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The Evolution of Development of Vascular Cambial Variants in a Large Genus of Neotropical Lianas: *Paullinia* (Sapindaceae)

By

Joyce G Chery

A dissertation submitted in partial satisfaction of the

requirements for the degree of

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in

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Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Chelsea Specht, Co-Chair
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Abstract

The Evolution of Development of Vascular Cambial Variants in a Large Genus of Neotropical Lianas: *Paullinia* (Sapindaceae)

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Doctor of Philosophy in Integrative Biology

University of California, Berkeley

Professor Chelsea D. Specht, Co-Chair

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Paullinia L. is a genus of ~220 species of neotropical lianas, with one species extending to tropical Africa. This genus is a part of the large monophyletic Paullinieae tribe of lianas, together comprising 1/3 of the species diversity in Sapindaceae. *Paullinia* and the five other genera, *Cardiospermum*, *Serjania*, *Urvillea*, *Lophostigma,* and *Thinouia*, are united by their climbing habit, paired inflorescence tendrils, and stipulate compound leaves. Vegetatively quite similar, these genera are best distinguished from each other by their fruit, which range from hard or papery capsules to schizocarp samaras with dorsal or ventral wings. Several stem developmental trajectories are also present across the tribe, ranging from the regular stem development typical of trees and shrubs, but also including a collection of vascular cambial variants. Recent phylogenetic analyses based on two loci, have confirmed the monophyly of Paullinieae, however the relationships among genera were largely unresolved.

Paullinia is distinct in the Paullinieae in having hardened septifragal capsular fruits. These fruits open to display three brown-black glossy seeds enveloped by a white fleshy aril. Within *Paullinia*, variations on the pericarp morphology have been the main focus for infrageneric classification, resulting in the classic 13-section system established in 1895 by Ludwig Radlkofer. Capsule fruits with wings (alate), without wings (exalae), or with spiny projections (echinate) are all present and are thought to have systematic value. Another feature of interest is the presence of vascular camvia variants that in *Paullinia* include phloem wedges, lobed xylem, compound stems, and successive cambia. The presence and type of cambial variant has been highlighted by previous authors in connection to a larger evolutionary correlation of the liana habit with unusual wood morphologies, which are thought to aid in the climbing habit. All vascular cambial variants in *Paullinia* are additionally found in other liana lineages, except the compound wood type that is restricted to the Sapindaceae lianas.

In this dissertation, I develop the first molecular phylogeny of *Paullinia* and explore the evolution of fruit morphologies, and the evolution of development of cambial variants in a phylogenetic context.

In the first chapter, I develop a bioinformatic pipeline that leverages publicly available genomic and transcriptomic data to target informative single-copy intron nuclear markers and demonstrate its efficacy in generating data for species-level phylogenetics across the Paullinieae. First, transcriptome reads from *Dimocarpus lognan* (Sapindaceae) are aligned to single isoform genes from the *Cirtus sinensis* (Rutaceae) genome with introns of a desired size (500-1100). Second, single-nucleotide polymorphisms are called, and at these positions, the base pair is changed to the majority rule base pair–this generates a set of consensus sequences ("pseudoreferences") that are "closer" to Paullinieae. Next, several filters are applied to meet the criteria of single-copy nuclear loci (i.e. reciprocal BLAST to remove paralogs; BLAST to ribosomal, transposons mitochondrial, chloroplast to remove non-nuclear genes; removal of low coverage sequences (<20x average gene coverage; removal of RepeatMasker hits). Finally, I designed primers in the conserved coding sequences of these putative single copy nuclear markers flanking the targeted introns. Using this pipeline, I developed nine novel and variable (53.7–94.3% pairwise identity) molecular markers.

In the second chapter, I generate a robust molecular phylogeny of *Paullinia* and of the infrageneric relationships across the Paullinieae tribe using nine single-copy nuclear markers developed from the bioinformatic pipeline outlined in Chapter 1, plus two commonly used variable markers (ITS and *trn*H-*psb*A). To generate sequence data, I utilized microfluidics PCR to amplify loci using Fluidigm™ technology, then sequenced those amplicons on an Illumina MiSeq. Given this novel phylogenetic hypothesis, I: 1) discuss the taxonomic implications in relation to the traditional infrageneric classification, and 2) conduct an ancestral state estimation of fruit morphologies along the tree. *Paullinia* is supported as monophyletic and is sister to *Cardiospermum* L., which together are sister to *Serjania* Mill + *Urvillea* Kunth. I discuss seven major clades are discussed that largely correspond to sections defined by morphology. The ancestral condition of fruit morphology in *Paullinia* is reconstructed as exalate, and seven transitions are inferred: five transition from exalate to alate, one transition from exalate to echinate, and one reversal from alate to exalate. Although the differences in fruit morphologies suggest changes in dispersal mode, because it is the seed (as opposed to the fruit) that is the dispersal diaspore and most species are dehiscent, I conclude that the repeated transitions in fruit morphology represents various strategies to enhance visual display to attract animal dispersers, as opposed to a shift from animal to wind dispersal.

In the third chapter, I describe six stem ontogenies that capture the diversity observed in *Paullinia* by studying three stages of stem development (primary growth, intermediate (onset of secondary growth), and mature wood) in 18 species. Most *Paullinia* species are angular in cross-sectional view at the shoot apex, which becomes reinforced by the unequal distribution of vascular bundles around the circumference of the young stem. Although rare among woody plants, this is the basic bauplan of primary growth in *Paullinia*, from which five of the six mature stem types develop. To explore the evolution of stem ontogenies in *Paullinia* and across the other Sapindaceae lianas, I employed phylogenetic comparative methods to reconstruct the ancestral primary plant body shape and the ancestral mature stem type across the Paullinieae tribe. Additionally, I tested the hypothesis that the evolution of cambial variants is contingent on first evolving the irregular angular primary growth confirmation. The results identify a critical relationship between primary and secondary growth in both the development of cambial variants of an individual plant, and the emergence of these novel forms through evolutionary time.

Dedication

To my parents, Elsina L. Chery and Guynemer Chery, who let me be a weird kid.

Motivating Quotes

"Doubtless many botanists would like to understand the functional and evolutionary nature of xylem in angiosperms and other groups of vascular plants, but feel excluded because the complexity of xylem and the diversity of literature on xylem make entry into this field difficult for those who cannot devote large amounts of professional time to developing an understanding of xylem structure and diversity."

-Sherwin Carlquist, 2009

"I came to win, to fight, to conquer, to thrive
I came to win, to survive, to prosper, to rise
To fly
To fly"

-Nicki Minaj featuring Rihanna, 2010

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I first would like to thank my research funding sources. All molecular lab work, genomic DNA extractions, PCR optimization, and gel electrophoresis in Chapter 1 was supported by an NSF DEB (1208666) awarded to Chelsea Specht.

I was supported to visit Panama during two dry seasons, totally ~ 3 months, by the Specht Lab and a National Science Foundation (NSF) Graduate Student Internship Program at the Smithsonian Tropical Research Institute on Barro Colorado Island. These two sources enabled me to pay for housing and food, and to purchase field equipment for collecting; including clothes, binoculars (crucial for collecting lianas), a GPS, a machete, and riteInTheRain notebooks.

A Smithsonian Predoctoral Fellowship and American Society of Plant Taxonomist Graduate Research Award supported subsequent molecular lab work to generate the *Paullinia* phylogeny, as well as all stem anatomical studies (e.g. histological stains, slides, PEG 1500).

The Society of Systematic Biologist and funding from the Rothfels Lab supported the generation of sequence data for the phylogeny–microfluidics PCR and Illumina Miseq ¼ sequence run.

Throughout graduate school, I have been supported by three fellowships (stipend and tuition): Berkeley Chancellor's Fellowship, NSF Graduate Research Fellowship, and the UC Dissertation Year fellowship.

But funding is only half of the story! I have been professionally and emotionally supported by many people. I want to thank Pedro Acevedo for sharing all of his silica dried collections of *Paullinia*, allowing me to sample herbarium vouchers, and giving me his stem collections for my projects. I thank Marcelo Pace for patiently training me in wood anatomy techniques, and intellectually supporting me in the form of our ongoing reading group. I thank my qualifying exam (David Ackerly, Bruce Baldwin, Kipling Will, and Cindy Looy) and dissertation committee (Chelsea Specht, Carl Rothfels, Paul Fine, and Kipling Will) for the intellectual support and feedback.

Throughout this journey, I learned on my fellow graduate students' friends, notably Roxy Marie Cruz de Hoyos, Carrie Tribble, Riva Breunn, Jenna Ekwealor , Ashley Smiley, Betsabe Castro, Clarissa Fonte, Claire Willing, Sara Elshafie, and Shanique Alabi. These women have helped me through the darkest days and have rejoiced with me in the light (typically at EastBay Spice Company!). Outside of Berkeley, I was always surrounded by the love of my childhood friend, Dominique Desilus, my brother Jeffrey, and my loving parents Elsina L. Chery and Guynemer Chery.

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Introduction

In coming to graduate school, I knew I wanted to generate the phylogeny of *Paullinia* L. (Sapindaceae). As an undergraduate researcher, I evaluated leaf architecture traits as potential characters of systematic value and entered these data into a large existing database of fine-scale characters of the genus. In seeing the great strides taken by my colleagues to elucidate species delimitations in *Paullinia*, I became aware of the need to generate a phylogeny and place these characters in a phylogenetic framework to better understand the macroevolutionary history of the genus.

As a first-year graduate student, I had one major goal: figure out my dissertation aims! In the search for my research questions, I read a broad array of literature relevant to *Paullinia*. I was most excited to learn there was a resurgence of research interest in liana ecology (due to the increasing liana abundance in tropical forests) and liana biology (due to the biological innovation hypothesis). The convergence of these two threads in the literature captured my curiousity: *why are lianas so successful?* To approach this question, I first had to define the liana habit and how it differs from self-sustaining plants, then I learned specifically how those differences are potentially adaptive and contribute to their ecological and evolutionary. One of the most cited traits correlated with the liana habit is the presence of atypical stem conformations–vascular cambial variants. The presence and type of cambial variant has been highlighted by previous authors in connection to a larger evolutionary correlation of the liana habit with unusual stem morphologies, which are thought to be adaptations to climbing. To my pleasant surprise, there were numerous reports of cambial variants in *Paullinia* and in related genera of lianas. Just as I was learning this fact (June 2015), I enrolled in a two-week intensive course in plant anatomy at the Arnold Arboretum at Harvard University. It was the first time I learned about plant anatomy from the perspective of how these cells and tissues developed, evolved, and contributed to the diversity of plant disparity. Up until that point, I could only describe attributes of cell and tissue types, not think of their collective and coordinated functions throughout development and in the evolution of vascular plants. On the last day of the course, I rented an AirBnB in Boston, Massachusetts, locked myself away for two days, and wrote the first draft of my dissertation proposal. It was June 2015– I had accomplished my first-year aim!

Sapindaceae Jussieu, *nom. cons.* is a large family of ca. 1900 species of trees, shrubs and lianas, most with a tropical to subtropical distribution, and some genera in temperate Asia and North America (Acevedo-Rodríguez et al. 2011). Modern systematic treatments of the family recognized four subfamilies—Sapindoideae, Hippocastanoideae, Dodonaeoideae and the monotypic Xanthoceroideae (Harrington et al., 2005). Molecular systematics has revealed that all 12 of the 13 infrafamilial tribes proposed by Radlkofer (1895; 1931) were non-monophyletic, except the lineage of lianas: the Paullinieae tribe. This lineage comprises six genera (*Serjania, Cardiospermum, Paullinia, Thinouia, Lophostigma,* and *Urvillea*), collectively containing one-third of the species diversity in the family, with the two largest genera being *Paullinia* (c. 220) and *Serjania* (c. 240). The tribe is united by several synapomorphies, including their climbing habit, paired inflorescence tendrils, and stipulate compound leaves. Vegetatively quite similar, these genera are best distinguished from each other by their fruit types, which range from hard or papery capsules to schizocarp samaras with dorsal or ventral wings. Various stem ontogenies and morphologies are also present across the tribe, ranging from the regular stems typical of trees and shrubs, but also including a collection of vascular cambial variants. More recent work, based on two loci, has confirmed the monophyly of Paullinieae, however the relationships among genera have remained unresolved, and are improved upon in this dissertation.

Paullinia is distinct in the Paullinieae tribe in having hardened septifragal capsular fruits. These fruits open to display three brown-black glossy seeds enveloped by a white fleshy aril. Within *Paullinia*, variations on the pericarp morphology have been the main focus for infrageneric classification, resulting in the classic 13 section system established in 1895 by Ludwig Radlkofer. Capsule fruits with wings (alate), without wings (exalae), or with spiny projections (echinate) are all present and have historically thought to have systematic value. Notably, another feature of interest is the presence of both regular stem ontogenies as well as four different cambial variants: phloem wedges, lobed xylem, compound stems, and successive cambia. Anatomical studies of *Paullinia* cambial variants are numerous, however the absence of a phylogeny has restricted the discussions in a phylogenetic comparative framework.

To address questions concerning the systematics and the evolution of key traits in *Paullinia*, I leveraged anatomical techniques, molecular phylogenetics and phylogenetic comparative methods. In this dissertation, I generate the first molecular phylogeny of *Paullinia*, and tested the traditional infrageneric classification by Radlkofer (1895, 1931), reconstructed the evolution of fruit morphologies, and teased apart the evolution of development of cambial variants in a phylogenetic context.

The first chapter is published in a peer-reviewed journal and chapters 2 and 3 are in preparation for publication.

Chapter 1. Development of single copy nuclear intron markers for species-level phylogenetics: Case Study with Paullinieae (Sapindaceae)

Chery J.G., Sass C., Specht C.D. 2017. Development of Single-Copy Nuclear Intron Markers for Species-Level Phylogenetics: Case Study with Paullinieae (Sapindaceae). Applications in Plant Sciences 2017 5(9): 1700051

1.1 Abstract

• Premise of study: We developed a bioinformatic pipeline that leverages a publicly available genome and published transcriptomes to design primers in conserved coding sequences flanking targeted introns of single copy nuclear loci. Paullinieae (Sapindaceae) is used to demonstrate the pipeline.
• Methods and Results: Transcriptome reads phylogenetically closer to the lineage of interest are aligned to the closest genome. SNPs are called generating a 'pseudoreference' closer to the lineage of interest. Several filters are applied to meet the criteria of single copy nuclear loci with introns of a desired size. Primers are designed in conserved coding sequences flanking introns. Using this pipeline, we developed 9 single copy nuclear intron markers for Paullinieae.
• Conclusions: This pipeline is highly flexible and can be used for any group with available genomic and transcriptomic resources. This pipeline led to the development of 9 variable markers for phylogenetic study without generating sequence data de novo.

Key words: nuclear marker development; Paullinieae; Sapindaceae; introns.

1.2 Introduction

Rapidly evolving introns of low copy nuclear markers have the potential to generate robust species level phylogenetic hypotheses (Sang, 2002).With current high throughput sequencing technologies, genomic and transcriptomic datasets are becoming available to utilize in developing informative markers for phylogenetic utility. In the most fortunate of cases, the available genome or transcriptome is within the targeted lineage of interest or is closely related (Curto et al., 2012; Mendoza et al., 2015; Stockenhuber et al., 2015). In other cases, authors generate transcriptome data of members of the targeted group (Tonnabel et al. 2014; Stockenhuber et al. 2015). Combining these data, authors aim to target low copy nuclear markers for phylogenetic utility. This strategy is very promising, however whole genomes are not typically available for non-model systems and generating transcriptome data de novo is expensive and may be unnecessary given existing data (e.g. One Thousand Plants Consortium 1KP; www.onekp.com).

Here, we present a bioinformatic pipeline that leverages a publicly available genome and published transcriptome reads to identify conserved regions in single-copy nuclear loci and to design primers for amplification of associated introns. Benefits of this pipeline include 1) reduced cost by not generating sequence data de novo and 2) targeting nuclear introns, which are expected to have high sequence variation even among closely related species. This pipeline can be powerful in cases where published transcriptomes are phylogenetically closer to the targeted lineage than the available genome, and in cases where researchers are interested in single copy nuclear introns for phylogenetic resolution. It is useful for researchers interested in using small scale sequencing efforts (i.e. Sanger sequencing) to identify relatively few (1-20) informative nuclear loci that can be amplified by PCR, but could be scaled up to include larger sets of loci by relaxing parameters and/or reducing the number of filtering steps in the pipeline. The final set of loci can be used to design baits for HybSeq Next Generation Sequencing (Weitemier et al., 2014), homemade in-solution capture (Peñalba et al., 2014) or microfluidic PCR primers (Uribe-Convers et al., 2016).

We demonstrate the utility of this bioinformatic pipeline to design primers to amplify single copy nuclear introns in the tribe Paullinieae Kunth (Sapindaceae Juss), a neotropical lineage of ~ 500 liana species (Acevedo-Rodríguez et al., 2011). A previous phylogenetic analysis of the Paullinieae tribe was strictly morphological (Acevedo-Rodríguez, 1993) and only at the generic level. Most recently, Acevedo-Rodríguez et al. (2017) aimed to resolve generic and species level relationships in Paullinieae using ITS and the trnL-intron. Although important tribal relationships were resolved, these 2 markers resulted in a polytomy of *Serjania* Mill., *Paullinia* L., *Urvillea* Kunth and *Cardiospermum* L. Thus, more phylogenetically informative molecular markers are needed to improve the resolution of generic and species-level relationships of Paullinieae. Here, we describe a bioinformatic pipeline that leverages publicly available genomic data from distantly related lineages (within the order and within the family but distant to the tribe of interest) to successfully design primers for a specific tribe. We demonstrate the pipeline using the annotated *Citrus sinensis* (L.) Osbeck (Rutaceae Juss: Sapindales Bercht. & J. Presl) genome with two transcriptomes, *Dimocarpus longan* Lour. and *Litchi chinensis* Sonn., that are within Sapindaceae but outside of the Paullinieae tribe of interest. The estimated pairwise divergence time between the transcriptome and genome is 94 MYA (www.timetree.org). By using two sets of transcriptome reads from species in the Sapindaceae family, we were able to find conserved regions where primers could be designed for amplification of single copy nuclear introns.

1.3 Methods and Results

1.3.1 Finding single copy nuclear markers

To generate nuclear intron markers, the annotated genome of *Citrus sinensis* (Rutaceae) provided the coding sequences, intron positions, and estimated intron size while the transcriptomes of more closely related Sapindaceae species, *Dimocarpus longan* and *Litchi chinensis,* provided the best estimate of the gene sequence from which primers could be designed for the target lineage. By using this combination of available data, we hoped to identify single copy gene regions to avoid amplification of unidentified paralogs, and design primers that would have a high likelihood of amplification success across the Paullinieae (Sapindaceae).

First, the genome is processed- genome coding sequences (CDS) were downloaded and filtered for single isoform mRNA strands for ease of processing and genes with introns of 500-1,100bps were selected so they would be easily amplified by traditional PCR and still contain a sufficient number of characters to have phylogenetic utility. The number of base pairs could be changed to include greater or fewer regions as desired. Second, the transcriptome reads are cleaned to remove adapters, low-complexity sequences, contamination, and PCR duplicates (Singhal, 2013). Third, a series of steps are applied to use the genome data to obtain homologous sequences from the more closely related transcriptome without generating a transcriptome assembly *de novo* as in Sass et al. (2016). (1) Cleaned transcriptome reads are aligned to the filtered genome coding sequences using NovoAlign v3.01 (http://novocraft.com) with -t 480, a lenient value to allow highly divergent sequences to map. (2) SNPs are called using SAMtools v0.1.19 (Li et al. ,2009) and new consensus sequences are generated based on SNPs called. (3) Transcriptome reads are aligned to the new consensus sequences created from the first alignment using NovoAlign –t 90 (more stringent). (4) SNPs are called, and the final consensus sequences are created to serve as a pseudoreference for primer design. The iterative alignment and SNP calling enables more distantly related transcriptome reads to align to the genome. The following filters are applied to all pseudoreference sequences (Figure 1) in this order: 1) Retain sequences that only BLAST to self with default settings (i.e. exclusively BLAST to the *Citrus* CDS from which the pseudoreference was generated); 2) Remove sequences that BLAST to plastid, chloroplast, ribosomal, transposon or mitochondrial loci using Megablast and Organism: Spermatophyta NCBI database; 3) Retain only genes with at least 20x average read coverage (Nielsen et al., 2011); 4) Remove sequences with hits to Repeat Masker (http://repeatmasker.org/)- i.e. interspersed repeats and low complexity DNA sequences. After applying the above filters, these sequences fit the criteria of single copy nuclear genes containing introns between 500-1,100 base pairs.

1.3.2 Primer Design

To verify that primer regions were conserved within the breadth of phylogenetic interest, a second transcriptome within the family of interest was aligned to the pseudoreference. This step increases the chances that the primers will be conserved and utilizable across the breadth of the lineage of interest. This step could be eliminated if a second transcriptome is not available, however further primer testing would likely be necessary. *Litchi chinensis* (Sapindaceae) transcriptome reads were aligned to the *Dimocarpus* pseudoreferences using the Map to Reference tool in Geneious v8.0.4 (Figure 1; Appendix A). Primers were then designed at a conserved coding sequence flanking intron positions using Primer3 v.0.4.0 (http://bioinfo.ut.ee/primer3-0.4.0/; Koressaar and Remm 2007; Untergasser et al. 2012).

1.3.3 DNA Extraction & Primer Testing

Several taxa were chosen that represent the phylogenetic breadth of the target lineage. These taxa were used to test the PCR primers with the aim that the primers would work in all samples in the tribe. Initial PCRs utilized a temperature gradient for the annealing step with a single taxon to determine the optimal annealing temperature for each primer pair. Once the annealing temperature and number of cycles were determined, the optimal PCR conditions were applied to all samples. Only primer pairs that yielded a single band across all samples were sequenced. The sequences were assembled, edited, and aligned and pairwise identity and parsimony informative scores were generated for each marker to determine phylogenetic utility.

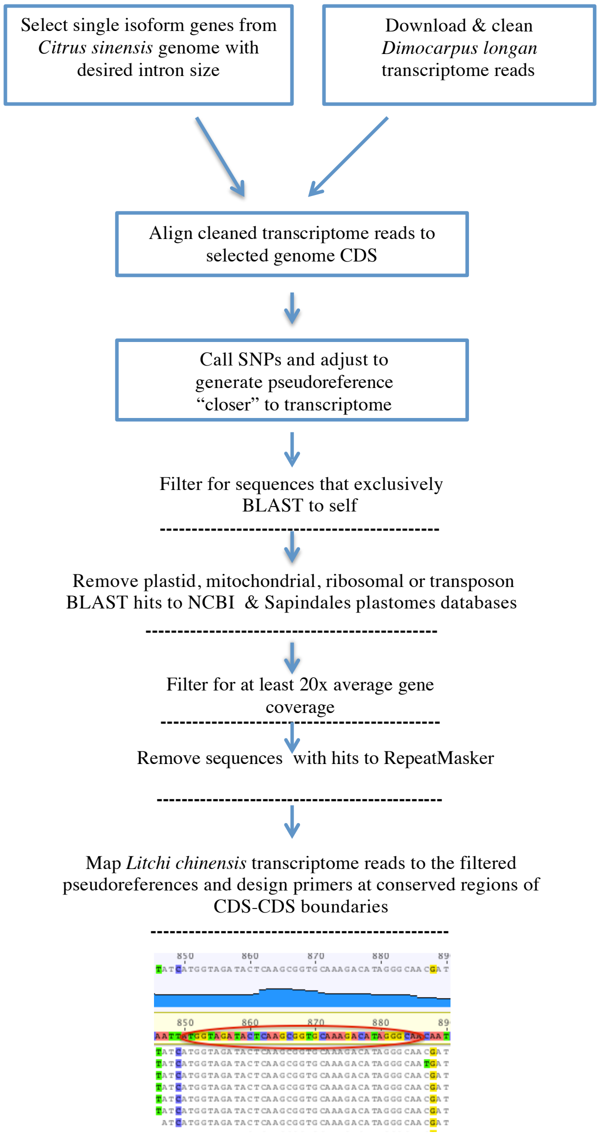

Figure 1. Bioinformatic pipeline to target single copy nuclear intron markers.

1.3.4 Case Study– targeting single copy nuclear introns of Paullinieae (Sapindaceae)

The '*Citrus sinensis* CDS' (46,147 sequences) and genome annotation files were downloaded from the Citrus Genome Website Csinensis v1.0 (www.citrusgenomedb.org-accessed on August 22, 2015; (Wu et al. 2014). Sequences in this CDS file are mature mRNA strands void of introns and untranslated regions. Of these 46,147 mature mRNAs, 18,384 were single isoform mRNAs and of those, 2159 had introns of 500-1,100 base pairs. The *Dimocarpus longan* transcriptome paired end reads (SRR412534) were downloaded from http://www.ncbi.nlm.nih.gov/ using the NCBI SRA Toolkit v. 2.4.5-2 and cleaned to remove adapters, low-complexity sequences, contamination, and PCR duplicates (Singhal, 2013). Of the 64,876,258 *D. longan* transcriptome reads, 39,701,810 remained after cleaning. Of the cleaned reads, 573,149 aligned to the 2,159 genes from *C. sinensis* in the final alignment. In transforming the *C. sinensis* reference to a *Dimocarpus* pseudoreference, 103,088 SNP positions were changed. After removing low coverage genes, 1,547 pseudoreference sequences remained. The following filters were applied (Figure 1) in this order: 1) Retain sequences that only BLAST to self (by BLAST to both the entire *Citrus* CDS and against all pseudoreferences) – 315 removed; 2) Remove sequences that BLAST to plastid, chloroplast, ribosomal, transposon or mitochondrial loci using Megablast and Organism: Spermatophyta NCBI database and compiled Sapindales plastomes (downloaded from https://www.ncbi.nlm.nih.gov/genbank) – 150 removed; 3) Retain only genes with at least 20x average read coverage – 793 removed; 4) Remove sequences with hits to Repeat Masker (http://repeatmasker.org/) using default settings- i.e. interspersed repeats and low complexity DNA sequences – 243 removed. After applying the above filters, a total of 46 sequences were isolated that fit the criteria of single copy, single isoform nuclear genes containing introns between 500-1,100 base pairs. To verify that primer design regions were conserved within the family, a second set of Sapindaceae transcriptome reads, from *L. chinensis* (NCBI: SRX258094; Li et al., 2013), was aligned to the pseudoreference using the Map to Reference tool in Geneious v8.0.4 low sensitivity and 2 iterations (Figure1). Geneious v8.04 was utilized at this step because the number of loci remaining made computation on a desktop computer possible and visualization manageable. Of the 53,437,444 *L. chinensis* reads, 88,779 mapped to the 46 genes of interest. Primers were designed using Primer3 v.0.4.0 (http://bioinfo.ut.ee/primer3-0.4.0/; Koressaar and Remm, 2007; Untergasser et al. 2012) by randomly selecting twenty-one conserved Sapindaceae CDS-CDS boundaries within the Geneious mapped alignment. The *L. chinensis* reads were not filtered for our purposes, because if primers designed in conserved regions from this second alignment resulted in amplification of multiple PCR products, marker development of these loci were not pursued. However, best practices are to clean all transcriptome reads prior to mapping. See Appendix A for step-by-step instructions.

1.3.5 Taxon Sampling, DNA Extraction & Primer Testing

DNA was extracted from silica dried leaf material from twelve Sapindaceae samples representing 9 species (Appendix B, Table 3) using the CTAB (Doyle and Doyle, 1987) method with minor modifications. Eleven ingroup samples (*Serjania paucidentata* DC., *Serjania pyramidata* Radlk., *Serjania atrolineata* C.Wright, *Serjania Mexicana* (L.) Willd., *Paullinia turbacensis* Kunth (3 individuals), *Paullinia bracteosa* Radlk. (2 individuals), *Paullinia sp*., and *Paullinia glomerulosa* Radlk. and one outgroup species *Allophylus psilospermus* Radlk. were used. After testing annealing temperatures over a temperature gradient (62-43°C) for each primer pair in a representative species, optimized PCR conditions were applied to all samples. Loci were amplified using Phire hot start II DNA polymerase (Thermo Fisher Scientific, Pittsburgh, Pennsylvania) with a 5 minute initial denaturing step at 98°C, loci specific cycles of 5 seconds at 98°C, 5 seconds at loci-specific annealing temperature and 20 seconds at 72°C and a final 1 minute 72 extension. Optimal annealing temperatures are reported in Table 1. Only primer pairs that yielded a single band across all samples were sequenced. Cycle sequencing was performed using BigDye V3.1. Sanger Sequencing was done at UC Berkeley Evolutionary Genetics Lab on an Applied Biosystems 3730x DNA analyzer. Reads were assembled, edited in Geneious v8.0.4 and aligned using MAFFT v7.271 (Katoh and Standley, 2013). The Paullinieae introns were consistently smaller than expected based on the *C. sinensis* intron sizes. Pairwise identity was calculated in Geneious v8.0.4 and parsimony informative sites were calculated in PAUP* 4.0b10 (Table 2). Of the 21 primer sets tried, 7 resulted in multiple products, 2 failed to amplify, and 12 resulted in single PCR products. An additional 3 markers were removed due to inconsistent amplification success across all samples. Amplification success of primer pairs are presented in Appendix B Table 4 and summary statistics of each loci are presented in Table 2. To check the identity of each sequenced loci, the consensus sequence of the Sanger reads and their primers were mapped back to their respective pseudoreference and the original *Citrus* gene. For loci with more internally designed primers (i.e. almost entirely intronic), their primers were mapped to both references. In all cases, either the CDS region of the Sanger sequence read itself or the primers aligned to their respective references.

Table 1. Primer sequences for the nine putative single-copy nuclear markers developed to amplify across Paullinieae (Sapindaceae).

Locus[a]	**Primer sequences (5′–3′)**	**Expected Bases (bps)**	**Aligned Bases (bps)**	**T_a (°C)**	**Putative Citrus Protein Homolog**	**GenBank ID**
orange1.1g002083m (intron2)	F: CATATGCAGTTACAGCACTAATGA R: AATCTCAACAGCATGAGCATC	537	358	44.3	Ap-4 complex subunit epsilon-1	KY770939-KY770948
orange1.1g047192m (intron9)	F: AGGTGCTTCACCTGAAATGG R: TTGGTTCACCATTTTCACC	755	665	44.3	Nad(p)-binding rossmann-fold superfamily protein	KY770927-KY770938
orange1.1g045023m (intron7)	F: AGGGCCCTTGAACTTGTTT R: CAGAGAGAACCTTGAGCATCTG	452	814	58.6	Sf27 - adaptin, alpha/gamma/epsilon	KY770919-KY770926
orange1.1g045023m (intron6)	F: GGGCCCTTTTACGAATAGAA R: AGGGCCCTTGAACTTGTTT	651	301	50.9	Sf27 - adaptin, alpha/gamma/epsilon	KY770913-KY770918
orange1.1g028997m (intron4)	F: AAAGAGTCCAAACCAACAATTC R: TAAGCAGCACTTTTCCCACA	885	852	58.6	Nuclear pore complex protein nup50	KY770903-KY770912
orange1.1g015495m (intron8)	F: CTGCTGGAAATGCCTCTAGC R: CTGAGCAGCGTCAGCATATC	151	156	49.0	Acetyl-coa c-acetyltransferase / acetoacetyl-coa thiolase	KY770891-KY770902
orange1.1g027952m (intron5)	F: TGGTTTTGATTGATGCAAGTG R: GCATCTTCCCACCAAGGATA	533	495	58.6	Sf825 - hydrolase, alpha/beta fold family protein	KY770879-KY770890
orange1.1g022777m (intron3)	F: GGAGGATTTCAATGAGGCTCT R: TCTCAGCATAATCAGACCTGTG	482	781	58.6	Coatomer subunit epsilon	KY770868-KY770878
orange1.1g009973m (intron5)	F: AGTGGAACTGCTTCGCAAGT R: TGCATATGGGTTATAGCCTTGA	855	470	49	Sf155 - kh domain containing rna binding protein	KY770861-KY770867

Note: T_a= annealing temperature.

[a] Reference locus in *Citrus sinensis* v1.0 genome.

Table 2. Summary statistics of the 9 putative single-copy nuclear markers developed to amplify across Paullinieae (Sapindaceae).

Reference Locus in *Citrus sinensis* v1.0 Genome	**# Taxa in Alignment**	**Parsimony informative Sites**	**Total Characters in multiple sequence alignment**	**Pairwise Identity (%)**
orange1.1g002083m (intron2)	10	15	358	88.3
orange1.1g047192m (intron9)	12	45	665	84.5
orange1.1g045023m (intron7)	8	52	814	94.3
orange1.1g045023m (intron6)	6	8	301	87.7
orange1.1g028997m (intron4)	10	50	852	71.8
orange1.1g015495m (intron8)	12	11	156	94.3
orange1.1g027952m (intron5)	12	29	495	94.2
orange1.1g022777m (intron3)	11	52	781	69.8
orange1.1g009973m (intron5)	7	64	470	53.7
ITS (for comparison)	7	34	708	69.1

1.3.6 Phylogenetic Analysis

The concatenated alignment of 9 markers with a total of 4,892 aligned characters was used to generate phylogenetic hypotheses under maximum likelihood. Gaps and the ends of shorter sequences were treated as missing data. ML trees were generated with the GTR sequence evolution model in RAxML-HPC on XSEDE 8.2.8 (Stamatakis, 2014) using the CIPRES Scientific Gateway (Miller et al. 2010) (Figure 2). Support was evaluated with 100 bootstrap replicates. Additionally, gene trees were generated using the GTRGAMMA model in RAxML with 1000 bootstraps on XSEDE 8.2.8 (Stamatakis, 2014). These gene trees were used as input into statistical binning (Mirarab et al., 2014), after which final gene trees were run under a partitioned RAxML run and were used as input in Astral-II (Appendix C). The two liana genera, *Paullinia* L. and *Serjania* Mill., each form monophyletic groups with moderate to high bootstrap support. Multiple individuals of *Paullinia turbacensis* and *Paullinia bracteosa* were included in the tree and formed monophyletic groups. The long branch of the *Paullinia* + *Serjania* group is explained by the relatively distantly related outgroup, *Allophylus psilospermus*. The Astral-II optimal tree differed from the RAxML tree only in the order of the 3 *Paullinia turbacensis* specimens (Figure 2), providing evidence that these markers are of appropriate length to be informative. Given the sequence variation, moderate to high bootstrap support across nodes and recovery of monophyly of major groups, we expect these markers to be highly informative with more inclusive sampling.

