DIVERSITY, PHYLOGENY, AND EVOLUTION IN THE MONOCOTYLEDONS
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Phylogenetic Relationships and Species Delimitation in *Canna* (Cannaceae)

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Abstract—Canna lilies are a conspicuous component of tropical and subtropical humid Neotropics where they are native, and the Asian Paleotropics where they have been introduced. Cannas have been cultivated as a food item (rhizome), for wrapping (leaves), and as beads (seeds) for millennia by indigenous people. In both tropical and temperate regions they have a long history as ornamental plants as well. With only a few dozen taxa in a single genus, Cannaceae has much lower generic and species diversity than its sister family, Marantaceae (550 species in 31 genera). Parsimony and Bayesian analyses of nuclear ribosomal internal transcribed spacer (ITS) and chloroplast non-coding sequence data (*trnE-T* intergenic spacer and *rpL16* intron) were used to infer evolutionary relationships among species. Potential causes of non-monophyly of nuclear ITS haplotypes and conflict between nuclear and plastid phylogenies for some samples are discussed. Chloroplast (*rbcL*, *ndhF*) DNA data indicate a North American taxon, *Canna flaccida*, is sister to all other species in the genus. Phylogenetic analyses are consistent with the hypothesis of a South American origin for the genus, followed by dispersal and migration to North and Central America, and the Caribbean.

Keywords—Bayesian inference, biogeography, cpDNA, ITS, molecular phylogeny.

Cannaceae has long been recognized as a distinct, monophyletic group closely allied to the Marantaceae whether based on results of phylogenetic analyses of morphological (Kress 1990) or molecular (Kress et al. 2001) data. Like Marantaceae, cannas grow in tropical and subtropical climates around the world with a native distribution restricted to the Neotropics, sometimes reaching into warm temperate regions. The family consists solely of the New World genus *Canna* L. Cannaceae (including their sister family Marantaceae) were recognized in 1821 by Link based on their distinctive floral morphology (single stamen, separate style).

Cannas form a strongly cohesive group based on morphological characteristics, yet the infrageneric taxonomy has proved difficult. Rogers (1984) described the group as, “a distinctive monogeneric family with a problem-free circumscription, in contrast with its internal taxonomy and nomenclatural disarray.” Various characters have been used by taxonomists to discriminate between the species, including the number of staminodes, the length of the corolla tube (including the adnate staminodes), flower color, the presence of spots on the flowers, rhizome diameter, etc. Species have been named based on flower color alone or for variation in the number of staminodia, a character that is plastic and controlled by only a few ‘Mendelian factors’ (Honing 1939 in Rogers 1984).

Kranzlin revised the group in 1912 and recognized ~59 species plus a list of *insertae sedis*, however, many species were based on a single herbarium sheet. Kubitzki (1998) provides a general review of the group, but more modern, synthetic treatments are available. Tanaka recognized 19 species plus a number of subspecific taxa within *C. discolor* and *C. indica* in his 2001 revision, again recognizing several species based on a single specimen. Additional newly described species include *C. ascendens* (Ciciarelli 2007), *C. tulianensis* (Tanaka 2008) and *C. variegata* (Ciciarelli 1995). Maas-van de Kamer and Maas (2008) published the most recent monograph in which they provide an extensive review of the nomenclatural and taxonomic history, vegetative and floral morphology, and fruit, seed, and seedling morphology, pollination biology, distribution, and ecology. They recognize ten species including a large *Canna indica* complex. Several fossil taxa have also been described (e.g. Knowlton 1924, Becker 1969, Daghlian 1982), but few can be assigned to the family with confidence with the exception of a leaf from the Eocene of Texas (Daghlian 1982).

Taxonomic difficulties have been exacerbated by cultivation, selection, and dispersal by humans. As previously stated, cannas are native to the New World with a center of diversity in South America. Some of the earliest *Canna* collections from Asia were described as new to science even though the plants had morphologically similar or seemingly identical New World counterparts. Current theory is that these plants represent very early human dispersal events. Cannas are grown primarily as a food item by indigenous peoples of the New World (Schmeda-Hirschmann 1994, Cereda and Vilpoux 2001; Leonel et al. 2002). Archaeological evidence suggests *Canna* was one of the first plants to be cultivated during incipient civilization of Peru and Argentina (2500 BC; Bird 1948). Cannas are also cultivated extensively in Asia where starch extracted from the rhizome is used in the preparation of cellophane noodles (Prain 1993; Hermann 1996; Tanaka et al. 2006). For a more detailed review of *Canna* domestication and cultivation in the New World, see Gade (1966), Pickersgill and Heiser (1977), Ugent et al. (1984), Pickersgill (2007), and Pearsall (2008). Additional information on cultivation in the Old World can be found in the numerous publications of Hermann (Hermann 1994, 1999; Hermann et al. 1997, 1999) and Tanaka (2004).

Canna indica was introduced to Britain as early as 1596 (Cooke 2001) and first illustrated in *Florilegium Renovatum* in 1612. Horticultural interest is currently experiencing significant growth with over 500 cultivars available today. Several hundred more were known from the first cultivation boom of the mid 1800's through the early 1900's (Cooke 2001). Cannas have long been cultivated and hybridized to produce ornamentals, some of which are sterile. These plants are so popular they have the dubious distinction of being listed by the Global Invasive Species Database (www.issg.org/database/) as invasive throughout the islands of the Pacific.

The continuing disagreement over the number and circumscription of species (10-22; see Table 1) using morphological characters may be exacerbated by poor preservation of significant characters on herbarium specimens and the long history of use and selection (on flower color and rhizome characteristics in particular) by indigenous people. Additionally, a large number of hybrids have been generated, both sterile and fertile, by the horticultural trade that are now cultivated and naturalized around the globe. Reports of the utility of molecular data to resolve relationships among cannas are also conflicting. Tanaka (2001) found support for several segregate species of *C. indica* using RFLP markers and Hermann (1999) was able to distinguish *C. edulis* (=*C. indica*) cultivars using RAPDs, yet Patra et al. (2008) were unable to distinguish cultivars of *C. indica* using either ISSR or RAPD markers.

This study used parsimony and likelihood methods to assess phylogenetic relationships within the genus. DNA sequence data from protein coding regions (*ndhF*, *rbcL*) were used to identify a functional outgroup taxon from within the ingroup because these regions present minimal alignment ambiguity. Relationships within the genus were estimated based on results of analyses of chloroplast markers *trnE-trnT* intergenic region (IGS), and *rpL16* intron, plus the nuclear internal transcribed spacer region (ITS). The phylogenetic reconstructions were used to assess the monophyly of the taxa sampled. They were also examined for conflict between the chloroplast and nuclear trees. Finally, the likely region of origin and paths of migration based on current biogeographic patterns were explored in relation to levels of genetic diversity observed.

Materials and Methods

Taxa Examined—A total of 27 ingroup and 24 outgroup samples were sequenced for the chloroplast protein coding regions *ndhF* and *rbcL*. Data from the chloroplast non-coding regions *trnE-T* IGS and *rpL16* intron were also collected from up to 39 ingroup samples, representing 11 of the 22 currently recognized species in the genus, plus one ornamental plant, *C.* × *orchiodes* (=*C. flaccida* male parent by *C.* × *generalis* female parent) as indicated in Table 1. Nuclear ribosomal internal transcribed spacer data (ITS) for the same 39 ingroup samples were also generated. *Canna* × *orchiodes* was included because it is a hybrid

