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Systematics of *Cuscuta chinensis* species complex (subgenus *Grammica*, Convolvulaceae): evidence for long-distance dispersal and one new species

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Abstract *Cuscuta chinensis* species complex is a small clade from subgenus *Grammica* (Cuscuta, Convolvulaceae). Many species of this clade exhibit crest- or dome-like multicellular appendages with stomata on the midvein/carena of calyx lobes. Basic morphology, scanning electron microscopy, and DNA sequence data from the plastid *trnL-F* region and the nuclear internal transcribed spacer (ITS) were used to investigate the phylogenetic relationships within the group and test the species limits. Based on their morphological and molecular similarly, *C. chinensis* and *C. applanata* represent one single species, and the latter was retained as a variety of the former. While the clade is centered in the southern USA and Mexico, *C. chinensis* var. *chinensis* has a disjunct distribution in Australia and Asia, which is likely the result of relatively recent long-distance dispersal. *Cuscuta alata* from Mexico, previously considered synonymous to *C. applanata*, was found to be a distinct species based on both molecular and morphological evidence. *Cuscuta potosina* var. *potosina* and var. *globifera* segregate into two different subclades of the *C. chinensis* species complex. Because these two taxa are also different morphologically, var. *globifera* is described as a new species, *C. azteca*. *Cuscuta campestris*, an almost cosmopolitan weed species from another clade ("Clade B"; "*C. pentagona* complex"), is commonly misidentified in Asia as *C. chinensis*, which raises questions about the identity of the *Cuscuta* plants used in widely commercialized medicinal herbal mixtures. A taxonomic treatment with an identification key, descriptions, and illustrations is provided.

Keywords *Cuscuta chinensis* · Dodder · Medicinal plant · Molecular phylogeny · ITS · *trnL-F* · SEM · Systematics · Long-distance dispersal

Introduction

Genus *Cuscuta* (dodders) includes ca. 200 species of stem parasites that grow in a wide variety of climates and ecosystems on all the continents except Antarctica (Yuncker 1932; Costea 2007-onwards). Because some 15 of its species can produce considerable damage to agricultural crops, *Cuscuta* is considered the third most detrimental group of parasitic plants worldwide after *Striga* and *Orobanche* (Parker and Riches 1993; Costea and Tardif 2006). However, less known is that numerous dodder species are not noxious, but are rare or threatened with extinction (Costea and Stefanović 2009a).

The most recent monograph of the genus was published by Truman G. Yuncker in 1932. The highest diversity of species (ca. 140–150) is encountered in subg. *Grammica*, distributed primarily in North and South America. Within this subgenus, Yuncker (1932) distinguished 2 sections and 24 subsections. However, a recent broad-scale phylogeny of subg. *Grammica* revealed 15 major clades that bear little correspondence to Yuncker's sections and subsections (Stefanović et al. 2007). Subsequent to this "bottom-down" phylogenetic approach, we have initiated a series of targeted studies intended to revise the species-level systematics of each of these clades. To date, a total of seven subg. *Grammica* clades have been examined:

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four that comprise mostly species from the territory covered by Flora of North America (Costea et al 2005, 2006a,b,c, 2009; Costea and Stefanović 2009b), and two with predominantly Mexican dodders (Costea et al. 2008; Costea and Stefanović 2010).

We continue this series of studies with “clade H” (Stefanović et al. 2007), named here the “*Cuscuta chinensis* complex” in the absence of a formal section name (to be published with a new infrageneric classification of the genus; M. Costea et al., in prep.). The current species composition of this clade (Stefanović et al. 2007; Stefanović and Costea 2008) comes as a surprise because its members were included by Yuncker (1932, 1965) in three different subsections and two sections: *C. applanata* and *C. chinensis* in subsect. *Tinctoriae*; *C. potosina* in subsect. *Odontolepisae* (both subsections classified in sect. *Eugrammica*; Yuncker 1932), and *C. yucatana* in subsect. *Acutae* (sect. *Cleistogrammica*; Yuncker 1932). This would suggest that, from a morphological point of view, the clade exhibits a high degree of divergence among its species. Indeed, based on Yuncker descriptions, *C. yucatana* is the only species in the group with umbellate inflorescences and indehiscent capsules; C. *applanata* and *C. chinensis* have carinate calyx lobes, and *C. potosina* has 4-merous flowers (the rest of species have 5-merous flowers; Yuncker 1932, 1935, 1965). In addition, while *C. chinensis* is distributed widely in Australia and Asia, the remaining species are confined to the southern United States (US) and Mexico. Interestingly, despite the morphological heterogeneity and biogeographical disjunction, previous molecular studies resulted in largely unresolved phylogenies (Stefanović et al. 2007; Stefanović and Costea 2008). To build on these interesting preliminary results, we have sampled numerous additional collections in an attempt to test species limits based on their evolutionary relationships. The specific objectives of this study are to: (1) recover the evolutionary history of the *C. chinensis* clade based on plastid (*trnL-F)* and nuclear (ITS) sequences; (2) investigate the morphology and micromorphology of the taxa involved; and (3) revise the taxonomy of the clade with the description a new species, *C. azteca* and one nomenclatural combination.

Materials and methods

Taxon sampling

Numerous specimens belonging to the *C. chinensis* species complex (Appendix 1) as well as other groups were studied in connection with the upcoming treatments of *Cuscuta* for *Flora of North America*, *Flora Mesoamericana*, *Flora Neotropica*, and a future monograph of the genus. A subset of 16 accessions, representing five ingroup species of the *C. chinensis* complex, was used for the molecular phylogenetic analyses (Appendix 2). Efforts were made to sample multiple accessions of broadly distributed and morphologically variable species (e.g., *C. chinensis*). As a result, one to eight individuals are included in the molecular analyses for all species. *Cuscuta alata*, known from only two herbarium specimens and considered by Yuncker (1932) synonymous to *C. applanata*, was sampled and examined for the first time in this study. *Cuscuta sandwichiana* is a species of putative hybrid origin (Stefanović and Costea 2008), with the maternal parent belonging to the *C. pentagona* clade (“clade B”; Stefanović et al. 2007) and paternal to the *C. chinensis* clade (“clade H”; Stefanović et al. 2007). Three samples of this species were included in our expanded ITS dataset to further investigate its relationship with other members of the *C. chinensis* species complex. In addition, based on our previous broad phylogenetic analyses of *Cuscuta* subg. *Grammica* (Stefanović et al. 2007; Stefanović and Costea 2008), we selected two species, *C. victoriana* (“clade G”) and *C. americana* (“clade I”), to serve as close outgroups (Appendix 2).

Morphology and micromorphology

Descriptions and measurements are based on rehydrated herbarium material (Appendix 1). We examined the morphology of inflorescences, flowers, seeds and capsules under a Nikon SMZ1500 stereomicroscope equipped with a PaxCam Arc digital camera and Pax-it 7.2 software (MIS, Villa Park, IL). For scanning electron microscopy (SEM), we substituted the critical dry point with a hexamethydisilazane treatment (HMDS). Rehydrated herbarium samples (Appendix 1) were dehydrated using a series of ethanol (70%, 80%, 95% and 100%; each step 1 h), immersed for 1 h in 1:1 ethanol and HMDS, and passed through an overnight change of 100% HMDS. Samples were air-dried, mounted on stubs, and coated with 30 nm gold using an Emitech K 550 sputter coater (http://www.quorumtech.com/). Examination was performed and photos taken at 10 kV using a Hitachi SU1510 variable pressure SEM equipped with Quartz PCI software (Quartz Imaging, Vancouver, BC) for measurements and image analysis.

Pollen data were provided by Welsh et al. (2010) for all taxa except *C. alata*. We included here pollen of the latter species and increased the sampling for all other taxa (Appendix 1). Terminology regarding the micromorphology of flowers, seeds, and capsules follows that of Costea et al. (2006a); pollen terminology uses Welsh et al. (2010). Numerous microphotographs that illustrate the morphology of the floral parts, pollen, fruit and seeds for all the species, including the types, are available on the *Digital Atlas of Cuscuta* (Costea 2007-onwards). The geographical distribution of taxa, and information about phenology, elevation and host ranges were extracted from herbarium specimen labels.

Molecular methods and alignments

In addition to the DNA samples used in previous studies (Stefanović et al. 2007; Stefanović and Costea 2008), total genomic DNA was isolated from newly obtained specimens by the modified CTAB method (Doyle and Doyle 1987) and purified using Wizard® minicolumns (Promega, Madison, Wisconsin). DNA fragments for the regions of interest were obtained via PCR from total genomic DNA. The plastid genome (ptDNA) region containing the *trnL* intron, 3′ *trnL* exon, and intergenic spacer between this exon and *trnF* (i.e., the *trnL-F* region) was amplified using primers designed by Taberlet et al. (1991). The internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) containing ITS1, 5.8S, and ITS2 (here called ITS) was obtained using primers described by Baldwin (1992). PCR reagents and conditions as well as amplicon purification and sequencing procedures followed the protocols detailed in Stefanović et al. (2007). Sequences newly generated in this study are deposited with GenBank (accession numbers JN234819–JN234832; see Appendix 2). Sequences were aligned manually using Se-Al v.2.0a14001 (Rambaut 2002). The final alignment and trees obtained have been deposited with TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S11892).

Phylogenetic analyses

For each matrix separately as well as the combined dataset, phylogenetic analyses were conducted using PAUP* v.4.0b10 (Swofford 2002) under both parsimony and maximum likelihood criteria.

