

# The Phylogeny of *Ayenia*, *Byttneria*, and *Rayleya* (Malvaceae s. l.) and its Implications for the Evolution of Growth Forms

Barbara A. Whitlock<sup>1,3</sup> and Amanda M. Hale<sup>2</sup>

<sup>1</sup>Department of Biology, University of Miami, Coral Gables, Florida 33124, U. S. A.

<sup>2</sup>Department of Biological Sciences, Texas Christian University, Fort Worth, Texas 76129, U. S. A.

<sup>3</sup>Author for correspondence (whitlock@bio.miami.edu)

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**Abstract**—Tropical plants show a diversity of growth forms, yet few phylogenies are available to examine the transitions between trees, shrubs, and lianas. Here, we reconstruct relationships among 37 species of *Ayenia*, *Byttneria*, and *Rayleya* with cpDNA sequences, and use the resulting phylogeny to test hypotheses on the evolution of climbing plants and to examine biogeography of these plants. Results show that *Rayleya* is sister to a combined clade of *Ayenia* and *Byttneria*; *Byttneria* is paraphyletic with *Ayenia* nested within it. The common ancestor of the combined *Ayenia*/*Byttneria*/*Rayleya* clade is reconstructed as a neotropical tree or shrub. Within the *Ayenia*/*Byttneria* clade, there is a single transition to unarmed lianas, with subsequent radiations into the Asian and African tropics. A second independent transition from trees or shrubs to spiny, semi-scandent shrubs occurred in the neotropics. We found no evidence of transitions from lianas to any other growth form; however, we recovered a reversal from semi-scandent shrubs to fully upright plants. Within the neotropics, there are two independent radiations into seasonal, open habitats. Additional sampling of African species of *Byttneria* may yield more complicated scenarios in both biogeography and the evolution of growth forms.

**Keywords**—Byttnerieae, Byttnerioideae, biogeography, cpDNA, lianas, tropical plants.

*Byttneria* and *Ayenia* have long fascinated botanists due to their tiny yet extraordinarily complex flowers, as well as the diversity of growth forms found in the two genera. While *Ayenia* is restricted to the New World, *Byttneria* is pantropical. Species of both genera grow in warm subtropical and tropical regions, and may be subshrubs, shrubs, small trees or, in the case of *Byttneria*, lianas. Although species of both genera are most diverse in tropical forests, in the New World, both *Ayenia* and *Byttneria* have radiated into dry, open habitats. A phylogenetic analysis of the chloroplast gene *ndhF* that included five species of *Ayenia* and *Byttneria* (Whitlock et al. 2001) suggested that all *Ayenia* sampled were nested within *Byttneria*, sister to neotropical species of *Byttneria*. If upheld, these results have implications for the evolution of the floral characters used to separate *Ayenia* and *Byttneria*, and intriguingly suggest that biogeography and growth forms are more indicative of phylogenetic relationships than floral morphology. Here, we present the results of expanded phylogenetic analyses of *Ayenia* and *Byttneria*, with morphologically and geographically diverse representatives of both genera, to test hypotheses on the evolution of growth forms and examine biogeographic relationships among lineages.

*Ayenia* and *Byttneria*, together with the recent segregates *Rayleya* and *Megatritheca*, have unusual constricted, or “clawed,” petals that insert at the base of the androecium and arch up to rejoin the staminal column at its apex, partially concealing minute antipetalous anthers (Cristóbal 1960, 1965, 1976, 1981). This morphology gives the flowers a lantern-like appearance. The complexity of petal form has proven challenging to describe and common terminology such as “claw” and “lamina” may be applied differently by different authors (see Dorr 1996 and references therein). We follow the terminology of Dorr (1996) and use “claw” to refer to the narrow base of the petal, from the point of insertion to where it rejoins the staminal column. The morphology of the flowers, their reddish or purplish coloration, small size, and the fetid odor reported from some species (Cristóbal 1960, 1976) are all generally associated with fly pollination (Faegri and van der Pijl 1971) although pollination biology has apparently not been studied in these species. Androecium and petal morphology separate the approximately 135 species of *Byttneria* from the ca. 70

species of *Ayenia*, as well as delimit sections in the most recent revisions of the genera (Cristóbal 1960, 1976). Both genera are usually interpreted as having five stamens, with dithecate anthers in *Byttneria* and trithecate anthers in *Ayenia*. The two genera also differ in the degree of constriction of the petal claw: in *Ayenia*, the petal is narrow for most of its length, whereas in *Byttneria* the petal is narrow at the base but expands in width as it meets the staminal column. Petals in *Byttneria* species generally have prominent ligules or projecting appendages (see Dorr 1996 for a discussion of the terminology) on the abaxial surface where the petals rejoin the staminal column. Similar appendages occur in some *Ayenia* species but are usually inconspicuous. Flowers of *Ayenia* also have a short androgynophore that is lacking in *Byttneria*. Because of the small size of *Ayenia* flowers (usually < 1 cm in diameter), the androgynophore is often barely discernible.

In the course of her work with *Byttneria* and *Ayenia*, Cristóbal (1965, 1981) described two small genera, *Megatritheca* and *Rayleya*, that are clearly closely related to *Byttneria* and *Ayenia* but include species that cannot be assigned with confidence to either genus as currently circumscribed. *Rayleya*, with one species from southeastern Brazil, has five dithecate anthers and pronounced petal appendages similar to those found in *Byttneria*, but the delicate, narrow petal claws and androgynophore of *Ayenia* (Cristóbal 1981). *Rayleya bahiensis* differs from all species of *Byttneria* and *Ayenia*, as well as *Megatritheca* (see below), in having a presumably glandular protuberance on the adaxial surface of the petals at the point of insertion. Cristóbal hypothesized that this protuberance may serve a similar function in pollination to the small tufts of glandular hairs found at the base of the sepals in some *Byttneria* and *Ayenia* species.

The genus *Megatritheca* consists of two Central African species previously placed in *Byttneria* (Cristóbal 1965). As with *Rayleya*, the two species of *Megatritheca* show a combination of features usually associated with either *Byttneria* or *Ayenia*, as well as some novel characters. The petal claw of *Megatritheca* is similar to what is seen in *Byttneria*, with a constricted base, expanded apex, and a prominent abaxial appendage. In addition, both species of *Megatritheca*, like some *Byttneria*, occur in tropical Africa, whereas *Ayenia* is restricted to the Americas.

