Evolutionary history and taxonomy of the Cuscuta umbellata complex (Convolvulaceae): Evidence of extensive hybridization from discordant nuclear and plastid phylogenies

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Abstract The Cuscuta umbellata complex is one of the 15 major clades recently circumscribed in C. subg. Grammica. Most of its members occur in North America and the Caribbean (C. desmouliniana, C. lacerata, C. leptantha, C. liliputana, C. odontolepis, C. polyanthemos, C. tuberculata, C. umbellata), but three species (C. acuta, C. membranacea, C. umbellata) grow in South America, and one (C. hyalina) is found as a native species in India, Pakistan and Eastern to South Africa. Basic morphology, scanning electron microscopy and sequence data from the nuclear internal transcribed spacer (ITS) and the plastid trnL-F region were used to reconstruct the phylogeny, gain a better understanding of the evolutionary history, and determine species boundaries. Our results show that in its currently accepted delimitation C. umbellata is polyphyletic. Discordances between phylogenies derived from plastid and nuclear data strongly suggest that at least four independent hybridization events have occurred in the evolution of this species group, rendering relationships among its members more complex than previously thought. One of these reticulation events involves C. umbellata var. reflexa, a taxon that has been considered synonymous to C. umbellata var. umbellata in the last decades. This hybrid is morphologically intermediate but distinct from its putative parents, C. odontolepis or C. acuta on the maternal side, and C. umbellata (var. umbellata) on the paternal side, which supports its treatment as a new species, C. legitiima. Cuscuta umbellata is further redefined to exclude C. umbellata var. dubia, which is merged into C. desmouliniana. A new classification is provided, together with an identification key, descriptions, illustrations, and geographical distributions for the twelve species of the clade.

Keywords Convolvulaceae; Cuscuta; hybridization; ITS; molecular phylogeny; morphology; taxonomy; trnL-F

INTRODUCTION

With more than 200 species and over 70 varieties Cuscuta L. (dodders) is one of the most diverse and challenging groups of parasitic plants. The genus is nearly cosmopolitan, but the highest diversity of species (ca. 140–160) is encountered in the Americas (Yuncker, 1932; García & Martín, 2007; Stefanović & al., 2007). Dodders are important both economically and ecologically. A few species are among the most damaging pests worldwide, being capable of producing considerable losses to agricultural crops (Parker & Riches, 1993; Holm & al., 1997; Costea & Tardif, 2006). Surprisingly, however, numerous other species in this genus are presumed extinct or require conservation measures (Costea & Stefanović, 2009a).

Almost eight decades after Truman G. Yuncker’s monograph (Yuncker, 1932), systematics of Cuscuta is receiving a renewed interest. Two of the three accepted subgenera, C. subg. Cuscuta and subg. Grammica, have been recently the subject of broad-scale phylogenetic studies (García & Martín, 2007; Stefanović & al., 2007). In C. subg. Grammica, the largest and most complicated infrageneric group, 15 major lineages have been circumscribed (Stefanović & al., 2007), with little correspondence to Yuncker’s sections and subsections. In parallel, a series of focused systematic studies have been initiated to investigate these clades at species level. To date, a total of five major Grammica clades have been examined: four that comprise mostly species from the territory covered by Flora of North America (Costea & al., 2006a–c; Costea & Stefanović, 2009b; Costea & al., 2009), and one with predominantly Mexican dodders (Costea & al., 2008).

Our present study continues this series by investigating “clade L” (Stefanović & al., 2007), referred to here as the “Cuscuta umbellata complex” in the absence of a formal section name (to be published with a new infrageneric classification of the genus; Costea & al., in prep.). The core of this group is represented by species of the former C. subsect. Umbellatae in which Yuncker (1932, 1965) included nine species characterized by umbellate inflorescences and dehiscent capsules: C. umbellata, C. deltoidea, C. desmouliniana, C. fasciculata, C. gracillima, C. hyalina, C. lacerata, C. macvaughii, C. saccharata, and C. serruloba. However, recent molecular results (Stefanović & al., 2007; Stefanović & Costea, 2008) have shown that the composition of the clade is radically different. Five other species previously included by Yuncker (1932) in various sections and subsections of C. subg. Grammica are also part of the C. umbellata complex (Stefanović & al., 2007; Stefanović & Costea, 2008). Two of these, C. acuta and C. membranacea, have indehiscent capsules (previously included in C. sect. Eugrammica subsect. Acuta); Yuncker, 1932; Hunziker, 1949), while the rest have circumsissile capsules: C. odontolepis (formerly in C. sect. Eugrammica subsect. Odontolepisae; Yuncker 1932), C. tuberculata, C. leptantha, and C. polyanthemos (formerly
in C. sect. Eugrammarica subsect. Leptanthae; Yuncker, 1932). In addition, C. lilliputana, a new species that is nested within this group, has been recently described (Stefanović & Costea, 2008). In contrast, C. gracilicima, C. sidarum (= C. saccharata), C. deltoidea (= C. serruloba) do not belong to the C. umbellata clade as previously thought by Yuncker (1932, 1965), but form a separate group (Stefanović & al., 2007; Costea & al., 2008).

Most of the members of the C. umbellata complex are known to occur in southwestern U.S.A. and Mexico, but three species (C. umbellata, C. acuta, C. membranacea) grow in South America, and another one (C. hyalina) can be found in India, Pakistan and Africa. Recent phylogenetic studies have clearly indicated that the boundaries of some species (e.g., C. umbellata and C. desmouliniana) must be reconsidered (Stefanović & al., 2007; Stefanović & Costea, 2008). These studies have also revealed discordant nuclear and plastid phylogenies which suggested that at least two of the species, C. lilliputana and C. desmouliniana, likely have a hybrid origin (Stefanović & Costea, 2008). These cases of reticulation, together with the disjoint distribution of the taxa, have shown that the evolutionary history of the C. umbellata clade is much more complex than previously thought. In view of these interesting preliminary findings based on relatively limited sampling within the C. umbellata complex, we have studied numerous herbarium specimens that have accumulated worldwide since Yuncker’s monographs (Yuncker, 1932, 1965). The aims of this study are to (1) recover the evolutionary history of the C. umbellata complex based on nuclear ITS and plastid trnL-F sequences and further investigate the extent of reticulate evolution known to occur in this group; (2) investigate the morphology and micromorphology of the taxa involved; and (3) provide a new classification of the C. umbellata complex with the description of a new species, C. legitima.

### MATERIALS AND METHODS

#### Taxon sampling.
- We have studied specimens from over 100 herbaria in connection with the upcoming treatments of Cuscuta for Flora of North America Project, Flora Neotropica, and a future monograph of the genus. A subset of 34 accessions, representing eleven ingroup species of the C. umbellata complex, was used for the molecular phylogenetic analyses (Appendix 1). Efforts were made to sample multiple accessions, particularly for those species spanning large biogeographical ranges (e.g., C. hyalina) and/or those with variable morphology (e.g., C. umbellata). As a result, two to nine individuals are included in the molecular analyses for all but one rare species, C. membraacea, known only from its type locality. In addition, several taxa (C. lacerata, C. fasciculata, C. hyalina var. subiana, C. umbellata var. dubia) are known only from their type specimens (Yuncker, 1921, 1932), and C. umbellata var. desertorum from two historical collections (Engelmann, 1859; Yuncker, 1932). Hence, these taxa could not be sampled for the molecular analyses. Based on our previous, more inclusive phylogenetic analyses of Cuscuta subg. Grammica (Stefanović & al., 2007; Stefanović & Costea, 2008), we selected two species from the C. gracillima clade as outgroup (Appendix 1).

#### Morphology and micromorphology.
- Descriptions are based on herbarium material (Appendix 2). We examined the basic morphology of rehydrated flowers and capsules under a Nikon SMZ1500 stereomicroscope equipped with a PaxCam Arc digital camera and Pax-it 7.0 software (MIS Inc., Villa Park, Illinois, U.S.A.). Micromorphological measurements and pictures were taken at 10 kV using a Hitachi SU1510 scanning electron microscope. Herbarium samples (Appendix 2) were coated with 30 nm gold using an Emitech K 550 sputter coater. The terminology regarding the micromorphology of flowers, seeds, and pollen follows Costea & al. (2006a). Hundreds of photographs that illustrate details of the floral parts, pollen and fruit morphology for all the species (including the types) are available on the Digital Atlas of Cuscuta (Costea, 2007 onwards). The geographical distribution of taxa, phenology, elevation and host ranges are based on observation made from herbarium specimens.

