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DELIMITATION OF MAJOR LINEAGES WITHIN *CUSCUTA* SUBGENUS *GRAMMICA* (CONVOLVULACEAE) USING PLASTID AND NUCLEAR DNA SEQUENCES¹

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Subgenus *Grammica*, the largest and most diverse group in the parasitic genus *Cuscuta*, includes ~130 species distributed primarily throughout the New World, with Mexico as its center of diversity. To circumscribe the subgenus and assess the relationships among its major lineages, we conducted the first phylogenetic study of *Grammica* using plastid *trnL F* and nrITS sequences from a wide taxonomic sampling covering its morphological, physiological, and geographical diversity. With the exception of one species belonging elsewhere, the subgenus was found to be monophyletic. The results further indicate the presence of 15 well supported major clades within *Grammica*. Some of those lineages correspond partially to earlier taxonomic treatments, but the majority of groups are identified in this study for the first time. The backbone relationships among major clades, however, remain weakly supported or unresolved in some cases. The phylogenetic results indicate that the fruit dehiscence character is homoplastic, thus compromising its value as a major taxonomic and evolutionary feature. While several striking cases of long distance dispersal are inferred, vicariance emerges as the most dominant biogeographical pattern for *Cuscuta*. Species placed within one of the clades with a predominantly South American distribution are hypothesized to have substantially altered plastid genomes.

Key words: Convolvulaceae; *Cuscuta*; *Grammica*; molecular phylogeny; nuclear ribosomal ITS; parasitic plants; plastid *trnL F*.

The parasitic mode of life arose at least 11 times independently during the evolution of flowering plants (Nickrent, 2002; APG II, 2003) and is found in approximately 4000 plant species (Nickrent et al., 1998) representing ~1% of the known angiosperm diversity. Parasitism is frequently associated with the extreme reduction or modification of vegetative structures as well as rampant convergence with other parasitic taxa, rendering an assessment of homology with other plant lineages quite hard (Kuijt, 1969). For these reasons, parasitic plants in general, and holoparasites in particular have been notoriously difficult to study from a systematic and taxonomic point of view. The resulting lack of knowledge of relationships within parasitic lineages as well as their precise relationships to autotrophic relatives hampers our ability to conduct detailed comparative studies and to understand the sequence of events that have shaped the evolution of these fascinating plants (Nickrent et al., 1998; Futuyma, 2004).

The genus *Cuscuta* represents one such taxonomically problematic group. Comprising some 165–175 currently described species, *Cuscuta* is nearly cosmopolitan in distribution with its species found on every continent (except Antarctica), ranging from the 60th parallel north in Europe and Asia, to the Cape region of South Africa, and as far south

as the 47th parallel in Argentina and Chile (Yuncker, 1932; Hunziker, 1950; Mabberley, 1997). All members of this genus are vines with twining, slender, pale stems, with reduced, scale-like leaves, and no roots. These stem parasites are attached to the host by haustoria and depend entirely (or nearly so) on their hosts to supply water and nutrients (Kuijt, 1969; Dawson et al., 1994). Most *Cuscuta* species are also characterized by reduced amounts or the complete absence of chlorophylls (van der Kooij et al., 2000) even though some species are capable of limited and localized photosynthesis (Dawson et al., 1994; Hibberd et al., 1998). Various species (commonly known as dodders) are capable of parasitizing a wide range of herbaceous and woody crop plants, but for the most part they do not cause significant agricultural losses due to the effectiveness of currently available methods of control (reviewed by Dawson et al., 1994; Costea and Tardif, 2006). Members of this genus were recently implicated as vectors in the horizontal transfer of mitochondrial genes in plants (Mower et al., 2004).

Traditional classifications largely ignored the question of *Cuscuta*'s precise relationships with nonparasitic relatives, owing mainly to the lack of useful taxonomic characters. An association with the Convolvulaceae was recognized early on, based on reproductive morphology, but few attempts were made to propose a more detailed scheme of relationships between *Cuscuta* and nonparasitic members of the family. The approaches taken have fallen into two categories, either recognition of *Cuscuta* as a separate monotypic family, implying a sister-group relationship to the rest of Convolvulaceae or placement of *Cuscuta* within Convolvulaceae under various taxonomic ranks (but without any further implications on its possible relationships). Examples of the former approach include the classifications by Dumortier (1829) and Roberty (1952, 1964), followed by most major synoptic works on flowering plants (e.g., Cronquist, 1988; Takhtajan, 1997). The latter approach includes recognition of *Cuscuta* as tribe Cuscuteae (Choisy, 1845; Bentham and Hooker, 1873; Baillon,

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than qualitative, difficult to discern, or subjective and open to interpretation. The general difficulty with these characters, combined with the large number of species in subgenus *Grammica*, explains in part why an updated revision of *Cuscuta* has not appeared in more than 70 years since Yuncker's (1932) seminal work on this subject.

Given the size and complexity of *Cuscuta* subgenus *Grammica*, the present study takes a "bottom-up" phylogenetic approach and focuses primarily on the first three of the aforementioned goals, i.e., the circumscription of major lineages within the subgenus, relationships among them, as well as an assessment of the monophyly of this group overall. To answer these questions, we generated a new molecular data set consisting of plastid and nuclear noncoding DNA sequences. This study presents the first phylogenetic analysis that includes members of all sections and subsections of *Cuscuta* subgenus *Grammica*.

MATERIALS AND METHODS

Taxon sampling A total of 265 accessions representing 99 species were used in this study. Species names, sources, voucher information, and corresponding DNA extraction numbers are provided in the Appendix. Yuncker's (1932) intrageneric classification is followed here because it is the most widely used one and represents the only comprehensive work at the generic level. The 96 ingroup taxa, on which our analyses are primarily focused, include members of two traditionally recognized sections within *Cuscuta* subgenus *Grammica* and all 24 recognized subsections. Due to the difficulties in distinguishing many of these species morphologically, effort was made to sample multiple accessions of each. Approximately two thirds of the species examined here are represented by more than one individual. Special attention was paid to morphologically variable species containing more than one subspecies/variety (e.g., *C. salina*, *C. indecora*, *C. umbellata*) and to those with wide geographic range (e.g., *C. campestris*, *C. gronovii*, *C. californica*). These species were represented by upward of 7–10 individuals from across their respective morphological/geographical range. The remaining one third of the species is represented by a single individual mainly because they are either rare or locally abundant but known only from their type localities or otherwise restricted areas or because they are underrepresented in collections. The latter is an especially significant factor for many South American species. Three species (*C. nitida*, *C. europaica*, and *C. approximata*) from the putative sister subgenus *Cuscuta* were selected as outgroup taxa.

Molecular techniques Total genomic DNA from silica dried or herbarium material was extracted using a modified hexadecyltrimethylammonium bromide (CTAB) technique from Doyle and Doyle (1987) and purified using Wizard minicolumns (Promega, Madison, Wisconsin, USA). The polymerase chain reaction (PCR) was used to obtain the double stranded DNA fragments of interest. The plastid genome (ptDNA) region containing the *trnL* intron, 3' *trnL* exon, and intergenic spacer between this exon and *trnF* (hereafter called *trnL-F*) was amplified using the C and F primers described by Taberlet et al. (1991). The internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) containing ITS1, 5.8S, and ITS2 (hereafter called nrITS) was obtained using primers ITS5 and ITS4 described by White et al. (1990). PCR was carried out in 50 μ L volumes with annealing temperatures of 50–55°C. Amplified products were cleaned by polyethylene glycol/NaCl precipitations. Cleaned products were sequenced directly, including both strands to ensure accuracy, using the DYEnamic ET dye terminator sequencing kit (GE Healthcare, Baie d'Urfé, Quebec, Canada) on an Applied Biosystems model 377 automated DNA sequencer (PE Biosystems, Foster City, California, USA). PCR products for which polymorphism was detected during direct sequencing (mostly ITS) were cloned into the pSTBlue1 AccepTor vector (EMD Biosciences, San Diego, California, USA), and multiple clones were sequenced. Sequence data were proofed, edited, and contigs assembled using Sequencher v.3.0 (Gene Codes Corp., Ann Arbor, Michigan, USA). Sequences generated in this study are in GenBank (accession numbers EF194288, EF194718 and EF202557, EF202563; Appendix).

Phylogenetic analyses Sequences were aligned manually using the program Se Al v.2.0a11 (Rambaut, 2002). Although numerous gaps had to be introduced in the alignments, the sequences were readily alignable among the ingroup taxa in both plastid and nuclear matrices. Regions that could not be unambiguously aligned were excluded from subsequent analyses. Gaps in the alignments were treated as missing data. Phylogenetic analyses were conducted using parsimony and Bayesian inference methods.

Parsimony analyses Heuristic searches and estimates of clade support were conducted for each matrix separately as well as for a combined data set. Nucleotide characters were treated as unordered, and all changes were equally weighted. Searches for most parsimonious (MP) trees were performed using a two stage strategy with PAUP* version 4.0b10 (Swofford, 2002). First, the analyses involved 1000 replicates with stepwise random taxon addition, tree bisection reconnection (TBR) branch swapping saving no more than 10 trees per replicate, and MULTREES option off. The second round of analyses was performed on all trees in memory with the same settings except with the MULTREES option on. Both stages were conducted to completion or until 100,000 trees were found. In addition, other searches were conducted using the parsimony "ratchet" analysis (Nixon, 1999) as implemented in NONA (Goloboff, 1999) with the WinClada interface (Nixon, 2002). Ten consecutive tree searches were conducted using 200 iterations per search, one tree held for each iteration, 10% of total characters sampled, and amb poly (no swapping on ambiguously supported nodes), but they did not find shorter trees. Relative support for clades was inferred by nonparametric bootstrapping (Felsenstein, 1985) as implemented in PAUP* using 500 pseudoreplicates, each with 20 random sequence addition cycles, TBR branch swapping, and MULTREES option off (DeBry and Olmstead, 2000). Conflict between data sets was evaluated by visual inspection, looking for the presence of strongly supported yet conflicting topologies from individual matrices.

Bayesian analyses The general time reversible (GTR) model (Yang, 1994) of DNA substitution, with rate variation among nucleotides following a discrete gamma distribution and assuming a portion of invariant sites (GTR + G + I), was selected as the best fit by both the hierarchical likelihood ratio test (hLRT) and Akaike information criterion (AIC), as implemented in ModelTest version 3.7 (Posada and Crandall, 1998). Bayesian phylogenetic inferences were performed using MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003) on the combined data set only. Two runs starting from random trees were carried out using the GTR + G + I substitution model. All model parameters were treated as unknown variables with uniform prior probabilities and were estimated as part of the analysis together with tree topologies. Metropolis coupled Markov chain Monte Carlo algorithm was used with four simultaneous chains, set at two million generations, and sampled every 100 generations. To determine the burn in cut off point, we plotted the \ln likelihood scores against generation time for both runs. After discarding all preasymptotic samples, remaining data points were analyzed separately in PAUP* to compute the 50% majority rule consensus tree. Because no significant differences between the two runs were detected, the reported topologies and posterior probabilities (PP) are based on trees pooled from both independent Bayesian analyses. Only the nodes receiving ≥ 0.95 PP were considered statistically significantly supported, given the assumptions of DNA sequence evolution (Rannala and Yang, 1996).

Testing of alternative topologies Alternative topologies, mainly designed to investigate the evolution of characters defining some traditional taxonomic groups, were constructed and their cost in parsimony was assessed using PAUP* (Swofford, 2002). To statistically compare resulting alternative phylogenetic hypotheses, we conducted one tailed Shimodaira Hasegawa tests (SH tests; Shimodaira and Hasegawa, 1999; Goldman et al., 2000) using the aforementioned substitution model and likelihood settings. The SH tests were conducted with PAUP* using 1000 replicates and full parameter optimization of the model.

RESULTS

Sequences and alignments Characteristics of the sequenced regions as well as statistics of MP trees derived from separate and combined analyses are summarized in Table 1.

The total aligned length of the *trnL-F* region is 689 bp, while

TABLE 1. Summary descriptions for sequences included in, and maximum parsimony trees derived from, individual and combined analyses of *Cuscuta* subgenus *Grammica*.

Description	<i>trnL-F</i> (plastid)	nrITS (nuclear)	Combined data
Number of individuals sequenced ^a	223	207	265
Number of OTUs analyzed ^b	141	153	161
Sequence characteristics:			
Aligned length	689	717	1406
Analyzed length ^c	525	676	1201
Variable sites	241	450	691
Parsimony informative sites	189	402	591
Mean AT content (%)	63	50	55 ^d
Base frequency homogeneity ($\chi^2/df/P$)	80.1/420/1.0	279.2/456/1.0	146.4/384/1.0 ^d
Tree characteristics:			
Number of trees	>100 000	>100 000	>100 000
Length	790	1965	2776
CI/RI	0.52/0.89	0.447/0.886	0.465/0.885

^a Excluding the outgroup taxa that could not be aligned with the ingroup

^b After individuals with identical sequence for both regions were aggregated into a single terminal taxon

^c After excluding portions of alignments corresponding to primer sites and ambiguously aligned regions

^d Including only OTUs for which both sequences are available; CI, consistency index; df, degrees of freedom; OTU, operational taxonomic unit; RI, retention index

individual sequences varied from 480 to 680 bp in length (480–510 bp for the ingroups alone). Most of the *Cuscuta* species under investigation were readily amplifiable for the *trnL-F* region with the same universal set of primers (C and F) used routinely for many other groups across angiosperms (Taberlet et al., 1991). However, this plastid region could not be obtained for a number of species belonging to several closely related subsections sensu Yuncker (1932; *Grandiflorae*, *Odoratae*, *Acutilobae*, and *Ceratophorae*), despite the fact that the same DNA accessions produced nrITS fragments without difficulty. Several attempts to amplify smaller fragments with internal and/or alternative primers failed as well. Sequences were easily aligned across most of the *trnL-F* region for all but one ingroup species sampled in this study. However, the spacer between 3'-*trnL* and *trnF* is evolving more rapidly than the *trnL* intron in terms of length and point mutations (as noted previously for Convolvulaceae in general; Stefanović et al., 2002) and a portion of 120 bp was excluded from analyses. Three outgroup species from subgenus *Cuscuta* (*C. nitida*, *C. europaea*, and *C. approximata*) yielded sequences significantly longer than those found within ingroup taxa. Except for short segments corresponding to *trnL* and *trnF* genes themselves, these sequences could not be aligned with the ingroups and hence could not be used in analyses to root trees. Surprisingly, one putative ingroup species, *C. appendiculata* from South Africa, was also found to have a longer sequence, unalignable with other ingroup species. Furthermore, while *C. europaea* and *C. approximata* (both Eurasian in distribution) had significant levels of similarity and were easily alignable with each other, *C. nitida* was divergent to the point that it could not be aligned with the other two outgroups. This South African species had high similarity only to *C. appendiculata*, and their sequences were easily alignable with each other.

