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Cuscuta sect. *Californicae* (Convolvulaceae) Revisited: 'Cryptic' Speciation and Host Range Differentiation

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Abstract—*Cuscuta californica* complex (sensu lato, s. l.) is a western North American group of species in which the infrastaminal scales are reduced, making the morphological delimitation of species particularly challenging. A revision of this group was prompted by the discovery of an apparent new species from central California based primarily on molecular means. Driven by this finding, the morphological limits of *C. californica* s. l. species were comprehensively re-evaluated through a morphometric study. DNA sequences from plastid (*trnL–F* region and *rbcL*), nuclear ribosomal ITS and 26S rDNA, as well as a low-copy nuclear pentatricopeptide repeat (PPR) gene were used to reconstruct evolutionary relationships among taxa. Last but not least, the host range of relevant taxa was determined using herbarium specimens. Molecular results strongly supported the new species, ***C. difficilis***, which was found to be morphologically separated from *C. brachycalyx* only by subtle calyx lobe and corolla tube shape differences. Despite sharing some of the hosts, all the members of *C. californica* s. l. exhibited a differentiation of their host ranges. An identification key and description of the new species were provided together with a discussion on the systematics and host range of *C. californica* s. l.. Hybridization, accompanied by plastid capture, was suggested as a possible mechanism of speciation for *C. brachycalyx*.

Keywords—dodder, host specificity, hybridization, morphometry, new species, parasitic plants, phylogeny, plastid capture.

Cuscuta section *Californicae* is a western North American clade of subg. *Grammica* (Costea et al. 2015b) which includes Yuncker's subsections *Californicae* and *Subinclusae* (Yuncker 1932). The merging of the two subsections into section *Californicae* was based on both morphological features (Costea et al. 2006) and molecular phylogenies (Stefanović et al. 2007; García et al. 2014). Section *Californicae* is the only clade of subg. *Grammica*, and the genus *Cuscuta* more broadly, in which flowers exhibit a reduction trend for the infrastaminal scales (Riviere et al. 2013). Infrastaminal scales are digitate or lobed structures with defense role found at the base of the stamens, which have provided some of the most important taxonomic characters at species level (reviewed by Riviere et al. 2013). In the taxa of *C. californica* species "complex" (sensu lato, s. l.), originally included by Yuncker (1932) in subsect. *Californicae*, the infrastaminal scales have completely disappeared. Historically, the morphological delimitation of *C. californica* s. l. has proven to be especially challenging because the absence of the scales eliminates a significant number of characters from an already depleted array of potentially examinable floral features (e.g. Costea et al. 2009; Riviere et al. 2013; Costea et al. 2015a). Within *C. californica* s. l., Yuncker (1932) recognized three species: *C. brachycalyx, C. californica*, and *C. occidentalis*. Beliz (1993) accepted only one species, a broadly defined *C. californica*, in which she also included *C. suksdorfii*. Costea et al. (2009), based on a combination of strongly supported molecular and corroborating morphological evidence, reinstated the original species circumscriptions of Yuncker (1932), which were also maintained in the taxonomic treatment of *Cuscuta* for the flora of California (Costea and Stefanović 2012).

Extensive field work conducted in California during 2013–2017, and further herbarium examination in connection with a future monograph of the genus, revealed yet another species within this group. The field-collected specimens of this new species were initially identified morphologically as belonging to *C. brachycalyx*, *C. californica*, or a form intermediate between *C. californica* and *C. occidentalis*, but the molecular data revealed their distinctiveness as a new lineage. In turn, this apparently new cryptic species prompted a comprehensive reevaluation of the tenuous morphological borders separating these taxa. In addition, because a recent study in sect. *Denticulatae* (García et al. 2018) revealed diverging host range patterns among species, we questioned whether the taxa of *C. californica* s. l. also possess different host ranges.

The aims of this study are to: 1) describe the new species; 2) examine its evolutionary relationships among *C. californica* s. l. taxa based on multilocus sequences from plastid and nuclear genomes; 3) compare the morphology of the new species to that of *C. californica*, *C. brachycalyx*, and *C. occidentalis* by means of a morphometric study; and 4) determine the host range of taxa within *C. californica* s. l. and analyze the extent of host range overlaps versus host specificity.

Materials and Methods

Taxon Sampling and Outgroup Selection—Over 2000 herbarium specimens, from approximately 50 herbaria, were identified, annotated, and examined for basic morphology, as well as host and geographical range for species in the *Cuscuta californica* s. l. group. In addition, we conducted a series of targeted field trips to the areas of particular interest for this section in the spring/summer of 2013–2017. Efforts were made to ensure sampling from localities across most of the known geographical range of each species (Costea et al. 2006; Costea and Stefanović 2012).

From these collections, a total of 139 specimens were selected for the morphometric analyses: 58 for *C. californica* (46 var. *californica* and 12 var. *papillosa*); 30 for *C. occidentalis*; 37 for *C. brachycalyx*; and 13 for the newly described species, *C. difficilis* (Appendix 1). *Cuscuta californica* var. *apiculata* Engelm. was not included in the study because of insufficient quantity and quality of material available (Costea et al. 2006). This taxon diverges morphologically from those included in this study because of its ovoid capsules with only one seed (Yuncker 1932; Costea et al. 2006; Costea and Stefanović 2012). From these samples, a subset of 42 specimens was used for the molecular phylogenetic analyses. Compared to our previous studies (Costea and Stefanović 2009a, b; Costea et al. 2009), we substantially improved our population-level sampling across all species of the *C. californica* group, representing the breadth of their morphological diversity and geographical range. In addition to the 15 DNA samples used in the past, total genomic DNA was isolated from 27 newly obtained localities, coming from additional herbarium material and field-collected (Appendix 1). Based on our previous, more inclusive phylogenetic analyses of *Cuscuta* sect. *Californicae* (Costea and Stefanović 2009a, b; Costea et al. 2009), several, progressively more distant species from this section were used as outgroups, with multiple representatives each (*C. subinclusa, C. jepsonii*, *C. pacifica*, *C. howelliana*, and *C. suksdorfii*).

Molecular Techniques—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). Double-stranded 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 *trnLF* region) was amplified using primers designed by Taberlet et al. (1991) while the *rbcL* gene was amplified using primers published by Olmstead et al. (1992). 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). To amplify a ca. 950 bp portion at the 5′ end of the nuclear large-subunit ribosomal DNA (26S rDNA), primers described by Kuzoff et al. (1998) were used. PCR reagents and conditions as well as amplicon purification followed the protocols detailed in Costea and Stefanović (2009b). An additional unlinked and low-copy nuclear gene, from the pentatricopeptide repeat (PPR) family of genes, was used. An approximately 770 bp portion at the 3′ end of the AT4G01570 PPR ortholog (sensu Yuan et al. 2009) was amplified following protocol described in Yuan et al. (2010). Using *Cuscuta*-specific primers PPR1447F (5′-ARGGAGAGGCTTATGAAGCA-3′) and PPR2216R (5′-TTGTAAGTGACAACATCAGG-3′) designed by Miguel A. García, PPR amplicons were obtained from 17 individuals, proportionally subsampled across all four ingroup species, plus seven outgroup individuals. Our sampling strategy sought to fully capture the allelic variation for this nuclear locus within individuals. Thus, multiple clones (3–8) per sample were sequenced, but for fewer individuals/populations compared to plastid and ribosomal matrices. Purified PCR products were cloned for all the species using the pGEM-T Easy Vector II cloning kit (Promega, Madison, Wisconsin). All cleaned PCR products were sequenced, including both strands to ensure accuracy, at the McGill University and Génome Québec Innovation Centre (Canada). Sequences newly generated for this study were deposited in GenBank (accession numbers MT070162-MT070401; see Appendix 1).

Sequence Alignment and Phylogenetic Analyses—Sequencher 4.8 (Gene Codes Corp., Ann Arbor, Michigan) was used to assemble and edit chromatograms of complementary strands. Sequence alignments were performed manually using the program Se-Al v. 2.0a11 (Rambaut 2002). Albeit sequences of all accessions were readily alignable in both the plastid and nuclear matrices, a number of gaps had to be introduced in the alignments. Preliminary phylogenetic analyses were conducted to explore the distribution of phylogenetic signal in the individual matrices with and without coded gaps. Neither resolution nor support was affected in a substantial way by inclusion of gaps (results not shown), and therefore gaps in the alignments were treated as missing data in subsequent analyses. Phylogenetic analyses were conducted under a variety of distance- and character-based methods.

To start exploring relationships among and within species and populations of the *C. californica* s. l. species group, we initially employed parsimony for each individual dataset similar to Stefanović and Costea (2008; see their 'Phylogenetic analyses' for details). Support for clades were inferred by nonparametric bootstrapping (Felsenstein 1985), using 500 heuristic bootstrap replicates, each with 20 random addition cycles, TBR branch swapping, and MULTREES option off. Conflict between datasets was evaluated by visual inspection, looking for strongly supported yet conflicting tree topologies resulting from individual data matrices.

Maximum likelihood analysis was performed using RAxML-HPC2 v. 8.2.10 (Stamatakis 2014) and run on the XSEDE computing cluster using the CIPRES Science Gateway v. 3.3 (Miller et al. 2010). The program MrModeltest v. 2.3 (Nylander 2004) was used to determine the model of sequence evolution that fits best the combined plastid and nuclear ribosomal data. We used the GTR + G model of sequence evolution, and 1000 rapid bootstrap replicates to assess branch support.

For the PPR clones, the planar phylogenetic networks were constructed using a neighbor-net (NN) algorithm (Bryant and Moulton 2004) as implemented in SplitsTree v. 4.11.3 (Huson and Bryant 2006). Prior to network analyses, sequences were corrected by imposing K80 + G model of DNA evolution, as selected by MrModeltest v. 2.3 (Nylander 2004).

Morphology and Morphometric Analyses—To examine the pollen of *C. difficilis*, anthers were removed from flowers of all the herbarium specimens available for this species (Appendix 1), mounted on aluminum stubs, squashed, and sputter-coated with 30 nm of gold using Emitech K550 sputter coater. Examination, imaging, and measurements of pollen grains were made using a Hitachi SU1510 scanning electron microscope (SEM) at 10 kV.

Based on initial molecular results (individual parsimony trees not shown), four operational taxonomic units (OTUs) corresponding to *C. difficilis*, *C. californica*, *C. brachycalyx*, and C. *occidentalis* were included in the morphometric analyses to test their morphological distinctiveness (Appendix 1). For *C. californica* we used specimens of both var. *californica* and var. *papillosa*. A previous morphometric study within a different group of species of sect. *Californicae* (*C. salina-C. pacifica*; Costea et al. 2009) provided a preliminary list of useful characters. However, a defining characteristic of the taxa examined in this study is the total reduction of infrastaminal scales, which are an important source of characters in *Cuscuta* (e.g. Riviere et al. 2013; Costea et al. 2015a). Additionally, only ca. 15% of the specimens examined had mature capsules and seeds, which further reduced the number of potential characters. Thus, in total, only 24 characters (18 quantitative and 6 binary; Table 1) were formulated (25% less than in other *Cuscuta* studies; e.g. 32 characters in Costea et al. 2009; 2015a; García et al. 2018).

Flowers removed from herbarium specimens were steeped in gradually warmed 50% ethanol, which was then allowed to boil for a few seconds to rehydrate tissues. Ethanol hardens the tissues, which are very delicate in *Cuscuta* flowers. For basic morphology, flowers were dissected under a Nikon SMZ1500 stereomicroscope and imaged with PaxCam Arc digital camera (MIS Inc. 2019, Villa Park, Illinois) equipped with a Pax-it 8.2 imaging software. Numerous photographs illustrating details of the floral and fruit morphology for all taxa, including their type collections, are made available on the Digital Atlas of *Cuscuta* website (Costea 2007).

To determine the extent of morphological variation, the data were visualized with both clustering and ordination methods using PAST (version 3.22; Hammer et al. 2009). Principal coordinates analysis (PCoA or metric multidimensional scaling) and unweighted pair-group average (UPGMA) were both conducted using the Gower's coefficient of similarity. Analyses were first carried out on the entire dataset, and then on two partitions with only three species (*C. difficilis* + *C. brachycalyx* + *C. californica* and *C. difficilis* + *C. californica* + *C. occidentalis*).