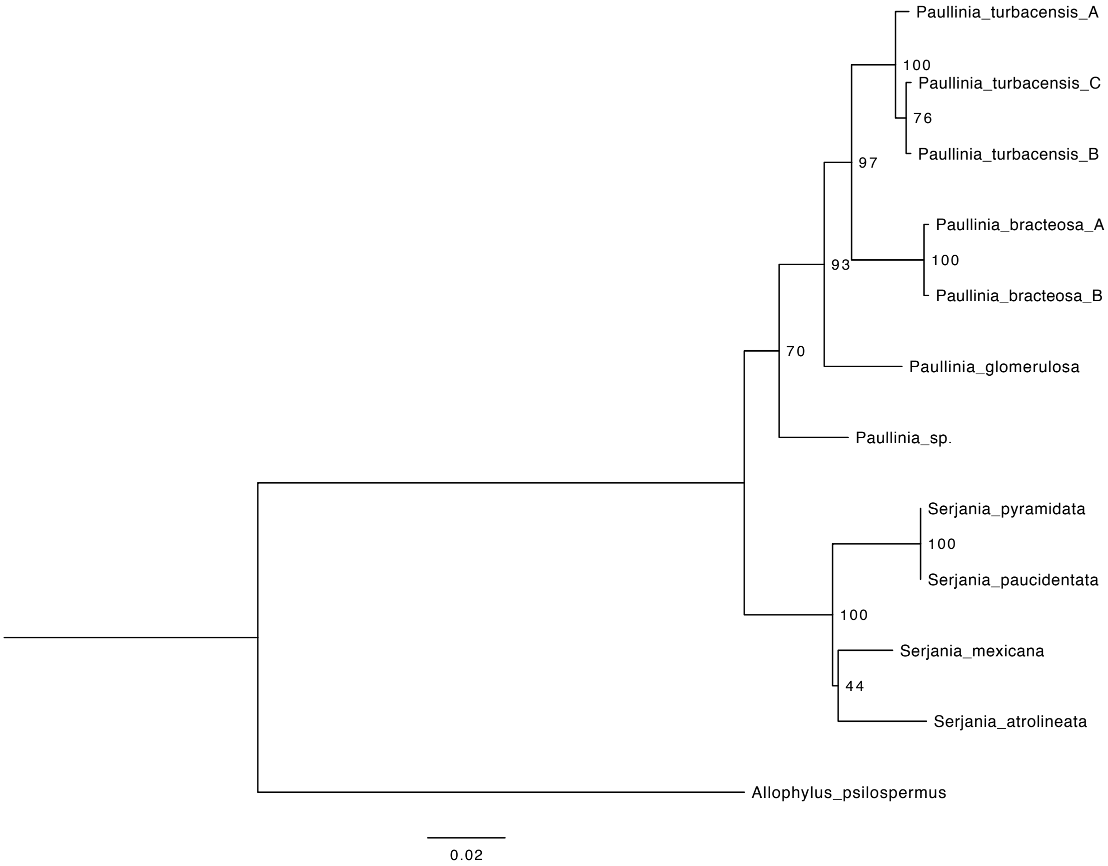

Figure 2: Maximum likelihood phylogenetic tree of 9 Sapindaceae species using concatenated alignment of 9 newly developed putative single-copy nuclear markers.

1.4 Conclusions

This bioinformatic pipeline utilizes publicly available genomic and transcriptomic resources to design primers in coding sequences flanking targeted introns of single copy nuclear loci without generating sequence data *de novo*. A fully annotated genome provides the information to determine approximate intron size and location, and two more closely related transcriptomes provide the best estimate of gene sequence to optimize primer design. By using this combination of data and through primer validation for exclusively single band PCR products, we increase the chances of targeting and successfully amplifying orthologous single copy nuclear introns in the lineage of interest. Although many steps were taken to obtain orthologous loci- only included genes which BLAST exclusively to self, only proceeded with markers that yielded a single PCR products, only proceeded with markers that sequenced a single PCR product and finally mapped Sanger sequences and primers back to their respective pseudoreference and original *Citrus* gene, orthology

could also be further assessed by constructing and analyzing gene trees with greater taxon sampling.
It is important to note that the number of potential target loci can easily be increased by customizing and relaxing parameters or filters throughout the pipeline (e.g. filters for single isoform genes, average gene coverage, desired intron length). By using the closest sequenced genome available, we had the best estimate of intron size and location. This method also worked in Zingiberales Griseb. (Sass et al., 2016) for designing markers from individuals distant from the genome by approximately 100my. Together with increasing transcriptome availability from publicly available sequencing projects like One Thousand Plant Transcriptomes, this pipeline might be increasingly available for many different plant groups. However, if reasonably close genomic and/or transcriptomic resources are unavailable, MarkerMiner (Chamala et al., 2015) is an easy to use alternative tool to develop primers spanning introns without the need for a closely related genome. Key differences between MarkerMiner and the bioinformatic pipeline presented here is our use of transcriptome reads rather than a full transcriptome assembly, explicitly targeting introns of a desired size, and the intentional use of the closest available genome.

Several authors present methods to target low copy nuclear makers for phylogenetic utility (Duarte et al. 2010; Curto et al. 2012; Tonnabel et al. 2014; Stockenhuber et al. 2015; Sass et al. 2016). Using published genomes from across angiosperms, Duarte et al. (2010) recovered 959 genes that were determined to be single-copy across *Arabidopsis thaliana (L.) Heynh.*, *Populus trichocarpa* Torr. & A. Gray, *Oryza sativa* L. and *Vitis vinifera* L. (APOV). Interestingly, of these 959 APOV genes, only 201 were found in the *Citrus sinensis* genome and of those only 24 were determined to be single-copy under our criteria. When specifically looking for those 24-remaining single-copy genes following our developed pipeline, we determined that 23 of the 24 APOV genes were removed through various steps specific to our filtering pipeline (i.e. intron size specific filters, 20x coverage filter, RepeatMasker filter). Considering copy number in *Arabidopsis thaliana* as a reference, (Curto et al., 2012) had success developing nuclear markers in Lamiaceae, however given the low number of single copy APOVs found in *C. sinensis* due to lineage-specific gene loss and duplication, the APOV markers were not appropriate for our purposes. By using phylogenetically closer genomic and transcriptomic data, we were able to test all markers for copy number prior to including them in phylogenetic analysis. Stockenhuber et al. (2015) and Tonnabel et al. (2014) efficiently detected low copy nuclear markers for Brassicaceae Burnett and Proteaceae Juss., however these authors generated transcriptome sequences of members of the lineage of interest. Sass et al (2016) detected low copy nuclear markers using publically available data but targeted exons. The pipeline presented here is cost efficient in that is does not generate sequence data de novo. Rather it utilizes publicly available genomic and transcriptomic resources spanning the breadth of the plant order Sapindales to design intron markers at conserved coding sequence boundaries. Our pipeline is cost-efficient in that it utilizes only publicly available datasets. Using the presented pipeline, amplification of 9 novel primer pairs was successful in generating phylogenetically informative markers from 9 Sapindaceae species, including amplification in the designated outgroup. Sequence variation within these markers ranges from 53.7-94.3% pairwise identity, making them promise for generating a robust data matrix to resolve species-level phylogenetic relationships within Paullinieae, especially when combined with other highly variable markers (e.g. ITS). This flexible marker development pipeline could be applied to any group with appropriate genomic resources. Identified regions of interests can be used in a variety of ways- amplified by PCR and sequenced using Sanger Sequencing or as baits for a hyb-seq NGS approach.

1.5 Funding Sources

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Chapter 2. Phylogeny of *Paullinia* L. (Paullinieae: Sapindaceae), a diverse genus of lianas with rapid fruit evolution

2.1 Abstract

Paullinia L. is a genus of c. 220 mostly Neotropical forest-dwelling lianas that displays a wide diversity of fruit morphologies. *Paullinia* resembles other members of the Paullinieae in being a climber with stipulate compound leaves and paired inflorescence tendrils. However, it is distinct in having capsular fruits with woody, coriaceous, or crustaceous pericarps. While consistent in this basic plan, the pericarps of *Paullinia* fruits are otherwise highly variable—in some species they are winged, whereas in others they are without wings or covered with spines. With the exception of the water-dispersed indehiscent spiny fruits of some members of *Paullinia* sect. *Castanella*, all other species are dehiscent, opening their capsules while they are still attached to the branch, revealing arillate animal-dispersed seeds. Here I present a molecular phylogeny of *Paullinia* derived from 11 molecular markers, including nine novel single-copy nuclear markers amplified by microfluidics PCR. This is the first broadly-sampled molecular phylogeny for the genus. *Paullinia* is supported as monophyletic and is sister to *Cardiospermum* L., which together are sister to *Serjania* Mill + *Urvillea* Kunth. This novel phylogenetic hypothesis is then used to test previous infrageneric classifications and to uncover that unwinged fruits represent the ancestral condition, from which there were repeated evolutionary transitions and reversals. However, because the seeds of both winged and unwinged fruits are all dispersed by animals, I conclude that the repeated transitions in fruit morphology may relate to visual display strategies to attract animal dispersers, and do not represent transitions to wind dispersal.

Keywords: Sapindaceae, lianas, fruit evolution, microfluidic PCR, single-copy nuclear markers, high-throughput sequencing

2.2 Introduction

Paullinia L. (Paullinieae: Sapindaceae) is a genus of c. 220 lianas native to the Neotropics with one species tropical sub-saharan Africa to Zimbabwe (Radlkofer, 1933; Irvine, 1961; Medeiros et al., 2016; Acevedo-Rodríguez and Somner 2018). The Amazon region is the center of diversity of the genus (Medeiros et al., 2016) and contains 44% of the described species. Members of *Paullinia* can be identified based on their habit (lianas or vines—seldom erect shrubs), their alternate compound leaves with a terminal leaflet, and their septifragal capsular fruits enclosing arillate seeds. Within this basic morphology, there is great variation, particularly in fruit morphology (Radlkofer, 1933, 1895; Acevedo-Rodríguez et al., 2017), degree of leaf dissection, and presence or absence of wood with cambial variants (Bastos et al. 2016; Cunha Neto et al. 2018; Pellissari et al. 2018).

In addition to its taxonomic diversity, morphological disparity, and ecological significance as a prominent component of Neotropical forests (Gentry, 1991), the genus has an extensive history of human utilization. Almost 20% of *Paullinia* species are reported to have ethnobotanical uses by the indigenous peoples of Central and South America, primarily as fish poisons, medicines, and caffeine-rich stimulants (Beck, 1990), and stem cross-sections are used in Brazilian marquetry (Tamaio et al., 2009). The greatest economic impact, however, is from the caffeine-rich seeds of *P. cupana* var *sorbilis* (Mart.) Ducke, known colloquially as *guaraná*, which is an important international export commodity for Brazil (Erickson et al., 1984).

What we now recognize as *Paullinia* was introduced by Plumier (1693) as "*Clematis*", and subsequently transferred to *Cururu* (Plumier, 1703). Although Plumier (1703) recognized *Paullinia* to be distinct from closely related *Serjania*, Linneaus (1753), however, included members of *Serjania* within his concept of *Paullinia*. Miller (1754) described *Serjania* Mill. as separate from *Paullinia*, and this treatment was further supported by Schumacher (1794), who called for the recognition of fruit morphologies as an "essential character"; *Serjania* is easily distinguished from *Paullinia* by its samaroid fruits (rather than capsules). These two genera were placed in section Paullinieae along with *Urvillea* Kunth and *Cardiospermum* L. (Kunth 1821); this section was later transferred to the rank of tribe by de Candolle (1824) and Radlkofer (1890).

More than a century after *Paullinia* was published by Linnaeus (1753), Radlkofer (1895, 1933) reviewed all 91 published names and described a total of 148 species. He subdivided the genus into 13 sections based heavily on fruit characters (Table 5, Figure 4), with the first couplet of his *Conspectus Sectionum* (Radlkofer, 1895, 1933) dividing the genus according to whether the capsules are alate (i.e., winged) vs. exalate (i.e., without wings; Figure 4 and 5). Finer-scale divisions relied on pericarp morphology and anatomy, inflorescence groupings, number and degree of connation of sepals, and presence of mucilage in leaves (Radlkofer, 1895, 1933). A century later, however, Beck, in his unpublished dissertation (1991), concluded that Radlkofer's (1895, 1933) system was unstable and lacking clear structure. He proposed, instead, that *Paullinia* sensu stricto be reduced to 60 spp., and placed the remaining species into five smaller genera (Beck, 1991). His generic system (Table 5) begins by separating taxa based on seed attachment, then by pericarp morphology and venation. The two large genera, *Paullinia* and *Prancea*, were organized into sections based on pericarp wing and aril characters, respectively. However, as Beck (1991) was never published, Radlkofer's (1895, 1933) infrageneric classification remains in effect today.

Until the advent of molecular systematics, which enabled a broad sampling of taxa across the Sapindaceae, the monophyly of Paullinieae and the relationships among its genera remained untested. The earliest molecular-based phylogenetic analysis including Sapindaceae (Gadek et al., 1996) did not sample Paullinieae species, but later analyses supported the monophyly of the tribe (Harrington et al., 2005; Buerki et al., 2009; Buerki et al., 2010) and recovered similar relationships to those inferred from cladistic analyses of vegetative and reproductive characters (Acevedo-Rodríguez, 1993) and a hierarchical analysis of wood anatomy using Ward's clustering algorithm (Klaassen 1999). Recently, Acevedo-Rodríguez et al., (2017) confirmed the monophyly of Paullinieae (comprising *Thinouia*, *Lophostigma*, *Urvillea*, *Cardiospermum*, *Serjania,* and *Paullinia*) as one of the four successively nested clades within the greater supertribe Paulliniodae. Paullinieae members are united by their climbing habit, stipulate leaves, and paired inflorescence tendrils (de Candolle, 1824; Radlkofer, 1890; Acevedo-Rodríguez et al., 2017), and can artificially be divided into the "*Paullinia* group" (*Urvillea*, *Cardiospermum*, *Paullinia*) and the "*Serjania* group" (*Serjania*, *Lophostigma* and *Thinouia*), based on whether their fruit are capsules or samaras (Acevedo-Rodríguez 1993; this hypothesis refuted in Acevedo et al., 2017).

The early history of taxonomic confusion surrounding *Paullinia* and its relatives reveals a lack of macroevolutionary understanding of this lineage, and the reliance on fruit characters highlights the long-standing interest in fruit evolution in the group. However, despite these charismatic features, and the economic and ecological importance of the genus, we still lack a robust phylogenetic framework for *Paullinia*. In this study, I aim to 1) infer the first broadly sampled molecular phylogeny of *Paullinia*; 2) explore taxonomic implications of this phylogeny, and; 3) infer the patterns of fruit evolution within the genus. Specifically, the diversity of fruit morphologies—dehiscent winged and unwinged capsules, and indehiscent fruits with echinate pericarps—are hypothesized to be adaptive features aiding in dispersal (Acevedo-Rodríguez et al., 2017); this *Paullinia* phylogeny allows the reconstruction of transitions among these fruit morphologies and to explore their implications this species-rich Neotropical genus.

Glossary: Alated = winged; Exalated = without wings; Echinate fruit = spiny protrusions on pericarp layer

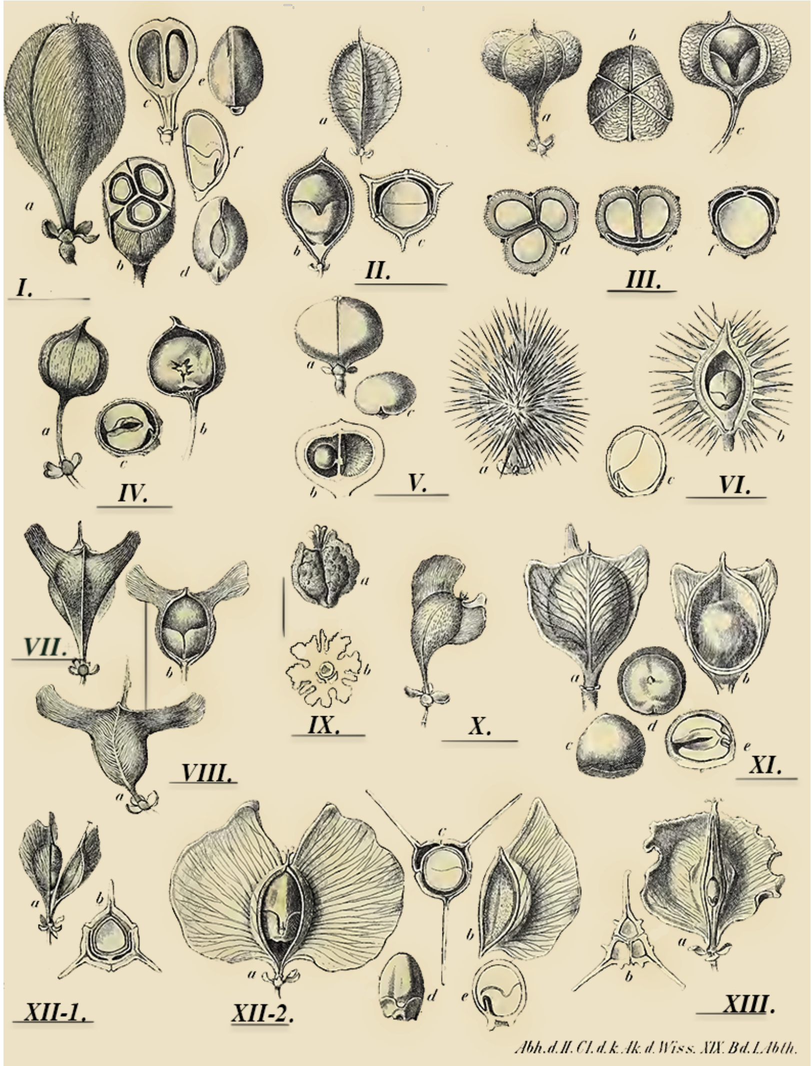

Figure 4. Diversity in fruit morphologies in *Paullinia*; plate modified from Radlkofer (1895) representing his classification system. I. Section. *Neurotoechus*, II. Section. *Diphtherotoechus*, III.Section. *Pleurotoechus*, IV.

Section. *Pachytoechus*, V. Section. *Enourea*, VI. Section. *Castanella*, IX. Section. *Cryptoptilon*, VIII. Section. *Neuroptilon*, VII. Section. *Xyloptilon*, X. Section. *Anisoptilon*, XI. Section. *Isoptilon*, XII. Section. *Caloptilon*, XIII. Section. *Phygoptilon*.

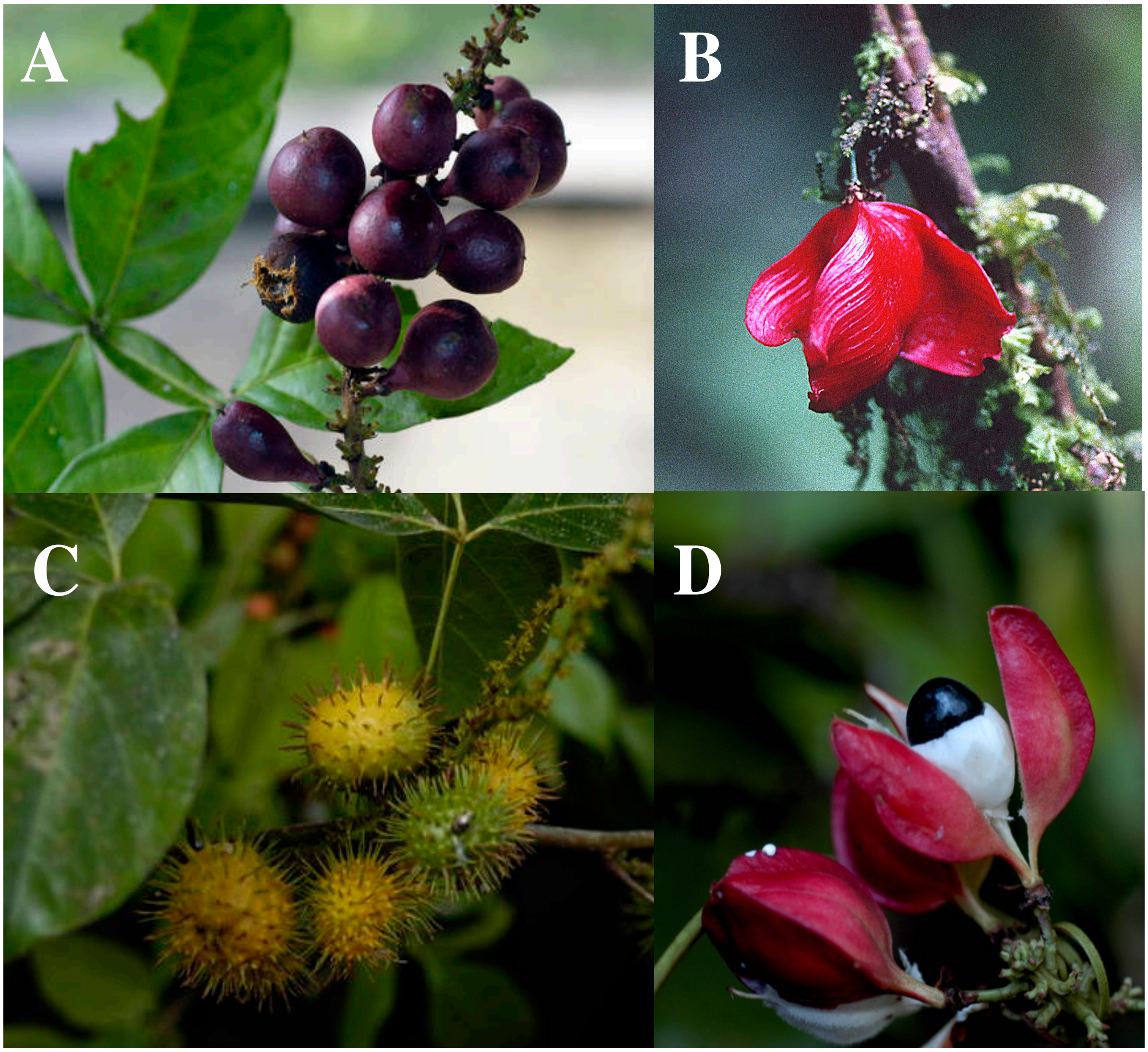

Figure 5. Diversity in fruit morphologies in *Paullinia*. **(A)** dehiscent exalate fruits of *P. imberbis* Acevedo 14849, **(B)** dehiscent alate fruits of *P. serjaniaefolia* Acevedo 7398, **(C)** in dehiscent echinate fruits of *P. paullinoides* Acevedo 14860, **(D)** open capsule of the dehiscent alate fruits of *P. caloptera* Acevedo 1431, capsule open along septa to reveal black glossy seed covered by a white fleshy aril. All photos by Pedro Acevedo-Rodriguez from collections.nmnh.si.edu.

Radlkofer (1895; 1931)	**Beck (1991) ***	**Sampled in this Phylogeny (sensu Radlkofer (1895; 1931)**
Paullinia sect. *Neurotoechus* (26)	*Paullinia* sect. *Paullinia* (19)	14
Paullinia sect. *Diphtherotoechus* (6)	*Prancea* sect. *Diphtherotoechus* (5)	1
Paullinia sect. *Pleurotoechus* (28)	*Chimborazoa* (1)	8
	Prancea sect. *Pleurotoechus* (4)	
	Prancea sect. *Ischnotoechus* (4)	
	Timothea (3) *	
Paullinia sect. *Pachytoechus* (11)	*Prancea* sect. *Prancea* (21)	2
Paullinia setc. *Enourea* (9)	*Enourea* (4)	4
Paullinia sect. *Castanella* (5)	*Castanella* (4)	2
Paullinia sect. *Cryptoptilon* (1)		0
Paullinia sect. *Neuroptilon* (3)	*Paullinia* sect. *Neuroptilon* (6)	0
Paullinia sect. *Xyloptilon* (3)		1
Paullinia sect. *Anisoptilon* (3)		0
Paullinia sect. *Isoptilon* (4)		1
Paullinia sect. *Caloptilon* (37)	*Paullinia* sect. *Caloptilon* (48)	11

Table 5. Comparison of the two major classification schemes for *Paullinia*. The number of recognized species are in parentheses. * Beck (1991) classification of Paullinia s.s. plus five smaller genera: *Enourea*, *Castanella*, *Prancea*, *Timothea* and *Chimborazoa*.

2.3 Materials and Methods

2.3.1 *Target Selection and Primer Design*

Novel single-copy nuclear markers were designed using the Chery et al. (2017) bioinformatic pipeline, utilizing publicly available genomic and transcriptomic resources within Sapindales. Primers were designed in Primer3 (Koressaar and Remm, 2007; Untergasser et al. 2012) with an optimal Tm of 57°C using the SantaLucia (1998) calculator. These primer pairs were modified by addition of conserved sequence tags for amplification on Fluidigm Access Array (Fluidigm San Francisco, California, USA). Chloroplast primers were taken from the literature (Taberlet et al. 1991; Demesure et al. 1995; Fazekas et al. 2008). In total, 87 primer pairs were tested, targeting a total of 50 single-copy nuclear exons, 24 single-copy nuclear introns, and 13 previously-published chloroplast markers. Primers were ordered through Eurofins MWG Operon, LLC (Huntsville Alabama, USA). Two modifications to the Fluidigm protocol (Fluidigm PN 100-3770 J1, San Francisco, California, USA) were carried out to enhance amplification success: annealing temperature of 57°C rather than 60°C and use of Phire Hot Start II DNA Polymerase reagents (ThermoFisher Scientific, Pittsburgh, Pennsylvania, USA) rather than the FastStart™ High Fidelity PCR System, dNTPack (Millipore Sigma, St. Louis, Missouri, USA).

2.3.2 Sampling scheme, DNA extraction

Taxon sampling prioritized testing Radlkofer's classification (1895, 1933) and spanning the morphological variation in *Paullinia*, while taking advantage of all available silica-dried leaf material which yield the highest quality DNA extractions. Genomic DNA was extracted from 191 samples (43 herbarium vouchers and 148 silica-dried leaves). CTAB DNA extractions were performed by an Autogen 965 at the Smithsonian Institution Support Center. Extraction quantity was measured by Qubit™ dsDNA HS Assay Kit (ThermoFisher Scientific, Waltham, Massachusetts, USA) at the UC Berkeley DNA sequencing facility. All extractions were diluted to a maximum of 50ng/ul as recommended by the iBEST Genome Resource Core (Moscow, Idaho). Extraction quality of all samples was tested by PCR amplification of ITS, which was sanger-sequenced on an Applied Biosystems 3730xl DNA analyzer at the UC Berkeley DNA Sequencing Facility. Sanger-sequenced reads were trimmed and cleaned in Geneious v.8.0.5 (Biomatters Ltd., Auckland, New Zealand) and aligned using MAFFT (Katoh and Standley, 2013).

2.3.3 Primer validation

Primer pairs were tested for amplification in four accessions (*Paullinia turbacensis* Chery 13, *Paullinia sp*. Breedlove 72699, *Paullinia carpopodea* Pace 317 and *Paullinia hystrix* Acevedo 14408) that span the phylogenetic breadth of the genus according to a preliminary ITS phylogeny. Thirty loci that successfully amplified under identical PCR conditions were further pursued. PCR products for these loci were sequenced directly on an ABI 3730x at the Evolutionary Genomics Laboratory at UC Berkeley. Primers were manually removed from sequences and the cleaned reads were aligned using MAFFT (Katoh and Standley 2013) implemented in Geneious v.8.0.5 (Biomatters Ltd., Auckland, New Zealand) to test for sequence variation among taxa. Two of the thirty loci generated two PCR products and were not pursued further. The final set of target loci included four chloroplast markers ((Taberlet et al. 1991; Demesure et al. 1995; Fazekas et al. 2008), fourteen novel single-copy nuclear exon loci, four novel single-copy nuclear intron markers, and six intron markers developed by Chery et al. (2017; see Appendix D).

2.3.4 Sequencing and data processing

DNA extractions, primers, and PCR reagents were sent to the iBEST Genome Core Facility (Moscow, Idaho) for amplification by microfluidic PCR and Illumina sequencing. Samples were run through a Fluidigm 192.24 chip with the standard protocol except for Phire Hot Start II reagents and annealing TM of 57°C. Amplicons were pooled and gel-purified, then run on a fragment analyzer to verify quality. qPCR was performed to determine the quantity of sequenceable libraries, and these were sequenced on 25% of a MiSeq lane.

Illumina Miseq raw reads were trimmed of reverse primer sequences and demultiplexed by dbcAmplicon (github.com/msettles/dbcAmplicons) by iBEST and additionally cleaned of forward primers and low quality reads with Trimmomatic v.38 (Bolger et al., 2014). Clean reads were processed through the Fluidigm2PURC pipeline (Blischak et al., 2018). This pipeline is specifically tailored to process Illumina data generated from amplicons and accounts for PCR error and Illumina sequencing errors to predict the likely haplotype(s) for each accession at each locus.

The first step merges cleaned paired-end reads using FLASH 2 (Magoc and Salzberg, 2011). These merged reads are processed through PURC (Rothfels et al. 2017), which iteratively clusters reads with the USEARCH cluster_fast algorithm (Edgar, 2010), and detects chimeras using UCHIME's USEARCH function (Edgar et al., 2011) to generate sequences of haplotype(s) for each accession at each locus. Finally, a maximum likelihood estimate of the number of haplotypes for each accession at each locus is generated by the *crunch cluster* script (Blischak et al., 2018). The output of the Fluidigm2PURC analysis is a MUSCLE alignment (Edgar, 2004) of all haplotypes for each locus. To validate the repeatability of inferred haplotypes (Rothfels et al., 2017), three different PURC regimes were run (denominated "A", "B", and "C"), each with four clustering and chimera-killing iterations and a minimum of 10 reads required for a cluster to be retained at each step (corresponding to ~10x mean coverage). For example, the regime A clustering criteria were .975, .995, .995, .995, meaning that in the first iteration of this regime sequences must be 97.5% identical in order to be clustered together, and these haplotypes (the consensus sequence of each cluster plus any as-yet unclustered sequences) are then fed into the second iteration requiring 99.5% identity, followed by two additional iterations of 99.5% identity each. The following three regimes were implemented: A=.975 .995 .995 .995; B= .995 .995 .995 .975; C= .995 .995 .995 .995). For each locus, maximum likelihood gene trees were inferred in RAxML V. 7.2.8 (Stamatakis, 2014) from the output alignment and putative contaminants and paralogs were removed according to the following workflow: 1) if a taxon had multiple haplotypes that formed a monophyletic group, one of these sequences was selected at random; 2) if a putative lineage-specific duplication led to two clades that did not share precisely the same set of taxa (due to inadequate sequencing coverage or other factors), all accessions involved in the duplication were removed from that alignment and; 3) if two or more accessions had identical sequences in all loci these were treated as contaminants (one for the other) and removed.

2.3.5 Phylogenetic Inference

The final concatenated alignment of 11 loci (nine single-copy nuclear markers, plus plastid *psb*A-*trn*H, and ITS; for accession list see Appendix E [1] and for sequences/accessions see Appendix E[2]) was analyzed by PartitionFinder2 (Lanfear et al., 2016) implemented in CIPRES (Miller et al., 2010) to select the best-fit partitioning scheme and models of evolution for the data (model=mrbayes, linked branch lengths, BIC model selection metric, search algorithm=all). The input to the PartitionFinder2 analysis was the full alignment with each locus designated as its own data subset. A partitioned Bayesian analysis with two runs each of four chains (one cold, three hot; temp=.02) was performed in MrBayes v.3.2.6, sampling every 1000 generations for 10 million generations (Ronquist et al., 2012). The analysis converged with a standard deviation of split frequencies = .008 and the estimated sample size (ESS) of all parameters exceeded 3000. TreeAnnotator v1.10.4 (Bouckaert et al., 2014) was utilized to generate the maximum clade credibility tree using the post burning trees from the combined MrBayes runs (Figure 6, 7, 8).

2.3.6 Trait Evolution

Paullinia fruit morphologies were categorized as alate (Figure 5B, D), exalate (Figure 5A), or echinate (Figure 5C). Fruit morphology was determined by examining the voucher of each accession in the phylogeny if fruits were present and/or voucher images were available on the Smithsonian Institution Herbarium web database (collections.nmnh.si.edu/search/botany/; accessed 27 February 2019) and from species description if this is described. If fruits were absent from the voucher, but species identity was confirmed by phylogenetic results, other vouchers of that same taxon were evaluated to score fruit morphology for that accession. The fruit morphology of 12 accessions were unknown and thus were excluded from all trait evolution analyses.

To infer patterns of fruit evolution, I estimated ancestral states and the total number of fruit morphology transitions across the phylogeny. Ancestral states and the number of state changes were estimated and visualized by stochastic character mapping using the best fit model (=symmetric rates, p=.05) along the branches of the Bayesian maximum clade credibility tree using the make.simmap function in the phytools package (Revell, 2012) in R (R Core Team, 2018). Topological and branch length uncertainties were accounted for in the estimation of total average fruit transitions by mapping character histories along the branches of 100 randomly sampled trees from the MrBayes (Ronquist et al. 2012) posterior distribution of trees, and the results were summarized using the describe.simmap function in the phytools package (Revell 2012) in R (R Core Team, 2018). All trees in these analyses were first rendered ultrametric with a relaxed clock model, then pruned down to *Paullinia* tips with fruit morphology data (=102) (using the chronos and keep.tip functions, respectively) in the APE package in R (Paradis et al. 2004; R Core Team, 2018)

2.4 Results

2.4.1 *Target Selection and Primer validation*

DNA extractions ranged from 2.47–50ug/ul with an average of 26.3 ug/ul (after diluting all extractions to a maximum of 50ng/ul). Primer validation resulted in 35% success (30 of the 87 primer pairs amplified successfully in the set of test species). The final set of loci selected for microfluidics PCR was 28: four chloroplast, nine single-copy nuclear introns, and 15 single-copy nuclear exons.

