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and not part of supertribe Paullinioidae. The following 14 new combinations are made to incorporate the results of our findings: Serjania biternata, Serjania cristobalitae, Serjania dissecta, Serjania guerrerensis, Serjania macrolopha, Serjania serjanoides, Serjania sonorensis, Serjania spinosa, Serjania tortuosa, Thinouia trifoliata, Urvillea cuchujaquense, Urvillea oliveirae, Urvillea procumbens, and Urvillea pterocarpa.

Response to Reviewers: Figure 2 has been replaced with a tiff file. All other supplementary figures and data have been uploaded in Dryad.
Dear editors,

Herein we addressed the comments received after we submitted the revision to our paper.

"A significant concern that I have is that separate plastid and ITS analyses were not reported in the manuscript. I think it is important that such results are included (the figures can be included as supplements if desired) and discussed. If the two trees are congruent, then I think it is fine to present the combined analyses, but it is impossible to determine this from the manuscript."

We had included this statement at the beginning of the Results section:

"Separate analyses of each locus did not reveal any strongly supported groupings that would indicate incongruence, although resolution in trnL was poor due to the low number of informative characters."

We have emended this to more clearly indicate what was done with this observation:

"Separate analyses of each locus did not reveal any strongly supported groupings that would indicate incongruence, although resolution in trnL was poor due to the low number of informative characters. Based on this broad congruence, we combined the loci for the total evidence approach on which we base our Discussion"

Given the poor resolution in trnL, it is something of a moot point to belabor the issue in the text or present rather uninformative trees as graphics.

"Please include phylogram(s) of the ML tree(s) you present. Phylograms are extremely useful ways to visually interpret evolutionary rates. It is fine if you prefer not to include them as primary figures, and in that case then please include them as supplementary figures."

We have included a phylogram as supplemental figure. The phylogram we chose to include is one of our alternative analysis (125-tip data set) that included Cardiospermum integerrimum to show it's exceptionally long branch that may affect its placement. Since the manuscript is already rich in graphics, we do not believe that it adds much value for the space to the main text. We have a revised supplement but the question of what to do with it remains. Our intent was to fit it into Dryad, although ideally it would be part of the article. The policy of such supplements in Systematic Botany generally precludes what we had in mind and what is standard for other journals. When looking at a recent model Gostel et al. (41:67-81, 2016), they refer to supplemental trees in the manner we do, but those supplements do not appear to be available through the journal.

"Lastly, there is no indication in the manuscript that the trees themselves have been deposited in Dryad (or TreeBASE). Please include these, or if they have already been included in the Dryad submission, please indicate so in the revised manuscript."

We deposited two data matrixes and three supplemental figures. The following DOI has been assigned: doi:10.5061/dryad.p2g6p
Generic Relationships and Classification of Tribe Paullinieae (Sapindaceae) with a New Concept of Supertribe Paullinioidae

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Abstract—The current study examines all genera of Sapindaceae tribe Paullinieae sensu Acevedo-Rodríguez et al. (2011). Based on molecular phylogenetic analyses of trnL intron and ITS sequence data along with critical evaluation of morphology, supertribe Paulliniodae is newly recognized and morphologically characterized by zygomorphic flowers, thyrses with lateral cincinni, corollas of 4 petals, and alternate leaves with a well-developed distal leaflet. Paulliniodae contains four successively nested subclades designated as tribes Athyaneae, Bridgesieae, Thouinieae, and Paullinieae. Athyaneae contains Athyana and Diatenoptyryx, and is composed of trees with exstipulate pinnately compound leaves, and isopolar, spherical, colporate pollen grains. Bridgesieae contains the monospecific shrub genus Bridgesia with exstipulate, simple leaves, and isopolar, spherical, tricolporate pollen grains. Thouinieae is resurrected and amended to include three genera of trees or shrubs with exstipulate trifoliolate or unifoliolate leaves. Paullinieae is circumscribed to include only six genera (Thinouia, Lophostigma, Cardiospermum, Paullinia, Serjania and Urvillea) of climbers or climber-derived shrubs with stipulate leaves and a pair of inflorescence tendrils. Guindilia, previously considered a member of this clade, is shown to be more distantly related and not part of supertribe Paulliniodae. The following 14 new combinations are made to incorporate the results of our findings: Serjania biternata, Serjania cristobaliae, Serjania dissecta, Serjania guerrerensis, Serjania macrolopha, Serjania serjanioides, Serjania sonorense, Serjania spinosa, Serjania tortuosa, Thinouia trifoliata, Urvillea cuchujaquense, Urvillea oliveira, Urvillea procumbens, and Urvillea pterocarpa.

Keywords—Athyaneae, Bridgesieae, Diatenoptyryx, Guindilia, molecular phylogenetics, Thouinieae.
Since the publication of the most recent taxonomic treatment of Sapindaceae (Acevedo-Rodríguez et al. 2011), where four subfamilies (Xanthoceroideae, Hippocastanoideae, Dodonaeoideae, and Sapindoideae) and 142 genera were recognized, four new genera (Alatococcus Acev.-Rodr., Allophylastrum Acev.-Rodr., Balsas J. Jiménez Ram. & K. Vega, and Gereaua [Capuron] Buerki & Callm.) have been described. Following the description of these new genera and the results of this analysis, we currently recognize 144 genera with ~1,900 species within the family. According to Acevedo-Rodríguez et al. (2011), the Sapindaceae in the New World contains about 925 species, of which ~475 belong to the Paullinieae (i.e., Paullinieae + Thouinieae, sensu Radlkofer 1890, 1931). Paullinieae are primarily Neotropical with the exception of Paullinia pinnata L., Cardiospermum corindum L., and C. halicacabum L., which in addition to their Neotropical ranges, are naturally distributed in parts of the Paleotropics (Brncic et al. 2007; Gildenhuys et al. 2015). Thouinieae is by far more diverse at the generic level in the Neotropics than in the Paleotropics. However, at the species level, there are many more species (~197 species) in the Paleotropics, all of which belong to the genus Allophylus (Coelho 2014).

The Paullinieae was proposed by Kunth as a “section” of Sapindaceae in 1821 to accommodate Cardiospermum L., Paullinia L., Serjania Mill., and Urvillea Kunth. This group of herbaceous or woody climbers with tendrils was characterized by the presence of well-developed petal appendages, unilateral nectaries with distinct lobes, and 3-carpellate gynoecia with uniovular locules. In 1824, De Candolle designated Kunth’s Paullinieae as a tribe, which was later adopted by Radlkofer in 1890 in his tribal classification of Sapindaceae that included 14 tribes. Radlkofer further characterized Paullinieae by the presence of stipules, and compound
leaves with a fully developed distal leaflet. The presence of stipules is unique within Sapindaceae to the Paullinieae, while the latter two characters are shared with members of the closely related Thouinieae. Radlkofer divided Paullinieae into the subtribes, Eupaullinieae and Thinouineae. The Eupaullinieae was essentially the same group as the one recognized by Kunth but further characterized by the presence of zygomorphic flowers with hood-shaped petal appendages. Subtribe Thinouineae solely contained Thinouia Triana & Planch., described in 1862, and was characterized by the presence of actinomorphic flowers with marginal or bifid petal appendages and an annular disc. Additional genera were later included in the Eupaullinieae: Lophostigma Radlk. was described in 1897 and is characterized by the presence of short petals and heteropolar, prolate pollen grains; Houssayanthus Hunz. was described in 1978 and is characterized by mericarps with a circumferential wing; and Chimborazoa H. T. Beck was described in 1992 and is characterized by wingless spherical mericarps.