Aligned sequences of nrITS used here were 717 bp in length with the individual sequences varying between 560 and 600 bp. The length variation was more or less equally distributed throughout the entire region. The nrITS sequences could not be obtained for all investigated individuals/species. However, the unsuccessful amplifications were randomly distributed (i.e., not part of any particular taxonomic group, unlike in the case

described for *trnL-F*) and were probably due to the poor quality of the DNA extracted from older herbarium specimens. For the majority of DNA accessions, the direct sequencing approach yielded results without apparent polymorphism. In some cases, however, it becomes clear that the PCR product contained more than one type of nrITS sequence, and for those the cloning approach was followed. In most of those cases, the “polymorphism” was caused by the presence of fungal DNA resulting either from the natural presence of fungal epi- and endophytes in *Cuscuta* species or from fungal tissue contamination. Fungal sequences were easily separated from *Cuscuta* nrITS sequences and were excluded from further analyses. In several cases a genuine polymorphism within *Cuscuta* nrDNA was detected, caused by point mutations and/or length variants. However, preliminary phylogenetic analyses in all of those cases (results not shown) indicated that these paralogous sequences were most closely related to each other, suggesting relatively recent duplication events or minor DNA polymerase error, and only one, randomly chosen, sequence was used to represent the given individual. The nrITS sequences were relatively easily alignable across all ingroup species, and this whole region was included in the phylogenetic analyses. However, in a way similar to that described for the *trnL-F* sequences, none of the three a priori chosen outgroup taxa from subgenus *Cuscuta* could be aligned with ingroup species for the more variable ITS1 and ITS2 regions. Only the highly conservative (and least informative) 5.8S was alignable between ingroups and outgroups, and consequently only this region could be used to root the nrITS trees. The same was true for a putative ingroup species, *C. appendiculata*, which had the most sequence similarity with *C. nitida* for nrITS region as well.

For phylogenies aimed at resolving species-level relationships, it is of paramount importance to incorporate within-species variability and take into account possible biological phenomena that can confound results (such as lineage sorting, deep coalescence). For these reasons, most of the species in the study were represented by multiple individuals, sampled from geographically distinct areas and encompassing morphological variability. However, the addition of terminal taxa results in a sharp increase of computational burden (Felsenstein, 1978).

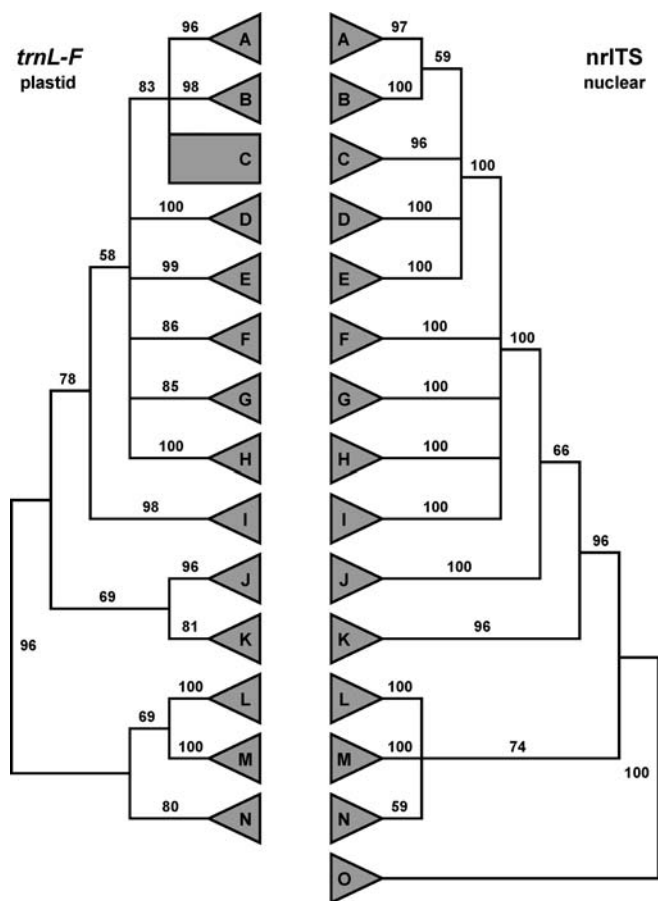


Fig. 2. Overview and comparison of strict consensus trees derived from separate *trnL-F* and nrITS parsimony analyses. Fifteen major groups are labeled A–O, and their parsimony bootstrap support values are indicated above branches. Plastid sequences could not be obtained for members of the O clade. Species relationships within the major clades are not shown (see Appendices S1 and S2 for detailed trees in Supplemental Data accompanying online version of this article). Trees are tentatively rooted using the L–O clades as functional outgroups (see Results for full explanation).

Therefore, to facilitate the phylogenetic analyses, individuals of the same species having both the *trnL-F* and nrITS sequences identical to each other were grouped into a single operational taxonomic unit (OTU). Following this procedure, the 223 individuals from *trnL-F* matrix were aggregated into 141 OTUs, 207 from nrITS matrix into 153, and 265 individuals used in the combined data set were aggregated into 161 OTUs (Table 1). No significant heterogeneity in base composition was detected within any of these data matrices across all taxa.

Tree topologies A number of distinct phylogenetic analyses were conducted using parsimony and Bayesian approaches to explore the distribution of phylogenetic signal in the different matrices. All analyses produced trees of remarkably similar topology although resolution and branch support varied. Tree characteristics from MP searches are summarized in Table 1.

Individual data set analyses The *trnL-F* and nrITS matrices produced >100 000 trees, 790 and 1965 steps in

length, respectively. Schematic consensus trees from parsimony analyses are presented in Fig. 2. The overview of relationships among the major groups also allows for topological comparison of results between the two data sets (Fig. 2). The detailed trees obtained from separate analyses of the data sets are presented in Appendices S1 and S2 (see Supplemental Data accompanying online version of this article). A total of 15 major clades, labeled A–O, were resolved within *Cuscuta* subgenus *Grammica* with nrITS sequences. Fourteen of the same groups, A–N, were also recovered with *trnL-F* data. However, none of the sequences belonging to clade O, a lineage consisting almost exclusively of South American species from subsections *Odoratae*, *Grandiflorae*, and *Acutilobae*, could be obtained for *trnL-F*. This plastid region could not be amplified either for several species within clade K (e.g., *C. erosa*, *C. boldinghii*), even though the same DNA accessions yielded good PCR products for nrITS. Nevertheless, other members of the K clade were sequenced for *trnL-F* and were available as placeholders in the phylogenetic analyses. Most of the 15 major clades received moderate (70–85%) to strong bootstrap support (>85%) from both of the individual matrices. However, some groups were found to be weakly supported (<70%) by one of the data sets while receiving moderate to strong support from the other in a mutually complementary fashion. For example, clade N was supported only by 59% BS with nrITS data, but it received 80% BS from *trnL-F* data. In a complementary fashion, clade C obtained <50% BS for with plastid sequences, yet the same clade was supported with 96% BS with nuclear data. The overall strong support for the circumscription of these 15 major clades stands in contrast to the less-resolved backbone relationships within *Cuscuta* subgenus *Grammica* based on separate analyses. The *trnL-F* phylogeny has only two well-supported backbone relationships, a group consisting of A–C clades (receiving 83% BS) and a group consisting of L–N clades (which received 96% BS). The nrITS consensus tree was somewhat more resolved, having three highly supported backbone nodes (100% BS for a group composed of A–E clades, 100% BS for a group consisting of A–I clades, and 96% BS for a group composed of A–K clades). Given the current taxonomic sampling, the only topological disagreement observed between the plastid and nuclear phylogenies involved clades J and K. With plastid data, these two clades were placed as each other's sister-group, whereas nuclear data resolved them as a successively diverging grade. However, these alternative topologies are weakly supported (<70% BS) in both cases. In addition, this difference represents only a slight topological distortion (a nearest-neighbor interchange) most likely caused by sampling discrepancies between *trnL-F* and nrITS matrices within the K clade (as described before). Taking all of this into account, we deemed these two matrices congruent and combined them into one data set.

Analyses of combined data sets The trees produced by the total-evidence approach had better resolution and overall support relative to those produced by independent analyses. Therefore, we have based our discussions on the analyses of the combined data sets. The parsimony analysis using this matrix resulted in >100 000 MP trees, each 2776 steps in length. Figures 3–4 present the strict consensus of those equally parsimonious trees and one of them, randomly selected, was chosen to illustrate the branch lengths (Fig. 5). Both Bayesian analyses, each initiated from a random starting tree,

converged on similar In likelihood scores and reached an asymptotic plateau at no later than 200 000 generations. The relationships inferred through the Bayesian analyses are topologically identical with those derived under the parsimony criterion (Figs. 3, 4).

As expected based on individual analyses, the same 15 clades were recovered using the combined data, with very high levels of support levels. Only one group, clade N, received weak support (69% BS; <0.95 PP). This relatively low-level of support is due to ambiguous placement of one species, *Cuscuta mcvaughii*. The rest of the N clade, without *C. mcvaughii*, is supported at 100% BS and ≥ 0.95 PP. In addition, the combined data set resolved with higher support more of the backbone relationships.

Neither of the two groups historically defined within subgenus *Grammica*, sections *Cleistogrammica* and *Eugrammica*, were found to be monophyletic. The presence of indehiscent vs. dehiscent capsules, the defining character states for these sections, was inferred to have occurred at least 10 times independently (solid bars; Figs. 3, 4). To segregate all the species into two groups based on the type of dehiscence, multiple well-supported nodes, most of them at 100% BS and ≥ 0.95 PP, would have to be collapsed. Not surprisingly, the enforced monophyly of *Cleistogrammica* and *Eugrammica* resulted in trees 460 steps longer than the most parsimonious trees and was rejected as a significantly worse solution by the SH test ($P < 0.01$). Another alternative topology concerning the fruit dehiscence was tested as well. Members of the *C. indecora* complex (i.e., clade M) all have indehiscent capsules, yet they were found on the MP trees surrounded by clades with predominantly dehiscent fruits. We wanted to determine the cost in parsimony and its significance for the alternative in which the M clade would be in closer association with the clades that have almost exclusively indehiscent capsules (clades A–E). Although much less stringent than the previous one, this constraint nevertheless yielded trees 46 steps longer than the most parsimonious trees and was also rejected as significantly different from the best solution by the SH test ($P < 0.01$). Even though the two character states clearly originated more than once (and hence their corresponding sections are not monophyletic), a boundary can still be drawn between two groups, one predominantly with indehiscent capsules and the other composed mostly of species with dehiscent capsules. The former group includes clades A–E and the latter clades G–O. Clade F cannot be unequivocally assigned to either of these groups at present due to the uncertainties regarding the capsule character states (open bars; Fig. 3).

Of 24 subsections defined within *Grammica* by Yuncker (1932; Fig. 1), three are monotypic (*Cephalanthe*, *Lobostigmae*, and *Prismaticae*). Only one subsection containing more than one species, subsection *Californicae*, was found to be a monophyletic group, nested in clade A, although a couple of its morphologically distinct species were not included in present analyses. None of the remaining 20 subsections were found to be monophyletic. In some instances, albeit not forming a monophyletic group, all members of a particular subsection were still found within one of the labeled clades and hence relatively closely related. For example, *Arvenses* is not monophyletic, but all species classified in this subsection are found only in clade B. Correspondingly, species classified in *Subulatae* are found only in clade G, species classified in *Leptanthae* are found only in clade L, etc. However, most of the subsections are divided among different major groups, with

their species dispersed throughout the tree, and clearly do not constitute natural assemblages of taxa.

Monophyly and rooting of the subgenus *Grammica* As indicated earlier, the sequence differences between ingroup and outgroup taxa were too great to unambiguously assess the primary homology between the two. This was the case for both *trnL-F* and nrITS sequences except for their highly conservative portions. The preliminary analyses, designed to verify the monophyly of the subgenus *Grammica* and to explore alternative placements of the root, were conducted using only the regions alignable between the ingroup and outgroup taxa (resulting trees not shown). When plastid data were restricted to include only the *trnL* and *trnF* exons, the results strongly supported the monophyly of *Grammica* (100% BS; excluding only *C. appendiculata*) and suggested placement of the root on the branch separating A–K clades on one side and L–N clades on the other (Fig. 2). Analyses limited to the more conservative 5.8S rDNA sequences indicated the L–O grade as sister to the remainder of the subgenus *Grammica* (Fig. 2). The monophyly of subgenus *Grammica* received 85% BS, a moderate yet significant value given the conservative nature of the region included in this analysis. Finally, the midpoint rooting conducted with ingroup taxa only but encompassing the entire *trnL-F* region and/or entire nrITS region indicated the same basal split into two major groups, the first including species from clades A–K and the second including members of clades L–O. Taking all of these results in aggregate, we used species from the L–O clades (L–M for *trnL-F* data alone) as functional outgroups to provide directionality for the inferred underlying phylogenetic network (Figs. 2–5).