2.4.2 Sequencing and data processing

The Illumina Miseq data (25% of a lane) generated 6,500,757 sequences spanning 17 of the 28 target loci (the remaining 11 loci did not yield sequence). After additional cleaning with Trimmomatic v.38 (Bolger et al. 2014), 6,279,352 sequences remained. Of the 191 samples, 189 produced sequences for at least one locus (Appendix D). Only loci with at least 9% success rate across all accessions were pursued. This resulted in 10 loci generated by Miseq and the inclusion of all ITS sequences generated by PCR and Sanger sequencing. The inferred sequences generated by each of the three PURC regimes were consistent (as visualized by maximum likelihood gene trees of all regime haplotypes), suggesting repeatability of the inferred haplotypes across the regimes. PURC regime A typically inferred haplotypes for more accessions so was preferred for nine of the 10 loci. Where regime A generated an overwhelmingly unnecessary number of haplotypes, regime C was preferred (1/10 loci). Two loci were too long for the Miseq paired-end reads to overlap so their alignments had a region of missing data concentrated in the middle. These regions were treated as missing data ("?").

2.4.3 Phylogenetic Inference

The final concatenated alignment contained 148 OTUs, 814 sequences, and 5881 base pairs across ten single-copy nuclear markers, *psb*A-*trn*H, and ITS. The PartitionFinder2 best scheme favored four partitions as follows: partition one (orange1.1g002083m intron9, orange1.1g027952m intron5, orange1.1g009973m intron5, orange1.1g030977m intron1, orange1.1g036770m intron27): HKY +G; partition two (orange1.1g015495m intron8, orange1.1g016982m intron11, *psb*A-*trn*H): HKY +G; partition three (orange1.1g022777m intron3, orange1.1g019384m intron3); and partition four (ITS): GTR+ G. Gene names are adopted from the *Citrus sinensis* (Rutaceae) v.1.0 genome (Wu et al. 2014) and Chery et al. (2017).

The maximum clade credibility tree (Figure 6, 7, 8) is well resolved with 75% of the nodes having support with ≥ 95% posterior probability (PP). Rooted with *Cupania* and *Matayaba, Allophylus* is sister to the Paullinieae. The first diverging lineage of Paullinieae is *Thinouia*, followed by *Lophostigma*, which is followed by a *Urvillea* + *Serjania* clade that is sister to *Cardiospermum* + *Paullinia*.

Paullinia infrageneric relationships are resolved with good support. Seven clades are described, which roughly correspond to sections sensu Radlkofer (1895, 1931; Figure 6, 7, 8): Clade I (100 PP) = sect. *Castanella*, sect. *Caloptilon*, sect. *Pleurotoechus*, and sect. *Neurotoechus*; Clade II (100 PP) = sect. *Enourea*; Clade III (77 PP) = sect. *Phygoptilon*; Clade IV (95 PP) = sect. *Pachytoechus*; Clade V (100 PP) = sect. *Pleurotoechus*, *P. rubiginosa* sect. *Diphtherotoechus*, *P. rufescens* sect. *Isoptilon*, and *P. sphaerocarpa* sect. *Enourea*; Clade VI (100 PP) = sect. *Caloptilon*; and Clade VII (92 PP) = sect. *Neurotoechus* and *P. turbacensis* sect. *Xyloptilon*.

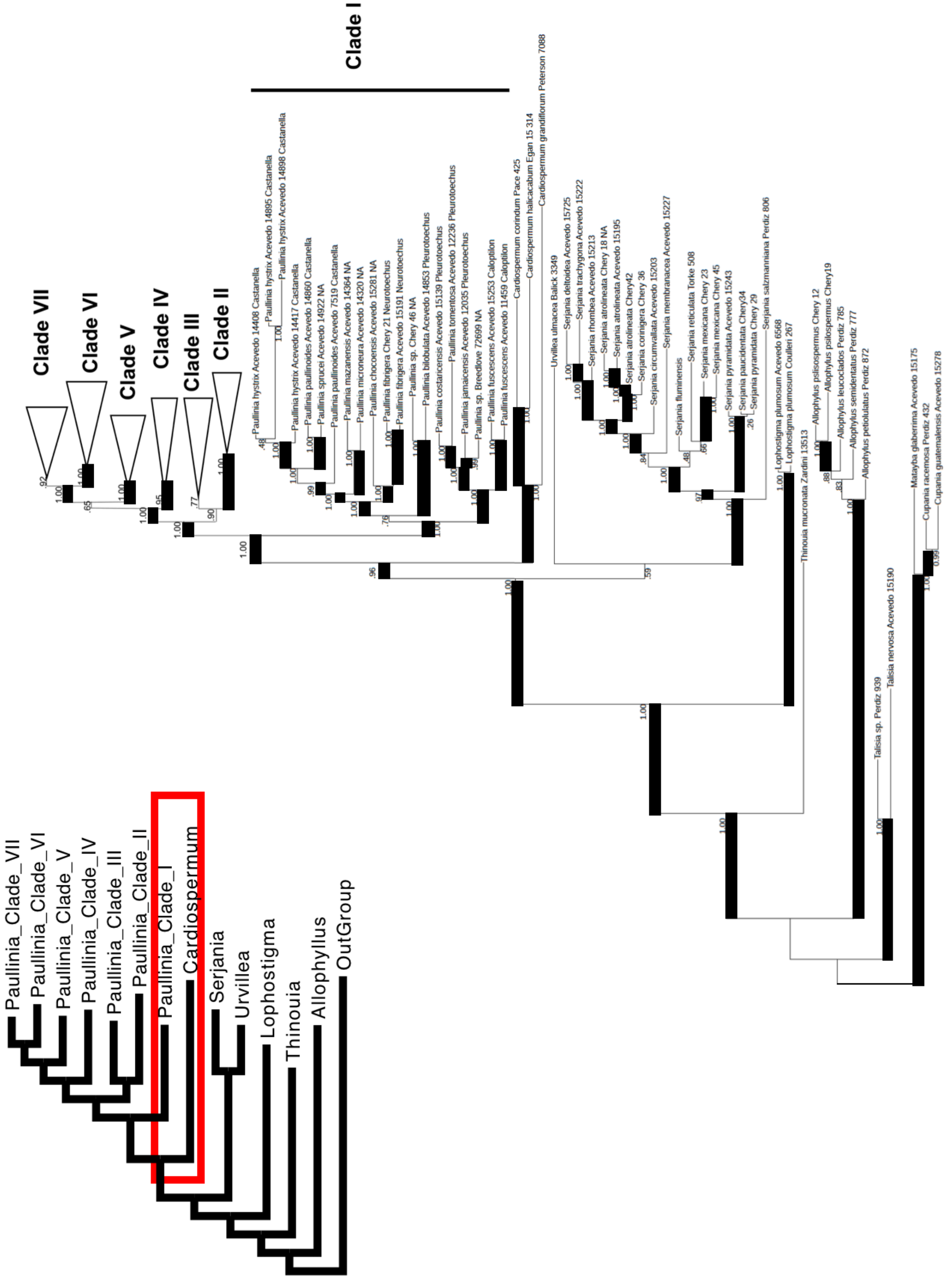

Figure 6. Outgroup and *Paullinia* Clade I on Bayesian maximum clade credibility tree.

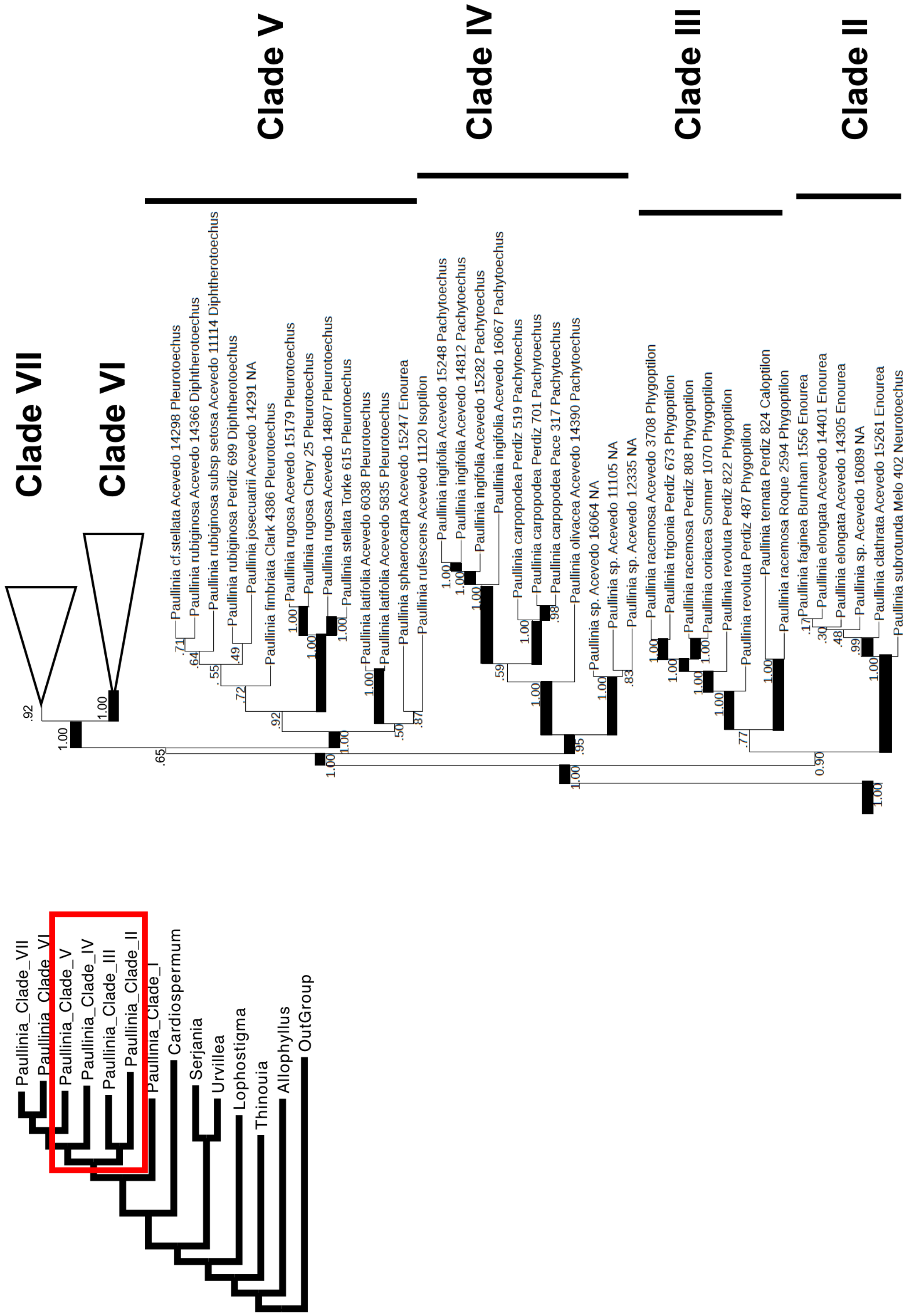

Figure 7. *Paullinia* Clade II, III, IV and V on Bayesian maximum clade credibility tree.

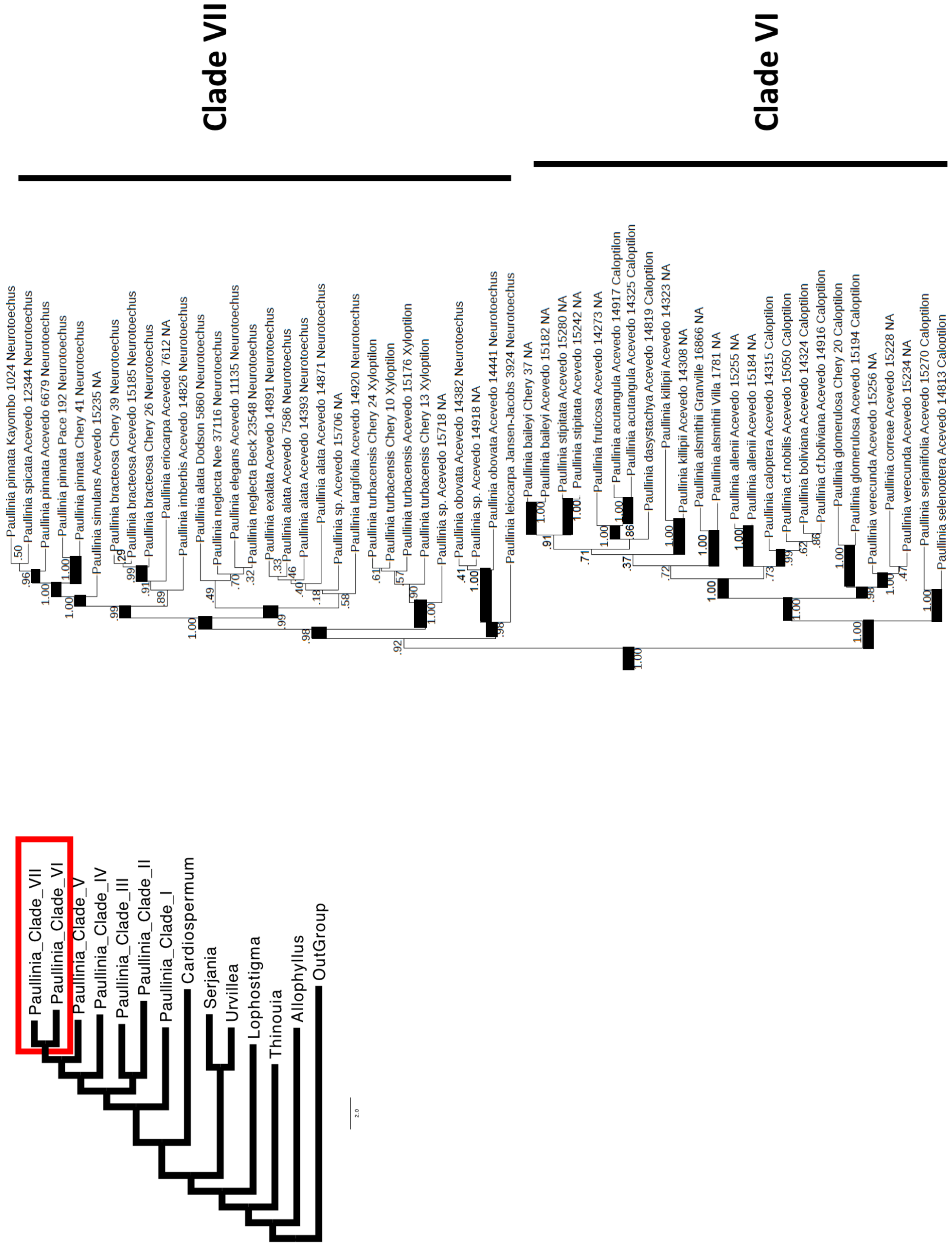

Figure 8. *Paullinia* Clade VI and VII on Bayesian maximum clade credibility tree.

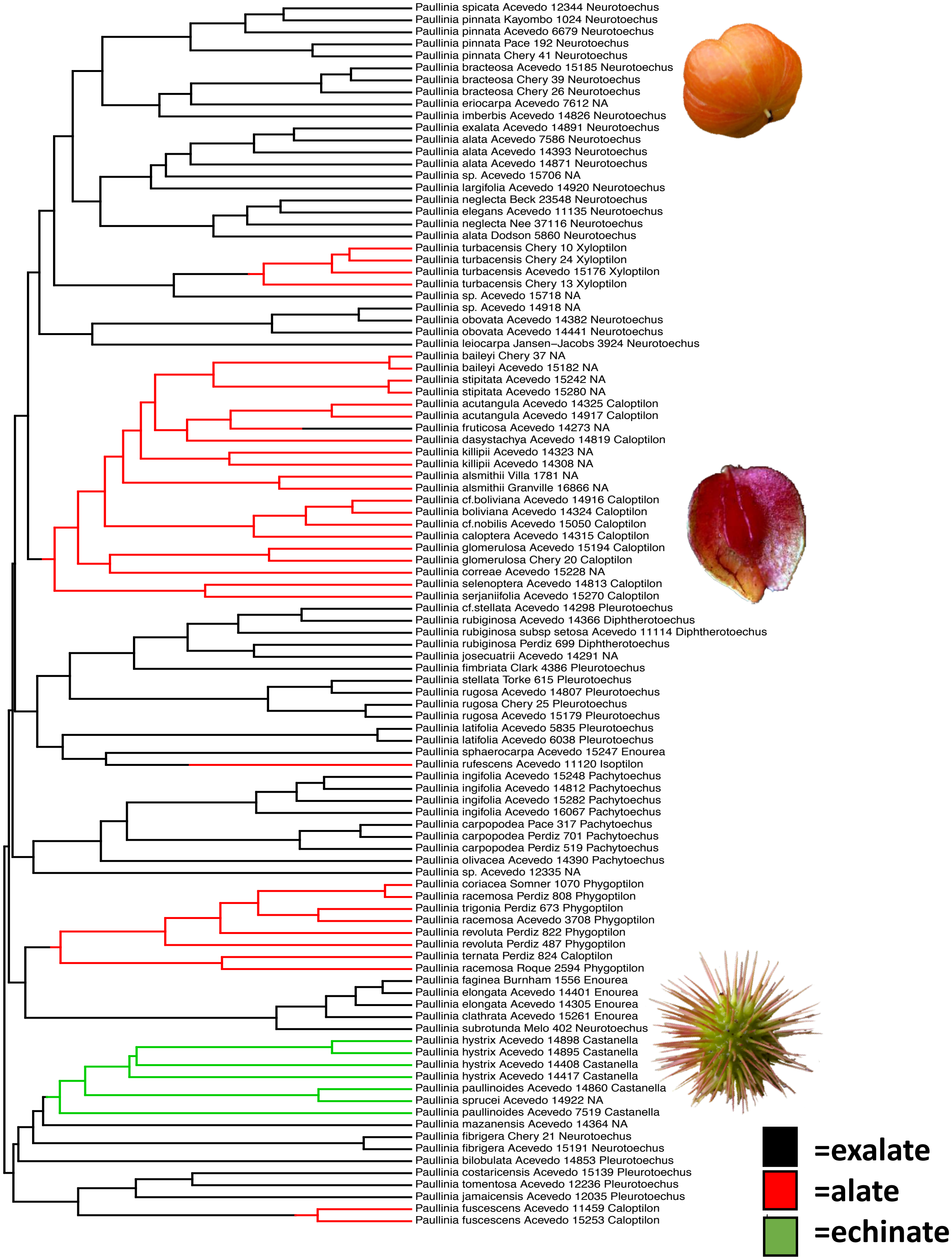

Figure 9. Ancestral State estimations of fruit morphology on *Paullinia* maximum clade credibility tree.

2.4.4 Trait Evolution

The ancestral condition of fruit morphology was reconstructed as exalate (Figure 6). Seven fruit transitions are observed on the maximum clade credibility tree: five transitions from exalate to alate in Clades I, III, IV, VI, and VII, one transition from exalate to echinate at the base of the sect. *Castanella* group in Clade I, and one reversal from alate to exalate in Clade VI. The average number of fruit transitions increases to 8.17 after accounting for topological and branch length uncertainties when character histories of 100 randomly selected trees from the posterior distribution were sampled.

2.5 Discussion

2.5.1 Taxonomic implications

The utilization of microfluidics PCR and Illumina sequencing facilitated the generation of the first broadly sampled molecular phylogeny for the genus *Paullinia*. Given the lack of resolution from chloroplast markers for species level relationships in the genus (Acevedo-Rodríguez et al., 2017; Chery, unpublished), it was necessary to employ rapidly evolving loci in order to generate robust phylogenetic hypotheses to investigate taxonomy and explore the evolution of morphologies. This phylogeny is an improvement from the first molecular phylogeny of the Paullinieae, which utilized ITS and the *trnL* intron, where the relationships between *Serjania*, *Paullinia,* and *Urvillea* were unresolved, and *Cardiospermum* was not monophyletic (Acevedo-Rodríguez et al., 2017). Given the incomplete sampling of this genus, I was unable to test the monophyly of *Cardiospermum*.

The bulk of *Paullinia* sections (sensu Radlkofer 1895, 1933; Table 5) were recovered as monophyletic, thus revealing that many of the morphological characters utilized by classical botanists are synapomorphies for clades and thus carry evolutionary signal as well as being useful for field identification. Some lineages are united by fruit morphology while others are united by various combinations of vegetative characters. Although the analysis presents a great improvement to our macroevolutionary understanding of *Paullina*, increased taxon sampling and/or including more molecular markers is needed to confirm infrageneric relationships and to make new infrageneric circumscriptions. I will not propose a revised classification until these additional data are incorporated.

The first diverging lineage of *Paullinia*, Clade I contains sect. *Castanella*, several members of sect. *Pleurotoechus*, and single species from sect. *Neurotoechus* and sect. *Caloptilon*. Clade I is the most diverse in fruit morphology, containing echinate fruits of sect. *Castanella*, exalate fruits of sect. *Neurotoechus* and sect. *Pleurotoechus*, and alate fruits of sect. *Caloptilon*. Here, there is a clear case of the transition in fruit types between closely related species: *P. fuscescens* and *P. costaricensis* have been described as “difficult to separate vegetatively” (Flora de Nicaragua, accessed on tropicos.org on 11 March 2019), the former with alate fruits and the latter with exalate. All members in this vegetative and reproductively disparate clade display regular wood (i.e., without cambial variants).

Clade II contains most of sampled sect. *Enourea* (with the exception of *P. subrotunda* sect. *Neurotoechus*). Section *Enourea* members have exalate fruits, 5-foliate leaves and simple wood. Sister to Clade II, Clade III represents a monophyletic sect. *Phygoptilon*, except for the inclusion of *P. ternata* of sect. *Caloptilon*. Clade III members all have alate fruits and some members have been reported to have successive cambia (Neto et al. 2018). Clade IV consists of a monophyletic sect. *Pachytoechus* with exalate fruit.

Clade V comprises half of sampled sect. *Pleurotoechus*, *P. rubiginosa* sect. *Diphtherotoechus*, *P. rufescens* sect. *Isoptilon*, and *P. josecuatrii*. Fruit morphology varies from exalate and sharply triangular (*P. rubiginosa* sect. *Diphtherotoechus*), to exalate and globose (sect. *Pleurotoechus*), to alate with large wings (*P. rufescens* sect. *Isoptilon*). The group is united by the presence of the phloem wedge cambial variant in most members.

Clade VI houses most of the sampled sect. *Caloptilon* species. All species in this clade have alate fruits and compound leaves that are 5-foliate and pinnate or of higher dissection (e.g. *P. glomerulosa*). In some lineages within Clade VI, there are transitions in vegetative characters between closely related species. For example, the 5-foliate and phloem wedge cambial variant species *P. baileyi* was recovered as sister to the ternate-pinnate (term sensu Croat 1976) and compound-wood species *P. stipitata*.

Clade VII houses most of the sampled sect. *Neurotoechus* and the only sampled representative of sect. *Xyloptilon* (*P. turbacensis*). This clade is united by the presence of ternate or 5-foliate pinnate compound leaves and compound or phloem wedge cambial variants. Embedded within this group is a clade characterized by the presence of cauliflorous inflorescences (*P. alata, P. exalata, P. largifolia*). All species in Clade VII have pyriform exalate fruits except *P. turbacensis* sect. *Xyloptilon* that has fruits with small wings.

2.5.2 Fruit morphology

Fruit morphology has been the most important character used to distinguish *Paullinia* from closely related genera and to identity and place taxa within the genus, however the patterns of evolution of *Paullinia* fruit have not been explored until now. The trait evolution reconstruction posits that the ancestral condition fruit morphology is exalate, and 7 transitions are observed on the maximum clade credibility tree: five transitions from exalate to alate, one transition from exalate to echinate, and one reversal from alate to exalate. A parsimonious hypothesis is that the repeated gain of wings suggests transitions from animal to wind dispersal. This would be consistent with the controversial hypothesis that wind dispersed fruits promote species diversification (Areces-Berazain and Ackerman 2017), which would conflict with the long standing hypothesis that the radiation of angiosperms, relative to gymnosperms, is due in part by the evolution of animal dispersed diaspores that facilitate an increased likelihood of propagules founding new populations where speciation by isolation can occur (challenged by Herrera, 1989 and reviewed by Eriksson and Bremer, 1991). However, although the differences in fruit morphologies in *Paullinia* appear meaningful for shifts in dispersal syndromes, field observations reveal that all *Paullinia* fruits open and dehisce their seeds while still attached to the branch, with the exception of some members of the "riparia group" (sect. *Castanella*), which have fruits that are echinate, indehiscent (in at least one species), and water dispersed (Acevedo-Rodríguez, personal communication). The pericarp of the dehiscent capsules are usually reddish (Weckerle and Rutishauser, 2005), and open to display three black glossy seeds covered by a fleshy white aril for consumption. The conspicuousness of the color contrast of the pericarp-seed-aril complex against the forest canopy attracts bird dispersers (van der Pijl, 1982; Howe and Smallwood, 2003; Schmidt et al., 2004). Experiments with wild-caught and hand-raised birds demonstrated significant preference for red fruits (Duan et al., 2014). This exact color contrast is also exhibited by the ovuliferous cones of the conifer genus *Phyllocladus* (Podocarpaceae), which is also bird dispersed (Contreras et al., 2017). If the alate fruits of *Paullinia* were wind dispersed, I would except there to be no aril, such as in *Serjania*; however, the seeds of all the dehiscent-capsuled species of *Paullinia* seeds are arillate. Other animal dispersers such as rodents are reported for *P. sphaerocarpa* Rich. ex Juss. (Weckerle and Rutishauser, 2005). The stability of the color-contrast and arillate fruit syndromes across the dehiscent species in *Paullinia* supports the observation that both alate and exalate fruits are animal dispersed, most probably by birds.

If the dispersal unit is the arillate seed rather than the capsule, then it follows that the fruit wings may also function as a bird attractant. For example, perhaps the wings function to increase the size of the fruit for a more attractive visual display to dispersers. Aside from the benefit of having a broader pool of dispersers, attracting a diversity of birds, potentially insectivorous ones, provides protection of their understory leaves from insect herbivory, as demonstrated in *Paullinia ingifolia* in La Selva, Costa Rica (Michel et al., 2014). The mutualistic relationship between *Paullinia* and birds is heightened in the dry season when most other plants are not producing fruit (Barro Colorado Island, Panama: Croat, 1978; Leigh, 1999). By reaching peak fruiting at this relatively scarce season, birds are most likely to take advantage of this available food resource (Leigh, 1999), facilitating successful dispersal of seeds.

Water dispersal may also be important in *Paullinia*. At least one species, *P. hystrix* sect. *Castanella*, has been reported to be water dispersed, containing green echinate capsules with septa that are slightly thickened and consisting of spongy tissue to allow the seed to float on water (Weckerle and Rutishauser 2005). Another member of this section, *P. paullinioides*, however, has red dehiscent echinate capsules. The dispersal syndrome of other *Castanella* members is unknown, however the presence of yellow to green capsules (Figure 5C) in several members is notable because they do not display the optimal color/color contrast for bird dispersal (Duan et al., 2014). Water dispersal has also been observed in distantly related *P. clathrata* (Weckerle and Rutishauser, 2005; Acevedo personal communication)

The repeated evolution of fruit morphologies across the phylogeny suggest an advantage associated with dispersal, however in the absence of direct field observations of the preferences of birds for alate vs. exalate fruits, and the dispersal success difference between birds vs. water dispersal, this remains an open question.

2.6 Conclusions

Here I present the first molecular phylogeny of *Paullinia* based on a large molecular dataset consisting of a set of rapidly evolving single-copy nuclear markers and two commonly used markers. *Paullinia* is recovered with high support as monophyletic and sister to *Cardiospermum*, which together are sister to *Serjania* + *Urvillea*. The ancestral condition of fruit morphology in *Paullinia* is reconstructed as exalate, and at least 7 transitions are estimated. Although it is tempting to ascribe the evolutionary trend of gain of wings to a transition from animal to wind dispersal, the arillate seed of both alate and exalate fruits are dispersed by animals Therefore, we conclude that the repeated transitions in fruit morphology represent different strategies to enhance visual display to attract a diversity of bird dispersers. Future research confirming precise bird preferences of different *Paullinia* fruits could uncover the advantages conferred by diverse fruit morphologies.

2.7 Funding Sources

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Chapter 3. Evolution of development of nature's strangest wood: Insights from *Paullinia* (Sapindaceae)

3.1 Abstract

Distantly related plant groups have converged upon the "liana syndrome"— a unique suite of anatomical traits that work in a coordinated fashion maximizing conductance, strength, and flexibility, which are critical to the climbing habit. Extreme elaborations of this liana syndrome can give rise to "vascular cambial variants", which are unusual stem conformations derived from the alternative activity of the vascular cambium in the formation of secondary xylem and phloem. The large neotropical liana genus, *Paullinia* L. (Sapindaceae), has a great diversity of stem conformations, with some species displaying the regular stem morphology and development reminiscent of self-sustaining plants (i.e., trees and shrubs), while others display one of a range of cambial variants. In this study, I integrate classical stem ontogeny techniques with phylogenetic comparative methods to explore the evolution of cambial variant development in *Paullinia*. I describe six stem ontogenies in *Paullinia* by studying three stages of development (primary growth, the onset of secondary growth, and the mature stems). Most *Paullinia* species are angular in cross-sectional view at the shoot apex, which becomes reinforced by the unequal distribution of vascular bundles around the circumference of the young stem. Although rare among woody plants, this is the basic bauplan of primary growth in *Paullinia*, from which five of the six mature stem types develop. To explore the evolution of stem ontogenies in *Paullinia* and across the other Sapindaceae lianas, I employed phylogenetic comparative methods to reconstruct the ancestral primary plant body shape and the ancestral mature stem type across the Paullinieae tribe. Additionally, I tested the hypothesis that the evolution of cambial variants is contingent on first evolving the irregular angular primary growth confirmation. My ontogenic studies reveal that all cambial variants arise from an angular primary body, and the ancestral reconstructions reveal that variant forms exclusively evolve in lineages with an angular primary body. These results identify a critical relationship between primary and secondary growth in both the development of cambial variants of an individual plant, and the emergence of these novel forms through evolutionary time.

Key words: stem development, vascular cambium, evolution of development, lianas, Sapindaceae, wood, secondary phloem

3.2 Introduction

When biologists observe traits that evolve independently multiple times across the tree of life, we wonder what adaptive advantage these traits might confer. The evolutionary shift to the liana habit (i.e., woody vines) is a well-noted example of such a case, first appearing in the fossil record in Paleozoic pteriodosperms (Burnham, 2015), and today present in one-third of extant seed plant families (Gentry, 1991; Burnham, 2015). This habit is distinct from self-sustaining woody plants (i.e., trees and shrubs) by its reliance on a host tree, to which they attach themselves by tendrils, adventitious roots, hooks, or the twining of their stems or leaves for structural support (Darwin 1867).

Twenty-four years after Putz and Mooney's seminal work, The Biology of Vines (1991), Schnitzer et al. (2015) reviewed the exciting resurgence of research interest in the ecology and biology of lianas. On the evolutionary front, the climbing habit (sensu lato) has been proposed to be a key innovation that promotes species diversification by Gianoli (2004, 2015) who reported that climbers were more species-rich than their non-climbing sister lineages. In compliment to this evolutionary perspective, numerous studies report on the ecological success of lianas evident by their increasing abundance in the neotropical forest of Barro Colorado Island, Panama (Schnitzer and Bongers, 2011), Central Panama (Wright et al., 2004; Ingwell et al., 2010), Costa Rica (Yorke et al., 2013), French Guiana (Chave et al., 2008), and Amazonia (Phillips et al., 2002). In contrast, other studies report a decline of lianas, for example in Gabon (Caballé and Martin, 2001) and the Democratic Republic of Congo (Ewango, 2010), or the lack of significant changes in abundance (Central Amazonia: Gerolamo et al., 2018; temperate United States: Londré and Schnitzer, 2006). To improve our understanding of lianas, it is critical to approach questions of their evolutionary success and their shifting ecological abundance from the perspective of their distinct stem anatomy, as this is a fundamental difference that sets them apart from trees and shrubs.

The term *liana syndrome* has emerged to describe the unique suite of stem anatomy traits that distantly related liana lineages have converged upon to maximize conductance, strength, and flexibility (Carlquist, 1991; Rowe and Speck, 2004; Angyalossy et al., 2012, 2015); this anatomy allows lianas to transport large quantities of water, while maintaining a stem compliant enough to twist and resilient enough not to break (Mooney and Gartner, 1991; Wyka et al., 2013). Consistent across lianas sensu lato, including pteridophytes, gymnosperms, monocots, and eudicots, the liana syndrome is even diagnostic for identifying lianas in the fossil record (Krings et al., 2006; Burnham, 2009; Jud and Nelson, 2017). Compared with trees and shrubs, lianas compensate for their narrow cross-sectional area by producing larger vessels and increase flexibility by decreasing the amount of fibers and increasing the abundance of non-lignified ray and axial parenchyma (Carlquist, 1985, 1991; Rowe and Speck, 2004; Angyalossy et al., 2012, 2015; Wyka et al., 2013).