Given the moderate number of terminal units, the parsimony searches were conducted with a Branch-and-Bound algorithm, ensuring recovery of all of the most parsimonious (MP) trees. Matrix characters were treated as unordered (Fitch 1971), and all changes were weighted equally.

ModelTest ver. 3.7 (Posada and Crandall 1998) was used to determine the model of sequence evolution that best fit the data by the hierarchical likelihood ratio test (hLRT), starting with the parsimony-derived tree. The Hasegawa-Kishino-Yano (HKY) model of DNA substitution (Hasegawa et al. 1985), with rate variation among nucleotides following a discrete gamma distribution (HKY + G), was selected as the best-fit for all three data matrices. The full heuristic searches for maximum likelihood (ML) trees were performed using corresponding models of DNA evolution, each involving 100 replicates with stepwise random taxon addition, tree bisection-reconnection (TBR) branch swapping, and MULTREES option on.

Under both criteria, support for clades was inferred by nonparametric bootstrapping (Felsenstein 1985), using 200 heuristic bootstrap pseudoreplicates, TBR branch swapping, and MULTREES option on. Nodes receiving bootstrap (BS) values <60%, 60–75, and >75% were considered weakly, moderately, and strongly supported, respectively. Potential conflict between datasets was evaluated by visual inspection, looking for the presence of strongly supported but conflicting topologies from individual data partitions.

Results

General morphology and micromorphology

Unlike other major groups of subg. *Grammica* (Stefanović et al. 2007), *C. chinensis* species complex lacks a strong set of unifying floral or fruit morphological traits. With the exception of C. *yucatana*, the inflorescence is glomerulate, as in the *C. pentagona* species complex (“clade B”; Stefanović et al. 2007). Pollen grains belong to the most common type encountered in subg. *Grammica* and are similar in all taxa examined: 3(−4)-zonocolpate, ± prolate, with an imperforate tectum (Welsh et al. 2010). Calyx and corolla lobes are either acute or obtuse to rounded, depending on the species. One common discrete feature, however, is the presence of fleshy multicellular protuberances on the calyx lobes. Such microscopic structures have evolved multiple times in subg. *Grammica* but their morphology can help to distinguish among some *Grammica* clades and/or species. For example, in *C. chapalana* group (“clade K”; Stefanović et al. 2007) multicellular protuberances develop in the form of subapical horn-like appendages on the calyx and/or corolla lobes (Costea et al. 2011). Multicellular formations in *C. chinensis* clade originate from thickened, fleshy carinas of the calyx lobes in *C. chinensis* (incl. *C. applanata*) and *C. alata* (Fig. 1a–f; Fig. 2a–e), or they appear as isolated, dome-like structures distributed along the calyx lobes midveins in *C. potosina*, *C. azteca* and *C. yucatana* (Fig. 1g–i; Fig. 2f–g). The carinate calyx lobes resemble those encountered in *C. tuberculata*, while the dome-like protuberances are very similar to those of *C. desmouliniana* and *C. liliputana*, all three species being part of *C. umbellata* clade (Costea and Stefanović 2010). In *C. chinensis* (incl. *C. applanata*), in addition to the thickened, fleshy carenas that correspond to the midveins of calyx lobes, secondary thickened radial lines can present in the area of the sinuses between the lobes (Fig. 1a–b). Corolla lobes are also carinate in *C. alata* but while in *C. chinensis* (incl. *C. applanata*) the fleshy multicellular protuberances are restricted to the calyx lobes, in *C. alata* they are present both on the calyx and corolla lobes (Fig. 1c,f; Fig. 2c–e). Multicellular protuberances are mostly isolated in *C. chinensis* (Fig. 2a–b) or they form extensive crests or wings in *C. alata* (Fig. 2c–e, h, j). In all the taxa, multicellular protuberances begin to develop early in the bud, and bear one or several stomata. As in other *Cuscuta* species (Costea et al. 2009), their physiological role is unknown.

Sequences and alignments

Summary descriptions for sequences obtained from *trnL-F* and ITS 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 et al. 2007; Stefanović and 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 et al. 2007; Stefanović and Costea 2008), in which large portions of the *trnL-F* spacer 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), ITS sequence could not be obtained from one individual (*C. chinensis* #459, indicated by a dash in Appendix 2), presumably due to the poor quality of the DNA extracted from the herbarium specimen.

Phylogenetic analyses

Initial phylogenetic analyses were conducted on individual matrices. For both of these analyses, the strict consensuses of equally parsimonious trees (not shown) resulted in

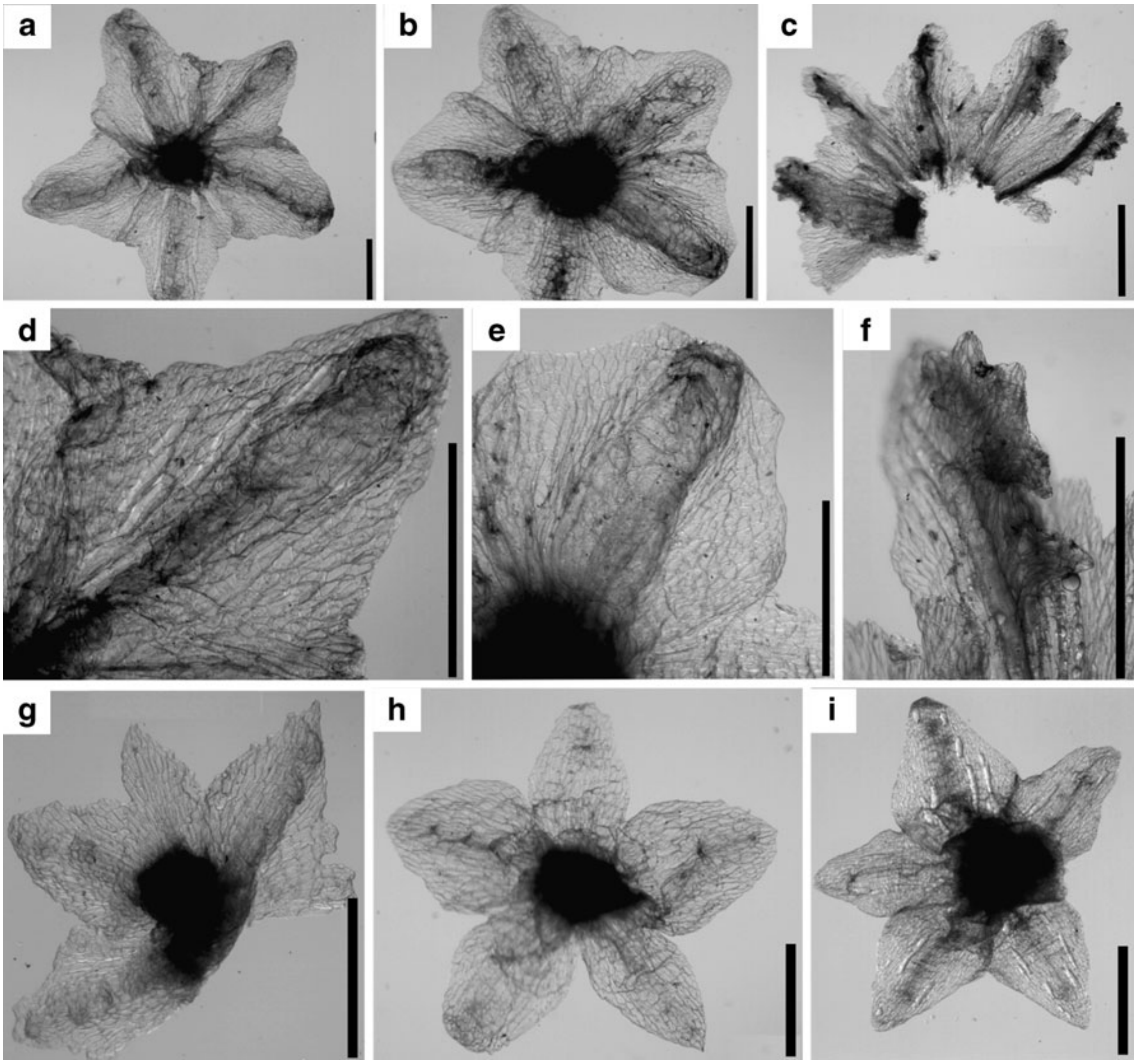

Fig. 1 **a–i** Morphology of dissected calyx in species of *Cuscuta chinensis* complex. **a**,**d** *Cuscuta chinensis* var. *chinensis*. **b**,**e** *C. chinensis* var. *applanata*. **c**,**f** *C. alata*. **g** *C. potosina*. **h** *C. azteca*. **i** *C. yucatana*. *Bars* 1 mm

