

# MOLECULAR SYSTEMATICS OF THE CATESBAEEAE-CHIOCOCCEAE COMPLEX (RUBIACEAE): FLOWER AND FRUIT EVOLUTION AND BIOGEOGRAPHIC IMPLICATIONS<sup>1</sup>

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The classification of the Catesbaeeae and Chiococceae tribes, along with that of the entire Rubiaceae, has long been debated. The Catesbaeeae-Chiococceae complex (CCC) includes approximately 28 genera and 190 species primarily concentrated in the Greater Antilles (nearly 70% of the species), Central and South America, and in the western Pacific (three genera). Previous molecular studies, with broad sampling of the Rubiaceae, have shown the CCC to be a monophyletic group. The present study is a more detailed examination of the generic relationships within the CCC using two data sets, the nuclear ribosomal ITS regions and the *trnL-F* chloroplast intron and spacer. Maximum parsimony analyses lend further support to the previous hypotheses that the CCC is monophyletic and sister to *Strumpfia maritima*. However, within the complex several genera do not form monophyletic groups. Previous studies of the Rubiaceae suggest that the ancestral fruit type in the CCC is a multiseeded capsule. Indehiscent, fleshy fruits appear to have evolved three to four times within this lineage. Changes in floral morphologies within the complex tend to correspond to cladogenesis among and within genera. Finally, molecular analyses suggest one or possibly two long-distance dispersals from the Americas to the western Pacific.

**Key words:** biogeography; Caribbean; Catesbaeeae; Chiococceae; flower evolution; fruit evolution; islands; ITS; Neotropics; Pacific; Rubiaceae; systematics; *trnL-F*.

The Rubiaceae is one of the largest and most diverse families of flowering plants, with approximately 650 genera and 13 000 species (Delprete, 2004), mostly of pantropical distribution. Historically fruit and seed characters have been used to infer the classification of the family and in several cases to define subfamilies (de Candolle, 1830; Hooker, 1873; Schumann, 1891). The subfamilial and tribal classification went through several minor modifications until Bremekamp (1966), using many additional characters, divided the family into eight

subfamilies and 43 tribes. The last comprehensive system of classification was proposed by Robbrecht (1988, 1993), in which he recognized four subfamilies and 44 tribes. Recent phylogenetic evidence based on results of molecular studies with broad sampling throughout the family have revealed that the Rubiaceae should be best treated as three subfamilies—Cinchonoideae, Ixoroideae, and Rubioideae (e.g., *rbcL*, Bremer et al., 1995; *rps16*, Andersson and Rova, 1999, *rbcL* and *ndhF*, Bremer et al., 1999; *trnL-F*, Rova et al., 2002). In addition, it has been demonstrated that fleshiness of the mesocarp, placentation, and ovule number are variable within monophyletic groups at the tribal level and above (Bremer, 1992; Delprete, 1996; Bremer and Manen, 2000).

Historically, the tribes Catesbaeeae, Condamineae, and Chiococceae have been variously circumscribed. Hooker (1873) divided the Rubiaceae into three series: Series A, species with many ovules per locule; Series B, species with two ovules per locule; and Series C, species with a single ovule per locule. Following these criteria, he placed the tribes Condamineae and Catesbaeeae in Series A, the tribe Chiococceae in Series C, and further divided the Condamineae on the basis of fruit types into the subtribes Condamineae, Pinckneyinae, and Portlandiinae.

Verdcourt (1958) treated the tribe Condamineae sensu Hooker (1873) as a subtribe of the Rondeletieae, without recognizing any further subdivisions, and placed the Rondeletieae with the Chiococceae and the Catesbaeeae, among other tribes, in the subfamily Cinchonoideae. Robbrecht (1988) moved the Chiococceae to the subfamily Antirrhoeoideae, the Condamineae as defined by Hooker (1873) in the subfamily Cinchonoideae, and treated the Catesbaeeae as *tribus incertae sedis* (for a more complete discussion of the taxonomic history see Delprete, 1996).

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TABLE 1. Genera of the Catesbaeeae-Chiococceae complex (CCC), with approximate number of species per genus, flower type, fruit type, and approximate number of species per geographic region. Symbols used in the table: FL = Flower types: C = *Chiococca* type, E = *Exostema* type, and P = *Portlandia* type; FR = Fruit types: B = baccate, C = capsular, and D = drupaceous; Geographic regions: B = The Bahamas, C = Cuba, CA = Central America, F = Florida, H = Hispaniola, J = Jamaica, M = Mexico, NC = New Caledonia, P = Puerto Rico, SA = South America, and WP = Western Pacific (except New Caledonia).

| Genera                          | Species no. | FL  | FR | F | B  | C  | H  | J  | P  | M  | CA | SA | WP | NC    |
|---------------------------------|-------------|-----|----|---|----|----|----|----|----|----|----|----|----|-------|
| <i>Asemnantha</i> Hook f.       | 1           | C   | D  | — | —  | —  | —  | —  | —  | 1  | 1  | —  | —  | —     |
| <i>Badusa</i> A. Gray           | 3           | E   | C  | — | —  | —  | —  | —  | —  | —  | —  | —  | 3  | —     |
| <i>Bikkia</i> Reinw.            | 20          | P   | C  | — | —  | —  | —  | —  | —  | —  | —  | —  | 12 | 9     |
| <i>Catesbaea</i> L.             | 16          | P/C | B  | 1 | 3  | 7  | 5  | 1  | 2  | —  | —  | —  | —  | —     |
| <i>Ceratopyxis</i> Hook f.      | 1           | P   | C  | — | —  | 1  | —  | —  | —  | —  | —  | —  | —  | —     |
| <i>Ceuthocarpus</i> A. Aiello   | 1           | P   | C  | — | —  | 1  | —  | —  | —  | —  | —  | —  | —  | —     |
| <i>Chiococca</i> P. Br.         | 20          | C   | D  | 2 | 3  | 2  | 2  | 2  | 3  | 4  | 10 | 6  | —  | —     |
| <i>Coutaportia</i> Urb.         | 2           | P   | C  | — | —  | —  | —  | —  | —  | 2  | —  | —  | —  | —     |
| <i>Coutarea</i> Aubl.           | 2           | P   | C  | — | —  | —  | —  | —  | —  | 1  | 1  | 2  | —  | —     |
| <i>Cubanola</i> A. Aiello       | 2           | P   | C  | — | —  | 1  | 1  | —  | —  | —  | —  | —  | —  | —     |
| <i>Erithalis</i> P. Br.         | 6           | C   | D  | 1 | 3  | 2  | 3  | 3  | 1  | —  | —  | 1  | —  | —     |
| <i>Exostema sensu</i> Borhidi   | 13          | E   | C  | 1 | 1  | 7  | 6  | 3  | 3  | 3  | 1  | 3  | —  | —     |
| <i>Hintonia</i> Bullock         | 3           | P   | C  | — | —  | —  | —  | —  | —  | 3  | 3  | —  | —  | —     |
| <i>Isidorea</i> A. Rich.        | 17          | P   | C  | — | —  | 10 | 8  | —  | —  | —  | —  | —  | —  | —     |
| <i>Lorencea</i> Borhidi         | 1           | P   | C  | — | —  | —  | —  | —  | —  | —  | 1  | —  | —  | —     |
| <i>Morierina</i> Vieill.        | 1 (2)       | E   | C  | — | —  | —  | —  | —  | —  | —  | —  | —  | —  | 1 (2) |
| <i>Nernstia</i> Urb.            | 1           | P   | C  | — | —  | —  | —  | —  | —  | 1  | 1  | —  | —  | —     |
| <i>Osa</i> A. Aiello            | 1           | P   | C  | — | —  | —  | —  | —  | —  | —  | 1  | —  | —  | —     |
| <i>Phialanthus</i> Griseb.      | 18          | C   | D  | — | 1  | 14 | 1  | 3  | 1  | —  | —  | —  | —  | —     |
| <i>Phyllacanthus</i> Hook       | 1           | C   | B  | — | —  | 1  | —  | —  | —  | —  | —  | —  | —  | —     |
| <i>Portlandia</i> P. Br.        | 7           | P   | C  | — | —  | —  | —  | 7  | —  | —  | —  | —  | —  | —     |
| <i>Salzmannia</i> DC            | 1           | C   | D  | — | —  | —  | —  | —  | —  | —  | —  | 1  | —  | —     |
| <i>Schmidtottia</i> Urb.        | 16          | P   | C  | — | —  | 16 | —  | —  | —  | —  | —  | —  | —  | —     |
| <i>Scolosanthus</i> Vahl        | 20          | C   | D  | — | 1  | 17 | 7  | 1  | 2  | —  | —  | —  | —  | —     |
| <i>Shaferocharis</i> Urb.       | 3           | C   | D  | — | —  | 3  | —  | —  | —  | —  | —  | —  | —  | —     |
| <i>Siemensia</i> Urb.           | 1           | P   | C  | — | —  | 1  | —  | —  | —  | —  | —  | —  | —  | —     |
| <i>Solenandra sensu</i> Borhidi | 12          | E   | C  | — | —  | 11 | 2  | —  | —  | 1  | 1  | —  | —  | —     |
| <i>Thogsennia</i> A. Aiello     | 1           | P   | D  | — | —  | 1  | —  | —  | —  | —  | —  | —  | —  | —     |
| Total no. of species/region     | 191         |     |    | 5 | 12 | 95 | 35 | 20 | 12 | 16 | 20 | 13 | 15 | 10    |

Based on phylogenetic analyses using molecular and morphological data Bremer (1992) transferred the subtribe Portlandiinae, characterized by multiseeded capsules, into the tribe Chiococceae, characterized by two-seeded, drupaceous fruits. Subsequently, Delprete (1996), using a morphology-based phylogeny, included the Portlandiinae in the Catesbaeeae, placed the Chiococceae as the sister tribe and created an informal *Exostema* group (including *Badusa*, *Exostema*, and *Morierina*). In the same work, he merged the two remaining subtribes of Hooker of the Condamineae into the Rondeletieae. Phylogenetic studies in the Rubiaceae using *rbcl* (Bremer et al., 1995), *rps16* (Andersson and Rova, 1999), and *trnL-F* (Rova et al., 2002) have corroborated some of the ideas presented by Delprete's (1996) tribal circumscription, but contradicted his circumscription of the tribes Catesbaeeae and Chiococceae, and instead revealed that the two groups are intermixed in a single monophyletic lineage.