In order to maximize the functions of conductance, strength and flexibility, many liana lineages have evolved unusual stem conformations and ontogenies that conspicuously deviate from that typical of self-sustaining plants; these atypical stems are called "vascular cambial variants" (Carlquist, 2001). Although cambial variants occur in self-sustaining plants (e.g., successive cambia in *Avicennia* mangrove trees: Robert et al. 2011), even characterizing entire lineages (intraxylary phloem in the Myrtales; Angiosperm Phylogeny Website- accessed 3 May 2019), they are much more common in lianas (Schenk, 1893; Obaton, 1960, Caballé, 1993; Carlquist, 1985, 1991; Angyalossy et al., 2012, 2015; Neotropical Lianas Project–accessed 26 April 2019). Cambial variants have been shown to facilitate various functions in lianas, including injury repair (Fisher and Ewers, 1989, 1991) and the partitioning of conductance and storage (Pace et al., 2011; Carlquist, 2013).

An ideal comparative system to study the evolution of stem ontogenies is the diverse genus *Paullinia* L. (Sapindaceae), which comprises ca. 220 species of lianas, climbing shrubs, and occasional erect shrubs that occur mostly in the Neotropics with one species in Africa (Acevedo-Rodríguez et al., 2011; Medeiros et al., 2016; Acevedo-Rodríguez and Somner, 2018). *Paullinia* species display the typical stem ontogeny of trees and shrubs or one of the following cambial variants: phloem wedges (Bastos et al., 2016; Pellissari et al., 2018), lobed xylem (Bastos et al., 2016; Pellissari et al., 2018), compound stems (Schenk, 1893; Obaton, 1960, Pfeiffer, 1926; Radlkofer 1895, 1933; Obaton, 1960; Van der Walt et al.,1970) and successive cambia (Neto et al. 2018). The diversity of stem morphologies expands if the other five genera of the Paullinieae tribe—all of which are lianas—are considered (Radlkofer 1895, 1933; Tamaio and Angyalossy, 2009; Tamaio and Somner, 2010; Tamaio, 2011; Tamaio et al. 2011; Lopes et al. 2017).

Several previous studies have described the wood anatomy and/or stem gross morphology of multiple species (e.g., Klaassen, 1999; Tamaio, 2011; Pellissari et al., 2018) or the full stem ontogeny of a few species (Van der Walt, et al., 1973; Neto et al., 2018), however an exploration of the evolution of development in a phylogenetic context is lacking. This approach allows a link between the development and evolution of complex traits and can be used to test adaptive hypotheses and/or illuminate synapomorphies and instances of convergent evolution. In this study I aim to: 1) describe and characterize ontogenies of *Paullinia* stems by studying their development in three stages (primary growth, the onset of secondary growth, and mature stems); 2) reconstruct the evolution of these ontogenies across the phylogeny; and 3) test if the evolution of cambial variants is contingent on the evolution of primary growth configurations.

3.3 Materials and Methods

3.3.1 Molecular dataset and phylogenetic analysis

Molecular sequences of the Paullinieae tribe from Chapter 2 and Acevedo et al. (2017) were utilized to generate a Paullinieae-wide molecular dataset. From the Acevedo et al. (2017) ITS and *trn*L dataset, I sampled 76 OTUs (members of *Serjania* Mill., *Cardiospermum* L. sensu stricto, *Urvillea* Kunth., *Lophostigma* Radlk., *Thinouia* Triana & Planch., *Thouinia* Poit, and *Allophyllus* L.), and I utilized the full dataset from Chapter 2), which consists of nine single copy nuclear markers, *trn*H-psbA, and ITS. The ITS sequences from Acevedo et al. (2017) and Chapter 2 were realigned using MUSCLE (Edgar, 2004) implemented in Geneious v.8.0.5 (Biomatters Ltd., Auckland, New Zealand) and the alignments of the other 11 loci were not realigned.

The final concatenated alignment of 12 loci (*trn*L intron, ITS, and nine single-copy nuclear markers, plus plastid *trn*H-*psbA*) across 224 OTUs was analyzed by PartitionFinder2 (Lanfear et al., 2016) implemented in CIPRES (Miller et al., 2010) to select the best-fit partitioning scheme and models of evolution (model= Mrbayes, linked branch lengths, BIC model selection metric, search algorithm=all). The input to the PartitionFinder2 analysis was the full alignment with each locus designated as its own data subset. A partitioned Bayesian analysis with two runs each of four chains (one cold, three hot; temp=.02) was performed in MrBayes v.3.2.6 (Ronquist et al., 2012), sampling every 1000 generations for 15 million generations. The analysis converged with estimated sample sizes (ESS) for all parameters exceeding 2000. TreeAnnotator v1.10.4 (Bouckaert et al., 2014) was utilized to generate the maximum clade credibility (MCC) tree using the post-burnin trees from the combined MrBayes runs. This MCC tree was rendered ultrametric with a relaxed clock model, then pruned down to only those OTUs that had primary and mature stem data available, using the chronos and keep.tip functions, respectively, in the APE package in R (Paradis et al., 2004; R Core Team, 2018). The final dataset comprises 188 terminal taxa (see accessions list in Appendix F). The maximum clade credibility tree (Appendix H) is moderately supported with 58% of the nodes having ≥ 95% posterior probability (PP). Rooted with *Cupania* L. and *Matayba* Aublet, *Allophyllus* is sister to the Paullinieae. Sister to the remaining Paullinieae is *Thinouia*, followed by *Lophostigma*, then *Urvillea*, then *Serjania,* which is the sister genus to

Cardiospermum + *Paullinia*. These relationships are all represented in Chapter 2, except in that tree, *Serjania* + *Urvillea* form a clade sister to *Cardiospermum* + *Paullinia*.

3.3.2 *Paullinia* sampling, embedding, and sectioning

To determine the stem developmental trajectories in *Paullinia*, I studied the ontogeny of 18 species at three developmental stages: primary growth (at the shoot apical meristem), intermediate (the onset of secondary growth), and mature stem. These stages were roughly approximated by stem diameters of 2mm, 4mm, and > 4mm respectively. Samples included stems that were immediately fixed in formaldehyde-acetic acid-alcohol then subsequently stored in 70% ethanol, and stems obtained by destructive sampling of herbarium vouchers at the US National Herbarium. Stems from herbarium vouchers of both the intermediate and mature developmental stages were rehydrated by boiling in a solution of 1ml of glycerin + 100ml of water. Primary growth material cannot be sampled and rehydrated successfully from herbarium vouchers due to the degradation of nonlignified tissues upon drying, therefore primary growth material was restricted to available ethanol collections.

All stems were gradually embedded in polyethylene glycol 1500 (Rupp, 1964). Stems were sectioned with a sliding microtome and each section was coated with a polystyrene foam solution (Barbosa et al. 2010). Sections were stained with Safrablau (nine parts 1% Astra Blue in 50% ethanol to one part 1% Safranin O in 50% ethanol; Bukatsch 1972) and mounted with Eurokitt® quick-hardening mounting medium to prepare permanent slides. Primary growth stems were sectioned in transverse plane, while the intermediate and mature woods were sectioned in transverse, tangential, and radial planes. Slides were analyzed under a Zeiss Stemi 508 stereo microscope and a Zeiss AxioImager light microscope and imaged with a Canon EOS M3 and a QImaing 5MPix MicroPublisher camera respectively. Slides are available upon request.

3.3.3 Developmental Character Matrices of *Paullinia* and the Paullinieae tribe

Stem ontogeny characters were scored at three developmental stages (Appendix G, Table 9) from the study of 18 species of *Paullinia* through development (see accessions list in Appendix G, Table 8). Primary growth characters scored are stem shape (including pith shape), vascular bundle distribution, width variation of the pericyclic fiber around the stem circumference, and location of laticifers. In the absence of a primary stem, these characters can also be reliably scored from the transition stem (e.g., variations in the widths of pericycle fibers indicates an angular pith). Intermediate stage characters include stem shape, stem type (presence/absence and type of cambial variant), vessel dimorphism, vessel groupings (for both small and large vessels). Mature stage characters include the presence/absence and type of cambial variants and pith shape. This *Paullinia*-specific character scheme allowed me to track how distinct regions of the stem changed throughout development. In addition to these stem ontogeny characters, I scored 30 discrete and continuous wood anatomy characters from the IAWA Committee (1989) and five additional *Paullinia*-specific characters: large vessel groupings, small vessel groupings, small ray width, and injuries.

For all non-*Paullinia* members included in the phylogeny (species of *Serjania*, *Urvillea*, *Cardiospermum*, *Lophostigma*, *Thinouia, Thouinia*, and *Allophyllus*), only two characters were considered: the primary body shape and mature stem type. These two characters were scored by analyzing multiple vouchers per taxon; the exact voucher matching the OTU was analyzed if stem and wood characters were retained and/or voucher images were available for viewing on

Smithsonian Institution Herbarium web database. These data are available in Appendix G, Table 11.

3.3.4 Phylogenetic Comparative Methods

To explore the evolution of stem anatomy across *Paullinia* and the Paullinieae, I performed two sets of analyses. In the first set, I sought to trace the evolution of each type of stem ontogeny present in *Paullinia* by estimating ancestral states for primary body shape (0= circular, 1= lobed) and mature stem type (0=regular, 1=phloem wedges, 2=lobed xylem, 3= compound stem, 4= successive cambia). Ancestral states were estimated and visualized using stochastic character mapping with the make.simmap function in the phytools R package (Revell, 2012; R Core Team, 2018) under the best fit model of evolution for each character; primary growth (=equal rates) and mature stem (=symmetric rates) on the MCC tree pruned down to *Paullinia* species with stem data (n=96; see Appendix G, Table 10). To account for the different possible character histories and to estimate the average number of state changes across this distribution, 100 character histories were simulated and the results were summarized with the describe.simmap and countSimmap functions in phytools (Revell, 2012; R Core Team, 2018) (Figure 16).

In the second set of analyses, I sought to trace the evolution of cambial variants as a general category across the Paullinieae tribe; this grouping represents species with atypical stem development and irregular final conformations that functionally separate them from regular stems. For these analyses, I scored primary growth (0= circular, 1= angular) and mature stem type (0= regular, 1= cambial variant). Ancestral states of stem ontogenies were estimated and visualized by simulating 100 character histories for both characters using stochastic character mapping with the make.simmap function in the phytools R package under the best fit model of evolution (=equal rates for both characters); these results were summarized by the describe.simmap and countSimmap functions in phytools (Revell, 2012; R Core Team, 2018) (Figure 17). To test the hypothesis that the evolution of mature stem type is contingent on the evolution of primary body shape, I performed a correlated evolution test (Pagel, 1994) using the fitPagel function in phytools with the following settings: model= equal rates and dependent variable= mature stem type. These analyses were performed on the MCC tree pruned down to members of the Paullinieae tribe (188) with stem data (Appendix G, Table 11).

3.4. Results

3.4.1 Developmental Trajectories

Stem ontogenies differed among the 18 *Paullinia* species studied, however there are clear developmental trajectories shared among groups of species. Six developmental trajectories are here described that capture the diversity of stem development in *Paullinia* (Appendix G, Table 9, 10). These developmental trajectories fall into one of the two large categories sensu Angyalossy et al. (2015): those that originate from a single vascular cambium or from multiple cambia.

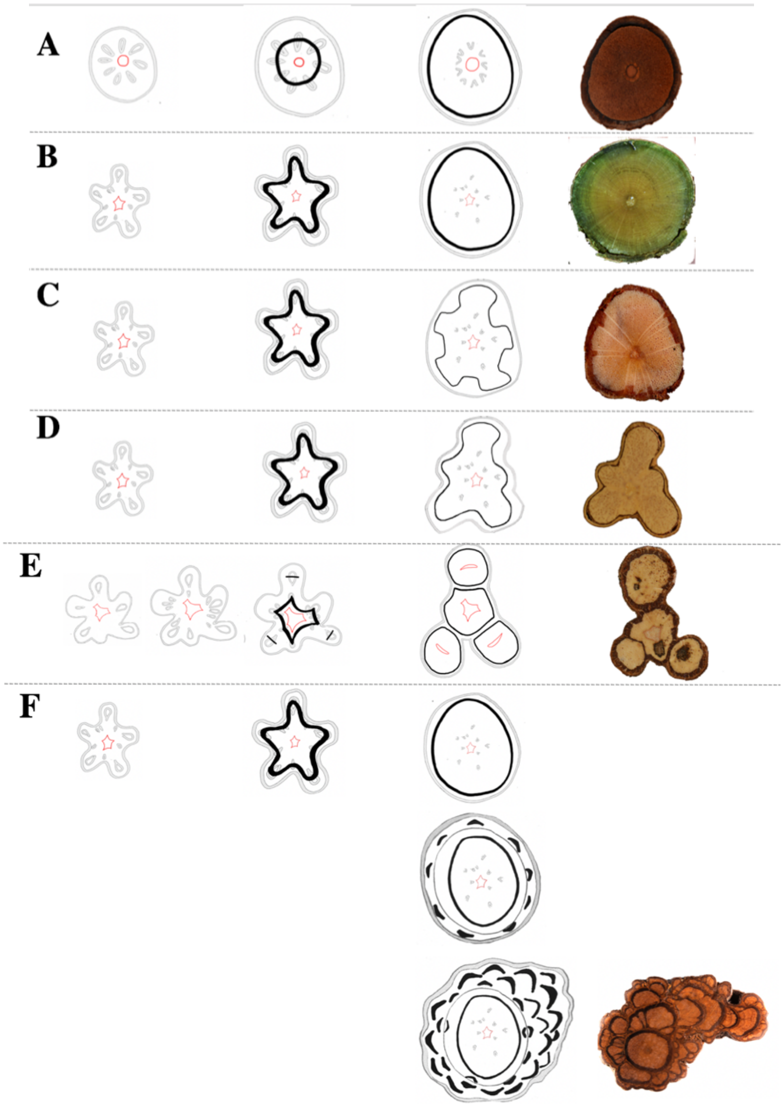

Figure 10. Six types of stem ontogenies observed in *Paullinia* illustrated from primary growth at the shoot apex to secondary growth in the mature stems. A–C: Ontogenies that arise from a single cambium. (A) Regular I (ontogeny 1) begins with a circular primary body and a continuous ring of vascular bundles that connect at the onset of secondary growth by the vascular cambium, which proceeds to produces equal amounts of cambial products (xylem and phloem) round the stem circumference (image of *P. capreolata* Acevedo 6065). (B) Regular II (ontogeny 2) begins with an angular primary body and an unequal distribution of vascular bundles that become connected by the vascular cambium, which through differential cambial activity of the interfascicular and fascicular cambia "rounds out" the stem then proceeds to produce equal amounts of secondary products (image of *P. bilobulata* Acevedo 17004). (C) Phloem wedges (ontogeny 3) begins with the same primary growth state as regular II, however at the onset of secondary growth the interfascicular cambium produces elevated amounts of phloem relative to the fascicular cambium, thus producing phloem wedges (image of *P. clavigera* Acevedo 16388); (D) Lobed xylem (ontogeny 4) begins with the same primary growth state as regular II, however at the onset of secondary growth the fascicular cambium produces elevated amounts of xylem relative to the interfascicular cambium (image of *P. caloptera* Acevedo 7535). E–F: Stem ontogenies that arise from multiple cambia. (E) Compound stems (ontogeny 5) begins with an angular primary body, that quickly adopts a and a polycyclic eustele; at the onset of secondary growth the central cylinder vascular cambium connects some but not all vascular bundles at the exclusion of three peripheral regions which become the sites of the peripheral cylinders (image of *P. alsmithii* Acevedo 7621). (F) Successive cambia (ontogeny 6) completes the regular II ontogeny, then after a period of considerable secondary growth, new cambia arcs arise externally and internally to the pericycle (image of *P. pseudota* modified with permission from Neto et al (2018).

I. Originating from a single vascular cambium

Regular I (stem ontogeny 1):

The primary body and pith are circular at the shoot apical meristem (Figure 10A). Vascular bundles are equally distributed around the stem circumference, each bundle accompanied by a pericyclic fiber cap (Figure 11A, 11B). The vascular cambium connects all bundles following the round outline, and the fascicular and interfascicular cambia produce equal amounts of cambial products Figure 11C). The final mature form is a round stem with a round pith (Figure 10A, 11C); this is the same as in self-sustaining trees and shrubs.

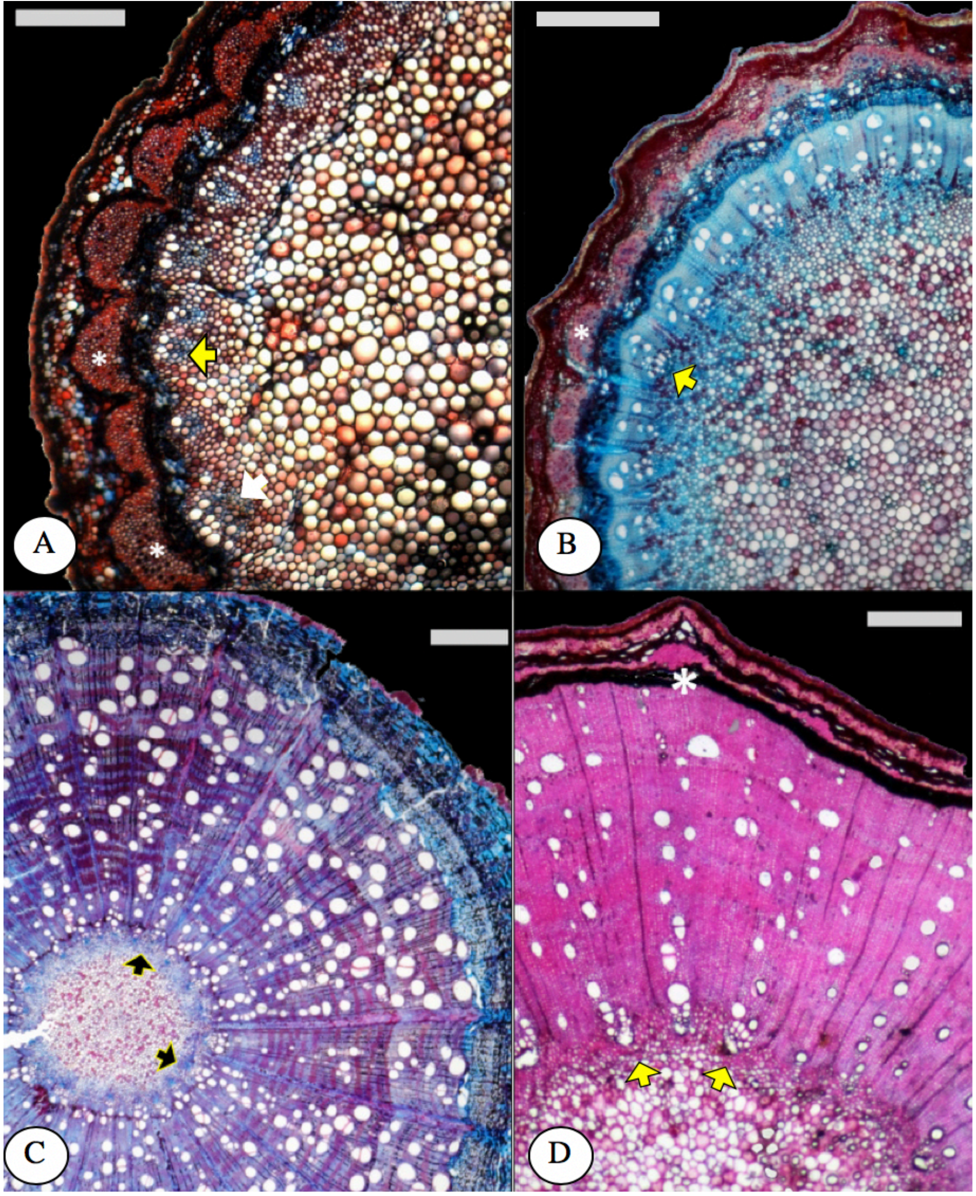

Figure 11. Regular I and regular II stem ontogenies in *Paullina*. A-C: Regular I stem ontogeny in *P. fibrigera* Chery 16 and Chery 21. (A) Circular stem outline in primary growth with a continuous ring of equally distributed vascular bundles, each associated with a pericyclic fiber cap. (B) The onset of secondary growth. (C) Mature form displaying a circular stem and circular pith. D: Regular II in *P. clathrata* Grandez 1722: secondary growth retaining a large pericyclic fiber cap that was once associated with a mass of vascular bundles in the lobes of the angular primary body. *=pericyclic fiber caps, arrow= vascular bundles. Scale bar= (A) 200µm, (B) 400µm, (C) 1mm, (D) 300µm.

Regular II (stem ontogeny 2):

The primary body and pith are angular at the shoot apex (Figure 10B). In the ridges of the angular primary body (the site of future fascicular cambia), masses of vascular bundles are present, each accompanied by a large cap of pericyclic fibers. In the furrows of the angular body (the site of the future interfascicular cambium), vascular bundles are nearly absent, and this region is accompanied by a thin layer of pericyclic fibers (Figure 11D). At the onset of secondary growth, the interfascicular cambium enters a brief period of elevated secondary xylem production, relative to the fascicular cambium. This differential secondary growth between the interfascicular and fascicular cambium progressively attenuates the furrows and “rounds out” the cambium and stem outline. Once the stem is round, the vascular cambium produces equal amounts of secondary products around the stem’s circumference. The final mature form is a round stem with an angular pith (Figure 10B).

Phloem wedges (stem ontogeny 3):

The primary body and pith are angular at the shoot apex (Figure 10C, 12A). At the ridges of the angular primary body (the site of the future fascicular cambium), masses of vascular bundles form, each accompanied by a large cap of pericyclic fibers (Figure 12B). In the furrows of the angular primary body (the site of the future interfascicular cambium), vascular bundles are nearly absent, and this region is accompanied by a thin layer of pericyclic fibers (Figure 12C). At the onset of secondary growth, the interfascicular cambium produces elevated levels of secondary phloem relative to normal activity of the fascicular cambium. This differential growth between the interfascicular and fascicular cambium progressively attenuates the furrows by filling these previously depressed regions with phloem wedges (Figure 12C). The mature form is a round to slightly lobed stem outline, angular pith, and phloem wedges (Figure 10C, 12C, 13B).

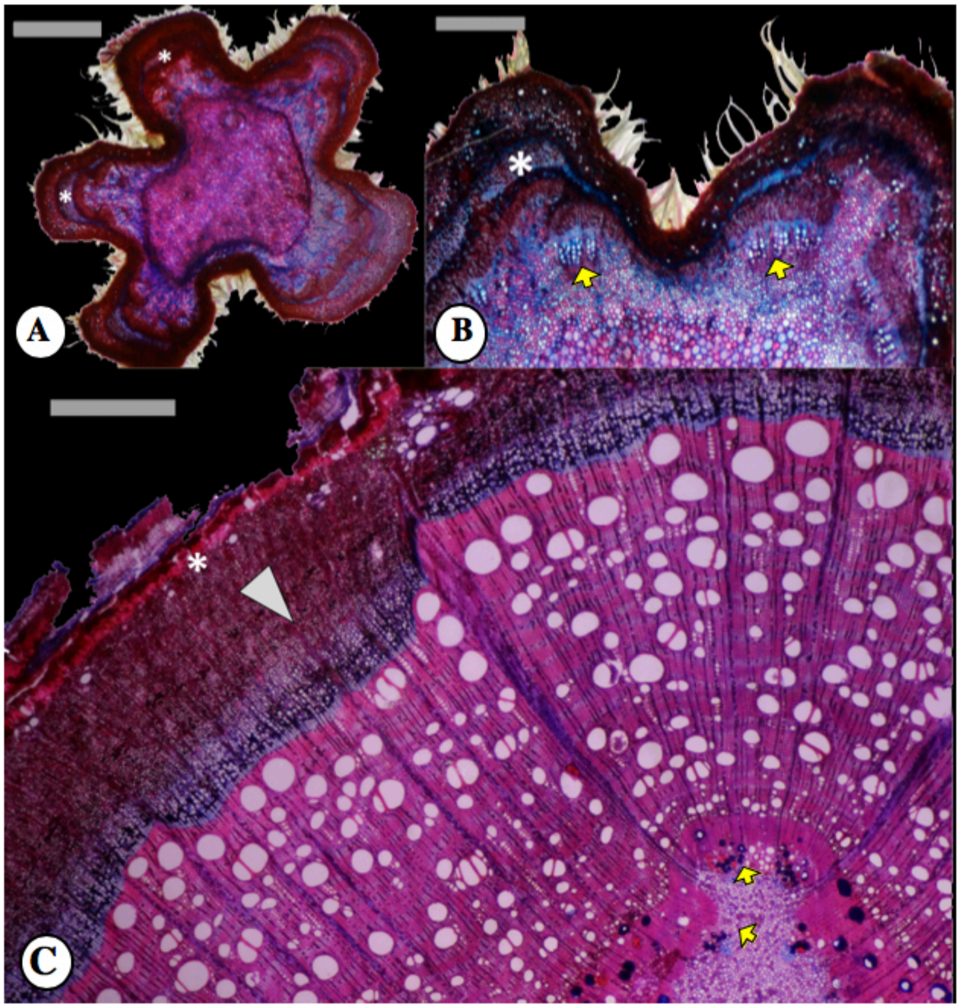

Figure 12. Phloem wedge ontogeny in *Paullinia rugosa* Chery 10. (A) Angular stem outline at the shoot apex in primary growth. (B) Close up of two adjacent lobes in primary growth with masses of vascular bundles clustered in the lobes (note the absence of bundles in the furrows). (C) Mature stem with a phloem wedge (note the appearance of a phloem wedge is parallel to the furrows of the pith). *=pericyclic fiber caps, arrow= vascular bundles, arrow head= phloem wedge. Scale bar= (A) 1mm, (B) 500µm, (C) 0.5cm.

Lobed xylem (stem ontogeny 4):

The primary body and pith are angular at the shoot apex (Figure 10D). At the ridges of the angular primary body (the site of the future fascicular cambium), masses of vascular bundles form, each accompanied by a large cap of pericyclic fibers. In the furrows of the angular primary body (the site of the future interfascicular cambium), vascular bundles are nearly absent, and this region is accompanied by a thin layer of pericyclic fibers (Figure 10D). At the onset of secondary growth, the interfascicular cambium produces elevated levels of secondary phloem and normal levels of xylem, while the fascicular cambium produces elevated secondary xylem and normal amounts of phloem. This differential secondary growth between the interfascicular and fascicular cambium generates mature stems that are deeply lobed, with phloem wedges in between the large xylem lobes and large rays at these junctures to accommodate growth, (Figure 10D, 13A) and an angular pith. Intermediates between the strict phloem wedge type described above and the lobed xylem variant are common.

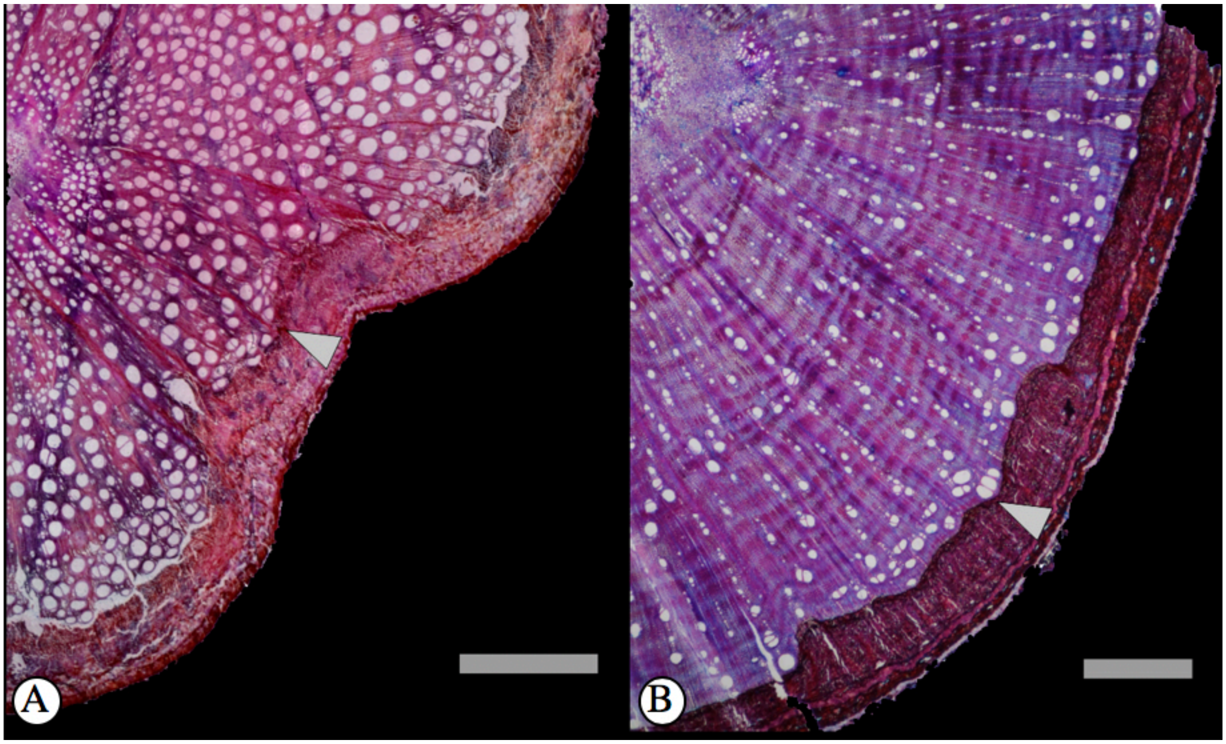

Figure 13. Comparison of lobed and phloem wedge variants in *Paullinia*. Phloem wedges in the lobed xylem cambial variant of *Paullinia obovata* Mathais 3585 (A) and in the strict phloem wedge type in *Paullinia dasystachya* Acevedo 14295 (B). Scale bar= (A) 2mm and (B)1mm.

I. Originating from a multiple vascular cambia

Compound stems (stem ontogeny 5):
The primary body and pith are angular at the shoot apex (Figure 10E, 14A, 14B). At the ridges of the angular primary body (the site of the future fascicular cambium), masses of vascular bundles form, each accompanied by a large cap of pericyclic fibers (Figure 14A, 14B). A second ring of vascular bundles is present internally (Figure 14B). At the onset of secondary growth, the first vascular cambium forms by connecting all bundles of the internal ring plus some bundles of the external rings; the trace of this central cylinder vascular cambium excludes three equidistant regions (Figure 10E). These excluded peripheral regions each develop their own cambium that produces secondary products normally (Figure 14C). The final mature form is a lobed stem outline with four cylinders, each producing secondary growth; the central cylinder containing an angular pith and the peripheral cylinders with very small arcs of ground meristem pith (Figure 14D).

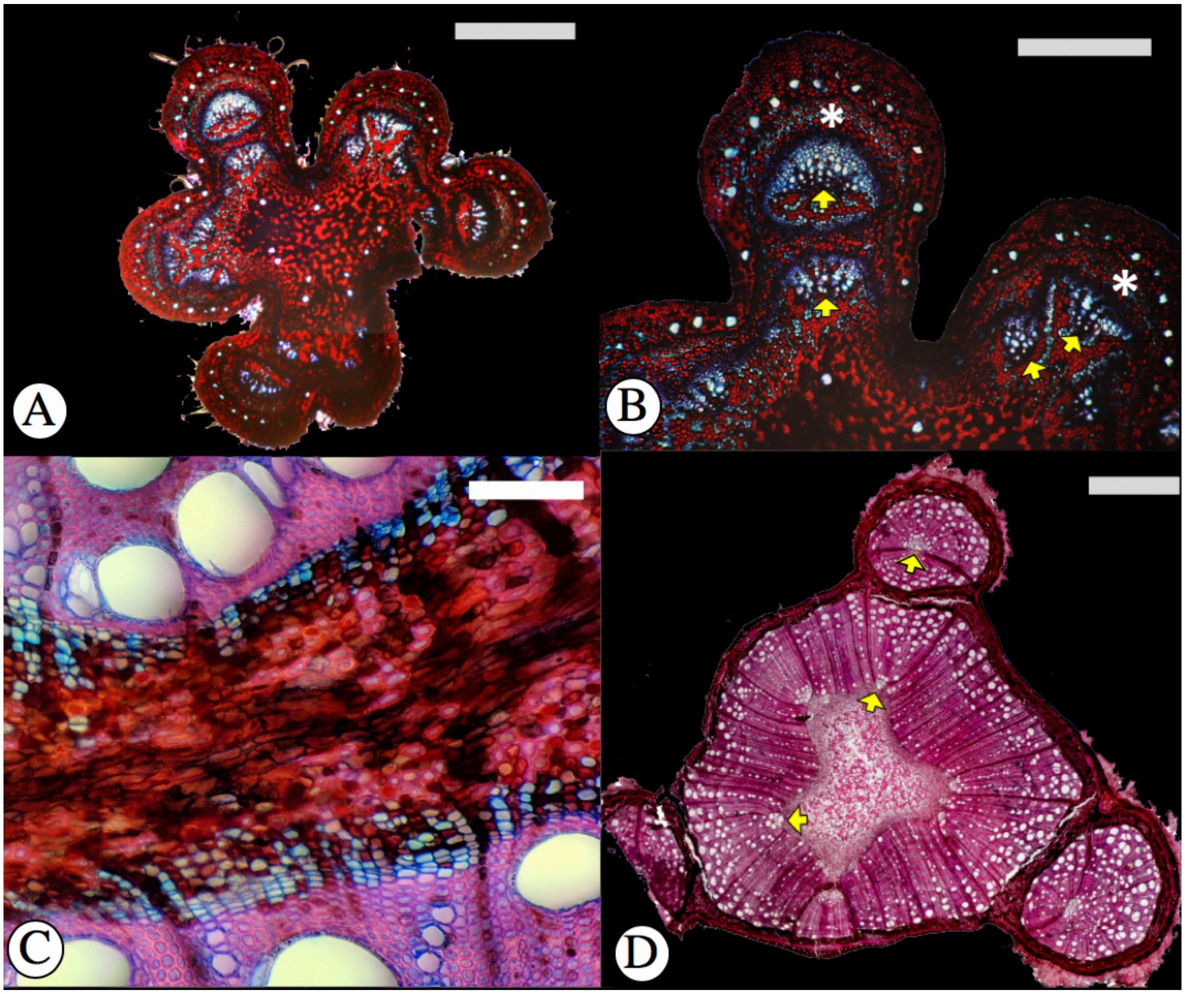

Figure 14. Compound stem ontogeny in *Paullinia*. (A) Angular stem outline at the shoot apex of *P. pinnata* Chery 30 in primary growth. (B) Close up of two adjacent primary body lobes, note the early isolation of a lobe that will form a peripheral cylinder later in development (left). (C) Juncture of the central cylinder and a peripheral cylinder (note their respective cambia–flattened cells stained blue) in *P. alata* Pace 594. (D) Mature compound stem in *P. alata* Brandbyge 32478. *= pericyclic fibers, Arrow= vascular bundles. Scale bar= (A) 500µm, (B) 600µm, (C) 0.25cm.

