SYSTEMATICS AND PHYLOGENY

Phylogenomics of *Brosimum* (Moraceae) and allied genera, including a revised subgeneric system

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Abstract We present a phylogenomic study of *Brosimum* (Moraceae) and the allied genera *Trymatococcus* and *Helianthostylis*, with near-complete taxon sampling. Distributed from Mexico and the Greater Antilles to the Amazon, this clade contains the underutilized crop ramón (bread nut) (*Brosimum alicastrum*) as well as other species valued for timber or medicinal uses. Target enrichment for 333 genes produced a well-resolved phylogenetic tree and showed that *Trymatococcus* and *Helianthostylis* are nested within *Brosimum*. We present a revised subgeneric classification of *Brosimum* (19 spp.) based on phylogenetic and morphological considerations, including the reduction of *Trymatococcus* and *Helianthostylis* to subgenera. The monophyletic subgenera can be diagnosed based on stipule, pistillode, and cotyledon synapomorphies. Divergence date estimates suggest a Miocene origin for *Brosimum*, and ancestral area reconstruction indicated that all four subgenera originated and initially diversified in Amazonia before dispersing into other parts of South and Central America.

Keywords bread nut; Brosimeae; *Brosimum*; Dorstenieae; *Helianthostylis*; HybSeq; Maya nut; Moraceae; phylogenetics; ramón; target enrichment; *Trymatococcus*

Supporting Information may be found online in the Supporting Information section at the end of the article.

■ INTRODUCTION

The mulberry family (Moraceae Gaudich.) has approximately 1100 species and 39 genera, with a worldwide distribution and a center of diversity in the tropics. It includes economically and ecologically important species such as breadfruit and jackfruit (*Artocarpus* J.R.Forst. & G.Forst.), mulberries (*Morus* L.) and figs (*Ficus* L.). Moraceae are characterized by latex in all parenchymatous tissue and tiny inconspicuous, unisexual flowers arranged in a wide variety of inflorescence forms, ranging from simple spikes to condensed heads, discs and the unique fig syconium.

While monophyly of Moraceae is not in doubt (Datwyler & Weiblen, 2004; Zerega & al., 2005; S. Zhang & al., 2011; Q. Zhang & al., 2019b), diverse morphology and widespread homoplasy within Moraceae has made the establishment of a robust and stable sub-familial taxonomy problematic (Corner, 1962; Berg, 1977, 2001; Rohwer, 1993; Clement & Weiblen, 2009; Gardner & al., in press). The most recent family-wide phylogenetic studies recognized seven tribes based on molecular and morphological evidence, with inflorescence morphology mostly reflecting the tribe-level clades (Artocarpeae Lam. & DC., Chlorophoreae Juss., Dorstenieae Dumort., Ficeae Dumort., Moreae Gaudich., Olmedieae Trécul, Parartocarpeae Zerega & E.M.Gardner) (Clement & Weiblen, 2009; Zerega & Gardner, 2019; Gardner & al., in press). The Dorstenieae, however, are something of an exception to that trend; this heterogenous tribe contains species with both unisexual and bisexual inflorescences ranging from spikes (*Sloetia* Teijsm. & Binn.) to condensed heads (*Brosimum* Sw.) or

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flattened discs (*Dorstenia* L.). This has led to conflicting views of phylogenetic relationships within the tribe (Berg, 2001; Clement & Weiblen, 2009; Zerega & al., 2010; Gardner & al., in press). A recent phylogenomic study of Dorstenieae, based on a target enrichment approach (Q. Zhang & al., 2019a), confirmed the monophyly of Dorstenieae, which includes *Brosimum* together with 15 other genera (Zerega & Gardner, 2019; Gardner & al., in press). These include members of the now-obsolete Brosimeae as well as others (*Bleekrodea* Blume, *Broussonetia* L'Hér. ex Vent., *Fatoua* Gaudich., *Sloetia*, *Sloetiopsis* Engl.) that were previously assigned to Moreae. Relationships within and between the genera comprising the former Brosimeae remain unclear.

Brosimum sensu Berg (Fig. 1) comprises 15 Neotropical species whose distribution extends from Mexico and the Greater Antilles to southern Brazil. The morphology and taxonomic history of the genus were most recently reviewed in detail by Berg (1972). Berg (1970) united the poorly differentiated genera *Brosimum*, *Galactodendrum* Kunth ex Humb. & Bonpl., *Ferolia* Aubl., and *Piratinera* Aubl. into *Brosimum* as currently circumscribed, based on inflorescence characters, maintaining *Ferolia* (Aubl.) C.C.Berg as a subgenus. The genera *Brosimum*, *Helianthostylis* Baill. (2 spp.) and *Trymatococcus* Poepp. & Endl. (2 spp.) comprised the tribe Brosimeae Trécul (Berg, 1972), later included within Dorstenieae (Berg, 2001) but used in this study as an informal clade name. The species in these three genera are all trees, native to habitats ranging from wet to seasonally dry forest. As is the case with most members of the Dorstenieae, inflorescences in the "Brosimeae" can be bisexual (but are not always so), and species may be monoecious, dioecious, or androdioecious. The inflorescence morphology is unique within Dorstenieae, typically consisting of a capitate inflorescence covered with many staminate (male) flowers and one (to several) central pistillate (female) flower(s) immersed in the receptacle-like inflorescence axis, visible only by virtue of its exserted style (Fig. 1B,C). Unisexual inflorescences follow the same general plan, the staminate ones sometimes with an abortive central pistillate flower. The immature inflorescence is initially covered completely by peltate bracts, which may persist in fruit (Fig. 1B,D,F,H). In fruit, the inflorescence axis becomes fleshy, surrounding the seed(s).

Berg (1970, 1972) divided *Brosimum* into two subgenera: *B*. subg. *Brosimum*, with non-amplexicaul stipules (Fig. 1D) and more or less globose-capitate inflorescences, and *B*. subg. *Ferolia*, with fully amplexicaul stipules (Fig. 1J) and often with lobed inflorescences resembling small cauliflower heads. *Trymatococcus* and *Helianthostylis* closely resemble *Brosimum*, differing in the presence of pistillodes (always lacking in *Brosimum*), as well as inflorescence sexuality (always bisexual in *Trymatococcus* and always bisexual or staminate in *Helianthostylis*), the presence of a well-developed perianth in staminate flowers (usually vestigial or lacking in *Brosimum*) and the number of stamens in the flower. *Helianthostylis*, remarkable for its long pistillodes, protruding up to 2 cm from the staminate flowers, can also have unisexual staminate inflorescences.

Several *Brosimum* species produce edible parts. The most well-known species, *B. alicastrum* Sw. (ramón, bread nut, Maya nut, Fig. 1A–D) has a large nutritious seed; a traditional famine food, it has recently been promoted for its crop potential (Peters & Pardo-Tejeda, 1982; Gillespie & al., 2004; Lander & Monro, 2015). Additionally, several species have copious latex that is consumed as milk, including *B. utile* Pittier (palo de vaca, Fig. 1I–L) (Berg, 1972). *Brosimum rubescens* Taub. (bloodwood) is a valuable timber tree, and *B. gaudichaudii* has been exploited by the pharmaceutical industry as a source of psoralens for the treatment of immunologic disorders (Palhares & al., 2007).

Previous phylogenetic work including *Brosimum* includes a 2-locus family-level study (with 5 *Brosimum* species) based on nuclear ribosomal and chloroplast DNA (Zerega & al., 2005; Clement & Weiblen, 2009) and a targeted study (12 *Brosimum* species) based on a single chloroplast locus (Silva, 2007). They found that *Trymatococcus* and *Helianthostylis* were nested within *Brosimum*. Uncertainty as to phylogenetic relationships, morphological evolution, and biogeographic history remained, however, due to the small number of sequences and incomplete taxon sampling employed in previous studies. As part of a broader investigation into relationships within the Dorstenieae (Q. Zhang & al., 2019a), we employed a phylogenomic approach to investigate the evolutionary history of the genera in the "Brosimeae".

In order to resolve the relationships between these three genera, we employed target enrichment sequencing (HybSeq) to capture 333 genes previously developed for phylogenetic work in Moraceae (Gardner & al., 2016; Johnson & al., 2016), with nearly complete species-level sampling. The HybSeq method allows for efficient capture of hundreds of loci and is suitable for both fresh material and degraded DNA from herbarium material (Hart & al., 2016; Villaverde & al., 2018; Brewer & al., 2019), which comprises much of the material employed in this study. This work aimed to test the monophyly of *Brosimum* and allied genera, determine synapomorphies for generic and subgeneric taxonomic levels, and revise *Brosimum* taxonomy accordingly. Additionally, we used divergence date estimates and geographical distributions within the "Brosimeae" in order to reconstruct the biogeographic history and ancestral range of the clade. While some species are widespread, others are restricted to the Amazon or the Guiana Shield. A recent study found that Amazonia was the primary source of Neotropical biodiversity (Antonelli & al., 2018). In addition, recent studies in Moraceae have tended to reveal a strong correlation between biogeography and phylogeny (Gardner & al., in press).

■ MATERIALS AND METHODS

Sampling and DNA preparation. — We sampled all 19 species of "Brosimeae" but were unable to obtain usable sequences from *Brosimum glaziovii* Taub., *B. melanopotamicum* C.C.Berg, and *Helianthostylis steyermarkii* C.C.Berg,

with final taxon sampling consisting of 16/19 species including both subspecies of *B. alicastrum* (Appendix 1). Outgroups included at least one species from most other genera within Dorstenieae (11 additional genera, missing only *Bleekrodea* and *Sloetiopsis*), one sample per tribe for the remaining six Moraceae tribes, and *Trema orientale* (L.) Blume (Cannabaceae Martinov). We prepared 21 new sequencing libraries for this study, combining those samples with reads from Johnson & al. (2016), Q. Zhang & al. (2019a), and the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) to complete the outgroup sampling. All new reads have been deposited in the SRA (BioProject PRJNA322184).

