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A Phylogenetic Study of *Cuphea* (Lythraceae) Based on Morphology and Nuclear rDNA ITS Sequences

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ABSTRACT. *Cuphea* is an endemic genus of the New World and the most speciose member of the Lythraceae with ca. 260 species classified in two subgenera and 13 sections. As a first attempt to construct a phylogenetic framework for the genus, data from morphology and nuclear ITS sequences for 53 species and four outgroup taxa were analyzed. Independent results employing morphological and molecular datasets confirmed *Cuphea* as monophyletic with *Pleurophora* as sister. The morphological strict consensus tree was substantially unresolved. The ITS parsimony and maximum likelihood phylogenies indicated South America as the initial center of diversification and identified a deep trichotomy, one branch of which was equivalent to subg. *Cuphea*. The ITS analyses also recognized seven well-supported clades, each composed of members from two to four taxonomic sections. Species of section *Melvilla* appear in five of the seven clades, supporting the hypothesis that the large, intensely-colored, bird-pollinated floral tubes that define the section are convergent, having evolved from smaller, more promiscuously-pollinated, green-tubed flowers. Sixteen endemic North American species have a single South American origin and form the core of a secondary center of *Cuphea* diversification in Mexico. The ITS analyses provide initial phylogenetic hypotheses for the genus that clarify relationships previously obscured by the highly homoplastic nature of the morphological taxonomic characters.

KEYWORDS: classification, dispersal, polyphyly, South America.

Cuphea is a New World genus and the largest of the 32 genera of Lythraceae (Graham et al. 2005) with about 260 species of herbaceous perennials and small shrubs. The genus is recognized by the ribbed floral tube which terminates in 6 deltate calyx lobes, and especially by a unique seed dispersal mechanism. Unlike all other Lythraceae, which retain seeds within a fruit surrounded by a persistent floral tube, *Cuphea* seeds are exposed for dispersal on a placenta that becomes exserted through matching longitudinal slits in the adaxial (dorsal) wall of the capsule and floral tube. Other synapomorphies for the genus are: 11 stamens; a unilateral free-standing nectariferous organ, the ''disc'', at the base of the ovary; septal walls reduced to two thin threads; oblate pollen (i.e., flattened from pole to pole as opposed to the elongated prolate form found in the rest of the family), and seed oils that vary in fatty acid composition depending on the species.

The monographer Emil Koehne considered *Cuphea* most closely related to *Woodfordia* (1886, 1903). In recent family-level cladistic analyses using morphology and molecular sequences (Graham et al. 2005) including up to four species of *Cuphea*, the genus was monophyletic with close relationships to *Adenaria*, *Pehria*, *Koehneria*, *Woodfordia*, and *Pleurophora*, but the sister group remained unclear. Additional sequences for a more complete representation of the genus, together with those of the most closely related genera, were needed to confirm the monophyly of *Cuphea* and to establish with certainty its nearest relative in the family.

The large number of species constituting the genus are divided between two major geographical centers: one in North America, mostly in western and southern Mexico; and the other in South America, mostly in eastern Brazil. The regions share few species in common. Most *Cuphea* occupy open mesophytic to marsh habitats, true to the propensity of the family for moist to aquatic habitats. On each continent, a small number of species have extensive distributions and are frequently found on disturbed or degraded lands, while the majority are narrowly distributed endemics, often localized in specialized habitats such as white sand savannas, rocky limestone outcrops, seasonally burned campos, or swamplands. Vegetative morphology is most diverse in South America, whereas floral morphology has diversified most extensively in the mountains of Mexico. The North American center is assumed to be secondary in origin, derived from one or more South American ancestors (Graham 1988, 1998a), but this premise has not been critically tested since relationships among the species are uncertain or unknown.

Taxonomically, *Cuphea* is divided into two subgenera and thirteen sections, defined by one to a few ''key'' characters in a now largely outdated monograph (Table 1; Koehne 1877, 1881, 1903). The genus has grown since 1903 by nearly 40% and new species continue to be discovered (Cavalcanti and Graham 2005). As the genus has become better known through sectional revisions (Graham 1988, 1989a, 1998a; Lourteig 1959, 1986, 1987, 1988), the classification has become more difficult to apply and it is increasingly apparent that relationships among the taxa implied by the classification are unsupportable. New information, particularly from previously uninvestigated pollen and seed characters, indicates the presence of a number of species groups that are at variance with the present taxonomy.

Diversity in pollen morphology, unequalled at the species level in other genera of the family or in any other family in the order (Patel et al. 1984), points to relationships that conflict with those based on the key sectional characters. Pollen diversity in section *Melvilla*, for example, strongly suggests that this large section is a gathering of species based on convergent floral morphology related to pollinator specialization. Species sharing similar elongate, thick-bodied, intensely-colored floral tubes, a floral syndrome under selection to attract bird and large bee pollinators (Raven 1972; Proctor et al. 1996), fall into at least four very different pollen categories, each known from or characteristic of other sections (Graham and Graham 1971).

Other relationships implied by the taxonomy are likely inaccurate due to convergent floral morphology related to change in the breeding system from outcrossing to self-fertilization. Section *Brachyandra* comprises species having pale green, very small flowers (3–8 mm long) with deeply included anthers and stigma, characters suggestive of self-fertilization. Several species of the section have been confirmed as facultatively self-fertilizing and a morphological phylogenetic analysis indicated that at least four distinct lineages constitute the section. Each lineage was defined by a unique combination of pollen and seed morphology, but shared the same homoplastic floral features (Graham 1998b).

The questions of species relationships raised by the morphological data, the degree to which the classification reflects natural lineages, and hypotheses about the historical biogeography of the genus have yet to be examined using a data source other than morphology. This study initiates examination of the phylogenetic relationships in *Cuphea* using parsimony analysis of the morphological data and parsimony and maximum likelihood (ML) analysis of sequences from the nuclear ITS region, including ITS-1, the 5.8 S gene, and ITS-2. The study also aims to assess the current classification in light of phylogenetic hypotheses produced by these data. The goal is to provide a preliminary view of the relationships and biogeographic history of the North and South American taxa and the evolution of the very diverse floral, pollen, seed oil, and other features of the genus. The large number of morphologically superficially similar species in *Cuphea* will require more extensive sampling of taxa and genes than is presented here. These results are an introduction to a more comprehensive molecular systematics survey of the genus that is currently underway (Ghebretinse and Barber, pers. comm.).

Materials and Methods

Taxon Sampling. Fifty-seven taxa were sampled, including 53 species of *Cuphea*. Four outgroups, *Lythrum lineare* L., *Pehria compacta* (Rusby) Sprague, *Pleurophora saccocarpa* Koehne, and *Woodfordia fruticosa* (L.) S. Kurz , were selected based on results of a recent family-level molecular/morphological phylogenetic study (Graham et al. 2005). The species of *Cuphea* sampled represent the full morphological range of the genus and include representatives of the two subgenera, 11 of 13 sections, and 14 of the 18 subsections (Table 1; the characters employed by Koehne (1903) to define the taxonomic categories are included in the table). Missing are representatives of section *Melicyathium* Koehne (one rare species from Brazil) and section *Amazoniana* Lourt. The latter, with 21 narrowly circumscribed species of northwestern South America, may possibly be represented by *Cuphea repens*. Although described in section *Brachyandra*, this species morphologically conforms fully to section *Amazoniana*.

Morphological Analysis. A matrix of 57 species by 46 characters was generated for the morphological analyses (Appendices 1 and 2). Six characters are vegetative, five are inflorescence characters, 24 are floral, six are from pollen, and five are seed characters. Missing data constitute 0.6% of the total data matrix. Parsimony analyses were performed in PAUP* 4.0b10 (Swofford 2002) using heuristic search algorithms. Multistate characters were treated as unordered and equally weighted. Analyses were conducted under the following parameters: 1000 random addition sequences, 10 trees held per random addition replicate, tree-bisection-reconnection branch swapping (TBR) with Multrees on to keep all equally most parsimonious trees, and steepest descent off. The program was set to collapse branches when minimum length was zero (amb-). Confidence in parsimony tree topologies of the unweighted data set was assessed using jackknife analysis (JK) with 10,000 replicates, holding 2 trees each replicate, TBR, Multrees on, with each character having a probability of deletion of $1/e$ (Farris et al. 1996). Bootstrap support (BS; Felsenstein 1985) for the maximum likelihood tree was calculated using full heuristic search with 100 bootstrap replicates, TBR and Multrees on.

Molecular Analysis. Fresh and silica-gel dried leaves, and air-dried leaves from herbarium specimens, were used for extraction following the CTAB method of Doyle and Doyle (1987). Results were best from fresh leaves and poor to absent from herbarium material. Extraction of DNA in *Cuphea*

TABLE 1. Current infrageneric classification of *Cuphea* based on Koehne (1903) with subsequent changes and additions incorporated (Graham 1988, 1989a, 1990, 1998a; Lourteig 1959, 1986, 1987, 1988). Key characters of the subgenera and sections as defined by Koehne are provided; species employed in this study are listed by section and subsection.

