Further Support for the Crotoneae Phylogeny: A New Species of *Brasiliocroton* (Euphorbiaceae) Based on Morphological, Geographical, and Molecular Evidence

Further Support for the Crotoneae Phylogeny: A New Species of *Brasiliocroton* (Euphorbiaceae) Based on Morphological, Geographical, and Molecular Evidence

Ricarda Riina,[1,5] Daniela S. Carneiro-Torres,[2] Jess A. Peirson,[3] Paul E. Berry,[3] and Inês Cordeiro[4]

[1]Real Jardín Botánico, RJB-CSIC, Plaza de Murillo 2, 28014 Madrid, Spain.
[2]Universidade Estadual Feira de Santana, Depto Ciências Biológicas, Avenida Transnordestina,
s/n Novo Horizonte, 44036-900, Feira de Santana-Bahia, Brazil.
[3]Department of Ecology and Evolutionary Biology and University of Michigan Herbarium, 3600 Varsity Drive,
Ann Arbor, Michigan 48108-2228, U. S. A.
[4]Instituto de Botânica, Seção de Curadoria do Herbário, Caixa Postal 3005, 001061-970, São Paulo- São Paulo, Brazil.
[5]Author for correspondence: (rriina@rjb.csic.es)

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Abstract—*Brasiliocroton muricatus* is a new species from eastern Brazil that is described, illustrated, and placed in a phylogenetic context. It is only the second known species of *Brasiliocroton*. Its phylogenetic position was inferred based on sequences from the nrITS and plastid *trnL-F* markers, using a sampling of closely related genera in tribe Crotoneae. *Brasiliocroton muricatus* was recovered within tribe Crotoneae, forming a clade with *B. mamoninha*. This clade is in turn sister to the large genus *Croton,* giving additional support to previous phylogenetic studies including *B. mamoninha*. The most obvious morphological characters distinguishing the new species from *B. mamoninha* are the unisexual, axillary inflorescences and the smaller fruits with muricate surface and white, stellate trichomes, as opposed to bisexual, terminal inflorescences and bigger fruits with smooth surface and ochraceous to brown dendritic trichomes in *B. mamoninha*. Both species of *Brasiliocroton* have the filaments of the staminate flowers erect in bud, which is the main character state distinguishing them from the closely related *Croton* and *Astraea.* Additional morphological features distinguishing *Brasiliocroton* and *Croton* from the rest of tribe Crotoneae are also discussed.

Keywords—*Acidocroton*, *Astraea*, Bahia, Brazil, *Croton*, *Ophellantha*.

The recently described genus *Brasiliocroton* P. E. Berry & Cordeiro (Euphorbiaceae) from eastern Brazil included only one species, *B. mamoninha* P. E. Berry & Cordeiro (Berry et al. 2005a). *Brasiliocroton mamoninha* was first included in a molecular phylogeny by Wurdack et al. (2005) in their family level study of uniovulate Euphorbiaceae. This study placed *Brasiliocroton* at the base of *Croton* L. Likewise, the first phylogenetic analysis of *Croton* and members of tribe Crotoneae, using sequences from nrITS and from the plastid *trnL* intron and *trnL-F* spacer, placed *B. mamoninha* as the closest relative to the large genus *Croton* (Berry et al. 2005b). In this phylogeny other Crotoneae genera, such as *Astraea* Klotzsch, *Ophellantha* Standl., and *Acidocroton* Griseb., formed a clade sister to the *Brasiliocroton-Croton* clade. Subsequent phylogenetic analyses of *Croton* DNA sequences using different sets of sampled taxa and molecular markers confirmed *B. mamoninha's* phylogenetic position as the closest relative of *Croton* (Van Ee et al. 2008, 2011; Riina et al. 2010; Caruzo et al. 2011). Berry et al. (2005a) indicated that the main character state distinguishing *Brasiliocroton* from *Croton* was the presence of erect stamen filaments in bud.

While conducting taxonomic studies of Neotropical *Croton,* we came across several undetermined specimens of a crotonoid species from Brazil. After close morphological examination of the available herbarium material, new field observations, and phylogenetic analyses of DNA sequence data, we assigned the specimens to a new taxon in the formerly monotypic genus *Brasiliocroton*. This new species, *B. muricatus*, is described, illustrated, and placed into a phylogenetic context.

Materials and Methods

Taxon Sampling—The sequences of the 34 species included in this study (Appendix 1), along with the new sequences of *Brasiliocroton muricatus*, represent a subset of the taxon sampling used in previous phylogenetic studies of tribe Crotoneae and *Croton* (e.g. Berry et al. 2005b; Van Ee et al. 2008; Caruzo et al. 2011). We included representatives from the four *Croton* subgenera proposed by Van Ee et al. (2011). Outgroup taxa were selected following the work of Berry et al. (2005a).

Molecular Data—DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing of ITS and *trnL−F* for the three accessions of *Brasiliocroton muricatus* followed the laboratory procedures described in Riina et al. (2009). The new sequences were assembled and edited using Staden Package v.2003.0b1 (Staden 1996) and were deposited in GenBank (Appendix 1). Previously published sequences were obtained from NCBI GenBank (www.ncbi.nlm.nih.gov). Sequences were aligned manually with MacClade v.4.08a (Maddison and Maddison 2005), following the similarity criterion as suggested by Simmons (2004). The final alignment of each matrix (ITS, *trnL-F*) was end-trimmed to remove most of the characters with missing data. The two matrices were combined into a single data set with two partitions corresponding to each molecular marker (ITS, *trnL-F*), following the previous analysis of tribe Crotoneae (Berry et al. 2005b). Three additional partitions (ITS1, 5.8S, and ITS2) were delimited within ITS according to Baldwin et al. (1995). The number of parsimony-informative sites was estimated in PAUP* v.4.0b.10 (Swofford 2003). The molecular data matrix and the Bayesian 50% majority rule consensus tree are available from TreeBASE under the number 14445.