#### Molecular techniques and alignments.
- To infer phylogenetic relationships among species of the C. umbellata complex, sequences for the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) as well as trnL-F intron/spacer region from the plastid genome (ptDNA) were obtained. In addition to the DNA samples used in previous studies (Stefanović & al., 2007; Stefanović & Costea, 2008), total genomic DNA was isolated from newly obtained specimens as well. DNA extractions, polymerase chain reaction (PCR) reagents and conditions, ampiclon purifications, cloning, as well as sequencing procedures followed the protocols detailed in Stefanović & al. (2007). Initial sequencing of nuclear and plastid amplicons was done directly. However, in the cases where significant polymorphism was detected, the PCR product was cloned and multiple clones per individual were sequenced. Sequences generated in this study are deposited in GenBank (accession numbers HM748863–HM748905; see Appendix 1). Sequences were aligned manually using Se-Al v.2.0a11 (Rambaut, 2002).

#### Phylogenetic analyses.
- Phylogenetic analyses were conducted under parsimony and Bayesian optimality criteria; summary descriptions of these analyses, for individual as well as combined datasets, are provided in Table 1.

Under the parsimony criterion, nucleotide characters were treated as unordered and all changes were equally weighted. Depending on the number of operational taxonomic units (OTUs) included, different search strategies were employed for the different matrices, using PAUP* v.4.0b10 (Swofford, 2002). For the ITS matrix, searches for most parsimonious (MP) trees were performed using a two-stage strategy. First, the analyses involved 10,000 replicates with stepwise random taxon addition, tree bisection-reconnection (TBR) branch swapping saving no more than 10 trees per replicate, and MULTREES on. The second round of analyses was performed on all trees in memory with the same settings except with 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 one million trees were found. For the trnL-F matrix, a full heuristic search was performed, involving 1000 replicates with stepwise...
random taxon addition, TBR branch swapping, and MULTREES option on. Given the relatively moderate number of terminal units included in the combined dataset, we performed a Branch-and-Bound search, therefore ensuring recovery of all MP trees. In all three cases, support for clades was inferred by nonparametric bootstrapping (Felsenstein, 1985), using 500 heuristic bootstrap replicates, each with 20 random addition cycles, TBR branch swapping, and MULTREES option off (DeBry & Olmstead, 2000). Nodes receiving bootstrap (BS) values <60%, 60%–75%, and >75% were considered weakly, moderately, and strongly supported, respectively.

Bayesian phylogenetic inferences were performed using MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003). ModelTest v.3.7 (Posada & Crandall, 1998) was used to determine the model of sequence evolution that best fit the data by the Akaike Information Criterion (AIC), starting with the parsimony-derived tree rather than the neighbor-joining default. The Tamura-Nei (TrN) model of DNA substitution (Tamura & Nei, 1993), with rate variation among nucleotides following a discrete gamma distribution (TrN + G), was selected as the best-fit for the ITS sets. For the trnL-F matrix, the F81 (Felsenstein, 1981) model was selected. Each Bayesian analysis consisted initially of two runs of one million generations from a random starting tree using the default priors and four Markov chains sampled every 100 generations. If needed, the run lengths were increased until the standard deviation of split frequencies between two runs was well below 0.01 (see Table 1 for details on MrBayes settings and number of generations used for each of three analyses). Convergence of the chains was determined by examining the plot of all parameter values and the –lnL against generation using Tracer v.1.3 (Rambaut & Drummond, 2004). Stationarity was assumed when all parameter values and –lnL had stabilized. Burn-in trees were discarded and the remaining trees and their associated parameters were saved. Because no significant differences between two runs were detected (for each of the three separate Bayesian analyses; Table 1), the reported topologies and posterior probabilities (PP) are based on trees from pooled runs. Only the nodes receiving ≥0.95 PP were considered statistically significantly supported (Rannala & Yang, 1996).

**Topological incongruence and alternative hypothesis testing.** — Conflict between datasets was first evaluated by visual inspection, by searching for the presence of conflicting and strongly supported topologies from individual matrices. For all the cases where such conflicts were found, reciprocally constrained topologies were constructed using MacClade v.4.06 (Maddison & Maddison, 2003) and their cost in parsimony was assessed using PAUP* (Swofford, 2002). In

### Table 1. Summary descriptions for sequences included in, phylogenetic analyses conducted on, and trees derived from, individual and combined datasets of the *Cuscuta umbellata* complex.

<table>
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<th>trnL-F (plastid)</th>
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<td>F81 (nst = 2; rates = equal)</td>
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<td>1 × 10⁶ (2)</td>
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<td>32,000</td>
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CI, consistency index (excluding parsimony uninformative characters); df, degrees of freedom; nst, number of substitution states; OTU, operational taxonomic unit; RI, retention index; RSA, random sequence addition; TBR, tree bisection and reconnection.
this fashion, for each case of the strongly supported incongruence between the two datasets, one randomly chosen MP tree representing topological results obtained from nuclear data was imposed on plastid data and vice versa. To evaluate the significance among these alternative phylogenetic hypotheses, we implemented the one-tailed Shimodaira-Hasegawa tests (SH tests; Shimodaira & Hasegawa, 1999; Goldman & al., 2000) in PAUP*. The test distributions were obtained using the re-estimated log likelihoods (RELL; Kishino & Hasegawa, 1989) with 10,000 bootstrap replicates.

### Results

**General morphology and micromorphology.** — Although the clade of *C. umbellata* is relatively easy to distinguish from other major groups of *Cuscuta* (see Stefanović & al., 2007), most of its species are notoriously difficult to separate from each other. The overall morphology of this clade reflects its phylogenetic affinities with the *C. gracillicima* clade ("clade N" in Stefanović & al., 2007). The loose, umbellate inflorescence encountered in most species, the shape of the calyx lobes as well as the morphology of the capsules are relatively similar to the species of the *C. gracillicima* complex (Costea & al., 2008). However, unlike in the latter group, the stems of species in the *C. umbellata* clade are persistent at maturity and inflorescences do not emerge directly from the host's stems (Costea & al., 2008).

Papillae are present on the perianth of some species, both on the calyx and corolla (*C. liliputana*, *C. leptantha*, *C. desmouliniana*; Fig. 1D, G, I), or only on the corolla (*C. odontolepis*, *C. tuberculata*, *C. umbellata*; Fig. 1A, E). *Cuscuta desmouliniana*, *C. tuberculata* and sometimes *C. liliputana* are the members of this clade that have multicellular protuberances with stomata on the bracts and calyx lobes (Fig. 1G–H). Similar structures with an unknown role have been also reported from *C. gracillicima* in the *C. californica* clade (Costea & Stefanović, 2009b). Infrafamilial scales with laticifers in the fimbriae are present in most species (Fig. 1B–C), except for *C. hyalina* in which scales are reduced or absent. The pollen is relatively uniform among species, comparable to that of *C. indecora* and *C. gracillicima* clades (Costea & al., 2006b, 2008). Pollen grains are 3(–4)-zonoocolpate, subspheroidal to prolate with perforate or imperforate tectum (for images see Costea, 2007 onwards; for descriptions see Welsh & al., 2010). In most species, capsules dehisce by a circular line at the base of the fruit. The capsules of the Caribbean form of *C. umbellata* var. *umbellata* dehisce late by a more or less irregular line (also found at *C. indicata* and *C. gracillicima* resultates). The loose, umbellate inflorescence, the shape of the calyx lobes, the scale and calyx type, and the morphology of the capsules are relatively similar to the species of the *C. gracillicima* complex (Costea & al., 2008). However, unlike in the latter group, the stems of species in the *C. umbellata* clade are persistent at maturity and inflorescences do not emerge directly from the host's stems (Costea & al., 2008).

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**Sequences and alignments.** — Summary descriptions for sequences obtained from ITS and trnL-F regions are presented in Table 1. Sequences newly generated for this study were incorporated together with the relevant portions of the alignments used in our previous analyses (Stefanović & al., 2007; Stefanović & Costea, 2008). Although these two non-coding regions exhibited length variation, the alignments among the ingroup taxa were straightforward throughout the entire length of these matrices and were used in their entirety for phylogenetic analyses. This is in contrast to the higher-level phylogenetic study of *Cuscuta* subg. *Grammica* (Stefanović & al., 2007; Stefanović & Costea, 2008) in which large portions of trnL-F could not be aligned across major clades, and these consequently had to be excluded from the analyses. Despite repeated attempts (including efforts to amplify the fragments in two parts), sequence data could not be obtained for one or the other region from a few individuals, presumably due to the poor quality or limited quantity of the DNA extracted from some older herbarium specimens.

