Phylogenetics of the Antillean Goetzeoideae (Solanaceae) and Their Relationships within the Solanaceae based on Chloroplast and ITS DNA Sequence Data

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Abstract. *Coeloneurum, Espadaea, Henoonia,* and *Goetzea* are shrubs and trees that are endemic to the islands of Cuba, Hispaniola, and Puerto Rico in the Greater Antilles. A phylogenetic analysis was conducted to elucidate the evolutionary relationships among them and with other major lineages of the Solanaceae. DNA sequences of the chloroplast genes *ndhF, rbcL,* and *trnL-trnF* intron and intergenic spacer were obtained for twenty two taxa and sequences of the nuclear rDNA ITS region were obtained for eight taxa comprising a data set of over 5,000 bp. The inferred phylogeny groups the Antillean genera together with the South American *Metternichia* and *Duckeodendron* in a clade within the Solanaceae, pointing to a broader circumscription of the Goetzeoideae. Both chloroplast and nuclear datasets find the following relationships among the Antillean taxa: (*Coeloneurum* (*Henoonia* (*Espadaea, Goetzea*))). The South American genera *Metternichia* and *Duckeodendron* are the first and second sister groups, respectively, to the Antillean genera. The close relationship of *Metternichia* to the Antillean genera also is supported by pollen morphology. Phylogenetic inference suggests that the Antillean taxa first occupied xeric environments and evolved into more mesic habitats. Floral characteristics indicate evolution of pollination systems from nocturnal, insect-pollination in *Duckeodendron* and *Metternichia* to diurnal, bird-pollination in the Antillean genera. *Duckeodendron* and the Antillean genera produce drupes, but their contrasting morphology and anatomy suggest that these fruit types originated from separate evolutionary events.

Coeloneurum Radlk., *Espadaea* A. Rich., *Goetzea* Wydler, and *Henoonia* Griseb. are tropical plant genera distributed in three of the four islands of the Greater Antilles, the archipelago composed of the islands of Cuba, Hispaniola, Jamaica, and Puerto Rico. The Hispaniolan genus *Coeloneurum* and the Cuban genera *Espadaea* and *Henoonia* are monotypic, whereas *Goetzea* comprises two species, one endemic to Hispaniola, and the other endemic to Puerto Rico. None of the species in these genera occurs in Jamaica. The systematic placement of these taxa (in this work referred to as "the Antillean genera") has been problematic since the first member of the group was described by Wydler (1830). Wydler initially placed *G. elegans* in the Ebenaceae. *Espadaea* was subsequently described by Richard (1850) in the Verbenaceae, and *Henoonia* was named by Grisebach (1866) in the Sapotaceae. Miers (1869) was the first author to suggest a placement for the Antillean genera in their own tribe or family.

Subsequent studies brought these four Antillean genera into or near the Solanaceae. A study by Radlkofer (1888) on the vegetative anatomy of *Henoonia* found that the presence of crystal sand and intraxylary phloem, and the lack of lactifers suggested that they belong to the Solanaceae. Wettstein (1895) included the Antillean genera in the subtribe Goetzeinae of tribe Cestreae in the Solanaceae. D'Arcy and Keating (1973) studied the leaves of *Goetzea* and other members of the Solanaceae and argued for the validity of the Goetzeaceae. A study by Gentry (1986) on

the pollen morphology of selected genera to elucidate tribal and generic relationships of the Cestreae (sensu Wettstein 1895) found differences between the pollen of *Goetzea* and *Coeloneurum* and the other solanaceous genera examined, leading Gentry to conclude that the Antillean genera "clearly do not belong in the Solanaceae." Further, Hunziker (1979) suggested the validity of the Goetzeaceae as a satellite of the Solanaceae, a hypothesis supported by Carlquist (1988) on the basis of wood anatomy. Studies on the leaf anatomy of the Antillean genera (Zona 1989; Vales and Fuentes 1990) found a set of features that together suggest a strong solanaceous affinity. These are the presence of glandular trichomes and uniseriate trichomes with long terminal cells, sinuous anticlinal epidermal walls, crystal sand and druses, and intraxylary phloem.

The application of molecular data has allowed new ways to tackle the systematic questions of these four Antillean genera. In a phylogenetic analysis of the Solanaceae (Olmstead et al. 1999), trees based on DNA sequences of the chloroplast genes *ndhF* and *rbcL* place *Goetzea* (the only one of the Antillean genera included in the analysis) as one of the earliest emerging lineages in the Solanaceae, and not belonging to tribe Cestreae. On the basis of this relationship, Olmstead et al. (1999) proposed the placement of all the Antillean genera under the new subfamily Goetzeoideae. None of the other taxa of the Solanaceae included in the analysis came out as sister to *Goetzea*,

TABLE 1. Taxa included in this study. Subfamilial classification follows Olmstead et al. (1999), modified to include *Duckeodendron* and *Metternichia* in Goetzeoideae. BIRM refers to the University of Birmingham Solanaceae seed collection.