However, in each of the molecular studies of Catesbaeeae and Chiococceae, the relationships among genera were not fully resolved and the generic sampling was incomplete. As a result, the monophyly of most genera included in these tribes has never been tested, and delimitations of several genera are still debated. Aiello (1979), in her treatment of the *Portlandia* complex, segregated several genera from *Portlandia*, reducing it to a Jamaican endemic genus. The separation of *Cubanola*, *Nernstia* (= *Cigarilla* A. Aiello), *Osa*, and *Thogsennia* from *Portlandia* was based principally on fruit and seed characters, and has been supported by recent morphological studies (Ochoterena, 2000), but has not yet been tested with molecular

characters. Molecular studies have been conducted on two genera in the tribes, *Exostema* s.l. (McDowell and Bremer, 1998; McDowell et al., 2003) and *Erithalis* (Negrón-Ortiz and Watson, 2002), but the relationships of these genera within the context of the entire tribe are unresolved.

Based on recent systematic studies, the Catesbaeeae-Chiococceae Complex (CCC) encompasses 28 genera and about 190 species (Table 1; Borhidi and Acuña, 1971; Jérémie and Hallé, 1976; Borhidi and Muñiz, 1975; Borhidi et al., 1977; Borhidi, 1980, 2002, 2003; Ridsdale, 1982; Aiello and Borhidi, 1986; Lorence, 1986; Villareal, 1987; Delprete and Nee, 1997; Huysmans et al., 1999).

The species of the CCC occupy a very intriguing geographic distribution. The center of diversity is in the Greater Antilles where 135 species, or about 70% of the CCC diversity occurs. Thirty species are found in Mexico and Central America, of which six species are endemic, nine widespread, and a few ranging into South America. Twenty-five species are endemic to the Western Pacific (Table 1; Fig. 1). The eastern limit of the Pacific taxa is along the edge of the Pacific Plate, which corresponds approximately to the western boundaries of the Andesite Line (Born, 1932; MacDonald, 1949; Fig. 1), a zoological, biogeographic boundary defining the easternmost limit of Indo-Pacific species, situated along a zone where the Pacific Plate abuts the Philippine and Indo-Australian Plates (Springer, 1982). The result is a large biogeographic disjunction among the western Pacific and American taxa. Only two widespread species of the CCC occur on the Pacific Plate, *Bikkia tetandra*, with an easternmost range extending to Niue Island and *Chio-*

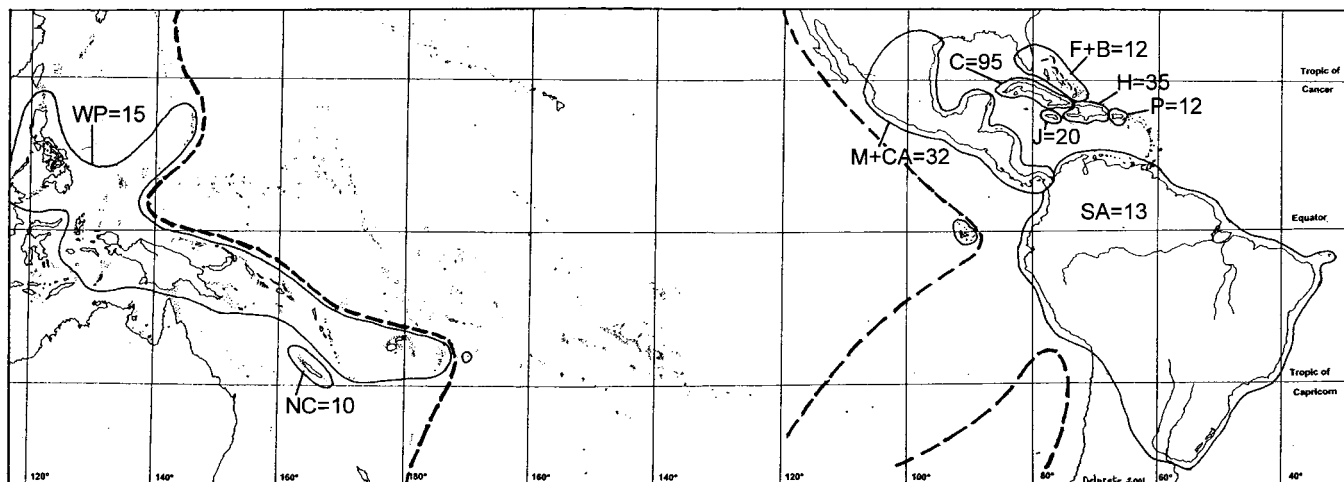


Fig. 1. Distribution map of Catesbaeae-Chiococceae complex (CCC) species. Circled areas indicate geographic regions with approximate numbers of species per region, B = The Bahamas, C = Cuba, CA = Central America, F = Florida, H = Hispaniola, J = Jamaica, M = Mexico, NC = New Caledonia, P = Puerto Rico, SA = South America, and WP = Western Pacific (except New Caledonia). Dashed line indicates the Andesite Line.

*cocca alba* which reaches the Galapagos Islands (Fig. 1). There is no other lineage of flowering plants that appears to have a similar distribution pattern as the CCC.

The objectives of this study were to: (1) re-evaluate the generic relationships within the CCC using combined sequence data from both the nuclear ribosomal internal transcribed spacers and the 5.8s gene (collectively, ITS) and chloroplast *trnL-F* intron and spacer (*trnL-F*); (2) examine flower and fruit evolution in the CCC using a phylogenetic framework; and (3) to test three biogeographic hypotheses to gain a better understanding of the origins of the biogeographic disjunction between the Caribbean and Pacific genera.

## MATERIALS AND METHODS

**Taxon sampling**—Four outgroup taxa were selected from the subfamily Cinchonoideae (*Strumpfia*, *Guettarda*, *Chione*, and *Cinchona* species). These taxa were selected based on previous results of Rova et al. (2002). Among the ingroup taxa, 61 species are included (plus four duplicate accessions of four polymorphic taxa) from 24 of the 28 genera included in the CCC (See Data Supplement accompanying online version of this article). The four genera not included are *Nernstia* from Central America and Mexico and the Cuban endemics, *Thogsennia*, *Ceuthocarpus*, and *Shaferocharis*; all but the last, with three species, are monotypic. Additionally, the genera *Molopanthera* Turcz., *Mastixiodendron* Melch., Merrill & Perry, *Placocarpa* Hook.f., and *Werhamia* S. Moore which have been included in the CCC, but excluded from this group based on evidence from recent studies (Delprete, 1996; Delprete and Nee, 1997; Huysmans et al., 1999; Rova et al., 2002), were not sampled. Only species from which both ITS and *trnL-F* data were available were included in the analyses presented here. Most large genera are represented by several species; however, some genera that are species rich in Cuba (*Phialanthus*, *Schmidtottia* and *Scolosanthus*) are under-represented.

**DNA extraction**—Leaf samples were collected either in silica gel or from herbarium sheets. Genomic DNA was extracted from approximately 1 cm<sup>2</sup> of dried leaf tissue using a modified CTAB methodology. Leaf material was ground in a lysing matrix "A" tube (Qbiogene, Carlsbad, California, USA) and pulverized for 15 s in a Fastprep machine FP-120 (Qbiogene) bead mill at speed 5. Subsequently, 500  $\mu$ L of Carlson Lysis Buffer (2 g CTAB, 8.18 g NaCl, 0.745 g EDTA, 10 mL 1 mol/L Tris/HCl pH 7.0, nanopure water to 100 mL, verified to pH 9.5, autoclaved, with 1 g PEG 4000 added when cool) and 75  $\mu$ L of  $\beta$ -mercaptoethanol were added to each tube and incubated at

74°C with occasional shaking for 60–90 min. Following incubation, 575  $\mu$ L of SEVAG (24 : 1 chloroform : isoamyl alcohol) were added to all tubes which were placed on a tipping board for 30 min at room temperature. The tubes were then centrifuged at 14000 rpm for 1 min, and  $\sim$ 350  $\mu$ L of supernatant were removed and added to new tubes containing 1050  $\mu$ L of NaI solution, 20  $\mu$ L Glassmilk, and 4  $\mu$ L TBE modifier (Qbiogene). The tubes were placed on a tipping board for 30 min at room temperature. Afterwards, the tubes were centrifuged at 14000 rpm for 1 min, and all of the supernatant was discarded. Next, each Glassmilk pellet was washed three times with 800  $\mu$ L and once with 150  $\mu$ L of ice cold New Wash solution (Qbiogene). After the final wash, all of the New Wash was aspirated from the Glassmilk pellet and 50  $\mu$ L of 10 mmol/L Tris-Cl (pH 8.5) elution buffer were added to re-suspend the DNA. The tubes were incubated at  $\sim$ 55°C for  $\sim$ 10 min and then centrifuged for 1 min at 14000 rpm. The supernatant containing the DNA was removed and transferred to new tubes and stored at  $-20^{\circ}\text{C}$ .

