EDINBURGH JOURNAL OF BOTANY 69 (3): 413–440 (2012)

doi:10.1017/S096042861200025X

PHYLOGENY, BIOGEOGRAPHY AND CHARACTER EVOLUTION OF *DORSTENIA* (MORACEAE)

T. M. Misiewicz[1,2,3] & N. C. Zerega[1,2]

Dorstenia, the second largest genus (105 species) within the Moraceae, is the only genus in the family with woody, herbaceous and succulent species. All but one species of *Dorstenia* are restricted to the Neotropics or Africa, and it is the only genus in the family with an almost equal transatlantic distribution. This work presents the first molecular phylogeny and the first evolutionary study to examine origin and diversification within the genus. We inferred the phylogeny with ITS sequence data using Bayesian and maximum likelihood approaches. We tracked the evolution of distinct morphological characters and tested for correlated evolution in multiple characters. Time and place of *Dorstenia*'s origin were estimated to test a post-Gondwanan versus a Gondwanan origin hypothesis using fossil calibrations, Bayesian molecular dating, and maximum likelihood-based ancestral range reconstructions. Our phylogenetic analysis supports the monophyly of *Dorstenia*; previous subgeneric classifications are polyphyletic and must be re-evaluated. Woody habit, phanerophytic life form, macrospermy, and lack of storage organs are ancestral traits found in African *Dorstenia*. Evolution of woodiness and macrospermy are correlated. *Dorstenia* appears to have originated in Africa, radiated into the Neotropics and subsequently re-colonised Africa. Whether or not the extant distribution is the result of vicariance or dispersal is equivocal.

Keywords. Biogeography, character evolution, diversification rate, *Dorstenia*, Moraceae.

Introduction

Dorstenia L. holds a fascinating position within the Moraceae (mulberry family). With 105 species, *Dorstenia* is the second largest genus within the family, second only to *Ficus* L. (figs, c.750 species), and is the only Moraceae genus with herbaceous, succulent and woody species (Berg & Hijman, 1999; Berg, 2001). All but one species of *Dorstenia* are restricted to the Neotropics or Africa, and it is the only group in the family with an almost equal distribution of species on either side of the Atlantic Ocean (Berg & Hijman, 1999). The Moraceae have been particularly well studied in part due to the unique pollination syndromes present in the family (Bawa *et al.*, 1985; Sakai *et al.*, 2000; Berg, 2001; Sakai, 2001; Zerega *et al.*, 2004) and it has been hypothesised

[1] Chicago Botanic Garden, 1000 Lake Cook Road, Glencoe, IL 60022, USA.
[2] Northwestern University, Plant Biology and Conservation, 2205 Tech Drive, Evanston, IL 60208, USA.
[3] Current address: University of California, Berkeley, Department of Integrative Biology, 3060 Valley Life Sciences Building #3140, Berkeley, CA 94720, USA. E-mail: tmisiewicz@berkeley.edu

that *Dorstenia* may represent an intermediate form in the evolution from open inflorescence and wind pollination in *Morus* L. (mulberries) to the specialised syconium and obligate pollination mutualism of *Ficus*. In spite of *Dorstenia*'s unique distribution, morphology and evolutionary position within the Moraceae family, little is known about its evolutionary history, biogeography or reproductive ecology.

The Moraceae are comprised of 37 genera and c.1100 species (Berg, 2001). The family exhibits a cosmopolitan distribution, with the majority of extant species found in the Old World tropics (Berg, 2001). Molecular studies have shown that Moraceae is a well-supported monophyletic group, although taxon sampling for *Dorstenia* within the study was very poor, including only two out of 105 species (Datwyler & Weiblen, 2004; Zerega *et al.*, 2005; Clement & Weiblen, 2009). The family is thought to have radiated during the mid-Cretaceous with an estimated date of 89.1 (72.6–110.0) million years ago (mya), suggesting that it probably diversified after the break-up of Africa and South America (105 mya) (Zerega *et al.*, 2005).

Dorstenia species are distinct from the majority of the Moraceae family in morphological characteristics as well as geographic distribution. Ninety per cent of *Dorstenia* species are herbaceous, as compared to the entire Moraceae family in which 90% of species exhibit a woody habit and only 10% are herbaceous. Life forms within the genus are variable, with geophytes (defined by the presence of underground storage organs), hemicryptophytes (plants with perennating buds located at the soil surface) and phanerophytes (in which the perennating buds are positioned well above the soil surface). Stems range from caulescent (well developed and above ground) to acaulescent (having only a very short below-ground stem or lacking one altogether) (Raunkiaer, 1934). Reproductively, *Dorstenia* is characterised by discoid inflorescences that are primarily bisexual, with tiny reduced male and female flowers. The size and shape of the inflorescence varies among species and many are surrounded by a diversity of vegetative appendages, which differ greatly among taxa (Fig. 1). The endosperm of the seed is either microspermous (< 5 mm long or broad) or macrospermous (> 5 mm long or broad) and the fruits are drupes that dehisce explosively (Berg & Hijman, 1999; Berg, 2001).

Berg & Hijman (1999) and Berg (2001) prepared the most recent and comprehensive taxonomic treatments of *Dorstenia* in regional floras (Africa and the Neotropics, respectively). These treatments brought together scattered and previously unpublished taxonomic information and not only provide morphological and geographic information for *Dorstenia*, but also pose hypotheses regarding character evolution and dispersal mechanisms, and present a sectional classification for species in the genus (Table 1; Berg & Hijman, 1999).

The genus is currently divided into nine sections based primarily on morphological characters pertaining to habit and life form (Table 1). While other sectional classifications proposed in the past included reproductive morphology, most of these divisions were proposed prior to the discovery of the majority of African species (Berg & Hijman, 1999). Berg & Hijman (1999) were the first to propose a subdivision of the genus in its entirety.

FIG. 1. *Dorstenia ciliata* Engl.: close-up of inflorescence. Photo by Tracy Misiewicz.

Because *Dorstenia* is distinct from almost all other genera in the Moraceae with regard to habit, Berg & Hijman (1999) posited that traits associated with woodiness, which is shared with most of the rest of the Moraceae, must be primitive, and characters associated with herbaceousness are derived. Based on morphological assessments Berg & Hijman (1999) hypothesised that *Dorstenia* species have evolved

TABLE 1. Distribution of defining morphological characters for the sectional classification proposed by Berg & Hijman (1999). Geographic distribution of species for each section is defined as Old World (OW), New World (NW) or both (NW & OW)

	Character states																			
Section	Woody	Herbaceous	Succulent	Microspermous	Macrospermous	Pistillate flowers 1–7	Pistillate flowers >7	Receptacle bracteate	Receptacle ebracteate	Petiole long (>5 cm)	Petiole short (<5 cm)	Internode short	Internode long	Caulescent	Acaulescent	Subcaulescent	Tuberous	Rhizomatous	No storage organ
Nothodorstenia (OW, 5 spp.)	X				X	X		X			X		X	X					X
Xylodorstenia (OW, 6 spp.)	X				X	X			X		X		X	X					X
Acauloma (OW, 3 spp.)		X		X			X		X	X		X			X		X		
Bazzemia (OW, 1 sp.)		X		X			X		X	X		X			X			X	
Lomatophora (OW, 24 spp.)		X		X			X		X		X		X	X				X	X
Kosaria (OW, 16 spp.)		X	X	X			X		X		X		X	X			X	X	
Emygodia (NW, 18 spp.)		X		X			X	X			X	X	X	X		X		X	
Lecanium (NW & OW, 22 spp.)		X		X			X	X			X		X	X				X	
Dorstenia (NW & OW, 8 spp.)		X		X			X	X		X		X	X	X		X		X	X

from phanerophytes to hemicryptophytes to geophytes with stems evolving from caulescent to subcaulesent to acaulescent. Herbaceousness is thought to have evolved from woody habits (Berg & Hijman, 1999).

Since Raven & Axelrod (1974) first proposed the shifting of continental landmasses as a major driver in the shaping of spatial and temporal patterns of plant diversity there has been a debate over the importance of vicariance versus dispersal. The break-up of Gondwana in particular is a common explanation for disjunctions seen in many tropical floras (Raven & Axelrod, 1974; Burnham & Graham, 1999; Muellner *et al.*, 2006; Clayton *et al.*, 2009). As a result of *Dorstenia*'s southern hemisphere distribution and a lack of apparent means for long distance dispersal, the vicariance hypothesis has been specifically applied to the genus (Berg & Hijman, 1999). This distribution along with the equal species diversity in both the Old and the New World has led to the suggestion that *Dorstenia* originated at least 105 mya, the last time South America and Africa were connected (McLoughlin, 2001), and that *Dorstenia* species on each continent subsequently underwent separate diversifications (Berg & Hijman, 1999; Berg, 2001). Alternatively, *Dorstenia* could have originated post-Gondwanan break-up and dispersed or migrated from one continent to the other, a more plausible hypothesis given that the Moraceae divergence is estimated to have occurred after Africa and South America split (Zerega *et al.*, 2005).

Work by Zerega *et al.* (2005) also suggested that *Dorstenia* is less than 20 million years old and proposed that the genus originated in the Old World and consequently migrated to the New World. However, that study included only 95 of the 1100 Moraceae taxa and only two Neotropical and no Afrotropical *Dorstenia* species, limiting the ability to calculate divergence dates for *Dorstenia* accurately or to determine whether the genus evolved in the New or the Old World.

This study reconstructs the first molecular phylogeny of the genus *Dorstenia* by analysing DNA sequence data from the ITS region using Bayesian and maximum likelihood approaches. This phylogeny in turn provides an evolutionary framework to test the current sectional classification of the genus, which is based solely on morphological characters, and estimate the time and place of *Dorstenia*'s origin. We test a post-Gondwana versus Gondwanan origin hypothesis and map the progression of distinct morphological characters, including the hypothesised evolution from woody to herbaceous habits, and test for correlations in the evolution of multiple characters.