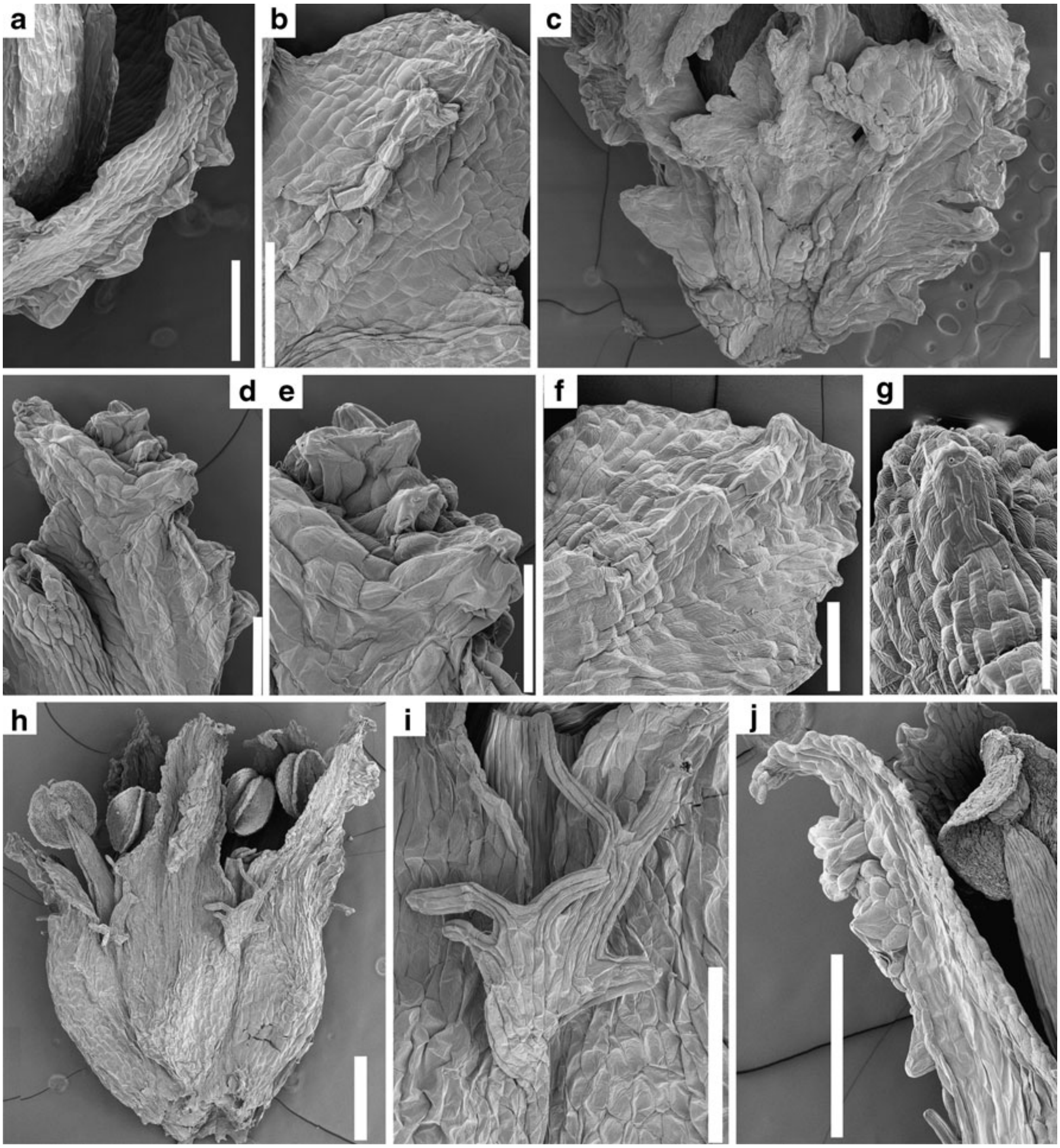

Fig. 2 **a–g** Scanning electron micrographs of multicellular protuberances on the calyx of *Cuscuta chinensis* species complex. **a** *C. chinensis* var. *chinensis*. **b** *C. chinensis* var. *applanata*. **c–e** *C. alata*. **f** *C. potosina*. **g** *C. azteca*. **h–j** Multicellular protuberances (**h**, **j**) and external scales (**h**, **i**) on the corolla of *C. alata*. *Bars* **a–d** 0.5 mm; **e–g** 0.25 mm; **h** 1 mm; **i** 0.3 mm; **j** 0.5 mm

relationships that were topologically identical or nearly identical to the respective results derived under the ML criterion (Fig. 3a–b). Also, clades recovered in each analysis were congruent with the tree structure recovered using data from the other matrix. Unlike the *trnL-F* phylogeny, the ITS tree included *C. sandwichiana*, representatives of which were found as a clade sister to the rest of the *C. chinensis* complex (Fig. 3b). This position is congruent with our previous studies on the hybrid origin of *C. sandwichiana* (Stefanović and Costea 2008). The inclusion/exclusion of *C. sandwichiana* ITS sequences from analyses did not affect phylogenetic relationships among members of the *C. chinensis* group (results not shown).

Because there were no strongly supported yet conflicting topologies from individual data partitions, we proceeded with a combined analysis. The ML analysis using the total-evidence approach resulted in a single best tree with greater resolution and stronger support compared to individual analyses. The phylogram illustrating the inferred relationships, as well as branch lengths, is shown in Fig. 3c. This tree is entirely congruent with the strict consensus tree resulting from the parsimony analysis (Table 1; tree not shown).

Table 1 Summary descriptions for sequences included in and trees derived from, individual and combined datasets of *Cuscuta chinensis* complex. *CI* Consistency index (excluding parsimony uninformative characters), *OTU* operational taxonomic unit, *RI* retention index

	trnL-F (plastid)	ITS (nuclear)[a]	Combined data
Number of OTUs included	18	21[a]	18
Sequence characteristics:			
Aligned length	508	645	1,153
Variable sites	56	106	160
Parsimony informative sites	20	36	50
Mean AT content (%)	64	50	56
MP search[b]:			
Number of trees	2	11	10
Length	65	124	183
CI/RI	0.954/0.917	0.96/0.933	0.956/0.902
ML search[c]:			
Number of trees	1	6	1
-lnL	1066.63	1561.8315	2681.8827

[a] Including three individuals of *C. sandwichiana*—a hybrid species whose plastid sequences belong within “clade B” of *Cuscuta* (Stefanović and Costea 2008)

[b] All parsimony searches were done under the Branch-and-Bound algorithm

[c] All likelihood searches were done under HKY + G model of DNA evolution

The topology resulting from the combined datasets revealed that the *C. chinensis* species complex is monophyletic, and strongly supported as such (100% BS; Fig. 3c). The first split within the *C. chinensis* group occurs between the *C. alata* lineage on one side, and the remainder of this complex on the other. Within the larger clade, two major groups were recovered. The first subclade is strongly supported (98% BS; Fig. 3c), and consists of two species, *C. yucatana* and *C. azteca*. The second subclade groups together all members of *C. chinensis* (including *C. applanata*) and the sole representative of *C. potosina*. While some resolution for the backbone relationships within this subclade is recovered, support remains weak (<60% BS; Fig. 3c).

Discussion

Cuscuta chinensis and *Cuscuta applanata*: one species with disjunct distributions

In the identification key of his monograph, Yuncker (1932) separated *C. chinensis* and *C. applanata* as follows: *C. chinensis*—“flowers mostly 3–3.5 mm long, styles commonly stoutish and not especially exserted”; *C. applanata*—“flowers mostly about 2–2.5 mm long; styles commonly slender and exserted”. In the text, however, he described *C. chinensis* flowers to be 2–3.5 mm long with “stoutish or slender” styles, while *C. applanata* had 2- to 3-mm-long flowers (the thickness of the styles was not mentioned). After a detailed examination of the types and numerous herbarium specimens we can affirm that the morphological distinction between *C. chinensis* and *C. applanata* is tenuous at best. Although we did not perform a morphometric analysis, the morphology and micromorphology of the flowers are remarkably similar in both *C. chinensis* and *C. applanata* (Figs. 1, 2; Costea 2007-onwards). Flowers and seeds tend to be slightly larger in *C. chinensis*, but the ranges of variation overlap with those of *C. applanata* (particularly of the flowers; see the description of *C. chinensis*). Indeed, without knowing their geographical provenence, many herbarium specimens of these two taxa would be difficult, if not impossible, to identify as *C. chinensis* or *C. applanata*. Together with the phylogenetic data, the morphological similarity strongly suggests that *C. chinensis* and *C. applanata* represent one single species with two geographical varieties. Therefore, *C. applanata* is retained as a variety of *C. chinensis*, and a new nomenclatural combination is proposed.

Cuscuta chinensis clade is one of the eight major groups of subg. *Grammica* that have originated and diversified in Mexico and the adjacent areas (Stefanović et al. 2007), and all other species of this complex are restricted to Mexico and southern US. Therefore, long-distance dispersal is the mostly likely explanation for the presence of var. *chinensis* in Australasia, Indo-Malaysia and the Asian part of Palearctic. However, in the absence of more data, the timeline and route of dispersal are difficult to hypothesize. Several other strongly supported cases of long-distance dispersal have been uncovered by the phylogeny of *Cuscuta* subg. *Grammica* (Stefanović et al. 2007), but nearly all these events are likely to be older because they are associated with completed speciation events. For example *C. victoriana* and *C. tasmanica* are endemic to Australia yet they belong to a large Mexican and Central American clade of subg. *Grammica* (“clade G”; Stefanović et al 2007). Similarly, C. *hyalina* from Eastern Africa, India and Pakistan is the only species of the *C. umbellata* complex, a clade otherwise distributed from the southern US to northern South America (Costea and Stefanović 2010). While these, presumably older, long-distance dispersal events also await an explanation, at the opposite end of the time scale are cases of dodder species that have managed to spread over large geographical areas in the last two centuries following anthropogenic pathways. One such example is *C. campestris*. This weedy species is inferred to be North American in origin but has dispersed successfully