**DNA amplification**—DNA was amplified using the polymerase chain reaction (PCR; Mullis and Faloona, 1987). PCR reactions were performed in a 25  $\mu$ L mixture consisting of 2.5  $\mu$ L 10 $\times$  buffer with MgCl<sub>2</sub> (Perkin Elmer, Foster City, California, USA), 9.3  $\mu$ L autoclaved water, 2.5  $\mu$ L BSA (bovine serum albumin), 2.5  $\mu$ L dNTP, 1  $\mu$ L each of two 20  $\mu$ mol/L primers, 5  $\mu$ L betaine, 0.2  $\mu$ L Taq polymerase (Qiagen Valencia, California, USA), and 1  $\mu$ L of genomic DNA. All PCR and cycle sequencing reactions were run on a Gene Amp PCR system 9600 (Applied Biosystems, Foster City, California, USA). Amplification of *trnL-F* region utilized external primers "c" (5'-CG AAATCGGTAGACGCTACG-3') and "f" (5'-ATTTGAACTGGTGACAC GAG-3') and the internal primers "e" (5'-GGTTCAACTCCCTCTATCCC-3') and "d" (5'-GGGGATAGAGGGACTTGAAC-3') (Taberlet et al., 1991). The PCR conditions for amplification of the *trnL-F* region were: 1 cycle 94°C for 3 min; 32 cycles of 94°C for 45 s, 52°C for 30 s, 72°C for 1 min 30 s; and 1 cycle 74°C for 7 min, hold 4°C. The ITS region was amplified using forward (5'-CCTTATCATTAAAGAGGAAGGAG-3') and reverse (5'-TATG CTTAAAYTCAGCGGGT-3') primers and when necessary two additional internal primers were employed, (5'-GCTACGTTCTTCATCGATGC-3') and (5'-GCATCGATGAAGAACGTAGC-3') (modified from White et al., 1990; Baldwin, 1992). The PCR conditions for amplification of the ITS region were: 1 cycle 97°C for 50 s; 30 cycles of 97°C for 50 s, 53°C for 50 s, 72°C for 1 min 50 s; and 1 cycle 72°C for 7 min, hold 4°C.

**DNA sequencing**—To detect successfully amplified products and the possible contamination of negative controls, PCR products were examined on 1% agarose gels stained with ethidium bromide and visualized under ultraviolet light. Amplified products were purified with spin columns from the QIAquick

PCR purification kit (Qiagen) following protocols provided by the manufacturer. Purified products were cycle sequenced with dye terminator ABI Prism Ready reaction mix (Applied Biosystems) using dRhodamine or Big Dye v1.0 (1/8 reaction) and 5% dimethyl sulfoxide. Cycle sequencing conditions were: 1 cycle 95°C for 1 min; 32 cycles of 96°C for 10 s, 50°C for 5 s, 60°C for 3 min; and hold 4°C. Products were purified via gel filtration over Sephadex G-50 (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA) and dehydrated in a Speed Vac (Savant Speed Vac Systems, Albertville, Minnesota, USA). The DNA was resuspended in 2.2 µL of formamide (83.5%) and EDTA blue-dextran loading dye (16.5%), heated at 95°C for 2 min and immediately placed on ice. Sequencing products were separated on 5% denaturing polyacrylamide gels on an ABI Prism 377XL DNA sequencer (Applied Biosystems).

**Sequence alignment**—Sequences were edited and aligned in Sequencher version 3.1.2 (Gene Codes, Ann Arbor, Michigan, USA) followed by manual refinement. Indels were treated as missing data. Additionally, in the *trnL-F* data set (and the *trnL-F* data in the combined analysis) indels of equal length that occurred in more than one sequence were considered as homologous and scored as separate binary characters added to the data matrix (Simmons and Ochoterena, 2000). Indels in the aligned ITS data for ingroup taxa which occurred in more than one sequence were 1 bp (base pair) (or 2 bp in a single case) in length and were not coded as characters, because these motifs are prone to sequencing, and reading errors (Goldenberg et al., 1993; Oxelman et al., 1997; Andersson and Rova, 1999).

**Phylogenetic analysis**—The alignment was analyzed using PAUP\* 4.0b10 (Swofford, 2000). Minimal length trees were generated using a heuristic search, with 1000 random addition sequence replicates, with Tree-bisection-reconnection (TBR) branch swapping, and multiple parsimonious trees option (MULPARS) in effect. Uninformative characters were included in analyses except, as noted, for the calculation of alternative tree statistics. Tree statistics included the consistency index (CI; Kluge and Farris, 1969) and retention index (RI; Farris, 1989). Relative internal branch support was estimated with bootstrap analysis (Felsenstein, 1985) with 1000 replicates with TBR branch swapping and simple taxon addition. Data sets (ITS and *trnL-F*) were analyzed independently and combined using a total evidence approach (Kluge, 1989; Chippindale and Wiens, 1994; Nixon and Carpenter, 1996). Bootstrap percentages are described as high (85–100%), moderate (75–84%), and low (50–74%). Furthermore, Branch Support analysis using AutoDecay version 4.0 (Eriksson, 1998) based on comparing suboptimal trees with minimum-length ones (Bremer, 1994) were used to provide an additional measure of branch support.

## RESULTS

The *trnL-F* data set had an aligned length of 1061 nucleotides, of which 105 were parsimony informative. Ten parsimony informative indels were scored as additional characters; three were present in multiple genera, four were shared by species within a single genus, and three were present in multiple accessions of a single species. Most indels ranged between 5 and 10 bps in length, except for a single 200-bp deletion that was present in both *Exostema acuminatum* accessions. Additionally, a poly-T microsatellite varying from 1 to 16 bp in length (beginning at bp 945 character position) was excluded from the analysis. Analysis of the *trnL-F* data resulted in 59 049 most parsimonious trees (MPT) of 298 steps in length, a CI = 0.876, and a RI = 0.934 (Fig. 2). Analysis of the *trnL-F* data without indels produced >100 000 MPTs 10 steps shorter with a very similar topology. Differences in the bootstrap consensus tree without the indel data from the tree consensus tree with indel data were that *Coutarea* was unresolved, and that the *Exostema caribaeum-Solenandra mexicana* clade and the polytomy supporting the *Erithalis* and

*Chiococca* Clades lacked support. Furthermore, the *Exostema acuminatum* clade was weakly supported (50% bootstrap) in a polytomy with the *Portlandia-Catesbaea* clade and a *Bikkia-Chiococca* clade was resolved, a clade not retrieved in the *trnL-F* indel analysis (results not shown). The ITS data set had an aligned length of 692 nucleotides, of which 216 were parsimony informative. Fifty-nine indels were present in the data set, 25 (ranging from 1 to 14 bps in length) were restricted to outgroup taxa. Of the remaining 34 (ranging from 1 to 4 bps in length) 19 were parsimony uninformative. The informative indels were, except for one 2 bp indel, only 1 bp in length. Analysis of the ITS data resulted in 64 515 MPT of 957 steps in length, a CI = 0.518, and RI = 0.740 (Fig. 2).

The strict consensus trees for the two analyses are congruent for most clades. There were three areas of incongruence between the two data sets. The first was among the taxa causing the polyphyly of the *Chiococca* subclade, which includes the genus *Chiococca* along with *Asemnantha pubescens*. In the *trnL-F* data analyses *C. filipes* is not resolved within the clade and in the ITS data analyses *C. pubescens* is excluded from the clade. The second area of incongruence is the alternative position of *Siemensia pendula* as either sister to a New Caledonian clade in the ITS analyses vs. in a trichotomy with the coastal *Bikkia* clade (which does not include the endemic, New Caledonia species) and a large *Scolosanthus/Erithalis/Chiococca* clade in the *trnL-F* analyses. The final area of incongruence is the placement of *Salzmannia nitida* as sister to *Scolosanthus* (*trnL-F*) or weakly supported (52% bootstrap) as sister to *Erithalis* (ITS). The two data sets generally provide slightly different levels of resolution, with *trnL-F* providing more information about generic relationships (deeper nodes) and ITS providing greater resolution at the tips (infrageneric relationships). The higher levels of homoplasy present in the ITS data causes many of the deeper nodes to collapse in the bootstrap consensus analyses. However, because the two data sets provide nearly congruent results we have taken a total evidence approach and combined the data sets. The combined analyses of the ITS and *trnL-F* data resulted in 94 742 MPT of 1278 steps in length, a CI = 0.591, and a RI = 0.7795 (Fig. 3).

The strict consensus of the combined data set shows a strongly supported (100% bootstrap) CCC group with *Strumpfia* as its sister. The CCC clade contains five supported subclades. Within the CCC, the genus *Exostema* sensu McDowell (1996, including *Solenandra*), does not form a supported monophyletic group but rather four, largely unresolved groups. *Coutarea* and *Coutaportia* are also unresolved, and the two species of *Hintonia* form a strongly supported clade (100% bootstrap).

The species of *Exostema* sect. *Exostema* sensu McDowell sampled in this study (*E. acuminatum*, *E. caribaeum*, *E. nitens*, and *E. spinosa*) are, with the exception of the *E. nitens-E. spinosa* clade, mostly unresolved. The remaining *Exostema* species form a weakly supported clade (Fig. 3, clade A). Within this clade are two subclades: one clade contains *Exostema* species placed in section *Pitonia* and the other clade contains species of section *Brachyantha* (McDowell, 1996; McDowell and Bremer, 1998) or more recently the genus *Solenandra* sensu Borhidi (2002) with the exception of *S. selleana*. The two clades have bootstrap support of 100 and 71%, respectively. *Exostema selleana* was placed by McDowell in section *Brachyantha* (McDowell, 1996; McDowell and Bremer, 1998) and included by Borhidi (2002) within *Solenandra*, but in our