Materials and Methods

DNA extraction, amplification and sequencing

Individuals representing 42 taxa were sequenced for the ITS region (including internal transcribed spacers 1 and 2 and the 5.8S region) of nuclear rDNA. Seven outgroup taxa were included from seven different genera and six different tribes in the Moraceae family (*Antiaropsis* K.Schum., *Artocarpus* J.R.Forst. & G.Forst.,

Brosimum P.Browne, *Ficus*, *Maclura* Nutt., *Morus* and *Treculia* Decne. ex Trécul) and 35 ingroup taxa, representing all but one monotypic section of *Dorstenia*. Material was mostly taken from herbarium specimens or, in a few cases, silica-gel-dried leaves. Vouchers and GenBank accession numbers are listed in Appendix 1 (along with taxon authorships). DNA was extracted from leaf tissue using a modified CTAB extraction method (Chaudhry *et al.*, 1999). Nine chloroplast primer pairs (pl32-trnL(UAG), trnQ(UUG)-5′rps16, 3′trnV(UAC)-ndhC, ndhF-rpl32, psbD-trnT (GGU), psbJ-petA, 3′rps16-5′trnK(UUU), atpI-atpH, and petL-psbE) described in Shaw *et al.* (2007) were tested but could not be included in this study due to inconsistent amplification and sequencing failure. Accordingly, all subsequent work was conducted using ITS. DNA was PCR-amplified using forward primer ITS 5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and reverse primer ITS 4 (5′-TCCTCCGCTTATTGATATGC-3′) (White *et al.*, 1990). PCR amplifications were performed in a 25 μl reaction containing approximately 25 ng of genomic DNA, 0.55 μl 25 mM $MgCl_2$, 2.5 μl 10× *TaKaRa Ex Taq* buffer (Otsu, Shiga, Japan), 2 μl 2.5 μM dNTP, 1 μl 10 mg/ml BSA, 1.25 μl 10 μM forward primer, 1.25 μl 10 μM reverse primer, and 1.25U *TaKaRa Ex Taq* DNA polymerase (Otsu). The PCR protocol consisted of an initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 2 min, followed by a final extension period of 72°C for 10 min. While ITS has a number of potentially confounding factors associated with it (multiple copies of nrDNA, pseudogenes, and the potential for imperfect concerted evolution) most of these problems can be overcome with cloning and phylogenetic analyses of variable DNA amplicons (Alvarez & Wendel, 2003; Bailey *et al.*, 2003; Feliner & Rossello, 2007). Two bands were detected and PCR products were cloned in order to isolate the gene region of interest. Transformation and ligation of PCR products were performed using the Invitrogen TOPO TA Cloning Kit for Sequencing and Invitrogen One Shot TOP10 Chemically Competent *E. coli* (Invitrogen Carlsbad, CA, USA). Colonies were screened using a 20 μl PCR reaction using 10 μM of primer t7, 10 μM of primer m13rev, 0.2 μM dNTP, 2 μl 10× *Ex Taq* buffer and 1.25U *Ex Taq*. Samples were cycled at 94°C for 10 min followed by 24 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and a final extension of 72°C for 10 min. PCR products were cleaned using a Qiagen Qia-Quick PCR Purification Kit (Valencia, CA, USA). Four amplicons per sample were selected for sequencing. Sequencing was performed in 10 μl reactions using Big Dye sequencing reagents and protocols (Applied Biosystems, Foster City, CA, USA), and data were collected on an ABI 377XL automated DNA sequencer (Applied Biosystems). The ITS region was sequenced in both directions using ITS primers. Sequences were edited using CodonCode Aligner (CodonCode Corporation, Dedham, MA, USA) and alignment was performed using MUSCLE (Edgar, 2004) for initial alignment, and Se-Al 2.0a11 (Rambaut, 2002) was used for manual adjustments using a similarity criterion (Simmons, 2004).

Phylogenetic analyses

Phylogenetic reconstruction was based solely on ITS sequence data. Bayesian MCMC analyses (Yang & Rannala, 1997) were conducted using MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003). Gaps were treated as missing data. MrModeltest v2.3 (Nylander, 2004) was used to find the best-fitting model of nucleotide substitution among those examined. The GTR+I+G model was selected and applied (shape parameter = 1.0801, proportion of invariant sites = 0.1971). Three heated chains and one cold chain were run for 1,000,000 generations, with sampling every 100 generations. A 50% majority rule consensus tree was generated after 2500 burn-in trees were excluded to create the final Bayesian phylogeny. Analyses were repeated a second time to verify tree topology convergence.

Maximum likelihood (ML; Felsenstein, 1973) analyses were performed with RAxML v7.0.2 (Stamatakis, 2006). Analyses were performed on the San Diego Supercomputing Center's computer cluster as part of the 'Cyber Infrastructure for Phylogenetic RESearch' (CIPRES) project. RAxML exclusively uses the GTRGAMMA model for approximating rate heterogeneity. Model parameter values were estimated in RaxML, and ML searches were performed using 500 bootstrap replicates (Felsenstein, 1985; Stamatakis *et al.*, 2008).

Phylogenetic signal

The topology based on the ML and Bayesian analyses was consistent (as described in the Results section) so the ML tree was used for all subsequent analyses. A matrix of 15 characters was compiled from the taxonomic literature (Oliver, 1868; Zohary, 1966; Berg & Hijman, 1999; Berg, 2001; Conn *et al.*, 2006+) (Appendix 2) and scored as follows: *Habit* (0 = woody, 1 = herbaceous), *Geophyte* (0 = phanerophytic, 1 = geophytic), *Stem* (0 = caulescent, 1 = acaulescent), *Leaf margin* (0 = lobate, 1 = entire), *Petiole length* (0 = long, 1 = short), *Pistillode* (0 = present, 1 = absent), *Distribution* (0 = Old World, 1 = New World), *Leaf variegation* (0 = present, 1 = absent), *Endosperm size* (0 = microspermous, 1 = macrospermous), *Inflorescence bracts* (0 = present, 1 = absent), *Storage unit* (0 = tuber or rhizome present, 1 = absent), *Leaf apex* (0 = triangular, 1 = rounded, 2 = variable), *Leaf base* (0 = triangular, 1 = rounded, 2 = variable), *Stipule* (0 = persistent, 1 = subpersistent, 2 = caducous), *Staminate flower arrangement* (0 = evenly dispersed, 1 = peripheral). Traits were tested for conservatism by determining whether there are significantly fewer steps in the evolution of a character on the ML phylogeny than one would expect for a random character that is not associated with the phylogeny. The number of steps in the evolution of each trait was calculated on the ML phylogeny and then compared to the number of steps from a null model generated by randomising characters across the phylogeny 1000 times. The null model was generated using randomised characters instead of randomised trees because changing the topology may reflect an effect of our phylogeny as opposed to our character. Randomising the

data over the same tree eliminated this potential complication. The actual distributions were then compared to the phylogenetically random distributions; a one-tailed Monte Carlo test was performed and variances significantly lower than the null (95% of the random data sets) indicated phylogenetic signal for a trait.

Ancestral state reconstruction

Ancestral states were reconstructed using Mesquite 2.6 (Maddison & Maddison, 2009). Ancestral states for all characters that showed significant phylogenetic signal were reconstructed using both parsimony and maximum likelihood methods. The likelihood analysis used the Markov k-state 1 parameter model (Mk1) that assumes some stochastic rate of change from one character state to another along a branch. This rate depends on the branch length and rate of change.

Correlated character evolution

Morphological information was obtained from the literature (Oliver, 1868; Zohary, 1966; Berg & Hijman, 1999; Berg, 2001; Conn *et al.*, 2006+). In order to test for correlated changes in discrete characters, Pagel's (1994) correlation test was performed using Mesquite 2.6 (Maddison & Maddison, 2009), which uses likelihood scores. The null hypothesis for this test is that all characters evolve independently of one another. There are four rate changes: a forward and reverse rate for each character in the pair being compared. A more complex model assumes the rate of change of each character is dependent on the character state of the other character, therefore having eight different rates. The simple model, which represents the null, of independent rate change is nested within the more complex model of dependent rate change and a likelihood ratio test is used to compare the likelihood scores of these two models and ultimately test if the likelihood of the complex model is greater enough than the simple model to justify its use.

Molecular dating

A likelihood ratio test for rate constancy was performed using PAUP* 4.0 (Swofford, 2003) and results indicated that the gene region was not evolving in a clock-like manner. As a result molecular dating analyses were performed using BEAST v1.4 (Drummond & Rambaut, 2007). The BEAST interface BEAUti was used to create a BEAST input file. The analyses were performed with a relaxed molecular clock model assuming a lognormal distribution (Drummond *et al.*, 2006). The Yule speciation process was used as a tree prior and the GTR+G+I model was applied. Eight gamma categories were used for the approximation of the gamma distribution. *Antiaropsis*, *Artocarpus*, *Brosimum*, *Ficus*, *Morus* and *Treculia* were constrained as outgroup taxa and node priors for the root; *Ficus* and *Morus* were set to a uniform

distribution and calibrated with the minimum and maximum ages of Moraceae fossils as used in Zerega *et al.* (2005). When a time period was assigned to a fossil, Zerega *et al.* (2005) used the oldest date from that time. Fossil calibrations were placed at the crown and included *Ficus* achenes and *Morus* fruit. For *Ficus* achenes the upper age constraint was set to 55 mya and the lower age constraint to 40 mya. For *Morus* fruits the upper age constraint was set to 65 mya and the lower age constraint to 23 mya (Collinson, 1989; Zerega *et al.*, 2005). The root node of the tree was calibrated with a lower age constraint of 23 mya, the lower age constraint of our youngest Moraceae fossil, and an upper age constraint of 135 mya based on the oldest known angiosperm fossil (Magallon & Sanderson, 2001). Operators were tuned using the auto-optimisation option and the final results were viewed in Tracer (Rambaut & Drummond, 2007) for all estimated parameters, and estimated sample size for all nodes was well above 200, indicating a sufficient posterior distribution quality.