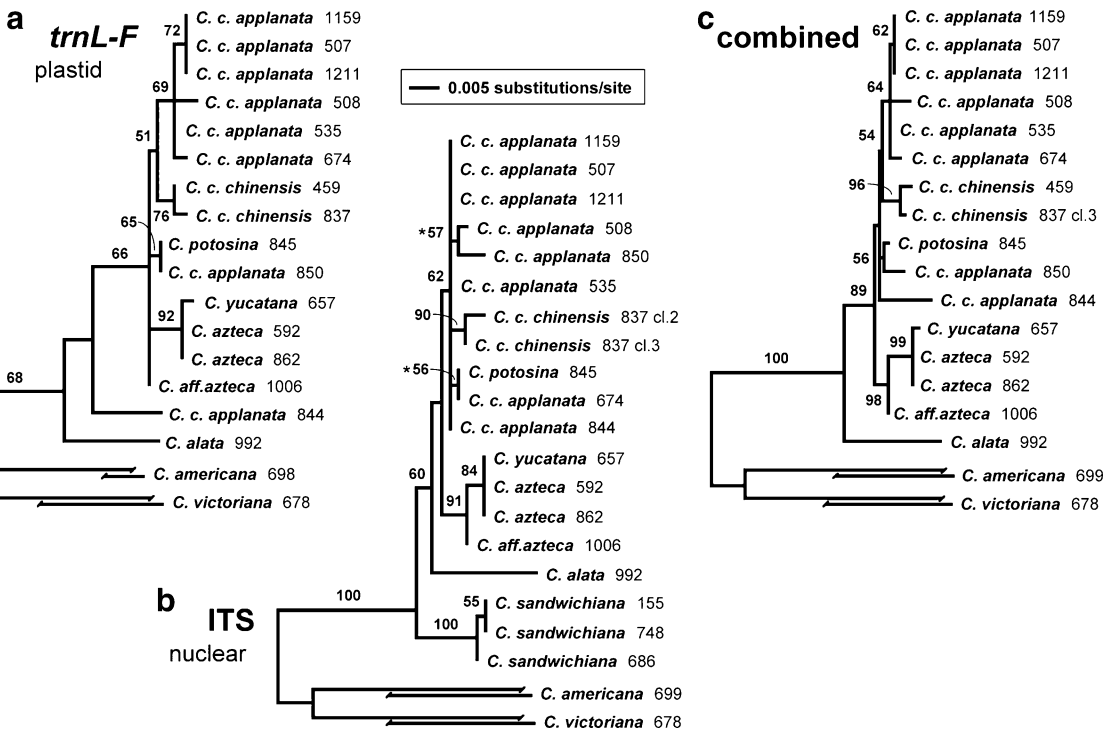

Fig. 3 Phylogenetic relationships among species of the *Cuscuta chinensis* (*C. c.*) complex obtained from maximum likelihood (ML) analyses of individual *trnL-F* (**a**) and ITS (**b**) as well as combined datasets (**c**), all under the HKY + G model of DNA evolution. *Asterisks* indicate nodes that collapsed in a strict consensus of equally likely trees (ITS). Closely related *C. americana* and *C. victoriana* are used as outgroups. Branch lengths are drawn at the same scale for all three phylograms. ML bootstrap values are indicated for nodes supported ≥50%. Numbers following species names correspond to DNA accessions (see Appendix 2)

worldwide despite the protection and quarantine legislative measures adopted by most countries (reviewed by Costea and Tardif 2006). Yet there is no indication that populations of *C. campestris* found on different continents have acquired sufficient genetic and/or morphological divergence to justify even the recognition of infraspecific taxa (Costea et al. 2006a). Instances of putative long-distance dispersal events that involve allopatric varieties are less common and not as distant geographically as the *C. chinensis* case. For instance, *C. obtusiflora* var. *obtusiflora* is encountered in South America while *C. obtusiflora* var. *glandulosa* and the majority of the clade members ("clade B", Stefanović et al 2007) are centered in North America. In the case of *C. chinensis*, more sampling from Asia-Australia and the use of faster evolving molecular markers would be necessary to recover the routes of migration that led to its extensive geographical distribution.

Cuscuta alata is a "good" species

Cuscuta alata described by Brandegee (1909) from Sinaloa was considered by Yuncker (1921) a "strongly keeled form" of *C. applanata* and the author considered the two names synonymous, which is how *C. alata* has been viewed since. Although it shares some morphological similarities with *C. chinensis* (incl. var. *applanata*), *C. alata* is the most dissimilar species of this clade both from a molecular and morphological point of view (Figs. 1, 2, 3). Multicellular projections in the form of crests or wings develop on the keels of both the calyx and the corolla lobes (Figs. 1, 2). In addition, the corolla lobes in *C. alata* are lanceolate and acute to acuminate, while in *C. chinensis* they are ovate and obtuse to rounded. More remarkably, flowers of *C. alata* often develop small external infrastaminal scales with 1–2 fimbriae on the dorsal part of the corolla tube, below the sinuses of the corolla lobes (Fig. 2h–i). In view of these findings, *C. alata* is reinstated as a species.

The former *Cuscuta potosina* includes two species

Cuscuta potosina var. *globifera* and *C. yucatana* formed a well-supported clade in the *C. chinensis* species complex, while *C. potosina* (var. *potosina*) was part of the sister clade that included both varieties of *C. chinensis* (Fig. 3). *Cuscuta potosina* var. *globifera* is also clearly distinct morphologically from *C. potosina* (var. *potosina*), *C.*

chinensis, and *C. yucatana* (see identification key and descriptions below). Consequently, *C. potosina* var. *globifera* is described as a new species, *C. azteca*. *Cuscuta globifera* W.Schaffn. ex Yunck. is an invalid name, and we preferred to avoid a new combination based on the basyonim of var. *globifera* Yunck. because several other very similar species epithets are already in use (e.g., *C. globosa* Ridl.; *C. globulosa* Benth., *C. globiflora* Engelm.).

Cuscuta potosina (var. *potosina*) was similar to *C. chinensis* var. *applanata* from a molecular point of view. However, it stands apart in this complex of species through its 4-merous flowers and 1-seeded capsules (see the identification key and descriptions). In this latter case, we considered appropriate to maintain *C. potosina* as a species based on its morphological distinction.

Cuscuta yucatana and *C. acuta*: convergent or reticulate evolution?

Cuscuta yucatana not only exhibits the same type of umbelliform inflorescence that characterizes the *C. umbellata* species complex ("clade L", Costea and Stefanović 2010), but it also resembles morphologically *C. acuta*—a species that belongs to the latter clade and grows in the Galapagos Islands and the Pacific coast of Ecuador and Peru. Both species have indehiscent fruit in clades with dehiscent (or preponderantly dehiscent) capsules. The similarity between the two species was also noted by Yuncker (1935), who included *C. yucatana* in the same subsection as *C. acuta* (subsect. *Acutae*) and differentiated the former species by the smaller flowers, longer pedicels, and obovoid shape of its capsules. Homoplasy is observed for many morphological characters in *Cuscuta*; for example, fruit indehiscence (Stefanović et al. 2007), pollen features (Welsh et al. 2010), and numerous gynoecium characters (Wright et al. 2011). Species from different clades of subg. *Grammica* may share some elements of morphology (e.g., the same type of inflorescence, flower parts or capsule). Unfortunately, too little is known about the ecology and host range of the two species to attempt a biological explanation of convergent evolution. One common ecological factor may be the halophytic habitat—coastal (*C. acuta*) or mainland (*C. yucatana*)—that these species apparently inhabit. Alternatively, the morphological similarity between *C. acuta* and *C. yucatana* may be explained through putative hybridization involving species from both clades. The evolutionary history of the *C. umbellata* clade has been marked by extensive reticulation among its members (Costea and Stefanović 2010), and hybridization involving the *C. chinensis* complex and species from another major clade of subg. *Grammica* ("clade B") have also been documented (Stefanović and Costea 2008). Although reticulation involving species from *C. chinensis* and *C. umbellata* clades was not been detected with the molecular markers used in this study, it may be revealed in the future by low-copy nuclear genes (Stefanović and Costea 2008).

Taxonomic treatment

Identification key for species of *Cuscuta chinensis* clade

1. Calyx lobes carinate with obtuse to rounded lobes; styles 0.9–2.2 mm long..2
1. Calyx lobes not carinate or weakly so (carina not visible without a microscope) with acute to mucronate lobes; styles 0.3–0.7 mm long.....................................3
2. Both calyx and corolla lobes with dorsal crests or wings; calyx lobes lanceolate, acute to cuspidate; infrastaminal scale bridge 0.3–0.8 mm..............*C. alata*
2. Crests or wings absent on the corolla; corolla lobes ovate, obtuse to acute; infrastaminal scale bridges 0.2–0.4 mm..*C. chinensis*
3. Flowers 4(−5) merous; capsules ovoid with 1(−2) seeds ....*C. potosina*
3. Flowers 5-merous; capsules depressed-globose with 3–4 seeds...4
4. Inflorescence umbellate; capsules indehiscent or irregularly dehiscent by a jagged line at the base of the capsules when pressure is applied..............*C. yucatana*
4. Inflorescence glomerulate; capsules regularly dehiscent at base of capsules...*C. azteca*

1. Cuscuta alata Brandegee, Univ. Calif. Publ. Bot. 3: 388. 1909.

Type: Mexico, Sinaloa, vicinity of Culiacan, 24 Aug 1904, *Brandegee s.n.* (holotype: UC).

