

Species delimitation, phylogenetic relationships, and two new species in the *Cuscuta gracillima* complex (Convolvulaceae)¹

Mihai Costea, Fiona Aiston, and Saša Stefanović

Abstract: Basic morphology, scanning electron microscopy, and DNA sequence data from the plastid *trnL-F* region and the nuclear internal transcribed spacer (ITS) regions were used to delimit the species of a recently circumscribed clade of *Cuscuta* (Convolvulaceae) and to investigate their phylogenetic relationships. This clade comprises the *Cuscuta gracillima* complex from Mexico, Central and northern South America, a group which is characterized by inflorescences that appear to emerge directly from the host stem. Eight lineages are recognized, with two of them described here as new species: *Cuscuta punana* Costea & Stefanović, sp. nov. from Ecuador and *Cuscuta vandevenderi* Costea & Stefanović, sp. nov. from Mexico. *Cuscuta colombiana* Yunck is redefined to include *Cuscuta aristeguietae* Yunck., and *Cuscuta deltoidea* Yunck. is broadened to encompass *Cuscuta serruloba* Yunck. A taxonomic treatment with an identification key, descriptions, and illustrations is provided; the biogeography and conservation status of the eight species are also discussed.

Key words: *Cuscuta*, molecular phylogeny, ITS, *trnL-F*, SEM, new species, taxonomy, conservation.

Résumé : Afin de délimiter les espèces d'un clade de *Cuscuta* récemment circonscrit et d'en examiner les relations phylogénétiques, les auteurs ont utilisé la morphologie de base, la microscopie électronique par balayage, ainsi que les données de séquençage de l'ADN de la région plastidique *trnL-F* et de l'espaceur interne transcrit (ITS) nucléique. Ce clade comprend le complexe *Cuscuta gracillima* du Mexique, de l'Amérique Centrale et du nord de l'Amérique du Sud, un groupe qui se caractérise par des inflorescences qui semblent émerger directement de la tige de l'hôte. On reconnaît huit lignées, dont deux espèces nouvellement décrites ici même; *Cuscuta punana* Costea & Stefanović, sp. nov. de l'Équateur et *Cuscuta vandevenderi* Costea & Stefanović, sp. nov. du Mexique. On redéfinit le *Cuscuta colombiana* Yunck. en incluant le *Cuscuta aristeguietae* Yunck., et on étend le *Cuscuta deltoidea* Yunck. pour inclure le *Cuscuta serruloba* Yunck. On présente un traitement taxonomique accompagné d'une clé, ainsi que des descriptions et des illustrations, et on discute le statut biogéographique et l'état de conservation des huit espèces.

Mots-clés : *Cuscuta*, phylogénie moléculaire, ITS, *trnL-F*, SEM, nouvelles espèces, taxonomie, conservation.

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Introduction

Cuscuta L. (dodders) includes about 180 species of holoparasitic herbs that have an almost cosmopolitan distribution, but with the majority of species (ca. 140) in the Americas. Although only relatively few species attack crops (Dawson et al. 1994; Costea and Tardif 2006), dodders are commonly regarded as noxious weeds or invasive plants. It is less well known that numerous *Cuscuta* species are endangered or even threatened with extinction (Costea et al. 2006a, 2006b, 2006c).

Nearly seven decades after Yuncker's (1932) comprehen-

sive monograph, our understanding of *Cuscuta* is undergoing a renaissance through systematic studies of its evolutionary relationships, character evolution, biogeography, and speciation. This research, ultimately, is laying the foundation for a new, phylogeny-based classification of the genus. Stefanović et al. (2007) circumscribed the major lineages of the subgenus *Grammica* (Lour.) Yunck., which is the largest (ca. 130 species) and most complicated infrageneric taxon of *Cuscuta*. Among the 15 major lineages recognized, only 4 have been studied recently from a taxonomic point of view across the geographic territory covered by the Flora of North America (Costea et al. 2005; Costea et al. 2006a, 2006b, 2006c). The remaining 11 clades, which comprise mostly Mexican, Central American, and South American taxa, are the most challenging, as the scarcity of herbarium material has added to other difficulties posed when studying this genus (Costea et al. 2006a; Stefanović et al. 2007). One of these poorly known lineages is "clade N" (Stefanović et al. 2007), named here as the "*Cuscuta gracillima* complex" in the absence of a formal section name (which will be published in a new infrageneric classification of the genus; M. Costea and S. Stefanović, unpublished data). This group is essentially a segregate of the former subsection *Umbellatae* Yunck. (Yuncker 1932), with which it shares phyloge-

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M. Costea² and F. Aiston.³ Department of Biology, Wilfrid Laurier University, Waterloo, ON N2L 3C5, Canada.

S. Stefanović. Department of Biology, University of Toronto at Mississauga, Mississauga, ON L5L 1C6, Canada.

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²Corresponding author (e-mail: mcostea@wlu.ca).

³Present address: 174 Loon Valley Lane, Stonecliff, ON K0J 2K0, Canada.

netic affinities (Stefanović et al. 2007). It is also related to the *Cuscuta indecora* complex (Costea et al. 2006b; labelled as “clade M” in Stefanović et al. 2007). The group is characterized by inflorescences that seem to emerge directly from the host stem (Stefanović et al. 2007). Most of the species were described by Yuncker from very scarce herbarium material. Among them, *Cuscuta macvaughii* Yunck., *Cuscuta serruloba* Yunck., and *Cuscuta aristaeguietae* Yunck. were known only from their type specimens (Yuncker 1942, 1961), and *Cuscuta choisiana* Yunck., *Cuscuta deltoidea* Yunck., and *Cuscuta colombiana* Yunck. from two collections each (Yuncker 1921, 1932, 1946). *Cuscuta gracillima* Engelm. and *Cuscuta sidarum* Liebm., the only two species of the group not described by Yuncker, are better represented in herbaria. The aims of this study are (i) to infer a phylogeny for the complex to aid in species delimitation, and (ii) to provide a taxonomic treatment for the species in the complex, making conservation recommendations where appropriate. We also describe and illustrate two new species that belong to this clade.

Material and methods

Taxon sampling and outgroup selection

We studied specimens from over 100 herbaria for collections pertaining to this complex. These specimens were annotated, examined for basic morphology, and used as a basis for SEM studies (a total of 36 specimens; vouchers noted in Appendix A). A subset of 18 collections was used for the molecular phylogenetic analyses. Taking into account the difficulties in separating many of these species from their closest relatives, we made an effort to sample multiple accessions, in particular for those with large biogeographical ranges and (or) diverse morphology. Three out of nine ingroup species are represented by three to five individuals each for the molecular analyses. Based on our previous, more inclusive phylogenetic analyses, we selected *Cuscuta warneri* Yunck. as an outgroup, a species which belongs to the closely related *Cuscuta indecora* complex (Stefanović et al. 2007).

Morphology and micromorphology

Our descriptions are based on herbarium material. 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 6.8 software (MIS Inc., Villa Park, Ill.). Hundreds of photographs that illustrate details of the floral and fruit morphology for all the species (including the new species) are available on the Digital Atlas of *Cuscuta* (Costea 2007).

Micromorphology measurements and pictures were taken using scanning electron microscopes (Hitachi S-570 or LEO1530FE-SEM) at 15 kV. Herbarium samples were coated with 30 nm of gold using an Emitech K 550 sputter coater. The terminology regarding the micromorphology of flowers, seeds and capsules, and pollen follows Costea et al. (2006a). The conservation status was assessed using NatureServe (2006) ranks and criteria.

Phylogenetic analysis

To infer phylogenetic relationships among the species of

the *Cuscuta gracillima* complex, sequences from the plastid genome (ptDNA) region containing the *trnL* intron, 3' *trnL*-UAA exon, and intergenic spacer between this exon and *trnF*-GAA (the entire region hereinafter referred to as *trnL-F*), as well as the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) containing ITS1, the 5.8S rDNA locus, and ITS2 (hereinafter referred to as ITS) were obtained. DNA extractions, polymerase chain reaction (PCR) reagents and conditions, amplicon purifications, cloning, and sequencing procedures follow Stefanović et al. (2007). The sequences generated in this study have been submitted to GenBank (accession numbers EU426953–EU426967; see Appendix).

Sequences were aligned manually using Se-AL version 2.0a11 (Rambaut 2002). Although numerous gaps had to be introduced in the alignments, the sequences were readily alignable among all the taxa in both matrices. Gaps in the alignments were treated as missing data. However, the gaps were scored automatically using SeqState version 1.32 (Müller 2005), coded as simple indel characters (Simmons and Ochoterena 2000), and appended to the sequence matrix as binary characters.

Parsimony searches were conducted for each individual region separately, as well as for the combined dataset. In all cases, the searches were done with and without coded indels. Matrix characters were treated as unordered (Fitch 1971), and all changes, including gap characters when used, were equally weighted. Given the moderate number of terminal units, we performed a Branch-and-Bound search using PAUP* version 4.0b10 (Swofford 2002), ensuring recovery of all of most parsimonious (MP) trees. Support for clades was inferred by nonparametric bootstrapping (Felsenstein 1985), again using Branch-and-Bound searching. 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

The overall morphology of this clade reflects its phylogenetic affinities. The shape of the calyx and corolla lobes and the morphology of papillae encountered in several species (e.g., *C. sidarum*, *C. colombiana*, *Cuscuta punana* sp. nov.) are similar to flowers of species from the *C. indecora* complex (Costea et al. 2006b). The predominantly dehiscent capsule, which has a translucent pericarp and is capped by a persistent corolla, is similar to that of species in the former subsection *Umbellatae* (Yuncker 1932).