However, as the name of the genus suggests, the stamens of *Megatritheca* are trithecate, as in *Ayenia*. The genus differs from all species of *Ayenia*, *Byttneria*, and *Rayleya* in its decurrent stigmas (vs. capitate), tricolporate pollen (vs. porate, though other Byttnerioideae are mostly tricolporate), large and oblong anthers (vs. small and suborbicular), and the barely developed staminodes that are below the level of the anthers so that the petal apices are adnate to the antipetalous anther-bearing parts of the staminal tube (rather than the antisepalous staminodes as in other genera).

The diversity of growth forms in *Byttneria*, and to a lesser extent in *Ayenia*, has long been appreciated. However, as is true for most tropical plants, detailed studies on the architecture, functional morphology, and ecology in *Ayenia* and *Byttneria* are lacking. Although Cristóbal (1960, 1976) did not emphasize vegetative characters in her revisions, she noted correspondences between growth forms and the sections that she recognized within both genera. These observations provide a baseline for investigations into evolutionary transitions between forms. Many species of *Ayenia* and *Byttneria*, as well as *Rayleya*, and all close outgroups (Whitlock et al. 2001), are erect, or self-supporting, shrubs or small trees. Most species of *Ayenia* are < 1 m tall and, in especially dry regions, may be low with procumbent or decumbent branches, typically malvaceous palmately-veined and toothed leaves. Only two species of *Ayenia* have been described as small trees (four to eight m) with a well-defined trunk (Cristóbal 1960). Species of *Byttneria* show remarkably more variation in growth form. Approximately 60 species of *Byttneria*, all members of sect. *Vahihara*, are unarmed lianas that rely on other woody plants for support. This group includes all species in Madagascar and southeast Asia, as well as many species from the Americas and Africa. At least 30 species of *Byttneria* (many though not all species in sects. *Urticifolia*, *Crassipetala*, and *Incasica*) are shrubs that may be leaning or semi-scandent at some stages of development, often with long curving branches covered in prickles or more rarely spines that are used to scramble over nearby vegetation. We adopt the terminology of functional studies of climbing plants (Speck and Rowe 1999) and refer to these plants as semi-self-supporting, although functional studies of *Byttneria* are lacking, and this group of plants likely exhibits substantial variation. Species of the exclusively neotropical sect. *Byttneria* consist of erect shrubs or subshrubs that differ in details of their growth forms from shrubby species of *Byttneria* and *Ayenia*. These plants may be geophytes, hemicryptophytes, or chamaephytes (Cristóbal 1976) with most branching occurring close to ground level; branches are erect, with rigid linear, lanceolate, or sagittate leaves. Plants with this last growth form occur in the llanos, cerrado, along seasonally flooded rivers, and in other open and wet areas of South America. Only four species of *Byttneria* have been described as small, erect trees, to ca. 8 m, two in sect. *Vahihara* and two in sect. *Crassipetala* (Cristóbal 1976; Barnett and Dorr 1990).

The variation found among these species presents an opportunity to test hypotheses on the evolution of growth forms, particularly of lianas. Climbing plants are taxonomically widespread, in one estimate occurring in 133 vascular plant families (Gentry 1991, although family circumscriptions have changed since this publication); such observations have led to the hypothesis that climbing growth forms have evolved many times independently. Despite this diversity, there are few examples of families that include both climbing

and self-supporting growth forms in which the climbing habit is inferred to be basal (Rowe and Speck 2005). The *Ayenia*/*Byttneria*/*Rayleya* clade is a promising group to search for such a reversal, because it includes several species of erect trees and shrubs as well as 60 + species of true lianas. In addition, the many leaning and scrambling species of *Byttneria* provide an additional phylogenetic test for the hypothesis that such semi-self-supporting plants represent an intermediate form between lianas and fully self-supporting trees and shrubs.

Within the Americas, *Ayenia* and *Byttneria* have similar distributions, at least at a broad geographic scale, extending from the southern U. S. A. (*Ayenia*) or Sonora, Mexico (*Byttneria*), to Argentina and Uruguay. Within this range, however, species of *Ayenia* tend to be more limited to drier habitats, whereas species of *Byttneria* can be found in both dry and mesic environments. Cristóbal (1960) recognized two centers of diversity in *Ayenia*: (1) northern Central America, Mexico, and the Antilles, with at least 38 endemic species distributed in all three sections that she recognized; and (2) Bolivia, Paraguay, and Eastern Brazil, with 10 endemic species all in section *Ayenia* subsect. *Ayenia*. She hypothesized that *Ayenia* originated in the former because of the greater number of species that currently occur there, as well as the greater morphological and taxonomic diversity. Because 60% of the species in *Byttneria* are American with representatives of five of the six sections, Cristóbal (1976) hypothesized a neotropical origin for this genus.

Here, we analyze sequences from three chloroplast regions from nine species of *Ayenia*, 27 species of *Byttneria*, and *Rayleya bahiensis*, with good representation of morphological diversity in both genera and sampling from throughout their geographic ranges. Our first goals are additional tests of the monophyly of *Ayenia* and *Byttneria* with greater taxonomic sampling of both genera, and an examination of the evolution of characters used to delimit both genera. In addition, we survey recently collected herbarium specimens to corroborate reports in the literature of growth forms found in *Ayenia* and *Byttneria*. We then test specific hypotheses on the evolution of growth forms and biogeography, including (1) there are no reversals from a lianescent growth form to a self-supporting, erect growth form; (2) semi-scandent trees and shrubs represent a phylogenetically intermediate form between self-supporting plants and fully scandent lianas; (3) neotropical species of both *Ayenia* and *Byttneria* form a clade; and (4) species of *Ayenia* and *Byttneria* (i.e. *Byttneria* sect. *Byttneria*) occurring in seasonal open habitats represent two independent radiations. A final goal is to provide a phylogenetic framework that can be used to identify appropriate candidates for comparative studies of functional morphology in the future.

