American Journal of Botany 92(9): 1520–1534. 2005.

MOLECULAR PHYLOGENETICS OF THE GIANT GENUS *CROTON* AND TRIBE CROTONEAE (EUPHORBIACEAE SENSU STRICTO) USING ITS AND *TRNL-TRNF* DNA SEQUENCE DATA[1]

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Parsimony, likelihood, and Bayesian analyses of nuclear ITS and plastid *trnL-F* DNA sequence data are presented for the giant genus *Croton* (Euphorbiaceae s.s.) and related taxa. Sampling comprises 88 taxa, including 78 of the estimated 1223 species and 29 of the 40 sections previously recognized of *Croton*. It also includes the satellite genus *Moacroton* and genera formerly placed in tribe Crotoneae. *Croton* and all sampled segregate genera form a monophyletic group sister to *Brasiliocroton*, with the exception of *Croton* sect. *Astraea*, which is reinstated to the genus *Astraea*. A small clade including *Moacroton*, *Croton alabamensis*, and *C. olivaceus* is sister to all other *Croton* species sampled. The remaining *Croton* species fall into three major clades. One of these is entirely New World, corresponding to sections *Cyclostigma*, *Cascarilla*, and *Velamea* sensu Webster. The second is entirely Old World and is sister to a third, also entirely New World clade, which is composed of at least 13 of Webster's sections of *Croton*. This study establishes a phylogenetic framework for future studies in the hyper-diverse genus *Croton*, indicates a New World origin for the genus, and will soon be used to evaluate wood anatomical, cytological, and morphological data in the Crotoneae tribe.

Key words: *Astraea*; *Croton*; Crotoneae; Euphorbiaceae; giant genus; ITS; molecular phylogenetics; *trnL-F*.

Croton (Euphorbiaceae s.s.) is one of the largest genera of flowering plants, with between 1200 and 1300 species of herbs, shrubs, trees, and occasionally lianas that are ecologically prominent and important elements of secondary vegetation in the tropics and subtropics worldwide (Webster, 1993; Govaerts et al., 2000; Fig. 1). There is horticultural confusion with *Codiaeum*, a small and distantly related Malesian genus of Euphorbiaceae whose common name ''croton'' refers to the worldwide cultivated ornamental varieties of *Codiaeum variegatum* (L.) A. Juss. In the field, *Croton* is usually readily recognizable by a suite of characters that includes conspicuous stellate or scalelike trichomes, narrow or condensed inflorescences of unisexual flowers, watery to colored sap, frequent petiolar glands, and senescent leaves that turn orange before dehiscing. The diverse array of extrafloral nectaries in *Croton* plays an important role in ant–plant interactions (viz., DeVries and Baker, 1989). Pollination of the slender inflorescences with clusters of unisexual flowers shifts between insects and wind, and breeding systems vary from monoecy to dioecy (Domínguez and Bullock, 1989; Bullock, 1994; Decker and Pilson, 2000). There is considerable variation in trichome morphology (Webster et al., 1996), pollen (Nowicke, 1994), and chromosome numbers (Hans, 1973; Urbatsch et al., 1975). *Croton* is rich in secondary metabolites including alkaloids and terpenoids (Rizk, 1987), the latter including irritant cocarcinogenic phorbol esters (Phillipson, 1995). The red sap of several South American species, known as ''sangre de drago'' or dragon's blood, is used medicinally at the local level as well as in the international herbal supplements market (Meza, 1999).

Croton has traditionally been classified with Euphorbiaceae s.l. and is retained, subsequent to the recent recognition of four segregate families (i.e., APG II, 2003), in the narrower circumscription of the family as containing only the uniovulate lineages (Wurdack et al., 2005). *Croton* belongs to subfamily Crotonoideae, which is characterized by mostly lactiferous taxa having pollen with an unusual (crotonoid) exine pattern of triangular supratectal elements attached to a network of muri with short columellae (Nowicke, 1994). Most of the subfamily, including *Croton*, is also characterized by inaperturate pollen, which is an unusual condition in the angiosperms. The subfamily has been divided into as many as 12 tribes (Webster, 1975, 1994; Radcliffe-Smith, 2001), but the circumscription and relationships of tribe Crotoneae have been characterized as particularly ''shaky'' (Webster, 1994, p. 110), and molecular evidence has shown that the subfamily and many of its tribes are not monophyletic (Wurdack et al., 2005). In addition to the generalized characters mentioned, the main morphological synapomorphy that characterizes *Croton* is the inflexed conformation of the tips of the staminal filaments in bud, which causes the anthers to be introrsely inverted until anthesis. *Croton* has been variously circumscribed in the past, with numerous proposed segregate genera (e.g., Klotzsch, 1841), but few

[1] Manuscript received 11 December 2004; revision accepted 2 May 2005.

The authors thank the following individuals who have provided plant material: Hans-Joachim Esser (M), Paul Forster (BRI), Dylan Hannon (RSA), and Victor Steinmann (IEB). We also thank the curators of BRIT, DAV, MO, NY, RSA, and US for allowing us to sample herbarium and/or living material. This work was funded in part by the National Science Foundation under Grant No. DEB-0212481. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation. Additional funding came from the University of Wisconsin–Madison Graduate School. Support for K. J. W. was provided by the Smithsonian Institution and by the Lewis B. and Dorothy Cullman Program for Molecular Systematic Studies at The New York Botanical Garden. We thank Hajo Esser for helpful comments on an earlier draft of this paper.

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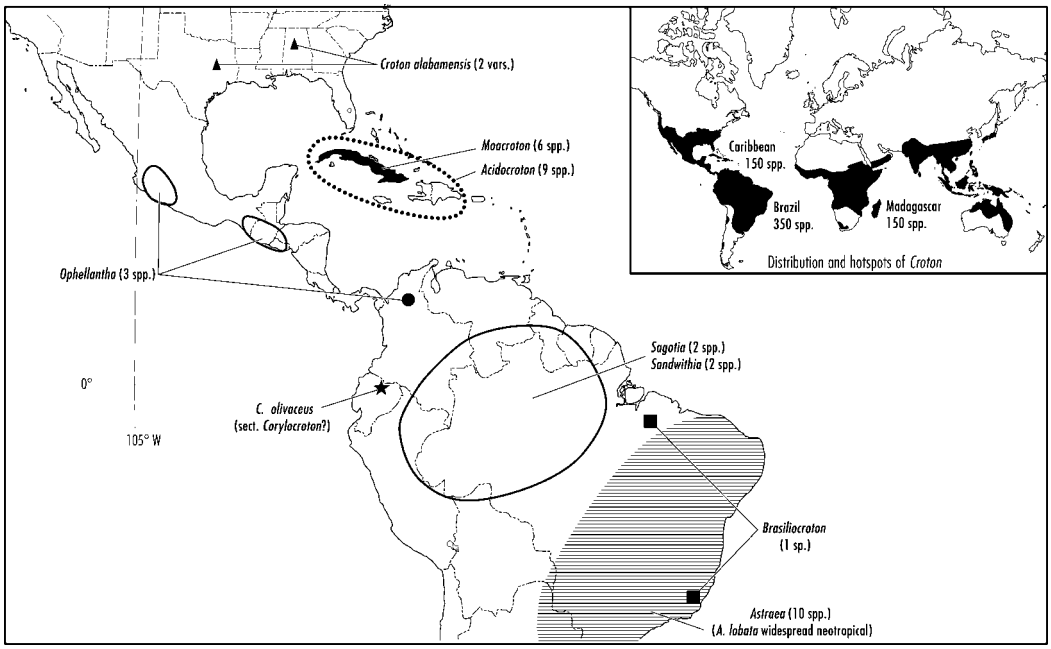

Fig. 1. Map of the worldwide distribution of *Croton* (insert), and the distribution of sister genera of *Croton* in the revised Crotoneae, including members of the small sister clade within *Croton* (clade C-1 in Fig. 5).

of these were maintained by the last synoptic monographer of the genus, Johannes Müller (1866, 1873). *Croton* has been rather broadly treated in recent circumscriptions (Webster, 1993, 1994; Govaerts et al., 2000), but some segregates were resurrected in the latest classification of the family (i.e., *Crotonopsis*, *Eremocarpus*, *Julocroton*; Radcliffe-Smith, 2001) and an additional segregate, *Colobocarpos*, was recently described by Esser and Welzen (2001). The most recent attempt at a sectional synopsis of *Croton* was the publication of Webster (1993), in which he recognized 40 sections. There was no attempt in Webster's system, however, to place the sections in any kind of phylogenetic framework, and such past workers in the genus as Croizat (1944) and Burger and Huft (1995) have bemoaned the taxonomic difficulty of the genus.

Croton is a ''giant genus,'' with 1223 species accepted in *The World Checklist and Bibliography of Euphorbiaceae* by Govaerts et al. (2000). Using the latest taxonomic estimates, 57 angiosperm genera contain more than 500 species each, and the total in these genera of over 53 000 species accounts for about 15% of all extant species (Frodin, 2004). *Astragalus* alone comprises between 2500 and 3000 species, or roughly 1% of all flowering plant species (Sanderson and Wojciechowski, 1996). Molecular studies have supported the monophyly of several of these large genera, for example *Solanum* (Bohs and Olmstead, 1997) and *Astragalus* (Wojciechowski et al., 1999). In other cases, however, large genera like *Acacia*, *Euphorbia*, and *Salvia* have been shown to be polyphyletic or paraphyletic and deserving of major generic recircumscription (Miller and Bayer, 2001; Steinmann and Porter, 2002; Walker et al., 2004). These giant genera pose huge taxonomic challenges as well as unparalleled opportunities to study phenomena such as changes in evolutionary diversification rates, adaptive radiations, key innovations, and chromosomal rearrangements.

This study is the first detailed molecular survey within *Croton*. We present sequences from the ITS and 5.8S regions of nuclear ribosomal DNA and the plastid *trnL-F* region, the latter including the *trnL* exon, intervening intron, and the 3′ intergenic spacer. Both loci are well known to be appropriate for species level phylogenies, as has been shown for other Euphorbiaceae lineages (Krähenbühl et al., 2002; Steinmann and Porter, 2002; Wurdack et al., 2005). The main goals of the study are to determine to what degree the genus as currently defined is monophyletic and to better define sister relationships so that we can reassess the circumscription of the tribe Crotoneae. We also make initial evaluations of the monophyly of Webster's (1993) sections as well as of relationships among them. Moreover, data presented elucidate the biogeography of a major clade of Euphorbiaceae s.s., particularly regarding the phylogenetic position of the Old World and New World taxa.

