PHYTOOLS. The colored scale on the left depicts seed size categories based on clustering analysis. On the right, representative seeds are shown in face and side view (see Fig. 3.1 for accession information).

Figure 3.S1 Primer map for new (*KEA1* introns 11 and 17, *TEB* exon 17) and redesigned (ETS, *matK*) molecular markers.

Figure 3.S2 Shortest maximum likelihood tree for incongruence analysis of individual markers: (a) ETS, (b) ITS, (c) *KEA1* intron 11, (d) *KEA1* intron 17, (e) *TEB* exon 17, (f) *matK*, and

(g) *ndhF*. Bootstrap percentages based on 500 replicates. Well-supported branches (> 85% maximum likelihood bootstrap percentage; MLBP) are in bold.

Figure 3.S3 Bayesian maximum clade credibility tree based on (a) plastid DNA (cpDNA) two marker, 74 accession dataset, and (b) nuclear DNA (nDNA) five marker, 86 accession dataset, for *Plukenetia* and Plukenetiinae outgroups. Maximum parsimony bootstrap percentage (MPBP) and Bayesian posterior probability (PP) support values > 50% are indicated on each branch. Branches in bold indicate strong support (≥ 85 MPBP and ≥ 0.95 PP). Grey boxes highlight strongly supported topological incongruences.

Figure 3.S4 BEAST chronogram of *Plukenetia* and Plukenetiinae outgroups inferred from the combined seven marker (cpDNA and nDNA), 83 accession dataset and two normal-distribution priors (indicated in red) based on previous subfamily Acalyphoideae estimates using three fossil calibrations. Numbers at each node indicate mean age estimates, and blue bars the 95% highest posterior density confidence interval.

Figure 3.S5 BIOGEOBEARS ancestral range estimation probabilities for each corner and node under (a) DEC+J and (b) DEC.

Figure 3.S6 UPGMA clustering analysis of $\log_{10}$ transformed seed dimensions (length, width, thickness) for 190 accessions of *Plukenetia*.

Figure 3.S7 Principal components analysis of $\log_{10}$ transformed seed dimensions (length, width, thickness) for 190 accessions of *Plukenetia*.

Figure 3.S8 Posterior distributions of the correlation coefficients (*r*) between the liabilities of seed size and (a) plant size, (b) fruit type, (c) seedling ecology, (d) fire tolerance, and (e) biome type, under the threshold model.

Figure 4.1 Summary cladogram of the relationships recovered by Bayesian analysis of combined and partitioned ETS, ITS, *KEA1* introns 11 and 17, *TEB* exon 17, *matK*, and *ndhF* DNA sequence data of *Plukenetia* and *Romanoa* (modified from Cardinal-McTeague *et al.*, in review), highlighting our revised sectional classification of *Plukenetia*. Maximum parsimony bootstrap percentage (MPBP) and Bayesian posterior probability (PP) support values > 50% are indicated on each branch. Branches in bold indicate strong support (≥ 85 MPBP and ≥ 0.95 PP). Subclade numbering system (P1–P5) follows Cardinal-McTeague & Gillespie (2016). Sections in the pinnately-veined clade (P1 + P2) are indicated by light grey boxes, sections in the palmately-veined clade (P3–P5) by dark grey boxes. (a–b) Leaf clearings demonstrating leaf architecture (images from Gillespie 1993): (a) *P. stipellata* (*Gillespie 413,* US); (b) *P. supraglandulosa*, (*Granville 3626*, CAY). (c–d) Scanning electron micrographs demonstrating pollen tectum morphology (images from Gillespie, 1994b): (c) *P. stipellata* (*Gillespie 418*, DAV); (d) *P. loretensis* (*Maguire & Politi 27371*, US).

Figure 4.2 Representative staminate flower diversity of *Plukenetia* and its sister genus *Romanoa*. (a) *Romanoa tamnoides* (*Krapovickas & Schinini 36265*, MO), stamens with slender-cylindrical filaments. (b) *P.* sect. *Fragariopsis*: *P. serrata* (*Davidse et al. 10480*, MO), anthers sessile and loosely packed on a large globose receptacle. (c–e) *P.* sect. *Penninerviae*: (c) *P. loretensis* (*Freitas et al. 155*, MO), anthers sessile and densely packed on a small globose receptacle; (d) *P. penninervia* (*van der Werff et al. 3173*, MO), and (e) *P. supraglandulosa* (*Granville 3626* CAY, US), most anthers sessile and densely packed on a small globose receptacle, with a dimorphic outer whorl of ~4 stamens with filaments, and often with a 4–lobed annular nectary. (f–i) *P.* sect. *Plukenetia*: (f) *P. polyadenia* (*Croat et al. 20285*, MO), stamens with slender-cylindrical filaments and ligulate interstaminal nectaries; (g) *P. stipellata* (*Cardinal-McTeague 8*, CAN), stamens with slender-cylindrical filaments and small irregularly shaped interstaminal nectary segments; (h) *P. carolis-vegae* subsp. *nectarifera* (*Woytkowski 6670*, MO), stamens with slender filaments and large irregularly shaped interstaminal nectary segments; (i) *P. volubilis* (*Wurdack s.n.*, US), stamens with short-conical filaments. (j) *P.* sect. *Angostylidium*: *P. conophora* (*Avio et al. 1621*, MO), stamens with short-conical filaments and slender-cylindrical interstaminal nectaries (in pink). (k) *P.* sect. *Hedraiostylus*: *P. corniculata* (*Chin See Chung 2712*, L), stamens with short-conical filaments (sometimes appearing sessile). (l) *P.* sect. *Madagascarienses*: *P. madagascariensis* (*Villiers et al. 4889*, MO), anthers more or less sessile, densely to somewhat loosely packed on an elongate receptacle. Photos (a–d, f–h, j, l) by W.Cardinal-McTeague; (i) by K.Wurdack. Line drawing (e) by Alice Tangerini, (k) by Anita Walsmit Sachs, used with permission from the Nationaal Herbarium Nederland. (Abbreviations: an = annular nectary, d = dimorphic filamentous stamens, in = interstaminal nectaries, r = receptacle. Scale bars = 1 mm).

Figure 4.3 Representative pistillate flower diversity of *Plukenetia* and its sister genus *Romanoa*. (a) *Romanoa tamnoides* (*Krapovickas & Schinini 36265*, MO), styles 70–80% connate into a cylindrical column. (b) *P.* sect. *Fragariopsis*: *P. serrata* (*Davidse et al. 10480*, MO), styles entirely connate into an obovoid column. (c–f) *P.* sect. *Penninerviae*: (c) *P. brachybotrya* (*Fuentes et al. 5398*, MO) and (d) *P. verrucosa* (*Prance et al. 11255*, DAV), styles entirely connate into a globose column; (e) *P. loretensis* (*Silva et al. 4750*, MO), styles entirely connate into a slender-cylindrical column, dilated at the apex; (f) *P. penninervia* (*Wallnöfer et al. 5996*, MO), styles entirely connate into a stout-cylindrical column. (g–k) *P.* sect. *Plukenetia*: (g) *P. polyadenia* (*Aulestia & Grefa 230*, MO), (h) *P. stipellata* (*Cardinal-McTeague 8*, CAN), (i) *P. volubilis* (*Wurdack s.n.*, US), and (j) *P. carolis-vegae* subsp. *nectarifera* (*Woykowski 6670*, MO), styles 70–95% connate into a cylindrical column; (k) *P. lehmanniana* (*Rangel et al. 5734*, MO), styles 20–25% connate into a cylindrical column, free style arms 2–fid near the tips. (l) *P.* sect. *Angostylidium*: *P. conophora* (*Thomas 5455*, MO), styles 75–(90)% connate into a funnel-shaped column, free style arms conspicuously dilated and spreading. (m) *P.* sect. *Hedraiostylus*: *P.*

corniculata (*Brink 5771*, L), styles entirely connate into a depressed-globose column with cross-shaped stigmas. (n–o) *P.* sect. *Madagascarienses*: (n) *P. ankaranensis* (*Gillespie 4076*, CAN), styles entirely connate into an obconic or obovoid column; (o) *P. madagascariensis* (*Andrianjafy et al. 1648*, MO), styles 55–60% connate into a cylindrical column, free style arms slender and tapered. Photos (a–c, e–h, j–l) by W.Cardinal-McTeague, (i) by of K.Wurdack, (n) by L.Gillespie, (o) by P.Phillipson. Line drawing (d) by Cathy Pasquale, (k) by Anita Walsmit Sachs, used with permission from the Nationaal Herbarium Nederland. (Scale bars = 1 mm).

Figure 4.4 Bayesian maximum clade credibility tree based on an 84 accession nrDNA ETS dataset for *Plukenetia* and Plukenetiinae outgroups. Maximum likelihood bootstrap percentage (MLBP) and Bayesian posterior probability (BPP) support values > 50% are indicated on each branch. Branches in bold indicate strong support (≥ 80 MLBP and ≥ 0.95 BPP). Subclades P1–P5 were defined in Cardinal-McTeague & Gillespie (2016). Sections in the pinnately-veined clade (P1 + P2) are indicated by light grey boxes, sections in the palmately-veined clade (P3–P5) by dark grey boxes.

Figure 4.5 *Plukenetia brevistyla* sp. nov. (a) Branch with mature leaves, inflorescences, and fruit. (b) Capsule. (c) Pistillate flower. (d) Abaxial leaf blade with laminar extrafloral nectaries. (e) Adaxial leaf blade with basilaminar extrafloral nectaries. (f) Close-up of inflorescences. (g) Close-up of staminate cymules. (h) Staminate flower. Photos by W.Cardinal-McTeague. Source: (a, c, f–g) *Lowrie et al.* 30 (MO, NY); (b) *Silva et al. 1150* (NY); (d–e, h) *Oliveira 4513* (NY). (Abbreviations: an = annular nectary; o = ovary; s = style. Scale bars: [a] = 1 cm; [b–h] = 1 mm).

Figure 4.6 *Plukenetia megastyla* sp. nov. (a) Branch with mature leaves and inflorescences. (b) Capsule. (c) Capsule segment with seed. (d) Columella. (e) Seed, lateral view (left), ventral view (right). (f) Close-up of staminate cymules. (g) Staminate flower. (h) Pistillate flower. (i) Adaxial leaf blade with basilaminar extrafloral nectaries. (j) Abaxial leaf blade with laminar extrafloral nectaries. (k) Close-up of inflorescence. Photos by W.Cardinal-McTeague. Source: (a) G. *Silva et al. 6161* (NY); (b, i–j) *Plowman 8741* (NY); (c–e) *Ledezma et al. 921* (CAN); (f, h, k) *Nee 38682* (NY). (Abbreviations: an = annular nectary; o = ovary; s = style. Scale bars: [a] = 1 cm; [b–k] = 1 mm).

Figure 4.7 Distribution map of our new species in sect. *Penninerviae*, *P. brevistyla* and *P. megastyla*, and members of the high elevation species complex in sect. *Plukenetia*, *P. carolis-vegae* subsp. *carolis-vegae*, *P. carolis-vegae* subsp. *nectarifera*, and *P. huayllabambana*. (Map data ©2018 Google, Imagery ©2018 NASA, TerraMetrics).

Figure 5.1 Inflorescence diversity of Euphorbiaceae vines (tribe Plukenetieae). Subtribe Dalechampiinae (a–b), bilaterally symmetrical pseudanthia composed of staminate and pistillate cymules, sometimes with resiniferous glands, subtended by two large often

showy bracts: (a) *Dalechampia websteri* Armbr. (*Cardinal-McTeague 7*, CAN), (b) *D.* aff. *tamifolia* Lam. (*Gillespie et al. 10658*, CAN). Subtribes Plukenetiinae (c–d) and Tragiinae (e–i), racemose thyrses or racemes: (c) *Plukenetia loretensis* Ule (*Grandez 19608*, US), (d) *P. volubilis* L. (*Wurdack s.n.*, US), (e) *Megistostigma cordata* Merr. (*Davidson 12936*), (f) *Tragia furialis* Bojer ex Prain (*Gillespie et al. 10648*, CAN), (g) *T. urens* L. (*Wurdack s.n.*, US), (h) *T. urticifolia* Michx. (*Wurdack s.n.*, US), (i) *T. volubilis* L. (*Medeiros & Cardinal-McTeague 556*, R). Photographs by: W.Cardinal-McTeague (a–b, f, i), C.Davidson (e), K.Wurdack (c–d, g–h).

Figure 5.2 Summary cladograms of the alternative backbone topologies of Plukenetieae recovered from: (a) cpDNA; (b) ETS; (c) ITS; (d) *TEB* exon 17. Blue nodes are congruent with the Concatenated dataset (Fig. 5.3C), yellow nodes are incongruent; grey clades with solid edges are congruent, grey clades with dashed edges are partially congruent but have alternative branching patterns. Bold branches indicate strong support (≥ 85% maximum likelihood bootstrap percentage [MLBP] and ≥ 0.95 Bayesian posterior probability [BPP]); dashed branches indicate low support (< 60% MLBP and < 0.8 BPP). An asterisk (*) indicates a member of the clade was not sampled. See Fig. 5.3 for an explanation of abbreviations.

Figure 5.3 Summary cladograms of the alternative backbone topologies of Plukenetieae recovered from (a-b) coalescence-based and (c) total evidence methods. (a) ASTRAL maximum quartet support (MQS) species tree topology based on Bayesian maximum clade credibility (MCC) trees from all six regions. Node support is based on 200 multilocus bootstrap replicates. (b) *BEAST MCC topology based on the reduced five region, "no-gap" dataset (177 terminals). (c) Concatenated dataset Bayesian MCC topology. Blue nodes are congruent with the Concatenated dataset (Fig. 5.3C), yellow nodes are incongruent; grey clades with solid edges are congruent, grey clades with dashed edges are partially congruent but have alternative branching patterns. Bold branches indicate strong support (≥ 85% Multilocus bootstrap percentage [BP] or maximum likelihood BP [MLBP] and/or ≥ 0.95 Bayesian posterior probability [BPP]); dashed branches indicate low support (< 60% Multilocus BP or MLBP, and/or < 0.8 BPP).

Figure 5.4 Ancestral range estimation (ARE) on the Plukenetiaeae BEAST chronogram using BioGeoBEARS (DEC+J model). Areas of tip species are shown left of taxon names, colour-coded for the seven biogeographical areas depicted on the inset map. Boxes at each node and corner are colour-coded for the area or combined area (up to five allowed) with the highest likelihood probability. Pie charts indicate the probability of each area and are included if there was < 75% confidence for a single area. Numbers at each node indicate mean age estimates, blue bars the 95% highest posterior density (HPD) confidence interval, and yellow bars the 95% HPD of calibrated nodes estimated under constraint. Arrows on the inset map indicate the direction of major trans-oceanic range

movements, along with the mean age of the events. Arrows with dotted lines indicate ARE patterns with ambiguous reconstructions and low support.

Figure 5.5 BAMM analysis of net diversification (*r*) and rate shifts within Plukenetieae. (a) Phylorate plot of the best rate shift configuration (out of 30) showing three significant shifts: 1. Stem of the African clade (subclade T3); 2. Stem of *Tragia* sect. *Tragia* (subclade T10); 3. Stem of the *Dalechampia* 5-armed clade. Colour intensity across branches is proportional to changes in net diversification. (b) Rate through time analysis of net diversification rates in Plukenetieae over the last 10 Myr at the start of the Late Miocene cooling period. Top of figure: Representation of temperature estimates through time based on global deep sea $\delta^{18}O$ isotope concentration data from Zachos *et al.* (2001; accessed from Marcot *et al.*, 2016).

Figure 5.6 Net diversification rates (*r*) and character state reconstructions under the best fitting models from HISSE (see Table 5.4) for three putative innovative traits in Plukenetieae: (a) twining growth form; (b) stinging hair plant defences; and (c) bilaterally symmetrical pseudanthial inflorescences. Outer coloured lines depict net diversification rates (scales comparable between figures), and inner black and white lines indicate character state reconstructions. Histograms show the posterior distribution of net diversification rates (*r*) and black and white bars the frequency of character states at each terminal.

Figure 5.S1 Detailed cladograms and/or phylograms of Plukenetieae from individual and combined datasets labeled with maximum likelihood bootstrap percentages (MLBP) and/or Bayesian posterior probabilities (BPP): (a) ETS, (b) ITS, (c) *KEA1* intron 11, (d) *TEB* exon 17, (e) *matK*, and (f) *ndhF*, (g) cpDNA, (h) Concatenated, and (i) Concatenated and fully annotated.

Figure 5.S2 Coalescence-based ASTRAL maximum quartet support (MQS) species tree of Plukenetieae based on Bayesian maximum clade credibility (MCC) topologies of all six regions, labeled with multilocus bootstrap percentage (Multilocus BP).

Figure 5.S3 Coalescence-based *BEAST maximum clade credibility (MCC) species tree of Plukenetieae based on reduced five region, "no-gap" dataset (177 terminals), labeled with Bayesian posterior probabilities (BPP).

Figure 5.S4 Maximum clade credibility (MCC) BEAST chronogram of Plukenetieae based on the reduced six region, "one-terminal" dataset (147 terminals), including mean common ancestor ages and 95% highest posterior density (HPD) confidence interval bars (yellow = calibrated estimate; blue = free estimate).

Figure 5.S5 Results of the BIOGEOBEARS ancestral range estimations (ARE) under (a) DEC+J and (b) DEC models.

Figure 5.S6 BAMM 95% credible shift set phylorate plots (the largest nine of 30) including the frequency (f) of each configuration in the posterior distribution.

List of Appendices

Appendix 2.1. 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 (*).

Appendix 4.1. List of species and vouchers used in the ETS phylogeny (Fig. 4.4), arranged by: ***Species*** Authority. COUNTRY. Collector & Number (Herbarium Code), GenBank accession number. Most sequences are from Cardinal-McTeague *et al*. (in review); a single new ETS sequence is published for *P. brevistyla* Card.-McTeag. & L.J.Gillespie sp. nov., indicated by an asterisk (*).

List of Supplemental Data

Supplemental data 3.1 Python script for LITE BLUE DEVIL v0.3.

Supplemental data 3.2 Setting file for LITE BLUE DEVIL v0.3.

Supplemental data 3.3 Seed size measurements for *Plukenetia* and Plukenetiinae outgroups.

Instructions: Upon the publication of Chapter 3, these files will be available from DataDryad (https://datadryad.org). A hard copy of each file is recorded at the end of the dissertation.

Chapter 1
Introduction

Systematics is the study of the classification and diversification of life on earth, past and present. It involves several intertwining disciplines in taxonomy, evolution, ecology, phylogenetics, paleobiology, and biogeography. It is a foundational science that provides important context to the field of biology, including names of organisms, how they are related to one another, and the history in which they evolved. All of this information is essential to understanding the biodiversity of the world we live in, and undeniably rely on. Our collective future is faced with growing challenges of climate change, biodiversity loss, and overpopulation, so having a strong understanding of the biological world is an asset to developing creative solutions for long-term sustainability and survival. Whether it is characterizing undescribed biodiversity to inform conservation planning, determining the drivers of organismal diversification to predict lineage expansions, or illuminating the genetic controls of valuable traits to improve food quality and production, systematics will continue to provide important contributions to scientific knowledge and the human experience.

Relevance

The tropics, regions near the equator that are typically warm and wet year-round, harbour the richest plant biodiversity resources on the planet. They are at risk from human-driven deforestation (Gibson *et al.*, 2011) and ecological upset from climate change (Williams *et al.*, 2003; Brodie *et al.*, 2012), and their loss would have negative impacts for human life on earth. As well, the tropics are the primary sources of the world's plant biodiversity (Donoghue & Edwards, 2014), so even plants of the Canadian Arctic and Boreal forest can trace their roots to ancient tropical ecosystems and we can benefit from knowing their evolutionary history. Currently, we are faced with a challenge in understanding the diversity of the tropics: tropical ecosystems are extremely diverse and complex (Hubbell, 2013) and there are not enough people studying them in the western scientific perspective (i.e., excluding Indigenous knowledges). Most nations in the tropics are burdened with a disparity of wealth and resources from a legacy of historical and ongoing colonialism, which compounds their ability to conduct research programs on their own (although recently some progress has been made, such as international

investment in systematics and conservation training and infrastructure in Madagascar). While more work needs to be done to develop sustainable tropical research programs led by nations in the tropics, studies by non-tropical nations help to build global support networks invested in protecting and understanding the world's most incredible natural resource. In Canada, the study of tropical plant systematics is a relevant contribution to some of the most pressing questions of our time, and aids the country's profile as an active member in the international scientific community.

Background

My dissertation contributes to the field of tropical plant systematics research by exploring the phylogeny, evolution, taxonomy, and diversification of an economically valuable but understudied lineage of tropical vines, Euphorbiaceae tribe Plukenetieae. The spurge family (Euphorbiaceae), collectively called euphorbs, includes several well-known and economically important species, such as the latex producing rubber tree (*Hevea brasiliensis* Müll.Arg.), castor bean (*Ricinus communis* L.) and physic nut (*Jatropha curcas* L.) oilseed crops, cassava/manioc (*Manihot esculenta* Crantz) edible root crops, and ornamental poinsettias (*Euphorbia pulcherrima* Willd. ex Klotzsch). Euphorbiaceae s.s. (Wurdack *et al.*, 2005) contains ~6,500 species (Govaerts *et al.*, 2015), several of which are known locally for their uses as food, medicine, building materials, and for cultural purposes. The family is characterized by typically small unisexual flowers, ovaries with one ovule per locule, and typically 3–carpellate schizocarpic capsules (i.e., a capsule that breaks into cocci or mericarps, which subsequently dehisce loculicidally) with a persistent ligneous columella (Wurdack *et al.*, 2005). Plukenetieae belongs to subfamily Acalyphoideae, which lacks the milky or coloured latex that subfamilies Crotonoideae and Euphorbioideae are known for (Webster, 1994, 2014; Radcliffe-Smith, 2001).

Plukenetieae is a diverse pantropical lineage with ~365 species and 18 genera that presents a valuable group for evolutionary studies. It is divided into three subtribes (Table 1.1): Dalechampiinae, with a single species-rich genus (~130 species) of stinging vines or rarely subshrubs; Plukenetiinae, with five genera (~29 species, most of which belong to *Plukenetia*) of non-stinging lianas, vines, and rarely small trees or shrubs; and Tragiinae, with 12 genera (~206 species, most of which belong to *Tragia*) of stinging vines and subshrubs, or rarely larger shrubs.

Table 1.1 Species number, growth form, and geographic distribution of genera in Euphorbiaceae tribe Plukenetieae.

Taxon	Species	Growth form	Distribution
Subtribe Dalechampiinae	~130		
Dalechampia L.	~130	Vines (subshrubs)	Pantropical, primarily New World
Subtribe Plukenetiinae	~29		
Angostylis Benth.	1	Small trees/shrubs	Amazonian Brazil
Astrococcus Benth.	1	Small trees/shrubs	Amazonian Brazil and Amazonian Venezuela
Haematostemon Pax & K.Hoffm.	2	Small trees/shrubs	Guyana and Amazonian Venezuela
Plukenetia L.	~24	Lianas, vines (subshrubs)	Pantropical, primarily New World
Romanoa Trevis.	1	Vines	Eastern Brazil, Paraguay, and Bolivia
Subtribe Tragiinae	~206		
Acidoton Sw.	5	Shrubs	Hispaniola, Jamaica
Bia Klotzsch	5	Vines	Costa Rica to South America
Cnesmone Blume	12	Vines	Southeast Asia
Ctenomeria Harv.	2	Vines	South Africa
Gitara Pax & K.Hoffm.	1	Shrubs	Central and northwestern South America
Megistostigma Hook.f.	5	Vines	Southeast Asia
Pachystylidium Pax & K.Hoffm.	1	Vines	Southeast Asia
Platygyna P.Mercier	7	Vines	Cuba
Sphaerostylis Baill.	2	Vines	Madagascar
Tragia L.	~160	Vines, subshrubs	Pantropical to warm temperate
Tragiella Pax & K.Hoffm.	4	Vines	Central and Southern Africa
Zuckertia Baill.	2	Vines	Mexico and Central America

Besides the presence of stinging hairs (notably absent in Plukenetiinae), the subtribes are distinguished by their inflorescence morphology, with specialized pseudanthial inflorescences composed of condensed staminate and pistillate cymules subtended by large involucral bracts in Dalechampiinae, or plesiomorphic racemose thyrses and racemes in Plukenetiinae and Tragiinae (Fig. 1.1).

The pollen morphology of Plukenetieae is notably diverse and possesses the greatest variation of any tribe in subfamily Acalyphoideae, if not the entire family (Gillespie, 1994b; Nowicke & Takahashi, 2002). Pollen morphology in the tribe is especially variable in exine (semi-tectate and coarsely or finely reticulate; tectate and rugulate, foveolate, or punctate; intectate and baculate/clavate) and aperture condition (tricolporate with an endocingulate endopore, tricolpate, weakly tricolporate, weakly tri-aperturate, and inaperturate). Tragiinae pollen exhibits a trend towards aperture reduction and loss, which is intriguing given that aperture loss should reduce the effectiveness of pollen tube germination and impact reproductive fitness (Furness, 2007). The importance of pollen morphology on plant ecology and evolution is becoming more apparent (Edlund *et al.*, 2004; Prieu *et al.*, 2016; Williams & Mazer, 2016)

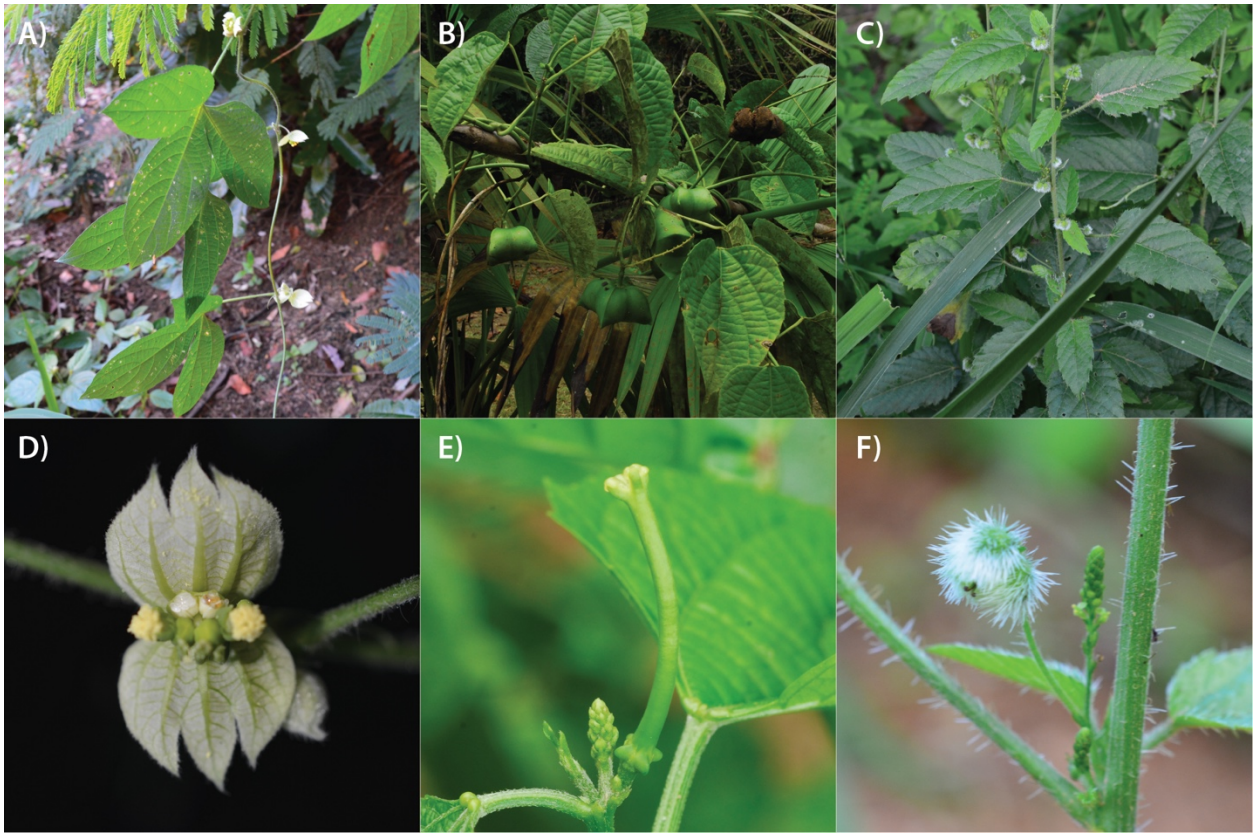


Figure 1.1 Representative habit (a–c) and inflorescence (d–f) diversity for the subtribes of Plukenetieae. Dalechampiinae: (a) stinging twining vines, (d) pseudanthial inflorescence composed of pistillate and staminate cymules and resiniferous glands subtended by two white involucral bracts (*Dalechampia* sp.; *Medeiros & Cardinal-McTeague 562*, R). Plukenetiinae: (b) non-stinging twining vines and slender lianas, (e) racemose thyrse with a proximal pistillate flower and two to three flowered distal staminate cymules in bud (*Plukenetia volubilis* L.; *Wurdack s.n.*, US). Tragiinae: (c) stinging twining vines and subshrubs, (f) raceme with proximal pistillate flower and distal staminate flowers (*Tragia volubilis* L.; *Medeiros & Cardinal-McTeague 556*, R).

suggesting studies on Plukenetieae could shed light on the selective pressures of exine type and pollen aperture reduction and loss.

Seeds of Plukenetieae are typically small and unremarkable, however the seeds of *Plukenetia* are notably variable in size and several species are economically valuable. At least four species of *Plukenetia* are traditionally cultivated for their large edible oil-rich seeds: *P. huayllabambana* Bussmann et al. (Mountain Sacha Inchi), *P. polyadenia* Müll.Arg. (Compadre-do-azeite), and *P. volubilis* L. (Sacha Inchi or Inca peanut) in South America, and *P. conophora* Müll.Arg. (Awusa or African walnut) in West Africa. Economic demand for these traditionally cultivated seeds is growing (Bussmann *et al.*, 2009, 2013), and agricultural interest and research are positioning the genus to be a useful model of seed trait evolution, e.g., the effects of elevation

on seed oil profiles (Cai *et al.*, 2012), differences in germination biology (Nwosu, 2006; Cardoso *et al.*, 2015; Silva *et al.*, 2016), and the genetic pathways of oil accumulation and polyunsaturated fatty acid synthesis (Wang *et al.*, 2012; Wang & Liu, 2014). Furthermore, while the general selective pressures of seed size are known (Leishman *et al.*, 2000), there are surprisingly few studies demonstrating the evolutionary pressures of seed size within a clade, suggesting *Plukenetia* could be a useful system to study seed size evolution.

The widespread pantropical distribution of Plukenetieae is also notable and raises questions about the biogeographical history of the tribe. All three subtribes are pantropically distributed (Table 1.1), suggesting each lineage underwent parallel biogeographic processes, but potential similarities and differences are unclear. Over the past decade, biogeographic studies have flourished, providing insight into the timing and direction of ancient plant movements and information on the evolution of specific tropical biomes (e.g., the Caribbean; Nieto-Blázquez *et al.*, 2017). Previously, pantropical distributions were thought to have developed by the vicariance of ancient Gondwanan distributions following the breakup of the continents (Raven & Axelrod, 1974), however most evidence now supports the prevalence of frequent and numerous long-distance dispersals since the Oligocene (Renner & Meyer, 2001; Givnish *et al.*, 2004; Renner, 2004a; Sytsma *et al.*, 2004; Clayton *et al.*, 2009; Berger *et al.*, 2016). As such, long-distance dispersal is a likely explanation for the pantropical distribution of Plukenetieae*,* however a formal analysis could shed light on predominant patterns, and improve our understanding of the diversification of each subtribe. Furthermore, detailed biogeography of certain genera/lineages could reveal insight into the neotropical diversification patterns, such as the significance of the Andes mountain barrier (Pérez-Escobar *et al.*, 2017b) and the open vegetational diagonal (Costa, 2003; Werneck, 2011).

Lastly, Plukenetieae possesses three innovative traits that were likely important to the successful diversification of the tribe. The first, twining vine growth form, is present in all three subtribes (Table 1.1). Climbing growth forms are known to promote diversification by providing greater access to light gradients and ecological niches, increasing defences by exposure to a greater number of herbivores, and investing resources into productive biomass rather than support structures (Gianoli, 2004). Second is stinging hair defences, found in Dalechampiinae and Tragiinae, more prominently in the latter (Webster, 2014). Stinging hairs composed of crystalline cell(s) and irritating chemical compounds are known from four plant families,

Urticaceae, Euphorbiaceae, Loascaceae, and Hydrophyllaceae (Thurston & Lersten, 1969). While the presence of stinging hairs is not a striking key innovation in of itself, plant defences are often associated with increased diversification rates (e.g., latex and resin, Farrell *et al.*, 1991; defence mutualisms, Weber & Agrawal, 2014). Third is the origin of pseudanthial inflorescences in Dalechampiinae, which includes innovations of floral symmetry and specialized pollination most often by resin collecting bees, but also fragrance and pollen collecting bees (Armbruster, 1984, 1993, Armbruster *et al.*, 1989, 1992). Bilaterally symmetrical (or zygomorphic) flowers are known to facilitate diversification by increasing reproductive isolation through floral modifications (Sargent, 2004) as do specialized pollination syndromes and pollinator shifts (van der Niet & Johnson, 2012). General trends of plant diversification are not yet established and there are few studies that examined how multiple innovative traits overlap, suggesting Plukenetieae could provide valuable insight.

Despite the ecological, evolutionary, and economic value of Plukenetieae, molecular phylogenetic studies of the tribe have been limited to a few representative taxa in family wide studies (Wurdack *et al.*, 2005; Tokuoka, 2007) or in-depth studies of *Dalechampia* (Armbruster *et al.*, 2009, 2013). Family-wide phylogenies have confirmed the monophyly of Plukenetieae and most of the subtribes, with the exception of one study that recovered the anomalous pairing of *Dalechampia* with the rare Amazonian small tree genus *Astrococcus* (Wurdack *et al.*, 2005), suggesting Plukenetiinae was paraphyletic. Excluding this relationship, most evidence suggests Plukenetiinae is the earliest diverging lineage, sister to Dalechampiinae + Tragiinae, but taxon sampling was limited and node support was low (Wurdack *et al.*, 2005; Tokuoka, 2007). Pollen morphology presents an alternative hypothesis, suggesting that the tricolporate pollen of Dalechampiinae is plesiomorphic for the tribe and that Plukenetiinae + Tragiinae form a monophyletic group based on the shared loss of endopores and similarities in pollen shape and size (Gillespie, 1994b). Furthermore, analysis of pollen morphology diversity proposed several novel species groups that have yet to be tested, and suggests that the large genus *Tragia* is likely paraphyletic (Gillespie, 1994b). As such, a detailed molecular phylogenetic investigation is needed to establish the generic and subtribal relationships within the tribe.

Aims

Through the course of this research portfolio, I explored the evolutionary history of tribe Plukenetieae to identify key species relationships, patterns of morphological evolution and biogeography, and general trends of plant diversification. Overall, my goals were: (1) develop a well-sampled molecular phylogeny of Plukenetieae to use as a framework for evolutionary studies; (2) establish baseline systematic relationships in the tribe, including testing species relationships based on pollen morphology; (3) study patterns of pollen exine and aperture evolution across the tribe; (4) study patterns of seed size evolution in *Plukenetia*, a genus with traditionally cultivated oilseed species; (5) revise the sectional classification of *Plukenetia* in light of molecular and morphological data; (6) reconstruct the biogeographical history of the tribe and determine patterns that resulted in pantropical disjunct distributions; and (7) investigate how multiple overlapping innovative traits (twining growth form, stinging hair plant defences, and pseudanthial inflorescences, including floral symmetry and specialized pollination biology) contributed to the diversification of the tribe.

Motivation and approach

In chapter two, I aimed to produce the first densely-sampled molecular phylogeny for Plukenetieae and explore pollen morphology evolution. To outline the generic and subtribal relationships in the tribe, and test species groups predicted by pollen morphology (Gillespie, 1994b), I constructed a phylogeny that sampled 154 terminals (93 of ~365 species) and 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. I then interpreted species groups on the phylogeny using molecular clades, vegetative, floral, and pollen morphology, and geographic distribution. Pollen morphology evolution was inferred from the phylogeny, with the goal of identifying trends in exine and aperture evolution.

In chapter three, I investigated the seed size evolution and biogeography of *Plukenetia*. My goals were to define seed size categories in *Plukenetia*, estimate ancestral seed size across its phylogeny, identify the number of origin(s) of large seeds, and test the relative contribution of plant size, fruit type/dispersal mechanism, seedling ecology, fire tolerance, and biome type to seed size evolution using phylogenetic regression. I also aimed to study neotropical and

pantropical diversification patterns using ancestral range estimation (ARE). To elucidate seed size evolution and biogeographic patterns, I developed an improved molecular phylogeny for *Plukenetia* (83 terminals, 20 of ~23 *Plukenetia* species) using two plastid (*matK*, *ndhF*) and five nuclear (ETS [external transcribed spacer], ITS, *KEA1* introns 11 and 17, *TEB* exon 17) markers. Both *KEA1* and *TEB* were used for phylogenetic inference for the first time, with *KEA1* identified here using the transcriptome/genome data-mining pipeline LITE BLUE DEVIL v0.3.

In chapter four, I produced a revised sectional classification for *Plukenetia* and described three new taxa from South America. The taxonomic history of *Plukenetia* has been complicated by shifts between recognizing several smaller genera (e.g., Pax & Hoffmann, 1919a) and a single broader genus united by synapomorphic 4–carpellate ovaries (Gillespie, 1993, 2007). To accommodate the breadth of morphological variation in the expanded generic concept, three new sections were formally described. The sections are supported by molecular phylogeny and defined by a combination of androecium morphology, style morphology, fruit type, and seed size, in addition to leaf venation and pollen tectum. I also described two new species from the Amazon basin and a new subspecies from Andes of Peru based on phylogenetic and morphological evidence. A taxonomic treatment with dichotomous keys to the sections and species are included.

Lastly, in chapter five, I investigated the biogeographical history and drivers of diversification in Plukenetieae. A combination of innovative traits (such as floral symmetry, specialized pollination biology, and beneficial growth forms) and abiotic processes (such as mountain building and aridification) are known to contribute to increased diversification in plants (Sargent, 2004; Givnish *et al.*, 2014, 2015; Hernández-Hernández *et al.*, 2014; Reyes *et al.*, 2015; Lagomarsino *et al.*, 2016), but less is known about how overlapping innovative traits interact, and general trends in diversification still need to be established. Here, I tested the relative contribution of overlapping innovative traits in Plukenetieae, (i) twining growth form, (ii) stinging hair defences, and (iii) specialized inflorescence structure + pollination biology, predicting that species with all three traits would exhibit the greatest diversification rates. Second, I used ARE to reconstruct the biogeographical history of the tribe, with special attention to determining the pattern and timing of plant movements that resulted in pantropical distributions. Furthermore, it allowed me to test if the arrival to another continent was associated with enhanced diversification through adaptation to new ecological niches (e.g., Richardson *et*

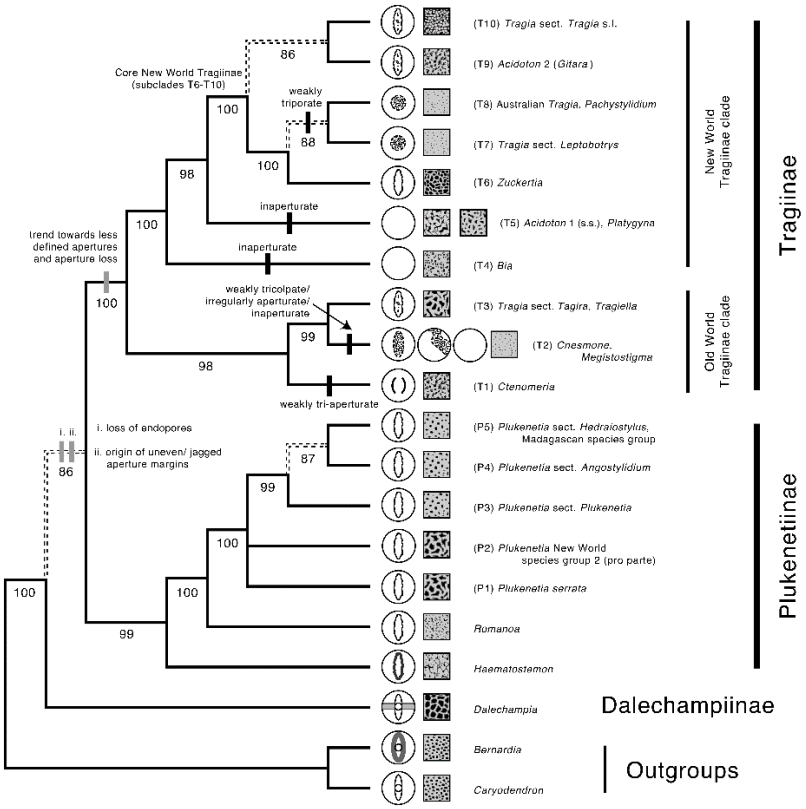
al., 2001; Federman *et al.*, 2015). To test these hypotheses, I developed an improved phylogeny for the tribe (289 terminals, 109 of ~365 Plukenetieae species) that sampled two plastid (*matK*, *ndhF*) and four nuclear markers (ETS, ITS, *KEA1* intron 11, *TEB* exon 17). Diversification patterns were determined using both time- and trait-dependent statistical modeling.

Chapter 2
Molecular Phylogeny and Pollen Evolution of Euphorbiaceae Tribe Plukenetieae*

Warren M. Cardinal-McTeague[1,2] and Lynn J. Gillespie[1,2]

1. Department of Biology, University of Ottawa, Gendron Hall, Room 160, 30 Marie Curie, Ottawa, Ontario, K1N 6N5, Canada.
2. Research & Collections, Canadian Museum of Nature, PO Box 3443, Station D, Ottawa, Ontario, K1P 6P4, Canada.

* Manuscript adapted from: Cardinal-McTeague, W.M., and Gillespie, L.J. (2016) Molecular phylogeny and pollen evolution of Euphorbiaceae tribe Plukenetieae. *Systematic Botany* 41(2): 329–347. http://dx.doi.org/10.1600/036364416X691759



Phylogeny and pollen morphology evolution in Euphorbiaceae tribe Plukenetieae.

Abstract

- **Background:** 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.
- **Results:** 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 ~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.
- **Conclusions:** 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.

Background

Tribe Plukenetieae (Benth.) Hutch. (Euphorbiaceae, Acalyphoideae) is a diverse pantropical lineage of ~17 genera and 350 species of twining vines and lianas, scandent to erect perennial herbs and subshrubs, and rarely shrubs and small trees (Gillespie, 1994b; 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* L., known for its unique pseudanthial inflorescence and specialized pollination biology (Webster & Webster, 1972; Webster & 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, 1994b; 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. 2.1). 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, 1994b; Nowicke & Takahashi, 2002).

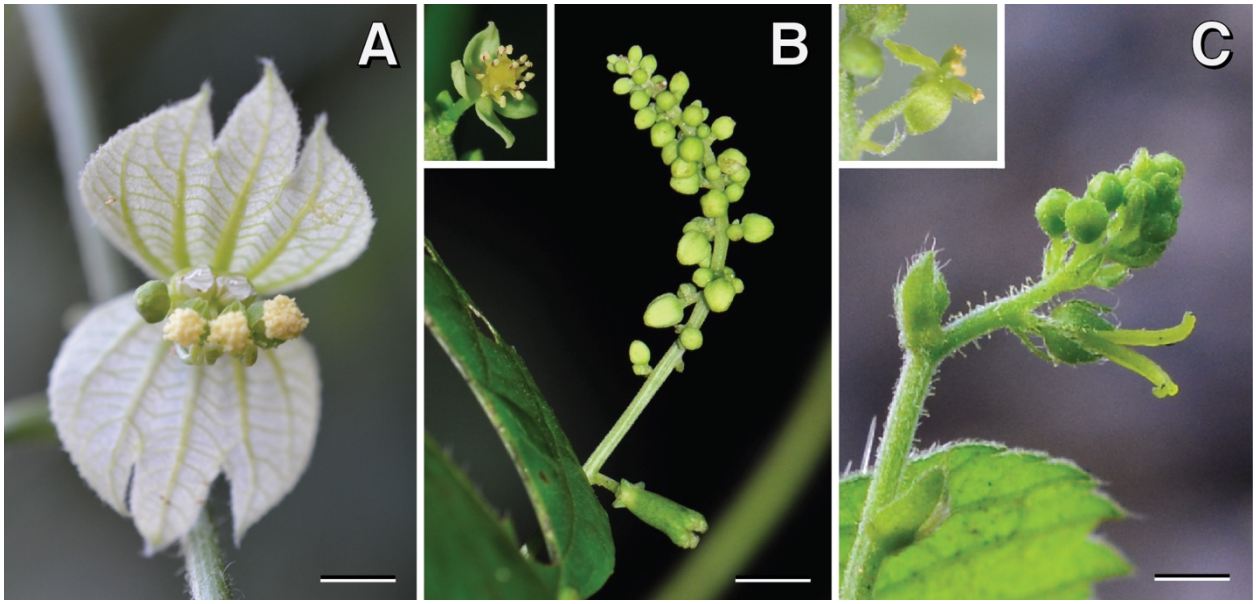


Figure 2.1 Representative inflorescences for the subtribes of Plukenetieae. (a) Dalechampiinae: pseudanthial inflorescence of *Dalechampia* sp. (*Medeiros & Cardinal-McTeague 562*, R), composed of pistillate and staminate cymules and resiniferous glands subtended by two white involucral bracts (scale 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 & Cardinal-McTeague 561*, R) with proximal pistillate flower and distal staminate flowers (scale bar = 3 mm). Inset, staminate flower.

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 & Hoffmann, 1919, as Acalypheae subtribe Plukenetiinae; Hutchinson, 1969; Webster, 1975), but later included *Dalechampia* (previously in the monogeneric tribe Dalechampieae; Müller, 1864a, 1865; Pax & 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 2.1.

Subtribe Dalechampiinae

Dalechampiinae is a monogeneric subtribe containing *Dalechampia* (Table 2.2), a pantropically distributed and species-rich genus (~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. 2.1A), which contributes to a suite of resin-, fragrance-, and

Table 2.1 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 (glomerules)	Racemes (racemose thyrses with reduced simple cymes), 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

pollen-gathering insect pollination strategies (Armbruster, 1984, 1993, Armbruster *et al.*, 1989, 1992, 2009; Armbruster & Baldwin, 1998).

Subtribe Plukenetiinae

Plukenetiinae is a small subtribe of five genera and ~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 2.2; Gillespie, 1994b). 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* (~21 species) is the largest and only pantropically distributed genus in the subtribe (Table 2.2), and is unique in having four-carpellate ovaries and often winged or

Table 2.2 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 L.	~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.	~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 L.	~150 (50)	Pantropical to warm temperate (primarily New World and Africa)
Tragiella Pax & K.Hoffm.	4 (3)	Central and S Africa
Zuckertia Baill.	2 (1)	Mexico and Central America
Selected Outgroups		
Bernardia Houst. ex Mill.	~70 (3)	North and South America
Caryodendron H.Karst.	4 (2)	Central and South America

tubercled 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 2.3).

Subtribe Tragiinae

Tragiinae is the largest subtribe of Plukenetieae and comprises a diverse lineage of ~11 genera and 195 species (Table 2.2). 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 2.1). 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* L. (~150 species) is pantropically distributed and the sixth largest genus

Table 2.3 Infrageneric classifications (including informal species groups) of *Plukenetia* (sensu Gillespie, 1993, 2007) and *Tragia* (sensu Pax & Hoffmann, 1919a, with modifications by: Miller & Webster, 1967; Leandri, 1971; Gillespie, 1994a; Webster, 2007, 2014). *Tragia* sects. *Leucandra* and *Ratiga* are regarded as synonyms of sect. *Tragia* (Miller & Webster, 1967; Múlgura de Romero & 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 & 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 & Hoffmann, 1919a; Forster, 1994).

Classification	Species number (species sampled)	Geographic distribution
Plukenetia	~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	~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

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 2.3. Floral and pollen morphology suggest that *Tragia* is paraphyletic, with the other genera of Tragiinae embedded within it (Gillespie, 1994b). 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, 1994b) 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, 1994b; 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 & Webster, 1972; Gillespie, 1994a,b; Nowicke & Takahashi, 2002) and is useful in differentiating among the subtribes (Table 2.1).

Interpreting pollen morphology variation has provided important hypotheses for the relationships of the Plukenetieae subtribes and genera (Gillespie, 1994b). 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, 1994b). Aperture variation in Tragiinae provides hypotheses for the relationships of species groups and genera and strongly suggests that *Tragia* is paraphyletic (Gillespie, 1994b). 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. 2.2).

Molecular phylogenetic analyses have also been conducted on *Dalechampia*, with a focus on evolutionary and ecological questions (Armbruster & 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.

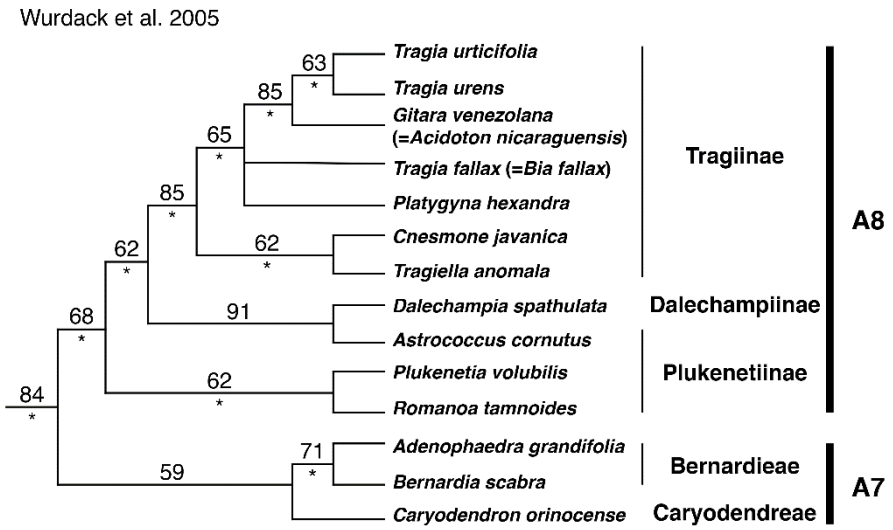


Figure 2.2 Hypothesis for the relationships of Plukenetieae and its close relatives, Bernardieae and Caryodendreae, based on parsimony analysis of *trnL-F* data (previously the best taxon sampled dataset), modified from Wurdack *et al.* (2005). Numbers above branches are parsimony bootstrap percentages ≥ 50%; an asterisk (*) below branches indicates Bayesian posterior probabilities ≥ 95%.

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 (1) elucidate the relationships of the subtribes and genera of Plukenetieae, (2) clarify generic circumscriptions and recommend taxonomic changes consistent with a phylogenetic classification based on molecular and morphological evidence, (3) evaluate evolutionary hypotheses inferred from pollen morphology, and (4) elucidate patterns of pollen aperture and exine evolution.

Methods

Taxon sampling for phylogenetic analysis

We sampled a total of 154 accessions representing ~93 species of Plukenetieae and selected outgroups (taxonomy and voucher data are provided in Appendix 2.1). 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 2.2). Sampling of the large genera *Plukenetia* and *Tragia* attempted to represent their sectional diversity and geographic distribution

(Table 2.3). *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 ~50 species) across seven sections/species groups (excluding sects. *Lassia* and *Monadelphae*, and subg. *Mauroya*, material not available). The remaining 14 genera were sampled for 31 accessions across 18 species (Table 2.2). 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.*, 2006) 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 & 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 & 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 & 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 & 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 & 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 & 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 (https://doi.org/10.5061/dryad.8nj00).

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, 1985b) 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 & Doyle (1975), with Plukenetieae specific aperture terms from Gillespie (1994b) and general pollen morphology terms from Punt *et al.* (2007). Exine terminology varies among authors (e.g., Gillespie, 1994b; Nowicke & 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 ~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, 1994b),

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 (1994b), 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 2.4). 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.

Table 2.4 Characteristics for individual and combined DNA sequence (ITS, *psbA-trnH*) and *psbA-trnH* indel gap-scored (including one hand-scored *psbA-trnH* inversion character) datasets.

Character	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

Phylogenetic reconstructions

The Bayesian maximum clade credibility tree is presented with MP, ML, and BI support values in Figs. 2.3A and 2.3B. Evidence of strong branch support was interpreted as ≥ 85% maximum parsimony 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. 2.3A; MLBP = 51, BPP = 86).

Plukenetiinae and its genera were recovered as monophyletic (Fig. 2.3A; 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. 2.3A; 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. 2.3A). 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 the subclades had mostly strong support, except in subclade P2 (Fig. 2.3A).

Tragiinae was resolved as a monophyletic lineage with strong support (Fig. 2.3A; 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. 2.3A, 2.3B), with the exception of subclade T3 (Fig. 2.3A; 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. 2.3A; MLBP = 71, BPP = 98); and (ii) a primarily New World clade comprised of T4–T10 (Fig. 2.3B; MPBP = 83, MLBP = 92, BPP = 100).

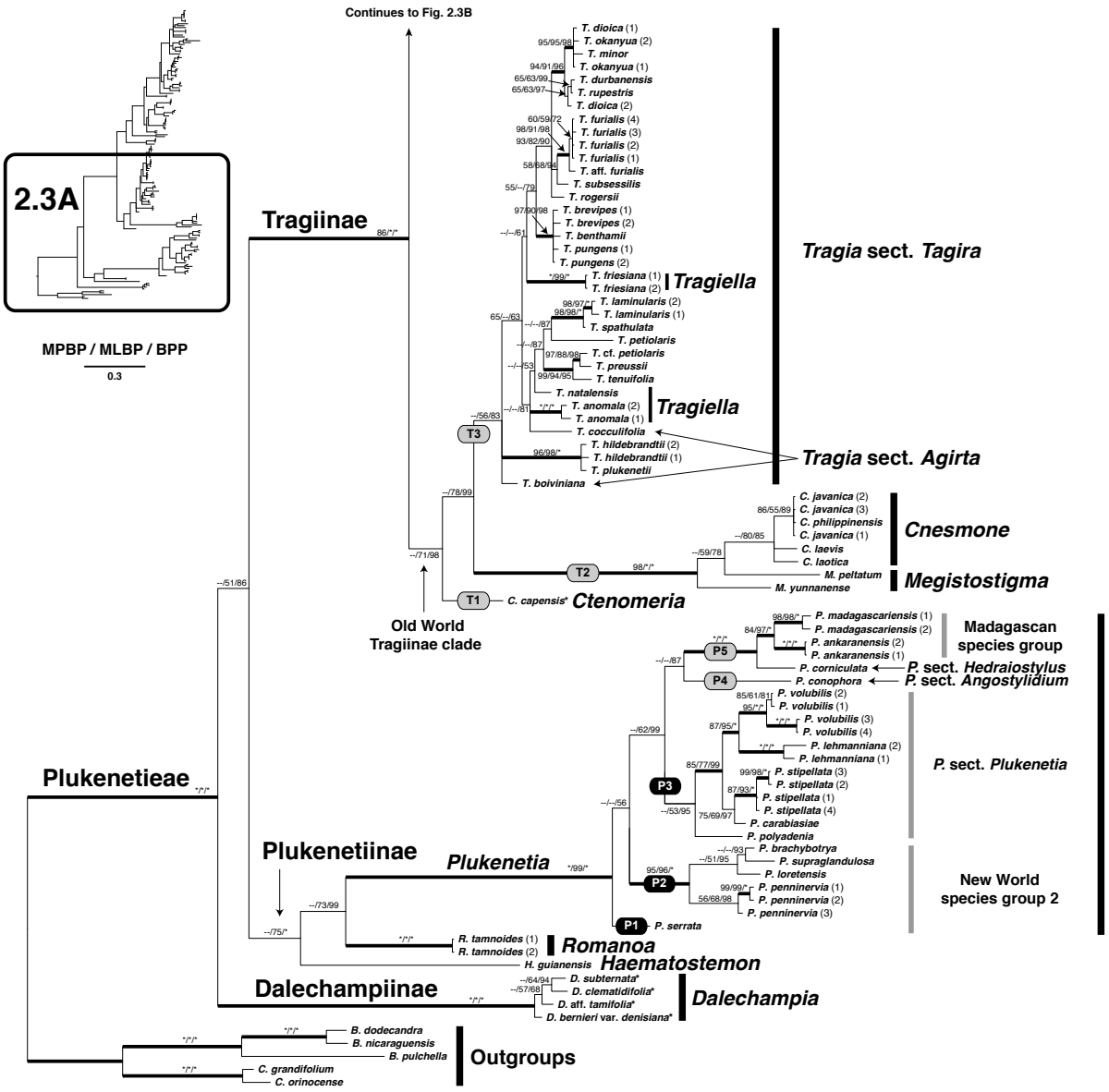


Figure 2.3A Bayesian inference (BI) maximum clade credibility tree based on combined and partitioned ITS, *psbA-trnH*, and indel data of Plukenetieae and selected outgroups. Support values > 50% are indicated on each branch for maximum parsimony (MP) and likelihood (ML) bootstrap analyses, and BI Markov chain Monte Carlo (MCMC) analysis, respectively (* indicate 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. 2.3B.

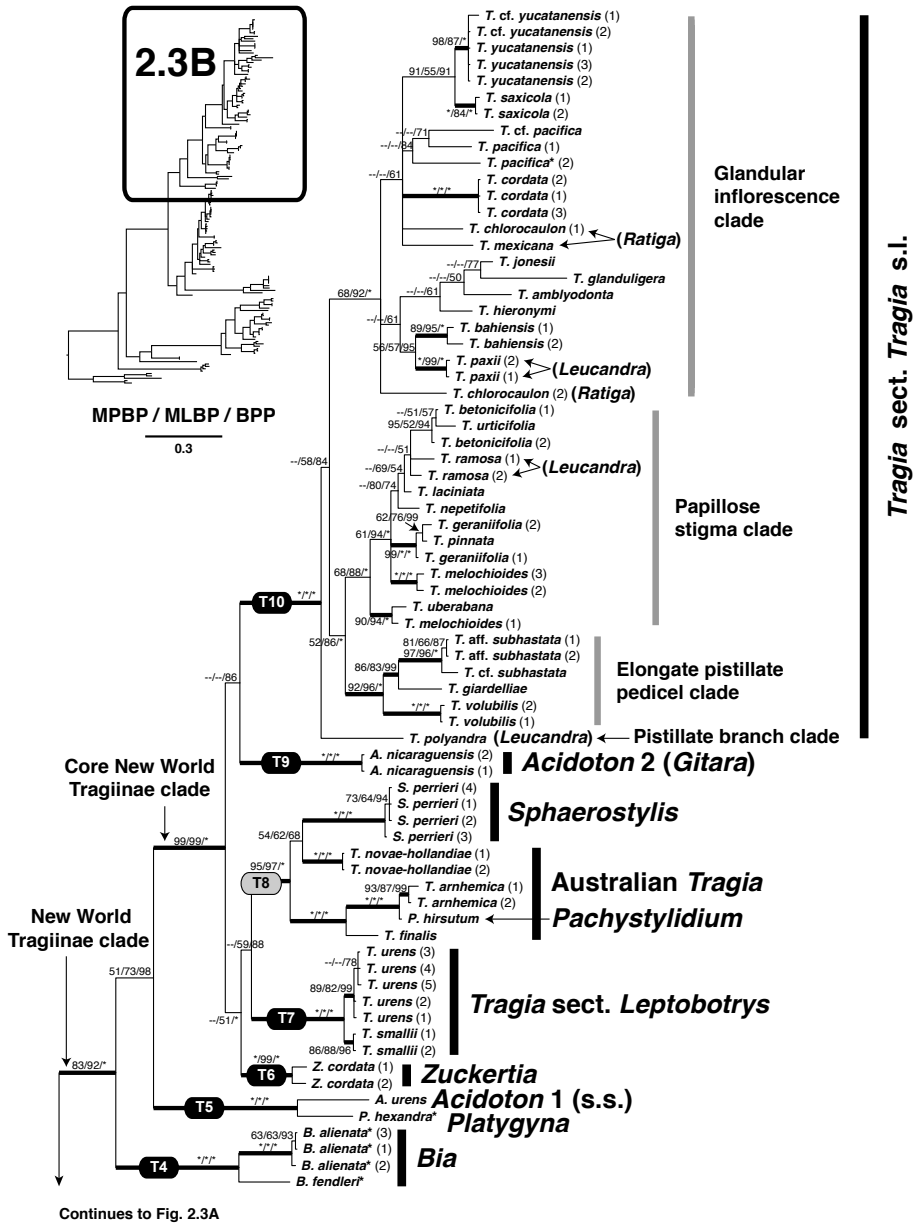


Figure 2.3B Continuation of Fig. 2.3A. Bayesian inference (BI) maximum clade credibility tree based on combined and partitioned ITS, *psbA-trnH*, and indel data of Plukenetieae and selected outgroups. Support values > 50% are indicated on each branch for maximum parsimony (MP) and likelihood (ML) bootstrap analyses, and BI Markov chain Monte Carlo (MCMC) analysis, respectively (* indicate 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.

Subclade relationships in the Old World Tragiinae clade (T1–T3) had moderate support (Fig. 2.3A; 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. 2.3B) 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. 2.3B; 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. 2.3B; BPP = 86), included T9 (*Acidoton* group 2) and T10 (*T.* sect. *Tragia*, including sects. *Leucandra* and *Ratiga*). Species relationships in subclade T10 were well resolved, although most internal nodes were not strongly supported (Fig. 2.3B). *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. 2.3B).

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 2.2) 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, 1994b; 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 (Webster, 1994, 2014; Radcliffe-Smith, 2001).

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 ~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. 2.1; 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, 1994b) that suggest Plukenetiinae and Tragiinae form a lineage based on the shared loss of endopores and uneven/jagged aperture margins. Previous studies recovered part of Plukenetiinae (*Plukenetia* and *Romanoa*) as the earliest diverging lineage with moderate to low support (Fig. 2.1; 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, 1994b; 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, 1994b). 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 & 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, 1994b), suggesting a close relationship. Our analyses resolved *Haematostemon* at the base of Plukenetiinae, sister to *Plukenetia* + *Romanoa* (Fig. 2.3A). Wurdack *et al.*'s (2005) analyses included only *Astrococcus* and resolved it in a clade with *Dalechampia* (Fig. 2.1; 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 (~20 compared to four) and pollen that lacks thickened aperture margins and elongate exine chambers characteristic of the other two genera (Gillespie, 1994b). The small tree and shrub genera are united by habit, pinnately veined oblanceolate leaves, and finely foveolate-rugulate pollen tectum (Gillespie, 1994b), 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 southern 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 ~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, 1994b).

***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, 1994b). The remaining species form a weakly supported clade (MLBP = 62, BPP = 99) united by foveolate pollen exine that includes New World 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 & 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, 1994b).

PLUKENETIA SUBCLADE P3

Subclade P3 was only moderately supported but includes the strongly circumscribed *Plukenetia* sect. *Plukenetia* (seven species, five sampled here). Species of 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. 2.3A) 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 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. 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 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. 2.1). 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 & 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, 1994b).

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 & Gillespie, 2008). Both genera have weakly tricolpate pollen with apertures covered with dense sexine islands, which is a unique combination in Tragiinae (Gillespie, 1994b). 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, 1994b). 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, 1994b; 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, 1994b), 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. 2.3A), although resolution and node support need improvement before taxonomic boundaries are revised.

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

Tragia sect. *Agirta* is a small lineage (~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 & Hoffmann, 1919a; Leandri, 1938a), which suggests that sect. *Agirta* might have resolved separately from sect. *Tagira* and *Tragiella*. Instead, poorly supported relationships imply that the origin of sect. *Agirta* is closely associated with or within the mainland African lineage. We suspect that the non-monophyly of sect. *Agirta* is an artifact of limited taxon sampling for the section and non-overlapping marker coverage of *T. boiviniana* and *T. cocculifolia* (Appendix 2.1).

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 & Hoffmann, 1919a; Múlgura de Romero

& 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. 2.1; 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, 1994b; 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, 1994b).

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 & 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, concave, planar, or semi-globose receptacle (absent in *A. nicaraguensis* [Hemsl.] G.L.Webster), and anther connectives with tufts of minute stinging hairs (Webster, 1967, 1994, 2014). *Acidoton* 1 is likely to contain all of the Caribbean species with inaperturate pollen (*Acidoton* pollen type 2 in Gillespie, 1994b) 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, ~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 & 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 & Hoffmann, 1919a; Gillespie, 1994b; Webster, 2007; Medeiros *et al.*, 2013). A second species associated with *Zuckertia* was recently described from the Sierra Madre del Sur, Mexico (Steinmann & 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 sect. *Leptobotrys* are most clearly differentiated from other New World *Tragia* by having two stamens rather than three or more (Pax & Hoffmann, 1919a; Miller & Webster, 1967; Gutiérrez de Sanguinetti & Múlgura de Romero, 1986), and by weakly triporate pollen with poorly defined circular apertures covered with fragmented sexine (Gillespie, 1994b). This pollen type closely resembles those of Old World taxa resolved in the sister subclade T8 (described below). Phylogeny and pollen associations suggest that 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, 1994b). 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 & Hoffmann, 1919a; Leandri, 1938b; Airy Shaw, 1969; Forster, 1994, 1997; Li & 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, 1994b). 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 sects. *Tragia* (~33 species, 20 sampled here), *Leucandra* (12 species, three sampled here), and *Ratiga* (five species, two sampled here) sensu Pax & Hoffmann (1919). 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, 1994b).

Species placed in sects. *Leucandra* and *Ratiga* (sensu Pax & Hoffmann, 1919) are labeled on the phylogeny (Fig. 2.3B); 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 & Webster, 1967; Múlgura de Romero & Gutiérrez de Sanguinetti, 1989), pollen (Gillespie, 1994b), and molecular evidence (Fig. 2.3B), and we recognize the clade as *Tragia* sect. *Tragia* s.l. Section *Ratiga* was differentiated by introrse anthers and incurved stamen orientation (Pax & Hoffmann, 1919a) and a more resolved and strongly supported phylogeny might show it to be a cohesive species group within sect. *Tragia* s.l. Section *Leucandra* was defined by having 4–20 stamens (Pax & Hoffmann, 1919a) but has not been considered a section worthy of recognition since stamen number is a weak taxonomic character (Miller & Webster, 1967). Species with 4–20 stamens are found in at least three different lineages in subclade T10 (see species labeled *Leucandra* in Fig. 2.3B), which supports 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 sect. *Leptorhachis* (Klotzsch) Müll.Arg. (not recognized by Gillespie, 1994b), which was recircumscribed by Múlgura de Romero & 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). Section *Leptorhachis* (sensu Múlgura de Romero & Gutiérrez de Sanguinetti, 1989) included a subset of species that were previously placed in 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 sect. *Tragia* s.l. The revised 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 & Hoffmann, 1919a; Múlgura de Romero & 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 & Hoffmann, 1919a; Lourteig & O'Donell, 1941; Miller & Webster, 1967; Múlgura de Romero & 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. 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.

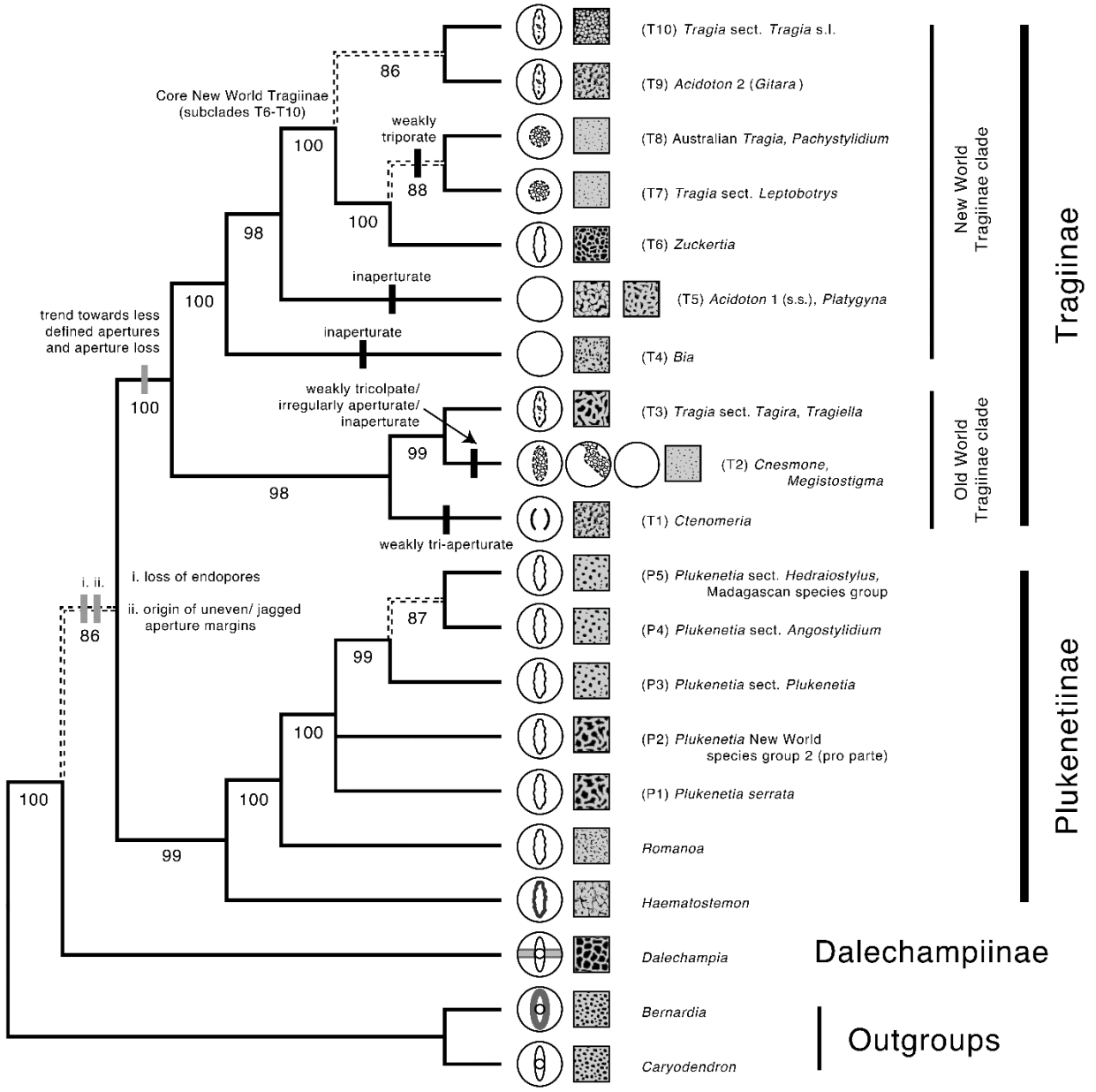
Correlation of pollen morphology with molecular phylogeny

Relationships in Plukenetieae predicted by pollen morphology (Gillespie, 1994b) were mostly congruent with the molecular phylogeny (Fig. 2.4). 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. 2.3A; shown as a polytomy in Fig. 2.4 when poorly supported branches are collapsed).

EXINE MORPHOLOGY EVOLUTION

Exine condition in Plukenetieae is diverse (Punt, 1962; Gillespie, 1994b; Nowicke & Takahashi, 2002) and does not appear to exhibit clear patterns of variation (Fig. 2.4). Tectate-perforate exine is the most common condition in Plukenetieae (Gillespie, 1994b), 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 & Takahashi, 2002; punctate by their definition), and finely foveolate-rugulate in *Haematostemon* (interpreted as 'microcrotonoid' by Nowicke & 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, 1994b). *Acidoton urens* (*Acidoton* group 1, subclade T5) and some species of *Platygyna* (not sampled here) have rugulate tecta with broad rugae (Gillespie, 1994b, 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, 1994b, 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. 2.4). 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 sect. *Agirta* is not known). Nowicke & 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, 1994b).



←

Figure 2.4 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 (1994b) for Plukenetiinae and Tragiinae, Nowicke & 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.

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. *Tragia*, *Leucandra*, and *Ratiga* (Gillespie, 1994b), including sect. *Leptorhachis* sensu Múlgura de Romero & 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 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, 1994b), and do not appear to be correlated with exine morphology (Fig. 2.4). 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, 1994b). *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, 1994b).

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, 1994b). 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. 2.4). 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, 1994b; Nowicke & 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, 1994b), 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, 1994b, 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, 1994b, 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 & 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, 1994b). Furthermore, individual specimens of *Megistostigma* often contain a range of aperture types (Gillespie, 1994b; Nowicke & 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, 1994b). *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. 2.4). *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, 1994b) 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, 1994b, 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, *R. 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 a San Felipe, en selvas, 15–29 May 1920, *H.F. 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, *H.F. 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 & 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 (~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 2.5). 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 ~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.

Table 2.5 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, concave 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

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: P00076087!).
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, *V.W. 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 name *T. bailloniana* (Müller, 1865), which would become rooted in the scientific literature (Pax & Hoffmann, 1919a; Webster & Huft, 1988; Gillespie, 1994b; Burger & 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 & 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 & 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 & 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.

APPENDIX 2.1 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*

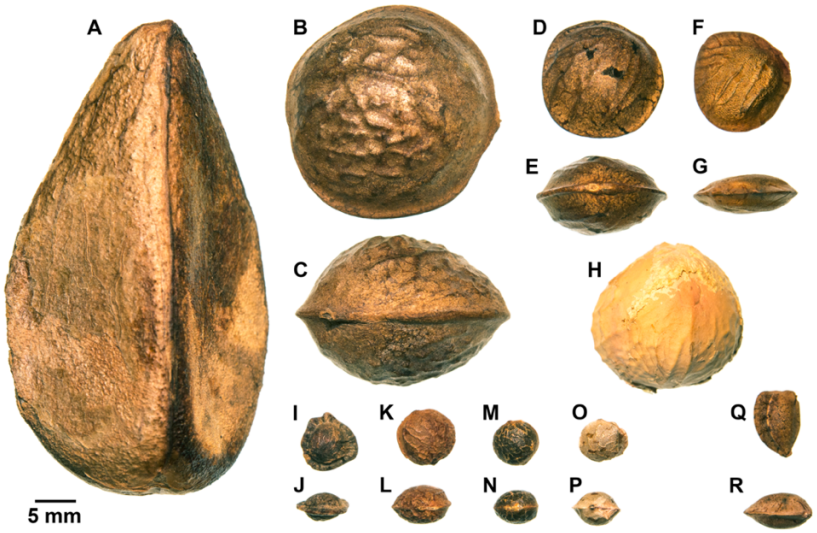
(MO), KP794386, KP794537. ***T. saxicola*** (2). U. S. A. *Kral 51842* (MO), KP794387, KP794538. ***T. smallii*** (1) Shinners. U. S. A. *McDaniel 28193* (MO), KP794401, —. ***T. smallii*** (2). U. S. A. *McDaniel 28698* (MO) , KP794402, —. ***T. spathulata*** Benth. TOGO. *Breteler 7083* (MO), KP794350, 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.

Chapter 3
Seed Size Evolution and Biogeography of *Plukenetia* (Euphorbiaceae), A Pantropical Genus with Traditionally Cultivated Oilseed Species*

Warren M. Cardinal-McTeague[1,2,3], Kenneth J. Wurdack[3], Erin M. Sigel[3,4] and Lynn J. Gillespie[1,2]

1. Department of Biology, University of Ottawa, Gendron Hall, Room 160, 30 Marie Curie, Ottawa, Ontario, K1N 6N5, Canada.
2. Research & Collections, Canadian Museum of Nature, PO Box 3443, Station D, Ottawa, Ontario, K1P 6P4, Canada.
3. Department of Botany, National Museum of Natural History, Smithsonian Institution, MRC-166, PO Box 37012, Washington, DC 20013-7012, USA.
4. Department of Biology, University of Louisiana at Lafayette, Billeaud Hall, Room 108, 410 E. St. Mary Blvd, Lafayette, Louisiana, 70503, USA.

* Manuscript under second review at BMC Evolutionary Biology.



Seed size variation in *Plukenetia* L.

Abstract

- **Background:** *Plukenetia* is a small pantropical genus of lianas and vines with variably sized edible oil-rich seeds that presents an ideal system to investigate neotropical and pantropical diversification patterns and seed size evolution. We assessed the biogeography and seed evolution of *Plukenetia* through phylogenetic analyses of a 5,069 character molecular dataset comprising five nuclear and two plastid markers for 86 terminals in subtribe Plukenetiinae (representing 20 of ~23 *Plukenetia* species). Two nuclear genes, *KEA1* and *TEB*, were used for phylogenetic reconstruction for the first time. Our goals were: (1) produce a robust, time-dependent evolutionary framework for *Plukenetia* using BEAST; (2) reconstruct its biogeographical history with ancestral range estimation in BIOGEOBEARS; (3) define seed size categories; (4) identify patterns of seed size evolution using ancestral state estimation; and (5) conduct regression analyses with putative drivers of seed size using the threshold model.
- **Results:** *Plukenetia* was resolved into two major groups, which we refer to as the pinnately- and palmately-veined clades. Our analyses suggest *Plukenetia* originated in the Amazon or Atlantic Forest of Brazil during the Oligocene (28.7 Mya) and migrated/dispersed between those regions and Central America/Mexico throughout the Miocene. Trans-oceanic dispersals explain the pantropical distribution of *Plukenetia*, including from the Amazon to Africa in the Early Miocene (17.4 Mya), followed by Africa to Madagascar and Africa to Southeast Asia in the Late Miocene (9.4 Mya) and Pliocene (4.5 Mya), respectively. We infer a single origin of large seeds in the ancestor of *Plukenetia*. Seed size fits a Brownian motion model of trait evolution and is moderately to strongly associated with plant size, fruit type/dispersal syndrome, and seedling ecology. Biome shifts were not drivers of seed size, although there was a weak association with a transition to fire prone semi-arid savannas.
- **Conclusions:** The major relationships among the species of *Plukenetia* are now well-resolved. Our biogeographical analyses support growing evidence that many pantropical distributions developed by periodic trans-oceanic dispersals throughout the Miocene and Pliocene. Selection on a combination of traits contributed to seed size variation, while movement between forest edge/light gap and canopy niches likely contributed to the seed size extremes in *Plukenetia*.

Background

Plukenetia L. (Euphorbiaceae subfamily Acalyphoideae) is a small pantropical genus of about 24 species of twining vines and lianas (in rare cases prostrate subshrubs) that represents an ideal system to study tropical biogeography and seed size evolution. Species are found throughout tropical regions of Mexico, Central and South America, Africa, Madagascar, and Southeast Asia (Gillespie, 1993, 2007), making the genus suitable for addressing questions on neotropical diversification patterns and the formation of pantropical distributions. *Plukenetia* seeds exhibit two main qualities that make them desirable for study. First, several species have large edible seeds that are rich in omega-3 and omega-6 polyunsaturated fatty acids and protein content (Akintayo & Bayer, 2002; Chirinos *et al.*, 2015; Mota *et al.*, 2015), making them high-interest crops for both domestic consumption and growing international markets. Second, species of *Plukenetia* exhibit remarkable seed size variation for a genus (Fig. 3.1), which provides a unique opportunity to investigate the genetic controls and ecological drivers of seed size in a well-defined group of species. Presently, the phylogeny of *Plukenetia*, based on two molecular markers and indel gap-coded data, is missing a significant component of species diversity and requires improved resolution and branch support (Cardinal-McTeague & Gillespie, 2016), all of which must be addressed before evolutionary studies can take place on this clade. Here, we develop an improved phylogenetic hypothesis for *Plukenetia* using near-exhaustive taxon sampling across five nuclear and two plastid molecular markers, including two novel low-copy nuclear genes we developed for phylogenetic analysis. This approach allows us to produce a robust time-calibrated phylogeny to examine global patterns of plant biogeography and provide novel insight into the patterns and drivers of seed size evolution among closely related species.

Seed size is an important life-history trait related to mechanisms of dispersal, germination, seedling survival, and the overall reproductive success of plants (Leishman *et al.*, 2000), as well as of economic interest. There have been several macroevolutionary (Moles *et al.*, 2005b, 2007; Beaulieu *et al.*, 2007; Eriksson, 2008; Igea *et al.*, 2017) and microevolutionary (Gómez, 2004; Halpern, 2005; Galetti *et al.*, 2013) seed size studies, but relatively few taxonomic groups with variably sized seeds have been identified and studied on an intermediate level among species or genera (however, see Rojas-Aréchiga *et al.*, 2013; El-ahmir *et al.*, 2015; Krahulcová *et al.*, 2017). It is unclear if seed size is conserved among most closely related taxa or if it is merely a trait that has been poorly documented at that level. Here, we highlight

Plukenetia as a tractable, small-sized genus with a growing number of seed-related resources (e.g., oil composition (Akintayo & Bayer, 2002; Chirinos *et al.*, 2015; Mota *et al.*, 2015), germination rate (Awodoyin *et al.*, 2000; Nwosu, 2006; Cardoso *et al.*, 2015; Silva *et al.*, 2016)) that shows promise for understanding how seed size variation develops among closely related species.

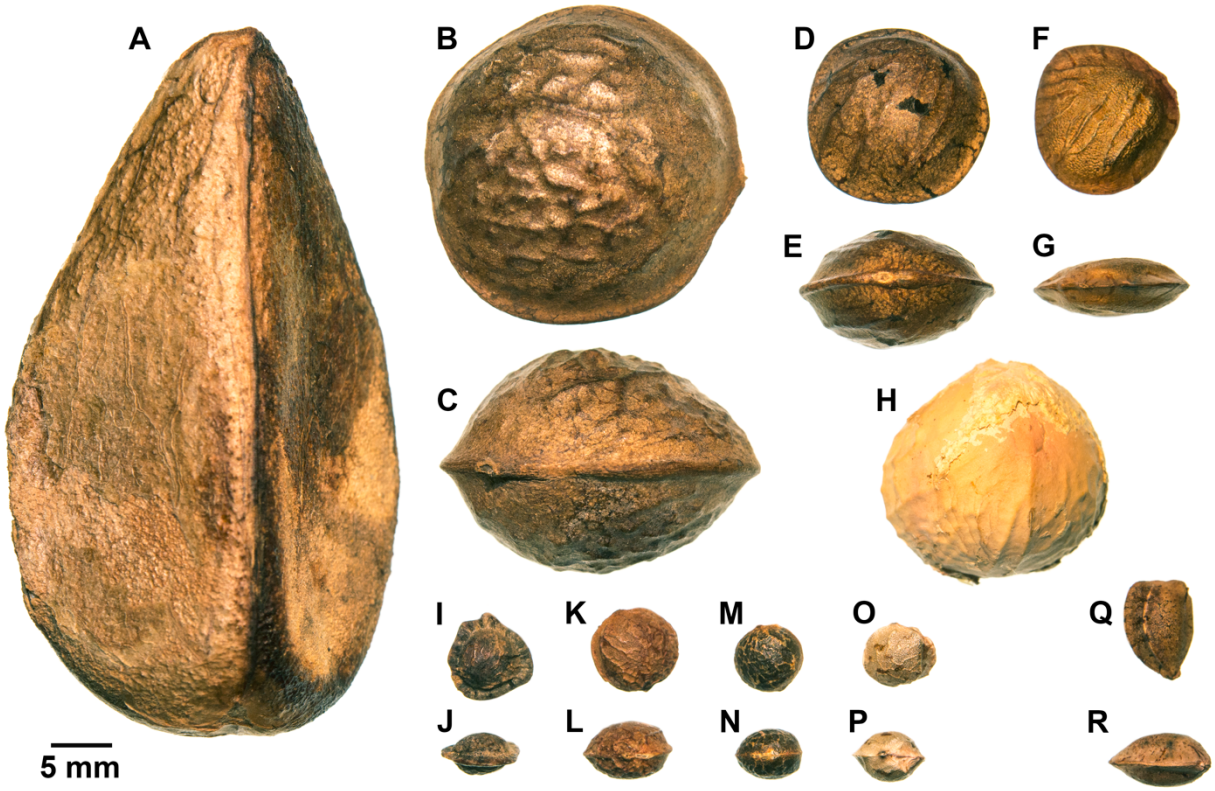


Figure 3.1 Seed size variation in *Plukenetia* and *Romanoa*. (a) *Plukenetia polyadenia* (*van der Werff 16350*, MO); (b–c) *P. huayllabambana* (*Gruhn 84*, MO); (d–e) *P. volubilis* (*Nee 35694*, MO); (f–g) *P. stipellata* (*Zambraver 1047*, MO); (h) *P. lehmanniana* (*Daniel 4943*, US); (i–j). *P. africana* (*Bartsch 1859*, MO); (k–l) *P. supraglandulosa* (*Granville 10783*, US); (m–n) *P. loretensis* (*Krukoff 1031*, MO); (o–p) *P. verrucosa* (*Barrabé & Crozier 145*, US); (q–r) *Romanoa tamnoides* (*Zardini & Cardozo 44939*, MO). Dual orientations (face and side) for most species to show flattened lenticular shape. Source: Vouchers in parentheses. Single photograph taken with a Nikon D800.

Seed size is influenced by a number of selective pressures including seedling and shade competition, predation, dormancy/persistence, drought tolerance, fire tolerance, and dispersal mechanism (Leishman *et al.*, 2000; Gómez, 2004; Lahoreau *et al.*, 2006). Far less is known about how these selective pressures contribute to patterns of seed size evolution within clades. So far, we know that the seed size of *Hakea* (Proteaceae) is correlated with seed production and

post-fire regeneration strategy, but not fruit size, plant height, or the length of time seeds were kept on the plant (El-ahmir *et al.*, 2015). By comparison, seed size in *Aesculus* (Sapindaceae) is associated with genome size (Krahulcová *et al.*, 2017), while in cacti the drivers are not yet known but exclude the amount of light required for germination (Rojas-Aréchiga *et al.*, 2013). Given that closely related species may exhibit phylogenetic constraints on seed size (Kang & Primack, 1999), it appears that variation develops through a complex set of interactions between ecological selection and genetic background. Moving forward we should aim to identify the similarities and differences among the numerous contexts in which seed size variation develops.

Seeds of *Plukenetia* are narrowly to broadly lenticular, subglobose, or obovoid and then laterally compressed (Gillespie, 1993, 2007) (Fig. 3.1) and several traits are potentially correlated with seed size. The most notable trend is the association of smaller seeds in herbaceous vines and slender lianas, and the largest seeds in thick stemmed canopy lianas (Gillespie, 1993). In addition, smaller seeded species tend to be associated with light gap and forest edge habitats, whereas the larger seeds of canopy liana species likely germinate and establish under shaded conditions. Fruit type and inferred dispersal mechanism also show potential associations. Smaller seeds are formed in dry, likely explosively dehiscent capsules (as is typical of most euphorbs), which locally disperse high energy seeds that then can be more efficiently dispersed by scatter hoarding rodents (Forget, 1990; Jansen *et al.*, 2002; Wang & Yang, 2014). In contrast, larger seeds are often associated with green or brown thinly fleshed indehiscent berries, which are likely dispersed by large mammals such as primates that may carry away seeds to eat and occasionally drop a few far from the parent plant (Poulsen *et al.*, 2002; Chapman & Russo, 2006). Lastly, while most *Plukenetia* species occur in wet and seasonally moist forests, some species have transitioned into drier habitats, where we find notable seed size reduction associated with fire-prone savanna ecosystems (Gillespie, 2007).

Within *Plukenetia*, five species have been traditionally cultivated for food and medicine in tropical regions of the Andes (*P. volubilis*, Sacha Inchi or Inca Peanut; *P. carolis-vegae* and *P. huayllabambana*, collectively Mountain Sacha Inchi), the Amazon (*P. polyadenia*, Compadre-de-azeite), and Africa (*P. conophora*, Awusa or African walnut). Despite agricultural interests, the evolutionary origin of large edible oil-rich seeds in *Plukenetia* has not been hypothesized or tested. Large, sometimes oil-rich, seeds have evolved independently in other members of the family [e.g., *Aleurites* J.R. & G.Forst., *Caryodendron* H.Karst., *Hura* L., *Joannesia* Vell., tribe

Micrandeae (Mull. Arg.) G.L.Webster, *Omphalea* L.], however most Euphorbiaceae have relatively small seeds. The continental separation of the cultivated species suggests there were at least two independent ancient domestication and/or semi-domestication events in *Plukenetia*. Since large seeded species are present in most taxonomic groupings of *Plukenetia*, we hypothesize that there was a single origin of large seeds in the ancestor of the genus. This hypothesis invokes at least three shifts back to smaller seeds based on the current *Plukenetia* phylogeny (Cardinal-McTeague & Gillespie, 2016). Presently, seed size concepts in *Plukenetia* are informal and oversimplified (e.g., "small versus large") considering the breadth of variation (Fig. 3.1). Therefore, measurement-based quantification of seed size variation could help develop a more nuanced interpretation of seed evolution.

The biogeography of *Plukenetia* has not been investigated but could contribute to our understanding of neotropical diversification patterns and the formation of pantropical distributions. There are currently no explicit hypotheses regarding the centre of origin of *Plukenetia*, but it is reasonable to predict that it diversified in South America given that the remaining genera of Plukenetiinae are endemic to that continent. Recent biogeographical analysis of subfamily Acalyphoideae (Cervantes *et al.*, 2016) suggests the ancestor of *Plukenetia* was most likely distributed in Central and South America. However, that analysis included only two neotropical species of *Plukenetia* and used biogeographic regions that suited their focus on the Caribbean. Here, we conduct species-level biogeography using more finely parsed South American regions, which may shed light on the impacts that the Andes mountains (Pérez-Escobar *et al.*, 2017b) and open vegetational diagonal (Werneck, 2011) had on species movement and diversification patterns. Moreover, biogeographical analysis of *Plukenetia* allows us to test the timing, directionality, and inferred method of movement (e.g., continental migration versus long-distance dispersal) that resulted in its pantropical distributions.

Under its current circumscription, *Plukenetia* is divided into five sections/informal species groups that are found primarily in wet or moist tropical forests (unless otherwise indicated) (Gillespie, 1993, 2007). They include: (1) sect. *Plukenetia*, containing about eight species from Mexico to Central and South America; (2) a second neotropical species group with about nine species, defined as New World species group "2" by Gillespie (Gillespie, 1993) (henceforth referred to as NWSG2); (3) sect. *Angostylidium*, containing *P. conophora*, distributed in Africa; (4) sect. *Hedraiostylus*, containing two species (one with an uncommon

subshrub growth form) from semi-arid savannas of Africa, and *P. corniculata* from Southeast Asia; and (5) an informal species group from Madagascar (Gillespie, 2007), containing three species found in dry tropical forest, with two species found exclusively on tsingy limestone. Molecular phylogenetic analysis of Plukenetieae largely supports the morphology-based classification of *Plukenetia*, although species group relationships need improved resolution and support (Cardinal-McTeague & Gillespie, 2016).

In this study, we developed a comprehensive phylogeny for *Plukenetia* and closely related genera in subtribe Plukenetiinae to investigate patterns of seed size evolution and biogeography. To produce a well-resolved and well-supported phylogeny, we sampled two plastid and five nuclear DNA markers, including two novel low-copy nuclear genes, one of which was identified using the modified genome/transcriptome data-mining pipeline LITE BLUE DEVIL v0.3. Our goals were to: (1) develop a robust, time-calibrated phylogeny of *Plukenetia* for broad use as an evolutionary framework; (2) reconstruct the biogeographical history of *Plukenetia* and examine hypotheses of neotropical diversification and pantropical disjunct distributions; (3) empirically define seed size categories for *Plukenetia*; (4) perform ancestral seed size estimation to test if there was a single origin of large seeds and identify patterns of seed size variation; and (5) use phylogenetic regression with the threshold model to test putative drivers of seed size evolution.

Methods

Taxon sampling for phylogenetic analysis

Our taxon sampling included 86 terminals, 81 of which belong to *Plukenetia* (voucher information and GenBank accessions are provided in Table 3.S1). We aimed for a comprehensive survey of all known *Plukenetia* species and included multiple accessions of taxa with morphologically diverse species complexes (e.g., *P. brachybotrya*, *P. penninervia*, and *P. volubilis*; Gillespie, 1993) and accessions that may represent undescribed species. In total, we sampled 20 of ~23 species representing 83% of the total diversity. Species that could not be sampled were either rare and known only from type collections (*P. multiglandulosa*, *P. procumbens*) or material was unavailable (*P. carolis-vegae*). We used five accessions of two closely related taxa in Plukenetiinae (*Haematostemon guianensis* and *Romanoa tamnoides*) as outgroups (Cardinal-McTeague & Gillespie, 2016). The relationship of *Romanoa* as the sister

genus of *Plukenetia* is well established with molecular and morphological evidence (Gillespie, 1993; Wurdack *et al.*, 2005; Cardinal-McTeague & Gillespie, 2016).