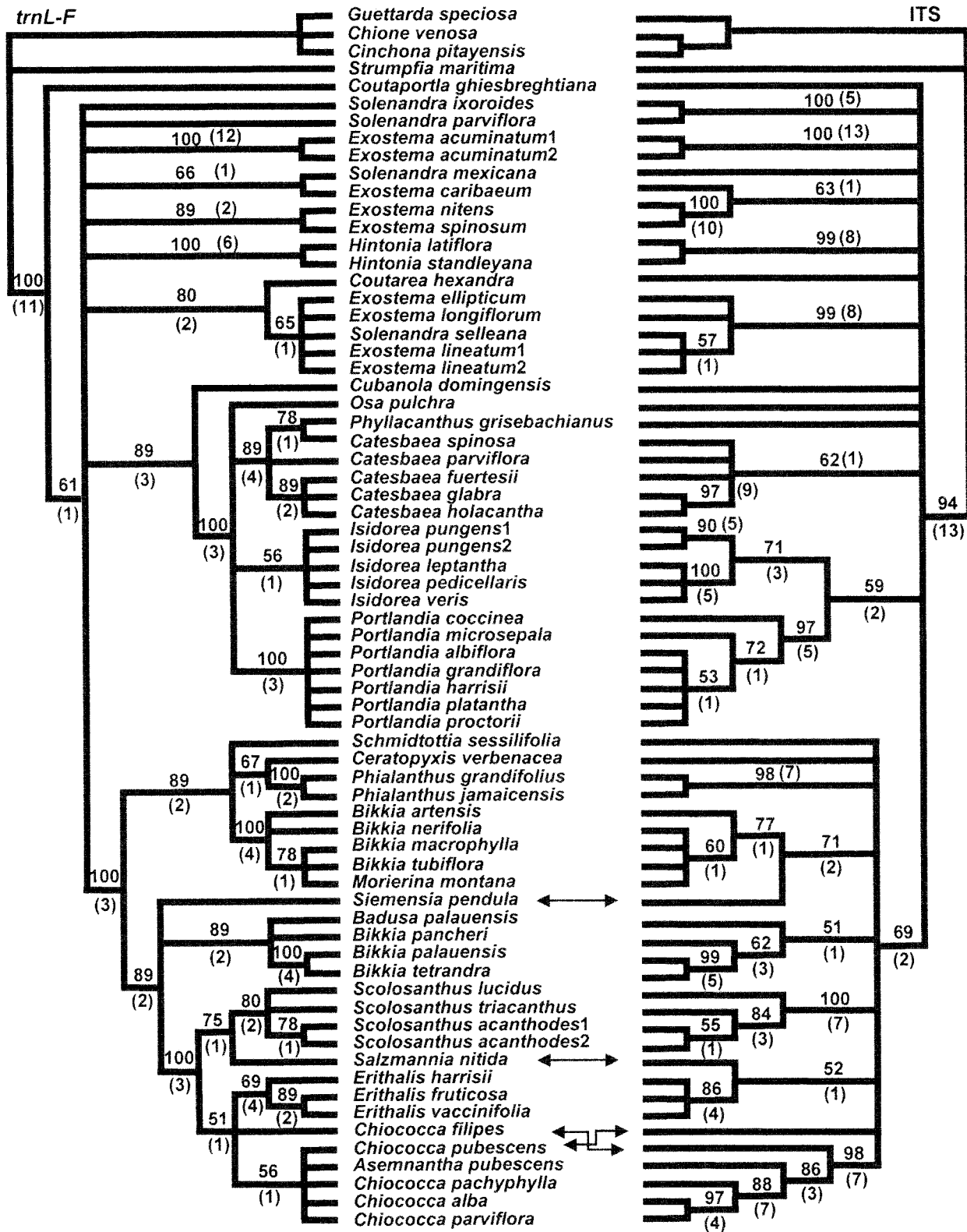


Fig. 2. The strict consensus trees from the two independent analyses. The tree on the left is the strict consensus of 59049 most parsimonious trees (length = 298 steps, CI = 0.876, RI = 0.934) obtained from the *trnL-F* data set. The tree on the right is the strict consensus of in 64515 most parsimonious trees (length = 957 steps, CI = 0.518, RI = 0.740) obtained from the ITS data set. Numbers above the branches are bootstrap values, numbers in parentheses are decay values, and arrows indicate the areas of incongruency in results from the chloroplast and nuclear data sets.

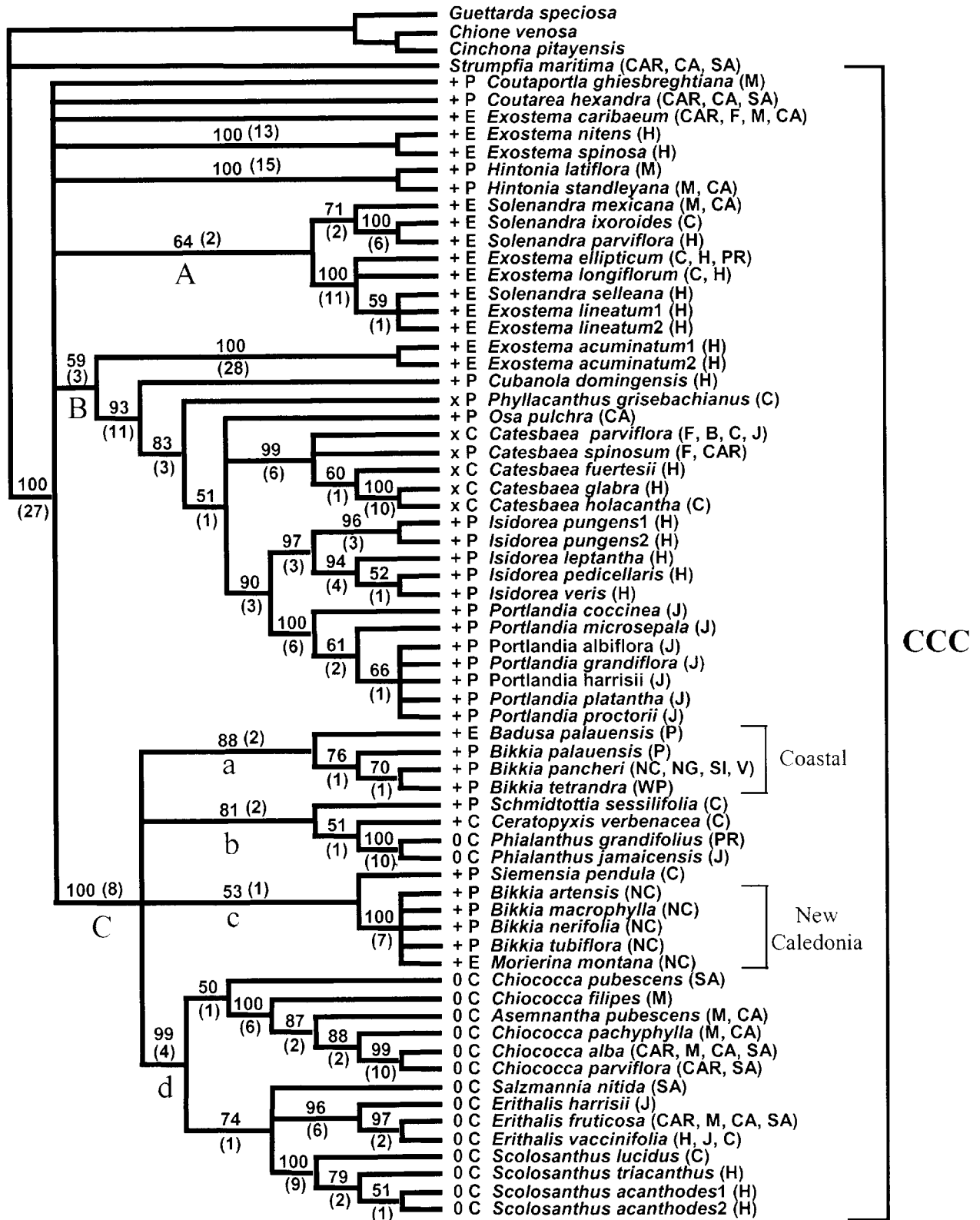


Fig. 3. The strict consensus tree of 94,742 most parsimonious trees (length = 1278 steps, CI = 0.591, RI = 0.7795) from the combined (*trnL-F* and ITS) data set. Numbers above branches are bootstrap values and numbers in parentheses are decay values. The members of the monophyletic Catesbaeae-Chiococceae complex (CCC) is indicated by the rightmost bracket and the two Pacific clades are indicated by the smaller brackets. The symbols prior to the terminal names indicate fruit types (+ = capsular; 0 = drupaceous; x = baccate) and flower types (C = *Chiococca* type; E = *Exostema* type; P = *Portlandia* type). Letters in parentheses following terminal names indicate distribution ranges of the species: B = The Bahamas, C = Cuba, CA = Central America, CAR = widespread Caribbean, F = Florida, H = Hispaniola, J = Jamaica, M = Mexico, NC = New Caledonia, NG = New Guinea, P = Palau, PR = Puerto Rico, SA = South America, SI = Solomon Islands, V = Vanuatu, and WP = Western Pacific (except New Caledonia). Letters below the branches indicate subclades, the uppercase letters designate three of the five subclades in the CCC and the lowercase letters designate the four subclades within subclade C.

combined analysis *Solenandra selleana* (= *E. selleanum*) is nested within *Exostema* section *Pitonia*. This species was included in the first two phylogenetic studies of *Exostema* (McDowell, 1996; McDowell and Bremer, 1998), but was not included in the more recent study that included broader sampling and provided better resolution (McDowell et al., 2003). This unexpected result, the placement of *S. selleana* within the *Exostema* section *Pitonia* clade, has been checked with laboratory notes and the voucher specimen is consistent with the type material of *S. selleana*.

The *Portlandia-Catesbaea* clade (Fig. 3, clade B), in general contains species with large, campanulate flowers (except for the 12 small-flowered species of *Catesbaea*). *Exostema acuminatum*, which is weakly supported (59%) as sister to the other members of the clade, is also an exception to this trend. *Cubanola domingensis* is sister to *Phyllacanthus grisebachianus*, which is sister to a trichotomy of clades each containing species in the genera *Osa*, *Catesbaea*, and *Portlandia-Isidorea*. The *Osa* clade, like the genus, is monotypic. The *Catesbaea* clade has 99% bootstrap support and is also a unresolved trichotomy. Two clades are represented by *C. parviflora* and *C. spinosa*, and the third clade contains three species with *C. fuertesii* being sister to *C. glabra* and *C. holacantha*. *Portlandia* and *Isidorea* are strongly supported (90% bootstrap support) as sister taxa and each genus is strongly supported as monophyletic, confirming the results presented by Delprete and Motley (2003).