Radlkofer’s system of classification of 1890 placed Paullinieae in the vicinity of the Thouinieae, a tribe that included the genera Guindilia Gillies ex Hook. & Arn., Bridgesia Bertero ex Cambess., Athyana Radlk., Diatenopteryx Radlk., Thouinia Poit., and Allophylus L. The Thouinieae was characterized by an arboreal or shrubby habit and zygomorphic flowers in lateral cincinni (or a simplification of it). Radlkofer’s concept of Paullinieae had survived through much of the 20th century and was only modified by Acevedo-Rodríguez (1993) who proposed placing Thinouia in the Cupanieae near Allosanthus Radlk. More recently, Thinouia was reinstated in tribe Paullinieae following recent molecular phylogenetic analyses (Acevedo-Rodríguez, unpubl. data; Acevedo-Rodríguez et al. 2011). At the same time, Allosanthus was treated as congeneric with Thinouia as its only differentiating character, i.e., the spicate inflorescence, was not considered worthy of generic recognition (Acevedo-Rodríguez et al. 2011).
Cladistic analyses of the Paullinieae and Thouinieae, based on morphological characters, indicated a monophyletic Paullinieae nested within a paraphyletic Thouinieae (Acevedo-Rodríguez 1993). Analyses of eight DNA sequence regions from the plastid and nuclear genomes by Buerki et al. (2009, 2010, 2011) recovered a “Paullinia group” of genera assignable to the Paullinieae and Thouinieae. Following the conclusions suggested in Acevedo-Rodríguez (1993) and Buerki et al. (2009), Acevedo-Rodríguez et al. (2011) placed the genera of Thouinieae in a newly circumscribed Paullinieae, which encompassed 12 genera. That classification made sense in light of the then sparse molecular phylogenetic taxon-sampling, but now the results of more comprehensive analyses call for a tribal reclassification.

Our study includes genera (Balsas and Allophylastrum) assignable to the Paullinieae that were published after the treatment of Sapindaceae by Acevedo-Rodríguez et al. (2011) as well as Sapindus oligophyllus Merr. & Chun, proposed by Buerki et al. (2009, 2010, 2011) as a member of their Paullinia group. Balsas was described to account for a species considered closely related to Paullinia, but which is distinguished by its exarillate seeds and hemi-tricolpate pollen grains. Allophylastrum, which vegetatively closely resembles Allophylus, was described due to its actinomorphic, apetalous, solitary (or on short racemes) flowers, and 4–5-porate pollen grains. The position of Sapindus oligophyllus as sister to Thouinia acuminata S. Watson (Buerki et al. 2009) was clarified by analyzing sequence data from its type collection. In addition, the validity of Chimborazoa, already reduced to synonymy in Serjania (Acevedo-Rodríguez 1998), was further tested in our analyses.

The objectives of our study were to analyze molecular and morphological evidence to: (1) evaluate the current circumscription of tribe Paullinieae (sensu Acevedo-Rodríguez et al.
2011), (2) test the monophyly of the genera currently included in this tribe, and (3) elucidate the relationships among the genera once considered to belong in the Paullinieae.

**MATERIALS AND METHODS**

**Sampling**—We sampled all genera of tribe Paullinieae (here recognized as supertribe Paulliniodae) sensu Acevedo-Rodríguez et al. (2011), and where possible multiple species per genus (see Appendix 1). The outgroups selected based on the family-wide phylogenies (i.e., Buerki et al. 2009, 2011), included 16 genera of subfamily Sapindoideae, which represent a successive series of nested sister clades to Paullinieae. The plastid *trnL* intron and nuclear ribosomal internal transcribed spacer, ITS, were selected as molecular phylogenetic markers because they have proven useful in Sapindaceae (e.g., Buerki et al. 2009).

**Molecular Methods**—DNA extractions, amplifications, and Sanger sequencing for the 184 newly generated sequences were conducted largely according to standard methods as outlined here. Silica-gel dried or herbarium leaf tissue (ca 1.0 cm²) was pulverized with glass and steel beads in a TissueLyser (Qiagen, Hilden, Germany). DNA extractions from the resulting tissue powder were mostly performed with the Qiagen DNeasy Plant Mini Kit according to the manufacturer’s protocol. The herbarium extractions of *Allophylastrum, Chimborazoa*, and *Sapindus oligophyllus* included an overnight incubation with proteinase K, and each sample was not co-processed (i.e., from DNA extractions through sequencing) with any other Sapindaceae. We believe the data for each of these is authentic and not derived from contamination based on the extra care taken during the extractions, expected correlation of DNA quality and amplification success, and the unique sequences obtained. The *trnL* intron was amplified and sequenced using primers “c” and “d” (Taberlet et al. 1991), and ITS was amplified using ITS5a
(Stanford et al. 2000) and ITS4 (White et al. 1990). The amplification reactions were conducted in a final volume of 25 µl with 2.5 µl of Bioline ammonium reaction buffer (10×), 2.0 µl of dNTP mix (10 mM), 1.25 µl of magnesium chloride (50 mM), 1.0 µl of each primer (10 nM), 0.5 µl of bovine serum albumin (10 mg/ml), 14.05 µl of water, 0.2 µl of Biolase DNA polymerase (5U/µl; Bioline USA, Taunton, Massachusetts) and 2.5 µl of DNA extraction (~20 ng/µl).

Amplification conditions were 95ºC for 3 min, followed by 35 cycles of 94ºC for 40 sec, 55ºC for 40 sec and 72ºC for 1 min 40 sec, with a 10 min final extension at 72ºC. PCR products were treated with ExoSAP-IT (Affymetrix-USB, Santa Clara, California) prior to sequencing.

Sequencing reactions were conducted using 8 µl of a cycle sequencing reaction mixture (including 0.8 µl BigDye Terminator v3.1 [Applied Biosystems, Foster City, California], 1.0 µl of 1 µM primer, 2.0 µl of 5× buffer, 4.2 µl water) combined with 4 µl of DNA template. The cycle sequencing conditions were 30 cycles of 95ºC for 30 sec, 50ºC for 30 sec and 60ºC for 4 min. The cycle sequencing products were purified using Sephadex G-50 (GE Healthcare Bio-Sciences, Pittsburgh, Pennsylvania) and run on an ABI 3730xl DNA Analyzer (Applied Biosystems) at the Smithsonian. The resulting reads were assembled and edited using Sequencher ver. 5.2.4 (Gene Codes, Ann Arbor, Michigan).

**Phylogenetic Analyses** — We assembled and did preliminary analyses on a complete data set that included our new data plus additional published trnL and/or ITS sequences for Paulliniodae (i.e., from Buerki et al. 2009, 2011; Urdampilleta et al. 2013; Gildenhuys et al. 2015). In our final data set we included some of this published data that complemented our taxon sampling, did not cause unnecessary taxon redundancy, and where we could confirm the identifications. The trnL alignment was straightforward with most indels representing tandem repeats while that of the ITS was complex with many ambiguous regions within ITS1 and ITS2.
Alignments were generated using MAFFT ver.7 (Katoh 2013) under the Q-INS-i refinement method (alternative MAFFT iterative refinement methods did not greatly improve the alignment) and/or using Clustal Omega (Sievers et al. 2011). For the trnL alignment we first inferred the secondary structure of one outgroup with the ViennaRNA package (Lorenz et al. 2011), which was then applied as a constraint to improve the alignment using RNAsalsa (Stocsits et al. 2009). Our concatenated datasets included a 125-tip sampling with some taxa having missing data in one partition (i.e., incorporating published data such as that for Cardiospermum), had manual refinement of the alignment using a similarity criterion, and had a manual character deletion set made by removing matrix columns with >50% missing data. This method eliminated overlapping indels with clearly noisy characters and reduced missing data, but did not remove all ambiguously aligned regions. We also created a 99-tip sampling that minimized missing data (i.e., by removing most tips that were missing one of the partitions and which especially impacted Cardiospermum), was not manually refined, and used Noisy (Dress et al. 2008) as an automated approach to remove “noisy” sites.

To further assess the monophyly of Paullinieae – in particular the effect of previously unsampled Lophostigma and Thinouia on the backbone of that subclade – and to assess the suspected misidentification of Sapindus oligophyllus in Buerki et al. (2011), we manually added our data (trnL and ITS) for those taxa to their 8-marker family-wide data set archived in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S10580). Two new gaps (insertions relative to rest of the data set) were added to trnL and we manually refined the ITS partition to improve alignment consistency within genera of Paullinionidae and within Harpullia Roxb., Lepisanthes Blume, and Llagunoa Ruiz & Pav. This combined taxon sampling contained 247 tips, including five new taxa and deleting their chimeric Haplocoelum foliosum (Hiern.)
Bullock (i.e., \textit{trnL}, EU721250, clusters with the “Dodonaea group” and is likely mislabeled). Our concatenated matrices, along with supplemental figures are archived in the Dryad Digital Repository (http://dx.doi.org/10.5061/dryad.p2g6p).