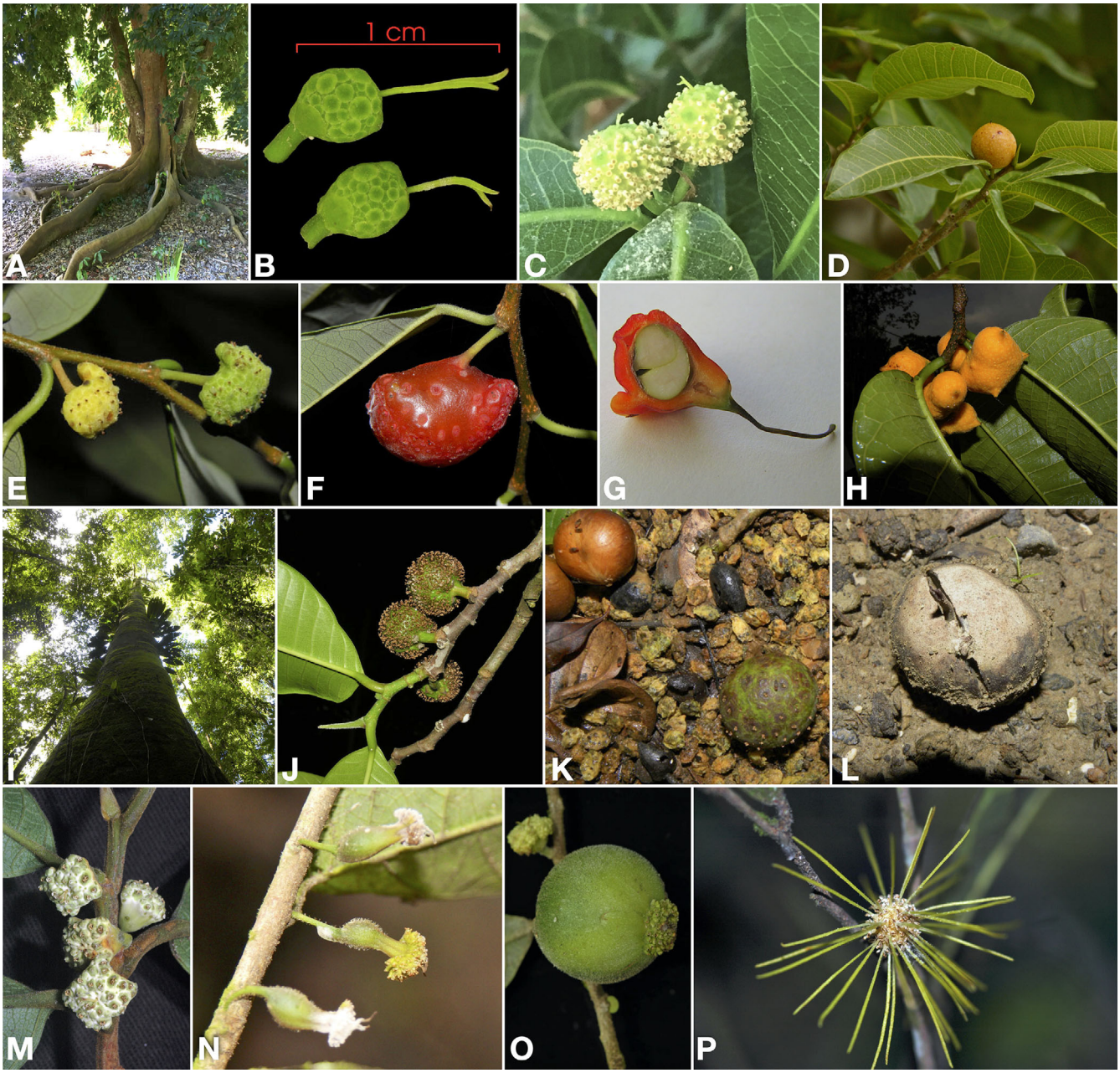

Fig. 1. A–D, *Brosimum alicastrum* Sw. buttresses, inflorescences, staminate inflorescences with central pistillate flower (the latter likely abortive), and infructescence on shoot showing lateral stipule typical of *B*. subg. *Brosimum*; **E–G**, *B. guianense* inflorescences, infructescence, and infructescence in section showing seed with unequal cotyledons typical of *B*. subg. *Brosimum*; **H**, Multi-seeded infructescences of *B. lactescens*; **I–L**, *B. utile* bole, shoot with bisexual inflorescences showing amplexicaul stipule typical of *B*. subg. *Ferolia*, seeds and infructescence showing persistent stamens, and germinating seed showing equal cotyledons typical of *B*. subg. *Ferolia*; **M–O**, Inflorescences and infructescence of *B. amazonicus* (*B*. subg. *Trymatococcus*); **P**, Inflorescence of *B. sprucei* showing long pistillodes typical of *B*. subg. *Helianthostylis*. — Photos by E. Gardner (A, C), S. Patton (B), Siddarth Machado (D), Reinaldo Aguilar (E, F, I–L), Alex Popovkin (G, H), J.E.L. da Silva Ribeiro (M, P), W. Milliken (N) bioweb.bio (O); photos D–L are reproduced under a CC BY-NC-SA 2.0 license, and O under a CC BY-NC-ND 4.0 license.

Sample preparation followed the approach detailed in Hale & al. (2020). DNA was extracted from ca. 0.5 cm^2 of leaf tissue (in almost all cases taken from a herbarium sheet) using a modified CTAB protocol (Doyle & Doyle, 1987), with overnight incubation for the initial lysis step as well as DNA precipitation. After assessing DNA fragment size on an agarose gel, samples with average fragment sizes more than 550 bp were sonicated to a mean insert size of 550 bp using a Covaris M220 (Covaris, Wobum, Massachusetts, U.S.A.). Libraries were prepared with the Illumina TruSeq Nano HT DNA Library Preparation Kit (Illumina, San Diego, California, U.S.A.) or the KAPA Hyper Prep (KAPA Biosystems, Cape Town, South Africa) following the manufacturer's protocol, except that reactions were performed in one-third volumes to save reagent costs. Libraries were combined into pools of 6–24 (sometimes with samples from other studies in Moraceae) and enriched for the 333 target genes (Gardner & al., 2016) with MYbaits custom probes (Arbor Biosciences, Ann Arbor, Michigan, U.S.A.) following the manufacturer's protocol, with 14 PCR cycles. Products were sequenced on an Illumina MiSeq (2 × 300 bp, version 3 chemistry) at the Field Museum of Natural History alongside samples for other studies in multiplexed runs of 30–70 samples each.

Sequence assembly and phylogenetic analysis. — Annotated workflow scripts detailing sequence assembly, alignment, and analysis parameters appear in supplementary Appendix S1A–D. We trimmed demultiplexed reads using Trimmomatic v.0.36 to remove low-quality bases (with these parameters: "ILLUMINACLIP: TruSeq3-PE.fa:2:30:10 HEADCROP:3 LEADING:30 TRAILING:25 SLIDINGWINDOW:4:25 MINLEN:20") (Bolger & al., 2014) and assembled them with HybPiper, which produces gene-by-gene, reference-guided, *de novo* assemblies. Briefly, a set of reference sequences is used to sort reads by gene, and then reads for each gene are assembled *de novo*; the reference is then used to predict coding sequences within the *de novo* assemblies (Johnson & al., 2016). Here we used the *Morus* and *Artocarpus* reference sequences developed by Gardner & al. (2016). The default output of HybPiper is the predicted coding sequence for each gene (exon sequences). We used the HybPiper script "intronerate.py" to build "supercontig" sequences for each gene, consisting of exons as well as any assembled flanking non-coding sequences (intronic or intergenic).

For each target gene, exon sequences were filtered to remove sequences less than 25% of the average length for that gene. Filtered sequences were then aligned using MAFFT v.7.453 (Katoh & Standley, 2013), and columns with more than 75% gaps were removed with trimAl v.1.4 (Capella-Gutiérrez & al., 2009). Single-gene phylogenies for each of the 333 genes were estimated using RAxML v.8.2.12 under the GTRGAMMA model, with 500 rapid bootstrap replicates (Stamatakis, 2006). After collapsing nodes with less than 30% bootstrap support (C. Zhang & al., 2018) using SumTrees v.4.4.0 (Sukumaran & Holder, 2010), we used these gene trees to estimate a species tree with a coalescent-based approach implemented in ASTRAL v.5.7.1 (Mirarab & Warnow, 2015; C. Zhang & al., 2018). Bootstrap (160 replicates, resampling across genes) and local posterior probability (representing quartet support) were calculated for each node. A maximum likelihood tree was also calculated with RAxML based on a concatenated supermatrix of all 333 genes using a mixed-partition (one partition per gene) GTRGAMMA model, with 500 rapid bootstrap replicates. The supermatrix, gene tree, and species tree analyses were then repeated with the "supercontig" sequences using the same parameters.