SUBGENUS *CUPHEA* (subg. *Lythrocuphea* Koehne) - Bracteoles none; floral tube 4–14 mm long.
SECTIONS
1. *Archocuphea* Koehne 3 spp. - Pedicels partly opposite, partly alternate. *C. mimuloides* Cham. & Schltdl., *C. pascuorum* Mart. ex Koehne.
2. *Cuphea* 16 spp. - Pedicels all opposite.
   Subsect. *Cuphea* – Dorsal petals smaller than the others. *C. decandra* Ait., *C. denticulata* Kunth, *C. flavisetula* Bacig., *C. gaumeri* Koehne, *C. utriculosa* Koehne.
   Subsect. *Notodynamia* Koehne – Dorsal petals larger than the others. *C. racemosa* (L.f.) Spreng.

SUBGENUS *BRACTEOLATAE* S. A. Graham (subg. *Eucuphea* Koehne) - Bracteoles 2, sometimes small; flowers alternate or paired at verticillate-leaved nodes, except sect. *Heteranthus*; floral tube 3–11, rarely −17 mm long, then without internal wings or enlarged dorsal lobe, or 12–40 mm long. If 5–12 mm, then with internal wings or enlarged dorsal lobe.
SECTIONS
3. *Heteranthus* Koehne 8 spp. - Flowers decussate, in terminal racemes, with hypsophylloid bracts. *C. epilobiifolia* Koehne.
4. *Melicyathium* Koehne 1 spp. - Disc cup-shaped; ovules 50–90.
5. *Brachyandra* Koehne 16 spp. - Disc dorsal, ovules 2–32; stamens not as long as the floral tube.
   Subsect. *Microcuphea* Koehne- Leaves 3–5-whorled. *C. repens* Koehne.
   Subsect. *Melanium* Koehne - Leaves not verticillate; perennial herbs or shrubs. *C. calophylla* Cham. & Schltdl., *C. melanium* (L.) R.Br. ex Steud., *C. urens* Koehne.
   Subsect. *Micranthium* Koehne - Annual herbs; ovules 3.
   Subsect. *Lythrocupheopsis* Koehne - Annual herbs; racemes distinct; ovules 4, seeds tuberculate. *C. circaeoides* Sm. ex R. Sim.
   Subsect. *Balsamonella* Koehne - Annual herbs, inflorescence foliate; ovules 4–11, seeds smooth. *C. carthagenensis* (Jacq.) Macbr., *C. parsonsia* (L.) R. Br., *C. pseudosilene* Griseb.
6. *Euandra* Koehne 91 spp. - Disc dorsal, plane or concave above, convex below; stamens at anthesis nearly equalling or exceeding the floral tube.
   Subsect. *Platypterus* Koehne - Small shrubs, seed margin sharp, frequently with broad thin encircling wing. *C. acinifolia* A. St.-Hil., *C. glutinosa* Cham. & Schltdl., *C. ingrata* Cham. & Schltdl., *C. strigulosa* Kunth, *C. thymoides* Cham. & Schltdl.
   Subsect. *Hyssopocuphea* Koehne - Small shrubs, seed margin obtuse or retuse, seeds less than 2 mm long.
   Subsect. *Pachypterus* Koehne- Small shrubs, seed margin very thick and retuse, seeds 2 mm long or more.
   Subsect. *Hilariella* Koehne - Small shrubs, seed margin obtuse, not or only slightly thickened, seeds 2 mm long or more. *C. disperma* Koehne, *C. pseudovaccinium* A. St.-Hil.
   Subsect. *Oidemation* Koehne - Perennial herbs with thick tuberous rhizome to 4.5 cm diameter. *C. brachypoda* T.B. Cavalc., *C. spermacoce* A. St.-Hil.
7. *Trispermum* Koehne 13 spp. - Disc strongly deflexed, subglobose above, excavated ventrally; floral tube 5–11 mm long; ovules 3, rarely 4 or 6. *C. bahiensis* T. B. Cavalc. & S.A. Graham, *C. ericoides* Cham. & Schltdl., *C. flava* Spreng., *C. sessilifolia* Mart.
8. *Pseudocircaea* Koehne 5 spp. - Petals persistent after fruit dehiscence; disc plane or concave above, convex ventrally. *C. lutescens* Koehne, *C. sessiliflora* A. St.-Hil.
9. *Amazoniana* Lourteig 20 (or 21) spp. - Malpighiaceous hairs common; the two dorsal petals generally smaller and wider than the ventral four.
10. *Heterodon* Koehne 28 spp. - Dorsal calyx lobe greatly enlarged; floral tube not bialate within, 12–40 mm long, never true red in color. *C. angustifolia* Jacq. ex Koehne, *C. llavea* Lex., *C. procumbens* Ortega, *C. wrightii* A.Gray.
11. *Melvilla* Koehne 48 spp. - Floral tube thick, 11–33 mm long, often intense red or yellow, calyx lobes often shorter than the appendages.
   Subsect. *Melvilla* - Racemes simple with distinct hypsophylloid bracts, not leaf-like as in the rest of the section. *C. melvilla* Lindl., *C. splendida var. viridiflava S. A. Graham*.
   Subsect. *Pseudolobelia* Koehne - Floral tube 11–15 mm; ovules 48–53.
   Subsect. *Polyspermum* Koehne - Floral tube 20–31 mm; ovules 60–120. *C. micropetala* Kunth.
   Subsect. *Paramelvilla* Koehne - Ovules 14–15; appendages prominent, much longer than the calyx lobes. *C. salvadorensis* (Standley) Standley, *C. schumannii* Koehne.
   Subsect. *Pachycalyx* Koehne - Ovules 4–26; appendages obsolete or shorter than the calyx lobes. *C. fuchsiifolia* A. St.-Hil., *C. pulchra* Moric., *C. teleandra* Lourt.
   Subsect. *Erythrocalyx* Koehne - Calyx lobes bearing long hairs thickened at the base; floral tube 16–28 mm long, always distinctly spurred. *C. heterophylla* Benth., *C. ignea* A. DC., *C. subuligera* Koehne, *C. watsoniana* Koehne.
12. *Leptocalyx* Koehne 7 spp. - Floral tube slender, very slender at the base, 13–34 mm long, often red or yellow. *C. appendiculata* Benth., *C. axilliflora* Koehne.
13. *Diploptychia* Koehne 14 spp. - Floral tube distinctly bialate within dorsally, 10–24 mm long. *C. cyanea* DC., *C. nitidula* Kunth, *C. spectabilis* S. A. Graham.

encountered the same problem of excessive mucilage in the extract that is experienced in nearly all members of the Lythraceae and its sister family Onagraceae (Graham et al. 2005). Difficulties in isolating DNA from the polysaccharides were reduced by use of 6X CTAB following methods cited in Smith et al. (1991). The higher percentage of CTAB and precipitation with ethanol and sodium chloride yielded better results, as did use of liquid nitrogen-ground fresh leaves stored under ultracold conditions for extended periods of one or more years. Some extractions utilized

DNeasy Kits (Qiagen Corp., Valencia, California) following manufacturer's directions. The ITS region was chosen after preliminary sequencing indicated that it provided resolution at the species level in *Cuphea*. The region was amplified using primers 5 and 4 of White et al. (1990) and included sequences from ITS1, 5.8 s, and partial ITS2. Amplification reactions were performed as in Freudenstein (1999), except that the buffer contained 100 mM Tris-HCL (pH 9.2), 30 mM $MgCl_2$, 250 mM KCL and 5% DMSO. The sequencing reactions used the BigDye kit from Applied Biosystems according to the manufacturer's instructions and were run on an ABI Model 377 sequencer. Contigs were assembled using Sequencher (Gene Codes Corporation, Inc., Ann Arbor, MI), aligned in Clustal X (Thompson et al., 1997) and manual adjustments were made in Se-Al (Rambaut 1996, 2000) and in MacClade 4.0 (Maddison and Maddison 2000). No indels were coded. All cladistic analyses were run excluding bases 1–35 and 764–803 at the 5′ and 3′ ends of the sequences to eliminate excessive missing data. A second analysis also excluded bases 70–106 and 234–247 in ITS-1 where alignments were ambiguous. Parsimony analyses were performed in PAUP* 4.0b10 using the same heuristic search strategy as with the morphological data. Branch support was assessed as for the morphological analysis using parsimony jackknife analyses. Model settings used for the maximum likelihood analysis (ML) were selected by the Akaike Information Criterion (AIC) in Modeltest version 3.06 (Posada and Crandall 1998, 2001). An Incongruence Length Difference test (ILD; Farris et al. 1994) was performed in PAUP under the Partition Homogeneity Test option to consider whether the morphological and molecular data sets were sufficiently congruent to be combined for analysis. Morphological character transformation was plotted on one of the 220 trees generated by ITS parsimony analysis using MacClade (Maddison and Maddison 2000). Sequences were deposited in GenBank (Appendix 3) and data matrices and trees were deposited in TreeBASE (study number S1536).

