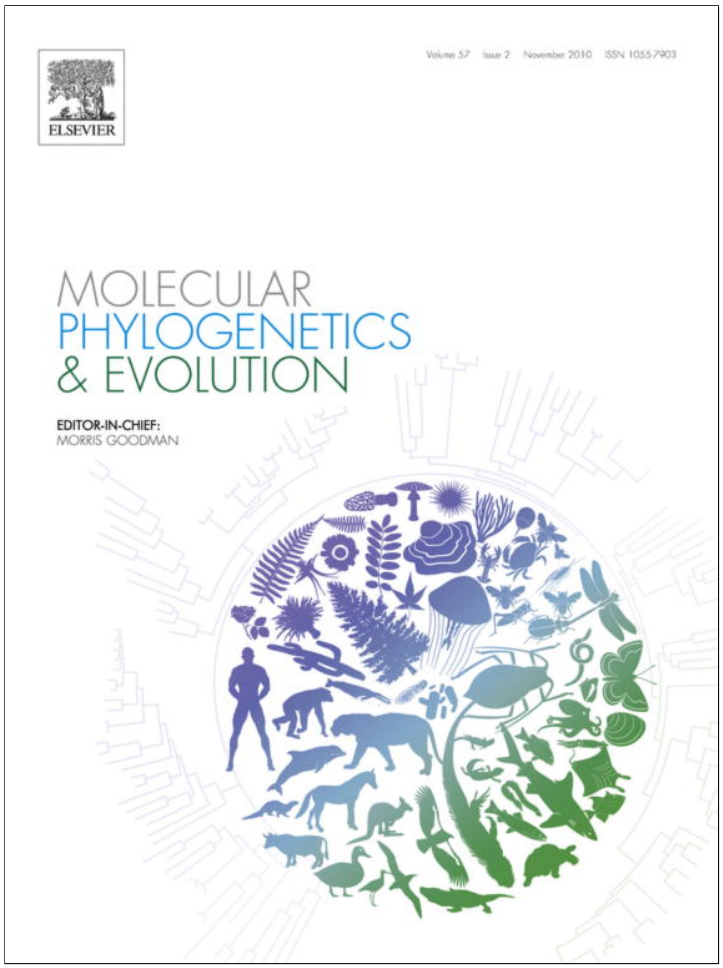

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Diversification in species complexes: Tests of species origin and delimitation in the *Bursera simaruba* clade of tropical trees (Burseraceae)

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ABSTRACT

Molecular phylogenies are invaluable for testing morphology-based species delimitation in species complexes, as well as for examining hypotheses regarding the origination of species in these groups. Using five nucleotide markers, we reconstructed the phylogeny of the *Bursera simaruba* species complex of neotropical trees to test the notion that four "satellite" species originated from populations of the most widely distributed member of the genus, *B. simaruba*, which the satellites strongly resemble. In addition to molecular phylogenetic reconstruction, we tested species delimitation of *B. simaruba* and the satellites using multivariate analyses of morphological and ecological characters. The analyses evaluated the taxonomic value of these traditional characters and pinpointed those in need of further study, such as the expression of pubescence. Phylogenetic data rejected the origin of three satellite species from their purported ancestor, *B. simaruba*, and we ascribe their morphological similarity to convergence or parallelism. The fourth satellite species likely represents one end of a spectrum of inflorescence length variation within *B. simaruba* and is conspecific. Despite its marked morphological variability, we recovered *B. simaruba* as a single valid species, which implies that it maintains genetic cohesion among distant populations throughout its vast range.

1. Introduction

Species are the working units in most of biology, yet delimiting species is fraught with complexity (Barraclough and Nee, 2001; Sites and Marshall, 2003, 2004). In a phylogenetic framework, the species of traditional taxonomy can be viewed as hypotheses tested by recovering either non-polyphyletic or polyphyletic groupings. Most species concepts view monophyly as congruent with a species hypothesis (e.g. Baum and Shaw, 1995; Shaw, 1998), and some also recognize the possibility of paraphyletic species (Coyne and Orr, 2004; Crisp and Chandler, 1996; Harrison, 1998; Templeton, 1989). However, virtually all concepts view polyphyly as a rejection of a species hypothesis. Because molecular phylogenetic hypotheses can readily identify polyphyletic taxa, they are an excellent aid in delimiting species (Knowles and Carstens, 2007; Wiens and Penkrot, 2002), especially in the case of species complexes, whose continuously varying morphological characters often confound morphology-based taxonomies (e.g. Campbell et al., 2004; Leliaert et al., 2009). In this work, we test species hypotheses in the *Bursera simaruba* complex, a group of neotropical trees with a knotty taxonomic history resulting from the strong morphological similarity and overlapping geographic distributions of its species (Rzedowski et al., 2007).

The history of species delimitation in the *simaruba* complex exemplifies central issues in species complex systematics. Species complexes may contain widespread, morphologically variable species, as in the painted turtle (Starkey et al., 2003), or multiple ecologically distinct species that are morphologically similar, as in the *Rana pipiens* complex (Hillis, 1988). Both the single and the multiple species scenarios have been proposed by taxonomists for entities of the *simaruba* complex (Bullock, 1936; McVaugh and Rzedowski, 1965; Rzedowski et al., 2007; Standley, 1923), especially for *B. simaruba*, the species from which the complex derives its name (Fig. 1A). Commonly known as the gumbo-limbo tree or palo mulato, *B. simaruba* is a conspicuous element in virtually all

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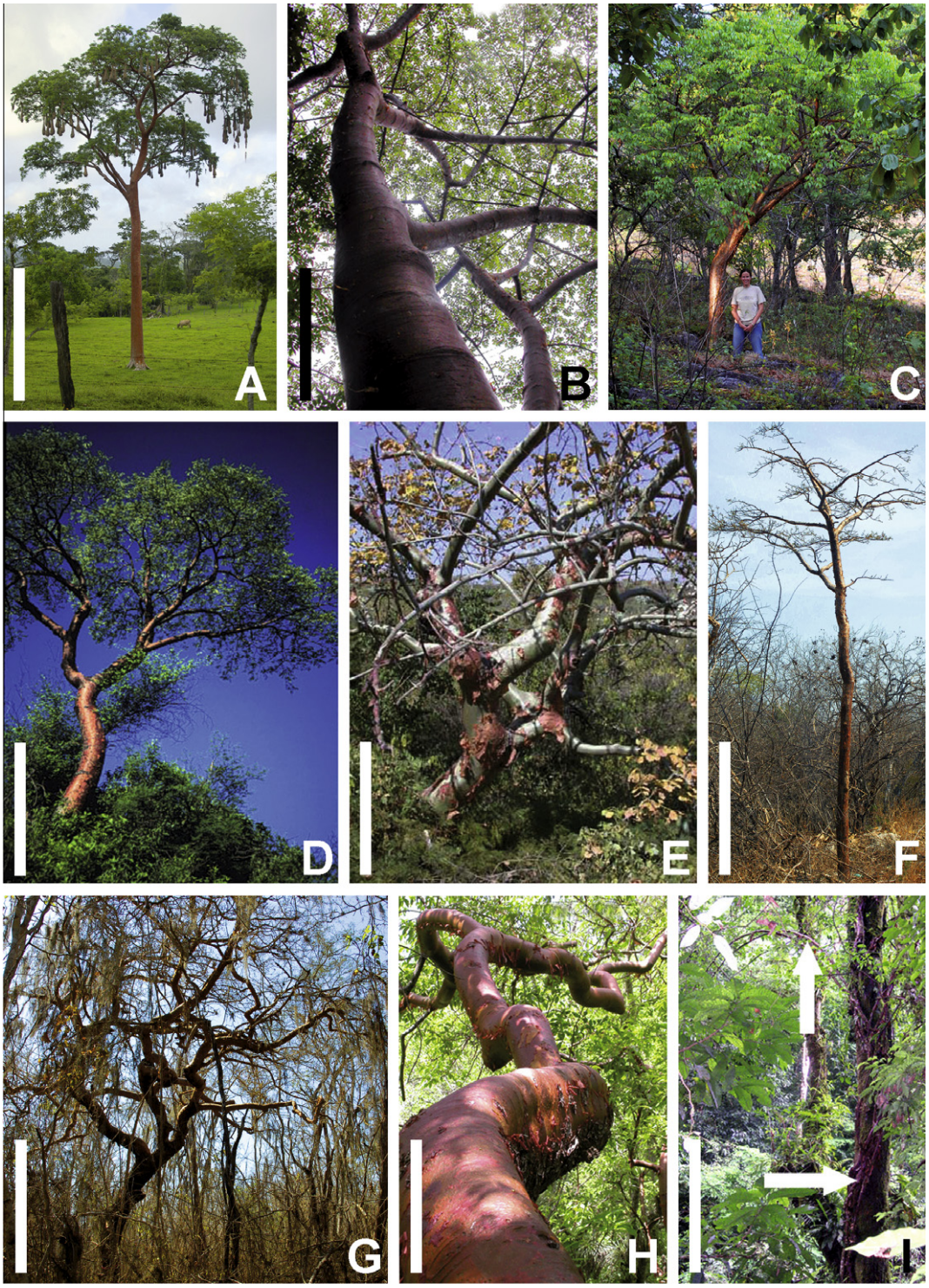

Fig. 1. Habit diversity in the *Bursera simaruba* species complex. This figure shows the red to copper colored peeling outer bark that characterizes the group, and that, for its size, the clade spans an exceptional array of life forms. (A–C) *Bursera simaruba* and "satellites." (A) *B. simaruba* remnant in a pasture cut from lowland rainforest. Scale = 10 m. (B) *B. attenuata* in a subdeciduous tropical forest canyon. Scale = 1.5 m. (C) *B. ovalifolia* resembles a smaller, intricately branched *B. simaruba*. Person = 1.90 m. (D–H) Representatives of the *simaruba* complex from tropical deciduous forests. (D) *B. longipes*, one of the smallest members of the clade, growing in very dry tropical deciduous forest. Scale = 2.5 m. (E) *B. grandifolia* is a widespread dryland shrub to medium tree, distinguished by its waxy green trunk shedding ragged copper sheets of outer bark. Scale = 6 m. (F) *B. laurihuertae* is endemic to the low, dense woods of the southern Isthmus of Tehuantepec. Scale = 1.5 m. (G) *B. instabilis* is unique for its long, slender branches, which in large individuals become lianescent. Scale = 1.5 m. (H) *B. cinerea*, a water-storing tree from tropical deciduous forest canyons. Scale = 0.75 m. (I) *B. standleyana*, the hemiepiphytic member of the genus, growing at 20 m above the floor of the rainforest. The lower arrow indicates the long lianescent roots that bring water from the soil; the upper arrow singles out arching branches. Scale = 1.5 m.

tropical forests below 1400 m from Mexico to northern South America, Florida, and the West Indies (Fig. 2). It varies from small trees of subdeciduous forest to towering rainforest emergents, and in the amount and distribution of leaf pubescence, leaflet shape and number, and bark color (Rzedowski et al., 2007; Rzedowski and Kruse, 1979). Although this variation has often been inter-

Fig. 2. Distribution of *B. simaruba* (in gray) and its satellite species *B. attenuata*, *B. itzae*, *B. ovalifolia*, and *B. roseana*.

preted as phenotypic plasticity, it could also indicate that what is now recognized as a single polymorphic species in fact includes several, one of the central questions of species complex taxonomy.