Divergence time estimation. — We time-calibrated the *Brosimum* phylogenetic tree with three fossils (for full details, see Q. Zhang & al., 2019a) and two secondary calibrations. The fossil wood of *Artocarpoxylon deccanensis* Mehrotra & al. (Mehrotra & al., 1984) was used as a minimum age constraint of 64.0 Ma for the stem node of *Artocarpus*, represented in our trees here by the split of *Artocarpus heterophyllus* Lam. and *Paratrophis glabra* (Merr.) Steenis. The fossil endocarps of *Broussonetia rugosa* Chandler (Chandler, 1961) were used to constrain the stem node of *Broussonetia* s.str. (represented here by the most recent common ancestor of *Allaeanthus luzonicus* (Blanco) Fern.-Vill., *Malaisia scandens* (Lour.) Planch. and *Broussonetia papyrifera* (L.) L'Hér. ex Vent.) to at least 33.9 Ma. The fossil achenes of *Ficus* (*F. lucidus* Chandler) (Chandler, 1962) were used as a minimum age constraint of 56.0 Ma for the stem node of *Ficus*, represented in our trees by the split of *F. macrophylla* Roxb. & Buch.-Ham. ex Sm. and *Antiaropsis decipiens* K.Schum. Lastly, the estimated ages for the crown node of Moraceae (73.2–84.7 Ma) and the most recent common ancestor of Moraceae and Cannabaceae (81.7–93.3 Ma) obtained from a recent family-wide molecular dating (Q. Zhang & al., 2019a) were used to secondarily calibrate the age of Moraceae and the root.

We estimated divergence times with both penalized likelihood (PL) and Bayesian relaxed clock approaches. PL was conducted in r8s v.1.7 (Sanderson, 2003) using the tree from the RAxML analysis of the concatenated supermatrix (because that tree had branch lengths in substitutions per site) with strict minimum (fossils) and maximum (root nodes) age constraints as described above. The best smoothing value was obtained using cross validation by testing 21 values of smoothing parameter scaling from 0.1 to 1000. The best value (i.e., with the lowest chi-square) of 2.6 was then applied as the smoothing parameter in divergence time estimation.

We used MCMCTree as implemented in the PAML v.4.9 package (Yang, 2007) to estimate the divergence times with a Bayesian relaxed clock, using the same topology used for the r8s analysis (RAxML analysis of the concatenated supermatrix). Two steps are needed for estimating divergence times by the approximate likelihood approach in MCMCTree. We first estimated the gradient and Hessian of branch lengths, and we then used them to estimate divergence times. A rough mean of several parameters was estimated by baseml in PAML v.4.9 at first. Then we set the parameters in MCMCTree according to the results from baseml. We launched the Bayesian analysis with a chain length of 22 million generations

with the first 10% of the chain length discarded as burn-in, sampling a total of 10,000 generations at a frequency of once every 2000 generations. To confirm convergence and to test the influence of the prior, two independent runs with the same settings were conducted. To further test the influence of the prior on the estimate, we used another prior setting (program defaults), followed the same process as described above, and then ran the chain for 16.5 million generations, sampling a total of 10,000 generations at a frequency of once every 1500 generations. An additional run was conducted for each setting but without data to confirm that the posterior was different from the prior. The convergence of the runs were checked using Tracer v.1.7 (Rambaut & al., 2018). After confirming all the effective sample size (ESS) values were over 100, we combined the results of the two independent runs for each prior setting. The entire dataset was treated as a single partition to avoid extremely long running times. The substitution model used in MCMCTree was GTR with gamma.

Ancestral state reconstructions. — A morphological matrix of 16 categorical characters was constructed based on examination of herbarium specimens at the Royal Botanic Gardens Kew (K) (Appendix 2) and the published literature (Berg, 1972, 2001) (suppl. Appendix S2A). We selected specimens in K that had been determined by C.C. Berg in order to maintain consistency in species delimitation with his monographic revision (Berg, 1972) and supplement (Berg, 2001). Characters included both vegetative and reproductive traits, including the stipule and pistillode characters used to differentiate the genera of the "Brosimeae" and the subgenera of *Brosimum* (suppl. Appendix S2A). We pruned the Bayesian time-calibrated tree to include only the Dorstenieae s.str. (*Brosimum*, *Helianthostylis*, *Trymatococcus*, *Bosqueiopsis* De Wild. & T.Durand, *Dorstenia*, *Scyphosyce* Baill., *Treculia* Decne. ex Trécul, *Trilepisium* Thouars, *Utsetela* Pellegr.) and carried out ancestral state reconstruction under maximum-likelihood using the rayDISC function in corHMM v.1.22, choosing the best-fitting model between "ER", "SYM", and "ARD" based on the corrected Akaike information criterion (AICc) for each trait (Beaulieu & al., 2013). Manipulation and plotting of trees was carried out using ape and phytools in R (Revell, 2012; Paradis & Schliep, 2019; R Core Team, 2019).

We assembled biogeographic matrices for the ingroup species based on the maps from Berg (1972) (suppl. Appendix S2B). For the first matrix, areas were based on the 10 Neotropical regions proposed by Antonelli & al. (2018), eight of which contained ingroup species: AMA (Amazonia), AGL (Andean Grasslands), ATF (Atlantic Forests), CAA (Caatinga), CEC (Cerrado and Chaco), DNO (Dry Northern South America), MES (Mesoamerica), and WIN (West Indies). Ranges were coded as present (1) or absent (0) and were compared to the "Brosimeae" records from that study, which originated from the Global Biodiversity Information Facility (GBIF). For the second matrix, we combined some areas that were adjacent and co-occurring and thus redundant and added the Guiana Shield as a separate area, as some ingroup species are concentrated there. The result contained four areas: Guiana Shield (including DNO), Amazonia (not including the Guiana Shield), Mesoamerica (including WIN), and Atlantic Forest (including CAA and CEC). For each matrix, we estimated ancestral ranges under the dispersal-extinction-cladogenesis (DEC) model using BioGeoBEARS v.1.1.1 in R (Matzke, 2018), setting the maximum number of allowed areas to 4 (equal to the maximum number of areas currently occupied by any single species).

■ RESULTS

Phylogenetic analyses. — Sequencing and assembly were successful for all taxa except *Brosmium glaziovii*, *B. melanopotamicum*, and *Helianthostylis steyermarkii*; the number of loci recovered per sample appear in Appendix 1. The analyses were generally concordant. The inclusion of non-coding sequences did not affect the topology at all, and differences in the ingroup between the supermatrix and species-tree analyses were limited to a single rearrangement in the clade containing *B. paranarioides* Ducke, *B. longifolium* Ducke, and *B. potabile* Ducke; among outgroups, the positions of *Dorstenia*, *Scyphosyce*, and *Utsetela* also differed (Fig. 2). In all analyses the "Brosimeae" as circumscribed by Berg (1972) were monophyletic, but *Brosimum* was not, as *Helianthostylis* and *Trymatococcus* were nested within it. However, the subgenera were monophyletic. The sister group to the "Brosimeae" was *Treculia africana* Decne. ex Trécul.

Divergence time estimation. — The stem- and crown-group ages of *Brosimum* s.l. were estimated as 22.09–35.35 Ma (early Miocene) and 18.49–29.62 Ma (Oligocene to early Miocene), respectively. Estimation from two different prior settings in MCMCTree showed similar results (Table 1, suppl. Fig. S1). The ages estimated with a PL approach were consistent with the results from MCMCTree (suppl. Fig. S2C) as well. Estimates from control runs without input data differed from results based on our dataset, suggesting the estimates were not determined by the priors.

Ancestral state reconstructions. — Trait mapping revealed several synapomorphic traits of taxonomic value in distinguishing the four subgeneric clades treated below (Figs. 3, 4, suppl. Fig. S2). Amplexicaul stipules (always co-occurring with long stipules) are diagnostic of *Brosimum* subg. *Ferolia*. Pistillodes characterize only the *Trymatococcus* + *Helianthostylis* clade, but only *Trymatococcus* has protuberances on the pistillate inflorescences, and within that clade, only *Helianthostylis* has equal cotyledons (a character shared with *Ferolia*). Only *B*. subg. *Brosimum* lacks both amplexicaul stipules and pistillodes. Other traits appear to be homoplastic within the subgeneric taxa and therefore not taxonomically informative at that level. For ancestral state reconstruction, model testing indicated that the "ER" model was preferred for all characters (suppl. Appendix S2C). It revealed that the common ancestor of the "Brosimeae" most likely was a monoecious tree with non-amplexicaul stipules, bisexual, more or less globose inflorescences with peltate bracts and a single