Host Range Assessment—Host range was determined based on herbarium specimens. Among the over 2000 herbarium sheets examined, only 614 *Cuscuta* specimens had the host(s) indicated in the label or included sufficient plant material to allow host identification. "False" hosts, used by dodders only for support, were eliminated based on the lack of haustorial penetration observed under a Nikon SMZ1500 stereomicroscope. The taxonomy and nomenclature from *The Jepson Manual* second edition were followed for the hosts (Baldwin et al. 2012). In some cases, host identification was possible only to genus level. In total, the host range assessment was based on 434 specimens of *C. californica* (397 var. *californica* and 37 var. *papillosa*), 102 for *C. occidentalis*, 66 for *C. brachycalyx*, and 13 for *C. difficilis*). To examine the host-parasite relationship, a host frequency indicator (HFI) was determined at three taxonomic levels (family, genus, and species/subspecies/variety) for each of the five *Cuscuta* taxa. HFI shows the percentage of the number of instances *Cuscuta* taxa were found on each of the hosts (family, genus, species) from the total host occurrences recorded for a particular dodder taxon. The HFI matrix was then mapped into a directed bipartite network using Cytoscape (Shannon et al. 2003). The lists

TABLE 1. Characters scored for the morphometric study.

Continuous characters. 1. Pedicel length (mm). 2. Length of receptacle prolongation ("fleshiness") on pedicel (mm). 3. Flower length (mm; measured from base of receptacle to the tip of corolla lobes). 4. Calyx lobe length (mm). 5. Length of acuminate part of calyx. 6. Calyx lobe width (mm, measured at the base). 7. Calyx tube length (mm). 8. Angle formed by the margins of calyx lobes at the apex (arc degrees). 9. Corolla lobe length (mm). 10. Corolla lobe width (measured at the base, mm). 11. Corolla tube length (mm). 12. Corolla tube circumference (mm). 13. Angle formed by corolla lobes at the apex (arc degrees). 14. Stamen filament length (mm). 15. Anther length (mm). 16. Anther width (mm). 17. Length of longest style (mm). 18. Length of shortest style (mm).

Qualitative characters. 19. Calyx glossy: present (1), absent (0). 20. Calix lobes rounded: present (1), absent (0). 21. Calix lobes acute: present (1), absent (0). 22. Calix lobes acuminate: present (1), absent (0). 23. At least 2 calyx lobes are overlapping at the base: present (1), absent (0). 24. Three or more calyx lobes are overlapping at the base: present (1), absent (0). 25. Calyx papillae: present (1), absent (0). 26. Corolla papillae: present (1), absent (0).

of family, genus, and species/infrageneric hosts were also imported into InteractiVenn (Heberle et al. 2015) to summarize shared versus unique hosts as Venn diagrams. To analyze the overall relationship among the host ranges of the five *Cuscuta* taxa, the HFI data were also clustered using UPGMA and the Euclidian coefficient of similarity. Morphometric and host data generated in the study, as well as supplemental figures, were deposited in Dryad (Costea et al. 2020).

RESULTS

Molecular Phylogenies—Several preliminary phylogenetic analyses were conducted to explore the distribution of phylogenetic signal in the different individual matrices, with and without coded gaps. Clades recovered in each analysis were congruent with the tree structure recovered using data from the other matrices derived from the same genome. Being present on the same organellar genome, *trnLF* and *rbcL* are on the same linkage group and thus were treated as one locus (ptDNA). Similarly, nuclear ITS and 26S rDNA sequences were treated as one locus (nrDNA). An additional low-copy gene from the pentatricopeptide repeat (PPR) family, albeit nuclear in origin, was treated separately because it is unlinked to the nuclear ribosomal arrays (Yuan et al. 2009). We present here only the maximum likelihood results for the combined ptDNA data and the combined nrDNA data (Fig. 1). The trees

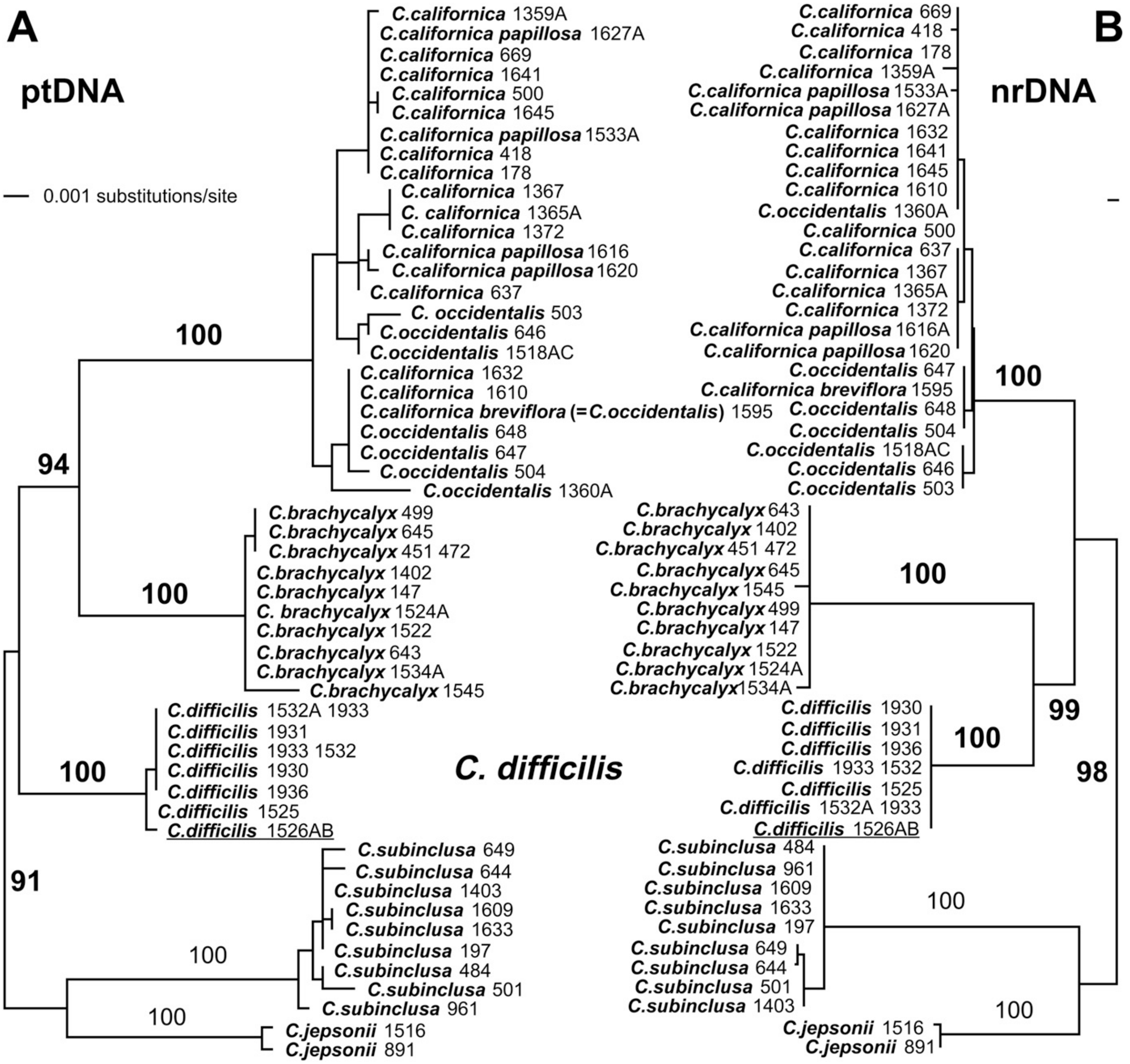

FIG. 1. Phylograms derived from maximum likelihood analyses of plastid DNA data (A. *trnLF* plus *rbcL*) and nuclear ribosomal DNA data (B. ITS plus 26S rDNA) from the *Cuscuta californica* s. l. complex. The lineages corresponding to four species currently circumscribed within this group are shown, including the newly described *C. difficilis* (type specimen underlined). The trees are rooted using closely related species from section *Californicae* as outgroups. Maximum likelihood bootstrap values are indicated for nodes supported at ≥ 80%. Numbers associated with nodes represent bootstrap values for some major groups (≥ 80%). Numbers following species names correspond to DNA accession numbers (see Appendix 1). One specimen of *C. occidentalis* (*Bjork* 3056, WTU) had been kept as *C. californica* var. *breviflora*, which is a synonym of the former.

from individual matrix analyses are not shown, except for the PPR sequences (Supplemental Fig. S1, Costea et al. 2020). The relationships derived under the parsimony criterion are topologically congruent with those inferred through the ML approach (Fig. 1) and have received similar levels of support (not shown).

All of the analyses conducted here show the *C. californica* s. l. to be a strongly supported monophyletic group (91%, 98%, and 99% BS; Fig. 1, Fig. S1). Within this complex, three major lineages can be delimited based on a combination of their individual strong support (100% BS) and molecular distinctiveness, as evidenced by the long branches subtending them (Fig. 1). The first lineage consists of all individuals and populations belonging to *C. difficilis*, a species newly described here. The second lineage comprises two morphologically very similar species, *C. californica* s. s. and *C. occidentalis*. Results reveal that reciprocal monophyly in this group has not yet been achieved, with individuals/populations of one species being nested within representatives of the other. The extent of sequence interspersal across representatives for these two species is best illustrated by the lack of support for their monophyly in the PPR data (Fig. S1). The third lineage consists of a single species, *C. brachycalyx*. Morphologically closely allied, yet quite distinct using molecular data, *C. brachycalyx* is found to be sister to the *C. californica* plus *C. occidentalis* clade with plastid-derived data (Fig. 1A; 94% BS). However, a different position for this species is recovered with nuclear data. Both nuclear ribosomal sequences and PPR sequences place *C. brachycalyx* as sister to *C. difficilis*, with strong (99% BS; Fig. 1B) to moderate (60%; Fig. S1) support, respectively. The points of congruences and discordances among different unlinked gene trees, and the correspondence between rooted and unrooted phylogenetic hypotheses for the *C. californica* s. l. group, are summarized in Fig. 2.

Morphometric Analyses—Principal coordinates analysis (PCoA) of the entire morphometric dataset resulted in two distinct groups of specimens: one that corresponded to *C. brachycalyx* and *C. difficilis*, and one for *C. californica* (including var. *papillosa*) and *C. occidentalis* (Fig. 3A). In this data visualization, *C. difficilis* was inseparable from *C. brachycalyx*, while *C. californica* and *C. occidentalis* overlapped in some of their specimens (Fig. 3A). However, PCoA of the dataset without *C. occidentalis* separated *C. difficilis* and *C. brachycalyx* (Fig. 3B). In this latter case, the first coordinate axis accounted for 41.3% of the variance and distinguished *C. californica* from *C. brachycalyx* + *C. difficilis* (Fig. 3B). The second coordinate accounted for 9.216% of the variation and parted *C. difficilis* from *C. brachycalyx* (Fig. 3B). The PCoA of the dataset without *C. brachycalyx* improved marginally the distinction between *C. californica* and *C. occidentalis*, which still overlapped in a few specimens (Fig. 3C).

UPGMA analysis of the entire dataset also resulted into two main clusters, but *C. difficilis* and *C. brachycalyx* formed two distinct sub-clusters (Fig. S2; cophenetic correlation = 0.866). Although the same was largely true for *C. californica* and *C. occidentalis*, three samples of the latter were distributed among clusters of *C. californica* (Fig. S2). Partitioned datasets consisting only of three species, as used in the PCoA analyses, produced clear species clusters in both cases (results not shown).