Genome/transcriptome data-mining for novel low-copy nuclear markers

Identification of low-copy nuclear markers largely followed a top-down approach for genome/transcriptome data-mining (Rothfels *et al.*, 2013). This approach operates by conducting a BLAST (Altschul *et al.*, 1990) search of a candidate gene on a designated database of genome/transcriptome sequences followed by alignment and tree building methods to screen for copy number. Potential low-copy nuclear loci were selected from a list of 1,083 highly conserved genes identified from the annotated genomes of seven angiosperm species and one moss (Zhang *et al.*, 2012). We compiled nine previously published genome/transcriptome assemblies for six Euphorbiaceae species: the draft genomes of *Jatropha curcas* (Sato *et al.*, 2011) and *Ricinus communis* (both coding sequence and gene) (Chan *et al.*, 2010), five transcriptomes from the 1000 Plants Initiative (i.e., 1KP: *Croton tiglium*, *Euphorbia mesembryanthemifolia* (both juvenile and mature), *Manihot grahamii*, and *R. communis* (Matasci *et al.*, 2014; 1000 Plants (1KP) Project, 2015)), and the seed transcriptome of *Plukenetia volubilis* (Wang *et al.*, 2012) (Table 3.S2). We used the python script LITE BLUE DEVIL (Additional files 3.1 and 3.2; modelled after BLUE DEVIL v0.6, Rothfels *et al.*, 2013) to detect the longest open reading frames (ORFs) in a series of query sequences (i.e., the list of 1,083 highly conserved genes), search for those ORFs within our pool of genome/transcriptome assemblies using BLAST, align the resulting hits using MUSCLE (Edgar, 2004), and conduct RAXML (Stamatakis, 2014) BestTree searches on alignments that returned four or more sequences. LITE BLUE DEVIL allows for blastn or blastp searches (Altschul *et al.*, 1990) with specified cut-off values. We used blastn with an e-value threshold of 1e-6. Alignments of potentially low-copy loci were imported into GENEIOUS v8.1.9 (Biomatters, Auckland, New Zealand) and screened for regions of suitable size (400–750 basepairs; bp) with conserved flanking regions to facilitate primer design and amplification. Of the 1,083 candidate genes surveyed (see results), AT1G01790, an anticipated potassium (K+) efflux antiporter 1, chloroplastic gene (*KEA1*), demonstrated the highest potential for phylogenetic utility and was carried forward in our study.

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from herbarium or silica gel desiccated leaf material using a DNeasy Plant Mini Kit (Qiagen, Valencia, U.S.A.) following the manufacturer's instructions or with a modified 12 hr proteinase K incubation (Wurdack *et al.*, 2004, 2005). Marker selection included our newly designed regions and those with previously demonstrated phylogenetic utility in Euphorbiaceae. In total, we sampled seven markers from the nuclear and plastid genomes. The nuclear ribosomal external and internal transcribed spacers (ETS, ITS) and partial *matK* regions have been used within Plukenetieae (Armbruster *et al.*, 2009; Cardinal-McTeague & Gillespie, 2016), while full or partial *matK* and *ndhF* regions have been broadly applied across Euphorbiaceae and Acalyphoideae (Horn *et al.*, 2012, 2014; van Welzen *et al.*, 2014; Cervantes *et al.*, 2016). Low-copy nuclear markers *KEA1* introns 11 and 17 (designed here) and *TEB* exon 17 (designed earlier by KJW) have not been previously used for phylogenetic analyses. *TEB* (AT4G32700) was originally screened but found unsatisfactory (i.e., erratic gene recovery) in preliminary work on broad Malpighiales phylogenetics (see Wurdack & Davis, 2009). We also redesigned Euphorbiaceae-specific primers for ETS and *matK*. The ETS primers were designed from ribosomal DNA assemblies from whole genome libraries of *Haematostemon guianensis* run on the Ion Torrent platform (Thermo Fisher Scientific) (KJW, unpublished data). The *matK* primers were designed by identifying conserved regions on an alignment with representatives of *Acalypha*, *Bernardia*, *Caryodendron*, *Ricinus*, and genera from tribe Plukenetieae. Table 3.S3 outlines the primers and parameters used for amplification and sequencing (White *et al.*, 1990; Olmstead *et al.*, 1993; Stanford *et al.*, 2000; Steinmann & Porter, 2002; Beilstein *et al.*, 2006; Cheng *et al.*, 2016). Figure 3.S1 illustrates new or redesigned markers and their relative primer positions. PCR products were treated with an exonuclease I (Exo) and shrimp alkaline phosphatase (SAP) procedure (MJS Biolynx, Brockville, Canada) or with ExoSAP-IT (Fisher Scientific, Fair Lawn, U.S.A.), followed by Sanger sequencing with BigDye Terminator v3.1 chemistry (Applied Biosystems, Foster City, U.S.A.). Sequencing reaction products were cleaned with a sodium acetate/ethanol precipitation or Sephadex G-50 (GE Healthcare Bio-Sciences, Pittsburg, U.S.A.) and run on an ABI 3130xl Genetic Analyzer (Applied Biosystems) at the Laboratory of Molecular Biodiversity (Canadian Museum of Nature) or at the Laboratories of Analytical Biology (Smithsonian Institution, National Museum of Natural History). All consensus sequences were assembled and edited using GENEIOUS.

Sequence alignment and model selection

Sequences were aligned using the auto-select algorithm of MAFFT v7.017 (Katoh & Standley, 2013) in GENEIOUS, then manually adjusted using a similarity criterion (Simmons, 2004). Optimal models of molecular evolution were selected for each marker by ranking the maximum likelihood (ML) scores of 24 nucleotide substitution models under PHYML (Guindon *et al.*, 2010) BEST tree searches using the Akaike information criterion (AIC; (Akaike, 1974)) implemented in JMODELTEST v2.1.10 (Darriba *et al.*, 2012). The GTR+I+G model was selected for ITS and *ndhF*, GTR+G for *KEA1* intron 11 and *matK*, HKY+G for ETS, and HKY+I for *KEA1* intron 17 and *TEB* exon 17.

Phylogenetic analyses

Prior to conducting phylogenetic analyses on combined datasets, we tested for well-supported incongruence between individual markers using ML bootstrap analyses (Felsenstein, 1985b) conducted in GARLI v2.01 on XSEDE (Zwickl, 2006) through the CIPRES SCIENTIFIC GATEWAY v3.3 (Miller *et al.*, 2010) (all other analyses were run with desktop programs). For each marker we generated 500 bootstrap replicates, implementing two independent runs starting from random trees, terminating after 20,000 generations without topological improvement, and estimating the values of each model of molecular evolution. A 50% majority-rule consensus tree was made from the optimal trees recovered from each replicate using the Consensus Tree Builder in GENEIOUS. Individual marker bootstrap values were mapped onto the optimal ML tree recovered under the same search conditions. Topologies from individual analyses were evaluated by pairwise comparisons, searching for well-supported conflicting relationships (interpreted as ≥ 85 ML bootstrap percentage; MLBP).

We implemented parsimony and Bayesian approaches for the analysis of concatenated datasets. Parsimony analyses were executed in PAUP* v4.0b10 (Swofford, 2002) with characters treated as unordered and equally weighted (Fitch, 1971). Branch support was evaluated using 1,000 bootstrap replicates, each with 10 random-addition replicates, applying tree-bisection-reconnection (TBR) swapping, saving multiple shortest trees each step (Multrees), with each replicate limited to the first 100 shortest trees. Bayesian Markov chain Monte Carlo (MCMC) analyses were conducted in MRBAYES v3.2.2 (Ronquist *et al.*, 2012) on concatenated datasets partitioned by marker and with independently estimated models. Two independent runs of four-

chained searches were performed for three million generations, sampling every 1,000 generations, with the remaining search parameters at their default settings. Independent runs were considered converged when the standard deviation of split frequencies were < 0.005, potential scale reduction factors (PSRF) were near 1.0, and effective sample size (ESS) values of each parameter were > 200 (determined using TRACER v1.6 (Rambaut *et al.*, 2014)). The first 25% of each run (750 trees) was discarded as burn-in prior to summarizing a maximum clade credibility tree and calculating posterior probabilities (PP).

Divergence date estimation

Molecular dating analyses were assessed under a relaxed molecular clock using Bayesian methods in BEAST v1.8.0 (Drummond *et al.*, 2012). XML files were prepared in BEAUTI v1.8.0 as a partitioned seven-marker dataset with independently estimated models of nucleotide evolution. We used two uncorrelated lognormal (UCLN) relaxed clock models separated into nuclear and plastid divisions, independently estimating rates of molecular evolution and rate variation parameters. The UCLN mean rate priors were set as a uniform distribution (0 to 1.0e100) with an initial value of 1.0. A single tree was modeled starting from a random tree and using the Yule process of speciation (Yule, 1925). Since there are no known *Plukenetia* fossils, we used two secondary 'time to most recent common ancestor' (TMRCA) calibration priors based on molecular dating estimates of subfamily Acalyphoideae that used three fossil calibrations (Cervantes *et al.*, 2016). We used normal-distribution priors to constrain the root of the tree and crown of Plukenetiinae (which are the same node) to 36.43 Mya (SD = 4.0) and the crown of *Plukenetia* to 15.21 Mya (SD = 4.0). We initiated three independent MCMC runs for 21 million generations, sampling every 10,000 generations. Runs were assessed for convergence and ESS > 200 using TRACER. When convergence and ESS thresholds were met, runs were combined after excluding the first million generations as burn-in using LOGCOMBINER v1.8.0. A maximum clade credibility tree with mean ages was summarized in TREEANNOTATOR v1.8.0 with a PP limit of 0.95.

Ancestral range estimation

We evaluated potential historical distributions patterns using ancestral range estimation (ARE). We limited biogeographical analyses to species lineages by pruning replicate species tips off the

maximum clade credibility BEAST tree using the drop.tip function of PHYTOOLS (Revell, 2012) in R v3.3.2 (R Core Development Team, 2016). Species distributions were gathered from literature (Jiménez Ramírez, 1993; Gillespie, 1993, 2007; Gillespie & Armbruster, 1997; Bussmann *et al.*, 2009, 2013) and online (Global Biodiversity Information Facility, 2017; Missouri Botanical Garden, 2017) resources. We categorized six areas of distribution: (1) Mexico, Central America, and Northwestern South America (north and west of the Andes); (2) the Amazon biome, including the Guiana shield; (3) the Atlantic Forest biome (Mata Atlântica, Brazil); (4) tropical wet and semi-arid Africa; (5) Madagascar; and (6) Southeast Asia (Table 3.S4). Our neotropical areas were adapted from Morrone (Morrone, 2014) to subdivide the South American floral kingdom, whereas our paleotropical areas were largely defined by the African and Indo-Pacific floral kingdoms (Cox, 2001), except with the recognition of Madagascar as a distinct region from Africa.

We conducted ARE on the modified BEAST chronogram using the maximum-likelihood approach in BIOGEOBEARS (Matzke, 2013, 2014) implemented in R. Our analyses used the dispersal-extinction-cladogenesis (DEC) model from LAGRANGE (Ree & Smith, 2008) in addition to the founder-event parameter (+J) developed in BIOGEOBEARS. The +J parameter allows for "jump speciation", in which cladogenic dispersals can occur outside of the parental area. For recent and ongoing discussion over criticisms of the DEC+J model, see Ree & Sanmartín (2018). Dispersal probabilities between pairs of areas were specified by distance for a single timeslice (Table 3.S5) following similar analyses (Buerki *et al.*, 2011; Berger *et al.*, 2016; Cardinal-McTeague *et al.*, 2016; Givnish *et al.*, 2016). To facilitate clear patterns, inferred ancestral ranges were allowed to occupy up to two areas. We conducted two independent runs with the DEC and DEC+J models and used a likelihood-ratio test to determine the model of best fit.

Defining seed size categories

To empirically define seed size categories, we compiled seed dimension measurements (length, width, and thickness) for all species of *Plukenetia* using literature reports (Jiménez Ramírez, 1993; Gillespie, 1993, 2007; Gillespie & Armbruster, 1997; Bussmann *et al.*, 2009, 2013) and/or mature seeds of (usually) dehisced fruits from dried herbarium specimens. The dimensions were converted into estimated volumes based on the formula for an ellipsoid ($v = 4/3\ \pi$ abc).

We used clustering analysis as an objective approach to identify seed size categories using PAST v3.14 (Hammer *et al.*, 2001). We clustered individual seeds based on their $\log_{10}$ transformed dimension measurements using Euclidean distance and the unweighted pair-group method using arithmetic averages (UPGMA). Clusters were defined by a distance of ~0.35. We visualized the resulting clusters using a three-dimensional principal components analysis (PCA).

Models of continuous trait evolution

We examined patterns of seed size evolution by fitting the modified BEAST chronogram with the mean $\log_{10}$(estimated seed volumes) for each species under three models of trait evolution: (1) Brownian motion; (2) Ornstein-Uhlenbeck (OU); and (3) Early Burst (EB). *Plukenetia huayllabambana* was included in seed evolution analyses by using a placeholder that shared the same phylogenetic position within the nuclear tree. We fit the data to each model using the fitContinuous function in the R package GEIGER (Harmon *et al.*, 2008) and then ranked the best model using ΔAIC and Akaike weights (w_i; (Wagenmakers & Farrell, 2004)). We assessed the phylogenetic signal of our data with Blomberg's *K* (Blomberg *et al.*, 2007) and Pagel's λ (Pagel, 1999) using the phylosig function of PHYTOOLS.

Ancestral state estimation

We performed maximum likelihood ancestral state estimation of $\log_{10}$(seed size volume) as a continuous character on the modified BEAST chronogram using the contMap function in PHYTOOLS. The contMap function uses ML to estimate the ancestral state of internal nodes under Brownian motion, then interpolates the states along the edges of each branch with Felsenstein's equation (3) (Felsenstein, 1985a; Revell, 2013). We also visualized seed size evolution through time by plotting the $\log_{10}$(estimated seed volume) on the modified BEAST chronogram using the phenogram function in PHYTOOLS.

Phylogenetic regression analyses

We used phylogenetic regression to test the correlation between seed size and five putative drivers of seed evolution: plant size, fruit type/dispersal mechanism, seedling ecology, fire tolerance, and biome type. Due to limited quantitative data, we simplified the complexity of each trait into a binary character (Table 3.S6). Plant size was divided into species with slender (0)

versus robust to thick (1) stems; fruit type/dispersal mechanism into dry and dehiscent (0) versus fleshy and indehiscent (1); seedling ecology by light gap and forest edge establishment (0) versus a shade avoidance canopy liana strategy (1); fire tolerance by species that are non-fire adapted (0) and fire adapted (1); and biome type by species that occupy wet dominate biomes (0) versus biomes with a significant dry component (1).

Correlation analyses were conducted with the threshold model (Felsenstein, 2012) using the threshBayes function in PHYTOOLS. The threshold model includes an unobserved quantitative character called the liability, where a trait is determined by whether the liability passes an arbitrary threshold. This method uses BI to measure the covariation of trait liabilities, allowing for the comparison of both continuous and discrete binary data. We ran MCMC analyses for 3 million generations sampling every 1,000th step and applied a 20% burnin prior to summarizing posterior distribution values for the correlation coefficient (*r*). We assessed the ESS of the coefficient using the effectiveSize function in the R package CODA (Plummer *et al.*, 2006).

Results

Genome/transcriptome data-mining results

Of the 1,083 candidate conserved genes queried through LITE BLUE DEVIL, 222 were returned and six were identified as potentially appropriate low-copy markers: AT1G01790, AT1G06820, AT1G08520, AT1G13820, AT2G26680, AT3G11830, AT3G54670. We designed 12 primer pairs across those candidate genes using *Plukenetia* and *Ricinus* database sequences as templates. After preliminary tests of amplification success, low-copy status (i.e., single band and few polymorphisms), and nucleotide variation, AT1G01790 (*KEA1*) was identified as most suitable for use in phylogenetic reconstructions.

Phylogenetic relationships

Table 3.1 presents dataset characteristics for each molecular marker. Comparison of individual markers' bootstrap analyses did not recover any well supported topological conflicts (Fig. 3.S2). As such, further analyses were conducted on three concatenated datasets: (i) combined plastid (cpDNA; *matK*, *ndhF*) including 74 accessions; (ii) combined nuclear (nDNA; ETS, ITS, *KEA1* introns 11 and 17, *TEB* exon 17) including 86 accessions; and (iii) total combined (all seven markers, plastid + nuclear) including 83 accessions. The total combined matrix had an aligned

Table 3.1 Molecular dataset characteristics.

Dataset	n ITS	n ETS	n *KEA1* intron 11	n *KEA1* intron 17	n *TEB* exon 17
No. terminals	85	84	73	57	45
Aligned length	803	468	435	396	558
Variable characters	304	241	150	88	141
Parsimony informative characters (%)	266 (33%)	199 (43%)	121 (28%)	68 (17%)	90 (16%)
Nucleotide model	GTR+I+G	HKY+G	GTR+G	HKY+I	HKY+I

Dataset	cp *matK*	cp *ndhF*	Combined plastid	Combined nuclear	Total combined
No. terminals	70	70	74	86	83
Aligned length	1,654	755	2,409	2,660	5,069
Variable characters	175	128	303	924	1,227
Parsimony informative characters (%)	115 (7%)	94 (13%)	209 (9%)	744 (28%)	953 (19%)
Nucleotide model	GTR+G	GTR+I+G	—	—	—

length of 5,069 characters, 2,660 of nDNA and 2,409 of cpDNA (Table 3.1). Comparison of cpDNA and nDNA topologies revealed two instances of well supported conflicting topologies (Fig. 3.S3), resulting in the removal of all *Plukenetia huayllabambana* accessions (*Téllez 4*, *Quipuscoa 381*) and one accession of *P. loretensis* (*Solomon 7972*) from the total combined dataset. Aside from these shallow incongruences, topologies were consistent across analyses and datasets, with greatly increased node support in total combined analyses (Fig. 3.2). Hereafter, the results and discussion focus on the total combined dataset (Fig. 3.2) and refer to the *Plukenetia* subclade naming system established by Cardinal-McTeague and Gillespie (Cardinal-McTeague & Gillespie, 2016).

General relationships across subtribe Plukenetiinae are strongly supported (interpreted as maximum parsimony bootstrap percentage [MPBP] ≥ 85, PP ≥ 0.95, indicated by bold branches) with only three internal nodes with low support (Fig. 3.2). *Romanoa*, *Plukenetia*, and each of the *Plukenetia* subclades (P1–P5) are monophyletic with strong support (MPBP = 100, PP = 1.0). *Plukenetia* is resolved into two main clades with strong support (MPBP = 100, PP = 1.0) that we formally name (i) the pinnately-veined clade (P1 + P2), containing NWSG2, and (ii) the palmately-veined clade (P3–P5), including sect. *Plukenetia* (P3) + the Old World *Plukenetia* lineage (P4 + P5). The latter comprises sect. *Angostylidium* (P4) and sect. *Hedraiostylus* + the Madagascar species group (P5).

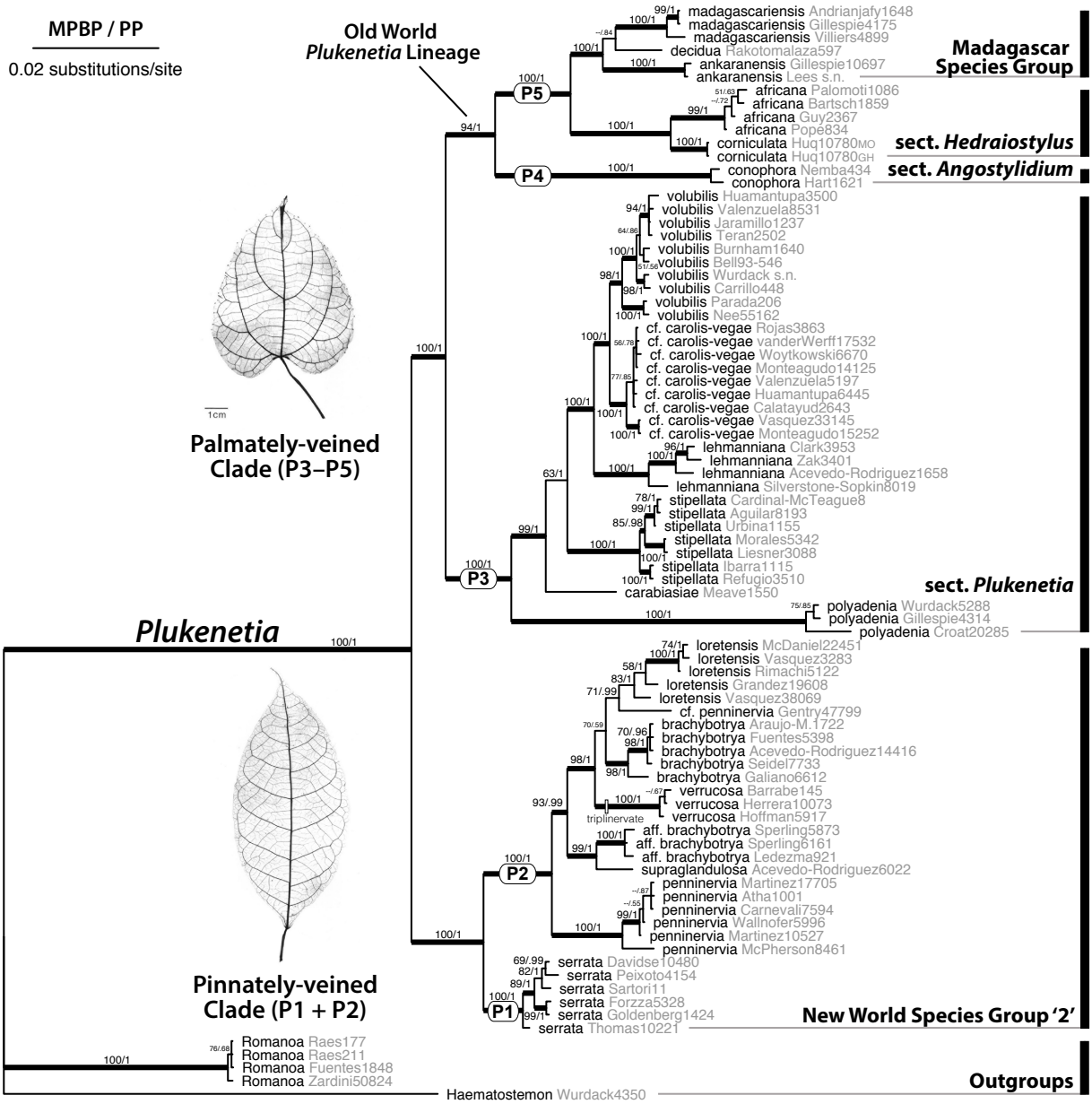


Figure 3.2 Bayesian maximum clade credibility tree based on the combined seven marker (cpDNA and nDNA), 83 accession dataset for *Plukenetia* and Plukenetiinae outgroups. Maximum parsimony bootstrap percentage (MPBP) and Bayesian posterior probabilities (PP) support values > 50% are indicated on each branch. Branches in bold indicate strong support (≥ 85 MPBP and ≥ 0.95 PP). Subclade numbering system (P1–P5) follows Cardinal-McTeague & Gillespie (2016). Inset, leaf clearings demonstrating pinnately-veined (*P. supraglandulosa*, *Granville 3626*, CAY) and palmately-veined (*P. stipellata*, *Gillespie 413*, US) leaf architecture.

Species relationships are well-resolved and a majority are strongly supported (Fig 3.2). Within the pinnately-veined clade, subclade P1 (*P. serrata*) and subclade P2 (the remaining members of NSWG2) form a strongly supported sister group (MPBP = 100, PP = 1.0). The earliest diverging lineage of subclade P2, *P. penninervia*, is sister to two strongly supported clades composed of: (i) *P.* aff. *brachybotrya* + *P. supraglandulosa*; and (ii) a functional polytomy containing *P. brachybotrya*, *P. verrucosa*, and a weakly supported clade (MPBP = 70, PP = 0.59) of *P.* cf. *penninervia* + *P. loretensis*. Within the palmately-veined clade, subclade P3 (sect. *Plukenetia*) is sister to P4 + P5 (the Old World *Plukenetia* lineage) with strong support (MPBP = 94 or 100, PP = 1.0). Sect. *Plukenetia* (P3) is monophyletic (MPBP = 100, PP = 1.0) and resolved into a successive grade of *P. polyadenia*, *P. carabiasiae*, *P. stipellata*, and *P. lehmanniana*, of which the latter is sister to *P.* cf. *carolis-vegae* + *P. volubilis*. The Old World *Plukenetia* lineage (P4 + P5) is monophyletic with strong support (MPBP = 94, PP = 1.0), with sect. *Angostylidium* (P4; *P. conophora*) sister to sect. *Hedraiostylus* (P5; *P. africana* + *P. corniculata*) + the Madagascar species group (P5; a polytomy of *P. ankaranensis*, *P. decidua*, and *P. madagascariensis*), all with strong support (MPBP = 100, PP = 1.0).

Divergence dating

The BEAST chronogram is presented with mean age estimates and 95% highest posterior density (HPD) confidence bars for nodes with ≥ 0.95 PP (Fig. 3.3). A simplified BEAST chronogram is illustrated in Fig. 3.S4. The crown of Plukenetiinae is estimated (under constraint) to have diverged in the Oligocene (31.94 Mya, HPD = 39.01–24.42) with *Romanoa* and *Plukenetia* diverging at 28.71 Mya (HPD = 36.0–21.12). The crown of *Plukenetia* (estimated under constraint) diverged in the Early Miocene (19.1 Mya, HPD = 24.11–14.07) with sect. *Plukenetia* (P3) and the Old World *Plukenetia* lineage (P4 + P5) diverging at 17.39 Mya (HPD = 22.38–12.62). Species of *Plukenetia* diverged continuously from the Middle Miocene (16.0 to 11.6 Mya) into the Pliocene (5.3 to 2.6 Mya).

Reconstructing biogeographical history

ARE analyses using the DEC and DEC+J models recovered similar overall patterns but with less resolved deep-node estimates under the DEC model. A likelihood-ratio test between the two

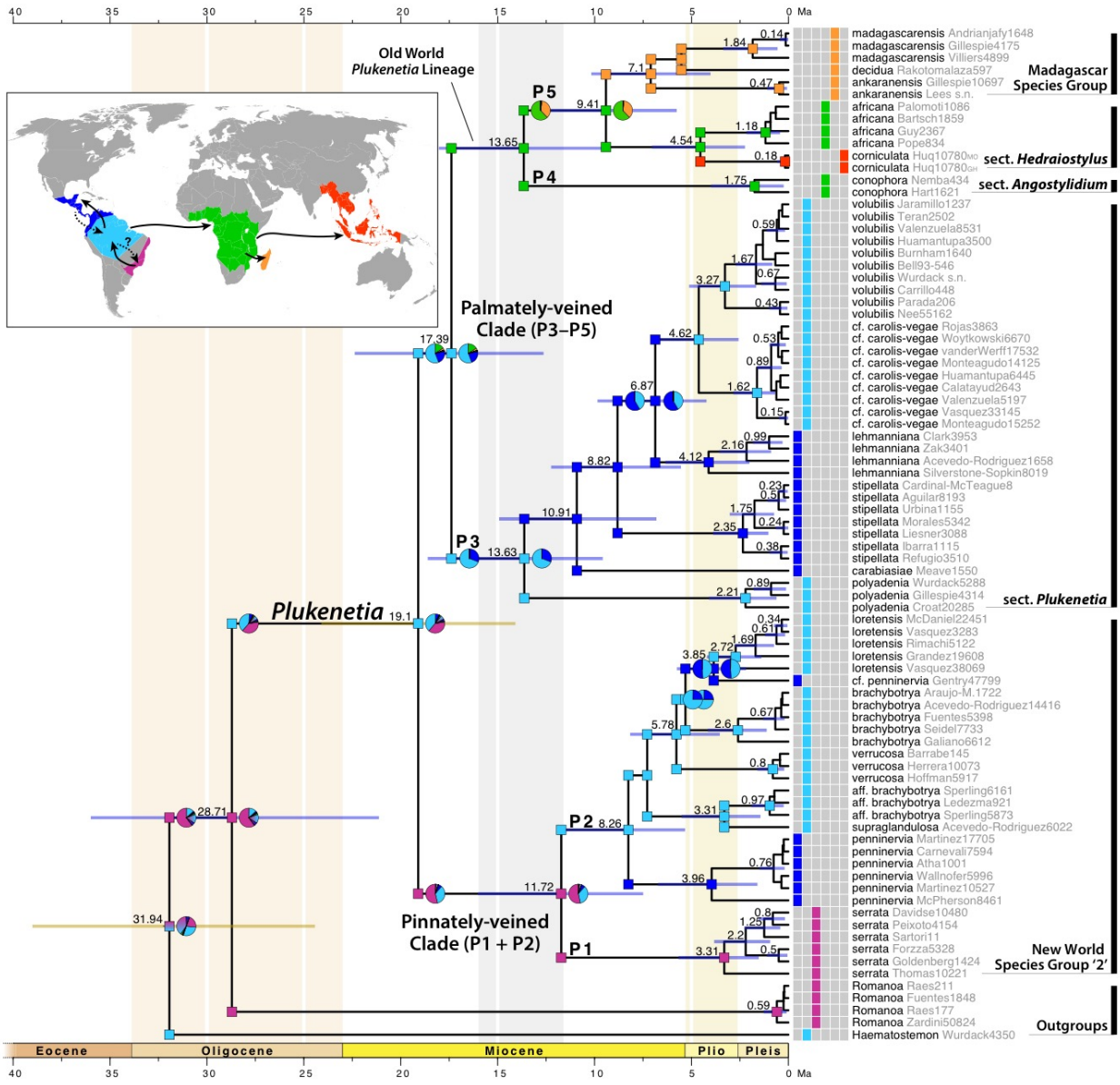


Figure 3.3 Ancestral range estimation on the *Plukenetia* BEAST chronogram using BIOGEOBEARS (DEC+J model). Areas of tip species are shown left of taxa names, color-coded for the six biogeographical areas depicted on the map inset. Boxes at each node and corner are color-coded for the area (or combined area, up to two allowed) with the highest maximum likelihood probability. Pie charts indicate the probability of each area and are included when there is < 75% confidence for a single area. Numbers at each node indicate mean age estimates, blue bars the 95% highest posterior density (HPD) confidence interval, and yellow bars the 95% HPD of calibrated nodes estimated under constraint. Arrows on the map indicate the general direction of range movement in *Plukenetia*; dashed lines indicate area reversals.

models revealed inclusion of the +J "jump speciation" parameter significantly improved likelihood scores (DEC lnL = -46.67, DEC+J lnL = -27.26, $\chi^2(1) = 38.83$, p = 4.6e-10). As such, only the DEC+J results are presented (see Fig. 3.S5 for complete DEC+J and DEC outputs, and Ree & Sanmartín [2018] for recent criticisms of the DEC+J model). The resulting parameters of the DEC+J model included: anagenetic dispersal rate *d* = 1e-5, extinction rate *e* = 1e-5, and cladogenetic dispersal rate *j* = 0.3997152. ARE on the *Plukenetia* BEAST chronogram using BIOGEOBEARS is shown in Fig. 3.3, illustrating the area or combined areas with highest probability for each node and corner. Nodes/corners with low area probabilities (< 0.75 PP) include pie charts depicting the proportion of probable areas (Fig. 3.3).

Biogeographical hypotheses inferred from ARE are based on several nodes with high probability, although the proportion of probable areas for the common ancestor of *Plukenetia* is split between two regions (Fig. 3.3). Biogeographical history at the crown of Plukenetiinae has low resolution but involved the Amazon and Atlantic Forest regions, either separately or as a combined area. The stem lineages of each genus diverged during the Oligocene (33.9 to 23.0 Mya) after becoming isolated in the Amazon (*Haematostemon*) or Atlantic Forest (common ancestor of *Plukenetia* + *Romanoa*) regions. The crown of *Plukenetia* diverged rapidly in the Early Miocene (23.0 to 16.0 Mya) most probably in the Amazon or Atlantic Forest.

ARE within *Plukenetia* revealed frequent migrations/dispersals within the pinnately- (P1 + P2) and palmately-veined (P3–P5) clades. Two biogeographical histories are highly likely in the ancestor of *Plukenetia*. In one scenario, *Plukenetia* originated after its ancestor migrated from the Atlantic Forest into the Amazon during the Oligocene, after which the pinnately-veined clade (P1 + P2) diverged and returned to the Atlantic Forest in the Early Miocene. In the other scenario, *Plukenetia* originated in the Atlantic Forest followed by the ancestor of the palmately-veined clade (P3–P5) migrating into the Amazon in the Early Miocene. Within the pinnately-veined clade (P1 + P2) the ancestor of subclade P1 remained in the Atlantic Forest, while the ancestor of subclade P2 migrated/dispersed to the Amazon by the end of the Middle Miocene followed by two independent migrations/dispersals to Central and NW South America in the Late Miocene (11.6 to 5.3 Mya) and Pliocene. Within sect. *Plukenetia* (P3) an early diverging lineage migrated/dispersed into Central and NW South America in the Middle Miocene and subsequently diversified. The common ancestor of *P.* cf. *carolis-vegae* + *P. volubilis* migrated back into the Amazon during the Pliocene. The ancestor of the Old World *Plukenetia* lineage (P4

+ P5) most likely underwent a long-distance trans-Atlantic Ocean dispersal from the Amazon to tropical Africa during the Early Miocene. Within subclade P5 an early diverging lineage underwent a short-distance dispersal into Madagascar during the Late Miocene, with a later diverging lineage undergoing a long-distance trans-Indian Ocean dispersal to Southeast Asia in the Pliocene.

Seed measurements and seed-size categories

In total, we sampled 122 vouchers of *Plukenetia*, *Romanoa*, and *Haematostemon* and produced a dataset of 212 individual seed measurements (Additional file 3.3), which are summarized for each species in Table 3.2.

Clustering analysis identified five size-based groups in *Plukenetia* (Fig. 3.S6) that can be classified by estimated seed volume: (S) small, 25–100 mm^3; (M) medium, 100–500 mm^3; (L) large, 500–3,000 mm^3; (XL) extra-large 3,000–13,000 mm^3; and (Max) maximum 26,000–38,000 mm^3. PCA analysis revealed the first component (PC1) accounted for 95% of the observed variance, suggesting limited overlap on the x-axis is a good measure of discreteness (Fig. 3.S7). We note that other seed size categories could be defined based on clustering or PCA analysis, but these volume-based categories provide intuitive boundaries based on the seed size variation of *Plukenetia*. The seed size variance of most species could be attributed to a single category, with the exception of *P. africana* (evenly split between S and M) and *P. volubilis* (mostly L but rarely M).

Patterns of seed size evolution

Seed size exhibited strong phylogenetic signal under Blomberg's K (1.0256; $p = 0.001$) and Pagel's λ (0.9999; $p = 0.0004$), with statistical values strongly suggestive of the Brownian motion model of trait evolution. AIC also identified Brownian motion as the best model of evolution ($w_i = 0.66$), rather than OU or EB ($w_i = 0.17$, each) (Table 3.S7).

Table 3.2 Summary of seed measurements for *Plukenetia*, *Romanoa*, and *Haematostemon* (min–max [mean]). Size categories: (S) small, 25–100 mm^3; (M) medium, 100–500 mm^3; (L) large, 500–3,000 mm^3; (XL) extra-large, 3,000–13,000 mm^3; (Max) maximum, 26,000–38,000 mm^3. † = measurements based solely on the species description; n/a = seeds not known.

Taxon	No. seeds	Length (mm)	Width (mm)	Thickness (mm)	Estimated volume (mm^3)	Size category
Haematostemon guianensis Sandw.	1	4	4	4.5	38	S
Plukenetia africana Sond.	18	5.5–8 (6.6)	5–7.5 (6.5)	2.5–4 (3.4)	47–126 (79)	S–M
P. ankaranensis L.J.Gillespie	6	15–18 (16.5)	16–17 (16.4)	14–17 (15.4)	1,929–2,623 (2,187)	L
P. brachybotrya Müll.Arg.	5	5–6 (5.7)	4–5.5 (4.9)	4–5 (4.5)	42–79 (67)	S
P. aff. *brachybotrya*	4	5.5–5.6 (5.6)	4–4.9 (4.6)	3.3–4.1 (3.7)	37–59 (49)	S
P. carabiasiae J.Jiménez Ram.	†	24–27	21–27	14–16	3,695–6,107	XL
P. carolis-vegae Bussmann, Paniagua & C.Téllez	1	27	25	20	7,069	XL
P. cf. *carolis-vegae*	2	19–20	17–18.6	13–15.8	2,221–2,981 (2,601)	L
P. conophora Müll.Arg.	3	25–29 (27)	25–27 (26.3)	25–28 (26)	8,181–10,688 (9,706)	XL
P. corniculata Sm.	27	8–10.5 (9.3)	6–8 (7.3)	4–8 (5.5)	123–272 (197)	M
P. decidua L.J.Gillespie	2	13.1	11.1–11.2 (11.2)	11.2–11.8 (11.5)	853–907 (880)	L
P. huayllabambana Bussmann, C.Téllez & A.Glenn	2	25–28 (26.5)	22–27 (24.5)	17–20 (18.5)	4,896–7,917 (6,627)	XL
P. lehmanniana (Pax & K.Hoffm.) Huft & L.J.Gillespie	3	28–34.3 (30.8)	20–24 (21.7)	24–30 (26)	7,037–12,919 (9,289)	XL
P. loretensis Ule	12	4.5–6 (5.1)	4–5.1 (4.5)	3–5 (4)	28–79 (48)	S
P. madagascariensis Leandri	n/a	n/a	n/a	n/a	n/a	n/a
P. multiglandulosa Jabl.	n/a	n/a	n/a	n/a	n/a	n/a
P. penninervia Müll.Arg.	8	5–6 (5.3)	5–6 (5.6)	4–5 (4.5)	60–79 (70.6)	S
P. cf. *penninervia*	2	7	6.3–6.5	5–5.2	115–124 (120)	M
P. polyadenia Müll.Arg.	19	49–56 (51.3)	33–37 (34.8)	30–36 (32.7)	26,177–37,322 (30,684)	Max
P. procumbens Prain	n/a	n/a	n/a	n/a	n/a	n/a
P. serrata (Vell.) L.J.Gillespie	2	15–15.5 (15.3)	15.5–16 (15.8)	15–16 (15.5)	1,887–2,011 (1,949)	L
P. stipellata L.J.Gillespie	47	10–14 (11.8)	8–13.5 (10.2)	2.5–6 (4.7)	144–484 (301)	M
P. supraglandulosa L.J.Gillespie	3	7–7.3 (7.2)	6.8–7.1 (6.9)	4.6–4.8 (4.7)	117–125 (122)	M
P. verrucosa Sm.	10	5.3–6 (5.9)	3.7–5.5 (5.1)	3.5–5 (4)	55–69 (62)	S
P. volubilis L.	14	13–22 (17)	11.8–18 (15.2)	5–9 (7.2)	480–1,659 (997)	(M) L
Romanoa tamnoides (A.Juss.) Radcl.-Sm.	21	6.3–8 (7.3)	5–6 (5.6)	3–4.2 (3.9)	63–101 (83)	S

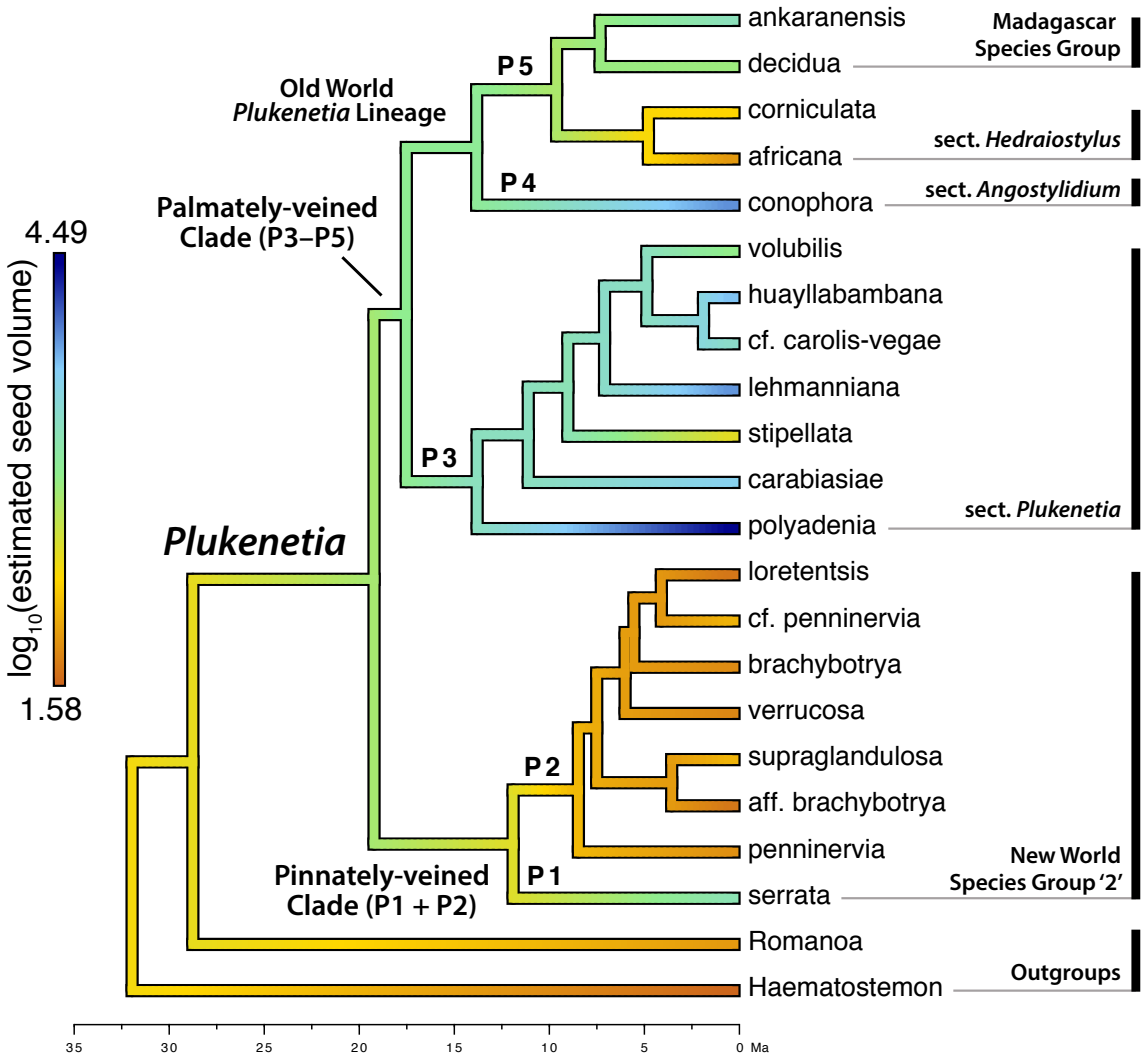


Figure 3.4 Ancestral state estimation of $\log_{10}$(estimated seed size volume) on the modified *Plukenetia* BEAST chronogram using the ContMap function of PHYTOOLS.

Maximum likelihood continuous-character ancestral state estimation (Fig. 3.4) revealed the ancestor of Plukenetiinae most likely had M seeds, with S seeds becoming established in *Haematostemon* and *Romanoa*. The ancestor of *Plukenetia* likely had L seeds, followed by a transition to M seeds in the ancestor of the pinnately-veined clade (P1 + P2) while retaining L seeds in the ancestor of the palmately-veined clade (P3–P5). Within the pinnately-veined clade (P1 + P2), L seeds became fixed in *P. serrata* (P1) and S seeds in the ancestor of the remaining species of NWSG2 (P2). Section *Plukenetia* (P3) is characterized by four independent increases (XL: *P. carabiasiae*, *P. huayllabambana*, *P. lehmanniana*; Max: *P. polyadenia*) and one

decrease (M: *P. stipellata*) from L seeded ancestors. The ancestor of the Old World *Plukenetia* lineage (P4 + P5) most likely had L seeds, with an inferred increase to XL in *P. conophora* (P4) and successive reductions to M and S–M seeds in *P. corniculata* and *P. africana*, respectively (P5). Traitgram analysis recovered similar results as ML ancestral state estimation and shows repeated divergences to larger and smaller seeds through time (Fig. 3.5).

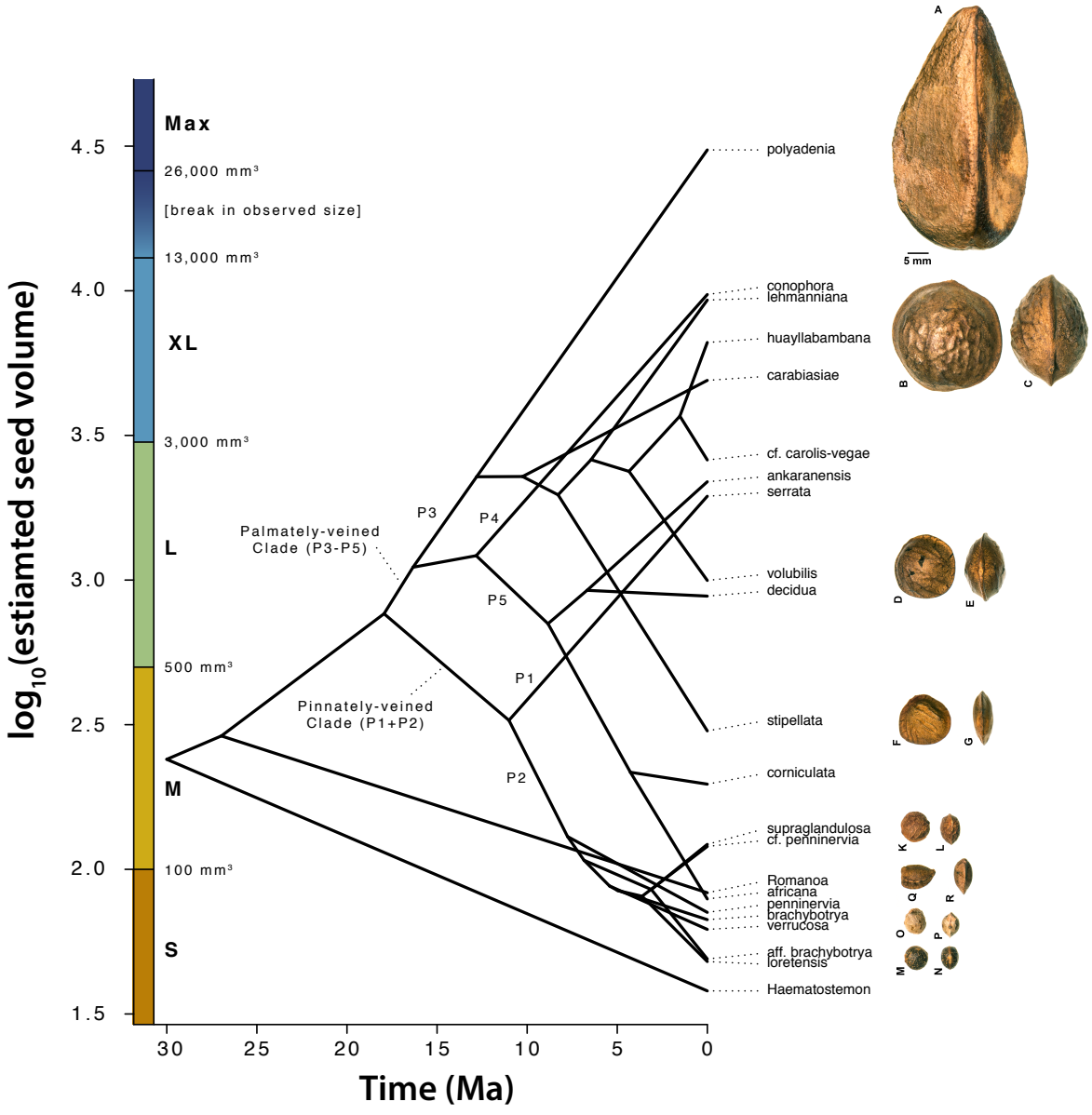


Figure 3.5 Traitgram of $\log_{10}$(estimated seed volume) mapped on the modified *Plukenetia* BEAST chronogram using the phenogram function of PHYTOOLS. The colored scale on the left depicts seed size categories based on clustering analysis. On the right, representative seeds are shown in face and side view (see Fig. 3.1 for accession information).

Correlates with seed size evolution

Phylogenetic regression under the threshold model indicate that seed size had moderate to strong positive relationships with plant size (r = 0.72), fruit type/dispersal mechanism (r = 0.66), and seedling ecology (r = 0.57), a weak negative association with fire tolerance (r = -0.36), and negligible association with biome type (r = -0.08) (Table 3.3). Plots of the posterior distribution for each coefficient (r) are available in Fig. 3.S8.

Table 3.3 Results of phylogenetic regression analysis using the threshold model.

Model	Coefficient (r) mean	Coefficient (r) 95% HPD
Seed size ~ Plant size	0.72	0.39 to 0.95
Seed size ~ Fruit type/dispersal mechanism	0.66	0.24 to 0.95
Seed size ~ Seedling ecology	0.57	0.09 to 0.96
Seed size ~ Biome	-0.08	-0.65 to 0.51
Seed size ~ Fire tolerance	-0.36	-0.87 to 0.31

Discussion

Low-copy nuclear gene identification

Our approach for identifying low-copy nuclear genes was effective but only moderately successful for our purposes. We recovered six promising low-copy genes, but the limited number of genomes/transcriptomes made it difficult to assess for lineage-specific paralogy until after sequencing. PCR amplification was challenging for low-copy nuclear genes compared to multiple-copy plastid and nuclear ribosomal DNA markers, particularly for low-yield DNA extractions common in tropical herbarium specimens, which was the vast majority of our sampling (81/86 accessions). Future applications of a top-down bioinformatics approach to marker development like the LITE BLUE DEVIL pipeline should consider the potential limitations of DNA quality and if there are sufficient genome/transcriptome resources.

Our successful candidate was *KEA1*, a potassium (K+) efflux antiporter gene with putative function in maintaining K+ homeostasis and lowering osmotic potential (Zheng *et al.*, 2013). It belongs to the cation/proton antiporter 2 (CPA2) supergene family, which includes the diverse cation-H+ exchanger (CHX) gene family (Brett *et al.*, 2005). The KEA gene family is highly conserved across green plants and is divided into three subtypes each comprising a variable number of genes (Chanroj *et al.*, 2012). Although unverified, *KEA1* is likely the only gene in the KEA-Ia subtype for *Ricinus communis, Plukenetia*, and by extension a majority of

subfamily Acalyphoideae. At the species level, *KEA1* exons are highly conserved, therefore we designed primers for two introns that had higher nucleotide variation.

Outside of our genome/transcriptome data-mining pathway, we included the single-copy nuclear gene *TEB* for phylogenetic analysis. *TEB* encodes for the helicase and polymerase-containing protein TEBICHI, which is a putative plant homolog of mammalian DNA polymerase θ and has broad function in plant DNA repair and cell differentiation (Inagaki *et al.*, 2006). Little is known about the diversity and function of this gene in plants outside of *Arabidopsis*. Here we designed primers for a large and moderately variable exon, which is located outside of the functional helicase and polymerase domains of the gene.

Phylogenetic relationships and systematic implications

Our results agree with the only other study examining the phylogenetic relationships of *Plukenetia* (Cardinal-McTeague & Gillespie, 2016) but provide a more detailed and strongly supported hypothesis for species relationships in the genus. Here, *Plukenetia* is resolved into two main clades, the pinnately-veined clade (P1 + P2) comprising NWSG2, and the palmately-veined clade (P3–P5) containing sect. *Plukenetia* (P3) + the Old World *Plukenetia* lineage (P4 + P5).

The pinnately- (P1 + P2) and palmately-veined (P3–P5) clades overlap in androecium and gynoecium characters, but are differentiated by pollen tectum and leaf morphology. The pinnately-veined clade (P1 + P2) is equivalent to NWSG2, which was defined by Gillespie (Gillespie, 1993) to include species with "entirely connate styles, all or mostly sessile anthers, pollen with reticulate tectum, small dry capsules (except *P. serrata*), and elliptic pinnately-veined leaves (except *P. verrucosa*)". Of those characters, pollen with reticulate tectum and elliptic pinnately-veined leaves are diagnostic features. The palmately-veined clade (P3–P5) contains the remaining four sections/species groups, sect. *Plukenetia*, sect. *Angostylidium*, sect. *Hedraiostylus*, and the Madagascan species group, of which the latter three compose the Old World lineage. Although species of the palmately-veined clade are morphologically diverse, especially in floral variation of the Old World lineage (Gillespie, 2007), they are united by foveolate pollen tectum and cordiform, ovate, or broadly elliptic leaves with palmate or triplinerved venation (Gillespie, 1993, 2007). Sect. *Plukenetia* has larger pollen grains (50.5–56 x 56.5–64.5 μm) than the Old World lineage (30.5–39 x 36.5–45.5 μm), although similar pollen size variation is found in the pinnately-veined clade (Gillespie, 1994b).

Within the pinnately-veined clade (P1 + P2) we recovered novel support that *Plukenetia serrata* (P1) is sister to the remaining species of NWSG2 (P2). Cardinal-McTeague and Gillespie (Cardinal-McTeague & Gillespie, 2016), using ITS and *psbA-trnH* with insertion/deletion gap-coded data, recovered subclades P1 and P2 in a functional polytomy with the palmately-veined clade (P3–P5), causing doubt over the monophyly of NWSG2. This study strongly supports the monophyly of a pinnately-veined clade (P1 + P2) and validates Gillespie's (Gillespie, 1993) original hypothesis that reticulate pollen exine and pinnate leaf venation are synapomorphies for the lineage. Gillespie (Gillespie, 1993) also hypothesized that *P. verrucosa* was sister to the remainder of NWSG2 since it has plesiomorphic triplinerved leaves. However, here we recover *P. verrucosa* nested inside the pinnately-veined clade suggesting that its leaf morphology is a reversal to a more palmate-like condition.

Within the palmately-veined clade (P3–P5), we provide new evidence for a strongly-supported, monophyletic Old World lineage and backbone topology, where sect. *Angostylidium* (P4) is sister to sect. *Hedraiostylus* + the Madagascar species group (P5). In Cardinal-McTeague and Gillespie (Cardinal-McTeague & Gillespie, 2016), the monophyly of the Old World lineage was not well-supported (PP = 0.87), which presented the possibility that sects. *Plukenetia* (P3) and *Angostylidium* (P4) could form a clade based on their similar leaf, floral, and fruit morphology (Gillespie, 2007). Alternatively, our results suggest that the shared morphology of sects. *Plukenetia* (P3) and *Angostylidium* (P4) is plesiomorphic for the palmately-veined clade (P3–P5) and that the morphology of sect. *Hedraiostylus* and the Madagascar species (P5) is derived.

Putative hybridization events in *Plukenetia*

An unexpected result of our study was evidence for two putative hybridization events between closely related species of *Plukenetia*. The first is a proposed ancient or recent hybridization event that resulted in the introgression of a *P. volubilis* plastid genome into both accessions of *P. huayllabambana* (*Téllez et al. 4*, *Quipuscoa 381*). *Plukenetia huayllabambana* is part of a high elevation species complex related to *P. volubilis*, which also comprises *P. carolis-vegae* and *P.* cf. *carolis-vegae*. *Plukenetia huayllabambana* and *P. carolis-vegae* were recently described from the Amazonas region of northern Peru and are noted for the economic significance of their

Table 3.4 Diagnostic characters of the high elevation species complex (*Plukenetia carolis-vegae*, *P.* cf. *carolis-vegae*, *P. huayllabambana*) and *P. volubilis*. Most characters were updated from species descriptions; † = unverified and potentially inaccurate.

Character	*P. carolis-vegae* Bussmann, Paniagua & C.Téllez	*P.* cf. *carolis-vegae*	*P. huayllabambana* Bussmann, C.Téllez & A.Glenn [putative cf. *carolis-vegae* × *volubilis*]	*P. volubilis* L.
Sampled in phylogeny	No	Yes	Yes	Yes
Geographic range in Peru	Amazonas	Cusco, Junin, Pasco	Amazonas, Cajamarca	Amazonas, Cusco, Junin, Loreto, Madre de Dios, Pasco, San Martin
Elevation	1,850 m	1,450–2,250 m	1,200–2,150	130–900(–1,800) m
Leaf stipels	1–2 thick stipels	1–2 thick stipels	1–2 thick stipels	1 knob (thick stipel)
Staminate sepals	4 (rarely 5) †	5 (sometimes 4 + 1 very thin, rarely 4)	4	4
Staminate nectaries	Absent †	Large irregularly shaped segments intermixed between stamens	Large irregularly shaped segments intermixed between stamens	Absent
Filament length	0.5–1 mm †	1–1.5(2) mm	0.1–0.5(1) mm	~0.5 mm
Style length	4–7 mm †	5–12 mm	6–7.5 mm	(9–)15–35 mm
Seed size measurements (length x width x thickness)	27 x 25 x 20 mm	19–20 x 17–18.6 x 13–15.8 mm	25–28 x 22–27 x 17–20 mm	13–22 x 11.8–18 x 5–9 mm
Seed size category	XL	L	XL	(M) L

cultivated oil-rich XL seeds (Bussmann *et al.*, 2009, 2013). In contrast, *Plukenetia* cf. *carolis-vegae* appears to comprise non-cultivated populations with L seeds that are widespread in the Cusco, Junín, and Pasco regions of central and southern Peru. Members of the high elevation species complex share similar morphology but their distinguishing characters are breaking down as more collections become available (Table 3.4). Our data suggests that *P. huayllabambana* is a hybrid between *P.* cf. *carolis-vegae* and *P. volubilis* on the basis of plastome introgression (Fig. 3.S3) and intermediate staminate floral morphology (Table 3.4). A paratype of *P. huayllabambana* (*Téllez et al. 4*, sampled here) possesses the introgressed plastome, which implies that the similar looking holotype was also based on hybrid material. Moving forward, we need to examine more material of the high elevation species complex from northern Peru, clarify if *P. carolis-vegae* is a naturally occurring or (semi-)domesticated species, and determine if it is distinct from the central/southern population of *P.* cf. *carolis-vegae*.

The second event is less clear but we infer there may have been hybridization and/or incomplete lineage sorting within subclade P2 (Fig. 3.S3). There are multiple weakly supported incongruences between the plastid and nuclear relationships of subclade P2, as well as a strongly supported incongruence in the placement of accession *Solomon 7972*. This specimen is morphologically attributed to *Plukenetia loretensis* (WCM, LJG, personal observations) but does not resolve with that species in either plastid or nuclear analyses. *Solomon 7972* is strongly supported as belonging to the *P. brachybotrya* clade in nDNA analyses, and is unresolved but strongly-supported outside of the *P. brachybotrya* clade by cpDNA. Chromosome number is variable within individuals of *P. volubilis* (Cai *et al.*, 2013), and high and variable ploidy levels are suspected in other Plukenetieae genera (*Dalechampia* and *Tragia*) (Miller & Webster, 1967; Vanzela *et al.*, 1997). Together this suggests that hybridization and possible allopolyploidization could be additional contributing factors to the incongruent relationships and evolutionary forces within *Plukenetia*.

Neotropical biogeography and the origin of *Plukenetia*

The biogeographical history presented here is the first detailed analysis for *Plukenetia* and Plukenetiinae. Using secondary node calibrations based on subfamily age estimates (Cervantes *et al.*, 2016), we find that Plukenetiinae and its genera diverged in the Oligocene (33.9 to 23.0 Mya) during the transitional period between the warm humid Eocene and the cooler drier Miocene (Zachos *et al.*, 2001). By the Oligocene, the continents had already diverged and were well-separated by oceans and seas (McLoughlin, 2001), precluding a Gondwanan vicariance explanation for the pantropical distribution of *Plukenetia*. BIOGEOBEARS analyses indicate the ancestor of Plukenetiinae occupied a broad distribution comprising the Amazon and Atlantic Forest regions (Fig. 3.3) at a time when they likely formed a large continuous forest in the process of being divided by the open vegetation diagonal (now composed of the Chaco, Cerrado, and Caatinga biomes (Werneck, 2011)). The open vegetation diagonal is an important feature of South American biogeography that acts as a barrier for moist forest plants and animals that cannot adapt and enter the drier biome conditions. The precise timing of the open vegetation diagonal's formation is ambiguous but divergence dates between Amazon and Atlantic forest clades of suboscine birds (Batalha-Filho *et al.*, 2013), shield frogs (Fouquet *et al.*, 2012), and spectacled lizards (Pellegrino *et al.*, 2011) suggest the barrier was present by the Oligocene and

Early Miocene. Yet, some plant lineages did not diversify in these open vegetation biomes until the Late Miocene and Pliocene, as is suggested by a Late Miocene radiation of orchids in the Campos Rupestres (rocky savannas) (Antonelli *et al.*, 2010) and a Pliocene radiation of several fire-adapted genera (including *Mimosa*) in the Cerrado (Simon *et al.*, 2009). Our data agree with an Oligocene origin for the open vegetation diagonal barrier (Fig. 3.3), which suggests there was a putative transitional stage between when the open vegetation diagonal barrier formed (Oligocene to Middle Miocene) and when modern Chaco, Cerrado, and Caatinga communities developed (Late Miocene to Pliocene).

Plukenetia and its sister genus, *Romanoa*, are suggested to have diverged in the Atlantic Forest, presumably after it was isolated from the Amazon (Fig. 3.3). *Romanoa* is estimated to have remained in the Atlantic Forest, while the common ancestor of *Plukenetia* either remained in the Atlantic Forest or migrated into the Amazon. Either scenario would invoke two dispersals or migrations between the Amazon and Atlantic Forests, which suggests that there were periodic connections across the open vegetation biogeographical barrier that allowed for biotic exchange. The marine ingression by the Paranaense Sea into northern Argentina, Paraguay, and southern Brazil (Hernández *et al.*, 2005) may have facilitated a forest connection along its coast during the Middle and Late Miocene (originally proposed by Costa 2003). The timing of Paranaense Sea coincides with the ancestor of subclade P2 migrating from the Atlantic Forest into the Amazon (Fig. 3.3). Older migrations during the Oligocene and Early Miocene do not have geological explanations and must invoke short distance dispersals or migrations across a putative mosaic of wet forest fragments. Similar explanations exist for Pliocene and Pleistocene crossings (Costa, 2003; Batalha-Filho *et al.*, 2013). Further examination of plant groups that exhibit an Amazon-Atlantic Forest disjunction (Martini *et al.*, 2007) could shed additional light onto the formation of the open vegetation diagonal and the timing of past biotic exchanges. We could also investigate when neighbouring wet forest plants shifted into the seasonally dry biome of the open vegetation diagonal and converge on a general pattern of its history and formation.

Following entry into the Amazon, a common biogeographical pattern of New World *Plukenetia* lineages was their repeated dispersal across the Andes mountain barrier and beyond into Central America and Mexico. The first transition occurred in subclade P3 (after the divergence of *P. polyadenia*) during the Middle Miocene (Fig. 3.3). At this time the Isthmus of Panama was not fully formed (Hoorn *et al.*, 2010), implying a short-distance dispersal between

South and Central America. The second occurred in subclade P2 in the ancestor of *P. penninervia* during the Late Miocene. These patterns agree with previous indications that many plant lineages dispersed between Central and South America prior to the formation of the Isthmus of Panama (Erkens *et al.*, 2007; Cody *et al.*, 2010; Bacon *et al.*, 2015).

A dispersal event across the Andes and back into the Amazon is revealed in the ancestor of *Plukenetia* cf. *carolis-vegae* and *P. volubilis* during the Pliocene (Fig. 3.3). The timing of re-entry into the Amazon coincides with the highest uplift of the Andes and a period of rapid plant diversification along elevational gradients (Hoorn *et al.*, 2010). *Plukenetia* did not rapidly diversify in response to the uplift of the Andes, but adaptation to either low-to-medium (100–800 m; *P. volubilis*) or high (1500–2500 m; *P.* cf. *carolis-vegae*) elevation environments likely contributed to the speciation of those taxa. A second dispersal across the Andes, from the Amazon into northwestern South America, is inferred in *P.* cf. *penninervia* during the Pliocene (Fig. 3.3). Together, our data supports increasing evidence that tropical plants dispersed across the Andes mountains after achieving their maximum elevation in the Pliocene (Pérez-Escobar *et al.*, 2017b).

Trans-oceanic dispersals explain the pantropical distribution of *Plukenetia*

The pantropical distribution of *Plukenetia* is best explained by multiple trans-oceanic dispersal events throughout the Miocene and Pliocene (Fig. 3.3). The ancestor of the Old World lineage likely underwent a trans-Atlantic Ocean crossing from the Amazon to tropical Africa during the Early Miocene. Current hypotheses suggest recent South American and African disjunctions are the result long-distance dispersal by trade winds or tangled plant mats crossing the Atlantic Ocean on equatorial currents (Renner, 2004a). *Plukenetia* most likely dispersed by water along the north equatorial countercurrent, and joins the growing list of taxa that dispersed in the less-frequently inferred direction from South America to Africa during the Miocene, e.g., *Pitcairnia* (Bromeliaceae) (Givnish *et al.*, 2004, 2011), Melastomeae (Melastomataceae) (Renner & Meyer, 2001; Renner *et al.*, 2001; Berger *et al.*, 2016), *Vanilla* (Orchidaceae) (Givnish *et al.*, 2016), *Maschalocephalus* (Rapateaceae) (Givnish *et al.*, 2004), and *Erismadelphus* (Vosychiaceae) (Sytsma *et al.*, 2004; Berger *et al.*, 2016).

We infer two further dispersal events from Africa into Madagascar and Southeast Asia within *Plukenetia* subclade P5 (Fig. 3.3). The Madagascar species group diverged in the Late

Miocene and likely experienced a short-distance dispersal event across the Mozambique channel from Africa, at a time when once widespread mainland rainforests were transitioning to woodlands and savanna (Jacobs, 2004). Increasing molecular evidence suggests plant diversity in Madagascar has been influenced by multiple arrivals from Africa during and since the Miocene, e.g., *Uvaria* (Annonaceae) (Zhou *et al.*, 2012), *Boscia*, *Cadaba*, *Thilachium* (Capparaceae) (Cardinal-McTeague *et al.*, 2016), Cyatheaceae (Korall & Pryer, 2014), Hibisceae (Malvaceae) (Koopman & Baum, 2008), Rubiaceae (Wikström *et al.*, 2010), and *Rinorea* (Violaceae) (van Velzen *et al.*, 2015).

The ancestor of *Plukenetia corniculata* most likely underwent a trans-Indian Ocean long-distance dispersal from Africa into Southeast Asia in the Pliocene (Fig. 3.3). Africa-to-Asia long-distance dispersals are still poorly understood but are indicated for several taxa starting from the Oligocene, including *Begonia* (Begoniaceae) (de Wilde *et al.*, 2011), *Exacum* (Gentianaceae) (Yuan *et al.*, 2005), *Osbeckia* (Melastomataceae) (Renner, 2004b), *Eurycoma* (Simaroubaceae) (Clayton *et al.*, 2009), and *Cissus* (Vitaceae) (Liu *et al.*, 2013). The Pliocene divergence of *P. corniculata* post-dates migration or step-wise dispersal via the Indian subcontinent or Eocene boreotropical forest, which emphasizes the probable role of the Indian Ocean equatorial countercurrent in transporting tangled plant mats from Africa to Southeast Asia (Schott *et al.*, 2009; Zhou *et al.*, 2012).

Seed size classification and the origin of large seeds

Clustering analysis of seed dimension data provided a more objective and finely parsed seed size classification for *Plukenetia* (Table 3.2). The prior subjective "small versus large" informal groupings have been replaced with five discrete categories that additional seeds can be measured and compared against. The former "small" category is divided into S and M seeds, while the "large" category now comprises L, XL, and Max seeds.

Our new seed size classification allows us to present a more nuanced interpretation of seed evolution in *Plukenetia* (Figs. 3.4 and 3.5). We found pronounced seed size differences in both the pinnately-veined (P1 + P2) and palmately-veined (P3–P5) clades, with more variation in the latter. Our results suggest that seed size evolution can be dynamic and responsive to ecological selection, although phylogenetically conserved in some cases (e.g., pinnately-veined

subclade P2). The rest of tribe Plukenetieae is fairly uniform in having S to M seeds (data not shown), suggesting usually strict genetic controls of seed size have been relaxed in *Plukenetia*.

The ancestor of *Plukenetia* is estimated as having L seeds, suggesting there was a single origin in the genus. Under this scenario, there was likely a reduction to M seeds in the ancestor of the pinnately-veined clade (P1 + P2) followed by continued size reduction in NWSG2 (P2) and a return to L seeds in *P. serrata* (P1) (Fig. 3.4). The molecular mechanisms controlling seed size are not known in *Plukenetia* but could be studied by comparing seed transcriptomes between divergent sister group pairings.

The ancestor of the palmately-veined clade (P3–P5) most likely had L seeds, which suggests that XL seeds evolved independently up to five times in sects. *Plukenetia* (P3) and *Angostylidium* (P4). Max seeds evolved once in the ancestor of *P. polyadenia*, the earliest diverging lineage of sect. *Plukenetia* (P3). *Plukenetia polyadenia* seeds are substantially larger than all other species (Fig. 3.1A) and are suggested to have evolved over a long period of time (since the Middle Miocene) in the Amazon. This species appears especially adapted for low light seedling establishment and uses considerable stored reserves to send out a long leafless leader (LJG, KJW, personal observations). The size, shape, and colour of *P. polyadenia* fruits and seeds are compatible with an extinct South American megafauna dispersal syndrome (Doughty *et al.*, 2016), although its widespread distribution contradicts expected range reduction following the loss of such dispersers.

Multiple traits correlated with seed size evolution

Our study presents, to our knowledge, one of the first analyses of seed size variation across a small, densely-sampled phylogenetic lineage (~23 species). While trends in seed size evolution have been studied within species (Gómez, 2004; Halpern, 2005; Galetti *et al.*, 2013) and on a macroevolutionary scale (Moles *et al.*, 2005b, 2007; Beaulieu *et al.*, 2007; Eriksson, 2008), evolutionary patterns among groups of species have not been well documented. Studies on entire clades allow us to understand the context in which seed size variation develops, as well as directs future comparative research on seed ecology and genetics.

We found that seed size in *Plukenetia* is associated with a combination of traits (plant size, fruit type/dispersal mechanism, seedling ecology), which suggests that the evolution of substantial seed size variation relied on multiple selective pressures rather than a single driving

force. By comparison, a study of *Hakea* (Proteaceae), a diverse group of 150 species of small trees and shrubs that largely occur in fire-prone ecosystems in southwestern Australia, found that seed size was correlated with a different set of traits (fecundity/seed production and postfire regeneration) (El-ahmir *et al.*, 2015). As more clade-based studies emerge we will be able to identify common trends in seed size evolution and how they relate or differ across growth forms and habitats.

Seed size trends in *Plukenetia*

Within *Plukenetia* we observe a strong association between smaller seeds in herbaceous vines and slender lianas up to the largest seeds in thick stemmed canopy lianas (Table 3.5). Our results are consistent with broader trends in seed plants in which plant size and seed mass are strongly correlated, more so than temperature, forest cover, and dispersal mechanism (Moles *et al.*, 2005a). One explanation is that larger plants require more time to reach reproductive maturity, which could drive selection for larger seeds with increased survival rates to reproductive age (Moles & Westoby, 2004). However, *Plukenetia* species start reproducing within one or two years regardless of plant size, suggesting that other ecological factors are driving seed and plant size. Furthermore, while smaller plants may produce more seeds early on, larger plants tend to occupy more canopy space and live longer, resulting in largely equivocal total lifetime seed production (Moles *et al.*, 2004). Comparative analysis of plant size, seed mass, lifespan, and total seed production among *Plukenetia* species with different seed sizes could shed more light on the association between seed and plant size.

Most *Plukenetia* species inhabit evergreen and seasonally moist forests, where seed size was likely driven by competing selection among plant size, dispersal mechanism, and seedling ecology. Although moist forest species of *Plukenetia* are widespread, they do not form a dominant component of forest vegetation or fit into early successional communities. Rather, they form a small but common element of primary and secondary forest edges and light gaps associated with treefall disturbances and natural light breaks from rivers and rocky outcrops (Table 3.5). Moist forest *Plukenetia* seeds have a high probability of being dispersed into shaded areas so there should be a trade-off between producing many smaller seeds that have a higher probability of being dispersed into favourable light gaps, and fewer larger seeds that may not be

Table 3.5 Traits associated with seed size for species of *Plukenetia* and outgroup genera (arranged by size). DDF = dry deciduous forest; ERF = evergreen rain forest; SMF = seasonal moist forest.

Species/ species group	Sub-clade	Biome	Habitat	Growth form	Stem size	Fruit type	Seed size category	Seedling ecology
P. polyadenia	P3	ERF	Lowland forest; riparian forest	Canopy liana	Thick	Fleshy	Max	Shade
P. conophora	P4	ERF, SMF	Lowland forest	Canopy liana	Thick	Fleshy	XL	Shade
P. lehmanniana	P3	ERF, SMF	Lowland to montane forest edges, light gaps	Slender to robust liana	Thick	Fleshy	XL	Light gap
P. huayllabambana	P3	ERF, SMF	Montane forest edges, ravines, light gaps	Slender to robust liana	Thick	Dry	XL	Light gap
P. carabiasiae	P3	ERF	Montane forest	Canopy liana	Thick	Dry	XL	Shade
P. cf. *carolis-vegae*	P3	ERF, SMF	Montane forest edges, ravines, light gaps	Slender to robust liana	Thick	Dry	L	Light gap
P. serrata	P1	SMF	Pre-montane to montane forest edges	Slender to robust liana	Thick	Fleshy	L	Light gap
Madagascan species	P5	DDF	Forest on limestone or sand; spiny forest scrub	Robust liana	Thick	Dry	L	Light gap
P. volubilis	P3	ERF, SMF, (semi-humid savanna)	Lowland to pre-montane forest edges, ravines, light gaps (seasonally flooded savanna woodland)	Vine to slender liana	Thin	Dry	(M) L	Light gap
P. stipellata	P3	ERF, SMF	Lowland forest edges, light gaps	Vine to slender liana	Thin	Dry	M	Light gap
P. corniculata	P5	ERF, SMF	Lowland forest edges, light gaps	Vine to slender liana	Thin	Dry	M	Light gap
P. supraglandulosa	P2	SMF	Lowland to submontane forest edges, light gaps	Vine to slender liana	Thin	Dry	M	Light gap
P. cf. *penninervia*	P2	ERF	Lowland pluvial forest edges, light gaps	Vine to slender liana	Thin	Dry	M	Light gap
P. africana	P5	Semi-arid savanna	Savanna woodland on sandy soil	Vine with thick rootstock	Thin	Dry	S–M	Light gap
Romanoa	n/a	SMF	Semi-deciduous lowland forest, rocky outcrops,	Vine to slender liana	Thin	Dry	S	Light gap

			light gaps; stony soil caatinga					
NWSG2 pro parte (excluding *P. serrata*, *P.* cf. *penninervia*, *P. supraglandulosa*)	P2	ERF, SMF, (DDF)	Lowland to pre-montane (montane) forest edges, rocky outcrops, light gaps; white sand forest	Vine to slender liana	Thin	Dry	S	Light gap
Haematostemon	n/a	ERF	Lowland forest; riparian forest	Small tree/ shrub	Thin	Dry	S	Light gap

dispersed far but produce more resilient seedlings that can tolerate or avoid low light conditions (Coomes & Grubb, 2003). The largest *Plukenetia* seeds are associated with canopy liana species (i.e., *P. conophora*, *P. polyadenia*), which have the added challenge of fueling seedling growth upwards to reach as much light as possible in the understory. It seems likely that movement into different niche spaces (i.e., light gap versus canopy) is the driving force of seed size extremes in *Plukenetia*, and that variation therein is a result of a balance between those competing selective pressures.

Biome shifts were not correlated with seed size changes, except in the transition to semi-arid savannas in *Plukenetia africana*. In this case, increasing aridification, forest fragmentation, seasonality, and fire regimes (Jacobs, 2004) are thought to have selected for smaller plants with perennial woody rootstocks and short-lived seasonal stems (Gillespie, 2007). This smaller seasonal growth form is better adapted to survive prolonged dry seasons and facilitates resprouting after fires (Clarke *et al.*, 2013). We recovered a weak negative association between fire tolerance and seed size, but with a sample size of one it is difficult to draw a strong conclusion. Furthermore, while there is typically a positive association between increased seed size and embryo survival in fire-prone systems (Lahoreau *et al.*, 2006; Tavşanoğlu & Serter Çatav, 2012; Ribeiro *et al.*, 2015), seed size can be constrained by conflicting selective pressure from seed predation (Gómez, 2004; Tavşanoğlu & Serter Çatav, 2012) or by a close association with plant size as is suggested by our data. We note that traits other than seed size can compensate for survival in fire-prone systems, such as seed pubescence, shape, and pericarp thickness (Gómez-González *et al.*, 2011), although these do not appear of relevance in *P. africana*.

Dispersal syndromes in *Plukenetia* are not yet well-documented but can be predicted based on fruit colour, dehiscence, and seed size and content. Species with S and M seeds usually

have dry brown capsules that explosively dehisce and release their seeds to the ground below within a few meters of the parent. This fruit type is typical for Euphorbiaceae in general and is likely plesiomorphic in the genus. Assuming that all *Plukenetia* species have seeds with high fatty acid content, we hypothesize that S and M seeds would be secondarily dispersed by scatter hoarding rodents that preferentially search for valuable high energy seeds (Jansen *et al.*, 2002; Wang & Yang, 2014). Scatter hoarding is a reliable short distance dispersal mechanism in neotropical forests (Forget, 1990), which could favour smaller seeds since larger seeds are more likely to be preyed upon (Gómez, 2004). *Plukenetia* species with XL and Max seeds often have green or brown, thinly fleshed, indehiscent berries. These large, high energy fruits could be part of the diverse diet of larger mammals such as primates (Poulsen *et al.*, 2002; Chapman & Russo, 2006). If dispersed by large mammals, we suspect that a majority of XL and Max seeds would be heavily preyed upon and inefficiently dispersed, although occasionally they could be transported further distances than possible by scatter hoarding rodents. Some L and XL seeds are intermediate and have semi-indehiscent, somewhat fleshy capsules that dry slowly and eventually dehisce to disperse their seeds. In this case, they are possibly dispersed by a combination of scatter hoarding and larger mammal syndromes.

Conclusions

Here, we identified two novel low-copy nuclear genes for phylogenetic analysis (*KEA1* and *TEB*), resolved the backbone and a majority of the species relationships in *Plukenetia*, and produced a robust chronogram for time-dependent evolutionary analysis of the genus. We found support for the monophyly of two major clades, the pinnately-veined clade (P1 + P2) composed of NWSG2, and the palmately-veined clade (P3–P5) composed of sects. *Plukenetia* (P3), *Angostylidium* (P4), and *Hedraiostylus* + the Madagascar species group (P5). Molecular dating and biogeographical analyses suggest that *Plukenetia* originated in either the Amazon or Atlantic Forest of Brazil during the Oligocene. The early biogeographical history of *Plukenetia* is equivocal between these two areas, but suggests a general trend of migration across the open vegetation diagonal during the Oligocene and Early to Mid Miocene. Ancestors in the Amazon underwent at least two independent dispersals into Central America and Mexico prior to the formation of the Isthmus of Panama in the Mid and Late Miocene. The ancestor of *P. volubilis* and *P.* cf. *carolis-vegae* was the only lineage to return to the Amazon from Central and NW

South America, by an inferred dispersal over the Andes during the Pliocene. The pantropical distribution of *Plukenetia* is best explained by trans-oceanic long-distance dispersals, first to Africa in the Early Miocene and then independently to Madagascar and Southeast Asia, during the Late Miocene and Pliocene. We estimate that there was a single origin of L seeds in the ancestor of *Plukenetia*. Within *Plukenetia*, seed size evolution is dynamic and correlated with plant size, fruit type (including inferred dispersal mechanism), and seedling ecology. Biome shifts were not associated with seed size, however, the transition to a seasonal, fire-regimented savanna recovered a weak association with seed size reduction. Quantitative seed ecology studies are needed to elaborate on the trends we identified in *Plukenetia*, and would serve as groundbreaking clade-based investigations into the drivers of seed size variation.

Supplemental data (available at end of dissertation)

Supplemental data 3.1: Python script for LITE BLUE DEVIL v0.3.

Supplemental data 3.2: Setting file for LITE BLUE DEVIL v0.3.

Supplemental data 3.3: Seed size measurements for *Plukenetia* and Plukenetiinae outgroups.

Table 3.S1 Accession table for vouchers used in phylogenetic analyses (bolded text indicates new GenBank accessions; [a] = silica dried specimens).

No.	Species	Voucher	Country	ITS	ETS	*KEA1* intron 11	*KEA1* intron 17	*TEB* exon 17	*matK*	*ndhF*
1	*Haematostemon guianensis* Sandwith	Wurdack 4350 (US) [a]	Guyana	KP794434	**MF502427**	—	—	**MF502846**	**MF502706**	**MF502776**
2	*Plukenetia africana* Sond.	Bartsch 1859 (US)	Namibia	**MF502513**	**MF502431**	**MF502579**	**MF502651**	**MF502848**	**MF502710**	**MF502780**
3	*P. africana*	Guy 2367 (MO)	Botswana	**MF502514**	—	—	—	—	—	**MF502781**
4	*P. africana*	Palomoti 1086 (MO)	Botswana	**MF502515**	**MF502432**	—	—	—	**MF502711**	**MF502782**
5	*P. africana*	Pope et al. 834 (MO)	Botswana	**MF502516**	**MF502433**	**MF502580**	—	—	**MF502712**	**MF502783**
6	*P. ankaranensis* L.J.Gillespie	Gillespie et al. 10697 (CAN) [a]	Madagascar	KP794438	**MF502434**	**MF502581**	**MF502652**	**MF502849**	**MF502713**	**MF502784**
7	*P. ankaranensis*	Lees s.n. (CAN)	Madagascar	KP794437	**MF502435**	—	**MF502653**	**MF502850**	**MF502714**	**MF502785**
8	*P. brachybotrya* Müll.Arg.	Acevedo-Rodriguez 14416 (NY)	Peru	**MF502517**	**MF502436**	**MF502582**	**MF502654**	**MF502851**	**MF502715**	**MF502786**
9	*P. brachybotrya*	Araujo-M. et al. 1722 (MO)	Bolivia	KP794452	**MF502437**	**MF502583**	**MF502655**	**MF502852**	**MF502716**	**MF502787**
10	*P. brachybotrya*	Fuentes & Torrico 5398 (MO)	Bolivia	**MF502518**	**MF502438**	**MF502584**	—	—	**MF502717**	**MF502788**
11	*P. brachybotrya*	Galiano et al. 6612 (MO)	Peru	**MF502519**	**MF502439**	—	—	—	**MF502718**	**MF502789**
12	*P. brachybotrya*	Seidel & Vaquiata 7733 (MO)	Bolivia	**MF502520**	**MF502440**	**MF502585**	**MF502656**	**MF502853**	**MF502719**	**MF502790**
13	*P. aff. brachybotrya*	Ledezma et al. 921 (CAN)	Bolivia	**MF502511**	**MF502428**	**MF502576**	**MF502649**	—	**MF502707**	**MF502777**
14	*P. aff. brachybotrya*	Sperling et al. 5873 (MO)	Brazil	**MF502512**	**MF502429**	**MF502577**	**MF502650**	**MF502847**	**MF502708**	**MF502778**
15	*P. aff. brachybotrya*	Sperling et al. 6161 (CAN)	Brazil	—	**MF502430**	**MF502578**	—	—	**MF502709**	**MF502779**
16	*P. carabiasiae* J.Jiménez Ram.	Meave et al. 1550 (MO)	Mexico	**MF502521**	**MF502441**	**MF502586**	—	—	**MF502720**	—
17	*P. cf. carolis-vegae*	Calatayud et al. 2643 (CAN)	Peru	**MF502527**	**MF502448**	**MF502592**	—	—	—	—
18	*P. cf. carolis-vegae*	Huamantupa et al. 6445 (CAN)	Peru	**MF502528**	**MF502449**	**MF502593**	**MF502660**	—	**MF502724**	—
19	*P. cf. carolis-vegae*	Monteagudo et al. 14125 (MO)	Peru	**MF502529**	**MF502450**	**MF502594**	**MF502661**	—	**MF502725**	**MF502793**
20	*P. cf. carolis-vegae*	Monteagudo et al. 15252 (MO)	Peru	**MF502530**	**MF502451**	—	—	—	**MF502726**	**MF502794**
21	*P. cf. carolis-vegae*	Rojas et al. 3863 (CAN)	Peru	**MF502532**	**MF502453**	**MF502595**	—	—	—	—

22	*P. cf. carolis-vegae*	Valenzuela et al. 5197 (CAN)	Peru	**MF502534**	**MF502455**	**MF502597**	**MF502663**	—	—	—
23	*P. cf. carolis-vegae*	van der Werff et al. 17532 (CAN)	Peru	**MF502535**	**MF502456**	**MF502598**	**MF502664**	**MF502856**	**MF502729**	**MF502797**
24	*P. cf. carolis-vegae*	Vasquez et al. 33145 (MO)	Peru	**MF502536**	**MF502457**	**MF502599**	**MF502665**	**MF502857**	**MF502730**	**MF502798**
25	*P. cf. carolis-vegae*	Woytkowski 6670 (MO)	Peru	**MF502537**	**MF502458**	**MF502600**	**MF502666**	**MF502858**	**MF502731**	**MF502799**
26	*P. conophora* Müll.Arg.	Hart 1621 (MO)	Democratic Republic of the Congo	**MF502523**	**MF502443**	**MF502588**	—	—	—	—
27	*P. conophora*	Nemba & Thomas 434 (MO)	Cameroon	KP794457	**MF502444**	**MF502589**	**MF502657**	—	**MF502722**	**MF502792**
28	*P. corniculata* Sm.	Huq & Haroon 10780 (GH)	Bangladesh	**MF502524**	**MF502445**	**MF502590**	**MF502658**	**MF502854**	—	—
29	*P. corniculata*	Huq & Haroon 10780 (MO)	Bangladesh	**MF502525**	**MF502446**	**MF502591**	**MF502659**	**MF502855**	**MF502723**	—
30	*P. decidua* L.J.Gillespie	Rakotomalaza 597 (CAN)	Madagascar	**MF502526**	**MF502447**	—	—	—	—	—
31	*P. huayllabambana* Bussmann, C.Tellez & A.Glenn	Quipuscoa 381 (MO)	Peru	**MF502531**	**MF502452**	—	—	—	**MF502727**	**MF502795**
32	*P. huayllabambana*	Tellez et al. 4 (MO)	Peru	**MF502533**	**MF502454**	**MF502596**	**MF502662**	—	**MF502728**	**MF502796**
33	*P. lehmanniana* (Pax & K.Hoffm.) Huft & L.J.Gillespie	Acevedo-Rodriguez & Daly 1658 (MO)	Ecuador	KP494443	**MF502459**	**MF502601**	**MF502667**	**MF502859**	**MF502732**	**MF502800**
34	*P. lehmanniana*	Clark 3953 (MO)	Ecuador	**MF502538**	**MF502460**	**MF502602**	**MF502668**	**MF502860**	**MF502733**	**MF502801**
35	*P. lehmanniana*	Silverstone-Sopkin & Giralda-Gesini 8019 (MO)	Colombia	**MF502539**	**MF502461**	**MF502603**	—	**MF502861**	**MF502734**	**MF502802**
36	*P. lehmanniana*	Zak & Jaramillo 3401 (MO)	Ecuador	**MF502540**	**MF502462**	**MF502604**	**MF502669**	**MF502862**	**MF502735**	**MF502803**
37	*P. loretensis* Ule	Grandez 19608 (AMAZ) [a]	Peru	KP794453	**MF502463**	**MF502605**	**MF502670**	**MF502863**	**MF502736**	**MF502804**
38	*P. loretensis*	McDaniel & Rimachi 22451 (MO)	Peru	**MF502541**	**MF502464**	**MF502606**	—	—	**MF502737**	**MF502805**
39	*P. loretensis*	Rimachi 5122 (MO)	Peru	**MF502542**	**MF502465**	**MF502607**	—	—	**MF502738**	**MF502806**
40	*P. loretensis*	Solomon 7972 (MO)	Bolivia	**MF502543**	**MF502466**	**MF502608**	—	**MF502864**	**MF502739**	**MF502807**
41	*P. loretensis*	Vásquez & Jaramillo 3283 (MO)	Peru	**MF502544**	**MF502467**	**MF502609**	—	—	—	—
42	*P. loretensis*	Vasquez et al. 38069 (MO)	Peru	**MF502545**	**MF502468**	**MF502610**	**MF502671**	**MF502865**	**MF502740**	**MF502808**

43	*P. madagascarensis* Leandri	Andrianjafy 1648 (CAN)	Madagascar	**MF502546**	**MF502469**	**MF502611**	**MF502672**	—	**MF502741**	—
44	*P. madagascarensis*	Gillespie 4175 (CAN)	Madagascar	**MF502547**	**MF502470**	**MF502612**	**MF502673**	**MF502866**	**MF502742**	**MF502809**
45	*P. madagascarensis*	Villiers et al. 4899 (MO)	Madagascar	**MF502548**	**MF502471**	**MF502613**	**MF502674**	**MF502867**	**MF502743**	**MF502810**
46	*P. penninervia* Müll.Arg.	Atha et al. 1001 (MO)	Belize	KP794455	**MF502472**	**MF502614**	**MF502675**	**MF502868**	**MF502744**	**MF502811**
47	*P. penninervia*	Carnevali & Duno 7594 (MO)	Mexico	**MF502549**	**MF502473**	**MF502615**	**MF502676**	**MF502869**	**MF502745**	**MF502812**
48	*P. penninervia*	Martínez 10527 (MO)	Mexico	KP794456	**MF502474**	**MF502616**	**MF502677**	**MF502870**	**MF502746**	**MF502813**
49	*P. penninervia*	Martinez 17705 (DAV)	Mexico	**MF502550**	**MF502475**	**MF502617**	**MF502678**	**MF502871**	**MF502747**	**MF502814**
50	*P. penninervia*	McPherson 8461 (MO)	Panama	KP794454	**MF502476**	**MF502618**	**MF502679**	**MF502872**	**MF502748**	**MF502815**
51	*P. penninervia*	Wallnöfer & Frisch 5996 (MO)	Guatemala	**MF502551**	**MF502477**	**MF502619**	—	**MF502873**	**MF502749**	**MF502816**
52	*P. cf. penninervia*	Gentry 47799 (MO)	Colombia	**MF502522**	**MF502442**	**MF502587**	—	—	**MF502721**	**MF502791**
53	*P. polyadenia* Müll.Arg.	Croat 20285 (MO)	Peru	**MF502552**	—	—	—	—	**MF502750**	**MF502817**
54	*P. polyadenia*	Gillespie 4314 (CAN)	Smithsonian Greenhouse ex French Guiana	**MF502553**	**MF502478**	**MF502620**	**MF502680**	**MF502874**	**MF502751**	**MF502818**
55	*P. polyadenia*	Wurdack 5288 (US)	Guyana	**MF502554**	**MF502479**	**MF502621**	**MF502681**	**MF502875**	**MF502752**	**MF502819**
56	*P. serrata* (Vell.) L.J.Gillespie	Davidse 10480 (MO)	Brazil	**MF502555**	**MF502480**	—	—	—	**MF502753**	**MF502820**
57	*P. serrata*	Forzza et al. 5328 (RB)	Brazil	**MF502556**	**MF502481**	**MF502622**	**MF502682**	—	**MF502754**	**MF502821**
58	*P. serrata*	Goldenberg et al. 1424 (RB)	Brazil	**MF502557**	**MF502482**	**MF502623**	**MF502683**	—	**MF502755**	**MF502822**
59	*P. serrata*	Peixoto et al. 4154 (MO)	Brazil	KP794458	**MF502483**	**MF502624**	—	—	**MF502756**	**MF502823**
60	*P. serrata*	Sartori & Pardo 11 (RB)	Brazil	**MF502558**	**MF502484**	**MF502625**	**MF502684**	—	**MF502757**	**MF502824**
61	*P. serrata*	Thomas 10221 (NY)	Brazil	**MF502559**	**MF502485**	**MF502626**	**MF502685**	—	—	**MF502825**
62	*P. stipellata* L.J.Gillespie	Aguilar 8193 (MO)	Costa Rica	KP794448	**MF502486**	**MF502627**	**MF502686**	**MF502876**	**MF502758**	**MF502826**
63	*P. stipellata*	Cardinal-McTeague 8 (CAN) [a]	Costa Rica	**MF502560**	**MF502487**	**MF502628**	**MF502687**	**MF502877**	**MF502759**	**MF502827**
64	*P. stipellata*	Ibarra Manriquez & Sinaca 1115 (MO)	Mexico	**MF502561**	**MF502488**	**MF502629**	**MF502688**	**MF502878**	—	**MF502828**

65	*P. stipellata*	Liesner 3088 (MO)	Costa Rica	KP794451	**MF502489**	**MF502630**	**MF502689**	**MF502879**	—	**MF502829**
66	*P. stipellata*	Morales & Rojas 5342 (MO)	Costa Rica	KP794450	**MF502490**	**MF502631**	**MF502690**	**MF502880**	**MF502760**	**MF502830**
67	*P. stipellata*	Refugio Cedillo Trigas 3510 (MO)	Mexico	**MF502562**	**MF502491**	**MF502632**	**MF502691**	**MF502881**	**MF502761**	**MF502831**
68	*P. stipellata*	Urbina 1155 (MO)	Nicaragua	KP794449	**MF502492**	**MF502633**	**MF502692**	**MF502882**	**MF502762**	**MF502832**
69	*P. supraglandulosa* L.J.Gillespie	Acevedo-Rodriguez 6022 (US)	Suriname	**MF502563**	**MF502493**	**MF502634**	**MF502693**	**MF502883**	**MF502763**	**MF502833**
70	*P. verrucosa* Sm.	Barrabe & Crozier 145 (US)	French Guiana	**MF502564**	**MF502494**	**MF502635**	**MF502694**	**MF502884**	**MF502764**	**MF502834**
71	*P. verrucosa*	Herrera & Kaemar 10073 (CAN)	Suriname	**MF502565**	**MF502495**	—	—	—	—	—
72	*P. verrucosa*	Hoffman 5917 (US)	Suriname	**MF502566**	**MF502496**	**MF502636**	**MF502695**	**MF502885**	**MF502765**	**MF502835**
73	*P. volubilis* L.	Bell 93-546 (US)	Peru	KP794447	**MF502497**	**MF502637**	**MF502696**	**MF502886**	**MF502766**	**MF502836**
74	*P. volubilis*	Burnham & Krings 1640 (MO)	Ecuador	KP794446	**MF502498**	**MF502638**	**MF502697**	**MF502887**	**MF502767**	**MF502837**
75	*P. volubilis*	Carrillo & Reyes 448 (MO)	Ecuador	**MF502567**	**MF502499**	**MF502639**	—	—	—	—
76	*P. volubilis*	Huamantupa 3500 (CAN)	Peru	**MF502568**	**MF502500**	**MF502640**	**MF502698**	—	—	—
77	*P. volubilis*	Jaramillo et al. 1237 (MO)	Bolivia	**MF502569**	**MF502501**	**MF502641**	**MF502699**	—	**MF502768**	**MF502838**
78	*P. volubilis*	Nee 55162 (MO)	Bolivia	KP794445	**MF502502**	**MF502642**	**MF502700**	**MF502888**	**MF502769**	**MF502839**
79	*P. volubilis*	Parada et al. 206 (CAN)	Bolivia	KP794444	**MF502503**	**MF502643**	**MF502701**	**MF502889**	**MF502770**	**MF502840**
80	*P. volubilis*	Teran et al. 2502 (MO)	Bolivia	**MF502570**	**MF502504**	**MF502644**	**MF502702**	—	**MF502771**	**MF502841**
81	*P. volubilis*	Valenzuela 8531 (MO)	Peru	**MF502571**	**MF502505**	—	—	—	—	—
82	*P. volubilis*	Wurdack s.n. (US)[a]	Smithsonian Greenhouse ex Peru	**MF502572**	**MF502506**	**MF502645**	**MF502703**	**MF502890**	**MF502772**	**MF502842**
83	*Romanoa tamnoides* (A.Juss.) Radcl.-Sm.	Fuentes 1848 (MO)	Bolivia	**MF502573**	**MF502507**	—	—	—	—	—
84	*R. tamnoides*	Raes & Terceros 177 (MO)	Bolivia	KP794435	**MF502508**	**MF502646**	**MF502704**	—	**MF502773**	**MF502843**
85	*R. tamnoides*	Raes et al. 211 (MO)	Bolivia	**MF502574**	**MF502509**	**MF502647**	**MF502705**	—	**MF502774**	**MF502844**
86	*R. tamnoides*	Zardini & Chaparro 50824 (MO)	Paraguay	**MF502575**	**MF502510**	**MF502648**	—	—	**MF502775**	**MF502845**

Table 3.S2 Accession table of genomes/transcriptomes used in data-mining analyses.