Species are characterized by thin stems that tend to disappear at maturity. As a result, at this stage the parasite is often represented only by the spherical inflorescences that emerge directly from the host stem.

The pollen is relatively uniform among species (Fig. 1), comparable with that of the *C. indecora* (Costea et al. 2006b) and *C. umbellata* complexes (M. Costea and S. Stefanović, unpublished data). Grains are 3(–4)-zonocolpate, spheroidal to prolate with tectum perforatum or imperforatum (sexine generally tends to persist in the small puncta apertures). Puncta are 50–200 nm (*C. gracillima*, *C. sidarum*, *C. colombiana*, *C. punana*, and *C. macvaughii*)

Fig. 1. Pollen of *Cuscuta colombiana* (a) scale bar = 5 μm ; (b) scale bar = 100 nm; *C. gracillima* (c) scale bar = 5 μm ; (d) scale bar = 100 nm; *C. deltoidea* (e) scale bar = 5 μm ; (f) scale bar = 1 μm . Note the differences in puncta size and shape of supratectal ornamentation.

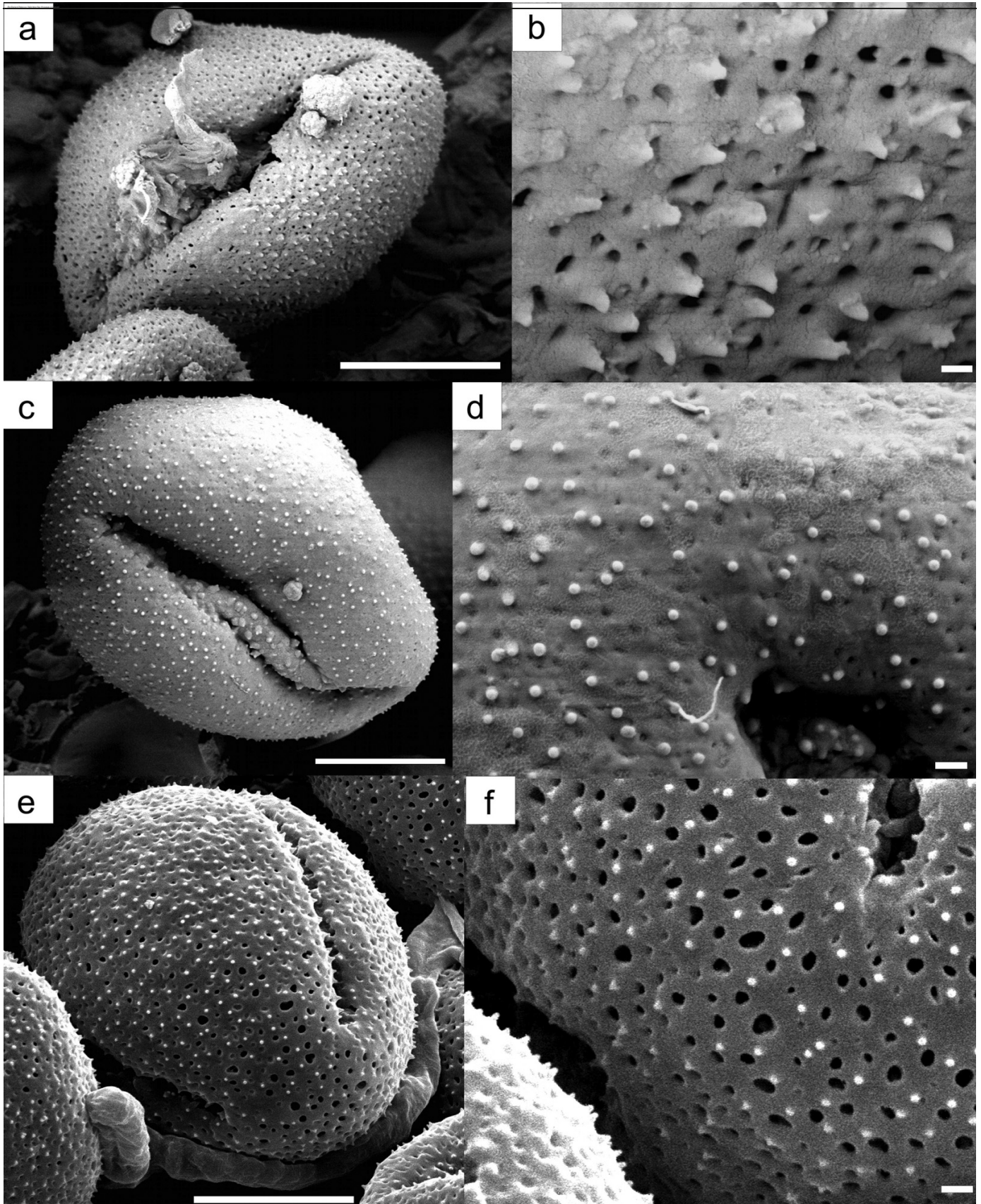
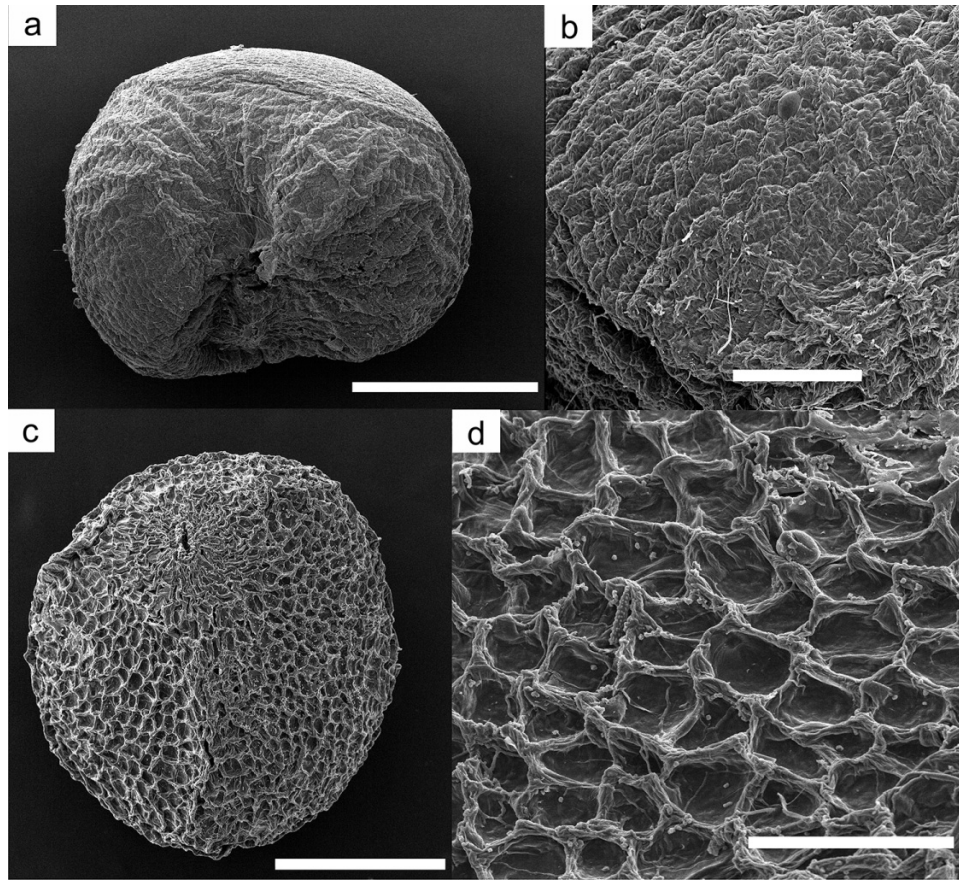


Fig. 2. Seed of *Cuscuta macvaughii* (a) scale bar = 0.38 mm; (b) scale bar = 86 μ m; and *C. punana* (c) scale bar = 0.3 mm; (d) scale bar = 86 μ m).



or 250–800 nm (*C. deltoidea* and *Cuscuta vandevenderi* sp. nov.) in diameter. Supratectal ornamentations in most species are conical and acute; in *C. gracillima* and *C. macvaughii* they are more or less granular-cylindric and rounded.

Capsules dehisce by a more or less irregular line at the base of the fruit in most species, and by a perfect circular line (also at the base of the capsule) in *C. macvaughii*. The fruit is indehiscent in *C. vandevenderi*, although the capsule may split by an irregular interstylar line when pressure is applied, as it does in other *Cuscuta* species with indehiscent capsules (e.g., the *Cuscuta pentagona* clade, Costea et al. 2006a).