Successive cambia (summarized from Neto et al., 2018; stem ontogeny 6)

The primary body and pith are angular at the shoot apical meristem (Figure 10F, 15A). At the ridges of the angular primary body (the site of the future fascicular cambium), masses of vascular bundles form, each accompanied by a large cap of pericyclic fibers. In the furrows of the angular body (the site of the future interfascicular cambium), vascular bundles are nearly absent, and this region is accompanied by a thin layer of pericyclic fibers (Figure 15B). At the onset of secondary growth, the interfascicular cambium enters a brief period of elevated secondary xylem production, relative to the fascicular cambium. This differential secondary growth between the interfascicular and fascicular cambium progressively attenuates the furrows and "rounds out" the cambium and stem outline. Once the stem is round, the vascular cambium produces equal amounts of secondary products around the stem's circumference. After a substantial period of regular secondary growth, new arcs of cambia form from the parenchymatous pericycle in both the direction of the cortex and the pith (Figure 15C, 6D) (Neto et al. 2018). The final mature form is a stem with multiple cambia and an angular pith (Figure 10F).

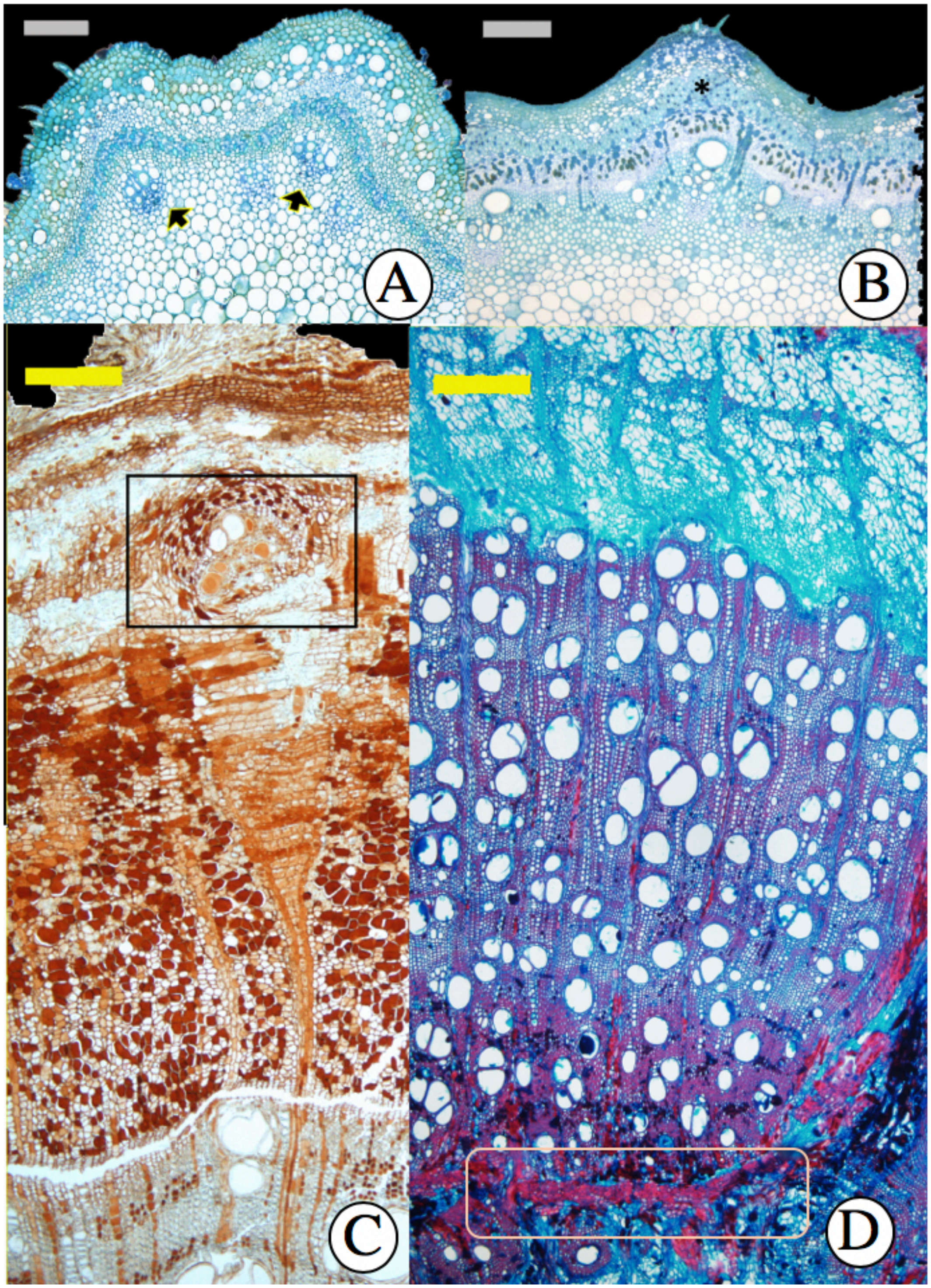

Figure 15. Successive cambia stem ontogeny in *Paullinia* (A) Angular stem outline in primary growth in *P. weinmannifolia*, (B) onset of secondary growth in *P. trigonia*; (C) formation of a cambial arc from the outermost pericycle cells in *P. micrantha,* rectangle around cambial arc; (D) formation of a cambial arcs both externally and internally to the pericycle in in *P. weinmanifolia,* rectangle around pericycle. Scale bar= (A) 30μm, (B)100μm, (C) 200μm, (D) 500μm. All images modified with permission from Neto et al., (2018).

3.4.2 Phylogenetic Comparative Methods

The ancestral state of stem ontogeny in *Paullinia* is reconstructed as regular II (angular primary body and regular mature stems; Figure 16). The average state changes in primary body shape across the 100 simulated character histories is 4.32; an average of 0.23 transitions from circular to angular and an average of 4.09 shifts from angular to circular primary body shape, which is to be interpreted as reversals to the ancestral state at the base of *Allophylus*, *Thouinia* + Paullinieae (see below; Figure 17).

The average state changes in mature stem type across 100 simulated character histories is 19.03 (the remaining shifts are the average across 100 simulated histories); 5.09 transitions to phloem wedges, 5.43 transitions to lobed xylem, 5.7 transitions to compound stems, 1.11 transition to successive cambia, and 1.73 reversals back to regular I, which is ancestral in Paullinieae (see below; Figure 17). With the exception of successive cambia, which is the most developmentally complex ontogeny, all other cambia variants have evolved multiple times across the genus.

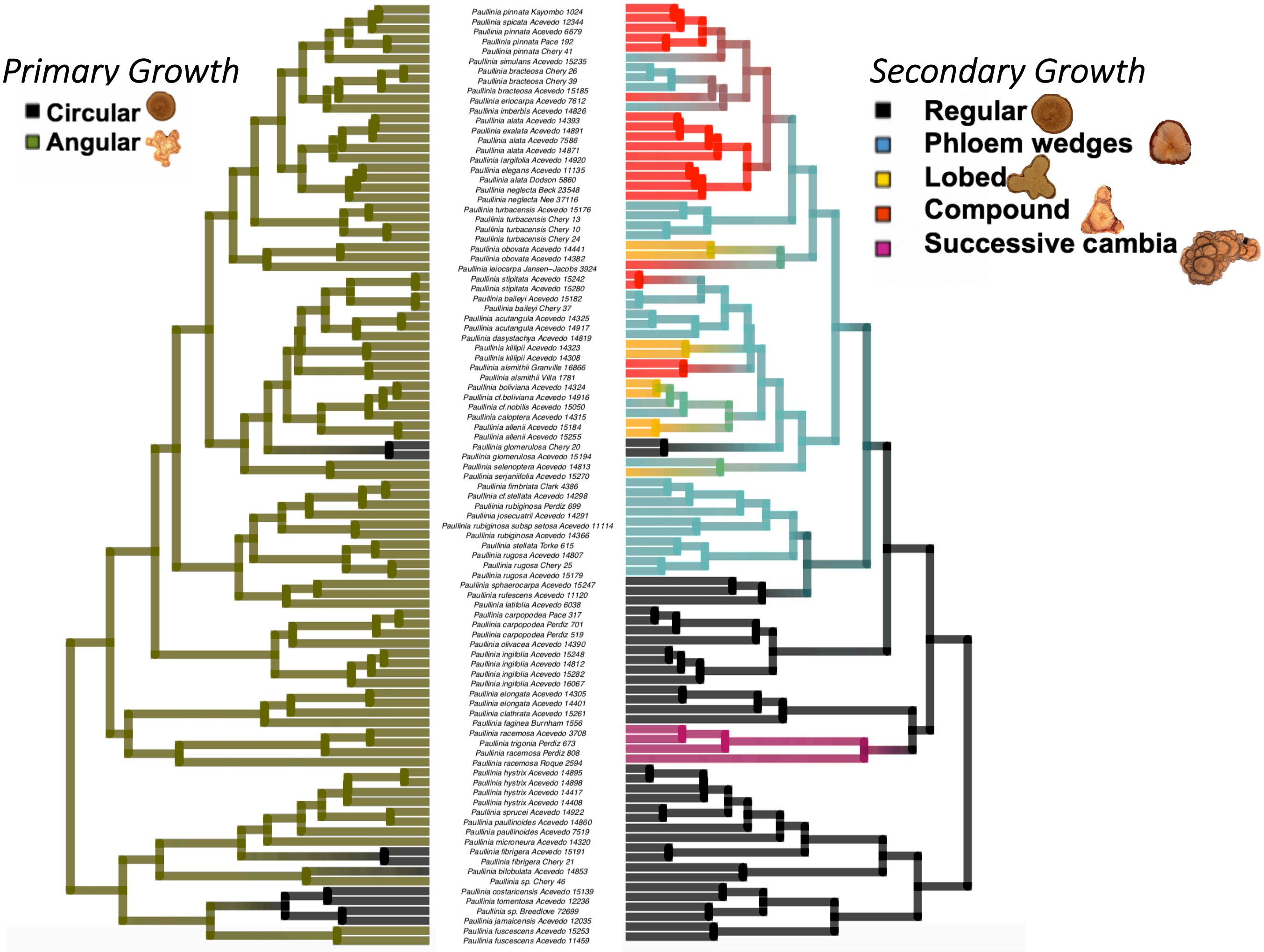

Figure 16. Ancestral state reconstructions of primary body shape and mature stem type in *Paullinia* (summary of 100 simulated character histories on the MCC tree).

All cambial variants in *Serjania*, *Urvillea,* and *Thinouia* also begin development with an angular primary body. The regular II ontogeny present in *Paullinia* was observed in *Cardiospermum*, while the regular I was observed in *Lophostigma* and within the self-sustaining (trees and shrubs) outgroup lineages, *Allophyllus* and *Thouinia*. The ancestral state of Paullinieae + the immediate outgroup genera of trees and shrubs (*Allophylus*, *Thouinia*, *Bridgesia* and *Athyana*), is reconstructed as having the regular I ontogeny typical of trees and shrubs (Figure 17). The ancestral state of the Paullinieae tribe was reconstructed as having a cambial variant and angular primary growth (Figure 17). The average state changes in primary body across 100 simulated character histories is 6.53: 1.54 transitions to angular and 4.99 reversals back to circular. An average of 9.38 state changes in mature stem type are observed corresponding to 4.69 transitions from regular to variant stems and 4.69 reversals from variant to regular stems.

The Pagel's (1994) test for correlated evolution found significant support (p= .038) in favor of the dependent model, which indicates that the evolution of mature stem types is contingent on the evolution of primary body shape. Specifically, the evolution of cambial variants depends on the evolution of an angular primary body.

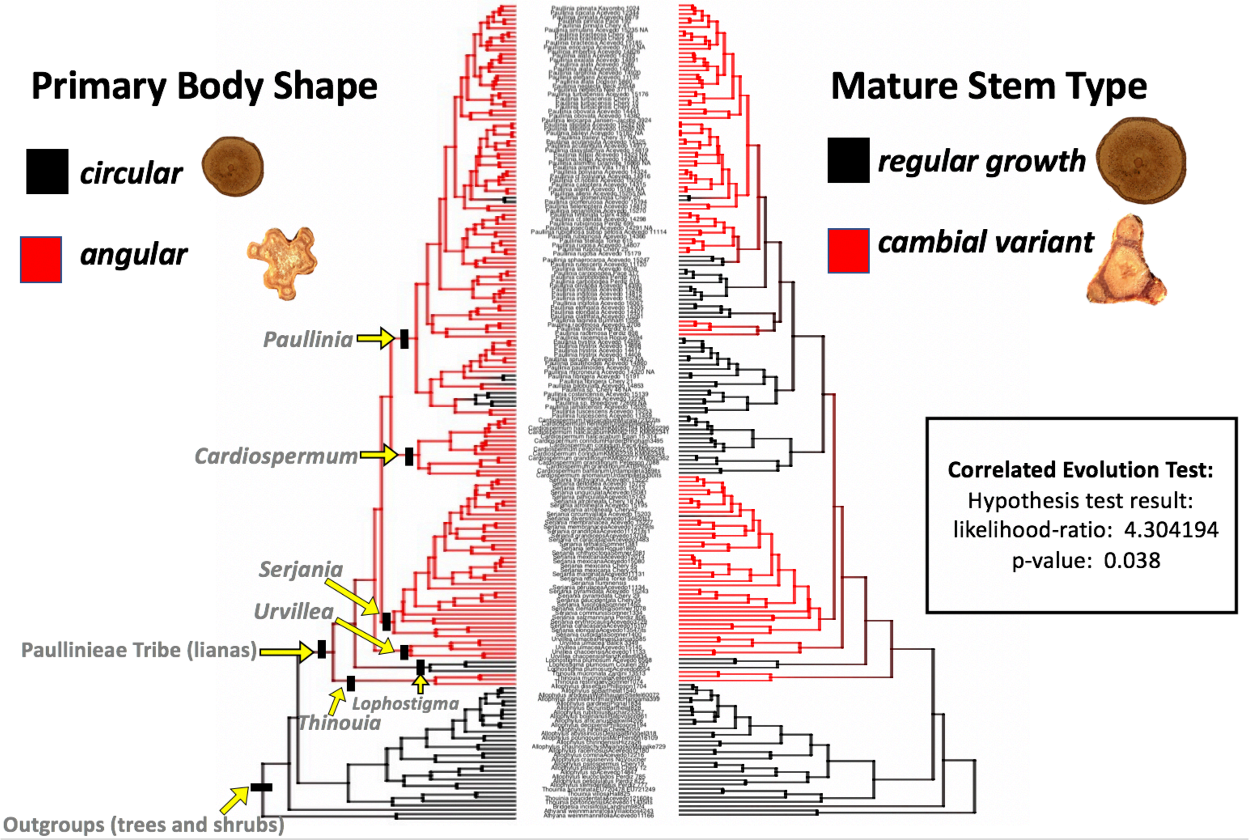

Figure 17. Ancestral state reconstructions of primary body shape and mature stem type across the Paullinieae tribe (summary of 100 simulated character histories on the MCC tree) and the results from the correlated evolution test, which favors the dependent model of evolution.

3.5 Discussion

3.5.1 Linking stem development with evolution

The six ontogenetic trajectories that I describe here (regular I, regular II, phloem wedges, lobed xylem, compound stems, or successive cambia) highlights a developmental and evolutionary connection between the structure of the plant body during primary growth and the expression of vascular cambial variants during secondary growth. The primary plant body of most *Paullinia* species is angular and this morphology is reconstructed as the ancestral state for the genus (Figure 16 & 17). This irregular shape is already present at the shoot apex and is reinforced by the unequal distribution of vascular bundles laid down by the procambium across the stem's circumference in primary growth. Although unusual among woody plants, which typically have a continuous circular ring of bundles (Evert 2006), this angular primary body shape is the basic bauplan of primary growth in *Paullinia*, from which five of the six mature stem types directly develop: regular II, phloem wedges, lobed xylem, compound, and successive cambia. These findings identify a direct link between variant primary body shape as the developmental precursor to all variant mature stem conformations.

In exploring the relationship between primary and secondary growth in *Paullinia* in a phylogenetic context, the stochastic character mapping analyses reveals that all cambial variants evolved from within clades with an angular primary body shape as the ancestral state. Additionally, these analyses reveal that each type of cambial variant, except the successive cambia type, has evolved multiple times, which suggests that these forms are more evolutionarily labile than I initially expected given the complexity of their ontogenies. Scaling these analyses up to a larger evolutionary context across the Paullinieae tribe, the statistically significant correlated evolution between primary plant body shape and mature stem type reveals that the evolution of cambial variants across the Paullinieae is contingent on the evolution of an angular primary body. Taken together, these results identify a critical relationship across primary and secondary growth both in the development of cambial variants in an individual plant, and the emergence of these novel forms through evolutionary time.

In the evolution of the Paullinieae tribe, the angular primary body has been leveraged to construct the diversity of cambial variants. However, given the fact that angular primary bodies are observed in self-sustaining trees and shrubs across Sapindaceae (e.g., *Cupania*, *Arytera*, *Dimocarpus*), so the co-opting of this existing trait in the evolution of the lianas represents an exaptation for climbing (Gould and Vrba, 1982). In light of the role of exaptation in the evolution of cambial variants, this result generates new research questions surrounding the anatomical evolutionary precursors that need to be present in a lineage for it to evolve lianas. These questions can be addressed by combining the homology and the convergence approaches discussed by Coddington (1994), which first places trait data within the phylogenetic context of an individual lineage (homology approach), then compares the inferred precursor anatomies across distantly related groups (the convergence approach). Such a study can be done using lineages with a diversity of woody plant habits (trees, shrubs and lianas) such as Sapindaceae, Fabaceae, and Rubiaceae– accessed 3 May 2019).

3.5.2 Function of variant stems throughout development

In Paullinieae, the variant forms in both primary (angular stems) and secondary growth (cambial variants) may indicate a benefit of these morphologies at their respective developmental stages.

For example, in primary growth, the irregular conformation may work in a manner analogous to a small cable, allowing the searcher shoots to twist while searching for a host tree to climb.

At a later stage in development, the presence of cambial variants in the mature stems add elaborations onto the liana syndrome anatomy to promote conductance, strength and flexibility (de Candolle 1824; Carlquist 1991; Rowe, Nick and Speck 2004; Angyalossy et al. 2012, 2015). In Paullinieae, each of the cambial variants, with the exception of the compound stems, has evolved multiple times in distantly related liana lineages (Schenk, 1893; Obaton, 1960, Caballé, 1993; Carlquist, 1985, 1991; Angyalossy et al., 2012, 2015; Neotropical Lianas Project–accessed 26 April 2019)) suggesting that the evolution of cambial variants is a common solution for lianas to efficiently access light in a competitive landscape. The lobed and compound stems function as a cable structure aiding the stem in twisting through the canopy, similar to what I posit for the angular primary body. Additionally, compound stems have been shown to allow more orderly vertical splitting of stem segments and faster vascular healing at wound sites (Fisher and Ewers 1989, 1991). The successive cambia variant has been proposed as a means of redundancy of essential tissues for regeneration and damage repair, and to protect the secondary xylem (Carlquist et al., 2007; Carlquist, 2013). In the phloem wedge cambial variant, perhaps the Paullinieae converge on other lianas with a regular and variant phloem in the division of labor with storage provided by the normal phloem and conductance by the variant phloem (Pace et al. 2011; Carlquist 2013).

3.5.3 Evolution of stem ontogenies by modularity and heterochrony

By studying the extreme ontogenies of lianas, we gain new insights into fundamental processes involved in stem development. In *Paullinia*, at least two mechanisms emerge to explain the evolution of stem ontogenies: modularity and heterochrony. In *Paullinia*, the differential activity of distinct spatially and tissue-specific regions demonstrates that different regions of the stem are in part operating independently from each other, but collectively evolving as an integrated and complex modular assemblage of developmental processes to achieve consistent gross morphologies (Wagner, 1996; Tomescu and Groover, 2018). The modularity of stem development has been noted at both the tissue developmental level (Pace, 2009) and the gene regulatory levels (Etchells et al. 2013; Tomescu and Groover, 2018). By slightly tweaking the activity of each unit (e.g., the fascicular or the interfascicular cambia), of the module (the overall stem) the developmental and evolutionary transitions between the stem ontogenies observed in *Paullinia* become linked. For example, the lobed xylem ontogeny can be contrasted with the phloem wedge variant; in the ontogeny of lobed xylem, the fascicular cambium indefinitely produces elevated amounts of xylem, while in the phloem wedge variant, the interfascicular cambium indefinitely produces elevated amounts of phloem. In these two ontogenies, a variant mature stem conformation could not be achieved without the coordination of differential cambial activity across the circumference of the stem.

In addition to modularity, the evolution of stem ontogenies in Paullinieae can also be explained by heterochrony–the developmental change in timing or rate of events (Buendía-Monreal and Gillmor 2018). In the successive cambia ontogeny observed in *Paullinia*, the stem first completes the entire ontogeny of regular II, then after considerable secondary growth, new cambial arcs arise from the pericycle (Neto et al., 2018). Interestingly, the successive cambia evolved from a regular II ancestor (Figure 17); meaning that the evolution of successive cambia in *Paullinia* arose by terminal developmental additions to the ancestral state. Pace (2009) found this pattern– that ontogeny recapitulates phylogeny– in the step-like evolution of phloem wedges in Bignonieaceae, supporting the views of Carlquist (2009) and Olson and Rosell (2006) that heterochrony is a key element in understanding evolution of woody growth habits.

3.6 Conclusion

The integration of phylogenetic comparative methods with classical stem anatomy techniques allowed me to elucidate that the development and the evolution of cambial variants are both contingent on variant primary growth in *Paullinia* and across the Paullinieae tribe. In *Paullinia*, the ancestral stem ontogeny is reconstructed as regular II (angular primary body and regular mature stems), and each cambial variant has evolved multiple times across the phylogeny, except the single origin of successive cambia type which is the most complex ontogeny. Across the Paullinieae, the presence of a cambial variant is ancestral, from which an average of 6.53 transitions are estimated across the six genera of lianas. The presence of angular primary bodies in self-sustaining trees and shrubs in Sapindaceae suggest that in the evolution of the Paullinieae lianas, this variant trait was co-opted for novel functions relating to the climbing habit (exaptation). Given the prevalence of variant morphologies in both primary and secondary growth of the lianas, may reflect that selection is acting on two developmental levels: the angular primary body aiding searcher shoots to find and wrap around host trees and the cambial variants of adults adding elaborations to the liana syndrome anatomy to aid in conductance, flexibility and/or strength.

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Appendix A: Supplemental materials from Chapter One

This document outlines the bioinformatic pipeline to isolate single copy nuclear introns of a desired size by use of a genome and two sets of transcriptome reads as described in Chery et al. (2017), followed by the primer design and primer testing protocols. All scripts are included in the corresponding directory on https://github.com/joycechery/Sapindaceae or the link is provided in the text. In addition, command syntax for external software, a bash command file, or a description of a process is given.

1. Genome Data Preparation

The following commands and scripts select mRNAs from the genome CDS file that contain introns of a desired size.

- a. Download CDS fasta files and genome annotation files
  from www.citrusgenomedb.org

- b. Select mrna names from the genome annotation file:
  ```
  grep 'mRNA' Csinensis_v1.0_gene.gff3 | awk '{print $9}' | awk
  'BEGIN{FS= ";"}{print $4}' | sed 's/Parent=//'| sed 's/m.g/m/' >
  mrnaNames
  ```

- c. Extract intron sizes by use of the genome annotation file:
  ```
  perl get_intron_size.pl Csinensis_v1.0_gene.gff3 > intronsize
  ```
  --`get_intron_size.pl` (https://gist.github.com/baihezimu/4163862)

- d. Associate mrna names with their intron sizes:
  ```
  paste mrnaNames intronsize > mrnaIntronSize
  ```

- e. Select single isoform genes from the “mrnaIntronSize”:
  ```
  sort mrnaNames | uniq -c | sort | awk '$1<2' | awk '{print $2}' >
  singlemrnaNames

  grep -f singlemrnaNames mrnaIntronSize > singlemRNAintrons
  ```

- f. Count the number of columns (i.e. each represent an intron size):
  ```
  awk '{print NF}' singlemRNAintrons | sort -nu | tail -n 1
  ```

- g. Search each column for introns between 500-1100bps in length:
  ```
  awk '$4>500&&$4<1000' singlemRNAintrons >>
  singlemRNAintrons_small

  awk '$5>500&&$5<1100' singlemRNAintrons >>
  singlemRNAintrons_small
  ```
  ...for as many columns as necessary

Appendix A Continued.

- h. Sort through the list of mrnas with introns of 500-1100bps to generate a list of sequence names to align transcriptome reads to in the next steps:

  ```
  sort singlemRNAintrons_small | uniq | awk '{print $1}' >
  singlemRNAintrons_small_uniq
  ```

- i. Convert the genome CDS fasta file from interleaved to sequential to be in the right format to run scripts in next steps:

  ```
  perl -MBio::SeqIO -e 'my $seqin = Bio::SeqIO->new(-fh => \*STDIN,
  -format => 'fasta'); while (my $seq = $seqin->next_seq) { print
  ">",$seq->id,"\n",$seq->seq,"\n"; }' < Csinensis_v1.0_cds.fa >
  Csinensis_v1.0_cds_seqs.fa
  ```

- j. Alter mrna names for ease of processing downstream:

  ```
  awk '{FS ="|"}{print $1}' Csinensis_v1.0_cds_seqs.fa >
  Csinensis_v1.0_cds_orangeNames.fa
  ```

- k. Select sequences from the list of single isoform mrnas with introns between 500-1100bps:

  `--selectSeqs.pl`
  (http://raven.iab.alaska.edu/~ntakebay/teaching/programming/perl-scripts/perl-scripts.html)

  ```
  perl selectSeqs.pl -in Csinensis_v1.0_cds_orangeNames.fa -out
  SelectedCitrusSeqs.fa -idfile singlemRNAintrons_small_uniq

  mv InSelectedCitrusSeqs.fa SelectedCitrusSeqs.fa
  ```

2. Transcriptome Read Processing

The following commands and scripts clean the transcriptome reads.

- a. Download the paired end reads of the phylogenetically closest transcriptome to the Paullinieae, Dimocarpus longan from http://www.ncbi.nlm.nih.gov/ and split using the NCBI SRA Toolkit v.2.4.5-2.

- b. Fix read names to run 2-scrubReads.pl in the next step:

  ```
  sed '/^@SRR412534/ s/SRR412534.[0-9]* //' SRR412534_1.fastq |
  sed'/^@A807H/ s/length=90/\/1/g' | sed '/^+SRR412534/ s/.*/\+/' >
  JCSR_R1.fq

  sed '/^@SRR412534/ s/SRR412534.[0-9]* //' SRR412534_2.fastq
  |sed'/^@A807H/ s/length=90/\/2/g' | sed '/^+SRR412534/ s/.*/\+/'
  > JCSR_R2.fq
  ```

Appendix A Continued.

- c. Clean all reads to remove adapters, low-complexity sequences, contamination, and PCR duplicates using protocol in (Singhal, 2013). This script removes PCR duplicates by removing all reads with identical sequences. Required files: (download NC_012947.1 Escherichia coli from GenBank as a negative filter and use adapters.fa, library.txt found: (https://github.com/joycechery/Sapindaceae/tree/master/TranscriptomeProcessing)

  --`scrubReads.sh`
  (https://github.com/joycechery/Sapindaceae/blob/master/TranscriptomeProcessing/scrubReads.sh

  --`2-scrubReads.pl`
  (https://github.com/MVZSEQ/SCPP/blob/master/2-scrubReads.pl)

3. Align transcriptome Reads to Genome

This script is used to align cleaned transctiptome reads to the selected *Citrus sinensis* mrnas (single isoform mrnas with introns of 500-1100bps) using NovoAlign v3.01 (http://novocraft.com) with -t 480 to allow highly divergent sequences to map. PCR duplicates are additionally removed by identical mapping location using MarkDuplicates in Picard. SNPs are called using SAMtools v0.1.19 and new consensus are generated based on SNPs called. A second iteration aligned reads to the first alignment consensus sequences using NovoAlign –t 90. The rate of multiple mapping is 0.5% in the first alignment and 0.2% in the second alignment with these data. SNPs are called again to generate a set of final consensus sequences- these serve as pseudoreferences from which primers are designed.

--`AlignFix.sh`
(https://github.com/joycechery/Sapindaceae/blob/master/AlignReadstoCDS/AlignFix.sh)

--`AlignFix.pl`
(https://github.com/joycechery/Sapindaceae/blob/master/AlignReadstoCDS/AlignFix.pl)

4. Filter Pseudoreference Sequences

The following commands and scripts filters the pseudoreferences to 1) retain only sequences that BLAST to self, 2) have at least 20x average gene coverage and 3) removes seqs with hits to Repeat Masker.

- a. Retain only sequences that exclusively BLAST to self:
  --`BlastToRemove_Paralogs`

(https://github.com/joycechery/Sapindaceae/blob/master/FilterSequences/BlastToRemove_Paralogs)

Appendix A Continued.

- b. Remove sequences that have BLAST hits to ribosomal, plastid, chloroplast, mitochondrial or transposons:

  --`BlastToRemove_nonnuclear`

  (https://github.com/joycechery/Sapindaceae/blob/master/FilterSequences/BlastToRemove_nonnuclear)

- c. Removed genes with less than 20x average gene coverage.

  i. Create a list of pseudoreferences names:

```
grep '>' LocalBlastRemoveNCBIRemove.fa | sed 's/>//' >
LocalBlastRemoveNCBIRemove_Names
```

  ii. Create a coverage per position file from the .bam file produced by "Alignfix.pl":

```
module load bedtools/2.22.1

bedtools genomecov -ibam JCSR.new2.bam -d -split >
JCSR.positioncoverage
```

  iii. Create a coverage per position file for filtered pseudoreference sequences:

```
grep -f LocalBlastRemoveNCBIRemove_Names JCSR.positioncoverage >
           LocalBlastRemoveNCBIRemove_PositionCoverage
```

  iv. Create an average gene coverage file:

  --`AverageGeneCoverage.pl`

  (https://github.com/joycechery/Sapindaceae/blob/master/FilterSequences/AverageGeneCoverage.pl)

  v. Select genes with more than 20x average gene coverage:

```
awk '$2> 20' CoverageFile_PerGene > CoverageFile_MoreThan20x

awk '{print $1}' CoverageFile_MoreThan20x > 20Xgenes

perl selectSeqs.pl -in LocalBlastRemoveNCBIRemove.fa –out
LocalBlastRemoveNCBIRemove20XCov.fa -idfile 20Xgenes

mv InLocalBlastRemoveNCBIRemove20XCov.fa
LocalBlastRemoveNCBIRemove20XCov.fa
```

- c. Run all sequences through RepeatMasker using default settings except Search engine: rmblast: http://www.repeatmasker.org/cgi-bin/WEBRepeatMasker. Compile a list of gene names with hits "RepeatMaskerGenestoRemove" and remove them:

```
perl selectSeqs.pl -in LocalBlastRemoveNCBIRemove20XCov.fa -out
LocalBlastRemoveNCBIRemove20XCovRM.fa -idfile
RepeatMaskerGenestoRemove
```

Appendix A Continued.

```
mv NotLocalBlastRemoveNCBIRemove20XCovRM.fa
LocalBlastRemoveNCBIRemove20XCovRM.fa
```

5. Primer Design

To verify that primer design regions are conserved across the desired phylogenetic breadth, Litchi chinensis transcriptome reads are mapped to the filtered pseudoreferences using the Map to Reference tool in Geneious v8.0.4 low sensitivity and 2 iterations. This second alignment is optional and if users choose to do so, it is recommended to clean the transcriptome reads prior to mapping. Primers are designed using Primer3 v.0.4.0 (http://bioinfo.ut.ee/primer3-0.4.0/) at conserved CDS-CDS boundaries to target introns. The input sequence consist of at least 30 nucleotides from the first CDS followed by 100 'X''s (representing the unknown intron sequence), followed by at least 30 nucleotides of the second CDS. The X's are excluded by use of the 'Excluded Region' option.

To design primers at the CDS-CDS boundaries, intron sizes from "singlemRNAintrons_small" were used and CDS sizes within each mrna are extracted:

--`parse_gff_to_bed.pl`
(https://github.com/chodon/zingiberales/blob/master/2MusaCDSprocess/parse_gff_to_bed.pl)

6. Primer Testing

- a. Extract DNA from silica material using CTAB (Doyle and Doyle, 1987).
- b. Conduct PCR temperature gradients (62-43°C) with a representative species to determine the optimal annealing temperature for each primer pair. PCR conditions: Phire hot start II DNA polymerase (Thermo Fisher Scientific, Pittsburgh, Pennsylvania); 5 minute initial denaturing step at 98°C, loci specific cycles of 5 seconds at 98°C, 5 seconds at loci-specific annealing temperature, and 20 seconds at 72°C and a final 1 minute 72 extension.
- c. Apply optimal PCR conditions to all samples.
- d. Only Sequence PCR products that yield a single band across all samples. Sequencing was done on a Biosystems 3730x DNA analyzer.
- e. Assemble, edit and align Sanger reads using MAFFT v7.271 in Geneious v8.0.4.
- f. Calculate pairwise identity in Geneious v8.0.4 and calculate parsimony informative sites in PAUP* 4.0b10.