Phylogenetic Analyses—The concatenated matrix with two data partitions was analyzed using a Bayesian approach. MrModeltest v.3.7 (Nylander 2004) was used to estimate the most appropriate model of sequence evolution for each data partition under the Akaike Information Criterion (AIC; Akaike 1974). The GTR + G + I model was selected for ITS1, SYM + I for 5.8S, GTR + G for both ITS2 and the *trnL-F* region. Bayesian inference, based on a Markov Chain Monte Carlo (MCMC) approach (Yang and Rannala 1997), was conducted in MrBayes v.3.2.1 (Ronquist and Huelsenbeck 2003). Base equilibrium frequencies, instantaneous substitution rates, and among-site rate variation values were estimated independently for each partition on shared topologies. Two runs of 10 million generations were conducted, and trees were sampled every 1,000 generations. Each run consisted of four independent Markov chains initiated from a random starting tree and using the default temperature (0.2). The resultant Ln likelihood and model parameters from the MCMC runs were inspected using Tracer v.1.5 (Rambaut and Drummond 2007) to determine run convergence and stationarity as indicated by the effective sample size (ESS) values, which should be higher than 100. One-fourth of the MCMC samples from each run was discarded as "burn-in." The remaining trees were

pooled into a 50% majority rule consensus tree with clade credibility values. The consensus tree was visualized and edited in FigTree v.1.3.1 (Rambaut 2010). Five hundred maximum likelihood (ML; Felsenstein 1985) bootstrap replicates, using the model GTR + γ and the same data partitions as in the Bayesian analysis above, were implemented in RAxML v.7.0.3 (Stamatakis et al. 2008) to generate an additional measure of clade support.

Taxonomic Treatment—Comparative morphological study of the new species was conducted using collections from the herbaria listed in the Acknowledgements. The protologue and type specimens of *B. mamoninha* were studied and compared with the new species. Morphological characters used in recent species treatments of *Croton* and *Brasiliocroton* (Berry et al. 2005a; Caruzo et al. 2010; Carneiro-Torres et al. 2011) were examined and described. Trichome terminology followed Webster et al. (1996). Information about habit, habitat, and distribution was taken from specimen labels and field observations. The conservation status was evaluated using IUCN criteria (IUCN 2001).

Results

Summary statistics for the three datasets analyzed in this study are provided in Table 1. The concatenated dataset combining the two markers contained 1,422 aligned positions, of which 331 were parsimony-informative. The ITS dataset had more variable and parsimony-informative characters than the *trnL-F* data set. There were several regions of the ITS matrix with alignment-ambiguous nucleotide sites, but preliminary analyses of the individual ITS dataset with those regions included/excluded produced trees with congruent topologies (data not shown). Although some branches were slightly shorter in the tree resulting from the matrix with ambiguously aligned regions excluded, we retained all molecular characters since we were mainly concerned with the phylogenetic position of the new species. Sequences of the *trnL-F* spacer for the three accessions of *B. muricatus* were identical. The three ITS sequences were nearly identical and differed in only three positions, one position differing in *Thomas 13584* and individual ambiguities in separate parts of the alignment (coded as "?" in the matrix) in each of *Carneiro-Torres 1000* and *1001*. Bayesian analyses of the two individual datasets did not show evidence of topological incongruence between the two genetic markers (results not shown). However, the *trnL-F* topology provided better resolution at deeper nodes of the phylogeny. In contrast, the ITS phylogeny lacked resolution at the *Brasiliocroton-Croton* node but gave better resolution at shallow nodes of the phylogeny (results not shown). Given the lack of obvious conflict between the topologies resulting from the individual matrices, we analyzed the combined dataset (ITS + *trnL-F*).

The Bayesian consensus tree resulting from the analysis of the concatenated matrix shows fully resolved phylo-

genetic relationships within tribe Crotoneae (Fig. 1). *Sagotia racemosa* is sister to a group formed by three successive sister clades, namely the species-poor clades of *Acidocroton-Ophellantha-Astraea* and *Brasiliocroton*, followed by the species-rich *Croton* clade (Fig. 1). The *Croton* clade includes representatives from all four subgenera established by Van Ee et al. (2011), and its phylogenetic structure is similar to previous published phylogenies with a broad sampling across *Croton* (Berry et al. 2005b; Van Ee et al. 2008, 2011; Riina et al. 2009, 2010).

Our analysis recovers the three sampled accessions of *B. muricatus* as an exclusive lineage that is sister to the clade formed by the two accessions of *B. mamoninha*. The sister-group relationship between the two species of *Brasiliocroton* has moderate to high clade support (0.71 Bayesian posterior probability / 97% maximum likelihood bootstrap). There is a notable difference in branch length between *Brasiliocroton* and the rest of the Crotoneae clades, with *Brasiliocroton* having the shortest branch of all genera in the context of this taxon sampling (Fig. 1).