Subfamily Cestroideae *Browallia speciosa* Hook. BIRM S.0416 (*Olmstead S-7* WTU) *rbcL* AY206719, *ndhF* AY206739, *trnL/F* AY206753; *Cestrum nocturnum* L. Matthaei Botanical Garden #21314 (No voucher) *rbcL* AY206721, *ndhF* AY206741, *trnL/F* AY206755, *trnL/F* AY206723, ITS AY206731; *Salpiglossis sinuata* Ruíz & Pav. BIRM S.0181 (*R. Olmstead S-71* WTU) *rbcL* U08618, *ndhF* U08928, *trnL/F* AY206765; *Sessea corymbiflora* Goudot ex Taylor et Phillips Venezuela (*Benítez de Rojas 5373* MY) *ndhF* AY206750, *trnL/F* AY206768; *Vestia lycioides* Willd. BIRM S.0105 (*BIRM S.0105* BIRM) *ndhF* AY206751, *trnL/F* AY206769
Subfamily Goetzeoideae *Coeloneurum ferrugineum* Radlk. Hispaniola (Dominican Republic) (*Santiago 93-201* MAPR) *ndhF* AY206742, *trnL/F* AY206756, AY206724, ITS AY206732; *Duckeodendron cestroides* Kuhlm. Brazil (*E. Ribeiro 1189* K) *rbcL* Y14760, *ndhF* AY206743, *trnL/F* AY206757, AY206725, ITS AY2206733; *Espadaea amoena* A. Rich. Cuba (*Santiago 93-202* UPR, WTU) *rbcL* AY206722, *ndhF* AY206744, *trnL/F* AY206758, AY206726, ITS AY206734; *Goetzea ekmanii* O.E. Schulz Hispaniola (Dominican Republic) (*Santiago 96-2a* WTU) *ndhF* AY206745, *trnL/F* AY206759, AY206727, ITS AY206735; *Goetzea elegans* Wydler Puerto Rico (*Santiago 89-6* MAPR, WTU) *rbcL* AF035738, *ndhF* AY206746, *trnL/F* AF206760, ITS AY206736; *Henoonia myrtifolia* Griseb. Cuba (*Santiago 96-15* WTU) *ndhF* AY206747, *trnL/F* AY206761, AY206728, ITS AY206737; *Metternichia princeps* Mik. Brazil (*Schnoor 88* RB, MO) *rbcL* AF022182, *ndhF* AY206748, *trnL/F* AY206763, AY206729, ITS AY206738
Subfamily Nicotianoidea *Nicotiana tabacum* L. Matthaei Botanical Garden (No voucher) *rbcL* Z00044, *ndhF* L14953, *trnL/F* Z00044; *Anthocercis viscosa* R. Br. Australia (*Symon 14835* AD) *rbcL* U08608, *ndhF* U08914, *trnL/F* AY206752
Subfamily Petunioideae *Petunia axillaris* (Lam.) B.S.P. BIRM S.0367 (*R. Olmstead S-60* WTU) *rbcL* X04976, *ndhF* U08926, *trnL/F* AY098702; *Brunfelsia americana* L. Matthaei Botanical Garden #840215 (No voucher) *rbcL* AY206720, *ndhF* AY206740, *trnL/F* AY206754
Subfamily Schizanthoideae *Schizanthus pinnatus* Ruíz & Pav. BIRM S.0224 (*R. Olmstead S-72* WTU) *rbcL* U08619, *ndhF* U08929, *trnL/F* AY206766
Subfamily Schwenckioideae *Schwenckia lateriflora* (Vahl) Carvalho Venezuela (*Benítez de Rojas 3901* MO) *rbcL* AF035739, *ndhF* AY206749, *trnL/F* AY206767
Subfamily Solanoideae *Solanum lycopersicum* L. Michigan USA (cult.) (No voucher) *rbcL* L14403, *ndhF* U08921, *trnL/F* AY098703; *Lycium cestroides* Schltdl. BIRM S.0368 (*R. Olmstead S-34* WTU) *rbcL* U08613, *ndhF* U08920, *trnL/F* AB036578, AB036607
Outgroups *Ipomoea coccinea* Rottl. Beal Botanical Garden (*R. Olmstead 88-015* WTU) *rbcL* L14400, *ndhF* U08918, *trnL/F* AY206762; *Montinia caryophyllacea* Thunb. South Africa (*Williams 2833* MO) *rbcL* L11194, *ndhF* AF130178, *trnL/F* AY206764