We determined nucleotide substitution models for four data partitions (i.e., ITS1, ITS2, 5.8S, and \textit{trnL}) in jModelTest ver. 2.1.2 (Darriba et al 2012) using the Akaike information criterion (AIC; Akaike 1974). For both individual and concatenated matrix analyses we performed parsimony, maximum likelihood (ML), and Bayesian analyses. Parsimony analyses on the 99-tip data set were performed with TNT ver.1.5 (Goloboff 2000) with a two-stage search using 100,000 replicates of TBR followed by SPR, and keeping the best 10 trees for each replicate (maximum of 100 million trees). Parsimony non-parametric bootstrap (Felsenstein 1985) analyses included 1 million replicates, using the same approach as in the tree search. ML analyses of the 99-tip data set were made with RAxML ver. 8.0.0 (Stamatakis 2014) on an unpartitioned matrix and 20 simultaneous runs, each followed by bootstrap analysis with 100,000 replicates. ML analyses of the 125-tip Paullinieae and 247-tip Sapindaceae data set were with GARLI ver.2.1 (Zwickl 2006) on an unpartitioned matrix; GARLI-ML bootstrap analysis included 500 replicates. Finally, Bayesian analyses under the GTR + I + \Gamma model were performed on the 99-tip data set using MrBayes (Ronquist and Huelsenbeck 2003) with four simultaneous runs, each with four Markov chains (three cold and one heated) for a total of 16 simultaneous chains including 1,000,000 generations. Phylogenetic trees were produced using Figtree ver. 1.4.0 (Rambaut 2015), Dendroscope ver.3 (Huson and Scornavacca 2012), and/or Mesquite ver. 3.04 (Maddison and Maddison 2015).

\textit{Morphology}—Morphological characterization of the genera is based on the treatment of the Sapindaceae by Acevedo-Rodríguez et al. (2011), Ferrucci (1991), and further study of
collections at the U.S. National Herbarium (US). Pollen morphology applies Erdtman’s terminology (Erdtman 1966) and the studies of van der Ham (Acevedo-Rodríguez et al. 2011), van der Ham and Tomlik (1994), and Ferrucci and Anzótegui (1993). Chromosome data come from Ferrucci (2000a), de Freitas et al. (2007), Urdampilleta et al. (2013), and the Index to Plant Chromosome Numbers. We have adopted the terms ‘anterior’ and ‘posterior’, as opposed to traditional terminology, to refer to specific floral parts as done by Ferrucci and Somner (2010), and Ferrucci and Urdampilleta (2011a, 2011b). Anterior is the side where the 3rd and 5th sepals are located; and posterior is the side on which the 4th sepal is found (Fig. 3c). Other floral parts are described according to their relative position between these two points of reference.

RESULTS

Phylogenetic Analyses—Data set statistics are summarized in Table 1 and results of our primary combined 99-tip analyses are shown in Figure 1, with additional analyses in Suppl. Figs. 1–3. Separate analyses of each locus did not reveal any strongly supported groupings that would indicate incongruence, although resolution in trnL was poor due to the low number of informative characters. Based on this broad congruence, we combined the loci for the total evidence approach on which we base our discussion. The 99- and 125-tip analyses had different approaches to data set polishing (automated vs. manual) and software (RAxML vs. GARLI). Both topologies are highly congruent but the 99-tip sampling is better resolved at deeper nodes and serves as the point of reference for our discussion. The long branches we observed (Suppl. Fig. 2) in Cardiospermum integerrimum Radlk. suggest a need for caution regarding the placement of that taxon, although its inclusion or exclusion did not greatly change the topology, or bootstrap values in neighboring subclades. Inspection of the ITS matrix indicated a
substitution bias that resulted in a GC content reduction to 45% in *Cardiospermum integerrimum* from an average across the rest of the data of 61% (range 58–65%). Data consistency between our sequence and that of Urdampilleta et al. (2013) contraindicates a lab-specific artifact. Our combined 99-tip DNA analyses show strong support (Bayesian posterior probability (PP)=1; ML bootstrap percentage (MLBP)=96) for a clade containing most taxa placed by Acevedo-Rodríguez et al. (2011) in the Paullinieae. This clade, here called supertribe Paulliniodae, contains four supported monophyletic groups, which are here classified as tribes *Athyaneae*, *Bridgesieae*, *Thouinieae* and *Paullinieae*. Tribe *Athyaneae* containing *Athyana* and *Diatenopteryx* is strongly supported (PP=1; MLBP=100) as basal within Paulliniodae. Tribe *Bridgesieae* with a single species (*Bridgesia incisifolia* Cambess.) is sister to the *Thouinieae* + *Paullinieae* (PP=1; MLBP=98). Tribe *Thouinieae* is strongly supported (PP=1; MLBP=100) as a clade including *Thouinia* as sister to *Allophylus* plus *Allophylastrum*. *Allophylastrum* is weakly embedded in a paraphyletic *Allophylus* (PP<60; MLBP<50) and requires additional data to be resolved. Tribe *Paullinieae* has strong support (PP=1; MLBP=94) and a basal clade formed by *Thinouia*, successively followed by *Lophostigma* and then a poorly resolved crown group of three subclades containing *Serjania*, *Paullinia*, *Urvillea*, *Cardiospermum*, *Balsas*, and *Houssayanthus*. Although *Serjania* is poorly resolved, it is clearly paraphyletic because of deeply embedded *Balsas*, *Chimborazoa* and *Houssayanthus*. *Cardiospermum* as currently circumscribed (Ferrucci 2000b) is polyphylectic, with species groping with *Serjania*, *Paullinia*, and *Urvillea*. The core *Cardiospermum* s.s. subclade (containing the type species *C. halicacabum*; sampled in the 125-tip analysis; Suppl. Fig. 1) is sister to *Paullinia* (PP=1; MLBP=70). *Guindilia* is among the outgroup taxa, and although support is not strong in the 99-tip analyses as to an exact placement, it is clearly excluded from Paulliniodae.
Our analyses of the 247-tip family-wide data set show (Suppl. Fig. 3) broad topological similarity among the sampled Paulliniodae to our focused 2-gene results, with two notable exceptions: (1) Bridgesia is sister to Thouinieae (MLBP=93) rather than sister to Thouinieae + Paullinieae, and (2) Guindilia is sister to the rest of Paulliniodae (MLBP=87) rather than farther out. Sapindus oligophyllus clearly groups (MLBP=100) with Lepisanthes, an affiliation proposed by Xia and Gadek (2007) who transferred S. oligophyllus to Lepisanthes. Therefore, the anomalous position of this taxon within the Paullinia group by Buerki et al. (2009, 2010, 2013) is due to the misidentification of their sample, which is likely a species of Allophylus.

**DISCUSSION**

**Characterization of the Paulliniodae and Nested Tribes**—Our best estimate (Fig. 1) of phylogenetic relationships of Paullinieae and its close relatives is incompletely resolved but makes considerable progress in a group that has been poorly sampled in prior phylogenetic studies. The examination of chromosome numbers, and important reproductive and vegetative characters (i.e., potential morphological synapomorphies) in the context of our trees reveals patterns of character state change that deserve closer examination (Fig. 2; Table 2). Supertribe Paulliniodae is characterized by the following morphological synapomorphies: leaves with a fully developed distal leaflet, thyrses with lateral cincinni, zygomorphic flowers with 4–5 sepals, 4 petals, unilateral nectary discs, schizocarpic fruits with divaricate mericarps, and reduced chromosome numbers ($2n \leq 30$).

Tribe Athyaneae, although distinct based on molecular data, is difficult to define with synapomorphic morphological characters. However, the tribe can be recognized by pinnately compound leaves in addition to the features that characterize the Paulliniodae. Athyana and
Diatenopteryx can be distinguished by a combination of a 3-carpellate gynoecium and an indumentum lacking glandular hairs in Athyana, and the 2-carpellate gynoecium and indumentum with glandular hairs in Diatenopteryx. These two genera are currently restricted to southern South America, but according to the fossil pollen record, Athyana once was present in North America during the Oligocene (MacGinitie 1953).