The seeds are angled; the seed coat cells are alveolate when dry and papillose when hydrated. They are 20–55 μ m in diameter, except in *C. macvaughii* which has more or less dorsoventrally compressed seeds and a mixture of polygonal, jigsaw-puzzle-like cells and alveolate/papillose cells, as in the *C. indecora* complex (Costea et al. 2006b). Epicuticular wax on the seed surface is present only in *C. macvaughii* and *C. colombiana*. The hilum region is terminal and round, and the vascular scar of the funiculus is short and vertical (Fig. 2).

Sequence data and alignment

The entire sequence length of both regions was included in the subsequent phylogenetic analyses. This is in contrast to the higher-level phylogenetic study of *Cuscuta* by Stefa-

nović et al. (2007), in which large portions of the *trnL-F* could not be aligned across major groups (clades), and which consequently had to be excluded from the analyses. ITS sequence data could not be obtained for *C. colombiana* (accession No. 1068) owing to the poor quality of the DNA extracted from this specimen. Alignments in Nexus format are deposited in TreeBASE (study accession No. S1981).

Tree topologies

A number of distinct preliminary phylogenetic analyses were conducted to explore the distribution of phylogenetic signal in the different matrices, with and without coded indels. Statistics of MP trees derived from these separate and combined analyses are summarized in Table 1. Clades recovered in each analysis were congruent with the tree structure recovered using data from the other genome (trees not shown). Hence, we combined all data and present only these analyses here. The trees produced from the combined matrix had better resolution and overall support relative to those produced from the individual matrices. The parsimony analysis resulted in three MP trees, one of which was randomly selected to illustrate the inferred relationships as well as branch lengths (Fig. 3). The only differences among the MP trees involved the relative branching order among individuals of *C. gracillima* (a branch that collapses in the strict consensus is indicated with an asterisk in Fig. 3).

The topology resulting from the combined *trnL-F* and ITS datasets revealed two major subclades that together

Table 1. Summary descriptions for sequences included in individual and combined analyses of the *Cuscuta gracillima* complex.

	<i>trnL-F</i>	ITS	Combined data
Number of individuals sequenced	18	17	18
Aligned sequence length	537	657	1,194
Number of gaps coded as binary characters	26	32	58
Variable characters ^a	97	244	341
Parsimony informative sites ^a	35	95	130
Mean AT content (%)	65	47	55 ^b
Base frequency homogeneity ($\chi^2/df/P$)	4.15/51/1.0	22.81/48/0.99	12.64/48/0.99 ^b
Number of MP trees	2	6	3
Tree length	117	317	435
CI/RI	0.92/0.93	0.91/0.92	0.91/0.92

Note: CI, consistency index; df, degrees of freedom; MP, most parsimonious; RI, retention index.

^aIncluding coded gaps.

^bIncluding only OTUs for which both sequences are available.

form a sister group to *C. macvaughii* (Fig. 3). Within one subclade, four species (*C. sidarum*, *Cuscuta aristeguietae* Yunck., *C. colombiana*, and *C. gracillima*) collectively form the sister group to the new species from Ecuador, *C. punana*. The sequences for *C. aristeguietae* and *C. colombiana* are virtually identical to one another, and together appear to be weakly nested within *C. sidarum*. The second subclade contains a representative of *C. deltoidea* and an assemblage of Sonoran forms described in this study as the new species, *C. vandevenderi*. The internal support for all of these major relationships was very strong ($\geq 95\%$ bootstrap support; Fig. 3).

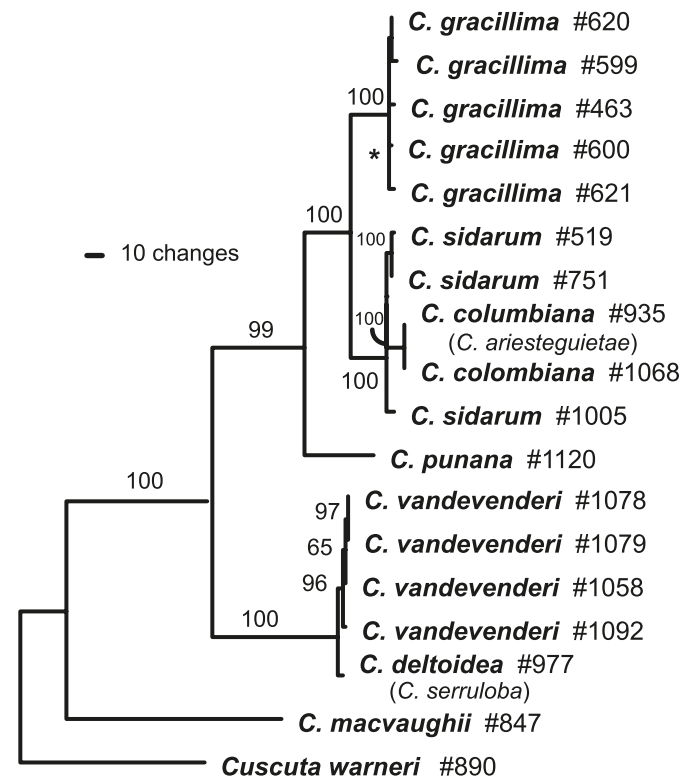
Species delimitation

As a general rule, we recognized species as distinct when both the morphological and molecular variation supported their separation. However, in one case, concerning the separation of *C. colombiana* (redefined here to include *C. aristeguietae*) from *C. sidarum*, we reached a decision based solely on morphological information (see below). In the two instances when DNA data were not available, a conclusion had to be reached solely based on morphology. For instance, *C. choisiana* was maintained as a species because it differs from *C. gracillima* in some characteristics (but pending further investigation, see below), whereas we merged *C. serruloba* into *C. deltoidea* because the morphological variation did not support their distinctiveness (see below).

Cuscuta sidarum, *Cuscuta colombiana*, and *Cuscuta aristeguietae*

The latter is one of last species described by Yuncker from a unique (Venezuelan) collection. He describes it as being “very distinctive because of the fleshy, papillate flowers on comparatively long pedicels” and having indehiscent capsules (Yuncker 1961). When discussing its relationships to other dodders, Yuncker (1961) indicated only that “it belongs to section *Cleistogrammica*” in which he had included all the species of the subgenus *Grammica* with indehiscent capsules (as opposed to section *Eugrammica*, which has species with dehiscent capsules; Yuncker 1932). He must have noticed that none of the 12 subsections of *Cleistogrammica*

Fig. 3. Phylogenetic relationships among species of the *Cuscuta gracillima* complex derived from the combined *trnL-F* and ITS maximum parsimony analyses. Trees are rooted using *C. warneri*, a species from the closely related *Cuscuta indecora* complex. Numbers following species names correspond to DNA accessions (see Appendix). One of three equally parsimonious trees (L = 435; CI = 0.91; RI = 0.92) was chosen to illustrate the amount and distribution of inferred change. Branch lengths are drawn proportionally to the number of changes. An asterisk indicates nodes that collapse in the strict consensus. Bootstrap values are indicated beside branches.



can include this species, but since in his view, fruit dehiscence (indehiscence) was a character of utmost importance in the evolution and taxonomy of *Cuscuta*, he did not connect *C. aristeguietae* to *C. gracillima* and *C. sidarum* (the

latter two having dehiscent fruits) as he had done earlier with *C. colombiana* (Yuncker 1946), which has an “irregular line of circumscission” at the base of the capsules. Our reexamination of herbarium material (including the type collection) revealed that *C. aristeguietae* has the same type of dehiscent capsules as *C. colombiana*, with a jagged basal dehiscence line and a thin pericarp that may tear irregularly. In fact, *C. aristeguietae* is almost identical to *C. colombiana* from both a molecular and morphological point of view. While the type of *C. aristeguietae* has flowers with ovate to subrotund calyx lobes, other collections from the same geographical area have triangular to lanceolate calyx lobes, as *C. colombiana* does. Therefore, we broaden the concept of *C. colombiana* to include *C. aristeguietae*. As previously indicated, when newly circumscribed in this way, *C. colombiana* is potentially nested in *C. sidarum*. Although similar in many respects, the characteristic cup and saucer floral morphology of *C. colombiana* distinguishes this species from *C. sidarum*.

Cuscuta gracillima and *C. choisiana*

Yuncker (1921) cited only two collections when he published *C. choisiana*: *Purpus* 4971 the type, from San Luis Potosí, and *Purpus* 5036 from Chapala (Yuncker 1921). Although Brandegee had identified the type collection as *C. gracillima*, and had added that it was merely a form with shorter stamens (“*forma staminibus brevioribus*”; Brandegee, in herbarium (GH, UC, and US), Yuncker (1932) placed his newly described species in a different group, subsection *Odontolepisae*, which included, in his view, a mixture of Mexican and South American species (among others: *Cuscuta purpusii* Yunck., *Cuscuta potosina* Schaffn., *Cuscuta partita* Choisy, *Cuscuta cockerellii* Yunck.). Our examination of the collections available indicates that *C. choisiana* is indeed related to *C. gracillima* (as noted by Brandegee on the type herbarium specimen), from which it differs essentially in having overlapping calyx lobes (vs. nonoverlapping calyx lobes in *C. gracillima*), and in having stamens that are shorter than corolla lobes (vs. stamens longer than corolla lobes in *C. gracillima*). Given both the scarcity of material available for *C. choisiana* and our inability to extract DNA from it, we cannot yet reach a decision on its status. Therefore, we maintain *C. choisiana* as a species until more plants can be collected and studied. *Cuscuta gracillima* var. *esquamata* was described by Yuncker (1921) from Baja California, and is known only from the type collection. The most prominent feature noted by Yuncker (1921) is the reduction of its infrastaminal scales, “lacking or reduced to but a few short processes” (Yuncker 1921, pp. 43). Our closer inspection of the holotype revealed not only that the scales are present and relatively well developed, but that calyx lobes are entire to serrulate, and that the capsules are indehiscent. We have found similar specimens from Sonora, and based on morphological and molecular data, we came to the conclusion that they represent a new species, *C. vandevenderi*, which is more closely related to *C. deltoidea* than to *C. gracillima* (see below).

