Reticulate evolution in the parasitic genus *Cuscuta* (Convolvulaceae): over and over again[1]

Saša Stefanović and Mihai Costea

Abstract: The frequency and relative importance of hybridization in plants has been an area of intense debate. Although this evolutionary phenomenon has received considerable attention from plant biologists, there are no well-supported cases of reticulate evolution involving parasitic plants, to date. Recent molecular phylogenetic analyses revealed that the subgenus *Grammica*, the largest and most diverse group of the stem-parasitic genus *Cuscuta* (dodder), consists of 15 major clades. We describe here five cases of strongly supported discordance between phylogenies derived from plastid and nuclear data, and interpret them as results of five independent hybridization events. Three of these cases could represent relatively recent reticulations, as each of them involves more closely related species, always confined within the same major clade as their putative parental species, and are currently sympatric or parapatric with them. The two remaining cases involve species whose potential progenitors are derived from different major groups of *Grammica*, and which are allopatric in their present distribution. A series of statistical tests was conducted to assess and further explore the significance of this phylogenetic incongruence. Alternative explanations for discordant gene topologies are explored. ***Cuscuta liliputana*** sp. nov., a new Mexican species of hybrid origin is described.

Key words: Convolvulaceae, *Cuscuta*, *Cuscuta liliputana* sp. nov., molecular phylogeny, parasitic plants, reticulate evolution.

Résumé : La fréquence et l'importance relative de l'hybridation chez les plantes soulèvent d'intenses débats. Bien que ce phénomène évolutif ait reçu beaucoup d'attention de la part des phytobiologistes, à ce jour, on ne connaît pas de cas bien établi d'évolution réticulée impliquant des plantes parasites. Des analyses phylogénétiques récentes révèlent que le sous-genre *Grammica*, le groupe le plus important et le plus diversifié du parasite caulinaire *Cuscuta* (cuscute), comporte 15 clades principaux. Les auteurs décrivent cinq cas de discordance bien établis entre les phylogénies dérivées de données plastidiques et nucléiques; ils les interprètent comme les résultats de cinq évènements d'hybridation indépendants. Trois de ces cas pourraient représenter des réticulations relativement récentes, puisque chacun d'eux implique des espèces plus étroitement apparentées, toujours confinées au même clade principal que leurs parents, et présentement sympatriques ou parapatriques avec eux. Les deux autres cas impliquent des espèces dont les progéniteurs potentiels dérivent de groupes majeurs distincts du *Grammica*, et qui sont actuellement allopatriques. Les auteurs ont conduit une série de tests statistiques pour évaluer et explorer la signification de cette inadéquation phylogénétique. Ils explorent des explications alternatives à ces discordances dans la distribution topologique des gènes. Ils décrivent le ***Cuscuta liliputana*** sp. nov., une nouvelle espèce mexicaine d'origine hybride.

Mots-clés : Convolvulaceae, *Cuscuta*, *Cuscuta liliputana* (sp. nov.), phylogénie moléculaire, plantes parasites, évolution réticulée.

[Traduit par la Rédaction]

Introduction

The frequency and importance of hybridization in plants has been an area of intense debate for a long time, especially when coupled with polyploidization (Stebbins 1959; Grant 1981; Arnold 1992; Soltis and Soltis 1993; Rieseberg 1995, 1997; Ramsey and Schemske 2002). The advent of molecular systematics and the use of DNA-based markers have helped to significantly accelerate the rate at which potential cases of hybridization and introgression have been documented (Rieseberg 1995). Some well-studied examples of hybridization in plants include *Helianthus* (Rieseberg et al. 1990), *Gossypium* (Wendel et al. 1995), *Paeonia* (Sang et al. 1995), *Penstemon* (Wolfe et al. 1998), *Dendrochilum* (Barkman and Simpson 2002), and *Sideritis* (Barber et al. 2007). However, there are very few documented examples of reticulate evolution in parasitic plants. Virtually all published cases involve hemiparasites from Santalales, the sandalwood order. For example, in Loranthaceae, hybridization has been described between *Loranthus* and *Tupeia* (Thomson 1949), within *Amyema* (Bernhardt and Calder 1981), and within *Tristerix* (Amico et al. 2007). Also, in Santalaceae, a potential case of hybridization was suggested in *Santalum* by Harbaugh and Baldwin (2007), although alternative explanations could not be excluded. There are no well-established cases of hybridization in holoparasites, sug-

Received 11 September 2007. Published on the NRC Research Press Web site at botany.nrc.ca on 29 August 2008.

S. Stefanović.[2] Department of Biology, University of Toronto, Mississauga, ON L5L 1C6, Canada.
M. Costea. Department of Biology, Wilfrid Laurier University, Waterloo, ON N2L 3C5, Canada.

[1]This paper is one of a selection of papers published in the Special Issue on Systematics Research.
[2]Corresponding author (e-mail: sasa.stefanovic@utoronto.ca).

 doi:10.1139/B08-033

gesting that this process is either understudied or rare in these plants.

The parasitic plant genus *Cuscuta*, members of which are commonly known as dodders, contains some 180 species, is nearly cosmopolitan in distribution, and occurs in a wide range of habitats (Yuncker 1932; Mabberley 1987). Engelmann (1859) recognized three groups within *Cuscuta*, based primarily on the morphology of stigma and styles, which were assigned subgeneric ranks by Peter (1897) and adopted by Yuncker (1932) in his seminal monograph on this genus. *Cuscuta* subgenera *Cuscuta* and *Grammica* are characterized by two distinct styles, and are distinguishable by their stigma morphology (elongated or short and capitate, respectively). *Cuscuta* subgenus *Monogyna* has partially or completely united styles, with capitate, conical, or ovate stigmas. *Cuscuta* subgenus *Grammica* is by far the largest group of *Cuscuta*, accounting for approximately three-quarters of its species diversity (135–140 spp.). While few members of this group are widespread, the vast majority of species occur only in the Americas, with Mexico and adjacent regions as a centre of diversity (Yuncker 1932).

Because of its heterotrophic life-style and a diversity of photosynthetic ability among its species, *Cuscuta* has been the focus of many scientific studies. Plastids of several species have been the subject of extensive molecular analyses (reviewed in Stefanović and Olmstead 2005) and recently the entire plastid genomes of four species have been sequenced (*Cuscuta campestris*, *Cuscuta obtusiflora*, *Cuscuta exaltata*, and *Cuscuta reflexa*; Funk et al. 2007; McNeal et al. 2007*a*). In addition, a substantial body of literature deals with the ecology and pest control of different dodder species (Dawson et al. 1994; Costea et al. 2006). Because this branch parasite is amenable to culture and direct experimental manipulation, it is also frequently used as a model system for developmental research, especially of haustorial initiation and formation (e.g., Lee and Lee 1989; Subramaniam and Mahadevan 1994). A recent review by García and Castroviejo (2003) summarized our knowledge on chromosome numbers and ploidy levels in *Cuscuta*, accumulated over a period of almost a century. The chromosome numbers in *Cuscuta* range from $2n = 8$ to $2n = 56$, indicating that polyploidy likely plays an important role in the evolution of this genus. Although much is known about its morphology and molecular evolution, very little is known about the natural history of *Cuscuta* species generally. One important exception is the study on life history and reproductive biology of *Cuscuta attenuata* by Prather and Tyrl (1993). From their crossing studies, these authors concluded that this species is primarily autogamous, although it is also capable of allogamy, and that interspecific crosses with several more or less distantly related species failed to produce seed set (Prather 1990). Also, some *Cuscuta* members were recently implicated as vectors in the horizontal transfer of mitochondrial genes in plants (Mower et al. 2004).

Cuscuta has been the subject of two broad molecular phylogenetic studies (Stefanović et al. 2007; García and Martín 2007). Both of those studies were based on plastid (pt) *trnL*-UAA/*trnF*-GAA and nuclear ribosomal (nr) ITS sequences from a wide taxonomic sampling, covering the morphological, physiological, and geographical diversity of *Cuscuta* subgenus *Grammica* (Stefanović et al. 2007) and subgenus *Cuscuta* (García and Martín 2007), respectively. In addition, McNeal et al. (2007*b*) conducted a study on a more limited taxon sampling, but included representatives from across the entire genus, and used a combination of several pt protein-coding genes (*rbcL*, *rps2*, *matK*), as well as nrITS. Within *Cuscuta* subgenus *Grammica*, the results of Stefanović et al. (2007) indicated the presence of 15 well-supported major clades. Stefanović et al. (2007) also noted several cases of conflict between plastid- and nuclear-derived phylogenies, indicative of either technical problems (e.g., incorrect identification, DNA contamination, sequence error, spurious phylogenetic reconstruction) or underlying organism-level phenomena (such as lineage sorting, orthology/paralogy conflation, horizontal gene transfer, or reticulation). Due to the inadequate taxon sampling, these problematic cases were excluded from the previous analyses. In the present study, we expand our existing *trnL-F* and ITS matrices through addition of multiple sequences for species of putatively hybrid origin, as well as other relevant taxa. Here, we formally analyze the cases of strong phylogenetic discordance between the gene trees, with the following goals in mind: (*i*) to demonstrate and document the first cases of reticulate evolution in *Cuscuta* subg. *Grammica* and to discuss in detail the molecular phylogenetic evidence for their hybrid origin, (*ii*) to assess the directionality of hybridization, and (*iii*) to investigate the potential alternative scenarios as well as evaluate their relative merits.

Materials and methods

Taxon sampling

A total of 286 accessions representing 105 species of *Cuscuta* were analyzed in this study (Appendix A). The taxon sampling strategy used originally to delimit major lineages within *Cuscuta* subgenus *Grammica* and to infer the overall relationships among those major lineages is detailed in Stefanović et al. (2007). To this backbone phylogeny with 15 well-supported clades we added here those taxa that showed topological incongruence in preliminary analyses. Efforts were made to sample multiple accessions for species with strongly supported phylogenetic conflict. Hence, two or more individuals are included for all but one rare species, which is known only from its type locality. To further increase the sampling density for critical groups, additional individuals or species were included for most of the affected clades (e.g., clades B, C, and L; see below).

Molecular techniques

DNA extractions, polymerase chain reaction (PCR) reagents and conditions, cloning, amplicon purifications, as well as sequencing procedures followed the protocols detailed in Stefanović et al. (2007). Sequences generated in this study are deposited in GenBank (accession numbers EU288331–EU288370; see Appendix A).

Phylogenetic analyses

DNA alignment and substitution model selection

Sequences were aligned manually using Se-Al version 2.0a11 (Rambaut 2002). Although numerous gaps had to be introduced in the alignments, the sequences could be readily

aligned among the ingroup taxa in both plastid and nuclear matrices. However, owing to the overwhelming prevalence of complex overlapping gaps, the indels in the alignments were not coded and were treated as missing data. Regions that could not be unambiguously aligned were excluded from subsequent analyses.

The general time-reversible 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).

Parsimony analyses

Heuristic searches and estimates of clade support were conducted for each matrix separately. 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 off. The second round of analyses was performed on all trees in memory with the same settings except with MULTREES on. Both stages were conducted to completion or until 100 000 trees were found. The 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 off (DeBry and Olmstead 2000).

Topological incongruence and alternative hypothesis testing

Conflict between datasets was evaluated by visual inspection, by searching for the presence of strongly supported yet conflicting topologies from individual matrices. For all the cases where such conflicts were found, reciprocally constrained topologies were constructed using MacClade version 4.06 (Maddison and Maddison 2003) and their cost in parsimony assessed using PAUP* (Swofford 2002). In this fashion, for each case of strongly supported incongruence between the two data sets, one randomly chosen MP tree representing topological results obtained from plastid data was imposed on nuclear data and vice versa.

To evaluate the significance among these alternative phylogenetic hypotheses, two types of statistical tests were conducted using the selected DNA substitution model. First, we implemented the one-tailed Shimodaira–Hasegawa tests (SH tests; Shimodaira and Hasegawa 1999; Goldman et al. 2000) in PAUP*. The test distributions were obtained using the re-estimated log likelihoods (RELL; Kishino and Hasegawa 1989) with 1000 bootstrap replicates. Second, we also conducted the Approximately Unbiased tests (AU tests; Shimodaira 2002). This test is recommended for general tree comparison because it is considered to be less biased than other methods employed for these purposes and is hence, less conservative than, for example, the SH test (Shimodaira 2002). For each data set, the total log likelihoods and sitewise log likelihoods of the tested tree topologies were computed with PAUP* before being subjected to the AU test. The *P*-values of the AU test were calculated using CONSEL version 0.1i (Shimodaira and Hasegawa 2001). Ten repetitions of multiscale bootstrapping, each consisting of 10 sets with 10 000 bootstrap replicates, were used to ensure small sampling error.

Results

Sequences and alignments

Summary descriptions for sequences obtained from plastid *trnL-F* and nuclear ITS regions are presented in Table 1. Plastid sequences could not be obtained for members of the O clade, a group of *Cuscuta* species hypothesized to have substantially altered plastid genomes (Stefanović et al. 2007). In addition, presumably due to the poor quality of the DNA extracted from some older herbarium specimens, sequences could not be obtained for a number of individuals for either *trnL-F* or ITS (Appendix A).

Sequences newly generated for this study were incorporated in the alignments used in our previous analyses (Stefanović et al. 2007). The assessment of primary homology was essentially unambiguous throughout the entire length of the ITS matrix. For the majority of DNA accessions, the direct sequencing approach yielded results without apparent polymorphism. However, in several cases a polymorphism was detected, caused by point mutations or length variants, and multiple cloned paralogues were included in the analyses. Sequences were also easily aligned across the *trnL* intron, as well as the *trnL* and *trnF* genes themselves. However, the spacer between 3′-*trnL* and *trnF* evolves more rapidly, both in terms of length and point mutations (Stefanović et al. 2007), and consequently a portion of 120 bp had to be excluded from the analyses. While the aligned lengths of the two matrices were similar in size, the analyzed length and the number of variable and parsimony informative sites was substantially smaller for the plastid matrix compared to the nuclear data set because of this exclusion (Table 1). No significant heterogeneity in base composition was detected within any of these matrices across all taxa. Alignments (in Nexus format) are deposited in TreeBASE (study accession number S1929).

Unconstrained topologies and overall levels of support

The *trnL-F* and ITS matrices each produced >100 000 trees, 798 and 2014 steps in length, respectively (Table 1). The overview of relationships among the major groups allows for an overall topological comparison of results between the two datasets (Fig. 1).

Within *Cuscuta* subg. *Grammica,* a total of 15 major clades labeled A–O were resolved with ITS sequences. Fourteen of the same groups, A–N, were also recovered with *trnL-F* data (as indicated earlier, none of the sequences belonging to the clade O could be obtained for *trnL-F*). Most of the 14–15 major clades received moderate (70%–85%) to strong (>85%) bootstrap support from both of the individual matrices. However, some groups were found to be weakly supported (<70%) by one of the datasets while receiving moderate to strong support from the other in a mutually complementary fashion (e.g., compare the support for the C and N clades between the two data sets; Fig. 1). Taken together, the analyses of separate plastid and nuclear matrices produced trees of remarkably similar topologies, with

Table 1. Characteristics of sequences included in, and maximum parsimony trees derived from, phylogenetic analyses of two data sets.

