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Phylogenetics, Conservation, and Historical Biogeography of the West Indian Members of the Adelieae (Euphorbiaceae)

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

PHYLOGENETICS, CONSERVATION, AND HISTORICAL BIOGEOGRAPHY OF
THE WEST INDIAN MEMBERS OF THE ADELIEAE (EUPHORBIACEAE)

A dissertation submitted in partial fulfillment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY

in

BIOLOGY

by

Brett Jestrow

2010

To: Dean Kenneth Furton
College of Arts and Sciences

This dissertation, written by Brett Anthony Jestrow, and entitled Phylogenetics, Conservation, and Historical Biogeography of the West Indian Members of the Adelieae (Euphorbiaceae), having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Date of Defense: November 12, 2010

The dissertation of Brett Jestrow is approved.

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Florida International University, 2010

DEDICATION

This dissertation is dedicated to my family, not to mention the dog.

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ABSTRACT OF THE DISSERTATION

PHYLOGENETICS, CONSERVATION, AND HISTORICAL BIOGEOGRAPHY OF
THE WEST INDIAN MEMBERS OF THE ADELIEAE (EUPHORBIACEAE)

by

Brett Jestrow

Florida International University, 2010

Miami, Florida

Professor Javier Francisco-Ortega, Major Professor

The Caribbean Island Biodiversity Hotspot is the largest insular system of the New World and a priority for biodiversity conservation worldwide. The tribe Adeliae (Euphorbiaceae) has over 35 species endemic to this hotspot, representing one of the most extraordinary cases of speciation in the West Indies, involving taxa from Cuba, Hispaniola, Jamaica, and the Bahamas. These species form a monophyletic group and traditionally have been accommodated in two endemic genera: *Lasiocroton* and *Leucocroton*. A study based on: (1) scanning electron microscopy of pollen and trichomes, (2) macromorphology, and (3) molecular data, was conducted to reveal generic relationships within this group. Phylogenies were based on parsimony and Bayesian analyses of nucleotide sequences of the ITS regions of the nuclear ribosomal DNA and the non-coding chloroplast DNA spacers *psbM-trnD* and *ycf6-pcbM*. One species, *Lasiocroton trelawniensis*, was transferred from the tribe into the genus *Bernardia*. Of the remaining species, three major monophyletic assemblages were revealed, one was restricted to limestone areas of Hispaniola and was sister to a clade with two monophyletic genera, *Lasiocroton* and *Leucocroton*. Morphological,

biogeographical, and ecological data provided additional support for each of these three monophyletic assemblages. The Hispaniolan taxa were accommodated in a new genus with four species: *Garciadelia*. *Leucocroton* includes the nickel hyperaccumulating species from serpentine soils of Cuba, while the rest of the species were placed in *Lasiocroton*, a genus restricted to limestone areas. The geographic history of the islands as well as the phylogenetic placement of the *Leucocroton*-alliance, allows the research to include the historical biogeography of the alliance across the islands of the Caribbean based on a dispersal-vicariance analysis. The alliance arose on Eastern Cuba and Hispaniola, with *Lasiocroton* and *Leucocroton* diverging on Eastern Cuba according to soil type. Within *Leucocroton*, the analysis shows two migrations across the serpentine soils of Cuba. Additional morphological, ecological, and phylogenetic analyses support four new species in Cuba (*Lasiocroton gutierrezii*) and Hispaniola (*Garciadelia abbottii*, *G. castilloae*, and *G. mejiae*).

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CHAPTER I

Lasiocroton trelawniensis (Euphorbiaceae), a critically endangered species from the Cockpit Country of Jamaica, belongs to *Bernardia* (Euphorbiaceae)

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ABSTRACT

A taxonomic study based on scanning electron microscopy of pollen, macro-morphology, and a molecular phylogenetic analysis of the chloroplast DNA regions *trnL-F* and *rbcL* shows that *Lasiocroton trelawniensis* belongs to the Neotropical genus *Bernardia*. The main morphological differences between *Bernardia* and *Lasiocroton* are given, and the new combination ***Bernardia trelawniensis*** is provided.

Key Words: Antilles, Caribbean, conservation, DNA phylogenetics, SEM, West Indies

INTRODUCTION

Conservation without clear knowledge of the taxonomy and phylogenetic status of the target taxa can be deceptive. Phylogenetic reconstructions constitute a necessary aspect of the framework required for assigning conservation priorities (Rodrigues et al., 2005), and phylogenetic trees are fundamental for understanding the taxonomy of endangered species (Sinclair et al., 2005). A species misplaced in a particular genus can have a definite impact on the establishment of conservation and management strategies (Rodrigues et al., 2005). A case in point takes place in the Cockpit Country of Jamaica. This region consists primarily of mesic forest over steep limestone hills and is well known as a major “hot spot” for endemic plant species in the West Indies (Iremonger, 1997). While the Cockpit Country only accounts for 4% the landmass of Jamaica, approximately 60% of the island-endemic vascular plants occur in the region (Iremonger, 1997). *Lasiocroton* Griseb., a genus of euphorbiaceous trees, is diverse on Jamaica, with four of the six species endemic to the island (Adams, 1972). All species found on Jamaica are listed by the IUCN (2007) from the Endangered (E) to the Lower Risk (LR) categories. *Lasiocroton trelawniensis* C.D. Adams, the species of discussion for this paper, is endemic to a single hilltop, “Island View Hill,” in the Cockpit Country (Adams, 1970), and I consider it to be in the Critically Endangered category (CR, based on criteria B1 + D, i.e. present from only one locality with a population size estimated to number fewer than 50 mature individuals) of the red list guidelines of the World Conservation Union (IUCN, 2001). *Lasiocroton trelawniensis* was incorrectly assigned to the Endangered category by the IUCN (2007).

Webster (1994) placed *Lasiocroton* in the tribe Adelleae s.l. (subfamily Acalyphoideae) on the basis of morphology. Recently, Wurdack et al. (2005) and De-Nova et al. (2006) published molecular phylogenies of the Euphorbiaceae. The study by Wurdack et al. (2005) focused on the chloroplast regions *rbcL* and *trnL-F* and proposed a new circumscription for the Acalyphoideae s.s. This subfamily has two major lineages, named as the “core acalyphoids” and the “alchorneoids.” The core acalyphoids comprise eight distinct clades, which were labeled from A1 to A8. *Lasiocroton* belongs to A6, and this clade is sister to a monophyletic assemblage composed of Clades A7 and A8. Clade A5 is sister to the group formed by Clades A6, A7, and A8. The genus *Bernardia* belongs to Clade A7. (Fig. 3.).

These previous molecular studies also changed the tribal placements of some genera within the Acalyphoideae. The tribe Adelleae no longer contains the genera *Enriquebeltrania* Rzed. and *Crotonogynopsis* Pax, results also supported by palynological data (Nowicke et al., 1999). The removal of these two genera restricts the Adelleae to the New World. *Philyra* Klotzsch, a monotypic South American genus treated by Webster (1994) in the tribe Chrozophoreae subtribe Ditaxinae, was found to be closely allied to the Adelleae. Therefore I consider the Adelleae s.s. to include four genera: *Adelia* L., *Lasiocroton*, *Leucocroton* Griseb., and *Philyra*. These genera form a monophyletic group, which represent half of the genera of Clade A6 from Wurdack et al. (2005). In addition, I also propose that *Adenophaedra* (Müll. Arg.) Müll. Arg., *Bernardia* Houst. ex Mill., and *Caryodendron* H. Karst. comprise the tribe Bernardieae s.s. (Clade A7 from Wurdack et al., 2005). These two tribes, as recognized in my study, are strongly supported by bootstrap values higher than 95% (Wurdack et al., 2005). However, certain representatives of the two

tribes are morphologically similar, exemplified by the lectotype of *Bernardia* was initially described as *Adelia bernardia* L. For example, recently *Adelia cinera* (Wiggins & Rollins) A. Cerv., V. W. Steinm. & Flores Olvera was moved from *Bernardia* (Cervantes et al., 2003).

As part of an ongoing project on the systematics of the West Indian genera *Lasiocroton* and *Leucocroton*, I investigate the taxonomic placement of *Lasiocroton trelawniensis* based on morphological, palynological, and DNA evidence. My study shows that this Jamaican endemic does not belong to the Adelieae, but to the Bernardieae where I have placed in *Bernardia*, a neotropical genus with approximately 70 species (Radcliffe-Smith, 2001) and seven sections (Müller Argovienensis, 1866; Pax and Hoffman, 1912). Pax and Hoffman (1912) provided the most recent published taxonomic treatment for this genus. Hereafter, I will refer to this species as *B. trelawniensis* (see taxonomic treatment below).

The lectotype of this genus is *Bernardia carpinifolia* Griseb. (= *B. dichotoma* (Willd.) Müll. Arg.) (Webster, 1994), and the description was published by Grisebach in the 19th century (Grisebach, 1859). Later Müller Argovienensis (1866) published *B. dichotoma*, based on the earlier name *Croton dichotomus* Willd. (Willdenow, 1805). *Bernardia dichotoma* is the name currently used in the West Indian Floras and *B. carpinifolia* is considered a synonym (e.g., Adams, 1972; Correll and Correll, 1982; Howard, 1989; Liogier, 1953, 1986; Liogier and Martorell, 2000). The type species is endemic to the Caribbean Islands, and it belongs to the section *Tyria* (Klotzsch) Müll. Arg., a taxon with 26 species (Cervantes, 2006). Because this section includes the type of the *Bernardia*, according to the rules of botanical nomenclature, I will refer to this taxon as section *Bernardia*. Pax and Hoffman

(1912) morphologically differentiated this group as shrubs having an indumentum with stellate or fasciculate trichomes, glandulous receptacles, and lacinulate-lacerate styles.

MATERIALS AND METHODS

Pollen Study. Pollen from *Bernardia trelawniensis* and *Lasiocroton macrophyllus* (Sw.) Griseb. was removed from herbarium specimens collected in the wild in Jamaica (*B. trelawniensis*: Jestrow 1004 (FTG); *L. macrophyllus*: Jestrow 1003 (FTG) and studied with Scanning Electron Microscopy (SEM) in the Florida Center for Analytical Electron Microscopy (FCAEM) of Florida International University (FIU) using the instrument JEOL JSM 5900LV. Untreated pollen was coated with gold-palladium using a SPI-Module Sputter Coater at 367Å for four minutes. Although pollen for other species of *Bernardia* were not examined in my study, I made comparisons with SEM images previously published by Nowicke et al. (1999) and Takahashi et al. (2000).

DNA Study. My study includes a phylogenetic analyses of nucleotide sequences of the chloroplast regions *rbcL* and *trnL-F*, and uses both data available in GenBank and published by Wurdack (2005), as well as new nucleotide sequences generated for this study. The ingroup included all the sequences available from GenBank for those taxa placed in Clades A6, A7, and A8 of the “core acalyphoid” group as defined by Wurdack et al. (2005) (24 species in 20 genera for *trnL-F*, and 19 species in 18 genera for *rbcL*). The sequence of *Bernardia* available for the *trnL-F* in GenBank is for *B. scabra* Müll. Arg., but it only had the half of the 3' end. The *Bernardia* GenBank (GB) sequence for *rbcL* is for *B. myricifolia* (Scheele) Watson. The ingroup had the following sequences obtained by us. For *rbcL*: *Bernardia dichotoma* (GB: EF470580; Jestrow 1001 (FTG)) and *B. trelawniensis* (GB:

EF470579; *Jestrow 1004* (FTG)). For *trnL-F*: *Bernardia dichotoma* (GB: EF470582 and EF470584; *Jestrow 1001* (FTG)), *B. myricifolia* (GB: EF470581 and EF470583; *Barron S. Rector 335* (FTG)), and *B. trelawniensis* (GB: EF107750 and EF107751; *Jestrow 1004* (FTG)).

I was unable to sample broadly within *Bernardia*. However I included *Bernardia dichotoma*. The lectotype of the genus (*B. carpinifolia*) is a synonym of *B. dichotoma*. My *Bernardia* sampling further included *B. myricifolia*, a species also belonging to section *Bernardia* with *B. dichotoma*. *Bernardia scabra* belongs to section *Polyboea* (Klotzsch) Müll. Arg. and was included in my analyses in order to have in my ingroup as many species as possible for this genus. The outgroup included two species (*Seidelia triandra* (E. Mey.) Pax and *Leidesia procumbens* (L.) Prain), belonging to Clade A5 (as defined by Wurdack et al. (2005)); based on previous phylogenetic studies this group is sister to all the genera of the ingroup (Wurdack et al. 2005). The nucleotide sequences of the outgroup were also retrieved from Wurdack et al. (2005) via GenBank.

Plant DNA was extracted from silica-dried material using a Qiagen DNeasy Plant Mini kit. PuReTaq Ready-To-Go PCR beads were used for the PCR amplification reaction mix. For both regions the cycle program is as follows: 80°C: 5 min, (94°C: 1 min, 50°C: 1 min, 72°C: 2 min) x35, 72°C: 5 min. The *trnL-F* amplification was obtained from four primers in two separate fragments because of difficulties in amplifying the region as a whole. The first fragment was amplified using primers “c” and “d” from Taberlet et al. (1991). Primers “e” and “f” were used to amplify the second fragment (Taberlet et al, 1991). The *rbcL* amplification was obtained using forward primer, *rbcL* RH1, and reverse primer, *rbcL* 3’ZC (Zurawski and Clegg, 1987). Cycle sequencing was performed in both directions with

the ABI PRISM™ BigDye™ v.3.1 Terminator Cycle Sequencing Kit (Perkin Elmer) following the manufacturers instructions. The primers used for PCR amplification were also utilized for the cycle sequencing reactions. Nucleotide sequences were visualized on a PE Applied Biosystems 377 automated sequencer at the Florida International University (FIU) DNA Core Facility.

All sequences were assembled and visually aligned, using Sequencher 3.1.1 (Gene Codes, Michigan). Both regions, *rbcL* and *trnL-F*, were found to be alignable and no regions were omitted in the analysis. Maximum Parsimony analysis was performed using PAUP* 4.0b10 (Swofford, 2003) using the heuristic algorithm with equal weights and unordered characters, tree-bisection-reconnection (TBR) swapping, and MulTrees “on.” Gaps were coded as uninformative. Phylogenetic support for each clade was evaluated through bootstrap analysis (Felsenstein, 1985) using 100 replicates in PAUP* with the same settings, except MulTrees was set to “off” in order to shorten calculation time for both the *trnL-F* and combined bootstrap analyses. Consistency index (CI; excluding uninformative characters) and retention index (RI) were also calculated (Kluge and Farris, 1969; Farris, 1989).

I conducted three different phylogenetic analyses, the first based on the *rbcL* data matrix (23 taxa), the second one on the *trnL-F* data set (29 taxa), and the third one on a combined matrix that included data from *trnL-F* and *rbcL*. The combined analyses only included those species for which data were available for both markers (22 taxa), with the only exception being for the genus *Dysopsis* Baill. As the genus *Dysopsis* included different species for the *rbcL* and the *trnL-F* analyses, the sequences from the two different species were combined as *Dysopsis sp.* for the combined analysis. This approach was also followed by Wurdack et al. (2005). I feel this is justified given the very close associations of the two

taxa, both were historically considered varieties of the same species, and presently some authorities consider *Dysopsis* to be a monotypic genus (Radcliffe-Smith, 2001). Also for the combined analysis, both the *rbcL* and *trnL-F* regions for each taxa were included with equal weighting according to individual base characters.

RESULTS AND DISCUSSION

Pollen Data. *Bernardia trelawniensis* has a punctate tectum with a psilate surface and lacks an operculum (Fig. 1). These are characteristics of *Bernardia* pollen that are not known to occur in *Lasiocroton* (Nowicke et al., 1999; Takahashi et al., 2000). In contrast, the pollen of *Lasiocroton* is strikingly different with both a fusiform operculum and a crotonoid tectum (Fig. 1, Table 1). Additional distinctive micromorphological features previously recorded by Nowicke et al. (1999) and Takahashi (2000) (i.e., margo and costa ectocolpi) are also indicated in Table 1. The presence or absence of margo and costa ectocolpi of *B. trelawniensis* were not observed. Nowicke et al. (1999) included nine species of *Bernardia* and Takahashi et al. (2000) included three species of *Lasiocroton*, allowing for robust confidences in their conclusions about pollen structure. The palynological data clearly supports the placement of my target species in *Bernardia* and not in *Lasiocroton*.

Molecular phylogenetic data--

***rbcL*.** The final aligned matrix for the *rbcL* parsimony analysis was 1446 nucleotide characters in length. There were 137 variable characters, with 54% (74) of these parsimony-informative. This phylogenetic analysis yielded 64 most-parsimonious trees of 230 steps each (CI = 0.5409; RI = 0.7137). The strict consensus of the 64 most parsimonious trees is

shown in Fig. 2. *Bernardia trelawniensis* forms a polytomy with the other two species of *Bernardia* included in the analyses, with 100% bootstrap support.

***trnL-F*.** The final aligned matrix for the *trnL-F* parsimony analysis had 1419 nucleotide characters. There were 319 variable characters, with 42% (135) of these being parsimony-informative. The parsimony analysis yielded 18 most-parsimonious trees of 433 steps each (CI = 0.7031; RI = 0.8455). The strict consensus of all 18 most parsimonious trees is shown in Fig. 2. The strict consensus shows *Bernardia trelawniensis* as sister to *B. dichotoma* with bootstrap support of 83%, and this clade is sister to *B. myricifolia* (bootstrap support of 90%).

The *trnL-F* study also has some additional phylogenetic implications for the family. Wurdack et al. (2005) showed that the tribe Bernardieae s.s. (Clade A7) to form a strongly supported monophyletic group after the phylogenetic analyses of the *rbcL* data alone and of the combined *rbcL* and *trnL-F* data set. However, the *trnL-F* phylogeny obtained by Wurdack et al. (2005) weakly supported the recognition of the tribe Bernardieae s.s. (Clade A7 after Wurdack et al. (2005)) with a bootstrap value of 59%. However, my phylogeny with the inclusion of three complete *Bernardia* sequences increased the support for this clade (to 96%). Therefore my molecular analysis provides additional strong support for the recognition of this tribe.

Combined: The parsimony analysis yielded tree of 582 steps (CI = 0.6347; RI = 0.7794). This tree is shown in Fig. 3. The three *Bernardia* species in the analysis form a monophyletic clade with a bootstrap of 100%. The combined analysis nests *Bernardia trelawniensis* into *Bernardia* sect. *Bernardia* with a bootstrap value of 73%. The molecular data support the palynological results of placing *Bernardia trelawniensis* within *Bernardia*

and also provide additional support for the phylogenetic conclusions of Wurdack et al. (2005).

Morphological evidence. During my initial field studies in Jamaica (May 2005), I noticed morphological features that were in conflict with the placement of *Bernardia trelawniensis* in the Antillean genus *Lasiocroton* (Radcliffe-Smith, 2001; Webster, 1994). *Bernardia trelawniensis* has leaves with denticulate margins, whereas, all other species in *Lasiocroton* have leaves with entire to sinuate margins (Radcliffe-Smith, 2001; Webster, 1994). In fact, no species has leaves with denticulate margins in the tribe Adelieae s.s. (Webster, 1994; Radcliffe-Smith, 2001). The only species in the Adelieae s.s. with leaves even approaching denticulation is the widespread Antillean *L. bahamensis* Pax & K. Hoffm., with some individuals from Cuba having sinuate leaves. In addition, a single pistillate flower was found on a primarily staminate plant of *B. trelawniensis*, and it is known that monoecy occurs in *Bernardia* but not in *Lasiocroton* (Radcliffe-Smith, 2001; Webster, 1994).

My initial morphological observations for *Bernardia* were supplemented by those provided by Radcliffe-Smith (2001) and Cervantes et al. (2003). I also found additional morphological characters that show that this taxon belongs to *Bernardia* (Table 1). All species of *Bernardia* have glands near the base of the leaf whereas no species in the Adelieae s.s. have glands on their leaves. *Bernardia trelawniensis* has glands numbering from one to three, though these glands are not obvious, particularly on immature leaves. Furthermore, the glands exhibited by *B. trelawniensis*, are abaxial on the lamina and quite flat which is typical for *Bernardia* sect. *Bernardia*. The glandular condition is quite different from that of *B. corensis* (Jacq.) Müll. Arg. of sect. *Polyboea* which has a pair of tuberculate glands at the joining of the lamina and petiole. In addition, *Bernardia* species have stipules, while

Lasiocroton species are exstipulate. *Bernardia trelawniensis* has small, 1-2 mm long subulate stipules, similar to other members of *Bernardia* sect. *Bernardia*.

Trichomes also provide important diagnostic characters. *Bernardia trelawniensis* has strikingly stellate trichomes, densely arranged with long, erect and narrow radii of a glass-like appearance. *Lasiocroton*, which literally translates to “wooly croton,” has stellate trichomes with thick and rugose radii that are typically brown in color. No species of *Lasiocroton* has trichomes similar to those of *B. trelawniensis*. While not all species of *Bernardia* have such trichomes, stellate trichomes are typical of *Bernardia* sect. *Bernardia*. Plants of *B. trelawniensis* have staminate flowers born on a short (5-10 mm) spicate inflorescence. In contrast, the staminate inflorescence of *Lasiocroton* is racemose to subpaniculate with a rachis ranging from 5 to 25 cm. Three additional floral structures (i.e., anthers, male disk glands, and pistillodes) also have consistent differences between the two genera. The main morphological features that distinguish *Lasiocroton* from *Bernardia* are listed in Table 1. These two genera are readily distinguishable, even from sterile specimens, on the basis of several morphological features.

TAXONOMIC IMPLICATIONS

One reason *Bernardia trelawniensis* was assigned to *Lasiocroton* is because the original description only included pistillate specimens (Adams, 1970). Male specimens provide the most obvious characters to distinguish the two genera. In addition, *B. trelawniensis* has some general features found in species of *Lasiocroton*. Traits shared by these two taxa include a pronounced abaxial leaf venation, a yellowish indumentum on recent

growth, flowers with a single whorl of perianth, pistillate inflorescences of similar length and structure, a woody habit, and technically “stellate trichomes.”

My morphological observations suggest that *Bernardia trelawniensis* is closely related to the type species of the genus. I am aware that my phylogenetic analyses had limited sampling within *Bernardia*, and that future studies might prove this genus not to be monophyletic. Two sections of *Bernardia*, *Passaea* (Baill.) Müll. Arg. and *Traganthus* (Klotzsch) Müll. Arg. were not included in my study, both have been suggested to possibly warrant generic status because of their unique morphologies (Webster, 1994; Radcliffe-Smith, 2001). As the morphology of the genus is not clearly understood, I cannot rule out the possibility of the paraphyly of *Bernardia*. However, even if future studies find *Bernardia* to be paraphyletic, *B. trelawniensis* will still remain part of *Bernardia* s.s. because of the species morphological similarities to the type species of this genus.

Bernardia dichotoma is widely distributed on the Caribbean Islands (Correll and Correll, 1982; Liogier, 1953, 1986). After a preliminary study of specimens of *B. dichotoma* from Jamaica, the consistent traits that differentiate these two species are trichome density and color. The trichomes of *B. dichotoma* growing on Jamaica are dense and give a tomentose appearance with a whitish hue. In contrast, *B. trelawniensis* has significantly fewer trichomes of a yellowish hue. Cervantes (2006) included only one variety of *B. dichotoma* in her thesis and suggested that the species boundaries of *B. dichotoma* are not well established. However, on Jamaica, *B. trelawniensis* is readily identifiable from *B. dichotoma* on the basis of my own observations in the field and of herbarium specimens.

Bernardia dichotoma seems to represent a taxonomic complex with a poorly understood morphology, and I cannot rule out that *B. trelawniensis* could be a part of this

complex. As Pax and Hoffman (1912) distinguished three varieties of *B. dichotoma*, clearly further research is needed to interpret morphological variation within *B. dichotoma* and its implications for the relationship between *B. trelawniensis* and *B. dichotoma*.

Bernardia trelawniensis is Critically Endangered because of its precarious locality, thus placing the species at the forefront of conservation importance. A recent botanical expedition (2006) to the Cockpit Country, headed by Fairchild Tropical Botanic Garden, found the species growing on all elevations of Island View Hill but identified less than 50 individuals (Lauren Raz, pers. comm.). These additional field observations further support that *B. trelawniensis* is Critically Endangered.

The phylogenetic component of this paper has no impact on the assignment of Critically Endangered according to the guidelines of the IUCN. However, the phylogeny would have an impact on management strategies, if these strategies were based on phylogenetic diversity. The conservation priority of a species closely related to a widespread species has a lower quantitative value of phylogenetic diversity when compared to a species with a highly differentiated morphology belonging to a restricted endemic genus (Rodrigues et al., 2005). This means, that while *Bernardia trelawniensis* was thought to have an unusual evolutionary history in *Lasiocroton* -with denticulate margins, glands, and stipules as autapomorphies- now the total amount of evolutionary change has been reduced by its placement in the morphologically similar *Bernardia*. *Bernardia trelawniensis* is Critically Endangered, but no longer has the prestige of being the only denticulate, glandular, and stipulate member of its genus.

TAXONOMIC TREATMENT

Bernardia trelawniensis (C.D. Adams) Jestrow & G. R. Proctor, **comb. nov.**

Lasiocroton trelawniensis C.D. Adams, *Phytologia* 20(5): 312. 1970. TYPE:

JAMAICA, Trelawny Parish, 1.5 miles north of Warsop, Wilson Valley District, Island View Hill, collected on wooded limestone hilltop, 2000-2200 feet, 26

Mar 1960, *G.R. Proctor 20746* (holotype IJ).

Additional specimen examined. JAMAICA. Trelawny Parish: 1.5 miles north of Warsop, Wilson Valley District, Cockpit Country, Island View Hill, limestone hilltop, 670m, 18°17'13.8"N, 77°34'33.3"W, *Jestrow 1004* (FTG).

SPECIES DESCRIPTION

Shrubs 1-4 m tall, dioecious with occasional deviations to monoecy, indumentum with stellate trichomes, latex absent. Leaves alternate, simple, elliptic to lanceolate, shortly rounded at base, glands numbering from 1-3 placed abaxially near base of lamina, lamina 3-10 cm long and 1-4 cm wide, denticulate margins, penninerved, prominent abaxial reticulate-pinnate venation, developing leaves yellowish abaxially, petiole 0.5-1cm long. Subulate stipules 1-2 mm long. Staminate inflorescence spicate, axillary, 4-6 mm long. Staminate flowers with single whorl of poorly defined perianth, 5-merous perianth ~1 mm long. Pistillate inflorescence as terminal racemes, erect in fruit, 3-6 cm in length, 5-11 flowers, flowers sessile. Pistillate flowers with a persistent single whorl of 5-merous perianth ~2 mm long. Fruits 3-sectioned schizocarps, oblong-globose, 6-8 mm long, olive-green, tawny-tomentose.