Host Range—The overall host range of *C. californica* s. l. included 237 species and varieties/subspecies, which belonged to 120 genera and 32 families (Figs. 4, 5; Figs. S3, S4). The top most common hosts at family-, genus-, and species-level are summarized in Table 2. Only one family (Asteraceae; Fig. 5; Fig. S3) and genus (*Centaurea*; Fig. 5; Fig. S4) were common hosts for all the *Cuscuta* species, but no single species was shared among all the parasites (Figs. 4, 5). For each species, the number of exclusive hosts is much higher compared to that of the shared hosts, which indicates that each *Cuscuta* species possesses a different host range (Fig. 5). *Cuscuta californica* has the most extensive host range (78 genera, 149 species/subspecies/varieties), followed by *C. occidentalis* (52 genera, 76 species), *C. brachycalyx* (32 genera, 47 species) and *C. difficilis* (3 genera, 3 species) (Figs. 4, 5). Most hosts are shared between *C. californica* and *C. occidentalis* (17

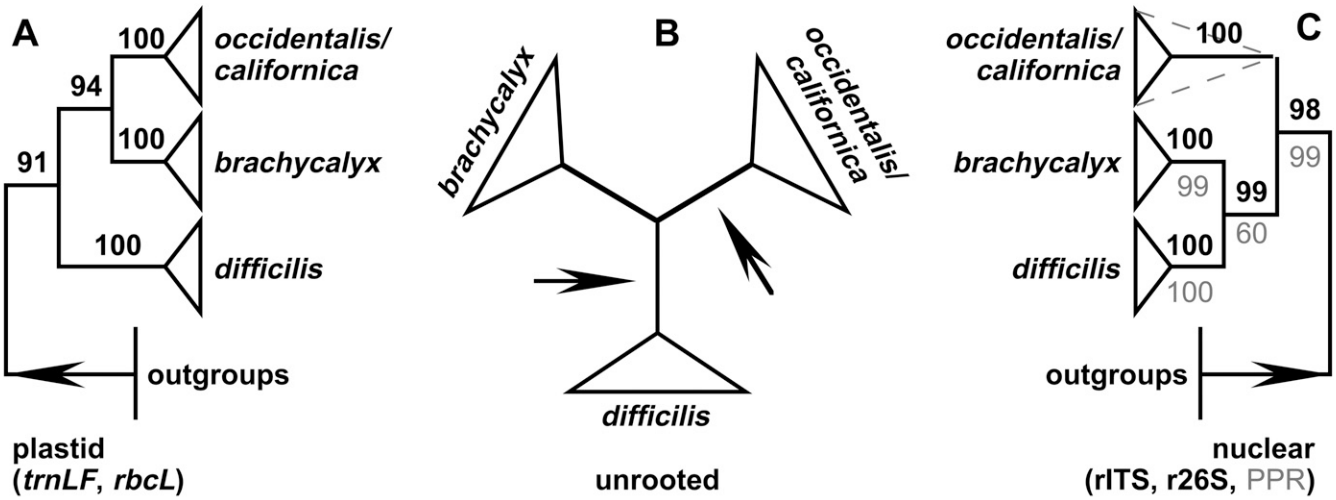

FIG. 2. Correspondence between competing rooted and unrooted phylogenetic hypotheses for the *Cuscuta californica* s. l. group, showing the same underlying topology when outgroups are excluded. A. Schematic representation of the plastid sequence derived trees (see Fig. 1A, whose bootstrap values are shown here). B. Unrooted network, with arrow showing alternative rootings as in A and C. C. Schematic representation of the nuclear sequence-derived trees. Bootstrap values above branches (in bold) are those from Fig. 1B (nrITS and nr26S); values below branches are from the PPR (At4G01570 ortholog) neighbor-net split graph (compare with Fig. S1). Note that the *Cuscuta occidentalis/californica* clade is strongly recovered with nuclear ribosomal sequences but not with the PPR sequences (dotted lines).

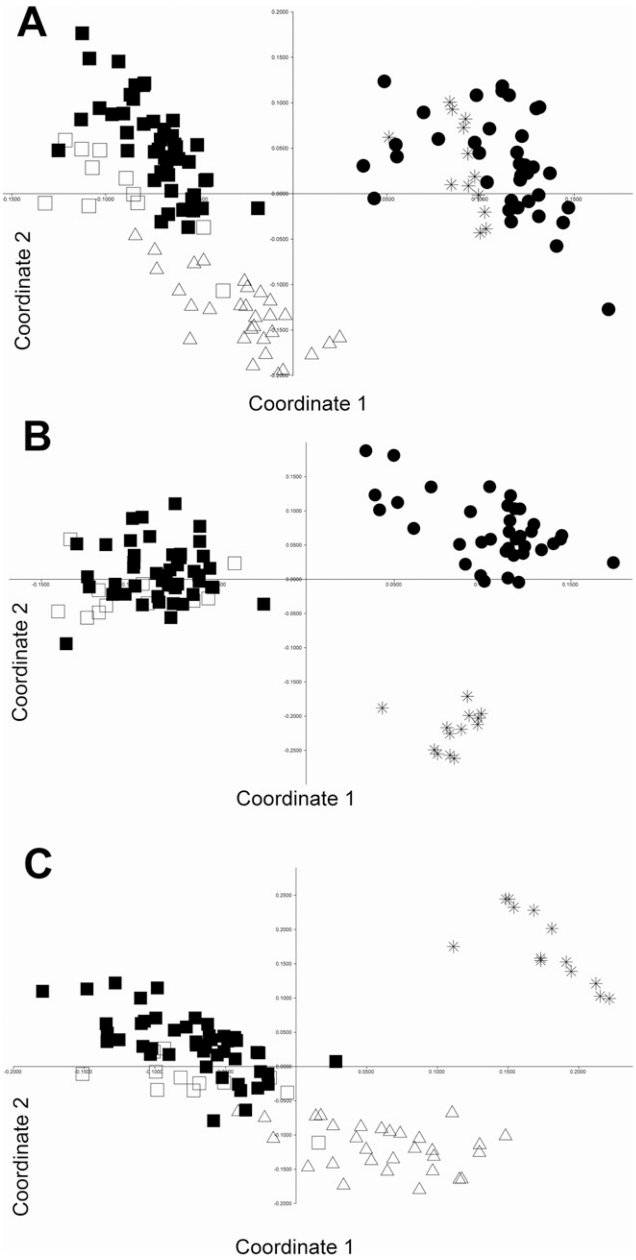

FIG. 3. Principal coordinates analysis (PCoA) of various data sets. A. Complete dataset (four species). First coordinate (33.206% of the variance) separated *C. californica* (including var. *papillosa*) and *C. occidentalis* from *C. difficilis* together with *C. brachycalyx*. B. Dataset without *C. occidentalis*. First coordinate axis accounted for 41.3% of the variance and distinguished *C. californica* from *C. brachycalyx* together with *C. difficilis*; the second coordinate (9.216% of the variation) parted *C. difficilis* from *C. brachycalyx*. C. Dataset without *C. brachycalyx*. First coordinate axis (29.479%) separated almost entirely *C. occidentalis* from *C. californica*. Filled squares = *C. californica* var. *californica*; empty squares = *C. californica* var. *papillosa*; triangles = *C. occidentalis*; circles = *C. brachycalyx*; stars = *C. difficilis*.

genera, 20 species) followed by *C. californica* and *C. brachycalyx* (7 genera, 9 species) and *C. brachycalyx* and *C. occidentalis* (3 genera, 3 species) (Fig. 5). Although *C. californica* and *C. occidentalis* share most host species, these hosts represent only 8.43% of the combined host range of the two species. With the exception of *C. difficilis*, all other dodders in this group have in common genus *Eriogonum*. Although no single host species is common among all the *Cuscuta* species, *Acmispon americanus* (Fabaceae) and *Abronia umbellata* (Nyctaginaceae) are shared with different frequencies by *C. californica*, *C. brachycalyx*, and *C. occidentalis*, while *Centaurea solstitialis* (Asteraceae) is a mutual host for *C. brachycalyx*, *C. occidentalis*, and *C. difficilis*. The latter species has the narrowest host range and occurs with a high frequency on *Centromadia parryi* ssp. *rudis*. Host ranges of *Cuscuta* also seem to differentiate at an infraspecific level. Although more than half of the hosts of *C. californica* var. *papillosa* were shared with var. *californica*, the former still possessed eight exclusive host species (not shown). The UPGMA dendrogram using HFI data resulted in three clusters (Fig. S5): one that grouped together the varieties of *C. californica*, one for *C. occidentalis* and *C. brachycalyx*, and one for *C. difficilis*, which was the most dissimilar. This clustering reflects not only the presence on common hosts (with different frequencies), but also the extent of occurrence on exclusive hosts (Fig. S5).

TAXONOMIC TREATMENT

Cuscuta difficilis Stefanović & Costea, sp. nov. TYPE: USA. California, Glenn Co.: Sacramento National Wildlife Refuge, along trail in vernal pool/alkali meadow, 39°25′33.6″N 122°11′16.9″W, 3 Jun 2015, *Stefanović SS-15–09* (holotype: TRTE!, isotypes: WLU!, NY!)

Cuscuta difficilis resembles closest *C. brachycalyx* but differs from it in the calyx lobes with rounded to subacute apices. In addition, corolla is campanulate and it becomes globose in fruit (in *C. brachycalyx*, corolla is campanulate-tubulose and it becomes urceolate in fruit). Superficially it also resembles *C. californica* from which it contrasts in the calyx shorter than the corolla tube and the same calyx lobe characters that separate it from *C. brachycalyx*.

Stems slender to medium, yellow-orange. **Inflorescences** dense to loose, racemiform-umbelliform cymes of 2–5 flowers, often confluent in larger inflorescences; pedicels 0.4–3.2 mm long; one bract at the base of cymes, membranous, ovate, 0.9–1.4 × 0.4–0.6 mm, margins entire, apex rounded to subacute. **Flowers** 5-merous, 3.3–4.8 mm long, fleshy; papillae absent; laticifers sometimes visible in the calyx and corolla, elongated; receptacle ± fleshy, brown when dried; calyx 0.7–1.4 mm long, membranous and golden-yellow or brownish when dried, ± finely reticulate and shiny, cupulate, not angled, 1/2–3/4 the length of the corolla tube, divided ca. 2/3 to the base, tube 0.4–0.6 mm long, lobes 0.7–1.1 mm long, ± equal, ovate, not overlapping and usually forming sinuses at base, margins entire, rounded to sub-acute; corolla 3–4.6 mm long, white when fresh, creamy-white when dried, the tube 1.7–2.4 mm long, initially campanulate becoming globose; lobes 1.7–2.3 mm long, oblong, equaling the corolla tube, initially erect, later patent or reflexed, not overlapping at the base, apex acute to sub-acute, at least in some lobes inflexed or cucullate when reflexed; stamens exerted when the flowers are completely open, anthers oblong, 0.7–1.2 × 0.4–0.64 mm, filaments 0.7–1.2 mm long; infrastaminal scales reduced but their bridges are visible at the base of corolla tubes, 0.3–5 mm long; styles evenly filiform, 1.2–3 mm long, longer than the ovary, which is spherical, not thickened nor enlarged into a collar apically. **Pollen** 3(–4)-zonocolpate (16–) 27.23–29.6 (–33.93) μm, perprolate to prolate, rounded at poles, tectum imperforatum or with a few puncta, 0.2–0.3 μm in diameter, granulate.