Cuscuta punana

This new species is sister to the clade consisting of *C. gracillima*, *C. sidarum*, and *C. columbiana* (Fig. 3); it

clearly represents a deep phylogenetic split in the complex. It grows on Puná Island, off the coast of southern Ecuador, opposite to the mouth of the Guayas River, and hence it represents the southernmost member of this complex known to date. It differs from *C. gracillima*, *C. sidarum*, and *C. colombiana* in having larger flowers with reflexed corolla lobes that are two times longer than the corolla tube.

Cuscuta deltoidea, *Cuscuta serruloba*, and *Cuscuta vandevenderi*

Yuncker described *C. deltoidea* and *C. serruloba* in 1932 and 1942, respectively, from Manzanillo, Colima State, Mexico. He only had the type specimens available for *C. serruloba*, which he noted was “not abundant” and without capsules (Yuncker 1942). At that time, Yuncker did not realize that he had already described *C. serruloba* as a variety (var. *serrulata*) of *C. deltoidea* back in 1935 (Yuncker 1935). In 1946, he stated that the specific status for this taxon was clearer, after examining additional material (Yuncker 1946). We could not find any additional collections of *C. serruloba* or *C. deltoidea* annotated by Yuncker, and in the subsequent treatment of *Cuscuta* for Flora of North America, he indicated that *C. serruloba* was known only from the type locality (Yuncker 1965, p. 35). The main distinctions between *C. serruloba* and *C. deltoidea* noted by Yuncker (1942) concern the morphology of the calyx lobes and the length of the styles. *Cuscuta serruloba* was said to have nonoverlapping, acute and serrate calyx lobes, and *C. deltoidea* obtuse, overlapping, and entire calyx lobes. However, a closer inspection of the type collections reveals that the flowers of *C. deltoidea* may have serrate, acute calyx lobes, while flowers of *C. serruloba* may have overlapping calyx, obtuse lobes, thus obscuring the distinction between the two forms. Other collections from Michoacan (including the only additional specimen cited by Yuncker as *C. deltoidea* at the publication of the binomial, *Pringle* 5350) exhibit serrate and overlapping calyx lobes. We find that the length of the styles, the only other character mentioned by Yuncker (1942; as being “shorter (?) and stouter” in *C. serruloba*), varies continuously even within the same inflorescence in both forms. Based on the type specimens alone, *C. deltoidea* tends to have somewhat broader calyx lobes (0.7–1.1 mm wide) than *C. serruloba* (0.55–0.9 mm wide). Nonetheless, these overlapping values are not enough, in our view, to clearly separate the specimens as different species or varieties. Consequently, we redefine *C. deltoidea* to include *C. serruloba*, although we currently lack molecular evidence for this combination. Recent collections from Sonora (Appendix), and the type of *C. gracillima* var. *esquamata* from Baja California, exhibited the same range of variation in the calyx morphology observed in *C. deltoidea* (including *C. serruloba*), with combinations of characters that varied from serrate and overlapping calyx lobes, to entire and nonoverlapping. These collections clearly differ morphologically from *C. deltoidea* by having denser inflorescences and larger, indehiscent capsules with a conspicuous interstylar opening. A certain amount of genetic diversity among specimens was observed (Fig. 3), but this variation could not be correlated with discernable morphological patterns. As these collections form a strongly supported clade (96%), and their morphology distinguishes them not only from *C. deltoidea*, but

also from other members of the *C. gracillima* complex, we describe them here as a new species, *C. vandevenderi*.

Taxonomic treatment

Cuscuta macvaughii Yunck., Brittonia 12: 40. 1960

TYPE: Mexico, Michoacán de Ocampo, in pasture one mile west of San Juan, elevation 275 m; locally abundant; plant erect, orange; flowers cream color, 17 September 1958, McVaugh 17970.

HOLOTYPE: MICH.

ISOTYPE: NY.

INFLORESCENCES: corymbiform cymes of 3–12 flowers further arranged in lax fasciculated inflorescences in which all the axes are visible; sparse papillae present on inflorescence axes; pedicels 0.5–5 mm.

FLOWERS: 5-merous, 4–5.2 mm, cream-coloured when fresh, brown–reddish when dried; papillae sometimes present on corolla; laticifers visible on corolla, mostly isolated and elongated. CALYX: 2.2–2.4 mm, campanulate, divided ca. 1/2, tube 0.8–1.6 mm, lobes 0.6–1.2 mm, triangular, equal, not overlapping or overlapping only at the base, not carinate but midveins sometimes with a few multicellular projections, apex acute, margins entire. COROLLA: 1.8–3.8 mm tube

campanulate to suburceolate, 1.3–1.9 mm, lobes 1.5–1.9 mm, narrowly lanceolate, spreading to reflexed, acute to acuminate, margins entire. STAMENS: equalling corolla lobes, anthers oblong to lanceolate, 0.85–1 mm × 0.35–0.45 mm, filaments 0.5–0.9 mm. POLLEN GRAINS: subspheroidal to round, 20–25 µm, puncta 50–200 nm, ornamentations granular; INFRASTAMINAL SCALES: oblong–obovate, 1.5–1.7 mm, shorter than corolla tube, bridged at 0.6–0.75 mm, apex rounded, fimbriae 0.1–0.3 mm. STYLES: 3–4.1 mm, much longer than the ovary. CAPSULES: dehiscent, globose to globose–obovoid, 0.8–1.2 mm × 0.7–0.9 mm, thickened and raised around the inconspicuous interstylar aperture; capped by persistent corolla and falling with it. SEEDS: 2–4 per capsule, subround to broadly elliptic, 0.75–1 mm × 0.65–0.8 mm, seed coat cells alveolate/papillate to jigsaw-puzzle-like, often with epicuticular wax, hilum area 0.2–0.4 mm, vascular scar 0.02–0.04 mm (Figs. 2a and 2b).

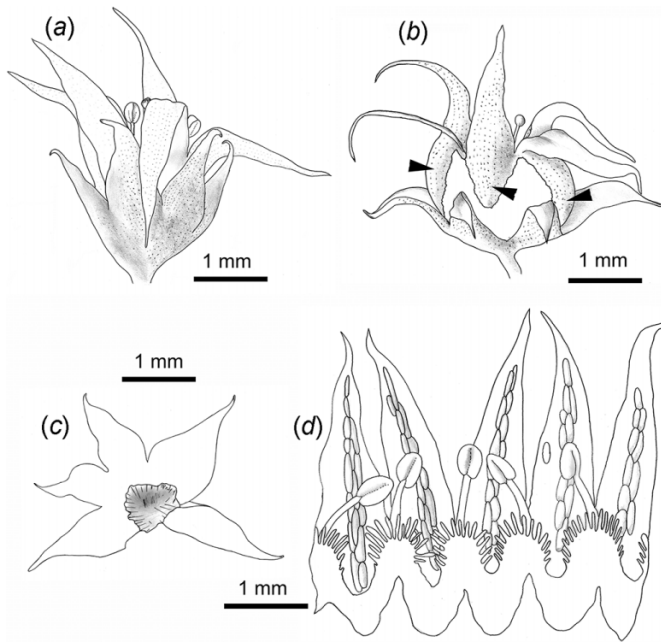
DISTRIBUTION, HABITAT, AND PHENOLOGY: Mexico, Michoacán, Distrito Apatzingan; the only two known specimens were collected either from road margins or from pastures, 275–400 m elevation, parasitic on *Okenia* Schtdl. & Cham. (Nyctaginaceae). Flowering August–September.

CONSERVATION STATUS: G1 (critically imperiled: at very high risk due to extreme rarity).

Identification key

- 1a Inflorescence axes with sparse papillae; anthers 0.85–1 mm long *Cuscuta macvaughii*
 1b Inflorescence axes without papillae; anthers 0.3–0.7 mm long 2
- 2a Flowers 1.6–2.6 mm 3
 2b Flowers 2.5–5.5 mm 4
- 3a Capsules indehiscent, surrounded at the base by persistent corolla *Cuscuta vandevenderi* sp. nov.
 3b Capsules dehiscent, capped by persistent corolla and falling with it *Cuscuta deltoidea*
- 4a Corolla lobes 2 times longer than the tube, reflexed *Cuscuta punana* sp. nov.
 4b Corolla lobes equalling the tube, erect to slightly spreading 5
- 5a Papillae present on calyx and corolla; calyx divided ca. 3/4 6
 5b Papillae absent; calyx divided 1/2–2/3 7
- 6a Calyx and corolla having a characteristic cup and saucer appearance *Cuscuta columbiana*
 6b Calyx and corolla not having a cup and saucer appearance *Cuscuta sidarum*
- 7a Calyx lobes not overlapping at base; stamens longer than corolla lobes *Cuscuta gracillima*
 7b Calyx lobes overlapping at the base; stamens shorter than corolla lobes *Cuscuta choisiana*

Fig. 4. *Cuscuta punana*, sp. nov: (a) flower; (b) maturing capsule capped by persistent corolla (indicated with arrowheads); (c) dissected calyx; (d) dissected corolla showing infrastaminal scales and stamens, note the laticifers in the corolla.