Taxon	Distribution	Comments	Number Included
C. *amabilis* T. Koyama & Nb. Tanaka*	**SA:** Argentina	Like C. *glauca* but staminodes narrower and red, seeds smaller. Known from a single locality.	--
C. *ascendens* Ciciarelli*	**SA:** Argentina	Like C. *indica* but flowers orange, labellum reflexed. Low to mid elevations.	--
C. *bangii* Kraenzl.	**SA:** Bolivia, Peru	Orange, pedunculate flowers. 1400-1800 m elev.	3-Bolivia 2-Peru 1-Ex Hort.
C. *coccinea* Mill.*	**SA:** Argentina	Like C. *indica* but larger bracted, 1-5 seeds per capsule.	--
C. *compacta* Roscoe*	**SA:** Argentina	Like C. *indica* but larger bracted, and inflorescence compact. Commonly cultivated.	1- Ex Hort.
C. *discolor* Lindl.*	**CA:** Honduras, Mexico **CULT/NAT:** Broadly in Asia	Sterile triploid, several color variants.	1-China
C. *flaccida* Salisb.	**NA:** USA	Flaccid, yellow flowers with reflexed petals. Report of specimen from Dominica probably in error (=C. x *orchioides*). Coastal plain (<300 m).	3-USA (2-Florida, 1-Alabama)
C. *glauca* L.	**Car:** Jamaica, Trinidad **NA:** Mexico **CA:** Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua **SA:** Argentina, Bolivia, Brazil, Columbia, Ecuador, Paraguay, Surinam, Uraguay, Guiana **CULT/NAT:** widely in Asia	Conspicuously glaucous, frequently growing in wet places. Reports from the USA are probably of cultivated plants. Generally lower elev. (<1100 m).	1-Argentina 1-Costa Rica 1-Guatemala

Taxon	Distribution	Comments	Number Included
C. *indica* L.	**Car:** Cuba, Dominica, Jamaica, Puerto Rico, West Indies **CA:** Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Panama **NA:** Mexico **SA**: Argentina, Brazil, British Guiana, French Guiana, Peru, Venezuela **CULT/NAT:** Broadly in Asia	Several color variants. Reports from the USA are probably of cultivated plants. Broadly distributed at low to mid elev. (<2000 m).	1-Costa Rica 5-Ex Hort.
C. *iridiflora* Ruiz & Pav.	**SA:** Peru	Pendant, purple-red flowers. Mid to high elev. (1800-2850 m).	1-Peru
C. *jacobiniflora* T. Koyama & Nb. Tanaka	**SA:** Argentina	Tubular corolla tube, purple-red bract. Known from a single locality.	--
C. *jaegeriana* Urb.	**Car:** Dominica, Haiti, Puerto Rico **SA:** Columbia, Ecuador, Peru	Curved, orange flowers. Lower to mid elev. (750-2000 m)	1-Ecuador
C. *liliiflora* Warsz. ex Planch.	**SA:** Bolivia, Peru	Stout, white flowers with reflexed petals. Mid to high elev. (2000-2800 m)	6-Bolivia
C. *paniculata* Ruiz & Pav.	**SA:** Argentina, Bolivia, Brazil, Ecuador, Peru, Venezuela	Single staminode. Low to mid elev. (150-2000 m)	1-Brazil 1-Peru
C. *patens* (W.T. Aiton) Roscoe	**SA:** Brazil **CULT/NAT:** Occasionally in Asia	Few-flowered inflorescences, yellow margin to staminodes.	--
C. *pedunculata* Sims	**SA:** Brazil	Distinctly pedunculate, small, yellow flowers. Narrowly distributed in SE Brazil.	--
C. *plurituberosa* T. Koyama & Nb. Tanaka*	**SA:** Argentina	Like C. *coccinea* but with a shorter, denser rhizome. Staminodes = functional stamen in length. Type locality only known population.	--
C. *speciosa* Roscoe ex Sims*	**SA:** Argentina, Brazil **CULT/NAT:** India, Thailand, Japan	Tall stature (2-4 m) with creeping rhizomes.	--

Taxon	Distribution	Comments	Number Included
C. *stenantha* Nb. Tanaka	**SA:** Argentina, Paraguay	Similar to C. *speciosa* but much smaller stature. Swampy habitats. Only known from two localities.	--
C. *tuerckheimii* Kraenzl.	**NA:** Mexico **CA:** Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Panama **SA:** Brazil, Ecuador, Peru	Tall stature (2-4.5 m), branched inflorescence, orange-red flowers. Low to mid elev. (500-2000 m)	2-Costa Rica 1-Ecuador 1-Mexico 1-Peru 1-Ex Hort.
C. *tulianensis* Nb. Tanaka*	**CA:** Honduras	Like C. *tuerckheimii* but flowers more erect, staminodes patent, and labellum recurved. Known from type locality only.	--
C. *variegatifolia* Ciciarelli*	**SA:** Argentina	Like C. *adscendens* but foliage variegated, inflorescence more highly branched. Urban species.	--
C. × *generalis* L. H. Bailey	Worldwide		1-Ex Hort. USA (Florida)
C. × *orchioides* L. H. Bailey	Worldwide		1-Ex Hort. USA (California)

Table 1. List of taxa currently recognized in *Canna* (Cannaceae), and sampling employed in this study. Geographic distribution information was taken from Tanaka (2001, 2008) and Ciciarelli (1995, 2007). * Indicate taxa included in the C. *indica* complex by Maas-Van de Kamer and Maas (2008). Distribution abbreviations: CA=Central America, NA=North America, SA=South America, Car=Caribbean, CULT/NAT=Cultivated or Naturalized, -- = not sampled.

of known parentage first developed in 1893 (Khoshoo and Guha n.d., in Cooke 2001). It was included to test the ability to detect and identify both parents and to assess the potential for amplification and detection of multiple ITS haplotypes. DNA extraction, voucher, and GenBank accession numbers are provided in Appendix 1.

DNA Extraction and Amplification—General use plastid primers were synthesized from descriptions in the literature or were designed by the author. Published primers used include *ndhF* 32F, 451F, 451R, 1318F, 1383R, 1600R, and 2110R (Terry et al. 1997); rbcL 1F*, 481F, 486R, 892F, 892R, 1370R (Prince and Kress 2006); *trnT-E* IGS trnTr, trnE (Doyle et al. 1992); and *rpL16* intron 1516R (Kelchner and Clark 1997). Newly designed primers used for this study included a number for the *trnT-trnE* IGS region (IF: ACGAATACCCTCATCACATG; IF-SL: TATCGTTATTGAATTTTCTG; IF-CZ: TTGCTTCSYTCATTGTTCAGAT; IR: GACCGACTCCACTTGTTTAC; IR-SL: CCCGCTTAATTTCTTTTCTTCA; IR-CZ: ACCAATCTGACACTGACATA) and for the *rp*L16 intron (F: GGAGTATTAGGAGTTAAAATTTGG; IF-Zing: ATTAATGGAGAAGCTATGGG; IR-Zing: AAATAAAGGTTTCGCGGGCG). Samples were amplified using standard methodologies as described in Prince and Kress (2006). The nuclear ITS region was amplified using either primers 5a and 241R (Prince and Kress 2006) or 18SF and 26SR (CGATTGAATGGTCCGGTGAAG and AGGACGCTTCTACAGACTACAA respectively) at an annealing temperature of 58-62C in 25 µL reactions and sequenced in both directions using those two primers plus 5.8S-F and 5.8S-R (Prince and Kress 2006) to ensure at least 90% sequence confirmation. Taq polymerase (Promega, Madison, Wisconsin, USA) was used with an ammonium sulfate buffer. Reactions included 5-10% DMSO to improve product yield and specificity. All amplification products were cleaned using an abbreviated version of the PEG precipitation protocol published by Johnson and Soltis (1995).

ITS samples that produced noisy chromatograms or those with more than 2 polymorphic bases per contig were subsequently cloned using Topo TA Kits (Invitrogen Life Technologies, Carlsbad, California, U. S. A.). Four to six colonies were screened per cloning reaction. Minipreps of liquid cultures were cleaned using Eppendorf Plasmid MiniPrep Kits (Westbury, New York, U. S. A.) and quantified in a Biospec-1601 spectrophotometer (Shimadzu Biotech, Columbia, Maryland, U. S. A.). Cycle sequencing used 1/8 concentration ABI BigDye III (Applied Biosystems, Inc., Foster City, California, U. S. A.) on PTC-100 themal cyclers (MJ Research Inc., Reno, Nevada, U. S. A.) for all regions except much of the *rbc*L data, which were generated using manual sequencing methods (Prince and Parks 2001). M13F and M13R primers were used for sequencing cloned products. Samples were visualized on an ABI 3100 or 3130xl Genetic Analyzer (Applied Biosystems, Inc., Foster City, California, U. S. A.) at Rancho Santa Ana Botanic Garden (Claremont, California, U. S. A.).