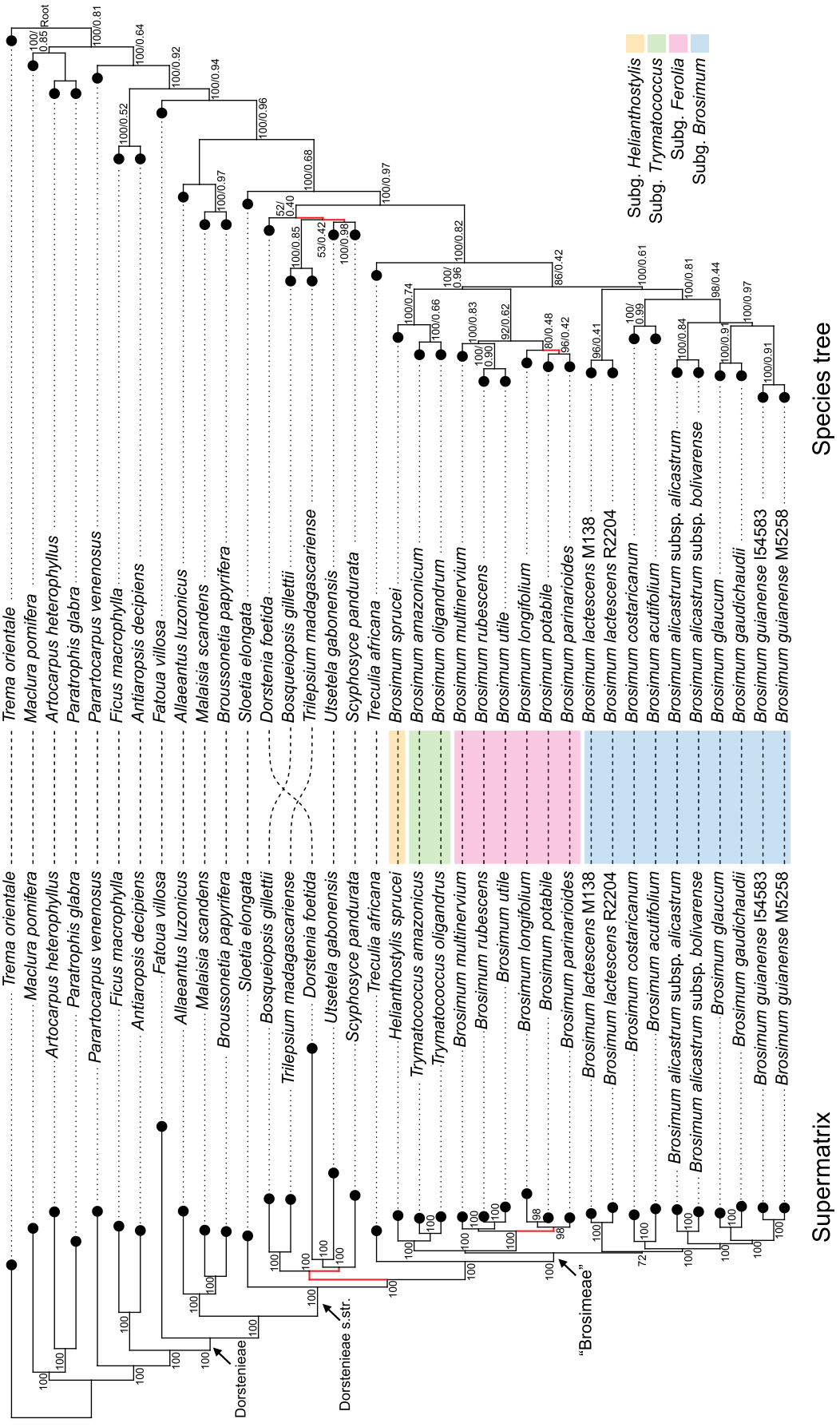

Fig. 2. Left, Maximum-likelihood tree based on a supermatrix of all sequences (exon dataset), showing bootstrap support, with previously accepted names and branch lengths proportional to substitutions. **Right**, ASTRAL species tree based on 333 gene trees (exon dataset), showing bootstrap/local posterior probability, with revised names and branch lengths proportional to coalescent units. Disagreeing branches appear in red. The "supercontig" topologies were identical (and therefore not shown).

pistillate flower, staminate flowers lacking both well-developed perianth and a pistillode, and unequal cotyledons. The well-developed staminate perianth was likely regained three times, once each in *Trymatococcus* + *Helianthostylis*, *B. lactescens* (S.Moore) C.C.Berg, and *B. costaricanum* Liebm.

Both DEC analyses (Fig. 5, suppl. Fig. S3) estimated Amazonia as the ancestral range of all four ingroup clades (*B.* subg. *Brosimum* and subg. *Ferolia*, *Trymatococcus*, *Helianthostylis*), with dispersals into additional areas starting approximately 15 Ma (8-area analysis: lnL = −46.24084, nparams = 2, d = 0.007252254, e = 1e-12, 4-area analysis: lnL = −40.27165, nparams = 2, d = 0.02100957, e = 0.002998425). The 4-area analysis, which considered the Guiana Shield separately, estimated the Amazonia + Guiana Shield as the ancestral range for *Trymatococcus* but estimated Amazonia not including the Guiana Shield as the ancestral range for the other three lineages, with subsequent dispersals to the Guiana Shield in the common ancestor of *B. potabile*, *B. longifolium*, and *B. parinarioides* (ca. 12 Ma) as well as even more recent dispersals in other individual species.

Table 1. Estimated divergence times (Ma) for select clades for all three analyses. 95% HPD values for the MCMCTree analyses appear in brackets.

Crown node	r8s	MCMCTree default	MCMCTree prior
"Brosimeae" + *Treculia*	23.5187	29.21 [22.29–36.83]	28.21 [22.09–35.35]
"Brosimeae"	19.8118	24.71 [18.79–31.47]	23.74 [18.49–29.62]
Brosimum subg. *Brosimum*	18.8775	23.62 [17.83–30.18]	22.71 [17.61–28.5]
Brosimum subg. *Ferolia* + *Helianthostylis* + *Trymatococcus*	19.0847	23.10 [17.38–29.6]	22.22 [17.1–27.92]
Brosimum subg. *Ferolia*	14.3197	15.04 [10.47–20.74]	14.35 [10.21–19.31]
Trymatococcus + *Helianthostylis*	12.9741	16.06 [10.09–22.7]	15.42 [9.81–21.64]

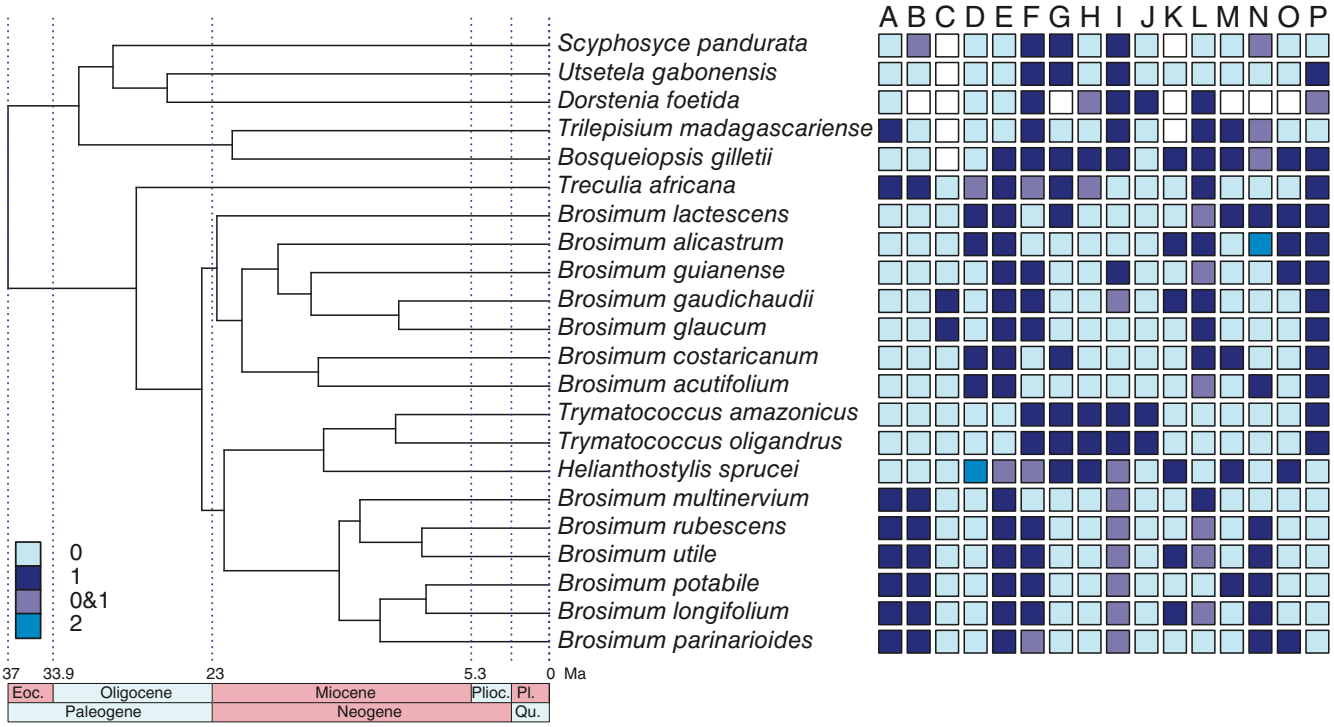

Fig. 3. Sixteen characters mapped onto the pruned, time-calibrated phylogeny, with revised nomenclature, including subgenera. From left: A, Stipules fully amplexicaulous (0, 1); B, Longer stipules ≤15 mm (0), >15 mm (1); C, Lateral veins loop-connected close (0) or far (1) from margin; D, Breeding system monoecious (0), dioecious (1), androdioecious (2); E, Interfloral bracts peltate (0, 1); F, Inflorescences unisexual (0), bisexual (1); G, Staminate perianth well developed (1) or vestigial / lacking (0); H, Pistillode absent (0), present (1); I, Pistillate inflorescence shape globose to ellipsoid (0) or turbinate, cylindrical, or hemispherical (1); J, Pistillate inflorescence surface with notable protuberances (1) or smooth (0); K, Bracts ≤1.5 mm (0), >1.5 mm (1); L, Pistillate flowers solitary (0) or multiple (1); M, Stigma equal or shorter than style (0), longer than style (1); N, Stigma disposition angle from vertical: under 45 (0), 45–90 (1), over 90 (2); O, Stigma weakly curved (0), sigmoid (1); P, Cotyledons unequal (1) or equal (0). Eoc., Eocene; Plioc., Pliocene; Pl., Pleistocene; Qu., Quaternary.