Tribe Bridgesieae is strongly supported by molecular data and is recognized by its simple, exstipulate leaves in addition to the same floral characters as in Paulliniodae and by short, axillary cymes (a single, few-flowered cincinnus) or flowers solitary. Simple leaves and reduced cincinni are considered morphological synapomorphies for the Bridgesieae. Monospecific Bridgesia is currently restricted to the Andean region of Chile; however, fossil pollen records indicate its presence in North America during the Eocene (Graham 2011).

Tribe Thouinieae is characterized by trifoliolate (or trifoliolate-derived) leaves, zygomorphic flowers, thyrses with lateral cincinni and by the oblate to subglobose, porate pollen grains (Figs. 6a–b). Thouinia, which is sister to Allophylus and Allophylastrum, does not seem to have any morphological apomorphies, but can be differentiated from the latter two genera by its schizocarpic fruit with 3 distally winged mericarps (Fig. 4g). The subclade containing Allophylastrum and Allophylus is defined by fruits with coccate monocarps (Fig. 4h–i). Although Allophylastrum is easily distinguished by unique morphological features (see treatment below), its phylogenetic position is poorly resolved with respect to Allophylus with individual trees indicating it as either sister to, or alternatively embedded within, Allophylus. While our sampling of Allophylus is broad, sequence variation is low and additional loci are needed to resolve this point. Except for Allophylus, the genera of Thouinieae are restricted to the New World. Thouinia is currently known from the Greater Antilles (except Jamaica), Mexico and northern Central
America but it has been documented in pollen samples from the Eocene to the Oligocene of North America (Muller 1981; Graham 2011). Accordingly, Thouinieae evolved in the New World, as the Neotropical genus Thouinia is the first diverging lineage of this clade and possibly shared a common ancestor with Allophylus and Allophylastrum. Hence, Allophylus evolved in the New World and later dispersed to Africa, Madagascar, and Asia. Poorly known Allophylastrum is restricted to northern South America, while Allophylus has a pantropical distribution. Allophylus has been documented as early as the middle Eocene from fossil pollen from the Gatuncillo Formation in Panama (Graham 1987).

Tribe Paullinieae is essentially Neotropical and the most species diverse of the four tribes. It is characterized by the presence of a climbing habit, stipules, and tendrils that seem to be homologous to the lower cincinni of the inflorescence (Darwin 1897). Floral symmetry for the most part is zygomorphic (Fig. 3a) with the exception of Thinouia, which has actinomorphic flowers (Fig. 3b). Actinomorphic flowers are plesiomorphic within the Paullinioidae and their occurrence in the Paullinieae can be best explained as a likely reversal. Thinouia is the first diverging lineage of this clade and although it does not have any morphological apomorphies, it is recognizable (in addition to the characters that define the Paullinioidae) by the presence of actinomorphic flowers, isopolar tricolporate pollen grains, and mericarps with distal wings (Figs. 3b, 4a and 5f). The next inclusive clade is formed by Lophostigma in a sister position to a clade containing Serjania, Paullinia, Urvillea, and Cardiospermum. The presence of heteropolar hemi-tri-syncolporate pollen grains (or its transformation) is considered synapomorphic in this clade (Figs. 5b–e). Lophostigma stands alone by the presence of prolate heteropolar 4-aperturate pollen grains and petals shorter than the sepals (Fig. 5e). The most inclusive clade is defined as a polytomy uniting Serjania, Paullinia, Urvillea, and Cardiospermum. This clade is characterized
by the presence of petals that are as long as, or longer than, the sepals, and by oblate pollen grains (Figs. 3a and 5b-d).

The greater species richness of Paullinieae has been hypothesized to be the result of the ecological specializations in its innovative climbing habit and development of zygomorphic flowers (Buerki et al. 2013). This suggestion however, does not explain finer differences in species richness, where *Cardiospermum*, *Lophostigma*, and *Urvilla* have far fewer species than *Paullinia* and *Serjania*. Flower zygomorphism has evolved in all four subfamilies of Sapindaceae presumably from ancestors with actinomorphic flowers. Although most large genera in Sapindaceae have zygomorphic flowers, this character alone cannot explain differences in species richness. For example, *Acer* L. with about 125 species and *Dodonaea* Miller with about 68 species have actinomorphic flowers. In addition, from a total of 61 genera of Sapindaceae with zygomorphic flowers, only five have more than 30 species and 24 contain a single species. Thus, the poor correlation of habit and floral symmetry with species richness in the Sapindaceae does not seem to explain taxonomic richness in the Paullinieae.

Novel seed dispersal mechanisms in Paullinieae could have played an important role in the evolution, specialization, and diversification of *Paullinia* and also perhaps of *Serjania*. *Paullinia* is largely a forest dweller while *Serjania* favors open savannah-like formations. The presence of bird and water dispersed seeds in *Paullinia* seems to be an adaptation for dispersal throughout the extensive Amazon biome, allowing *Paullinia* to reach new micro-niches and eventually to diversify into distinct species (Figs. 7a–d and 8a–b). Species richness in *Serjania*, however, cannot be explained solely by its wind-dispersed mericarps, as this feature also occurs in the much less diverse lianoid genera *Thinouia* and *Lophostigma* (Figs. 4a, c). *Serjania* may have capitalized on this dispersal mechanism by becoming a dry habitat specialist in more open
environments (Acevedo-Rodríguez 1990), while Thinouia is mostly found in moister habitats where wind dispersed species are less abundant (Mori and Brown 1994), and Lophostigma is restricted to high elevations. The poor species diversity observed in Cardiospermum and Urvillea may be due to the lack of effective dispersal mechanisms which otherwise would allow these genera to reach and radiate into novel habitats. In both genera, the seeds remain attached to the septa of the dehisced capsules and are only liberated by gravity after the decay of the septa (Fig. 8c). The suggestion that capsules of Cardiospermum may be water or wind dispersed (Harden 2002) is highly unlikely, as it has not been documented and because the capsule dehisces long before the fruit abscises from the inflorescence.

**Chromosome Number**—Recent studies on chromosome structure, size, and number provide additional insights into the evolution of the Paulliniodae (Ferrucci 2000a; Urdampilleta et al. 2013; Index to Plant Chromosome Numbers 1979—). When chromosome number is examined in the context of our trees, a pattern emerges of reduction from 2n = 32 (Talisia Aubl. and Matayba Aubl.) to 2n ≤ 30 in Paulliniodae (Fig. 2), with occasional reports of polyploidy. This trend is evident in the Athyaneae (Diatenopteryx) where 2n = 30 and in the closely related Thouinieae (Allophylus) and the basal taxa of Paullinieae (Thinouia and Lophostigma) where the number is further reduced to 2n = 28. The most derived clade (Cardiospermum, Paullinia, Serjania, and Urvillea) is characterized by 2n ≤ 24, with even further reduction to 2n ≤ 22 in Cardiospermum and Urvillea.

**Exclusion of Guindilia from Paulliniodae**—Guindilia, which heretofore has been considered a member of the Paullinieae (Thouinieae sensu Radlkofher 1931), is placed in our 99-tip phylogeny among the outgroup taxa, and clearly outside of the strongly supported Paulliniodae. While this suggests that it does not belong in the supertribe, its exact position
within the tree may be an artifact of our limited outgroup sampling. A richer phylogenetic context still excludes *Guindilia* from Paulliniodae with placement as either sister to the supertribe (i.e., our 247-tip results in Suppl. Fig. 3; also Buerki et al. 2011) or a placement further out and roughly similar to our 99-tip results (i.e., divergence dating in Buerki et al. 2013). Our data from a new accession of *Guindilia* agrees with that of Buerki et al. (2011). Morphologically, this genus differs from other Paulliniodae by the presence of opposite, simple leaves. Although the disc in *Guindilia* flowers is unilateral, it is roughly pyramidal-shaped and two-lobed, a feature that is not present in the Paulliniodae. We defer to assign *Guindilia* to any group until its phylogenetic position is better resolved.