The *Bikkia-Chiococca* clade (Fig. 3, clade C) is a well-supported clade (100% bootstrap support) composed of four subclades in an unresolved polytomy: the coastal *Bikkia* subclade (subclade a), the Cuban subclade (subclade b), the New Caledonia *Bikkia* subclade (subclade c), and the *Chiococca* subclade (subclade d). *Bikkia*, like *Exostema*, is polyphyletic. The coastal *Bikkia* clade has high bootstrap support (88%) and includes *Badusa palauensis*, which is sister to *Bikkia palauensis*, *Bi. pancheri*, and the widespread species *Bi. tetandra*. Typically, these *Bikkia* species are from coastal habitats and have similar morphologies (solitary flowers with white funnel-shaped corollas). The New Caledonia *Bikkia* clade contains *Siemensia pendula* (Cuban endemic) as a weakly supported (53% bootstrap) sister to an unresolved clade of *Bikkia* species endemic to New Caledonia and often occurring on ultramafic soils and *Morierina*, a monotypic genus, endemic to New Caledonia. The clade containing the endemic New Caledonian *Bikkia* species and *Morierina* clade is strongly supported (100%). The Cuban subclade (81% support) consists of the genera *Schmidtottia*, *Ceratopyxis*, and *Phialanthus*. *Schmidtottia* is sister to the latter two genera.

The *Chiococca* subclade (subclade d) contains most of the indehiscent-fruited CCC genera apart from *Catesbaea* and *Phialanthus*. It comprises two groups—a paraphyletic *Chiococca* lineage, which includes *Asemnantha*, and an unresolved *Erithalis-Salzmannia-Scolosanthus* lineage. The monophyly of the subclade containing *Chiococca* is weakly supported (50% bootstrap) due to the tenuous grouping of *C. pubescens* with the other species of *Chiococca*; however, the inclusion of *Asemnantha* inside of the *Chiococca* clade is strongly supported (100%). The relationship among the genera of the *Erithalis-Salzmannia-Scolosanthus* subclade is unresolved (due to incongruence between the two data sets); however, both *Erithalis* (96%) and *Scolosanthus* (100%) are strongly supported as monophyletic genera. In the ITS analyses *Salzmannia* is weakly supported (52% bootstrap) as sister to *Erithalis*,

but in the *trnL-F* data analysis *Salzmannia* is placed as sister (75% bootstrap support) to *Scolosanthus*.

## DISCUSSION

**Systematic relationships**—*Tribal phylogeny of the Catesbaeae-Chiococceae complex*—The phylogenetic relationships among the genera of the Catesbaeae and Chiococceae tribes and the identification of the two tribes as a single monophyletic assemblage (Rova, 1999; Rova et al., 2002) was further substantiated by the more rigorous sampling of the ingroup taxa and combined analysis of sequence data from both the nrDNA ITS and cpDNA *trnL-F* regions used in this study. No previous taxonomic treatments of the tribes have monophyletic circumscriptions. However, Bremer et al. (1995) came the closest to defining the genera in the lineage when they suggested a modification of Chiococceae sensu Bremer (1992) to possibly include the members of the Catesbaeae.

Historically, Hooker (1873) first circumscribed the Chiococceae as a tribe of 11 genera. *Allenanthes* Standley, *Chione* D C., and *Placocarpa*, which he included in the tribe, have all been shown in numerous later studies (Bremer, 1992; Bremer et al., 1995; Delprete, 1996; Huysmans et al., 1999; Rova et al., 2002) to not be closely related to the other genera in the lineage. They are morphologically easily distinguished by a combination of floral and pollen characters. The remaining eight genera included by Hooker (*Asemnantha*, *Ceratopyxis*, *Chiococca*, *Erithalis*, *Phialanthus*, *Salzmannia*, and *Scolosanthus*, excluding the unsampled *Shaferocharis*) are paraphyletic. In our study these genera form two (subclades b and d) of the four subclades producing a polytomy in the *Bikkia-Chiococca* clade (clade C). Bremer (1992) circumscribed the tribe Chiococceae as a much broader unit. She included the genera of the subtribe Portlandiinae (*Bikkia*, *Ceuthocarpus*, *Coutaportia*, *Coutarea*, *Cubanola*, *Isidorea*, *Nernstia*, *Osa*, *Portlandia*, *Schmidtottia*, *Siemensia*, and *Thogsennia*), all formerly placed in the Condamineae by Robbrecht (1988). Additionally, her circumscription contained *Hintonia*, the *Exostema* group (including *Badusa* and *Morierina*), and the eight genera included by Hooker, (listed above), all of which have basal stamen attachment. However, *Phialanthus* was excluded from this circumscription because the anthers are ovate rather than linear and the stamens, while having basal attachment, are free rather than fused into a basal ring (Bremer, 1992). Furthermore, members of the Catesbaeae and Chiococceae (*Catesbaea*, *Phyllacanthus*, and *Shaferocharis*) were not included in Bremer's original (1992) circumscription, but in subsequent studies (Bremer and Struwe, 1992; Bremer et al., 1995) the latter strongly suggesting the inclusion of the Catesbaeae or as sister to the Chiococceae. Later, Delprete (1996), based on a phylogenetic analysis using morphological data, emended the Catesbaeae sensu Hooker to include the Portlandiinae. He also recognized the Chiococceae sensu Hooker and an informal *Exostema* group (*Badusa*, *Exostema*, and *Morierina*), which, based on morphological analyses, were not included in either of the two monophyletic tribes. The *trnL-F* and ITS data independently and combined support the hypothesis that the Chiococceae sensu Bremer, the Catesbaeae sensu Hooker, and *Phialanthus* combined are a monophyletic lineage. Only a combination of two morphological characters define the CCC, anther attachment at base of corolla tube and spinulose pollen, but neither one is unique to this group. It is perhaps the lack of a single synapomorphic character that hindered the previous

tribal treatments from defining a monophyletic, generic circumscription of the lineage.

*Phylogenetic position of Strumpfia*—*Strumpfia* has long been a genus with uncertain affinities in the Rubiaceae (Hooker, 1873; Schumann, 1891; Bremekamp, 1966; Bridson and Robbrecht, 1985; Robbrecht, 1988; Igersheim, 1993). Igersheim (1993), in a detailed morphological and anatomical study of the genus, noted that the characters of *Strumpfia* do not fit well into any of Robbrecht's (1988) subfamilies or tribes and identified the characters in *Strumpfia* that overlap with the characters defining the groups in Robbrecht's hierarchical classification. Although a monotypic tribe was suggested, Igersheim (1993) felt the formal recognition of a tribe was premature and would be "too easy a solution" to the problem. However, molecular studies (Rova, 1999; Rova et al., 2002) have shown that *Strumpfia* belongs to the subfamily Cinchonoideae and based on *trnL-F* and *rps16* data is sister to the CCC (Rova, 1999; Rova et al., 2002). In their work the question of whether it should be included as a member of the CCC was still unsettled. In all analyses, both independent and combined, our data support the work of Rova (1999) and Rova et al. (2002), in that *Strumpfia* is sister to the CCC. The combination of morphological characteristics of the androecium, plurilocular pyrenes, and verrucose pollen, present in *Strumpfia*, further differentiate it from the CCC and other genera of the Cinchonoideae (Igersheim, 1993). Based on these apomorphies we opt not to treat it as a member of the CCC but instead as a monotypic tribe.

*The Exostema complex*—In a recent study McDowell et al. (2003) showed that *Exostema* is a paraphyletic genus with respect to *Chiococca*, *Coutarea*, and *Erithalis*, contrary to the monophyly accepted in earlier investigations (McDowell, 1996; McDowell and Bremer, 1998). *Exostema* is here shown to be a polyphyletic group; however, a large paraphyletic lineage cannot be completely ruled out. In general, three of the four *Exostema* clades retrieved by McDowell et al. (2003) correspond to the clades retrieved in the combined analysis (except for the collapse of the *E. caribaeum* branch from the *E. spinosum-E. nitens* clade, and the fact that the South American taxa, which formed an independent clade in the McDowell study, but were not included here). Borhidi (2002) transferred the Caribbean taxa of section *Brachyantha* to the genus *Solenandra* prior to the study of McDowell et al. (2003), who were apparently unaware of the taxonomic change.

In our study, section *Exostema* sensu McDowell (1996), represented by *E. caribaeum*, *E. nitens*, and *E. spinosum*, was monophyletic in the ITS analysis (and weakly supported as sister to *E. mexicanum* in the *trnL-F* data set), but formed two groups in the combined analyses due to the collapse of the branch resolving *E. caribaeum* as sister to the other two species. Section *Exostema* was circumscribed by McDowell (1996) as having axillary inflorescences with one or a few flowers.

The other two *Exostema* sections sensu McDowell, section *Brachyantha* (*Solenandra ixoroides*, *S. mexicana*, *S. parviflora*, and *S. selleana*) and section *Pitonia* (*E. ellipticum*, *E. lineatum*, and *E. longiflorum*), both have terminal inflorescences with many flowers. They differ from each other by the presence of smaller flowers, with diurnal floral fragrance and basipetally arranged seeds in the former section, and the presence of larger flowers with nocturnal floral fragrance and acrope-

tally arranged seeds in the latter section. In our combined analyses, these two sections formed sister clades in a monophyletic lineage, as was the case in the recent phylogeny of McDowell et al. (2003). However, in our study, *Solenandra selleana* was nested within the *Pitonia* clade of *Exostema* with 100% bootstrap support. Unfortunately, this taxon was not included in the most recent study of McDowell et al. (2003), and, upon re-examining the voucher specimen and sequence data, our placement of this species within the section *Pitonia* is confirmed and indicates the genus *Solenandra* is not monophyletic. Furthermore, in the McDowell et al. (2003) study, the South American *Exostema* species and *Coutarea* form an unresolved sister clade to this lineage. Our data set does not include samples of the South American *Exostema* species; however, in the *trnL-F* analyses, *Coutarea* is sister to a clade containing members of sect. *Pitonia* and *S. selleana*, a relationship not supported in either the ITS or combined analyses.