In addition to allowing us to examine the issue of species delimitation, species complexes are also natural model systems for understanding how new species arise (Kopp and Barmina, 2005; Kornfield and Smith, 2000; Losos, 1994). Scattered within the vast distribution of *B. simaruba* are four species (Fig. 2), which are so similar in habit, habitat, and leaflet number, shape, size, and pubescence that they have been termed "satellite" species (Rzedowski et al., 2007). These species, *B. attenuata*, *B. itzae*, *B. roseana*, and *B. ovalifolia* (Fig. 1A–C), could have originated from isolated populations of *B. simaruba*. In phylogenetic terms this hypothesis would translate into each satellite appearing nested within a paraphyletic *B. simaruba* (Crisp and Chandler, 1996; Funk and Omland, 2003; Graybeal, 1995; Hedin, 1997; Omland et al., 2000; Patton and Smith, 1994; Slade and Moritz, 1998; Talbot and Shields, 1996). Alternatively, if sufficient time has elapsed and genetic loci have attained reciprocal monophyly (Avise and Ball, 1990; Funk and Omland, 2003; Omland et al., 2006; Rieseberg and Brouillet, 1994; Shaw, 1998), the paraphyletic pattern of *B. simaruba* could be erased, leaving the set of satellites as a clade sister to *B. simaruba* or a grade basal to it. Finding that satellites are more closely related to other species and not *B. simaruba* would reject the hypothesis that they are derived from the gumbo-limbo and would make the satellites likely examples of morphological convergence or parallelism.

1.1. Species delimitation in the simaruba complex: genetics, phenetics, and ecology

The *simaruba* complex is part of an abundant, species-rich American genus of trees and shrubs whose center of diversity is Mexico. The complex comprises 15 species spanning a remarkable array of life form and ecology (Fig. 1, Table 1). They are distinguished from other *Bursera* by their reddish peeling phellem (outer bark), compound leaves without wings on the rachis, ovate to broadly elliptic entire leaflets with mostly acuminate apices and brochidodromous venation, and three-valved fruits with reddish pseudarils covering the endocarp completely. Although the complex has long been recognized as distinct (McVaugh and Rzedowski, 1965; Rzedowski and Kruse, 1979), and is well supported in molecular phylogenies (Becerra, 2003; Becerra and Venable, 1999), species limits and relationships within the group have always been regarded as problematic (Daly, 1993; Rzedowski et al., 2005, 2007). Molecular phylogenies have included only a few species of the complex and provide little resolution within the group (Becerra, 2003; Becerra and Venable, 1999).

As in many plant groups, the taxonomy of the *simaruba* complex employs combinations of plastic and potentially evolutionarily labile characters, rather than unique synapomorphies (Daly, 1993; Appendix B, Supplementary material). Years of taxonomic confusion have resulted, reaching a zenith in the satellite species, in which barely diagnostic combinations of leaflet number, pubescence patterns, and fruit size are used to delimit species, with taxonomic realignments continuing to the present day (Bullock, 1936; McVaugh and Rzedowski, 1965; Rzedowski et al., 2007; Standley, 1923). To test morphologically-based species delimitations, we include in our phylogenetic reconstruction two or more samples of eight of the species in the complex, including the four satellite species. In addition, we performed the first phenetic study of the morphological characters used in the *simaruba* complex taxonomy and compare it to our molecular phylogeny, an approach that we use to rank a wide array of traditional characters in their diagnostic effectiveness.

In addition to morphological and molecular characters, ecology can also help delimit species (Ruiz-Sanchez and Sosa, 2010). Therefore, we test for ecological differentiation between *B. simaruba* and its satellites through multivariate analyses of environmental variables (Stockman and Bond, 2007). We assume that different niches between species would reject the hypothesis of ecological exchangeability, which, along with genetic exchangeability, is one of two main aspects of the cohesion species concept, which we follow here (Templeton, 1989).

Table 1
Habitat and distribution of the 15 species in the *B. simaruba* species complex and its nine related Antillean species, plus an undescribed Cuban endemic. Elevation is included for *B. simaruba* and its satellite species. TDF: tropical deciduous forest, TSDF: tropical subdeciduous forest, TRF: tropical rainforest.

Simaruba complex species	Habitat and distribution
B. arborea (Rose) L. Riley[c]	TDF, Mexican Pacific coast and Durango
B. attenuata (Rose) L. Riley[a,c]	TDF and TSDF, 600–1000 m, western Mexican Pacific coast and Durango
B. cinerea Engl.[c]	TDF, Mexico: upper Papaloapan river basin
B. grandifolia (Schltdl.) Engl.[c]	TDF, western and central Mexico, Balsas Depression
B. instabilis McVaugh & Rzed.[c]	TDF, Mexican Pacific coast
B. inversa Daly	TRF, Panama, Colombia and Venezuela
B. itzae Lundell[a,b]	TRF, N shore of Lake Petén Itzá, Guatemala
B. krusei Rzed.[b,c]	TDF, Mexico: Guerrero and Oaxaca
B. laurihuertae Rzed. & Calderón[b,c]	TDF, Mexico: southeastern Oaxaca
B. longipes (Rose) Standl.[c]	TDF, Mexico: eastern Balsas Depression
B. ovalifolia (Schltdl.) Engl.[a,b,c]	TDF and TSDF, 0–1500 (1850) m, Pacific coast from Mexico to Costa Rica
B. permollis Standl. & Steyerm.[b]	TDF, El Salvador, Guatemala, Honduras, Nicaragua
B. roseana Rzed., Calderón & Medina[a,c]	TDF and TSDF, 1200–1900 m, Western and southern Mexican Pacific coast, western central Mexico
B. simaruba (L.) Sarg.[c]	TSDF and TRF, <1400 m, southern Florida to northern Brazil, including both Mexican coasts, the Caribbean, and Central America
B. standleyana L. O. Williams & Cuatrec.[b]	TRF, hemiepiphytic tree, Costa Rica (San José and Puntarenas Provinces)
Antillean species	
B. aromatica Proctor	TDF on limestone, northwestern Jamaica
B. frenningae Correll	TDF, Bahamas (Great Exhuma, Cat Island, Long Island)
B. hollickii Fawc. & Rendle	TDF on limestone, southern central coast of Jamaica
B. inaguensis Britton	TDF on limestone, Bahamas (Little Inagua) and Cuba
B. nashii Urb.	TDF on limestone, Haiti and the Dominican Republic
B. spinescens (Urb.) Urb. & Ekman	TDF on limestone, Haiti and the Dominican Republic
B. shaferi Urb.[b]	TDF on limestone, western Cuba
B. sp. *nov.*[b]	TDF on limestone, eastern Cuba
Commiphora angustata (Grieseb.) Moncada[b]	TSDF, western and central Cuba
C. glauca (Grieseb.) Moncada[b]	TDF on limestone, eastern Cuba

[a] Hypothesized "satellite" species.
[b] Species in this paper not included in previous *Bursera* phylogenies.
[c] Species included in morphometric analyses.

Finally, we test the membership in the *simaruba* complex of some six species from Mexico and Central America, which have never been included in prior phylogenetic analyses (Table 1). In our phylogenetic reconstruction, we also include eight mostly narrow endemic Antillean species. These species are closely related to the *simaruba* complex, with which they share the tree habit, reddish peeling outer bark, and compound leaves with entire leaflets, but differ in having tougher, smaller leaflets with a thicker waxy cuticle (Daly, 1993). However, it is not clear whether these species form a clade, perhaps sister to or within the *simaruba* complex (Weeks and Simpson, 2004), or, for example, a polyphyletic group derived from repeated invasions of the Antilles from the mainland. In summary, our phylogenetic reconstruction of the complex and the Antillean group includes DNA sequence data from five chloroplast and nuclear markers for 71 samples; 50% of the sampled species and over 90% of the DNA sequences are new contributions to *Bursera* phylogeny reconstructions (Appendix A).

2. Materials and methods

2.1. Taxon sampling

We sampled all known species in the *simaruba* complex except for *B. inversa* (Table 1). From the related Antillean group, we included all species except *B. hollickii* and *B. aromatica.* Sampling for *B. simaruba* included morphologically and environmentally contrasting individuals from Mexico, Central America, and Cuba (Appendix A), along with published sequences from the Dominican Republic and Florida (Weeks and Simpson, 2004). The samples we collected at the type locality of *B. itzae* were sterile, so they could not be assigned to species with certainty and are thus referred to as *B.* "*itzae*". Of the Antillean species group, we included an undescribed Cuban species, three poorly known Cuban endemics, which had been transferred to *Commiphora* based on pollen morphology (Moncada-Ferrara, 1989), and four previously sequenced Caribbean species (Weeks and Simpson, 2004; Appendix A). Our outgroup sampling included species of the two subgenera of *Bursera, Bursera* and *Elaphrium*, as well as three species of *Commiphora*, the sister taxon to *Bursera* subg. *Bursera* (Weeks et al., 2005) or perhaps to all of *Bursera* (Becerra, 2003). We downloaded outgroup sequences from GenBank and sequenced missing markers from our collections. GenBank accession numbers are listed in Appendix A.