MATERIALS AND METHODS

Taxon sampling—A total of 97 accessions and 88 taxa were sequenced for this study (Appendix). All sequences were newly generated for this study, except for 16 *trnL-F* sequences that were jointly used for a broader analysis of uniouvulate Euphorbiaceae s.s. (Wurdack et al., 2005). Taxa within *Croton* were selected to broadly represent the morphological and geographic range of the genus, and to include a wide range of the traditionally recognized sections, subgenera, or segregate genera (Webster, 1993; Radcliffe-Smith, 2001). Outgroups (*Jatropha* and *Paracroton*) were selected based on the family-wide analysis of *rbcL* and *trnL-trnF* by Wurdack et al. (2005) and in part by the previous tribal delimitation of Webster (1994).

DNA extraction, PCR, and sequencing—DNA was extracted from fresh, silica-dried, or herbarium tissue using a DNeasy Plant Mini kit (Qiagen, Valencia, California, USA). ITS-1, ITS2, and 5.8S were amplified by polymerase chain reaction (PCR) using primers ITS-I (Urbatsch et al., 2000) and ITS4 (White et al., 1990) in 50-μL reactions containing ultra pure deionized water with 2.5 μM $MgCl_2$, 5 μL 10 × $MgCl_2$-free *Taq* buffer, 0.5 μL bovine serum albumin, 5% dimethyl sulfoxide, 0.20 μM of each primer, 1.25 units *Taq* DNA polymerase, and 1 to 2 μL of template DNA. The *trnL-trnF* region was

amplified using primers ''c'' and ''f'' of Taberlet et al. (1991); for some individuals with highly degraded DNA, amplification was performed in two pieces, using primers ''c'' and ''d'' for the *trnL* intron and primers ''e'' and ''f'' for the *trnL-trnF* spacer. Sequencing was done on ABI 377 or ABI 3100 automated sequencers (Applied Biosystems, Foster City, California, USA) largely at the University of Wisconsin–Madison Biotechnology Center's DNA facility. ITS-I and ITS4 primers were used to sequence the ITS region in both directions, with additional sequences from internal primers ITS3B (Baum and Sytsma, 1994) and ITS2 (White et al., 1990) for taxa in which ITS-I and ITS4 sequences did not provide double coverage. Primers ''c,'' ''e,'' and ''f'' were sufficient to sequence the *trnL-trnF* region for most taxa sampled. Individuals in which amplification was conducted in two pieces were sequenced using the additional internal primer ''d.'' Sequences were edited and assembled in Sequencher 3.0 (GeneCodes Co., 1991–1995), then aligned in Clustal X v1.8, the Windows interface to Clustal W 1.8 (Thompson et al., 1994). Alignments were adjusted manually in BioEdit 5.0.9 (Hall, 1999). The full data matrix is archived in TreeBASE (www.treebase.org/treebase), and sequences are deposited in GenBank.

Potentially informative indels were scored as separate characters at the end of the data matrix. Indels that were not nested or overlapping were scored as binary characters. Nested indels—those in which overlapping indels of different sizes could not be unambiguously distinguished from one another—were scored as multistate characters. Several sequence regions in both data partitions were informative at some phylogenetic levels, but ambiguously aligned at other levels. Ambiguously aligned regions were identified and coded as two character exclusion sets: (1) regions ambiguously aligned among ingroup taxa (''core'' *Croton*, excluding the *Croton alabamensis* clade and all taxa outside of that group), and (2) ambiguities involving taxa outside of core *Croton*. Preliminary Bayesian analyses were conducted on the combined data excluding and including ambiguities to determine whether their inclusion had any effect on topology or support levels.

Analysis—Parsimony analyses were conducted in PAUP* v4.0b10 (Swofford, 2002) using 100 random addition replicates with tree-bisection-reconnection (TBR) branch-swapping, MULTREES activated, and steepest descent option not activated. Analysis of the *trnL-trnF* data set could not be run to completion due to computational limits and the large number of most parsimonious trees recovered. Analysis of this data set was conducted using 100 random addition replicates with 1000 trees saved in each random addition replicate (chuckscore = 1, nchuck = 1000). A strict consensus of the resulting trees was used as a constraint in a second search of 10 000 random addition replicates with one tree saved at each replicate (nchuck = 1), saving only trees not compatible with the consensus. This method should ensure that no shorter trees exist and that the strict consensus recovered in this study represents the set of all most parsimonious trees (Catalan et al., 1997). Bootstrap analyses were conducted using 200 heuristic search replicates of 100 random sequence addition replicates each, saving one tree per sequence addition replicate for the individual data sets and two trees per replicate for the combined data set.

Bayesian analyses of the sequence data were conducted in MrBayes v3.0b4 (Huelsenbeck and Ronquist, 2001). Models of nucleotide substitution were selected for each partition using hierarchical likelihood ratio tests (hLRT) in the program MrModeltest v2 (Nylander, 2004) with $\alpha = 0.01$, using the default hLRT. Each data partition was first analyzed separately in MrBayes using the best-fit likelihood model. Data sets were then analyzed in combination, with model parameters fit independently to the separate data partitions. All parameters except for topology and branch length were unlinked between the two partitions. Prior probability distributions were assigned as follows: topologies equiprobable; branch lengths unconstrained (non-clocklike) with an exponential distribution (exponent = 10.0); gamma distribution shape parameter uniform on the interval (0.05, 50.0); nucleotide frequencies and substitution rates uniform on the dirichlet distribution. Three independent analyses of four linked chains were each run for 1 000 000 generations. The default temperature parameter (0.2) was used to increase the rate of mixing among chains via metropolis coupling (Altekar et al., 2004). Convergence was assessed by comparing topology and posterior probabilities for the independent runs. Likelihoods were graphed against generation number in Excel and the ''burn-in'' was visually determined to be confined to the initial 200 000 generations for each run. Trees from these generations were excluded from analysis. Posterior probabilities reported here are based on analysis of post burn-in trees from a single run.

Likelihood analyses of the combined data were conducted in PAUP*. The topology with highest posterior probability based on Bayesian analysis was used as the start tree for model selection in Modeltest 3.5 (Posada and Crandall, 1998). The likelihood model was selected from among the 56 evaluated in Modeltest using both the Akaike information criterion (AIC) and hLRT at $\alpha = 0.01$. Model parameters were fixed during heuristic likelihood searches with random sequence addition starting trees followed by TBR branch swapping. Analysis was allowed to run for 15 to 60 h before being stopped and was repeated five times. The ''full'' branch-length likelihood-ratio test implemented in PAUP* was used to evaluate whether branch lengths were significantly greater than zero.

Sequence partitions (nuclear vs. plastid) and indel partitions were initially analyzed separately. Congruence between data partitions was evaluated by inspection, and the hypothesis that the sequence data derive from a homogeneous sample was tested using the parsimony-based incongruence length difference (ILD) test (Farris et al., 1994) as implemented in PAUP*. Although the ILD test has been criticized for its sensitivity to heterogeneity in evolutionary parameters that are not related to genealogical congruence, the weight of evidence suggests that it is a conservative first test of data partition homogeneity, provided adequate amounts of sequence data are available (Hipp et al., 2004). Tests were conducted using 499 partition homogeneity replicates, heuristic searches of 100 random addition replicates with one tree saved in each replicate, excluding uninformative characters and indels. Although this heuristic search regimen is not likely to recover all most parsimonious trees—each search can recover no more than 100 MP trees and is likely to miss MP trees in many replicates—repeated tests on both data partitions separately and on combined data found that the method invariably recovered at least one MP tree in each search. Because the ILD test depends only on the length of the MP trees and not on recovering all of them, the relatively low computational demand of this search regimen permits an increased number of ILD replicates and thus a lower variance in ILD P value.

RESULTS

Data set and search characteristics are presented in Table 1.

Analysis of trnL-trnF—The aligned *trnL-trnF* data matrix is 1520 base pairs in length. Ambiguously aligned regions between *Croton* (as defined by clades C-1 to C-11 in Fig. 5) and the remainder of the taxa total 220 base pairs. Ambiguities within the ingroup alone total 312 base pairs. Pairwise corrected distances (HKY + G) excluding ambiguities range to 0.098 among all taxa, to 0.049 among *Croton* species only. The 47 potentially informative indels range from 1 to 147 base pairs in length. Analyzed alone as unordered characters, they recover a tree that is largely congruent with the tree recovered using sequence data alone. Analyzed in combination, the sequence data and indels recover more than 100 000 trees of 1039 steps (with all characters included, CI = 0.687, RI = 0.826; with informative characters only, L = 804 and CI = 0.596). Heuristic searches using the strict consensus as a reverse constraint recover only trees of 1040 steps or longer, providing evidence that the strict consensus recovered is identical to the consensus of all most parsimonious trees for the *trnL-trnF* data set (see Fig. 2).

The HKY + G model was selected using hLRT ($\alpha = 0.01$). Akaike weight of that model is only 0.0544, with GTR + G and GTR + I + G sharing a cumulative weight of 0.9065. In an analysis of 56 models using Modeltest v3.5, 95% of the cumulative Akaike weight is shared by seven models, sug-

TABLE 1. Data set and parsimony-based tree characteristics for ITS and *trnL-trnF* analyses.

Parameter	ITS	*trnL-trnF*	Combined (excluding *C. setiger*)		
			ITS	*trnL-trnF*	ITS + *trnL-trnF*
No. accessions	96	97	92	92	92
Aligned length	738	1520	738	1520	2258
Length of ambiguous regions: ingroup	31	312[a]	31	312	343
Length of ambiguous regions: outgroup	335	220	335	220	555
Variable characters	428	473	412	456	868
Potentially informative characters	350	253	339	228	567
Number of potentially informative indels	41	47	40	42	82
Number of trees	910	>100 000	n/a	n/a	6
MP tree length	2119	1039	n/a	n/a	3195
CI (informative characters only)	0.363	0.596	n/a	n/a	0.425
RI	0.679	0.826	n/a	n/a	0.712

[a] This includes a 96-bp indel.

gesting a good deal of uncertainty in model selection. Bayesian analysis using the HKY + G model (not shown) recovers the same major clades as the parsimony analysis.

Analysis of ITS—The aligned ITS data matrix is 738 base pairs long, with 335 sites of ambiguous alignment between the ingroup and the outgroup and 31 sites ambiguously aligned among ingroup taxa. The maximum pairwise corrected genetic distance (GTR + G) among all taxa is 0.161. Within *Croton*, maximum pairwise divergence is 0.105. The 41 potentially informative indels range from 1 to 27 base pairs in length. Analyzed alone, the indel data show little phylogenetic structure and diverge strongly from sequence data at several levels in the phylogeny, although they do support the monophyly of *Croton* + *Moacroton*, excluding *Brasiliocroton* and *Astraea*. Bootstrap support within *Croton* decreases with the addition of ITS indels, which are consequently not included in the analyses presented in this paper. Parsimony analysis of ITS sequence data recovers 910 trees of 2119 steps, RI = 0.679 (with all characters included, CI = 0.390; with informative characters only, L = 2029, CI = 0.363; see Fig. 3). In the ITS tree, unlike in the *trnL-trnF* tree, *C. setiger* Hook. and *C. insularis* Baill. form a weakly supported clade within *Croton* that is sister to all of the Old World members of *Croton* sampled and all of the New World members except for *C. alabamensis* E. A. Smith ex Chapman, *C. olivaceus* Müll. Arg., and *Moacroton*.