Discussion

The topology of the Bayesian consensus tree (Fig. 1) shows similar phylogenetic relationships to those found by the previous study of tribe Crotoneae using the same two molecular markers (Berry et al. 2005b). It also gives further support to the phylogenetic position of *Brasiliocroton* as the closest relative of *Croton*, although the short branch subtending the two species of *Brasiliocroton* shows moderate Bayesian support (0.71 BPP) but high ML bootstrap support (97%). In phylogenetic studies the opposite pattern, higher (inflated) BPP than ML or MP bootstrap values, is often the rule. This unusual pattern of support for the *Brasiliocroton* branch is probably an artifact of the shortcuts implemented by the rapid bootstrapping of RAxML (Simmons and Norton 2013). The Bayesian support better reflects the fact that in the individual ITS Bayesian consensus tree (not shown) the two species of *Brasiliocroton* are unresolved and form a trichotomy with *Croton*. The topology within *Croton* differs slightly from the most recently published phylogeny by Van Ee et al. (2011), but this could be due to the reduced taxon sampling used in the present study compared with previous phylogenies with a more comprehensive sampling of *Croton* lineages (Berry et al. 2005b, Van Ee et al. 2008, 2011; Riina et al. 2009; Caruzo et al. 2011).

The sister relationship between *Croton* and *Brasiliocroton* is also supported by morphological features and geography. *Brasiliocroton* shares several morphological character states with many *Croton* lineages, including the presence of stellate and dendritic trichomes, petiolar glands, and glands along the leaf margin. As in *Brasiliocroton*, several of the early diverging *Croton* lineages are arborescent (clades in subg. *Quadrilobi* (Müll. Arg.) Pax, sensu Van Ee et al. 2011) and are distributed in South America and especially in eastern Brazil (e.g. *Croton* sect. *Sampatik* (G. L. Webster) Riina).

The classification of these two sister clades (*Brasiliocroton* and *Croton*) as different genera was primarily justified by the long branch leading to the *Croton* crown in the phylogeny and the different morphology of the stamens in the flower bud. *Croton* species have the filaments incurved

TABLE 1. Summary statistics from ITS, *trnL−F*, and combined ITS + *trnL−F* datasets for the species in tribe Crotoneae analyzed in this study.

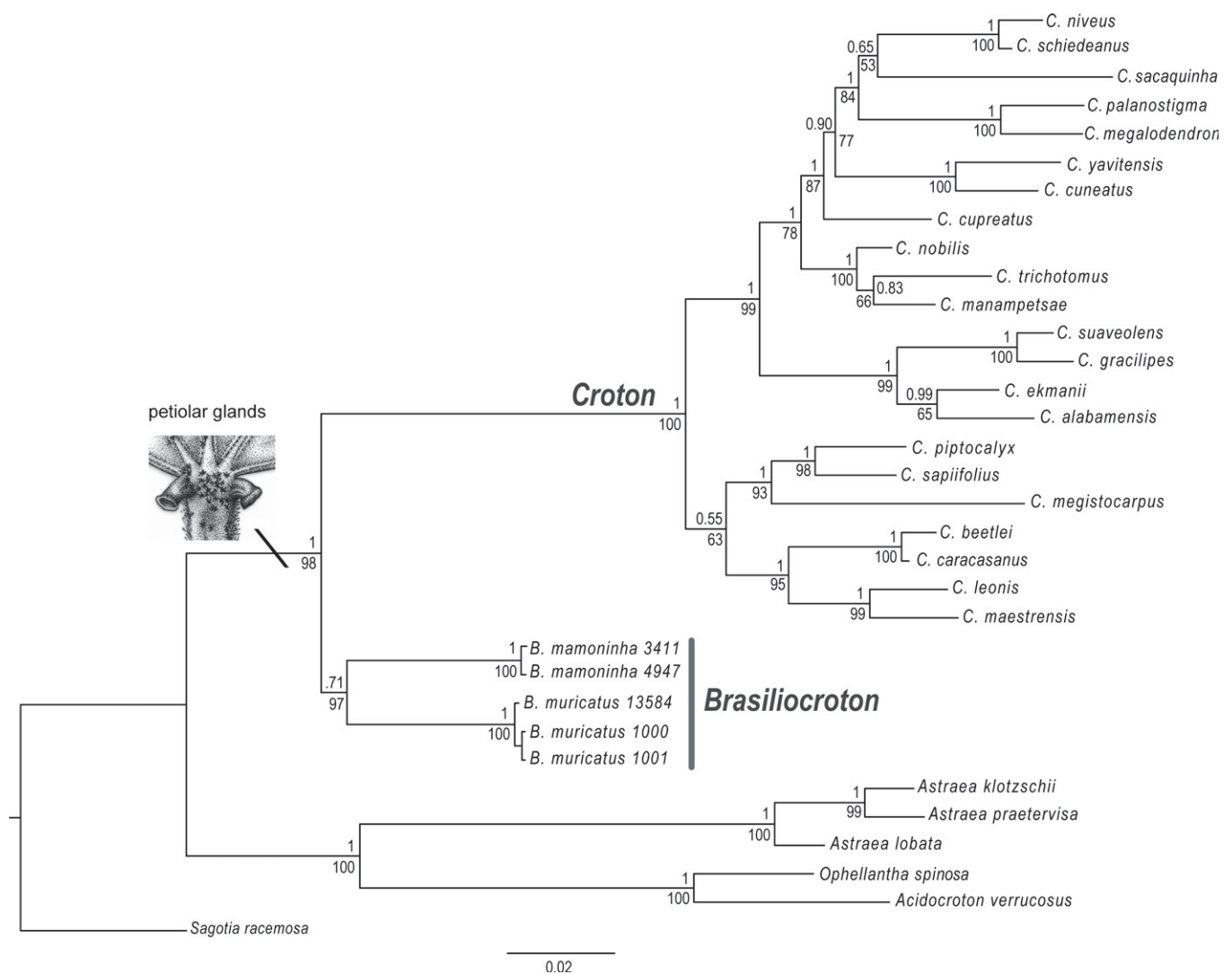|	ITS	*trnL−F*	Combined ITS + *trnL−F*
Total no. of species	33	33	33
Unaligned length [bp]	622–655	618–668	1252–1318
Aligned length [bp]	693	729	1422
Variable characters (%)	325 (46.9)	171 (24.7)	496 (34.8)
Missing data [%]	0.08	3.9	3.98
Parsimony-informative characters	253	81	334