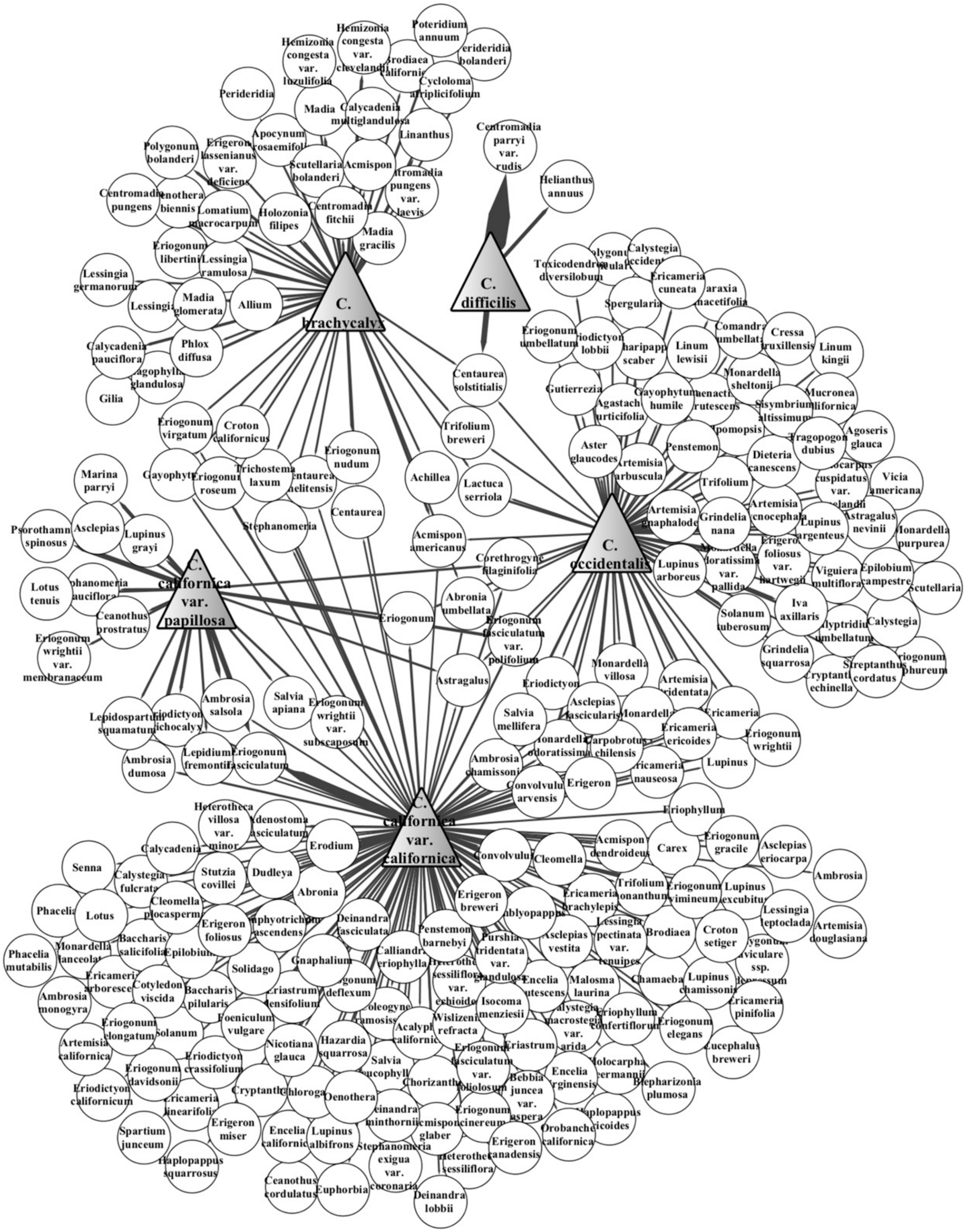

FIG. 4. Species and variety level host range in *Cuscuta californica* s. l. visualised as a bipartite directed network. Five *Cuscuta* taxa nodes represented as triangles are connected with the corresponding nodes (circles) of their species/variety hosts. The frequency of particular *Cuscuta*-host species associations is indicated by the proportionally thickened lines (edges) using the host-frequency indicator (HFI). The number of edges shared by *Cuscuta* taxa and their host species indicates the amount of overlap or separation of the host ranges.

Capsules indehiscent, globose to globose-depressed, 2.2–3 × 1.6–2 mm, surrounded by the withered, white-papery corolla; corolla lobes at this stage patent or reflexed; interstylar aperture 0.5–0.7 mm long, initially not visible but as the capsules develop, the corolla stretches around them, it becomes visible. **Seeds** 2–4 per capsule, angled, subround to broadly elliptical, 1.1–1.4 × 1–1.3 mm, seed coat cells alveolate/papillate, hilum area 0.2–0.45 mm in diameter, scar 0.14–0.2 mm long. Figure 6.

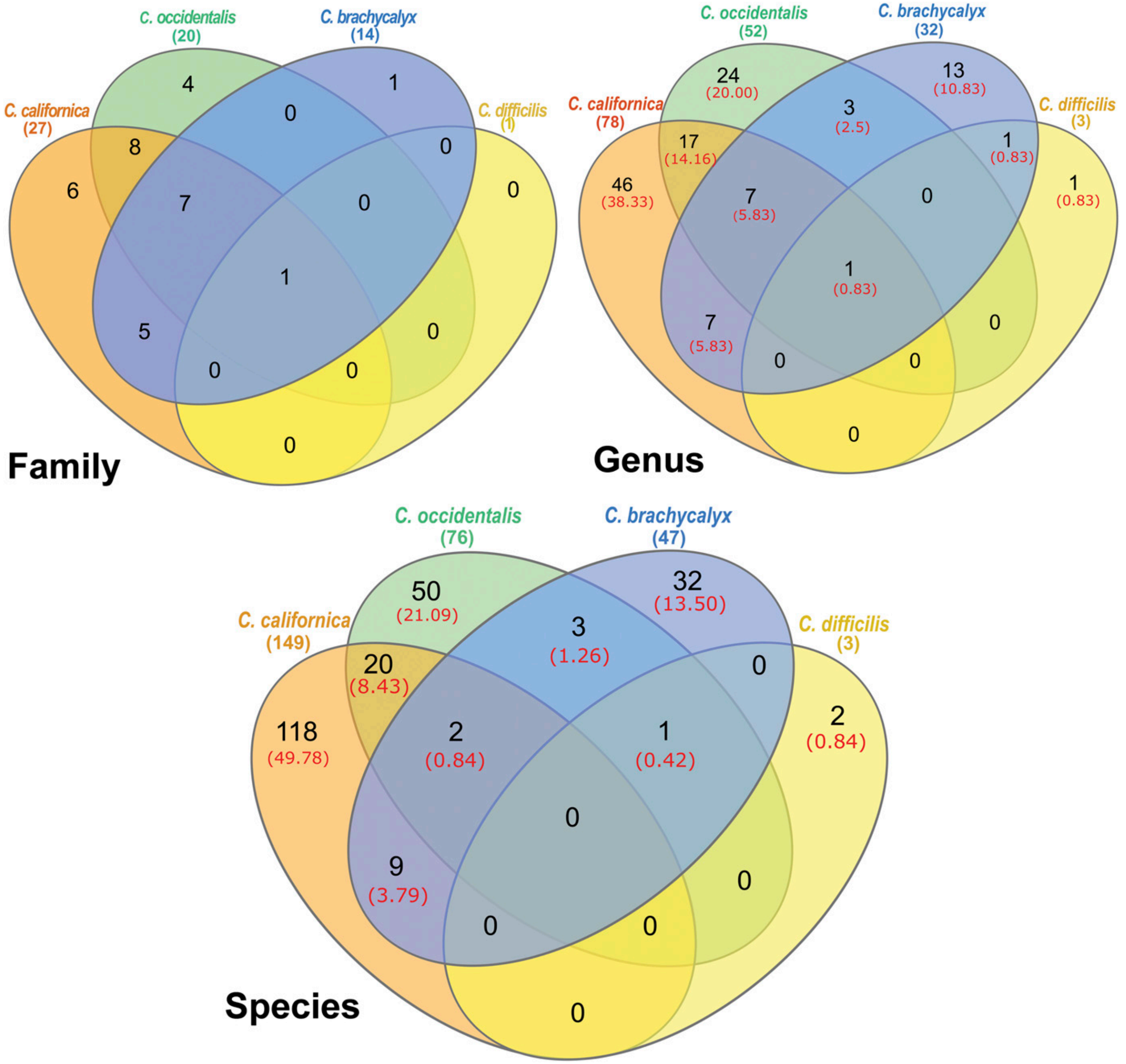

FIG. 5. Host range summaries in *Cuscuta californica* s. l. represented as Venn diagrams. Different host taxonomic levels (family, genus, and species) are depicted. Species level did not separate var. *papillosa* within *C. californica*. Numbers under each *Cuscuta* species indicate the total number of hosts while the numbers within the Venn diagrams show the number of shared or exclusive hosts. Red numbers in parenthesis represent the percentage of the total host range of *C. californica* s. l.

Distribution—USA: California, mostly in the Sacramento and San Joaquin Valleys.

Phenology and Ecology—Flowering June to October; grows at 20–335 m elevation in grasslands, vernal pools, edges of marshes, and disturbed sites. The host is usually *Centromadia parryi* subsp. *rudis* (formerly *Hemizonia parryi* subsp. *rudis*) but it was also collected growing on *Centaurea solstitialis* and *Helianthemum annum*. The latter two species are most likely secondary hosts (on which the parasite attached after it had become established on *Centromadia*).

Etymology—From the Latin "difficilis" = "difficult", to reflect the initial difficulties encountered when trying to place these plants into a species and likely of future field botanists when they will try to identify it.

Notes—The species is less common and abundant compared to *C. brachycalyx* and *C. californica* but incomplete knowledge of the geographical distribution and biodiversity data prevent recommending a conservation status.

Representative Specimens Examined—See Appendix 1.

Table 2. Top hosts of *C. californica s. l.* at family, genus, and species level. HFI is the frequency (%) each parasite was encountered on a particular host.

Cuscuta taxon	Host family	HFI%	Host genus	HFI%	Host species, ssp. or var.	HFI%
C. difficilis	Asteraceae	100	*Centromadia* Greene	76.923	*Centromadia parryi* Greene ssp. *rudis* (Greene) B.G. Baldwin	76.923
			Centaurea L.	15.384	*Centaurea solstitialis* L.	15.383
			Helianthus L.	7.692	*Helianthus annuus* L.	7.692
C. californica var. *californica*	Polygonaceae	42.569	*Eriogonum* Michx.	42.065	*Eriogonum fasciculatum* Benth.	29.974
	Asteraceae	20.906	*Salvia* L.	5.037	*Salvia mellifera* Greene	3.778
	Lamiaceae	8.312	*Ambrosia* L.	3.778	*Adenostoma fasciculatum* Hook. & Arn.	3.022
	Fabaceae	6.549	*Eriodictyon* Benth.	3.526	*Acmispon glaber* (Vogel) Brouillet	2.518
	Boraginaceae	4.534	*Ericameria* Nutt.	3.274	*Ambrosia dumosa* (A.Gray) W.W. Payne	2.267
C. californica var. *papillosa*	Fabaceae	29.729	*Eriogonum*	27.027	*Eriogonum fasciculatum*	13.513
	Polygonaceae	27.027	*Ambrosia* L.	18.918	*Psorothamnus spinosus* (A. Gray) Barneby	13.513
	Asteraceae	27.027	*Psorothamnus* Rydb.	13.513	*Ambrosia salsola* (Torr. & A. Gray) Strother & B. G. Baldwin	10.810
	Boraginaceae	5.405	*Marina* Liebm.	8.108	*Ambrosia dumosa*	8.108
	Lamiaceae	2.702	*Eriodictyon* Benth.	5.405	*Marina parryi* (Torr. & A. Gray) Barneby	8.108
C. occidentalis	Asteraceae	31.372	*Monardella* Benth.	13.725	*Monardella*	6.862
	Lamiaceae	20.588	*Eriogonum* Michx.	6.862	*Agastache urticifolia* (Benth) Kuntze	3.921
	Fabaceae	12.745	*Lupinus* L.	5.88	*Convolvulus arvensis* L.	2.941
	Polygonaceae	8.823	*Ericameria* Nutt.	4.901	*Iva axillaris* Pursh	2.941
	Convolvulaceae	5.882	*Artemisia* L.	3.921	*Salvia melifera*	1.960
C. brachycalyx	Asteraceae	48.484	*Eriogonum* Michx.	7.575	*Hemizonia congesta* DC. ssp. *clevelandii* (Greene) Babc. & H. M. Hall	6.060
	Polygonaceae	12.121	*Centromadia* Greene	7.575	*Acmispon americanus* (Nutt.) Rydb.	4.545
	Fabaceae	7.575	*Hemizonia* DC.	7.575	*Calycadenia pauciflora* A. Gray	4.545
	Polemoniaceae	6.060	*Madia* Molina	7.575	*Polygonum bolanderi* W. H. Brewer	4.545
	Apiaceae	4.545	*Acmispon* Raf.	6.060	*Eriogonum nudum* Douglas ex Benth.	3.030