RESULTS

Morphological Analysis. Parsimony analysis, based on 44 informative characters, generated 18,319 trees with tree length = 277, CI = 0.34, RI = 0.65. The strict consensus tree (Fig. 1) presented a monophyletic *Cuphea* with *Pleurophora* as sister and *Woodfordia*, *Pehria*, and *Lythrum* more distantly related. Jacknife values ≥ 50% were limited in *Cuphea* to six small clades. *Cuphea epilobiifolia* appears as sister to the rest of the genus but with less than 50% jacknife support. One large, partially resolved clade (*C. angustifolia* - *C. spermacoce*) comprises 16 endemic North American taxa together with four South American species. A clade of four South American and Caribbean species is sister to the North American clade. There are five other small clades of two to five members. The relationships of 12 species remain unresolved at the penultimate basal node of the genus.

Molecular Analysis. The aligned ITS sequences comprise 803 characters of which 303 are parsimony informative. The ITS region varies from 506 to 629 bp in length, including 158–239 bp for ITS-1 and 133–252 bp for partial ITS-2 sequences. Seventy-five base pairs were excluded in all analyses, 35

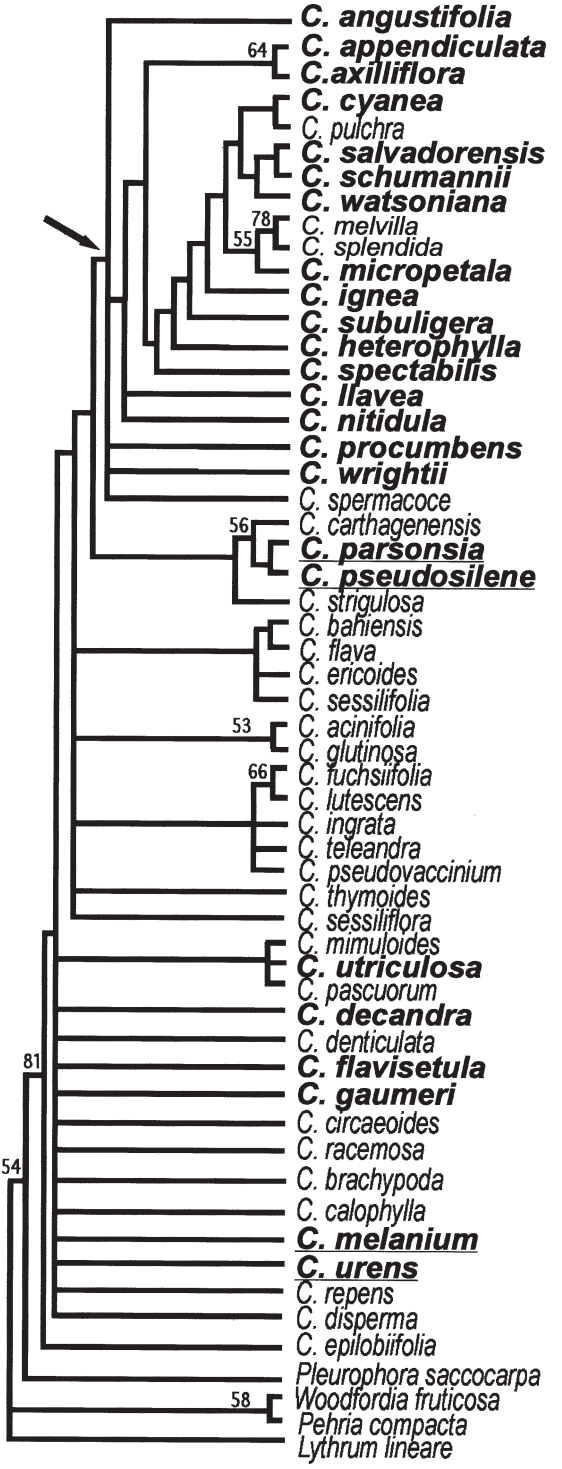

FIG. 1. Strict consensus of 18,319 parsimony trees based on a heuristic search of the morphological data set. Tree length = 277, CI = 0.34, RI = 0.65. Species endemic to North America are in bold typeface, South American species are in plain typeface, and species endemic to the Caribbean are underlined; arrow points to the base of a primarily North American clade. Numbers above the branches are jackknife values ≥ 50%.

at the 5′ end and 40 at the 3′ end, to eliminate excessive missing data. The cladistic analysis yielded 220 most parsimonious trees of length 1739, CI = 0.40, and RI = 0.61. The analysis excluding two small areas totalling 51 base pairs that were difficult to align produced 1044 trees and a consensus tree identical to the more inclusive run.

The strict consensus tree of 220 ITS trees (Fig. 2) differs from that based on morphology by the presence of trichotomies at the base and penultimate base of the genus. Seven clades, with jackknife values between 83% and 100%, are identified. Members of Clade 1 were largely dispersed on the morphological consensus tree. Clade 2 (99% JK) comprises the same 16 North American taxa united in the morphological analysis. The four South American members of the clade present in the morphological analysis move to Clade 3 (*C. spermacoce*), Clade 4 (*C. pulchra*) and Clade 7 (*C. melvilla* and *C. splendida*) in the ITS analysis. The multiple polytomies of the morphological analysis are mostly well resolved with moderate to full support by the ITS data as Clades 3–7, whereas relationships among the North American taxa of Clade 2 remain unknown. *Cuphea epilobiifolia* is associated with *C. melvilla* and *C. splendida* in Clade 7. The three species share a unique synapomorphic 36 base pair deletion; the deletion is not present in *C. repens* or elsewhere among species studied. This was the only apparent informative indel in the aligned data set.

The ITS maximum likelihood tree (Fig. 3) was generated using the GTR+G+I model selected from among 56 models by Modeltest 3.06. Base frequencies were : A = 0.2541, C = 0.2757, G = 0.2439, T = 0.2263; substitution rates A–C = 0.9239, A–G = 1.4533, A–T = 1.4102, C–G = 1.0402, C–T = 2.7876, G–T = 1.000; and gamma distribution shape parameter = 0.8039. The single ITS ML tree (-lnL = 10165.6064) is largely congruent with the strict consensus ITS parsimony tree; clade membership and most sister relationships are identical. The basal trichotomous division of the parsimony consensus tree is absent in the ML tree, where only Clade 1 finds very weak support (53%). The bootstrap support value for Clade 2 (65%) is also much lower than in the consensus parsimony tree (99%) and within Clade 2 all but four branches lack ≥50% support. Clade 3 in the ML tree, comprising five South American/Caribbean species, is sister to the North American Clade 2 but with ≤ 50% bootstrap support. Clades 4–7 have equivalent membership and relationships are congruent in the parsimony and maximum likelihood analyses.

Although the ILD test indicated that the morphological and molecular data sets were significantly incongruent ($p = 0.01$), they were combined and parsimony analysis was performed to inspect the outcome (results not illustrated). Four trees were recovered (tree length = 2251, CI = 0.43, RI = 0.58) that differed only in the relationship of *C. gaumeri* to *C. utriculosa*−*C. decandra* in Clade 1 and relationships among *C. schumannii*, *C. angustifolia*, and *C. llavea* + *C. procumbens* in Clade 2. The strict consensus tree of the combined data was otherwise identical to the ITS ML tree. ILD tests were then run to determine if the datasets were congruent when each of the problematic species was excluded in turn. The morphological and ITS datasets remained incongruent in each instance, suggesting that differences were widespread and not limited to one or a few taxa.

Discussion

Cuphea is monophyletic in all analyses and *Pleurophora* is sister genus. The ITS data, which here provide the first molecular-based assessment of *Cuphea*, reveal a deep branching event represented by a trichotomy of moderately- to well-supported branches and the presence of seven well-supported major clades (Fig. 2). The ITS phylogenies are substantially at odds with the relationships inferred by Koehne's classification (1903). Clade 1 consists of the two sections that constitute subgenus *Cuphea* (83% JK) plus one species, *C. circaeoides*, from subg. *Bracteolatae*. The second branch of the trichotomy (71% JK), which includes the majority of the species investigated, and the third branch (99% JK) include species of the alternative subgenus *Bracteolatae*. Sections *Brachyandra*, *Euandra*, and *Melvilla* are exceptionally polyphyletic, the members of the sections being widely dispersed in four, three, and five clades respectively (Figs. 2, 3).

