Brunfelsia (Solanaceae): A genus evenly divided between South America and radiations on Cuba and other Antillean islands

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A B S T R A C T

Hallucinogenic or toxic species of *Brunfelsia* (Solanaceae: Petunieae) are important in native cultures throughout South America, and the genus also contains several horticulturally important species. An earlier morphological revision of the c. 50 species recognized three main groups, one consisting of the 23 Antillean species, another of southern South American and Andean species, and a third of species from the Amazon Basin and Guiana Shield. Based on plastid and nuclear DNA sequences from up to 65 accessions representing 80% of the species, we generated a phylogeny and a calibrated chronogram for *Brunfelsia* to infer clade expansion and shifts in pollinators and fruit types. *Brunfelsia* flowers offer nectar, and attract lepidoptera, hummingbirds, or bees; the fruits are dry or fleshy. Our results imply that *Brunfelsia* is 16–21 Myr old and entered the Antilles from South America early during its history, with subsequent expansion along the island arc. The ancestor of the Antillean clade was hawk-moth-pollinated and had fleshy capsules, perhaps facilitating dispersal by birds. The only shift to hummingbird pollination occurred on Cuba, which also harbors the largest single radiation, with 11 species (10 included in our study) that apparently arose over the past 4 Myr. Jamaica, Hispaniola, and Puerto Rico each sustained smaller radiations. The data also reveal at least one new species.

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1. Introduction

The medium-sized family Solanaceae, with ca. 2500 species in 100 genera, most of them in the Neotropics, is well known for its many biologically active alkaloids and saponins. Among the most important hallucinogenic or toxic plants used by indigenous groups throughout South America are species in the genus *Brunfelsia* L. (Schultes, 1979; Bennett, 1992; Plowman, 1998; Kloucek et al., 2005; Singh et al., 2008), and a saponin from *B. grandiflora* has potent leishmanicidal activity (Fuchino et al., 2008). *Brunfelsia* also has horticultural importance, with species being sold under the names "lady of the night" or "yesterday–today–tomorrow" for their nocturnally scented and color-changing flowers, which during anthesis turn from dark purple over mauve to white (Fig. 1E). The decrease in anthocyanin concentration in these flowers is extremely rapid and occurs at a specific and well-defined stage (Vaknin et al., 2005). In total, *Brunfelsia* comprises some 50 species that occur between Panama and northernmost Uruguay and on the Greater and Lesser Antilles. Most are medium-sized shrubs. Molecular data place the genus in the tribe Petunieae (Olmstead et al., 2008), which besides *Petunia* L. (14 species)

includes *Bouchetia* Dunal (3 species), *Calibrachoa* La Llave & Lex (24 species), *Fabiana* Ruiz & Pav. (15 species), *Hunzikeria* D'Arcy (3 species), *Leptoglossis* Benth. (7 spp.), *Nierembergia* Ruiz & Pav. (20 species), and *Plowmania* Hunz. & Subilis, a monotypic genus.

Brunfelsia has two centers of species diversity. One is in South America, especially south-central and eastern Brazil, where about half the species are native. The other is in the Antilles, with 23 species overall, distributed in Cuba, Jamaica, Hispaniola, and Puerto Rico. The *Flora de Cuba* lists 13 species, two cultivated and 11 endemic (León and Alain, 1974). Jamaica has six endemic species (Adams, 1972), Hispaniola two endemics and two widespread species (Liogier, 1994), Puerto Rico three endemics and the same two widespread species (Liogier, 1995), and the Lesser Antilles are thought to share a single species from St. Lucia to Hispaniola (Plowman, 1979). Most Antillean brunfelsias occur in specialized habitats, such as serpentine outcrops in Puerto Rico (*B. densifolia*), limestone at sea level in northern Jamaica (*B. undulata*), or cloud forest in the Blue Mountains of eastern Jamaica (*B. jamaicensis*).

Besides the Antillean species group (section *Brunfelsia*), a revision of the genus in South America recognized two other sections differing in flower morphology, *Franciscea* with 24 species in the eastern Andes and southeastern Brazil and *Guianenses* with six species in the Amazon basin and the Guayana region (Plowman, 1978, 1979, 1998). The three species occurring in coastal Ecuador, the Pacific Colombian Chocó region, and Panama have different flower

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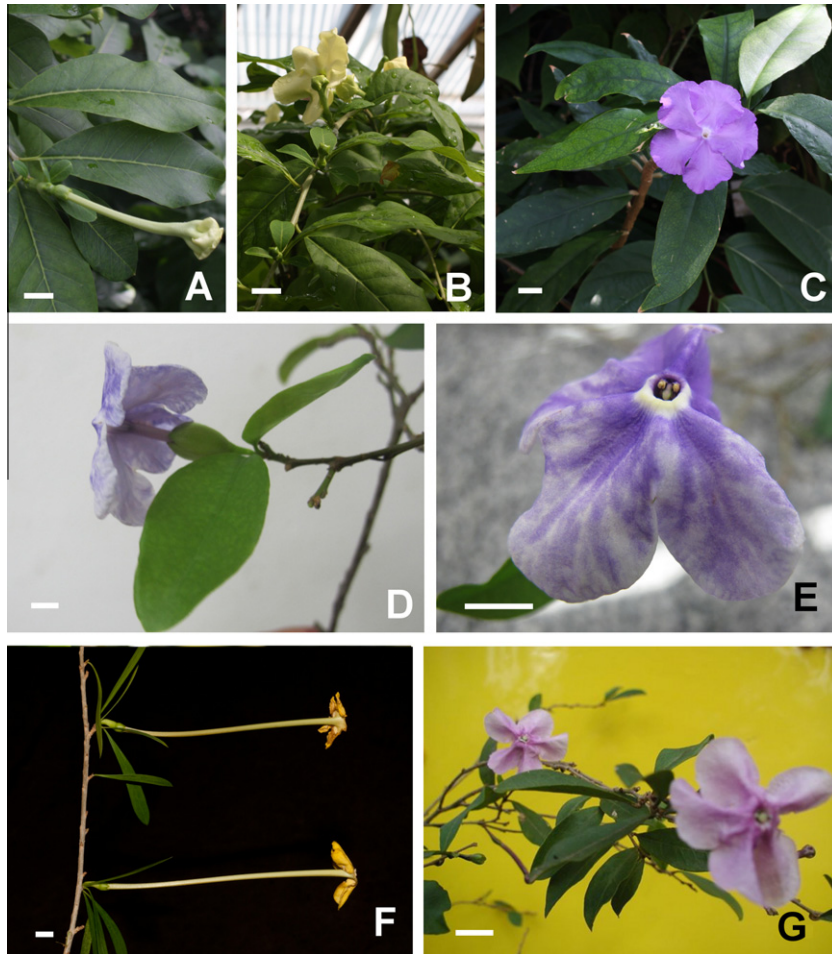*E-mail address:* nata@gumed.edu.pl (N. Filipowicz).

Fig. 1. Flowers of *Brunfelsia*. The scale bar corresponds to 1 cm. (A) *B. americana* bud, (B) open flower, Lesser Antilles; (C) *B. pauciflora* 1st-day flower, coastal lowland forest from Espiritu Santo to Santa Catarina; (D and E) *B. uniflora* 2nd or 3rd day flowers changing color from violet to pale lavender; eastern coast of Brazil; (F) *B. densifolia* endemic to Puerto Rico; (G) *B. cestroides* endemic to Cuba. Photographs by: N. Filipowicz (A–C), R. Teixeira de Queiroz (D and E), M. Nee (F), V. Fuentes (G). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

shapes and were therefore partly assigned to section *Franciscea*, partly to section *Guianenses.* The typical *Brunfelsia* habitat in South America is lowland rain forest, but a few species grow in riverine habitats, *Araucaria* forests, or the Brazilian shrub savanna "cerrado" (Plowman, 1998). Based on fieldwork and hundreds of herbarium specimens, Plowman (1979, 1998) hypothesized that the range of *Brunfelsia* in South America was once much wider and that climatic deterioration led to the disjunctions in the genus range seen today (our Fig. 2, inset B). He also thought that *Brunfelsia* arrived from South America to the Lesser Antilles and did not re-disperse to South or Central America. These hypotheses are now testable with a dated molecular phylogeny.