DISCUSSION

This study represents the most complete molecular phylogenetic hypothesis for *Cuscuta* subgenus *Grammica* yet made. It is based on plastid and nuclear noncoding sequences obtained for an extensive sampling of species from a broad taxonomic and geographic range. The resulting phylogenetic inferences are well resolved and robust, including significant support for some of the higher level relationships along the spine of the tree.

Circumscription of major clades within *Cuscuta* subgenus *Grammica* Combined data analyses resulted in a phylogenetic hypothesis (Figs. 3–5) featuring many resolved and well-supported clades. We circumscribe here 15 of those groups, labeled informally as clades A–O, and discuss them in some detail later. A formal classification will be provided elsewhere, along with the taxonomic revisions for the whole genus. Several factors were taken into account in deciding which clades are well enough defined to warrant labeling. Priority was given to molecular analyses in which labeled clades were both well supported and distinct (Figs. 3–4), as evidenced by their relative branch lengths (Fig. 5). Morphological distinctiveness, geographical distribution, as well as correspondence with previously circumscribed taxa were also considered.

Current knowledge of morphology, ecology, distribution, and other biologically relevant information on these 15 different species groups is uneven. Species from clades that occur primarily in North America are collected more frequently; thus their taxonomy is best understood. Several

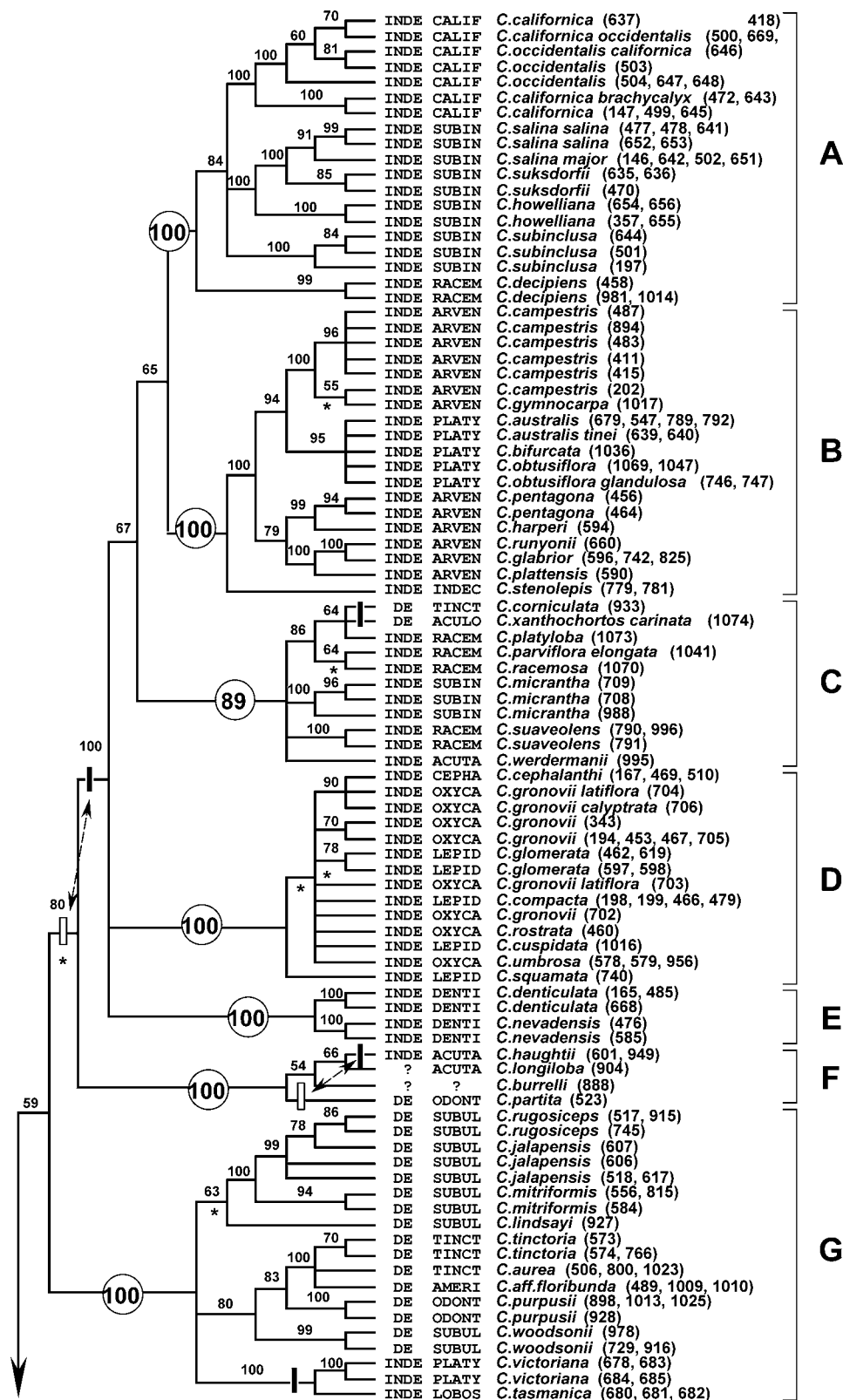


Fig. 3. Distal portion of the strict consensus tree derived from the combined *trnL* *F* and nrITS maximum parsimony analysis comprising species from all traditionally recognized *Cuscuta* subgenus *Grammica* sections and subsections. Major clades referred to in the text are labeled and their bootstrap support is encircled. Bootstrap values for relationships within major clades as well as for the backbone relationships are indicated above branches (for support $\geq 50\%$). Asterisk indicates branches with Bayesian posterior probability < 0.95 ; all other interior branches have posterior probability ≥ 0.95 . Capsule indehiscence (INDE) and dehiscence (DE), corresponding to species' circumscription into sections *Cleistogrammica* and *Eugrammica*,

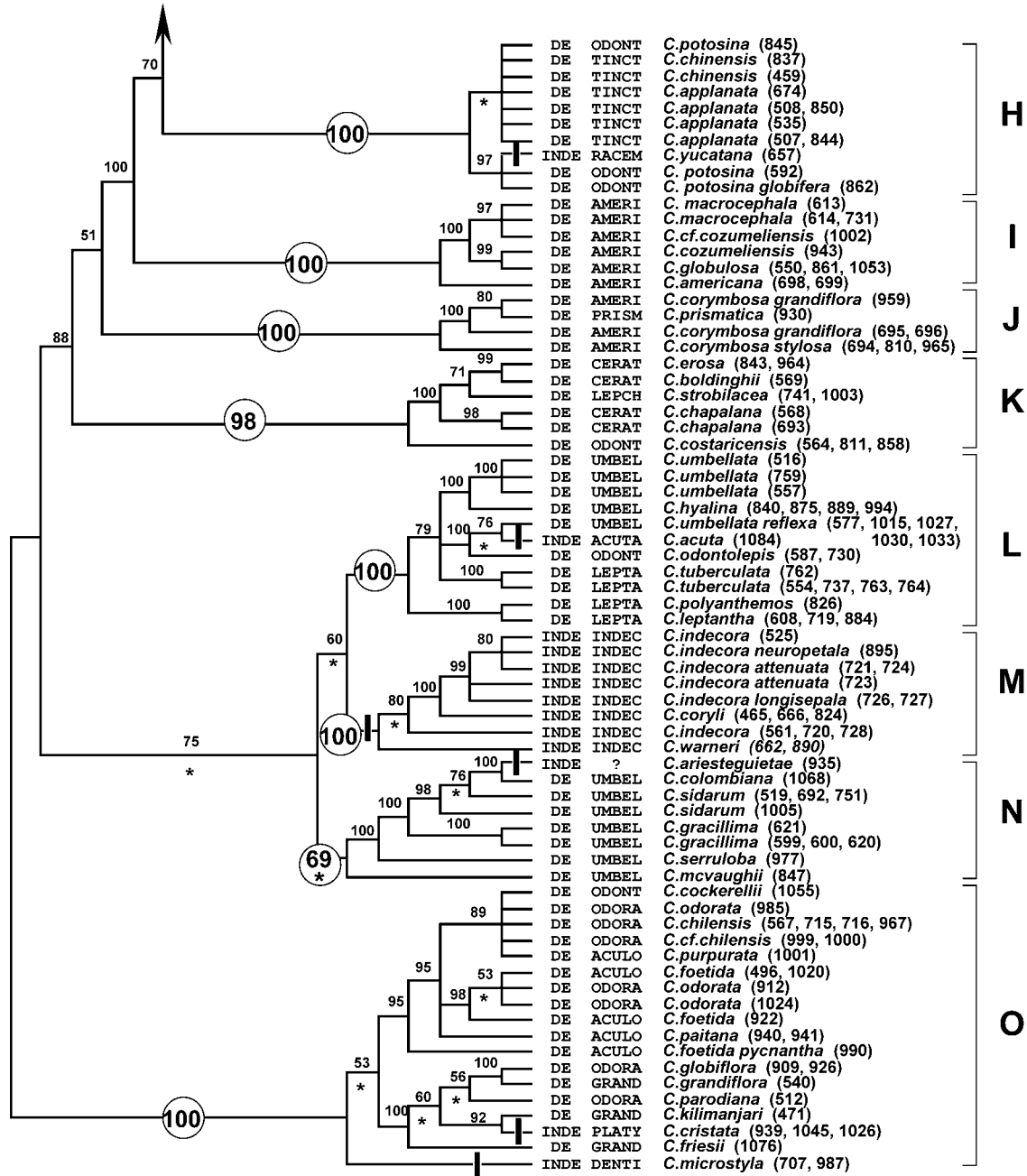


Fig. 4. Proximal portion of the strict consensus tree derived from the combined *trnL F* and nrITS maximum parsimony analysis comprising species from all traditionally recognized *Cuscuta* subgenus *Grammica* sections and subsections. For further explanations, see Fig. 3.

recent treatments have provided a wealth of information on North American species but have focused either on a particular geographic area (Beliz, 1986, 1993; Austin, 1986; Musselman, 1986; Prather and Tyrl, 1993) or a taxonomic subset (Costea et al., 2005, 2006a d). Treatments covering Central and South

America are comparatively fewer in number and older (Hunziker, 1947a, b, 1949, 1950), despite the fact that these regions are the centers of biodiversity for *Cuscuta* (and in particular subgenus *Grammica*). By defining a number of previously unsuspected relationships among South American

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respectively, are indicated before the species name, along with the subsection to which it traditionally belongs. The five letter abbreviations of subsection names follow those from Fig. 1. Solid bars depict position of changes in fruit dehiscence optimized on the tree. Open bars indicate two equally parsimonious reconstructions, and their alternatives are indicated with dotted arrows. Numbers in parentheses following species names correspond to DNA accessions (see Appendix).

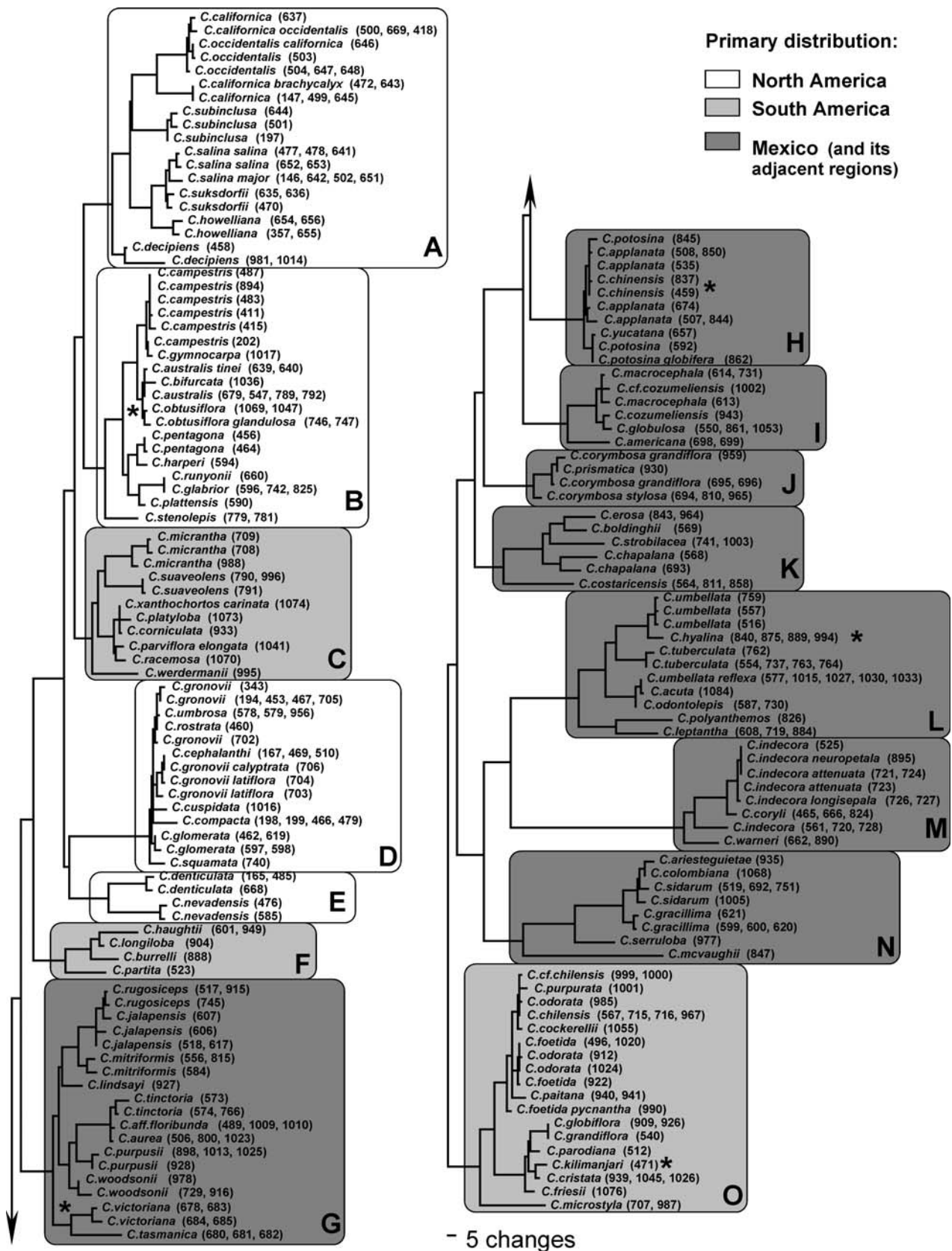


Fig. 5. One of equally parsimonious trees derived from the combined *trnL F* and nrITS analysis, including species from all traditionally recognized *Cuscuta* subgenus *Grammica* sections and subsections, chosen to illustrate branch lengths. Branch lengths are drawn proportionally to the number of

species and pointing out their overall importance for understanding the systematics and biogeography of the genus, our present study provides new impetus to rectify this obvious disparity.