Within Clade 1, *C. mimuloides* and *C. pascuorum* represent section *Archocuphea*, regarded by Koehne (1886, 1903) as the ''primitive'' base of the genus. In the ITS phylogeny these species occupy derived positions at the apex of an unsupported grade subtended by species from sections *Cuphe*a and *Brachyandra*. The terminal branches of the *C. pascuorum* - *C. circaeoides* clade are among the longest (Fig. 3), suggesting either great age or rapid evolution in the lineage. The very short branches of the *C. utriculosa* − *C. gaumeri* clade, on the other hand, suggest more recent evolution following introduction of this lineage into North America. *Cuphea circaeoides* in Clade 1 was considered by Koehne (1903: 121) to be the most distinctive species of the genus and morphologically intermediate between the subgenera. He

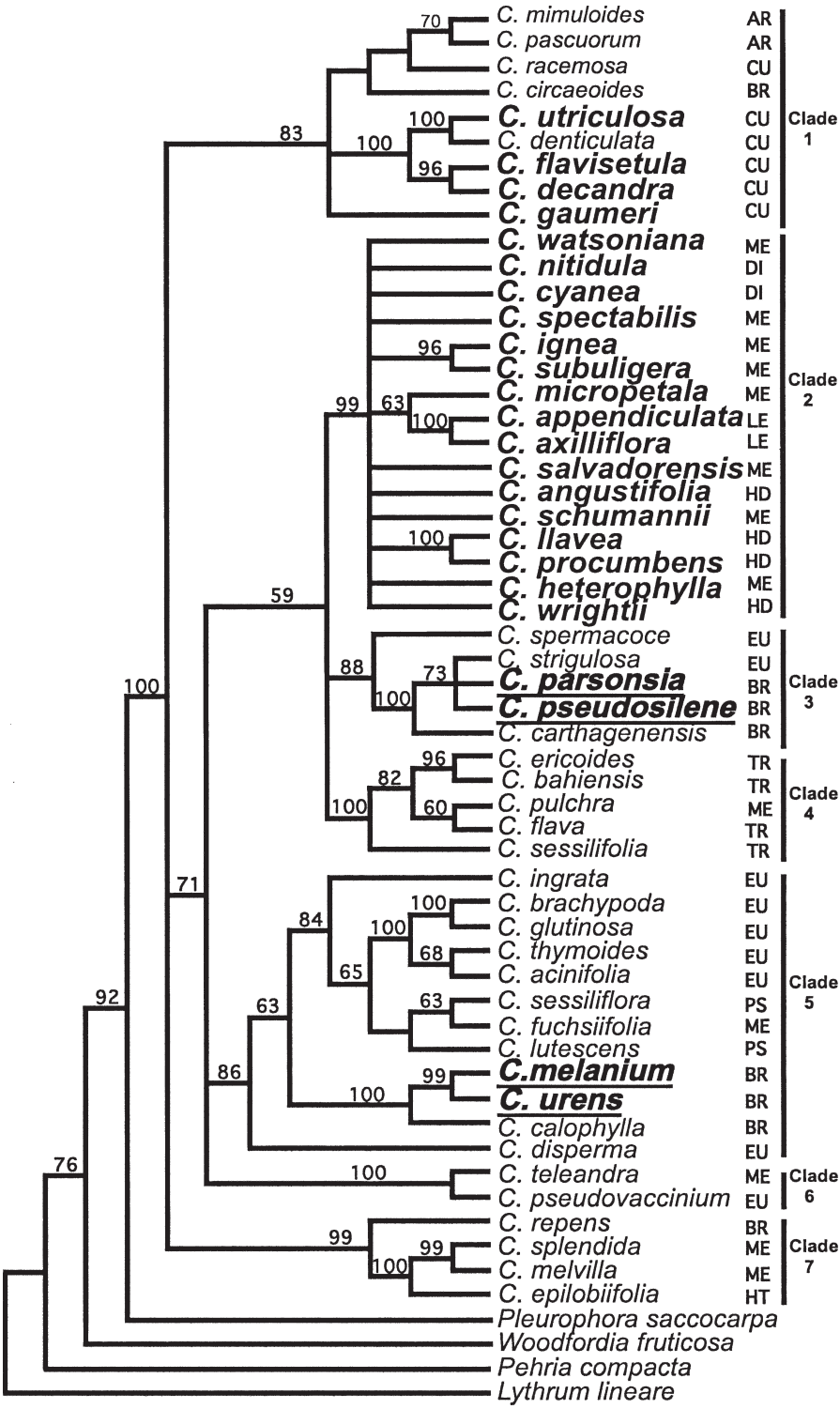

FIG. 2. Strict consensus of 220 parsimony trees based on a heuristic search of the ITS data set. Tree length = 1739, CI = 0.40, RI = 0.61. Species endemic to North America are in bold typeface, South American species are in plain typeface, and species endemic to the Caribbean are underlined. Numbers above the branches are jackknife values ≥ 50%. Taxonomic sections are: AR= *Archocuphea*; CU = *Cuphea*; ME = *Melvilla*; DI = *Diploptychia*; LE = *Leptocalyx*; HD = *Heterodon*; EU = *Euandra*; BR = *Brachyandra*; TR = *Trispermum*; PS = *Pseudocircaea*; HT = *Heteranthus*.

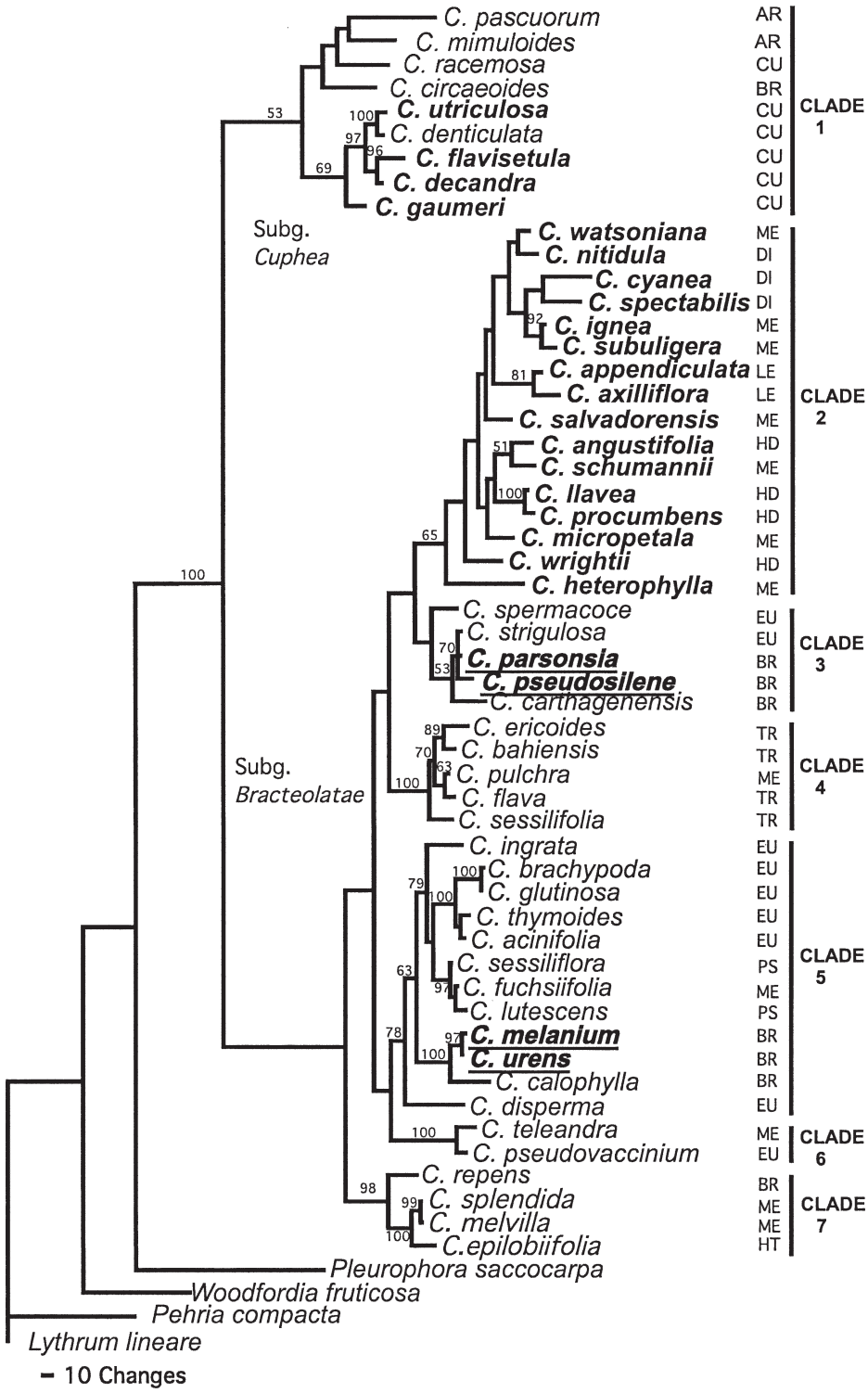

FIG. 3. Phylogram of the single tree derived from a maximum likelihood search of the ITS data using the GTR + G + I model of evolution. Species endemic to North America are in bold typeface, South American species are in plain typeface, and species endemic to the Caribbean are underlined. Numbers above the branches are bootstrap values ≥ 50%. Taxonomic sections are: AR= *Archocuphea*; CU = *Cuphea*; ME = *Melvilla*; DI = *Diploptychia*; LE = *Leptocalyx*; HD = *Heterodon*; EU = *Euandra*; BR = *Brachyandra*; TR = *Trispermum*; PS = *Pseudocircaea*; HT = *Heteranthus*.

assigned the species to subg. *Bracteolatae* section *Brachyandra* rather than to subg. *Cuphea* because it possessed minute bracteoles (Table 1). Placement in subg. *Cuphea* (Clade 1) based on ITS brings it together with the other small, partially opposite-flowered, many-seeded species that characterize the subgenus, close to *C. mimuloides* and *C. pascuorum*, with which it shares the derived basic chromosome number of 11 (Graham and Cavalcanti 2001).