Besides Plowman's explicit hypotheses about the geographic history of *Brunfelsia*, his classification indirectly points to a role of flower size and morphology, and hence pollinator shifts, in the evolution of the genus. This is apparent from Plowman's use of flower structure as a sectional character, while fruit morphology was judged too labile for grouping species. The capsular fruits of *Brunfelsia* can be dehiscent or indehiscent, thin-walled or thick-walled, fleshy or dry, with brown or orange colors (Plowman, 1998). The flowers are salveriform and differ in the relative proportions of corolla tube length and calyx size (Fig. 1A, D, and F). Most South American species have flowers with narrow, medium-length tubes with a circular nectar guide near the tube opening (Fig. 1C, and E), diurnal anthesis, and petals that change color during anthesis. Most Antillean species, by contrast, have long-tubed flowers

that are pale colored, open at night, and are strongly scented (Fig. 1A, B, and F; Cocucci, 1995; Plowman, 1998). Two Cuban species, however, have purplish flowers with medium-length corolla tubes (Fig. 1G; Plowman, 1998).

We here use plastid and nuclear DNA sequences for some 80% of the *Brunfelsia* species (sampling multiple populations for widespread species), molecular clock dating, and ancestral trait and area reconstruction to (i) infer species relationships in *Brunfelsia*, (ii) date the biogeographical events that led to the group's disjunct distribution, and (iii) infer shifts in pollination and fruit type in a biogeographic context. *Brunfelsia* is among the few genera that have almost as many species in the Antilles as in South America (see Francisco-Ortega et al., 2007), making it an interesting system for addressing questions about the time and direction of clade expansion into or out of the Antilles or among islands. Given the group's distribution and Plowman's work (1998), our expectation was that the Antillean clade would be nested inside one of the two South American sections, *Franciscea* or *Guianenses*.

2. Materials and methods

2.1. Taxon sampling

Table 1 provides a list of the material used, with species and author names, voucher information, geographic origin, and
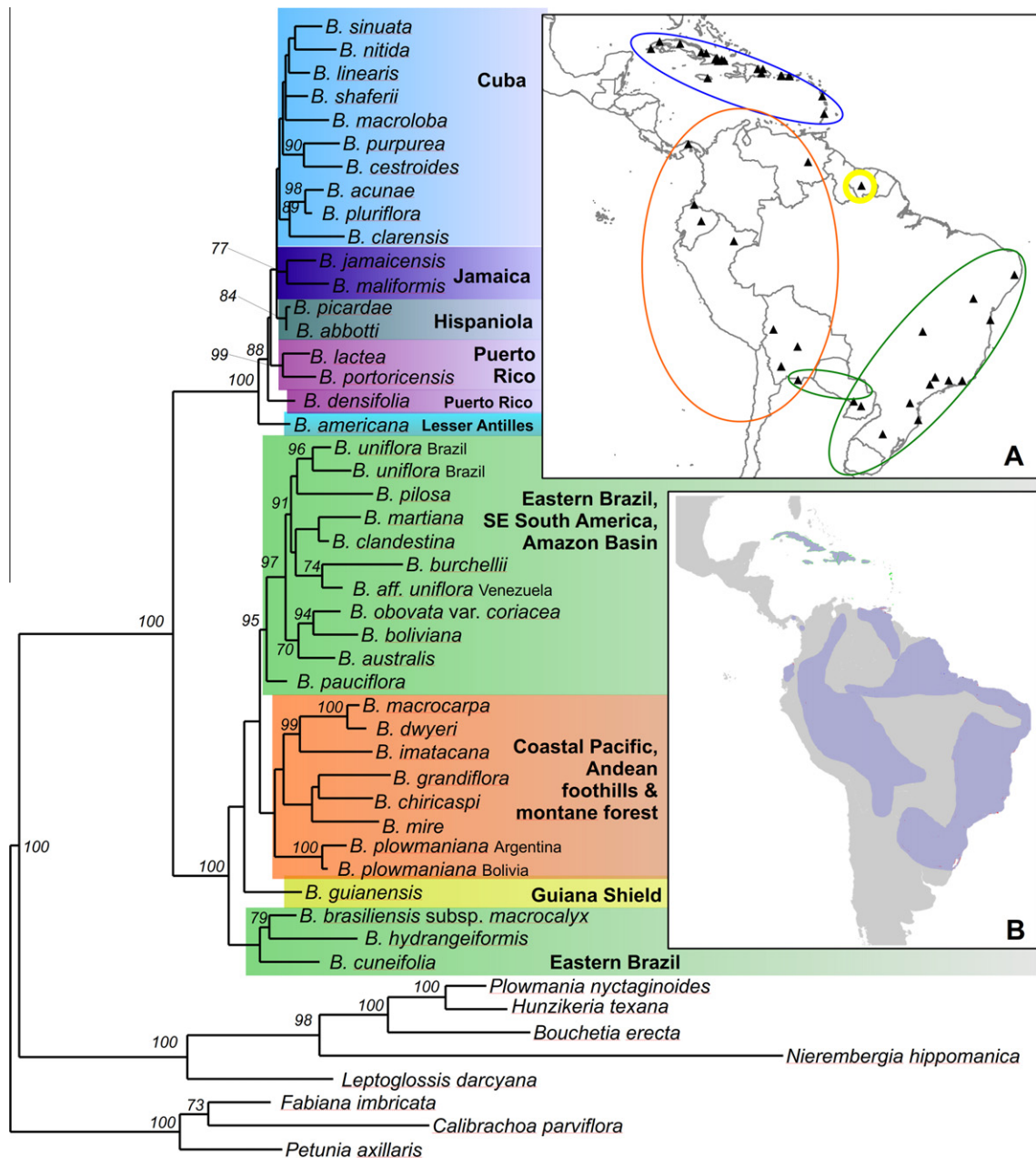
Fig. 2. Maximum likelihood phylogram for *Brunfelsia* based on combined plastid and nuclear sequences (3754 aligned nucleotides) and rooted based on the results of Olmstead et al. (2008). Numbers above branches refer to ML bootstrap support ⩾70%. (A) Map showing the geographic origin of the sequenced plants with the main clades color-coded: blue stands for Antilles, orange for Andean, green for Brazil, SE South America and Amazon Basin, yellow for Guyana Shield; (B) distribution map based on Plowman's revision (1998). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

GenBank accession numbers for all sequences. A total of 159 sequences were generated for this study. Material came mostly from specimens deposited in the herbaria B, GH, HAC, JE, LPB, M, MO, NYBG, PR, ROIG and S (herbarium acronyms follow Thiers, 2011). Of the ingroup *Brunfelsia*, we included 18 of the 23 Antillean species and 19 of the 24 South American species described so far. Specifically, we sampled 10 of the 11 Cuban species (*B. grisebachii* is missing), two of the six Jamaica species (missing are *B. splendida, B. plicata, B. membranacea* and *B. undulata*) and all endemic species from Puerto Rico and Hispaniola. As outgroups we included representatives of *Bouchetia, Calibrachoa, Fabiana, Hunzikeria, Leptoglossis, Nierembergia, Petunia*, and *Plowmania*, that is, all genera of Petunieae (Olmstead et al., 2008). The oldest herbarium collections that yielded useable DNA were collections made by Martius 1817–1820 and Regnell (c. 1865).

2.2. DNA isolation, amplification, sequencing, and alignment

Total genomic DNA was isolated from herbarium specimens and silica-dried leaves using the NucleoSpin plant kit (Macherey–Nagel, Düren, Germany). DNA amplification, fragment purification and cycle sequencing were applied following protocols described in Kocyan et al. (2007). The plastid *trnL* intron and *trnL–F* intergenic spacer were amplified using the primers of Taberlet et al. (2012) and the *ndhF* region with the primers of Olmstead and Sweere (1994). The nuclear ribosomal DNA internal transcribed spacers ITS1 and ITS2, together with intervening 5.8S gene, were amplified using the Balthazar et al. primers (2000). Where ITS sequences had ambiguous base calls, cloning was conducted using pGEM-T Easy Vector System (Promega, USA) and DH5α competent cells (Invitrogen Corporation, USA), following suppliers' protocols.

Table 1
List of plant material included in this study, with species authors, vouchers and their geographic origin, and GenBank accession numbers for all sequences. Herbarium acronyms follow Thiers (2011).