**Unconstrained analyses and overall levels of support.** — Preliminary phylogenetic analyses were conducted on individual matrices with the inclusion of the outgroup taxa (trees not shown). Those analyses indicated that the first split within the *C. umbellata* group occurs between the *C. leptantha*/*C. polyanthemos* clade on one side, and the remainder of this complex on the other, in agreement with our previous broad-scale results ("clade L" in Stefanović & al., 2007; Stefanović & Costea, 2008). Taking this into account, in all subsequent analyses we used *C. leptantha* and *C. polyanthemos* as functional outgroup (Figs. 2–4), allowing for the full usage of all available nuclear and plastid data. Summary descriptions of trees derived from individual and combined datasets are presented in Table 1. For all these three analyses, the strict consensus of equally parsimonious trees (not shown) resulted in relationships that were topologically identical or nearly identical to the respective results derived under the Bayesian criterion (Figs. 2–3).

Four major clades labeled A–D were resolved within the *Cuscuta umbellata* complex with nuclear ITS sequences (Fig. 2, left). All of these clades have branches with substantial length subtending them and have received strong bootstrap support (97%–100%) as well as significant posterior probabilities (≥0.95). Clade A groups all the accessions/clones of *C. umbellata*, *C. hyalina*, *C. membranacea*, *C. liliputana*, and *C. desmouliniana*. In addition, this lineage contains some but not all the clones derived from the *C. legitima* accessions. In contrast to the strong support for this clade, the relationships within it remained mostly unresolved and unsupported, with a couple of exceptions. Namely, all the members of *C. hyalina* are found grouped together and sister to the sole representative of *C. membranacea*. Both of these results received strong support (98% BS; ≥0.95 PP). Clade B consists of all the representatives of *C. acuta* and *C. odontolepis* plus the remainder of the clones obtained from *C. legitima* accessions. Similarly to the situation described above for clade A, the relationships within clade B are mainly unresolved as well. Moderate support was observed only for a clade grouping all the *C. acuta* clones (52% BS; ≥0.95 PP), while the representatives of the other two species are found interspersed among each other. Clade C contains exclusively *C. tuberculata* individuals, and clade D groups together two
Fig. 1. Morphological and micromorphological features of species from the *Cuscuta umbellata* complex. **A**, *Cuscuta odontolepis* flower; **B**, infrastaminal scale of *C. umbellata* (var. *umbellata*); **C**, laticifers (arrowheads) in the infrastaminal scales of *C. umbellata* (var. *umbellata*); **D**, *C. leptantha* flower; **E**, *C. tuberculata* flower; **F**, capsule and persistent corolla in *C. desmouliniana*; **G**, flower of *C. desmouliniana* with multicellular protuberances on the calyx (arrowheads); **H**, multicellular protuberance with stomata; **I**, papillae on the corolla of *C. desmouliniana*. Bars: A, D–G, 1 mm; B, 0.5 mm; C, 100 µm; H, 30 µm; I, 60 µm.
Fig. 2. Phylogenetic relationships among species of the *Cuscuta umbellata* complex derived from separate Bayesian analyses of nuclear and plastid sequences. Majority-rule consensus trees with mean branch lengths are drawn at the same scale for both phylograms. Four major lineages are labeled A–D. Prime and double-prime symbols indicate clades exhibiting substantial topological incongruence between nuclear and plastid results. Species with conflicting positions are depicted in bold. Parsimony bootstrap values are indicated for nodes supported ≥50%. Asterisks indicate branches with Bayesian posterior probability <0.95; all other branches have posterior probability ≥0.95 (thick lines). Numbers following species names correspond to DNA accessions (see Appendix 1); in addition, for the ITS data different clones are labeled, if applicable.
remaining species from this complex, C. leptantha and C. polyanthemos; all these results received maximum support (100% BS; ≥0.95 PP).

Analyses of plastid trnL-F matrix also recovered four major, well-defined, and well-supported lineages (A–D; Fig. 2, right). However, the composition of two of those lineages, clades A and B, differs substantially compared to that obtained from the nuclear matrix. With plastid data, clade A contains only individuals of C. umbellata and C. hyalina. Members of these two species are reciprocally monophyletic and both are moderately supported (71% and 68% BS, respectively; ≥0.95 PP for both clades). Similarly to the ITS results, clade B contains C. acuta and C. odontolepis. However, in addition to these two species, and unlike in the results obtained with nuclear data, all the individuals of C. membranacea, C. liliputana, C. desmoulini ana, and C. legitima are also confined to clade B, with strong support (100% BS; ≥0.95 PP). Relationships within clade B are only weakly supported with the exception of the C. liliputana/desmoulini ana subclade (83% BS; ≥0.95 PP). The remaining two major groups, clades C and D, are identical in composition between the nuclear and plastid matrices, and consist of C. tuberculata and C. leptantha/polyanthemos individuals, respectively.

The combined analyses were conducted on a dataset in which nuclear and plastid sequences were concatenated but the accessions with strongly supported conflicting positions in individual analyses excluded. Not surprisingly, the same basic underlying tree structure containing four major clades was recovered (Fig. 3). In addition, the combined data provided some support for the backbone relationships among these four lineages. Clades B and C were found sister to each other with stronger support (60% BS; ≥0.95 PP) than in individual analyses. Together, these two clades are sister to clade A. As previously indicated, the preliminary analyses including the outgroup placed C. leptantha and C. polyanthemos (i.e., clade D) as sisters to the rest of the C. umbellata complex, with strong support (100% BS; ≥0.95 PP; trees not shown).

Tests of alternative tree topologies. — The analyses of separate nuclear and plastid matrices produced trees of remarkably similar topologies, with the exception of four striking and strongly supported conflicts (Figs. 2 and 4) whose topological discordances span across two major clades, A and B. According to the nuclear data, C. membranacea, C. liliputana, C. desmoulini ana as well as a number of clones from C. legitima accessions belong to the strongly supported clade A. In contrast, the plastid haplotypes place all these four species within clade B. Using nuclear data yet enforcing trnL-F results on these species and constraining them individually or in combination to clade B, with C. acuta and C. odontolepis, produced trees 17–22 steps longer than the optimal trees. All these length differences were deemed strongly significant and were rejected based on the SH tests (Table 2). Similarly, constraining these species to group in a clade with C. umbellata and C. hyalina (following the ITS results) with plastid data yielded trees 5–6 steps longer. Despite the relatively small length penalty, these results were also rejected as significantly worse solutions by the SH tests (Table 2).

DISCUSSION

Evidence for hybridization in the Cuscuta umbellata complex and alternative explanations for the observed plastid-nuclear discordance. — Instances of reticulate evolution in plants can be detected through detailed analyses of discordance among different unlinked gene trees (Rieseberg, 1995; Sang & Zhong, 2000). When the pDNA tree is compared with an independently derived phylogenetic tree (from morphology or molecular data), conflicting position of a taxon between phylogenies may be taken as evidence for the hybrid origin of this taxon (Sang & Zhong, 2000; an illustration of this principle is depicted in Fig. 4). We present here evidence for four cases of strongly supported yet conflicting phylogenetic signals between ITS and trnL-F sequence data for four species of the Cuscuta umbellata complex. Two of these cases, C. desmoulianana and C. liliputana, were already described and discussed in detail in our previous broad-scale assessment of hybridization in Cuscuta subg. Grammica (Stefanović & Costea, 2008). The other two putative cases of hybridization involving C. membranacea and C. legitima are documented here for the first time.

In addition to hybridization, strongly conflicting gene trees can result from several other biological phenomena (e.g., Madison, 1997; Wendel & Doyle, 1998), as discussed in detail for the Cuscuta cases by Stefanović & Costea (2008). While
A disproportionately large number of the reported horizontal gene transfer (HGT) events involve parasitic plants (Davis & Wurdack, 2004; Mower & al., 2004; Nickrent & al., 2004; Davis & al., 2005), the discordances discussed here are at lower phylogenetic levels, where hybridization is expected to occur, and they involve nrDNA and ptDNA, for which there are virtually no known cases of HGT in land plants despite extensive amounts of available data (Rice & Palmer, 2006).