making the authors infer that subfamily Goetzeoideae comprised only the four Greater Antillean genera. In further phylogenetic analysis of *rbcL* sequence data (Fay et al. 1998), *Goetzea* was sister to the Brazilian monotypic genus *Metternichia* Mikan (Solanaceae). This lineage was one node apart from the node containing the lineage of the Brazilian monotypic *Duckeodendron* Kuhlmann. The placements of both *Metternichia* and *Duckeodendron* have also been problematic. *Metternichia* was first described by Mikan in 1823 in the Convolvulaceae. Meisner (1836–1843) placed it in the Bignoniaceae (Carvalho 1986). It was placed in tribe Metternichieae in the Solanaceae by Miers (1846) and, more recently, by Hunziker (1979) and D'Arcy (1991) in tribe Cestreae. The genus *Duckeodendron* was originally described in the Solanaceae, but was transferred to the Boraginaceae on the basis of structural characteristics of the fruit (Kuhlmann 1930). Studies of wood anatomy led Record (1933) to propose its inclusion in the Apocynaceae. It was later placed in the monotypic family Duckeodendraceae (Kuhlmann 1947), and most recently back in the Solanaceae (Fay et al. 1998). The Antillean genera and *Duckeodendron* have drupaceous fruit, not found elsewhere in the Solanaceae, where other members develop either capsules or berries. The difference in the fruit type has been considered as a major feature for the proposition of familial status of these groups (Miers 1869; Kuhlmann 1947; Hunziker 1979). Given the complex taxonomic history of this group and uniqueness of mor-

phological features, further molecular studies are needed to determine if the Antillean genera are a monophyletic group and to determine the relationship between them and *Metternichia* and *Duckeodendron*. In addition to clarifying evolutionary relationships, a phylogeny will help to interpret patterns of morphological evolution and biogeographic history of the taxa mentioned above, in relation to the rest of the Solanaceae.

MATERIALS AND METHODS

Taxonomic Sampling. This study was designed to test of the monophyly of the Antillean Genera, *Metternichia*, and *Duckeodendron*, by focusing on subfamilial lineages near the base of the family. Twenty-two taxa were included (Table 1), representing all subfamilies (sensu Olmstead et al. 1999) of Solanaceae and all members of the Goetzeoideae. Complete sequences for *trnL-trnF* and *ndhF* were obtained for all 22, whereas *rbcL* was missing for five taxa. The taxa missing *rbcL* sequences represent three Antillean species of Goetzeoideae and two species of Cestreae, where *rbcL* sequences are not expected to vary substantially. One representative each of Convolvulaceae (*Ipomoea*) and Montiniaceae (*Montinia*) were selected as outgroups on the basis of previous results (Olmstead et al. 1999, 2000) that suggest these families are the closest to the Solanaceae. The Antillean genera together with *Metternichia*, *Duckeodendron* and *Cestrum* L. were sequenced for ITS to increase resolution within Goetzeoideae.

DNA Isolation and Sequencing. DNA was obtained from fresh leaves and silica gel-dried leaves with the CTAB extraction procedure of Doyle and Doyle (1987). Some samples used for sequencing were from the same source as that of Olmstead et al. (1999). Sequences of *Duckeodendron cestroides* were obtained from the same DNA sample of Fay et al. (1998). All regions were amplified by using the polymerase chain reaction

(PCR) in a mix of 10 mM Tris-HCl, pH8.3, 50 mM KCl, 3 mM MgCl$_2$, 2.5 mM dNTP, 0.0125 units of *Taq* polymerase, 0.05 mM of each primer, and 1–5 μl of total DNA template in 50 μl of reaction. The chloroplast gene *ndhF* was amplified in two overlapping parts with primer pairs 5' and 1318R and, 972F and 3' (Olmstead and Sweere 1994). Amplification conditions were: 92°C for 1 min, 45°C for 1 min, and 72°C for 1.5 min over 35 cycles. The gene *rbcL* was amplified using standard protocols (Olmstead et al. 1992). Amplification conditions were: 92°C for 3 min, followed by 35 cycles at 92°C for 1 min, and 50°C for 45 sec, and 72° for 90 sec. The region including both the *trnL* intron and *trnL-trnF* spacer was amplified with primers c, d, e, f (Taberlet et al. 1991) in the following conditions: 92°C for 1 min, 50°C for 1 min, 72°C for 2 min over 35 cycles. The 5.8S nrDNA and flanking ITS regions were amplified with primers ITS-N18L18 (Hershkovitz and Zimmer 1996), ITS3, ITS4, ITS5 (White et al. 1990) under the following conditions: initial step of 93°C for 3 min, then 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min over 35 cycles. The purified PCR products were sequenced with the cycle sequencing procedure by using the ABI PRISM Dye Terminator Cycle Sequencing (Perkin Elmer ABI, Foster City, CA). Both strands were obtained for all accessions sequenced, except for ITS in *Coeloneurum ferrugineum*. Sequences were edited with the computer program Sequencher, version 3.0 (Gene Codes Corporation, Ann Arbor MI).