Stems slender, yellow to creamy. **Inflorescences** glomerulate, often confluent; pedicels 0–0.4 mm; bracts 1 at the base of clusters, 1–2.4 mm long, ovate, carinate and winged, margins entire to denticulate, apex ± acute. **Flowers** 5-merous, 3–3.4 mm, membranous, white-creamy when fresh, creamy-yellow upon drying; papillae absent; laticifers visible in the bracts, calyx, corolla, anthers and ovary, isolated or in rows, ovoid to elongated; **calyx** 1.5–2 mm, straw-yellow, reticulate and shiny, shallowly cupulate, ca. as long as the corolla tube, divided ca. 1/2 to the base, tube 0.7–1.2 mm, lobes 0.8–1.2 mm, broadly overlapping and forming longitudinal folds at sinuses, broadly ovate-triangular, carinate and with multicellular protuberances along midveins that form longitudinal wings or crests (Fig. 1c, f; Fig. 2c–e), margins entire or irregular, apex obtuse, rounded to subacute; **corolla** 2.5–3 mm, tube 1.3–1.5 mm, campanulate but becoming ovoid or subglobose at fructification, lobes 1–1.5 mm, spreading, ca. as long as the tube, lanceolate, carinate and with multicellular protuberances along midveins that form longitudinal wings or crests (Fig. 2h,j) margins entire or irregular denticulate, apex acute to cuspidate, ± incurved (but not inflexed); **stamens** exserted, shorter than the lobes, anthers 0.4–0.6×0.25–0.35 mm, elliptical, filaments 0.5–0.8 mm; **infrastaminal scales** 1.4–1.8 mm long, equal or longer than corolla tube, bridged at 0.3–0.8 mm, oblong to obovate, long fringed, fimbriae 0.2–0.4 mm; sometimes small external infrastaminal scales with 1–2 fimbriae are present on the dorsal part of the corolla tube, below the sinuses of the corolla lobes (Fig. 2h–i); **styles** 1.6–2.2 mm, longer than the ovary, evenly filiform. **Capsules** circumscissile, 2–2.4×1.3–1.7 mm, subglobose to somewhat depressed, not thickened or risen around the small interstylar aperture, translucent, surrounded and capped by the withered corolla. **Seeds** 3–4 per capsule, 0.8–1.1×0.7–1.1 mm, angled, broadly-elliptic to subround, seed coat cells alveolate/papillate.

Distribution and ecology—Flowering Nov–Jan; elevation ca. 450 m; hosts: *Acacia*; Mexico: SW Chihuahua, Sinaloa.

2. Cuscuta azteca Costea and Stefanović, **sp. nov.**

Type: Mexico, San Luis Potosi, 1877, *Schaffner 780* (holotype: GH, isotypes: CAS, F, K, NY). Fig. 4.

≡ *Cuscuta potosina* Schaffner ex S. Watson, Proc. Amer. Acad. Arts 18: 124. 1883 var. *globifera* Schaffner ex Yuncker, Illinois Biol. Monogr. 6: 130. 1921. Type: Mexico, San Luis Potosi, 1877, *Schaffner 780* (holotype: GH, isotypes: CAS, F, K, NY).

Cuscuta yucatana similis, sed inflorescentia glomerulata et capsula dehiscentibus, depressa. Ab *Cuscuta chinensis* (*C. applanata* inclusa) different floribus minoribus et calycis lobi acuti, non carinati.

Stems slender, orange-yellow. **Inflorescences** glomerulate, often confluent; pedicels 0.4–1.3 mm; bracts 1 at the base of clusters and 0–1 at the base of pedicels, ovate, 1.2–1.8 mm long, margins entire, apex acute. **Flowers** 5-merous, 2.0–2.6 mm, membranous, white when fresh, creamy-brownish when dried, papillae absent; laticifers visible in the corolla and ovary/capsule, isolated, elongated; **calyx** 1.4–1.7 mm, straw-yellow, membranous, finely reticulate and shiny, cupulate, equalling corolla tube, divided ca. 2/3 the length, tube 0.3–0.5 mm, lobes 0.8–1.3 mm, not basally overlapping or only slightly so, ovate, not carinate but occasionally with a few multicellular protuberances along midveins (Fig. 1h; Fig. 2g), margins entire, apex acute; **corolla** 1.5–2.1 mm, the tube 0.8–1.2 mm, campanulate but becoming globose in fruit, lobes 0.8–1.2 mm, erect to spreading, shorter to equalling the tube, ovate-triangular, margins entire or with a few irregular teeth, apex acute, straight to slightly incurved; **stamens** ± exserted, shorter than corolla lobes, anthers 0.25–0.3×0.25–0.3 mm, ovate to subround, filaments 0.3–0.6 mm; **infrastaminal scales** 0.9–1.3 mm long, equalling to longer than corolla tube, bridged at 0.1–0.25 mm, oblong, fringed in the distal 1/2, fimbriae 0.15–0.3 mm; **styles** 0.4–0.7 mm, shorter than the ovary, evenly filiform. **Capsules** circumscissile, 1.2–2.5×1–1.8 mm, depressed-globose, not thickened or risen around the moderately large interstylar aperture, translucent, loosely surrounded by the withered corolla. **Seeds** 3–4 per capsule, 0.8–1×0.7–0.9 mm, angled, broadly elliptic to subround, seed coat cells alveolate/papillate.

Distribution and ecology—Flowering Jul–Nov; elevation 370–2,200 m; herbaceous hosts: *Ayenia*, *Cosmos*, *Dalea*, *Heterosperma*, *Evolvulus*, and others; US: Arizona and New Mexico; Mexico.

3. Cuscuta chinensis Lam., Encycl. Méthodique, Botanique 2(1): 229. 1786. Type: from a plant accidentally grown at "Jardin du Roi" (Jardin des Plantes) in Paris from seeds presumably brough from China, 1784, collector unknown, (lectotype: P, fragments at NY, Yuncker 1932).

= *Cuscuta ciliaris* Hohen. ex Boiss. Diagn. Pl. Orient., ser. 2, 3: 129–130. 1856. Type: (currently) Iraq: in cultis aridis prope Mossul, 1 Sep 1841, *Kotschy 431* (lectotype: MO; isolectotypes: MO, P; Yuncker 1932). ≡ *Cuscuta chinensis* var. *ciliaris* (Hohen. ex Boiss.) Engelm., Trans. Acad. Sci. St. Louis 1(3): 480. 1859.

= *Cuscuta carinata* R. Br. Prodr. 491. 1810. Type: Australia, "New Holland" (Bay of Inlets, Cape Grafton; New South Wales; Australia), 1770, *Banks & Solander s.n.* (lectotype here designated: BM). ≡ *Cuscuta chinensis* var. *carinata* (R. Br.) Engelm. Trans. Acad. Sci. St. Louis 1(3): 480. 1859.

Stems thin, yellow. **Inflorescences** glomerulate to dense paniculiform, often confluent; pedicels 0.4–3 mm; bracts 1 at the base of the clusters, 1.6–3.3 mm long, subround, ovate to lanceolate, carinate, margins entire to denticulate, apex obtuse to rounded. **Flowers** 5-merous, 2.5–4 mm, membranous, white-creamy when fresh, creamy-yellow upon drying; papillae absent; laticifers evident in the bracts, calyx, corolla, anthers and ovary, isolated or in rows, ovoid to elongated; **calyx** 1.5–2.5 mm, straw-yellow, reticulate and shiny, shallowly cupulate, ca. as long as the corolla tube, divided ca. 1/2 to the base, tube 0.8–1.2 mm, lobes 0.8–1.4 mm, basally overlapping, ovate to broadly ovate-triangular, carinate and with irregular multicellular protuberances along midveins (Fig. 1a, d, b, e; Fig. 2a–b) margins entire or irregular, apex obtuse to rounded; **corolla** 2–3.8 mm, tube 1–1.9 mm, campanulate but becoming ovoid to globose in fruit, lobes 0.9–2 mm, spreading, ca. as long as the tube, ovate, margins entire or irregular, apex acute to obtuse, ± incurved (but not inflexed); **stamens** exserted, shorter than the lobes, anthers 0.4–0.6×0.4–