Paralogy (i.e., gene duplication followed by differential deletion) is also not likely to be the cause for the topological discrepancy between plastid and nuclear phylogenies detected in our study. Although nrDNA is present in multiple copies in plants, it generally evolves in unison through the process of concerted evolution (Zimmer & al., 1980; Buckler & al., 1997). However, despite our intensive cloning efforts, ITS sequences from putative hybrids either were not different within a given species or showed only limited amount of 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 paralogs (Stefanović & al., 2007; Stefanović & Costea, 2008). The only significant departure from this was found in the C. legitima case. In this species, almost all accessions yielded two substantially distinct sets of clones, phylogenetically segregated into two clades (A and B in Figs. 2 and 4). The presence of two ribotypes is interpreted here as an additional evidence for the hybrid origin of this species. The additive pattern observed in nrDNA arrays of C. legitima is likely due to a recent hybridization event following which concerted evolution did not have time to homogenize towards one of the parental types (e.g., Sang & al., 1995 in Paeonia; Ainouche & Bayer, 1997 in Bromus; reviewed by Álvarez & Wendel, 2003).

On the other side, the trnL-F sequences used here are located in the large single-copy region of Cuscuta plastids (Funk & al., 2007; McNeal & al., 2007) and hence are likely to be orthologous. No polymorphism for this ptDNA region was observed within any individual and very little, if any, polymorphism was seen among different individuals from the same species. Lineage sorting represents potentially the strongest alternative explanation but for the cases presented here we still

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**Table 2.** Results of the Shimodaira-Hasegawa (SH) tests for comparison between highly supported yet incongruent topologies recovered from nuclear and plastid datasets of the *Cuscuta umbelata* species complex. Probabilities below 0.05 (i.e., tree topology rejected as significantly worse) are indicated in bold.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Constrained topology</th>
<th>Length</th>
<th>Length difference</th>
<th>SH test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear (ITS)</td>
<td>Optimal tree (Figs. 2, 4; left)</td>
<td>376</td>
<td>Best</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td><em>C. membranacea</em> constrained to clade B</td>
<td>394</td>
<td>18</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td></td>
<td><em>C. legitima</em> constrained to clade B</td>
<td>398</td>
<td>22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td><em>C. liliputana</em> and <em>C. desmouliniana</em> constrained to clade B</td>
<td>393</td>
<td>17</td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td>Plastid (trnL-F)</td>
<td>Optimal tree (Figs. 2, 4; right)</td>
<td>48</td>
<td>Best</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td><em>C. membranacea</em> constrained to clade A</td>
<td>53</td>
<td>5</td>
<td><strong>0.025</strong></td>
</tr>
<tr>
<td></td>
<td><em>C. legitima</em> constrained to clade A</td>
<td>54</td>
<td>6</td>
<td><strong>0.014</strong></td>
</tr>
<tr>
<td></td>
<td><em>C. liliputana</em> and <em>C. desmouliniana</em> constrained to clade A</td>
<td>53</td>
<td>5</td>
<td><strong>0.025</strong></td>
</tr>
</tbody>
</table>
favor hybridization for a number of reasons. First, because the plastid genome is unparentally inherited and haploid, the plastid haplotype tree has a substantially higher probability of shorter coalescence time, leading to the relatively rapid elimination of any polymorphism (Moore, 1995). Second, like the majority of Cuscuta species (Yuncker, 1932), the four species of putative hybrid origin included in our study have relatively narrow geographic distributions. Of these, C. membranacea is the only species represented by a single individual because it is known only from its type locality. By contrast, each of the other three putative hybrids are represented by multiple individuals (3–5), spanning their respective distribution ranges. Yet, in all cases, the results were identical (or nearly so) among all the individuals included. Third, perhaps as a result of the multiple hybridization events, hybrids and their putative parental species are so closely allied that their morphological intermediacy is readily apparent (see below for details).

Phylogenetic analyses of additional, independently inherited sequence data, such as low-copy nuclear genes, as well as critically needed cytological information (the chromosome number is known only from C. hyalina—Vij & al., 1974) will help to further support the extent and importance of hybridization in the evolution of Cuscuta. However, in light of currently available molecular phylogenetic evidence for hybrid origins we discuss below the delineation of species within this complex.

Basis for species delimitation. — Due to the taxonomic difficulty of this clade, neither morphological nor molecular data alone allowed a corroboration of taxon boundaries. In some instances we had to make a decision based on one type of data alone. For example, in the cases where taxa were known from historical collections only (e.g., C. lacerata, C. fasciculata, C. umbellata var. desertorum), and DNA could not be extracted, a conclusion had to be reached solely based on morphology. In contrast, the evolutionary relationships and putative cases of hybridization inferred from molecular data lead us to maintain some species in the absence of a clear morphological distinctiveness. This is exemplified by the case of C. membranacea and C. acuta, two species from South America that are not only very similar morphologically to one another, but also difficult to separate from the morphotype of C. umbellata var. umbellata that grows on the Cape Verde islands, the Caribbean Islands and the N to NE coast of South America (see below).

Redefinition of Cuscuta umbellata and C. desmouliniiana. — The delimitation of C. umbellata represents the prerequisite for the taxonomy of the entire clade. Kunth’s protologue (Bonpland & al., 1818), and later Choisy (1841) referred to a plant collected from central Mexico by Humboldt and Bonpland. Subsequently, Engelmann (1859) expanded C. umbellata to include other forms “from many localities along the United States and Mexican boundary line from northern Mexico, and from the Antilles”. He also included here as a variety (‘ß??) C. desertorum, described on a herbarium specimen by Martius from Brazil, expanding therefore the geographical distribution of C. umbellata to South America (Engelmann, 1859). Yuncker (1921) increased even more the complexity of C. umbellata by adding two more varieties: C. umbellata var. reflexa from southern U.S.A. and northern Mexico and C. umbellata var. dubia from Sonora. In this study, we show that C. umbellata circumscribed with four varieties is polyphyletic and that only Engelmann’s delimitation (1859) reflects the reconstructed phylogenetic relationships among taxa.

Cuscuta umbellata var. reflexa was described as a variety of C. californica from Texas by Coulter (1890) and was later transferred to C. umbellata by Yuncker (1921), who maintained this combination until 1965 when he reconsidered and merged it with the type variety (Yuncker, 1965). Consequently, subsequent North American overviews (e.g., Kartesz, 1999; USDA- NRCS, 2010) did not differentiate between the two entities, and C. umbellata var. reflexa has been generally considered synonymous to the type variety. However, our results have determined that C. umbellata var. umbellata and var. reflexa segregate in different clades of the complex and have different evolutionary histories (Figs. 2 and 4). In addition, C. umbellata var. reflexa differs from var. umbellata in larger flowers with acuminate calyx and corolla lobes (Fig. 5). Based on its molecular and morphologic distinction, C. umbellata var. reflexa is redefined as a species, and since the binomial C. reflexa is not available (C. reflexa Roxb., Pl. Coromandel 2: 3, pl. 104. 1798), we prefer to describe it as a new species, C. legitima (Fig. 5A–D).

Cuscuta umbellata var. dubia was described by Yuncker (1921, 1932, 1965) from a single, poor specimen collected in Sonora and is characterized by a calyx with revolute lobe bases and more or less angled sinuses. Revolute bases of calyx lobes can also be observed in typical C. desmouliniiana, but the angled feature is not as conspicuous because the calyx lobes are usually narrower. Molecular data together with other morphological features (e.g., infrastaminal scales shorter than the corolla tube, papillae on both sides of corolla lobes, and the presence of stomata on the calyx) firmly indicate that C. umbellata var. dubia is within the variation range of C. desmouliniiana, where we transfer it without further recognition at infraspecific level.