Phylogenetic Analysis. Edited sequences were aligned with the DNA sequence alignment program Clustal W (Thompson et al. 1994), after which they were visually inspected and adjusted if needed by using the manual alignment editor Se-Al (A. Rambaut, University of Oxford). Parsimony analyses were carried out for each individual data set with PAUP* version 4.0b10 (Swofford 2002). Parsimony analyses were performed by using the heuristic search method with 100 replicates using random addition sequence starting trees, TBR branch swapping, and MULTREES. All nucleotide substitutions were weighted equally (Olmstead et al. 1998) and shared alignment gaps were scored as separate binary characters. We conducted an incongruence length difference test (ILD) (Farris et al. 1994) to test whether the various chloroplast sequence regions differ significantly from random partitions of the combined data set (implemented in PAUP*). Both chloroplast and ITS datasets were bootstrapped with 1,000 replicates by using TBR swapping.

RESULTS

Molecular Data. The combined chloroplast data comprise 4,748 bases of aligned sequence that contain 450 parsimony-informative characters. Missing data, not counting the missing *rbcL* sequences, represent 1.4% of the total matrix. Sequences of *ndhF* ranged from 2,095 to 2,111 nucleotides (nt) long (positions 24 to 2,114 in tobacco). The aligned sequence was 2,140 nt and required six gaps in the alignment, all but one of which were unique to individual taxa. One gap of six nt was shared only by *Metternichia* and the Antillean genera. Sequences of the gene *ndhF* provided 617 variable characters, of which 261 are parsimony-informative (55.8% of all parsimony informative characters in the cpDNA dataset). Sequences of *rbcL* were 1,408 nt (positions 27 to 1,434 in tobacco) and required no alignment gaps. The gene *rbcL* provided 100 parsimony-informative characters (21.4% of cpDNA characters) along with 145 variable but uninformative characters. Sequences of the *trnL* intron and *trnL-trnF* spacer range from 815 to 999 nt long; the aligned sequence was 1,200 nt

and required numerous gaps for alignment, of which 18 were parsimony-informative and were included as binary characters. The boundaries of the sequence strand include the last five bases of the 5' portion of the *trnL* exon, the entire 3' *trnL* exon (50 nt), and the first 41 nt of the 5' end of *trnF*. *trnL* intron sequences ranged from 341 to 533 nt long; those of the *trnL-trnF* spacer ranged from 282 to 432 nt long. A total of 52 sites in three sections of the *trnL-trnF* region were excluded from analysis because the alignment was ambiguous. The *trnL-trnF* region provides 89 parsimony-informative nucleotide sites plus the 18 gap characters for a total of 107 characters (22.9% of cpDNA characters).

Boundaries of the internal transcribed spacers and the nrDNA coding region in the Goetzeoideae and in *Duckeodendron* and *Cestrum* were identified by comparison with those of tomato, *Solanum lycopersicum* L. (Kiss et al. 1988). The sequences obtained ranged from 693 to 703 nucleotides long. The aligned sequence was 713 nt, requiring 18 gaps. The 5.8 nrDNA was 160 nucleotides. ITS1 sequences ranged from 258 to 266 nt; the ITS2 ranged from 207 to 210 nucleotides. We were unable to sequence ITS1 of *Coeloneurum* and ITS2 of *Duckeodendron*. Missing data, excluding missing regions of *Coeloneurum* and *Duckeodendron* account for 1.1% of the total matrix. The ITS region contained 145 variable characters, 40 (5.6% of the total) of which were informative.

Phylogenetic Analyses. The ILD test indicated that three chloroplast DNA partitions are not significantly different (P = 0.42), so only combined cpDNA analyses are reported here. The combined chloroplast DNA analysis yielded three most-parsimonious trees of 1,805 steps (CI = 0.791; RI = 0.715). The results of the combined analysis (Fig. 1) identified strongly supported clades (100% bootstrap support) comprising the Antillean genera and the Antillean genera plus *Metternichia*. The combined analysis provides moderate support (78%) for the Goetzeoideae as a clade, including *Duckeodendron*. Although the combined chloroplast DNA sequence data resolved critical nodes in the phylogeny, the >4,700 nucleotides provided only 31 parsimony-informative sites in the Goetzeoideae and the results provide only weak support with respect to species-level relationships among the Antillean taxa.