***Cuscuta punana* Costea & Stefanović, sp. nov.**

TYPE: Ecuador, in Guayas, Isla Puná, El Placer, 0–5 km on path toward Río Hondo, 02°48'S, 80°00'W; semideciduous forest and beach, 8 September 1987, *Madsen 63936*.

HOLOTYPE: AAU (Fig. 4).

ISOTYPE: QCA.

Ad species *Cuscuta gracillima*, *C. sidarum*, et *C. colombiana* similes, sed floribus majoribus (3.8–4.5 mm) et lobis corollae reflexis tubo duplo longioribus differt.

INFLORESCENCES: dense cymes of 3–7 flowers further grouped in glomerules with numerous flowers; pedicels 0.4–2 mm.

FLOWERS: (4–)5-merous, 3.8–4.5 mm, white when fresh, brownish creamy when dried; papillae present on calyx and corolla; laticifers visible, especially in the corolla lobes where they are elongated, articulate and form long rows; in calyx they are present, articulated or isolated, ovoid or elongated, but not conspicuous.

CALYX: 1.9–2.7 mm, campanulate, divided ca. 1/3, tube 0.4–0.7 mm, lobes 1.3–2.2 mm, triangular-lanceolate, more or less unequal, not overlapping at base, not carinate, apex acuminate, recurved, margins entire. COROLLA: 2.8–3.5 mm, tube campanulate to globose-suburceolate, 0.8–1 mm, lobes 2–2.4 mm, linear-lanceolate, initially erect, then becoming reflexed, acuminate, margins entire. STAMENS: shorter than corolla lobes, anthers elliptic 0.3–0.4 mm × 0.2–0.25 mm, filaments 0.5–0.7 mm. POLLEN GRAINS: subprolate, 21–24 μm, puncta 50–200 nm, ornamentations conical. INFRASTAMINAL SCALES: ovate, longer than corolla tube, 1.2–1.4 mm, bridged at 0.4–0.6 mm, apex rounded, fimbriae 0.15–0.2 mm; styles 1.2–1.6 mm. CAPSULES: dehiscent, 1.3–1.8 mm × 0.8–1 mm, depressed, interstylar aperture inconspicuous; capsule capped by persistent corolla and falling with it. SEEDS: four per capsule, subrotund, 0.6–0.8 mm × 0.6–0.75 mm, hilum area 0.20–0.30 mm, vascular scar 0.025–0.04 mm (Figs. 2c and 2d).

ETYMOLOGY: the specific epithet refers to Puná Island, where the species was found.

DISTRIBUTION, HABITAT, AND PHENOLOGY: Ecuador, known only from Puná Island; semideciduous forests and beach vegetation; elevation 0 m a.s.l.; herbaceous hosts unknown. Flowering June–September.

CONSERVATION STATUS: G1 (critically imperiled).

Cuscuta gracillima Engelm., *Trans. Acad. Sci. St. Louis* 1: 488. 1859

TYPE: Mexico (unknown location and date), *Mornay 299*.

LECTOTYPE: G.

ISOLECTOTYPE: MO.

Cuscuta gracillima Engelm. var. *subtilis* Yunck., *Illinois Biol. Monogr.* 6: 43. 1921

TYPE: Mexico, Colima and Manzanillo, *Palmer 949*.

HOLOTYPE: MO.

INFLORESCENCES: corymbiform cymes of 5–15 flowers further arranged in dense, spherical inflorescences 1.5–3 cm in diameter, with hundreds of flowers; pedicels 1.5–5 mm.

FLOWERS: 5-merous, 3–3.7(–4) mm, white when fresh, yellowish, brown–gray when dried; papillae absent; laticifers present in both calyx and corolla, but inconspicuous. CALYX: campanulate, 1.3–1.8 mm, divided 1/2–2/3, tube 0.5–1.2 mm, lobes 0.75–1.4 mm, triangular, more or less equal, not overlapping, not carinate, triangular, apex acute, margins entire. COROLLA: 1.5–2.7 mm, tube campanulate, 0.8–1.6 mm, lobes 0.7–1.1 mm, triangular to triangular-lanceolate, equaling or shorter than corolla tube, erect to slightly spreading, apex acute, margins entire. STAMENS: longer than corolla lobes, anthers elliptic to oblong, 0.5–0.7 mm × 0.25–0.4 mm, filaments 1.4–2 mm. POLLEN GRAINS: subspheroidal to subprolate (the former more common), 18–22 μm, puncta 50–200 nm, ornamentations granular (Figs. 1c–1d). INFRASTAMINAL SCALES: elliptic-obovate, 1.25–1.5 mm, equalling corolla tube or longer, bridged at 0.25–0.45 mm, apex rounded, fimbriae 0.15–0.3 mm. STYLES: 1.5–2.5 mm, longer than the ovary. CAPSULES: dehiscent, globose to globose-depressed, 0.8–1.4 mm × 0.72–1 mm, interstylar aperture inconspicuous; capsule hidden inside the persistent corolla and falling together. SEEDS: 2–4 per capsule, subrotund to broadly elliptic, 0.6–0.8 mm × 0.5–0.9 mm, hilum area 0.20–0.28 mm, vascular scar 0.03–0.05 mm.

DISTRIBUTION, HABITAT, AND PHENOLOGY: west Mexico, from Sinaloa to Guerrero, and in the states of Mexico and Morelos. It grows on numerous herbs and shrubs from a variety of habitats: humid montane vegetation, volcanic slopes, tropical deciduous forests, alluvial flats and river shores, pastures, and ruderal vegetation on road margins and in urban settlements; elevation 25–2500 m a.s.l. Flowering October–February.

CONSERVATION STATUS: G4 (apparently secure. Uncommon but not rare; some cause for long-term concern due to declines or other factors).

Cuscuta choisiana Yunck., *Illinois Biol. Monogr.* 6: 38. 1921

TYPE: Mexico, San Luis Potosí, Minas de San Rafael, 'forma staminibus brevioribus', November 1940, *Purpus 4971*.

HOLOTYPE: US.

ISOTYPES: GH, UC.

INFLORESCENCES: dense cymes of 3–9 flowers further grouped in dense, spherical glomerules; pedicels 0.5–3 mm.

FLOWERS: 5-merous, 3–4 mm, yellow when dried; papillae absent; laticifers visible in the corolla and calyx, articulated or isolated, ovoid and elongated. CALYX: 1.4–2 mm, campanulate, divided ca. 1/2, tube 0.5–0.6 mm, lobes 1–1.2 mm, ovate-lanceolate, equal, overlapping at base, not carinate, apex acute, somewhat spreading, margins entire. COROLLA: 3–3.5 mm, tube campanulate, 1.6–2 mm, lobes 1.6–2 mm, lanceolate, spreading, acuminate, margins entire. STAMENS: shorter than corolla lobes, anthers elliptic-oblong, 0.5–0.7 mm × 0.25–0.3 mm, filaments 0.5–0.7 mm. POLLEN GRAINS: subspheroidal to subprolate, 18–25 µm, puncta 50–200 nm, ornamentations granular. INFRASTAMINAL SCALES: oblong, 1.95–2 mm, equalling corolla tube, bridged at 0.7–0.8 mm, apex rounded, fimbriae 0.15–0.3 mm. STYLES: 1.6–2.2 mm, much longer than the ovary. CAPSULES: dehiscent, 1.1–1.4 mm × 0.75–1 mm, globose, interstylar aperture inconspicuous; capsule hidden inside the persistent corolla and falling with it. SEEDS: not seen.

DISTRIBUTION AND HABITAT: Central Mexico, San Luis Potosí, Jalisco, and Puebla; known only from three collections with incomplete labels; the host, *Syncephalantha decipiens* Bartl. (Asteraceae), is discernible in only one collection (Vease 337, MEXU). Flowering September–November.

CONSERVATION STATUS: an assessment of the conservation status requires a better understanding of the systematics of this plant.

Cuscuta sidarum Liebm., Förh. Skand. Naturf. Möte 1844: 196–197. 1847

TYPE: Mexico, Oaxaca, St. Jago Estate, Nov 1842, *Liebmann* 12359.

LECTOTYPE: designated here, K.

ISOLECTOTYPES: GH, US.

Cuscuta gracillima var. *saccharata* Engelm., Trans. Acad. Sci. St. Louis 1: 489. 1859; Syst. Arrang. Sp. *Cuscuta*, 39. 1859

TYPE: Mexico, Oaxaca, St. Jago Estate, November 1842, *Liebmann* 12359.