Key to Species of Cuscuta californica Complex

1\. Calyx equaling corolla tube; calyx lobes acuminate to attenuate 2
    2\. Pedicels 0.7–3.5 mm long; stamen filaments 0.7–1.1 mm long; anthers 0.6–1.2 mm long; short styles 1.1–3 mm long .... *C. californica*
    2\. Pedicels 0.2–0.8 mm long; stamen filaments 0.4–0.7 mm long; anthers 0.3–0.5 mm; short styles 0.3–0.7(–1) mm long .... *C. occidentalis*
1\. Calyx shorter than corolla tube; calyx lobes rounded to acute 3
    3\. Calyx lobes rounded to subacute; corolla tube campanulate, equal in length to corolla lobes; corolla tube becomes globose at fructification .... *C. difficilis*
    3\. Calyx lobes acute; corolla tube campanulate-tubulose, longer than the lobes; corolla tube becomes urceolate at fructification .... *C. brachycalyx*

Discussion

Systematics of Cuscuta californica s. l.—*Cuscuta occidentalis* and *C. californica* individuals formed a single, intermixed lineage, which suggests that they may be conspecific. However, we consider it premature to include *C. occidentalis* into *C. californica*. First, the statistical support for the lack of monophyly of *C. occidentalis* was moderate. Based on the phylogeny, a varietal status of *C. occidentalis* would also be equivocal. Second, *C. occidentalis* and *C. californica* are usually morphologically recognizable, ecologically distinct (Costea et al. 2006; Costea and Stefanović 2012), and, as shown in this study, they have different host ranges. Thus, "lumping" *C. occidentalis* into *C. californica* may lead to loss of valuable biological and biogeographical information. The easily recognizable *C. californica* var. *papillosa* is in a similar situation, but the implications of not accepting it as a taxon are less dramatic. However, we also suggest maintaining var. *papillosa* until a definitive answer is reached. *Cuscuta californica* var. *apiculata*, which was not included in this study, is quite distinct morphologically from other *C. californica* s. l. because of its ovoid capsules with only one seed. This feature is reminiscent of *C. salina* and *C. pacifica*, which belong to a different clade of section *Californicae* (Costea and Stefanović 2009b; García et al. 2014). In general, more sampling and methodological approaches are necessary to solve the systematics of *C. californica* s. l., but this complex of morphotypes and lineages offers an excellent opportunity to study speciation mechanisms and host range divergence in *Cuscuta* (see also next sections).

Cuscuta difficilis was initially revealed exclusively by the molecular data and thus thought to be a cryptic species (see also Introduction). Subsequently, however, the morphometric study uncovered a couple of subtle morphological differences that permitted the separation of *C. difficilis* from *C. brachycalyx*, *C. californica*, and *C. occidentalis*. It has been beyond the scope of this study to discuss the different views on cryptic species (e.g. Struck et al. 2018; Korshunova et al. 2019). Without a doubt, however, the discovery of *C. difficilis*, whether we call it 'cryptic' or not, further increases the identification difficulties within this already challenging group.

Plastid Capture in Cuscuta brachycalyx—The products of gene sequence analyses are gene phylogenies, which infer relationships between genes or genomes. These gene trees do not necessarily have to agree with the true evolutionary pathways of the taxa under investigation owing to a variety of analytical and biological natural phenomena (Doyle 1992; Sang and Zhong 2000). Our phylogenetic results brought up a situation where there is strongly supported yet discordant position of lineages between trees derived from plastid versus nuclear sources: in a rooted context, plastid data place *C. brachycalyx* with the *C. californica/occidentalis* clade (Fig. 1A), while nuclear data show *C. brachycalyx* as sister to *C. difficilis* (Fig. 1B, Fig. S1). This topological incongruence is summarized in Fig. 2.

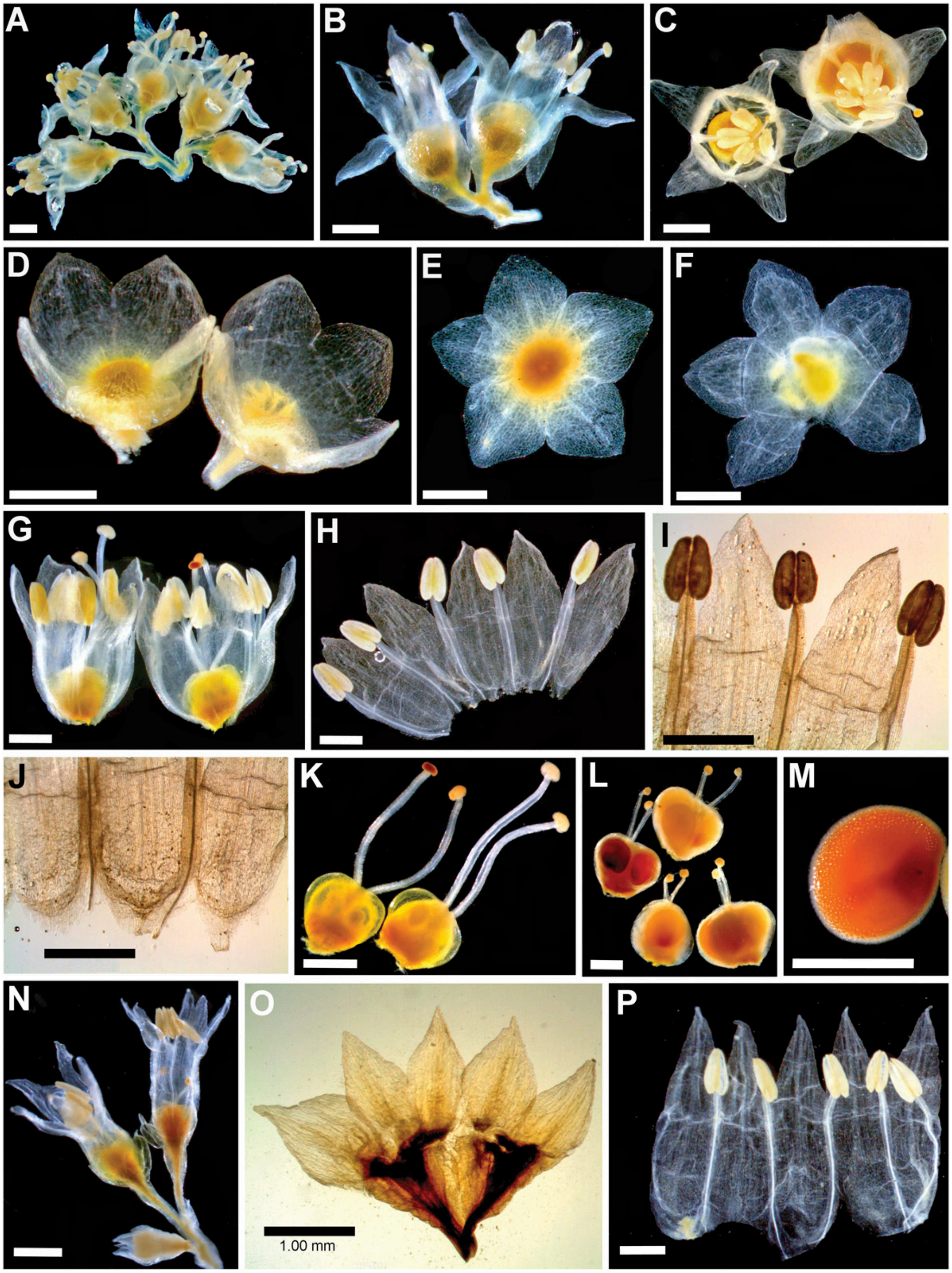

FIG. 6. Morphology of *C. difficilis* and *C. brachycalyx*. A–M. *Cuscuta difficilis*. A. Fragment of inflorescence. B. Mature flowers (corolla lobes patent to reflexed; *SS-15–09*, WLU). C. Mature flowers viewed from the top (*SS-17–82*, WLU). D. Calyx before dissection (*SS-17–82*, WLU). E–F. Variation of dissected calyx (dorsal view; E = *Keil 18008*, UCR; F = *SS-17–18*, WLU). Note the rounded to subacute calyx lobes. G. Corolla at flowering (campanulate), before dissection (*SS-17–81*, WLU). H. Dissected corolla. I. Detail of corolla lobes and stamens. J. Detail of corolla tube showing vasculature of staminal filaments prominent in the corolla tube and remnants of infrastaminal scale bridges (*SS-15–09*, WLU). K. Gynoecia (*SS-17–81*, WLU). L. Capsules. M. Seed (*SS-17–84*, WLU). N–P. *Cuscuta brachycalyx* (*Twisselmann 12742*, UCR). N. Flowers; note the cylindrical corolla. O. Dissected calyx; note the acute lobes. P. Dissected corolla. Scale bars = 1 mm.

From the analytical point of view, even a simple topological distortion, such as nearest-neighbor interchange (NNI), would result in trees compatible between plastid and nuclear data (Fig. 2). Yet, such a spurious transformation, caused for example by the long-branch attraction (Felsenstein 1978), is not likely to explain observed topological differences because the branches 1) have been corrected by corresponding models of DNA evolution, and 2) are not significantly different in length (neither the internal branches nor the root subtending the clade; see phylograms in Fig. 1).

Strongly conflicting gene trees can also result from several biological phenomena (e.g. Maddison 1997; Wendel and Doyle 1998). At low (species) phylogenetic levels, these most frequently include lineage sorting (i.e. random sorting of ancestral polymorphism or "deep coalescence"; Avise 1986; Wu 1991; Doyle 1992) and hybridization/introgression (Rieseberg and Soltis 1991; Rieseberg 1995; Sang and Zhong 2000), two processes that are hard to distinguish because of their similar phylogenetic pattern (Wendel and Doyle 1998; Yi et al. 2015). While lineage sorting cannot be completely eliminated as an alternative, we still propose to consider the random sorting of ancestral polymorphism as an unlikely explanation for the observed topological discrepancies involving *C. brachycalyx* for a couple of reasons. First, in *Cuscuta*, as is the case for the majority of flowering plants, plastomes are maternally transmitted to the next generation (Corriveau and Coleman 1988; Reboud and Zeyl 1994; Mogensen 1996). Because the plastid genome is both uniparentally inherited and haploid, it has a significantly smaller effective population size when compared to nuclear loci (Moore 1995). Hence, the plastid haplotype tree has a substantially higher probability of more rapid coalescence time, leading to the relatively rapid elimination of any polymorphism. Second, like the majority of *Cuscuta* species (Yuncker 1932), the four ingroup species included in our study have relatively narrow geographic distributions, all essentially confined to the California Floristic Province and Desert Province (Baldwin et al. 2012). In our sampling, each species was represented by 7–17 individuals, spanning their entire respective distribution ranges, to increase the chance of finding polymorphic alleles, indicative of lineage sorting. No evidence of polymorphism in the most recent common ancestor has been found. In these four cases, the plastid and especially nuclear ribosomal sequences were very similar among all the individuals included (Fig. 1). Similarly, the clones of PPR sequences showed comparatively little polymorphism (Fig. S1).

Taken together, most of the data seem to point to hybridization or introgression as the likely explanation for the incongruent phylogenetic position of *C. brachycalyx* (Figs. 1, 2). Over the last decade, a dozen cases of reticulate evolution have been discovered and described in *Cuscuta* subgenus *Grammica*, including both inter- and intra-sectional hybridization (Stefanović and Costea 2008; Costea and Stefanović 2010; García et al. 2014; Costea et al. 2015a; García et al. 2018). In several of those cases for which the corroborating cytological information is available, it appears clear that hybridization is linked with polyploidization (García et al. 2018, 2019; Ibiapino et al. 2019). For example, *C. veatchii* and C. *psorothamnensis*, two species of hybrid origin in sect. *Denticulatae* and both allotetraploids, with $2n = 60$ (García et al. 2018; Ibiapino et al. 2019). *Cuscuta sandwichiana*, an intersectional hybrid between species in sections *Cleistogrammica* and *Grammica*, appears to be a decaploid, with $2n = 150$ (García et al. 2019). However, the available cytological information for species across the entire *Cuscuta* sect. *Californicae*, as well as specifically for all four ingroup species analyzed in this study, indicates that all of them are diploids, including *C. brachycalyx*, with $2n = 30$ (García et al. 2019). This argues against the polyploid hybridization in the case of *C. brachycalyx*, but leaves the possibility of hybrid speciation without change in ploidy level (i.e. "diploid" hybrid speciation) accompanied by "plastid capture," as a viable alternative.