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TABLE 1

MORPHOLOGICAL AND PALYNOLOGICAL DISTINCTIONS BETWEEN *Bernardia*

AND *Lasiocroton*, ADAPTED FROM CERVANTES ET AL. (2003) AND JESTROW

(UNPUBLISHED)

	<i>Bernardia</i>	<i>Lasiocroton</i>
Leaf		
Glands	present	absent
Margin	dentate	entire to sinuate
Stipules	present	absent
Trichomes	simple, fasciculate, stellate	rugose-stellate
Flower		
Anthers	basifixed	subdorsifixed
Male disk glands	interstaminal or none	annular
Pistillode	absent	filiform or absent
Inflorescence		
Pistillate inflorescence	racemose to spicate	racemose to subpaniculate
Pistillate rachis length	1 mm to 20 cm	5 cm to 25 cm
Pollen		
Operculum	absent	fusiform
Tectum	punctate to reticulate	crotonoid
Costa ectocolpi	present	absent
Margo	present	absent

FIG. 1. Pollen SEM. **A.** *Bernardia trelawniensis* (From *Jestrow 1004*). **B.** *Lasiocroton macrophyllus* (From *Jestrow 1003*).

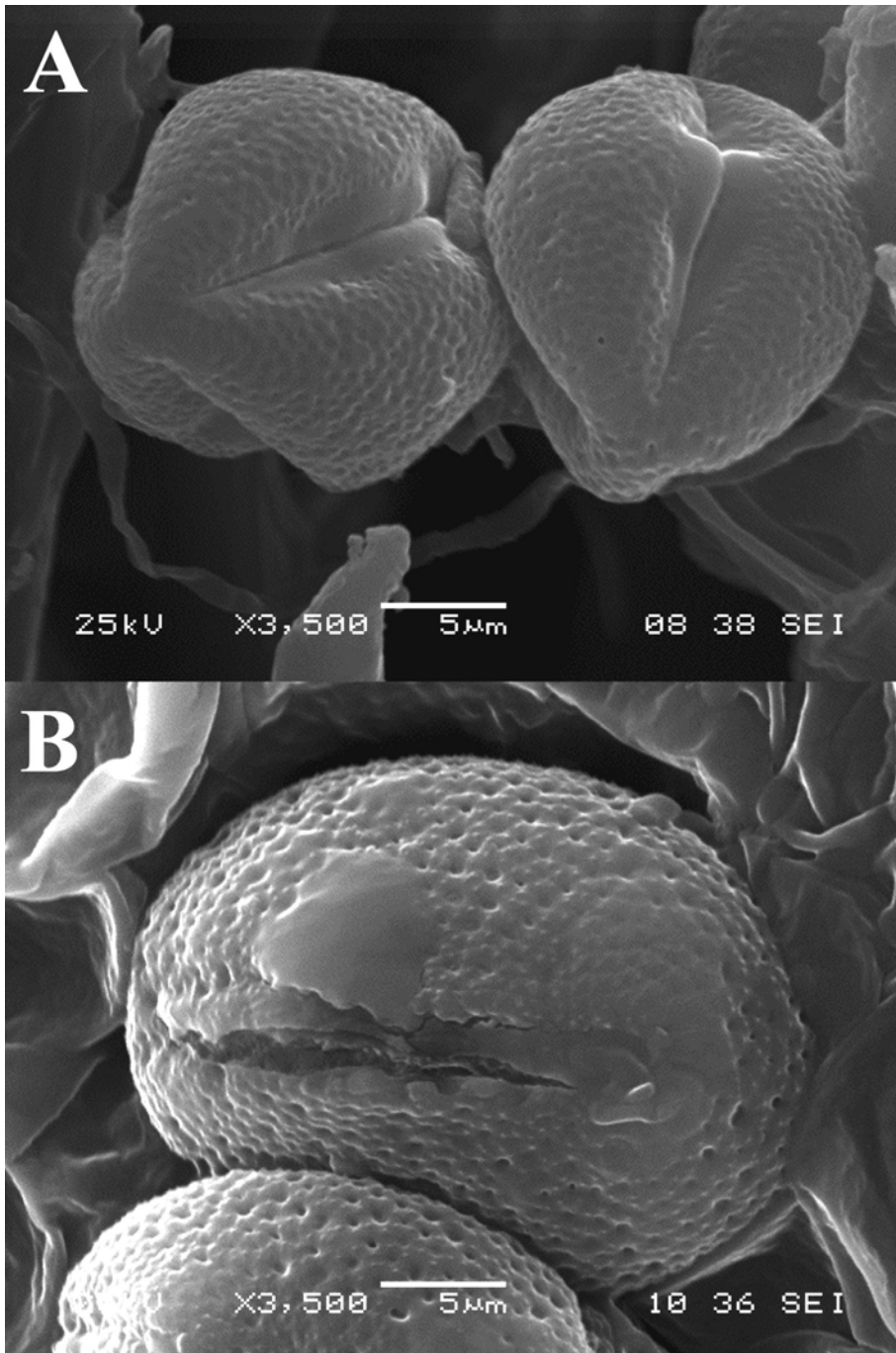


FIG. 2. Strict consensus tree obtained after the maximum parsimony phylogenetic analyses of the subset from the Acalyphoideae using *rbcL* and *trnL-F* nucleotide sequences. On the left is the *rbcL*, on the right is the *trnL-F*. Bootstrap values are shown above the nodes.

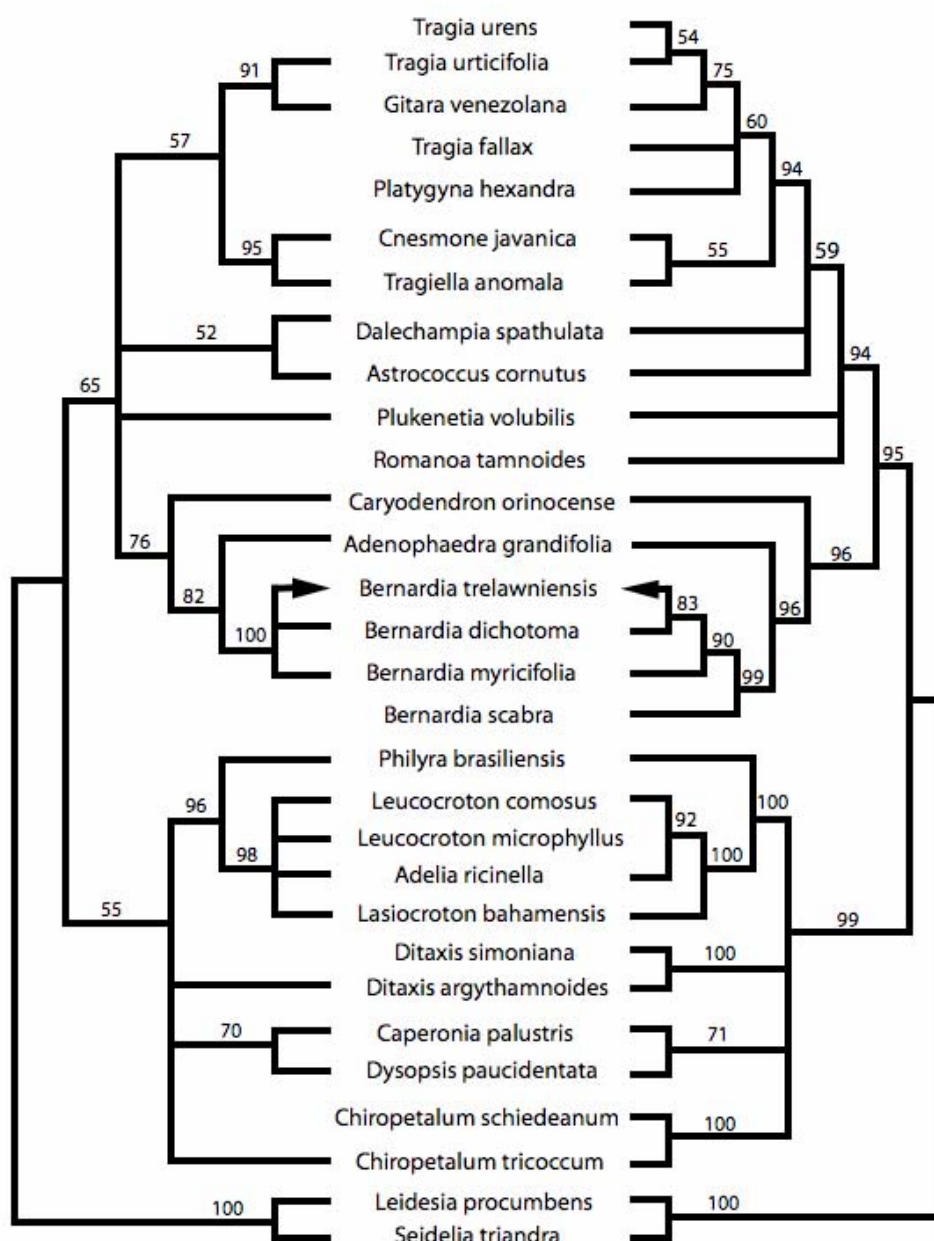
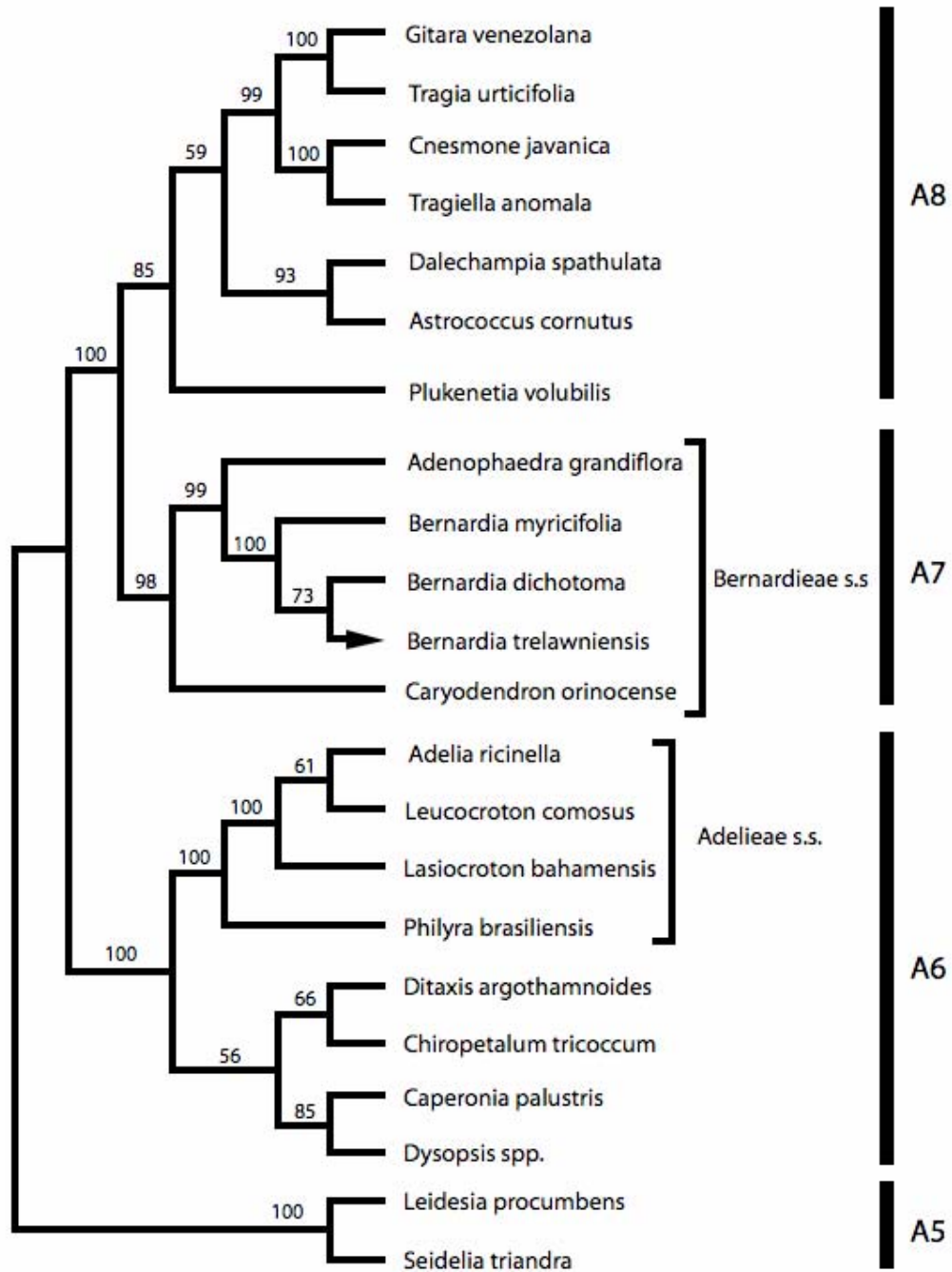


FIG. 3. Strict consensus tree obtained after the maximum parsimony phylogenetic analysis of the subset from the Acalyphoideae using combined *rbcL* and *trnL-F* nucleotide sequences.

Bootstrap values are shown above the nodes.



CHAPTER II

The generic delimitations of the Antillean members of tribe Adelleae (Euphorbiaceae) and the description of the Hispaniolan endemic genus *Garciadelia*

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ABSTRACT

The Caribbean Island Biodiversity Hotspot is the largest insular system of the New World and a priority for biodiversity conservation worldwide. The tribe Adeliae (Euphorbiaceae) has over 35 species endemic to this hotspot, representing a prime example of speciation in the West Indies, involving taxa from Cuba, Hispaniola, Jamaica, and the Bahamas. These species form a monophyletic group and traditionally have been accommodated in two endemic genera: *Lasiocroton* and *Leucocroton*. A study was conducted on: (1) scanning electron microscopy of pollen, (2) macromorphology, and (3) molecular phylogenies, to reveal generic relationships within this group. Phylogenies were based on parsimony and Bayesian analyses of nucleotide sequences of the ITS regions of the nuclear ribosomal DNA and the non-coding chloroplast DNA spacers *psbM-trnD* and *ycf6-pcbM*. Three major monophyletic assemblages were revealed; one of them is restricted to Hispaniola and is accommodated in a new genus, *Garciadelia*, with four species. This genus is sister to a clade comprising two monophyletic groups. The first of these two groups includes all species of *Leucocroton* and is restricted to serpentine soils of Cuba. The second group includes the species of *Lasiocroton*, with species occurring in Cuba, Hispaniola, Jamaica, and the Bahamas. Morphological, biogeographical, and ecological data provided additional support for each of these three monophyletic assemblages. Two new combinations (i.e., *Lasiocroton microphyllus* from Cuba and *Garciadelia leprosa* from Hispaniola) are made. Four new species are described (*Lasiocroton gutierrezii* from Cuba, and *Garciadelia abbottii*, *G. castilloae*, and *G. mejiae* from Hispaniola).

INTRODUCTION

The Caribbean Islands Biodiversity Hotspot, as defined by Smith & al. (2004), includes the Greater and Lesser Antilles, as well as the Bahamas and most of the islands off the Venezuelan coast. This insular system includes over 8,000 endemic plant species (Santiago-Valentín & Olmstead, 2004), and is ranked among the eight most important biodiversity hotspots based on endemism, massive habitat loss, and vulnerability to extinction (Myers & al., 2000). The threatened nature of such a high-diversity area has raised both conservation and scientific concerns in the region (Maunder & al., 2008). Of particular interest to this research are *Lasiocroton* Griseb. and *Leucocroton* Griseb. (Euphorbiaceae); two of the approximately 184 endemic seed plant genera restricted to the hotspot (Francisco-Ortega & al., 2007, 2008; Acevedo & Strong, 2008; Schaeffer & al., 2008). *Wallenia* Sw. (40 spp.) (Myrsinaceae), *Calycogonium* DC. (36 spp.) (Melastomataceae), and *Dendropemon* (Blume) Rchb. (36 spp.) (Loranthaceae) are the only endemic genera that have a larger number of species than the focal genera of this study (reviewed by Francisco-Ortega & al., 2007, 2008).

Lasiocroton has long been considered closely allied to *Leucocroton* (Grisebach, 1861; Pax & Hoffman, 1914; Webster, 1994), and a sister relationship of these two genera is strongly supported by recent molecular phylogenetic studies (Wurdack & al., 2005; De-Nova & Sosa, 2007). *Lasiocroton* and *Leucocroton* together constitute one of the most important examples of species radiation for the Caribbean seed-plant genera. Only three additional monophyletic assemblages of multiple endemic genera, which include more than 20 species, are known. The first one is in the Rubiaceae: *Isidorea* DC. (17 spp.), a genus endemic to Cuba

and Hispaniola, and the Jamaican endemic *Portlandia* P. Browne (7 spp.) are sister taxa (Motley & al., 2005). The second case is found in the Orchidaceae as the endemic genera *Broughtonia* R. Br. (6 spp.), *Psychilis* Raf. (15 spp.), *Quisqueya* Dod (4 spp.), and *Tetramicra* Lindl. (13 spp.) form a monophyletic group (Van Den Berg & al., 2000, 2005). Finally, the tribe Senecioneae (Asteraceae) has possibly the largest Caribbean endemic clade including ten genera: *Antillianthus* B. Nord. (17 spp.), *Elekmania* B. Nord. (10 spp.), *Ekmaniopappus* Borhidi (2 spp.), *Herodotia* Urb. & Ekman (1 sp.), *Leonis* B. Nord. (1 sp.), *Lundinia* B. Nord. (1 sp.), *Mattfeldia* Urb. (1 sp.), *Nesampelos* B. Nord. (3 spp.), *Oldfeltia* B. Nord. & Lundin (1 sp.), and *Zemisia* B. Nord. (1 sp.) (Pelser & al., 2007). Further endemic genera not included in the Pelsers & al. (2007) phylogenetic study, but which are closely allied members of the Senecioneae include: *Herreranthus* B. Nord. (1 sp.), *Ignurbia* B. Nord. (1 sp.), *Odontocline* B. Nord. (6 spp.), and *Shafiera* Greenm. (1 sp.) (Nordenstam, 2006).

Lasiocroton and *Leucocroton* belong to tribe Adelleae (subfamily Acalyphoideae). The type genus for this tribe, *Adelia* L., comes from Greek [α , not, and δῆλος, (= *delos*) visible] a name chosen by Linnaeus (1759) presumably for the difficulties in interpreting this taxon. The members of the Adelleae have undergone considerable taxonomic changes, particularly during the last five years. While the tribe formerly included five genera (Webster, 1975, 1994; Radcliffe-Smith, 2001), molecular phylogenies show that two of them, *Crotonogynopsis* Pax and *Enriquebeltrania* Rzed., are no longer considered to be part of this taxon (Wurdack & al., 2005; De-Nova & al., 2006). Currently *Adelia*, *Lasiocroton*, and *Leucocroton* form the central core of the Adelleae (Jestrow & al., 2008), and recent DNA phylogenetic studies also support the monospecific genus *Philyra* Klotzsch as belonging to the tribe (Wurdack & al., 2005). Therefore in my study the Adelleae include *Adelia*,

Lasiocroton, *Leucocroton*, and *Philyra* as suggested by Jestrow & al. (2008). *Adelia* and *Philyra* are found in the continent from Mexico to Brazil with a single species, *A. ricinella* L., reaching the Caribbean Islands.

The first infrageneric molecular phylogeny for the tribe was constructed for *Adelia* (De-Nova & al., 2007) with representative taxa from *Lasiocroton* and *Leucocroton*. That phylogenetic study built on the previous *rbcL* and *trnL-F* phylogeny of Wurdack & al. (2005) by adding nucleotide sequence data from the internal transcribed spacers (ITS1 and ITS2) of the nuclear ribosomal DNA and morphological data.

Available taxonomic keys for *Lasiocroton* and *Leucocroton* are difficult to follow and do not provide good discriminatory characters among the genera and their species (e.g., Borhidi, 1991; Webster, 1994; Radcliffe-Smith, 2001). For example, the keys of Webster (1994) and Radcliffe-Smith (2001) defined *Leucocroton* as having 4-colpate pollen; however, my micro-morphological observations show that this genus has 3-colpate pollen.

Species of *Leucocroton* have been the subject of ecophysiological studies, because of their extraordinary ability to hyperaccumulate nickel (Berazaín & al., 2007). All 26 species of this genus found on the serpentine soils of Cuba are known to sequester nickel (Reeves & al., 1996). Indeed, *Leucocroton* has more nickel hyperaccumulating species than any other genus endemic to the West Indies (Reeves & al., 1996, 1999).

The last significant taxonomic revision of *Leucocroton* was completed by Borhidi (1991), who listed 28 species comprising three sections (i.e., *Adeliocroton* Borhidi, *Lasiocrotonopsis* Borhidi, and *Leucocroton*). In addition, seven new species of the genus were described in his study. *Leucocroton* section *Adeliocroton* included only two species, and are the only ones not found on serpentine soils. Borhidi (1991) segregated this section

based on free stamens, as opposed to the connate stamens of the remaining two sections of the genus. Of the two non-serpentine species, *L. microphyllus* is confined to coastal limestone scrub of Cuba. The second one, *L. leprosus* (Willd.) Pax & K. Hoffm. (transferred to *Garciadelia* in the present study, see below), is the only member of the genus found on the island of Hispaniola. The other two sections, *Leucocroton* (19 spp.) and *Lasiocrotonopsis* (7 spp.), comprise the majority of the taxa and were segregated from each other by Borhidi (1991) according to leaf venation and, marginally, by stamen number.

In this study I used molecular data from the nuclear and chloroplast genomes to investigate phylogenetic relationships between the West Indian genera of the Adelleae. The resulting phylogeny was interpreted in the light of micro- and macromorphological data either specifically obtained for my research or reported in previous studies (i.e., Jestrow & al., 2008 and Takahashi & al., 2000). Section *Adeliocroton* is a main focus of this research, both in the proposing of the new genus *Garciadelia* (including *Leucocroton leprosus*) and in the new combination *Lasiocroton microphyllus* (formerly in *Leucocroton*). The ultimate goal of this study was to analyze the monophyly of the genera and what, if any, consistent generic groups exist within this group. In order to avoid confusion, the new taxonomic nomenclature (see below) will be followed throughout this work, with what I have named the “*Leucocroton* alliance” consisting of three genera: *Garciadelia*, *Lasiocroton*, and *Leucocroton*. All of the species of *Garciadelia* and *Lasiocroton* grow on limestone soils and form two monophyletic groups; likewise all *Leucocroton* taxa are endemic to serpentine areas of Cuba and form a distinct lineage (see below).

MATERIALS AND METHODS

Taxon sampling. -- A total of 19 taxa were sampled from 17 previously recognized species. The most distant outgroup was *Bernardia dichotoma* from the tribe Bernardieae of subfamily Acalyphoideae (Jestrow & al., 2008). The outgroup also included *Caperonia palustris* from the tribe Chrozophoreae. A previous phylogenetic study showed these two genera to be closely related to the Adeliae (Wurdack & al., 2005). The ingroup consisted of representatives from both the *Leucocroton* alliance and the rest of the tribe, including the monospecific genus *Philyra* and three species of *Adelia* representing the main lineages of this genus. My study included the type of *Lasiocroton*, *L. macrophyllus* (Webster, 1994; Radcliffe-Smith, 2001), as well as three species from each of the main lineages of this genus (Jestrow & al., unpublished): *L. microphyllus* [moved from *Leucocroton* section *Adeliocroton* in this study (see below)], the widely-distributed *L. bahamensis*, and the Jamaican endemic, *L. harrisii*. Six species from *Leucocroton* were selected as representatives of the five main clades of this genus as identified in a separate phylogenetic study (Jestrow & al., unpublished), including the type of the genus, *L. wrightii* (Webster, 1994; Radcliffe-Smith, 2001). Four of them belong to the section *Leucocroton* (i.e., *L. comosus*, *L. linearifolius*, *L. pachyphyllus*, and *L. wrightii*) and two to section *Lasiocrotonopsis* (i.e., *L. moncadae* and *L. virens*) as described by Borhidi (1991). As for the new genus *Garciadelia* (including the former *Leucocroton leprosus*), the three new species described in this study (see below) were used (i.e., *G. abbottii*, *G. castilloae*, and *G. mejiae*). Unfortunately, I did not include the Haitian endemic *G. leprosa* because I was unable to obtain samples of this extremely rare species.

DNA extraction, amplification, and sequencing. -- DNA of plant samples was extracted from silica-dried material using a DNeasy Plant Mini Kit (Qiagen, Valencia, California). PuReTaq Ready-To-Go PCR Beads (GE Healthcare Life Sciences, Piscataway, New Jersey) were used for the PCR amplification reaction mix. For all regions, the cycle program used is as follows: (1) 80°C for 5 min; (2) 94°C for 1 min; (3) 50°C for 1 min; (4) 72°C for 2 min; 35 cycles from step (2); and (5) 72°C for 5 min. Chloroplast noncoding regions, *psbM-trnD* and *ycf6-pcbM* were amplified with primers previously published by Shaw & al. (2005). The ITS1, 5.8s, and ITS2 were amplified as one region using primers ITS 5 (Downie & Katz-Downie, 1996) and ITS 4 (White & al., 1990). For members of *Garciadelia*, the total ITS regions were cloned prior to sequencing. Cloning was needed because of the poor quality of sequences obtained after direct sequencing for the species of this genus (see Results below). There was no need to clone the sequences obtained for the rest of the taxa because direct sequencing yielded clean products that were easily read and interpreted. I used TOPO TACloning (Invitrogen, Carlsbad, California), following the manufacturer's guidelines. Cycle sequencing was performed in both directions with the PRISM BigDye v.3.1 Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, California) following the manufacturers instructions. The primers used for PCR amplification were also utilized for the cycle sequencing reactions. Nucleotide sequences were visualized on a PRISM 377 Automated DNA Sequencer (Applied Biosystems, Foster City, California) at the Florida International University (FIU) DNA Core Facility. All sequences were submitted to GenBank (see Appendix).

Phylogenetic analyses. -- All sequences were assembled using the automated alignment option of Sequencher 3.1.1 (Gene Codes Corporation, Ann Arbor, Michigan) and

visually adjusted using MacClade 4.06 (Madison & Madison, 2000). A single region in the *ycf6-pcbM* alignment was removed because of a large “T” repeat within the ingroup (20-40 bp), this repeat also coincided with a large (254 bp) insertion in *Bernardia dichotoma* and was also eliminated from the alignment. All regions were gap-coded using the Simple Indel Coding method (Simmons & Ochoterena, 2000). For the ITS regions, only the ingroup was scored for gaps because of the alignment ambiguities because of the level of saturation with both *B. dichotoma* and *Caperonia palustris*. These gap characters were scored as missing data for the outgroup.