Fig. 1. Majority rule consensus phylogram obtained from the combined Bayesian analysis of ITS and *trnL-F* sequences. The image inserted on the left (petiolar glands) indicates a synapomorphy for the *Croton-Brasiliocroton* clade. Values above and below branches are Bayesian posterior probabilities and maximum likelihood bootstrap percentages, respectively. The scale bar indicates the mean number of nucleotide substitutions per site.

in the staminate flower buds, whereas the two *Brasiliocroton* species have the filaments erect in bud. The genus *Astraea* has filaments incurved in bud as well, but it also displays character states that are absent in *Croton*, such as the occurrence of moniliform trichomes on the petals of staminate flowers (De Paula et al. 2011). *Brasiliocroton* differs from both *Croton* and *Astraea* in other distinctive features, such as highly branched and expanded inflorescences and conspicuously verrucose stigmatic surfaces.

In the context of tribe *Crotoneae*, the presence of glands (extrafloral nectaries) at the junction of the petiole with the lamina (petiolar or basilaminar glands) can be regarded as a synapomorphy for the *Brasiliocroton-Croton* clade (Fig. 1), since petiolar glands are absent in the rest of the genera of tribe Crotoneae (*Astraea*, *Acidocroton*, *Ophellantha*, *Sagotia*, and *Sandwithia*). However, although the vast majority of *Croton* species bear petiolar glands, these secretory structures have been lost multiple times within the genus. An

Table 2. Main morphological characters distinguishing *Brasiliocroton muricatus* from *B. mamoninha*.

	B. muricatus	*B. mamoninha*
Indumentum on young branches and inflorescence axes	Ferrugineous	Whitish to ochraceous
Position of inflorescences	Axillary	Terminal
Sexuality of inflorescences	Unisexual, rarely bisexual	Always bisexual
Sepals of pistillate flowers	Free	Connate for 1/2–2/3 their length
Fruit shape	Spherical, slightly flattened	Spherical-trigonous
Fruit size	5–7 × 5–7 mm	15–20 × 10–15 mm
Fruit surface and indumentum	Strongly muricate, with whitish stellate trichomes	Smooth, with ochraceous to brown dendritic trichomes

anatomical study by Sá-Haiad et al. (2009) indicated the presence of petiolar glands in three species of *Astraea*, but a more recent study has shown that these authors misinterpreted the agglomeration of colleters on the adaxial side of the petiole as petiolar glands (Vitarelli, 2013). Colleters, like nectaries, are secretory structures present in different vegetative and reproductive organs across many angiosperm families (Thomas, 1991). De-Paula et al. (2011) also failed to recognize the presence of colleters on the pistillate flowers of the six species of *Croton* included in their study, but they recognized these structure for *Astraea lobata*. Only a few genera (none of them Crotoneae) of Euphorbiaceae were known to bear colleters at the time of Thomas' review, but Vitarelli (2013) has since found colleters of the standard type, following the classification of Thomas (1991), on the leaves of *Astraea lobata*, *Brasiliocroton mamoninha* and several species of *Croton*. Preliminary observations of specimens of *Acidocroton*, *Ophellantha*, *Sagotia* and *Sandwithia* using a stereomicroscope suggest the presence of colleters in those genera as well, although more detailed anatomical studies are needed to confirm these observations.

Another morphological change supporting the *Brasiliocroton-Croton* clade is the evolution of more complex trichome types (from stellate to lepidote, and all the degrees of forms in between) in relation to the other genera in the tribe. The other Crotoneae genera have long simple trichomes, with the exception of some species of *Astraea* that can have, besides the simple trichomes, the basic form of stellate trichomes (i.e. a porrect central ray with several horizontal, very short rays at the base situated in the same plane).

Brasiliocroton muricatus shares many morphological character states with its sister species, but it can be distinguished from *B. mamoninha* mainly by the inflorescence position, the surface and size of the fruit, and several other reproductive characters shown in Table 2.

Although the staminate and pistillate inflorescences of *Brasiliocroton muricatus* are always axillary, there is a clear temporal gap in their development. We have observed plants bearing pistillate inflorescences with flowers in anthesis (or with young fruits) on the younger parts of branches and (at the same time) staminate inflorescences with most flowers still in bud on the older parts of branches. For this reason, some herbarium specimens could give the wrong impression that the species is dioecious.

Brasiliocroton muricatus is known from a few localities in the states of Bahia, Espírito Santo, and Minas Gerais in eastern Brazil. There is some overlap with the distribution of *B. mamoninha* in Bahia, where the two species are probably sympatric. This is indicated by several collections of both species occurring in the Atlantic rain forest of the Municipality of Teixeira de Freitas (Bahia).

Taxonomic Treatment

Brasiliocroton muricatus Riina & Cordeiro, sp. nov.—TYPE: BRAZIL. Bahia: Município de Cruz das Almas, Reserva Florestal do Instituto Baiano do Fumo, 12 Nov 1983, *H. P. Bautista et al. 1223* (holotype: HUEFS!; isotypes: ALCB!, RB!, MBM!).

Brasiliocroton muricatus differs from *B. mamoninha* by its usually unisexual (rarely bisexual) and axillary inflorescences, the smaller muricate fruits with white-stellate trichomes (vs. always bisexual and terminal inflorescences and bigger fruits with smooth surface and ochraceous to brown dendritic trichomes in *B. mamoninha*).