Species	Voucher	Geographic origin	GenBank accession		
			trnL intron, *trnL–F* spacer	*ndhF*	*ITS1, 5.8S rRNA, ITS2*
Brunfelsia abbotti Leonard	E. Santiago-Valentín s.n.	Greater Antilles: Hispaniola, Samana Penisula	JQ081071	JQ081124	–
Brunfelsia acunae Hadač	E. Hadač 1567 (PR)	Greater Antilles: Cuba oriental	JQ081072	JQ081125	JQ081177
Brunfelsia americana L.	C. Whiteford 7319 (M)	Lesser Antilles: Dominica	JQ081073	JQ081126	JQ081178
Brunfelsia americana L.	D.C. Wasshausen & E.S. Ayensu 384 (B)	Lesser Antilles: Dominica, St. John	–	JQ081128	JQ081179
Brunfelsia americana L.	T. Plowman 3370 (M)	Lesser Antilles: St. Vincent	–	JQ081127	–
Brunfelsia australis Benth.	A. Bornmüller 219 (M)	Brazil: Rio Grande do Sul	JQ081074	JQ081129	JQ081180
Brunfelsia australis Benth.	E. Zardini & E. Velazquez 19183 (B)	Paraguay	JQ081075	JQ081130	JQ081181
Brunfelsia boliviana Plowman	S. Beck 9379 (LPB)	Bolivia: Chuquisaca, Monteagudo	JQ081076	JQ081131	JQ081182
Brunfelsia brasiliensis subsp. *brasiliensis* (Spreng.) L.B. Sm. & Downs	A.F. Regnell I375 (M)	Brazil	JQ081077	JQ081132	JQ081183
Brunfelsia brasiliensis subsp. *macrocalyx* (Dusén) Plowman	T. Plowman 2891 & D. Sucre 5191 (M)	Brazil: Rio de Janeiro state	JQ081078	JQ081133	JQ081184
Brunfelsia burchellii Plowman	M.R.C. Sales 74 (MO)	Brazil: Pernambuco	JQ081079	JQ081134	–
Brunfelsia cestroides A. Rich.	W. Greuter et al. 25553 (B)	Greater Antilles: Cuba, Pinar del Rio	JQ081080	JQ081135	JQ081185
Brunfelsia cestroides A. Rich.	V. Fuentes s.n. (HAC 452581)	Greater Antilles: Cuba, cult.	JQ081081	JQ081136	JQ081186
Brunfelsia cestroides Griseb.	V. Fuentes s.n. (ROIG 4842)	Greater Antilles: Cuba, cult.	JQ081114	JQ081167	JQ081219
Brunfelsia chiricaspi Plowman	C.E. Ceron 995 (MO)	Ecuador	JQ081082	JQ081137	JQ081187
Brunfelsia clandestina Plowman	T. Plowman et al. 10087 (MO)	Brazil: Bahia	JQ081083	JQ081138	JQ081188
Brunfelsia clarensis Britt. & Wils.	B. Leon & F. R. Casanás 5928 (NY)	Greater Antilles: Cuba, Mordazo	JQ081084	JQ081139	JQ081189
Brunfelsia cuneifolia J.A. Schmidt	M. Kummrow 2432 (MO)	Brazil: Parana, Serra do Puruna	JQ081085	–	JQ081190
Brunfelsia densifolia Krug & Urb.	T. Plowman 3365 (M)	Greater Antilles: Puerto Rico	JQ081086	JQ081140	JQ081191
Brunfelsia densifolia Krug & Urb.	R. Olmstead ID 380 E unvouchered	Greater Antilles: Puerto Rico	JQ081087	JQ081141	JQ081192
Brunfelsia dwyeri D'Arcy	C. Galdames 6641 (MSB)	Panama: Cerro Jeffe	JQ081088	JQ081142	JQ081193
Brunfelsia guianensis Benth. DC.	B. Hammel et al. 21244 (MO)	Suriname	JQ081089	–	JQ081194
Brunfelsia *guianensis* Benth. DC.	S.A. Mori et al. 27377 (NY)	Guyane	JQ081090	JQ081143	JQ081195
Brunfelsia guianensis Benth. DC.	S.A. Mori et al. 22207 (NY)	Guyane	JQ081091	JQ081144	–
Brunfelsia grandiflora D. Don.	J. Revilla 2693 (MO)	Peru: Loreto	JQ081092	JQ081145	JQ081196
Brunfelsia hydrangeiformis (Pohl) Benth.	W.W. Thomas et al. 11207 (MO)	Brazil: Bahia	JQ081093	JQ081146	JQ081197
Brunfelsia hydrangeiformis subsp. *capitata* (Benth.) Plowman	P. von Luetzelburg 6495 (M)	Brazil: Rio de Janeiro, Serra dos Orgaos	JQ081094	JQ081147	JQ081198
Brunfelsia imatacana Plowman	A. Gentry & P. Berry 15034 (MO)	Venezuela: Bolívar	JQ081095	JQ081148	JQ081199
Brunfelsia jamaicensis (Benth.) Griseb.	T. Plowman 2970 (GH)	Greater Antilles: Jamaica, Blue Mountains	JQ081096	JQ081149	JQ081200
Brunfelsia jamaicensis (Benth.) Griseb.	Matthaei Botanical Garden, University of Michigan, unvouchered	Greater Antilles: Jamaica	JQ081097	JQ081150	JQ081201
Brunfelsia lactea Krug & Urb.	L. Struwe & C. Specht 1143 (NY)	Cult.	JQ081098	JQ081151	JQ081202
Brunfelsia lactea Krug & Urb.	E. Santiago-Valentín ESV1 unvouchered	Greater Antilles: Puerto Rico	JQ081099	JQ081152	JQ081203
Brunfelsia linearis Ekman	E.L. Ekman 7394 (S)	Greater Antilles: Cuba oriental	JQ081100	JQ081153	JQ081204
Brunfelsia macrocarpa Plowman	C. Aulestia et al. 526 (MO)	Ecuador: Esmeraldas	JQ081101	JQ081154	JQ081205
Brunfelsia macroloba Urb.	Fuentes s.n. (ROIG 4841)	Greater Antilles: Cuba, cult.	JQ081102	JQ081155	JQ081206
Brunfelsia maliformis Urb.	C.D. Adams 6414 (M)	Greater Antilles: Jamaica, St. Andrew	JQ081103	JQ081156	JQ081207
Brunfelsia martiana Plowman	C.F.P. von Martius s.n. (M)	Brazil: Bahia	JQ081104	JQ081157	JQ081208
Brunfelsia mire Monach.	J.C. Salomon 17671 (MO)	Bolivia	JQ081105	JQ081158	JQ081209
Brunfelsia nitida Benth.	V. Fuentes s.n. (HAC 42584)	Greater Antilles: Cuba, cult.	JQ081106	JQ081159	JQ081210
Brunfelsia nitida Benth.	A. Alvarez de Zayas et al. s.n. (B, HFC43519)	Greater Antilles: Cuba, Pinar del Rio	–	JQ081160	JQ081211
Brunfelsia obovata var. *coriacea* J.A. Schmidt	P. von Luetzelburg 4019 (M)	Brazil: Jatoba	JQ081107	JQ081161	JQ081212
Brunfelsia pauciflora (Cham. & Schltdl.) Benth.	S.S. Renner & N. Filipowicz 2836 (M)	Cultivated in Botanical Garden in Munich	JQ081108	JQ081162	JQ081213
Brunfelsia picardae Krug. & Urb.	R. Bastardo et al. 177 (B)	Greater Antilles: Hispaniola, Duarte	JQ081109	JQ081163	JQ081214
Brunfelsia pilosa Plowman	E. Zardini 14783 (MO)	Paraguay	JQ081110	–	JQ081215
Brunfelsia plowmaniana N. Filipowicz & M. Nee	L.J. Novara 7124 (B)	Argentina: De Salta	JQ081121	JQ081174	JQ081226
Brunfelsia plowmaniana N. Filipowicz & M. Nee	L. Arroyo et al. 3569 (M)	Bolivia: Santa Cruz, Manuel Maria Caballero	JQ081122	JQ081175	JQ081227
Brunfelsia pluriflora Urb.	J. Bisse et al. s.n. (B, HFC 44802)	Greater Antilles: Cuba, Holguin	JQ081111	JQ081164	JQ081216
Brunfelsia portoricensis Krug & Urb.	R. Olmstead, extracted 28 June 1995, unvouchered	Greater Antilles: Puerto Rico, Bosque Estatal De Guajataca	JQ081112	JQ081165	JQ081217
Brunfelsia purpurea Griseb.	A. Alvarez et al. s.n. (J 27168)	Greater Antilles: Cuba, Holguin, Baracoa	JQ081113	JQ081166	JQ081218
Brunfelsia Shaferii Britt. & Wils.	J. Bisse & E. Köhler s.n. (JE 007796)	Greater Antilles: Cuba, Holguin, Sierra de Nipe	JQ081115	JQ081168	JQ081220
Brunfelsia sinuata A. Rich.	A. Alvarez de Zayas et al. s.n. (B, HFC 43794)	Greater Antilles: Cuba, Camaguey, Sierra de Cubitas	JQ081116	JQ081169	JQ081221
Brunfelsia sinuata A. Rich.	W. Bonet et al. 25275 (B)	Greater Antilles: Cuba, Las Tunas	JQ081117	JQ081170	JQ081222
Brunfelsia sinuata A. Rich.	V. Fuentes 372 (HAC 42580)	Greater Antilles: Cuba, Las Tunas	JQ081118	JQ081171	JQ081223
Brunfelsia uniflora (Pohl) D. Don.	A.F. Regnell I374 (M)	Brazil: Minas Gerais, Caldas	JQ081119	JQ081172	JQ081224
Brunfelsia uniflora (Pohl) D. Don.	R.T. Queiroz 1364 (M)	Brazil: São Paulo, Campinas	JQ081120	JQ081173	JQ081225
Brunfelsia aff. *uniflora*	T. Plowman & L. Aristeguieta 1911 (GH)	Venezuela: Guarico, Paso Real	JQ081123	JQ081176	JQ081228