Clade A This clade corresponds largely to the *C. salina-californica* complex, as defined by Costea et al., (2006d) and groups together species distributed in North America, west of the Rockies, from the Pacific Northwest to the northern Mexican states. Molecular data revealed four distinct lineages within this clade. The first group contains two species, *C. californica* and *C. occidentalis*, both belonging to the subsection *Californicae* (Yuncker, 1932). In most cases, these two species can be distinguished by a combination of characters (Costea et al., 2006d), but morphologically intermediate individuals are consistently, if rarely, encountered. Most of the sequences obtained from individuals representing *C. californica* and *C. occidentalis* segregated into two separate groups. One group corresponds entirely to *C. californica*, including *C. brachycalyx*, an entity described by Yuncker (1932, 1965) as a separate species but treated here as conspecific with *C. californica*. The other corresponds predominantly to *C. occidentalis*. However, the intermediate plants included into our analyses grouped also with *C. occidentalis*. It is not clear if this could be attributed to introgression/hybridization between these sympatric species or to incomplete lineage sorting at both the molecular and morphological level. Subsection *Californicae*, characterized by a complete reduction of infrastaminal scales, are the only traditionally defined subsection (Yuncker, 1932) that appears monophyletic in our results. It should be noted, however, that two morphologically distinct species also classified in *Californicae*, *C. sandwichiana* and *C. jepsonii*, are not included in the present analyses. At least one of them, *C. sandwichiana*, is believed to belong to the *C. pentagona* complex (our clade B) based on its branching characteristics (Costea et al., 2006c). If true, this would render *Californicae* nonmonophyletic as well. The second and third groups within the A clade belong traditionally to subsection *Subinclusae*. Here, two species, *C. salina* and *C. suksdorfii*, with infrastaminal scales reduced to ridges in one and lateral wings in the other, form a well-supported group. They have *C. howelliana*, a species with well-developed infrastaminal scales, as their sister, supporting the hypothesis that these scales underwent a gradual reduction in this group (Costea et al., 2006b). The exact relationship of *C. subinclusa* to the other groups is not resolved. The fourth group in clade A consists of several individuals belonging to *C. decipiens*. This Mexican species, traditionally circumscribed in subsection *Racemosae*, is found as an isolated and well-defined lineage in a sister-group position to the rest of the A clade.

Clade B Taxonomically, this clade contains all sampled members of subsection *Arvenses* (*C. pentagona*- complex) with several additional species traditionally classified into *Platycarpae* (Yuncker, 1932) nested within it. Together, this group is characterized by depressed-globose capsules, with mostly short and subulate styles, and relatively large interstylar

apertures (Costea et al., 2006c). Its monophyly is strongly supported by molecular data (100% BS) in a position as sister group to *C. stenolepis*, a species from Ecuador originally classified in *Indecorae* (Yuncker, 1932). Albeit morphologically entangled with other species from subsection *Arvenses* to the point of being treated sometimes as conspecific with *C. pentagona* (e.g., Beliz, 1986), *C. campestris* was found to be molecularly quite distinct from this group and more closely related to those members of *Platycarpae* found in the B clade.

Several species of clade B have their distributions spanning multiple continents and represent some of the most frequently encountered and best-known dodders worldwide. For example, the native distributional range of *C. australis* includes Asia, Australia, and Europe, while that of *C. obtusiflora* spans the entire western hemisphere. In addition, *C. campestris* is one of the most successful parasitic weeds, spreading worldwide through contaminated crop seed, especially that of forage legumes (e.g., alfalfa, clovers; Dawson et al., 1994). Originally, however, this species is native to North America, where it is second in abundance only to *C. gronovii* (Yuncker, 1932). Even though the B clade is today essentially cosmopolitan (due to *C. australis* and *C. obtusiflora* with worldwide distributions), its origin and diversification is still deduced to be primarily North American. This inference is supported by the derived position of taxa with wide geographic distribution, nested as a monophyletic group within a grade of species with largely North American distribution. It is worth noting that the species with the widest geographical distributions and ecological amplitudes (*C. australis*, *C. obtusiflora*, *C. campestris*) also form a monophyletic group, nested within clades containing species with narrow (*C. runyonii*, *C. plattensis*, *C. harperi*) to moderate (*C. glabrior*, *C. pentagona*, *C. stenolepis*) distribution ranges. This implies that their physiological capability to use hundreds of genera as hosts probably arose once, in their common ancestor. This capability thus allowed them to spread over large geographic areas, either naturally or as consequence of anthropogenic influences.

Clade C The existence and composition of this clade, first identified here, emerges as one of the biggest surprises in this study. Based on substantial morphological differences, the eight currently sampled species belonging to this clade were previously dispersed among five different subsections. The unusual level of heterogeneity is best illustrated by an example. Two species with dehiscent capsules, *C. corniculata* and *C. xanthochortos*, are not only nested within the C clade, where all other species have indehiscent capsules, but are also nested within an even larger group, comprising five clades (A–E), all members of which are characterized by indehiscent fruits. In a context of the rooted phylogeny (discussed later), this represents the only case of a reversal from indehiscent to dehiscent fruit in *Cuscuta*. All the species found in the C clade are distributed exclusively in South America, mainly east of the Andes.

Clade D All of the species traditionally assigned to subsections *Oxycarpae*, *Lepidanchae*, and *Cephalanthae*

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changes. Shaded boxes indicate the primary geographic distribution of clades by regions. Asterisks depict species (or groups of species) that are inferred to have a major disjunction in distribution compared to the other members of their respective clades (indicative of long distance dispersal events). Numbers in parentheses following species names correspond to DNA accessions (see Appendix).

(Yuncker, 1932) are found only within this clade. Furthermore, according to Yuncker's (1932) phylogenetic scheme, these three subsections are each other's closest relatives (i.e., they are expected to be monophyletic, using modern terminology). Morphologically, this entire group is supported by the presence of an ovary/capsule that thickens apically, forming, in some species in its most pronounced form, a beak-like stylopodium, and also corresponds to a broadly circumscribed subsection *Oxycarpae* sensu Engelmann (1859). The monophyly of this entire group is well supported in all molecular analyses and under all optimality criteria, and the long branch leading to this clade further highlights its molecular distinctiveness (Fig. 5). In these respects, clade D represents one of the very few cases in which molecular and traditional classifications are in full accord (compare with Fig. 1). The distinctiveness and support for the D clade, however, is in sharp contrast with very short branch lengths and almost complete lack of resolution within it. It is therefore not clear whether either of the subsections (other than monotypic *Cephalanthae*) is monophyletic or not. Additional, faster evolving data will be necessary to answer this question. Most species of this clade are found from the Great Plains to the Rockies. In addition, some common species, such as *C. gronovii*, are distributed throughout North America (Yuncker, 1932).

Clade E Subsection *Denticulatae* traditionally includes four species (Yuncker, 1932, 1943). Two of those, *C. denticulara* and *C. nevadensis*, constitute a distinct and well-supported clade, labeled E in this study. One species, *C. veatchii*, was not sampled here, but there is strong evidence that it forms a natural group with the former two, based on a few distinctive morphological characters (Costea et al., 2005). The most unusual of these is the seed with a "thickened" embryo, where the embryo's radicular end is enlarged in a ball-like structure. This feature is unique among dodder species. Also, these three species are characterized by a distinctively reticular calyx surface. Similarly to the A clade distribution, they occur in North America, west of the Rockies, from the Pacific Northwest to the northern Mexican states. Among them, *C. denticulata* has the broadest geographical distribution, encompassing this entire region. *C. nevadensis* is sympatric with *C. denticulata*, but it has a narrower range and is found in southern California, Nevada, and perhaps Arizona. *Cuscuta veatchii* is restricted in distribution to Baja California in Mexico and is parapatric with *C. denticulata*. Morphological differences among these species are subtle, yet discontinuous and consistent (Costea et al., 2005), and thus in agreement with the substantial differences in branch lengths observed between sampled species (Fig. 5). Yuncker (1932) also included *C. microstyla* in *Denticulatae*, based on its overall similarity to the other species in this subsection. However, only material from the type locality was available to him at the time, and it contained neither capsules nor seed, thus preventing him from observing the defining embryo feature. This Chilean species is found elsewhere on the tree, as a distinct lineage within the O clade, together with the other species with South American distribution.

Clade F Little is known about the four species found in this clade, the second of three lineages with exclusively South American distribution revealed in this study. Even though Yuncker noted the overall resemblance between *C. haughtii* and *C. partita* in his monograph (1932), he nevertheless placed

these species into two different sections, based on their fruit dehiscence. The other two species from the F clade, *C. longiloba* and *C. burrellii*, both of which were described more recently (Yuncker, 1957, 1961), are known only from very few collections and are even more obscure. Their capsules were not observed, and the state of dehiscence is not clear at present. These uncertainties leave open the question whether this clade is primarily dehiscent, with convergent evolution of indehiscence in one of its species (*C. haughtii*) or whether it is primarily indehiscent with reversion to dehiscence in *C. partita*.

Clades G N The common theme for these eight clades, discussed here together, is their fruit dehiscence and geographic distribution. Almost all species within these groups, resolved as a grade, feature dehiscent capsules. In a few exceptional cases where capsules are found to be indehiscent, those species were usually nested within the given clade in a derived position (with the exception of clade M). In the context of rooted phylogeny, this indicates a convergent evolution toward an indehiscent character state from a plesiomorphic dehiscent state. Also, all these clades are distributed primarily or exclusively in Mexico and its adjacent regions, comprising Southwest USA bordering Mexico, Central America, and/or West Indies. However, three long-distance dispersals are inferred from within three of these clades (G, H, and L; marked on Fig. 5 with asterisks).

Clade G comprises species traditionally included in subsections *Subulatae*, *Tinctoriae*, *Lobostigmae*, and *Platycarpae* (Yuncker, 1932). It is characterized by relatively large, thick flowers and subulate or thick styles, which become more obvious as the fruit matures. Capsules are primarily dehiscent, and infrastaminal scales are thick and fleshy. While most of its species are distributed in Mexico and Central America, a well-supported subclade, consisting of two species with indehiscent capsules (*C. victoriana* and *C. tasmanica*), is found in SW Australia and Tasmania, strongly implying long-distance dispersal.

The four species of the H clade were originally included in three different sections (Yuncker, 1932); however, they share some common morphological characteristics. Their calyx lobes are often carinate or with longitudinal protuberances along the midvein, and their capsules are surrounded at the base by the withered corolla. *Cuscuta yucatanana* was described by Yuncker as having an indehiscent fruit, while the remaining species have dehiscent capsules. Three species occur in Mexico and/or the southern USA, whereas *C. chinensis* is clearly disjunct from the rest of the clade and is found in SE Asia, Australia, and Africa.

Most of the species confined to clades I and J were traditionally included in subsection *Americanae* (Yuncker, 1932). These species have dehiscent capsules and more or less cylindrical flowers and are distributed in Mexico, the West Indies, Central America, and northern South America. Although morphologically very similar, these two clades are well supported by molecular data as distinct and monophyletic.

Clade K, which includes a group of several Mexican and Central American species generally possessing various appendages on their calyx and/or corolla lobes, corresponds closely to section *Ceratophorae* of Yuncker (1932). As already indicated, the relative positions of this clade and the J clade represent the only point of topological conflict between the plastid and nuclear data sets, albeit weakly supported in both

cases. With *trnL-F* sequences, these two clades were found as each other's sisters (69% BS; Fig. 2), yet were resolved as diverging consecutively, with the nrITS data (66% BS). Because the *trnL-F* sequences could not be obtained for several accessions of the K clade (compare Appendices S1 and S2; see Supplemental Data accompanying online version of this article), this conflict can be accounted for, in large part, as an artifact derived from sampling differences between the two data sets.

Clade L includes mostly species circumscribed by Yuncker (1932) in subsections *Umbellatae* and *Leptanthae* plus a few species that were traditionally classified elsewhere (subsections *Odontolepisae* and *Acutae*). Altogether, these species are characterized by loose, umbellate inflorescences and flowers with acute calyx and corolla lobes. Most species have dehiscent capsules and occur in Mexico and the SW USA. However, *C. acuta*, endemic to the Galapagos Islands, has indehiscent capsules. Additionally, *C. hyalina*, with its disjunct populations found in India and W South Africa, is also nested in this clade.