Monoecious trees 4–9 (–20) m high, diameter of main trunk to 30 cm, bark gray, exfoliating in irregular plates, sap colorless. Young branches with a dense ferrugineous indumentum of stellate or stellate-porrect trichomes. Stipules subulate, deciduous, 0.5–0.7 mm long, with an apical gland, indumentum of stellate trichomes. Leaves alternate; lamina ovate, 5–15 × 2.5–7 cm; base rounded; apex acute to acuminate; margin entire or slightly crenate, with a conical gland in each sinus; venation pinnate, 3-nerved at base, secondary veins 4 or 5 per side; adaxial and abaxial leaf sides glabrous to densely pubescent (when young), trichomes mostly concentrated along the veins, sessile to stipitate, stellate or stellate-porrect; petioles 1–5 cm long, glabrous to pubescent with stellate-porrect trichomes; acropetiolar glands 2, dark, patelliform, 0.6–0.9 mm diam, sessile to shortly stipitate, on the adaxial side of the petiole. Inflorescences of axillary panicles 2–15 cm long, on either young or old branches, usually unisexual, rarely bisexual; indumentum densely ferrugineous with sessile to stipitate, stellate trichomes; bracts triangular, 1–2 mm long, prophylls 2, one on each side of the bract base, ca. 0.3 mm long. Staminate flowers pedicellate, pedicels 2–9 mm long; sepals 5 (rarely 4), valvate, unequal, narrowly triangular-ovate to broadly ovate, 2–3 × 1.1–1.5 mm, thick, adaxial surface with trichomes toward the apex and margin, abaxial surface with a dense ferrugineous indumentum of stellate trichomes; petals 5, narrowly elliptic to slightly rhomboidal, 3.0–3.5 × 0.8–1 mm, scattered stellate trichomes on both surfaces to nearly glabrous, apex and margin lanate; receptacle short-villous; nectary disk with 5 glands opposite the sepals; stamens (14–20) 24, filaments glabrous, yellowish-green, 2.5–3 mm long, erect in bud, anthers 0.8–1.1 × 0.7–1.1 mm. Pistillate flowers with pedicel 5–7 mm long, with stellate or stellate-porrect trichomes; sepals 5, free, recurved at anthesis, unequal, ovate, 2–2.8 × 1–1.5 mm, ca. 0.3 mm thick, adaxial surface with stellate trichomes, abaxial surface with a dense ferrugineous indumentum of stellate trichomes; petals absent; nectary disk entire, ovary globose, ca. 3 mm diam, densely muricate with pyramidal projections up to 0.3 mm long, covered by whitish stellate trichomes, 3-locular, locules 1-ovulate; styles 3, bifid, with two basal lobules at the bifurcation of each branch, 2–2.3 mm long, patent, glabrous to pilose at base. Fruit a spherical schizocarp, slightly trigonous, flattened apically, 5–7 mm diam, with persistent styles, surface projections accrescent up to 1 mm long, with a whitish indumentum of stellate trichomes; columella 4–5 mm long, angled, with three projections at the tip; seeds brown, smooth, ovoid, ca. 4 × 3 mm (in young fruit), dorsally angled; caruncle whitish, smooth. Figures 2, 3.

Etymology—The specific epithet refers to the muricate surface of both the ovary and the fruit (Figs. 2D, 2H, 3D).

Habitat and Distribution—The species grows as a small to medium-sized tree in southwestern Bahia, northeastern Minas Gerais, and Espírito Santo states of eastern Brazil (Fig. 4), at the edge of rain forests, seasonally dry forests, and secondary forests, at elevations of 200–800 m.