Six cloned accessions each contained several ITS haplotypes, but these differed only in single nucleotide substitutions and always clustered together in phylogenetic trees. We therefore used the g2cef software to compute consensus sequences, based on the majority state of the clones (Göker and Grimm, 2008). For all non-cloned sequences, forward and reverse strands were sequenced and consensus sequences generated with the Sequencher software (version 4.1.2, GeneCodes Corp.). Sequence alignment relied on MAFFT (Katoh et al., 2002), followed by minor manual adjustments in Mesquite version 2.71 (Maddison and Maddison, 2010).

2.3. Phylogenetic analyses

Maximum likelihood (ML) tree searches and bootstrapping (using 100 replicates) relied on RAxML version 7.0.4 (Stamatakis et al., 2008) with the GTR + Γ model and 25 rate categories. Nuclear ITS and plastid data sets were analyzed independently and produced congruent phylogenetic estimates, with all areas of discordance being restricted to branches with low support (i.e., bootstrap proportions <70%). The data sets were therefore concatenated, yielding a matrix of 65 plant accessions. Since our coding and non-coding sequences were relatively short, we did not partition the data to avoid overparametrization, which can decrease precision in estimates of branch lengths (Cunningham et al., 1998). For molecular clock dating, ancestral area reconstructions, and trait inference reduced matrices were used as described below.

2.4. Molecular clock analyses

Calibration of Solanaceae genetic distances is difficult because of the family's poor fossil record (Collinson et al., 1993). To estimate the crown age of *Brunfelsia* we used three approaches, aiming at cross validation. Our first calibration point was the 38 (26–49 SD) Myr age inferred for the *Petunia/Duckeodendron* split by Bell et al. (2010, their node 14 in Fig. S11). Next, we instead used different ITS substitution rates for woody species from the survey of Kay et al. (2006) to transform ITS distances in *Brunfelsia* into absolute time. Thirdly, we used an average substitution rate for angiosperm plastid DNA of 0.7 substitutions/site/year $\times$ 10^{-9} (Palmer, 1991) to transform *ndhF* and *trnL–trnF* genetic distances into absolute time. Dating relied on BEAST version 1.6.1 (Drummond and Rambaut, 2007), using strict and relaxed clock models. All runs employed a Yule tree prior and the GTR + Γ model with four rate categories. Markov chain Monte Carlo (MCMC) chains were run for 10 million generations, sampling every 1000th generation. Of posterior trees, we left out the first c. 20% as burn-in and then checked convergence using Tracer version 1.4.1 (Rambaut and Drummond, 2007); effective sample sizes were always well above 300. Maximum clade credibility trees were edited in FigTree version 1.3.1 (A. Rambaut; http://tree.bio.ed.ac.uk/software/figtree/).

2.5. Ancestral area reconstruction

To infer ancestral areas we used two approaches. One was the Continuous Time Monte Carlo (CTMC) model of Lemey et al. (2009), which assumes that ancestral populations have a relatively small range and that dispersal is the dominant process explaining species distribution. This model fits *Brunfelsia* well because under a vicariance assumption, the genus would initially have inhabited all Antillean islands plus South America, which is geologically nonsensical. For the CTMC analysis, carried out in BEAST, we used a reduced matrix of 36 accessions (taking out near-identical sequences to reduce rate heterogeneity from zero-length branches), a Yule tree prior, and the GTR + Γ model with four rate categories. MCMC chains were run for 10 million generations, sampling every

1000th generation. The other approach to biogeographic reconstruction was an S-DIVA analysis as implemented in *Reconstruct Ancestral States in Phylogenies* (RASP 1.107; Yu et al., 2010). The default assumption under this model is vicariance, which makes it appropriate mostly for South American *Brunfelsia*. The input file for RASP consisted of 10,000 post-burn-in trees obtained from BEAST for the 36-accession matrix. Biogeographic regions followed Antonelli et al. (2009) and Givnish et al. (2011), with minor *Brunfelsia*-specific modification (Plowman, 1979, 1998): (A) Antilles, (B) Eastern Brazil, southeastern South America and Amazon Basin, (C) Coastal Pacific, (D) Andean foothills and montane forest, (E) Guiana Shield, and (F) ambiguous (mostly for poorly sampled outgroup genera). For the tips representing the outgroup genera (*Calibrachoa, Nierembergia, Petunia, Fabiana, Leptoglossis*), we scored the distribution of the entire genus, where possible taking into account centers of distribution and diversification. The micro-genera with scattered distribution (*Plowmania, Hunzikeria* and *Bouchetia*) were coded as "ambiguous." All codings are shown in Table 2.

2.6. Ancestral state reconstruction of pollination modes and fruit types

To infer evolutionary shifts in pollination mode and fruit type, we used a ML tree obtained with RAxML from a reduced data set of 47 accessions, appropriately representing all species. The pollination trait had five states: (0) hawk-moth (Sphingidae), (1) butterfly (diurnal), (2) bee, (3) hummingbird, (4) settling moths (nocturnal), and (5) ambiguous, and species were scored as shown in Table 2 based on published studies, personal communications from colleagues, and flower traits as visible in herbarium specimens or mentioned on specimen labels. *Petunia* was coded as bee pollinated, the ancestral state for the genus (Gübitz et al., 2009). All Petunieae have capsules (Knapp, 2001), which in *Brunfelsia* differ in pericarp texture and color. We coded capsule types as (0) dry, (1) fleshy, (2) ambiguous. Ancestral states at nodes of interest (that had statistical support) were inferred in Mesquite version 2.73 (Maddison and Maddison, 2010) using the Markov k-state one-parameter model. We let Mesquite estimate the transition parameters of the model, using either a phylogram or an ultrametric version of the ML tree.

3. Results

3.1. Phylogenetic relationships in Brunfelsia

The nuclear matrix comprised 56 accessions and 708 aligned nucleotides, the plastid matrix 65 accessions and 3060 nucleotides, and the concatenated matrix 3754 aligned nucleotides and up to 65 accessions (for full tree see TreeBase under acc. No. 12245). The maximum likelihood trees obtained from the nuclear or plastid data (Fig. 2; OSM Fig. S1) confirm the monophyly of *Brunfelsia* and reveal two well-supported clades (ML bootstrap values of 100%) consisting, respectively, of the South American and the Antillean species. Neither of the two South American sections recognized by Plowman is recovered. Instead, *B. martiana*, *B. clandestina*, and *B. burchellii*, which were part of his section Guianenses, group far from *B. guianensis*, the type of this section (Fig. 2). The remaining South American brunfelsias fall into two clades, one of seven species from the eastern slopes of the Andes, coastal Ecuador, Panama, and southeastern Venezuela, the other with ten species from coastal Venezuela, Brazil, and Bolivia.