The last *Exostema* clade contains *E. acuminatum*, which is weakly supported as sister to the *Portlandia-Catesbaea* clade in the combined analyses. This species, along with *E. salicifolium*, formerly placed in section *Exostema*, was also resolved by McDowell et al. (2003) as a separate clade, but there are few or no morphological characters separating these taxa from the rest of the section (McDowell et al., 2003). Our examinations of specimens of *E. acuminatum* and *E. caribaeum* (both in *Exostema* section *Exostema*, sensu McDowell), which sometimes occur sympatrically, could only discern two morphological differences among the species. *Exostema acuminatum* flowers are reddish-pink at later stages of anthesis (not yellow), and the corolla is more shallowly lobed (less than half corolla length). An additional difference was that the two *E. acuminatum* accessions both had a 200-bp deletion in the *trnL-F* sequences that was absent from all other *Exostema* species. While it is tempting to elevate all three sections of *Exostema* and the *E. acuminatum* clade to generic level, we prefer to refrain from doing so until further morphological and molecular evidence is available and all species are represented in the study.

*The Pacific genera*—*Bikkia* is a genus of approximately 20 species (Darwin, 1985). Eleven species occur in New Caledonia, 10 of which are endemic to the main island (Jérémie and Hallé, 1976). The remaining species are distributed from New Guinea, Philippines, the Moluccas, Micronesia, Fiji, Tonga, and Niue to the Wallis Islands (Airy Shaw, 1973). Our analyses show that *Bikkia*, as presently treated, is a polyphyletic genus. One clade consists of species endemic to New Caledonia with colorful, campanulate corollas and the monotypic genus *Morierina*, which is also endemic to New Caledonia (Vieillard, 1865; Brongnart and Gris, 1871). The other *Bikkia* species form the coastal *Bikkia* clade, which is sister to *Badusa palauensis*, the only representative sampled of the Pacific genus *Badusa* (a widespread genus of three species; Ridsdale, 1982; Soejarto et al., 1996). The *Bikkia* species in this clade are typically coastal species with white, funnel-shaped corollas. The species of this group included in these analyses include: *B. tetandra*, a widespread species throughout the Mariana Islands, Fiji, and Western Polynesia, which is the type species of the genus; *B. pancheri* from the Isle of Pines, New Caledonia (which also occurs in the New Hebrides, Solomon Islands, and New Britain); and *Bikkia palauensis*.

The New Caledonia endemic species of *Bikkia* are typically found in the inland forests and have been treated in the past

as part of separate genera (*Cormigonus* Rafin. nom. nud., *Thiollierea* Montr., and *Grisia* Brongn.; Jérémie and Hallé, 1976), so the formal segregation of the two *Bikkia* clades is not unprecedented. However, the placement of *Morierina* within the endemic New Caledonia *Bikkia* clade is surprising. *Morierina* differs from *Bikkia* in having narrow, tubular flowers with exerted anthers and nonreduplicate corollas, typical in *Exostema* type flowers, rather than the *Portlandia* type of *Bikkia* (Vieillard, 1865; Delprete, 1996). Additionally, *Morierina montana* is a large tree found in densely forested areas (T. Motley, personal observations), whereas, the *Bikkia* species in New Caledonia are shrubs on ultrabasic soils. This relationship could represent a case of morphological diversification, in which case *Morierina* should be a member of the New Caledonian *Bikkia* species. This hypothesis is suggested by the congruent placement (although poorly supported) in the individual analyses (Fig. 2) where *Morierina* is nested inside of the *Bikkia* clade. An alternate hypothesis is that *Morierina* is simply sister to the New Caledonian *Bikkia* species, a case that gains support from reduced resolution in this clade in the combined analyses (Fig. 3). In either case, *Morierina* represents a morphological change, perhaps driven by a shift to a different ecological niche and/or pollination syndrome. The sister relationship of *Badusa* to the other coastal *Bikkia* clade also suggests an adaptive pollinator shift as in the case of *Morierina*. Thus a shift in floral morphology is perhaps not an uncommon evolutionary event. *Badusa* flowers, although shorter in the length of the corolla tube, are similar in shape to *Morierina* (*Exostema* type) and the *Bikkia* species have the *Portlandia* type flowers (Ridsdale, 1982; Darwin, 1985; Delprete, 1996). *Badusa* is a genus of three species (Ridsdale, 1982; Soejarto et al., 1996) endemic to the South Pacific, and represented here by only one species, *B. palauensis*.

*Chiococca*, *Asemnantha*, and *Salzmannia*—*Chiococca* is a Neotropical genus of about 20 species, with the greatest species diversity in Mexico and Central America, but with a few species extending into the Caribbean and South America (Standley, 1934). The species are scandent shrubs or vines, with axillary inflorescences and campanulate to slightly urceolate corollas. They have bilocular ovaries with axial placentation, and a single, apically attached ovule per locule, and their fruits are drupaceous, with woody pyrenes (Standley, 1934). The most widespread and polymorphic species of the genus is *Chiococca alba*, a scandent shrub that can grow into a vine up to 15 m tall and ranges from southern North America (Florida Keys) to Argentina. *Asemnantha* is a monotypic genus of small shrubs endemic to southern Mexico and northern Central America, and differs from *Chiococca* by having the stamens fused at the base to form a ring, rather than being free (Standley, 1934). In both the separate and combined analyses *Asemnantha* is strongly supported as a member of the *Chiococca* clade (Fig. 3, clade C, subclade d).

*Salzmannia* is a monotypic genus of scandent shrubs and woody vines from the coastal forests of Brazil, traditionally associated with *Chiococca* because of their morphological similarities, although its distinctness has been questioned (Schumann, 1889, 1891). Morphologically *Salzmannia* differs from *Chiococca* and *Asemnantha* only by the presence of glabrous filaments, corolla lobes not recurved, and persistent, leaf-like bracts subtending the inflorescence (Hooker, 1873; Schumann, 1891). In this study, *Salzmannia* was found to be more closely related to genera principally from the Greater and

Lesser Antilles (i.e., *Scolosanthus* and *Erithalis*, the latter also occurring in Florida) than to *Asemnantha* with which it shares many morphological synapomorphies (Bremer, 1992). *Salzmannia* was weakly supported (52%) as sister to *Erithalis* in the ITS tree and more strongly supported sister to *Scolosanthus* (75%) in the *trnL-F* tree. These three genera form a trichotomy (74%) in the combined analysis, which supports the recognition of *Salzmannia* as a monotypic genus.

*Erithalis* and *Scolosanthus*—*Erithalis* is a genus of 8–10 species distinguished by ovaries with 3–5 locules, rather than 2, as in the rest of the CCC. The results of this study correspond to those of Negrón-Ortiz and Watson (2002), which supported the monophyly of this genus. *Scolosanthus* a genus of 20 species (Liogier, 1962, 1995) represented by four species in this study, is well supported (100% bootstrap) as a monophyletic genus.

*Catesbaea* and *Phyllacanthus*—*Catesbaea* is a genus of about 16 species distributed throughout the Greater Antilles, with one species in the Bahamas and southern Florida, and one species in the Lesser Antilles (Liogier, 1995). Our study sampled five species, which formed a strongly supported clade (99%), confirming the monophyly of the genus. This genus has the most variable corollas in the CCC, both in size and in shape. For example, of the species sampled, *C. fuertesii*, *C. glabra*, *C. holacantha*, and *C. parviflora* have small, campanulate corollas (less than 2 cm long), whereas *C. spinosa* (the type species of the genus) has large, funnel-shaped corollas 4–17 cm long (Liogier, 1995; Delprete, 1996). Although the basal lineage within *Catesbaea* is not resolved, comparison with sister groups suggests that the campanulate corollas are derived from funnel-shaped corollas within *Catesbaea*.

*Phyllacanthus grisebachianus* is a species endemic to Cuba (Liogier, 1962), is probably now extinct due to habitat loss caused by sugar cane cultivation (Oviedo et al., 1988). *Phyllacanthus* shares many morphological similarities with *Catesbaea*, but it can be distinguished from *Catesbaea* by having large, flattened, triangular thorns and carpels with uniseriate ovules (vs. terete, needle-like thorns and multiseriate ovules; Liogier, 1962; Delprete, 1996). Rova (1999) succeeded in extracting DNA from this species by grinding one thorn from a specimen more than 50 yr old, which is the most recent of the two known collections ever made of this taxon. In our *trnL-F* analysis, *Phyllacanthus* nested within the *Catesbaea* clade (a result also recovered by Rova et al., 2002); however, this relationship collapsed in the ITS and combined analyses. Because the *Phyllacanthus* sequences are incomplete due to degraded DNA, its relationship to *Catesbaea* is still uncertain. Morphologically, *Phyllacanthus* flowers are nearly identical in size, shape, and color to those of *C. flaviflora* Urb. Furthermore, based on the *trnL-F* data (analyses both here and in Rova et al., 2002) and the morphological similarities with *Catesbaea* we suspect that it is best to include *Phyllacanthus* in *Catesbaea*, and return to its original binomial, *Catesbaea phyllacantha* Hook. f. (Hooker, 1871).

*Portlandia*, and the related genera *Isidorea*, *Osa*, and *Cubanola*—Aiello (1979) circumscribed *Portlandia* as a genus of five species endemic to Jamaica with large, funnel-shaped corollas, imbricate aestivation, basal insertion of stamen, basal fusion of filaments, anthers basifixed and linear, and seeds horizontally arranged and wingless. In a recent revision (Delprete

and Motley, 2003), *Portlandia* was shown to be a monophyletic genus of seven Jamaican species. *Isidorea*, which is also shown here to be monophyletic, is sister to *Portlandia*. These genera share the morphological features listed above, and the only difference between the two is the presence of pungent apices on the leaves and stipules of *Isidorea* (Aiello, 1979). While all the recognized species of *Portlandia* were sampled in this study, only four species of *Isidorea* from the Dominican Republic were sampled (the Cuban species were unsampled); however, the two genera are strongly supported as sister monophyletic lineages in the combined analyses.