#### MATERIALS AND METHODS

**Taxonomic Sampling**—We sequenced chloroplast markers from representatives of nine species of *Ayenia* with representatives from all three sections recognized by Cristóbal (1960), 27 specimens of *Byttneria* with representatives from four of six sections (Cristóbal 1976), plus the monotypic *Rayleya bahiensis* (Appendix 1). We made several attempts to extract DNA from the few herbarium specimens available of *Megatritheca*, but without success. For one species of *Byttneria* with a disjunct distribution across the tropics, *B. catalpifolia*, we included a Central African collection as well as a South American collection. In addition, we used sequences from five other Byttnerioideae as outgroups (Appendix 1), including two closely related Byttnerieae (*Abroma* and *Kleinhowia*), two Lasioptaleae

(*Guichenotia* and *Rulingia*) and one Theobromeae (*Theobroma*), following results of Whitlock et al. (2001).

**Molecular Methods**—Total genomic DNA was extracted from leaf material that was silica-gel-dried, air-dried, or from herbarium specimens using DNeasy columns (Qiagen Inc., Valencia, California) or a 6% PVP method (Kim et al. 1997). We sequenced three chloroplast markers using two sets of PCR primers: the 3' end of *matK*, the *trnL* intron and the adjacent *trnL-trnF* spacer. For the *matK* region, we used primers *matK4F* and *matK9R* (Manos and Steele 1997) that amplified a 930 bp product. This product was sequenced directly with the PCR primers. The *trnL* intron and *trnL-trnF* spacer usually amplified in a single 1.2 kb piece using primers *c* and *f* (Taberlet et al. 1991). In a few cases of specimens with degraded DNA, internal PCR primers were used to amplify two smaller fragments: primers *c/d* and *e/f* (Taberlet et al. 1991). The 21 sites representing the internal primers were excluded from all analyses.

PCR products were cleaned with QIAquick columns (Qiagen Inc.) or a standard exo-SAP procedure. Double-stranded products were sequenced in both directions using ABI BigDye dye-terminators and cycle-sequencing protocols. Sequencing reactions were run on an ABI 377 or 3730xl DNA analyzer. Sequences were assembled and inspected for frame shifts and stop codons in coding regions using Sequencher 3.0 (GeneCodes Corp., Ann Arbor, Michigan). The boundaries of adjacent regions were identified by comparison to published sequences of *Nicotiana* (Sugita et al. 1985). All sequences are available in GenBank (accession numbers HM488369-HM488452).

**Phylogenetic Analyses**—All sequences were aligned by eye and analyzed with both maximum parsimony and Bayesian analyses. Unambiguously aligned insertions and deletions (indels) > one bp were coded as binary characters and included in all searches. Because all the sequences used were from the chloroplast, all analyses were performed on a concatenated dataset combining *matK*, the *trnL* intron, and *trnL-F* spacer. A combined alignment is available in TreeBASE (study number S10616).

The parsimony analysis was performed with PAUP\* 4.0b10 (Swofford 2002) using heuristic searches with TBR and 100 random addition sequence replicates. Bootstrap support (BS; Felsenstein 1985) was estimated with 100 bootstrap replicates, each with 100 random addition sequence searches.

Bayesian analyses were implemented with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The likelihood ratio test (Huelsenbeck and Crandall 1997) was used initially to test the fit of four evolutionary models (JC, F81, HKY, GTR) for each of the three datasets (*matK*, *trnL*, and *trnL-trnF*), using one of the most parsimonious trees, with empirical nucleotide frequencies and rate parameters estimated from the data when appropriate. Across site rate heterogeneity was then incorporated with a gamma distribution (four rate categories and the shape parameter alpha estimated from the data) and I, the proportion of invariant characters, estimated from the data. A binary model was applied to 14 coded indels. Model parameters were derived from default prior distributions and were unlinked among the four partitions. A variable rate prior was used among partitions. We conducted two simultaneous MCMC runs each with four linked chains for  $1 \times 10^6$  generations, sampling trees every 100 generations. Three of the four chains were heated (sequential heat = 0.2) to improve mixing. Convergence of runs was indicated by average standard deviation of split frequencies between the two runs of less than 0.01 which were obtained after  $8.9 \times 10^5$  generations. The first 2,500 trees were discarded as burn-in after visual examination of the likelihood-by-generation plot. The remaining trees were used to produce a majority-rule consensus tree and to calculate posterior probabilities (PP).

**Biogeography and Growth Form Evolution**—Information on the growth forms of *Ayenia*, *Byttneria*, *Rayleya* and outgroups was obtained from previously published reports, supplemented by observations from recently collected herbarium specimens (primarily from MO, NY, GH, and A), and data from herbarium specimen labels. Three character states were scored that appear to correspond to the variation observed: (1) erect trees, shrubs, and subshrubs; (2) semi-scandent trees and shrubs; and (3) lianas. A second character describing geographic description was established with the following states: (1) New World; (2) Asian tropics; (3) African tropics; and (4) Madagascar. All characters were optimized on the pool of most parsimonious tree using parsimony (implemented in MacClade 4.08; Maddison and Maddison 2005).

## RESULTS

The aligned matrix includes 938 bp from *matK* and 1,224 bp from the *trnL-trnF* region with 103 (11%) and 124 (10%) parsimony-informative characters, respectively, and 0.8% of

matrix cells scored as missing data. Two indels of 6bp and 9bp were coded from *matK* and 13 from the *trnL/trnF* region, 2–145bp in length. Likelihood ratio tests selected GTR + gamma for each partition of the sequence data.

Unweighted parsimony analysis of the 42 sequences including 15 indels resulted in 243 equally parsimonious trees of 569 steps (C. I. = 0.81, R. I. = 0.91; Fig. 1). The strict consensus of the parsimony analysis was identical to the majority-rule consensus revealed by the Bayesian analysis (Fig. 1). In both analyses, all *Byttneria*, *Ayenia*, and *Rayleya* together form a well-supported clade (BS = 100, PP = 100). *Rayleya* is sister to all sampled representatives of *Ayenia* and *Byttneria*, although support is not strong (BS = 62, PP = 58). As was found in the *ndhF* analysis (Whitlock et al. 2001), *Ayenia* is monophyletic (BS = 100, PP = 100) and nested within a clade of exclusively neotropical *Byttneria*. Within *Ayenia*, the two representatives sampled from sect. *Leiaayenia* form a clade that is nested within a paraphyletic sect. *Cybiostigma*. The position of one representative of sect. *Ayenia*, *A. praeclara*, is unresolved within the genus. Our analyses do not show that it forms a clade with the two other members of the section that were sampled, although we cannot reject this possibility.