We could not reach a final decision about the taxonomic identity of C. umbellata var. desertorum described by Engelmann from Brazil (Piauí and Ceará) because of the poor quality of the only two herbarium specimens available (Appendix 2). Morphologically, this taxon appears to be quite similar to the C. umbellata plants encountered in Cape Verde islands, the Caribbean Archipelago, and N to NE littoral of South America, which are slightly different from those growing in Mexico and the U.S.A. This Atlantic form of C. umbellata has fewer-flowered, loose inflorescences, and often plants become dark-brownish upon drying (the U.S.-Mexican C. umbellata has many-flowered, dense inflorescences and plants commonly remain straw-yellow when dried). The only difference between the Atlantic form and C. umbellata var. desertorum resides in the more reduced, with fewer fimbria or rarely bifid infrastaminal scales encountered in the latter. Engelmann himself mentioned that a specimen of C. umbellata from Antigua (Wulfschlagel 352, MO) was very similar to C. umbellata var. desertorum (Engelmann, 1859). In view of this unresolved issue, we have not distinguished a new variety of C. umbellata from the Pacific, and we maintained C. umbellata var. desertorum until more plants can be collected and studied from Brazil.
Fig. 5. A–D, *Cuscuta legitima*: A, Flower; B1–B2, calyx variation; C, corolla dissected; D, gynoecium. E–H, *C. umbellata* (var. *umbellata*): E, Flower; F1–F2, calyx variation; G, corolla dissected; H, gynoecium. Bars: 1 mm.
Late and occasionally irregular dehiscence of capsules is another subtle morphological difference that distinguishes the Atlantic form of *C. umbellata* from those that grow in Mexico and the U.S.A., in which capsules dehisce by a regular circular line even when they are still immature. The late dehiscence of capsules represents also the only character that separates the Atlantic *C. umbellata* from the morphologically very similar *C. acuta* and *C. membranacea* (see below). The decision to maintain these three species distinct is based on the evolutionary-ary relationships observed among them (Figs. 2−3) and the complex reticulation patterns inferred (Fig. 4).

*Cuscuta fasciculata*, known from a single specimen (Yuncker, 1932), is morphologically identical to typical *C. umbellata*, and the former name is therefore considered a heterotypic synonym.

**Cuscuta acuta** and **C. membranacea**. — Since its description in 1859, *C. acuta* has been largely considered endemic to Galapagos Islands (Engelmann, 1859; Yuncker, 1921, 1932; Hunziker, 1949). Austin (1982) reported first *C. acuta* from mainland Ecuador and observed that “*C. acuta* is very similar to *C. membranacea* [...] and perhaps a single species is involved” (Austin, 1982). The doubt about the distinctiveness of the two species was prompted by the fact that the mainland Ecuador collection cited by Austin as a voucher of *C. acuta* in *C. membranacea* (S) or “*C. membranacea* vel. aff.” (US). As pointed out by Yuncker’s identification (keep in mind that *C. membranacea* was described by Yuncker, 1939), these *C. acuta* plants are morphologically different from the “typical” *C. acuta* found in the Galapagos in that they exhibit some common features with *C. membranacea*. Similarly to *C. membranacea*, these dodders display a globose capsule with a small interstaminal aperture and erect styles (*C. acuta* has a globose-depressed capsule with a relatively large interstaminal aperture and divergent styles). Notwithstanding their apparent intermediary, these mainland Ecuadorian *C. acuta* plants are slightly different morphologically from both *C. membranacea* and the “typical” *C. acuta* in their larger flowers, which remind more of *C. legitima*. We have found additional specimens of this continental *C. acuta* from Peru, where it has been generally identified as “*C. umbellata*”. The difficulty to distinguish *C. acuta* and *C. membranacea* morphologically certainly makes the idea of a single species appealing, but this approach is unsupported by molecular data. *Cuscuta membranacea* is morphologically so close to *C. acuta* because it is most likely a hybrid species, and *C. acuta* is the putative maternal progenitor (see the plastid haplotype tree; Fig. 2, right; Fig. 4).

## TAXONOMIC TREATMENT

Identification Key for species of the *Cuscuta umbellata* clade

1. Capsules indehiscent .......................... 2
2. Flowers 2.5−3.6(−4.0) mm; calyx 1.6−2.3 mm; infrastaminal scales uniformly dense-fringed; styles slightly subulate at the base .......................... 1. *C. acuta*
3. Calyx and corolla lobes lacerate .......................... 4. *C. lacerata*
4. Infrastaminal scales absent or represented by scarcely dentate ridges .......................... 5. *C. hyalina*
5. Inflorescences dense, paniculiform-glomerulate; calyx lobes basally overlapping; capsules 2.9−4.0 × 3.0−3.5 mm, not translucent; seeds 1.00−1.25 × 0.65−0.80 mm .......................... 9. *C. odontolepis*
6. Infrastaminal scales equaling or slightly longer than the calyx tube .......................... 10. *C. polyanthemos*
7. Flowers 5.0−7.5 mm; corolla lobes ca. 1/2 the tube .......................... 10. *C. tuberculata*
8. Flowers 5-merous; calyx lobes carinate; corolla lobes spreading to reflexed .......................... 9
9. Calyx equaling corolla tube; infrastaminal scales bridged at 0.1−0.3 mm .......................... 7. *C. hiliputana*
10. Infrastaminal scales ca. 3/4 of the corolla tube .......................... 2. *C. desmoulinaea*
11. Infrastaminal scales equaling or slightly longer than the corolla tube .......................... 11
12. *C. umbellata*
11. Flowers 4.0−5.5(−6.0) mm; calyx lobes acuminate .......................... 5. *C. legitima*
12. *C. umbellata*


**Stems** slender, yellow-orange. **Inflorescences** dense-umbelliform, confluent; pedicels 0.6−3.0 mm; bracts 1 at the base of clusters and 0−1 at the base of pedicels, 1.5−2.8 mm long, triangular-ovate, margins entire, apex acuminate. **Flow- ers** 5-merous, 2.5−3.6(−4.0) mm, membranous, white when fresh, creamy-brown when dried; papillae absent, laticifers evident in the bracts, calyx, corolla, tips of infrastaminal scale fimbriae, and ovary, isolated or in rows, ovoid to elongate; calyx 1.6−2.3(−2.6) mm, straw-yellow to brown, not reticulate or shiny, campanulate, longer than corolla tube, divided ca. 2/3 the length, tube 0.4−0.8 mm, lobes (unequal) 0.7−1.5 mm,
not basally overlapping, ovate-triangular, not carinate, margins entire, apex acuminate; corolla 1.8–3.3 (–3.8) mm, tube 0.9–1.7 mm, campanulate, lobes 0.9–1.9 mm, initially erect, later reflexed, ca. as long or slightly longer than the tube, ovate-lanceolate, margins entire, apex acute to acuminate, straight; stamens exserted, shorter than the lobes, anthers 0.33–0.40 × 0.20–0.25 mm, broadly ovate to oblong, filaments 0.4–0.7 mm; infrastaminal scales extremely thin, 0.9–1.7 mm long, equaling the tube, bridged at 0.15–0.30 mm, obvate to oblong, uniformly dense-fringed, fimbriae 0.15–0.30 mm; styles 0.4–1.1 mm, shorter or equaling the ovary, slightly subulate at the base, filiform in the rest. Capsules indehiscent, 1.7–2.5 × 0.9–1.4 mm, globose-depressed, thickened around the moderate interstylar aperture, translucent, surrounded by the withered corolla. Seeds 4 per capsule, 0.7–1.2 × 0.7–1.0 mm, subround to round.

Distribution and ecology. – Galapagos Islands and the Pacific Coast of Ecuador and Peru; flowering Jan.–July; elevation 40–160 m; hosts: Alliannthera, Coldenia, Boerhavia, Euphorbia, Ipomoea, Pectis, Portulaca, Rhyynosia and Tribulus.


Stems slender, yellow-orange. Inflorescences loose, umbelliform, confluent; pedicels 2–4 mm; bracts 1 at the base of clusters and 0–1 at the base of pedicels, 2.0–3.6 mm long, narrow triangular lanceolate, margins entire, apex long-acuminate. Flowers 5-merous, 2.5–4.2 mm, membranous, creamy-white when fresh, creamy-yellow when dried; papillae absent, laticifers evident in the bracts, calyx, corolla lobes, and ovary, isolated, ovoid to elongate; calyx 2.0–3.2 mm, straw-yellow, not reticulate, slightly shiny, campanulate, much longer than corolla tube, divided 1/2–2/3 the length, tube 0.9–1.4 mm, lobes 1.5–2.0 mm, not basally overlapping, triangular-lanceolate, not carinate, margins entire, apex acuminate, ± reflexed; corolla 2.3–4.0 mm, tube 1.0–1.4 mm, campanulate, lobes 1.5–2.5 mm, initially erect, later reflexed, longer than the tube, triangular-lanceolate, margins entire, apex acuminate, straight; stamens exserted, shorter than the lobes, anthers 0.4–0.6 × 0.3–0.4 mm, elliptic, filaments 0.5–0.7 mm; infrastaminal scales absent or represented by scarcely dentate ridges; styles 0.9–2.0 mm, equaling or longer than the ovary, evenly filiform. Capsules circumscissile, 2.0–3.0 × 2.0–2.5 mm, globose, thickened and slightly risen around the inconspicuous interstylar aperture, translucent, surrounded by the withered corolla. Seeds 2–4 per capsule, 1.3–1.5 × 0.9–1.1 mm, elliptic.