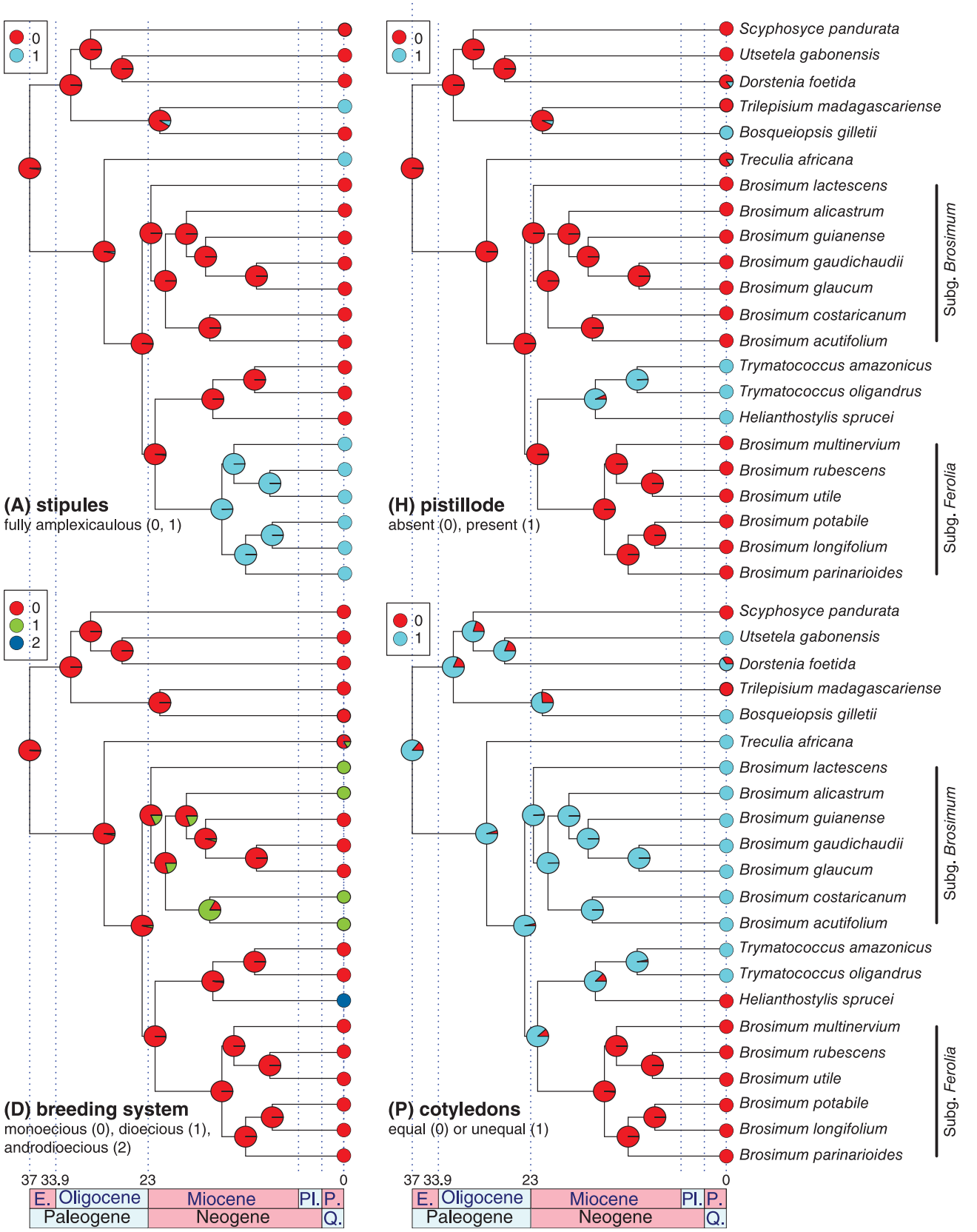

Fig. 4. Maximum-likelihood ancestral reconstructions for four select characters, labeled to align with the complete set of characters appearing in Fig. 3 and suppl. Fig. S2. **A**, Stipules fully amplexicaulous (0, 1); **D**, Breeding system monoecious (0), dioecious (1), androdioecious (2); **H**, Pistillode absent (0), present (1); **P**, Cotyledons equal (0) or unequal (1).

■ DISCUSSION

Phylogenetic relationships. — The phylogenetic trees presented here, based on hundreds of genes with near-complete species sampling, were consistent with previous findings (Zerega & al., 2005; Silva, 2007; Clement & Weiblen, 2009; Q. Zhang & al., 2019a) that *Brosimum* is paraphyletic, encompassing *Trymatococcus* and *Helianthostylis* (Fig. 2). The morphological affinities between these genera, in particular the inflorescence architecture, led previous authors to treat them together as the "Brosimeae", and notwithstanding the rank of *Trymatococcus* and *Helianthostylis*, our results match the higher divisions outlined by Berg in his morphology-based treatment of the "Brosimeae" (Berg, 1972). Sister to the ingroup was the African genus *Treculia* (3 species native to Afrotropics), whose inflorescences, although larger, are also covered with peltate bracts that strongly resemble those of *Brosimum*. This sister placement of *Treculia* is consistent with previous studies (Zerega & al., 2005; Silva, 2007; Clement & Weiblen, 2009; Misiewicz & Zerega, 2012; Q. Zhang & al., 2019a).

Our results highlight the value of the 333 loci employed here for resolving and clarifying the systematics of Moraceae. Phylogenomic studies based on these markers have so far produced robust phylogenetic hypotheses for Artocarpeae (Gardner & al., 2020), Dorstenieae (Q. Zhang & al., 2019a), Moreae (Gardner & al., in press), and Parartocarpeae (Zerega & Gardner, 2019) and therefore show promise in resolving longstanding taxonomic problems in the family.

Divergence times and historical biogeography. — Divergence time estimates (Table 1, suppl. Fig. S1) were consistent, but slightly younger than the results obtained in a Dorstenieae-wide analysis in which stem- and crown-group ages for *Brosimum* s.l. were estimated between 19.4 and 42.9 Ma and 14.7 and 31.7 Ma, respectively (Q. Zhang & al., 2019b). These differences may have resulted from sparser sampling outside the "Brosimeae" in the present study. Africa and South America were last connected approximately 105 million years

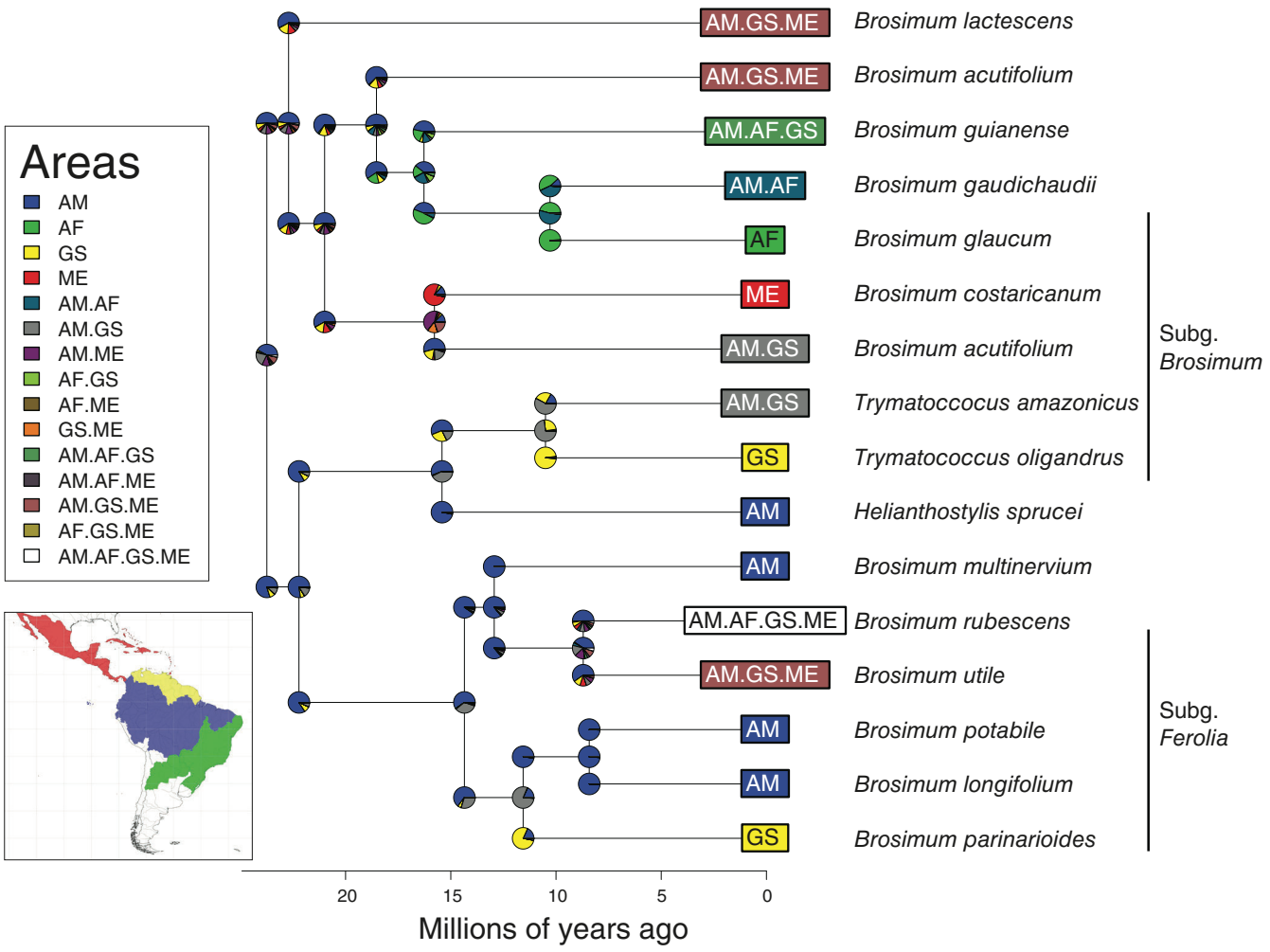

Fig. 5. Results of DEC analysis using four simplified biogeographic areas. Pie charts indicate relative probabilities of different scenarios. Areas are AM (Amazonia, not including the Guiana Shield), GS (Guiana Shield and the adjacent Dry Northern South America), AF (Atlantic Forest and the adjacent Cerrado and Caatinga), and ME (Mesoamerica and West Indies).

ago (McLoughlin, 2001), but *Brosimum* seed dispersers include birds and bats (Monterrubio-Rico & al., 2009; Poelchau & Hamrick, 2012). If the clade had its origins in Africa as reconstructed by Q. Zhang & al. (2019a), the arrival of the ancestor of *Brosimum* in the Neotropics might therefore be explained by long-distance dispersal. In assembling the area matrix, the distributions we coded based on the *Flora Neotropica* (Berg, 1972) maps did not materially differ from the distributions coded from the cleaned GBIF data from Antonelli & al. (2018) (suppl. Appendix S2B). Estimates of ancestral ranges indicate that all four major lineages of "Brosimeae" originated in Amazonia and initially diversified there, first dispersing to other areas, such as northern South America, and Central America in the middle Miocene (*B*. subg. *Brosimum*) and to the Guiana Shield in the late Miocene (*B*. subg. *Ferolia*, subg. *Trymatococcus*, and subg. *Helianthostylis*) (Fig. 5, suppl. Fig. S3). These findings are consistent with overall patterns of Neotropical diversification, which are overwhelmingly centered in Amazonia (Antonelli & al., 2018).