Plastid capture is a phenomenon in which the plastid of one species is introgressed into another plant species via initial interspecific hybridization and subsequent rounds of backcrosses. Unlike the recombinational/diploid hybridization, this evolutionary process seems to be relatively common in plants (e.g. Rieseberg and Soltis 1991; Soltis and Kuzoff 1995; Acosta and Premoli 2010; Yuan et al. 2010; Yi et al. 2015). It seems that the introgression of plastomes occurs generally quite frequently in plants as a result of biological properties (e.g. uniparental inheritance, lack of recombination), as well as because of low selective constraint (Rieseberg and Wendel 1993; Martinsen et al. 2001; Avise 2004), a factor particularly pertinent when it comes to parasitic (nonphotosynthetic) plants.

The preponderance of evidence, including morphometric data showing a nearly complete morphological overlap between *C. brachycalyx* and *C. difficilis* (Fig. 3), is consistent with the nuclear trees rather than the plastid tree. Although several phenomena could allow for incongruence between these phylogenies (as reviewed in Stefanović and Costea 2008, García et al. 2014), we favor plastid capture as the most likely explanation for the phylogenetic discordance observed in the *C. brachycalyx* case. Overall, the results presented here provide initial evidence for an important role of plastid introgression ("capture") in the evolution of the parasitic genus *Cuscuta*. Nonetheless, some alternatives, and in particular diploid hybrid speciation, could not be eliminated. Phylogenetic analyses of additional, independently inherited loci, derived from whole-genome sequencing, as well as analyses of multilocus data using the multispecies coalescent approaches (e.g. Heled and Drummond 2010) will help to resolve these outstanding questions.

Host Range—The challenge of defining the host range in dodders was insightfully articulated initially by Engelmann (1843): "[…] many Cuscutae prefer some plants to others, but the same species often grows upon a great variety of different plants." Subsequently only the second part of his statement gained a broad acceptance, leading to the current characterization of dodders as "generalists" (e.g. Dean 1934; Atsatt 1983; Meulebrouck et al. 2007). However, based on this study and the ~20 species for which basic data were published (Dean 1934; Gaertner 1950; Costea and Stefanović 2009a; Barath and Csiky 2012; Sarma et al. 2012; García et al. 2018), the host range in *Cuscuta* varies from one/few species within a genus to hundreds of species belonging to many genera and families. It appears that a continuum exists between "specialist" and "generalist" host range strategies, but even in the case of the latter, some species are more "generalist" than others. A recent evolutionary study of sect. *Denticulatae* (García et al. 2018), which is another western N American clade of subg. *Grammica* (Stefanović et al. 2007), revealed similar divergence patterns among parasite host ranges to those observed in this study. The evolution of different *Cuscuta* host ranges in both García et al. (2018) and the current study entails different habitats and ecology for the hosts. This divergence of host ranges may potentially lead to reproductive isolation and spur different evolutionary trajectories for the emerging dodder lineages, which would be an interesting future direction for experimental approaches to studying speciation in parasitic plants generally, and *Cuscuta* in particular.

The different host ranges may also be a reflection of the different geographical distribution range and rarity of the dodder species involved. *Cuscuta californica* is the most common and widespread species among those under investigation in this study, followed by *C. occidentalis* and *C. brachycalyx* (Costea et al. 2006; Costea and Stefanović 2012). Similarly, the narrow host range observed for *C. difficilis* may be a consequence of its confinement to the Great Central Valley of California (both of its subregions, the Sacramento and San Joaquin Valleys; sensu Baldwin et al. 2012). Last but not least, the different number of host species observed within the host ranges of *C. californica* s. l. taxa may have also been influenced by the sampling constraints. Additional sampling may reveal additional host overlaps, as well as new exclusive hosts. Western N America is one the floristically best known areas, yet this is all the host data we could gather. We would like to take this opportunity to encourage collectors to also harvest/make notes of the host when collecting *Cuscuta* because understanding the host range will likely provide an insight into the diversification of dodders.

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Author Contributions

MC examined and annotated all the herbarium material used in the study; did the morphometric analyses; compiled the host data, did the Venn diagrams, and wrote the bulk of the manuscript. HEM dissected and imaged most of the flowers used in the morphometric study. RF helped to generate the molecular data. CF produced the host networks. SS did the field work and found the new species, conducted the phylogenetic analyses, and wrote the corresponding sections, as well as edited the rest of manuscript.

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APPENDIX 1. List of herbarium specimens examined for morphometric and molecular analyses of the *Cuscuta californica* s. l. Country, locality details, date, collector(s) and collection number, and herbaria in which the specimens are deposited are provided for all specimens. In addition, for material used in molecular phylogenetic analyses, DNA extraction and GenBank accession numbers (plastid *trnL-F* and *rbcL*, nuclear ITS rDNA, 26S rDNA, At4G01570 PPR ortholog) are given in square brackets. Newly generated sequences are indicated in bold; 'n/a' indicates sequences that are not available. Abbreviations of herbaria follow Index Herbariorum (Thiers 2019).

Cuscuta brachycalyx Yunck.: **USA. California.** Butte Co.: Along Humboldt Rd., Butte Meadows, 1450 m, 16 Aug 1983, *Ahart 4282* (CHSC); Ca. 0.25 mi NE of the Carr Mine, 1798 m, 26 Jul 1992, *Ahart 6858* (CHSC); N side Humboldt Rd., 1325 m, 5 Jul 1990, *Oswald & Ahart 4348* (CHSC); 1.5 mi S of Hurleton, 487 m, 19 Jul 1998, *Ahart 8048* (CHSC); Mooreville Ridge, Jackass Flat, ca. 1 mi NW Lost Creek Reservoir Dam, 1158 m, 20 Jun 1993, *Ahart 7001* (CHSC); E side of Old Humboldt Rd., ca. 4.5 min NE of Butte Meadows turnoff from Hwy. 32, 1326 m, 17 Aug 1977, *Taylor 1457* (CHSC); W of Lunt Rd., ca. 0.5 mile NW of Hwy 70, 548 m, 10 Aug 1988, *Ahart 6170* (CAS). Colusa Co.: on Hwy 20, just E of county line, 39°00′39.2″N, 122°24′27.7″W, 3 Jun 2015, *Stefanović 15-07* (TRTE, WLU), [**1524A**; **MT070387**, **MT070359**, **MT070214**, **MT070180**, n/a]. Contra Costa Co.: Near Antioch, 7.6 m, 8 Oct 1937, *Munz 12200* (NY). Kern Co.: Mountain Mesa, Poplar St., S of Hwy 178 35°38′10″N, 118°24′02″W, 24 Jul 2017, *Stefanović 17-95* (TRTE, WLU); Poso Creek, 0.5 mi S of the junction of the Granite and Woody Rds., 823 m, 26 Aug 1966, *Twisselmann 12742* (UCR); 1 mi E of Bodfish Gap, 1311 m, 17 Jun 1971, *Howell & True 48051* (UCR); 1.5 mi N of Kernville, 814 m, 25 Sep 1962, *Howell 38877* (CAS). Lake Co.: N of Upper Lake, Rd. #301 (M1) to Lake Pillsbury, mi 26.5, 39°24′11.1″N, 122°57′57.4″W, 2 Jun 2015, *Stefanović 15-05* (TRTE, WLU), [**1522**; **MT070386**, **MT070358**, **MT070213**, **MT0701179**, **MT070284-MT070290**]; Eel River Forest Rd., between Hullville and Potter Valley, 39°22′36.4″N, 123°03′48.9″W, 2 Jun 2015, *Stefanović 15-06* (TRTE, WLU). Mariposa Co.: Merced River Canyon, W of Yosemite National Park, Incline Rd., 37.670246°N, 119.821710°W, 26 Jun 2013, *Stefanović 13-41* (TRTE, WLU), [**1402**; **MT070388**, **MT070360**, **MT070215**, **MT0701181**, **MT070280-MT070283**]; Chowchilla Mts., Iron Mt., 8 Aug 1975, *Reveal 3920* (NY); Yosemite National Park, N slope of Merced River Canyon, on N side of Hwy. 140, 869 m, 16 Jun 2004, *Colwell AC 04-31* (YM, TRTE, WLU), [**451, 472**; EF194484, EU883441, EF194699, EU883489, n/a]. Merced Co.: 1.5 mi S of Delhi along Hwy 99, 46 m, 15 Aug 1954, *Crampton 2261* (UCR); *Colwell AC 04-305* (YM, TRTE), [**643**; EF194485, EU883443, EF194700, EU883491, n/a]. Monterey Co.: along Hwy 198, halfway between San Lucas and Coalinga, 36°10′18.3″N, 120°40′13.3″W, 6 Jun 2015, *Stefanović 15-21* (TRTE, WLU), [**1534A**; **MT070389**, **MT070361**, **MT070216**, **MT070182**, n/a]. Placer Co.: Sierra Nevada Foothills, 3.5 mi NE of Auburn, E of Elder's Corner on Dry Creek Road, 412 m, 2 Jul 1977, *Crampton 9512* (UCR). Plumas Co.: Northern High Sierra Nevada, 1890 m, 14 Aug 1996, *Oswald & Ahart 8150* (CHSC); Hot Springs terrace on the S side of the meadow at Drakesbad, 1731 m, 11 Sep 1991, *Oswald 4864* (CHSC); Ridge ca. 0.25 mile N of the intersection of Lumpkin Ridge Rd. and rd. to Tamarack Flat, 39°44′32.9″N, 121°01′29.5″W, 1743 m, 18 Jul 2002, *Ahart 9856* (CHSC, JEPS), [**499**; EF194487, EU883442, EF194697, EU883490, n/a]; Along Pacific Crest Trail, ca. 1 mi NW of A-Tree, 2134 m, 11 Aug 1982, *Taylor & Swanson 4964* (CHSC); Ca. 1 mi NW of Little Grass Valley Reservoir, 1737 m, 28 Aug 1998, *Castro & Janeway 825* (CHSC); NE of a small branch of Lights Creek, E side of Forest Road 27N54, 40°12′31.0″N, 120°42′43.7″W, 1822 m, 10 Aug 2005, *Ahart & Guardino 12303*, (CHSC); Cleghorn Bar Rd., about 1.5 mi W of La Porte Quincy Rd., about 2 mi NW of Pilot Peak, 1768 m, 21 Jul 1981, *Ahart 2971* (CHSC, NY), [**645**; EF194488, EU883444, EF194698, EU883492]; Kelly Mtn., ca. 0.3 mi SW of the end of Kelly Mtn. Rd., 1813 m, 6 Jul 1992, *Oswald & Ahart 5063* (CHSC). San Benito Co.: San Benito Canyon, 10 mi NW of Hernandez, 7 Sep 1931, *Howell 8066* (CAS). San Bernardino Co.: Amboy Rd., S of Hwy. 66, 23 Apr 2013, *Stefanović 13-21* (TRTE, WLU). San Diego Co.: Wash, upper Mason Valley, near mouth of Oriflamme Canyon, 823 m, 30 Jan 1940, *Munz 15822* (UC). Tehama Co.: Inner North Coast Ranges, 39°55′40″ N, 122°39′52″ W, 1073 m, 30 Jun 2000, *Janeway 6869* (CHSC); About 9 mi W of Paskenta, 762 m, 8 Jun 2001, *Ahart* 8771 (CHSC); NW of the Nomalaki Trail, 39°50′11.3″ N, 122°39′51.4″ W, 553 m, 11 Jun 2004, *Ahart 11017* (CHSC); Cascade Range foothills, 40°25′30.2″ N, 122°03′17.19″ W, 176 m, 12 Jun 2003, *Dittes & Ahart 2003*-68 (CHSC); Dales Lake Ecological Reserve, 213 m, 30 Jun 1995, *Oswald 6952* (CHSC). Trinity Co.: On top of Blue Point Ridge, just W. of Dubakella Mt., 1646 m, 21 Aug 1980, *Taylor & Nelson 3294* (CHSC); ca. 1 air mi WNW of White Rock Forest Station (above Rd 28N10, SE ridge of Pony Buck Peak), Pony Buck Peak, 1500 m, 2 Aug 1988, *Dean 159* (UC), [**1545**; **MT070385**, **MT070357**, **MT070212**, **MT0701178**, n/a]. Tulare Co.: Sequoia National Forest, Mountain Hwy. 99, 1 mi W of intersection with Sherman Pass Rd., 35°58′27″N, 118°29′ 55″W, 23 Jul 2017, *Stefanović 17-94* (TRTE, WLU). **Oregon.** Josephine Co.: 1998, *Stefanović 98-59* (TRTE, WTU), [**147**; EF194486, EU883440, EF194696, **MT0701177**, n/a].