Analysis of geographic range evolution

Lagrange (Likelihood analysis of geographic range evolution) (Ree *et al.*, 2005; Ree & Smith, 2008) was used to estimate the likelihood of ancestral species ranges throughout the phylogeny using an unconstrained model. Lagrange calculates the likelihood that any particular ancestral node occupies an area given the ranges of the two daughter nodes and their branch lengths to the ancestral node. Using this calculation the algorithm calculates the likelihood for all ancestral nodes across the entire phylogeny using Monte Carlo methods. Extinction and dispersal parameters are varied throughout the calculations in order to optimise for the maximum likelihood estimates of extinction and dispersal rate.

The time-calibrated maximum likelihood phylogeny for *Dorstenia* and presence/absence data for Old and New World distributions were input into the Lagrange configurator, a web application that creates a python script that can be run in Lagrange.

RESULTS

Phylogenetic analysis

The aligned sequence data obtained from 42 taxa included 985 base pairs of the ITS region. No amplicon variation was observed within any individuals. The alignment is deposited in TreeBASE (Study Accession S11222). MrModeltest identified the GTR+I+G model as the best-fitting model of sequence evolution according to the Akaike Information Criterion (Akaike, 1974).

Bayesian and ML analyses of the sequence data revealed trees with similar topologies and support values. The ML tree is shown without any collapsed nodes at locations with low bootstrap values because many of our subsequent analyses could not be performed on trees with unresolved branching (Fig. 2). The data presented

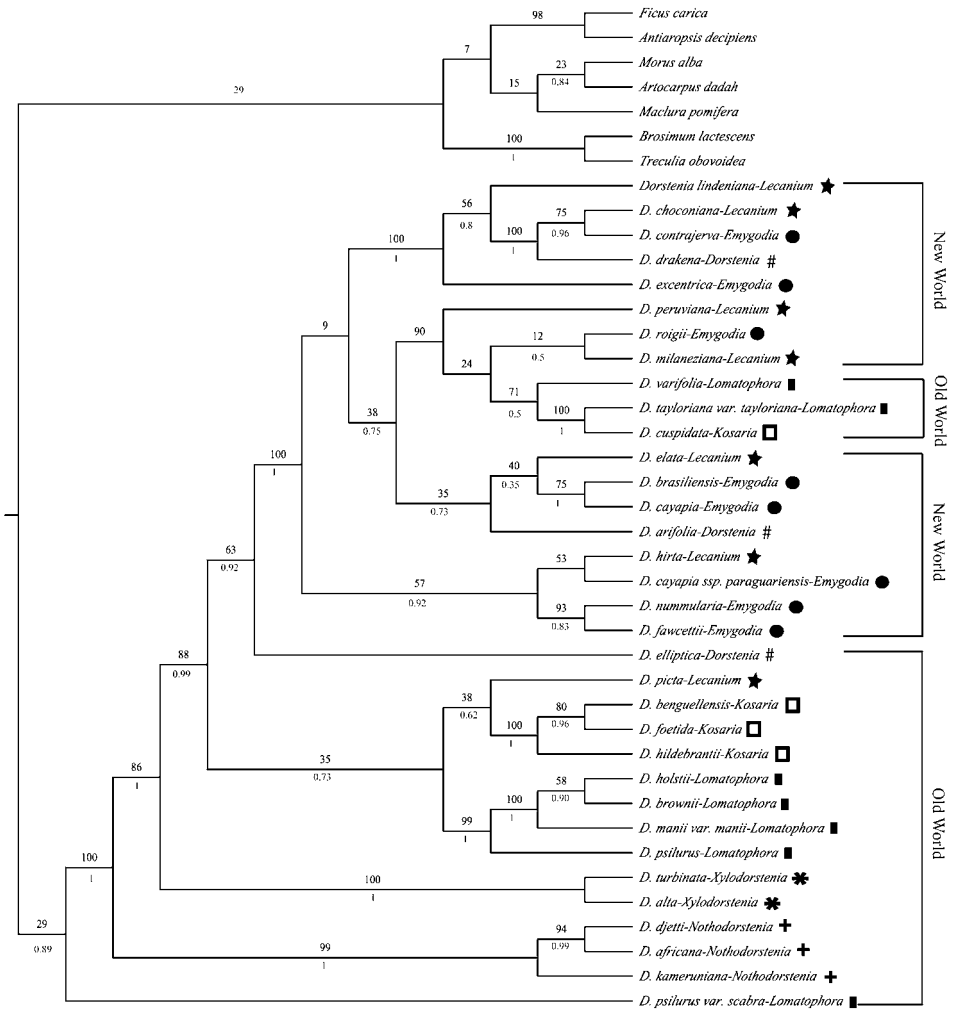


FIG. 2. Best scoring maximum likelihood tree resulting from 500 bootstrap replicates from ITS sequences. Bootstrap support values are indicated above branches and posterior probabilities from the 50% majority rule consensus Bayesian tree are indicated below branches. Posterior probabilities indicating less than 50% support are not reported. *Dorstenia* species are assigned shapes corresponding to their sections. Terminals have been pruned to one individual per species and labelled with their sectional classification as determined by Berg & Hijman (1999).

here support a monophyletic *Dorstenia*, however, with low support due to disparate confidence values calculated by the Bayesian and ML estimates for the position of *D. psilurus* var. *scabra* at the base of the clade (0.89 posterior probability [PP] and 29% bootstrap support [BS], respectively). Apart from this taxon there is strong support for the monophyly of the genus *Dorstenia* (1 PP, 100% BS). Also, both trees exhibit a grade of Old World *Dorstenia* species basal to a well-supported clade of primarily New World species (0.89 PP, 100% BS) with an Old World clade nested

within it (0.5 PP, 71% BS). With the exception of two sections, the sectional classification was not supported by the phylogeny. *Dorstenia* sect. *Xylodorstenia* and *Dorstenia* sect. *Nothodorstenia* were both monophyletic with high support (100% BS, 99% BS, respectively) (Fig. 2).

Phylogenetic signal

Phylogenetic signal ($P < 0.05$) was found in nine of the 15 characters tested. Thus, for characters labelled as habit, life form, geophyte, stem, leaf margin, petiole length, endosperm size, distribution, inflorescence bracts and storage unit, there were fewer evolutionary steps along the phylogeny than would be expected if they had evolved without respect to the phylogeny.

Ancestral state reconstruction

Parsimony and likelihood ancestral state reconstructions for each character are congruent to the extent that there are no instances where opposing states are unambiguously reconstructed at a node. Discrepancies in state reconstructions at a given node are infrequent for most characters and occur as instances where one of the two optimisation methods yielded an equivocal reconstruction. Results are as follows, with the most relevant results from the historical reconstructions shown in Fig. 3 and summarised in Table 2.

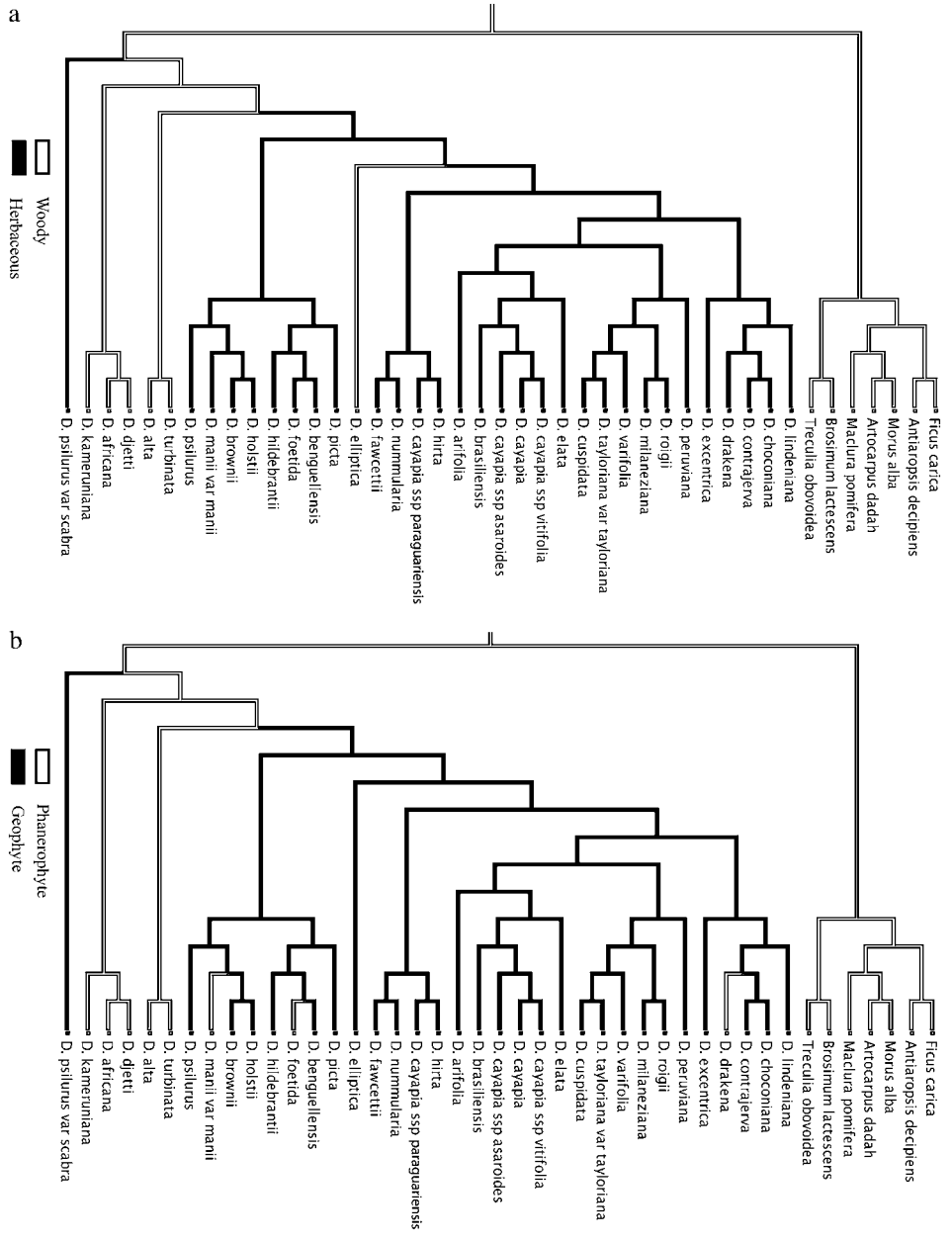
1. *Habit:* The character state reconstruction showed woody habit as the plesiomorphic state. There have been two independent instances of evolution to a herbaceous habit in the Old World grade and one instance of evolution to herbaceousness in the New World clade, the latter instance being conserved throughout the clade including the nested Old World species (Fig. 3a).