The parsimony analyses for the ITS matrix was based on a heuristic search with 10,000 random replicates saving no more than 100 trees for each step with the TBR and Multrees options. Clade support was assessed with bootstrap analysis but searches were conducted using a 10,000 replicate heuristic approach with starting trees from random addition. For the chloroplast and combined data sets, parsimony analysis was based on a Branch and Bound search using PAUP* 4.0b10 (Swofford, 2003). Statistical support for each clade was evaluated through bootstrap analysis (Felsenstein, 1985) with Branch and Bound searches using 10,000 replicates in PAUP*. Consistency index (CI; excluding uninformative characters), retention index (RI), and rescaled consistency index (RC) were calculated (Kluge & Farris, 1969; Farris, 1989). Incongruence Length Difference (ILD) tests were run between the three data sets (ITS, *psbM-trnD*, and *ycf6-pcbM*). Branch lengths were assigned by the Deltran algorithm. For the combined analyses (see Results below) I chose one ITS clone per *Garciadelia* species based on the minimum number of autapomorphic characters. This selection allowed us to avoid any false inflation of the branch lengths. Therefore the

following ITS clones were included in this combined analysis; *G. abbottii*, GU000002; *G. castilloae*, GU000008; and *G. mejiae*, GU000012.

Bayesian methods were also used for phylogenetic inferences. For this analysis, I did not include gap-coding because of uncertainties regarding molecular evolution modeling. Each region (ITS1, 5.8s, ITS2, *psbM-trnD*, *ycf6-pcbM*) were run separately through Modeltest v.3.06 (Posada & Crandall, 1998) to identify the models and parameters. Models were chosen based on the Akaike Information Criterion as explained in Posada and Crandall (1998). I used the following models for the Bayesian analyses: ITS 1, GTR+I+G; 5.8s, TRNef+I; ITS2, TRN+G; *psbM-trnD*, K81uf+I; and *ycf6-pcbM*, TVM+G. Bayesian inferences were conducted using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001) with two MCMC runs of four linked chains for 1,000,000 generations, sampling every 100 generations. The four chains included one cold, and the other three with incremental heating as per the default of MrBayes. Of the 10,001 trees produced per MCMC run, the first 25% of them were removed as burnin, resulting in a total of 15,000 trees. The burnin of 25% was determined to be adequate from the likelihood values, as the values leveled off by 100,000 generations for the datasets. All Bayesian analyses produced split frequencies of less than 0.015, showing convergence between the paired runs. I then used PAUP* to compute the majority consensus tree for the total data to give the posterior probabilities. For the Bayesian analysis of the total combined data set, the estimated rates of changes per character were saved for each branch in order to compare the relative branch lengths.

SEM studies. -- Pollen from the species of the *Leucocroton* alliance included in the phylogenetic analysis were removed from herbarium specimens collected from the wild or from the living collections of Fairchild Tropical Botanic Garden (see Appendix). They were

studied with Scanning Electron Microscopy (SEM) at the Florida Center for Analytical Electron Microscopy (FCAEM) of FIU using the JSM 5900LV (JEOL, Tokyo, Japan) instrument. Untreated samples were coated with gold-palladium using a SPI-Module Sputter Coater (SPI Supplies, West Chester, Pennsylvania) at 367Å for four minutes. I examined an additional species for pollen, *Lasiocroton fawcetti* Urb., that was not included in the phylogenetic analysis. I also made comparisons with pollen SEM images previously published by Jestrow & al. (2008) and Takahashi & al. (2000).

RESULTS

ITS analyses. -- The aligned ITS data matrix was 764 nucleotide positions in length and included 175 parsimony informative (PI) nucleotide positions along with five PI coded gaps (Table 2). Of these, approximately half (89 nucleotides and three coded gaps) were PI within the *Leucocroton* alliance. The strict consensus tree of the 14 most parsimonious trees shows *Adelia cinerea* to be sister to the *Leucocroton* alliance, although with low bootstrap support (Fig. 4). Within the *Leucocroton* alliance, *Garciadelia* was found to be sister to a clade formed by *Lasiocroton* and *Leucocroton*, but with low bootstrap support. However, the monophyly of the three genera of the *Leucocroton* alliance was strongly supported with bootstrap values ranking between 100% (*Garciadelia* and *Leucocroton*) and 99% (*Lasiocroton*). Interestingly, the two species of *Leucocroton* section *Lasiocrotonopsis* (i.e., *L. moncadae* and *L. virens*) were not found to be sister taxa.

Putative ITS sequences of *Garciadelia abbottii*, *G. castilloae*, and *G. mejiae*, were cloned because it was difficult to produce clean sequences by direct sequencing. Through

GenBank searches I blasted the cloned sequences of these three species and found that a few of them matched (up to 88% of the query coverage) ITS accessions of endophytic Ascomycetes. This explained my difficulties to obtain readable ITS sequences of this genus from direct PCR products. The fungal clones from the *Garciadelia* species were quite similar to each other, and were easy to align manually. These initial GenBank searches also showed that the majority of the cloned sequences matched ITS accessions of the *Leucocroton* alliance. I was able to recover five ITS clone sequences for each of these three *Garciadelia* species. The five clones for a given species differed from each other by no more than two mutations (Fig. 4). The cladistic analysis showed the multiple ITS clones of *G. abbottii* and *G. castilloae* forming two distinct monophyletic groups with bootstrap supports of 61% and 93% respectively. A single nucleotide change supported the clade of clones of *G. abbottii*. Three mutations were synapomorphies for the ITS clone clade of *G. castilloae*. However, no synapomorphies supported the clones of *G. mejiae* as a distinct clade (Fig. 3).

The Bayesian analysis produced a tree without any conflict to the parsimony strict consensus topology. However, this analysis did not find support for a sister relationship between *Adelia cinerea* and the *Leucocroton* alliance, but a polytomy with the species of *Adelia* (Fig. 3). Bayesian posterior probabilities for all three genera were 100%.

Chloroplast analyses. -- The aligned *psbM-trnD* and *ycf6-psbM* data matrices were 1065 and 1222 nucleotide positions in length, respectively (Table 2). The data set of *psbM-trnD* included 38 PI nucleotide positions along with 15 PI coded gaps, and the *ycf6-psbM* data matrix had 43 PI nucleotide positions and 16 PI coded gaps. Within the *Leucocroton* alliance, the *psbM-trnD* data set yielded eight PI nucleotide positions with ten PI coded gaps, while the *ycf6-psbM* alignment had 16 PI nucleotide positions with seven PI coded gaps. One

of the six most parsimonious trees of *psbM-trnD* conflicted with the strict consensus at a single node, which collapsed in the bootstrap analysis (Fig. 5). The *ycf6-psbM* search produced a single most parsimonious tree.

In contrast to the results produced by the parsimony analyses of the ITS and *ycf6-psbM* data, the *psbM-trnD* phylogeny found a clade formed by *Adelia ricinella* and *A. vaseyi* as sister to the *Leucocroton* alliance; however, this relationship was poorly supported with a bootstrap value of 71%. The *ycf6-psbM* tree conflicted with both the ITS and *psbM-trnD* topologies by nesting *Philyra* within the *Adelia* clade, this relationship was strongly supported with a bootstrap value of 100%. Within the *Leucocroton* alliance, both the *psbM-trnD* and the *ycf6-psbM* trees found *Garcia delia* to be sister to the *Lasiocroton* and *Leucocroton* clade with high bootstrap support greater than 83% and 92%.

Bayesian and parsimony analyses conflicted only at a single node of the *psbM-trnD* tree (represented by the “#” in Fig. 5). The Bayesian topology placed *Philyra* between *Caperonia* and *Bernardia*, although with a low Bayesian posterior probability of 69%. The Bayesian analysis of the *ycf6-psbM* did not find support for a sister relationship between *Adelia cinerea* and the *Leucocroton* alliance, but a polytomy with the species of *Adelia* (Fig. 5).

Combined analyses. -- The ILD tests showed conflicts between the *ycf6* and ITS ($P=0.01$) and between the *trnD* and *ycf6* ($P=0.01$) data sets. The ITS-*trnD* ILD test had less conflict ($P=0.07$). I found *Philyra brasiliensis* to be the prime culprit in creating the incongruence, and when I removed this taxon from the analyses, I resolved the conflicts [i.e., ITS-*trnD* ($P=0.38$), ITS-*ycf6* ($P=0.11$), and *trnD-ycf6* ($P=0.52$)]. When *Adelia cinerea* was not included, then I found an even higher rate of congruence, especially between the two

chloroplast regions [*trnD-ycf6* (P=1.00)]. Clearly these two species constituted the source for incongruence, but whether included or not in the phylogenetic analyses, they did not affect the topology of the *Leucocroton* alliance. I decided to keep both *A. cinerea* and *P. brasiliensis* in my combined analyses, but remain aware of the difficulty in ascertaining their proper placement in the tribe. Looking at the combined phylogeny, *Lasiocroton*, *Leucocroton*, and *Garciadelia* form highly supported monophyletic clades, according to both Bayesian and parsimony approaches.

My combined analysis also showed a sister relationship of *Garciadelia* to the rest of the alliance (Fig. 6). The combined parsimony analysis found *Adelia cinerea* to be sister to the *Leucocroton* alliance with a bootstrap support of 90%, however the Bayesian analysis did not resolve this relationship. The placement of *A. cinerea* is problematic as already indicated by De-Nova & al. (2007), and I do not feel justified in granting this species with generic status without complete agreement between both phylogenetic analyses.

Pollen SEM analyses. -- All pollen of the *Leucocroton* alliance had three colpi, opercula, and a perforated testa, in contrast with earlier reports by Webster (1994) that indicated that some of these taxa had pollen with four colpi. The palynological study of Takahashi & al. (2000) recognized these three traits as synapomorphies of the *Leucocroton* alliance; however, these authors had a limited taxonomic sampling, and did not report any pollen autapomorphies for *Garciadelia* as I found in this study.

Garciadelia pollen has a conspicuously reticulate-perforated incomplete testa (Fig. 7A, 7B, 7C). In contrast, *Leucocroton* pollen has considerably smaller pores in their testa, with *L. virens* (Fig. 7H) having the largest pores I have observed in the genus, including those images published by Takahashi & al. (2000). The Jamaican species of *Lasiocroton* had

a nearly complete testa, while *L. bahamensis* and *L. microphyllus* had considerably larger pores. Polar-equatorial length ratio (P/E) patterns were concordant with the resulting phylogeny. Low P/E ratios are common in *Leucocroton*, as shown in *L. linearifolius* and *L. pachyphyllus* with pollen grains wide and flat in shape. In contrast, the pollen grains of *Garciadelia* and *Lasiocroton* are generally elongate, with high P/E ratios, approaching a spherical shape as shown in *L. microphyllus*. Likewise, the pollen opercula of all of the *Leucocroton* species are wider and more pronounced than the other genera, with the single exception of *Lasiocroton microphyllus*; a species that exhibited a unique mosaic of character traits also found in the other two genera. The pollen of *Adelia cinerea* (shown by Cervantes & al., 2003) has a similar operculum and shape to *Garciadelia*, though the perforations are not as dramatic.

***Garciadelia* as a distinct monophyletic genus.** -- Criteria to define new genera have been a central focus in plant systematic and taxonomy, even in specific reference to the Euphorbiaceae (Humphreys & Linder, 2009; Judd & al., 2008; Berry & al., 2005). One only needs to look at the abundance of published, only to be abandoned generic names in the family, to recognize it.

After my research, I was left with two possibilities, either describing a large inclusive genus or dividing the group into three genera that correspond with the major clades identified in my study. By going the route of the large genus, I will not recognize the major ecological, physiological, biogeographical, and morphological differences behind the three major monophyletic groups found in my study.

With three genera, the taxonomy reflects clearly the evolutionary history of this group, which is one of the central tenets of modern taxonomy (Judd & al., 2008). My study

shows that *Leucocroton* includes 26 species, all of which are endemic to serpentine areas of Cuba and have the ability to hyperaccumulate nickel. No other endemic plant genus from the Caribbean Islands displays this extraordinary radiation along the naturally fragmented landscape of serpentine soils found across Cuba. *Leucocroton* represents the paradigm of speciation on this unique environment of the neotropics. These physiological traits are not found in any member of *Garciadelia* or *Lasiocroton*. Importantly, the three genera recognized in my study are morphologically distinct (see taxonomic treatment below). I also found that *Garciadelia* has pollen with large perforations as an autapomorphic trait. In addition, this genus is the earliest branch of the alliance and its pollen has similar operculum and shape to *Adelia cinerea*. Interestingly this Mexican species is supported as sister to the *Leucocroton* alliance in the parsimony analyses of the ITS, *ycf6-pcbM*, and combined data sets.

Accepting these three genera elucidates the biogeographic knowledge present in the phylogeny, namely that Cuban and Hispaniolan species each form a separate clade, with all these groups following the rules of monophyly. Finally, from a conservation viewpoint, *Garciadelia* has some of the most endangered species of the dry lowland shrub of the biodiversity hotspot (see below). From this perspective, to dissolve this genus into another would be to eliminate any ability to recognize quickly the evolutionary and conservation significance of the genus.

For a single all-encompassing genus, *Lasiocroton*, would take taxonomic precedence, as it is the earliest published genus of the clade. Therefore, all 28 species formerly included in *Leucocroton* would have to be described under the new combinations. This change would take significant amount of effort, and would ultimately only serve to remove information

from the evolutionary history as explained above. The situation is made much simpler with the naming of the new genus *Garciadelia*. I needed to move only one species, *Leucocroton microphyllus*, to *Lasiocroton*, while the species *Leucocroton leprosus* is transferred to *G. leprosa*. I publish three new species in *Garciadelia*, but these are new descriptions and hold no sway as to how I divide the genera.

TAXONOMIC TREATMENT

Key to the genera of the *Leucocroton* alliance

1. Stamens mostly connate; restricted to serpentine soils on Cuba; nickel-hyperaccumulators*Leucocroton*
1. Stamens free; restricted to limestone soils; not nickel-hyperaccumulators 2
2. Leaf lamina width/length ratio greater than 0.5 or leaves less than 25 mm in length, pistillate disk thick.....*Lasiocroton*
2. Leaf lamina width/length ratio less than 0.5 and leaves more than 25 mm in length, pistillate disk absent.....*Garciadelia*

Lasiocroton microphyllus (A. Rich.) Jestrow, **comb. nov.** -- *Adelia microphylla* A. Rich. in Ramón de la Sagra: Hist. Fis. Cuba 11: 209. 1850 \equiv *Bernardia microphylla* (A. Rich.) Müll. Arg. in Linnaea 34: 172. 1865 \equiv *Leucocroton microphyllus* (A. Rich.) Pax & K. Hoffm. in Engler: Pflanzenr. IV.147.VII. 64. 1914 -- Type: Cuba. Legit Ramón de la

Sagra, P00635080. Holotype (P!, photo of herbarium specimen examined), isotypes (G-DC!, photos of two herbarium specimens examined).

= *Bernardia lycioides* Müll. Arg. ex Pax & K. Hoffm. in Engler: Pflanzenr. IV. 147. VII. 64. 1914, nom. nud.

Shrubs 0.5--2 m, dioecious, indumentum of rugose-peltate trichomes. Stems stout and heavily branched, 1.5--4 mm thick, branches typically terminating in stout stem-spines. Leaves alternate, simple, oblong to obovate, 5--25 mm long, 2--5 mm wide, mucronate with a rounded apex, bluish-green, no vein impression, exstipulate, no glands; petioles poorly defined to 1 mm long. Staminate inflorescence spicate, 5--10 flowered, 10--65 mm long; staminate flowers with a single whorl of 5 sepals, 10 stamens. Pistillate inflorescence racemes, 1--3 flowered, 10--20 mm long; pistillate flowers with 5 sepals, pedicel 3--6 mm, disk thick, style 3--branched and compactly lacinate. Fruit a trilobed schizocarp, 2--3 mm diam.

Distribution, habitat, and collection history. -- I have examined a photo of a specimen of this species collected by Sagra in the herbarium of A. Richard located at P. The protologue refers to: "Crescit circa Havanam, in locis saxosis (*Ramon de la Sagra*) et in *Vuelta de abajo*, (J.-M. Valenzuela)" (Richard, 1850). Borhidi (1991) interprets "*Sagra 58* (P)" as the holotype for this name, however *Sagra 58* is located in G. Therefore I am not certain if Borhidi (1991) was referring to material found in P or in G. The herbarium of P has a single Sagra collection, without any collection number. This specimen was labeled as "HOLOTYPUS" by F. Zimmermann in 1991. I concord with Zimmermann's typification, as the specimen belonged to the herbarium of Richard, was collected by Sagra, and it follows the original description by Richard (1850). Therefore I consider this material to be the

holotype of *Adelia microphylla* A. Rich. Pax & Hoffmann (1914) listed *Bernardia lycioides* Müll. Arg. as a synonym of *Leucocroton microphyllus*, but this name was never published, so it is a *nomen nudum*.

Lasiocroton microphyllus is endemic to Cuba and is restricted to coastal scrub on limestone soils. After *Lasiocroton bahamensis*, which is more abundant and has a broader distribution area, it is the second most common and widely distributed species of the *Leucocroton* alliance. Most collections are from three distinct areas of the island (i.e., the northern coast of Habana province from El Mariel to Cojimar, the southern coast of Cienfuegos, and the coastlines of Santiago-Guantánamo-Holguín to the northern coasts and islands of Las Tunas-Camagüey-Ciego de Avila provinces). The coastal limestone scrub is also known as “manigua costera” and this ecosystem directly follows the distribution of *L. microphyllus*, with the single exception of the extreme southwestern coastal area of Pinar del Río, where *L. microphyllus* is unknown (Castro, 1978). *Lasiocroton microphyllus* is widespread, but is nowhere common or dominant. Many of the previous collections, particularly those in and near the City of Havana and the Havana province, have not been collected in the last fifty years. This species warrants the IUCN (2007) status of Near Threatened because of its apparent rarity.

***Lasiocroton gutierrezii* Jestrow, sp. nov.** -- Type: Shady place, limestone rocks, Sierra de Grillo, Madruga. No. 5289. Feb. 24, 1956. Col. Bro. Alain, HAC17403 -- Holotype (HAC!), isotype (GH!).

Lasiocroton microphyllus affinis sed caulibus gracilibus et sine spinis.

Shrubs to 1.5 m tall, dioecious, indumentum of rugose-peltate trichomes. Stems narrow and trailing, 0.5--1.5 mm thick, lacking stem-spines. Leaves alternate, simple, narrowly obovate, 10--30 mm long, 2--5 mm wide, mucronate with a rounded apex, bluish-green, no vein impression, exstipulate, no glands; petioles poorly defined to 1 mm long. Staminate inflorescence spike, 5--10 flowered, 10--25 mm long; staminate flowers with 5 sepals. Pistillate inflorescence panicle, 3--10 flowered; pistillate flowers with 5 sepals, disk thick, style 3--branched and compactly lacinate. Fruit a trilobed schizocarp. Figure 10.

Distribution, habitat, and collection history. -- Among the herbarium specimens that I initially assigned to *Lasiocroton microphyllus*, all but one was from the dry, coastal, scrub areas of Cuba. This single exception (*Alain 5289*) is from an inland locality of the Grillo Range, located in the center of the island, midway between north and south coasts of Havana province. The specimen immediately stood out because of its long, narrow, trailing stems, and lack of stem spines. While examining photographs of specimens from HAC, an additional specimen had the same morphological traits and was also collected from an inland site outside the “manigua costera” (see above). This second locality was from the Escaleras de Jaruco, which constitutes the northwestern edge of the limestone highlands of the provinces of Havana and Matanzas, while the former locality occurs near the southeastern edge of these hills (Fig. 9). This additional collection was of pistillate material and showed inflorescences with 3--10 flowers, unlike *L. microphyllus*, which has 1--3 flowers.

Lasiocroton gutierrezii, now known from only two collections, is therefore justified by both morphology and distribution. The two localities are typical of the semideciduous forests of central Cuba as shown by Castro (1978). I honor Dr. Jorge Gutiérrez of the Jardín Botánico Nacional, Universidad de Habana, with the eponym, for his life long dedication to

the flora of Cuba and his particular contributions to the taxonomy of the Euphorbiaceae. I consider this species as Endangered according to the IUCN criteria [B1ab(i)].

Garciadelia Jestrow & Jiménez Rodr., **gen. nov.** -- Type: *Croton leprosus* Willd. Sp. Pl., ed. 4, 4 (1): 553. 1805.

Arbuscula et frutices, dioecius, indumentum stellatum. Folia alternata, simplex, cordata, penninervis, stellatopilosa, estipulata, eglandulata. Inflorescentia mascula subpaniculata. Flores masculini actinomorphi; perianthium univerticillatum, lobis triangularibus, stamina duplo quot lobi parvilobis. Inflorescentia feminea racemosa. Flores feminei actinomorphi, perianthium univerticillatum 5-lobi, sine disco, stylus trifurcatus. Fructus 3-lobi, schizocarpus, stellatopilosus. Semen orbicularis ecarunculata.

Small trees and shrubs 1--4 m tall, dioecious, indumentum of stellate trichomes. Leaves alternate, simple, cordate at base, penninerved, erect-stellate trichomes, estipulate, without glands. Staminate inflorescence subpaniculate. Staminate flowers actinomorphic; perianth 5--merous in a single whorl with triangular lobes; stamens twice as numerous as the perianth divisions; anthers dorsifixed and dehiscing longitudinally; staminate disk weakly lobed. Pistillate inflorescence a raceme. Pistillate flowers actinomorphic, perianth 5--merous, in a single whorl with triangular lobes, without disk, style 3--branched. Fruit a trilobed schizocarp, covered in stellate trichomes. Seeds orbicular and lacking a caruncle. Figure 11.

I have chosen to honor Ricardo G. Garcia, director of the Jardín Botánico Nacional Dr. Rafael M. Moscoso of Dominican Republic (JBSD), for his outstanding contributions and dedication to the botany of Hispaniola. I have suffixed his last name with the greek root, δῆλος (= *delos*), in reference to the type genus of the Adelineae (i.e., *Adelia*).

Key to the species of *Garciadelia*

1. Leaf margin revolute, leaf surface bullate from tertiary vein impression, trichomes on abaxial lamina yellowish- to golden-brown2
2. Leaves 25-45 mm long, ovate-oblong, apex rounded, entire surface bullate and strongly revolute*G. mejiae*
2. Leaves 65-120 mm long, lanceolate, apex acute; surface bullate primarily at base and not strongly revolute*G. leprosa*
1. Leaf margin entire or weakly revolute; leaf surface not bullate but possibly with some tertiary vein impression; trichomes on abaxial lamina white or with occasional browning along the midvein.....3
3. Leaves coriaceous, elliptic-oblong, symmetric, apex rounded-subacute, tertiary vein impression; trichomes on abaxial lamina with some browning on midvein.....*G. abbottii*
3. Leaves chartaceous, oblanceolate, asymmetric, apex acute, no tertiary vein impression; trichomes on abaxial lamina white*G. castilloae*

Garciadelia leprosa (Willd.) Jestrow & Jiménez Rodr., **comb. nov.** \equiv *Croton leprosum*

Willd. Sp. Pl., ed. 4, 4 (1): 553. 1805 \equiv *Adelia ferruginea* Baill. in Étude Euphorb.

418. 1858, nom. superfl. \equiv *Bernardia leprosa* (Willd.) Müll. Arg. Linnaea 34: 172.

1865 \equiv *Leucocroton leprosus* (Willd.) Pax & K. Hoffm. in Engler: Pflanzenr.

IV.147.VII 64. 1914 \equiv *Adelia leprosa* (Willd.) Moscoso in Cat. Fl. Domingensis 302.

1943 -- Type: *Croton leprosum* foliis lanceo latis integerrimis cordatis subtus

tomentosis. Habitat in St. Domingo, B-W17896. Holotype (B-W!), photo of herbarium

specimen examined), isotypes [BM!, CGE!, G! (photo of herbarium specimen examined), G-DC! (photo of herbarium specimen examined), P! (photo of herbarium specimen examined)].

= *Croton leprosus* Spreng. ex Griseb. Fl. Brit. W. I. 38. 1859, nom. illegit.

Shrubs or small trees. Leaves, coriaceous, lanceolate, mucronate and acute apex, cordate base, lamina 6--10 cm long and 1--2.5 cm wide, entire to revolute margins, penninerved, adaxial tertiary venation impressed and weakly bullate proximally, brownish-yellow to white erect-stellate trichomes, petiole 5--20 mm long. Staminate inflorescence subpaniculate, 5--10-flowered, 25--40 mm long. Staminate flowers, petals absent, single whorl of 5-sepals, 2--3 mm long. Pistillate inflorescence raceme, 1--2 flowered, 20--30 mm long, articulate with 1--2 bracts on pedicel 1--2 mm long. Pistillate flowers with single whorl of 5 sepals, 2--3 mm long, pedicel 2--5 mm, style 3--branched. Fruit a trilobed schizocarp, 4--5 mm diam. Figure 11A.

Distribution, habitat, and collection history. -- The original description refers to a collection by Pierre Antoine Poiteau from “St. Domingo” without any additional locality (Willdenow, 1805). This was the name for the western half of the island of Hispaniola, now known as Haiti, where Poiteau was collected between 1798 and 1801 (Pennell, 1950). Borhidi (1991) identified the holotype as “POITEAU s.n. Haiti, (G)”. I disagree with this typification as it seems that this was not the original material studied by Willdenow. His herbarium (B-W) includes a Poiteau’s specimen having a label with the name *Croton leprosum* that also matches the protologue (Willdenow, 1805). The label of this specimen refers to “St. Domingo” as stated in the original protologue, suggesting this specimen to be

the holotype for this taxon. All of the other Poiteau collections have on their labels the name "*Adelia ferruginea*" and the locality "St. Domingue."

The greek noun, κρότος (= *crotos*), is unusual in nomenclature for being a masculine noun ending in "-on" in the third declension. Therefore the specific epithet should follow the masculine ending (e.g., -us) as opposed to the neuter (e.g., -um) (Manara, 1991). Sprengel (1826) recognized this and corrected the orthography by publishing the name as, "*Croton leprosus* W." All subsequent publications have followed Sprengel's lead, and the name, *Croton leprosum*, has been abandoned.

Sprengel (1826) assigned further localities and collections from Hispaniola, Martinique, Mexico, and Guadalupe to the name "*Croton leprosus* W." However, Grisebach (1859) considered that Sprengel (1826) was referring to *Croton flocculosus* Geis. [accepted name *C. flavens* L. (Acevedo-Rodriguez & Strong, 2010)] and not to *C. leprosum* Willd. In Grisebach's (1859) treatment for *C. flocculosus* this taxon is listed as "*Croton leprosus* Spreng (non W.)"; therefore, I am certain that *C. leprosus* sensu Sprengel does not refer to a taxon of the *Leucocroton* alliance.