Conservation Status—This species can be considered vulnerable (VU B1a) because its geographic range is estimated to
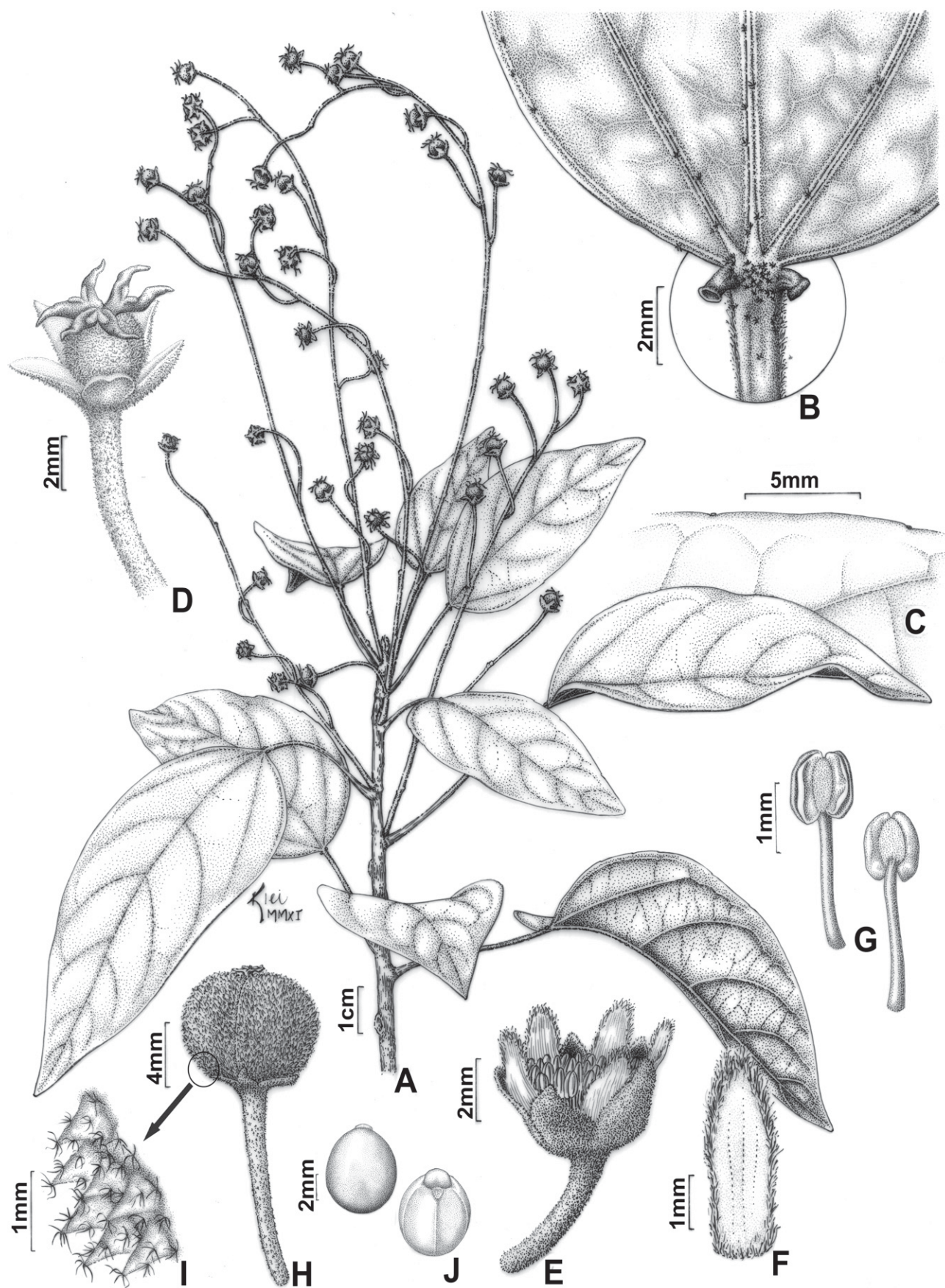
FIG. 2. Morphology of *Brasiliocroton muricatus*: A. Branch with young fruits. B. Leaf base with stipitate glands. C. Leaf margin with glands. D. Pistillate flower. E. Staminate flower. F. Petal of staminate flower. G. Stamens: dorsal and ventral views. H. Young fruit. I. Detail of fruit surface. J. Seed: dorsal and ventral views. Vouchers: A, C–E (*Bauptista 1223*), B (*Macedo 381*), D, G (*Cordeiro 3408*), F (*Carvalho 2527*), H (*Carneiro-Torres 1010*).
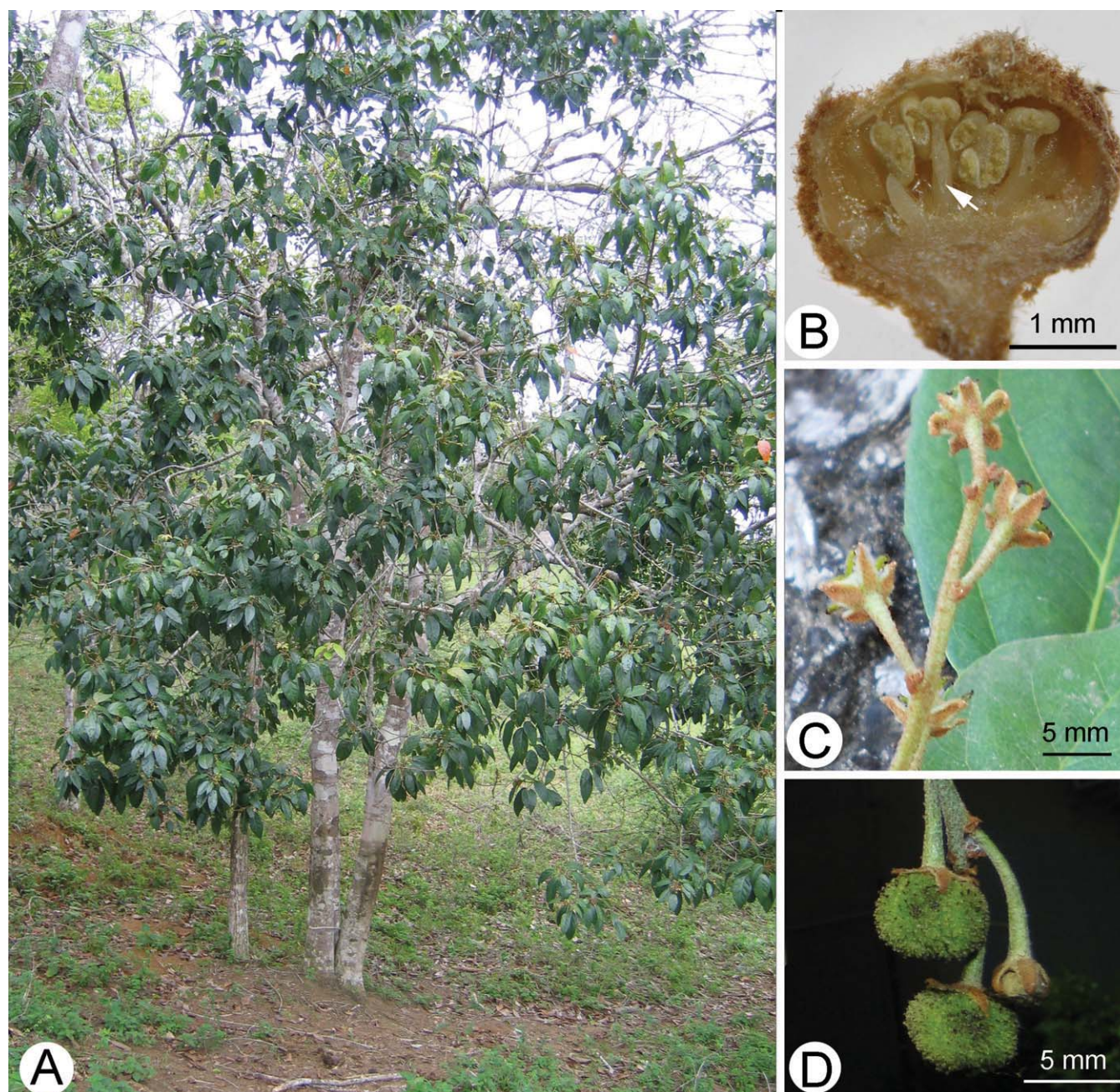
Fig. 3. *Brasiliocroton muricatus*: A. Individuals in the field showing the arborescent habit. B. Longitudinal section of staminate flower bud (3 × 3 mm) showing the erect filaments. C. Branch of a young inflorescence with pistillate flowers. D. Well-developed fruits showing the muricate surface. Vouchers: A, D (*Carneiro-Torres 1010*), B, C (*Cordeiro & Carneiro-Torres 3406*).