Description	Plastid (*trnL-F*)	Nuclear (ITS)
Number of individuals sequenced	240	226
Number of OTUs analyzed[a]	155	176[b]
Sequence characteristics:		
Aligned length	688	715
Analyzed length[c]	524	673
Variable sites	242	455
Parsimony informative sites	190	406
Mean AT content (%)	63	50
Base frequency homogeneity (χ^2/df/*P*)	84.65/459/1.0	330.21/522/1.0
Tree characteristics:		
Number of trees	>100 000	>100 000
Length	798	2014
CI/RI	0.52/0.90	0.44/0.90

Note: CI, consistency index; df, degrees of freedom; OTU, operational taxonomic unit; RI, retention index.

[a]After individuals with identical sequence for both regions were aggregated into a single terminal taxon.

[b]Including seven individuals represented by multiple, distinct clones.

[c]After excluding portions of alignments corresponding to primer sites and ambiguously aligned regions.

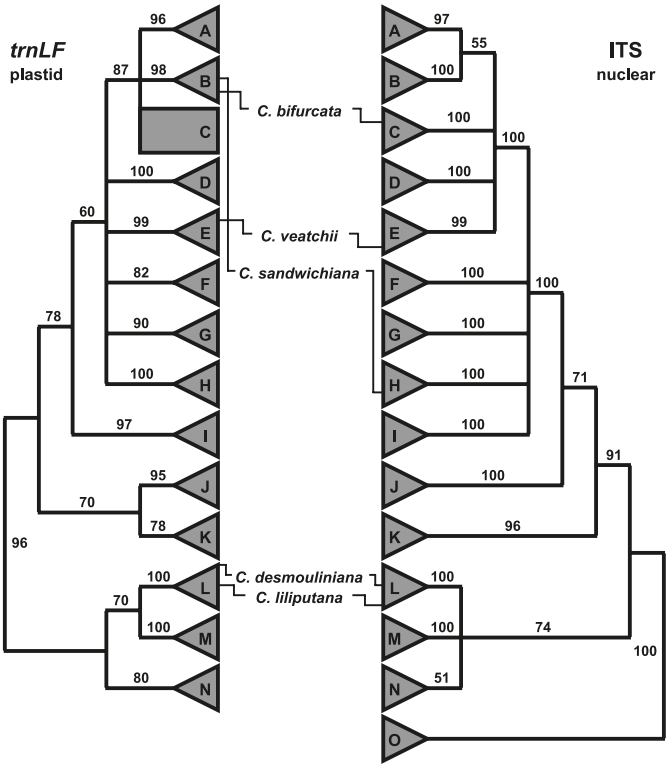


Fig. 1. Schematic overview of the conflicting phylogenetic position for the five species of putatively hybrid origin belonging to *Cuscuta* subg. *Grammica*. Phylogenetic relationships are inferred from separate parsimony analyses of plastid and nuclear sequences (Stefanović et al. 2007). Fifteen major groups are labeled A–O on these bootstrap consensus trees, and their supports are indicated (plastid sequences could not be obtained for members of the O clade). To facilitate topological comparison, the unrooted phylogenetic networks are rooted using the L–O clades as functional outgroups. Species relationships within the major clades are not shown (see Figs. 2 and 3 for detailed trees).

the exception of five striking and strongly supported conflicts (Fig. 1).

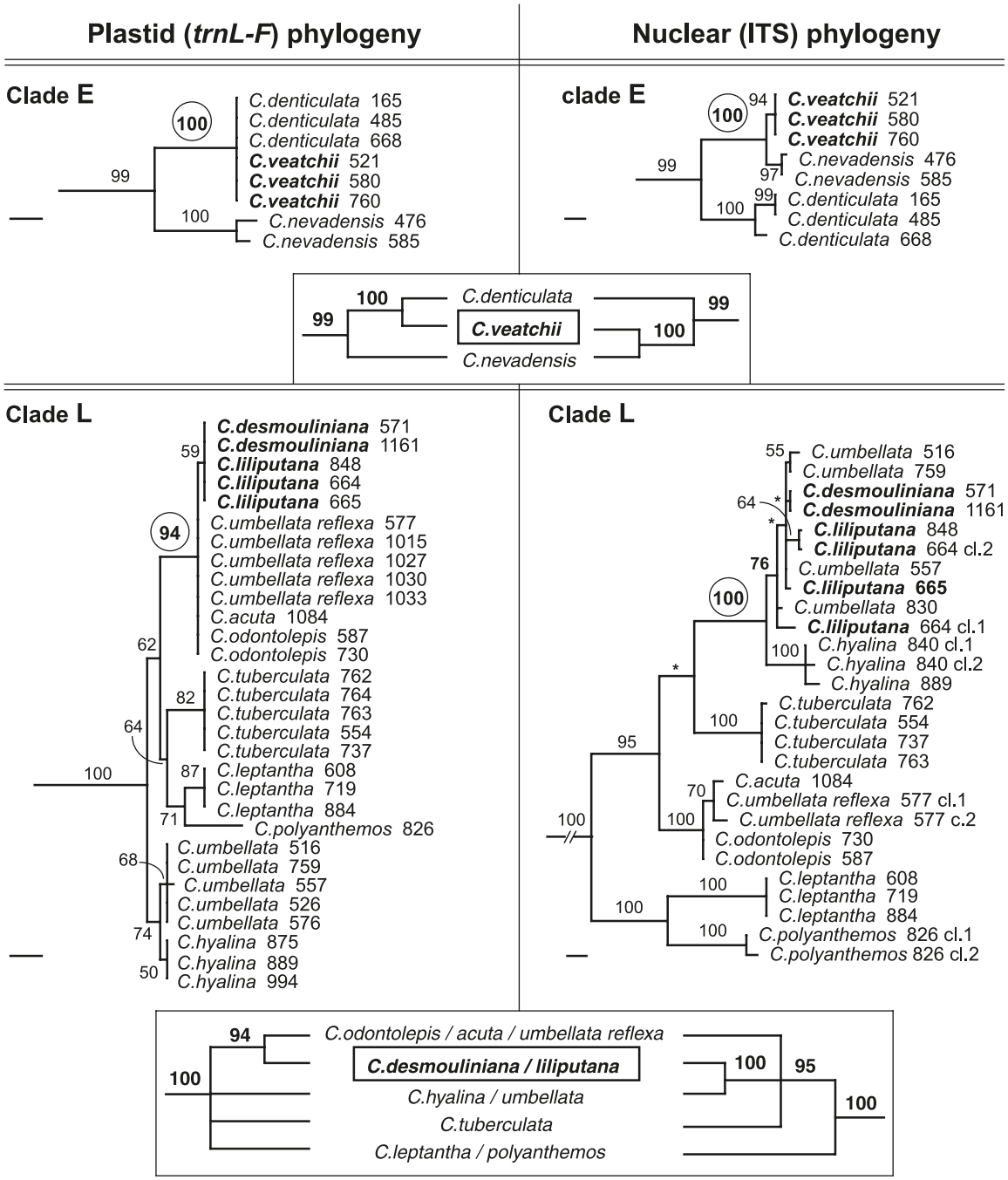
The first three cases of well-supported incongruence involve species whose topological discordances are limited to a given major clade (Fig. 2; clades E and L). Plastid data place *Cuscuta veatchii* Brandegee within the E clade, as sister to *Cuscuta denticulata* Engelm., while nuclear data resolve *C. veatchii* also within the E clade, but as sister to *Cuscuta nevadensis* I.M. Johnst. (Fig. 2). Both of these results received 100% BS. In a similar fashion, *Cuscuta desmouliniana* Yunck. and ***Cuscuta liliputana*** sp. nov. were recovered nested within the L clade. However, each of these two species was found to be closely associated with *Cuscuta umbellata* H.B. & K. var. *reflexa* Yunck., *Cuscuta odontolepis* Engelm., and *Cuscuta acuta* Engelm. with plastid data (94% BS; Fig. 2) while the nuclear data show them in a strongly supported clade with *C. umbellata* and *Cuscuta hyalina* Roth (100% BS; Fig. 2).

The remaining two cases of topological incongruence between plastid and nuclear data involve species whose topological discordances span across different major clades. According to the plastid data, both *Cuscuta bifurcata* Yunck. and *Cuscuta sandwichiana* Choisy belong to the strongly supported B clade (98% BS; Fig. 3). *Cuscuta bifurcata* forms a group with *Cuscuta australis* Hook. f. and *Cuscuta obtusiflora* H.B. & K. (87% BS; Fig. 3), while the position of *C. sandwichiana* is not well resolved with *trnL-F* data. In contrast, the nuclear data place *C. bifurcata* as nested within the C clade and *C. sandwichiana* as part of the H clade (both at 100% BS; Fig. 3). Within the C clade, *C. bifurcata* is strongly supported as sister to *Cuscuta werdermanii* Hunz., while *C. sandwichiana* is sister to the rest of the well-supported H clade (Fig. 3).

Tests of alternative tree topologies

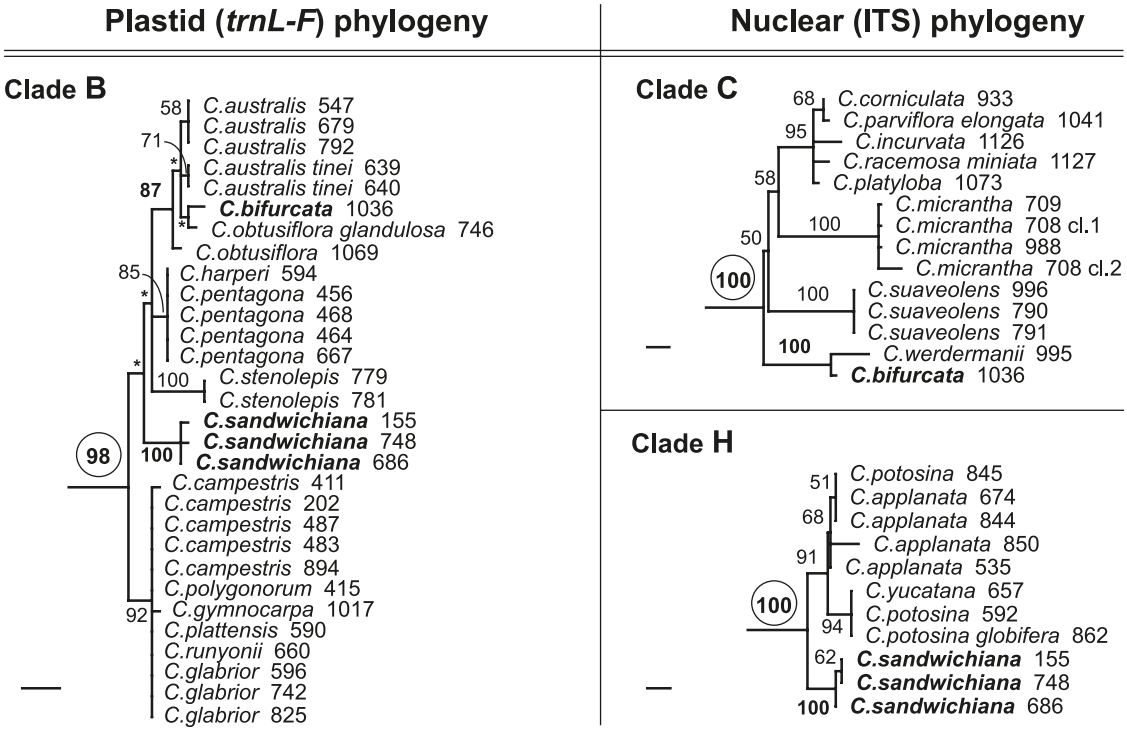
For the three cases where incongruence was confined within major clades (i.e., *C. veatchii*, *C. desmouliniana*, and *C. liliputana*; Figs. 1 and 2), the results of the SH and AU

Fig. 2. Portions of one of equally parsimonious trees derived from separate maximum parsimony analyses of plastid and nuclear sequences showing strong incongruence for phylogenetic placements of *Cuscuta veatchii* (within the E clade), as well as *C. desmouliniana* and *C. liliputana* (within the L clade). Branch lengths are drawn proportionally to the number of changes (bars indicate five changes; note the different scale for plastid and nuclear phylograms). Asterisks indicate nodes that collapse in the strict consensus. Bootstrap values are provided and those most relevant for supporting the conflicting topologies are emphasized (in bold and encircled). Numbers following species names correspond to DNA accessions (see Appendix A). Insets schematically illustrate the conflicting topologies indicative of hybrid origins of these species (boxed). For simplicity, only the strongly supported backbone nodes (>85% bootstrap) are shown as resolved.



tests were mixed, regarding both the species studied and the method used (Table 2). Using plastid data and enforcing *C. veatchii* to be sister to *C. nevadensis* (following the ITS results) produced trees 10 steps longer than the optimal trees, but this solution was not significantly different based on the SH and AU tests (although it approaches significance for the latter case; $P = 0.051$). However, imposing *C. veatchii* to be sister to *C. denticulata* (following the *trnL-F* results), using nuclear data resulted in trees 13 steps longer than the MP trees. This difference was deemed significant according to the AU test but not the SH test. Similarly, constraining either *C. desmouliniana* or *C. liliputana* to group in a clade with *C. umbellata* and *C. hyalina* (following the ITS results) with plastid data yielded trees which were found to be significantly

Fig. 3. Portions of one of equally parsimonious tree derived from separate maximum parsimony analyses of plastid and nuclear sequences showing strong incongruence for phylogenetic placements of *Cuscuta bifurcata* and *C. sandwichiana.* Plastid phylogenies place both of these species within the B clade. However, nuclear-derived phylogenies place *C. bifurcata* within the C clade, while *C. sandwichiana* is recovered within the H clade (compare with the overview in Fig. 1). Branch lengths are drawn proportionally to the number of changes (bars indicate five changes; note the different scale for plastid and nuclear phylograms). Asterisks indicate nodes that collapse in the strict consensus. Bootstrap values are provided and those most relevant for supporting the conflicting topologies are emphasized (in bold and encircled). Numbers following species names correspond to DNA accessions (see Appendix A).



different according to the AU tests but not according to the SH tests. The reverse constraints, placing *C. desmouliniana* or *C. liliputana* in a clade with *C. odontolepis*, *C. acuta*, and *C. umbellata* var. *reflexa* (following the *trnL-F* results) and using nuclear data were rejected as significantly worse solutions by both SH and AU tests.

For the two cases where the incongruence spanned different major clades (i.e., *C. bifurcata* and *C. sandwichiana*; Figs. 1 and 3), the results of the SH and AU tests were more uniform, unanimously rejecting the alternatives as significantly different from the best respective solutions in all comparisons (Table 2). This is not surprising given that in all of those cases multiple well-supported nodes (most of them at 100% BS) had to be collapsed to impose respective alternative topologies.