No.	Taxon	Type	Assembly file	Reference
1	*Croton tiglium* L.	Transcriptome	VVPY_Croton_tiglium_-SOAPdenovo-Trans-assembly.fa	1KP Project
2	*Euphorbia mesembryanthemifolia* Jacq.	Transcriptome	LSLA_Euphorbia_mesembryanthemifolia_juv_-SOAPdenovo-Trans-assembly.fa	1KP Project
3	*E. mesembryanthemifolia*	Transcriptome	VPDX_Euphorbia_mesembryanthemifolia_mat_-SOAPdenovo-Trans-assembly.fa	1KP Project
4	*Jatropha curcas* L.	Draft genome	GCF_000696525.1_JatCur_1.0_rna.fna	Sato et al. 2011
5	*Manihot grahamii* Hook.	Transcriptome	XNLP_Manihot_grahamii_-SOAPdenovo-Trans-assembly.fa	1KP Project
6	*Plukenetia volubilis* L.	Transcriptome	PlutentiaAssemGADC01000001-GADC01066310.fasta	Wang et al. 2012
7	*Ricinus communis* L.	Draft genome	TIGR_castorWGS_release_0.1.cds.fsa	Chan et al. 2010
8	*R. communis*	Draft genome	TIGR_castorWGS_release_0.1.gene.fsa	Chan et al. 2010
9	*R. communis*	Transcriptome	PAZJ_Ricinus_communis_1kp_-SOAPdenovo-Trans-assembly.fa	1KP Project

Table 3.S3 List of molecular markers, primer sequence, and amplification/sequencing protocols.

Name	Primer (5' to 3')	Reference	Recommended Protocol
Nuclear DNA (nDNA)			
External Transcribed Spacer (ETS)			
ETS F1	GTT ATG TTT GGY GTT TCG GSA ATG CT	Designed here	Amplification and sequencing: ETS Pluk F2 + 18S 5' R
ETS F2	ATG ATC GTT TGS TTT GGC AGG CTC	Designed here	Annealing temperature: 52ºC
18S 5' R	GAA TTA GTT CAT ACT TAC ACA TGC ATG	Designed here	
Internal Transcribed Spacer (ITS)			
ITS 5a [F]	CCT TAT CAT TTA GAG GAA GGA G	Stanford et al. 2000	Amplification and sequencing were done for the Full region (ITS 5a + u4) or in two overlapping fragments: ITS1 (ITS 5a + u2) and ITS2 (ITS p3 + u4). Sometimes ITS p2 and 4 were used as slightly internal sequencing primers. Annealing temperature: 55ºC
ITS u2 [R]	GCG TTC AAA GAY TCG ATG RTT C	Cheng et al. 2016	
ITS p2 [R]	GCC RAG ATA TCC GTT GCC GAG	Cheng et al. 2016	
ITS p3 [F]	YGA CTC TCG GCA ACG GAT A	Cheng et al. 2016	
ITS u4 [R]	RGT TTC TTT TCC TCC GCT TA	Cheng et al. 2016	
ITS 4 [R]	TCC TCC GCT TAT TGA TAT GC	White et al. 1990	
KEA1 intron 11			Amplification and sequencing: KEA1-i11 F + R Annealing temperature: 55ºC
KEA1-i11 F	GAA TCA GAT ATT GCK CCA TAT CGT G	Designed here	
KEA1-i11 R	AGA AGC TTT GGA TCA ATA GAC ATT C	Designed here	
KEA1 intron 17			Amplification and sequencing: KEA1-i17 F + R Annealing temperature: 65ºC
KEA1-i17 F	AGG TCC TTC ATA AAG TTG GTG CWG A	Designed here	
KEA1-i17 R	GTG CAA GAA CAG CAG CTG CCA ACT G	Designed here	
TEB exon 17			
TEB F	GCR AAG AAR TCA AGA ATG GTG CWC G	Designed here	Amplification and sequencing: TEB F + R2 Annealing temperature: 55ºC
TEB R1	CTC TCY TCA TCA GGC CAM GAA TCC A	Designed here	
TEB R2	ATT SAT AGG ACC CTT CTC ACA GGC A	Designed here	
Plastid DNA (cpDNA)			
matK (full coding sequence)			
matK F1	AAT CCT CTA TCC TTG CTT CAA	Designed here	Amplification and sequencing were designed to capture the full coding region in three overlapping fragments, an effective protocol for poor quality DNA. matK Pluk F1 + R1, F2 + R2, and F3 + R3. Annealing temperature: 55ºC
matK R1	CTC ATG AAG AAA GAG TYG TAA TAA A	Designed here	
matK F2	AAT ACC TTA CCC CAT CCA TCT A	Designed here	
matK R2	ATT GGA ACT ATT GTA TCG AGT TTC	Designed here	
matK F3	GTA CGG AGT CAA ATG YTA GAA	Designed here	
matK R3	CCT TAC TCG TAT ACT GTA TGA GC	Designed here	
ndhF 3' (partial coding sequence)			Amplification and sequencing: ndhF 1318F + 2110Ri (or 1703R, to obtain a partial sequence for poorer quality samples) Annealing temperature: 49ºC
ndhF 1318F	GGA TTA ACY GCA TTT TAT ATG TTT CG	Olmstead and Sweere 1994	
ndhF 1703R	GGC TCC AAT AAA YAA AGT	Beilstein et al. 2006	
ndhF 2110Ri	TCA ATT ATT CGT TTA TCA A	Steinmann and Porter 2002	

All PCR amplifications used BIOLASE DNA Polymerase (Bioline) in 15uL reactions. Recipes included: 9.2uL H_2O; 1.5uL 10x NH_4 Reaction Buffer; 0.6uL 50mM $MgCl_2$ Solution [2.0mM]; 0.3uL 10mM dNTP Mix [0.2mM]; 0.375uL 10uM Primer (each) [0.25uM]; 1.5uL 1mg/mL Bovine Serum Albumin (BSA; Sigma Aldrich) [0.1 mg/mL]; 0.15uL 5U/uL BIOLASE DNA Polymerase [0.05U/uL]; 1uL Genomic DNA. Nuclear markers included 3uL 5M Betaine (Sigma Aldrich) [1M], in which case H_2O was reduced to 6.2uL. Thermocycler protocols followed: 3min 94ºC Initiation; 35 cycles of (i) 30s 94ºC Denaturing, (ii) 1m (see table) Annealing, (iii) 30s 72ºC Extension; 10min 72ºC Final extension.

Table 3.S4 Biogeographical distribution matrix (A = Mexico to NW South America, B = Amazon/Guiana, C = Atlantic Forest, D = Tropical Africa, E = Madagascar, F = SE Asia).

Taxon	A	B	C	D	E	F
Haematostemon guianensis	0	1	0	0	0	0
Plukenetia africana	0	0	0	1	0	0
Plukenetia ankaranensis	0	0	0	0	1	0
Plukenetia brachybotrya	0	1	0	0	0	0
Plukenetia aff. *brachybotrya*	0	1	0	0	0	0
Plukenetia carabiasiae	1	0	0	0	0	0
Plukenetia cf. *carolis-vegae*	0	1	0	0	0	0
Plukenetia conophora	0	0	0	1	0	0
Plukenetia corniculata	0	0	0	0	0	1
Plukenetia decidua	0	0	0	0	1	0
Plukenetia lehmanniana	1	0	0	0	0	0
Plukenetia loretensis	0	1	0	0	0	0
Plukenetia madagascariensis	0	0	0	0	1	0
Plukenetia penninervia	1	0	0	0	0	0
Plukenetia cf. *penninervia*	1	0	0	0	0	0
Plukenetia polyadenia	0	1	0	0	0	0
Plukenetia serrata	0	0	1	0	0	0
Plukenetia stipellata	1	0	0	0	0	0
Plukenetia supraglandulosa	0	1	0	0	0	0
Plukenetia verrucosa	0	1	0	0	0	0
Plukenetia volubilis	0	1	0	0	0	0
Romanoa tamnoides	0	0	1	0	0	0

Table 3.S5 Manual dispersal matrix between biogeographical areas (adjacent = 1.0, short-distance dispersal = 0.5, long-distance dispersal = 0.1).

0 to 35 Mya	Mex. to NW South Am. A	Amazon/ Guiana B	Atlantic Forest C	Trop. Africa D	Madagascar E	SE Asia F
Mex. to NW South Am.	1	-	-	-	-	-
Amazon/ Guiana	1.0	1	-	-	-	-
Atlantic Forest	0.1	0.5	1	-	-	-
Trop. Africa	0.1	0.1	0.1	1	-	-
Madagascar	0.1	0.1	0.1	0.5	1	-
SE Asia	0.1	0.1	0.1	0.1	0.1	1

Table 3.S6 Trait matrix used in phylogenetic regression analysis with seed size. Plants size (0 = slender stems; 1 = robust to thick stems), seedling ecology (0 = light gap or forest edge; 1 = shade avoidance, canopy liana strategy), fruit type/ hypothesized dispersal mechanism (0 = dry, dehiscent/ scatter hoarding rodents; 1 = fleshy, indehiscent/ mammal predation), biome (0 = wet dominant; 1 = dry component), fire tolerance (0 = non-fire adapted; 1 = fire adapted).

Taxon	Plant size	Seedling ecology	Fruit type/ dispersal	Biome	Fire tolerance
Haematostemon guianensis	1	0	0	0	0
Romanoa tamnoides	0	0	0	0	0
Plukenetia africana	0	0	0	1	1
Plukenetia ankaranensis	1	0	0	1	0
Plukenetia brachybotrya	0	0	0	0	0
Plukenetia aff. *brachybotrya*	0	0	0	0	0
Plukenetia carabiasiae	1	1	0	0	0
Plukenetia cf. *carolis-vegae*	1	0	0	0	0
Plukenetia conophora	1	1	1	0	0
Plukenetia corniculata	0	0	0	0	0
Plukenetia decidua	1	0	0	1	0
Plukenetia huayllabambana	1	0	0	0	0
Plukenetia lehmanniana	1	0	1	0	0
Plukenetia loretensis	0	0	0	0	0
Plukenetia penninervia	0	0	0	0	0
Plukenetia cf. *penninervia*	0	0	0	0	0
Plukenetia polyadenia	1	1	1	0	0
Plukenetia serrata	1	0	1	0	0
Plukenetia stipellata	0	0	0	0	0
Plukenetia supraglandulosa	0	0	0	0	0
Plukenetia verrucosa	0	0	0	0	0
Plukenetia volubilis	0	0	0	0	0

Table 3.S7 The best evolutionary model for seed size based on ΔAIC and Akaike weights (w_i).

Model	AIC_c	ΔAIC	w_i
Brownian motion	50.03442	0.0	0.6584417
Early Burst	52.73257	2.69815	0.1708530
Ornstein-Uhlenbeck	52.73430	2.69988	0.1707053

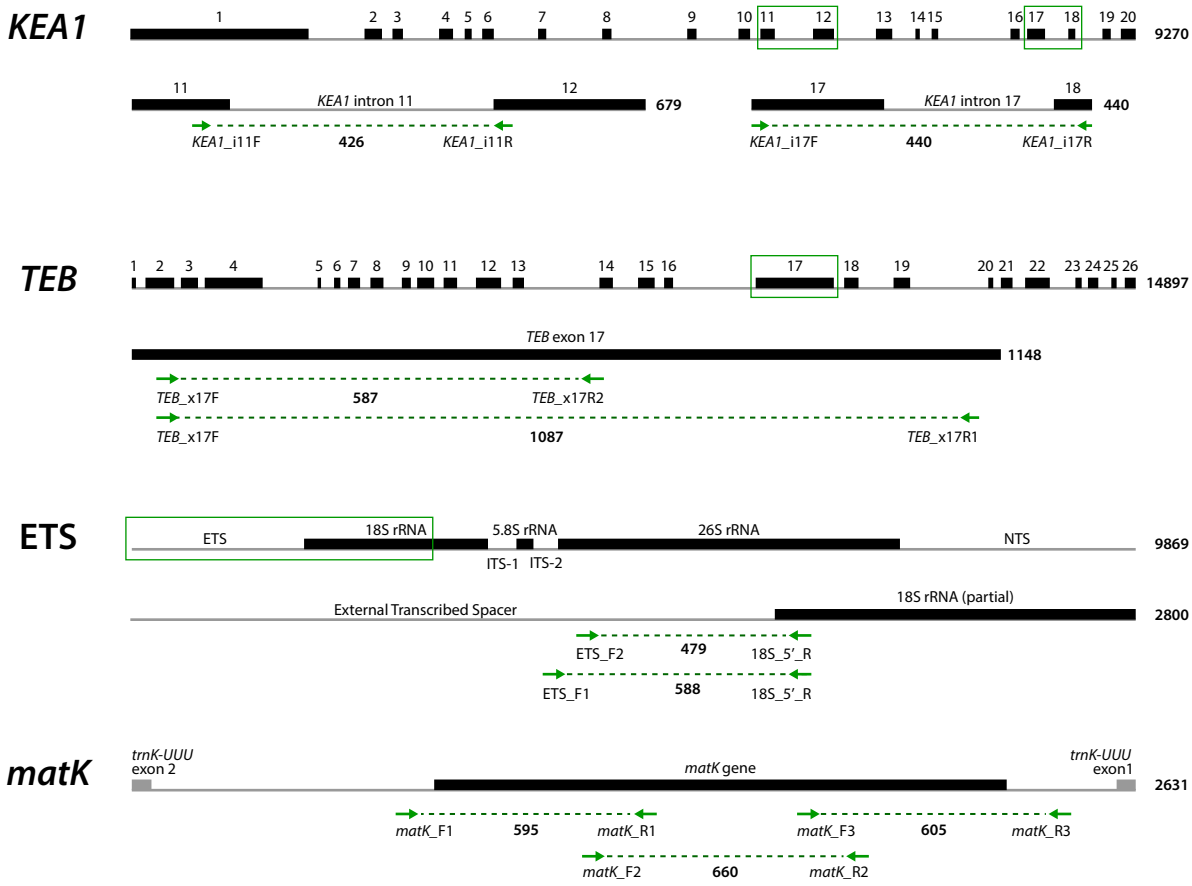


Figure 3.S1 Primer map for new (*KEA1* introns 11 and 17, *TEB* exon 17) and redesigned (ETS, *matK*) molecular markers.

Fig. 3.S2(A)
nrDNA ETS
HKY+G
500 ML Bootstrap Replicates

Figure 3.S2A Shortest maximum likelihood tree for incongruence analysis of individual markers: (a) ETS, (b) ITS, (c) *KEA1* intron 11, (d) *KEA1* intron 17, (e) *TEB* exon 17, (f) *matK*, and (g) *ndhF*. Bootstrap percentages based on 500 replicates. Well-supported branches (> 85% maximum likelihood bootstrap percentage; MLBP) are in bold.

Fig. 3.S2(B)
nrDNA ITS
GTR+I+G
500 ML Bootstrap Replicates

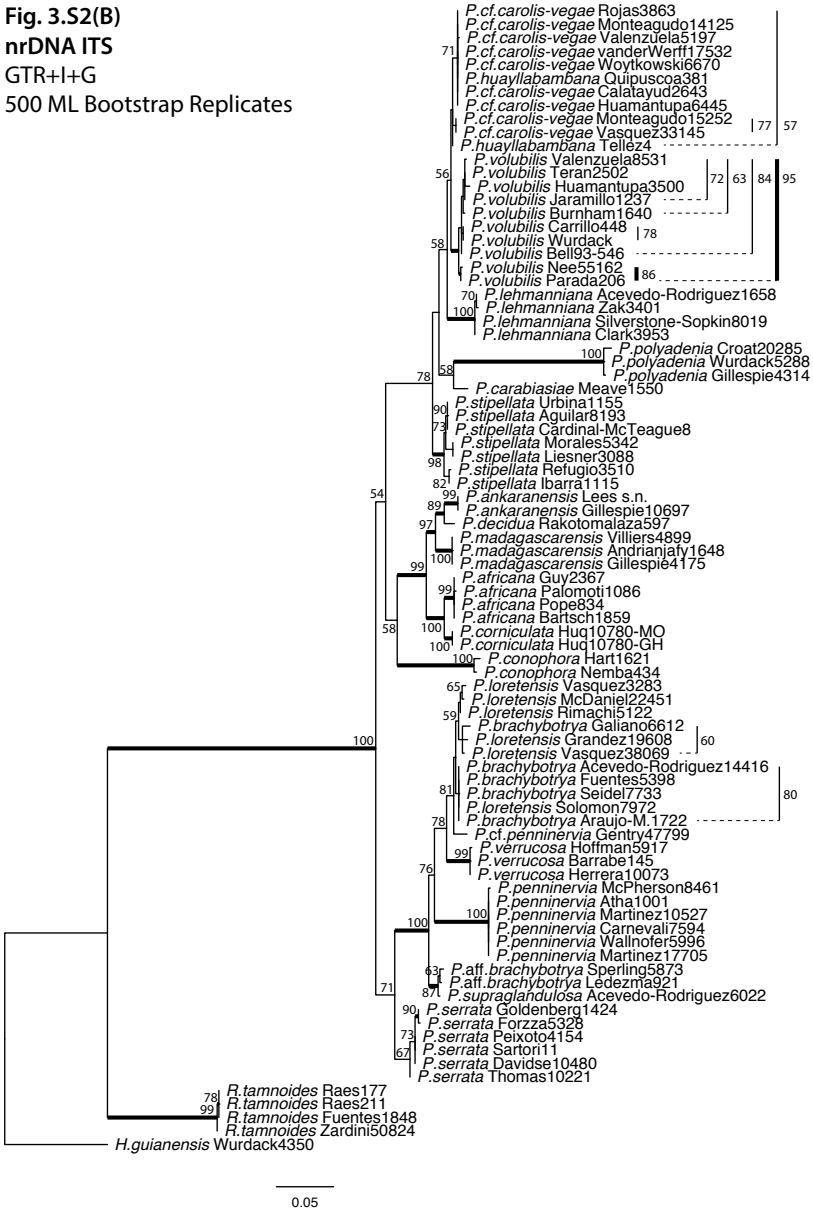


Figure 3.S2B Shortest maximum likelihood tree for incongruence analysis of individual markers: (a) ETS, (b) ITS, (c) *KEA1* intron 11, (d) *KEA1* intron 17, (e) *TEB* exon 17, (f) *matK*, and (g) *ndhF*. Bootstrap percentages based on 500 replicates. Well-supported branches (> 85% maximum likelihood bootstrap percentage; MLBP) are in bold.

Figure 3.S2C Shortest maximum likelihood tree for incongruence analysis of individual markers: (a) ETS, (b) ITS, (c) *KEA1* intron 11, (d) *KEA1* intron 17, (e) *TEB* exon 17, (f) *matK*, and (g) *ndhF*. Bootstrap percentages based on 500 replicates. Well-supported branches (> 85% maximum likelihood bootstrap percentage; MLBP) are in bold.

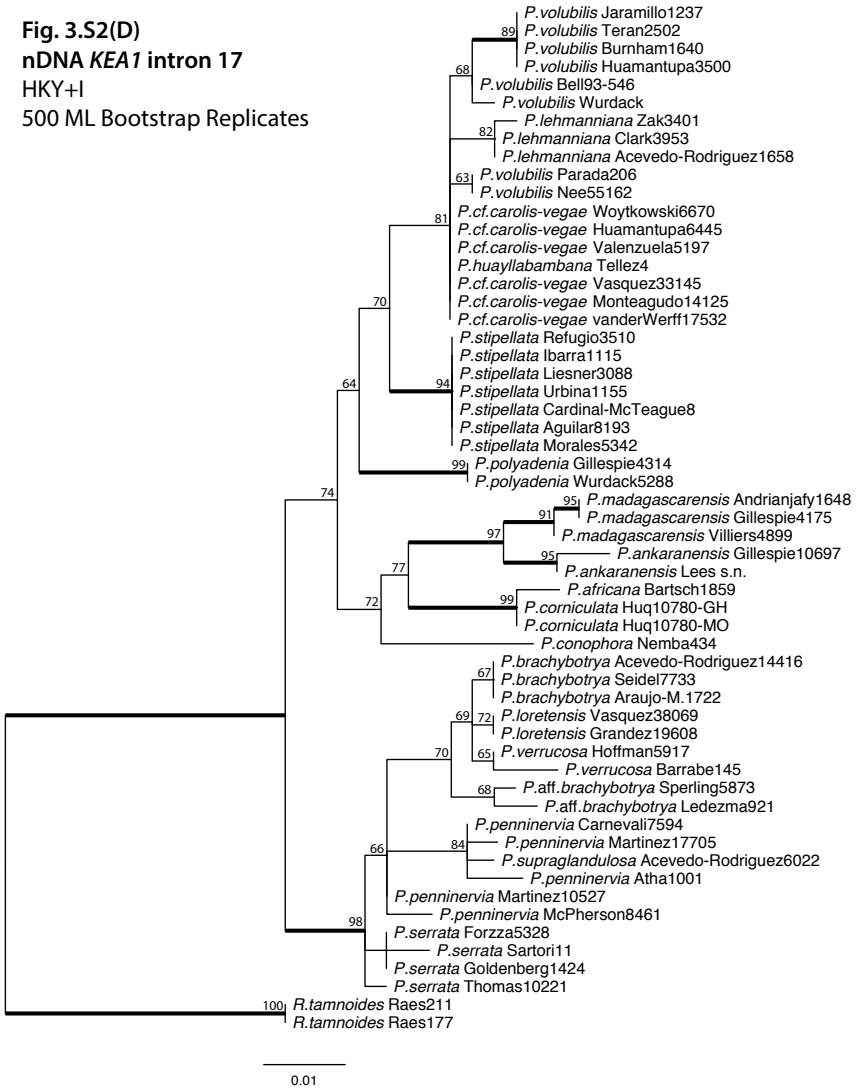


Figure 3.S2D Shortest maximum likelihood tree for incongruence analysis of individual markers: (a) ETS, (b) ITS, (c) *KEA1* intron 11, (d) *KEA1* intron 17, (e) *TEB* exon 17, (f) *matK*, and (g) *ndhF*. Bootstrap percentages based on 500 replicates. Well-supported branches (> 85% maximum likelihood bootstrap percentage; MLBP) are in bold.

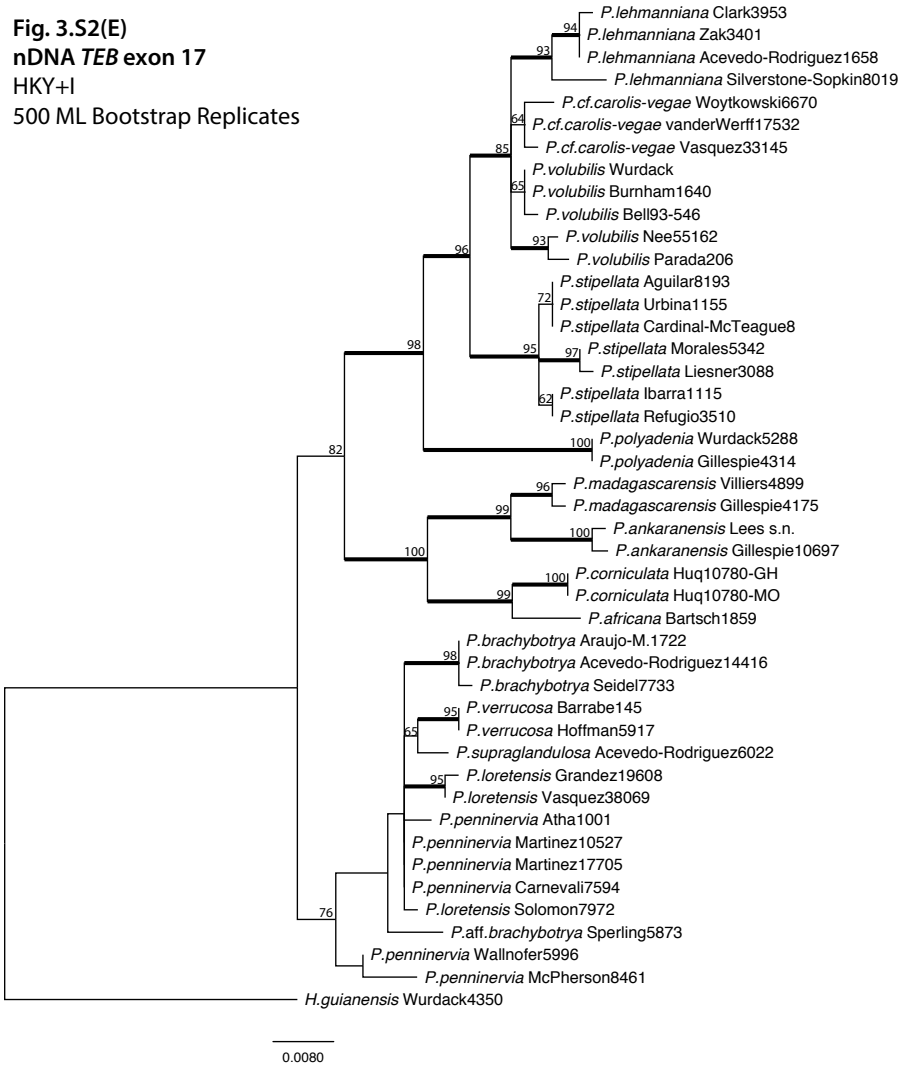


Figure 3.S2E Shortest maximum likelihood tree for incongruence analysis of individual markers: (a) ETS, (b) ITS, (c) *KEA1* intron 11, (d) *KEA1* intron 17, (e) *TEB* exon 17, (f) *matK*, and (g) *ndhF*. Bootstrap percentages based on 500 replicates. Well-supported branches (> 85% maximum likelihood bootstrap percentage; MLBP) are in bold.

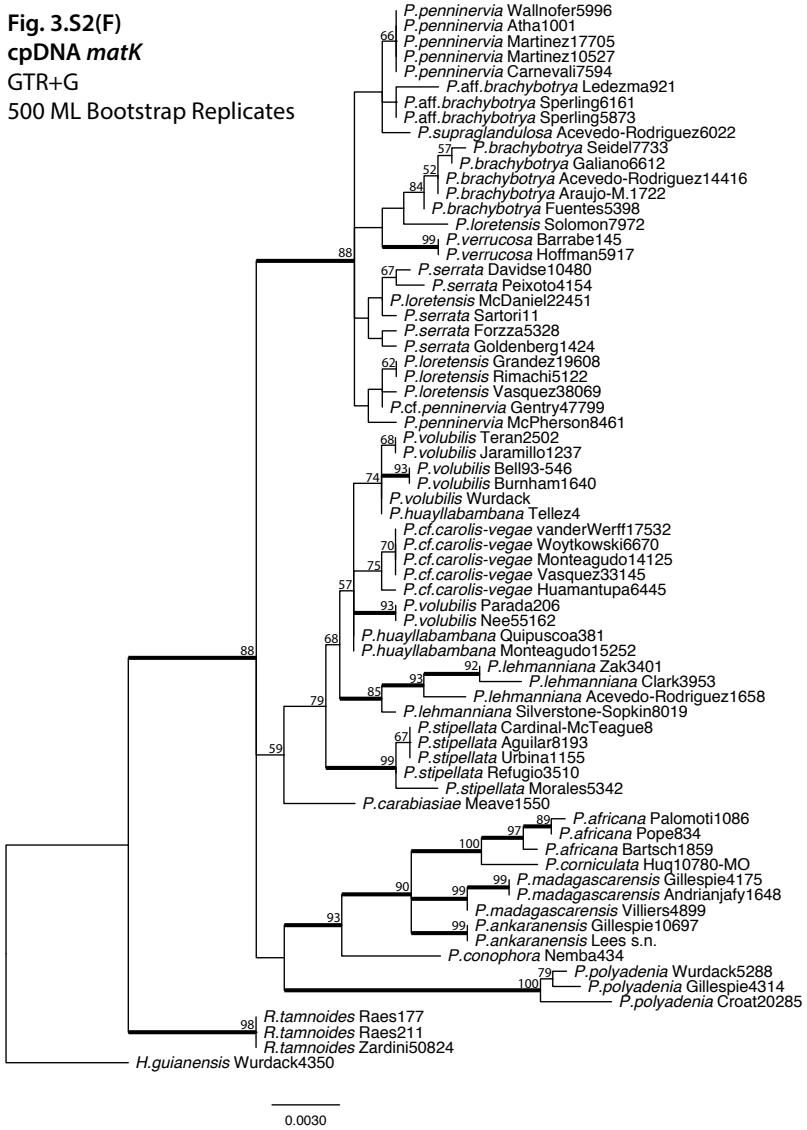


Figure 3.S2F Shortest maximum likelihood tree for incongruence analysis of individual markers: (a) ETS, (b) ITS, (c) *KEA1* intron 11, (d) *KEA1* intron 17, (e) *TEB* exon 17, (f) *matK*, and (g) *ndhF*. Bootstrap percentages based on 500 replicates. Well-supported branches (> 85% maximum likelihood bootstrap percentage; MLBP) are in bold.

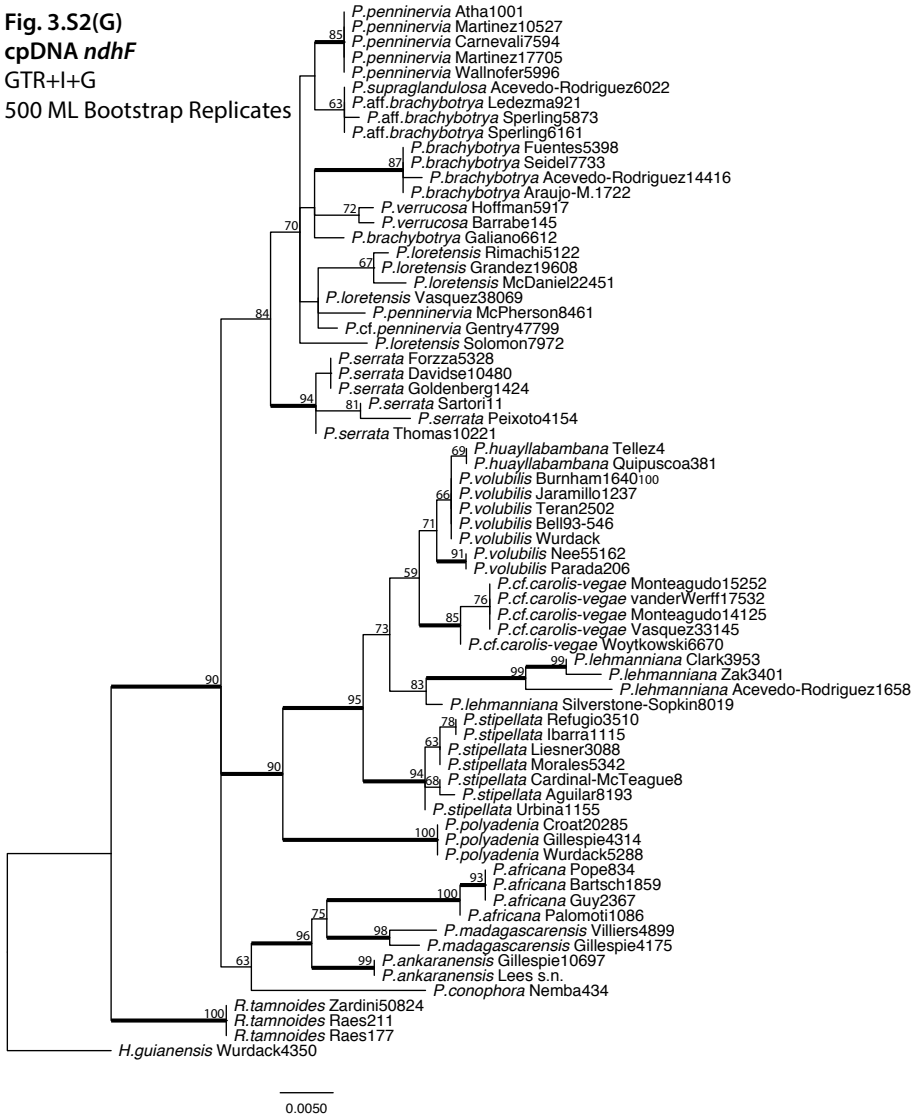


Figure 3.S2G Shortest maximum likelihood tree for incongruence analysis of individual markers: (a) ETS, (b) ITS, (c) *KEA1* intron 11, (d) *KEA1* intron 17, (e) *TEB* exon 17, (f) *matK*, and (g) *ndhF*. Bootstrap percentages based on 500 replicates. Well-supported branches (> 85% maximum likelihood bootstrap percentage; MLBP) are in bold.

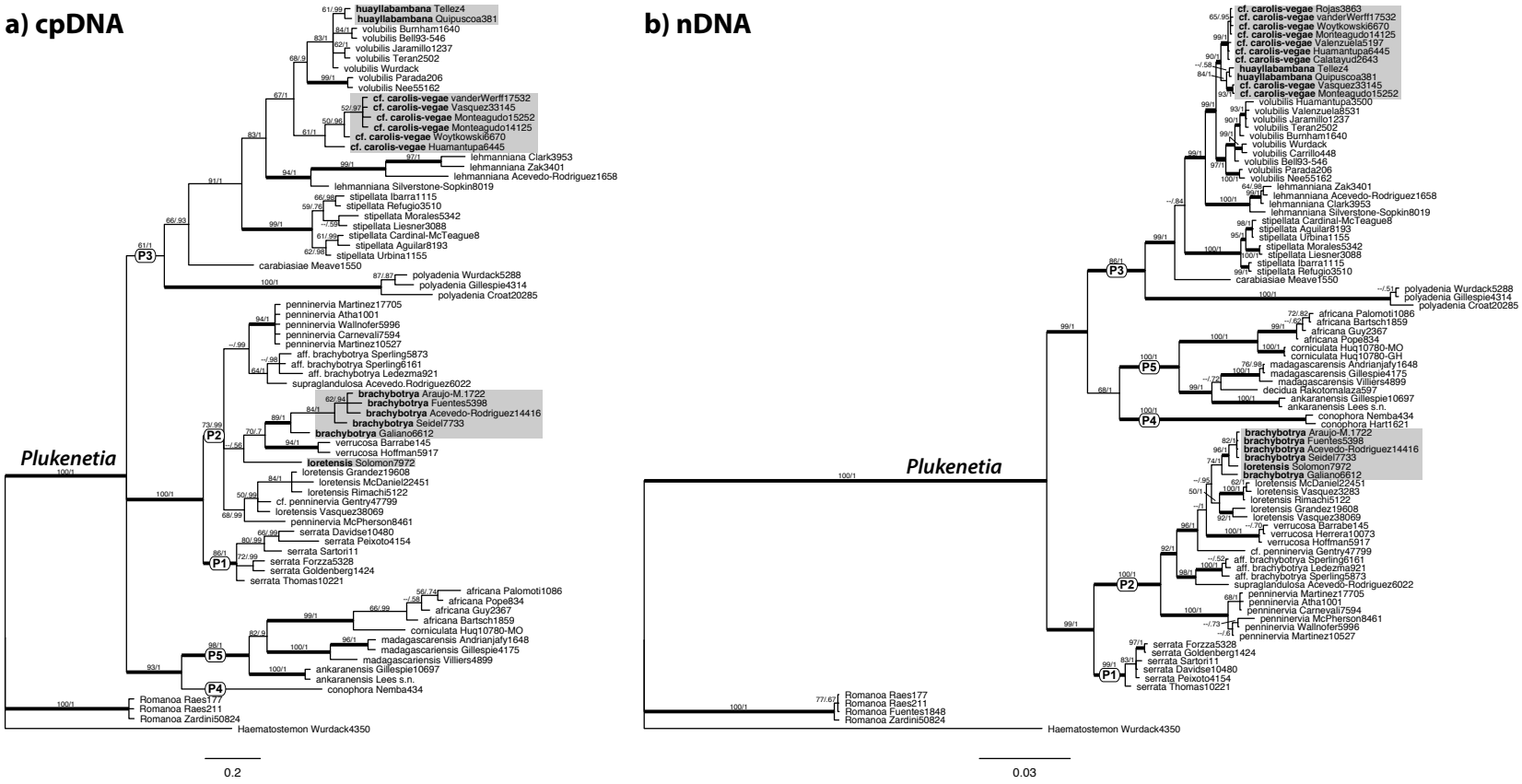


Figure 3.S3 Bayesian maximum clade credibility tree based on (a) plastid DNA (cpDNA) two marker, 74 accession dataset, and (b) nuclear DNA (nDNA) five marker, 86 accession dataset, for *Plukenetia* and Plukenetiinae outgroups. Maximum parsimony bootstrap percentage (MPBP) and Bayesian posterior probability (PP) support values > 50% are indicated on each branch. Branches in bold indicate strong support (≥ 85 MPBP and ≥ 0.95 PP). Grey boxes highlight strongly supported topological incongruences.

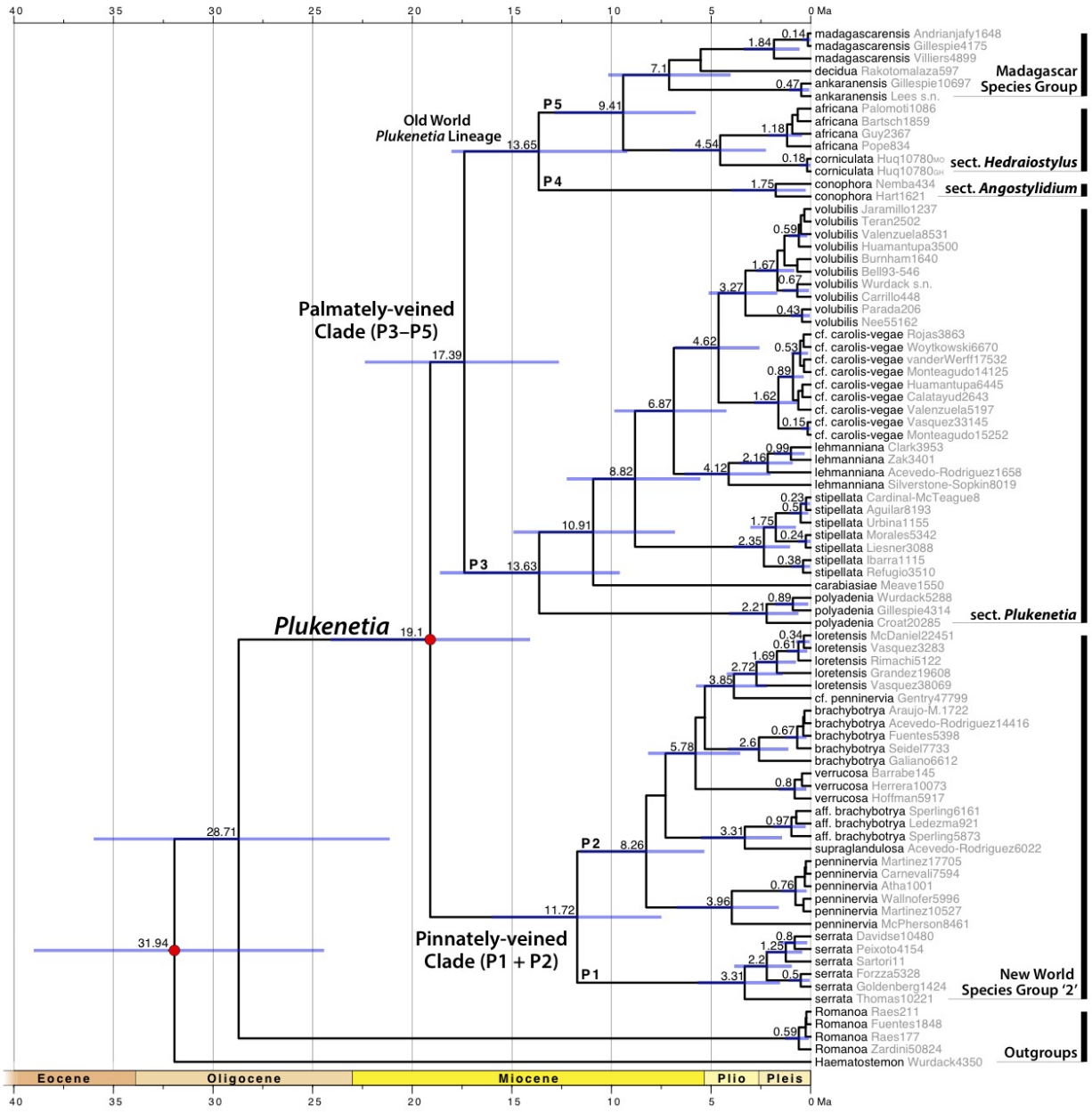


Figure 3.S4 BEAST chronogram of *Plukenetia* and Plukenetiinae outgroups inferred from the combined seven marker (cpDNA and nDNA), 83 accession dataset and two normal-distribution priors (indicated in red) based on previous subfamily Acalyphoideae estimates using three fossil calibrations. Numbers at each node indicate mean age estimates, and blue bars the 95% highest posterior density confidence interval.

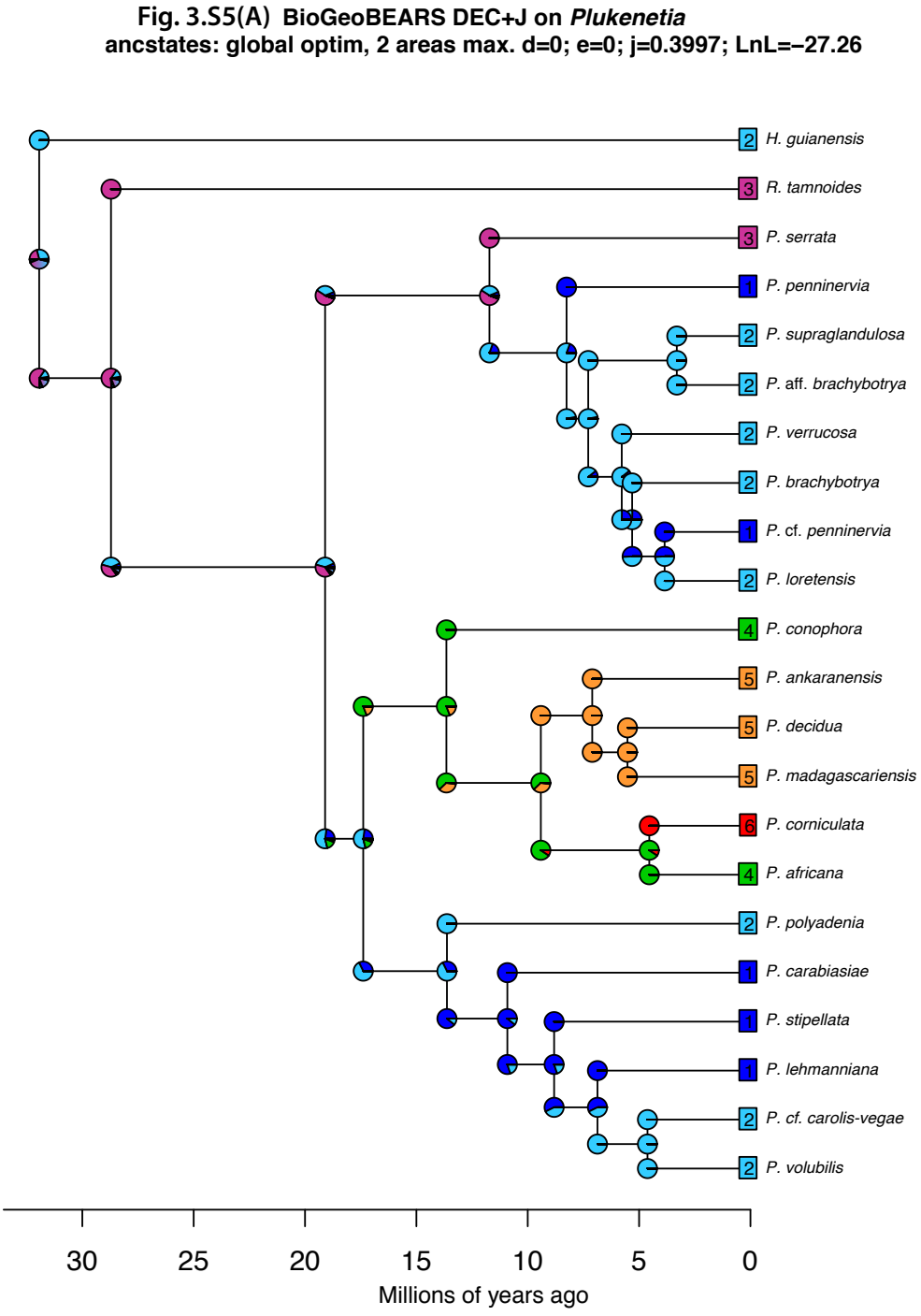


Figure 3.S5A BIOGEOBEARS ancestral range estimation probabilities for each corner and node under (a) DEC+J and (b) DEC.

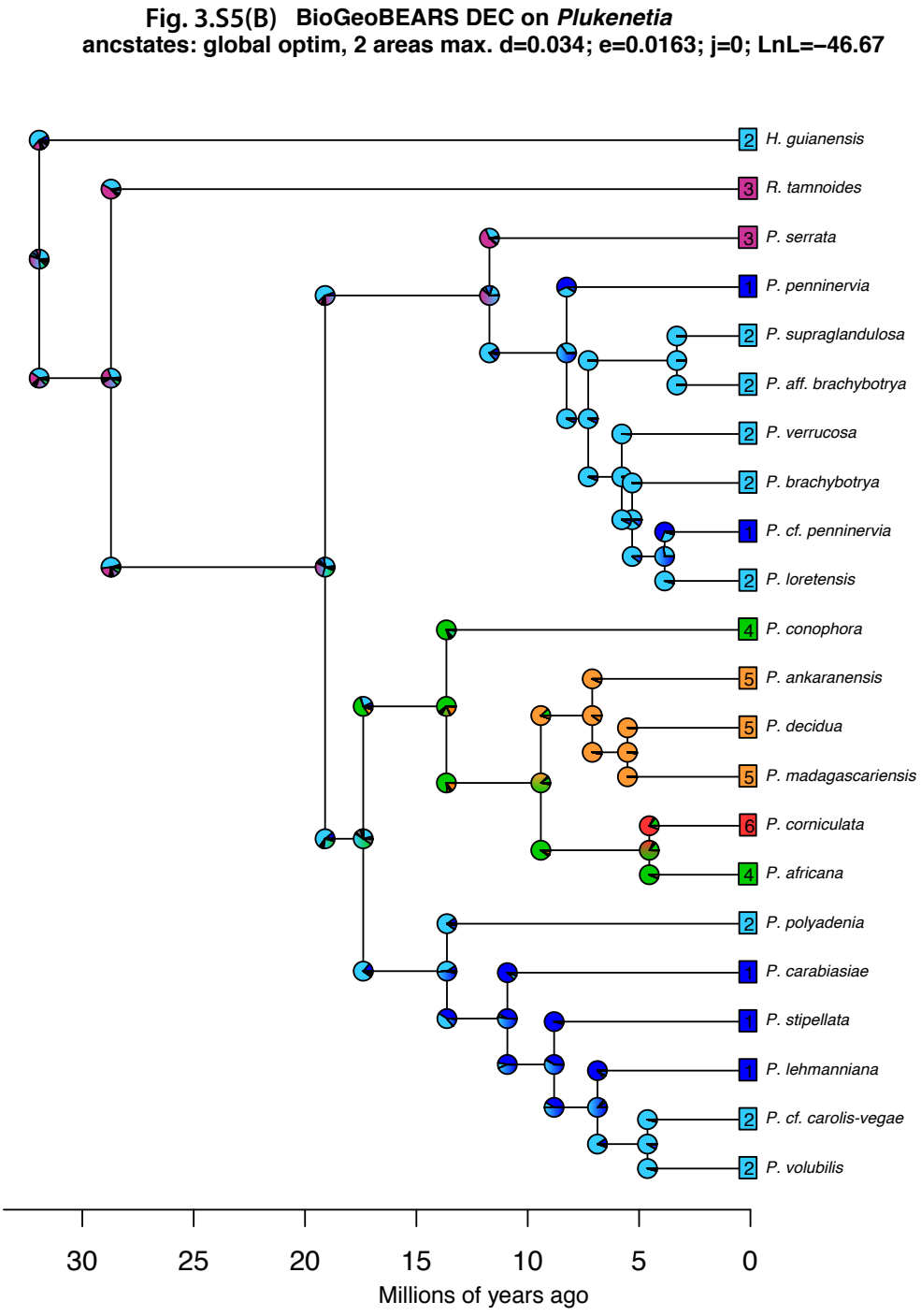


Figure 3.S5B BIOGEOBEARS ancestral range estimation probabilities for each corner and node under (a) DEC+J and (b) DEC.

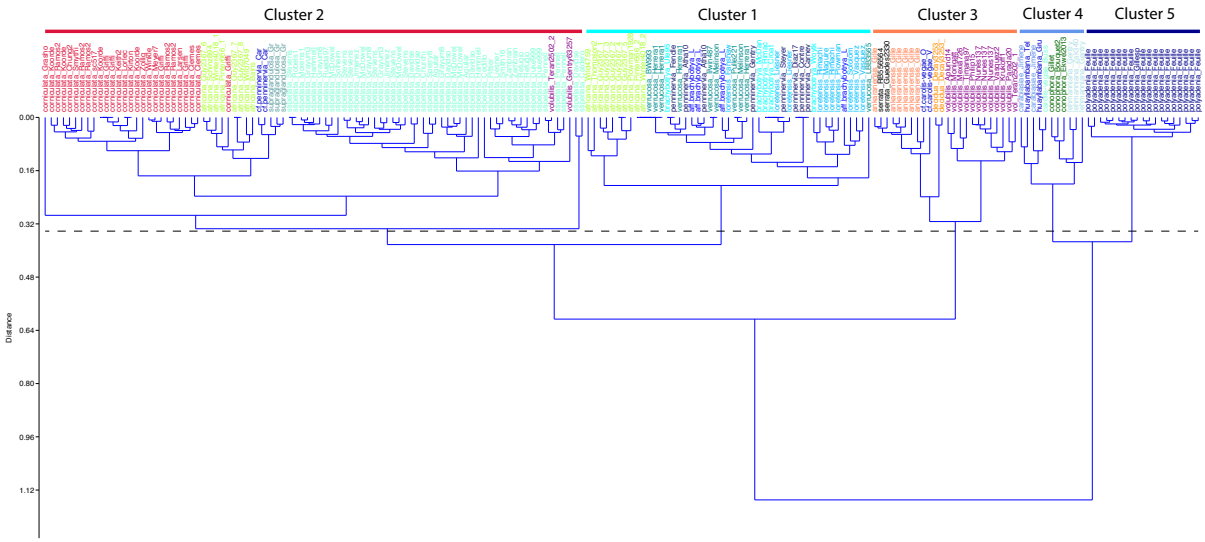


Figure 3.S6 UPGMA clustering analysis of $\log_{10}$ transformed seed dimensions (length, width, thickness) for 190 accessions of *Plukenetia*.

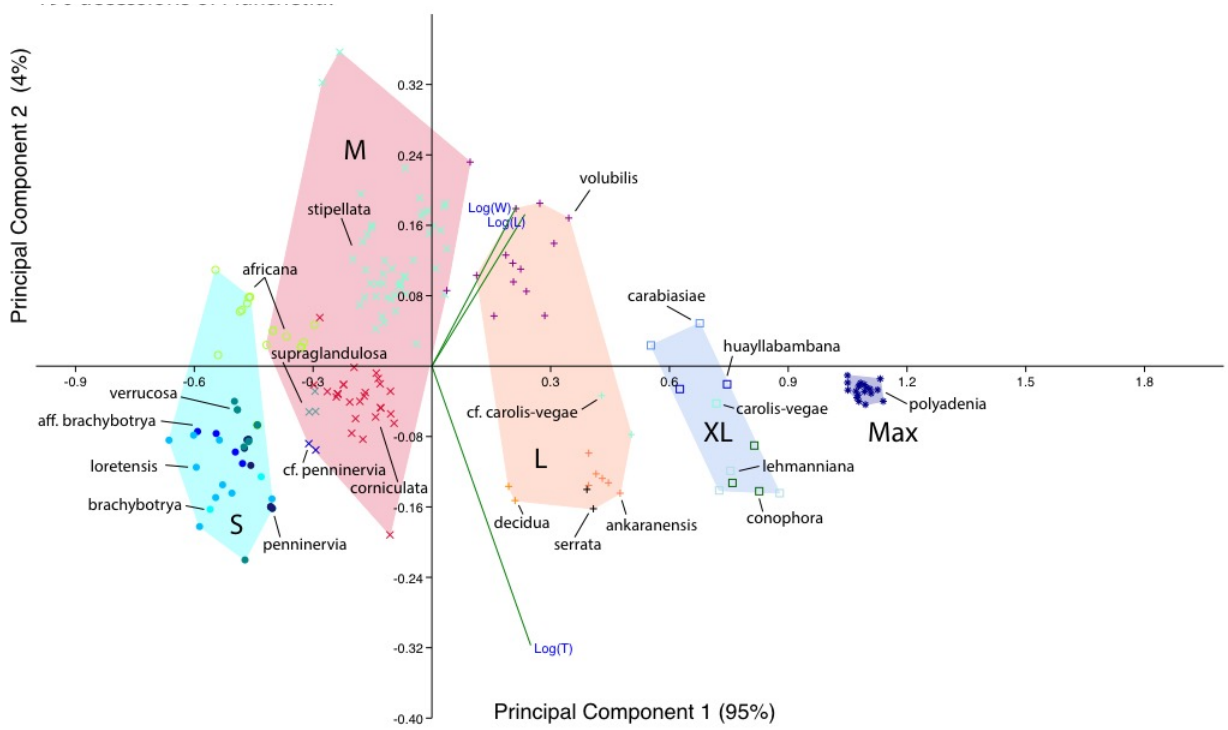


Figure 3.S7 Principal components analysis of $\log_{10}$ transformed seed dimensions (length, width, thickness) for 190 accessions of *Plukenetia*.

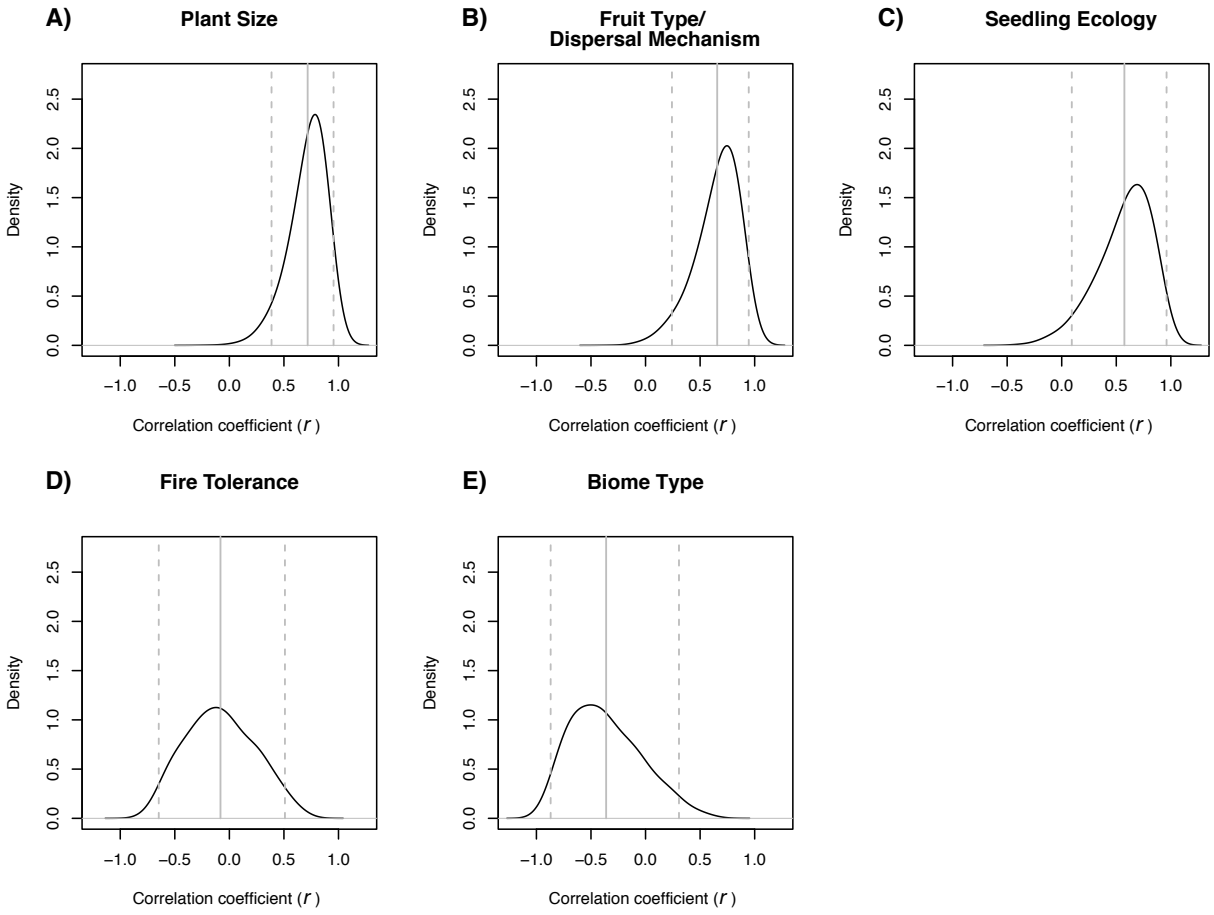


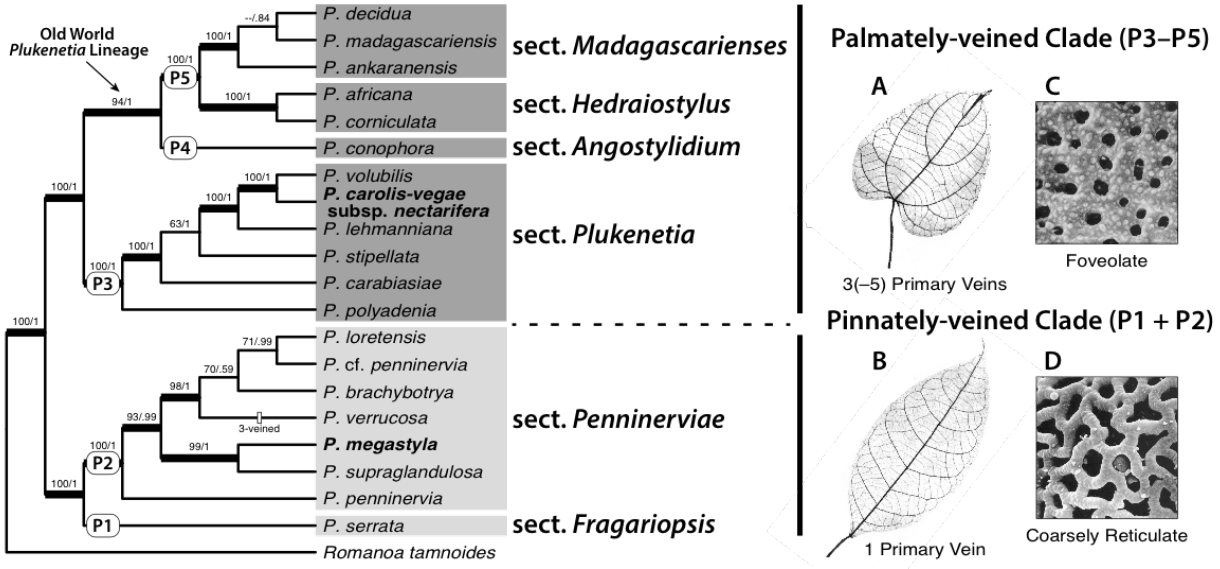
Figure 3.S8 Posterior distributions of the correlation coefficients (*r*) between the liabilities of seed size and (a) plant size, (b) fruit type, (c) seedling ecology, (d) fire tolerance, and (e) biome type, under the threshold model.

Chapter 4
A Revised Sectional Classification of *Plukenetia* L. (Euphorbiaceae, Acalyphoideae) with Three New Taxa from South America*

Warren M. Cardinal-McTeague[1,2] and Lynn J. Gillespie[1,2]

1. Department of Biology, University of Ottawa, Gendron Hall, Room 160, 30 Marie Curie, Ottawa, Ontario, K1N 6N5, Canada.
2. Research & Collections, Canadian Museum of Nature, PO Box 3443, Station D, Ottawa, Ontario, K1P 6P4, Canada.

* Manuscript in prep, to be submitted to Systematic Botany. (Note: the new names presented here are not to be considered effectively published)



Phylogeny with updated sectional classification of *Plukenetia* L.

Abstract

- **Background:** We present a phylogenetic classification for *Plukenetia* (Euphorbiaceae subfamily Acalyphoideae) based on morphology and molecular phylogenetic studies using nuclear (ETS, ITS, *KEA1* introns 11 and 17, *TEB* exon 17) and plastid (*matK*, *ndhF*, *psbA-trnH*) DNA sequence data.
- **Results/Conclusions:** *Plukenetia* comprises 23 species divided into six sections, with three new sections, two new species, and a new subspecies described here. The circumscription of *Plukenetia* is unaltered from recent treatments and we continue to recognize *Romanoa* as a separate genus. The sections of *Plukenetia* correspond with the subclade system proposed by Cardinal-McTeague and Gillespie (2016): P1 = ***P.* sect. *Fragariopsis***; P2 = ***P.* sect. *Penninerviae***; P3 = *P.* sect. *Plukenetia*; P4 = *P.* sect. *Angostylidium*; and P5 = *P.* sect. *Hedraiostylus* + ***P.* sect. *Madagascarienses***. The sections are distinguished by a combination of pistillate flower number, androecium morphology, style morphology, fruit type, and seed size. We also describe two new species from the Amazon basin belonging to ***P.* sect. *Penninerviae***: ***Plukenetia brevistyla*** and ***Plukenetia megastyla***. Both new species are morphologically similar to *P. brachybotrya* and are distinguished by their style shape and size. An ETS phylogeny supports the new species as distinct. We also identify the wild progenitor of the cultivated species *P. carolis-vegae* (sect. *Plukenetia*) and describe it as ***Plukenetia carolis-vegae* subsp. *nectarifera***. This new subspecies differs by its smaller seeds and fruits and broader distribution in central and southern Peru. We provide keys to the sections and species of *Plukenetia* and designate 11 lectotypes in *Plukenetia* and *Romanoa*.

Background

The circumscription of *Plukenetia* L. (Euphorbiaceae tribe Plukenetieae) has changed considerably over the last four centuries, particularly in repeated shifts between recognizing multiple genera and a single morphologically diverse pantropical genus. The genus was first named *Pluknetia* by French botanist Charles Plumier (1703) to honour English botanist Leonard Plukenet, who is regarded for his influential publications on New World plants, *Phytographia* vols. 1–4 (Plukenet, 1691a,b, 1692, 1696a) and *Almagestum botanicum* (Plukenet, 1696b). Plumier's name was validated by Linnaeus (1753) as *Plukenetia* and was chosen over a second pre-Linnaean genus from Southeast Asia, *Sajor* Rumph. (type = *Plukenetia corniculata* Sm.), which he treated as a synonym. *Plukenetia* now comprises 24 species of non-stinging twining lianas, vines, and rarely subshrubs. The most well-known species, *P. volubilis* L. (Sacha Inchi or Inca Peanut), has been traditionally cultivated in Peru for its oil-rich seeds, which have gained in popularity and consumption over the past 20 years. The genus was most recently revised by Gillespie (1993, 2007) and outside of those treatments three new species have been described, one from Oaxaca, Mexico (Jiménez Ramírez 1993) and two from the Andes of northern Peru (Bussmann *et al.*, 2009, 2013). A recent phylogenetic study has resolved most relationships in *Plukenetia* and analyzed the historical biogeography and seed size evolution of the genus (Cardinal-McTeague *et al*., in review). Here, we use those phylogenetic hypotheses to present a revised sectional classification and updated species list for *Plukenetia*, including two new species from the Amazon basin and a new subspecies that is the wild progenitor of the cultivated species *P. carolis-vegae*.

Plukenetia is a member of tribe Plukenetieae, a distinctive lineage noted for its twining vine growth forms and stinging hair defences (Webster, 1994; Wurdack *et al.*, 2005; Cardinal-McTeague & Gillespie, 2016). *Plukenetia* belongs to the non-stinging subtribe Plukenetiinae, which is divided into two informal groups comprising rare small tree and shrub (*Angostylis*, *Astrococcus*, and *Haematostemon*) and twining vine and liana genera (*Plukenetia* and *Romanoa*) (Gillespie, 1994b; Cardinal-McTeague & Gillespie, 2016). The other genera of Plukenetiinae contain only one or two species each, making *Plukenetia* the most diverse and species-rich member of the clade. *Plukenetia* is readily distinguished by its 4–carpellate fruits/ovaries (compared to 3–carpellate in other Plukenetieae and most other Euphorbiaceae) and by the

presence of paired basilaminar extrafloral nectaries (sometimes called ‘glands’) on the adaxial surface of the leaf blade (Gillespie, 1993, 2007).

History of generic and sectional classifications

The early taxonomic history of *Plukenetia* was complicated by the creation of multiple generic names for the same taxon (Table 4.1). In particular, several genera were described for *P. corniculata* Sm. (*Sajor* Rumph., nom. inval. pre 1753, *Pterococcus* Hassk., nom. cons., *Hedraiostylus* Hassk., *Ceratococcus* Meisn., nom. illeg., *Sajorium* Endl., nom. illeg.) and for *P. serrata* (Vell.) L.J.Gillespie (*Vigia* Vell., *Fragariopsis* A.St.-Hil., *Accia* A.St.-Hil., *Botryanthe* Klotzsch).

Table 4.1 Generic and sectional classifications of *Plukenetia* and *Romanoa* (NWSG2 = New World species group “2”; [a] = genera described by Pax & Hoffmann, 1919a).

Molecular phylogeny	Prior to Baillon	Baillon (1858)	Müller (1864, 1865, 1866)	Pax and Hoffmann (1919)	Webster (1975)	Gillespie (1993, 2007)	This treatment
Plukenetia subclade P1	*Vigia* (Vell. 1832) *Fragariopsis* (A.St.-Hil. 1840) *Accia* (A.St.-Hil. 1840) *Botryanthe* (Klotzsch 1841)	*Fragariopsis*	*Fragariopsis*	*Fragariopsis*	*Fragariopsis*	NWSG2	***P.* sect. *Fragariopsis*** stat. nov.
Plukenetia subclade P2	*Plukenetia* (L. 1753)	*Sajorium* sect. *Pluknetia*	*P.* sect. *Euplukenetia*	*P.* sect. *Euplukenetia* *Apodandra*[a]	*Plukenetia*	NWSG2	***P.* sect. *Penninerviae*** sect. nov.
Plukenetia subclade P3	*Pluknetia* (Plum. 1703) *Plukenetia* (L. 1753)	*Sajorium* sect. *Pluknetia*	*P.* sect. *Euplukenetia* *P.* sect. *Cylindrophora*	*P.* sect. *Cylindrophora* *Eleutherostigma*[a] *Elaeophora* (Ducke, 1925)	*Plukenetia* *Eleutherostigma*[a]	*P.* sect. *Plukenetia*	*P.* sect. *Plukenetia*
Plukenetia subclade P4	n/a	n/a	*P.* sect. *Angostylidium*	*Tetracarpidium* (Pax 1899) *Angostylidium*[a]	*Plukenetia*	*P.* sect. *Angostylidium*	*P.* sect. *Angostylidium*
Plukenetia subclade P5	*Sajor* (Rumph. 1750) *Pterococcus* (Hassk. 1842) *Hedraiostylus* (Hassk. 1843) *Ceratococcus* (Meisn. 1843) *Sajorium* (Endl. 1843)	*Sajorium* sect. *Hedraiostylus*	*P.* sect. *Sajor* *P.* sect. *Hedraiostylus*	*Pseudotragia* (Pax 1908) *Pterococcus*	*Plukenetia*	*P.* sect. *Hedraiostylus*	*P.* sect. *Hedraiostylus*
Plukenetia subclade P5	n/a	n/a	n/a	n/a	*Plukenetia*	Madagascan species group	***P.* sect. *Madagascarienses*** sect. nov.
Romanoa	*Anabaena* (A.Juss. 1824) *Romanoa* (Trev. 1848)	*Sajorium* sect. *Anabaena*	*P.* sect. *Anabaena*	*Anabaenella*[a]	*Anabaena*	*Romanoa*	*Romanoa*

Baillon (1858) was the first to recognize a broader circumscription for *Plukenetia* and unified three previously distinct genera under Endlicher's (1843) illegitimate replacement name *Sajorium* (Table 4.1). Müller Argoviensis (1864, 1865, 1866) built on Baillon’s classification but

applied it under the earliest legitimate genus, *Plukenetia*. Müller's work provided many of the sectional names used today (Table 4.1). However, Müller mistakenly described *P. peruviana* Müll.Arg., a synonym of the type species *P. volubilis*, and placed each in two different sections. The type species of *Plukenetia*, *P. volubilis*, was placed in sect. *Euplukenetia* alongside most other neotropical species known at the time (*P. brachybotrya*, *P. penninervia*, and *P. verrucosa*). The synonym, *P. peruviana*, was placed its own sect. *Cylindrophora* on the basis of having a long cylindrical stylar column, despite sharing this exact morphology with *P. volubilis*. Even after the synonymy of *P. peruviana* with *P. volubilis* was identified, Pax and Hoffmann (1919) perpetuated this error and placed *P. volubilis* in sect. *Cylindrophora* (= sect. *Plukenetia*) and misapplied sect. *Euplukenetia* (= **sect. *Penninerviae*** sect. nov.) to a subset of neotropical species united by short connate styles and mostly pinnately-veined leaves (*P. brachybotrya*, *P. penninervia*, *P. verrucosa*).

Both Baillon and Müller upheld *Fragariopsis* (= *P. serrata*) as a distinct genus, Baillon (1858) for its unique androecium morphology, high and variable stamen count, and thick stylar columns, and Müller (1866) because it had fleshy indehiscent fruits rather than dry dehiscent capsules. High stamen number, thick stylar columns, and fleshy fruits would be shared with subsequently described species of *Plukenetia*, but its androecium morphology remains distinct.

Five decades after Baillon and Müller, Pax (1899, 1908) and Pax and Hoffmann (1919) dismantled *Plukenetia* and in the process described several new genera (Table 4.1). Many genera in the Pax and Hoffmann (1919) treatment were poorly defined and included obvious errors, such as placing synonyms of *P. brachybotrya* in both sect. *Euplukenetia* (= **sect. *Penninerviae*** sect. nov.) and their new genus *Apodandra*. Over time, Croizat (1941), Webster (1975, 1994), and Gillespie (1993, 1994, 2007) challenged Pax and Hoffmann's treatment, reuniting these genera under *Plukenetia* on the basis of their numerous overlapping characters and synapomorphic 4–carpellate ovaries.

Analysis of leaf, floral, and pollen morphology provided many characters to develop species group hypotheses (Gillespie, 1993, 1994b, 2007), but major taxonomic changes were withheld pending molecular phylogenetic study. Recent phylogenetic analyses (Cardinal-McTeague and Gillespie 2016; Cardinal-McTeague *et al*., in review) support many of Gillespie's hypotheses and provide a robust framework to formalize a revised classification for *Plukenetia* (Fig. 4.1, Tables 4.1, 4.2).

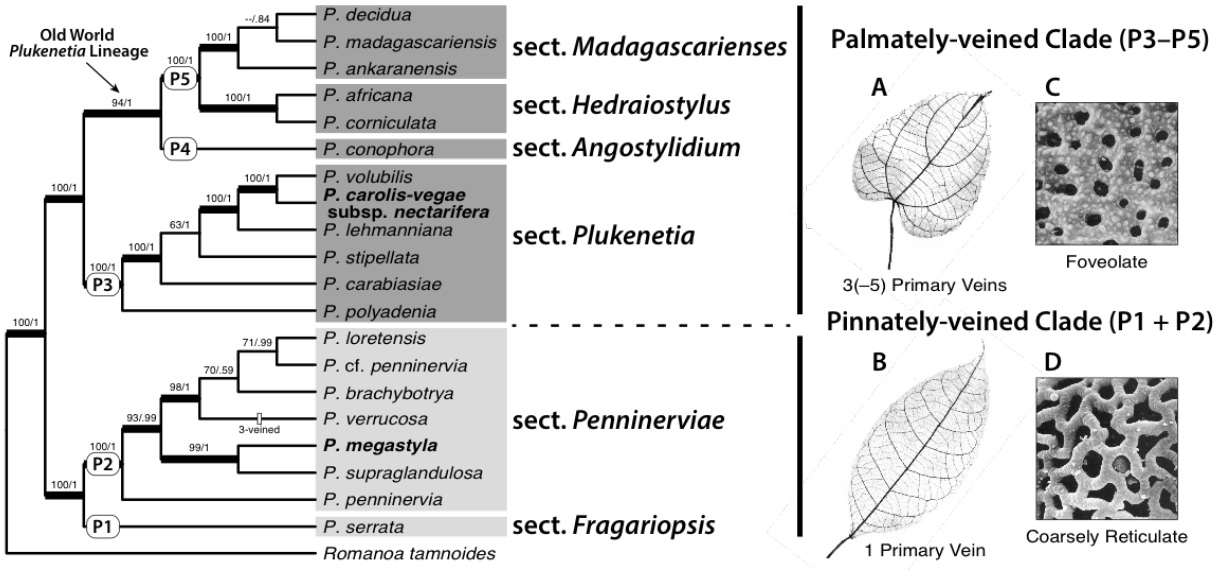


Figure 4.1 Summary cladogram of the relationships recovered by Bayesian analysis of combined and partitioned ETS, ITS, *KEA1* introns 11 and 17, *TEB* exon 17, *matK*, and *ndhF* DNA sequence data of *Plukenetia* and *Romanoa* (modified from Cardinal-McTeague *et al.*, in review), highlighting our revised sectional classification of *Plukenetia*. Maximum parsimony bootstrap percentage (MPBP) and Bayesian posterior probability (PP) support values > 50% are indicated on each branch. Branches in bold indicate strong support (≥ 85 MPBP and ≥ 0.95 PP). Subclade numbering system (P1–P5) follows Cardinal-McTeague & Gillespie (2016). Sections in the pinnately-veined clade (P1 + P2) are indicated by light grey boxes, sections in the palmately-veined clade (P3–P5) by dark grey boxes. (a–b) Leaf clearings demonstrating leaf architecture (images from Gillespie 1993): (a) *P. stipellata* (*Gillespie 413,* US); (b) *P. supraglandulosa*, (*Granville 3626*, CAY). (c–d) Scanning electron micrographs demonstrating pollen tectum morphology (images from Gillespie, 1994b): (c) *P. stipellata* (*Gillespie 418*, DAV); (d) *P. loretensis* (*Maguire & Politi 27371*, US).

Table 4.2 Revised sectional classification of *Plukenetia* with species number, distribution, habitat, and growth forms of each section, including *Romanoa*.

Taxon	Species	Distribution	Habitat	Growth form
P. sect. *Angostylidium*	1	Central and Western Africa	Lowland forest	Canopy lianas; stems thick
P.* sect. *Fragariopsis	1	Atlantic Forest of Brazil	Pre-montane to montane forest edges	Lianas; stems slender to thick
P. sect. *Hedraiostylus*	3	Southern Africa, Southeast Asia	Lowland forest edges and light gaps or savanna woodland on sandy soil	Vines, lianas, or herbs; stems slender, twining or procumbent
P.* sect. *Madagascarienses	3	Madagascar	Forest on limestone or spiny forest scrub	Lianas; stems slender to thick
P.* sect. *Penninerviae	9	Mesoamerica, Central America, northwestern and Amazonian South America, and the Lesser Antilles	Lowland to pre-montane (montane) forest edges, rocky outcrops, and light gaps or white sand forest	Vines or lianas; stems slender
P. sect. *Plukenetia*	7	Mesoamerica, Central America, northwestern and Amazonian South America, and the Lesser Antilles	Lowland to pre-montane forest edges, ravines, and light gaps	Vines, lianas, or canopy lianas; stems slender to thick
Romanoa	1	Atlantic Forest of Brazil and Southwest Amazon (Bolivia, Brazil, Paraguay)	Lowland forest, rocky outcrops, and light gaps or stony soil caatinga	Vines or lianas; stems slender

Taxonomically informative characters

In the phylogeny of Cardinal-McTeague *et al*. (in review), *Plukenetia* was divided into two major clades, the pinnately-veined clade (subclades P1 + P2) and the palmately-veined clade (subclades P3–P5) (Fig. 4.1). These clades are united by their leaf blade venation, with 1 primary vein in the pinnately-veined clade (3–veined in *P. verrucosa*) and 3(–5) primary veins in the palmately-veined clade. The pinnately-veined clade was previously called New World species group "2" (sensu Gillespie 1993) and was determined to be further united by entirely connate styles, all or mostly sessile anthers, and coarsely reticulate pollen tecta. Some Old World species in the palmately-veined clade share entirely connate styles (*P. ankaranensis*, *P. corniculata*) and sessile anthers (species in Madagascar), rendering those characters non-exclusive to the pinnately-veined clade. However, in addition to leaf venation, members of the palmately-veined clade are united by foveolate pollen tecta.

A combination of androecium morphology, style morphology, fruit type, and seed size can be used to differentiate the sections (Table 4.3). Useful androecium characters include the presence of filaments and nectaries, nectary type, and receptacle shape (Fig. 4.2). Style size and morphology varies considerably, with styles ranging from partly to entirely connate, and stylar columns either cylindrical or variously shaped (Fig. 4.3). Seeds range from "small" (~5 x 5 x 4 mm) to "medium", "large", "extra-large", and "maximum" (~50 x 35 x 30 mm) sized (for more detail see Cardinal-McTeague *et al*., in review). Smaller seeds ("small", "medium", "large") tend to be associated with dry dehiscent capsules, while larger seeds ("large", "extra-large", "maximum") are from fleshy indehiscent berries or dry semi-dehiscent capsules.

Methods

Morphology

Sectional descriptions of *Plukenetia* build on previous treatments (Gillespie 1993, 2007) and new species descriptions (Jiménez Ramírez, 1993; Bussmann *et al.*, 2009, 2013). Herbarium material from CAN, GH, MO, NY, and US was consulted for morphology. Pollen morphology and measurement data are based on Gillespie (1993, 1994, 2007) and Nowicke and Takahashi (2002). Pollen morphology for new taxa were investigated by soaking staminate flowers with a simple rehydrating solution (100 mL water with a drop of liquid soap), isolating pollen grains

onto a glass slide, and making observations with a compound light microscope. Keys to the species of *Plukenetia* were modified from Gillespie (1993, 2007).

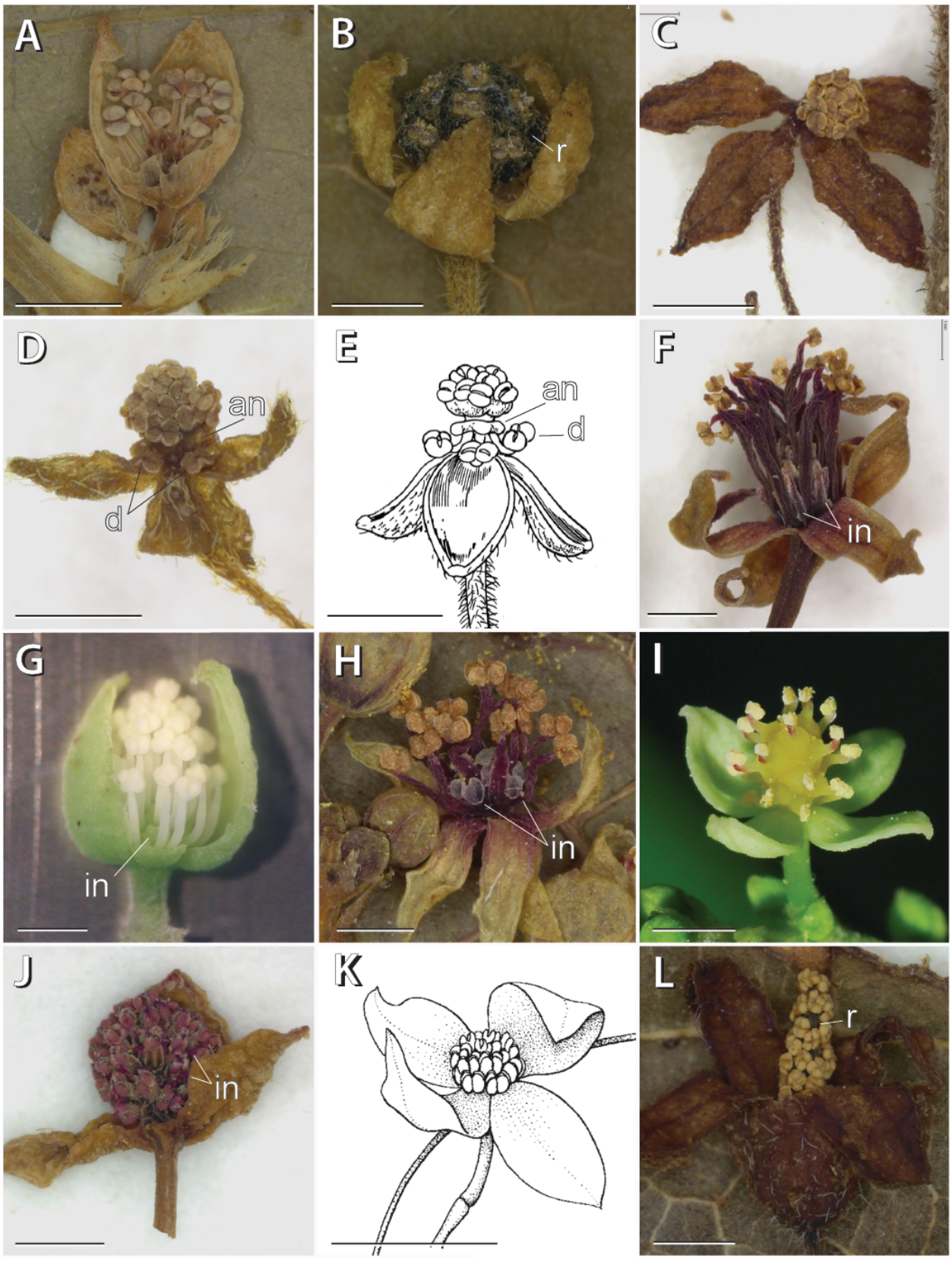
Table 4.3 Morphological differences between the sections of *Plukenetia*, including *Romanoa*.

Taxon	Primary veins	Pistillate flowers	Staminate receptacles	Staminate nectaries	Filament length	Pollen tecta	Carpels	Style connation	Style length	Stylar column shape	Fruit type	Fruit diameter	Seed size
P. sect. *Angostylidium*	3	1–2(3)	Subglobose	Interstaminal	< 0.5 mm	Foveolate	4	75(–90)%	4–7.5 mm	Funnel-shaped	Fleshy	4.5–7.5 cm	XL
P.* sect. *Fragariopsis	1	1–10	Globose	Absent	Absent	Coarsely reticulate	4	Entire	3–3.5 mm	Obovoid	Fleshy	4–5 cm	L
P. sect. *Hedraiostylus*	3(–5)	1	Convex, subglobose, or globose	Absent	< 0.5 mm	Foveolate	4	~70% or entire	0.5–1.8 mm	Cylindrical or depressed-globose	Dry	1.1–2 cm	S, M
P.* sect. *Madagascarienses	3(–5)	1 or 1–2(3)	Ellipsoid, oblong-cylindrical, or ovoid-conical	Absent	Absent	Foveolate	4	55–60% or entire	3.5–16 mm	Cylindrical or obconic or obovoid	Dry	2.3–4 cm	L
P.* sect. *Penninerviae	1(3)	1	Subglobose or globose	Absent or extrastaminal	Absent, sometimes with an outer whorl 0.3–1 mm	Coarsely reticulate	4	Entire	0.3–4.5 mm	Cylindrical, depressed-subglobose, globose, obovoid, or oblong-obovoid	Dry	0.9–1.5 cm	S, M
P. sect. *Plukenetia*	3	1 or 1–2(3)	Convex or subglobose	Interstaminal (absent)	0.5–2 mm	Foveolate	4	(20–40) 70–95%	5.6–35 mm	Cylindrical	Dry or fleshy	3.5–11 cm	M, L, XL, Max
Romanoa	3–5	1	Subglobose	Absent	0.5–1 mm	Fossulate-foveolate	3	70–80%	5.6–10 mm	Cylindrical	Dry	1–1.7 cm	S

ETS phylogeny

A phylogenetic analysis is included to demonstrate the relationships of our proposed new species *P. brevistyla* and *P. megastyla*, and subspecies *P. carolis-vegae* subsp. *nectarifera*. Only the nrDNA ETS region was successfully amplified for *P. brevistyla*, hence we show only a single marker phylogeny. Accessions of *P. megastyla* and *P. carolis-vegae* subsp. *nectarifera* were previously published as *P.* aff. *brachybotrya* and *P.* cf. *carolis-vegae*, respectively (Cardinal-McTeague *et al*., in review; Fig. 4.1). See Cardinal-McTeague *et al*. (in review) for methods on DNA extraction, primers, and PCR amplification and sequencing protocols. A single sequence was manually added to the ETS alignment from Cardinal-McTeague *et al*. (in review, available from DataDryad), with taxon sampling of 22 out of 24 *Plukenetia* species.

Phylogenetic relationships were reconstructed using maximum likelihood (ML) and Bayesian inference (BI) through the CIPRES Science Gateway v3.3 (https://www.phylo.org). ML analysis executed 1000 rapid bootstrap replicates under default search parameters using RAxML-HPC v8 on XSEDE (Stamatakis, 2014). BI analysis was conducted in MrBayes v3.2.6 on XSEDE (Ronquist *et al.*, 2012), implementing two independent MCMC runs, each with four chains, run for five million generations, and sampling every 1000 generations. We applied a


A
B
r
C
D
an
d
E
an
d
F
in
G
in
H
in
I
J
in
K
L
r

←

Figure 4.2 Representative staminate flower diversity of *Plukenetia* and its sister genus *Romanoa*. (a) *Romanoa tamnoides* (*Krapovickas & Schinini 36265*, MO), stamens with slender-cylindrical filaments. (b) *P.* sect. *Fragariopsis*: *P. serrata* (*Davidse et al. 10480*, MO), anthers sessile and loosely packed on a large globose receptacle. (c–e) *P.* sect. *Penninerviae*: (c) *P. loretensis* (*Freitas et al. 155*, MO), anthers sessile and densely packed on a small globose receptacle; (d) *P. penninervia* (*van der Werff et al. 3173*, MO), and (e) *P. supraglandulosa* (*Granville 3626* CAY, US), most anthers sessile and densely packed on a small globose receptacle, with a dimorphic outer whorl of ~4 stamens with filaments, and often with a 4–lobed annular nectary. (f–i) *P.* sect. *Plukenetia*: (f) *P. polyadenia* (*Croat et al. 20285*, MO), stamens with slender-cylindrical filaments and ligulate interstaminal nectaries; (g) *P. stipellata* (*Cardinal-McTeague 8*, CAN), stamens with slender-cylindrical filaments and small irregularly shaped interstaminal nectary segments; (h) *P. carolis-vegae* subsp. *nectarifera* (*Woytkowski 6670*, MO), stamens with slender filaments and large irregularly shaped interstaminal nectary segments; (i) *P. volubilis* (*Wurdack s.n.*, US), stamens with short-conical filaments. (j) *P.* sect. *Angostylidium*: *P. conophora* (*Avio et al. 1621*, MO), stamens with short-conical filaments and slender-cylindrical interstaminal nectaries (in pink). (k) *P.* sect. *Hedraiostylus*: *P. corniculata* (*Chin See Chung 2712*, L), stamens with short-conical filaments (sometimes appearing sessile). (l) *P.* sect. *Madagascarienses*: *P. madagascariensis* (*Villiers et al. 4889*, MO), anthers more or less sessile, densely to somewhat loosely packed on an elongate receptacle. Photos (a–d, f–h, j, l) by W.Cardinal-McTeague; (i) by K.Wurdack. Line drawing (e) by Alice Tangerini, (k) by Anita Walsmit Sachs, used with permission from the Nationaal Herbarium Nederland. (Abbreviations: an = annular nectary, d = dimorphic filamentous stamens, in = interstaminal nectaries, r = receptacle. Scale bars = 1 mm).

single model of nucleotide evolution on an unpartitioned dataset, selected using the Akaike information criterion (AIC) following default ML Best tree searches conducted in jModelTest2 on XSEDE (Darriba *et al.*, 2012). Independent MCMC runs were considered converged after the standard deviation of split frequencies was < 0.05, estimated sample sizes (ESS) were > 2000, and potential scale reduction factors (PSRF) were ~1.00. A 25% burn-in was used prior to summarizing parameter and tree statistics and a maximum clade credibility (MCC) tree.