2. *Life form:* A phanerophytic character state was shown to be plesiomorphic, with the geophytic form evolving twice independently followed by three reversals (one in the New World and two in the Old World) back to the phanerophytic life form (Fig. 3b).

3. *Stem:* A caulescent stem was shown to be the ancestral character state on the phylogeny with four independent instances of evolution to acaulescence for New World taxa.

4. *Leaf margin:* No clear ancestral state could be determined between plants with lobed and entire leaf margins. There are eight independent instances of evolution to lobate leaves in the *Dorstenia* clade.

5. *Petiole length:* No clear ancestral state could be determined between plants with short (< 5 cm) or long petioles. Short petioles are conserved throughout the Old World grade. Long petioles evolved once for the New World lineage with three reversals back to short petioles.


a
Woody
Herbaceous
D. psilurus var scabra
D. kameruniana
D. africana
D. djetti
D. alta
D. turbinata
D. psilurus
D. manii var manii
D. brownii
D. holstii
D. hildebrantii
D. foetida
D. benguellensis
D. picta
D. elliptica
D. fawcettii
D. nummularia
D. cayapia ssp paraguariensis
D. hirta
D. arifolia
D. brasiliensis
D. cayapia ssp asaroides
D. cayapia
D. elata
D. cayapia ssp vitifolia
D. cuspidata
D. taylorana var taylorana
D. variifolia
D. milaneziana
D. roigii
D. peruviana
D. excentrica
D. drakena
D. contrajerva
D. choconiana
D. lindeniana
Treculia obovoidea
Brosimum lactescens
Maclura pomifera
Artocarpus dadah
Morus alba
Antiaropsis decipiens
Ficus carica
b
Phanerophyte
Geophyte
D. psilurus var scabra
D. kameruniana
D. africana
D. djetti
D. alta
D. turbinata
D. psilurus
D. manii var manii
D. brownii
D. holstii
D. hildebrantii
D. foetida
D. benguellensis
D. picta
D. elliptica
D. fawcettii
D. nummularia
D. cayapia ssp paraguariensis
D. hirta
D. arifolia
D. brasiliensis
D. cayapia ssp asaroides
D. cayapia
D. elata
D. cayapia ssp vitifolia
D. cuspidata
D. taylorana var taylorana
D. variifolia
D. milaneziana
D. roigii
D. peruviana
D. excentrica
D. drakena
D. contrajerva
D. choconiana
D. lindeniana
Treculia obovoidea
Brosimum lactescens
Maclura pomifera
Artocarpus dadah
Morus alba
Antiaropsis decipiens
Ficus carica

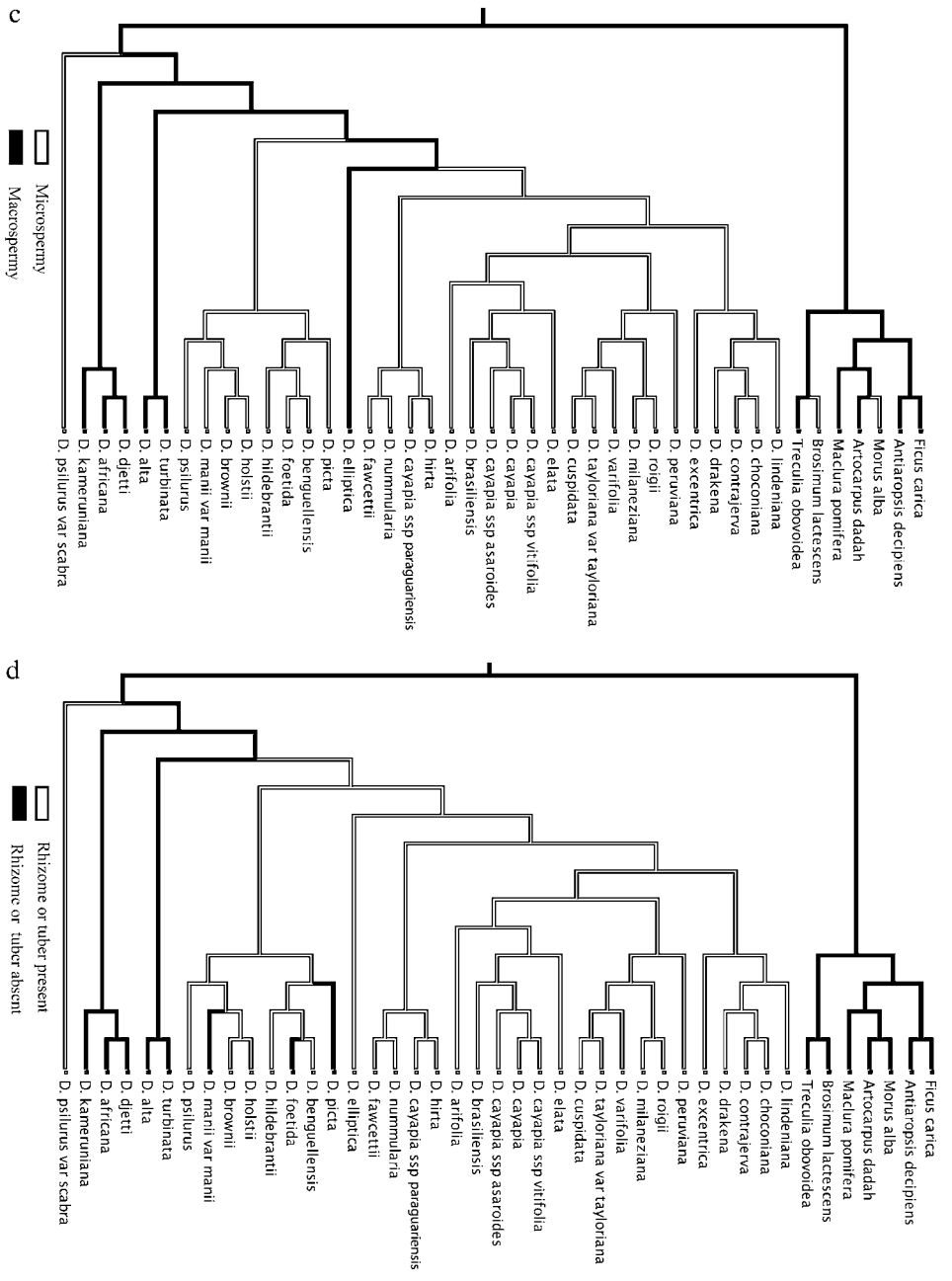


FIG. 3. Ancestral state reconstruction for the following characters: a, habit; b, life form; c, endosperm size; d, storage organ.

6. *Endosperm size:* Macrospermy was determined to be the ancestral character for endosperm size. Microspermy evolved independently up to three times (Fig. 3c).

7. *Bracts:* There was no clear distinction as to whether the presence or absence of inflorescence bracts was the ancestral state.

8. *Storage unit:* An absence of a storage unit such as a rhizome or tuber was shown to be the ancestral character state and it evolved independently up to five times among taxa found in the Old World (Fig. 3d).

Character correlation

Of the 36 pairwise comparisons between the nine morphological characters with significant phylogenetic signal, five comparisons were significantly correlated ($P < 0.05$) after Bonferoni corrections. Habit was strongly correlated with endosperm size, life form, and the presence or absence of a storage organ. Life form and the presence or absence of bracteate inflorescences is correlated with the presence or absence of a storage organ (Table 3).

Molecular dating

The likelihood ratio test for rate constancy rejected the null hypothesis that *Dorstenia* is evolving under a global molecular clock ($P < 0.01$). Dates for both

TABLE 2. Ancestral state reconstruction results for characters showing phylogenetic signal. Emboldened character states are unambiguous reconstructions supported by both parsimony and likelihood methods

Character	Parsimony ancestral state	Likelihood ancestral states (proportional likelihoods)
Habit	**Woody (3 steps)**	**Woody (0.985)** Herbaceous (0.0151)
Life form	**Phanerophytic (5 steps)**	**Phanerophytic (0.951)** Geophytic (0.049)
Stem	**Caulescent (5 steps)**	**Caulescent (0.995)** Acaulescent (0.005)
Leaf margin	Entire (10 steps)	Lobate (0.5) Entire (0.5)
Petiole length	Short (9 steps)	Long (0.5) Short (0.5)
Endosperm size	**Macrospermous (5 steps)**	**Macrospermous (0.925)** Microspermous (0.075)
Inflorescence bracts	**Absent (10 steps)**	**Absent (0.693)** Present (0.307)
Storage unit (rhizome or tuber)	**Absent (5 steps)**	**Absent (0.968)** Present (0.032)

TABLE 3. Significant results for Pagel's (1994) correlation analysis

Character X	Character Y	*P*-value	*P*-value after Bonferoni correction
Habit	Endosperm	0.000	0.000
Habit	Life form	0.001	0.027
Habit	Storage organ	0.001	0.027
Life form	Storage organ	0.000	0.000
Inflorescence bract	Storage organ	0.001	0.027

the divergence (stem) and radiation (crown) were calculated for the New World *Dorstenia* clade. Unfortunately, due to poorly supported nodes, it was not possible to calculate the divergence dates accurately for the genus as a whole or the nested Old World clade. For these groups only the radiation (crown) dates were calculated. The mean estimate for the radiation of the genus *Dorstenia* is 112.3 mya. The mean estimate for the divergence of New World *Dorstenia* is 67.2 mya and the mean estimate for the radiation of New World *Dorstenia* is 30.3 mya. The radiation for the Old World clade nested within the New World clade is dated with a mean estimate at 13.6 mya (Table 4 and Fig. 4).