Morphological evolution. — Stipule amplexicauly and equal cotyledons are derived states within the "Brosimeae" and comprise a synapomorphy for *Brosimum* subg. *Ferolia* (Fig. 4). In "Brosimeae", stipule amplexicaulity always co-occurred with longer stipules, perhaps due to geometric constraints because an amplexicaul stipule clasps the full circumference of the stem as well as the developing leaf (Fig. 1J). In the broader Dorstenieae s.str., the trait is homoplastic and likely evolved independently twice outside our ingroup, in *Treculia*—sister to the "Brosimeae"—and in *Trilepisium*. The role of amplexicauly relative to lateral stipules is unclear. They may facilitate the production of large leaves through a protective and/or photosynthetic role, and the clade comprising species with amplexicaul stipules does comprise the larger-leaved ingroup taxa. The role of unequal cotyledons (Fig. 1G) is unclear. They do not correlate with seed size so may instead relate to seedling establishment. As with amplexicaul stipules, equal cotyledons are homoplastic within the Dorstenieae s.str. and present also in *Trilepisium*, *Scyphosyce*, and *Dorstenia* (p.p.).

The presence of a pistillode is a synapomorphy for the *Trymatococcus* + *Helianthostylis* clade and is apparently derived within the Dorstenieae s.str., otherwise appearing only in *Bosqueiopsis* (Fig. 4). The presence of a well-developed pistillode in Moraceae may facilitate the positioning of inflexed stamens that ballistically release pollen at anthesis (Corner, 1962), and it is therefore not surprising that most members of the Dorstenieae s.str.—which never have ballistic pollen release—would lack a pistillode. In that case, there is likely another function for the pistillode in *Trymatococcus* and *Helianthostylis*, and it is possible that at least in the latter, the long showy pistillodes may relate to pollinator attraction. However, little is known about pollination in the Dorstenieae (Berg, 2001).

Berg (1972) separated the three genera of his "Brosimeae" based on a suite of characters (stipule amplexicaulity, pistillode, inflorescence sexuality, staminate perianth, stamen number) that align with our results, requiring only a change of rank for *Trymatococcus* and *Helianthostylis*, highlighting the durability of his careful morphological studies. The stipule and pistillode characters may be readily observed in fertile specimens. However, the other characters, while informative in studying broader patterns of evolution, are less reliable for diagnosing individual specimens. Inflorescence sexuality can vary within a species, sometimes within the same individual (Peters, 1991), leading Berg to cast doubt on its reliability as a diagnostic trait (Berg, 1972). The presence of a well-developed staminate perianth, while readily observable, was recovered as homoplastic and is thus diagnostic only in combination with other characters. In contrast to other tribes in Moraceae, stamen number is remarkably variable within the genera of the Dorstenieae. Although *Trymatococcus* and *Helianthostylis* never have fewer than three stamens, there otherwise exists some overlap in stamen number between the subgenera treated here. We therefore find that the most reliable and phylogenetically consistent diagnostic characters for distinguishing these clades are stipule amplexicaulity, the presence of a pistillode, and equal/unequal cotyledons (although the latter can be difficult to determine from herbarium specimens) (Fig. 4).

■ TAXONOMIC REVISIONS

Based on these analyses, we reduce *Trymatococcus* and *Helianthostylis* to subgenera of *Brosimum*. This expanded genus unites all the woody members of Dorstenieae species in the New World, all based on a shared inflorescence plan. We include *B. multinervium* C.C.Berg in *B*. subg. *Ferolia*, although it was described after Berg's (1972) treatment of that subgenus; the fully amplexicaul stipules and its phylogenetic position leave no doubt as to its correct placement. Likewise, we maintain Berg's (1972) placement of the taxa we were unable to include in our phylogenetic analyses based on unambiguous morphological synapomorphies. The fully amplexicaul stipules of *B. melanopotamicum* leave no doubt about its proper placement in *B*. subg. *Ferolia*, where it also appeared, with strong support, in a previous single-locus phylogenetic study (Silva, 2007). The lateral stipules and unequal cotyledons of *B. glaziovii* place it within *B*. subg. *Brosimum*, even in the absence of sequence data. Finally, the non-amplexicaul stipules, equal cotyledons, and well-developed pistillodes of *H. steyermarkii* confirm its proper placement in *B*. subg. *Helianthostylis*. Below follows an updated phylogenetic classification and taxonomic summary for the expanded genus *Brosimum*. A short-form description and summary of the key characters defining each group is provided. For complete taxonomic histories, synonymies, and descriptions, the reader should refer to C.C. Berg's comprehensive treatments of *Brosimum*, *Trymatococcus*, and *Helianthostylis* in *Flora Neotropica* monographs 7 and 83 (Berg, 1972, 2001).

Brosimum Sw., Prodr.: 12. 1788, nom. cons. – Type: *B. alicastrum* Sw., typ. cons.

= *Alicastrum* P.Browne, Civ. Nat. Hist. Jamaica: 372. 1765, nom. rej.
= *Piratinera* Aublet, Hist. Pl. Guiane 2: 888. 1775, nom. rej. – Type: *P. guianensis* Aubl. (≡ *Brosimum guianense* (Aubl.) Huber).
= *Ferolia* Aublet, Hist. Pl. Guiane 2, Suppl.: 7. 1775, nom. rej. – Type: *F. guianensis* Aubl. (= *Brosimum rubescens* Taub.).
= *Galactodendrum* Kunth in Humboldt & Bonpland, Voyage, Relat. Hist. 2: 108. 1819 – Type: *G. utile* Kunth (≡ *Brosimum utile* (Kunth) Oken).
= *Trymatococcus* Poepp. & Endl., Nov. Gen. Sp. Pl. 2: 30. 1838 – Type: *T. amazonicus* Poepp. & Endl.
= *Helianthostylis* Baill. in Adansonia 11: 299. 1875 – Type: *H. sprucei* Baill.
= *Brosimopsis* S.Moore in Trans. Linn. Soc. London, Bot. 4: 473. 1895 – Type: *B. lactescens* S.Moore (≡ *Brosimum lactescens* (S.Moore) C.C.Berg).

Monoecious or dioecious trees. *Leaves* distichous, pinnately veined. Stipules free or connate, lateral or fully amplexicaul. *Inflorescences* unisexual or bisexual, axillary, solitary or paired, globose, hemispherical, cylindrical, or turbinate, interfloral bracts usually peltate. Staminate flowers few to many, perianth well developed or lacking/vestigial, stamens 1–4, straight in bud, pistillode present or absent. Pistillate flowers one to several, immersed in the center of the inflorescence axis with the style exserted. *Fruits* adnate to the enlarged and fleshy inflorescence axis, to at least 2 cm in diameter; cotyledons equal or unequal.

Species: 19, restricted to the Neotropics; the only woody Dorstenieae in that region (Fig. 6).

Key to the subgenera

1. Pistillode absent, plants monoecious or dioecious, stipules amplexicaul or not 2
1. Pistillode present, plants monoecious or androdioecious, stipules non-amplexicaul 3
2. Stipules non-amplexicaul, cotyledons unequal, plants monoecious or dioecious ..... subg. ***Brosimum***
2. Stipules fully amplexicaul, cotyledons equal, plants monoecious ..... subg. ***Ferolia***
3. Pistillode minute; cotyledons unequal, inflorescences with protuberances, plants monoecious ..... subg. ***Trymatococcus***
3. Pistillode usually well-developed, cotyledons equal, inflorescences lacking protuberances, plants androdioecious ..... subg. ***Helianthostylis***

Brosimum subg. ***Brosimum***

Monoecious or dioecious trees, stipules lateral, inflorescences unisexual or bisexual, usually ± globose, staminate perianth usually not well developed, pistillode absent, cotyledons unequal.

Distribution: Mexico and the Greater Antilles to northern South America and Amazon basin (Colombia, Venezuela, Ecuador, Peru, Brazil, Bolivia, French Guiana, Costa Rica, Panama, Mexico, Guatemala, Nicaragua, Paraguay; Fig. 6).