Clade M corresponds very closely to subsection *Indecorae*, a group of species characterized primarily by fleshy, papillose or glandular flowers. According to Yuncker (1932, 1965), *Indecorae* comprise four species, two North American (*C. coryli* and *C. warnerii*), one South American (*C. stenolepis*), and one widely spread species spanning the Americas (*C. indecora* with its varieties). An additional species (*C. attenuata*) was recognized within *Indecorae* by Prather and Tylr (1993). Albeit morphologically very similar to *C. indecora*, the two species were treated as separate, based on failure of artificial crosses to yield fruit/seed and their apparent reproductive isolation (Prather and Tylr, 1993). However, emphasizing the morphological similarities with *C. indecora* and taking into account the degree of morphological differences found within this species, *C. attenuata* was recently reduced to a variety of *C. indecora* (Costea et al., 2006a). All taxa traditionally classified into subsection *Indecorae* (Yuncker, 1932), except for *C. stenolepis*, are found in the M clade, with high support. Flowers of *C. stenolepis* are not fleshy, and a combination of other characters was used to place it with *Indecorae*. As previously noted, this species is a member of clade B, where it forms an isolated lineage positioned as sister group to the rest of that clade.

While the composition of clade M is not surprising, its phylogenetic placement is, both from morphological and geographical points of view. All the species grouped here have indehiscent capsules, yet they are nested deeply within several distinct clades all of which are primarily or exclusively with dehiscent capsules. Bringing the M clade in a closer association with other groups characterized by indehiscent capsules (clades A–E) requires dissolving a number of internal branches, some of which are highly supported. This scenario was formally tested and rejected as significantly worse topological solution. A similar, albeit less clear-cut, argument can be made from the biogeographical standpoint. Most taxa in the M clade, as currently delimited, are North American in distribution. *Cuscuta warnerii* is narrowly limited to the Four Corners region of the USA (i.e., southern Utah and Colorado and northern Arizona and New Mexico). *Cuscuta attenuata* is also limited in its distribution and is found only in Kansas, Oklahoma, and northern Texas. *Cuscuta coryli* is much more widespread in distribution, but still confined to North America, occurring throughout most of the USA and southern Canada, east of the Rockies. Yet, all the other groups in the immediate

vicinity of the M clade are primarily Mexican in distribution. It has to be pointed out, however, that *C. indecora*, also a member of the M clade, is distributed throughout the Americas, from southern Canada to Argentina and Chile, and is rendering the geographical distinctiveness of this group with its phylogenetic surroundings more blurry compared to the morphological.

The morphological and geographical uniqueness of the M clade is equaled by its molecular distinctiveness. As indicated in the phylogram (Fig. 5), this group has one of the longest branches on the tree, due primarily to more divergent nrITS sequences. Within this clade, both *C. coryli* and *C. attenuata* are nested within *C. indecora*. *Cuscuta coryli* seems to have achieved the reciprocal monophyly, based on samples from several individuals, and is distinct molecularly (as well as morphologically) from *C. indecora*. *Cuscuta attenuata* is both morphologically and molecularly more similar to *C. indecora*, in accordance with keeping these two entities conspecific (Costea et al., 2006a). *Cuscuta warnerii*, a strikingly distinct dodder with calyx lobes apically extended into horn-like projections, forms a separate lineage, sister to *C. indecora* s.l.

Clade N represents another segregate from the subsection *Umbellatae*; hence it is not surprising that it is morphologically very similar to clade L. However, it differs from the L clade by the tendency to have no stems at flowering/fructification time. Consequently, the inflorescences appear as tufts emerging directly from the stems of their hosts. Most species found here are poorly known. For example, *C. serruloba*, *C. mcvaughii*, and *C. aristeguietae* have been known only from their type collections, and *C. columbiana* is extremely rare. All species have dehiscent capsules except for *C. aristeguietae*, which has indehiscent fruits. This species is also one of the very few that Yuncker (1932) did not place in any of the 24 subsections he described in the subgenus *Grammica*.

Clade O This group is almost exclusively of South American distribution and represents the largest and most diverse clade in subgenus *Grammica* (and perhaps in the entire genus). Its species belong, for the most part, to three relatively closely related subsections, *Odoratae*, *Grandiflorae*, and *Acutilobae* (Fig. 1; Yuncker, 1932). Based on good support and sequence divergence, molecular data also reveal three major lineages within the O clade. Those groups, however, have a taxonomic make-up differing from Yuncker's subsections. The first subclade consists of some *Odoratae* and all of the sampled species belonging to *Acutilobae* (95% BS; ≥ 0.95 PP). With large, thick flowers, cylindrical or subulate styles longer than the ovary, and often large, convoluted stigmas, its members superficially resembles species from the G clade. Its species are distributed primarily along the Andes (Ecuador, Peru, Bolivia, Chile). The second subclade includes taxonomically the rest of *Odoratae* and all of the sampled species belonging to subsection *Grandiflorae* (100% BS; ≥ 0.95 PP). It is characterized by flowers with rotate or globose corollas, anthers often curved toward the ovary, styles absent or shorter than the ovary/capsule, and large, convoluted stigmas. Among the species of this subclade, *C. cristata* can be interpreted as a case of incomplete evolution of indehiscence. Its pericarp does not split along a definite line in the dehiscence area. Instead, because it is very thin, it breaks when mechanical pressure is applied. Although both Yuncker (1932, p. 188) and Hunziker (1949, p. 1159) considered the capsule of *C. cristata* indehiscent, Hunziker labeled it also as rather "pseudoinde-

hiscent.” He even described a variety of this species, *C. cristata* var. *chacoensis* (not sampled in our study) with almost perfectly dehiscent capsules. The majority of the species confined to this group occur east of the Andes (Argentina, Uruguay, Brazil), and a few are found along the Andes (Colombia to Chile). However, one species represents another striking case of relatively recent long-distance dispersal. *Cuscuta kilimanjari*, found in eastern Africa, is nested within this otherwise exclusively South American clade. Finally, *C. microstyla* represents the third distinct lineage within the O clade. Compared to the previous two subclades, this Chilean species has smaller flowers and clearly indehiscent fruits. The relationships among the three major lineages of clade O are not resolved.

Monophyly of *Cuscuta* subgenus *Grammica* In addition to the primary emphasis on the circumscription and relationships of major groups within *Grammica*, our data are also pertinent to the question of monophyly of this subgenus. According to analyses including portions of sequences alignable between the ingroup and outgroup, subgenus *Grammica* is monophyletic and supported by high to moderate bootstrap values (100% BS with *trnL-F*; 85% BS with nrITS). The single origin for this subgenus is further supported by the presence of two distinct styles with globose, nonlinear stigmas. However, there is one notable exception to this result, given our current species sampling. *Cuscuta appendiculata* features globose stigmas and indehiscent capsules. Both of these characteristics are typical of subgenus *Grammica* to which this South African species was traditionally assigned. Yet *C. appendiculata* has both its plastid and nuclear sequences more similar to those of the outgroups, and it is found to group with other South African species currently classified in subgenus *Cuscuta* with high bootstrap supports (95% BS with *trnL-F*; 100% with nrITS; resulting trees not shown).

Our results, to a limited extent, also bear on the issue of monophyly of *Cuscuta* subgenus *Cuscuta*. They are consistent with the notion that this subgenus is not monophyletic (McNeal, 2005) and consists of two quite distinct groups, one South African in distribution, corresponding in large part to subsection *Africanae*, and the other containing the rest of the species belonging traditionally to this subgenus (García and Martín, in press).

Multiple origins of fruit indehiscence in *Cuscuta* Yuncker (1932) considered species with the indehiscent capsules to be more “primitive” as judged by their central-basal position in his phylogenetic scheme for the genus (Fig. 1). Furthermore, the evolution “from capsules which remain closed to those which are regularly and definitely circumscissile when mature” was explicitly listed as one of the major evolutionary tendencies within *Cuscuta* (Yuncker, 1932, p. 115). Our phylogenetic results disagree with both the taxonomic implications as well as the directionality for proposed scenario of evolution of this character.

Regardless of the position of the root, the switch from dehiscent to indehiscence was inferred to have occurred at least 10 times independently within subgenus *Grammica* (solid bars; Figs. 3–4). An attempt to group all the species according to their fruit characteristics was very costly in terms of parsimony steps (460 additional steps) and was also rejected by the SH test. Even a much less stringent attempt to bring together some of the clades with indehiscent capsules for

reasons other than morphology (e.g., for biogeographical reasons) was also rejected. This refutes the taxonomic hypothesis of monophyly of two sections, *Cleistogrammica* and *Eugrammica*. In addition, the relative ease by which this character shifts its states, as indicated by the inferred number of changes, suggests relatively simple genetics for this trait, possibly regulated by only one or only a few genes. In some other plant groups featuring similar polymorphisms regarding fruit dehiscence (e.g., *Amaranthus*, Amaranthaceae), the dehiscent/indehiscent character states were also found to shift easily, and a similarly simple genetic determinism was suggested (Costea et al., 2001). As Cronquist (1988) proposed, the dehiscence mechanism may be easily lost because the genetic causes of indehiscence are not selected against and the advantages of indehiscent vs. dehiscent capsules, if any, are obscure.

Because the unrooted phylogenetic networks indicate the location of the changes but not their directions, we used two approaches to assess the polarity of fruit (in)dehiscence. The first line of evidence comes from comparison with the states found in outgroups (the relative apomorphy rule; Wiley et al., 1991). While the ingroup species have both indehiscent and dehiscent character states, almost all the outgroups from subgenera *Cuscuta* and *Monogyna* feature dehiscent capsules. Hence, the dehiscence is deduced to be a primitive character for *Grammica*. The second line of evidence comes from the position of the root. The root for *Grammica* could not be determined unambiguously due to the alignment problems between the ingroup and outgroup taxa. However, the outgroup rooting, using limited conservative portions of alignment, as well as the mid-root rooting approach, indicated that the first split within *Grammica* occurred among lineages with predominantly dehiscent capsules (clades J–O; Fig. 4). Both of these arguments support the conclusion that dehiscence is a plesiomorphic condition and that the apomorphic indehiscence evolved several times independently. This is contrary to Yuncker’s (1932) hypotheses developed for character evolution in *Cuscuta*, but it supports views put forward for the fruit evolution in flowering plants generally (Cronquist, 1988).

Biogeographical implications While Yuncker’s (1932) monograph of *Cuscuta* did take the distribution of species into account in some cases, his overall taxonomic circumscription and proposed phylogenetic arrangement was based almost entirely on morphology with comparatively little attention given to biogeography. Consequently, in subgenus *Grammica* alone, a minimum of 14 long-distance dispersals has to be implicitly assumed to accommodate this traditional classification. The phylogenetic relationships inferred here portray a significantly different biogeographic scenario. Diversification through vicariance, as opposed to long-distance dispersal, clearly emerges as the more dominant pattern for the species of *Grammica*. Of its 15 major lineages, four are primarily or entirely of North American distribution (A, B, D, and E), three are South American in distribution (C, F, and O), and the rest (eight major clades in total: G–N) are found in Mexico and adjacent regions. All these inferences are evident from the unrooted phylogenetic network within this subgenus and are not dependent on the exact position of its root. On the other hand, the most closely related outgroup taxon to *Grammica*, subgenus *Cuscuta*, and in particular its section *Africanae*, all occur in the Old World and are most abundant in the Mediterranean region and southern Africa. Hence, there is

little doubt that the initial jump had to occur from the Old World, most likely Africa, to the New World. Given the rooting proposed here, the first major split and subsequent diversification of *Cuscuta* species in the New World most likely occurred between South America (clade O) on one side and Mexico on the other. Mexico (with its adjacent regions), where more than half of *Grammica* species occur, is clearly the center of biodiversity for *Cuscuta*. This region also represents a “staging ground” for the next major diversification. Namely, from within this Mexican grade, two clades (C and F) split off, and diversity back in South America and four clades (A, B, D, and E) spread throughout North America.

In addition to the clear preponderance of vicariant events that explain species distribution in *Cuscuta*, several striking cases of long-distance dispersal are also evident from the inferred phylogeny as well (indicated with asterisks in Fig. 5). For example, *C. kilimanjari*, an eastern African species, is nested deeply within an otherwise exclusively South American group (clade O). *Cuscuta hyalina* has a disjunct distribution, with populations found in southern Africa and India, yet this species is nested within the L clade, all other members of which are found in Mexico and adjacent regions. Similar arguments for long-distance dispersal can be made for *C. chinensis*, distributed in eastern Asia, and *C. victoriana* and *C. tasmanica*, both found in Australia. In both of these cases, their closest relatives, grouped in clades H and G, respectively, are of Mexican distribution. Finally, the most cosmopolitan of all dodders today is clade B, with several of its species spreading over multiple continents. However, its ancestral distribution is inferred to be North American, followed by one long-distance dispersal from within it. Nevertheless, all these cases taken together still account for less than half of long-distance dispersals implied by Yuncker’s classification (1932).