Analysis of geographic range evolution

Lagrange calculates the likelihood of range inheritance scenarios at each node. For most nodes the program only calculated one inheritance scenario. When more than one scenario is calculated, the scenario with the lowest log-likelihood is the most probable. If the second scenario is lower by at least two log-likelihood units it is considered to be outside the appropriate confidence range (Ree & Smith, 2008). Only four nodes on our phylogeny had more than one range scenario calculated, and only one of those four nodes had one scenario with significantly higher support than the other. The three nodes (denoted with an X in Fig. 4) with two equally probable range hypotheses all shared the same two scenarios, ancestors existing in either the Old World or the New World or ancestors existing in both the New and Old World. Because the dates at which these two scenarios are calculated occur well after the

TABLE 4. Molecular dating results using Bayesian methods

	Effective sample size	Mean divergence date	Standard error of the mean	95% highest probability density lower	95% highest probability density upper
Dorstenia radiation	780.6	112.3 mya	0.51	84.8 mya	132.0 mya
New World divergence	371.7	67.2 mya	0.92	32.4 mya	101.0 mya
New World radiation	458.5	30.3 mya	0.34	16.5 mya	44.8 mya
Nested Old World radiation	777.4	13.6 mya	0.16	5.5 mya	22.3 mya

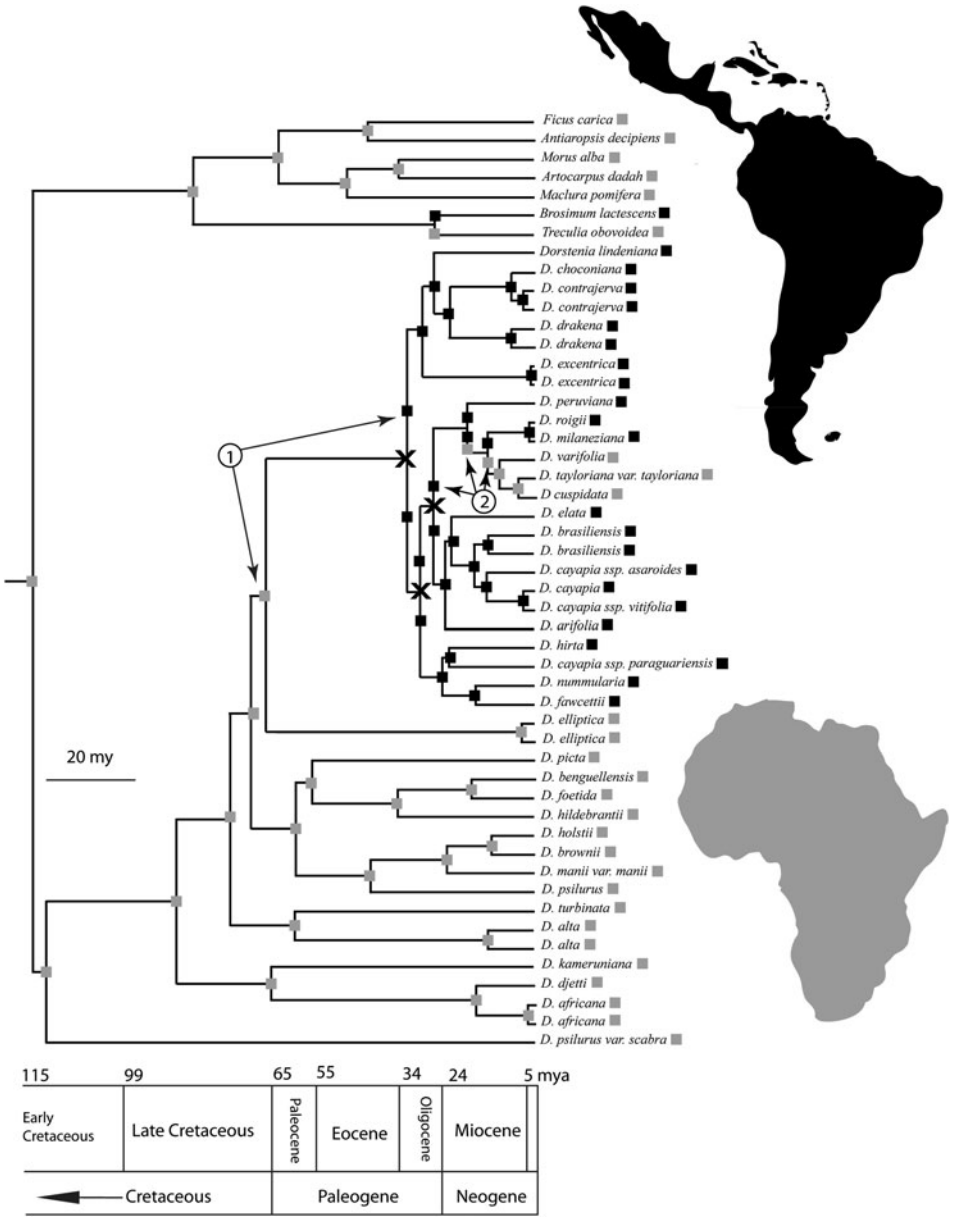


FIG. 4. Maximum likelihood tree with ancestral range reconstructions calculated in Lagrange and divergence date estimates. Grey blocks indicate ancestral range in the Old World, black blocks represent an ancestral range in the New World, and branches with grey and black blocks indicate ancestors living in both ranges. Nodes marked with X represent places where two scenarios were presented with less than two log-likelihood units difference and the most probable ranges based on continental locations at those dates were chosen (see text). #1 indicates a migration from the Old World to the New World and #2 indicates a migration from the New World back to the Old World.

break-up of South America and Africa (105 mya), the scenario with only one ancestral range per ancestor seems more intuitive and is what we present. Reconstructions reveal that an Old World origin for *Dorstenia* is most probable with one dispersal event to the New World and one back again to the Old World (Fig. 4).

Discussion

Dorstenia *phylogeny/sectional classification*

Both Bayesian and likelihood analyses of the ITS region reconstruct a monophyletic genus *Dorstenia*. Old World species comprise the basal grade of the phylogeny. The New World species are part of a well-supported clade with a small clade of three Old World species nested within it. Interestingly, *Dorstenia psilurus* var. *scabra* stands out in its placement on the phylogeny. It is not sister to its conspecific *Dorstenia psilurus* var. *psilurus*, from which it differs morphologically, ecologically, geographically and in chromosome number (*Dorstenia psilurus* var. *psilurus* $2n = 26$, *D. psilurus* var. *scabra* $2n = 40$). It is also distinguished from other closely related species because it does not display characters exhibited in basal lineages despite its position at the base of the tree. These differences along with *Dorstenia psilurus* var. *scabra*'s position on the phylogeny call for a closer investigation and possible revision of the species.

With the exception of *Dorstenia* sections *Xylodorstenia* and *Nothodorstenia*, which are the only two sections that exhibit woodiness, macrospermy and a phanerophytic life form, molecular data strongly suggest that the current classification does not reflect monophyletic lineages. While *Dorstenia* sections *Xylodorstenia* and *Nothodorstenia* are monophyletic sections as originally proposed, it is only the presence or absence of bracts on the inflorescence that distinguishes these two sections from each other. These results suggest that characters pertaining to reproductive morphology may be more useful for defining sections and that habit and life form are probably subject to convergent evolution, limiting their usefulness without support from other characters. Many of the sections and their defining characters are dispersed across the tree and complete sampling of the genus would not resolve polyphyletic sections.

Dorstenia has never been subjected to a global monograph. In the only treatment containing descriptions of all Old and New World *Dorstenia* species, Berg & Hijman (1999) compiled and summarised 'scattered' unpublished data which resulted in the inconsistent inclusion of morphological data across all species. Due to this discontinuity in species descriptions, it was exceedingly difficult to code alternative characters to those proposed for determining more natural subdivisions for the genus. Incongruence between the findings presented here and the traditional classification clearly calls for a global revision of the genus incorporating both morphological and molecular data. Future directions should include reconstructing the *Dorstenia* phylogeny with additional markers and more complete taxon sampling. More thorough species descriptions in the form of a full monograph would also aid in proposing a more natural sectional classification for the genus.

Ancestral state reconstruction and character correlation

Ninety per cent of the Moraceae are woody. The remaining 10% of taxa with herbaceous habits are found almost exclusively in the genus *Dorstenia*. Based on *Dorstenia*'s unique position in a primarily woody family it has been suggested that the woody habit is a primitive character in the genus (Berg & Hijman, 1999). Observations of other morphological characters that appear to be correlated with woody and herbaceous habits have led to hypotheses that additional character states in the genus are ancestral as well and have evolved directionally (Berg & Hijman, 1999). For example, acaulescent stems are thought to have evolved towards caulescent stems, phanerophytic forms are considered ancestral to geophytic forms, and petiole lengths are hypothesised to have evolved from short to long (Berg & Hijman, 1999; Berg, 2001). All of these hypotheses, with the exception of petiole length evolution, are well supported based on both parsimony and likelihood ancestral state reconstructions (Table 2).