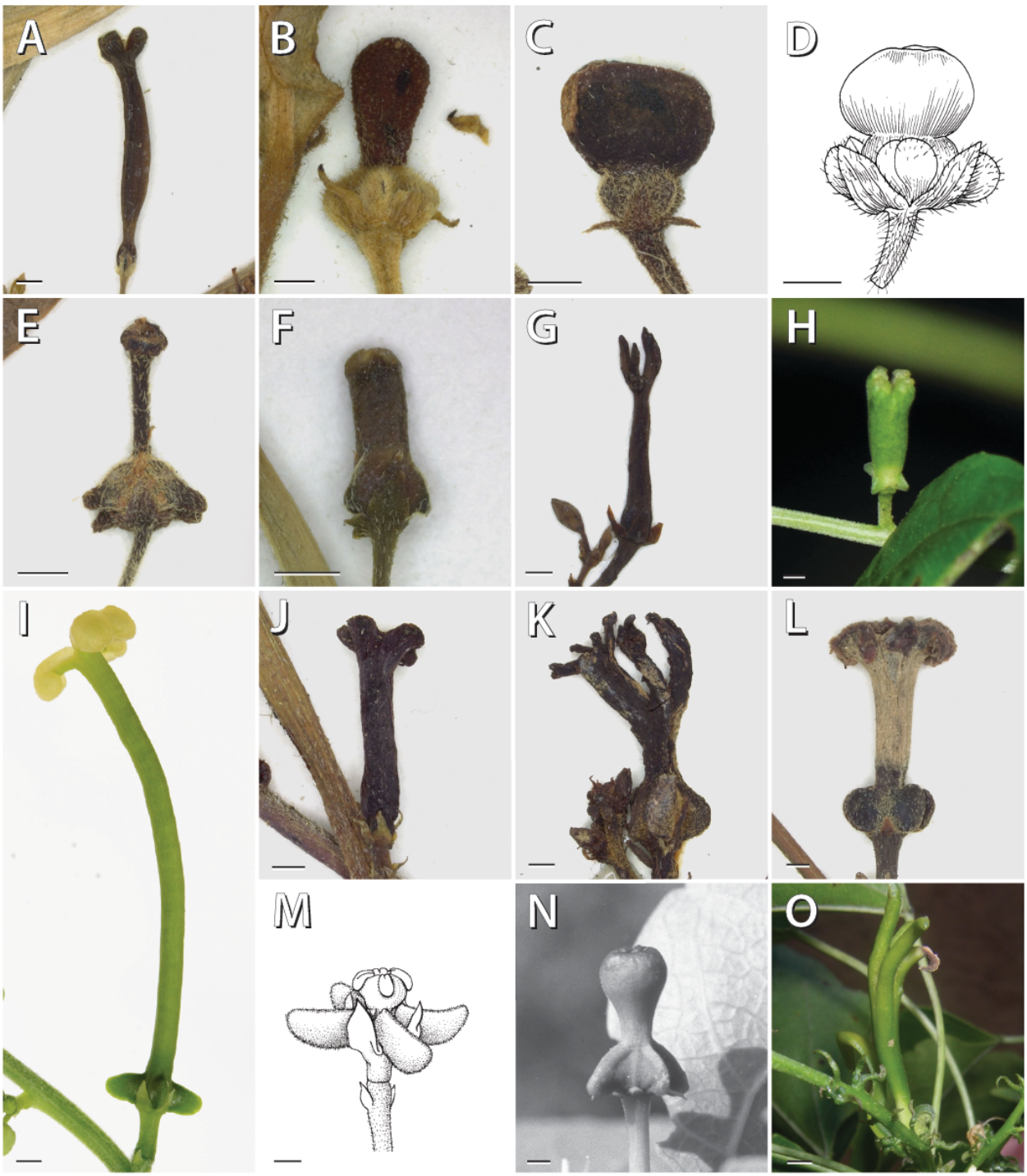
Distribution map

The base of a species distribution map was created using ggmap (Kahle & Wickham, 2013) and ggplot2 (Wickham, 2009) in R. The R script is available on DataDryad (http://datadryad.org).

Results

Dataset characteristics and phylogenetic relationships

In total 84 accessions were included in our ETS dataset (Appendix 4.1), resulting in an aligned length of 469 characters (228 constant, 241 variable, 199 [42.4%] parsimony informative). AIC identified TrN+I+G as the optimal model of molecular evolution; however, we selected the next best model that could be implemented in MrBayes, HKY+I+G (AIC delta = 1.6812). Our


A
B
C
D
E
F
G
H
I
J
K
L
M
N
O

←

Figure 4.3 Representative pistillate flower diversity of *Plukenetia* and its sister genus *Romanoa*. (a) *Romanoa tamnoides* (*Krapovickas & Schinini 36265*, MO), styles 70–80% connate into a cylindrical column. (b) *P.* sect. *Fragariopsis*: *P. serrata* (*Davidse et al. 10480*, MO), styles entirely connate into an obovoid column. (c–f) *P.* sect. *Penninerviae*: (c) *P. brachybotrya* (*Fuentes et al. 5398*, MO) and (d) *P. verrucosa* (*Prance et al. 11255*, DAV), styles entirely connate into a globose column; (e) *P. loretensis* (*Silva et al. 4750*, MO), styles entirely connate into a slender-cylindrical column, dilated at the apex; (f) *P. penninervia* (*Wallnöfer et al. 5996*, MO), styles entirely connate into a stout-cylindrical column. (g–k) *P.* sect. *Plukenetia*: (g) *P. polyadenia* (*Aulestia & Grefa 230*, MO), (h) *P. stipellata* (*Cardinal-McTeague 8*, CAN), (i) *P. volubilis* (*Wurdack s.n.*, US), and (j) *P. carolis-vegae* subsp. *nectarifera* (*Woykowski 6670*, MO), styles 70–95% connate into a cylindrical column; (k) *P. lehmanniana* (*Rangel et al. 5734*, MO), styles 20–25% connate into a cylindrical column, free style arms 2–fid near the tips. (l) *P.* sect. *Angostylidium*: *P. conophora* (*Thomas 5455*, MO), styles 75–(90)% connate into a funnel-shaped column, free style arms conspicuously dilated and spreading. (m) *P.* sect. *Hedraiostylus*: *P. corniculata* (*Brink 5771*, L), styles entirely connate into a depressed-globose column with cross-shaped stigmas. (n–o) *P.* sect. *Madagascarienses*: (n) *P. ankaranensis* (*Gillespie 4076*, CAN), styles entirely connate into an obconic or obovoid column; (o) *P. madagascariensis* (*Andrianjafy et al. 1648*, MO), styles 55–60% connate into a cylindrical column, free style arms slender and tapered. Photos (a–c, e–h, j–l) by W.Cardinal-McTeague, (i) by of K.Wurdack, (n) by L.Gillespie, (o) by P.Phillipson. Line drawing (d) by Cathy Pasquale, (k) by Anita Walsmit Sachs, used with permission from the Nationaal Herbarium Nederland. (Scale bars = 1 mm).

alignment and MrBayes tree file are available on DataDryad (http://datadryad.org).

The BI maximum clade credibility tree is presented with ML bootstrap percentage (MLBP) and Bayesian posterior probability (BPP) support values in Fig. 4.4. Evidence of strong branch support was interpreted as ≥ 80 MLBP and ≥ 0.95 BPP, indicated by bold branches. The ETS phylogeny is mostly congruent with the topology of the combined seven marker dataset recovered in Cardinal-McTeague *et al*. (in review), with the exception of subclade P4 moderately supported as embedded in subclade P3 (Fig. 4.4; MLBP = 69, BPP = 0.97).

Our proposed new species were recovered as distinct lineages in subclade P2. *Plukenetia brevistyla* is resolved as the earliest diverging lineage in a strongly supported clade sister to *P. penninervia* (MLBP = 84, BPP = 0.97). Accessions of *P. megastyla* were strongly supported as monophyletic (MLBP = 100, BPP = 1) and were resolved in a weakly supported clade (MLBP = 60, BPP = 0.8) composed of a functional polytomy with the remaining species of subclade P2. *Plukenetia carolis-vegae* subsp. *nectarifera* resolved into two clades in a strongly supported polytomy with *P. volubilis* (MLBP = 81, BPP =1.0). One of the clades is strongly supported (MLBP = 95, BPP = 1.0) and includes accessions of the putative hybrid species *P. huayllabambana*. Note that *P. carolis-vegae* subsp. *nectarifera* was strongly supported as monophyletic when additional molecular markers were sampled (Cardinal-McTeague *et al.*, in review).

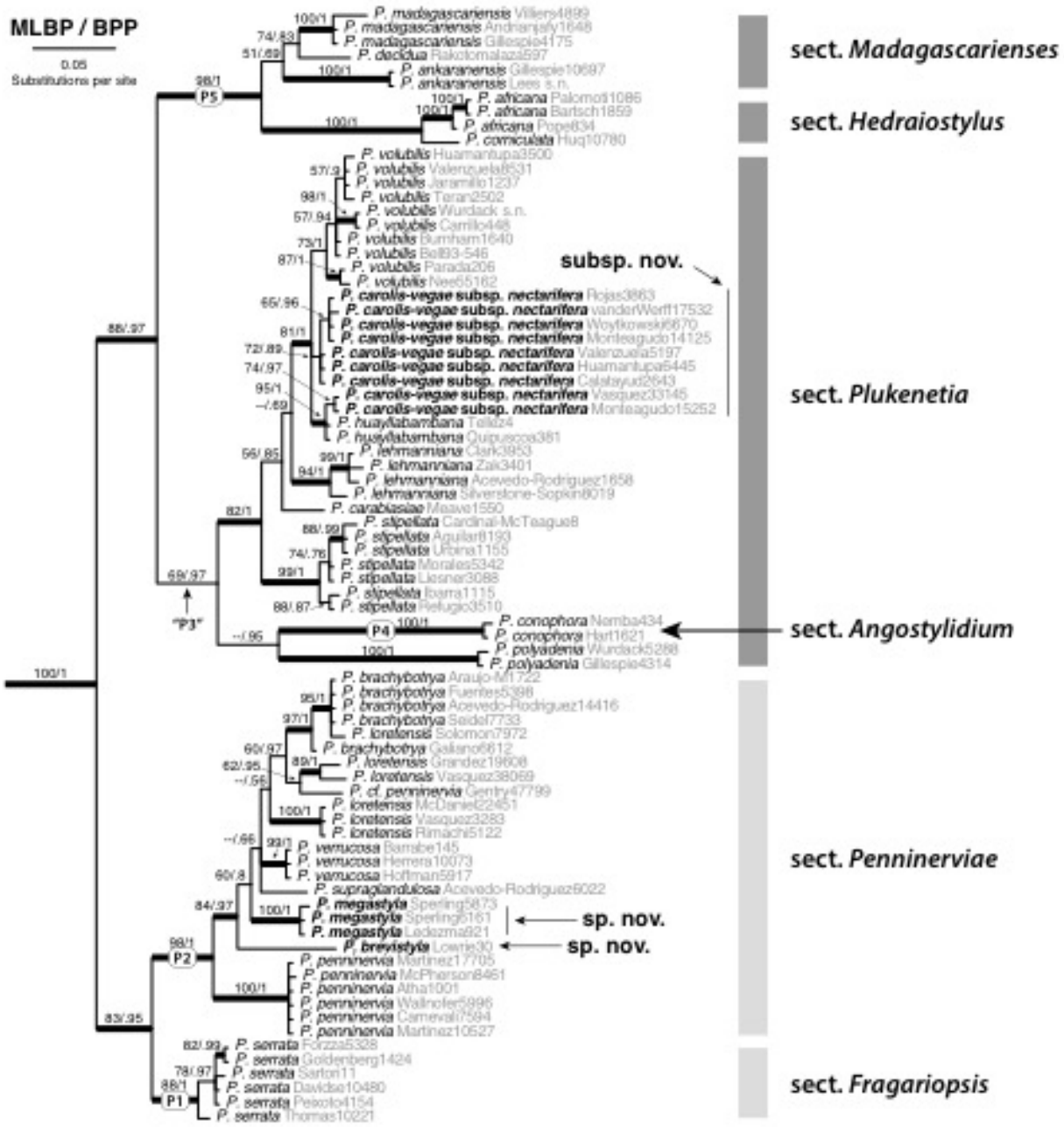


Figure 4.4 Bayesian maximum clade credibility tree based on an 84 accession nrDNA ETS dataset for *Plukenetia* and Plukenetiinae outgroups. Maximum likelihood bootstrap percentage (MLBP) and Bayesian posterior probability (BPP) support values > 50% are indicated on each branch. Branches in bold indicate strong support (≥ 80 MLBP and ≥ 0.95 BPP). Subclades P1–P5 were defined in Cardinal-McTeague & Gillespie (2016). Sections in the pinnately-veined clade (P1 + P2) are indicated by light grey boxes, sections in the palmately-veined clade (P3–P5) by dark grey boxes.

Taxonomic Treatment

Key to the genera of non-stinging vines and lianas in tribe Plukenetieae

1. Carpels 3; leaf blades 3–5–veined at the base; styles partly connate into a cylindrical column, 5.6–10 mm long; staminate sepals 5, stamens 10, filaments slender-cylindrical; pollen tecta fossulate-foveolate; distributed in Bolivia, southeast Brazil, and Paraguay. – ***Romanoa***

1. Carpels 4 (sometimes 5–7 in cultivated forms); leaf blades 1– or 3(–5)–veined at the base; styles entirely connate into a cylindrical, depressed-subglobose, globose, obconic, obovoid, or oblong-obovoid column, 0.3–5.5 mm long, or partly connate into a cylindrical column, 1–35 mm long; staminate sepals 3–5, stamens 6–60, filaments absent, short-conical, or slender-cylindrical; pollen tecta coarsely reticulate or foveolate; distributed pantropically. – ***Plukenetia***

PLUKENETIA L., Sp. Pl. 1 (2): 1192. 1753.—TYPE: *Plukenetia volubilis* L.

Vigia Vell., Fl. Flumin. Icon. 9: t.128. 1832.—TYPE: *Vigia serrata* Vell. [= *Plukenetia serrata* (Vell.) L.J.Gillespie]

Fragariopsis A.St.-Hil., Leçons Bot. 426. 1840.—TYPE: *Fragariopsis scandens* A.St.-Hil. [= *Plukenetia serrata* (Vell.) L.J.Gillespie]

Accia A.St.-Hil., Leçons Bot. 499. 1840, nom. illeg.—TYPE: *Accia scandens* A.St.-Hil. [= *Plukenetia serrata* (Vell.) L.J.Gillespie]

Botryanthe Klotzsch, Arch. Naturgesch. (Berlin) 7: 190. 1841.—TYPE (designated by Webster 1994): *Botryanthe discolor* Klotzsch [= *Plukenetia serrata* (Vell.) L.J.Gillespie]

Pterococcus Hassk., Flora 25 (2, Bleibl.): 41. 1842, nom. cons., non Pall. 1773.—TYPE: *Pterococcus glaberrimus* Hassk. [= *Plukenetia corniculata* Sm.]

Hedraiostylus Hassk., Tijdschr. Natuurl. Gesch. Physiol. 10: 141. [~September] 1843.—TYPE: *Hedraiostylus glaberrimus* Hassk. [= *Plukenetia corniculata* Sm.]

Sajorium Endl., Gen. Pl. Suppl. 3: 98. [October] 1843, nom. illeg.—TYPE: *Sajorium corniculatum* (Sm.) D.Dietr. [= *Plukenetia corniculata* Sm.]

Ceratococcus Meisn., Pl. Vasc. Gen. 2: 369. [2–4 November] 1843, nom. illeg.—TYPE: not designated

Tetracarpidium Pax, Bot. Jahrb. Syst. 26 (3–4): 329. 1899.—TYPE: *Tetracarpidium staudtii* Pax [= *Plukenetia conophora* Müll.Arg.]

Pseudotragia Pax, Bull. Herb. Boissier, ser. 2, 8: 635. 1908.—TYPE: *Pseudotragia scandens* Pax [= *Plukenetia africana* Sond.]

Eleutherostigma Pax & K.Hoffm. in A.Engler (ed.), Pflanzenr. IV, 147, IX (Heft 68): 11, t.3. 1919.—TYPE: *Eleutherostigma lehmanniana* Pax. & K.Hoffm. [= *Plukenetia lehmanniana* (Pax & K.Hoffm.) Huft & L.J.Gillespie]

Angostylidium (Müll.Arg.) Pax & K.Hoffm. in A.Engler (ed.), Pflanzenr. IV, 147, IX (Heft 68): 17. 1919. *Plukenetia* sect. *Angostylidium* Müll.Arg., Flora 47: 530. 1864.—TYPE: *Angostylidium conophorum* (Müll.Arg.) Pax & K.Hoffm. [= *Plukenetia conophora* Müll.Arg.]

Apodandra Pax & K.Hoffm. in A.Engler (ed.), Pflanzenr. IV, 147, IX (Heft 68): 20. 1919.—TYPE (designated by Webster 1994): *Apodandra loretensis* (Ule) Pax & K.Hoffm. [= *Plukenetia loretensis* Ule]

Elaeophora Ducke, Arch. Jard. Bot. Rio de Janeiro 4: 112. 1925.—TYPE: *Elaeophora abutifolia* Ducke [= *Plukenetia polyadenia* Müll.Arg.]

Notes—See (Gillespie, 2007) for genus description.

Key to the sections of *Plukenetia*

1. Leaf blades 1–veined at the base (3–veined in *P. verrucosa*); styles entirely connate into a cylindrical, depressed-subglobose, globose, obovoid, or oblong-obovoid column, 0.3–4.5 mm long; pollen tecta coarsely reticulate. (Pinnately-veined clade, subclades P1 + P2) – **(2)**

1. Leaf blades 3(–5)–veined at the base; styles partly connate into a cylindrical column, 1–35 mm long (entirely connate into a depressed-globose column 0.5–0.7 mm in *P. corniculata*, or an obconic or obovoid column 3.5–5.5 mm in *P. ankaranensis*); pollen tecta foveolate. (Palmately-veined clade, subclades P3–P5) – **(3)**

2. Fruits 4–5 cm in diam, fleshy; pistillate flowers 1–10 per inflorescence; all anthers sessile and loosely packed on a globose receptacle, receptacles 2 mm or more in diam, visible

between anthers; stipels 2, present adaxially at petiole apex; distributed in the Atlantic Forest region of Brazil. – **II. *P.*** sect. ***Fragariopsis***

2. Fruits 0.9–1.5 cm in diam, dry; pistillate flowers 1 per inflorescence; all or most anthers sessile and densely packed on a globose or subglobose receptacle, receptacles less than 1 mm in diam, not visible between anthers; stipels absent adaxially at petiole apex (2 in *P. verrucosa*); distributed in Mesoamerica, Central America, northwestern and Amazonian South America, and the Lesser Antilles. – **V. *P.*** sect. ***Penninerviae***

3. Styles 0.5–1.7 mm long, shorter than or nearly equal in length to the ovary; distributed in southern Africa and Southeast Asia. – **III. *P.*** sect. ***Hedraiostylus***

3. Styles 3.5–35 mm long, longer than the length of the ovary; distributed in tropical central and western Africa, Madagascar, or the Neotropics. – **(4)**

4. Staminate receptacles ellipsoid, oblong-cylindrical, or ovoid-conical; anthers sessile; nectaries absent; distributed in Madagascar. – **IV. *P.*** sect. ***Madagascarienses***

4. Staminate receptacles convex to subglobose; anthers on short-conical or slender-cylindrical filaments, 0.5–2 mm long; nectaries of interstaminal slender-cylindrical, ligulate, or small or large irregularly shaped segments (absent in *P. volubilis*); distributed in tropical central and western Africa or the Neotropics. – **(5)**

5. Stylar columns funnel-shaped, free style arms conspicuously dilated and spreading; distributed in tropical central and western Africa. – **I. *P.*** sect. ***Angostylidium***

5. Stylar columns cylindrical, free style arms more or less uniform in thickness or tapered and erect or spreading; distributed in the Neotropics. – **VI. *P.*** sect. ***Plukenetia***

I. PLUKENETIA sect. ANGOSTYLIDIUM Müll.Arg., Flora 47: 530. 1864. *Angostylidium* (Müll.Arg.) Pax & K.Hoffm. in A.Engler (ed.), Pflanzenr. IV, 147, IX (Heft 68): 17. 1919.— TYPE: *Plukenetia conophora* Müll.Arg.

Canopy lianas, stems thick. **Leaf blades** 3–veined at the base. **Inflorescences** unisexual or functionally unisexual; staminate inflorescences racemose thyrses, staminate flowers 3–7/node

in condensed cymules, sometimes with 1–2 apparently non-functional pistillate flowers at basal-most nodes; pistillate inflorescences short racemes, pistillate flowers solitary at 1–2(3) basal-most nodes, axes mostly aborted above (rarely with a few staminate flowers in cymules). **Staminate flowers:** receptacle subglobose; nectary of numerous interstaminal slender-cylindrical segments; stamens 25–50, densely packed; filaments conical, < 0.5 mm; pollen P = 34–38 μm, E = 41–46 μm, tectum foveolate. **Pistillate flowers:** styles 75(–90)% connate into a funnel-shaped column, entire style 4–7.5 mm. **Fruits** 4(5)–lobed or subglobose-quadrangular, capsule-like, fleshy but indehiscent, 4.5–7.5 cm in diam. **Seeds** broadly ovoid or subglobose, 25–29 x 25–27 x 25–28 mm ("extra-large" sensu Cardinal-McTeague *et al.*, in review).

Distributed in tropical central and western Africa.

Discussion—Section *Angostylidium* corresponds to subclade P4 (Fig. 4.1) and includes a single species, *P. conophora*, from tropical central and western Africa. This Old World species/section is morphologically similar to sect. *Plukenetia* of the Neotropics. Both sections have staminate flowers with interstaminal nectaries (absent in *P. volubilis* of sect. *Plukenetia*), partly connate styles, often larger fleshy fruits (although dry fruits are just as frequent in sect. *Plukenetia*), and often larger seeds (usually "large" and "extra-large"). Phylogenetic analysis suggests these characters are symplesiomorphic for the palmately-veined clade (P3–P5). Section *Angostylidium* is differentiated by its African wet tropical distribution, functionally unisexual inflorescences, and funnel-shaped stylar column with conspicuously dilated and spreading free style arms (Fig. 4.3L).

1. PLUKENETIA CONOPHORA Müll.Arg., Flora 47: 530 1864. *Angostylidium conophorum* (Müll.Arg.) Pax & K.Hoffm. in A.Engler (ed.), Pflanzenr. IV, 147, IX (Heft 68): 17. 1919. *Cleidion mannii* Baker, Bull. Misc. Inform. Kew 1910 (2): 58. 1910, nom. illeg. *Tetracarpidium conophorum* (Müll.Arg.) Hutch. & Dalziel, Fl. W. Trop. Afr. 1: 307. 1928.—TYPE: CAMEROON. River Cameroon, January 1863, *G. Mann 2202* (lectotype designated by Gillespie 2007: K000425658!; isolectotypes: K000425659!, K000425660!).

Mallotus preussii Pax, Bot. Jahrb. Syst. 23 (4): 525. 1897. *Cleidion preussii* (Pax) Baker, Bull. Misc. Inform. Kew 1910 (9): 343. 1910.—TYPE: CAMEROON. Barombistation, 25 August

1890, *P.R. Preuss 420* (holotype: B [destroyed]; lectotype designated here: K000252567!; isolectotype: BM000535904!).

Tetracarpidium staudtii Pax, Bot. Jahrb. Syst. 26 (3-4): 329. 1899.—TYPE: CAMEROON. Station Johann-Albrechtschöhe, 15 January 1897, *A. Staudt 802* (holotype: B [destroyed]; lectotype designated here: BM000535187!; isolectotypes: G00414564 [online!], sketch of holotype with floral fragments at K000425656!; MO1648824! [barcode MO-2289110], PH00030257 [online!]).

Notes—See Gillespie (2007) for species description.

Taxonomic Discussion—*Plukenetia conophora* and sect. *Angostylidium* were simultaneously described by Müller (1864). Subsequent authors described taxa that are now treated as synonyms, including *Mallotus preussii* (Pax, 1897) and the novel genus and species *Tetracarpidium staudtii* (Pax, 1899). Furthermore, Baker (1910) described *Cleidion mannii* from *P. conophora* syntype material ("River Cameroon", *G. Mann 2202*) but erroneously cited the type as "Cameroons river, *G. Mann 1202*" (that specimen is recorded as *Pittosporum viridiflorum* "mannii" Sims, "Mount Cameroon", *G. Mann 1202* K000106232). Baker appears to have described *Cleidion mannii* from the only *G. Mann 2202* sheet at Kew that was not annotated as Müller's *P. conophora* (K000425659).

The holotype of *Mallotus preussii* is assumed to have been in Berlin, where Pax worked and Preuss's types were housed. Presumably the type was destroyed, as such we designate a lecotype from the isotype housed at Kew, given it has abundant staminate flowers. Under a similar circumstance we designate a lectotype for *Tetracarpidium staudtii*, selecting the isotype with the most abundant leaf and pistillate flower material housed at the British Museum. Alois Staudt was the collection assistant of G. Zenker in Cameroon, so we assume his collections were part of Zenker's main set in Berlin.

II. **Plukenetia** sect. **Fragariopsis** (A.St.-Hil.) Card.-McTeag. & L.J.Gillespie, comb. et stat. nov.

Fragariopsis A.St.-Hil., Leçons Bot. 426. 1840.—TYPE: *Fragariopsis scandens* A.St.-Hil. [= *Plukenetia serrata* (Vell.) L.J.Gillespie]

Lianas, stems slender to thick. **Leaf blades** 1–veined at the base. **Inflorescences** bisexual racemose thyrses; pistillate flowers solitary at 1–10 basal-most nodes; staminate flowers 2(3)/node in condensed cymules. **Staminate flowers:** receptacle globose; nectaries absent; stamens 10(–20), loosely packed (receptacle clearly visible between anthers); filaments absent; pollen P = 32–46 μm, E = 42–55 μm, tectum coarsely reticulate. **Pistillate flowers:** styles entirely connate into an obovoid column, 3–3.5 mm. **Fruits** 4–lobed or subglobose-quadrangular, capsule-like, initially fleshy, apparently indehiscent or tardily splitting, 4–5 cm in diam. **Seeds** subglobose, 15–15.5 x 15.5–16 x 15–16 mm ("large" sensu Cardinal-McTeague *et al.*, in review).

Distributed in the Atlantic Forest of Brazil.

Etymology—The sectional epithet is derived from *Fragaria* (Latin, strawberry) and -*opsis* (Greek, appearing like or resembling), referring to the androecium, which has the appearance of a small strawberry. We chose *Fragariopsis* over other possible generic names because it describes the unique androecium morphology of the section and because, historically, it was the most frequently recognized genus used for *Plukenetia serrata*.

Discussion—Section *Fragariopsis* refers to subclade P1 (Fig. 4.1) and includes a single species, *P. serrata*, from the Atlantic Forest region of Brazil. This species/section has long been recognized as distinct genus (Table 4.1), but belongs within *Plukenetia* due to their numerous shared characters (Gillespie, 1993) and phylogenetic relationships (Cardinal-McTeague and Gillespie 2016; Cardinal-McTeague *et al.*, in review). Together, sects. *Fragariopsis* and *Penninerviae* constitute the pinnately-veined clade (P1 + P2), which is united by pinnate leaf venation, coarsely reticulate pollen tecta, all or mostly sessile anthers, and entirely connate styles. Section *Fragariopsis* is differentiated by its unique androecium composed of sessile anthers loosely packed on a globose receptacle (Fig. 4.2B), as well as by having 1–10 pistillate flowers, "large" seeds derived from fleshy fruits, and for being distributed in the Atlantic Forest region of Brazil.

2. PLUKENETIA SERRATA (Vell.) L.J.Gillespie, Syst. Bot. 18 (4): 587. 1993. *Vigia serrata* Vell., Fl. Flumin. Icon. 9: t.128. 1827 publ. 29 October 1831.—TYPE: BRAZIL. Illustration t.128 in Vellozo, Fl. Flumin. Icon. 9. 1827 publ. 29 October 1831.

Fragariopsis scandens A.St.-Hil., Leçons Bot. 426. 1840. *Plukenetia scandens* (A.St.-Hil.) Pax in Engler & Prantl (eds.), Nat. Pflanzenfam. 3 (5): 67. 1890.—TYPE: BRAZIL. 1816–1821, *A. de Saint-Hilaire 95* (lectotype designated here: P00072051!; isolectotype: P00072052!).

Accia scandens A.St.-Hil., Leçons Bot. 499. 1840, nom. illeg.—TYPE: not designated. [Likely based on the same syntypes as *Fragariopsis scandens*, therefore a nomenclatural synonym and an illegitimate name; one of the syntype collections, *A. de Saint-Hilaire D 72* (P00072053!, P00072054!), is annotated as both *Accia* and *Fragariopsis scandens*, but the first name appears to be a later addition.]

Botryanthe discolor Klotzsch, Arch. Naturgesch. (Berlin) 7 (1): 191, 204. tab. 9b. 1841. *Fragariopsis discolor* (Klotzsch) Baill., Étude Euphorb. 498. 1858.— TYPE: BRAZIL. *Sellow s.n.* (holotype: B [destroyed]; lectotype designated by Gillespie 1993: P00072049!; isolectotypes: F! [fragment], HBG516131 [online!]). [Klotzsch's species *Botryanthe concolor* given in the same publication was not validly published]

Fragariopsis polyandrus Baill., Étude Euphorb. 498. 1858. *Fragariopsis scandens* var. *polyandrus* (Baill.) Baill., Adansonia 5: 318. 1865.—TYPE: BRAZIL. Rio de Janeiro, Mont. Corcovado, Sainte-Thérèse, May 1839, *J.B.A. Guillemin cat. n. 798* (lectotype designated here: P00072048!; isolectotypes: G-DC! [pro parte, left specimen excluding packet; G00313646], P00072047!, P05564459 [as *J.-B. Houllet cat. n. 798*; online!]).

Fragariopsis warmingii Müll.Arg. in Martius (ed.), Fl. Bras. 11 (2): 338. 1874. *Plukenetia warmingii* (Müll.Arg.) Pax in Engler & Prantl (eds.), Nat. Pflanzenfam. 3 (5): 67. 1890.—TYPE: BRAZIL. Minas Gerais, Lagoa Santa, *E. Warming s.n.* (holotype: P00072055!; isotype: F! [fragment]; probable isotype: F!).

Notes—See Gillespie (1993) for species discussion.

Taxonomic Discussion—*Plukenetia serrata* was first described as *Vigia serrata* (Vellozo, 1831) but was widely known as *Fragariopsis scandens* (Saint-Hilaire, 1840) until the priority of Vellozo's name was identified by Gillespie (1993). In the same publication, Saint-Hilaire (1840) described *Accia scandens*, which is likely a superfluous name for *F. scandens* based on the same syntypes. The description of *Fragariopsis* only includes the staminate flowers, whereas *Accia* is only of the pistillate flowers, however both descriptions are indicative

of *P. serrata*. *Botryanthe* was independently described by Klotzsch (1841) and was synonymized along with *Accia* under *Fragariopsis* by Baillon (1858). Two additional species, *F. polyandrus* (Baillon, 1858) and *F. warmingii* (Müller, 1874), were described, but both are treated as synonyms of *P. serrata* (Gillespie, 1993). Phylogenetic data indicates there is substantial nucleotide variation in *P. serrata* (Cardinal-McTeague *et al*., in review), suggesting its species boundaries should be reassessed with better taxon sampling across its morphological and geographical range.

Saint-Hilaire (1840) did not cite a collection number in his original species description of *Fragariopsis scandens*, but two of his collections, each with a duplicate, are housed at P. All four sheets have sufficient vegetative and floral material to identify the species, however, to clarify the type status we designate the best sheet (*A. de Saint-Hilaire 95*; P00072051) as lectotype. Remaining syntype: BRAZIL. Minas Gerais, 1816–1821, *A. de Saint-Hilaire D 72* (P00072053!, P00072054!).

Fragariopsis polyandrus has a complicated type history. Baillon (1858) did not cite any collections or a type for *F. polyandrus* but indicated its species illustrations in Tab. XIII were based on material from Herb. Houllet (Atlas, p. 26). Baillon (1865) later cited a single specimen with *F. scandens* var. *polyandrus,* "*Guillemin* et *Houllet* (herb.), cat., n. 798", which matches *J.B.A. Guillemin cat. n. 798* specimens housed at P and G-DC. To complicate matters, another collection of *Fragariopsis scandens* was found at Paris, *J.-B. Houllet cat. n. 798*. Notably, the Houllet collection includes the locality information cited by Baillon (1865), which is missing from all known *J.B.A. Guillemin cat. n. 798* sheets. Guillemin and Houllet were collecting partners in Brazil and many of Guillemin's collections appear to have been brought to Paris by Houllet, who worked as the Jardinier en chef des Serres. Since the Guillemin and Houllet collections are of the same species, share the same catalogue number, and are associated together by Baillon (1865), we consider them the same collection. We designate a lectotype for *F. polyandrus* based on the specimen with the most abundant pistillate flower material.

III. PLUKENETIA sect. HEDRAIOSTYLUS (Hassk.) Müll.Arg., D.C. Prod. 15 (2): 772. 1866.

Hedraiostylus Hassk., Tijdschr. Natuurl. Gesch. Physiol. 10: 141. 1843. *Sajorium* sect. *Hedraiostylus* (Hassk.) Baillon, Étude Euphorb. 483. 1858.—TYPE: *Hedraiostylus glaberrimus* Hassk. [= *Plukenetia corniculata* Sm.]

Plukenetia sect. *Sajor* Müll.Arg., Linnaea 34: 159. 1865, nom. illeg.—TYPE: not designated

Plukenetia sect. *Pterococcus* (Hassk.) Benth. & Hook., Gen. Pl. 3, 1: 327. 1880. *Pterococcus* Hassk., Flora 25 (2, Bleibl.): 41. 1842, nom. cons., non. Pall. 1773.—TYPE: *Pterococcus glaberrimus* Hassk. [= *Plukenetia corniculata* Sm.]

Vines, lianas, or perennial herbs, stems slender, twining or sometimes procumbent. **Leaf blades** 3(–5)–veined at the base. **Inflorescences** bisexual racemes (rarely racemose thyrses); pistillate flowers solitary (rarely 2) at basal-most node(s); staminate flowers 1/node (sometimes 1–2/node in reduced condensed cymules). **Staminate flowers:** receptacle convex, subglobose, or globose; nectaries absent; stamens 8–20, densely packed; filaments conical, < 0.5 mm; pollen P = 34–40 μm, E = 40–50 μm, tectum foveolate. **Pistillate flowers:** styles entirely connate into a depressed-globose or stout-cylindrical column, 0.5–1.4 mm, or ~70% connate into a stout-cylindrical column with spreading free style arms, 1.3–1.7 mm. **Fruits** 4–lobed capsules, dry, dehiscent, 1.1–2 cm in diam. **Seeds** broadly lenticular, 5.5–10.5 x 5–8 x 2.5–8 mm ("small" or "medium" sensu Cardinal-McTeague *et al*., in review).

Distributed in southern Africa and Southeast Asia.

Discussion—Section *Hedraiostylus* refers to a strongly supported clade within subclade P5 (Fig. 4.1) and includes three species, *P. africana* and *P. procumbens* from southern Africa and *P. corniculata* from Southeast Asia. This section is weakly defined morphologically but appears to be united by short styles < 2 mm long, usually shorter than the length of the ovary (although slightly longer than the ovary in *P. procumbens*), staminate flowers with short-conical filaments and lacking nectaries, smaller dry dehiscent fruits, and "small" or "medium" seeds. The southern African species are unique for growing in seasonally dry wooded savannas and have evolved thick rootstocks that facilitate resprouting after fires.

Key to the species of *Plukenetia* sect. *Hedraiostylus*

1. Petioles > (1–)3 cm; leaf blades > (2–)4 cm wide, base deeply cordate, adaxial basilaminar extrafloral nectaries 2, stipels 2, present adaxially at petiole apex ~0.5–1 mm long; capsules with strap-shaped wing 6–12 mm long on each carpel lobe; distributed in Southeast Asia. – **4. *P. corniculata***

1. Petioles < 1 cm; leaf blades < 3.5 cm wide, base obtuse to truncate, hastate, or rarely sagittate, adaxial basilaminar extrafloral nectaries (0–)2–12(–20), sometimes minute, stipels 2, present adaxially at petiole apex, 0.1–0.6 mm long, or absent; capsules with tubercle or wing ≤ 3 mm long on each carpel lobe; distributed in southern Africa. – **(2)**
2. Leaf blades narrowly triangular, lanceolate or linear-lanceolate, 3–8 cm long, base often hastate; adaxial basilaminar extrafloral nectaries (0–)2, 0.2–0.3 mm in diam. – **3. *P. africana***
2. Leaf blades elliptic or ovate, 2–4.5 cm long, base obtuse to rounded; adaxial basilaminar extrafloral nectaries 2–12(–20), 0.2–0.8 mm in diam. – **5. *P. procumbens***

3. PLUKENETIA AFRICANA Sond., Linnaea 23: 110. 1858. *Pterococcus africanus* (Sond.) Pax & K.Hoffm. in A.Engler (ed.), Pflanzenr. IV, 147, IX (Heft 68): 22. 1919.—TYPE: SOUTH AFRICA. Magaliesberg, auf Grasfeldern, October, *C.L.P. Zeyher 1522* (holotype: S! [S-G-10556]; isotypes: BM!, G00441997!, K000425662!, MEL501283 [online!]).

Plukenetia hastata Müll.Arg., Flora 47: 469. 1864.—TYPE: MOZAMBIQUE. Between Shupanga & Senna, Portuguese East Africa, on the Lower Zambesi, 14°–19° S, January 1859, *J. Kirk s.n.* (holotype: K000425661!).

Pseudotragia schinzii Pax, Bull. Herb. Boissier, ser. 2, 8: 635. 1908.—TYPE: NAMIBIA. Amboland, Otjiheveta, 1886, *H. Schinz 895* (holotype: G; isotypes: K001044972!, Z000085917 [online!]).

Pseudotragia scandens Pax, Bull. Herb. Boissier, ser. 2, 8: 636. 1908.—TYPE: NAMIBIA. Amboland, Oohama, March 1886, *H. Schinz 894* (holotype: G; isotypes: K001044973! Z000085918 [online!]).

Notes—See Gillespie (2007) for species description.

Although Sonder did not explicitly cite holotypes, his types based on Ecklon and Zeyher collections from South Africa are presumed to be housed in Stockholm (Nordenstam 1980), with duplicates found in many other herbaria in Europe and Melbourne, Australia. Indeed, the specimen at Stockholm, which we consider the holotype, has the most abundant floral and fruit material and original labels.

4. PLUKENETIA CORNICULATA Sm., Nova Acta Regiae Soc. Sci. Upsal. 6: 4. 1799. *Pterococcus glaberrimus* Hassk., Flora 25 (2): 41. 1842. *Hedraiostylus glaberrimus* Hassk., Tijdschr. Natuurl. Gesch. Physiol. 10: 141. 1843. *Hedraiostylus corniculatus* (Sm.) Hassk., Cat. Hort. Bot. Bogor. 234. 1844. *Sajorium corniculatum* (Sm.) D.Dietr., Syn. Pl. 5: 331. 1852. *Sajorium corniculatum* (Sm.) Baill., Étude Euphorb. 484. 1858, nom. illeg. *Pterococcus corniculatus* (Sm.) Pax & K.Hoffm. in A.Engler (ed.), Pflanzenr. IV, 147, IX (Heft 68): 22. 1919.—TYPE: INDONESIA. Amboina, Bagulae Regione, Illustration t.79, fig. 2 in Rumphius, Herb. Amboin. 1: 193. 1750.

Notes—See Gillespie (2007) for species description.

Taxonomic Discussion—*Plukenetia corniculata* is one of the oldest names in *Plukenetia* and has a complicated taxonomic history. Originally described as the pre-Linnaean *Sajor volubilis* (Rumphius 1750), it was included as a synonym of *Plukenetia volubilis* by Linnaeus (1753). Smith (1799) gave Rumphius' species its first legitimate name, *P. corniculata*, but four other genera would be erected for *Sajor*: *Pterococcus* (Hasskarl, 1842), *Hedariostylus* (Hasskarl, 1843), *Sajorium* (Endlicher, 1843), and *Ceratococcus* (Meisner, 1843). Hasskarl's first name, *Pterococcus* (1842), was previously published by Pallas (1773) in Polygonaceae, so he proposed the replacement name *Hedraiostylus* in ~September 1843 (Stafleu & Cowan, 1979). Subsequent replacement names *Sajorium* and *Ceratoccocus*, both nomenclaturally superfluous, were published in October and 2–4 November of 1843, respectively (Stafleu & Cowan, 1976, 1981).

Baillon (1858) first treated *Hedraiostylus* as a section of the illegitimate genus *Sajorium* [= *Plukenetia*], giving *Hedraiostylus* priority as a sectional name. The fact that *Sajorium* was illegitimate precludes the rejection of sect. *Hedraiostylus* in favour of the autonym *Sajorium* sect. *Sajorium* (ICN 2012, Art. 22.5; McNeill et al. 2012). When Müller created the first sectional classification of *Plukenetia* he initially erected sect. *Sajor* (Müller, 1865), but quickly updated it to sect. *Hedraiostylus* (Müller, 1866). To complicate matters, *Pterococcus* Hassk. was conserved over *Pterococcus* Pall., but as a sectional name, *Hedraiostylus* retains priority.

5. PLUKENETIA PROCUMBENS Prain, Bull. Misc. Inform. Kew 1912 (5): 240. 1912. *Pterococcus procumbens* (Prain) Pax & K.Hoffm. in A.Engler (ed.), Pflanzenr. IV, 147, IX (Heft 68): 23. 1919.—TYPE: ANGOLA. Benhuella, Ganguella, on the Cubagano River at Princeza

Amelia, 1520 m, 27 January 1907, *J. Gossweiler 2540* (holotype: BM000535903!; isotypes: COI00072615!, K000425657! [fragments with floral illustration]).

Notes—See Gillespie (2007) for species description.

IV. **Plukenetia** sect. **Madagascarienses** Card.-McTeag. & L.J.Gillespie sect. nov.—TYPE: *Plukenetia madagascariensis* Leandri

Lianas, stems slender to thick. **Leaf blades** 3(–5)–veined at the base. **Inflorescences** bisexual racemes or racemose thyrses; pistillate flowers solitary at 1–2 basal-most nodes; staminate flowers 1/node or 3–5/node in lax or moderately condensed cymules with conspicuous and irregularly branched cyme axes. **Staminate flowers:** receptacle ellipsoid, oblong-cylindrical, or ovoid-conical; nectaries absent; stamens 15–60, densely or loosely packed; filaments absent; pollen P = 28–41 μm, E = 35–51 μm, tectum foveolate. **Pistillate flowers:** styles entirely connate into an obconic or obovoid column, 3.5–5.5 mm, or 55–60% connate into a cylindrical column, 8–16 mm, free style arms slender and tapered. **Fruits** 4–lobed capsules, dry, dehiscent, 2.3–4 cm in diam. **Seeds** broadly ellipsoid or subglobose, 13.1–18 x 11.1–17 x 11.2–17 mm ("large" sensu Cardinal-McTeague *et al*., in review).

Distributed in Madagascar.

Etymology—The sectional epithet is derived from the combination of Madagascar and -*ensis* (Latin, of a place), which reflects that all the species in the section are endemic to Madagascar.

Discussion—Section *Madagascarienses* refers to a strongly supported clade within subclade P5 (Fig. 4.1) and includes three species endemic to Madagascar. This section was originally defined as the Madagascan species group (Gillespie, 2007) and was noted for exhibiting sessile anthers similar to the pinnately-veined clade. Style morphology is variable in the section, including both partly (*P. decidua*, *P. madagascariensis*) and entirely connate (*P. ankaranensis*) styles (Fig. 4.3N–O). Section *Madagascarienses* is differentiated by having sessile anthers on a prominently elongated receptacle (Fig. 4.2L), larger dry dehiscent fruits, "large" seeds, and for being endemic to Madagascar. All three species occur in seasonally dry

environments, either dry forest on tsingy limestone (*P. ankaranensis*, *P. madagascariensis*) or in dry scrub (*P. decidua*).

Key to the species of *Plukenetia* sect. *Madagascarienses*

1. Styles entirely connate, 3.5–5.5 mm long, stylar column obconic or obovoid, free style arms absent; androecia 0.6–1 mm long, anthers 15–20; inflorescences thyrses, terminal and appearing leaf-opposed, staminate flowers in distinct cymules; glandular knobs absent at petiole apex. – **6. *P. ankaranensis***

1. Styles 55–60% connate, entire style 8–16 mm long, stylar column cylindrical, free style arms slender, tapered; androecia 1.6–4 mm long, anthers 18–60+; inflorescences very narrow thyrses or racemes, axillary or terminal, staminate flowers single per node or in condensed cymules; glandular knobs 1–2 at petiole apex, sometimes minute. – **(2)**

2. Inflorescences terminal racemes, staminate flowers single per node; bracts triangular, 1–2 mm long, sessile, eglandular; androecia 1.6–1.8 mm long, anthers 18–30 on oblong-ellipsoid receptacle; leaf blades triangular-ovate or ovate. – **7. *P. decidua***

2. Inflorescences axillary thyrses, staminate flowers in condensed cymules; bracts lanceolate, 3–8 mm long, usually petiolate and 2–glandular; androecia 3–4 mm long, anthers 35–60+ on narrowly conical receptacle; leaf blades broadly ovate or orbicular. – **8. *P. madagascariensis***

6. PLUKENETIA ANKARANENSIS L.J.Gillespie, Syst. Bot. 32 (4): 797. 2007.—TYPE: MADAGASCAR. Antsirinana Province: Special Reserve #3, Ankarana, ca. 7 km SE of Matsaborimanga, trail between Camp Anglais and river (ca. 3 km SW Camp Anglais), [-12.917°, 49.1°], 150 m, 28 November 1990, *L. Gillespie 4076* (holotype: MO6128360!; isotypes: CAN589544!, DAV182695!, G00386814!, K001044974!, L3784620!, NY01104761!, P00717080!, TAN!, US!; FAA preserved material at MO).

Notes—See Gillespie (2007) for species description.

7. PLUKENETIA DECIDUA L.J.Gillespie, Syst. Bot. 32 (4): 798. 2007.—TYPE: MADAGASCAR. Sud-Ouest, entre Ampanihy et Itrobiky (route Ampanihy-Androka), 4 July 1958, *R. Capuron 18682-SF* (holotype: P00586758!; isotypes: P00586759!, P00586760!).

Notes—See Gillespie (2007) for species description.

Although barcodes were not applied to the specimens at Paris when Gillespie (2007) described *P. decidua*, the author confirms that specimen P00586758, which has the most abundant vegetative and floral material, is correctly annotated as the holotype (LJG, personal communication).

8. PLUKENETIA MADAGASCARIENSIS Leandri, Bull. Bot. Soc. France 85: 527. 1938 publ. 1939.—TYPE: MADAGASCAR. Bois à Morataitra, rive droite de Betsiboka, est de Maevatanana (Boeny), Mars 1899, *H. Perrier 848* (lectotype designated by Gillespie 2007: P00586763!; isolectotypes: L0388568!, P00586764!, P00586765!).

Notes—See Gillespie (2007) for species description.

V. **Plukenetia** sect. **Penninerviae** Card.-McTeag. & L.J.Gillespie sect. nov.—TYPE: *Plukenetia penninervia* Müll.Arg.

Vines or lianas, stems slender. **Leaf blades** 1–veined at the base (3–veined in *P. verrucosa*). **Inflorescences** bisexual racemose thyrses (racemes in *P. brachybotrya*); pistillate flowers solitary at basal-most node; staminate flowers 1–3(–5)/node in condensed cymules, sometimes appearing short racemose, or 1/node. **Staminate flowers:** receptacle globose or subglobose; nectaries absent or an extrastaminal annular ring (4–lobed, or unlobed with an uneven or undulate upper surface); stamens 6–50, anthers sessile and densely packed on the receptacle, sometimes with an outer whorl ~4 filamentous stamens; filaments absent or slender-cylindrical 0.3–1 mm in outer whorl; pollen P = 31–62 μm, E = 39–76 μm, tectum coarsely reticulate. **Pistillate flowers:** styles entirely connate into a depressed-subglobose, globose, obovoid, oblong-obovoid, stout-cylindrical, or slender-cylindrical column with a dilated apex, 0.3–4.5 mm. **Fruits** 4–lobed capsules, dry, dehiscent, 0.9–1.5 cm in diam. **Seeds** globose,

subglobose, or broadly lenticular, 4.5–7.3 x 3.7–7.1 x 3–5.2 mm, ("small" or "medium" sensu Cardinal-McTeague *et al*., in review).

Distributed in Mesoamerica, Central America, northwestern and Amazonian South America, and the Lesser Antilles.

Etymology—The sectional epithet is derived from *penni-* (Latin, feather) and *-nervia* (Latin, -nerved or -veined), referring to the pinnately-veined leaves exhibited by most species in the section (excluding *P. verrucosa*, which is 3–veined at the base).

Discussion—Section *Penninerviae* refers to subclade P2 (Fig. 4.1) and includes eight species widespread in the Neotropics. This section was erroneously referred to as sect. *Euplukenetia* by Pax and Hoffmann (1919), but that name was both invalid and incorrectly attributed to a section excluding the type species, *P. volubilis*. Section *Penninerviae* forms a major component of the pinnately-veined clade (P1 + P2), and is distinguished by having staminate flowers with sessile anthers densely packed on a globose receptacle (Fig. 4.2C), frequently with an extrastaminal annular nectary (Figs. 4.5H, 4.6G) and/or an outer whorl of ~4 filamentous stamens (Fig. 4.2D–E). It differs from sect. *Fragariopsis* by having a single pistillate flower per inflorescence and "small" or "medium" seeds borne in small dry dehiscent capsules.

Key to the species of *Plukenetia* sect. *Penninerviae*

1. Leaf blades 3–veined at the base, base subcordate to truncate; stipels 2, present adaxially at petiole apex; stylar column globose, subglobose, or obovoid. – **16. *P. verrucosa***
1. Leaf blades 1–veined at the base, base rounded to attenuate; stipels absent adaxially at petiole apex; stylar column slender-cylindrical, stout-cylindrical, depressed-subglobose, globose, or oblong-obovoid. – **(2)**

2. Adaxial basilaminar extrafloral nectaries 1–5 pairs; stylar columns slender-cylindrical or stout-cylindrical. – **(3)**
2. Adaxial basilaminar extrafloral nectaries 1 pair; stylar columns depressed-subglobose, globose, or oblong-obovoid. – **(6)**

3. Stylar columns slender-cylindrical, 2.5–4.8 mm long; stamens of one type: 15–25 sessile anthers on a globose receptacle; staminate nectaries absent. – **11. *P. loretensis***

3. Stylar columns stout-cylindrical, 1–2(3) mm long; stamens of two types: 6–12 sessile anthers on a globose receptacle and an outer whorl of ~4 stamens with filaments; staminate nectaries annular, 4–lobed or absent. – **(4)**

4. Adaxial basilaminar extrafloral nectaries 3–5 pairs; young shoots and petioles densely hirsute; leaf blades hirsute abaxially. – **13. *P. multiglandulosa***
4. Adaxial basilaminar extrafloral nectaries 1–2(3) pairs; young shoots and petioles puberulent; leaf blades sparsely puberulous, glabrate, or glabrous abaxially. – **(5)**

5. Scattered laminar extrafloral nectaries absent or rarely 1–8 near margin on abaxial leaf surface only; leaf blades chartaceous to coriaceous, mostly subcoriaceous, margins distinctly serrulate and often distinctly glandular; inflorescences 1–3 cm long (to 6 cm only in Colombia). – **14. *P. penninervia***
5. Scattered laminar extrafloral nectaries numerous, present on both leaf surfaces; leaf blades chartaceous, margins minutely serrulate, appearing undulate, never distinctly glandular; inflorescences 2–8 cm long. – **15. *P. supraglandulosa***

6. Stylar columns globose; stamens: 30–50 sessile anthers; staminate nectaries absent. – **9. *P. brachybotrya***
6. Stylar columns depressed-subglobose or oblong-obovoid; stamens: 16–30 sessile anthers, sometimes ~3 basal-most stamens on short filaments; staminate nectaries an annular ring with an uneven or undulate upper surface, sometimes 3–segmented or absent. – **(7)**

7. Stylar columns depressed-subglobose, 0.3–1 mm long; staminate sepals 3, often recurved at anthesis; stamens 16–20 usually sessile anthers, sometimes ~3 basal-most stamens with short filaments; petioles generally of uniform thickness and colour; fruit carpels usually unornamented, sometimes with a short tubercle. – **10. *P. brevistyla***
7. Stylar columns oblong-obovoid, 2.6–4.4 mm long; staminate sepals 3–4, incurved to spreading at anthesis; stamens 20–30 sessile anthers; petioles with thickened, often purplish pulvinus-like regions at base and apex; fruit carpels usually with a short tubercle, sometimes unornamented. – **12. *P. megastyla***

9. PLUKENETIA BRACHYBOTRYA Müll.Arg., Linnaea 34: 158. 1865. *Apodandra brachybotrya* (Müll.Arg.) J.F.Macbr., Publ. Field Columb. Mus., Bot. Ser., 13 (3a, 1): 117. 1951.—TYPE: PERU. Peruvia, "*Herb. Pavon*" likely *H. Ruiz & J. Pavón s.n.* (holotype: G-BOIS! [G00441995]; isotypes: G! [G00441996], G-DC! [G00313654]).

Plukenetia buchtienii Pax, Repert. Spec. Nov. Regni Veg. 7: 110. 1909. *Apodandra buchtienii* (Pax) Pax & K.Hoffm. in A.Engler (ed.), Pflanzenr. IV, 147, IX (Heft 68): 21. 1919.—TYPE: BOLIVIA. Charopompa bei Mapiri, 570 m, November 1907, *O. Buchtien 1962* (lectotype designated by Gillespie 1993: US00096450!; isolectotypes: NY00273178!, Z000019739! [online!]).

Notes—See Gillespie (1993) for species discussion.

Müller cited the protologue of *P. brachybotrya* as "hb. Pavon! in hb. Boiss." suggesting the holotype was a Pavon collection housed in Boissier's herbarium. Given that there is only one such sheet in Boissier's herbarium (now housed at G), we maintain that it is a holotype and not in need of lectotypification.

10. **Plukenetia brevistyla** Card.-McTeag. & L.J.Gillespie sp. nov.—TYPE: BRAZIL. Amazonas, km 320 on Manaus-Humaitá road, 16 September 1980, *S.R. Lowrie, B. Lowy, D. Coelho, M. Morreira, & V.M. de Souza 30* (holotype: MO2926690!; isotypes: CAN!, NY!, US!).

Similar to *P. brachybotrya* Müll.Arg. and *P. megastyla* Card.-McTeag. & L.J.Gillespie, but with a short depressed-subglobose stylar column, 0.3–1 mm long.

Monoecious slender lianas; stems erect or twining; older stems dull light grey-brown or straw-coloured, to ~5 mm in diam, striate or weakly striate, glabrate with patches of exfoliating cuticle; younger stems smooth to striate, sparsely puberulous. **Leaves** alternate, evergreen; stipules narrowly triangular to deltoid, 0.4–1.1 mm, persistent; petioles generally of uniform thickness and colour, 0.4–3 cm, glabrate except sparsely puberulous when young, often with exfoliating cuticle; blades simple, narrowly elliptic to elliptic or oblanceolate, sometimes oblong-elliptic, 4.2–19.9 x 1.9–7.4 cm, subcoriaceous, both surfaces glabrate except sparsely puberulous on abaxial major veins when very young, base cuneate or obtuse, sometimes attenuate, margins

remotely serrulate, apex usually cuspidate, sometimes acuminate, tip 0.4–1.4 cm; primary veins 1–nerved at the base, secondary veins brochidodromous, (4)5–6(7) on each side of the midrib, tertiary veins percurrent and somewhat reticulate near the midrib; stipels and glandular knobs absent adaxially at petiole apex; adaxial basilaminar extrafloral nectaries 2, narrowly oblong-elliptic to elliptic, oblong-obovate to broadly obovate, or round-deltoid (rarely circular or irregularly obovate), 1.3–3 x 0.4–1.8 mm; abaxial laminar extrafloral nectaries (2–)4–7(–14) per side, 0.2–0.6 mm in diam, near the margins on distal 2/3rds of the blade, adaxial laminar nectaries absent. **Inflorescences** axillary, bisexual racemose thyrses (often appearing unisexual due to protogynous development), 0.5–3.5 cm long, 1–2/axil; peduncles absent; axes glabrate to sparsely puberulous; staminate bracts ovate to broadly ovate, 0.5–0.9 mm, glabrate to puberulous (sometimes only on the central vein and margin); pistillate bracts ovate, 0.6–0.9 mm, glabrate to puberulous (sometimes only along the margin); staminate flowers numerous, distal, 1–2(3)/node in reduced and condensed cymules (rarely proximal cymules bisexual with a terminal pistillate flower); pistillate flowers 1, basal (may appear to arise from the leaf axil), or rarely 2–3, 1/node in proximal bisexual cymules, usually fallen or in fruit when staminate flowers are at or near anthesis. **Staminate flowers:** pedicel (2.5)9–13 mm at anthesis, sparsely to densely puberulous; bud subglobose to ovoid, apex rounded; sepals 3, often recurved at anthesis, ovate, 1.2–1.5 x 0.8–1 mm, apex obtuse, abaxial surface sparsely to moderately puberulous (sometimes only near the apex); receptacle globose, fully covered with anthers; nectary an extrastaminal annular ring, sometimes appearing lobed or 3–segmented, or not evident; androecium subglobose, 0.5–0.6 x 0.6–0.8 mm, stamens 16–20, filaments absent (rarely present, stout, < 0.5 mm long, on ~3 basal-most stamens), anther sacs ellipsoid, dehiscing longitudinally. **Pistillate flowers:** pedicel 3–12.5 mm, glabrate to sparsely puberulous; sepals 4, triangular to broadly triangular, sometimes lanceolate, 0.5–0.7 x 0.3–0.6 mm, glabrate to sparsely puberulous; ovary 4–lobed, 0.8–2 x 1.3–3.4 mm, lobes rounded and laterally compressed, glabrate except often puberulous along midline of the lobe, conspicuous wings or tubercles absent; styles 4, entirely connate into a depressed-subglobose column, wider than long, sometimes donut-shaped, with a central dimple when young, 0.3–1 x 0.6–1.8 mm, unlobed distally, glabrate; stigmas 4, pale yellow or golden-brown when dry, round-deltoid (appearing clover-shaped as a group), 0.3–0.5 mm each, smooth. **Fruits** 4–lobed capsules, 0.5–0.8 x 0.9–1.3 cm, surface verrucose, glabrate to very sparsely puberulous, each carpel subglobose, usually unornamented, sometimes with a short tubercle, 0.9–1.1 mm;

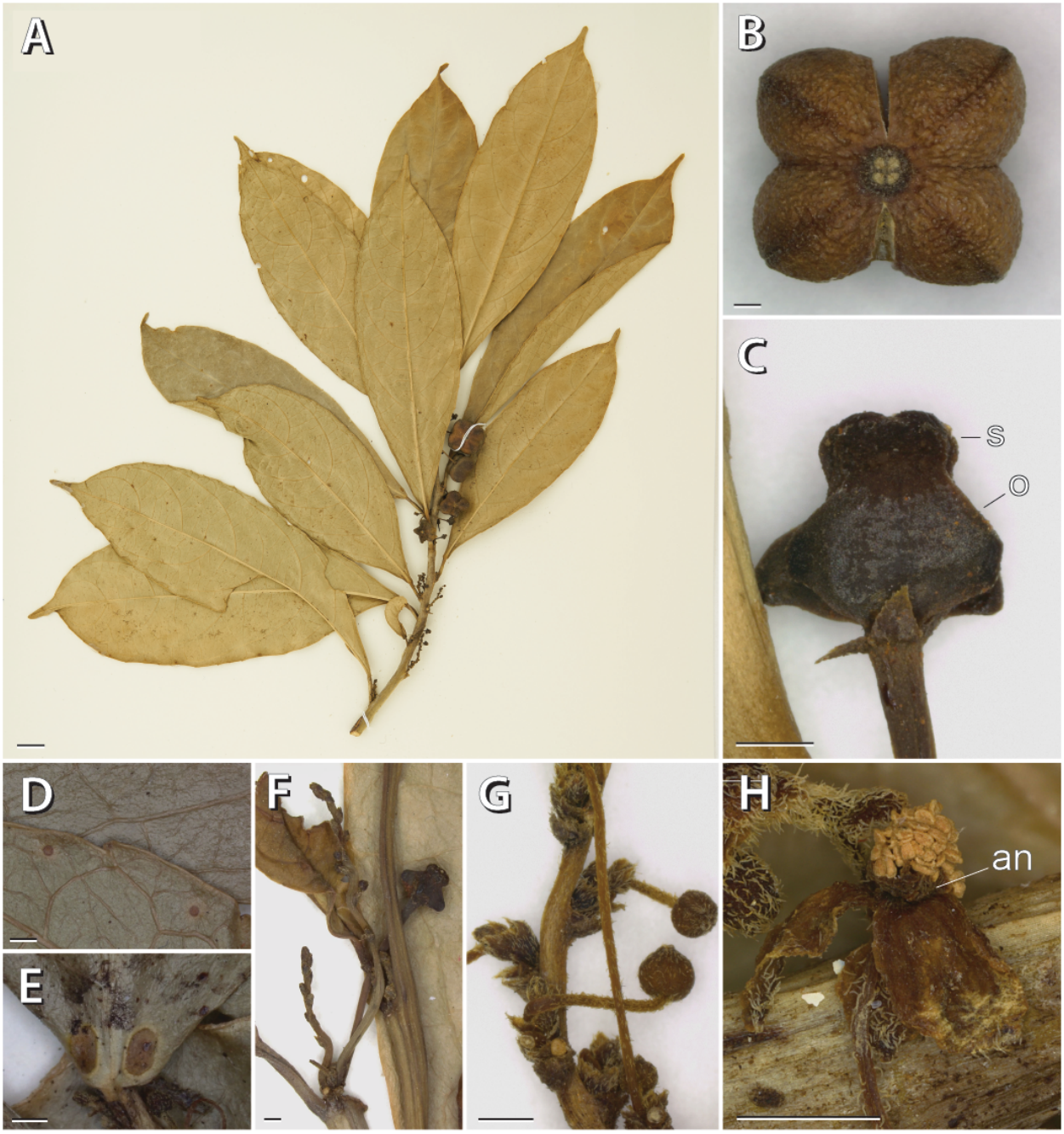


Figure 4.5 *Plukenetia brevistyla* sp. nov. (a) Branch with mature leaves, inflorescences, and fruit. (b) Capsule. (c) Pistillate flower. (d) Abaxial leaf blade with laminar extrafloral nectaries. (e) Adaxial leaf blade with basilaminar extrafloral nectaries. (f) Close-up of inflorescences. (g) Close-up of staminate cymules. (h) Staminate flower. Photos by W.Cardinal-McTeague. Source: (a, c, f–g) *Lowrie et al.* 30 (MO, NY); (b) *Silva et al. 1150* (NY); (d–e, h) *Oliveira 4513* (NY). (Abbreviations: an = annular nectary; o = ovary; s = style. Scale bars: [a] = 1 cm; [b–h] = 1 mm).

stylar columns persistent, depressed-subglobose with conspicuous clover-shaped stigmas, glabrate; pedicels 8.5–15 mm. **Seeds** not seen.

Pollen—Tricolpate, oblate spheroidal (P/E = 0.88–1.0), polar axis 29.1–32.7 µm, equatorial axis 29.1–37.1 µm; amb subcircular; colpi broad, margins uneven and jagged; tectum coarsely reticulate (voucher: *Lowrie et al. 6161* MO).

Etymology—The specific epithet is derived from *brevi-* (Latin, short or small) and *-styla* (Latin, -styled), and refers to the short stylar column that differentiates it from other neotropical species.

Distribution, Habitat, and Phenology—Known from the Rio Jarí region of northern Pará and a single collection from east-central Amazonas (Fig. 4.7), suggestive of a more widespread distribution in the central and eastern Amazon basin. Slender lianas climbing 3–6 m in terra firme rainforest, elevation not recorded (estimated < 100 m). Flowering and fruiting specimens collected from July to October.

Discussion—*Plukenetia brevistyla* is morphologically similar to *P. megastyla* but differs by having short depressed-subglobose stylar columns, 0.3–1 mm long (Fig. 4.5), petioles with more or less uniform thickness (sometimes darker in colour at the base and apex), and subcoriaceous leaf blades, compared to thick oblong-obovoid stylar columns, 2.6–4.4 mm long (Fig. 4.6), petioles with thickened, often purplish, pulvinus-like regions at the base and apex, and thick-chartaceous leaf blades in *P. megastyla*. Both species are distributed in the Amazon basin with *P. brevistyla* near the main stem of the Amazon River and *P. megastyla* along the southern boundary of the region (Fig. 4.7). The ETS phylogeny resolved *P. brevistyla* as an early diverging lineage in sect. *Penninerviae*, distinct from *P. megastyla* (Fig. 4.4). Both *P. brevistyla* and *P. megastyla* are vegetatively similar to *P. brachybotrya*, but the latter differs in its short massive globose stylar columns, 2–2.5 mm long (Fig. 4.3C), solitary staminate flowers (compared to condensed cymules), and primarily western Amazon distribution.

Staminate flowers of *P. brevistyla* show variation that falls within the typical range of sect. *Penninerviae*. All have sessile anthers densely packed on a globose or subglobose receptacle; however, variation was noted in the presence of a nectary and outer whorl of stamens with short filaments. An annular nectary was either consistently (*Lowrie et al. 30*) or variably present (*Silva 2392*). A third collection (*Oliveira 4513*) had a distinctly 3–lobed annular nectary

and an outer whorl of ~3 stamens with very short filaments. Additional collections are needed to understand this variation in staminate flower morphology.

The NY sheet of *Lowrie et al. 30* is unusual for having inflorescences with 2–3 pistillate flowers arising from the basal-most node, as well as for having proximal bisexual cymules that terminate with a pistillate flower. Remaining sheets of the same collection (CAN, MO, US) have a single pistillate flower per inflorescence, which is typical for the species.

The collection *Oliveira 3603* (NY01461552) co-occurs with *P. brevistyla* near Monte Dourado, Pará, but differs by having oblong-elliptic leaf blades and petioles with slightly thickened pulvinus-like regions at the base and apex, which is more typical of *P. megastyla*. There is insufficient floral material to confidently determine its identification, but we tentatively place this collection with *P. brevistyla* based on its location.

***Specimens Examined*—Brazil**.—PARÁ: Rio Jarí, Monte Dourado, ao lado do Campo de Aviação, *Oliveira 4513* (NY); Rio Jarí, Monte Dourado, *Silva 1105* (CAN, NY, US); Jari, Estrada do Munguba, km 10, *Silva 2392* (NY).

11. PLUKENETIA LORETENSIS Ule, Verh. Bot. Vereins Prov. Brandenburg 50: 81. 1908.
Apodandra loretensis (Ule) Pax & K.Hoffm. in A.Engler (ed.), Pflanzenr. IV, 147, IX (Heft 68): 21. 1919.—TYPE: PERU. Iquitos, April 1903, *E. Ule 6837* (holotype: B [destroyed]; lectotype designated here: L0137704 [online!]; isolectotypes: F0042476F! [fragment ex G], G00441994! [photo at F, neg. number 24572!], HBG515850 [online!], MG006665 [online!]).

Notes—See Gillespie (1993) for species discussion and Gillespie and Armbruster (1997) for species description. We report two additions to the distribution given in Gillespie (1993): Colombia (*Idrobo 9020*) and Pará, Brazil (*Ramos 1105*).

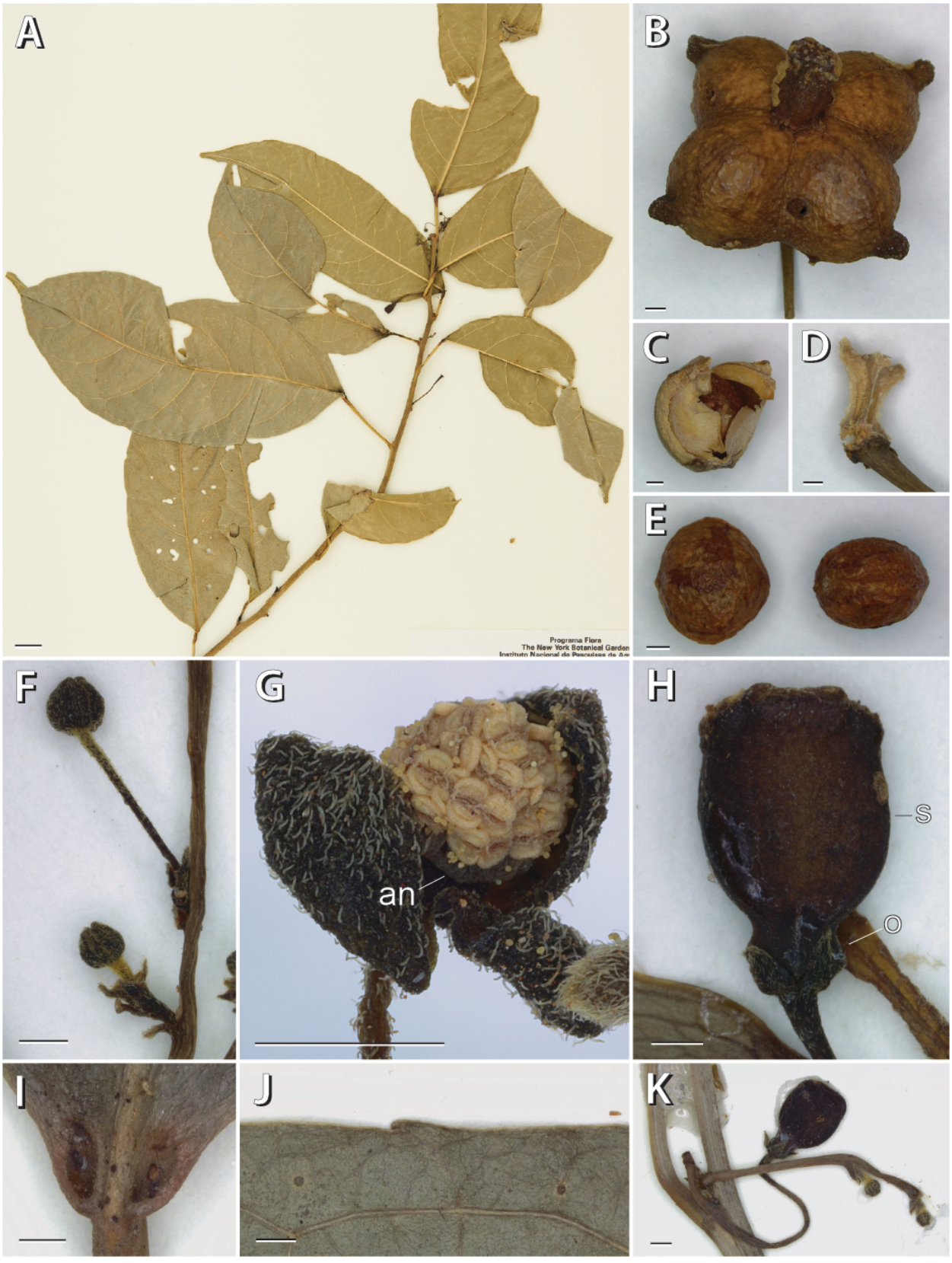
The holotype was destroyed at B so a new lectotype is designated from isotype material housed at L. The specimen we selected as lectotype has the most abundant and intact floral and vegetative material among the isotypes.

12. **Plukenetia megastyla** Card.-McTeag. & L.J.Gillespie sp. nov.—TYPE: BOLIVIA. Depto. Santa Cruz, Prov. Guarayos (formerly Prov. Nuflo de Chavez), 4 km N of Perseverencia,

[-14.683º, -62.800º], 275 m, 9 September 1990, *M. Nee 38682* (holotype: NY02286564!; isotype: MO3816603!).

Similar to *P. brachybotrya* Müll.Arg. and *P. brevistyla* Card.-McTeag. & L.J.Gillespie, but differs by having an oblong-obovoid stylar column with a truncate apex, 2.6–4.4 mm long.

Monoecious vines to slender lianas; stems erect or twining; older stems light brown and tan when dry, to ~5 mm in diam, striate, glabrate; younger stems smooth to striate, puberulous. **Leaves** alternate, evergreen; stipules narrowly triangular to deltoid, 0.3–1 mm, persistent; petioles with thickened, often purplish, pulvinus-like regions at the base and apex, entire petiole 0.7–3.5 cm, glabrate except sparsely puberulous when young; blades simple, narrowly elliptic to elliptic, oblong-elliptic, oblanceolate-elliptic, or obovate-elliptic (rarely ovate-elliptic), 7–19.8 x 1.8–7.5 cm, thick-chartaceous, both surfaces glabrate except sparsely puberulous on abaxial major veins when very young, base cuneate to obtuse, margins remotely serrulate, apex usually cuspidate, sometimes acuminate or attenuate, tip 0.4–1.6 cm; primary veins 1–nerved at the base, secondary veins weakly brochidodromous, 5–7(–9) on each side of the midrib, tertiary veins percurrent, sometimes reticulate towards the midrib; stipels and glandular knobs absent adaxially at petiole apex; adaxial basilaminar extrafloral nectaries 2, narrowly to broadly oblong-elliptic, obovate, round-deltoid, or sometimes irregularly obtriangular, rarely with an additional smaller nectary above, 1.0–2.4 x 0.4–1.7 mm; abaxial laminar extrafloral nectaries (3–)6–14 per side (rarely absent), near the margins on distal 9/10th to 4/5th of the blade, 0.2–0.7 mm in diam, adaxial laminar nectaries absent. **Inflorescences** axillary, bisexual racemose thyrses, 0.6–3 cm, 1(2)/axil; peduncles absent (rarely 0.2–0.3 mm); axes very sparsely to moderately puberulous throughout (sometimes more puberulous distally); staminate bracts ovate to broadly ovate, 0.4–1.1 mm, puberulous (sometimes only along the margins and at the apex); pistillate bracts ovate, sometimes deltoid, (0.3–)0.7–1 mm, sparsely to densely puberulous; staminate flowers numerous, distal, 1–3(4)/node in condensed cymules (appearing short racemose if 3–4 flowers); pistillate flowers 1, basal (may appear to arise from the leaf axil), usually fallen or in fruit when staminate flowers are at or near anthesis. **Staminate flowers:** pedicel 12.8–14.8 mm at anthesis, sparsely puberulous (densely when young); bud subglobose to ovoid, sometimes depressed and wider than long, apex rounded; sepals 3–4, incurved to spreading at anthesis, ovate to broadly ovate, 1–2.4 x 0.5–1.7 mm, apex obtuse, abaxial surface sparsely to moderately puberulous;


A
B
C
D
E
F
G
an
H
S
O
I
J
K

←

Figure 4.6 *Plukenetia megastyla* sp. nov. (a) Branch with mature leaves and inflorescences. (b) Capsule. (c) Capsule segment with seed. (d) Columella. (e) Seed, lateral view (left), ventral view (right). (f) Close-up of staminate cymules. (g) Staminate flower. (h) Pistillate flower. (i) Adaxial leaf blade with basilaminar extrafloral nectaries. (j) Abaxial leaf blade with laminar extrafloral nectaries. (k) Close-up of inflorescence. Photos by W.Cardinal-McTeague. Source: (a) G. *Silva et al. 6161* (NY); (b, i–j) *Plowman 8741* (NY); (c–e) *Ledezma et al. 921* (CAN); (f, h, k) *Nee 38682* (NY). (Abbreviations: an = annular nectary; o = ovary; s = style. Scale bars: [a] = 1 cm; [b–k] = 1 mm).

receptacle subglobose to globose, fully covered with anthers; nectary an extrastaminal annular ring, upper surface sometimes undulate or uneven; androecium subglobose, 0.6–0.9 x 0.8–1.3 mm, stamens 20–30, filaments absent, anther sacs ellipsoid, dehiscing longitudinally. **Pistillate flowers:** pedicel 12.9–24.7 mm, glabrate to sparsely puberulous; sepals 4, triangular, sometimes deltoid to broadly triangular, 0.5–1.2 x 0.4–0.7 mm, puberulous; ovary 4–lobed, 0.8–1.7 x 1–2 mm, lobes rounded and laterally compressed, glabrate except puberulous along midline of the lobe, conspicuous wings or horns absent; styles 4, entirely connate into an oblong-obovoid column, 2.6–4.4 x 2.6–3.7 mm, base constricted and 0.9–1.6 mm wide, apex truncate, with a central dimple when young, unlobed distally, glabrate; stigmas 4, light yellow-brown/tan or purple-red when dry, round-deltoid to circular, 0.8–1.1(2) mm each, smooth. **Fruits** 4–lobed capsules, 0.7–1.1 x 1–1.6 cm, surface irregularly verrucose, glabrous or glabrate, each carpel lobe subglobose, usually with a short tubercle 0.4–1.4 mm, sometimes unornamented; stylar columns persistent, oblong-obovoid, apex truncate and dilated; pedicels (12)25–35 mm long. **Seeds** broadly lenticular, laterally compressed, elliptic to circular in outline, 4.8–5.2 x 5–5.9 x 3.8–4.2 mm, surface light to golden brown, sometimes with dark brown irregular splotches, weakly rugulose; testa persistent.

Pollen—Tricolpate, oblate spheroidal (P/E = 0.88–1.1), polar axis 32.9–45 µm, equatorial axis 31–51 µm; amb subcircular; colpi broad, margins uneven and jagged; tectum coarsely reticulate (voucher: *Sperling et al. 6161* CAN).

Etymology—The specific epithet is derived from *mega-* (Greek, large or great) and *-styla* (Latin, -styled), and refers to the large stylar columns that differentiate this from similar species.

Distribution, Habitat, and Phenology—Known from two disjunct ranges in the southern Amazon basin: (i) Brazil, in southeastern Pará; and (ii) Bolivia, in eastern Beni and Santa Cruz, and Brazil, in western Mato Grosso (Fig. 4.7). Slender lianas climbing on trees in terra firme rainforest with high canopy, growing in primary or disturbed primary forest along trails or forest

edges, on flat lands or low hills from 140–738 m. Flowering and fruiting specimens collected from February to November in Pará and August to November in Mato Grosso and Bolivia.

Discussion—*Plukenetia megastyla* is morphologically similar to *P. brevistyla* but differs by having thick oblong-obovoid stylar columns, 2.6–4.4 mm long (Fig. 4.6), petioles with thickened, often purplish, pulvinus-like regions at the base and apex, and thick-chartaceous leaf blades, compared to short depressed-subglobose stylar columns, 0.3–1 mm long (Fig. 4.5), petioles with more or less uniform thickness (sometimes darker in colour at the base and apex), and subcoriaceous leaf blades in *P. brevistyla*. Additionally, the annular nectary of *P. megastyla* appears to be consistently present (Fig. 4.6G), whereas the nectary of *P. brevistyla* is variable and can be annular, 3–lobed, or not evident. Both species are distributed in the Amazon basin, with *P. megastyla* along the southern boundary of the region and *P. brevistyla* near the main stem of the Amazon River (Fig. 4.7). A previous molecular phylogeny based on two plastid (*matK*, *ndhF*) and five nuclear (ETS, ITS, *KEA1* introns 11 and 17, *TEB* exon 17) markers resolved *P. megastyla* (referred to as *P.* aff. *brachybotrya*; *Ledezma et al. 921*, *Sperling et al. 5873*, *6161*) in a strongly supported clade with *P. supraglandulosa* (Cardinal-McTeague *et al*., in review; summarized in Fig. 4.1). The ETS phylogeny of this study recovered *P. megastyla* in a poorly resolved polytomy within sect. *Penninerviae*, distinct from *P. brevistyla* (Fig. 4.4). Both *P. megastyla* and *P. brevistyla* are vegetatively similar to *P. brachybotrya*, but the latter differs in its short massive globose stylar columns, 2–2.5 mm long (Fig. 4.3C), solitary staminate flowers (compared to condensed cymules), and primarily western Amazon distribution.

Sperling et al. 5873 (NY1461553) is unusual for having an aberrant pistillate flower with a slender-cylindrical stylar column and dilated apex. A younger pistillate flower on the sheet appears to have the typical oblong-obovoid stylar column.

***Specimens Examined*—Bolivia**.—BENI: Prov. Iténez, Canton Mateguá, Campamento móvil Cerro Azul ubicado a 30 km de la Comunidad de Tiquin, [-13.816º, -62.761º], *Ledezma et al. 921* (CAN, MO6057037).—SANTA CRUZ: Prov. Velasco, Los Fierros, la senda hacia la meseta adentro bosque alto, [-14.558º, -60.861º], *Jardim et al. 3336* (MO04850338). **Brazil**.—MATO GROSSO: Mun. Vila Bela da Santíssima Trindade, 41 km NNW of Pontes e Lacerda on BR364 to Vilhena, [-14.950º, -59.583º], *Thomas et al. 4732* (NY010064912).—PARÁ, Mun. Conceição do Araguaia, range of low hills ca. 20 km west of Redenção, near São João and Troncamento Santa Teresa, approx. [-8.050º, -50.167º], *Plowman et al. 8741* (NY01461555);

Mun. Conceição do Araguaia, 100 km south of Redenção on road (PA-150) to Barreiras dos Campos, Fazenda Inajaporã between Rio Inajazinho and Rio Inajá, approx. [-8.750º, -50.417º], *Plowman et al. 8908* (NY01461551); Serra dos Carajás, 1–4 km along road from camp AZUL toward AMZA camp N-1, [-6.100º, -50.283º], *Sperling et al. 5873* (MO4249342, NY1461553); Serra dos Carajás, 2–10 km southeast of ferry crossing on Rio Itacaiúnas, [-5.917º, -50.483º], *Sperling et al. 6161* (CAN, MO4247783, NY, US).

Figure 4.7 Distribution map of our new species in sect. *Penninerviae*, *P. brevistyla* and *P. megastyla*, and members of the high elevation species complex in sect. *Plukenetia*, *P. carolis-vegae* subsp. *carolis-vegae*, *P. carolis-vegae* subsp. *nectarifera*, and *P. huayllabambana*. (Map data ©2018 Google, Imagery ©2018 NASA, TerraMetrics).

13. PLUKENETIA MULTIGLANDULOSA Jabl., Mem. New York Bot. Gard. 17 (1): 143. 1967.—TYPE: VENEZUELA. Amazonas: Cerro Parú, base of escarpment southward from camp 2 km, 1,800 m, 12 February 1951, *R.S. Cowan & J.J. Wurdack 31400* (holotype: NY00273179!; isotypes: F0057060F [online!], NY00273180!, US00096451!).

Notes—See Gillespie (1993) for species discussion.

14. PLUKENETIA PENNINERVIA Müll.Arg., Linnaea 34: 158. 1865.—TYPE: VENEZUELA. Near Biscaina, 3000 ft [~900 m], 1860, *A. Fendler 2412* (lectotype designated here: G-DC! [G00313655; photo at F!, neg. number 7110]; isolectotypes: GH00048692!, GOET003663 [online!], K000600747!, PH00030713 [online!]).

Plukenetia angustifolia Standl., Publ. Field Columb. Mus., Bot. Ser., 4 (8): 314. 1929.—TYPE: HONDURAS. Lancetilla Valley, near Tela, Department of Atlántida, 20–600 m, 6 December 1927–20 March 1928, *P.C. Standley 56708* (holotype: F! [V0057059F]; isotypes: A!, K!, US00096449!). [This name was published alongside two paratypes: *H. von Tuerckheim 11.372*, and *A. Schipp 156* (GH [HUH00048690; online!], K000600751 [online!]). The original description did not indicate the holotype but a specimen annotated as such was deposited at Standley's home herbarium at F.]

Notes—See Gillespie (1993) for species discussion.

Taxonomic Discussion—A recent phylogenetic study (Cardinal-McTeague *et al*., in review) resolved *P. penninervia* in two distinct clades (Fig 4.1). The first group, composed of accessions of typical *P. penninervia* from Mesoamerica and Central America, was strongly supported as sister to the remaining species of sect. *Penninerviae*. The second was a single accession from Chóco, Colombia (referred to as *P.* cf. *penninervia*), which was well supported as sister to *P. loretensis*. Specimens of *P.* cf. *penninervia* are distinguished by having 3–6 flowers per staminate cymule (appearing distinctly short racemose), as well as by having staminate flowers with an annular nectary and pistillate flowers with a stout-cylindrical stylar column and noticeably dilated apex. By comparison, typical *P. penninervia* has 1–3 staminate flowers per condensed cymule, lacks a staminate annular nectary, and has a stout-cylindrical stylar column

with an undilated apex (Gillespie 1993). *Plukenetia* cf. *penninervia* likely represents a new species and will be described in the officially published version of this treatment.

To complicate matters, there is evidence of a putative ancient hybridization event that resulted in the introgression of the ancestral plastid genome of *P.* cf. *penninervia* + *P. loretensis* into southern populations of *P. penninervia*. In nDNA analyses, *McPherson 8461* (Panama) forms a strongly supported clade with other Mesoamerican and Central American *P. penninervia*, whereas in cpDNA analyses it is resolved as sister to an intermixed clade of *P.* cf. *penninervia* and *P. loretensis* with moderate support (Cardinal-McTeague *et al*., in review; Fig. 3.S3). It is unclear if the Venezuelan populations of *P. penninervia* share the same putative hybridization event. Additionally, weakly supported incongruence between both individual genes and nuclear and plastid datasets are more frequent in sect. *Penninerviae* than in other sections (Cardinal-McTeague *et al*., in review), suggesting incomplete lineage sorting could be an additional obstacle to resolving the relationships of the *P. penninervia* complex.

Two syntypes were listed in the description of *P. penninervia*, *A. Fendler 2412* from Venezuela and a "*hb. Pavon*" collection from Mexico (Müller 1865). Ruiz and Pavón collected exclusively in Chile and Peru, suggesting the Mexican specimen came from a different collector. Madrid has five sheets of *P. penninervia* under *Sessé & Mociño 4212*, which is a likely candidate for the Mexican syntype. At the time of writing, it is not known if a specimen of *Sessé & Mociño 4212* is housed at Müller's working herbarium, G. Although the Mexican syntype is likely identified, we designate *A. Fendler 2412* as the lectotype given that it is explicitly cited and includes fruits and seeds associated with the species description (notably absent on all *Sessé & Mociño 4212* sheets). All sheets of *A. Fendler 2412* are of similar limited quality, so we lectotypify the specimen at G-DC since it was likely Müller's selected type. Remaining syntype: MEXICO. "*hb. Pavon*", likely *M. Sessé & J. Mociño 4212* (MA602300 [online!], MA602301 [online!], MA602302 [online!], MA602303 [online!], MA602304 [online!]).

15. PLUKENETIA SUPRAGLANDULOSA L.J.Gillespie, Syst. Bot. 18 (4): 598. 1993.—TYPE: FRENCH GUIANA. Sommet Tabulaire, zone centrale, versant occidental, ca. 40 km SE de Saül, 650 m, 27 August 1980, *J.-J. de Granville 3626* (holotype: US00433067!; isotypes: CAY!, U0002068!, US00646987!).

Notes—See Gillespie (1993) and Gillespie and Armbruster (1997) for species descriptions. Described originally from French Guiana and Amapá, Brazil (Gillespie 1993), a recent collection (*Clark & Hoffman 622* US) extends the range west to Guyana.

16. PLUKENETIA VERRUCOSA Sm., Nova Acta Regiae Soc. Sci. Upsal., ser. 2, 6: 4. 1799.—TYPE: SURINAME. *Linn. Collection of Surinam plants, No. 146* (holotype: LINN-HS 1489.1 [online!]).

Plukenetia volubilis L.f., Suppl. Pl. 421. 1781 publ. April 1782, nom. illeg.—TYPE: SURINAME. Herb. Alstroemeri 1848, *C.G. Dahlberg s.n.* (holotype: S09-18958 [online!]).

Plukenetia integrifolia Vahl, Eclog. Amer. 3: 43. 1807.—TYPE: GUYANA. Demerari, *J.P. von Rohr 86* (holotype: C10011347 [online!]).

Notes— See Gillespie (1993) and Gillespie and Armbruster (1997) for species descriptions.

Taxonomic Discussion—Linnaeus f. (1781 publ. 1782) was the first to suggest there was second species of *Plukenetia* from the Neotropics when he described the novel morphology of a new collection of *P. volubilis* from Surinam. However, there was no indication that Linnaeus f. meant for this to be a new species and it appears that he was expanding the concept of *P. volubilis* with the morphological variation found in Suriname (*Dalberg s.n.* S). Regardless, if we treat *P. volubilis* L.f. as a new species, it would be a homonym of *P. volubilis* L. and therefore illegitimate. As such, Smith (1799) was the first to to provide a legitimate name, *P. verrucosa* Sm., for the new species from Suriname. Smith (1799) recognized that the description of *P. volubilis* in Linnaeus f.'s *Supplementum Plantarum* (1781 publ. 1782) was associated with *P. verrucosa*, but by convention, type status would be conferred to the specimen listed in Smith's herbarium (*Linn. collection of Surinam plants No. 146* LINN-HS).

VI. PLUKENETIA sect. PLUKENETIA—TYPE: *Plukenetia volubilis* L.

Sajorium sect. *Pluknetia* Baillon, Étude Euphorb. 483. 1858, nom. illeg. TYPE: not designated

Plukenetia sect. *Cylindrophora* Müll.Arg., Linnaea 34: 157. 1865.—TYPE: *Plukenetia peruviana* Müll.Arg. [= *Plukenetia volubilis* L.]

Lianas or canopy lianas, stems slender to thick. **Leaf blades** 3–veined at the base. **Inflorescences** bisexual racemose thyrses (rarely racemes); pistillate flowers solitary at 1–2(3) basal-most nodes; staminate flowers (1–2)3–10/node in condensed cymules, glomerules, or moderately condensed cymules with conspicuous and irregularly branched cyme axes (rarely 1/node). **Staminate flowers:** receptacle conical or globose; nectaries of interstaminal slender-cylindrical, ligulate, or small or large irregularly shaped segments (absent in *P. volubilis*); stamens 12–40, densely packed; filaments short-conical or slender-cylindrical, 0.5–2 mm; pollen P = 48–60 μm, E = 53–69 μm, tectum foveolate. **Pistillate flowers:** styles (20–40)70–95% connate into a cylindrical column, 5.6–13.4(–35) mm. **Fruits** 4(–7)–lobed or subglobose-quadrangular capsules, dry, dehiscent or semi-dehiscent, (1.6–)2.5–10 cm in diam, or subglobose-quadrangular berries, fleshy, indehiscent, 3.5–11 cm in diam. **Seeds** broadly lenticular to lenticular (ovoid or triangular-ovoid in *P. polyadenia*), 10–34.3(49–56) x 8–27(33–37) x (2.5–9)14–36 mm ("medium", "large", "extra-large", or "maximum", sensu Cardinal-McTeague *et al*., in review).

Distributed in Mesoamerica, Central America, northwestern and Amazonian South America, and the Lesser Antilles.

Discussion—Section *Plukenetia* refers to subclade P3 (Fig. 4.1) and includes six species distributed in the Neotropics. The section is morphologically similar to sect. *Angostylidium* but differs in its neotropical distribution and having cylindrical stylar columns with free style arms that are more or less uniform in thickness or tapered and erect or spreading (Fig. 4.3G–K). Section *Plukenetia* is also distinct in having staminate flowers with long slender-cylindrical filaments and interstaminal nectaries (although with short-conical filaments and without nectaries in *P. volubilis*), and exhibiting the widest range of fruit type (dry or fleshy, and dehiscent, semi-dehiscent, or indehiscent) and seed size ("medium" to "maximum" sized).