2.2. DNA extraction, PCR amplification, sequencing, and alignment

We extracted DNA with DNeasy Plant Mini Kits (Qiagen, Valencia, CA) from living and herbarium material (Appendix A). We sequenced five markers, two low-copy nuclear regions (the fourth intron of the phosphoenolpyruvate carboxylase gene, *PEPC*, and the third intron of the nuclear nitrate reductase gene, *NIAi3*), two multiple-copy regions (the internal and external transcribed spacer regions of the nuclear ribosomal DNA, *ITS* and *ETS*), and one from the chloroplast (the *psbA-trnH* intergenic spacer).

We used published primers and protocols to amplify *psbA-trnH* (Sang et al., 1997; Weeks and Simpson, 2004), *PEPC* (Olson, 2002), and *ETS* (Baldwin and Markos, 1998; Weeks and Simpson, 2004). We increased specificity for *PEPC* by raising the annealing temperature to 62 °C for eight samples. We amplified *ITS* with the external and internal primers of Fine et al. (2005), with the following protocol: 97 °C/3 min; nine cycles of 97 °C/1 min, with a touchdown starting at 56 °C/1 min and decreasing 1 °C every cycle, 72 °C/45 s + 4 s/cycle; 30 additional cycles with 48 °C as annealing temperature and a final extension at 72 °C/7 min. Finally, the protocol for *NIAi3* (Howarth and Baum, 2002) started at 94 °C/3 min; 14 cycles of 94 °C/1 min, with a touchdown starting at 62 °C/2 min and decreasing 1 °C each cycle, 72 °C/3 min; 21 additional cycles with

48 °C as annealing temperature and a final extension at 72 °C/7 min. PCR volumes of 25 μL included 10–100 ng of template DNA, 2.5 μL of 10× PCR buffer, 0.5 μL of 10 μM dNTPs in an equimolar ratio, 0.75 μL of 50 μM $MgCl_2$, 0.63 μL of each of the 10 μM primers, 5 μL of Q solution (Qiagen), and 1 U of *Taq* polymerase added after the initial denaturation step.

When necessary, we cloned PCR products by ligation into pGEM-T vector (Promega, Madison, WI) and transformation of competent *Escherichia coli* (JM109). We sequenced three positively transformed colonies for each sample cloned. Due to the presence of more than one product, we cloned the *NIAi3* PCR products of six samples, one of *B. permollis* (J. Linares 7326), two samples of *B. grandifolia* (Rosell 50 and Olson 1026) and three of *B. simaruba* (Rosell 46, Rosell 51, and Olson 1063). The other nuclear markers *ETS*, *ITS*, and *PEPC* were not cloned due to the observed congruence between their tree topologies; previous studies have shown coalescence within samples in closely related species for *ETS* (Weeks and Simpson, 2004).

PCR products were purified with QIAquick PCR purification kit (Qiagen), and sequenced bidirectionally using the BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) in final volumes of 10 μL. Sequencing products were cleaned with Sephadex G-50 (GE Healthcare Bio-Sciences, Piscataway, NJ) and run on an ABI 3100 automated DNA sequencer (Applied Biosystems). Sequences were edited using Sequencher v.4.6 (Gene Codes, Ann Arbor, MI) and aligned using Se-Al v.2.0a11 Carbon (Rambaut, 2002).

2.3. Maximum parsimony analyses (MP)

We analyzed data matrices by marker, in combined analyses with congruent markers as assessed by the incongruence length difference test (ILD, Farris et al., 1995), and also the five loci together. We included potentially informative characters only, which were unordered and weighted equally. We treated gaps as missing data and performed analyses in NONA 2.0 (Goloboff, 1999) using the parsimony ratchet (Nixon, 1999) with WinClada v.1.00.08 (Nixon, 2002) as a shell program. We conducted three searches with different starting seeds using 300 iterations (100 trees held per iteration). We sampled 10% of the characters for reweighting during the parsimony ratchet and calculated a strict consensus from most parsimonious trees. To evaluate support, we used TNT (Goloboff et al., 2008) running 1000 replicates with the "traditional search" approach with TBR set to 100 replications holding 50 trees, and saving the consensus of each resampling matrix. We assessed congruence between each marker using ILD tests as implemented in WinClada v.1.00.08, running 1000 replicates.

2.4. Bayesian analyses (BA)

We used ModelTest v.3.7 (Posada and Crandall, 1998) to determine the model of evolution that best fit each of the five markers and, as for parsimony, performed Bayesian analyses of each marker, combined data of congruent markers, and finally of the five loci combined. We used MrBayes v.3.2 (Huelsenbeck and Ronquist, 2001) run on the CIPRES portal v.2.2 (Miller et al., 2009). For combined datasets, we performed partitioned analyses, applying a different model of evolution to each marker (Table 2), and ran four Markov chains simultaneously for 15 million generations, starting with a random tree, setting the heating parameter to 0.01 to improve the probability of exchange between chains, which was very low with the default value of 0.2, and sampling trees every 200 generations. After 15 million generations, average split deviations were <0.01 and the plot of the likelihood score vs. MCMC generation suggested that stationarity had been reached after 3.75 million generations. Majority-rule consensus trees were derived from 56,250 trees after a 25% burn-in. Analyses by marker were run for five million generations under the same conditions as combined analyses, reaching average split deviations <0.007.

2.5. Species delimitation using molecular characters

Using combined analyses and analyses by marker derived from MP and BA, we assessed whether species were mono-, para-, or polyphyletic, or whether monophyly or paraphyly could not be rejected because of polytomies in the phylogenetic tree. In assessing the poly-, para-, or mono-phyletic status of a species, we considered nodes with parsimony bootstrap values (PB) $\geqslant$70% (Hillis and Bull, 1993) or posterior probabilities (PP) $\geqslant$0.95 to be strongly supported.

2.6. Species delimitation using morphological characters

We measured 476 herbarium specimens of eleven Mexican species of the *simaruba* complex, including all satellites except *B. itzae*, for which material was not available (Table 1). We measured 24 morphological variables of taxonomic importance (Table 3) on specimens deposited in the National Herbarium of Mexico (MEXU) and the Herbarium of the Centro Regional del Bajío (IEB). We included minimum and maximum values per specimen for continuous variables to reflect intraspecific variation. To evaluate the effectiveness and relative importance of these variables in separating species, we performed discriminant analyses in R v.2.9.2 (R Development Core Team, 2009). We log-transformed some variables to achieve normality and assessed homoscedasticity graphically; no variables had correlations >0.9. We centered and scaled all variables and there were no missing data. We repeated the discriminant analysis including only *B. simaruba* and its satellites to assess which variables were useful in discriminating these four morphologically similar species.

2.7. Species delimitation using ecological characters

To evaluate ecological differentiation between all satellites, except for *B. itzae*, and *B. simaruba*, we performed a principal

Table 2
Summary of parsimony analyses for each marker and combined markers (congruent markers *PEPC* + *ETS* + *ITS* and all five markers), and models of sequence evolution used for Bayesian analyses.

	No. maximum parsimonious trees (length)	Aligned length (bp)	No. parsimony informative characters (%)	CI	RI	RC	Model of sequence evolution
psbA-trnH	336 (84)	564	61 (11)	0.82	0.96	0.787	TVM+Γ
PEPC	71,174 (137)	588	88 (15)	0.77	0.93	0.716	HKY+Γ
ETS	49,090 (210)	389	84 (22)	0.56	0.86	0.482	TVM+I+Γ
ITS	58,220 (539)	754	164 (22)	0.48	0.76	0.365	GTR+I+Γ
NIAi3	645 (216)	679	135 (20)	0.81	0.95	0.770	TVM+I
PEPC+ETS+ITS	57,690 (993)	1731	336 (19)	0.48	0.78	0.374	–
Five markers	58,737 (1309)	2974	532 (18)	0.55	0.83	0.457	–

Table 3
Characters and results of morphometric analysis including all species and that including only *B. simaruba* and satellites. Only the first two discriminant functions (DF) are presented. *L*, length; min, minimum; max, maximum; *W*, width. Variables with high contribution to the DF in bold.

	All species		*B. simaruba* + satellites	
	DF1	DF2	DF1	DF2
Leaf *L* min	−0.048	0.26	−0.096	**−0.556**
Leaf *L* max	−0.131	−0.138	0.235	0.296
Leaf *W* min	0.172	−0.242	−0.238	0.041
Leaf *W* max	−0.234	0.176	−0.049	0.365
Petiole base pubescence[a]	−0.075	**−0.691**	**0.654**	−0.019
Petiole shaft pubescence[a]	−0.014	0.349	−0.027	−0.083
Petiole *L* min	−0.116	−0.135	0.09	0.463
Petiole *L* max	0.197	0.225	−0.334	−0.297
Petiolule *L* terminal leaflet min	0.133	−0.134	0.208	0.019
Petiolule *L* terminal leaflet max	0.041	0.122	−0.444	0.112
Petiolule *L* lateral leaflet min	0.089	−0.231	0.182	**−0.618**
Petiolule *L* lateral leaflet max	−0.048	−0.308	0.015	−0.255
Petiolule pubescence[a]	−0.206	−0.424	0.277	**−0.682**
Leaflet number min	−0.097	**−1.172**	**0.722**	**0.73**
Leaflet number max	**0.507**	**−0.557**	−0.124	−0.179
Leaflet upper surface pubescence[b]	**−1.474**	0.005	0.342	**−0.912**
Leaflet lower surface pubescence[c]	0.193	**0.56**	**−0.75**	**1.293**
Infructescence *L* min	0.103	−0.118	−0.218	0.082
Infructescence *L* max	0.11	0.226	0.016	−0.207
Infructescence robustness[d]	−0.037	**−0.628**	**0.953**	0.131
Fruit *L* min	−0.092	−0.339	0.037	0.234
Fruit *L* max	0.126	0.039	**0.583**	−0.03
Fruit apex[e]	0.103	0.19	0.095*	0.332*
Fruit pubescence[f]	**−2.663**	**−0.717**		

All characters were continuous, except [a]absent, scarce, abundant; [b]absent, present only on midvein, scarce on main veins, abundant on main veins; [c]absent, present only on midvein, present in two basal tufts, scarce on main veins, abundant on main veins; [d]slender, medium, robust; [e]obtuse, acute, acuminate; [f]present, absent; *excluded character.

components analysis (PCA) on 19 climatic variables extracted from WorldClim 1.4 (Table 4; Hijmans et al., 2005) based on 462 locality data from specimens at MEXU and IEB. Following Stockman and Bond (2007), we performed a multiple analysis of variance (MANOVA) on PC scores to assess differences between those species appearing as sister taxa according to our phylogenetic results. We assessed homoscedasticity of dependent variables before applying the MANOVA, and when this assumption was violated, we performed a non-parametric MANOVA (Anderson, 2001). To assess which PC accounted for the overall difference detected by the parametric and non-parametric MANOVAs, we performed *t*- or Mann–Whitney tests per species pairs, adjusting alpha levels to account for multiple tests. We performed these analyses with R.