Poiteau's collection of this taxon was the basis for the description of *Adelia ferruginea* Baill. However, Baillon (1858) did not realize that this species had already been described by Willdenow (1805) 50 years earlier. Because Baillon's description of *A. ferruginea* was based on the same collections used by Willdenow (1805) to describe *Croton leprosus*, I consider the name *A. ferruginea* as a *nomen superfluum*.

While Poiteau (1815a, b) collected in the of Cap-Haïtien region of Haiti, the exact locality is unknown (Fig. 8). The reason for my conjecture of the Cap-Haïtien region as the original collection locality is related to the record of Erik Ekman. In 1924, Ekman was the

second and last botanist to collect *Garciadelia leprosa*. His collection information details the locality as at the foot of precipices of Eocene limestone near the town Haut-du-Cap, a small town just southwest of Cap-Haïtien. Ekman's material also contains both staminate and pistillate material, making his collections the only known source of pistillate material.

Given the species has not been collected in over eighty years from the single known locality, it has been assigned the IUCN [B1ab(iii)+B2ab(iii)] conservation status of Critically Endangered (IUCN, 2007). I have not considered the species as extinct, because no recent plant exploration has been made to this region to confirm its occurrence.

Garciadelia abbottii Jestrow & Jiménez Rodr., **sp. nov.** -- Type: Guettarda. Small tree.

Lajana, Samaná Peninsula; altitude about 100 meters. No. 1306 W. L. Abbott, Collector Apr 11, March 27 [crossed out], 1921, US1079094. Holotype (US!), isotypes (BM!, GH!, JBSD!).

Garciadelia leprosa affinis foliis subellipticis obtusis differt.

Shrubs or small trees. Leaves, elliptic-oblong to ovate-oblong, apex rounded to subacute, base weakly cordate, lamina 4--10 cm in length, margins entire, penninerved, adaxial primary and secondary venation impressed and not bullate, yellow to white trichomes, petiole 7--15 mm long. Staminate inflorescence margins subpaniculate, 10--12 flowered, 20--30 mm long Staminate flowers actinomorphic, single whorl of 5 sepals 2--3 mm long and ivory-colored adaxially, 10 stamens. Pistillate inflorescence raceme, 1--2 flowered, 30--40 mm long, articulate with 2--3 bracts on pedicel 1--2 mm long. Pistillate flowers actinomorphic with 5 sepals 2--3mm long, pedicel 1--5mm, style 3--branched. Fruit trilobed schizocarp, 7--9 mm diam., densely stellate. Figure 11E.

Distribution, habitat, and collection history. -- This species is the most widely collected of the genus with seven known collections. Prior to my study it was identified as *Leucocroton leprosus* by Urban (1923), R. Howard (1950), Alain (1966), and Liogier (1970). The first collection was by W.L. Abbott in 1921, with the label stating from Lajana, an area of the Samaná Peninsula northeastern Hispaniola. However, because this was the only known collection from the Samaná Peninsula, I researched this locality further. Zanoni (1986) explained that from the 5th - 12th of April, Abbott sailed from the Samaná Peninsula to the southern coast of Samaná Bay. This trip of Abbott coincides with the collection date, and I believe that this serves as confirmation that this first collection is not from the Samaná Peninsula but from coastal areas of Los Haitises, the area from where the species has been collected by the rest of the collectors (Fig. 8). I have chosen the holotype from among four duplicate specimens of Abbott, based on its quality and the high number of staminate inflorescences.

Collecting sites for this species range in altitude from near sea level to ca. 500 m. The low elevation localities are limestone outcrops on the Bahía de Samaná, and even includes red mangroves (*Rhizophora mangle* L., Rhizophoraceae) as an associated species on a collection label [*T. Zanoni, M. Mejía, J. Pimentel 21209* (NY)]. I have found only three living plants of this species (two juveniles and one mature staminate individual) in a single site of Los Haitises, at a karst hill covered with humid evergreen forests.

The species is certainly rare, but I cannot rule out that more populations exist in unexplored areas of this region. Bolay (1997) wrote about Los Haitises National Park, “Slash-and-burn cultivation goes on and the park cannot be considered well protected. The original territory of 208 sq km was reduced to only 70 sq km in 1980.” This information

allowed us to classify the species as Endangered, according to the IUCN criteria, [B1ab(i,iii)], although I have not been able to visit all known localities of the species.

Garciadelia mejiae Jestrow & Jiménez Rodr., **sp. nov.** -- Type: Shrub, 1.75. high; the whole plant golden yellow, aromatic; on limestone rocks, Hoyo de Pelempito, Bahoruco Mts. Alt. 1000 m. Bro. Alain H. Liogier 326 Feb 1971 6, US2649059. Holotype (US!), isotype: (GH!).

Garciadelia leprosa affinis foliis brevis revolutissimis bullatissimis differt.

Shrubs 1--2 m tall. Leaves, coriaceous, narrow-elliptic, mucronate and rounded apex, weakly cordate, no glands, petiole 3--6 mm, lamina 20--40 x 5-10 mm, margins revolute, penninerved, tertiary venation impressed forming a bullate surface, golden-orange erect-stellate trichomes, petiole 0.5--1 cm long, exstipulate. Staminate inflorescence compact, subpaniculate, 5--8 flowered, 20--25 mm long; staminate flowers actinomorphic, sepals 5, 2--3 mm long, ivory-colored adaxially, 10 stamens. Pistillate inflorescence axillary racemes, 1-3 flowered, 30--40 mm long with 2--3 bracts on pedicel 1--2 mm long; pistillate flowers actinomorphic with 5 sepals 2--3mm long, pedicel 1--5 mm, style 3--branched. Fruit a trilobed schizocarp, 7--9 mm diam., densely stellate. Figures 11B, 11C, and 11F.

Distribution, habitat, and collection history. -- I first identified the species from two duplicate specimens from a single collection made by Alain Liogier in 1971. These specimens came from a previously unrecorded locality for this genus, in the Bahoruco Mountains, quite distant from the known localities at northwestern (*Garciadelia leprosa*) and northeastern (*G. abbottii*) Hispaniola (Fig. 7). Curiously, although Liogier identified these

specimens as *Leucocroton leprosus*, he did not include this locality in his Flora of Hispaniola (Liogier, 1986).

When I contacted R. Garcia (JBSD), about these two collections, he informed us about a curious plant he had just recently collected resembling *Garciadelia leprosa*. Although R. Garcia found this plant in a different site, in the Parque Nacional Sierra de Bahoruco, the locality was close to that of the designed holotype. In 2008, I traveled to the site and collected additional specimens; the collections were morphologically identical to the ones made by Liogier. This population is the largest I have seen for the genus, although numbering fewer than 20 individuals. The altitude was twice that of the other species at ca. 1000 meters. The habitat is dry deciduous forests on karst in a transition towards higher elevation humid evergreen forests. The plants were flowering, and all were dioecious. Only two localities are known for the species, one of which has not been collected since 1971. This species is considered as Endangered according to the IUCN criteria [B1ab(ii)+2ab(ii); D]. I am honoring Milciades Manuel Mejía, former director and botanist of the JBSD, with the eponym *Garciadelia mejiae*.

Garciadelia castilloae Jestrow & Jiménez Rodr., **sp. nov.** -- Type: Sierra del Bahoruco, Provincia Barahona, Municipio La Ciénaga, Sección La Filipina, márgenes de la Cañada La Baliza, bosque húmedo con *Piper spp.*, *Persea sp.* *Reinhardtia paiewonskiana* 17 06' 57" N, 70 06' 27" W, Elev. 600 800 m, 7 Diciembre 2006. T. Clase, B. Peguero & C. De los Santos, JBSD116910. Holotype (JBSD!)
Garciadelia leprosa affinis foliis chartaceis asymmetricis sine impressivenis, trichomatibus albidus abaxialis

Small spindly trees 3--4 m tall. Leaves, chartaceous, oblanceolate and asymmetric, mucronate and acute apex, weakly cordate, petiole 10--25 mm, lamina 5--15 cm long and 20--45 mm wide, entire margins, penninerved, only primary venation impressed, white trichomes abaxially. Staminate inflorescence compact, subpaniculate, 5--10 flowered, 50--70 mm long. Staminate flowers actinomorphic, single whorl of 5 sepals 2--3 mm long, 10 stamens. Pistillate material not known. Figure 11D.

Distribution, habitat, and collection history. -- This species was first discovered in 2006 by T. Clase, B. Peguero, and C. de los Santos and was then tentatively identified as *Leucocroton leprosus*. While both *Garciadelia mejiae* and *G. castilloae* are found in the Baboruco Mountains, they occur in separate provinces with strikingly different ecosystems and morphologies. *Garciadelia castilloae* is located in Barahona Province, inland from the town La Filipina, along a roadside surrounded by slash and burn farming. About a dozen mature dioecious plants were seen growing on the side of a steep ravine, with a dense canopy covering a dense underbrush, typical of a humid evergreen forest. Given the slash-and-burn tactics taking place within a mile or two, *G. castilloae* is one of the most critically endangered species in the *Leucocroton* alliance. I consider this species to have the Critically Endangered conservation category according to the IUCN guidelines [B1ab(ii)+2ab(ii); C1; D]. I have decided to honor the late Daisy Castillo, botanist of JBSD, with the eponym *Garciadelia castilloae*.

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Fig. 4. One of the 14 most parsimonious trees from the analysis of ITS nucleotide sequences. This tree was identical to the 50% majority rule consensus tree inferred from the Bayesian analysis except for the branch indicated with “*”. This branch collapsed in the Bayesian topology. Number of steps along each branch are indicated in italics. Bootstrap values from parsimony analysis are above branches, clade credibility values from Bayesian analysis are below branches. This tree was identical to the strict consensus tree obtained after the parsimony analysis.

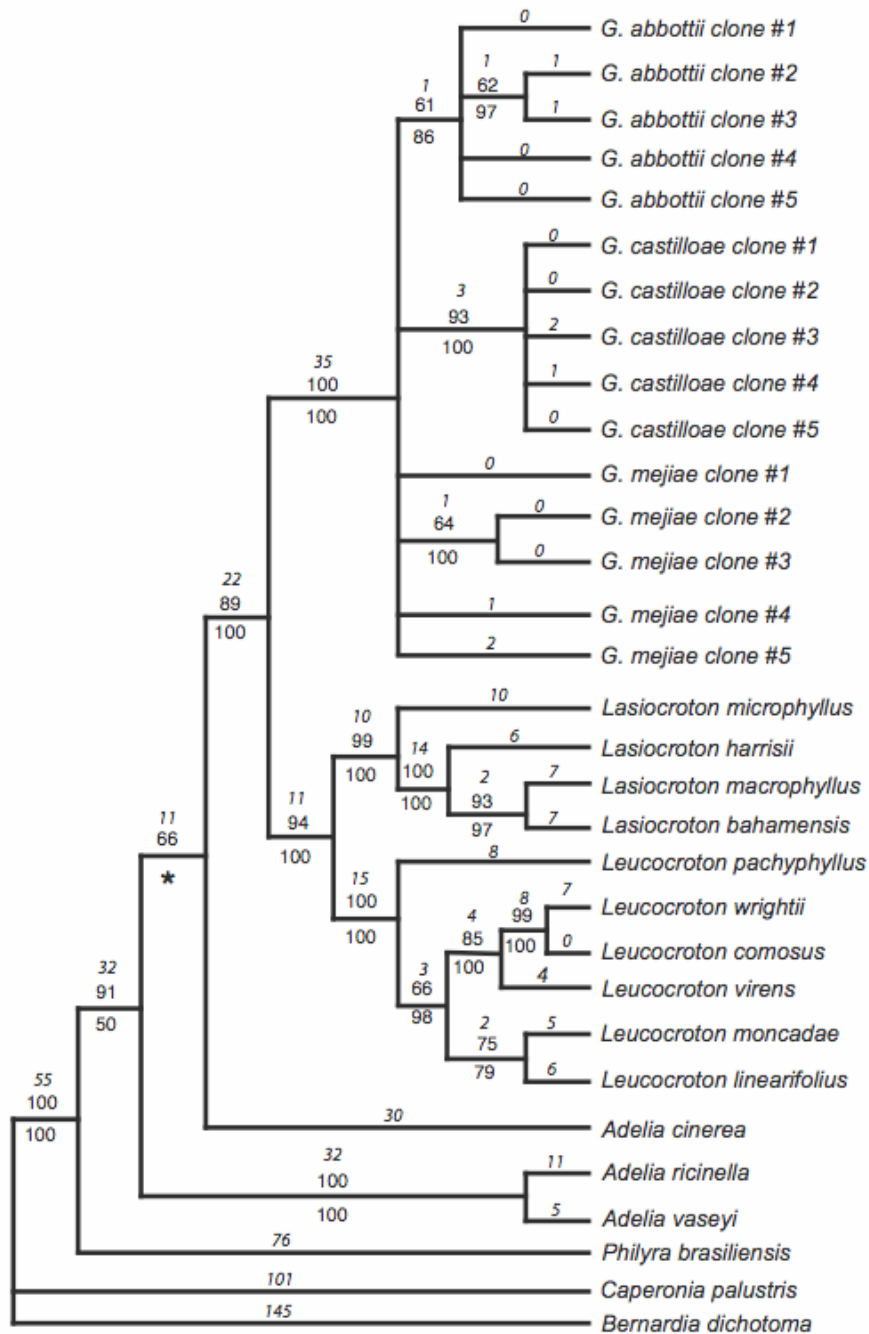


Fig. 5. Topologies recovered after parsimony analyses of nucleotide sequences of the two regions of the chloroplast genome. **Top,** One of the six most parsimonious trees from the analysis of *psbM-trnD* nucleotide sequences. The branch that collapses in the strict consensus tree is indicated with an arrow. **Bottom,** The single most parsimonious tree from the analysis of *ycf6-pcbM* nucleotide sequences. Trees were identical to the 50% majority rule consensus tree inferred from the Bayesian analyses except for the branches indicated with “*”. These branches collapsed in the Bayesian topologies. Numbers of steps for each branch are indicated in italics. Bootstrap values (>50%) from parsimony analysis are above branches, clade credibility values from Bayesian analysis are below branches. An additional conflict between the Bayesian and parsimony topologies (*pcbM-trnD* data set) was detected for the branch indicated with “#”.

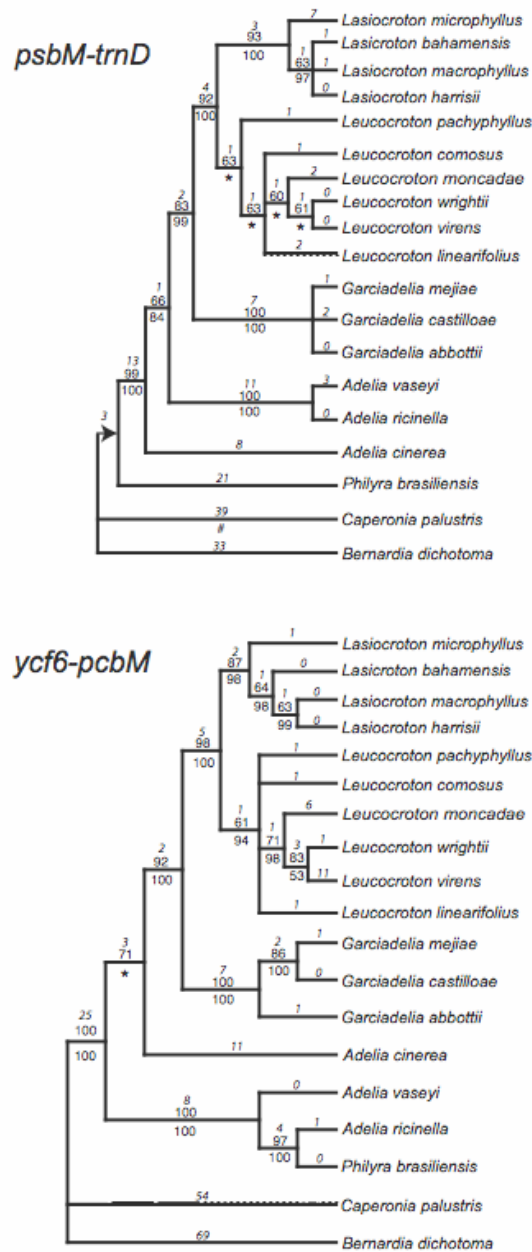


Fig. 6. The single most parsimonious tree from the analysis of the combined datasets. This tree was identical to the 50% majority rule consensus tree inferred from the Bayesian analysis except for the branch indicated with “*”. This branch collapsed in the Bayesian topology. Bootstrap values (>50%) from parsimony analysis are above branches, clade credibility values from Bayesian analysis are below branches. An additional conflict between the Bayesian and parsimony topologies was detected for the branch indicated with “#”. Number of steps along each branch, according to the Deltran algorithm, are indicated above the bootstrap values in italics. The expected rate of change per character (x1000 for scale) from the Bayesian analysis is shown in italics below the clade credibility values.

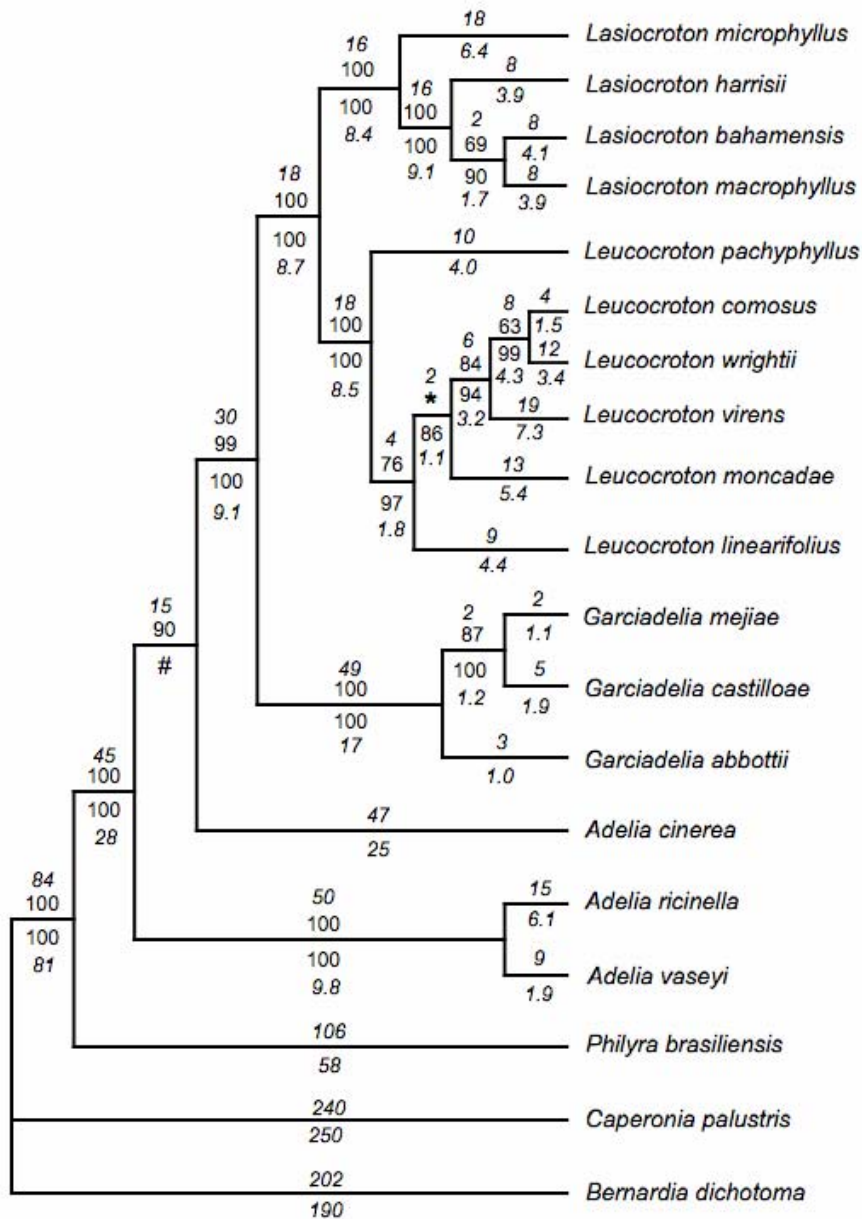


Fig. 7. SEM of pollen of the *Leucocroton* alliance. **A**, *Garciadelia mejiae*. **B**, *G. castilloae*. **C**, *G. abbottii*. **D**, *Lasiocroton bahamensis*. **E**, *L. harrisii*. **F**, *L. microphyllus*. **G**, *Leucocroton pachyphyllus*, **H**, *L. virens*, **I**, *L. linearifolius*.

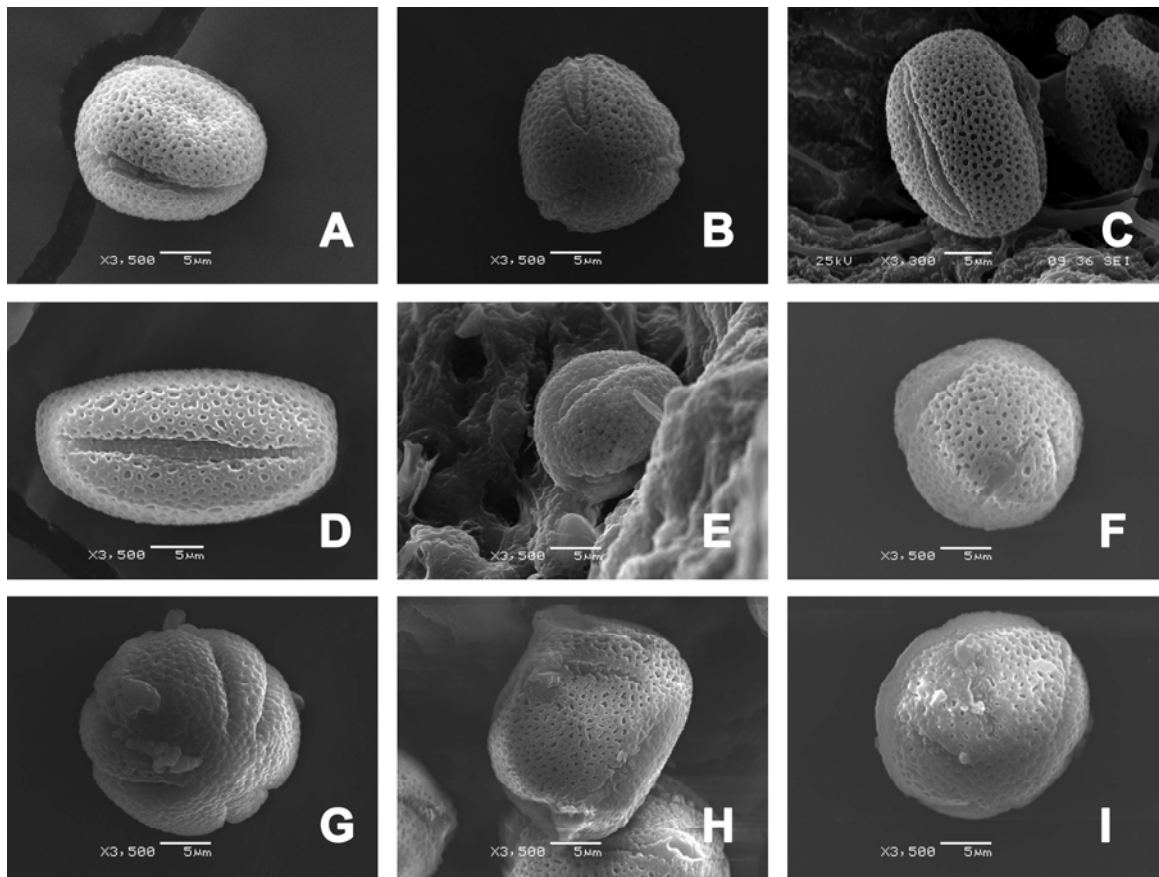


Fig. 8. Map of Hispaniola showing the known localities of the four species of *Garciadelia*. **Triangle**, *G. abbottii*. **Square**, *G. castilloae*. **Circle**, *G. leprosa*. **Star**, *G. mejiae*.

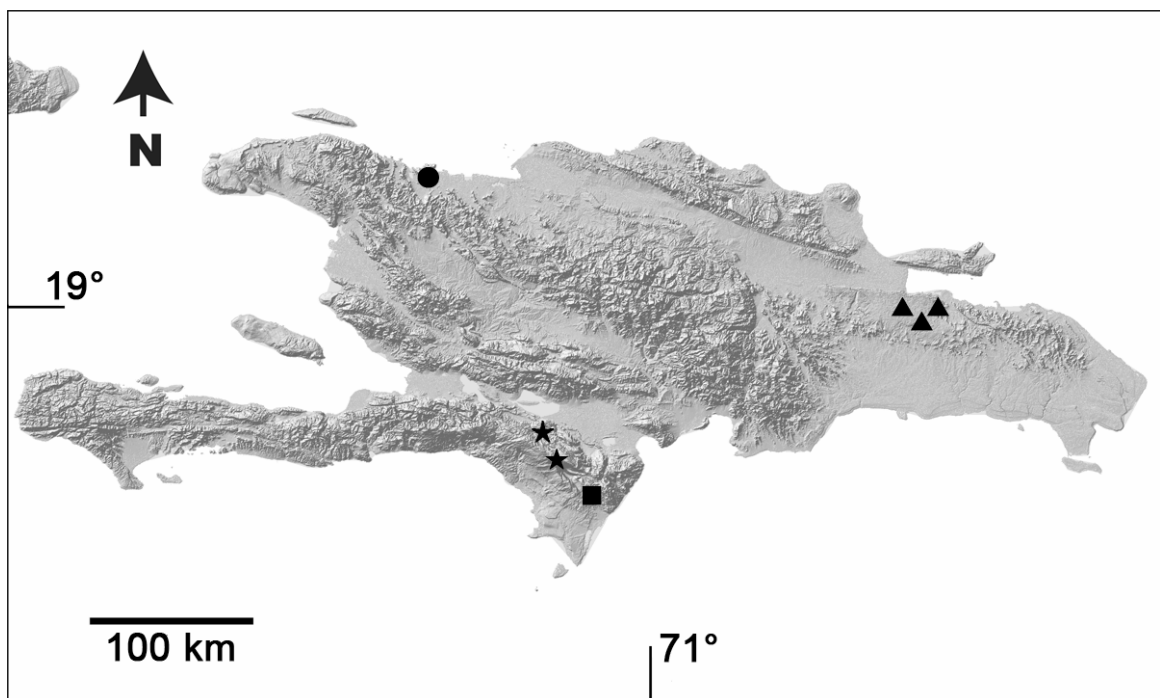


Fig. 9. Map of Cuba showing the known localities for the new species of *Lasiocroton* described in this study. **Diamond**, *L. gutierrezii*.