Note. – Cuscuta hyalina var. nubiana Yunck. in Mem. Torrey Bot. Club 18(2): 236. 1932 [Type: Sudan, Gef. bei Suakin. Nubische Küste, Jun 1864, Schweinfurth 964 (isotype: K)] has flowers with scales occasionally represented by ridges.

Distribution and ecology. – Asia: Pakistan and India; Africa: Ethiopia, Sudan, Botswana, Zimbabwe, Uganda, Kenya, Ruanda, Burundi, South Africa, Namibia; flowering July–Nov.; Dec.–Mar.; elevation 700–1500 m; hosts: Cyperus bulbosus, Portulaca sp., Tribulus terestris, and Zaleya pentandra.


Stems slender, yellow-orange. Inflorescences loose, umbellate, often confluent; pedicels 1.5–4.0 mm; bracts 1 at the base of clusters and 0–1 at the base of pedicels, 0.6–1.2 mm long, lanceolate, margins entire or irregularly dentate, apex

short-fringed, fimbriae 0.05–0.15 mm; styles 1.2–2.1 mm, longer than the ovary, evenly filiform. Capsules circumscissile, 1.2–2.0 × 0.9–1.7 mm, globose to globose-depressed, slightly thickened and risen, or with a few protuberances around the inconspicuous interstylar aperture, translucent, capped by the withered corolla. Seeds 2–4 per capsule, 0.75–0.90 × 0.70–0.80 mm, subround to broadly elliptic.

Distribution and ecology. – Mexico: Baja California and Sonora; flowering Aug.–Sept., Dec.–Mar.; elevation 30–300 m; hosts: usually on Chamaeysce, rarely on Boerhaavia or Pectis.
acuminate. Flowers 5-merous, 3.0–4.4 mm, membranous, white when fresh, creamy-white when dried, papillae absent; laticifers visible in the calyx and corolla lobes, isolated, ovoid to elongate; calyx 1.8–2.4 mm, yellow, ± reticulate or shiny, campanulate, longer than the corolla tube, divided 1/4–1/3 the length, tube 0.3–0.6 mm, lobes 1.5–1.8 mm, not overlapping, lanceolate, not carinate, not forming angled sinuses, margins with a few irregular teeth (especially toward apex), apex long acuminate, reflexed; corolla 2.8–4.2 mm, tube 1.0–1.4 mm, campanulate, lobes 2.0–1.6 mm, initially erect, later spreading or reflexed, longer than the tube, linear, margins with a few large, irregular teeth, sometimes involute upon drying and appearing very narrow, apex long acuminate, capillary; stamens exerted, shorter than corolla lobes, anthers 0.5–0.6 × 0.2–0.3 mm, ovate to oblong, filaments 0.6–1.0 mm; infrastaminal scales 1.0–1.6 mm long, equaling or longer than corolla tube, bridged at 0.16–0.25 mm, oblong-spellumate, medium-fringed, fimbriae 0.12–0.25 mm; styles 1.8–2.4 mm, longer than the ovary, evenly filiform. Capsules circumscissile, 1.2–1.6 × 1.0–1.7 mm, globose, with a ring of protuberances around the inconspicuous interstylar aperture, translucent, capped by the withered corolla. Seeds 1–4 per capsule, 0.9–1.0 × 0.8–0.9 mm, angled, subrotund.

Distribution and ecology. – Known only from the type collection from Cuicatlan, Mexico; flowering July; elevation and host unknown.

5. Cuscuta legitima Costea & Stefanović, sp. nov. – Type: Mexico. Sonora: Northwest side of Río Yaqui at MEX 15 near Esperanza, ca. 9 km north of Ciudad Obregón, 27°35′45″ N 109°56′ W, ca. 40 m elevation, locally common parasite on Boerhavia coccinea (Nyctaginaceae), flowers white, stems yellow, 10 Sep 1994, Van Devender 94-458 & al. (holotype: ARIZ; isotypes: ASU, MEXU, UC, UCR, WLU). Figure 5A–D.


Cuscuta umbellatae similis, sed flores 4.0–5.5(–6.0) mm longi; calyx 2.5–3.2 mm longus, lobis acuminatis; corolla 3.8–5.2(–5.6) mm longa. Cuscutae acutae et C. membranacea similis, sed floribus majoribus et capsulae deshiscibentibus.

Stems slender, yellow-orange. Inflorescences dense to loose, umbelliform, confluent; pedicels 2–10 mm; bracts 1 at the base of clusters and 0–1 at the base of pedicels, 2.0–3.6 mm long, broadly triangular-ovate, margins entire, apex acuminate. Flowers 5-merous, 4.0–5.5(–6.0) mm, membranous, white when fresh, creamy-white when dried; papillae absent, laticifers evident in the bracts, calyx, corolla, tips of infrastaminal scale fimbriae, and ovary, isolated, ovoid; calyx 2.5–3.2 mm, straw-yellow, finely reticulate, slightly shiny, campanulate, longer than corolla tube, divided ca. 2/3 the length, tube 0.6–1.0 mm, lobes 1.5–2.2 mm, not basally overlapping, ovate-lanceolate, not carinate, margins entire, apex acuminate; corolla 3.8–5.2(–5.6) mm, tube 1.6–2.1 mm, campanulate, lobes 1.8–3.0 mm, initially erect, later reflexed, longer than the tube, linear-lanceolate, margins entire, apex acuminate, straight; stamens exserted, shorter than the lobes, anthers 0.50–0.70 × 0.24–0.36 mm, elliptic to oblong, filaments 0.6–1.0 mm; infrastaminal scales 1.8–2.2 mm long, equaling or slightly longer than the tube, bridged at 0.2–0.4 mm, spathulate to obovate, uniformly dense-fringed, fimbriae 0.2–0.5 mm; styles 0.9–2.5 mm, longer than the ovary, evenly filiform. Capsules circumscissile, 2.3–3 × 1–2 mm, depressed, irregularly thickened and slightly risen around the inconspicuous interstylar aperture, translucent, surrounded or capped by the withered corolla. Seeds 2–4 per capsule, 0.9–1.2 × 0.8–0.9 mm, broadly elliptic to subround.


Stems slender, yellow-orange. Inflorescences loose, umbellate, confluent; pedicels (1–)2–7 mm; bracts 1 at the base of clusters and 0–1 at the base of pedicels, 0.75–1.00 mm long, triangular ovate, margins entire, apex acute. Flowers 4-merous, 3.5–4.5(–5.0) mm, membranous, white when fresh, creamy-white when dried, papillae usually present on the pedicels and perianth; laticifers not visible; calyx 1.5–1.8 mm, straw-yellow, not reticulate or shiny, campanulate, 1/3–1/2 of the corolla tube, divided ca. 1/2 the length, the tube 0.5–0.8 mm, lobes 0.8–1.0 mm, not basally overlapping, triangular-ovate, not carinate, margins entire, apex acute; corolla 3–4 mm, tube 1.5–2.5 mm, cylindric, lobes 1.5–2.0 mm, initially erect, later spreading or reflexed, as long as the tube, lanceolate, margins entire often involute upon drying and corolla lobes appearing narrow, apex acute, ± cucullate; stamens short-exserted, shorter than corolla lobes, anthers 0.40–0.60 × 0.35–0.45 mm, subround to broadly elliptic, filaments 0.3–0.6 mm; infrastaminal scales 1.3–2.1 mm long, ca. 1/2 of the corolla tube, bridged at 0.4–0.8 mm, oblong, uniformly short-fringed, fimbriae 0.05–0.15 mm; styles 1.2–2.1 mm, longer than the ovary, evenly filiform. Capsules circumscissile, 1.5–2.0 × 1.6–1.9 mm, globose, slightly thickened and risen or with a few protuberances around the inconspicuous interstylar aperture, translucent, capped by the withered corolla. Seeds 2–4 per capsule, 0.75–0.90 × 0.70–0.80 mm, angled, subrotund to broadly elliptic.

Distribution and ecology. – U.S.A.: Texas, New Mexico; Mexico: Baja California, Sonora, Sinaloa; flowering Nov.–May; elevation 10–125 m; host: Chamaesyce sp.