Species (8): *B. acutifolium* Huber, *B. alicastrum* Sw., *B. costaricanum* Liebm., *B. gaudichaudii* Trécul, *B. glaucum*

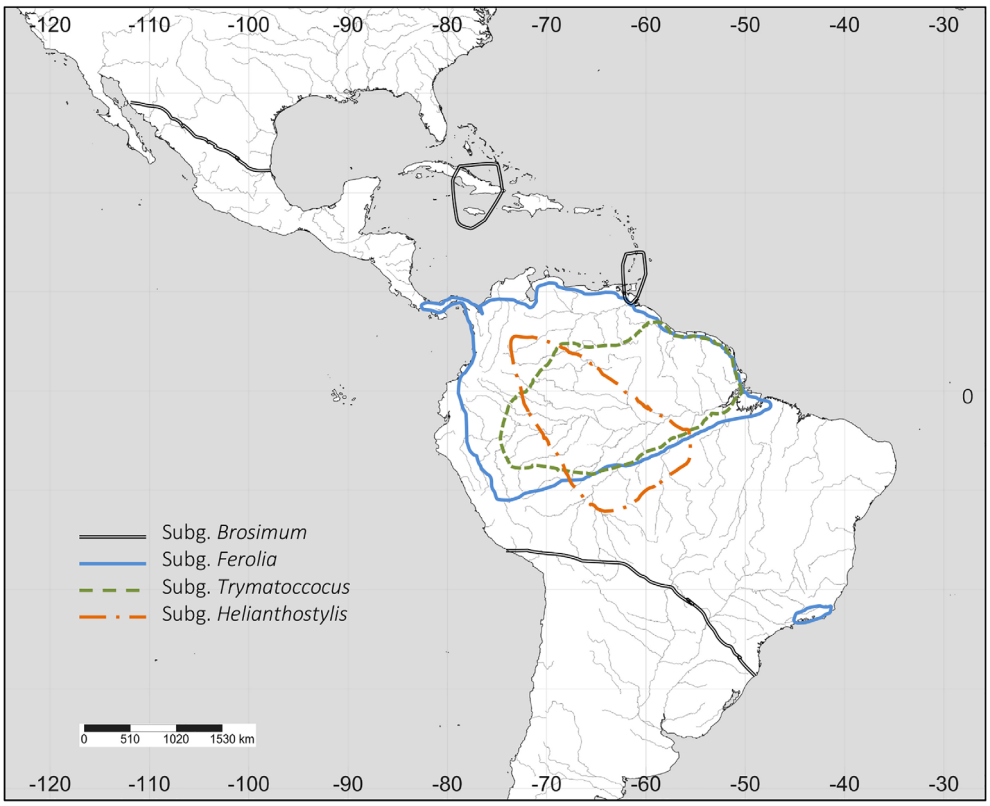

Fig. 6. Distributions of the four subgenera of *Brosimum*, following the revisions outlined here.

Taub., *B. glaziovii* Taub., *B. guianense* (Aubl.) Huber ex Ducke, *B. lactescens* (S.Moore) C.C.Berg.

Brosimum subg. ***Ferolia*** (Aubl.) C.C.Berg in Acta Bot. Neerl. 19: 327. 1970 ≡ *Ferolia* Aubl., Hist. Pl. Guiane 2, Suppl.: 7. 1775 – Type: *F. guianensis* Aubl. (= *Brosimum rubescens* Taub.).

Monoecious trees, stipules amplexicaul, inflorescences usually bisexual, globose to, hemispherical, cylindrical, or turbinate, sometimes irregularly lobed, staminate perianth not well developed, pistillode absent, cotyledons equal.

Distribution: Members of this subgenus are largely concentrated in the northern part of the South American continent distinctly associated with the Guiana Shield (Panama, Colombia, Venezuela, Ecuador, Peru, Brazil, Bolivia, Guyana, French Guiana; Fig. 6).

Species (7): *B. longifolium* Ducke, *B. melanopotamicum* C.C.Berg, *B. multinervium* C.C. Berg, *B. parinarioides* Ducke, *B. potabile* Ducke, *B. rubescens* Taub., *B. utile* (Kunth) Oken.

Brosimum subg. ***Trymatococcus*** (Poepp. & Endl.) E.M. Gardner & Zerega, **comb. & stat. nov.** ≡ *Trymatococcus* Poepp. & Endl., Nov. Gen. Sp. Pl. 2: 30. 1838 – Type: *T. amazonicus* Poepp. & Endl.

Monoecious trees, stipules lateral, inflorescences bisexual, turbinate, with protuberances, staminate perianth well developed, pistillode present but minute, cotyledons unequal.

Distribution: Upper Amazon basin to the Guianas (Colombia, Venezuela, Guyana, French Guiana, Ecuador, Peru, Brazil; Fig. 6).

Species (2):

Brosimum amazonicum (Poepp. & Endl.) E.M.Gardner & Zerega, **comb. nov.** ≡ *Trymatococcus amazonicus* Poepp. & Endl., Nov. Gen. Sp. Pl. 2: 30. 1838.

Brosimum oligandrum (Benoist) E.M. Gardner & Zerega, **comb. nov.** ≡ *Lanessania oligandra* Benoist in Bull. Mus. Natl. Hist. Nat. 27: 199. 1921.

Brosimum subg. ***Helianthostylis*** (Baill.) E.M.Gardner & Zerega, **comb. & stat. nov.** ≡ *Helianthostylis* Baill. in Adansonia 11: 299. 1875 – Type: *H. sprucei* Baill.

Monoecious or androdioecious trees, stipules lateral, inflorescences bisexual or staminate, globose to turbinate, staminate perianth well developed, pistillode well developed and often showy, cotyledons equal.

Distribution: Amazon Basin (Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, Venezuela; Fig. 6).

Species (2):

Brosimum sprucei (Baill.) E.M.Gardner & Zerega, **comb. nov.** ≡ *Helianthostylis sprucei* Baill. in Adansonia 11: 299. 1875.

Brosimum steyermarkii (C.C.Berg) E.M.Gardner & Zerega, **comb. nov.** ≡ *Helianthostylis steyermarkii* C.C.Berg in Acta Bot. Neerl. 21: 99, fig. 1. 1972.

■ CONCLUSION

The analyses and revisions presented above result in a well-defined and monophyletic *Brosimum* with four diagnosable subgenera, providing a framework for further work on this understudied genus, including phylogenetic and biogeographic investigations at the subspecific level.

■ AUTHOR CONTRIBUTIONS

EMG led the writing of the manuscript, did field, lab, and herbarium work, and conducted all analyses except the divergence time estimation. LA did herbarium sampling and lab work for the ingroup. QZ did herbarium sampling and lab work for the outgroups and conducted the divergence time analyses. HS acquired funding for outgroup sequencing and supervised overall work on Dorstenieae. AKM provided overall guidance on *Brosimum* and was responsible for collecting the morphological data including reviewing herbarium specimens and assembling the character matrix. NJCZ initiated the project, provided overall supervision, and acquired funding for ingroup sequencing. All authors commented on and contributed to the manuscript. — EMG, https://orcid.org/0000-0003-1133-5167; LA, https://orcid.org/0000-0003-0690-4476; QZ, https://orcid.org/0000-0003-4927-1867; HS, https://orcid.org/0000-0001-8305-3236; AKM, https://orcid.org/0000-0003-4013-3804; NJCZ, https://orcid.org/0000-0003-1132-4943

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Appendix 1. Accessions used for the phylogenetic analyses, listing taxon, geographic origin, voucher information, number of loci recovered, and GenBank SRA accession number. Asterisks denote samples newly sequenced for this study.

Allaeanthus luzonicus (Blanco) Fern.-Vill., Philippines, 2012, *K.F. Chung 2016* (HAST), 283, SRR13092433*. ***Antiaropsis decipiens*** K.Schum., Papua New Guinea, 2003, *Zerega & al. 281* (CHIC), 273, SRR3907583. ***Artocarpus heterophyllus*** Lam., Malaysia, Borneo (cult.), 2014, *Gardner & al. 98* (F), 330, SRR3907497. ***Bosqueiopsis gilletii*** De Wild. & T.Durand, Mozambique, 2009, *Timberlake 5767* (P), 286, SRR13188663. ***Brosimum acutifolium*** subsp. ***obovatum*** (Ducke) C.C.Berg, Bolivia, 1992, *Killeen 4457* (F), 278, SRR12282976*. ***Brosimum alicastrum*** Sw. subsp. ***alicastrum***, U.S.A. (Florida), 2013, *Gardner 23* (F), 309, SRR12283030*. ***Brosimum alicastrum*** subsp. ***bolivarense*** (Pittier) C.C.Berg, Panama, 1905, *G.P. Cooper 441* (F), 311, SRR12282975*. ***Brosimum amazonicum*** (Poepp. & Endl.) E.M.Gardner & Zerega, Brazil, 2002, *G. Weiblen 1522* (F), 243, SRR13089487*. ***Brosimum costaricanum*** Liebm., Costa Rica, 1988, *C. Kernan 27* (F), 317, SRR12282973*. ***Brosimum gaudichaudii*** Trécul, Brazil, 2002, *Mendes & al. 332* (F), 249, SRR12283029*. ***Brosimum glaucum*** Taub., Brazil, 1981, *Mori & al. 13882* (NY), 250, SRR12282977*. ***Brosimum guianense*** (Aubl.) Huber ex Ducke, Suriname, 1963, *Irwin & al. 54583* (F), 302, SRR12282974*; Costa Rica, 1996, *Morales & Ureña 5258* (F), 300, SRR12282972*. ***Brosimum lactescens*** (S.Moore) C.C.Berg, Bolivia, 2008, *Moya & al. 138* (MO), 300, SRR13089488*; Costa Rica, 1994, *Rivera & al. 2204* (F), 236, SRR12282971*. ***Brosimum longifolium*** Ducke, Peru, 1987, *Vasquez & al. 4044* (F), 28, SRR12282970*. ***Brosimum multinervium*** C.C. Berg, Peru, 1997, *Vasquez & Chavez 25075* (F), 289, SRR12282969*. ***Brosimum oligandrum*** **(**Benoist) E.M. Gardner & Zerega, French Guiana, 1989, *W. Han 3649* (F), 296, SRR12282963*. ***Brosimum parinarioides*** subsp. ***ampticoma*** (Ducke) C.C.Berg, Peru, 1983, *Vasquez & Jaramillo 3817* (F), 301, SRR12282967*. ***Brosimum potabile*** Ducke, Brazil, 1989, *Maciel & Rosario 1552* (F), 284, SRR12282966*. ***Brosimum rubescens*** Taub., Panama, 2009, *McPherson 20944* (F), 312, SRR12282965*. ***Brosimum sprucei*** (Baill.) E.M.Gardner & Zerega, Brazil, 1977, *Prance & al. P 25416* (F), 279, SRR12282964*. ***Brosimum utile*** (Kunth) Oken, Panama, 2000, *Galdames 4403* (F), 301, SRR12283028*. ***Broussonetia papyrifera*** (L.) L'Hér. ex Vent., –, –, – (–), 249, SRR1477753. ***Dorstenia foetida*** Schweinf., Ethiopia, 1963, *Burger 2844* (F), 210, SRR13188662. ***Fatoua villosa*** (Thunb.) Nakai, U.S.A., Florida (cult.), 2013, *E. Gardner 27* (CHIC), 292, SRR13188661. ***Ficus macrophylla*** Pers., U.S.A., Florida (cult.), 2013, E. Gardner 30 (CHIC), 326, SRR3907044. ***Maclura pomifera*** (Raf.) C.K.Schneid., U.S.A., Illinois (cult.), 2014, *E. Gardner 139* (CHIC), 321, SRR3907028. ***Malaisia scandens*** (Lour.) Planch., Malaysia, Borneo, 2014, *E. Gardner 122* (F), 313, SRR12283025*. ***Parartocarpus venenosus*** (Zoll. & Moritzi) Becc., Malaysia, Borneo, 2013, *N. Zerega 874* (F), 228, SRR3907334. ***Paratrophis glabra*** (Merr.) Steenis, Malaysia, Borneo, 2013, *E. Gardner 78* (F), 326, SRR3907307. ***Scyphosyce pandurata*** Hutch., Cameroon, 1987, *Thomas 6869* (P), 297, SRR13188660. ***Sloetia elongata*** (Miq.) Koord., Malaysia, 1992, *Thomas s.n.* (P), 313, SRR13188659. ***Treculia africana*** Decne. ex Trécul, Malaysia, Borneo (cult.), 2013, *Zerega & al. 909* (F), 312, SRR13188658. ***Trema orientale*** (L.) Blume, –, –, – (–), 246, SRR5674478. ***Trilepisium madagascariense*** DC., Madagascar, 2013, *Ranktonirina 197* (P), 52, SRR13188657. ***Utsetela gabonensis*** Pellegr., Gabon, 1997, *Breteler 14096* (P), 265, SRR13188656.