Woodiness is scattered throughout the angiosperm phylogeny and considered to be a trait shared by the ancestors of extant herbaceous angiosperms (Barghoorn, 1964). More recent studies on angiosperm evolution have pointed to an ancestral condition of woody growth (Field *et al.*, 2004). However, while ancient angiosperms may have exhibited a woody habit, the evolution from woodiness to herbaceousness is not unidirectional. Studies have shown that woodiness in some species has arisen from herbaceous progenitors after a migration from a continental mainland onto islands (Böhle *et al.*, 1996; Kim *et al.*, 1996).

Berg & Hijman (1999) hypothesised that the presence of a woody habit and macrospermy were correlated, as was the presence of the herbaceous habit and microspermy. This hypothesis was tested along with tests for correlations among woodiness, a lack of a storage organ, and a phanerophytic life form as well as herbaceousness, the presence of a storage organ, and a geophytic life form. Based on Pagel's (1994) correlation analysis, all of these traits are significantly correlated. These correlations not only corroborate Berg & Hijman's (1999) evolutionary hypotheses, but are also consistent with recent studies demonstrating very strong correlations between woodiness and macrospermy and herbaceousness and microspermy in global data (Moles *et al.*, 2007). The strong correlation between growth form and seed size has been suggested to result from coordination between life-history variables. Shifts towards a woody and macrospermous life history have been observed in tropical plant species as they move away from the equator towards less tropical areas (Moles *et al.*, 2007). *Dorstenia*'s adherence to this pattern in shifting correlated characters could possibly suggest that its ancestor originated in the subtropics and migrated towards more equatorial regions.

Dorstenia exhibits high levels of variation across the genus, and habitat descriptions suggest a wide range of ecological preferences. The majority of taxa sampled in this study are found in wet forests though a few have been noted to prefer drier forests, more open areas or limestone cliffs. The descriptions from herbarium

specimens and taxonomic treatments used in this study were not specific enough to perform evolutionary tests, however; future studies should explicitly examine the relationship between morphology and ecology.

Molecular dating

The dating analysis places the radiation of the genus *Dorstenia* at 112.3 mya; the New World divergence was dated at 67.2 mya and the radiation was dated at 30.3 mya. The Old World clade nested within the New World radiated at 13.6 mya (Table 4; Fig. 4). These dates differ from estimates determined in a previous study by Zerega *et al.* (2005), which used combined data from different gene regions (*ndhF* and the 26S nuclear rDNA subunit as opposed to ITS used in this study). Zerega *et al.* (2005) estimated a radiation of 3.5–18.4 mya for the genus *Dorstenia.* The discrepancy in dates may also have occurred because Zerega *et al.* (2005) included only two *Dorstenia* species, both of which were New World taxa that are among the more recently diverged species in the present study (*D. arifolia* and *D. choconiana*).

Analysis of geographic range evolution

The break-up of Gondwana began about 180 mya with the current continents of South America and Africa splitting about 105 mya in the mid-Cretaceous (McLoughlin, 2001). It has been previously suggested that the distribution both of the family Moraceae and the genus *Dorstenia* is the result of vicariance caused by the break-up of the Gondwanan landmass (Corner, 1967; Berg & Hijman, 1999; Berg, 2001). Recent studies have suggested a more recent radiation date for the family (72.6–110 mya) (Datwyler & Weiblen, 2004; Zerega *et al.*, 2005). However, our study indicates that *Dorstenia* radiated about 112 mya in the Old World, which suggests that the distribution of the genus could be the result of vicariance due to the separation of South America and Africa. It also suggests that a re-evaluation of Moraceae divergence dates using denser taxon sampling may yield an older age for the family.

Another possibility based on the present data is long distance dispersal or migration of *Dorstenia* between South America and Africa. Dispersal across the ocean seems unlikely given that *Dorstenia* has no obvious means for long distance dispersal, and seeds have been observed to be viable for only a limited period (Berg & Hijman, 1999). Nonetheless, studies suggest that long distance dispersal may be much more common than previously assumed even in flora lacking mechanisms for long distance dispersal (Pennington & Dick, 2004; Renner, 2004). Long distance migration over land may also be a possible dispersal scenario. The estimated date of divergence for New World *Dorstenia* from Old World *Dorstenia* is 67.2 mya, which coincides with one of the collisions between Africa and Eurasia at about 60 mya (McLoughlin, 2001). *Dorstenia* could have migrated from Africa through Eurasia and into North America via the Laurasian land bridge, which existed during the Eocene (c.55–33 mya) when climates supported more tropical environments than

today (Tiffney, 1985). As continents continued to shift, *Dorstenia* would eventually go extinct in North America and Eurasia as temperatures became more temperate. This would have put the genus in South America at about the time the New World radiation was calculated to have occurred (30.3 mya) and explain why we don't find *Dorstenia* in temperate areas today. Fossil evidence of *Dorstenia* in Eurasia or North America would support this hypothesis. Unfortunately, the authors have found no reliable record of *Dorstenia* fossil evidence. The explanation of a North Atlantic land bridge as a migration route into the New World, however, would not explain the apparent migration back to Africa (5.46–22.27 mya) based on our dates for the radiation of the nested Old World clade, suggesting that perhaps long distance dispersal may play an important role in determining the distribution of the genus. However, since we were unable to calculate accurately the date of divergence of the nested Old World clade from its New World ancestor, which would be older than the radiation, there is a possibility that *Dorstenia* could have migrated back across the North Atlantic land bridge to Africa again and subsequently diversified.

Conclusions

This study represents the first molecular reconstruction of the *Dorstenia* phylogeny with substantial taxon sampling. It allows for the re-evaluation in an evolutionary framework of hypotheses based on morphological characters. The evolutionary trajectory that was proposed for the majority of characters pertaining to life form, such as woodiness and herbaceousness, appears to be subject to convergent evolution and cannot, therefore, be used to define natural lineages. The vicariance hypothesis proposed by Berg & Hijman (1999) to explain the diversification of the genus was tested with molecular dating and estimates of ancestral geographic ranges using likelihood methods. Based on the results it is inconclusive whether dispersal or vicariance is responsible for the distribution of the genus. *Dorstenia* appears to be old enough to support a Gondwanan hypothesis, although the confidence intervals around the dates fail to eliminate the possibility of a migration through Eurasia into the New World across a North Atlantic land bridge.

Acknowledgements

The authors thank the Royal Botanic Gardens, Kew, the Harvard University Herbaria, the Field Museum of Natural History Herbarium, the Cameroonian National Herbarium and the Missouri Botanical Garden for access to plant material and herbarium specimens; M. Cheek, J. M. Onana, R. Ree and S. Wagenius for guidance in different aspects of this project; and J. Fant and T. K. Misiewicz for their invaluable assistance in the laboratory and field. This research was partially funded by the Botanical Society of America Graduate Research Award, the Society of Systematic Biologists Graduate Student Award, Northwestern Plant Biology and Conservation Research Award, the Federated Garden Club of Maryland Scholarship, the Central

Atlantic Region Garden Club Scholarship, and the Shaw Fellowship for Plant Conservation and Biology.

References

AKAIKE, H. (1974). A new look at the statistical model identification. *IEEE Transactions of Automatic Control* 19: 716–723.

ALVAREZ, I. & WENDEL, J. F. (2003). Ribosomal ITS sequences and plant phylogenetic inference. *Molec. Phylogenet. Evol.* 29: 417–434.

BAILEY, C. D., CARR, T. G., HARRIS, S. A. & HUGHES, C. E. (2003). Characterization of angiosperm nrDNA polymorphism, paralogy, and pseudogenes. *Molec. Phylogenet. Evol.* 29: 435–455.

BARGHOORN, E. (1964). Evolution of cambium in geologic time. In: ZIMMERMANN, M. H. (ed.) *The Formation of Wood in Forest Trees*, pp. 3–17. New York and London: Academic Press.

BAWA, K. S., PERRY, D. R. & BEACH, J. H. (1985). Reproductive biology of tropical lowland rainforest trees. I. Sexual systems and incompatibility mechanisms. *Amer. J. Bot.* 72: 331–410.

BERG, C. C. (2001). *Moreae, Artocarpeae, and* Dorstenia *(Moraceae)*. New York: New York Botanical Garden Press.

BERG, C. C. & HIJMAN, M. E. E. (1999). The genus *Dorstenia* (Moraceae). *Ilicifolia* 2: 1–211.

BÖHLE, U. R., HILGER, H. H. & MARTIN, W. F. (1996). Island colonization and evolution of the insular woody habit in *Echium* L. (Boraginaceae). *Proc. Natl. Acad. Sci. USA* 93: 11740–11745.

BURNHAM, R. J. & GRAHAM, A. (1999). The history of neotropical vegetation: New developments and status. *Ann. Missouri Bot. Gard.* 86: 546–589.

CHAUDHRY, B., YASMEEN, A., HUSNAIN, T. & RIAZUDDIN, S. (1999). Mini-scale genomic DNA extraction from cotton. *Plant Mol. Biol. Rep.* 17: 1–7.

CLAYTON, J. W., SOLTIS, P. S. & SOLTIS, D. E. (2009). Recent long-distance dispersal overshadows ancient biogeographical patterns in a pantropical angiosperm family (Simaroubaceae, Sapindales). *Syst. Biol.* 58: 395–410.

CLEMENT, W. L. & WEIBLEN, G. D. (2009). Morphological evolution in the mulberry family (Moraceae). *Syst. Bot.* 34: 530–552.

COLLINSON, M. E. (1989). The fossil history of the Moraceae, Urticaceae (including Cecropiaceae) and Cannabaceae. In: CRANE, P. R. & BLACKMORE, S. (eds) *Evolution, Systematics and Fossil History of the Hamamelidae, Vol. 2: 'Higher' Hamamelidae*, pp. 319–339. Oxford: Clarendon Press.