Cuba, Hispaniola, Jamaica, and Puerto Rico each harbor endemic clades of *Brunfelsia*, but the direction of spread throughout the island chain cannot be inferred with confidence (Fig. 2). The heterogeneous and supposedly widespread South American species *B. uniflora* turns out to be polyphyletic, with two accessions from Bolivia and Argentina (representing a new species, *B. plowmaniana*

Table 2

Brunfelsia floral traits, pollinators, fruit types, geographic ranges, and habitats. Pollinators were coded as follows: 0 = hawk-moths, 1 = butterflies, 2 = bees, 3 = hummingbirds, 4 = settling moths, 5 = ambiguous. Fruit types are coded as follows: 0 = dry capsule, 1 = fleshy capsule, 2 = ambiguous. Area codes for both ancestral area analyses (S-DIVA and CTMC) were: (A) Antilles, (B) Eastern Brazil, Southeastern South America and Amazon Basin, (C) Coastal Pacific, (D) Andean foothills and montane forest, (E) Guiana Shield, (F) ambiguous.

Species	Flower traits	Pollinator	Type of capsule	Range and habitat	Reference
B. acunae	Unknown	Unknown, 5	Dry, 0	Cuba: Hologuin A	Hadač (1970)
B. americana	White or cream-like, nocturnal anthesis, fragrant[c]	Hawk-moth, 0	Fleshy, yellow, 1	Smaller Antilles reaching Hispaniola A	Plowman (1998), Cocucci (1995)
B. australis	Showy, violet fading to white with age, fragrant[a]	Butterfly, 1	Dry, indehiscent, 0	Brazil, Paraguay, Argentina, Uruguay; Low elevation forests, near the rivers B	Plowman (1998)
B. boliviana	Light violet fading to white with age[a]	Butterfly, 1	Dry, 0	The eastern Andes of Southern Bolivia; forest and chaco vegetation up to 1200 m B	Plowman (1981)
B. brasiliensis	Violet fading to lavender/white, scentless[b]	Butterfly, 1	Dry, sparingly dehiscent, 0	Southeastern Brazil; subsp. brasiliensis: grassland, gallery forests; subsp. macrocalyx: wet montane cloud forests, high elevation grassland B	Plowman (1998)
B. burchelli	Unknown	Unknown, 5	Unknown, 2	Brazil: Tocantins and Maranhão; known only from few collections B	Plowman (1981)
B. cestroides	Violet[c]	Hummingbird, 3	Fleshy, 1	Cuba: Guantánamo, Pinar del Río, Artemisa, Holguín, Camagüey Coastal and serpentine moorlands A	León and Alain (1974), Plowman (1979); V. Fuentes, pers. comm., April 2010, April 2011
B. chiricaspi	Sky blue to violet fading white[b]	Butterfly, 1	Dry, 0	Colombia, Ecuador; Humid primary forest (300–500 m) C	Plowman (1973)
B. clandestina	White[a]	Settling moth, 4	Dry, 0	Brazil: Bahia, Espiritu Santo; Moist coastal forest B	Plowman (1981, 1998)
B. clarensis	Unknown	Unknown, 5	Fleshy, 1	Cuba: Villa Clara; A	León and Alain (1974)
B. cuneifolia	Violet fading to white[b]	Butterfly, 1	Unknown, 2	Southeastern Brazil; Araucaria and gallery forests, secondary forests (400 – 900 m) B	Plowman (1998)
B. densifolia	White fading yellow[c]	Hawk-moth, 0	Fleshy, orange, 1	Eastern Puerto Rico; Forests at high elevations A	Liogier (1994)
B. dwyeri	Purple fading to white[b]	Butterfly, 1	Dry, 0	Panama: Cerro Jefe; Cloud forests C	Plowman (1998)
B. guianensis	White[a]	Settling moth, 4	Fleshy, yellow, 1	Surinam, Guyane, Brazil; Lowland tropical forest E	Plowman (1998)
B. grandiflora	Violet fading to white, scentless[b]	Butterfly (Nymphalidae, Pieridae, Lycaenidae, Hesperidae), 1	Dry, 0	Eastern slopes of Andes (from Colombia to Bolivia); subsp. grandiflora humid montane rain forest (650–2000 m), subsp. schultesii primary and secondary forests (100–900 m) D	Plowman (1998)
B. hydrangeiformis	Purple fading to white, fragrant in the evening[b]	Butterfly, 1	Dry, sparingly dehiscent, 0	Eastern Brazil; Moist primary forests (700–2000 m) B	Plowman (1998), Cocucci (1995)
B. imatacana	Light violet fading to white, fragrant[b]	Butterfly, 1	Dry, 0	Venezuela: Bolívar state; Open woodland with sandy soil E	Plowman (1981)
B. jamaicensis	White or yellowish[c]	Hawk-moth, 0	Fleshy, green, 1	Jamaica; Forest at high elevations A	Adams (1972)
B. lactea	Pale yellow or whitish[c]	Hawk-moth, 0	Flashy, 1	Eastern Puerto Rico; Forests at high elevations A	Liogier (1994)
B. linearis	Unknown	Unknown, 5	Unknown, 2	Cuba: Holguin, Sierra de Nippe A	León and Alain (1974)
B. macrocarpa	Showy, violet fading white, fragrant[b]	Butterfly, 1	Fleshy, yellow-ochre, 1	Ecuador: Gorgona Island; Understory of primary forest C	Plowman (1973)
B. macroloba	Cream white[c]	Hawk-moth, 0	Fleshy, 1	Cuba: Pinar del Rio province, Cuba oriental; Mountain habitat A	León and Alain (1974)
B. maliformis	Primrose yellow[c]	Hawk-moth, 0	Fleshy, 1	Jamaica; Woodland and limestone cliffs (200–1100 m) A	Adams (1972)
B. martiana	Greenish white[a]	Settling moth, 4	Unknown, 2	Brazil: Amazonas, Para, Bahia; Guyana; Lowland tropical forest B	Plowman (1974)
B. mire	Showy, violet fading white[b]	Butterfly, 1	Dry, tardily dehiscent, 0	Peru, Brazil, Bolivia; Humid swampy forests (400–1400 m) D	Plowman (1998)
B. nitida	White[c]	Hawk-moth, 0	Fleshy, brightly colored, 1	Cuba: Matanzas, La Habana, Pinar del Rio A	León and Alain (1974)
B. obovata	Bluish to lilac fading to white[b]	Butterfly, 1	Dry, sparingly dehiscent, 0	Brazil: Goiás, Minas Gerais, São Paulo; Aquatic and semiaquatic environment (300–1800 m) B	Plowman (1998)
B. pauciflora	Showy, reddish purple fading to pale lavender/white, the white spot at mouth sometimes edged with violet[b], scentless	Butterfly, 1	Dry, tardily dehiscent, 0	Southeastern Brazil; Coastal, low elevation rain forest B	Plowman (1998), Bertrand et al. (2005)
B. picardae	Color unknown[c]	Unknown, 5	Fleshy, 1	Hispaniola; Calcareous rocks at low altitude A	Liogier (1994)

Table 2 (continued)