LECTOTYPE: K.

ISOLECTOTYPES: GH, US.

Cuscuta saccharata (Engelm.) Yunck. Mem. Torrey Bot. Club 18: 239. 1932

TYPE: Mexico, Oaxaca, St. Jago Estate, November 1842, *Liebmann* 12359.

HOLOTYPE: K.

ISOTYPES: GH, US.

INFLORESCENCES: corymbiform cymes of 5–15 flowers further arranged in dense, spherical inflorescences 1.5–3 cm in diameter with hundreds of flowers; pedicels 1.5–5 mm.

FLOWERS: 5-merous, 2.5–3.2 (–3.5) mm, brownish when dried; papillae present and dense on calyx and corolla; laticifers visible in the corolla and calyx, primarily isolated on calyx and articulated on corolla, ovoid and elongated. CALYX: 1.3–1.6 mm, campanulate, divided ca. 3/4, tube 0.25–0.5 mm, lobes 0.8–1.2 mm, triangular to lanceolate, commonly unequal, not overlapping, not carinate, apex acute, margins entire apex. COROLLA: 2.2–3.2 mm, tube campanulate, 1.4–1.6 mm, lobes (0.8–)1.2–1.6 mm, triangular to oblong-lanceolate, erect to slightly spreading, apex acute, margins entire. STAMENS: shorter than corolla lobes, anthers

elliptic-oblong, 0.4–0.6 mm × 0.25–0.4 mm, filaments 0.5–0.7 mm. POLLEN GRAINS: subprolate to prolate, 23–26 µm, puncta 50–200 nm, ornamentations conical. INFRASTAMINAL SCALES: oblong, equalling corolla tube, 1.3–1.5 mm, bridged at 0.6–0.75 mm, apex rounded, fimbriae 0.15–0.3 mm. STYLES: 1.2–1.7 mm, much longer than the ovary. CAPSULES: dehiscent, globose, to globose-depressed, 1.1–1.7 mm × 1.1–1.5 mm; interstylar aperture inconspicuous; capsule hidden inside the persistent corolla and falling together. SEEDS: 2–4 per capsule, 0.65–0.85 mm × 0.6–0.8 mm, subrotund, hilum area 0.25–0.3 mm, vascular scar 0.05–0.7 mm.

DISTRIBUTION AND HABITAT: Mexico in Jalisco, Colima, Michoacan, Guerrero, Oaxaca; Central America in Guatemala, Honduras, Nicaragua, Costa Rica; it grows mostly on herbs from deciduous tropical forests, thickets along rivers, pastures, and ruderal habitats on the margins of the roads and villages; elevation 20–1000 m a.s.l. Flowering October–February.

CONSERVATION STATUS: G3 (vulnerable, at moderate risk of extinction due to a restricted range, relatively few populations (often 80 or fewer), recent and widespread declines, or other factors).

Cuscuta colombiana Yunck., Bull. Torrey Bot. Club 73: 6. 1946

TYPE: Colombia, Departamento de Magdalena, open grassland at Alto de Minas, elevation about 300 m a.s.l., on *Euphorbia* sp. 10 October 1944, *Haught* 4400.

HOLOTYPE: US.

Cuscuta aristeguietae Yunck., Acta Biol. Venez. 3: 6. 1961

TYPE: Venezuela, Guárico. Parque “Lazo Martí”, La Aguada, Calabozo, planta parásita de color blanco-cremoso parasita sobre una Acanthaceae [parasitic plant of white-creamy colour, parasitizing on an Acanthaceae], Febrero 1961, *Aristeguieta* 4487.

HOLOTYPE: NY.

INFLORESCENCES: corymbiform cymes of 5–15 flowers further arranged in dense, spherical inflorescences 2.5–4 cm in diameter with hundreds of flowers; pedicels 0.5–3 mm.

FLOWERS: (4–)5-merous, 2.5–3.2 mm, white-cream when fresh, yellow to chestnut brown when dried; papillae present on calyx and corolla lobes; laticifers prominent on both calyx and corolla, articulated or isolated, ovoid or elongated. CALYX: 1–1.4 mm long, cupulate, together with the corolla having a characteristic cup-and-saucer appearance (lobes ± patent or spreading, not enclosing the corolla tube), divided for ca. 3/4 of length, tube 0.25–0.5 mm, lobes 0.8–1.2 mm, varying in size, not basally overlapping, not carinate, ovate, subround to triangular, margins entire, apex acute to rounded. COROLLA: 2–2.6 mm, tube campanulate to urceolate, 0.9–1.2 mm, lobes triangular to lanceolate, 1.2–1.4 mm, erect to slightly spreading, apex acute to acuminate, initially cucullate, later flat. STAMENS: shorter than corolla lobes, anthers subround to elliptic 0.35–0.4 mm × 0.2–0.25 mm, filaments 0.5–0.6 mm. POLLEN GRAINS: subprolate to subspheroidal, 18–25 µm, puncta 50–200 nm, ornamentations conical (Figs. 1a and 1b). INFRASTAMINAL SCALES: ovate to oblong ovate, 1.1–1.25 mm, equalling or longer than corolla tube, bridged at 0.35–0.55 mm, apex rounded, fimbriae 0.1–0.15 mm. STYLES: 0.6–1.3 (–1.5) mm, much longer than the ovary. CAPSULES: dehiscent, globose to depressed, 0.7–1.2 mm × 0.95–1.6 mm, interstylar aperture inconspicuous; capsule hidden inside the persistent corolla and falling to-

gether. SEEDS: 2–4 per capsule, round to broader than long, 0.79–0.9 mm × 0.9–1 mm, seed coat cells more or less jigsaw-puzzle-like, with epicuticular wax, hilum area 0.25–0.3 mm, vascular scar 0.04–0.06 mm.

DISTRIBUTION AND HABITAT: North Colombia and Venezuela; habitat data were not available on the herbarium labels, and the fragments of host could not be identified. Venezuelan collections grow on herbs from Acanthaceae, and the Colombian ones on “drying herbs, vines and undershrubs” (Haught 4535); elevation 30–100 m a.s.l. Flowering December–March.

CONSERVATION STATUS: G2 (imperiled).

Cuscuta deltoidea Yunck., Illinois Biol. Monog. 6: 44. 1921

TYPE: Mexico. Colima. Manzanillo, 1891, Palmer 948.

HOLOTYPE: US.

ISOTYPES: GH, NY.

Cuscuta deltoidea var. *serrulata* Yunck., Bull. Torrey Bot. Club 62: 512. 1935

TYPE: Mexico. Colima. Manzanillo, 21 October 1910, Orcutt 4457.

HOLOTYPE: F.

Cuscuta serruloba Yunck., Bull. Torrey Bot. Club 69: 542. 1942

TYPE: Mexico. Colima. Manzanillo, 20 October 1919, Orcutt sp. nov.

HOLOTYPE: GH.

ISOTYPE: MEXU.

INFLORESCENCES: loose corymbiform cymes of 3–9 flowers further arranged in larger loose inflorescences in which all the axes are visible; pedicels 0.7–5 mm.

FLOWERS: (4–)5-merous, 1.6–2.3 mm, white when fresh, creamy to light brown when dried; papillae absent; laticifers prominent on calyx and corolla, articulated or isolated, rectangular, ovoid to elongated. CALYX: broadly campanulate, 0.85–1.5 mm long, divided 1/2–1/3, tube 0.25–0.6 mm, lobes 0.4–0.6 mm, more or less equal, not overlapping to overlapping, carinate, triangular to broadly triangular, two exterior lobes usually auriculate, apex acute to obtuse, margins more or less entire to serrulate. COROLLA: 1.4–2 mm, tube campanulate, 0.8–1.2 mm, lobes 0.6–1 mm, triangular, erect or slightly spreading, apex obtuse to acute. STAMENS: equalling to longer than corolla lobes, anthers broadly elliptic, 0.3–0.5 mm × 0.2–0.25 mm, filaments 0.4–0.7 mm. POLLEN GRAINS: subprolate to subspheroidal, 17–19 μm, puncta 250–800 nm, ornamentations conical (Figs. 1e and 1f). INFRASTAMINAL SCALES: oblong-ovate to obovate, equalling corolla tube, 0.65–1.2 mm, bridged at 0.25–0.5 mm, fimbriae 0.1–0.2 mm. STYLES: 0.5–1.2 mm, longer than the ovary. CAPSULES: dehiscent, 0.75–1.2 mm × 0.5–0.9 mm, globose to slightly depressed, interstylar aperture inconspicuous; capsule hidden inside the persistent corolla and falling with it. SEEDS: 3–4 per capsule, subround, 0.65–0.8 mm × 0.65–0.7 mm, hilum area 0.2–0.3 mm, vascular scar 0.02–0.04 mm.