CONN, B. J., BANKA, R. & LEE, L. L. (2006+). Plants of Papua New Guinea (www.pngplants.org; 30 June 2009).

CORNER, E. J. H. (1967). *Ficus* in the Solomon Islands and its bearing on the Post-Jurassic history of Melanesia. *Philos. Trans., Ser. B* 254: 23–159.

DATWYLER, S. & WEIBLEN, G. (2004). On the origin of the fig: Phylogenetic relationships of Moraceae from *ndhF* sequences. *Amer. J. Bot.* 91: 767–777.

DRUMMOND, A. J. & RAMBAUT, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *Biomed. Cent. Evol. Biol.* 7: 214.

DRUMMOND, A. J., HO, S. Y. W., PHYLLIPS, M. J. & RAMBAUT, A. (2006). Relaxed phylogenetics and dating with confidence. *PLoS Biol.* 4: e88.

EDGAR, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32: 1792–1797.

FELINER, G. N. & ROSSELLO, J. A. (2007). Better the devil you know? Guidelines for insightful utilization of nrDNA ITS in species-level evolutionary studies in plants. *Molec. Phylogenet. Evol.* 44: 911–919.

FELSENSTEIN, J. (1973). Maximum likelihood and minimum-steps methods for estimating evolutionary trees from data on discrete characters. *Syst. Zool.* 22: 240–249.

FELSENSTEIN, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

FIELD, T. S., ARENS, N. C., DOYLE, J. A., DAWSON, T. E. & DONOGHUE, M. J. (2004). Dark and disturbed: a new image of early angiosperm ecology. *Paleobiology* 30: 82–107.

KIM, S. C., CRAWFORD, D. J., FRANCISCO-ORTEGA, J. & SANTOS-GUERRA, A. (1996). A common origin for woody *Sonchus* and five related genera in the Macaronesian islands: molecular evidence for extensive radiation. *Proc. Natl. Acad. Sci. USA* 93: 7743–7748.

MADDISON, W. P. & MADDISON, D. R. (2009). Mesquite: a modular system for evolutionary analysis, v2.6: http://mesquiteproject.org

MAGALLON, S. & SANDERSON, M. J. (2001). Absolute diversification rates in angiosperm clades. *Evolution* 55: 1762–1780.

MCLOUGHLIN, S. (2001). The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. *Austral. J. Bot.* 49: 271–300.

MOLES, A. T., ACKERLY, D. D., TWEDDLE, J. C., DICKIE, J. B., SMITH, R., LEISHMAN, M. R. *et al.* (2007). Global patterns in seed size. *Global Ecol. Biogeogr.* 16: 109–116.

MUELLNER, A. N., SAVOLAINEN, V., SAMUEL, R. & CHASE, M. W. (2006). The mahogany family "Out-of-Africa": Divergence time estimation, global biogeographic patterns inferred from plastid rbcL DNA sequences, extant, and fossil distribution of diversity. *Molec. Phylogenet. Evol.* 40: 236–250.

NYLANDER, J. A. A. (2004). MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

OLIVER, D. (1868). *Flora of Tropical Africa*, vol. 6. London: L. Reeve.

PAGEL, M. (1994). Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proc. Roy. Soc. London, Ser. B, Biol. Sci.* 255: 37–45.

PENNINGTON, R. T. & DICK, C. W. (2004). The role of immigrants in the assembly of the South American rainforest tree flora. *Philos. Trans., Ser. B* 359: 1611–1622.

RAMBAUT, A. (2002). Se-Al: Sequence Alignment Editor: http://evolve.zoo.ox.ac.uk

RAMBAUT, A. & DRUMMOND, A. J. (2007). Tracer v1.4: http://beast.bio.ed.ac.uk/Tracer

RAUNKIAER, C. (1934). *The life-forms of plants and statistical plant geography*. Oxford: Oxford University Press.

RAVEN, P. H. & AXELROD, D. I. (1974). Angiosperm biogeography and past continental movements. *Ann. Missouri Bot. Gard.* 61: 539–673.

REE, R. H. & SMITH, S. A. (2008). Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Syst. Biol.* 57: 4–14.

REE, R. H., MOORE, B. R., WEBB, C. O. & DONOGHUE, M. J. (2005). A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. *Evolution* 59: 2299–2311.

RENNER, S. S. (2004). Plant dispersal across the tropical Atlantic by wind and sea currents. *Int. J. Pl. Sci.* 165: S23–S33.

RONQUIST, F. & HUELSENBECK, J. P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

SAKAI, S. (2001). Thrips pollination of androdioecious *Castilla elastica* (Moraceae) in a seasonal tropical forest. *Amer. J. Bot.* 88: 1527–1534.

SAKAI, S., KATO, M. & NAGAMASU, H. (2000). *Artocarpus* (Moraceae)–gall midge pollination mutualism mediated by a male-flower parasitic fungus. *Amer. J. Bot.* 87: 440–445.

SHAW, J., LICKEY, E. B., SCHILLING, E. E. & SMALL, R. L. (2007). Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *Amer. J. Bot.* 94: 275–288.

SIMMONS, M. P. (2004). Independence of alignment and tree search. *Molec. Phylogenet. Evol.* 31: 874–879.

STAMATAKIS, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.

STAMATAKIS, A., HOOVER, P. & ROUGEMONT, J. (2008). A rapid bootstrap algorithm for the RAxML web servers. *Syst. Biol.* 57: 758–771.

SWOFFORD, D. L. (2003). *PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4*. Sunderland, MA: Sinauer Associates.

TIFFNEY, B. H. (1985). Perspectives on the origin of the floristic similarity between Eastern Asia and eastern North America. *J. Arnold Arbor.* 66: 73–94.

WHITE, T. J., BRUNS, T., LEE, S. & TAYLOR, J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: INNIS, M. A., GELFAND, D. H., SNINSKY, J. J. & WHITE, T. J. (eds) *PCR Protocols: A Guide to Methods and Applications*, pp. 315–322. New York: Academic Press, Inc.

YANG, Z. & RANNALA, B. (1997). Bayesian phylogenetic inference using DNA sequences: a Markov Chain Monte Carlo method. *Molec. Biol. Evol.* 14: 717–724.

ZEREGA, N. J. C., MOUND, L. A. & WEIBLEN, G. D. (2004). Pollination in the New Guinea endemic *Antiaropsis decipiens* (Moraceae) is mediated by a new species of thrips, *Thrips antiaropsidis* (Thysanoptera: Thripidae). *Int. J. Pl. Sci.* 165: 1017–1026.

ZEREGA, N. J. C., CLEMENT, W. L., DATWYLER, S. L. & WEIBLEN, G. D. (2005). Biogeography and divergence times in the mulberry family (Moraceae). *Molec. Phylogenet. Evol.* 37(2): 402–416.

ZOHARY, M. (1966). *Flora Palaestina* 1: 37–38. Jerusalem: The Israel Academy of Sciences and Humanities.

Received 19 November 2011; accepted for publication 5 July 2012

APPENDIX 1

Collection information

Taxon; ITS GenBank accession; *Voucher specimen*; Collection locale; Herbarium.

Dorstenia africana (Baill.) C.C.Berg, HQ214078, *Carvalho* 5308, Equatorial Guinea, NY. ***Dorstenia africana*** (Baill.) C.C.Berg, HQ214077, *X.M. van der Burgt et al.* 527, Cameroon, MO. ***Dorstenia alta*** Engl., HQ214081, *A. Ntemi Sallu* 638, Tanzania, MO. ***Dorstenia alta*** Engl., HQ214082, *Ndangalasi & I. Rajabu*, Tanzania, F. ***Dorstenia arifolia*** Lam., HQ214101, *A.M. de Carvalho* 7169, Brazil, NY. ***Dorstenia benguellensis*** Welw., HQ214102, *A.S. Mkeya et al.* 1109, Tanzania, MO. ***Dorstenia brasiliensis*** Lam., HQ214110, *Paulo Heringer et al.* 735, Brazil, NY. ***Dorstenia brasiliensis*** Lam., HQ214111, *M.J. Jansen-Jacobs et al.* 4436, Guyana, NY. ***Dorstenia brownii*** Rendle, HQ214094, *M.A. Mwangoka et al.* 3606, Tanzania, MO. ***Dorstenia cayapia*** Vell., HQ214109, *G.T. Prance et al.* 19219, Brazil, NY. ***Dorstenia cayapia*** Vell. ssp. ***asaroides*** (Hook.) C.C.Berg, HQ214107, *M. Nee* 42307, Bolivia, NY. ***Dorstenia***