Species	Flower traits	Pollinator	Type of capsule	Range and habitat	Reference
B. pilosa	Deep violet fading to white[b]	Butterfly, 1	Dry, tardily dehiscent, 0	Southeastern Brazil, Paraguay, Argentina B	Plowman (1974)
B. plowmaniana	Purple flowers that later become pleasant smelling white[1] young flowers blue, old white[2]	Unknown, 5	Dry, 0	[1]Bolivia, Santa Cruz: Manuel Maria Caballero (2700 m) [2]Argentina, De Salta: Los Toldos (1600–1700 m) D	Label information on [1]Arroyo et al. 3569 (M); [2]Novara 7124 (B)
B. pluriflora	Unknown	Unknown, 5	Unknown, 2	Cuba: Holguin, Sierra de Nippe A	León and Alain (1974)
B. portoricensis	White[c]	Hawk-moth, 0	Fleshy, 1	Eastern Puerto Rico; Mountain forest (500–600 m) A	Liogier (1994)
B. purpurea	Violet[c]	Hummingbird, 3	Fleshy, 1	Cuba: Guantánamo; Pluvial, gallery and pine forests A	León and Alain (1974); V. Fuentes, pers. comm., April 2010, April 2011
B. shaferii	Cream white[c]	Hawk-moth, 0	Fleshy, 1	Cuba: Holguin, Sierra de Nippe A	León and Alain (1974)
B. sinuata	White[c]	Hawk-moth, 0	Flashy, 1	Cuba: Camagüey, La Habana and Villa Clara A	León and Alain (1974)
B. uniflora	Violet fading white, emitting sweet scent in the evening[b]	Butterfly or/and moth, 5	Dry, tardily dehiscent, 0	Southeastern Brazil; Coastal rain forest B	Plowman (1998), Cocucci (1995) (see Fig. 7), R. Teixeira de Queiroz, pers. comm., April 2011
Outgroups[d]					
Bouchetia erecta	White, creamy, lilac or purple	Bee, 2	Dry, 0	Southwestern USA, Mexico, Guatemala (B. erecta), South America: Brazil, Paraguay, Uruguay, Argentina F	D'Arcy (1978), Hunziker (2001), Knapp (2010)
Calibrachoa parviflora	Funnel-form, purple, red, pink, or whitish	Bee, 2	Dry, 0	Southern Brazil, Peru, Chile B	Ando et al. (2001), Stehmann and Semir (2001) (observations for C. elegans)
Fabiana imbricata	Whitish or lilac/bluish, sometimes yellow with violet/red streaks, fragrant	Hawk-moth, 0	Dry, 0	Western South America; Mountain areas (1000–4900 m), with two lowland exceptions D	Hunziker (2001)
Hunzikeria texana	Pink or purple, funnel-form	Butterfly, 1	Dry, 0	Southwestern USA, Mexico, Venezuela F	D'Arcy (1978), Hunziker (2001), Knapp (2010) (for genus)
Leptoglossis darcyana	Variously colored, salveriform, producing nectar	Butterfly, 1	Dry, 0	Coastal Peru and Argentina D	D'Arcy (1978), Cocucci (1995) (observation for L. linifolia)
Nierembergia hippomanica	Blue, violet, pink, white, corolla tube well developed, nectarless, oil production	Bee, 2	Dry, 0	Argentina (center of diversification) D	D'Arcy (1978), Cosacov et al. (2008) (observation for N. lineariifolia); Tate et al. (2009)
Petunia axillaris	Funnelform, campanulate, or salveriform; purple, red, pink, or white	Bee, 2	Dry, 0	Southern Brazil, Paraguay, Bolivia, Argentina, Uruguay B	D'Arcy (1978), Stehmann et al. (2009), Gübitz et al. (2010) (bee pollination ancestral for genus)
Plowmania nyctaginoides	Corolla up to 5 cm long, showy, tube orange, lobes vermilion scarlet, orifice golden-yellow	Hummingbird, 3	Dry and leathery, 0	Southern Mexico, Guatemala; Mountain rain forest (1800–3800 m) F	Hunziker (2001), Knapp (2010)

The following general information comes from Plowman's revision (1998):

[a] Corolla tube up to 3× as long as the calyx, with deeply cleft limb and notched lobes, producing nectar at its base.
[b] Corolla tube ca. 5× as long as the calyx with a white eye at its throat and a spreading landing platform, producing nectar at its base.
[c] Corolla tube 6–24× longer than the calyx, producing nectar at its base, nocturnal anthesis, nocturnal scent emission.
[d] Area, pollinator and fruit coding for outgroups do not necessarily represent the state in the particular species sequenced, but instead the entire genus.

in Figs. 2 and 3) falling far from Brazilian accessions (from near the type locality) as well as one from Venezuela (labeled *B.* aff. *uniflora* in Figs. 2 and 3). The monophyly of the likewise widespread (and cultivated) Antillean species *B. americana* also is doubtful. The species hybridizes with Puerto Rican *B. lactea* (compare Section 4.1; nuclear and plastid phylograms in OSM Fig. S1). An accession of *B. americana* from near the type locality on Martinique may represent the true species, while the two other plants sequenced likely are hybrids (Fig. 2).

3.2. Divergence times and ancestral area reconstruction

Of our three calibration approaches (see Section 2), the first, namely secondary calibration yielded a *Brunfelsia* crown age of 18 Myr (9–28 Myr 95% confidence interval) under a relaxed clock and 16 Myr (9–22) under a strict clock model. The second, namely using an average nuclear DNA substitution rates for woody species yielded ages for the *Brunfelsia* crown group of 13, 17 and 30 Myr (calculated with rates of 3.30, 1.99, or 1.44 substitutions/site/

year × 10^{-9}), and the third, using an average plastid rate, yielded an age of 17 Myr (14–21) under a strict clock model and of 21 Myr (15–35) under a relaxed clock model. A chronogram with the ancestral area reconstruction obtained under the CTMC model is shown in Fig. 3, and a reconstruction obtained with the S-DIVA approach in Fig. S2. With vicariance between the Antilles and South America precluded, S-DIVA inferred an ambiguous ancestral range for the most recent common ancestor of *Brunfelsia* (Fig. S1). The CTMC model revealed eastern Brazil, southeastern South America, and the Amazon Basin (proto-Amazonia coded as B in Figs. 3 and S2) as the ancestral region for the genus, but only with 48% posterior probability (Fig. 3), followed by dispersal to the Lesser Antilles. The radiations on Cuba, Hispaniola, Jamaica, and Puerto Rico are dated to the Late Miocene or Early Pliocene (9–4 million years ago (Ma)), with Puerto Rico perhaps colonized twice, at c. 6 and 4 Ma. The Guiana Shield (turquoise coding in Fig. 3 and S2) was reached from Brazil/Amazon Basin about 12 Ma and a second time from the Andes/coastal Pacific about 7 Ma (giving rise to *B. imatacana* in the state of Bolívar in Venezuela). The split of Andean
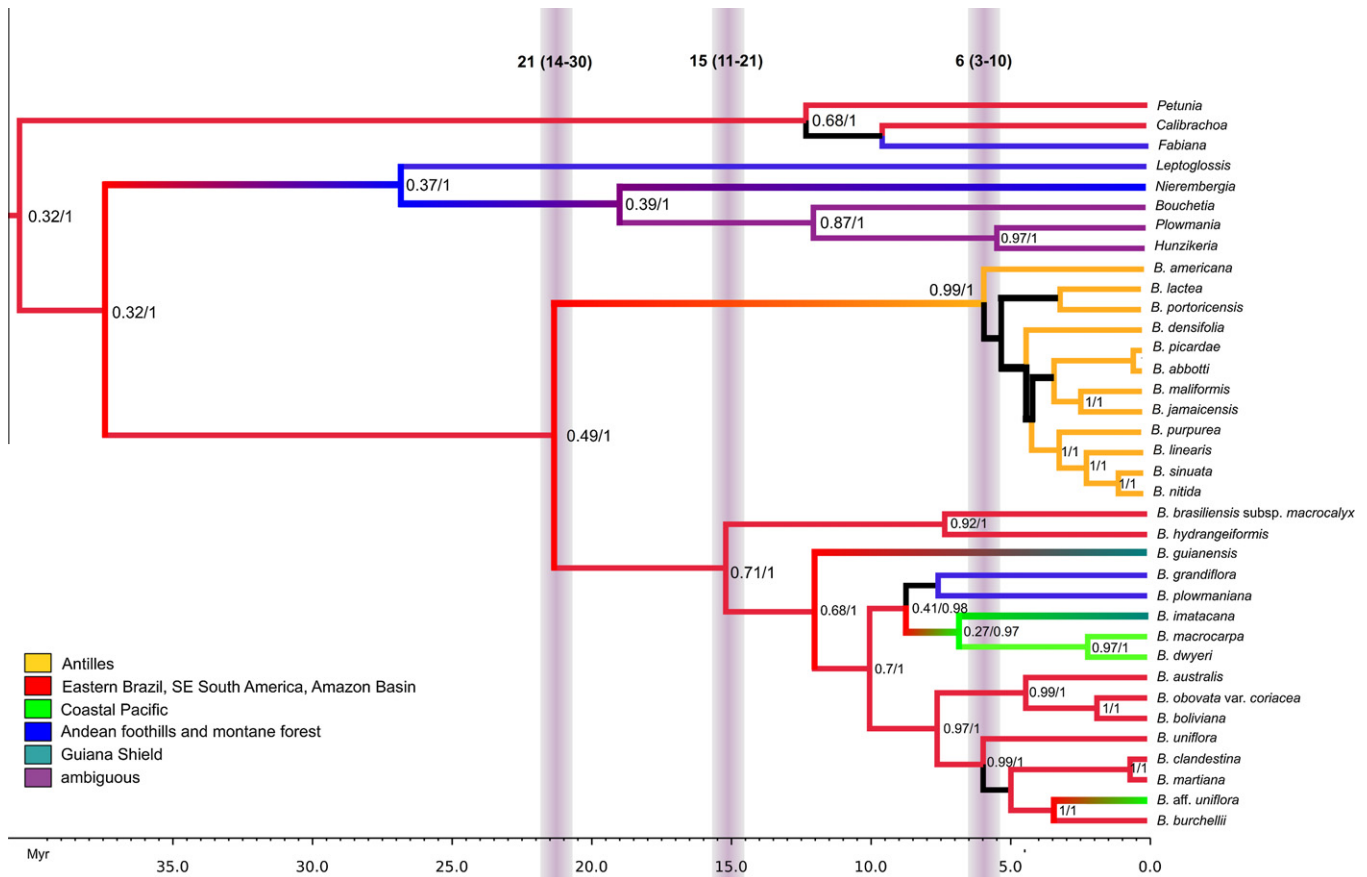
Fig. 3. Chronogram and CTMC ancestral area reconstruction obtained from a plastid data matrix representing 28 species of *Brunfelsia* and eight outgroups. The absolute times shown here were obtained using an average substitution rate for angiosperm plastid genomes (0.7 substitutions/site/year $\times 10^{-9}$; Section 2, approach 3). The branches are colored according to the most probable state inferred for their descendant nodes, with the coding explained in the inset. The node labels show the probability values for particular areas and posterior probability values. Vertical purple bars mark the estimated ages of the most recent common ancestor of the genus *Brunfelsia*, the South American clade, and the Antillean clade. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and coastal Pacific lineages from the Brazilian ones took place about 10 Ma.