All specimens of *Byttneria* sampled form three well-supported clades. (1) All species sampled of *Byttneria* sect. *Vahihara* are a clade (BS = 100, PP = 100). Within this clade, the five Malagasy specimens form a subclade that is well-supported (BS = 93, PP = 100); the four Asian specimens also form a subclade that has high posterior probability (PP = 94) but low bootstrap support (BS = 51) despite containing nearly identical sequences. One specimen of *B. catalpifolia*, the only representative from tropical Africa in this study, is sister to a conspecific specimen collected from Peru (BS = 100, PP = 100). Relationships among these taxa and other neotropical representatives of sect. *Vahihara* are unresolved. (2) *Byttneria morii* and *B. schunkei* (BS = 100, PP = 100) are both neotropical representatives of sect. *Crassipetala*. (3) A large exclusively neotropical clade includes the remaining species of sect. *Crassipetala* sampled, all representatives of sect. *Urticifolia* and sect. *Byttneria* sampled (BS = 100, PP = 100). Of these, only sect. *Byttneria* is monophyletic (BS = 100, PP = 100), while sects. *Crassipetala* and *Urticifolia* both appear polyphyletic. This last neotropical clade is sister to *Ayenia* in the strict parsimony consensus; however, support for this relationship is low (BS = 51, PP < 50).

Observations from recently collected herbarium specimens confirm Cristóbal's (1960, 1976) descriptions of these plants, in particular in distinguishing lianas from semi-scandent or scrambling shrubs. Specimens of *Byttneria* sect. *Vahihara* are almost always described as lianas, often in trees > 10 m tall, agreeing well with Cristóbal's (1976) descriptions. Specimens from sects. *Crassipetala* and *Urticifolia*, in contrast, are almost always described as leaning, scandent, scrambling or climbing shrubs, again agreeing with Cristóbal (1976). The vegetative morphology and architecture of these semi-scandent plants also differ from the lianas of sect. *Vahihara*, most significantly in the presence of prickles and spines that enable these plants to climb and scramble over nearby vegetation. The growth form of these species were therefore coded with the character state "semi-scandent trees and shrubs."

All reconstructions of the character describing growth forms were similar across all 243 most parsimonious trees. The common ancestor of the combined *Ayenia/Byttneria/Rayleya* clade is inferred to be an erect tree or shrub. Within the combined



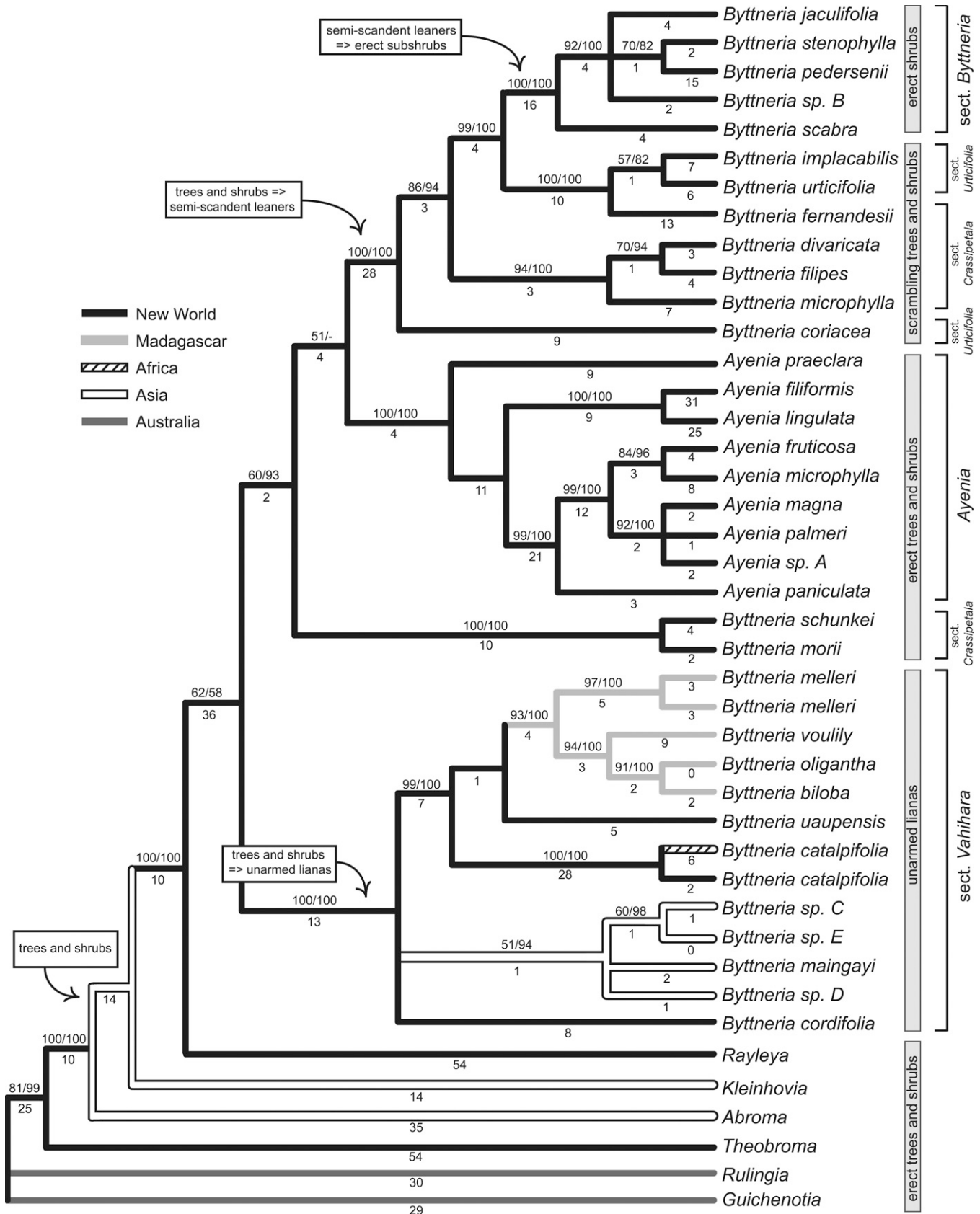


FIG. 1. Phylogeny of *Ayenia*, *Byttneria*, and *Rayleya*. One of 243 most parsimonious trees. Nodes appearing in the parsimony strict consensus and Bayesian majority-rule consensus show parsimony bootstrap values/Bayesian posterior probabilities ( $\times 100$ ) above branches; parsimony branch lengths are below. Shading of branches indicates inferred geographic distribution in the most basal nodes is uncertain; however, all close outgroups to the combined *Ayenia/Byttneria/Rayleya* clade are paleotropical. Growth forms of included taxa are shown on the right margin. The plesiomorphic growth form for the *Ayenia/Byttneria/Rayleya* clade is inferred as self-supporting trees and shrubs; inferred transitions are indicated on the tree. Sections of *Byttneria* following Cristóbal (1976) are on the far right.