Sequence Alignment—Sequences were edited in Sequencher v. 4.2.8 (Gene Codes Corp., Ann Arbor, Michigan, U. S. A.) after vector fragments (cloned

ITS only) and primer sequences were trimmed. Edited sequences were aligned manually using Se-Al v. 2.0a11 (Rambaut 2001) and exported into NEXUS files for analysis. Previously published sequences were downloaded from GenBank and used to fill out the data matrices. Gaps were not coded but were treated as missing data. Data matrices are available through TREEBASE or from the author.

Phylogenetic Analyses—Maximum parsimony analyses were conducted using PAUP*4.0 (Swofford 2002). Individual (*ndhF*, *rbcL*, *rpL16* intron, *trnE-T* IGS, and ITS) and combined (*ndhF* + *rbcL*, *rpL16* intron + *trnE-T* IGS) data matrix analyses were conducted under heuristic search options with 1,000 random addition replicates, tree bisection and regrafting (TBR) branch swapping, holding 4 trees, saving all shortest trees (except ITS and combined chloroplast data sets: 10,000 random addition replicates, limit of 10 trees per replicate). Branch support was calculated using parsimony bootstrap (BS) in PAUP* and posterior probabilities (PP) in MrBayes v3.2.1 (Ronquist and Huelsenbeck 2003). Bootstrap values were based on 1,000 pseudoreplicates, each with 100 random addition replicates, TBR branch swapping, saving a maximum of 10 trees per random addition replicate, hold=4 trees. Posterior probabilities were estimated using 1-10 million generations depending on the splits values where all trees with average standard deviation of the split frequencies equal to or higher than 0.01 were discarded as burn-in.

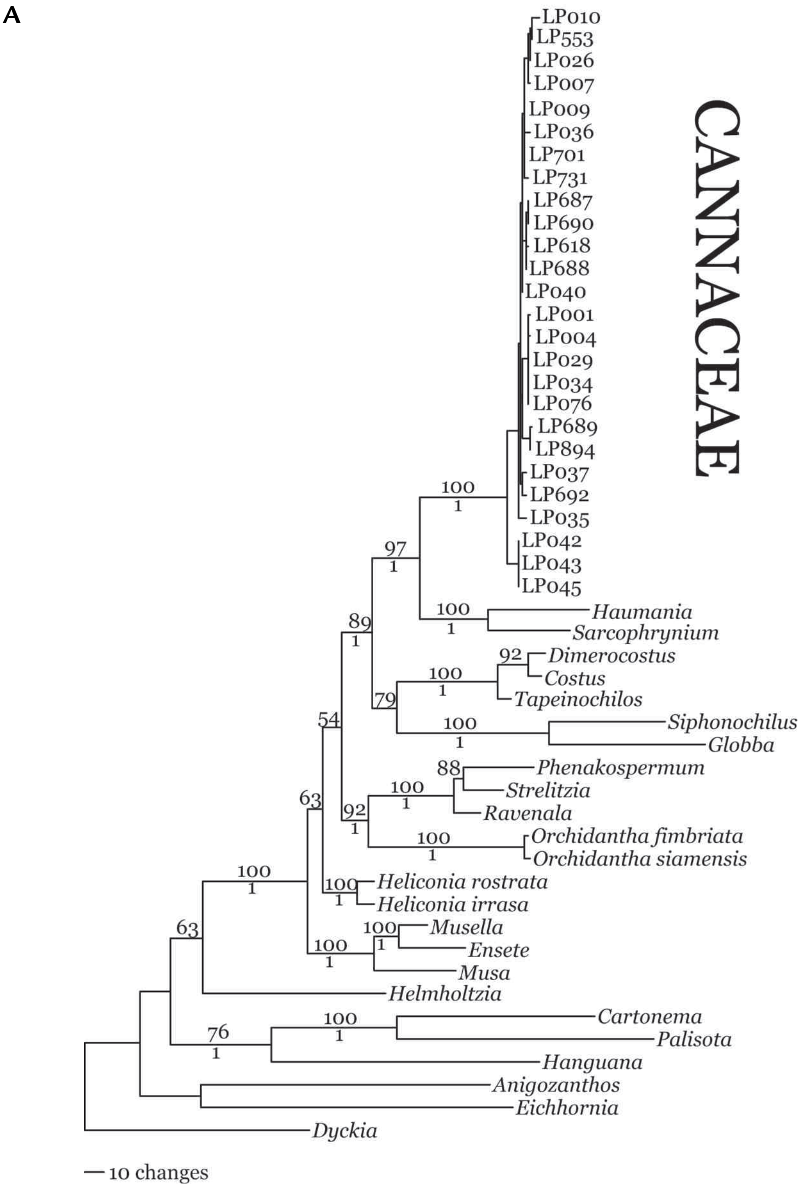
Results

Summary information on the characteristics of all data sets and analyses is provided in Table 2 including number of characters in the data matrix, number of potentially parsimony informative characters, indices values, burn-in times, etc. Bayesian analyses required between 1 and 10 million generations to stabilize. In general, combined analyses required less accurate search methods (save limit of 10 trees per replicate). For example, the *ndhF* 1,000 random addition replicate analysis ran to completion and found 750 shortest trees, the *rbcL* analysis also ran to completion and found 3,419 shortest trees, but the combined chloroplast coding data analysis ran for 10,000 random addition replicates, saving only 10 trees per replicate. The analysis produced 57,922 shortest trees. This is likely due to missing data from unequal taxon sampling between the two data sets.

Protein Coding Data—*ndhF* and *rbcL* were analyzed to identify an ingroup taxon that could serve as a functional outgroup for analyses of the non-coding regions. These two regions provided 480 and 163 potentially parsimony informative characters each (see Table 2), and both resolved *Canna* as a monophyletic lineage with branch support of 100% BS and 1.00 PP. The combined analysis yielded the same results as shown in the tree illustrated in Fig. 1. Topologies differed in the support and arrangement of some *Canna* clades and in the relationship of Heliconiaceae to Strelitziaceae + Lowiaceae (62% BS for sister

Data Set	ALL OTU	Ingroup OTU	# Char.	# PIC	# Trees	L	CI	RI	RC	Burn-in
ndhF	48	25	2097	480	750	1302	0.53	0.75	0.39	773,000
rbcL	37	13	1322	163	4812	433	0.49	0.68	0.34	480,000
Combined coding	50	26	3419	643	57922	1739	0.52	0.73	0.38	863,000
rpL16 intron	30	30	868	16	54,402	28	0.64	0.86	0.55	594,000
trnE-trnT IGS	39	39	899	12	149	15	0.87	0.96	0.83	1,560,000
Combined non-coding	39	39	1755	28	66,010	45	0.69	0.88	0.61	5,410,000
ITS	39	39	713	105	78,257	204	0.63	0.95	0.60	4,045,000

Table 2. Summary information of the characteristics for all data sets and analyses employed for estimation of phylogenetic relationships of *Canna* (Cannaceae). OTU=Operational Taxonomic Unit, L=Length, CI=Consistency Index, RI=Retention Index, RC=Rescaled Consistency Index, Burn-in=number of generations discarded from construction of 50% majority rule tree for Bayesian analyses.