**Origin of the Paulliniodae and Nested Tribes** —Our analyses do not suggest a clear affinity of the Paulliniodae with any of the outgroup taxa. However, based on pollen morphology, van der Ham and Tomlik (1994) suggested the parallel evolution of Paullinieae and Thouinieae from a *Cupania*-like ancestor. This hypothesis posits that the hemi-syncolporate pollen found in *Serjania, Cardiospermum*, and *Urvillea* is necessarily derived from a syncolporate or parasyncolporate pollen type that is found in the Cupanieae. Support for this claim is found in the sporadic presence of distal vestigial colpi in the pollen of these three genera, and fine traces of a parasyncolporate condition in *Athyana* (van der Ham and Tomlik 1994; Acevedo-Rodríguez 2011). From the floral morphology point of view, this hypothesis is plausible as some Cupanieae (e.g., species of *Guioa* Cav.) have zygomorphic flowers with unilateral, nectary discs similar to those in the Paulliniodae. The alternative hypotheses of derivation from an ancestral group with colporate pollen such as *Sapindus* L. (Acevedo-Rodríguez 1993) or *Thinouia*-like (Ferrucci and Anzótegui 1993) would have to account for the
unlikely transformation of tricolporate into the hemi-tri-syncolporate pollen present in *Serjania* and allied genera.

Buerki et al. (2013) offered a hypothesis for the origin of the Paulliniodae (their “Paullinia group”) as a result of a migration event of ancestral Sapindaceae from Australia via Antarctica during the Middle Eocene. Although this hypothesis and the proposed timing of events is not supported by the available fossil record, it is possible that such a migratory route existed much earlier as suggested by the wide occurrence of *Cupaniopsis* pollen (Cupanieae) during the middle Cretaceous in Antarctica, Africa (Gabon), South America (Brazil), and North America (Coetzee and Muller 1984; Muller 1981). Although no specific ancestor is mentioned by Buerki et al. (2013), their consensus tree has *Blomia* Miranda and the Melicocceae closer to the Paulliniodae than to the Cupanieae. *Blomia* and the Melicocceae have colporate pollen grains and could share a common ancestor with the Paulliniodae.

The constraints of the van der Ham and Tomlik (1994) hypothesis do not preclude a common ancestor with isopolar colporate pollen like those of *Talisia,* and *Melicoccus* P. Browne although it would require the additional evolutionary step of becoming syncolporate. Our analyses do not provide support for either scenario but it would be an interesting hypothesis to test by expanding the molecular sampling coupled with examining the pollen of *Blomia* and the Melicocceae for vestigial Cupanieae-like pollen characters. From a palynological point of view, *Paullinia* could have been derived from a common ancestor with *Cardiospermum, Serjania,* and *Urvillea.* Its isopolar, triporate pollen (Fig. 4c) is seen as derived from the type found in *Serjania.* The presence of subisopolar pollen grains in a few species of *Paullinia* is seen as a vestigial character that adds weight to this argument. In addition, the presence of capsular fruits with thick walls and seeds without sarcotesta in *Balsas* (herein considered a *Serjania*) is
interpreted as a step in the derivation of the capsules in *Paullinia* from an ancestor with schizocarpic, thin-walled fruits and non-sarcodeal seeds.

The persistence of plesiomorphic characters such as the trigonous-subglobose, tricolporate, striate pollen grains (*Athyaneae, Bridgesieae, Allophyclus, Thinouia, and Lophostigma*) and actinomorphic flowers (*Allophylastrum* and *Thinouia*) are indicative of mosaic-like character evolution in the Paulliniodae. In addition, the occurrence of transitional plesiomorphic characters such as the sub-zygomorphic flowers in *Lophostigma* and some species of *Allophylus* provide further evidence for mosaic evolution and provide possible links between genera. For example, striate pollen and sub-zygomorphic flowers in *Lophostigma* are seen as intermediate between *Thinouia* and the remaining genera (*Serjania, Cardiospermum, Urvillea, and Paullinia*) of Paullinieae. The presence of sub-actinomorphic flowers within *Allophylus* suggests an incipient reversal (from zygomorphic to actinomorphic flowers) and provides a connection with *Allophylastrum* where actinomorphic flowers are found. This notion also supports the interpretation of actinomorphic flowers in *Thinouia* as a reversal. Instances of intermediate or incipient characters are also found in *Serjania* where a few species show features (pseudo-hilum, and capsules) that are characteristic of *Cardiospermum, Urvillea, and Paullinia*. While our resolution of major subclades within supertribe Paulliniodae is incomplete, an examination of morphological characters with reference to the persistence of plesiomorphic and intermediate morphological characters, suggests links among genera and testable hypotheses of evolutionary trends.

Pollen fossils identified as *Allophylus, Bridgesia, Cardiospermum, Paullinia, Serjania,* and *Thouinia* indicate the wide establishment of supertribe Paulliniodae in the Americas by the late Eocene (Gray 1960; Muller 1981; Graham 1987, 2011). Moreover, the fact that *Allophylus*
fossils have only been found in the Old World in recent geological times further supports the hypothesis that *Allophylus* evolved in the Americas and later dispersed to Africa and other regions of the Old World.

**A New Classification**—The recognition of Supertribe Paullinioidae allows for the retention of tribes that are recognizable from a suite of morphological characters. Tribe Athyaneae although similar to the Thouinieae, contains trees with exstipulate, pinnately compound leaves with fully developed distal leaflets. Tribe Bridgesieae contains shrubs with simple, exstipulate leaves, and short, axillary cymes (a single few-flowered cincinni) or solitary flowers. Tribe Thouinieae deviates from previous classifications by excluding *Athyana, Bridgesia, Diatenopteryx, and Guindilla* and contains trees or shrubs with trifoliolate or unifoliolate, exstipulate leaves, and zygomorphic flowers. Finally, tribe Paullinieae agrees with previous classifications (De Candolle 1824; Radlkofer 1890), and contains climbers or climber-derived shrubs with stipulate leaves, and tendrils. We have made limited taxonomic changes toward the goal of recognizing only monophyletic genera, although problems still remain with regard to *Allophylastrum* and *Cardiospermum* (i.e., *Cardiospermum integerrimum, C. urvilleoides*). Not all of these changes are based on direct molecular phylogenetic placement, but rather some are inferred based on close morphological similarity to our sampled taxa.

**TAXONOMIC TREATMENT**

Supertribe **PAULLINIOIDEAE**

Lianas, vines, trees shrubs, or hemicryptophytes. Leaves alternate, with a fully developed distal leaflet; thyrses with lateral cincinni; flowers zygomorphic, with 4–5 sepals, 4 petals,
unilateral nectary disc (or exceptionally actinomorphic), and schizocarpic (or schizocarpic derived) fruits.

KEY TO THE TRIBES

1. Lianas, climbing shrubs, or less often erect or hemicryptophytic shrubs; stipules minute to foliaceous; tendrils paired at base of inflorescence rachis ........................................... Paullinieae

1. Trees or erect shrubs; stipules and tendrils wanting

2. Leaves pinnately compound .................................................................................. Athyaneae

2. Leaves trifoliolate, unifoliolate or simple

3. Leaves trifoliolate or unifoliolate (pulvinulate at the base of blade); fruits bicoccate or schizocarpic with non-bladder-like mericarps, apically elongated into a long (distal) wing; pollen porate ................................................................................................. Thouinieae

3. Leaves simple; fruit schizocarpic, with bladder-like mericarps, transversely elongated into a short (dorsal) wing; pollen tricolporate .......................................................... Bridgesieae

Tribe Athyaneae Acev.-Rodr., tribus nova—TYPE: Athyana

Exstipulate, small trees, leaves alternate imparipinnate with fully developed distal leaflet; flowers zygomorphic; pollen isopolar, obtusely triangular in polar view, subspherical in equatorial view, tricolporate, with colpi nearly reaching the poles.
Small trees; exstipulate. Leaves alternate, imparipinnate, distal leaflet fully developed or exceptionally rudimentary. Inflorescences of thyrses with flowers in lateral cincinni; pedicel articulate (abscission zone) below the middle. Flowers zygomorphic, structurally or functionally unisexual; calyx 4–5-merous, sepals valvate or imbricate; corolla of 4 distinct petals, each with an adaxial, basal, hood-shaped appendage; disc semiannular; stamens (6–)8, filaments usually of different lengths, anther dorsifixed; gynoecium 2- or 3-carpellate with a single ovule per carpel.

**Distribution**—Two genera distributed in southern South America.

**Key to the Genera**

1. Gynoecium 3-carpellate; leaf rachis winged .................................................. *Athyana*

1. Gynoecium 2-carpellate; leaf rachis unwinged .................................................. *Diatenopteryx*

**Athyana** (Griseb.) Radlk. in T. Durand, Index Gen. Phan. 73. 1887 [“1888”].

—TYPE: *Athyana weinmanniifolia* (Griseb.) Radlk.