Table 4
Results for the PCA of climatic variables. *T*, temperature; qtr, quarter; ppt, precipitation; Max, maximum; Min, minimum. Variables with high loadings in bold.

Variable	PC1	PC2	PC3
Annual mean *T*	**0.366**	−0.025	−0.039
Mean diurnal range	−0.174	0.228	0.075
Isothermality	0.167	0.194	**0.402**
T seasonality	−0.194	−0.118	**−0.481**
Max *T* warmest month	0.254	0.026	−0.243
Min *T* coldest month	**0.359**	−0.074	0.063
T annual range	−0.278	0.102	−0.212
Mean *T* wettest qtr	**0.332**	−0.04	−0.173
Mean *T* driest qtr	**0.352**	−0.044	0.04
Mean *T* warmest qtr	**0.331**	−0.064	−0.205
Mean *T* coldest qtr	**0.362**	0.024	0.139
Annual ppt	−0.045	**−0.37**	0.188
Ppt wettest month	−0.078	**−0.317**	0.277
Ppt driest month	−0.008	**−0.368**	−0.115
Ppt seasonality	−0.037	0.282	0.288
Ppt wettest qtr	−0.086	**−0.305**	0.309
Ppt driest qtr	−0.004	**−0.374**	−0.104
Ppt warmest qtr	−0.091	−0.222	0.289
Ppt coldest qtr	−0.004	**−0.369**	−0.031

3. Results

Alignment was unambiguous, except for a portion of *PEPC*, which we excluded from the analyses (positions 836–886 of the matrix with concatenated markers). Alignments are available from TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S10354).

3.1. Analyses of single markers and ILD tests

The clones of *ETS* downloaded from GenBank (Appendix A), and the clones of *NIAi3* of *B. permollis* (J. Linares 7326), and two of the three samples of *B. simaruba* (Rosell 46 and Olson 1063) coalesced per sample under MP, so consensus sequences were used for terminal taxa. The remaining three samples for which we cloned *NIAi3* did not coalesce. Two sequences of *NIAi3*, differing in five nucleotides, were present in two samples of *B. grandifolia* (Rosell 50 and Olson 1026) and grouped with other *B. grandifolia* collections but not with each other. To streamline combined analyses, whose main purpose was to identify relationships among *simaruba* complex members rather than within *B. grandifolia*, we generated a consensus sequence. One sample of *B. simaruba* (Rosell 51) had two different sequences differing mainly in a 26 bp deletion and 14 nucleotides. The other 27 *B. simaruba* samples had only one or the other of these two sequences. Stop codons in the coding portion (the third exon), which would indicate loss of function, were absent in both sequences, so we decided to include the two *NIAi3* sequences of *B. simaruba* as separate terminal taxa (* in Figs. 3 and 4) duplicating all the other markers.

We include the lengths of the datasets and results from MP by marker in Table 2. Each marker separately recovered only certain groups of species. Fig. 3 includes the results of *psbA-trnH* and *NIAi3* in isolation, and of the combined analyses of the other nuclear markers, which were congruent according to ILD tests. Pairwise ILD tests showed that significant incongruence was mostly restricted to *psbA-trnH* and *NIAi3* with respect to all the other markers. No significant incongruence was found between *PEPC*

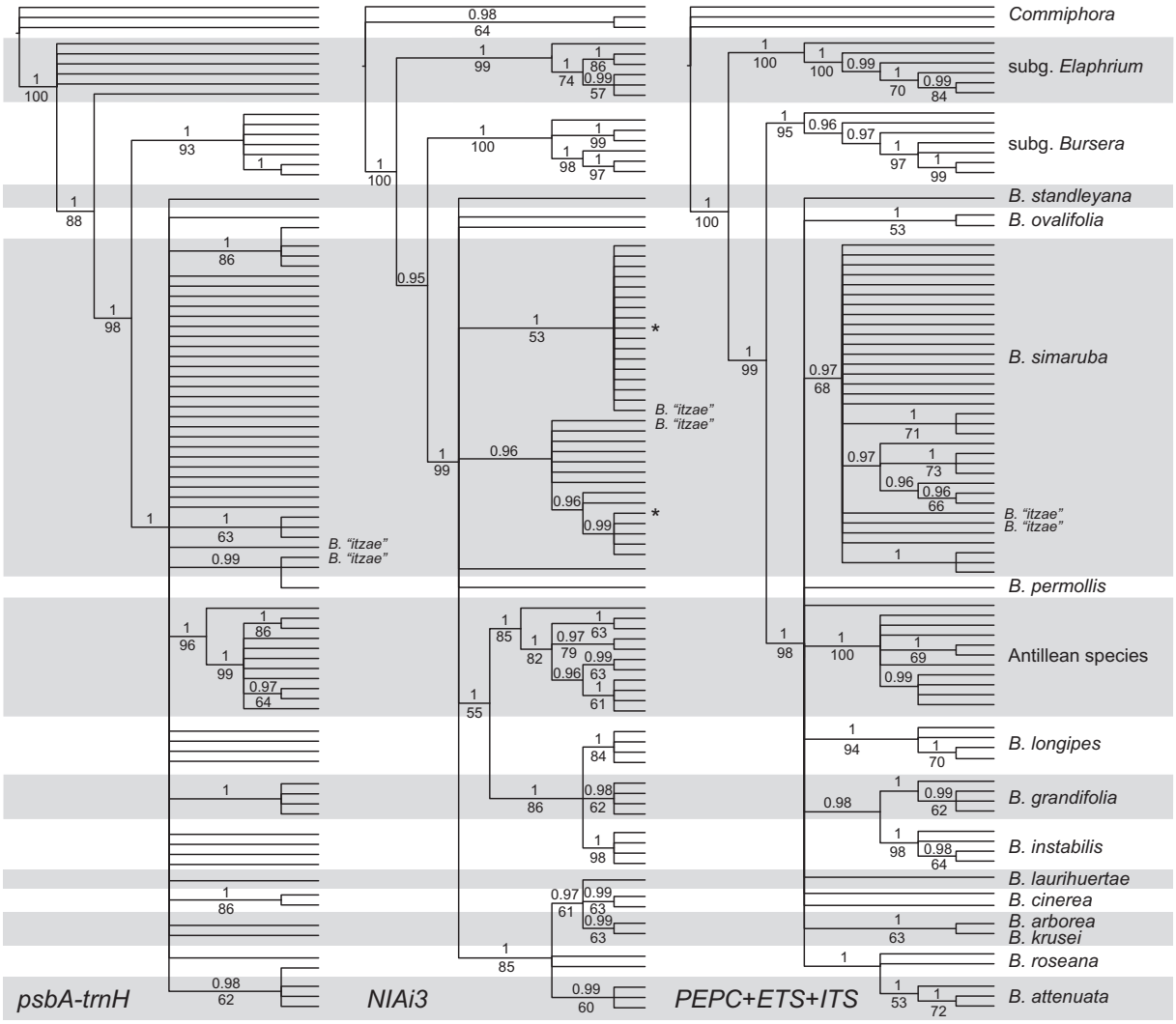

Fig. 3. Congruence between markers in the phylogenetic reconstruction of the *simaruba* complex and its related Antillean species. Bayesian 95% majority-rule consensus cladograms based on *psbA-trnH*, *NIAi3*, and the combined analysis of *PEPC*, *ETS*, *ITS*. Posterior probabilities are shown above branches, and parsimony bootstrap values >50 below branches; ∗: clones of the *B. simaruba* sample with two different versions of *NIAi3*.

and *ETS* ($p = 0.393$), *PEPC* and *ITS* ($p = 0.114$), *ETS* and *ITS* ($p = 0.173$), and between *psbA-trnH* and *ITS* ($p = 0.06$).

The chloroplast marker mostly informed regarding the deepest relationships, poorly resolving the complex itself (Fig. 3). All markers showed maximum phylogenetic support (PP = 1.0, PB = 100) for the genus *Bursera* and most recovered clades of its two subgenera. A well-supported clade including the *simaruba* complex species and the Antillean species was recovered by all markers with BA (PP $\geqslant$ 0.98) and by *PEPC*, *ETS* and *NIAi3* under MP (PB $\geqslant$ 82). All markers recovered a well-supported clade including all Antillean species (PP = 1.0, PB $\geqslant$ 85), but with *ETS* and *PEPC* the clade excluded *B. spinescens*. With most markers, this Antillean clade appeared in a polytomy with clades of the *simaruba* complex, but with *NIAi3* it was recovered well within the *simaruba* complex as sister to the *grandifolia-longipes* clade with strong Bayesian support (Fig. 3). Two well-supported clades of *B. simaruba* samples were recovered by *NIAi3*, each composed of one of the two different sequences found in the cloned *B. simaruba* sample (Fig. 3).

3.2. Analyses from combined-marker datasets

The aligned combined dataset had a length of 2974 bp. In combination, the five loci strongly supported a clade including the species of the *B. simaruba* complex and also of the Antillean group (Fig. 4). Although major clades in the phylogeny had good support, deeper relationships were not fully resolved. The Antillean clade was maximally supported, but its position was unresolved, as was the case also for *B. permollis* and *B. standleyana* (Fig. 4). Satellite species *B. attenuata* and *B. roseana* grouped in a clade that did not include *B. simaruba*, whereas *B. "itzae"* samples appeared intercalated among *B. simaruba* samples. Finally, *B. ovalifolia* appeared as sister of *B. permollis,* but this relationship had low support (Fig. 4).