A
LP010
LP553
LP026
LP007
LP009
LP036
LP701
LP731
LP687
LP690
LP618
LP688
LP040
LP001
LP004
LP029
LP034
LP076
LP689
LP894
LP037
LP692
LP035
LP042
LP043
LP045
CANNACEAE
100
1
97
1
89
1
Haumania
Sarcophrynium
100
1
92
Dimerocostus
Costus
Tapeinochilos
100
1
79
Siphonochilus
Globba
100
1
54
88
Phenakospermum
Strelitzia
Ravenala
100
1
92
1
63
Orchidantha fimbriata
Orchidantha siamensis
100
1
100
1
Heliconia rostrata
Heliconia irrasa
100
1
Musella
Ensete
100
1
Musa
63
Helmholtzia
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1
Cartonema
Palisota
76
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Hanguana
Anigozanthos
Eichhornia
Dyckia
10 changes

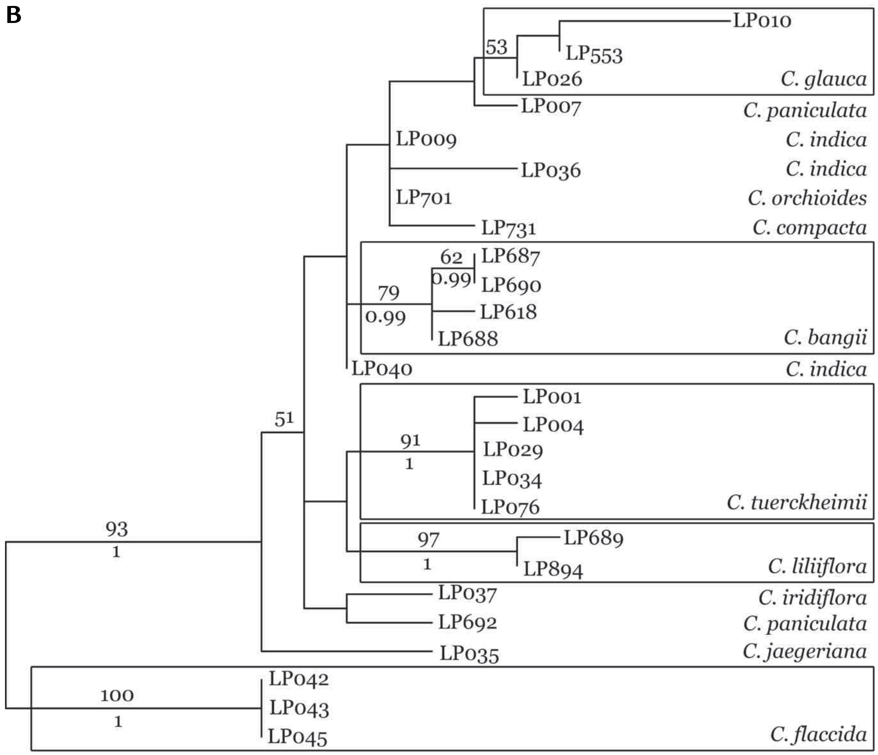


Fig. 1. One of 57,922 most parsimonious trees from analysis of the combined chloroplast region *ndhF* and *rbcL* sequences to identify functional outgroup taxon in *Canna* (Cannaceae). Length = 1739 (excluding parsimony uninformative characters). Bootstrap values above the branch, posterior probabilities below. (A) Entire phylogram, (B) Cannaceae shown exaggerated.

relationship; unresolved for PP). Results of these data analyses also indicated *Canna flaccida* (samples LP042, LP043, and LP045) was monophyletic (BS 100%, PP 1.00; individual and combined analyses) and sister to all other *Canna* species (BS 76% in *ndhF*, 98% in *rbcL*, and 93% in combined; PP 1.00 in *rbcL* and combined). *Canna bangii* (combined data: BS 79%, PP 0.99), *C. liliiflora* (combined data: BS 97%, PP 1.00), *C. tuerckheimii* (combined data: BS 91%, PP 1.00) were also identified as monophyletic. Other relationships within the genus were poorly resolved or only weakly supported. Sequence diversity was extremely low within the genus for these two markers.

Chloroplast non-coding data—The *rpL16* intron and *trnE-T* IGS data sets were analyzed independently and in combination. More potentially parsimony informative characters were obtained from the *rpL16* data set, but analyses of this region provided lower tree indices than the *trnE-T* data set. Neither of the separate analyses resulted in highly resolved or supported trees (data not shown), and the combined analysis is only slightly more informative (Fig. 2).

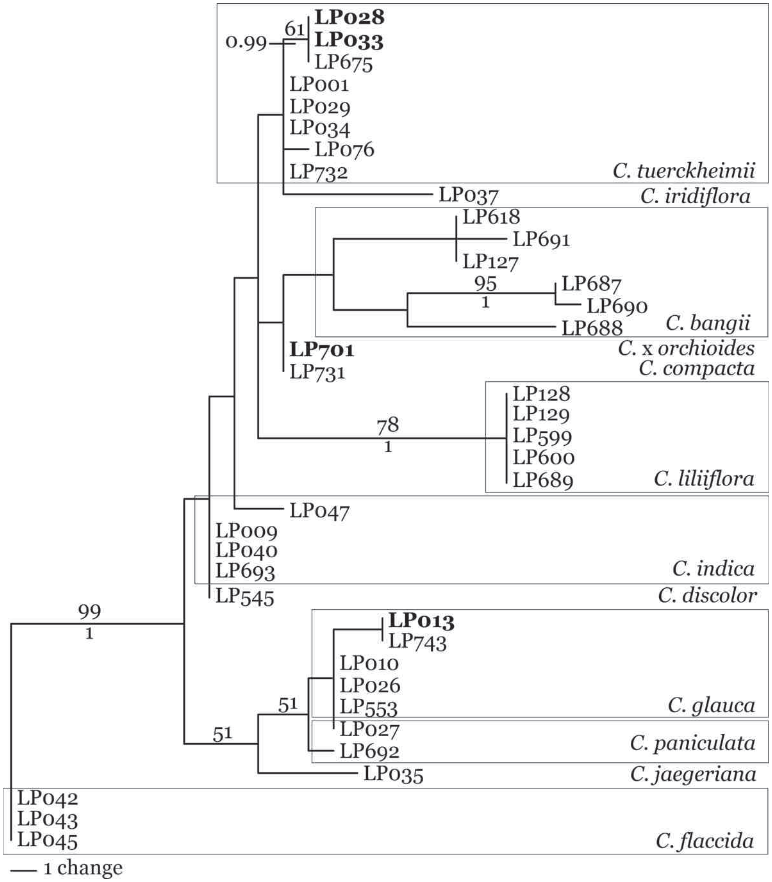


Fig. 2. One of 66,010 most parsimonious trees from analysis of the combined chloroplast non-coding regions *rpL16* intron and *trnE-T* intergenic spacer region sequences, rooted using *Canna flaccida*, to assess phylogenetic relationships among species of *Canna* (Cannaceae). Length = 45 (excluding parsimony uninformative characters). Bootstrap values above the branch, posterior probabilities below. Bold typeface indicates taxa with placement in hard conflict with results of ITS data.

Posterior probabilities were estimated from the last 4.6 million generations of a 10 million generation analysis. Relationships were identical in the BS and PP consensus trees for the separate analyses and only slightly different in the combined analysis (placement of LP688 relative to LP687 + LP690; PP 0.96 for sister relationship, unresolved in BS). Results of combined chloroplast non-coding data analysis resolve two of the four monophyletic lineages identified by the chloroplast coding data *C. flaccida* clade (BS 99%, PP 1.00) and *C. liliiflora* (BS 78%, PP 1.00). Sequence diversity (based on branch length) was low for almost all taxa except for *C. bangii*.