Relationships in Clade 2 among the North American species classified in sections *Heterodon*, *Leptocalyx*, and *Diploptychia* and four subsections of section *Melvilla* remain unresolved or with low support values. Efforts are currently focused on expanding the number of taxa and gene sequences within this large clade and its South American relatives in order to clarify relationships and decipher the history of the radiation of *Cuphea* in North America (Ghebretinse and Barber pers. comm.).

Clades 3–6 illustrate the polyphyletic nature of sections *Euandra* and *Brachyandra*, and their close relationship to sections *Trispermum* and *Pseudocircaea*, as suggested by earlier morphologically-based studies (Graham, 1988, 1998b; Graham and Graham 1971). Species of the large section *Euandra* occur in Clades 3, 5, and 6 (Fig. 2) together with species of sections *Brachyandra*, *Pseudocircaea*, and *Melvilla*. Members of section *Brachyandra* appear in Clades 1, 3, 5, and 7 with species from sections *Archocuphea*, *Cuphea*, *Euandra*, and *Melvilla*. The majority of species currently classified in sections *Euandra* and *Brachyandra* have a generalized *Cuphea* floral form: small, predominantly green floral tubes with six subequal petals and limited or no spur development. Taxonomically, the two sections are separated only by the deep stamen insertion and included stigma of section *Brachyandra* (Table 1). Stamen and stigma levels (Chars. 29 and 30) reflect the type of breeding system in *Cuphea* since predominantly outcrossing species have long-exserted stamens and stigma and predominantly selfing species have included stamens and an anther-level stigma. Results from the ITS analysis support evidence from pollen and seed morphology (Graham 1998b) that suggests species of section *Brachyandra* have been classified together on the basis of convergent floral characters that are the result of repeated evolution of autogamy within this basically xenogamous genus. Section *Pseudocircaea* is embedded within Clade 5 as sister to part of section *Euandra* with which it shares all the plesiomorphic characteristics defining section *Euandra*. It differs in having persistent petals, the single synapomorphy by which the section is recognized.

Clade 4 comprises the species of a paraphyletic section *Trispermum*. Unlike other sections, section *Trispermum* is clearly defined morphologically by a suite of unique morphological synapomorphies: cordiform seed shape, presence of a nectary cavity, interaperturate thickenings on the inner pollen wall, and three seeds per capsule. The support value for the clade in both parsimony and maximum likelihood analyses is 100%. *Cuphea pulchra*, currently classified in section *Melvilla* because of its elongate red-orange floral tubes, appears exotic in section *Trispermum*, but its alliance with the small, green to purple-flowered species of section *Trispermum* was indicated prior to the molecular results based on possession of all the sectional morphological synapomorphies excepting that seeds number 4–7 (Graham 1990). The ITS data validate the relationship of *C. pulchra* with species of section *Trispermum*.

Section *Melvilla*, defined by thick, long, intensely-colored floral tubes, is the most polyphyletic of the sections, with species dispersed in Clades 2, 4, 5, 6, and 7. Seven species compose part of the monophyletic North American Clade 2. The rest of the representatives of this section, *C. pulchra*, *C. fuchsiifolia*, *C. teleandra*, *C. melvilla* and *C. splendida*, find nearest relatives among species with smaller green- or green/fuchsia-tubed flowers. In Clade 5, *Cuphea fuchsiifolia* as sister to *C. lutescens* or *C. sessiliflora* is supported morphologically by shared presence of persistent petals and diporate pollen. In Clade 6, *Cuphea teleandra*, with the elongated red floral tubes of section *Melvilla*, also possesses the deeply inserted stamens of section *Brachyandra*. ITS results, however, place it with the small green-flowered *C. pseudovaccinium* in section *Euandra*. The disposition of members of section *Melvilla* in the ITS analyses among several clades dominated by smaller, green-flowered species demonstrates that larger red floral tubes are not restricted to a single lineage but have evolved repeatedly in the genus from species with smaller, green-tubed flowers; in this study large red tubes evolved a minimum of four times (Fig. 3 and 5A; Graham 1990: 27). Convergent development of thick, long, red-tubed flowers is most certainly related to selection for large bee and bird pollination from the more prevalent, less conspicuous flowers that are pollinated by a variety of small-insect visitors.

Clade 7 includes two members of section *Melvilla* and *Cuphea epilobiifolia* in section *Heteranthus*. They share a unique 36 bp deletion in ITS-1. The sister relationship is surprising given their very different floral morphology, habit, and habitat. *Cuphea repens*, also in Clade 7, lacks the 36 bp deletion. Although described in section *Brachyandra*, it con-

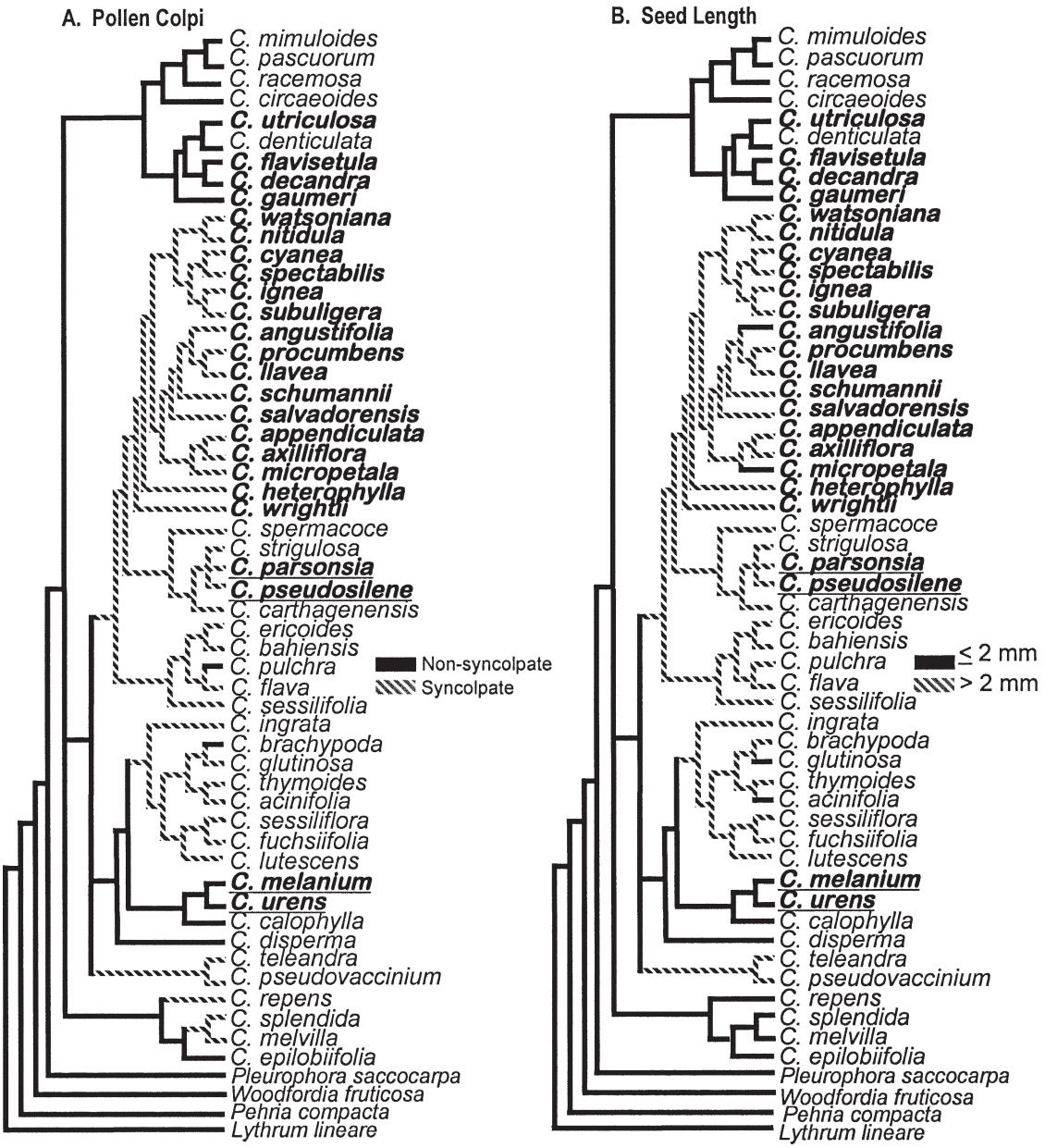

FIG. 4. A, B. Optimization mapping of selected morphological characters on one of 220 ITS parsimony trees. A. Pollen colpi. B. Seed length.

forms morphologically to section *Amazoniana*. The position of *C. repens* in Clade 7 and Clade 7 itself may be the result of long branch attraction due to underrepresentation of sections *Heteranthus* and *Amazoniana* in the phylogeny.