3.3. Shifts in pollinator and fruit type

State reconstructions that used a phylogram or an ultrametric tree gave statistically undistinguishable results. The most recent common ancestor of *Brunfelsia* may have been butterfly-pollinated (but the proportional likelihood value was only 0.54; Fig. 4), with two shifts from butterflies to settling moths in South America, one in the common ancestor of *B. martiana* and *B. clandestina* and the second in the *B. guianensis* ancestor. The ancestral state in the Antillean clade is hawk-moth pollination (Figs. 1A, F and 4), with a single switch to hummingbird pollination in the common ancestor of the Cuban species *B. purpurea* and *B. cestroides* (Fig. 1G). The ancestral capsule type in *Brunfelsia* probably was dry (likelihood value 0.77 for dry capsules and 0.2 for fleshy ones, Fig. S3), and the ancestor of the Antillean clade then appears to have evolved a fleshy pericarp. In the South American clade, fleshy fruits evolved at least twice (in *B. guianensis* and *B. macrocarpa*).

4. Discussion

4.1. Species relationships in Brunfelsia

Brunfelsia in its traditional circumscription (Plowman, 1998) is monophyletic, and contrary to our expectation, the Antillean clade

is not nested inside any South American clade. Instead, it is sister to the South America clade, indicating that the spread to the Antilles occurred early during the evolution of *Brunfelsia*, with extinction long since having eliminated the mainland population from which the Antillean clade originated. The traditional sections *Franciscea* and *Guianenses* (Plowman, 1998) are para- and polyphyletic, implying that the flower differences used by Plowman to characterize these sections evolved independently, probably in adaptation to similar pollinators (see Section 4.4). Plowman's section *Brunfelsia*, with all Caribbean species, however is monophyletic.

The eastern slopes of the Andes harbor four species, *B. grandiflora*, *B. chiricaspi*, *B. mire* and *B. plowmaniana*, all included in our study. The latter grows in humid or cloud forests from 1500 up to 3200 m alt. in Bolivia and Argentina (Filipowicz et al., 2012), while the others occur at lower altitudes in southeastern Peru, western Brazil and Bolivia (*B. mire*), southeastern Colombia and northern Ecuador (*B. chiricaspi*), or are widespread (*B. grandiflora*), occurring from Venezuela to Bolivia. The western side of the Andes harbors *B. macrocarpa* in western Ecuador and on Gorgona Island and *B. dwyeri* on Cerro Jefe in Panama. The two are sister species as hypothesized by Plowman (1998) and are close to the Venezuelan *B. imatacana* restricted to Serrania de Imataca and Serrania de Nuria in Bolívar (Fig. 2). A last species growing in the Colombian Chocó and adjacent Panama, *B. chocoensis*, could not be included in this study, but based on the thick-walled fruits and solitary flowers (Plowman, 1998) it is expected to group with *B. macrocarpa* and *B. dwyeri*.

Among the insights coming from this study is that one, and possibly two, widespread species are not monophyletic. *Brunfelsia uniflora* sensu Plowman consists of three entities, one from Minas Gerais and São Paulo, a second from Bolivia and Argentina, and a third from southern Venezuela, Guyana, and adjacent Roraima. Plowman had noted morphological differences in plants from these locations, but found them within "the normal range of a polymorphic species." (Plowman, 1998: 117). The type of the name *B. uniflora* was collected in the state of Rio de Janeiro, and a new name was thus assigned for the Andean species, *Brunfelsia plowmaniana* N. Filipowicz & N. Nee (Figs. 2–4; Filipowicz et al., 2012). The second possibly polyphyletic species is *B. americana*, inhabiting several islands from St. Lucia in the southern part of the Lesser Antilles arc up to the eastern tip of Hispaniola (Plowman, 1979). We sequenced three accessions of *B.*"americana" (shown in Fig. S1) of which C. Whiteford 7319 (M) from Dominica likely represents true *B. americana* because Dominica is close to Martinique from which the type of the name originates (Lamarck, 1783). The two remaining accessions came from St. Thomas and Dominica and appear to be hybrids of *B. americana* and *B. lactea*, the latter described from Puerto Rico and morphologically similar to *B. americana*. Experiments conducted by Plowman show that these species can be crossed (a voucher of *B. americana × B. lactea*, T. Plowman 10966, is deposited at MO). Two suspected hybrid plants group with C. Whiteford 7319 in the plastid tree, but with Puerto Rican *B. lactea* in the ITS tree (shown in blue in OSM Fig. S1). Deciding on the exact species boundary of *B. americana* will require further sampling.

4.2. Historical biogeography of Brunfelsia

The oldest solanaceous diaspore is dated to the Lower Eocene, 56–49 Ma (Collinson et al., 1993), and molecular dating efforts have placed the most recent common ancestor of the extant Solanaceae at 35–51 Ma (Wikström et al., 2001; Paape et al., 2008; Dillon et al., 2009; Bell et al., 2010), with the results of Wikstroem et al. and Bell et al. largely congruent (39 and 37 Ma, respectively). Since the Petunieae (to which *Brunfelsia* belongs) have no fossil record, we used three dating approaches (Section 2) in an attempt to cross-validate the resulting estimates. The approaches yielded crown ages of 16–18 Ma (approach 1), 13, 17, or 30 Ma (approach 2, depending on the substitution rate), or 17–21 Ma (approach 3), suggesting that *Brunfelsia* may have evolved sometime in the Early Miocene, a time when the geotectonic changes in the Amazon basin associated with the uplift of the Eastern Cordillera in the Central Andes were just starting (Antonelli et al., 2009; Hoorn et al., 2010).

The earliest inferred divergence events in *Brunfelsia* occurred around 15 Ma, giving rise to the lineage inhabiting southeastern Brazil (*B. brasiliensis*, B. cuneifolia, B. hydrangeiformis*; Figs. 2 and S2) and the larger South American clade. Between 12 and 14 Ma was the time of the middle Miocene climate optimum, when plant diversity increased after an Early Miocene low (Jaramillo et al., 2006). From the proto-Amazonian region, *Brunfelsia* then expanded its range west to the Andean foothills, where *B. plowmaniana*, a new species (Filipowicz et al., 2012) occurs at altitudes of up to 3200 m in cloud forests and north to the Guiana Shield (see Figs. 3 and S2). The Pacific clade (*B. dwyerii*, B. macrocarpa*, and probably *B. chocoensis*) and the Venezuelan *B. imatacana* diverged from trans-Andean relatives roughly 7 Ma (based on the average plastid DNA substitution rate), at a time of peak orogeny of the Colombian Andes at 7–11° N (Gregory-Wodzicki, 2000; Hoorn et al., 2010), and *B. dwyerii* (Panama) and *B. macrocarpa* (Ecuador) diverged from each other c. 3 Ma (Fig. S1), matching the closure of the Panamanian Isthmus at 3–3.5 Ma (Farris et al., 2011).

The expansion of *Brunfelsia* to the Antilles early during its evolution plausibly involved seed dispersal by birds since all Antillean species have fleshy, brightly colored capsules, and this is also the ancestral state inferred for the insular brunfelsias (Fig. S3). Fleshy capsules may be the key innovation that permitted successful expansion among islands as suggested by Plowman (1998), while most South American species of *Brunfelsia* retained the dry, usually many-seeded capsule that is plesiomorphic for the Petunieae.