Appendix 2. Accessions examined for morphological characters, listing taxon, geographic origin, and voucher information.

Brosimum acutifolium Huber subsp. ***acutifolium***, Brazil: *Irwin & Westra 47744* (K); French Guiana: Irwin, *Pires & Westra 48433* (K); ***Brosimum acutifolium*** subsp. ***interjectum*** C.C.Berg, Brazil: *Ducke s.n. 'HJBR 19477'* (K); ***Brosimum acutifolium*** subsp. ***obovatum*** (Ducke) C.C.Berg, Brazil: *Ducke s.n. 'HJBR 23624'* (K), *Krukoff 5378* (K), *5645* (K); Guyana: *Smith 2617* (K). ***Brosimum alicastrum*** Sw. subsp. ***alicastrum***, Belize: *Lundell 492* (K), Peck 811 (K); Cuba: Ekman 16192b (K); El Salvador: *Tucker 952* (K); Grenadine Islands: *Beard 543* (K); Jamaica: *Prior 860* (K); Mexico: *Gaumer 23287* (K), *Hinton 5661* (K), *Palmer 471* (K), *Schiede & Deppe 1117* (K); Saint Vincent: *Smith & Smith 1755* (K); Trinidad: *Broadway 7267* (K), *Russell 12431* (K); ***Brosimum alicastrum*** subsp. ***bolivarense*** (Pittier) C.C.Berg, Colombia: *Agostini 91257* (K), *Triana 1861* (K); Ecuador: *Eggers 15721* (K), *Ule 9324* (K); Guyana: *Jenman 1541* (K); Panama: *Cooper 441* (K). ***Brosimum amazonicum*** (Poepp. & Endl.) E.M.Gardner & Zerega, Brazil: *Ducke s.n. 'HJBR 23973'* (K), *Spruce 1825* (K), *2859* (K), *Traill 709* (K); Peru: *Klug 2718* (K), *Kuhlmann s.n. 'HJBR 18262'* (K), *Spruce 3895* (K). ***Brosimum gaudichaudii*** Trécul, Bolivia: *Steinbach 6412* (K), *7233* (K); Brazil: *Burchell 1984* (K), *5131* (K), *5261* (K), *5851* (K), *6076* (K), *7377* (K), *Ducke s.n. (HJBR 35671)* (K), *Dusen 10564* (K), *Glaziou 11572* (K), *12170* (K), *16349* (K), *22149* (K), *Malme 1668* (K); Paraguay: *Hassler 4641* (K), *10558* (K). ***Brosimum glaucum*** Taub., Brazil: *Glaziou 15428* (K). ***Brosimum glaziovii*** Taub., Brazil: *Glaziou 8081* (K), *13496* (K). ***Brosimum guianense*** (Aubl.) Huber ex Ducke, Belize: *Gentle 2891* (K), *3144* (K), *Schipp 1094* (K), *1246* (K); Bolivia: *Krukoff 10769* (K), *11109* (K), *Steinbach 6658* (K); Brazil: *Ducke 316* (K), *Ducke s.n. 'HJBR 12511'* (K), *s.n. 'HJBR 18279'* (K), *Froes 21447* (K),

Appendix 2. Continued.

Glaziou 1158 (K), *2704* (K), *5990* (K), *20487* (K), *Krukoff 1681* (K), *5198* (K), *5477* (K), *6385* (K), *8179* (K), *8546* (K), *Mexia 5314* (K), *Siguiera s.n. (HAMP 4066)* (K); French Guiana: *Klug 2562* (K); Guyana: *Anderson s.n. 'FD 308'* (K), *s.n. 'FD 406'* (K), *Fanshawe 630* (K), *688* (K), *733* (K), *739* (K), *2238* (K), *2280* (K), *Gleason 346* (K), *Hohenkerk s.n. 'FD 467'* (K), *Maguire & Fanshawe 23490* (K), *Schomburgk 549* (K), *Smith 3623* (K); Mexico: *Williams 9391* (K); Panama: *Pittier 4336* (K); Suriname: *Maguire 24358* (K). ***Brosimum lactescens*** (S.Moore) C.C.Berg, Belize: *Gentle 1737* (K), *Schipp 522* (K); Brazil: *Ducke s.n. 'HJBR 12720'* (K), *Krukoff 5377* (K), *Kuhlmann s.n. 'HJBR 19808'* (K), *Moore 366* (K), *Prance*, *Pena & Ramos 8293* (K); Guyana: *Forest Dept. 6184* (K), *Smith 2876* (K); Mexico: *Williams 8350* (K). ***Brosimum longifolium*** Ducke, Brazil: *Ducke 1457* (K), *s.n. 'HJBR 23621'* (K). ***Brosimum oligandrum*** (Benoist) E.M. Gardner & Zerega, French Guiana: *Martin s.n.* (K); Suriname: *Lanjouw & Lindeman 2297* (K). ***Brosimum parinarioides*** Ducke, Brazil: *Ducke s.n. 'HJBR 23921'* (K), *s.n. 'HJBR 23922'* (K), *s.n. 'HJBR 12517'* (K), *s.n. 'HJBR 12521'* (K); Guyana: *Smith 2901* (K). ***Brosimum potabile*** Ducke, Brazil: *Ducke 1159* (K), *Krukoff 6685* (K), ***Brosimum rubescens*** Taub., Brazil: *Ducke 1794* (K), *1916* (K), *s.n. 'HJBR 12536, HAMP 16594'* (K), *s.n. 'HJBR 12569, HAMP 16991'* (K), *s.n. 'HJBR 18276'* (K), *s.n. 'HJBR 23612'* (K), *s.n. 'HJBR 23622'* (K), *Glaziou 12169* (K), *Krukoff 1384* (K), *7181* (K), *7986* (K); Guyana: *Fanshawe 980* (K), *1788* (K), *2296* (K); Peru: *Fox 93* (K), Suriname: *Lanjouw & Lindeman 2411* (K). ***Brosimum sprucei*** (Baill.) E.M.Gardner & Zerega, Brazil: *Ducke 71* (K), *s.n. 'HJBR 18284'* (K), *s.n. 'HJBR 23613'* (K), *s.n. 'HJBR 23971'* (K), *s.n. 'HJBR 23972'* (K), *Spruce 2097* (K), *2219* (K), *2242* (K), *3375* (K); Colombia: *Mutis 5360* (K). ***Brosimum utile*** subsp. ***darienense*** C.C.Berg, Panama: *Dawe 879* (K); ***Brosimum utile*** subsp. ***magdalenense*** C.C.Berg, Colombia: *Lawrence 765* (K), *Scultes & Villareal 5320* (K); ***Brosimum utile*** subsp. ***occidentale*** C.C.Berg, Ecuador: *Little 6212* (K), *6213* (K), *6245* (K), *6247* (K); **subsp. *ovatifolium*** (Ducke) C.C.Berg, Brazil: *Ducke 367* (K), *1451* (K), *Krukoff 6656* (K); Venezuela: *Spruce 3297* (K); ***Brosimum utile*** (Kunth) Oken subsp. ***utile***, Venezuela: *Fendler 2018* (K), *Porter s.n.* (K).