The GTR + G model is selected for the ITS data using hLRT in MrModeltest ($\alpha = 0.01$). In an evaluation of 56 models in Modeltest, Akaike weight for the GTR + I + G model is 0.9759, suggesting little uncertainty in model selection. The Bayesian ITS topology based on GTR + G differs from the MP topology primarily in degree of resolution and in the placement of *Croton setiger* and *C. insularis*. In the Bayesian tree, these taxa fall in the same major clade as they do in the *trnL-trnF* trees.

Congruence between data partitions—The most obvious topological difference between the chloroplast and nuclear ribosomal data partitions is the position of *Croton setiger* and *C. insularis*, which in the ITS tree form a clade wedged between the *C. alabamensis* clade and the remainder of *Croton*, while in the chloroplast tree *C. insularis* falls within the Old World clade, and *C. setiger* is positioned at the base of one of the New World clades. The ILD test was performed on (1) the complete set of taxa, (2) the complete set of taxa minus outgroups, (3) the complete set of taxa minus outgroups and *C. setiger*, (4) the complete set of taxa minus outgroups and *C. insularis*, and (5) the complete set of taxa minus outgroups, *C. insularis*, and *C. setiger*. The data partitions show significant incongruence with outgroups ($p = 0.038$) and nearly significant incongruence with outgroups removed ($p = 0.066$). Removal of either *C. insularis* or *C. setiger* strongly increases congruence between data partitions ($p = 0.232$ without *C. setiger*, $p = 0.295$ without *C. insularis*, $p = 0.214$ without both taxa), suggesting that *C. setiger* and *C. insularis* contribute substantially to heterogeneity in the data set.

Analysis of combined data—Parsimony analysis of the combined data, excluding ITS indels but including *trnL-trnF* indels, recovered six most parsimonious trees in a single island (with all characters: L = 3053, CI = 0.487, RI = 0.715; with only informative characters: L = 2730, CI = 0.426). The resulting strict consensus tree (not shown) is resolved for all nodes except for a trichotomy connecting the three *C. elegans* Kunth accessions and a trichotomy connecting *C. martinianus* V. W. Steinm., *C. glandulosus* L., and *C. tiarensis* P.E. Berry and Riina. There is strong support (bootstraps > 90%) for the nodes along the spine leading to *Croton*. *Croton setiger* and *C. insularis* fall sister to one another and sister to all *Croton* species except the *C. alabamensis* + *Moacroton* clade, but support for this branch is low (61%).

Parsimony analysis with *C. setiger* removed also produces a resolved consensus that is almost identical in topology to the strict consensus with all taxa included (Fig. 4). However, *C. insularis* moves from the position sister to most of *Croton* to a position within an entirely Old World clade. This is the same clade in which it is found in the Bayesian analyses and in the parsimony analysis of *trnL-trnF*. With *C. setiger* removed, six most parsimonious trees were recovered from a single island (with all characters: L = 2995, CI = 0.491, RI = 0.717; with informative characters only: L = 2665, CI = 0.429).

Bayesian analysis of the combined data was conducted using two separate models (GTR + G for ITS, HKY + G for *trnL-trnF*), with all parameters unlinked except for topology and branch lengths (Fig. 5). *Croton setiger* is positioned at the base of the New World clade (C-5 to C-11) that falls sister to the Old World clade C-4 under both data partitions analyzed separately and in the combined analysis. The same resolution is recovered under the combined analysis with both regions modeled together using the GTR + I + G model, all param-

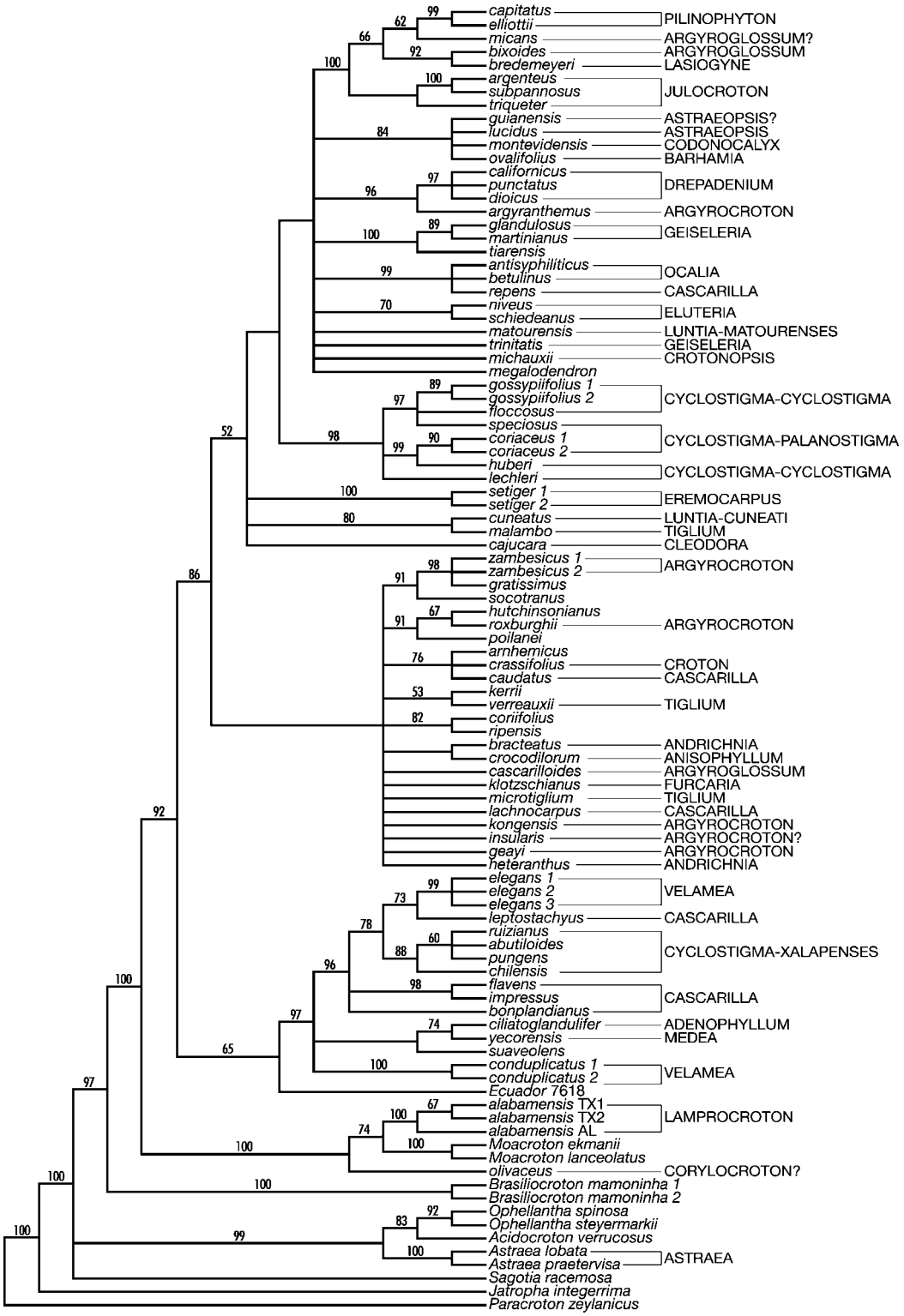

Fig. 2. Strict consensus of 1.0×10^5 minimal length trees resulting from parsimony analysis of *trnL-F* data. Numbers above branches are bootstrap percentages >50%. Names on the right in all caps refer to the section (or section–subsection) of *Croton* as assigned for the species in Webster (1993). Note that not all species were assigned to a section in that publication.

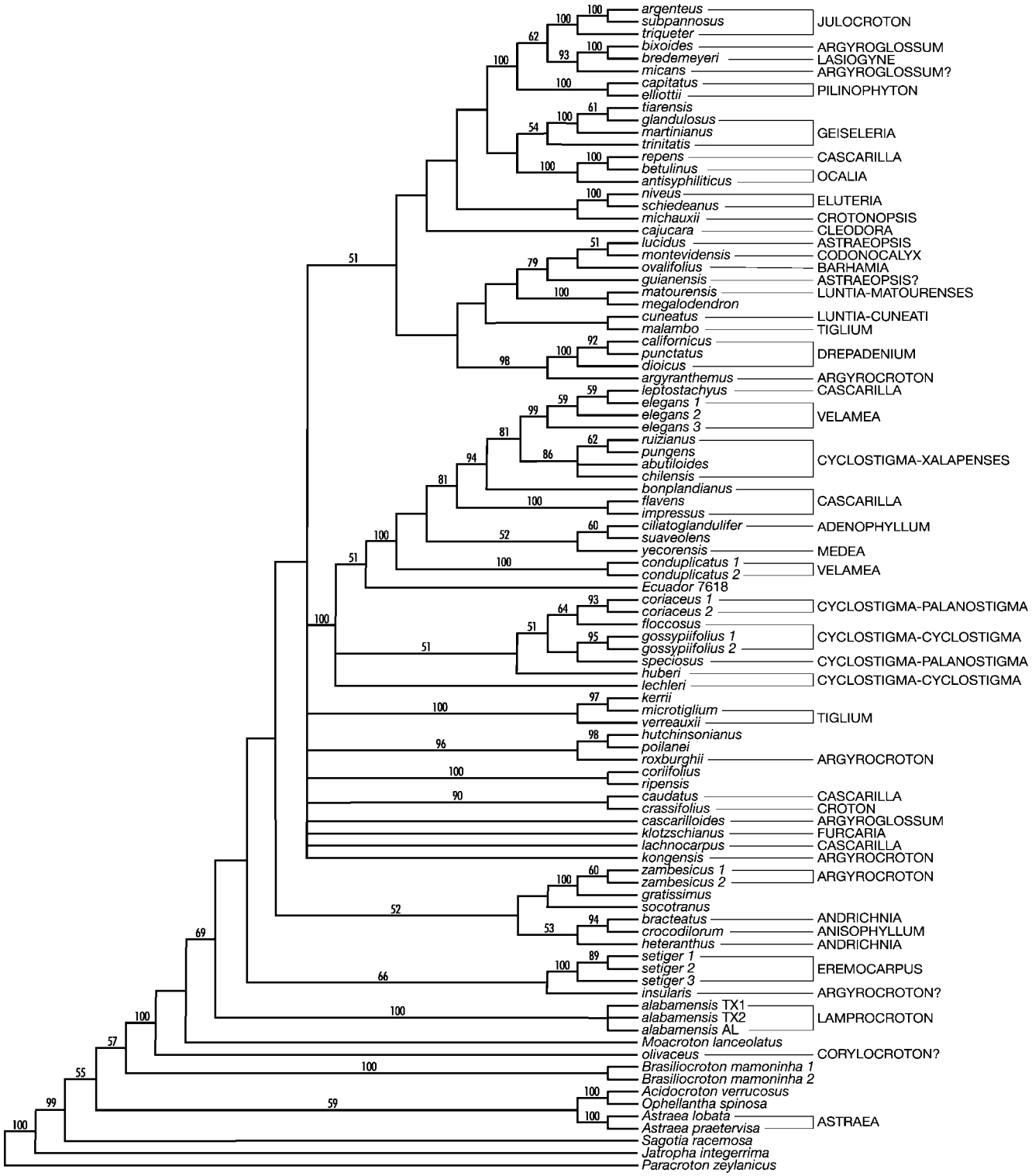

Fig. 3. Strict consensus of 910 minimal length trees resulting from parsimony analysis of ITS data. Numbers above branches are bootstrap percentages >50%. Names on the right in all caps refer to the section (or section–subsection) of *Croton* as assigned for the species in Webster (1993).

eters linked (tree not shown). In all cases, posterior probability for the branch connecting *C. setiger* to the New World clade that includes it is 1.00.