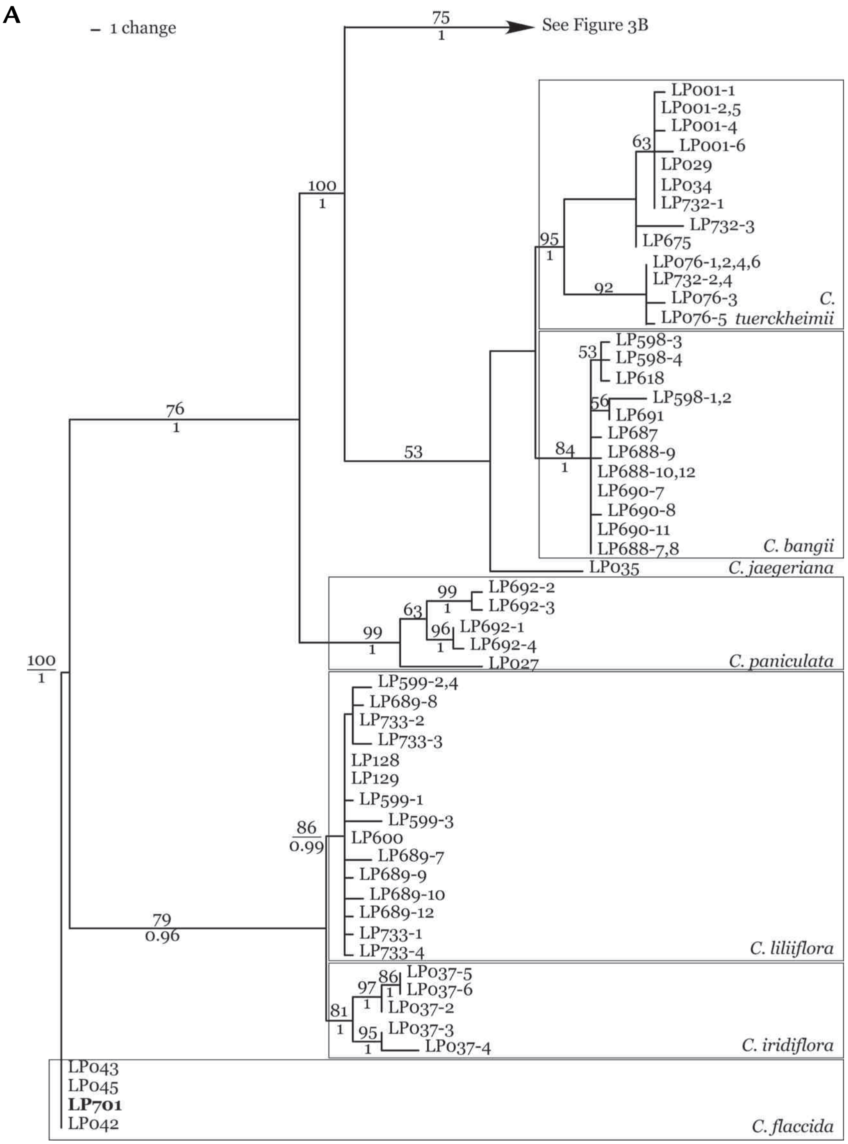
Nuclear Ribosomal ITS Data—The largest number of potentially parsimony informative characters (110) were obtained from the ITS data set. With just under 100 OTUs, the tree indices were higher than those obtained for the chloroplast regions (see Table 2). The maximum parsimony analysis required less thorough search options and produced 78,257 shortest trees, one of which is shown in Fig. 3. Topologies were similar for the BS and PP trees except as noted below. Seven clades, roughly corresponding to seven species (*C. bangii*, *C. flaccida*, *C. glauca*, *C. indica, C. liliiflora*, *C. paniculata*, and *C. tuerckheimi*), received moderate support (BS ≥ 75%, PP ≥ 0.95). *Canna iridiflora* was represented by multiple clones of a single accession so was not a test of monophyly for the species. Resolution within those clades was generally poor and unsupported.

Clones that failed to coalesce but were all placed within the broadly defined *C. indica* clade include samples LP028, LP033, LP040, LP545, and LP731. All had some branch support separating the clones. Samples LP545 and LP731 resolved as a basal grade in the *C. indica* clade (BS 73%, PP 1.00) with the exception of one LP731 clone 3 which was firmly embedded in *C. indica sensu stricto*. Inferred sequence diversity was highest in the broadly defined *C. indica* lineage and non-existent in *C. flaccida*. Diversity was also low in the *C. liliiflora* and *C. bangii* clades. Some of the variation observed among clones of the same sample may be due to Taq polymerase error. Future amplification and cloning work should employ a polymerase with better proof-reading capabilities.

Comparison of Chloroplast nrITS—Comparing the non-coding chloroplast and ITS phylogenies allowed for the identification of a number of hard conflicts. *Canna* × *orchiodes* (LP701) was included in the study because it is a hybrid of known parentage, *C. flaccida* (pollen donor) and *C.* × *generalis* and was generated no longer than 120 years ago. *Canna* × *generalis* is in turn a hybrid of either *C. indica* × *C. glauca* or *C. indica* × *C. iridiflora*. ITS data were identical to one of the parents, *C. flaccida*. Chloroplast results place it in an unresolved position distinctly separate from *C. flaccida*. Samples LP028 and LP033 grouped with *C. tuerckheimii* in the cpDNA tree (without support) but with *C. indica* in the ITS tree (BS 89%). Sample LP013 grouped with *C. glauca* and *C. paniculata* in the cpDNA tree (BS 51%), but with *C. indica* in the ITS tree (BS 89%).

Discussion

All recent morphological and molecular data analyses, including the one presented here, identify Marantaceae as sister to the Cannaceae. Phylogenetic analyses of these data provided the first broadly sampled estimate of relationships within the genus *Canna* and were used to assess the monophyly of the taxa sampled, to explore genetic diversity within and among taxa, and to explore biogeographic patterns. Analyses of both chloroplast and nuclear ITS data allowed for the identification of hard conflicts among the phylogenetic reconstructions. Conflict may be due to a number of processes such as lineage


A
1 change
75
1
See Figure 3B
LP001-1
LP001-2,5
LP001-4
63
LP001-6
LP029
LP034
LP732-1
LP732-3
95
1
LP675
LP076-1,2,4,6
LP732-2,4
92
LP076-3
C.
LP076-5
tuerckheimii
100
1
LP598-3
53
LP598-4
LP618
LP598-1,2
56
LP691
LP687
84
1
LP688-9
LP688-10,12
LP690-7
LP690-8
LP690-11
LP688-7,8
C. bangii
76
1
53
LP035
C. jaegeriana
99
LP692-2
1
LP692-3
63
96
LP692-1
99
1
LP692-4
1
LP027
C. paniculata
100
1
LP599-2,4
LP689-8
LP733-2
LP733-3
LP128
LP129
LP599-1
LP599-3
86
0.99
LP600
LP689-7
LP689-9
LP689-10
LP689-12
79
0.96
LP733-1
LP733-4
C. liliiflora
86
LP037-5
97
1
LP037-6
81
1
LP037-2
1
95
LP037-3
1
LP037-4
C. iridiflora
LP043
LP045
LP701
LP042
C. flaccida

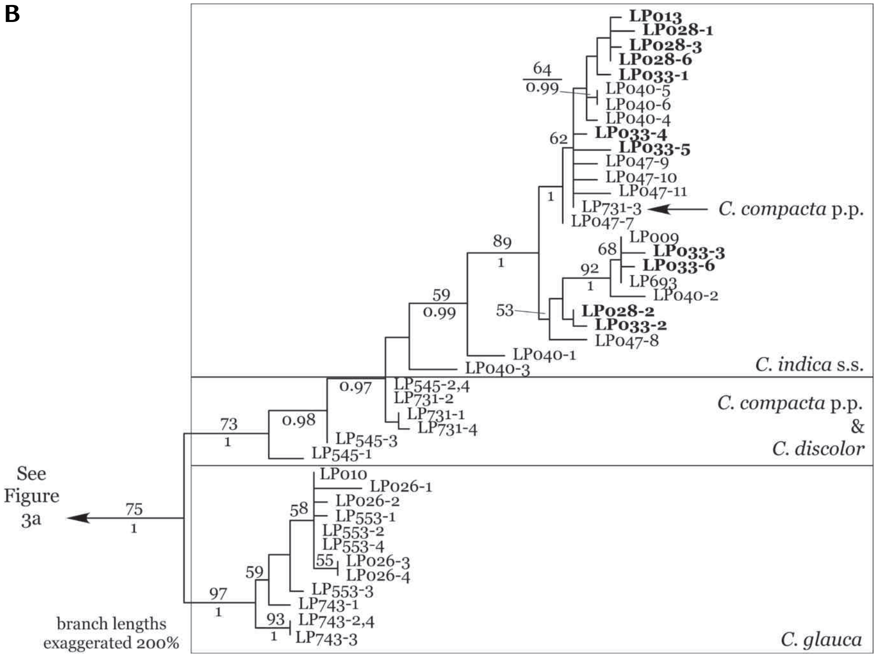


Fig. 3. One of 78,257 most parsimonious trees from analysis of the ITS sequences, rooted using *Canna flaccida*, to assess phylogenetic relationships in *Canna* (Cannaceae). The lower portion of the phylogeny (*C. indica* and *C. glauca*) is shown for both the best Bayesian tree (upper left) and maximum parsimony (right) because of differing topology and support values. Cloned data shown with clone number following the DNA extraction number (e.g. LP010-6,9,12 = LP010 clones 6, 9, and 12). Maximum parsimony tree length = 204 (excluding parsimony uninformative characters). Bootstrap values above the branch, posterior probabilities below. Bold typeface indicates taxa with placement in hard conflict with results of chloroplast non-coding data. Arrow indicates outlier clone of *C. compacta*. (A) Lower portion of phylogram, (B) upper portion of phylogram.

sorting, lateral transfer, and reticulation. These issues will be discussed after each taxon is reviewed below.