Discussion

Evidence for hybridization in *Cuscuta*

Instances of reticulate evolution in plants can be detected through careful analyses of discordance among different unlinked gene trees (Rieseberg and Soltis 1991; Rieseberg 1995; Sang and Zhong 2000). Because the plastid genome is maternally inherited in the majority of flowering plants (Corriveau and Coleman 1988; Reboud and Zeyl 1994; Mogensen 1996), the plastid-derived phylogeny will usually trace maternal genealogy. When the ptDNA tree is compared with an independently derived phylogenetic tree (from morphology or other molecular data), conflicting position of a taxon between phylogenies may be taken as evidence for the hybrid origin of this taxon (Sang and Zhong 2000; a simple illustration of this principle is depicted in the insets of Fig. 2). We present here evidence for five cases of strongly supported yet conflicting phylogenetic signals between *trnL-F* and ITS sequence data for five species of *Cuscuta*. In addition, in preliminary phylogenetic analyses of the entire genus, based on *rbcL* and 26S nrDNA sequences, the same five cases of hybridization events are evident, with equally strong support (S. Stefanović, M. Kuzmina, M. Costea, unpublished data). For each of these putative cases of hybridization, here documented for the first time in a group of holoparasitic plants, we discuss in detail the molecular phylogenetic evidence for hybrid origin, directionality of hybridization, and provide some alternative evolutionary explanations.

The C. veatchii *case*

According to both the *trnL-F* and ITS data (Figs. 1 and 2), *C. veatchii* is found in the E clade (Stefanović et al. 2007), together with *C. denticulata* and *C. nevadensis*, as expected from the traditional taxonomy (*Cuscuta* subg. *Grammica* sect. *Cleistogrammica* subsect. *Denticulatae*; Yuncker 1932, 1943) and recent morphological analyses (Costea et al. 2005). Members of this group share a seed with a "thickened" embryo, where the embryo's radicular end is enlarged in a ball-like structure that increases in vol-

Table 2. Results of the Shimodaira-Hasegawa (SH) and the approximately unbiased (AU) tests for comparison between highly supported yet incongruent topologies recovered from plastid and nuclear data sets of *Cuscuta* subg. *Grammica* species.

Data set	Constrained topology	Length	Δ length	SH test	AU test
Plastid (*trnL-F*)	Optimal (MP) tree (Fig. 1, left)	798	Best	1.000	0.967
	C. veatchii sister to *C. nevadensis* (Fig. 2)	808	10	0.525	0.051
	C. desmouliniana sister to *C. umbellata/hyalina* (Fig. 2)	804	6	0.148	**0.034**
	C. liliputana sister to *C. umbellata/hyalina* (Fig. 2)	805	7	0.148	**$7 \cdot 10^{-5}$**
	C. bifurcata part of the C clade (Fig. 3)	812	14	**0.028**	**$9 \cdot 10^{-5}$**
	C. sandwichiana part of the H clade (Fig. 3)	816	18	**0.016**	**0.003**
Nuclear (ITS)	Optimal (MP) tree (Fig. 1, right)	2014	Best	1.000	0.995
	C. veatchii sister to *C. denticulata* (Fig. 2)	2027	13	0.173	**0.002**
	C. desmouliniana sister to *C. odontolepis/acuta/umbellata_reflexa* (Fig. 2)	2037	23	**0.035**	**$2 \cdot 10^{-4}$**
	C. liliputana sister to *C. odontolepis/acuta/umbellata_reflexa* (Fig. 2)	2036	22	**0.036**	**0.012**
	C. bifurcata part of the B clade (Fig. 3)	2039	25	**0.002**	**$5 \cdot 10^{-11}$**
	C. sandwichiana part of the B clade (Fig. 3)	2051	37	**$1 \cdot 10^{-4}$**	**$1 \cdot 10^{-78}$**

Note: SH, probabilities of the Shimodaira-Hasegawa test; AU, probabilities according to the approximately unbiased test. Boldface, $P < 0.05$ (i.e., tree topology rejected as significantly worse).

ume during seed maturation. This feature is unique among dodder species. Also, these three species are characterized by a distinctively reticular calyx surface. Morphological differences among these species are subtle, yet discontinuous and consistent (Costea et al. 2005). The *trnL-F* sequences from representatives of *C. veatchii* are identical to those of *C. denticulata* (three individuals for each; Fig. 2), while their ITS sequences are very similar (but not identical) to those of *C. nevadensis*. We posit that these strongly supported and disagreeing phylogenetic results are indicative of reticulate evolution involving two progenitor species, *C. denticulata* and *C. nevadensis*, yielding their putative hybrid derivative, *C. veatchii*, with support for *C. denticulata* as the maternal parent.

All three of these species occur in North America, west of the Rockies. Among them, *C. denticulata* has the broadest geographic distribution, occurring from the Pacific Northwest to northern Mexican states. The other putative parent, *C. nevadensis*, has a much narrower range and is found in southwestern California, Nevada, and perhaps Arizona (USA), yet it is fully sympatric with *C. denticulata*. The putative hybrid, *C. veatchii*, is restricted in distribution to Baja California (Mexico), from the San Felipe Desert south to the Vizcaino Desert. *Cuscuta denticulata* reaches Lower California, at the border of USA–Mexico, but, based on the currently available data, there is no overlap in its distribution with *C. veatchii*. While this geographic separation may exclude this hybrid species from competition with its progenitors today, it is likely that additional mechanisms were involved in the past to allow for the establishment of a persistent hybrid lineage. From the theoretical standpoint, to overcome the minority cyotype exclusion (Levin 1975; Husband 1998), hybrids must remove themselves from the random mating pool and diverge ecologically from parental species (Coyne and Orr 2004). Unfortunately, the ploidy level of these three species is not known. Hence, it is not clear whether postzygotic isolation could have been achieved through triploid hybrid sterility. It is known, however, that *Cuscuta* species are primarily autogamous (Prather and Tyrl 1993; Stefanović and Olmstead 2005; Costea and Tardif 2006) and selfing could have served as a preadaptation necessary for escaping minority cyotype exclusion in this case. Additionally, unlike the two putative progenitor species, *C. veatchii* is host specific, and is thus ecologically divergent from them as well. Specifically, *C. denticulata* parasitizes primarily *Artemisia* (Asteraceae), *Chrysothamnus* (Asteraceae), and *Larrea* (Zygophyllaceae), but it also grows on a wide variety of other desert plants, such as *Ambrosia* (Asteraceae), *Atriplex* (Chenopodiaceae), *Eriogonum* (Polygonaceae), *Lepidospartum* (Asteraceae), and others. Hosts of *C. nevadensis* are also diverse desert herbs and shrubs, mainly *Ambrosia*, *Atriplex*, *Psorothamnus* (Fabaceae), and *Xylorhiza* (Asteraceae). However, *C. veatchii* is narrowly restricted to *Pachycormus* [=*Veatchia*] *discolor* Coville (rarely also *Bursera*; both small trees in Anacardiaceae) as a host.

While these biogeographical, life history, and ecological data are consistent with a hybrid origin of *C. veatchii*, an in-depth exploration of alternative possibilities is warranted (see below for a general discussion of this topic). Because the E clade includes only three species and a root (Fig. 2; see the inset), a simple topological distortion, such as nearest-neighbor interchange (NNI), would result in trees compatible between plastid and nuclear data. Yet, a spurious phylogenetic reconstruction due to the long-branch attraction (Felsenstein 1978) is not likely to explain observed topological differences because the branches involved are not significantly different in length (neither the internal branches nor the root subtending the E clade; Fig. 2). However, the simplicity of an NNI swap could explain why the SH and AU tests have failed (in three out of four tests) to find the significance in observed length differences between the optimal and constrained trees for both plastid and nuclear data sets (Table 2). In addition, multiple individuals from all three species were included in the analysis to increase the chance of finding polymorphic alleles, indicative of explanations alternative to hybridization, such as lineage sorting. No evidence of ancestral polymorphism has been found. Yet, caution is still necessary when interpreting these results, because there are only three (extant) species in this clade. Thus, the possibility of an ancestral polymorphism and its transmission through only one speciation event cannot be excluded at present. Phylogenetic analyses of additional, independently inherited genes will help to ascertain this issue with more confidence.

Given that the E clade contains only three species, topological distortion and lineage sorting are more difficult to

eliminate as alternatives for the *C. veatchii* putative hybrid compared with the other four (see below). Despite this, we still suspect that our strongly conflicting phylogenetic results represent evidence for hybrid origin of this species and that there is solid additional corroborative evidence provided by biogeographic and natural history data for this particular evolutionary interpretation of discordance between plastid and nuclear trees.

The C. desmouliniana *and* C. liliputana *cases*

Cuscuta desmouliniana, as well as *C. liliputana*, a new species described here, are found nested within the L clade (Figs. 1 and 2). This clade, first explicitly defined by Stefanović et al. (2007), includes mostly species circumscribed by Yuncker (1932) in *Cuscuta* subg. *Grammica* sect. *Eugrammica* subsections *Umbellatae* and *Leptanthae* plus a few species that were traditionally classified elsewhere (*Cuscuta* subg. *Grammica* subsections *Odontolepisae* and *Acutae*). Morphologically, these species are characterized by loose, umbellate inflorescences and flowers with acute calyx and corolla lobes. Most species occur in Mexico and the southwest USA. However, in the context of a rooted phylogeny for *Cuscuta* subg. *Grammica*, two potential cases of long-distance dispersal have been inferred within this clade (Stefanović et al. 2007). *Cuscuta acuta* is endemic to the Galapagos Islands and *C. hyalina*, with its disjunct populations found in tropical India, east Africa uplands, and western South Africa, is nested within this otherwise predominantly north Mexico – southwest USA clade.

While both the nuclear and plastid sequences place *C. desmouliniana* and *C. liliputana* deeply within the L clade, the more precise relationships of these two species with other taxa differ according to different data sets (Fig. 2). The ITS phylogeny resolves *C. desmouliniana* in a clade with the typical variety of *C. umbellata*,[3] and with *C. hyalina* as sister to these two taxa together (76% and 100% BS, respectively; Fig. 2). In contrast, the *trnL-F* data place *C. desmouliniana* in a clade with *C. acuta*, *C. odontolepis*, and *C. umbellata* var. *reflexa* (94% BS; Fig. 2). These well-supported and discordant topologies are indicative of a hybridization event involving two groups of potential parental species. The first group includes *C. acuta*, *C. odontolepis*, or *C. umbellata* var. *reflexa* as a putative maternal progenitor and the other includes *C. umbellata* or *C. hyallina* as putative paternal progenitor. The present phylogenetic resolution does not permit us to choose among these different parental species within each paternal group with more precision. However, *C. desmouliniana* occurs only in the state of Sonora (Mexico) and adjacent areas. Given this relatively restricted distribution of the hybrid species, the involvements of either *C. acuta* or *C. hyalina* in reticulation seem unlikely, and thus the potential progenitors can probably be narrowed down to three taxa: *C. umbellata* on the paternal side and *C. odontolepis* or *C. umbellata* var. *reflexa* on the maternal side.

As part of our ongoing morphological and molecular investigations of *Cuscuta* for its treatments in the *Flora of North America* and the forthcoming revision of *The Jepson Manual*, we discovered several morphologically distinct specimens belonging to a previously undescribed species. Based on a combination of morphological and molecular data, we recognize these individuals as a new species and describe it here as *C. liliputana* sp. nov. (Fig. 4; see Taxonomic treatment). Morphologically, *C. liliputana* generally resembles *C. desmouliniana* and *C. leptantha* Engelm. (another species in the L clade; Fig. 2). However, the individuals belonging to *C. liliputana* also have several well-defined morphological features, and are easily distinguishable from its closest and most similar relatives. The most noticeable among these is the number of flower parts. Instead of five-parted flowers, usually found in *C. desmouliniana*, *C. liliputana* has predominantly four-parted and sometimes even three-parted flowers (Figs. 4*a* and 4*b*). Furthermore, *C. liliputana* has a cylindric calyx tube and more or less revolute calyx lobes, while *C. desmoulinana* has a campanulate calyx tube and flat lobes; also the former has larger flowers and seeds compared to the latter. With respect to the 4-merous flowers and its host preferences (*Chamaesyce*; Euphorbiaceae), *C. liliputana* is more similar to *C. leptantha* and *C. polyanthemos* W. Shaffn. ex Yunck., but *C. liliputana* never forms a clade with either species (Fig. 2). *Cuscuta liliputana* can be distinguished from these species through various floral features commonly used to differentiate *Cuscuta* species: e.g., the calyx tube equaling corolla tube (1/2–1/3 in *C. leptantha* and *C. polyanthemos*), infrastaminal scales and corolla tube ratios, and other more subtle characters. From a molecular phylogenetic standpoint, *C. liliputana* exhibits patterns of relationships with other species within the L clade identical to those described for *C. desmouliniana* (including similar support values; Fig. 2). Hence, this species also shows the same conflict between plastid and nuclear data, indicative of reticulate evolution, involving the same combination of putative progenitors and directionality. However, because of discontinuous and consistent morphological differences observed between *C. liliputana* and *C. desmouliniana*, we conclude that these are two separate and well-defined species. Two distinct evolutionary scenarios could account for these observations. We infer either two independent hybrid origins for these two species, each from the same (or similar) potential combination, or alternatively, a single hybrid origin followed by a speciation event. Due to the significant morphological differences observed between the two species, we favour the former hypothesis, but the evidence regarding these two alternatives remains equivocal.

No data are available in the literature regarding the chromosome numbers and ploidy levels for any of the species in the L clade. While selfing may serve as a preadaptation facilitating the prezygotic isolation from their respective progenitors and allowing the establishment of lineages after hybridization, the biogeographic and ecological (host) data are not as distinctive and supportive for the *C. desmouliniana*

[3] *Cuscuta umbellata* is not monophyletic. As traditionally circumscribed, this species has four varieties (Yuncker 1932). Two of those were sampled, the typical variety *C. umbellata* and *C. umbellata* var. *reflexa*, but were found as two distinct segregates within the L clade (Fig. 2; see also Stefanović et al. 2007). A new status and name have to be assigned to *C. umbellata* var. *reflexa*. However, these taxonomical issues are beyond the scope of this paper and will be dealt with elsewhere.

and *C. liliputana* cases as they were for *C. veatchii*. According to the presently available data, *C. desmouliniana* seems to be restricted to Sonora and Baja California (Mexico), and *C. liliputana* grows in southern New Mexico, Arizona, and southwest Texas (USA). While these two putative hybrid species appear to be distinct with respect to their distributions, the three potential parental taxa co-occur in the same general area, desert and semidesert regions across central portions of southwest USA and northern Mexico, and overlap with both hybrids. The hybrids appear to be host-restricted. *Cuscuta liliputana* parasitizes only on *Chamaesyce*, and *C. desmouliniana* grows primarily on *Chamaesyce*, but can be also encountered on *Boerhaavia* (Nyctaginaceae) and *Pectis* (Asteraceae). Among the potential progenitors, only *C. odontolepis* is host specific, and it is known to grow only on *Amaranthus* species. The other two putative parents, the typical *C. umbellata* and *C. umbellata* var. *reflexa*, are not host-specific and occur on a large number of herbaceous desert plants, primarily caryophyllids (e.g., *Alliona*, *Alternanthera*, *Amaranthus*, *Atriplex*, *Boerhavia*, *Portulacca*, *Polygonum*, *Salsola*, *Suaeda*, *Sesuvium*, *Tidestromia*, etc.), but also species of Zygophyllaceae (*Kallstroemia*), Crassulaceae (*Kalanchoe*), Convolvulaceae (*Evolvulus*), Solanaceae (*Chamaesaracha* and *Solanum*), and others. According to Yuncker (1932), their hosts also include *Euphorbia* [=*Chamaesyce*], but we have not encountered any of the putative parental species parasitizing on *Chamaesyce* in our herbarium surveys.