Appendix A Continued.

7. Phylogenetic Analysis

- a. A concatenated alignment of all loci was used to generate phylogenetic hypotheses under maximum likelihood GTR sequence evolution model in RAxML-HPC on XSEDE 8.2.8 (https://www.phylo.org/portal2/home.action). Support was evaluated with 100 bootstrap replicates.

- b. An ASTRAL- II analysis was run using statistically binned gene trees generated in RAxML using the GTRGAMMA model and 1000 bootstraps.
  `--ASTRAL-II-Commands`
  (https://github.com/joycechery/Sapindaceae/blob/master/ASTRAL-II/ASTRAL-II-Commands

Appendix B: Supplemental Tables from Chapter One

Table 3. Silica Dried Specimens Used in this study. Collections P3-P13 represent personal collection numbers by Chery.

Sample ID	Species name	Collection No. [a]	Locality	Latitude	Longitude
P0	*Paullinia sp.*	UCBG No. 92.0509	Provenance: Chiapas State, Mexico, North America	-	-
P1	*Paullinia bracteosa*_A	NTBG No. 760259	Provenance: Venezuela	-	-
P3	*Serjania mexicana*	Chery 23	Barro Colorado Island, Panama	09°09.914 'N	079°50.213 'W
P5	*Serjania paucidentata*	Chery 34	Barro Colorado Island, Panama	09°09.905 'N	079°50.202 'W
P6	*Serjania pyramidata*	Chery 29	Barro Colorado Island, Panama	09°10.867 N	079°49.444 'W
P7	*Paullinia glomerulosa*	Chery 20	Barro Colorado Island, Panama	09°09.896 'N	079°50.294 'W
P8	*Paullinia turbacensis*_A	Chery 13	Barro Colorado Island, Panama	09°09.924 'N	079°50.188 'W
P9	*Paullinia turbacensis*_B	Chery 24	Barro Colorado Island, Panama	09°09.830 'N	079°50.270 'W
P10	*Paullinia turbacensis*_C	Chery 10	Barro Colorado Island, Panama; Donata Trail	-	-
P11	*Serjania atrolineata*	Chery 42	Barro Colorado Island, Panama	09°10.705 N	079°50.819 'W
P12	*Paullinia bracteosa*_B	Chery 26	Barro Colorado Island, Panama; Lake # 4 Trail Marking	-	-
P13	*Allophylus psilospermus*	Chery 19	Barro Colorado Island, Panama	09°09.910 'N	079°50.287 'W

Note: Sample ID P1-P13 are deposited in the University of Panama Herbarium.

Appendix B: Supplemental Table from Chapter One

Table 4. Report of amplification success for each marker (i.e. orange) for each sample (i.e. P#) [a]

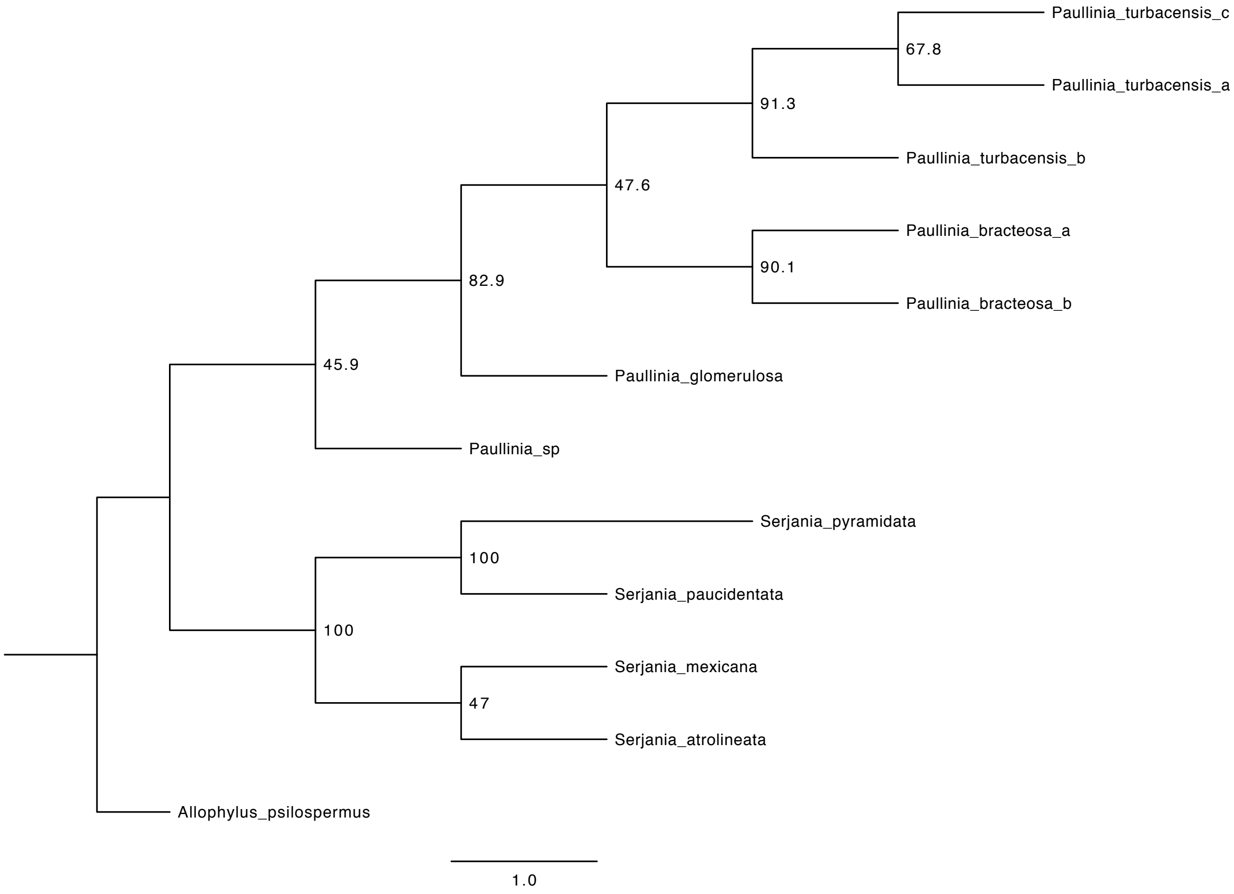
	P0	P1	P3	P5	P6	P7	P8	P9	P10	P11	P12	P13
orange1.1g002083m (intron2)	+	+	+	-	-	+	+	+	+	+	+	+
orange1.1g047192m (intron9)	+	+	+	+	+	+	+	+	+	+	+	+
orange1.1g045023m (intron7)	+	+	-	+	+	+	+	+	+	-	-	-
orange1.1g045023m (intron6)	+	+	-	+	+	-	-	-	-	+	-	+
orange1.1g028997m (intron4)	+	+	+	-	+	-	+	+	+	+	+	+
orange1.1g015495m (intron8)	+	+	+	+	+	+	+	+	+	+	+	+
orange1.1g027952m (intron5)	+	+	+	+	+	+	+	+	+	+	+	+
orange1.1g022777m (intron3)	+	-	+	+	+	+	+	+	+	+	+	+
orange1.1g009973m (intron5)	+	+	+	+	+	-	-	-	-	-	+	+

Notes: +=successful amplification & sequencing; -=no amplification or unsuccessful sequencing

[a] Species code, names and collection information listed in Appendix .

Appendix C: Supplemental Figure from Chapter One

Figure 3. Astral-II tree generated from binned genes and a partitioned RAxML analysis of 9 newly developed putative single-copy nuclear markers.



Appendix D: Supplemental Tables from Chapter Two

Table 6. Locus characteristics, PCR amplification and sanger sequence primer validation results, and MiSeq sequencing success results.

Locus[a]	**Region**	**Primer Validation/ Sanger Sequenced Successfully**	**Illumina MiSeq Reads Attained**	**Locus Included in Final Alignment***	**%Sequence Identity**	**Alignment Length**	**#OTUs**
orange1.1g002083m (intron9)	Nuclear Intron	+	+	+	7.70%	662	107
orange1.1g015495m (intron8)	Nuclear Intron	+	+	+	40.50%	237	15
orange1.1g027952m (intron5)	Nuclear Intron	+	+	+	19.20%	605	55
orange1.1g022777m (intron3)	Nuclear Intron	+	+	+	39.60%	847	36
orange1.1g016982m (intron11)	Nuclear Intron	+	+	+	43.80%	176	130
orange1.1g009973m (intron5)	Nuclear Intron	+	+	+	32.30%	316	41
orange1.1g030977m (intron1)	Nuclear Intron	+	+	+	26.70%	306	85
orange1.1g036770m (intron27)	Nuclear Intron	+	+	+	11.40%	703	95
orange1.1g019384m (intron3)	Nuclear Intron	+	+	+	10.90%	693	36
*psb*A-*trn*H	chloroplast	n/a		+	13.10%	624	123
ITS	nrDNA	+		+	32.60%	763	129
orange1.1g028997m (intron4)	Nuclear Intron	+	+				
orange1.1g016982m (intron11)	Nuclear Intron	+	+				
orange1.1g022600m	Nuclear Exon	+					
orange1.1g011087m	Nuclear Exon	+	+				
orange1.1g039733m	Nuclear Exon	+					
orange1.1g001405m	Nuclear Exon	+	+				
orange1.1g028318m	Nuclear Exon	+	+				
orange1.1g004101m	Nuclear Exon	+					
orange1.1g008050m	Nuclear Exon	+	+				
orange1.1g000832m	Nuclear Exon	+					
orange1.1g004904m	Nuclear Exon	+	+				
orange1.1g013475m	Nuclear Exon	+					
orange1.1g022288m	Nuclear Exon	+					
orange1.1g000428m	Nuclear Exon	+	+				
orange1.1g011916m	Nuclear Exon	+					
*rpo*B	chloroplast	NA					
*trn*D-T	chloroplast	NA					
*trn*L-F	chloroplast	NA					

Table 7. Forward and Reverse primers of loci that successfully amplified PCR + sanger sequenced.

Locus	**Forward Primer**	**Reverse Primer**	**Citation**
orange1.1g002083m (intron9)	CATATGCAGTTACAGCACTAATGA	AATCTCAACAGCATGAGCATC	Chery et al. 2017
orange1.1g015495m (intron8)	CTGCTGGAAATGCCTCTAGC	CTGAGCAGCGTCAGCATATC	Chery et al. 2017
orange1.1g027952m (intron5)	TGGTTTTGATTGATGCAAGTG	GCATCTTCCCACCAAGGATA	Chery et al. 2017
orange1.1g022777m (intron3)	GGAGGATTTCAATGAGGCTCT	TCTCAGCATAATCAGACCTGTG	Chery et al. 2017
orange1.1g016982m (intron11)	CATTCCGTGATTTGCCTCTT	TCCATATTCCTGTTTCATCTGC	Chery et al. 2017
orange1.1g009973m (intron5)	AGTGGAACTGCTTCGCAAGT	TGCATATGGGTTATAGCCTTGA	Chery et al. 2017
orange1.1g030977m (intron1)	ACCGCCTCCCTATTACAGACTCTACA	TGGGTAAAGCTGACGCACTCCTTG	Chery et al. 2017
orange1.1g036770m (intron27)	TGAAGCCATTTTCCAGTGCACATT	ACCGAATCAATGCAGGAAAACAGTGA	This study
orange1.1g019384m (intron3)	TGCATTCAAATGTCACCGAAAATCA	ACCATCACATCCTCCAGTAGCAAA	This study
*psb*A-*trn*H	CGCGCATGGTGGATTCACAATCC	GTTATGCATGAACGTAATGCTC	(Sang et al. 1997; Tate and Simpson 2003)
ITS	TCC TCC GCT TAT TGA TAT GC	CCT TAT CAT TTA GAG GAA GGA G	(Whites et al. 1990; Stanford et al. 2000)
orange1.1g028997m (intron4)	AAAGAGTCCAAACCAACAATTC	TAAGCAGCACTTTTCCCACA	Chery et al. 2017
orange1.1g016982m (intron11)	GACCAAATCATTTCTGGGATAGAC	TAGCCTAAGGATAACAAGGATGG	This study
orange1.1g022600m	TTCGTTGTCTATCAATTCTGAA	TTAGAAGCAGTGTGAGTAATTT	This study
orange1.1g011087m	ATGATGCAGGACATGATAAAG	TCTTTATAATTCCCATCATCTCTC	This study
orange1.1g039733m	GATTTAAAATGTGATTGTCAACAT	ATTTGCTAAAAACCCTAAAGTTTA	This study
orange1.1g001405m	CATGTACTGGATAGCATCTAC	CTTAAGTCCAAAACTCCTCTC	This study
orange1.1g028318m	TGTGATGATATTAACTTGGATTTT	AGGTCTATGATAAAATTATCCCAA	This study
orange1.1g004101m	TTACACAGAGTCAGAAATTTTTAC	TACTTCCAAGTTACTGAAATGG	This study
orange1.1g008050m	CTTGAGTGGAGTTGTTAAGATAT	CTTCTAATGCAAGAATACTTCTC	This study

orange1.1g000832m	ATTGCATTTACTTTTGGACTTG	ATTAAACTGGAAAGTTCGAATATC	This study
orange1.1g004904m	CGTAATGCTTTGTATAACATACA	CAACTTGTTCTTCTGACAAAG	This study
orange1.1g013475m	ATGGATCAATCAATCTGCATAT	ATAGAATTCTCACTCATATTGACT	This study
PACid_18132828	CATCTCTTAGATCTGATATTGAGA	AGTTTTCAATTCTAGGTCAGTT	This study

Appendix E[1]: Voucher Table for Chapter Two

Taxon	**Collector**	**Collection No.**	**Herbarium**	**Herbarium Catalog_No.**	**Country**
Allohylus psilosermus	Chery	19	PMA	-	Panama
Allophylus leucoclados	Perdiz	785	US	3627807	Brazil
Allophylus petiolulatus	Perdiz	872	US	3627806	Brazil
Allophylus pslisospermus	Chery	12	PMA	-	Panama
Allophylus semidentatus	Perdiz	777	US	3627813	Brazil
Cardiospermum corindum	Pace	425	US	3677380	Mexico
Cardiospermum grandiflorum	Peterson	7088	US	3594402	Panama
Cardiospermum halicacabum	Egan	15-314	US	3678585	United States
Cupania guatemalensis	Acevedo	15278	US	3668963	Panama
Cupania racemosa	Perdiz	432	US	3627812	Brazil
Lophostigma plumosum	Coulleri	267	US	3628138	Bolivia
Lophostigma plumosum	Acevedo	6568	US	3295140	Bolivia
Matayba glaberrima	Acevedo	15175	US	3691476	Panama
Paullinia acutangula	Acevedo	14325	US	3635026	Peru
Paullinia acutangula	Acevedo	14917	US	-	Brazil
Paullinia alata	Acevedo	7586	US	3330090	Ecuador
Paullinia alata	Acevedo	14393	US	3620749	Peru
Paullinia alata	Dodson	5860	US	2843911	Ecuador
Paullinia allenii	Acevedo	15184	US	3691471	Panama
Paullinia allenii	Acevedo	15255	US	3683522	Panama
Paullinia alsmithii	Villa	1781	US	3472337	Ecuador
Paullinia alsmithii	Granville	16866	US	3523305	French Guiana
Paullinia baileyi	Chery	37	PMA	-	Panama
Paullinia baileyi	Acevedo	15182	US	3691470	Panama
Paullinia bilobulata	Acevedo	14853	US	-	Brazil
Paullinia boliviana	Acevedo	14324	US	3620721	Peru
Paullinia bracetosa	Chery	39	PMA	-	Panama
Paullinia bracteosa	Acevedo	15185	US	3691469	Peru
Paullinia bracteosa	Chery	26	PMA		Panama
Paullinia caloptera	Acevedo	14315	US	3635021	Peru

Paullinia carpopoda	Perdiz	701	CEPEC		Brazil
Paullinia carpopodea	Perdiz	519	US	3627811	Brazil
Paullinia carpopodea	Pace	317	US		
Paullinia cf. alata	Acevedo	14871	US	3680205	Brazil
Paullinia cf. boliviana	Acevedo	14916	US	-	Brazil
Paullinia cf. stellata	Acevedo	14298	US	3629599	Peru
Paullinia cf.nobilis	Acevedo	15050	US	3680336	Brazil
Paullinia chocoensis	Acevedo	15281	US	3668966	Panama
Paullinia clathrata	Acevedo	15261	US	3683515	Panama
Paullinia coriacea	Somner	1070	RBR	30803	Brazil
Paullinia correae	Acevedo	15228	US	3691477	Panama
Paullinia costaricensis	Acevedo	15139	US	3582595	Mexico
Paullinia dasystachya	Acevedo	14819	US	3680192	Brazil
Paullinia elegans	Acevedo	11135	US	-	Bolivia
Paullinia elongata	Acevedo	14305	US	3625010	Peru
Paullinia elongata	Acevedo	14401	US	3620757	Peru
Paullinia eriocarpa	Acevedo	7612	US	3330104	Ecuador
Paullinia exalata	Acevedo	14891	US	3680199	Brazil
Paullinia faginea	Burnham	1556	US	3381558	Ecuador
Paullinia fibrigera	Chery	21	PMA	-	Panama
Paullinia fibrigera	Acevedo	15191	US	3668964	Panama
Paullinia fimbriata	Clark	4386	US	3698347	Ecuador
Paullinia fruticosa	Acevedo	14273	US	-	Peru
Paullinia fuscescens	Acevedo	11459	US	3429796	U.S.Virgin Islands
Paullinia fuscescens	Acevedo	15253	US	3683516	Panama
Paullinia glomerulosa	Acevedo	15194	US	3691466	Panama
Paullinia glomerulosa	Chery	20	PMA	-	Panama
Paullinia hystrix	Acevedo	14408	US	3620767	Peru
Paullinia hystrix	Acevedo	14417	US	3620775	Peru
Paullinia hystrix	Acevedo	14895	US	3680198	Brazil
Paullinia hystrix	Acevedo	14898	US	3680197	Brazil
Paullinia imberbis	Acevedo	14826	US	3680269	Brazil
Paullinia ingifolia	Acevedo	16067	US	-	French Guiana

Paullinia ingifolia	Acevedo	15248	US	3668951	Panama
Paullinia ingifolia	Acevedo	14812	US	-	Brazil
Paullinia ingifolia	Acevedo	15282	US	3668969	Panama
Paullinia jamaicensis	Acevedo	12035	US	3590021	Jamaica
Paullinia josecuatrii	Acevedo	14291	US	3629595	Peru
Paullinia killipii	Acevedo	14308	US	3625007	Peru
Paullinia killipii	Acevedo	14323	US	3620718	Peru
Paullinia largifolia	Acevedo	14920	US	-	Brazil
Paullinia latifolia	Acevedo	6038	US	3676038	Suriname
Paullinia latifolia	Acevedo	5835	US	3526316	Suriname
Paullinia leiocarpa	Jansen-Jacovs	3924	US	3359942	Guyana
Paullinia mazanensis	Acevedo	14364	US	3630454	Peru
Paullinia neglecta	Nee	37116	US	3174683	Bolivia
Paullinia neglecta	Beck	23548	US	3476039	Bolivia
Paullinia obovata	Acevedo	14441	US	3592867	Peru
Paullinia obovata	Acevedo	14382	US	3630472	Peru
Paullinia obovata	Acevedo	14918	US	-	Brazil
Paullinia olivacea	Acevedo	14390	US	3620745	Peru
Paullinia paullinoides	Acevedo	7519	US	3330097	Ecuador
Paullinia paullinoides	Acevedo	14860	US	3680190	Brazil
Paullinia pinnata	Chery	41	PMA	-	Panama
Paullinia pinnata	Acevedo	6679	US	3580781	Bolivia
Paullinia pinnata	Kayombo	1024	US	3316618	Tanzania
Paullinia pinnata	Pace	192	US	3677377	Brazil
Paullinia racemosa	Acevedo	3708	US	3212304	Brazil
Paullinia racemosa	Perdiz	808	CEPEC	128946	Brazil
Paullinia racemosa	Roque	2594	ALCB	93954	Brazil
Paullinia revoluta	Perdiz	487	US	3627818	Brazil
Paullinia revoluta	Perdiz	822	CEPEC	128960	Brazil
Paullinia rubiginosa	Acevedo	14366	US	3630452	Peru
Paullinia rubiginosa	Perdiz	699	-	-	
Paullinia rubiginosa subsp. *setosa*	Acevedo	11114	US	3569426	French Guiana
Paullinia rufescens	Acevedo	11120	US	3569432	French Guiana
Paullinia rugosa	Chery	25	PMA	-	Panama

Paullinia rugosa	Acevedo	15179	US	3691473	Panama
Paullinia rugosa	Acevedo	14807	US	3680195	Brazil
Paullinia selenoptera	Acevedo	14813	US	-	Brazil
Paullinia serjaniifolia	Acevedo	15270	US	3668972	Panama
Paullinia simulans	Acevedo	15235	US	3668974	Panama
Paullinia sp.	Breedlove	72699	UCBG	92.0509	Mexico
Paullinia sp.	Chery	46	PMA	-	Panama
Paullinia sp.	Acevedo	16064	US	-	French Guiana
Paullinia sp.	Acevedo	16089	US	-	French Guiana
Paullinia sp.	Acevedo	15718	US	-	Colombia
Paullinia sp.	Acevedo	15706	US	-	Colombia
Paullinia sp.	Acevedo	12335	US	3526309	French Guiana
Paullinia sp.	Acevedo	11105	US	3569445	French Guiana
Paullinia sphaerocarpa	Acevedo	15247	US	3668952	Panama
Paullinia spicata	Acevedo	12344	US	3526306	French Guiana
Paullinia sprucei	Acevedo	14922	US	-	Brazil
Paullinia stellata	Torke	615	MO	6709351	Bolivia
Paullinia stipitata	Acevedo	15280	US	3668967	Panama
Paullinia stipitata	Acevedo	15242	US	3668954	Panama
Paullinia subrotunda	Melo	402	US	3682609	Brazil
Paullinia ternata	Perdiz	824	CEPEC	128962	Brazil
Paullinia tomentosa	Acevedo	12236	US	-	Mexico
Paullinia trigonia	Perdiz	673	CEPEC?	-	Brazil
Paullinia turbacensis	Acevedo	15176	US	3691475	Panama
Paullinia turbacensis	Chery	10	PMA	-	Panama
Paullinia turbacensis	Chery	13	PMA	-	Panama
Paullinia turbacensis	Chery	24	PMA	-	Panama
Paullinia verecunda	Acevedo	15256	US	3683512	Panama
Paullinia verecunda	Acevedo	15234	US	3668959	Panama
Serjania atrolineata	Acevedo	15195	US	3691465	Panama
Serjania atrolineata	Chery	42	PMA		Panama
Serjania circumvallata	Acevedo	15203	US	3691463	Panama
Serjania corinigera	Chery	36	PMA	112573	Panama
Serjania deltoidea	Acevedo	15725			Colombia
Serjania fluminensis	-	-	-	-	Brazil

Serjania membranacea	Acevedo	15227	US	3691479	Panama
Serjania mexicana	Chery	23	PMA	-	Panama
Serjania mexicana	Chery	45	PMA	-	Panama
Serjania paucidentata	Chery	34	PMA	-	Panama
Serjania pyramidata	Chery	29	PMA	112578	Panama
Serjania reticulata	Torke	508	US	3677589	Bolivia
Serjania rhombea	Acevedo	15213	US	3691459	Panama
Serjania salzmanniana	Perdiz	806	US	3627810	Brazil
Serjania sp.	Chery	18	PMA	-	Panama
Serjania trachygona	Acevedo	15222	US	3668956	Panama
Serjania pyramidata	Acevedo	15243	US	3668953	Panama
Talisia nervosa	Acevedo	15190	US	3683511	Panama
Talisia sp.	Perdiz	939	US	3627815	Brazil
Thinouia mucronata	Zardini	13513	US	3239039	Paraguay
Urvillea ulmacea	Balick	3349	US	3296216	Belize

Appendix E[2]: Sequences represented in the phylogeny alignment

*= Sanger Sequence; all others derived from Ilumina MiSeq

Taxon	Col. No.	orange1.1g019384m (intron3)	orange1.1g036770m (intron27)	orange1.1g030977m (intron1)	orange1.1g009973m (intron5)	orange1.1g016982m (intron11)	orange1.1g022777m (intron3)	orange1.1g027952m (intron5)	orange1.1g015495m (intron8)	orange1.1g002083m (intron9)	*psbA-trnH*	ITS	Totals
Paullinia													
P. acutangula	Acevedo 14325			+		+				+			3
P. acutangula	Acevedo 14917		+	+		+						+	4
P. alata	Acevedo 14393			+		+				+		+	4
P. alata	Acevedo 7586			+		+		+		+	+	+	6
P. alata	Dodson 5860										+	+	2
P. alata	Acevedo 14871			+		+		+		+			4
P. allenii	Acevedo 15255					+				+	+	+	4
P. allenii	Acevedo 15184		+	+		+					+	+	5
P. alsmithii	Villa 1781		+	+		+	+			+	+	+	7
P. alsmithii	Granville 16866		+			+	+			+	+	+	6
P. baileyi	Chery 37		+			+	+			+		+	5
P. baileyi	Acevedo 15182		+			+				+		+	4
P. bilobulata	Acevedo 14853	+	+	+	+	+				+			6
P. boliviana	Acevedo 14324										+	+	2
P. bracteosa	Chery 39			+		+				+	+	+	5
P. bracteosa	Acevedo 15185		+	+		+				+	+	+	6
P. bracteosa	Chery26				+*		+*	+*	+*	+*			5

P. caloptera	Acevedo 14315		+	+		+				+	+		5
P. carpopodea	Pace 317		+*	+*			+*				+		4
P. carpopodea	Perdiz 519		+			+				+	+	+	5
P. carpopodea	Perdiz 701		+			+				+		+	4
P. cf boliviana	Acevedo 14916		+			+				+		+	4
P. cf nobilis	Acevedo 15050		+	+		+	+				+	+	6
P. cf stellata	Acevedo 14298		+					+		+		+	4
P. chocoensis	Acevedo 15281	+	+		+	+		+		+	+	+	8
P. clathrata	Acevedo 15261		+	+		+		+		+	+	+	7
P. coriacea	Somner 1070			+	+	+						+	4
P. correae	Acevedo 15228	+	+		+	+	+					+	6
P. costaricensis	Acevedo 15139	+				+		+		+	+	+	6
P. dasystachya	Acevedo 14819		+	+		+	+			+		+	6
P. elegans	Acevedo 11135		+	+		+		+		+			5
P. elongata	Acevedo 14305		+			+			+	+		+	5
P. elongata	Acevedo 14401	+	+	+		+				+	+	+	7
P. eriocarpa	Acevedo 7612			+		+				+	+	+	5
P. exalata	Acevedo 14891		+	+		+	+			+		+	6
P. faginea	Burnham 1556										+	+	2
P. fibrigera	Chery 21		+		+	+		+		+	+	+	7
P. fibrigera	Acevedo 15191		+		+	+		+		+	+	+	7
P. fimbriata	Clark 4386					+					+	+	3
P. fruticosa	Acevedo 14273		+			+	+			+	+	+	6
P. fuscescens	Acevedo 15253	+	+	+	+	+		+		+	+	+	9
P. fuscescens	Acevedo 11459		+	+	+	+		+		+		+	7
P. glomerulosa	Acevedo 15194		+	+		+	+			+		+	6
P. glomerulosa	Chery20						+*	+*	+*	+*			4
P. hystrix	Acevedo 14408	+	+			+				+	+	+	6
P. hystrix	Acevedo 14417		+			+				+	+	+	5
P. hystrix	Acevedo 14895											+	1
P. hystrix	Acevedo 14898		+	+		+						+	4
P. imberbis	Acevedo 14826	+		+		+				+		+	5

P. ingifolia	Acevedo 16067	+	+	+	+	+			+	+	+	8
P. ingifolia	Acevedo 15248	+	+	+	+	+	+	+	+		+	9
P. ingifolia	Acevedo 15282		+	+	+	+	+	+	+	+	+	9
P. ingifolia	Acevedo 14812		+	+	+	+		+	+			6
P. jamaicensis	Acevedo 12035		+			+		+	+	+		5
P. josecuatrii	Acevedo 14291					+				+	+	3
P. killipii	Acevedo 14308			+		+	+			+	+	5
P. killipii	Acevedo 14323			+		+				+	+	4
P. largifolia	Acevedo 14920			+		+		+	+			4
P. latifolia	Acevedo 6088									+	+	2
P. latifolia	Acevedo 5835					+			+		+	3
P. leiocarpa	Jansen-Jacobs 3924	+		+		+			+	+	+	6
P. mazanensis	Acevedo 14364			+		+			+	+	+	5
P. microneura	Acevedo 14320		+	+	+	+			+	+		6
P. neglecta	Beck 23548	+		+		+			+	+		5
P. neglecta	Nee 37116			+	+	+			+	+		5
P. obovata	Acevedo 14441			+		+				+	+	4
P. obovata	Acevedo 14382			+		+			+	+	+	5
P. olivacea	Acevedo 14390		+			+		+		+	+	5
P. paullinoides	Acevedo 7519		+	+	+	+			+	+	+	7
P. paullinoides	Acevedo 14860	+				+			+			3
P. pinnata	Chery 41	+		+		+		+	+	+	+	7
P. pinnata	Acevedo 6679					+				+	+	3
P. pinnata	Kayombo 1024					+				+	+	3
P. pinnata	Pace 192	+	+	+		+		+	+	+		7
P. racemosa	Acevedo 3708			+		+		+	+	+	+	6
P. racemosa	Perdiz 808		+	+	+	+		+		+	+	7
P. racemosa	Roque 2594		+	+	+	+		+	+		+	7
P. revoluta	Perdiz 487		+			+			+	+	+	5
P. revoluta	Perdiz 822	+		+	+	+	+	+	+	+	+	9
P. rubiginosa	Acevedo 14366		+			+		+		+		4
P. rubiginosa	Perdiz 699		+			+			+	+	+	5

P. rubiginosa subsp setosa	Acevedo 11114		+		+	+		+					4
P. rufescens	Acevedo 11120		+	+						+		+	4
P. rugosa	Chery 25		+	+	+	+	+	+		+	+	+	9
P. rugosa	Acevedo 15179			+	+	+		+		+	+	+	7
P. rugosa	Acevedo 14807		+	+		+		+		+		+	6
P. selenoptera	Acevedo 14813	+	+	+		+				+			5
P. serjaniifolia	Acevedo 15270		+			+				+	+	+	5
P. simulans	Acevedo 15235	+	+	+		+		+		+		+	7
P. sp.	Chery 46	+	+	+	+	+		+			+		7
P. sp.	Acevedo 16089										+	+	2
P. sp.	Acevedo 16064		+		+	+					+	+	5
P. sp.	Acevedo 15718	+		+		+					+	+	5
P. sp.	Acevedo 15706	+	+	+		+				+	+		6
P. sp.	Acevedo 12335		+	+		+		+				+	5
P. sp.	Acevedo 11105		+					+		+		+	4
P. sp.	Acevedo 14918			+		+				+		+	4
P. sp.	Breedlove 72699		+	+	+*	+*	+*	+	+*	+*			8
P. sphaerocarpa	Acevedo 15247	+	+	+	+			+			+	+	7
P. spicata	Acevedo 12344	+				+	+			+	+		5
P. sprucei	Acevedo 14922			+		+						+	3
P. stellata	Torke 615		+			+					+	+	4
P. stipitata	Acevedo 15280		+	+		+			+	+	+	+	7
P. stipitata	Acevedo 15242		+	+		+	+			+	+	+	7
P. subrotunda	Melo 402					+					+	+	3
P. ternata	Perdiz 824		+	+	+	+				+		+	6
P. tomentosa	Acevedo 12236		+	+		+		+		+		+	6
P. trigonia	Perdiz 673					+					+	+	3
P. turbacensis	Acevedo 15176	+		+		+				+	+	+	6
P. turbacensis	Chery10						+*	+*	+*	+*			4
P. turbacensis	Chery13		+*	+*				+*	+*	+*			5
P. turbacensis	Chery24					+*	+*	+*	+*	+*			5
P. verecunda	Acevedo 15256		+	+		+	+			+	+	+	7

P. verecunda	Acevedo 15234	+	+	+		+	+				+	+	7
Cardiospermum													
C. corindum	Pace 425	+		+		+				+			4
C. grandiflorum	Peterson 7088	+	+	+		+		+		+		+	7
C. halicacabum	Egan 15-314					+				+	+	+	4
Serjania													
S. atrolineata	Chery 18		+	+	+	+				+	+	+	7
S. atrolineata	Acevedo 15195		+	+	+	+		+		+	+	+	8
S. atrolineata	Chery42						+*	+*	+*	+*			4
S. circumvallata	Acevedo 15203		+	+		+				+			4
S. corinigera	Chery 36		+	+	+	+		+		+	+	+	8
S. deltoidea	Acevedo 15725		+		+	+		+		+		+	6
S. fluminensis			+	+		+				+	+	+	6
S. membranacea	Acevedo 15227	+	+	+	+	+		+			+	+	8
S. mexicana	Chery 23		+	+	+	+	+*	+	+*	+*	+	+	10
S. mexicana	Chery 45		+			+				+	+	+	5
S. paucidentata	Chery34						+*	+*	+*				3
S. pyramidata	Chery 29	+	+	+	+	+	+	+*	+*	+	+	+	11
S. pyramidata	Acevedo 15243		+	+		+					+	+	5
S. reticulata	Torke 508					+						+	2
S. rhombea	Acevedo 15213		+	+		+						+	4
S. salzmanniana	Perdiz 806	+	+			+		+		+		+	6
S. trachygona	Acevedo 15222		+			+		+		+	+		5
Lophostigma													
L. plumosum	Acevedo 6568		+	+	+	+	+				+	+	7
L. plumosum	Coulleri 267		+	+	+	+	+					+	6
Thinouia mucronata	Zardini 13513					+				+	+	+	4
Urvillea ulmacea	Balick 3349	+	+	+		+		+		+	+	+	8
Allophylus													
A. leucoclados	Perdiz 785										+	+	2

A. petiolulatus	Perdiz 872											+	1
A. psilospermus	Chery19				+*		+*	+*	+*				4
A. pslisospermus	Chery 12	+	+				+			+		+	5
A. semidentatus	Perdiz 777						+					+	2
Matayba glaberrima	Acevedo 15175		+		+	+	+		+	+	+	+	8
Cupania guatemalensis	Acevedo 15278	+	+		+	+	+	+	+	+		+	9
Cupania racemosa	Perdiz 432		+		+	+	+	+		+	+	+	8
Talisia nervosa	Acevedo 15190										+	+	2
Talisia sp.	Perdiz 939										+	+	2
Totals		33	90	83	40	124	36	55	15	101	89	116	3 3

Appendix F: Vouchers list for Chapter Three

This voucher list includes all of the individuals included in the phylogeny in Chapter Two (Appendix E[1]) plus ITS sequences from 76 individuals from the Acevedo et al., 2017 phylogeny.