*Cubanola* is a genus of two species, one endemic to Hispaniola and the other to Cuba (Aiello, 1979). In the combined and *trnL-F* analyses (but unresolved in the ITS analyses) *Cubanola* is sister to a clade containing *Catesbaea*, *Phyllacanthus*, *Portlandia*, *Isidorea*, and *Osa* (a monotypic genus endemic to the Peninsula de Osa, Costa Rica). The four large-flowered genera, with corollas of some species exceeding 25 cm in length, along with *Catesbaea*, *Phyllacanthus*, and perhaps *Exostema acuminatum*, represent a monophyletic group of closely related genera. The internal relationships within this clade for the most part support the morphological work of Aiello (1979), who proposed their generic segregation.

**Relationships of additional Cuban genera**—Among the other genera of the CCC not previously discussed, four are principally Cuban or are endemic to Cuba. *Schmidtottia* (16 species endemic to serpentine soils of eastern Cuba), *Ceratopyxis* (a single species occurring in the limestone haystack mountains of eastern Cuba), and *Phialanthus* (17 of 20 species occurring in Cuba) (Liogier, 1962) form a well-supported clade (81%) in the combined analyses. The species in this clade encompass a diverse assemblage floral and fruit morphologies. *Schmidtottia* has capsular fruits and medium-sized, narrowly campanulate to narrowly infundibuliform corollas (*Portlandia* type). *Ceratopyxis* also has capsular fruits and small, narrowly funnel-shaped flowers. In contrast, the species of *Phialanthus* have drupaceous fruits and minute, narrowly campanulate corollas (*Chiococca* type) (Liogier, 1962). In our study, *Schmidtottia* was sister to *Ceratopyxis* and two accessions of *Phialanthus*. Although sampling within the large genera of this clade was limited, our analyses indicate an independent derivation of fleshy fruits from capsules in this Cuban lineage.

*Siemensia* is a monotypic genus endemic to the limestone haystack mountains of the Province of Pinar del Rio, western Cuba (Liogier, 1962). In the ITS phylogeny, *Siemensia* was placed as sister to the New Caledonian *Bikkia* clade, whereas in the *trnL-F* analysis it was placed in a polytomy with the coastal *Bikkia* clade and the *Chiococca* clade. In the combined analysis *Siemensia* was sister to the New Caledonian *Bikkia* clade. Although it was poorly supported (53%), this evidence suggests a possible close relationship of this Cuban endemic species to the Pacific taxa.

**Relationships of genera from Mexico and Central and South America**—*Hintonia* is a genus of three species (Ochoterena, 2000) endemic to Mexico and northern Central America. *Coutarea* is a genus of two species—*C. hexandra*, ranging from Mexico to Argentina, and *C. andrei* Standl., endemic to the Loja Province of Southern Ecuador (Delprete, 1999). These two genera are isolated in our strict consensus trees. *Coutaportia* is a genus of three or four species from Mexico and Central America (Lorence, 1986, 1999; Villarreal, 1987). Re-

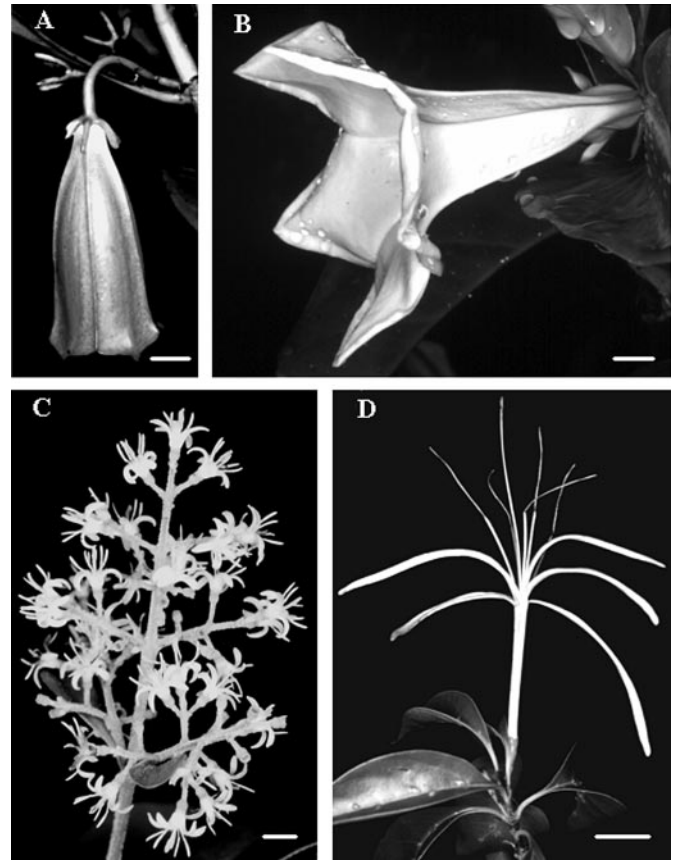


Fig. 4. Flower types in the Catesbaeeae-Chiococceae complex (CCC). The *Portlandia* type (A–B) with either large (A) long, campanulate (*Bikkia macrophylla*) or (B) tubular-funnelform corollas (*Portlandia platantha*). (C) The *Chiococca* type (*Erithalis harrisii*) with short, campanulate, deeply lobed corollas. (D) The *Exostema* type with short or long, narrowly tubular, lobed corollas (*Exostema caribaum*). Scale bars = 1 cm.

cently, Borhidi (2003) transferred *C. guatemalensis* (Standl.) Lorence to his new monotypic genus *Lorencea* (not sampled). Because only one species of *Coutaportia* was sampled in this study, we cannot discuss this segregation of *Lorencea* based on these data.

**Fruit and flower evolution**—The second objective of this study was to examine flower and fruit evolution in the CCC using a phylogenetic framework. Flower characters uniting the CCC are stamen attachment at the base of the corolla (Bremer, 1992; Delprete, 1996), pollen with perforate and microechinate tectum, and a smooth, often perforated layer under the inner nexine layer (Huysmans et al., 1999). Floral morphology varies within the CCC mainly in size, shape, and merosity. Delprete (1996) described three flower types (Table 1; Fig. 4): (1) the *Portlandia* type with long ([5–]10–27 cm), campanulate (Fig. 4A) to tubular-funnelform (Fig. 4B) and shallowly lobed corollas, linear anthers, and a long style with four-parted, adnate, elongate-clavate stigma; (2) the *Chiococca* type (Fig. 4C) with short (0.3–2 cm long), campanulate, and deeply lobed corollas, oblong anthers, and a short style and two-lobed stigmas; and (3) the *Exostema* type (Fig. 4D) with short or long (2–20 cm), narrowly tubular, reflexed-lobed corollas, linear, exerted anthers, and a long style with capitate or linear stigmas. Fruits vary greatly among the members of the CCC

(Table 1). The mesocarp can be dry, leathery, or fleshy; placentation is axial or apical; dehiscence is loculicidal, septicidal or absent; and seeds are winged or unwinged, and flattened or globose. The genera of the CCC can exhibit almost any combination of these fruit characters.

As a preliminary overview of the flower and fruit evolution in the CCC, we have taken a compartmental approach to classifying the flower types (*Portlandia*, *Exostema*, or *Chiococca* types) and fruit types (baccate, drupaceous, and capsular) based on overall morphological similarities. We are aware of homology problems with simple typological classification of fruits, especially drupaceous forms, with indehiscent forms, which if structurally or developmentally examined might be very different. Nevertheless, it is obvious that the axial multi-ovulate, leathery, baccate fruits of *Catesbaea* have a different evolutionary and developmental history than the bilocular drupaceous fruits of *Chiococca* and *Phialanthus* and the multi-locular and drupaceous fruits of *Erithalis*.

Presuming the ancestral condition in the CCC included capsular fruits, as is the case in most of the subfamily and closely related taxa (Rova et al., 2002), drupaceous fruits seem to have evolved two separate times within the CCC (Fig. 3), once in *Phialanthus* and a second time in the *Chiococca* clade (including *Chiococca*, *Asemnantha*, *Salzmannia*, *Erithalis*, and *Scolosanthus*). The baccate fruit type has evolved at least one time and possibly twice in *Catesbaea* and *Phyllacanthus*. The genus *Chiococca* has one of the broadest geographical distributions in the CCC, and its spread probably due to endochorous dispersal by birds eating the small, drupaceous fruit. *Scolosanthus*, *Erithalis*, and *Phialanthus*, also have similar drupaceous fruits and *Catesbaea*, with baccate fruits, all tend to have widespread distributions that are facilitated by animal dispersal (Table 1). However, *Coutarea hexandra*, *Exostema caribaeum*, the coastal *Bikkia* species, and *Badusa* are also widespread and have capsular fruits and wind-dispersed seeds. Thus no clear trend is evident that would suggest one fruit type is more advantageous over another for dispersability.

The differing floral morphologies probably reflect adaptive shifts for pollinator specialization. Unfortunately, actual pollination data for the CCC are lacking for this group, although several species have been the subject of field observations. The *Exostema* type flowers (narrowly, tubular, lobed corollas; linear, exserted anthers; and a long style) are typical of moth or butterfly pollination; the *Chiococca* type flowers (short, campanulate, and deeply lobed corollas; oblong anthers; and a short style) are typical of entomophilous (particularly bee) pollination; and the *Portlandia* type flowers (long, campanulate to tubular-funnelform corollas; linear anthers; and a long style) are characteristic of bird- and bat-pollinated flowers (Faegri and Pijl, 1979; Delprete and Motley, 2003).