Stems slender, yellow to pale orange. Inflorescences loose, umbelliform; pedicels (1–)2–3–(5) mm; bracts 1 at the base of clusters and 0–1 at the base of pedicels, 0.7–1.0 mm long, ovate-lanceolate, margins entire, apex acute. Flowers (3–)4–merous, 2.8–4.0 mm, fleshy, white when fresh, creamy when dried; papillae usually present on pedicels, calyx and corolla; laticifers hardly visible only in the midveins of the corolla lobes, elongate; calyx 1.3–1.7 mm, straw-yellow, somewhat reticulate and shiny, equaling the corolla tube, divided ca. 3/4 the length, tube 0.3–0.7 mm, cylindric, lobes 1.00–1.35 mm, not basally overlapping, ovate-triangular, not carinate but sometimes with multicellular protuberances bearing stoma on the midveins, apex acute to acuminate, margins entire; corolla 3.0–3.6 mm, tube 1.5–2.0 mm, cylindric, lobes 1.30–1.65 mm, initially erect, later spreading or reflexed, equaling the tube, lanceolate, margins entire, apex acute to acuminate; stamens exerted, shorter than the lobes, anthers 0.35–0.50 × 0.20–0.35 mm, broadly to short acuminate, filaments 0.5–0.8 mm; styles 1.6–2.6 mm long, triangular–ovate, margins entire, apex acute to acuminate, filaments 1–3 mm; bracts 1 at the base of clusters and 0–1 at the base of pedicels, 0.7–1.0 mm long, ovate-triangular, not carinate margins entire, apex acute to acuminate; stamens exerted, shorter than the lobes, anthers 0.70–1.10 × 0.25–3.00 mm, oblong, styles 0.10–0.18 mm; infrastaminal scales 0.6–0.8 mm long, 1/4–1/3 of the corolla tube, divided ca. 1/3 the length, the tube 0.4–0.8 mm, lobes 1.5–2.0 mm, initially erect, later reflexed, ovate-triangular, not carinate, margins entire, apex acute to acuminate; stigma 3.0–3.6 mm, ovate-triangular, outer 2 lobes auriculate, basally overlapping, not carinate, margins entire, apex acute to short acuminate; corolla 3.5–4.5 mm, tube 2.2–2.8 mm, cylindric, lobes 1.6–2.0 mm, initially erect, later reflexed, ovate-triangular, margins entire, basally overlapping, apex acute to short acuminate, straight; stamens barely exerted, shorter than corolla lobes, anthers 0.70–1.10 × 0.25–3.00 mm, oblong, filaments 0.3–0.7 mm; infrastaminal scales 2.0–2.5 mm long, 1/2 to equaling the corolla tube, bridged at 0.25–0.50 mm, oblong-spathulate to obovate, rounded, fringed in the distal 1/2, filaments 0.2–0.3 mm; styles 2.8–4.0 mm, longer than the corolla, evenly filiform. Capsules circumscissile, 2.9–4.0 × 3.0–3.5 mm, globose to globose-depressed, thickened and raised around the inconspicuous interstyal aperture, translucent, loosely surrounded and clogged by the withered corolla. Seeds 2–4 per capsule, 0.90–1.26 × 0.80–1.10 mm, subround to broadly elliptic.


9. **Cuscuta odontolepis** Engel. in Trans. Acad. Sci. St. Louis 1: 486. 1859 – Type: U.S.A. Arizona, Santa Rita Mts., south of Tucson, 1851–1852, Wright 1624 (holotype: MO; isotypes: GH, K, NY). Figure 1A.

Stems slender, yellowish. Inflorescences dense, paniciform-glomerulate; pedicels to 1 mm; bracts 1 at the base of cymes and 0–1 at the base of pedicels/flowers, 2–3 mm long, subround to broadly ovate, margins entire, apex acute to short acuminate. Flowers 5-merous, 4.5–5.0 mm, membranous, white when fresh, creamy-white when dried; papillae present on the bracts, calyx and corolla lobes; laticifers poorly visible only in the corolla lobes, isolated, elongated; calyx 2.0–2.5 mm, straw-yellow, finely reticulate, not shiny, campanulate, 1/2–3/4 as long as the corolla tube, divided ca. 2/3, tube 0.5–0.9 mm, lobes 1.3–1.5 mm, ovate-triangular, outer 2 lobes auriculate, basally overlapping, not carinate, margins entire, apex acute to short acuminate; corolla 3.5–4.5 mm, tube 2.2–2.8 mm, cylindric, lobes 1.6–2.0 mm, initially erect, later reflexed, ovate-triangular, margins entire, basally overlapping, apex acute to short acuminate, straight; stamens barely exerted, shorter than corolla lobes, anthers 0.70–1.10 × 0.25–3.00 mm, oblong, filaments 0.3–0.7 mm; infrastaminal scales 2.0–2.5 mm long, 1/2 to equaling the corolla tube, bridged at 0.25–0.50 mm, oblong-spathulate to obovate, rounded, fringed in the distal 1/2, filaments 0.2–0.3 mm; styles 2.8–4.0 mm, longer than the corolla, evenly filiform. Capsules circumscissile, 2.9–4.0 × 3.0–3.5 mm, globose to globose-depressed, thickened and raised around the inconspicuous interstyal aperture, translucent, loosely surrounded and clogged by the withered corolla. Seeds 2–4 per capsule, 1.00–1.25 × 0.65–0.80 mm, broadly elliptic.


Stems slender, yellow. Inflorescences loose, corymbiform or umbellate, often confluent; pedicels 4–15(–20) mm; bracts 1 at the base of clusters, 0.8–1.2 mm long, ovate triangular to lanceolate, margins entire, apex acute. Flowers 5-merous, 5.0–7.5 mm, membranous, white both when fresh and dry, papillae present on the corolla lobes; laticifers not visible; calyx 2.0–2.5 mm, straw-yellow, not reticulate or shiny, cylindric campanulate, 1/4–1/3 of the corolla tube, divided ca. 1/3 the length, the tube 0.4–0.8 mm, lobes 1.5–2.0 mm, not basally overlapping, triangular-ovate to lanceolate, not carinate but with small protuberances on the midveins, margins entire, apex acute; corolla 5–7 mm, tube 4–5 mm, cylindric, lobes 2.0–2.5 mm, initially erect, later spreading or reflexed, 1/2 as long as the tube, triangular lanceolate, margins entire, apex acute to...
acuminate; stamens short-exserted, shorter than corolla lobes, anthers 0.60–1.00 × 0.35–0.45 mm, oblong elliptic, filaments 0.5–0.8 mm; infrastaminal scales 2.0–2.5 mm long, bridged at 0.25–0.40 mm, 1/3–1/2 of the corolla tube, oblong, sparsely short-fringed, fimbriae 0.05–0.20 mm; styles 4–5 mm, much longer than the ovary, evenly filiform. Capsules circumscissile, 1.0–2.0 × 0.8–1.2 mm, globose, thickened and risen around the inconspicuous interstylar aperture, translucent, capped by the withered corolla. Seeds 2–3 per capsule, 1.00–1.20 × 0.70–0.85 mm, subrotund to broadly ovate.

**Distribution and ecology.** – Mexico: Sonora and Sinaloa; flowering Aug.–Sep.; elevation ca. 635 m; host: Chamaesyce sp.

**11. Cuscuta tuberculata** Brandegee in Univ. Calif. Publ. Bot. 3: 389. 1909 – Type: Mexico, 6 Mar 1889, Brandegee s.n. (holotype: UC). Figure 1E.

Stems filiform, yellow-orange. Inflorescences loose, umbelliform or racemiform, confluent; pedicels 2–3(–5) mm; bracts 1 at the base of clusters, usually absent at the base of peduncles, 0.50–0.75 mm long, ovate-lanceolate, margins entire, apex acute. Flowers 5-merous, 2.5–4.0 mm, membranous, white-creamy when fresh, creamy when dried; papillae present especially at the base of the corolla tube, laticifers barely visible in the corolla, isolated, ovoid to elongated; calyx 0.5–1.5 mm, yellow, not or finely reticulate, ± glossy, campanulate-angular, 1/3–1/2 as long as the corolla tube, divided almost to the base, tube 0.2–0.5 mm, lobes 1.0–1.3 mm, not basally overlapping, triangular to lanceolate, carinate and with multicellular protuberances bearing stomata on the midveins, margins entire, acute to acuminate; corolla 2.0–3.5 mm, tube 1.5–2.2 mm, cylindric, lobes 1.2–2.0 mm, erect, about equaling the tube, triangular lanceolate, margins entire, apex acute, straight; stamens barely exserted, shorter to almost equaling the corolla tubes, anthers 0.50–0.80 × 0.25–0.30 mm, ovate to oblong, filaments 0.4–0.7 mm; infrastaminal scales 0.5–1.0 mm long, ca. 1/2 the length of the corolla tube, bridged at 0.3–0.5 mm, ovate, uniformly short-fringed, fimbriae 0.05–0.15 mm; styles 1.5–3.0 mm, longer than the ovary, evenly filiform. Capsules circumscissile, globose, 1.3–2.2 × 1.0–2.3 mm, slightly thickened and risen around the small interstylar aperture, translucent, capped by the withered corolla. Seeds usually 4 per capsule, 0.6–0.9 × 0.3–0.5 mm, elliptic-oblong.