Taxon	**Collector**	**Collection No.**	**Herbarium**	**Voucher Catalog_No.**	**Country**
Allohylus psilosermus	Chery	19	PMA	-	Panama
Allophylus leucoclados	Perdiz	785	US	3627807	Brazil
Allophylus petiolulatus	Perdiz	872	US	3627806	Brazil
Allophylus pslisospermus	Chery	12	PMA	-	Panama
Allophylus semidentatus	Perdiz	777	US	3627813	Brazil
Cardiospermum corindum	Pace	425	US	3677380	Mexico
Cardiospermum grandiflorum	Peterson	7088	US	3594402	Panama
Cardiospermum halicacabum	Egan	15-314	US	3678585	United States
Cupania guatemalensis	Acevedo	15278	US	3668963	Panama
Cupania racemosa	Perdiz	432	US	3627812	Brazil
Lophostigma plumosum	Coulleri	267	US	3628138	Bolivia
Lophostigma plumosum	Acevedo	6568	US	3295140	Bolivia
Matayba glaberrima	Acevedo	15175	US	3691476	Panama
Paullinia acutangula	Acevedo	14325	US	3635026	Peru
Paullinia acutangula	Acevedo	14917	US	-	Brazil
Paullinia alata	Acevedo	7586	US	3330090	Ecuador
Paullinia alata	Acevedo	14393	US	3620749	Peru
Paullinia alata	Dodson	5860	US	2843911	Ecuador
Paullinia allenii	Acevedo	15184	US	3691471	Panama
Paullinia allenii	Acevedo	15255	US	3683522	Panama
Paullinia alsmithii	Villa	1781	US	3472337	Ecuador

Paullinia alsmithii	Granville	16866	US	3523305	French Guiana
Paullinia baileyi	Chery	37	PMA	-	Panama
Paullinia baileyi	Acevedo	15182	US	3691470	Panama
Paullinia bilobulata	Acevedo	14853	US	-	Brazil
Paullinia boliviana	Acevedo	14324	US	3620721	Peru
Paullinia bracetosa	Chery	39	PMA	-	Panama
Paullinia bracteosa	Acevedo	15185	US	3691469	Peru
Paullinia bracteosa	Chery	26	PMA		Panama
Paullinia caloptera	Acevedo	14315	US	3635021	Peru
Paullinia carpopoda	Perdiz	701	CEPEC		Brazil
Paullinia carpopodea	Perdiz	519	US	3627811	Brazil
Paullinia carpopodea	Pace	317	US		
Paullinia cf. alata	Acevedo	14871	US	3680205	Brazil
Paullinia cf. boliviana	Acevedo	14916	US	-	Brazil
Paullinia cf. stellata	Acevedo	14298	US	3629599	Peru
Paullinia cf.nobilis	Acevedo	15050	US	3680336	Brazil
Paullinia chocoensis	Acevedo	15281	US	3668966	Panama
Paullinia clathrata	Acevedo	15261	US	3683515	Panama
Paullinia coriacea	Somner	1070	RBR	30803	Brazil
Paullinia correae	Acevedo	15228	US	3691477	Panama
Paullinia costaricensis	Acevedo	15139	US	3582595	Mexico
Paullinia dasystachya	Acevedo	14819	US	3680192	Brazil
Paullinia elegans	Acevedo	11135	US	-	Bolivia
Paullinia elongata	Acevedo	14305	US	3625010	Peru
Paullinia elongata	Acevedo	14401	US	3620757	Peru
Paullinia eriocarpa	Acevedo	7612	US	3330104	Ecuador
Paullinia exalata	Acevedo	14891	US	3680199	Brazil
Paullinia faginea	Burnham	1556	US	3381558	Ecuador
Paullinia fibrigera	Chery	21	PMA	-	Panama

Paullinia fibrigera	Acevedo	15191	US	3668964	Panama
Paullinia fimbriata	Clark	4386	US	3698347	Ecuador
Paullinia fruticosa	Acevedo	14273	US	-	Peru
Paullinia fuscescens	Acevedo	11459	US	3429796	U.S.Virgin Islands
Paullinia fuscescens	Acevedo	15253	US	3683516	Panama
Paullinia glomerulosa	Acevedo	15194	US	3691466	Panama
Paullinia glomerulosa	Chery	20	PMA	-	Panama
Paullinia hystrix	Acevedo	14408	US	3620767	Peru
Paullinia hystrix	Acevedo	14417	US	3620775	Peru
Paullinia hystrix	Acevedo	14895	US	3680198	Brazil
Paullinia hystrix	Acevedo	14898	US	3680197	Brazil
Paullinia imberbis	Acevedo	14826	US	3680269	Brazil
Paullinia ingifolia	Acevedo	16067	US	-	French Guiana
Paullinia ingifolia	Acevedo	15248	US	3668951	Panama
Paullinia ingifolia	Acevedo	14812	US	-	Brazil
Paullinia ingifolia	Acevedo	15282	US	3668969	Panama
Paullinia jamaicensis	Acevedo	12035	US	3590021	Jamaica
Paullinia josecuatrii	Acevedo	14291	US	3629595	Peru
Paullinia killipii	Acevedo	14308	US	3625007	Peru
Paullinia killipii	Acevedo	14323	US	3620718	Peru
Paullinia largifolia	Acevedo	14920	US	-	Brazil
Paullinia latifolia	Acevedo	6038	US	3676038	Suriname
Paullinia latifolia	Acevedo	5835	US	3526316	Suriname
Paullinia leiocarpa	Jansen-Jacovs	3924	US	3359942	Guyana
Paullinia mazanensis	Acevedo	14364	US	3630454	Peru
Paullinia neglecta	Nee	37116	US	3174683	Bolivia
Paullinia neglecta	Beck	23548	US	3476039	Bolivia
Paullinia obovata	Acevedo	14441	US	3592867	Peru

Paullinia obovata	Acevedo	14382	US	3630472	Peru
Paullinia obovata	Acevedo	14918	US	-	Brazil
Paullinia olivacea	Acevedo	14390	US	3620745	Peru
Paullinia paullinoides	Acevedo	7519	US	3330097	Ecuador
Paullinia paullinoides	Acevedo	14860	US	3680190	Brazil
Paullinia pinnata	Chery	41	PMA	-	Panama
Paullinia pinnata	Acevedo	6679	US	3580781	Bolivia
Paullinia pinnata	Kayombo	1024	US	3316618	Tanzania
Paullinia pinnata	Pace	192	US	3677377	Brazil
Paullinia racemosa	Acevedo	3708	US	3212304	Brazil
Paullinia racemosa	Perdiz	808	CEPEC	128946	Brazil
Paullinia racemosa	Roque	2594	ALCB	93954	Brazil
Paullinia revoluta	Perdiz	487	US	3627818	Brazil
Paullinia revoluta	Perdiz	822	CEPEC	128960	Brazil
Paullinia rubiginosa	Acevedo	14366	US	3630452	Peru
Paullinia rubiginosa	Perdiz	699	-	-	
Paullinia rubiginosa subsp. *setosa*	Acevedo	11114	US	3569426	French Guiana
Paullinia rufescens	Acevedo	11120	US	3569432	French Guiana
Paullinia rugosa	Chery	25	PMA	-	Panama
Paullinia rugosa	Acevedo	15179	US	3691473	Panama
Paullinia rugosa	Acevedo	14807	US	3680195	Brazil
Paullinia selenoptera	Acevedo	14813	US	-	Brazil
Paullinia serjaniifolia	Acevedo	15270	US	3668972	Panama
Paullinia simulans	Acevedo	15235	US	3668974	Panama
Paullinia sp.	Breedlove	72699	UCBG	92.0509	Mexico
Paullinia sp.	Chery	46	PMA	-	Panama
Paullinia sp.	Acevedo	16064	US	-	French Guiana

Paullinia sp.	Acevedo	16089	US	-	French Guiana
Paullinia sp.	Acevedo	15718	US	-	Colombia
Paullinia sp.	Acevedo	15706	US	-	Colombia
Paullinia sp.	Acevedo	12335	US	3526309	French Guiana
Paullinia sp.	Acevedo	11105	US	3569445	French Guiana
Paullinia sphaerocarpa	Acevedo	15247	US	3668952	Panama
Paullinia spicata	Acevedo	12344	US	3526306	French Guiana
Paullinia sprucei	Acevedo	14922	US	-	Brazil
Paullinia stellata	Torke	615	MO	6709351	Bolivia
Paullinia stipitata	Acevedo	15280	US	3668967	Panama
Paullinia stipitata	Acevedo	15242	US	3668954	Panama
Paullinia subrotunda	Melo	402	US	3682609	Brazil
Paullinia ternata	Perdiz	824	CEPEC	128962	Brazil
Paullinia tomentosa	Acevedo	12236	US	-	Mexico
Paullinia trigonia	Perdiz	673	CEPEC?	-	Brazil
Paullinia turbacensis	Acevedo	15176	US	3691475	Panama
Paullinia turbacensis	Chery	10	PMA	-	Panama
Paullinia turbacensis	Chery	13	PMA	-	Panama
Paullinia turbacensis	Chery	24	PMA	-	Panama
Paullinia verecunda	Acevedo	15256	US	3683512	Panama
Paullinia verecunda	Acevedo	15234	US	3668959	Panama
Serjania pyramidata	Acevedo	15243	US	3668953	Panama
Serjania atrolineata	Acevedo	15195	US	3691465	Panama
Serjania atrolineata	Chery	42	PMA		Panama
Serjania atrolineata.	Chery	18	PMA	-	Panama
Serjania circumvallata	Acevedo	15203	US	3691463	Panama

Serjania corinigera	Chery	36	PMA	112573	Panama
Serjania deltoidea	Acevedo	15725			Colombia
Serjania fluminensis	-	-	-	-	Brazil
Serjania membranacea	Acevedo	15227	US	3691479	Panama
Serjania mexicana	Chery	23	PMA	-	Panama
Serjania mexicana	Chery	45	PMA	-	Panama
Serjania paucidentata	Chery	34	PMA	-	Panama
Serjania pyramidata	Chery	29	PMA	112578	Panama
Serjania reticulata	Torke	508	US	3677589	Bolivia
Serjania rhombea	Acevedo	15213	US	3691459	Panama
Serjania salzmanniana	Perdiz	806	US	3627810	Brazil
Serjania trachygona	Acevedo	15222	US	3668956	Panama
Talisia nervosa	Acevedo	15190	US	3683511	Panama
Talisia sp.	Perdiz	939	US	3627815	Brazil
Thinouia mucronata	Zardini	13513	US	3239039	Paraguay
Urvillea ulmacea	Balick	3349	US	3296216	Belize

Taxon	**Collector**	**Collection No.**	**Herbarium**	**Country**	**GenBank#(ITS Sequence)**	**GenBank#(trnLSequence)**
Allophylus abyssinicus	Desissa & Binggeli	318	MO	Ethiopia	KX584886	-
Allophylus africanus	Balkwill	4206	MO	South Africa	KX584887	KX584984
Allophylus arboreus	Wohlhauser&Stiefel	60072	MO	Madagascar	KX584888	KX584985
Allophylus bicruris	Barthelat	828	MO	Mayotte	KX584889	KX584986
Allophylus bojerianus	Ratovoson	961	MO	Madagascar	KX584890	KX584987
Allophylus chaunostachys	Mwangoko	729	MO	Tanzania	KX584891	KX584988
Allophylus chirindensis	Hizza	26	MO	Tanzania	KX584892	KX584989

Allophylus cominia	Acevedo	12216	US	Mexico	KX584893	KX584990
Allophylus crassinervis	-	-	-	Puerto Rico	KX584894	KX584991
Allophylus decipiens	Phillipson	4194	MO	South Africa	KX584895	KX584992
Allophylus dissectus	Phillipson	1704	MO	Madagascar	KX584896	KX584993
Allophylus gardineri	Pignal	1834	MO	Mayotte	KX584897	KX584994
Allophylus hirtellus	Cheek	5059	-		KX584898	KX584995
Allophylus pervillei	Hoffmann	399	MO	Mayotte	KX584899	KX584996
Allophylus poungouensis	McPherson	16109	MO	Gabon	KX584900	KX584997
Allophylus puberulus	Somner	1069	US	Brazil	KX584901	KX584998
Allophylus racemosus	Acevedo	12180	US	Mexico	KX584902	KX584999
Allophylus rubifolius	Kuchar	23357	MO	Tanzania	KX584903	KX585000
Allophylus sp.	Acevedo	14847	NY	Brazil	KX584904	KX585001
Allophylus sp.	Barthelat	1540	MO	Mayotte	KX584905	KX585002
Athyana weinmanniifolia	Acevedo	11166	US	Bolivia	KX584906	KX585003
Athyana weinmanniifolia	Villalobos	4243	MO	Bolivia	KX584907	KX585004
Bridgesia incisifolia	Landrum	9824	NY	Chile	KX584909	KX585006
Cardiospermum anomalum	Urdampilleta	330	UEC	Brazil	KX584910	-
Cardiospermum bahianum	Urdampilleta	389	UEC	Brazil	KX584911	-
Cardiospermum corindum	JLR	291	-	-	KM062239	KM062345
Cardiospermum corindum	Harder &Bringham	3495	MO	Zambia	KX584912	KX585007
Cardiospermum grandiflorum	ATBP	603	MO	Uganda	KX584915	KX585009
Cardiospermum grandiflorum	Gildenhuys	H1?	-	Hawaii	KM062277	KM062362
Cardiospermum halicacabum	Gildenhuys	280	-	Argentina	KM062192	KM062341
Cardiospermum halicacabum	CSM	8	-	South America	KM062194	KM062296

Cardiospermum halicacabum	Murata	72327	MO	Japan	KX584916	-
Cardiospermum heringeri	Urdampilleta	437	US	Brazil	KX584917	KX585010
Cardiospermum pechuelii	Gildenhuys	-	-	Namibia	KM062216	KM062299
Cupania dentata	Acevedo	12241	US	Mexico	KX584924	KX585015
Cupania emarginata	Somner	1068	RBR	Brazil	KX584925	KX585016
Lophostigma plumosum	Acevedo	6554	US	Bolivia	KX584929	KX585020
Matayba guianensis	Acevedo-Rodríguez	12342	US	French Guiana	EU720527	EU721294
Serjania altissima	Acevedo	14953	US	Brazil	KX584945	KX585036
Serjania ampelopsisa	Acevedo	11181	US	Bolivia	KX584946	KX585037
Serjania caracasana	Acevedo	15107	US	Mexico	KX584947	KX585038
Serjania cf. *caracasana*	Acevedo	3483	US	Guyana	KX584948	KX585039
Serjania clematidifolia	Somner	1078	RBR	Brazil	KX584949	KX585040
Serjania communis	Somner	1334	US	Brazil	KX584950	KX585041
Serjania cuspidata	Somner	1400	US	Brazil	KX584951	KX585042
Serjania diversifolia	Acevedo	13462	US	Puerto Rico	KX584952	-
Serjania elongata	Acevedo	13547	US	Brazil	KX584953	-
Serjania emarginata	Acevedo	15135	US	Mexico	KX584954	KX585043
Serjania erythrocaulis	Acevedo	3729	US	Brazil	KX584955	KX585044
Serjania eucardia	Somner	1072	RBR	Brazil	KX584956	KX585045
Serjania fuscifolia	Somner	1455	RBR	Brazil	KX584957	KX585046
Serjania grandiceps	Acevedo	13704	US	Brazil	KX584958	KX585047
Serjania grandifolia	Acevedo	11121	US	French Guiana	KX584959	-
Serjania ichthyoctona	Somner	1081	RBR	Brazil	KX584960	KX585048
Serjania lethalis	Roque	1860	ALCB	Brazil	KX584961	KX585049

Serjania lethalis	Somner	1381	RBR	Brazil	KX584962	KX585050
Serjania marginata	Acevedo	11131	US	Bolivia	KX584963	KX585051
Serjania membranacea	Acevedo	12329	US	French Guiana	KX584964	-
Serjania mexicana	Acevedo	12014	US	Jamaica	KX584965	KX585052
Serjania mexicana	Acevedo	15080	US	Mexico	KX584966	KX585053
Serjania paniculata	Acevedo	15143	US	Mexico	KX584967	KX585054
Serjania perulacea	Acevedo	11134	US	Bolivia	KX584968	KX585055
Serjania pyramidata	Acevedo	15243				
Serjania unguiculata	Acevedo	15081	US	Mexico	KX584969	KX585056
Serjania yucatanensis	Acevedo	12183	US	Mexico	KX584970	KX585057
Thinouia mucronata	Keller	6919	US	Argentina	KX584971	KX585058
Thinouia myriantha	Acevedo	12359	US	French Guiana	-	KX585059.
Thinouia restingae	Somner	1074	RBR	Brazil	KX584972	KX585060
Thouinia acuminata	Liston	633-2	-	-	EU720478	EU721249
Thouinia paucidentata	Acevedo	12160	US	Mexico	KX584973	-
Thouinia portoricensis	Acevedo	11435	US	Puerto Rico	KX584974	-
Thouinia villosa	Hall	825	US	Mexico	KX584975	KX585062
Urvillea chacoensis	Acevedo	11133	US	Bolivia	KX584976	KX585063
Urvillea chacoensis	Keller	6834	US	Argentina	KX584977	KX585064
Urvillea rufescens	Somner	1073	RBR	Brazil	KX584978	KX585065
Urvillea ulmacea	Acevedo	15145	US	Mexico	KX584979	KX585066
Urvillea ulmacea	Reyes-Garcia	5585	MO	Mexico	KX584980	KX585067

Appendix G: Stem and Wood anatomy data in Chapter Three

Table 8. List of accessions used in *Paullinia* stem ontogeny studies.

Species	**Collection Number**
Paullinia alata	Brandbyge 3247 Pace 594
Paullinia sp.	Chery 46
Paullinia baileyi	Chery 37
Paullinia bracteosa	Chery 26 Chery 39
Paullinia dasystachya	Acevedo 14295
Paullinia echinata	Schunke 2310
Paullinia exalata	Couvreur 207
Paullinia fibrigera	Chery 16 Chery 21
Paullinia fimbriata	Day 9005
Paullinia latifolia	Acevedo 5835 Brasil 1528
Paullinia obovata	Mathais 3585
Paullinia pinnata	Chery 30 Chery 41
Paullinia rubiginosa	Somner 1849 Acevedo 14366
Paullinia rugosa	Chery 10
Paullinia spicata	Oldeman 1248
Paullinia sprucei	Acevedo 7519 Schunke 15402
Paullinia turbacensis	Acevedo 15176 Chery 13
Paullinia weinmannifolia	Acevedo 3698

Appendix G: Stem and Wood anatomy data in Chapter Three

Table 9. Stem ontogeny characters of *Paullinia* at three developmental stages: primary growth, intermediate and mature secondary growth. International Association of wood anatomist (1989) wood characters for mature secondary growth.

	PRMARY BODY (at the shoot apex)			
Species	**Stem shape including pith**	**Vascular bundle distribution**	**Laticifer location(s)**	**Pericyclic fibers width variation**
P. alata	Angular	Unequal (polycyclic eustele)	Cortex	Continuous in size
P. sp.	Angular	Unequal	Cortex	Large masses associated with vascular bundles
P. baileyi	Angular	Unequal	Cortex	Large masses associated with vascular bundles
P. bracteosa	Angular	Unequal	Cortex and phloem	Continuous in size
P. dasystachya	Angular	Unequal	Cortex	Large masses associated with vascular bundles
P. echinata	Circular	Unequal	Cortex and phloem	Large masses associated with vascular bundles
P. exalata	Angular	Equal	Cortex	Continuous in size
P. fibrigera	Circular	Equal	Cortex and phloem	Large masses associated with vascular bundles
P. fimbriata	Angular	Unequal	?	?
P. latifolia	Angular	Unequal	Cortex and phloem(?)	Continuous in size
P. obovata	Angular	Unequal	Cortex(?)	Large masses associated with vascular bundles
P. pinnata	Angular	Unequal	Cortex	Large masses associated with vascular bundles
P. rubiginosa	Angular	Unequal	Cortex	?
P. rugosa	Angular	Unequal	Cortex	Large masses associated with vascular bundles
P. spicata	Angular	Unequal	Cortex	Continuous in size
P. sprucei	Circular	Equal	Cortex	Continuous in size
P. turbacensis	Angular	Unequal	Cortex	Continuous in size
P. weinmannifolia	Angular	Unequal	Cortex	Large masses associated with vascular bundles

Appendix G: Stem and Wood anatomy data in Chapter Three

Table 9 Continued

INTERMEDIATE (Onset of Secondary Growth)

Species	**Stem shape**	**Stem type**	**Vessel dimorphism**	**Large Vessel grouping**	**Small Vessel grouping**
P. alata	Angular	Compound (3 peripheral cylinders)	+	Solitaire and tangential multiples of 2	Solitaire and radials of 3-5
P. sp.	Angular	Regular	+	Solitaire and tangential multiples of 2	Solitaire and radial of 2-4
P. baileyi	Angular	Regular	+	Solitaire	Solitaire and radials of 4-6
P. bracteosa	Angular	Phloem wedges	+	Solitaire	Solitaire and radial of 2-4
P. dasystachya	Angular	?	?	?	?
P. echinata	Circular	Regular	+	Solitaire and tangential multiples	Radials of 2-4
P. exalata	Angular	Compound (4 peripheral cylinders)	+	Solitaire and tangential multiples of 2	Solitaire and radials of 4-6
P. fibrigera	Circular	Regular	+	Solitaire and tangential multiples of 2	Solitaire and radial multiples of 2-3
P. fimbriata	Angular	?	?	?	?
P. latifolia	Angular	Regular	+	Solitaire and tangential multiples of 2	radials of 6-11
P. obovata	Angular	lobed	?	?	?
P. pinnata	Angular	Compound (3 peripheral cylinders)	?	?	?
P. rubiginosa	Angular	Phloem wedges	?	?	?
P. rugosa	Angular	Phloem wedges	+	Solitaire and tangential multiples of 2	Radials of 2-5
P. spicata	Angular	Compound (3 peripheral cylinders)	+	Solitaire	Solitaire and radials of 2-4
P. sprucei	Circular	Regular	+	Solitaire and radials of 2	Solitaire and radials of 2
P. turbacensis	Angular	Phloem wedges	+	Solitaire	Radials of 2-4
P. weinmannifolia	Angular	Regular	+	Solitaire	Solitaire and radials of 2-4

Appendix G: Stem and Wood anatomy data in Chapter Three

Table 9 Continued

MATURE SECONDARY GROWTH

Species	**Stem outline**	**Pith shape**	**Vascular cambial variant**	**Growth rings**	**Cell type demarking growth rings**
P. alata	Triangle	Triangle	Compound (3 peripheral cylinders)	-	NA
P. sp.	Circular - lobed	Circular	NA	+	Flattened Fibers
P. baileyi	Circular	Slightly 5-point star angular	Phloem wedges (as per Acevedo designation on Smithsonian liana project)	-	NA
P. bracteosa	Triangle	Triangle		-	NA
P. dasystachya	Triangle(?)	Circular- slightly	Phloem wedges	-	NA
P. echinata	Circular	Circular	NA	-	NA
P. exalata	Triangle	Triangle	Compound (4 peripheral cylinders)	-	NA
P. fibrigera	Circular	Circular	NA	+	Flattened Fibers
P. fimbriata	Triangle	Triangle	Phloem wedges	-	NA
P. latifolia	Circular	5-point angular	Very Shallow phloem wedges	-	NA
P. obovata	Triangle	Triangle	Lobed xylem	-	NA
P. pinnata	Triangle	Triangle- circular	Compound (3 peripheral cylinders)	-	NA
P. rubiginosa	5-point angular	5-point angular	Phloem wedges	-	NA
P. rugosa	5-point angular	5-point angular	Phloem wedges	-	NA
P. spicata	Triangle	5-point angular	Compound (3 peripheral cylinders)	-	NA
P. sprucei	Circular	Circular	NA	-	NA
P. turbacensis	Triangle	5-point angular	Phloem wedges	+	Flattened Fibers
P. weinmannifolia	Circular	Circular	Successive cambia (Neto et al., 2018)	-	NA

Appendix G: Stem and Wood anatomy data in Chapter Three

Table 9 Continued

MATURE SECONDARY GROWTH

Species	**Porosity**	**Vessel Dimorphism present**	**Large Vessel groupings**	**Small Vessel groupings**	**Solitary Vessel outline**
P. alata	Diffuse	+	Solitaire & tangential multiples of 2	Radials of 2-4 and mostly 9-13	Circular
P. sp.	Diffuse	+	Solitaire & tangential multiples of 2	Radials of 2-4 and 6-9	Circular
P. baileyi	Diffuse	+	Solitaire	Solitaire and Radials of 2-4	Circular
P. bracteosa	Diffuse	+	Solitaire & tangential multiples of 2	Radials of 6-8 and > 9 (15)	Circular
P. dasystachya	Diffuse	+	Solitaire, tangential & radial multiples of 2	Radials of 2-4 and 6-9	Circular
P. echinata	Diffuse	+	Mostly solitaire with some tangential & radial multiples of 2	Radials of 2-4	Circular
P. exalata	Diffuse	+	Solitaire & tangential multiples of 2	Radials of 2-4 and 6-9	Circular
P. fibrigera	Diffuse	+	Solitaire & tangential multiples of 2	Solitaire and radials of 2-6	Circular
P. fimbriata	Diffuse	+	Solitaire & tangential multiples of 2	Radials of 6-9	Circular
P. latifolia	Diffuse	+	Solitaire & tangential multiples of 2	Radials of 3-4	Circular
P. obovata	Diffuse	+	Solitaire, tangential & radial multiples of 2	Radials of 3-4	Circular
P. pinnata	Diffuse	+	Solitaire & tangential multiples of 2	Radials of 3-4	Circular
P. rubiginosa	Diffuse	+	Solitaire & tangential multiples of 2	Radials of 3-4	Circular
P. rugosa	Diffuse	+	Solitaire & tangential multiples of 2	Radials of (2)4-13	Circular
P. spicata	Diffuse	+	Solitaire & tangential multiples of 2	Radials of 2-4 and 6-9	Circular
P. sprucei	Diffuse	+	Solitaire & tangential multiples of 2	Solitaire and Radials of 2-4	Circular
P. turbacensis	Diffuse	+	Solitaire & tangential multiples of 2	Radials of 2-4 and 6-9	Circular
P. weinmannifolia	Diffuse	+	Solitaire and radials of 2	Radials of 6-9	Circular

Appendix G: Stem and Wood anatomy data in Chapter Three

Table 9 Continued

MATURE SECONDARY GROWTH

Species	**Perforation plates**	**Intervessel pits**	**Vestured pits**	**Vessel-ray pitting**	**Small vessels lumen diameter (µm) [n≥ 300]**	**Large vessels lumen diameter(µm) [n≥ 300]**
P. alata	Simple	Alternate	-	Same	27.27028	106.369
P. sp.	Simple	Alternate	-	Same	27.7133	59.4604
P. baileyi	Simple	Alternate	-	?	?	?
P. bracteosa	Simple	Alternate	-	Same	29.70932	97.8808
P. dasystachya	Simple	Alternate	-	Same	37.80272	95.623
P. echinata	Simple	Alternate	-	Same	29.97363	158.612
P. exalata	Simple	Alternate	-	Same	?	?
P. fibrigera	Simple	Alternate	-	Same	20.30446	105.949
P. fimbriata	Simple	Alternate	-	Same	26.8266	87.6233
P. latifolia	Simple	Alternate	-	Same	38.35175	156.094
P. obovata	Simple	Alternate	-	Same	?	?
P. pinnata	Simple	Alternate	-	Same	?	?
P. rubiginosa	Simple	Alternate	-	Same	?	?
P. rugosa	Simple	Alternate	-	Same	28.80972	112.968
P. spicata	Simple	Alternate	-	Same	32.52374	93.2917
P. sprucei	Simple	Alternate	-	Same	19.28282	61.5984
P. turbacensis	Simple	Alternate	-	Same	30.73455	100.778
P. weinmannifolia	Simple	Alternate	-	Same	23.0456	87.2367

Appendix G: Stem and Wood anatomy data in Chapter Three

Table 9 Continued

MATURE SECONDARY GROWTH

Species	Gums in vessels	Septate fibers present	Non-septate fibers present	Parenchyma-like fibre bands alternating with ordinary fibres	Fiber pits	Parenchyma arrangement	Axial parenchyma strand length
P. alata	-	+	+	+	Simple	Scanty to Vasicentric	?
P. sp.	+	+	+	+	Simple	Scanty	?
P. baileyi	+	+	+	+	?	?	?
P. bracteosa	-	+	+	+	Simple	Scanty	?
P. dasystachya	-	+	+	+	Simple	Vasicentric	2
P. echinata	+	+	+	-	Simple	Vasicentric	2
P. exalata	+	+	+	+	Simple	Scanty	?
P. fibrigera	-	+	+	+	Simple	Vasicentric	2
P. fimbriata	+	+	+	+	Simple	Scanty	2
P. latifolia	-	+	+	-	Simple	Scanty to Vasicentric	2
P. obovata	+	+	+	-	Simple	Vasicentric	2
P. pinnata	+	+	+	+	Simple	Vasicentric	?
P. rubiginosa	+	+	+	+	Simple	Vasicentric	?
P. rugosa	+	+	+	+	Simple	Scanty	?
P. spicata	-	+	+	+	Simple	Scanty	2
P. sprucei	-	+	+	+	Simple	Vasicentric	2
P. turbacensis	+	+	+	+	Simple	Vasicentric	2
P. weinmannifolia	+	+	-	-	Simple	NA	?