The TrN + I + G model was selected for the combined data under the hLRT ($\alpha = 0.01$) as well as AIC. Akaike weight for this model is 0.657, with a weight of 0.2961 on the TIM + I + G model (0.9531 cumulative weight). These models differ by only one parameter (1 vs. 2 transversion rates). A single ML tree was recovered in two of the five independent runs ($-\ln L = 17882.33425$; not shown). The other three runs

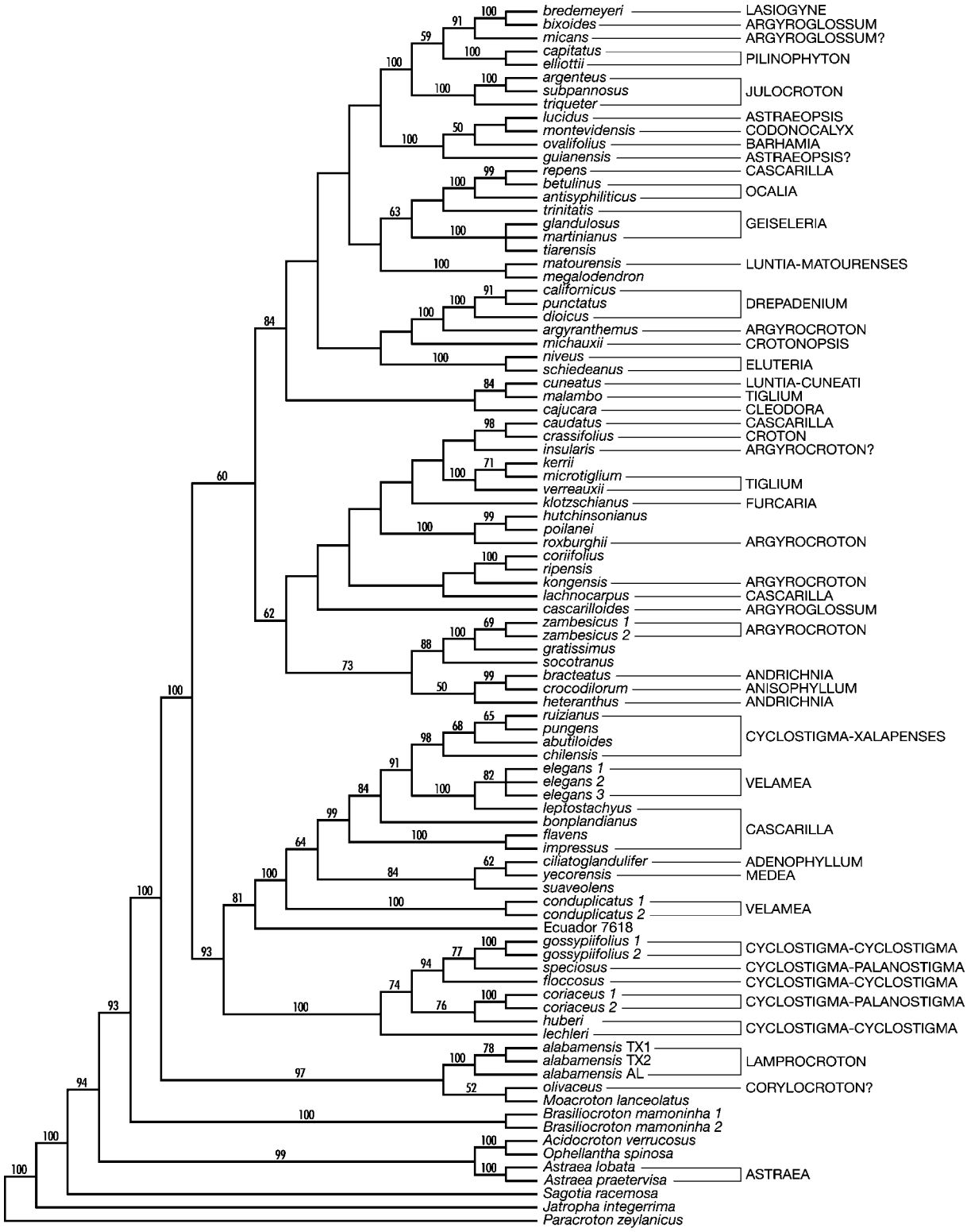

Fig. 4. Strict consensus of six minimal length trees resulting from parsimony analysis of combined *trnL-trnF* and ITS data, with *Croton setiger* excluded (see text). Numbers above branches are bootstrap percentages >50%. Names on the right in all caps refer to the section (or section–subsection) of *Croton* as assigned for the species in Webster (1993).

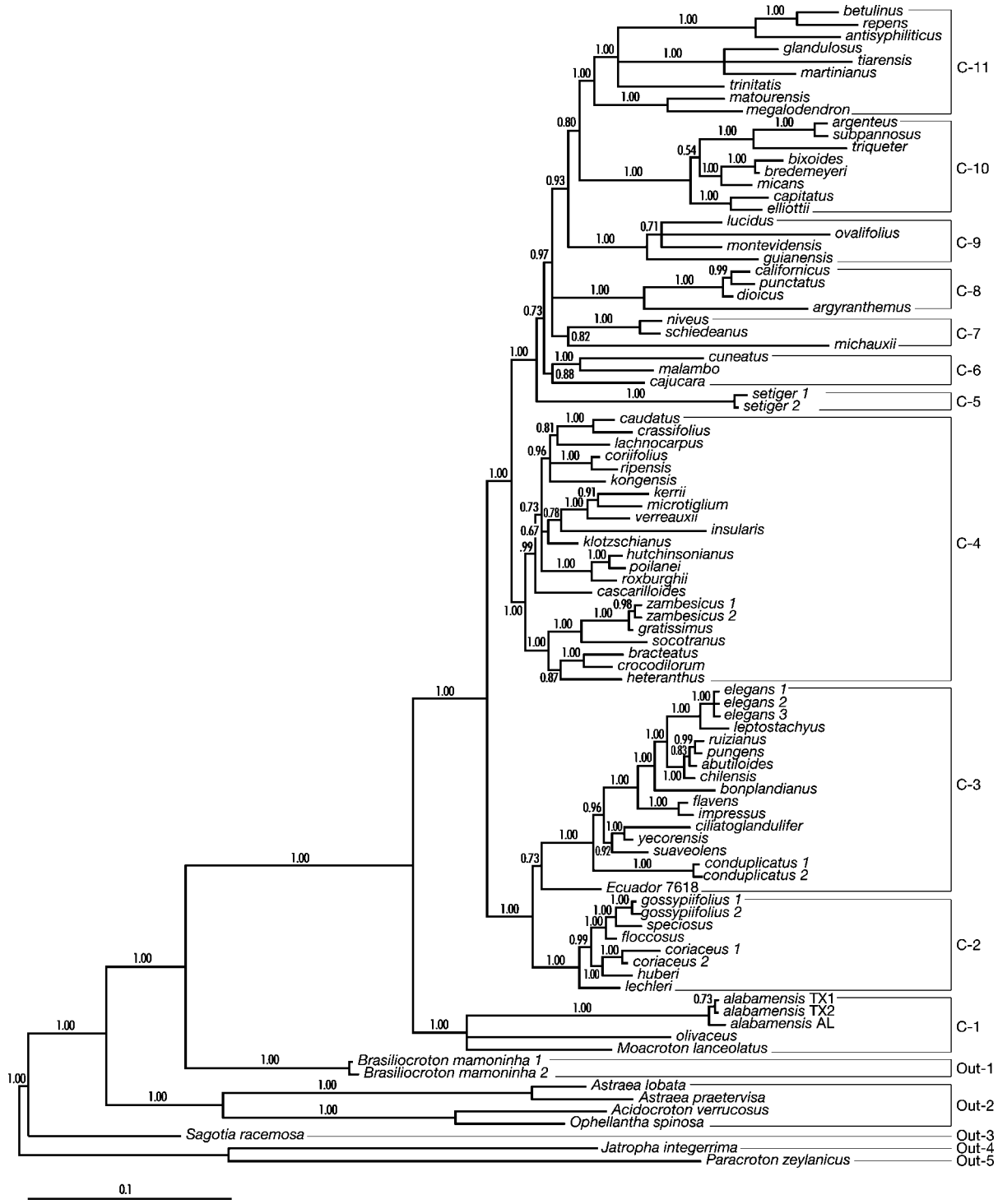

Fig. 5. Phylogram of Bayesian analysis of combined *trnL-F* and ITS data. Numbers above branches are posterior probabilities. Groups on the right are clades referred to in the text.

recovered a tree only slightly less optimal ($-\ln L = 17884.14032$). The ML topology is highly compatible with the Bayesian consensus. All clades labeled on the Bayesian topology are recovered in the ML tree, and relationships between those clades are identical to relationships between clades on the Bayesian tree, with the exception that relationships between clades C-7 and C-8 are resolved by a very short branch. Relationships are the same within all labeled clades except for C-4, which differs only in the degree of resolution and in the placement of *C. lachnocarpus* Benth.

Bayesian analyses conducted after excluding aligned nucleotides ambiguous among the outgroups recovered topologies identical in outgroup resolution to trees recovered using all data (not shown). Exclusion of ragged ends and ingroup am-

biguities resulted in two minor topological changes: collapse of the branch between *Brasiliocroton* and clade C-1, and reversal of positions between *Croton glandulosus* and *C. martinianus* (clade C-11). Average posterior probability for all nodes ≥ 0.50 in the Bayesian consensus with all ambiguities removed is 0.9565. With ambiguities included, average posterior probability is 0.9571. Trees shown in this paper include all ambiguously aligned regions, as described in the Materials and Methods.