Key to the species of *Plukenetia* sect. *Plukenetia*

1. Styles (9–)15–35 mm long; filaments short-conical, ~0.5 mm long; interstaminal nectaries absent. – **23. *P. volubilis***
1. Styles 4–12 mm long; filaments slender-cylindrical, 0.5–1.5(2) mm long; interstaminal nectaries present (small and sometimes overlooked in *P. stipellata*). – **(2)**

2. Fruits 1.6–3 cm diam, dry; interstaminal nectaries of small irregularly shaped segments; stipels 2 and glandular knob absent adaxially at petiole apex. – **22. *P. stipellata***

2. Fruits 2.5–11 cm diam, dry or fleshy; interstaminal nectaries slender-cylindrical, ligulate (strap-shaped), or large irregularly shaped segments; stipels 0–2 and glandular knob 0 or 1 adaxially at petiole apex. – **(3)**

3. Interstaminal nectaries of large irregularly shaped segments; leaves: lateral primary veins arching towards the margins ≤ 1/2 the length of the blade, stipels 1–2 and glandular knob absent adaxially at petiole apex; typically growing in montane rainforest (580–)1280–2440 m. – **(4)**

3. Interstaminal nectaries slender-cylindrical or ligulate (strap-shaped); leaves: lateral primary veins arching towards the apex ≥ 1/2 the length of the blade, stipels absent and glandular knob 1 adaxially at petiole apex; growing in lowland to pre-montane rainforest 0–1000 m (montane rainforest to 2100 m in *P. lehmanniana*). – **(6)**

4. Fruits 2.8–3.7 cm in diam, seeds "large", 19.2–19.6 x 17–18.5 x 13–15.7 mm; staminate sepals 5, usually of equal size but sometimes one much narrower, rarely 4; distributed in central and southern Peru (Cusco, Junín, Pasco). – **18a. *P. carolis-vegae*** subsp. ***nectarifera***

4. Fruits 4–10 cm in diam, seeds "extra-large", 27–50 x 25–40 x 15–35 mm; staminate sepals 4–5; distributed in northern Peru (Amazonas, Cajamarca). – **(5)**

5. Filaments 0.5–1 (perhaps to ~1.8) mm long; staminate cymules densely packed on inflorescence, axis not clearly visible. – **18b. *P. carolis-vegae*** subsp. ***carolis-vegae***

5. Filaments 0.1–0.5(1) mm long; staminate cymules loosely packed on inflorescence, axis clearly visible. – **19. *P. huayllabambana***

6. Styles 20–40% connate, column 3–6 mm long, free style arms 3–6 mm long; fruits squarish in profile; distributed north and west of the Andes in the Pacific coastal and montane regions of Colombia and Ecuador. – **20. *P. lehmanniana***

6. Styles 70–95% connate, column 3–14 mm long, free style arms 1–2.5(3) mm long; fruits ovoid in profile; distributed south and east of the Andes in the Amazon basin, the Guianas, and eastern Venezuela, or in southern Mexico. – **(7)**

7. Seeds broadly lenticular, "large" to "extra-large", 24–27 x 21–27 x 14–16 mm; fruits 5.8–7 cm in diam, capsules, at least semi-dehiscent; styles 12–14 mm long; distributed in southern Mexico. – **17. *P. carabiasiae***

7. Seeds ovoid, "maximum" sized, 49–56 x 33–37 x 30–36 mm; fruits 5–11 cm in diam, berries, indehiscent; styles 3–8 mm long; distributed in the northern Amazon basin, the Guianas, and eastern Venezuela. – **21. *P. polyadenia***

17. PLUKENETIA CARABIASIAE J.Jiménez Ram., Anales Inst. Biol. Univ. Nac. Autón. México, Bot. 64 (2): 55. 1993.—TYPE: MEXICO. Oaxaca: Distrito Tuxtepec, Municipio San Felipe Usila, Cerretera de Arroyo Tambor a Cerro Verde, [17.833°, -96.467°], 500 m, 9 April 1992, *J. Ismael Calzada 17733* (holotype: FCME; isotype: MEXU).

Notes—See Jiménez Ramírez (1993) for species description. This species was described after Gillespie (1993) and is known only from a few collections from the Tuxtepec district of Oaxaca, Mexico (*J. Ismael Calzada 16965* FCME, *17733* FCME, MEXU; *Meave et al. 1550* MO4786568!, MO6030707!). It appears to be morphologically similar to *P. polyadenia* but with smaller, at least semi-dehiscent fruit, "large" to "extra-large" broadly lenticular seeds, and longer styles. The staminate flower morphology of *P. carabiasiae* is not yet known.

18. PLUKENETIA CAROLIS-VEGAE Bussmann, Paniagua & C.Téllez, Econ. Bot. 67 (4): 388. 2013.—TYPE: PERU. Amazonas: Provincia de Rodríguez de Mendoza, Distrito Leymebamba [as "Limabamba"], finca of Sr. Rodríguez in Monte Allegre, [-6.586°, -77.532°], 1854 m, 19 August 2012, *R. Bussmann et al. 17132* (holotype: HAO (not seen); isotypes: MO6605774 [sheet 1 of 2; online!], MO6605775 [sheet 2 of 2; online!]; INBIAPERU [Instituto para el Desarrollo Local Sostenible y la Conservación Biológica y Cultural Andino-Amazónica, Trujillo]). [The two isotype sheets at MO could not be

found at the time of writing. They are mistakenly labelled as holotypes according to images on Tropicos.org]

Discussion—Together, *P. carolis-vegae* and *P. huayllabambana* form the high elevation species complex sister to *P. volubilis*. Members of the species complex grow in high elevation montane rainforest in the Andes, 1280–2440 m, and possess staminate flowers with large irregularly shaped interstaminal nectary segments, short styles partly connate into a cylindrical column usually < 10 mm long, larger fruits and seeds, ~3–10 cm diam and "large" or "extra-large", and 1–2 thick stipels at their petiole apex. They differ by having typically 5 staminate sepals, longer filaments (0.5)1–1.5(2) mm long, and inflorescences densely packed with staminate cymules in *P. carolis-vegae*, compared to 4 staminate sepals, shorter filaments 0.1–0.5(1) mm long, and loosely packed with staminate cymules in *P. huayllabambana*.

There are at least two subspecies within *P. carolis-vegae*. The species was first described based on a cultivated form (subsp. *carolis-vegae*), which we infer was derived from natural populations (subsp. *nectarifera* subsp. nov.), as is suggested by its very large fruits and seeds and only being found in cultivation (Bussmann *et al.*, 2013). Since the cultivated form differs in morphology and ecology from the natural populations, a new taxon appears warranted. Here, we choose the rank of subspecies because it follows the convention of other cultivated species, such as maize (*Zea mays* L. subsp. *mays* derived from natural populations of *Z. mays* subsp. *parviglumis* Iltis & J.F.Doebley) and manioc (*Manihot esculenta* Crantz subsp. *esculenta* derived from natural populations of *M. esculenta* subsp. *flabellifolia* (Pohl) Cif.). The subspecies differ by fruit and seed size, 4–10 cm diam and "extra-large" in subsp. *carolis-vegae* and 2.8–3.7 cm in diam and "large" in subsp. *nectarifera*, but are similar in other morphological aspects.

18a. PLUKENETIA CAROLIS-VEGAE Bussmann, Paniagua & C.Téllez subsp. CAROLIS-VEGAE.

Distribution, Habitat, and Phenology—Presently only known from its type collection from the Amazonas region of northern Peru (Fig 4.7). Twining lianas 4–6 m long, in cultivation at 1855 m. Flowering and fruiting specimen collected in August.

Discussion—We hypothesize that subsp. *carolis-vegae* is a cultivated form derived from natural populations of subsp. *nectarifera*. Subspecies *carolis-vegae* is only known from its type

specimen (*Bussmann et al. 17132*), which was collected from a farmer's field. It is distinguished by its larger fruits and seeds but overlaps with subsp. *nectarifera* in all other morphological characters. The original description of subsp. *carolis-vegae* characterized its filaments as relatively short, 0.5–1 mm long, and its staminate flowers as lacking nectaries, but the type illustration (Bussmann et al. 2013, fig. 1C) suggests its filaments can be as long as ~1.8 mm and that large irregularly shaped nectary segments are present. The MO isotype sheets of subsp. *carolis-vegae* could not be located at the time of writing, but are needed to verify these staminate morphology characters.

Subspecies *carolis-vegae* is very similar to *P. huayllabambana*. Both taxa are primarily distributed in the Rodríguez de Mendoza Province in northern Peru (Fig. 4.7), have large fruits 4–10 cm in diam with "extra-large" seeds, and appear to be fully or semi-domesticated with possible reintroductions into forests adjacent to farmland. Subspecies *carolis-vegae* differs by having longer filaments, 0.5–1 mm long (perhaps as long as ~1.8 mm) and inflorescences densely packed with staminate cymules. By comparison, *P. huayllabambana* has short filaments, 0.1–0.3 mm long in its type description (Bussmann et al. 2009) and 0.4–0.5(1) mm long in a specimen we independently measured (*Quipuscoa 381* MO5294603), and inflorescences loosely packed with staminate cymules.

18b. ***Plukenetia carolis-vegae*** subsp. ***nectarifera*** Card.-McTeag. & L.J.Gillespie subsp. nov.—
TYPE: To be determined in official publication.

Similar to *Plukenetia carolis-vegae* Bussmann, Paniagua & C.Téllez subsp. *carolis-vegae* but with smaller fruits and seeds and found in natural/non-cultivated populations from central and southern Peru.

Monoecious vines or lianas; stems erect. **Leaves** alternate, evergreen; stipules triangular, 1–2 mm, persistent; petioles 3–6.5 cm; blades simple, ovate to broadly ovate, 9–13.5 x 4.2–9 cm, chartaceous, adaxial surface puberulent with veins puberulous, abaxial surface puberulent with major veins pubescent, base truncate to subcordate, margins minutely serrulate, apex acuminate, tip 0.9–1.2 cm; primary veins 3–nerved at the base, secondary veins weakly brochidodromous, 4–6 originating from the midrib near the middle and apex, tertiary veins percurrent; stipels 1–2 adaxially at petiole apex, ligulate, 0.6–1 mm; adaxial basilaminar extrafloral nectaries 2,

narrowly-elliptic to subcircular, 1–2 x 0.5–1 mm; adaxial laminar extrafloral nectaries not absent or 1–2, 0.4–0.6 mm in diam, abaxial laminar nectaries absent or 1–2, remote, 0.2–0.3 mm in diam. **Inflorescences** axillary, bisexual racemose thyrses, 2–12 cm, 1/axil; peduncles 3.5–8 mm; staminate bracts narrowly triangular, 0.5–1 mm; pistillate bracts deltoid to broadly deltoid, 0.5–1 mm; staminate flowers numerous, distal, 3–7/node in condensed cymules; pistillate flowers 1–3, basal. **Staminate flowers:** pedicel 1–2.5 mm at anthesis, puberulous; bud subglobose, apex rounded; sepals (4)5, spreading to reflexed at anthesis, ovate, 2.5–3.5 x 1.5–2.5 mm, usually of equal size but sometimes 1 much narrower, apex acute, abaxial surface sparsely puberulous; receptacle convex to subglobose; androecium subglobose, 0.6–0.9 x 0.8–1.3 mm; interstaminal nectaries large irregularly shaped segments; stamens 25–35, filaments slender-cylindrical, 1–1.5(2) mm, anther sacs ellipsoid, dehiscing longitudinally. **Pistillate flowers:** pedicel 1.3–2.9 mm; sepals 4, narrowly triangular to lanceolate, 1.1–1.4 x 0.3–0.6 mm; ovary 4–lobed, 0.8–1.6 x 0.9–1.4 mm, lobes rounded and laterally compressed, conspicuous wings or horns absent; styles 4, partly connate into a cylindrical column, 6.6–15.6 x 1.2–2.2 mm, 4–lobed distally, free style lobes erect or spreading, 1.2–2.2 mm, sometimes slightly 2–fid; stigmas 4, smooth to short-papillose. **Fruits** 4–lobed capsules, 1.8–2.3 x 3–3.4 cm, surface microverrucose, glabrescent, each carpel lobe subglobose, carinate, keel 0.5–1.4 mm; stylar columns persistent, often broken off; pedicels 7–10 mm long. **Seeds** broadly lenticular, laterally compressed, elliptic-circular in outline, 17–19.3 x 17.5–18.5 x 13.4–15.9 mm, surface dull golden brown or light to dark brown, with irregular ridges and reticulations; testa persistent.

Etymology—The specific epithet is derived from *nectari-* (Latin, nectary) and *-fera* (Latin, -bearing), referring to the large irregularly shaped interstaminal nectary segments, which differentiates members of the high elevation species group (*P. carolis-vegae* subsp. *carolis-vegae*, *P. carolis-vegae* subsp. *nectarifera*, and *P. huayllabambana*) from all other species of *Plukenetia*.

Distribution, Habitat, and Phenology—Known from two disjunct populations from central (Junín and Pasco) and southern (Cusco) regions of Peru (Fig. 4.7). Twining vines and lianas climbing to 10 m in primary and secondary montane forest, sometimes partly disturbed and along roadsides, forest edges, or riparian forest, (580–)1280–2440 m. Flowering specimens collected in February, March and May through September, fruiting specimens from September through February.

Discussion—*P. carolis-vegae* subsp. *nectarifera* is morphologically similar to subsp. *carolis-vegae* but differs primarily by its smaller fruits and seeds. Subspecies *nectarifera* is also widespread in central and southern Peru and found in natural habitats, while subsp. *carolis-vegae* is narrowly distributed in northern Peru and so far only known in cultivation.

***Specimens Examined*—Peru.**—CUSCO: La Convención, Dist. Santa Ana, Poromate, 2118 m, [-12.917º, -72.783º], 16 June 2003, *Calatayud et al. 1470* (CAN, MO); La Convención, Dist. Vilcabamba, Espiritupampa, 1544 m, [-12.914º, -72.212º], 23 July 2004, *Calatayud et al. 2643* (CAN, MO); La Convención, Dist. Santa Ana, Tunquimayo, 2110 m, [-12.909º, -72.813º], 20 September 2004, *Calatayud et al. 2746* (MO04837341, MO04837342); La Convención, Distrito Santa Ana, Localidad Tunquimayo, 2007 m, [-12.907º, -72.821º], 19 October 2007, *Farfán et al. 1820* (CAN, MO); La Convención, Dist. Echarati, Monte Cristo, 1447 m, [-13.5º, -72.317º], 29 July 2005, *Huamantupa et al. 6445* (CAN, MO); Ca. 5 km N of Aguas Calientes (km. 116 on railroad), ca. 2000 m, 7 June 1977, *Solomon 3166* (MO2637789); La Convención, Dist. Occobamba, Santa Elena, 1995 m, [-12.45º, -72.167º], 24 February 2005, *Valenzuela et al. 5197* (CAN, MO); La Convención, Dist. Santa Ana, Bosque del Chuyapi, 2100 m, [-12.949º, -72.785º], 19 July 2006, *Valenzuela et al. 7297* (CAN, MO); La Convención, Dist. Ocobamba, Versalles, Santa Elena, 1942 m, [-12.783 º, -72.305 º], 24 November 2007, *Valenzuela et al. 10453* (CAN, MO); La Convención, Distrito Santa Ana, Tunqui Mayo, 1870 m, [-12.901º, -72.762º], 2 November 2007, *Vasquez et al. 33145* (MO).—JUNÍN: Yaupi, dept. Junin, 1600 m, 19 July 1961, *Woytkowski 6670* (MO2154072).—PASCO: Oxapampa, Distrito Huancabamba, Zona de Amortiguamiento del Parque Nacional Yanachaga-Chemillén, sector Tunqui, 1753 m, [-10.287º, -75.523º], 13 September 2007, *Castillo et al. 965* (CAN, MO); Cordillera Yanachaga, E of Oxapampa, lumber road to Chacas microwave station, 10 km E of main road, 2040-2110 m, [-10.583º, -75.25º], 2 March 1982, *Gentry & Smith 35906* (MO2983786); Oxapampa, Distrito Oxapampa, Parte alta de la quebrada San Luis, 2440 m, [-10.565º, -75.345º], 30 May 2007, *Monteagudo et al. 14125* (CAN, MO); Oxapampa, Distrito Huancabamba, Parque Nacional Yanachaga-Chemillén, Sector Tunqui, 1790 m, [-10.289º, -75.518º], 22 September 2007, *Monteagudo et al. 15253* (CAN, MO); Oxapampa, Dist. Villa Rica, Palma Centro Bocaz, camino a Alto Atarraz-Zona de Amortiguamiento, Parque Nacional Tanachaga-Chemillén, 1515 m, [-10.65º, -75.193º], 14 January 2005, *Ortiz et al. 191* (CAN, MO); Oxapampa, Distrito Villa Rica, Localidad Centro Bocaz, 1280 m, [-10.633º, -75.167º], 17 September 2003, *Perea et al. 282*

(CAN, MO); Oxapampa, Dist. Oxapampa, Abra Villa Rica, 2000 m, [-10.4º, -75.183º], 26 August 2005, *Rojas et al. 3863* (CAN, MO); Oxapampa, Distrito Pozuzo, Camino de Puente Victoria hacia la Comunidad Nativa Alto Lagarto, 700 m, [-10.1º, -75.433º], 29 September 2007, *Rojas et al. 4648* (MO6126857); Oxapampa, Dist. Palcazú, Comunidad Nativa de Alto Lagarto, 700 m, [-10.199º, -75.356º], 3 December 2007, *Rojas et al. 4827* (CAN, MO); Oxapampa, Dist. Pozuzo, Alto Lagarto a Puente Victoria, 700 m, [-10.1º, -75.433º], 28 December 2007, *Rojas & Ortiz 5152* (CAN, MO); Oxapampa, Dist. Palcazú, Comunidad nativa Alto Lagarto - Reserva Comunal Yanesha., 584 m, [-10.152º, -75.392º], 30 October 2009, *Rojas & Ortiz 7102* (CAN, MO); Oxapampa, Road from Oxapampa to San Alberto, 2250 m, [-10.533º, -75.35º], 20 June 2003, *van der Werff et al. 17532* (CAN, MO); Oxapampa, Along old road Oxapampa-Villa Rica, 2000 m, [-10.633º, -75.367º], 25 June 2003, *van der Werff et al., 17759* (CAN, MO), *17760* (CAN, MO); Oxapampa, Distrito Bermudez, Bosque Protección San Matias-San Carlos, Sector Unión-Shimaki, 1382 m, [-10.75º, -74.917º], 12 February 2003, *Vasquez et al. 27906* (MO5709828).

19. PLUKENETIA HUAYLLABAMBANA Bussmann, C.Téllez & A.Glenn, Nordic J. Bot. 27 (4): 313. 2009.—TYPE: PERU. Región Amazonas: Provincia de Rodríguez de Mendoza, El Cedro-Cruzpata, [-6.404°, -77.449°], 1676 m, 5 July 2008, *C. Téllez, C. Vega, and L. Cabrera 2* (holotype: INBIAPERU [Instituto para el Desarrollo Local Sostenible y la Conservación Biológica y Cultural Andino-Amazónica, Trujillo]; isotype: K001089689 [online!]). [The isotype was originally published as housed at MO (Bussmann *et al.*, 2009), but only one sheet of a different collection (*Téllez et al. 4* MO6116702!) incorrectly labeled as an isotype could be found. The isotype has since been found at Kew (*Téllez et al. 2* K001089689 [online!]) and it appears to be the isotype that was originally deposited at MO ("Ex Herbarium" MO6116701).]

Discussion—Together, *P. huayllabambana* and *P. carolis-vegae* form the high elevation species complex sister to *P. volubilis*. Members of the species complex grow in high elevation montane rainforest in the Andes, 1280–2440 m, and possess staminate flowers with large irregularly shaped interstaminal nectary segments, short styles partly connate into a cylindrical column usually < 10 mm long, and 1–2 thick stipels at their petiole apex. Members of the species

complex also have larger fruits and seeds, 4–10 cm diam and "extra-large" seeds in *P. huayllabambana* and *P. carolis-vegae* subsp. *carolis-vegae*, and 2.8–3.7 cm in diam and "large" seeds in *P. carolis-vegae* subsp. *nectarifera*.

This species is a putative hybrid between *P. carolis-vegae* subsp. *nectarifera* × *P. volubilis*, which is supported by the presence of a *P. volubilis* plastid genome in accessions of *P. huayllabambana* (Cardinal-McTeague *et al*., in review; Fig. 3.S3) and their intermediate staminate floral morphology. Both *P. huayllabambana* and *P. volubilis* have 4 staminate sepals and stamens with short filaments, 0.1–0.5(1) mm long, compared to typically 5 staminate sepals and longer filaments, (0.5)1–1.5(2) mm long, in *P. carolis-vegae*.

P. huayllabambana is distributed in the Amazonas and Cajamarca regions of northern Peru (Fig. 4.7). It is known from only a few collections, mostly in the Rodríguez de Mendoza Province, but is likely more common in cultivation. Its staminate flowers were originally described as having 5 (rarely 4) sepals and [nectary] disc absent (Bussmann *et al.*, 2009), but our observations found 4 staminate sepals, short filaments, 0.4–0.5(1) mm long, and the presence of large irregularly shaped interstaminal nectary segments (*Quipuscoa 381* MO5294603). The original type illustration only shows an immature staminate flower dissected from a bud, and the staminate flower at anthesis is aberrant and likely from a non-euphorb species (Bussmann et al. 2009, figs. 1J and 1H, respectively).

***Specimens Examined*—Peru.**—AMAZONAS: Chachapoyas District, Distrito Leymebamba, Los Chichos/San Lucas, 2150 m, [-6.701º, -77.586º], 19 June 2000, *Gruhn et al. 84* (MO6406258); Rodriguez de Mendoza District, Michina, 1608 m, [-6.378º, -77.527º], 5 June 2008, *Téllez et al. 4* (MO6116702).—CAJAMARCA: San Ignacio, Distrito San José de Lourdes, Villarrica, Nororiental del Marañón RENOM, 1200–1420 m, [-4.917º, -78.833º], 28 October 1995, *Quipuscoa 381* (MO5294603).

20. PLUKENETIA LEHMANNIANA (Pax & K.Hoffm.) Huft & L.J.Gillespie, Syst. Bot. 18 (4): 584. 1993. *Eleutherostigma lehmannianum* Pax & K.Hoffm. in A.Engler (ed.), Pflanzenr. IV, 147, IX (Heft 68): 11, t.3. 1919.—TYPE: COLOMBIA. Nariño, Ricaurte, 1000–1400 m, *F.C. Lehmann 5158* (holotype: B [destroyed]; lectotype designated by Gillespie 1993: K000600748!; isolectotype: K000600749!).

Plukenetia chapoensis Croizat, Caldasia 2: 431. 1944.—TYPE: COLOMBIA. Boyaca, region of Mt. Chapón, 3600 ft, 29 June 1932, *A.E. Lawrance 276* (holotype: A00048691!; isotypes: BM!, F [V0057061F; online!], F [V0057062F; online!], K000600750!, MO!, NY00579365!, US00386091!).

Notes—See Gillespie (1993) for species discussion.

21. PLUKENETIA POLYADENIA Müll.Arg. in Martius (ed.), Fl. Bras. 11 (2): 334. 1874. *Elaeophora polyadenia* (Müll.Arg.) Ducke, Arch. Jard. Bot. Rio de Janeiro 5: 146. 1930.—TYPE: PERU. Maynas, *E.F. Poeppig 2385* (*2585*?) (lectotype designated here: W [photo at F!, neg. number 32544]; isolectotypes: F! [fragment], G00441993! [fragment]; possible isotypes (labeled as *Poeppig 2585*): HAL0140191 [online!], NY00273181!).

Elaeophora abutifolia Ducke, Arch. Jard. Bot. Rio de Janeiro 4: 112. 1925. *Plukenetia abutifolia* (Ducke) Pax & K.Hoffm. in Engler & Prantl (eds.), Nat. Pflanzenfam., ed. 2, 19c: 141. 1931.—TYPE: BRAZIL. Breves Antonio Lemos, margem inundada do Rio Tajapuru, 5 May 1923, *A. Ducke s.n., RB 17893* (lectotype designated here: RB00283479 [online!]; isolectotypes: G00441992 [online!], IAN50621 [online!], K000600770 [online!], P00645482!, S [S-R-10641; online!], U0002066 [online!], US1442272 [online!]).

Notes—See Gillespie (1993) for species discussion and Gillespie and Armbruster (1997) for species description.

There is sufficient ambiguity over the type of *P. polyadenia* to warrant designating a lectotype. The specimen at Geneva, where Müller worked, is limited to a leaf and inflorescence fragment, suggesting the detailed type description was based on additional material. The specimen at Vienna is a likely candidate given that Poeppig's types are often housed there (Stafleu & Cowan, 1983), however we cannot assume that Müller based his description on that specimen. Since the holotype is unclear, we designate a lectotype based on the specimen from Vienna, which has the most abundant floral and vegetative material.

Of the three syntypes cited in the protologue of *Elaeophora abutifolia*, *A. Ducke s.n., RB 17893* has the best floral material. Overall, the Utrecht specimen (now housed at Leiden) most clearly demonstrates the pistillate morphology, but by convention we lectotypify the original

syntype housed in Ducke's herbarium at the Jardim Botânico do Rio de Janeiro. Remaining syntypes: BRAZIL. Pará: Belém Marutucú, capoeira úmida, 18 October 1923, *A. Ducke s.n., RB 17892* (RB00283480 [online!]); São Félix do Xingu Estrada da Victório ao Forte, 21 April 1924, *J.G. Kuhlmann 2051, RB 17895* (RB00724931 [online!], RB00287343 [online!], S [S-R-10855; online!], U0002067!).

22. PLUKENETIA STIPELLATA L.J.Gillespie, Syst. Bot. 18 (4): 588. 1993.—TYPE: COSTA RICA. Herederia: Finca La Selva, OTS Field Station on the Río Puerto Viejo, just E of junction with the Río Sarapiqui, [10.400°, -84.000°], 100 m, 13 September 1983, *L.J. Gillespie 413* (holotype: US00432786!; isotypes: CR!, MO!, NY00022625!; FAA preserved material at US!).

Notes—See Gillespie (1993) for species description.

23. PLUKENETIA VOLUBILIS L., Sp. Pl. 1 (2): 1192. 1753. *Sajorium volubile* (L.) Baill., Étude Euphorb. 483. 1858.—TYPE: WEST INDIES. Illustration t.13 (lower half) in Plumier, Nov. Pl. Amer. 1703.

Plukenetia peruviana Müll.Arg., Linnaea 34: 157. 1865.—SYNTYPES: PERU. hb. Pavon (G!, G-DC! [G00313656; photo at F!, neg. number 7111]); Prov. Maynas, *E.F. Poeppig 2110* (not seen).

Plukenetia macrostyla Ule, Verh. Bot. Vereins Prov. Brandenburg 50: 80. 1908.—TYPE: BRAZIL. Amazonas: Rio Juruá sup., September 1901, *E. Ule 5864* (holotype: B [destroyed]; lectotype designated here: G00441991! [photo at F!, neg. number 24573]; isolectotypes: F! [fragment], HBG515849 [online!], MG005766 [online!]).

Fragariopsis paxii Pittier, J. Wash. Acad. Sci. 19: 351. 1929.—TYPE: VENEZUELA. Hacienda Puerto La Cruz, Coastal Range, 1000 m, 28 August–4 September 1918, *H. Pittier 8109* (holotype: VEN6906 [online!]; isotype: GH00048014!, US00096452!).

Notes—See Gillespie (1993) for species discussion and Gillespie and Armbruster (1997) for species description.

Taxonomic Discussion—The *P. volubilis* species complex includes a main group of typical *P. volubilis*, plus two smaller groups that differ in morphology and phylogeny and may warrant their own taxonomic rank. The first is an open savanna species group found on the Moxos Plains of Bolivia. This group has broad leaf blades with subcordate bases similar to the typical moist or wet forest *P. volubilis*, but differs in its habitat, generally thicker leaf blades, and smaller seeds/fruit ("medium" compared to "large" sensu Cardinal-McTeague *et al*., in review). Accessions of the open savanna group (known only from *Nee 55162* MO and *Parada et al. 206* CAN, MO) form a strongly supported clade sister to the remaining moist or wet forest *P. volubilis* (Cardinal-McTeague *et al*., in review), suggesting this group could represent a distinct taxon on the basis of ecology and phylogeny. Floral morphology of the open savanna group is not yet known and additional collections are needed to verify if there are sufficient characters to warrant a new species.

The second is a mid-elevation species group from the Andes of Bolivia and Peru. Individuals in this group are found at higher elevations, (200)500–900(1800) m, than the typical moist or wet forest species group (usually to 500 m). The mid-elevation group is known from many collections across Bolivia (*Jaramillo et al. 1237* MO [208 m]; *Teran et al. 2006* MO [800 m]; *Teran et al. 2502* MO [1800 m]) and Peru (*Foster & Wachter 7404* MO [500–600 m]; *Gentry et al. 40071* MO [830–900 m]; *Huamán. 188* MO [290 m]; *Huamantupa et al. 3500* MO [780 m]; *Nùñez 13775* MO [640 m]; *Rojas et al. 4349* MO [800 m], *4593* MO [800 m], *5222* MO [600 m], *5525* MO [1500 m], *5665* MO [600 m], *8491* MO [500 m]; *Rojas & Ortiz 6726* MO [500 m]; *Valenzuela et al. 8531* MO [739 m], *9242* MO [1200 m], *10968* MO [1000 m], *12029* MO [375–635 m]; *van der Werff et al. 18445* MO [700 m]). These collections are differentiated by their narrower leaf blades and cuneate or truncate bases, compared to broader leaf blades with subcordate or cordate bases in typical *P. volubilis*. Other characters such as style length and fruit/seed size share the same breadth of variation observed in the typical *P. volubilis* group. Phylogenetic analyses suggest mid-elevation accessions (*Huamantupa et al. 3500, Jaramillo et al. 1237, Teran et al. 2502, Valenzuela et al. 8531*) form a strongly supported clade embedded within typical *P. volubilis* (Cardinal-McTeague *et al*., in review). Although the mid-elevation group appears to form a cohesive lineage within *P. volubilis*, it exhibits considerable geographical overlap and gradation of leaf shape characters with the typical lowland moist and

wet forest group, and might be better recognized as an ecological variant without taxonomic status.

Additional work is needed to clarify species group boundaries in the *P. volubilis* complex. Future studies should test the resolution of our proposed groups with improved taxon sampling across the geographic range of *P. volubilis*, specifically from the central and eastern Amazon basin, the Guianas, and the Lesser Antilles.

The presumed holotype of *P. macrostyla* was destroyed in Berlin. Although each of the isotypes are in good condition and demonstrate exemplary pistillate flowers, we lectotypify the specimen at Geneva since it includes staminate flowers near anthesis.

ROMANOA Trevis., Sagg. Algh. Coccot. 99. 1848. *Anabaena* A.Juss., Euphorb. Gen. 46, 1824, nom. rej., non. Bory ex Bornet & Flahault 1888. *Sajorium* sect. *Anabaena* (A.Juss.) Baill., Étud. Euphorb. 484. 1858. *Plukenetia* sect. *Anabaena* (A.Juss.) Müll.Arg., Linnaea 34: 158. 1865. *Anabaenella* Pax & K.Hoffm. in A.Engler (ed.), Pflanzenr. IV, 147, IX (Heft 68): 27. 1919.—TYPE: *Anabaena tamnoides* A.Juss. [= *Romanoa tamnoides* (A.Juss.) Radcl.-Sm.]

Taxonomic Discussion—The genus *Romanoa* Trev. 1848 is the earliest published replacement name for *Anabaena* A.Juss. 1824, nom. rej., after the well-known genus of cyanobacteria, *Anabaena* Bory ex Bornet & Flahault 1888, was conserved. The replacement name *Anabaenella* Pax & K.Hoffm. 1919 was used for much of the 20th century until the lesser-known *Romanoa* was re-discovered by Punt (1962) and nomenclaturally corrected by Radcliffe-Smith (1980). *Romanoa* was briefly treated as a synonym of *Plukenetia*, first as *Sajorium* sect. *Anabaena* (Baillon, 1858) then *Plukenetia* sect. *Anabaena* (Müller, 1865), but has often been regarded as a separate genus for having plesiomorphic 3–carpellate ovaries, compared to 4–carpellate in *Plukenetia*. Pollen morphology further supported the recognition of *Romanoa* (Gillespie, 1994b) and molecular phylogeny suggests they are distinct and form sister genera (Cardinal-McTeague & Gillespie, 2016).

1. ROMANOA TAMNOIDES (A.Juss.) Radcl.-Sm., Kew Bull. 34 (3): 589. 1980. *Anabaena tamnoides* A.Juss., Euphorb. Gen. 115, t.15. 1824. *Sajorium tamnoides* (A.Juss.) Baill.,

Étude. Euphorb. 484. 1858. *Plukenetia tamnoides* (A.Juss.) Müll.Arg., Linnaea 34: 158. 1865. *Anabaenella tamnoides* (A.Juss.) Pax & K.Hoffm. in A.Engler (ed.), Pflanzenr. IV, 147, IX (Heft 68): 27. 1919. *Anabaenella tamnoides* var. *genuina* Pax & K.Hoffm. in A.Engler (ed.), Pflanzenr. IV, 147, IX (Heft 68): 27. 1919, nom. illeg.—TYPE: BRAZIL. Brésil, 1819, *Leandro di Saccramento s.n.* (lectotype designated here: P00645483 [online!]).

Croton scandens Vell., Fl. Flumin. Icon. 10: t.72. 1831.— TYPE: illustration in t.72.

Plukenetia sinuata Ule, Bot. Jahrb. Syst. 42 (2-3): 217. 1908. *Anabaenella tamnoides* var. *sinuata* (Ule) Pax & K.Hoffm. in A.Engler (ed.), Pflanzenr. IV, 147, IX (Heft 68): 27. 1919. *Romanoa tamnoides* var. *sinuata* (Ule) Radcl.-Sm., Kew Bull. 34 (3): 589. 1980.—TYPE: BRAZIL. Bahia: Serra do São Ignacio, February 1907, *E. Ule 7445* (holotype: B [destroyed]; lectotype designated here: L0137705!; isolectotype: HBG515848 [online!]). [Both isotypes have abundant floral material; our selected lectotype also includes fruit.]

Taxonomic Discussion—Originally, two syntypes were designated for *Anabaena tamnoides,* "Species unica brasiliensis (in Herb. Mus. et J.)" (Jussieu 1824, p. 47), which refer to *Leandro di Saccramento s.n.* (P [P0064583]) and *herb. Dombey s.n.* (P-JU [P00678933]). Radcliffe-Smith (1980) inaccurately cited *Dombey s.n.* as the holotype of *A. tamnoides*, which did not qualify as a valid lectotype designation. Both sheets are relatively poor in quality, *herb. Dombey s.n.* with better preserved leaves and a 3–carpellate fruit, and *Leandro di Saccramento s.n.* with degraded leaves but more abundant floral material, including intact stylar columns. We select *Leandri di Saccramento s.n.* as the lectotype since it demonstrates the distinctive pistillate floral morphology of the species and genus. Remaining syntype: BRAZIL. Brésil, *herb. Dombey s.n.* (P-JU [P00678933; online!]).

Both syntype collections of *Anabaena tamnoides* were annotated as "*Plukenetia occidentalis*" Leandro (in mss.). Jussieu (1824) and Baillon (1858) listed *P. occidentalis* as a synonym of *A. tamnoides*; however, neither case resulted in valid publication of that name (ICN 2012, Art. 36.1; McNeill et al. 2012).

Appendix 4.1 List of species and vouchers used in the ETS phylogeny (Fig. 5.4), arranged by: ***Species*** Authority. COUNTRY. Collector & Number (Herbarium Code), GenBank accession number. Most sequences are from Cardinal-McTeague *et al*. (in review); a single new ETS sequence is published for *P. brevistyla* Card.-McTeag. & L.J.Gillespie sp. nov., indicated by an asterisk (*).

Ingroup: ***Plukenetia africana*** Sond. BOTSWANA. Palomoti 1086 (MO), MF502432; Pope et al. 834 (MO), MF502433. NAMIBIA. Bartsch 1859 (US), MF502431. ***P. ankaranensis*** L.J.Gillespie. MADAGASCAR. Gillespie 10697 (CAN), MF502434; Lees s.n. (CAN), MF502435. ***P. brachybotrya*** Müll.Arg. BOLIVIA. Araujo-M. et al. 1722 (MO), MF502437; Fuentes & Torrico 5398 (MO), MF502438; Seidel & Vaquiata 7733 (MO), MF502440. PERU. Acevedo-Rodriguez 14416 (NY), MF502436; Galiano et al. 6612 (MO), MF502439. ***P. brevistyla*** Card.-McTeag. & L.J.Gillespie sp. nov. BRAZIL. Lowrie et al. 30 (MO), MH119142*. ***P. carabiasiae*** J.Jiménez Ram. MEXICO. Meave et al. 1550 (MO), MF502441. ***P. carolis-vegae*** Bussmann, Paniagua & C.Téllez. PERU. Calatayud et al. 2643 (CAN), MF502448; Huamantupa et al. 6445 (CAN), MF502449; Monteagudo et al. 14125 (MO), MF502450; Monteagudo et al. 15252 (MO), MF502451; Rojas et al. 3863 (CAN), MF502453; Valenzuela et al. 5197 (CAN), MF502455; van der Werff et al. 17532 (CAN), MF502456; Vasquez et al. 33145 (MO), MF502457; Woytkowski 6670 (MO), MF502458. ***P. conophora*** Müll.Arg. DEMOCRATIC REPUBLIC OF THE CONGO. Hart 1621 (MO), MF502443. CAMEROON. Nemba & Thomas 434 (MO), MF502444. ***P. corniculata*** Sm. BANGLADESH. Huq & Haroon 10780 (MO), MF502446. ***P. decidua*** L.J.Gillespie. MADAGASCAR. Rakotomalaza 597 (CAN), MF502447. ***P. huayllabambana*** Bussmann, C.Téllez & A.Glenn. PERU. Quipuscoa 381 (MO), MF502452; Téllez et al. 4 (MO), MF502454. ***P. lehmanniana*** (Pax & K.Hoffm.) Huft & L.J.Gillespie. COLOMBIA. Silverstone-Sopkin & Giralda-Gesini 8019 (MO), MF502461. ECUADOR. Acevedo-Rodriguez & Daly 1658 (MO), MF502459; Clark 3953 (MO), MF502460; Zak & Jaramillo 3401 (MO), MF502462. ***P. loretensis*** Ule. BOLIVIA. Solomon 7972 (MO), MF502466. PERU. Grandez 19608 (AMAZ), MF502463; McDaniel & Rimachi 22451 (MO), MF502464; Rimachi 5122 (MO), MF502465; Vásquez & Jaramillo 3283 (MO), MF502467; Vasquez et al. 38069 (MO), MF502468. ***P. madagascarensis*** Leandri. MADAGASCAR. Andrianjafy 1648 (CAN), MF502469; Gillespie 4175 (CAN), MF502470; Villiers et al. 4899 (MO), MF502471. ***P. megastyla*** Card.-McTeag. & L.J.Gillespie sp. nov. BOLIVIA. Ledezma et al. 921 (CAN), MF502428. BRAZIL. Sperling et al. 5873 (MO), MF502429; Sperling et al. 6161 (CAN), MF502430. ***P. penninervia*** Müll.Arg. BELIZE. Atha et al. 1001 (MO), MF502472. GUATEMALA. Wallnöfer & Frisch 5996 (MO), MF502477. MEXICO. Carnevali & Duno 7594 (MO), MF502473; Martínez 10527 (MO), MF502474; Martinez 17705 (DAV), MF502475. PANAMA. McPherson 8461 (MO), MF502476. ***P.* cf. *penninervia***. COLOMBIA. Gentry 47799 (MO), MF502442. ***P. polyadenia*** Müll.Arg. GUYANA. Wurdack 5288 (US), MF502479. SMITHSONIAN GREENHOUSE EX FRENCH GUIANA. Gillespie 4314 (CAN), MF502478. ***P. serrata*** (Vell.) L.J.Gillespie. BRAZIL. Davidse 10480 (MO), MF502480; Forzza et al. 5328 (RB), MF502481; Goldenberg et al. 1424 (RB), MF502482; Peixoto et al. 4154 (MO), MF502483; Sartori & Pardo 11 (RB), MF502484; Thomas 10221 (NY), MF502485. ***P. stipellata*** L.J.Gillespie. COSTA RICA. Aguilar 8193 (MO), MF502486; Cardinal-McTeague 8 (CAN), MF502487; Liesner 3088 (MO), MF502489; Morales & Rojas 5342 (MO), MF502490. MEXICO. Refugio Cedillo Trigas 3510 (MO), MF502491; Ibarra Manriquez & Sinaca 1115 (MO), MF502488. NICARAGUA. Urbina 1155 (MO), MF502492. ***P. supraglandulosa*** L.J.Gillespie. SURINAME. Acevedo-Rodriguez 6022 (US), MF502493. ***P. verrucosa*** Sm. FRENCH GUIANA. Barrabe & Crozier 145 (US), MF502494. SURINAME. Herrera & Kaemar 10073

(CAN), MF502495; Hoffman 5917 (US), MF502496. ***P. volubilis*** L. BOLIVIA. Jaramillo et al. 1237 (MO), MF502501; Nee 55162 (MO), MF502502; Parada et al. 206 (CAN), MF502503; Teran et al. 2502 (MO), MF502504. ECUADOR. Burnham & Krings 1640 (MO), MF502498; Carrillo & Reyes 448 (MO), MF502499. SMITHSONIAN GREENHOUSE EX PERU. Wurdack s.n. (US), MF502506. PERU. Bell 93-546 (US), MF502497; Huamantupa 3500 (CAN), MF502500; Valenzuela 8531 (MO), MF502505.

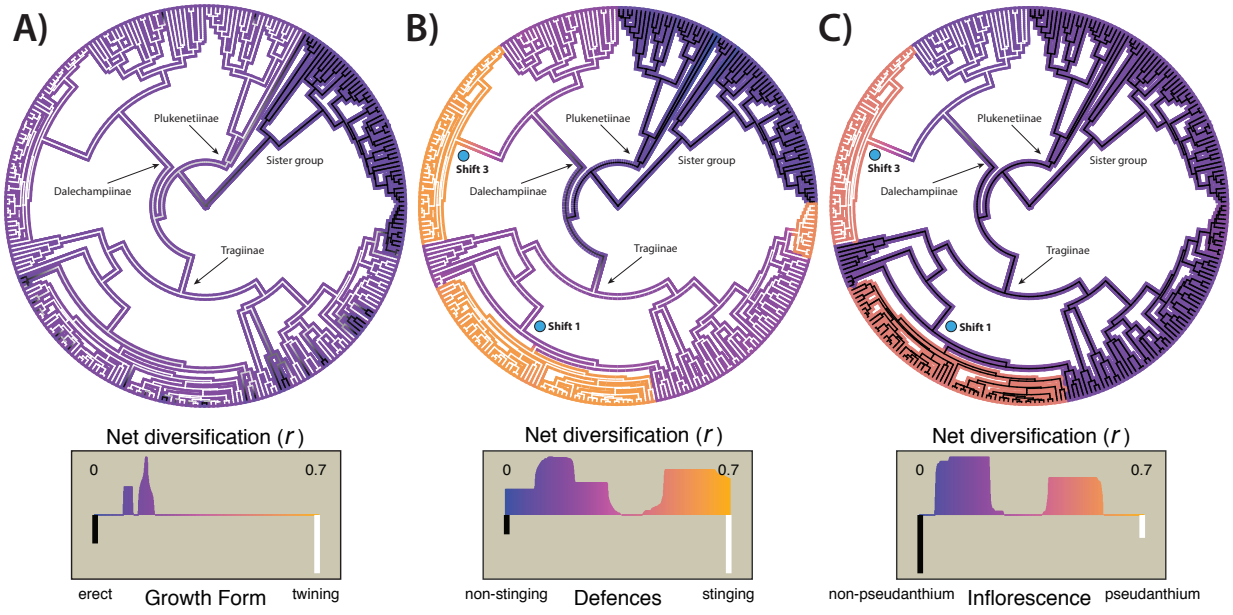
Outgroup: ***Haematostemon guianensis*** Sandwith. GUYANA. Wurdack 4350 (US), MF502427. ***Romanoa tamnoides*** (A.Juss.) Radcl.-Sm. BOLIVIA. Fuentes 1848 (MO), MF502507; Raes & Terceros 177 (MO), MF502508; Raes et al. 211 (MO), MF502509; PARAGUAY. Zardini & Chaparro 50824 (MO), MF502510.

Chapter 5
Biome Shifts, Not Innovative Traits or Biogeography, Drive Diversification Rates of Pantropical Euphorbiaceae Vines (Tribe Plukenetieae)*

Warren M. Cardinal-McTeague[1,2,3], Kenneth J. Wurdack[3] and Lynn J. Gillespie[1,2]

1. Department of Biology, University of Ottawa, Gendron Hall, Room 160, 30 Marie Curie, Ottawa, Ontario, K1N 6N5, Canada.
2. Research & Collections, Canadian Museum of Nature, PO Box 3443, Station D, Ottawa, Ontario, K1P 6P4, Canada.
3. Department of Botany, National Museum of Natural History, Smithsonian Institution, MRC-166, PO Box 37012, Washington, DC 20013-7012, USA.

* Manuscript in prep, to be submitted to New Phytologist.



Trait-dependent diversification analyses of Euphorbiaceae tribe Plukenetieae.

Abstract

- **Background:** Plant diversity is often enhanced by biotic traits (e.g., specialized growth forms, floral symmetry, and pollination syndromes) and/or abiotic processes (e.g., mountain building, aridification).While specific drivers of diversification are known, general patterns and processes still need to be established, including how multiple innovative traits interact.
- **Methods:** We studied the contribution of overlapping innovative traits, including twining growth form, stinging hair defences, and pseudanthial inflorescences (including floral symmetry and specialized pollination biology), as well as biogeographic history, on the diversification of pantropical Euphorbiaceae vines (tribe Plukenetieae). We developed a comprehensive time-calibrated phylogeny of Plukenetieae to use as a framework for estimating ancestral ranges and identifying time and trait-dependent shifts in diversification.
- **Results:** The early diverging relationships of the tribe were incongruent among datasets and remain unresolved. The pantropical distribution of Plukenetieae was best explained by multiple, periodic long-distance dispersals through the Oligocene to Pliocene. We detected 2–3 rate shifts during the Late Miocene cooling period, none of which were associated with innovative traits. We inferred that diversification rates increased in clades that shifted out of tropical moist forests into drier open habitats, sometimes spurred by habitat expansion.
- **Conclusions:** Contrary to our expectations, neither biogeography nor innovative traits were directly associated with diversification in the tribe. Our study highlights the importance of biome shifts and climate-mediated habitat expansions in diversification processes.

Background

A major goal of evolutionary biology is to understand the generation and maintenance of biodiversity. In recent years, botanical research has identified several processes associated with increased diversification, most notably the evolution of biotic traits such as specialized growth forms, plant defences, floral symmetry modifications, specialized pollination, alternative photosynthetic pathways (i.e., CAM and C_4), and whole genome duplications (WGD) (Farrell *et al.*, 1991; Gianoli, 2004; Sargent, 2004; Quezada & Gianoli, 2011; Silvestro *et al.*, 2013; Bouchenak-Khelladi *et al.*, 2014; Givnish *et al.*, 2014; Horn *et al.*, 2014; Weber & Agrawal, 2014; Breitkopf *et al.*, 2015; Tank *et al.*, 2015; Roalson & Roberts, 2016; Serrano-Serrano *et al.*, 2017). However, a growing number of abiotic drivers have also been described, such as arid landscapes, elevational gradients, and mountain building (Drummond, 2008; Loera *et al.*, 2012; Bouchenak-Khelladi *et al.*, 2014; Spriggs *et al.*, 2014; Reyes *et al.*, 2015; Sundue *et al.*, 2015; Lagomarsino *et al.*, 2016; Pérez-Escobar *et al.*, 2017a). It is becoming clear that these traits are context specific and not always associated with increased species diversity, and that often a combination of traits are driving diversification processes.

Recent studies found that diversification rates increased in many lineages by a combination of traits/processes, such as CAM photosynthesis and tank habit in bromeliads (Silvestro *et al.*, 2013), plant height and bird/bat pollination in cacti (Hernández-Hernández *et al.*, 2014), WGD and phytochemical innovation in plant defences in the mustard-oil order (Edger *et al.*, 2015), and elevational gradients and mountain building in concert with bird pollination and seed dispersal in Andean bellflowers (Lagomarsino *et al.*, 2016). Broad analyses of the diverse orchid and bromeliad families (Givnish *et al.*, 2014, 2015) recovered the important interaction between key landscapes like mountains and trait innovations such as epiphytism, specialized pollination, and distinctive morphology (i.e., orchid pollinia or bromeliad tank habit). The influence of the abiotic environment and biome shifts is also becoming apparent (Donoghue & Edwards, 2014) and may even be more significant than innovative traits. For example, in Proteaceae, a morphologically and ecologically diverse lineage in the southern hemisphere, species diversity is most strongly correlated with entry into arid Mediterranean biomes, rather than floral symmetry modifications (Reyes *et al.*, 2015). Biogeographic history can also be an important factor, although in many cases increased diversification appears to be associated with secondary biotic and abiotic drivers encountered in new regions (e.g., Richardson *et al.*, 2001;

Federman *et al.*, 2015). Moving forward, we should characterize regional and global patterns and processes of diversification across a greater number of groups, with the aim of identifying general trends that can serve as predictive frameworks for ecology and conservation research and the maintenance of present and future biodiversity.

Here, we aim to disentangle the interactive effects of overlapping key innovations within a single clade, using Euphorbiaceae vines (tribe Plukenetieae) as a study system. Plukenetieae (subfamily Acalyphoideae) is a pantropically distributed clade of ~330 species of morphologically diverse vines and lianas, less frequently subshrubs, and rarely shrubs and small trees (Cardinal-McTeague & Gillespie, 2016). The tribe includes three distinct groups, the non-stinging subtribe Plukenetiinae (~29 species), subtribe Dalechampiinae (~130 species) with unique pseudanthial inflorescences, and the species-rich stinging vine subtribe Tragiinae (~200 species). All of the subtribes are pantropically distributed and have their greatest diversity in the Neotropics, although Tragiinae stands out for its high species richness in Africa. Plukenetieae is unique in Euphorbiaceae for the combination of twining growth form and stinging hair defences. It also has diverse floral morphology, including inflorescences that range from racemes or racemose thyrses in Plukenetiinae and Tragiinae, to bisexual pseudanthia with large, bilaterally symmetrical involucral bracts in Dalechampiinae (Fig. 5.1). Dalechampiinae is also well known for its specialized pollination biology associated with resin, fragrance, and pollen pollinator reward systems, floral/pollinator defences, and shifts between specialized and generalized syndromes (Armbruster *et al.*, 2009 and references therein). Given its pantropical distribution and possession of multiple innovative traits, Plukenetieae presents an ideal group to examine the interaction of biogeography and potential drivers of diversification.

The goals of our study are to develop the first well-sampled, time-calibrated phylogeny of Plukenetieae and apply several statistical models to investigate patterns of biogeography and diversification in the group. Specifically, we test if three innovative traits, (i) twining habit, (ii) stinging hair defences, and (iii) bilaterally symmetrical pseudanthial inflorescences, contributed to increased diversification in Plukenetieae. We also reconstruct the tribe's biogeographic history to test if entry into new continents is associated with diversification, as well as identify patterns that resulted in pantropical distributions. We predict that there should be an additive effect of innovative traits, and that dispersal into new continents enhanced diversification through adaptation to new ecological niches. Our aim is to determine how innovative traits and

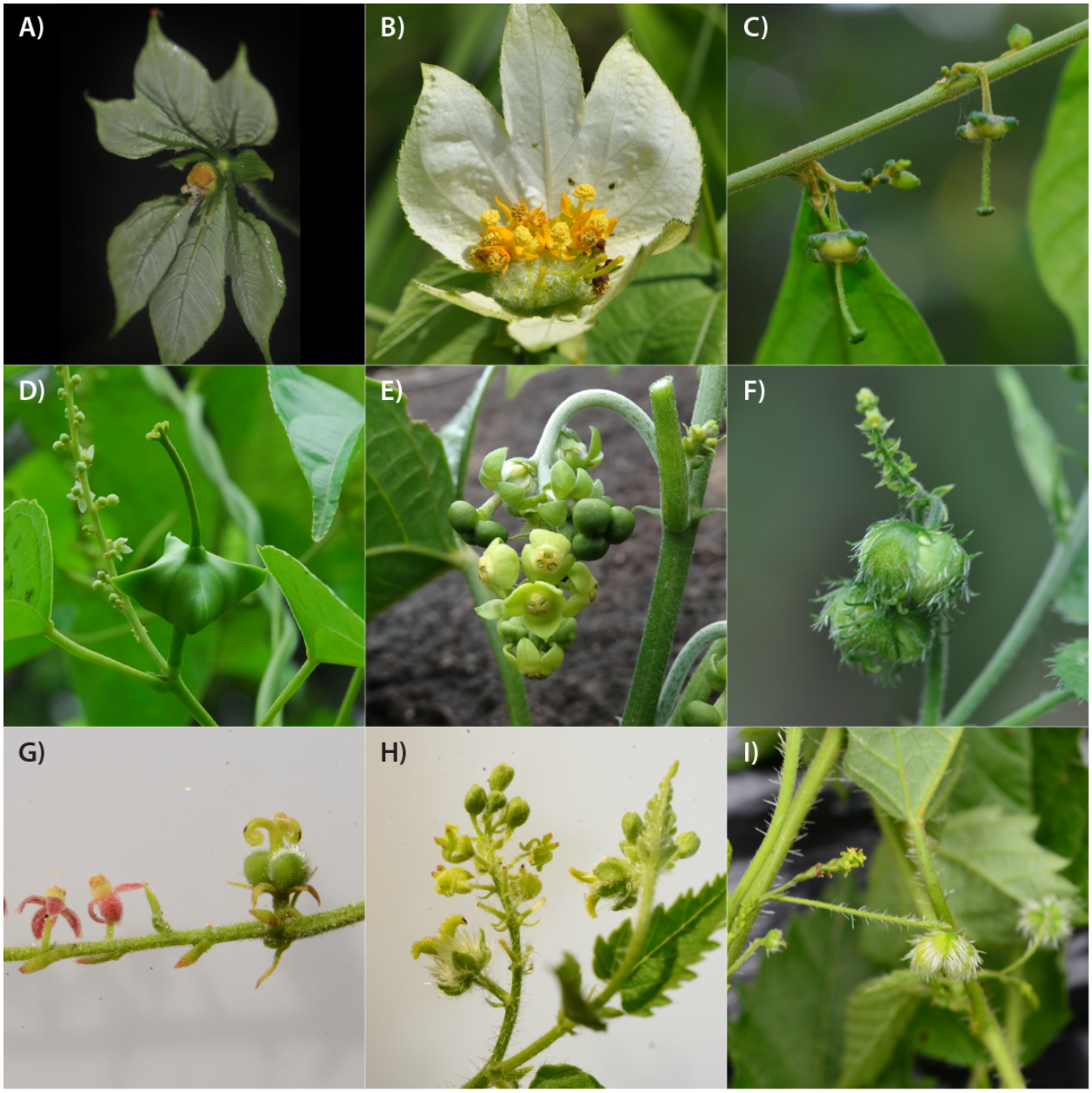


Figure 5.1 Inflorescence diversity of Euphorbiaceae vines (tribe Plukenetieae). Subtribe Dalechampiinae (a–b), bilaterally symmetrical pseudanthia composed of staminate and pistillate cymules, sometimes with resiniferous glands, subtended by two large often showy bracts: (a) *Dalechampia websteri* Armbr. (*Cardinal-McTeague 7*, CAN), (b) *D.* aff. *tamifolia* Lam. (*Gillespie et al. 10658*, CAN). Subtribes Plukenetiinae (c–d) and Tragiinae (e–i), racemose thyrses or racemes: (c) *Plukenetia loretensis* Ule (*Grandez 19608*, US), (d) *P. volubilis* L. (*Wurdack s.n.*, US), (e) *Megistostigma cordata* Merr. (*Davidson 12936*), (f) *Tragia furialis* Bojer ex Prain (*Gillespie et al. 10648*, CAN), (g) *T. urens* L. (*Wurdack s.n.*, US), (h) *T. urticifolia* Michx. (*Wurdack s.n.*, US), (i) *T. volubilis* L. (*Medeiros & Cardinal-McTeague 556*, R). Photographs by: W.Cardinal-McTeague (a–b, f, i), C.Davidson (e), K.Wurdack (c–d, g–h).

biogeography, either individually or combined, contributed to increased diversification in this lineage.

Methods

Taxon sampling and DNA methods

In total we sampled 289 terminals representing ~33% of the species diversity of Plukenetieae, ~10% of Dalechampiinae (13/130), ~79% of Plukenetiinae (23/29), ~35% of Tragiinae (73/206), including representatives of most genera except the rare monotypic Amazonian genera *Angostylis* Benth. and *Astrococcus* Benth. (Table 5.1; Table 5.S1). Our sampling included all or most taxonomic sections of *Plukenetia* and *Tragia* (Cardinal-McTeague and Gillespie, 2016), and representative lineages of *Dalechampia* (Armbruster *et al.*, 2009). We assembled molecular DNA datasets for four nuclear (ETS, ITS, *KEA1* intron 11, *TEB* exon 17) and two plastid (*matK*, *ndhF*) regions, with a total aligned length of 5,160 bp (Table 5.2). DNA extraction, amplification, sequencing methods, and alignment protocols followed Cardinal-McTeague *et al*. (in review). Outgroup sampling included nine species from Plukenetieae's sister clade, including Bernardieae, Caryodendreae, and *Avellanita* (Wurdack *et al.*, 2005; Cervantes *et al.*, 2016). Finally, four representatives from Acalyphoideae subclade A6 (Wurdack *et al.*, 2005) were used to root the phylogeny. All newly created sequences were deposited in GenBank and voucher information for all accessions is available in Table 5.S1.

Phylogenetic analysis and alternative topologies

Prior to analyzing combined datasets we searched for well supported incongruence among individual markers using maximum likelihood (ML) bootstrap analyses (Felsenstein, 1985b). We initiated 1000 rapid bootstrap replicates under the default model and search parameters in RAxML-HPC2 on XSEDE (Stamatakis, 2014; all XSEDE programs were run through the CIPRES Scientific Gateway v3.3, Miller *et al.*, 2010) and compared consensus trees for strongly supported conflicting relationships (interpreted as ≥ 85 ML bootstrap percentage; MLBP). Preliminary assessment revealed several moderately to strongly supported conflicting topologies along the backbone of the phylogeny (Fig. 5.S1), prompting us to examine the competing hypotheses in greater detail.

Table 5.1 Genera of tribe Plukenetieae and its sister group (including tribes Bernardieae, Caryodendreae, and *Avellanita*).

Taxon	Species number	Species sampled	Proportion sampled	Geographic distribution
Dalechampiinae				
Dalechampia L.	~130	13	0.1	Pantropical
Plukenetiinae				
Angostylis Benth.	1	0	0	Amazonian Brazil
Astrococcus Benth.	1	0	0	Amazonian Brazil and Amazonian Venezuela
Haematostemon Pax & K.Hoffm.	2	1	0.5	Guyana and Amazonian Venezuela
Plukenetia L.	~24	22	0.92	Pantropical
Romanoa Trevis.	1	1	1.0	Eastern Brazil, Paraguay, and Bolivia
Tragiinae				
Acidoton Sw.	5	2	0.4	Hispaniola, Jamaica
Bia Klotzsch	5	3	0.6	Costa Rica to South America
Cnesmone Blume	12	4	0.3	Southeast Asia
Ctenomeria Harv.	2	1	0.5	South Africa
Gitara Pax & K.Hoffm.	1	1	1.0	Central and northwestern South America
Megistostigma Hook.f.	5	2	0.4	Southeast Asia
Pachystylidium Pax & K.Hoffm.	1	1	1.0	Southeast Asia
Platygyna P.Mercier	7	2	0.29	Cuba
Sphaerostylis Baill.	2	1	0.5	Madagascar
Tragia L.	~160	71	0.44	Pantropical to warm temperate
Tragiella Pax & K.Hoffm.	4	3	0.75	Central and Southern Africa
Zuckertia Baill.	2	2	1.0	Mexico and Central America
Sister group				
Adenophaedra (Müll.Arg.) Müll.Arg.	4	1	0.25	Central and South America
Avellanita Phil.	1	0	0	Chile
Bernardia Houst. ex Mill.	~70	4	0.06	North to South America
Caryodendron H.Karst.	~6	4	0.67	Central and South America

We reconstructed four alternative topologies (ETS, ITS, *TEB*, and a cpDNA dataset composed of *matK* + *ndhF*), including the concatenated dataset with all six regions for comparison, using Bayesian inference (BI) through MRBAYES v3.2.3 on XSEDE (Ronquist *et al.*, 2012). Optimal models of nucleotide evolution were determined using JMODELTEST2 on XSEDE (Darriba *et al.*, 2012) under default settings. Four-chained Metropolis-coupled Markov chain Monte Carlo ($[MC]^3$) analyses were run for 5 million generations sampling every 1000^{th} generation, with the temperature parameter reduced to 0.005 promote swapping among chains. Runs were considered converged when the standard deviation of split frequencies were < 0.01, potential scale reduction factors were near 1.0, and effective sample size (ESS) values of each

parameter were > 200 (determined by TRACER v1.6; Rambaut *et al.*, 2014). The first 25% of each run (1,250 trees) was discarded as burn-in prior to summarizing posterior probabilities (PP) on the maximum clade credibility (MCC) tree. We calculated RAXML bootstrap values for the cpDNA and concatenated datasets using the same search parameters as above.

To assess whether the four alternative backbone topologies had significantly worse likelihood scores than the concatenated dataset we employed the approximately unbiased (AU) test (Shimodaira, 2002, 2004) in CONSEL v0.2 (Shimodaira & Hasegawa, 2001). We calculated the site likelihood inputs for CONSEL by constraining the backbone of the concatenated dataset to each of the alternative topologies (ETS, ITS, *TEB* exon 17, and cpDNA; Fig 5.2), using the best of two unpartitioned default ML searches from GARLI v2.01 (Zwickl, 2006).

Coalescence-based species tree estimation

To reconcile the discordant relationships among gene trees we applied coalescent-based methods, which were designed to address incomplete lineage sorting and hybridization commonly encountered in phylogenomic data (Degnan & Rosenberg, 2009). Although species tree estimation is more accurate with higher gene sampling (e.g., Mirarab & Warnow, 2015; Sayyari & Mirarab, 2016), these methods provide a useful comparison with our concatenated data.

First, we used quartet-based species tree estimation in ASTRAL-III v5.5.6 (Mirarab *et al.*, 2014; Mirarab & Warnow, 2015; Zhang *et al.*, 2018). The quartet method takes unrooted gene trees and searches for the topology with the most shared quartets (i.e., bifurcations before and after each branch), and it allows for incomplete taxon sampling between trees (Mirarab *et al.*, 2014). We searched for the maximum quartet support (MQS) species tree using the Bayesian MCC trees estimated from all six individual regions, and evaluated node uncertainty using 200 multilocus bootstrap replicates (Seo *et al.*, 2005; Seo, 2008; Mirarab *et al.*, 2014) starting from the pool of 1000 rapid bootstrap trees calculated for each region by RAXML.

Second, we used a Bayesian application of the multispecies-coalescence model (*BEAST; Heled & Drummond, 2010) in BEAST v1.8.4 on XSEDE (Drummond & Rambaut, 2007; Suchard & Rambaut, 2009). *BEAST is computationally demanding for large datasets so we conducted analyses on a reduced dataset with 177 terminals and no missing data (Table 5.2). *KEA1* intron 11 was excluded because it was missing sequence data for the entire Old World

Tragiinae clade (subclades T1–T3). We ran the multispecies coalescence model on unlinked site, clock, and tree models, and estimating each parameter throughout the run. Site models were specified by AIC and we implemented uncorrelated log-normal relaxed clock models, a Yule species tree prior (Yule, 1925), and piecewise constant population size prior. We ran four independent runs for 100 million generations, sampling every 10,000th generation, and evaluated parameter convergence and ESS using TRACER. Runs that converged were combined using LOGCOMBINER v1.8.4 on XSEDE after excluding the first 10% of trees as a burn-in (1,000 trees), and then summarized a MCC tree with mean node heights using TREEANNOTATOR v1.8.4 on XSEDE (Drummond & Rambaut, 2007).

Given that topological conflicts along the backbone could be attributed to phylogenetic uncertainty (see results), and that there was broad congruence among the rest of the tree, we conducted the remaining analyses on the concatenated dataset.

Divergence date estimation

Molecular dating was conducted in BEAST under similar parameters as the *BEAST analyses, except where noted. The concatenated dataset was reduced to one terminal per species or divergent lineage (Table 5.2), tree models were linked, and the tree prior was set to a birth-death process (Gernhard, 2008). No fossils are known from Plukenetieae or the outgroups sampled, so we applied secondary node calibrations based on a dating analysis of subfamily Acalyphoideae that used three reliable fossil calibrations (Cervantes *et al.*, 2016). The calibration priors were set as normal distributions approximated to the 95% highest posterior density (HPD) estimates from Cervantes *et al.* (2016). We constrained the root of the tree (the crown of subclades A6 + A7 + A8 sensu Wurdack *et al.*, 2005) to 59.29 Mya (SD = 5.3), the divergence node of Plukenetieae and its sister group to 45.39 Mya (SD = 5.3), and the crown age of Plukenetieae to 36.53 Mya (SD = 4.5). We initiated two independent MCMC runs for 50 million generations, sampling every 5,000 generations. After assessing for convergence, a 20% burn-in (2000 trees) was applied to each run and an MCC tree was annotated with common ancestor age heights.

Ancestral range estimation

We used ancestral range estimation (ARE) to reconstruct potential biogeographic histories of the tribe. Species distributions were gathered from herbarium labels, literature (Radcliffe-Smith,

1987, 2001; Webster, 2014; Levin & Gillespie, 2016), and online resources (GBIF, https://www.gbif.org; Tropicos, http://www.tropicos.org). In total, we categorized seven areas (Table 5.S2): (1) South America, including Central America and tropical Mesoamerica; (2) arid, subtropical, and warm temperate North America; (3) the Caribbean islands; (4) Africa, south of the Saharo-Arabian area; (5) Madagascar; (6) Southeast Asia, including tropical South Asia; and (7) Australasia. Inclusion of unoccupied areas like temperate North America, Eurasia and Antarctica, did not affect ARE (data not shown) and were excluded. Most species were coded by the area they occupied, except for Madagascan accessions of *Dalechampia*, which we coded to represent the full distribution of the Old World *Dalechampia* clade (Armbruster & Baldwin, 1998; Armbruster *et al.*, 2009).

ARE was conducted on the BEAST MCC chronogram using the maximum-likelihood approach of the BIOGEOBEARS package (Matzke, 2013, 2014) in R v3.3.2 (R Core Development Team, 2016). Our analyses used the dispersal-extinction-cladogenesis (DEC) model from LAGRANGE (Ree & Smith, 2008) in addition to the founder-event parameter (+J) from BIOGEOBEARS. The +J parameter allows for "jump speciation", in which cladogenic dispersals can occur outside of the parental area. For recent and ongoing discussion over criticisms of the DEC+J model, see Ree & Sanmartín (2018). Dispersal probabilities between pairs of areas were specified by distance for two timeslices (Table 5.S3) following similar analyses (Buerki *et al.*, 2011; Berger *et al.*, 2016; Cardinal-McTeague *et al.*, 2016; Givnish *et al.*, 2016), and ancestral ranges were permitted to occupy up to five areas. We conducted independent runs under the DEC and DEC+J models and used a likelihood-ratio test to determine the model of best fit.

Time-dependent diversification analysis

We used BAMM v2.5.0 (Rabosky, 2014) to estimate speciation (λ), extinction (μ), and net diversification (r) rates, and identify shifts in diversification rates across the branches of the Plukenetieae phylogeny. BAMM uses a reversible jump Markov chain Monte Carlo (rjMCMC) BI approach to explore numerous models of lineage diversification and detect rate heterogeneity. BAMM can also account for non-random taxon sampling by specifying the proportion of terminals sampled for each clade. The proportion of species we sampled for each designated terminal (Table 5.S4) was based on counts from published sources (Radcliffe-Smith, 2001;

Webster, 2014; Cardinal-McTeague & Gillespie, 2016; Levin & Gillespie, 2016) with updates based on personal knowledge of missing and undescribed diversity (Table 5.1).

We determined our initial priors using the R package BAMMTOOLS v2.1.6 (Rabosky *et al.*, 2014) and then ran two independent four-chained $(MC)^3$ runs for 5 million generations sampling every 2,500th step, with additional replicates exploring the effect of the Poisson rate prior equal to 10, 1, or 0.1 expected shifts. We tested for run convergence by plotting log likelihood scores following a 10% burn-in. After convergence was confirmed, we proceeded to analyze the first run and checked for sufficient ESS using the R package coda v0.19-1 (Plummer *et al.*, 2006). Using BAMMTOOLS, we calculated BayesFactors to determine the model of best fit, made phylorate plots of both the 95% credible set of rate shifts and best rate shift configuration, and plotted speciation, extinction, and net diversification rates through time.

There is debate over some theoretical and practical aspects of BAMM (Moore *et al.*, 2016; Rabosky *et al.*, 2017). Major criticisms include sensitivity of the posterior distribution to the Poisson rate prior and claims that BAMM's likelihood function is incorrect because it does not account for rate shifts that occurred on extinct lineages (Moore *et al.*, 2016). While most of these concerns have been refuted (Rabosky *et al.*, 2017), we acknowledge the limitations of current time-dependent diversification models and aim to confirm any results with alternative (i.e., trait-dependent) models.

Trait-dependent diversification analysis

We modeled the impact of trait evolution on Plukenetieae diversification by estimating net turnover (τ = speciation + extinction) and extinction fraction (ε = extinction/speciation) rates using the Hidden State Speciation and Extinction model (HiSSE; Beaulieu & O'Meara, 2016). The HiSSE model is an important improvement of the Binary State Speciation and Extinction model (BiSSE; Maddison *et al.*, 2007) because it accommodates the possibility that high diversification rates associated with a focal trait could be attributed to unmeasured/hidden traits within a subset of the clade (Beaulieu & O'Meara, 2016).

Our traits of interest, growth form (0 = erect, 1 = twining), plant defence (0 = non-stinging, 1 = stinging), and inflorescence type (0 = non-pseudanthium, 1 = pseudanthium), were coded using herbarium specimens and taxonomic treatments (Radcliffe-Smith, 1987, 2001; Webster & Armbruster, 1991; Gillespie, 1993, 2007; Gillespie & Armbruster, 1997; Cardinal-

McTeague & Gillespie, 2016; Levin & Gillespie, 2016) (Table 5.S5). To overcome incomplete taxon sampling in our phylogeny, we randomly attached terminals to notably species rich but under-sampled lineages of the BEAST MCC tree using the R package PHYTOOLS (Revell, 2012). Phylogenetic uncertainty introduced by this approach has a smaller effect on detecting rate shifts than inaccurate estimates from incomplete sampling (Kuhn *et al.*, 2011). We added 36 terminals to *Bernardia*, 33 to Tragiinae subclade T3, and in *Dalechampia*, 41 to the 5-armed clade and 24 to the 4-armed clade, such that their sampling proportion was equal to ~0.65, the highest sampling we achieved for a species-rich lineage (Tragiinae subclade T10).

Analyses were conducted in the R package HISSE (Beaulieu & O'Meara, 2016), where we explored 24 models of trait-dependent diversification (Table 5.S6) with a sampling fraction of 0.60. We included the BiSSE and HiSSE models, as well as two Character Independent (CID) null models, which explicitly assume binary traits evolved independently and that diversification rates vary across the tree (Beaulieu & O'Meara, 2016). We determined the model of best fit for each trait using the Akaike information criterion (Akaike, 1974) with transformed Akaike weights (w_i) (Wagenmakers & Farrell, 2004), and used the best fitting model to reconstruct ancestral states and net diversification rates using the 'MarginRecon' and 'plot.hisse.states' functions. Currently, there are performance issues with the 'MarginRecon' function under the CID-4 model, as such the second best model was implemented where indicated.

Results

Molecular phylogeny and incongruence

Our study produced 923 new DNA sequences (191 ETS, 84 ITS, 105 *KEA1*, 143 *TEB*, 195 *matK*, and 205 *ndhF*) resulting in a total aligned length of 5,160 bp. Dataset characteristics for each alignment are presented in Table 5.2.

The phylogenies recovered by individual nuclear marker and combined cpDNA datasets (Fig. 5.2) were generally consistent with previous phylogenetic studies (Cardinal-McTeague & Gillespie, 2016; Cervantes *et al.*, 2016). Although each dataset recovered moderately to strongly supported topological conflicts along the backbone of the phylogeny, species relationships within major clades were generally congruent (Fig. 5.S1). The backbone topology of the cpDNA dataset was most similar to that of the Concatenated dataset, which supports Dalechampiinae as sister to

Table 5.2 Molecular datasets and their characteristics.

Dataset	n ETS	n ITS	n *KEA1* intron 11	n *TEB* exon 17	cp *matK*
No. terminals	275	281	178	188	262
Marker recovery	0.95	0.97	0.62	0.65	0.91
Aligned length	538	873	481	579	1,835
Variable characters	428	520	340	446	613
Parsimony informative characters (%)	404 (75%)	472 (54%)	289 (60%)	361 (62%)	422 (23%)
Nucleotide model	GTR+I+G	GTR+I+G	GTR+I	GTR+G	GTR+I+G

Dataset	cp *ndhF*	cpDNA	Concatenated	*BEAST (no-gaps)	BEAST (one-term.)
No. terminals	275	279	289	177	147
Marker recovery	0.95	0.93	0.84	1.0	0.87
Aligned length	854	2,689	5,160	4,599	5,158
Variable characters	340	953	2,687	2,124	2,615
Parsimony informative characters (%)	245 (29%)	667 (25%)	2,193 (43%)	1,735 (38%)	1,905 (37%)
Nucleotide model	GTR+I+G	—	—	—	—
Combined regions	—	*matK*, *ndhF*	All six regions	Excluding *KEA1*	All six regions

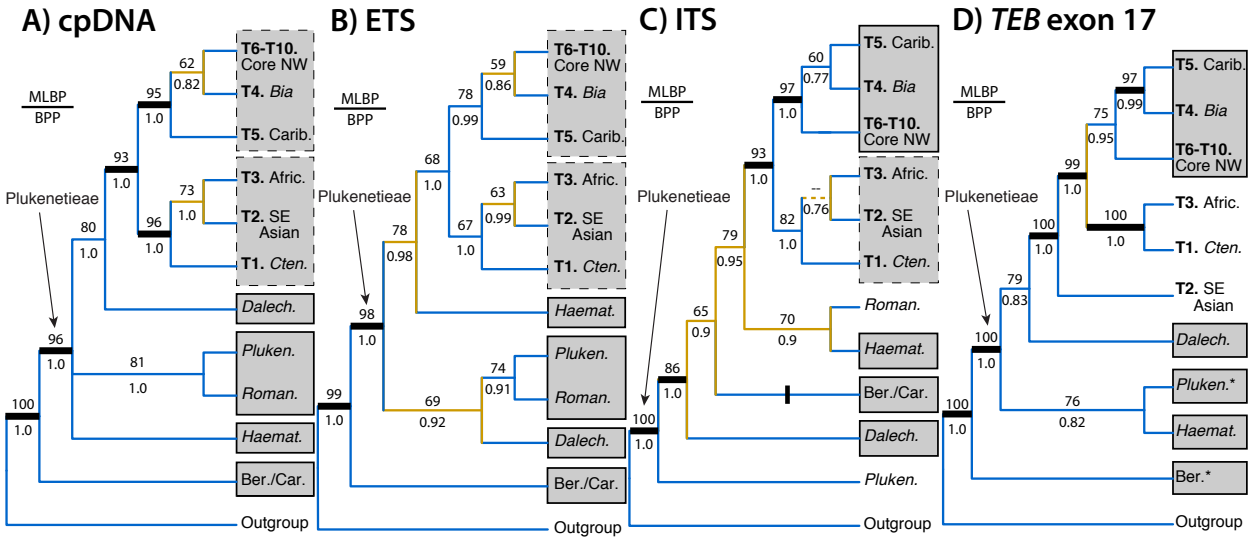


Figure 5.2 Summary cladograms of the alternative backbone topologies of Plukenetieae recovered from: (a) cpDNA; (b) ETS; (c) ITS; (d) *TEB* exon 17. Blue nodes are congruent with the Concatenated dataset (Fig. 5.3C), yellow nodes are incongruent; grey clades with solid edges are congruent, grey clades with dashed edges are partially congruent but have alternative branching patterns. Bold branches indicate strong support (≥ 85% maximum likelihood bootstrap percentage [MLBP] and ≥ 0.95 Bayesian posterior probability [BPP]); dashed branches indicate low support (< 60% MLBP and < 0.8 BPP). An asterisk (*) indicates a member of the clade was not sampled. See Fig. 5.3 for an explanation of abbreviations.

Tragiinae (*KEA1* had the same topology but is missing data for the entire Old World Tragiinae clade, subclades T1–T3). The ITS topology was most divergent and recovered *Plukenetia* and *Dalechampia* outside of the Plukenetieae + Bernardieae/Caryodendreae clade (Fig. 5.2C). There was elevated GC content in the ITS region of *Plukenetia* (63%) and *Dalechampia* (57%) compared to the rest of the tribe and our sampled outgroups (54%), suggesting DNA saturation and long-branch attraction are causing the irregular topology. The AU test revealed that among the competing backbone topologies only ITS was statistically different ($P < 0.0002$; Table 5.3).

Table 5.3 Results of the approximately unbiased (AU) test examining if enforced competing gene tree backbone topologies are significantly worse than the unconstrained Concatenated dataset.

Topology	-lnL	AU *P*-value	Conclusion
cpDNA	-62317.4	0.218	not reject
ETS	-62322.6	0.119	not reject
ITS	-62397.0	***0.0002***	**reject**
TEB exon 17	-62307.2	0.456	not reject
Unconstrained	-62303.5	0.748	not reject

Coalescence-based species tree methods identified nodes of uncertainty on the backbone of the Plukenetieae phylogeny (Fig. 5.3). The ASTRAL MQS species tree (Fig. 5.S2) recovered the same topology as ETS, but multilocus bootstrap support at the crown of the tribe is so low that it should be interpreted as a polytomy. The *BEAST MCC species tree (Fig. 5.S3) resolved a unique topology with low to moderate support along the backbone; the crown was also collapsed to a functional polytomy. Overall, our phylogenetic analyses suggest there is substantial uncertainty among the early diverging relationships of the tribe, but that none of the combined datasets are statistically different enough to be rejected (*P*-values > 0.05; Table 5.3). As such, we proceeded with the remaining analyses using the Concatenated dataset, recognizing that our results are conditional on this topology and that certain nodes may need to be reassessed if a strongly supported alternative topology is produced.

Divergence date estimation and biogeography

Results of the divergence date estimation analyses are presented in Fig. 5.S4. Based on our secondary calibrations from Cervantes et al. (2016), we estimate that the crown of Plukenetieae diverged in the late Eocene, *c.* 34.8 Mya (95% HPD: 29.5–40.1 Mya). Dalechampiinae and Tragiinae diverged shortly after in the Oligocene, *c.* 32.8 Mya (95% HPD: 27.7–38.2 Mya). The crown groups of major lineages diverged throughout the Miocene, starting with New World

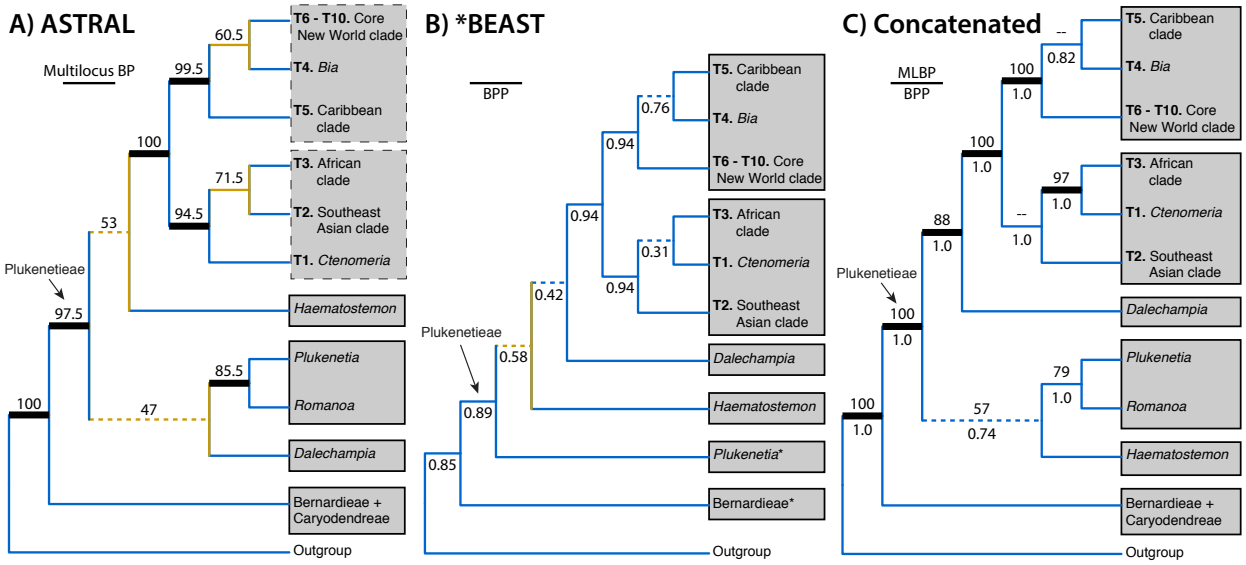
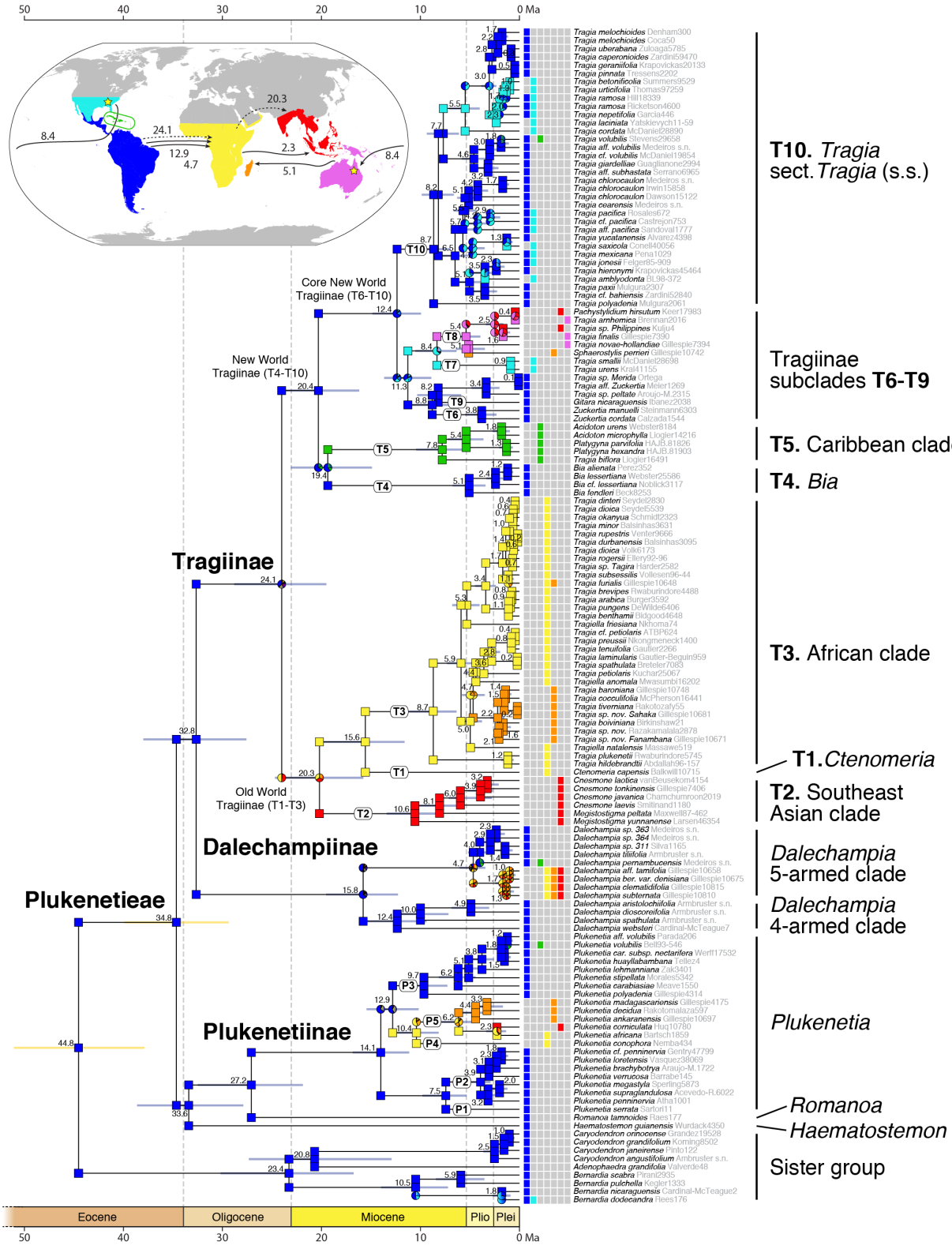


Figure 5.3 Summary cladograms of the alternative backbone topologies of Plukenetieae recovered from (a-b) coalescence-based and (c) total evidence methods. (a) ASTRAL maximum quartet support (MQS) species tree topology based on Bayesian maximum clade credibility (MCC) trees from all six regions. Node support is based on 200 multilocus bootstrap replicates. (b) *BEAST MCC topology based on the reduced five region, "no-gap" dataset (177 terminals). (c) Concatenated dataset Bayesian MCC topology. Blue nodes are congruent with the Concatenated dataset (Fig. 5.3C), yellow nodes are incongruent; grey clades with solid edges are congruent, grey clades with dashed edges are partially congruent but have alternative branching patterns. Bold branches indicate strong support (≥ 85% Multilocus bootstrap percentage [BP] or maximum likelihood BP [MLBP] and/or ≥ 0.95 Bayesian posterior probability [BPP]); dashed branches indicate low support (< 60% Multilocus BP or MLBP, and/or < 0.8 BPP).

Tragiinae subclades T4–T10 *c*. 20.4 Mya (95% HPD: 16.4–24.5 Mya), Old World Tragiinae subclades T1–T3 *c.* 20.3 Mya (95% HPD: 15.8–24.8 Mya), *Dalechampia c.* 15.8 Mya (95% HPD: 12.3–19.6 Mya), and *Plukenetia c.* 14.1 Mya (95% HPD: 11.2–16.9 Mya). Most species diversity appears to have accumulated during the Pliocene and Pleistocene (5.33–0.01 Mya).

ARE analyses under the DEC and DEC+J models recovered similar overall patterns (complete outputs are available in Fig. 5.S5). A likelihood-ratio suggests the DEC+J model significantly improved likelihood scores (DEC lnL = -196.43, DEC+J lnL = -189.85, $\chi^2(1) = 13.17$, $p < 0.0003$), as such only the DEC+J results are discussed (Fig. 5.4). The resulting parameters of the DEC+J model included: anagenetic dispersal rate (*d*) = 0.0182, extinction rate (*e*) = 1e-5, and cladogenetic dispersal rate (*j*) = 0.0273.

The ancestor of Plukenetieae was strongly supported as distributed in Central and South America (Fig. 5.4). There were four major long-distance dispersals (LDD) to the Old World, starting with the ancestor of the Old World Tragiinae subclades T1–T3 in the Oligocene (*c.* 24.1


50
40
30
20
10
0 Ma
8.4
24.1
12.9
4.7
20.3
2.3
5.1
8.4
Core New World
Tragiinae (T6-T10)
New World
Tragiinae (T4-T10)
Old World
Tragiinae (T1-T3)
Tragiinae
Dalechampiinae
Plukenetieae
Plukenetiinae
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
P1
P2
P3
P4
P5
Tragia melochioides Denham300
Tragia melochioides Coca50
Tragia uberabana Zuloaga5785
Tragia caperonioides Zardini59470
Tragia geraniifolia Krapovickas20133
Tragia pinnata Tressens2202
Tragia betonicifolia Summers9529
Tragia urticifolia Thomas97259
Tragia ramosa Hill18339
Tragia ramosa Ricketson4600
Tragia nepetifolia Garcia446
Tragia laciniata Yatskievych11-59
Tragia cordata McDaniel28890
Tragia volubilis Stevens29658
Tragia aff. volubilis Medeiros s.n.
Tragia cf. volubilis McDaniel19854
Tragia giardelliae Guaglianone2994
Tragia aff. subhastata Serrano6965
Tragia chlorocaulon Medeiros s.n.
Tragia chlorocaulon Irwin15858
Tragia chlorocaulon Dawson15122
Tragia cearensis Medeiros s.n.
Tragia pacifica Rosales672
Tragia cf. pacifica Castrejon753
Tragia aff. pacifica Sandoval1777
Tragia yucatanensis Alvarez4398
Tragia saxicola Conell40056
Tragia mexicana Pena1029
Tragia jonesii Felger85-909
Tragia hieronymi Krapovickas45464
Tragia amblyodonta BL98-372
Tragia paxii Mulgura2307
Tragia cf. bahiensis Zardini52840
Tragia polyadenia Mulgura2061
Pachystylidium hirsutum Keer17983
Tragia armhemica Brennan2016
Tragia sp. Philippines Kulju4
Tragia finalis Gillespie7390
Tragia novae-hollandiae Gillespie7394
Sphaerostylis parrieri Gillespie10742
Tragia smallii McDaniel28698
Tragia urens Krall1155
Tragia sp. Merida Ortega
Tragia aff. Zuckertia Meier1269
Tragia sp. peltate Aroujo-M.2315
Gitara nicaraguensis Ibanez2038
Zuckertia manuelli Steinmann6303
Zuckertia cordata Calzada1544
Acidoton urens Webster8184
Acidoton microphylla Liogier14216
Platygyna parvifolia HAJB.81826
Platygyna hexandra HAJB.81903
Tragia biflora Liogier16491
Bia alienata Perez352
Bia lessertiana Webster25586
Bia cf. lessertiana Noblick3117
Bia fendleri Beck8253
Tragia dinteri Seydel2830
Tragia dioica Seydel5539
Tragia okanyua Schmid2323
Tragia minor Balsinhas3631
Tragia rupestris Venter9666
Tragia durbanensis Balsinhas3095
Tragia dioica Volk6173
Tragia rogersii Ellery92-96
Tragia sp. Tagira Harder2582
Tragia subsessilis Vollesen96-44
Tragia funalis Gillespie10648
Tragia brevipes Rwaburindore4488
Tragia arabica Burger3592
Tragia pungens DeWilde6406
Tragia benthamii Bidgood4648
Tragiella lnesiana Nkhoma74
Tragia cf. petiolaris ATBP624
Tragia preussii Nkongmeneck1400
Tragia lenulifolia Gaultier2266
Tragia laminularis Gautier-Beguin959
Tragia spathulata Breteler7083
Tragia petiolaris Kuchar25067
Tragiella anomala Mwasumbi16202
Tragia baroniana Gillespie10748
Tragia cocculifolia McPherson16441
Tragia liverniana Rakotozafy55
Tragia sp. nov. Sahaka Gillespie10681
Tragia boiviniana Birkinshaw21
Tragia sp. nov. Razakamalala2878
Tragia sp. nov. Fanambana Gillespie10671
Tragiella natalensis Massawe519
Tragia plukenetii Rwaburindore5745
Tragia hildebrandtii Abdallah96-157
Ctenomeria capensis Balkwill10715
Cnesmone laotica vanBeusekom4154
Cnesmone tonkinensis Gillespie7406
Cnesmone javanica Chamchumroon2019
Cnesmone laevis Smitinand1180
Megistostigma peltata Maxwell87-462
Megistostigma yunnanense Larsen46354
Dalechampia sp. 363 Medeiros s.n.
Dalechampia sp. 364 Medeiros s.n.
Dalechampia sp. 311 Silva1165
Dalechampia tiliifolia Armbruster s.n.
Dalechampia pernambucensis Medeiros s.n.
Dalechampia aff. lamiifolia Gillespie10658
Dalechampia ber. var. denisiana Gillespie10675
Dalechampia clematidifolia Gillespie10815
Dalechampia subternata Gillespie10810
Dalechampia ariifolia Armbruster s.n.
Dalechampia dioscoreifolia Armbruster s.n.
Dalechampia spathulata Armbruster s.n.
Dalechampia websteri Cardinal-McTeague7
Plukenetia aff. volubilis Parada206
Plukenetia volubilis Bell93-546
Plukenetia car. subsp. nectarifera Werff17532
Plukenetia huayllabambana Tellez4
Plukenetia lehmanniana Zak3401
Plukenetia stipellata Morales5342
Plukenetia carabiasiae Meave1550
Plukenetia polyadena Gillespie4314
Plukenetia madagascariensis Gillespie4175
Plukenetia decidua Rakotomalaza597
Plukenetia ankaranensis Gillespie10697
Plukenetia corniculata Hug10780
Plukenetia africana Bartsch1859
Plukenetia conophora Nemba434
Plukenetia cf. penninervia Gentry47799
Plukenetia loretensis Vasquez38069
Plukenetia brachybotrya Araujo-M.1722
Plukenetia verrucosa Barrabe145
Plukenetia megastyla Sperling5873
Plukenetia supragladulosa Acevedo-R.6022
Plukenetia penninervia Atha1001
Plukenetia serrata Sartori11
Romanoa tamnoides Raes177
Haematostemon guianensis Wurdack4350
Caryodendron orinocense Grandez19528
Caryodendron grandifolium Koming8502
Caryodendron janeirense Pinto122
Caryodendron angustifolium Armbruster s.n.
Adenophaedra grandifolia Valverde48
Bernardia scabra Pirani2935
Bernardia pulchella Kogler1333
Bernardia nicaraguensis Cardinal-McTeague2
Bernardia dodecandra Rees176
Eocene
Oligocene
Miocene
Plio
Plei
T10. Tragia sect. Tragia (s.s.)
Tragiinae subclades T6-T9
T5. Caribbean clade
T4. Bia
T3. African clade
T1.Ctenomeria
T2. Southeast Asian clade
Dalechampia 5-armed clade
Dalechampia 4-armed clade
Plukenetia
Romanoa
Haematostemon
Sister group

←

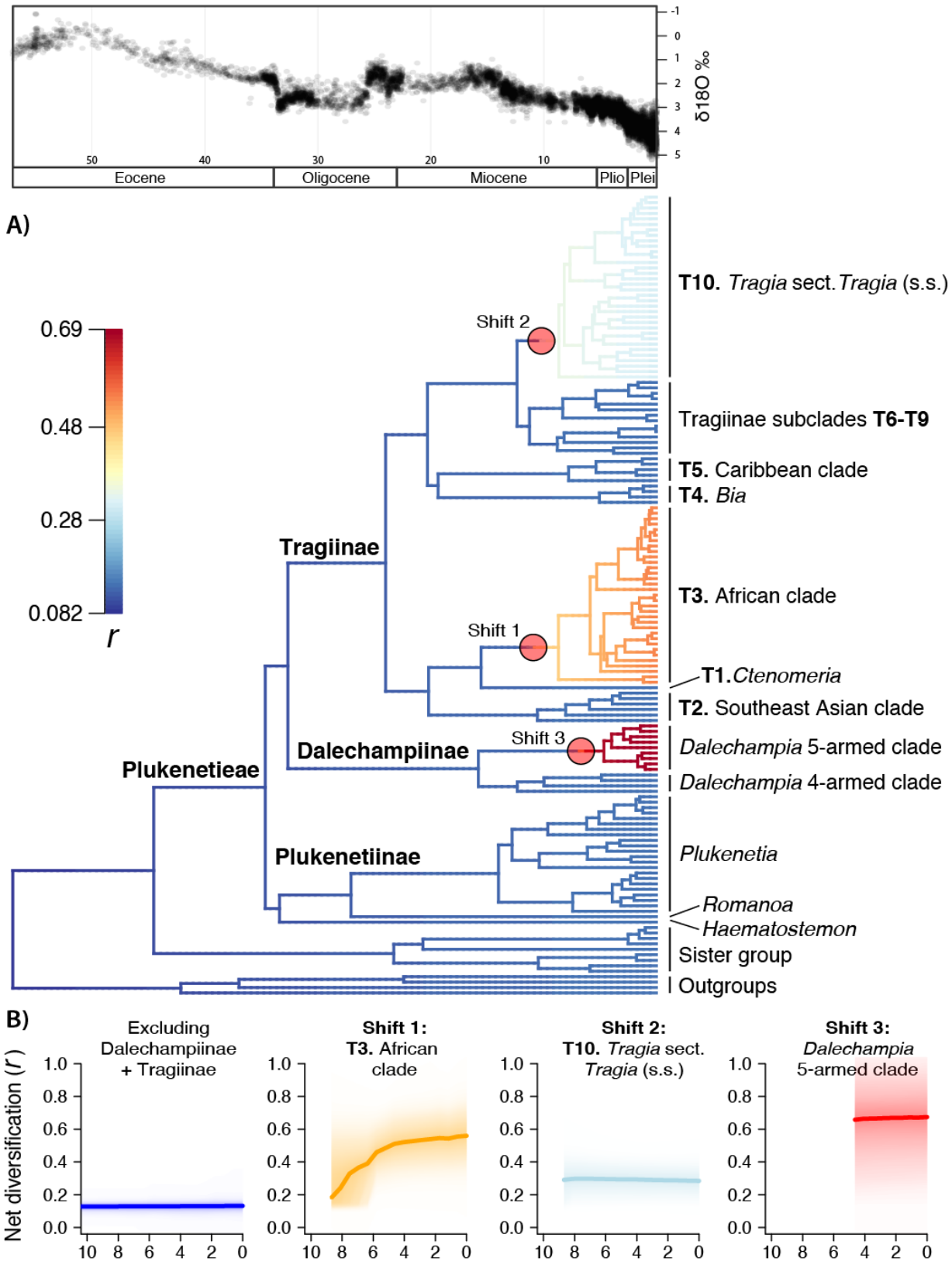
Figure 5.4 Ancestral range estimation (ARE) on the Plukenetiaeae BEAST chronogram using BIOGEOBEARS (DEC+J model). Areas of tip species are shown left of taxon names, colour-coded for the seven biogeographical areas depicted on the inset map. Boxes at each node and corner are colour-coded for the area or combined area (up to five allowed) with the highest likelihood probability. Pie charts indicate the probability of each area and are included if there was < 75% confidence for a single area. Numbers at each node indicate mean age estimates, blue bars the 95% highest posterior density (HPD) confidence interval, and yellow bars the 95% HPD of calibrated nodes estimated under constraint. Arrows on the inset map indicate the direction of major trans-oceanic range movements, along with the mean age of the events. Arrows with dotted lines indicate ARE patterns with ambiguous reconstructions and low support.