Phylogeny—Results based upon chloroplast data analyses, coding and non-coding, resulted in less resolved and less strongly supported phylogenies than the ITS data analyses, but a number of significant conclusions can be drawn. Eleven of the 22 recognized species were included in this study. Multiple accessions for seven species were available, confirming the monophyly of *C. bangii*, *C. glauca*, *C. flaccida*, *C. indica, C. liliiflora, C. paniculata*, and *C. tuerckheimii* with moderate to strong support (i.e. BS ≥ 75%, PP ≥0.95 for at least the ITS phylogeny). Relationships among species were generally well resolved and supported in the ITS phylogeny (Fig. 3) so only that phylogeny will be discussed unless otherwise noted.

Canna flaccida, a North American endemic species, is sister to the rest of the genus based on chloroplast coding data analyses (Fig. 1). This taxon was designated as the outgroup for analyses of all non-coding regions to minimize alignment ambiguity. The sister relationship should be confirmed using data from nuclear markers. *Canna flaccida* inhabits swampy regions of the coastal plain of the U.S.A is distributed from southern Virginia to Texas. The large, fragrant yellow flowers open in the evening, are open for only one night, and presumably moth pollinated. *Canna* × *orchioides* (LP701) is an ornamental hybrid of *C. flaccida* (male parent) and *C.* × *generalis* (female parent, hybrid of *C. indica* × *C. glauca* or *C. indica* × *C. iridiflora*). *Canna* × *orchioides* (LP701) grouped with *C. flaccida* in the ITS phylogeny (Fig. 3). The sequence data was generated from direct sequencing methods and produced clean chromatograms (no polymorphic bases) identical in sequence to the three *C. flaccida* samples, one from Alabama and two from Florida (0% sequence divergence). The chloroplast data resolve this sample with *C. indica* + *C. glauca* (coding, unsupported). The data strongly support the exclusion of this sample from *C. flaccida* (coding Fig. 1 BS 100%, PP 1.00; non-coding Fig. 2. BS 99%, PP 1.00). Thus these data confirmed the male parentage and were not in conflict with the female parentage of this taxon. The generation of clean ITS sequences for *C.* × *orchioides* was surprising given the tri-parental nature of the hybrid. It is possible that cloning of the PCR product might have revealed the other copies. It is also possible that amplification bias resulted in one dominant copy from a mixed parental pool. Alternatively, concerted evolution may proceed quickly in this taxon, resulting in a uniform ITS sequence pool in as little as 120 years.

Canna iridiflora and *C. liliiflora*, two large flowered, South American species diverge next in the phylogeny and form a sister pair (BS 79%, PP 0.96). A single specimen of *Canna iridiflora* was included. ITS sequences included a number of polymorphisms, so the PCR product was cloned. All clones coalesced into a moderately supported clade (BS 81%, PP 1.00). *Canna iridiflora* is restricted to the moist mountains of five states in Peru, from 1800 to 2850 m elevation. Flowers are reddish-purple, conspicuously pendant, and presumably hummingbird pollinated. *Canna liliiflora* occurs at similar elevation and in similar habitats to *C. iridiflora* but is even more narrowly distributed, with populations in Cuzco, Peru, and Bolivia (Cochabamba and La Paz). As the name implies, *C. liliiflora* bears white flowers. The flowers produce nectar and are sturdy enough to support the weight of the bats that pollinate them (Vogel 1969). All samples (and clones) coalesced into a well supported clade (chloroplast coding BS 97%, PP 1.00; chloroplast non-coding BS 78%, PP 1.00; ITS BS 86%, PP 0.99).

Canna paniculata, the only species bearing a single staminode, is the next taxon to diverge (BS 76%, PP 1.00). The two samples included here, one from Brazil and the other from Peru, coalesce with 99% BS and 1.00 PP support. The flowers are salmon colored and displayed in a panicle-like inflorescence. This taxon is sometimes placed in the subgenus *Distemon* because of the reduced number of staminodes. Recognition of this subgenus would render subgenus *Canna* paraphyletic and so is not supported here. *Canna paniculata* occurs in scattered localities at low to mid elevation (150-2000 m) throughout the wet Neotropics from Panama to southern Argentina.

Canna bangii, *C. jaegeriana*, and *C. tuerckheimii* form the next diverging clade but with little branch support (BS 53%). *Canna bangii* is distributed in similar habitats as *C. iridiflora* and *C. liliiflora*, but is distinctly different in morphology with smaller red flowers borne erectly. Samples included here (ex hort., Bolivia, Peru) coalesce with strong support (chloroplast coding BS 79%, PP 0.99; ITS BS 84%, PP 1.00). *Canna jaegeriana* is common in the Greater Antilles and in western South America at mid to higher elevations (750-2000 m). Flowers are curved, tubular, and orange. *Canna tuerckheimii* is commonly found at mid to higher elevations (500-2000 m) in Central America but also extends south to Colombia and Ecuador. Flowers are bright orange and the plant superficially resembles *C. bangii* but the pedicle is naked in *C. tuerckheimii* and tuberculate in *C. bangii*. Here six samples of *C. tuerckheimii*, with representatives from all three Americas, coalesce with high branch support (BS 95%, PP 1.00).

The last clade (BS 75%, PP 1.00) includes *C. compacta*, *C. discolor*, *C. glauca*, and *C. indica*. *Canna glauca* and *C. indica* are both widely distributed (and cultivated) in the Neotropics. *Canna glauca* grows in very wet to marshy areas, bears yellow flowers, and has distinctly glaucous leaf surfaces. *Canna indica* grows in a more diverse array of habitats including disturbed sites, bears red (occasionally yellow) flowers, and may also be glaucous. *Canna indica* displays a wide variety of leaf variegations and leaf colors, sometimes with magenta or reddish striping. It is not clear whether the coloring and glaucous nature of *C. indica* leaves is a naturally occurring phenomenon or whether these plants are actually a result of hybridization with other species such as *C. glauca* and *C. iridiflora*. Sequences of each of these taxa coalesce with strong support (*C. glauca* BS 97%, PP 1.00; *C. indica* BS 73%, PP 1.00) if *C. compacta* and *C. discolor* are not recognized. *Canna compacta* (LP731) and *Canna discolor* (LP545) formed an unresolved polytomy at the base of the *C. indica* clade in the ITS tree (Fig. 3) but clones of each accession failed to coalesce. Additionally, one clone of *C. compacta* (LP731-3) was nested well within the *C. indica* s.s. clade. At present, these data are insufficiently resolved and relationships too weakly supported to draw any firm conclusions. Additional study will be required to tease apart relationships among these closely related taxa.

Half of the described species were not available for this study due to their very limited distribution. These include *C. amabilis, C. ascendens, C. compacta, C. jacobiniflora, C. patens, C. pedunculata, C. plurituberosa, C. speciosa, C. stenantha, C. tulianensis,* and *C. variegatifolia*. Almost all of these species (except *C. pedunculata*) are considered synonymous with *C. indica* (Maas-van de Kamer and Maas 2008). Several are known from only a single herbarium sheet or a single population thus future molecular studies should focus on inclusion of these rare, and putatively distinct taxa.