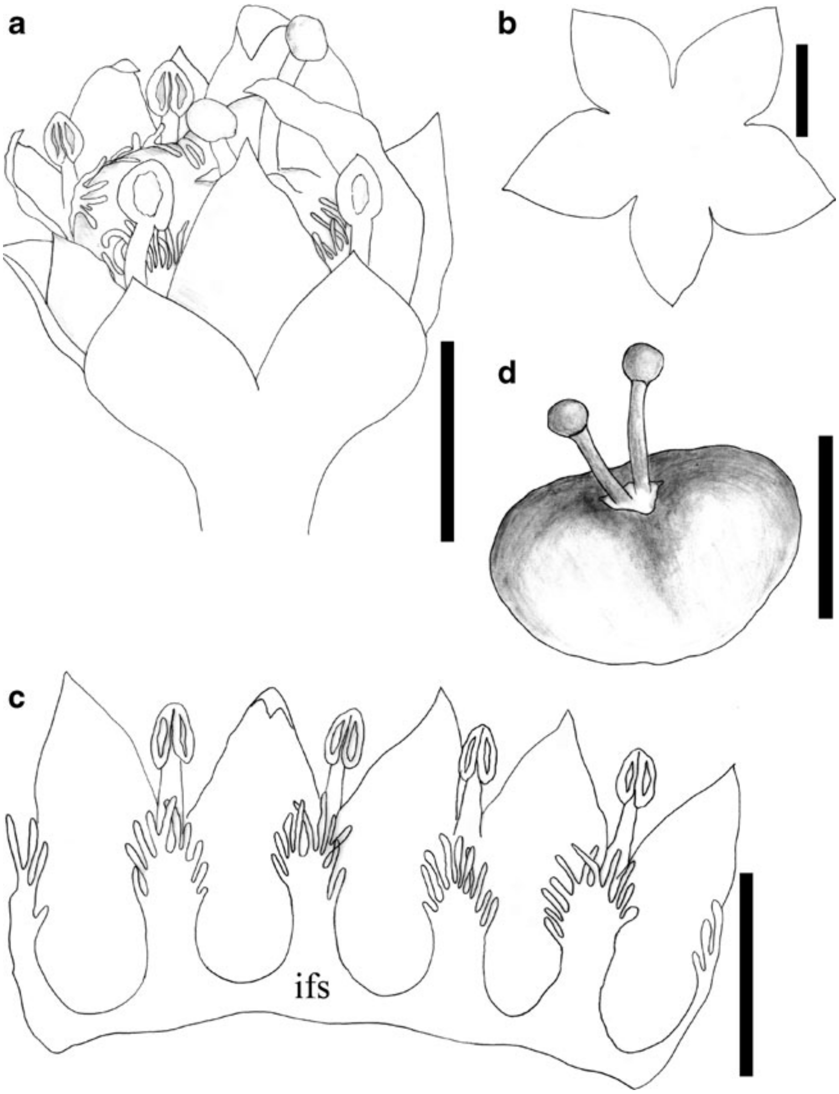

Fig. 4 Morphology of *Cuscuta azteca*. **a** Flower. **b** Outline of dissected calyx. **c** Dissected corolla showing infrastaminal scales (ifs). **d** Capsule. *Bars* 1 mm

0.5 mm, broadly ovate to subround, filaments 0.4–0.8 mm; **infrastaminal scales** 1.2–2.2 mm long, equal or longer than corolla tube, bridged at 0.2–0.4 mm, obovate, long fringed, fimbriae 0.2–0.4 mm; **styles** 0.8–1.8 mm, equal or longer than the ovary, evenly filiform. **Capsules** circumscissile, 1.8–2.8×0.8–2 mm, subglobose to slightly depressed not thickened or risen around the small interstylar aperture, translucent, surrounded and capped by the withered corolla. **Seeds** 3–4 per capsule, 0.85–1.4×0.8–1.2 mm, angled, broadly-elliptic, seed coat cells alveolate/papillate.

Identification key for varieties

1. Inflorescence glomerulate; bracts ovate to lanceolate; flowers 2.6–4 mm; calyx 1.7–2.5 mm, lobes ovate; corolla 2.2–3.8 mm, tube 1–1.9 mm, lobes 1–2 mm; apex acute to obtuse; infrastaminal scales 1.4–2.2 mm; capsules 1.8–2.8×0.8–2 mm; seeds 1.1–1.4×1–1.2 mm..*C. chinensis* var. *chinensis*

1. Inflorescences glomerulate to dense paniculiform, bracts ovate to subround; flowers 2.5–3.5 mm; calyx 1.5–2 mm, lobes ovate to broadly-ovate; corolla 2–3.3 mm; tube 1–1.5 mm, lobes 0.9–1.3 mm, apex obtuse to rounded; infrastaminal scales 1.2–1.8 mm; capsules 1.8–2.5×0.8–1.6 mm; seeds 0.85–1.2×0.8–1.1 mm..*C. chinensis* var. *applanata*

2.1. Cuscuta chinensis var. **chinensis** (Figs. 1 a, d; 2a)

Distribution and ecology—Flowering Jun–Oct; Dec–Mar; Feb–May. Africa: Ethiopia; Western Asia: Afghanistan, Iran, Iraq; Middle Asia: Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan; Mongolia; Russia; China; Tropical Asia: India, Sri Lanka; Indonesia; Eastern Asia: Korea, Japan, Taiwan, Thailand; Australasia: Australia. Generalist dodder parasitizing hundreds of mostly herbaceous hosts from numerous families (e.g., see Patel and Patel 2010); 200–3,000 m.

Note—Numerous herbarium collections from Asia labeled "*C. chinensis*" are in fact *C. campestris*—a North American species that has become almost cosmopolitan (Costea and Tardif 2006). *Cuscuta campestris* belongs to "clade B" (Stefanović et al. 2007) and can be distinguished easily from *C. chinensis* by its non-carinate calyx lobes, corolla lobes with inflexed lobes and indehiscent capsules. Distinguishing these two species is important because *C. chinensis* is used widely as a medicinal plant in Asia (e.g., Bao et al. 2002), and the medicinal efficacy, or even safety of *C. campestris*, has not been tested.

2.2. Cuscuta chinensis var. **applanata** (Engelm.) Costea and Stefanović, **comb. et stat. nov.** Basionym: *Cuscuta applanata* Engelm., Trans. Acad. Sci. St. Louis 1: 479. 1859.

Type: US Arizona, 1851–1852, Wright 1623 (lectotype: MO, isolectotypes K, NY, US). (Fig. 1b,e; Fig. 2b).

Distribution and ecology—Flowering Jun–Oct; elevation 400–2,200 m, hosts: various herbaceous species, including *Amaranthus*, *Ambrosia*, *Anisacanthus*, *Bahia*, *Baileya*, *Boerhavia*, *Chamaecrista*, *Chamaesaracha*, *Coldenia*, *Croton*, *Dalea, Flaveria*, *Ipomoea*, *Parthenium*, *Sanvitalia*, *Solanum*, *Tragia*, *Viguiera*, and probably others. US: Arizona, New Mexico, Texas and Utah; Mexico.

4. Cuscuta potosina Schaffner ex S. Watson, Proc. Amer. Acad. Arts 18: 124. 1883.

Type: Mexico, San Luis Potosí, in arenosis circa urbem, 1876, *Schaffner 779* (holotype: GH, fragment NY; isotypes: K, MO, NY).

Stems slender, orange-yellow. **Inflorescences** small glomerules; pedicels 0.2–1 mm; bracts 1 at the base of clusters and 0–1 at the base of pedicels, ovate, 0.8–1.3 mm long, weakly carinate and with a few multicellular protuberances, margins entire, apex acute. **Flowers** 4(−5)-merous, 1.5–2.2 mm, membranous, white when fresh, creamy-brownish when dried, papillae present on corolla and sometimes calyx lobes; laticifers visible mostly in the ovary/capsule, isolated, elongated; **calyx** 0.8–1.3 mm, straw-yellow, membranous, ± shiny, campanulate, equalling corolla tube, divided ca. 2/3 the length, tube 0.3–0.4 mm, lobes 0.6–0.8 mm, not basally overlapping, triangular-ovate, weakly carinate (Figs. 1g; 2f), margins irregular, apex acute to mucronate; **corolla** 1–1.7 mm, the tube 0.4–0.6 mm, campanulate becoming urceolate in fruit, lobes 0.4–0.8 mm, erect, shorter to equalling the tube, triangular, margins entire to irregularly denticulate, apex acute to mucronate, slightly inflexed; **stamens** not exerted, shorter than corolla lobes, anthers 0.2–0.3 × 0.25–0.3 mm, round to broadly ovate, filaments 0.2–0.3 mm; **infrastaminal scales** 0.6–0.9 mm long, equalling to longer than corolla tube, bridged at 0.1–0.2 mm, oblong to slightly obovate, fimbriae scarce, distal, 0.1–0.2 mm long; **styles** 0.3–0.6 mm, shorter than the ovary, evenly filiform. **Capsules** circumscissile, 1.2–1.5 × 0.9–1.2 mm, ovoid, not thickened or risen around the inconspicuous interstylar aperture, translucent, capped by the withered corolla. **Seeds** 1(−2) per capsule, 0.7–0.9 × 1–1.16 mm, not angled (subspherical), wider than long, subround, seed coat cells alveolate/papillate.

Distribution and ecology.—Flowering Jul–Oct; elevation 1,850–2,350 m; hosts herbaceous, generally not identified in the herbarium specimens. Central Mexico.

5. Cuscuta yucatana Yunck., Bull. Torrey Bot. Club 62: 511. 1935.

Type: Mexico, Yucatan, Progresso, in salt marsh, August 11–15, 1932, *W.C. Steere 3077* (holotype: F; isotype: MICH).

Stems slender to moderate, orange-yellow. **Inflorescences** umbellate; pedicels 0.5–3 mm; bracts 1 at the base of clusters and 0–1 at the base of pedicels, ovate to lanceolate, 1–2 mm long, margins entire or irregulary denticulate, apex acute. **Flowers** 5-merous, 1.8–2.4 mm, membranous, white when fresh, creamy-white when dried, papillae present on corolla lobes; laticifers visible in the calyx and corolla, isolated, elongated; **calyx** 1–1.5 mm, yellow, membranous, finely reticulate and shiny, campanulate, equalling to longer than corolla tube, divided ca. 2/3 the length, tube 0.4–0.6 mm, lobes 0.6–1 mm, not basally overlapping or only slightly so, ovate, not carinate but sometimes with a few multicellular protuberances along midveins (Fig. 1i), margins entire or with a few irregular teeth, apex acute to mucronate; **corolla** 1.5–2.2 mm, the tube 0.6–1 mm, campanulate, lobes 0.8–1.1 mm, reflexed, equalling or somewhat longer than the tube, lanceolate, margins entire, apex acute, slightly incurved; **stamens** exserted, shorter than corolla lobes, anthers 0.2–0.3 × 0.2–0.25 mm, subround to round, filaments 0.4–0.6 mm; **infrastaminal scales** 0.8–1 mm long, equalling to longer than corolla tube, bridged at ca. 0.1 mm, obovate, fringed in the distal 1/2, fimbriae 0.1–0.3 mm; **styles** 0.4–0.6 mm, ± equalling the ovary, evenly filiform. **Capsules** indehiscent or sometimes opening by a jagged line at the base of the capsules when pressure is applied, 2–3 × 1–1.6 mm, depressed-globose, not thickened or risen around the moderately large interstylar aperture, translucent, surrounded by the withered corolla. **Seeds** 3–4 per capsule, 0.8–1.1 × 0.7–0.9 mm, angled, broadly elliptic to subround, seed coat cells alveolate/papillate.