Mya). AREs for this node were split between Africa and Southeast Asia, although there was a slightly higher probability for Africa. Under that scenario, there was subsequent movement from Africa to Southeast Asia in the ancestor of subclade T2 in the Early Miocene (*c.* 20.3 Mya). The second LDD occurred within *Plukenetia* from Central and South America to Africa in the Middle Miocene (*c.* 12.9 Mya) and then from Africa to Southeast Asia in the Pleistocene (*c.* 2.3 Mya). The third LDD suggests an anomalous route from North America to Australia in subclades T7 + T8 in the Late Miocene (*c.* 8.4 Mya), followed by Australia to Madagascar in the Pliocene (*c.* 5.1 Mya). The fourth LDD event occurred in the 5–armed clade of *Dalechampia* from Central and South America to Africa during the Pliocene (*c.* 4.7 Mya).

ARE also identified several short-distance dispersal (SDD) events and repeated migrations between North and South America. First, there was a SDD from Central and South America into the Caribbean in the ancestor of subclade T5 in the Early Miocene (*c.* 19.4 Mya). There were also at least three other recent and minor arrivals into the Caribbean in each of the subtribes during the Pleistocene (2.58–0.01 Mya). Second, SDD events from Africa to Madagascar were recovered in each of the subtribes, including at least three events in Tragiinae, all of which occurred from the Late Miocene (*c.* 6.2 Mya) into the Pleistocene (*c.* 1.1 Mya). Lastly, movement from Central and South America into North America started in the ancestor of subclade T7 + T8 in the Late Miocene (*c.* 11.3 Mya). There were also numerous migrations between those regions in subclade T10 from the Late Miocene (*c.* 7.7 Mya) through to the Pleistocene (*c.* 1.1 Mya).

Diversification analyses

Time-dependent diversification analyses in BAMM support rate heterogeneity in Plukenetieae. Exploring alternative poissonRatePriors resulted in similar estimates and shift configurations, as such we present the BAMMTOOLS recommended prior of 1.0. The best rate shift configuration


δ18O ‰
-1
0
1
2
3
4
5
50
40
30
20
10
Eocene
Oligocene
Miocene
Plio
Plei
A)
0.69
0.48
0.28
0.082
r
Shift 2
Shift 1
Shift 3
Tragiinae
Dalechampiinae
Plukenetieae
Plukenetiinae
T10. Tragia sect. Tragia (s.s.)
Tragiinae subclades T6-T9
T5. Caribbean clade
T4. Bia
T3. African clade
T1. Ctenomeria
T2. Southeast Asian clade
Dalechampia 5-armed clade
Dalechampia 4-armed clade
Plukenetia
Romanoa
Haematostemon
Sister group
Outgroups
B)
Net diversification (r)
Excluding Dalechampiinae + Tragiinae
Shift 1: T3. African clade
Shift 2: T10. Tragia sect. Tragia (s.s.)
Shift 3: Dalechampia 5-armed clade
1.0
0.8
0.6
0.4
0.2
0.0
10
8
6
4
2
0

←

Figure 5.5 BAMM analysis of net diversification (*r*) and rate shifts within Plukenetieae. (a) Phylorate plot of the best rate shift configuration (out of 30) showing three significant shifts: 1. Stem of the African clade (subclade T3); 2. Stem of *Tragia* sect. *Tragia* (subclade T10); 3. Stem of the *Dalechampia* 5-armed clade. Colour intensity across branches is proportional to changes in net diversification. (b) Rate through time analysis of net diversification rates in Plukenetieae over the last 10 Myr at the start of the Late Miocene cooling period. Top of figure: Representation of temperature estimates through time based on global deep sea $\delta^{18}O$ isotope concentration data from Zachos *et al.* (2001; accessed from Marcot *et al.*, 2016).

identified three shifts that occurred in the Late Miocene (11.6–5.33 Mya): shift 1 along the stem of subclade T3 (African clade; r = 0.58 events/Myr; 95% HPD = 0.28–0.89), shift 2 along the stem of subclade T10 (*Tragia* sect. *Tragia*; r = 0.29 events/Myr; 95% HPD = 0.15–0.42), and shift 3 along the stem of the *Dalechampia* 5–armed clade (r = 0.66 events/Myr; 95% HPD = 0.18–1.10) (Fig. 5.5). The 95% credible set of rate shifts included 30 configurations with rate shifts in similar positions on the phylogeny (Fig. 5.S6). Excluding Dalechampiinae and Tragiinae, the background net diversification rate was relatively constant through time (r = 0.12 events/Myr; 95% HPD = 0.08–0.16).

Trait-dependent diversification analyses in HiSSE suggest that neither growth form, plant defence, or inflorescence type were associated with increased diversification in Plukenetieae. HiSSE analyses of each putative innovative trait rejected the standard BiSSE model of diversification and performed best under hidden-state and character independent (CID) models (Table 5.S6). Ancestral state estimations under the best fitting model(s) determined by ΔAIC and Akaike weights (Table 5.4) resulted in similar shift placements as the BAMM analyses. Shift 1 and shift 3 were recovered in plant defence and inflorescence type models, while the best growth form model resulted in a relatively constant diversification rate across the phylogeny (Fig. 5.6).

Table 5.4 Best fitting models of trait-dependent diversification for growth form, plant defence, and inflorescence type, based on ΔAIC and Akaike weights (w_i). Traits with multiple models of good fit (ΔAIC < 2) are shown. Models used for 'MarginRecon' analysis are indicated by an asterisk (*); note that reconstructions under CID-4 are currently not permitted, so the second best model was used for inflorescence type.

Best model	Type	Net turnover (τ)	Extinction fraction (ε)	Transition rate (q)	np	-lnL	AIC	ΔAIC	w_i
Growth form:									
Model 21*	HiSSE	τ0A=τ0B	ε0A=ε0B	q0B1B = 0, q1B0B = 0, all other q's equal	7	-772.0	1558.0	0.00	0.982
Plant defence:									
Model 17*	HiSSE	τ's free	ε's equal	q0B1B = 0, q1B0B = 0, all other q's equal	9	-664.5	1347.0	0.00	0.517
Model 8	CID-4	τ's free	ε's equal	q's equal	6	-667.6	1347.3	0.03	0.445
Inflorescence type:									
Model 8	CID-4	τ's free	ε's equal	q's equal	6	-664.9	1341.9	0.00	0.947
Model 6*	CID-2	τ's free	ε's equal	q's equal	5	-670.4	1348.7	6.86	0.031

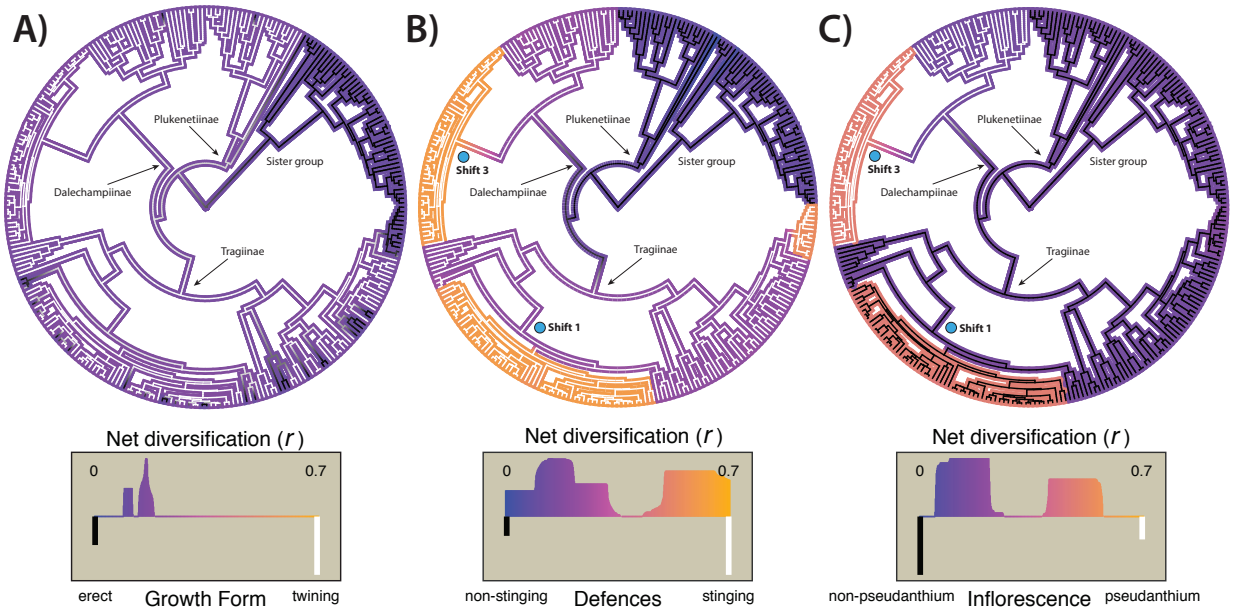


Figure 5.6 Net diversification rates (*r*) and character state reconstructions under the best fitting models from HISSE (see Table 5.4) for three putative innovative traits in Plukenetieae: (a) twining growth form; (b) stinging hair plant defences; and (c) bilaterally symmetrical pseudanthial inflorescences. Outer coloured lines depict net diversification rates (scales comparable between figures), and inner black and white lines indicate character state reconstructions. Histograms show the posterior distribution of net diversification rates (*r*) and black and white bars the frequency of character states at each terminal.

Discussion

Increased plant diversification is often associated with key biotic traits (e.g., specialized growth forms, floral symmetry modifications, specialized pollination biology; Gianoli, 2004; Sargent, 2004; Givnish *et al.*, 2014; Hernández-Hernández *et al.*, 2014; Serrano-Serrano *et al.*, 2017) and abiotic processes (e.g., mountain building, aridification; Bouchenak-Khelladi *et al.*, 2014; Reyes *et al.*, 2015; Lagomarsino *et al.*, 2016; Pérez-Escobar *et al.*, 2017), but regional and global diversification trends still need to be established. Our study investigates the drivers of diversification in the pantropically distributed clade of Euphorbiaceae vines (tribe Plukenetieae), and explores the effects of multiple overlapping innovative traits. Contrary to our expectations, innovative traits and biogeography were not directly associated with increased diversity. Instead, we suspect that biome shifts and climate-driven landscape changes played an important role in diversification of the tribe.

Early history of the tribe unresolved

Here, we produced the largest phylogeny of Plukenetieae and developed a temporal framework for investigating biogeographical history and shifts in diversification rates. Increased taxon and molecular sampling resulted in well supported species relationships consistent with previous hypotheses (Cardinal-McTeague & Gillespie, 2016), but the backbone of the tribe was strongly incongruent among nuclear and plastid datasets (Fig. 5.2). Recent phylogenies of Plukenetieae recovered either a functional polytomy of the subtribes (Cardinal-McTeague & Gillespie, 2016), or sampled only plastid regions and recovered the same topology of Plukenetiinae sister to Dalechampiinae + Tragiinae (Cervantes *et al.*, 2016). Results of coalescence-based species tree methods (based on relatively limited molecular sampling) highlight that early branching patterns of the tribe are very poorly supported (Fig. 5.3). Incongruence is likely the result of incomplete lineage sorting among nuclear genes, compounded by saturation in ITS, suggesting that greater character sampling and coalescence-based methods using phylogenomics would be an efficient approach to resolving the backbone of the tribe.

Pantropical distributions the result of steady periodic dispersals

The biogeographical history presented here is the first detailed analysis of Plukenetieae, and includes near complete sampling of the biogeographic diversity in the tribe, except for finer details within Dalechampiinae (Fig. 5.4). Using secondary node calibrations based on subfamily age estimates (Cervantes *et al.*, 2016), we find that the crown of the tribe diverged at the end of the Eocene (*c.* 34.8 Mya) when the warm Eocene climate was rapidly transitioning into the cooler Oligocene (Zachos *et al.*, 2001). The subtribes diverged by the Oligocene (33.9–23.0 Mya) and were most likely distributed in Central and South America (Fig. 5.4). In the Oligocene, the modern continents had mostly formed and were separated by seas and oceans (McLoughlin, 2001), precluding a Gondwanan vicariance hypothesis for pantropical distributions.

The pantropical distribution of Plukenetieae is best explained by LDDs that started in the Oligocene and continued steadily into the Pleistocene (Fig. 5.4). Major patterns include LDDs from Central and South America to Africa in each of the subtribes, during the Oligocene in Tragiinae, the Miocene in Plukenetiinae, and the Pliocene in Dalechampiinae. These patterns add to the growing number of lineages that dispersed in the less frequent direction from the

Neotropics to Africa, presumably along the Atlantic equatorial countercurrent (Renner & Meyer, 2001; Renner *et al.*, 2001; Givnish *et al.*, 2004, 2011, 2016; Sytsma *et al.*, 2004; Berger *et al.*, 2016). There is ambiguity in the ARE of Old World Tragiinae (subclades T1–T3), which suggests that a LDD from Central and South America into Southeast Asia was also likely (Fig. 5.4). However, most dataset topologies suggest the South African genus *Ctenomeria* (subclade T1) was the earliest diverging lineage in Old World Tragiinae (subclades T1–T3) (Figs. 5.2 and 5.3), which otherwise might support a LDD to Africa. In either scenario, there was an inferred migration between Africa and Southeast Asia during the Early Miocene (*c.* 20.3 Mya), which could have been facilitated by tropical forest connections between Africa and West Asia (Zhou *et al.*, 2012). By comparison, recent Pleistocene movement (*c.* 2.3 Mya) of *Plukenetia* from Africa to Southeast Asia is best explained by LDD across Indian Ocean countercurrents (Cardinal-McTeague *et al*., in review), as is suggested in several other plant groups (Renner, 2004b; Yuan *et al.*, 2005; Clayton *et al.*, 2009; de Wilde *et al.*, 2011; Liu *et al.*, 2013).

The most anomalous biogeographic pattern we recovered was a LDD from North America to Australia in the ancestor of Tragiinae subclades T7 + T8 (Fig. 5.4). Subclade T7 includes two species of subshrubs endemic to the southeastern USA, whereas subclade T8 contains about five species of tropical vines distributed primarily in Australia and Southeast Asia. A LDD directly from the Southeast USA to eastern Australia seems unlikely, but could have been possible through the Central American seaway prior to the formation of the Isthmus of Panama. Alternatively, the ancestor could have been more widespread in North America and possibly Central America, dispersing from a more likely location on the west coast, and then becoming isolated in the Southeast USA. North American and Australian disjunctions are uncommon but have been noted in the aquatic genus *Myriophyllum* (Chen *et al.*, 2014). Within subclade T8 there was a second LDD from Australasia to Madagascar across the Indian Ocean during the Pliocene (*c.* 5.1 Mya). Trans-Indian Ocean LDDs from Southeast Asia/Australasia to Madagascar are indicated in many plant lineages (Michalak *et al.*, 2010; Renner *et al.*, 2010; Viljoen *et al.*, 2013).

Besides global plant migration patterns, analysis of Plukenetieae revealed regional biogeographic patterns in the Caribbean, Madagascar, and subtropical North America (Fig. 5.4). First, there was a SDD from Central and South America into the Caribbean in the ancestor of the Tragiinae Caribbean clade (subclade T5) in the Early Miocene (*c.* 19.4 Mya), followed by recent

minor arrivals in each of the subtribes during the Pleistocene (2.58–0.01 Mya). A Miocene entry into the Caribbean is consistent with other euphorb lineages (Cervantes *et al.*, 2016), as well as orchids, bromeliads, and a majority of endemic Caribbean seed plant genera (Givnish *et al.*, 2011; Nieto-Blázquez *et al.*, 2017; Pérez-Escobar *et al.*, 2017a). Second, there were numerous SDDs from Africa to Madagascar through the Late Miocene and Pliocene, with one to three events in each of the subtribes. Repeated entries into Madagascar since the Miocene is emerging as a dominant biogeographic pattern for the island (Koopman & Baum, 2008; Wikström *et al.*, 2010; Zhou *et al.*, 2012; Korall & Pryer, 2014; van Velzen *et al.*, 2015; Cardinal-McTeague *et al.*, 2016). Lastly, there were several interchanges between Central and South America and subtropical North America in New World Tragiinae (subclades T4–T10) from the Miocene into the Pleistocene. Transitions from tropical to temperate American biomes are noted in the Paleocene and Eocene in Anacardiaceae (Weeks *et al.*, 2014) and Miocene in Rubiaceae (Janssens *et al.*, 2016), suggesting out-of-tropic transitions have been ongoing since the Paleocene and perhaps intensified as cooling increased in the Late Miocene.

Biome shifts, not innovative traits, associated with increased diversity

Contrary to our expectations that innovative traits would exhibit additive effects on species diversification, we found two to three shifts in Plukenetieae that were not directly associated with the specified traits or biogeography. Time-dependent BAMM analyses found three rate shifts that occurred in the Late Miocene in the African clade (subclade T3; shift 1) and *Tragia* sect. *Tragia* (subclade T10; shift 2) of Tragiinae and in the *Dalechampia* 5–armed clade (shift 3) of Dalechampiinae (Fig. 5.5). Trait-dependent HiSSE analyses recovered two of the same shifts, in the African clade (subclade T3) and the *Dalechampia* 5–armed clade (Fig. 5.6). Our results provide insight into the nuanced interaction between innovative traits and diversification, and support the influence of biome shifts and climate change on plant diversification.

Biome shifts are the most likely driver of the 'hidden' diversification shifts in Plukenetieae. Species with the largest rate shifts (shifts 1 and 3) occur primarily in a mix of seasonal and semi-arid open tropical/subtropical habitats, like wooded savannas, grasslands and scrublands (Radcliffe-Smith, 1987; Armbruster *et al.*, 2009; Urtecho, 2016), compared to evergreen and moist tropical forests in the rest of the tribe. Increased diversification associated with arid and open habitats is found in other lineages like Proteaceae (Reyes *et al.*, 2015),

Verbenaceae (Olmstead, 2013), and caesalpinioid legumes (Souza-Neto *et al.*, 2016). Species in *Tragia* sect. *Tragia* (shift 2) inhabit a mix of seasonal tropical, subtropical, and temperate open habitats, including arid deserts (Múlgura de Romero & Gutiérrez de Sanguinetti, 1989; Urtecho, 2016). Greater variability and reversals in biome preference could be one reason why net diversification rates were lower in shift 2. Future studies could identify stronger patterns of biome-associated diversification by reconstructing ancestral biomes and habitats across the tribe.

Climate change and open habitat expansion likely contributed to diversification rate increases in Plukenetieae. Rate shifts occurred during the Late Miocene when open and arid biomes expanded across the globe, suggesting that Plukenetieae diversification was aided by emerging ecological opportunities. This effect is especially apparent in African Tragiinae (shift 1), where Late Miocene and Pliocene expansion of savannas coincides with a notable upswing in diversification rates (Fig. 5.5B). In this case, biogeographic history was not directly associated with increased diversification since the ancestor of African Tragiinae arrived in Early Miocene (*c.* 20.3 Mya) and did not diversify until open and drier landscapes became dominant, although arrival to Africa was an important precursor. American grasslands were established by the start of Miocene (Edwards *et al.*, 2010) and as a result may have provided fewer novel ecological opportunities than Africa and produced lower diversification rates in *Tragia* sect. *Tragia* (shift 2). Our results reinforce growing evidence that, in certain contexts, abiotic processes associated with niche expansion (e.g., mountain building, increasing aridity and open habitat expansion) had a positive effect on plant diversification. We note that other potential abiotic drivers of diversification, Andes mountain building in *Plukenetia* and shifts to cold temperature tolerance in *Tragia*, were not associated increased species-richness. Furthermore, rapid evolution of floral and plant defences in the *Dalechampia* 5–armed (e.g., nocturnal closure of involucral bracts, glochidial spines on pistillate sepals, resin secretion by leaves, stipules, and bracts) is likely a contributing factor of its increased diversification rates (Armbruster *et al.*, 2009).

Conclusion

Biogeographic analyses revealed that pantropical distributions formed by steady periodic LDDs through the Oligocene to Pleistocene, and that dispersals were not directly associated with diversification. Contrary to our expectations, we did not find that increased diversification in Plukenetieae was associated with any of the overlapping innovations in twining growth form,

stinging hair defences, and bilaterally symmetrical pseudanthial inflorescences. Instead, increased species richness was driven in two to three subclades that appear to have shifted biomes from primarily moist tropical forests to drier open habitats. Diversification in these subclades was likely enhanced by climate change and the expansion of arid and open habitats associated with the Late Miocene and Pliocene cooling.

Table 5.S1 Accession table with voucher information for taxa used in phylogenetic analyses.

No.	Taxon	Voucher	Country	ETS	ITS	*KEA1* intron 11	*TEB* exon 17	*matK*	*ndhF*
1	*Acidoton microphyllus* Urban	Liogier 14216 (NY)	Dominican Republic	GenBank	GenBank	—	GenBank	GenBank	GenBank
2	*Acidoton urens* Sw.	Webster 8184 (DAV)	Jamaica	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
3	*Acidoton urens*	Thorne 48242 (MO)	Jamaica	GenBank	KP794316	—	—	—	GenBank
4	*Adenophaedra grandofolia* (Klotzsch) Müll.Arg.	Valaverde 48 (NY)	Costa Rica	GenBank	GenBank	—	—	—	GenBank
5	*Bernardia dodecandra* (Sessé ex Cav.) McVaugh	Rees 176 (MO)	Belize	GenBank	KP794418	GenBank	GenBank	GenBank	GenBank
6	*Bernardia nicaraguensis* Standl. & L.O.Williams	Cardinal-McTeague 2 (CAN)	Costa Rica	GenBank	GenBank	—	GenBank	GenBank	GenBank
7	*Bernardia nicaraguensis*	Stevens 30168 (MO)	Nicaragua	GenBank	KP794419	—	GenBank	GenBank	GenBank
8	*Bernardia pulchella* (Baill.) Müll.Arg.	Kegler 1333 (MO)	Brazil	GenBank	KP794420	GenBank	—	GenBank	GenBank
9	*Bernardia scabra* Müll.Arg.	Pirani et al. 2935 (CAN)	Brazil	GenBank	GenBank	—	—	GenBank	GenBank
10	*Bia alienata* Didr.	Stevens et al. 31232 (MO)	Paraguay	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
11	*Bia alienata*	Pérez 352 (MO)	Paraguay	GenBank	KP794318	GenBank	GenBank	GenBank	GenBank
12	*Bia alienata*	Zardini 11174 (MO)	Paraguay	GenBank	KP794319	GenBank	GenBank	GenBank	GenBank
13	*Bia alienata*	Carretero 1237 (MO)	Bolivia	GenBank	KP794320	GenBank	GenBank	GenBank	GenBank
14	*Bia fendleri* Müll.Arg.	Webster 23296 (DAV)	Peru	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
15	*Bia fendleri*	Seidel and Hezog 7516 (DAV)	Bolivia	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
16	*Bia fendleri*	Beck 8253 (MO)	Bolivia	GenBank	KP794321	GenBank	GenBank	GenBank	GenBank
17	*Bia lessertiana* Baill.	Webster 25586 (DAV)	Brazil	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
18	*Bia cf. lessertiana*	Noblick 3117 (DAV)	Brazil	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
19	*Caperonia palustris* (L.) A.St.-Hil.	Wurdack D073 (US)	USA	GenBank	GenBank	—	GenBank	GenBank	GenBank
20	*Caryodendron angustifolium* Standl.	(Scott Armbruster)	Costa Rica	GenBank	GenBank	—	—	GenBank	GenBank
21	*Caryodendron grandifolium* (Müll.Arg.) Pax	Korning 8502 (MO)	Ecuador	GenBank	KP794421	—	—	GenBank	GenBank
22	*Caryodendron janeirense* Müll.Arg.	Pinto et al. 122 (RB)	Brazil	GenBank	GenBank	—	—	GenBank	GenBank

23	*Caryodendron orinocense* Karst.	Gentry 70541 (MO)	Bolivia	—	KP794422	—	—	GenBank	GenBank
24	*Caryodendron orinocense*	Grandez 19528 (AMAZ)	Peru	GenBank	GenBank	—	—	GenBank	GenBank
25	*Cnesmone javanica* Blume	Chamchumroon 2019 (L)	Thailand	GenBank	KP794430	—	GenBank	GenBank	GenBank
26	*Cnesmone javanica*	Huq 10189 (US)	Bangladesh	GenBank	GenBank	—	GenBank	—	GenBank
27	*Cnesmone javanica*	Larsen 44027 (MO)	Thailand	GenBank	KP794427	—	GenBank	GenBank	GenBank
28	*Cnesmone javanica*	Larsen 44147 (MO)	Thailand	GenBank	KP794428	—	—	GenBank	GenBank
29	*Cnesmone laevis* (Ridl.) Airy Shaw	Smitinand 1180 (L)	Thailand	—	—	—	—	GenBank	GenBank
30	*Cnesmone laotica* (Gagnep.) Croizat	van Beusekom 4154 (L)	Thailand	GenBank	KP794431	—	GenBank	GenBank	GenBank
31	*Cnesmone tonkinensis* (Gagnep.) Croizat	Gillespie et al. 7406 (CAN)	Vietnam	GenBank	GenBank	—	GenBank	GenBank	GenBank
32	*Ctenomeria capensis* (Thunb.) Harv. ex Sond.	Balkwill and Balkwill 10715 (MO)	South Africa	GenBank	GenBank	—	GenBank	GenBank	GenBank
33	*Ctenomeria capensis*	Hugo 2107 (MO)	South Africa	GenBank	KP794322	—	—	GenBank	GenBank
34	*Dalechampia* aff. *tamifolia* Lam.	Gillespie et al. 10658 (CAN)	Madagascar	—	KP794423	GenBank	—	GenBank	GenBank
35	*Dalechampia aristolochiifolia* Kunth	(Scott Armbruster)	Greenhouse ex Peru	GenBank	GenBank	GenBank	—	GenBank	GenBank
36	*Dalechampia bernieri* var. *denisiana* Leandri	Gillespie et al. 10675 (CAN)	Madagascar	—	KP794424	GenBank	GenBank	GenBank	GenBank
37	*Dalechampia clematidifolia* Bojer ex Baill.	Gillespie et al. 10815 (CAN)	Madagascar	GenBank	KP794426	GenBank	GenBank	GenBank	GenBank
38	*Dalechampia dioscoreifolia* Peopp.	(Scott Armbruster)	Costa Rica	GenBank	GenBank	GenBank	—	GenBank	GenBank
39	*Dalechampia scandens* L.	Silva et al. 1165 (CAN)	Brazil	GenBank	—	—	—	GenBank	GenBank
40	*Dalechampia sp. (Field)*	Medeiros and Cardinal-McTeague 555 (R)	Brazil	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
41	*Dalechampia sp. (River)*	Medeiros and Cardinal-McTeague 562 (R)	Brazil	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
42	*Dalechampia sp. (Serra Jua)*	Medeiros et al. # (R)	Brazil	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
43	*Dalechampia spathulata* (Scheidw.) Baill.	(Scott Armbruster)	Greenhouse ex Mexico	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
44	*Dalechampia subternata* Müll.Arg.	Gillespie et al. 10810 (CAN)	Madagascar	—	KP794425	GenBank	GenBank	GenBank	GenBank

45	*Dalechampia tiliifolia* Lam.	(Scott Armbruster)	Costa Rica	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
46	*Dalechampia websteri* Armbr.	Cardinal-McTeague 7 (CAN)	Costa Rica	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
47	*Dalechampia websteri*	(Scott Armbruster)	Costa Rica	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
48	*Ditaxis argothamnoides* (Bertol. ex Spreng.) Radcl.-Sm. & Govaerts	Wurdack D105 (US)	USA	GenBank	GenBank	—	—	GenBank	GenBank
49	*Gitara nicaraguensis* (Hemsl.) Card.-McTeag. & L.J.Gillespie	Berg et al. 729 (CAN)	Brazil	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
50	*Gitara nicaraguensis*	Stevens 33392 (MO)	Nicaragua	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
51	*Gitara nicaraguensis*	Ibañez 2038 (MO)	Panama	GenBank	KP794397	GenBank	GenBank	GenBank	GenBank
52	*Gitara nicaraguensis*	Korning 47437 (MO)	Ecuador	GenBank	KP794398	GenBank	GenBank	GenBank	GenBank
53	*Haematostemon guianensis* Sandwith	Wurdack 4350 (US)	Guyana	MF502427	KP794434	—	MF502846	MF502706	MF502776
54	*Lasiocroton bahamensis* Pax & K.Hoffm.	Wurdack D058 (US)	Cult. USA, Fairchild Tropical Garden 66629b	GenBank	GenBank	—	—	FJ670013	FJ670084
55	*Megistostigma peltatum* (J.J.Sm.) Croizat	Maxwell 87-462 (L)	Thailand	GenBank	GenBank	—	—	—	—
56	*Megistostigma yunnanense* Croizat	Larsen 46354 (L)	Thailand	GenBank	GenBank	—	—	GenBank	GenBank
57	*Pachystylidium hirsutum* (Blume) Pax & K.Hoffm.	Keer 17893 (L)	Thailand	GenBank	KP794408	—	—	GenBank	GenBank
58	*Pachystylidium sp. Philippines*	Kulju 4 (CAN)	Philippines	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
59	*Philyra brasiliensis* Klotzsch	Webster 25536 (NY)	Brazil	GenBank	GenBank	—	—	GenBank	GenBank
60	*Platygyna hexandra* (Jacq.) Müll.Arg.	Acevedo-Rodríguez 5566 (NY)	Cuba	GenBank	KP794317	—	—	GenBank	GenBank
61	*Platygyna hexandra*	HAJB 81903 (MICH)	Cuba	GenBank	GenBank	—	GenBank	GenBank	GenBank
62	*Platygyna hexandra*	HAJB 81961 (MICH)	Cuba	GenBank	GenBank	—	GenBank	GenBank	GenBank
63	*Platygyna parvifolia* Alain	HAJB 81826 (MICH)	Cuba	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
64	*Plukenetia africana* Sond.	Palomoti 1086 (MO)	Botswana	MF502432	MF502515	—	—	MF502711	MF502782
65	*Plukenetia africana*	Bartsch 1859 (US)	Namibia	MF502431	MF502513	MF502579	MF502848	MF502710	MF502780

66	*Plukenetia africana*	Pope et al. 834 (MO)	Botswana	MF502433	MF502516	MF502580	—	MF502712	MF502783
67	*Plukenetia ankaranensis* L.J.Gillespie	Gillespie et al. 10697 (CAN)	Madagascar	MF502434	KP794438	MF502581	MF502849	MF502713	MF502784
68	*Plukenetia ankaranensis*	Lees *s.n.* (CAN)	Madagascar	MF502435	KP794437	—	MF502850	MF502714	MF502785
69	*Plukenetia brachybotrya* Müll.Arg.	Araujo-M. et al. 1722 (MO)	Bolivia	MF502437	KP794452	MF502583	MF502852	MF502716	MF502787
70	*Plukenetia brachybotrya*	Galiano et al. 6612 (MO)	Peru	MF502439	MF502519	—	—	MF502718	MF502789
71	*Plukenetia brachybotrya*	Fuentes and Torrico 5398 (MO)	Bolivia	MF502438	MF502518	MF502584	—	MF502717	MF502788
72	*Plukenetia brachybotrya*	Acevedo-Rodriguez 14416 (NY)	Peru	MF502436	MF502517	MF502582	MF502851	MF502715	MF502786
73	*Plukenetia brachybotrya*	Seidel and Vaquiata 7733 (MO)	Bolivia	MF502440	MF502520	MF502585	MF502853	MF502719	MF502790
74	*Plukenetia carabiasiae* J.Jiménez Ram.	Meave et al. 1550 (MO)	Mexico	MF502441	MF502521	MF502586	—	MF502720	—
75	*Plukenetia carolis-vegae* subsp. *nectarifera* Card.-McTeag. & L.J.Gillespie	Valenzuela et al. 5197 (CAN)	Peru	MF502455	MF502534	MF502597	—	—	—
76	*Plukenetia carolis-vegae* subsp. *nectarifera*	Huamantupa et al. 6445 (CAN)	Peru	MF502449	MF502528	MF502593	—	MF502724	—
77	*Plukenetia carolis-vegae* subsp. *nectarifera*	Rojas et al. 3863 (CAN)	Peru	MF502453	MF502532	MF502595	—	—	—
78	*Plukenetia carolis-vegae* subsp. *nectarifera*	van der Werff et al. 17532 (CAN)	Peru	MF502456	MF502535	MF502598	MF502856	MF502729	MF502797
79	*Plukenetia carolis-vegae* subsp. *nectarifera*	Calatayud et al. 2643 (CAN)	Peru	MF502448	MF502527	MF502592	—	—	GenBank
80	*Plukenetia carolis-vegae* subsp. *nectarifera*	Woytkowski 6670 (MO)	Peru	MF502458	MF502537	MF502600	MF502858	MF502731	MF502799
81	*Plukenetia carolis-vegae* subsp. *nectarifera*	Vasquez et al. 33145 (MO)	Peru	MF502457	MF502536	MF502599	MF502857	MF502730	MF502798
82	*Plukenetia carolis-vegae* subsp. *nectarifera*	Monteagudo et al. 14125 (MO)	Peru	MF502450	MF502529	MF502594	—	MF502725	MF502793
83	*Plukenetia carolis-vegae* subsp. *nectarifera*	Monteagudo et al. 15252 (MO)	Peru	MF502451	MF502530	—	—	—	MF502794
84	*Plukenetia cf. penninervia*	Gentry et al. 47799 (MO)	Colombia	MF502442	MF502522	MF502587	—	MF502721	MF502791
85	*Plukenetia conophora* Müll.Arg.	Hart 1621 (MO)	Democratic Republic of Congo	MF502443	MF502523	MF502588	—	—	—

86	*Plukenetia conophora*	Nemba and Thomas 434 (MO)	Cameroon	MF502444	KP794457	MF502589	—	MF502722	MF502792
87	*Plukenetia corniculata* Sm.	Huq and Haroon 10780 (GH)	Bangladesh	MF502445	MF502524	MF502590	MF502854	—	—
88	*Plukenetia corniculata*	Huq and Haroon 10780 (MO)	Bangladesh	MF502446	MF502525	MF502591	MF502855	MF502723	—
89	*Plukenetia decidua* L.J.Gillespie	Rakotomalaza 597 (CAN)	Madagascar	MF502447	MF502526	—	—	—	—
90	*Plukenetia huayllabambana* Bussmann et al.	Tellez et al. 4 (MO)	Peru	MF502454	MF502533	MF502596	—	MF502728	MF502796
91	*Plukenetia huayllabambana*	Quipuscoa 381 (MO)	Peru	MF502452	MF502531	—	—	MF502727	MF502795
92	*Plukenetia lehmanniana* (Pax & K.Hoffm.) Huft & L.J.Gillespie	Silverstone-Sopkin and Giralda-Gesini 8019 (MO)	Colombia	MF502461	MF502539	MF502603	MF502861	MF502734	MF502802
93	*Plukenetia lehmanniana*	Clark 3953 (MO)	Ecuador	MF502460	MF502538	MF502602	MF502860	MF502733	MF502801
94	*Plukenetia lehmanniana*	Zak and Jaramillo 3401 (MO)	Ecuador	MF502462	MF502540	MF502604	MF502862	MF502735	MF502803
95	*Plukenetia lehmanniana*	Acevedo-Rodriguez and Daly 1658 (MO)	Ecuador	MF502459	KP494443	MF502601	MF502859	MF502732	MF502800
96	*Plukenetia loretensis* Ule	Grandez 19608 (AMAZ)	Peru	MF502463	KP794453	MF502605	MF502863	MF502736	MF502804
97	*Plukenetia loretensis*	McDaniel and Rimachi 22451 (MO)	Peru	MF502464	MF502541	MF502606	—	—	MF502805
98	*Plukenetia loretensis*	Vásquez and Jaramillo 3283 (MO)	Peru	MF502467	MF502544	MF502609	—	—	GenBank
99	*Plukenetia loretensis*	Rimachi 5122 (MO)	Peru	MF502465	MF502542	MF502607	—	MF502738	MF502806
100	*Plukenetia loretensis*	Solomon 7972 (MO)	Bolivia	MF502466	MF502543	MF502608	MF502864	—	MF502807
101	*Plukenetia loretensis*	Vasquez et al. 38069 (MO)	Peru	MF502468	MF502545	MF502610	MF502865	MF502740	MF502808
102	*Plukenetia madagascarensis* Leandri	Villiers et al. 4899 (MO)	Madagascar	MF502471	MF502548	MF502613	MF502867	MF502743	MF502810
103	*Plukenetia madagascarensis*	Andrianjafy 1648 (CAN)	Madagascar	MF502469	MF502546	MF502611	—	MF502741	—
104	*Plukenetia madagascarensis*	Gillespie 4175 (CAN)	Madagascar	MF502470	MF502547	MF502612	MF502866	MF502742	MF502809
105	*Plukenetia megastyla* Card.-McTeag. & L.J.Gillespie	Sperling et al. 5873 (MO)	Brazil	MF502429	MF502512	MF502577	MF502847	MF502708	MF502778
106	*Plukenetia megastyla*	Sperling et al. 6161 (CAN)	Brazil	MF502430	—	MF502578	—	MF502709	MF502779
107	*Plukenetia megastyla*	Ledezma et al. 921 (CAN)	Bolivia	MF502428	MF502511	MF502576	—	—	MF502777

108	*Plukenetia penninervia* Müll.Arg.	Martinez 17705 (DAV)	Mexico	MF502475	MF502550	MF502617	MF502871	MF502747	MF502814
109	*Plukenetia penninervia*	McPherson 8461 (MO)	Panama	MF502476	KP794454	MF502618	MF502872	MF502748	MF502815
110	*Plukenetia penninervia*	Atha et al. 1001 (MO)	Belize	MF502472	KP794455	MF502614	MF502868	MF502744	MF502811
111	*Plukenetia penninervia*	Wallnöfer and Frisch 5996 (MO)	Guatemala	MF502477	MF502551	MF502619	MF502873	MF502749	MF502816
112	*Plukenetia penninervia*	Carnevali and Duno 7594 (MO)	Mexico	MF502473	MF502549	MF502615	MF502869	MF502745	MF502812
113	*Plukenetia penninervia*	Martínez 10527 (MO)	Mexico	MF502474	KP794456	MF502616	MF502870	MF502746	MF502813
114	*Plukenetia polyadenia* Müll.Arg.	Wurdack 5288 (US)	Guyana	MF502479	MF502554	MF502621	MF502875	MF502752	MF502819
115	*Plukenetia polyadenia*	Gillespie 4314 (CAN)	Smithsonian Greenhouse ex French Guiana	MF502478	MF502553	MF502620	MF502874	MF502751	MF502818
116	*Plukenetia polyadenia*	Neill et al. 8370 (MO)	Ecuador	—	GenBank	—	—	—	GenBank
117	*Plukenetia polyadenia*	Croat 20285 (MO)	Peru	—	MF502552	—	—	MF502750	MF502817
118	*Plukenetia serrata* (Vell.) L.J.Gillespie	Thomas 10221 (NY)	Brazil	MF502485	MF502559	MF502626	—	—	MF502825
119	*Plukenetia serrata*	Forzza et al. 5328 (RB)	Brazil	MF502481	MF502556	MF502622	—	MF502754	MF502821
120	*Plukenetia serrata*	Goldenberg et al. 1424 (RB)	Brazil	MF502482	MF502557	MF502623	—	MF502755	MF502822
121	*Plukenetia serrata*	Sartori and Pardo 11 (RB)	Brazil	MF502484	MF502558	MF502625	—	MF502757	MF502824
122	*Plukenetia serrata*	Davidse 10480 (MO)	Brazil	MF502480	MF502555	—	—	MF502753	MF502820
123	*Plukenetia serrata*	Peixoto et al. 4154 (MO)	Brazil	MF502483	KP794458	MF502624	—	MF502756	MF502823
124	*Plukenetia stipellata* L.J.Gillespie	Cardinal-McTeague 8 (CAN)	Costa Rica	MF502487	MF502560	MF502628	MF502877	MF502759	MF502827
125	*Plukenetia stipellata*	Ibarra Manriquez and Sinaca 1115 (MO)	Mexico	MF502488	MF502561	MF502629	MF502878	—	MF502828
126	*Plukenetia stipellata*	Refugio Cedillo Trigas 3510 (MO)	Mexico	MF502491	MF502562	MF502632	MF502881	MF502761	MF502831
127	*Plukenetia stipellata*	Morales and Rojas 5342 (MO)	Costa Rica	MF502490	KP794450	MF502631	MF502880	MF502760	MF502830
128	*Plukenetia stipellata*	Aguilar 8193 (MO)	Costa Rica	MF502486	KP794448	MF502627	MF502876	MF502758	MF502826
129	*Plukenetia stipellata*	Urbina 1155 (MO)	Nicaragua	MF502492	KP794449	MF502633	MF502882	MF502762	MF502832
130	*Plukenetia stipellata*	Liesner 3088 (MO)	Costa Rica	MF502489	KP794451	MF502630	MF502879	—	MF502829

131	*Plukenetia supraglandulosa* L.J.Gillespie	Acevedo-Rodriguez 6022 (US)	Suriname	MF502493	MF502563	MF502634	MF502883	MF502763	MF502833
132	*Plukenetia verrucosa* Smith	Barrabe and Crozier 145 (US)	French Guiana	MF502494	MF502564	MF502635	MF502884	MF502764	MF502834
133	*Plukenetia verrucosa*	Herrera and Kaemar 10073 (CAN)	Suriname	MF502495	MF502565	—	—	—	—
134	*Plukenetia verrucosa*	Hoffman 5917 (US)	Suriname	MF502496	MF502566	MF502636	MF502885	MF502765	MF502835
135	*Plukenetia volubilis* L.	Bell 93-546 (US)	Peru	MF502497	KP794447	MF502637	MF502886	MF502766	MF502836
136	*Plukenetia volubilis*	Huamantupa 3500 (CAN)	Peru	MF502500	MF502568	MF502640	—	—	—
137	*Plukenetia volubilis*	Valenzuela 8531 (MO)	Peru	MF502505	MF502571	—	—	—	—
138	*Plukenetia volubilis*	Jaramillo et al. 1237 (MO)	Bolivia	MF502501	MF502569	MF502641	—	MF502768	MF502838
139	*Plukenetia volubilis*	Teran et al. 2502 (MO)	Bolivia	MF502504	MF502570	MF502644	—	MF502771	MF502841
140	*Plukenetia volubilis*	Wurdack s.n. (US)	Smithsonian Greenhouse ex Peru	MF502506	MF502572	MF502645	MF502890	MF502772	MF502842
141	*Plukenetia volubilis*	Carrillo and Reyes 448 (MO)	Ecuador	MF502499	MF502567	MF502639	—	—	GenBank
142	*Plukenetia volubilis*	Burnham and Krings 1640 (MO)	Ecuador	MF502498	KP794446	MF502638	MF502887	MF502767	MF502837
143	*Plukenetia aff. volubilis*	Parada et al. 206 (CAN)	Bolivia	MF502503	KP794444	MF502643	MF502889	MF502770	MF502840
144	*Plukenetia aff. volubilis*	Nee 55162 (MO)	Bolivia	MF502502	KP794445	MF502642	MF502888	MF502769	MF502839
145	*Romanoa tamnoides* (A.Juss.) Radcl.-Sm.	Raes and Terceros 177 (MO)	Bolivia	MF502508	KP794435	MF502646	—	MF502773	MF502843
146	*Romanoa tamnoides*	Raes et al. 211 (MO)	Bolivia	MF502509	MF502574	MF502647	—	MF502774	MF502844
147	*Romanoa tamnoides*	Zardini and Chaparro 50824 (MO)	Paraguay	MF502510	MF502575	MF502648	—	MF502775	MF502845
148	*Romanoa tamnoides*	Fuentes 1848 (MO)	Bolivia	MF502507	MF502573	—	—	—	GenBank
149	*Sphaerostylis perrieri* Leandri	Labat 3441 (MO)	Madagascar	GenBank	KP794412	—	—	GenBank	GenBank
150	*Sphaerostylis perrieri*	Gillespie 10738 (CAN)	Madagascar	GenBank	KP794413	GenBank	GenBank	GenBank	GenBank
151	*Sphaerostylis perrieri*	Gillespie 10739 (CAN)	Madagascar	GenBank	KP794414	—	—	GenBank	GenBank
152	*Sphaerostylis perrieri*	Gillespie 10742 (CAN)	Madagascar	GenBank	KP794415	GenBank	GenBank	GenBank	GenBank
153	*Tragia aff. furialis* Bojer ex Prain	Lovett 3707 (MO)	Tanzania	GenBank	KP794344	—	GenBank	GenBank	GenBank
154	*Tragia aff. pacifica*	Sandoval 1777 (MO)	El Salvador	GenBank	KP794388	GenBank	GenBank	GenBank	GenBank

155	*Tragia aff. subhastata*	Serrano 6965 (MO)	Bolivia	GenBank	KP794357	GenBank	GenBank	GenBank	GenBank
156	*Tragia aff. subhastata*	Serrano 6929 (MO)	Bolivia	GenBank	KP794358	GenBank	GenBank	GenBank	GenBank
157	*Tragia amblyodonta* (Müll.Arg.) Pax & K.Hoffm.	B.L. 98-372 (MO)	USA	GenBank	KP794376	GenBank	—	GenBank	GenBank
158	*Tragia arabica* (Müll.Arg.) Baill. ex Prain	Burger 3592 (US)	Ethiopia	GenBank	GenBank	—	—	GenBank	GenBank
159	*Tragia arnhemica* P.I.Forst.	Brennan 2016 (DNA)	Australia	GenBank	KP794410	GenBank	GenBank	GenBank	GenBank
160	*Tragia arnhemica*	Russel-Smith 5235 (DNA)	Australia	GenBank	KP794409	GenBank	GenBank	GenBank	GenBank
161	*Tragia bahiensis* Müll.Arg.	Medeiros and Cardinal-McTeague 561 (R)	Brazil	—	—	—	—	GenBank	GenBank
162	*Tragia baroniana* Prain	Gillespie 10723 (CAN)	Madagascar	GenBank	GenBank	—	GenBank	GenBank	GenBank
163	*Tragia baroniana*	Gillespie 10748 (CAN)	Madagascar	GenBank	GenBank	—	GenBank	GenBank	GenBank
164	*Tragia benthamii* Baker	Bidgood 4648 (MO)	Tanzania	GenBank	KP794328	—	GenBank	GenBank	GenBank
165	*Tragia betonicifolia* Nutt.	Summers 9529 (MO)	USA	GenBank	KP794363	GenBank	GenBank	GenBank	GenBank
166	*Tragia betonicifolia*	Smith 3940 (MO)	USA	GenBank	KP794364	GenBank	GenBank	GenBank	GenBank
167	*Tragia biflora* Urb. & Ekman	Liogier 16491 (NY)	Dominican Republic	GenBank	GenBank	—	GenBank	GenBank	GenBank
168	*Tragia boiviniana* Müll.Arg.	Birkinshaw 21 (MO)	Madagascar	GenBank	—	—	—	GenBank	GenBank
169	*Tragia brevipes* Pax	Bidgood 4686 (MO)	Tanzania	GenBank	KP794325	—	—	GenBank	GenBank
170	*Tragia brevipes*	Rwaburindore 4488 (MO)	Uganda	GenBank	KP794329	—	—	GenBank	GenBank
171	*Tragia caperonoides* Pax & K.Hoffm.	Zardini and Gamarra 59470 (MO)	Paraguay	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
172	*Tragia cearensis* Pax & K.Hoffm.	Medeiros et al. # (R)	Brazil	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
173	*Tragia cearensis*	Medeiros et al. # (R)	Brazil	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
174	*Tragia cearensis*	Medeiros et al. # (R)	Brazil	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
175	*Tragia cf. bahiensis*	Zardini 52840 (MO)	Paraguay	—	KP794378	GenBank	GenBank	GenBank	GenBank
176	*Tragia cf. bahiensis*	Zardini 54517 (MO)	Paraguay	—	—	GenBank	—	—	GenBank
177	*Tragia cf. cocculifolia*	McPherson and van der Werff 16441 (CAN)	Madagascar	GenBank	GenBank	—	GenBank	GenBank	GenBank
178	*Tragia cf. pacifica*	Castrejón 753 (MO)	Mexico	GenBank	KP794392	GenBank	GenBank	GenBank	GenBank
179	*Tragia cf. petiolaris*	ATBP 624 (MO)	Uganda	GenBank	KP794351	—	GenBank	GenBank	GenBank
180	*Tragia cf. pohlii*	Irwin 5514 (NY)	Brazil	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank

181	*Tragia cf. subhastata*	Serrano 5264 (MO)	Bolivia	GenBank	KP794359	—	—	GenBank	GenBank
182	*Tragia cf. volubilis*	McDaniel and Rimachi 19854 (MO)	Peru, Loreto	GenBank	GenBank	—	—	GenBank	GenBank
183	*Tragia cf. yucatanensis*	Wallnöfer 5898 (MO)	Guatemala	GenBank	KP794381	—	—	GenBank	GenBank
184	*Tragia cf. yucatanensis*	Álvarez 6623 (MO)	Mexico	GenBank	KP794383	—	GenBank	GenBank	GenBank
185	*Tragia chlorocaulon* Baill.	Dawson 15122 (MO)	Brazil	GenBank	KP794390	GenBank	GenBank	GenBank	GenBank
186	*Tragia chlorocaulon*	Irwin 15858 (MO)	Brazil	GenBank	KP794391	GenBank	GenBank	GenBank	GenBank
187	*Tragia chlorocaulon*	Medeiros et al. # (R)	Brazil	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
188	*Tragia cocculifolia* Prain	Razanatsoa 250 (MO)	Madagascar	GenBank	KP794331	—	—	GenBank	GenBank
189	*Tragia cordata* Michx.	McDaniel 28890 (MO)	USA	GenBank	KP794395	GenBank	GenBank	GenBank	GenBank
190	*Tragia cordata*	Thomas 171769 (MO)	USA	GenBank	KP794396	GenBank	GenBank	GenBank	GenBank
191	*Tragia cordata*	Thomas 76208 (CAN)	USA	GenBank	KP794394	GenBank	GenBank	GenBank	GenBank
192	*Tragia dinteri* Pax	Seydel 2830 (L)	Namibia	GenBank	GenBank	—	—	GenBank	GenBank
193	*Tragia dioica* Sond.	Volk 6173 (MO)	Namibia	GenBank	KP794332	—	—	GenBank	GenBank
194	*Tragia dioica*	Seydel 5539 (MO)	Namibia	GenBank	KP794335	—	GenBank	GenBank	GenBank
195	*Tragia durbanensis* Kuntze	Balsinhas 3095 (MO)	South Africa	GenBank	KP794333	—	—	GenBank	GenBank
196	*Tragia finalis* P.I.Forst.	Gillespie 7390 (CAN)	Australia	GenBank	KP794411	GenBank	GenBank	GenBank	GenBank
197	*Tragia furialis* Bojer ex Prain	Kayombo 5098 (MO)	Tanzania	—	KP794340	—	—	—	GenBank
198	*Tragia furialis*	Barthlet 171 (MO)	Mayotte	—	KP794341	—	—	GenBank	GenBank
199	*Tragia furialis*	Nusbaumer 2725 (MO)	Madagascar	GenBank	KP794343	—	GenBank	GenBank	GenBank
200	*Tragia furialis*	Gillespie 10648 (CAN)	Madagascar	GenBank	KP794342	—	GenBank	GenBank	GenBank
201	*Tragia furialis*	Gillespie 10700 (CAN)	Madagascar	GenBank	GenBank	—	GenBank	GenBank	GenBank
202	*Tragia geraniifolia* Klotzsch ex Baill.	Krapovickas 20133 (MO)	Argentina	GenBank	KP794368	GenBank	GenBank	GenBank	GenBank
203	*Tragia geraniifolia*	Peña-Chocarro 2240 (MO)	Paraguay	GenBank	KP794370	GenBank	GenBank	GenBank	GenBank
204	*Tragia giardelliae* M.M.Gutiérrez & M.E.Múlgura	Mulgura 399 (NY)	Argentina	GenBank	GenBank	—	—	—	—
205	*Tragia giardelliae*	Guaglianone 2994 (MO)	Argentina	GenBank	KP794360	GenBank	GenBank	GenBank	GenBank
206	*Tragia glanduligera* Pax & K.Hoffm.	Tenorio 21300 (MO)	Mexico	—	—	—	—	GenBank	GenBank

207	*Tragia hieronymi* Pax & K.Hoffm.	Krapovickas 45464 (MO)	Paraguay	GenBank	—	GenBank	GenBank	GenBank	GenBank
208	*Tragia hieronymi*	Mendoza 474 (NY)	Bolivia	GenBank	GenBank	GenBank	—	GenBank	GenBank
209	*Tragia hildebrandtii* Müll.Arg.	Abdallah 96/157 (MO)	Tanzania	GenBank	KP794355	—	GenBank	GenBank	GenBank
210	*Tragia jonesii* Radcl.-Sm. & Govaerts	Felger 85-909 (MO)	Mexico	GenBank	KP794377	GenBank	GenBank	GenBank	GenBank
211	*Tragia laciniata* (Torr.) Müll.Arg.	Yatskievych 11-59 (MO)	Mexico	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
212	*Tragia laminularis* Müll.Arg.	Gautier-Béguin 959 (MO)	Côte d'Ivoire	GenBank	KP794348	—	GenBank	GenBank	GenBank
213	*Tragia laminularis*	Gautier-Béguin 592 (MO)	Côte d'Ivoire	GenBank	KP794349	—	GenBank	GenBank	GenBank
214	*Tragia melochioides* Griseb.	Zuloaga 4896 (MO)	Argentina	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
215	*Tragia melochioides*	Lozano 1784 (MO)	Bolivia	GenBank	KP794372	GenBank	GenBank	GenBank	GenBank
216	*Tragia melochioides*	Coca 50 (MO)	Bolivia	GenBank	KP794373	GenBank	GenBank	GenBank	GenBank
217	*Tragia melochioides*	Denham et al. 300 (SI)	Uruguay	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
218	*Tragia mexicana* Müll.Arg.	Peña 1029 (MO)	Belize	GenBank	KP794393	GenBank	GenBank	GenBank	GenBank
219	*Tragia minor* Sond.	Balsinhas 3631 (MO)	South Africa	GenBank	KP794338	—	GenBank	GenBank	GenBank
220	*Tragia nepetifolia* Cav.	García 446 (MO)	Mexico	GenBank	KP794366	GenBank	GenBank	GenBank	GenBank
221	*Tragia novae-hollandiae* Müll.Arg.	Gillespie 7394 (CAN)	Australia	GenBank	KP794417	GenBank	GenBank	GenBank	GenBank
222	*Tragia novae-hollandiae*	Bower S1 (CAN)	Australia	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
223	*Tragia okanyua* Pax	Schmidt 2323 (MO)	Zambia	GenBank	KP794337	—	GenBank	GenBank	GenBank
224	*Tragia okanyua*	Long 386 (MO)	Botswana	GenBank	KP794336	—	GenBank	GenBank	GenBank
225	*Tragia pacifica* McVaugh	Rosales 672 (MO)	El Salvador	GenBank	KP794389	GenBank	GenBank	GenBank	GenBank
226	*Tragia paxii* Lourteig & O'Donnell	Múlgura 2307 (MO)	Argentina	GenBank	KP794379	GenBank	GenBank	GenBank	GenBank
227	*Tragia paxii*	Johnson 920 (MO)	Argentina	GenBank	KP794380	GenBank	GenBank	GenBank	GenBank
228	*Tragia petiolaris* Radcl.-Sm.	Kuchar 25067 (MO)	Tanzania	GenBank	KP794354	—	GenBank	GenBank	GenBank
229	*Tragia petiolaris*	Greenway and Polhill 11556 (L)	Tanzania	GenBank	GenBank	—	GenBank	GenBank	GenBank
230	*Tragia pinnata* (Poir.) A.Juss.	Tressens 2202 (MO)	Argentina	GenBank	KP794369	GenBank	—	GenBank	GenBank
231	*Tragia plukenetii* Radcl.-Sm.	Rwaburindore 5745 (MO)	Uganda	GenBank	KP794356	—	GenBank	GenBank	GenBank

232	*Tragia plukenetii*	Burger 3768 (US)	Ethiopia	GenBank	GenBank	—	GenBank	GenBank	GenBank
233	*Tragia pohlii* Müll.Arg.	Eiten 4836 (UBC)	Brazil	GenBank	GenBank	GenBank	—	GenBank	GenBank
234	*Tragia polyandra* Vell.	Múlgura 2061 (MO)	Argentina	GenBank	KP794375	GenBank	—	GenBank	GenBank
235	*Tragia preussii* Pax	Nkongmeneck 1400 (MO)	Cameroon	GenBank	KP794352	—	GenBank	GenBank	GenBank
236	*Tragia pungens* (Forssk.) Müll.Arg.	De Wilde 6406 (MO)	Ethiopia	GenBank	KP794327	—	—	GenBank	GenBank
237	*Tragia pungens*	Bally 16026 (MO)	Somalia	GenBank	KP794326	—	—	GenBank	GenBank
238	*Tragia pungens*	Spellenberg 7547 (L)	Yemen	GenBank	GenBank	—	—	GenBank	GenBank
239	*Tragia ramosa* Torr.	Hill 18339 (MO)	USA	GenBank	KP794371	GenBank	GenBank	GenBank	GenBank
240	*Tragia ramosa*	Ricketson 4600 (MO)	USA	GenBank	KP794367	GenBank	GenBank	GenBank	GenBank
241	*Tragia rogersii* Prain	Ellery 92/96 (MO)	South Africa	GenBank	KP794339	—	GenBank	GenBank	GenBank
242	*Tragia rupestris* Sond.	Venter 9666 (MO)	South Africa	GenBank	KP794334	—	GenBank	GenBank	GenBank
243	*Tragia saxicola* Small	Conell 40056 (MO)	USA	GenBank	KP794386	—	GenBank	GenBank	GenBank
244	*Tragia saxicola*	Kral 51842 (MO)	USA	GenBank	KP794387	—	GenBank	GenBank	GenBank
245	*Tragia smallii* Shinners	McDaniel 28193 (MO)	USA	GenBank	KP794401	—	—	GenBank	GenBank
246	*Tragia smallii*	McDaniel 28698 (MO)	USA	GenBank	KP794402	GenBank	—	GenBank	GenBank
247	*Tragia sp. aff. Zuckertia*	Meier 1269 (MO)	Venezuela	GenBank	GenBank	—	—	GenBank	GenBank
248	*Tragia sp. Merida*	Ortega s.n. (CAN)	Mexico	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
249	*Tragia sp. peltate*	Aroujo-M. et al. 2315 (MO)	Bolivia	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
250	*Tragia sp. Ratiga*	Rodriguez and Tejada 2150 (MO)	El Salvador	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
251	*Tragia sp. Tagira*	Harder and Bingham 2582 (CAN)	Zambia	GenBank	GenBank	—	GenBank	GenBank	GenBank
252	*Tragia sp. nov. Fanambana*	Gillespie 10671 (CAN)	Madagascar	—	GenBank	—	GenBank	GenBank	GenBank
253	*Tragia sp. nov. Fanambana*	Ranirison and Nusbaumer 456 (G)	Madagascar	GenBank	GenBank	—	—	GenBank	GenBank
254	*Tragia sp. nov. Razakamalala*	Razakamalala 2878 (MO)	Madagascar	GenBank	GenBank	—	—	GenBank	GenBank
255	*Tragia sp. nov. Sahaka*	Gillespie 10681 (CAN)	Madagascar	GenBank	GenBank	—	GenBank	GenBank	GenBank
256	*Tragia spathulata* Benth.	Breteler 7083 (MO)	Togo	GenBank	KP794350	—	—	GenBank	GenBank
257	*Tragia subsessilis* Pax	Vollesen 96/44 (MO)	Tanzania	GenBank	KP794345	—	—	GenBank	GenBank
258	*Tragia tenuifolia* Benth.	Gautier 2266 (MO)	Côte d'Ivoire	GenBank	KP794353	—	GenBank	GenBank	GenBank

259	*Tragia tiverneana* Leandri	Rakotozafy et al. 55 (CAN)	Madagascar	GenBank	GenBank	—	GenBank	GenBank	GenBank
260	*Tragia tiverneana*	Hanitrarivo 325 (CAN)	Madagascar	GenBank	GenBank	—	GenBank	GenBank	GenBank
261	*Tragia tristis* Müll.Arg.	Anderson 9117 (NY)	Brazil	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
262	*Tragia uberbana* Müll.Arg.	Zuloaga 5785 (MO)	Argentina	GenBank	KP794374	GenBank	GenBank	GenBank	GenBank
263	*Tragia urens* L.	Thomas 55051, 1745, 830 (CAN)	USA	GenBank	KP794403	GenBank	GenBank	GenBank	GenBank
264	*Tragia urens*	Thomas 100075 (MO)	USA	GenBank	KP794404	GenBank	—	GenBank	GenBank
265	*Tragia urens*	Thomas 124328 (MO)	USA	GenBank	KP794405	GenBank	GenBank	GenBank	GenBank
266	*Tragia urens*	Kral 41155 (MO)	USA	GenBank	KP794406	GenBank	GenBank	GenBank	GenBank
267	*Tragia urens*	Anderson 7188 (MO)	USA	GenBank	KP794407	GenBank	—	GenBank	GenBank
268	*Tragia urens*	Mayfield 2006 (MO)	USA	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
269	*Tragia urens*	Abbott 22604 (MO)	USA	GenBank	GenBank	GenBank	—	GenBank	GenBank
270	*Tragia urticifolia* Michx.	Thomas 97259 (CAN)	USA	GenBank	KP794365	GenBank	GenBank	GenBank	GenBank
271	*Tragia volubilis* L.	Stevens 29658 (MO)	Nicaragua	GenBank	KP794361	GenBank	GenBank	GenBank	GenBank
272	*Tragia volubilis*	Hammel 19108 (MO)	Costa Rica	GenBank	KP794362	GenBank	GenBank	GenBank	GenBank
273	*Tragia volubilis*	Medeiros and Cardinal-McTeague 556 (R)	Brazil	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
274	*Tragia volubilis*	Medeiros and Cardinal-McTeague 563 (R)	Brazil	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
275	*Tragia volubilis*	Stevens and Montiel 34954 (MO)	Nicaragua	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
276	*Tragia volubilis*	Peñarando et al. 651 (MO)	Bolivia	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
277	*Tragia volubilis*	Villalobos et al. 1251 (MO)	Bolivia	GenBank	GenBank	—	GenBank	GenBank	GenBank
278	*Tragia volubilis*	Medeiros et al. # (R)	Brazil	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
279	*Tragia volubilis*	Medeiros et al. # (R)	Brazil	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank
280	*Tragia yucatanensis* Millsp.	Christenhusz 5678 (MO)	Guatemala	GenBank	KP794384	—	GenBank	GenBank	GenBank
281	*Tragia yucatanensis*	Lira 420 (MO)	Mexico	GenBank	KP794385	—	GenBank	GenBank	GenBank
282	*Tragia yucatanensis*	Alvarez 4398 (MO)	Mexico	GenBank	KP794382	GenBank	GenBank	GenBank	GenBank
283	*Tragiella anomala* (Prain) Pax & K.Hoffm.	Mwasumbi 16202 (MO)	Tanzania	GenBank	KP794323	—	GenBank	GenBank	GenBank
284	*Tragiella anomala*	Luke 11120 (MO)	Tanzania	GenBank	KP794324	—	GenBank	GenBank	GenBank

285	*Tragiella friesiana* (Prain) Pax & K.Hoffm.	Nkhoma 74 (MO)	Zambia	GenBank	KP794347	—	GenBank	GenBank	GenBank
286	*Tragiella natalensis* (Sond.) Pax & K.Hoffm.	Massawe 519 (MO)	Tanzania	GenBank	KP794330	—	GenBank	GenBank	GenBank
287	*Zuckertia cordata* Baill.	González 1045 (MO)	Costa Rica	GenBank	KP794399	GenBank	GenBank	GenBank	GenBank
288	*Zuckertia cordata* Baill.	Calzada 1544 (MO)	Mexico	GenBank	KP794400	GenBank	GenBank	GenBank	GenBank
289	*Zuckertia manuelii* (V.W.Steinm. & Ram.-Amezcua) Card.-McTeag. & L.J.Gillespie	Steinmann et al. 6303 (CAN)	Mexico	GenBank	GenBank	GenBank	GenBank	GenBank	GenBank

Table 5.S2 Biogeographical distribution matrix for BIOGEOBEARS analyses of Plukenetieae (A = Central + South America, B = North America, C = Caribbean, D = Africa + South Arabia, E = Madagascar, F = South + Southeast Asia; G = Australasia).

No.	Taxon	A	B	C	D	E	F	G
1	*Acidoton microphylla*	0	0	1	0	0	0	0
2	*Acidoton urens*	0	0	1	0	0	0	0
3	*Adenophaedra grandifolia*	1	0	0	0	0	0	0
4	*Bernardia dodecandra*	1	1	0	0	0	0	0
5	*Bernardia nicaraguensis*	1	0	0	0	0	0	0
6	*Bernardia pulchella*	1	0	0	0	0	0	0
7	*Bernardia scabra*	1	0	0	0	0	0	0
8	*Bia alienata*	1	0	0	0	0	0	0
9	*Bia* cf. *lessertiana*	1	0	0	0	0	0	0
10	*Bia fendleri*	1	0	0	0	0	0	0
11	*Bia lessertiana*	1	0	0	0	0	0	0
12	*Caperonia palustris*	1	1	1	1	1	0	0
13	*Caryodendron angustifolia*	1	0	0	0	0	0	0
14	*Caryodendron grandifolium*	1	0	0	0	0	0	0
15	*Caryodendron janeirense*	1	0	0	0	0	0	0
16	*Caryodendron orinocense*	1	0	0	0	0	0	0
17	*Cnesmone javanica*	0	0	0	0	0	1	0
18	*Cnesmone laevis*	0	0	0	0	0	1	0
19	*Cnesmone laotica*	0	0	0	0	0	1	0
20	*Cnesmone tonkinensis*	0	0	0	0	0	1	0
21	*Ctenomeria capensis*	0	0	0	1	0	0	0
22	*Dalechampia* aff. *tamifolia*	0	0	0	1	1	1	0
23	*Dalechampia aristolochiifolia*	1	0	0	0	0	0	0
24	*Dalechampia bernieri* var. *denisiana*	0	0	0	1	1	1	0
25	*Dalechampia clematidifolia*	0	0	0	1	1	1	0
26	*Dalechampia dioscoreifolia*	1	0	0	0	0	0	0
27	*Dalechampia pernambucensis*	1	0	1	0	0	0	0
28	*Dalechampia* sp. 311	1	0	0	0	0	0	0
29	*Dalechampia* sp. 363	1	0	0	0	0	0	0
30	*Dalechampia* sp. 364	1	0	0	0	0	0	0
31	*Dalechampia spathulata*	1	0	0	0	0	0	0
32	*Dalechampia subternata*	0	0	0	1	1	1	0
33	*Dalechampia tiliifolia*	1	0	0	0	0	0	0
34	*Dalechampia websteri*	1	0	0	0	0	0	0
35	*Ditaxis argothamnoides*	1	1	1	0	0	0	0

36	*Gitara nicaraguensis*	1	0	0	0	0	0	0
37	*Haematostemon guianensis*	1	0	0	0	0	0	0
38	*Lasiocroton bahamensis*	1	1	1	0	0	0	0
39	*Megistostigma peltatum*	0	0	0	0	0	1	0
40	*Megistostigma yunnanense*	0	0	0	0	0	1	0
41	*Pachystylidium hirsutum*	0	0	0	0	0	1	0
42	*Philyra brasiliensis*	1	0	0	0	0	0	0
43	*Platygyna hexandra*	0	0	1	0	0	0	0
44	*Platygyna parvifolia*	0	0	1	0	0	0	0
45	*Plukenetia* aff. *volubilis*	1	0	0	0	0	0	0
46	*Plukenetia africana*	0	0	0	1	0	0	0
47	*Plukenetia ankaranensis*	0	0	0	0	1	0	0
48	*Plukenetia brachybotrya*	1	0	0	0	0	0	0
49	*Plukenetia carabiasias*	1	0	0	0	0	0	0
50	*Plukenetia carolis-vegae* subsp. *nectarifera*	1	0	0	0	0	0	0
51	*Plukenetia* cf. *penninervia*	1	0	0	0	0	0	0
52	*Plukenetia conophora*	0	0	0	1	0	0	0
53	*Plukenetia corniculata*	0	0	0	0	0	1	0
54	*Plukenetia decidua*	0	0	0	0	1	0	0
55	*Plukenetia huayllabambana*	1	0	0	0	0	0	0
56	*Plukenetia lehmanniana*	1	0	0	0	0	0	0
57	*Plukenetia loretensis*	1	0	0	0	0	0	0
58	*Plukenetia madagascariensis*	0	0	0	0	1	0	0
59	*Plukenetia megastyla*	1	0	0	0	0	0	0
60	*Plukenetia penninervia*	1	0	0	0	0	0	0
61	*Plukenetia polyadenia*	1	0	0	0	0	0	0
62	*Plukenetia serrata*	1	0	0	0	0	0	0
63	*Plukenetia stipellata*	1	0	0	0	0	0	0
64	*Plukenetia supraglandulosa*	1	0	0	0	0	0	0
65	*Plukenetia verrucosa*	1	0	0	0	0	0	0
66	*Plukenetia volubilis*	1	0	1	0	0	0	0
67	*Romanoa tamnoides*	1	0	0	0	0	0	0
68	*Sphaerostylis perrieri*	0	0	0	0	1	0	0
69	*Tragia chlorocaulon*	1	0	0	0	0	0	0
70	*Tragia* aff. *pacifica*	1	1	0	0	0	0	0
71	*Tragia* aff. *subhastata*	1	0	0	0	0	0	0
72	*Tragia* aff. *volubilis*	1	0	0	0	0	0	0
73	*Tragia* aff. *Zuckertia*	1	0	0	0	0	0	0
74	*Tragia amblyodonta*	0	1	0	0	0	0	0

75	*Tragia arabica*	0	0	0	1	0	0	0
76	*Tragia arnhemica*	0	0	0	0	0	0	1
77	*Tragia baroniana*	0	0	0	0	1	0	0
78	*Tragia benthamii*	0	0	0	1	0	0	0
79	*Tragia bentonicifolia*	0	1	0	0	0	0	0
80	*Tragia biflora*	0	0	1	0	0	0	0
81	*Tragia boiviniana*	0	0	0	0	1	0	0
82	*Tragia brevipes*	0	0	0	1	0	0	0
83	*Tragia caperonioides*	1	0	0	0	0	0	0
84	*Tragia cearensis*	1	0	0	0	0	0	0
85	*Tragia* cf. *bahiensis*	1	0	0	0	0	0	0
86	*Tragia* cf. *pacifica*	1	1	0	0	0	0	0
87	*Tragia* cf. *petiolaris*	0	0	0	1	0	0	0
88	*Tragia* cf. *volubilis*	1	0	0	0	0	0	0
89	*Tragia chlorocaulon*	1	0	0	0	0	0	0
90	*Tragia chlorocaulon*	1	0	0	0	0	0	0
91	*Tragia cocculifolia*	0	0	0	0	1	0	0
92	*Tragia cordata*	0	1	0	0	0	0	0
93	*Tragia dinteri*	0	0	0	0	0	0	0
94	*Tragia dioica*	0	0	0	1	0	0	0
95	*Tragia dioica*	0	0	0	1	0	0	0
96	*Tragia durbanensis*	0	0	0	1	0	0	0
97	*Tragia finalis*	0	0	0	0	0	0	1
98	*Tragia furialis*	0	0	0	1	1	0	0
99	*Tragia geraniifolia*	1	0	0	0	0	0	0
100	*Tragia giardelliae*	1	0	0	0	0	0	0
101	*Tragia hieronymi*	1	0	0	0	0	0	0
102	*Tragia hildebrandtii*	0	0	0	1	0	0	0
103	*Tragia jonesii*	1	1	0	0	0	0	0
104	*Tragia laciniata*	0	1	0	0	0	0	0
105	*Tragia laminularis*	0	0	0	1	0	0	0
106	*Tragia melochioides*	1	0	0	0	0	0	0
107	*Tragia melochioides*	1	0	0	0	0	0	0
108	*Tragia mexicana*	1	1	0	0	0	0	0
109	*Tragia minor*	0	0	0	1	0	0	0
110	*Tragia natalensis*	0	0	0	1	0	0	0
111	*Tragia nepetifolia*	1	1	0	0	0	0	0
112	*Tragia novae-hollandiae*	0	0	0	0	0	0	1
113	*Tragia okanyua*	0	0	0	1	0	0	0

114	*Tragia pacifica*	1	1	0	0	0	0	0
115	*Tragia paxii*	1	0	0	0	0	0	0
116	*Tragia petiolaris*	0	0	0	1	0	0	0
117	*Tragia pinnata*	1	0	0	0	0	0	0
118	*Tragia plukenetii*	0	0	0	1	0	0	0
119	*Tragia polyandra*	1	0	0	0	0	0	0
120	*Tragia preussii*	0	0	0	1	0	0	0
121	*Tragia pungens*	0	0	0	1	0	0	0
122	*Tragia ramosa*	1	1	0	0	0	0	0
123	*Tragia ramosa*	1	1	0	0	0	0	0
124	*Tragia rogersii*	0	0	0	1	0	0	0
125	*Tragia rupestris*	0	0	0	1	0	0	0
126	*Tragia saxicola*	0	1	0	0	0	0	0
127	*Tragia smallii*	0	1	0	0	0	0	0
128	*Tragia* sp. Merida	1	0	0	0	0	0	0
129	*Tragia* sp. nov. Fanambana	0	0	0	0	1	0	0
130	*Tragia* sp. nov. Razakalamala	0	0	0	0	1	0	0
131	*Tragia* sp. nov. Sahaka	0	0	0	0	1	0	0
132	*Tragia* sp. peltate	1	0	0	0	0	0	0
133	*Tragia* sp. Philippines	0	0	0	0	0	1	0
134	*Tragia* sp. *Tagira*	0	0	0	1	0	0	0
135	*Tragia spathulata*	0	0	0	1	0	0	0
136	*Tragia subsessilis*	0	0	0	1	0	0	0
137	*Tragia tenuifolia*	0	0	0	1	0	0	0
138	*Tragia tiverniana*	0	0	0	0	1	0	0
139	*Tragia uberabana*	1	0	0	0	0	0	0
140	*Tragia urens*	0	1	0	0	0	0	0
141	*Tragia urticifolia*	0	1	0	0	0	0	0
142	*Tragia volubilis*	1	0	1	0	0	0	0
143	*Tragia yucatanensis*	1	0	0	0	0	0	0
144	*Tragiella anomala*	0	0	0	1	0	0	0
145	*Tragiella friesiana*	0	0	0	1	0	0	0
146	*Zuckertia cordata*	1	0	0	0	0	0	0
147	*Zuckertia manuelii*	1	0	0	0	0	0	0

Table 5.S3 Manual dispersal matrix between biogeographical areas for two time periods for BIOGEOBEARS analyses of Plukenetieae (adjacent = 1.0, short-distance dispersal = 0.5, long-distance dispersal = 0.1).

Modern Day 30–0 Mya	Neotropics + South America A	North America B	Caribbean C	Africa + South Arabia D	Madagascar E	South + Southeast Asia F	Australasia G
Neotropics + South America	1	-	-	-	-	-	-
North America	1.0	1	-	-	-	-	-
Caribbean	0.5	0.5	1	-	-	-	-
Africa + South Arabia	0.1	0.1	0.1	1	-	-	-
Madagascar	0.1	0.1	0.1	0.5	1	-	-
Southeast Asia	0.1	0.1	0.1	0.5	0.1	1	-
Australasia	0.1	0.1	0.1	0.1	0.1	1.0	1

Tropical fragments 60–30 Mya	Central + South America A	North America B	Caribbean C	Africa + South Arabia D	Madagascar E	South + Southeast Asia F	Australasia G
Central + South America	1	-	-	-	-	-	-
North America	0.5	1	-	-	-	-	-
Caribbean	0.5	0.5	1	-	-	-	-
Africa + South Arabia	0.1	0.1	0.1	1	-	-	-
Madagascar	0.1	0.1	0.1	0.5	1	-	-
Southeast Asia	0.1	0.1	0.1	0.1	0.1	1	-
Australasia	0.5	0.1	0.1	0.1	0.1	0.1	1

Table 5.S4 Proportion of extant species sampled per designated terminal for BAMM analysis of Plukenetieae.

No.	Taxon	Terminal	Proportion of terminal sampled
1	*Acidoton microphylla*	Acid	0.4
2	*Acidoton urens*	Acid	0.4
3	*Adenophaedra grandifolia*	Aden	0.25
4	*Bernardia dodecandra*	Bern	0.057
5	*Bernardia nicaraguensis*	Bern	0.057
6	*Bernardia pulchella*	Bern	0.057
7	*Bernardia scabra*	Bern	0.057
8	*Bia alienata*	Bia	0.8
9	*Bia* cf. *lessertiana*	Bia	0.8
10	*Bia fendleri*	Bia	0.8
11	*Bia lessertiana*	Bia	0.8
12	*Caperonia palustris*	Cape	0.029
13	*Caryodendron angustifolia*	Cary	0.667
14	*Caryodendron grandifolium*	Cary	0.667
15	*Caryodendron janeirense*	Cary	0.667
16	*Caryodendron orinocense*	Cary	0.667
17	*Ctenomeria capensis*	Cten	0.5
18	*Dalechampia* aff. *tamifolia*	Dale	0.108
19	*Dalechampia aristolochiifolia*	Dale	0.108
20	*Dalechampia bernieri* var. *denisiana*	Dale	0.108
21	*Dalechampia clematidifolia*	Dale	0.108
22	*Dalechampia dioscoreifolia*	Dale	0.108
23	*Dalechampia pernambucensis*	Dale	0.108
24	*Dalechampia* sp. 311	Dale	0.108
25	*Dalechampia* sp. 363	Dale	0.108
26	*Dalechampia* sp. 364	Dale	0.108
27	*Dalechampia spathulata*	Dale	0.108
28	*Dalechampia subternata*	Dale	0.108
29	*Dalechampia tiliifolia*	Dale	0.108
30	*Dalechampia websteri*	Dale	0.108
31	*Ditaxis argothamnoides*	Dita	0.013
32	*Tragia* sp. Merida	GenMer	1
33	*Tragia* sp. peltate	GenPel	1
34	*Tragia* aff. *Zuckertia*	GenZuc	1
35	*Gitara nicaraguensis*	Gita	1
36	*Haematostemon guianensis*	Haem	0.25
37	*Lasiocroton bahamensis*	Lasio	0.019

38	*Tragia smallii*	LepSma	1
39	*Tragia urens*	LepUre	1
40	*Plukenetia brachybotrya*	P2	0.778
41	*Plukenetia* cf. *penninervia*	P2	0.778
42	*Plukenetia loretensis*	P2	0.778
43	*Plukenetia megastyla*	P2	0.778
44	*Plukenetia penninervia*	P2	0.778
45	*Plukenetia supraglandulosa*	P2	0.778
46	*Plukenetia verrucosa*	P2	0.778
47	*Pachystylidium hirsutum*	PacHir	1
48	*Philyra brasiliensis*	Phil	1
49	*Platygyna hexandra*	Plat	0.286
50	*Platygyna parvifolia*	Plat	0.286
51	*Plukenetia africana*	PluAfr	0.5
52	*Plukenetia ankaranensis*	PluAnk	1
53	*Plukenetia* aff. *volubilis*	PluBol	1
54	*Plukenetia carabiasias*	PluCar	1
55	*Plukenetia conophora*	PluCon	1
56	*Plukenetia corniculata*	PluCor	1
57	*Plukenetia decidua*	PluDec	1
58	*Plukenetia huayllabambana*	PluHua	1
59	*Plukenetia lehmanniana*	PluLeh	1
60	*Plukenetia madagascariensis*	PluMad	1
61	*Plukenetia carolis-vegae* subsp. *nectarifera*	PluNec	1
62	*Plukenetia polyadenia*	PluPol	1
63	*Plukenetia serrata*	PluSer	1
64	*Plukenetia stipellata*	PluSti	1
65	*Plukenetia volubilis*	PluVol	1
66	*Romanoa tamnoides*	Roma	1
67	*Cnesmone javanica*	SEAsia	0.353
68	*Cnesmone laevis*	SEAsia	0.353
69	*Cnesmone laotica*	SEAsia	0.353
70	*Cnesmone tonkinensis*	SEAsia	0.353
71	*Megistostigma peltatum*	SEAsia	0.353
72	*Megistostigma yunnanense*	SEAsia	0.353
73	*Sphaerostylis perrieri*	SphPer	1
74	*Tragia arabica*	Tagira	0.33
75	*Tragia baroniana*	Tagira	0.33
76	*Tragia benthamii*	Tagira	0.33

77	*Tragia boiviniana*	Tagira	0.33
78	*Tragia brevipes*	Tagira	0.33
79	*Tragia* cf. *petiolaris*	Tagira	0.33
80	*Tragia cocculifolia*	Tagira	0.33
81	*Tragia dinteri*	Tagira	0.33
82	*Tragia dioica*	Tagira	0.33
83	*Tragia dioica*	Tagira	0.33
84	*Tragia durbanensis*	Tagira	0.33
85	*Tragia furialis*	Tagira	0.33
86	*Tragia hildebrandtii*	Tagira	0.33
87	*Tragia laminularis*	Tagira	0.33
88	*Tragia minor*	Tagira	0.33
89	*Tragia natalensis*	Tagira	0.33
90	*Tragia okanyua*	Tagira	0.33
91	*Tragia petiolaris*	Tagira	0.33
92	*Tragia plukenetii*	Tagira	0.33
93	*Tragia preussii*	Tagira	0.33
94	*Tragia pungens*	Tagira	0.33
95	*Tragia rogersii*	Tagira	0.33
96	*Tragia rupestris*	Tagira	0.33
97	*Tragia* sp. nov. Fanambana	Tagira	0.33
98	*Tragia* sp. nov. Razakalamala	Tagira	0.33
99	*Tragia* sp. nov. Sahaka	Tagira	0.33
100	*Tragia* sp. *Tagira*	Tagira	0.33
101	*Tragia spathulata*	Tagira	0.33
102	*Tragia subsessilis*	Tagira	0.33
103	*Tragia tenuifolia*	Tagira	0.33
104	*Tragia tiverniana*	Tagira	0.33
105	*Tragiella anomala*	Tagira	0.33
106	*Tragiella friesiana*	Tagira	0.33
107	*Tragia arnhemica*	TraArn	1
108	*Tragia biflora*	TraBif	1
109	*Tragia finalis*	TraFin	1
110	*Tragia chlorocaulon*	Tragia	0.586
111	*Tragia* aff. *pacifica*	Tragia	0.586
112	*Tragia* aff. *subhastata*	Tragia	0.586
113	*Tragia* aff. *volubilis*	Tragia	0.586
114	*Tragia amblyodonta*	Tragia	0.586
115	*Tragia bentonicifolia*	Tragia	0.586

116	*Tragia caperonioides*	Tragia	0.586
117	*Tragia cearensis*	Tragia	0.586
118	*Tragia* cf. *bahiensis*	Tragia	0.586
119	*Tragia* cf. *pacifica*	Tragia	0.586
120	*Tragia* cf. *volubilis*	Tragia	0.586
121	*Tragia chlorocaulon*	Tragia	0.586
122	*Tragia chlorocaulon*	Tragia	0.586
123	*Tragia cordata*	Tragia	0.586
124	*Tragia geraniifolia*	Tragia	0.586
125	*Tragia giardelliae*	Tragia	0.586
126	*Tragia hieronymi*	Tragia	0.586
127	*Tragia jonesii*	Tragia	0.586
128	*Tragia laciniata*	Tragia	0.586
129	*Tragia melochioides*	Tragia	0.586
130	*Tragia melochioides*	Tragia	0.586
131	*Tragia mexicana*	Tragia	0.586
132	*Tragia nepetifolia*	Tragia	0.586
133	*Tragia pacifica*	Tragia	0.586
134	*Tragia paxii*	Tragia	0.586
135	*Tragia pinnata*	Tragia	0.586
136	*Tragia polyandra*	Tragia	0.586
137	*Tragia ramosa*	Tragia	0.586
138	*Tragia ramosa*	Tragia	0.586
139	*Tragia saxicola*	Tragia	0.586
140	*Tragia uberabana*	Tragia	0.586
141	*Tragia urticifolia*	Tragia	0.586
142	*Tragia volubilis*	Tragia	0.586
143	*Tragia yucatanensis*	Tragia	0.586
144	*Tragia novae-hollandiae*	TraNov	1
145	*Tragia* sp. Philippines	TraSp	1
146	*Zuckertia cordata*	ZucCor	1
147	*Zuckertia manuelii*	ZucMan	1

Table 5.S5 Trait matrix for HISSE analysis of Plukenetieae: Growth form (0 = non-twining; 1 = twining), plant defences (0 = non-stinging; 1 = stinging), pseudanthial inflorescences (0 = non-pseudanthial; 1 = pseudanthial). Accessions with a number were randomly added to the BEAST maximum clade credibility tree to balance undersampled clades (see methods).