Given the susceptibility of parsimony to analytical artifacts such as long-branch attraction (Felsenstein, 1987) and the fact that topological incongruence in this study between the nrDNA and cpDNA data partitions is lower in the likelihood and Bayesian trees than in the parsimony tree, we interpret the Bayesian topology (Fig. 5) as representing our best estimate of phylogenetic relationships within *Croton* and its outgroups. Most of the discussion in this paper will be focused on the Bayesian topology, except when taxa were only sampled for one of the single-gene analyses.

DISCUSSION

Generic circumscription and outgroup relationships—The sectional synopsis of *Croton* by Webster (1993) offers an abundant source of hypotheses concerning groups of related species (although only exemplars and not all taxa of the genus were explicitly placed within specific sections). However, it provides few clues as to how the 40 sections relate to each other or to potential outgroups among the Euphorbiaceae. Our sampling and that of Wurdack et al. (2005) form a solid basis on which to propose a redefinition of tribe Crotoneae. *Paracroton* (referred to under its synonym *Fahrenheitia* by Webster, 1994) and *Mildbraedia*, which Webster (1994) included in a weakly defined Crotoneae, are not close relatives of *Croton* but instead belong to a sister clade of largely Old World inaperturate-pollen Crotonoideae that is characterized by a unique *trnL-trnF* spacer deletion (Fig. 4 in Wurdack et al., 2005). This finding is also supported by our individual and combined analyses (Figs. 2–5). Members of tribe Jatropheae, including *Jatropha* sampled here (Out-4 in Fig. 5) and *Joannesia* sampled by Wurdack et al. (2005), emerge as a well-supported sister group to a new Crotoneae clade. The circumscription of tribe Jatropheae remains to be fully evaluated with molecular data, but the tribe appears to be paraphyletic based on a sampling of three of the eight genera (Wurdack et al., 2005). Some, if not all, of the five exclusively Old World genera formerly assigned to Jatropheae by Webster (1994) belong with the largely Old World inaperturate-pollen clade (Wurdack et al., 2005), leaving *Jatropha*, *Joannesia* and possibly *Vaupesia* to comprise the Jatropheae. The predominantly New World composition of this reduced group may indicate that Jatropheae and Crotoneae together had a New World origin.

A well-supported grade leading from *Jatropha* to *Croton* includes *Sagotia* (together with *Sandwithia*, not sampled here, but see Wurdack et al., 2005; Out-3 in Fig. 5), *Acidocroton*, *Ophellantha*, *Astraea* (Out-2 in Fig. 5), and *Brasiliocroton* (Out-1 in Fig. 5). We propose that tribe Crotoneae be modified to include the members of this grade. We acknowledge that no morphological characters have yet been identified to define this enlarged Crotoneae, but there is also no great morphological discontinuity along the grade. *Astraea* was treated by Webster (1993) as a section of *Croton*, as has been historically done because of its combination of inflexed anthers in bud and the presence of some stellate as well as simple trichomes. Although the inflexed anthers are supposedly the main morphological synapomorphy defining *Croton*, both the sister group to *Astraea* (*Acidocroton* + *Ophellantha*), and the next clade in the Crotoneae grade (*Brasiliocroton*), have anthers erect in bud, thus making inflexed anthers a potentially homoplasious character between *Astraea* and *Croton*. *Astraea* has other characters that distinguish it from *Croton*, including highly divided slender-cylindrical styles; leaves that are usually deeply lobed; male flowers with imbricate perianth and normally glabrous receptacle; seeds that are quadrangular, rugulose, and strongly carunculate; and an apparently unique chromosome number for the tribe of $n = 9$ (Miller and Webster, 1966).

The recently described *Brasiliocroton* (Berry et al., 2005) emerges here as the closest relative of the main *Croton* clade. It has a much more branched inflorescence than *Croton*, with the female flowers terminal on the side branches, and male flowers that resemble *Croton* except for the anthers remaining erect in bud. The genus has an interesting disjunct distribution in eastern Brazil between the southern Atlantic coastal forests and the sub-Amazonian liana forests of Maranhão state (Fig. 1).

Relationships within a redefined Croton—The remaining large clade (clades C-1 to C-11, Fig. 5) comprises a partially recircumscribed genus *Croton*, which contains the segregate genera *Crotonopsis*, *Eremocarpus*, and *Julocroton*. Radcliffe-Smith and Govaerts (1997) treated these groups as part of *Croton* but designated each of them as a new subgenus, due to one or more distinctive, autapomorphic characters. *Colobocarpos* (not sampled here) also belongs in *Croton* (Wurdack et al., 2005), and its separation from *Croton* based on erect anthers in bud (Esser and van Welzen, 2001) was due to an error in interpretation (K. Wurdack, unpublished). The representatives sampled from these groups in our analysis are all well embedded within *Croton* (clades C-5, C-6, and C-10 in Fig. 5), making their continued recognition as genera or even subgenera untenable from a phylogenetic perspective. The status of *Moacroton* and *Cubacroton* needs further investigation, but they also appear to be embedded within *Croton*. In our combined tree (Fig. 5), *Croton* consists of a small sister group designated as C-1 and a much larger clade that includes various subclades that we have designated as C-2 to C-11.

Clade C-1—This group, which includes *Croton alabamensis* from the southern United States, *Moacroton* (and possibly *Cubacroton* as well) from Cuba, and *Croton olivaceus* from Ecuador, is clearly separate from and sister to the rest of the large genus *Croton*. *Croton alabamensis* was treated by Webster (1993) as a member of the otherwise South American *Croton* sect. *Lamprocroton* because of its lepidote trichomes and petals in the female flowers. In our analyses, *C. alabamensis* emerges in an isolated position in the genus, on a long branch of the C-1 clade (Fig. 5). The species has a disjunct distribution between Texas and Alabama, recognized as separate varieties (both sampled), and two separate, localized populations within each state (N. Jelinksi et al., University of Wisconsin–Madison, unpublished data). *Moacroton*, with six species endemic to serpentine outcrops in Cuba, has a reduced number of stamens (three to six) and anthers that are sessile and therefore do not show the character of inflexed stamens in bud. *Cubacroton*, which Radcliffe-Smith (2001) subsumed into

Croton without any explanation, occurs near the summit of the high Sierra Maestra of Cuba on igneous substrates; *Cubacroton* also has a reduced number of stamens (two or three), but the filaments are inflexed in bud as in other species of *Croton*. The remaining species sampled in clade C-1 is *C. olivaceus*. This species appears to be allied with sect. *Corylocroton*, which includes about 10 species centered in and around the Caribbean, but also extending as far south as northern Argentina.

Clades C-2 and C-3—Within the remainder of *Croton* (clades C-2 to C-11), there are three major clades that account for the majority of species in *Croton*, as well as all of the Old World members sampled so far in the genus. The first clade, comprising C-2 and C-3, is entirely New World as far as the current sampling is concerned and includes three large sections recognized by Webster (1993), namely *Cyclostigma*, *Cascarilla*, and *Velamea*. Clade C-3 is largely composed of members of sections *Velamea* and *Cascarilla*, two of the most species-rich sections defined by Webster (1993). He distinguished the two sections by the presence of basal leaf or petiolar glands in sect. *Cascarilla* and their absence in sect. *Velamea*, while acknowledging that the two sections intergrade. Our results indicate that there is no real separation between these two groups; the petiolar gland character in these groups varies from prominent to imperceptible, with intermediate conditions along the entire spectrum. Therefore, the two sections should be merged into one, with sect. *Cascarilla* having nomenclatural priority over *Velamea*.

An unexpected result in clade C-3 is the presence of an embedded subclade of four species that were treated by Webster (1993) as members of sect. *Cyclostigma*, namely *C. abutiloides* Kunth, *C. chilensis* Müll. Arg., *C. pungens* Jacq., and *C. ruizianus* Müll. Arg. Section *Cyclostigma* was characterized by Webster (1993) as having the lower nodes of the inflorescence with bisexual cymules, stellate pubescence, and a shrubby to arborescent habit. He modified this circumscription, however, when he described sect. *Cyclostigma* subsect. *Xalapenses* (Webster, 2001), to accommodate species lacking bisexual cymule and with sessile or subsessile pistillate flowers, including *C. chilensis* and *C. pungens*. Our molecular data indicates that *C. abutiloides*, *C. chilensis*, *C. pungens*, and *C. ruizianus* are all embedded in sect. *Cascarilla*, and this may apply to the other Mexican and Central American species that Webster (2001) included in subsect. *Xalapenses*. Each of the four species sampled in this group are small to medium-sized shrubs, and the group is widely distributed from Venezuela south to Chile along the Andes. The closely related genus *Brasiliocroton*, as well as *Astraea*, both have basal bisexual cymules, so this is likely a plesiomorphic condition in the tribe.

There are two other species in clade C-3 that were not assigned to sections *Cascarilla* or *Velamea* by Webster (1993). These include *Croton ciliatoglandulifer* Ortega, which Webster assigned to sect. *Adenophyllum* based on its stalked glands on the leaf margins and its glandular stipules and pistillate calyx lobes. The other species is *C. yecorensis* V.W. Steinm. and Felger, which Steinmann and Felger (1998) assigned tentatively to sect. *Medea*, based on its glandular or laciniate stipules and pistillate sepals. Because they described the species as having stipitate glands along the leaf margin, it actually fits much better morphologically into Webster's sect. *Adenophyllum*, together with *C. ciliatoglandulifer* (Webster, 2001). We sampled four other taxa with glandular stipules and calyces, and all emerged in clade C-9, part of an entirely different major clade in the genus (see discussion for that clade). The main morphological differences between the *C. yecorensis* + *C. ciliatoglandulifer* clade in C-3 and those sections represented in clade C-9 appear to be the stipitate-glandular leaf margins in the C-3 subclade. Webster (1993) listed eight species in his sect. *Adenophyllum*. If further sampling of these species supports their position alongside *C. ciliatoglandulifer* and *C. yecorensis*, sect. *Cascarilla* would be paraphyletic unless sect. *Adenophyllum* were subsumed into an even larger sect. *Cascarilla*.