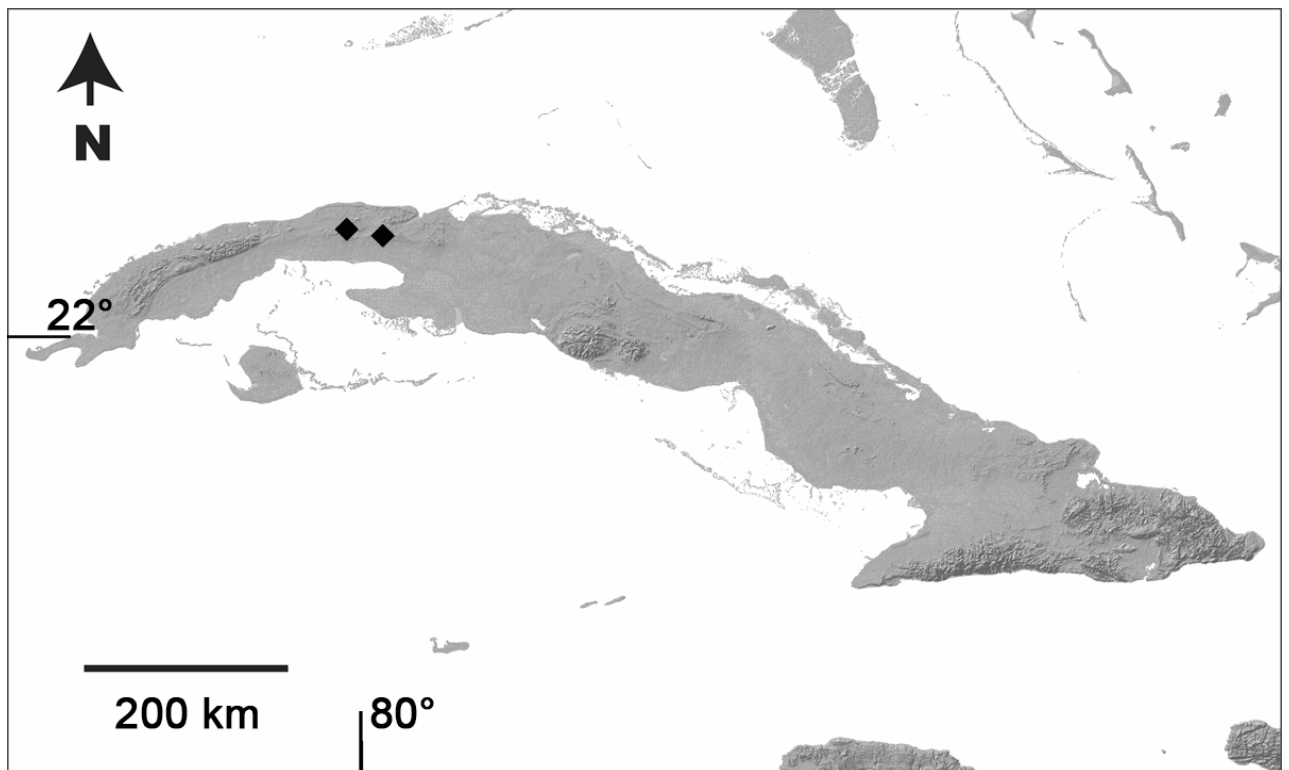


Fig. 10. *Lasiocroton gutierrezii*. **A**, Flower, pistillate. **B**, Flower, staminate. **C**, Branch, fertile pistillate. **D**, Branch, fertile staminate. (A, C, from *J. Acuña & Roig 16912*; B, D, from *Alain 5289*).

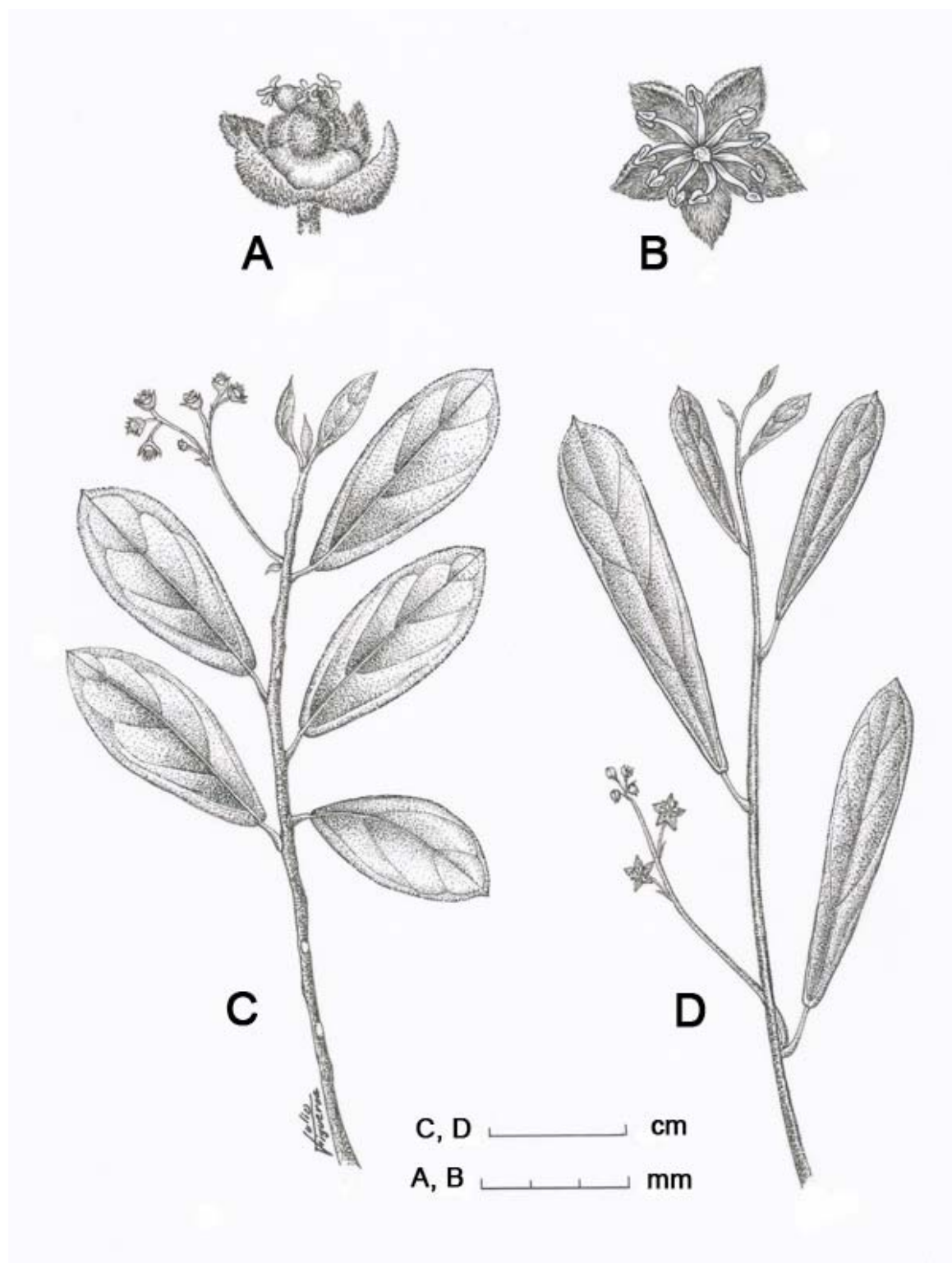


Fig. 11. *Garciadelia*. **A**, *G. leprosa*, Branch, staminate. **B**, *G. mejiae*, Inflorescence, pistillate. **C**, *G. mejiae*, Flower, staminate. **D**, *G. castilloae*, Leaf. **E**, *G. abbottii*, Leaf. **F**, *G. mejiae*, Leaf. (**A**, from Poiteau s.n.; **B**, from Jestrow & F. Jiménez 1019; **C**, from Jestrow & F. Jiménez 1018; **D**, from Jestrow & Jiménez 1020; **E**, from W.L. Abbott 1306; **F**, Liogier 326).

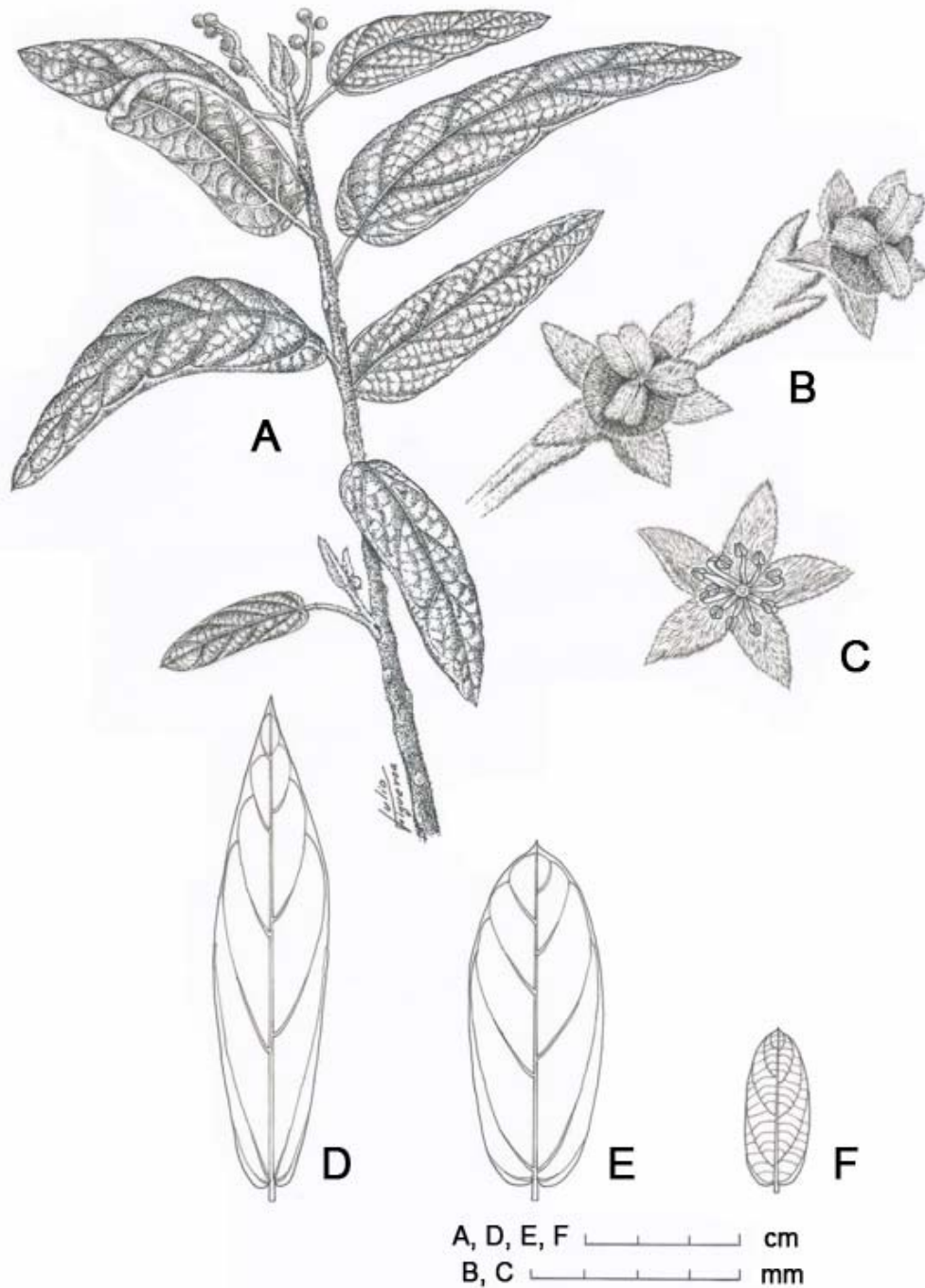


Table 2. Parsimony statistics of tree topologies.

DNA region	AL	N	Total PI	LA PI	MPT	CI/RI/RC
ITS	764 (7)	19 (15)	175 (5)	89 (3)	14 (689)	0.66/0.81/0.62
<i>pcbM-trnD</i>	1065(42)	19	38 (15)	8 (10)	6 (171)	0.78/0.86/0.78
<i>ycf6-pcbM</i>	1222(45)	19	43 (16)	16 (7)	1 (224)	0.83/0.91/0.85
Combined data set	3051 (94)	19	252 (36)	109 (20)	1 (1056)	0.67/0.75/0.61

AL = alignment length (number of coded gaps); N = number of taxa (number of sequenced clones); Total PI = total parsimony informative nucleotides (number of parsimony informative coded gaps); LA PI = parsimony informative nucleotides within the *Leucocroton* alliance (number of PI coded gaps); MPT = number of most parsimonious trees (tree score); CI/RI/RC = consistency index excluding uninformative characters, retention index, and rescaled consistency index

Appendix. Species names, voucher information, specimens examined (* = photo of herbarium specimen was examined), pollen voucher specimens (#), and Genbank accession numbers for the sequences used in the molecular analyses (ITS, *pcbM-trnD*, *ycf6-pcbM*, respectively).

Adelia cinerea (Wiggins & Rollins) A. Cerv., V. W. Steinm. & Flores Olv.; Mexico; *Steinmann 971* (RSA); GU000027; GU000042; GU000048. *Adelia ricinella* L.; Cuba; *HABJ 81949* (FTG); GU000023; GU000045; GU000049. *Adelia vaseyi* (J.M. Coult.) Pax & K.Hoffm.; Mexico; *De-Nova 191* (XAL); GU000026; GU000043; GU000050. *Bernardia dichotoma* Müll. Arg.; Jamaica; *Jestrow 1001* (FTG); GU000029; GU000047; GU000051. *Caperonia palustris* A. St.-Hil.; USA; GU000030; GU000046; GU000052. *Garcia delia abbottii* Jestrow & Jiménez Rodr.; Dominican Republic; *W.L. Abbott 1306* (BM, GH, JBSD, US); *P. Acevedo-Rodríguez. 8571* (JBSD); *Ekman 15393* (US); *F. Jiménez, A. Veloz & B. Peguero 3577* (FTG#); clone1 GU000002; clone2 GU000003; clone3 GU000004; clone4 GU000005; clone5 GU000006; GU000041; GU000055; *A. H. Liogier 14495* (GH, NY); *T. Zanoni, M. Mejía & J. Pimentel 21209* (NY); *T. Zanoni, M. Mejía, J. Pimentel & R. García 34204* (JBSD). *Garcia delia castilloae* Jestrow & Jiménez Rodr.; Dominican Republic; *T. Clase, B. Peguero & C. De los Santos 4341* (JBSD); *Jestrow & Jiménez 1020* (FTG#); clone1 GU000007; clone2 GU000008; clone3 GU000009; clone4 GU000010; clone5 GU000011; GU000040; GU000053. *Garcia delia leprosa* (Willd.) Jestrow & Jiménez Rodr.; Haiti. *Ekman 2726* (IJ, K, NY, S, US); *Poiteau s.n.* (B-W*, BM, CGE, G*, G-DC*, P*). *Garcia delia mejiae* Jestrow & Jiménez Rodr.; Dominican Republic; *R. García, G. Caminero & D. Höner 4409-A* (JBSD); *Jestrow & F. Jiménez 1018* (FTG*); clone1 GU000012; clone2

GU000013; clone3 GU000014; clone4 GU000015; clone5 GU000016; GU000039;
 GU000054; *Jestrow & F. Jiménez 1019* (FTG); *Liogier 326* (GH, NY); *F. Jiménez, R. García & R. Rodríguez 4152* (FTG, JBSD). ***Lasiocroton bahamensis*** Pax & K. Hoffm.;
 Bahamas; *W. Gillis 10451 ex situ* (FTG#); GU000024; GU000032; GU000056. ***Lasiocroton gutierrezii*** Jestrow; Cuba. *Alain 5289* (GH, HAC); *J. Acuña & Roig 16912* (HAC).
Lasiocroton harrisii Britton; Jamaica; *Jestrow 1009* (FTG); GU000018; GU000033;
 GU000057; *Jestrow 1010* (FTG#). ***Lasiocroton macrophyllus*** (Sw.) Griseb.; Jamaica;
Jestrow 1002 (FTG); HM185116; HM185117; HM185118. ***Lasiocroton microphyllus*** (A. Rich) Jestrow; Cuba; *J. Acuña 17846* (HAC*); *J. Acuña & Correll 18667* (HAC*, HAJB*);
Bro. Alain 5289 (GH); *Bisse & al. HAJB 29791* (HAC*); *Britton & al. 5619* (F, NY); *A. Areces & al. 31330 HAJB* (HAC*, HAJB*); *Britton & al. 5675* (F, NY); *Britton & al. 5980*
 (F, NY); *Britton & al. 6244* (F, NY); *Britton & al. 13979* (NY); *G. Bucher* (NY); *I. Castañeda & I. Alemán 1363* (HAC*); *Bro. Clemente 1223* (HAC*); *Bro. Clemente 3039*
 (HAC*, HAJB*, NY, US); *Bro. Clemente 3259* (HAC*); *Bro. Clemente 5724* (GH, HAC*, US); *Bro. Clemente 7223* (GH, US); *Bro. Clemente 7534* (GH, HAC*); *R. Comb. 575* (F, GH, K, NY); *Ekman 398* (S); *Ekman 334* (S); *Ekman 1100* (F, MGE, S); *Ekman 8920* (K);
Ekman 13317 (S); *Ekman 13659* (S); *Ekman 15534* (NY, S, US); *Greene 4* (K*); *J. Grudzinskaya & N. Tuchanitzkaja 454* (HAC*); *Gutiérrez & al. 81915* (FTG); *Gutiérrez & al. 81947* (FTG#); GU000017; GU000031; GU000058; *Gutiérrez & al. 81839* (FTG); *R. Howard & al. 4* (A); *E.P. Killip 13826* (US); *Bro. León 2908* (NY, HAC*); *Bro. León 6865*
 (NY); *Bro. León 7177* (GH, HAC*, NYBG); *Bro. León 9084* (NY); *Bro. León 10655* (HAC*); *M. López 61* (HAJB*, US); *M. López 62* (HAC*); *M. López 173* (HAC*, HAJB*, US); *M. López 1048* (HAC*, HAJB*); *M. López 1138* (HAJB*); *M. López 1177* (HAC*,

HABJ*); *Moldruke, Bro. León, Bro. Alain & J. Acuña 16913* (HAC*); *G. Proctor 16424* (A, IJ); *R. de la Sagra* (P*, G-DC*, G-DC*); *G.L. Webster 83* (A); *G.L. Webster 4046* (GH, MGR, NY, US); *G.L. Webster 4047* (US, MGR); *C. Wright 1977* (BM, F, G-DC*, GH, HAC*, HABJ*, K, NY, S). ***Leucocroton comosus*** Urb.; Cuba; *Gutiérrez & al. 81722* (FTG); GU000020; GU000035; GU000059. ***Leucocroton linearifolius*** Britton; Cuba; *Berazáin 71542* (US#); *Gutiérrez & al. 81807* (FTG); GU000022; GU000038; GU000060.

Leucocroton moncadae Borhidi; Cuba; *Gutiérrez & al. 84363* (HABJ); GU000021; GU000036; GU000061. ***Leucocroton pachyphyllus*** Urb.; Cuba; *Gutiérrez & al. 81896* (FTG); GU000019; GU000034; GU000062; *Shafer 4164* (US#). ***Leucocroton virens*** Griseb.; Cuba; *Bro. Alain & Bro. Clemente 1002* (US#); *Gutiérrez & al. 81851* (FTG); GU000025; GU000037; GU000063. ***Leucocroton wrightii*** Griseb.; Cuba; *Bennet 7590* (FTG); HM185119; HM185120; HM185121. ***Philyra brasiliensis*** Klotzsch.; Brazil; *Thomas & al. 12565* (NY); GU000028; GU000044; GU000064.

CHAPTER III

Islands within Islands; a Molecular Phylogenetic Study of a Plant Radiation across the Serpentine Archipelago of Cuba

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ABSTRACT

Aim My aim was to investigate the historical biogeography of the three genera, *Garciadelia* Jestrow & Jiménez Rodr., *Lasiocroton* Griseb., and *Leucocroton* Griseb. (Euphorbiaceae) known collectively as the *Leucocroton* alliance.

Location *Garciadelia* (endemic to Hispaniola) and *Lasiocroton* (restricted to the Bahamas, Cuba, and Jamaica) thrive on limestone soils. *Leucocroton* is endemic to serpentine soil regions across Cuba.

Methods Members of the *Leucocroton* alliance along with representatives from tribe Adelieae (*Adelia* L. and *Philyra* Klotzsch.) were included in a molecular phylogenetic analysis based upon nucleotide sequences of the internal transcribed spacers of the nuclear ribosomal DNA and the non-coding chloroplasts regions, *psbM-trnD* and *ycf6- pcbM*. I included 37 collections representing 28 species.

Results Phylogenetic analyses support the monophyly of the three genera. Incongruence tests identified conflicts among the three data sets; the major topological incongruence concerned the placement of *Lasiocroton macrophyllus* (Sw.) Griseb. The chloroplast phylogenies and the Bayesian analyses of the combined ITS-cpDNA data set placed this taxon as sister to two other Jamaican species (i.e., *L. fawcettii* Urb., and *L. harrisii* Britton). In contrast the other phylogenetic analyses supported a clade formed by this species and the widespread *L. bahamensis* Pax & K.Hoffm. Multiple accessions of five species formed distinct clades, supporting them as distinct taxa. In contrast, multiple accessions for two

species did not form two distinct monophyletic groups. Phylogenetic relationships within these groups, however, received low statistical support. The ancestral area of the *Leucocroton* alliance is Eastern Cuba and Hispaniola. Ancestral forms of *Leucocroton* arose on eastern Cuba and underwent two migrations across the island. The ancestor of *Lasiocroton* also originated on Eastern Cuba followed by later dispersal and speciation events to the other islands. My study also suggests that ancestral forms of the *Leucocroton* alliance most likely occurred on limestone soils.

Main conclusions My study concurs with previous hypotheses suggesting that the flora of serpentinite regions of the Caribbean derives from other types of soils. The serpentine endemics of the *Leucocroton* alliance have a single origin and represent one of the most extraordinary examples of speciation in this unique environment of the New World. The high colonization success achieved by the members of *Leucocroton* on serpentine soils was not attained by the other genera of the alliance, which occur on limestone areas.

Keywords

Caribbean Islands, *Adelia*, *Garciadelia*, *Lasiocroton*, *Leucocroton*, nickel, hyperaccumulator, phylogeography, serpentine, ultramafic

INTRODUCTION

Most molecular phylogenetic studies concerning plant adaptive radiation of insular systems focus on remote volcanic oceanic islands such as the Galápagos, the Hawaiian Archipelago, Juan Fernández, Macaronesia, and St. Helena (Andrus *et al.*, 2009; Eastwood *et al.* 2004; Francisco-Ortega *et al.*, 1996; Price & Wagner, 2004; Ruiz *et al.*, 2004; Trusty *et al.*, 2005;). Fewer studies have targeted larger tropical systems such as the Caribbean Islands, Indonesia, Malesia, and the Philipines. As opposed to the volcanic oceanic islands, these other tropical islands have complex geologies and broad soil diversities as they follow the ophiolitic regions of the orogenic belts (Brooks, 1987).

With over 1,000 islands and islets, the West Indies are unique because of their ancient isolation from the mainland. According to the Gaarlandia hypothesis, the most recent land-bridge between these islands and the mainland dates to the Eocene-Oligocene boundary (~34 Ma) and connected the Greater Antilles with South America through the Aves Ridge (Iturralde-Vinent & MacPhee, 1999), a geographic connection significantly older than the well known land-bridges that joined Indonesia, Malesia, and the Philippines with the Asian continent during the Pleistocene (Vorisi, 2000).

Another point of interest of the West Indies is the presence of ultramafic rocks, rocks which consist of more than 70% mafic (i.e., magnesium-iron based) silicate minerals. The vast majority of these rocks are intrusive and are either metamorphic (e.g., serpentinite) or igneous (e.g., peridotite). The ultramafic rocks occur on all large tropical island systems in disproportion to their occurrence on continents, unlike the oceanic volcanic islands, where this rock type is virtually nonexistent (Révillon *et al.*, 2000). While less than 1% of the earth's terrestrial surface consists of ultramafic rock (Brooks, 1987), Cuba is covered by

about 7% of ultramafic rock (Reeves *et al.* 1999). New Caledonia is an exceptional example with nearly a third of its geological substrate composed of ultramafic rocks (Jaffré, 1980). Within the Greater Antilles, ultramafic rocks occur on Cuba, Hispaniola, Jamaica, and Puerto Rico (Lewis *et al.*, 2006), while a single ultramafic area of Jamaica encompasses a very small patch of 0.25 km² (Abbott *et al.*, 1999). In tropical regions, nickel lateritic soils usually develop from ultramafic rocks through weathering processes. Depending on the specific mineral constituents of the ultramafic substrate, these soils are identifiable by their high magnesium to calcium ratios as well as high levels of the metals nickel and cobalt; these characteristics exert selective pressures on the plants growing on them. High selection pressure is the typical case for the Caribbean Island ultramafic laterites, generally referred to as serpentine soils in biological literature (Brooks, 1987).

Botanists have recognized the correlation of ultramafic substrates with unusual floras for hundreds of years (Brooks, 1987). Morphological tendencies such as sclerophylly, microphyllly, and other traits related to xeromorphism are typical for plants inhabiting these soils in the tropics (Borhidi, 1996). Despite the dramatic morphologies, few biogeographical studies have used molecular tools to understand the origin and evolution of insular floras thriving on this unique habitat. Most such studies have focused on continental regions with small radiations over serpentine areas, as in the Mediterranean, Asia Minor, and California (e.g., Cecchi & Selvi, 2009; Nguyen *et al.*, 2008). Serpentine radiations within tropical islands are even more poorly researched. The remote island of New Caledonia has been the subject of two recent studies of members of the Cunoniaceae. One study focused on *Codia* J.R. Forst & G. Forst with seven serpentine obligate endemic species (Pillon *et al.*, 2009a), and the other was on *Spiraeanthemum* A. Gray with three serpentine obligate endemic

species (Pillon *et al.*, 2009b, 2009c). These studies focused on describing the reticulate evolution and hybridization within the respective genera. A similar paucity of research exists for the Caribbean Islands, with most relevant studies being taxonomic in nature with little or no reference to soil types (see Discussion below). As far as I am aware, no molecular phylogenies focusing on large radiations of West Indian plants endemic to serpentine soils have been produced, though many potential genera exist. For instance, *Calyptranthes* Sw. (Myrtaceae), *Eugenia* L. (Myrtaceae), *Ossaea* DC. (Melastomataceae), and *Rondeletia* L. (Rubiaceae) each have over twenty species of serpentine obligates within Cuba (Reeves, 1999).