No.	Taxon	Growth form	Plant defences	Pseudanthial inflorescence
1	*Acidoton microphylla*	0	1	0
2	*Acidoton urens*	0	1	0
3	*Adenophaedra grandifolia*	0	0	0
4	*Bernardia - 01*	0	0	0
5	*Bernardia - 02*	0	0	0
6	*Bernardia - 03*	0	0	0
7	*Bernardia - 04*	0	0	0
8	*Bernardia - 05*	0	0	0
9	*Bernardia - 06*	0	0	0
10	*Bernardia - 07*	0	0	0
11	*Bernardia - 08*	0	0	0
12	*Bernardia - 09*	0	0	0
13	*Bernardia - 10*	0	0	0
14	*Bernardia - 11*	0	0	0
15	*Bernardia - 12*	0	0	0
16	*Bernardia - 13*	0	0	0
17	*Bernardia - 14*	0	0	0
18	*Bernardia - 15*	0	0	0
19	*Bernardia - 16*	0	0	0
20	*Bernardia - 17*	0	0	0
21	*Bernardia - 18*	0	0	0
22	*Bernardia - 19*	0	0	0
23	*Bernardia - 20*	0	0	0
24	*Bernardia - 21*	0	0	0
25	*Bernardia - 22*	0	0	0
26	*Bernardia - 23*	0	0	0
27	*Bernardia - 24*	0	0	0
28	*Bernardia - 25*	0	0	0
29	*Bernardia - 26*	0	0	0
30	*Bernardia - 27*	0	0	0
31	*Bernardia - 28*	0	0	0
32	*Bernardia - 29*	0	0	0
33	*Bernardia - 30*	0	0	0
34	*Bernardia - 31*	0	0	0
35	*Bernardia - 32*	0	0	0

36	*Bernardia - 33*	0	0	0
37	*Bernardia - 34*	0	0	0
38	*Bernardia - 35*	0	0	0
39	*Bernardia - 36*	0	0	0
40	*Bernardia dodecandra*	0	0	0
41	*Bernardia nicaraguensis*	0	0	0
42	*Bernardia pulchella*	0	0	0
43	*Bernardia scabra*	0	0	0
44	*Bia alienata*	1	1	0
45	*Bia* cf. *lessertiana*	1	1	0
46	*Bia fendleri*	1	1	0
47	*Bia lessertiana*	1	1	0
48	*Caryodendron angustifolia*	0	0	0
49	*Caryodendron grandifolium*	0	0	0
50	*Caryodendron janeirense*	0	0	0
51	*Caryodendron orinocense*	0	0	0
52	*Cnesmone javanica*	1	1	0
53	*Cnesmone laevis*	1	1	0
54	*Cnesmone laotica*	1	1	0
55	*Cnesmone tonkinensis*	1	1	0
56	*Ctenomeria capensis*	1	1	0
57	*Dal 4-armed clade - 01*	1	1	1
58	*Dal 4-armed clade - 02*	1	1	1
59	*Dal 4-armed clade - 03*	1	1	1
60	*Dal 4-armed clade - 04*	1	1	1
61	*Dal 4-armed clade - 05*	1	1	1
62	*Dal 4-armed clade - 06*	1	1	1
63	*Dal 4-armed clade - 07*	1	1	1
64	*Dal 4-armed clade - 08*	1	1	1
65	*Dal 4-armed clade - 09*	1	1	1
66	*Dal 4-armed clade - 10*	1	1	1
67	*Dal 4-armed clade - 11*	1	1	1
68	*Dal 4-armed clade - 12*	1	1	1
69	*Dal 4-armed clade - 13*	1	1	1
70	*Dal 4-armed clade - 14*	1	1	1
71	*Dal 4-armed clade - 15*	1	1	1
72	*Dal 4-armed clade - 16*	1	1	1
73	*Dal 4-armed clade - 17*	1	1	1
74	*Dal 4-armed clade - 18*	1	1	1

75	*Dal 4-armed clade - 19*	1	1	1
76	*Dal 4-armed clade - 20*	1	1	1
77	*Dal 4-armed clade - 21*	1	1	1
78	*Dal 4-armed clade - 22*	1	1	1
79	*Dal 4-armed clade - 23*	1	1	1
80	*Dal 4-armed clade - 24*	1	1	1
81	*Dal 5-armed clade - 01*	1	1	1
82	*Dal 5-armed clade - 02*	1	1	1
83	*Dal 5-armed clade - 03*	1	1	1
84	*Dal 5-armed clade - 04*	1	1	1
85	*Dal 5-armed clade - 05*	1	1	1
86	*Dal 5-armed clade - 06*	1	1	1
87	*Dal 5-armed clade - 07*	1	1	1
88	*Dal 5-armed clade - 08*	1	1	1
89	*Dal 5-armed clade - 09*	1	1	1
90	*Dal 5-armed clade - 10*	1	1	1
91	*Dal 5-armed clade - 11*	1	1	1
92	*Dal 5-armed clade - 12*	1	1	1
93	*Dal 5-armed clade - 13*	1	1	1
94	*Dal 5-armed clade - 14*	1	1	1
95	*Dal 5-armed clade - 15*	1	1	1
96	*Dal 5-armed clade - 16*	1	1	1
97	*Dal 5-armed clade - 17*	1	1	1
98	*Dal 5-armed clade - 18*	1	1	1
99	*Dal 5-armed clade - 19*	1	1	1
100	*Dal 5-armed clade - 20*	1	1	1
101	*Dal 5-armed clade - 21*	1	1	1
102	*Dal 5-armed clade - 22*	1	1	1
103	*Dal 5-armed clade - 23*	1	1	1
104	*Dal 5-armed clade - 24*	1	1	1
105	*Dal 5-armed clade - 25*	1	1	1
106	*Dal 5-armed clade - 26*	1	1	1
107	*Dal 5-armed clade - 27*	1	1	1
108	*Dal 5-armed clade - 28*	1	1	1
109	*Dal 5-armed clade - 29*	1	1	1
110	*Dal 5-armed clade - 30*	1	1	1
111	*Dal 5-armed clade - 31*	1	1	1
112	*Dal 5-armed clade - 32*	1	1	1
113	*Dal 5-armed clade - 33*	1	1	1

114	*Dal 5-armed clade - 34*	1	1	1
115	*Dal 5-armed clade - 35*	1	1	1
116	*Dal 5-armed clade - 36*	1	1	1
117	*Dal 5-armed clade - 37*	1	1	1
118	*Dal 5-armed clade - 38*	1	1	1
119	*Dal 5-armed clade - 39*	1	1	1
120	*Dal 5-armed clade - 40*	1	1	1
121	*Dal 5-armed clade - 41*	1	1	1
122	*Dalechampia* aff. *tamifolia*	1	1	1
123	*Dalechampia aristolochiifolia*	1	1	1
124	*Dalechampia bernieri* var. *denisiana*	1	1	1
125	*Dalechampia clematidifolia*	1	1	1
126	*Dalechampia dioscoreifolia*	1	1	1
127	*Dalechampia pernambucensis*	1	1	1
128	*Dalechampia* sp. 311	1	1	1
129	*Dalechampia* sp. 363	1	1	1
130	*Dalechampia* sp. 364	1	1	1
131	*Dalechampia spathulata*	0	1	1
132	*Dalechampia subsessilis*	1	1	1
133	*Dalechampia tiliifolia*	1	1	1
134	*Dalechampia websteri*	1	1	1
135	*Gitara nicaraguensis*	0	1	0
136	*Haematostemon guianensis*	0	0	0
137	*Megistostigma peltatum*	1	1	0
138	*Megistostigma yunnanense*	1	1	0
139	*Pachystylidium hirsutum*	1	1	0
140	*Platygyna hexandra*	1	1	0
141	*Platygyna parvifolia*	1	1	0
142	*Plukenetia* aff. *volubilis*	1	0	0
143	*Plukenetia africana*	0	0	0
144	*Plukenetia ankaranensis*	1	0	0
145	*Plukenetia brachybotrya*	1	0	0
146	*Plukenetia carabiasias*	1	0	0
147	*Plukenetia carolis-vegae* subsp. *nectarifera*	1	0	0
148	*Plukenetia* cf. *penninervia*	1	0	0
149	*Plukenetia conophora*	1	0	0
150	*Plukenetia corniculata*	1	0	0
151	*Plukenetia decidua*	1	0	0
152	*Plukenetia huayllabambana*	1	0	0

153	*Plukenetia lehmanniana*	1	0	0
154	*Plukenetia loretensis*	1	0	0
155	*Plukenetia madagascariensis*	1	0	0
156	*Plukenetia megastyla*	1	0	0
157	*Plukenetia penninervia*	1	0	0
158	*Plukenetia polyadenia*	1	0	0
159	*Plukenetia serrata*	1	0	0
160	*Plukenetia stipellata*	1	0	0
161	*Plukenetia supraglandulosa*	1	0	0
162	*Plukenetia verrucosa*	1	0	0
163	*Plukenetia volubilis*	1	0	0
164	*Romanoa tamnoides*	1	0	0
165	*Sphaerostylis perrieri*	1	1	0
166	*Tra Subclade T3 - 01*	1	1	0
167	*Tra Subclade T3 - 02*	1	1	0
168	*Tra Subclade T3 - 03*	1	1	0
169	*Tra Subclade T3 - 04*	1	1	0
170	*Tra Subclade T3 - 05*	1	1	0
171	*Tra Subclade T3 - 06*	1	1	0
172	*Tra Subclade T3 - 07*	1	1	0
173	*Tra Subclade T3 - 08*	1	1	0
174	*Tra Subclade T3 - 09*	1	1	0
175	*Tra Subclade T3 - 10*	0	1	0
176	*Tra Subclade T3 - 11*	0	1	0
177	*Tra Subclade T3 - 12*	1	1	0
178	*Tra Subclade T3 - 13*	1	1	0
179	*Tra Subclade T3 - 14*	0	1	0
180	*Tra Subclade T3 - 15*	1	1	0
181	*Tra Subclade T3 - 16*	1	1	0
182	*Tra Subclade T3 - 17*	0	1	0
183	*Tra Subclade T3 - 18*	0	1	0
184	*Tra Subclade T3 - 19*	1	1	0
185	*Tra Subclade T3 - 20*	0	1	0
186	*Tra Subclade T3 - 21*	1	1	0
187	*Tra Subclade T3 - 22*	1	1	0
188	*Tra Subclade T3 - 23*	1	1	0
189	*Tra Subclade T3 - 24*	1	1	0
190	*Tra Subclade T3 - 25*	0	1	0
191	*Tra Subclade T3 - 26*	0	1	0

192	*Tra Subclade T3 - 27*	1	1	0
193	*Tra Subclade T3 - 28*	1	1	0
194	*Tra Subclade T3 - 29*	1	1	0
195	*Tra Subclade T3 - 30*	1	1	0
196	*Tra Subclade T3 - 31*	0	1	0
197	*Tra Subclade T3 - 32*	0	1	0
198	*Tra Subclade T3 - 33*	1	1	0
199	*Tragia* aff. *pacifica*	1	1	0
200	*Tragia* aff. *subhastata*	1	1	0
201	*Tragia* aff. *volubilis*	1	1	0
202	*Tragia* aff. *Zuckertia*	1	1	0
203	*Tragia amblyodonta*	0	1	0
204	*Tragia arabica*	1	1	0
205	*Tragia arnhemica*	1	1	0
206	*Tragia baroniana*	1	1	0
207	*Tragia benthamii*	1	1	0
208	*Tragia bentonicifolia*	0	1	0
209	*Tragia biflora*	0	1	0
210	*Tragia boiviniana*	1	1	0
211	*Tragia brevipes*	1	1	0
212	*Tragia caperonioides*	0	1	0
213	*Tragia cearensis*	1	1	0
214	*Tragia* cf. *bahiensis*	0	1	0
215	*Tragia* cf. *pacifica*	1	1	0
216	*Tragia* cf. *petiolaris*	1	1	0
217	*Tragia* cf. *volubilis*	1	1	0
218	*Tragia chlorocaulon*	1	1	0
219	*Tragia chlorocaulon*	1	1	0
220	*Tragia chlorocaulon*	1	1	0
221	*Tragia cocculifolia*	1	1	0
222	*Tragia cordata*	1	1	0
223	*Tragia dinteri*	1	1	0
224	*Tragia dioica*	0	1	0
225	*Tragia dioica*	0	1	0
226	*Tragia durbanensis*	1	1	0
227	*Tragia finalis*	1	1	0
228	*Tragia furialis*	1	1	0
229	*Tragia geraniifolia*	1	1	0
230	*Tragia giardelliae*	1	1	0

231	*Tragia hieronymi*	0	1	0
232	*Tragia hildebrandtii*	0	1	0
233	*Tragia jonesii*	0	1	0
234	*Tragia laciniata*	0	1	0
235	*Tragia laminularis*	1	1	0
236	*Tragia melochioides*	0	1	0
237	*Tragia melochioides*	0	1	0
238	*Tragia mexicana*	1	1	0
239	*Tragia minor*	0	1	0
240	*Tragia natalensis*	1	1	0
241	*Tragia nepetifolia*	0	1	0
242	*Tragia novae-hollandiae*	1	1	0
243	*Tragia okanyua*	1	1	0
244	*Tragia pacifica*	1	1	0
245	*Tragia paxii*	1	1	0
246	*Tragia petiolaris*	1	1	0
247	*Tragia pinnata*	0	1	0
248	*Tragia plukenetii*	0	1	0
249	*Tragia polyandra*	1	1	0
250	*Tragia preussii*	1	1	0
251	*Tragia pungens*	0	1	0
252	*Tragia ramosa*	0	1	0
253	*Tragia ramosa*	0	1	0
254	*Tragia rogersii*	0	1	0
255	*Tragia rupestris*	1	1	0
256	*Tragia saxicola*	0	1	0
257	*Tragia smallii*	0	1	0
258	*Tragia* sp. *Merida*	1	1	0
259	*Tragia* sp. nov. Fanambana	1	1	0
260	*Tragia* sp. nov. Razakalamala	1	1	0
261	*Tragia* sp. nov. Sahaka	1	1	0
262	*Tragia* sp. peltate	1	1	0
263	*Tragia* sp. Philippines	1	1	0
264	*Tragia* sp. *Tagira*	1	1	0
265	*Tragia spathulata*	1	1	0
266	*Tragia subsessilis*	0	1	0
267	*Tragia tenuifolia*	0	1	0
268	*Tragia tiverniana*	1	1	0
269	*Tragia uberabana*	0	1	0

270	*Tragia urens*	0	1	0
271	*Tragia urticifolia*	0	1	0
272	*Tragia volubilis*	1	1	0
273	*Tragia yucatanensis*	0	1	0
274	*Tragiella anomala*	1	1	0
275	*Tragiella friesiana*	0	1	0
276	*Zuckertia cordata*	1	1	0
277	*Zuckertia manuelii*	1	1	0

Table 5.S6 The fit of alternative models of trait-dependent diversification in Plukenetieae, with best models selected by ΔAIC and Akaike weights (w_i) in bold.

Growth Form:									
Model No.	Model Type	Net turnover (τ)	Extinction fraction (ε)	Transition rate (*q*)	np	lnL	AIC	ΔAIC	w_i
1	BiSSE	τ's free	ε's free	*q*'s free	6	-801.6	1615.2	57.20	0.000
2	BiSSE	τ's free	ε0=ε1	*q*'s free	5	-803.5	1617.0	59.01	0.000
3	BiSSE	τ's free	ε's free	*q*'s equal	5	-803.6	1617.3	59.26	0.000
4	BiSSE	τ's free	ε0=ε1	*q*'s equal	4	-803.9	1615.9	57.90	0.000
5	CID-2	τ's free	ε's free	*q*'s equal	5	-786.1	1582.2	24.20	0.000
6	CID-2	τ's free	ε's equal	*q*'s equal	4	-788.8	1585.5	27.53	0.000
7	CID-4	τ's free	ε's free	*q*'s equal	9	-775.5	1568.9	10.94	0.004
8	CID-4	τ's free	ε's free	*q*'s equal	6	-779.7	1571.4	13.41	0.001
9	HiSSE	τ's free	ε's free	*q*'s equal	9	-787.8	1593.7	35.67	0.000
10	HiSSE	τ's free	ε's equal	*q*'s equal	6	-790.6	1593.2	35.17	0.000
11	HiSSE	τ0A=τ1A=τ0B	ε0A=ε1A=ε0B	*q*'s equal	5	-798.3	1606.6	48.62	0.000
12	HiSSE	τ0A=τ1A=τ0B	ε's equal	*q*'s equal	4	-796.5	1600.9	42.92	0.000
13	HiSSE	τ0A=τ0B	ε0A=ε0B	*q*'s equal	7	-790.2	1594.3	36.34	0.000
14	HiSSE	τ0A=τ0B	ε's equal	*q*'s equal	5	-790.7	1591.3	33.34	0.000
15	HiSSE	τ0A=τ1A	ε0A=ε1A	*q*'s equal	7	-788.0	1590.0	31.96	0.000
16	HiSSE	τ0A=τ1A	ε's equal	*q*'s equal	5	-791.6	1593.3	35.29	0.000
17	HiSSE	τ's free	ε's free	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	9	-775.7	1569.4	11.39	0.003
18	HiSSE	τ's free	ε's equal	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	6	-779.9	1571.8	13.77	0.001
19	HiSSE	τ0A=τ1A=τ0B	ε0A=ε1A=ε0B	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	5	-790.5	1591.0	33.01	0.000
20	HiSSE	τ0A=τ1A=τ0B	ε's equal	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	4	-790.7	1589.4	31.42	0.000
21	**HiSSE**	**τ0A=τ0B**	**ε0A=ε0B**	***q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal**	**7**	**-772.0**	**1558.0**	**0.00**	**0.982**
22	HiSSE	τ0A=τ0B	ε's equal	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	5	-778.8	1567.6	9.63	0.008
23	HiSSE	τ0A=τ1A	ε0A=ε1A	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	7	-786.8	1587.7	29.66	0.000
24	HiSSE	τ0A=τ1A	ε's equal	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	5	-786.7	1583.5	25.46	0.000

Plant Defences:

Model No.	Model Type	Net turnover (τ)	Extinction fraction (ε)	Transition rate (*q*)	np	lnL	AIC	ΔAIC	w_i
1	BiSSE	τ's free	ε's free	*q*'s free	6	-686.8	1385.6	38.59	0.000
2	BiSSE	τ's free	ε0=ε1	*q*'s free	5	-692.1	1394.3	47.26	0.000
3	BiSSE	τ's free	ε's free	*q*'s equal	5	-687.6	1385.1	38.12	0.000
4	BiSSE	τ's free	ε0=ε1	*q*'s equal	4	-688.0	1384.1	37.09	0.000
5	CID-2	τ's free	ε's free	*q*'s equal	5	-672.3	1354.6	7.62	0.011
6	CID-2	τ's free	ε's equal	*q*'s equal	4	-676.3	1360.6	13.57	0.001
7	CID-4	τ's free	ε's free	*q*'s equal	9	-667.7	1353.3	6.35	0.022
8	CID-4	τ's free	ε's free	*q*'s equal	6	-667.6	1347.3	0.30	0.445
9	HiSSE	τ's free	ε's free	*q*'s equal	9	-685.4	1388.8	41.80	0.000
10	HiSSE	τ's free	ε's equal	*q*'s equal	6	-685.8	1383.6	36.61	0.000
11	HiSSE	τ0A=τ1A=τ0B	ε0A=ε1A=ε0B	*q*'s equal	5	-694.3	1398.6	51.57	0.000
12	HiSSE	τ0A=τ1A=τ0B	ε's equal	*q*'s equal	4	-694.1	1396.2	49.21	0.000
13	HiSSE	τ0A=τ0B	ε0A=ε0B	*q*'s equal	7	-686.9	1387.8	40.77	0.000
14	HiSSE	τ0A=τ0B	ε's equal	*q*'s equal	5	-687.4	1384.8	37.85	0.000
15	HiSSE	τ0A=τ1A	ε0A=ε1A	*q*'s equal	7	-685.4	1384.9	37.90	0.000
16	HiSSE	τ0A=τ1A	ε's equal	*q*'s equal	5	-690.4	1390.7	43.75	0.000
17	**HiSSE**	**τ's free**	**ε's free**	***q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal**	**9**	**-664.5**	**1347.0**	**0.00**	**0.517**
18	HiSSE	τ's free	ε's equal	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	6	-675.2	1362.4	15.45	0.000
19	HiSSE	τ0A=τ1A=τ0B	ε0A=ε1A=ε0B	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	5	-673.6	1357.2	10.18	0.003
20	HiSSE	τ0A=τ1A=τ0B	ε's equal	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	4	-675.4	1358.8	11.78	0.001
21	HiSSE	τ0A=τ0B	ε0A=ε0B	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	7	-684.5	1383.0	35.96	0.000
22	HiSSE	τ0A=τ0B	ε's equal	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	5	-685.3	1380.6	33.61	0.000
23	HiSSE	τ0A=τ1A	ε0A=ε1A	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	7	-681.4	1376.8	29.77	0.000
24	HiSSE	τ0A=τ1A	ε's equal	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	5	-682.9	1375.9	28.89	0.000

Inflorescence Type:									
Model No.	Model Type	Net turnover (τ)	Extinction fraction (ε)	Transition rate (*q*)	np	lnL	AIC	ΔAIC	w_i
1	BiSSE	τ's free	ε's free	*q*'s free	6	-689.6	1391.1	49.25	0.000
2	BiSSE	τ's free	ε0=ε1	*q*'s free	5	-695.8	1401.5	59.63	0.000
3	BiSSE	τ's free	ε's free	*q*'s equal	5	-689.4	1388.7	46.83	0.000
4	BiSSE	τ's free	ε0=ε1	*q*'s equal	4	-689.2	1386.4	44.48	0.000
5	CID-2	τ's free	ε's free	*q*'s equal	5	-674.9	1359.7	17.85	0.000
6	CID-2	τ's free	ε's equal	*q*'s equal	4	-670.4	1348.7	6.86	0.031
7	CID-4	τ's free	ε's free	*q*'s equal	9	-665.7	1349.5	7.60	0.021
8	**CID-4**	**τ's free**	**ε's free**	***q*'s equal**	**6**	**-664.9**	**1341.9**	**0.00**	**0.947**
9	HiSSE	τ's free	ε's free	*q*'s equal	9	-676.7	1371.4	29.51	0.000
10	HiSSE	τ's free	ε's equal	*q*'s equal	6	-685.6	1383.3	41.38	0.000
11	HiSSE	τ0A=τ1A=τ0B	ε0A=ε1A=ε0B	*q*'s equal	5	-694.3	1398.6	56.76	0.000
12	HiSSE	τ0A=τ1A=τ0B	ε's equal	*q*'s equal	4	-692.2	1392.4	50.54	0.000
13	HiSSE	τ0A=τ0B	ε0A=ε0B	*q*'s equal	7	-689.2	1392.4	50.56	0.000
14	HiSSE	τ0A=τ0B	ε's equal	*q*'s equal	5	-689.1	1388.3	46.40	0.000
15	HiSSE	τ0A=τ1A	ε0A=ε1A	*q*'s equal	7	-671.2	1356.4	14.58	0.001
16	HiSSE	τ0A=τ1A	ε's equal	*q*'s equal	5	-673.8	1357.6	15.73	0.000
17	HiSSE	τ's free	ε's free	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	9	-679.0	1376.0	34.11	0.000
18	HiSSE	τ's free	ε's equal	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	6	-678.3	1368.6	26.68	0.000
19	HiSSE	τ0A=τ1A=τ0B	ε0A=ε1A=ε0B	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	5	-679.5	1369.0	27.13	0.000
20	HiSSE	τ0A=τ1A=τ0B	ε's equal	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	4	-692.1	1392.3	50.40	0.000
21	HiSSE	τ0A=τ0B	ε0A=ε0B	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	7	-689.7	1393.4	51.48	0.000
22	HiSSE	τ0A=τ0B	ε's equal	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	5	-688.0	1386.1	44.19	0.000
23	HiSSE	τ0A=τ1A	ε0A=ε1A	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	7	-674.2	1362.4	20.57	0.000
24	HiSSE	τ0A=τ1A	ε's equal	*q*0B1B = 0, *q*1B0B = 0, all other *q*'s equal	5	-682.6	1375.3	33.39	0.000

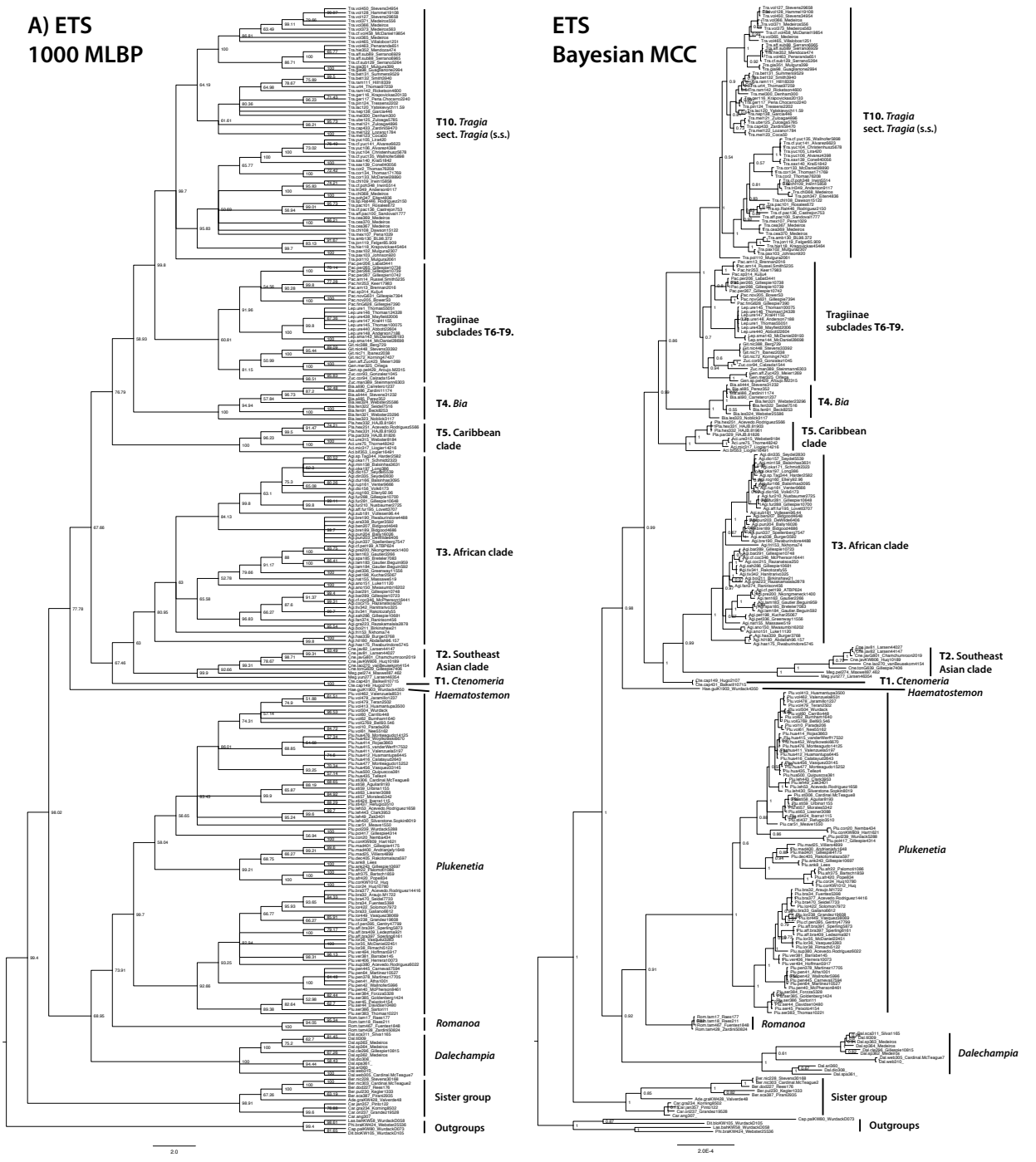


Figure 5.S1A Detailed cladograms and/or phylograms of Plukenetieae from individual and combined datasets labeled with maximum likelihood bootstrap percentages (MLBP) and/or Bayesian posterior probabilities (BPP): (a) ETS, (b) ITS, (c) *KEA1* intron 11, (d) *TEB* exon 17, (e) *matK*, and (f) *ndhF*, (g) cpDNA, (h) Concatenated, and (i) Concatenated and fully annotated.

Figure 5.S1B Detailed cladograms and/or phylograms of Plukenetieae from individual and combined datasets labeled with maximum likelihood bootstrap percentages (MLBP) and/or Bayesian posterior probabilities (BPP): (a) ETS, (b) ITS, (c) *KEA1* intron 11, (d) *TEB* exon 17, (e) *matK*, and (f) *ndhF*, (g) cpDNA, (h) Concatenated, and (i) Concatenated and fully annotated.

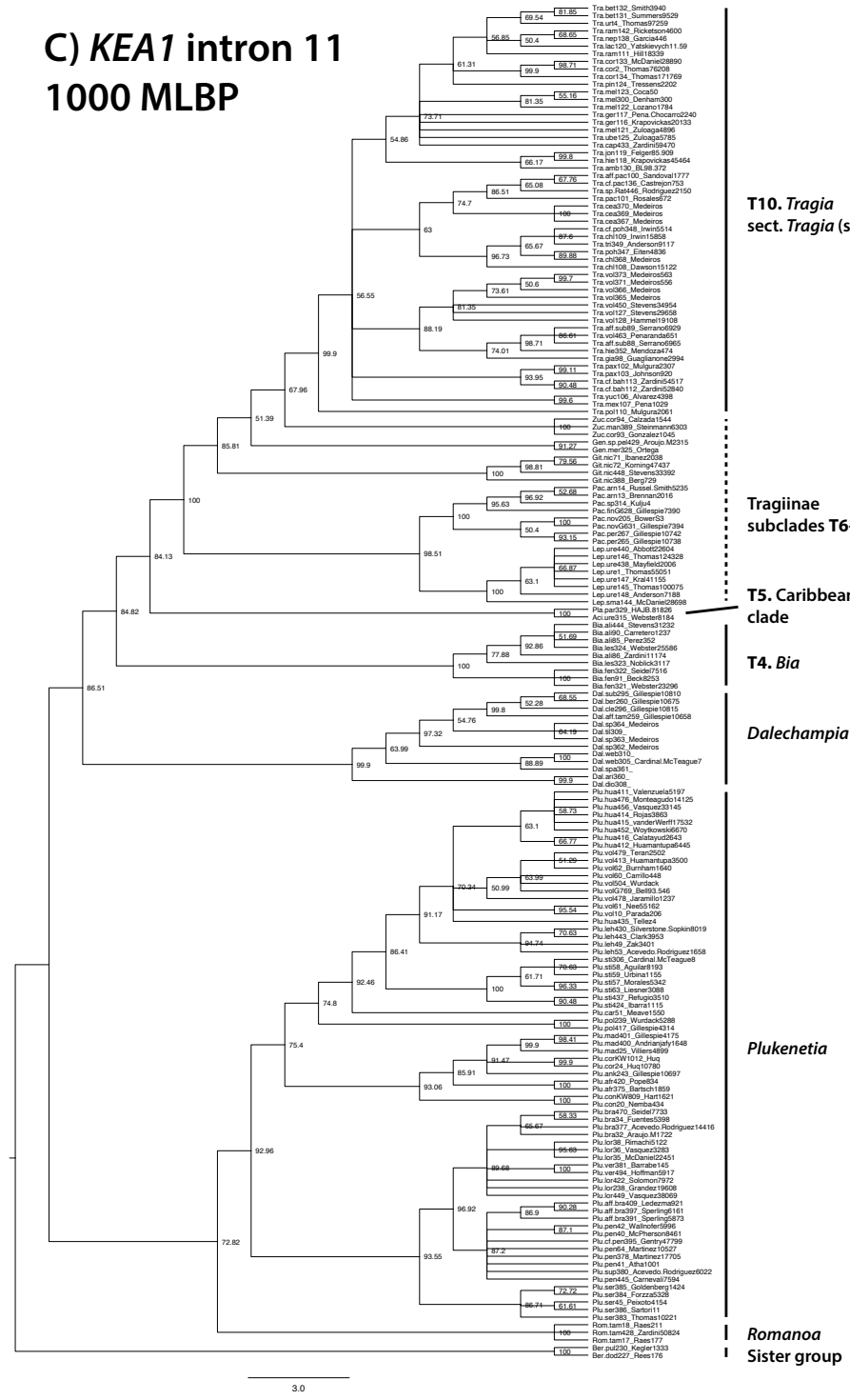


Figure 5.S1C Detailed cladograms and/or phylograms of Plukenetieae from individual and combined datasets labeled with maximum likelihood bootstrap percentages (MLBP) and/or Bayesian posterior probabilities (BPP): (a) ETS, (b) ITS, (c) *KEA1* intron 11, (d) *TEB* exon 17, (e) *matK*, and (f) *ndhF*, (g) cpDNA, (h) Concatenated, and (i) Concatenated and fully annotated.

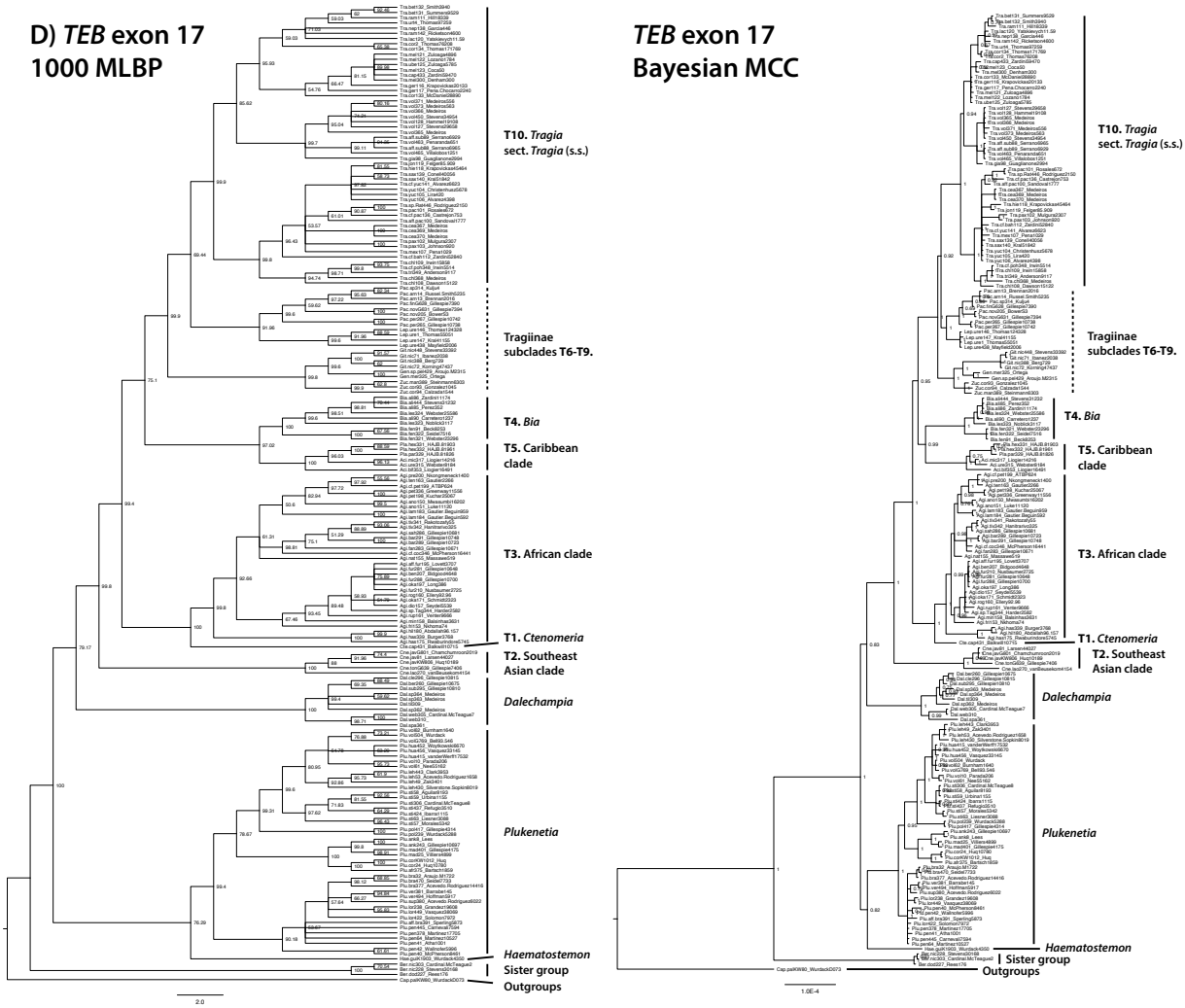


Figure 5.S1D Detailed cladograms and/or phylograms of Plukenetieae from individual and combined datasets labeled with maximum likelihood bootstrap percentages (MLBP) and/or Bayesian posterior probabilities (BPP): (a) ETS, (b) ITS, (c) *KEA1* intron 11, (d) *TEB* exon 17, (e) *matK*, and (f) *ndhF*, (g) cpDNA, (h) Concatenated, and (i) Concatenated and fully annotated.

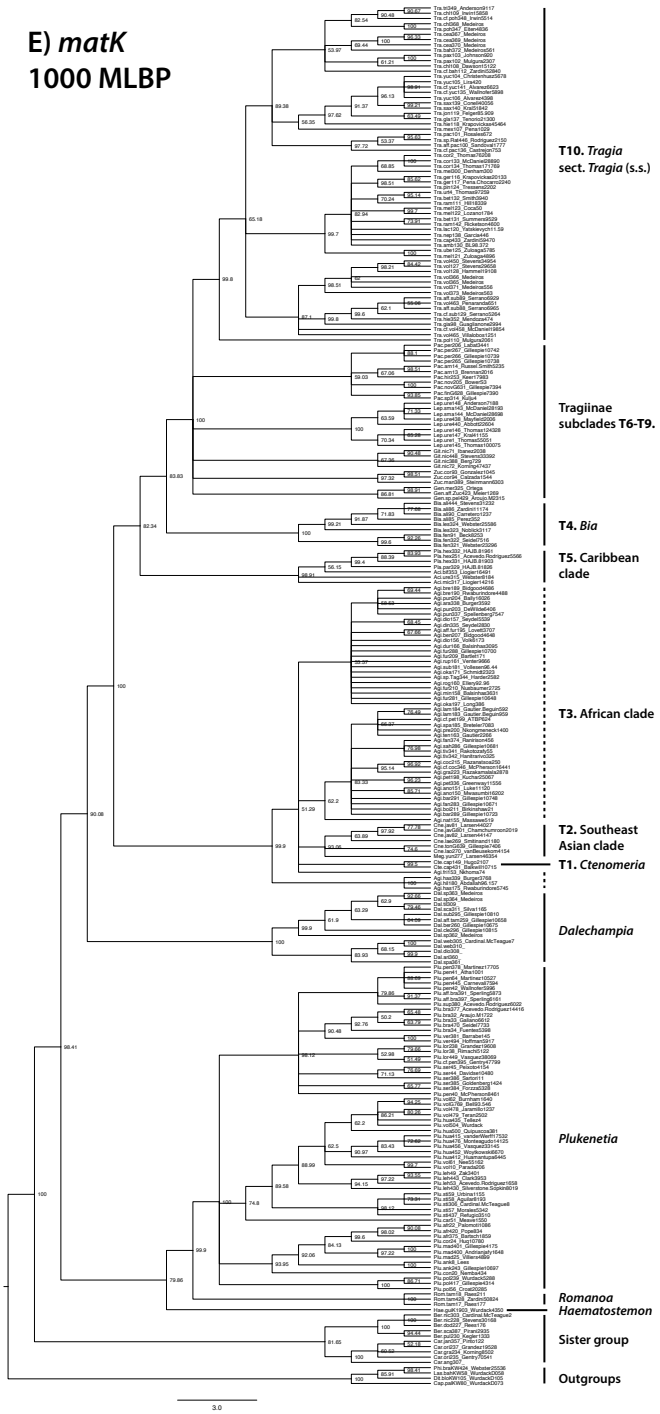


Figure 5.S1E Detailed cladograms and/or phylograms of Plukenetieae from individual and combined datasets labeled with maximum likelihood bootstrap percentages (MLBP) and/or Bayesian posterior probabilities (BPP): (a) ETS, (b) ITS, (c) *KEA1* intron 11, (d) *TEB* exon 17, (e) *matK*, and (f) *ndhF*, (g) cpDNA, (h) Concatenated, and (i) Concatenated and fully annotated.

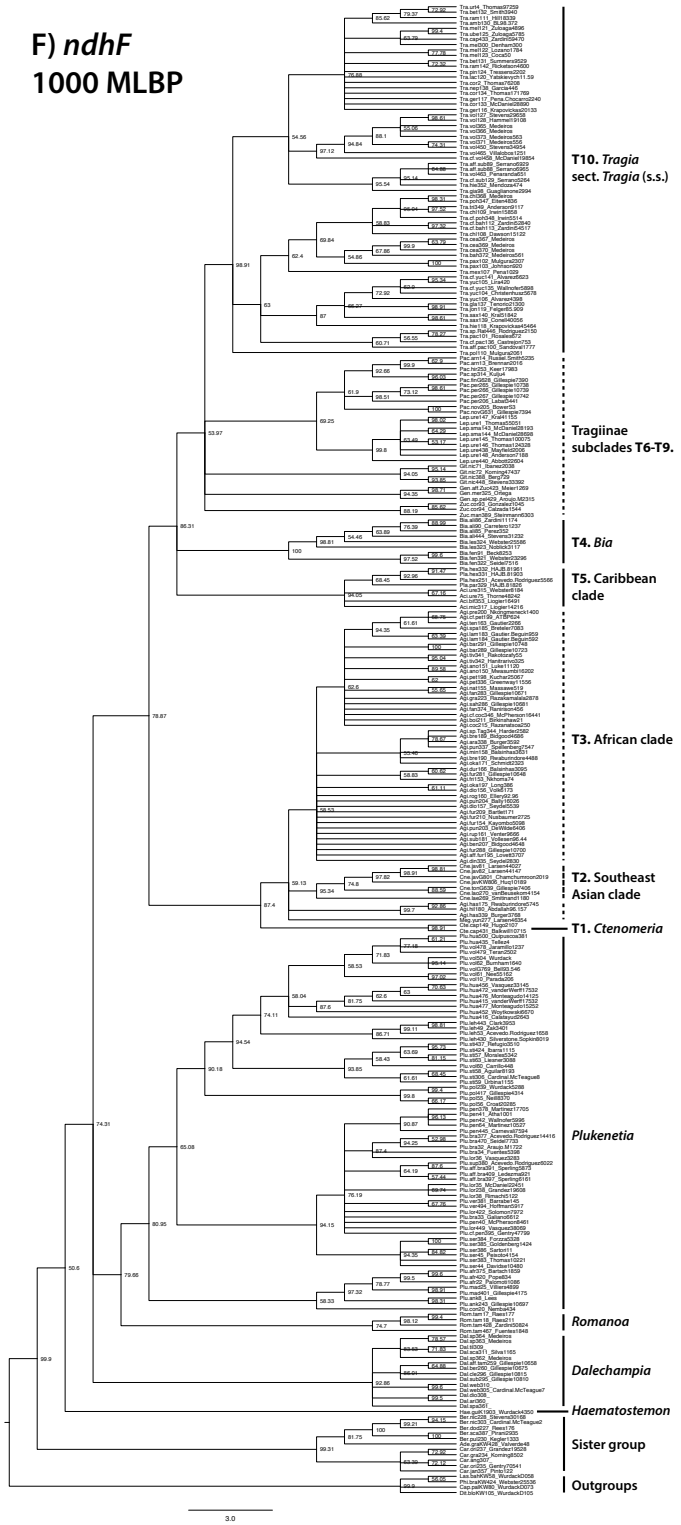


Figure 5.S1F Detailed cladograms and/or phylograms of Plukenetieae from individual and combined datasets labeled with maximum likelihood bootstrap percentages (MLBP) and/or Bayesian posterior probabilities (BPP): (a) ETS, (b) ITS, (c) *KEA1* intron 11, (d) *TEB* exon 17, (e) *matK*, and (f) *ndhF*, (g) cpDNA, (h) Concatenated, and (i) Concatenated and fully annotated.

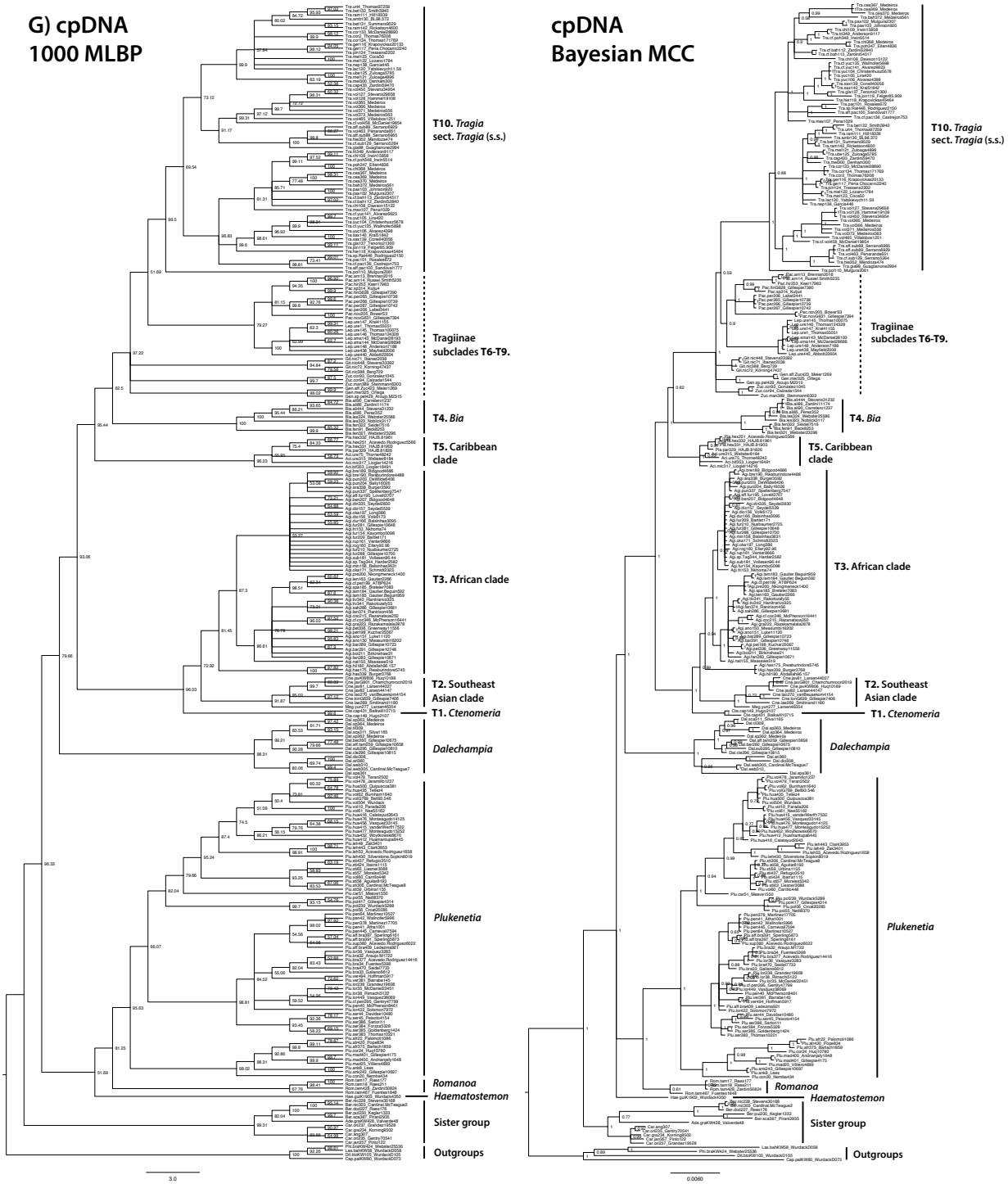


Figure 5.S1G Detailed cladograms and/or phylograms of Plukenetieae from individual and combined datasets labeled with maximum likelihood bootstrap percentages (MLBP) and/or Bayesian posterior probabilities (BPP): (a) ETS, (b) ITS, (c) *KEA1* intron 11, (d) *TEB* exon 17, (e) *matK*, and (f) *ndhF*, (g) cpDNA, (h) Concatenated, and (i) Concatenated and fully annotated.

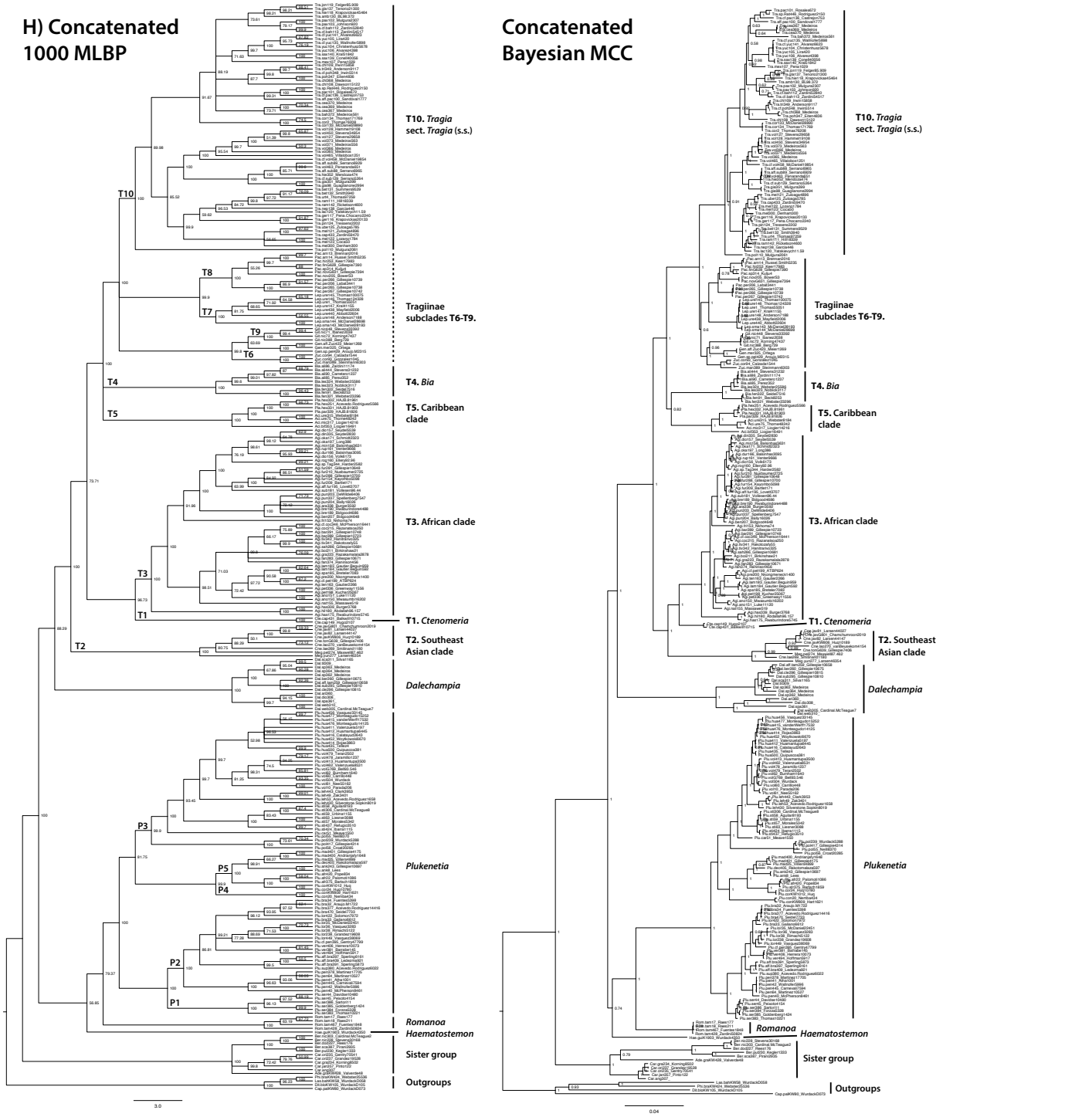


Figure 5.S1H Detailed cladograms and/or phylograms of Plukenetieae from individual and combined datasets labeled with maximum likelihood bootstrap percentages (MLBP) and/or Bayesian posterior probabilities (BPP): (a) ETS, (b) ITS, (c) *KEA1* intron 11, (d) *TEB* exon 17, (e) *matK*, and (f) *ndhF*, (g) cpDNA, (h) Concatenated, and (i) Concatenated and fully annotated.

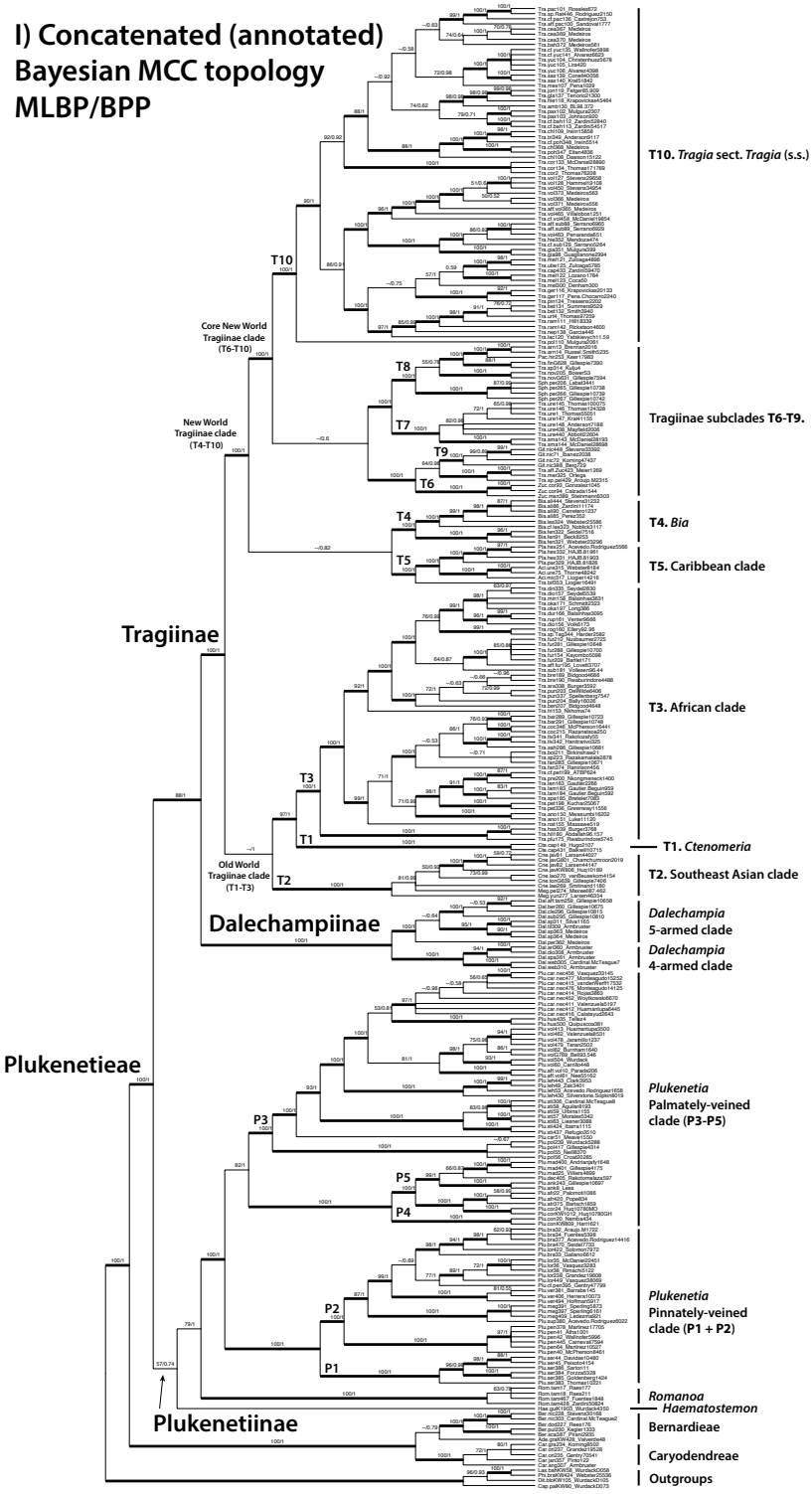


Figure 5.S1I Detailed cladograms and/or phylograms of Plukenetieae from individual and combined datasets labeled with maximum likelihood bootstrap percentages (MLBP) and/or Bayesian posterior probabilities (BPP): (a) ETS, (b) ITS, (c) *KEA1* intron 11, (d) *TEB* exon 17, (e) *matK*, and (f) *ndhF*, (g) cpDNA, (h) Concatenated, and (i) Concatenated and fully annotated. Bold branches indicate strong support (≥ 85% maximum likelihood bootstrap percentage [MLBP] and ≥ 0.95 Bayesian posterior probability [BPP]).

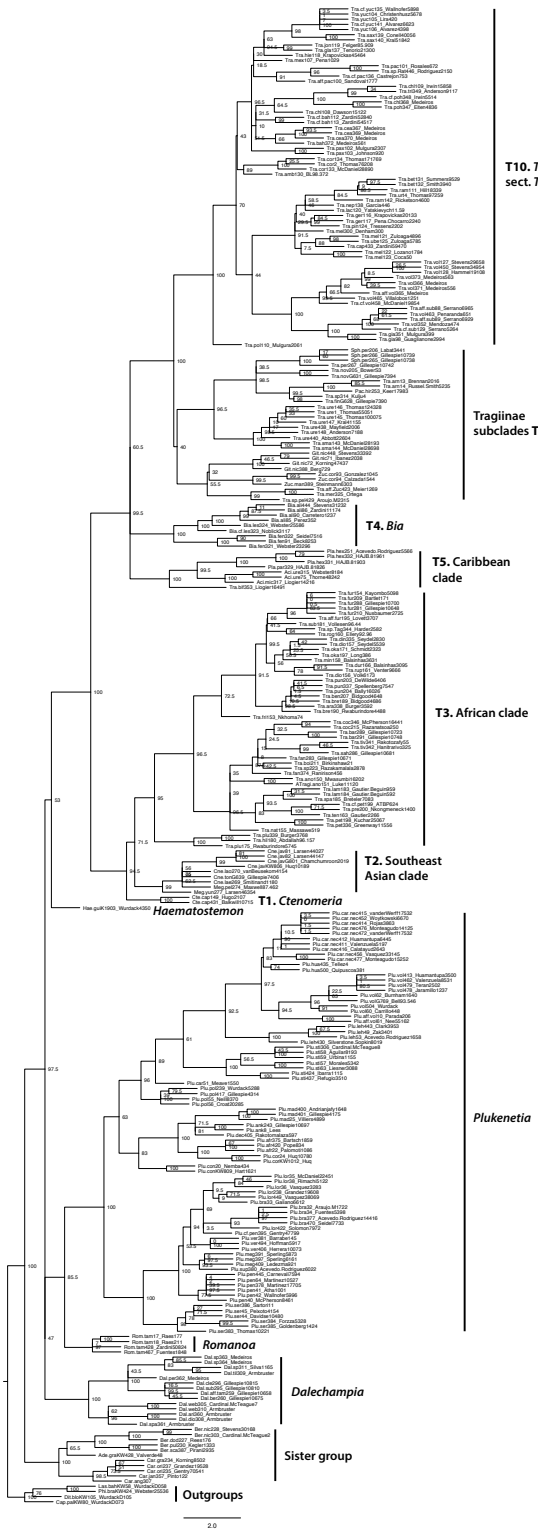


Figure 5.S2 Coalescence-based ASTRAL maximum quartet support (MQS) species tree of Plukenetieae based on Bayesian maximum clade credibility (MCC) topologies of all six regions, labeled with multilocus bootstrap percentage (Multilocus BP).

Figure 5.S3 Coalescence-based *BEAST maximum clade credibility (MCC) species tree of Plukenetieae based on reduced five region, "no-gap" dataset (177 terminals), labeled with Bayesian posterior probabilities (BPP).

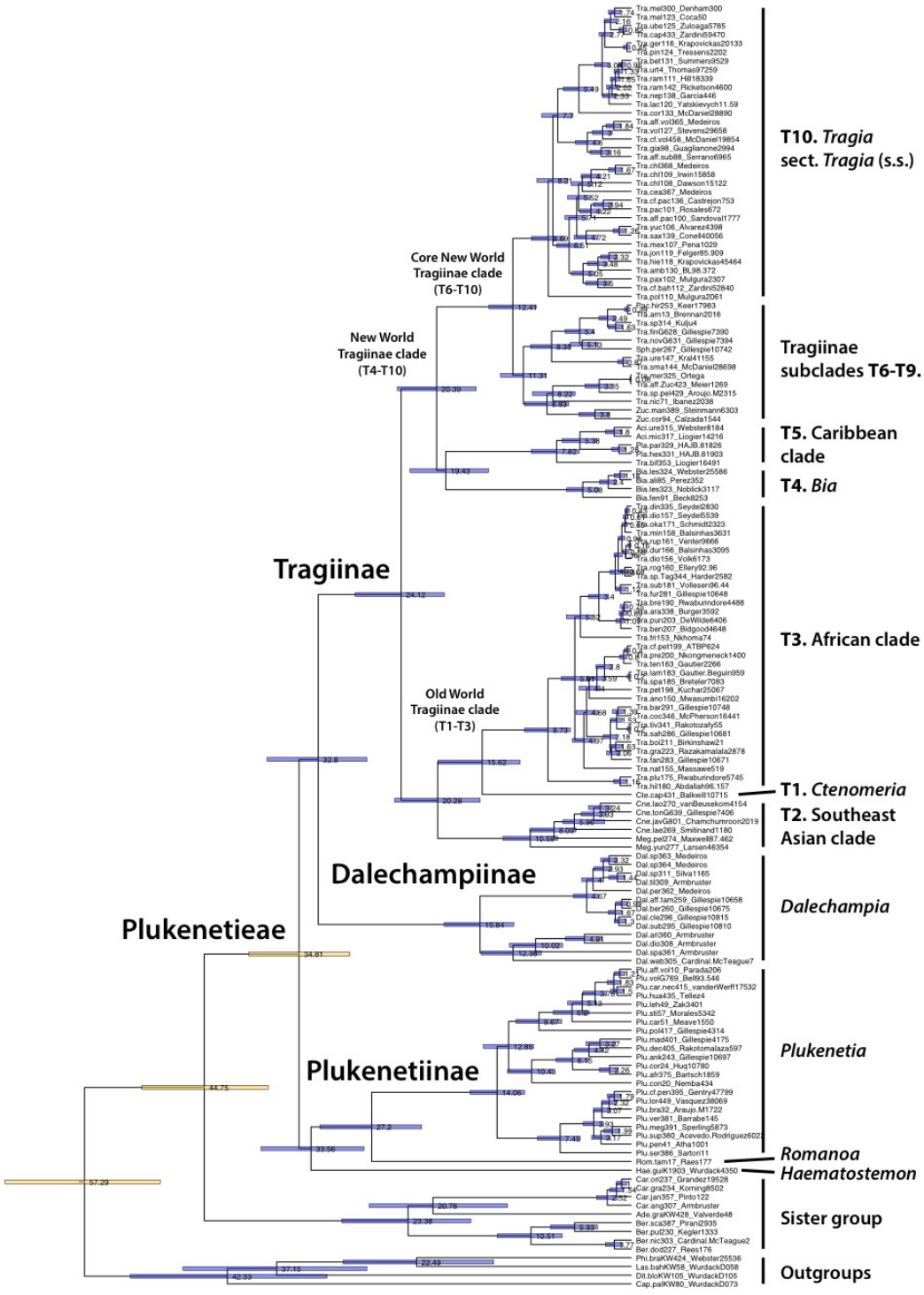


Figure 5.S4 Maximum clade credibility (MCC) BEAST chronogram of Plukenetieae based on the reduced six region, "one-terminal" dataset (147 terminals), including mean common ancestor ages and 95% highest posterior density (HPD) confidence interval bars (yellow = calibrated estimate; blue = free estimate).

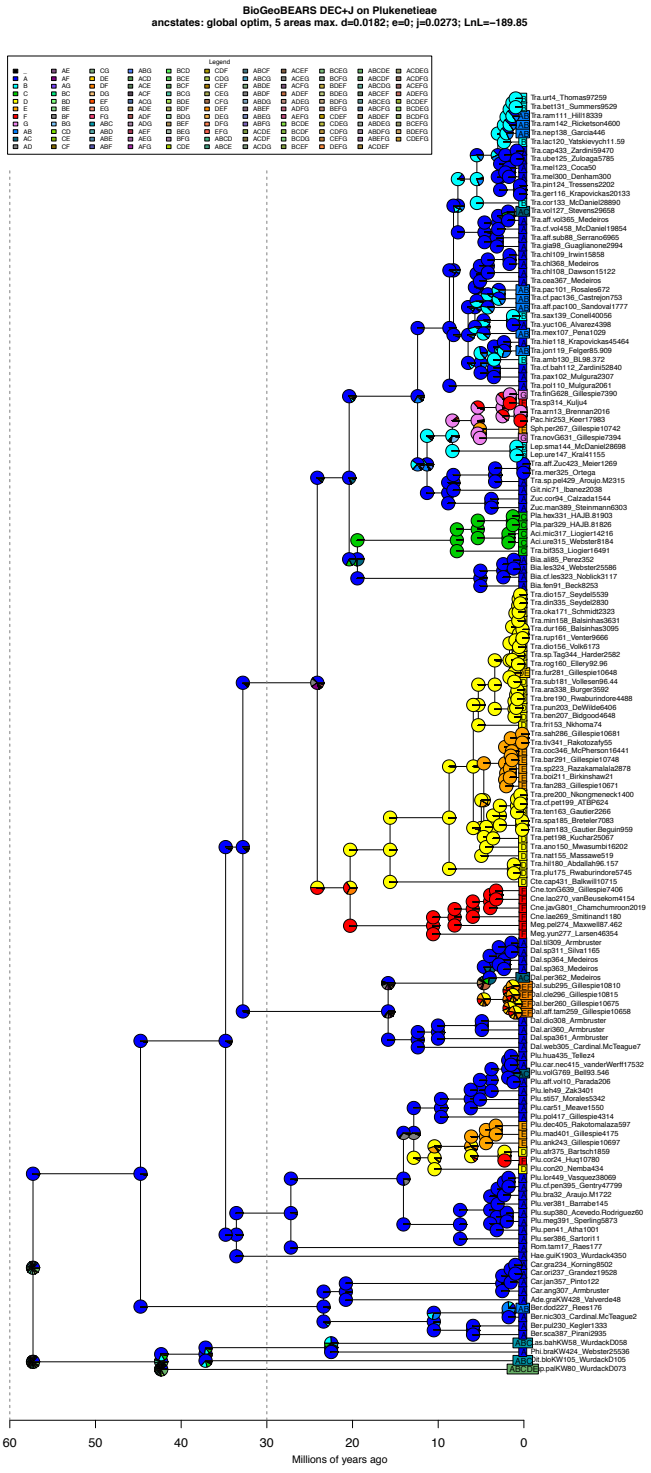


Figure 5.S5A Results of the BIOGEOBEARS ancestral range estimations (ARE) under (a) DEC+J and (b) DEC models.

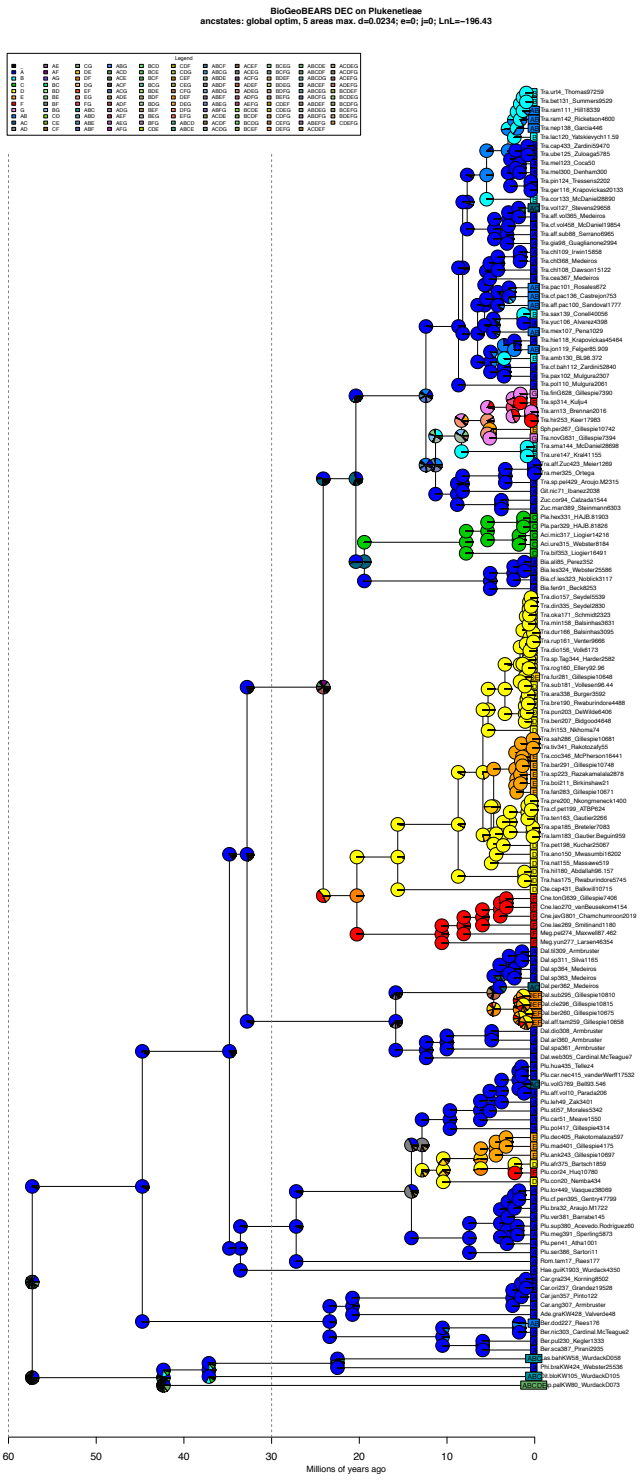


Figure 5.S5B Results of the BIOGEOBEARS ancestral range estimations (ARE) under (a) DEC+J and (b) DEC models.

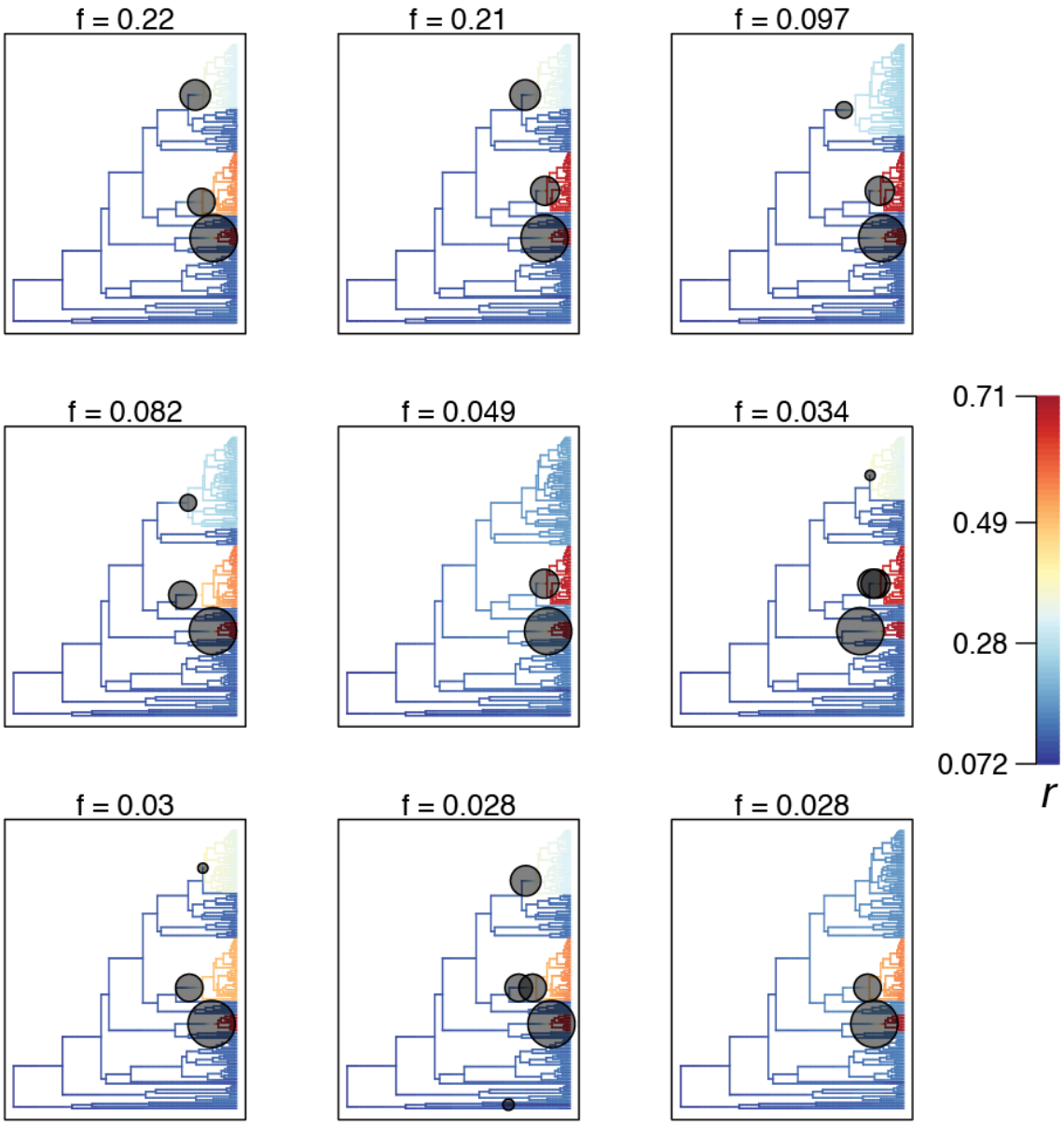


Figure 5.S6 BAMM 95% credible shift set phylorate plots (the largest nine of 30) including the frequency (f) of each configuration in the posterior distribution.

Chapter 6
Conclusions

The aim of my dissertation was to resolve the evolutionary history of a diverse but underutilized clade of tropical vines, Euphorbiaceae tribe Plukenetieae. Through the use of molecular phylogenetics and statistical analysis, we now have a strong understanding of the species relationships, pollen evolution, biogeographical history, and drivers of diversification in Plukenetieae. I also investigated the molecular phylogeny and drivers of seed size evolution in *Plukenetia*, one of the first small, well-characterized clades with notable seed size variation, and presented a revised sectional classification for the genus including three new taxa. Together, my research illuminated general trends of biogeography, seed evolution, and diversification, while contributing a vast amount of phylogenetic information on Plukenetieae, which positions the tribe as a useful system for future studies in pollen, seed, and biome evolution.

Prior to chapter two, there was a relatively limited understanding of the species group relationships in Plukenetieae. Previous research outlined that the tribe formed a monophyletic group (Wurdack *et al.*, 2005; Tokuoka, 2007), but generic boundaries were not yet tested and pollen morphology suggested traditional circumscriptions were paraphyletic (Gillespie, 1994b). Furthermore, the pollen morphology diversity found in Plukenetieae is uncommon and presented a convenient model to study pollen exine and aperture evolution. Using molecular phylogenetics and statistical inference, I produced the first densely-sampled phylogeny of Plukenetieae and described novel species group relationships based largely on phylogeny, morphology, and geographic location (Cardinal-McTeague & Gillespie, 2016). Although certain nodes of the phylogeny were poorly supported (e.g., early diverging relationships), many subclades were strongly supported and provided a foundation for future research on the tribe. One of our main conclusions was that *Tragia* was para- and/or polyphytletic and should be divided into smaller monophyletic genera. In terms of pollen morphology, exine evolution was dynamic, sporadic, and without obvious trends. Within Tragiinae, aperture evolution exhibited several parallel transitions from tricolpate pollen to inaperturate and intermediate weakly defined aperture states. This suggests Tragiinae 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). Moving forward,

I aim to produce a revised generic classification for Tragiinae, including a new infrageneric classification for the New World clade of *Tragia* sect. *Tragia* (s.l.). Future comparative studies of pollination biology, floral development, and pollen germination between Tragiinae (small pollen with trends in aperture reduction and loss) and *Dalechampia* (large pollen with well-developed apertures) could illuminate the evolutionary processes that favoured divergent strategies of pollen size, aperture type, and germination efficiency.

In chapter three, I conducted one of the first investigations into the drivers of seed size evolution in a small sized clade (*Plukenetia*, ~23 species). Prior to this, macro- and micro-evolutionary pressures of seed size evolution were known (Gómez, 2004; Moles *et al.*, 2005b, 2007; Halpern, 2005; Beaulieu *et al.*, 2007; Eriksson, 2008; Galetti *et al.*, 2013; Igea *et al.*, 2017) but had rarely been demonstrated within clades (although see El-ahmir *et al.*, 2015). Our findings suggest that seed size evolution was dynamic and correlated with plant size, fruit type (including inferred dispersal mechanism), and seedling ecology. Biome shifts were not associated with seed size, however, the transition to a seasonal, fire-regimented savanna recovered a weak association with seed size reduction. In general, a combination of selective pressures appeared to be necessary to develop seed size variation in *Plukenetia*. The same selective pressures are not apparent in other members of the tribe, which may account for their constrained smaller sized seeds. *Plukenetia* is now well-positioned to serve as a model for seed size evolution. For instance, transcriptomic comparison of differently sized species could identify genes and pathways involved with seed size and oil biosynthesis, which could be beneficial in developing improved oilseed crops. Furthermore, quantitative seed ecology studies could elaborate on the evolutionary trends we identified in *Plukenetia*, aided by the growing number of studies documenting oil content and germination rates in the edible oilseed species (Awodoyin *et al.*, 2000; Akintayo & Bayer, 2002; Nwosu, 2006; Cardoso *et al.*, 2015; Chirinos *et al.*, 2015; Mota *et al.*, 2015; Silva *et al.*, 2016).

In chapter four I present a revised sectional classification for *Plukenetia*, including the description of three new taxa from South America. The classification was largely built on previous morphological evidence outlined by Gillespie (1993, 1994, 2007), but was informed and supported by the molecular phylogenies developed in chapters two and three (Cardinal-McTeague & Gillespie, 2016; Cardinal-McTeague *et al.*, in review) and updated morphological descriptions. We now recognize six sections in *Plukenetia*, which are distinguished by a

combination of pistillate flower number, androecium morphology, style morphology, fruit type, and seed size. I also described two new species from the Amazon basin, *P. brevistyla* and *P. megastyla*, which is a small indicator of how much biodiversity is still undescribed in that region. The newly described *P. carolis-vegae* subsp. *nectarifera* is significant because it settles part of the complicated taxonomic history of the *Plukenetia* high elevation species complex. Previously described species in this complex, *P. huayllabambana* and *P. carolis-vegae*, are traditionally cultivated oilseed crops with substantial economic potential due to their extra-large oil-rich seeds (Bussmann *et al.*, 2009, 2013). We determined that these cultivated species are likely derived from natural populations (*P. carolis-vegae* subsp. *nectarifera*) that have smaller fruits and seeds. Understanding the evolutionary history of the high elevation species complex could be useful for future crop development and provide insight into the history of plant domestication by the Incas.

Chapters three and five included biogeographical analyses and discussion of neotropical and pantropical diversification patterns. Over the past decade, biogeographical studies have concluded that a majority of pantropical distributions developed through long-distance dispersals since the Late Oligocene (28.1 Mya) (Renner & Meyer, 2001; Renner *et al.*, 2001; Givnish *et al.*, 2004, 2011; Sytsma *et al.*, 2004; Renner, 2004b; Yuan *et al.*, 2005; Clayton *et al.*, 2009; Liu *et al.*, 2013; Berger *et al.*, 2016). Our results in *Plukenetia* (chapter three) and Plukenetieae (chapter five) confirm this trend and highlight the role of long-distance dispersals out of Central and South America throughout the Oligocene to Pleistocene. We also recovered an anomalous long-distance dispersal from North America to Australia in the Late Miocene (*c*. 8.4 Mya), which has only been identified at least one other time, in the opposite direction, in the aquatic genus *Myriophyllum* (Chen *et al.*, 2014). Within *Plukenetia* we found evidence for connections between the Atlantic Forest of Brazil and the Amazon during the Oligocene and Miocene, which is some of the first evidence of plants migrating across the open vegetational diagonal (Werneck, 2011). The Central American seaway between Central and South America, and the uplift of the Andes, were also found to be relatively poor biogeographic barriers that were frequently overcome by dispersal (Erkens *et al.*, 2007; Cody *et al.*, 2010; Bacon *et al.*, 2015; Pérez-Escobar *et al.*, 2017b). Looking forward, studies on the biogeography of *Tragia* sect. *Tragia* s.l. (subclade T10) could shed additional light on the formation of amphitropical disjunctions between temperate regions of North and South America (Simpson *et al.*, 2017).

Lastly, in chapter five I aimed to investigate the relative contribution of innovative traits on the diversification rates in Plukenetieae. A combination of biotic traits (e.g., floral symmetry, specialized pollination biology, and beneficial growth forms) and abiotic processes (e.g., mountain building, aridification) have been associated with increased diversification in plants (Gianoli, 2004; Sargent, 2004; Spriggs *et al.*, 2014; Reyes *et al.*, 2015; Roalson & Roberts, 2016; Lagomarsino *et al.*, 2016; Serrano-Serrano *et al.*, 2017). Although the same set of traits are not always beneficial across groups, a combination of traits and processes have often been found to increase lineage diversification (Silvestro *et al.*, 2013; Givnish *et al.*, 2014, 2015; Hernández-Hernández *et al.*, 2014; Lagomarsino *et al.*, 2016). Our study expanded on this subject by exploring the interactions of multiple overlapping innovative traits (twining growth form, stinging hairs, and inflorescence structure), with the expectation that species with all three traits would experience the highest diversification rates. Contrary to our ideas, we found three major shifts that were not associated with any of the innovative traits. Instead, increased species richness was driven in two to three subclades that appear to have shifted biomes from primarily moist tropical forests to drier open habitats. Diversification in these subclades was likely enhanced by climate change and the expansion of arid and open habitats associated with the Late Miocene and Pliocene cooling. Other plant groups were found to have diversified alongside the expansion of arid and open habitats (Bouchenak-Khelladi *et al.*, 2010; Hammer *et al.*, 2015; Reyes *et al.*, 2015) or fire-prone C_4 grasslands (Simon *et al.*, 2009; Bouchenak-Khelladi *et al.*, 2014; Linder, 2014), suggesting it was a significant process of plant diversification. Future studies should investigate how other climate-mediated biome expansions/contractions affected plant diversification, with the goal of anticipating shifts in biodiversity anthropogenic climate change.

Literature Cited

1000 Plants (1KP) Project. **2015**. 1KP: The One Thousand Transcriptomes Project [https://sites.google.com/a/ualberta.ca/onekp/]. Accessed 28 May 2016.

Airy Shaw HK. **1969**. Notes on Malesian and other Asiatic Euphorbiaceae. CXII. Notes on the subtribe Plukenetiinae Pax. *Kew Bulletin* **23**: 114–121.

Akaike H. **1974**. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* **19**: 716–723.

Akintayo ET, Bayer E. **2002**. Characterisation and some possible uses of *Plukenetia conophora* and *Adenopus breviflorus* seeds and seed oils. *Bioresource Technology* **85**: 95–97.

Alexander PJ, Rajanikanth G, Bacon CD, Bailey CD. **2006**. Recovery of plant DNA using a reciprocating saw and silica-based columns. *Molecular Ecology Notes* **7**: 5–9.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. **1990**. Basic local alignment search tool. *Journal of Molecular Biology* **215**: 403–10.

Angiosperm Phylogeny Group. **2009**. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* **161**: 105–121.

Antonelli AE, Verola CF, Parisod CN, Gustafsson ALS. **2010**. Climate cooling promoted the expansion and radiation of a threatened group of South American orchids (Epidendroideae: Laeliinae). *Biological Journal of the Linnean Society* **100**: 597–607.

Armbruster WS. **1984**. The role of resin in angiosperm pollination: Ecological and chemical considerations. *American Journal of Botany* **71**: 1149–1160.

Armbruster WS. **1993**. Evolution of plant pollination systems: Hypotheses and tests with the Neotropical vine *Dalechampia*. *Evolution* **47**: 1480–1505.

Armbruster WS, Baldwin BG. **1998**. Switch from specialized to generalized pollination. *Nature* **394**: 632.

Armbruster WS, Herzig AL, Clausen TP. **1992**. Pollination of two sympatric species of *Dalechampia* (Euphorbiaceae) in Suriname by male euglossine bees. *American Journal of Botany* **79**: 1374–1381.

Armbruster WS, Keller S, Matsuki M, Clausen TP. **1989**. Pollination of *Dalechampia magnoliifolia* (Euphorbiaceae) by male euglossine bees. *American Journal of Botany* **76**: 1279–1285.

Armbruster WS, Lee J, Baldwin BG. **2009**. Macroevolutionary patterns of defense and pollination in *Dalechampia* vines: Adaptation, exaptation, and evolutionary novelty. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 18085–18090.

Armbruster WS, Lee J, Edwards ME, Baldwin BG. **2013**. Floral paedomorphy leads to secondary specialization in pollination of Madagascar *Dalechampia* (Euphorbiaceae). *Evolution* **67**: 1196–1203.

Awodoyin RO, Egunjobi JK, Ladipo DO. **2000**. Biology, germination and prospects for the

domestication of the conophor nut, *Plukenetia conophora* Mull. Arg. [Syn. *Tetracarpidium conophorum* (Mull. Arg.) Hutch. & Dalz.]. *Journal of Tropical Forest Research* **16**: 30–38.

Bacon CD, Silvestro D, Jaramillo C, Smith BT, Chakrabarty P, Antonelli A. **2015**. Biological evidence supports an early and complex emergence of the Isthmus of Panama. *Proceedings of the National Academy of Sciences of the United States of America* **112**: 6110–6115.

Baillon H. **1858**. *Etude Générale du Groupe des Euphorbiacées*. Paris: Victor Masson.

Baillon H. **1865**. Species Euphorbiacearum. Euphorbiacées Américaines. Première partie (suite). Amérique austro-orientale. *Adansonia* **5**: 305–360.

Baker JG. **1910**. Diagnoses Africanae: XXXIV. *Bulletin of Miscellaneous Information Kew* **1910**: 55–59.

Batalha-Filho H, Fjeldså J, Fabre P-H, Miyaki CY. **2013**. Connections between the Atlantic and the Amazonian forest avifaunas represent distinct historical events. *Journal of Ornithology* **154**: 41–50.

Beaulieu JM, Moles AT, Leitch IJ, Bennett MD, Dickie JB, Knight CA. **2007**. Correlated evolution of genome size and seed mass. *New Phytologist* **173**: 422–437.

Beaulieu JM, O'Meara BC. **2016**. Detecting hidden diversification shifts in models of trait-dependent speciation and extinction. *Systematic Biology* **65**: 583–601.

Beilstein MA, Al-Shehbaz IA, Kellogg EA. **2006**. Brassicaceae phylogeny and trichome evolution. *American Journal of Botany* **93**: 607–619.

Berger BA, Kriebel R, Spalink D, Sytsma KJ. **2016**. Divergence times, historical biogeography, and shifts in speciation rates of Myrtales. *Molecular Phylogenetics and Evolution* **95**: 116–136.

Blomberg SP, Garland T, Ives AR. **2007**. Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution* **57**: 717–745.

Borchsenius F. **2009**. FastGap v1.2. Published online at [http://www.aubot.dk/FastGap_home.htm].

Borhidi A. **1972**. La taxonomía del género Platygyne Merc. *Annales Historico-Naturales Musei Nationalis Hungarici* **64**: 89–94.

Bouchenak-Khelladi Y, Maurin O, Hurter J, van der Bank M. **2010**. The evolutionary history and biogeography of Mimosoideae (Leguminosae): An emphasis on African acacias. *Molecular Phylogenetics and Evolution* **57**: 495–508.

Bouchenak-Khelladi Y, Muasya AM, Linder HP. **2014**. A revised evolutionary history of Poales: origins and diversification. *Botanical Journal of the Linnean Society* **175**: 4–16.

Breitkopf H, Onstein RE, Cafasso D, Schlüter PM, Cozzolino S. **2015**. Multiple shifts to different pollinators fuelled rapid diversification in sexually deceptive Ophrys orchids. *New Phytologist* **207**: 377–389.

Brett CL, Donowitz M, Rao R. **2005**. Evolutionary origins of eukaryotic sodium/proton exchangers. *American Journal of Physiology, Cell Physiology* **288**: C223–C239.

Brodie J, Post E, Laurance WF. **2012**. Climate change and tropical biodiversity: A new focus. *Trends in Ecology & Evolution* **27**: 145–150.

Buerki S, Forest F, Alvarez N, Nylander JA, Arrigo N, Sanmartín I. **2011**. An evaluation of new parsimony-based versus parametric inference methods in biogeography: A case study using the globally distributed plant family Sapindaceae. *Journal of Biogeography* **38**: 531–550.

Burger W, Huft M. **1995**. Flora Costaricensis: Family #113 Euphorbiaceae. *Fieldiana, Botany n.s.* **36**: 1–169.

Bussmann RW, Paniagua Zambrana N, Téllez C. **2013**. *Plukenetia carolis-vegae* (Euphorbiaceae) - A new useful species from northern Peru. *Economic Botany* **67**: 387–392.

Bussmann RW, Téllez C, Glenn A. **2009**. *Plukenetia huayllabambana* sp. nov. (Euphorbiaceae) from the upper Amazon of Peru. *Nordic Journal of Botany* **27**: 313–315.

Cai ZQ, Jiao DY, Tang SX, Dao XS, Lei YB, Cai CT. **2012**. Leaf photosynthesis, growth, and seed chemicals of Sacha Inchi plants cultivated along an altitude gradient. *Crop Science* **52**: 1859–1867.

Cai ZQ, Zhang T, Jian HY. **2013**. Chromosome number variation in a promising oilseed woody crop, *Plukenetia volubilis* L. (Euphorbiaceae). *Caryologia* **66**: 54–58.

Cardinal-McTeague WM, Gillespie LJ. **2016**. Molecular phylogeny and pollen evolution of Euphorbiaceae tribe Plukenetieae. *Systematic Botany* **41**: 329–347.

Cardinal-McTeague WM, Sytsma KJ, Hall JC. **2016**. Biogeography and diversification of Brassicales: A 103 million year tale. *Molecular Phylogenetics and Evolution* **99**: 204–224.

Cardoso AA, Obolari A de MM, Borges EE de L e, Silva CJ da, Rodrigues HS. **2015**. Environmental factors on seed germination, seedling survival and initial growth of sacha inchi (Plukenetia volubilis L.). *Journal of Seed Science* **37**: 111–116.

Cervantes A, Fuentes S, Gutierrez J, Magallon S, Borsch T. **2016**. Successive arrivals since the Miocene shaped the diversity of the Caribbean Acalyphoideae (Euphorbiaceae). *Journal of Biogeography* **43**: 1773–1785.

Chan AP, Crabtree J, Zhao Q, Lorenzi H, Orvis J, Puiu D, Melake-Berhan A, Jones KM, Redman J, Chen G, *et al.* 2010. Draft genome sequence of the oilseed species Ricinus communis. *Nature Biotechnology* **28**: 951–6.

Chanroj S, Wang G, Venema K, Zhang MW, Delwiche CF, Sze H. **2012**. Conserved and diversified gene families of monovalent cation/H(+) antiporters from algae to flowering plants. *Frontiers in Plant Science* **3**: 25.

Chapman CA, Russo SE. **2006**. Primate seed dispersal: Linking behavioural ecology with forest community structure. In: Primates in Perspective. 510–525.

Chen L-Y, Zhao S-Y, Mao K-S, Les DH, Wang Q-F, Moody ML. **2014**. Historical biogeography of Haloragaceae: An out-of-Australia hypothesis with multiple intercontinental dispersals. *Molecular Phylogenetics and Evolution* **78**: 87–95.

Cheng T, Xu C, Lei L, Li C, Zhang Y, Zhou S. **2016**. Barcoding the kingdom Plantae: New PCR primers for ITS regions of plants with improved universality and specificity. *Molecular Ecology Resources* **16**: 138–149.

Chirinos R, Pedreschi R, Domínguez G, Campos D. **2015**. Comparison of the physico-chemical and phytochemical characteristics of the oil of two Plukenetia species. *Food Chemistry* **173**: 1203–1206.

Clarke PJ, Lawes MJ, Midgley JJ, Lamont BB, Ojeda F, Burrows GE, Enright NJ, Knox KJE. **2013**. Resprouting as a key functional trait: how buds, protection and resources drive persistence after fire. *New Phytologist* **197**: 19–35.

Clayton JW, Soltis PS, Soltis DE. **2009**. Recent long-distance dispersal overshadows ancient biogeographical patterns in a pantropical angiosperm family (Simaroubaceae, Sapindales). *Systematic Biology* **58**: 395–410.

Cody S, Richardson JE, Rull V, Ellis C, Pennington RT. **2010**. The great American biotic interchange revisited. *Ecography* **33**: 326–332.

Coomes DA, Grubb PJ. **2003**. Colonization, tolerance, competition and seed-size variation within functional groups. *Trends in Ecology and Evolution* **18**: 283–291.

Costa LP. **2003**. The historical bridge between the Amazon and the Atlantic Forest of Brazil: A study of molecular phylogeography with small mammals. *Journal of Biogeography* **30**: 71–86.

Cox B. **2001**. The biogeographic regions reconsidered. *Journal of Biogeography* **28**: 511–523.

Croizat L. **1941**. The tribe Plukenetiinae of the Euphorbiaceae in eastern tropical Asia. *Journal of the Arnold Arboretum* **22**: 417–431.

Croizat L. **1945**. New or critical Euphorbiaceae from the Americas. *Journal of the Arnold Arboretum* **26**: 181–196.

Darriba D, Taboada GL, Doallo R, Posada D. **2012**. jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods* **9**: 772–772.

Degnan JH, Rosenberg NA. **2009**. Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in Ecology & Evolution* **24**: 332–340.

Dobritsa AA, Coerper D. **2012**. The novel plant protein INAPERTURATE POLLEN1 marks distinct cellular domains and controls formation of apertures in the *Arabidopsis* pollen exine. *The Plant Cell* **24**: 4452–4464.

Donoghue MJ, Edwards EJ. **2014**. Biome shifts and niche evolution in plants. *Annual Review of Ecology, Evolution, and Systematics* **45**: 547–572.

Doughty CE, Wolf A, Morueta-Holme N, Jørgensen PM, Sandel B, Violle C, Boyle B, Kraft NJB, Peet RK, Enquist BJ, *et al.* 2016. Megafauna extinction, tree species range reduction, and carbon storage in Amazonian forests. *Ecography* **39**: 194–203.

Douzery EJP, Pridgeon AM, Kores P, Linder HP, Kurzweil H, Chase MW. **1999**. Molecular phylogenetics of Diseae (Orchidaceae): A contribution from nuclear ribosomal ITS sequences. *American Journal of Botany* **86**: 887–899.

Drummond CS. **2008**. Diversification of *Lupinus* (Leguminosae) in the western New World:

Derived evolution of perennial life history and colonization of montane habitats. *Molecular Phylogenetics and Evolution* **48**: 408–421.

Drummond AJ, Rambaut A. **2007**. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**: 214.

Drummond AJ, Suchard MA, Xie D, Rambaut A. **2012**. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**: 1969–1973.

Ducke A. **1925**. Plantes nouvelles ou peu connues de la région amazonienne (IIIe partie). *Archivos do Jardim Botânico do Rio de Janeiro* **4**: 1–208.

Edgar RC. **2004**. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**: 1792–1797.

Edger PP, Heidel-Fischer HM, Bekaert M, Rota J, Glöckner G, Platts AE, Heckel DG, Der JP, Wafula EK, Tang M, *et al.* 2015. The butterfly plant arms-race escalated by gene and genome duplications. *Proceedings of the National Academy of Sciences of the United States of America* **112**: 8362–8366.

Edlund AF, Swanson R, Preuss D. **2004**. Pollen and stigma structure and function: The role of diversity in pollination. *The Plant cell* **16 Suppl**: S84-97.

Edwards EJ, Osborne CP, Strömberg CAE, Smith SA. **2010**. The origins of C4 grasslands: Integrating evolutionary and ecosystem science. *Science* **328**: 587–591.

El-ahmir SM, Lim SL, Lamont BB, He T. **2015**. Seed size, fecundity and postfire regeneration strategy are interdependent in Hakea. *PLoS ONE* **10**: e0129027.

Endlicher SL. **1843**. *Generum Plantarum, Supplementum 3*. Vindobonae [Vienna]: Apud Fridericum Beck.