Morphological Support for Clades. Among the clades recognized by ITS, Clade 1 is most easily characterized morphologically although only two character states, absence of bracteoles (char. 11) and presence of seed oils with C18:2 as the predominant fatty acid (char. 44), are uniquely synapomorphic. Opposite flowers (char. 9) occur in all species of Clade 1 but appear as well in *C. spermacoce* and *C. epilobiifolia* in Clades 3 and 7 (subg. *Bracteolatae*) as a polymorphic state. Clade 1 otherwise is supported by a suite of homoplastic character states that are found in one or more additional clades among the species classified in subgenus *Bracteolatae*. These characters include: opposite, green-tubed flowers less than 9 mm long,

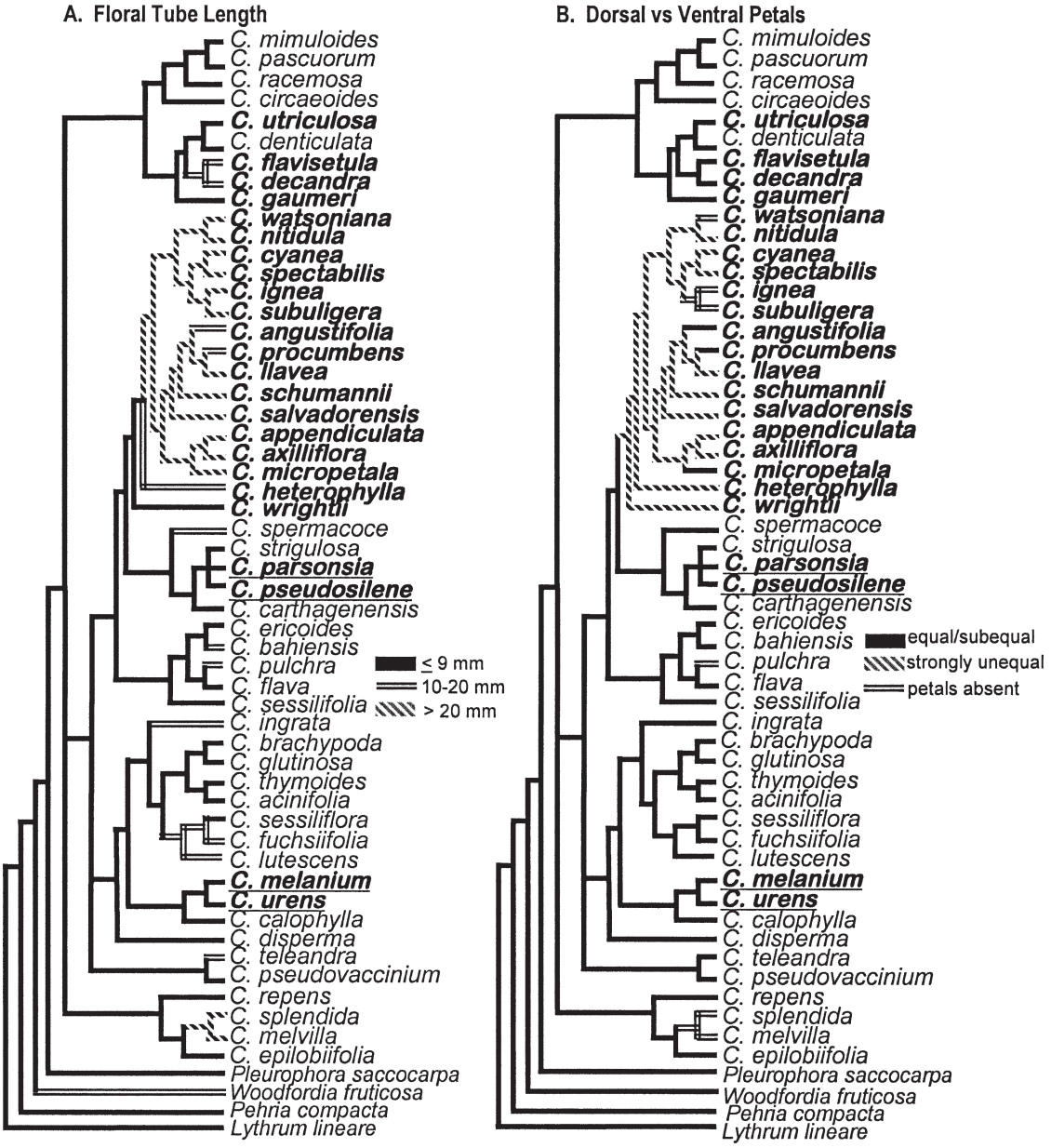

FIG. 5. A, B. Optimization mapping of selected morphological characters on one of 220 ITS parsimony trees. A. Floral tube length. B. Dorsal vs. ventral petals; length of dorsal petals equal or subequal to length of ventral petals or much larger than the ventral petals, or all petals absent.

floral spurs absent or poorly developed, petals equal or subequal in size, non-syncolpate pollen, and numerous small seeds (Fig. 4B).

Clades 2–7 encompass greater morphological diversity than Clade 1, but all characters are homoplastic. Syncolpate pollen (char. 34; Fig. 4A) and seeds greater than 2 mm long (char. 40; Fig. 4B) were gained at the base or near the base of the genus and are held by the majority of the species. Changes along the branch to the endemic North American Clade 2 and many of the changes within Clade 2 relate to increase in size of vegetative and floral parts over those of South American species, e.g., the enlargement of leaves and longer petioles, pedicels, epicalyx appendages, and floral tubes (Fig. 5A). Dorsal and ventral petals (char. 22) in Clade 2 become disparate in size and orientation (Fig. 5B). Dorsal petals become larger and are held erect at right angles to the floral tube while ventral petals are reduced and positioned

horizontally in the same plane as the floral tube, or are lost. Petals are lost in several red, large-flowered species of Clades 2 and 7 (i.e., in *C. ignea*, *C. melvilla*, *C. splendida*, *C. pulchra*, *C. subuligera*, and *C. watsoniana*), although, rarely, minute petals may occur within a population. The enhanced color of the floral tubes presumably replaces petals as a pollinator attractant. Elongation of the floral tube (char. 12) occurred multiple times in Clades 2–7 on both continents. Red floral tube color (char. 14) evolved at least four times among the species in this study (in Clades 2, 4, 6, and 7) indicating a greater lability of flower color than is implied by the current taxonomic classification in which species with red floral tubes are grouped in section *Melvilla*.

Short, deeply inserted stamens and stigmas (chars. 25, 29, 30), linked to acquisition of facultative self-fertilization, evolved independently minimally five times, once in Clade 1 and four times in Clades 2–7 (not illustrated).

Pollen morphology varies extensively in *Cuphea* (A. Graham et al. 1985; Graham 1998b; Graham and Graham 1971). Clade I is characterized by non-syncolpate, exceptionally small, psilate grains lacking protruding pores. (A. Graham et al. 1985; Graham 1998b; Graham and Graham 1971). This type is also found in some members of Clades 4 and 5. Syncolpate, coarsely-striated pollen grains with protruding pores occur in 10 of 16 taxa of Clade 2 and link the clade to Clade 3. Interaperturate wall thickenings (char. 33) are restricted to Clade 4, although they are faint in *C. pulchra* and lost in *C. bahiensis*. Diporate pollen (char. 31) is synapomorphic for Clade 6 and for subclade *C. ingrata*-*C. lutescens*, excepting *C. brachypoda* of Clade 5. Additional diporate taxa in section *Melvilla* subsection *Pachycalyx* need to be included in future molecular analyses to confirm or refute the homoplastic nature of the diporate pollen condition. An earlier morphological cladistic analysis recognized a single origin for this unusual character state (Graham and Graham 2000).

Small seeds (char. 40) characterize the small flowers (≤ 9 mm long) of Clade 1 and at the opposite extreme also occur in some large-flowered (20–35 mm long) species of Clades 2, 5, 7 (Fig. 4B). Small seeds are ancestral in Clade 1 but represent reversals from larger seeds elsewhere in the genus. Narrowly winged seeds are infrequent in *Cuphea*, being synapomorphic for *C. strigulosa*-*C. carthagenensis*, but they also occur in Clade 1 in *C. racemosa* and *C. circaeoides* and in Clade 5 in *C. acinifolia* and *C. glutinosa*, thus limiting the use of this character state for predicting close phylogenetic relationship.

The seeds of *Cuphea* are unique in Lythraceae and among all angiosperms for the diversity of seed oils produced (Graham and Kleiman 1987). Seed oil composition predominates in medium-chain, fully saturated fatty acids ranging from caprylic acid (C8:0) to linolenic acid (C18:3) depending on the species (Graham 1989b). Although the character state is still unknown for ca. one-third of the species in this study, data are sufficient to indicate that linoleic acid (C18:2), the type common to most angiosperms, prevails in seed oils of species in Clade 1 and in no other part of the genus; lauric acid (C12:0) characterizes Clades 5 and 6; lauric acid and myristic acid (C14:0) co-dominate in Clade 3, and the greatest diversity of fatty acids and shortest fatty acid chain lengths occur in Clade 2. In Clade 2, seeds produce either C12:0 or C10:0 fatty acid as the dominant component of the seed oil, excepting in *C. cyanea* (C8:0), *C. salvadorensis* (C14:0), and *C. spectabilis* (C18:3). Clades 4 and 7 are too poorly known to be characterized. Because chain length and saturation of fatty acids are affected by temperature (Crane et al. 2003), the selective force leading to the array of fatty acids in seed oils in the North American clade may be the different temperatures that prevail at different altitudes in mountainous western Mexico. In contrast, fatty acids are less diverse among *Cuphea* that have evolved in the more climatically and topographically uniform cerrado of eastern Brazil.