be less than 2,000 km^2, and it is known from fewer than ten locations (IUCN 2001). Furthermore, populations of *B. muricatus* are isolated from each other, and some occur in populated areas that are subject to development.

Additional Specimens Examined—BRAZIL. Bahia: Município de Baixa Grande, Fazenda Olhos D'Água, 12°0′29″ S, 40°17′22″W, 350 m, 9 Jun 1999, *F. França et al. 2985* (HUEFS); Município Boa Nova, Fazenda Cotemaia, entrance 1.2 km E of Boa Nova on road to Dario Meira, 14°22′23″S, 40°11′18″W, 790 m, 25 Oct 2001, *W. W. Thomas et al. 12639* (CEPEC, MA, MICH, MO, NY); Município de Barra do Choça, entrada que liga São Sebastião, 21 Nov 1978, *S. A. Mori et al. 11270* (DAV, MO); Município Cruz das Almas, Reserva Florestal Instituto do Fumo, dentro da cidade, 26 Mar 2010, *D. S. Carneiro-Torres 1000* (HUEFS); 16 May 2011, *D. S. Carneiro-Torres 1010* (HUEFS); 19 Nov 2012, *I. Cordeiro & D. S. Carneiro-Torres 3406* (HUEFS, SP); Município de

Itarantim, Mata do Boi Rajado, ca. 14 km da Sede de Bandeira, na divisa com Minas Gerais, 15°48′18.7″S 40°30′59.8″W, 620–800 m, 08 Oct 2003, *A. Salino et al. 9151* (BHCB, SP); Município Jequié, Fazenda Brejo Novo, a 10.5 km da Av. Otávio Mangabeira pela Exupério Miranda no Bairro do Mandacaru, 13°56′41.4″S, 40°06′33.9″W, 617–750 m, 22 Nov 2003, *G. E. L. Macedo 381 et al.* (PEUFR, SP); Serra do Brejo, Fazenda of Francisco Brito, 10.5 km S of Mandacaru on road to Serra do Brejo (road that goes past stadium), 13°56′49″S, 40°06′40″W, 600–700 m, 26 Jul 2003, *W. W. Thomas et al. 13584* (CEPEC, MICH, MO, NY); passando da ponte sobre o Rio de Contas entra no sentido Mandacarú, passa na frente do estádio Waldomiro Borges, segue direto pela estrada de chão até a fazenda do Francisco, ca. 10.5 km, Serra do Brejo Novo, 13°56′49″S 40°06′40″W, alt 600–700 m, 20 May 2010, *D. S. Carneiro-Torres 1001* (HUESB); 24 Nov 2010, *D. S. Carneiro-Torres 1004* (HUEFS); Fazenda Brejo Novo, a 10.5 km da Av. Otávio Mangabeira pela Exupério Miranda, no Bairro do Mandacaru, 13°56′41.4″S 40°06′33.9″W, alt 617 a
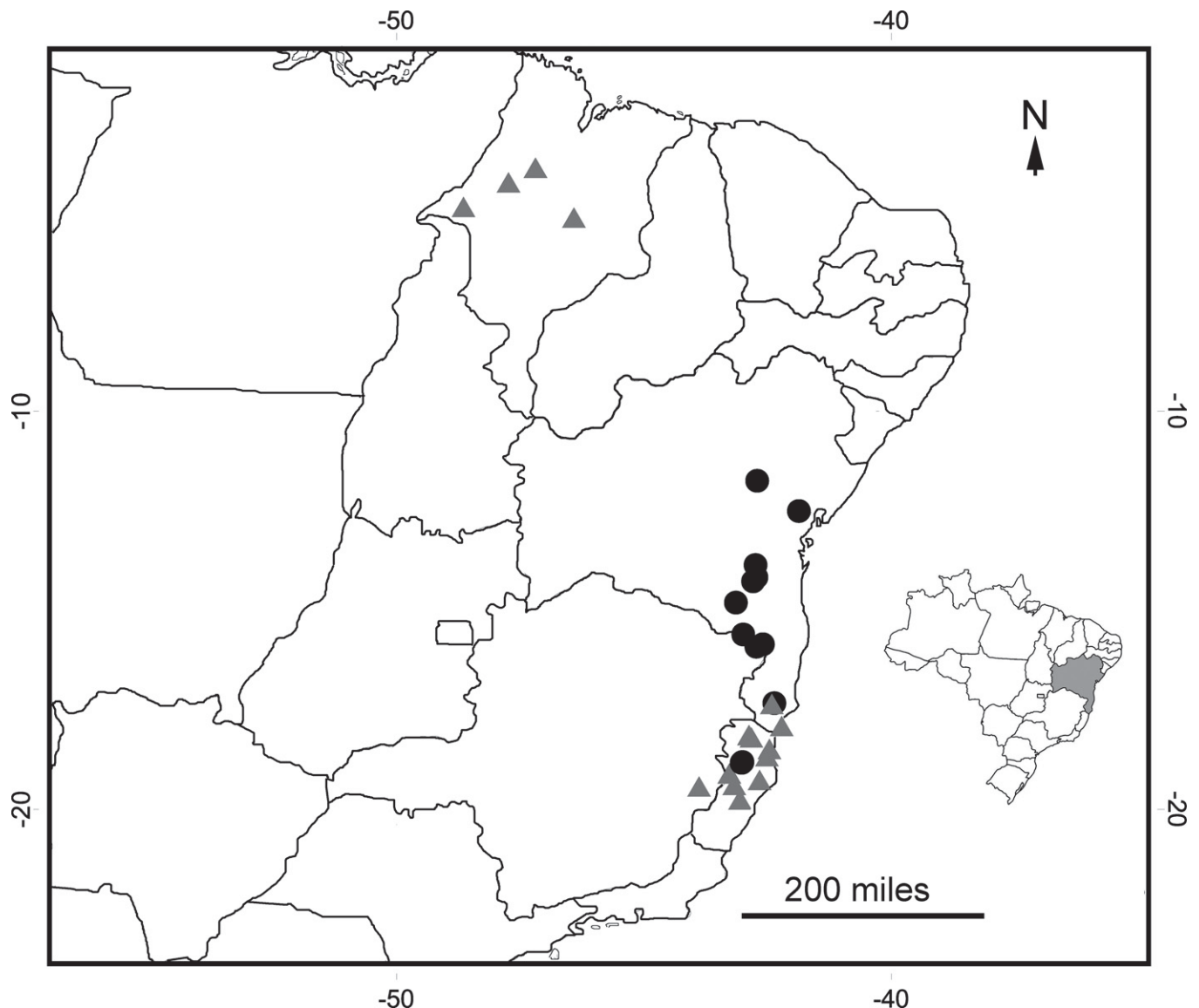
Fig. 4. Distribution map of known specimens of *B. muricatus* (circles) and *B. mamoninha* (triangles).