Clade C-2 corresponds to the core of sect. *Cyclostigma* and includes the type of that section, *C. gossypiifolius* Vahl. Overall, 10 of the 59 species assigned to sect. *Cyclostigma* by Webster (1993) were sampled in our analyses, including eight species of subsect. *Cyclostigma* and two of subsect. *Palanostigma*, but none from subsect. *Sampatik*. Four of the eight species from subsect. *Cyclostigma* appear in clade C-2, including *C. gossypiifolius*, *C. floccosus* B.A. Smith, *C. huberi* Steyerm., and *C. lechleri* Müll. Arg. These are all medium to large trees that produce reddish sap in the trunks and are referred to as the true ''sangre de drago'' or ''sangre de grado'' of medicinal fame in Mexico, Central America, the Andes, the Chaco, and the mountains of southeastern Brazil. Currently, *Croton lechleri* is the primary source, both locally and commercially, for South American dragon's blood remedies (Meza, 1999; Milanowski et al., 2002). The other four members that were either assigned by Webster to subsect. *Xalapenses* or else fit his description of the subsection now appear in clade C-3 as part of sect. *Cascarilla* (*C. abutiloides* + *chilensis* + *pungens* + *ruizianus*). These species are all characterized by bifid styles, a character shared between sect. *Cascarilla* and sect. *Cyclostigma* subsects. *Cyclostigma* and *Xalapenses*. Each member of this group is shrubby, with yellow (not red) sap in the stems, and their female flowers are sessile to subsessile, whereas all other members of sect. *Cyclostigma* outside of subsect. *Xalapenses* have pedicellate female flowers.

The two remaining species in clade C-2, *C. coriaceus* Kunth and *C. speciosus* Müll. Arg., were assigned by Webster (1993) to subsect. *Palanostigma*, which is characterized by multifid styles and reduplicate calyx lobes (i.e., lobes that are strongly valvate, with the adjacent edges folding outwards into an obvious flange). In our analyses, however, they do not appear as sister species, but are rather embedded within other members of subsect. *Cyclostigma*. We have yet to sample *C. palanostigma* Benth., the subsectional type, but the current circumscription of subsect. *Palanostigma* is not supported by the limited sampling reported here. Molecular phylogenetic studies with a much denser sampling of the entire *Cyclostigma* clade are currently underway by R. Riina.

Clade C-4—This clade is strongly supported in the combined Bayesian analysis, but with a relatively short branch length (Fig. 5). It includes exclusively Old World taxa of *Croton*, and with its well-supported position deep within New World lineages of *Croton* and sister groups, this suggests that *Croton* had a New World origin and subsequently diverged in the Old World. It is important to note, however, that the sampling for Old World taxa is still sparse and does not adequately cover the morphological variety of *Croton* in the Old World, particularly taxa from Africa and Madagascar that have been assigned by Webster (1993) to sect. *Cyclostigma*. Further sam-

pling needs to be done to determine if all Old World taxa form a single large clade or whether there are multiple lineages independently allied to different groups from the New World. In his 1993 sectional synopsis, Webster acknowledged that his conspectus of the genus was heavily biased towards the New World taxa with which he was more familiar. Most taxonomists who have since worked on *Croton* for Old World floras have not adopted the Webster sectional circumscriptions because ''some [Old World] species appear to have combinations of characters that transgress Webster's sections, many of which appear to be artificial and do not correlate well with some of the New World taxa included'' (Forster, 2003, p. 352). In Radcliffe-Smith's revision of Malagasy *Croton* species (A. Radcliffe-Smith, Royal Botanic Garden, Kew, unpublished manuscript), he takes a similar approach and designates his own informal groupings rather than adopting the Webster-defined sections. The results of our molecular analyses largely confirm these authors' suspicions, as far as most Old World species of *Croton* are concerned.

Within clade C-4, there is a basal dichotomy that is highly supported in the combined Bayesian analysis (posterior probability = 1.00, parsimony bootstrap = 62%). The smaller sister clade (the lowermost six species in Fig. 5) is entirely African and Malagasy. Within this group, there are two subgroups, the lowermost one with three opposite-leaved species from Madagascar that correspond to Webster's (1993) sections *Anisophyllum* and *Andrichnia*. In our trees *C. crocodilorum* Leandri, assigned by Webster to sect. *Anisophyllum*, is nested between two species he assigned to sect. *Andrichnia*, which suggests that they should probably be grouped under a single, more inclusive section instead. Within the other subgroup, *C. socotranus* Balf. f. from the island of Socotra adjacent to the Horn of Africa and south of Yemen, is sister to two species from mainland Africa.

The second subclade in C-4 includes 15 species that are all native to the southeastern Asian region, including China, Thailand, India, Sri Lanka, Australia, Micronesia, and Borneo. There is poor phylogenetic resolution among these species, but we can distinguish a strongly supported group of *C. roxburghii* N.P. Balakr., *C. poilanei* Gagnep., and *C. hutchinsonianus* Hosseus that are native to southeast Asia, all of which are trees with large leaves and either lepidote (*C. roxburghii*) or stellate indumentum. They seem to correspond most closely to Webster's sect. *Argyrocroton*, which has bifid styles and glandular-based leaves. Another more weakly supported clade includes five species, with *C. klotzschianus* (Wight) Thw. from Sri Lanka, *C. insularis* and *C. verreauxii* Baill. from Australia, *C. microtiglium* Burkill from Tonga, and *C. kerrii* Airy Shaw from Thailand. At least two of these, *C. verreauxii* and *C. microtiglium*, have very sparse pubescence and were placed by Webster (1993) in sect. *Tiglium*. *Croton kerrii* is very similar to *C. verreauxii* and shares with it stipitate basal leaf glands (H.-J. Esser, Munich Botanical Garden, personal communication). A final, well-supported group of six species include four from China, Indochina, and Thailand; one from Micronesia (*C. ripensis* Kaneh. & Hatus.); and one from Borneo (*C. coriifolius* Airy Shaw). Of these six, *C. caudatus* Geiseler, *C. lachnocarpus*, and *C. kongensis* Gagnep. were included by Webster in the mainly New World sect. *Cascarilla*. Although these may share some of the same characters as that group, they are most likely plesiomorphic characters, and the Old World species do not belong in the same section as the New World taxa. *Croton coriifolius* was placed by Webster in sect. *Tiglium*, while he included *C. crassifolius* Geiseler in sect. *Croton*.

Clade C-5—Croton setiger is a weedy annual species native to western North America and was treated by Webster (1993) as the sole member of *Croton* sect. *Eremocarpus*. It has been segregated (e.g., Radcliffe-Smith, 2001) as a monotypic genus, *Eremocarpus* Benth., because of its highly reduced flowers, unilocular ovaries, and annual habit. The position of *Croton setiger* at the base of the second major New World clade (clades C-5 to C-11) is supported with a posterior probability of 100%, despite placement in parsimony analyses of ITS alone and of the combined data as sister to core *Croton* (i.e., excluding clade C-1). The conflict between the two data partitions regarding the position of this taxon is restricted to the parsimony analysis, however, suggesting that the ITS placement of this species is an artifact of analysis. Incongruence regarding the placement of the species in the parsimony analysis is evidenced by the dramatic difference in ILD test results with and without *C. setiger*. Incongruence is also suggested, though less strongly, by the increase in parsimony bootstrap support for several nodes with the removal of *C. setiger*. Support for clade C-4 is less than 50% with *C. setiger* included (data not shown); without it, support rises to 62% (Fig. 4). Thus the data shown here are incongruent when analyzed with parsimony, as suggested by the ILD test and by the topological incongruence between the two parsimony trees. When analyzed using Bayesian or likelihood methods, however, combining data appears to be an appropriate means of recovering an accurate phylogeny. We consequently accept the placement of *C. setiger* within the C-5 to C-11 clade, although its placement as sister to clades C-6 to C-11 is not strongly supported.

Clade C-6—Although this clade represents very limited sampling from three different sections of Webster (1993), it clearly suggests that *C. cuneatus* Kl., the type species of sect. *Luntia* subsect. *Cuneati*, is not sister to *C. matourensis* Aubl., which is the type of sect. *Luntia* subsect. *Luntia*. Instead, *C. matourensis* emerges in the same New World clade, but in group C-11 together with *C. megalodendron* Müll. Arg., which was not formerly assigned to any section. The sister group relationship of *C. cuneatus* to *C. malambo* Karst. was unexpected, because Webster (1993) placed *C. malambo* in sect. *Tiglium*, a mostly Old World assemblage of species with very sparse pubescence. Many of the members of subsect. *Cuneati*, however, also have sparse, lepidote or stellate-lepidote pubescence and inflated, sessile capsules like *C. malambo*, and all have biglandular leaf bases.

Croton cajucara Benth. is a lowland riverine tropical forest species that was placed by Webster in sect. *Cleodora* because of its strongly connate pistillate calyx. Denser sampling in this clade, which includes a number of taxa from seasonally flooded forests in the Amazon basin, will help further clarify the relationships of this largely arborescent clade. We predict that some other as yet unplaced Amazonian tree species belong here, including *C. roraimensis* Benth., *C. yavitensis* Croizat, and an undescribed riverine species from Bolivia. They share with *C. cuneatus* both basal and marginal leaf glands, similar venation, and arillate (vs. carunculate) seeds.

Clade C-7—Croton michauxii G. L. Webster and the closely related (perhaps conspecific) but unsampled *C. willdenowii* G. L. Webster inhabit southeastern North America and together

form sect. *Crotonopsis*, which has also been recognized at the subgeneric or generic level (Radcliffe-Smith and Govaerts, 1997; Radcliffe-Smith, 2001). Like *C. setiger*, these are annual, monoecious herbs, with reduced flowers and fruits reduced to a single locule, but unlike that species, they have silvery stellate-lepidote pubescence, pinnately veined leaves, a branched stigma, and indehiscent fruits. Webster (1993) hypothesized that this section was a derivative of sect. *Gynamblosis*, an American group of five species including the North American *C. monanthogynus* Michx., but we have not yet sampled any species in this section.

The sister group to *C. michauxii* includes two species from sect. *Eluteria*, *C. niveus* Jacq. and *C. schiedeanus* Schltdl. This section is morphologically one of the most distinctive groups within *Croton*, with axillary inflorescences, eglandular leaves, stellate-lepidote pubescence, petals in flowers of both sexes, and multifid styles.

Clade C-8—Webster (1993) placed the North American shrub *C. argyranthemus* Michx. and two other species from South America as the sole American members of sect. *Argyrocroton*, which otherwise includes a dozen or so arborescent species from Africa, Madagascar, and Malesia, including the type of the section, *C. menyharthii* Pax. Four of the Old World species of the section were sampled in our analysis, and all four fall out in clade C-4, but not together. Therefore, this appears to be an artificial sectional alignment, and *C. argyranthemus* should no longer be treated as belonging to sect. *Argyrocroton*. Instead, it is strongly supported as sister to clade C-10, which includes three species all belonging to *Croton* sect. *Drepadenium*. This section is very well defined morphologically, with an herbaceous-subshrubby habit and about a dozen species that lack petals in flowers of both sexes and have eglandular leaves and multifid styles. Several species of sect. *Drepadenium* have been examined cytologically, and they all have the otherwise unique chromosome numbers in the genus of $n = 14$ or 28 (Urbatsch et al., 1975; Turner, 2004).