Many of the species found on these soils are serpentine obligates, but only a subset of species are nickel hyperaccumulators. Plants described as Ni-hyperaccumulators have over 1,000 µg/g nickel per dry plant material; this is between one and two orders of magnitude higher than a typical serpentine obligate (Reeves *et al.*, 1999). Nickel, though a required nutrient, is typically found in very low concentrations in non-serpentine land plants, and is considered toxic at the high levels found in many serpentine soils. Physiologically, serpentine plants must deal with the heavy metal either by hyperaccumulation or by inhibiting their uptake at the root-soil interfaces (Verbruggen *et al.*, 2008). The world total of Ni-hyperaccumulators has last been tallied at only ~ 390 species (Verbruggen *et al.*, 2008), although new hyperaccumulators are routinely identified (Ghaderian *et al.*, 2007; Reeves *et al.*, 2007). Other metal hyperaccumulators are known (~ 450 hyperaccumulating species in total; Verbruggen *et al.*, 2008), but those dealing with nickel (~ 90%) represent the majority. From a biogeographic perspective, several studies consider Ni-hyperaccumulation as a syndrome of paleoendemism, arguing that the required complex metabolic pathways for

hyperaccumulation imply significant evolutionary change and can only have originated in ancient groups (Borhidi, 1996; Reeves *et al.*, 1999).

Cuba, with over 150 species of Ni-hyperaccumulators, ranks highly in global importance for diversity of hyperaccumulators. While Ni-hyperaccumulation has been identified in many different genera within the flora of this island, three are particularly species-rich. Two of them, (i.e., *Buxus* L. (Buxaceae) and *Phyllanthus* L. (Phyllanthaceae)), have wide distributions outside Cuba (Reeves *et al.*, 1999). These are taxonomically difficult genera, with a large number of endemic species on Cuba (*Buxus*: 37 species; *Phyllanthus*: 53 species and subspecies) and occurring on a wide array of soils and ecosystems. *Buxus* has 17 Ni-hyperaccumulating endemic species, whereas *Phyllanthus* has 25. The third genus, the focus of this research, is the Cuban endemic taxon *Leucocroton* Griseb. (Euphorbiaceae) (Fig. 1). *Leucocroton* has more Ni-hyperaccumulating species than any other genus within the Caribbean Islands (Bordács & Borhidi, 1993; Borhidi, 1991, 1996; Borhidi *et al.* 1992; Reeves *et al.*, 1996, 1999). The present delimitation of *Leucocroton* (Jestrow *et al.*, in press) includes 26 species; all are Ni-hyperaccumulators restricted to the serpentine soil areas across Cuba.

Lewis *et al.* (2006) divide the ultramafic substrates of Cuba into ten primary massifs distributed from the western to the eastern extremes of the island (Fig. 2). All of the massifs are metamorphic in origin and have undergone varying amounts of serpentinization, therefore the ultramafic rocks of Cuba can be broadly classified as serpentinite. On the basis of their developmental age, the soils formed on these massifs are divided into two major groups. Finko *et al.* (1967) identify the western massif region, Cajalbana and the two Eastern massifs, Mayarí-Cristal and Moa-Baracoa, as having mature soils ranging from 10-30 Ma.

The other massifs of central Cuba are considered to be much younger at about 1 Ma or less (Finko *et al.*, 1967). However, Lewis *et al.* (2006) suggest that the soil development of the central Cuban massif Camagüey to date to the Miocene (5-20 Ma), with the laterites of Camagüey having undergone significant erosion into neighboring sedimentary basins. While the proposed ages and constituent minerals of these massifs differ, species of *Leucocroton* are known to occur on eight of these massifs from western, central, and eastern Cuba (Fig. 2). This serpentine obligate genus has diversified throughout the serpentine archipelago of Cuba, representing a unique evolutionary model system within the Caribbean Islands.

Leucocroton together with *Garciadelia* Jestrow & Jiménez Rodr. and *Lasiocroton* Griseb., constitute the *Leucocroton* alliance, a monophyletic group endemic to the West Indies (Francisco-Ortega *et al.*, 2007) (Fig. 1). *Garciadelia* is restricted to Hispaniola, whereas *Lasiocroton* has a wider distribution of the Bahamas, Cuba, Hispaniola, and Jamaica (Fig. 3). Members of this alliance are woody, long-lived, evergreen plants ranging from small shrubs to canopy trees (Fig. 1). All members of the alliance are dioecious. Though no field pollination study exists for the group, I consider most of the alliance putatively to be wind-pollinated, as most of the species exhibit many of the wind-pollination syndromes described by Freidman and Barrett (2009) (e.g., many flowers, small or absent petals, greenish or white flowers, unscented flowers, few ovules per flower, unvarying pollen size, massive pollen production, lack of pollen ornamentation, unisexual flowers, synchronous flowering, and occurrence in open habitats with high conspecific density).

In a prior taxonomic study, I defined the *Leucocroton*-alliance as a clade within the tribe Adelleae (Jestrow *et al.*, 2008, in press). This tribe is composed of two additional genera: *Adelia* L., and *Phylira* Klotzsch. The former is found on the continent from Mexico

south to Brazil with a single species, *A. ricinella* L., reaching the Caribbean Islands. *Philyra* is native to Eastern South America.

Because *Garciadelia* (four species) and *Lasiocroton* (seven species) are restricted to limestone areas, while *Leucocroton* is endemic to serpentine soils, the *Leucocroton* alliance has a unique biogeography, including taxa occurring on several islands and on two major soil types. In this paper I use molecular phylogenies to understand the historical biogeographical patterns of this taxon both across the Caribbean Islands (i.e., within the *Lasiocroton* alliance) and across the serpentinite archipelago of Cuba (i.e., within *Leucocroton*).

MATERIALS AND METHODS

Taxon Sampling

A total of 37 individual collections were sampled from 28 recognized species (Table 1). The most distant outgroup was *Philyra brasiliensis*. This monospecific genus has been shown to represent the earliest diverging lineage of the tribe (Wurdack *et al.*, 2005; Jestrow *et al.*, 2008). The outgroup also included three species of *Adelia*, the sister genus to the *Leucocroton*-alliance. (De-Nova & Sosa, 2007). Two of these species, *A. ricinella* and *A. vaseyi* represent the two primary clades of the genus (De-Nova & Sosa, 2007). The third one, *A. cinerea* is a distinctive species of uncertain phylogenetic placement. De-Nova & Sosa (2007) found this taxon to be the earliest diverging lineage of *Adelia*, while other studies place *A. cinerea* as sister to the *Leucocroton* alliance (De-Nova & Sosa., 2007; Jestrow *et al.*, in press).

The ingroup consisted of representatives from the *Leucocroton* alliance. It included five of the seven known species of *Lasiocroton* (Jestrow *et al.*, 2008, in press) (Table 1).

Lasiocroton gracilis Britton & P. Wilson was not part of my study. This extremely rare taxon is known from a single collection dating from 1910, possibly representing a rare hybrid of *L. bahamensis* and *L. microphyllus* (see Discussion below). The other omitted species is the recently described *L. gutierrezii* Jestrow; this taxon is known from only two collections dating from 1949 and 1956, and is closely related to *L. microphyllus* (Jestrow *et al.*, in press); however, during my field studies in Cuba, I was unable to find this species. I included two accessions from different localities of *L. bahamensis*, *L. harrisii*, and *L. microphyllus* (see Table 1.)

Formerly, *Leucocroton* was divided into three sections (Borhidi, 1991): *Adeliocroton* Borhidi (2 spp.), *Lasiocrotonopsis* Borhidi (7 spp.), and *Leucocroton* Borhidi (19 spp.). The two species belonging to section *Adeliocroton* are no longer included in the genus because one has been moved to *Lasiocroton* as *L. microphyllus*, and the other has been transferred to *Garciadelia* as *G. leprosa* (Willd.) Jestrow & Francisco Rodr. Sixteen of the 26 species of *Leucocroton* were selected to represent its morphological variability and geographical distribution. Thirteen of them belonged to the section *Leucocroton* and three to the section *Lasiocrotonopsis* (i.e., *L. moncadae*, *L. subpeltatus*, *L. virens*) as described by Borhidi (1991). I included the type of the genus, *L. wrightii* (Webster, 1994; Radcliffe-Smith, 2001).

The ten species of *Leucocroton* that were not included in this study have a limited collection history with poorly known geographical distributions and few collection localities. On the basis of my taxonomic studies, minor morphological characters distinguish these enigmatic species from those included in the present study. For example, *L. longibracteatus* Borhidi, a taxon morphologically similar to *L. wrightii*, was described on the basis of a single collection from Taco Bay of the Moa-Baracoa massif dating from 1952. I traveled to this

locality and were unable to find a single exemplar of this species. When examining herbarium collections, I did not find additional localities for this taxon, nor any specimens collected after the type material. In spite of these difficulties in collecting these poorly known taxa of *Leucocroton*, I have included all species occurring in Western and Central Cuba. In Eastern Cuba, I have collected the two species from the Holguín massif (*L. anomalus* and *L. virens*). I am missing only nine enigmatic taxa (*L. acunae*, *L. brittonii*, *L. cordifolius*, *L. discolor*, *L. incrustatus*, *L. longibracteosus*, *L. moaensis*, *L. obovatus*, and *L. pallidus*) from the species-rich massifs of Mayarí-Cristal and Moa-Baracoa (Fig. 2). For a few of the taxa, I was unable to collect samples for every massif where they have been reported. For example, while *L. moncadae* is recorded for three different massifs, this taxon is presently known from a single population of the Habana-Matanza massif. I have included multiple collections of four taxa (i.e., *L. comosus*, *L. ekmanii*, *L. linearifolius*, and *L. pachyphyllus*), as I was able to find these species from different localities.

As for the recently described genus *Garciadelia* (formerly in *Leucocroton* section *Adeliocroton*), I included three species (i.e., *G. abbottii*, *G. castilloae*, and *G. mejiae*). Unfortunately, I did not study the Haitian endemic *G. leprosa* (Willd.) Jestrow & Jiménez Rodr. because I was unable to obtain samples of this extremely rare species, last collected in 1924 (Jestrow *et al.*, in press).

DNA extraction, amplification, and sequencing

Plant DNA was extracted from silica-dried material using a DNeasy Plant Mini Kit (Qiagen, Valencia, California). I used PuReTaq Ready-To-Go PCR Beads (GE Healthcare Life Sciences, Piscataway, New Jersey) for the PCR amplification reaction mix. For all regions,

the cycle program used was as follows: (1) 80°C for 5 min; (2) 94°C for 1 min; (3) 50°C for 1 min; (4) 72°C for 2 min; 35 cycles from step (2); and (7) 72°C for 5 min. The chloroplast DNA (cpDNA) noncoding regions, *psbM-trnD* and *ycf6- pcbM* were amplified with primers previously published by Shaw *et al.* (2005). The ITS1, 5.8s, and ITS2 of the nuclear ribosomal DNA were amplified as one region using primers ITS 5 (Downie & Katz-Downie, 1996) and ITS 4 (White *et al.*, 1990). For members of *Garciadelia* and the species *Leucocroton subpeltatus*, the total ITS regions were cloned prior to sequencing. Cloning was required because of the poor quality of sequences obtained after direct sequencing (see Results below) for these particular taxa only. I used TOPO TACloning (Invitrogen, Carlsbad, California), following the manufacturer's guidelines. Cycle sequencing was performed in both directions with the PRISM BigDye v.3.1 Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, California) following the manufacturers instructions. The primers used for PCR amplification were also utilized for the cycle sequencing reactions. Nucleotide sequences were visualized on a PRISM 377 Automated DNA Sequencer (Applied Biosystems, Foster City, California) at the Florida International University (FIU) DNA Core Facility. All new sequence data were submitted to GenBank (see Table 1).

Phylogenetic analysis

All sequences were assembled and visually aligned, using Sequencher 3.1.1 (Gene Codes Corporation, Ann Arbor, Michigan). A single region in the *ycf6-pcbM* alignment was removed because of a large "T" repeat (30-40 bp) in both the ingroups and outgroups, which could lead to slippage of the DNA polymerase. All regions were gap-coded using the Simple Indel Coding method (Simmons & Ochoterena, 2000).

When analyzing the ITS and the ITS-cpDNA combined data sets, the parsimony analyses were conducted as a two-step heuristic search in PAUP*4.0b10 (Swofford, 2003). First, trees were found using a 10,000 replicate heuristic search, saving no more than ten trees per replicate with Multrees off. The second step used the trees previously saved as the starting trees for another heuristic search with Multrees on.

The chloroplast data were run similarly, but as a result of the large number of seed trees, Multrees was not engaged for the second step. I conducted three analyses of the chloroplast data set; (1) *psbM-trnD*, (2) *ycf6-pcbM*, and (3) the combined two regions. Branch lengths for the total combined dataset were calculated according to the Deltran algorithm. Clade support was assessed with bootstrap analysis (Felsenstein, 1985) and searches were conducted using a 10,000 replicate heuristic approach with starting trees from random addition. The consistency index excluding uninformative characters (CI), retention index (RI), and rescaled consistency index (RC) were also calculated (Kluge and Farris, 1969; Farris, 1989). Incongruence Length Difference (ILD) tests were run between the three major data sets (ITS, *psbM-trnD*, and *ycf6-pcbM*).

Bayesian methods were also used for phylogenetic inferences in order to identify possible long branch attraction. However with the Bayesian analyses, I did not include gap-coding data given the uncertainties regarding molecular evolution modeling. Each region (ITS1, 5.8s, ITS2, *psbM-trnD*, *ycf6-pcbM*) was run separately through Modeltest v.3.06 (Posada & Crandall, 1998). Models were chosen on the basis of the Akaike Information Criterion (AIC) following Posada and Crandall (1998). I used the following models for the Bayesian analyses: ITS 1, GTR+G; 5.8s, K80; ITS2, TIM+G; *psbM-trnD*, TIM+I; and *ycf6-pcbM*, K81uf+I. Bayesian inferences were conducted using MrBayes 3.1.2 (Huelsenbeck &

Ronquist, 2001) with two Markov chain Monte Carlo (MCMC) runs of four linked chains for 1,000,000 generations sampling every 100 generations. The four chains included one cold, and the other three with incremental heating as per the default of MrBayes. Of the 10,001 trees produced per MCMC run, the first 25% of these trees were removed as burnin, resulting in a total of 15,000 trees. The burnin of 25% was determined to be adequate from the likelihood values, as the values leveled off at between 100,000 and 200,000 generations for the datasets. All Bayesian analyses produced split frequencies of less than 0.02, showing convergence between the paired runs. I then used PAUP* to compute the majority consensus tree for the total data to give the posterior probabilities.

Ancestral analysis

For the ancestral area analyses, the members of the *Leucocroton* alliance were placed into six areas: (1) Western Cuba, (2) Central Cuba, (3) Eastern Cuba, (4) the Bahamas, (5) Jamaica, and (6) Hispaniola. The division of Western and Central Cuba was drawn between the two massifs, Havana-Matanza and Villa Clara, while Central and Eastern Cuba were separated between the Camagüey and Holguín massifs (Fig. 2). Three of the outgroup taxa do not occur on the Caribbean Islands, therefore they were assigned to either Central or South America. The exception was *Adelia ricinella* as this taxon is present in Cuba, Hispaniola, Jamaica, and South America.

Ancestral area analyses were conducted via two different methods: a cladistic approach described by Bremer (1992) and the dispersal-vicariance (DIVA) procedure of Ronquist (1997a). Both methods were applied to area and soil types using both Bayesian and parsimony tree topologies based on the ITS-cpDNA combined dataset. As both methods

require fully bifurcating topologies, I randomly selected four fully resolved trees from both the parsimony and Bayesian methods as the basis to perform Bremer and DIVA ancestral areas reconstructions. As the cladistic method of Bremer can be calculated only for a particular node in a phylogeny, I chose two nodes to analyze my data. The first one was the basal node of the *Leucocroton* alliance (see Results below) and the second was the node defining the *Lasiocroton* clade (see Results below). As the parsimony and Bayesian topologies conflicted (see below), I calculated the Bremer values for these two nodes along both topologies. Ronquist's approach was implemented with the program DIVA 1.1 (Ronquist, 1997b) using the same topologies that were utilized for Bremer's cladistic method.

RESULTS

ITS analysis

The aligned ITS data matrix was 678 nucleotide positions in length and included 135 parsimony informative (PI) nucleotide positions along with 15 coded gaps, ten of which were PI (Table 2). Of these, 109 nucleotides and five coded gaps were PI within the *Leucocroton* alliance. The cladistic analysis yielded 5880 most parsimonious trees. The strict consensus tree shows *Adelia cinerea* to be sister to the *Leucocroton* alliance, with bootstrap support of 91% (Fig. 4a). Within the *Leucocroton* alliance, the Hispaniolan genus *Garciadelia* was found to be sister to a clade formed by *Lasiocroton* and the Cuban genus *Leucocroton*, with bootstrap support of 87%. The monophyly of the three genera of the *Leucocroton* alliance was strongly supported with bootstrap values exceeding 99%. Within *Leucocroton*, *Leucocroton* section *Lasiocrotonopsis* (i.e., *L. moncadae*, *L. subpeltatus*, and *L. virens*) was

found to be paraphyletic. The Jamaican endemic *Lasiocroton macrophyllus* formed a clade with *L. bahamensis*, with bootstrap value equal to 89%. The Bayesian analysis produced a tree without any conflict to the parsimony strict consensus tree. Bayesian posterior probabilities for the monophyly of each genus were 100%. The Bayesian posterior probabilities were higher than the bootstrap values for all clades, except for a single node that had the lowest bootstrap value of the phylogeny (60%) (see asterisk, Fig. 4a).

The ITS regions of *Garciadelia abbottii*, *G. castilloae*, *G. mejiae*, and *Leucocroton subpeltatus* were cloned because of difficulties with direct sequencing. I blasted the cloned sequences of these four species through GenBank and found that some matched ITS accessions of endophytic Ascomycetes, which explains my difficulties in obtaining readable ITS sequences from direct PCR products. The fungal clones from the *Garciadelia* species were quite similar to each other, while the fungal clone from *L. subpeltatus* was significantly different. My initial GenBank searches showed that most of the cloned sequences matched ITS accessions of the *Leucocroton* alliance. I was able to recover five ITS clone sequences for each of these five taxa. Within each species, the five cloned sequences were extremely similar (Jestrow *et al.* in press), and, therefore, for my phylogenetic analyses I decided to include one representative clone per taxon. I chose the clones based on the minimum number of autapomorphic characters, thus allowing us to avoid false inflation of the branch lengths.

Chloroplast analyses

The aligned *psbM-trnD* and *ycf6-psbM* data matrices were 1070 and 1204 nucleotide positions in length, respectively (Table 2). The data set of *psbM-trnD* included 23 PI nucleotide positions along with 14 PI coded gaps. The *ycf6-psbM* data matrix had 35 PI

nucleotide positions and 13 PI coded gaps. Within the *Leucocroton* alliance, the *psbM-trnD* data set yielded 12 PI nucleotide positions with ten PI coded gaps. The *ycf6-psbM* alignment had 27 PI nucleotide positions and ten PI coded gaps. The ILD test identified incongruence between the two chloroplast data sets ($p=0.01$). The *ycf6-psbM* tree conflicted with the *psbM-trnD* topology by nesting the outgroup taxon *Philyra* within the *Adelia* clade. The incongruence was resolved when this species was removed ($p=0.47$). The inclusion or exclusion of *P. brasiliensis* did not change the topology of the ingroup. The combined chloroplast cladistic analyses yielded 6646 most parsimonious trees.

In contrast to the results produced by the analyses of the ITS data, the combined chloroplast phylogeny found the *Leucocroton* clade poorly supported with a bootstrap value of only 56%. However, the *Garciadelia* and *Lasiocroton* clades received strong support, with bootstrap values of 100% and 99%, respectively. Within *Leucocroton*, resolution of the species relationships was weak. Only three clades were recovered within the genus, receiving bootstrap values below 55%.

The main differences between the ITS and cpDNA topologies concerned the placement of *Lasiocroton macrophyllus*. The chloroplast phylogeny placed this taxon within a clade formed exclusively of taxa endemic to Jamaica (Fig. 4b). The Jamaican assemblage was weakly supported by a 63% bootstrap, although it received a higher support from the Bayesian analyses (see below).

Bayesian and parsimony analyses conflicted in the position of a single taxon, *Leucocroton comosus*. The Bayesian analysis placed (78% posteriori probability) this Eastern Cuban species as sister to another species from that region of the island, *L. saxicola*. In contrast, the cladistic analyses weakly supported (54% bootstrap value) a sister relationship

between *L. comosus* and *L. moncadae*; the latter is restricted to the Central and Western Cuba. Interestingly, the clade of *Lasiocroton* species endemic to Jamaica received a stronger support from the Bayesian analyses (100% a posteriori probability), but had a low bootstrap value of 63% after the parsimony analyses.

Combined analysis

The ILD tests showed conflicts between the ITS with both *psbM-trnD* and *ycf6-psbM* regions ($P=0.01$). After removing *Philyra brasiliensis* and *Lasiocroton macrophyllus*, the conflict between the ITS and chloroplast regions remained. I analyzed the ITS-cpDNA combined data in spite of this incongruence, in order both to include the largest sample of informative characters and to compare the combined with the separate nuclear and chloroplast datasets. I followed the strategy of Wiens (1998) by including both the combined and separate ITS and cpDNA datasets, and discuss the differences and similarities found among them.

The parsimony analyses of the ITS-cpDNA combined data set confirmed the monophyly of the genera endemic to Cuba (*Leucocroton*, 100% bootstrap value) and Hispaniola (*Garciadelia*, 100% bootstrap value). The species of the third genus of the alliance, *Lasiocroton*, also formed a monophyletic group supported by a bootstrap value of 99%. This cladistic analysis found *Adelia cinerea* of Northern Mexico to be sister to the *Leucocroton* alliance, a relationship that was supported with a 100% bootstrap value.

The ITS and the ITS-cpDNA combined data sets had similar phylogenies, and they both recovered the same major clades within each of the three genera. Two major clades were found within *Leucocroton*. The first one had the two accessions of the Eastern Cuba endemic, *L. pachyphyllus*. The second clade received a 73% bootstrap support and had the

remaining 15 taxa of the genus. This second clade had three lineages that collapsed in a polytomy. The first lineage had a single species, *L. subpeltatus* of Eastern Cuba. The second lineage had 12 species, ten of them are restricted to Eastern Cuba and they formed a monophyletic group strongly supported with a 94% bootstrap value. The remaining two taxa of this lineage formed another monophyletic group (80% bootstrap value). One of these taxa is restricted to Western Cuba (i.e., *L. flavicans*) whereas the second one (i.e., *L. virens*) has a widespread distribution on serpentine areas of Central and Western Cuba. The third lineage was weakly supported with a 53% bootstrap value and had seven species. Three of them (i.e., *L. havanensis*, *L. moncadae*, and *L. revolutus*) are from Western and/or Central Cuba and formed a strongly supported clade with a 100% bootstrap value. The remaining four species are Eastern Cuban endemics, but only three of them formed a monophyletic group (i.e., *L. anomalus*, *L. stenophyllus*, and *L. saxicola*) that was supported with a 99% bootstrap value. The fourth, *L. linearifolius*, was the earliest divergent branch of the third lineage.

Lasiocroton was composed of two major clades. The first one had the two accessions of the Cuban endemic *L. microphyllus*. The second clade was supported with a bootstrap value of 100% and had two lineages. The first one comprised the two Jamaican endemics *L. fawcettii* and *L. harrisii* (bootstrap support of 95%). The second lineage had the other two species of this genus, *L. bahamensis* (a species with a widespread distribution in the Bahamas, Cuba, and Hispaniola) and *L. macrophyllus* (endemic in Jamaica). However, a sister relationship between these two taxa was weakly supported with a 68% bootstrap value.

The two species of *Garciadelia* from Southern Hispaniola formed a monophyletic group (supported with a 96% bootstrap value) that was sister to *G. abbottii*, an endemic in the Northeastern part of the island (Fig. 3d).

The topologies from parsimony and Bayesian methods conflicted within the genus *Leucocroton* at three nodes, but these nodes lacked support (<50% bootstrap value). The only other conflict between the parsimony and Bayesian reconstructions concerned *Lasiocroton macrophyllus*. The Bayesian topology (51% clade credibility) grouped this taxon as sister to the Jamaican species, but the parsimony topology placed this species with *L. bahamensis* (68% bootstrap value).

Ancestral area analysis

For the *Leucocroton*-alliance node, the cladistic method (Bremer, 1992) strongly favored Eastern Cuba (1.00 AA value) as the ancestral area in both parsimony and Bayesian topologies (Table 3). This method yielded Hispaniola as a second choice (0.38) for the Bayesian topology, while the parsimony reconstruction found Hispaniola, Jamaica and Western Cuba as second in importance (0.36). I found that these cladistic historical biogeography reconstructions were sensitive to the distribution scoring of a single species, *Lasiocroton bahamensis*, known from Hispaniola by a single collection from Haiti (see Discussion below). The omission of “Hispaniola” from the distribution scoring of *L. bahamensis* significantly increases the statistics for Hispaniola as an ancestral area for the alliance for both parsimony (0.50) and Bayesian reconstructions (0.57), though Cuba was still the first choice (1.00) (see asterisks Table 3).

For the *Lasiocroton* clade, Bremer’s method yielded different results along the Bayesian and parsimony topologies (Table 4). The parsimony tree supported the three areas of Cuba and Jamaica as equally likely ancestral areas (1.00) for the genus. In contrast, reconstruction along the Bayesian tree found the three areas of Cuba as the ancestral area

(1.00). Removal of “Hispaniola” from the distribution scoring of *L. bahamensis* did not change the results of the cladistic method for the *Lasiocroton* clade, as *L. bahamensis* was the only member of the clade to occur on the island.

Dispersal-vicariance analysis produced similar results to Bremer’s method. For the *Leucocroton*-alliance, DIVA shows Eastern Cuba and Hispaniola as the ancestral area, according to both parsimony and Bayesian trees (Fig. 6). Furthermore, both topologies found the ancestral area of *Garciadelia* to be Hispaniola and of *Leucocroton* to be Eastern Cuba. The only discrepancies between the two topologies in the DIVA analysis are found within the *Lasiocroton* clade. Based on the parsimony reconstruction, Eastern Cuba or Eastern Cuba – Jamaica are the ancestral areas for this genus. In contrast, DIVA selected only Eastern Cuba as the ancestral area along the Bayesian tree. Interestingly, within the *Lasiocroton* clade, the Bayesian topology produced an output with fewer ancestral areas than the parsimony topology as it identified the Jamaican taxa as a monophyletic group (Fig. 6).