Analysis of the ITS region yielded a single most parsimonious tree of 179 steps (CI = 0.950; RI = 0.816; Fig. 2). The tree is topologically identical to one of the three cpDNA trees (depicted in Fig. 1) and is fully resolved with bootstrap values over 70% for each node. The analysis of all data combined for the seven ingroup taxa with *Cestrum* as the outgroup yielded a single tree of 570 steps (CI = 0.958; RI =
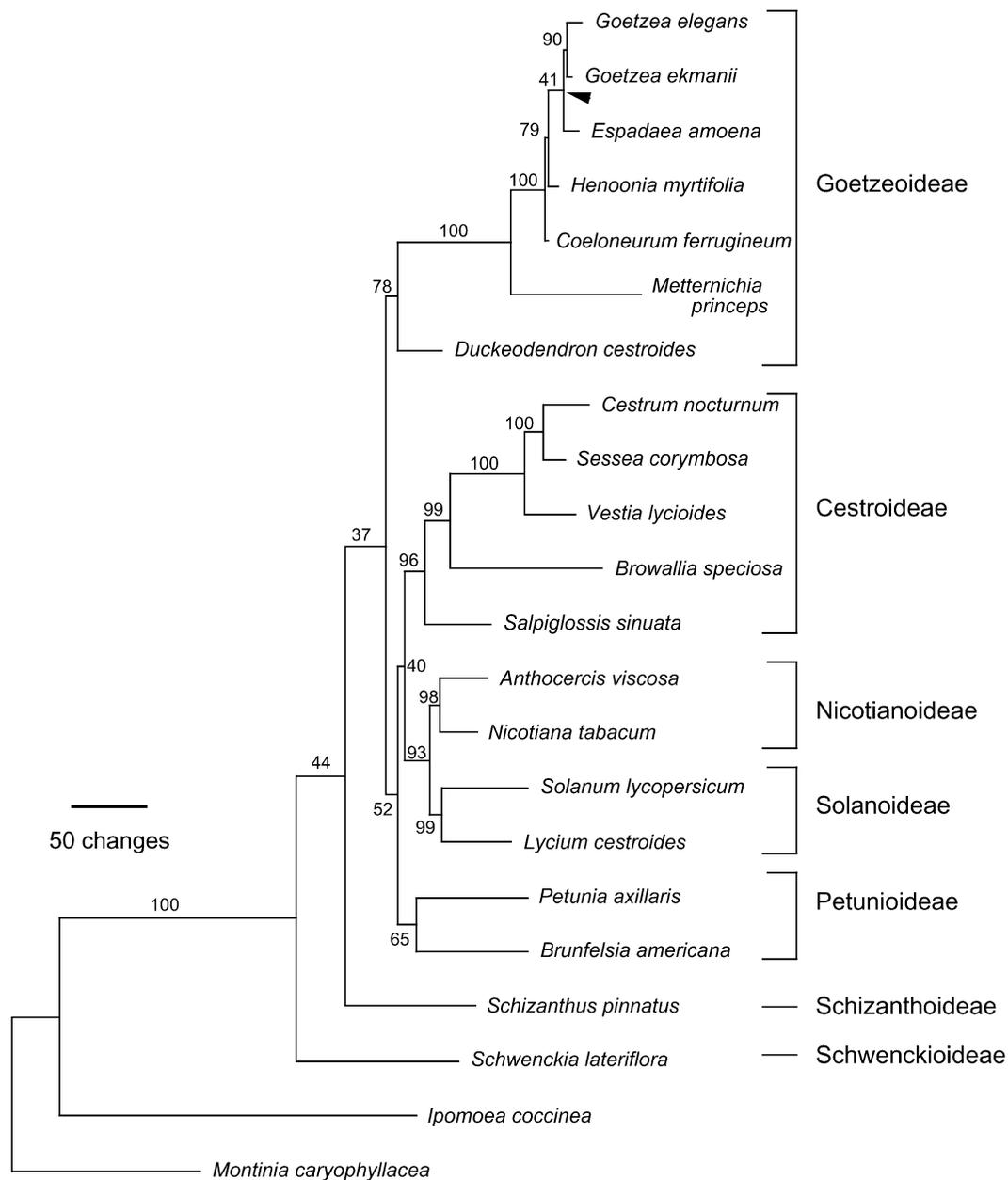
Fɪɢ. 1. One of three most-parsimonious trees based on the chloroplast DNA sequence data drawn with branch lengths proportional to inferred change in DNA sequences (scale provided to left). Arrow indicates the single internode that collapses in the strict consensus of the three trees. Bootstrap values are indicated for each clade. Subfamilial classification according to Olmstead et al. (1999).

0.872) with bootstrap values 85% or higher for all nodes (Fig. 2).

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Phylogeny and Systematics. Our chloroplast DNA and nuclear ITS sequence data support a clade comprising the Antillean genera *Coeloneurum, Espadaea,*

Henoonia, and *Goetzea,* and also support the more inclusive clade of the Antillean genera plus *Metternichia* and *Duckeodendron,* thus identifying the closest continental relatives to the Antillean genera. These clades were recovered in the combined analysis of the chloroplast regions, even though it is unresolved in all of the individual data sets (results not shown), probably due to the paucity of informative characters. Increasing
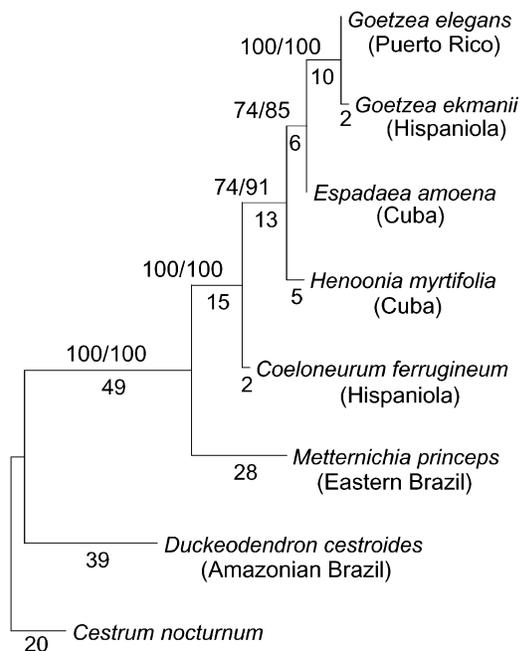
FIG. 2. The single most-parsimonious tree based on ITS sequences. Topology is identical to the cpDNA tree. Branch lengths based on ITS sequences indicated below the branches; bootstrap values for ITS and the combined ITS and chloroplast sequences indicated above the branches.