Cuscuta californica Hook. & Arn.: **Mexico. Baja California.** San Martin Island near 30°29′N, 116°6.5′W, 10 m, 11 Apr 1963, *Moran 10541* (SD); **U.S.A. Arizona.** Mohave Co.: Hwy. 95, 1 mi S of I-40, 34°42′51″N, 114°18′ 30″W, 5 Apr 2016, *Stefanović 16-26* (TRTE, WLU). **California.** *Colwell s.n.* (no voucher), [**178**; EU883435, EU883445, EU883480, EU883493, n/a]; 1986, *Pinzl 7238a* (NY), [**637**; EF194475, EU883448, EF194688, EU883496, n/a]; 2000, *Stefanović 00-59* (TRTE), [**418**; EF194480, EU883446, EF194692, EU883494, **MT070250**]. Butte Co.: ca 3 mi E Philbrook Reservoir, 2027 m, 19 Jul 1990, *Oswald & Ahart 4368* (CHSC). Los Angeles Co.: Liebre Mountains, Knapp Ranch area at upper end of Castaic Creek drainage in broad alluvial valley at head of Cienaga Canyon, S of Liebre Mt., 28 May 1997, *Boyd 9839* (JEPS), [**500**; EF194478, EU883447, EU883481, EU883495, n/a]; Transverse Ranges, Verdugo Mts.: N side of La Tuna Canyon Rd., 518 m, 24 Jun 2002, *Gross 548* (CHSC); Big Tujunga Wash, 200 m NW of corner of Wescott Av. and Grove St., 34°16′14.8″N, 118°19′11.2″W, 385 m, 19 Jan 2001, *Keefe 30971* (CHSC); SW slope of Verdugo Mts., 34°13′23.02″N, 118°20′28.55″W, 24 Dec 2003, *Dittes 856* (CHSC); San Gabriel Mts., Charlton Flats, roadside at parking area #18, 14 Sep 1971, *Schlising 2999* (CHSC); Chaney Trail ca. 1/2 way up, 20 Nov 1973, *Wheeler s.n.* (RSA); Western San Gabriel/ Liebre Mts., Santa Clara area, ca. 0.5 mi, NW of community of Mint Canyon, 34°26′N, 118°26′ 54″W, 610 m, 24 May 2005, *Sanders & Salvato 30270* (UCR); Newhall Ranch, hills SW and W of Magic Mt. amusement park, 34°25′N, 118°36′W, 366–457 m, 18 Jun 2002, *Sanders & Elvin 25122* (UCR); Del Valle area, between San Martinez Grande Canyon Rd. and (San Martinez) Chiquito Canyon Rd., N of Hwy 126, 34°25′10″N, 118°39″50″W, 305 m, 20 Jun 2003, *Sanders & Marsden 26986* (UCR); Blue Ridge, San Gabriel Mts., Angeles National Forest, 2256 m, 10 Oct 1967, *Throrne & Dourley 37351* (RSA); Point Dume, 15 m, 11 Aug 1971, *Martin s.n.* (RSA). Orange Co.: Hwy 74, 8 mi NE of I-5, 33°31′51″N, 117°33′04″W, 7 Apr 2016, *Stefanović 16-40* (TRTE, WLU); Hwy 133, Laguna Coast Wilderness Park, Sycamore trail, 33°36′31.5″N, 117°45′56″W, 7 Apr 2016, *Stefanović 16-41* (TRTE, WLU), [**1641**; **MT070373**, **MT070344**, **MT070197**, **MT0701165**, **MT070251-MT070254**]; Orange Park Acres, 1/4 mi N of Twin Lakes, 396 m, 17 Apr 1971, *Cerf s.n.* (UCR); Lomas de Santiago, Santiago Creek Canyon 0.5–0.8 mi above the hwy. bridge, 33°44′34″N, 117°40′11″W, 300 m, 18 Sep 2008, *Sanders 36097* (UCR); Plumas Co.: Southside of the Pacific Crest Trail, N of Mt. Etna, 39.74888°N, 120.83694°W, 1981 m, 22 Jul 1994, *Ahart 7506* (UCR). San Bernardino Co.: Cajon Pass, Cajon Wash, ~1–2 mi S of Hwy. 138 crossing, May 1997, *White 5033* (ASU), [**669**; EF194479, EU883449, EF194691, EU883497, **MT070255-MT070260**]; San Bernardino National Forest, Hwy 38, 20 Apr 2013, 34.103660° N, 117.009580°W, *Stefanović 13-04* (TRTE, WLU), [**1359A**; **MT070370**, **MT070341**, **MT070191**, **MT0701162**, **MT070274-MT070279**]; Historic Rt. 66, 1 mi S of exit 129 on I-15 S, intersection with Swarthout Canyon Rd., 34°16′29″N, 117°27′04″W, 25 Jul 2017, *Stefanović 17-105* (TRTE, WLU); Cajon Blvd. (Rte. 66), under Palm Ave. bridge, 34°11′18″N, 117°21′55″W, 25 Jul 2017, *Stefanović 17-106* (TRTE, WLU); Hwy 66, 15 mi W of Essex, 15 mi E of Amboy, 34.574634°N, 115.474954°W, 23 Apr 2013, *Stefanović 13-18* (TRTE, WLU); 2 mi W of intersection US 95/Hwy 66, 34.936243°N, 114.865908°W, 22 Apr 2013, *Stefanović 13-15* (TRTE, WLU), [**1367**; **MT070380**, **MT070352**, **MT070204**, **MT0701172**, **MT070263-MT070267**]; intersection US 95/Hwy 66, 34.942251°N, 114.824549°W, 22 Apr 2013, *Stefanović 13-13* (TRTE, WLU), [**1365A**; **MT070382**, **MT070354**, **MT070206**, **MT0701174**, **MT070268-MT070273**]; intersection US 95 with Hwy. 66, 34.942251°N, 114.824549°W, 22 Apr 2013, *Stefanović 13-14* (TRTE, WLU); Amboy-Kelso Rd (Kelbaker Rd.), 1 mi N of Hwy. 66, 34.584810°N, 115.645928°W, 23 Apr 2013, *Stefanović 13-20* (TRTE, WLU), [**1372**; **MT070381**, **MT070353**, **MT070205**, **MT0701173**, **MT070261-MT070262**]; Cajon Wash, 300 m, W of Cajon Rd. and Kenwood Ave. junction, 692 m, 22 Jun 1993, *Swinney 2296* (UCR); San Antonio Mts., Pine Mt., 34°18′30″N, 117°38′34″W, 2439 m, 22 Aug 1917, *Johnston 1686* (UCR); San Gabriel Mts., Stoddard Flat, S of Barret Village, 1366 m, 25 Jul 1995, *Swinney 4295* (UCR); San Bernardino Mts., Cleghorn Canyon, 34°17′N, 117°26′W, 1341 m, 21 Jul 1993, *White 1771* (UCR); Jurupa Hills, Mary Vagl Museum and Nature Center, Cypress St., Fontana, 8 Apr 1997, *Wisura 5044* (UCR); Eastern Mojave Desert, Bristol Mts. along Kelbaker Rd., ca. 4 mi N of rt. 66 Junction, 489 m, 19 Nov 2005, *Andre 5631* (UCR); San Bernardino Valley, 366 m, 25 Jun 1907, *Parish 6404* (NMC); San Diego Co.: Hwy 94, Dulzura, 32°38′28″N, 116°46′22″W, 3 Apr 2016, *Stefanović 16-08* (TRTE, WLU); Japatul Rd., 5 mi W of intersection w/ Japatul Valley and Lyons Valley Rds., 32°47′49″N, 116°44′48″W, 6 Apr 2016, *Stefanović 16-32* (TRTE, WLU), [**1632**; **MT070377**, **MT070349**, **MT070201**, **MT0701169**, n/a]; Hwy 94, Potrero, 32°35′55″N, 116°34′04″W, 3 Apr 2016, *Stefanović 16-10* (TRTE, WLU), [**1610**; **MT070379**, **MT070351**, **MT070203**, **MT0701171**, n/a]; Peninsular Range, Palomar Mtn., Plaisted Creek Ecological Reserve, 33°18′08″N, 116°54′35″W, 793 m, 9 Jul 2004, *Sanders et al. 28485* (UCR); Deerhorn Valley, ca. 832 m, 11 Sep 1998, *Bell 357* (UCR); Sweetwater River Watershed, Dehesa Rd. SW of Alpine, 32°48′N, 116°48′W, 305 m, 23 Jul 1976, *Sanders 286* (UCR); La Jolla, Summer Canyon, 24 m, 17 May 1964, *Miller 6452* (UCR); Alpine, 1/2 mile N of Victoria Drive, 32.8543°N, 116.7514°W, 707 m, 17 Jul 2004, *Gregory 1049* (SD); Walker Canyon Ecological Reserve, 32.6614°N, 116.2339°W, 4 May 2004, *Rebman 10044* (SD); Santa Barbara Co.: San Antonio Rd., cross to Lompoc/Casmalia Rd., 34°47′00″N, 120°32′18″W, 19 Jul 2017, *Stefanović 17-78* (TRTE, WLU); San Luis Obispo Co.: Oceana, 28 Aug 1932, *Purer 4853* (UCR); Tulare Co.: Sequoia National Forest, Hwy. 198, 1 mi E from intersection with Ten Mile Rd., 36°43′01″N, 118°53′49″W, 22 Jul 2017, *Stefanović 17-92* (TRTE, WLU); Ventura Co.: Santa Monica Mts., Conejo Grade, along Hwy 101, 34.19851°N, 118.95790°W, 10 May 1959, *Raven & Thompson 14187* (RSA); Hwy 1, Point Mugu State Park, 34°05′20″N, 119°03′52″W, 8 Apr 2016, *Stefanović 16-45* (TRTE, WLU), [**1645**; **MT070374**, **MT070345**, **MT070198**, **MT0701166**, n/a]; CSU Channel Islands Campus 34°09′42″N, 119°02′17″W, 9 Apr 2016, *Stefanović 16-46* (TRTE, WLU); Oxnard, N Harbor Blvd., 34°12′19″N, 119°14′47″W, 8 Apr 2016, *Stefanović 16-44* (TRTE, WLU); Point Mugu State Park, Hwy 1, 34°05′20″N, 119°03′52″W, 18 Jul 2017, *Stefanović 17-72* (TRTE, WLU).

Cuscuta californica Hook. & Arn. *var.* ***papillosa*** Yunck.: **USA. California.** Merced Co.: SW of Los Banos, 234 m, 17 May 1986, *Janeway & Janeway s.n.* (CHSC); Riverside Co.: Hwy 74, 1 mi E of Hemet, 6 Apr 2016, 33°44′12″N, 116°49′40″W, *Stefanović 16-27* (TRTE, WLU), [**1627A**; **MT070372**, **MT070343**, **MT070193-MT070196**, **MT0701164**, n/a]; Indio Hills, Dillon Rd., 33°50′29″N, 116°14′17″W, 4 Apr 2016, *Stefanović 16-18* (TRTE, WLU); Indio Hills, Dillon Rd., 2 mi N on Thousand Palms Canyon Rd., 33°53′10″N, 116°16′48″W, 4 Apr 2016, *Stefanović 16-20* (TRTE, WLU), [**1620**; **MT070384**, **MT070356**, **MT070211**, **MT0701176**, n/a]; Peninsular Range: summit ridge of Alberhill Mt., 33°43′09″N, 117°22′29″W, 503 m, 18 May 2004, *Sanders et al. 27901* (UCR); Hills on S side of San Jacinto Valley, 488 m, 10 Jun 1972, *Ziegler 480* (UCR); San Benito Co.: Pinnacles National Park, along Hwy 146, 36°29′59.2″N, 121°08′27.0″W, 6 Jun 2015, *Stefanović 15-20* (TRTE, WLU), [**1533A**; **MT070371**, **MT070342**, **MT070192**, **MT0701163**, **MT070242-MT070249**]; San Bernardino Co.: San Bernardino Mts., Union Flat, 28 Aug 1978, *Krantz s.n.* (UCR); Mouth of Devil Canyon, Cajon Pass area, 518 m, 12 Jun 1987, *Sanders 7125* (UCR); Reche Canyon, near Riverside County line, 396 m, 5 Sep 1968, *Clarke s.n.* (UCR); Big Morongo Canyon near the Riverside Co. line, 2 mi below the Big Morongo Preserve headquarters, 701 m, 29 Apr 1984, *Helmkamp A40* (UCR); San Bernardino Valley, 22 July 1905, *Parish 5224* (OSU); San Diego Co.: Borrego Valley near school, 12 Dec 1939, *Gander 7833* (RSA); Hwy 78, ca. 2 mi E of intersection with S3, 33°07′59″N, 116°18′48″W, 4 Apr 2016, *Stefanović 16-16* (TRTE, WLU), [**1616**; **MT070383**, **MT070355**, **MT070207-MT070210**, **MT0701175**, n/a].