Duodichogamous trees; exstipulate. Leaves imparipinnate; leaflets (5) 7–15, serrate; distal leaflet fully developed; rachis winged. Inflorescences axillary or pseudoterminal, simple or double thyrses; flowers on lateral, reduced cincinni (seemingly a depauperate dichasium); pedicels articulate. Flowers zygomorphic; calyx actinomorphic, sepals 5, valvate, of similar size, with the 2 anterior sepals connate at base; petals 4, as long as the sepals, with a single basal, hood-shaped, crested appendage; disc semiannular, lobed; stamens (7)8, filaments of unequal lengths, anthers dorsifixed; pollen isopolar, obtusely triangular in polar view, subspherical in equatorial view, tricolporate, with colpi nearly reaching the poles (Fig. 6d), finely perforate (also
faintly parasyncolporate fide van der Ham and Tomlik 1994); ovary 3-carpellate; stigma capitate. Fruit schizocarpic, splitting into 3 samaroid mericarps with a dorsal-distal, ascending wing; seed exarillate. A single species found in Peru, Bolivia, Paraguay, and Argentina.


Duodichogamous small to large trees; exstipulate. Indumentum of simple hairs. Leaves imparipinnate or paripinnate; leaflets dentate or serrate; distal leaflet fully developed; rachis unwinged. Inflorescences terminal or axillary thyrses, with flowers in lateral cincinni; pedicel articulate. Flowers zygomorphic, functionally unisexual; sepals 4, free, imbricate, the anterior sepal larger; petals 4, up to four times as long as the sepals, each with a basal hood-shaped, crested appendage; disc semiannular, crenate; stamens (6–)8, of unequal length, anthers dorsifixed; pollen isopolar, obtusely triangular in polar view, subspherical in equatorial view, tricolporate, with elongated colpi nearly reaching the poles, rugulate (Fig. 6c); ovary 2-carpellate, v-shaped; style filiform; stigma bifid. Fruits schizocarpic, splitting into 2 samaroid divaricate, mericarps with a long dorsal wing; seed exarillate. Two species from Brazil, Bolivia, Paraguay, and Argentina. 2n= 30.

Tribe **Bridgesieae** Acev.-Rodr., tribus nova—**TYPE:** *Bridgesia*

Exstipulate, shrubs; leaves alternate simple; flowers zygomorphic; pollen isopolar, subspherical in polar and equatorial views, tricolporate.
Characters as in *Bridgesia*.

**Distribution**—A single, monospecific genus distributed in Chile, South America.


Duodichogamous shrubs; exstipulate. Leaves simple, deeply trilobed or serrate. Inflorescence a short, axillary cyme (a single few-flowered cincinni) or flowers solitary; pedicels articulate. Flowers zygomorphic; sepals 5, imbricate, lanceolate, nearly equal, the 2 anterior sepals partly connate; petals 4, pink, with a single basal, hood-shaped crested appendage; disc semiannular, 4-lobed; gynophore with 4 tiny lobes; stamens 8, filaments of unequal lengths, anthers dorsifixed; pollen isopolar, spherical in polar and equatorial view, tricolporate, striate (Fig. 6e); ovary 3-carpellate; style filiform with 3 stigmatic branches. Fruit schizocarpic, splitting into 3 samaroid, bladder-like, papery, divaricate mericarps with a short dorsal wing; seed exarillate, black, with a large hilum. A single species from Andean Chile.

**Tribe Thouinieae** Blume, Rumphia 3: 186. 1847.

Small trees or shrubs; exstipulate. Leaves alternate, trifoliolate or unifoliolate. Inflorescences of thyrses with flowers in lateral cincinni, or less often solitary or in short racemes; pedicel articulate (abscission zone) below the middle or non-articulate (in *Allophylastrum*). Flowers zygomorphic or less often actinomorphic, structurally or functionally unisexual; calyx 4(5)-merous, sepals with quincuncial imbrication; corolla of 4 distinct petals (or missing in
Allophylastrum), usually with an adaxial or marginal petaloid appendage; disc semiannular, 2–4 lobed, or less often annular; stamens (6)8, filament usually of different lengths, anther dorsifixed; gynoecium (2)3-carpellate with a single ovule per carpel.

**Distribution**—All genera Neotropical, except for Allophylus that is primarily Paleotropical.

**Key to the Genera**

1. Fruit schizocarpic, of 3, distally-winged mericarps ......................................................... Thouinia

1. Fruits unicoccate, bicoccate or seldom tricoccate

2. Flowers zygomorphic, petaliferous, on lateral cincinni in racemiform or paniculate thyrses; nectary disc unilateral, semiannular, 2- to 4-lobed, or rarely annular; pedicels articulate, 1–2 times as long as the calyx; pollen 3(4)-porate, triangular (seldom cuadrangular) in polar view ................................................................................................................................................... Allophylus

2. Flowers actinomorphic, apetalous, solitary, or in short axillary racemes; nectary disc cupular; pedicels non-articulate, >4 times as long as the calyx; pollen 4- or 5-porate, 4- or 5-angled in polar view .......................................................... Allophylastrum


—TYPE: Thouinia simplicifolia Poit.

Duodichogamous shrubs or small trees; exstipulate. Leaves trifoliolate or unifoliolate. Inflorescences axillary, racemiform thyrses with flowers in reduced lateral cincinni; pedicels articulate. Flowers zygomorphic; sepals 4, imbricate, the outer two usually smaller; petals 4, with an adnate, ventral, 2-lobed appendage; disc unilateral, 4-lobed; stamens 8, filaments of equal
length; pollen isopolar, obtusely triangular, quadrangular, pentagonal or hexagonal in polar view, oblate in equatorial view, 3–6-porate, rugulate; ovary 3-carpellate, with a single ovule per carpel; style filiform, with 3 stigmatic branches. Fruit schizocarpic, splitting into 3 samaroid mericarps, each bearing a dorsal ascending wing; seeds exarillate. About 30 species from the Greater Antilles (excluding Jamaica), Bahamas, Mexico, and northern Central America.


Duodichogamous, rarely dioecious, erect shrubs, trees, or rarely scandent shrubs (in Malaysia); exstipulate. Leaves alternate, trifoliolate or less often unifoliolate or digitately 5-foliolate (few Asian species); leaflets serrate, dentate or entire; petioles elongated. Inflorescences axillary, racemiform or paniculate thyrses, flowers on lateral cincinni; pedicels articulate. Flowers zygomorphic; sepals 4(5), free, imbricate, the outer distinctly smaller than the inner ones; petals 4, with a single, adnate, 2-lobed, or a basal hood-shaped, non-crested appendage, or these represented by an elongation of petal’s margins, exceptionally wanting; disc unilateral, semiannular or divided into 2 or 4 lobes, or exceptionally annular; stamens 8, filaments of unequal lengths, with posterior three shorter, anthers dorsifixed; pollen isopolar, obtusely triangular (seldom quadrangular) in polar view, oblate in equatorial view, 3(4)-porate (Fig. 5a) or less often brevicolporate, rugulate, sometimes striate, reticulate or psilate (Cruz and Melhem 1984) (pollen of *A. guaraniticus* has been shown also as quadrangular in polar view and 4-porate (da Luz and Barth 1999; in *A. edulis*, > 60% of pollen grains are reported as 4-porate by Anzótegui and Ferrucci 1998; Fig. 6a); ovary 2(3)-carpellate; style with 2–3 stigmatic branches. Fruit unicoccate (with a
rudimentary coccus) or bicoccate, endocarp crustose, exocarp fleshy; seeds exarillate, with papery testa. A circumtropical genus with about 255 species (Coelho 2014). 2n = 28; 56.