**Distribution and ecology.** – U.S.A: Arizona and New Mexico; Mexico: Baja California and Sonora; flowering Aug.–Nov.; elevation 70–700 m; host usually Boerhavia, rarely Amaranthus and genera of Euphorbiaceae.


Stems slender, yellow-orange. Inflorescences dense to loose, umbelliform, confluent; pedicels 2–10 mm; bracts 1 at the base of clusters, usually absent at the base of pedicels, 0.5–2.0 mm long, triangular-ovate, margins entire, apex acute. Flowers 5-merous, 2–3 mm, membranous, white when fresh, creamy-white or dark brown when dried; papillae sometimes present but only on the adaxial face of corolla lobes; laticifers evident in the bracts, calyx, corolla, tips of infrastaminal scale fimbriae, and ovary, isolated, ovoid; calyx 0.8–1.4 mm, straw-yellow, finely reticulate, slightly shiny, campanulate, equaling the corolla tube, divided ca. 2/3 the length, tube 0.25–0.60 mm, lobes 0.5–0.9 mm, not basally overlapping, broadly triangular-ovate, not carinate, margins entire, apex obtuse to acute, initially cucullate, later straight; corolla 2.0–2.5 mm, tube 0.6–1.2 mm, campanulate, lobes 0.8–1.5 mm, initially erect, later reflexed, equaling or slightly longer than the tube, oblong to lanceolate, margins entire, apex obtuse to acute, straight; stamens exerted, shorter than the lobes, anthers 0.40–0.60 × 0.24–0.30 mm, elliptic to oblong, filaments 0.4–0.7 mm; infrastaminal scales 0.8–1.2 mm long, equaling or slightly longer than the tube, bridged at ca. 0.1 mm, subspathulate to obovate, uniformly dense-fringed, fimbriae 0.15–0.32 mm; styles 0.8–1.7 mm, equaling or longer than the ovary, evenly filiform. Capsules circumscissile, 1.0–2.5 × 0.5–1.2 mm, depressed, irregularly thickened and slightly risen around the inconspicuous interstylar aperture, translucent, surrounded or capped by the withered corolla. Seeds 4 per capsule, 0.80–1.20 × 0.65–0.80 mm, broadly elliptic to subround.

**Note.** – *Cuscuta umbellata* var. desertorum Engelm. in Trans. Acad. Sci. St. Louis 1(3): 488. 1859, differs in having more reduced infrastaminal scales, with fewer fimbriae (rarely bifid, see the holotype morphology gallery in Costea, 2007 onwards).

**Distribution and ecology.** – North and Central America: U.S.A, Mexico, Panama; Caribbean: Cuba, Haiti, Jamaica, Turks and Caicos, Virgin Islands; South America: Venezuela, Guyana, Suriname, Brazil; Cape Verde. Flowering June–Dec.; Dec.–Mar.; elevation 10–2700 m; sometimes on saline soils; hosts: Acleisanthes, Alternanthera, Allionia, Amaranthus, Atropa, Boerhavia, Gilia, Iresine, Kallstroemia, Phyllokerus, Sesuvium, Salsola, Selinocarpus, Suaeda, Tidestromia, Trianthema, Tribulus.

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Appendix 1. Taxa, DNA accession numbers, sources of plant material from which DNA was extracted, and GenBank accession numbers for sequences used in this study. DNA extraction numbers are indicated on the phylogenetic trees in the main text following species names. GenBank accession numbers are given in the following order: trn-L-F, ITS (if applicable, multiple clones are separated by forward slash). Sequences newly generated for this study are indicated in bold. A dash indicates the sequence was not obtained. Abbreviations of herbaria in which the vouchers are deposited follow Index Herbariorum indicated in bold. A dash indicates the sequence was not obtained. Abbreviations of herbaria in which the vouchers are deposited follow Index Herbariorum.

Cuscuta acuta: 1084, Foxberg 44967 (US), EF149330, EF149565; HM748867/HM748868/HM748869; 1188, Ferreyra et al. 10665 (USM), HM748863, HM748870/HM748871; C. desmouliniana: 571, Porter 224 (GH), EU288341, EU288339; 1162, Christiansen 1274 (URC), HM748864, HM748872; 1161, Wider 06-368 (WLU), EU288342, EU288360; C. hyalina: 840, Bosh 25022 (BOL), –; EF149561/EU288365/875, Hardy & de Winter 1392 (PRE), EF149318, –; 889, Parvati s.n. (RSA), EF149319, EF149562; 994, Mikhano 45 (ARIZ), HM748873; C. legitima: –; C. umbellata var. reflexa: 577, Spellenberg & Zucker 12966 (NMC), HM748925, HM748863/EU288370; 1015, Van Derven 94-458 (TEX), EF149326, HM748874/HM748875/HM748876; 1027, Austin & Austin 7585 (ASU), EF149327, HM748877/HM748878/HM748879/HM748880; 1030, Van Derven & al. 94-458 (ASU), EF149328, HM748881/HM748882/HM748883/HM748884; 1033, Daniel 2445 (ASU), EF149229, HM748885/HM748886/HM748877/HM748888/HM748889; C. leptontha: 608, Wiggins 20889 (MICH), EF149322, EF149569; 719, Wiggins 14668 (GH), EF149323, EF149340, 884, Fritsch & Fritsch 1337 (RSA), EF149342, EF149571; C. pilulifera: 664, Sivinski 5689 (NY), EU288343, EU288363/EU288366/665, Neesse.s.n. (NY); EU288344, EU288362/848, Metcalf 1290 (NY), EU288345, EU288361; C. sanguinea: 1185, Hanzawa 4969 (MO), HM748865, HM748890; C. odontolobus: 587, White 2730 (GH), EF149311, EF149563/EU288389/HM748892/HM748893/HM748895; 730, Hartman 52 (GH), EF149322, EF149564/HM748894/HM748895/HM748896; C. polyanthemos: 826, Robbles 123 (XL), EF149321, EF149562/EU288366; 1162, Van Derven 2006-809 (WLU); C. tuberculata: 554, Lody 8543 (ARIZ), EF149334, EF149567, EF149568; 673, Stevens & Fairhurst 2052 (MICH), EF149336, EU288368, 764, Carter & Kellogg 3085 (GH), EF149337, HM748866, HM748867; C. umbellata (var. umbellata): 516, Fletcher 5875 (USM), EF149345, EF149558/HM748899; 526, Ward & Spellenberg 81-167 (ASU), EU288346; –; 557, Blankenhorn 216 (ARIZ), EF149317, EF149560/EU288389; C. vandevenderi: 830, Nee & Taylor 29575 (XAL), –; EF149441/1033, EF149338, EU288389; 1189, Canarranza 7045 (WLU), 1190, Canarranza 7919 (WLU); GB, Medina s.n. (MA), AJ428053, EF192271; Outgroup: C. serruloba Yunck.: 977, Oorrent 4457 (MEXU), EF149313, EF149555; C. vandevenderi Costea & Stefanović: 1058, Gentry & al. 19423 (US), EU426964, EU426989.
Costea & Stefanović • Systematics of the Cuscuta umbellata complex

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Costea & Stefanović • Systematics of the Cuscuta umbellata complex (CAS).

Appendix 2. Continued.


ckley & Pinkava 3864 UNM); just W of Ft. Bliss, 1100 m, 12 Oct 1950, (ARIZ); 0.5 km E of Río Sonoyta, 4 Oct 1985, 19.8 mi S of Desemboque Río San Ignacio, 14 May 1966, TINA.


Costea & Stefanović • Systematics of the Cuscuta umbellata complex (CAS).

Appendix 2. Continued.


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