Unlike the Bremer reconstruction which was sensitive to the scoring of *Lasiocroton bahamensis*, DIVA was resilient and consistent. The DIVA analysis, when including Hispaniola in the scored distribution of *L. bahamensis*, produced a single change with the parsimony topology. This subtlety is seen as the inclusion of Hispaniola as one of many ancestral areas for the *L. bahamensis* clade (Fig. 6).

Concerning *Leucocroton*, DIVA yielded identical results both along the Bayesian and parsimony topologies. Eastern Cuba is the ancestral area for the genus. Indeed, most of the clades have this region as an ancestral area, with two exceptions: (1) the assemblage of *L. flavicans* and *L. virens* had the three areas of Cuba as a potential ancestral area; and (2) the clade of *L. havanensis*, *L. moncadae*, and *L. revolutus* had Western Cuba as its ancestral area.

DISCUSSION

Biogeographical patterns across islands and soils

All of the analyses agreed that the ancestral forms of the *Leucocroton* alliance originated in Eastern Cuba and/or Hispaniola. These two regions are geographically close (75 km between Cajobabo in Eastern Cuba and Môle St. Nicolas in Haiti), and they formed a single geographical unit until the early Miocene (~16 Ma) when they started to separate through tectonic processes (Iturralde-Vinent, 2006; Pindell *et al.*, 2006; Pindell & Kennan, 2009).

The earliest split of the alliance yielded two major groups, one of them restricted to limestone regions of Hispaniola (i.e., *Garciadelia* clade) with only four species. Ancestral forms of this group originated on this island. Notably, none of the species of this genus colonized the available serpentine areas of Hispaniola (i.e., Loma Caribe and North Coast Belt, Fig. 3d). The second speciation group comprised two clades. The members of the first of these clades, *Lasiocroton*, successfully colonized limestone areas of Cuba (three endemic species) and Jamaica (three endemic species), with *L. bahamensis* reaching the Bahamas, Cuba, and Hispaniola (a single and uncertain record, see below). None of the members of this clade have colonized serpentine regions. My historical biogeography reconstructions primarily supported Eastern Cuba as the ancestral area for the *Lasiocroton* clade.

The second clade (i.e., *Leucocroton*), although limited to the serpentine areas of Cuba, has the highest number of species (26 spp.) and provides the most extensive example of speciation within the alliance. None of the species of this clade thrive on the limestone areas of Cuba. Ancestral area reconstructions identified Eastern Cuba as the region where this genus originated.

A study of ancestral soil types using Bremmer's method and DIVA (data not presented) showed that limestone areas represent the ancestral soil type for the alliance. Several molecular phylogenies have included West Indian endemics that are restricted to serpentine soils (e.g., McDowell & Bremer, 1998, McDowell *et al.*, 2003; Lavin *et al.*, 2001; Loockerman *et al.*, 2003; Liu *et al.*, 2004; Namoff *et al.*, 2007; Pelser *et al.*, 2007; Cariaga *et al.*, 2008; Francisco-Ortega *et al.*, 2008; Michelangeli *et al.*, 2008, pers. comm.; van Ee *et al.*, 2008; Rova, 2009). However, most of these studies focus on plant systematics and rarely discuss speciation events and soil adaptation in a historical biogeographical context. An exception was provided by Liu *et al.* (2004) who reported forms from serpentine soils of Cuba as ancestral types to the *Sachsia* Griseb. – *Rhodogeron* Griseb. group (Asteraceae). These authors indicated that the evolutionary history of these genera has followed a colonization track from serpentine to limestone areas. My results indicate a different evolutionary pathway for the *Leucocroton* alliance, and concurs with Borhidi (1996) who suggested that plant colonization in Cuba has occurred from non-serpentine areas towards those with ultramafic soils. This direction of colonization is also supported by results for the Neotropical genus *Exostema* (Pers.) Bonpl. (Rubiaceae) (MacDowell & Bramwell, 1998; MacDowell *et al.*, 2003), which showed that the five serpentine species endemic to Cuba and Hispaniola not only had three independent origins but also were part of the terminal branches of the phylogeny of the genus.

My results support a single origin for the serpentine species of *Leucocroton*. A similar pattern has been reported for endemics of *Croton* L. subgenus *Moacroton* (Croizat) van Ee & P.E. Berry (van Ee *et al.*, 2008). However, multiple origins for

serpentine endemics have also been found not only within *Exostema* (see above), but within the “Caribbean clade” of the tribe Miconieae (Melastomataceae) (Michelangeli *et al.*, 2008, pers. comm.).

I included multiple collections for each of seven species of the *Leucocroton* alliance. For five of them, these multiple accessions formed distinct monophyletic groups, therefore my phylogeny supports them being distinct taxa that have differentiated from the rest of the members of the alliance. In contrast, the multiple accessions of *L. cosmosus* and of *L. ekmanii* did not form two distinct monophyletic groups. However, phylogenetic relationships within these collections and their sister taxa received low statistical support and therefore it is premature to draw conclusions concerning the phylogenetic placement of these accessions and the monophyly of these two species.

***Leucocroton* and the serpentine islands of Cuba**

Leucocroton arose in Eastern Cuba on the large ultramafic massifs. The lateritic soils derived from these massifs date between 10-30 million years ago (Ma), and are about the same age as the small serpentine area of Western Cuba, at Cajalbana (Finko *et al.*, 1967; Borhidi, 1996; Lewis *et al.*, 2006). The serpentine areas between these extremities are ca. 1 Ma (Finko *et al.*, 1967; Borhidi, 1996). Paleogeographical studies suggested that Eastern and Western Cuba were two separate islands until approximately 6 Ma when a land-bridge was established between them (Iturralde-Vinent, 2006). Groups with this biogeographical disjunction have been identified in the tribe Tageteae (Asteraceae) (i.e., *Lescaillea* Griseb. and *Harnackia* Urb.), *Heptanthus* Griseb. (Asteraceae), *Spathelia* L. (Rutaceae), and *Purdiaea* Planch. (Cyrillaceae) (reviewed by Bordács & Borhidi, 1993;

Borhidi, 1996). My molecular phylogenies do not detect two clades supporting a strict Eastern-Western Cuba split. The biogeographical situation of *Leucocroton* follows a more complex pattern. All taxa restricted to a particular area do not group in a single separate clade. For instance, the nine species from Eastern Cuba belong to three different monophyletic groups. Likewise the two species confined to Western Cuba belong to two different clades. These results suggest that the genus has undergone multiple introductions to each of the “islands” that form the serpentine archipelago of Cuba.

Although, some of the nodes within the *Leucocroton* clade have low statistical support, it appears that at least two migrations across the island occurred in the evolutionary history of this genus. The first one was supported with a 88% a posteriori probability and included three species from Eastern Cuba (i.e., *L. anomalus*, *L. saxicola*, and *L. stenophyllus*) and three from Western and/or Central Cuba (i.e., *L. havanensis*, *L. moncadae*, and *L. revolutus*). Historical biogeographical analyses identified Eastern-Western Cuba as the ancestral area for this assemblage. The second migration group received a 99% posteriori probability and included five species from the Eastern portion of the island, one from Central-Eastern Cuba, and one restricted to Western Cuba. This group had Eastern Cuba as an ancestral area.

The historical biogeographical study suggested Eastern Cuba as the major center of diversification of *Leucocroton* species and supported a general east to west migration route. Interestingly, Borhidi (1996) in his seminal work on Cuban phytogeography indicated that Eastern Cuba is the “starting point for the most important migrations” on the island. My results appear to confirm this hypothesis.

Conflicting topologies: biogeographical implications

Within the *Leucocroton* alliance, the placement of the Jamaican endemic *Lasiocroton macrophyllus* is the primary culprit of the main topological conflicts between the nuclear and chloroplast phylogenies. The ITS topology placed this species as sister to the widespread *L. bahamensis*. In contrast, the cpDNA data supported this taxon as part of a clade composed exclusively of Jamaican endemics. Likewise, the parsimony and Bayesian analyses of the combined data set did not agree about the placement of this taxon. The former supported *L. macrophyllus* as sister to *L. bahamensis* (bootstrap support of 68%), whereas the Bayesian analyses placed this taxon within the Jamaican clade with a low a posteriori probability of 51%.

Both lineage sorting between the nuclear and chloroplast regions and/or hybridization could explain this conflict. *Lasiocroton bahamensis* does not reach Jamaica, and although I cannot rule out that this species occurred on the island in the past, the available evidence suggests that hybridization is not the best explanation for the conflicting placement of *L. macrophyllus*. Morphologically this species is very similar to *L. bahamensis*, with only subtleties of branching habit, leaf apex, and stigma structure differentiating them. *Lasiocroton bahamensis* was initially identified as *L. macrophyllus* (Northrop, 1902) before being formally described as a distinct species (Pax & K. Hoffm., 1914). In addition, both species occur on coastal limestone. I believe that *L. macrophyllus* evolved in the lowlands of Jamaica as a transitional form between *L. bahamensis* and the two other Jamaican endemics. These two other taxa are confined to montane environments of this island (Fig. 3c). Therefore, I hypothesize that lineage sorting

between the nuclear and chloroplast markers, rather than hybridization, provides the best explanation for the conflictive phylogenetic placement of *L. macrophyllus*.

Because of the conflict concerning the placement of *Lasiocroton macrophyllus*, reconstruction of ancestral areas for the *Lasiocroton* clade using DIVA yielded different results along the Bayesian and parsimony topologies (Fig. 6). However, I favor the results along the Bayesian phylogeny, as this reconstruction agrees with my hypothesis of *L. macrophyllus* as a taxon with its ancestor in Jamaica (see above). In contrast, results along the parsimony topology yielded five putative ancestral areas for the ancestor of *L. macrophyllus*.

In my study I am not arguing that hybridization has not been relevant in the evolutionary history of the *Leucocroton* alliance. A good example of its importance is provided by the Cuban endemic *Lasiocroton gracilis*. Known from a single collection from southern Cuba in 1902, this taxon exhibits intermediate characters of *L. bahamensis* and the Cuban endemic *L. microphyllus*. I suggest that the best interpretation of *L. gracilis* is that it arose from a rare hybrid between these two morphologically-distinct species (Jestrow, in preparation). Unfortunately, during my field trips to Eastern Cuba I was unsuccessful in trying to locate plants of this putative hybrid species.

***Lasiocroton bahamensis*: the biogeography of a widespread species**

Lasiocroton bahamensis is unique as it is the only species in the *Leucocroton* alliance to occur on more than one island. While originating in Eastern Cuba (Fig. 6), the species has dispersed to the Bahamas and the northeastern tip of Hispaniola. Within the Bahamas it has been collected from four islands: Eleuthera, Great Ragged Island, Great Exuma, and

South Andros (Fig. 3b). These four Bahamian islands are part of the Great Bahama Bank which formed a large landmass during the last glacial maximum, approximately 18,000 yr BP (Hearty & Kaufman, 2000). This ancient land mass was separated from Cuba by only ca. 20 kilometers and had approximately the same area of current Hispaniola. While *L. bahamensis* dispersed to the Bahamas, the other coastal limestone species of Cuba, *L. microphyllus*, is only found on Cuba. One difference between these two coastal limestone species is fruit production. Plants of *Lasiocroton bahamensis* produce copious amounts of fruit per inflorescence and they are held high in the trees. In contrast, *L. microphyllus* has only 1-3 fruits and they are typically held near the ground. This morphological difference suggests a more limited ability for dispersal by *L. microphyllus* and may help explain why *L. bahamensis* has migrated across the narrow strait between Cuba and the Bahamas. However, the distribution of *L. bahamensis* on Hispaniola raises other questions. The single collection of the species on the island was made by the great plant collector Erik Ekman in 1925 (located at S) near “Môle St. Nicholas.” Ekman’s notes for this specimen states “obs. only one poor individual found!” Ekman was familiar with the species, having collected broadly in Cuba, and clearly looked specifically for more plants of this species at the Haitian locality, without success. Môle St. Nicolas is a port on a bay and was a hub of commerce in the Caribbean from the late 15th century until its eventual decline in the late 1800’s (Barskett, 1818). The long history of commerce, combined with knowledge that only a single plant has ever been collected from this site, raises the question if this represents a record for a human-based introduction or for a truly native Hispaniolan species.

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Figure 1 Photographs of staminate inflorescences in the left column and pistillate in the right of species of the *Leucocroton* alliance. (a) *Garciadelia abbottii* in Los Haitises, Dominican Republic (photograph by Brett Jestrow). (b) *G. mejiae* in Sierra de Bahoruco, Dominican Republic (photograph by Francisco Jiménez). (c) *Lasiocroton microphyllus*, living collections of the National Botanical Garden of Cuba (photograph by Benjamin van Ee). (d) *L. bahamensis* in coastal Guantánamo, Cuba (photograph by Benjamin van Ee). (e) *Leucocroton pachyphyllus* in Minas Iberia, Holguín, Cuba (photograph by Benjamin van Ee). (f) *L. comosus* Sierra del Nipe, Holguín, Cuba (photograph by Benjamin van Ee).

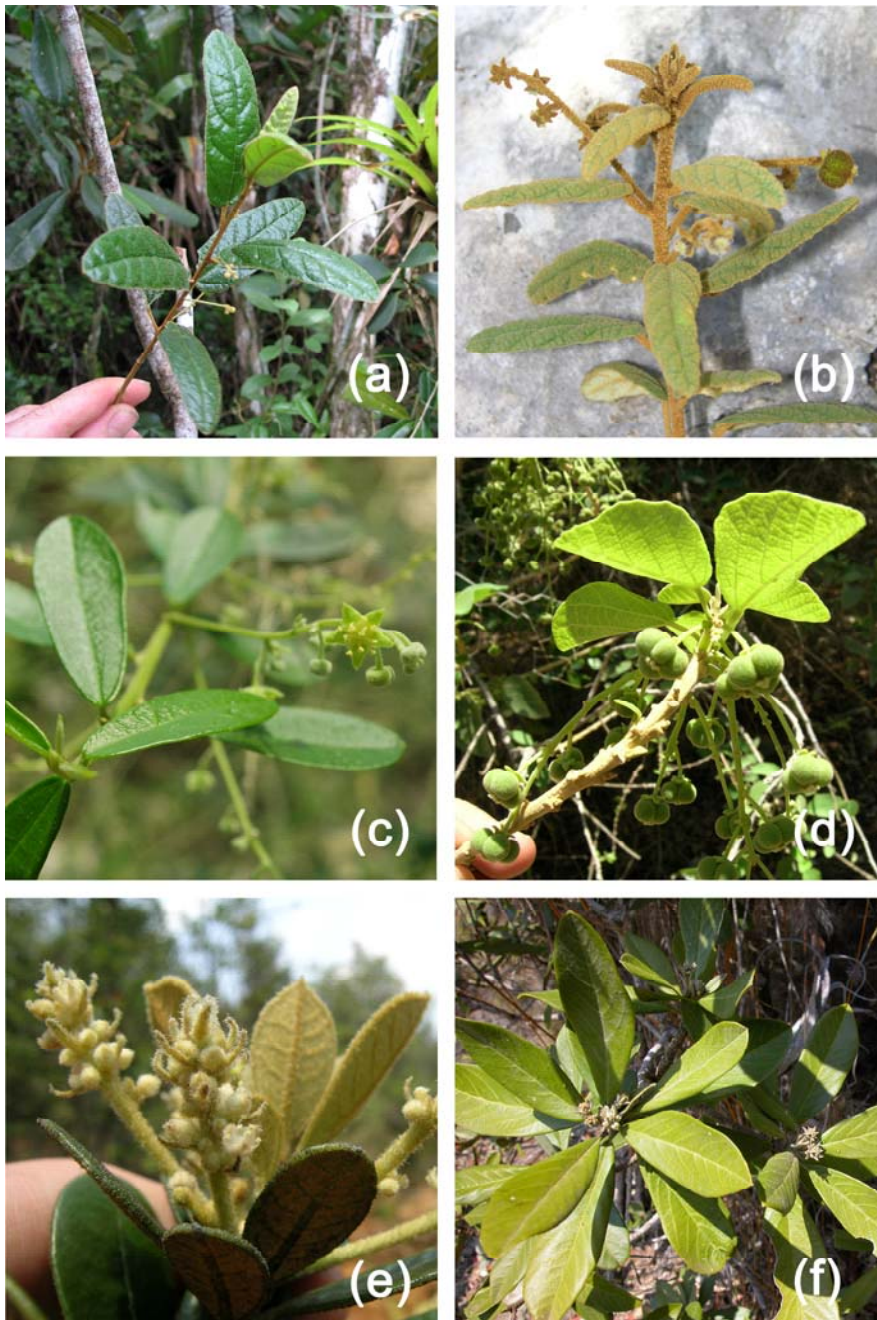


Figure 2 The nine major ultramafic massifs of Cuba showing the distribution of *Leucocroton*. A tenth small massif, Alto de la Corea, is not shown separate from the large Mayarí-Cristal massif which it borders. Species are listed for every massif where they have been recorded. Underlined species were not included in the molecular analyses. (adapted from Lewis et al., 2006 and Reeves *et al.*, 1999).

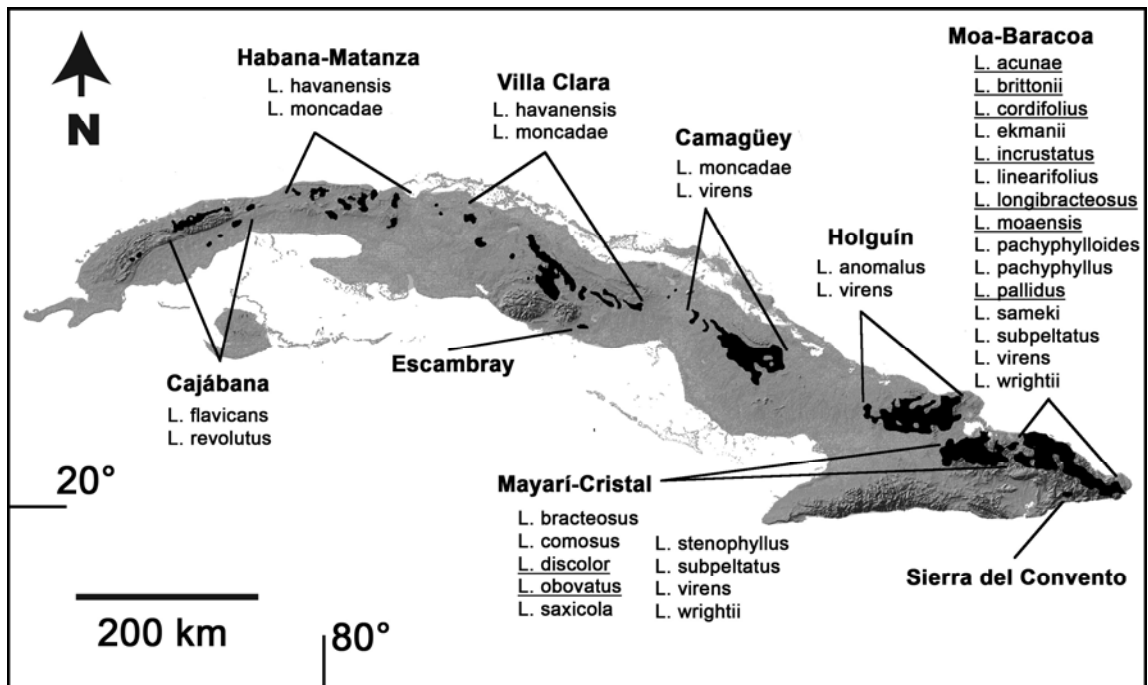


Figure 3 Geographical distribution of species of *Lasiocroton* and *Garciadelia*. (a) Cuba; (b) the Bahamas; (c) Jamaica; (d) Hispaniola. The ultramafic massifs for Hispaniola (adapted from (adapted from Draper & Barros, 1994, Draper *et al.*, 1996, and Lewis *et al.*, 2006) and Jamaica (adapted from Abbott *et al.*, 1999 and Lewis *et al.*, 2006) are labeled. Species coded as follows: (◇) *G. abbottii*, (□) *G. castilloae*, (Π) *G. leprosa*, (○) *G. mejiae*, (λ) *L. bahamensis*, (τ) *L. fawcettii*, (6) *L. gracilis*, (v) *L. gutierrezii*, (H) *L. harrisii*, and (σ) *L. microphyllus*. Where *L. bahamensis* and *L. microphyllus* occur together, the symbols have been overlapped.

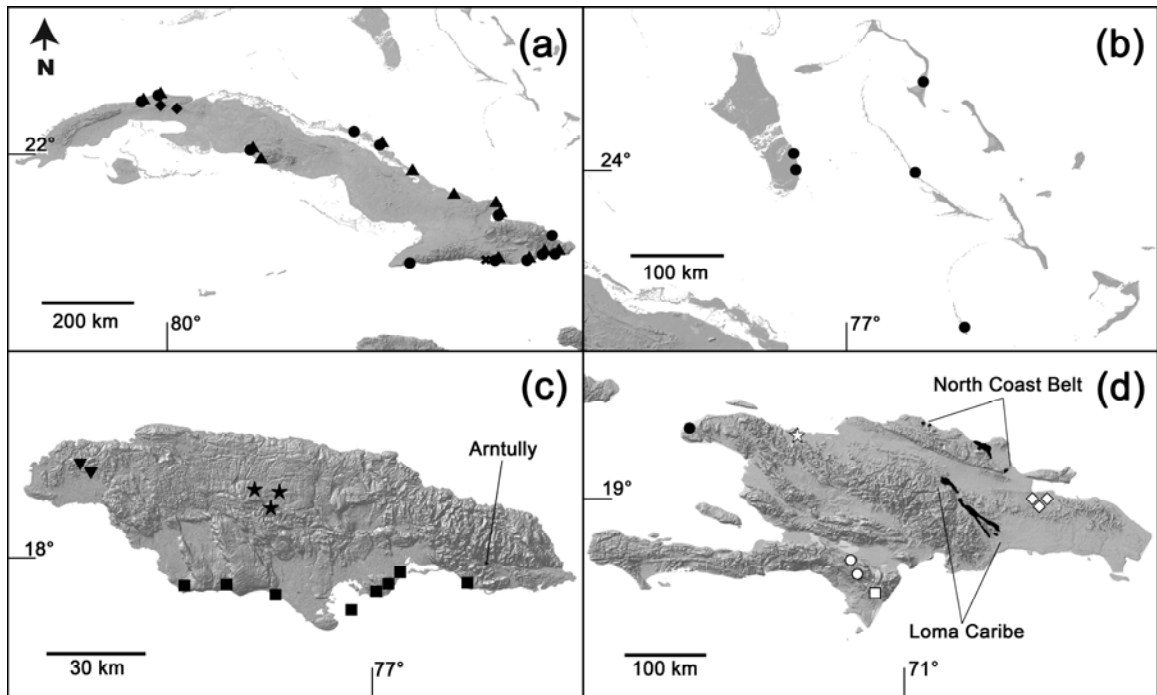
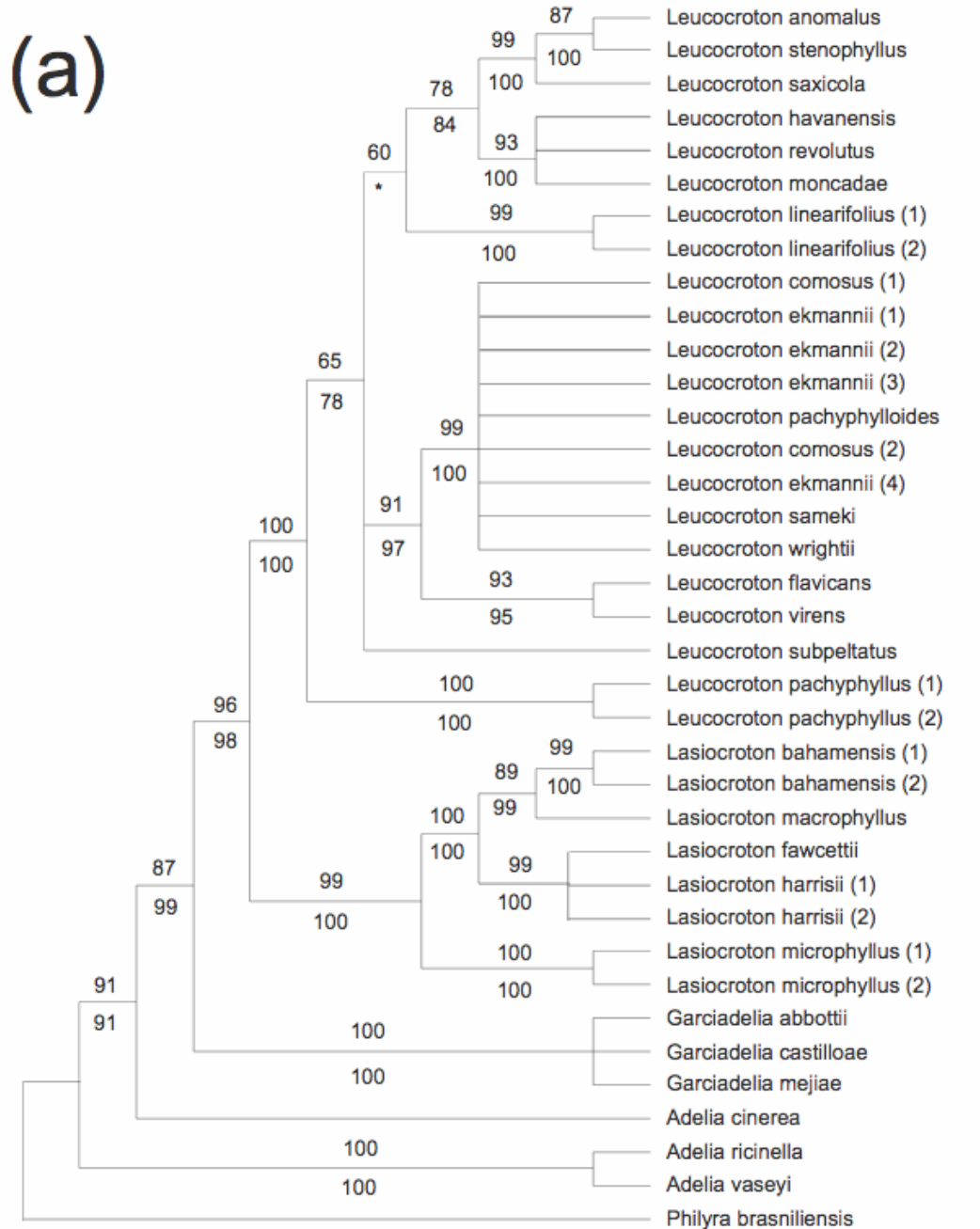


Figure 4 The strict consensus trees of the ITS and combined cpDNA datasets. Top topology, the ITS strict consensus tree of the 5880 most parsimonious trees. Bottom topology, the combined cpDNA strict consensus tree of the 6646 most parsimonious trees. Bootstrap values (>50%) from parsimony analysis are above branches, clade credibility values from Bayesian analysis are below branches. For both analyses, support values of less than 50% are denoted with an asterisk. The two conflicts between the Bayesian and parsimony topologies (cpDNA data set) are indicated with “#”.