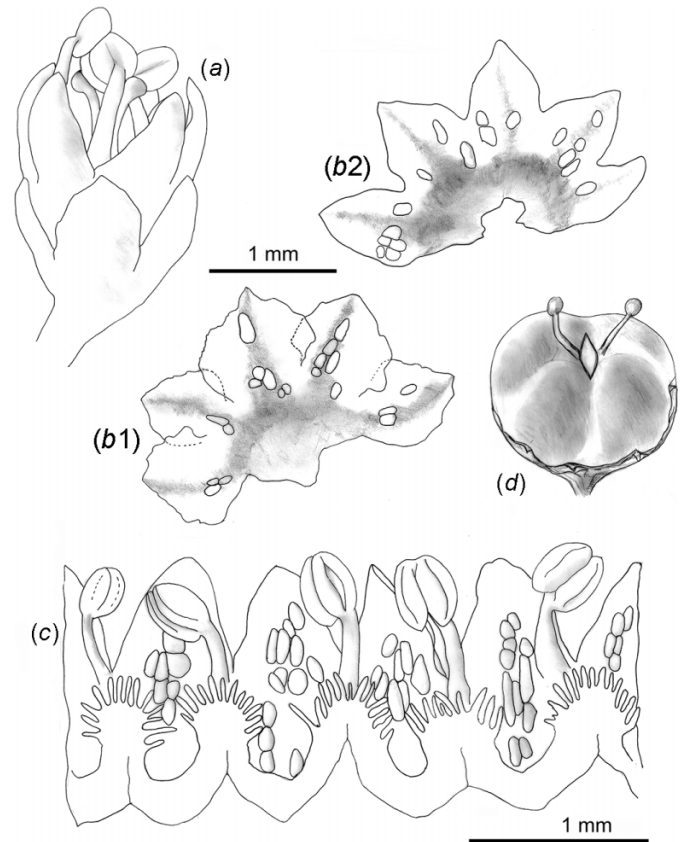
DISTRIBUTION AND HABITAT: Mexico, Colima and Michoacan. Flowering October–November.

CONSERVATION STATUS: G2 (imperiled).

Cuscuta vandevenderi Costea & Stefanović, sp. nov.

TYPE: Mexico, Sonora, Municipio de Yécora, Santa Ana,

Fig. 5. *Cuscuta vandevenderi*, sp. nov.: (a) flower; (b1, b2) variation of calyx; (c) dissected corolla showing infrastaminal scales and stamens; (d) capsule, note the laticifers in the calyx and corolla.



28°22'40"N, 109°09'W, elevation 850 m a.s.l, common parasite on *Sida rhombifolia* (Malvaceae); flowers starting white, turning yellowish, 20 September 1998, Van Devender et al. 98–1434.

HOLOTYPE: ARIZ.

ISOTYPES: MEXU, NY, WLU (Fig. 5).

Cuscuta gracillima var. *esquamata* Yunck., Illinois Biol. Monogr. 6 (2–3): 43. 1921

TYPE: Mexico, Baja California, El Taste, 16 September 1893, Brandegee sp. nov.

HOLOTYPE: UC.

Ad species Cuscuta deltoidea similes, sed inflorescentiis densioribus, capsulis majoribus indehiscentibus apertis interstylaribus, et corollis persistentibus basim capsulae cingentibus differt.

INFLORESCENCES: corymbiform cymes of 3–9 flowers further arranged in larger dense globose inflorescences, 1–3 cm in diameter, in which first order axes are not visible; pedicels 0.7–6 mm.

FLOWERS: (4–)5-merous, 2–2.6 mm, white turning cream-yellowish when fresh, creamy–light brown when dried; papillae absent; laticifers prominent in the calyx and corolla, articulated or isolated, rectangular, ovoid to elongated. CALYX: campanulate, 0.9–1.6 mm long, divided 1/2–1/3, tube 0.4–0.8 mm, lobes 0.5–1.2 mm, more or less equal, not overlapping to overlapping, carinate, triangular to broadly triangular, apex obtuse, acute to acuminate, margins more or less

entire to serrulate. COROLLA: 1.5–2.3 mm, tube campanulate, 0.9–1.3 mm, lobes triangular to triangular-lanceolate, erect to slightly spreading, 0.6–1.3 mm, margins entire to slightly serrulate, apex obtuse to acute. STAMENS: equalling to longer than corolla lobes, anthers subround to broadly elliptic, 0.3–0.5 mm × 0.3–0.4 mm, filaments 0.6–1.5 mm. POLLEN GRAINS: prolate, 22–25 µm, puncta 250–800 nm, ornamentations conical. INFRASTAMINAL SCALES: oblong-ovate to truncate, equalling corolla tube, 1–1.2 mm, bridged at 0.4–0.5 mm, fimbriae 0.1–0.2 mm. STYLES: 0.8–1.2 mm, longer than the ovary. CAPSULES: indehiscent, globose-depressed to globose-obovoid 1.8–2.4 mm × 0.8–1.2 mm, interstylar aperture relatively large; persistent corolla surrounding the base of capsules. SEEDS: 3–4 per capsule, subrotund 0.9–1.1 mm × 1–1.2 mm, hilum area 0.35–0.45 mm, vascular scar 0.04–0.06 mm.

Cuscuta vandevenderi includes a substantial amount of morphological diversity; however, taking into account the discreet and continuous nature of this variation, we did not consider it feasible or useful to distinguish additional taxa. The plant was first described by Yuncker in 1921 as a variety (var. *esquamata*) of *C. gracillima*. However, contrary to the protologue, the 4-merous flowers appear to be an exception, and the infrastaminal scales are regularly present and well developed. The study of additional material from Sonora supports its description as a species that belongs to the same subclade as *C. deltoidea*. It is the only species of the complex that has evolved indehiscent capsules, a characteristic not mentioned by Yuncker. A valid binomial, *Cuscuta insquamata*, Yuncker refers to a Brazilian species.

ETYMOLOGY: the specific epithet honours Thomas Roger Van Devender, accomplished naturalist at the Arizona – Sonora Desert Museum (Tucson, Ariz.) who has collected extensively in Sonora and who made possible the description of this new species.

DISTRIBUTION AND HABITAT: Mexico, Sonora, in tropical deciduous forest, oak woodland, and pine-oak forests (sometimes in openings), 350–1550 m a.s.l., parasitizing various herbs, e.g., *Ayenia* L. (Sterculiaceae), *Chamaecrista* Moench (Caesalpiniaceae), *Cosmos* Cav. (Asteraceae), *Euphorbia* L. (Euphorbiaceae), *Sida* L. (Malvaceae), *Evolvulus* L. (Convolvulaceae). Flowering September–December.

CONSERVATION STATUS: G3 (vulnerable).

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References

- Costea, M. 2007 onwards. Digital Atlas of *Cuscuta* (Convolvulaceae). Wilfrid Laurier University Herbarium, Waterloo, Ont. [Online]. Available from www.wlu.ca/page.php?grp_id=2147&p=8968 [accessed 26 December 2007; updated 28 April 2008].
- Costea, M., and Tardif, F.J. 2006. Biology of Canadian weeds. *Cuscuta campestris* Yunck., *C. gronovii* Willd. ex Schult., *C. umbrosa* Beyr. ex Hook., *C. epithymum* (L.) L. and *C. epilinum* Weihe. Can. J. Plant Sci. **86**: 293–316.
- Costea, M., Nesom, G.L., and Tardif, F.J. 2005. Taxonomic status of *Cuscuta nevadensis* and *C. veatchii* (Convolvulaceae). Brittonia, **57**: 264–272. doi:10.1663/0007-196X(2005)057[0264:T SOCNA]2.0.CO;2.
- Costea, M., Nesom, G.L., and Stefanović, S. 2006a. Taxonomy of the *Cuscuta pentagona* complex (Convolvulaceae) in North America. SIDA Contrib. Bot. **22**: 151–175.
- Costea, M., Nesom, G.L., and Stefanović, S. 2006b. Taxonomy of the *Cuscuta indecora* (Convolvulaceae) complex in North America. SIDA Contrib. Bot. **22**: 209–225.
- Costea, M., Nesom, G.L., and Stefanović, S. 2006c. Taxonomy of the *Cuscuta salina californica* complex (Convolvulaceae). SIDA Contrib. Bot. **22**: 176–195.
- Dawson, J.H., Musselman, L.J., Wolswinkel, P., and Dörr, I. 1994. Biology and control of *Cuscuta*. Rev. Weed Sci. **6**: 265–317.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, **39**: 783–791. doi:10.2307/2408678.
- Fitch, W.M. 1971. Toward defining the course of evolution: minimum change for a specified tree topology. Syst. Zool. **20**: 406–416. doi:10.2307/2412116.
- Müller, K. 2005. SeqState — primer design and sequence statistics for phylogenetic DNA data sets. Appl. Bioinformatics, **4**: 65–69. doi:10.2165/00822942-200504010-00008. PMID:16000015.
- NatureServe. 2006. NatureServe Explorer: an online encyclopedia of life [web application]. Version 6.1. NatureServe, Arlington, Virginia. Available from www.natureserve.org/explorer [accessed 17 May 2007].
- Rambaut, A. 2002. Se-AL sequence alignment editor, version 2.0a11. University of Oxford, Oxford, UK.
- Simmons, M.P., and Ochoterena, H. 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. **49**: 369–381. doi:10.1080/10635159950173889. PMID:12118412.
- Stefanović, S., Kuzmina, M., and Costea, M. 2007. Delimitation of major lineages within *Cuscuta* subgenus *Grammica* (dodders; Convolvulaceae) using plastid and nuclear DNA sequences. Am. J. Bot. **94**: 568–589. doi:10.3732/ajb.94.4.568.
- Swofford, D.L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. Sinauer, Sunderland, Mass.
- Yuncker, T.G. 1921 (1970). Revision of the North American and West Indian species of *Cuscuta*. Ill. Biol. Monogr. **6**: 91–231.
- Yuncker, T.G. 1932. The genus *Cuscuta*. Mem. Torrey Bot. Club, **18**: 113–331.
- Yuncker, T.G. 1935. Three new *Cuscutas*. Bull. Torrey Bot. Club, **62**: 511–513. doi:10.2307/2481191.
- Yuncker, T.G. 1942. Three new *Cuscutas* from Western North America. Bull. Torrey Bot. Club, **69**: 541–543. doi:10.2307/2481629.
- Yuncker, T.G. 1946. Nomenclatural notes and a new species of *Cuscuta*. Bull. Torrey Bot. Club, **73**: 570–571. doi:10.2307/2481342.
- Yuncker, T.G. 1961. A new species of *Cuscuta* from Venezuela. Acta Biol. Venez. **3**: 99–101.