Phylogeny Conflict—Several hard conflicts between the chloroplast and nuclear phylogenies were identified. These hard conflicts may be due to incomplete lineage sorting, lateral gene transfer, or incomplete concerted evolution (in the ITS). The failure of the cloned haplotypes to coalesce and the conflicting position of samples between the chloroplast and nuclear phylogenies indicate one or more of the above processes have played a role in the evolutionary history of *Canna*. As discussed above, a recently generated hybrid (*C.* × *orchioides*, LP701) showed no indication of multiple ITS haplotypes (i.e. clean, unambiguous direct sequence chromatograms) that were identical to the putative male parent (*C. flaccida*). It is possible laboratory methods preferentially amplified one haplotype over another, but this did not seem to be the case for samples of most other species, thus it is reasonable to infer that concerted evolution can occur in under 125 years. Five samples included here (LP028, LP033, LP040, LP545, and LP731) produced ITS haplotypes that failed to coalesce. A failure of ITS haplotypes to coalesce could indicate recent hybridization events where recombination events are still in the process of homogenizing the ITS region. Alternatively, incomplete lineage sorting of a polymorphic population could be responsible. Widespread incomplete lineage sorting seems unlikely since the majority of the haplotypes coalesced within a recognized taxon clade. Samples LP545 and LP731 differed from LP028, LP033 and LP040 in the nature of sequence substitutions. LP545 and LP731 ITS sequences (excluding LP731 clone 3) shared specific base substitutions with both of the putative parents (e.g. a T at bp 50 shared with *C. indica* samples, and a C at bp 166 shared *with C. glauca*). Lateral gene transfer seems probable since many of these taxa co-occur, are cultivated, and have been successfully hybridized by horticulturalists. There is an expectation that approximately ½ of any hybrid samples will exhibit hard conflicts between the nuclear and chloroplast phylogenies (assuming maternal inheritance of the chloroplast genome) due to the random process of concerted evolution, sometimes converting to the female ITS haplotype (undetectable) and sometimes the male ITS haplotype (conflict). In this study, samples LP013, LP028, LP033, and LP701 (*C.* × *orchioides*) fell in contradictory locations depending on the data set. *Canna* × *orchioides* has already been discussed above. The three remaining samples, LP013, LP028, and LP033 fall within the *C. indica* clade (sensu lato) of the ITS phylogeny and are strongly supported as such (BS 89% PP 1.00), yet two of these samples (LP028 and LP033) are strongly supported (BS 61%, PP 0.99) as a part of *C. tuerckheimii* by chloroplast non-coding data. Examination of the ITS data showed no indication of recombination of the ITS haplotypes of *C. indica* and *C. tuerckheimii* for these two samples. Samples LP028 and LP033 shared several *C. indica* specific substitutions but did not share any species-specific substitutions with *C. tuerckheimii*. The final sample, LP013, is placed in the *C. glauca* clade (unsupported) by chloroplast data. This method was not successful in identifying another suspected hybrid sample, LP010, which is morphologically similar to *C. indica* (red flower color, broad leaf, etc.) but consistently groups with *C. glauca*

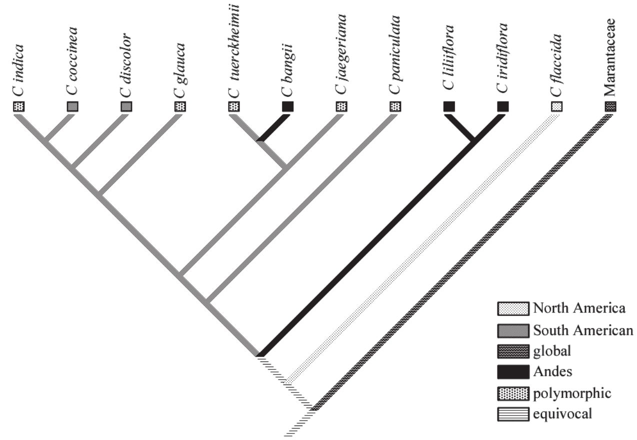


Fig. 4. Parsimony character state tracing of biogeography for *Canna*.

in both the ITS (BS 97%, PP 1.00) and chloroplast (chloroplast coding BS 53%) phylogenies. This is a sample of unknown origin that was acquired from Duke University greenhouses under the name *C. edulis*.

Biogeography—The center of diversity for *Canna* is South America so one might predict a South American origin for the genus. Results of DNA sequence data analyses (*ndhF* and *rbcL*) identify *C. flaccida,* a North American taxon, as sister to all other species sampled, however, the next two nodes lead to Andean and South American taxa (see Fig. 4). Simple unordered character state tracing (using MacClade v. 4.08; Maddison and Maddison 2005) was equivocal for geography at the base of the tree, but the vast majority of the tree was South American. Thus these data are consistent with a South American origin for *Canna* and imply a long distance dispersal to North America to account for the position of *C. flaccida* as the first branch in the phylogeny. Surprisingly there was no detectable DNA sequence variation was found in *C. flaccida* (two samples from Florida and one from Alabama) for any of the markers screened, including the highly variable chloroplast *psbA-trnH* IGS region (Prince unpublished data). This genic region was not included in the present study because of difficulty aligning sequences among species. Low sequence divergence within *C. flaccida* might be due to rapid, recent expansion in North America post introduction from a southern progenitor, a recent bottleneck, or significant selection pressure.

As stated above, character state tracing was equivocal for geography at the base of the tree below the branch leading to the two Andean endemic taxa *C. iridiflora* and *C. liliiflora*. The distribution of these taxa may be indicative of the early evolution in the genus or may represent more recent specialization. A few critical nodes are unsupported, so few additional conclusions may be drawn.

It is clear from the ITS phylogeny that Caribbean taxa (*C. glauca, C. indica*, *C. jaegeriana*) represent multiple, independent dispersals from South (or Central?) America, and that cannas have probably been migrating north through Central America into southern North America (Mexico). The most widely distributed taxa, *C. indica* and *C. glauca*, are of relatively recently origin. The extent to which man has influenced the current perceived natural distribution of these two species is unclear. More detailed analyses of biogeographical hypotheses requires finer resolution and more strongly supported phylogenetic reconstructions than those presented here, especially of the wide spread species *C. indica* and *C. glauca*, and for the relationship among *C. bangii*, *C. jaegeriana*, and *C. tuerckheimii*.

The current study identified an appropriate ingroup taxon (*C. flaccida*) for use as a functional outgroup. The use of a functional outgroup will allow the analysis of more variable data sets while minimizing error due to alignment ambiguity. *Canna* evolutionary history has likely been obscured by ancient and ongoing use by humans and the identification of natural populations may be difficult. Better phylogenies that include more taxa and more appropriately variable chloroplast sequence data are required. A combination of phylogenetic and population genetic approaches will likely be the best way to tease apart the evolutionary history of this difficult group.

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Appendix 1

List of voucher specimens used in analyses of *Canna*. Specimens are arranged in alphabetical order by genus and species within family. *Canna* DNA extraction number is indicated by LP###. GenBank accession numbers are in order *ndhF*, *rbcL*, *rpL16* intron, *trnE-trnT* IGS, ITS. * Indicates specimens from living collections only (no voucher yet).