Implications for plastid DNA evolution in Cuscuta

Previous analyses of plastid genomes of *Cuscuta* species (e.g., Bömmmer et al., 1993; Haberhausen and Zetsche, 1994; Stefanovic et al., 2002) have pointed out the unexpectedly conservative nature of *Cuscuta* ptDNA evolution, especially when compared to those of its close nonparasitic relatives (Stefanovic and Olmstead, 2005). The profound morphological and physiological modifications that the ancestors of this genus experienced during the transition from an autotrophic to parasitic mode of life are therefore contrasted with their relatively unaltered plastid genomes (Stefanovic and Olmstead, 2005). For example, the *trnL-F* region included in this study was amplified with the same set of primers used for nonparasitic taxa across flowering plants. However, this plastid region was unamplifiable for some DNA accessions. The simple explanation of poor DNA quality could be excluded as unlikely in this case because the very same extractions were used consistently with success to amplify the nrITS region. Furthermore, multiple attempts to amplify smaller fragments using different combinations of internal and/or alternative primers were also unsuccessful. Taken collectively, these data indicate that the *trnL-F* region either experienced a significant acceleration in substitution and thus attained sequence divergence at priming sites used in PCR, or it was altogether lost from the plastid genome of these species. The phylogenetic analyses, based on nrITS data, revealed that the majority of these species belong to a single group, the O clade (Figs. 2, 4). While the negative PCR results (i.e., the lack of amplifications) are hard to explain unequivocally, in this case, they are

consistent with results obtained by van der Kooij et al. (2000). These authors showed the presence of different degrees of degradation in photosynthetic apparatus across *Cuscuta*. The variability of plastid functionality was documented using a combination of southern, northern, and western hybridizations on six *Cuscuta* species (five of which were from *Grammica*). Their results suggested the absence of the *rbcL* gene itself in *C. odorata* as well as the absence of transcripts and proteins in *C. odorata* and *C. grandiflora* (van der Kooij et al., 2000). It is clear from our phylogenetic results that both of these factors affected species belonging to the O clade, the same group of South American species for which *trnL-F* could not be amplified. In conjunction with newly inferred phylogenetic relationships, the simultaneous absence of both protein-coding genes and/or their products (*rbcL*, Rubisco large subunit) as well as noncoding regions (*trnL-F*), indicates a putatively genome-wide phenomenon for a whole clade rather than a localized exception for a particular species and/or particular plastid region. Based on these two lines of evidence, we hypothesize that most, if not all, species of the O clade will have much more significantly altered plastid genomes in comparison to what has been documented up to this point (for a review, see Stefanović and Olmstead, 2005), with many plastid genes and regions absent or divergent to the point of being undetectable by methods such as PCR and hybridization.

Another, smaller group of species for which the *trnL-F* sequences could not be obtained was also detected. Both of these species, *C. erosa* and *C. boldinghii*, belong traditionally in section *Ceratophorae*, and are found nested within the K clade, with several well-supported nodes leading to them (Fig. 4). Similar to the situation described for the O clade, the nrITS sequences were easily amplifiable from the same DNA accessions. In this case, however, the independent data regarding the presence or absence of other plastid regions are not currently available. Hence, it remains to be seen if this represents a second lineage of species with highly altered plastid genomes or a simple case of PCR amplification difficulties due to primer mismatches or poor quality DNA.

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APPENDIX. Taxa, DNA accession numbers, sources of plant material from which DNA was extracted, and GenBank accession numbers for sequences used in this study. Infrageneric classification follows Yuncker (1932). Numbers in square brackets indicate sampled and estimated numbers of species belonging to corresponding subsections, respectively.

SUBGENUS

Section	Subsection	Species	DNA accession ^a	Voucher ^b	Country ^c	Clade ^d	GenBank accession	
							<i>trnL-F</i>	nrITS
<i>GRAMMICA</i> (Lour.) Yunck.								
<i>Cleistogrammica</i> Engelm.								
<i>Acutae</i> Yunck. [4/6]								
		<i>Cuscuta acuta</i> Engelm.	1084	Fosberg 44965; US	Ecuador, Galapagos	L	EF194330	EF194565
		<i>C. appendiculata</i> Engelm.	528	Burrows 4666; J	South Africa, Mpumalanga	OG	EF202559	EF202563
		<i>C. haughtii</i> Yunck.	949	Haught s.n.; F	Peru, Negritos	F	EF194351	EF194590
		<i>C. haughtii</i> Yunck.	601	Svenson 11281; QFA	Ecuador, Guayas	F	EF194350	
		<i>C. werdermanii</i> Hunz.	995	Reiche s.n.; SGO	Chile	C	EF194444	EF194655
<i>Arvenses</i> Yunck. [7/9]								
		<i>C. campestris</i> Yunck.	483	Pitzer 3765; ASU	USA, CA	B	EF194453	EF194661
		<i>C. campestris</i> Yunck.	487	Baker & Wright 11575 1; ASU	USA, AZ	B	EF194452	EF194659
		<i>C. campestris</i> Yunck.	415	Solomon 17192; IND	USA, MO	B	EF194455	EF194677/ EF194680
		<i>C. campestris</i> Yunck.	456	Lakela 26019; IND	USA, FL	B	EF194465	EF194678/ EF194664
		<i>C. campestris</i> Yunck.	894	Alava 11039; RSA	Iran, Razavi Khorasan	B	EF194454	EF194660
		<i>C. campestris</i> Yunck.	411	SS 03 103; TRTE	Serbia, Belgrade	B	EF194450	EF194663
		<i>C. campestris</i> Yunck.	202	Ownboy s.n.; WTU	USA	B	EF194451	EF194665
		<i>C. glabrior</i> (Engelm.) Yunck.	596	Palmer 723; GH	Mexico, COA	B	EF194470	EF194684
		<i>C. glabrior</i> (Engelm.) Yunck.	742	Cory 42164; NY	USA, TX	B	EF194471	EF194685
		<i>C. glabrior</i> (Engelm.) Yunck.	825	Villarreal & Vasquez 6154; XAL	Mexico, COA	B	EF194472	EF194686
		<i>C. gymnocarpa</i> Engelm.	1017	Mears & Andersen 5288; TEX	Ecuador, Galapagos	B	EF194456	EF194666
		<i>C. harperi</i> Small	594	Demaree 46295; NY	USA, AL	B	EF194464	EF194681
		<i>C. pentagona</i> Engelm.	464	Taylor 5765; IND	USA, MO	B	EF194467	EF194679
		<i>C. plattensis</i> A. Nelson	590	Dorn 5470; NY	USA, WY	B	EF194468	EF194682
		<i>C. runyonii</i> Yunck.	660	Flyr 368; TEX/LL	USA, TX	B	EF194469	EF194683
<i>Californicae</i> Yunck. [2/5]								
		<i>C. californica</i> Choisy	669	White 5033; ASU	USA, CA	A	EF194479	EF194691
		<i>C. californica</i> Choisy	499	Ahart 9856; JEPS	USA, CA	A	EF194487	EF194697
		<i>C. californica</i> Choisy	500	Boyd 9839; JEPS	USA, CA	A	EF194478	
		<i>C. californica</i> Choisy	637	Pinzl 7238a; NY	USA, NV	A	EF194475	EF194688
		<i>C. californica</i> Choisy	645	Ahart 2971; NY	USA, CA	A	EF194488	EF194698
		<i>C. californica</i> Choisy	147	SS 98 59; TRTE	USA, OR	A	EF194486	EF194696

APPENDIX. Continued.

SUBGENUS							
Section							
Subsection		DNA accession ^a	Voucher ^b	Country ^c	Clade ^d	GenBank accession	
Species						<i>trnL-F</i>	nrITS
<i>C. californica</i> Choisy var. <i>brachycalyx</i> Yunck.	472		SS 04 140/AC 04 31; TRTE	USA, CA	A	EF194484	EF194699
<i>C. californica</i> Choisy var. <i>brachycalyx</i> Yunck.	643		Colwell AC 04 305; YM/WLU	USA, CA	A	EF194485	EF194700
<i>C. californica</i> Choisy var. <i>californica</i>	418		SS 00 59; TRTE	USA, CA	A	EF194480	EF194692
<i>C. occidentalis</i> Millsp.	503		Erter 7326; NY	USA, CA	A	EF194477	EF194690
<i>C. occidentalis</i> Millsp.	504		Tiehm 12257; NY	USA, NV	A	EF194481	EF194693
<i>C. occidentalis</i> Millsp.	647		Tiehm 14108; NY	USA, NV	A	EF194482	EF194694
<i>C. occidentalis</i> Millsp.	648		Schoolcraft et al. 2220; NY	USA, CA	A	EF194483	EF194695
<i>C. occidentalis/californica</i>	646		Ahart 9116; JEPS	USA, CA	A	EF194476	EF194689
<i>Cephalanthae</i> Yunck. [1/1]							
<i>C. cephalanthi</i> Engelm.	469		Deam 51439; IND	USA, IN	D	EF194413	EF194632
<i>C. cephalanthi</i> Engelm.	510		Hill 29748; NY	USA, IL	D	EF194414	EF194633
<i>C. cephalanthi</i> Engelm.	167		Raven 27211; WTU	USA	D	EF194412	EF194631
<i>Denticulatae</i> Yunck. [3/4]							
<i>C. denticulata</i> Engelm.	485		Tiehm 13319; ASU	USA, NV	E	EF194410	EF194627
<i>C. denticulata</i> Engelm.	668		Baier et al. 10732; ASU	USA, AZ	E	EF194411	EF194628
<i>C. denticulata</i> Engelm.	165		Beck & Caplan 94051; WTU	USA, CA	E	EF194409	EF194626
<i>C. microstyla</i> Engelm.	707		Muñoz et al. 3575; SGO	Chile, III	O		EF194538
<i>C. microstyla</i> Engelm.	987		Vargas & Farah 80; SGO	Chile, Santiago	O		EF194537
<i>C. nevadensis</i> I.M. Johnst.	476		Pinkava et al 12181; ASU	USA, CA	E	EF194407	EF194629
<i>C. nevadensis</i> I.M. Johnst.	585		Morefield 2119a; NY	USA, CA	E	EF194408	EF194630
<i>Indecorae</i> Yunck. [4/4]							
<i>C. coryli</i> Engelm.	824		Boivin & Champagne 13869; ALTA	Canada, MB	M	EF194290	
<i>C. coryli</i> Engelm.	465		Deam 51589; IND	USA, IN	M	EF194288	EF194539
<i>C. coryli</i> Engelm.	666		Bartholomew 0 923; NY	USA, WV	M	EF194289	EF194540
<i>C. indecora</i> Choisy	561		Worthington 26947; ARIZ	USA, TX	M	EF194300	EF194549
<i>C. indecora</i> Choisy	728		Spellenberg & Spurrier 8256; NY	USA, NM	M	EF194302	
<i>C. indecora</i> Choisy	525		Wagner & Powell 2493; UNM	USA, NM	M	EF194293	EF194543
<i>C. indecora</i> Choisy <i>attenuata</i>	721		Horr 4410; NY	USA, KS	M	EF194295	EF194546
<i>C. indecora</i> Choisy <i>attenuata</i>	723		Tyrl 1648; OKLA	USA, OK	M	EF194297	EF194547
<i>C. indecora</i> Choisy <i>attenuata</i>	724		Waterfall 17191; OKLA	USA, OK	M	EF194296	EF194545
<i>C. indecora</i> Choisy <i>longisepala</i> Yunck.	726		Runyon 2819; NY	USA, TX	M	EF194298	
<i>C. indecora</i> Choisy <i>longisepala</i> Yunck.	727		Lean 7964/208; NY	Argentina, LP	M	EF194299	EF194548
<i>C. indecora</i> Choisy <i>neuropetala</i> (Engelm.) Hitchc.	720		Spellenberg et al 3427; NY	USA, NM	M	EF194301	
<i>C. indecora</i> Choisy <i>neuropetala</i> (Engelm.) Hitchc.	895		DeDecker 5383; RSA	USA, CA	M	EF194294	EF194544
<i>C. stenolepis</i> Engelm.	779		Ollgaard 99142; QCNE	Ecuador, Pichincha	B	EF194473	EF194687
<i>C. stenolepis</i> Engelm.	781		Nunez et al. 034; QCNE	Ecuador, Pichincha	B	EF194474	
<i>C. warneri</i> Yunck.	662		Peterson 98 699; NMC	USA, NM	M	EF194291	EF194542
<i>C. warneri</i> Yunck.	890		Warner s.n.; RSA	USA, UT	M	EF194292	EF194541
<i>Lepidanche</i> Engelm. [4/4]							
<i>C. compacta</i> Juss.	479		Kerby 7; ASU	USA, GA	D	EF194426	
<i>C. compacta</i> Juss.	466		Deam 58335; IND	USA, IN	D	EF194425	
<i>C. compacta</i> Juss.	198		Laing 411; WTU	USA	D	EF194423	
<i>C. compacta</i> Juss.	199		Eggert s.n.; WTU	USA	D	EF194424	EF194640
<i>C. cuspidata</i> Engelm.	1016		Carr 13221; TEX	USA, TX	D	EF194429	EF194643
<i>C. glomerata</i> Choisy	619		Stevens 2546; DAO	USA, ND	D	EF194431	
<i>C. glomerata</i> Choisy	462		McClain 2448; IND	USA, IN	D	EF194430	
<i>C. glomerata</i> Choisy	597		Freeman 293; NY	USA, KS	D	EF194432	
<i>C. glomerata</i> Choisy	598		Freeman 2235; NY	USA, KS	D	EF194433	EF194644
<i>C. squamata</i> Engelm.	740		Anderson & Brice 8057; NMC	USA, NM	D	EF194434	EF194645
<i>Lobostigmae</i> Engelm. [1/1]							
<i>C. tasmanica</i> Engelm.	680		Craven s.n.; CANB	Australia, NSW	G	EF194387	
<i>C. tasmanica</i> Engelm.	681		Lepschi 908/909; CANB	Australia, NSW	G	EF194388	EF194612
<i>C. tasmanica</i> Engelm.	682		Taws 729; CANB	Australia, NSW	G	EF194389	EF194613

APPENDIX. Continued.