the number of characters can reveal a consistent phylogenetic signal that may be hidden by the noise in each individual data set (Mishler 1994; Olmstead and Sweere 1994). Although heterogeneity present in the data sets may influence phylogenetic reconstruction and combining data might be misleading (de Queiroz et al. 1995), the strong congruence of the data sets, as indicated by the non-significant ILD test result, suggests this is not the case here.

Goetzeoideae (sensu Olmstead et al. 1999), here expanded to include *Metternichia* and *Duckeodendron*, constitute one of the most early-diverging lineages within the family. Given the strong affinity found in all our analyses between *Metternichia* and the Antillean genera, we propose to transfer *Metternichia* from tribe Cestreae to subfamily Goetzeoideae. Based on *rbcL* data alone, Fay et al. (1998) inferred a placement for *Goetzea* and *Metternichia* as sister group to the rest of the Solanaceae and inferred *Duckeodendron* to be the next diverging lineage, but without strong support. Their placement of Goetzeoideae at the base of the family is at odds with other studies based on more data (Olmstead et al. 1999) and with the placement found here. Our complete sampling of the Goetzeoideae and the inclusion of much more informative sequence data from *ndhF* and *trnL-trnF* suggests that their results suffered from inadequate sampling of taxa and characters

(although perfectly adequate to their goal of determining whether *Duckeodendron* belongs in the Solanaceae).

Morphological Evolution. Our results allow assessment of the morphological evolution of the Goetzeoideae. A reexamination of Gentry's (1986) study on pollen morphology supports our results placing *Metternichia* close to the Antillean genera. Gentry found that the pollen of *Metternichia* was different from that of *Cestrum* and *Vestia*, the two other representatives of the Cestreae in his study. The pollen of *Cestrum* and *Vestia* is tricolporate with a smooth exine, whereas that of *Metternichia* is tricolpate with an echinate exine sculpturing and perforate tectum. Furthermore, the pollen of *Coeloneurum* and *Goetzea* are tricolpate with echinate exine sculpturing and perforate tectum. These features were considered remarkably different from the rest of the Solanaceae, the only exception being *Metternichia*, which shared those general characteristics and looked "similar to *Goetzea*" (Gentry 1986). Although the morphological distinctiveness of the pollen of the Antillean genera was offered as evidence for the validity of the Goetzeaceae, no conclusion or inference was drawn that the palynological similarities between the Antillean genera and *Metternichia* were the product of close relationship or homology. The genus *Tsoala* Bosser & D'Arcy from Madagascar has been reported to have pollen similar to *Metternichia* (Bosser et al. 1992). *Tsoala* has not been collected in forty years (D'Arcy 1992, pers. comm.). This genus should be included in future phylogenetic studies as material becomes available.

In the Goetzeoideae, *Duckeodendron* and the Antillean genera are characterized by drupaceous fruit, but their structure appears to contrast morphologically. The drupe of *Duckeodendron* is pyriform with an orange exocarp and a peculiar thick fibrous mesocarp (Fay et al. 1998). The fruits of the Antillean members of Goetzeoideae have a fleshy, smaller, usually round to pyriform, shiny orange drupe, but these lack the thick fibrous covering found in the drupe of *Duckeodendron*. *Metternichia* is the only member of the Goetzeoideae that produces dry capsular fruits, as in the earliest diverging solanaceous genera *Schizanthus* and *Schwenckia* and most of the Convolvulaceae. Assuming that *Duckeodendron* is the first split in the Goetzeoideae and that the Antillean genera are derived, it could be inferred that the species in the ancestral lineage that gave rise to the Goetzeoideae produced dry capsules, and that drupes evolved independently in *Duckeodendron* and in the common ancestor of the Antillean genera. Anatomical and morphological studies comparing the drupe of *Duckeodendron* to that of the Antillean genera are needed.