Yuncker, T.G. 1965. *Cuscuta*. N. Am. Flora, Ser. 2, 4: 1–51.

Appendix A

Herbarium vouchers for scanning electron microscopy (SEM) and molecular studies. A subset of plant material from which DNA was extracted is indicated. DNA extraction numbers and GenBank accession numbers (*trnL-F*; ITS) for sequences used in this study are provided. The symbol “****” indicates missing data.

Cuscuta macvaughii, MEXICO. Michoacan, Distrito Apatzingan, 360 m a.s.l., 25 Aug 1939, *Hinton 12098* (NY) (SEM; DNA No. 847; EF194314; EF194557).

Cuscuta warneri, USA. Utah, Millard County, 24.14 km west of Fillmore, 1957, *Warner* sp. nov. (RSA) (for SEM see Costea et al. 2006b; DNA No. 890; EF194292; EF194541).

Cuscuta gracillima, MEXICO, Colima: Municipio Comala, Rancho El Jabalí, north of Colima, 19°26'N, 103°41.8'W, 1500 m a.s.l., 7 November 1991, *Sanders et al. 11784* (UCR) (SEM); Guerrero, along Hwy 51 at kilometres 100–103, a few kilometres west of El Aguacate, 18°23'N, 100°03'W, 25 November 1971, *Iltis and Cochran 149* (MICH) (SEM; DNA No. 599; EF194303; EU426954⁴); Lago Tres Palos (Acapulco), 15 December 1966, *Boege 490* (GH) (DNA No. 620; EF194305; EU426955⁴); Jalisco, Municipio La Huerta, Rancho Cuixmata, 19°23'N, 104°58'45"W, 11 January 1991, *Lott et al. 3107* (UCR) (SEM); ca. 3 km southeast of Puerto Vallarta, 50 m a.s.l., 12 November 1963, *Dieterle 3093* (MICH) (SEM); Mexico State, Municipio Tejupilco, 17 km northeast of Tejupilco, 1530 m a.s.l., 13 October 1982, *Koch and Fryxell 82253* (NY) (SEM; DNA No. 600; EF194304; EF194551); 3 km west of Ixtapan del Oro on the road to Zitacuaro, 19°15'N, 100°16'W, 1900 m a.s.l., 3 December 1973, *Solheim and Benz 1073* (NY) (SEM); Michoacan, Municipio Aquila, road between Aquila and Ostula, 600 m a.s.l., 16 Dec 2006, *García Ruiz 7334* (CIMI, WLU) (SEM); Oaxaca, 10 km north of Pachutla Road to Oaxaca, 190 m a.s.l., 24 October 1982, *Martínez 2395* (MEXU) (SEM); near kilometre post 238, ca. 3.2 km north of Ixtapa on Hwy 125, 27 December 1983, *Yatskievych et al. 83-437* (IND) (DNA No. 463; EU426962⁴; EU426953⁴); Sinaloa, 91.7 km south of Mazatlán, along Hwy 15, just north of Escuinapa, 30 December 1968, *Clarke et al. 681230-17* (MICH) (SEM; DNA No. 621; EF194306; EF194550); Morelos, by the lanes of Cuernavaca, 1500 m a.s.l., 1895, *Pringle 6189* (F) (SEM).

Cuscuta choisiana Mexico: Puebla, 10 km to Metamoros, 5 September 1966, *Véase 490* (MEXU).

Cuscuta punana Ecuador, Guayas, Isla Puná, Río Hondoto la Florida, 02°49'S, 80°01'W, 0 m a.s.l., 7 June 1987, *Madsen 63850* (AAU) (SEM; DNA No. 1120; EU426963⁴; EU426957⁴); El Placer, 0–5 km on path toward Río Hondo, 02°48'S, 80°00'W, 8 September 1987, *Madsen 63936* (AAU) (SEM).

Cuscuta sidarum Costa Rica, Puntarenas, 10°00'00"N,

84°42'00"W, 100 m a.s.l., 31 January 1993, *Hammel 18763* (F) (SEM; DNA No. 519; EF194308; EF194552). Guatemala, Departamento Zacapa, vicinity of Zacapa, 200 m a.s.l., 7–16 October 1940, *Standley 74614* (NY) (SEM). Honduras, Departamento de Choluteca, vicinity of Choluteca, 20 m a.s.l., 31 October – 9 November 1949, *Standley 24370* (F) (SEM). Mexico, Colima, near km 293, 24.1 km southeast of Manzanillo, 3 December 1959, *McVaugh and Koelz 1630* (MICH) (SEM); Jalisco, Municipio La Huerta, Laguna La Virgencita, 2 km northwest of the Biological Station, Chamela, 7 November 1986, *Ayala 1054* (TEX & LL) (SEM; DNA No. 1005; EF194307; EF194553); Michoacan, hills between Río Tepalcatepec and Arteaga, along Hwy S, 350 m a.s.l., 24 February 1965, *McVaugh 22526* (MICH) (SEM). Nicaragua, Departamento de Managua, Managua, near Parque de las Madres, 12°08'N, 86°16'W, 80 m a.s.l., 30 November 1981, *Stevens and Krukoff 20950* (GH) (SEM; DNA No. 751; EF194310; EU426956⁴).

Cuscuta colombiana, Colombia, Departamento Magdalena, near Riohacha, 23 December 1944, *Haught 4535* (US, NY) (SEM; DNA No. 1068; EF194312; ***). Venezuela: Guarico, Parque Lazo Marti, La Aguada, Calabozo, March 1961, *Aristeguieta 4568* (F) (SEM; DNA No. 935; EF194311; EF194554); February 1961, *Aristeguieta 4500* (VEN); May 1961, *Tamayo 4432* (VEN) (SEM).

Cuscuta deltoidea, Mexico, Colima, Manzanillo, 21 October 1910, *Orcutt 4457* (MEXU) [SEM; DNA No. 977; EF194313; EF194555]; Michoacan, Monte Leon, 11 November 1892, *Pringle 5350* (US); 1 km north of Zinaparo, 1600 m a.s.l., 17 October 1988, *García Ruiz 2516* (CIMI).

Cuscuta vandevenderi, Mexico, Sonora, Sierra Tecurahui, 1200–1500 m a.s.l., 26–28 October 1961, *Gentry et al. 19423* (US) (SEM; DNA No. 1058; EU426964⁴; EU426958⁴); Municipio de Álamos, 3.9 km above Rancho El Palmarito, 23.9 km, east-northeast of Álamos, 27°03'04"N, 108°45'51"W, 516 m a.s.l., 1 October 2006, *Van Devender et al. 2006-983* (WLU) (SEM); Santa Ana de Yécora; 28°22'40"N, 109°09'W, 850 m a.s.l., 20 September 1998, *Van Devender et al. 98-1434* (ARIZ, MEXU, NY, WLU) (SEM; DNA No. 1078; EU426965⁴; EU426960⁴); Sierra de Mazatán, Rancho El Flauta, 29°06'N, 110°12'50"W, 1260 m a.s.l., 9 October 2004, *Reina et al. 2004-1224* (USON, WLU) (SEM; DNA No. 1079; EU426966⁴; EU426959⁴); Cañada La Ventana (Arroyo El Otro Lado), 2.5 km (by air) east-southeast of Yécora; 28°21'38"N, 108°53'55"W, 1520 m a.s.l., 18 September 1998, *Van Devender et al. 98-1334* (WLU) (SEM); El Guayabo Crossing of Río Cuchujaquí, 14 km (by air) east-southeast of Álamos, 27°00'05"N, 108°47'08"W, 370 m a.s.l., 21 November 1993, *Steinmann et al. 93-349* (ASU) (SEM; DNA No. 1092; EU426967⁴; EU426961⁴).

References

Costea, M., Nesom, G.L., and Stefanović, S. 2006b. Taxonomy of the *Cuscuta indecora* (Convolvulaceae) complex in North America. *SIDA Contrib. Bot.* 22: 209–225.

⁴Sequence newly generated for this study.