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Section						
Subsection	DNA accession ^a	Voucher ^b	Country ^c	Clade ^d	GenBank accession	
Species					<i>trnL-F</i>	nrITS
<i>Oxycarpae</i> Engelm. [3/3]						
<i>C. gronovii</i> Willd.	705	Garneau & Roy 89 626 M; DAO	Canada, QC	D	EF194422	EF194639
<i>C. gronovii</i> Willd.	343	SS 02 03; TRTE	USA, IN	D	EF194418	EF194637
<i>C. gronovii</i> Willd.	453	SS 04 143A; TRTE	USA, IN	D	EF194420	EF194638
<i>C. gronovii</i> Willd.	467	SS 04 161; TRTE	USA, IN	D	EF194421	
<i>C. gronovii</i> Willd.	702	Hinds et al 11582; UNB	Canada, NB	D	EF194427	EF194641
<i>C. gronovii</i> Willd.	194	Demaree 18594; WTU	USA	D	EF194419	
<i>C. gronovii</i> Willd. var. <i>caliptrata</i> Engelm.	706	Cory 52529; TEX/LL	USA, TX	D	EF194416	EF194635
<i>C. gronovii</i> Willd. var. <i>latiflora</i> Engelm.	703	Catling s.n.; DAO	Canada, ON	D	EF194417	EF194636
<i>C. gronovii</i> Willd. var. <i>latiflora</i> Engelm./ <i>C. cephalanthi</i> Engelm.	704	Bewick 108; DAO	USA, WI	D	EF194415	EF194634
<i>C. rostrata</i> Shuttlw. ex Engelm. & A. Gray	460	Bozeman et al. 45268; IND	USA, NC	D	EF194428	EF194642
<i>C. umbrosa</i> Beyrich ex Hook.	578	Fields s.n.; DAO	Canada, MB	D	EF194435	EF194646
<i>C. umbrosa</i> Beyrich ex Hook.	956	Hutchinson 2262; RSA	USA, NM	D	EF194437	EF194648
<i>C. umbrosa</i> Beyrich ex Hook.	579	Hudson 5082; USAS	Canada, SK	D	EF194436	EF194647
<i>Platycarpae</i> Engelm. [5/8]						
<i>C. australis</i> Hook. f.	547	Sykes 99; CHR	China, Guangxi Zhuang	B	EF194457	EF194667
<i>C. australis</i> Hook. f.	679	Hosking 938; CANB	Australia, NSW	B	EF194458	EF194668
<i>C. australis</i> Hook. f.	789	Beaughlehole 83203; MEL	Australia, VIC	B		EF194669
<i>C. australis</i> Hook. f.	792	Curtis 124; MEL	Australia, VIC	B		EF194670
<i>C. australis</i> Hook. f. var. <i>tinei</i> (Ins.) Yunck.	639	Thiebaut 3098; NY	France	B	EF194460	EF194671
<i>C. australis</i> Hook. f. var. <i>tinei</i> (Ins.) Yunck.	640	Simonkoi 2635; NY	Hungary	B	EF194459	EF194672
<i>C. bifurcata</i> Yunck.	1036	Paterson 578; PRE	South Africa, Cape	B	EF194461	
<i>C. cristata</i> Engelm.	939	Riggs 100; F	Argentina, CA	O		EF194529
<i>C. cristata</i> Engelm.	1045	Hunziker 5047; US	Argentina, LR	O		EF194530
<i>C. cristata</i> Engelm.	1026	Landrum 3057; ASU	Argentina, BA	O		EF194531
<i>C. obtusiflora</i> H.B. & K.	1047	Pedersen 3688; US	Argentina, CR	B		EF194673
<i>C. obtusiflora</i> H.B. & K.	1069	Skolnik & Barkley 19ANL23; US	Colombia, Antioquia	B	EF194463	EF194674
<i>C. obtusiflora</i> H.B. & K. var. <i>glandulosa</i> Engelm.	746	Mitchell 3387; NY	USA, OK	B	EF194462	EF194675
<i>C. obtusiflora</i> H.B. & K. var. <i>glandulosa</i> Engelm.	747	Lundell & Lundell 11717; NY	USA, TX	B		EF194676
<i>C. victoriana</i> Yunck.	678	Cowie 9624; CANB	Australia, NT	G	EF194383	EF194616
<i>C. victoriana</i> Yunck.	683	Mitchell 6089; CANB	Australia, WA	G	EF194384	
<i>C. victoriana</i> Yunck.	684	Latz 14050; CANB	Australia, NT	G	EF194385	EF194614
<i>C. victoriana</i> Yunck.	685	Smyth 261; CANB	Australia, SA	G	EF194386	EF194615
<i>Racemosae</i> Yunck. [6/8]						
<i>C. decipiens</i> Yunck.	458	Tharp 46072; IND	USA, TX	A	EF194508	
<i>C. decipiens</i> Yunck.	981	Henrickson 13394; MEXU	Mexico, ZAC	A	EF194509	
<i>C. decipiens</i> Yunck.	1014	Henrickson 22781; TEX	Mexico, ZAC	A	EF194510	EF194718
<i>C. parviflora</i> Engelm. var. <i>elongata</i> Engelm.	1041	Oliveira et al. 745; US	Brazil, GO	C	EF194448	EF194657
<i>C. platyloba</i> Prog.	1073	Sehnm 5597; PACA	Brazil, South	C	EF194447	EF194658
<i>C. racemosa</i> Mart.	1070	Rambo 53990; PACA	Brazil, South	C	EF194449	
<i>C. suaveolens</i> Ser.	790	Paget 2579; MEL	Australia, VIC	C	EF194441	EF194652
<i>C. suaveolens</i> Ser.	791	Chesterfield & Bush 2378; MEL	Australia, VIC	C	EF194443	EF194654
<i>C. suaveolens</i> Ser.	996	Castillo 98 74; SGO	Chile, I	C	EF194442	EF194653
<i>C. yucatanica</i> Yunck.	657	Alava 1341; NY	Mexico, CHI	H	EF194364	EF194598
<i>Subinclusae</i> Yunck. [5/5]						
<i>C. howelliana</i> Rubtzoff	654	Oswald & Ahart 7978; JEPS	USA, CA	A	EF194504	
<i>C. howelliana</i> Rubtzoff	655	Ahart 8044; JEPS	USA, CA	A	EF194507	EF194717
<i>C. howelliana</i> Rubtzoff	656	Reino & Alava 6809; JEPS	USA, CA	A	EF194505	EF194715
<i>C. howelliana</i> Rubtzoff	357	Tank s.n.; no voucher	USA, CA	A	EF194506	EF194716
<i>C. micrantha</i> Choisy	708	Muñoz et al. 2914; SGO	Chile, III	C	EF194439	EF194651
<i>C. micrantha</i> Choisy	709	Teillier & Faundez 3844; SGO	Chile, IV	C	EF194438	EF194649
<i>C. micrantha</i> Choisy	988	Teillier 498; SGO; SGO	Chile, II	C	EF194440	EF194650

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Section		GenBank accession					
Subsection	Species	DNA accession ^a	Voucher ^b	Country ^c	Clade ^d	<i>trnL-F</i>	nrITS
	<i>C. salina</i> Engelm. var. <i>major</i> Yunck.	502	Standley 777; NY	USA, WA	A	EF194499	EF194710
	<i>C. salina</i> Engelm. var. <i>major</i> Yunck.	642	Halse 4961; NY	USA, OR	A	EF194498	EF194709
	<i>C. salina</i> Engelm. var. <i>major</i> Yunck.	651	Kennedy & Ganders 4947; UBC	Canada, BC	A	EF194500	EF194711
	<i>C. salina</i> Engelm. var. <i>major</i> Yunck.	146	Dudley s.n.; WTU	USA	A	EF194497	
	<i>C. salina</i> Engelm. var. <i>salina</i>	477	Tiehm 12744; ASU	USA, NV	A	EF194492	EF194704
	<i>C. salina</i> Engelm. var. <i>salina</i>	478	Tiehm 13405; ASU	USA, NV	A	EF194493	EF194705
	<i>C. salina</i> Engelm. var. <i>salina</i>	641	Tiehm & Bair 12744; GH	USA, NV	A	EF194494	EF194706
	<i>C. salina</i> Engelm. var. <i>salina</i>	652	Hammond 10349; NY	USA, AZ	A	EF194495	EF194707
	<i>C. salina</i> Engelm. var. <i>salina</i>	653	Felger & Fenn s.n.; NY	USA, AZ	A	EF194496	EF194708
	<i>C. subinclusa</i> Durand & Hilg.	501	Raz & Boyd 15; NY	USA, CA	A	EF194491	EF194701
	<i>C. subinclusa</i> Durand & Hilg.	644	Anderson 3248; NY	USA, CA	A	EF194490	EF194702
	<i>C. subinclusa</i> Durand & Hilg.	197	Munz & Balls 17942; WTU	USA	A	EF194489	EF194703
	<i>C. suksdorfii</i> Yunck.	635	Ahart 9885; JEPS	USA, CA	A	EF194501	EF194712
	<i>C. suksdorfii</i> Yunck.	636	Ahart 3949; JEPS	USA, CA	A	EF194502	EF194713
	<i>C. suksdorfii</i> Yunck.	470	Cobwell AC 04 159; YM/TRTE	USA, CA	A	EF194503	EF194714
Uncertain							
	<i>C. aristeguietae</i> Yunck.	935	Aristeguieta 4568; F	Venezuela, Guarico	N	EF194311	EF194554
Eugammica Yunck.							
<i>Acutilobae</i> Yunck. [4/8]							
	<i>C. foetida</i> H.B. & K.	496	Ollgaard & Balsev 8960; F	Ecuador, Chimborazo	O		EF194512
	<i>C. foetida</i> H.B. & K.	922	Steyermark 53255; F	Ecuador, Azuay	O		EF194513
	<i>C. foetida</i> H.B. & K.	1020	Sparre 16952; TEX	Ecuador, Pichincha	O		EF194511
	<i>C. foetida</i> H.B. & K. var. <i>pyncnantha</i> Yunck.	990	Lira 13; SGO	Chile, Tarapaca	O		EF194527
	<i>C. paitana</i> Yunck.	940	Haught 63; F	Peru, Parinas	O		EF194516
	<i>C. paitana</i> Yunck.	941	Weberbauer 7762; F	Peru, Piura	O		EF194517
	<i>C. purpurata</i> Phil.	1001	Biese 2918; SGO	Chile, Coquimbo	O		EF194526
	<i>C. xanthochortos</i> Mart. ex Engelm. var. <i>carinata</i> (Yunck.) Yunck.	1074	Aperecida et al. 4333; US	Brazil, GO	C	EF194446	
Americanae Yunck. [6/7]							
	<i>C. aff. cozumeliensis</i> Yunck.	1002	Fernandez & Acosta 2131; MEXU	Mexico, QRO	I	EF194358	EF194596
	<i>C. aff. floribunda</i> H.B. & K.	489	Grimaldo 492; F	Mexico, MIC	G	EF194396	
	<i>C. aff. floribunda</i> H.B. & K.	1009	Prather & Soule 1221; TEX	Mexico, MIC	G	EF194397	
	<i>C. aff. floribunda</i> H.B. & K.	1010	King & Soderstrom 5053; TEX	Mexico, MIC	G	EF194398	EF194619
	<i>C. americana</i> L.	699	Buswell 6231; NY	USA, FL	I		EF194597
	<i>C. americana</i> L.	698	Garneau et al. 1470; TRT	Grenada	I	EF194363	
	<i>C. corymbosa</i> Ruiz & Pav. var. <i>grandiflora</i> Engelm.	695	Ilitis & Guzman 29077; MICH	Mexico, JAL	J	EF194343	EF194584
	<i>C. corymbosa</i> Ruiz & Pav. var. <i>grandiflora</i> Engelm.	696	Mendez Ton & de Lopez 9608; MICH	Mexico, CHI	J	EF194344	EF194585
	<i>C. corymbosa</i> Ruiz & Pav. var. <i>grandiflora</i> Engelm.	959	Tellez 9976; RSA	Mexico, NAY	J	EF194345	EF194586
	<i>C. corymbosa</i> Ruiz & Pav. var. <i>stylosa</i> Engelm.	965	Rzedowski 28752; ASU	Mexico, MEX	J	EF194348	EF194587
	<i>C. corymbosa</i> Ruiz & Pav. var. <i>stylosa</i> Engelm.	694	Medrano et al 7965; GH	Mexico, HGO	J	EF194347	EF194588
	<i>C. corymbosa</i> Ruiz & Pav. var. <i>stylosa</i> Engelm.	810	Gutierrez 2801; XAL	Mexico, PUE	J	EF194349	
	<i>C. cozumeliensis</i> Yunck.	943	Standley 62142; F	Guatemala, Suchitepequez	I	EF194359	EF194592
	<i>C. globulosa</i> Benth.	550	Axelrod & Axelrod 1875; UPRRP	China, Guangxi	I	EF194360	EF194593
	<i>C. globulosa</i> Benth.	861	Axelrod 1154; UPRRP	USA, PR	I	EF194361	
	<i>C. globulosa</i> Benth.	1053	Liogier 10138; US	USA, PR	I	EF194362	
	<i>C. macrocephala</i> W. Schaffn. ex Yunck.	731	Palmer 141; GH	Mexico, BCA	I	EF194357	EF194595
	<i>C. macrocephala</i> W. Schaffn. ex Yunck.	614	Gentry 1145; MICH	Mexico, SON	I	EF194356	
	<i>C. macrocephala</i> W. Schaffn. ex Yunck.	613	Alexander 1241; NY	Mexico, CHI	I	EF194355	EF194594
Ceratophorae Yunck. [3/4]							
	<i>C. boldinghii</i> Urb.	569	Breedlove 37373; NY	Mexico, CHI	K		EF194575