Our results indicate that *Espadaea* is sister to *Goetzea*, these two genera are sister to *Henoonia*, and *Coeloneurum* is sister to the other three Antillean genera. These

relationships are consistent with the two groups into which the Antillean Goetzeoideae can be divided on the basis of morphology. One group includes *Goetzea* and *Espadaea*, which exhibit flowers with long peduncles, conspicuous funnel-shaped corollas, and broad leaf blades. These two genera grow in mesic conditions (Santiago-Valentín 1995). *Henoonia* and *Coeloneurum*, in contrast, have nearly sessile flowers, with a much smaller corolla that is deeply lobed nearly to the base, have narrow, spinescent leaves, and occur in extremely dry habitats. *Duckeodendron* is a tall rainforest tree of the central Amazon and *Metternichia* occurs in east and northeast Brazil, where it grows in dry regions (Carvalho 1986). This distrubution suggests that the ancestor of the Antillean genera occured in dry habitats and, by inference, evolved to occupy more mesic habitats in the ancestor of *Goetzea* and *Espadaea*. Zona (1989) described adaptation to arid environments in *Henoonia*, such as thick cuticle, inrolled leaf margin, and vascular bundle surrounded by fibrous bundle sheaths. Although *Coeloneurum* was not included in the anatomical studies by Zona, its morphological and ecological similarity to *Henoonia* suggests the presence of such adaptations as well (Zona 1989). *Espadaea* and *Goetzea* exhibit less adaptation to dry environments. In *Goetzea*, for instance, the thin leaf lamina, thin cuticle and higher frequency of stomata all indicate that it does not exhibit strong adaptations to aridity. *Metternichia* does not exhibit the suite of traits described by Zona (1989), suggesting that the early Antillean members evolved these traits as they adapted to more xeric habitats than their ancestors in Brazil occuppied.

Additional interpretations of morphological and ecological characteristics can be made for the Goetzeoideae. All the genera of the Goetzeoideae are woody, a derived characteristic in the Solanaceae (Olmstead and Palmer 1992). Flowers have also evolved to play a role in more than one pollination syndrome. The flowers of *Duckeodendron* are tubular, with a whitish green corolla, open at night, and have a rather heavy, deeprose odor (M. Hopkins, pers. comm.). The flowers of *Metternichia* are lightly fragrant, with a white or rose funnel-shaped corolla. Both *Duckeodendron* and *Metternichia* are very likely pollinated by night-flying moths. In contrast, the flowers of the Antillean genera are bright orange, have no fragrance, and usually open at dawn (E. S.-V., pers. obs.). Birds play an important role in plant interactions in the Antilles today, both as flower visitors, and as seed dispersers. Hummingbirds and honey creepers are very likely the natural pollinators of *Goetzea elegans* (Santiago-Valentin 1995). Unidentified passerine birds have also been seen visiting flowers of *Espadaea amoena* (E. S.-V., pers. obs.). It is possible that the shift in the pollination syndrome of the ancestral lineage of the Antillean Goetzeoideae from entomophily to ornithophily was promoted at least in part

because of the absence of appropriate insect pollinators in the islands. In addition, adopting more xeric habitats in the early diversification on the Antilles (*Coeloneurum* and *Henoonia*) may have promoted bird visitation by selecting against the extremely long corollas observed in the continental taxa of the Goetzeoideae. Bird interaction must have also increased with the appearance of yellow-orange color in the corollas. Fleshy fruits, on the other hand seem counterintuitive for plants belonging to a plant family where dry capsules are predominant, and for a lineage that apparently was initially adapted to dry situations. In these dry-habitat taxa (*Henoonia* and *Coeloneurum*), however, fruits are much smaller than in the mesic-habitat taxa (*Espadaea*, *Goetzea*). Here, selection also may have favored fleshy fruits because of the effectiveness of bird dispersal.

Biogeographic Assessment. Our phylogenetic reconstructions allow several explicit biogeographical interpretations of the Antillean Goetzeoideae. First, the closest extant relatives of the Antillean genera are South American, with most of the diversity and most of the early diverging lineages found there (Olmstead and Palmer 1992; Olmstead et al. 1999). The Goetzeoideae represent an early-diverging lineage in the family and, with the extant continental taxa found only in Brazil, the inference is strong that they originated in that part of South America and colonized the Antilles from there. Second, the monophyly of the Antillean genera implies that their radiation began after the common ancestor of these taxa appeared somewhere in the islands. Third, the change in pollination syndrome (from entomophily in the continental taxa to ornithophily in the Antillean taxa) and fruit morphology (presence of drupes in all the Antillean taxa) must have occurred before lineage radiation. Fourth, the ancestral stock originated in land portions of what is now Cuba or Hispaniola. Fifth, the Puerto Rican taxon (*Goetzea elegans*) is derived from a Hispaniolan ancestor. An important assumption to these interpretations is that no lineage extinction has occurred in the Goetzeoideae, and that the Antillean genera have existed only in the same islands they do today. The few species present today and the relatively low levels of genetic divergence among them suggest that speciation has been rare, thus the assumption of few extinction events is reasonable.