**Allophylastrum** Acev-Rodr., Phytokeys 5: 40. 2011. —**TYPE:** *Allophylastrum frutescens* Acev.-Rodr.

Dioecious (?), small trees or shrubs; exstipulate. Leaves trifoliolate; leaflets serrate. Flowers solitary, axillary or in short racemes, actinomorphic, unisexual with staminate flowers sometimes bearing a rudimentary 2-locular gynoecium; pedicels elongated, non-articulate; calyx 4-merous, sepals of similar length, in two whorls; petals wanting; disc cupular; stamens (5)6, the filaments of nearly equal length, connate at base; pollen isopolar, obtusely 4- or 5-angled in polar view, subspherical in equatorial view, 4–5-porate, with rugulate ornamentation (Fig. 6b); ovary 2-carpellate; style with 2 stigmatic branches. Fruit unicoccate (with a rudimentary coccus) or bicoccate, endocarp semi-woody, exocarp fleshy; seeds exarillate, with papery testa. A single, poorly collected species from Guyana and Brazil (Roraima).

**Tribe Paullinieae** (Kunth) DC., Prodr. 1: 601. 1824. —**TYPE:** *Paullinia*

Lianas, vines or climbing shrubs, exceptionally erect or hemicryptophytic shrubs, with a short, axillary shoot bearing a distal pair of coiled tendrils; stipules minute to foliaceous. Leaves alternate, with a well-developed distal leaflet, variously pinnate, trifoliolate, biteminate, bipinnate or seldom unifoliolate. Inflorescences of thyrses with flowers in lateral cincinni; pedicel usually articulate below the middle. Flowers zygomorphic or less often actinomorphic (in *Thinouia*), structurally or functionally unisexual; calyx 4(5)-merous, sepals with quincuncial imbrication;
corolla of 4(5) distinct petals, with an adaxial petaloid appendage; disc unilateral, 2- or 4-lobed, or less often annular (in Thinouia); stamens 8, filament usually of three different lengths, anther dorsifixed; gynoecium 3-carpellate with a single ovule per carpel.

**Distribution**—Neotropical, with a couple of species naturally extending into Africa and Madagascar.

**Key to the Genera***

1. Leaves trifoliolate; stipules minute; flowers actinomorphic; pollen subspherical to oblate-spherical in equatorial view ................................................................. Thinouia

1. Leaves variously compound; stipules minute to foliaceous; flowers zygomorphic (sub-zygomorphic in Lophostigma); pollen oblate, peroblate or prolate in equatorial view.

2. Petals much shorter than sepals; petal appendages about ½ the length of petals, bifid;
stamens of equal lengths; pollen striate, prolate or prolate-spherical in equatorial view, 4-hemicolporate, 2 colpi always diorate ................................................................. Lophostigma

2. Petals much longer than sepals; petal appendages as long or nearly as long as the petals, hood-shaped; stamens of unequal length; pollen reticulate, perforate or psilate, oblate or peroblate in equatorial view.

3. Lianas, climbing shrubs or seldom erect shrubs; fruit always capsular, woody, coriaceous or crustaceous; seed with a partial to complete arilode (sarcotesta); pollen isopolar or subisopolar, 3-porate ................................................................. Paullinia
3. Lianas, climbing shrubs, herbaceous vines or less often erect shrubs or prostrate herbs; fruit schizocarpic or if capsular, membranaceous; seeds lacking an arillode; pollen heteropolar, hemi-syncolporate.

4. Fruits schizocarpic with 3 winged mericarps or exceptionally capsular; seeds without a pseudo-hilum or if present then trigonous ............................................ Serjania

4. Fruits capsular, membranaceous, with seeds persistent on septa after dehiscence; seeds with a heart-shaped, reniform, or crescent-shaped pseudo-hilum.

5. Sepals 5; nectary disc 4-lobed; anthers usually with a more or less conspicuous apical connective expansion ................................................................. Urvillea

5. Sepals 4 or 5, when 5 nectary disc of 2 corniform lobes; anthers without an apical connective expansion .................................................................Cardiospermum

*Cardiospermum urvilloides and C. integerrimum are weakly associated with Serjania in our analyses. However, because they are morphologically indistinguishable from Cardiospermum (sensu stricto), we defer creating new genera until additional data supporting their placement are found.


Allosanthis Radlkofer in A. Engler, Pflanzenr. IV. 165 (Heft 98): 1157. 1933.

Lianas. Early stems and branches with a single vascular cylinder; older stems occasionally with neoformed cortical vascular cylinders. Leaves trifoliolate; stipules minute. Inflorescences of umbelliform (racemiform in T. trifoliata) thyrses, seldom bearing tendrils,
axillary or aggregate into terminal thyroid inflorescences with flowers in cincinni; pedicels articulate. Flowers actinomorphic, 5-merous; calyx cup-shaped, the sepals valvate, equal; petals 5, obovate to spatulate, with a pair of short, marginal appendages (a prolongation of petal’s margins); disc annular; stamens 6–8, of unequal lengths, anthers dorsifixed; pollen isopolar, obtusely triangular in polar view, subspherical in equatorial view, tricolporate, with elongated colpi nearly reaching the poles, striate (Fig. 5f); ovary 3-carpellate; style elongated with 3 stigmatic branches. Fruit schizocarpic, splitting into 3 mericarps (Fig. 4a), each with a distal wing; seeds trigonous ellipsoid, or lenticular-ellipsoid, exarillate, with a small hilum. About 10 species from Mexico, Central America, and South America. 2n = 28 (Urdampilleta et al. 2008a).

New Combination—


Duodichogamous woody vines. Cross section of stem with a single vascular cylinder. Leaves trifoliolate; stipules minute. Inflorescences axillary racemiform thyrses with a pair of tendrils at base of rachis and flowers in lateral cincinni. Flowers sub-zygomorphic; sepals 5, unequal, imbricate; petals 4, shorter than the sepals, with very short, bifid, basally adnate appendages; disc unilateral, 4-lobed; stamens 8, with short, equal, filaments, anthers dorsifixed (seemingly basifixed); pollen heteropolar, subspherical or subspherical-trilobed in polar view, prolate or prolate-spherical in equatorial view, 4-aperturate (possibly a lengthy variation hemi-
tri-syncolporate, 2 colpi always diorate, striate; ovary 3-carpellate (Fig. 5e); style with 3 stigmatic branches. Fruit a schizocarp splitting into 3 mericarps with an elongated proximal wing (Fig. 4c); seeds lenticular, exarillate, with a small trigonous or ellipsoid hilum. Two species, one from Ecuador and Peru, the other from Bolivia. 2n = 28.


Duodichogamous woody or herbaceous vines, often producing milky sap. Cross section of stem with a single or with a central vascular cylinder and (1–2)3–10 peripheral vascular cylinders. Leaves ternately compound (trifoliolate, biternate, or triternate) or 5-pinnately compound; stipules generally small, persistent, deciduous or ephemeral. Thyrse axillary and solitary with a pair of tendrils at the base of the rachis, or distal, aggregated into terminal synflorescences bearing no tendrils. Flowers in lateral cincinni, zygomorphic; sepals 4–5, unequal, imbricate; petals 4, with a basal, hood-shaped, crested appendage; disc unilateral, 2- or 4-lobed; stamens 8, filaments of unequal lengths, anthers dorsifixed; pollen heteropolar, obtusely triangular in polar view, oblate in equatorial view, hemi-tri-syncolporate proximally, sometimes with short distal demicolpi as well, perforate, reticulate, psilate or faintly rugulate (Fig. 5d); ovary 3-carpellate; style with 3 stigmatic branches. Fruit schizocarpic, splitting into three, winged mericarps (Fig. 4b) [wing proximal, sometimes vestigial or lacking (e.g. S. lachnocarpa (Radlk.) Acev.-Rodr., S. cissoides Radlk.), or less often circumferential (e.g. S. biternata, S. dissecta and S. macrolopha)], or fruits sometimes capsular by dissolution of septa (i.e., S.
cristobaliae, S. guerrerensis, S. tortuosa); seeds lenticular to nearly globose, exarillate, with a small trigonous or ellipsoid hilum or sometimes with a pseudo-hilum (e.g., S. glabrata Kunth). About 230 species native to tropical and subtropical America. 2n = 24.

New Combinations—


**Serjania cristobaliae** (Ferrucci & Urdampilleta) Ferrucci & Steinmann, comb. nov.;

*Cardiospermum cristobaliae* Ferrucci & Urdampilleta, Brittonia 63: 479. 2011. Fruit in this species are apparently dehiscent (along the connection of the fruit wall to the partitioning wall), with flattened, circumferential winged locules; seeds lack a pseudo-hilum.