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Section							
Subsection	DNA accession ^a	Voucher ^b	Country ^c	Clade ^d	GenBank accession		
Species					<i>trnL-F</i>	nrITS	
	568	<i>Mc Vaugh 22042</i> ; MICH	Mexico, JAL	K	EF194338	EF194578	
	693	<i>Mc Vaugh 26593</i> ; MICH	Mexico, JAL	K		EF194579	
	964	<i>Lehto & Lehto L49371</i> ; ASU	Mexico, SON	K		EF194574	
	843	<i>Kearney & Publes 14988</i> ; NY	USA, AZ	K		EF194573	
<i>Grandiflorae</i> Yunck. [3/5]							
	1076	<i>Cabrera et al. 21399</i> ; LP	Argentina, Jujuy	O		EF194536	
	540	<i>Hutchinson & Wright 4305</i> ; F	Peru, Ancash	O		EF194535	
	471	<i>Knox 5020</i> ; TRTE	Kenya	O		EF194528	
<i>Lepidanchoptis</i> Yunck. [1/4]							
	741	<i>Gentry 5291</i> ; GH	Mexico, DGO	K		EF194576	
	1003	<i>Gentry 5291</i> ; MEXU	Mexico, DGO	K	EF194339	EF194577	
<i>Leptanthae</i> Yunck. [3/3]							
	719	<i>Wiggins 14668</i> ; GH	Mexico, BAC	L	EF194323	EF194570	
	608	<i>Wiggins 20889</i> ; MICH	Mexico, BAC	L	EF194322	EF194569	
	884	<i>Fritsch & Fritsch 1337</i> ; RSA	Mexico, BAC	L	EF194324	EF194571	
	826	<i>Robles 123</i> ; XAL	Mexico, SIN	L	EF194321	EF194572	
	737	<i>Wiggins 15153</i> ; GH	Mexico, BAC	L	EF194335		
	764	<i>Carter & Kellogg 3085</i> ; GH	Mexico, BAC	L	EF194337		
	763	<i>Stevens & Fairhurst 2052</i> ; MICH	Mexico SON	L	EF194336		
	762	<i>Daniel & Butterwick 4341</i> ; NY	USA, AZ	L	EF194333	EF194567	
	554	<i>de la Luz 8543</i> ; ARIZ	USA, PR	L	EF194334	EF194568	
<i>Odontolepisae</i> Yunck. [6/10]							
	1055	<i>Straw 2267</i> ; US	Peru, Arequipa	O		EF194518	
	564	<i>Chazaro et al. 7527</i> ; MICH	Mexico, MIC	K	EF194340	EF194580	
	858	<i>Gonzalez 145</i> ; NY	Mexico, DGO	K	EF194342	EF194582	
	811	<i>Chazaro 7537</i> ; XAL	Mexico, JAL	K	EF194341	EF194581	
	730	<i>Hartman 52</i> ; GH	Mexico, SON	L	EF194332	EF194564	
	587	<i>White 2730</i> ; GH	Mexico, SON	L	EF194331	EF194563	
	523	<i>Cardenas 2555</i> ; F	Bolivia, Chaco	F	EF194353	EF194591	
	592	<i>Medina 2493</i> ; MICH	Mexico, HGO	H	EF194365	EF194599	
	845	<i>Rose et al. 9650</i> ; NY	Mexico, QRO	H	EF194367	EF194601	
	862	<i>Axelrod & Hernandez 2242</i> ; NY	Mexico, PUE	H	EF194366	EF194600	
	1025	<i>Correll & Johnston 19796</i> ; ASU	Mexico, NLE	G	EF194401		
	928	<i>Purpus 5444</i> ; F	Mexico, SLP	G	EF194402	EF194623	
	898	<i>Henrickson 6608</i> ; RSA	Mexico, NLE	G	EF194399	EF194622	
	1013	<i>Hinton et al 23503</i> ; TEX	Mexico, COA	G	EF194400		
<i>Odoratae</i> Yunck. [4/6]							
	999	<i>Hichins & Muñoz s.n.</i> ; SGO	Chile, Quilhota	O		EF194525	
	1000	<i>Teüller et al 2489</i> ; SGO	Chile	O		EF194524	
	967	<i>Landrum 3392</i> ; ASU	Chile, Valparaiso	O		EF194523	
	715	<i>Arroyo et al. 996099</i> ; SGO	Chile, Linares	O		EF194521	
	716	<i>Morales & Cordoba s.n.</i> ; SGO	Chile, IV	O		EF194522	
	567	<i>Ledingham 4455</i> ; USAS	Chile, Santiago	O		EF194520	
	909	<i>Vargas 684</i> ; F	Peru, Cuzco	O		EF194533	
	926	<i>Buchtien 133</i> ; F	Bolivia, La Paz	O		EF194534	
	912	<i>Hutchinson 1055</i> ; F	Peru, Junin	O		EF194514	
	1024	<i>Asplund 7737</i> ; TEX/LL	Ecuador, Chimborazo	O		EF194515	
	985	<i>Muñoz & Meza 2202</i> ; SGO	Chile, IV	O		EF194519	
	512	<i>Krapovickas 37354</i> ; F	Argentina, Tucuman	O		EF194532	
<i>Prismaticae</i> Yunck. [1/1]							
	930	<i>Mille 112</i> ; F	Ecuador, Guayaquil	J	EF194346	EF194583	
<i>Subulatae</i> Yunck. [5/5]							
	518	<i>Nee & Hansen 18685</i> ; F	Mexico, VER	G	EF194379		
	607	<i>Ton & Lopez 9826</i> ; MICH	Mexico, CHI	G	EF194377	EF194609	
	606	<i>Lorence & Irigos 4076</i> ; NY	Mexico, OAX	G	EF194378	EF194608	
	617	<i>Breedlove & Thorne 31083</i> ; NY	Mexico, CHI	G	EF194380		
	927	<i>Wiggins 13185</i> ; F	Mexico, SIN	G	EF194406	EF194625	
	556	<i>Eastoe & Clothier s.n.</i> ; ARIZ	Mexico, BAC	G	EF194381		
	815	<i>Wardlee 146728</i> ; CHR	New Zealand	G		EF194610	

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SUBGENUS						
Section						
Subsection	DNA accession ^a	Voucher ^b	Country ^c	Clade ^d	GenBank accession	
Species					<i>trnL-F</i>	nrITS
<i>C. mitrififormis</i> Engelm. ex Hemsl.	584	<i>R. Carrillo 356</i> ; CIIDIR	Mexico, DGO	G	EF194382	EF194611
<i>C. rugosiceps</i> Yunck.	517	<i>Cosminsky 71</i> ; F	Guatemala, Guatemala	G	EF194374	
<i>C. rugosiceps</i> Yunck.	915	<i>Williams et al. 41476</i> ; F	Guatemala, Totonicapan	G	EF194375	EF194606
<i>C. rugosiceps</i> Yunck.	745	<i>Brenckle 47 269</i> ; NY	Guatemala	G	EF194376	EF194607
<i>C. woodsonii</i> Yunck.	916	<i>Standley 81878</i> ; F	Guatemala, Huehuetenango	G	EF194405	EF194624
<i>C. woodsonii</i> Yunck.	729	<i>Davidson 967</i> ; GH	Panama, Chiriqui	G	EF194404	
<i>C. woodsonii</i> Yunck.	978	<i>Spellenberg et al. 8359</i> ; MEXU	Mexico, HGO	G	EF194403	
<i>Tinctoriae</i> Yunck. [5/9]						
<i>C. applanata</i> Engelm.	535	<i>Johnston 8826</i> ; F	Mexico, COA	H	EF194372	EF194605
<i>C. applanata</i> Engelm.	844	<i>Shreve 9323</i> ; GH	Mexico, SLP	H		EF194602
<i>C. applanata</i> Engelm.	507	<i>Spellenberg & Mahrt 10680</i> ; NMC	USA, NM	H	EF194373	
<i>C. applanata</i> Engelm.	850	<i>Corral Biaz 3912</i> ; NMC	Mexico, CHH	H		EF194604
<i>C. applanata</i> Engelm.	508	<i>Torreillas 237</i> ; NY	Mexico, DGO	H	EF194371	
<i>C. applanata</i> Engelm.	674	<i>Rodrigues 653</i> ; XAL	Mexico, COA	H	EF194370	EF194603
<i>C. aurea</i> Liebm.	506	<i>Chiang et al. 2161</i> ; MICH	Mexico, PUE	G	EF194391	EF194620
<i>C. aurea</i> Liebm.	1023	<i>King 2281</i> ; TEX	Mexico, PUE	G	EF194390	
<i>C. aurea</i> Liebm.	800	<i>Hernandez & Arias 21117</i> ; XAL	Mexico, PUE	G	EF194392	EF194621
<i>C. chinensis</i> Lam.	837	<i>Carter 628</i> ; CANB	Australia, WA	H	EF194368	
<i>C. chinensis</i> Lam.	459	<i>Surapat 137</i> ; IND	Thailand	H	EF194369	
<i>C. corniculata</i> Engelm.	933	<i>Stannard et al 51861</i> ; F	Brazil, BA	C	EF194445	EF194656
<i>C. tinctoria</i> Mart. ex Engelm.	574	<i>Ortega 149</i> ; GH	Mexico, GRO	G	EF194394	EF194618
<i>C. tinctoria</i> Mart. ex Engelm.	766	<i>Moore & Wool 3879</i> ; MICH	Mexico, HGO	G	EF194395	
<i>C. tinctoria</i> Mart. ex Engelm.	573	<i>Ortega s.n.</i> ; NY	Mexico, HGO	G	EF194393	EF194617
<i>Umbellatae</i> Yunck. [7/10]						
<i>C. colombiana</i> Yunck.	1068	<i>Haught 4535</i> ; US	Colombia, Magdalena	N	EF194312	
<i>C. gracillima</i> Engelm.	620	<i>Boege 490</i> ; GH	Mexico, GRO	N	EF194305	
<i>C. gracillima</i> Engelm.	599	<i>Iltis & Cochrane 149</i> ; MICH	Mexico, GRO	N	EF194303	
<i>C. gracillima</i> Engelm.	621	<i>Clarcke et al. 681230 17</i> ; MICH	Mexico, SIN	N	EF194306	EF194550
<i>C. gracillima</i> Engelm.	600	<i>Fryxell 82257</i> ; NY	Mexico, MEX	N	EF194304	EF194551
<i>C. hyalina</i> Roth	994	<i>Mkharme 34</i> ; ARIZ	India, Delhi	L	EF194320	
<i>C. hyalina</i> Roth	840	<i>Bosch 25022</i> ; BOL	Namibia, Windhoek	L		EF194561
<i>C. hyalina</i> Roth	875	<i>Hardy & de Winter 1392</i> ; PRE	Namibia, Walvis Bay	L	EF194318	
<i>C. hyalina</i> Roth	889	<i>Parvati s.n.</i> ; RSA	India, Delhi	L	EF194319	EF194562
<i>C. macvaughii</i> Yunck.	847	<i>Hinton 12098</i> ; NY	Mexico, MIC	N	EF194314	EF194557
<i>C. serruloba</i> Yunck.	977	<i>Orcutt 4457</i> ; MEXU	Mexico, COL	N	EF194313	EF194555
<i>C. sidarum</i> Leibm.	692	<i>Stevens & Krukoff 20950</i> ; CANB	Nicaragua, Managua	N	EF194309	
<i>C. sidarum</i> Leibm.	519	<i>Hammel 18763</i> ; F	Costa Rica, Puntarenas	N	EF194308	EF194552
<i>C. sidarum</i> Leibm.	751	<i>Austin 20956</i> ; GH	Nicaragua, Managua	N	EF194310	
<i>C. sidarum</i> Leibm.	1005	<i>Ayala 1054</i> ; TEX	Mexico, JAL	N	EF194307	EF194553
<i>C. umbellata</i> H.B.&K.	557	<i>Blankenhorn 216</i> ; ARIZ	USA, NM	L	EF194317	EF194560
<i>C. umbellata</i> H.B.&K.	759	<i>Bleakey 4662</i> ; NMC	USA, NM	L	EF194316	EF194559
<i>C. umbellata</i> H.B.&K.	516	<i>Fletcher 5857</i> ; UNM	USA, NM	L	EF194315	EF194558
<i>C. umbellata</i> H.B. & K. var. <i>reflexa</i> Yunck.	1027	<i>Austin & Austin 7585</i> ; ASU	USA, AZ	L	EF194327	
<i>C. umbellata</i> H.B. & K. var. <i>reflexa</i> Yunck.	1030	<i>Van Devender et al. 94 458</i> ; ASU	Mexico, SON	L	EF194328	
<i>C. umbellata</i> H.B. & K. var. <i>reflexa</i> Yunck.	1033	<i>Daniel 2445</i> ; ASU	Mexico, BCA	L	EF194329	
<i>C. umbellata</i> H.B. & K. var. <i>reflexa</i> Yunck.	577	<i>Spellenberg & Zucker 12966</i> ; NMC	USA, AZ	L	EF194325	EF194566
<i>C. umbellata</i> H.B. & K. var. <i>reflexa</i> Yunck.	1015	<i>Van Devender 94 458</i> ; TEX	Mexico, SON	L	EF194326	
<i>Uncertain</i>						
<i>C. longiloba</i> Yunck	904	<i>Krapovickas & Schinini 31255</i> ; F	Bolivia, Chuquisaca	F	EF194352	
<i>C. burrellii</i> Yunck.	888	<i>Dawson 14278</i> ; RSA	Brazil, GO	F	EF194354	EF194589
CUSCUTA						
Pachystigma Yunck.						
<i>Africanae</i> Yunck.						
<i>C. nitida</i> E.Mey.	625	<i>Helme 3042</i> ; SAM	South Africa, Western Cape	OG	EF202558	EF202562

APPENDIX. Continued.

SUBGENUS						
Section						
Subsection	DNA accession ^a	Voucher ^b	Country ^c	Clade ^d	GenBank accession	
Species					<i>trnL-F</i>	nrITS
Eucuscuta Engelm.						
<i>Europaeae</i> Yunck.						
<i>C. europaea</i> L.	166	<i>Alanko 94416</i> ; H	Finland, Uusimaa	OG	AY101169	EF202560
<i>Planiflorae</i> Yunck.						
<i>C. approximata</i> Bab.	509	<i>Lomer 93 204</i> ; UBC	Canada, BC	OG	EF202557	EF202561

^a Extraction numbers following species names are indicated on the phylogenetic trees.

^b Abbreviations of herbaria in which the vouchers are deposited follow Index Herbariorum.

^c If known, lower administrative units within a country (e.g., states, provinces, regions, departments) are indicated and are customary abbreviations for a given country.

^d Letters correspond to major clades as they are labeled in Figs. 3–5 within the main text and Appendices S1–S2.