Distribution and ecology.—Flowering Jul–Nov; elevation 20–2,700 m; herbaceous halophytic hosts not identified in the herbarium specimens; South Mexico and Guatemala.

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HUFU, IAC, IEB, IND, J, JEPS, LL, LP, LPB, LPS, K, MEL, MERL, MEXU, MICH, MO, NMC, NY, OAC, OKLA, OSC, OXF, PACA, PRE, QCNE, QFA, P, PACA, RB, RSA, SAM, S, SD, SGO, SI, SPF, TEX, TRT, TRTE, UB, UBC, UCR, UCT, UNB, UNM, UPRRP, UPS, US, USAS, ZEA, WTU and XAL for supplying plant material. Miguel García and Guy Nesom kindly provided comments that improved the quality of the article. Ignacio García Ruiz, Eleazar Carranza and Tom Van Devender sent us additional plant material. This research was supported by NSERC of Canada Discovery grants to M.C. (327013) and S.S. (326439).

Appendix 1

Herbarium vouchers used for taxa description and examined for micromorphology. Species accession code: country, region, location, elevation (m a.s.l.), date, collector(s), herbarium acronym

1. *Cuscuta alata*. MEXICO. Sinaloa. La Cofradía, 26 Oct 1904, *Brandegee s.n*. (MO); Cerro Colorado, 450 m, 10 Dec 1939, *Gentry 992* (ARIZ). Mpio. Culiacán, on Rd at Varejonal, 10 Km from the international Hwy, 12 Oct 1984, *Ochoa Avalos & Bojórguez 196* (MEXU). **Chihuahua.** South Western Chihuahua, Aug–Nov 1885, *Palmer 142* (GH, K, MEXU). **2. *Cuscuta azteca*. U.S.A. Arizona**. Cochise Co.: Mule Mts., W side, 18 Sep 1961, *Goodding 290–61* (ASU). Greenlee Co.: U.S. 666, 7.6 mi S of Hog Trail Saddle, 7 Sep 1968, *Pinkava et al. 13685* (ASU). **New Mexico**. Otero Co.: Caballero Canyon, 2103 m, 13 Jul 1997, *Fletcher 2348* (UNM). Mogollon Mts., Sep 1881, *Rusby 295* (MO). **MEXICO. Chihuahua**. Mpio. Temosachi: Nabogame, 28°30′N 108°30′W, 19 Sep 1987, *Laferrière 1054* (MEXU). **Distrito Federal**. Close to San Pablo, 7 Sep 1952, *Rzedowski 1742* (MEXU). **Durango**. Mpio. de Mezquital, Santa María de Ocotán, 27 Aug 1984, *Gonzáles 1465* (MEXU). **Federal District**. Valley of Mexico, Pedrigal, 2225 m, 8 Oct 1896, *Pringle 6575* (CAS, F, K, MEXU, MO, S). **Hidalgo**. Mpio. de Hidalgo: near Tula (de Allende), 2072 m, 14 Oct 1902, *Pringle 11305* (F, K). Mpio. Pachuca de Soto: Presa Jaramillo, 4 Km N of Pachuca, 2750 m, 2 Nov 1983, *Medina 2493* (F, MICH). **Morelos**. Cuernavaca, 31 Aug 1910, *Orcutt 3874* (CAS, MO). **Oaxaca**. Vicinity of Oaxaca, Juárez Hill, 25 Jul 1947, *Kenoyer 1504* (GH). Mpio. San Lorenzo Albarradas: Hierve el Agua, 1757 m, 23 Oct 2003, *Torres & Soriano Silva 2360* (MEXU). **Puebla**. Mpio. Caltepec: vicinity of San Luis Atolotitlán (formerly San Luis Tultitlanapa), 9 Jul 1908, *Purpus 3554* (F, MO). Mpio. Tehuacán: El Riego, Aug 1905, *Rose & Painter 9888* (K); San Diego, 5 Km from Tehuacàn on Rd to Teotitlan, 1460 m, 12 Dec 1963, *McKee 11042* (K); near Cerro el Pinar, 28 Aug 1945, *Alexander & Hernández 2242* (NY). **Querétaro**. Mpio. Pinal de Amoles, 21º04′59.6N 99º36′20.5″, 1546 m, 27 Aug 2007, *Ramírez-Amezcua 1099* (IEB). Mpio. Tequisquiapan: Tequisquiapan, 1 Nov 1950, *Matuda 18565* (MEXU). **San Luis Potosí**. Mpio. de Santa María del Río: Paso de Tortugas, San José Albuquerque, 2000 m, 6 Oct 1952, *Takaki 10102* (ENCB, MEXU); Sierra de San Miquelito, 2200 m, 10 Sep 1954, *Rzedowski 4443* (MEXU); 5 Km al NE de Laguna Seca, 2000 m, 30 Aug 1955, *Rzedowski 6298* (MEXU). **Sonora.** Mpio. Álamos: El Guayabo crossing of Río Cuchujaqui, 2.6 km NE of Sabinito Sur, 14 km (by air) E-SE Álamos; 27°00′05″N 108°47′08″W, 370 m, 21 Nov 1993, *Steinmann 93–349 et al*. (ARIZ, ASU, UC, UCR). Mpio. Yécora: Restaurant Puerto de la Cruz, 14 km W of Yécora on MEX 16, 28°22′30″N 109°01′42″W, 1920 m, 6 Sep 1996, *Van Devender 96–451 et al.* (ARIZ, USON, WLU). **3.1. *C. chinensis*** var. ***chinensis*. AUSTRALIA.** Northern Territory, 7 May 1948, *Sprecht 390* (CANB)**;** Western Australia: at NE end of airstrip One Arm Point, N Dampier Peninsula, W Kimberley, 16º27′S 123º04′ E, 9 Apr 1993, *Carter 628* (CANB, PERTH); 9 Mar 1989, *Keighery 11100* (PERTH); Queensland, 23 Feb 1934, *Garvey 71* (BRI). **AFGANISTAN.** Chanabad, 7 Oct 1927, Bukinitach 7 (NY). **CHINA**. Anhui Prov.: Qing-hill Dongzhi, 300 m, 9 Sep 1992, *Yao 12002* (PE, OSU). Beijing Municipality: Miyunxian, 8 Sep 1951, *Wang Web-Tsai 2378* (RSA); Xianshan, 11 Jun 1956, *Zhao Fu et al. 1425* (PE, RSA); Xizhimenwai, 30 Jun 1953, *Zhao Fu et al. 338* (PE, RSA); near Beijing, Nov 1874, *Bushell s.n* (NY). Inner Mongolia Autonomous Region, Yin Shan Mts., 22 Jul 1990, *Boyd et al. 4379* (RSA). Jiangsu Prov., Yuntaishan, 20 Aug 1981, *Yao 8388* (NY). Shantung Prov.: Tsinanfu, 4 Sep 1930, *Chiao 3033* (GH, NY); Lao Shan, 16 Aug 1930, *Chiao 2931* (GH, NY). Xinjiang Prov.: 3–5 Km N of Dushanzi, 16 Jun 1989, *Liston 823–21* (NY, RSA). **NORTH KOREA**. Rikiho, Sep 1930, *Dorsett & Morse* 6336 (NY). **THAILAND**. Nakornrajsima, 3 Mar 1959, *Surapat 137*(IND). **3.2. *C. chinensis*** var. ***applanata*. U.S.A. Arizona**. Cochise Co.: Pearce, 14 Aug 1996, *McGill 6746* (ASU); vicinity of Tombstone, 12 Sep 1969, *García 348* (MEXU); Huachuca Mountains, Miller Canyon, 1 Oct 1929, *Jones s.n.* (RSA). **New Mexico**. Bernalillo Co.: NW of Albuqueque, 1555 m, 24 Sep 1966, *Britt 23* (UNM); Albuquerque, 28 Sep 1932, *Casteller 7339* (UNM). Dona Ana Co.: White Sands Missile Range, 1290 m, 23 Sep 1990, *Spellenberg & Mahrt s.n.* (NMC, NY, RSA, UC). Sierra Co.: 2 Mi W of Hillsboro, 1676 m, 9 Sep 1904, *Metcalfe 1288* (NMC); Kingston, 2011 m, 13 Sep 1904, *Metcalfe 1300* (CAS). **Texas**. Deaf Smith Co.: Glenorio, 3 Sep 1967, *Waller 1537* (ASU); 1 mi S of Glenorio, 3 Sep 1967, *Waller 1540* (ASU). Presidio Co.: 9.3 mi S of Shafter along Hwy 67, 29°43′N 104°21′W, 1020 m, 24 Jul 1973, *Henrickson 11268* (RSA). **MEXICO. Aguascalientes**. Mpio. Calvillo, NW of Escondida, 19 Sep 2002, *García 5083* (MEXU). **Chihuahua**. Mpio de Chihuahua: near Chihuahua, 2 Sep 1886, *Pringle 784* (F, G, K, MEXU, MO, S, UPS, US); Sierra Mapula, W base of Pico Chihuahua Viejo, 28°33′N 105°51′30″W, 1500–1675 m, 20 Jul 1977, *Lehto 21612* (ASU). Mpio. Jiménez: Biosphere Reserve