3.3. Species delimitation using molecular characters

In the five-marker analyses, *B. simaruba* samples formed a clade with strong support, which also included *B. "itzae"* samples (Fig. 4). Well-supported groups with geographic affinity were recovered within the clade, including a Costa Rican Pacific coast group, a Caribbean group (with collections from the Dominican Republic, and most of those from the Yucatan and Cuba), and the Mexican Pacific coast group (Nayarit, Colima, and all but one Jalisco sample; Fig. 4). *NIAi3* recovered two well-supported clades of *B. simaruba*, but left out one sample (Fig. 3). A clade with all but one sample of *B. simaruba* and *B. "itzae"* was recovered by *ITS* (PP = 0.94) and

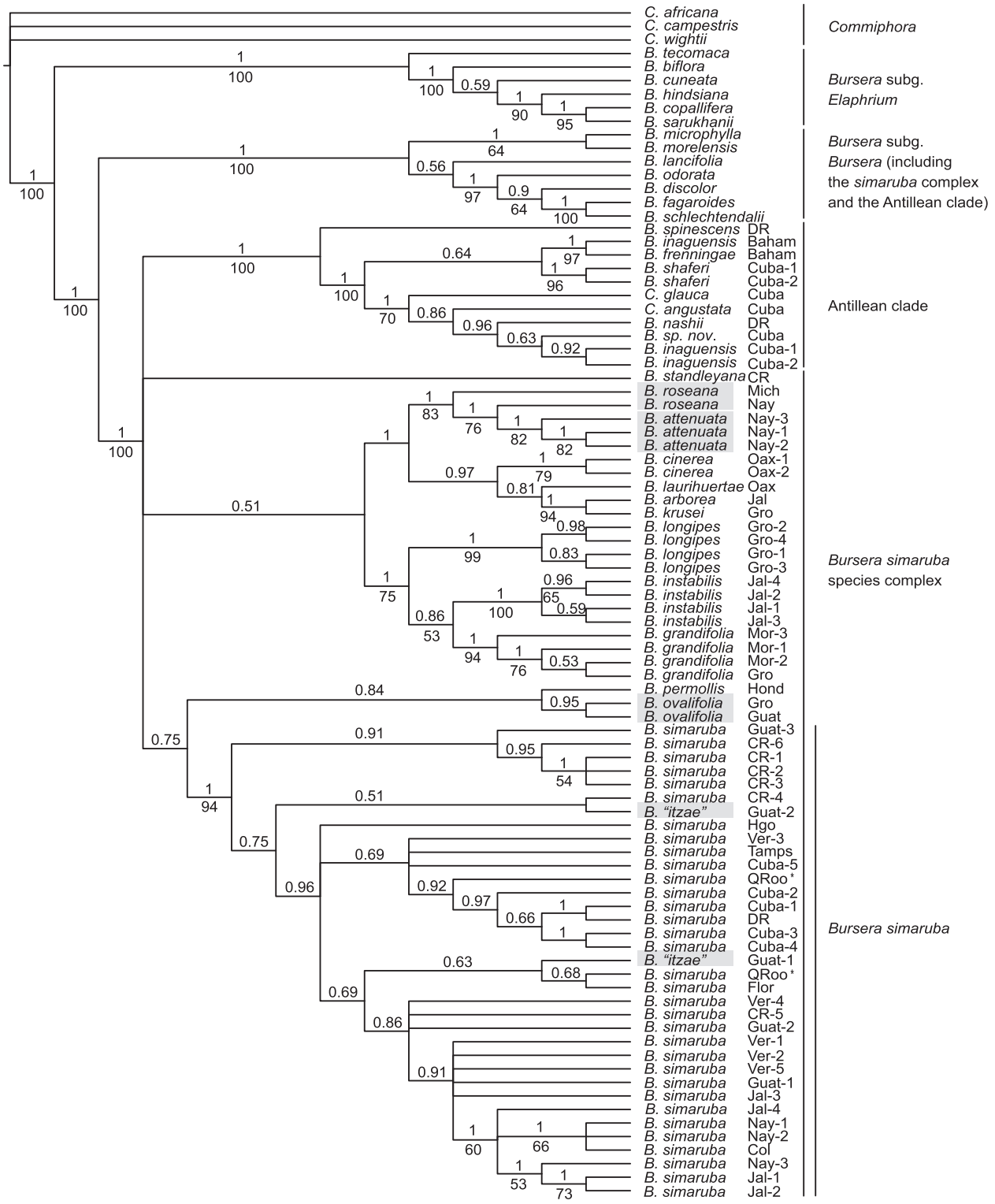

Fig. 4. Phylogeny of the *Bursera simaruba* species complex and its related Antillean species based on five markers. Bayesian 50% majority-rule consensus cladogram is shown with posterior probabilities above branches, and parsimony bootstrap values > 50 below branches. Labels on terminals indicate voucher locality (Appendix A); gray boxes highlight *simaruba* satellite species; ∗: clones of the *B. simaruba* sample with two different versions of *NIAi3*.

also with *ETS* (PP = 0.98) but included *B. arborea* and *B. krusei*. When *ITS*, *PEPC*, and *ETS* were combined, a well-supported clade of *B. simaruba* samples was recovered (Fig. 3).

Two of the four satellites were non-mono-phyletic, while the other two were recovered as strongly supported clades. In the five-marker analyses, *B. roseana* was paraphyletic in a clade that also included a strongly-supported group of *B. attenuata* samples (Fig. 4). The *B. attenuata* group was recovered by *ITS* (PP ⩾ 0.97, PB = 54) and *NIAi3* (Fig. 3), but included also a *B. roseana* accession in the case of *PEPC* and *psbA-trnH* (Fig. 3). The other *B. roseana* collection usually appeared in a polytomy at the next highest level in the tree. The two *B. ovalifolia* samples were recovered as a clade with

strong support in the combined analyses (Fig. 4) and by *ITS* (PP = 1.0, PB = 70). The other markers did not reject its monophyly, except for *psbA-trnH*, which recovered a *B. ovalifolia* sample in a strongly supported clade with *B. simaruba* samples (Fig. 3). In contrast, the two samples from the type locality of *B. itzae* were recovered as polyphyletic within the clade of *B. simaruba* samples in the combined analyses and with *NIAi3* and *psbA-trnH* (Fig. 3), and in a polytomy including these samples with all other individual markers.

Finally, *B. inaguensis* appeared polyphyletic, with Cuban samples included with other Cuban species and *B. nashii*, whereas *B. inaguensis* from the Bahamas was sister to *B. frenningae* (Fig. 4). Under BA, *ETS, ITS*, and *NIAi3* also supported the polyphyletic status of *B. inaguensis*, but its potential monophyly was not rejected under MP or by the remaining markers. All other species represented by two or more samples appeared monophyletic in the combined analysis (Fig. 4) or their monophyly could not be rejected by individual markers (Fig. 3).

3.4. Species delimitation using morphological characters

The first two discriminant functions (DF) of the analysis including all species explained 69% of the observed variance (DF1 = 55%, DF2 = 14%). DF1 indicated that fruit and upper leaflet surface pubescence and the maximum number of leaflets are the most important characters for discriminating between species, followed by the minimum number of leaflets, petiole base and lower leaflet surface pubescence, and infructescence robustness, characters with high coefficients for DF2 (Table 3). A plot of these two DFs shows *B. grandifolia* and *B. krusei* close to one another due to their pubescent fruits and leaflets (Fig. 5A), but because of its more numerous leaflets, *B. grandifolia* was lower on the vertical axis. Specimens of *B. cinerea*, a species with pubescent leaflets but glabrous fruits, appeared to the right and close to *B. arborea*, which has pubescent to glabrous leaflets. Another cluster was formed by *B. instabilis* and *B. laurihuertae*, which share the glabrous one to three-foliolate condition, although some specimens of *B. instabilis* showed scant pubescence and are higher on the vertical axis. A large group formed by *B. simaruba* and its satellites fell to the right because of their glabrous fruits and upper leaflet surfaces, and higher maximum leaflet number. *Bursera longipes* overlapped with this group, but is readily distinguished by its bluish waxy upper cuticle and small habit. Some specimens fell far from the species they belong to, e.g. the pubescent-fruited specimens of *B. simaruba* (Fig. 5A). Leave-one-out classification tables revealed that species were classified correctly >92% of the time except for *B. simaruba*, which was misclassified 10% of the time, *B. attenuata* 12%, *B. ovalifolia* 12%, *B. roseana* 28%, *B. arborea* 18%, and *B. instabilis* 38% (Appendix C, Supplementary material). Misclassifications of *B. simaruba* and its satellites were mostly due to assignment to another of these four taxonomically difficult species. Specimens of *B. instabilis* were often misclassified as *B. laurihuertae*, a situation that could be avoided by including leaflet apex and shape, which readily distinguish these species.

In the discriminant analysis of *B. simaruba* and its satellites, fruit pubescence was removed because it was present in only four specimens. The first two DF explained 93% (DF = 72%, DF2 = 21%) of total observed variance. To discriminate between *B. simaruba* and its satellites, infructescence robustness, leaflet lower surface and petiole base pubescence, minimum number of leaftlets, and maximum fruit length were useful characters, followed by leaflet upper surface and petiolule pubescence, minimum lateral leaflet petiolule length, and minimum leaf length (Table 3). Although these characters tended to separate satellites, some specimens overlapped, highlighting the continuous ranges of some of these characters (Fig. 5B). DF1 separated *B. simaruba* with its robust infructescences, more numerous leaflets, large fruits, and pubescent petiole bases. On the left side of the graph, *B. attenuata* has the opposite characteristics. The other two satellites, *B. ovalifolia* and *B. roseana*, could not be separated on the basis of DF1 alone because of their wide ranges in leaflet lower surface pubescence and infructescence robustness (Appendix B). In contrast, DF2 helped separate *B. ovalifolia* because of its long lateral leaflet petiolules. Classification of *B. simaruba* and *B. ovalifolia* tended to be correct (>92%), but not of *B. attenuata* (88%) and *B. roseana* (62%, Appendix C).