Despite the lack of decisive corroborative evidence from distribution and ecology, the evidence for hybrid origin(s) of *C. desmouliniana* and *C. liliputatana* from strongly supported gene tree discordances is significant. For example, in both of these two cases, the simplest topological distortion, NNI, cannot result in concordant plastid and nuclear phylogenies. The substantial differences of alternative tree topologies are further underlined by the results of the SH and AU tests. Out of eight tests conducted in total for these two cases (four for *C. desmouliniana* and four for *C. liliputana*), only those involving the plastid data set with the SH tests were found not to be significant (Table 2). Two factors can account for the lack of significance in these two particular cases. First, because the analyzed length was shorter and general variability lower for the *trnL-F* sequences than for ITS, the plastid matrix contains approximately only half the number of variable and parsimony informative sites compared with the nuclear matrix (Table 1). Second, only the SH test failed to show significance; the AU test returned significant *P*-values for both data sets, despite the short length differences between the optimal and constrained trees (Table 2). The SH test is known to be more conservative than the AU test (Goldman et al. 2000; Shimodaira 2002). In addition, given the number of species in the L clade and relative placements of *C. desmouliniana* and *C. liliputana*, lineage sorting does not seem to be a strong alternative either. The ancestral polymorphisms would have had to survive through a minimum of three speciation events, making this alternative to hybrid origin for each of these two species progressively less likely.

The C. bifurcata *case*

This species was described by Yuncker (1932) based only on two specimens from South Africa (Cape and KwaZulu-Natal Provinces). Owing to its indehiscent capsules and several other more subtle morphological features (capsule shape, infrastaminal scales, corolla shape, etc.), Yuncker (1932) placed this species in *Cuscuta* subg. *Grammica* sect. *Cleistogrammica* subsect. *Platycarpae*, together with species such as *C. obtusiflora*, *C. australis*, *C. polygonorum* Engelm., etc. Our plastid-derived phylogeny is in complete agreement with the traditional taxonomic placement of this species. The sole representative of *C. bifurcata* available for molecular studies was found nested within the B clade, with high support (98% BS; Fig. 3). The B clade, as defined by Stefanović et al. (2007) contains members of *Cuscuta* subg. *Grammica* sect. *Cleistogrammica* subsect. *Platycarpae*, nested within representatives of *Cuscuta* subg. *Grammica* sect. *Cleistogrammica* subsect. *Arvenses* (including *C. polygonorum*). Together, this whole group is characterized by depressed-globose capsules, with mostly short and subulate styles, and relatively large interstylar apertures (Costea et al. 2006). Furthermore, within the B clade, *C. bifurcata* is most closely related to *C. australis* and *C. obtusiflora*, and together these three species form a well-supported subclade (87% BS; Fig. 3). Some of these taxa span in their distribution multiple continents and represent some of the most frequently encountered dodders. The native distribution range of *C. australis* includes Asia, Australia, and Europe, while that of *C. obtusiflora* spans the entire western hemisphere. These are also the only two *Cuscuta* species potentially native to Oceania (Yuncker 1932).

The nuclear-derived phylogeny, however, supports quite a different evolutionary scenario for *C. bifurcata*. According to the ITS data, *C. bifurcata* is completely detached from the B clade and is instead found within the C clade (Figs. 1 and 3). The C clade was first identified by Stefanović et al. (2007) and, given its composition, came as one of the biggest surprises of that study. Species traditionally classified in up to five different subsections (Yuncker 1932) were found in this morphologically diverse clade. Within the C clade, ITS sequence of *C. bifurcata* was the most similar to a representative of a South American (Chilean) species, *C. werdermanii* (100% BS; Fig. 3). Although quite similar, the ITS sequences for these two species are not identical, thereby eliminating contamination as a possible explanation for these unexpected results (compare branch lengths in Fig. 2).

Taken together, these results are indicative of reticulate evolution. Given the overall concordance of plastid phylogeny with morphological features and, by extension, with numerous genes encoding those features, these results are consistent in particular with the introgression of (at least) nrDNA. Taking into account the number of strongly supported clades that would need to be dissolved to impose a nuclear-derived topology for *C. bifurcata* onto plastid data and vice versa, it does not come as a surprise that those alternatives were rejected by both the SH and AU tests (Table 2). Also, sorting of ancestral polymorphism across multiple clades with many speciation events is much less likely as an explanation for the observed discordance.

Two different evolutionary scenarios can be proposed to explain the existence and distribution of this hybrid taxon. The first scenario involves hybridization–introgression

between the two South American species, such as *C. obtusiflora* and *C. werdermanii*, followed by a long-distance dispersal of the hybrid species and its establishment in South Africa. Stefanović et al. (2007) concluded, based on overall phylogenetic relationships in *Cuscuta* subg. *Grammica*, that diversification through vicariance, as opposed to long-distance dispersal, emerged as the more dominant pattern for this group. Nevertheless, several striking cases of long-distance dispersal were inferred, some of which involved species from eastern Africa nested deeply within otherwise exclusively South American clade (e.g., *C. kilimanjari* within the O clade; Stefanović et al. 2007). The *C. bifurcata* case seems to represent one additional example of such long-distance dispersal, with the same directionality. This scenario would also imply a relatively recent event, which is supported by the small amount of observed differences in sequences between *C. bifurcata* and *C. werdermanii* (ITS) and *C. bifurcata* and *C. obtusiflora* (*trnL-F*; see phylograms in Fig. 2). An alternative would be the hybridization of a *C. werdermanii*-like paternal progenitor with a *C. australis*- or *C. obtusiflora*-like maternal progenitor before the break-up of Gondwana and separation of South America from Africa (~100–140 million years ago; Raven and Axelrod 1974; Scotes 2001; Jokat et al. 2003), followed by differential extinctions. This vicariance scenario is deemed less likely because it would imply not only that subgenus *Grammica*, but also *Cuscuta* as a whole, as well as Convolvulaceae, are much older than the oldest known microfossils attributed to this family (Lower Eocene, ~55–60 million years ago; Cronquist 1988). Also, the relatively small amounts of observed sequence differences among species involved are not consistent with this alternative.

Despite the search through copious amounts of *Cuscuta* specimens from several major South African herbaria (e.g., BOL, J, PRE), we were unable to find additional specimens of *C. bifurcata*. Hence, its current conservation status is unknown, but this remarkable taxon could be critically imperiled or possibly extinct.

The C. sandwichiana *case*

This species was placed by Yuncker (1932) in *Cuscuta* subg. *Grammica* sect. *Cleistogrammica* subsect. *Californicae* because it exhibits a reduction of the infrastaminal scales similar to the other species classified in this group (the A clade of Stefanović et al. 2007). However, in his later treatments, Yuncker (1965) omitted it without any explanation and, based on morphological characters, others questioned whether this species is allied to the *C. californica* complex (Beliz 1986; Costea et al. 2006). Owing to the particular growth and branching pattern encountered in *C. sandwichiana*, shared with species such as *C. pentagona* Engelm. and *C. campestris* Yunck., as well as additional similarities in flower and seed features, Costea et al. (2006) proposed closer evolutionary ties of this species with the *C. pentagona* complex (*Cuscuta* subg. *Grammica* sect. *Cleistogrammica* subsect. *Arvenses*; the B clade of Stefanović et al. 2007). According to *trnL-F* sequences, three individuals of *C. sandwichiana* sampled in our study are resolved as members of the B clade, with high support (98% BS; Figs. 1 and 3). All three representatives of this Hawaiian endemic form a well-supported and distinct lineage within the B clade, but its relationships with other members of this group, widely distributed throughout North America and beyond (e.g., *C. pentagona*, *C. campestris*, *C. australis*, *C. obtusiflora*), remain unresolved (Fig. 3).

In contrast to inferences from morphology and plastid data, our ITS sequences place *C. sandwichiana* as sister to the species of the H clade (Figs. 1 and 3). Bootstrap support for this sister-group relationship as well as for the monophyly of the H clade itself are both high (100% and 91% BS, respectively; Fig. 3). The H clade, as defined by Stefanović et al. (2007) consists of four species (*Cuscuta yucatana* Yunck., *Cuscuta potosina* W. Schaffn. ex S. Wats., *Cuscuta applanata* Engelm., and *Cuscuta chinensis* Lam.), sharing some common morphological features, such as calyx lobes with longitudinal protuberances and capsules surrounded at the base by the withered corolla. These characters are not encountered in *C. sandwichiana*. Also, unlike *C. sandwichiana*, most members of the H clade (except *C. yucatana*) have dehiscent fruits. Three species of this clade occur in Mexico or the southern USA, whereas *C. chinensis* is disjunct from the rest of this clade and is found in southeast Asia, Australia, and Africa (but not Hawaii).

This striking and strongly supported phylogenetic conflict is also consistent with reticulation involving a maternal progenitor from the B clade and a paternal progenitor from the H clade. As with the *C. bifurcata* case, the hybridization–introgression could have occurred in sympatry, probably somewhere in southwest North America where species from the B and H clades co-occur, followed by dispersal from the continent and establishment of a persistent hybrid lineage in Hawaii. However, the amount of differences accumulated for both *trnL-F* and ITS between *C. sandwichiana* individuals and representatives of its putative parental species is consistent with a relatively more ancient hybridization event. Long-distance dispersal between North America and Hawaii has been documented in other plant groups as well (e.g., Baldwin et al. 1991; Baldwin 1997). Imposing a nuclear-derived topology for *C. sandwichiana* onto plastid data and vice versa resulted in the most costly alternatives in terms of additional steps needed to accommodate them, and consequently were strongly rejected as alternatives (Table 2). Other explanations for the observed discordance, such as potential contaminations or lineage sorting, can also be excluded with confidence. Multiple individuals of *C. sandwichiana*, with independent DNA extractions, were used in this study, and yielded identical topological results, reinforcing each other. Taking into account the number of nodes through which it would have to persist, lineage sorting is also unlikely.

Alternative explanations for the observed plastid-nuclear discordance

In addition to hybridization, strongly conflicting gene trees can result from several other biological phenomena (e.g., Maddison 1997; Wendel and Doyle 1998). These include horizontal gene transfer (HGT; Kidwell 1993; Avise 2004), gene duplication followed by differential deletion (i.e., paralogy; Fitch 1970; Doyle 1992), and lineage sorting

(i.e., random sorting of ancestral polymorphism or "deep coalescence"; Avise 1986; Wu 1991; Doyle 1992).

Recently, plant mitochondrial (mt) genes have been shown to be transmitted horizontally across mating barriers at a surprisingly high rate (Won and Renner 2003; Bergthorsson et al. 2003, 2004; for a review, see Richardson and Palmer 2007 and references therein). Moreover, a disproportionately large number of the reported HGT events involve parasitic plants (Mower et al. 2004, Davis and Wurdack 2004; Nickrent et al. 2004; Davis et al. 2005), providing evidence for direct plant-to-plant transmission of DNA from parasite to host as one potential mechanism of HGT in plants. While some *Cuscuta* species were explicitly involved in one of those events of HGT (Mower et al. 2004), it is highly unlikely that any of the five instances of incongruence encountered in the present study could be explained by these means. The case involving HGT in *Cuscuta* spanned much deeper phylogenetic distances, from *Cuscuta* to the members of only remotely related genus *Plantago* (Plantaginaceae), too genetically distant to allow for hybridization as a potential explanation and it involved frequently horizontally transmitted mtDNA. In contrast, the discordances discussed here are at lower (i.e., species) phylogenetic levels, where hybridization can be expected to occur, and they involve ptDNA and nrDNA for which there are virtually no known cases of HGT in land plants despite extensive amounts of available data (Rice and Palmer 2006).

Paralogy is also unlikely to be the root cause of the striking pyhlogenetic discrepancies between plastid and nuclear phylogenies detected in our study. Except for the genes located in the inverted repeat, other genes from the haploid plastid genome exist only in a single copy (Palmer 1991). The *trnL-F* sequences used here are located in the large single-copy region of *Cuscuta* plastids (Funk et al. 2007; McNeal et al. 2007*a*) and hence are likely to be orthologous (i.e., related by direct descent only). In contrast, assessing the othology–paralogy for nrDNA can be more challenging. Although nrDNA is present in multiple copies in plants, it generally evolves in unison through the process of concerted evolution (Zimmer et al. 1980; Buckler et al. 1997). Nevertheless, paralogy in nrDNA repeats and the presence of multiple independent loci or pseudogenes could potentially lead to spurious phylogenetic reconstructions in some plant groups (Álvarez and Wendel 2003; Bailey et al. 2003; Feliner and Rosselló 2007). However, despite our intensive cloning efforts, ITS sequences from putative hybrids either were not different within a given species or showed very little polymorphism. When present, the paralogous sequences were most closely related to each other, consistent with either relatively recent duplication events or minor DNA polymerase errors rather than with the divergent ancestral paralogues (Stefanović et al. 2007).

Lineage sorting represents potentially the strongest alternative explanation for the observed topological discrepancies. Regardless, for the cases presented here we still favour hybridization for a number of reasons. First, in *Cuscuta*, as is the case for the majority of flowering plants, the ptDNA is maternally transmitted to the next generation (Corriveau and Coleman 1988; Reboud and Zeyl 1994; Mogensen 1996). Because the plastid genome is both uniparentally inherited and haploid, it has a significantly smaller effective population size when compared to nuclear loci (Moore 1995). Hence, the plastid haplotype tree has a substantially higher probability of more rapid coalescence time, leading to the relatively rapid elimination of any polymorphism. Second, like the majority of *Cuscuta* species (Yuncker 1932), the five species of putative hybrid origin included in our study have relatively narrow geographic distributions. Of these, *C. bifurcata* is the only species represented by a single individual because it is known only from its type locality. By contrast, each of the other four putative hybrids is represented by two to three individuals, spanning their respective distribution ranges. In these four cases, the *trnL-F* sequences were identical (or nearly so) among all the individuals included. Similarly, as discussed above, the clones of ITS sequences from putative hybrids either had no differences at all within a given species or showed very little polymorphism. Third, to account for discordance between the gene trees, the ancestral polymorphism would have had to persist through a minimum of three and up to six speciation events, depending on the case (with the exception of the *C. veatchii* case). Notwithstanding the genes under long-term balancing selection, such as the major histocompatibility complex genes in animals (e.g., Edwards et al. 1997; Garrigan and Hedrick 2003) or self-incompatibility genes in plants (e.g., Richman et al. 1996; Lu 2001), the survival of such a polymorphism, spanning multiple speciation events, is progressively more unlikely. The combination of these reasons, each of which is compelling individually, is inconsistent with the random sorting of ancestral polymorphism as a likely explanation for the observed topological discrepancies.