Clade C-9—The four species sampled in this clade share a suite of characters that include some degree of viscid-glandular trichomes on the stipules and/or calyx lobes, as well as eglandular petioles and leaf bases, and multifid stigmas. These species were placed by Webster (1993) in several different but presumably related sections. *Croton lucidus* L. is the type species of sect. *Astraeopsis*, in which *C. guianensis* Aubl. was tentatively placed as well by Webster. *Croton montevidensis* Spreng. belongs to sect. *Codonocalyx*, which differs from sect. *Astraeopsis* only in the looser (vs. appressed) indumentum. Lastly, *C. ovalifolius* Vahl was placed by Webster as intermediate between sections *Barhamia* and *Micranthis*, because of its habit intermediate between the shrubby species of sect. *Barhamia* and the mostly procumbent species of sect. *Micranthis*. The results here suggest that many of the members of sections with laciniate/glandular-toothed calyces (sections *Barhamia*, *Medea*, *Micranthis* with valvate calyces, and sections *Astraeopsis*, *Codonocalyx*, and *Decalobium* with reduplicate calyces), belong to the same general clade, but considerably more sampling will be needed to determine if there are well supported subclades present or not.

Clade C-10—This group forms a strongly supported clade, but internally there are three poorly resolved subclades, with only 0.54 posterior probability support for two of them. The sister subclade, with *C. capitatus* Michx. and *C. elliottii* Chapm., includes two of the three species of sect. *Pilinophytum*, a group of North American annual herbs with stellate pubescence, leaves with an eglandular base, sepals mostly seven or eight, and multifid styles. Webster (1993) hypothesized that this group was close to sects. *Velamea* and *Gynamblosis*, but our molecular analyses do not support a close relationship to sect. *Velamea* (part of sect. *Cascarilla* in clade C-3), and no members of sect. *Gynamblosis* were sampled in this study.

The remaining species sampled in this clade include a group of three species with strongly reduplicate-valvate calyx lobes in the pistillate flowers, eglandular leaf bases, and multifid styles. *Croton micans* Sw. and *C. bixoides* Vahl were both placed by Webster (1993) in sect. *Argyroglossum*, which has a shiny-lepidote indumentum on the lower leaf surface. The third species, *C. bredemeyeri* Müll. Arg., has stellate or stellate-lepidote indumentum and was therefore placed by Webster in sect. *Lasiogyne*. Its strongly supported position nested within the two species sampled of sect. *Argyroglossum* suggests that indumentum type may be variable in this clade or that we need a denser sampling among the different sections that Webster recognized.

The final subclade includes three species out of the estimated 50 that were formerly treated in the segregate genus *Julocroton* (including the type species), but which were transferred to *Croton* sect. *Julocroton* by Webster (1967). This is a morphologically well-defined group with often dense stellate indumentum, eglandular leaves, and compact inflorescences with pistillate flowers having strongly unequal and deeply divided calyx lobes. The species sampled here form a strongly supported clade, and the remaining unsampled species of the section would be expected to belong here as well.

Clade C-11—The position and members of the sister subclade to the rest of this clade, namely *C. matourensis* and *C. megalodendron*, are important because *C. matourensis* is the type of sect. *Luntia* subsect. *Matourenses* (Webster, 1993), yet it is quite distant from *C. cuneatus* (clade C-6), which is the type of Webster's sect. *Luntia* subsect. *Cuneati*. The two subsections are supposedly related by their lepidote indumentum, multifid styles, biglandular leaf bases, and arborescent habit, but *C. matourensis* shows a much denser indumentum that is present only on the undersides of the leaves. *Croton megalodendron* was previously unplaced as to section, and its position here as sister to *C. matourensis* is consistent with its tree habit, partly gamosepalous calyx, closely parallel secondary venation, and highly divided stigmas.

The rest of this clade forms an unresolved trichotomy among the species sampled. Although *C. trinitatis* Millsp. is not placed within the strongly supported subclade that includes *C. glandulosus*, *C. martinianus*, and *C. tiarensis*, they all morphologically resemble sect. *Geiseleria*. These are all herbs to small shrubs with stellate indumentum, leaves that are palmately veined and dentate with basal, often stipitate glands, sepals that are unequal and often well spaced from each other, and bifid styles. This grouping would be inclusive of what Webster (1993) recognized as a separate sect. *Podostachys*, characterized by multifid styles and a noticeable gap on the inflorescence between the staminate and pistillate flowers. Likewise, it could also include sect. *Octolobium*, which differs from sect. *Podostachys* only in the greater number of sepals (6–8 vs. five).

The final subclade includes *C. antisyphiliticus* Mart., the type species of sect. *Ocalia*, as well as *C. betulinus* Vahl, which Webster (1993) also placed in this section, which is characterized by multifid styles, stellate pubescence, and leaves that are basally glandular and with doubly dentate margins. The Mexican and Central American *C. repens* Schltdl. was placed by Webster (1993) in sect. *Cascarilla*, based on the description of bifid styles, but examination of herbarium specimens from Mexico shows that most specimens of *C. repens* have quadrifid stigmas, as well as saucer-shaped glands near the sinuses of the larger leaf lobes on the lower leaf surface.

Unsampled groups—Missing from our analyses so far are *Colobocarpos*, *Cubacroton*, and members from 11 of the sections recognized by Webster (1993): *Anadenocroton*, *Decapetalon*, *Eutropia*, *Gynamblosis*, *Klotzschiphytum*, *Lamprocroton*, *Medea*, *Monguia*, *Octolobium*, *Podostachys*, and *Quadrilobus*. Two of these sections are monotypic (*Eutropia* and *Quadrilobus*), three are exclusively Old World (*Decapetalon*, *Klotzschiphytum*, and *Monguia*), and the affinity of several of these is fairly evident, as discussed before. There is a large contingent of *Croton* species that have never been classified to section, any of which could potentially occupy an unsuspected position in the phylogeny of *Croton*. Sampling of additional taxa and genes (such as *ndhF*) is underway to allow the creation of more robust phylogenies that can be used to understand evolutionary trends and allow a new phylogenetic classification of *Croton*.

Nomenclatural and taxonomic changes—*Astraea praetervisa* (Müll. Arg.) P.E. Berry, **comb. nov.** Basionym: *Croton praetervisus* Müll. Arg. in Mart., Fl. Bras. 11(2): 240. 1873.

Croton sect. *Cascarilla* Griseb., Fl. Brit. W. I. 38. 1859.—*Croton* sect. *Velamea* Baill., Adansonia 4: 316. 1864, **syn. nov.**

Conclusions—We found in general a high congruence between the results obtained from ITS and *trnL-trnF*, suggesting that adding other genomic regions of suitable variability will increase resolution in tribe Crotoneae, especially as our sampling of taxa increases. The results reported here support the transfer of the species in *Croton* sect. *Astraea* into their own genus. The placement of the southeastern U.S. endemic shrub *Croton alabamensis* in a small sister clade to the rest of the genus together with the Cuban *Moacroton* indicates an isolated position with regards to other species of *Croton* in the southern United States. It now appears that sect. *Cyclostigma* as defined by Webster (1993) is excessively broad, with a number of species belonging instead to sect. *Cascarilla*, and the three subsections recognized by Webster will likely turn out not to be monophyletic. The emergence of an entirely Old World clade (C-4) embedded within the two major New World clades of *Croton* was surprising, although this clade has a short branch length, and there are many still unsampled Old World species, some of which may yet fall inside one of the otherwise strictly New World clades. The main morphological diversification of *Croton* appears to have occurred within the second New World clade (C-5 to C-11 in Fig. 5). Broader sampling in this group should enable us to make more robust conclusions concerning the monophyly of morphologically well-defined lineages such as sect. *Drepadenium* and sect. *Eluteria*. Of more general interest is the fact that *Croton* is a nearly ubiquitous member of many tropical habitats and represents a significant portion of higher plant diversity, yet it has been largely avoided because of its taxonomic complexity. This paper develops the first phylogenetic framework of *Croton*, which will help substantially to overcome this taxonomic impediment. With continued study, we should be able to learn which are the closest relatives of the known pharmacologically active species in *Croton*, better understand the complex biogeography of a giant genus, and eventually tackle the question of why this lineage of plants has become so diverse.

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APPENDIX. Taxa, vouchers, localities, and GenBank accession numbers for all sequences analyzed.

Taxon; *voucher*; locality; *trnL-F*; ITS.

Acidocroton verrucosus Urb.; *Webster 8463* (DAV); Jamaica; AY971265; AY971171. ***Astraea lobata*** Kl.; *Riina 1268* (WIS); Nueva Esparta, Venezuela; AY794689; AY971172. ***Astraea praetervisa*** (Müll. Arg.) P.E. Berry; *Pirani 2938* (SPF); Bahia, Brazil; AY971266; AY971173.

Brasiliocroton mamoninha P.E. Berry & I. Cordeiro ''1''; *Lobo 340* (NY); Maranhão, Brazil; AY794691; AY971174. ***Brasiliocroton mamoninha*** P.E. Berry & I. Cordeiro ''2''; *Pirani 3411* (NY); Espirito Santo, Brazil; AY971267; AY971175.