3.5. Satellite species delimitation using ecological characters

The first three PCs of environmental variables accounted for 82% of the variability (PC1 = 38%, PC2 = 33%, PC3 = 11%). Six temperature variables had high loadings on PC1, six precipitation variables on

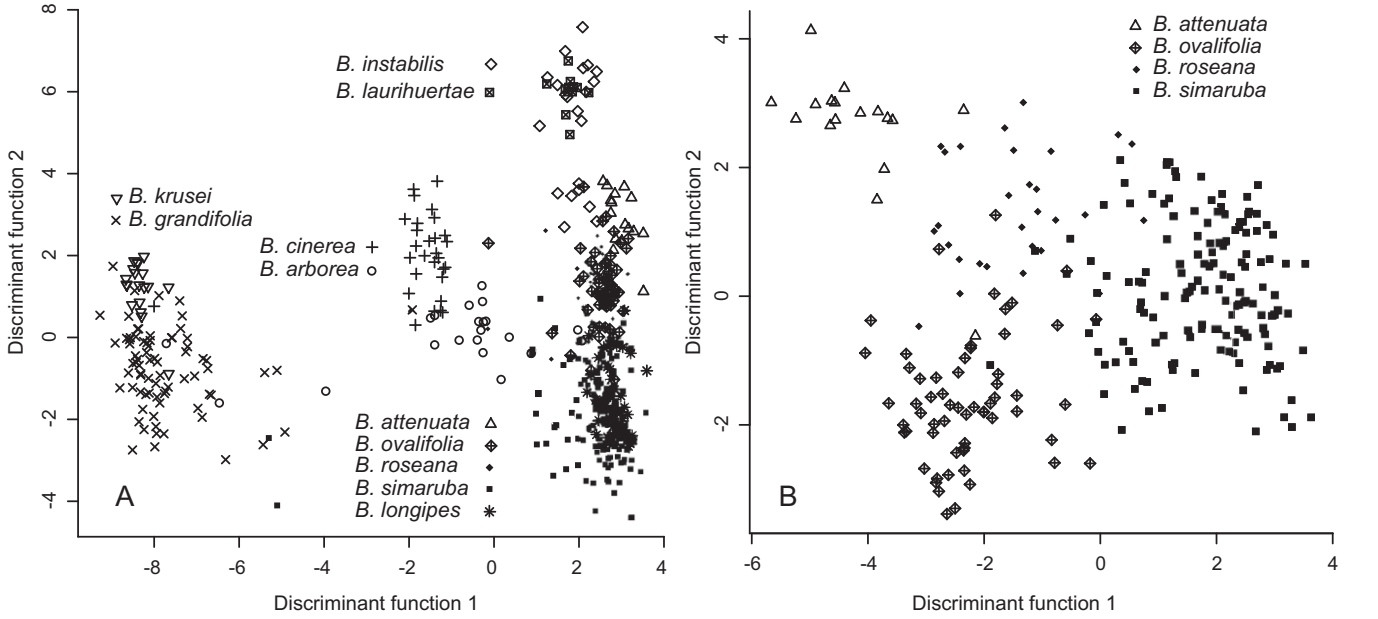

Fig. 5. Morphological phenetic analysis of the Mexican members of the *simaruba* complex. Distribution of specimens according to the first two discriminant functions including all *simaruba* complex species (A) and only *B. simaruba* and its satellite species (B), showing marked overlap between species, especially the satellites.

PC2, and isothermality and temperature seasonality on PC3 (Table 4). We compared ecologically *B. attenuata* and *B. roseana*, which appeared as sister taxa in our phylogeny, and also *B. simaruba* and *B. ovalifolia*, a relationship resulting from one of the possible locations of *B. ovalifolia*, given its unresolved position in the phylogeny, but the only one that would be of interest for this comparison given our goal of using ecological information to delimit satellite species from *B. simaruba*. The MANOVA of the first three PCs rejected the null hypothesis of no ecological differences between *B. attenuata* and *B. roseana* ($F_{3,78} = 11.07$, $p < 0.001$), but only PC2, which was related to precipitation, showed significant differences ($t = -4.717$, $p < 0.001$). A non-parametric MANOVA of the first three PCs rejected the hypothesis of no differences between *B. ovalifolia* and *B. simaruba* ($F_{1,378} = 52.17$, $p < 0.001$). Mann–Whitney tests found differences between these two species regarding temperature (PC1: $W = 18360.5$, $p < 0.001$), precipitation (PC2: $W = 22111.5$, $p < 0.001$), and seasonality (PC3: $W = 19012.5$, $p < 0.001$).

4. Discussion

4.1. Species delimitation and the taxonomy of the simaruba complex

We recovered the two most widespread species of the complex as monophyletic. Given its morphological variability and wide range, along with the confusing taxonomy of the complex, we fully expected to recover a paraphyletic *B. simaruba*. However, not only was the species monophyletic but the apparently weak geographic structuring of its populations (Fig. 4) implies genetic cohesion between distant populations via pollen and seed dispersal (Dunphy and Hamrick, 2007; Greenberg et al., 1995; Sousa, 1969). *Bursera simaruba* monophyly is based on considering *B. itzae* a likely synonym of *B. simaruba*, an issue discussed below. Like *B. simaruba*, and despite a long history of nomenclatural changes and wide distribution (Rzedowski et al., 2007; Table 1, Fig. 2), *B. ovalifolia* collections from distant localities formed a single clade. Although one of the possible positions of *B. ovalifolia* is as sister to *B. simaruba* and their ranges overlap (Fig. 2), their ecological conditions differ in temperature, precipitation, and seasonality, suggesting they are not ecologically interchangeable and supporting the validity of *B. ovalifolia* as a separate species.

The three species recovered as non-monophyletic highlight the need for taxonomic revision of these taxa. The paraphyletic pattern of *B. roseana* could suggest that *B. attenuata* is derived from this species and reciprocal monophyly has not been attained, or that both species are in fact the same taxon. *Bursera attenuata* is conceived of as a lowland entity and *B. roseana* its highland counterpart, so we expected their environmental conditions to differ strongly. Surprisingly, only precipitation differed (PC2). Given that their pubescence patterns overlap considerably, and to a lesser extent their distributions (Fig. 2), it may be that the two species are a single entity that varies with elevation (McVaugh and Rzedowski, 1965). With regard to *B. itzae*, it was polyphyletically embedded within *B. simaruba* (Fig 4). *Bursera itzae* is distinguished from *B. simaruba* by its relatively long inflorescences (15–20 cm; Lundell, 1968). Not known when *B. itzae* was described, however, is that this characteristic varies considerably within *B. simaruba*, especially in Guatemala (M. Véliz, pers. comm.) and adjacent Mexico, where we have observed inflorescences up to 22 cm long. Although our results await confirmation based on unequivocally identified fertile material, we suspect that *B. itzae* simply represents a variant of *B. simaruba* with long inflorescences. Finally, Cuban and Bahamian collections of *B. inaguensis* appeared in two different places in the phylogeny (Fig. 4). Our Cuban collections differ markedly from Bahamian material in leaf and inflorescence features, making it likely that Cuban *B. inaguensis* represents a distinct species.

As was the case with molecular characters, species of the *simaruba* complex could for the most part be reliably distinguished in discriminant analyses of morphological characters (Fig. 5, Appendix C), despite their pronounced morphological plasticity (Appendix B). Although pubescence patterns were crucial for discriminating species (Table 3), the ranges and patterns of expression of indumentum are poorly understood in the complex. Bewilderingly, pubescence may vary between populations, individuals, and even leaflets of the same leaf. In addition, the expression of pubescence in one part of the leaf may not be independent of its production in other parts. For example, the development of trichomes on the lower surface of leaflets seems necessary for production on the upper surface. Ascertaining the independence or lack thereof of pubescence states will determine how they can be coded and interpreted in future analyses. Because of high levels of variation, accurate identification of some species in the clade, particularly the satellites, would seem to require extensive collections from different parts of individual trees, as well as from numerous individuals of the same population. In cases of unusual combinations of morphological characters, identification via molecular characters may be the only alternative at present.

4.2. The origin of satellite species

Our results do not support the origin of the satellites *B. attenuata, B. ovalifolia*, and *B. roseana* from *B. simaruba*, suggesting instead that their similarity is due to convergence or parallelism. Distant *B. attenuata* and *B. roseana* are clearly not derived from *B. simaruba* (Fig. 4). As for *B. ovalifolia,* support for its position as sister to *B. permollis* in the analyses with all markers was low, so one of its possible positions is as sister to *B. simaruba* (Fig. 4), making the origin of *B. ovalifolia* from *B. simaruba* a possibility. Nevertheless, support for a *ovalifolia-permollis* clade becomes strong in the absence of the chloroplast marker (see Section 4.3 and Fig. 7, Supplementary material), a topology that would translate into rejecting the origin of *B. ovalifolia* from *B. simaruba*. The similarity between *B. simaruba* and the satellites is in a suite of features, many of which occur in other species but all of which tend to be found together only in these four species (Fig. 6, Appendix B). Given that most species in the genus are trees of tropical deciduous forests or shrubs of xerophytic scrubs, it is likely that the massive size of some populations of *B. simaruba* and its satellites derives from their repeated invasions of moister forests (Fig. 6).

4.3. Congruence and testing membership in the simaruba complex

Although the recovery of some species as members of the *simaruba* complex clade reaffirmed previous results (Becerra, 2003), the placement of others was heretofore uncertain. This was the case of the unifoliolate Oaxacan endemic *B. laurihuertae* (Rzedowski and Calderón, 2000), whose position among species with several leaflets makes it another example of the changes in leaflet number widespread in the genus (Rzedowski and Kruse, 1979). Likewise, the Central American *B. standleyana* and *B. permollis* were confirmed as belonging to the complex. Although unresolved, the distant placement of *B. permollis* from *B. grandifolia* suggests that the synonymy proposed for these two species (Daly, 1993; Porter and Pool, 2001) is unnecessary.