Appendix G: Stem and Wood anatomy data in Chapter Three

Table 9 Continued

MATURE SECONDARY GROWTH

Species	Ray dimorphism	Large ray width	Small ray width	Merging rays	Ray cellular composition	Perforated ray cells	Injury cells that resemble oil cells in the ray
P. alata	+	6-10	1-2	+	Mixed	-	-
P. sp.	+	3-4	1-2	+	Mixed, mostly upright and square	-	-
P. baileyi	+	6	1-2	+	Mixed, most procumbent and square	-	?
P. bracteosa	+	5	1-2	+	Mixed, mostly upright	-	+
P. dasystachya	+	5	1-2	+	Mixed	-	-
P. echinata	+	3-5	1-2	+	Mixed	-	-
P. exalata	+	3	1-2	+	Mixed	-	+
P. fibrigera	+	5	1-2	+	Mixed	-	-
P. fimbriata	+	5-6	1-2	+	Mixed, mostly upright and square	-	+
P. latifolia	+	6-10	1	+	Mixed, mostly procumbent and upright	-	-
P. obovata	+	5	1-2	+	?	-	-
P. pinnata	?	?	?	?	Mixed, mostly upright and square	-	-
P. rubiginosa	?	?	?	?	Mixed	?	?
P. rugosa	+	5	1-2	+	Mixed	-	+
P. spicata	+	6	1-2	+	Mixed	-	-
P. sprucei	-	1-2	1-2	-	Mixed	-	-
P. turbacensis	+	5	1-2	+	Mixed	-	-
P. weinmannifolia	+	5	1	+	Mixed	-	-

Appendix G: Stem and Wood anatomy data in Chapter Three

Table 9 Continued

MATURE SECONDARY GROWTH

Species	Storied structure	Oil or mucilage cells	Stained (unidentified) substance in the rays	Intercellular canals	Laticifer location(s)	Prismatic crystals in ray parenchyma	Prismatic crystals in axial parenchyma
P. alata	-	-	+	-	Cortex	+	+
P. sp.	-	-	-	-	Cortex	-	+
P. baileyi	-	-	+	-	?	?	?
P. bracteosa	-	-	+	-	Cortex and early formed secondary phloem	-	+
P. dasystachya	-	-	-	-	Cortex	+	-
P. echinata	-	-	-	-	Cortex and early formed secondary phloem	+	-
P. exalata	-	-	?	-	Cortex	-	+
P. fibrigera	-	-	+	-	Cortex and early formed secondary phloem	-	+
P. fimbriata	-	?	?	-	Cortex and early formed secondary phloem	+	-
P. latifolia	-	-	+	-	?	+	+
P. obovata	?	-	+	-	Cortex and early formed secondary phloem	+	+
P. pinnata	-	-	+	-	?	-	+
P. rubiginosa	-	?	+	-	Cortex	-	-
P. rugosa	-	-	-	-	Cortex and early formed secondary phloem	-	-
P. spicata	-	-	+	-	Cortex and not sure if in phloem	+	+
P. sprucei	-	-	-	-	Cortex	+	+
P. turbacensis	-	-	-	-	Cortex	+	-
P. weinmannifolia	-	-	+	-	NA	-	-

Appendix G: Stem and Wood anatomy data in Chapter Three

Table 10. Primary body shape and mature stem type data for *Paullinia;* data utilized for ancestral state reconstruction in Figure 16

Taxon	**Collection**	**Primary body Shape (0= circular, 1=angular)**	**Mature Stem Type (0= regular, 1=phloem wedges, 2= lobed xylem, 3=compound, 4=successive cambia)**
P. acutangula	Acevedo 14325	1	1
P. acutangula	Acevedo 14325	1	1
P. acutangula	Acevedo 14917	1	1
P. alata	Acevedo 14393	1	3
P. alata	Acevedo 14871	1	3
P. alata	Acevedo 7586	1	3
P. alata	Dodson 5860	1	3
P. allenii	Acevedo 15184	1	2
P. allenii	Acevedo 15255	1	2
P. alsmithii	Granville 16866	1	3
P. alsmithii	Villa 1781	1	3
P. baileyi	Acevedo 15182	1	1
P. baileyi	Chery 37	1	1
P. bilobulata	Acevedo 14853	0	0
P. boliviana	Acevedo 14324	1	2
P. bracteosa	Acevedo 15185	1	1
P. bracteosa	Chery 26	1	1
P. bracteosa	Chery 39	1	1
P. caloptera	Acevedo 14315	1	1
P. carpopodea	Pace 317	1	0
P. carpopodea	Perdiz 519	1	0
P. carpopodea	Perdiz 701	1	0
P. cf.boliviana	Acevedo 14916	1	2
P. cf.nobilis	Acevedo 15050	1	1
P. cf.stellata	Acevedo 14298	1	1
P. clathrata	Acevedo 15261	1	0

Appendix G: Stem and Wood anatomy data in Chapter Three

Table 10 Continued

P. costaricensis	Acevedo 15139	0	0
P. dasystachya	Acevedo 14819	1	1
P. elegans	Acevedo 11135	1	3
P. elongata	Acevedo 14305	1	0
P. elongata	Acevedo 14401	1	0
P. eriocarpa	Acevedo 7612	1	3
P. exalata	Acevedo 14891	1	3
P. faginea	Burnham 1556	1	0
P. fibrigera	Acevedo 15191	0	0
P. fibrigera	Chery 21	0	0
P. fimbriata	Clark 4386	1	1
P. fuscescens	Acevedo 11459	1	0
P. fuscescens	Acevedo 15253	1	0
P. glomerulosa	Acevedo 15194	0	0
P. glomerulosa	Chery 20	0	0
P. hystrix	Acevedo 14408	1	0
P. hystrix	Acevedo 14417	1	0
P. hystrix	Acevedo 14895	1	0
P. hystrix	Acevedo 14898	1	0
P. imberbis	Acevedo 14826	1	1
P. ingifolia	Acevedo 14812	1	0
P. ingifolia	Acevedo 15248	1	0

Appendix G: Stem and Wood anatomy data in Chapter Three

Table 10 Continued

P. ingifolia	Acevedo 15282	1	0
P. ingifolia	Acevedo 16067	1	0
P. jamaicensis	Acevedo 12035	0	0
P. josecuatrii	Acevedo 14291	1	1
P. killipii	Acevedo 14308	1	2
P. killipii	Acevedo 14323	1	2
P. largifolia	Acevedo 14920	1	3
P. latifolia	Acevedo 6038	1	0
P. leiocarpa	Jansen-Jacobs 3924	1	3
P. microneura	Acevedo 14320	1	0
P. neglecta	Beck 23548	1	3
P. neglecta	Nee 37116	1	3
P. obovata	Acevedo 14382	1	2
P. obovata	Acevedo 14441	1	2
P. olivacea	Acevedo 14390	1	0
P. paullinoides	Acevedo 14860	1	0
P. paullinoides	Acevedo 7519	1	0
P. pinnata	Acevedo 6679	1	3
P. pinnata	Chery 41	1	3
P. pinnata	Kayombo 1024	1	3
P. pinnata	Pace 192	1	3
P. racemosa	Acevedo 3708	1	4
P. racemosa	Perdiz 808	1	4
P. racemosa	Roque 2594	1	4
P. rubiginosa	Acevedo 14366	1	1
P. rubiginosa	Perdiz 699	1	1
P. rubiginosa	subsp setosa	1	1
P. rufescens	Acevedo 11120	1	0
P. rugosa	Acevedo 14807	1	1

P. rugosa	Acevedo 15179	1	1
P. rugosa	Chery 25	1	1
P. selenoptera	Acevedo 14813	1	1
P. serjaniifolia	Acevedo 15270	1	2
P. simulans	Acevedo 15235	1	1
P. sp.	Breedlove 72699	0	0
P. sp.	Chery 46	1	0
P. sphaerocarpa	Acevedo 15247	1	0
P. spicata	Acevedo 12344	1	3
P. sprucei	Acevedo 14922	1	0
P. stellata	Torke 615	1	1
P. stipitata	Acevedo 15242	1	3
P. stipitata	Acevedo 15280	1	3
P. tomentosa	Acevedo 12236	0	0
P. trigonia	Perdiz 673	1	4

Appendix G: Stem and Wood anatomy data in Chapter Three

Table 11. Primary body shape and mature stem type data for the Paullinieae tribe; data utilized for ancestral state reconstruction in Figure 17.

Taxon	**Collection**	**Primary Body Shape (0=circular, 1=angular)**	**Mature Stem Type (0= regular, 1=cambial variant)**
Allophylus abyssinicus	Desissa & Binggeli 318	0	0
Allophylus africanus	Balkwill 4206	0	0
Allophylus arboreus	Wohlhauser & Stiefel 60072	0	0
Allophylus bicruris	Barthelat 828	0	0
Allophylus bojerianus	Ratovoson 961	0	0
Allophylus chaunostachys	Mwangoko 729	0	0
Allophylus chirindensis	Hizza 26	0	0
Allophylus comina	Acevedo 12216	0	0
Allophylus crassinervis	No voucher	0	0
Allophylus decipiens	Phillipson 4194	0	0
Allophylus dissectus	Phillipson 1704	0	0
Allophylus gardineri	Pignal 1834	0	0
Allophylus hirtellus	Cheek 5059	0	0
Allophylus leucoclados	Perdiz 785	0	0
Allophylus pervillei	Hoffmann 399	0	0
Allophylus petiolulatus	Perdiz 872	0	0
Allophylus poungouensis	McPherson 16109	0	0
Allophylus psilospermus	Chery 10	0	0
Allophylus pslisospermus	Chery 12	0	0
Allophylus racemosus	Acevedo 12180	0	0
Allophylus rubifolius	Kuchar 23357	0	0
Allophylus semidentatus	Perdiz 777	0	0
Allophylus sp	Acevedo 14847	0	0
Allophylus sp	Barthelat 1540	0	0
Athyana weinnmanniifolia	Acevedo 11166	0	0
Athyana weinnmanniifolia	Villalobos 4243	0	0
Bridgesia incisiifolia	Landrum 9824	0	0
Cardiospermum anomalum	Urdampilleta 330	1	1
Cardiospermum bahianum	Urdampilleta 389	1	1
Cardiospermum corindum	Pace 425	1	1

Cardiospermum corindum	Harder Bringham 3495	1	1
Cardiospermum corindum	KM062239	1	1
Cardiospermum grandiflorum	Peterson 7088	1	1
Cardiospermum grandiflorum	ATBP 603	1	1
Cardiospermum grandiflorum	Gildenhuys H1(?)	1	1
Cardiospermum halicacabum	Egan 15	1	1
Cardiospermum halicacabum	Gildenhuys 280	1	1
Cardiospermum halicacabum	CSM 8	1	1
Cardiospermum halicacabum	Murata 72327	1	1
Cardiospermum heringeri	Urdampilleta 437	0	0
Cardiospermum pechuelii	KM062216	1	1
Lophostigma plumosum	Acevedo 6568	1	1
Lophostigma plumosum	Coulleri 267	1	1
Lophostigma plumosum	Acevedo 6554	1	1
Paullini ingifolia	Acevedo 16067	1	0
Paullini selenoptera	Acevedo 14813	1	1
Paullini serjaniifolia	Acevedo 15270	1	1
Paullini simulans	Acevedo 15235	1	1
Paullini sp.	Breedlove 72699	0	0
Paullini sp.	Chery 46	1	0
Paullini sphaerocarpa	Acevedo 15247	1	0
Paullini spicata	Acevedo 12344	1	1
Paullini sprucei	Acevedo 14922	1	0
Paullini stellata	Torke 615	1	1
Paullini stipitata	Acevedo 15242	1	1
Paullini stipitata	Acevedo 15280	1	1
Paullini tomentosa	Acevedo 12236	0	0
Paullini trigonia	Perdiz 673	1	1
Paullini turbacensis	Acevedo 15176	1	1
Paullini turbacensis	Chery 10	1	1
Paullini turbacensis	Chery 13	1	1
Paullini turbacensis	Chery 24	1	1
Paullinia acutangula	Acevedo 14325	1	1
Paullinia acutangula	Acevedo 14917	1	0
Paullinia alata	Acevedo 14393	1	0
Paullinia alata	Acevedo 14871	1	0
Paullinia alata	Acevedo 7586	1	1
Paullinia alata	Dodson 5860	1	1
Paullinia allenii	Acevedo 15184	1	1
Paullinia allenii	Acevedo 15255	1	0

Paullinia alsmithii	Granville 16866	0	0
Paullinia alsmithii	Villa 1781	1	1
Paullinia baileyi	Acevedo 15182	1	1
Paullinia baileyi	Chery 37	1	0
Paullinia bilobulata	Acevedo 14853	1	0
Paullinia boliviana	Acevedo 14324	1	1
Paullinia bracteosa	Acevedo 15185	1	1
Paullinia bracteosa	Chery 26	1	1
Paullinia bracteosa	Chery 39	1	1
Paullinia caloptera	Acevedo 14315	1	1
Paullinia carpopodea	Pace 317	1	0
Paullinia carpopodea	Perdiz 519	1	0
Paullinia carpopodea	Perdiz 701	1	0
Paullinia cf.boliviana	Acevedo 14916	1	1
Paullinia cf.nobilis	Acevedo 15050	1	1
Paullinia cf.stellata	Acevedo 14298	1	1
Paullinia clathrata	Acevedo 15261	1	0
Paullinia costaricensis	Acevedo 15139	0	0
Paullinia dasystachya	Acevedo 14819	1	1
Paullinia elegans	Acevedo 11135	1	1
Paullinia elongata	Acevedo 14305	1	0
Paullinia elongata	Acevedo 14401	1	0
Paullinia eriocarpa	Acevedo 7612	1	1
Paullinia exalata	Acevedo 14891	1	1
Paullinia faginea	Burnham 1556	1	0
Paullinia fibrigera	Acevedo 15191	0	0
Paullinia fibrigera	Chery 21	0	0
Paullinia fimbriata	Clark 4386	1	1
Paullinia fuscescens	Acevedo 11459	1	0
Paullinia fuscescens	Acevedo 15253	1	0
Paullinia glomerulosa	Acevedo 15194	0	0
Paullinia glomerulosa	Chery 20	0	0
Paullinia hystrix	Acevedo 14408	1	0
Paullinia hystrix	Acevedo 14417	1	0
Paullinia hystrix	Acevedo 14895	1	0
Paullinia hystrix	Acevedo 14898	1	0
Paullinia imberbis	Acevedo 14826	1	1
Paullinia ingifolia	Acevedo 15248	1	0
Paullinia ingifolia	Acevedo 15282	1	1
Paullinia ingifolia	Acevedo 16067	1	1

Paullinia jamaicensis	Acevedo 12035	1	1
Paullinia josecuatrii	Acevedo 14291	1	1
Paullinia killipii	Acevedo 14308	1	1
Paullinia killipii	Acevedo 14323	1	1
Paullinia largifolia	Acevedo 14920	1	1
Paullinia latifolia	Acevedo 6038	1	1
Paullinia leiocarpa	Jansen-Jacobs 3924	1	1
Paullinia microneura	Acevedo 14320	1	1
Paullinia neglecta	Beck 23548	1	0
Paullinia neglecta	Nee 37116	1	1
Paullinia obovata	Acevedo 14382	1	1
Paullinia obovata	Acevedo 14441	1	1
Paullinia olivacea	Acevedo 14390	1	1
Paullinia paullinoides	Acevedo 14860	1	1
Paullinia paullinoides	Acevedo 7519	1	1
Paullinia pinnata	Acevedo 6679	0	0
Paullinia pinnata	Chery 41	1	0
Paullinia pinnata	Kayombo 1024	1	0
Paullinia pinnata	Pace 192	1	1
Paullinia racemosa	Acevedo 3708	1	0
Paullinia racemosa	Perdiz 808	1	1
Paullinia racemosa	Roque 2594	1	1
Paullinia rubiginosa	Acevedo 14366	1	1
Paullinia rubiginosa	Perdiz 699	0	0
Paullinia rubiginosa subsp *setosa*	Acevedo 11114	1	1
Paullinia rufescens	Acevedo 11120	1	1
Paullinia rugosa	Acevedo 14807	1	1
Paullinia rugosa	Acevedo 15179	1	1
Paullinia rugosa	Chery 25	1	1
Serjania atrolineata	Acevedo 15195	1	1
Serjania atrolineata	Chery 18	1	1
Serjania atrolineata	Chery42	1	1
Serjania caracasana	Acevedo 15107	1	1
Serjania cf.*caracasana*	Acevedo 3483	1	1
Serjania circumvallata	Acevedo 15203	1	1
Serjania clematidifolia	Somner 1078	1	1
Serjania communis	Somner 1334	1	1
Serjania cuspidata	Somner 1400	1	1
Serjania deltoidea	Acevedo 15725	1	1

Serjania diversifolia	Acevedo 13462	1	0
Serjania elongata	Acevedo 13547	1	1
Serjania erythrocaulis	Acevedo 3729	1	1
Serjania fluminensis	no voucher	1	1
Serjania fuscifolia	Somner 1455	1	1
Serjania grandiceps	Acevedo 13704	1	1
Serjania grandifolia	Acevedo 11121	1	1
Serjania ichthyoctona	Somner 1081	1	1
Serjania lethalis	Roque 1860	1	1
Serjania lethalis	Somner 1381	1	1
Serjania marginata	Acevedo 11131	1	1
Serjania membranacea	Acevedo 15227	1	1
Serjania membranacea	Acevedo 12329	1	1
Serjania mexicana	Chery 23	1	1
Serjania mexicana	Chery 45	1	1
Serjania mexicana	Acevedo 12014	1	1
Serjania mexicana	Acevedo 15080	1	1
Serjania paniculata	Acevedo 15143	1	1
Serjania paucidentata	Chery 34	1	0
Serjania perulacea	Acevedo 11134	1	0
Serjania pyramidata	Acevedo 15243	1	0
Serjania pyramidata	Chery 29	1	0
Serjania reticulata	Torke 508	1	0
Serjania rhombea	Acevedo 15213	1	0
Serjania salzmanniana	Perdiz 806	1	0
Serjania trachygona	Acevedo 15222	1	0
Serjania unguiculata	Acevedo 15081	1	0
Thinouia mucronata	Zardini 13513	1	0
Thinouia mucronata	Keller 6919	1	0
Thinouia restingaev	Somner 1074	1	0
Thouinia acuminata	Liston 633-2	1	0
Thouinia paucidentata	Acevedo 12160	1	0
Thouinia portoricensis	Acevedo 11435	0	0
Thouinia villosa	Hall 825	0	0
Urvillea chacoensis	Acevedo 11133	0	0
Urvillea chacoensis	Keller 6834	0	0
Urvillea ulmacea	Balick 3349	0	0
Urvillea ulmacea	Acevedo 15145	0	0
Urvillea ulmacea	Reyes-Garcia 5585	0	0

Taxon	Collection	Primary Body Shape (0=circular, 1=angular)	Mature Stem Type (0= regular, 1=cambial variant)
Allophylus abyssinicus	Desissa & Binggeli 318	0	0
Allophylus africanus	Balkwill 4206	0	0
Allophylus arboreus	Wohlhauser & Stiefel 60072	0	0
Allophylus bicruris	Barthelat 828	0	0
Allophylus bojerianus	Ratovoson 961	0	0
Allophylus chaunostachys	Mwangoko 729	0	0
Allophylus chirindensis	Hizza 26	0	0
Allophylus comina	Acevedo 12216	0	0
Allophylus crassinervis	No voucher	0	0
Allophylus decipiens	Phillipson 4194	0	0
Allophylus dissectus	Phillipson 1704	0	0
Allophylus gardineri	Pignal 1834	0	0
Allophylus hirtellus	Cheek 5059	0	0
Allophylus leucoclados	Perdiz 785	0	0
Allophylus pervillei	Hoffmann 399	0	0
Allophylus petiolulatus	Perdiz 872	0	0
Allophylus poungouensis	McPherson 16109	0	0
Allophylus psilospermus	Chery 10	0	0
Allophylus pslisospermus	Chery 12	0	0
Allophylus racemosus	Acevedo 12180	0	0
Allophylus rubifolius	Kuchar 23357	0	0
Allophylus semidentatus	Perdiz 777	0	0
Allophylus sp	Acevedo 14847	0	0
Allophylus sp	Barthelat 1540	0	0
Athyana weinnmanniifolia	Acevedo 11166	0	0
Athyana weinnmanniifolia	Villalobos 4243	0	0
Bridgesia incisiifolia	Landrum 9824	0	0
Cardiospermum anomalum	Urdampilleta 330	1	1
Cardiospermum bahianum	Urdampilleta 389	1	1
Cardiospermum corindum	Pace 425	1	1
Cardiospermum corindum	HarderBringham 3495	1	1
Cardiospermum corindum	KM062239	1	1
Cardiospermum grandiflorum	Peterson 7088	1	1
Cardiospermum grandiflorum	ATBP 603	1	1
Cardiospermum grandiflorum	Gildenhuys H1(?)	1	1

Cardiospermum halicacabum	Egan 15	1	1
Cardiospermum halicacabum	Gildenhuys 280	1	1
Cardiospermum halicacabum	CSM 8	1	1
Cardiospermum halicacabum	Murata 72327	1	1
Cardiospermum heringeri	Urdampilleta 437	0	0
Cardiospermum pechuelii	KM062216	1	1
Lophostigma plumosum	Acevedo 6568	1	1
Lophostigma plumosum	Coulleri 267	1	1
Lophostigma plumosum	Acevedo 6554	1	1
Paullinia acutangula	Acevedo 14325	1	1
Paullinia acutangula	Acevedo 14917	1	0
Paullinia alata	Acevedo 14393	1	0
Paullinia alata	Acevedo 14871	1	0
Paullinia alata	Acevedo 7586	1	1
Paullinia alata	Dodson 5860	1	1
Paullinia allenii	Acevedo 15184	1	1
Paullinia allenii	Acevedo 15255	1	0
Paullinia alsmithii	Granville 16866	0	0
Paullinia alsmithii	Villa 1781	1	1
Paullinia baileyi	Acevedo 15182	1	1
Paullinia baileyi	Chery 37	1	0
Paullinia bilobulata	Acevedo 14853	1	0
Paullinia boliviana	Acevedo 14324	1	1
Paullinia bracteosa	Acevedo 15185	1	1
Paullinia bracteosa	Chery 26	1	1
Paullinia bracteosa	Chery 39	1	1
Paullinia caloptera	Acevedo 14315	1	1
Paullinia carpopodea	Pace 317	1	0
Paullinia carpopodea	Perdiz 519	1	0
Paullinia carpopodea	Perdiz 701	1	0
Paullinia cf.boliviana	Acevedo 14916	1	1
Paullinia cf.nobilis	Acevedo 15050	1	1
Paullinia cf.stellata	Acevedo 14298	1	1
Paullinia clathrata	Acevedo 15261	1	0
Paullinia costaricensis	Acevedo 15139	0	0
Paullinia dasystachya	Acevedo 14819	1	1
Paullinia elegans	Acevedo 11135	1	1
Paullinia elongata	Acevedo 14305	1	0
Paullinia elongata	Acevedo 14401	1	0
Paullinia eriocarpa	Acevedo 7612	1	1

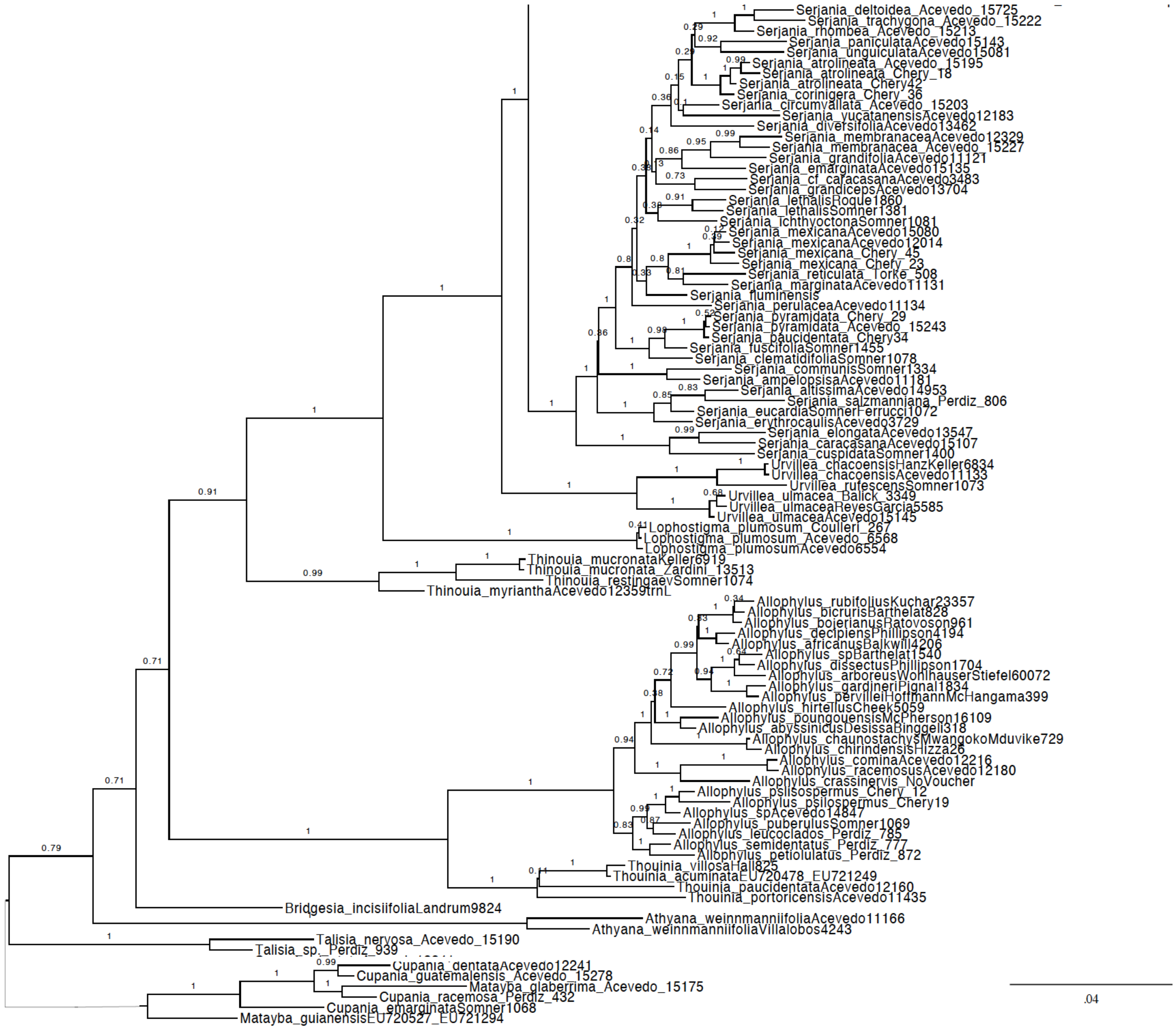
Paullinia exalata	Acevedo 14891	1	1
Paullinia faginea	Burnham 1556	1	0
Paullinia fibrigera	Acevedo 15191	0	0
Paullinia fibrigera	Chery 21	0	0
Paullinia fimbriata	Clark 4386	1	1
Paullinia fuscescens	Acevedo 11459	1	0
Paullinia fuscescens	Acevedo 15253	1	0
Paullinia glomerulosa	Acevedo 15194	0	0
Paullinia glomerulosa	Chery 20	0	0
Paullinia hystrix	Acevedo 14408	1	0
Paullinia hystrix	Acevedo 14417	1	0
Paullinia hystrix	Acevedo 14895	1	0
Paullinia hystrix	Acevedo 14898	1	0
Paullinia imberbis	Acevedo 14826	1	1
Paullinia ingifolia	Acevedo 15248	1	0
Paullinia ingifolia	Acevedo 15282	1	1
Paullinia ingifolia	Acevedo 16067	1	1
Paullinia jamaicensis	Acevedo 12035	1	1
Paullinia josecuatrii	Acevedo 14291	1	1
Paullinia killipii	Acevedo 14308	1	1
Paullinia killipii	Acevedo 14323	1	1
Paullinia largifolia	Acevedo 14920	1	1
Paullinia latifolia	Acevedo 6038	1	1
Paullinia leiocarpa	Jansen-Jacobs 3924	1	1
Paullinia microneura	Acevedo 14320	1	1
Paullinia neglecta	Beck 23548	1	0
Paullinia neglecta	Nee 37116	1	1
Paullinia obovata	Acevedo 14382	1	1
Paullinia obovata	Acevedo 14441	1	1
Paullinia olivacea	Acevedo 14390	1	1
Paullinia paullinoides	Acevedo 14860	1	1
Paullinia paullinoides	Acevedo 7519	1	1
Paullinia pinnata	Acevedo 6679	0	0
Paullinia pinnata	Chery 41	1	0
Paullinia pinnata	Kayombo 1024	1	0
Paullinia pinnata	Pace 192	1	1
Paullinia racemosa	Acevedo 3708	1	0
Paullinia racemosa	Perdiz 808	1	1
Paullinia racemosa	Roque 2594	1	1
Paullinia rubiginosa	Acevedo 14366	1	1

Paullinia rubiginosa	Perdiz 699	0	0
Paullinia rubiginosa subsp *setosa*	Acevedo 11114	1	1
Paullinia rufescens	Acevedo 11120	1	1
Paullinia rugosa	Acevedo 14807	1	1
Paullinia rugosa	Acevedo 15179	1	1
Paullinia rugosa	Chery 25	1	1
Serjania grandifolia	Acevedo 11121	1	1
Serjania ichthyoctona	Somner 1081	1	1
Serjania lethalis	Roque 1860	1	1
Serjania lethalis	Somner 1381	1	1
Serjania marginata	Acevedo 11131	1	1
Serjania membranacea	Acevedo 15227	1	1
Serjania membranacea	Acevedo 12329	1	1
Serjania mexicana	Chery 23	1	1
Serjania mexicana	Chery 45	1	1
Serjania mexicana	Acevedo 12014	1	1
Serjania mexicana	Acevedo 15080	1	1
Serjania paniculata	Acevedo 15143	1	1
Serjania paucidentata	Chery 34	1	0
Serjania perulacea	Acevedo 11134	1	0
Serjania pyramidata	Acevedo 15243	1	0
Serjania pyramidata	Chery 29	1	0
Serjania reticulata	Torke 508	1	0
Serjania rhombea	Acevedo 15213	1	0
Serjania salzmanniana	Perdiz 806	1	0
Serjania trachygona	Acevedo 15222	1	0
Serjania unguiculata	Acevedo 15081	1	0
Thinouia mucronata	Zardini 13513	1	0
Thinouia mucronata	Keller 6919	1	0
Thinouia restingaev	Somner 1074	1	0
Thouinia acuminata	Liston 633-2	1	0
Thouinia paucidentata	Acevedo 12160	1	0
Thouinia portoricensis	Acevedo 11435	0	0
Thouinia villosa	Hall 825	0	0
Urvillea chacoensis	Acevedo 11133	0	0
Urvillea chacoensis	Keller 6834	0	0
Urvillea ulmacea	Balick 3349	0	0
Urvillea ulmacea	Acevedo 15145	0	0
Urvillea ulmacea	Reyes-Garcia 5585	0	0
Paullini selenoptera	Acevedo 14813	1	1

Paullini serjaniifolia	Acevedo 15270	1	1
Paullini simulans	Acevedo 15235	1	1
Paullini sp.	Breedlove 72699	0	0
Paullini sp.	Chery 46	1	0
Paullini sphaerocarpa	Acevedo 15247	1	0
Paullini spicata	Acevedo 12344	1	1
Paullini sprucei	Acevedo 14922	1	0
Paullini stellata	Torke 615	1	1
Paullini stipitata	Acevedo 15242	1	1
Paullini stipitata	Acevedo 15280	1	1
Paullini tomentosa	Acevedo 12236	0	0
Paullini trigonia	Perdiz 673	1	1
Paullini turbacensis	Acevedo 15176	1	1
Paullini turbacensis	Chery 10	1	1
Paullini turbacensis	Chery 13	1	1
Paullini turbacensis	Chery 24	1	1
Serjania atrolineata	Acevedo 15195	1	1
Serjania atrolineata	Chery 18	1	1
Serjania atrolineata	Chery42	1	1
Serjania caracasana	Acevedo 15107	1	1
Serjania cf.*caracasana*	Acevedo 3483	1	1
Serjania circumvallata	Acevedo 15203	1	1
Serjania clematidifolia	Somner 1078	1	1
Serjania communis	Somner 1334	1	1
Serjania cuspidata	Somner 1400	1	1
Serjania deltoidea	Acevedo 15725	1	1
Serjania diversifolia	Acevedo 13462	1	0
Serjania elongata	Acevedo 13547	1	1
Serjania erythrocaulis	Acevedo 3729	1	1
Serjania fluminensis	no voucher	1	1
Serjania fuscifolia	Somner 1455	1	1
Serjania grandiceps	Acevedo 13704	1	1
Paullini ingifolia	Acevedo 16067	1	0

Appendix H: Paullinieae maximum clade credibility tree from Chapter Three

(phylogeny displayed in two parts for best visualization)


Serjania_deltoidea_Acevedo_15725
Serjania_trachygona_Acevedo_15222
Serjania_rhombea_Acevedo_15213
Serjania_paniculataAcevedo15143
Serjania_unguiculataAcevedo15081
Serjania_atrolineata_Acevedo_15195
Serjania_atrolineata_Chery_18
Serjania_atrolineata_Chery42
Serjania_corinigera_Chery_36
Serjania_circumvallata_Acevedo_15203
Serjania_yucatanensisAcevedo12183
Serjania_diversifoliaAcevedo13462
Serjania_membranaceaAcevedo12329
Serjania_membranacea_Acevedo_15227
Serjania_grandifoliaAcevedo11121
Serjania_emarginataAcevedo15135
Serjania_cf_caracasanaAcevedo3483
Serjania_grandicepsAcevedo13704
Serjania_lethalisRoque1860
Serjania_lethalisSomner1381
Serjania_ichthyoctonaSomner1081
Serjania_mexicanaAcevedo15080
Serjania_mexicanaAcevedo12014
Serjania_mexicana_Chery_45
Serjania_mexicana_Chery_23
Serjania_reticulata_Torke_508
Serjania_marginataAcevedo11131
Serjania_fluminensis
Serjania_perulaceaAcevedo11134
Serjania_pyramidata_Chery_29
Serjania_pyramidata_Acevedo_15243
Serjania_paucidentata_Chery34
Serjania_fuscifoliaSomner1455
Serjania_clematidifoliaSomner1078
Serjania_communisSomner1334
Serjania_ampelopsisAcevedo11181
Serjania_altissimaAcevedo14953
Serjania_salzmanniana_Perdiz_806
Serjania_eucardiaSomnerFerrucci1072
Serjania_erythrocaulisAcevedo3729
Serjania_elongataAcevedo13547
Serjania_caracasanaAcevedo15107
Serjania_cuspidataSomner1400
Urvillea_chacoensisHanzKeller6834
Urvillea_chacoensisAcevedo11133
Urvillea_rufescensSomner1073
Urvillea_ulmacea_Balick_3349
Urvillea_ulmaceaReyesGarcia5585
Urvillea_ulmaceaAcevedo15145
Lophostigma_plumosum_Coulleri_267
Lophostigma_plumosum_Acevedo_6568
Lophostigma_plumosumAcevedo6554
Thinouia_mucronataKeller6919
Thinouia_mucronata_Zardini_13513
Thinouia_restingaevSomner1074
Thinouia_myrianthaAcevedo12359trnL
Allophylus_rubifoliusKuchar23357
Allophylus_bicrurisBarthelat828
Allophylus_bojerianusRatovoson961
Allophylus_decipiensPhillipson4194
Allophylus_africanusBalkwill4206
Allophylus_spBarthelat1540
Allophylus_dissectusPhillipson1704
Allophylus_arboreusWohlhauserStiefel60072
Allophylus_gardineriPignal1834
Allophylus_pervilleiHoffmannMcHangama399
Allophylus_hirtellusCheek5059
Allophylus_poungouensisMcPherson16109
Allophylus_abyssinicusDesissaBinggeli318
Allophylus_chaunostachysMwangokoMduvike729
Allophylus_chirindensisHizza26
Allophylus_cominaAcevedo12216
Allophylus_racemosusAcevedo12180
Allophylus_crassinervis_NoVoucher
Allophylus_psilospermus_Chery_12
Allophylus_psilospermus_Chery19
Allophylus_spAcevedo14847
Allophylus_puberulusSomner1069
Allophylus_leucoclados_Perdiz_785
Allophylus_semidentatus_Perdiz_777
Allophylus_petiolulatus_Perdiz_872
Thouinia_villosaHall825
Thouinia_acuminataEU720478_EU721249
Thouinia_paucidentataAcevedo12160
Thouinia_portoricensisAcevedo11435
Bridgesia_incisifoliaLandrum9824
Athyana_weinmanniifoliaAcevedo11166
Athyana_weinmanniifoliaVillalobos4243
Talisia_nervosa_Acevedo_15190
Talisia_sp._Perdiz_939
Cupania_dentataAcevedo12241
Cupania_guatemalensis_Acevedo_15278
Matayba_glaberrima_Acevedo_15175
Cupania_racemosa_Perdiz_432
Cupania_emarginataSomner1068
Matayba_guianensisEU720527_EU721294
.04