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*Ayenia*/*Byttneria*/*Rayleya* clade, there is one transition to a lianescent growth form in the branch leading to the clade that includes all species of *Byttneria* in sect. *Vahihara*, with no evidence of reversals to erect trees and shrubs. There is a second transition from erect trees and shrubs to a semi-scandent growth form within the neotropics that appears to have arisen independently from lianas of sect. *Vahihara*. Within this group of semi-scandent trees and shrubs, there is a reversal to the erect shrubs and subshrubs found in *Byttneria* sect. *Byttneria*. The common ancestor of the *Ayenia*, *Byttneria*, and *Rayleya* is inferred to be neotropical.

#### DISCUSSION

The finding that *Ayenia* is nested within *Byttneria* suggests complex patterns of evolution of the floral characters that have been used to circumscribe genera and sections with *Ayenia* and *Byttneria*. Additional sampling is predicted to reveal more complicated histories of these plants.

**Implications for Floral Evolution**—Morphology of the androecium, particularly stamen number, has been emphasized in delimiting families and genera of Malvales, including members of the “Sterculiaceae” (e.g. Hutchinson 1967). Molecular phylogenetic analyses have shown that many of these taxa are nonmonophyletic (e.g. Alverson et al. 1999; Whitlock et al. 2001), raising questions on the usefulness of stamen number as a character. Our results suggest additional homoplasy in stamen number within one small clade of plants. All potential outgroups in our study have either fifteen (*Kleinhovia*, *Abroma*, *Scaphopetalum*) or ten (*Leptonychia*) dithecate stamens, whereas all basal lineages of the ingroup (*Rayleya* and *Byttneria*) have five dithecate stamens. There is thus evidence for a reduction in stamen number in the lineage leading to the common ancestor of *Ayenia*, *Byttneria*, and *Rayleya*. The position of *Ayenia* nested within *Byttneria* indicates a secondary proliferation of the androecium in this lineage that can be interpreted alternately as an increase in theca number (from ten to fifteen) or a doubling of stamen number (from five to ten, with five dithecate and five unithecate stamens in *Ayenia*).

The homology of constricted petal claws and androgynophores, both found in *Ayenia* and *Rayleya* but not in *Byttneria*, also needs to be revisited in light of our phylogenetic results. *Ayenia* and *Rayleya* are not closely related in the context of this clade (Fig. 1), suggesting that both characters are independently derived in these two lineages. The almost thread-like petal claws appear to be unique to *Ayenia* and *Rayleya*; however, an androgynophore is also present in *Kleinhovia*, probably the nearest sister group to the *Ayenia*/*Byttneria*/*Rayleya* clade (Whitlock et al. 2001).

Floral developmental studies may help to illuminate patterns of petal evolution and to identify homology in these complex three dimensional structures. For example, Leinfellner (1960) examined petal development in five species of *Ayenia*, six *Byttneria*, and 13 other Byttnerioideae, and identified commonalities at early stages across these taxa. A similar approach may be needed to interpret the morphology of the petals and staminal column at their apices where these structures meet. Such an approach has provided many characters used to delimit sections in *Ayenia* and *Byttneria*. The form of both structures varies subtly and in concert with one another across species, with various grooves, ridges, and projections that affect the complex three-dimensional fit of the

petals to the staminal column. The nonmonophyly of at least two sections in each genus (Appendix 1; Fig. 1) suggests that this complex morphology may have equally complicated patterns of evolution.

**Evolution of Growth Forms**—Our results suggest that a self-supporting common ancestor of *Ayenia* and *Byttneria* gave rise to a lineage of lianas and a second independent lineage of semi-self-supporting, scrambling shrubs (Fig. 1). We found no evidence of a reversal from lianas to self-supporting trees and shrubs, or to any other growth form. All species of lianas sampled form a well-supported clade, corresponding to *Byttneria* sect. *Vahihara*, that does not include plants with any other growth form or from any other section (Fig. 1). However, our results show a reversal from what appear to be primarily semi-scandent plants to erect shrubs and subshrubs in the branch leading to *Byttneria* sect. *Byttneria* (Fig. 1).

There are few documented examples of reversals from lianas to self-supporting growth forms and perhaps none from semi-self-supporting plants, apart from this study. Speck et al. (2003) state that in most examples of clades that include both climbing and self-supporting plants, the plesiomorphic condition is almost always inferred to be self-supporting. This is consistent with Gentry’s (1991) observations of the taxonomic distribution of climbing species who notes that, while some families consist almost entirely of climbers (e.g. Convolvulaceae), many climbers are in families that are otherwise made up almost entirely of trees and shrubs (and thus the climbing habit is presumably derived). The generality of such observations is limited by the small number of well-sampled phylogenetic analyses of groups that include substantial numbers of both climbing and nonclimbing species. In some exceptional examples, however, there may be multiple reversals from climbing forms to self-supporting growth forms within a single clade of closely related species. In one group of Apocynaceae, for example, there have been five independent transitions from lianas to a shrub-like habit (Lahaye et al. 2005). Because semi-self-supporting plants are rarely distinguished from lianas or vines, little is known about their taxonomic distribution or frequency, or transitions to or from this growth form.