Although we recovered a single clade including all Antillean species (Figs. 3 and 4), its position as sister to or part of the *simaruba* complex could not be resolved. Based on our five marker reconstruction, the placement of the Antillean clade as sister to the whole *simaruba* complex (Weeks and Simpson, 2004) or to the *grandifolia-longipes* clade, as suggested by *NIAi3*, results in a tree with the same number of steps. Because of the numerous synapomorphies supporting the Antillean clade itself, it seems

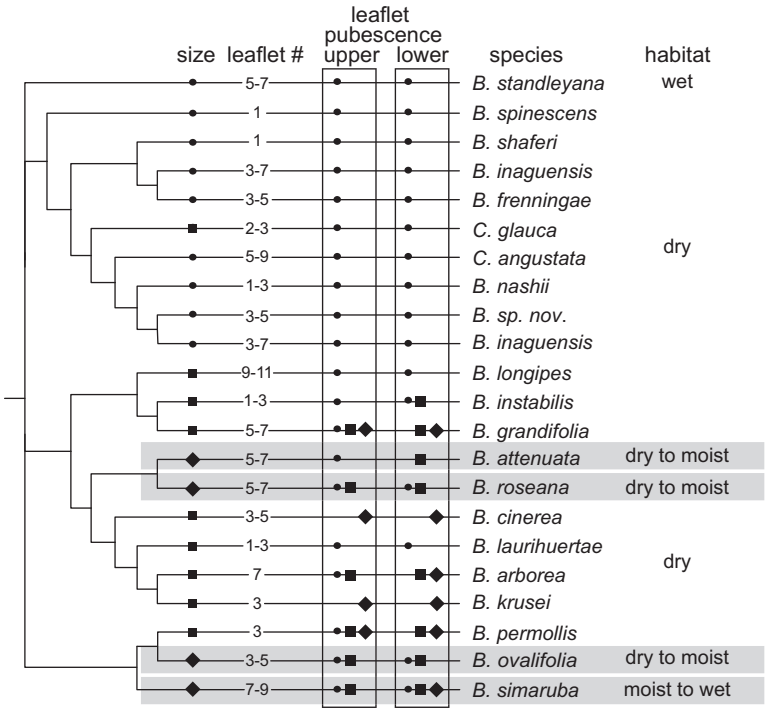

Fig. 6. Convergence/parallelism in the *simaruba* complex. Similarity between *B. simaruba* and its satellites mapped on the phylogeny of the *simaruba* complex and its related Antillean clade, illustrating the similarity between satellites and *B. simaruba* in a suite of features. Size: circle = small tree or shrub (<8 m), square = small to medium tree (<16 m), diamond = small to large tree (>16 m); Pubescence: circle = glabrous, square = sparsely pubescent, diamond = pubescent; Habitat: dry = tropical deciduous forest, moist = tropical subdeciduous forest; wet = tropical rainforest.

unlikely that its ambiguous placement is due to hybridization or lineage sorting, but rather that more characters are needed. This lack of information could also explain the unresolved position of *B. standleyana*, whose molecular changes were also mostly autapomorphic. The position of *B. permollis* within a strongly supported clade of *B. simaruba* samples in the analyses of *psbA-trnH* contrasts with its unresolved position in the analyses by other markers (Fig. 3), and also with its strongly supported location as sister to *B. ovalifolia* when *psbA-trnH* is excluded from analysis (Fig. 7, Supplementary material). This incongruence between the chloroplast and nuclear markers could suggest genetic influence of *B. simaruba* in this sample.

The two main clades within *B. simaruba* reflect two *NIAi3* sequence variants that differ mainly in a characteristic deletion. In general, each *B. simaruba* sample had one or the other of these variants, but both occurred together in one sample. These two sequence types may represent different alleles or paralogues. Differences between the sequences are comparable to those found between alleles in *Scaevola* (Howarth and Baum, 2005), which, together with the absence of stop codons, would suggest that the versions are alleles rather than pseudogenes. At the same time, duplications of *NIAi3* have been found in the tribe Canarieae within Burseraceae (Weeks, 2009), and in other families (Hamman-Khalifa et al., 2007; Yi et al., 2008), although the sequences found in *B. simaruba* did not correspond to those in Canarieae. Whether they represent alleles or paralogues with potential associated lineage sorting, in the genus these sequences seem to be restricted to *B. simaruba* (J.A. De-Nova, unpublished results), and they would not affect the phylogenetic reconstruction of the *simaruba* complex.

5. Conclusion

As in many species complexes, molecular tools proved invaluable in our analysis of the *simaruba* clade, in which high levels of intraspecific morphological plasticity coexist with marked overlap between species. Results pinpointed priority species that require further sampling for molecular and morphological work, and also characters that need to be reexamined to refine the taxonomy of the group. The molecular phylogeny of the *simaruba* complex allowed us to reject the origin of three satellite species from their purported ancestor, *B. simaruba*, and to ascribe their morphological similarity as likely being due to convergence or parallelism. Disentangling the effects of similarity due to common ancestry, convergence, or parallelism, if they can in fact be separated, is a major challenge of species complex biology, especially as evolutionary developmental biology blurs some of these distinctions (Abouheif, 2008; Kim et al., 2003). The *simaruba* complex appears to provide excellent examples for studies of adaptive divergence, convergence, or parallelism, and our phylogeny offers an essential framework for any such effort using the *simaruba* complex as a model system.

Acknowledgments

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Appendix A.

Voucher information, sample labels and GenBank accession numbers (*psbA-trnH*, *PEPC*, *ETS*, *ITS*, *NIAi3*) for the species sampled. All vouchers are deposited in MEXU, unless otherwise stated. Mx: Mexico; NA: not available. GenBank accession numbers of previously published sequences are designated by a superscript corresponding to the following publications: [a]Becerra (2003), [b]Weeks and Simpson (2004), [c]Weeks et al. (2005), [d]Weeks and Simpson (2007).

B. arborea: **Jal** = Chamela, Jalisco, Mx (Rosell 16), GQ377870, GQ377934, GQ378006, GQ378070, GQ378140.

B. attenuata: **Nay-1** = Tepic, Nayarit, Mx (R. Medina 3412), GQ377871, GQ377935, GQ378007, GQ378071, GQ378141; **Nay-2** = Santa Ma. Del Oro, Nayarit, Mx (R. Medina 3417), GQ377872, GQ377936, GQ378008, GQ378072, GQ378142; **Nay-3** = Santa Ma. Del Oro, Nayarit, Mx (R. Medina 3423), GQ377873, GQ377937, GQ378009, GQ378073, GQ378143.

B. cinerea: **Oax-1** = Jayacatlán, Oaxaca, Mx (Rosell 40), GQ377874, GQ377938, GQ378010, GQ378074, GQ378144; **Oax-2** = Teotitlán, Oaxaca, Mx (R. Medina 1281), GQ377875, GQ377939, GQ378011, GQ378075, GQ378145.

B. frenningae: **Baham** = Fairchild Tropical Garden, Bahamas (79410F, TEX), AY309392[b], AY309366[b], AY309311–AY309313[b], GQ378076, GQ378152.

B. grandifolia: **Mor-1** = Alpuyeca, Morelos, Mx (Rosell 50), GQ377876, GQ377940, GQ378012, GQ378077, GQ378146-GQ378147; **Mor-2** = Atlacholoaya, Morelos, Mx (Rosell 5), GQ377877, GQ377941, GQ378013, GQ378078, GQ378148; **Mor-3** = Atlacholoaya, Morelos, Mx (Olson 1026), GQ377878, GQ377942, GQ378014, GQ378079, GQ378149-GQ378150; **Gro** = Taxco, Guerrero, Mx (R. Medina 3977), GQ377879, GQ377943, GQ378015, GQ378080, GQ378151.

B. inaguensis: **Cuba-1** = Rafael Freyre, Holguín, Cuba (42572, HAC), GQ377880, GQ377944, GQ378016, GQ378081, GQ378153; **Cuba-2** = Bahía de Naranjos, Holguín, Cuba (42569, HAC), GQ377881, GQ377945, GQ378017, GQ378082, GQ378154; **Baham** = Inagua, Fairchild Tropical Garden (64269M, TEX), AY309393[b], AY309367[b], AY309314-AY309316[b], GQ378083, GQ378155.

B. instabilis: **Jal-1** = Chamela, Jalisco, Mx (Rosell 11), GQ377882, GQ377946, GQ378018, GQ378084, GQ378156; **Jal-2** = Chamela, Jalisco, Mx (Rosell 12), GQ377883, GQ377947, GQ378019, GQ378085, GQ378157; **Jal-3** = Chamela, Jalisco, Mx (Rosell 13), GQ377884, GQ377948, GQ378020, GQ378086, GQ378158; **Jal-4** = Chamela, Jalisco, Mx (J.C. Montero 923), GQ377885, GQ377949, GQ378021, GQ378087, GQ378159.

B. "itzae": **Guat-1** = Lake Petén Itzá, Guatemala (J.C. Montero 1029), GQ377886, GQ377950, GQ378022, GQ378088, GQ378160; **Guat-2** = Lake Petén Itzá, Guatemala (J.C. Montero 1030), GQ377887, GQ377951, GQ378023, GQ378089, GQ378161.

B. krusei: **Gro** = Río Papagayo, Guerrero, Mx (R. Medina 4045), GQ377888, GQ377952, GQ378024, GQ378090, GQ378162.

B. laurihuertae: **Oax** = Tehuantepec, Oaxaca, Mx (Olson 1119), GQ377889, GQ377953, GQ378025, GQ378091, GQ378163.

B. longipes: **Gro-1** = La Organera, Guerrero, Mx (Olson 1029), GQ377890, GQ377954, GQ378026, GQ378092, GQ378164; **Gro-2** = Xalitla, Guerrero, Mx (Cervantes 5), GQ377891, GQ377955, GQ378027, GQ378093, GQ378165; **Gro-3** = Xochipala, Guerrero, Mx (Olson 1028), GQ377892, GQ377956, GQ378028, GQ378094, GQ378166; **Gro-4** = Xochipala, Guerrero, Mx (R. Medina P-6), GQ377893, GQ377957, GQ378029, GQ378095, GQ378167.