Croton abutiloides Kunth; *Berry 7617* (MO, WIS); Chimborazo, Ecuador; AY971268; AY971176. ***Croton alabamensis*** E.A. Smith ex Chapman **var. *alabamensis***; *Wurdack D008* (US); Cultivated from plants in Bibb Co., Alabama, USA; AY794692; AY971177. ***Croton alabamensis* var. *texensis*** Ginzbarg; *Carr 17733* (BRIT); Coryell Co., Texas, USA; AY971269; AY971178. ***Croton alabamensis* var. *texensis*** Ginzbarg; *Nesom 7850* (NY); Travis Co., Texas, USA; AY971270; AY971179. ***Croton antisyphiliticus*** Mart.; *Macedo 5635* (NY); Brazil; AY971271; AY971180. ***Croton argenteus*** L.; *Diaz 3943* (GUYN); Bolívar, Venezuela; AY971272; AY971181. ***Croton argyranthemus*** Michx.; *Wurdack D656* (US); Georgia, USA; AY971273; AY971182. ***Croton betulinus*** Vahl; *Steinmann 2028* (RSA); St. Croix, US Virgin Islands; AY971274; AY971183. ***Croton bixoides*** Vahl; *Webster 24015* (MO); Dominica, West Indies; AY971275; AY971184. ***Croton bonplandianus*** Baill.; *Killeen 4272* (MO); Santa Cruz, Bolivia; AY971276; AY971185. ***Croton bracteatus*** Lam.; *Schatz 3185* (MO); Fianarantsoa, Madagascar; AY971277; AY971186. ***Croton bredemeyeri*** Müll. Arg.; *van der Werff 3514* (MO); Falcón, Venezuela; AY971278; AY971187. ***Croton cajucara*** Benth.; *Hopkins 653* (MO); Roraima, Brazil; AY971279; AY971188. ***Croton californicus*** Müll. Arg.; *Merello 1919* (MO); Arizona, USA; AY971280; AY971189. ***Croton capitatus*** Michx.; *Miller 9085* (MO); Florida, USA; AY971281; AY971190. ***Croton cascarilloides*** Raeusch.; *Esser 2001–1* (WIS); Chanthaburi, Thailand; AY971282; AY971191. ***Croton caudatus*** Geiseler; *Soejarto 7728* (MO); Palawan, Philippines; AY971283; AY971192. ***Croton chilensis*** Müll. Arg.; *Berry 7713* (WIS); Cultivated at Rancho Santa Ana Botanic Garden, California; native to Chile; AY971284; AY971193. ***Croton ciliatoglandulifer*** Ortega; *Ramírez 480* (MO); Nayarit, Mexico; AY971285; AY971194. ***Croton conduplicatus*** Kunth ''1''; *Berry 5542* (MO); Bolívar, Venezuela; AY794695; AY971195. ***Croton conduplicatus*** Kunth ''2''; *Riina 1266* (WIS); Nueva Esparta, Venezuela; AY971286; AY971196. ***Croton coriaceus*** Kunth ''1''; *Berry 7603* (WIS); Pichincha, Ecuador; AY971287; AY971197. ***Croton coriaceus*** Kunth ''2''; *Berry 7620* (WIS); Chimborazo, Ecuador; AY971288; AY971198. ***Croton coriifolius*** Airy Shaw; *Eerwog* s.n. (photo); Sarawak, Borneo, Malaysia; AY971289; AY971199. ***Croton crassifolius*** Geiseler; *Esser 98–157* (A); Nong Khai, Thailand; AY971290; AY971200. ***Croton crocodilorum*** Leandri; *Eboroke 929* (MO); Toliara, Madagascar; AY971291; AY971201. ***Croton cuneatus*** Kl.; *Berry 7589* (PORT); Apure, Venezuela; AY794698; AY971202. ***Cro-***

ton dioicus Cav.; *Brant 1934* (MO); Texas, USA; AY971292; AY971203. ***Croton*** sp. Ecuador 7618; *Berry 7618* (WIS); Chimborazo, Ecuador; AY971293; AY971204. ***Croton elegans*** Kunth ''1''; *Berry 7600* (WIS); Pichincha, Ecuador; AY971294; AY971205. ***Croton elegans*** Kunth ''2''; *Berry 7676* (WIS); Imbabura, Ecuador; AY971295; AY971206. ***Croton elegans*** Kunth ''3''; *Berry 7675* (WIS); Imbabura, Ecuador; AY971296; AY971207. ***Croton elliottii*** Chapm.; *Wurdack D455* (US); Florida, USA; AY971297; AY971208. ***Croton flavens*** L. **var. *rigidus*** Müll. Arg.; *Steinmann 2015* (WIS); St. Croix, US Virgin Islands; AY971298; AY971209. ***Croton floccosus*** B.A. Smith; *Berry 7610* (WIS); Pichincha, Ecuador; AY971299; AY971210. ***Croton glandulosus*** L.; *Riina 1267* (WIS); Nueva Esparta, Venezuela; AY971300; AY971211. ***Croton gossypiifolius*** Vahl ''1''; *Riina 1261* (WIS); Trujillo, Venezuela; AY971301; AY971212. ***Croton gossypiifolius*** Vahl ''2''; *Berry 5806* (WIS); Miranda, Venezuela; AY971302; AY971213. ***Croton gratissimus*** Burch.; *Wurdack D536* (US); Cultivated, New York Botanical Garden, USA; native to Africa; AY794696; AY971214. ***Croton guianensis*** Aubl.; *Berry 5535* (MO); Bolívar, Venezuela; AY971303; AY971215. ***Croton heteranthus*** DC.; *Gereau 5773* (MO); Toamasina, Madagascar; AY971304; AY971216. ***Croton huberi*** Steyerm.; *Berry 7679* (WIS); Distrito Federal, Venezuela; AY971305; AY971217. ***Croton hutchinsonianus*** Hosseus; *Esser 99–8* (WIS); Kamphaeng Phet, Thailand; AY971306; AY971218. ***Croton impressus*** Urb.; *Judd 6119* (NY); Puerto Rico, USA; AY971307; AY971219. ***Croton insularis*** Baill.; *Berry 7682* (WIS); cultivated, Sydney Botanical Garden, Australia; native to Queensland; AY971308; AY971220. ***Croton kerrii*** Airy Shaw; *Esser 98–233* (WIS); Uttaradit, Thailand; AY971309; AY971221. ***Croton klotzschianus*** (Wight) Thw.; *Wambeek 2633* (US); Sri Lanka; AY971310; AY971222. ***Croton kongensis*** Gagnep.; *Esser 98–156* (A); Chaiyaphum, Thailand; AY971311; AY971223. ***Croton lachnocarpus*** Benth.; *Newmann 1186* (E); Thailand; AY971312; AY971224. ***Croton lechleri*** Müll. Arg.; *Berry 7622* (WIS); Morona-Santiago, Ecuador; AY971313; AY971225. ***Croton leptostachyus*** Kunth; *Riina 1260* (WIS); Trujillo, Venezuela; AY971314; AY971226. ***Croton lucidus*** L.; *Nee 44194* (MO); Puerto Rico, USA; AY794701; AY971227. ***Croton malambo*** Karst.; *Zarucchi 3856* (MO); Bolívar, Colombia; AY971315; AY971228. ***Croton martinianus*** V.W. Steinm.; *Steinmann 606* (RSA); Sonora, Mexico; AY971316; AY971229. ***Croton matourensis*** Aubl.; *Chavez 248* (MO); Pucallpa, Peru; AY971317; AY971230. ***Croton megalodendron*** Müll. Arg.; *Riina 1273* (WIS); Miranda, Venezuela; AY971318; AY971231. ***Croton micans*** Sw.; *Riina 1269* (WIS); Nueva Esparta, Venezuela; AY971319; AY971232. ***Croton michauxii*** G.L. Webster; *Wurdack* s.n. (US); North Carolina, USA; AY794702; AY971233. ***Croton microtiglium*** Burkill; *Drake 375* (BISH); Tonga; AY971320; AY971234. ***Croton montevidensis*** Spreng.; *Scur 332* (US); Rio Grande do Sul, Brazil; AY971321; AY971235. ***Croton niveus*** Jacq.; *Berry 7596* (WIS); Oaxaca, Mexico; AY971322; AY971236. ***Croton olivaceus*** Müll. Arg.; *Neill 11163* (MO); Napo, Ecuador; AY794694; AY971237. ***Croton ovalifolius*** Vahl; *Riina 1271* (WIS); Nueva Esparta, Venezuela; AY971323; AY971238. ***Croton poilanei*** Gagnep.; *Esser 98–162* (BKF); Nong Khai, Thailand; AY971324; AY971239. ***Croton punctatus*** Jacq.; *Bradley 149* (MO); Texas, USA; AY971325; AY971240. ***Croton pungens*** Jacq.; *Riina 1272* (WIS); Aragua, Venezuela; AY971326; AY971241. ***Croton repens*** Schltdl.; *Steinmann 1062* (RSA); Nayarit, Mexico; AY971327; AY971242. ***Croton ripensis*** Kaneh. & Hatus.; *Rinehart LR19676* (US); Pohnopei, Micronesia; AY971328; AY971243. ***Croton roxburghii*** N.P. Balakr.; *Esser 99–11* (WIS); Nakhon Sawan, Thailand; AY971329; AY971244. ***Croton ruizianus*** Müll. Arg.; *Berry 7616* (WIS); Chimborazo, Ecuador; AY971330; AY971245. ***Croton schiedeanus*** Schltdl.; *Aguilar 886* (MO); Puntarenas, Costa Rica; AY971331; AY971246. ***Croton setiger*** Hook. ''1''; *Walker 2548* (WIS); California, USA; AY971332; AY971247. ***Croton setiger*** Hook. ''2''; *Hughey* s.n. (US); California, USA; AY794697; AY971249. ***Croton setiger*** Hook. ''3''; *Berry 7710* (WIS); California, USA; —; AY971248. ***Croton socotranus*** Balf. f.; *Lavranos & James 31042* (RSA); cultivated at Rancho Santa Ana Botanical Garden, California; plants from Socotra, Yemen; AY971333; AY971250. ***Croton speciosus*** Müll. Arg.; *Berry 7590* (MO, WIS); Distrito Federal, Venezuela; AY794699; AY971251. ***Croton suaveolens*** Torr.; *Devender 96257* (RSA); Coahuila, Mexico; AY971334; AY971252. ***Croton subpannosus*** Müll. Arg. ex Griseb.; *Webster 25374* (MO); Mato Grosso do Sul, Brazil; AY971335; AY971253. ***Croton tiarensis*** P.E. Berry & R. Riina; *Riina 1274* (WIS); Aragua, Venezuela; AY971336; AY971254. ***Croton trinitatis*** Millsp.; *Berry 7586* (WIS); Amazonas, Venezuela; AY971337; AY971255. ***Croton triqueter*** Lam.; *Nee 40034* (NY); Santa Cruz, Bolivia; AY794700; AY971256. ***Croton verreauxii*** Baill.; *Berry 7683* (WIS); cultivated, Sydney Botanical Garden, Australia; native to New South Wales, Australia; AY971338; AY971257. ***Croton yecorensis*** V.W. Steinm. & Felger; *Devender 95469* (RSA); Sonora, Mexico; AY971339; AY971258. ***Croton zambesicus*** Müll. Arg. ''1''; *Berry 5781* (WIS); cultivated, Sydney Botanical Garden, Australia; native to Africa; AY971340; AY971259. ***Croton zambesicus*** Müll. Arg. ''2''; *Zimba 901* (MO); Songwe Gorge, Zambia; AY971341; AY971260.

Jatropha integerrima Jacq.; *Wurdack D047* (US); cultivated, Fairchild Tropical Gardens, Florida, USA; native to Neotropics; AY794685; AY971261.

Moacroton ekmanii (Urb.) Croizat; *Axelrod 10371* (US); Holgún, Cuba; AY971343; —. ***Moacroton lanceolatus*** Alain; *Figueiras 274* (US); Holgún, Cuba; AY971342; AY971262.

Ophellantha spinosa Standl.; *Gentry 74385* (MO); Jalisco, Mexico; AY971344; AY971263. ***Ophellantha steyermarkii*** Standl.; *Breedlove 46994* (NY); Chiapas, Mexico; AY794690; —.

Paracroton zeylanicus (Müll. Arg.) N.P. Balakr. & Chakrab.; *Annable 3575* (NY); cultivated, Hawaii, USA; native to India and Sri Lanka; AY794719; AY972074.

Sagotia racemosa Baill.; *Smith 253* (US); Madre de Dios, Peru; AY794687; AY971264.