750 m, 22 Nov 2003, *G. E. L. Macedo 381* (PEUFR, SP); Município de Teixeira de Freitas, ca. 56 km na estrada de Teixeira de Freitas para Itamaraju, BR 101, Mata higrófila sul Bahiana, 6 Sep 1989, *A. M. Carvalho et al. 2527* (CEPEC, SP). Espírito Santo: Município de São Gabriel da Palha, Sítio dos Lorenzoni, 27 Apr 1995, *G. F. Arbocz 1386* (SP). Minas Gerais: Município de Salto da Divisa, Fazenda Santana, 16°03′20.7″S 40°01′59.5″W, alt. 199 m, 21 Aug 2003, *J. A. Lombardi et al. 5337* (BHCB, SP).

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APPENDIX 1. Species sampled with GenBank accession numbers for *trnL-F* and ITS sequences, respectively.

Acidocroton verrucosus Urb. AY971265, AY971171. **Astraea klotzschii** Didr. HM564209, HM564073. **A. lobata** (L.) Klotzsch. EU586999, EU586945. **A. praetervisa** (Müll. Arg.) P. E. Berry. AY971266, AY971173. **Brasiliocroton mamoninha** P. E. Berry & Cordeiro. *Pirani 4947* EU586998, EU586944, *Pirani 3411* AY971267, AY971175. **B. muricatus** Riina & Cordeiro. BRAZIL Bahia: *Thomas 13584* (MICH), KF208631, KF208628; *Carneiro-Torres 1000* (HUESF), KF208632, KF208629; *Carneiro-Torres 1001* (HUESF), KF208633, KF208630. **Croton alabamensis** E. A. Smith ex Chapman var. **texensis** Ginzbarg. AY971270, AY971179. **C. beetlei** Croizat. EF408090, EU586916. **C. caracasanus** Pittier. DQ227557, DQ227525. **C. cuneatus** Klotzsch. EU497710, EU497735. **C. cupreatus** Croizat. EU586974, EU586919. **C. ekmanii** Urb. EF408145, EF421761. **C. gracilipes** Baill. EU586962, EU586909. **C. leonis** (Croizat) van Ee & P. E. Berry. EF408140, EF421758. **C. maestrensis** (Alain) van Ee & Berry. EF408127, EF421753. **C. manampetsae** Leandri. HM044777, HM044796. **C. megalodendron** Müll. Arg. EU586996, EU586942. **C. megistocarpus** J. A. González & Poveda. EU587004, EU586953. **C. niveus** Jacq. EU478155, EU478046. **C. nobilis** Baill. HM044778, HM044797. **C. palanostigma** Klotzsch. EU586997, EU586943. **C. piptocalyx** Müll. Arg. EF408148, EF421791. **C. sacaquinha** Croizat. HM044782, HM044802. **C. sapiifolius** Müll. Arg. EF408150, EF421754. **C. schiedeanus** Schltdl. AY971331, AY971246. **C. suaveolens** Torr. AY971334, AY971252. **C. trichotomus** Geiseler. HM044787, HM04480. **C. yavitensis** Croizat. EU586973, EU586918. **Ophellantha spinosa** Standl. AY971344, AY971263. **Sagotia racemosa** Baill. AY794687, AY971264.