The transition from semi-scandent plants to the erect shrubs and subshrubs of *Byttneria* sect. *Byttneria* may be the first documented example of a reversal from a semi-self-supporting growth form. However, the taxa that we score as semi-scandent likely include some upright shrubs, thus there may be additional transitions between these growth forms. Nevertheless, the unusual architectures, leaf morphologies, and ecology of *Byttneria* sect. *Byttneria* mark a divergence from its close relatives. Section *Byttneria* is a morphologically cohesive group of over 20 species, all native to South America that are erect, often rhizomatous shrubs or subshrubs mostly occurring in open, seasonally dry and/or flooded habitats (Cristóbal 1976). The lineage sister to the remainder of the ingroup sampled here, *Byttneria scabra* (as well as an unsampled species *B. dentata*), is an erect shrub, 2.5 m tall, with broad, palmately-veined, toothed leaves, and prickly stems similar to what is seen in closely related semi-scandent shrubs such as *B. urticifolia* and *B. divaricata* (Fig. 1). All other species of sect. *Byttneria* are generally shorter, have unarmed stems and entire, coriaceous leaves with prominent, often parallel secondary venation, and leaf shapes ranging from linear, lanceolate, to sagittate, all uncommon in Malvaceae. Branching generally occurs at or close to ground level, with plants dying

back to the ground in adverse conditions such as drought or fire. All species are also united in having uniapecturate foliar nectaries (vs. multipecturate in other species of *Byttneria* and a few species of *Ayenia*).

The result that *Byttneria scabra* is sister to all other members of sect. *Byttneria* suggests that the transition to a self-supporting growth form preceded a reduction in stature and the modification in leaf morphology associated with invasion of more open and seasonal habitats. Lahaye et al. (2005) speculated that transitions to more shrub-like habits in Apocynaceae that grow in open dry habitats were due to reduced competition and fewer hosts for lianas. The remarkably monocot-like shrubs and subshrubs of *Byttneria* sect. *Byttneria* may reflect similar adaptations. A second invasion of dry, open habitats occurs in *Ayenia*. All species of *Ayenia* sampled in this study are upright self-supporting plants, although many other species are low with procumbent or decumbent branches, suggesting that *Ayenia* underwent different morphological innovations in an independent invasion of dry, seasonal, and open habitats.

The cambial variants in secondary growth seen in many lianas may pose developmental or ecological constraints against transitions from lianas to self-supporting growth forms (Rowe and Speck 2004). Alternatively, because lianas often have a self-supporting stage as seedlings or reiterations, with anatomy similar to what is seen in mature self-supporting species, a simple retention of juvenile characteristics may provide a mechanism by which lianas could evolve into self-supporting forms (Speck et al. 2003). Application of these ideas to semi-self-supporting plants is limited again by a general lack of information about them. It is not clear if semi-self-supporting plants as a class tend to have cambial variants. Rowe and Speck (2004) suggest that semi-self-supporting plants may not exhibit developmental changes in mechanical properties seen in lianas or the structural novelties found in mature liana shoots, and instead may simply lean on surrounding vegetation and interlock the branches. If this is the case, then transitions from semi-self-supporting growth forms may face different developmental and physiological constraints (if any) than lianas.

To our knowledge, the anatomy of secondary growth has never been investigated in any species of *Ayenia* or *Byttneria*. The current phylogeny, however, provides a powerful system with which to explore anatomical and architectural similarities and differences between closely related species with differing growth forms, in particular to compare lianas and semi-scandent shrubs, semi-scandent species of *Byttneria* with species of *Ayenia* with procumbent and decumbent growth forms, and self-supporting species of *Ayenia* with the independently derived subshrubs of *Byttneria* sect. *Byttneria*.

Perhaps the most uncommon growth form in this clade is a tree, or an upright woody plant with a single, well-defined trunk. Although our sampling is not complete, our results suggest the common ancestor of this clade was a tree, with subsequent trends towards reduced stature and/or increased branching. Both of the closest outgroups, *Abroma* and *Kleinhovia*, are small trees. It appears that only two species of *Ayenia* and four of *Byttneria* are arborescent. We sampled from three of these tree species, *B. morii* from French Guiana, *B. schunkei* from the Peruvian Amazon, and *A. praeclarae* from Guyana. *Byttneria morii* and *B. schunkei* appear to be together a relatively basal lineage within the *Ayenia/Byttneria/Rayleya*

clade. Relationships of *A. praeclarae* are unresolved; however, a basal position within the *Ayenia* clade is supported by the presence of foliar nectaries, a trait found in all sections of *Byttneria* but limited to seven species in *Ayenia*, (Cristóbal and Arbo 1971; Cristóbal et al. 1992; Dorr 1996). The other arborescent species of *Ayenia*, *A. stipularis*, also has foliar nectaries and is hypothesized to be closely related to *A. praeclarae* (Cristóbal et al. 1992). The other species of *Byttneria* that have been described as trees, *B. fruticosa* and *B. glabra*, both from east Africa, are also unarmed and have been both placed in sect. *Vahihara*, on the basis of floral morphology. If growth form is indeed an indicator of phylogenetic relationships, we expect that future analyses that include these African taxa will show these lineages near the base of the *Ayenia/Byttneria/Rayleya* clade, although this may result in more complicated biogeographic patterns (see below).

**Biogeography**—The *Ayenia/Byttneria/Rayleya* clade is unusual in Byttnerieae in that many species occur in the Americas. *Rayleya* and *Ayenia* only occur in the new world, and the apparently paraphyletic *Byttneria* is most diverse there, both morphologically and in the number of species. All other genera of Byttnerieae are exclusively paleotropical (Whitlock et al. 2001). Although the closest outgroups of this clade are two small Australasian genera, *Kleinhovia* (monotypic) and *Abroma* (1–2 species), the basal lineages of the *Ayenia/Byttneria/Rayleya* clade are predominantly neotropical, suggesting that the common ancestor of these plants was neotropical as well (Fig. 1), consistent with Cristóbal's (1976) prediction based on the diversity within *Byttneria*. This conclusion, however, depends on relationships among basal lineages within the clade that are not strongly supported in our analyses.

All paleotropical taxa sampled within the *Ayenia/Byttneria/Rayleya* clade are derived from within the clade of lianas, i.e. *Byttneria* sect. *Vahihara* (Fig. 1). Although relationships among lineages within the liana clade are not fully resolved, there appear to be at least two independent radiations in the paleotropics, in Madagascar and Southeast Asia. The sole specimen that we were able to sample from Africa, *B. catalpifolia*, may represent a third paleotropical radiation. In Madagascar, *Byttneria* displays a remarkable radiation of approximately 27 species of lianas, many with unusual leaf morphologies and dramatic heterophyly. In contrast, *Byttneria* in Southeast Asia appears to be less diverse morphologically. Although many names exist for Asian taxa, there are no comprehensive keys to species for the region, and it is possible that species diversity is low.