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Cannaceae. ***Canna bangii*** Kraenzl.: BOLIVIA Clark 6618 (US), LP687, FJ861163, ns, FJ939418, FJ939452, FJ939527; BOLIVIA Clark 6744 (US), LP688, FJ861164, ns, FJ939419, FJ939453, FJ939528-FJ939530; BOLIVIA Clark 6849 (US), LP690, FJ861166, ns, FJ939421, FJ939455, FJ939536-FJ939538; PERU Peyton & Peyton 1200 (MO), LP127/LP598, ns, ns, ns, FJ939443, FJ939518-FJ939520; PERU Maas 4638 (U), LP618, FJ861162, ns, FJ939416, FJ939450, FJ939525; EX HORT USA Prince (*), LP691, ns, ns, FJ939422, FJ939456, FJ939539. ***C. compacta*** Roscoe: EX HORT Prince 523 (RSA), LP731, FJ861169, ns, ns, FJ939460, FJ939546-FJ939549. ***C. discolor*** Lindl.: CHINA Kress 00-6788 (US), LP545, ns, ns, FJ939415, FJ939446, FJ939511-FJ939513. ***C. flaccida*** Salisb.: USA (Alabama) Deramus D981 (UNA), LP045, FJ861159, ns, FJ939398, FJ939427, FJ939500; USA (Florida) Adams 605 (FSU), LP042, FJ861157, ns, FJ939396, FJ939425, FJ939498; USA (Florida) Siebert 1403 (MO), LP043, FJ861158, FJ861136, FJ939397, FJ939426, FJ939499. ***C. glauca*** L.: ARGENTINA Ocampo et al. 1543 (RSA), LP743/LP751, ns, ns, ns, FJ939462, FJ939557-FJ939559; COSTA RICA Prince 1995-239 (RSA), LP026, FJ861150, AF378774, FJ939403, FJ939432, FJ939470-FJ939473; GUATEMALA Kress 77-738 (US), LP553, FJ861161, ns, ns, FJ939447, FJ939514- FJ939517. ***C. indica*** L.: COSTA RICA Kress 89-2849 (US), LP036/LP047, FJ861155, AF378763, FJ939410, FJ939439, FJ939501-FJ939505; USA (Florida) Godfrey 60501 (RSA), LP040, ns, FJ861135, FJ939412, FJ939441, FJ939492-FJ939497; EX HORT USA Duke 88-124 (*), LP009, FJ861148, FJ861130, FJ939400, FJ939429, FJ939467; EX HORT USA Duke 66-314 (*), LP010, FJ861149, FJ861131, FJ939401, FJ939430, FJ939468; EX HORT USA Prince 1995-223 (RSA), LP013, ns, ns, FJ939402, FJ939431, FJ939469; EX HORT USA Prince 1995-210 (RSA), LP004/ LP028/LP046, FJ861151, FJ861132, FJ939405, FJ939434, FJ939474- FJ939477; EX HORT USA Prince 1995-222 (RSA), LP033, ns, ns, FJ939407, FJ939436, FJ939479-FJ939484; EX HORT USA Prince (*), LP693, ns, ns, FJ939424, FJ939458, FJ939544. ***C. iridiflora*** Ruiz & Pav.; PERU Plowman & Davis 4753 (US), LP037, FJ861156, FJ861134, FJ939411, FJ939440, FJ939487-FJ939491. ***C. jaegeriana*** Urb.; ECUADOR Kress 89-2884 (US), LP035, FJ861154, FJ861133, FJ939409, FJ939438, FJ939486. ***C. liliiflora*** Warsz. Ex. Pl.; BOLIVIA Gentry & Solomon 44739 (MO), LP128, ns, ns, ns, FJ939444, FJ939509; BOLIVIA Croat 51443

(MO), LP129, ns, ns, ns, FJ939445, FJ939510; BOLIVIA Solomon et al. 11906 (MO), LP599, ns, ns, ns, FJ939448, FJ939521-FJ939523; BOLIVIA Solomon 13129 (MO), LP600, ns, ns, ns, FJ939449, FJ939524; BOLIVIA Clark 6829 (US), LP689, FJ861165, ns, FJ939420, FJ939454, FJ939531-FJ939535; BOLIVIA Refulio 227 (RSA), LP733/LP894, FJ861170, ns, ns, ns, FJ939553-FJ939556. ***C.* × *orchioides*** L. H. Bailey: EX HORT USA Prince 2003-498 (RSA), LP701, FJ861168, ns, ns, FJ939459, FJ939545. ***C. paniculata*** Ruiz & Pav.: BRAZIL Heringer & Eiten 15219 (US), LP692, FJ861167, ns, FJ939423, FJ939457, FJ939540-FJ939543; PERU Plowman & Kennedy 5700 (US), LP006/LP027/LP041, AY656106, AY656132, FJ939404, FJ939433, AY673069. ***C. tuerckheimii*** Kraenzl.; COSTA RICA Kress 89-2853 (US), LP034, FJ861153, AF378764, FJ939408, FJ939437, FJ939485; COSTA RICA Prince 1995-240 (RSA), LP076, FJ861160, ns, FJ939414, FJ939442, FJ939506-FJ939508; ECUADOR Maas 4707 (U), LP029, FJ861152, ns, FJ939406, FJ939435, FJ939478; MEXICO Duke 95-008 (*, Possibly = Kress 77-720), LP635/LP675, ns, ns, FJ939417, FJ939451, FJ939526; PERU USGH 98-001 (*), LP732, ns, ns, ns, FJ939461, FJ939550-FJ939552; EX HORT USA Duke 85-034 (*), LP001, FJ861147, FJ861129, FJ939399, FJ939428, FJ939463-FJ939466. COSTACEAE. ***Costus pulverulentus*** C. Presl.: Duke GH 86-043 (*), AY656107, AF278776, ns, ns, ns. ***Dimerocostus strobilaceus*** Kuntze: Kress 94-3601 (US), AY124997, AF243838, ns, ns, ns. ***Tapeinochilos ananassae*** K. Schum.: Kress 90-2984 (US), AY124996, AF243840, ns, ns, ns. HELICONIACEAE. ***Heliconia irrasa*** Lane ex R. R. Sm.: Kress 76-519 (DUKE), AY656108, AF378778, ns, ns, ns. ***Heliconia rostrata*** Ruiz & Pav.: Duke GH 81-030 (*), FJ861145, AF378767, ns, ns, ns. LOWIACEAE. ***Orchidantha fimbriata*** Holttum: Kress 87-2159 (US), AY656109, AF243841, ns, ns, ns. ***Orchidantha siamensis*** K. Larsen; Kress 94-3718 (US), FJ861146, AF378771, ns, ns, ns. MARANTACEAE. ***Haumania* sp.**: DJ Harris 6672 (RBGE), AY656091, AY656119, ns, ns, ns. ***Sarcophrynium brachystachys*** (Benth.) K. Schum.: Kress 01-7007 (US) AY656100, AY656126, ns, ns, ns. MUSACEAE. ***Ensete ventricosum*** (Welw.) E. E. Cheesman: Kress 96-5372 (US), AY124993, AF243843, ns, ns, ns. ***Musa ornata*** Roxb.: Duke GH 88-110 (*), FJ861144, AF378779, ns, ns, ns. ***Musella lasiocarpa*** (Franch.) C. Y. Wu: Kress 94-3709 (US), AY124992, AF243844, ns, ns, ns. STRELITZIACEAE. ***Phenakospermum guyannense*** (L. C. Rich.) Endl. ex Miq.: Kress 86-2099D (US), AY124995, AF243845, ns, ns, ns. ***Ravenala madagascariensis*** Sonnerat: Kress 92-3504 (US), AY124994, FJ861128, ns, ns, ns. ***Strelitzia nicolai*** Regel & Körn.: Duke GH 78-044D (*), FJ861143, AF243846, ns, ns, ns. ZINGIBERACEAE. ***Globba curtissii*** Holttum: Kress 99-6247 (US), AY125001, AF243847, ns, ns, ns. ***Siphonochilus kirkii*** B. L. Burtt: Kress 94-3692 (US), AY124999, FJ861127, ns, ns, ns. PONTEDERIALES. ***Anigozanthos* sp.**: Prince 2003-499 (RSA), FJ861140, FJ861123, ns, ns, ns. ***Cartonema philydroides*** F. Muell.: DNA from T. Evans (voucher unknown) FJ861138, FJ861121, ns, ns, ns. ***Eichhornia crassipes*** (Mart.) Solms: (DNA from S. Graham), Barrett 814 (TRT), FJ861142, FJ861126, ns, ns, ns. ***Hanguana* sp.**: Kress 99-6325 (US), AY125006, FJ861125, ns, ns, ns. ***Helmholtzia glaberrima*** (Hook. f.) Caruel: Prince 2003-007 (*, University of California-Berkeley Botanic Garden #95-0250), FJ861141, FJ861124, ns, ns, ns. ***Palisota ambigua*** (P. Beauv.) C. B. Clarke: (DNA from T. Evans) Faden 86/55 (*), FJ861139, FJ861122, ns, ns, ns. POALES. ***Dyckia marnier-lapostollei*** *L.B. Sm.:* T. World 97686 (*), FJ861137, FJ861120, ns, ns, ns.