Although each of the three biological phenomena (HGT, undetected paralogy, and lineage sorting) invoked to explain the topological incongruences documented in our study is possible, these alternative hypotheses are more complex than the possibility of hybridization or introgression, and no corroborating evidence exists to support them. Phylogenetic analyses of additional, independently inherited sequence data, such as low-copy nuclear genes, as well as critically needed cytological information will help to resolve these outstanding questions. Overall, the results presented here provide strong initial evidence for an important role of hybridization in the evolution of the parasitic genus *Cuscuta*.

Frequency of hybridization in *Cuscuta*

In this study, we analyzed 105 species of *Cuscuta* subg. *Grammica*, representing over 75% of known diversity in this group (Yuncker 1932; 1965). A total of five of those species demonstrated strong evidence for discordance among gene regions between different genomes, interpreted here as resulting from hybridization events. Hence, the estimated rate of hybridization in *Cuscuta*, calculated from currently available data, is at about 5%. However, this frequency is likely to be an underestimation due to several factors.

First, there is a lack of resolution at the species level within some large and geographically widespread groups of *Cuscuta* (e.g., the D and O clades; Stefanović et al. 2007). Well-supported resolution of relationships among these numerous closely related species may point out presently "hidden" cases of relatively recent hybridization. Second, ancient hybridization events are difficult to detect owing to

the increased chance of fixation and loss of recognizable intermediacy through genetic drift (Rieseberg and Soltis 1991; Wendel and Doyle 1998; Sang and Zhong 2000). In addition, following a hybridization event, the two distinct sets of nrDNA arrays originating from paternal species may experience different fates following their merger in a single genome (Wendel 2000). Two of these evolutionary outcomes, the maintenance of both arrays in parallel or their recombination to various degrees into chimeric sequences (Álvarez and Wendel 2003), would leave behind a potentially recognizable signature of hybridization, and are therefore relatively easily detectable (e.g., Sang et al. 1995; Campbell et al. 1997; Barkman and Simpson 2002; Beardsley et al. 2004). The third outcome involves retention of one and the loss of the other parental nrDNA array as a consequence of concerted evolution mechanisms (e.g., Brochmann et al. 1996; Fuertes Aguilar et al. 1999). When the retention bias favours the paternal array, the nrDNA phylogeny can produce trees with a strong topological disagreement to those derived from maternally inherited organellar genes, and hence point out putative reticulation events. This evolutionary scenario is inferred to be the most likely for the five *Cuscuta* hybridization cases. However, if concerted evolution is biased toward the maternal nrDNA array, there will be no discrepancies with the organellar-derived phylogenies. In these cases, the nrDNA phylogeny alone will not be enough to invoke hybridization. Because there is no theoretical reason for concerted evolution to favour a priori one parental set of arrays over the other, the chance of fixation of one array and elimination of the other is essentially equal. We hypothesize, therefore, that the frequency of hybridization in *Cuscuta* is substantially higher than calculated from the evidence provided here. Further investigations, resulting in more resolved species-level relationships and including multiple low-copy nuclear genes, unlinked to the nrDNA, are necessary to test this prediction.

Taxonomic treatment

Cuscuta liliputana Costea & Stefanović, sp. nov.

TYPE: USA, New Mexico, Sierra County, 3 miles (ca. 5 km) out of Hillsboro, 5500 feet (1676 m a.s.l.), 9 September 1904, *O. B. Metcalfe 1290*.

HOLOTYPE: UNM.

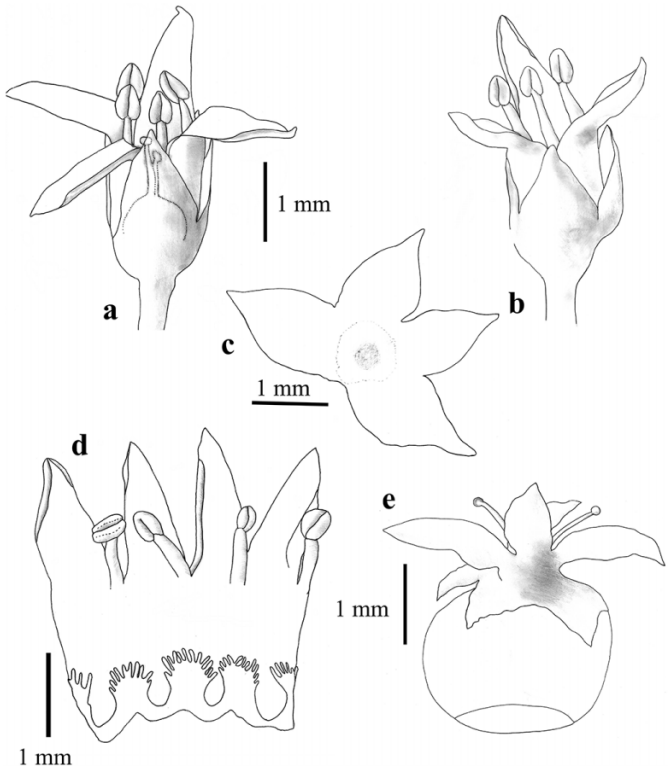
ISOTYPES: ARIZ, MO, NMC, NY, UNM, WLU, Figure 4.

Species haec, inter species subgeneris Grammica, *ad* C. desmouliniana *accedens, sed floribus (3–)4-merus et squamis infrastaminaribus valde brevibus ab ea differt. Pariter, species nova ad* C. leptantha *similis, sed calycibus tubis corollarum aequilongis et squamis infrastaminaribus valde brevibus praecipue differt*.

DESCRIPTION: STEMS slender, yellow to pale orange. INFLORESCENCES umbelliform cymes of (1–)2–11 flowers; bracts 1 at the base of clusters and 0–1 at the base of pedicels, 0.7–1 mm long, fleshy, ovate-lanceolate, margins entire, apex acute; pedicels (1–)2–3(–5) mm long. FLOWERS (3–)4-merous, 2.8–4 mm long, white-cream when fresh, cream when dried, fleshy, papillae usually present on pedicels, calyx and corolla; laticifers not visible or hardly so in the midveins of the corolla lobes, elongate; CALYX 1.3–1.7 mm, straw-yellow, somewhat reticulate and shiny, cylindric, equalling the corolla tube, divided ca. 3/4 the length, tube 0.3–0.7 mm long, lobes 1–1.35 mm long, ovate-triangular, not overlapping, apex acute to acuminate, margins entire; corolla white, 3–3.6 mm long, tube 1.5–2 mm long, cylindric; lobes 1.3–1.65 mm long, initially erect, later spreading and reflexed, lanceolate, margins entire, apex acute; epicuticular wax with a pattern of longitudinally reticulate rodlets; stamens exerted, shorter than corolla lobes, anthers broadly to narrow elliptic, 0.35–0.5 mm × 0.2–0.35 mm, filaments 0.5–0.8 mm long; POLLEN GRAINS 3-zonocolpate, prolate, 24–28 μm long, the tectum imperforatum or with a few puncta, the ornamentations granular-conical; INFRASTAMINAL SCALES truncate to slightly obovate, 1/4–1/3 of the corolla tube, 0.6–0.8 mm long, bridged at 0.1–0.2 mm, fimbriae 0.1–0.18 mm long; STYLES evenly filiform, 0.8–2.5 mm long, longer than the ovary; STIGMAS capitate, globose. CAPSULES circumscissile, 1.5–2.2 mm × 0.75–1.5 mm, globose to globose-depressed, thicken and slightly risen, or with 2–4 protuberances around the small interstylar aperture, translucent, capped by the withered corolla. SEEDS 2–4 per capsule, angled, subrotund to broadly elliptic, 0.8–1.15 mm × 0.7–0.85 mm, seed coat cells alveolate–papillate; hilum suterminal, hillum area 0.15–0.18 mm in diameter, vascular scar linear, oblique to vertical, 0.025–0.03 mm long.

ETYMOLOGY: the specific epithet alludes to the small size of this plant and its flowers (deliberately modified from Lilliput, one of the imaginary countries in "Gulliver's Travels" by Jonathan Swift).

Fig. 4. *Cuscuta liliputana* Costea & Stefanović, sp. nov. (*a*) Typical tetramerous flower; (*b*) trimerous flower; (*c*) dissected calyx; (*d*) dissected corolla showing infrastaminal scales and stamens; (*e*) maturing capsule capped by a persistent corolla. All drawings are from the holotype (*Metcalfe 1290*, UNM).



DISTRIBUTION, HABITAT, AND PHENOLOGY: southern New Mexico, Arizona, and southwest Texas. The species is also likely to occur in some of the adjacent Mexican territory. It parasitizes *Chamaesyce* (Euphorbiaceae) species that grow in disturbed places of desert wash, in sand and fine gravel; it was collected at 1250–1680 m a.s.l. in New Mexico, 730 m a.s.l. in Arizona, and only 30 m a.s.l. in Texas. Flowering July–November; November–February. Compared with other *Cuscuta* species in the area, it is less common, and it may require conservation measures; therefore, a G2–G3 (Imperiled–Vulnerable) NatureServe (2006) conservation status is proposed.

COLLECTIONS EXAMINED: USA. Arizona, Pima County, ca. 15 miles (ca. 24 km) southeast of Tucson, along Haughton Road., 1 mile (ca. 1.6 km) north of I-10, 731 m a.s.l., *Larrea–Palo verde* community, 20 October 1982, *Neese s. n.* (NY) [SEM + DNA accession]. New Mexico, De Baca County, Hwy 20, just south of Conejo Creek, ca. 24 miles (38.6 km) southwest of Fort Sumner, T1S R24E Sec 29 Ne1/4, 1250 m a.s.l., 26 September 2002, *Sivinski 5689* (NMC, NY, TEX) [SEM + DNA accessions from NY and NMC]; Doña Ana County, White Sands Missile Range (WSMR), 29 km north-northeast of las Cruces, 3 km south of US Hwy 70, on entrance road to WSMR headquarters area, disturbed roadside, west edge of Section 7, T22S, RSE; UTM 360900E, 3586500W, 1300 m a.s.l., 27 August 1990, *Spellenberg & Brozka 10526* (NMC, ID, UC) [SEM from NMC]; Sierra County, 3 miles (ca. 5 km) south of Hillsboro, 1680 m a.s.l., 9 September 1904, *O. B. Metcalfe 1290* (ARIZ, MO, NY, SD, UNM, WLU) [SEM + DNA accession from NY]. Texas, Hidalgo County, low ground about 4.5 miles (ca. 7 km) S of San Juan, 9 Feb 1969, *Correll 36759* (TEX).

Acknowledgements

The authors warmly thank Alison Colwell, Tom Van Devender, Thierry Deroin, Ignacio García, Miguel García, and David Tank, as well as the curators/directors of the herbaria ALTA, ARIZ, ASU, BOL, CANB, CHR, CIIDIR, CTES, DAO, F, GH, IND, JEPS, LL, LP, MEL, MEXU, MICH, NMC, NY, OKLA, PACA, PRE, QCNE, QFA, RSA, SGO, TEX, UBC, UNB, UNM, UPRRP, US, USAS, WTU, and XAL for supplying plant material. We are indebted to Maria Kuzmina for technical support in generating sequences. Critical comments from Dave Tank, Dick Olmstead, and Yuan Yaowu, as well as Rodger Evans and an anonymous reviewer, further improved the presentation of our results. This work was supported by Natural Sciences and Engineering Research Council of Canada Discovery grants to S. Stefanović (326439-06) and M. Costea (327013-06), as well as University of Toronto Connaught Grants to S. Stefanović.

References

Álvarez, I., and Wendel, J.F. 2003. Ribosomal ITS sequences and plant phylogenetic inference. Mol. Phylogenet. Evol. **29**: 417–434. doi:10.1016/S1055-7903(03)00208-2. PMID:14615184.

Amico, G.C., Vidal-Russell, R., and Nickrent, D.L. 2007. Phylogenetic relationships and ecological speciation in the mistletoe *Tristerix* (Loranthaceae): the influence of pollinators, dispersers, and hosts. Am. J. Bot. **94**: 558–567. doi:10.3732/ajb.94.4.558.

Arnold, M.L. 1992. Natural hybridization as an evolutionary process. Annu. Rev. Ecol. Syst. **23**: 237–261. doi:10.1146/annurev.es.23.110192.001321.

Avise, J.C. 1986. Mitochondial DNA and evolutionary genetics of higher animals. Philos. Trans. R. Soc. Lond. B Biol. Sci. **312**: 325–342. doi:10.1098/rstb.1986.0011. PMID:2870525.

Avise, J.C. 2004. Molecular markers, natural history, and evolution. 2nd ed. Sinauer, Sunderland, Mass.

Bailey, C.D., Carr, T.G., Harris, S.A., and Hughes, C.E. 2003. Characterization of angiosperm nrDNA polymorphism, paralogy, and pseudogenes. Mol. Phylogenet. Evol. **29**: 435–455. doi:10.1016/j.ympev.2003.08.021. PMID:14615185.

Baldwin, B.G. 1997. Adaptive radiation of the Hawaiian silversword alliance: congruence and conflict of phylogenetic evidence from molecular and non-molecular investigations. *In* Molecular evolution and adaptive radiation. *Edited by* T.J. Givnish and K.J. Sytsma. Cambridge University Press, Cambridge, UK. pp. 103–128.

Baldwin, B.G., Kyhos, D.W., Dvorak, J., and Carr, G.D. 1991. Chloroplast DNA evidence for a North American origin of the Hawaiian silversword alliance (Asteraceae). Proc. Natl. Acad. Sci. U.S.A. **88**: 1840–1843. doi:10.1073/pnas.88.5.1840. PMID:11607157.

Barber, J.C., Finch, C.C., Francisco-Ortega, J., Santos-Guerra, A., and Jansen, R.K. 2007. Hybridization in Macaronesian *Sideritis* (Lamiaceae): evidence from incongruence of multiple independent nuclear and chloroplast sequence datasets. Taxon, **56**: 74–88.

Barkman, T.J., and Simpson, B.B. 2002. Hybrid origin and parentage of *Dendrochilum acuiferum* (Orchidaceae) inferred in a phylogenetic context using nuclear and plastid DNA sequence data. Syst. Bot. **27**: 209–220.

Beardsley, P.M., Schoening, S.E., Whittall, J.B., and Olmstead, R.G. 2004. Patterns of evolution in western North American *Mimulus* (Phrymaceae). Am. J. Bot. **91**: 474–489. doi:10.3732/ajb.91.3.474.

Beliz, T. 1986. A revision of *Cuscuta* section *Cleistogrammica* using phenetic and cladistic analyses with a comparison of reproductive mechanisms and host preferences in species from California, Mexico, and Central America. Ph.D. thesis, University of California, Berkeley, Calif.

Bergthorsson, U., Adams, K.L., Thomason, B., and Palmer, J.D. 2003. Widespread horizontal transfer of mitochondrial genes in flowering plants. Nature (London), **424**: 197–201. doi:10.1038/nature01743. PMID:12853958.

Bergthorsson, U., Richardson, A.O., Young, G.J., Goertzen, L.R., and Palmer, J.D. 2004. Massive horizontal transfer of mitochondrial genes from diverse land plant donors to the basal angiosperm *Amborella.* Proc. Natl. Acad. Sci. U.S.A. **101**: 17747–17752. doi:10.1073/pnas.0408336102. PMID:15598737.