4.3. Plant radiations in the Antilles

Studies of the Antillean flora have found that it is comprised of North American and South American elements, and this is supported by the few molecular phylogenies with sufficiently dense species sampling (Santiago-Valentín and Olmstead, 2003, 2004; Francisco-Ortega et al., 2007). The floristic affinities of the Lesser Antilles are mostly with South America, those of Western Cuba with the Yucatan Peninsula (Acevedo-Rodríguez and Strong, 2008). *Brunfelsia* does not follow this pattern. Instead, the Cuban radiation, which comprises species in Western and Eastern Cuba, is part of a larger expansion that may have progressed from the Lesser Antilles to the Greater Antilles, although the low statistical support in our phylogeny (Fig. 2) precludes inference of the direction of geographic expansion between islands. None of the 63 molecular phylogenies that have included Antillean species (Francisco-Ortega et al., 2007) appear to have applied molecular dating, so that it is not possible to compare the c.4 Myr-old 10-species radiation of *Brunfelsia* on Cuba to other clades. The 11th Cuban endemic, *B. grisebachii* from western Cuba, is not sampled here, but based on morphology it resembles *B. cestroides* and *B. purpurea*, and may be a polyploid form of one of them (Amshoff, 1956).

The Antilles region has a complex, still incompletely understood geologic history (Graham, 2003). However, the *Brunfelsia* radiations on Cuba, Jamaica, Puerto Rico, and Hispaniola all post-date the known above-water exposure of these islands, with inferred ages of 4 Myr for the Cuban radiation, 3 Myr for Jamaica (but only two of six Jamaican endemics are sampled), 1 Myr for Hispaniola, and 3–5 Myr for Puerto Rico (assuming it was reached twice, Fig. 3). The islands' geographical distances (ca. 80 km between Cuba and Hispaniola, 110 km between Puerto Rico and Hispaniola, and ca. 180 km between Hispaniola and Jamaica) apparently were sufficiently large to prevent gene flow among islands so that each of the larger islands now harbors an *in situ* radiation. The only species that supposedly occurs from St. Lucia in the southern Lesser Antilles to the eastern tip of Hispaniola, *B. americana*, is widely cultivated and hybridizes at least with Puerto Rican *B. lactea* (see Section 4.1).

4.4. Pollinator shifts involving hawk moths, hummingbirds, and settling moths

The most recent common ancestor of *Brunfelsia* is inferred as having been butterfly-pollinated (Fig. 4), with a shift to hawk-moth pollination in the ancestor of the Antillean clade, while butterfly pollination persisted in South America. Antillean species have white or creamy-yellow flowers with long straight nectar tubes (6–24 times the calyx length; our Fig. 1A, B, and F) and an intensive perfume (Plowman, 1998), traits fitting with moth pollination (Oliveira et al., 2004). Only the Cuban species *B. purpurea* and *B. cestroides* have purplish, scentless flowers with relatively long corolla tubes (León and Alain, 1974, our Fig. 2G) suggesting bird pollination (Plowman, 1998; Knapp, 2010). The first of these species is restricted to open habitats in Guantánamo province, while the second grows in gallery forests and pine forests in the western and eastern part of the island (V. Fuentes, Instituto de
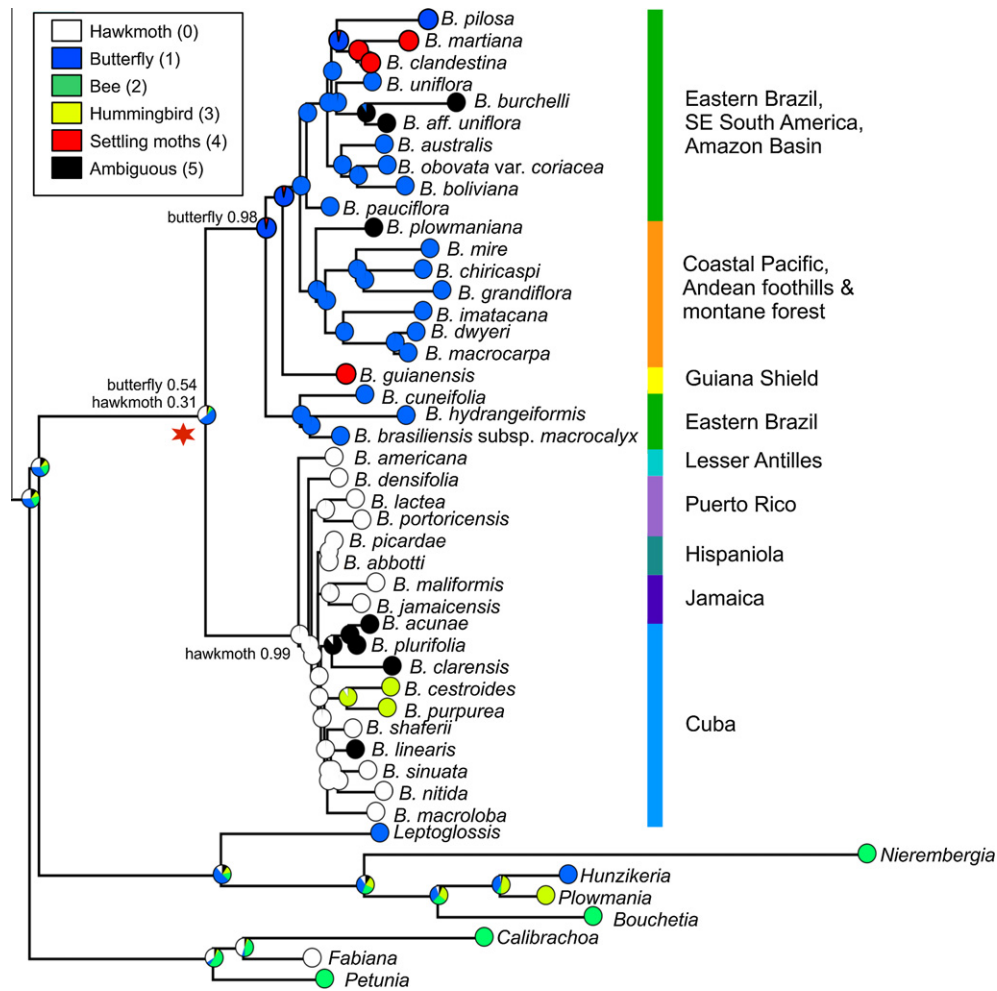
Fig. 4. Evolution of *Brunfelsia* flower traits associated with pollinator type, as inferred on a maximum likelihood phylogram under maximum likelihood optimization. Color codes for the character states are shown in the legend, and pie diagrams at nodes represent likelihoods. The asterisk indicates the most recent common ancestor of the genus. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Investigaciones en Fruticultura Tropical, Havanna, Cuba, personal communication, April 2011). Switches between hawk-moth pollination and hummingbird pollination do not require drastic morphological changes since both animals forage on the wing (hence require not landing platforms), exploit long-tubed flowers, and prefer sucrose-rich nectar.

Compared to the Antillean species, South American brunfelsias have shorter, thinner corolla tubes, usually with a spreading rim, more often with nectar guides in their centers and/or with color change during anthesis. Butterflies from four families have been observed visiting such flowers (Plowman, 1998 for *B. grandiflora*). Switches from butterfly to moth pollination apparently occurred in species from coastal moist forest of Bahía and Espírito Santo (*B. clandestina*) and eastern Brazil to Guyana (*B. martiana*). The smallest *Brunfelsia* flowers are visited by settling moths (Noctuidae, Geometridae, or Pyralidae), which have low metabolic rates and seek nectar by landing and walking on the inflorescences (Cocucci, 1995; Oliveira et al., 2004). Transitions between butterfly and moth pollination are suggested by the observation that *B. uniflora*, with morphologically butterfly-adapted flowers, nevertheless emits a heavy sweet scent in the evening (Cocucci, 1995; R. Teixeira de Queiroz, Instituto de Biologia, Universidade Estadual de Campinas, Brazil, personal communication 18 April 2011).

To our knowledge, *Brunfelsia* with 23 species on the Antilles archipelago is the first plant clade for which rapid radiations on these islands, most importantly Cuba, has been addressed with a dated molecular phylogeny. Its 4-Ma-10-species radiation on Cuba is also the only dated plant diversification on that island, which simply illustrates the difficulty of accessing suitable Cuban material. Clearly, all divergence times inferred here have to be regarded as rough due to the indirect calibrations, and denser population-level sampling, using nuclear data, is needed to infer dispersal directions among islands.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2012.02.026.

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