Mapimí, 26°52′49″N 103°53′9.8″W, 1110 m, 6 Jul 1997, *García 2637* (MEXU). Mpio. Madera: vicinity of Nicolás Bravo, 2200 m, 18 Aug 1994, *Quintana et al. 3126* (NMC). **Coahuila**. Ca. 45 Km W of Cuatro Ciénagas, 1100 m, 4–5 Sep 1941, *Johnston 8826* (F, GH); 71 Km SSW of Cuatro Cienegas, on Rd 30 to Torreón at Km 159, 26°34′N 102°24′W, 820 m, 13 Sep 1978, *Iltis & Lasseigne 70* (F, K, MEXU); 25 mi E of Americanos, 22 Aug 1937, *Wynd 754* (GH, IND, K, NY, MO, S). **Durango**. Mpio. San Juan del Río: San Juan del Rio, 20 Sep 1946, *Xolocotzi et al. 2615* (MEXU). Mpio. Gómez Palacio: Dinamita, 2 Aug 1984, *Pacheco 67* (MEXU). Mpio. Mapimí: 8 Km SE of Mapimí, 25°47′N 103°46′W, 1999 m, 8 Sep 1983, *Torrecillas 237* (MEXU, NY). Mpio. Tlahualilo, Biosphere Reserve Mapimí, 26°34′2.2″N 103°45′0.6″W, 1160 m, 18 Sep 1999, *García 3576* (MEXU). **Guanajuato**. Mpio. San Miguel de Allende: 8 mi N of San Miguel de Allende, 6 Jul 1963, *Harris 25806* (GH). **Hidalgo**. 8 km SSW of Alfajayucan, 2000 m, 6 Sep 1965, *Quintero 3026* (MEXU). **Oaxaca**. Mpio. Villa Tepelmeme de Morelos: La Mesa del Calvario, 18°01′N 97°21′W, 1920 m, 18 Sep 1994, *Salinas & Martíz-Correa 7390* (MEXU). **Puebla**. Mpio. Caltepec: Cerro Coatepec, Sof San Luis Atotitlán, 18°09′N 97°27′W, 2115 m, 9 Sep 1993, *Salinas et al. 7541* (MEXU). Mpio Tehuacán: near Tehuacán, 20 Aug–3 Sep 1905, *Rose et al. 10275* (US); Tehuacán, El Riego, 30 Aug 1905, *Rose et al. 9888* (MEXU, US); 4 Km E of San Pablo Tepetzingo, 24 Jul 1979, *Chiang et al. 61* (MEXU); 1 Sep 1979, *Chiang et al. 315* (MEXU). Mpio. Zapotitlán: "El Potrero", 2–3 Km S of Zapotitlán de las Salinas, 1500 m, 23 Jul 1992, *Salinas & Juárez-Jaimes 6977* (MEXU). **Querétaro**. Mpio. Pinal de Amoles, 13.8 Km at SE of Mex 120, 21°04′59.6″N 99°36′20.5″W, 1546 m, 24 Aug 2007, *Ramírez-Amezcua 1099* (IEB). **San Luis Potosí**. Mpio. de San Luis Potosí: 18 Km E of San Luis Potosí, 1900 m, 8 Jul 1959, *Rzedowski 11200* (MEXU); Mpio. de Matehuala: Matehuala, 29 Jun 1970, *Clarke 137* (UCR); Charcas, Jul–Aug. 1934, *Lundell 5493* (MICH); 2 mi S of Venado, 1950 m, 6 Sep 1939, *Shreve 9323* (GH). **Sonora**. Mpio. Ónavas: Rancho La Mula, 28.2 km SE of Río Yaqui on MEX 16 (KM 195 E of Hermosillo), 28°28′50″N 109°22′W, 790 m, 30 Aug 2000, *Van Devender & Reina-G. 2000–506* (ARIZ, WLU). Mpio. Santa Cruz: 0.8 km S of Santa Cruz on road to San Lázaro, 31°11′23″N 110°36′22″W, 1340 m, 17 Aug 2001, *Van Devender et al. 2001–710* (ARIZ, USON, WLU). **Zacatecas**. 35 mi NNW of Concepción del Oro, 24°38′N 101°56′W, 1706 m, 2 Sep 1971, *Henrickson 6353* (MEXU). **4. *C potosina*. MEXICO**. **Aguascalientes**. Mpio. de Calvillo: extreme N of Temazcal, 1900 m, 11 Oct 1987, *García 2834* (IEB). **Jalisco**. Mpio. Ojuelos de Jalisco: Mesa el Toro, W of Ojuelos, 21°56′33″N 101°70′50″W, 2127 m, 28 Aug 2002, *Machuca et al. 8820* (IBUG). **San Luis Potosí**. Mpio. de Santa María del Río, 1850 m, 27 Aug 1954, *Rzedowski 3894* (MEXU); San Luis Potosí, 1879, *Schaffner 379* (B, MEXU); [same area], 1878, *Parry & Palmer 431* (MO). **Estado de Mexico**. Rd Ameca-Cautla (exit to Tlamaca), 31 Jul 1989, *Arreola Gonzáles 13* (MEXU); Mpio Tequiquiac: Cerro Colima Ladera Norte, 8 Nov 1980, 2350 m, *Romero-Rojas 825*(MEXU). **Guanajuato**. Mpio. de San Diego de la Unión, 11 Sep 1997, *Pérez et al. 3707* (IEB). **Puebla** (?). Loma del Ciervo, 20 Aug 1906, *Altamirano 1606* (MEXU; "Pue" was added to the label at a later date but we could not find this locality anywhere in Mexico). **Querétaro**. Hacienda Ciervo, 20 Aug 1905, *Rose 9650* (MEXU, NY, US). **5. *C. yucatana*. MEXICO**. **Chiapas**. Mpio of Tenejapa: Paraje Tenejapa, 2700 m, 7 Nov 1971, *Breedlove & Sith 22017* (MEXU). Mpio. San Cristóbal Las Casas, 2200 m, 2 Sep 1974, *Breedlove 37107* (MEXU); out of San Cristóbal, along Hwy to Comiton, 20 Aug 1957, *Alava 1341* (NY); Mpio. Zinacantán, near the summit of Cerro Huitepec, 2600 m, 15 Aug 1976, *Breedlove 39587* (MO). **Hidalgo**. Presa Jaramillo, 4 Km N of Pachuca, 2800 m, 29 Jul 1978, *Rzedowski 25758* (G, MEXU). **Puebla**. Mpio. Tepeyahualco, Laguna Salado, 6 Km N of Laguna Alchichica, 19°28′N 97°25′W, 2300 m, 20 Feb 1980, *Nee & Taylor 29575* (F, MO, XAL). **Veracruz**. Llanos de Alchichica, 2200 m, 20 Jul 1968, *Gómez-Pompa 3809* (MO). **Yucatán**. Valladolid, 22 m, 2 Jul 1932, *Steere 1695* (F, NY). **GUATEMALA**. Dept. Jalapa: vicinity of Soledad, Miramundo Mt., between Jalapa and Mataquescuintla, 2000–2500 m, 4 Dec 1939, *Steyermark 32648* (F).

Appendix 2

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 for *trnL-F* followed by ITS. Sequences newly generated for this study are indicated in bold. A dash (—) indicates missing data. Abbreviations of herbaria in which the vouchers are deposited follow *Index Herbariorum*

Cuscuta alata: **992**, *Gentry 5188* (ARIZ), **JN234819**, **JN234825**. ***C. azteca***, **592**, *Medina 2493* (MICH), EF194365, EF194599; **862**, *Axelrod & Hernandez 2242* (NY), EF194366, EF194600; **1006**, *Chiang et al. 2187* (TEX/LL), **JN234820**, **JN234826**. ***C. chinensis* var. *chinensis***: **459**, *Surapat 137* (IND), EF194369, —; **837**, *Carter 628* (CANB), EF194368, **JN234827/JN234828**. ***C. chinensis* var. *applanata***: **507**, *Spellenberg & Mahrt 10680* (NMC), EF194373, **JN234829**; **508**, *Torrecillas 237* (NY), EF194371, **JN234830**; **535**, *Johnston 8826* (F), EF194372, EF194605; **674**, *Rodrigues 653* (XAL), EF194370, EF194603; **844**, *Shreve 9323* (GH), **JN234821**, EF194602; **850**, *Corral-Diaz 3912* (NMC), **JN234822**, EF194604; **1159**, *Valdés 611* (IEB), **JN234823**, **JN234831**; **1211**, *Valdés 621* (ENCB), **JN234824**, **JN234832.** ***C. potosina***: **845**, *Rose et al. 9650* (NY), EF194367, EF194601. ***C. 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. ***C. yucatana*** Yunck.: **657**, *Alava 1341* (NY), EF194364, EF194598. **Outgroups:** ***C. americana*** L.: **698**, *Garneau et al. 1470* (TRT), EF194363, –-; **699**, *Buswell 6231* (NY), –-, EF194597. ***C. victoriana*** Yunck.: **678**, *Cowie 9624* (CANB), EF194383, EF194616.

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