Bernhardt, P., and Calder, D.M. 1981. Hybridization between *Amyema pendulum* and *Amyema quandang* (Loranthaceae). Bull. Torrey Bot. Club, **108**: 456–466. doi:10.2307/2484446.

Brochmann, C., Nilsson, T., and Gabrielsen, T.M. 1996. A classic example of postglacial allopolyploid speciation re-examined using RAPD markers and nucleotide sequences: *Saxifraga osoensis* (Saxifragaceae). Symb. Bot. Ups. **31**: 75–89.

Buckler, E.S., Ippolito, A., and Holtsford, T.P. 1997. The evolution of ribosomal DNA: divergent paralogues and phylogenetic implications. Genetics, **145**: 821–832. PMID:9055091.

Campbell, C.S., Wojciechowski, M.F., Baldwin, B.G., Alice, L.A., and Donoghue, M.J. 1997. Persistent nuclear ribosomal DNA se-

quence polymorphism in the *Amelanchier* agamic complex (Rosaceae). Mol. Biol. Evol. **14**: 81–90. PMID:9000756.

Corriveau, J.L., and Coleman, A.W. 1988. Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. Am. J. Bot. **75**: 1443–1458. doi:10.2307/2444695.

Costea, M., and Tardif, J.F. 2006. Biology of Canadian weeds. *Cuscuta campestris* Yuncker, *C. gronovii* Willd. ex Schult., *C. umbrosa* Beyr. ex Hook., *C. epithymum* (L.) L. and *C. epilinum* Weihe. Can. J. Pl. Sci. **86**: 293–316.

Costea, M., Nesom, G.L., and Tardif, J.F. 2005. Taxonomic status of *Cuscuta nevadensis* and *C. veatchii* (Convolvulaceae). Brittonia, **57**: 264–272. doi:10.1663/0007-196X(2005)057[0264:TSOCNA]2.0.CO;2.

Costea, M., Nesom, G.L., and Stefanović, S. 2006. Taxonomy of the *Cuscuta pentagona* complex (Convolvulaceae) in North America. Sida, **22**: 151–175.

Coyne, J.A., and Orr, H.A. 2004. Speciation. Sinauer, Sunderland, Mass.

Cronquist, A. 1988. The evolution and classification of flowering plants. The New York Botanical Garden, Bronx, N.Y.

Davis, C.C., and Wurdack, K.J. 2004. Host-to-parasite gene transfer in flowering plants: phylogenetic evidence from malpighiales. Science (Wash.), **305**: 676–678. doi:10.1126/science.1100671. PMID:15256617.

Davis, C.C., Anderson, W.R., and Wurdack, K.J. 2005. Gene transfer from a parasitic flowering plant to a fern. Proc. R. Soc. Lond. B Biol. Sci. **272**: 2237–2242. doi:10.1098/rspb.2005.3226.

Dawson, J.H., Musselman, L.J., Wolswinkel, P., and Dörr, I. 1994. Biology and control of *Cuscuta.* Rev. Weed Sci. **6**: 265–317.

DeBry, R.W., and Olmstead, R.G. 2000. A simulation study of reduced tree-search effort in bootstrap resampling analysis. Syst. Biol. **49**: 171–179. doi:10.1080/10635150050207465. PMID:12116479.

Doyle, J.J. 1992. Gene trees and species trees: molecular systematics as one-character taxonomy. Syst. Bot. **17**: 144–163. doi:10.2307/2419070.

Edwards, S.V., Chesnut, K., Satta, Y., and Wakeland, E.K. 1997. Ancestral polymorphism of Mhc class II genes in mice: implications for balancing selection and the mammalian molecular clock. Genetics, **146**: 655–668. PMID:9178014.

Engelmann, G. 1859. Systematic arrangement of the species of the genus *Cuscuta* with critical remarks on old species and descriptions of new ones. Trans. Acad. Sci., St. Louis **1:** 453–523.

Feliner, G.N., and Rosselló, J.A. 2007. Better the devil you know? Guidelines for insightful utilization of nrDNA ITS in species-level evolutionary studies in plants. Mol. Phylogenet. Evol. **44**: 911–919. doi:10.1016/j.ympev.2007.01.013. PMID:17383902.

Felsenstein, J. 1978. Case in which parsimony or compatibility methods will be positively misleading. Syst. Zool. **27**: 401–410. doi:10.2307/2412923.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, **39**: 783–791. doi:10.2307/2408678.

Fitch, W.M. 1970. Distinguishing homologous from analogous protein. Syst. Zool. **19**: 99–113. doi:10.2307/2412448. PMID:5449325.

Fuertes Aguilar, J., Rosselló, J.A., and Nieto Feliner, G. 1999. Nuclear ribosomal DNA (nrDNA) concerted eolution in natural and artificial hybrids of *Armeria* (Plumbaginaceae). Mol. Ecol. **8**: 1341–1346. doi:10.1046/j.1365-294X.1999.00690.x. PMID:10447874.

Funk, H.T., Berg, S., Krupinska, K., Maier, U.G., and Krause, K. 2007. Complete DNA sequences of the plastid genomes of two parasitic flowering plant species, *Cuscuta reflexa* and *Cuscuta gronovii.* BMC Plant Biol. **7**: 45. doi:10.1186/1471-2229-7-45. PMID:17714582.

García, M.A., and Castroviejo, S. 2003. Estudios citotaxónomicos en las especies ibéricas del género *Cuscuta* (Convolvulaceae). Anales Jard. Bot. Madrid, **60**: 33–44.

García, M.A., and Martín, M.P. 2007. Phylogeny of *Cuscuta* subgenus *Cuscuta* (Convolvulaceae) based on nrDNA ITS and chloroplast *trnL* intron sequences. Syst. Bot. **32**: 899–916.

Garrigan, D., and Hedrick, P.W. 2003. Perspective: detecting adaptive molecular polymorphism: lessons from the MHC. Evolution, **57**: 1707–1722. PMID:14503614.

Goldman, N., Anderson, J.P., and Rodrigo, A.G. 2000. Likelihood-based tests of topologies in phylogenetics. Syst. Biol. **49**: 652–670. doi:10.1080/106351500750049752. PMID:12116432.

Grant, V. 1981. Plant speciation. 2nd ed. Columbia Univ. Press, New York, N.Y.

Harbaugh, D., and Baldwin, B.G. 2007. Phylogeny and biogeography of the sandalwoods (*Santalum*, Santalaceae): repeated dispersals throughout the Pacific. Am. J. Bot. **94**: 1028–1040. doi:10.3732/ajb.94.6.1028.

Husband, B. C. 1998. Constraints on polyploid evolution: a test of the minority cytotype exclusion principle. Proc. Royal Soc. Biol. Sci. Lond. **267:** 217–223.

Jokat, W., Boebel, T., König, M., and Meyer, U. 2003. Timing and geometry of early Gondwana breakup. J. Geophys. Res. **108**(B9): 2428. doi:10.1029/2002JB001802.

Kidwell, M.G. 1993. Lateral transfer in natural populations of eukaryotes. Annu. Rev. Genet. **27**: 235–256. doi:10.1146/annurev.ge.27.120193.001315. PMID:8122903.

Kishino, H., and Hasegawa, M. 1989. Evaluation of the maximum-likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. J. Mol. Evol. **29**: 170–179. doi:10.1007/BF02100115. PMID:2509717.

Lee, K.B., and Lee, C.D. 1989. The structure and development of the haustorium in *Cuscuta australis.* Can. J. Bot. **67**: 2975–2982. doi:10.1139/b89-381.

Levin, D.A. 1975. Minority cytotype exclusion in local plant populations. Taxon, **24**: 35–43. doi:10.2307/1218997.

Lu, Y. 2001. Roles of lineage sorting and phylogenetic relationship in the genetic diversity at the self-incompatibility locus of Solanaceae. Heredity, **86**: 195–205. doi:10.1046/j.1365-2540.2001.00823.x. PMID:11380665.

Mabberley, D.J. 1987. The plant book. Cambridge Uniersity Press, Cambridge, UK.

Maddison, D.R., and Maddison, W.P. 2003. MacClade: analysis of phylogeny and character evolution, version 4.06. Sinauer, Sunderland, Mass.

Maddison, W.P. 1997. Gene trees in species trees. Syst. Biol. **46**: 523–536. doi:10.2307/2413694.

McNeal, J.R., Kuehl, J.V., Boore, J.L., and dePamphilis, C.W. 2007*a*. Complete plastid genome sequences suggest strong selection for retention of photosynthetic genes in the parasitic plant genus *Cuscuta.* BMC Plant Biol. **7**: 57. doi:10.1186/1471-2229-7-57. PMID:17956636.

McNeal, J.R., Arumugunathan, K., Kuehl, J.V., Boore, J.L., and de Pamphilis, C.W. 2007*b*. Systematics and plastid genome evolution of the cryptically photosynthetic parasitic plant genus *Cuscuta* (Convolvulaceae). BMC Biol. **5**: 55. doi:10.1186/1741-7007-5-55. PMID:18078516.

Mogensen, H.L. 1996. The hows and whys of cytoplasmic inheritance in seed plants. Am. J. Bot. **83**: 383–404. doi:10.2307/2446172.

Moore, W.S. 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. Evolution, **49**: 718–726. doi:10.2307/2410325.

Mower, J.P., Stefanović, S., Young, G.J., and Palmer, J.D. 2004. Gene transfer from parasitic to host plants. Nature (London), **432**: 165–166. doi:10.1038/432165b. PMID:15538356.

NatureServe. 2006. NatureServe Explorer: An online encyclopedia of life [web application]. Version 6.1. NatureServe, Arlington, Virginia. Available from www.natureserve.org/explorer [accessed: 17 May 2007].

Nickrent, D.L., Blarer, A., Qiu, Y.L., Vidal-Russell, R., and Anderson, F.E. 2004. Phylogenetic inference in Rafflesiales: the influence of rate heterogeneity and horizontal gene transfer. BMC Evol. Biol. **4**: 40. doi:10.1186/1471-2148-4-40. PMID: 15496229.

Palmer, J.D. 1991. Plastid chromosomes: structure and evolution. *In* Cell culture and somatic cell genetics of plants, vol. 7A. *Edited by* L. Bogorad, and I.K. Vasil. Academic Press, San Diego, Calif. pp. 5–53.

Peter, A. 1897. Convolvulaceae. *In* Die Natürlichen Pflanzenfamilien. *Edited by* A. Engler and K. Prantl. **4:** 375–377 (Appendix).

Posada, D., and Crandall, K.A. 1998. ModelTest: testing the model of DNA substitution, v.3.7. Bioinformatics, **14**: 817–818. doi:10.1093/bioinformatics/14.9.817. PMID:9918953.

Prather, L.A. 1990. The biology of *Cuscuta attenuata* Waterfall. M.Sc. thesis, Department of Botany, Oklahoma State University, Stillwater, Okla.

Prather, L.A., and Tyrl, R.J. 1993. The biology of *Cuscuta attenuata* Waterfall (Cuscutaceae). Proc. Okla. Acad. Sci. **73**: 7–13.

Rambaut, A. 2002. Se-Al sequence alignment editor, version 2.0a11. University of Oxford, Oxford, UK.

Ramsey, J., and Schemske, D.W. 2002. Neopolyploidy in flowering plants. Annu. Rev. Ecol. Syst. **33**: 589–639. doi:10.1146/annurev.ecolsys.33.010802.150437.

Raven, P.H., and Axelrod, D.I. 1974. Angiosperm biogeography and past continental movements. Ann. Mo. Bot. Gard. **61**: 539–673. doi:10.2307/2395021.

Reboud, X., and Zeyl, C. 1994. Organelle inheritance in plants. Heredity, **72**: 132–140. doi:10.1038/hdy.1994.19.

Rice, D.W., and Palmer, J.D. 2006. An exceptional horizontal gene transfer in plastids: gene replacement by a distant bacterial paralog and evidence that haptophyte and cryptophyte plastids are sisters. BMC Biol. **4**: 31. doi:10.1186/1741-7007-4-31. PMID: 16956407.

Richardson, A.O., and Palmer, J.D. 2007. Horizontal gene transfer in plants. J. Exp. Bot. **58**: 1–9. doi:10.1093/jxb/erl148. PMID:17030541.

Richman, A.D., Uyenoyama, M.K., and Kohn, J.R. 1996. Allelic diversity and gene genealogy at the self-incompatibility locus in the Solanaceae. Science (Wash.), **273**: 1212–1216. doi:10.1126/science.273.5279.1212. PMID:8703052.

Rieseberg, L.H. 1995. The role of hybridization in evolution: old wine in new skins. Am. J. Bot. **82**: 944–953. doi:10.2307/2445981.

Rieseberg, L.H. 1997. Hybrid origin of plant species. Annu. Rev. Ecol. Syst. **28**: 359–389. doi:10.1146/annurev.ecolsys.28.1.359.

Rieseberg, L.H., and Soltis, D.E. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. Evol. Trends Plants, **6**: 65–84.

Rieseberg, L.H., Beckstrom-Sternerg, S., and Doan, K. 1990. *Helianthus annuus* ssp. *texanus* has chloroplast DNA and nuclear ribosomal RNA genes of *Helianthus debilis* ssp. *cucumerifolius.* Proc. Natl. Acad. Sci. U.S.A. **87**: 593–597. doi:10.1073/pnas.87.2.593. PMID:11607056.

Sang, T., and Zhong, Y. 2000. Testing hybridization hypotheses based on incongruent gene trees. Syst. Biol. **49**: 422–434. doi:10.1080/10635159950127321. PMID:12116420.

Sang, T., Crawford, D.J., and Stussey, T.F. 1995. Documentation of reticulate evolution in peonies (*Paeonia*) using internal transcribed spacer sequences of nuclear ribosomal DNA: implications for biogeography and concerted evolution. Proc. Natl. Acad. Sci. U.S.A. **92**: 6813–6817. doi:10.1073/pnas.92.15.6813. PMID:7624325.

Scotes, C.R. 2001. Atlas of earth history. Vol. 1., Paleography. PALEOMAP project, Arlingtom, Tx.

Shimodaira, H. 2002. An approximately unbiased test of phylogenetic tree selection. Syst. Biol. **51**: 492–508. doi:10.1080/10635150290069913. PMID:12079646.

Shimodaira, H., and Hasegawa, M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inferrence. Mol. Biol. Evol. **16**: 1114–1116.

Shimodaira, H., and Hasegawa, M. 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. Bioinformatics, **17**: 1246–1247. doi:10.1093/bioinformatics/17.12.1246. PMID: 11751242.

Soltis, D.E., and Soltis, P.S. 1993. Molecular data and the dynamic nature of polyploidy. Crit. Rev. Plant Sci. **12**: 243–273. doi:10.1080/713608048.

Stebbins, G.L. 1959. The role of hybridization in evolution. Proc. Am. Philos. Soc. **103**: 231–251.

Stefanović, S., and Olmstead, R.G. 2005. Down the slippery slope: plastid genome evolution in Convolvulaceae. J. Mol. Evol. **61**: 292–305. doi:10.1007/s00239-004-0267-5. PMID:15999247.