Unexpectedly, the Malagasy clade of *Byttneria* is sister to a neotropical lineage in all reconstructions. In a recent review of the biogeographic origins of the Malagasy biota, Yoder and Nowak (2006) found that the most common pattern was a sister group relationship between lineages from Madagascar and Africa, most likely the result of recent Cenozoic dispersal. We cannot rule out the possibility of a close relationship between African and Malagasy lineages of *Byttneria* because African taxa are undersampled here and because relationships among lineages within the liana clade are poorly supported (Fig. 1). The one specimen from Africa that we were able to sample belongs to the most widely distributed species in the genus, *B. catalpifolia* and forms a well-supported clade with a conspecific specimen from Peru. The uncertain position of *B. catalpifolia* may be due to the extraordinarily long branch leading up to these two specimens (Fig. 1).



Although *Byttneria* is not diverse in Africa, additional sampling of African taxa will likely lead to more complicated patterns of biogeography, and to further examples of transitions in growth forms. In addition to *B. catalpifolia*, there are two species of *Byttneria* in sect. *Vahihara* from East Africa, three poorly collected species in sect. *Crassipetala* endemic to West Africa, and two species in the segregate genus *Megatritheca* from Central Africa (Cristóbal 1965, 1976). The African species of the nonmonophyletic sect. *Crassipetala* appear to be spiny, scrambling shrubs and may represent an independent radiation in the African tropics if they are closely related to neotropical lineages with similar morphology. While the floral morphology of the two East African species in sect. *Vahihara* is consistent with other members of the section, they differ in that both are small, unarmed and upright trees. If an arborescent growth form is plesiomorphic within the *Ayenia*/*Byttneria* clade, these two taxa should diverge at the base of the clade or perhaps sister to the remaining taxa of sect. *Vahihara*, representing an additional lineage in the paleotropics. Alternatively, if these two species are closely related to the African *B. catalpifolia*, they may represent a reversal from true lianas to self-supporting trees.

The unsampled and rarely collected central African genus *Megatritheca* promises to provide additional complications to our understanding of morphological evolution and biogeography within this group. If *Megatritheca* is closely related to the exclusively American species of *Ayenia*, as its unusual trithecate anthers suggest, it would represent an additional paleotropical lineage. Other aspects of its floral morphology are consistent with the paraphyletic *Byttneria* sect. *Crassipetala*, such as the broad petal claws and prominent fleshy petal appendages (Cristóbal 1965, 1976). Little is known of the morphology or growth form of the two species of *Megatritheca*. One species is described as a shrub, whereas the other is described as sarmentose, or creeping.

Although the timing of shifts in distribution between the neotropics and paleotropics is of great interest, assigning divergence times in the *Ayenia*/*Byttneria*/*Rayleya* clade is challenging because of a lack of suitable calibrations as well as substantial rate heterogeneity (Fig. 1). There are no known fossils of *Byttneria*, *Ayenia*, or *Rayleya*, and the few assignable to Byttnerioideae are mostly of recent origin. However, the wide distribution of a few species of *Byttneria* argues in favor of at least some long distance dispersal events, probably recently, between the neotropics and paleotropics. *Byttneria catalpifolia*, for example, occurs in the neotropics, from Mexico to Argentina, as well as in tropical Africa, and Tahiti (Cristóbal 1976). Our finding that two widely separated specimens that we sampled are closely related with nearly identical sequences (Fig. 1), supports this species concept. Although the parsimony optimizations of geographic distribution suggest that dispersal within *B. catalpifolia* occurred from the neotropics to Africa, additional sampling within this species and of other African species may yet reveal an African origin, with subsequent dispersal to the neotropics. Regardless of the directionality of dispersal, *B. catalpifolia* provides an additional example of a recent disjunction across the tropical Atlantic (Thorne 1973; Renner 2004). A second widely distributed species not sampled here, *B. aculeata* of sect. *Crassipetala*, is found from Mexico to Bolivia, with disjunct populations in Tuamotu in French Polynesia.

Long distance dispersal is somewhat unexpected in *Byttneria* given its fruit morphology. *Byttneria* fruits are five-

seeded capsules or five one-seeded mericarps, ranging in size from 0.5–4 cm in diameter. They are dry and woody, often armed with hooks or spines. There is no obvious adaptation for dispersal by endozoochory, wind, or water, the processes most often invoked in long distance dispersal. However, it is conceivable that some fruit may be dispersed by attachment to the feathers or fur of birds and mammals, although neither has been reported. Once dispersed, the weedy nature of at least some *Byttneria* species may facilitate establishment. *Byttneria aculeata*, for example, is a serious weed in Mexico and Central America and the wide distribution of this species may reflect in part its weedy habit (Cristóbal 1976). The general ability for many lianas to thrive in disturbed habitats may also facilitate establishment in new areas after a rare dispersal event.

Within the neotropics, members of the *Ayenia*/*Byttneria*/*Rayleya* clade are found in a wide range of habitats, from deserts to tropical rain forests. Information on the habitat occurrences for most species is incomplete and thus difficult to quantify. However, our phylogenetic results suggest that plants that grow in open habitats with pronounced dry seasons represent two independent lineages. Many species of *Ayenia* occur in deserts and scrublands in Mexico, the southern U. S. A., and the Caribbean. A second independent radiation into seasonal savannahs of South America occurs within *Byttneria* sect. *Byttneria*. In both lineages, plants have evolved different growth forms associated with more seasonal conditions.

Phylogenetic analyses of closely related species provide an opportunity to examine how plants with differing growth forms contribute to diversity at multiple geographic scales. Do changes in growth form allow a lineage to invade a previously uninhabitable region? Are plants with some growth forms more likely to disperse to and establish in new regions? Are plants with some growth forms more likely to diversify within a region? We are just beginning to address such questions in the *Ayenia*/*Byttneria*/*Rayleya* clade. Our results show that a change in growth form preceded invasion of seasonal, open habitats of South America in *Byttneria* sect. *Byttneria*. At a broader geographic scale, lianas appear more successful at establishing and radiating into new continental landmasses. Within the *Ayenia*/*Byttneria*/*Rayleya* clade, lianas account for the majority of the species diversity, suggesting that this growth form may have an increased speciation rate, with some regions showing a remarkable radiation of lianas, such as Madagascar. Expanded sampling of species, combined with comparative anatomical and developmental studies, may allow us to add a new dimension to floristic studies and shed light on the evolutionary processes that underlie large-scale patterns of tropical plant diversity.