(b)

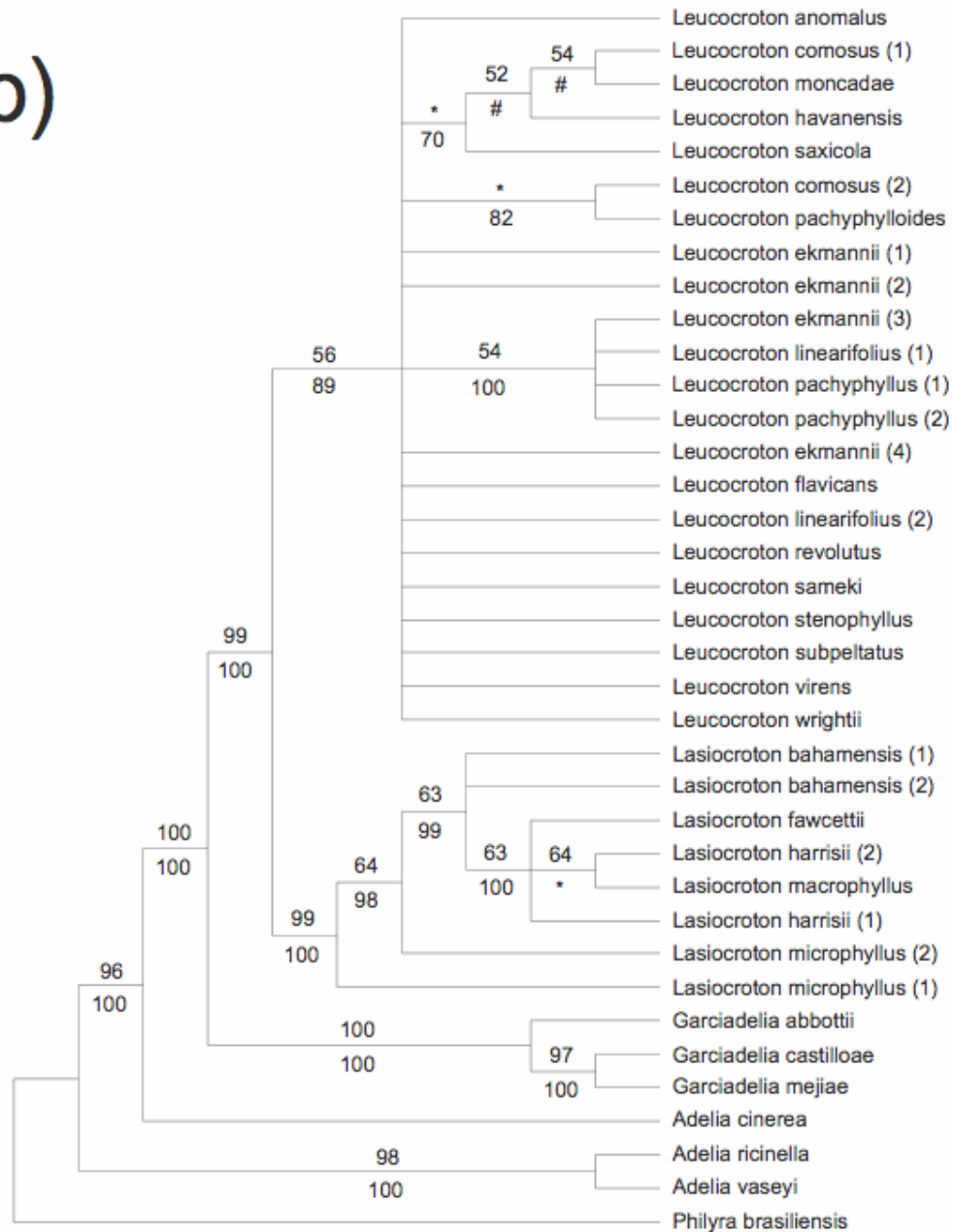


Figure 5 One of the twelve most parsimonious trees from the total combined ITS—cpDNA dataset. Bootstrap values (>50%) from parsimony analysis are above branches, clade credibility values from Bayesian analysis are below branches. Bootstrap values of less than 50% are denoted with an asterisk. The three conflicts between the Bayesian and parsimony topologies are indicated with “#”. Number of steps along each branch are indicated in italics. The branch that collapses in the strict consensus tree is indicated with an arrow.

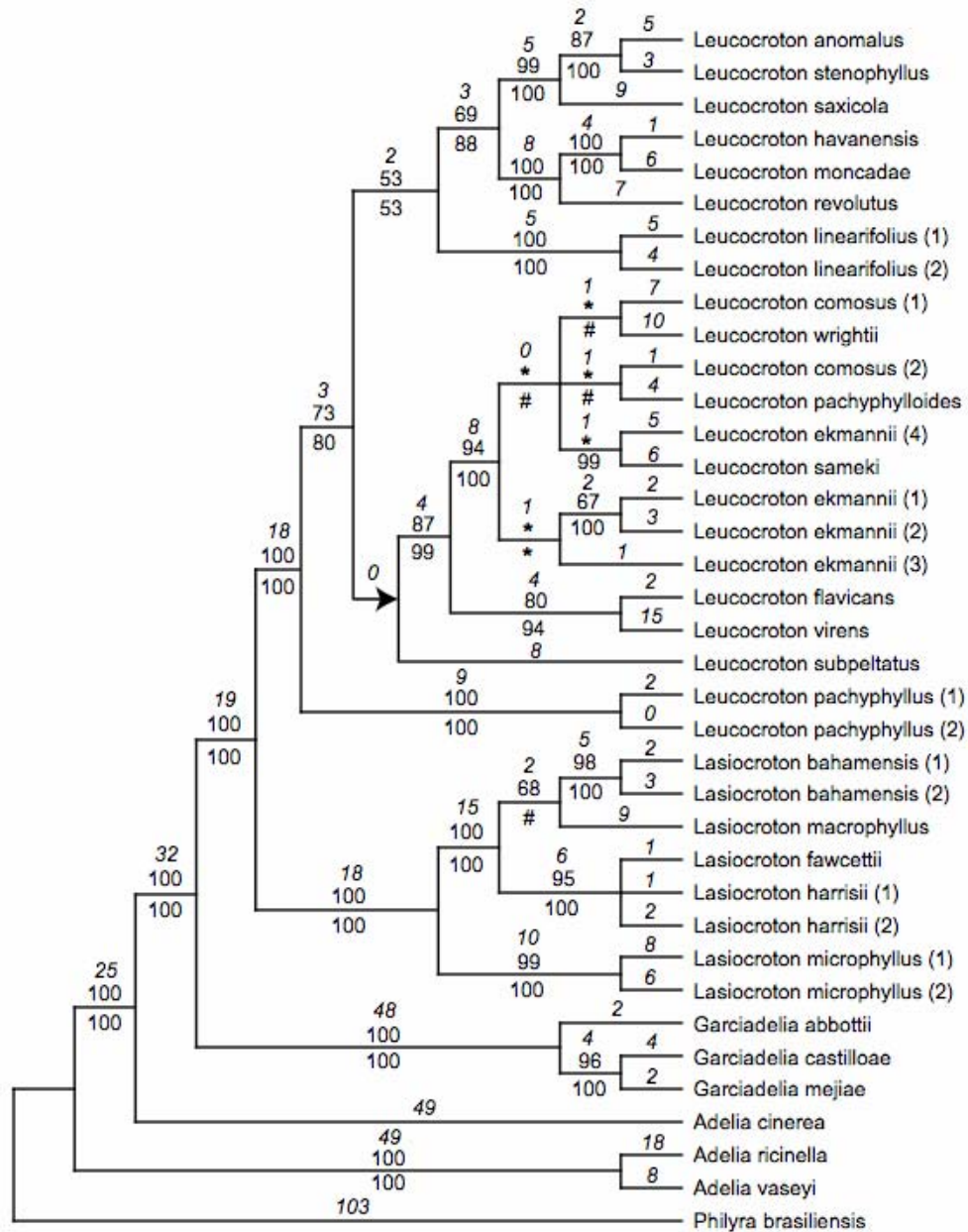


Figure 6 Reconstruction of ancestral areas using DIVA area analyses along topologies obtained after phylogenetic analyses of the ITS-cpDNA combined data set. Top topology, reconstruction along one of the most parsimonious trees yielded by parsimony. Bottom topology, reconstruction along one of the trees yielded by Bayesian inferences. Notice that the Bayesian tree only shows reconstructions along the *Lasiocroton* clade as the parsimony and Bayesian topologies were identical for the remaining branches.

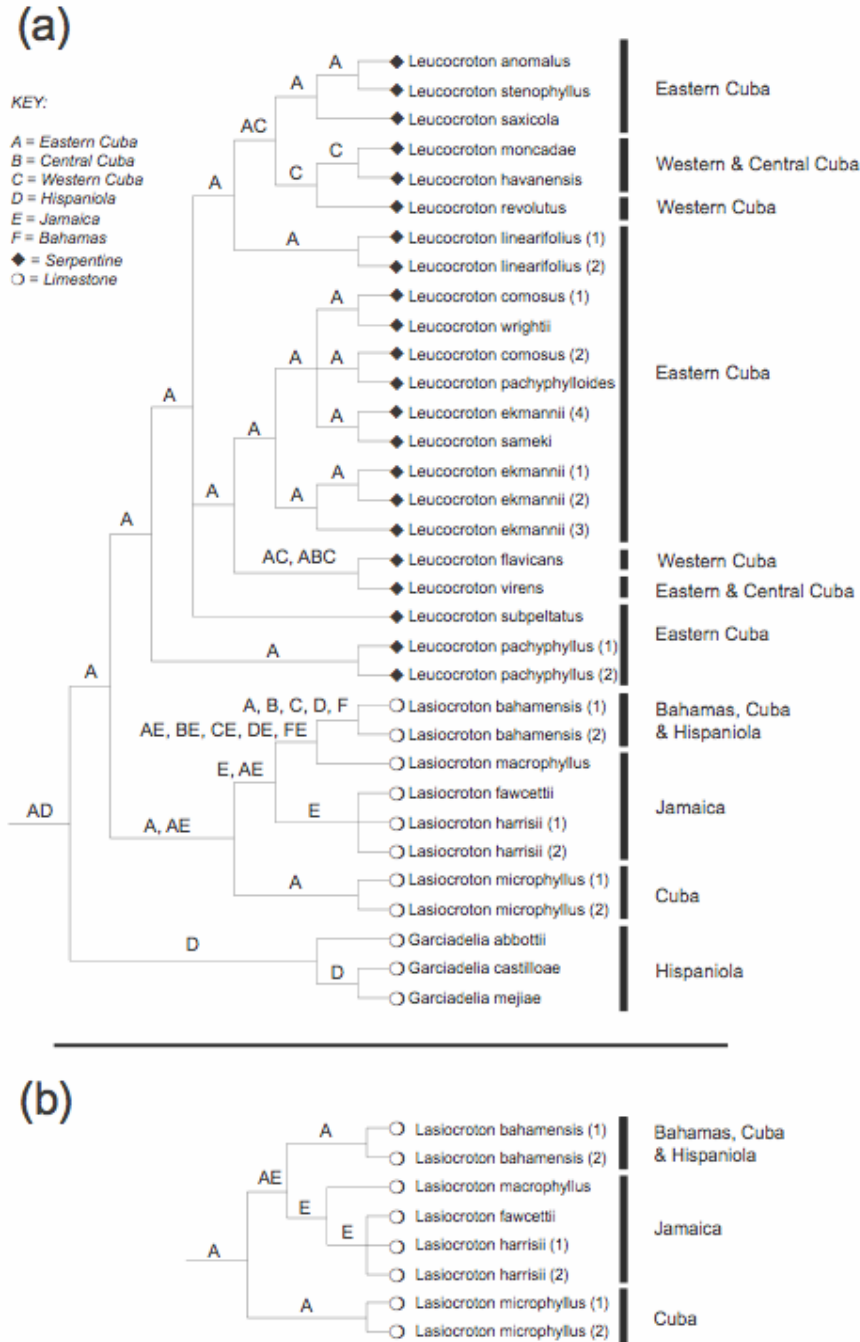


Table 1 Plant Collections: localities, collectors, and genbank numbers.

Species (reference #)	Collection Locality	Genetic Voucher	GenBank (ITS, <i>psbM-trnD</i> , <i>ycf6-pcbM</i>)
<i>Garciadelia abbottii</i> Jestrow & Jiménez Rodr.	Los Haitises, Hato Mayor, Hispaniola	Jiménez 3577 (FTG)	GU000002 GU000041, GU000055
<i>Garciadelia castilloae</i> Jestrow & Jiménez Rodr.	Sierra del Batoruco, Barahona, Hispaniola	Jestrow & Jiménez 1020 (FTG)	GU000008 GU000040, GU000053
<i>Garciadelia mejiae</i> Jestrow & Jiménez Rodr.	Sierra del Batoruco, Independencia, Hispaniola	Jestrow & Jiménez 1018 (FTG)	GU000012 GU000039, GU000054
<i>Lasiocroton bahamensis</i> Pax & K.Hoffm. (1)	South Andros, Bahamas	W. Gillis 10451 (<i>ex situ</i> FTBG)	GU000024, GU000032, GU000056
<i>Lasiocroton bahamensis</i> Pax & K.Hoffm. (2)	Punta Macambo, Guantánamo, Cuba	HABJ 81950 (FTG)	HM223459, HM223460, HM223461
<i>Lasiocroton fawcettii</i> Urb.	Dolphin Head, Hanover, Jamaica	Jestrow 1004 (FTG)	HM223462, HM223463, HM223464
<i>Lasiocroton harrisii</i> Britton (1)	Peckham Woods, Clarendon, Jamaica	Jestrow 1009 (FTG)	GU000018, GU000033, GU000057
<i>Lasiocroton harrisii</i> Britton (2)	Aboukir, St. Ann, Jamaica	Jestrow 1016 (FTG)	HM223465, HM223466, HM223467
<i>Lasiocroton macrophyllus</i> (Sw.) Griseb.	Hellshire Hills, St Catherine, Jamaica	Jestrow 1002 (FTG)	HM185116, HM185117, HM185118
<i>Lasiocroton microphyllus</i> (A.Rich.) Jestrow (1)	Baitiquiri, Guantánamo, Cuba	HABJ 81947 (FTG)	GU000017, GU000031, GU000058
<i>Lasiocroton microphyllus</i> (A.Rich.) Jestrow (2)	Playa Caletica, Holguín, Cuba	HABJ 81839 (FTG)	HM223468, HM223469, HM223470
<i>Leucocroton anomalus</i> Borhidi	Melones, Holguín, Cuba	HABJ 81831 (FTG)	HM223471, HM223472, HM223473
<i>Leucocroton comosus</i> Urb. (1)	Sierra de Nipe, Holguín, Cuba	Bennet 7608 (FTG)	HM223474, HM223475, HM223476
<i>Leucocroton comosus</i> Urb.(2)	Sierra de Nipe, Holguín, Cuba near the base of Mina Iberia,	HABJ 81722 (FTG)	GU000020, GU000035, GU000059
<i>Leucocroton ekmanii</i> Urb. (1)	Guantánamo, Cuba near the peak of Mina Iberia,	HABJ 81859 (FTG)	HM223477, HM223478, HM223479
<i>Leucocroton ekmanii</i> Urb. (2)	Guantánamo, Cuba	HABJ 81864 (FTG)	HM223480, HM223481, HM223482
<i>Leucocroton ekmanii</i> Urb. (3)	La Melba, Holguín, Cuba	HABJ 81794 FTG)	HM223483, HM223484, HM223485
<i>Leucocroton ekmanii</i> Urb. (4)	Baracoa, Guantánamo, Cuba	R. Oviedo 24/10/04 (HAC)	HM223486, HM223487, HM223488
<i>Leucocroton flavicans</i> Müll.Arg. in DC.	Cajalbana, Pinar del Río, Cuba	HABJ 81972 (FTG)	HM223489, HM223490, HM223491
<i>Leucocroton havanensis</i> Borhidi	Canasí, Matanzas, Cuba	HABJ 82009 (FTG)	HM223492, HM223493, HM223494
<i>Leucocroton linearifolius</i> Britton (1)	La Melba, Holguín, Cuba	HABJ 81793 (FTG)	HM223495, HM223496, HM223497

<i>Leucocroton linearifolius</i> Britton (2)	La Melba, Holguín, Cuba	HAJB 81807 (FTG)	GU000022, GU000038, GU000060
<i>Leucocroton moncadae</i> Borhidi	Loma de Coca, La Habana, Cuba	HAJB 84363 (HAJB)	GU000021, GU000036, GU000061
<i>Leucocroton pachyphylloides</i> Borhidi	La Melba, Holguín, Cuba	HAJB 81806 (FTG)	HM223498, HM223499, HM223500
<i>Leucocroton pachyphyllus</i> Urb. (1)	Mina Iberia, Guantánamo, Cuba	HAJB 81896 (FTG)	GU000019, GU000034, GU000062
<i>Leucocroton pachyphyllus</i> Urb. (2)	La Melba, Holguín, Cuba	HAJB 81808 (FTG)	HM223501, HM223502, HM223503
<i>Leucocroton revolutus</i> C.Wright in Sauvalle	Cajálbana, Pinar del Río, Cuba	HAJB 81968 (FTG)	HM223504, HM223505, HM223506
<i>Leucocroton sameki</i> Borhidi	Yamanigüey, Holguín, Cuba	HAJB 81781 (FTG)	HM223507, HM223508, HM223509
<i>Leucocroton saxicola</i> Britton	Sierra de Nipe, Holguín, Cuba	HAJB 81744 (FTG)	HM223510, HM223511, HM223512
<i>Leucocroton stenophyllus</i> Urb.	Sierra de Nipe, Holguín, Cuba	HAJB 81748 (FTG)	HM223513, HM223514, HM223515
<i>Leucocroton subpeltatus</i> (Urb.) Alain	Sierra de Nipe, Holguín, Cuba	Bennet 7609 (FTG)	HM223516, HM223517, HM223518
<i>Leucocroton virens</i> Griseb.	Baracoa, Guantánamo, Cuba	HAJB 81851 (FTG)	GU000025, GU000037, GU000063
<i>Leucocroton wrightii</i> Griseb.	Moa, Holguín, Cuba	Bennet 7590 (FTG)	HM185119, HM185120, HM185121
<hr/>			
<i>Adelia cinerea</i> (Wiggins & Rollins) A.Cerv., V.W.Steinm. & Flores Oliv.	Sonora, Mexico	Steinmann 971 (RSA)	GU000027, GU000042, GU000048
<i>Adelia ricinella</i> L.	Guantánamo, Cuba	HAJB 81949 (FTG)	GU000023, GU000045, GU000049
<i>Adelia vaseyi</i> (J.M.Coult.) Pax & K.Hoffm.	Tamaulipas, Mexico	De Nova 191 (XAL)	GU000026, GU000043, GU000050
<i>Philyra brasiliensis</i> Klotzsch.	Bahia, Brazil	Thomas <i>et al.</i> 12565 (NY)	GU000028, GU000044, GU000064

Table 2 Parsimony statistics of trees and characters.

DNA Region	AL	Total PI	LA PI	MPT	CI/RI/RC
ITS	678 (15)	135 (10)	109 (5)	5880 (397)	0.65/0.86/0.62
<i>psbM-trnD</i>	1070 (35)	23 (14)	12 (10)	9870 (102)	0.79/0.91/0.82
<i>ycf6-pcbM</i>	1204 (25)	35 (13)	27 (10)	236 (99)	0.74/0.88/0.73
Chloroplast	2274 (60)	58 (27)	39 (20)	6646 (211)	0.82/0.85/0.70
Total	2952 (75)	193 (37)	148 (25)	12 (624)	0.64/0.85/0.63

AL = alignment length (number of coded gaps); Total PI = total parsimony informative nucleotides (number of parsimony informative coded gaps); LA PI = parsimony informative nucleotides within the *Leucocroton* alliance (number of PI coded gaps); MPT = number of most parsimonious trees (tree score); CI/RI/RC = consistency index excluding uninformative characters, retention index, and rescaled consistency index

Table 3 Bremer's Ancestral Areas Analysis for the *Leucocroton* alliance clade according to both parsimony and Bayesian topologies. The asterisks denote the Bremer values with *Lasiocroton bahamensis* scored as absent from Hispaniola.

Parsimony				
	G	L	G/L	AA
Eastern Cuba	7	5	1.40	1.00
Central Cuba	4	10	0.40	0.29
Western Cuba	4	8	0.50	0.36
Hispaniola	2	4	0.50	0.36
Jamaica	2	4	0.50	0.36
Bahamas	1	5	0.20	0.10
Hispaniola*	1	1	1.00	0.50

Bayesian				
	G	L	G/L	AA
Eastern Cuba	7	4	1.75	1.00
Central Cuba	4	7	0.57	0.33
Western Cuba	4	7	0.57	0.33
Hispaniola	2	3	0.67	0.38
Jamaica	1	4	0.25	0.14
Bahamas	1	4	0.25	0.14
Hispaniola*	1	1	1.00	0.57

Table 4 The Bremer Ancestral Areas Analysis for the *Lasiocroton* clade according to both parsimony and bayesian topologies.

Parsimony				
	G	L	G/L	AA
Eastern Cuba	2	2	1	1
Central Cuba	2	2	1	1
Western Cuba	2	2	1	1
Hispaniola	1	3	0.33	0.33
Jamaica	2	2	1	1
Bahamas	1	3	0.33	0.33

Bayesian				
	G	L	G/L	AA
Eastern Cuba	2	1	2	1
Central Cuba	2	1	2	1
Western Cuba	2	1	2	1
Hispaniola	1	2	0.5	0.25
Jamaica	1	2	0.5	0.25
Bahamas	1	2	0.5	0.25

CHAPTER IV

CONCLUSION AND FUTURE RESEARCH DIRECTIONS

- Combined phylogenetic analyses of *trnL-F* and *rbcL* showed that the critically endangered Jamaican endemic, *Lasiocroton trelawniensis* (Euphorbiaceae; Adelieae) belongs to the genus *Bernardia*. Pollen SEM and macromorphological characters further support the finding. *Bernardia trelawniensis* (C.D. Adams) Jestrow & G.R. Proctor is a new taxonomic combination, placing the species in a different tribe. The new placement explains many of the previously misunderstood morphological characters (e.g., denticulate margins), which suggested that the species underwent significant evolutionary change. The placement in *Bernardia* now shows a species closely related the type of the genus, *B. dichotoma*, a taxon widely distributed across the Greater Antilles. My investigation further found that *Bernardia dichotoma* poses a possible species complex including, not only *B. trelawniensis* but, other varieties and synonyms from varying elevations of different islands. Further phylogenetic and population genetic studies are required to unravel the relationships of these Caribbean *Bernardia*.
- Members of the Adelieae, along with outgroups from neighboring tribes, were included in phylogenetic analyses of both chloroplast (i.e., *psbM-trnD* and *ycf6-pcbM*) and nuclear (i.e., ITS) regions. This showed that the Caribbean genera, *Lasiocroton* and *Leucocroton* were not monophyletic in respect to each other because of two species of *Leucocroton*. One of these was moved to *Lasiocroton* as *Lasiocroton microphyllus*, a taxonomic change further supported by

morphological characters (e.g. thick pistillate disk). The other species was found to represent ancestral lineage before the dichotomization of the two genera. This species, formally recognized as *Leucocroton leprosus*, was described as the basis of the new genus, *Garciadelia*. The separation of these two species from *Leucocroton* now defines the genus as a nickel hyperaccumulators, endemic to the serpentine areas of Cuba. As no other members of the tribe either occur on serpentine or hyperaccumulate nickel, this genus provides an example of an isolated evolutionary development towards heavy metal tolerance. As genetic methods improve, the identification of this radiation provides an ideal project into the physiology of heavy metal tolerance.

- The genus *Garciadelia* was published based on the aforementioned species, *Garciadelia leprosa*. This formed, together with *Lasiocroton* and *Leucocroton*, a monophyletic assemblage of three genera endemic to the Caribbean Islands, now known as the *Leucocroton*-alliance. Furthermore three new species of differing morphologies and from disparate localities were described. The genus is endemic to Hispaniola, and all species were classified according to the IUCN guidelines. The possibility of gene flow between these species is not supported by the phylogeny. As all species of the genus are represented by few individuals, the level of inbreeding depression is a concern. The genus represents an ideal subject of population genetics for critically endangered taxa of the Caribbean.
- The species *Lasiocroton gutierrezii* was described from two historical collections previously identified as *Lasiocroton microphyllus*. Unlike *L. microphyllus*, the new species is found on an upland habitat and is differentiable both by sterile

(e.g., lack of stem spines) and fertile (e.g., many flowered pistillate inflorescence) structures. Publishing this species bring attention to the field botanists of Cuba, in order to recollect this presumably rare species. If found, the two closely related species would be an ideal subject both for a common garden experiment and a genetic investigation to determine the genetic and phenotypic differences between these taxonomic entities.

- The historical biogeography of the *Leucocroton*-alliance was studied based on phylogenetic analyses of the same gene regions, though with a more rigorous sampling of taxa. Bremer and DIVA algorithms were used to reconstruct the ancestral areas. The alliance arose on Eastern Cuba and Hispaniola, with *Garciadelia* separating from the *Lasiocroton*-*Leucocroton* clade as a vicariant event. This suggests a time of divergence of ~15 Ma, though a later dispersal is still a possibility. Fossils are unknown within the alliance, however future studies of the Dominican amber lagerstätte could provide relevant pollen specimens for the dating of nodes.
- *Lasiocroton* and *Leucocroton* arose on Eastern Cuba. *Lasiocroton* dispersed across Cuba and also eventually to the Bahamas and Jamaica. The low elevation species of Jamaica, *L. macrophyllus*, represents a link between those of upland Jamaican and those of the Bahamas and Cuba. Within *Leucocroton*, two separate clades of the genus have representatives in Western Cuba. This gives evidence of two westward migrations across the serpentine islands of Cuba. While my phylogeny gave no evidence of hybridization, the species boundaries of the genus

require further investigation. This is could be approached through both population genetics, as well as a detailed field study of extant populations.

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PUBLICATIONS

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