Stefanović, S., Kuzmina, M., and Costea, M. 2007. Delimitation of major lineages within *Cuscuta* subgenus *Grammica* (Convolvulaceae) using plastid and nuclear DNA sequences. Am. J. Bot. **94**: 568–589. doi:10.3732/ajb.94.4.568.

Subramaniam, K., and Mahadevan, S. 1994. The cDNA sequence of cytochrome b5 associated with cytokinin-induced haustoria formation in *Cuscuta reflexa.* Gene, **149**: 375–376. doi:10.1016/0378-1119(94)90180-5. PMID:7959021.

Swofford, D.L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4.0b2a. Sinauer, Sunderland, Mass.

Thomson, W.A. 1949. A natural hybrid of *Loranthus micranthus* and *Tupeia antarctica.* Trans. R. Soc. N. Z. **77**: 208.

Wendel, J.F. 2000. Genome evolution in polyploids. Plant Mol. Biol. **42**: 225–249. doi:10.1023/A:1006392424384. PMID: 10688139.

Wendel, J.F., and Doyle, J.J. 1998. Phylogenetoc incongruence: window into genome history and molecular evolution. *In* Molecular plant systematics II. *Edited by* D.E. Soltis, P.S. Soltis, and J.J. Dolye. Kluwer, Boston, Mass. pp. 265–296.

Wendel, J.F., Schnabel, A., and Seelanan, T. 1995. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). Proc. Natl. Acad. Sci. U.S.A. **92**: 280–284. doi:10.1073/pnas.92.1.280. PMID:7816833.

Wolfe, A.D., Xiang, Q.-Y., and Kephart, S.R. 1998. Diploid hybrid speciation in *Penstemon* (Scrophulariaceae). Proc. Natl. Acad. Sci. U.S.A. **95**: 5112–5115. doi:10.1073/pnas.95.9.5112. PMID: 9560237.

Won, H., and Renner, S.S. 2003. Horizontal gene transfer from flowering plant to *Gnetum.* Proc. Natl. Acad. Sci. U.S.A. **100**: 10824–10829. doi:10.1073/pnas.1833775100. PMID: 12963817.

Wu, C.I. 1991. Inferences of species phyogeny in relation to segregation of ancestral polymorphism. Genetics, **127**: 429–435. PMID:2004713.

Yang, Z. 1994. Estimating the pattern of nucleotide substitution. J. Mol. Evol. **39**: 105–111. PMID:8064867.

Yuncker, T.G. 1932. The genus *Cuscuta.* Mem. Torrey Bot. Club, **18**: 113–331.

Yuncker, T.G. 1943. Nomenclatural changes in the genus *Cuscuta* and notes on some American species. Bull. Torrey Bot. Club, **70**: 61–67. doi:10.2307/2481684.

Yuncker, T. G. 1965. *Cuscuta*. N. Am. Fl., ser. II **4:** 1–51.

Zimmer, E.A., Martin, S.L., Beverley, S.M., Kan, Y.W., and Wilson, A.C. 1980. Rapid duplication and loss of genes coding for the alpha-chains of hemoglobin. Proc. Natl. Acad. Sci. U.S.A. **77**: 2158–2162. doi:10.1073/pnas.77.4.2158. PMID:6929543.

Appendix A

Taxa, DNA accession numbers, sources of plant material from which DNA was extracted, and GenBank accession numbers for sequences used in this study. Letters A–O correspond to major clades as they are labeled in Figs. 1-3. Extraction numbers (in bold) following species names are indicated on the phylogenetic trees. Abbreviations of herbaria in which the vouchers are deposited follow Index Herbariorum. N/a, not applicable – indicates accessions where plastid and nuclear data are found in different major clades (e.g., clades B and C or clades B and H). A dash indicates missing data. Order is as follows: clade, species name, and authority; DNA accessions (number); voucher information (herbaria); *trnL-F*, nrITS. **A.** *Cuscuta californica* Choisy: **147**, *Stefanović SS-98-59* (TRTE), EF194486, EF194696; **499**, *Ahart 9856* (JEPS), EF194487, EF194697; **500**, *Boyd 9839* (JEPS), EF194478, —; **637**, *Pinzl 7238a* (NY), EF194475, EF194688; **645**, *Ahart 2971* (NY), EF194488, EF194698; **669**, *White 5033* (ASU), EF194479, EF194691. *Cuscuta californica* Choisy var. *brachycalyx* Yunck.: **472**, *Stefanović SS-04-140/AC-04-31* (TRTE), EF194484, EF194699; **643**, *Colwell AC 04-305* (YM/WLU), EF194485, EF194700; **418**, *Stefanović SS-00-59* (TRTE), EF194480, EF194692. *Cuscuta decipiens* Yunck.: **458**, *Tharp 46072* (IND), EF194508, —; **981**, *Henrickson 13394* (MEXU), EF194509, —; **1014**, *Henrickson 22781* (TEX), EF194510, EF194718. *Cuscuta howelliana* Rubtzoff: **357**, *Tank s. n.*; no voucher, EF194506, EF194716; **654**, *Oswald & Ahart 7978* (JEPS), EF194504, —; **655**, *Ahart 8044* (JEPS), EF194507, EF194717; **656**, *Reino & Alava 6809* (JEPS), EF194505, EF194715. *Cuscuta occidentalis* Millsp.: **503**, *Ertter 7326* (NY), EF194477, EF194690; **504**, *Tiehm 12257* (NY), EF194481, EF194693; **647**, *Tiehm 14108* (NY), EF194482, EF194694; **648**, *Schoolcraft et al. 2220* (NY), EF194483, EF194695. *Cuscuta occidentalis/ californica*: **646**, *Ahart 9116* (JEPS), EF194476, EF194689. *Cuscuta salina* Engelm. var. *major* Yunck.: **146**, *Dudley s. n.* (WTU), EF194497, —; **502**, *Standley 777* (NY), EF194499, EF194710; **642**, *Halse 4961* (NY), EF194498, EF194709; **651**, *Kennedy & Ganders 4947* (UBC), EF194500, EF194711. *Cuscuta salina* Engelm. var. *salina*: **477**, *Tiehm 12744* (ASU), EF194492, EF194704; **478**, *Tiehm 13405* (ASU), EF194493, EF194705; **641**, *Tiehm & Bair 12744* (GH), EF194494, EF194706; **652**, *Hammond 10349* (NY), EF194495, EF194707; **653**, *Felger & Fenn s. n.* (NY), EF194496, EF194708. *Cuscuta subinclusa* Durand & Hilg.: **197**, *Munz & Balls 17942* (WTU), EF194489, EF194703; **501**, *Raz & Boyd 15* (NY), EF194491, EF194701; **644**, *Anderson 3248* (NY), EF194490, EF194702. *Cuscuta suksdorfii* Yunck.: **470**, *Colwell AC-04-159*; (YM/TRTE), EF194503, EF194714; **635**, *Ahart 9885* (JEPS), EF194501, EF194712; **636**, *Ahart 3949* (JEPS), EF194502, EF194713.

B. *Cuscuta australis* Hook. f.: **547**, *Sykes 99* (CHR), EF194457, EF194667; **679**, *Hosking 938* (CANB), EF194458, EF194668; **789**, *Beaughlehole 83203* (MEL), —, EF194669; **792**, *Curtis 124* (MEL), —, EF194670; *C. australis* Hook. f. var. *tinei* (Ins.) Yunck.: **639**, *Thiebaut 3098* (NY), EF194460, EF194671; **640**, *Simonkoi 2635* (NY), EF194459, EF194672. *Cuscuta bifurcata* Yunck.: **1036**, *Paterson 578* (PRE), EF194461, n/a. *Cuscuta campestris* Yunck.: **202**, *Ownboy s. n.* (WTU), EF194451, EF194665; **411**, *Stefanović SS-03-103* (TRTE), EF194450, EF194663; **415**, *Solomon 17192* (IND), EF194455, EF194677/ EF194680; **483**, *Pitzer 3765* (ASU), EF194453, EF194661; **487**, *Baker & Wright 11575-1* (ASU), EF194452, EF194659; **894**, *Alava 11039* (RSA), EF194454, EF194660. *Cuscuta glabrior* (Engelm.) Yunck.: **596**, *Palmer 723* (GH), EF194470, EF194684; **742**, *Cory 42164* (NY), EF194471, EF194685; **825**, *Villarreal & Vasquez 6154* (XAL), EF194472, EF194686. *Cuscuta gymnocarpa* Engelm.: **1017**, *Mears & Andersen 5288* (TEX), EF194456, EF194666. *Cuscuta harperi* Small: **594**, *Demaree 46295* (NY), EF194464, EF194681. *Cuscuta obtusiflora* H.B.&K.: **1047**, *Pedersen 3688* (US), —, EF194673; **1069**, *Skolnik & Barkley 19ANL23* (US), EF194463, EF194674. *Cuscuta obtusiflora* H.B. & K. var. *glandulosa* Engelm.: **746**, *Mitchell 3387* (NY), EF194462, EF194675; **747**, *Lundell & Lundell 11717* (NY), —, EF194676. *Cuscuta pentagona* Engelm.: **456**, *Lakela 26019* (IND), EF194465, EF194678/ EF194664; **464**, *Taylor 5765* (IND), EF194467, EF194679; **468**, *Deam 62612* (IND), EU288331, EU288348; **667**, *Fosberg 59604* (CHR), EU288332, —. *Cuscuta plattensis* A. Nelson, **590**, *Dorn 5470* (NY), EF194468, EF194682. *Cuscuta runyonii* Yunck.: **660**, *Flyr 368* (TEX/LL), EF194469, EF194683. *Cuscuta sandwichiana* Choisy: **155**, *Degener & Degener 36596* (WTU), EU288333, n/a; **686**, *Degener & Degener 35248A* (CANB), EU288334, n/a; **748**, *Sylva & Rumel s. n.* (NY), EU288335, n/a. *Cuscuta stenolepis* Engelm.: **779**, *Ollgaard 99142* (QCNE), EF194473, EF194687; **781**, *Nunez et al. 034* (QCNE), EF194474, —.

C. *Cuscuta bifurcata* Yunck.: **1036**, *Paterson 578* (PRE), n/a, EU288349. *Cuscuta corniculata* Engelm.: **933**, *Stannard et al. 51861* (F), EF194445, EF194656. *Cuscuta incurvata* Progel: **1126**, *Lopez et al. 243* (CTES), EU288336, EU288350. *Cuscuta micrantha* Choisy: **708**, *Muñoz et al. 2914* (SGO), EF194439, EF194651, EU288351; **709**, *Teillier & Faundez 3844* (SGO), EF194438, EF194649; **988**, *Teillier 498*; SGO (SGO), EF194440, EF194650. *Cuscuta parviflora* Engelm. var. *elongata* Engelm.: **1041**, *Oliveira et al. 745* (US), EF194448, EF194657. *Cuscuta platyloba* Prog.: **1073**, *Sehnem 5597* (PACA), EF194447, EF194658; *Cuscuta racemosa* Mart.: **1070**, *Rambo 53990* (PACA), EF194449, —; *Cuscuta racemosa* Mart. var. *miniata* Engelm.: **1127**, *Arbo et al. 5100* (CTES), EU288337, EU288352; *Cuscuta suaveolens* Ser.: **790**, *Paget 2579* (MEL), EF194441, EF194652; **791**, *Chesterfield & Bush 2378* (MEL), EF194443, EF194654; **996**, *Castillo 98-74* (SGO), EF194442, EF194653; *Cuscuta werdermanii* Hunz.:

995, *Reiche s. n.* (SGO), EF194444, EF194655. *Cuscuta xanthochortos* Mart. ex Engelm. var. *carinata* (Yunck.) Yunck.: **1074**, *Aperecida et al. 4333* (US), EF194446, —.

D. *Cuscuta cephalanthi* Engelm.: **167**, *Raven 27211* (WTU), EF194412, EF194631; **469**, *Deam 51439* (IND), EF194413, EF194632; **510**, *Hill 29748* (NY), EF194414, EF194633. *Cuscuta compacta* Juss.: **198**, *Laing 411* (WTU), EF194423, —; **199**, *Eggert s. n.* (WTU), EF194424, EF194640; **466**, *Deam 58335* (IND), EF194425, —; **479**, *Kerby 7* (ASU), EF194426, —. *Cuscuta cuspidata* Engelm.: **1016**, *Carr 13221* (TEX), EF194429, EF194643. *Cuscuta glomerata* Choisy: **462**, *McClain 2448* (IND), EF194430, —; **597**, *Freeman 293* (NY), EF194432, —; **598**, *Freeman 2235* (NY), EF194433, EF194644; **619**, *Stevens 2546* (DAO), EF194431, —. *Cuscuta gronovii* Willd.: **194**, *Demaree 18594* (WTU), EF194419, —; **343**, *Stefanović SS-02-03* (TRTE), EF194418, EF194637; **453**, *Stefanović SS-04-143A* (TRTE), EF194420, EF194638; **467**, *Stefanović SS-04-161* (TRTE), EF194421, —; **702**, *Hinds et al. 11582* (UNB), EF194427, EF194641; **705**, *Garneau & Roy 89-626-M* (DAO), EF194422, EF194639. *Cuscuta gronovii* Willd. var. *caliptrata* Engelm.: **706**, *Cory 52529* (TEX/LL), EF194416, EF194635. *Cuscuta gronovii* Willd. var. *latiflora* Engelm.: **703**, *Catling s. n.* (DAO), EF194417, EF194636. *Cuscuta gronovii* Willd. var.*latiflora* Engelm. / *Cuscuta cephalanthi* Engelm.: **704**, *Bewick 108* (DAO), EF194415, EF194634. *Cuscuta rostrata* Shuttlw. ex Engelm. & A.Gray: **460**, *Bozeman et al. 45268* (IND), EF194428, EF194642. *Cuscuta squamata* Engelm.: **740**, *Anderson & Brice 8057* (NMC), EF194434, EF194645. *Cuscuta umbrosa* Beyrich ex Hook.: **578**, *Fields s. n.* (DAO), EF194435, EF194646; **579**, *Hudson 5082* (USAS), EF194436, EF194647; **956**, *Hutchinson 2262* (RSA), EF194437, EF194648.

E. *Cuscuta denticulata* Engelm.: **165**, *Beck & Caplan 94051* (WTU), EF194409, EF194626; **485**, *Tiehm 13319* (ASU), EF194410, EF194627; **668**, *Baher et al. 10732* (ASU), EF194411, EF194628. *Cuscuta nevadensis* I.M. Johnst., **476**, *Pinkava et al. 12181* (ASU), EF194407, EF194629; **585**, *Morefield 2119a* (NY), EF194408, EF194630. *Cuscuta veatchii* Brandegee, **521**, *Thorne et al. 62616* (F), EU288338, EU288353; **580**, *Henrickson 2323* (MICH), EU288339, EU288354; **760**, *Thorne et al. 62616* (NY), EU288340, EU288355.