There is no fossil evidence of the Solanaceae in the Greater Antilles that establish the minimum time of appearance of this group in the islands (Graham, pers. comm.). However, because radiation and diversification of many angiosperm lineages began before the appearance of the Greater Antilles (Gentry 1982), many plant lineages might have arrived once the first land blocks of the islands formed, even before the isthmian connection of North and South America (Lavin

and Luckow 1993). One of these early arrivals could have been the lineage that gave rise to the Antillean Goetzeoideae. The interpretation of the age of the lineage, however, needs to be tested with further evidence that permits its timing, but without a fossil record, a molecular clock cannot be calibrated reliably. Thus, we present biogeographic interpretations considering both a scenario of an early arrival to the islands of the Antillean genera and the alternative scenario that they appeared once the Antilles acquired a configuration similar to that observed today.

In the Greater Antilles, all of the species are endemic to single islands, and all the major islands except Jamaica have at least one species. *Goetzea*, with one species on Puerto Rico and one on Hispaniola, is the only genus with species on more than one island. Both Cuba and Hispaniola have two endemic species of Goetzeoideae, but in neither case are the taxa occurring on one island sister species. The broad-leaved, mesic taxa are found on all three islands (*Espadaea* on Cuba and *Goetzea* on Hispaniola and Puerto Rico) and form a clade, whereas the xeric taxa are found on Hispaniola (*Coeloneurum*) and Cuba (*Henoonia*) and form a basal grade in the group.

Under an ''early-appearance'' scenario, the stock that resulted into the Antillean genera could have originated sometime between Late Creatceous and Early Tertiary, when only portions of the Greater Antilles were present in what is now the Caribbean region (Malfait and Dinkelman 1972; Pindell and Barrett 1990). At that time, the major land areas were land blocks belonging to the Greater Antillean islands that today are occupied by members of the Goetzeoideae: Eastern Cuba, North-Central Hispaniola, and Puerto Rico. These areas were very close to each other or possibly connected (Iturralde-Vinent 1994). During that period, Jamaica, Southwestern Hispaniola, and the Lesser Antilles were not present or were in early stages of formation (Pindell and Barrett 1990; Huebeck and Mann 1991). This difference in timing may explain why the Antillean genera are present only in Cuba, Hispaniola, and Puerto Rico, and not in Jamaica or the Lesser Antillean arc. If we consider the early appearance scenario and that the phylogeny reflects the result of strict vicariance, a minimum of two events of land separations and one land coalescence between Hispaniolan and Cuban areas need to be invoked. An early divergence of a land block of what is today part of Hispaniola and Cuba originated the lineage of *Coeloneurum* (in Hispaniola), and the lineage that leads towards *Henoonia* and *Espadaea* (in Cuba). Given that the lineages of the Cuban *Henoonia* and *Espadaea* are placed in the phylogeny in contiguous nodes, their origin can be interpreted as either the result of a second land split (that later fused back to become again part of Cuba), or as arising within the same land block (without land

splitting). Under either situation, an additional subsequent vicariant event of a Cuban land block must be invoked to explain the split of the sister lineages *Espadaea* (Cuba) and *Goetzea* (Hispaniola). The land comprising what would be the lineage of *Goetzea* must have coalesced to other Hispaniolan land blocks (including that with *Coeloneurum*, which was already formed). A last land disruption that separated Hispaniola and Puerto Rico would have given rise to the two species of *Goetzea*. Further approaches to test this vicariant hypothesis would require synchronizing the timing of lineage divergence in the phylogeny with a more refined understanding of the origin and development of the island land blocks, especially those related to the formation of Cuba and Hispaniola. The early-appearance scenario can also be assessed under dispersal mechanisms (see next paragraph) as well as the result of a mix of vicariance and dispersal. More data will be needed to decide between these mechanisms.

A ''recent-appearance'' scenario must only consider that radiation of the Antillean genera occurred via over-water dispersal. The ancestor of the Antillean genera could have been established first on either Cuba or Hispaniola with equal parsimony. If it originated first on Cuba, two dispersal events must be postulated to explain the Hispaniolan taxa (*Coeloneurum* and *Goetzea*). If it originated first on Hispaniola, either two dispersal events must be postulated to Cuba (one each for *Henoonia* and *Espadaea*), or one to Cuba (xeric-adapted ancestor of *Henoonia*) followed by one back to Hispaniola (*Goetzea*), probably after the evolution in Cuba of the ancestor of *Espadaea* and *Goetzea* in a more mesic habitat. The morphological and genetic similarity between the two species of *Goetzea* indicate a recent divergence and would suggest that Puerto Rico is the most recently colonized island (following a stepping stone model of island colonization). The recent colonization of Puerto Rico by over-water dispersal from Hispaniola probably makes the most sense, regardless of whether other distributions derive from vicariance or dispersal events.

The Antillean genera represent a case in which morphological and geographic diversification has proceeded to a degree sufficient for classical taxonomists to recognize most elements at the level of genus, yet divergence at the DNA sequence level remains small. This pattern is similar to many others found in oceanic island groups (Baldwin et al. 1998), but these results represent one of the first well documented cases among Antillean plant groups.

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