Biogeographic Implications. The ITS data support the hypothesis that the genus originated and first diversified in eastern Brazil. Lack of complete resolution at the deepest nodes prevents a full understanding of the earliest evolutionary pathways, however, early multiple branching events generated at least one lineage that gave rise to species that subsequently radiated into what is now southeastern Mexico (*C. utriculosa*−*C. gaumeri*, Clade 1), and another (Clade 2) that diversified in the topographically and climatically diverse mountain chains of southern and western Mexico. The occurrence of the genus in the Greater Antilles involves at least two dispersal events (see Clades 3 and 5), and possibly up to four or more (Graham 2003).

The members of Clade 3 show an exceptional capacity for geographic expansion. The weedy diploids *C. carthagenensis* ($2n = 16$) and *C. strigulosa* ($2n = 16$) are two of the most widespread species in the genus. Both occur in eastern and western South America and have invaded the wet, coastal areas of the southern United States after long-distance dispersal (Graham 1975; Wunderlin and Hansen 2003). *Cuphea carthagenensis* also has long been

known from the Hawaiian Islands and several islands of the South Pacific. *Cuphea parsonsia* ($2n = 32$) and *C. pseudosilene* ($2n = 64$) are self-fertilizing endemic species of the Antilles, considered probable polyploid descendants of *C. carthagenensis* or a similar ancestral species of the clade (Graham and Cavalcanti 2001; Graham 2003).

This first survey of molecular and morphological evolution in *Cuphea* demonstrates the utility of ITS data to test phylogenetic relationships predicted by morphology. The results confirm the monophyly of the genus with *Pleurophora* as sister and South America as the primary center of diversification. Phylogenetic analysis of the ITS sequences indicates multiple lineages diverged near the base of the genus but the exact pattern remains unclear. Several major clades, including a large monophyletic North American group, are well supported. The clades are not congruent with the classification of the genus. Although sampling of additional taxa and genes is needed to confidently hypothesize the phylogeny of the genus and produce a classification more in line with the relationships uncovered, these molecular data offer initial insight into the history of this most speciose representative of the Lythraceae.

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APPENDIX 1. List of morphological characters employed in construction of character matrix (Appendix 2) for cladistic analysis.

1. HABIT: 0 = perennial; 1 = annual.
2. HEIGHT: 0 = 0.75 m or more; 1 = less than 0.75 m.
3. PETIOLES OF LARGEST LEAVES: 0 = 2 mm or more; 1 = less than 2 mm.
4. LEAF BASE: 0 = acute to decurrent; 1 = acute to rounded; 2 = rounded to cordate.
5. LARGEST LEAF LENGTH: 0 = 40 mm or more; 1 = less than 40 mm.
6. MALPIGHIACEOUS HAIRS: 0 = absent; 1 = present.
7. INFLORESCENCE POSITION: 0 = terminal, distinct; 1 = axillary, indistinct.
8. HYPSOPHYLLOID INFLORESCENCE BRACTS: 0 = absent; 1 = present.
9. FLOWER ARRANGEMENT: 0 = opposite; 1 = alternate.
10. PEDICEL LENGTH: 0 = avg. 0–4 mm; 1 = avg. greater than 4 mm.
11. BRACTEOLES: 0 = present; 1 = absent.
12. FLORAL TUBE LENGTH: 0 = 9 mm or less; 1 = 10–20 mm; 2 = consistently greater than 20 mm.
13. FLORAL TUBE WIDTH AT ANTHESIS: 0 = 4 mm or less; 1 = greater than 4 mm.
14. FLORAL TUBE COLOR: 0 = green to purple-red; 1 = red, red-orange, or yellow.
15. FLORAL TUBE DORSAL SHAPE: 0 = horizontal to concave; 1 = convex.
16. FLORAL TUBE BASE: 0 = spurless, no measurable extension beyond the pedicel at 40x; 1 = spurred, floral tube extending 0.5 mm or more beyond the pedicel.
17. FLORAL TUBE DISTAL MARGIN: 0 = blunt; 1 = oblique, ventral margin extended beyond the dorsal margin.
18. CALYX LOBES: 0 = equal to subequal; 1 = unequal, the dorsal lobe longer than the other 5.
19. APPENDAGES: 0 = absent; 1 = present as thickenings, only margin free; 2 = elongate, 1/2 as long as calyx lobes or longer.
20. INTERNAL ALAE: 0 = absent; 1 = present.
21. PETALS: 0 = present, caducous; 1 = present, persistent; 2 = absent.
22. DORSAL VS. VENTRAL PETALS: 0 = equal or subequal; 1 = distinctly unequal; 2 = petals absent.
23. PETAL/FLORAL TUBE LENGTH: 0 = 1/1–1/3; 1 = less than 1/3.
24. DORSAL PETAL COLOR: 0 = white to light or dark purple or fuchsia; 1 = red-orange; 2 = yellow; 3 = petals absent.
25. STAMEN BASE: 0 = lower 1/2 of floral tube, near ovary; 1 = at 1/2–2/3 of the floral tube; 2 = higher than 2/3 of the floral tube.
26. STAMEN INSERTION: 0 = all 11 at same level; 1 = 2 dorsalmost deepest; 2 = 2 dorsalmost absent.
27. STAMEN NUMBER: 0 = 6 or 12; 1 = 11; 2 = variably 4–9.
28. STAMEN LENGTH: 0 = stamens of distinctly alternating lengths; 1 = stamens of approximately equal length.
29. ANTHER POSITION: 0 = 5–11 reaching or exceeding the sinuses of the calyx lobes; 1 = deep in floral tube, not reaching the sinuses.
30. STIGMA: 0 = surpassing anther level; 1 = at anther level.
31. POLLEN PORE NUMBER: 0 = 3; 1 = 2.
32. POLLEN PORE PROTRUSION: 0 = non-protruding; 1 = strongly protruding.
33. POLLEN INTERAPERTURATE THICKENINGS: 0 = absent; 1 = present.
34. POLLEN COLPI: 0 = non-syncolpate; 1 = syncolpate.
35. POLLEN EXINE: 0 = psilate to scabrate; 1 = rugulate to uniformly finely to moderately coarsely striate; 2 = coarsely striate at pores.
36. POLLEN DIAMETER: 0 = 21 μm or less; 1 = 22 μm or greater.
37. OVARY LOCULES: 0 = equal in size; 1 = one locule reduced.
38. PLACENTA: 0 = retained in capsule; 1 = emergent.
39. OVULE NUMBER: 0 = 25 or more; 1 = less than 25.
40. SEED LENGTH: 0 = 2 mm or less; 1 = greater than 2 mm.
41. SEED SHAPE: 0 = elongated pyramidal; 1 = bilateral compressed.
42. SEED MARGIN: 0 = rounded, non-winged; 1 = thinned, narrowly winged.
43. SEED HAIRS: 0 = straight; 1 = spirally twisted.
44. SEED OIL DOMINANT FATTY ACID: 0 = C18:2; 1 = C14:0; 2 = C12:0; 3 = C10:0; 4 = C8:0; 5 = C18:3.
45. NECTARIFEROUS TISSUE: 0 = part of inner lining of floral tube; 1 = separated from inner lining as a cup or ring surrounding base of ovary; 2 = separated from inner lining of floral tube as a free-standing unilateral organ, the "disc."
46. NECTARIFEROUS "DISC": 0 = absent; 1 = plane to deflexed away from or erect over the dorsal side of the ovary; 2 = subglobose with distinct ventral cavity.

APPENDIX 2. Matrix of morphological character states employed in cladistic analysis. a=0,1; b=1,2; c=0,2; ?= missing data.