F. *Cuscuta burrellii* Yunck.: **888**, *Dawson 14278* (RSA), EF194354, EF194589. *Cuscuta haughtii* Yunck.: **601**, *Svenson 11281* (QFA), EF194350, —; **949**, *Haught s. n.* (F), EF194351, EF194590. *Cuscuta longiloba* Yunck: **904**, *Krapovickas & Schinini 31255* (F), EF194352, —. *Cuscuta partita* Choisy: **523**, *Cardenos 2555* (F), EF194353, EF194591.

G. *Cuscuta* aff. *floribunda* H.B. & K.: **489**, *Grimaldo 492* (F), EF194396, —; **1009**, *Prather & Soule 1221* (TEX), EF194397, —; **1010**, *King & Soderstrom 5053* (TEX), EF194398, EF194619. *Cuscuta aurea* Liebm.: **506**, *Chiang et al. 2161* (MICH), EF194391, EF194620; **800**, *Hernandez & Arias 21117* (XAL), EF194392, EF194621; **1023**, *King 2281* (TEX), EF194390, —. *Cuscuta jalapensis* Schltd:, **518**, *Nee & Hansen 18685* (F), EF194379, —; **606**, *Lorence & Irigos 4076* (NY), EF194378, EF194608; **607**, *Ton & Lopez 9826* (MICH), EF194377, EF194609; **617**, *Breedlove & Thorne 31083* (NY), EF194380, —. *Cuscuta lindsayi* Wiggins: **927**, *Wiggins 13185* (F), EF194406, EF194625. *Cuscuta mitriformis* Engelm. ex Hemsl.: **556**, *Eastoe & Clothier s. n.* (ARIZ), EF194381, —; **584**, *R. Carrillo 356* (CIIDIR), EF194382, EF194611; **815**, *Wardlee 146728* (CHR), —, EF194610. *Cuscuta purpusii* Yunck.: **898**, *Henrickson 6608* (RSA), EF194399, EF194622; **928**, *Purpus 5444* (F), EF194402, EF194623; **1013**, *Hinton et al. 23503* (TEX), EF194400, —; **1025**, *Correll & Johnston 19796* (ASU), EF194401, —. *Cuscuta rugosiceps* Yunck.: **517**, *Cosminsky 71* (F), EF194374, —; **745**, *Brenckle 47-269* (NY), EF194376, EF194607; **915**, *Williams et al. 41476* (F), EF194375, EF194606. *Cuscuta tasmanica* Engelm.: **680**, *Craven s. n.* (CANB), EF194387, —; **681**, *Lepschi 908/909* (CANB), EF194388, EF194612; **682**, *Taws 729* (CANB), EF194389, EF194613. *Cuscuta tinctoria* Mart. ex Engelm.: **573**, *Ortega s. n.* (NY), EF194393, EF194617; **574**, *Ortega 149* (GH), EF194394, EF194618; **766**, *Moore & Wool 3879* (MICH), EF194395, —. *Cuscuta victoriana* Yunck.: **678**, *Cowie 9624* (CANB), EF194383, EF194616; **683**, *Mitchell 6089* (CANB), EF194384, —; **684**, *Latz 14050* (CANB), EF194385, EF194614; **685**, *Smyth 261* (CANB), EF194386, EF194615. *Cuscuta woodsonii* Yunck.: **729**, *Davidson 967* (GH), EF194404, —; **916**, *Standley 81878* (F), EF194405, EF194624; **978**, *Spelenberg et al. 8359* (MEXU), EF194403, —.

H. *Cuscuta applanata* Engelm.: **507**, *Spellenberg & Mahrt 10680* (NMC), EF194373, —; **508**, *Torrecillas 237* (NY), EF194371, —; **535**, *Johnston 8826* (F), EF194372, EF194605; **674**, *Rodrigues 653* (XAL), EF194370, EF194603; **844**, *Shreve 9323* (GH), —, EF194602; **850**, *Corral-Biaz 3912* (NMC), —, EF194604. *Cuscuta chinensis* Lam.: **459**, *Surapat 137* (IND), EF194369, —; **837**, *Carter 628* (CANB), EF194368, —. *Cuscuta potosina* W. Schaffn. ex S. Wats.: **592**, *Medina 2493* (MICH), EF194365, EF194599; **845**, *Rose et al. 9650* (NY), EF194367, EF194601. *Cuscuta potosina* W. Schaffn. ex S. Wats. var. *globifera* W. Schaffn.: **862**, *Axelrod & Hernandez 2242* (NY), EF194366, EF194600. *Cuscuta sandwichiana* Choisy: **155**, *Degener & Degener 36596* (WTU), n/a, EU288356; **686**, *Degener & Degener 35248A* (CANB), n/a, EU288357; **748**, *Sylva & Rumel s. n.* (NY), n/a, EU288358. *Cuscuta yucatana* Yunck.: **657**, *Alava 1341* (NY), EF194364, EF194598.

I. *Cuscuta* aff. *cozumeliensis* Yunck.: **1002**, *Fernandez & Acosta 2131* (MEXU), EF194358, EF194596. *Cuscuta americana* L., **698**, *Garneau et al. 1470*; TRT, EF194363, —; **699**, *Buswell 6231* (NY), —, EF194597. *Cuscuta cozumeliensis* Yunck.: **943**, *Standley 62142* (F), EF194359, EF194592. *Cuscuta globulosa* Benth.: **550**, *Axelrod & Axelrod 1875* (UPRRP), EF194360, EF194593; **861**, *Axelrod 1154* (UPRRP), EF194361, —. *Cuscuta globulosa* Benth.: **1053**, *Liogier 10138* (US), EF194362, —. *Cuscuta macrocephala* W. Schaffn. ex Yunck.: **613**, *Alexander 1241* (NY), EF194355, EF194594; **614**, *Gentry 1145* (MICH), EF194356, —; **731**, *Palmer 141* (GH), EF194357, EF194595.

J. *Cuscuta corymbosa* Ruiz & Pav. var. *grandiflora* Engelm.: **959**, *Tellez 9976* (RSA), EF194345, EF194586; **695**, *Iltis & Guzman 29077* (MICH), EF194343, EF194584; **696**, *Mendez-Ton & de Lopez 9608* (MICH), EF194344, EF194585. *Cuscuta corymbosa* Ruiz & Pav. var. *stylosa* En-

gelm.: **694**, *Medrano et al. 7965* (GH), EF194347, EF194588; **810**, *Gutierrez 2801* (XAL), EF194349, —; **965**, *Rzedowski 28752* (ASU), EF194348, EF194587. *Cuscuta prismatica* Pav. Ex Choisy: **930**, *Mille 112* (F), EF194346, EF194583.

K. *Cuscuta boldinghii* Urb.: **569**, *Breedlove 37373* (NY), —, EF194575. *Cuscuta chapalana* Yunck.: **568**, *Mc Vaugh 22042* (MICH), EF194338, EF194578; **693**, *Mc Vaugh 26593* (MICH), —, EF194579. *Cuscuta costaricensis* Yunck.: **564**, *Chazaro et al. 7527* (MICH), EF194340, EF194580; **811**, *Chazaro 7537* (XAL), EF194341, EF194581; **858**, *Gonzalez 145* (NY), EF194342, EF194582. *Cuscuta erosa* Yunck.: **843**, *Kearney & Publes 14988* (NY), —, EF194573; **964**, *Lehto & Lehto L49371* (ASU), —, EF194574. *Cuscuta strobilacea* Leibm.: **741**, *Gentry 5291* (GH), —, EF194576; **1003**, *Gentry 5291* (MEXU), EF194339, EF194577.

L. *Cuscuta acuta* Engelm.: **1084**, *Fosberg 44965* (US), EF194330, EF194565. *Cuscuta desmouliniana* Yunck.: **571**, *Poster 224* (GH), EU288341, EU288359; **1161**, *Wider 06-368* (WLU), EU288342, EU288360. *Cuscuta hyalina* Roth: **840**, *Bosch 25022* (BOL), —, EF194561, EU288365; **875**, *Hardy & de Winter 1392* (PRE), EF194318, —; **889**, *Parvati s. n.* (RSA), EF194319, EF194562; **994**, *Mkharme 34* (ARIZ), EF194320, —; *Cuscuta leptantha* Engelm.: **608**, *Wiggins 20889* (MICH), EF194322, EF194569; **719**, *Wiggins 14668* (GH), EF194323, EF194570; **884**, *Fritsch & Fritsch 1337* (RSA), EF194324, EF194571. *Cuscuta liliputana* Costea & Stefanović: **664**, *Sivinski 5689* (NY), EU288343, EU288363/EU288364; **665**, *Neese s. n.* (NY), EU288344, EU288362; **848**, *Metcalfe 1290* (NY), EU288345, EU288361. *Cuscuta odontolepis* Engelm.: **587**, *White 2730* (GH), EF194331, EF194563; **730**, *Hartman 52* (GH), EF194332, EF194564. *Cuscuta polyanthemos* W. Schaffn. ex Yunck.: **826**, *Robles 123* (XAL), EF194321, EF194572, EU288366. *Cuscuta tuberculata* Brandegee: **554**, *de la Luz 8543* (ARIZ), EF194334, EF194568; **737**, *Wiggins 15153* (GH), EF194335, EU288367; **762**, *Daniel & Butterwick 4341* (NY), EF194333, EF194567; **763**, *Stevens & Fairhurst 2052* (MICH), EF194336, EU288368; **764**, *Carter & Kellogg 3085* (GH), EF194337, —. *Cuscuta umbellata* H.B. & K.: **516**, *Fletcher 5857* (UNM), EF194315, EF194558; **526**, *Ward & Spellenberg 81-167* (ASU), EU288346, —; **557**, *Blankenhorn 216* (ARIZ), EF194317, EF194560; **576**, *Silversmith s. n.* (NMC), EU288347, —; **759**, *Bleakey 4662* (NMC), EF194316, EF194559; **830**, *Nee & Taylor 29575* (XAL), —, EU288369. *Cuscuta umbellata* H.B. & K. var. *reflexa* Yunck.: **577**, *Spellenberg & Zucker 12966* (NMC), EF194325, EF194566, EU288370; **1015**, *Van Devender 94-458* (TEX), EF194326, —; **1027**, *Austin & Austin 7585* (ASU), EF194327, —; **1030**, *Van Denender et al. 94-458* (ASU), EF194328, —; **1033**, *Daniel 2445* (ASU), EF194329, —.

M. *Cuscuta coryli* Engelm.: **465**, *Deam 51589* (IND), EF194288, EF194539; **666**, *Bartholomew 0-923* (NY), EF194289, EF194540; **824**, *Boivin & Champagne 13869* (ALTA), EF194290, —. *Cuscuta indecora* Choisy: **525**, *Wagner & Powell 2493* (UNM), EF194293, EF194543; **561**, *Worthington 26947* (ARIZ), EF194300, EF194549; **728**, *Spellenberg & Spurrier 8256* (NY), EF194302, —. *Cuscuta indecora* Choisy var. *attenuata* Waterfall: **721**, *Horr 4410* (NY), EF194295, EF194546; **723**, *Tyrl 1648* (OKLA), EF194297, EF194547; **724**, *Waterfall 17191* (OKLA), EF194296, EF194545. *Cuscuta indecora* Choisy var. *longisepala* Yunck.: **726**, *Runyon 2819* (NY), EF194298, —; **727**, *Lean 7964/208* (NY), EF194299, EF194548. *Cuscuta indecora* Choisy var. *neuropetala* (Engelm.) Hitchc.: **720**, *Spellenberg et al. 3427* (NY), EF194301, —; **895**, *DeDecker 5383* (RSA), EF194294, EF194544. *Cuscuta warneri* Yunck.: **662**, *Peterson 98-699* (NMC), EF194291, EF194542; **890**, *Warner s. n.* (RSA), EF194292, EF194541.

N. *Cuscuta aristeguietae* Yunck.: **935**, *Aristeguieta 4568* (F), EF194311, EF194554. *Cuscuta colombiana* Yunck.: **1068**, *Haught 4535* (US), EF194312, —. *Cuscuta gracillima* Engelm.: **599**, *Iltis & Cochrane 149* (MICH), EF194303, —; **600**, *Fryxell 82257* (NY), EF194304, EF194551; **620**, *Boege 490* (GH), EF194305, —; **621**, *Clarcke et al. 681230-17* (MICH), EF194306, EF194550. *Cuscuta macvaughii* Yunck,: **847**, *Hinton 12098* (NY), EF194314, EF194557. *Cuscuta serruloba* Yunck.: **977**, *Orcutt 4457* (MEXU), EF194313, EF194555. *Cuscuta sidarum* Leibm.: **519**, *Hammel 18763* (F), EF194308, EF194552; **692**, *Stevens & Krukoff 20950* (CANB), EF194309, —; **751**, *Austin 20956* (GH), EF194310, —; **1005**, *Ayala 1054* (TEX), EF194307, EF194553.

O. *Cuscuta* aff. *chilensis* Ker Gawl.: **999**, *Hichins & Muñoz s. n.* (SGO), —, EF194525; **1000**, *Teiller et al. 2489* (SGO), —, EF194524. *Cuscuta chilensis* Ker Gawl.: **567**, *Ledingham 4455* (USAS), —, EF194520; **715**, *Arroyo et al. 996099* (SGO), —, EF194521; **716**, *Morales & Cordoba s. n.* (SGO), —, EF194522; **967**, *Landrum 3392* (ASU), —, EF194523; *Cuscuta cockerellii* Yunck.: **1055**, *Straw 2267* (US), —, EF194518. *Cuscuta cristata* Engelm.: **939**, *Riggs 100* (F), —, EF194529; **1026**, *Landrum 3057* (ASU), —, EF194531; **1045**, *Hunziker 5047* (US), —, EF194530. *Cuscuta foetida* H.B. & K.: **496**, *Ollgaard & Balsev 8960* (F), —, EF194512; **922**, *Steyermark 53255* (F), —, EF194513; **1020**, *Sparre 16952* (TEX), —, EF194511. *Cuscuta foetida* H.B.& K. var. *pycnantha* Yunck.: **990**, *Lira 13* (SGO), —, EF194527. *Cuscuta friesii* Yunck.: **1076**, *Cabrera et al. 21399* (LP), —, EF194536. *Cuscuta globiflora* Engelm.: **909**, *Vargas 684* (F), —, EF194533; **926**, *Buchtien 133* (F), —, EF194534. *Cuscuta grandiflora* H.B. & K.: **540**, *Hutchinson & Wright 4305* (F), —, EF194535. *Cuscuta kilimanjari* Olive: **471**, *Knox 5020* (TRTE), —, EF194528. *Cuscuta microstyla* Engelm.: **707**, *Muñoz et al. 3575* (SGO), —, EF194538; **987**, *Vargas & Farah 80* (SGO), —, EF194537. *Cuscuta odorata* Ruiz & Pav.: **912**, *Hutchinson 1055* (F), —, EF194514; **985**, *Muñoz & Meza 2202* (SGO), —, EF194519; **1024**, *Asplund 7737* (TEX/LL), —, EF194515. *Cuscuta paitana* Yunck.: **940**, *Haught 63* (F), —, EF194516; **941**, *Weberbauer 7762* (F), —, EF194517. *Cuscuta parodiana* Yunck.: **512**, *Krapovickas 37354* (F), —, EF194532. *Cuscuta purpurata* Phil.: **1001**, *Biese 2918* (SGO), —, EF194526.