Cuscuta difficilis, *sp. nov.*: **USA. California.** Butte Co.: Gray Lodge Waterfowl Management Area, 25 May 1988, *Oswald 3339* (CHSC); ca. 0.25 mi W of Pennington Rd., E of Parking Area 5, 30 m, 6 Sep 1991, *Ahart 6711* (CHSC); Colusa Co.: Williams, W of intersection Hwy 20/I-5, 39°09′27.1″N, 122°09′59.7″W, 3 Jun 2015, *Stefanović 15-08* (TRTE, WLU), [**1525**; **MT070391**, **MT070362**, **MT070217**, **MT0701183**, **MT070295-MT070298**]; Fresno Co.: Hwy 180, between Trinity and Shasta Av., 1.5 mi W of Kerman, 36°44′05″N, 120°06′34″W, 21 Jul 2017, *Stefanović 17-87*

(TRTE, WLU), [**1936**; **MT070394**, n/a, **MT070221**, n/a, n/a]; Glenn Co.: Sacramento National Wildlife Refuge, ca. 7 mi S of Willows, 30 m, 5 Aug 1993, *Oswald 5772* (CHSC); At the Checking Station on the S side of Norman Rd., 24 m, 23 Jun 1993, *Oswald 5568* (CHSC); 39°25′33.6″N, 122°11′16.9″W, 3 Jun 2015, *Stefanović 15-09* (TRTE, WLU), [**1526A, B**; **MT070390**, **MT070363**, **MT070218**, **MT0701184**, **MT070291-MT070294**]; Merced Co.: Great Valley Grassland State Park, on Hwy 165, 1.5 mi S of intersection w/ Hwy 140, 37°17′29.9′N, 120°51′05.4″W, 6 Jun 2015, *Stefanović 15-18* (TRTE, WLU), [**1532A**; **MT070392**, **MT070364**, **MT070219**, **MT0701185**, **MT070299-MT070302**]; On Hwy. 165, 16 mi of Los Banos intersection w/ Hwy 33, 37°17′18″N, 120°51′26″W, 20 Jul 2017, *Stefanović 17-84* (TRTE, WLU), [**1933**; **MT070393**, n/a, **MT070220**, n/a, n/a]; San Luis Obispo Co.: Hwy 41, 5 mi of Atascadero, 35°30′20″N, 120°36′34″W, 20 Jul 2017, *Stefanović 17-82* (TRTE, WLU), [**1931**; **MT070396**, n/a, **MT070223**, n/a, n/a]; Atascadero, El Camino Real I1431 Rd., near cross with N Santa Margarita Rd., 35°26′21″N, 120°37′38″W, 19 Jul 2017, *Stefanović 17-81* (TRTE, WLU), [**1930**; **MT070395**, n/a, **MT070222**, n/a, n,a]; Just S of Creston and W of O'Donovan Rd., 341 m, 23 Oct 1983, *Keil 18008* (UCR); Santa Clara Co.: between Sunnyvale and Alviso, lower end of San Francisco Bay, 22 Aug 1933, *Keck 2473* (CAS).

Cuscuta occidentalis Millsp.: **USA. California.** Butte Co.: Upper Bidwell Park, Chico, 5 Jun 1983, *Oswald 637* (CHSC); Ca. 9 mi E of Chico via Hwy 32 and 2 mi E on Altatina Drive, 439 m, 18 May 1987, *Castro 107* (CHSC); Contra Costa Co.: Mount Diablo, July 1903, *Elmer 4543* (CAS); Mariposa Co.: Midpines, 4642 Buffalo Gulch Rd., 37.587129°N, 119.939093°W, 26 Jun 2013, *Stefanović 13-43* (TRTE, WLU); Mendocino Co.: Mackerricher State Park, 50 mi S of Inglenook Creek, 9 Jul 2013, *Sholars 1601* (WLU); Nevada Co.: Bowman Lake, N side of Meadow Lake Rd., 1706 m, 14 Aug 2001, *Ahart 9116* (JEPS), [**646**; EF194476, EU883458, EF194689, EU883508, **MT070228-MT070231**]; Plumas Co.: N of Forest Rd. 27N09, ca 6 mi NW of the Boulder Creek Work Center at Antelope Lake, 40°13′44.3″N, 120°43′07.0″W, 9 Aug 2005, *Ahart & Guardino 12277* (CHSC); Bucks Lake, 8 Aug 1975, *Mayer & Brink s.n.* (CHSC); Lakes Basin Recreation Area, S facing slope above Mud Lake, 2072 m, 1 Sep 1984, *Janeway 1129* (CHSC); San Bernardino Co.: Providence Mts., 9 mi SE of Kelso, 20 Jun 1973, *Henrickson 10805* (CAS); San Diego Co.: Anza-Borrego Desert State Park, Hwy. S2, mile 11.5, W side, 21 Apr 2013, *Stefanović 13-06* (TRTE, WLU), [**1360A**; **MT070376**, **MT070348**, **MT070200**, **MT0701168**, **MT070236-MT070240**]; San Luis Obispo Co.: Los Osos-Baywood Park area, between end of Martingale Ave. and Eto Lake, 46 m, 31 May 1986, *Keil 19626* (UCR); Sierra Co.: N side of Mt. Fillmore, ca. 0.75 mi S Gibsonville to Johnsville, 2133 m, 24 Sep 1993, *Ahart 7254* (CHSC); Siskiyou Co.: 2.2 mi N of Scott Mt. summit on Hwy 3, 1372 m, 26 July 1987, *Ertter 7326* (RSA, NY), [**503**; EF194477, **MT070347**, EF194690, EU883506, n/a]; Tehama Co.: A16 Ono-Platina Rd., 6 mi NE of stop #36, 40°22′59.8″N, 122°50′34.3″W, 3 Jun 2011, *Stefanović 15-11* (TRTE, WLU), [**1518A, C**; **MT070375**, **MT070346**, **MT070199**, **MT0701167**, **MT070241**]; 150 yards E of Colby Creek, 2 mi SE of Colby Mountain, 40°07′21.3″N, 121°29′20.4″W, 1525 m, 9 Sep 2006, *Ahart 13256* (CHSC). Trinity Co.: Near Trinity River, on W side of Hwy 137, 914 m, 22 Jul 1980, *Taylor 3165 A* (CHSC); E of Colby Creek, 40°07′07.4″N, 121°29′ 11.3″W, 1503 m, 15 Aug 2006, *Ahart 13199* (CHSC). Tulare Co.: Sierra Nevada, Kern River Drainage, on the Needles, Lloyd Meadows, 1798 m, 19 Jul 1972, *Shevock 2074* (SD); Kern Plateau, slopes E of Long Valley, 1859 m, 8 Aug 1967, *Howell & True 43581* (CAS, NY). Washoe Co.: ca 1 mi ENE of Grass Valley Reservoir, 1829 m, 14 Aug 1991, *Schoolcraft et al. 2220* (NY), [**648**; EF194483, EU883460, EF194695, EU883510, n/a]. **Nevada.** Douglas Co.: Antelope Valley at the E edge of Red Hill on the E side of the Gray Hills, 1521 m, 14 Jul 1997, *Tiehm 12257* (CAS), [**504**; EF194481, EU883457, EF194693, EU883507, n/a]. Eureka Co.: Roberts Mts., 1.7 mi N of Three Bars Ranch on the main rd. to Tonkin Summit, 1829 m, 22 Aug 2002, *Tiehm 14108* (NY), [**647**; EF194482, EU883459, EF194694, EU883509, **MT070232-MT070235**]. **Oregon.** Harney Co.: 5 mi. W. of Riley, 9 Jul 1925, *Peck 14304* (OSC). Jackson Co.: 2 mi. N of Central Point, Jun 1927, *Peck 14965* (OSC). Josephine Co.: Grants Pass, [no date], *Howell s.n.* (OSC); On bank of Rogue River, 5 Jul 1909, *Peck 3997* (OSC); Jun 1913, *Peck 3998* (OSC). Lake Co.: S end of Summer Lake, Jul 1927, *Peck 15685* (OSC); Fremont National Forest; just N of the California State Line along NFD Road 3915 above Dismal Creek, 2076 m, 4 Aug 2006, *Halse 7001* (OSC). **Washington.** Adams Co.: as *C. californica* var. *breviflora* Engelm.) Marcellus Nature Preserve, 47.23361° N, 118.40417° W, Sep 1997, *Bjork 3056* (WTU), [**1595**; **MT070378**, **MT070350**, **MT070202**, **MT0701170**, n/a].

Outgroups (used only in phylogenetic analyses). ***Cuscuta jepsonii*** Yunck.: **USA. California.** Mariposa Co.: Yosemite NP, Wawona, along Chowchilla Mt. Rd, 0.6 road miles from the junction with Hwy. 41, 9 Jul 2009, *Taylor et al. 20761b* (YOSE), [**1516**; **MT070401**, **MT070369**, **MT070227**, **MT0701190**, n/a]. Trinity Co.: North Yolla Bolly Mountains, 2.5 mi NW of Stuart Gap, 1402 m, 17 Jul 1951, *Munz 16294* (RSA), [**891**; FJ479700, FJ479699, FJ479698, FJ479697, n/a].

Cuscuta subinclusa Durand & Hilg.: **USA. California.** *Raz & Boyd 15* (NY), [**501**; EF194491, EU883469, EF194701, EU883520, n/a]. *Anderson 3248* (NY), [**644**; EF194490, EU883470, EF194702, EU883521, n/a]. *Ahart 7638* (JEPS), [**649**; **MT070397**, EU883471, EU883487, EU883522, n/a]. Mariposa Co.: Yosemite National Park, Hwy 41 (Wawona Rd.), 2 mi N of Chinquapin, 26 Jun 2013, *Stefanović 13-42* (TRTE, WLU), [**1403**; **MT070398**, **MT070366**, **MT070224**, **MT0701187**, n/a]. San Bernardino Co.: San Bernardino Mts., Pilot Road truck trail, Silverwood Lake, 1 Oct 1995, *Sanders et al. 17902* (RSA), [**961**; EU883439, EU883472, EU883488, EU883523, n/a]. San Diego Co.: 4 miles W. of Hwy 94 on road toward Otay Reservoir, N base of San Ysidro Mts, 20 Apr 1952, *Munz & Balls 17942* (RSA, WTU), [**197**; EF194489, EU883468, EF194703, EU883518, n/a]; Hwy 94, Dulzura, Marron Valley Rd., 32°37′20″N, 116°46′16″W, 3 Apr 2016, *Stefanović 16-09* (TRTE, WLU), [**1609**; **MT070399**, **MT070367**, **MT070225**, **MT0701188**, n/a], on Dehesa Rd., 4.5 mi E of Dehesa, 32°48′22″N, 116°47′35″W, 6 Apr 2016, *Stefanović 16-33* (TRTE, WLU), [**1633**; **MT070400**, **MT070368**, **MT070226**, **MT0701189**, n/a]. San Luis Obispo Co.: just W of junction of Huasna Rd. and Branch Mill Rd., E of Arroyo Grande, 25 Oct 1981, *Keil 14274-1* (ASU), [**484**; EU883438, **MT070365**, EU883486, **MT0701188**, n/a].