cayapia Vell. ssp. ***paraguariensis*** (Hassl.) C.C.Berg, HQ214108, *Shirley Ortiz* 218, Bolivia, NY. ***Dorstenia cayapia*** Vell. ssp. ***vitifolia*** (Gardner) C.C.Berg, HQ214106, *Roberto Fontes Vieira et al.* 554, Brazil, NY. ***Dorstenia choconiana*** S.Watson, HQ214085, *G. Weiblen* 1417, Costa Rica, MIN. ***Dorstenia contrajerva*** L., HQ214100, *E. Martinez S. et al.* 28913, Mexico, NY. ***Dorstenia contrajerva*** L., HQ214099, *Ulises Chavarria* 809, Costa Rica, F. ***Dorstenia cuspidata*** Hochst. var. ***humblotiana*** (Baill.) Leandri, HQ214105, *L. Gautier* & *D. Ravelonarivo* LG4138, Madagascar, MO. ***Dorstenia djettii*** Guillamet, HQ214076, *J. Amponsah et al.* 1413, Ghana, MO. ***Dorstenia drakena*** L., HQ214097, *S.D. Koch et al.* 87195, Mexico, NY. ***Dorstenia drakena*** L., HQ214098, *S. Salas et al.* 2105, Mexico, MO. ***Dorstenia elata*** Gardner, HQ214087, *R. Mello-Silva et al.* 1563, Brazil, NY. ***Dorstenia elliptica*** Bureau, HQ214075, *J. Nning* 20, Cameroon, MO. ***Dorstenia elliptica*** Bureau, HQ214074, *D.W. Thomas et al.* 6990, Cameroon, NY. ***Dorstenia excentrica*** Moric., HQ214113, *M. Nee* 22374, Mexico, F. ***Dorstenia excentrica*** Moric., HQ214112, *P.A. Fryxell et al.* 3445, Mexico, NY. ***Dorstenia fawcettii*** Urb., HQ214114, *M.R. Crosby et al.* 770, Jamaica, F. ***Dorstenia foetida*** Schweinf., HQ214104, *N. Zerega* 312, cultivated, Illinois USA, CHIC. ***Dorstenia hildebrandtii*** Engl., HQ214103, *N. Zerega* 311, cultivated, Illinois USA, CHIC. ***Dorstenia hirta*** Desv., HQ214088, *F.O. Souza et al.* 21, Brazil, NY. ***Dorstenia holstii*** Engl., HQ214091, *A. Ntemi Sallu* 645, Tanzania, MO. ***Dorstenia kameruniana*** Engl., HQ214079, *O.A. Kibure* & *H. Bofu* 1045, Tanzania, MO. ***Dorstenia lindeniana*** Bureau, HQ214083, *D. Alvarez* 2721, MO. ***Dorstenia mannii*** Hook.f. var. ***mannii***, HQ214095, *R.E. Gereau* 5593, Cameroon, MO. ***Dorstenia milaneziana*** Carauta et al., HQ214086, *W.W. Thomas et al.* 11078, Brazil, NY. ***Dorstenia nummularia*** Urb. & Ekman, HQ214115, *P. Acevedo Rodriguez et al.* 6439, Cuba, NY. ***Dorstenia peruviana*** C.C.Berg, HQ214084, *T. Plowman* 5904, Peru, F. ***Dorstenia picta*** Bureau, HQ214089, *R.E. Gereau et al.* 5189, Cameroon, MO. ***Dorstenia psilurus*** Welw., HQ214093, *L. Festo* & *W. Bayona* 859, Tanzania, MO. ***Dorstenia psilurus*** Welw. var. ***scabra*** Bureau, HQ214092, *G. Zenker* s.n., Cameroon, F. ***Dorstenia roigii*** Britton, HQ214116, *E.L. Eckman* 17973, Cuba, F. ***Dorstenia tayloriana*** Rendle var. ***tayloriana***, HQ214096, *M.A. Mwangoka* & *A. Kalage* 2673, Tanzania, MO. ***Dorstenia turbinata*** Engl., HQ214080, *M. Cheek* 11086, Cameroon, MO. ***Dorstenia variifolia*** Engl., HQ214090, *J.J. Lovett et al.* 2135, Tanzania, MO. ***Antiaropsis decipiens*** K.Schum., AY289284, *Weiblen* 1233, New Guinea, MIN. ***Artocarpus dadah*** Miq. FJ917051, *Zerega* 245, Malaysia, CHIC. ***Brosimum lactescens*** (S.Moore) C.C.Berg, AY289329, *Weiblen* 1513, Brazil, MIN. ***Morus alba*** L., AY289274, *Weiblen* 1173, Michigan, USA, MIN. ***Treculia obovoidea*** N.E.Br., FJ917004, *Leeuwenberg* 9700, Cameroon, US.

Appendix 2

Matrix of character states

Habit (0 = woody, 1 = herbaceous), *Geophyte* (0 = phanerophytic, 1 = geophytic), *Stem* (0 = caulescent, 1 = acaulescent), *Leaf margin* (0 = lobate, 1 = entire), *Petiole length* (0 = long, 1 = short), *Pistillode* (0 = present, 1 = absent), *Distribution* (0 = Old World, 1 = New World), *Leaf variegation* (0 = present, 1 = absent), *Endosperm size* (0 = microspermous, 1 = macrospermous), *Inflorescence bracts* (0 = present, 1 = absent), *Storage unit* (0 = tuber or rhizome present, 1 = absent), *Leaf apex* (0 = triangular, 1 = rounded, 2 = variable), *Leaf base* (0 = triangular, 1 = rounded, 2 = variable), *Stipule* (0 = persistent, 1 = subpersistent, 2 = caducous), *Staminate flower arrangement* (0 = evenly dispersed, 1 = peripheral).

	Habit	Geophyte	Stem	Leaf margin	Petiole length	Pistillode	Distribution	Leaf variegation	Endosperm size	Inflorescence bracts	Storage unit	Leaf apex	Leaf base	Stipule	Staminate flower arrangement
Antiaropsis decipiens K.Schum.	0	0	0	0	1	1	0	1	1	1	1	0	2	0	N/A
Artocarpus dadah Miq.	0	0	0	0	1	1	0	1	1	0	1	0	1	2	N/A
Brosimum lactescens (S.Moore) C.C.Berg	0	0	0	1	1	1	1	1	0	0	1	0	0	0	N/A
Ficus carica L.	0	0	0	0	0	1	0	1	1	0	1	1	0	0	N/A
Maclura pomifera (Raf.) C.K.Schneid.	0	0	0	1	1	1	1	1	1	1	1	0	1	0	N/A
Morus alba L.	0	0	0	0	1	1	0	1	0	1	1	2	2	0	N/A

	Habit	Geophyte	Stem	Leaf margin	Petiole length	Pistillode	Distribution	Leaf variegation	Endosperm size	Inflorescence bracts	Storage unit	Leaf apex	Leaf base	Stipule	Staminate flower arrangement
Treculia obovoidea N.E.Br.	0	0	0	1	1	1	0	1	1	0	1	0	0	1	N/A
Dorstenia africana (Baill.) C.C.Berg	0	0	0	1	1	1	0	1	1	0	1	2	2	2	0
D. alta Engl.	0	0	0	0	1	1	0	1	1	1	1	0	0	0	0
D. arifolia Lam.	1	1	1	0	0	1	1	1	0	1	0	1	1	0	0
D. benguellensis Welw.	1	1	0	1	1	1	0	1	0	1	0	0	0	0	0
D. brasiliensis Lam.	1	1	0	1	0	1	1	1	0	0	0	2	2	0	0
D. brownii Rendle	1	1	0	1	1	0	0	1	0	1	0	0	0	0	0
D. cayapia Vell.	1	1	1	0	0	1	1	0	0	0	0	2	1	0	0
D. cayapia ssp. *asaroides* (Hook.) C.C.Berg	1	1	1	0	0	1	1	0	0	0	0	2	1	0	0
D. cayapia ssp. *paraguariensis* (Hassl.) C.C.Berg	1	1	1	0	0	1	1	1	0	0	0	2	1	0	0

D. cayapia ssp. *vitifolia* (Gardner) C.C.Berg	1	1	1	0	0	1	1	1	0	0	0	2	1	0	0
D. choconiana S.Watson	1	1	0	0	1	1	1	1	0	0	0	1	1	0	0
D. contrajerva L.	1	1	1	0	0	1	1	0	0	0	0	2	2	0	0
D. cuspidata Hochst.	1	1	0	1	1	1	0	1	0	1	0	0	0	0	0
D. djetti Guillamet	0	0	0	1	1	1	0	1	1	0	1	0	2	1	0
D. drakena L.	1	0	1	0	0	1	1	1	0	0	0	2	2	0	1
D. elata Gardner	1	1	0	1	1	1	1	1	0	0	0	2	2	0	0
D. elliptica Bureau	0	1	0	1	1	0	0	1	1	1	0	0	0	1	0
D. excentrica Moric.	1	1	0	1	0	1	1	1	0	0	0	1	1	0	0
D. fawcettii Urb.	1	1	0	1	0	1	1	1	0	0	0	1	1	0	1
D. foetida Schweinf.	1	0	0	1	0	1	0	1	0	1	1	2	2	0	0
D. hildebrandtii Engl.	1	1	0	1	1	1	0	1	0	1	0	0	0	0	0
D. hirta Desv.	1	1	0	1	1	1	1	0	0	0	0	0	0	0	1
D. holstii Engl.	1	1	0	1	1	0	0	1	0	1	0	0	0	1	0
D. kameruniana Engl.	0	0	0	0	1	1	0	1	1	0	1	2	2	2	0
D. lindeniana Bureau	1	1	0	1	1	1	1	0	0	1	0	1	1	0	0

	Habit	Geophyte	Stem	Leaf margin	Petiole length	Pistillode	Distribution	Leaf variegation	Endosperm size	Inflorescence bracts	Storage unit	Leaf apex	Leaf base	Stipule	Staminate flower arrangement
D. manii Hook.f. var. *manii*	1	0	0	0	1	1	0	1	0	1	1	1	2	0	0
D. milaneziana Carauta et al.	1	1	0	1	1	1	1	0	0	0	0	1	1	0	1
D. nummularia Urb. & Ekman	1	1	0	1	0	0	1	1	0	0	0	2	2	0	1
D. peruviana C.C.Berg	1	1	0	1	1	1	1	1	0	0	0	1	1	0	1
D. picta Bureau	1	1	0	1	1	1	0	0	0	0	1	1	1	0	0
D. psilurus Welw.	1	1	0	1	1	0	0	1	0	1	0	2	2	1	0
D. psilurus var. *scabra* Bureau	1	1	0	1	1	0	0	1	0	1	0	2	2	1	0
D. roigii Britton	1	1	0	1	0	1	1	1	0	1	0	2	2	0	0
D. tayloriana Rendle var. *tayloriana*	1	1	0	1	1	0	0	1	0	1	0	1	2	2	0
D. turbinata Engl.	0	0	0	1	1	1	0	1	1	1	1	0	0	2	0
D. variifolia Engl.	1	1	0	0	1	1	0	1	0	1	0	0	0	0	0