	Character								
Species	5	10	15	20	25	30	35	40	46
OUTGROUPS									
Lythrum lineare	00110	0a0a0	00000	00020	00000	00aaa	00001	10000	000010
Pehria compacta	00010	01020	00000	00010	00000	00100	00000	10000	101000
Pleurophora saccocarpa	00100	01010	00000	00020	00000	01100	00000	110a0	101010
Woodfordia fruticosa	00120	01021	01010	01010	00110	00000	00000	10000	101000
CUPHEA									
C. acinifolia	01111	01010	00000	10010	00001	11000	10011	11110	111221
C. angustifolia	00100	01011	01000	10111	00001	11000	01012	01100	101321
C. appendiculata	00110	11011	02000	11020	01012	11000	01012	11111	101221
C. axilliflora	00110	11011	02000	11020	01012	11000	01012	11111	101221
C. bahiensis	01121	00110	01000	10010	00001	11000	00010	11111	101?22
C. brachypoda	01111	01011	00000	10010	00001	11000	00000	01111	101?21
C. calophylla	01ab0	0a010	00000	00010	00001	11a11	00000	01110	101221
C. carthagenensis	01011	01010	00000	10010	00001	11111	01012	11111	111221
C. circaeoides	11000	001a1	00001	10a10	00101	11111	00001	01110	111?21
C. cyanea	00020	00011	02010	11011	01102	11000	00011	01111	101421
C. decandra	01101	00000	a1000	00010	00001	11000	00001	11110	101021
C. denticulata	01101	00001	10000	00010	00001	11100	00001	11110	101021
C. disperma	01101	01010	00000	10010	00001	11000	00000	01110	101221
C. epilobiifolia	00100	101a0	00000	11020	00001	11000	00000	11110	101121
C. ericoides	01101	01010	0a000	11020	00001	11000	00110	11111	101?22
C. flava	01121	00010	00010	10010	00021	11000	00110	11111	101?22
C. flavisetula	01101	00001	11000	00010	00001	11001	00001	11110	101?11
C. fuchsiifolia	00020	01011	01000	10010	10001	11000	10011	11111	101?21
C. gaumeri	01111	01001	10000	10010	00001	11000	00001	11110	101?11
C. glutinosa	01111	01010	00000	11010	00001	11000	10011	11110	111221
C. heterophylla	00010	00011	01011	11010	01012	11000	00012	11111	101221
C. ignea	01001	01011	02011	11020	22132	11000	00012	11111	101321
C. ingrata	01101	01010	01000	10010	00001	11001	10011	11111	101221
C. llavea	01100	01010	02000	10120	01012	11000	01012	01111	101321
C. lutescens	00020	01011	01000	10020	10001	11000	10010	11111	101221
C. melanium	01121	01010	00000	00010	00001	11011	00000	01110	101221
C. melvilla	00000	10111	02111	1a020	22132	11000	00011	11100	101221
C. micropetala	00000	00011	02111	1a020	00102	11000	00011	11100	101321
C. mimuloides	11101	01001	10000	00000	00001	11111	00000	01100	101011
C. nitidula	00010	01011	02000	10021	01002	11000	01011	11111	101321
C. parsonsia	01111	01010	00000	00010	00001	12?11	01012	11111	111221
C. pascuorum	11121	010a1	10000	00010	00001	11000	00000	01100	101?11
C. procumbens	01010	01011	11000	101b0	00001	11000	01012	11111	101321
C. pseudosilene	01101	11011	00000	00010	00001	10111	01012	11111	111?21
C. pseudovaccinium	00121	01010	00000	10010	00001	11000	10011	11111	101221
C. pulchra	00120	00111	01011	11010	cc132	11000	01100	01111	101?21
C. racemosa	01010	01001	10a00	00a10	00001	11100	00001	11100	111021
C. repens	01101	11011	00000	10010	00001	11011	00010	01110	101?21
C. salvadorensis	00000	01011	02111	10020	01012	11000	00011	01111	101121
C. schumannii	00000	01011	02111	11020	01002	11000	00011	01111	101321
C. sessiliflora	01011	01010	00000	10010	10001	11001	10010	11111	101?21
C. sessilifolia	01111	01010	00000	10010	00010	11000	00110	11111	101122
C. spectabilis	00110	00011	02110	11a11	01012	11000	01012	11111	101521
C. spermacoce	01110	010a1	01000	10010	00001	11000	01011	11111	101?21
C. splendida	01000	10111	02111	10020	22132	11000	00011	11100	101?21
C. strigulosa	01101	01010	00000	10010	00001	11000	01012	11111	111121
C. subuligera	00000	00011	020a1	11020	0caa2	11000	01012	11111	101221
C. teleandra	00101	01010	01011	1a010	00011	11000	10011	11111	101?21
C. thymoides	01111	01010	00000	00101	00001	11000	10011	11111	101211
C. urens	01121	01011	00000	10010	00001	11011	00000	01110	101211
C. utriculosa	01101	01001	10000	00010	00001	01110	00000	01100	101001
C. watsoniana	00000	01011	02011	1a020	0ca02	11000	00011	01111	101?11
C. wrightii	11010	00010	00000	10110	01001	11000	01012	11111	101211

APPENDIX 3. Vouchers for species sequenced for this study with GenBank accession numbers. All vouchers are deposited at the Herbarium, Missouri Botanical Garden (MO) unless otherwise indicated. (CAS) = Herbarium, California Academy of Science; (CEN) = Herbário, CENARGEN, EMBRAPA, Brasília, Brazil; (MICH) = University of Michigan Herbarium; (NY) = Herbarium, New York Botanical Garden; (SYS) = Herbarium, Zhongshan University, Guangzhou, China; (US) = United States National Herbarium.

Cuphea acinifolia, Brazil: *Graham 951*, AY910696; *C. angustifolia*, Mexico: *Graham 677*, AY910730; *C. appendiculata*, Mexico: *Graham 1081*, AY910725; *C. axilliflora*, Guatemala: *Graham 1018*, AY910726; *C. bahiensis*, Brazil: *Cavalcanti et al. 2480*, AY910718; *C. brachypoda*, Brazil: *Cavalcanti et al. 2468*, AY910701; *C. calophylla*, Brazil: *Graham 923*, AY910724; *C. carthagenensis*, Mexico: *Graham 840*, AY910705; *C. circaeoides*, Brazil: *Mori et al. 9967* (NY), AY910715; *C. cyanea*, Mexico: *Graham 1060*, AY910732; *C. decandra*, Guatemala: *Graham 1015*, AY910743; *C. denticulata*, Venezuela: *Fryxell 4381*, AY910739; *C. disperma*, Brazil: *Cavalcanti et al. 2285*, AY910700; *C. epilobiifolia*, Costa Rica: *Graham 1097*, AY910710; *C. ericoides*, Brazil: *Cavalcanti 337* (CEN), AY910717; *C. flava*, Brazil: *Cavalcanti et al. 2321*, AY910720; *C. flavisetula*, Mexico: *Hernandez & Vasquez 53* (US), AY910742; *C. fuchsiifolia*, Brazil: *Cavalcanti et al. 2308*, AY910699; *C. gaumeri*, Mexico: *Cabrera 9093*, AY910741; *C. glutinosa*, USA: *Thieret s.n. in 1972*, AY910697; *C. heterophylla*, Mexico: *Graham 998*, AY910729; *C. ignea*, Cultivated, no voucher, AY910735; *C. ingrata*, Brazil: *Cavalcanti et al. 2282*, AY910703; *C. llavea*, Mexico: *Graham 906*, AY910712; *C. lutescens*, Brazil: *Cavalcanti et al. 2200*, AY910698; *C. melanium*, Dominican Republic: *Zanoni 31877*, AY910694; *C. melvilla*, Brazil: *Anderson 9268*, AY910711; *C. micropetala*, Mexico: *Graham 1051*, AY910727; *C. mimuloides*, Mexico: *McVaugh 25272* (MICH), AY910714; *C. nitidula*, Mexico: *Graham 1011*, AY910733; *C. parsonsia*, Cuba: *Stevens 23628*, AY910704; *C. pascuorum*, Brazil: *Cavalcanti et al. 2381*, AY910745; *C. procumbens*, Mexico: USDA PI-534709, AY910707; *C. pseudosilene*, Cuba: *Graham 1117*, AY910719; *C. pseudovaccinium*, Brazil: *Cavalcanti et al. 2276*, AY910738; *C. pulchra*, Brazil: *Cavalcanti et al. 323* (CEN), AY910722; *C. racemosa*, Mexico: *Graham 689*, AY910744; *C. repens*, Bolivia: *Gutiérrez 1186*, AY910713; *C. salvadorensis*, Mexico: *Graham 829*, AY910721; *C. schumannii*, Mexico: *Graham 1090*, AY910731; *C. sessiliflora*, Brazil: *Cavalcanti et al. 2212*, AY910723; *C. sessilifolia*, Brazil: *Cavalcanti 447* (CEN), AY910736; *C. spectabilis*, Mexico: *Reveal 4339*, AY910716; *C. spermacoce*, Brazil: *Cavalcanti et al. 2176*, AY910706; *C. splendida* var. *viridiflava*, Bolivia: *Graham 1109*, AY910709; *C. strigulosa*, Brazil: *Cavalcanti et al. 2324*, AY910702; *C. subuligera*, Mexico: *Breedlove 41116* (CAS), AY910728; *C. teleandra*, Brazil: *Cavalcanti et al. 2316*, AY910737; *C. thymoides*, Brazil: *Romero et al. 880*, AY 910695; *C. urens*, Dominican Republic: *Zanoni & Garcia 41811*, AY910693; *C. utriculosa*, Mexico; *Graham 1086*, AY910740; *C. watsoniana*, Mexico: *Graham 1043*, AY910734; *C. wrightii*, Mexico: USDA PI-534818, AY910708. *L. lineare* L., USA: *Brown s.n. in 1996*, AY910748. *Pehria compacta* (Rusby) Sprague, Venezuela: P. Berry s.n. in 1979, AY905430. *Pleurophora saccocarpa* Koehne, Argentina: *Schinini et al. 22640*, AY910746. *Woodfordia fruticosa* (L.) S. Kurz, Cultivated: *S. Tang 99070504* (SYS), AF201692.