Duodichogamous, herbaceous to woody vines. Stems terete and lenticellate, becoming trilobate at age, with a single vascular cylinder, producing milky or watery sap. Leaves trifoliolate; stipules minute, persistent or deciduous. Inflorescences axillary, thyrses with flowers in cincinni; pedicels articulate. Flowers zygomorphic; sepals 5, unequal, imbricate; petals 4, spatulate, with a basal hood-shaped, crested appendage on adaxial surface; disc unilateral, 4-lobed, receptacle enlarged into a short androgynophore; stamens 8; pollen heteropolar, obtusely triangular in polar view, oblate in equatorial view, hemi-tri-syncolporate proximally, sometimes with short distal demicolpi, perforate, indistinctly rugulate or reticulate; ovary 3-carpellate; style with 3 stigmatic branches. Fruit a 3-locular, thin, papery, semi-inflated capsule, mericarps flattened into a wing along dorsal margin (Fig. 4d); seeds 3 or 1, trigonous-ellipsoid, with a heart-shaped or reniform, white pseudo-hilum. About 21 species distributed in the United States (Texas), Mexico, Central America and South America. $2n = 20, 22, 24, 88$ (Urdampilleta et al. 2008b).
New Combinations—


**Cardiospermum L., Sp. Pl. 366. 1753. —TYPE: Cardiospermum halicacabum L.**

Duodichogamous herbaceous vines. Leaves trifoliolate or biternate; stipules usually minute and persistent. Inflorescences simple, axillary, racemiform or umbelliform thyrses bearing tendrils at base of rachis, with flowers in cincinni. Flowers zygomorphic; sepals 4(5), unequal, imbricate; petals 4, with a basal, hood-shaped, crested appendage; disc unilateral, 2- or 4-lobed; stamens 8, filaments of unequal lengths, anthers dorsifixed; pollen heteropolar, obtusely triangular in polar view, oblate in equatorial view, tri-syncolporate proximally, perforate to reticulate (Fig. 5b); ovary 3-carpellate; style with 3 stigmatic branches. Fruit a 3-locular septifragal-marginicidal, inflated capsule, generally with sub-chartaceous walls; seeds brown or black, with a small, round to heart-shaped, light-colored area around the hilum (pseudo-hilum sensu Van der Pijl 1957; Fig. 8d). Six or seven species native to the Neotropics, one of which is
also native in Africa, and 2 that are now widely distributed throughout the tropics. $2n= 14, 18, 20, 22, 36.$


Duodichogamous, woody vines, usually producing milky sap. Cross section of stem with a central and 3–5 peripheral vascular cylinders, or with a single vascular cylinder. Leaves trifoliolate, 5-foliolate pinnate, partly bipinnate, bipinnate, biternate, or variously dissected; stipules minute to foliaceous. Inflorescences axillary, solitary or fascicled thyrses, with a pair of tendrils at base of rachis when solitary, sometimes cauliflorous and fascicled and bearing no tendrils. Flowers zygomorphic; sepals 4–5, unequal, imbricate; petals 4, with a basal, hood-shaped, crested appendage; disc unilateral, 2- or 4-lobed; stamens 8, filaments of unequal lengths, anthers dorsifixed; pollen isopolar or subisopolar, obtusely triangular in polar view, oblate in equatorial view, triporate, perforate (Fig. 5c); ovary 3-carpellate; style with 3 stigmatic branches. Fruit a 1–3-locular, septifragal-marginicidal capsule, locules sometimes dorsally winged or exceptionally spiny; seeds globose, oblong or ellipsoid, with a partial to nearly complete sarcotesta, leaving a scar when removed (Fig. 7c & 8a–b). About 200 species native to the Neotropics, one species extending into Africa and Madagascar. $2n = 24, 210$ (de Freitas et al. 2007).

**Placement Pending**


Duodichogamous trees. Leaves simple, opposite, entire or tridentate at apex, exstipulate. Inflorescences of axillary cymes. Flowers zygomorphic, bisexual or functionally unisexual; sepals 5, imbricate; petals 4(5), with a hood-shaped, crested, ventral appendage; disk unilateral, 2-lobed-pyramidal; stamens 8; pollen colporate, striate (Fig. 6f); ovary 3-carpellate, with a single ovule per carpel; style filiform; stigma 3-lobed. Fruit schizocarpic, splitting into (1–)3 subglobose, crustose mericarps. Seed exarillate. Three species from southern South America.

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**LITERATURE CITED**


872–882.


San Diego, California: Academic Press.


TABLE 1. Data set characteristics for 99 and 125-tip phylogenetic analyses.

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<th>Characteristic</th>
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**Table 2. Morphological characters (synapomorphies) for clades in supertribe Paulliniodae.**

<table>
<thead>
<tr>
<th>1. Leaves with a fully developed distal leaflet; thyrses with lateral cincinni; zygomorphic flowers with 4–5 sepals; 4 petals; unilateral nectary disc; schizocarpic fruits with divaricate mericarps; seeds not arillate; chromosome number $2n \leq 30$.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Leaves trifoliolate or trifoliolate derived; seeds basal; chromosome number $2n \leq 28$.</td>
</tr>
<tr>
<td>3. Climbers with stipules and tendrils.</td>
</tr>
<tr>
<td>4. Pollen heteropolar.</td>
</tr>
<tr>
<td>5. Pollen oblate (compressed in polar area) with triangular outline; petals as long as or longer than the sepals; chromosome number $2n \leq 24$.</td>
</tr>
<tr>
<td>6. Seeds in center of axis, with pseudo-hilum; fruit capsular.</td>
</tr>
<tr>
<td>7. Pollen hemi-isopolar, triporate; capsules coriaceous to woody; seeds arillate or sarcotestal.</td>
</tr>
<tr>
<td>8. Imparipinnate leaves.</td>
</tr>
<tr>
<td>9. Leaves simple; cincinni reduced.</td>
</tr>
<tr>
<td>10. Subglobose to oblate porate pollen grains; chromosome number $2n \leq 28$.</td>
</tr>
<tr>
<td>11. Fruits with coccate monocarps; seeds covered by fleshy endocarp.</td>
</tr>
<tr>
<td>12. Actinomorphic flowers, a reversal.</td>
</tr>
<tr>
<td>13. Pollen prolate heteropolar, 4-aperturate; petals shorter than the sepals.</td>
</tr>
<tr>
<td>15. Capsule membranous; chromosome number mostly $2n &lt; 24$.</td>
</tr>
</tbody>
</table>
APPENDIX 1. Voucher and GenBank information for taxa included in the phylogenetic analyses. Listed as: taxon, collection, herbarium, place of origin, and GenBank accession numbers (ITS, trnL intron). Herbarium acronyms follow Index Herbariorum (Thiers, continuously updated). Accession numbers for new data begin with KX; an en-dash (–) indicates missing data.

*Allophylastrum frutescens* Acev.-Rodr., *Lima 812* (K), Brazil, KX584885, KX584982.

Cardiospermum procumbens Radlk., Urdampilleta 322 (UEC), Brazil, KX584920, —.

Cardiospermum pterocarpum Radlk., Urdampilleta 321 (US), Brazil, KX584921, KX585012.


Elattostachys apetala Radlk., Munzinger 692 (MO), New Caledonia, EU720537, EU721306.


FIGURE LEGENDS

FIG. 1. Bayesian 50% majority-rule consensus tree based on the combined two marker, 99-tip data set for Paulliniodae and outgroups. Bayesian posterior probability values ≥0.50 are indicated above branches, followed by RAxML bootstrap values ≥50%. (-) = value < 0.50/50%. np = an edge recovered in the Bayesian tree but not present with ML.

FIG. 2. Hypothetical relationships of genera in supertribe Paulliniodae, backbone based on DNA sequence and chromosome analyses; relationships within tribe Paullinieae based on morphological characters. Refer to Table 2 for synapomorphies.

FIG. 3. Flowers in Paullinieae. A. zygomorphic flower (*Paullinia stellata* Radlk.; *Acevedo-Rodríguez* 14964 (US), photo by P. Acevedo). B. actinomorphic flower (*Thinouia mucronata* Radlk.; photo by S. Maris Solís). C. Diagram of flower bud cross section of *Serjania salzmanniana* Schltdl. showing floral orientation (72°) in relation to inflorescence axis (e) and bracteole (b); numbers mark the different sepals (modified from Radlkofer 1931–34).