B. nashii: **DR** = Dominican Republic (Weeks 01-VIII-22–1, TEX), AY309389[b], AY309363[b], AY309302-AY309304[b], NA, GQ378168.

B. ovalifolia: **Gro** = Río Papagayo, Guerrero, Mx (Olson 1128), GQ377894, GQ377958, GQ378030, GQ378096, GQ378169; **Guat** = La Antigua, Sacatepéquez, Guatemala (Olson 1133), GQ377895, GQ377959, GQ378031, GQ378097, GQ378170.

B. permollis: **Hond** = Maraita, Francisco Morazán, Honduras (J. Linares 7326), GQ377896, GQ377960, GQ378032, GQ378098, GQ378171–GQ378172.

B. roseana: **Mich** = Río Cupatitzio, Michoacán, Mx (Rosell 25), GQ377899, GQ377963, GQ378035, GQ378101, GQ378175; **Nay** = El Cuarenteño, Nayarit, Mx (R. Medina 3406), GQ377900, GQ377964, GQ378036, GQ378102, GQ378176.

B. simaruba: **Col** = Tecomán, Colima, Mx (J. C. Montero 938), GQ377897, GQ377961, GQ378033, GQ378099, GQ378173; **Hgo** = Tolantongo, Hidalgo, Mx (R. Medina 4430), GQ377901, GQ377965, GQ378037, GQ378103, GQ378177; **Jal-1** = Chamela, Jalisco, Mx (Olson 1078), GQ377902, GQ377966, GQ378038, GQ378104, GQ378178; **Jal-2** = Chamela, Jalisco, Mx (Olson 1079), GQ377903, GQ377967, GQ378039, GQ378105, GQ378179; **Jal-3** = Chamela, Jalisco, Mx (Olson 1080), GQ377904, GQ377968, GQ378040, GQ378106, GQ378180; **Jal-4** = Tomatlán, Jalisco, Mx (J.C. Montero 932), GQ377898, GQ377962, GQ378034, GQ378100, GQ378174; **Nay-1** = Tepic, Nayarit, Mx (R. Medina 3413), GQ377924, GQ377989, GQ378061, GQ378127, GQ378204; **Nay-2** = Tepic, Nayarit, Mx (R. Medina 3414), GQ377925, GQ377990, GQ378062, GQ378128, GQ378205; **Nay-3** = Tepic, Nayarit, Mx (Olson 1072), GQ377905, GQ377969, GQ378041, GQ378107, GQ378181; **QRoo** = Cozumel, Quintana Roo, Mx (Rosell 51), GQ377906, GQ377970, GQ378042, GQ378108, GQ378182–GQ378183; **Tamps** = Ciudad Mante, Tamaulipas, Mx (Olson 1040), GQ377907, GQ377971, GQ378043, GQ378109, GQ378184; **Ver-1** = Los Tuxtlas, Veracruz, Mx (Rosell sn), GQ377908, GQ377972, GQ378044, GQ378110, GQ378185; **Ver-2** = Los Tuxtlas, Veracruz, Mx (Rosell 44), GQ377909, GQ377973, GQ378045, GQ378111, GQ378186; **Ver-3** = Los Tuxtlas, Veracruz, Mx (Rosell 46), GQ377910, GQ377974, GQ378046, GQ378112, GQ378187–GQ378189; **Ver-4** = Los Tuxtlas, Veracruz, Mx (Rosell 45), GQ377911, GQ377975, GQ378047, GQ378113, GQ378190; **Ver-5** = Isla, Veracruz, Mx (Olson 1034), GQ377912, GQ377976, GQ378048, GQ378114, GQ378191; **CR-1** = Palo Verde, Guanacaste, Costa Rica (A. Fernández & C. Hood 1128, MO), GQ377913, GQ377977, GQ378049, GQ378115, GQ378192; **CR-2** = Palo Verde, Guanacaste, Costa Rica (M.F. Quigley 856, MO), GQ377914, GQ377978, GQ378050, GQ378116, GQ378193; **CR-3** = Palo Verde, Guanacaste, Costa Rica (Olson 1046, INB), GQ377915, GQ377979, GQ378051, GQ378117, GQ378194; **CR-4** = La Selva, Heredia, Costa Rica (Whitson 343, MO), GQ377916, GQ377980, GQ378052, GQ378118, GQ378195; **CR-5** = Cahuita, Limón, Costa Rica (Olson 1063), GQ377917, GQ377981, GQ378053, GQ378119, GQ378196–GQ378197; **CR-6** = La Cangreja, San José, Costa Rica (Olson 1061, INB), GQ377918, GQ377982, GQ378054, GQ378120, GQ378198; **Cuba-1** = Bahía de Naranjos, Holguín, Cuba (42570, HAC), GQ377919, GQ377983, GQ378055, GQ378121, GQ378199; **Cuba-2** = Gibara, Holguín, Cuba (C. Martínez et al., 2284, RAS), GQ377920, GQ377984, GQ378056, GQ378122, NA; **Cuba-3** = Gibara, Holguín, Cuba (C. Martínez et al., 2285, RAS), GQ377921, GQ377985, GQ378057, GQ378123, GQ378200; **Cuba-4** = Gibara, Holguín, Cuba (42573, HAC), GQ377922, GQ377986,

GQ378058, GQ378124, GQ378201; **Cuba-5** = Havana-Pinar del Río hwy, Pinar del Río, Cuba (42563, HAC), NA, GQ377987, GQ378059, GQ378125, GQ378202; **Guat-1** = Ceibal, Guatemala (J.C. Montero 1028), GQ377926, GQ377991, GQ378063, GQ378129, GQ378206; **Guat-2** = Ciudad Vieja, Guatemala (J.C. Montero 1005), GQ377923, GQ377988, GQ378060, GQ378126, GQ378203; **Guat-3** = Lake Petén Itzá, Guatemala (J.C. Montero 1032), GQ377927, GQ377992, GQ378064, GQ378130, GQ378207; **DR** = Dominican Republic, AY309401[b], AY309379[b], AY309344–AY309346[b], NA, NA; **Flor** = Florida, AY309402[b], AY309378[b], AY309341–AY309343[b], NA, NA.

B. shaferi: **Cuba-1** = Viñales, Pinar del Río, Cuba (42561, HAC), GQ377929, GQ377993, NA, GQ378131, GQ378208; **Cuba-2** = Viñales, Pinar del Río, Cuba (42562, HAC), GQ377928, GQ377994, GQ378065, NA, GQ378209.

B. sp. nov.: **Cuba** = Gibara, Holguín, Cuba (42557, HAC), GQ377930, GQ377995, GQ378066, GQ378132, GQ378210.

B. spinescens: **DR** = Dominican Republic (Weeks 01-VIII-23-1, TEX), AY309403[b], AY309388[b], AY309356–AY309358[b], NA, GQ378211.

B. standleyana: **CR** = La Cangreja, San José, Costa Rica (J.F. Morales 1944, MO), GQ377931, GQ377996, GQ378067, GQ378133, GQ378212.

Commiphora angustata: **Cuba** = Trinidad, Sancti Spiritus, Cuba (42575, HAC), GQ377932, NA, GQ378068, GQ378134, GQ378213.

C. glauca: **Cuba** = Yateritas, Guantánamo, Cuba (42577, HAC), GQ377933, GQ377997, GQ378069, GQ378135, GQ378214.

Outgroup species: ***B. biflora***: UNAM Botanical Garden, Mx (J.C. Montero sn), AY831896[d], GQ377998, AY315039–AY315041[c], AF445807[a], GQ378215.

B. copallifera: Taxco, Guerrero, Mx (R. Medina 3975), AY831897[d], GQ377999, AY315042–AY315044[c], AF445833[a], GQ378216.

B. cuneata: La Pera, Morelos, Mx (J.C. Montero 808), AY831898[d], GQ378000, AY315045–AY315047[c], AF445825[a], GQ378217.

B. discolor: Chilpancingo, Guerrero, Mx (R. Medina 4028), AY309390[b], AY309364[b], AY309305–AY309307[b], AF445846[a], GQ378218.

B. fagaroides: Chirangangueo, Michoacán, Mx (R. Medina 4227), AY309391[b], AY309365[b], AY309308–AY309310[b], AF445843[a], GQ378219.

B. hindsiana: Sonora, Mx (J. Núñez-Farfán sn), AY831899[d], GQ378001, AY315048–AY315050[c], GQ378136, GQ378220.

B. lancifolia: Casa Verde, Guerrero, Mx (R. Medina 4007), AY309394[b], AY309368[b], AY309317–AY309319[b], AF445857[a], GQ378221.

B. microphylla: Sonora, Mx (J. Núñez-Farfán sn), AY309396[b], AY309370[b], AY309326–AY309328[b], AF445855[a], GQ378222.

B. morelensis: Cuicatlán, Oaxaca, Mx (R. Medina 3971), AY309397[b], AY309371[b], AY309329–AY309331[b], AF445852[a], GQ378223.

B. odorata: Mexico, AY309398[b], AY309372[b], AY309332–AY309334[b], AF445850[a], NA.

B. sarukhanii: San Jerónimo, Michoacán, Mx (R. Medina 4246), AY831900[d], GQ378002, AY315051–AY315053[c], AF445820[a], GQ378224.

B. schlechtendalii: Palo Verde, Guanacaste, Costa Rica (Olson 1050, INB), AY309400[b], AY309377[b], AY309323–AY309325[b], AF445847[a], GQ378225.

B. tecomaca: Guerrero, Mx (R. Medina 4512), AY309409[b], AY309362[b], AY309359–AY309361[b], AF445838[a], GQ378226.

Commiphora africana: South Africa (Weeks 02-XII-09-03, TEX), AY831901[d], GQ378003, AY831869[d], GQ378137, GQ378227.

C. campestris: Zimbabwe (Weeks 00-VI-24-3, TEX), AY831906[d], GQ378004, AY831873[d], GQ378138, GQ378228.

C. wightii: India (Weeks-00-VIII-18-3, TEX), AY831936[d], GQ378005, AY315081–AY315083[c], GQ378139, GQ378229.

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2010.08.004.

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