The *Exostema* type flowers seem to have evolved at least three times in the CCC. Because of the poor resolution among the clades of *Exostema* s.l. it is not possible to more precisely determine the number of events. However, among the *Exostema* clades the corolla color variation and differences in production of floral fragrances (diurnal vs. nocturnal) seem to reflect cladogenesis in these groups. This is especially evident between the sister clades corresponding to sections *Brachyantha* (= *Solenandra*) and *Pitonia* in McDowell's (1996) classification. Apart from the species of *Exostema* s.l., the *Exostema* type floral morphology seems to have evolved independently twice in the Pacific, once in *Badusa* and again in *Mor-*

*ierina*. Both genera are most closely related to species with *Portlandia* type flowers (Fig. 3).

The *Chiococca* type flower has apparently evolved three or four times (*Catesbaea* spp., *Phyllacanthus*, *Phialanthus*, and the *Chiococca* clade). These three events correspond to the same groups that have evolved fleshy, indehiscent fruits. This flower type is characteristic of mellitophily (Faegri and Pijl, 1979). *Catesbaea* is the genus with the most variable corollas in the complex, both in size and shape. Twelve species have *Chiococca* type flowers (<2 cm long, campanulate corollas), and four species have *Portlandia* type flowers (4–17 cm long, funnel-shaped corollas; Delprete, 1996). Because *Catesbaea* is within a clade (Fig. 3, clade B) of genera that principally have *Portlandia* type flowers, the change in flower type appears to be an adaptive shift from bird or bat pollination to entomophily. It will be interesting to determine in future analyses whether the two floral types in *Catesbaea* are resolved in sister clades in a manner similar to the species with floral differences seen in the *Exostema* clade (sections *Brachyantha* and *Pitonia*) described above. Because only a single large-flowered species was included in the present analysis, the effects of pollinator shifts in the evolution of the genus cannot be determined.

The *Portlandia* type flowers are widely distributed throughout the CCC phylogenetic tree and appear to have originated independently five or more times in the group. The corolla shape ranges from infundibuliform-salverform in *Portlandia*, *Isidorea*, *Hintonia*, *Coutaportia*, *Schmidtottia*, and the coastal *Bikkia* species, to campanulate in *Osa*, *Coutarea*, *Siemensia*, *Cubanola*, and four species of *Catesbaea*, and the New Caledonian *Bikkia* species. The differences in floral structure and color seen between the two *Bikkia* clades seems to reflect different pollination strategies. The coastal *Bikkia* species have white, upright, salverform flowers characteristic of flowers that attract bats and moths (Faegri and Pijl, 1979). The New Caledonian *Bikkia* species have brightly colored campanulate flowers (red, yellow, purple, and pink) like those of species adapted to ornithophily (Faegri and Pijl, 1979). The white-colored, campanulate flowers of *Osa*, *Cubanola*, *Siemensia*, and large-flowered *Catesbaea* species that have a long corolla tube, (9–12 cm) are most likely pollinated by long-tongued moths or possibly bats. The flowers of *Coutarea hexandra* vary from white to pink, purple, red, or yellow with a broad, obconical, and slightly asymmetrical floral tube that suggests an adaptation to a wide range of bird pollinators. Among the species in both *Portlandia* and *Isidorea*, there are also differences in floral changes in color, shape, and size, although the differences are not as pronounced as the variation in *Catesbaea*. Within *Portlandia* there are four red-flowered species and three white-flowered species (Delprete and Motley, 2003). Corolla length varies from 2.5–5.4 cm in *P. proctorii* to 10–22 cm in *P. grandiflora* (Aiello, 1979). The species with the smaller red corollas, *P. proctorii*, were observed being visited by the red-billed streamertail (*Trochilus polytomus polytomus*) (Delprete and Motley, 2003). Anecdotal evidence from foresters and field observations indicate the salverform, white corolla flowers of *P. grandiflora* were always collected in the field with damage due to aggressive floral visitors. The flowers open in the evening and produce large amount sweet-smelling floral fragrance (T. Motley and P. Delprete, personal observations), which strongly suggest bat pollination. No pollination data are available for the other species. However, the red flower morphology seems to be the ancestral state in *Portlandia*. *Portlandia coccinea* is sister to *P. microsepala*, both red-flow-

ered species, which are sister to an unresolved clade of both white- and red-flowered species (Fig. 3). Shifts in floral morphology and adaptation to various pollinators seem to have occurred often among and within the genera of the CCC.

In general, taxa with *Chiococca* type flowers have drupaceous fruits, with a single, pendulous, apical ovule per locule (*Ceratopyxis* is an exception to this trend in having woody, septicidal capsules), and those with *Exostema* or *Portlandia* type flowers have few to many axial ovules, and either woody, septicidal capsules or leathery, baccate fruits in *Catesbaea*. However, because many of the characters appear homoplasious, our preliminary compartmental classifications of fruit and fruit types are being re-evaluated through careful studies (H. Ochoterena, P. Delprete, and T. Motley, unpublished data), and, although our discussions on flower morphology and pollinators are based mostly on generalizations, we hope it will spur more detailed field studies in the future.

**Biogeography**—The third objective of this study was to better understand the origins of the biogeographic disjunction between the Caribbean and Pacific genera. The Greater Antilles is the most species-rich area and is likely the center of origin for the CCC (Fig. 1). However, alternate hypotheses (i.e., South American or Pacific origins) cannot be completely ruled out. Working under the assumption that the CCC is of American origin we attempt to determine the number of long-distance dispersal events that were involved in the evolution and establishment of the three Pacific genera (*Bikkia*, *Badusa*, and *Morierina*). We propose three biogeographic hypotheses, which we hope to test using the phylogeny of the CCC: (1) origin in the Greater Antilles, and prior to the formation of the Central American landbridge (ca. 60–80 my (million years ago); Briggs, 1994; Iturralde-Vinent and MacPhee, 1999), dispersal into the Pacific basin; (2) origin in the Greater Antilles, followed by a series of dispersals to South or Central America and subsequently to the Pacific; and (3) origin in Central or South America with dispersals to Caribbean and the Pacific. Unfortunately, due to the lack of resolution among the major clades of the lineages and the uncertain placement of several continental taxa, we are not able to determine the area of origin for the CCC. Additionally, dates based on molecular divergence of the Gentianales (60 my; Yuan et al., 2003) and estimates based on the earliest known Rubiaceae fossil (53 my; Wikström et al., 2001) may make the first hypothesis implausible. Nevertheless, a New World origin for the CCC is indicated by the distribution of the taxa in the basal polytomy of the combined analyses (Fig. 3). One or two dispersal events are required to account for the two western Pacific clades (Fig. 3, clades Ca and Cc), one event is required to give rise to the New Caledonia clade and the other the *Badusa*-coastal *Bikkia* clade. *Siemensia* from western Cuba was weakly supported as sister to the New Caledonian clade (ITS and combined analyses; Figs. 2 and 3) and in an unresolved clade with the coastal *Bikkia* clade and the *Chiococca* clade in the *trnL-F* analyses. Other plant groups or genera with similar distributions to the CCC (species in Central and South America, the Caribbean, and species in the Pacific Islands east of the Andesite line and absent in Australia, Asia, and Africa) seem to be lacking. We could identify just one other group with similar distribution: the genus *Augusta* sensu Kirkbride (Rubiaceae; Darwin, 1976; Delprete, 1997, 1999; Kirkbride, 1997), a rheophytic shrub with capsular fruits and minute seeds with one species each in

Central America, central and southern Brazil, Fiji, and New Caledonia (P. Delprete and T. Motley, unpublished data).

Capsular fruits with wind-dispersed seeds are present in most of the basal clades of the CCC, including all of the Pacific taxa and *Siemensia*, suggesting that anemochory was a successful strategy for long-distance dispersal across the vast disjunction formed by the Pacific Plate. Zoochory and/or hydrochory of the widespread indehiscent-fruited taxa of the CCC appear to be a more efficient dispersal strategy for crossing the relatively shorter distances among the islands of the Caribbean.

## CONCLUSIONS

The Catesbaeae-Chiococceae complex is a diverse, monophyletic assemblage that includes the genera whose previous circumscriptions are mostly in agreement with our results. Exceptions include *Bikkia*, *Exostema*, and *Solenandra*, which appear to be polyphyletic. Additionally, the monotypic genera *Asemnantha* and possibly *Phyllacanthus* should be included within *Chiococca* and *Catesbaea*, respectively. The anomalous genus *Strumpfia* is closely related to the CCC.

The genera of the CCC vary widely in flower and fruit morphology, and the lineage is distributed in Central and South America, the Caribbean (where the complex is most species rich), and the islands of the Pacific basin east of the Pacific Plate (Andesite line; Fig. 1). Fruit morphology in the complex was classified into drupaceous, baccate, and capsular fruits, and the former two types have arisen from the pleisomorphic capsular fruit type two times in the complex, whereas the leathery baccate fruits of *Catesbaea* and *Phyllacanthus* may have evolved one or two times. Genera with drupaceous or baccate fruits seem to be more successful in dispersing among the islands of the Caribbean; however, the small, wind dispersed seeds of the capsular-fruited species has been more successful for long-distance dispersal spanning the barrier of the Pacific Ocean. The dispersal into the Pacific may have been the result of either one or two separate events. The variation in flower morphology from extremely large funnel-shaped to narrow, tubular to small, campanulate flowers could be strongly correlated with shifts in pollination strategies, which in some instances appear correlated with cladogenesis in the CCC.

Presently we have only examined general trends in flower and fruit morphology based on similarities in gross morphology. Detailed morphological and anatomical studies are underway (P. Delprete, H. Ochoterena, and T. Motley, unpublished data) to examine character evolution, which varies within and among the flower and fruit classifications we have defined here. Additionally, the phylogenetic sampling is being expanded to include yet unsampled genera (*Ceuthocarpus*, *Nernstia*, *Shaferocharis*, and *Thogsennia*) and more complete sampling of several large genera (*Isidorea*, *Phialanthus*, *Schmidtottia*, and *Scolosanthus*), principally enhanced by recent fieldwork conducted by one of us, P. G. Delprete, in Cuba. This generic complex seems to be an ideal group for better understanding flower and fruit evolution, pollination, dispersal, and biogeography in the Rubiaceae.

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