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- APPENDIX 1. Specimens sampled for molecular phylogenetic analyses. Taxon sampled; geographic range of species; voucher information (collector, collection number, and herbarium where voucher is deposited); and GenBank accession numbers (*trnL-trnF* and *matK*, respectively). Geographic distribution and classification follow Cristóbal (1960, 1976, 1981, and 1985), Barnett and Dorr (1990), and Whitlock et al. (2001).
- Ayenia* sect. *Ayenia*.** *A. filiformis* S. Watson; Whitlock et al. 398 (GH); Southwestern U. S. A.; HM488369, HM488411; *A. lingulata* Grisebach; Cantino 691 (ARIZ); Argentina; HM488370, HM488412; *A. praeclara* Sandwith; Cid 9705 (NY); Guyana; HM488371, HM488413.
- Ayenia* sect. *Cybiostigma*.** *A. magna* L.; Arnoldo-Broeders 3874 (A); South America, Caribbean; HM488372, HM488414; *A. palmeri* Watson; Van Devender 95-1037 (ARIZ); Mexico; HM488373, HM488415; *A. paniculata* Rose; Goldberg 76-319 (ARIZ); Mexico; HM488374, HM488416.
- Ayenia* sect. *Cybiostigma*?** *Ayenia* sp. A; Boyle et al. 5550 (MO); Costa Rica; HM488375, HM488417.
- Ayenia* sect. *Leiayenia*.** *A. fruticosa* Rose; Jenkins 97-70 (GH); Mexico; HM488376, HM488418; *A. microphylla* A. Gray; Jenkins 97-71 (GH); Southwestern U. S. A., northern Mexico; HM488377, HM488419.
- Byttneria* sect. *Byttneria*.** *B. jaculifolia* Pohl; Hatschbach et al. 60306 (GH); Brazil; HM488378, HM488420; *B. pedersenii* Cristóbal; Arbo et al. 6159 (GH); Brazil, northern Argentina; HM488379, HM488421; *B. L.*; Troeis et al. 10272 (A); Widely distributed in South America; HM488380, HM488422; *B. stenophylla* Cristóbal; Cristóbal et al. 2384 (GH); Paraguay; HM488381, HM488423; *Byttneria* sp. B; Tsugaru et al. B-2500 (A); Brazil; HM488382, HM488424.
- Byttneria* sect. *Crassipetala*.** *B. divaricata* Benth.; Sette Silva 701 (GH); Widely distributed in South America; HM488383, HM488425; *B. fernandesii* Cristóbal; Mori et al. 11233 (NY); Brazil; HM488384, HM488426; *B. filipes* Mart. ex K. Schum.; Lopez & Vanni 110 (GH); Brazil, Paraguay, Argentina; HM488385, HM488427; *B. microphylla* Jacq.; Zannoni et al. 26254 (NY); Cuba, Hispaniola; HM488386, HM488428; *B. morii* L. Barnett & Dorr; Mori et al. 23755 (NY); French Guiana; HM488387, HM488429; *B. schunkei* Cristóbal; Schunke 4567 (GH); Perú; HM488388, HM488430.
- Byttneria* sect. *Urticifolia*.** *B. coriacea* Britton; Cid and Luna 3508 (NY); Amazonia; HM488389, HM488431; *B. implacabilis* Cristóbal; Hatschbach et al. 50359 (NY); Brazil; HM488390, HM488432; *B. urticifolia* K. Schum.; Tressens et al. 3314 (A); Brazil, Uruguay, Argentina; HM488391, HM488433.
- Byttneria* sect. *Vahihara*.** *B. biloba* var. *grandidieri* Arènes; Schatz 2985 (NY); Madagascar; HM488392, HM488434; *B. catalpifolia* Jacq. "Africa"; Whitlock et al. 403 (GH); Americas, Tropical Africa, Tahiti; HM488393, HM488435; *B. catalpifolia* Jacq. "South America"; Acevedo-Rodríguez 8968 (GH); Americas, Tropical Africa, Tahiti; HM488394, HM488436; *B. cordifolia* Sagot; Prevost 3392 (NY); French Guiana, Surinam, Brazil; HM488395, HM488437; *B. maingayi* Mast.; Gentry & La Frankie 66848A (A); Malesia; HM488396, HM488438; *B. melleri* Back.; Randrianasolo 572 (MO); Madagascar; HM488397, HM488439; *B. melleri* Baker; Miller and Randrianasolo 6217 (NY); Madagascar; HM488398, HM488440; *B. oligantha* J. Ar.; Phillipson 3066 (NY); Madagascar; HM488399, HM488441; *B. uau-pensis* Spruce ex K. Schum.; Jansen-Jacobs et al. 2331 (NY); Northern South America; HM488400, HM488442; *B. voolily* Baill.; Randrianasolo 520 (MO); Madagascar; HM488401, HM488443; *B. sp. C*; Ambriansyah et al. AA873 (A); Indonesia; HM488402, HM488444; *B. sp. D*; Laman et al. TL1378 (A); Indonesia; HM488403, HM488445; *B. sp. E*; Sidiyasa 1046 (A); Indonesia; HM488404, HM488446.
- Byttnerieae*.** *Rayleya bahiensis* Cristóbal; Guiletti et al. 7097 (US); Brazil; HM488405, HM488447; *Abroma augustum* (L.) L. f.; Whitlock 500 (FTG); Australasia; HM488406, HM488448; *Kleinhovia hospita* L.; Whitlock 501 (FTG); Australasia; HM488407, HM488449.
- Lasiopetaleae.** *Guichenotia ledifolia* J. Gay; Hansen s.n. (PERTH); Australia; HM488408, HM488450; *Rulingia magniflora* F. Muell.; Wilkins and Whitlock 1912 (PERTH); Australia; HM488409, HM488451.
- Theobromeae.** *Theobroma cacao* L.; Whitlock 361 (GH); tropical America; HM488410, HM488452.