Eriksson O. **2008**. Evolution of seed size and biotic seed dispersal in angiosperms: Paleoecological and neoecological evidence. *International Journal of Plant Sciences* **169**: 863–870.

Erkens RHJ, Chatrou LW, Maas JW, van der Niet T, Savolainen V. **2007**. A rapid diversification of rainforest trees (*Guatteria*; Annonaceae) following dispersal from Central into South America. *Molecular Phylogenetics and Evolution* **44**: 399–411.

Farrell BD, Dussourd DE, Mitter C. **1991**. Escalation of plant defense: Do latex and resin canals spur plant diversification? *The American Naturalist* **138**: 881–900.

Federman S, Dornburg A, Downie A, Richard AF, Daly DC, Donoghue MJ. **2015**. The biogeographic origin of a radiation of trees in Madagascar: Implications for the assembly of a tropical forest biome. *BMC Evolutionary Biology* **15**: 216.

Felsenstein J. **1985a**. Phylogenies and the comparative method. *The American Naturalist* **125**: 1–15.

Felsenstein J. **1985b**. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**: 783–791.

Felsenstein J. **2012**. A comparative method for both discrete and continuous characters using the threshold model. *The American Naturalist* **179**: 145–156.

Fitch WM. **1971**. Toward defining the course of evolution: Minimum change for a specific tree topology. *Systematic Biology* **20**: 406–416.

Forget P-M. **1990**. Seed-dispersal of *Vouacapoua americana* (Caesalpiniaceae) by caviomorph rodents in French Guiana. *Journal of Tropical Ecology* **6**: 459–468.

Forster PI. **1994**. A taxonomic revision of *Tragia* (Euphorbiaceae) in Australia. *Australian Systematic Botany* **7**: 377–383.

Forster PI. **1997**. *Tragia finalis* (Euphorbiaceae), a new species from northern Queensland. *Australian Systematic Botany* **10**: 863–866.

Fouquet A, Loebmann D, Castroviejo-Fisher S, Padial JM, Orrico VGD, Lyra ML, Roberto IJ, Kok PJR, Haddad CFB, Rodrigues MT. **2012**. From Amazonia to the Atlantic forest: Molecular phylogeny of Phyzelaphryninae frogs reveals unexpected diversity and a striking biogeographic pattern emphasizing conservation challenges. *Molecular Phylogenetics and Evolution* **65**: 547–561.

Furness CA. **2007**. Why does some pollen lack apertures? A review of inaperturate pollen in eudicots. *Botanical Journal of the Linnean Society* **155**: 29–48.

Galetti M, Guevara R, Côrtes MC, Fadini R, Von Matter S, Leite AB, Labecca F, Ribeiro T, Carvalho CS, Collevatti RG, *et al.* 2013. Functional extinction of birds drives rapid evolutionary changes in seed size. *Science* **340**: 1086–1090.

Gernhard T. **2008**. The conditioned reconstructed process. *Journal of Theoretical Biology* **253**: 769–778.

Gianoli E. **2004**. Evolution of a climbing habit promotes diversification in flowering plants. *Proceedings. Biological sciences / The Royal Society* **271**: 2011–2015.

Gibson L, Lee TM, Koh LP, Brook BW, Gardner TA, Barlow J, Peres CA, Bradshaw CJA, Laurance WF, Lovejoy TE, *et al.* 2011. Primary forests are irreplaceable for sustaining tropical biodiversity. *Nature* **478**: 378.

Gillespie LJ. **1993**. A synopsis of Neotropical *Plukenetia* (Euphorbiaceae) including two new species. *Systematic Botany* **18**: 575–592.

Gillespie LJ. **1994a**. A new section and two new species of *Tragia* (Euphorbiaceae) from the Venezuelan Guayana and French Guiana. *Novon* **4**: 330–338.

Gillespie LJ. **1994b**. Pollen morphology and phylogeny of the tribe Plukenetieae (Euphorbiaceae). *Annals of the Missouri Botanical Garden* **81**: 317–348.

Gillespie LJ. **1999**. *Acidoton* (Euphorbiaceae). In: Steyermark JA, Berry PE, Yatskievych K, Holst BK, eds. Flora of the Venezuelan Guayana vol. 5. St. Louis: Missouri Botanical Garden Press, 86–87.

Gillespie LJ. **2007**. A revision of Paleotropical *Plukenetia* (Euphorbiaceae) including two new species from Madagascar. *Systematic Botany* **32**: 780–802.

Gillespie LJ, Armbruster WS. **1997**. A contribution to the Guianan Flora: *Dalechampia*, *Haematostemon*, *Omphalea*, *Pera*, *Plukenetia*, and *Tragia* (Euphorbiaceae) with notes on subfamily Acalyphoideae. *Smithsonian Contributions to Botany* **86**: 1–48.

Givnish TJ, Barfuss MHJ, van Ee B, Riina R, Schulte K, Horres R, Gonsiska PA, Jabaily

RS, Crayn DM, Smith JAC, *et al.* 2014. Adaptive radiation, correlated and contingent evolution, and net species diversification in Bromeliaceae. *Molecular Phylogenetics and Evolution* **71**: 55–78.

Givnish TJ, Barfuss MHJ, Ee B Van, Riina R, Schulte K, Horres R, Gonsiska PA, Jabaily RS, Crayn DM, Smith JAC, *et al.* 2011. Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: Insights from an eight-locus plastid phylogeny. *American Journal of Botany* **98**: 872–895.

Givnish TJ, Millam KC, Evans TM, Hall JC, Pires JC, Berry PE, Sytsma KJ. 2004. Ancient vicariance or recent long-distance dispersal? Inferences about phylogeny and South American–African disjunctions in Rapateaceae and Bromeliaceae based on ndhF sequence data. *International Journal of Plant Sciences* **165**: S35–S54.

Givnish TJ, Spalink D, Ames M, Lyon SP, Hunter SJ, Zuluaga A, Doucette A, Caro GG, McDaniel J, Clements MA, *et al.* 2016. Orchid historical biogeography, diversification, Antarctica and the paradox of orchid dispersal. *Journal of Biogeography* **43**: 1905–1916.

Givnish TJ, Spalink D, Ames M, Lyon SP, Hunter SJ, Zuluaga A, Iles WJD, Clements MA, Arroyo MTK, Leebens-Mack J, *et al.* 2015. Orchid phylogenomics and multiple drivers of their extraordinary diversification. *Proceedings of the Royal Society B: Biological Sciences* **282**.

Global Biodiversity Information Facility. 2017. GBIF.org.

Gómez JM. 2004. Bigger is not always better: conflicting selective pressures on seed size in *Quercus ilex*. *Evolution* **58**: 71–80.

Gómez-González S, Torres-Díaz C, Bustos-Schindler C, Gianoli E. 2011. Anthropogenic fire drives the evolution of seed traits. *Proceedings of the National Academy of Sciences* **108**: 18743 LP-18747.

González J. 2010. Euphorbiaceae. In: Hammel BE, Grayum MH, Herrera C, Zamora N, eds. Manual de Plantas de Costa Rica vol. 5. St. Louis: Missouri Botanical Garden Press, 290–394.

Govaerts R, Barker S, Carter S, Davies S, Esser H-J, Fernandez Casas FJ, Gilbert M, Hoffmann P, Radcliffe-Smith A, Steinmann V, *et al.* 2015. World checklist of Euphorbiaceae. *Facilitated by the Royal Botanical Gardens, Kew*.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology* **59**: 307–321.

Gutiérrez de Sanguinetti MM, Múlgura de Romero ME. 1986. Una nueva especie de *Tragia* (Euphorbiaceae). *Darwiniana* **27**: 491–497.

Halpern SL. 2005. Sources and consequences of seed size variation in *Lupinus perennis* (Fabaceae): Adaptive and non-adaptive hypotheses. *American Journal of Botany* **92**: 205–213.

Hammer T, Davis R, Thiele K. 2015. A molecular framework phylogeny for *Ptilotus* (Amaranthaceae): Evidence for the rapid diversification of an arid Australian genus. *Taxon* **64**: 272–285.

Hammer Ø, Harper DAT, Ryan PD. **2001**. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4**: 1–9.

Harmon LJ, Weir JT, Brock CD, Glor RE, Challenger W. **2008**. GEIGER: Investigating evolutionary radiations. *Bioinformatics* **24**: 129–131.

Hasskarl JC. **1842**. Plantarum genera et species novae aut reforma tae javenses. *Flora* **25**: 1–56.

Hasskarl JC. **1843**. Adnotationes de plantis quibusdam Javanicis nonnullisque Japonicus, haud rite cognitis, e Catologo Horti Bogoriensis exceptae. Accedunt nonullae novae species (*). *Tijdschrift voor Natuurlijke Geschiedenis en Physiologie* **10**: 115–150.

Heled J, Drummond AJ. **2010**. Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution* **27**: 570–580.

Hemsley WB. **1883**. Euphorbiaceae. In: Godman FD, Salvina O, eds. Biologia Centrali-Americana, Botany vol. 3. London: R.H.Porter and Dalau & Co., 88–137.

Henke W, Herdel K, Jung K, Schnorr D, Loening SA. **1997**. Betaine improves the PCR amplification of GC-rich DNA sequences. *Nucleic Acids Research* **25**: 3957–3958.

Hernández-Hernández T, Brown JW, Schlumpberger BO, Eguiarte LE, Magallón S. **2014**. Beyond aridification: Multiple explanations for the elevated diversification of cacti in the New World Succulent Biome. *New Phytologist* **202**: 1382–1397.

Hernández RM, Jordan TE, Dalenz Farjat A, Echavarría L, Idleman BD, Reynolds JH. **2005**. Age, distribution, tectonics, and eustatic controls of the Paranense and Caribbean marine transgressions in southern Bolivia and Argentina. *Journal of South American Earth Sciences* **19**: 495–512.

Hoorn C, Wesselingh FP, ter Steege H, Bermudez MA, Mora A, Sevink J, Sanmartín I, Sanchez-Meseguer A, Anderson CL, Figueiredo JP, *et al.* 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science* **330**: 927–931.

Horn JW, van Ee BW, Morawetz JJ, Riina R, Steinmann VW, Berry PE, Wurdack KJ. **2012**. Phylogenetics and the evolution of major structural characters in the giant genus *Euphorbia* L. (Euphorbiaceae). *Molecular Phylogenetics and Evolution* **63**: 305–326.

Horn JW, Xi Z, Riina R, Peirson JA, Yang Y, Dorsey BL, Berry PE, Davis CC, Wurdack KJ. **2014**. Evolutionary bursts in *Euphorbia* (Euphorbiaceae) are linked with photosynthetic pathway. *Evolution* **68**: 3485–3504.

Hubbell SP. **2013**. Tropical rain forest conservation and the twin challenges of diversity and rarity. *Ecology and Evolution* **3**: 3263–3274.

Hutchinson J. **1969**. Tribalism in the family Euphorbiaceae. *American Journal of Botany* **56**: 738–758.

Igea J, Miller EF, Papadopulos AST, Tanentzap AJ. **2017**. Seed size and its rate of evolution correlate with species diversification across angiosperms. *PLoS Biology* **15**: e2002792.

Inagaki S, Suzuki T, Ohto M, Urawa H, Horiuchi T, Nakamura K, Morikami A. **2006**. Arabidopsis *TEBICHI*, with helicase and DNA polymerase domains, is required for regulated cell division and differentiation in meristems. *The Plant Cell* **18**: 879–892.

Jacobs BF. **2004**. Palaeobotanical studies from tropical Africa: relevance to the evolution of forest, woodland and savannah biomes. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **359**: 1573–1583.

Jansen P, Bartholomeus M, Bongers F, Elzinga J, den Ouden J, Van Wieren SE. **2002**. The role of seed size in dispersal by a scatter-hoarding rodent. In: Seed Dispersal and Frugivory: Ecology, Evolution and Conservation. 209–225.

Janssens SB, Groeninckx I, Block PJ De, Verstraete B, Smets EF, Dessein S. **2016**. Dispersing towards Madagascar: Biogeography and evolution of the Madagascan endemics of the Spermacoceae tribe (Rubiaceae). *Molecular Phylogenetics and Evolution* **95**: 58–66.

Jiménez Ramírez J. **1993**. Especie nueva de *Plukenetia* (Euphorbiaceae) del estado de Oaxaca, Mexico. *Anales del Instituto de Biologia Universidad Nacional Autonoma de Mexico Serie Botanica* **64**: 55–58.

Jorgensen PM, Nee MH, Beck SG. **2014**. *Catálogos de los Plantas Vasculares de Bolivia 2 Vols.* St. Louis: Missouri Botanical Garden Press.

Jussieu A de. **1824**. *De Euphorbiacearum Generibus*. Parisiis [Paris]: Ex Typis Didot Junioris.

Kahle D, Wickham H. **2013**. ggmap: Spatial visualization with ggplot2. *The R Journal* **5**: 144–161.

Kang H, Primack RB. **1999**. Evolutionary change in seed size among some legume species: The effects of phylogeny. *Plant Systematics and Evolution* **219**: 151–164.

Katoh K, Standley DM. **2013**. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.

Klotzsch JF. **1841**. Neue und weniger gekannte südamerikanische Euphorbiaceen-Gattungen. *Archiv für Naturgeschichte* **7**: 175–205.

Koopman MM, Baum DA. **2008**. Phylogeny and biogeography of tribe Hibisceae (Malvaceae) on Madagascar. *Systematic Botany* **33**: 364–374.

Korall P, Pryer KM. **2014**. Global biogeography of scaly tree ferns (Cyatheaceae): Evidence for Gondwanan vicariance and limited transoceanic dispersal. *Journal of Biogeography* **41**: 402–413.

Krahulcová A, Trávníček P, Krahulec F, Rejmánek M. **2017**. Small genomes and large seeds: Chromosome numbers, genome size and seed mass in diploid Aesculus species (Sapindaceae). *Annals of Botany* **119**: 957–964.

Kreader CA. **1996**. Relief of amplification inhibition in PCR with bovine serum albumin or T4 gene 32 protein. *Applied and Environmental Microbiology* **62**: 1102–1106.

Kuhn TS, Mooers AØ, Thomas GH. **2011**. A simple polytomy resolver for dated phylogenies. *Methods in Ecology and Evolution* **2**: 427–436.

Lagomarsino LP, Condamine FL, Antonelli A, Mulch A, Davis CC. **2016**. The abiotic and biotic drivers of rapid diversification in Andean bellflowers (Campanulaceae). *New Phytologist* **210**: 1430–1442.

Lahoreau G, Barot S, Gignoux J, Hoffmann WA, Setterfield SA, Williams PR. **2006**. Positive effect of seed size on seedling survival in fire-prone savannas of Australia, Brazil and West Africa. *Journal of Tropical Ecology* **22**: 719–722.

Lane DJ, Pace B, Olsen GJ, Stahl DA, Sogin ML, Pace NR. **1985**. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proceedings of the National Academy of Sciences of the United States of America* **82**: 6955–6959.

Leandri J. **1938a**. Le genre *Tragia* (Euphorbiacées) à Madagascar. *Bulletin Trimestriel de l'Académie Malgache, nouvelle série* **21**: 65–68.

Leandri J. **1938b**. Euphorbiacées malgaches nouvelles récoltées par M. H. Perrier de la Bathie. *Bulletin de la Société Botanique de France* **85**: 523–533.

Leandri J. **1971**. Un sous-genre malgache nouveau de *Tragia* (Euphorbiacees). *Adansonia, série 2* **11**: 437–439.

Lehtonen S, Myllys L, Huttunen S. **2009**. Phylogenetic analysis of non-coding plastid DNA in the presence of short inversions. *Phytotaxa* **1**: 3–20.

Leishman MR, Wright IJ, Moles AT, Westoby M, others. **2000**. The evolutionary ecology of seed size. In: Seeds: The Ecology of Regeneration in Plant Communities. CAB International Wallingford, 31–57.

Levin GA, Gillespie LJ. **2016**. Euphorbiaceae. In: Flora of North America Vol. 12 Magniliophyta: Vitaceae to Garryaceae. New York, Oxford: Oxford University Press, 156–324.

Lewis PO. **2001**. A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology* **50**: 913–925.

Li B, Gillespie LJ. **2008**. *Pachystylidium* (Euphorbiaceae). In: Wu Z, Raven PH, Hong D, eds. Flora of China vol. 11. St. Louis: Missouri Botanical Garden Press, 257–258.

Linder HP. **2014**. The evolution of African plant diversity. *Frontiers in Ecology and Evolution* **2**: 38.

Linnaeus C. **1753**. *Species Plantarum, vol. 2*. Holmiae [Stockholm]: Impensis Laurentii Salvii.

Linnaeus f. C. **1782**. *Supplementum Plantarum*. Brunsvigae [Braunschweig]: Impensis Orphanotrophei.

Liogier AH. **1952**. Estudios en Euforbiáceas Cubanas. *Contribuciones Ocasionales del Museo de Historia Natural del Colegio De La Salle* **11**: 1–12.

Liu X-Q, Ickert-Bond SM, Chen L-Q, Wen J. **2013**. Molecular phylogeny of *Cissus* L. of Vitaceae (the grape family) and evolution of its pantropical intercontinental disjunctions. *Molecular Phylogenetics and Evolution* **66**: 43–53.

Loera I, Sosa V, Ickert-Bond SM. **2012**. Diversification in North American arid lands: Niche conservatism, divergence and expansion of habitat explain speciation in the genus Ephedra. *Molecular Phylogenetics and Evolution* **65**: 437–450.

Lourteig A, O'Donell CA. **1941**. Tragiae Argentinae (Euphorbiaceae). *Lilloa* **6**: 347–380.

Lowry PP, Plunkett GM, Frodin DG. **2013**. Revision of *Plerandra* (Araliaceae). I. A synopsis

of the genus with an expanded circumscription and a new infrageneric classification. *Brittonia* **65**: 42–61.

Maddison WP, Midford PE, Otto SP. **2007**. Estimating a binary character's effect on speciation and extinction. *Systematic biology* **56**: 701–710.

Marcot JD, Fox DL, Niebuhr SR. **2016**. Data from: Late Cenozoic onset of the latitudinal diversity gradient of North American mammals. *Proceedings of the National Academy of Sciences of the United States of America*.

Martini AMZ, Fiaschi P, Amorim AM, Paixão JL da. **2007**. A hot-point within a hot-spot: A high diversity site in Brazil's Atlantic Forest. *Biodiversity and Conservation* **16**: 3111–3128.

Matamoro-Vidal A, Furness CA, Gouyon P-H, Wurdack KJ, Albert B. **2012**. Evolutionary stasis in Euphorbiaceae pollen: Selection and constraints. *Journal of Evolutionary Biology* **25**: 1077–1096.

Matasci N, Hung L-H, Yan Z, Carpenter EJ, Wickett NJ, Mirarab S, Nguyen N, Warnow T, Ayyampalayam S, Barker MS, *et al.* 2014. Data access for the 1,000 Plants (1KP) project. *GigaScience* **3**: 17.

Matzke NJ. **2013**. Probabilistic historical biogeography: New models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Ph.D. Thesis*.

Matzke NJ. **2014**. Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. *Systematic Biology* **63**: 951–970.

McLoughlin S. **2001**. The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. *Australian Journal of Botany* **49**: 271–300.

McNeill J, Barrie FR, Buck WR, Demoulin V, Greuter W, Hawksworths DL, Herendeen PS, Knapp S, Marhold K, Prado J, *et al.* 2012. International Code of Nomenclature for algae, fungi and plants (Melbourne Code) adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011. *Regnum Vegetabile* **154**: 1–240.

Medeiros D, Valle L de S, Alves RJV. **2013**. Revalidation of the genera *Bia* and *Zuckertia* (Euphorbiaceae) with *B. capivarensis* sp. nov. from Serra da Capivara, Brazil. *Nordic Journal of Botany* **31**: 595–602.

Meisner CF. **1843**. *Plantarum Vascularium Genera vol. 2*. Lipsiae [Leipzig]: Libraria Weidmannia.

Michalak I, Zhang L-B, Renner SS. **2010**. Trans-Atlantic, trans-Pacific and trans-Indian Ocean dispersal in the small Gondwanan Laurales family Hernandiaceae. *Journal of Biogeography* **37**: 1214–1226.

Miller MA, Pfeiffer W, Schwartz T. **2010**. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE). New Orleans, LA, 1–8.

Miller KI, Webster GL. **1967**. A preliminary revision of Tragia (Euphorbiaceae) in the United States. *Rhodora* **69**: 241–305.

Mirarab S, Reaz R, Bayzid MS, Zimmermann T, Swenson MS, Warnow T. **2014**. ASTRAL: Genome-scale coalescent-based species tree estimation. *Bioinformatics* **30**: i541–i548.

Mirarab S, Warnow T. **2015**. ASTRAL-II: Coalescent-based species tree estimation with many hundreds of taxa and thousands of genes. *Bioinformatics* **31**: i44–i52.

Missouri Botanical Garden. **2017**. Tropicos.org.

Moles AT, Ackerly DD, Tweddle JC, Dickie JB, Smith R, Leishman MR, Mayfield MM, Pitman A, Wood JT, Westoby M. **2007**. Global patterns in seed size. *Global Ecology and Biogeography* **16**: 109–116.

Moles AT, Ackerly DD, Webb CO, Tweddle JC, Dickie JB, Pitman AJ, Westoby M. **2005a**. Factors that shape seed mass evolution. *Proceedings of the National Academy of Sciences of the United States of America* **102**: 10540–10544.

Moles AT, Ackerly DD, Webb CO, Tweddle JC, Dickie JB, Westoby M. **2005b**. A brief history of seed size. *Science* **307**: 576–580.

Moles AT, Falster DS, Leishman MR, Westoby M. **2004**. Small-seeded species produce more seeds per square metre of canopy per year, but not per individual per lifetime. *Journal of Ecology* **92**: 384–396.

Moles AT, Westoby M. **2004**. Seedling survival and seed size: a synthesis of the literature. *Journal of Ecology* **92**: 372–383.

Moore BR, Höhna S, May MR, Rannala B, Huelsenbeck JP. **2016**. Critically evaluating the theory and performance of Bayesian analysis of macroevolutionary mixtures. *Proceedings of the National Academy of Sciences of the United States of America* **113**: 9569–74.

Morrone JJ. **2014**. Cladistic biogeography of the Neotropical region: identifying the main events in the diversification of the terrestrial biota. *Cladistics* **30**: 202–214.

Mota A, de Lima A, Albuquerque T, Silveira T, Nascimento J, Silva J, Ribeiro A, Maia J, Bastos G. **2015**. Antinociceptive activity and toxicity evaluation of the fatty oil from *Plukenetia polyadenia* Mull. Arg. (Euphorbiaceae). *Molecules* **20**: 7925–7939.

Múlgura de Romero ME, Gutiérrez de Sanguinetti MM. **1989**. Actualizacion taxonomica de *Tragia* (Euphorbiaceae) para Argentina y regiones limitrofes. *Darwiniana* **29**: 77–138.

Müller J. **1864a**. System der Euphorbiaceen. *Botanische Zeitung* **22**: 324.

Müller J. **1864b**. Neue Euphorbiaceen des Herbarium Hooker in Kew. *Flora* **47**: 529–544.

Müller J. **1865**. Euphorbiaceae. *Linnaea* **34**: 1–224.

Müller J. **1866**. Euphorbiaceae. In: de Candolle AP, ed. Prodromus Systematis Naturalis Regni Vegetabilis vol. 15(2). Parisiis [Paris]: Victoris Masson et Filii.

Müller J. **1874**. *Flora Brasiliensis, vol. 11(2)* (KFP von Martius, Ed.). Lipsiae [Leipzig]: Apud Frid. Fleischer in comm.

van der Niet T, Johnson SD. **2012**. Phylogenetic evidence for pollinator-driven diversification of angiosperms. *Trends in Ecology & Evolution* **27**: 353–361.

Nieto-Blázquez ME, Antonelli A, Roncal J. **2017**. Historical biogeography of endemic seed

plant genera in the Caribbean: Did GAARlandia play a role? *Ecology and Evolution* **7**: 10158–10174.

Nordenstam B. **1980**. The herbaria of Lehmann and Sonder in Stockholm, with special reference to the Ecklon and Zeyher collection. *Taxon* **29**: 279–288.

Nowicke JW, Takahashi M. **2002**. Pollen morphology, exine structure and systematics of Acalyphoideae (Euphorbiaceae), part 4: Tribes Acalypheae pro parte (Erythrococca, *Claoxylon, Claoxylopsis, Mareya, Mareyopsis, Discoclaoxylon, Micrococca, Amyrea, Lobanilia, Mallotus, Deuteromallotus*, … *Review of Palaeobotany and Palynology* **121**: 231–336.

Nowicke JW, Takahashi M, Webster GL. **1999**. Pollen morphology, exine structure and systematics of Acalyphoideae (Euphorbiaceae): Part 2. Tribes Agrostistachydeae (*Agrostistachys, Pseudagrostistachys, Cyttaranthus, Chondrostylis*), Chrozophoreae (*Speranskia, Caperonia, Philyra, Ditaxis, Argythamnia*, … *Review of Palaeobotany and Palynology* **105**: 1–62.

Nwosu M. **2006**. Studies on germination and seedling anatomy of *Plukenetia conophora* Muell.-Arg. (Euphorbiaceae). *Journal of Economic and Taxonomic Botany* **30**: 504–509.

Olmstead RG. **2013**. Phylogeny and biogeography in Solanaceae, Verbenaceae and Bignoniaceae: A comparison of continental and intercontinental diversification patterns. *Botanical Journal of the Linnean Society* **171**: 80–102.

Olmstead RG, Sweere JA, Wolfe KH. **1993**. Ninety extra nucleotide in *ndhF* gene of tobacco chloroplast DNA: A summary of revisions to the 1986 genome sequence. *Plant Molecular Biology* **22**: 1191–1193.

Pagel M. **1999**. Inferring the historical patterns of biological evolution. *Nature* **401**: 877.

Pallas PS. **1773**. *Reise durch Verschiedene Provinzen des Russischen Reichs, vol. 2*. St. Petersburg: Kayserlichen Academie der Wissenschaften.

Pax F. **1897**. Euphorbiaceae africanae. III. *Botanische Jahrbücher für Systematik* **23**: 518–536.

Pax F. **1899**. Euphorbiaceae africanae. IV. *Botanische Jahrbücher für Systematik* **26**: 325–329.

Pax F. **1908**. Beiträge zur Kenntnis der Afrikanischen-Flora XXI, Euphorbiaceae. *Bulletin de l'Herbier Boissier, série 2* **8**: 634–637.

Pax F, Hoffmann K. **1919a**. Euphorbiaceae-Acalypheae-Plukenetiinae. In: Engler A, ed. Das Pflanzenreich IV.147.IX. (Heft 68). Leipzig: Wilhelm Engelmann, 1–108.

Pax F, Hoffmann K. **1919b**. Euphorbiaceae-Dalechampieae. In: Engler A, ed. Das Pflanzenreich IV.147.XII. (Heft 68). Leipzig: Wilhelm Engelmann, 1–59.

Pax F, Hoffmann K. **1924**. Euphorbiaceae additamentum IV. In: Engler A, ed. Das Pflanzenreich IV.147.XVII. (Heft 85). Leipzig: Wilhelm Engelmann, 179–204.

Pellegrino KCM, Rodrigues MT, Harris DJ, Yonenaga-Yassuda Y, Jr. JWS. **2011**. Molecular phylogeny, biogeography and insights into the origin of parthenogenesis in the Neotropical genus *Leposoma* (Squamata: Gymnophthalmidae): Ancient links between the Atlantic Forest and Amazonia. *Molecular Phylogenetics and Evolution* **61**: 446–459.

Pérez-Escobar OA, Chomicki G, Condamine FL, Karremans AP, Bogarín D, Matzke NJ,

Silvestro D, Antonelli A. **2017a**. Recent origin and rapid speciation of Neotropical orchids in the world's richest plant biodiversity hotspot. *New Phytologist* **215**: 891–905.

Pérez-Escobar OA, Gottschling M, Chomicki G, Condamine FL, Klitgård BB, Pansarin E, Gerlach G. **2017b**. Andean mountain building did not preclude dispersal of lowland epiphytic orchids in the Neotropics. *Scientific Reports* **7**: 4919.

Perumal Samy R, Sethi G, Chow VT, Stiles BG. **2013**. Plant-based hydrocarbon esters from *Tragia involucrata* possess antimicrobial and anti-inflammatory activities. *Infectious Disorders - Drug Targets* **13**: 141–153.

Plukenet L. **1691a**. *Phytographia, vol. 1*. Londini [London]: Sumptibus Autoris.

Plukenet L. **1691b**. *Phytographia, vol. 2*. Londini [London]: Sumptibus Autoris.

Plukenet L. **1692**. *Phytographia, vol. 3*. Londini [London]: Sumptibus Autoris.

Plukenet L. **1696a**. *Phytographia, vol. 4*. Londini [London]: Sumptibus Autoris.

Plukenet L. **1696b**. *Almagestum Botanicum*. Londini [London]: Sumptibus Autoris.

Plumier C. **1703**. *Nova Plantarum Americanarum Genera*. Parisiis [Paris]: Apud Joannem Boudot.

Plummer M, Best N, Cowles K, Vines K. **2006**. CODA: Convergence diagnosis and output analysis for MCMC. *R News* **6**: 7–11.

Poulsen JR, Clark CJ, Connor EF, Smith TB. **2002**. Differential resource use by primates and hornbills: Implications for seed dispersal. *Ecology* **83**: 228–240.

Prieu C, Matamoro-Vidal A, Raquin C, Dobritsa A, Mercier R, Gouyon PH, Albert B. **2016**. Aperture number influences pollen survival in *Arabidopsis* mutants. *American Journal of Botany* **103**: 452–459.

Punt W. **1962**. Pollen Morphology of the Euphorbiaceae with special reference to taxonomy. *Wentia* **7**: 1–116.

Punt W, Hoen PP, Blackmore S, Nilsson S, Thomas A Le. **2007**. Glossary of pollen and spore terminology. *Review of Palaeobotany and Palynology* **143**: 1–81.

Qiu H, Gillespie LJ. **2008**. *Cnesmone*, Megistostigma (Euphorbiaceae). In: Wu Z, Raven PH, Hong D, eds. Flora of China vol. 11. St. Louis: Missouri Botanical Garden Press, 255–257.

Quezada IM, Gianoli E. **2011**. Crassulacean acid metabolism photosynthesis in Bromeliaceae: An evolutionary key innovation. *Biological Journal of the Linnean Society* **104**: 480–486.

Rabosky DL. **2014**. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. *PLoS ONE* **9**: e89543.

Rabosky DL, Grundler M, Anderson C, Title P, Shi JJ, Brown JW, Huang H, Larson JG. **2014**. BAMMtools: An R package for the analysis of evolutionary dynamics on phylogenetic trees (S Kembel, Ed.). *Methods in Ecology and Evolution* **5**: 701–707.

Rabosky DL, Mitchell JS, Chang J. **2017**. Is BAMM Flawed? Theoretical and practical concerns in the analysis of multi-rate diversification models. *Systematic Biology* **66**: 477–498.

R Core Development Team. **2016**. R: A language and environment for statistical computing.

Radcliffe-Smith A. **1980**. A note on *Romanoa* (Euphorbiaceae). *Kew Bulletin* **34**: 589–590.

Radcliffe-Smith A. **1987**. *Flora of Tropical East Africa. Euphorbiaceae (Part 1)* (RM Polhill, Ed.). Rotterdam, Boston: A.A. Balkema.

Radcliffe-Smith A. **2001**. *Genera Euphorbiacearum*. Royal Botanical Gardens, Kew.

Rambaut A, Suchard MA, Xie D, Drummond AJ. **2014**. Tracer v1.6. Published online at [http://beast.bio.ed.ac.uk/Tracer].

Raven PH, Axelrod DI. **1974**. Angiosperm biogeography and past continental movements. *Annals of the Missouri Botanical Garden* **61**: 539–673.

Ree RH, Sanmartín I. **2018**. Conceptual and statistical problems with the DEC+J model of founder-event speciation and its comparison with DEC via model selection. *Journal of Biogeography* **45**: 741–749.

Ree RH, Smith SA. **2008**. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology* **57**: 4–14.

Renner S. **2004a**. Plant dispersal across the tropical Atlantic by wind and sea currents. *International Journal of Plant Sciences* **165**: S23–S33.

Renner SS. **2004b**. Multiple Miocene Melastomataceae dispersal between Madagascar, Africa and India. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **359**: 1485–1494.

Renner SS, Clausing G, Meyer K. **2001**. Historical biogeography of Melastomataceae: The roles of Tertiary migration and long-distance dispersal. *American Journal of Botany* **88**: 1290–1300.

Renner SS, Meyer K. **2001**. Melastomeae come full circle: Biogeographic reconstruction and molecular clock dating. *Evolution* **55**: 1315–1324.

Renner SS, Strijk JS, Strasberg D, Thébaud C. **2010**. Biogeography of the Monimiaceae (Laurales): A role for East Gondwana and long-distance dispersal, but not West Gondwana. *Journal of Biogeography* **37**: 1227–1238.

Revell LJ. **2012**. phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3**: 217–223.

Revell LJ. **2013**. Two new graphical methods for mapping trait evolution on phylogenies. *Methods in Ecology and Evolution* **4**: 754–759.

Reyes E, Morlon H, Sauquet H. **2015**. Presence in Mediterranean hotspots and floral symmetry affect speciation and extinction rates in Proteaceae. *New Phytologist* **207**: 401–410.

Ribeiro LC, Barbosa ERM, van Langevelde F, Borghetti F. **2015**. The importance of seed mass for the tolerance to heat shocks of savanna and forest tree species. *Journal of Vegetation Science* **26**: 1102–1111.

Richardson JE, Pennington RT, Pennington TD, Hollingsworth PM. **2001**. Rapid diversification of a species-rich genus of neotropical rain forest trees. *Science* **293**: 2242–2245.

Roalson EH, Roberts WR. **2016**. Distinct processes drive diversification in different clades of Gesneriaceae. *Systematic Biology* **65**: 662–684.

Rojas-Aréchiga M, Mandujano MC, Golubov JK. **2013**. Seed size and photoblastism in species belonging to tribe Cacteae (Cactaceae). *Journal of Plant Research* **126**: 373–386.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. **2012**. Mrbayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539–542.

Rothfels CJ, Larsson A, Li FW, Sigel EM, Huiet L, Burge DO, Ruhsam M, Graham SW, Stevenson DW, Wong GKS, *et al.* 2013. Transcriptome-mining for single-copy nuclear markers in ferns. *PLoS ONE* **8**: e76957.

Rumphius GE. **1750**. *Herbarium Amboinense, vol. 1*. Amstelaedami [Amsterdam].

Saint-Hilaire A. **1840**. *Leçons de Botanique*. Paris: P.-J. Loss.

Sang T, Crawford DJ, Stuessy TF. **1997**. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany* **84**: 1120–1136.

Sargent RD. **2004**. Floral symmetry affects speciation rates in angiosperms. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **271**: 603 LP-608.

Sato S, Hirakawa H, Isobe S, Fukai E, Watanabe A, Kato M, Kawashima K, Minami C, Muraki A, Nakazaki N, *et al.* 2011. Sequence analysis of the genome of an oil-bearing tree, *Jatropha curcas* L. *DNA Research* **18**: 65–76.

Sayyari E, Mirarab S. **2016**. Fast coalescent-based computation of local branch support from quartet frequencies. *Molecular Biology and Evolution* **33**: 1654–1668.

Schott FA, Xie S-P, McCreary JP. **2009**. Indian Ocean circulation and climate variability. *Reviews of Geophysics* **47**: RG1002.

Seo T-K. **2008**. Calculating bootstrap probabilities of phylogeny using multilocus sequence data. *Molecular Biology and Evolution* **25**: 960–971.

Seo T-K, Kishino H, Thorne JL. **2005**. Incorporating gene-specific variation when inferring and evaluating optimal evolutionary tree topologies from multilocus sequence data. *Proceedings of the National Academy of Sciences of the United States of America* **102**: 4436–4441.

Serrano-Serrano ML, Rolland J, Clark JL, Salamin N, Perret M. **2017**. Hummingbird pollination and the diversification of angiosperms: An old and successful association in Gesneriaceae. *Proceedings of the Royal Society B: Biological Sciences* **284**.

Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder CT, Schilling EE, Small RL. **2005**. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* **92**: 142–166.

Shimodaira H. **2002**. An approximately unbiased test of phylogenetic tree selection. *Systematic Biology* **51**: 492–508.

Shimodaira H. **2004**. Approximately unbiased tests of regions using multistep-multiscale

bootstrap resampling. *Ann. Statist.* **32**: 2616–2641.

Shimodaira H, Hasegawa M. **2001**. CONSEL: For assessing the confidence of phylogenetic tree selection. *Bioinformatics* **17**: 1246–1247.

Silva GZ da, Vieira VAC, Boneti JEB, Melo LF, Martins CC. **2016**. Temperature and substrate on *Plukenetia volubilis* L. seed germination. *Revista Brasileira de Engenharia Agrícola e Ambiental* **20**: 1031–1035.

Silvestro D, Zizka G, Schulte K. **2013**. Disentangling the effects of key innovations on the diversification of Bromelioideae (Bromeliaceae). *Evolution* **68**: 163–175.

Simmons MP. **2004**. Independence of alignment and tree search. *Molecular Phylogenetics and Evolution* **31**: 874–879.

Simmons MP, Ochoterena H. **2000**. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* **49**: 369–381.

Simon MF, Grether R, de Queiroz LP, Skema C, Pennington RT, Hughes CE. **2009**. Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 20359–20364.

Simpson MG, Guilliams CM, Johnson LA. **2017**. Patterns and processes of American amphitropical disjunctions: New insights. *American Journal of Botany* **104**: 1597–1599.

Smith JE. **1799**. Dissertatio Botanica de *Plukenetia*. *Nova Acta Regiae Societatis Scientiarum Upsaliensis* **6**: 1–4.

Souza-Neto AC, Cianciaruso M V, Collevatti RG. **2016**. Habitat shifts shaping the diversity of a biodiversity hotspot through time: insights from the phylogenetic structure of Caesalpinioideae in the Brazilian Cerrado. *Journal of Biogeography* **43**: 340–350.

Spriggs EL, Clement WL, Sweeney PW, Madriñán S, Edwards EJ, Donoghue MJ. **2014**. Temperate radiations and dying embers of a tropical past: the diversification of *Viburnum*. *New Phytologist* **207**: 340–354.

Stafleu FA, Cowan RS. **1976**. Volume I: A-G. In: Taxonomic literature: A selective guide to botanical publications and collections with dates, commentaries and types. Utrecht: Bohn, Scheltema & Holkema.

Stafleu FA, Cowan RS. **1979**. Volume II: H-Le. In: Taxonomic literature: A selective guide to botanical publications and collections with dates, commentaries and types. Utrecht: Bohn, Scheltema & Holkema.

Stafleu FA, Cowan RS. **1981**. Volume III: Lh-O. In: Taxonomic literature: A selective guide to botanical publications and collections with dates, commentaries and types. Utrecht: Bohn, Scheltema & Holkema.

Stamatakis A. **2014**. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.

Stanford AM, Harden R, Parks CR. **2000**. Phylogeny and biogeography of *Juglans* (Juglandaceae) based on *matK* and ITS sequence data. *American Journal of Botany* **87**: 872–882.

Starr JR, Naczi RFC, Chouinard BN. **2009**. Plant DNA barcodes and species resolution in sedges (*Carex*, Cyperaceae). *Molecular Ecology Resources* **9**: 151–163.

Steinmann VW, Porter JM. **2002**. Phylogenetic relationships in Euphorbieae (Euphorbiaceae) based on ITS and *ndhF* sequence data. *Annals of the Missouri Botanical Garden* **89**: 453–490.

Steinmann VW, Ramírez-Amezcua Y. **2013**. *Bia manuelii* (Euphorbiaceae: Acalyphoideae), a new species from Sierra de Coalcomán, Michoacán, Mexico. *Revista Mexicana de Biodiversidad* **84**: 746–750.

Štorchová H, Olson MS. **2007**. The architecture of the chloroplast *psbA-trnH* non-coding region in angiosperms. *Plant Systematics and Evolution* **268**: 235–256.

Suchard MA, Rambaut A. **2009**. Many-core algorithms for statistical phylogenetics. *Bioinformatics* **25**: 1370–1376.

Sundue MA, Testo WL, Ranker TA. **2015**. Morphological innovation, ecological opportunity, and the radiation of a major vascular epiphyte lineage. *Evolution* **69**: 2482–2495.

Swofford D. **2002**. PAUP*: Phylogenetic analysis using parsimony (* and other methods). Version 4. Sunderland: Sinauer Associates.

Sytsma KJ, Litt A, Zjhra ML, Pires JC, Nepokroeff M, Conti E, Walker J, Wilson PG. **2004**. Clades, clocks, and continents: Historical and biogeographical analysis of Myrtaceae, Vochysiaceae, and relatives in the southern hemisphere. *International Journal of Plant Sciences* **165**: S85–S105.

Takahashi M, Nowicke JW, Webster GL, Orli SS, Yankowski S. **2000**. Pollen morphology, exine structure, and systematics of Acalyphoideae (Euphorbiaceae), part 3: Tribes Epiprineae (*Epiprinus, Symphyllia, Adenochlaena, Cleidiocarpon, Koilodepas, Cladogynos, Cephalocrotonopsis, Cephalocroton, Cephalomappa*), Adelieae (*Adelia*, … *Review of Palaeobotany and Palynology* **110**: 1–66.

Tank DC, Eastman JM, Pennell MW, Soltis PS, Soltis DE, Hinchliff CE, Brown JW, Sessa EB, Harmon LJ. **2015**. Nested radiations and the pulse of angiosperm diversification: Increased diversification rates often follow whole genome duplications. *New Phytologist* **207**: 454–467.

Tate JA, Simpson BB. **2003**. Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. *Systematic Botany* **28**: 723–737.

Tavşanoğlu Ç, Serter Çatav Ş. **2012**. Seed size explains within-population variability in post-fire germination of *Cistus salviifolius*. *Annales Botanici Fennici* **49**: 331–340.

Thurston EL, Lersten NR. **1969**. The morphology and toxicology of plant stinging hairs. *The Botanical Review* **35**: 393–412.

Tokuoka T. **2007**. Molecular phylogenetic analysis of Euphorbiaceae sensu stricto based on plastid and nuclear DNA sequences and ovule and seed character evolution. *Journal of Plant Research* **120**: 511–522.

Torrecilla P, Catalán P. **2002**. Phylogeny of broad-leaved and fine-leaved *Festuca* lineages (Poaceae) based on nuclear ITS sequences. *Systematic Botany* **27**: 241–251.

Urtecho RJ. **2016**. *Tragia* (Euphorbiaceae). In: Flora of North America Vol. 12 Magniliophyta: Vitaceae to Garryaceae. New York, Oxford: Oxford University Press, 184–191.

Vanzela ALL, Ruas PM, Marin-Morales MA. **1997**. Karyotype studies of some species of *Dalechampia* Plum. (Euphorbiaceae). *Botanical Journal of the Linnean Society* **125**: 25–33.

Vellozo JM da C. **1831**. *Tab. 128.* Parisiis [Paris]: Senefelder.

van Velzen R, Wahlert GA, Sosef MSM, Onstein RE, Bakker FT. **2015**. Phylogenetics of African *Rinorea* (Violaceae): Elucidating infrageneric relationships using plastid and nuclear DNA sequences. *Systematic Botany* **40**: 174–184.

Viljoen J-A, Muasya AM, Barrett RL, Bruhl JJ, Gibbs AK, Slingsby JA, Wilson KL, Verboom GA. **2013**. Radiation and repeated transoceanic dispersal of Schoeneae (Cyperaceae) through the southern hemisphere. *American Journal of Botany* **100**: 2494–2508.

Wagenmakers E-J, Farrell S. **2004**. AIC model selection using Akaike weights. *Psychonomic Bulletin & Review* **11**: 192–196.

Walker JW, Doyle JA. **1975**. The bases of angiosperm phylogeny: Palynology. *Annals of the Missouri Botanical Garden* **62**: 664–723.

Wang X, Liu A. **2014**. Expression of genes controlling unsaturated fatty acids biosynthesis and oil deposition in developing seeds of Sacha Inchi (*Plukenetia volubilis* L.). *Lipids* **49**: 1019–1031.

Wang X, Xu R, Wang R, Liu A. **2012**. Transcriptome analysis of Sacha Inchi (*Plukenetia volubilis* L.) seeds at two developmental stages. *BMC Genomics* **13**: 716.

Wang B, Yang X. **2014**. Teasing apart the effects of seed size and energy content on rodent scatter-hoarding behavior. *PLoS ONE* **9**: e111389.

Weber MG, Agrawal AA. **2014**. Defense mutualisms enhance plant diversification. *Proceedings of the National Academy of Sciences* **111**: 16442 LP-16447.

Webster G. **1967**. *Acidoton* (Euphorbiaceae) in Central America. *Annals of the Missouri Botanical Garden* **54**: 191.

Webster GL. **1975**. Conspectus of a new classification of the Euphorbiaceae. *Taxon* **24**: 593–601.

Webster GL. **1994**. Synopsis of the genera and suprageneric taxa of Euphorbiaceae. *Annals of the Missouri Botanical Garden* **81**: 33–144.

Webster GL. **2007**. Taxonomic and nomenclatural changes in American Euphorbiaceae sensu lato. *Contributions from the University of Michigan Herbarium* **25**: 235–239.

Webster GL. **2014**. Euphorbiaceae. In: Kubitzki K, ed. Flowering Plants. Eudicots: Malpighiales. Springer-Verlag Berlin Heidelberg, 51–216.

Webster GL, Armbruster WS. **1991**. A synopsis of the neotropical species of Dalechampia (Euphorbiaceae). *Botanical Journal of the Linnean Society* **105**: 137–177.

Webster GL, Huft MJ. **1988**. Revised synopsis of Panamanian Euphorbiaceae. *Annals of the*

Missouri Botanical Garden **75**: 1087–1144.

Webster GL, Webster BD. **1972**. The morphology and relationships of *Dalechampia scandens* (Euphorbiaceae). *American Journal of Botany* **59**: 573–586.

Weeks A, Zapata F, Pell SK, Daly DC, Mitchell JD, Fine PVA. **2014**. To move or to evolve: Contrasting patterns of intercontinental connectivity and climatic niche evolution in 'Terebinthaceae' (Anacardiaceae and Burseraceae). *Frontiers in Genetics* **5**: 409.

van Welzen PC, Strijk JS, van Konijnenburg-van Cittert JHA, Nucete M, Merckx VSFT. **2014**. Dated phylogenies of the sister genera *Macaranga* and *Mallotus* (Euphorbiaceae): Congruence in historical biogeographic patterns? *PloS ONE* **9**: e85713.

Werneck FP. **2011**. The diversification of eastern South American open vegetation biomes: Historical biogeography and perspectives. *Quaternary Science Reviews* **30**: 1630–1648.

White TJ, Bruns S, Lee S, Taylor J. **1990**. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR Protocols: A Guide to Methods and Applications. New York: Academic Press Inc., 315–322.

Whitlock BA, Hale AM, Groff PA. **2010**. Intraspecific inversions pose a challenge for the t*rnH-psbA* plant DNA barcode. *PLoS ONE* **5**: e11533.

Wickham H. **2009**. *ggplot2: Elegant graphics for data analysis*. New York: Springer-Verlag.

Wikström N, Avino M, Razafimandimbison SG, Bremer B. **2010**. Historical biogeography of the coffee family (Rubiaceae, Gentianales) in Madagascar: Case studies from the tribes Knoxieae, Naucleeae, Paederieae and Vanguerieae. *Journal of Biogeography* **37**: 1094–1113.

de Wilde JJFE, Hughes M, Rodda M, Thomas DC. **2011**. Pliocene intercontinental dispersal from Africa to Southeast Asia highlighted by the new species *Begonia afromigrata* (Begoniaceae). *Taxon* **60**: 1685–1692.

Williams SE, Bolitho EE, Fox S. **2003**. Climate change in Australian tropical rainforests: An impending environmental catastrophe. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **270**: 1887–1892.

Williams JH, Mazer SJ. **2016**. Pollen—Tiny and ephemeral but not forgotten: New ideas on their ecology and evolution. *American Journal of Botany* **103**: 365–374.

Wurdack KJ, Davis CC. **2009**. Malpighiales phylogenetics: Gaining ground on one of the most recalcitrant clades in the angiosperm tree of life. *American Journal of Botany* **96**: 1551–1570.

Wurdack KJ, Hoffmann P, Chase MW. **2005**. Molecular phylogenetic analysis of uniovulate Euphorbiaceae (Euphorbiaceae sensu stricto) using plastid *rbcL* and *trnL-F* DNA sequences. *American Journal of Botany* **92**: 1397–1420.

Wurdack KJ, Hoffmann P, Samuel R, De Bruijn A, Van Der Bank M, Chase MW. **2004**. Molecular phylogenetic analysis of Phyllanthaceae (Phyllanthoideae pro parte, Euphorbiaceae sensu lato) using plastid rbcL DNA sequences. *American Journal of Botany* **91**: 1882–1900.

Yuan Y-M, Wohlhauser S, Möller M, Klackenberg J, Callmander MW, Küpfer P. **2005**. Phylogeny and biogeography of *Exacum* (Gentianaceae): A disjunctive distribution in the Indian Ocean basin resulted from long distance dispersal and extensive radiation. *Systematic Biology* **54**: 21–34.

Yule GU. **1925**. A mathematical theory of evolution, based on the conclusions of Dr. J. C. Willis, F.R.S. *Philosophical Transactions of the Royal Society of London, Series B* **213**: 21–87.

Zachos J, Pagani M, Sloan L, Thomas E, Billups K. **2001**. Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* **292**: 686–693.

Zhang C, Rabiee M, Sayyari E, Mirarab S. **2018**. ASTRAL-III: Polynomial time species tree reconstruction from partially resolved gene trees. *BMC Bioinformatics* **19**: 153.

Zhang N, Zeng L, Shan H, Ma H. **2012**. Highly conserved low-copy nuclear genes as effective markers for phylogenetic analyses in angiosperms. *New Phytologist* **195**: 923–937.

Zheng S, Pan T, Fan L, Qiu Q-S. **2013**. A novel *AtKEA* gene family, homolog of bacterial K+/H+ antiporters, plays potential roles in K+ homeostasis and osmotic adjustment in Arabidopsis. *PLoS ONE* **8**: e81463.

Zhou L, Su YCF, Thomas DC, Saunders RMK. **2012**. 'Out-of-Africa' dispersal of tropical floras during the Miocene climatic optimum: evidence from *Uvaria* (Annonaceae). *Journal of Biogeography* **39**: 322–335.

Zwickl DJ. **2006**. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. *Ph.D. Dissertation*.

Supplemental Data

Supplemental data 3.1 Python script for LITE BLUE DEVIL v0.3.

```
#!/usr/bin/python
print
print """
-------------------------------------------------------------------------------------------------
|                                        Lite Blue Devil                                        |
|                                    version 0.3 blastn or blastp                               |
|                      inspired by BLast Utility and sequence Extraction DEVIsed by Li          |
|                                    Written by E. M. Sigel                                     |
|                                                                                               |
|If you use Lite Blue Devil or a modified version of it, please cite:                           |
| Cardinal-McTeague WM, Wurdack KJ, Sigel EM, Gillespie LJ. Seed size evolution and biogeography |
|       of Plukenetia (Euphorbiaceae), a pantropical genus with traditionally cultivated oilseed |
|       species. BMC Evolutionary Biology                                                       |
|                                                                                               |
| Programs used by Lite Blue Devil:                                                             |
| Cock PJ, Antao T, Chang JT, Chapman BA, Cox CJ, Dalke A, et al. Biopython: freely available   |
|       Python tools for computational molecular biology and bioinformatics. Bioinformatics.   |
|       2009;25:1422-1423.                                                                      |
| Edgar RC. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic |
|       Acids Res. 2004;32:1792-7.                                                              |
| Rothfels CJ, Larsson A, Li FW, Sigel EM, Huiet L, Burge DO, et al. Transcriptome-mining for   |
|       single-copy nuclear markers in ferns. PLoS One. 2013;8:e76957.                          |
| Stamatakis A. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large    |
|       phylogenies. Bioinformatics. 2014;30:1312-3.                                            |
-------------------------------------------------------------------------------------------------
"""
print
##############################################################################################
#*YOU NEED TO HAVE PYTHON, BIOPYTHON, MUSCLE, and RAXML (Sequential) INSTALLED*
#load in the python/biopython modules

import re #Regular expression
import sys #For using arguments
import subprocess
import os
import shutil
import csv
from Bio.Blast.Applications import NcbiblastnCommandline
from Bio.Blast.Applications import NcbitblastnCommandline
from Bio.Align.Applications import MuscleCommandline
from Bio.Phylo.Applications import RaxmlCommandline
from Bio import SeqIO
from Bio import AlignIO
from Bio.SeqRecord import SeqRecord
from Bio.Blast import NCBIWWW, NCBIXML
from Bio import AlignIO
from Bio.Alphabet import IUPAC, Gapped

##############################################################################################
#Change the below paths to accommodate the locals of your versions of Muscle and RaxML
#You can comment out this section if the paths in your path file (e.g., .bash_profile)
muscle_exe= '/Users/scripts/muscle'
raxml = '/Users/scripts/standard-RAxML-master/raxmlHPC'
##############################################################################################
#state the Usage instructions
if len(sys.argv) < 2:
sys.exit("""
You need to provide the bluedevil_settings file

Usage: litebluedevil.py litebluedevil_settings file
Example: litebluedevil.py litebluedevil_settings_phytochrome.txt

litebluedevil_settings file: This file allows you to modify up all major parameters and set up the run.
See the bluedevil_settings_template.txt for details. Note the file name must start with
'bluedevil_settings', for the program to recognize it.

         """)

elif len(sys.argv) >3:
         sys.exit("""
You need to provide the bluedevil_settings file

Usage: litebluedevil.py litebluedevil_settings file
Example: litebluedevil.py litebluedevil_settings_phytochrome.txt
```

```
litebluedevil_settings file: This file allows you to modify up all major parameters and set up the run.
See the bluedevil_settings_template.txt for details. Note the file name must start with
'bluedevil_settings', for the program to recognize it.

        """)

###############################################################################################
#If the bluedevil_settings file is provided, take in the parameters, and make output file names
elif sys.argv[1].startswith('litebluedevil_settings'):
        settings = open(sys.argv[1], 'rU')
        for line in settings:
                line = line.strip(' \t\n\r')
                line = line.replace(' ', '')
                line = line.replace('\t', '')
                line = line.split('#')[0]
                setting_line = line.find('=')
                if setting_line != -1:
                        setting = line.split('=')
                        setting_name = setting[0]
                        setting_argument = setting[1]
                        if setting_name == 'Blast_type':
                                Blast_type = setting_argument
                        elif setting_name == 'Max_blast_hits':
                                Max_hits = setting_argument
                                Max_hits = int(Max_hits)
                        elif setting_name == 'Query_sequence_file_name':
                                Seq_query = setting_argument
                        elif setting_name == 'Transcriptome_list':
                                Transcriptome_list = setting_argument.replace(' ', '').replace('\t',
'').split(',')
                        elif setting_name == 'BLAST_E_value':
                                BLAST_E_value = setting_argument
                        elif setting_name == 'RaxmL_Tree':
                                RaxmL_Tree = setting_argument

        Blast_results_filename = Seq_query.split('.')[0]+'_blast_results.txt'

###############################################################################################
#open up the query fasta file, create a dictionary for each query sequence, and

print '\nReading '+Seq_query+'...'
Query_infile = open(Seq_query, "rU")

##make a dictionary of the input/query fasta file, such that gene name is the key and the parsed fasta
record is the value
## gene name :parsed fasta record
##This dictionary will be used to make a copy of the input query fasta file in each trancriptome database
folder
input_fasta_Dict = SeqIO.to_dict(SeqIO.parse(Query_infile, "fasta"))
#print input_fasta_Dict
#print 'XXXXXX'

##Make an empty nested dictory that will hold all the query gene and hit sequence data you are about to
generate
##query_genes_Dict = {'query_gene_1_id':{'hit_sequence_1_id': 'hit sequence_1', 'hit_sequence_2_id': 'hit
sequence_2'}, 'query_gene_2_id':{'hit_sequence_1_id': 'hit sequence_1', 'hit_sequence_2_id': 'hit
sequence_2'}}
##query_genes_Dict = {'rbcL': {'Cystopteris_rbcl': 'ATGACGTAA', 'Polypodium_rbcl': 'ATGACGTGG'}, 'trnGR':
{'Cystopteris_trnGR': 'TTTNCGAT', 'Polypodium_trnGR': 'ATTTACGAT'}}
all_query_genes_Dict = {}

## populate the query_genes_DICT with the names of the query genes (called by their fasta record id)
Query_infile = open(Seq_query, "rU")
for record in SeqIO.parse(Query_infile, "fasta"):
        query_name = str(record.id)
        query_sequence = str(record.seq)
        all_query_genes_Dict[query_name] = {}
        #print all_query_genes_Dict

        # Then enter the query_name: query_sequence as the first entry under
all_query_genes_Dict[query_name]
        all_query_genes_Dict[query_name][query_name] = query_sequence
        #print all_query_genes_Dict
```

```
#############################################################################################
#copy the query file to each transcriptome folder and blast query file against the transcriptome
if Blast_type == 'blastn' or Blast_type == 'blastp':
         for transcriptome in Transcriptome_list:
                  #Go to each transcriptome folder
                  os.chdir(transcriptome)
                  #identify the transcriptome database
                  TranscriptomeID = transcriptome
                  TranscriptomeDB = TranscriptomeID + '.fa'

                  #write a copy of the input Query Sequence fasta files to each transcriptome file ex.
SQD1_input.txt
                  SeqIO.write(input_fasta_Dict.values(), Seq_query, 'fasta')
                  #os.chdir('..')

                  #Blastn or blastp the query sequences against the transcriptome
                  if Blast_type == 'blastn':
                           print '\nBlasting ' + Seq_query + ' against ' + TranscriptomeID + '...\n'
                           Blast_results_file = Seq_query.split('.')[0]+'_blastn_results.txt'
                           #print TranscriptomeDB
                           blastn_cline = NcbitblastnCommandline(cmd='blastn', query=Seq_query,
db=TranscriptomeDB, out=Blast_results_file, evalue=BLAST_E_value, outfmt = 6, max_target_seqs = Max_hits)
                           stdout, stderr = blastn_cline()
                  elif Blast_type == 'blastp':
                           print '\nBlasting ' + Seq_query + ' against ' + TranscriptomeID + '...\n'
                           Blast_results_file = Seq_query.split('.')[0]+'_blastp_results.txt'
                           #print TranscriptomeDB
                           blastp_cline = NcbitblastpCommandline(cmd='blastp', query=Seq_query,
db=TranscriptomeDB, out=Blast_results_file, evalue=BLAST_E_value, outfmt = 6, max_target_seqs = Max_hits)
                           stdout, stderr = blastp_cline()

                  #Open the blast output file, find the names of the subject/hit sequences and add then to
the appropriate query_gene_Dict
                  blast_hits_file = open(Blast_results_file, 'rU')
                  csvreader = csv.reader(blast_hits_file, delimiter='\t')
                  for row in csvreader:
                           query_name = row[0]
                           subject_name = row[1]
                           if query_name in all_query_genes_Dict:
                                    all_query_genes_Dict[query_name][subject_name] = []
                  #print all_query_genes_Dict
                  #print 'XXXXXXX'
                  blast_hits_file.close()

                  #Open the transcriptome/genome fasta file and add subject/hit sequences to
all_query_genes_Dict
                  transcriptome_fasta_Dict = {}
                  transcriptome_fasta_file = open(TranscriptomeDB, 'rU')
                  for record in SeqIO.parse(transcriptome_fasta_file, 'fasta'):
                           record_id = str(record.id)
                           record_seq = str(record.seq)
                           transcriptome_fasta_Dict[record_id] = record_seq
                  #print len(transcriptome_fasta_Dict)

                  for query_name in all_query_genes_Dict:
                           for subject_name in all_query_genes_Dict[query_name]:
                                    if subject_name in transcriptome_fasta_Dict:
                                             #print subject_name
                                             all_query_genes_Dict[query_name][subject_name] =
transcriptome_fasta_Dict[subject_name]
                                             #all_query_genes_Dict[query_name][subject_name] =
transcriptome_fasta_Dict[str(subject_name.record)]

                  #print all_query_genes_Dict
                  #print 'XXXXXXXXXXXXXXXXXXXXXXXXXXXXX'
                  os.chdir('..')
else:
         print "\n****ERROR: PLEASE SPECIFY blastn OR blastp IN YOUR litebluedevil_settings FILE****\n"

#print all_query_genes_Dict
#############################################################################################
#Create a fasta file of all subject/hit sequences for each query gene
##make a folder and make a fasta file for each query gene that has one or more hits
```

```
alignment_list = []
for query_name in all_query_genes_Dict:
         if len(all_query_genes_Dict[query_name]) > 1:
                  directory = str(query_name)
                  alignment_list.append(directory)
                  #make the folder if it does not already exist
                  if not os.path.exists(directory):
                           os.makedirs(directory)
                  #make the fasta file in each folder
                  query_fasta_file = str(query_name)+'.fasta'
                  with open(os.path.join(directory, query_fasta_file), 'wb') as fasta_file:
                           #sort subject/hit sequences alphabetically and then write them to the fasta
file
                           for subject_name,subject_seq in
sorted(all_query_genes_Dict[query_name].items()):
                                    fasta_file.write('>'+subject_name+'\n'+subject_seq+'\n')
                  fasta_file.close()

#############################################################################################
#Create an alignment for each output fasta file using muscle
#for alignments >3 sequences, the convert each output alignment fasta file to relaxed phylip format
#and generate a fast ML tree in Raxml

for query_name in alignment_list:
         print 'aligning sequences for query '+query_name+'...'
         os.chdir(query_name)
         infile = query_name+'.fasta'
         alignment_file = query_name+'.aligned.fasta'
         muscle_cline = (MuscleCommandline(muscle_exe, input = infile, out = alignment_file))
         #make the alignment and output the alignment file
         stdout, stderr = muscle_cline()

         #convert the output alignment fasta file to relaxed phylip format for build an nexus ML tree
         if RaxmL_Tree == 'yes':
                  outfile = query_name+'.aligned.phy'
                  alignment = AlignIO.read(alignment_file, "fasta")
                  if len(alignment) > 3:

                           #submitt the phylip alignment to raxml to build a ML tree
                           print "building ML tree in raxml"
                           tree_outfile = query_name+'.aligned.tre'
                           if Blast_type == 'blastn':
                                    #raxml_cmd = raxml +' -f a -p 12345  -x 12345 -N 100 -o %s -m GTRCAT
-s %s -n %s' % (query_name, alignment_file, tree_outfile)
                                    raxml_cmd = raxml +' -f d -p 12345 -o %s -m GTRCAT -s %s -n %s' %
(query_name, alignment_file, tree_outfile)
                                    print("raxml cmd: %s" % (raxml_cmd,) )
                                    result = subprocess.call(raxml_cmd, shell=True)
                                    if result != 0:
                                             raise Exception("raxml failed")
                                             print("raxml failed, continuing to next gene ...")
         os.chdir('..')
```

Supplemental data 3.2 Setting file for LITE BLUE DEVIL v0.3.

```
#blastn or blastp

Blast_type = blastn



#max number of hits from each blast database (e.g. transcriptome, genome, EST library)

Max_blast_hits = 10



#the name of your input fasta file (i.e. the fasta sequences for which you want to find blast hits);
nucleotide for blastn and peptide for blastp

Query_sequence_file_name = query_sequences.txt



#List of folders containing the blast databases that will be searched; databases should be nucleotide for
blastn and peptide for blastp

Transcriptome_list = Transcriptome1, Transcriptome2, Transcriptome3, Transcriptome4, Transcriptome5,
Transcriptome6, Transcriptome7, Transcriptome8



#The E-value threshold as a decimal value

BLAST_E_value = 0.000001



# yes = build a Raxml ML tree for an alignment of > 3 sequences; no = do not build a tree

RaxmL_Tree = yes
```

Supplemental data 3.3 Seed size measurements for *Plukenetia* and Plukenetiinae outgroups.

No.	Genus	Species	Voucher	Country	ID	L	W	T	Vol
1	*Haematostemon*	*guianensis*	Wurdack 4350 (US)	Guyana	KJW	4	4	4.5	37.7
2	*Plukenetia*	aff. *brachybotrya*	Ledezma et al. 921 (CAN) 3	Bolivia	WCM	5.6	4.8	3.9	54.89
3	*Plukenetia*	aff. *brachybotrya*	Ledezma et al. 921 (CAN) 4	Bolivia	WCM	5.6	4.9	4.1	58.91
4	*Plukenetia*	aff. *brachybotrya*	Ledezma et al. 921 (MO) 1	Bolivia	WCM	5.5	4	3.25	37.44
5	*Plukenetia*	aff. *brachybotrya*	Ledezma et al. 921 (MO) 2	Bolivia	WCM	5.5	4.5	3.5	45.36
6	*Plukenetia*	*africana*	Bains s.n. (K)	South Africa	LGB	7.5	6.5	3.7	94.44
7	*Plukenetia*	*africana*	Guy 2367 (MO) 1	Zimbabwe	WCM	6	7	3	65.97
8	*Plukenetia*	*africana*	Guy 2367 (MO) 2	Zimbabwe	WCM	5.5	7	3	60.48
9	*Plukenetia*	*africana*	Guy 2367 (MO) 3	Zimbabwe	WCM	6	6.5	3	61.26
10	*Plukenetia*	*africana*	Guy 2367 (MO) 4	Zimbabwe	WCM	6.5	6.5	3	66.37
11	*Plukenetia*	*africana*	Guy 2367 (MO) 5	Zimbabwe	WCM	6.5	6.5	3	66.37
12	*Plukenetia*	*africana*	Guy 2367 (MO) 6	Zimbabwe	WCM	7	7.5	4	109.96
13	*Plukenetia*	*africana*	Guy 2367 (MO) 7	Zimbabwe	WCM	6.5	7	3.5	83.38
14	*Plukenetia*	*africana*	Guy 2367 (MO) 8	Zimbabwe	WCM	6.5	7	3.5	83.38
15	*Plukenetia*	*africana*	Pinter 666 (BM) 1	Namibia	LGB	8	7.5	4	125.66
16	*Plukenetia*	*africana*	Pinter 666 (BM) 2	Namibia	LGB	6	5.5	4	69.12
17	*Plukenetia*	*africana*	Plowman 1928	Zambia	LGB	6.8	6	3	64.09
18	*Plukenetia*	*africana*	Schinz 894	Namibia	LGB	7.2	7.5	4	113.1
19	*Plukenetia*	*africana*	Thompson 2 (MO)	Mozambique	WCM	6	5	3	47.12
20	*Plukenetia*	*africana*	Wild 4759 (COMB)	Botswana	LGB	6.3	5.7	2.5	47.01
21	*Plukenetia*	*africana*	Wild 7049 (K)	Botswana	LGB	7	6	3.5	76.97
22	*Plukenetia*	*africana*	Winter 4018 (K) 1	Namibia	LGB	7.5	7	4	109.96
23	*Plukenetia*	*africana*	Winter 4018 (K) 2	Namibia	LGB	6	5.5	4	69.12
24	*Plukenetia*	*ankaranensis*	Gillespie 4076 1	Madagascar	LGB	15	16.375	15	1929.13
25	*Plukenetia*	*ankaranensis*	Gillespie 4076 2	Madagascar	LGB	18	16.375	17	2623.62
26	*Plukenetia*	*ankaranensis*	Gillespie 4076 3	Madagascar	LGB	16.5	17	16	2349.91
27	*Plukenetia*	*ankaranensis*	Gillespie 4076 4	Madagascar	LGB	16.5	16	14	1935.22
28	*Plukenetia*	*ankaranensis*	Gillespie 4076 5	Madagascar	LGB	16.5	16.5	15.5	2209.52
29	*Plukenetia*	*ankaranensis*	Gillespie 4076 6	Madagascar	LGB	16.5	16	15	2073.45
30	*Plukenetia*	*brachybotrya*	Croat 4732 (MO)	Brazil	WCM	6	5	5	78.54
31	*Plukenetia*	*brachybotrya*	McDaniel et al. 21461 (MO)	Peru	WCM	6	5	4.5	70.69
32	*Plukenetia*	*brachybotrya*	Rimachi 4015 (MO)	Peru	WCM	6	5	5	78.54
33	*Plukenetia*	*brachybotrya*	Ule 9539 (NY)	Peru	LGB	5.5	5.5	4	63.36
34	*Plukenetia*	*brachybotrya*	Woytkowski 5399 (MO)	Peru	WCM	5	4	4	41.89
35	*Plukenetia*	*carabiasiae*	Jimenez Ramirez 1993	Species description MIN	null	24	21	14	3694.51
36	*Plukenetia*	*carabiasiae*	Jimenez Ramirez 1993	Species description MAX	null	27	27	16	6107.26
37	*Plukenetia*	*carolis-vegae*	Bussmann et al. 17132 (MO)	Peru	WCM	27	25	20	7916.81
38	*Plukenetia*	cf. *carolis-vegae*	Ortiz et al. 191 (CAN)	Peru	WCM	19.6	18.5	15.7	2980.75
39	*Plukenetia*	cf. *carolis-vegae*	Vasquez et al. 33145 (CAN)	Peru	WCM	19.2	17	13	2221.73
40	*Plukenetia*	*conophora*	Bouquet 2460 (P)	Democratic Republic of the Congo	LG	27	27	28	10687.7
41	*Plukenetia*	*conophora*	Ekwe and Ihemje 2013	Species description	null	25	25	25	8181.23
42	*Plukenetia*	*conophora*	Gillet s.n. (P)	Democratic Republic of the Congo	LG	29	27	25	10249.45
43	*Plukenetia*	*corniculata*	Chin See chung 2712 (L)	Malaysia (Borneo)	LG	9	7	5.6	184.73
44	*Plukenetia*	*corniculata*	Clemes 21257 (US) 1	?	LGB	10	7.6	6	238.76

45	*Plukenetia*	*corniculata*	Clemes 21257 (US) 2	?	LGB	9.6	7.5	6	226.19
46	*Plukenetia*	*corniculata*	Grashoff 587 (BO)	Indonesia (Sumatra)	LG	10	6	8	251.33
47	*Plukenetia*	*corniculata*	Griffith (K)	India	LG	9	7	4	131.95
48	*Plukenetia*	*corniculata*	Griffith 4716 (K) 1	Malaysia	LG	8.5	7	5	155.77
49	*Plukenetia*	*corniculata*	Griffith 4716 (K) 2	Malaysia	LG	9.5	8	6	238.76
50	*Plukenetia*	*corniculata*	Griffith 4716 (K) 3	?	LGB	10	8	5.5	230.38
51	*Plukenetia*	*corniculata*	Griffith 4716 (K) 4	?	LGB	8.5	7	5	155.77
52	*Plukenetia*	*corniculata*	Keith T22 (K)	?	LGB	8.6	6.8	5	153.1
53	*Plukenetia*	*corniculata*	Kirton (T22)	Malaysia	LG	9	7	5	164.93
54	*Plukenetia*	*corniculata*	Koorders 40372 (L)	Indonesia (Java)	LG	8.5	7	5	155.77
55	*Plukenetia*	*corniculata*	Koorders 41507 (L)	Indonesia (Java)	LG	9	7	5.3	174.83
56	*Plukenetia*	*corniculata*	Koorders 41509 (L)	Indonesia (Java)	LG	8	7	4.8	140.74
57	*Plukenetia*	*corniculata*	Koorders 41720 (L)	Indonesia (Java)	LG	9	7.5	5.5	194.39
58	*Plukenetia*	*corniculata*	Larsen et al. 552 (L)	Thailand	LG	10.5	8	6	263.89
59	*Plukenetia*	*corniculata*	Lorleck 517 (K)	India	LG	9	7	5	164.93
60	*Plukenetia*	*corniculata*	Meyer 7319	Indonesia (Sumatra)	LGB	10	8	5.7	238.76
61	*Plukenetia*	*corniculata*	Ramos 24086 (K) 1	Philippines	LG	10	8	6.3	263.89
62	*Plukenetia*	*corniculata*	Ramos 24086 (K) 2	Philippines	LG	9.5	7.5	5.5	205.19
63	*Plukenetia*	*corniculata*	Ramos 24086 (K) 3	Philippines	LGB	10	8	6.5	272.27
64	*Plukenetia*	*corniculata*	Ramos 24086 (K) 4	Philippines	LGB	10	7	5.5	201.59
65	*Plukenetia*	*corniculata*	Ramos 24086 (K) 5	Philippines	LGB	9	7	6	197.92
66	*Plukenetia*	*corniculata*	s.coll. 517 (K)	?	LGB	10	7	5	183.26
67	*Plukenetia*	*corniculata*	Smythies 14073 (K)	Malaysia (Borneo)	LG	8.5	7	5.7	177.58
68	*Plukenetia*	*corniculata*	Winkler 3096 (L)	Indonesia (Borneo)	LG	10	8	5.6	234.57
69	*Plukenetia*	*corniculata*	Zollig 3167 (NY)	Indonesia (Java)	LGB	8	6.5	4.5	122.52
70	*Plukenetia*	*decidua*	Decary 3253 (P) 1	Madagascar	LGB	13.1	11.1	11.2	852.73
71	*Plukenetia*	*decidua*	Decary 3253 (P) 2	Madagascar	LGB	13.1	11.2	11.8	906.5
72	*Plukenetia*	*huayllabambana*	Gruhn et al. 84 (MO)	Peru	WCM	28	27	20	6627.02
73	*Plukenetia*	*huayllabambana*	Tellez et al. 4 (MO)	Peru	WCM	25	22	17	4895.65
74	*Plukenetia*	*lehmanniana*	Gentry 9701 (MO)	Ecuador	LGB	34.27	24	30	12919.49
75	*Plukenetia*	*lehmanniana*	Gentry et al. 24724 (MO)	Ecuador	LGB	28	20	24	7037.17
76	*Plukenetia*	*lehmanniana*	Zak and Jaramillo 3401 (MO)	Ecuador	WCM	29.98	21	24	7911.54
77	*Plukenetia*	*loretensis*	Fanshaw 7179 (G)	Guyana	LGB	5.2	4.8	3.6	47.05
78	*Plukenetia*	*loretensis*	Freitas et al. 155 (MO) 1	Brazil	WCM	5.25	4.25	4.25	49.65
79	*Plukenetia*	*loretensis*	Freitas et al. 155 (MO) 2	Brazil	WCM	5	4	3.75	39.27
80	*Plukenetia*	*loretensis*	Liesner 3475 (MO, NY)	Venezuela	LGB	6	5.1	4.9	78.51
81	*Plukenetia*	*loretensis*	Liesner and Funk 16526 (MO)	Venezuela	WCM	6	5	5	78.54
82	*Plukenetia*	*loretensis*	Maguire and Politi 27371	Venezuela	LGB	4.8	4.8	4	48.25
83	*Plukenetia*	*loretensis*	Plowman et al. 12298 (CAN)	Brazil	WCM	5.1	4.7	4.2	52.71
84	*Plukenetia*	*loretensis*	Rimachi 5122 (MO) 1	Peru	WCM	4.75	4.5	4	44.77
85	*Plukenetia*	*loretensis*	Rimachi 5122 (MO) 2	Peru	WCM	4.5	4	4	37.7
86	*Plukenetia*	*loretensis*	Solomon 7972 (MO)	Bolivia	WCM	4.5	4	3	28.27
87	*Plukenetia*	*loretensis*	Vasquez 3208 (MO) 1	Peru	WCM	4.75	4.25	3.5	37
88	*Plukenetia*	*loretensis*	Vasquez 3208 (MO) 2	Peru	WCM	4.75	4.5	3.25	36.37
89	*Plukenetia*	*penninervia*	Al Gentry 8180 (MO)	Belize	WCM	5	6	4	62.83
90	*Plukenetia*	*penninervia*	Atha et al. 1001 (MO) 1	Belize	WCM	5.75	5	4	60.21
91	*Plukenetia*	*penninervia*	Atha et al. 1001 (MO) 2	Belize	WCM	5.5	5.25	4.25	64.26
92	*Plukenetia*	*penninervia*	Elias Contreras 8361 (MO)	Guatemala	WCM	5	6	5	78.54

93	*Plukenetia*	*penninervia*	Fendler 2412 (MO)	?	LGB	5.5	5.5	4	63.36
94	*Plukenetia*	*penninervia*	G. Carnevali and R. Duno 7594 (MO)	Mexico	WCM	5	6	5	78.54
95	*Plukenetia*	*penninervia*	Steyermark and Espinuza 112709 (MO)	Venezuela	LGB	6	5	5	78.54
96	*Plukenetia*	*penninervia*	Wilmer Diaz 1742 (MO)	Venezuela	WCM	5	6	5	78.54
97	*Plukenetia*	cf. *penninervia*	Cardenas 1903 (MO)	Colombia	WCM	7	6.3	5	115.45
98	*Plukenetia*	cf. *penninervia*	Cardenas 1903 (MO)	Colombia	WCM	7	6.5	5.2	123.88
99	*Plukenetia*	*polyadenia*	Feuillet collection	? (CAY or US?)	LGB	55	36	36	37322.12
100	*Plukenetia*	*polyadenia*	Feuillet collection (FG) 1	?	LGB	52	34	32	29623.12
101	*Plukenetia*	*polyadenia*	Feuillet collection (FG) 10	?	LGB	50.5	33	30	26177.32
102	*Plukenetia*	*polyadenia*	Feuillet collection (FG) 11	?	LGB	51	35	32	29907.96
103	*Plukenetia*	*polyadenia*	Feuillet collection (FG) 12	?	LGB	52	36	33.5	32835.93
104	*Plukenetia*	*polyadenia*	Feuillet collection (FG) 13	?	LGB	49	33.5	30.5	26214.37
105	*Plukenetia*	*polyadenia*	Feuillet collection (FG) 14	?	LGB	56	36.5	34	36388.02
106	*Plukenetia*	*polyadenia*	Feuillet collection (FG) 15	?	LGB	52	35	32.5	30970.87
107	*Plukenetia*	*polyadenia*	Feuillet collection (FG) 16	?	LGB	50	34	32	28483.77
108	*Plukenetia*	*polyadenia*	Feuillet collection (FG) 17	?	LGB	49	33	31	26246.44
109	*Plukenetia*	*polyadenia*	Feuillet collection (FG) 2	?	LGB	51	33	32.5	28639.54
110	*Plukenetia*	*polyadenia*	Feuillet collection (FG) 3	?	LGB	51	36	33	31723.8
111	*Plukenetia*	*polyadenia*	Feuillet collection (FG) 4	?	LGB	52	34.5	33	30998.09
112	*Plukenetia*	*polyadenia*	Feuillet collection (FG) 5	?	LGB	51.5	34	34	31171.93
113	*Plukenetia*	*polyadenia*	Feuillet collection (FG) 6	?	LGB	49.5	34.5	32.5	29060.71
114	*Plukenetia*	*polyadenia*	Feuillet collection (FG) 7	?	LGB	50.5	37	34	33263.71
115	*Plukenetia*	*polyadenia*	Feuillet collection (FG) 8	?	LGB	53	37	34.5	35423.81
116	*Plukenetia*	*polyadenia*	Feuillet collection (FG) 9	?	LGB	50.5	33.5	33	29231.34
117	*Plukenetia*	*polyadenia*	Gillespie 4314 (CAN)	Guyana	WCM	50	35	32	29321.53
118	*Plukenetia*	*serrata*	Guedes et al. 2330 (RB)	Brazil	DM	15	16	16	2010.62
119	*Plukenetia*	*serrata*	RB 556564	Brazil	DM	15.5	15.5	15	1886.92
120	*Plukenetia*	*stipellata*	Aguilar 8193 (MO) 1	Costa Rica	WCM	10.5	8	4.5	197.92
121	*Plukenetia*	*stipellata*	Aguilar 8193 (MO) 2	Costa Rica	WCM	10.5	8.5	4	186.92
122	*Plukenetia*	*stipellata*	Calderon 4809 (A)	Mexico	LGB	12	11	2.5	172.79
123	*Plukenetia*	*stipellata*	Cedillo Trigos 3510 (MO)	Mexico	WCM	14	11	4	322.54
124	*Plukenetia*	*stipellata*	Churchill et al. 4149 (MO)	Panama	WCM	11	8	5	230.38
125	*Plukenetia*	*stipellata*	Davidse and Herrera 31266 (MO) 1	Costa Rica	LGB	10	9.5	4.3	213.89
126	*Plukenetia*	*stipellata*	Davidse and Herrera 31266 (MO) 2	Costa Rica	LGB	10	9.5	4.5	223.84
127	*Plukenetia*	*stipellata*	Davidson 6734 (US)	Costa Rica	LGB	11	10	2.5	143.99
128	*Plukenetia*	*stipellata*	Davidson et al. 6734 (MO) 1	Costa Rica	LGB	11.5	9.5	3.6	205.93
129	*Plukenetia*	*stipellata*	Davidson et al. 6734 (MO) 2	Costa Rica	LGB	11.5	9.5	4	228.81
130	*Plukenetia*	*stipellata*	Duke 9994 1	?	LGB	13.5	12	5	424.12
131	*Plukenetia*	*stipellata*	Duke 9994 2	?	LGB	13.5	11.5	5	406.44
132	*Plukenetia*	*stipellata*	Grayum 3511 (MO) 1	Costa Rica	LGB	11.3	10	5	295.83
133	*Plukenetia*	*stipellata*	Grayum 3511 (MO) 2	Costa Rica	LGB	10.7	8.9	4.7	234.35
134	*Plukenetia*	*stipellata*	Grayum 8737 (MO) 1	Costa Rica	LGB	11	9.5	5.2	284.52
135	*Plukenetia*	*stipellata*	Grayum 8737 (MO) 2	Costa Rica	LGB	11.6	10	5.2	315.83
136	*Plukenetia*	*stipellata*	Grayum et al. 8731 (MO)	Costa Rica	WCM	11	9	4	207.35
137	*Plukenetia*	*stipellata*	Hammell 16933 (MO) 1	Costa Rica	LGB	11.1	10	6	348.72
138	*Plukenetia*	*stipellata*	Hammell 16933 (MO) 2	Costa Rica	LGB	11.6	10.3	5	312.8
139	*Plukenetia*	*stipellata*	Jimenez 3611 (MO) 1	Costa Rica	LGB	11	9	4.6	238.45
140	*Plukenetia*	*stipellata*	Jimenez 3611 (MO) 2	Costa Rica	LGB	11	9.3	4.9	262.46

141	*Plukenetia*	*stipellata*	Liesner 2086 (US)	Costa Rica	LGB	14	13	5	476.47
142	*Plukenetia*	*stipellata*	McDowel 241 (MO) 1	Costa Rica	WCM	12	9	4	226.19
143	*Plukenetia*	*stipellata*	McDowel 241 (MO) 2	Costa Rica	WCM	12	9.5	5	298.45
144	*Plukenetia*	*stipellata*	McDowel 241 (MO) 3	Costa Rica	WCM	11.5	9	4	216.77
145	*Plukenetia*	*stipellata*	McDowel 241 (MO) 4	Costa Rica	WCM	11.5	9.5	4.5	257.41
146	*Plukenetia*	*stipellata*	McDowell 821 (GH)	Costa Rica	LGB	11	9.5	5	273.58
147	*Plukenetia*	*stipellata*	Morens and Sandino 14657 (MO)	Nicaragua	LGB	11.5	10	5	301.07
148	*Plukenetia*	*stipellata*	Pennell 4584 (NY) 1	Colombia	LGB	13	11.5	6	469.67
149	*Plukenetia*	*stipellata*	Pennell 4584 (NY) 2	Colombia	LGB	14	12	5.5	483.81
150	*Plukenetia*	*stipellata*	Pennell 4584 (NY) 3	Colombia	LGB	13	11.5	5.5	430.53
151	*Plukenetia*	*stipellata*	Pittier 324 (US)	Guatemala	LGB	13	13	5.2	460.14
152	*Plukenetia*	*stipellata*	Refugio 3510 (MO)	Mexico	WCM	13.5	11.75	4.5	373.75
153	*Plukenetia*	*stipellata*	Refugio 3510 (MO)	Mexico	WCM	13.5	12	4.75	402.91
154	*Plukenetia*	*stipellata*	Robels 1132 (MO)	Costa Rica	LGB	12	10	5	314.16
155	*Plukenetia*	*stipellata*	Rueda et al. 9777 (MO)	Nicaragua	WCM	12	9	5	282.74
156	*Plukenetia*	*stipellata*	Smith 1651 (US)	Guatemala	LGB	13.7	13.5	5	484.2
157	*Plukenetia*	*stipellata*	Solheim and Reisfiell (NY)	Mexico	LGB	12	11	5	345.58
158	*Plukenetia*	*stipellata*	Sperry 803 (GH)	Costa Rica	LGB	11	8	4.5	207.35
159	*Plukenetia*	*stipellata*	Stevens 12095 (MO)	Nicaragua	WCM	12	10	5	314.16
160	*Plukenetia*	*stipellata*	Stevens 23837 (MO) 1	Costa Rica	LGB	10.5	9	5.1	252.35
161	*Plukenetia*	*stipellata*	Stevens 23837 (MO) 2	Costa Rica	LGB	10.5	9	5	247.4
162	*Plukenetia*	*stipellata*	Stevens 6357 (MO) 1	Nicaragua	LGB	10.7	9.6	4.7	252.79
163	*Plukenetia*	*stipellata*	Stevens 6357 (MO) 2	Nicaragua	LGB	11.3	9.3	4.6	253.11
164	*Plukenetia*	*stipellata*	Stevens 6357 (MO) 3	Nicaragua	LGB	11.6	9.7	4.7	276.9
165	*Plukenetia*	*stipellata*	Zambrano (MO) 1	Mexico	LGB	12.7	12.1	4.7	378.17
166	*Plukenetia*	*stipellata*	Zambrano (MO) 2	Mexico	LGB	13	12.1	4.7	387.1
167	*Plukenetia*	*supraglandulosa*	Granvilla et al. 10783 (U, DAV, CAY) 1	French Guiana	LGB	7.3	7.1	4.6	124.84
168	*Plukenetia*	*supraglandulosa*	Granvilla et al. 10783 (U, DAV, CAY) 2	French Guiana	LGB	7.3	6.8	4.8	124.76
169	*Plukenetia*	*supraglandulosa*	Granvilla et al. 10783 (U, DAV, CAY) 3	French Guiana	LGB	7	6.8	4.7	117.14
170	*Plukenetia*	*verrucosa*	BW 993	Suriname	LGB	6	5.5	4	69.12
171	*Plukenetia*	*verrucosa*	Herrera and Koemar 10073 (CAN) 1	Suriname	WCM	6	5.5	4	69.12
172	*Plukenetia*	*verrucosa*	Herrera and Koemar 10073 (CAN) 2	Suriname	WCM	6	5	3.5	54.98
173	*Plukenetia*	*verrucosa*	Herrera and Koemar 10073 (MO) 3	Suriname	WCM	5.25	5.5	4	60.48
174	*Plukenetia*	*verrucosa*	Herrera and Koemar 10073 (MO) 4	Suriname	WCM	6	5.5	4	69.12
175	*Plukenetia*	*verrucosa*	Irwin 48796	Brazil	LGB	6	5	4	62.83
176	*Plukenetia*	*verrucosa*	LBB 3565	Suriname	LGB	6	3.7	5	58.12
177	*Plukenetia*	*verrucosa*	Melinson (P) 1	French Guiana	LGB	6	5	3.6	56.55
178	*Plukenetia*	*verrucosa*	Melinson (P) 2	French Guiana	LGB	6	5	4	62.83
179	*Plukenetia*	*verrucosa*	Pulle 221	Suriname	LGB	6	5	3.6	56.55
180	*Plukenetia*	*volubilis*	Aplund 14129 (US)	Peru	LGB	20	17	8	1424.19
181	*Plukenetia*	*volubilis*	Gentry et al. 63257 (MO)	Peru	WCM	17	14	5	623.08
182	*Plukenetia*	*volubilis*	Krukoff 10082 (A)	Bolivia	LGB	17	16	9	1281.77
183	*Plukenetia*	*volubilis*	M. Monigatti and F. Diaz V. 309 (MO) 5 carpels	Peru	WCM	22	18	8	1658.76
184	*Plukenetia*	*volubilis*	Mexia 7265 (US)	Peru	LGB	20	17	7	1246.17
185	*Plukenetia*	*volubilis*	Nunez 13775 (MO) 1	Peru	WCM	16.35	15.25	7.2	939.98
186	*Plukenetia*	*volubilis*	Nunez 13775 (MO) 2	Peru	WCM	16.4	14.8	6.9	876.91
187	*Plukenetia*	*volubilis*	Nunez 13775 (MO) 3	Peru	WCM	16.6	15.6	7.5	1016.93
188	*Plukenetia*	*volubilis*	Parada et al. 206 (CAN)	Bolivia	WCM	14.5	13.5	7.5	768.71

189	*Plukenetia*	*volubilis*	Philip et al. 1582 (US)	Colombia	LGB	16	15	7.5	942.48
190	*Plukenetia*	*volubilis*	Teran et al. 2502 (MO) 1	Bolivia	WCM	13.5	14.25	6.5	654.73
191	*Plukenetia*	*volubilis*	Teran et al. 2502 (MO) 2	Bolivia	WCM	13	11.75	6	479.88
192	*Plukenetia*	*volubilis*	Timana 979 (MO)	Peru	WCM	18	16	6.5	980.18
193	*Plukenetia*	*volubilis*	Vasquez et al. 22376 (MO)	Peru	WCM	17	15	8	1068.14
194	*Romanoa*	*tamnoides*	Zardini 45104 (MO)	Paraguay	WCM	8	6	4	100.53
195	*Romanoa*	*tamnoides*	Zardini 50824 (MO)	Paraguay	WCM	8	5	3	62.83
196	*Romanoa*	*tamnoides*	Zardini and Cardozo 44921 (CAN) 1	Paraguay	WCM	7.6	5.7	4.2	95.27
197	*Romanoa*	*tamnoides*	Zardini and Cardozo 44921 (CAN) 2	Paraguay	WCM	7	5.4	4	79.17
198	*Romanoa*	*tamnoides*	Zardini and Cardozo 44921 (CAN) 3	Paraguay	WCM	7.3	5.8	3.9	86.46
199	*Romanoa*	*tamnoides*	Zardini and Cardozo 44921 (CAN) 4	Paraguay	WCM	7	5.4	3.8	75.21
200	*Romanoa*	*tamnoides*	Zardini and Cardozo 44939 (CAN) 1	Paraguay	WCM	6.9	5.3	4.1	78.51
201	*Romanoa*	*tamnoides*	Zardini and Cardozo 44939 (CAN) 2	Paraguay	WCM	6.9	5.7	4.1	84.43
202	*Romanoa*	*tamnoides*	Zardini and Cardozo 44939 (CAN) 3	Paraguay	WCM	6.7	5.3	3.4	63.22
203	*Romanoa*	*tamnoides*	Zardini and Cardozo 44939 (CAN) 4	Paraguay	WCM	7.6	6	4.2	100.28
204	*Romanoa*	*tamnoides*	Zardini and Cardozo 44939 (CAN) 5	Paraguay	WCM	7.6	5.4	4.1	88.1
205	*Romanoa*	*tamnoides*	Zardini and Cardozo 44939 (CAN) 6	Paraguay	WCM	7.6	5.5	3.8	83.17
206	*Romanoa*	*tamnoides*	Zardini and Cardozo 44939 (CAN) 7	Paraguay	WCM	7.4	5.9	4.1	93.73
207	*Romanoa*	*tamnoides*	Zardini and Cardozo 44939 (CAN) 8	Paraguay	WCM	7	5.6	3.7	75.94
208	*Romanoa*	*tamnoides*	Zardini and Cardozo 45093 (CAN) 1	Paraguay	WCM	7.8	5.9	4	96.38
209	*Romanoa*	*tamnoides*	Zardini and Cardozo 45093 (CAN) 2	Paraguay	WCM	7.4	5.8	3.8	85.4
210	*Romanoa*	*tamnoides*	Zardini and Cardozo 45093 (CAN) 3	Paraguay	WCM	7.3	5.5	3.9	81.99
211	*Romanoa*	*tamnoides*	Zardini and Cardozo 45104 (CAN) 1	Paraguay	WCM	7.5	6	4	94.25
212	*Romanoa*	*tamnoides*	Zardini and Cardozo 45104 (CAN) 2	Paraguay	WCM	6.6	5.4	3.7	69.05
213	*Romanoa*	*tamnoides*	Zardini and Cardozo 45104 (CAN) 3	Paraguay	WCM	6.3	5.6	3.8	70.2
214	*Romanoa*	*tamnoides*	Zardini and Cardozo 45104 (CAN) 4	Paraguay	WCM	7.2	5.7	3.8	81.66

ID (person who conducted measurements) abbreviations: DM (Debora Medeiros), LG (Lynn Gillespie Syst.Bot. 2007 notes), LGB (Lynn Gillespie blue binder notes), KJW (Kenneth J. Wurdack), WCM (Warren Cardinal-McTeague).